Proceedings: Fourth International Zebra Mussel Conference

March 7-10, 1994
Madison, Wisconsin, U.S.A.

Sponsored by
American Water Works Association
American Water Works Association — Research Foundation
Electric Power Research Institute
Environment Canada — Fisheries and Oceans
Great Lakes Sea Grant Network
Province of Ontario
Ontario Hydro
U.S. Fish & Wildlife Service

Prepared by
University of Wisconsin Sea Grant Institute
Advisory Services

P R O C E E D I N G S:

F O U R T H I N T E R N A T I O N A L

Z E B R A M U S S E L C O N F E R E N C E

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ACKNOWLEDGMENTS

It takes many people to make a conference work well and foremost are the authors who prepare and present their papers. Thanks to them for providing the latest and best information available. Over fifty exhibitors presented their products and services during the conference and their efforts and contributions are greatly appreciated. Special thanks to members of the conference planning committee and the University of Wisconsin Sea Grant members who supervised its implementation.

Allen H. Miller
Conference Coordinator
Great Lakes Sea Grant Network
University of Wisconsin Sea Grant Institute

4th International Zebra Mussel Conference Committee

FOREWORD

The introduction of non-native species to eastern North America is a continuing concern to industries, coastal communities and state and federal resource managers. Since the 1800s over 136 different species have been introduced into the Great Lakes basin including 61 plants, 24 fish, 24 algae, 9 mollusks, and 7 oligochaets. None however, has received the notoriety of the now nationally infamous zebra mussel (*Dreissena polymorpha*). Zebra mussels have disrupted flows in water intakes and service lines of power and water utilities, and other industries and are beginning to reveal their impact on the natural environment, especially native clams. Navigation structures along the inland arteries are reporting zebra mussels on locks and in reservoirs. In a few short years the mussels have spread from the Great Lakes to the Hudson, Susquehanna, Ohio, Illinois, Mississippi, Arkansas and Tennessee Rivers. Zebra mussels can now be found in St. Paul, New Orleans, Chicago and Little Rock!

The response to this invader has been one of cooperation between industry, resource management agencies and the academic community in the United States and Canada. Over the past four years, information and ideas have been generously shared through personal contacts and through major conferences. Several year ago an agreement was reached to cosponsor one major international conference, alternating U.S. and Canadian locations. This year, cosponsorship of the conference expanded to add the American Water Works Association and the U.S. Fish and Wildlife Services to the existing network of American Water Works Association Research Foundation, Electric Power Research Institute, Environment Canada (Fisheries and Oceans), Great Lakes Sea Grant Network, Ontario Hydro and the Province of Ontario. We're please to be the first U.S. host and had to work hard to match the past hospitality of our Canadian colleagues.

Over 170 papers were presented during the four-day conference in oral and poster sessions, including papers by five scientists from France, Belorussia, the Ukraine, Germany, and the Netherlands. Participants learned about the first successful attempt to culture zebra mussel larvae in the laboratory under controlled conditions. Twenty papers were given on promising alternative controls including the use of coatings, removal of oxygen and the use of filtration devices, along with updates on potential microbial control agents. Several presentations chronicled the invasion of inland river systems and efforts to keep zebra mussels out of unique environments such as the St. Croix National Scenic Riverway. And, ten high school students who are researching zebra mussels attended the conference. from Oconto and Verona, Wisconsin and Bay City, Michigan, and participants heard of more than 200 High Schools throughout the midwest who are involved in zebra mussel monitoring.

Printed papers were solicited from all authors and 50 chose to publish in these proceedings.

Allen H. Miller
Conference Coordinator

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CONTROL: CHEMICAL

A Continuous-Flow Facility for Zebra Mussel Research and Control

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ABSTRACT

The extensive biofouling by zebra mussels (*Dreissena polymorpha* and *D. bugensis*) presented new demands on industrial water users in North America. Foremost among these was the need to develop and test the effectiveness of techniques that would prevent and control mussel fouling under realistic conditions. Unfortunately, testing facilities to undertake such research and development, using raw lakewater, natural populations of larvae and translocating mussels, were not available. We, therefore, report on a research facility, which was designed and remains in use at Ontario Hydro's Nanticoke Thermal Generating Station on Lake Erie to evaluate control measures for zebra mussels. The facility consists of a continuous-flow system, which supplies raw lakewater to 20 flow cells (12.7 cm diameter x 91 cm in length). The velocity through each flow cell can be controlled to relate treatments options to the biology of mussels, and to assist in the development of monitoring programs. This facility has provided valuable insight into zebra mussel biology and mitigation techniques including the optimization of chlorine treatment. It is believed that the present design provides a better model for such development versus static or open-channel test conditions.

INTRODUCTION

The introduction of zebra mussels (*Dreissena polymorpha* and *D. bugensis*) into North America has impacted residential, municipal, and industrial users of raw lakewater (Nalepa and Schloesser, 1993; Tsou and Mussalli, 1993; Claudi and Mackie, 1994). Principally these impacts have resulted from the colonization or biofouling of intake structures, piping systems, heat exchangers, etc., through the recruitment of veliger larvae and the translocation of adult mussels (Claudi and Ackerman, 1992). Not only do mussels restrict the flow through water-processing equipment, but their byssal attachment mechanisms may increase the potential for corrosion of the colonized surface. The real costs associated with zebra mussel biofouling in the Great Lakes is expected to reach some US $5 billion by the next century (Ludyanskiy *et al.*, 1993).

From the onset of the introduction (Hebert *et al.*, 1989), it became obvious that there was limited European and Soviet data on control strategies that could be scaled to those levels found in many North American Industries (Claudi and Mackie, 1994). For example, the service water at Ontario Hydro's Pickering Nuclear Generating Station uses $3.5 \cdot 10^4$ gal·min^{-1} with fluid residence times on the order of 30 min. In addition, it was evident that much of the data obtained under static conditions in the laboratory, were of limited value to the implementation of control measures within industrial plants. Such data can only be obtained from dynamic-flow-through experiments using raw lakewater containing natural populations of veligers (larvae) and translocating adults. Unfortunately, few designs are available for such facilities. We, therefore, present the following design for a zebra mussel research facility that has operated almost continuously for the past 3 years on Lake Erie. This facility was designed specifically to: 1) determine the settling and translocation behavior of zebra mussels; and 2) evaluate various chemical and physical control strategies to prevent or disrupt zebra mussel biofouling (Table 1). While the facility incorporates design criteria specific to zebra mussels, it will also be of interest to researchers in other fields (e.g., metallurgy, microbiology, etc.) who require dynamic-flow-through conditions.

MATERIALS AND METHODS

Site Description: The research was conducted in the forebay of Ontario Hydro's Nanticoke Thermal Generating Station (NTGS) on the northeast shore of Lake Erie at Nanticoke, Ontario. The forebay is essentially a rectangular channel (~7.4 m to ~ 20.4 m wide, ~780 m long, and ~8.4 m mean depth; Ontario Hydro, 1970) cut from the natural limestone. It runs parallel to the lake shore and is bounded by the concrete wall of the station to the north. Lakewater is drawn 485 m offshore, from a depth of 10 m, into 2 intake structures at the western and central portion of the forebay. There are no other sources of water inputs into the forebay, which serves as a source of unprocessed

Table 1. Design criteria for the continuous-flow facility for zebra mussel research.

Design Features: Physical Systems	**Purpose**
Centrifugal pump	To provide a continuous and large (4000 $l \cdot min^{-1}$) supply of raw lakewater for experiments.
Floating dock	To maintain constant depth in the event of wind-generated changes in lake levels.
Self-cleaning intake filter	To remove coarse material (> 852 μm) such as filamentous algae from system.
Separate banks of flow cells	To provide independence of operation and minimize cross contamination of experiments.
Vacuum break loops on inlet and return lines	To maintain wetted environment in case of malfunction.
Individual flow control on flow cells	To control and manipulate flow rates and fluid residency times experimentally.
Removable flow cells	To facilitate monitoring of experiments.
Flow cell inserts	To provide reliable substrates on which to monitor settlement.
Rotameter flow meters on each flow cell	To measure flow rates in individual flow cells
Temperature and Pressure Sensors	To measure physical condition in the facility.
Data acquisition system	To monitor and record physical and chemical data.
Design Features: Chemical Systems	**Purpose**
Auto-injection chemical feed	To provide continuous and direct feedback and control of chemical injection.
Remote chemical (e.g., chlorine) sensors	To monitor chemical levels in individual flow cells.
Separate upstream and downstream sampling ports	To evaluate within flow cell transport of chemicals.
Bench-top laboratory	To provide for on-site calibration and problem solving.

lakewater for cooling purposes. Additional lakewater is used at the eastern portion of the forebay for tempering the heated effluent discharge in an adjacent and separate channel. There is no backflow from the tempering channel to the forebay.

The average water velocity in the forebay is ~0.15 $m \cdot s^{-1}$, based on a ~155 m^2 cross-sectional area and 23.7 $m^3 \cdot s^{-1}$ flow through the pumping systems (Ontario Hydro, 1970). This regular and rapid flow through the forebay provides a steady supply of zebra mussel veliger larvae. The recruitment under these conditions would likely be similar to other energy intensive areas (e.g., shallow well mixed regions, rivers etc.), leading to ideal conditions in which to develop and test control strategies.

The Research Facility was located at the eastern end of the forebay ~ 9 m above the lake, just downstream of the tempering pumps. Lakewater was drawn through a 1 to 2 cm screen from a constant depth of ~1.5 m, which was achieved through the use of a floating platform. This platform was required because of the changes in water level caused by wind-driven storms and sieches in large basins like Lake Erie (Barnes and Mann, 1991). A single stage vertical turbine 10 Hp pump (Brier Hydraulics, Burlington, Ontario), mounted on the floating platform, pumped lakewater at a capacity of ~$2.2 \cdot 10^{-2}$ $m^3 \cdot s^{-1}$ with a pressure head of ~18 m. Lakewater was delivered through 24 m of 10 cm diameter steel pipe, elevated 1.5 m above the ground, to the Research Facility.

Facility Design: The Research Facility was installed in an air conditioned mobile trailer (3 m x 11 m x 1.85 m), which was divided into a dry lab/office and wet lab sections. The main intake from the floating platform entered the wet lab through a 4" NPT (National Pipe Thread) diameter main, which was connected to a 862 µm self-cleaning filter unit (Hayward Filter model 596; Elizabeth, NJ). This filter limited the introduction of filamentous algae, large zebra mussel translocators, and flotsam, into the water-delivery system. Zebra mussel larvae and small translocators were not, however, affected by the filter. All major piping systems downstream of the filter, including valves and fitting, were primarily PVC schedule 40 NPT. The supply line (3" NPT) passed through a check valve and vacuum break loop (a similar configuration was included on the return line), which maintained a wetted condition in the event of pump malfunction. The supply line delivered water to a supply manifold (3" NPT) which was connected to five separate banks of flow cells systems. Each bank consisted of a vertical supply line, four flow cell systems stacked vertically (~ 0.4 m spacing), and a vertical return line (2" NPT), which connected to a return manifold (3" NPT). The return manifold led into a return line (3" NPT), which passed through the bottom of facility and through a flexible rubber pipe (3" NPT) to the forebay. The banks were differentiated nominally by flow rates, through the use of different diameter vertical supply and return lines. There were three slow-flow banks (3/4" NPT), one medium-flow bank (1" NPT), and one high-flow

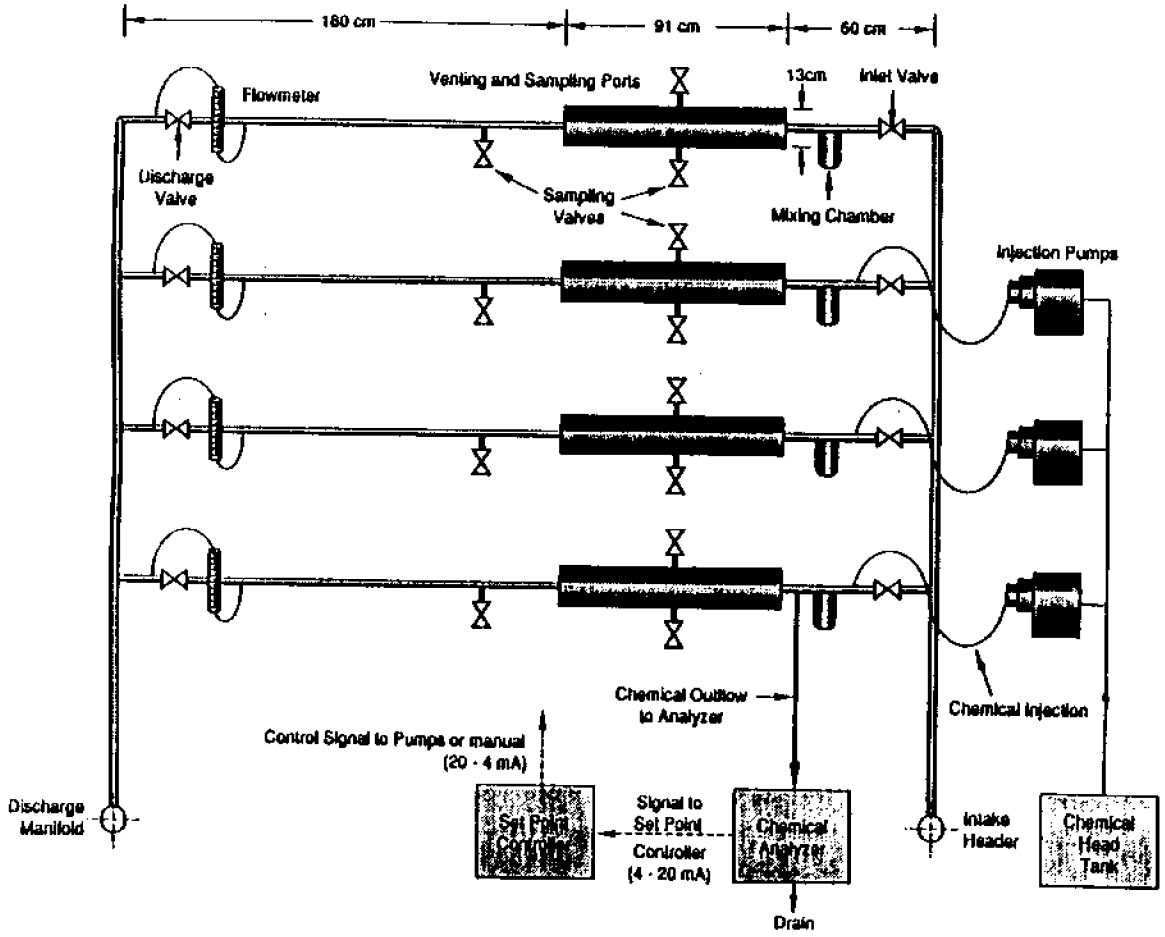

Figure 1. Schematic diagram of the Chemical Injection System included in the Flow Cell System.

bank (2" and 3" NPT). Ball valves at the supply and return lines allowed each bank to be isolated as required. In addition, a bypass line upstream of the supply manifold allowed flow to be diverted to the return line in the case of low flow and, hence minimize the load on the pump.

Flow Cells: The flow cell systems were essentially linear conduits ~3.3 m long (Fig. 1). They consisted of a 60 cm intake line, a 91 cm removable flow cell (12.7 cm diameter), and a 180 cm discharge line. The diameter of the intake and discharge lines depended on the bank on which they were located (see above). Rotameter flow meters were included in the discharge lines with the capacity dependent on the flow rate of the bank (i.e., 0 - 1 $l \cdot min^{-1}$ CalQFlo Flowmeter; Blue White Industries; Westminster, CA; 2 - 20 $gal \cdot min^{-1}$ Visi-Float Flowmeter; Dwyer Instruments; Michigan City, IN). Ball valves directly downstream of the vertical supply line and directly upstream of the vertical return line provided a mechanism for accurate flow control. Two sampling valves (1/4" NPT Thread x Hose Lab Cock) were included opposite on another at mid-length on the flow cell, and a third was included ~30 cm downstream on the discharge line. The flow cells were constructed out of a 91 cm section of 5" NPT pipe welded onto end flanges bored to the same inner diameter and fitted with an O-ring on their outer edge. Matching end flanges were welded onto the intake and discharge lines, which were bored to the respective diameter of the lines. The end flanges were clamped together with 5/16" stainless steel bolts and nut fasteners.

The flow cell systems were modified slightly for the evaluation of chemical control treatments (Fig. 1). In this case, a chemical injector pump (ProMinent, model gamma 4-I; Pittsburgh, PA) was used to introduce oxidants to a mixing chamber 13 cm upstream of the flow cell. The pump was remotely controlled and operated through the use of a chlorine monitoring system (Capital Controls, model 1870E; Colmar, PA) and a PC-based data logger/controller system (Labmate Data Acquisition and Control System; Scimetric Instruments; Ottawa, Ontario). In this way, the predetermined oxidant concentration was maintained throughout the study. Periodic titrations were used to calibrate the monitor.

The majority of the experiments were undertaken at ambient velocities of ~$9.2 \cdot 10^{-4}$ $m \cdot s^{-1}$, which corresponded to the 30 minute residence time for cooling water within generating stations. Based on these flow rates and the physical dimensions of the flow cell, the Reynolds number was ~$1.2 \cdot 10^{2}$ ($Re = d \cdot u / \nu$, where d is the cell diameter, u is the average velocity, and ν is the kinematic viscosity). This would indicate that the flow conditions were laminar, but not fully developed within the flow cell, as the computed entrance length was ~$8.9 \cdot 10^{-1}$ m ($\frac{L_e}{d} \sim 0.06Re$; White, 1986). The developing boundary layer (d) would, therefore, be a function of the length from the entrance (x) given approximately for a flat plate by, $\frac{\delta}{x} \sim \frac{5.0}{Re_x^{1/2}}$, where Re_x is the Re computed using x. Using this relationship, d increases from ~1.6 cm at x = 0.1 m to ~15 cm at x = 0.9 m, which is consistent with the entrance length concept given that overlapping d are indicative of

Table 2. Research Projects Undertaken in Research Facility
(A bibliography is available upon written request)

A) Zebra Mussel Biology.	
(i) Larval Biology:	- Life history characterization and morphometrics
	- Larval density
	- Larval recruitment dynamics
	- Larval recruitment mechanisms
(ii) Mussel Biology:	- Mussel attachment strength
	- Mussel density
	- Mussel contaminant load /toxicity
	- Mussel live/dead determination
B) Zebra Mussel Control.	
(i) Physical Controls:	- Acoustics
	- Anti-fouling coatings
	- Fluid dynamics
	- Mechanical filtration
	- UV radiation
(ii) Chemical Controls:	- Hypochlorite - Intermittent treatment
	- Continuous treatment
	- Semi-continuous treatment
	- Copper hypochlorite
	- Chlorine electrolysis
C) Non-biological Research.	- Hypochlorite metal corrosion
	- Under-deposit corrosion
	- Dechlorination with $Na_2S_2O_3$
	- Chlorine monitor evaluation
	- Water temperature and chemistry

fully-developed flow. Newly-recruited mussels would thus always exist within a boundary layer. Calculations indicated that the maximum oxygen demand by mussels would be easily offset by Fickian diffusion through the boundary layer (i.e., $J_y = -D \frac{\partial C}{\partial y}$, where J is the flux, D is the diffusivity, C is the concentration, and y is the height).

RESULTS AND DISCUSSION

The modular design of the research facility allowed the examination of a wide range of research topics, many of which were examined simultaneously. The precise number of investigations per season was limited by the number of flow cells and the need for experimental replication. Fortunately, it was possible, in some cases, to conduct several trials within a single field season, although variations in larval recruitment precluded this option in 1992 (see below). The complete range of topics examined over the past three years have included: A) aspects of zebra mussel larval and adult biology; B) physical and chemical zebra mussel control technologies; and C) a number of non-biological research topics (Table 2). Table 2 is provided as an illustration of the type of research that has been undertaken and is, therefore, not meant as a review. A complete bibliography of this research is available on request.

One of the most relevant biological parameters for zebra mussel control research is the presence of larvae in the water column. This provides an indication of the period when larvae may recruit onto substrates and the period during which experiments can be conducted. It should be noted, however, that zebra mussel veliger larvae pass through a number of developmental stages, the last of which involves the acquisition of foot -- the pediveliger stage (Ackerman *et al.*, In Press). While pediveligers are the only veligers competent to recruit onto substrates (Claudi and Ackerman, 1992; Ackerman *et al.*, In Press), few studies have differentiated among the different larval stages. Hence, presence/absence of veligers only provides a measure of the potential for larval recruitment. Important issues, such as the influence of fluid dynamics on recruitment are presented elsewhere (Ackerman and Sim, 1994; In Prep).

A comparison of the interannual variation in daily water temperatures measured in the intake water of the research facility is presented in Fig. 2A. Observation were made between May and November, although delays in the initial commissioning of the facility in 1991 limited the data set to the latter portion of the season. The daily water temperature data are similar for all years and show a seasonal pattern, with the highest temperatures observed in July and August. There were major deviations in these patterns as indicated by the relatively low temperatures in the end of September 1991, and the relatively high temperatures in May 1992. These and smaller-scale excursions were likely caused by ambient air temperatures as well as mixing due to storms, which may influence the temperatures of near-shore surface waters (Barnes and Mann, 1991). The temperatures in the spring of 1992 were relatively warmer than those of 1993, although July and August 1992 water temperatures were generally lower than those of 1991 and 1993. The maximum water temperatures during the summers were 25.8°C in 1991, 23.4°C in 1992,

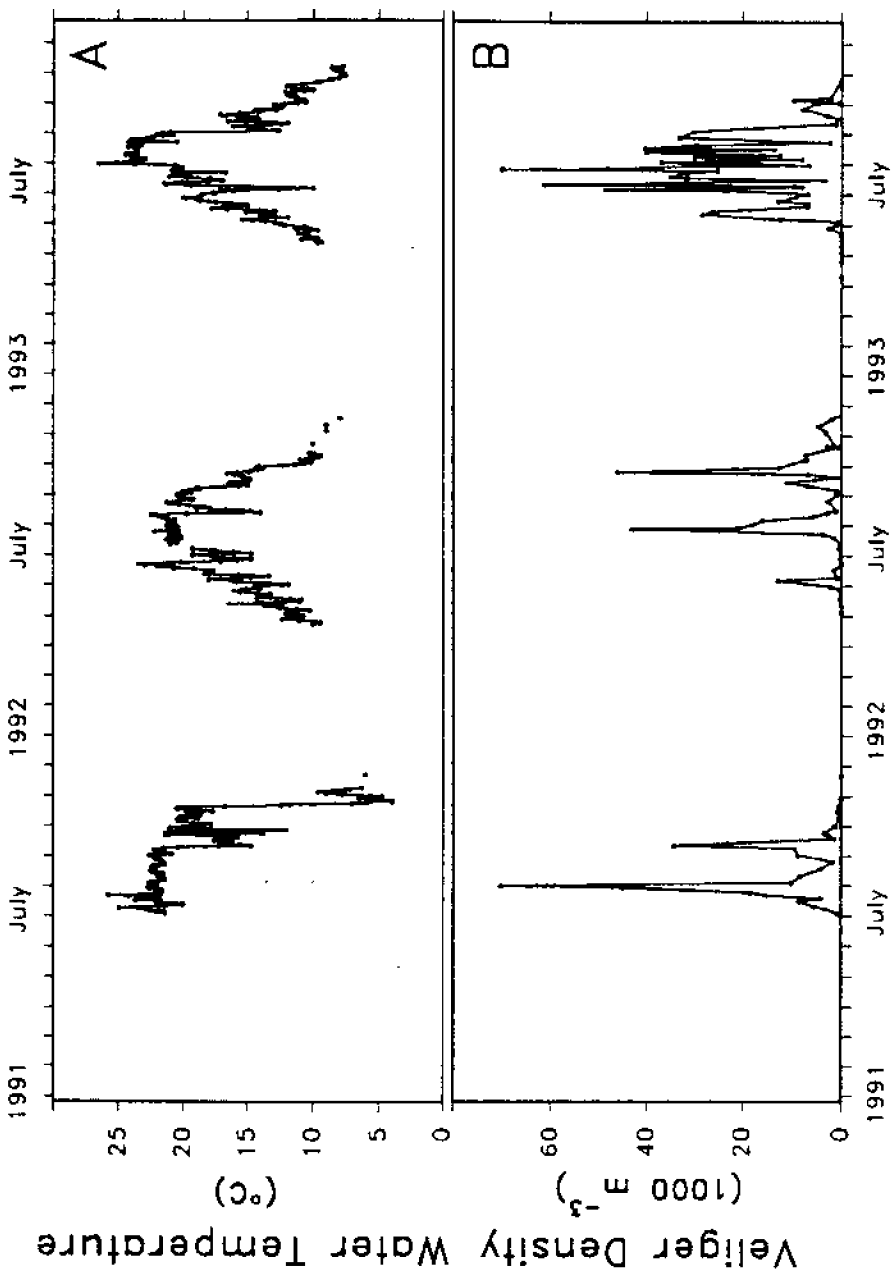

Figure 2. Interannual variation in (A) water temperature and (B) veliger density in the intake water of the Facility.

and 26.5°C in 1993. The fall water temperatures in 1991 were generally higher than those of 1992 and 1993, which were similar.

The abundance of veligers in the intake water of the research facility is presented in Fig. 2B. The average density of veligers varied among years from $11\pm3\cdot10^3$ (mean ± standard error; n = 29) veligers·m^{-3} in 1991, $5\pm1\cdot10^3$ (n = 54) veligers·m^{-3} in 1992, and $14\pm2\cdot10^3$ (n = 71) veligers·m^{-3} in 1993. These differences may have affected the level of recruitment (e.g., Ackerman *et al.*, 1993; In Review), and is an area that deserved additional attention. General annual patterns are more difficult to discern, although there were a number of peaks in each year when the veliger densities exceeded the background to levels as high as $70\cdot10^3$ veligers·m^{-3}. Overall, there were approximately three peaks corresponding to early July, early August, and mid to late September. Commissioning delays in 1991 precluded the identification of the first peak in 1991, while intensive sampling in 1993 indicated that the middle peak may have been more extensive in nature. The 1993 data also indicates that the day-to-day variation in veliger abundance can vary considerably at any location. The sudden appearance, and multiple number of peaks in veliger densities have also been seen in other years within Lake Erie (Garton and Haag, 1993). This would support the concept that the distribution and abundance of zebra mussel veligers varies both spatially and temporally similar to other planktonic organisms (Mann and Lazier, 1991).

CONCLUSIONS

- A modular design can be easily modified for a wide range of zebra mussel research and control topics.
- Trials should be conducted using natural populations of zebra mussel veligers and translocators
- Direct on-site observations of mussels can lead to important and significant findings.
- Larval densities in intake waters may vary temporally over short-time scales.
- Flow-through systems provide important results which are valid models for plant systems.

ACKNOWLEDGEMENTS

The following individuals are acknowledged for their assistance during the design, construction, deployment, and operation of the facility: 1) From NTGS - Bruno Powleski, Sumantha Jayabarathan, and John Walker; 2) From OHT - John Cocuzzi, A. Brennenstuhl, E.G. Leitch, and Bob Koopmans; 3) and Doug Barker from J.J. Downs Industrial Plastics. Support for this work was provided by research funds from Ontario Hydro's Environmental Protection Department.

LITERATURE CITED

Ackerman, J. D., C. R. Ethier, D. G. Allen, and J. K. Spelt. 1993. Patterns of recruitment and persistence of zebra mussels on a variety of materials. pp. 2:211-121 in J. L. Tsou and Y. G. Mussalli (eds.) *Proceedings, Third International Zebra Mussel Conference, 1993.* TR-102077, EPRI, Palo Alto, CA.

Ackerman, J. D. and B. Sim. 1994. Fluid dynamic influences on the recruitment of zebra mussels. *Abstracts, Fourth International Zebra Mussel Research Conference*, Madison, WI, March 1994.

Ackerman, J. D., B. Sim, S. J. Nichols, and R. Claudi. In Press. A review of the early life history of the zebra mussel (*Dreissena polymorpha*): Comparisons with marine bivalves. *Can. J. Zool.*

Ackerman, J. D., C. R. Ethier, D. G. Allen, and J. K. Spelt. In Review. Recruitment and persistence of zebra mussels onto various materials of differing surface roughness. *Environ. Sci. & Technol.*

Barnes, R. S. K. and K. H. Mann. 1991. *Fundamentals of Aquatic Ecology.* Blackwell, London, UK.

Claudi, R. and Ackerman J. D. 1992. Post settlement movements of juvenile zebra mussels and their potential impact on plant system. *Corrosion '92. NACE*, Houston, TX, 346/1-6.

Claudi, R. and Mackie, G. L. 1994. *Practical Manual for Zebra Mussel Monitoring and Control.* Lewis Press, Boca Raton, Florida, USA.

Garton, D. W. and W. R. Haag. 1993. Seasonal reproductive cycles and settlement patterns of *Dreissena polymorpha* in Western Lake Erie. pp. 111-128 in T. F. Nalepa and D. W. Schloesser (eds.) *Zebra Mussels: Biology, Impact, and Control.* Lewis Press, Boca Raton, Florida, USA.

Hebert, P. D. N., Muncaster, B. W. and Mackie, G. 1989. Ecological and genetic studies on *Dreissena polymorpha* (Pallas): a new mollusc in the Great Lakes (1989). *Can. J. Fish. Aquat. Sci.* 46:1587-1591.

Ludyanskiy, M. L., D. McDonald, and D. MacNeill. 1993. Impact of the zebra mussel, a bivalve invader. *Bioscience* 43:533-544.

Mann, K. H. and J. R. N. Lazier. 1991. *Dynamics of Marine Ecosystems.* Blackwell, London, UK.

Nalepa, T. and D. W. Schloesser. 1993. *Zebra mussels: Biology, Impacts and Control.* Lewis Press, Boca Raton, Florida, USA.

Ontario Hydro. 1970. *Ontario Hydro Nanticoke Generating Station Commissioning and Operating Guide.* Ontario Hydro, 700 University Avenue, Toronto, Ontario, Canada.

Tsou, J. L. and Y. G. Mussalli. 1993. *Proceedings, Third International Zebra Mussel Conference, 1993.* TR-102077, EPRI, Palo Alto, CA.

White, F. M. 1986. *Fluid Mechanics, 2nd Ed.* McGraw Hill, New York, USA.

Evaluation of Copper Ions and Aluminum Floc for Preventing Settlement of Zebra Mussels

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Abstract

The controlled electrolytic dissolution of copper and aluminum anodes to inhibit macrofouling in seawater intakes and piping systems has been employed for over 30 years. It was on this basis that we undertook development of this proven macrofouling control technology for preventing settlement of zebra mussels at the post-veliger stage.

The authors discuss the results of field evaluation conducted during the Summer 1993 at the City of Toledo's intake structure, which is located 2.9 miles offshore in Lake Erie. The copper and aluminum ions were electrolytically generated by specially designed cells supplied by *MacroTech Inc.*

Field testing was performed by Dr. Fraleigh. The test set-up consisted of 24 flow-thru bioboxes being fed by raw lake water. The bioboxes were divided among untreated, copper + aluminum at several concentrations, and copper only at several concentrations.

Weekly ambient veliger counts were taken and a slide from each biobox was removed for mussels settlement count. Chemical analyses were performed weekly by the City of Toledo.

INTRODUCTION

During the summer of 1988 large numbers of zebra mussels *Dreissena polymorpha* (Pallas, 1771) were first seen in Lake St. Clair, probably the result of a ship's ballast water being discharged into the lake a couple of years earlier. By the end of the 1989 growing season zebra mussels had established themselves in Lakes Erie, Ontario, Superior, Michigan and the St. Lawrence River causing enormous problems to electric utilities, water treatment plants, etc. The infestation has continued to spread into Lake Huron, the Mississippi and Ohio River systems, to the point where most bodies of water throughout the eastern U.S.A. and Canada are or soon will be infested, as shown in the New York Sea Grant's compilation *Sightings - North American Range of the Zebra Mussel.*

The adult zebra mussel, a native European bivalve (clam) is a prolific breeder and attaches itself to any substratum (rocks, boats, piers, pipes and each other) by secreting horny sticky threads by means of their byssus apparatus.

Egg fertilization and production occurs in the adult zebra mussel within hours of the water temperature rising above 12°C. After hatching, and as long as the water temperature stays between 14 and 24°C, the free swimming larvae or veligers (40-70 μm in diameter) will appear in the plankton. At this stage dispersal in the water is due primarily to water currents.

Development changes occur in the veligers and they grow rapidly to 150-250 μm in diameter, during which time they grow a rudimentary clam-like shell. When the shell grows too heavy to allow free swimming the zebra mussel settles and attaches itself to a suitable substratum by it's byssus threads. At this stage it is called a postveliger and by the end of the first growing season it has taken on the appearance of the adult mussel. Also, by the end of the first growing season or the beginning of the second year the female zebra mussel is sexually mature to produce 30,000 to 40,000 eggs.

FRESH WATER BIOFOULING PROBLEMS AND SOLUTIONS

The effectiveness of the copper ion as a toxin to marine organisms has been known for centuries. Controlled release of copper at parts-per-billion (ppb) levels effectively inhibits the attachment and growth of algae, mussels, oysters, barnacles, etc. Furthermore, maturing (live) organisms have a threshhold tolerance to copper and by exceeding ambient levels they will exfoliate, thereby gradually cleaning out an already fouled system.

The effectiveness of alum as a floculating agent for water purification is likewise well known. When dissolved in water a highly-hydrated aluminum hydroxide floc forms which, when used in conjunction with copper, complexes the ionic copper, settling it in those lower and laminar flow areas most susceptible to settlement, thereby maximizing its anti-fouling properties. It has also been postulated that the aluminum hydroxide floc may entrap veligers and prevent their attachment and growth.

Although non-toxic in itself, it inhibits the settlement of veligers by smothering these organisms in the gel and taking them out through the discharge back into the lake or river. A further advantage of the aluminum hydroxide is that it coats the surface of an enclosed system such as pipework, which discourages the initial bacteria/organism layer (biofilm) from forming, thus further inhibiting zebra mussel attachment.

Applying technology developed over 30 years ago, the Blume Antifouling System (BAS) employs the controlled electrolytic dissolution of copper and aluminum anodes, to produce the copper ions and aluminum floc, to inhibit macrofouling in seawater systems.[1, 2, 3] The BAS has been employed extensively on ships, offshore drilling rigs, oil & gas platforms, and coastal installations worldwide.

It was on the foregoing basis that *MACROTECH* undertook development of this proven macrofouling control technology for controlling the fresh water zebra mussel and asiatic clam.

Because of the conductivity of sea water it is possible to use the water as an effective electrolyte when used in conjunction with copper and aluminum anodes as described above. There are, however, a number of problems arising when trying to adapt the system to work in fresh water. Although this is a reasonably simple arrangement in sea water the same arrangement in fresh water would result in current being passed only in the milliamp range, when usually a current in the 0.5 to 2.5 amp range would be needed.

To employ the seawater design in this fresh water application would require increasing the voltage to uneconomically high levels. The problem of the low conductivity of fresh water has been overcome with the development of the *MACROTECH* Treatment Unit, which uses anodes and cathodes in a proprietary cell design.

Fresh water is pumped through the cell which, in conjunction with the electrical controller feeding current to the specially designed anodes, causes conductivity in the water and the anodes then produce the required copper ions and aluminum floc at reasonable voltage levels. The treated water is then injected into pipework requiring protection.

The unit is of modular design so that the number of cells can be increased to meet the demands of widely varying flowrates to be treated. Each standard cell module will treat a flowrate of 5,000 gpm. Also, smaller cells [1,000 - 5,000 gpm] can be supplied as required.

There is also the question of power supply to a large number of cells. The power requirement is $\leq$ 100 watts per cell so that a cell for treating 10,000 gpm would reqire $\leq$ 200 watts, a 20,000 gpm Unit would require $\leq$ 400 watts, and so on.

The *MACROTECH* Treatment Unit works much the same way as a sea water system and controls the fouling of pipework in two ways. First, by the electrolytic dissolution of toxic copper into the water, thus killing the veligers and inhibiting adult settlement by reducing their ability to attach to the substratum as they become increasingly unable to secrete byssus threads (their attachment structures). The target level of copper added to the water is 10 ppb above the background levels - an environmentally acceptable level.

FIELD DEVELOPMENT

1991

A prototype unit was installed at NYSEG's Kintigh Generating Station (Somerset, NY) in July, 1991 to test the performance of the system hardware and the effectiveness for zebra mussel control. Despite several minor component problems the performance data (i.e., evaluation of the controller and cell operation in fresh water) was very encouraging. These tests proved beyond any doubt that the cell will operate in fresh water at very acceptable power consumption levels.

Unfortunately several permitting delays resulted in the unit not being allowed to be continuously on line until mid-September. Since veliger counts being conducted for NYSEG by Beak Consultants had shown a sharp decline by then, insufficient growing season remained to develop conclusive zebra mussel control data in 1991.

Based on the encouraging results and experience gained with the unit at Kintigh, very significant design modifications were made over the following Winter.

1992

Our 1992 test program was designed to both evaluate the cell design modifications and to demonstrate the efficacy of the technology for inhibiting veliger settlement. Ontario Hydro agreed to include evaluation of the *MACROTECH* Fresh Unit in the 1992 test program for their zebra mussel field test rig[4] based at their Nanticoke Thermal Generating Station.

Permitting delays once again did not allow the unit to be fully operational in 1992. Fortunately, data developed during conditioning of the unit did confirm several orders of magnitude reduction in veliger settlement rates.

1993

This study, conducted at the City of Toledo's water intake in Lake Erie, investigated the effects of electrolytically produced copper and aluminum to inhibit settling of zebra mussels. The tests were conducted by introducing various amounts of copper solution into flow-through bioboxes [Figs. 1 & 2] and following settling on glass slides placed in the bioboxes. Samples were collected and analyzed for copper concentrations and slides were counted for densities of settled mussels.

During the first several weeks of the study (in August 1993) settlement was found to decrease with increasing total copper concentrations among the bioboxes treated with electrolytically produced copper and aluminum.

On August 25th two replicate slides from compartment 1 of each biobox were counted. This was 13 days after treatment was begun, which was the same day that the slides had been put in the bioboxes. Among the bioboxes arithmetic mean numbers settled per slide did not vary between slides 1 and 2 among the bioboxes, by two-way analysis of variance [Table 1]. In addition, natural logarithm transforms of the number per slide + 1, which is a commonly used transformation for densities, were analyzed by two-way analysis of variance and geometric means per biobox [= (antilog of the arithmetric mean of the transformed values) - 1] were calculated [Table 2].

On August 17th and August 23rd inflow and outflow samples were analyzed for total copper concentrations. For each day geometric means were calculated [Tables 4 & 6]. Among bioboxes geometric mean total total copper varied significantly among bioboxes on August 23rd [Table 6]. Using these geometric means for August 17th and August 23rd geometric means were found for each biobox [Table 7].

The geometric mean total copper for August 17th and August 23rd, using geometric means of inflows and outflows for each day and for each biobox, were compared to geometric mean settling on August 25th [Table 8 and Figs. 3 & 4]. Among the bioboxes, settling decreased as the total copper concentration increased. Geometric mean settling decreased linearly as the log of the total copper concentration increased, with a significant correlation coefficient [r = -.5986], as shown in Fig. 2. Using the regression relationship from this analysis, predicted number settled and percent of untreated [with a mean total Cu = 1.189 ppb] were calculated [Table 9]. With a total copper concentration of 10 ppb a density of 4.3/slide or 1,484/m², or 42.5 % of numbers in untreated bioboxes, settled on the slides.

Finally, a best fit of the copper concentration against veliger settlemetnt data was made covering the duration of the test program and is presented in Fig. 5. This also clearly shows the decrease in settlement as copper concentration increased.

Based on the foregoing results a 10 ppb operating level will achieve roughly 60% reduction in settlement during the Summer which (based on our marine experience), coupled with removal of settled juvenile veligers during the Winter as their absorbed copper level approaches toxic level resulting in exfoliation, should result in complete control of zebra mussel infestation.

1994

A full-scale installation is currently treating the 2,550 gpm service water system at NYSEG's Greenidge [Dresden, NY] Station on Seneca Lake. This plant experienced both zebra mussel and asiatic clam infestations in 1993.

ACKNOWLEDGEMENTS

We wish to express our deep appreciation to NYSEG and to the DEC for their interest in and support of our work during 1991. Particular notes of thanks go to Ray Tuttle for his assistance in making this project possible at NYSEG; to Kevin Robison, Jerry Morgan, Jack White and John Ahlstrom for the invaluable services they provided at Kintigh; and to Alan Fairbanks and Gary Keipper of Beak Consultants for their hands-on support throughout this project.

We also wish to express our deep appreciation to Ontario Hydro for their interest in and support of our work during 1992. Special thanks again goes to Ray Tuttle for his continuing encouragement and support; to Renata Claudi, Fred Spencer and Dave Evans for their support at Ontario Hydro, to Blair Sim for his invaluable field support, and to the Nanticoke Thermal Generating Station for allowing us to work at their facility.

Our 1993 work received invaluable support from the City of Toledo and from Bob Stevenson and the entire staff at the City's Collins Park Water Treatment Plant.

REFERENCES

1. William J. Blume & Barry J. Kirk, "An Electrolytic System for Controlling Marine Growth and Corrosion in Sea Water Service Systems," 1982 Offshore Technology Conference [Paper No. 4364].

2. William J. Blume, "Control of Marine Growth, Corrosion and Scale Formation in Sea Water Systems," The Society Of Naval Architects and Marine Engineers, Paper presented to the Metropolitan New York Section, January 12, 1988.

3. Proceedings: Third International Zebra Mussel Conference 1993, EPRI TR-102077 [sec. 3, pp. 295-306].

4. "Zebra Mussel Field Test Rig," Catalogue of Ontario Zebra Mussel Research Projects 1990-91, Report 91.5 (Page 81).

Table 1 Analysis of Variance of Total Copper

Numbers Per Slide for Two Reps on 8/25/93

Biobox	Dose	Slide 1	Slide 2	Total	Sum YY	CT	Mean	Variance	SE
B-1	0	34	8	42	1220	882	21	338	13
B-2	2	6	10	16	136	128	8	8	2
B-3	16	1	2	3	5	4.5	1.5	0.5	0.5
B-4	4	0	17	17	280	144.5	8.5	144.5	8.5
B-5	12	1	4	5	17	12.5	2.5	4.5	1.5
B-6	8	15	7	22	274	242	11	32	4
C-1	8	0	6	6	35	18	3	18	3
C-2	0	4	8	12	80	72	6	8	2
C-3	16	2	1	3	5	4.5	1.5	0.5	0.5
C-4	12	4	1	5	17	12.5	2.5	4.5	1.5
C-5	4	5	12	17	169	144.5	8.5	24.5	3.5
C-6	2	0	1	1	1	0.5	0.5	0.5	0.5
	n	12	12	24					
	Sum	72	77	149	2249	1665.5	74.5	583.5	
	SumYY	1480	769	2249					
	CT	432	494.08	926.08					
	Mean	6	6.4167	12.417					
	Variance	1048	274.92	1322.9					
	SE	9.35	4.79						

Summary	Numbers
total n	24
Sum Y	149
Sum YY	2249
grand CT	925.042
Total SS	1323.96

Columns		Rows
Sum CT	926.08	1665.5
SS col	1.0417	740.46
df col	1	11

ANOVA TABLE - 1 two-way

Source		df	SS	MS	F	
Rows		11	740.46	67.31	1.271	ns
Cols		1	1.042	1.042	0.02	ns
Error		11	582.46	52.95		
Total		23	1323.96			

ANOVA TABLE - one-way

Source		df	SS	MS	F	
among	rows	11	740.46	67.31	1.384	ns
within	rows	12	583.5	48.625		
	total	23	1323.96			

Table 2 Analysis of Variance of Total Copper										
Numbers Per Slide for Two Reps on 8/25/93						ln (Y+1) Values				
Biobox	Dose	Slide 1	Slide 2	Total	Sum YY	CT	Mean	Variance	SE	GM
B-1	0	3.555	2.197	5.7526	17.4683	16.546	2.8763	0.9222	0.6791	16.748
B-2	2	1.946	2.398	4.3438	9.5365	9.4343	2.1719	0.1	0.226	7.775
B-3	16	0.693	1.099	1.7918	1.6874	1.6052	0.8959	0.0822	0.2	1.449
B-4	4	0	2.89	2.8904	8.3642	4.1771	1.4452	4.1771	77.1.44 52	3.243
B-5	12	0.693	1.609	2.3026	3.0707	2.6509	1.1513	0.4198	0.4581	2.162
B-6	8	2.773	2.079	4.652	12.0113	11.7711	2.426	0.24	0.3468	10.314
C-1	8	0	1.946	1.9459	3.7866	1.8933	0.973	1.8933	0.973	1.646
C-2	0	1.609	2.197	3.8067	7.4181	7.2453	1.9033	0.1727	0.2939	5.708
C-3	16	1.099	0.693	1.7918	1.6874	1.6052	0.8959	0.0822	0.2	1.449
C-4	12	1.609	0.693	2.3026	3.0707	2.6509	1.1513	0.4198	0.4581	2.162
C-5	4	1.792	2.565	4.3567	9.7894	9.4905	2.1784	0.2989	0.3866	7.832
C-6	2	0	0.693	0.6931	0.48	0.24	0.3466	0.24	0.3466	0.414
	n	12	12	24						
	Sum	15.769	21.06	36.8299	78.3611	69.31	18.415	9.0509		
	SumYY	34.673	43.688	78.3611						
	CT	20.723	36.962	57.6849						
	Mean	1.31	1.76	3.07						
	Variance	13.95	6.726	20.676						
	SE	1.08	0.75							
	GM	2.721	4.784							
Summary	Number			ANOVA TABLE -1 two-way						
total n	24		Source		df	SS	MS	F		
Sum Y	36.8299		Rows		11	12.7918	1.1629	1.622	ns	
Sum YY	78.3611		Cols		1	1.66498	1.6649	1.627	ns	
grand CT	56.5184		Error		11	7.8844	0.7167			
Total SS	21.8427		Total		23	21.8427				
Columns		Rows								
Sum CT	57.6849	69.31		ANOVA TABLE - one-way						
SS col	1.1665	12.792		Source	df	SS	MS	F		
df col	1	11	among rows		11	12.792	1.1629	1.384	ns	
			within rows		12	9.0509	0.7542			
				total	23	21.8427				

Table 3 Analysis of Variance of Total Copper

Total Copper in PPB on 8/17/93									
Biobox	Dose	Inflow	Outflow	Total	Sum YY	CT	Mean	Variance	SE
B-1	0	1	1	2	2	2	1	0	0
B-2	2	7	107	114	11498	6498	57	5000	50
B-3	16	5	144	149	20761	11100.5	74.5	9660.5	69.5
B-4	4	5	110	115	12125	6612.2	57.5	5512.5	52.5
B-5	12	37	180	217	33769	23544.5	108.5	10224.5	71.5
B-6	8	5	6	11	61	60.5	5.5	0.5	0.5
C-1	8	3	5	8	34	32	4	2	1
C-2	0	1	1	2	2	2	1	0	0
C-3	16	11	1	12	122	72	6	50	5
C-4	12	10	11	21	221	220.5	10.5	0.5	0.5
C-5	4	28	60	88	4384	3872	44	512	16
C-6	2	1	30	31	901	480.5	15.5	420.5	14.5
	n	12	12	24					
	Sum	114	656	770	83880	52497	385	31383	
	SumYY	2510	81370	83880					
	CT	1083	35861	36944					
	Mean	9.5	54.667	64.167					
	Variance	1427	45508	46935					
	SE	10.9	61.58						

Summary	Number			ANOVA TABLE -1		two-way			
total n	24		Source		df	SS	MS	F	
Sum Y	770		Rows		11	27792.8	2526.6	1.452	ns
Sum YY	83880		Cols		1	12240	12240	7.034	ns
grand CT	24704		Error		11	19142.8	1740.3		
Total SS	59175.8		Total		23	59175.8			
Columns		Rows							
Sum CT	36944.3	52497		ANOVA TABLE - one-way					
SS col	12240.2	27792		Source	df	SS	MS	F	
df col	1	11	among	rows	11	27792.8	2526.6	0.966	ns
			within	rows	12	31383	2615.25		
				total	23	59175.8			

Table 4 Analysis of Variance of Total Copper

Total Copper in PPB on 8/17/93				ln transforms						
Biobox	Dose	Inflow	Outflow	Total	Sum YY	CT	Mean	Variance	SE	GM
B-1	0	0	0	0	0	0	0	0	0	1
B-2	2	1.946	4.673	6.6187	25.622	21.904	3.3094	3.718	1.3635	27.368
B-3	16	1.609	4.97	6.5793	27.2893	21.6433	3.2896	5.6461	1.6802	26.833
B-4	4	1.609	4.7	6.3099	24.6848	19.9075	3.155	4.7773	1.5455	23.452
B-5	12	3.611	5.193	6.8039	40.0055	38.7541	4.4019	1.2514	0.791	81.609
B-6	8	1.609	1.792	3.4012	5.8007	5.7841	1.7006	0.0166	0.0912	5.477
C-1	8	1.099	1.609	2.7081	3.7972	3.6668	1.354	0.13	0.2554	3.873
C-2	0	0	0	0	0	0	0	0	0	1
C-3	16	2.398	0	2.3979	5.7499	2.875	1.1989	2.875	1.1989	3.317
C-4	12	2.303	2.398	4.7005	11.0518	11.0473	2.3502	0.0045	0.0477	10.488
C-5	4	3.332	4.094	7.4265	27.8672	27.5768	3.7133	0.29	0.3811	40.988
C-6	2	0	3.401	3.4012	11.5681	5.7841	1.7006	5.7841	1.7006	5.477
	n	12	12	24						
	Sum	19.516	32.831	52.347	183.4366	158.943	26.174	24.494		
	SumYY	47.958	135.48	183.44						
	CT	31.741	89.82	121.56						
	Mean	1.63	2.74	4.36						
	Variance	16.218	45.657	61.874						
	SE	1.16	1.95							
	GM	5.085	15.424	78.434						
Summary	Number			ANOVA TABLE -1		two-way				
total n	24		Source		df	SS	MS	F		
Sum Y	52.347		Rows		11	44.7667	4.0697	2.617	nc	
Sum YY	183.436		Cols		1	7.3862	7.3862	4.749	nc	
grand CT	114.176		Error		11	17.108	1.5552			
Total SS	69.26		Total		23	69.26				
Columns		Rows								
Sum CT	121.562	158.94		ANOVA TABLE - one-way						
SS col	7.3862	44.766		Source	df	SS	MS	F		
df col	1	11	among	rows	11	44.767	4.0697	1.994	ns	
			within	rows	12	24.494	2.0412			
				total	23	69.261				

Table 5 Analysis of Variance of Total Copper

Total Copper in PPB on 8/23/93

Biobox	Dose	Inflow	Outflow	Total	Sum YY	CT	Mean	Variance	SE
B-1	0	2	1	3	6	4.5	1.5	0.5	0.5
B-2	2	2	2	4	8	8	2	0	0
B-3	16	6	57	63	3285	1984.5	31.5	1300.5	25.5
B-4	4	8	16	24	320	288	12	32	4
B-5	12	18	22	40	808	800	20	8	2
B-6	8	7	6	13	85	84.5	6.5	0.5	0.5
C-1	8	8	45	53	2089	1404.5	26.5	684.5	18.5
C-2	0	2	1	3	5	4.5	1.5	0.5	0.5
C-3	16	2	10	12	104	72	6	32	4
C-4	12	7	8	15	133	112.5	7.5	0.5	0.5
C-5	4	3	3	6	18	18	3	0	0
C-6	2	12	50	62	2644	1922	31	722	19
	n	12	12	24					
	Sum	77	221	298	9484	6703	149	2781	
	SumYY	755	8729	9484					
	CT	494.08	4070.1	4564.17					
	Mean	6.42	18.417	24.8333					
	Variance	260.92	4658.9	4919.83					
	SE	4.66	19.7						

Summary	Number
total n	24
Sum Y	298
Sum YY	9484
grand CT	3700.17
Total SS	5783.83

Columns		Rows
Sum CT	4564.17	6703
SS col	864	3002.8
df col	1	11

ANOVA TABLE -1 two-way

Source		df	SS	MS	F	
Rows		11	3002.83	272.98	1.566	ns
Cols		1	864	864	4.958	ns
Error		11	1917	174.27		
Total		23	5783.83			

ANOVA TABLE - one-way

Source	df	SS	MS	F	
among rows	11	3002.8	272.98	1.178	ns
within rows	12	2781	231.75		
total	23	5783.83			

Table 6 Analysis of Variance of Total Copper

Total Copper in PPB on 8/23/93				In transforms						
Biobox	Dose	Inflow	Outflow	Total	Sum YY	CT	Mean	Variance	SE	GM
B-1	0	0.693	0	0.693	0.45	0.24	0.3466	0.24	0.3466	1.414
B-2	2	0.693	0.693	1.3863	0.96	0.96	0.6931	0	0	2
B-3	16	1.792	4.043	5.8348	19.5567	17.0225	2.9174	2.5342	1.1256	18.493
B-4	4	2.079	2.773	4.852	12.0113	11.7711	2.426	0.24	0.3466	11.314
B-5	12	2.89	3.091	5.9814	17.9088	17.8887	2.9907	0.02	0.1	19.9
B-6	8	1.946	1.792	3.7377	6.997	6.9851	1.8688	0.0119	0.0771	6.481
C-1	8	2.079	3.807	5.8861	18.8148	17.3231	2.9431	1.4916	0.8636	18.974
C-2	0	0.693	0	0.693	0.48	0.24	0.3466	0.24	0.3466	1.414
C-3	16	0.693	2.303	2.9957	5.7824	4.4872	1.4979	1.2951	0.8	4.472
C-4	12	1.946	2.079	4.0254	8.1106	8.1017	2.0127	0.0069	0.0688	7.483
C-5	4	1.099	1.099	2.1972	2.4139	2.4139	1.0986	0	0	3
C-6	2	2.485	3.912	6.3969	21.4787	20.4604	3.1985	1.0183	0.7136	24.495
	n	12	12	24						
	Sum	19.089	25.591	44.6798	114.996	107.895	22.34	7.10089		
	SumYY	37.089	77.906	114.996						
	CT	30.366	54.575	84.94						
	Mean	1.59	2.13	3.72						
	Variance	6.724	23.332	30.056						
	SE	0.75	1.39							
	GM	4.907	8.437	41.402						
Summary	Number			ANOVA TABLE -1		two-way				
total n	24		Source		df	SS	MS	F		
Sum Y	44.6799		Rows		11	24.716	2.2469	4.629	**	
Sum YY	114.996		Cols		1	1.7615	1.7615	3.629	ns	
grand CT	83.1787		Error		11	5.339	0.4854			
Total SS	31.817		Total		23	31.817				
Columns		Rows								
Sum CT	84.94	107.89		ANOVA TABLE - one-way						
SS col	1.7615	24.7163		Source	df	SS	MS	F		
df col	1	11	among	rows	11	24.716	2.2469	3.797	*	
			within	rows	12	7.10089	0.5917			
				total	23	31.817				

Table 7 Analysis of Variance of Total Copper

Mean Concentration			(GM of	In & Out	Flows)				
Biobox	Dose	Aug17	Aug23	Total	Sum YY	CT	Mean	Variance	SE
B-1	0	1	1.414	2.414	2.9994	2.9137	1.2	0.1	0.2
B-2	2	27.368	2	29.368	753	431.24	14.7	321.8	12.684
B-3	16	26.833	18.493	45.326	1062	1027.22	22.7	34.8	4.17
B-4	4	23.452	11.314	34.766	678	604.337	17.4	73.7	6.069
B-5	12	81.61	19.9	101.51	7056.04	5152.04	50.8	1904	30.854
B-6	8	5.477	6.481	11.958	72	71.5	6	0.5	0.5
C-1	8	3.873	18.974	22.847	375.013	260.99	11.4	114	7.55
C-2	0	1	1.414	2.414	2.999	2.914	1.2	0.1	0.2
C-3	16	3.317	4.472	7.789	31	30.33	3.9	0.7	0.5775
C-4	12	10.488	7.483	17.971	165.99	161.48	9	4.5	1.5025
C-5	4	40.988	3	43.988	1689.016	967.47	22	721.5	18.994
C-6	2	5.477	24.495	29.972	630	449.16	15	180.8	9.509
	n	12	12	24					
	Sum	230.88	119.44	350.32	12518.08	9161.6	175.16	3356.475	
	SumYY	10557	1961	12518					
	CT	4442.2	1188.8	5631					
	Mean	19.24	9.953	29.19					
	Variance	6114.8	772.19	6887.04					
	SE	22.57	8.02						
Summary	Number			ANOVA TABLE -1		two-way			
total n	24		Source		df	SS	MS	F	
Sum Y	350.322		Rows		11	4048	368	1.426	ns
Sum YY	12518		Cols		1	517.47	517.47	2.005	ns
grand CT	5113.56		Error		11	2839	258.09		
Total SS	7404.5		Total		23	7404.5			
Columns		Rows							
Sum CT	5631	9161.6		ANOVA TABLE - one-way					
SS col	517.47	4048		Source	df	SS	MS	F	
df col	1	11	among	rows	11	4048	368	1.316	ns
			within	rows	12	3356.47	279.7		
				total	23	7404.5			

Table 8 Analysis of Variance of Total Copper

Mean Concentration (GM of In and Out Flows)						In Transforms				
Biobox	Dose	Aug17	Aug23	Total	Sum YY	CT	Mean	Variance	SE	GM
B-1	0	0	0.346	0.3464	0.12	0.06	0.1732	0.06	0.1732	1.169
B-2	2	3.309	0.693	4.0025	11.4324	8.0101	2.0013	3.4223	1.3081	7.396
B-3	16	3.29	2.917	6.207	19.3329	19.2636	3.1035	0.0693	0.1861	22.276
B-4	4	3.155	2.423	5.581	15.8394	15.5738	2.7905	0.2657	0.3645	16.289
B-5	12	4.402	2.991	7.3927	28.3215	27.3257	3.6963	0.9958	0.7	40.299
B-6	8	1.701	1.869	3.5694	6.3846	6.3704	1.7847	0.0142	0.0842	5.958
C-1	8	1.354	2.943	4.2971	10.4951	9.2325	2.1458	1.2625	0.7945	8.572
C-2	0	0	0.346	0.3464	0.12	0.06	0.1732	0.06	0.1732	1.189
C-3	16	1.199	1.498	2.6969	3.6813	3.6366	1.3484	0.0446	0.1494	3.851
C-4	12	2.35	2.013	4.3629	9.5743	9.5173	2.1814	0.057	0.1688	8.859
C-5	4	3.713	1.099	4.8119	14.9954	11.5772	2.4059	3.4182	1.3073	11.089
C-6	2	1.701	3.198	4.899	13.1221	12.0002	2.4495	1.1219	0.749	11.583
	n	12	12	24						
	Sum	26.174	22.339	48.513	133.419	122.63	24.257	10.7915		
	SumYY	79.47	53.947	133.419						
	CT	57.088	41.588	98.6765						
	Mean	2.18	1.86	4.04						
	Variance	22.383	12.359	34.7424						
	SE	1.37	1.01							
	GM	8.856	6.434	56.984						
Summary	Number			ANOVA TABLE -1		two-way				
total n	24		Source		df	SS	MS	F		
Sum Y	48.5133		Rows		11	24.5634	2.233	2.413	nc	
Sum YY	133.419		Cols		1	0.6125	0.6125	0.662	ns	
grand CT	98.6765		Error		11	10.179	0.9254			
Total SS	35.355		Total		23	35.355				
Columns		Rows								
Sum CT	98.6765	122.63		ANOVA TABLE - one-way						
SS col	0.6125	24.583		Source	df	SS	MS	F		
df col	1	11	among	rows	11	24.564	2.233	2.483	nc	
			within	rows	12	10.791	0.8993			
				total	23	35.355				

Table 9 Mean Settling vs. Total Copper

Biobox	Cu Target	Cu Actual	Mean Settling
B-1	0	1.189	16.748
B-2	2	7.398	7.775
B-3	16	22.276	1.449
B-4	4	16.289	3.243
B-5	12	40.299	2.162
B-6	8	5.958	10.314
C-1	8	8.572	1.646
C-2	0	1.189	5.708
C-3	16	3.851	1.449
C-4	12	8.859	2.162
C-5	4	11.089	7.832
C-6	2	11.583	0.414

The First Non-Oxidizing Molluscicide Treatment in Canada

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ABSTRACT

The introduction and virtual unchecked spread of *Dreissena polymorpha*, the Zebra Mussel, throughout the Great Lakes Basin has caused significant operating concerns for all water users. This paper discusses our industrial experience in early detections, chemical mitigation control techniques and the mitigation performance.

INTRODUCTION

The Stelco Steel, Hilton Works baywater system distributes 175,000 USGPM of raw water from the Hamilton harbour through an extensive underground distribution network. The water is an integral part of the steelmaking process as it is required for both process and cooling throughout the facility. The effluent is directly discharged back to the Hamilton harbour via five outfalls, located at the perimeter of the property.

Since their initial introduction in Lake St. Clair in the mid 1980's, the zebra mussel has spread at an alarming rate. Acknowledging, the potential risk to our operation, a comprehensive zebra mussel monitoring program was implemented in 1989. During the 1992 infestation season, monitoring results had indicated that a dramatic increase in the mussel densities had occurred. In addition, numerous sightings of adult zebra mussels throughout the baywater network were reported plant wide. Sightings included severe fouling in heat exchangers and full water restrictions to small diameter piping.

To prevent serious macrofouling and water flow restrictions to critical production equipment, it was concluded that a mitigation program would be required in 1993.

Several control strategies, both mechanical and chemical, were examined and assessed. Due to the facilities size and water requirements only two viable control alternatives were applicable, these being chlorination and a non-oxidizing molluscicide. A comprehensive review was conducted on each alternative.

The focus of this paper is to discuss the monitoring results, the selection of a control strategy, treatment obstacles, efficacy results, toxicity results and overall conclusions of the selected treatment which are derived from our industrial experience.

INFESTATION RESULTS - MONITORING SUMMARY

In 1989, a consultant was retained to assist in the implementation of a monitoring program that would measure the infestation rate throughout the facility. The approach taken was to install six bioboxes at various locations throughout the facility.

The bioboxes which act as settling chambers for the zebra mussels were used to identify the colonization rate of the mature mussel as well, indicate the population of unsettled mussels in the water column.

Additional water samples were collected at the pumphouse in order to indicate the mussel population entering the facility. This was achieved by using a modified plankton net constructed of 53 micron mesh. This allowed water and smaller organisms to pass through, while trapping mussel larvae. During each sampling event, three vertical tows were performed and the samples were concentrated and combined, thus increasing the likelihood of detecting larvae.

Sampling procedures were designed and implemented by a consultant and our own personnel. The procedure implemented required that sample retrieval occurred once a week during the period of the day at which we injected chlorine for algae control. At the time, the consultant believed that the injection of chlorine for slime control would be sufficient for the control of zebra mussels. Thus, it was logical for the samples to be collected and the residual chlorine levels monitored at the same time throughout the plant.

The monitoring program was implemented during the spring of every year, when the water temperature reached 12 degrees celsius and concluded in the fall of each year when the water temperature dropped below 12 degrees. At year end the bioboxes would be taken off-line and stored throughout the winter months.

The samples were delivered to the consultant, for analysis, at which time, both population and mortality values were determined.

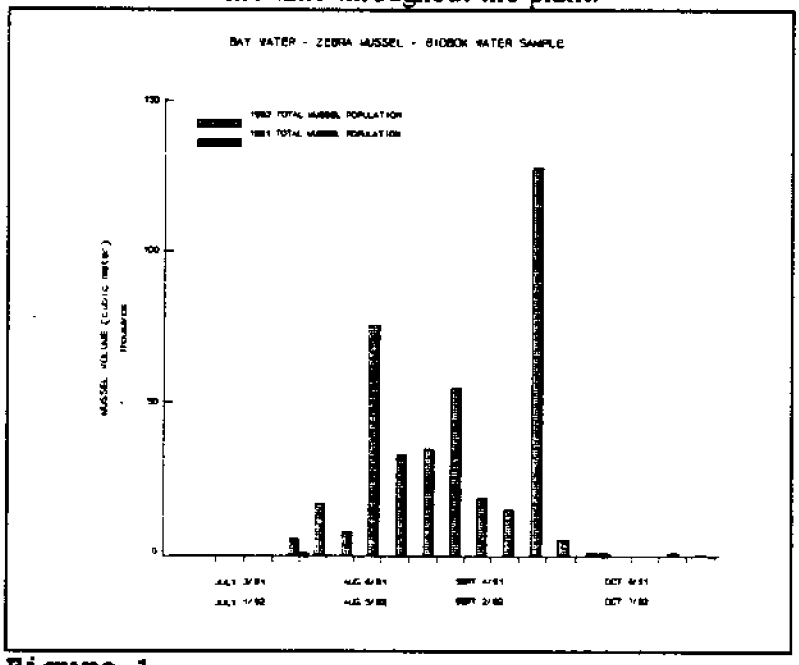

Figure 1

The following figure, figure 1, compares the 1991 and 1992 total zebra mussel population suspended in the water

column. The data was obtained from sample collection from the free flowing water through the bioboxes from all sample sites. The biobox water measures the amount of unsettled mussels in the water column and is a good indicator of the mussel infestation rate. Similarly, figure 2, compares the 1991 and 1992 total settled zebra mussel population. The data was obtained from the collected biobox culture plate scrapping samples from all monitoring sites. The culture plate sample is an excellent indicator of the degree of the settled mussel population.

The data revealed that the population within our water supply had increased between 1991 and 1992, by a factor of 30. Stating this, figure 3 illustrates the total mussel population and reported mortality levels. Examining this figure, the conclusion that would be drawn is that the chlorination program used for algae control was effective in the protecting the facilities water system.

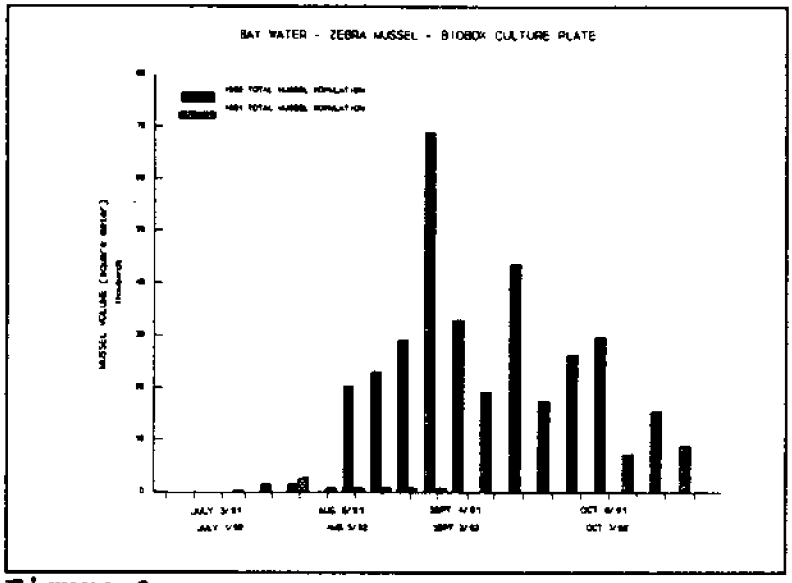

Figure 2

Suspicious with the results, Stelco reviewed the monitoring program that was implemented. It was discovered that the mortality values were misleading because the collected zebra mussel samples were taken during peak chlorination periods. Thus, the mussels were stored in chlorinated water containing high values of residual chlorine. As well, the samples were transported and stored for an average period of 48 hours prior to being analyzed. The exposure time to the chlorine was not representative to what was present in our system. As well, it was discovered that the mortality levels would increase when the mussels were disturbed from there natural environment, in this case the biobox.

To verify this speculation, personnel throughout the plant were instructed to open equipment and look for zebra mussels. The results were alarming. Mussels were detected plant wide in heat exchangers, pipes, and strainers. Various mussel sizes were found up to 3/4 of an inch.

We had a false sense of security. It was then recommended that a control strategy be implemented during the 1993 infestation season in order, to avoid any production problems. A minimum of three treatments per year were envisioned.

CONTROL STRATEGIES

Several control strategies, both mechanical and chemical, were examined and assessed. Due to the facilities size and water requirements only two viable control alternatives were applicable, these being continuous chlorination and a non-oxidizing molluscicide. A comprehensive review on each alternative was conducted.

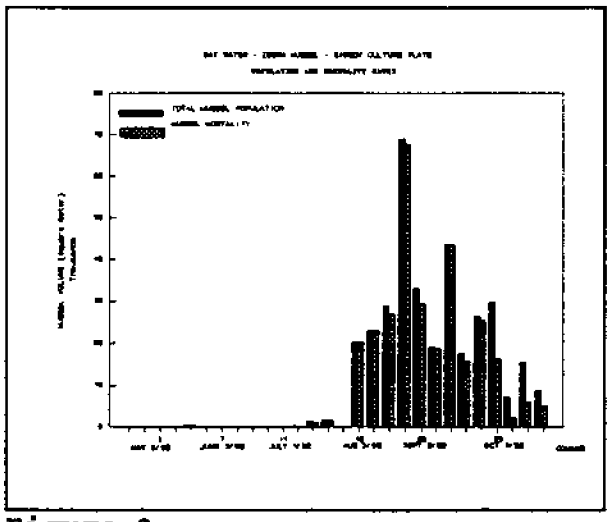

Figure 3

CHLORINATION

Our research, indicated that a continuous exposure to free chlorine in the range of 0.5 to 1.0 ppm for a duration of up to 21 days was required to mitigate adult zebra mussels from our baywater system. Reason being, the zebra mussel would sense the oxidant in the water and cease filtering.

Due to the large and variable chlorine demand for incoming baywater, ammonia concentrations of 0.58 to 0.75 ppm, a chlorine feed dosage in the range of 5.2 to 8.4 ppm (as NaOCl) was required to ensure the required free residual chlorine levels.

To ensure that the chlorine would be distributed uniformly across the plant, in-line chlorine analyzers were installed. Thus a measurement of residual chlorine concentrations, at strategic locations throughout the water network could be determined. The recorded data was then to be used to manually adjust the injection pumps and to achieve the required concentration throughout the facility.

Since the regulatory requirement at the point of discharge was to be less than 0.01 ppm of total residual chorine, which is the sum of free (HOCl, OCl) and combined (HN2Cl,NHCl2,NCl3,etc), a comprehensive dechlorination program would have to be implemented.

Our analysis indicated that, to destroy both the combined residual chlorine resulting from breakpoint chlorination of the ammonia and the free residual produced to kill the mussels, a stoichiometric dose of sodium metabisulphite, as NaHSO3, would be required in the range of 4.26 to 8.15 ppm at the outfalls.

In order to dechlorinate effectively, extensive trials were conducted on ORP controllers. The results obtained from these trials indicated very poor reliability for dechlorination purposes. Problem being, a very unstable signal was produced with which the dechlor process would have to be controlled. Therefore, to ensure compliance with the regulatory requirements, a 50 percent amount in excess of the stoichiometric dose of sodium metabisulphite, as

NaHSO4, dosage rate of 6.40 to 12.27 ppm, would be required.

NON-OXIDIZING MOLLUSCICIDE

At the same time that we were reviewing the chlorination treatment strategy, we began our investigation and review of the non-oxidizing molluscicide.

A detailed review of literature revealed several non-oxidizing molluscicides on the United States market place. Of interest to us was the success of molluscicide treatments performed throughout the United States, particularly those conducted using the Calgon H-130M molluscicide.

The H-130M is a member of a family of chemicals known as quaternary ammonium compounds or QUATS, similar to the QUATS used in fabric softeners and soaps. The H-130M itself, is a 10 carbon straight chain QUAT chemically known as a didecyl-dimethyl ammonium chloride (DDAC).

The DDAC works by coagulating the mucus layer on the gills of the zebra mussel, thus inhibiting the oxygen transfer. The result is suffocation of the zebra mussel. The key point to note, is that the zebra mussel does not detect the presence of the H-130M in the water. Thus the mollusk continues to filter water as normal in the presence of the molluscicide, permitting for high mortality rates in very short treatment periods, typically 24 hours. But very important to note, the H-130M must be deactivated prior to discharge since the mode of action is not specific for only zebra mussels.

Thus the CA-35-bentonite clay addition must be administered prior to effluent discharge at a ratio of 5 parts clay to one part QUAT. The clay acts by absorbing any excess H-130M in the water. Upon reaction with the clay the H-130M does not deabsorb. Naturally occurring bacteria degrades the H-130M product, by utilizing the nitrogen backbone of the QUAT.

TREATMENT COMPARISONS

Once all the background work was completed the two alternatives were compared. The comparison was based upon several assumptions;

1. A constant chemical composition of the supply water.

2. A theoretical chlorine demand which did not include allowances for the organic demand which are greatly effected by sunlight and temperature.

3. That a 50 percent amount in excess of the stoichiometric dose of the sodium metabisulfate would be required to ensure compliance.

4. The chlorine strategy required capitol costs for the purchase of dechlorination equipment.

5. The chemical costs of the Calgon control strategy included Calgons labor costs.

6. The comparison was based on the fact that three treatments per year would be required.

Although their was a significant cost advantage to the molluscicide treatment, there were more significant advantages to this approach. These being;

1. the treatment duration to obtain 100 percent mortality was 24 hours compared to the required 21 days for continuous chlorine.

2. a significantly less chemical usage; 1.9 tonne of H-130M and 9.3 tonne of clay versus 1230 tonne of hypochloride and 310 tonne of sodium metabisulfite.

3. less exposure to an environmental spill due to shorter treatment periods.

4. a reduced risk of pipe corrosion.

5. the elimination of the concern that employees would be exposed to chlorine odors across the facility. This was an important Health and Safety concern within our plant, because in certain mills there were existing complaints of chlorine odors during our daily injection of chlorine for algae control.

Although, there were some disadvantages in the molluscicide treatment strategy. These being;

1. it was a new chemical application in Canada and approvals, at the time had not been issued and,

2. literature reviews indicated a fast release of debris after treatment. A concern that could not be ignored, because of the possibility of a significant slough-off which may have effected operations.

PRE-TREATMENT ACTIVITIES

Regardless of the strategy that would be implemented, concurrent tasks needed to be addressed in order that a successful treatment could be implemented.

Water flow and distribution within the facility had to be understood as well, a plant wide awareness and education program would be required to avoid any interruption to production .

To determine the water distribution, a computer model used by our design groups was updated. The model allowed us to identify low flow areas within our network and predict potential areas in which large slough off rates may occur.

Also, the collected data from the model allowed us to revamp the monitoring program, so

that the degree of infestation could be better understood and measured.

A notification was sent to all operating units indicating the intention to implement a control program and of the potential risks involved. Several presentations were made across the plant at various levels. The intent was to educate the operating personnel of the potential risks and actions that could be taken in order to avoid any interruptions to the operations. To keep all personnel updated of the progress made towards implementing a control strategy, a bi-weekly newsletter was issued.

DECISION TO PROCEED

After reviewing the alternatives it was recommend to senior management to proceed with Calgon's approach providing that all necessary regulatory requirements could be met in time for the 1993 treatment season.

The objectives of the program were;

- to provide 100 percent mortality of the zebra mussels within the Hilton Works facility.
- to provide a treatment strategy that would not impact the environment.
- to ensure that all environmental guidelines were met.

ZEBRA MUSSEL TREATMENT

Although the H-130M was registered by the Environmental Protection Association (EPA) in the United States, it was not approved in Canada.

In order to apply the product and generate data to support registration in Canada, an application was made by Calgon Corporation to administer the product at the Stelco Hilton Works facility. After an extensive review of the application, permission was granted by Environment Canada for the administration of H-130M for zebra mussel control at the Stelco, Hilton Works facility.

However, following the approval at the federal level, provincial and regional approval had to be obtained from the Ontario Ministry of the Environment and Energy (MOEE). A Certificate of Approval was granted to Calgon, for the discharge of water from Stelco Hilton Works facility during the treatment period. The Certificate of Approval stipulated that the effluent concentrations for Total Suspended Solids from the facility could not exceed 15 parts per million. As well, the effluent streams from the facility had to be sampled every three hours for the measurement of the Didecyl Dimethyl Ammonium Chloride. Furthermore, composite samples were to be collected during the treatment so that aquatic toxicity testing could be performed.

The plan was to have Calgon perform two treatments at Stelco in 1993. One successful treatment, early in the infestation season and the second later in the season. The purpose

of the first treatment would be to flush the water system of any existing mussels. The second treatment would flush the mussels that would have infested the water system after the previous treatment. This would also insure minimum growth through the winter months of the mussels that may have infested the water network.

The scheduling of the treatments were dictated by the ambient influent water temperature. Since, the molluscicide is not as effective at water temperatures below 12 degrees celsius because the zebra mussel does not actively feed and reproduce. The first treatment was scheduled for mid July and the second for late September before the water dropped below 12 degrees.

The H-130M was applied at the baywater pumphouse, in a neat form at the prescribed rate of 1 ppm for 24 hours. To ensure proper dispersion through the facility, product residuals were confirmed throughout the plant by taking samples at several strategic locations.

Prior to effluent discharge, 5 ppm of CA-35 bentonite clay was added at the five outfalls of the plant. Administration of the clay was conducted using the Calgons' portable detoxification trailers. The trailers were designed achieve a consistent clay and water slurry prior to discharge. Residual testing at the outfalls confirmed that the molluscicide was absorbed on to the clay, thus the outfalls did not contain any free biocide residuals.

During both treatments, no major operating problems were encountered.

EFFICACY TESTING AND RESULTS

In order to determine the effectiveness of the treatment strategy, a third party consultant was retained to perform a bioassay study that would determine the mussel mortality rate.

The consultant seeded seven bioboxes, by placing several baskets containing 10 live mussels within the biobox, several days prior to the start of the each treatment. This procedure was conducted in order to allow the mussels to acclimate to the water. Prior to each treatment, the seeded bioboxes were inspected in order to determine the background mortality rates.

The background analysis revealed that for the first treatment, July 1993, 7 out of 654 mussels had died. The remaining baskets, containing the live zebra mussels were subjected to the water treated with the H-130M. After the 24 hour treatment, the consultant removed the mussels from the bioboxes and analyzed for initial "apparent" mortality at 24 and 48 hours following the initiation of the treatment. The criteria used for determining apparent mortality included mussel gaping and non-response during gentle prodding.

Since some mussels were not gaped open, they could not be considered dead. These mussels were brought back to a laboratory and placed into recovery tanks for an additional 24 hours. Thus, after 48 hours after the initiation of the treatment, the mussels were analyzed again. If the mussels remained closed or gaped open and did not respond to prodding, then they were considered dead.

The 48 hour analysis indicated that 100 percent mortality was achieved at all the test sites.

For the second treatment which was in September 1993, the consultant was again contracted to seed all the bioboxes. However, for this treatment it was requested that each biobox be seeded with both zebra and quagga mussels. Again, a background mortality analysis was conducted, which indicated that 3 out of 640 mussels had died.

Following the treatment, a 24 and 48 hour apparent mortality was conducted. The overall results determined by the 48 hour apparent mortality test indicated 100 percent mortality for both species.

EFFLUENT QUALITY

Water samples were collected from influent and each effluent stream prior to the molluscicide treatment. As well, composite samples were collected during the treatment at all effluent streams. The samples were submitted to an independent laboratory to perform the acute and chronic toxicity studies. These studies were to determine if the treated and untreated effluents would affect the survival, growth and reproduction of aquatic test organisms.

Acute lethality of effluent was determined by exposing a suitable number of test organisms of Rainbow Trout and Daphnia Magna to the test solutions. As well, the chronic lethality of the effluent was determined by using the larval, growth and survival of the Fathead Minnow and the effect of reproduction and survival of the Cladoceran Ceriodaphinia Dubia. The prescribe protocols, EPS 1/RM/14, EPS 1/RM/21 and EPS 1/RM/22 were indicated in the Certificate of Approval.

The Daphnia Magna 48 hour toxicity tests indicated that the samples were not acutely lethal to the daphnids. As well, the results of the 96 hour toxicity test using the rainbow trout indicated that all the test water was non-lethal to fish and there was no sublethal impairment observed during exposure time. Finally, there was virtually no indication that chemical treatment would impair growth of the Fathead Minnow, Fry or reproduction of Ceriodaphina.

Several composite samples were collected during each treatment and analyzed for total suspended solids. The concern was that the addition of the bentonite clay would significantly increase the effluent loadings.

The analysis of the collected samples indicated that there was an average increase of in the gross concentration of total suspended solids of 6 ppm. The result, all effluent discharges remained below the 15 ppm limit.

Finally, to ensure that the effluent streams were detoxified, samples were collected and analyzed for residual levels of the didecl-dimethyl ammonium chloride (DDAC). The recorded residual levels of the DDAC in the effluent streams were on average 0.0006 ppm greater than the background concentrations found in the influent water stream.

CONCLUSION

The implementation of both treatments in 1993 were successful, in which they had met our outlined objectives, that is;

- 100 percent mortality of the zebra mussels within the facility was achieved.
- a control strategy was implemented that had no impact on the environment.
- all environmental guidelines were met.

As well, there was minimal interruptions to the operating divisions. This success was mainly due to the fact that contingency plans throughout the plant were put into place, in the event of the loss of water. The plan included the installation and monitoring of water strainers and around the clock surveillance of the water network.

The effect of the treatment was seen within hours after the commencement of the chemical injection. Dead mussels began sloughing off and turning up at strainers throughout the plant. The volumes ranged from handfuls to shovelfuls.

It is actually, interesting to note that after treatment the water had a foul smell. It is believed that the mussels began to decay and decompose. This correlated with the discharge of slimy material from our system.

After the first treatment the slough-off continued for several weeks at different areas throughout the plant. In one case, at an open outfall, several cubic yards of shells could be seen.

The 1993 monitoring results are depicted in figure 4. Figure 4 illustrates that there was a significant reduction in the reported settled mussels within our facility when compared to the previous year. This may be attributed to the successful control strategy implemented during 1993. It is believed that the treatment had flushed our system of settled mussels thus eliminating the cumulative effect of measuring both the mussels that were brought into our system at the intake and the mussels that were breeding within the system. However, it is important to note that the zebra mussel population in the biobox water sample remained at relatively the same levels of those reported in 1992. Remembering that this result is used as an indicator to measure the degree of infestation, future control measures will need to be implemented.

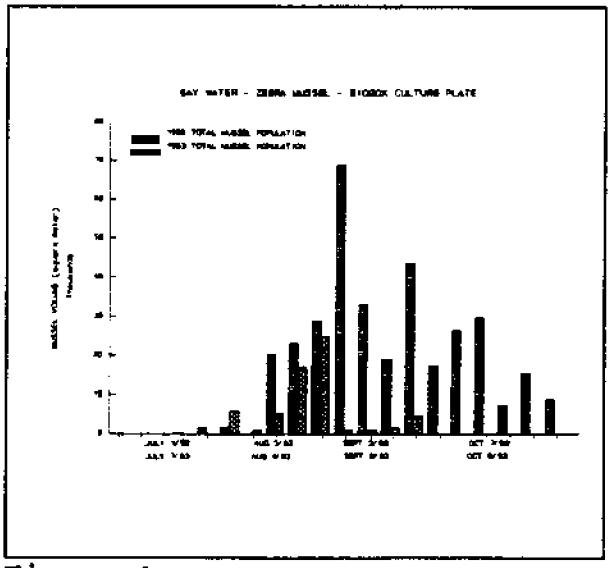

Figure 4

In the fall of 1993, the bioboxes were left in service and visually inspected throughout the course of the winter. As of yet virtually no mussels have been sighted within the bioboxes, and for that matter throughout the plant, thus the application of the second treatment has met all our expectations.

In conclusion, the turn key approach used by Calgon for the control of zebra mussels is not only cost effective but was also very viable.

In our water system, we are certain that the implementation of a chlorination and dechlorination program, would not have been as effective as an overall treatment because of the high ammonia levels in the intake water and possibly not even applicable because of the potential health and safety issues.

CARBON DIOXIDE AS A NARCOTIZING PRE-TREATMENT FOR CHEMICAL CONTROL OF DREISSENA POLYMORPHA

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Abstract

Measures of zebra mussel control to date have been wide ranging, including such strategies as the use of thermal treatment, protective coatings, filtration, mechanical removal, and the use of oxidizing and non-oxidizing molluscicides. While many of these measures have been shown to be successful, they often have limitations related to cost, incompatibility with facility design, regulatory resistance to biocide use, and stringently regulated discharge limits. Preliminary studies on another macrofouling organism (the asiatic clam, *Corbicula fluminea*) have demonstrated narcotizing effects of carbon dioxide at concentrations of 100 mg/l and toxic effects at concentrations of 500 mg/l. This investigation was conducted to evaluate the effectiveness of using carbon dioxide (CO_2)as a non-toxic treatment alternative for the control of the zebra mussel (*Dreissena polymorpha*). The study approach was developed to evaluate the effectiveness of treatment using carbon dioxide by itself and as a pre-treatment to chlorination on multiple size classes of mussels (1-5 mm, >5-10 mm, and >10-15 mm) under two temperature regimes (18 and 22°C) and varying application rates and exposure durations (48 and 96 hr). Results of these tests indicate that a narcotizing response that is identified by gaping of the mussel, can be induced by exposure to carbon dioxide in a relatively short period of time (less than 6 hours). Mortality has been observed to occur in tests using carbon dioxide by itself and as a pretreatment for chlorination in both 48 and 96 hour test durations. Consequently, this procedure may prove to be a valuable alternative treatment method for control of zebra mussels, particularly in areas having stringent regulatory discharge limits.

1.0 INTRODUCTION

The selection of the most appropriate zebra mussel control strategy can be a difficult process. Numerous strategies have been developed and are available for use. These include the use of thermal treatment, treatment by oxidizing and non-oxidizing chemicals, molluscicides, mechanical removal, filtration and the use of protective coatings, to name a few (Claudi and Mackie 1994, Edwards *et al* 1993).

In many cases, the configuration of the industrial system (i.e., intake location, type of intake, type of facility or process, facility design, flow characteristics, etc.) cost, water quality, and degree of infestation are important determinants of the control measures selected. Because of these factors, plant managers often select a combination of control measures to effectively treat different component systems within their facility (eg. main circulating water system vs. fire suppression system).

Chlorination is one treatment alternative that appears to be widely applied. In large part this is due to its familiarity with and acceptance by the regulatory community. However, because of environmental concerns regarding its use, and the cost of its application, minimization of chlorination requirements is often the goal of regulators as well as plant managers. In recent years, an increasing amount of research has been conducted to evaluate the use of chlorination as a zebra mussel control measure. These studies have been performed using hypochlorite, chlorine dioxide, and chloramines under both continuous and intermittent treatment regimes (Khalanski 1993, Jenner and Janssen-Mommen 1993, Fraleigh *et al*, 1993, Brooks *et al* 1993, Bardygula-Nonn 1993). Much of the research using hypochlorite has shown that chlorination can be effective, but because of a long dose-response time (10-30 days (Van Benschoten *et al*, 1993)), it requires application over a long time period.

Carbon dioxide is a gas that has been used in industrial processes for a number of years in controlling pH. At electric generating stations it has been used for the acidification of ash pond water in addition to being used to control scaling in house service water systems (Wine and Morrison, 1986). Preliminary studies performed by Commonwealth Edison Company in the early 1980's (CECo, unpublished) demonstrated that the application of carbon dioxide (CO_2) had a "narcotizing effect" on another biofouling organism, the asiatic clam (*Corbicula fluminea*). This effect, marked by a gaping response, was observed at concentrations of 100 mg/l, whereas, toxic effects were observed at concentrations of 500 mg/l.

The purpose of this investigation was to evaluate the effectiveness of using CO_2 as a non-toxic treatment alternative for the control of the zebra mussel (*Dreissena polymorpha*). Individuals responding to the application by exhibiting a gaping response may be more susceptible to treatment by oxidizing chemicals like hypochlorite at much reduced concentrations and for shorter durations. In order to evaluate this relationship, several specific study objectives were formulated:

- Determine the sub-lethal narcotizing concentrations of CO_2,
- Determine the lethal levels of CO_2 in a closed system,
- Evaluate the control effectiveness of NaOCl and CO_2 in combination,
- Evaluate the overall effectiveness of this combination at various test durations (48 and 96 hours),
- Determine the effects of temperature (18 or 22°C) on organism response, and
- Determine the size related effectiveness of the above test conditions.

For the purposes of this paper, detailed results of tests performed using only CO_2 will be presented for the 6-10 mm size class only. However, results from all size classes will be presented to illustrate size dependant response results as well as results of various treatments using the combined application of CO_2 and NaOCl.

2.0 METHODS AND MATERIALS

Test Organisms

Test specimens of *Dreissena polymorpha* were obtained by field collection from ESE's Ecology group, and maintained in-house at ESE's Aquatic Toxicology Laboratory throughout the duration of the research project. Collections of the 6-10 mm and 11-15 mm individuals were made in October 1992 at CECo's Waukegon Station on Lake Michigan, whereas individuals of the 1-5 mm size class were collected in October 1993 at CECo's Fisk Station on the North Branch of the Chicago River.

All mussels were cultured and tested in temperature-controlled areas at 18 ($\pm$1)°C and 22°C ($\pm$1). During the holding period, the mussels were fed a suspension of algae (*Selenastrum capricornutum* and *Ankistrodesmus falcatus*), which was supplemented with a flake food (Tetra® Conditioning Food) suspension. Only healthy, active mussels were selected for testing.

Test Apparatus

A half-liter proportional diluter system described by Mount and Brungs (1967), dosed with a Hamilton® Micro Lab 420 syringe dispenser, was used to intermittently introduce dilution water and Sodium Hypochlorite into the test chambers. A manifold constructed of PVC pipe, equipped with 5 brass needle valves and a two-stage gas regulator attached to a 50-pound CO_2 cylinder was used to continuously introduce CO_2 into the test chambers. The system contained six sets of two replicate 40-milliliter test chambers, designated as control, and level #1 through level #5. Flow-splitting boxes equipped with lids were used to thoroughly mix and divide each NaOCl and CO_2 concentration for delivery to the test chambers. The CO_2 was continuously dosed to each flow-splitting box (excluding the control level) via 1/4-inch tygon tubing attached to its respective needle valve located on the CO_2 manifold. To improve CO_2 saturation of the influent water, the tubing used to deliver the CO_2 to the flow-splitting boxes was equipped with an air diffuser to decrease CO_2 bubble size and increase the CO_2 surface area that contacted the

incoming water, improving the CO_2 dosing efficiency. To minimize turbulence, the influent water was introduced into the test chambers via 1/4-inch tygon tubing. Glass vials (40-mls) labeled A and B, were used as test chambers. These chambers had screw-on caps with influent and effluent tubing to allow adequate test solution exchange within the test vessels while preventing the mussels from escaping. The test mussels were placed in each of the duplicate chambers at the start of the study and observed at given test intervals. For the 48 hour test, observations were conducted at 0, 4, 24 and 48 hours, and for the 96 hour test, observations were made at 24 hour intervals.

The dilution water used in this study was a hard-blended water prepared to a total hardness between 160-180 mg/L as $CaCO_3$. The specific water hardness was obtained by blending ESE's well water with soft blended water (a combination of ESE's well water and lime-softened water). Water quality parameters of temperature, hardness, dissolved oxygen, pH, alkalinity, and conductivity were measured daily for the duration of each study. The dilution water was delivered intermittently to each test chamber at a rate of 500 mls every 8 minutes. This resulted in an average rate of 12.3 ml/min, an amount which was sufficient to replace the 40-ml test volume 443.8 times in a 24-hour period.

Concentrations of NaOCl were measured by means of spectrophotometric analysis. This analysis was accomplished using the DPD colorimetric method of analysis for chlorine as outlined in Standard Methods for the Examination of Water and Wastewater (American Public Health Association, 1989). This method was validated for the detection of sodium hypochlorite as free chlorine before study initiation.

Concentrations of CO_2 were measured at 0, 2, 4, 24, 48, 72, and 96-hours by means of titrametric analysis by titrating the sample with sodium hydroxide to a phenolphthalein end point. Samples were analyzed for the presence of CO_2 by means of a Hach model 16900-01 Digital Titrator equipped with a 3.636 N NaOH Titration Cartridge.

The test chambers were immersed in a temperature-controlled water bath held at 18°C ($\pm$2°C) or 22°C ($\pm$2°C) in order to meet specific test parameters. The drain system of the water bath was equipped with 41 μm nylon mesh (Spectra/Mesh® of Spectrum Medical Industries, Inc.) to prevent any veligers potentially released during the studies from leaving the test system and/or entering the municipal sewer system. Temperature was recorded continuously with an electronic Data Logger. The proportional diluter system used for the project was calibrated prior to testing by checking volume deliveries from the diluter mixing cell to obtain a dilution factor used for stock preparations. The diluter system was set to provide test levels at -50% dilutions of each other.

Test Design

As specified by the above study objectives, completion of the project was conducted in several phases. All testing was performed on individuals of three separate size classes (1-5 mm, 6-10 mm, and 11-15 mm) at 18 and 22°C. Tests using CO_2 and the combined application of CO_2 and NaOCl were conducted for 48 and 96-hour durations with a 24-

hour latent period to determine delayed mortality effects. In contrast, tests using only NaOCl were performed for the 96-hour duration only.

The initial phase of the investigation consisted of a series of tests designed to evaluate the response of *D. polymorpha* to exposure by CO_2. The response of zebra mussel to applications of CO_2 were evaluated for multiple test durations and application rates in order to determine lethal concentrations (LC_{50}) and effective concentrations (EC_{50}). The response considered to be an effect for these tests was the gaping response in which it was presumed that *D. polymorpha* would become more vulnerable to exposure to other control measures. A closed response, for the purposes of this investigation was not considered to be an effect of the CO_2 application. This work was scheduled to be conducted at two temperature regimes (18 and 22°C) for two test durations (48 and 96 hours). Determination of lethal and sub-lethal effects of CO_2 will be limited to tests with the following target concentrations: 50, 110, 180, 270, and 500 mg/l.

The second phase of the investigation consisted of the application of hypochlorite (NaOCl) to establish baseline data as to the lethal concentrations of NaOCl required for zebra mussel treatment. A geometric dilution series was used in the testing in which test concentrations were 5.0, 2.5, 1.25, 0.6, and 0.3 mg/l. Based upon recent literature however, it was recognized as unlikely that lethal concentrations would be achieved within either a 48 hour or 96 hour test duration (Van Benschoten, *et al*, 1993).

The final phase of the investigation involved combined application tests using CO_2 and NaOCl designed to evaluate the effectiveness of CO_2 in increasing the vulnerability of zebra mussels to chlorination. During these series of tests, a CO_2 concentration of 150 mg/l was applied to each treatment. Using this as a pretreatment, NaOCl was applied at 4 hours into the test using the same geometric dilution as the baseline studies.

Replicates for each test consisted of 5 individuals of zebra mussels of the same size class. This number was based on the ability to adequately observe each of the individuals of the replicate at various observation periods during the tests.

3.0 RESULTS AND DISCUSSION

CO_2 Treatment

As hypothesized, the effectiveness of using CO_2 as a pretreatment alternative to application by NaOCl is dependant upon its ability to induce a gaping response in tested individuals. In order to evaluate this relationship, tests were conducted for 48 and 96 hour durations at 18 and 22 °C for three separate size classes of test organisms. Results of these tests are presented in Figures 1 and 2, respectively for the 6-10 mm size classes. Graphs presented to the left of the figure illustrate the effectiveness of each CO_2 treatment in inducing a gaping response, whereas graphs on the right side of the figures illustrate lethal effects. As is evident in both the 48 and 96 hour test, a gaping response was evident in more than 50% of the test population. In addition, this response appeared to

be produced after only a short exposure duration (4 hours) as was evident from the 48 hour test. Both temperature and test duration appeared to influence the effective concentration. EC_{50} values for the 48 hour test were 162.0 mg/l and 253 mg/l at 18 and 22 °C, respectively. Similarly, EC_{50} values for the 96 hour test were much lower (74.0 and 82.0 mg/l), reflecting a greater effectiveness at more extended test durations. Lethal concentration was also variable with respect to temperature and test duration. LC_{50} values for the 48 hour test were 196 and 290 mg/l at 18 and 22 °C, respectively. Similarly, LC_{50} values were markedly reduced for the 96 hour test (74 mg/l and 81 mg/l at 18 and 22 °C, respectively).

Delayed mortality effects were also evident. In many cases, specimens that were observed to be gaping subsequently died during over the course of the next observation period. Potential causes of mortality appear to be water quality related. As is illustrated in Figure 3 for the 96 hour test at 18°C, dissolved oxygen and pH values were successively reduced with increasing CO_2 treatment levels. (This pattern was similar for other tests conducted for different durations and at different temperatures.) In fact, pH values during the 96 hour test were depressed below 6.0 for all CO_2 application levels (probably attributable to the formation of carbonic acid) and were as low as 5.2-5.3 for the 250 mg/l and 400 mg/l treatments. As presented by Vinogradov, *et al* (1993) pH values less than 7.0 disrupt ion exchange and interfere with calcium metabolism. Mortality evident in this study is similar to that reported by Vinogradov, *et al* (1993) in which 75% mortality was observed after 4 days of exposure to low pH. It is important to note however, that water quality conditions typically recovered to pre-test conditions shortly after the termination of the test, suggesting rapid recovery of the system.

Combined Application

The effectiveness of the combined treatment (i.e., CO_2 and NaOCl in combination) was evaluated by comparing responses of each combined application with the base responses obtained from tests using NaOCl only and with CO_2 applications at 150 mg/l. This value was the only CO_2 test concentration used in the comparison as this was the EC_{50} value administered as part of the combined application treatments. A comparison of the combined treatments is presented in Figure 4. Results of tests conducted at 18°C are presented on the left side of the figure, whereas test conducted at 22°C are illustrated on the right side of the figure.

Among the tests conducted at 18°C, responses to the combined application in all cases exceeded the responses to other treatments. A mortality rate of 10 percent was observed in the NaOCl treatment (5.0 mg/l) of the 1-5 mm and 6-10 mm size classes after 96 hours. No response to chlorination was evident among the 11-15 mm size class. By comparison, treatment using CO_2 by itself resulted in a mortality rate of 60-100% after 96 hours. Combined application in treatments however, typically resulted in 100% mortality after 96 hours and 60-80% mortality after 48 hours (48-hr LC_{50}=0.75 and 0.76 for the 6-10 and 11-15 mm size classes, respectively). In contrast, the LC_{50} value was

much greater for the 1-5 mm size class suggesting a differential vulnerability to treatment with respect to size.

Results of tests conducted at 22°C are presented for the 48 hour test in Figure 4. Patterns of response were similar to those established in the 18°C test in which combined applications typically resulted in 60-80% mortality after 48 hours for the 6-10 and 11-15 mm size classes. As was evident for the 18°C tests a lower response appeared to be evident among the 1-5 mm size class, again suggesting a differential response to treatment for this size class. Respective 48-hr LC_{50} values for the combined applications to the 1-5 mm, 6-10 mm, and 11-15 mm size classes at 22°C were 3.98, 0.42, and 0.48 mg/l, suggesting a slightly increased vulnerability at higher temperatures. An increase in the metabolic rate of *D. polymorpha* at higher temperatures may account for this effect.

Size-Dependant Response

An examination of the effect of size on vulnerability to treatment was made by comparing both gaping and mortality responses when test organisms were exposed to CO_2 by itself (180 mg/l)(Figure 5) and by comparing mortality responses to combined applications (Figure 6). As is evident in Figure 5, response to application by CO_2 appears to be greater among the 6-10 and 11-15 mm size classes. A gaping response of only 30% was exhibited for the 1-5 mm size after 4 hours of exposure at 18°C. No gaping was observed thereafter. In contrast, in tests conducted at 22°C, the gaping response of the 1-5 mm size class was more similar to that of the other size classes.

A lower incidence of gaping in Figure 5 between observation periods indicates a change in the response of the tested organisms from that of gaping to one in which they were either observed to be closed or were determined as dead. This change in response is most evident in Figure 5 among the 6-10 mm and 11-15 mm size classes in which reduction in gaping percentages are generally mirrored by increases in mortality. As suggested by Figure 5 the incidence of mortality among the 1-5 mm size class was low relative to that of other size classes (0% at 18°C, 10% at 22°C), suggesting a lower vulnerability to treatment by CO_2 and ultimately a greater tolerance to reductions in pH.

Size dependant response to the combined application of CO_2 and three of the five NaOCl treatment levels is presented in Figure 6 for the 6-10 mm size class under the 18°C and 22°C test conditions. The results of these tests confirm the reduced vulnerability of the 1-5 mm size class to treatment. No mortality was evident at either temperature in the 0.3 mg/l treatment; mortality was 20% in the 0.6 mg/l treatment under both temperatures; and mortality was 10% in the 1.25 mg/l treatment under the 22°C test conditions. In contrast, the 6-10 mm and 11-15 mm size classes exhibited a mortality rate of 100 and 80 percent under the same conditions. Individuals representing the smaller size class of *D. polymorpha* therefore, appear to demonstrate a distinct differential vulnerability to each of the treatment alternatives tested (Figures 4-6). This result however, may be in part, attributable to the use of specimens collected from a slightly different location (N. Branch of the Chicago River) and from different years (1922 vs. 1993). Repetition of the tests

using specimens within the 1-5 mm size class from multiple locations may provide information that more clearly describes this relationship.

4.0 CONCLUSIONS

The application of CO_2 represents an effective and viable treatment alternative to control resident zebra mussel populations. Sub-lethal narcotizing effects can be induced after only short exposure periods (4 hours), whereas lethal effects can be induced after 24 hours of exposure and can reach 100 percent after 72 hours. In addition, it appears that the treatment of *D. polymorpha* is size dependant, with individuals of the 1-5 mm size class notably less vulnerable to treatment. When used as a pre-treatment to chlorination with NaOCl, CO_2 effectively increases mortality rates and decreases required contact times. Consequently, for facilities currently using chlorination as a control measure, the duration and concentration of NaOCl treatments may effectively be reduced using a combined treatment approach using CO_2.

ACKNOWLEDGEMENTS

The authors would like to extend their thanks and appreciation to the funding support of the study sponsors: Commonwealth Edison Company, Chicago Illinois; National Sea Grant and local members of the Illinois-Indiana Sea Grant. In addition, we would like to acknowledge and thank Mr. Harry Bernhard and Mr. Bob Morrison for their support and guidance during the formative stages of this project.

LITERATURE CITED

American Public Health Association 1989. Standard Methods For the Examination of Water and Wastewater.

Bardygula-Nonn, L. G., G. Wojtowicz, R. A. Nonn, J. Savitz. 1993. A surveillance protocol for adjusting intermittent chlorine injections throughout the spawning and settlement season. pp. 3-51 through 3-66 in Proceedings: Third International Zebra Mussel Conference. Toronto, Canada. Feb. 23-26.

Brooks, G., G. Matisoff, and B.I. Bourland. 1993. Control of adult zebra mussels with chloric dioxide. pp. 3-139 through 3-154 in Proceedings: Third International Zebra Mussel Conference. Toronto, Canada. Feb. 23-26.

Claudi, R., G. Mackie. 1994. Practical manual for zebra mussel monitoring and control. Lewis Publishers. Ann Arbor, MI.

Edwards, R., J. Tsou, J. Mattice, and A. Armor. 1993. EPRI zebra mussel monitoring and control guide. pp. 1-1 through 1-15 in Proceedings: Third International Zebra Mussel Conference. Toronto, Canada. Feb. 23-26.

Fraleigh, P.C., W.R. VanCott, M.E. Wenning, and J.A. DeKam. 1993. Effects of hypochlorite, permanganate, chloric dioxide and chloramines on zebra mussel settling. pp. 3-23 through 3-36 in Proceedings: Third International Zebra Mussel Conference. Toronto, Canada. Feb. 23-26.

Khalanski, M. 1993. Testing of five methods for the control of zebra mussels in cooling circuits of power plants located on the Moselle River. pp. 3-1 through 3-20 in Proceedings: Third International Zebra Mussel Conference. Toronto, Canada. Feb. 23-26.

Mount, D.I. and W.A. Brungs. 1967. A Simplified Dosing Apparatus for Fish Toxicological Studies. Water Res. 1:21-29.

Sprung, M. 1993. The other life: an account of present knowledge of the larval phase of *Dreissena polymorpha*. In T.F. Nalepa and D.F. Schloesser eds. Zebra Mussels, Biology Impact and Control. Lewis Publishers. Ann Arbor, MI. pp. 39-53.

Strand, R.L. 1993. Chlorine and dechlor automation technique for zebra mussel mitigation and prevention programs. pp. 1-89 through 3-101 in Proceedings: Third International Zebra Mussel Conference. Toronto, Canada. Feb. 23-26.

Wine, R.D. and R.D. Morrison. 1986. Effective Use of Carbon Dioxide for pH Control in Utility Service and Waste Waters. Proceedings of the American Power Conference. Vol. 48, pp 1042-45.

Van Benschoten, J.E., J.N. Jansen, D. Lewis, and T.J. Brady. 1993. Chemical oxidants for controlling zebra mussels (*Dreissena polymorpha*): a synthesis of recent laboratory and field studies, in T.F. Nalepa and D.F. Schloesser eds. Zebra Mussels, Biology Impact and Control. Lewis Publishers. Ann Arbor, MI. pp. 599-619.

Vinogradov, G.A., Nataliya F. Smirnova, V.A. Sokolov, and A.A. Bruzintsky. 1993. Influence of chemical composition of the water on the mollusk *Dreissena polymorpha*. In T.F. Nalepa and D.F. Schloesser eds. Zebra Mussels, Biology Impact and Control. Lewis Publishers. Ann Arbor, MI. pp. 283-293.

Jenner, H.A. and J.P.M. Janssen-Monnen. 1993. Monitoring and control of *Dreissena polymorpha* and other macrofouling bivalves in the Netherlands. In T.F. Nalepa and D.F. Schloesser eds. Zebra Mussels, Biology Impact and Control. Lewis Publishers. Ann Arbor, MI. pp. 537-554.

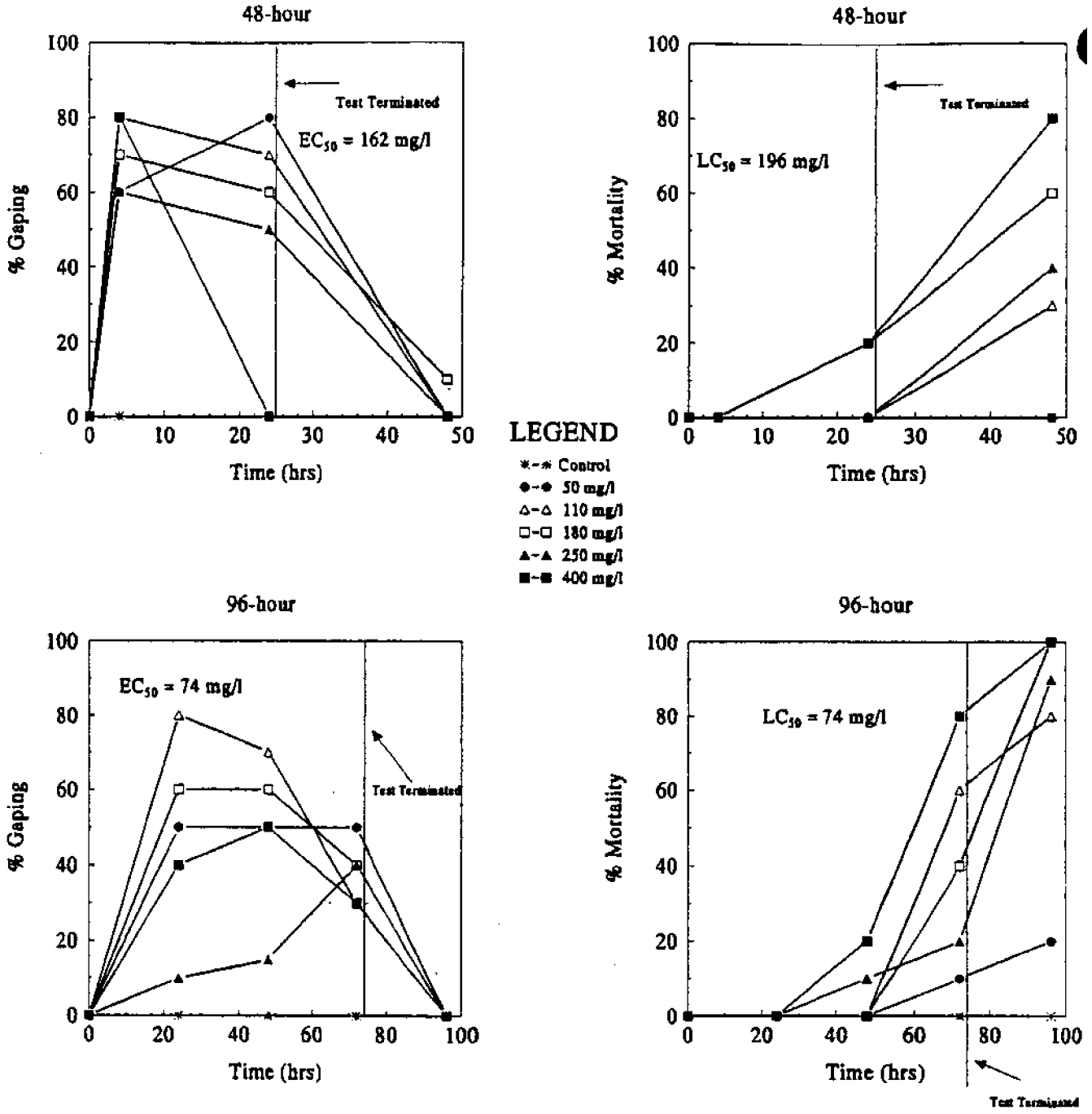

Figure 1. Effectiveness of CO_2 Treatment on 6-10 mm Size Class of Dreissena polymorpha *at 18 C*

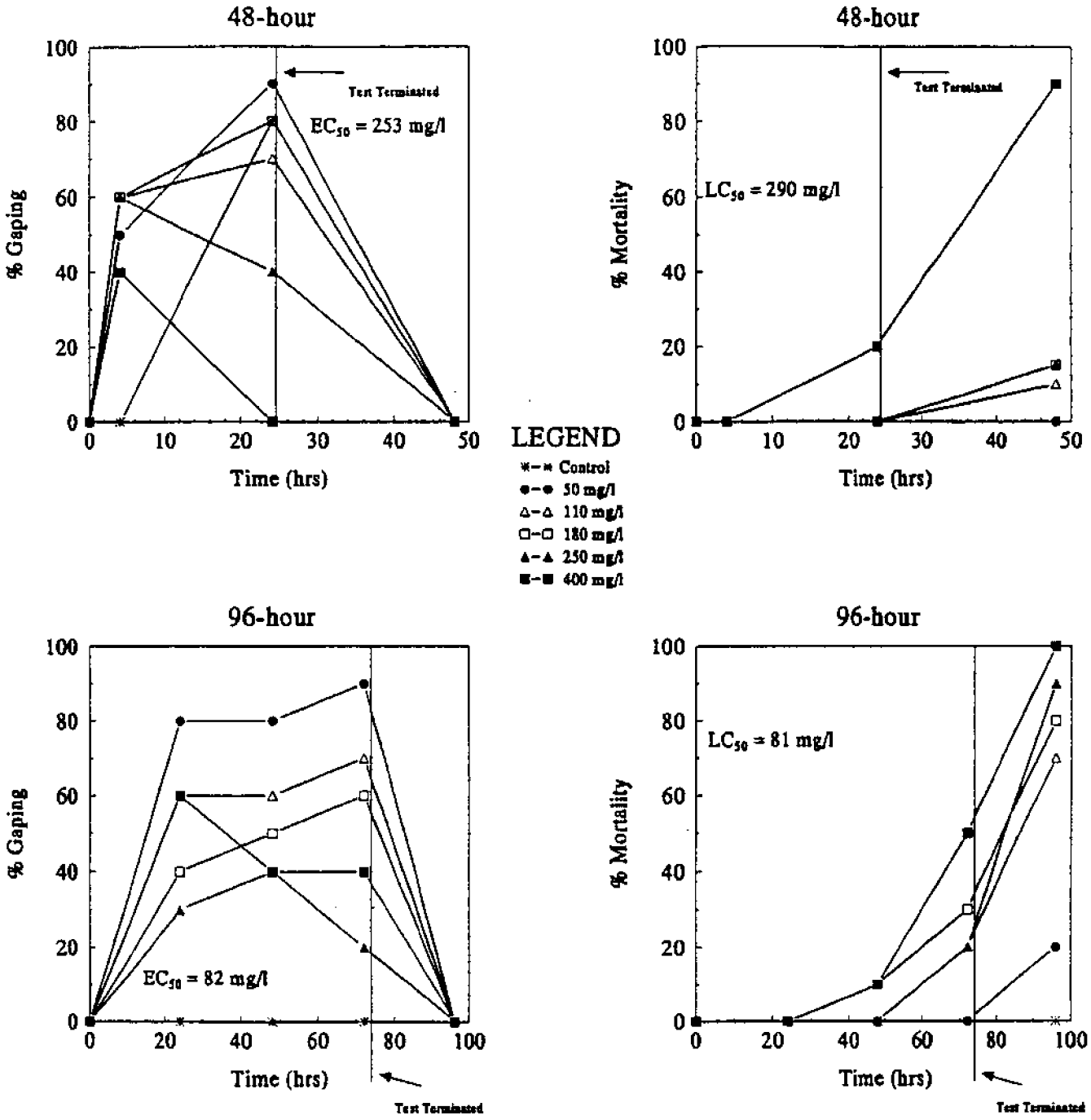

Figure 2. Effectiveness of CO_2 Treatment on 6-10 mm Size Class of Dreissena polymorpha *at 22 C*

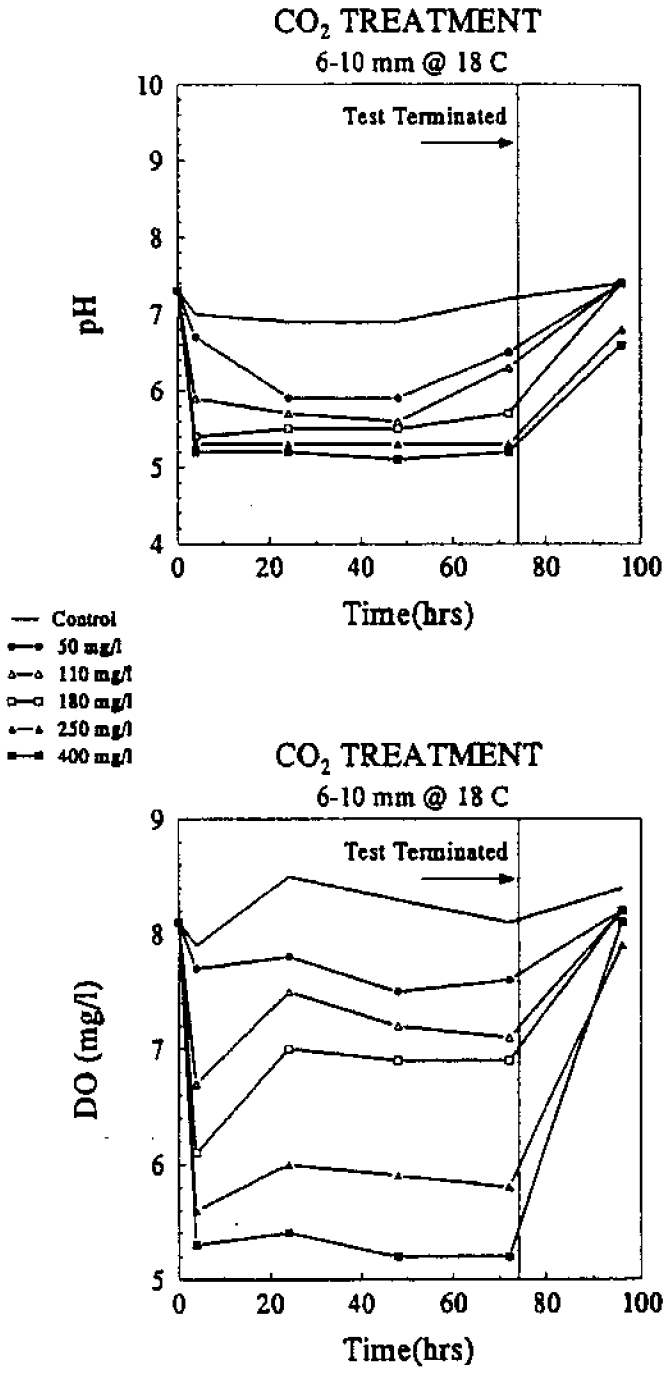

Figure 3. Effect of CO_2 Application on pH and Dissolved Oxygen

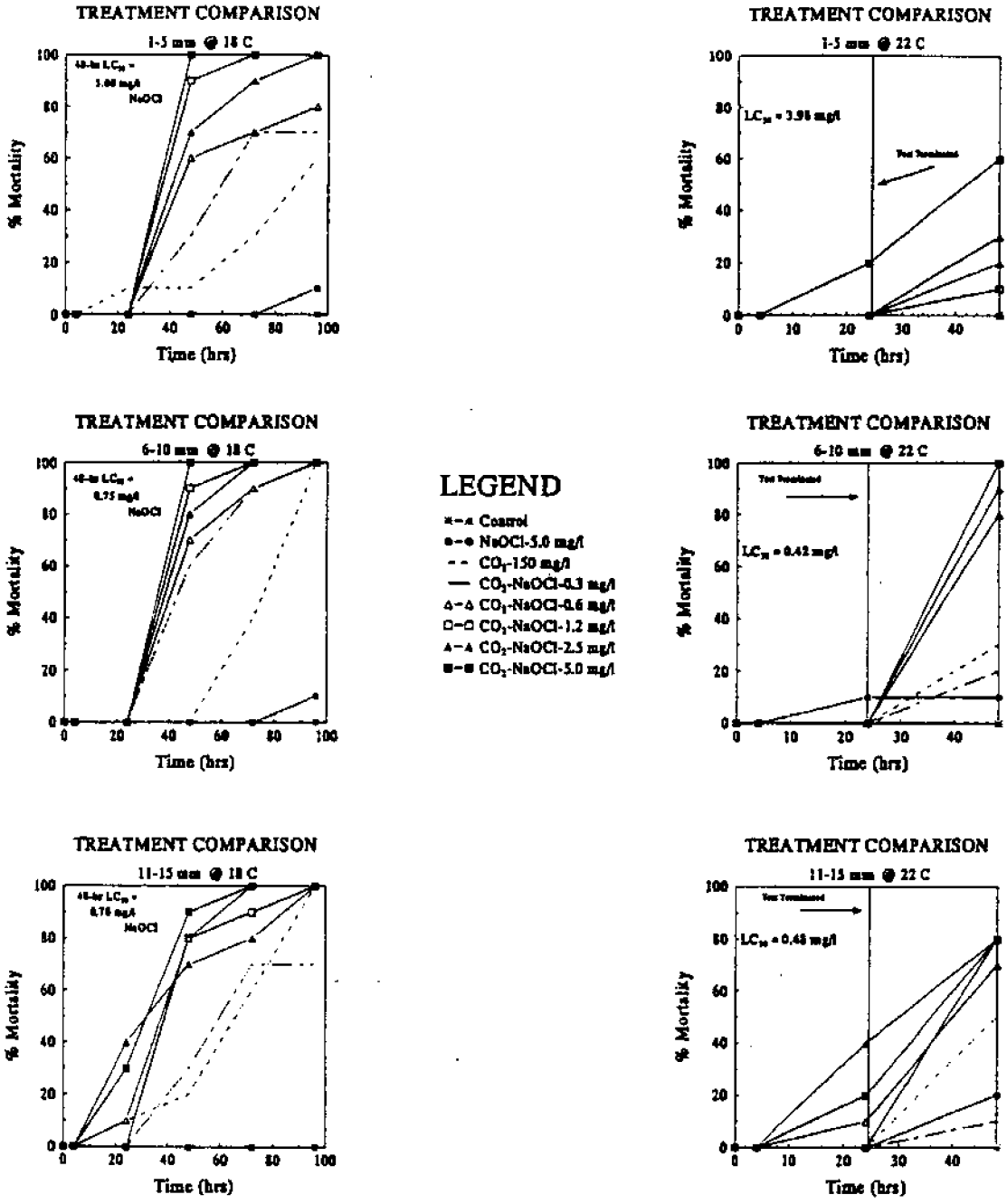

Figure 4. Effectiveness of the Different Treatment Applications on Dreissena polymorpha *at 18 and 22 C*

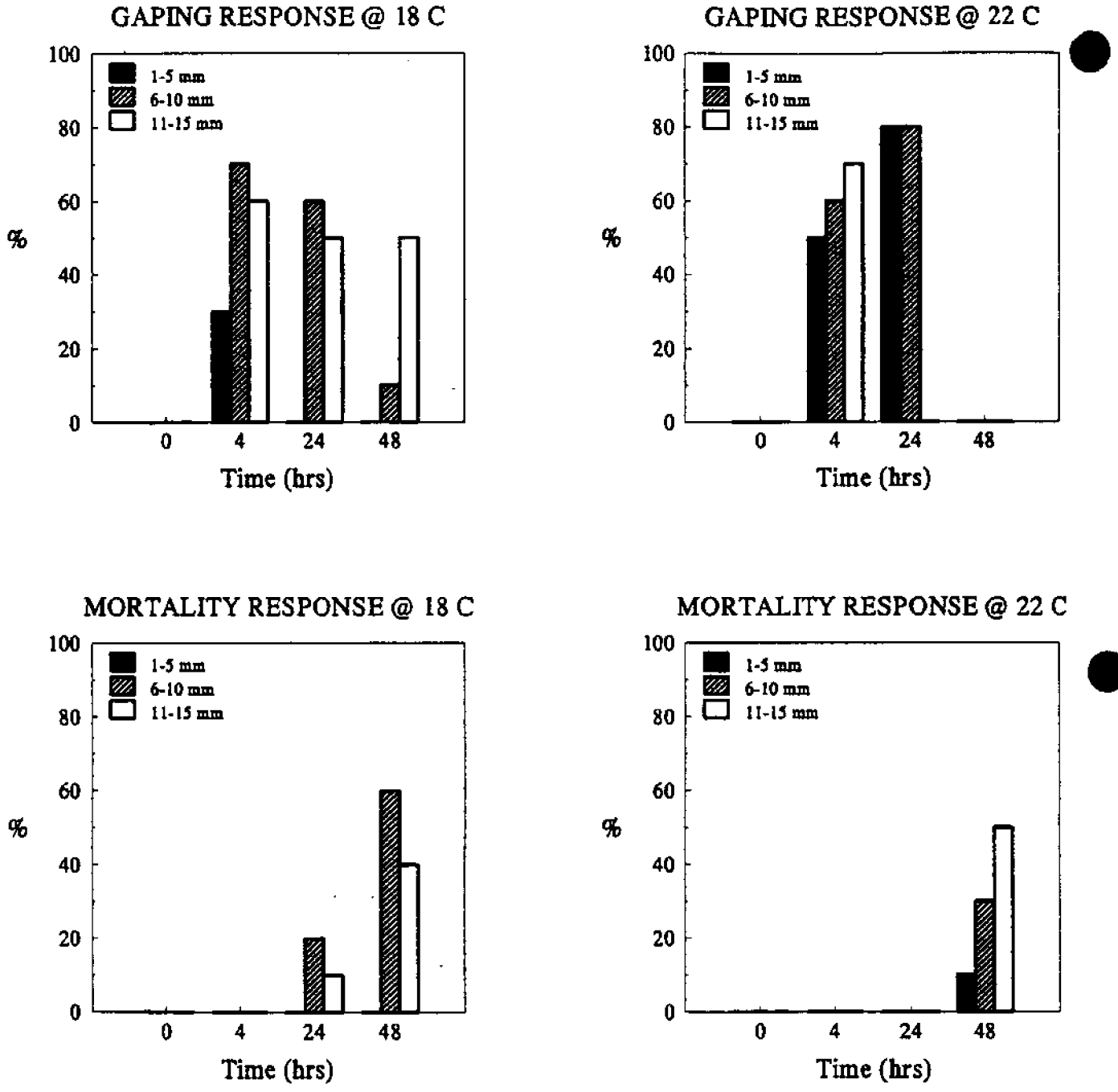

Figure 5. Effectiveness of CO_2 Application on Size of Dreissena polymorpha *at 18 and 22 C*

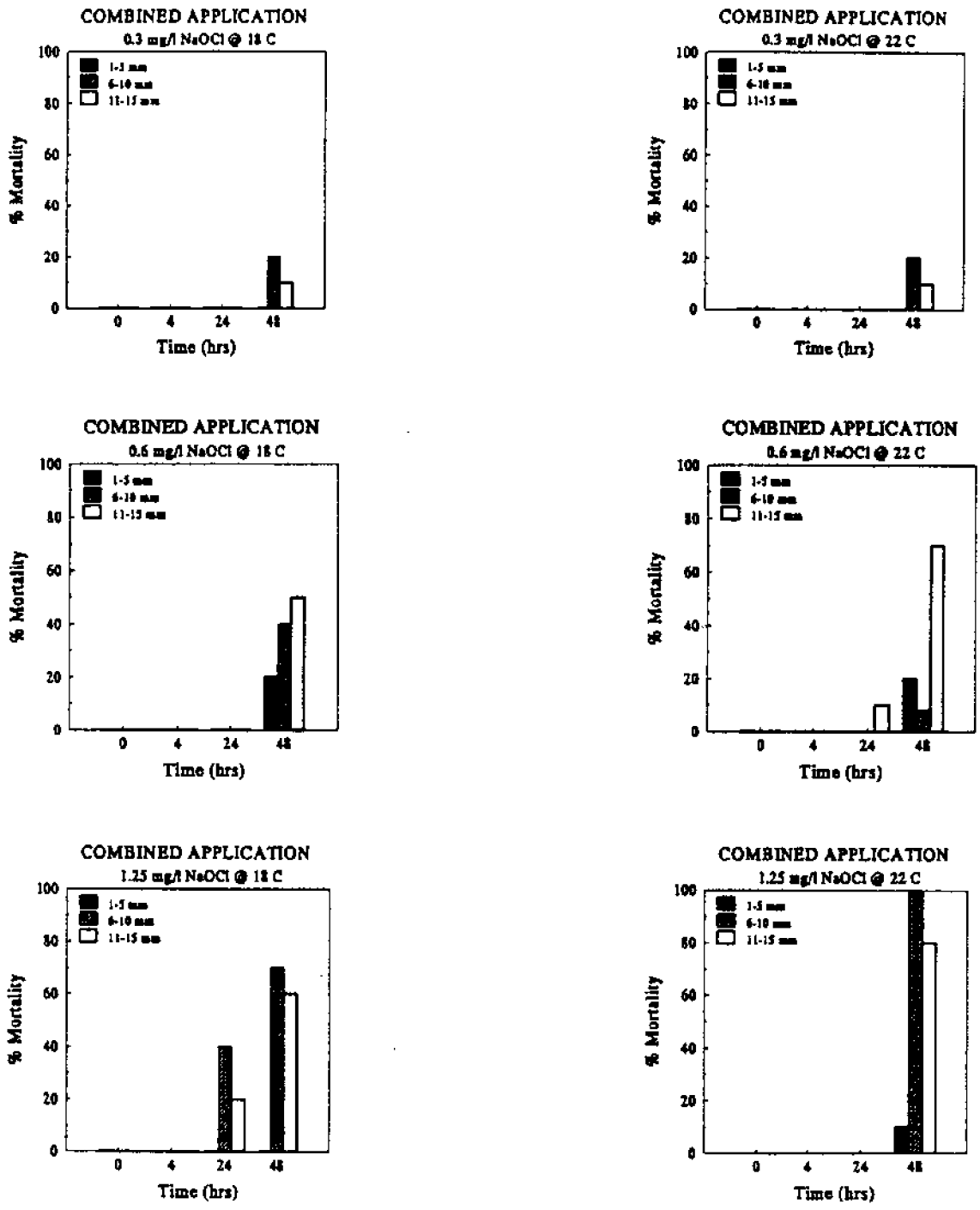

Figure 6. Variable Effectiveness of Combined Treatment on Size

"Birth Control" in Zebra Mussels: Inhibition and Sex-Specific Activation of Spawning by High Affinity Serotonergic Ligands

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Abstract--Zebra mussels (*Dreissena polymorpha*) can be stimulated to spawn with external application of serotonin (5-hydroxytryptamine, 5-HT). To develop methods of controlling zebra mussel spawning, the effects of methiothepin and metergoline, two compounds known to have high affinity for both vertebrate and invertebrate 5-HT receptors, on 5-HT-induced spawning in zebra mussels were tested.

To test for inhibitory effects, mussels were pre-treated with methiothepin or metergoline for two hours, after which they were exposed to 5-HT for four hours and scored for male or female spawning. Sex and maturity of all non-spawning animals were assessed by dissection at the end of the experiment.

Methiothepin significantly reduced spawning induced by 10^{-4} M and 10^{-3} M 5-HT in both males and females. Significant reductions in spawning were obtained with methiothepin concentration as low as 10^{-6} M, 100-fold less than the corresponding 5-HT concentration. Metergoline (10^{-4} M) reduced spawning elicited by 10^{-3} M 5-HT; however, at lower concentrations (10^{-8} M to 10^{-5} M), metergoline itself significantly induced spawning in male zebra mussels but not female zebra mussels. Metergoline (10^{-5} M)-induced male spawning was inhibited by 10^{-5} M methiothepin.

Thus, methiothepin had effects on spawning at concentrations as low as 10^{-6} M, and metergoline affected spawning, inducing it in males, at concentrations as low as 10^{-8} M. In other experiments (Fong et al., in press, b), methiothepin produced long-lasting inhibitory effects on female spawning latency at concentrations as low as 10^{-8} M. Methiothepin is the most effective inhibitor and metergoline the most powerful activator of spawning yet tested in zebra mussels. Similarities in the pharmacology of spawning in zebra mussels to the results of ligand-binding studies in the freshwater snail *Lymnaea* suggest that the receptor mediating spawning in zebra mussels may be related to 5HTlym, a serotonin receptor in *Lymnaea* that has been cloned by Sugamori et al. (1993).

INTRODUCTION

The zebra mussel is a bivalve that was recently introduced accidentally into North America (Hebert et al., 1989; Ram et al., 1992). Although best known for its biofouling effects, the zebra mussel has proven to be a highly suitable organism for studying neurotransmitter control of reproductive behavior in a bivalve. As described briefly below, there is excellent evidence for the physiological role of serotonin in the regulation of spawning in zebra mussels. In addition, several serotonergic agonists and antagonists of zebra mussel spawning have previously been identified; however, until now, no agonist or antagonist had been shown to be active in regulating spawning at concentrations lower than that of serotonin. It was the purpose of the present study to identify more effective agonists and antagonists of spawning.

Zebra mussels spawn in response to serotonin applied either by injection or by external application in the surrounding water (Ram et al., 1993a). As little as 5 min application of serotonin is enough to trigger a complete spawning episode (Fong et al., in press, a). At room temperature male zebra mussels usually spawn within 30 min; whereas, female mussels take an hour or more (Ram et al., 1993a; Fong et al., in press, a,b). Histological analysis shows that prior to serotonin application ripe oocytes are normally arrested at prophase I with large germinal vesicles. Following serotonin application, germinal vesicles break down and oocytes mature to metaphase I, with spindles clearly evident, prior to release from the animal (Kyozuka et al., 1993; Fong et al., in press,a). Spawned oocytes are almost uniformly at metaphase I and remain at that stage until fertilization. Studies of sperm motility and fertilization have shown that serotonin-released sperm are active, and oocytes are capable of fertilization and early embryonic development (Crawford et al., 1991; Kyozuka et al, 1993; Miller et al., 1994). Serotonin has been demonstrated immunohistologically and by HPLC analysis to be present in zebra mussel gonad, supporting its role as a physiological mediator of spawning and oocyte maturation (Ram et al., 1992).

The receptor mediating spawning has been characterized pharmacologically by determining effects of specific serotonergic agonists and antagonists. 8-OH-DPAT and serotonin were about equally effective at stimulating spawning (Ram et al., 1992; Fong et al., 1993); TFMPP, 2-methylserotonin, and alpha-methylserotonin were all less effective than 8-OH-DPAT and serotonin at inducing spawning (Fong et al., 1993). In studies of possible antagonists, ketanserin and propranolol had no effect; mianserin, NAN-190, and cyproheptadine had partial inhibitory effects (Fong et al., 1993). The pharmacological profile for inducing/blocking spawning does not fit readily into vertebrate serotonin receptor categories; however, Sugamori et al. (1993) has recently cloned a serotonin receptor, 5HTlym, from the freshwater snail Lymnaea for which the ligand-binding profile in the receptor in an expression system more nearly resembles that of the pharmacology of zebra mussel spawning. In their study, Sugamori et al. (1993) identified several compounds that had much higher binding affinities for 5HTlym than either serotonin or 8-OH-DPAT. The present study investigated two of these high affinity ligands, methiothepin and metergoline, for

effects on spawning in zebra mussels.

MATERIALS AND METHODS

Animal handling procedures

Zebra mussels were collected on May 28, 1993 from Lake Erie by scraping clumps of mussels from vertical steel bulkheads at the Detroit Edison plant in Monroe, MI. Animals were maintained in the laboratory with moderate feeding at 12°C in thermostatically controlled aquaria until needed for spawning experiments. Such animals have remained responsive to spawning inducers until late September (Ram et al., 1993a). Reproductive state of animals was monitored by sampling a group of animals by squash mounts or by testing responsiveness to serotonin (see below) on at least a biweekly basis either in the course of scheduled experiments or as a regularly scheduled maintenance activity.

Spawning bioassay

Procedures for testing effects of agonists and antagonists on spawning were similar to techniques previously published (Fong et al., 1993). Briefly, the procedures were as follows: Agonists: Ripe zebra mussels were each placed in 10 ml buffered aquarium water in individual vials at room temperature. Generally, control and experimental animals were tested in groups of 10 to 20 animals at a time. This number is large enough to give reliable and statistically significant differences between groups, with respect to spawning v. no spawning, as tested by Chi square or Fisher exact tests. In all experiments, groups having no chemical addition or only

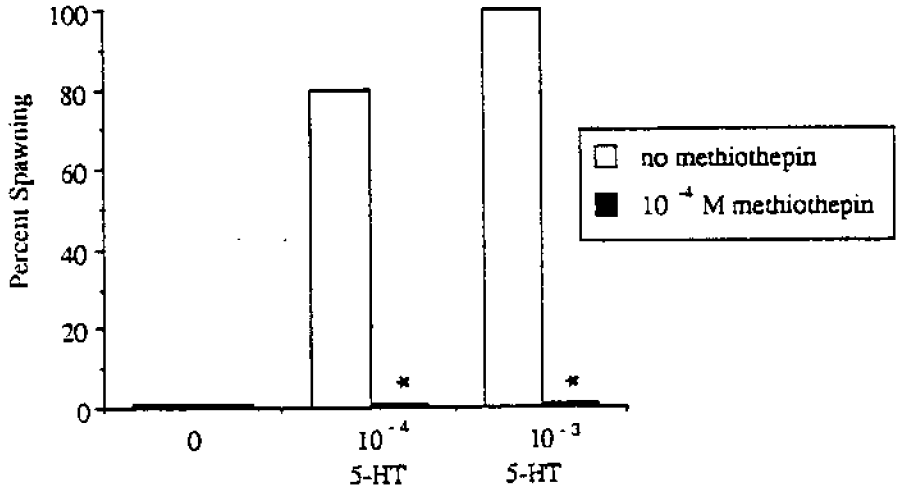

*Figure 1. Effect of 10^{-4} M methiothepin on spawning induced in zebra mussels by 10^{-4} and 10^{-3} M serotonin (5-HT). 0 group = aquarium water (negative control). Each group contained 10 animals. *, $p < 0.0007$, comparing 10^{-4} M methiothepin to no methiothepin. Reprinted by permission from Fong et al., 1994.*

vehicle added (negative control; 1% ethanol vehicle in the case of metergoline), or 10^{-3} M serotonin (positive control) were tested along with groups exposed to various concentrations of the chemical of interest. Spawning responses were assessed by sampling the water surrounding the mussel. Two drops were placed on a slide, covered with a coverslip, and searched under high power microscope for eggs or sperm. Frequently, the response was so massive that the surrounding water became cloudy with sperm or the bottom became carpeted with oocytes, in which case the microscopic assessment was merely for corroborating the response and determining sex. Observations of spawning response were usually made at least once every 15 min, to determine latency of response. The sex and maturity of all non-spawning animals were subsequently determined as described below. Antagonists: The procedure for testing antagonists was similar except that the chemical to be tested was added to the medium two hours before adding serotonin. The antagonist remained in the medium throughout the subsequent period (usually 4 hr) that serotonin was present. Control groups included the putative antagonist alone (i.e. not subsequently tested with serotonin) and no antagonist (with and without serotonin subsequently added=positive and negative control groups). As in the agonist studies, all non-spawning animals were subsequently assessed for sex and reproductive maturity.

Squash mounts were made by opening the shell, pushing gill tissue aside and lifting out a small amount of gonadal tissue (Y-shaped organ along midline) with fine forceps. The tissue was squashed between cover slip and slide, observed at high power (200-400X) under a microscope, and scored for sex and reproductive state.

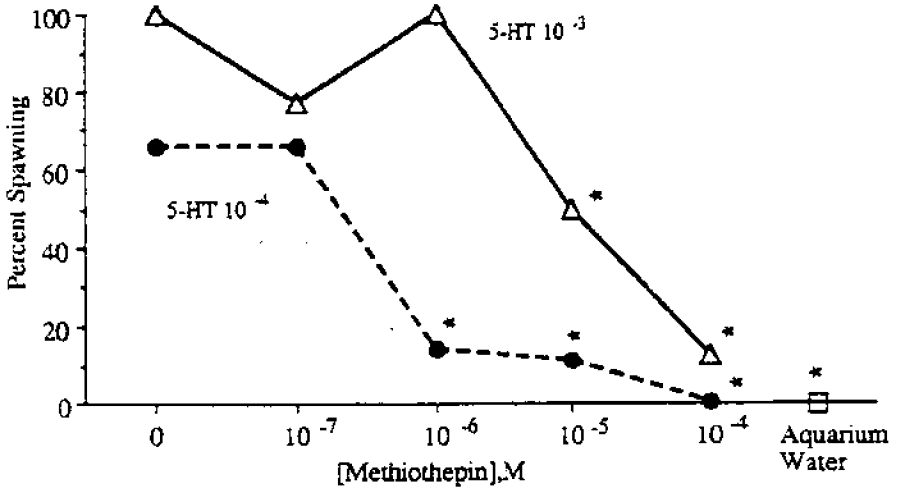

*Figure 2. Dose-response curves for zebra mussel spawning in 10^{-4} M and 10^{-3} M serotonin (5-HT) in the presence of varying concentrations of methiothepin. Each group contained 10 animals. *, $p<0.05$, compared to serotonin alone. Reprinted with permission from Fong et al., 1994.*

The reproductive state classification was as follows (Haag and Garton, 1992): 0, gonad spent; 1, immature; 2, initial stages of gametogenesis (first stage at which sex can be identified); 3, late immature with approximately 50% mature gametes; 4, ripe with $>>50\%$ mature gametes.

RESULTS

Methiothepin was a very effective antagonist. In the experiment illustrated in Figure 1, two-hour pretreatment with 10^{-4} M methiothepin completely blocked the activation of spawning by both 10^{-3} M and 10^{-4} M serotonin. Tested over a range of concentrations (Fig. 2), methiothepin produced significant reductions of spawning at concentrations up to two orders of magnitude lower than the corresponding concentration of agonist, serotonin.

Metergoline, at 10^{-4} M, partially blocked spawning induced by serotonin (Fig. 3); however, when tested at lower concentrations, metergoline proved to be an effective agonist for eliciting spawning in males. In the first of the three experiments summarized in Fig. 4, male mussels began spawning during the initial two hour period of the experiment. As a result of this serendipitous occurrence, the planned addition of serotonin was omitted, and resultant spawning from metergoline alone was assessed at the end of a total time of six hours. In this and subsequent replicates, metergoline significantly stimulated spawning in males at concentrations as low as

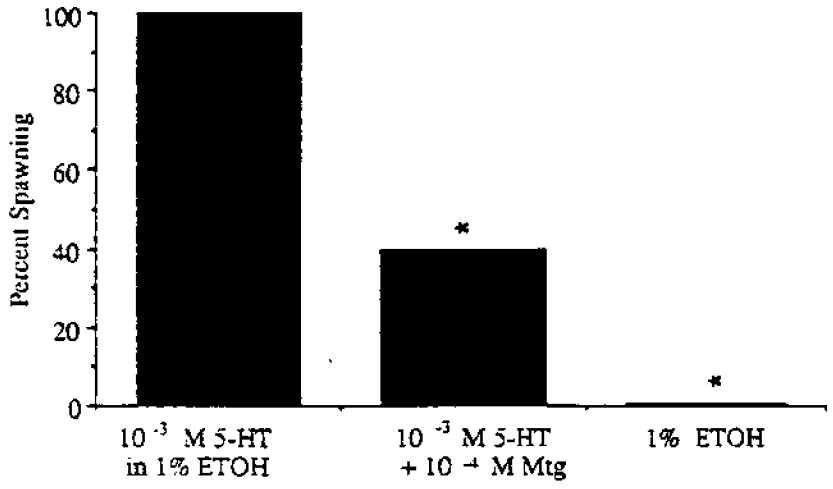

*Figure 3. Effect of 10^{-4} M metergoline (Mtg, dissolved in 1% ethanol) on spawning induced by 10^{-3} M serotonin (5-HT). Each group contained 10 animals. *$p<0.01$, compared to serotonin in 1% ethanol. Reprinted by permission from Fong et al., 1994.*

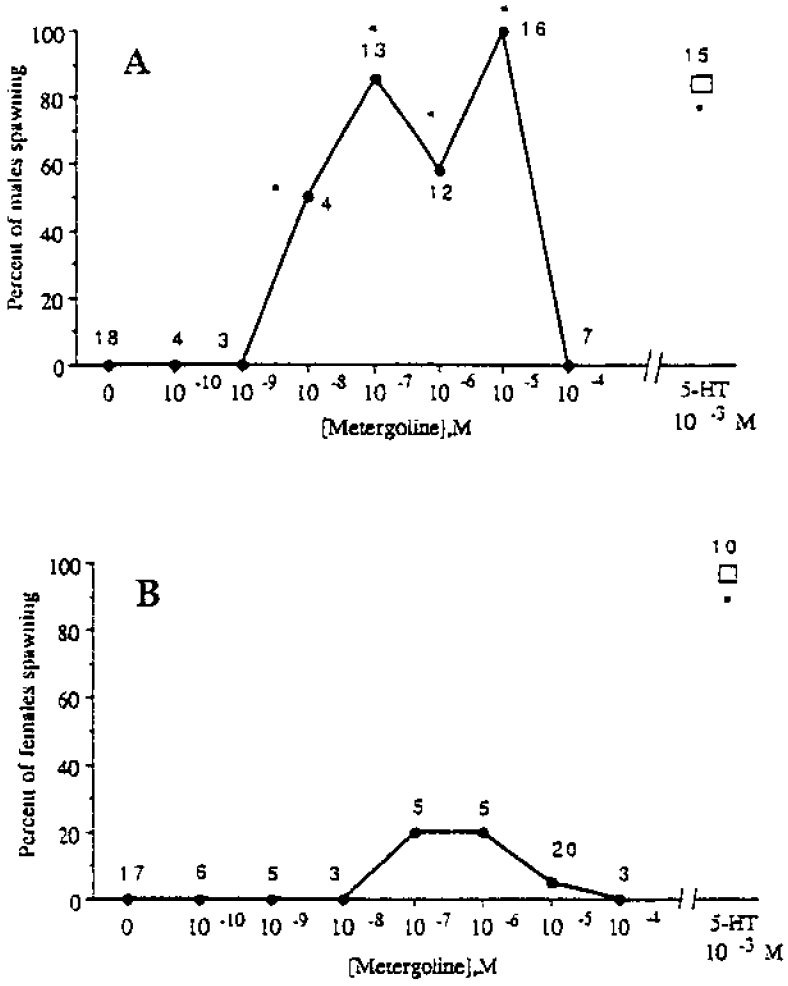

*Figure 4. Percent of males spawning (A) and females spawning (B) in varying concentrations of metergoline. Metergoline (10^{-4} M) was initially dissolved in 1% ethanol, then serially diluted to achieve desired concentrations. Data are pooled from three experiments. Numbers adjacent to each point show the total number of animals of that sex tested at the given concentration, out of 10 - 40 animals (of both sexes, since zebra mussel sex cannot be determined prior to spawning or post-experiment dissection) initially tested in each treatment. Percent spawning in 10^{-3} M serotonin (5-HT) tested simultaneously is given for comparison. *, $p < 0.05$, compared to no drug (group 0). Reprinted with permission from Fong et al., 1994.*

10^{-8} M. A few females also spawned at low concentrations of metergoline; however, the effect was not significant.

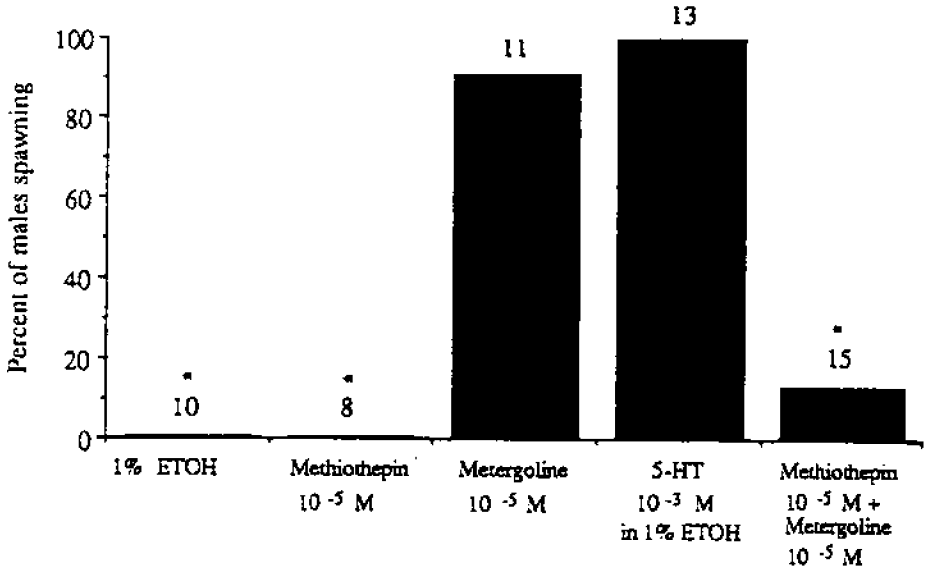

*Figure 5. Effect of 10^{-5} M methiothepin (Meth.) on 10^{-5} M metergoline (Mtg.)-induced male spawning. Methiothepin was present 2 hours before and during the 4 hour treatment with metergoline. No female spawned. Metergoline (10^{-5} M) was dissolved in 0.1% ethanol. Serotonin (5-HT, 10^{-3} M) was dissolved in 1% ethanol (ETOH). Above each bar are the numbers of males out of a total of 25 animals initially tested in each group. *, $p < 0.0002$ compared to 10^{-5} M metergoline alone.*

The stimulatory effect of metergoline on male spawning could be blocked by methiothepin. As illustrated in Fig. 5, no spawning was elicited by 1% ethanol (the vehicle for metergoline) or 10^{-5} M methiothepin, nor did 1% ethanol inhibit spawning elicited by 10^{-3} M serotonin. However, the high level (91%) of male spawning induced by 10^{-5} M metergoline was significantly reduced to 12% by the addition of 10^{-5} M methiothepin.

DISCUSSION

Methiothepin is the most effective antagonist and metergoline the most effective agonist yet demonstrated on spawning in zebra mussels. Metergoline had mixed agonist/antagonist properties, stimulating males to spawn at concentrations as low as 10^{-8} M, while blocking spawning at 10^{-4} M. Methiothepin produced significant inhibitory effects on serotonin-induced spawning at concentrations as low as 10^{-6} M. All previously studied serotonin agonists and antagonists required at least 10^{-4} M to cause significant activation or inhibition of spawning. This is also the first demonstration of a sex-specific stimulation of spawning at a time when both sexes were capable of spawning.

Although a large number of mammalian serotonin receptors have been

sequenced, as have several Drosophila sequences, only one molluscan serotonin receptor has been cloned. The molluscan sequence is 5HTlym, a G-protein coupled receptor cloned from a Lymnaea (snail) nervous system cDNA library (Sugamori et al., 1993). Competitive binding of 5HTlym expressed in COS-7 showed a pattern of affinities similar to ligands affecting spawning in zebra mussels; viz. very high affinity for methiothepin and metergoline, much lower but approximately equal affinity for serotonin and 8-OH-DPAT, and still lower affinity for ketanserin and propranolol.

Among other known serotonin receptor sequences, 5HTlym is closest to Drosophila 5HTdro2 sequences and to human 5HT1A and human 5HT1D sequences. 5HTdro2 receptors inhibit adenylyl cyclase and activate phospholipase C (Sandou et al., 1992). 5HT1A receptors may consist of a family of receptors some of which stimulate adenylyl cyclase and others of which inhibit adenylyl cyclase. The cloned 5HT1A receptor expressed in HeLa cells has been shown both to inhibit adenylyl cyclase and to stimulate phospholipase C metabolism (Fargin et al., 1989, 1991). G proteins mediate both actions of the cloned 5HT1A receptor; Gi proteins (especially Gi3) both inhibit adenylyl cyclase and stimulate phospholipase C (Fargin et al., 1991). Cloned 5HT1A receptors expressed in CHO cells have been reported to inhibit adenylyl cyclase, increase hydrolysis of inositol phosphates, cause a transient elevation of $[Ca^{2+}]_i$, and augment arachidonic acid release (Raymond et al., 1992). 5HT1D is another family of serotonin receptors, with one 5HT1D clone stimulating adenylyl cyclase (Maenhaut et al., 1991) and another (5HT1Dß) inhibiting adenylyl cyclase (Miller et al., 1992). Cloned 5HT1Dß receptors have also been shown to cause elevations of $[Ca^{2+}]_i$ and inositol phosphates (Zgombick et al., 1993). The 5HT1Dß receptor is very effectively inhibited by methiothepin, whereas metergoline acts as a full agonist at the 5HT1Dß receptor (Miller et al., 1992). As a result of the above considerations, we speculate that the serotonin receptor mediating spawning in zebra mussels will be a G-protein coupled receptor that resembles 5HTlym, stimulates inositol phosphate metabolism, and inhibits cyclic AMP synthesis.

Serotonin has previously been demonstrated to trigger spawning in a large number of marine bivalves (reviewed in Ram et al., 1992). Matsutani and Nomura (1982) first demonstrated serotonin induction of spawning, in scallops. Matsutani and Nomura (1987) also described an in vitro preparation of gonad fragments in which the release of oocytes could be triggered by serotonin and regulated by prostaglandins. Our studies, however, are the first to characterize pharmacologically the serotonin receptors involved in the spawning response.

In addition to the results described in the present paper, methiothepin has also been used to investigate the time course of effective stimulation of spawning by serotonin (Fong et al., in press,a). The critical receptor-activated event(s) mediating spawning by serotonin apparently occur within the first 5 - 10 min of serotonin exposure, since 5 min exposure to serotonin was sufficient to trigger spawning, and methiothepin reduced the spawning response when added 5 min after serotonin but not

when added after 10 min. Methiothepin has also been demonstrated to cause a long-lasting sex-specific reduction in serotonin-elicited spawning. Spawning by female zebra mussels was significantly reduced up to 12 days after a 5 hr exposure to 10^{-5} M methiothepin (Fong et al., in press, b). The same treatment with methiothepin had no long-lasting effect on male spawning. The occurrence of male-only spawning responses, as occurred in the present study with metergoline, and in the long-lasting effects of methiothepin (Fong et al., in press, b) may have a number of possible causes. It may indicate a difference in the receptors for 5-HT ligands between males and females, or that chemical/structural differences between males and females, such as the amount of lipids, may affect the efficacy, access, or retention of chemical modulators of reproductive behavior in zebra mussels.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Susan Jerrine Nicholsm, the U.S. Fish and Wildlife Service, and Detroit Edison for assistance in collecting zebra mussels. This publication is a result of work sponsored, in part, by the Michigan Sea Grant College Program under grants from the National Sea Grant College Program, National Oceanic and Atmospheric Administration (NOAA), U.S. Department of Commerce under grant No. NA16RC0417-01 and the State of Michigan. The U.S. Government is authorized to produce and distribute reprints for governmental purposes notwithstanding any copyright notation that may appear hereon. The views are those of the authors and do not necessarily reflect the views of NOAA or any of its subagencies. J.D. received support from a Summer Undergraduate Research Fellowship of the Department of Physiology. Illustrations are reprinted from Fong, et al. (1994), with kind permission from Experientia, published by Birkhauser Verlag AG, Basel, Switzerland.

REFERENCES

Crawford GW, Mojares JJ, Walker JU, and Ram JL. 1991. Zebra mussel spawning by serotonin: Video recording of sperm and egg ejection and subsequent fertilization and development. Proc. Second Int. Zebra Mussel Res. Conf. Rochester, NY. p. 50.

Fargin A, Raymond JR, Regan JW, Cotecchia S, Lefkowitz RJ. (1989) Effector coupling mechanisms of the cloned 5-HT1A receptor. J. Biol. Chem. 264:14848-14852.

Fargin A, Yamamoto K, Cotecchia S, Goldsmith PK, Spiegel AM, Lapetina EG, Caron MG, Lefkowitz RJ. (1991) Dual coupling of the cloned 5-HT1A receptor to both adenylyl cyclase and phospholipase C is mediated via the same Gi protein. Cellular Signalling. 3:547-557.

Fong PP, Duncan J, Ram JL (1994) Inhibition and sex specific induction of spawning

by serotonergic ligands in the zebra mussel Dreissena polymorpha (Pallas). Experientia 50:506-509.

Fong PP, Kyozuka K, Abdelghani H, Hardege JD, Ram JL (in press, a). In vivo and in vitro induction of germinal vesicle breakdown in a freshwater bivalve, the zebra mussel Dreissena polymorpha (Pallas). J. Exp. Zool. (in press).

Fong PP, Hardege JD, Ram JL (in press, b). Long-lasting, sex-specific inhibition of serotonin-induced spawning by methiothepin in the zebra mussel, Dreissena polymorpha (Pallas). J. Exp. Zool. (in press).

Fong PP, Wall DM, Ram JL (1993) Characterization of serotonin receptors in the regulation of spawning in the zebra mussel Dreissena polymorpha (Pallas). J. Exp. Zool. 267:475-482.

Haag WR, Garton DW. 1992. Synchronous spawning in a recently established population of the European zebra mussel, Dreissena polymorpha. Hydrobiologia. 234:103-110.

Hebert PDN, Muncaster BW, Mackie GL. 1989. Ecological and genetic studies on Dreissena polymorpha (Pallas): A new mollusc in the Great Lakes. Can. J. Fish Aquatic Sci. 46:1587-1591.

Kyozuka K, Fong PP, Ghani HA, Ram JL. 1993. Oocyte maturation and fertilization mechanisms in Dreissena polymorpha. Proceedings, Third International Zebra Mussel Conference.

Maenhaut C, Van Sande J, Massart C, Dinsart C, Libewrt F, Monferini E, Giraldo E, Ladinsky H, Vassart G, Dumont JE. (1991) The orphan receptor cDNA RDC4 encodes a 5-HT1D serotonin receptor. Bioc. Biop. Res. Comm. 180:1460-1468.

Matsutani T, Nomura T (1982) Induction of spawning by serotonin in the scallop, Patinopecten yessoensis (Jay) Mar Biol Lett 3:353-358.

Matsutani T, Nomura T (1987) In vitro effects of serotonin and prostaglandins on release of eggs from the ovary of the scallop, Patinopecten yessoensis. Gen. Comp. Endoc. 67:111-118.

Miller RJ, King A, Demchyshyn L, Niznik H, Teitler M (1992) Agonist activity of sumatriptan and metergoline at the human 5HT1D beta receptor: Further evidence for a role of the 5-HT1D receptor in the action of sumatriptan. Eur. J. Pharm. 227:99-102.

Miller RL, Mojares JJ, Stachecki J, Ram JL (1994) Species specific sperm attraction in zebra mussels and quagga mussels. Fourth Int. Zebra Mussel Conf., abstract.

Ram JL, Crawford GW, Walker JU, Mojares JJ, Patel N, Fong PP, Kyozuka K. (1993a). Spawning in the zebra mussel (Dreissena polymorpha): Activation by internal or external application of serotonin J. Exp. Zool. 265:587-598.

Ram JL, Fong P, Croll RP, Nichols SJ, Wall D (1992) The zebra mussel (Dreissena polymorpha), a new pest in North America: Reproductive mechanisms as possible targets of control strategies. Inv. Repro. Devel. 22:77-86.

Raymond JR, Kim J, Beach RE, Tisher CC (1993) Immunohistochemical mapping of cellular and subcellular distribution of 5HT1A receptors in rat and human kidneys. Am. J. Physiol. 264:F9-F19.

Sandou F, Boschert U, Amlaiky N, Plassat JL, Hen R. (1992) A family of Drosophila serotonin receptors with distinct intracellular signalling properties and expression patterns. Embo J. 11:7-17.

Sugamori KS, Sunahara RK, Guan H, Bulloch AG, Tensen CP, Seeman P, Niznik HB, and Van Tol HHM. (1993). Serotonin receptor cDNA cloned from Lymnaea stagnalis Proc. Natl. Acad. Sci. USA 90:11-15.

Zgombick JM, Borden LA, Cochran TL, Kucharewicz SA, Weinshank RL, Branchek TA. (1993) Dual coupling of cloned Human 5-HT1Dα and 5-HT1Dß receptors: Inhibition of cAMP and elevation in intracellular calcium ($[Ca^{2+}]_i$) via pertussis toxin (PT)-sensitive G proteins(s). Abs. Soc. Neurosci. 19:633.

Effects of MEXEL 432 on the Settling, Detachment and Mortality of Adult Zebra Mussels

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ABSTRACT

The zebra mussel *Dreissena polymorpha* is an unwelcome species in numerous countries as it is a organism with a high capacity of invasion of natural habitats as well as man-made structures such as water intake pipes. This colonization may cause adverse repercussions on both the aquatic environment and human activities. Biofouling attributed to this mussel has become a major problem for industrial facilities that withdraw raw water from areas that have been invaded by *D. polymorpha*.
To control the spread of zebra mussels as well as to remove existing colonies different strategies have been proposed. Although some of these methods are effective, they are often inappropriate under most conditions as they are non-selective and very expensive.
The aim of this study was to test a new molluscicide under low and intermittent doses during a variety of exposure time. The Mexel 432 is an organic surface-active agent.Because mussels attach themselves to diverse solid substrates by a byssus synthesized by the byssal gland, we have studied the effect of MEXEL 432 on the settling behavior of *D. polymorpha* on two different substrates.
A series of laboratory experiments were conducted to evaluate the effectiveness of different concentrations of this organic coumpound on byssal thread development, and on settling and detachment of adult mussels (18-23 mm shell length). Exposure regimes include 1.5 and 3 hours treatments each day for 1 to 30 days with molluscicide concentrations of 2, 6 and 10 mg L^{-1}. For all the treatments, mortality was monitored daily.
Results to date indicate that an intermittent injection of low concentrations has the potential for reducing molluscicide quantities while maintaining effectiveness. However, further investigations are needed to demonstrate whether or not this alternative method is suitable in terms of technical feasibility, economical cost and environmental acceptability.

INTRODUCTION

During the last decades, the freshwater mussel, *Dreissena polymorpha*, has colonized most of European rivers and lakes (Morton, 1969). After its introduction in Great Lakes, few years ago, zebra mussels have rapidely spread throughout North America (Griffiths et al., 1991)
Capacity to attach to many solid substrates with byssal threads makes the zebra mussel a major and sever biofouler of artificial raw water systems (Mackie, 1991; Jenner and Janssen-Mommen, 1993).
While a wide range of control strategies are now availaible against *Dreissena* fouling, chlorination remain the most used method for macrofouling problems.
All the existing strategies with oxidizing agent for controlling zebra mussels are not always effective and environmentally acceptable, and development of control technics with new molluscicides are required as fouling problems increase and widespread.
Khalanski (1993) has shown that continous injection of a new nonoxidizing chemical product was effective as molluscicide for control of zebra mussel. Organic agent tested (Mexel 432) consisted of open hydrocarbons chains with primary and secondary amines.
The aim of this study was to test the effectiveness of intermittent injection of Mexel 432 for controlling adult mussels. The objectives of laboratory experiments were not only to evaluate the capacity of this compound to kill the zebra mussels but also to prevent attachment of adult mussels. Because byssal attachment is indispensable for *Dreissena* colonizing hard substrates, prevent new byssal threads formation could be lead to a strategy of zebra mussel control. The effects of mexel 432 on byssal threads formation and byssal gland activity were studied.

MATERIALS AND METHODS

Materials

Organisms : Adult zebra mussels (18-23 mm shell length) were collected from the Moselle river (Northeastern France). The animals were maintained in the laboratory in continously aerated, dechlorinated.tap water (T : 18 ° C +/- 1 ° C, pH : 7.6-8.2) and were fed daily with frozen algae, *Chlamidomonas*. Frozen algae cubes were suspended over tank containing mussels. The algal cells were allowed to drip into water as the cube melted.

Compound : The product tested in these experiments, sold under the commercial name of Mexel 432 (Mexel Society, Haubourdin, France), contains no toxic organic or mineral sustances. It is composed of straight hydrocarbons chains with primary and secondary amines, emulsified in water. Concentrate manufactured compound contains 80 % water, and has a specific gravity of 0.998 g / mL.
In solution it forms a film on all surfaces which provides anti-corrosion and anti-fouling properties.
Mexel 4362 was determined by a colorimetric method, after extraction in an organic solvant.

In this study, all the concentrations of Mexel 432 are expressed as milligrams of the concentrated manufactured product per liter of water.

Methods

Mortality test : Efficacy of Mexel 432 were performed against adult zebra mussel in a flow throught exposure (100 ml / min) in 12 L tap water containers homogenized with a pump (6 L / min). One day prior the experiments, 50 mussels were allowed to attach themselves to different kind of supports used (glass slides and ceramic tiles).
Concentrations tested against mussels were 2 , 6 and 10 mg manufactured product per liter of water. Zebra mussels were exposed for 1.5 or 3 hours per day during one month.
Control mussels were placed in tank containing only dechlorinated tap water.
Mortality was recorded daily as no shell valve closure occured after tactile stimulus.

Attachment test and byssus formation : Exposure conditions were the same for this second test except for treatment duration and acclimatation. Seven mussels were exposed daily to Mexel 432 for 3 hours during 10 days. Concentrations of 2, 6 and 10 mg / L were tested.
In each containers, each zebra mussel, without old byssal threads, was placed individually on a Petri dish and was not allowed to attach to the support before the initiation of experiment. Number of new byssal threads was recorded daily by microscopical observations.
Control mussels were placed only in dechlorinated tap water.

Byssal gland histology : After ten days of experiment, the specimens were prepared following the usual procedure for histological examination. The whole soft body was removed from the shell, fixed in 10 % formalin for 24 hours and dehydrated throught a series of increasing concentrations of alcool and toluen. Tissues were embedded in paraffin and sectioned at 5 - 7 µm. Finally, the slides concerning byssal gland were stained using different procedures (e. g. hemalum- picro-indigo carmin, hematoxylin, trichrom, toluidin blue) (Humason, 1979) to histochemically distinguish between elastin and collagen fibers of byssal threads.
Activity of phenol gland, responsable of quinone- tanning system of threads, located in the foot of mussel was studied with catechol incubation following the procedure of Smyth (1954).
Mann-Whitney test was employed to compare all mean values of the experimental bivalves and control group.

RESULTS

Mortality of control mussels over exposure duration (31 days) was very low, ranging between 2 and 5 % (Fig. 1 A, B) indicated that mortality in exposed sample resulted from toxic effects of Mexel 432 exposure.
In these experiments, no detachment of adult mussels was observed. Specimens died and remained attached by byssal threads to the substrate.

Mortality test

Mexel 432 was toxic to adult zebra mussels at all intermittent concentrations tested. Mortality pourcents of *Dreissena* exposed to compound for 1.5 hours per day are reported in figures 1 A and B.
Mortality rates were different between the two substrates for mussels exposed to 6 mg / L Mexel 432. They reached 16 % and 73.8 % in samples of mussels fixed on ceramic tile or glass slide respectively.
After one month, mortality levels reached 61.7 and 77.3 % in population exposed to the highest concentration of 10 mg / L .
40 % of mussels died on glass slide after exposure for one month to 2 mg / L Mexel 432.
Results of mortality rates for *Dreissena* exposed for 3 hours per day, during one month, to different concentration of Mexel 432 are reported in figures 2 A and B.
The whole population of mussels died after 15 days and after one month when exposed to 10 mg / L and 6 mg / L respectively. In sample of zebra mussels exposed to the highest concentration, mean time to death decreased with increasing time exposure. LT 50 values (LT 50 = estimated time for 50 % sample death) ranged from 19 to 7 days respectively.

Attachment test and byssal threads formation

Total mean number of byssal threads newly secreted by zebra mussel exposed to intermittent concentrations of Mexel 432 are reported in figure 3.
After 10 days control mussels have secreted an average number of 30 new byssal threads (temporary and permanent threads) (figure 3). Thread formation was greatest during the first four days (figure 4). After this time, threads formation continued but remained lower. The numerous byssal threads, newly and rapidely secreted, provides zebra mussels a solid attachment to substrates.
Eckroat et al. (1993) also reported formation of a majority of threads on the early days in *D. polymorpha* (16-26 mm shell lenght), however the total number of threads remained lower after four weeks of experiment.
At the end of this experiment, mussels exposed to the highest concentrations, have formed much less new threads than the control group did (figure 3). The difference between the two experimental and the control populations were highly significant ($P < 0.001$ and $P < 0.002$ respectively).
Zebra mussels exposed to the lowest concentration of Mexel 432 formed one half of new threads than the control group did (figure 3). The number of threads secreted daily by each mussel remained irregular during the exposure.
In the samples of mussels exposed to the highest concentrations (6 and 10 mg / L), the number of threads newly secreted remained extremely low at the end of the experiemnt, because only one mussel of each population has secreted few byssal threads only at the beginning of the exposure (figure 4).

Different staining procedures of histological section indicated that the byssus of zebra mussel was histochemically formed of both elastin and collagenous fibers.

In the present study, secretion granules and elastin pool have been observed in byssus histological sections of control and experimentally exposed mussels. Microscopical observations have not allowed us to reveal histochemical differences between byssus of exposed and control *D. polymorpha*. Frisina and Eckroat (1992) observed the presence of secretion granules in the root and the stem of byssus of *D. polymorpha* reponsable of elastin secretion. Elastin pool was observed in the stem of byssus.
Catechol technic has been used by Smyth (1954) to demontsrate polyphenol oxydase, which catalized tanning reaction in byssal threads of *Mytilus edulis*.
In this experiment, polyphenol oxydase has been observed in byssus histological section of control and exposed mussels. Intermittent concentrations of Mexel 432 tested in this study have not change polyphenol oxydase activity in byssal gland of *Dreissena*.

CONCLUSIONS

These laboratory tests indicated that Mexel 432 has molluscicidal capacities against adult zebra mussels. Rapid mortality rates of *Dreissena* induced by intermittent injections of this compound suggest that it could be use as molluscicide in arificial water systems. Futhermore low intermittent injections (6 mg / L) were effective to kill the whole population in one month. Injection of 10 mg / L induced 100 % mortality only in 15 days.
Laboratory experiments indicated that Mexel 432 was also effective to prevent attachment of adult zebra mussels. Formation of new byssal threads was reduced or totally inhibited in few days when mussels were exposed to intermittent injections of low concentrations of Mexel.
However, further studies on byssus of *D. polymorpha* are required in order to understand the effects of Mexel 432 on byssus activity and to improve a strategy for controlling zebra mussel macrofouling.

REFERENCES

Eckroat, L. R., Masteller, E. C., Shaffer, J. C., and Steele, M. L. 1993. The byssus of the zebra mussel (*Dreissena polymorpha*): Morphology, byssal threads formation and detachment. *In* "zebra mussels: biology, impacts and control" (T. F. Napela, D. W. Schloesser, Eds), pp. 239-263. Lewis Puplication, Boca Raton.

Frisina, A. C., and Eckroat, L. R. 1992. Histological and morphological attributes of the byssus of the zebra mussel, *Dreissena polymorpha*(Pallas). J. Penn. Acad. Sci. 66, 63-67.

Griffiths, R. W., Schloesser, D. W., Leach, J. H., and Kovalak, W. P. 1991. Distribution and dispersal of the zebra mussel (*Dreissena polymorpha*) in the great lakes region. *Can. J. Fish. Aquat. Sci.* 48, 1381- 1388.

Jenner, H. A., and Janssen-Mommen, J. P. 1993. Monitoring and control of *Dreissena polymorpha* and other macrofouling bivalves in the Netherlands. *In* "zebra mussels: biology, impacts and control" (T. F. Napela, D. W. Schloesser, Eds), pp. 537-554. Lewis Puplication, Boca Raton.

Khalanski, M. 1993. Testing of five methods for the control of zebra mussels in cooling circuits of power plants located on the Moselle River. *In* "Proceedings: Third international zebra mussel conference", Toronto, Ontario, Canada., 1993. EPRI, TR-10 2077 : 3-1. pp. 3-20.

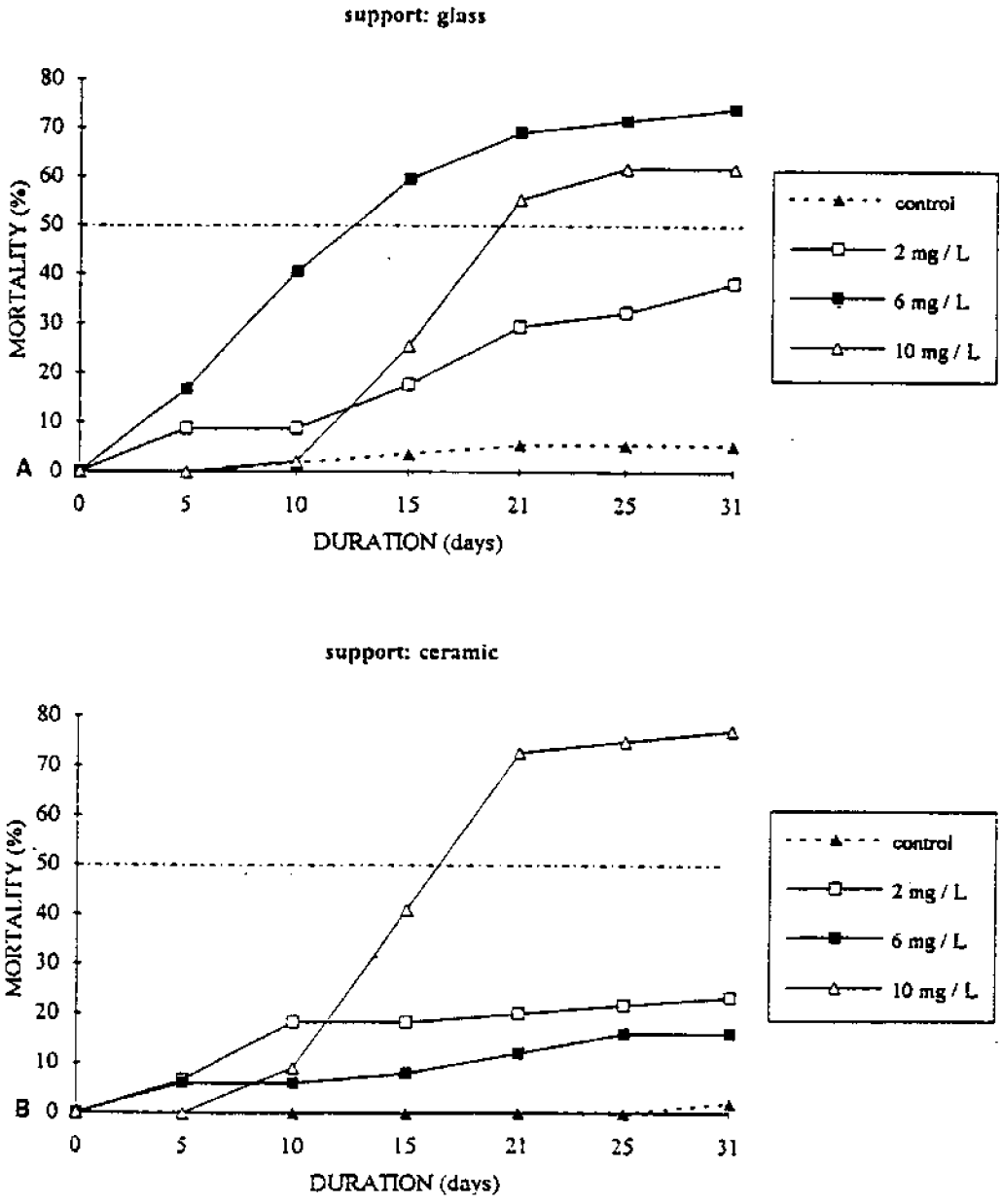
Mackie, G. L. 1991. Biology of the exotic the zebra mussel, *Dreissena polymorpha* in relation to native bivalves and its potential impacts in Lake St. Clair. *Hydrobiologia* 219, 251-268.

McMahon, R. F., Shipman, B. N., and Long, D. P. 1993. Laboratory efficacies of nonoxidizing molluscicides on zebra mussel (*Dreissena polymorpha*) and the asian clam (*Corbicula fluminae*). *In* "zebra mussels: biology, impacts and control" (T. F. Napela, D. W. Schloesser, Eds), pp. 575-598. Lewis Puplication, Boca Raton.

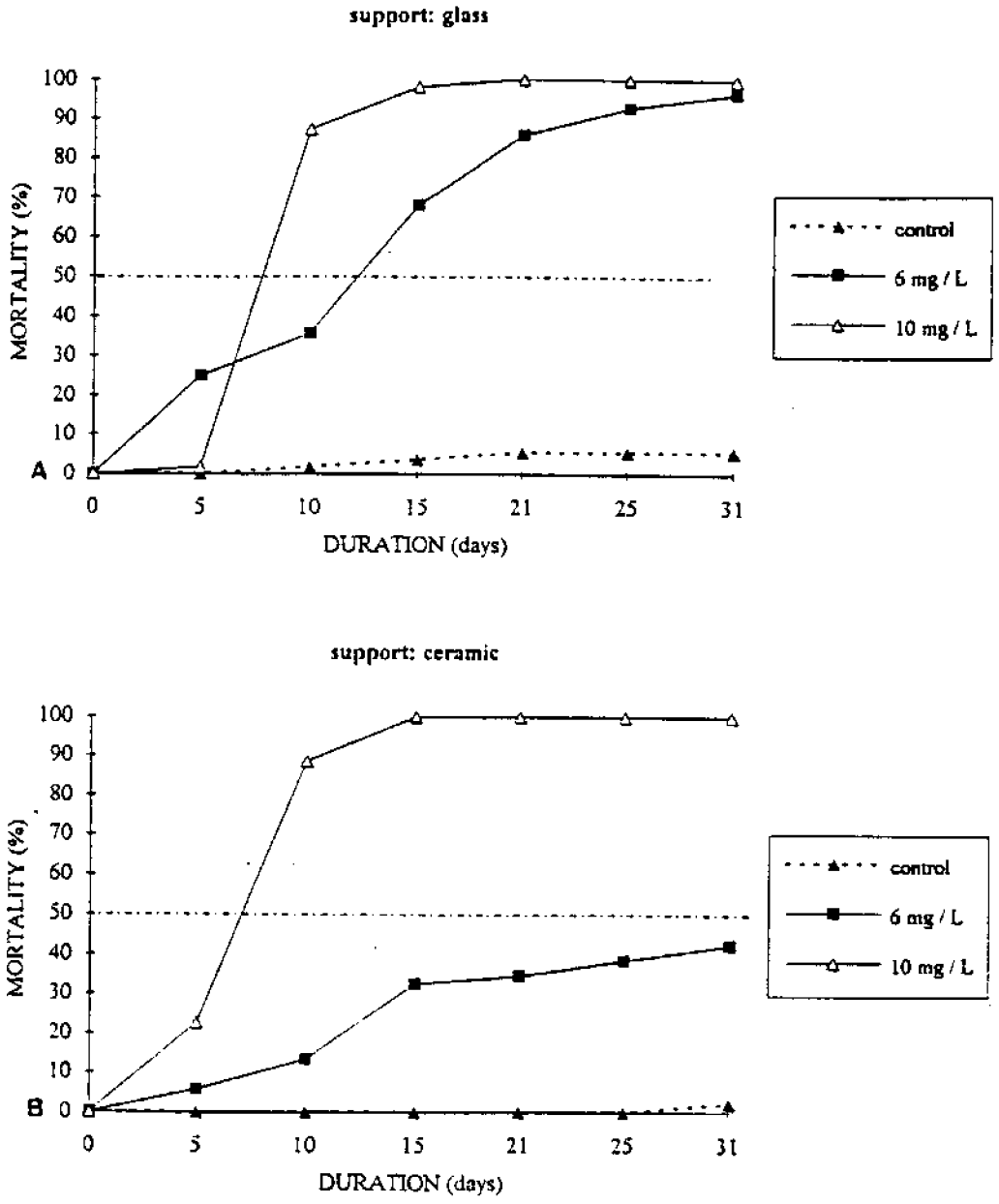
Morton, B. 1969. Studies on the biology of *Dreissena polymorpha* Pallas I. General anatomy and morphology. *Proc. malacol. Soc. Lond.* 38, 301-321.

Smyth, J. D. 1954. A technique for the histochemical demonstration of polyphenol oxidase and its application to egg-shell formation in Helminths and byssus formation in *Mytilus*.J. Micros. Sci. 92(2), 139-152.

Van Benschoten, J. E., Jensen, J. N., Lewis, D., and Brady, T. J. 1993. Chemical oxidants for controlling zebra mussels (*Dreissena polymorpha*) : a synthesis of recent laboratory and field studies. *In* "zebra mussels: biology, impacts and control" (T. F. Napela, D. W. Schloesser, Eds), pp. 599-619. Lewis Puplication, Boca Raton.



Figures 1: Mortality (%) of D. polymorpha exposed for 1.5 hours per day to Mexel 432 on glass slides (A) or ceramis tiles (B).



Figures 2 : Mortality (%) of D. polymorpha exposed for 3 hours per day to Mexel 432 on glass slides (A) or ceramis tiles (B).

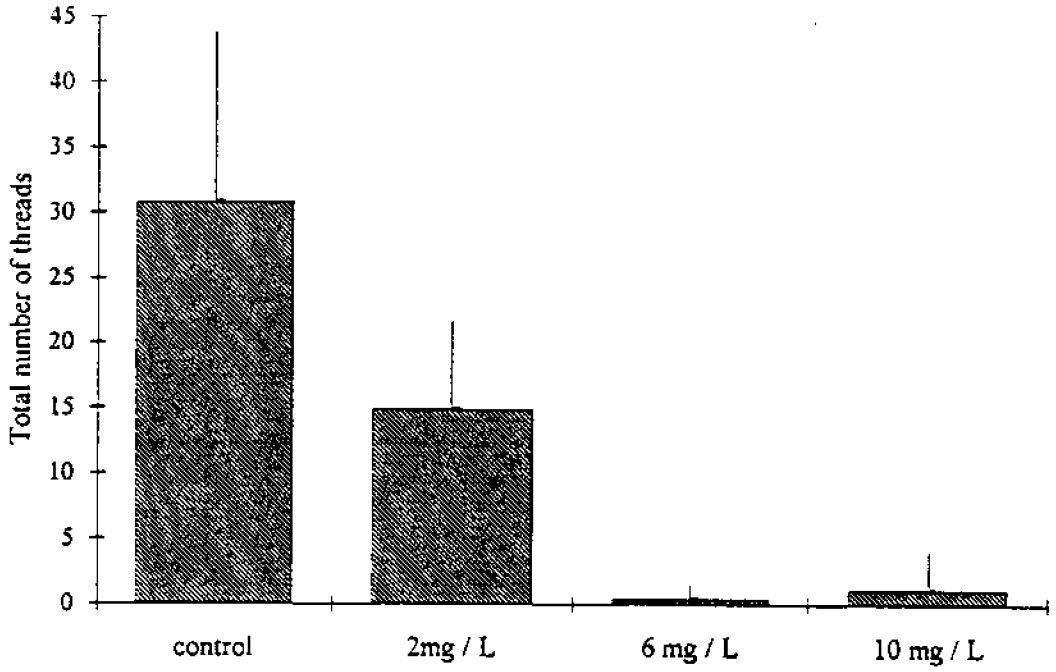


Figure 3 : Total mean number of byssal threads formed by D. polymorpha exposed to Mexel 432 for 10 days.

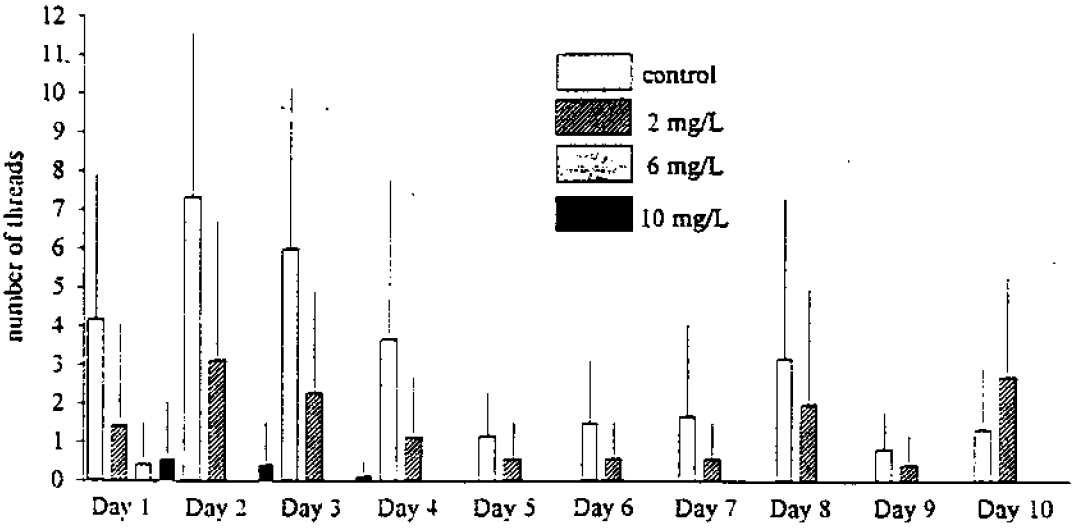


Figure 4 : Mean number of byssal threads secreted each day by D. polymorpha exposed to Mexel 432.

Development of Zebra Mussel Control Strategies for a Coalition of Vermont Water Suppliers on Lake Champlain

Cameron L. Lange, Therese M. Short (Acres International Corporation) and Edward Blake (Champlain Water District)

ABSTRACT

In early spring 1993, 12 raw water users on Lake Champlain formed the Lake Champlain Coalition in response to the likelihood of zebra mussel infestation in the lake and subsequently in their plants. The 12 coalition members, including 11 water treatment facilities and a fish hatchery, intended to pool their resources to develop a proactive strategy to control zebra mussels in their facilities without unnecessary redundant effort among facilities. The fish hatchery subsequently elected to independently develop a control strategy. In June 1993, Acres International Corporation was retained by the coalition to perform an evaluation aimed at developing zebra mussel control strategies for the 11 water treatment facilities. Goals of the evaluation were to:

- Review regulations, water quality data, plant characteristics, and proposed state monitoring programs;
- Group facilities sharing similar characteristics and potentially common recommended zebra mussel control solutions; and
- Evaluate feasible control strategies for each group and provide recommendations.

Based on key system similarities, the 11 facilities were separated into four groups. Various potential control strategies were evaluated for each group. For each group a matrix was developed to rank each of the evaluated controls for the various raw water components. Considerations in the ranking process included biological effectiveness, regulatory restrictions, costs, adaptability, operational impacts, and environmental impacts. Control strategies evaluated for the group raw water components included chlorination, dual potassium permanganate/chlorine injection, mechanical cleaning, hot water flushing, deoxygenation, installation of redundant intakes, or some combination of the above. Mechanical cleaning was recommended to control fouling on the submerged

intake screens or racks and dual potassium permanganate/chlorine injection systems were found to be workable and, in some cases, the preferable option for proactive protection of the intake piping at all facilities. Deoxygenation and mechanical cleaning were workable intake control options for two of the groups; however, further investigations would be required prior to final selection of these options.

INTRODUCTION

General

The zebra mussel (Dreissena polymorpha) is a freshwater macrofouling bivalve species that has recently been introduced to North America. This mussel has the potential to biofoul raw water intake systems, limit recreational use opportunities, and alter the aquatic environment. Potential effects on water treatment facilities include operational problems involving constriction or blockage of conduits and trashracks, as well as contributing to taste and odor problems. During the period 1989-1994, biofouling attributed to zebra mussels has been documented at a large number of water supplies and water-dependent facilities in the Great Lakes drainage basin. In July 1993, adult and juvenile zebra mussels were documented at the southern end of Lake Champlain in Vermont. This discovery placed the raw water users of the entire lake at a short-term zebra mussel fouling risk.

Lake Champlain runs south to north, is about 120 miles long and 12 miles across. Five distinct basins are present: the river-like South Lake; the cold deep Main Lake; the shallower Inland Sea; Malletts Bay; and Missiquoi Bay (LCRC, 1992). The water quality in each basin is quite different, so much that they can almost be considered different waterbodies for any zebra mussel analysis. Overall, the majority of the lake can be classified as mesotrophic or eutrophic. Each summer, a large seiche develops that affects the water transport in the lake. Canals connect the North and South Lakes to the St. Lawrence River and the Hudson River, respectively, each of which are heavily colonized by zebra mussels.

Zebra mussels can spread and be introduced into new areas via natural and/or human-mediated dispersal mechanisms (Carlton, 1993). Natural mechanisms are water currents, birds, and other animals. Human-mediated dispersal mechanisms include those related to waterways, vessels, and navigation; fishing activities; as well as a variety of miscellaneous mechanisms that include aquaria release and intentional introductions. Adults and juveniles can be transported by all mechanisms, but eggs and larvae can be transported by fewer means. Downstream movement is achieved by all of the mechanisms, while upstream dispersal can be achieved by all of the identified mechanisms except for current flow. By identifying the potential vectors that will introduce the zebra mussel into a new area, it may be possible to predict how soon the zebra mussel will significantly impact the new area.

As with any organism, the zebra mussel requires specific environmental parameters in order to survive and successfully reproduce. Salinity is limiting to zebra mussel growth at about 6.0 ppt, and survival is affected at 8.0 ppt, over a wide range of temperatures (Kilgour and Mackie, 1992). Salinities of this magnitude occur only in estuarine

situations, so chloride concentrations in freshwater systems would not limit mussel growth or survival. Other limiting factors that may be important in freshwater systems include calcium, pH, temperature, and chlorophyll a. Ongoing investigations are attempting to determine the critical limit of each parameter, but initial research indicates that the critical limit for each parameter depends on the condition (i.e., health) of the mussel population as well as synergistic effects of the various parameters. By evaluating water quality data for a particular water source, it is possible to predict the likelihood of zebra mussel survival and growth within that water source.

Background on 11 Water Treatment Facilities

Eleven Lake Champlain Coalition members draw water from the lake for municipal drinking water. These water suppliers, located on the eastern shore of Lake Champlain, in Vermont are (Figure 1):

- Champlain Water District;
- Tri-Town Water District;
- Burlington Water Resources Department;
- Village of Swanton Water Department;
- City of St. Albans/Maquam Shore Treatment Plant;
- Alburg Water Department;
- Vergennes/Panton Water District;
- South Hero Fire District No. 4;
- Grand Isle Fire District No. 1;
- Grand Isle Fire District No. 3; and
- Grand Isle Fire District No. 4.

All 11 water treatment facilities have at least one offshore intake, intake piping, a raw water pump station, and water treatment processes. Typical daily flow rates range from 8,000 gallons/day to 8.6 million gallons/day per facility. Submerged intake pipe lengths range from approximately 200 ft to 6,700 ft. Two facilities have redundant intake piping. Presently, the raw water within the intake pipes is untreated and no controls are in place for zebra mussel protection.

Grand Isle Fire Districts No. 1 and No. 3 are proposed to be replaced by a single facility by 1995. The new facility would possibly draw water from the nearby Ed Weed Fish Culture Station's deep intake sump. The hatchery is presently evaluating the vulnerability of their system to zebra mussel fouling and developing potential control strategies. The deep intake sump is gravity fed from the lake via 3,400 ft of 36-inch diameter intake piping. Since the mouth of the deep intake is located in approximately 180 ft of water, the water supply to the sump is relatively cold throughout the year. Since the cold water temperatures are not conducive to zebra mussel growth and

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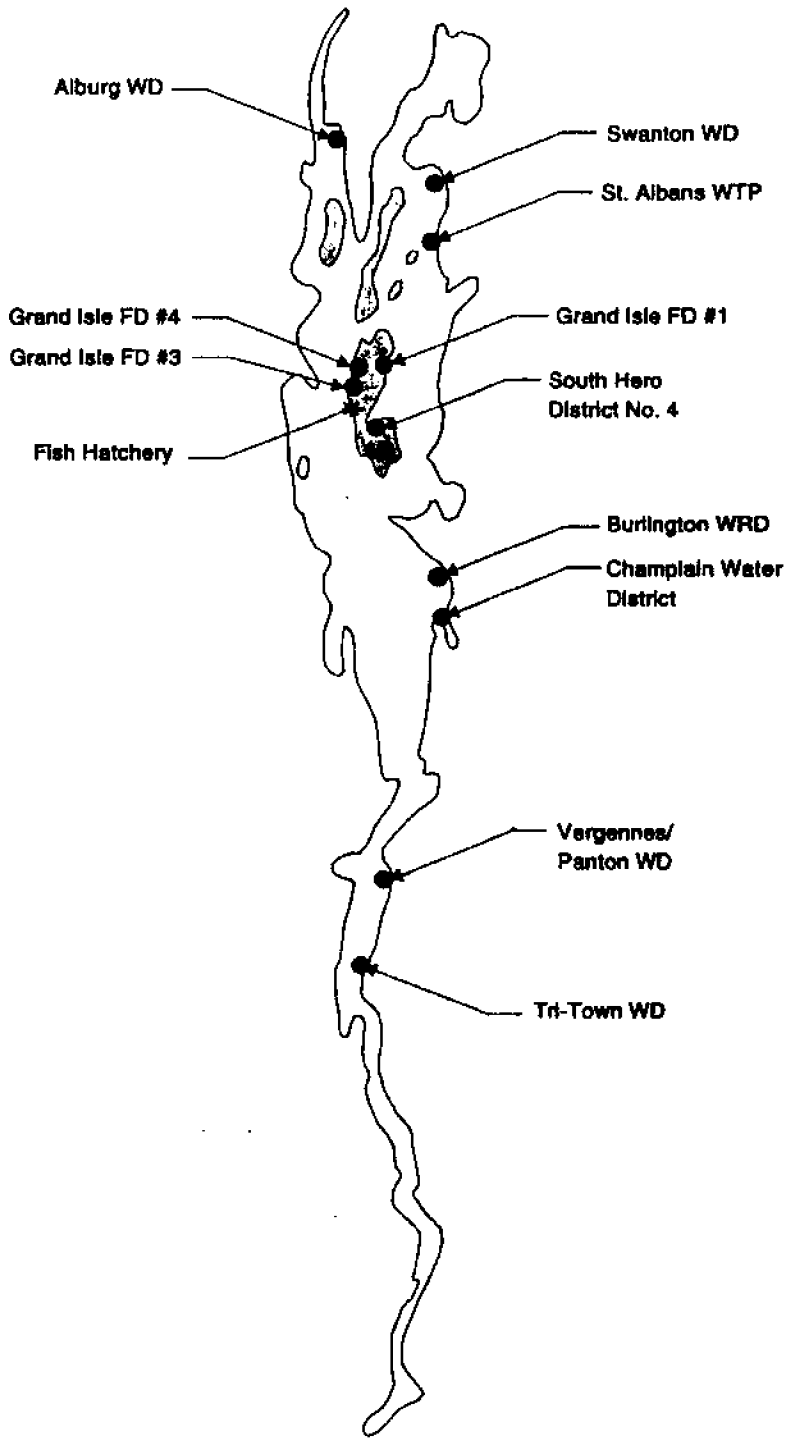

LOCATION OF COALITION
MEMBER'S WATER TREATMENT FACILITIES

Figure 1

attachment, it is possible that the fish hatchery will rely on this as their method of fouling control in the intake pipe. Other methods that may be considered are chlorine or ozone injection.

Background on Zebra Mussel Control Technologies

There are a variety of approaches to prevent and/or alleviate zebra mussel biofouling that have been proposed or are being used. These approaches fall under the broad categories of biological, physical, and chemical methods. Each approach has unique environmental impacts, biological effectiveness, and costs associated with its application; many have not yet been applied in-field so are considered unproven technologies. Some proven technologies available to combat the zebra mussel within raw water intakes and conduits for water treatment facilities may also be limited in site-specific applicability, when factors such as governmental, health, and environmental regulations, as well as plant operational constraints are considered.

Physical and chemical approaches to zebra mussel control can be categorized as proactive and reactive. To obtain the best possible protection, reactive strategies should only be used in situations where proactive control is not technically or economically feasible.

Chemical injection, to date, remains the most widely used proactive method of zebra mussel control in the United States and Canada.

METHODS

To be proactive in their zebra mussel response, the Lake Champlain Coalition desired to develop a plan for the control of zebra mussels before they became a widespread problem in Lake Champlain. To accomplish this goal, Acres was retained to complete the following tasks:

- Review available information regarding the zebra mussel and current applicable Federal and state regulations;
- Review existing water quality and sampling/monitoring data to aid in control planning;
- Evaluate feasible alternative control methods; and
- Define pilot-scale experiments to determine the likelihood of control success without undesirable impacts.

The first task was to determine whether the water users would be impacted by zebra mussels. A review was conducted of recreational activity on the lake, connecting waterbodies and nearby infested water sources to identify the possible transport mechanisms and thus to determine the likelihood of zebra mussel introduction into the lake. To determine the likelihood of zebra mussel survival in Lake Champlain, water quality parameters such as pH, temperature, calcium, chlorophyll a, and total organic carbon (TOC) were reviewed and compared with literature-based limits.

The next task was to review the raw water systems of each facility and combine facilities into logical groups based on the potential for common zebra mussel control solutions. Generally, facilities were grouped according to intake flows, allowed plant downtime, intake redundancy, and/or planned facility modifications.

A zebra mussel control alternative evaluation was conducted for each of the grouped facilities. Facility raw water systems are generally comprised of intakes and water transmission systems. Zebra mussel control for the intakes was evaluated independent of the water transmission systems, because some typical methods employed to protect the latter cannot be employed at the intake for environmental reasons. A matrix, which ranked the control alternatives for the intake and for the water transmission systems, was developed for each group. Considerations in the selection of the preferred control strategy included:

- Proven biological effectiveness;
- Possible regulatory restrictions, especially disinfection byproduct (DBP) levels;
- Relative capital and operations/maintenance costs;
- Adaptability to the existing system;
- Possible impact on plant operations; and
- Environmental impact.

A review of Federal and state regulations pertaining to zebra mussel control was incorporated into the control evaluation. The major concern for water treatment facilities is human health, which is regulated by the U.S. Environmental Protection Agency (USEPA) or its state designate. Many of the zebra mussel control strategies for water treatment facilities employ chemical addition, which may affect the water quality through formation of DBPs. Maximum Contaminant Levels (MCLs) for DBPs set by the USEPA as well as the course of future regulations on DBPs were reviewed. The Vermont Department of Environmental Conservation (VTDEC) permitting requirements for drinking water facilities were also reviewed.

RESULTS

General

Based on the review of potential dispersal mechanisms and water quality, Acres predicted that zebra mussels would eventually colonize and flourish in Lake Champlain. Human-mediated transport mechanisms, including transport by boat, were predicted to play a significant role in the dispersal of zebra mussels into Lake Champlain. Based on the analysis of potentially limiting water quality parameters of temperature, pH, calcium, and nutrients, Acres concluded that reproducing colonies of adult zebra mussels would likely be established throughout the near shore areas of Lake Champlain. Soon after Acres' analysis was completed, zebra mussels were, in fact, documented in Lake Champlain.

For the control evaluation, facilities were placed into four groups. Four facilities were placed into Group A: Champlain Water District; Tri-Town Water District; Village of Swanton Water Department; and Vergennes/Panton Water District. These are all large facilities with flows greater than 500,000 gallons per day. They each have a single intake and have a maximum allowable downtime of between three and 36 hours.

Two facilities, Burlington Public Works and the City of St. Albans, were placed into Group B. These two facilities have redundant intake systems that provide significant flexibility relative to downtime.

Three facilities were placed in Group C: Alburg Water Department; Grand Isle Fire District No. 4; and South Hero Fire District No. 4. These are moderate-sized facilities with flows between 30,000 and 100,000 gallons per minute. Each facility has a single intake and limited downtime.

The fourth group included two small facilities, Grand Isle Fire Districts No. 1 and No. 3, that are planning on consolidating into one new facility. The new facility will likely use one of the fish hatchery's intakes as a water source.

Group A Control Evaluation

The results of the evaluation showed favorable conditions for zebra mussel settlement and growth in the Group A facilities' raw water system components. Colder water temperatures, however, from Champlain Water District's 65-ft deep intake may slow rates of growth and settlement at that facility. It cannot, however, be assumed that the cold water will act as a preventative control. Control options selected for this group of facilities included potassium permanganate/ chlorine, mechanical cleaning, and chlorination.

For environmental reasons (i.e., uncontrollable release to Lake Champlain), the chemical options considered for controlling fouling in the intake piping could not be considered for controlling mussel buildup on the intake screens. Also, antifouling coatings are not approved for use in drinking water facilities, so could not be considered for the intake screens. The only option for zebra mussel control at the intake screens, therefore, is periodic, diver-assisted mechanical cleaning using manual scraping and/or a high-pressure water jet.

The recommended control option for protecting the intake piping at the Group A facilities is the continuous or semi-continuous injection of potassium permanganate at the intake and short-term periodic shocking with high doses of chlorine injected at the intake. The permanganate is intended to continuously control the veligers entrained in the intake flow, and the periodic chlorination is intended to kill any translocated adults. The efficacy of permanganate on zebra mussel veligers is still being studied and was found in at least one laboratory experiment to deactivate the mussels rather than cause mortality. Facilities presently using potassium permanganate for proactive zebra mussel control are satisfied with its effectiveness. There are no regulated byproducts associated with the injection of potassium permanganate in drinking water systems. Dosages of the chemical are, however, limited by discoloration of the water on formation of precipitates. Although potassium permanganate has not been proven in long-term applications, its reported effectiveness to date for zebra mussel applications is good. The overall intent of the dual chemical feed system is to minimize chlorine usage and subsequent trihalomethane (THM) formation while providing some degree of proactive control with the option to use chlorine as needed.

Chlorination used alone was ranked lower in preference because of THM concerns associated with continuous dosing. THM measurements for at least two of the four facilities in this group were close to 50 ppb. With increased chlorine contact time associated with injection at the intake, THM levels may approach future DBP limitations if continuous chlorination is used. Mechanical cleaning was also ranked lower. Pigging operations for cleaning these larger diameter pipes would be expensive, and long periods of plant shutdown would be required; in some cases longer than would be permissible at one time. If cleaning were used as a control, it would either have to be accomplished in stages or a redundant intake pipe (either temporary or permanent) would have to be installed. The Champlain facility, in particular, has some sharp bends in its piping and, therefore, it is questionable whether pigging, which is the preferred method for cleaning in this application, would be possible without the pig getting "hung up" in the pipe bends, which would jeopardize restart of plant operations.

For the recommended option, a potassium permanganate solution will be continuously injected at the intake via small diameter piping installed to the mouth of the intake. The chemical dosage will be regulated to maintain a residual of at least 0.2 ppm in the

vulnerable piping just prior to the existing water treatment processes. Another pipe will be installed alongside the permanganate piping for periodic delivery of a chlorine solution to the intake. It was recommended that the piping system be shocked with chlorine twice per year for two-week periods, in the spring and fall, at dosages regulated to produce a residual of 2 ppm at the end of pipe. Either the existing prechlorination equipment or all new equipment will be used. The chemical solution lines will either be installed within the existing intake pipe or alongside the intake pipe (lake bottom installation), encased in a carrier pipe to protect against chemical leakage to the surrounding water body. Although the pipe-within-a-pipe method is common for this application and is preferred to reduce environmental concerns, lake bottom installations are also possible if precautions are taken to reduce the potential for leakage. In some cases where a pipe-within-a-pipe installation was not practical, lake bottom construction was considered.

Mechanical cleaning of the intake screens will be accomplished by a diver using either mechanical scraping techniques or a high-pressure water jet. It was recommended that screens be cleaned at least once in the fall of every year. A diver inspection should also be conducted in the spring of every year, to determine whether a second cleaning is necessary. Since the frequency of required cleaning is dependent on zebra mussel loading and the water quality at the intake, this recommendation was based on an estimate derived from past experience at other facilities. For the Champlain Water District facility in particular, the depth of the intake screens is such that water temperatures may not be conducive to zebra mussel veliger settlement throughout most of the zebra mussel breeding season. At this facility, reattachment of translocated adults may be the more significant means of fouling. The required frequency of cleaning will ultimately be determined by studying actual fouling patterns at these particular facilities.

Group B Control Evaluation

For the Group B facilities, conditions with the plants' raw water system components are favorable for zebra mussel settlement and growth. For the Burlington facility, however, colder water temperatures from its 40-ft deep intake may slow rates of growth and settlement. It was not, however, assumed that the cold water would act as a preventative control. Control options selected for Group B facilities included deoxygenation, chlorination, potassium permanganate/chlorine injection, and mechanical cleaning. The characteristic unique to these facilities is intake pipe redundancy, which allowed control options to be considered that require shutdown of plant flow through the intake pipe being treated.

Again, the only practical option for zebra mussel control at the intake screens is periodic mechanical cleaning with a diver using manual scraping methods or a high-pressure water

jet, as described for Group A, since chemical options and antifouling coatings cannot be considered.

Periodic chemical deoxygenation was proposed as the most cost-effective technology for reactive treatment of the intake piping at the Group B facilities. Chemical oxygen scavengers are presently being used to successfully control zebra mussel fouling in redundant intake pipes at a water treatment facility on Lake Michigan. Recommendation of this alternative, however, would be contingent on its acceptability from an environmental standpoint. Also, although the reported effectiveness to date for deoxygenation in zebra mussel applications has been good, it has not been proven in many applications. Therefore, selection of such a method would be more of a gamble than a widely used treatment method such as chlorination. If for any of these reasons this method would not be preferred, a dual potassium permanganate/chlorine feed system would be the next favorable option. It was also recommended that normal plant flow be maintained with only the necessary number of intake pipes and that any additional pipes be capped and used only if reactive treatment on the operational pipe is required. In this case, treatment may never be required on the normally non-operational pipes, especially if treatments are properly scheduled in low breeding periods to avoid fouling in the temporary intakes.

The mass transfer oxygen stripping method is an economical option; however, this particular method for deoxygenation has never been used for zebra mussel or macrofouling control, so it was ranked fairly low. Mechanical pigging is ranked below oxygen scavengers because it would have a much higher associated annual cost.

For the proposed deoxygenation method, the pipe to be treated would be isolated from the other intake pipes and either sodium bisulfite or sodium meta-bisulfite would be injected at the mouth of the intake from a floating work station. Uniform mixing of the chemical over the length of the pipe would be accomplished by providing a small flow through the intake pipe to draw the chemical to the end of the pipe. An alternative method for chemical injection would be to install a parallel pipe for closed loop circulation of the chemical solution. In either case, the chemical solution would be held in the isolated pipe for two to four weeks to cause mortality of the attached adult mussels.

Since deoxygenation would protect only up to the intake pipe header, any piping downstream of the header, up to the treatment process, would also be susceptible to fouling. It was recommended that any existing pre-treatment chemical injection points be moved upstream to the pump station discharge to protect the additional piping.

Mechanical cleaning and inspection of the intake screens would be accomplished in the same manner as described for the other facilities.

Group C Control Evaluation

Control options selected for the Group C facilities included chlorination, potassium permanganate/chlorine injection, and mechanical cleaning. Again, the only practical option for zebra mussel control at the intake screens is periodic mechanical cleaning with a diver using manual scraping methods or a high-pressure water jet, since chemical options and anti-fouling coatings cannot be considered.

To protect the intake piping at these facilities, it was proposed that a temporary intake be set up and mechanical cleaning be performed periodically without interrupting the water supply. Although the long-term costs of annual mechanical cleaning could be higher than the dual feed chemical option, it was proposed because of uncertainty of present DBP levels for these facilities. Recommendation of this option would be contingent upon the results of DBP formation pilot-scale studies. If DBP formation is found to be reasonable, a dual potassium permanganate/chlorine feed system installed to the mouth of the intake would be recommended.

Mechanical cleaning of the intake pipes would be performed utilizing a high-pressure water jet. Water jet cleaning is possible for these smaller diameter intake pipes and is less costly than pigging operations which are required at facilities with larger diameter intake pipes. The redundant pipe will either be an identical parallel intake pipe or a shorter flexible-type hose.

Mechanical pipe cleaning operations would involve discharging a jet of high-pressure water at an angle to the inner pipe surfaces. The pump providing high-pressure water would be located at the pumping station. Depending on environmental regulations, the water jet would either be directed towards the intake and the debris discharged back to the lake, or the water would be carried to the mouth of the intake via small diameter piping installed within the intake pipe and the water jet would be directed back towards the pump station. The latter would be employed if it is required that the loosened debris be collected and landfilled rather than directly discharged to the lake. It is recommended that the pipes be cleaned at least once in the fall of every year, although spring cleaning may also be needed. Since the frequency of required cleaning is dependent on zebra mussel loading and water characteristics, this recommendation was based only on an estimate derived from past experience at other facilities. The frequency of cleaning required will ultimately be determined by studying actual fouling patterns at these particular facilities.

Mechanical cleaning and inspection of the intake screens will be accomplished in the same manner as for the other facilities.

Group D Control Evaluation

Control recommendations were given for both the two existing treatment facilities and for the single facility that is planned to be operating by the year 1995. Control recommendations for the existing facilities are temporary solutions intended to allow plant operation if zebra mussel fouling occurs before the new facility is installed. Recommendations for the proposed facility are based on assumed new plant characteristics.

The recommendation for the existing facilities was to install redundant intake piping and mechanically clean the non-operational intake pipes and screens at least twice per year. It was also recommended that diver inspections of the intake be performed in the interim to estimate the need for further cleaning, since the very small diameter intake piping will be more susceptible to fouling and clogging problems. Mechanical cleaning of the intake screens will be accomplished in the same manner described for the other facilities. Mechanical pipe cleaning operations would involve discharging a jet of high-pressure water at an angle to the inner pipe surfaces and would employ the same methods as described for the Group C facilities.

The control strategies selected for the new water treatment facility are dependent on the controls implemented for the hatchery's intake and the extent of raw water piping installed from the hatchery intake sump to the new treatment facility. If the hatchery relies on cold water to discourage attachment of entrained veligers within their intake piping, veligers will still survive and remain in the water system until conditions are more favorable for attachment. In this case, any raw water piping installed from the sump to the water treatment process could be vulnerable to fouling if the water warms up to a temperature that would be conducive to settlement. If prechlorination is to be part of the treatment process, it was recommended that the injection point be placed at the pump drawing from the deep sump, provided that precautions are taken to avoid contamination of the fish hatchery's water with the chlorine. Chlorine injection at the pumps will protect all raw water piping downstream to the treatment processes. It was also recommended that if chlorine is injected for fouling control, the treatment plant be placed as close to the hatchery as possible to reduce chlorine retention time, required chlorine dosages, and subsequently to reduce THM concerns. Other alternatives to protect raw water piping would be installing pipe wyes and redundant piping for cleaning, redundant piping for hot water flushing operations, or fine pre-filtration with automatic backwash filters for removal of the settlement-stage veligers. Control strategies should be incorporated into the new facility design. Specific recommendations cannot be made until plans for the new facility and for zebra mussel control at the fish hatchery are decided upon. It was recommended that the group of facilities concentrate on designing the new plant to facilitate the non-chemical control strategies such as hot

water and mechanical cleaning, since the future use of chemical zebra mussel control strategies is uncertain.

CONCLUSIONS

The formation of the coalition of water suppliers allowed the 11 facilities to be evaluated for zebra mussel protection in groups. Some additional effort may have been required in grouping the facilities to sort out commonality between facilities and find zebra mussel control solutions compatible with multiple facilities. The groupings, however, will likely save on overall design costs by eliminating redundant efforts in costly associated tasks such as producing drawings, selecting equipment, writing specifications, and performing water quality pilot-scale tests. Facilities in close proximity, considering chemical control with the same source water quality could perform one pilot-scale study for the range of required chemical retention time and dosages. To save on design costs and pilot-scale studies, groups of similar type facilities on other waterbodies should consider consolidating their zebra mussel control efforts.

Finally, 10 of the 11 coalition members are proposing a group design of a dual sodium hypochlorite/potassium permanganate feed system, in some cases with provisions for mechanical cleaning of the intake pipes. The eleventh member is independently designing a chemical injection system.

REFERENCES

Carlton, J.T. 1993. Dispersal Mechanisms of Zebra Mussel Dreissena In: Nalepa, T.F. and D.W. Schloesser (eds.) Zebra Mussels: Biology, Impacts and Control, Lewis Publishers. Ann Arbor. 810 pp.

Kilgour, B.W. and G.L. Mackie. 1992. Effects of Salinity on Growth and Survival of Zebra Mussels (Dreissena Polymorpha). Presented at the Second International Zebra Mussel Conference. Toronto, Ontario. February 1992.

Lake Champlain Research Consortium. 1992. A Research and Monitoring Agenda for Lake Champlain. Proceedings of a Workshop. Burlington, Vermont. December 17-19, 1991.

Performance Evaluation of a Raw Water System Specifically Designed for Mussel Control at Monroe, Michigan

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Abstract

The new Wilfred L. LePage Plant of the Monroe Water Department was formally placed in service in the spring of 1993. The award-winning installation provides western Lake Erie water to the treatment plant of the City of Monroe and, by the end of 1994, will also serve the treatment plant of the Charter Township of Frenchtown. The new plant was specifically designed to preclude colonization by the zebra mussel, Dreissena polymorpha.

The new facilities include a raw water intake structure and pipeline featuring offshore chemical treatment, a new pumping plant, chemical storage, application and monitoring apparatus, and related control and telemetry equipment. Facilities for screening, backflushing, and mechanical cleaning were provided as well as provisions for alternate chemical treatments.

Constant, multiple-point applications of sodium hypochlorite maintain free chlorine residuals at 0.15 to 0.20 mg/L throughout ten miles of raw water systems. The protected raw water system will be more than twelve miles in length by the end of 1994. Underwater inspections have confirmed the total effectiveness of the treatment.

The mussel control facilities are described in detail and evaluated based on initial operations data. Operation and maintenance costs are projected and comparisons of treatment options are discussed.

History

Monroe Water Works obtains water from the shallow extremities of the western basin of Lake Erie. The seasonally warm, nutrient-rich waters provide an ideal habitat for the zebra mussel. The region became heavily populated by the animals soon after their discovery in lower Lake St. Clair, which is upstream from Lake Erie in the Great Lakes system.

The mussels are believed to have entered the raw water piping of the Monroe system in 1988 but, possibly, as early as 1987, judging from the size and number of animals found in the treatment works in January of 1989.[1]

The infestation, the first in the United States, created havoc throughout 1989 until brought under control in 1990. Flow through the intake was interrupted numerous times during 1989, once for a period of fifty-six hours. Herculean efforts were required to regain possession of the raw water piping system. Initially, the nine-mile long transmission main was treated with chlorine which successfully cleared it of mussels but created troublesome debris problems downstream. The sixty-one hundred foot long offshore intake pipeline was successfully cleaned mechanically, but not without additional debris problems. Finally, chlorination was initiated at the intake crib which created even more debris problems before final success was declared and operations returned to normal.

Facilities Planning

New raw water facilities were already in the planning stage when the mussel infestation was discovered. Design was suspended temporarily in order to assure that any new construction would include all possible defenses against future zebra mussel intrusions. This prompted reconsideration of intake structure design and a search for the most effective and economical mitigation strategy.

Extensive investigations were carried out in search of the most effective and economical treatment for permanent use.[2] Chlorination was a front-runner because of initial successes with it plus the fact that its use was fully understood by the plant staff, it was readily available, and the equipment was already on hand. On the negative side was the fact that it would be applied from a remote controlled, unmanned facility in close proximity to a residential area. Understandably, the residents were uncomfortable with tons of chlorine unattended in their midst.

Investigations into the use of alternate treatments implied that while successful results might be obtained, the cost/benefit ratios remained most favorable with chlorine. Thus, chlorination emerged as the treatment of choice.

New Raw Water Facilities

The new raw water facilities were designed specifically for zebra mussel control. Included are a new raw water intake and additional raw water pump station, both equipped to accommodate an elaborate chlorination system. All operations in the new and existing stations are closely monitored and remotely controlled from the water treatment plant ten miles distant.

Much of the new system has been on stream for more than a year while some segments have been in service only about nine months at this writing. Nonetheless, adequate data have been accumulated to support a sound evaluation and long term projections.

The Raw Water Intake

The new raw water intake pipeline meets all design objectives. It is constructed of 42-inch (106.6 cm) diameter prestressed concrete cylinder pipe (PCCP) and extends to an intake structure 1,555 feet (474 m) offshore in approximately 20 feet (6 m) of depth. The pipeline is buried beneath the rocky lake floor with minimum cover of 3 feet (0.9 m).[Figure 1]

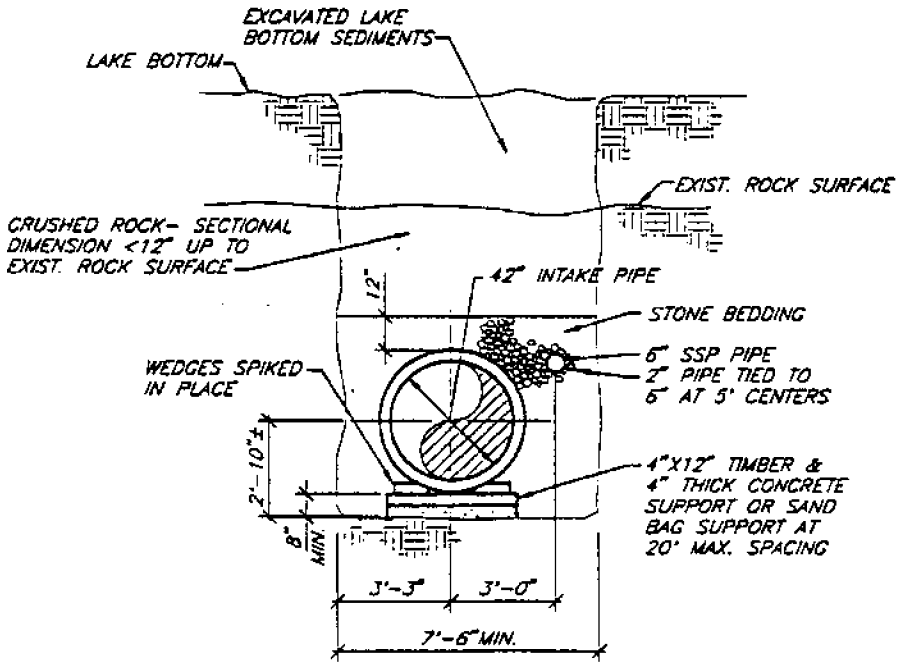

Figure 1. Detail of Marine Trench

Two access points have been provided on shore to ease insertion of scrubbing devices should the need arise. One is located near the pump station and the other is situated in line with the straight run of offshore pipe. The pipe trench is extended approximately 10 feet (3.05 m) beyond the blind flange sealing the offshore end of the intake pipe to permit easy retrieval of any such device.[Figure 2]

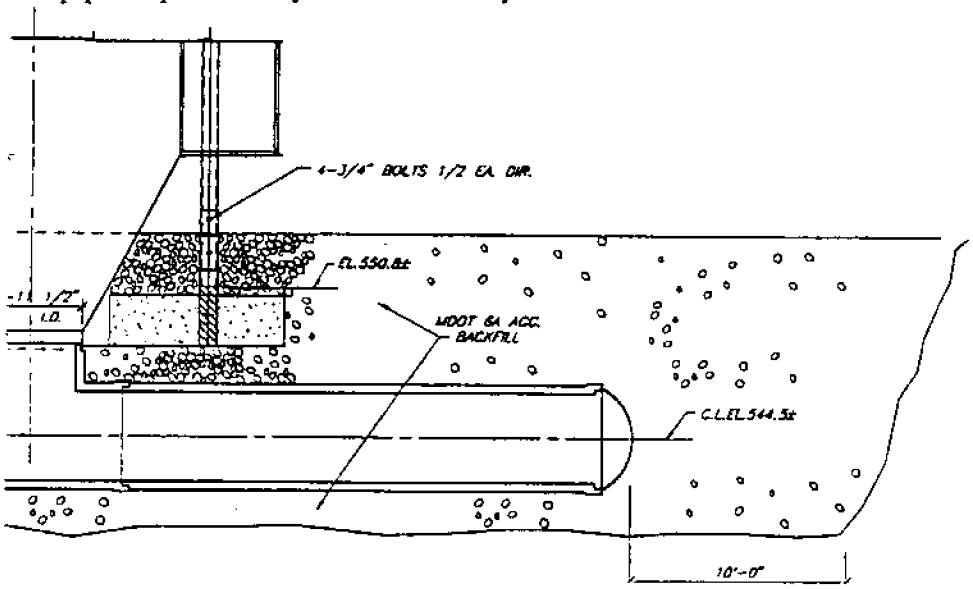

Figure 2. End Detail of Marine Trench

The entire intake system has been equipped with high capacity backflushing capability whereby one or more low service pumps may draw water through one intake and discharge it through the other. This feature may be applied to displace frazzle ice blockages, rid a pipeline of accumulated mussels or other debris, or to propel a scrubber through the intake system.

Two solution lines are buried adjacent to the large pipe in the same trench.[Figure 1] These include a 2-inch (5.08 cm) diameter high density polyethylene hose for the delivery of a chlorine solution, and a 6-inch (15.24 cm) diameter stainless steel pipe suitable for the delivery of a high volume sidestream, should alternate treatments become desirable. The 6-inch (15.24 cm) line is currently capped to prevent fouling.

Water enters the system through a unique, upflow inlet structure which is designed for the dual purposes of zebra mussel control and minimization of frazzle ice formation.[Figure 3] The structure, which is constructed of 0.5-inch (1.27 cm) thick steel, may be described as a hooded cone or funnel 11.5-feet (3.77 m) in diameter at the inlet tapering over a distance of 7-feet (2.13 m) to a throat diameter of 42-inches (106.6 cm) where it intersects the pipeline.

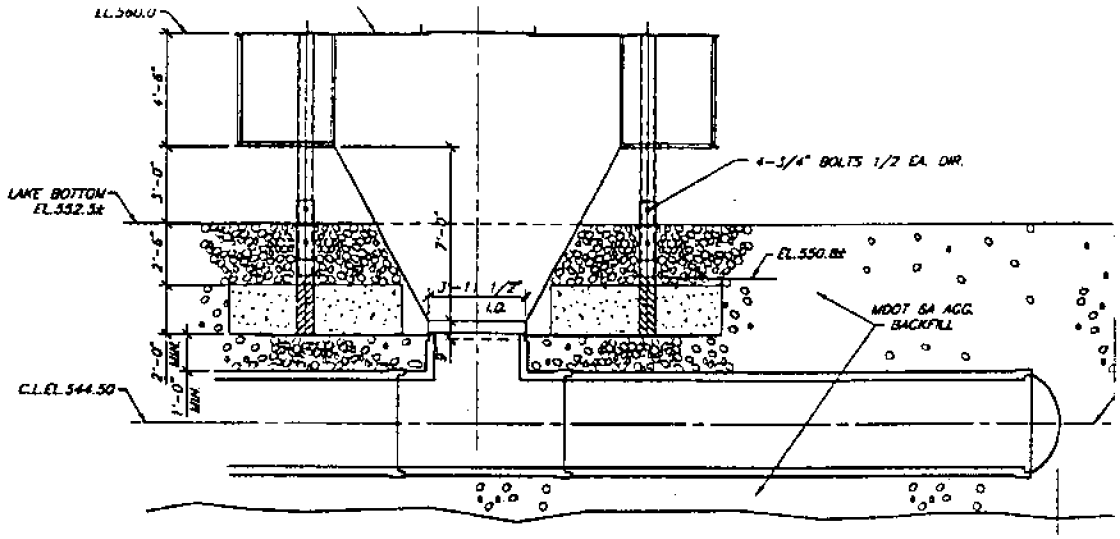

Figure 3. Detail of the Raw Water Inlet Structure

The funnel-like cone is capped by a flat-topped, steel umbrella with a diameter of 19-feet (5.79 m) with vertical sides extending downward a distance of 4.5-feet (1.37 m). Two-inch (5.08 cm) diameter circular chlorine diffusion rings are positioned inside the lip of the periphery of the hood, and around the periphery of the lip of the cone.[Figure 4)]

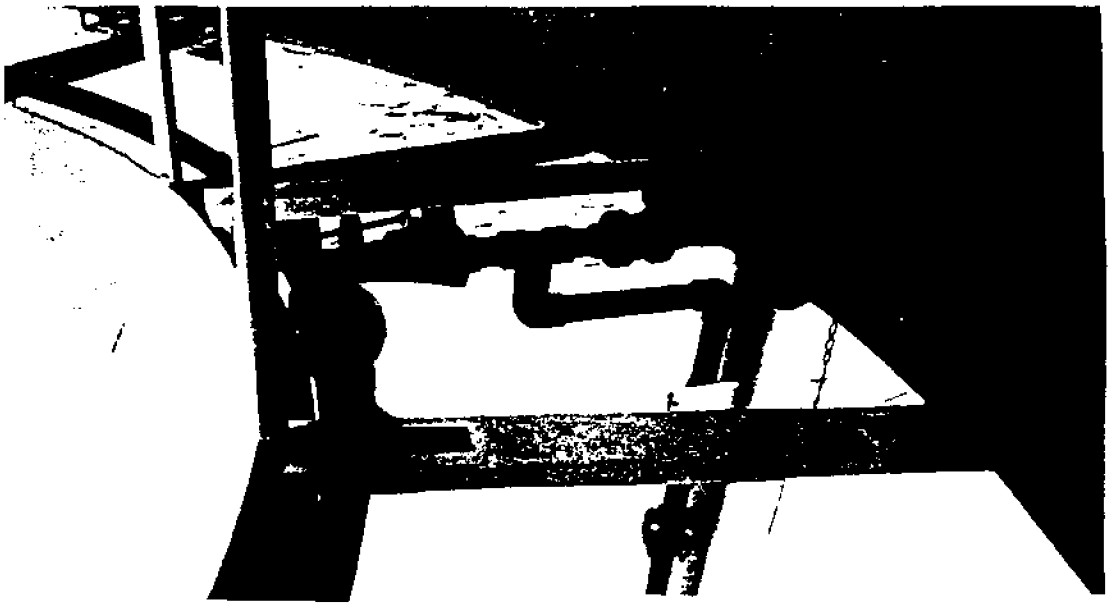

Figure 4. Inner and Outer Peripheral Diffusers

The circular area between the periphery of the funnel and the periphery of the vertical walls of the hood constitutes the inlet port. All water entering the inlet port must pass through the applied chemical (chlorine) plume.

The inlet ports are situated at a depth of approximately 16-feet (4.88 m) at low water which is about 4-feet (1.22 m) above the bottom. The top of the structure is approximately 12.5-feet (3.8 m) beneath the surface. The site is not frequented by vessels of sufficient draft to inflict damage.

Control valves to balance the flow through the diffusers were adjusted prior to immersion of the structure and checked again under operating conditions to assure uniform chemical dispersion. The escape of chlorinated water outside the structure is precluded by the umbrella during operation. If flow through the intake is interrupted for any reason, protective interlocks immediately shut down the chemical feed and delivery pumps.

Lake elevation, water elevations in the four screen chambers and pump suction wells are monitored continuously and displayed in the pump station and the treatment plant. Deviation alarms alert the operator if any critical parameter exceeds operating tolerance.

The intake pipeline and inlet structure have been in service since December, 1992. Under normal conditions, the new intake is used in concert with the older 30-inch (76 cm) pipeline to prevent colonization of what, otherwise, would be a dormant conductor. Currently, about 85% of the raw water arrives through the 42-inch (106.6 cm) pipe and about 15% of the total comes through the 30-inch (76 cm). That split, which is easily adjustable, or any desired split, remains constant over the total flow range.

Tests and operating experience indicate that all physical aspects of the intake structure and pipelines meet or exceed design expectations.

Frazzle Ice Mitigation

The low velocity of flow through the inlet ports and over clean surfaces in the new intake structure appears to preclude the formation of troublesome frazzle ice. At flows of 18 mgd (68 ML/d) velocity through the inlet ports is 0.15 ft/sec (0.045 m/sec). At the funnel entrance velocity increases to 0.27 ft/sec (0.08 m/sec) and to 2.89 ft/sec (0.88 m/sec) in the 42-inch (106.6 cm) diameter pipeline. Weather and sea conditions conducive to frazzle ice formation were recorded on several occasions during the winters of 1992-93 and 1993-94 without any evidence of the troublesome ice formations.

Mussel Control by Chlorination

Chlorine has been used for mussel control at Monroe since 1989. Its application has been refined over the years to achieve optimum protection and minimize impact on other treatment systems. As a result, chlorine doses have been carefully reduced to current levels while effectiveness continues to be measured by visual underwater inspections.

Chemical treatment was initiated at the new intake structure in January of 1993 using liquid chlorine. In July, 1993, the liquid chlorine system was placed in reserve and control was shifted to the hypochlorination system for both intakes. The shift was a precautionary measure to eliminate the risks associated with chlorine storage and use at a remote controlled, unmanned facility.

Description of the Hypochlorination System

The desired hypochlorite application rate is set on manual loading stations by the water treatment plant operator. That signal is transmitted by radio telemetry to the pump station where it is used to regulate the motor speed of the appropriate hypochlorite pump. Flow signals from the two discharge mains are utilized to derive flow pacing signals to regulate the stroke of the hypochlorite pumps. Sodium hypochlorite from the metering pumps is mixed with dilution/carrier water that delivers the solution to the diffusers at the inlet of each raw water intake. A sequestering agent[3] is added to the dilution/carrier water to preclude precipitate formation as a result of the elevated pH value following addition of the hypochlorite. The material is batch fed from drums by a package unit the output of which is paced to the metered dilution water flow. Electrical interlocks prevent operation of the system when raw water pumps are not in operation.

Each transmission main is equipped with a similar system, regulated by the respective destination plant operators.

All essential parameters such as chlorine residuals, pH values, head losses at key points, fluid elevations, and others, are transmitted to the respective treatment plant control rooms by radio telemetry signals with telephone line backup.

Enough hypochlorite is applied at each intake offshore to provide a free chlorine residuals of +/- 0.2 mg/L in water arriving at the pump station. The water leaving the pump station is chlorinated again to provide a maximum 0.2 residual on arrival at the Monroe treatment plant 9 miles (14.4 km) away.[Figure 5] The reason for the multi-point application is two-fold. It is intended to minimize excess chlorine in the screen chambers in order to suppress the liberation of noxious and corrosive fumes

in the station and, secondly, the new Frenchtown Water Treatment Plant will also receive raw water from this facility by the end of 1994. That plant is only 2.5 miles (4.0 km) from the pump station. The vast difference in distance and detention time dictates individually controlled applications in order to maintain desired terminal residuals. Underwater inspections of both intakes in November of 1993 verified the total effectiveness of the treatment.[Table 1]

Table 1.

Hypochlorite Applications for Mussel Control
July - December 1993

NaOCl Applied at Cribs and Trans. Main	Chlorine Residual 30" Intake	42" Intake	Chlorine Residual Arriving at WTP
2.10 mg/L	0.23 mg/L	0.20 mg/L	0.09 mg/L

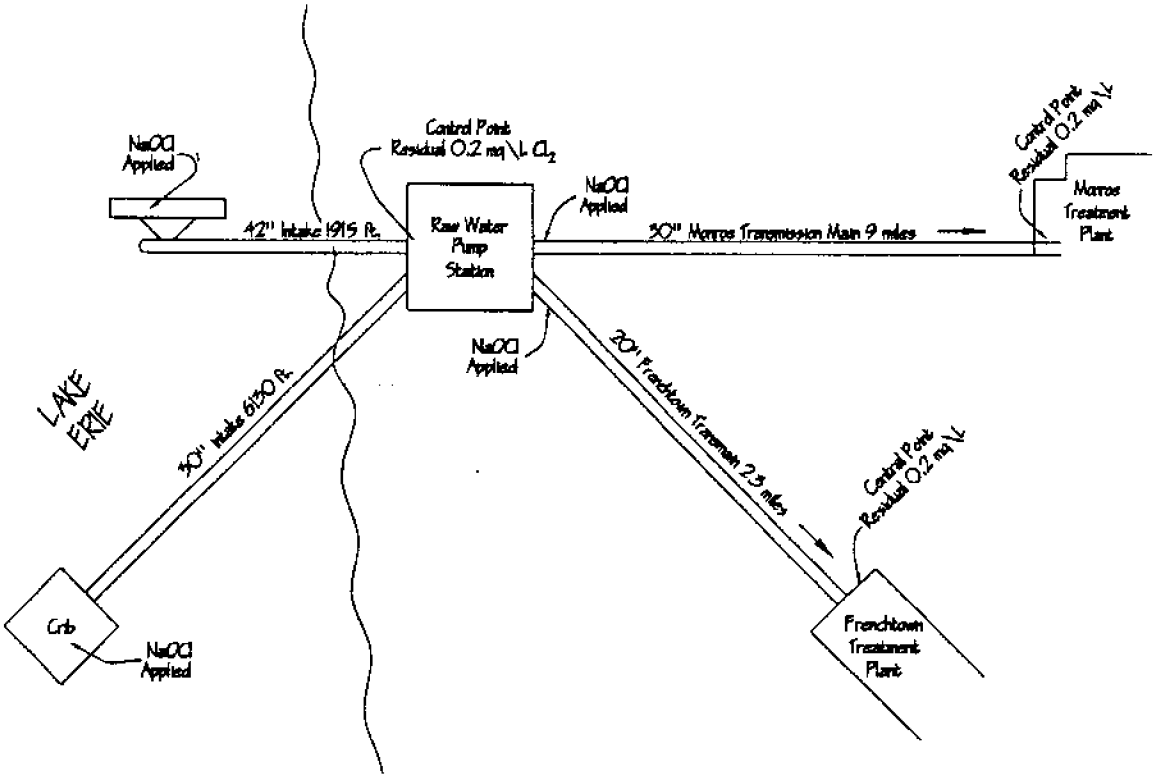

Figure 5. Chlorination Scheme for Zebra Mussel Control

Inspection of the 30-inch intake revealed substantial colonization of all surfaces adjacent to but not in the chemical discharge plume. The inlet structure on the 42-inch intake is exceptionally clean on the inside surfaces while the exterior already supports a substantial population of mussels.

Impacts on Water Quality

Prechlorination off shore has had no deleterious effect on the treatment process or finished water quality. In fact, a noteworthy improvement in the stability of chlorine residuals throughout the distribution system has been realized. The effectiveness of ozonation for taste and odor control has not been compromised; neither have trihalomethane levels increased appreciably.[Table 2] Compliance with the current Maximum Contaminant Level (MCL) of 80 ug/L for total trihalomethanes (TTHM) is easily achieved. Preliminary data implies that an even more stringent MCL for total trihalomethanes could be met. The shaded cells in Table 2 indicate periods during which water was being drawn from an emergency source in the River Raisin at the time samples were collected for quarterly THM analysis. Without the influence of river water, the annual averages might be expected to be below 40 ug/L TTHM.

Table 2

Total Trihalomethane Formation
With Treble Pre-Chlorination
1991 - 1993

Date	1991	1992	1993
March	29.3 ug/L	12.8 ug/L	28.5 ug/L
June	37.8 ug/L	33.0 ug/L	68.3 ug/L
September	51.0 ug/L	94.1 ug/L	52.2 ug/L
December	58.8 ug/L	26.9 ug/L	18.9 ug/L
Average	44.2 ug/L	41.7 ug/L	42.0 ug/L

Sodium Hypochlorite vs. Liquid Chlorine

The results obtained with hypochlorite are very satisfactory but there are several disadvantages connected with its use. The most notable, perhaps, is the additional cost compared to the cost of liquid chlorine.[Table 3] The higher cost for hypochlorite and the fact that hypochlorite ion is a weaker disinfectant than hypochlorous acid

has increased the chemical cost for mussel control about 54 percent. However, since the mussel control system is used year round, any chlorine demand thus satisfied reduces the amount of chlorination necessary at the treatment plant.

Table 3

Comparison of Chemical Costs for Mussel Control
Liquid Chlorine vs. Sodium Hypochlorite
July - December, 1993

Cost/MG: Chlorine	Cost/MG: Hypochlorite	Increase or Decrease
$3.87	$5.97	$2.10/MG = 54%

A sequestering agent, tetra pottasium pyrophosphate, must be added to the dilution water sidestream to prevent precipitation as a result of the elevated pH value following addition of the sodium hypochlorite.[Table 4] The cost of the sequestering agent equates to about $0.33/MG and increases the total chemical cost to $2.43/MG or 63% more than for doing the same job with liquid chlorine.

Table 4

Sequestering Agent Cost
Tetra Pottasium Pyrophosphate
(TPC 515H)
July - December, 1993

Cost/MG with Chlorine	Cost/MG with Hypochlorite	Cost/MG Increase or Decrease
NA	$0.33	+$0.33/MG = 100%

Another disadvantage of using hypochlorite is its rapid decomposition, especially during the hot weather months. Degradation of as much as one trade percent has been recorded by the time the material arrives at the plant site. That can represent a loss of as much as $350 before the material arrives. The decomposition curve is really quite steep initially and flattens out only after the material degrades or is diluted to about a ten percent solution. As an example, in the summer of 1993, a shipment that was 14.5 percent on arrival was down to 9.6 percent 35 days later. The storage system has since been modified to provide for dilution of the material to a more stable 10 percent solution upon arrival. As expected, this produces some fluffy precipitate which, if it proves troublesome, may be controlled by adding

another sequestering agent to the material in storage. Lacking a stable concentration, the material has to be assayed about every other day in order to accurately determine the amount of chlorine applied. Liquid chlorine was cheaper, more efficient, and far less work.

Economics

The raw water system improvements, as built, cost a total of $4.9 million. This does not include the cost of six large low service pumps, additional piping and valves and other ancillary equipment yet to be added. The pumps will be added in pairs at two year intervals.

More than $500,000 of the project cost was specifically related to zebra mussel control systems.(Table 5)

Previous expenditures for investigative and remedial actions, research, construction and operation of temporary mussel management facilities from 1989 to 1992 totaled approximately $321,795.(4) Added to the cost of the new mussel management facilities, the total expenditure for mussel control is approximately $801,960. The initial costs were absorbed solely by the water department of the City of Monroe without necessitating a water rate increase. The cost of construction and operation of the raw water system improvements will be shared by the City and the Charter Township of Frenchtown with the Township share being 4/15 of the total. The facility will be operated and maintained solely by the City.

Table 5

Estimated Costs
New Facilities for Mussel Management

Engineering design	$36,449
Chemical treatment building	30,000
Chemical storage tanks and accessories	16,000
Chemical feed systems, controls, instrumentation	295,316
Offshore chemical solution lines	77,500
Chemical diffusion systems at intake structure	10,000
Miscellaneous changes and additions	14,900
TOTAL Estimated Costs for New Facilities	$480,165

Preliminary data imply that operating costs for the new mussel control systems, excluding labor and unanticipated expenditures, may be expected to average approximately $10.00/MG treated.[Table 6]

Table 6

Projected Operating Costs
Mussel Control System
(Excluding Labor)

Parameter	Annual Cost
Sodium Hypochlorite	$21,200
Tetra Pottasium Pyrophosphate	920
Instrument Reagents	720
Electric Energy	9,000
Heating Fuel	700
TOTAL Operating Cost	$32,540 (10.01/mg)*

* Based on production of 3,250 MG

Start up and operation of the Frenchtown treatment facility is not expected to appreciably increase the cost of operation. Any chemical applied to the Frenchtown transmission main will effect a similar reduction in application to the Monroe main. Thus, additional costs will be limited to an almost negligible increase in electric energy consumption for the operation of one additional chemical feed pump and attendant instrumentation.

Summary

The new raw water facilities and chemical treatment system meet all design objectives for zebra mussel control. All mussel management features on the intakes, pump stations, transmission mains and their controls have been tested, accepted, and placed in operational status. Operation and maintenance costs for all mussel related systems equate to approximately $10/MG treated, which is considered reasonable.

Treatment regimes have been optimized for mussel control with sodium hypochlorite and with liquid chlorine. Sodium hypochlorite is the chemical of choice owing to safety concerns at the unmanned facility. Comparative studies, however, have established that chlorination with liquid chlorine is substantially more cost effective than with sodium hypochlorite.

No insurmountable difficulties have been encountered with the system as built, and no deleterious impacts on finished water quality have been recorded as a result of operations.

Underwater inspections have verified the total effectiveness of treatment with liquid chlorine and with sodium hypochlorite. Both intake pipelines and their inlet structures are totally free of zebra mussels. In addition to mussel control, the offshore treatment has reduced the biofilm in the old intake and effectively kept fish and other animals from entering the system.

The new facilities provide a blend of ample water supply with operating convenience and a formidable defense against mussel intrusion. The system is considered a success in all respects.

REFERENCES

1. LePage, W.L. The Impact of Dreissena polymorpha on Water Works Operations at Monroe, Michigan: A Case History. Zebra Mussels: Biology, Impacts and Control. Nalepa, T.F. and D.W. Schloesser, Eds. Lewis Publishers. (1993)

2. LePage, W.L. and L.J. Bollyky. A Proposed Treatment for Dreissena polymorpha. Proc. Symposium entitled New Developments: Ozone in Water and Wastewater Treatment. The International Ozone Association - Pan American Group, Stamford, CT. (1990)

3. TPC 515H (Tetra-Potassium Pyrophosphate). Technical Products Corporation, Portsmouth, Virginia.

4. LePage, W.L. Mussel Management at Monroe: Past, Present and Future. Proc. Third International Zebra Mussel Research Conference. The Electric Power Research Institute, Palo Alto, CA. (1993)

TD 2335: Laboratory and Field Efficacy Studies for Control of Zebra Mussels in Electric Power Plants

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Abstract

A series of laboratory efficacy trials against zebra mussels were conducted under static and static renewal conditions with TD 2335 and TD 2330. Results of these studies showed 48 hour LC50 concentration of 0.5 to 2.0 ppm for small mussels (0.5 to 0.8 cm) and 1.35 ppm for large mussels (2.0 to 2.5 cm). The 24 hour LC50 for veligers ranged from 0.19 to 0.71 ppm. Based on the results from these studies, a field efficacy study was conducted at the Toledo Edison Acme Station, Toledo, Ohio under the approval of the Ohio Environmental Protection Agency.

Clumps of viable zebra mussels were added to each of three bioboxes that were connected to the water flow pipes at separate sites within the power plant. Chemical injection points were located on the suction side of the raw water pumps and on service water header. Injection pumps were calibrated to deliver a constant TD 2335 concentration of 3 ppm. The study was planned for a 48 hour exposure with regular monitoring of zebra mussels. Water samples were taken from the bioboxes, the cooling water tank and two Maumee River effluent sites and concentrations of TD 2335 were analytically measured for each of these sites at various intervals. Bioassays in Ceriodaphnia dubia and fathead minnows were also conducted with effluent samples.

The study was terminated after 8 hours of exposure since all mussels within the bioboxes were gapped open and nonresponsive to external stimuli. Analytical results showed an average flow water concentration of 2.39 ppm with a range of 1.27 to 3.52 ppm (between 2 and 8 hours). Effluent samples from the discharge channel ranged from <0.01 to 0.12 ppm over a 36 hour period. The average concentration of TD 2335 in ash pond effluent samples was 0.173 ppm over the 84 hour collection period. None of the bioassay samples from the Maumee River effluent sites was acutely toxic to Ceriodaphnid or fathead minnows.

The Zebra mussel (Dresseina polymorpha) has created serious problems for electric power plants throughout the Great Lakes region of the United States and Canada. Chemical treatments to date are limited and it is apparent that new control products are required. TD 2335 is a new formulation of an EPA registered aquatic pesticide that has shown efficacy against the zebra mussel in static bioassays. These studies indicated that TD 2335 was toxic to mollusks at low concentrations. Although this product was also toxic to other aquatic species, environmental fate data indicate rapid degradation of TD-2335. Under electric power plant control procedures, rapid microbial degradation coupled with the high filtration and retention rates of the test chemical of the zebra mussel was expected to reduce potential discharge concentrations to relatively nontoxic levels. The purpose of this study was to explore the efficacy of TD 2335 under potential use conditions in an electric power plant.

METHODS

Testing Facility

This study was conducted on July 26-30, 1993 at the Toledo Edison Acme Station which is located approximately five miles east of downtown Toledo, Ohio, at the mouth of the Maumee River which empties into the western end of Lake Erie. A flow diagram of this facility is found in Figure I.

Test Chemical

Approximately 400 gallons of TD 2335 were shipped to Toledo Edison from the Elf Atochem facility in Bryan, TX. The sample was certified in regards to the concentration of the active ingredient and shipping papers. The active ingredient content was 53%.

Testing Procedures

Clumps of viable zebra mussels were added to each of three bioboxes that were connected to the water flow pipes at separate sites within the power plant. The bioboxes were equilibrated for several days prior to study initiation. The locations of the bioboxes (Boxes #1, #2, #3) are indicated on Figure I.

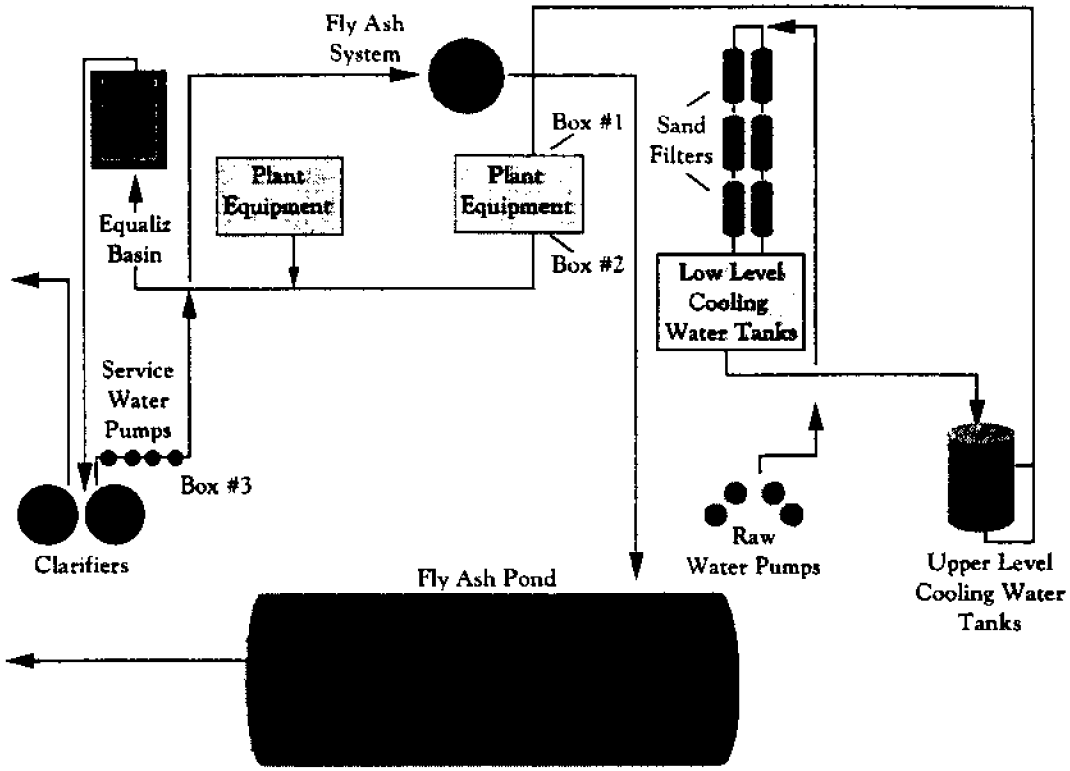
Chemical injection points were located on the suction side of the raw water pumps and on service water header. Injection pumps were calibrated to deliver a constant TD 2335 concentration of 3 ppm. The study was planned for a 48 hour exposure.

Zebra mussels were monitored for effects on survival, attachment, and behavior at initiation of the treatment and at 0, 1, 2, 4 and 8 hours after initiation of treatment.

Analytical Monitoring

Water samples were taken from the bioboxes, the cooling water tank and two Maumee River effluent sites and concentrations of TD 2335 were analytically measured for each of these sites at

Figure 1. Flow Diagram - Toledo Edison Acme Station



various intervals. The sites for analytical monitoring are indicated on Figure I and were as follows:

Site B Low Level Cooling Water Tanks
Site C Biobox #1
Site D Biobox #2
Site E Biobox #3
Site F Discharge channel into Maumee River
Site G Fly Ash Pond Effluent into Maumee River.

Samples were collected on the following schedule:

Site B 0, 1, 2, 4 and 8 hours
Site C 0, 1, 2, 4 and 8 hours
Site D 0, 1, 2, 4 and 8 hours
Site E 0, 1, 2, 4 and 8 hours
Site F 0, 4, 8, 12, 16, 24, 28, 32 and 36 hours
Site G 0, 4, 8, 12, 16, 24, 28, 32, 36, 44, 52, 60, 68, 76 and 84 hours

Samples were collected into appropriately sized bottles and handled according to procedures established by Elf Atochem for aquatic test samples. Sample handling procedures were completed by Elf Atochem personnel. After sampling, the water samples were frozen. Upon completion of the test, the samples were shipped on dry ice to McKenzie Laboratories for analysis. McKenzie Laboratories was selected on the basis that this laboratory had procedures in place for analyzing water samples for TD 2335.

Bioassay of Effluent Samples

Composite samples of effluent were collected at Sites E and F by staff from EnviroScience, Stow, Ohio, and transported to their facility for testing. Both sites were at study initiation and after 24, 48 and 72 hours post initiation of exposure. An intake water sample was taken at study initiation to serve as a control for the bioassays.

Static bioassays were conducted on these samples in Daphnia magna and fathead minnow (Pimephales promelas). These tests were standard 48 hour Daphnia magna and 96 hour fathead minnow studies as specified by the Ohio EPA Quality Assurance Manual and/or standard operating procedures promulgated by the U.S. EPA. The tests were conducted with undiluted effluent samples.

RESULTS

Static and semistatic bioassays

Static and semistatic bioassays were performed by Dr. Susan Fisher, Ohio State University. Two experimental formulations were used in these tests and were designated as TD 2330 and TD 2335. The results from these studies are summarized below. Small mussels were approximately 0.5 to 0.8 cm. while large mussels were approximately 2.0 to 2.5 cm. The veligers were 3 to 4 days old. The LC50 value is the test concentration that produces lethality in 50% of the test species. The LT50 is the time required to produce 50% lethality in a test specie at a given concentration.

TABLE 1

Laboratory Efficacy Trials: Summary of Results

Size of Mussel	TD 2330	TD 2335
Small Mussels -		
24 hour LC50	1.44 ppm	5.7 ppm
48 hour LC50	0.51 ppm	2.0 ppm
LT50 @ 1 ppm	72.0 hours	118.5 hours
LT50 @ 3 ppm	15.5 hours	38.2 hours
Large mussels -		
24 hour LC50	2.00 ppm	-
48 hour LC50	1.35 ppm	-
LT50 @ 0.5 ppm	-	66.0 hours
LT50 @ 1.0 ppm	33.0 hours	34.6 hours
Veligers		
24 hour LC50	0.19 ppm	0.71 ppm

Based on the results from these studies, it was determined that a 48 hour exposure at a concentration of 3 ppm was selected for the field trial.

Zebra mussel survival and observations

At 1, 2 and 4 hours after the initiation of treatment, the Zebra mussels appeared viable. At the 8 hour interval, all mussels within Bioboxes 1, 2 and 3 were gapped open and nonresponsive to external stimuli. The Bioboxes were opened and the mussels removed for further evaluation. All mussels from bioboxes 1 and 3 and most of the mussels from biobox 2 were considered nonviable while other mussels showed slow response and were not expected to survive. The study was considered completed at this time and TD 2335 treatment was stopped.

Analytical monitoring

As noted above the exposure level for TD 2335 was nominally selected to be 3 ppm. Results from the in-plant analyses are summarized in Table 2. In general, TD 2335 concentrations were generally within this expected range around the nominal concentration. The exception is the 4 hour sample from Biobox #1. The higher concentrations seen at Biobox #3 are considered to be combination of in-plant concentrations from the raw water addition and the "recharging" of TD 2335 by pumping at the service water header.

TABLE 2

Results from In-Plant Analysis for TD 2335

Sample site	Time Interval	Concentration
Cooling water tank (B)	1 hour	1.27 ppm
	2 hours	2.33 ppm
	4 hours	2.10 ppm
	8 hours	1.92 ppm
Biobox #1 (C)	1 hour	2.05 ppm
	2 hours	3.10 ppm
	4 hours	0.86 ppm
	8 hours	2.04 ppm
Biobox #2 (D)	1 hour	0.05 ppm
	2 hours	1.27 ppm
	4 hours	1.70 ppm
	8 hours	2.97 ppm
Biobox #3 (E)	1 hour	2.13 ppm
	2 hours	2.79 ppm
	4 hours	3.52 ppm
	8 hours	3.32 ppm

Concentrations of TD 2335 in effluent samples from the discharge channel and the fly ash pond are summarized in Table 3.

Transit time from the raw water pumps through to the discharge channel was expected to be 16 to 24 hours. However, the highest residue concentration (0.115 ppm) was seen at the 8 hour interval which suggests that the transit time may be shorter. By 16 hours, insignificant levels of TD 2335 were discharged in the channel.

TABLE 3

Effluent Concentrations of TD 2335

Sample site	Time Interval	Concentration
Discharge channel (F)	8 hours	0.12 ppm
	16 hours	<0.01 ppm
	24 hours	0.01 ppm
	36 hours	<0.01 ppm

TABLE 3 continued

Sample site	Time Interval	Concentration
Fly Ash Pond (G)	4 hours	<0.01 ppm
	8 hours	0.133 ppm
	16 hours	0.045 ppm
	24 hours	0.066 ppm
	36 hours	0.264 ppm
	52 hours	0.272 ppm
	68 hours	0.273 ppm
	76 hours	0.188 ppm
	84 hours	0.118 ppm

Discharge samples from the fly ash pond effluent ranged from 0.045 (16 hour sample) to 0.273 ppm (68 hour sample). The transit time from the service water header through to the fly ash pond discharge into the Maumee River was expected to be 24 to 48 hours. Thus, the 0.133 ppm values at the 8 hour interval is unusual. The average concentration of TD 2335 in ash pond effluent samples was 0.147 ppm over the 84 hour collection period.

Bioassay samples

None of the bioassay samples from the Maumee River effluent sites was acutely toxic to Ceriodaphnid or fathead minnows.

DISCUSSION AND CONCLUSIONS

Upon completion of the laboratory efficacy trials, it was decided that further laboratory trials would be conducted under flow through conditions. Flow through bioassays would better simulate the potential field use of TD 2335. The major purpose of the flow through studies would be elucidation of specific treatment concentrations and durations for field trials.

The opportunity to conduct the field efficacy trial at the Toledo Edison Acme Plant occurred shortly after the decision to conduct the flow-through studies was made. Since these data were not available, the determination of the appropriate treatment concentration and duration for the field trial had to be based on the limited static bioassay results. On the basis of the static tests, a treatment concentration of 1 to 1.5 ppm with a 48 hour treatment period was considered adequate. Upon evaluation of the Acme plant, however, it was decided that a higher concentration should be used. Initially, TD 2335 was intended to be pumped at suction side of the raw water pump only. Based on the design of the Acme Station, it was expected that at least some, if not a significant amount of TD 2335 would be taken up in the sand filters as well as by the large biomass of zebra mussels. Thus, declining concentrations of TD 2335 would occur as the water traversed through the power plant. The 3 ppm concentration was selected on the basis that this would permit a 50% loss of TD 2335 without a loss of efficacy.

In addition, it was determined that a "recharging" of the TD 2335 may be necessary. It was decided that a second pump would be placed in the service water header and with a 3 ppm recharge at that point.

Based on the in-plant analytical results, it appears that very little loss of TD 2335 occurred throughout the trial. The cooling water tank was located just after the sand filters. The concentrations in the cooling water tanks reached plateau concentration approximately 2 hours after pumping was initiated. Figure I shows Bioboxes #1 and # 2 in close proximity to each other. From an in-plant standpoint, these bioboxes were close, however, from a water flow prospective, they were separated by a substantial distance. The analytical results suggest that 3 to 4 hours were required for concentrations in biobox #2 to be comparable to those of biobox #1.

Biobox #3 was spatially located next to biobox #2. The water flow for biobox #3 was distinctly different from Biobox #2. The water source for biobox #3 was from the service water header which received the 3 ppm recharge. The TD 2335 concentration at this point consist of any residual TD 2335 from the raw water injection as well as the "recharge". The analytical results at 4 and 8 hours suggest that a smaller "recharge" could have been used.

From a flow standpoint, TD 2335 concentrations seen in the discharge channel are a direct reflection of addition of TD 2335 at the raw water header. Fly ash pond effluent concentrations reflect residual TD 2335 from application at the raw water header as well as the "recharge" at the service water header. Reduction in "recharge" concentrations would be expected to reduce effluent concentrations in other power stations of similar design.

In conclusion, an 8 hour application of TD 2335 at a nominal concentration of 3 ppm resulted in lethality to Zebra mussels at the Toledo Edison Acme Station. Effluent concentrations of TD 2335 from the power station into the Maumee river were low and did not produce mortality in either fathead minnow or Ceriodaphnia bioassays. This field efficacy trial provides substantial guidance for planned field efficacy trials to be conducted in 1994.

Enhanced Toxicity of Zebra Mussel Control Chemicals in Pure Water

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Abstract--Previous studies have shown that deionized water enhances the toxicity of potassium chloride (KCl) to zebra mussels approximately 10-fold, compared to its toxicity in normal aquarium water. In order to determine whether the enhanced toxicity caused by deionized water is a general effect or is specific to KCl, the effects of deionized water on the toxicity of other toxic chemicals was investigated. In addition, we tested whether purified water produced for use in a high pressure turbine drive system (high purity condensate (HPC) water) was as effective as laboratory deionized water in enhancing the effect of a toxic chemical.

Toxicity of sodium hypochlorite was enhanced approximately two orders of magnitude in deionized water: in aquarium water, 10 mg/L < LC50 < 100 mg/L; in deionized water, 0.1 mg/L < LC50 < 1 mg/L. A shift of two orders of magnitude in toxicity was also observed for ouabain: in aquarium water, 10^{-4} M < LC50 < 10^{-3} M; in deionized water, 10^{-6} M < LC50 < 10^{-5} M. Virtually no shift in toxicity due to deionized water was observed for niclosamide and for Endod. For niclosamide, LC50 in deionized water was slightly less than 10^{-5} mg/L and in aquarium water slightly above 10^{-5} mg/L. For Endod LC50 was between 5 and 10 mg/L in both media.

The largest shift in toxicity in deionized water occurred for Noxfish (rotenone), for which LC50 shifted from approximately 1 mg/L in aquarium water to less than 10^{-4} mg/L in deionized water. Thus, the enhancement of toxicity due to deionized water is not an additive effect, but varies with the toxic substance, probably according to the mechanism of toxicity.

HPC enhanced toxicity of KCl as effectively as did laboratory deionized water. KCl was approximately 10 times more toxic to zebra mussels in both HPC and laboratory deionized water than it was in aquarium water. Death occurred within 24 hours, and during an additional 24 hours after return of the mussels to aquarium water, mussels in sensitive groups continued to die. Thus, HPC may prove to be convenient for use in power plants' zebra mussel control applications in service water systems, since it is a by-product of steam-generated, supercritical power plants and is effective at enhancing the toxicity of chemicals that may be useful for controlling zebra mussel infestations.

INTRODUCTION

The viability of zebra mussels is greatly affected by the presence of purified water. This was first discovered serendipitously by Jim Selegean while studying the possible use of zebra mussels as a biofilter for waste water treatment (Selegean, 1993). In his initial experiment, investigating how well zebra mussels could survive in Detroit sewage, Selegean (1993) tested groups of mussels in various dilutions of sewage, diluting the sewage with deionized water. The mussels survived well in Detroit sewage, surviving quite well except in the highest concentration; however, the group that died most quickly was the control group in deionized water with no sewage. This result suggested that deionized water might be a non-polluting medium with which to kill zebra mussels, and subsequent experiments confirmed this (Ram and Walker, 1993a,b).

As shown by Ram and Walker (1993a,b), mussels placed in deionized water begin to die within a few days. In contrast, animals in normal aquarium water survived in most cases for 7 days or more in deionized water. Ram and Walker (1993a,b) also showed that animals are more sensitive to toxic chemicals when exposed to them in deionized water than in aquarium water. This was demonstrated to be the case using potassium, previously reported by Fisher et al. (1991) to be toxic to zebra mussels. In experiments by Ram and Walker (1993a,b), measuring the survival of mussels after 24 hours in various concentrations of KCl in deionized water and aquarium water, zebra mussels in aquarium water did not die until the concentration of KCl exceeded 100 mg/L; whereas, in deionized water, 30 mg/L was lethal within 24 hours. The shift in sensitivity to potassium was thus increased about 10-fold in purified water.

In the present study, the effect of purified water on the toxicity of other chemicals toxic to zebra mussels was investigated. Are the effects of all toxic chemicals similarly affected by purified water or is the increased sensitivity to potassium specific to potassium? If death were due just to a generalized stress or osmotic response of the animal, then we might expect the toxicity of all chemicals to be enhanced similarly, about 10-fold. This was investigated by examining the effects of purified water on the toxicity of several toxic chemicals, including sodium hypochlorite, one of the most frequently used control chemicals. This paper reviews data on selected data previously reported by Walker and Ram (1994). In addition, experiments were initiated to determine whether purified water produced by thermal power plants was pure enough to mimic the toxicity enhancing effects of laboratory deionized water. Purified water can be difficult and expensive to make; however, thermal power plants make large quantities (tens to hundreds of thousands of gallons per day) of it for use in their high pressure turbine drive systems. These experiments were initiated using potassium as the toxic test chemical.

MATERIALS AND METHODS

Mussels were placed individually in capped glass vials, filled with 10 ml of

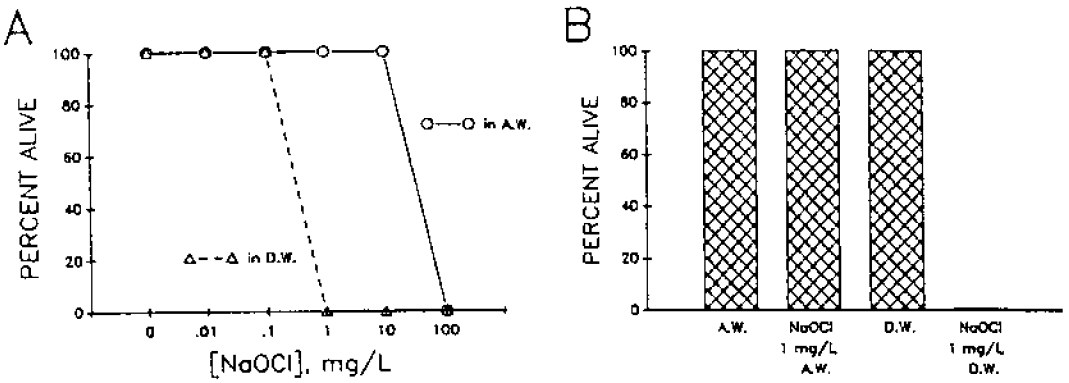

Fig. 1. Toxicity of NaOCl in aquarium water (A.W.) and deionized water (D.W.). (A) Dose-response curves for NaOCl in A.W. and D.W. Dose-response curves were determined in separate experiments for the two vehicles. Ten animals were tested at each concentration in each vehicle, and scored for viability at 24 hrs. (B) Viability after 24 hrs in A.W. and D.W. alone and in 1 mg NaOCl/L A.W. and D.W. 1 mg NaOCl/L was a concentration at which the dose-response curves in (A) diverged between A.W. and D.W. n=10 animals in each group. Reprinted with permission from Walker and Ram, 1994.

purified water or other solutions. In experiments that lasted more than one day the water in each vial is changed daily. In all experiments, groups of animals were also placed in normal aquarium water as a control for normal viability. For the experiments using laboratory deionized water, the water was MILLI-Q water with a specific resistivity of >14 Mohm, and all experiments were done at 18 ^{o}C by immersion of the vials in a thermostatically regulated water bath. Except where otherwise indicated, the animals being tested were young animals, about 1- 3 mm in length.

The effect of purified water on the toxicity of 5 chemicals was investigated: sodium hypochlorite (NaOCl), ouabain (which is an ion pump poison, Sigma), niclosamide (a toxic chemical also studied by Waller et al., 1993) and known also as Bayer 73 or Bayluscide; obtained from Sigma), Endod (a botanical product investigated previously by Lemma et al., 1991 and Lee et al., 1993; obtained from Lee), and rotenone, in its commercial formulation as Noxfish (Roussel Bio Corp., Englewood Cliffs, NJ).

Experiments with each toxic agent were conducted in three stages. First a concentration-response curve was generated with the toxic substance dissolved in aquarium water, measuring numbers of animals surviving after 24 hrs exposure. Second, a concentration-response curve in deionized water was determined. Finally, with the two concentration-response curves in hand, a single concentration was chosen at which a difference in sensitivity, if present, would be expected, and animals in both

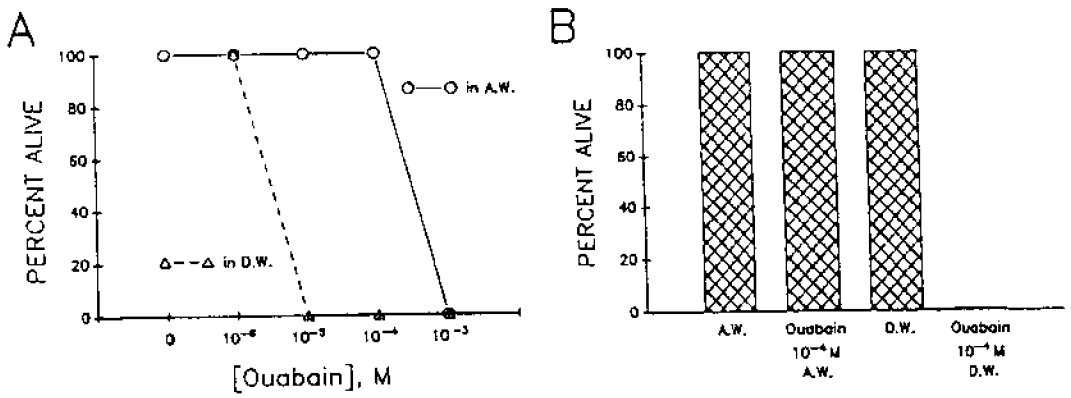

Fig. 2. Toxicity of ouabain in aquarium water (A.W.) and deionized water (D.W.). Design of experiment was the same as in Fig. 1. (A) Dose-response curves. (B) Direct comparison of effects of 10^{-4} M ouabain in A.W. and D.W. Reprinted with permission from Walker and Ram, 1994.

aquarium water and deionized water were tested at the same time.

To investigate the efficacy of purified water from a thermal power plant, purified water was obtained from Detroit Edison from their Monroe coal-fired power plant. This water is called "High purity condensate" (HPC). Mussels were immersed in either aquarium water, laboratory deionized water, or high purity condensate, with and without various concentrations of potassium. After 24 hours, the medium for all animals was replaced with aquarium water, to see if animals would recover from the observed toxic effects. The number of survivors was counted daily.

RESULTS

A typical result for sodium hypochlorite is shown in Fig. 1. The two concentration-response curves are shown in Fig. 1A. In aquarium water, it took 100 mg/L sodium hypochlorite to kill all mussels; whereas, in deionized water, only 1 mg/L was needed, a shift in sensitivity of 100-fold. With hypochlorite the shift in sensitivity is, thus, about 10-fold greater than with potassium. Similarly (Fig. 2), there was a 100-fold increase in sensitivity for ouabain. All mussels were dead in aquarium water at 10^{-3} M ouabain; whereas, 10^{-5} M ouabain was sufficient to kill all mussels in deionized water. A direct comparison at 10^{-4} M ouabain confirmed this difference.

However, deionized water did not increase the toxicity of all toxic chemicals. As described elsewhere (Walker and Ram, 1994), niclosamide showed practically no difference in sensitivity, with all animals dying in 10^{-6} M niclosamide in both deionized water and aquarium water. The only reliable difference in niclosamide toxicity was at 10^{-5} M niclosamide, at which all animals survived in aquarium water, but 60 - 70% died in aquarium water. Similarly, there was little, if any, enhancement

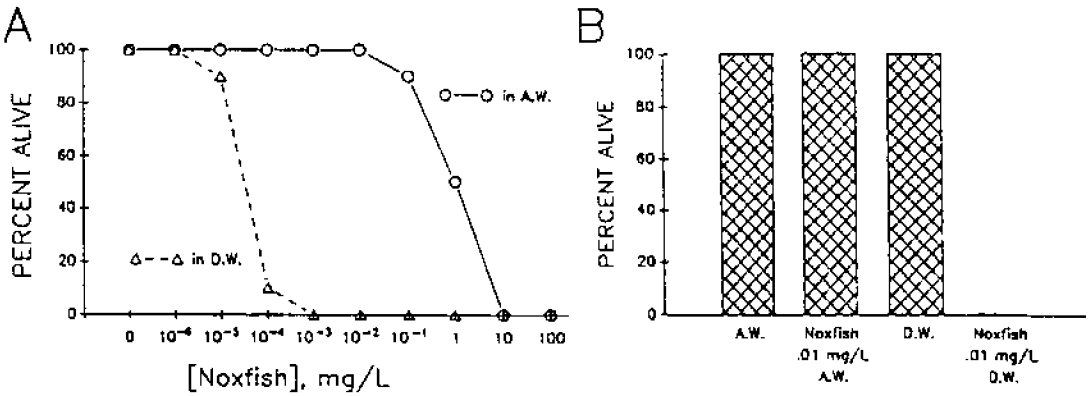

Fig. 3. Toxicity of Noxfish in aquarium water (A.W.) and deionized water (D.W.). Design of experiment was the same as in Fig. 1. Concentrations of Noxfish are calculated with respect to the amount of active ingredient, rotenone, in the solution being tested. (A) Dose-response curves. (B) Direct comparison of effects of 0.01 g/L Noxfish in A.W. and D.W. Reprinted with permission from Walker and Ram, 1994.

of toxicity of Endod. The concentration-response curves in deionized water and aquarium water were practically identical (LC50 of approximately 1 to 10 mg/L), and no significant differences between the two treatments could be detected at any concentration.

In contrast to these results, deionized water caused a greater enhancement in sensitivity to Noxfish (rotenone) than to any other toxic chemical previously studied. Data in Fig. 3 show that the sensitivity to rotenone was increased an extraordinary four to five orders of magnitude. Whereas, 10 mg of rotenone/L was necessary to kill mussels in ordinary aquarium water, in deionized water, all mussels died at 10^{-3} mg/L. A direct comparison at 10^{-2} mg/L confirmed this difference in sensitivity.

HPC does seem to be just as effective as laboratory deionized water at enhancing the toxicity of potassium. In the experiments in Figure 4, repeated three times with different groups of animals, concentrations of potassium greater than 100 mg/L were necessary to kill all animals in aquarium water. The sensitivity to potassium was enhanced about 10-fold in both laboratory water and HPC, so that only 10-30 mg/L was necessary to kill all mussels. Furthermore, to assure that animals classified as dead after 24 hours in these solutions were in fact dead and not merely paralyzed, all animals were put back into aquarium water for an additional 24 hours. As illustrated in Figure 4, the result was not only do they not recover in aquarium water, but mussels previously exposed to potassium continued to die even in this supposedly healthy environment. In fact, in all of our experiments in which literally hundreds of dead mussels have been observed, there has been only one case in which an apparently dead mussel did in fact recover in aquarium water. Additional

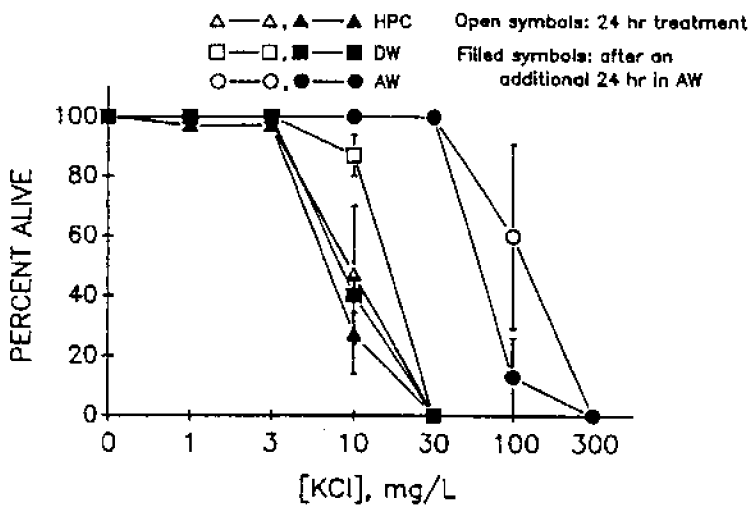

Fig. 4. Toxicity of KCl in high purity condensate (HPC) water, compared to its toxicity in deionized water (DW) and aquarium water (AW). Survival was assessed in various concentrations of KCl for 1 - 3 mm zebra mussels after 24 hr treatment (open symbols) and after an additional 24 hr (filled symbols) during which the medium for all mussels was changed to AW, with no added KCl. Points represent the means ± S.E. of percent of animals surviving in three replicate experiments.

experiments on effects of HPC alone or in combination with potassium on different sized animals are described in a recently submitted manuscript by Fong et al. (1994).

DISCUSSION

The initial series of experiments in this paper demonstrated that the enhancement of toxicity by deionized water is not specific to potassium. However, it also does not seem to be due to a generalized osmotic stress effect, or we would have seen the sensitivity to all toxic agents increased about the same amount. Instead, the enhancement ranged from no effect to 10-100,000-fold. The effect may in fact be dependent on specific mechanisms of toxicity in the animal and particularly the location at which the active agent works. Rotenone, the active agent in Noxfish, whose toxicity is enhanced the greatest, is a mitochondrial poison (Anderson et al., 1993; Chretien et al., 1990). We hypothesize that any mechanism, such as osmotic stress, that increases the uptake of chemicals into cells, may be expected to enhance its effect of any agent whose primary site of action is intracellular. Increased uptake, together with the increased energy demands on mitochondria caused by osmotic stress, may act synergistically to produce the extraordinary enhancement obtained with rotenone. In contrast, a surface active agent, for example, Endod, would not have its effect enhanced because it is already at its effective site in normal water.

These are hypotheses about mechanism which can be subjected to experimental test and may lead to even more effective enhancements of toxicity.

This paper has also demonstrated that purified water, such as is readily available in thermal power plants as makeup water for high pressure turbine systems is an effective vehicle for enhancing the toxicity of toxic chemicals to zebra mussels. The amounts of this water, while not sufficient for once-through cooling systems, is probably sufficient for service water systems such as fire control systems. Excess capacity in the makeup water system can be used to flush service water systems with or without low concentrations of toxic chemicals added. Furthermore, we have shown that the toxicity of a commonly used control chemical, hypochlorite, is enhanced 100-fold and the toxicity of rotenone, a chemical agent approved by EPA for other uses, is enhanced 10-100,000-fold.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Jill Duncan, Susan Jerrine Nichols, and the U.S. Fish and Wildlife Service for assistance in collecting zebra mussels, Professor Harold H. Lee, University of Toledo, for supplying Endod, and Roussel Bio Corp. for providing Noxfish. This publication is a result of work sponsored, in part, by the Michigan Sea Grant College Program under grants from the National Sea Grant College Program, National Oceanic and Atmospheric Administration (NOAA), U.S. Department of Commerce under grant No. NA16RC0417-01 and the State of Michigan. The U.S. Government is authorized to produce and distribute reprints for governmental purposes notwithstanding any copyright notation that may appear hereon. The views are those of the authors and do not necessarily reflect the views of NOAA or any of its subagencies. J.W. received support from NIH grant RR-08167. Figures 1 to 3 are reprinted from Walker and Ram (1994), with kind permission from Elsevier Science Ltd., The Boulevard, Langford Lane, Kidlington 0X5 1GB, UK.

REFERENCES

Anderson W.M., Delinck D.L., Benninger L., Wood J. M., Smiley S. T., and Chen L.B. (1993) Cytotoxic effect of thiacarbocyanine dyes on human colon carcinoma cells and inhibition of bovine heart mitochondrial NADH-ubiquinone reductase activity via a rotenone-type mechanism by two of the dyes. Biochem. Pharmcol. **45**, 691-696.

Chretien D., Bourgeron T., Rotig A., Munnich A. and Rustin P. (1990) The measurement of rotenone-sensitive NADH cytochrome c reductase activity in mitochondria isolated from minute amounts of human skeletal muscle. Biochem. Biophys. Res. Comm. **173**, 26-33.

Fisher S.W., Stromberg P., Bruner K.A., and Boulet L.D. (1991) Molluscicidal activity

of potassium to the zebra mussel, Dreissena polymorpha: toxicity and mode of action. Aquatic Toxicol. **20,** 219-234.

Fong P.P., Walker J.U., and Ram J.L. (submitted for publication) High purity condensate: An effective vehicle for potassium chloride in the control of zebra mussels.

Lee, H.H., Mesfin, M., and Mezul, J. (1993) Mitigation of zebra mussels with a botanical molluscicide Endod: Laboratory bioassay, non-target organisms and preliminary field studies. Proceedings: Third International Zebra Mussel Conference, 1993 pp. 3/307-3/321.

Lemma A., Wolde-Yohannes L., Fraleigh P.C., Klerks P.L., and Lee H.H. (1991) Endod is lethal to zebra mussels and inhibits their attachment. J. Shellfish Res. **10,** 361-365.

Ram J.L. and Walker J.U. (1993a) Effects of deionized water on viability of the zebra mussel, Dreissena polymorpha. Comp. Biochem. Physiol. **105C**, 409-414.

Ram J.L. and Walker J.U. (1993b) Deionized water, alone or in combination with other chemicals, used to kill zebra mussels. Abstracts, Third International Conference on Zebra Mussels, Toronto, Ont., Canada. p. 118.

Selegean, J.P. (1993) The use of Dreissena polymorpha (the zebra mussel) as a biofilter of municipal wastewater with special reference to the bioaccumulation of heavy metals. M.S. thesis. Wayne State University.

Walker J.U. and Ram J.L. (1994) Effects of deionized water on sensitivity of zebra mussels (Dreissena polymorpha) to toxic chemicals. Comp. Biochem. Physiol. **107C**, 353-358.

Waller, D.L., Rach, J.J., Cope, W.G., Marking, L. L., Fisher, S.W., and Dabrowska, H. (1993) Toxicity of candidate molluscicides to zebra mussels (Dreissena polymorpha) and selected nontarget organisms. J. Great Lakes Res. **19,** 695-702.

Chlorine Dioxide - A Molluscicidal Agent For Adult Zebra Mussel Eradication

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ABSTRACT

Dreissena polymorpha (zebra mussel) were exposed to several treatment levels of chlorine dioxide to determine the potential of this oxidant to be an effective molluscicidal agent. Zebra mussels were collected from Lake Erie and evaluated using Niagara River, NY water. Testing was performed on-site at an industrial plant in which adult zebra mussels were treated continuously with chlorine dioxide using a flow-through specimen containment box specifically designed for adult eradication studies. Treatment levels ranged from 0.25 ppm - 5.0 ppm above the oxidant demand of the river water utilized. Thirty adult mussels were exposed for each treatment level tested. All treatment levels produced 100% mortality ranging from 2.9 days - 8.8 days at a temperature averaging 14.3°C (57.7°F) for testing performed in early June. The control specimens exhibited no mortality during this 9 day test period. Additional testing performed in mid-August at treatment levels of 0.25 ppm - 0.50 ppm produced 100% mortality ranging from 1.6 days - 3.7 days at an average temperature of 25.8°C (74.8°F). A 13% control mortality was recorded for this test, however the results generated were statistically acceptable. The results of these studies established a temperature dependent response in relation to the increased efficacy of chlorine dioxide with increasing water temperature. A carefully structured zebra mussel mortality protocol used to determine specimen death was strictly adhered to during examinations which were made every two hours. Behavioral responses of adult zebra mussels to chlorine dioxide exposure were observed and used as an indicator to determine time of zebra mussel mortality. Histological studies to determine the mode of death were also performed. During testing water parameters were monitored as in pH, oxidation-reduction potential, chlorine dioxide residuals, dissolved oxygen, flow rate, temperature and conductivity. The 100% mortality results achieved in these studies indicate that chlorine dioxide is efficacious for control of water system macrofouling by adult zebra mussels. Chlorine dioxide was toxic to zebra mussels at concentration levels as low as 0.25 ppm above the oxidant demand of the raw water system tested.

INTRODUCTION

Zebra Mussel eradication studies were conducted at a test site located in Niagara Falls, NY from June 1 - 10, 1993 and August 16 - 20, 1993. The test site is a producer of liquid nitrogen, hydrogen, oxygen and argon for bulk transport, which requires tremendous quantities of cooling water. The source of this once through cooling water is the Niagara River, NY. The pumping capacity of this facility is 35 MGPD. This facility also supplies water to neighboring chemical manufacturers, therefore it is critical that efficiency loss be kept to a minimum by the control of Dreissena polymorpha (zebra mussel) infestation.

Dreissena polymorpha (referred to commonly as the zebra mussel) is a small, striped, freshwater mollusk which was first discovered in Lake Erie in 1988 and reported in New York State in 1989/1990.(1) Historically, the zebra mussel has been an inhabitant of the Northern Caspian Sea in what is now known as the Soviet Republics since the 1700s.(2) This extraordinarily hardy organism has in the past century invaded the entire European continent and subsequently has been recently introduced into North American waters by international shipping vessels emptying freshwater ballast into the Great Lakes Region.(1) It has also been observed that zebra mussels can tolerate both freshwater and marine environments as well as live for extended periods out of water which will lead to additional infestation to surrounding waterways due to recreational boating.(2)

The reproduction cycle of the zebra mussel is the primary key to the rapid and abundant infestation being displayed by this organism.(1) Eggs produced by the female are fertilized outside of the shell and hatch into free-swimming larvae called veligers. Veligers are capable of actively swimming for 1 to 2 weeks resulting in population dispersion with a considerable distance from parent colonies. Within 3 weeks of hatching the veligers reach the settling stage in which attachment to a substrate occurs. After attachment, the veligers undergo a metamorphosis to the shelled adult. Adults can vary in size from 1 to 1.5 inches. The adult remains attached to the hard surface which may be rock, metal, wood, plastic, vinyl, glass or rubber etc. by use of byssal threads produced by a gland in the mussel. If necessary, byssal threads can be severed by the mussel and regenerated for reattachment in a more conducive environment. With the environment as a variable, zebra mussels can have an average life span of 3.5 years and in some cases 5 years.(1,3)

The zebra mussel feeds and respires using filtration which is accomplished by water passing over the mussel allowing cilliary movement of mussel components acting as a filter. Each adult zebra mussel is capable of filtering up to one liter of water per day.(3,4) In this function the mollusk displays selectivity in the acquisition of food.(5) These organisms are not only causing severe macrofouling of water intake pipes but are also having an effect on plankton food supplies of the delicate ecosystem of the Great Lakes Region.(2)

The objective of these studies was to determine if chlorine dioxide would provide the needed efficacy for the mitigation, eradication and long term control of macrofouling in the raw water system of the industrial test site utilized.(6) This was attempted by evaluating a range of treatment concentration levels.

MATERIALS AND METHODS

SPECIMEN COLLECTIONS

Adult zebra mussels were collected from Lake Erie and transported in lake water to the test site in Niagara Falls, NY. The mussels were then habituated in 25 gallon aquaria that were continuously aerated three days prior to experimentation in order to insure specimen viability.(5)

EXPERIMENTAL MOLLUSCICIDE

Chlorine dioxide (ClO_2) was evaluated to determine if the oxidant would provide efficacious responses against adult zebra mussels. Chlorine dioxide used in these studies was generated on-site prior to this testing periods using a GENEROX™ 3 - Chlorine Dioxide Generator which incorporates a three pump method.(7,8) This generation required the reaction of sodium hypochlorite with the DREWCHLOR® precursor which is 25% sodium chlorite. This reaction is as follows:

$$NaOCl + H_2O \rightarrow NaOH + HOCL$$

$$HCl + HOCl + 2NaClO_2 \rightarrow 2ClO_2 + 2NaCl + H_2O$$

(DREWCHLOR®)

Chlorine dioxide is a yellow-green gas which is water soluble, a true dissolved gas with a pH range of 2.8-3.5.(7,8) A minimum available chlorine dioxide of 500 ppm, the target chlorine dioxide concentration needed to produce optimum results, was achieved through this generation. Once chlorine dioxide generation parameters were satisfied, injection was made continuously in separate feed levels ranging from 0.25 ppm - 5.0 ppm above the demand of the raw water. The chlorine dioxide demand of the raw water was determined to be 0.30 ppm. Chlorine dioxide was pumped into the individual specimen containment equipment via separate pumps for each treatment level. The desired residuals of chlorine dioxide were maintained by testing every two hours for the duration of each test using a DPD (N,N-diethyl-p-phenylenediamine) photometric and a colormetric field titration procedure. Oxidation-reduction potentials were also utilized to maintain target chlorine dioxide residuals. Testing of these parameters was crucial for maintaining the continuity of these evaluations. The controls (untreated raw water) were tested and did not contain any chemical contamination that would hinder or bias these evaluations.

PROTOCOL FOR DETERMINATION OF ZEBRA MUSSEL MORTALITY

The procedure for the determination of chlorine dioxide to be a chemical treatment able to produce adult zebra mussel mortality is as follows:

(A) As previously described, a stock of adult zebra mussels were carefully prepared for experimentation. This allowed the selection of only the most healthy and viable zebra mussels for these studies.
(B) Chlorine dioxide was generated on-site at the test location with an initial mean ppm concentration of 473 ppm chlorine dioxide.
(C) Equipment descriptions and assembly prior to testing:
(1) A specialized specimen containment system was utilized for each level of concentration tested as well as the untreated control. These specimen containers were constructed of a black plastic so that light conditions could mimic raw water distribution lines. The dimensions of these containers were 12 inches (H) x 12 inches (W) x 12 inches (L), thus forming a cube shaped container with a hinged lid, which swings open from the top. The intake bulkhead fitting is located on the side near the bottom of the specimen container. The discharge bulkhead fitting is located on the opposite side of the intake and is near the top of the specimen container. This assembly allows an appropriate flow rate of one gallon per minute through the specimen container. These specimen containers were placed in an area of the industrial test site where a constant raw water supply was present as well as suitable drainage to provide proper container function.
(2) Each specimen container was equipped with an enclosure that houses thirty adult zebra mussels in individual chambers for treatment effectiveness evaluations. The materials used to construct these enclosures are non-toxic, high density, polyethylene and PVC to provide a test environment free of metal contaminants to allow for accurate efficacy testing. These enclosures are 2 inches (H) with the width and length approximately 11 7/8 inches in order for the enclosure to rest horizontally on a 1/8 inch wide ledge along the perimeter of the specimen container. The chambers in which the zebra mussels are housed allow the raw water, treated or untreated, to pass through the chambers while the test specimens are confined so that progress can be tracked for each mussel during the test period.
(3) Thirty zebra mussels were placed into each of the individual chambers within the enclosure that were secured with wing-nut closures and placed inside a specimen container for exposure. The specimens were allowed to acclimate for 36 hours with untreated waterflow. No mortalities were observed in either of the two studies performed prior to treatment with chlorine dioxide.
(4) After acclimation, treatment with chlorine dioxide was initiated at levels of 0.25 ppm, 0.50 ppm, 1.0 ppm, 2.0 ppm and 5.0 ppm residual chlorine dioxide above the demand of 0.30 ppm. Treatment with chlorine dioxide was maintained on a continuous basis. Waterflow ratios were maintained through the monitoring of the untreated control specimen container for each study.
(D) The procedure for monitoring the effects of chlorine dioxide on the zebra mussels is detailed as follows:
(1) Every two hours (120 min.) in addition to the determination of zebra mussel mortality the following parameters were measured:

(a) pH measurements were taken for each specimen container both chlorine dioxide treated or untreated.
(b) ORP (Oxidation-Reduction Potential) readings were performed for all specimen containers.
(c) Chlorine dioxide residuals were determined by Drew's chlorophenol test kit which is a field titration method to determine chlorine dioxide residuals. The residuals obtained by this method were cross-checked utilizing a Hach modified DPD photometric test specific for chlorine dioxide. There was excellent agreement with the field titration method.
(d) The DO (dissolved oxygen) of the raw water was measured.
(e) The flow rate to the specimen containers was monitored to insure a 1.0 gpm flow rate.

(2) Other water parameters that were tested every eight hours during the test period were:
(a) Chlorine dioxide reservoir tank concentration was tested using the Hach DR 2000 spectrophotometer to be certain of the integrity of the chlorine dioxide being used in these evaluations.
(b) Chlorine dioxide pump draw downs were performed routinely to assure proper metering rates of chlorine dioxide into the specimen containers.
(c) Temperature of raw water.
(d) Conductivity of raw water.

(3) Every two hour period the inspection of the zebra mussels within the individual specimen enclosures were examined for mortality. This was accomplished by:
(a) Each of the thirty mussels in all specimen containers used in these studies were removed, one at a time to observe its appearance. These observations required efficiency and quickness to be sure that the mussels were not removed from the water for extended time intervals. All of the test organisms were observed and evaluated every two hours.
(b) The appearance check also involved in some cases physical stimuli. Expired mussels are obvious in that their valves are displayed wide open with internal organs clearly visible, however this may not occur in all cases.
(c) If doubt was present as to if a zebra mussel had expired, a probe was gently administered between the bi-valves. If the mussel was alive it would respond positively by tightly closing the shells. If no response occurred, the mussel was held in both hands using the thumbs and index fingers to gently twist the mussel longitudinally, so as to very slightly set the two shells ajar (<1mm). If the mussel responded by closing the shells, it was scored as alive and returned to the specimen container enclosure.
(d) If mortalities were recorded, the expired mussels were placed in examination/transport jars containing 10% buffered formalin solution for preservation, labeled and routed to Cornell University, Ithaca, NY for histological examination. Examinations of the soft tissue and internal organs of the expired mussels would show, in theory, lesions caused by chlorine dioxide exposure as a cause of death. This would give indication that chlorine dioxide caused zebra mussel mortality, not mortality due to starvation or suffocation.

(e) In order to statistically determine the effects of chlorine dioxide, the percent mortality was calculated by:

$$\frac{\text{Number of Expired Mussels} \times 100}{\text{Total Number of Mussels in Specific Enclosure}} = \text{Percent Mortality}$$

also to determine frequency of treatment:
Treatment Percent Mortality - Control Percent Mortality = Mortality Attributed to Treatment

RESULTS

WATER PARAMETERS

Measured water parameters during both test periods remained constant in all variables monitored. Since water parameters were tested every two hours of these evaluations, a very effective method of control was available to correct minor inconsistencies when apparent. These evaluations therefore had built-in quality control in order to maximize the quality of data generated.

The temperatures of the raw water for the early June study ranged from 13.5°C - 15.9°C with a mean of 14.3°C. Temperatures for the mid-August evaluation ranged from 24.6°C - 26.4°C with a mean of 25.8°C. All specimen containers had temperatures consistent with the control during testing. Dissolved oxygen readings of the raw water ranged from 9.4 mg/l - 10.6 mg/l with a mean of 10.1 mg/l. The DO levels were in a range consistent with very turbulent water as was evident at the test site. Chlorine dioxide residuals which were very important in maintaining the integrity of these studies did not deviate significantly from the assigned dose rates of 0.25 ppm, 0.50 ppm, 1.0 ppm, 2.0 ppm and 5.0 ppm. The pH in certain specimen containers decreased slightly when increased treatment of chlorine dioxide was applied, which was expected. The ability to maintain test treatment levels was primarily due to the stability of the chlorine dioxide reservoir which ranged in concentration from 452 ppm - 523 ppm with a mean of 483 ppm.

The level of chlorine dioxide treatment established an ORP (oxidation-reduction potential) range of 362 mv - 781 mv depending on dose rate. The ORP of the raw water was consistent at a range of 204 mv - 294 mv with a mean 241 mv. Flow rate of 1.0 gpm (gallon per minute) for each specimen container was required to maintain proper operating conditions in the test systems. Flow rate checks were diligent and adjustments were made if necessary. Lastly, conductivity measurements were made on the raw water with a mean value of 272 micromhos/cm.

As a result of the constant water parameters which existed through these studies it can be determined the parameters did not interfere with the test objective of the determination of chlorine dioxide toxicity. Therefore, water parameters in these studies did not have an effect on the experimental outcomes with the exception of temperature. Mortality of control zebra mussels did not occur over the test exposure period (211.0 hours), in the early June study. A low 13% mortality occurred on the control for the mid-August test period. These overall statistics would suggest that the mortality which was observed in the zebra mussels was a result of the toxicity of chlorine dioxide and not other extrinsic factors.

TOXICITY TO ZEBRA MUSSELS

Chlorine dioxide (ClO_2) proved 100% toxic to adult zebra mussels at all tested treatment concentrations. (Tables I & II)

TABLE I - SUMMARY OF TOXICITY TESTS OF CHLORINE DIOXIDE TO ADULT ZEBRA MUSSELS (AVERAGE TEMP - 14.3°C)

Treatment Level ppm Chlorine Dioxide Residuals	LT_{50} (Hours)	LT_{100} (Hours)	Time Needed to Achieve 100% Mortality After Reaching 50% Mortality in Hours
5.0	41.0	70.0	29.0
2.0	42.0	78.0	36.0
1.0	69.0	102.0	33.0
0.5	92.0	139.0	47.0
0.25	164.0	211.0	47.0
Control	(No Mortalities after 211.0 Hours)		

TABLE II - SUMMARY OF TOXICITY TESTS OF CHLORINE DIOXIDE TO ADULT ZEBRA MUSSELS (AVERAGE TEMP - 25.8°C)

Treatment Level ppm Chlorine Dioxide Residuals	LT_{50} (Hours)	LT_{100} (Hours)	Time Needed to Achieve 100% Mortality After Reaching 50% Mortality in Hours
0.50	24.0	40.0	16.0
0.25	40.0	89.0	49.0
Control	(13.0% (7) Mortalities After 89 Hours)		

BEHAVIORAL RESPONSES TO THE MOLLUSCICIDE

Through the duration of these studies, behavioral observations were made in an effort to understand the effects of chlorine dioxide on the adult zebra mussels tested. Based on behavioral responses, a correlation can be seen between the intensity of chlorine dioxide dose levels and zebra mussel behavioral patterns.

(1) Treatment levels of 2.0 ppm and 5.0 ppm provided very irritating conditions for the zebra mussels. The zebra mussels did not have open shell valves through the test period when exposed to chlorine dioxide. It was apparent that after approximately 48 hours, during which time the shell valves remained tightly shut for protection from toxicant exposure, the zebra mussels in these experiments were eventually forced to respire to avoid suffocation or starvation. Once respiration occurred, death was certain. However, stronger mussels held on for a longer period of time in the state of intense valve closure, therefore causing delayed mortality.

(2) The experiments treated at 0.25 ppm - 1.0 ppm of chlorine dioxide showed significantly different behavioral patterns in the specimens tested. Initial contact of chlorine dioxide again caused mussels to tightly close their valves. After 48 hours some of the specimens reverted back to a somewhat normal respiratory pattern in which the valves opened to filter water. An observation was made that not all of the zebra mussels at these treatment levels showed active siphoning, but rather restrictive functioning in activity. This would seem to indicate that although the zebra mussels observed at these dose levels showed sensitivity to chlorine dioxide, the severity of this sensitivity was not intolerable and allowed these mussels to attempt to resume restrictive respiration.

(3) The control groups of zebra mussels often remained with the shell valves open with the occurrence of fully extended siphons, engaging in very active respiration. Also notable is that after 72 hours of testing the control zebra mussels began to attach to the walls of the specimen container enclosure using byssal threads.(4) Neither active respiration nor byssal attachment was observed in the zebra mussels exposed to chlorine dioxide. The lack of these activities indicated that zebra mussels exposed to chlorine dioxide can only attempt to survive the treated environment, not to accept the conditions of chlorine dioxide exposure. This could be the direct result of a manifestation of sublethal impairments induced by exposure to chlorine dioxide.

ADDITIONAL TESTING

In addition to the two field trials described, histological evaluations of the expired specimens was performed. Cornell University, Ithaca, NY performed soft tissue examinations to determine the actual mechanism of kill exhibited by chlorine dioxide. The results of these studies indicated that chlorine dioxide is apparently readily absorbed into the internal tissue structure of exposed adult zebra mussels. Lesions were observed in the

mussels exposed to a residual as low as 0.25 ppm of chlorine dioxide. Target areas of chlorine dioxide were the digestive system and respiratory tract where lesions from moderate to severe were observed. Also a loss of cilia was also observed. As exposure time elapsed, lesions were also located on the ovaries and testes of the mussels. Also a correlation can be made in that the severity of the lesions on the soft tissues that were observed can worsen with increased exposure of chlorine dioxide.

Dreissena bugensis, (quagga mussel), was also intermittently tested to determine the effect of chlorine dioxide in order to provide quagga mussel toxicity. Although these tests were limited in frequency and size, results indicated that chlorine dioxide did cause high mortality rates in the quagga mussel populations when exposed to chlorine dioxide. Therefore this can be used as an indicator which shows chlorine dioxide can cause quagga mussel mortality under test conditions similar to that of zebra mussels.

DISCUSSION

The flow-through field tests indicated that chlorine dioxide has molluscicidal properties against adult zebra mussels. These studies therefore showed the potential of chlorine dioxide to be an effective toxicant for the control of raw water systems from macrofouling by zebra mussels. All of the tested treatment rates produced 100% mortality in reasonable time intervals, as presented in Tables I and II. The control (untreated) mussels in these studies were healthy and very active during the test periods indicating that all of the zebra mussels used were well adjusted and suitable for testing.

Due to zebra mussel sensitivity to the presence of chemical oxidants, a lag period usually was observed between the time of oxidant introduction and the time mortality was first observed.(9) During this lag time period the zebra mussels tightly close their valves to avoid exposure to chlorine dioxide, especially at higher treatment concentrations (>1.0 ppm residual chlorine dioxide).

The results of the studies completed also showed that with an increase in water temperature, the contact period required to achieve zebra mussel mortality will decrease due to a reduced lag time with the occurrence of warmer water temperatures.(10) The mussels seem to be unable to maintain prolonged valve closure in warmer water temperatures due to the necessity of the mussels to respire as to not produce a situation of either suffocation or starvation within this closed environment.

CONCLUSIONS

It is now a commonly known fact that zebra mussels are rapidly colonizing the Great Lakes and are likely to spread throughout North America, therefore, it is important that chlorine dioxide be tested as a possible eradication agent used to control zebra mussels.(9) The results of our studies indicate that at warmer water temperatures low concentrations of chlorine dioxide can provide a toxic environment for zebra mussels without causing

serious environmental implications. Finally, molluscicide applications involving chlorine dioxide need not involve strategies with the utilization of high dose levels to produce rapid kills. It would be more advantageous to use the lowest possible treatment level to attain 100% mortality, thus reducing the environmental and economic considerations.

REFERENCES

1. O'Neill, Charles R. and David B. MacNeill, *Dreissena polymorpha: An Unwelcome New Great Lakes Invader*, pp. 1-3, Brockport, New York: New York Sea Grant Extension Program, 1990.

2. Snyder, Fred L., *Zebra Mussels In Lake Erie And Its Implications*, pp. 1-2, Fact Sheet 045 Columbus, Ohio: Ohio Sea Grant College Program, 1989.

3. Garton, David W. and Wendell R. Haag, *Seasonal Reproductive Cycles And Settlement Of Dreissena polymorpha In Western Lake Erie*, pp. 111-115, from Zebra Mussels: Biology, Impacts And Control, Edited by Nalepa & Schloesser, Boca Raton, Florida: Lewis Publishing for CRC Press, 1993.

4. *Dreissena polymorpha, Structure And Formation Of The Byssus*, p. 4, Dreissena polymorpha Review, New York Zebra Mussel Information Clearinghouse, Vol. 2, Number 1, New York: New York Sea Grant Extension, Jan./Feb. 1991.

5. Nichols, S. Jerrine, *Maintenance Of The Zebra Mussel (Dreissena polymorpha) Under Laboratory Conditions*, pp.734-738, from Zebra Mussels: Biology, Impacts And Control, Edited by Nalepa & Schloesser, Boca Raton, Florida: Lewis Publishing for CRC Press, 1993.

6. Claudi, Renata and David W. Evans, *Chemical Addition Strategies For Zebra Mussel Dreissena polymorpha Control In Once Through Service Water Systems*, pp. 563-573, from Zebra Mussels: Biology, Impacts And Control, Edited by Nalepa & Schloesser, Boca Raton, Florida: Lewis Publishing for CRC Press, 1993.

7. Drew Industrial Division, Ashland Chemical Company, *Principles Of Industrial Water Treatment*, Eighth Edition, pp. 143-146, Boonton, New Jersey: Drew Industrial Division, 1985.

8. Drew Industrial Division, Ashland Chemical Company, *Principles Of Industrial Water Treatment*, Eighth Edition, pp. 102-103, Boonton, New Jersey: Drew Industrial Division, 1985.

9. Van Benschoten, et al, *Chemical Oxidants For Controlling Zebra Mussels: A Synthesis of Laboratory and Field Studies*, pp. 599-617, from Zebra Mussels: Biology, Impacts And Control, Edited by Nalepa & Schloesser, Boca Raton, Florida: Lewis Publishing for CRC Press, 1993.

10. Iwanyski, Stanley and Robert W. McCauley, *Upper Lethal Temperatures Of Adult Zebra Mussels, Dreissena polymorpha*, pp. 667-671, from Zebra Mussels: Biology, Impacts And Control, Edited by Nalepa & Schloesser, Boca Raton, Florida: Lewis Publishing for CRC Press, 1993.

Development of Zebra Mussel Control Programs Utilizing Chlorine, Acti-brom and Bromine

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Abstract

For zebra mussel control in New York State, the Department of Environmental Conservation (DEC) has allowed pre-evaluated zebra mussel treatment programs, commonly termed "generic modifications", for holders of SPDES Permits. Chemicals for which generic modifications are available include the oxidizing biocides chlorine and bromine. The bromine modification covers Acti-brom (a proprietary compound manufactured by Nalco Chemical Co.) and bromine, both utilized in combination with chlorine. For these chemicals the treatment programs allowed at three RG&E facilities consisted of continuous treatment for 18 or 30 days with a Total Residual Oxidant level of 0.1ppm within the plant discharge. To date, RG&E has conducted fifteen 18-day and five 30-day chlorine treatment studies, three 18-day and one 30-day Acti-brom studies, and one 30-day bromine study. In all treatment programs, mortality rates were determined by utilizing adult mussels in bioboxes designed by RG&E. These bioboxes were placed to receive sidestream water from circulating and/or service water systems and checked periodically throughout the study duration to determine mortalities.

Evaluations of the treatments conducted to date have shown the 18-day generic modification treatment program for chlorine to be relatively ineffective, with overall mortalities ranging from 0 to 20%, and the 30-day chlorine treatments resulting in great variabilities with mortalities varying from 0 to >90%. The Acti-brom treatments conducted to date have been more effective and consistent, with mortalities ranging up to 60% for the 18-day and 90-100% for the 30-day. Results of the 30-day bromine testing ranged from 0 to 10%, however water temperatures during these treatments were only in the teens (°C), and on the decline.

Water temperatures were found to greatly influence the effectiveness of all the chemicals, especially chlorine. When temperatures were below 20°C or extremely variable, chlorine mortalities were generally limited to <10%. Although Acti-brom was not found to be as temperature dependent, as demonstrated by substantial mortalities in waters below 20°C, a clear effect was observed when the water temperatures suddenly decreased and then rebounded. The inter-relationships amongst water temperature (both absolute and fluctuating), chemical concentration, and application duration should provide the basis for establishing treatment requirements for zebra mussel control using these chemicals.

INTRODUCTION

Rochester Gas and Electric (RG&E) has been treating raw water systems against zebra mussel biofouling within three power stations since the Fall of 1990. These treatments have utilized both chlorine and bromine compounds. This paper will discuss (1) the results of chlorine treatments, (2) the results of Acti-brom treatments compared to chlorine treatments and, (3) the results of generic bromine treatments. All of the treatment programs included in this paper were conducted by RG&E.

METHODS

Control treatment programs conducted by RG&E were approved by New York State Department of Environmental Conservation (DEC). During 1990 DEC developed a standardized zebra mussel chemical control treatment program utilizing chlorine, for raw water users possessing a State Pollution Discharge Elimination System (SPDES) Permit. This program, termed a "Generic Permit Modification", included the following conditions:

1) continuous chlorination for an 18-day period, with a TRC limitation at the point of discharge of 0.1ppm,
2) an interval of 45 days between treatments, and
3) a maximum of four treatments per year.

In addition, treatments for power stations located upon Lake Ontario were restricted to the May through October time period, as an added precaution to protect the winter salmonid fishery in the Lake. Based upon poor zebra mussel mortality rates found in 1991 and 1992, for 1993 DEC approved an extension of the treatment period from 18 to 30 days, and the decrease of the interval between treatments from 45 to 30 days.

Bromine, which is in the same class of chemicals as chlorine (halogens), has also been utilized for biofouling control. In addition, it is theoretically more chemically active than chlorine in higher pH waters such as Lake Ontario and the Genesee River (near pH.8). Due to the low zebra mussel mortality rates found in the chlorine treatments, Acti-brom, a proprietary bromine compound manufactured by Nalco Chemical Company, was selected for study at one RG&E station on Lake Ontario in hopes that a slightly more active compound would increase effectiveness enough to control zebra mussel biofouling. Acti-brom is a combination product of sodium bromide and a surfactant penetrant that is activated <u>in situ</u> by chlorine to form hypobromous acid and hypochlorous acid. Finally, "generic" bromine (hereafter referred to as bromine) was also utilized for one treatment program. This substance is chemically similar to Acti-brom except that it does not contain the surfactant penetrant found in Acti-brom. Due to the similar nature of chlorine, Acti-brom and bromine, the DEC treats these compounds similarly with respect to a generic permit modification. Thus the treatment applications for Acti-brom and bromine were exactly the same as that previously utilized for chlorine, i.e., 18- or 30-days at a concentration of 0.1ppm

TRO (Total Residual Oxidant, instead of Chlorine). Monitoring was also conducted in the same manner as during previous chlorine treatments. This standardization in both treatment and monitoring allows for a direct comparison between results of all three chemicals.

During 1991, 1992 and 1993, zebra mussel control treatments have been conducted at three RG&E power stations: two located on Lake Ontario and one located on the Genesee River in Rochester, NY. During 1991 RG&E conducted nine 18-day chlorine treatments while during 1992 six 18-day chlorine and three 18-day Acti-brom treatments were conducted. In 1993 five 30-day chlorine and one 30-day Acti-brom treatments were conducted. Along with these treatments, in situ studies of treatment effectiveness were conducted to quantify results. These studies monitor effectiveness under actual plant conditions, including normal operational variabilities (e.g., flow changes, temperature fluctuations, etc.). By placing monitoring stations at a number of locations throughout the station (such as intake waters, service water systems, and discharge effluent streams) and doing this over different seasons, a number of distinct tests were performed during each treatment and many different environmental situations were monitored. For example, temperatures ranging from 10°C to 30°C were able to be studied this way.

Monitoring performed consisted of a bio-assay, utilizing bioboxes which normally contained 100 zebra mussels per test location. Bioboxes were designed as flow-through systems receiving sidestream water from the water system being studied. Since trans-locating adult zebra mussels frequently enter the plant water systems, all studies were performed using adult zebra mussels. This approach will assure mortality of juvenile or newly settling mussels as well as adults.

CHLORINE STUDY RESULTS

The results of 25 tests utilizing chlorine per the conditions allowed by the original generic SPDES permit modification for chlorine, i.e., 18-days continuous chlorination at 0.1ppm TRC, are summarized by average test temperature in Figure 1. It should be noted that, per the permit, chlorine injection was ended on Day 18, thus any mortality beyond that time is latent mortality. Also, the number of tests conducted at each temperature are shown in the parentheses. A substantial difference in efficacy is shown between the lower temperatures of 10-20°C and the higher temperatures of 25 and 30°C. While mortalities approaching 60% were found at the higher temperatures (20% of which were latent mortalities), less than 10% mortality occurs within the lower temperature range. Since ambient water temperatures at these power stations rarely attains 25°C, the effectiveness of the 18-day generic permit modification is limited to less than 10% for virtually all of the power station locations, aside from the discharge canal itself. Based upon the literature, it was presumed that either an increase in TRC level or a longer duration of treatment at the 0.1ppm TRC level would result in higher mortalities. The DEC was amenable to longer duration

treatment, and therefore the 30-day treatment was established.

The database for the 30-day chlorine treatment evaluations consists of ten tests conducted during 1993, two at the Genesee River site and a total of eight at the two Lake Ontario sites. Figure 2, a summary of 30-day chlorine treatment results at 25°C, exemplifies the wide range of results found in all 30-day tests. A closer look at the data revealed that the River station tests achieved over 90% mortality, while the Lake station tests were consistently below 10%. Knowing that lower pH makes chlorine more effective, this was the first environmental variable to be investigated. River pH values however, were found to be slightly higher than the Lake during these treatments, so this could not explain the large difference in mortality rates. Temperature was then suspected as a factor affecting results. Figure 3 shows the results of the two River tests along with the daily temperature ranges which occurred throughout the treatments. It is apparent that temperatures did not fluctuate by more than 1°C on any day during the tests. In the Lake however, daily temperature fluctuations of up to 10°C had occurred during the summer, while the average mortality rate reached only 2.4% (Figure 4). Such extreme temperature fluctuations, caused by upwellings of the Lake's cold, hypolimnetic waters, can occur quite regularly throughout the summer, once the Lake is thermally stratified. Previous work had shown that absolute temperature influenced chlorine effectiveness, now the 1993 results suggest that extreme temperature variations will also reduce chlorine effectiveness. Overall, these results question the effectiveness of zebra mussel control using 30-day continual chlorination at 0.1ppm TRC in situations like the Lake Ontario sites due to the occurrence of these upwellings.

Although all quantitative testing was restricted to adult zebra mussel mortality, qualitative, yet very important observations, were made of zebra mussel settlement upon the walls of the biobox holding tanks. It was clearly evident that the control tank, receiving unchlorinated water, had a number of recently settled zebra mussels upon its walls, whereas the treated test tanks had no mussels at all attached to the tank walls. This finding suggests that even though this treatment program may not be effective in killing adult zebra mussels, such treatments may be sufficient to prevent zebra mussel settlement.

CHLORINE SUMMARY AND CONCLUSIONS

To conclude the chlorine treatment section of this analysis, the following concepts summarize the major findings:

1) An 18-day chlorine treatment at 0.1ppm TRC is generally ineffective in killing adult zebra mussels within power stations in the Great Lakes' Region.
2) A 30-day chlorine treatment at 0.1ppm TRC can be effective in killing adult zebra mussels under conditions of relatively stable water temperatures of above 20°C.

3) A 30-day chlorine treatment at 0.1ppm TRC should effectively control zebra mussel settlement under normal environmental conditions found in the Great Lakes' Region.

ACTI-BROM STUDY RESULTS

Figure 5 summarizes Acti-brom results compared to chlorine results for data collected prior to 1993. It appears that Acti-brom is relatively consistent at all three temperatures, and that at the lower water temperatures of 15 and 20°C Acti-brom is much more effective than chlorine. At 25°C these two chemicals showed generally similar results, although this observation includes latent mortalities for Acti-brom, while latent mortalities for chlorine could not be assessed. To confirm the effectiveness of Acti-brom at lower temperatures the Acti-brom treatment was repeated during Spring, 1993. This treatment, which was for 30-days, resulted in average mortalities of 90%, including latent mortality. Figure 6 compares 18-day and 30-day Acti-brom treatment results at 15°C, as well as showing chlorine results for both treatment programs at that temperature. It can be seen that even after 18-days the Spring 1993 Acti-brom results were well ahead of the Fall 1992 Acti-brom mortalities. As previously discussed, chlorine is not effective at all for killing adult zebra mussels in this temperature range.

Of particular interest in the Spring 1993 Acti-brom results was a leveling off of the mortality rate during the third week of the treatment (approximately Study Days 23-29). Upon investigation of water temperatures, as shown in Figure 7, this reduction in mortality rate corresponded perfectly with an upwelling in Lake Ontario that occurred at that time. It can be speculated that if this temperature drop did not occur, the final mortality of 90% may have been realized six or seven days earlier. This finding also shows that temperature has an impact upon Acti-brom effectiveness, although it does not appear to be as dramatic as temperature impacts upon chlorine.

ACTI-BROM SUMMARY AND CONCLUSIONS

To conclude the Acti-brom treatment section of this analysis, the following concepts summarize the major findings:

1) Acti-brom appears to be more consistent in effectiveness than chlorine when comparing water temperatures of 15, 20, and 25°C.
2) An Acti-brom 30-day treatment program appears to be effective in controlling adult zebra mussels.
3) Acti-brom is more effective at lower water temperatures than chlorine for controlling adult zebra mussels.
4) Acti-brom effectiveness is reduced by extreme water temperature fluctuation, although such temperature effects are not as dramatic as found for chlorine.

BROMINE STUDY RESULTS

In late Fall, 1993 a 30-day treatment utilizing bromine was conducted. Bromine was studied for two reasons: (1) its use would eliminate any question concerning the effect and/or the chemical fate of the surfactant contained in Acti-brom, and (2) not being a proprietary chemical, it may be more widely available, thus possibly leading to reduced costs.

Figure 8 shows the results of the bromine testing. Although mortalities of less than 10% are not encouraging, it is important to note that when these studies got underway the water temperatures were steadily declining throughout the study period and averaged only 10 - 17°C for the 30-day treatment. Previous temperature experiences suggest that no conclusions can be drawn at this time concerning the effectiveness of bromine, however it can be stated that the temperature regime studied in this case was outside of what would be considered a normal treatment situation. Further tests utilizing bromine are currently scheduled for Spring, 1994.

FINAL CONCLUSIONS

A few final conclusions concerning these treatment programs are worth noting:

1) Treatment programs utilizing 0.1ppm TRO for controlling adult zebra mussels border on the lower boundary of effectiveness. While it appears as though such treatments can be successful, subtle changes in environmental variables can cause marginal to poor results.
2) Temperature plays a very complex role in the effectiveness of these chemicals to kill zebra mussels. Temperature will effect chemical reactivity rates, zebra mussels' metabolic rates and biochemical rates.
3) Bromine chemistry (i.e., Acti-brom and bromine) provides more flexibility than chlorine for control of adult zebra mussels. This is because bromine appears to be more effective at the lower temperature ranges typically found at Great Lakes' facilities.
4) The 30-day continuous chlorine treatment does appear to be effective for control of zebra mussel settlement. Such control may be sufficient for facilities not subject to large influxes of trans-locating adult zebra mussels.

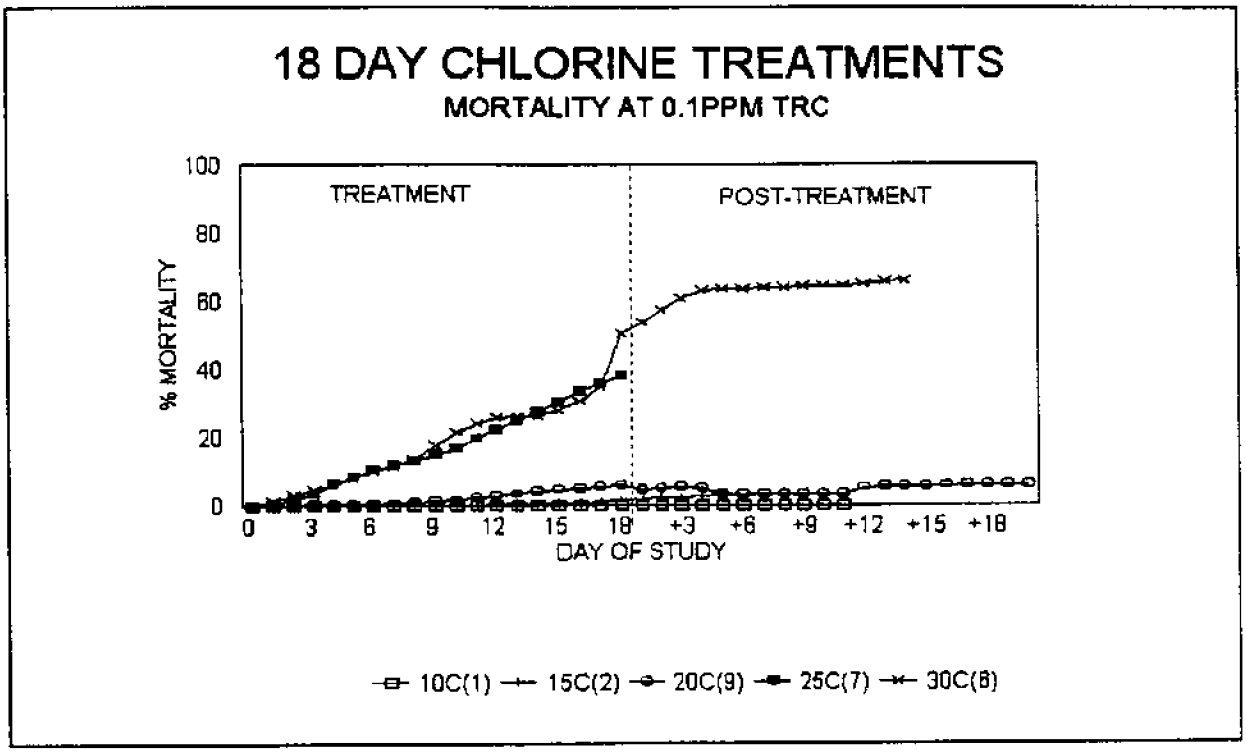

Figure 1

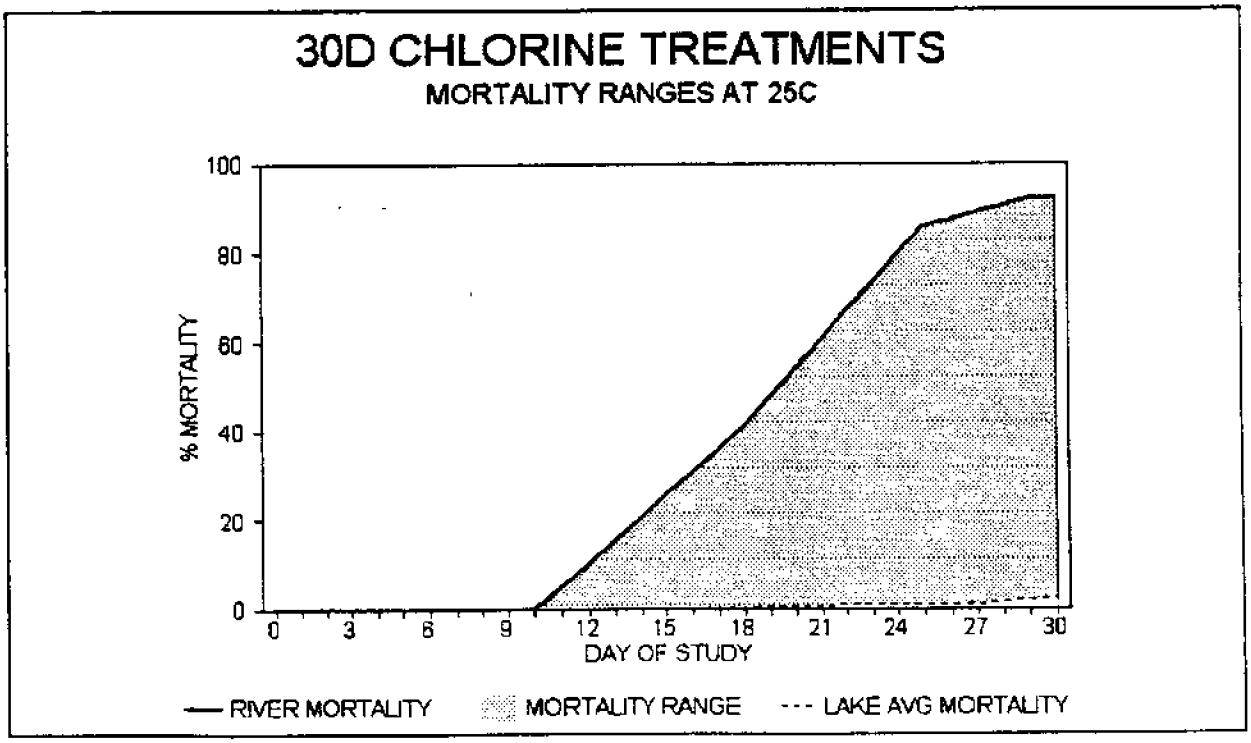

Figure 2

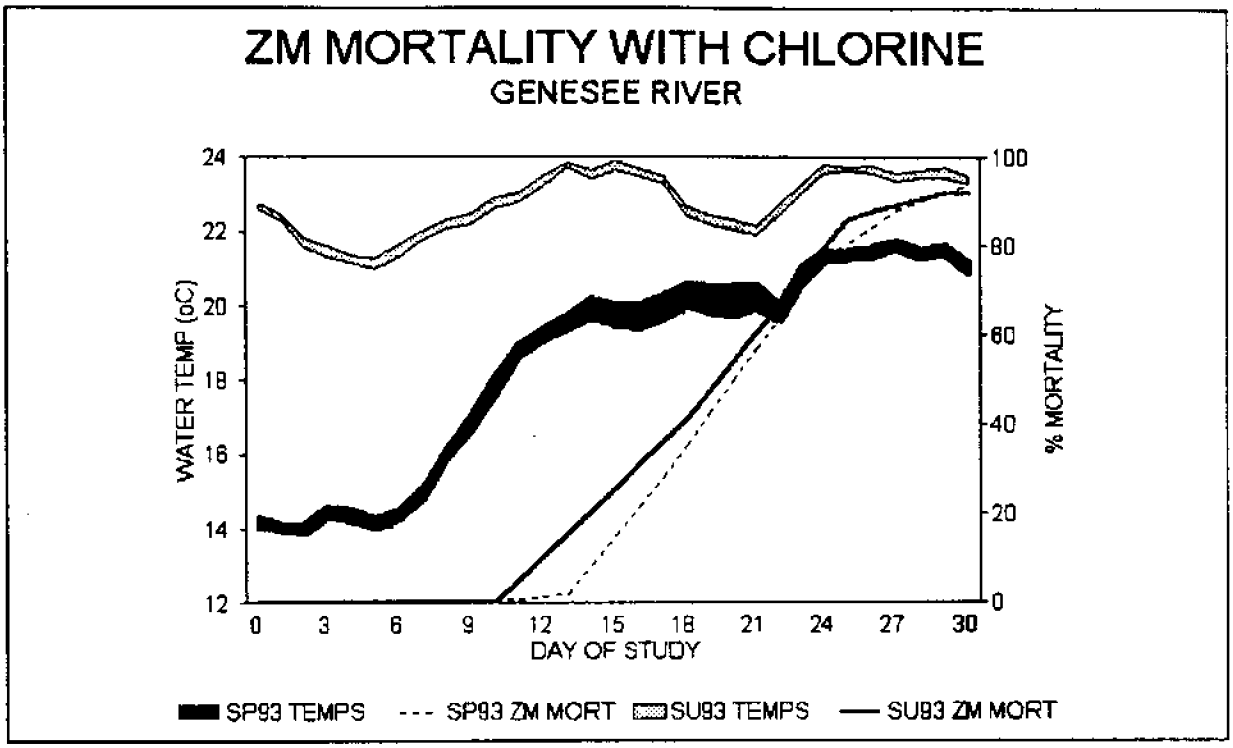

Figure 3

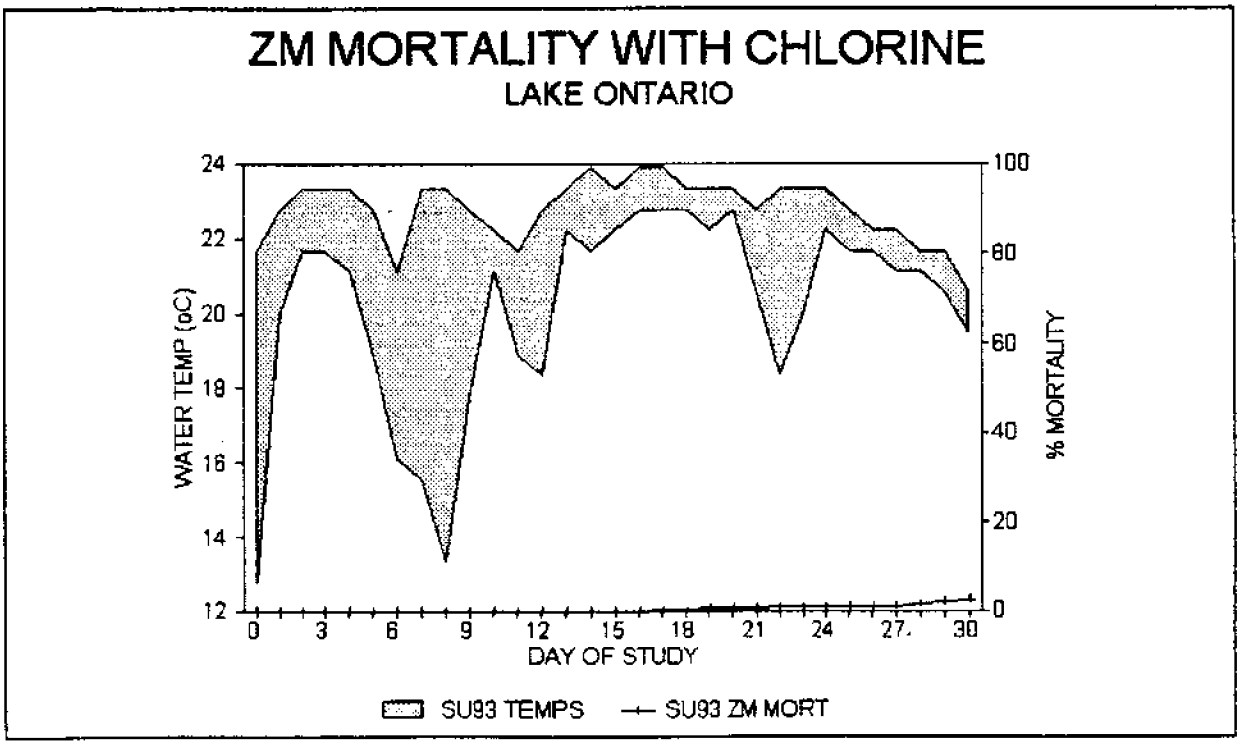

Figure 4

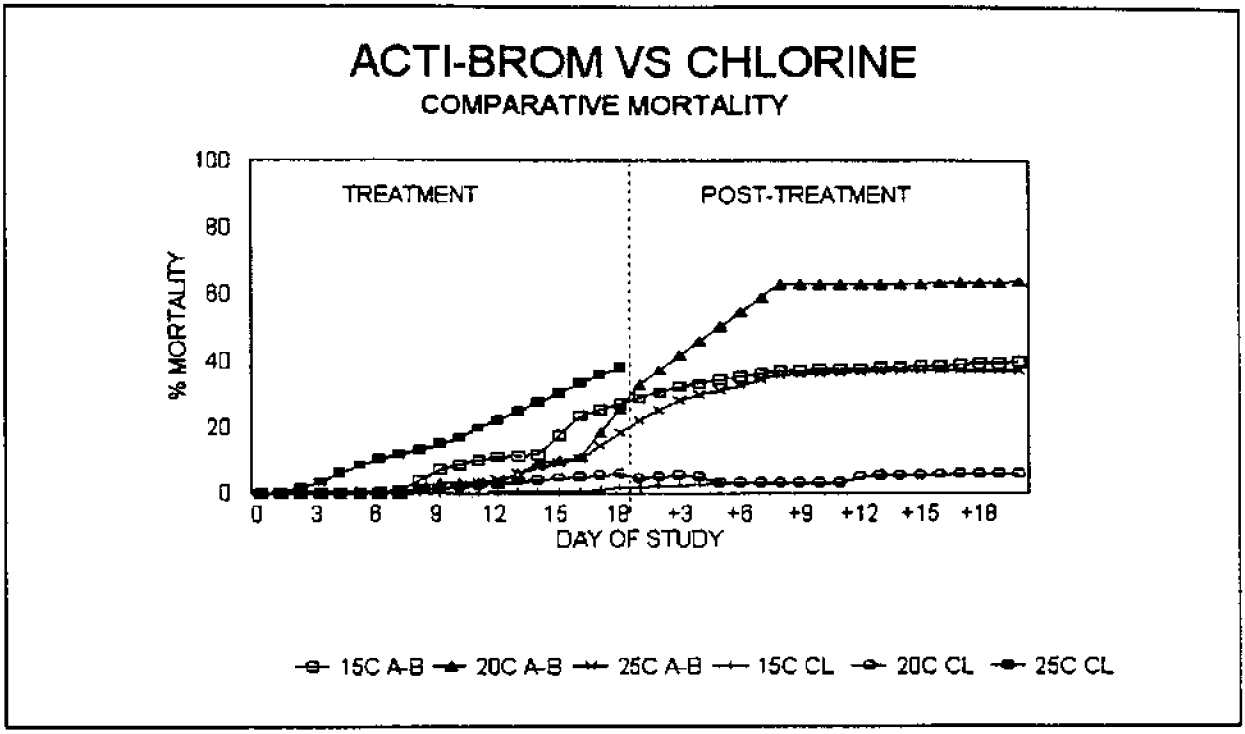

Figure 5

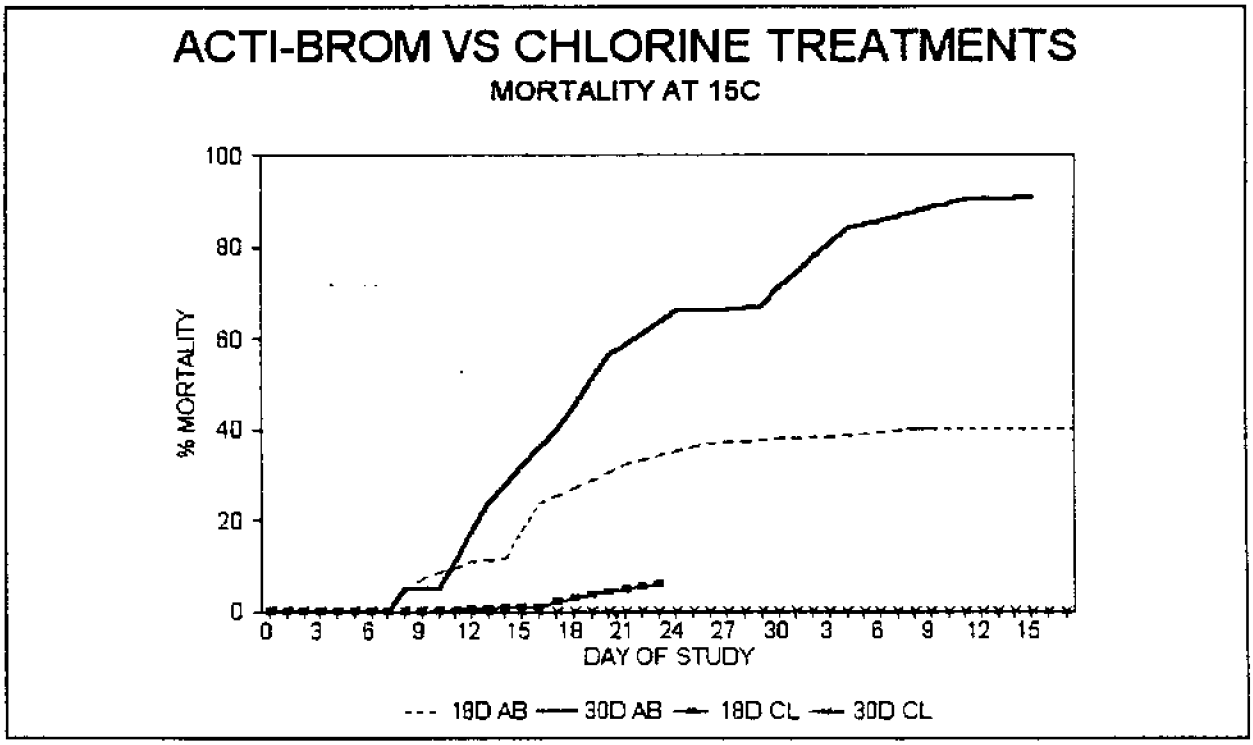

Figure 6

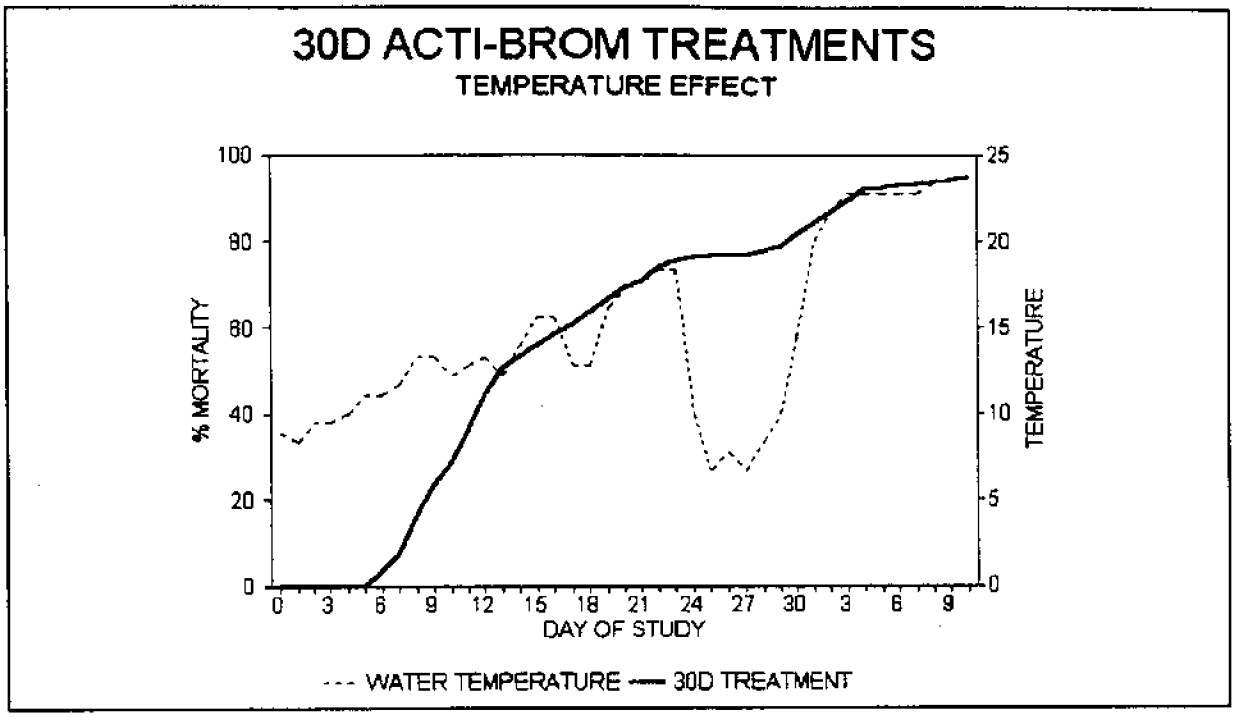

Figure 7

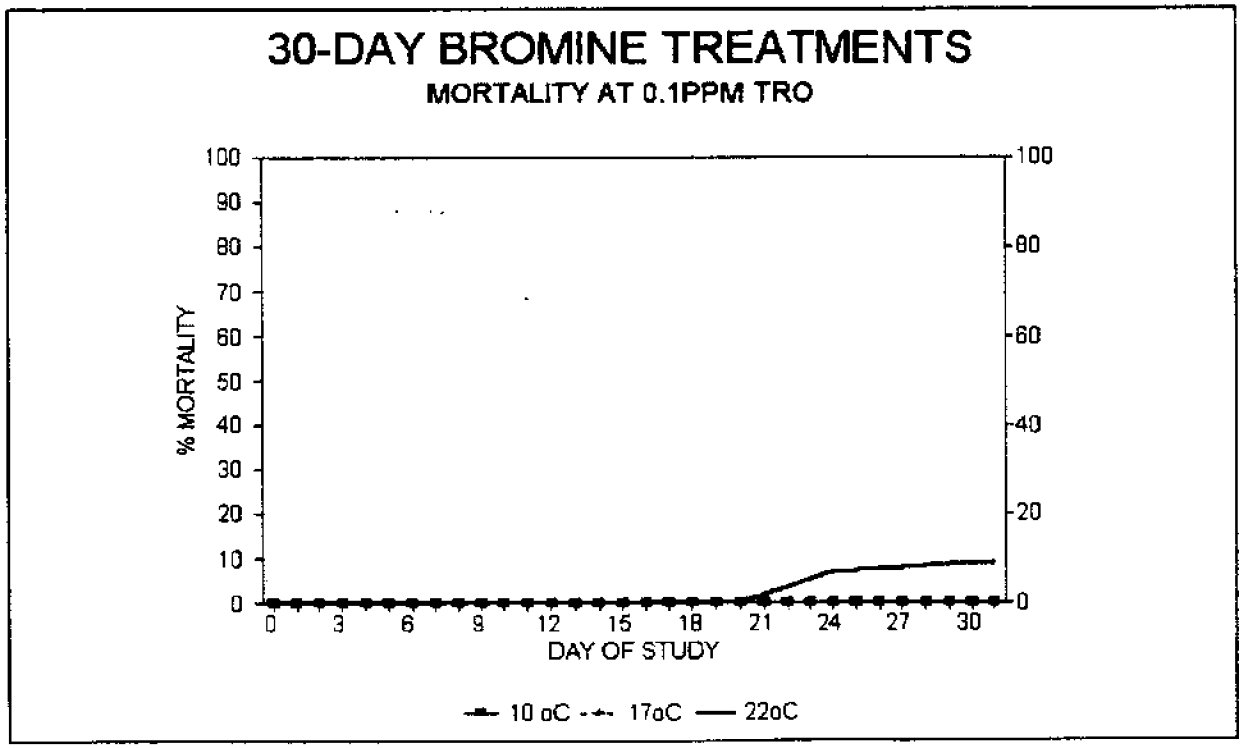

Figure 8

Control Strategy of Biofouling of Hydrotechnical Constructions by *Dreissena* (*Dreissena polymorpha* Pall., *D. bugensis* Andr.)

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During recent decades the geographical distribution of the bivalve mussels *Dreissena polymorpha* and in particular *D. bugensis* has increased. It is known that *D. polymorpha* is widespread in European reservoirs, and since the 1970s a northward expansion of this species has been observed. Currently, *D. bugensis* dominates in all reservoirs of the Dnieper cascade (Pligin, 1984). It has occupied canals in the southern Ukraine and reservoirs connected to the Dnieper River. In 1987 *D. bugensis* was found in the Severskiy Donets river (Azov Sea watershed), where it had penetrated from the Dnieperdzerzhinskoye reservoir, located in the river Dnieper, through the canal Dnieper-Donbass. In 1989-1991 *D. bugensis* became abundant in Dniester reservoir, constructed in the middle current of the Dniester River. This species has also appeared in the Laurentian Great Lakes in America (Rosenberg G., Ludyanskiy M., 1993).

Both *Dreissena* species, despite some biological and ecological differences, have similar functional features. Being active filterers-sedimenters, they diminish the anthropogenic load to reservoirs by increasing the sedimentation of particulate matter, including organic substances and heavy metals, thereby improving water quality.

At the same time *Dreissena*'s presence in reservoirs causes a complex of problems related to their tendency to colonize hard substrates used in hydrotechnical constructions. The most significant problems are related to the colonization of water pipelines. *Dreissena* fouling leads to a narrowing of the inner diameter of pipelines, resulting in a decrease in water delivery rate. As a result of such biofouling electric energy expenses required to maintain standard volumes of water delivery are increased.

Disturbances caused by *Dreissena* can be faced by pump stations and different enterprises using nonpurified water. Under the impact of biofouling the deterioration of hydrotechnical construction materials is speeded up. The corrosion rate of

unprotected steel structures under the influence of biofouling can increase 4 times, and the structure of concrete can also be changed. Following a sharp decrease of the water level in reservoirs mass die-offs of mussels can occur, leading to a deterioration of water quality and the appearance of strong odors.

Numerous studies have been devoted to *Dreissena*'s biology and methods of preventing biofouling. I propose a general strategy to deal with *Dreissena* fouling in hydrotechnical constructions (Table 1).

An example of such a strategy can be found in works carried out during the construction of water transport pipelines from the newly-built Krasnopavlovskoye reservoir in the canal Dnieper-Donbass (Table 2) to the City of Charkov, and the construction of pipelines for water-use in small settlements on the Dneister River.

It was known that in Dnieperdzerzhinskoye reservoir, leading to the Dnieper-Donbass Canal, and in the canal itself, significant populations of *D. bugensis* and *D. polymorpha* existed. Experts predicted that *Dreissena* would colonize all hard substrates in Krasnopavlovskoye Reservoir. Mollusk densities were anticipated to reach 100,000/m^2; the biomass was expected to reach 2000 g/m^2. Serious pipeline fouling was expected.

Data on reproductive timing, veliger dynamics, and growth rates obtained in similar water bodies were applied to this situation. In this reservoir *Dreissena*'s reproduction was expected to take place within the time period of April-October and within a temperature range of 12-23°C (Table 3). Expected veliger dynamics (with three peaks) and growth rates of newly settled mussels were expected to correspond to growth curves shown in Figures 1 and 2.

Studies of pipeline technical characteristics and water properties allowed us to make recommendations. These included constructing water intakes at a depth of 12-15 m, where *Dreissena* larvae are less abundant. We recommended that initial water chlorination levels range from 4-6 mg/l, so that the output water chlorination level would not be less than 0.5 mg/l. On the basis of biological and technical parameters chemical control programs must be implemented annually in June, August and October for 7-8 days.

These recommendations were included in the technical project design. Water pipelines were completed in 1988-1989, and fouling has not been observed.

For solving the problem of water intake protection in the Dniester River periodic chlorination was recommended, but the pipeline water purification system required technological modification. In that case chlorination of the water pipeline should not be conducted more than 2 times a year using a significantly larger dose of chlorine (10-17 mg/l) to reach the final concentration of 5 mg/l when the water gets to the water treatment plant. In this case the pipeline treatment has to occur over a 24-hour period. The use of mobile chlorinators has been recommended.

Another problem is the removal of accumulated masses of fouled *Dreissena*. During the 1960s and 1970s in the Ukraine a large network of underground irrigation pipelines was built without any protection from biofouling. As a result unpredictable

fouling has occurred, decreasing the water-carrying capacity of these systems, and leading to the blockage of irrigation devices.

Several control methods were unacceptable due to the toxicity of many ingredients to soil and agricultural crops. In addition, during the irrigation season it was impossible to turn off separate plots. Therefore an immediate need developed for controlling *Dreissena* in irrigation systems. Such a method needed to remove *Dreissena* without being toxic to the plants, without breaking the regime of irrigation for agricultural crops, and it had to be available for organizations serving irrigation systems.

In laboratory conditions the toxic action of different solutions of mineral fertilizers on *Dreissena* was tested. Finally, ammonia nitrate in a concentration of 300-600 mg/l was selected as an effective control method. This method of *Dreissena* control in irrigation systems has been patented. In Table 4 data from experimental systems, using this new control method, is shown. At the present time this technique is widely applied in Ukraine irrigation systems. Controlling *Dreissena*'s fouling in water systems, if not planned for ahead of time, presents many difficulties and requires additional expenses. Therefore, it is important to develop predictive techniques and practical control recommendations at the planning stage of hydrotechnical constructions.

Table 1. A strategy for the control of mollusks.

Initial data

I. Biological
1. *Dreissena* monitoring:
- spread in the reservoirs and constructions;
- reproductive season;
- veliger dynamics;
- growth rate.
2. Prediction of expected biodisturbance in the hydroconstructions.
3. The determination of the control measurement - nature and terms.

II. Technical
1. Data to be analyzed:
- hydroconstruction characteristics;
- some water quality indices;
- requirements for the exploitation service.
2. Analysis of the current control methods: physical, chemical, biological and mixed.
3. Choice of known control methods or development of a new one.

III. Development of control technology and recommendations to be used.
IV. Putting into practice.
V. Results.

Table 2. Development and putting into practice the control biofouling technology for the water pipelines in the drinking water purification station (Charkov City population - 1.5 million).

Initial data

I. Biological

1. Monitoring results:
   - the presence of *Dreissena polymorpha* and *Dreissena bugensis*;
   - the maximum density - 9600 ind/m^2;
   - the largest biomass - 3.4 kg/m^2;
   - reproduction season - April-October;
   - three veliger density peaks - June, July, August, September;
   - growth rate - max. length of the juveniles - 12 mm (October)

2. Prognosis:
   - probability that water pipe lines will be fouled by Dreissena;
   - essential biodisturbances.

3. The determination of the control measurement's nature and terms;
   - periodical character;
   - recommended terms - June, August, October.

II. Technical

1. Data to be analyzed:
   - the drinking water pipelines nature 1-22 km, d-1400-1600 mm, Q-10 m^3/dec;
   - a chlorine treatment station has been planned;
   - some water quality indexes in summer - t - 18-23°C, hardness - 350-480 mg/l, solidity - 4.1 eq/l, uptake of chlorine during two hours by water - 1.5-3.5 mg;

2. Analysis of the current control methods.

3. Choice of the control method:
   - device layer-by-layer, deeper intakes;
   - chlorination.

III. Technology

1. To take water samples from the depths to be suggested of 12-15 m - from May to October.
2. To conduct water chlorination in the water intake structures - annually, during 7-8 days in June, August, October.

IV. Putting into practice

1982 - proposing control measures for the project;
1988 - 1989 - implementing control measures.

V. Results

Biodisturbances have not been observed.

Table 3. Dreissena reproduction periods in water-bodies.

Water-body	Months								Author
	IV	V	VI	VII	VIII	IX	X	XI	
Water-body Kegums (watershed of Daugava river) 57° N.L.	-	+	*	*	+	+	+	+	Kachalova O., Sloka N., 1964
Uchinskoye 55.4° N.L.			+	*	*	+			Kachanova A., 1961
Kujbyeshevskoye 54° N.L.	-	-	+	*	*	+	-	+	Kirpichenko M., 1965
Zaporozskoye 48° N.L.	-	+	+	*	*	+	+	-	Dyga A., 1975
Canals Ukraina 45-46° N.L.	+	+	*	*	*	+	+	+	Shevtosova L., 1991

+ = single; * = mass; - = absent

Table 4. Development and putting into practice the control biofouling technology for the the irrigation water piping system in the south of Ukraine.

Initial data

I. Biological

1. Mussel's monitoring results:
   - the presence of Dreissena polymorpha and Dreissena bugensis; in irrigation water intakes in the Kachovskiy canal; separate local canal settlements many-cirded at the irrigation water piping;
   - the maximum density-10000-50000 ind/m^2;
   - the largest biomass - 1-1.5 kg/m^2;
   - reproduction season - April-October;
   - two veligers' density peaks - July, August.
2. Forecast:
   - forecast was asbent.
3. The defermination of the control measurement's nature and terms:
   - periodic character;
   - recommended terms - April, September, October

II. Technical

1. Data to be analized:
   - the irrigation water piping's nature 1-12km, d - 600 mm, working time-periodically fro April to October;
   - exploitation service's requirements:
     a) to keep standards of the field - watering;
     b) to restrict mechanical fragments quantity on the watering machines filters.
2. Analysis of the current control methods.
3. Choice of the control methods:
   - speed regime;
   - hermetization.
4. Development of modern methods:
   - experimental investigation of the mineral fertilizers, toxical influences on both Dreissena polymorpha and Dreissena bugensis (choice NH_4NO_3).

III. Suggested technology

1. Wash off irrigation of pipe lines by clean water - April.
2. Introduction NH4NO3 in dose 600 mg/l (for old attached colonies) or 400-500 mg/l (preserving measure) with following hermetization of the apparatus for 7 days.
3. Wash off irrigation of pipelines by water three times

IV. Putting into practice

1990 - 1992 - Putting into practice in irrigation systems in the South of Ukraine.

V. Results

Irrigation water piping system is without any biofoulers.

Figure 1

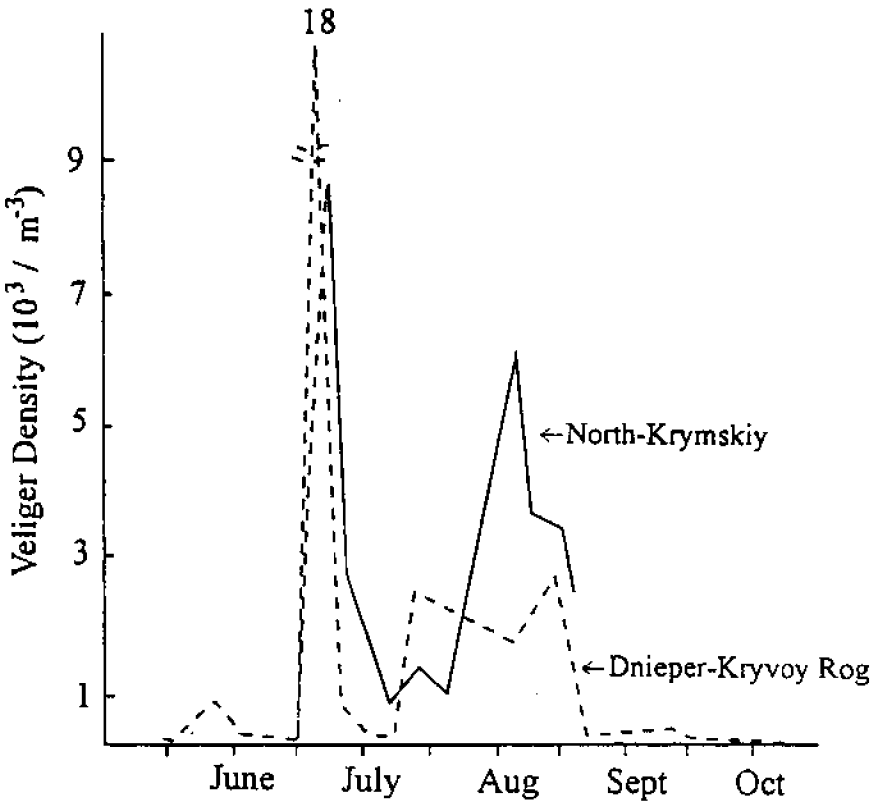
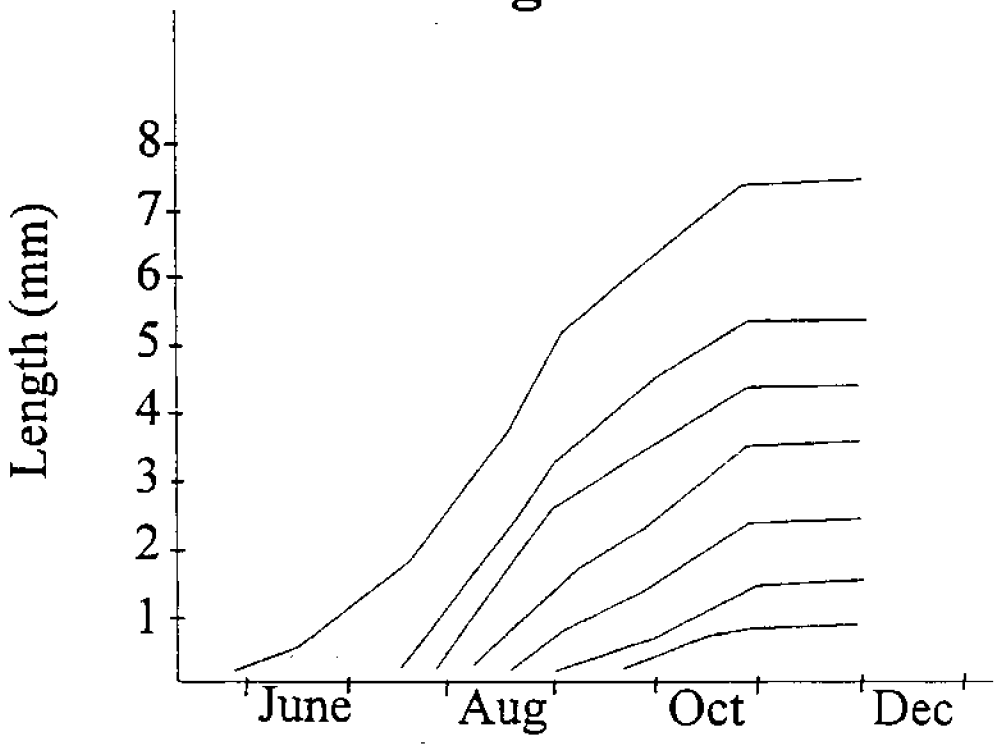

18
9
7
5
3
1
Veliger Density (10^3 / m^{-3})
←North-Krymskiy
←Dnieper-Kryvoy Rog
June
July
Aug
Sept
Oct

Figure 2



4th International Zebra Mussel Conference '94 Proceedings

CONTROL: NON-CHEMICAL

Influence of Wide-Range Ultraviolet Radiation upon Behavior and Mortality of *Dreissena polymorpha*

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[2] General Dynamics, Electric Boat Division

Abstract

Our previous work with low power UV sources has demonstrated the sensitivity of *Dreissena polymorpha* veligers to mid-range ultraviolet radiation (UV-B); however, adults are more resistant to UV-B exposure, requiring weeks of exposure to affect mortality. These earlier studies were performed using a relatively low power UV source (Westinghouse FS40 fluorescent lamps). The current study used a xenon arc lamp (500 watt) capable of delivering much higher levels of UV radiation over a greater portion of the UV spectrum. Various life stages of *D. polymorpha* (veligers, post-veligers, young adults, and adults) were exposed to one of three ranges of radiation:

white light + UV-A (320-400 nm);
white light + UV-A + UV-B (280-320 nm); and
white light + UV-A + UV-B + UV-C (radiation < 280 nm).

Our purpose was two-fold: first, we determined the total fluence needed to kill the various life stages of the mussel. Secondly, we studied the behavior of individual mussels of all age classes following exposure to radiation; in other words, would ultraviolet radiation stun individuals and thereby inhibit movement and settlement?

Veliger and post-veliger larvae are extremely sensitive to even short exposures of wide-range ultraviolet. Without exception, exposed larvae ceased all swimming or crawling motions following exposure to wide-range ultraviolet and UV-B radiation. In contrast, neither white light nor UV-A induced behavioral changes. Wide-range ultraviolet and UV-B were both effective in inducing 100% mortality in nearly every instance. Young adult and adult mussels are more resistant as their shells are opaque, yet they also can be killed if exposure periods to wide-range and UV-B radiation are long enough.

Introduction

Since 1986, when zebra mussels *(Dreissena polymorpha)* were first found in Lake St. Clair, they have colonized all of Lake Erie and spread to adjacent waters. Most larvae are born in July and August and settle in August and September, usually becoming reproductive adults the following year (Mackie, 1991). During the transition from a pelagic to a benthic habitat, *Dreissena* suffers a high mortality rate (Lewandowsky, 1983). Presumably, the physiological stress of this metamorphosis increases the organism's sensitivity to environmental stress. When the postveliger larvae settle, they secrete strong byssal threads which firmly attach the mussels to the substrate. If they settle in a suboptimal area, juvenile mussels can use their byssal threads as a means to climb over objects and as a means to drift. Although more sessile, adults can also dissolve old threads and use new ones to assist their movement (Griffiths *et al.*, 1991). Thus, even settled mussels can leave an area that has become less hospitable.

In response to the increasing fouling problem caused by *D. polymorpha*, various methods of control have been attempted with limited success. The critical point of preventative control is the settling stage of the planktonic larvae. Once settled, the mussels are difficult and expensive to remove from intake pipes and other structures. Prevention of settling would be the easiest and most cost-effective mechanism to remediate zebra mussel fouling problems.

A possible control method, which has shown promise in our recent studies, is exposure of the larvae to ultraviolet radiation. We have determined that broad spectrum ultraviolet radiation, consisting of UV-B (280-320 nm) wavelengths and some of the longer UV-C (< 280 nm) wavelengths, has a major negative impact upon movement and survival of *D. polymorpha*.

Biological Effectiveness of Ultraviolet Radiation

Concern over depletion of the earth's ozone layer has resulted in a great deal of information being gathered on the effects of UV-B radiation upon aquatic organisms, especially planktonic species. There is increasing evidence that naturally occurring UV-B is an important limiting ecological factor in zooplankton habitats. In particular, zooplankton exposed to UV-B typically show changes in movement patterns and/or physiological functions. Young larval stages are most susceptible to this type of radiation.

There is an inverse relationship between water transmissibility and biological effectiveness of ultraviolet radiation. UV-A (320-400 nm) is transmitted through water the most effectively, but is generally not associated with direct damage to biological systems. UV-B is also water-transmissible and has negative effects on aquatic organisms (reviewed in Hader *et al.*, 1989). Both UV-B and UV-C are lethal to living cells, as these wavelengths are absorbed by proteins and nucleic acids. Although

UV-C is more biologically active, it is also absorbed more completely by water and is therefore not a realistic method of control by itself (since it has little effect on aquatic organisms not in the immediate proximity of the source). Conversely, UV-B can penetrate through several meters of water but requires longer exposures to be effective. Broad spectrum ultraviolet, consisting of UV-B wavelengths and the longer UV-C wavelengths (near 280 nm), would theoretically have a major negative impact upon aquatic organisms due to the combination of high biological activity and good water transmittance.

Previous Studies with Ultraviolet Radiation

Ultraviolet radiation is proposed as a possible method of zebra mussel control, as previous studies in natural systems have found UV-B to be lethal to a variety of aquatic organisms. With few exceptions (Chalker-Scott, 1994), exposure to increased levels of UV-B results in increased mortality of plankton (Hader *et al.*, 1989; Scott, 1982; Worrest *et al.*, 1981). UV-B tolerant organisms often are highly pigmented (Chalker-Scott, 1994; Hader *et al.*, 1989), which is not true of *D. polymorpha* veligers. In a natural habitat, *Dreissena* larvae and other planktonic organisms could escape harmful radiation by changing their position in the water column. In a restricted area (such as a water intake pipe), however, escape from artificially-supplied ultraviolet radiation would be unlikely.

UV exposure has proven 100% effective in preventing settlement of barnacle and other larvae on transparent pipes in salt water systems (Plotner, 1968). As mentioned previously, the process of settling has a high mortality cost associated with it. This factor could be exploited by increasing environmental stress (i.e. adding ultraviolet radiation) and thereby killing or repelling the sensitive settling stages. In a biofouling study of marine *Chthamalus* larvae, mortality rates associated with ecdysis (metamorphosis) were increased following UV exposure (Hori *et al.*, 1990).

UV radiation is probably even more biologically effective in fresh water, where concentrations of dissolved UV-absorbing minerals are less. We have tested *Dreissena polymorpha* for its sensitivity to UV-B in the laboratory; like other most other planktonic species, the larvae are not resistant to this radiation (Chalker-Scott *et al.*, 1993). We also observed that larvae stop moving for a period of time after exposure to sublethal doses of UV-B. This observation suggested that prevention of accumulation of zebra mussels within restricted areas could be accomplished by one of at least two methods:

1) Supplying enough UV radiation to kill any juvenile zebra mussels which settle in or pass through the irradiated region; and/or

2) "Stunning" the larvae with enough UV radiation to inhibit movement and settlement, thereby allowing larvae to be swept through the area of interest.

Although veliger larvae of *D. polymorpha* are sensitive to low dosages of mid-range ultraviolet (UV-B), adults can survive radiation levels far above those that are lethal to the larvae (Chalker-Scott *et al.*, 1993). By virtue of their opaque shells, the adults are resistant to UV radiation; however, they must open their shells to feed and thus expose more sensitive tissues to environmental conditions (i.e. ultraviolet radiation). Presumably, irradiated adults are killed either by accumulating lethal doses of UV during feeding, or by starvation if they remain shut.

A major problem associated with our previous UV-B experiments was the use of low pressure mercury fluorescent bulbs as the radiation source. These bulbs are low wattage and therefore require relatively long exposure times to have significant effects on *Dreissena* mortality (Chalker-Scott *et al.*, 1993). This is a good system for decreasing the settling of mussels on specific surfaces, and for increasing avoidance behavior of particular areas, but is not satisfactory for situations requiring sterilization: in other words, 100% mortality. Only by using these bulbs in multi-arrays (as is currently being done by some companies) can biologically effective dosages be obtained in a practical setting, but the size and expense of the unit can be prohibitive in many instances.

In this paper, we establish the success of broad spectrum ultraviolet in killing all life stages of *Dreissena polymorpha* in the lab. Theoretically, UV treatment could kill all planktonic life given adequate exposure times.

Materials and Methods

During the summer months of 1993, we collected all life stages of *D. polymorpha* from Lake Erie. Veligers, post-veligers and young adults were found in the water column and were captured using a 135 μ mesh plankton net. Adults were collected from the walls surrounding the Black Rock locks in Buffalo, New York. All mussels were placed in aerated glass aquaria and briefly acclimated (24 hours) to laboratory conditions under natural temperature (20°C) and day length (14 hr light/10 hr dark) conditions, and supplied with natural phytoplankton cultures.

Adult Susceptibility to Ultraviolet Radiation

Following acclimation, groups of 10 adult mussels of similar size (approximately 4 cm) were placed in a 1000 ml graduated cylinder filled with filtered lakewater. The depths of air and water separating the mussels from the source were approximately 90 and 37 cm, respectively. The cylinder was placed into a test chamber, and mussels were exposed for various times to unfiltered UV radiation via a 500 watt xenon arc lamp (Photon Technologies). The fluence rate measured at the water surface with a spectroradiometer (Model IL-790, International Light, Inc.) was 8,180 $\mu W \cdot cm^{-2}$.

Mussels were removed after radiation treatment and held in aerated culture dishes with fresh filtered lake water for one week to determine percent mortality.

Determination of Ultraviolet Exposure Time for Juveniles

Following acclimation, sets of ten veligers were transferred to small petri dishes containing 10 ml filtered lake water and placed on a turntable in the test chamber. Dishes were covered with either Mylar (control) or cellulose acetate (partial UV) filters, or left uncovered. Tests were performed at two different distances from the source (18 and 102 cm) for varying time periods (1-5 minutes and 5-20 minutes respectively). UV radiation was delivered using the 500 watt xenon arc lamp described previously. Fluence rates for all treatments were as follows:

	Fluence rates ($\mu W \cdot cm^{-2}$)	
	18 cm distance	102 cm distance
Mylar	245	28
Cellulose acetate	1,867	212
Unfiltered	10,225	1,160

Following exposure, the dishes were returned to acclimation conditions for 24 hours. Individuals were then examined under the dissection scope and percent mortality was determined.

Effect of Ultraviolet Radiation on Larval Behavior

Following acclimation, sets of ten planktonic individuals (veligers, post-veligers, and young adults) were transferred to small petri dishes containing 10 ml filtered lake water and placed under a dissection scope. After mussels began their normal swimming or crawling movements, they were exposed to brief intervals (1-5 seconds) of UV radiation using the 500 watt xenon arc lamp described previously. Wavelengths were controlled using a variety of filters: Mylar (visible and UV-A), cellulose acetate (visible, UV-A and some UV-B), Acrylite OP-4 (visible, UV-A, UV-B and some UV-C), or no filter (visible, UV-A, UV-B, and UV-C) (Figure 1). Respective fluence rates under these filters were 120, 915, 3,210, and 5,010 $\mu W \cdot cm^{-2}$. During UV exposure and post-treatment, individuals were filmed with a video camera attached to the dissection scope ocular tube. After exposure, the individuals were visually monitored under the scope for 20 minutes to determine changes in behavior.

Effect of Ultraviolet Radiation on Larval Mortality

Following the 20 minute observation interval, the exposed individuals were allowed to incubate for 24 hours under acclimation conditions. Individuals were examined under the dissection scope and percent mortality was determined.

Results and Discussion

Adult Susceptibility to Ultraviolet Radiation

In contrast to our previous studies (Chalker-Scott *et al.*, 1993), adult zebra mussels were killed in a matter of hours rather than weeks (Figure 2). Radiation was attenuated through the water column, meaning that the exposure to the mussels was somewhat less. During exposure, mussels remained closed, presumably to avoid radiation damage to the soft tissues. While the opaque shells may have afforded some protection, enough radiation reached soft tissues to allow lethal damage to accumulate internally.

Determination of Ultraviolet Exposure Time for Juveniles

Veliger larvae exposed to unfiltered, broad spectrum ultraviolet radiation were uniformly killed after a few minutes of exposure (Figures 3 and 4). The lowest dose received under these conditions was 0.35 $J \cdot cm^{-2}$. In contrast, doses as high as 0.56 $J \cdot cm^{-2}$ under cellulose acetate filters were not as effective, as only a 60-70% mortality rate was found. Presumably, veligers under cellulose acetate filters were more protected, as no UV-C and little UV-B is transmitted; mortality increased with decreased distance from the radiation source (compare Figures 3 and 4). Veligers under Mylar filters were least affected by exposure (Figure 3), receiving only a maximum of 0.074 $J \cdot cm^{-2}$. Some mortality was present (10-20%), especially at closer proximity to the source (Figure 4).

Effect of Ultraviolet Radiation on Larval Behavior

Video tapes were analyzed to relate exposure time with behavioral changes (such as immobility). In previous work, behavior of barnacle larvae (rate and pattern of beating by appendages) was used to assess UV radiation damage (Hori *et al*, 1990). Such information can be used in conjunction with flow rates to determine ultraviolet dosages needed to prevent larvae from settling and allow them to exit the area of interest.

Without exception, veligers exposed for as little as one second to unfiltered UV or under the Acrylite OP-4 filter immediately ceased all swimming movements.

Although internal movements were still apparent, swimming movements did not resume even after 20 minutes of observation. The cessation of swimming was less consistent in those larvae protected by the cellulose acetate filter; although most exposed larvae would initially close their velums, many would resume movement within 5-10 minutes. Those that did resume swimming, however, often exhibited unusual behaviors (e.g. erratic jerks, swimming in circles) that had not been noticed previous to exposure. This would suggest that some damage had been imparted by the ultraviolet radiation (Hori *et al*, 1990). The movement of veligers under Mylar filters was unaffected by even longer exposures (5 seconds) of ultraviolet; this is important to note as it suggests that high intensity visible light does not inhibit veliger larvae.

Similar inhibition of movement was seen with post-veliger larvae exposed to 1-5 seconds of unfiltered UV or under the Acrylite OP-4 filter; the initial crawling motion would cease as the foot was retracted. Again internal movements were still apparent, but crawling did not resume even after 20 minutes of observation. Like the veliger larvae, the movements of post-veligers were less affected by the radiation passing through the cellulose acetate filter, and were uninterrupted by the radiation passing through the Mylar filter.

Young adults found in the plankton tows were also subjected to these same conditions. They appeared to be less affected by the radiation, usually withdrawing their foot but then resuming movement shortly after exposure ceased. These mussels, although small, already had an opaque shell and were presumably more protected from acute levels of ultraviolet radiation.

Effect of Ultraviolet Radiation on Larval Mortality

We were dissatisfied with our preliminary analysis of cellulose acetate as a UV-B transmitting filter (Figure 1). This was emphasized by the results found during our determination of exposure time. Transmittance of UV-B and longer wavelengths of UV-C was greatly increased using the Acrylite OP-4 filter (Figure 1). Radiation in the vicinity of 280 nm is particularly effective as this is the region where proteins absorb maximally. The UV-B radiation which transmits through the OP-4 filter is approximately 80% of that found in unfiltered conditions (Figure 5). In contrast, cellulose acetate and Mylar respectively transmit only 24% and 3% of the available UV-B radiation (Figure 5).

The amount of UV-C present does not seem to be as critical as the amount of UV-B in determining mortality rates; this is probably due to the poor transmittance of short wavelengths through water. The OP-4 filter only transmits 14% of the available UV-C (Figure 5), yet it is nearly as effective in killing the veligers as the unfiltered lamp (Figure 6). (It should be noted that although only a 90% mortality rate was found under the Acrylite OP-4 filter 24 hours post-exposure, 100% mortality was

found 24 hours later). Cellulose acetate is much less effective as we generally obtained a mortality of about 20% (Figure 6).

As we had previously reported (Chalker-Scott *et al.*, 1993), the older life stages of *D. polymorpha* are more resistant to acute dosages of ultraviolet radiation as their shells absorb much of these wavelengths. Veliger and post-veliger larvae were uniformly killed following a brief (5 second) exposure to ultraviolet radiation filtered through the OP-4 plastic, but young adults were unaffected (Figure 7). Longer exposure times, however, will result in the death of even mature adults (Figure 1).

Conclusions

Results obtained using various filters suggest that UV-B and longer wavelength UV-C radiation are the most effective wavelengths for killing *D. polymorpha*. Shorter wavelength UV-C, while undoubtedly biologically harmful, is too strongly absorbed by water to be of practical use. Lamps which emit a high percentage of energy between 280-320 nm should be the most effective in zebra mussel control. With high energy lamps, such as the one used in the current study, relatively short periods of exposure are required for killing all life stages of mussels, as opposed to the lower energy fluorescent bulbs previously used.

The use of ultraviolet radiation as a control for zebra mussel settlement is potentially superior to chemical control methods because it is non-polluting. It does not affect any of the biogeochemical cycles which are critical to the health of any ecosystem. It is not labor intensive to operate and after installation would require only routine maintenance. Most of the costs associated with such a unit would be in the initial purchase and installation, then occasional replacement of the UV source. Human health risks are minimized by the use of protective goggles, clothing, and sunscreen for those who would contact the unit (i.e. installation and maintenance personnel). Finally, its practicality is high as ultraviolet radiation is deadly to all life forms and would therefore have ubiquitous, lethal effects on all planktonic species. We would anticipate complete success in preventing zebra mussel fouling in any aquatic area (e.g. holding tanks or intake pipes) that does not allow *Dreissena* to escape environmental stress.

Literature Cited

Chalker-Scott, L. 1994. Survival, fecundity and F1 sex ratios of the marine copepod *Tigriopus californicus* following ultraviolet-B (290-230 nm) radiation. *Mar. Biol.* (in revision).

Chalker-Scott, L., J.D. Scott, R. Carnevale, and K. Smith. 1993. Comparison of acute and chronic mid-range ultraviolet radiation (280-320 nm) effects on adult zebra mussels, pp. 4:149-161. *In*: J.L. Tsou (ed.), Proceedings from the Third International Zebra Mussel Conference, Toronto, Ontario. Electric Power Research Institute.

Griffiths, R.W., D.W. Schloesser, J.H. Leach, and W.P. Kovalak. 1991. Distribution and dispersal of the zebra mussel (*Dreissena polymorpha*) in the Great Lakes region. *Can. J. Fish. Aq. Sci.* 48:1381-1388.

Hader, D.-P., R.C. Worrest, and H.D. Kumar. 1989. Aquatic Ecosystems. In: United Nations Environment Programme, Environmental Effects Panel Report (pursuant to Article 6 of the Montreal Protocol on Substances that Deplete the Ozone Layer), pp. 39-48.

Hallegraeff, G.M. and C.J. Bolch. 1992. Transport of diatom and dinoflagellate resting spores in ships' ballast water: implications for plankton biogeography and aquaculture. *J. Plankton Res.* 14:1067-1084.

Lewandowsky, K. 1983. The role of early developmental stages in the dynamics of *Dreissena polymorpha* (Bivalvia) populations in lakes. 2. Settling of larvae and dynamics of numbers of settle individuals. *Ekol. Pol.* 30:223-286.

Mackie, G.L. 1991. Biology of the exotic zebra mussel, *Dreissena polymorpha*, in relation to native bivalves and its potential impact in Lake St. Clair. *Hydrobiologia* 219: 251-268.

Plotner, R. 1968. Sensor fouling on deep submergence vehicles, pp. 267-295. In: Marine Sciences Instrumentation. Proc. Fourth Natl. Marine Sci. Symp., Vol. 4. Plenum Press, NY.

Scott, J.D. 1982. Physiological responses of the marine phytoplankton *Dunaliella tertiolecta* to UV-B enhanced solar radiation. Master's Thesis. Oregon State University, Corvallis, OR, 34 pp.

Worrest, R.C., K.U. Wolniakowski, J.D. Scott, D.L. Brooker, B.E. Thomson and H. Van Dyke. 1981. Sensitivity of marine phytoplankton to UV-B radiation: impact upon a model ecosystem. *Photochem. Photobiol.* 33(2): 223-228.

ACKNOWLEDGEMENT

This paper is a result of research funded by the National Oceanic and Atmospheric Administration award #NA26RG0401-01 to the Research Foundation of State University of New York for the New York Sea Grant Institute. The U.S. Government is authorized to produce and distribute reprints for governmental purposes notwithstanding any copyright notation that may appear hereon. The views expressed herein are those of the author(s) and do not necessarily reflect the views of NOAA or any of its sub-agencies.

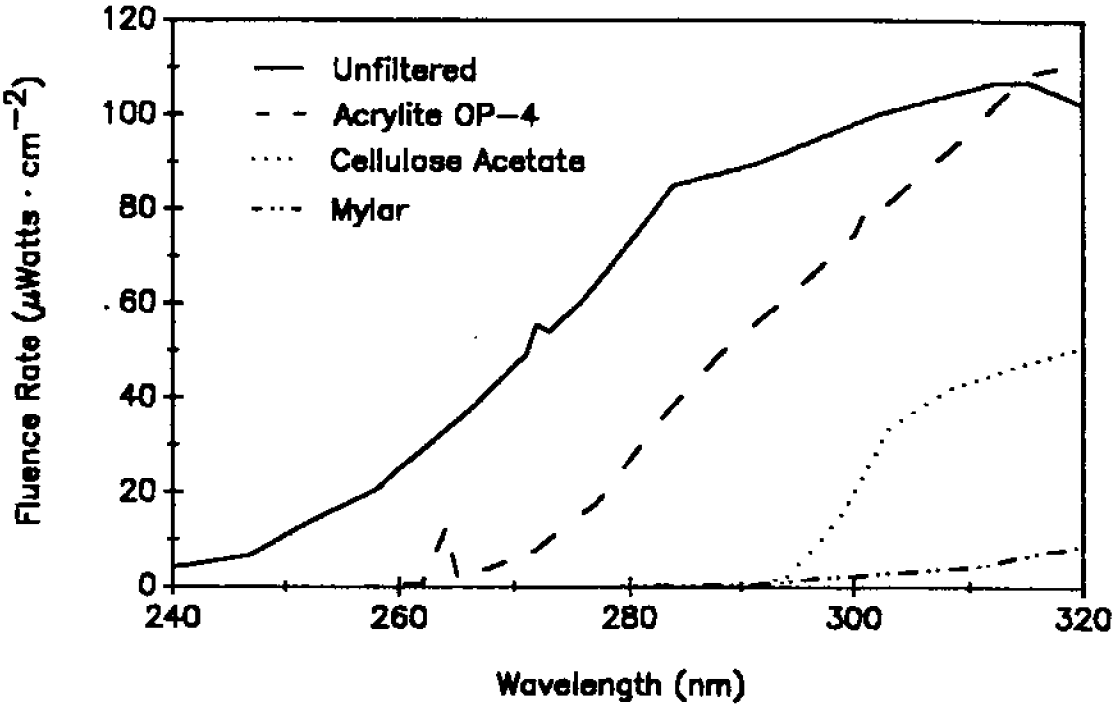

Figure 1. Broad-band UV spectrum of the 500 watt xenon arc lamp under filtered or unfiltered conditions.

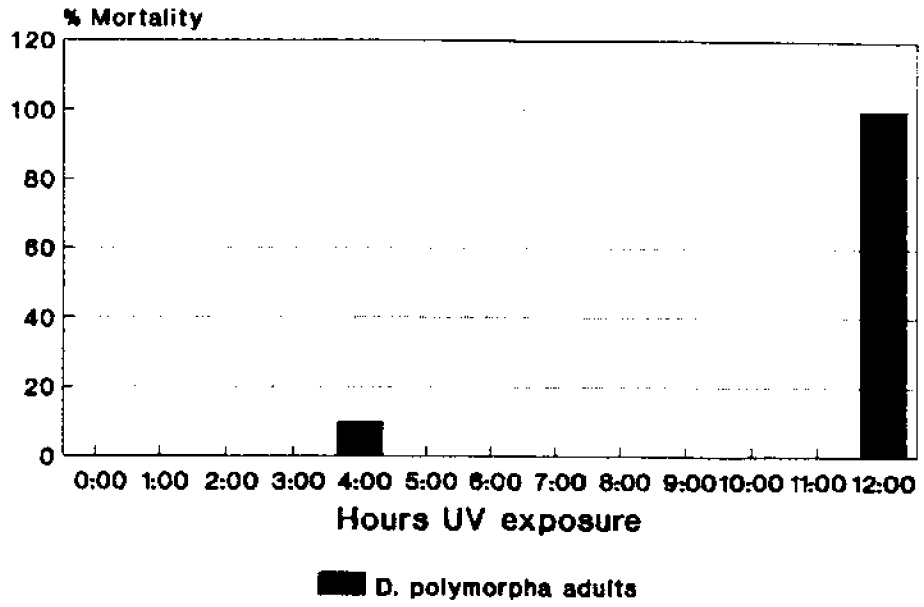

Figure 2. Percent mortality of adult *D. polymorpha* one week after exposure to broad spectrum UV radiation. Mussels were exposed for 0, 4, or 12 hours. Total radiation doses at the water surface during the 4 and 12 hour exposures were 118 and 354 $J \cdot cm^{-2}$, respectively.

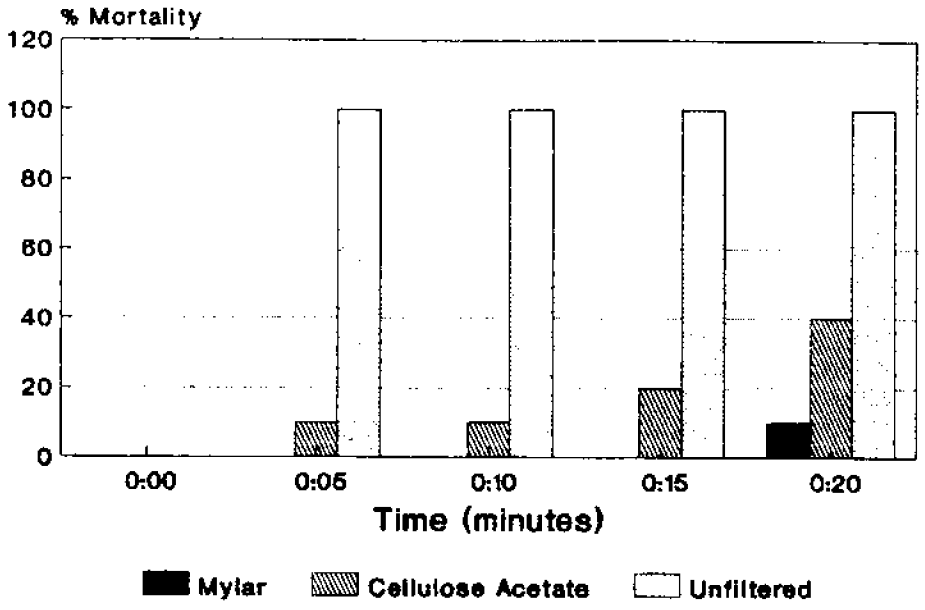

Figure 3. Percent mortality of *D. polymorpha* veligers 24 hours after exposure to UV-A (Mylar), UV-A + UV-B (cellulose acetate), or broad spectrum (unfiltered) UV radiation. Veligers were located 102 cm below the radiation source. (Total doses were calculated using the fluence rates found under each filter, multiplied by total seconds of exposure.)

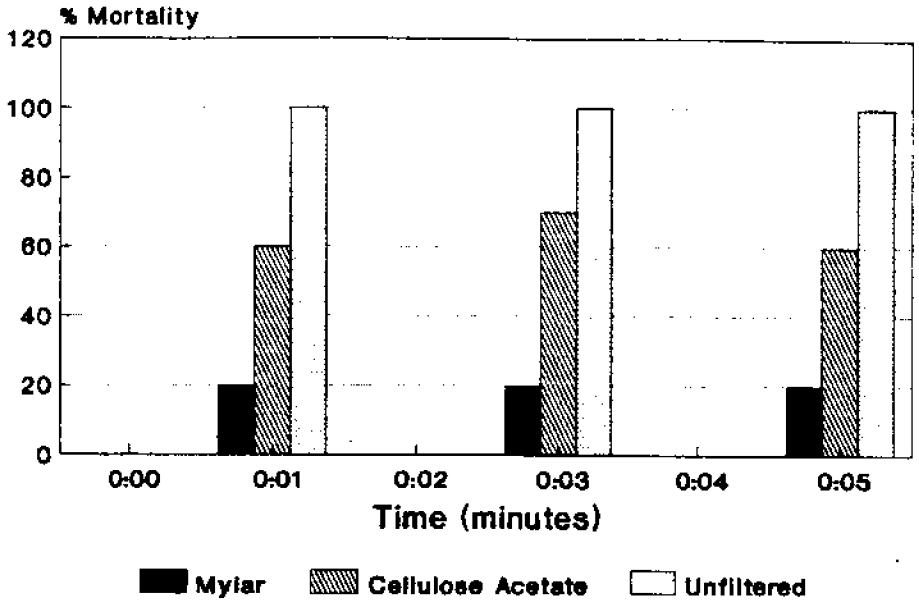

Figure 4. Percent mortality of *D. polymorpha* veligers 24 hours after exposure to UV-A (Mylar), UV-A + UV-B (cellulose acetate), or broad spectrum (unfiltered) UV radiation. Veligers were located 18 cm below the radiation source. (Total doses were calculated using the fluence rates found under each filter, multiplied by total seconds of exposure.)

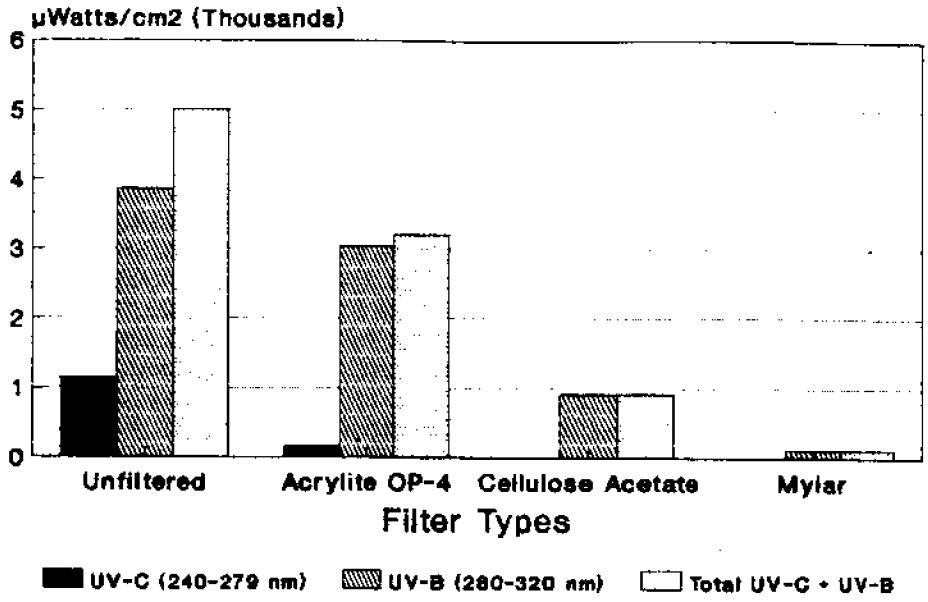

Figure 5. Relative UV-A, UV-B and UV-C transmission through various filters.

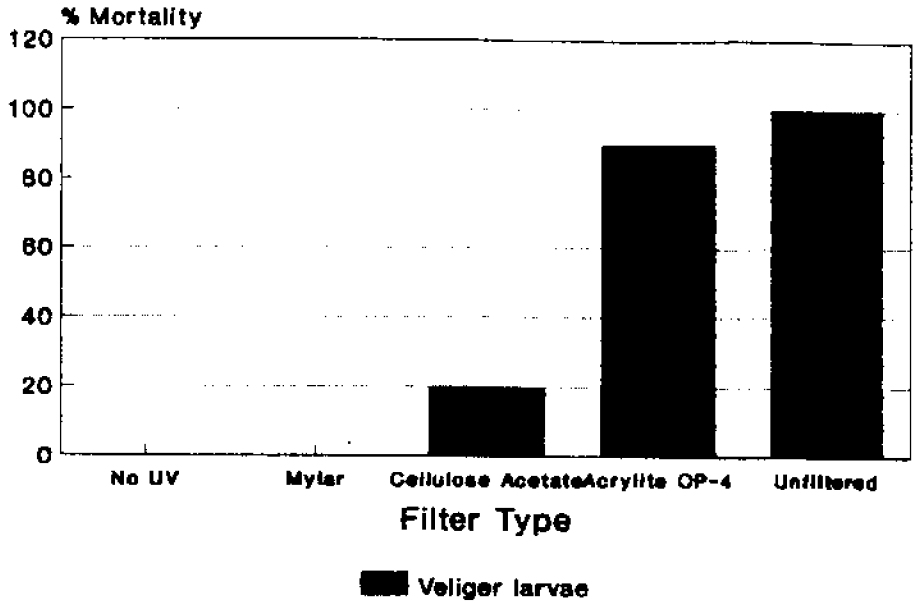

Figure 6. Percent mortality of *D. polymorpha* veligers 24 hours after exposure to UV-A (Mylar), UV-A + some UV-B (cellulose acetate), UV-A, UV-B, and some UV-C (Acrylite OP-4) or broad spectrum (unfiltered) UV radiation. Total fluences were 600, 4,600, 16,000, and 25,000 $\mu J \cdot cm^{-2}$, respectively.

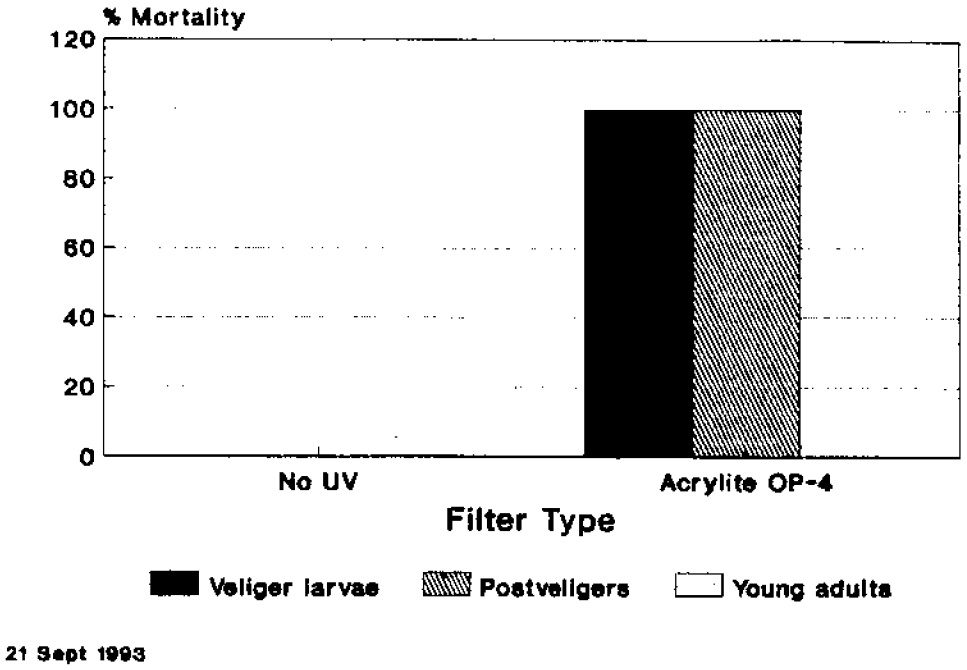

Figure 7. Percent mortality of *D. polymorpha* veligers, post-veligers, and young adults 24 hours after exposure to UV-A (Mylar) or UV-A, UV-B, and some UV-C (Acrylite OP-4). Young adults showed no mortality under either condition. Total fluences were 600 and 16,000 $\mu J \cdot cm^{-2}$, respectively.

New Infiltration Intake System for Zebra Mussel Control and Larval Exclusion

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ABSTRACT

The presence of matter in suspension form in the free water is becoming a major problem for installations withdrawing water from water bodies such as lakes, seas, and oceans.

The problem is growing fast as the demand for clean water is steadily increasing. Many remedial systems have been used such as extending intakes far offshore to subturbulent suspended matter-free depths or by constructing involved screening systems onshore which require skilled personnel for operation and maintenance. Elaborate pretreatment systems and filtration networks are employed in the fast growing reverse osmosis industry. To keep clean raw water flowing, some highly automated systems have been used. These require highly skilled personnel to operate and maintain them.

This paper presents a new concept for a filtration system with a uniquely engineered filter bed to keep zebra mussels, seaweed, and larvae from entraining into raw water systems.

INTRODUCTION

The increase of electrical energy consumption and the need for fresh water from the sea require newer, larger, and more efficient desalination and power plants. To fulfill the latter condition, these plants must have constant supply of water. To keep a constant uninterrupted supply of water, a suitable water body must exist in the vicinity of the plant site and a highly reliable water intake system must be constructed.

In this paper, withdrawal of clear and almost sediment-zebra mussel-seaweed-and larvae-free water from the sea is discussed.

Because of the growing costs of operation and maintenance of conventional seawater intake systems, which range from simple unreliable low availability onshore intake systems to advanced, sophisticated, complex systems that require high capital and running costs and skilled personnel for their operation and maintenance, simpler low-maintenance intake is required.

The quality of the water withdrawn from the sea is dictated by the purpose for which it is used. The quality is determined by the characteristics of the water body which in turn are determined by the geo-environmental state of the area in question.

Designers of intake systems face problems ranging from seaweeds and algae, to fish and shells, other organisms and suspended matter.

The disposal of phosphate and nitrate pollutants from sea and land-based sources contributes to the rapid expansion of seaweed infested areas. Finding a proper site for installing an intake system is becoming a major task. Locating a suitable site near a certain location, keeping the costs within feasible limits, and providing high reliability with maximum availability have made the design of an intake system a very difficult job.

THE SOLUTION

La Rbesch Limited has gone a long way in seawater system development. La Rbesch holds three patents in the subject so far, and two more are being researched and developed.

Design of an inexpensive, yet reliable onshore seawater intake system is the subject of this paper. It covers the analysis, design, and stability of a filtration intake system concealed from wave action under the sea bed by a few centimeters of site sand 5 to 10 meters into the sea from the shoreline.

The system is designed to deliver constant and steady supply of clean water all year round and around the clock, free of suspended matter, and sufficient to run any system capacity. The system performance improves with stormy conditions, and the flow rate increases when the waves are violently breaking on the beach.

The system is maintenance free and requires no backwash, cleaning, treatment, scrapping, replacement, rechanging, or rehabilitation. It is expendable without the need of system shutdown.

The systems require no settling basin since there is nothing to settle in the first place except for an equalizing pool where the pump intake pipes are placed and where the outlet of the intake system is located. A cement asbestos or fiberglass, 1-meter-diameter pipe section is sufficient for housing the filter bed outlet and the pump suction pipe inlet.

Artificial filter intakes have been considered for sometime, but applications and experiences are limited. The studies that have been done before dealt with small capacity intakes using relatively clean waters with minimal wave disturbance; backwashing was usually an integral part of these intakes. The maximum capacities obtained by these systems usually did not exceed 3 m^3/sec. This is adequate for cooling only 60 MW capacity electric power plant.

The intake system described herein is different in many ways from the types that have been studied or constructed before. This system utilizes the very natural forces that conventional intakes are protected from and uses gravity as the only flow-driving force. It makes use of the site resources in building the filtration system.

HYDRAULIC CONSIDERATION

Friction losses through the filtration systems are the major limiting factor in their design and usually backwashing is activated at certain minimum flow rates. If high flow rates are required, larger filter area must be made available.

But this new system requires no backwash. It utilizes special non-biodegradable, physically and chemically stable materials with high porosity and low friction losses. The filtration system is made in modular form to facilitate transportation and construction.

Model studies were conducted in two sites: one in the southern coast of the Mediterranean, in Tripoli, Libya, in an area infested with seaweeds; the other site is in Ajman, the United Arab Emirates, on the Arabian Gulf, where shallow water prevails and high concentrations of silt and algae are present.

The soil analysis of first and second sites showed that the soil D_{50} are 0.42 mm and 0.23 mm, respectively.

The system can be custom-designed for each site. Upon soil analysis of a certain prospective site, a filter system can be designed, and the filtration area can be decided. The filter modules are critically spaced in a hydraulically tuned form that maximizes the flow rate, eliminates filter clogging, and minimizes the required filtration area.

Table 1 and Figures 1 and 2 give an indication of the required filter area for each required flow rate. The combined hydraulic conductivities are 0.00275 m/s for the Southern Mediterranean site, and 0.00133 m/s for the Arabian Gulf site.

ECONOMIC ASPECTS

The costs of the system are almost limited to the initial costs of materials and labor since there are not running costs or maintenance and operation costs associated with this intake system.

The initial costs are estimated to be less than 30 percent of the costs of conventional intakes for major reverse osmosis and electric power plants, i.e., capacity over 10,000 m^3/d or 6 MW, respectively. For smaller capacity plants, the costs are much less.

This system is specifically suitable for reverse osmosis plants. It provides filtered water low in SDI and free of any suspended matter. This raw water quality eliminates or drastically minimizes the need for coagulation, flocculation, and filtration at the pretreatment stage of the reverse osmosis plant, thus cutting back chemical consumption. This also minimizes the need for large buffer tanks since small or no residence time is required.

This system also contributes to the reduction of operation and maintenance costs of electric power plants since it delivers clean water free of eroding materials such as sand which has a devastating effect on pumps and heat exchanger tubes. Biological fouling is also kept to a minimum since minimal quantities, or none at all, of microorganisms reach the cooling system.

The savings made by the lower costs of operation and maintenance of reverse osmosis and power plants were estimated to pay off the initial system costs in a period of 2 to 5 years after commissioning (see Figures 3, 4, and 5).

BIBLIOGRAPHY

1. C.I. Bretschneider. Topics in Ocean Engineering. Gulf, 1969.

2. R.L. Weigel. Oceanographical Engineering. Prentice-Hall, 1964.

3. AVALA, Hydraulic Laboratory. Wave Field Measurements for Khoms Power Plant, Libya. Preliminary Report No. 111, Belgrade, Yugoslavia, 1978.

4. P. F. Colon and H. L. Lee. Model Hydraulic Study (Link Belt Dual Flow Screens) Circulating Pump Structure for Long Island Lighting Company, Shoreham Nuclear Power Station, Unit No. 1. Alden Research Laboratories, Report No. 69-74/M10RF, July 1974.

Table 1

Required Filtration Area to Provide Sufficient Flow
to Run Electric and RO Plants of Various Capacities
in Both Sites in the Southern Mediterranean and in the Arabian Gulf

Power Plant Size, MW	RO Plant Capacity, m^3/d	Flow Rate Q, m^3/sec	Filter Bed Area, m^2			
			Site 1, Tripoli		Site 2, Ajman	
			A, m^2	LxB*, m	A, m^2	LxB*, m
0.1	100	0.004	0.18	3x1	5	3x2
0.5	500	0.019	0.9	3x1	18	3x6
1.0	1,000	0.039	1.8	3x1	37	3x12
2	2,000	0.077	3.5	3x2	72	6x12
5	5,000	0.193	9	3x3	180	6x30
7	7,000	0.270	12	3x4	254	7x28
10	10,000	0.386	17	3x6	360	12x30
15	15,000	0.578	26	3x9	543	15x36
20	20,000	0.772	35	6x6	725	16x48
25	25,000	0.964	43	6x7	906	18x50
30	30,000	1.16	52	6x8.6	1,090	21x52
40	40,000	1.54	69	6x12	1,450	24x60
50	50,000	1.93	87	6x14	1,815	27x67
70	70,000	2.70	122	6x18	2,540	33x77
100	100,000	3.86	174	6x28	3,630	36x100
160	170,000	6.5	293	9x22	6,100	42x145
175	and	7	315	9x35	6,500	45x146
200	over	8	360	12x30	7,500	52x144
225		9	400	12x33	8,400	
250		10	450	12x38	9,400	
275		11	500	15x42	10,300	
300		12	540	15x36	11,300	
325		13	585	15x39	12,200	
350		14	630	15x42	13,200	
375		15	675	15x45	14,100	
400		16	720	15x48	15,000	
425		17	765	15x51	16,000	

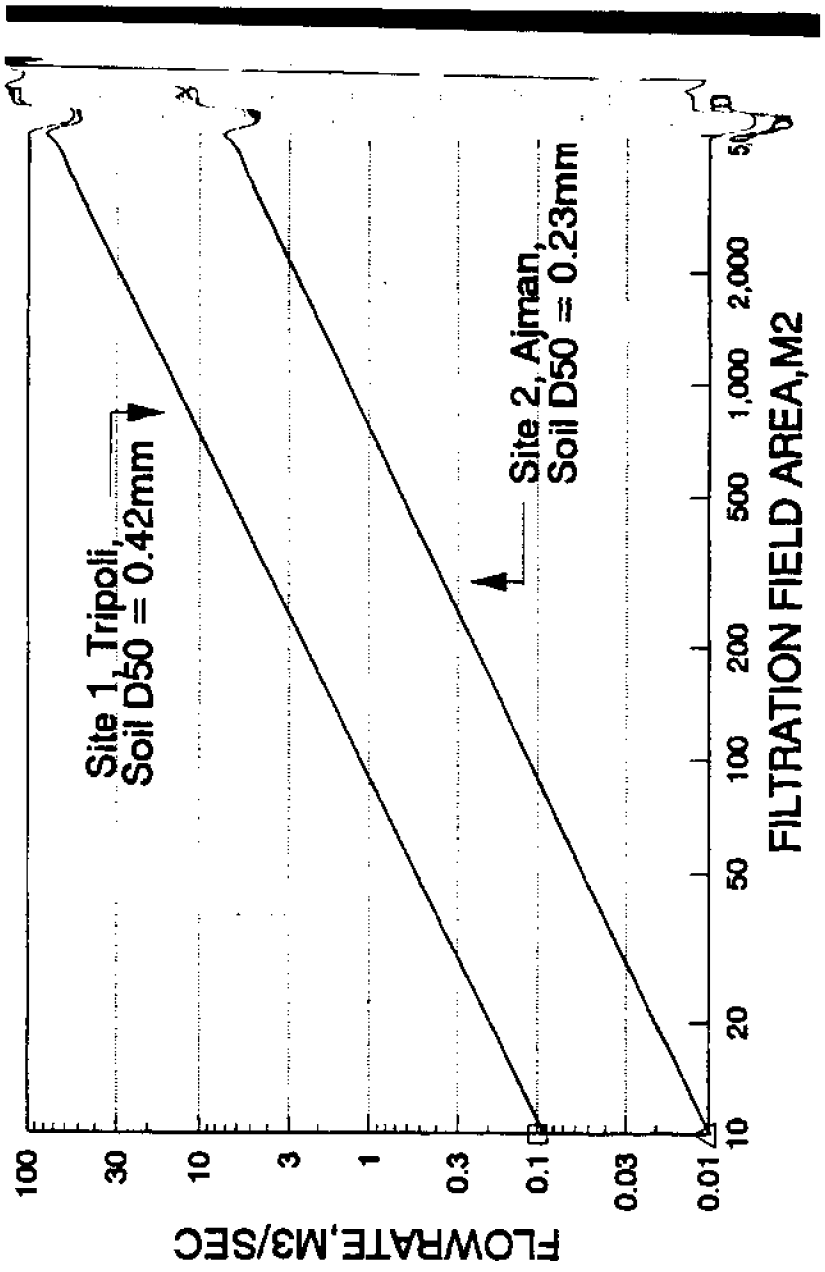


Figure 1. Flow Rate vs Filtration Field Area

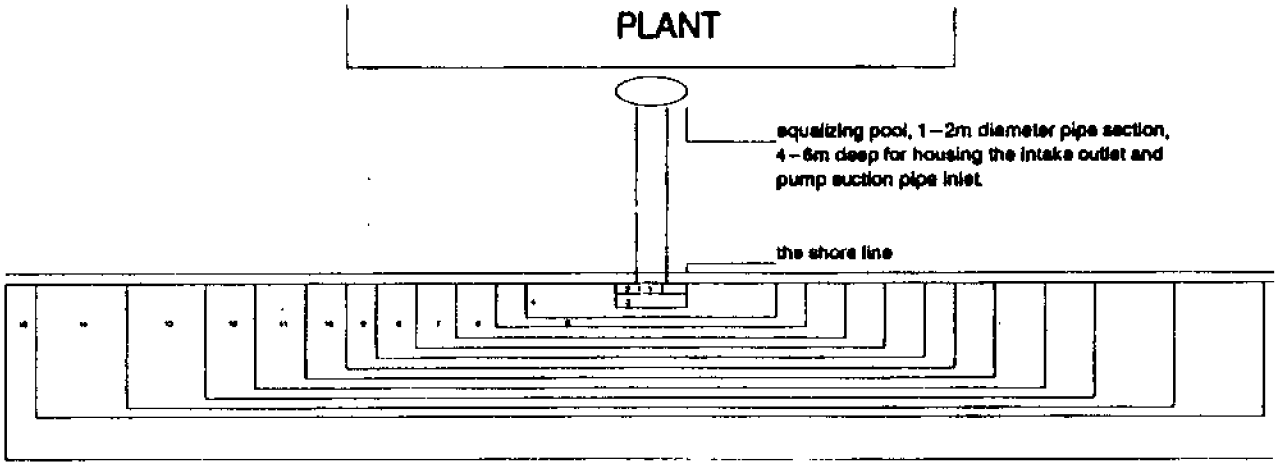


Figure 2. Required Area for Each Flow Capacity, About 5 to 10 m Away From the Shoreline Into the Sea

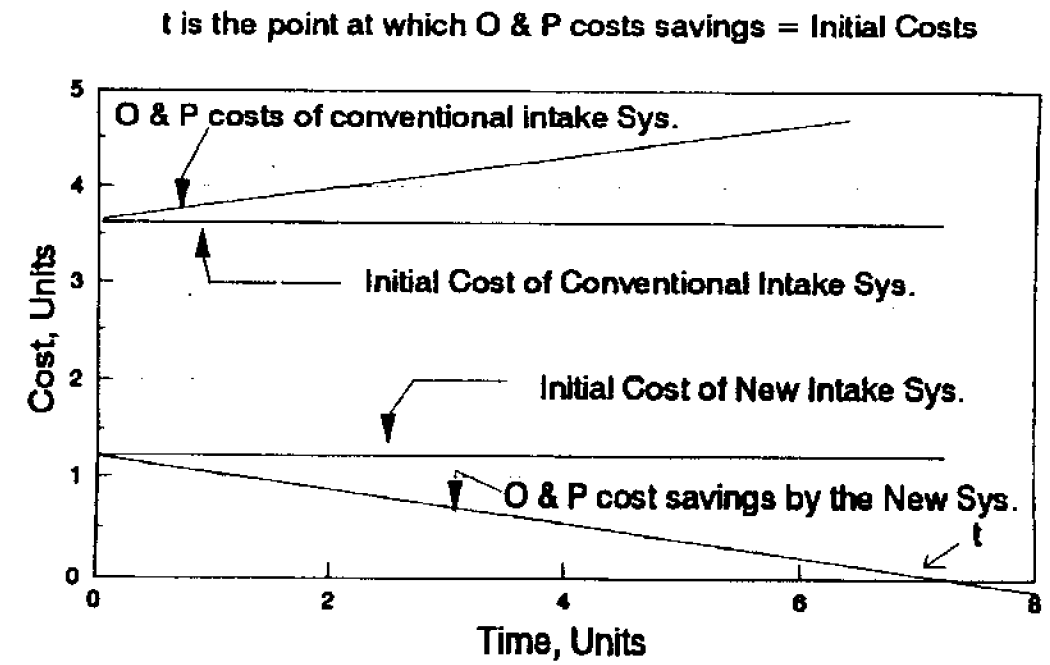


Figure 3. Costs of New Intake System vs Conventional Intake System of the Same Capacity

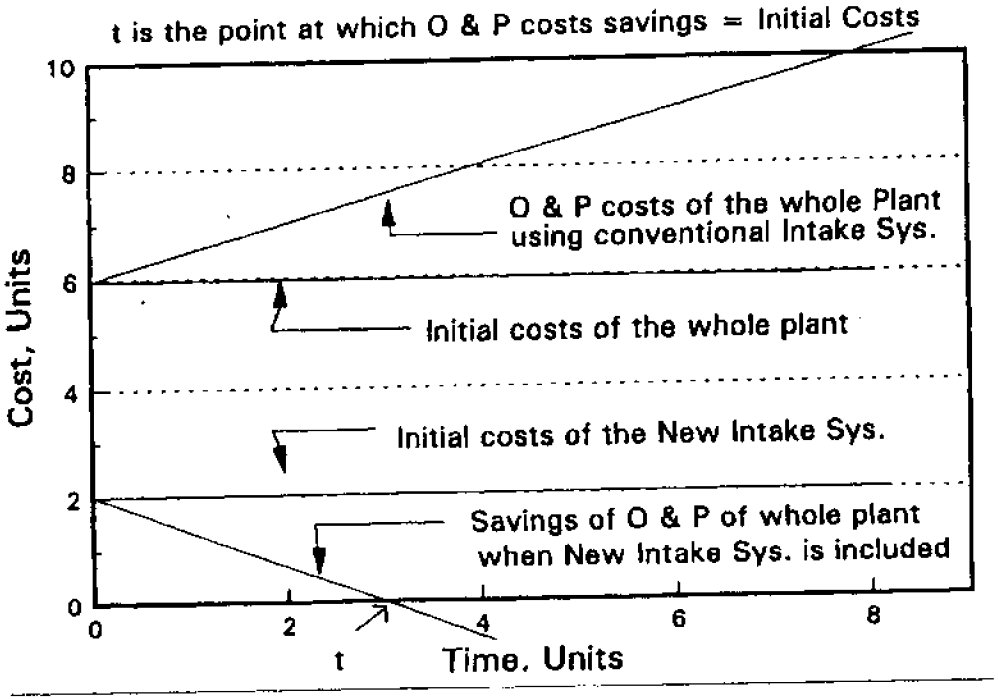
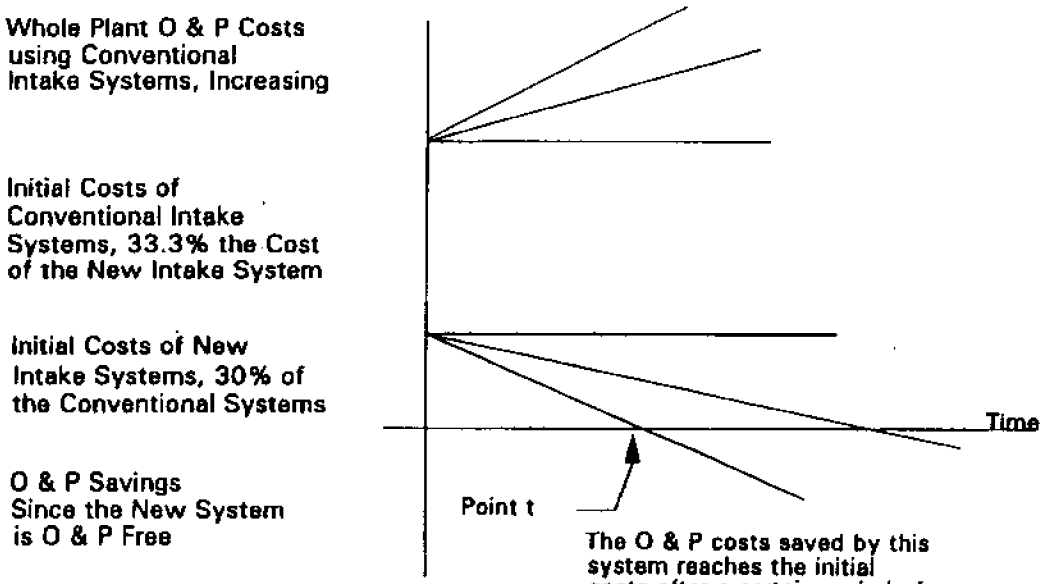


Figure 4. Costs of O&M of the Complete Plant When Using Conventional Intake System or New Intake System



SYSTEM ADVANTAGES

1. The system is installed onshore.
2. Costs are very low.
3. Requires no skilled personnel to install it.
4. Filter systems are made in modular form to facilitate transport and installation.

Figure 5. Comparison of Costs Between Conventional Intake System and The New One

Use of Low levels of Electric Current (AC) for Controling Zebra Mussels

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Abstract

The efficacy of low-voltage alternating currents for preventing attachment by adult zebra mussels was tested using both static and flow-through experimental designs, and using various conductor configurations. In static experiments with steel rods (4.5 cm or 2 in separation), significant reductions in attachment occurred near 3 v in^{-1}, while adult mussels failed to attach to glass surfaces when exposed to voltage potentials exceeding 8 v in^{-1}, v in^{-1} being defined as the voltage potential existing between adjacent conductors divided by the separation of the conductors in inches. After ten days mortality of adults exposed to 6 v in^{-1} was 15% and for adults exposed to 8 v in^{-1} it was 35%. In flow-through experiments, mussels did not attach to wood substrates when placed between either steel rods (0.6 cm diam x 60 cm long) or plates (0.6 cm thick x 15 cm long x 11.5 cm high) and exposed to either 6 or 8 v in^{-1}. After 14 d, all mussels exposed to 8 v in^{-1} between plates or rods were dead. Between plates at 6 v in^{-1} all mussels died after 14 d, but only 9% of the mussels died between rods. These data suggest that low-voltage alternating currents have potential to control attachment by adult mussels, with efficacy being improved by different conductor configurations. Although continuous low-voltage alternating current was used in these experiments, it should be possible to use pulsed low-voltage currents thereby reducing the power requirements necessary for protection.

INTRODUCTION

Currently, it is fairly routine to prevent fouling by zebra mussels in water intake or distribution systems with biocides. However, biocides cannot be used to protect external structures, such as intake cribs, because their use becomes an environmental hazard outside water intake systems.

Electricity may represent one alternative for some utilities. Some work has already been conducted to determine the effectiveness of high voltage potentials for killing the free-swimming larval stage (veliger) of zebra mussels. Although feasible, the technology is expensive. However, for water intake systems where biocides are currently being used to protect the water pipelines or distribution systems, it is not necessary to kill veliger larvae but merely to prevent settlement. As such, it may be feasible to use low voltage potentials for this purpose, but little work has been conducted with this objective in mind.

Recently, Smythe et al. (1991) tested the utility of low-voltage electrical fields for preventing settlement of veliger larvae. No attempt was made to induce mortality. The use of direct current resulted in increased settlement and attachment of post-veligers. Alternating current is known to cause greater physiological damage in fish than direct current (Vibert, 1967). Hence, alternating current may be more useful than direct current for preventing settlement of zebra mussels.

This paper describes a series of experiments designed to determine if attachment of adult mussels can be prevented using continuous low-voltage alternating current. Effects of low-voltage alternating current on mortality of adult mussels is also be described. The benefits of using adult mussels are: (1) it is possible to conduct studies in the laboratory and obtain quicker feedback from experiments; and, (2) they are larger allowing behavioral responses to be observed with less difficulty than with veliger larvae. It is uncertain whether the voltage potentials which prevent attachment and induce mortality in adults will be applicable to veliger larvae because of the size differences. Larger fish are more sensitive to voltage potentials than smaller fish because of a greater surface area (Vibert, 1967). As such, the potentials that prevent attachment and induce mortality of adults may be much lower than those needed for veliger larvae. However, microscopic observation of the veligers in the presence of low-voltage currents indicates severe spasmodic movement that may result in the inability of veligers to attach. The data presented will still be relevant for control purposes because if veligers do attach and grow on the protected surfaces, they will at some point attain the size at which the voltage potentials have significant effects on mortality.

OBJECTIVES

The specific objectives of this study were to:

1) determine the effect of low voltage potentials (A-C) on mortality of adult zebra mussels and the potentials required to prevent attachment.

2) test the relative efficacies of different configurations for preventing mussel attachment at low voltages.

3) determine the effect of temperature on low voltage potentials (A-C) for preventing attachment by adult zebra mussels on plates and rods.

4) determine if low volatge potentials could be used to detach adult mussels from steel plates.

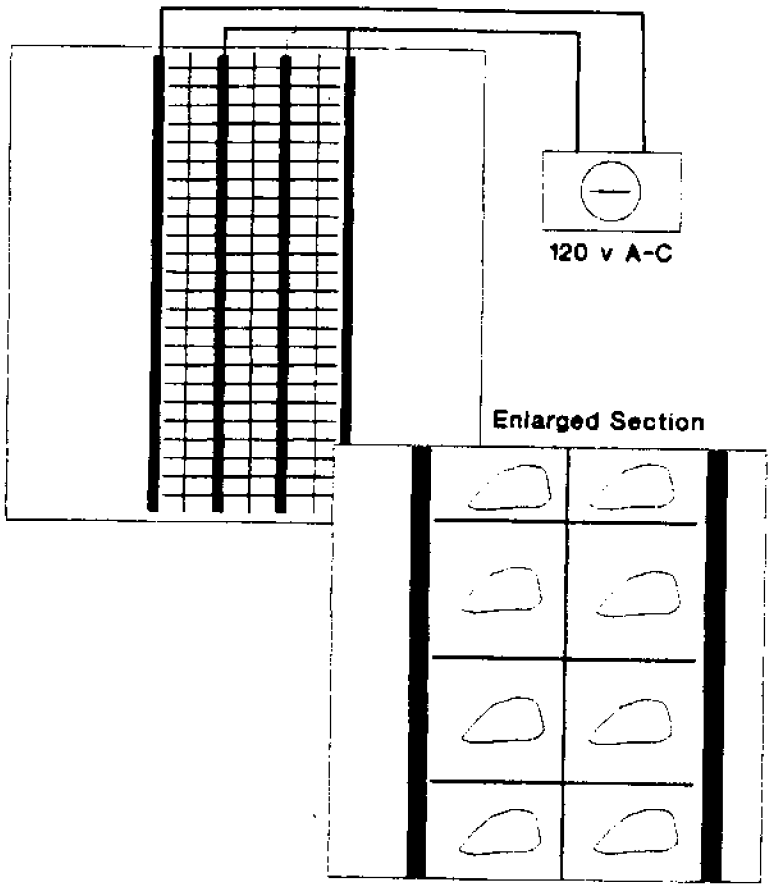
MATERIALS AND METHODS

VOLTAGES REQUIRED TO INDUCE MORTALITY AND PREVENT ATTACHMENT

In order to address Obective 1 preliminary testing with steel probes was used to provide an estimate of the voltage potential at which all adult zebra mussels responded. Valve closure was used as the response because it was assumed that when critical voltage potentials were reached, individuals would close involuntarily. This is the same principle used for electroshocking fish (Vibert, 1967). The preliminary work showed that all zebra mussels would close when exposed to voltage potentials $\geq$ 10 volts in^{-1}. As such, all experiments were conducted at voltage potentials less than 10 volts in^{-1}. **Volts $inch^{-1}$ is defined here as the voltage potential existing between adjacent conductors divided by the separation of the conductors in inches.**

For determining the minimum voltages for preventing attachment or inducing mortality of adult mussels, the physical experimental designs were similar and are schematically shown in Fig. 1. A-C voltage potentials were applied to four steel rods each separated by 5 cm (2 in) in 60-L aquaria. Between each pair of rods, a grid pattern of 2.54-cm (1-in) intervals was marked on the bottom so that the position of the adults could be recorded, as well as any movement over time. Aquaria were filled with 4 L of water (total hardness of 135 mg/L as $CaCO_3$) giving a depth of 2.4 cm. The rods were made of threaded galvanized steel rods, 0.63-cm (0.25-in) diam OD and 43.2 cm (17 in) long and were glued to the bottom of the aquarium with silicone. Tanks were aerated to maintain dissolved oxygen saturation at room temperature (20°C). The terminals from each tank were connected in parallel to a

Figure 1. Schematic diagram of experimental design.



120 v A-C variable power supply.

To determine the minimum voltage needed for preventing attachment and for inducing mortality of adults, groups of 10 adult mussels were exposed to one of a series of voltages between 0 and 8 volts in^{-1} (Fig. 4). For assessing effects on attachment, groups of mussels were exposed to the test voltage potentials for a 2-h period because preliminary work suggested that attachment of most adults occurred within the first 2 h.

To examine the effects of voltage potential on mortality, adults were exposed to the voltage potentials for ten days with mortality checks every 24 hours. Adult mussels averaged 7.7 mm (5.5-9.5) in shell length and were placed in the aquarium between rods as indicated in Fig. 1.

Effects of Temperature on Mussel Attachment at Low-voltages

In order to address Objectives 2 and 3, a jet pump was installed to supply water to four wooden aquaria and a plexiglass biobox by means of a 2.54-cm (1 in) I.D. diam pressure hose with a foot valve at the inflow end. A 2.54-cm diam hose was attached to the discharge side of the pump and a T-fitting was inserted in order to supply water to the 75-L biobox and the wooden aquaria. The biobox was used to monitor settlement of mussels on a weekly basis. However, weekly analyses showed that no settlement occurred throughout the study period (August to December).

The wooden aquaria were made of 1.90-cm (0.75-in) thick plywood. Two of the aquaria held 70 L of water each and had inside dimensions of about 45.7 cm wide x 66.2 cm long by 25.4 cm high (18 in x 26 in x 10 in). Each contained 8 stainless steel rods (0.6 cm diam x 60 cm long). One aquarium was used as a control without electric current and the other with electric current. The remaining two aquaria held approximately 50 L of water and had inside dimensions of about 40.6 cm wide x 66.2 cm long x 25.4 cm high (16 in x 26 in x 10 in). Each contained 12 steel plates with dimensions of 0.6 cm thick x 15 cm long x 11.5 cm high (0.25 in x 6 in x 4.5 in), but one was used as a control without electric current and the other was electrified. All tests were carried out in a trailer at the Canada Centre for Inland Waters using water from Hamilton Harbour.

Four trials were carried out, each trial at a different potential and temperature. After each trial (2 to 6 weeks duration), the aquaria were scrubbed cleaned and new mussels were placed in each aquarium with the long axis (front to back) of the mussel at right angles to the direction of the rods and plates and approximately midway between the plates and rods. This was done to help determine if some or all the mussels moved before reattaching themselves, if the mussels did reattach. In aquaria with metal plates, a single mussel was placed between each pair of 12 plates (total 11 mussels). In aquaria with rods, 3 rows of

mussels were used; each row had 7 mussels (i.e. 1 mussel between each pair of rods). Observations were made weekly. Table 1 gives the dates, temperatures, and voltages used for each trial with the rods and plates. All dead mussels were removed after each period of observation.

Table 1. Voltage potentials, temperature ranges, and dates for each trial.

Trial #	AC voltage (volts/in)	Temp. (°C)	Date
1	8	16.0-19.8	Sept 13-Sept 24
2	8	19.8-13.1	Sept 24-Oct 8
3	6	13.1-10.3	Oct 8-Nov 5 (Plates)
3	6	13.1-8.8	Oct 8-Nov 19 (Rods)
4	6 plates	10.3-6.7	Nov 5-Dec 3 (Plates)
4	4 rods	8.8-4.7	Nov 19-Dec 17 (Rods)

Detachment of Mussels from Steel Plates at Low Voltages

The previous experiments were designed to prevent attachment of adult zebra mussels using low voltages on plates and rods. The objective (No. 4) of these experiments was to determine if low voltages could be used to detach adult zebra mussels that were byssally attached to steel plates. Only plates were tested for the detachment studies. However, the setup described above for Objectives 2 and 3 was used for Objective 4. Two voltages were tried, 4 volts/inch and 8 volts/inch, each at a duration of 7 d.

Four plates were placed in an aquarium with small (2 to 6 mm shell length) mussels for one week. After one week a sufficient number of mussels had attached to the steel plates and were transferred to the wooden aquaria. Adult mussels that had attached to the wooden bases in the control tank from Trial 4 above were left in place but two of the four plates with new mussels were also added (slots D and I, Fig. 1 and photographs). The remaining two plates were placed in the electrified tank in slots B and I. A voltage of 4 volts/inch was applied for one week but since few mussels had detached the voltage was increased to 8 volts/inch. No "detachment" experiments were performed with the rods.

RESULTS AND DISCUSSION

VOLTAGES REQUIRED TO INDUCE MORTALITY AND PREVENT ATTACHMENT

Fig. 2 shows the variations in percentages of mussels attaching after a two-hour period at various voltage potentials. The data suggest that at voltages near 3.0 volts $inch^{-1}$, there is a significant decrease in the percentage of adults attaching. At 8 volts $inch^{-1}$, no attachment of adult mussels was observed.

Significant mortality of adult mussels occurred after 120 hours exposure to eight volts in^{-1} and 168 hours exposure to six volts in^{-1} (Fig. 3). After 10 days exposure to 8 volts in^{-1}, 35% of the adults died, whereas, at six volts in^{-1}, 15% of the adults died.

To determine if observed mortality may have been due to zinc or cadmium liberation from the surface of the galvanized rods, 4-d static bioassays were conducted with 1-L water samples collected after the 10-d experiments were finished. There was no mortality of mussels in the bioassays, suggesting that mortality of mussels exposed to 6 and 8 volts in^{-1} was not related to water-borne constituents (e.g. Cd or Zn).

These data suggest that voltages (A-C) as low as 6 volts in^{-1} are useful for preventing attachment, and voltages as low as 8 volts in^{-1} are useful for inducing mortality in adult mussels. The effects of these voltage potentials on veliger larvae and post-veligers are unknown, and subsequent studies will be designed to address these questions. However, even if these voltage potentials have little effect on larvae, significant effects on mortality would be expected as they grow.

EFFECTS OF TEMPERATURE ON MUSSEL ATTACHMENT AT LOW-VOLTAGES

A comparison of Figs. 4 and 5 shows that **the mortality rates of mussels tends to be faster between plates than between rods at a given voltage and temperature, especially at low temperatures.** Hence, **configuration of the conductors is important.**

Figs. 4 and 5 also show the effects of temperature on mortality rates at 4, 6, and 8 volts in^{-1} within each configuration. At 8 volts in^{-1} temperatures between 13 and 20°C have little effect on the mortality rate, with 100% mortality occurring within 14 d. Two mussels remained alive in the aquarium with rods at 8 volts in^{-1} after 14 days (Fig. 5, 80% mortality) but both mussels had been moved by the inflow current to positions where the current was < 1.2 volts in^{-1}. **Mortality rates appear to increase with increasing temperatures at 6 and 8 volts in^{-1}.** This is probably due to increased energy demands associated with contraction of adductor muscles when the mussels were being stimulated to remain closed. *This implies that the*

Figure 2.
Attachment trends of zebra mussels after a period of two hours

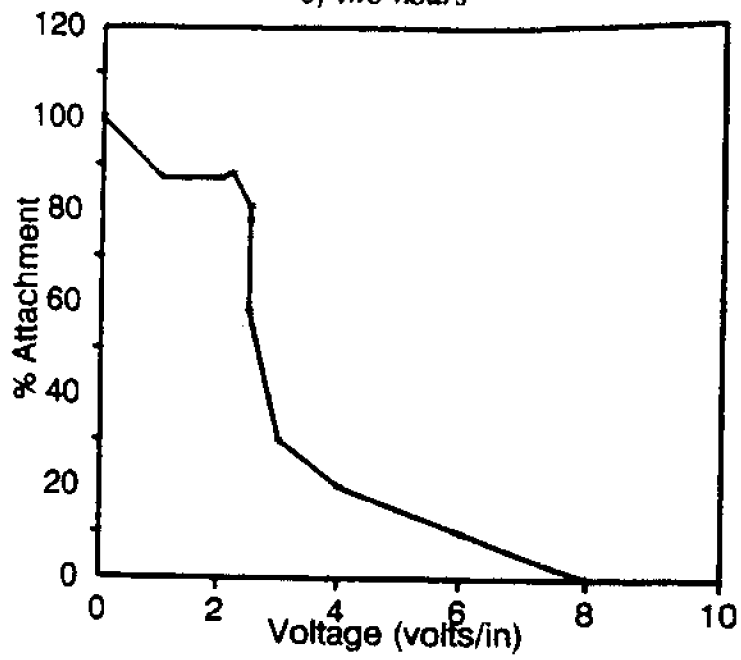


Figure 3.
Mortality of zebra mussels exposed to low A-C potentials

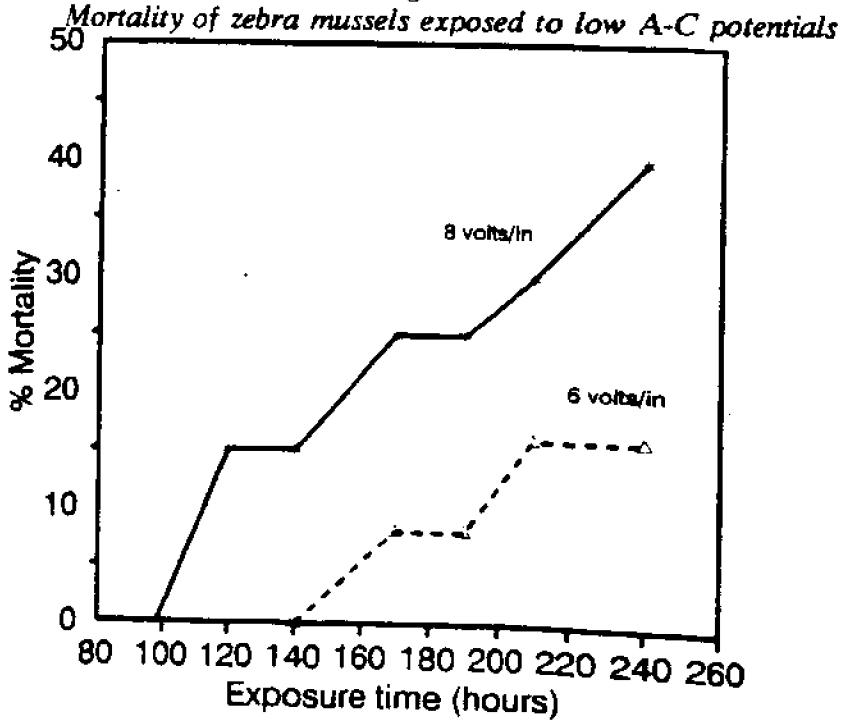


Figure 4.
Mortality of zebra mussels on plates at 6 and 8 volts/in at different temperatures

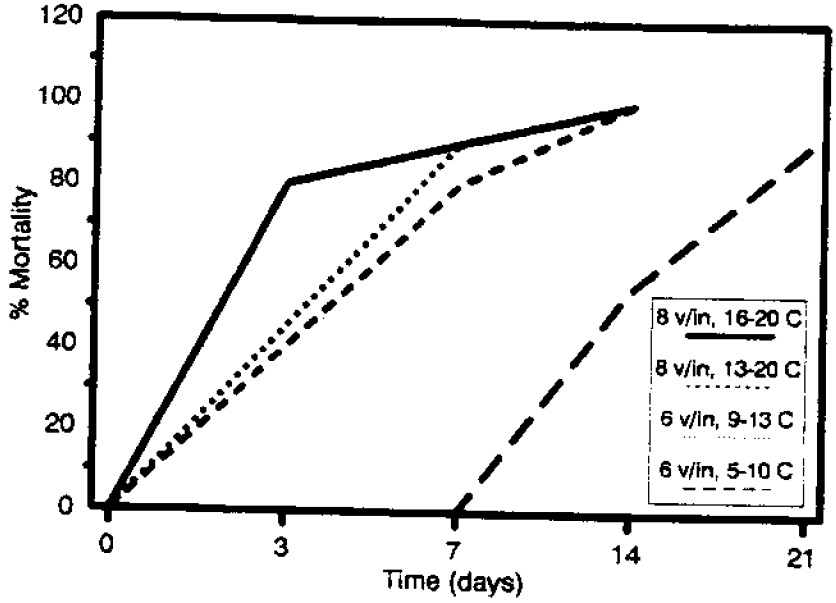
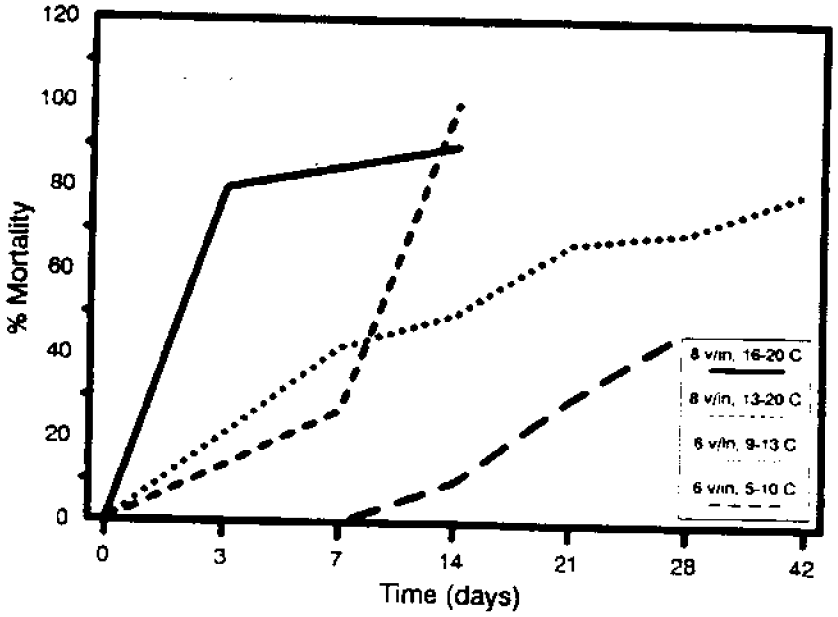


Figure 5.
Mortality of zebra mussels on rods at 6 and 8 volts/in at different temperature



summer is the best time to use this technology to reduce costs. Both 4 and 6 volts in^{-1} appear to be useful for killing adult mussels, but at least 6 weeks is required to induce total mortality. Longer term experiments are required to determine the time required to induce 100% mortality at these voltage levels.

These results are slightly different than obtained in the first experiments above, but those experiments ran for only 10 d.

A-C potentials as low as 4 and 6 volts in^{-1} is sufficient to prevent attachment at low temperatures (5-13°C). We were unable to test these voltages at higher temperatures and further experimentation is required.

Detachment of Mussels from Steel Plates at Low Voltages

A potential of 4 volts in^{-1} had little effect on the detachment of mussels after 1 week at 5-9°C; some mussels had detached on the electrified plates (3 on B; 4 on I) but some had also detached in the control tank (1 on D and 2 on I, Table 2). However, after one week at 8 volts/inch, there was 78% (B) and 72% (I) detachment compared to 39% (D) and 25% (I) on the control plates (Table 3). The numbers of mussels detaching from the control plates suggests that the mussels had secreted only a few byssal threads after allowing only one week for mussels to attach to the plates in the glass aquaria. **The results from this and previous experiments suggests that electrical currents not only inhibit byssal secretions (or else prevents extension of the foot or valve gapage) to make new threads but also alters the ability of the mussels to retain threads already secreted.**

These results suggest that it is possible to remove mussels attached to steel structures. In a reactive strategy, mussels could be allowed to settle and grow (to a small size) before applying current. This would reduce operating costs. Presumably, costs could be reduced even further if the current was applied at higher temperatures, although further studies are needed to verify this.

Table 2. Numbers of adult mussels attached to steel plates in both the control and electrified (4 v in^{-1}) aquaria on December 10, after one week.

	Plate No.	No. of mussels start	No. of mussels at 1 wk	°C	Temperature
Electrified plates	B	25	22	6.7	
	I	29	25	6.7	
Control plates	D	19	18	6.5	
	I	6	4	6.5	

Table 3. Numbers of adult mussels attached to steel plates in both the control and electrified (8 v in^{-1}) aquaria on December 17.

	Plate No.	No. of mussels at start	No. of mussels after 1 wk	Temperature °C
Electrified plates	B	22	5	4.6
	I	25	7	4.6
Control plates	D	18	11	4.7
	I	4	3	4.7

CONCLUSIONS

1. The potentials that prevent attachment and induce mortality of adults may be much lower than those needed for veliger larvae. Observation of the veligers in the presence of low-voltage currents indicates severe spasmodic movement that may result in the inability of veligers to attach.
2. Voltages (A-C) as low as 6 volts in^{-1} are useful for preventing attachment, and voltages as low as 8 volts in^{-1} are useful for inducing mortality in adult mussels.
3. Configuration of the conductors is important because mortality rates of mussels was faster between plates than between rods at a given voltage and temperature, especially at low temperatures.
4. Mortality rates appear to increase with increasing temperatures at 6 and 8 volts in^{-1}. This is probably due to increased energy demands associated with contraction of adductor muscles when the mussels were being stimulated to remain closed. This implies that the summer is the best time to use this technology to reduce costs.
5. **A-C potentials as low as 4 volts in^{-1} is sufficient to prevent attachment at low temperatures (5-13°C) with either plate or rod configurations.** Electrical currents not only seem inhibit byssal secretions (or else prevents extension of the foot or valve gapage) but also alters the ability of the mussels to retain threads already secreted.
6. The results suggest that low-voltage can be used as both proactive and reactive strategies to control mussel infestations. In the proactive strategy, low-voltage (A-C) can be applied continuously to prevent mussels from attaching. In the reactive strategy, mussels could be allowed to settle and grow (to a small size) before applying continuous, low-voltage alternating current. This would reduce operating costs. Costs could be reduced even further if the current was applied at higher temperatures or if pulsed low-voltage potentials were used, although the duration of the pulse needs to be investigated.

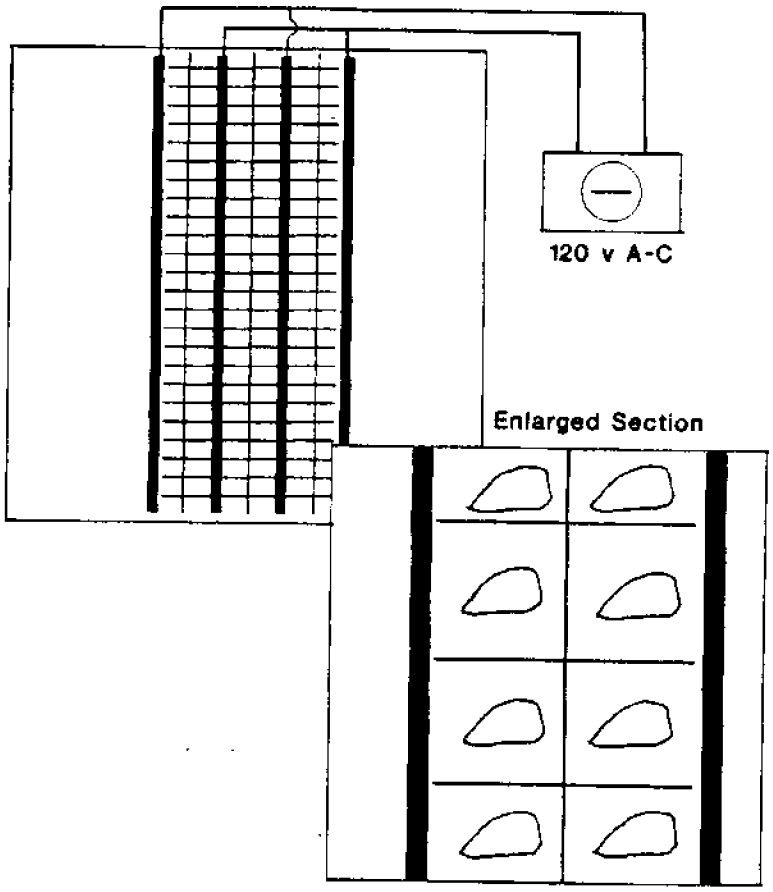
REFERENCES

Mackie, G.L., W.N. Gibbons, B.W. Muncaster, and, I.M. Gray. 1989. The zebra mussel, Dreissena polymorpha: A synthesis of European experiences and a preview for North America. Ontario Ministry of the Environment, Water Resources Branch, Great Lakes Section, Queens Printer, Toronto, Ontario.

Smythe, G.A. C.L. Lange, J.F. Doyle, and P.M. Sawyko. 1991. Application of low voltage electric fields to deter attachment of zebra mussels to structures. Abstract of a paper presented at the Second International Zebra Mussel Research Conference, Rochester, NY. November 1991.

Vibert, R. (ed.). 1967. Fishing with Electricity - Its Applications to Biology Management. European Inland Fish. Adv. Comm. FAO, U.N., Fishing News Ltd. London. 276 pp.

Figure 1. Schematic diagram of experimental design.



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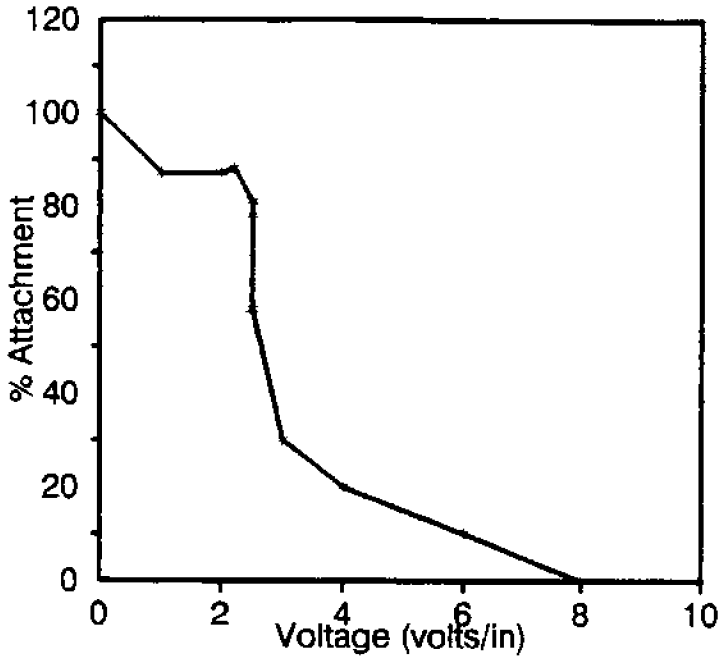
Attachment trends of zebra mussels after a period of two hours



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Mortality of zebra mussels exposed to low A-C potentials

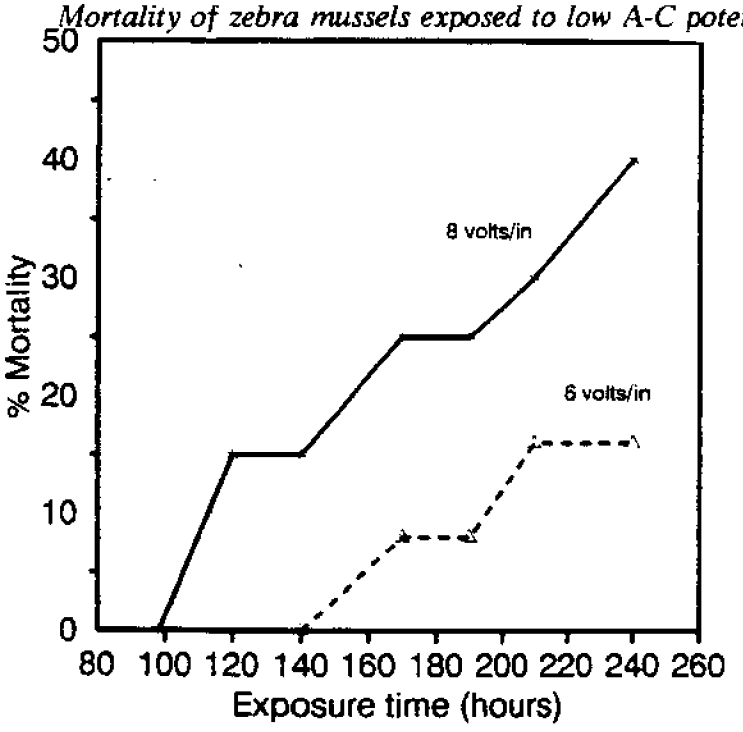


Figure 4.
Mortality of zebra mussels on plates at 6 and 8 volts/in at different temperatures

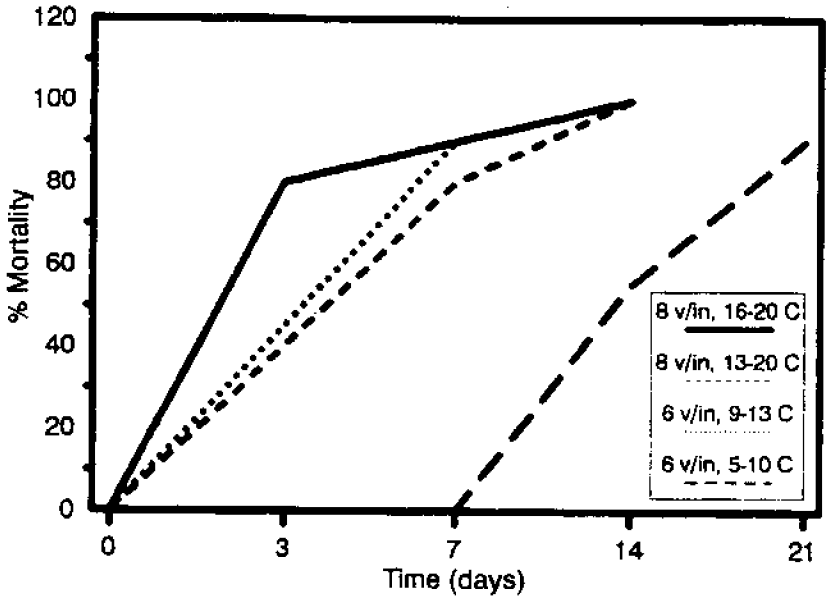
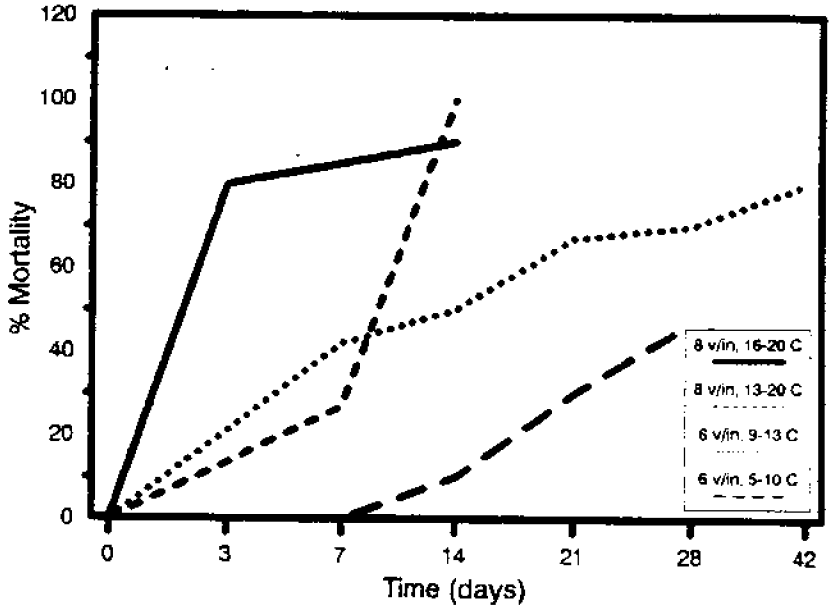


Figure 5.
Mortality of zebra mussels on rods at 6 and 8 volts/in at different temperature



Experience with Non-Fouling Coatings for Mussel Control

A. Christopher Gross
Long Island Lighting Company

ABSTRACT

Intake systems of the Long Island Lighting Company's power plants can foul with enormous quantities of the blue mussel (Mytilus edulis), which is similar in most respects except salinity tolerance to the zebra mussel (Dreissena polymorpha). Chlorination was found to be ineffective for mussel control unless applied continuously. As continuous chlorination is not authorized by discharge permits and no other control measures were practical, until recently, mussels could only be controlled by cleaning all underwater surfaces at least once per year.

Faced with this mussel problem, in 1981 LILCO initiated an investigation into the use of non-fouling coatings for mussel control. Testing of coatings began with evaluations of available antifouling coatings for ship bottoms. These proved successful and testing then moved to evaluations of new coating types on small test panels which were suspended into plant intakes. The best performers were then applied full-scale to entire intakes. This selection process continues today and has led to the identification of several coatings that provide multi-year protection. As a result of this program, approximately 135,000 square feet of underwater surfaces have been coated and the mussel problem is largely under control.

Two generic coating types have been found most effective at preventing fouling for periods of greater than five years: soft silicone rubber paints (a "foul-release" coating), and hard copper-containing epoxy paints.

The costs of applying these coatings, $8-10 per square foot, have been returned in less than two years in reduced cleaning costs alone, even without consideration of savings from outage reductions and efficiency improvements. These coatings can also reduce or eliminate the need for chemical control methods.

INTRODUCTION

Power stations that must rely on salt water for cooling are frequently faced with significant macrofouling problems. The most important contributor to these problems is often the blue mussel, Mytilus edulis. This mussel can grow in dense colonies attached to essentially any hard underwater substrate. This growth habit can lead to physical occlusion of cooling water conduits and, when clusters of mussels break loose, they can plug smaller pipes and heat exchange tubes downstream in the cooling water system. Effects on the plant can be major and acute, such as an unscheduled outage due to total loss of cooling water. Effects can also be chronic, but perhaps no less expensive in the long run, as backpressure and fuel costs rise due to partial clogging of small diameter heat exchanger and condenser tubes. Corrosion can also be increased and safety systems can be compromised. The growth habit of the blue mussel and the problems it causes are essentially identical to the freshwater zebra mussel, Dreissena polymorpha.

Mussel fouling has been difficult and expensive to control. Chemical control with chlorine injections into the cooling water is not possible unless application is continuous, a condition that is difficult to maintain and which, in most cases, is not authorized by discharge permits. The large volumes of cooling water that need to be treated require significant and costly quantities of chlorine, even at low permitted concentrations. While we have experimented with other chemical control methods, LILCO's first line of defense against the blue mussel has been manual cleaning of the intake systems at least annually. Full protection from condenser performance penalties imposed by mussel fouling requires two cleanings per year.

Cleaning is an arduous and expensive process that requires the unit to shut down (for a tunnel cleaning) or at least drop load (for an intake bay cleaning). Until recent experience proved that underwater cleaning with divers was economically feasible, cleaning was performed by plant personnel working with hand scrapers in dewatered bays and tunnels.

With such a weak arsenal of weapons to use on the mussel problem, LILCO turned to the experience of the shipping industry in using antifouling coatings to control macrofouling.

THE COATING RESEARCH PROGRAM

Phase 1, Ship Bottom Coatings

Ship bottom antifouling paint technologies before the late 1970's were generally limited to conventional rosin based paints, containing toxicants that leached out over

time. They generally had a useful life of not much more than one year. For a power plant intake system, a one year service life was not cost effective because the expense of dewatering, cleaning, painting labor and materials could not be recovered in less than two or more years.

By the late 1970's, however, improved antifouling paints became available to the marine industry. These were copolymer, ablative paints containing organotin and cuprous oxide as their active antifoulant components. Despite a higher initial applied cost, copolymer paints quickly achieved favor in the shipping industry because of their effectiveness and long life. Being ablative rather than leaching, multiple layers could be applied and the effective life was extended to greater than four years. Generally, each layer is good for at least one or more year's protection. The layers can be applied in alternating colors so erosion rates can be determined.

This technology seemed applicable to power plant intakes. Our program began by applying test strips of various ablative organotin ship paints inside concrete intake bays. After one year exposure these were judged successful and full scale applications began in intakes.

In all, four intakes, each with a coatable surface area of approximately 3000 square feet, were painted with products from International Paint Co., (SPC-9, 4 coats), M&T Chemical Co. (Poly-Flo 2000, 2 coats) and Pettit Paint Co. (Horizons, 4 coats). Results of all were acceptable with IP showing the greatest effective life (eight years) followed by Pettit (6-7 years) and M&T (2-3 years - due to an insufficient number of coats). These results, while excellent, are now academic because these coatings are not presently registered for use in stationary sources. However, a great deal of experience was gained with regard to application methodologies.

Phase 2. Test Panel Evaluations

While the ablative paints applications in the intakes were being evaluated over the long term, in 1987 we initiated testing of new candidate coatings on small test panels (approximately 12" x 12") which could be suspended in the water either in or close to plant intake systems. The benefit of the small panels, some of which were attached in series on racks, was that they could be pulled and inspected frequently without the necessity of dewatering an intake or conducting an underwater inspection by diver. Additionally, many materials and coatings could be evaluated in a relatively small space.

Panels coated and supplied by the various coating manufacturers were utilized in most cases to eliminate variables that might be introduced by coating the panels ourselves. Panel materials have included concrete, slate, steel, fiberglass and various

plastics. Through experience, we have learned that the panel substrate material has little or no effect on the effectiveness of the test coating; however, adhesion and corrosion protection will vary among the different materials.

When time allowed, the panels were inserted at least several months prior to the start of the mussel larvae settling season which generally starts in June and runs through September. Otherwise, the panels were inserted whenever received, but it is recognized that at least a short period of aging is necessary before the true characteristics of the coating material may become apparent. The panels were pulled briefly about once per month for visual inspection and photographing. The inspection procedure must be brief because air exposures of more than a few minutes (particularly during extremes of heat or cold) can cause attached mussels to abandon the panel when reinserted into the water. The panels were ranked on a scale of 1 to 5 monthly and at the end of each exposure year.

The results of the continuing panel tests are presented in Table 1.

As the data indicate, only three basic coating types have been successful. The first is the copolymer, ablative marine antifouling paint that utilizes either organotin or cuprous oxide as an active antifouling ingredient. The second type, copper/epoxy is a hard, non-ablative epoxy resin that contains metallic copper powder. The third type, silicone rubber paint, is based on the concept of a passive nonfouling rather than an antifouling surface. This is achieved with a coating that provides a low critical surface tension, i.e., a "slippery" surface, as is found with some silicone rubber coatings. These have been termed "foul-release" in that while the surface can become lightly fouled, the foulants should slough off before significant or harmful quantities can accumulate.

The successful organotin and cuprous oxide based paints are ablative in action; as mentioned above, the organotins are no longer allowed in power plant applications. Ablative cuprous oxides, which are designed to prevent fouling by means of a toxic surface reaction as the paint wears away, are now commonly used to protect boat bottoms in marine waters. Examples of successfully tested cuprous oxide ablative paints are Micron CSC (International Paint Co.), Horizons (Pettit Paint Co.), Awlstar (US Paint Co.), and TFA (CMP Chugoku Marine Paint Co.) While effective, because these paints release cuprous oxides into the cooling water discharge, albeit at low levels, they were not included in our studies of large scale applications.

The copper/epoxies differ from ablative or leaching type paints in that metallic copper powder is suspended in an epoxy or polyester matrix and the surface is "activated" by mechanical means to expose the copper particles. The resulting

surface finish is hard and does not readily or noticeably ablate or leach but still repels organisms. Two copper epoxies that have tested well on small panels are Epco-Tek 2000 (Hi-Tek Chemical Co.) and CopperClad (Ferro Corp.).

Nonfouling (foul-release) silicone rubber coatings that have proved worthy of large scale testing include Bioclean BC and Bioclean DX (CMP Chugoku), Biox (Kansai Paint Co.), and Exsil 2200 (GE Silicones). Two additional silicone rubbers that look promising, at least after short term exposures, are Intersleek (the latest version from Porter International - earlier versions were ineffective), and CSL 560 (CSL Silicones, Inc., previously Sermatech). Of these five silicone rubbers, the first to be found acceptable, from its very first panel tests, was Bioclean DX. The other brands had to go through one or more iterations before acceptable performance was achieved. The Bioclean is still providing "fair" protection after six years but, while the coating is still intact, its non-fouling characteristics are waning.

No other type of coating or material was found to offer lasting fouling protection including: hard silicones (e.g., Wearlon), teflons (Scatt), hard non-copper epoxies (Belzona, Arcor, Simsite), a copper epoxy that is not activated (Barnacl X), polyurethanes (Devcon), silicone grease (Slipstream), waxes and various plastics such as polyvinyl chloride and polyethylene. A number of additives with purported antifouling qualities have also been tested and found ineffective: an antibiotic (Compound X), and red, black and cayenne pepper (generic brands).

Phase 3. Large Scale Applications

The results of the small panel tests were used to select candidate coatings for application to full scale systems including intake bays, tunnels, cell blocks and trash racks. Based on its early success on panels, in 1988 Bioclean was applied to the interior surfaces of two concrete intake bays (approximately 3000 square feet each, with dimensions of 40'L x 14'W x 25'D), a concrete tunnel (6'D, 120'L, about 2300 square feet) and galvanized steel trash racks. These applications were inspected after one year. The bays were found to be approximately 95% clean, the tunnel 98% clean and the trash racks about 50% clean. It appeared that the galvanizing on the trash racks had effloresced, which caused the coating to flake away in patches. No adhesion loss was noted in the remainder of the systems.

Subsequent inspections of these systems have been made approximately yearly. Results for the next three to four years in the bays and tunnel were similar to the first, except that a slight decrease in performance became noticeable; however, cleanliness was good enough that it was not until after six years of exposure that the performance of the coating in the tunnel degraded enough to allow seasonal growth to affect plant performance. That tunnel was washed down and overcoated with two

more coats of Bioclean in the spring of 1994. Performance in the bays remained acceptable until that unit was placed in cold standby in 1992. No attempt was made to recoat the trash racks. (It was determined that monthly cleaning by a diver would be more effective than coatings because of to the amount of debris that must be removed.)

Encouraged by the results of these first full scale applications, additional large scale applications of Bioclean and other coatings that passed the panel test stage have been made. Bioclean was applied to six more tunnels (up to 6'D x 960'L) at three plants, 16 intake bays (3-4000 square feet each) at four plants, and six cell blocks (400 square feet each) at one plant. Results of these applications, over the same exposure period, are consistent with those first made in 1988. As other brands were proven in the panel test phase, they also were applied full scale. These include: Biox in two bays at one plant, Epco-Tek in three bays (two plants) and one tunnel, and Exsil 2200 in one bay.

Plant staffs have been very pleased with the results of the tunnel and bay applications. The annual cleaning requirement for tunnels coated with Bioclean has been eliminated, as the velocities (6 to 10 fps, depending on the tunnel design) maintain a clean surface. The intake bays, with average (and turbulent) velocities in the range of 0.5 to 1.0 fps, can go for two years or longer without cleaning when the coating is new. As it ages, light cleaning becomes necessary in generally the third year, but this requires much less effort and the removal of far less material than in uncoated bays. Bays coated with Biox have developed luxuriant mussel growths on walls and beams in a single growing season but these fell off of their own weight when the bay was dewatered. Biox coated bays have not yet gone beyond a single year's exposure without cleaning. The Epco-Tek and Exsil coated bays have endured their first year exposure with good resistance to fouling that did not require cleaning.

Additional prospects for future large scale applications are the latest version of Intersleek, Sermatech, and CopperClad.

COATING APPLICATION METHODS

All the materials tested to date are formulations that must be applied in the dry. (Hi-Tek has recently developed Epco-Tek 1000 U, which they claim can be applied underwater. We intend to make a test application later this year.) All (CMP Bioclean, Kansai Biox, Hi-Tek Epco-Tek 2000, GE Silicone) are applied in basically the same manner, to both concrete and steel:

1. A gate or stoplog is inserted in the mouth of the intake bay and standing water is pumped out of the system. Any leaks or drips are stopped to the

extent possible.

2. Major marine growth is scraped from the interior walls, beams, pumps, racks, screens and any other components that are to be coated. Waterblasting may be used to further clean the surfaces. This material is then removed, often by vacuum truck, and transported to a landfill.
3. Gritblasting is used to clean and help dry the surfaces, which are then blown down with air. The grit is removed.
4. Depending on the season and ambient conditions, heaters, dehumidifiers, tarps, etc. may be required to achieve the necessary environment for product application in accordance with the manufacturer's specs. Appropriate ventilation may be needed to remove solvent vapors.
5. The first seal coat (generally a two-part epoxy sealer specified by the manufacturer) is applied by spray gun. It has not been necessary to trowel on or otherwise apply any heavy filler materials to even deteriorated concrete, unless it is to fill a crack or repair damage.
6. Several (one to three, depending on the manufacturer) prime coats are applied by spray gun. These may or may not be epoxy materials.
7. The top coat(s) of the non-fouling material are sprayed on to achieve the desired mil thickness.
8. In the case of the Epco-Tek only, the final coat is now blasted with either grit or baking soda (which is preferred for easy clean up) to activate the surface.
9. Equipment is removed and the bay is reflooded and returned to service.

The entire coating process, starting with initial blasting, has been accomplished in as little as four days, generally limited by drying time intervals. The cleaning process is variable, depending on the amount of material to be removed, but usually can be accomplished in two to three days. Overcoating should only require a water wash of exposed surfaces in good condition, followed by the recommended number of top coats. Any competent commercial painting contractor should be able to apply these coatings, but he will probably have to comply with enclosed space regulations in many cases. Technical oversight by a representative from the coating manufacturer is recommended.

BENEFITS AND COSTS

In a typical unit's untreated intake system consisting of two intake bays and a tunnel (with a coatable surface area of 25,000 square feet), removal and disposal of a year's worth of macrofouling by conventional means costs an average of $120,000 and takes about five days per event. This does not include the cost of lost generation or purchased power should that unit have to be removed from service for that cleaning. Replacement power for a 375 MW unit averages more than $60,000 per day (range

is $20,000 to $185,000 or more per day); if cleaning must be done during an unplanned outage, the total cost for cleaning and purchased power could be over $1,000,000.

At an average cost of $8 to $10 per square foot (labor and materials) the cost of painting this typical unit's intake system would be about $200,000 to $250,000, which would be paid off in reduced cleaning costs in about two years. Additional savings in increased condenser performance would also be realized but are more difficult to quantify.

The coatings tested to date have shown an effective life of five or more years after which they can be overcoated without the need for the multi-step application process of the first application. We have just completed our first overcoat of Bioclean; only a surface washing followed by two new coats of Bioclean were required and the cost was about one-half that of the original job.

Figure 1 graphs several options for calculating costs of maintaining an intake system acceptably free of mussel fouling. Line A indicates the cost of the biennial preventative conventional cleaning (by plant personnel) of an uncoated system that would be required to prevent significant degradation of plant performance. At the end of the 13 year test period, the total cost would be $1,560,000. Line B shows the estimated cost of cleaning the uncoated system by an underwater diving method that was recently demonstrated in our intake bays. Total cost of this option would be $760,000, however, this assumes there will be no economic penalty for plant shutdown to clean the intake tunnel. Line C indicates the accumulating costs of initial paint application followed by recoats every six years; included in this is a small amount of conventional cleaning in the intake bays every two years (coated tunnels don't require periodic cleaning). Total cost of the coated system with conventional cleaning is $620,000. The dotted line demonstrates the consequence of inaction; in this case, the operators are seen reacting to an unplanned outage that could occur every few years when massive quantities of accumulated mussels break free and occlude the condensers. The cost of this catastrophic event includes purchased power as well as cleaning for a one time total of $1,336,000. It would probably not be allowed to occur a second time! (Note: Lines A and C are based on real data, extrapolated from seven years of experience; Line B is estimated from costs of underwater cleaning of coated intake bays; The dotted line is speculative but not unbelievable.)

CONCLUSIONS

Unprotected water intake systems in marine waters are rapidly colonized and fouled by a rich community of macrofouling organisms. Of these, the species that causes

the most trouble to power plant cooling systems is the blue mussel. Utilization of antifouling and nonfouling coatings has been demonstrated to prevent unwanted mussel and other macrofouling growth from adversely affecting intake systems.

Of the many coating types available, most are not effective. The two preferred effective coating types are the silicone rubber "foul release" paints and the hard copper/epoxies. These coatings have been proven in actual use to be effective and long-lived. Their application, while expensive, quickly produces savings in terms of reduced cleaning and disposal costs and reduces the possibility of an unplanned unit outage due to loss of intake water.

While we feel we have obtained a level of confidence in our ability to control mussels in our intake systems, we will continue to investigate, evaluate and sponsor the development of new coatings or other innovative macrofouling control methods in an effort to improve our plants' performance.

TABLE 1
PANEL TEST EVALUATIONS

Coating Type	Code[1]	Year(s) Installed	YEAR-END RATING[2] Year 1	Year 2	Year 3	Year 4	Year 5	Year 6
Copolymer Copper Paint	USC	1989	1	3	5	D	D	-
Copolymer Copper Paint	CTP	1988	1	1	1	2	2	2
Copolymer Copper Paint	CSC	1991	1	1	1	-	-	-
Copolymer Copper Paint	PHC	1991	1	1	2	-	-	-
Copolymer Tin Paint	POT	1987	1	1	1	D	D	D
Silicone Rubber Paint	CBC	1987	1	1	2	2	2	3
Silicone Rubber Paint	CDX	1987	1	1	1	2	2	3
Silicone Rubber Paint	IIS	1987	3	5	5	D	D	D
Silicone Rubber Paint	II2	1993	1	-	-	-	-	-
Silicone Rubber Paint	CSL	1993	1	-	-	-	-	-
Silicone Rubber Paint	KBS	1988	3	3	3	4	D	D
Silicone Rubber Paint	KB2	1990	1	1	2	3	-	-
Silicone Rubber Paint	GES	1991	2	2	3	-	-	-
Silicone Rubber Paint	GES	1992	1	1	-	-	-	-
Copper/Epoxy Paint	HTC	1989	2	2	2	3	4	-
Copper/Epoxy Paint	HT2	1991	1	1	1	-	-	-
Copper/Epoxy Paint	HT3	1993	1	-	-	-	-	-
Copper/Polyester Epoxy	CCF	1992	1	1	-	-	-	-
Copper/Epoxy Paint	BAX	1992	5	D	-	-	-	-
Epoxy	SMS	1990	5	5	5	D	-	-
Epoxy	BES	1990	5	5	5	5	-	-
Epoxy	AC1	1993	5	-	-	-	-	-

TABLE 1, Continued

Coating Type	Code[1]	Year(s) Installed	YEAR-END RATING[2] Year 1	Year 2	Year 3	Year 4	Year 5	Year 6
Polyurethane	DCI	1991	5	5	D	-	-	-
Teflon Paint	RST	1987/8	5	5	D	D	D	D
Teflon & Antibiotic	RSA	1988	5	5	D	D	D	D
Silicone Grease	SSG	1987	5	5	D	D	D	D
Copper, Tacky Paint	DSC	1988/9	5	5	D	D	D	D
Silicone Hard Paint	WDI	1993	4	-	-	-	-	-
Fiberglass Control	CON	Yearly	5	5	5	5	5	5

[2] Rating Key: 1 = Excellent; 2 = Good; 3 = Fair; 4 = Poor 5 = Failed; D = Discontinued

[1] Code:

USC = Awlstar, U. S. Paint
CTF = Tin-Free Antifouling, CMP Chugoku
CSC = Micron CSC, Interlux
PHC = Horizons, Pettit Paint
POT = Horizons, Pettit Paint
CBC = Bioclean BC, CMP Chugoku
CDX = Bioclean DX, CMP Chugoku
II1 = Intersleek (blue), International Paint
II2 = Intersleek (white), International Paint
CSL = CSL560, CSL Silicones
KBS = Biox, Kansai Paint
KB2 = Biox 1990, Kansai Paint
GES = Exsil 2200, GE Silicones
HTC = Epco-Tek 2000 (sanded), Hi-Tek Chemical Co.
HT2 = Epco-Tek 2000 (blasted), Hi-Tek Chemical Co.
HT3 = Epco-Tek 3000, Hi-Tek Chemical Co.
CCF = Copper Clad, Ferro Industries
BAX = Barnacl-X, Enviro Coatings
SMS = Simsite, Simsite Chemical Co.
BZS = Superglide, Belzona Chemical Co.
AC1 = Arcor, Arcor Chemical Co.
DCI = Irathane, Devcon Coatings
RST = Scatt, Release Industries
RSA = Scatt with Compound 'X' Antibiotic, Starbrite Co.
SSG = Slipstream, Synthetic Lubricants, Ltd.
DSC = Sea Coat, Donar Chemical Co.
WDI = Wearlon, Decora Industries
CON = Sanded Fiberglass Control Plate

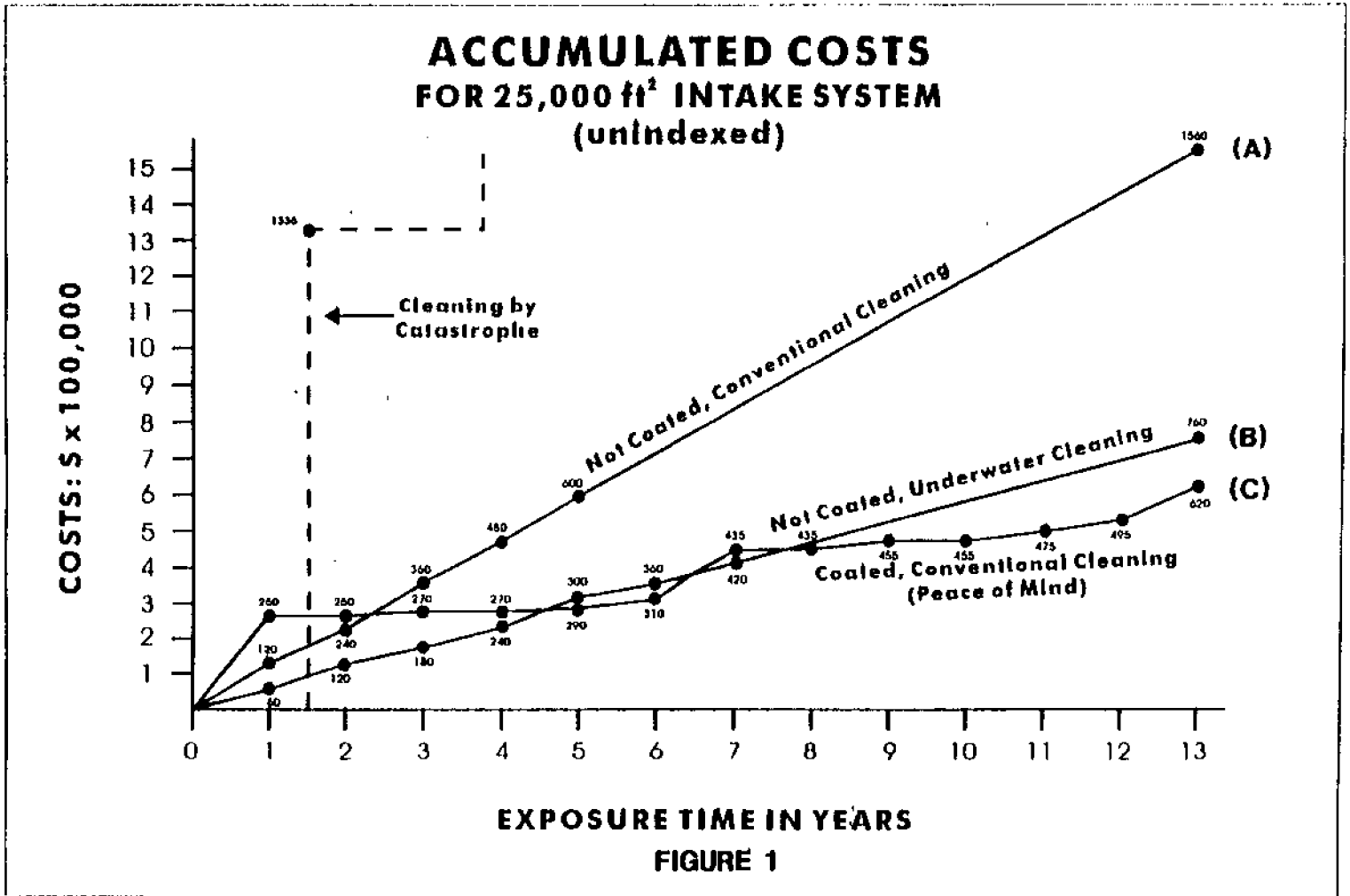

ACCUMULATED COSTS
FOR 25,000 ft² INTAKE SYSTEM
(unIndexed)
COSTS: $ x 100,000
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
1
2
3
4
5
6
7
8
9
10
11
12
13
EXPOSURE TIME IN YEARS
Cleaning by Catastrophe
1336
Not Coated, Conventional Cleaning
(A)
1560
600
480
360
260
120
60
Not Coated, Underwater Cleaning
(B)
760
435
420
360
310
300
290
270
270
240
240
180
Coated, Conventional Cleaning
(Peace of Mind)
(C)
620
495
475
455
455
435
260
240
120

FIGURE 1

Biological Control of Zebra Mussels *Dreissena Polymorpha* by Indigenous Bacteria and Their Products

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ABSTRACT

The use of indigenous bacteria and their products as biological control agents of zebra mussels *Dreissena polymorpha* was investigated. A large library of bacterial isolates was established from stressed zebra mussels in our laboratory, and four isolates HU-1, -2, -3, and -4 were used to examine the model of their antagonistic effects on adult zebra mussels. Bacterial isolates were grown to their mid-exponential phase, harvested by centrifugation, resuspended in phosphate buffer, and used to inoculate zebra mussels (inoculum of 10^7 bacterial cells in 10 μL phosphate buffer). The mortality of inoculated mussels was 90, 80, 100, and 60% for HU-1, -2, -3, and -4, respectively, within 5 days after administration. Control zebra mussels receiving only phosphate buffer did not show mortality. These results demonstrated that bacterial isolates can be effective antagonists against zebra mussels. We also concentrated microbial products from the bacterial cultures for molecular weights of 1-3, 3-10, and >10-kDalton (kDa) fractions, and introduced the obtained bacterial products of different fractions into zebra mussels for mortality test. Results of mussel mortality indicated that the fractions 1-3 and 3-10-kDa of HU-1 and -4 were effective in killing of over 60% of the mussels within a 10-day period. However, more promising results were obtained with a >10-kDa fraction of HU-2 and -3, over 80% mortality within 3-day period. Chemical analysis of the bacterial products revealed the presence of polysaccharides and proteins in all fractions of four isolates, but no DNA, 2-keto-3-deoxyoctanoic acid (KDO) or uronic acid. Preliminary results of this study suggested that the selective bacteria and their products may have potential applications in the development of antifouling coatings and bioactive mulluscides.

INTRODUCTION

The introduction and rapid spread of the zebra mussel, *Dreissena polymorpha,* in the freshwaters of North America have caused serious problems for water users, particularly in the Great Lakes area. The zebra mussel is a bivalve mollusk, native to the Caspian and Black Seas, which has adapted to low salt and freshwater conditions (Ludyanskiy *et al.*, 1993). Since the first report of zebra mussels in Lake St. Clair in 1988 (Hebert *et al.*, 1989), they have spread from the Great Lakes south as far as New Orleans on the Mississippi, and east as far as the Hudson River in New York (Ross, 1994). Such a rapid increase in the range of distribution is largely due to the high productivity of this mussel in combination with a swimming pelagic larval stage and the ability to attach onto vessels moving from infested to uninfested areas. In addition, the improved water quality in North America in the late 80's may also facilitate the colonization of this introduced species. As a result, damage has been observed in freshwater ecosystems, specifically a loss of indigenous species resulting in a decrease in biodiversity. Power plants and water treatment facilities have been in danger of having their water intakes blocked due to the fouling of zebra mussels around the Great Lakes. In addition, due to the consumption of primary producers by zebra mussels resulting in a decrease in the availability of these food sources to existing food chains, the fishing industry has suffered along with other ecologically and economically important organisms. The zebra mussel is also capable of colonizing fish and other mussel species that are of economic importance. If the spread continues, an economic loss has been estimated at over 5 billion dollars by the year 2000 in the Great Lakes alone (Ross, 1994).

The available control strategies include the use of chemicals, particularly chlorine and biocides that not only cause pollution of the freshwaters but also raise concern over their effect on the food chain. Chemical treatments by chlorination and biocides are often required to eradicate zebra mussel fouling, but intensive post-treatment cleaning is also needed due to the strong attachment of zebra mussel byssal threads to surfaces. Chemical treatments also have the disadvantages of leaving residues in the environment. In the case of chlorination, organic materials in freshwater may become chlorinated, generating toxic organic species in the ecosystem.

Another approach to control is biological, either by microorganisms or by their bioactive compounds. Previously, we established that the diversity of heterotrophic bacteria found in zebra mussels decreased over time when mussels were exposed to forms of environmental stress, including elevated temperature or starvation (Mitchell and Maki, 1993; Maki and Mitchell, 1994). The bacteria that become enriched during stress to the animals can be isolated and used in the biological control of zebra mussels (Mitchell and Maki, 19934). Though advancement in this area has been slow in the past, the tools of genetic manipulation make it possible to amplify production of microbial products which

can lead to their commercialization as biocontrol agents. Microorganisms and/or their bioactive compounds have been subjects of active research and development for use in the control of various plants and pests. The objective of our study were to 1) demonstrate that microorganisms can be used as antagonists to zebra mussels, and 2) examine the antagonistic effect of microbial products on mussels.

MATERIALS AND METHODS

Isolation of bacteria from stressed zebra mussels

Adult zebra mussels were collected in Lake Erie at Buffalo, New York and transported overnight to Cambridge, Massachusetts. These mussels were held in aquaria (10 gallon capacity) containing 10% artificial lake water (ALW) which consisted of artificial sea water (Instant Ocean, Aquarium Systems, Inc., Eastlake, OH) diluted to 10% (v/v) with distilled water. Water in the aquaria was aerated by using an air pump. The aquaria were kept in an environmental chamber at 4°C. Zebra mussels were not fed because we had determined they could survive extended period of starvation.

To isolate microorganisms, zebra mussels were taken out of the aquarium water, placed in sterile Petri dishes and left at room temperature. After 24 h at room temperature, 15 mL of ALW were added to the dish before incubation and observation of mortality. These mussels were stressed by being held at an elevated temperature (22°C) to induce the development of microbial infection. Once they were observed to be dead by failure to open or close shell valves (Leitch and Sim, 1991), isolation of the bacteria from tissues of the dead mussels was performed immediately. Dead mussels were transferred into a clean sterile Petri dish where dissection was conducted aseptically. The external shell surfaces of the mussels were sterilized by cleaning with an ethanol-saturated cotton swab and then dried. Mussel tissue was extracted from the shell and homogenized in a glass grinder containing 10 mL 0.0375 *M* phosphate buffer. The buffer was composed of (g liter^{-1} of deionized water): Na_2HPO_4 5.3 g, NaH_2PO_4 5.2 g, and Bacto-peptone (Difco Lab., Detroit, MI) 1.0 g. Following a series dilution in the same buffer, 100 µL of proper dilutions were used to inoculate spread plates of Plate Count Agar (PCA) (Difco Lab., Detroit, MI). The plates were incubated at 26°C. After at least 48 h, well developed colonies on PCA plates were streaked onto new PCA plates for a purity check and for use in a morphological description of the isolates.

When the purity of an isolate was confirmed by the appearance of colony type on PCA plates and by microscopic examination, Gram staining was carried out to determine which of the identification procedures would follow. The Biolog System with GN Microplates (Biolog, Hayward, CA) was used to identify Gram negative isolates. API NFT and/or

20E identification kits (bioMerieux Vitek. Inc., Hazelwood, MS) was used when no identification was available from the Biolog database. All bacterial isolates were preserved on PCA slants and kept at 4°C. Four isolates, designated HU-1, -2, -3, and -4, were selected to test as potential antagonists to zebra mussels.

Preparation of bacterial cells and inoculation of zebra mussels

Nutrient Broth medium (Difco lab, Detroit, MI) in 100 mL batches was prepared, sterilized, and inoculated with each of the bacterial isolates (HU-1 through 4) from either slants or plates. Inoculated flasks were cultured at 26°C on a water bath shaker. When the isolate culture reached its mid-exponential phase of growth (A_{600} = 0.9 - 1.1), cells in culture were harvested by centrifugation at 10,000 x *g*. After decanting the supernatant, pellet of bacterial cells was resuspended in 0.0375 *M* phosphate buffer containing 0.1 % peptone (Difco Lab., Detroit, MI) and centrifuged again. The supernatant was discarded and cells were resuspended in 50 mL phosphate buffer. The density of these cell suspension was determined by staining with acridine orange (0.01%, w/v) and a direct count with epifluorescence microscopy (Hobbie *et al.*, 1977).

Adult zebra mussels were taken out of the aquarium water and placed in sterile Petri dishes, 10 animals for each treatment. These animals were conditioned to room temperature for about 6 h, then placed in a sterile laminar flow hood to evaporate the external water for 2 h. After drying, mussels were injected with 10 µL of a bacterial suspension. The projected cell population (10^7 bacteria) in 10 µL was made through dilution of the cell suspension with phosphate buffer. All animals were held in the Petri dishes for 12 h. Afterwards, 10 mL of ALW were added into each Petri dish. All mussels were incubated at 22°C for observation of mortality. Zebra mussel death was confirmed by their inability to open or close their shells when mussels were touched with sterile tweezers (Leitch and Sim, 1991).

Separation of microbial extracellular materials

Bacterial isolates (HU-1 through 4) were cultured as described above to stationary phase and cells were separated from the culture by centrifugation at 10,000 x *g* for 30 min. The supernatant was then filtered though a 0.2 µm-pore-size membrane filter (Gelman Sciences, Ann Arbor, MI). The filtrate then was concentrated using an ultrafiltration stirred cell (Amicon Co., Danver, MA) with filters having molecular weight cut-offs of 10-kDa, 3-kDa, and 1-kDa in sequence to a volume of about 1.0-1.5 mL. This volume was used in testing the effects of bacterial products on mussels. After injecting the mussels with 10 µL of the concentrated bacterial products, they were incubated in ALW at 22°C. Mortality of zebra mussels was observed and recorded over incubation time at 24 h intervals.

Analysis of chemicals composition of bacterial products

Bacterial product concentrates were desalted with ultrapure water and lyophilized to obtain them in dry form. Chemical analyses were performed on 1 mg of the lyophilized material dissolved in a proper volume of solvent that was relevant for the analyses. Five categories of analysis were conducted: 1) protein with Coomassie blue (Bradford, 1976), 2) hexose sugars assayed by the anthrone method (Daniels *et al.*, 1994), 3) uronic acids by the *m*-phenylphenol method (Blumenkrantz and Asboe-Hansen, 1973), 4) cell wall components by thiobarbituric method for 2-keto-3-deoxyoctonate (Osborn, 1963), and 5) deoxyribonucleic acid (DNA) by diphenylamine method (Master, 1965).

RESULTS AND DISCUSSION

Bacterial isolates and mortality of zebra mussels

All four bacterial isolates (HU-1, -2, -3, and -4) grew in nutrient broth, and maximum growth was reached within 55 h at 26°C (data not shown). That these isolates grew better at 26°C than 37°C may be due to their origin in a freshwater habitat where the temperature is generally lower. All four isolates are Gram negative rods and were associated with dead zebra mussels after exposing the mussels to stress by incubation at room temperature (22°C). Bacteria are common causes of disease (Smith, 1977), and freshwater invertebrates may serve in some way as carriers or reservoirs of diseases causing microorganisms. Mitchell and Maki (1993) and Maki and Mitchell (1994) reported that a shift in microbial population diversity was evident when temperature or starvation stress was applied to zebra mussels. A range of diverse microorganisms that were morphologically distinct has been observed in tissues of natural zebra mussels by scanning electron microscopy (SEM) (J.-D. Gu and R. Mitchell, unpublished data).

Microorganisms isolated from dead zebra mussels may be effective antagonists against them. The mortality rates were 90, 80, 100, and 60% for HU-1, -2, -3, and -4, respectively, within 5 days after introduction (10^7 cells carried by 10 µL of phosphate buffer) (Fig. 1). Over the same period of incubation, control zebra mussels that were injected with the phosphate buffer alone did not show mortality. The antagonistic effects of bacterial isolates on zebra mussels were also temperature dependent. They were more pronounced when injected zebra mussels were incubated at 22°C than at 10°C (J.-D. Gu and R. Mitchell, unpublished data). Mussel mortality was not observed within the same period of time at 10°C, but a similar pattern of mortality was observed after switching the incubation temperature of the infected mussels from 10°C to 22°C. This suggested that mortality was temperature dependent as a result of biological activity. Bacterial infection was the primary mechanism by which zebra mussels were killed in this study.

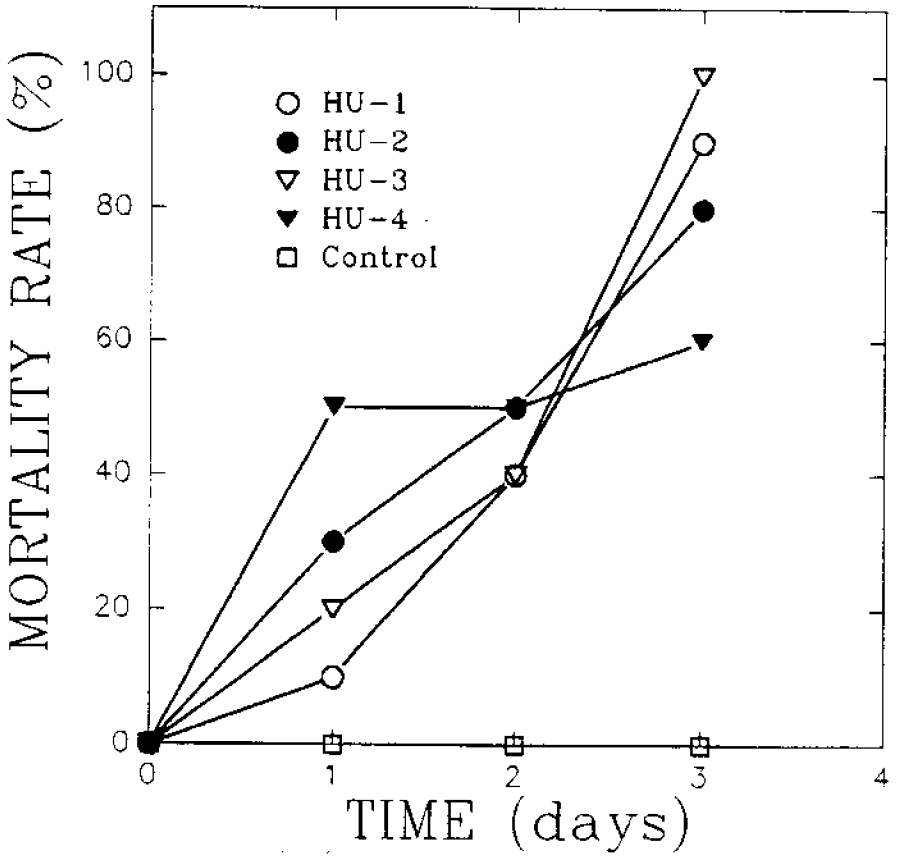

Fig. 1. Effects of bacterial isolates, HU-1, HU-2, HU-3, and HU-4, on mortality rates of adult zebra mussels incubated at 22°C. Bacterial cells were introduced at 10^7 in 10 µL phosphate buffer.

Bacterial products and mortality rates

These bacterial isolates were further studied with respect to the effect of their products on mortality of zebra mussels and the chemical composition of the these products. Bacterial products that were concentrated from stationary phase cultures were demonstrated to have lethal effects on adult zebra mussels. We found that the three fraction concentrates (1-3, 3-30, and >10-kDa) of each bacterial isolate exhibited varying effects on mussel mortality (Fig. 2). There were also varying effects on mortality between isolates. The effect on mussel mortality of different fractions was as follows: (1-3) > (3-10) > 10 for HU-1; 10 > (3-10) > (1-3) for HU-2; 10 > (3-10) > (1-3) for HU-3; and (1-3) = (3-10) >> (>10) for HU-4. Mitchell (1984) and Maki and Mitchell (1986) hypothesized that exopolymers of attached bacteria react with lectins on the larval surface triggering the settlement and possibly metamorphosis of larvae of the spirobid larvae *Janua brasiliensis*. Maki *et al.* (1990) reported that bacterial biofilms and/or bacterial exopolymers may be involved as associative cues in the attachment of cypris larvae of the barnacle *Balanus amphitrite*. Our results indicated that extracellular materials from bacteria may also contain toxins that appear to be responsible for the mortality of the zebra mussels we observed. An appealing approach for controlling the infestation of zebra mussel and other macro-fouling is the use of an impregnated coating with a biochemical component which is either toxic or repellent to the targeted animals (Mitchell and Maki, 1993).

Analyses to chemically characterize the bacterial products indicated the presence of polysaccharides and proteins, but not DNA, 2-keto-3-deoxyoctanoic acid (KDO) or uronic acid in all fractions of the four isolates. Concentration of each chemical component varied between the isolates and the fractions (data not shown). The chemical components we measured only accounted for less than 50% of the total material obtained. Elucidating the chemical composition of the remaining material and the identification of the active components are the foci of our current research. A heat stable extracellular component from a marine bacterium, less than 500 Da, kills larvae of *B. amphitrite* within a few hours of exposure (Holmstrom *et al.*, 1992). This biologically active component does not show any evidence of the presence of proteins or peptides (Holmstrom *et al.*, 1992). We also tested a methylene chloride extract of the supernatant from a bacterial culture and found that it caused adult zebra mussel mortality after 5 days of exposure (J.-D. Gu and R. Mitchell, unpublished data). The low molecular weight material from bacteria may also have a potential application in the development of a zebra mussel control strategy.

In conclusion, we have demonstrated that bacteria isolated from stressed zebra mussels can be used as antagonists and effectively cause mortality of adult zebra mussels. The extracellular products of these bacteria also exhibit lethal effects on zebra mussels. Varying degrees of mortality of adult mussels were observed after they were injected with either whole cells or extracellular products of the different isolates. The goals of this

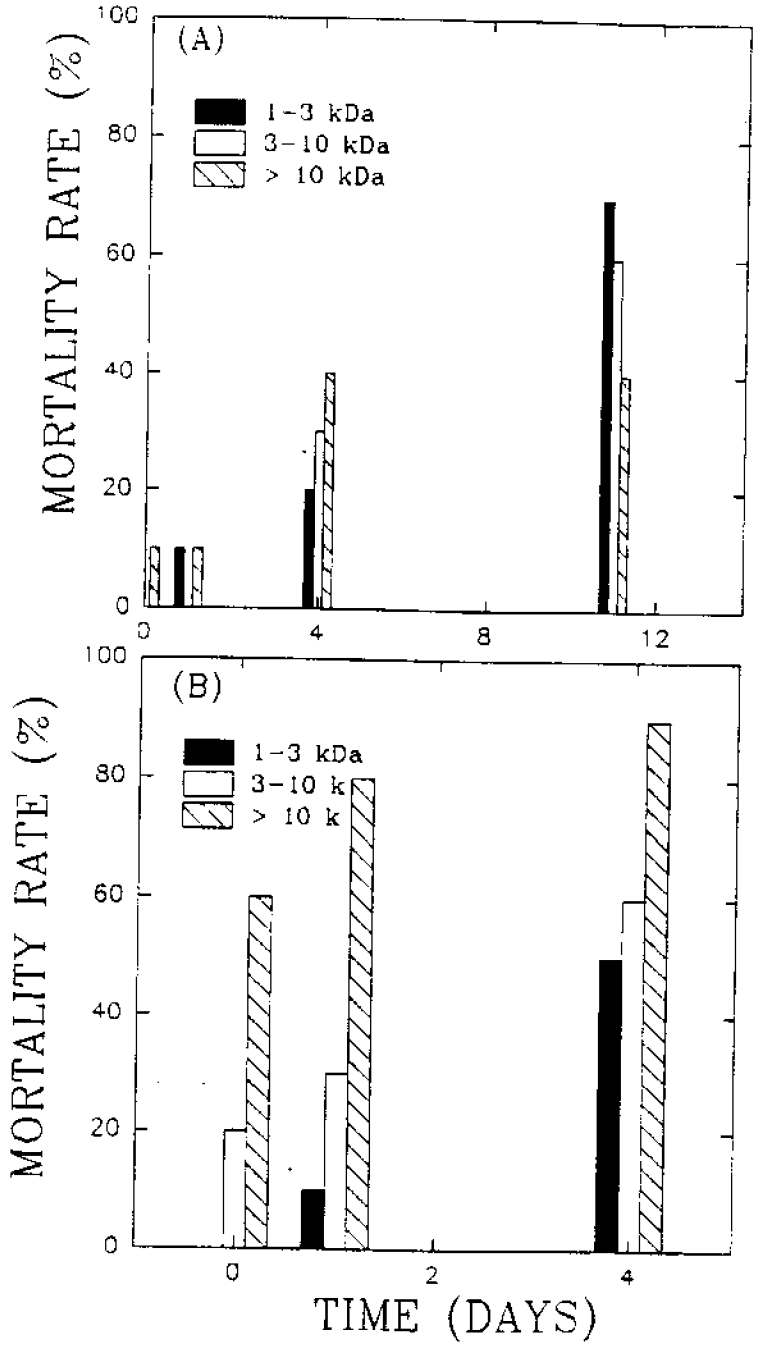

Fig. 2. Effects of bacterial product concentrates of HU-1 (a), HU-2 (b), HU-3 (c), and HU-4 (d) on zebra mussel mortality. Products were concentrated from their stationary phase cultures to fractions of 1-3, 3-10, and >10-kDa molecular weights. Incubation temperature was 22°C.

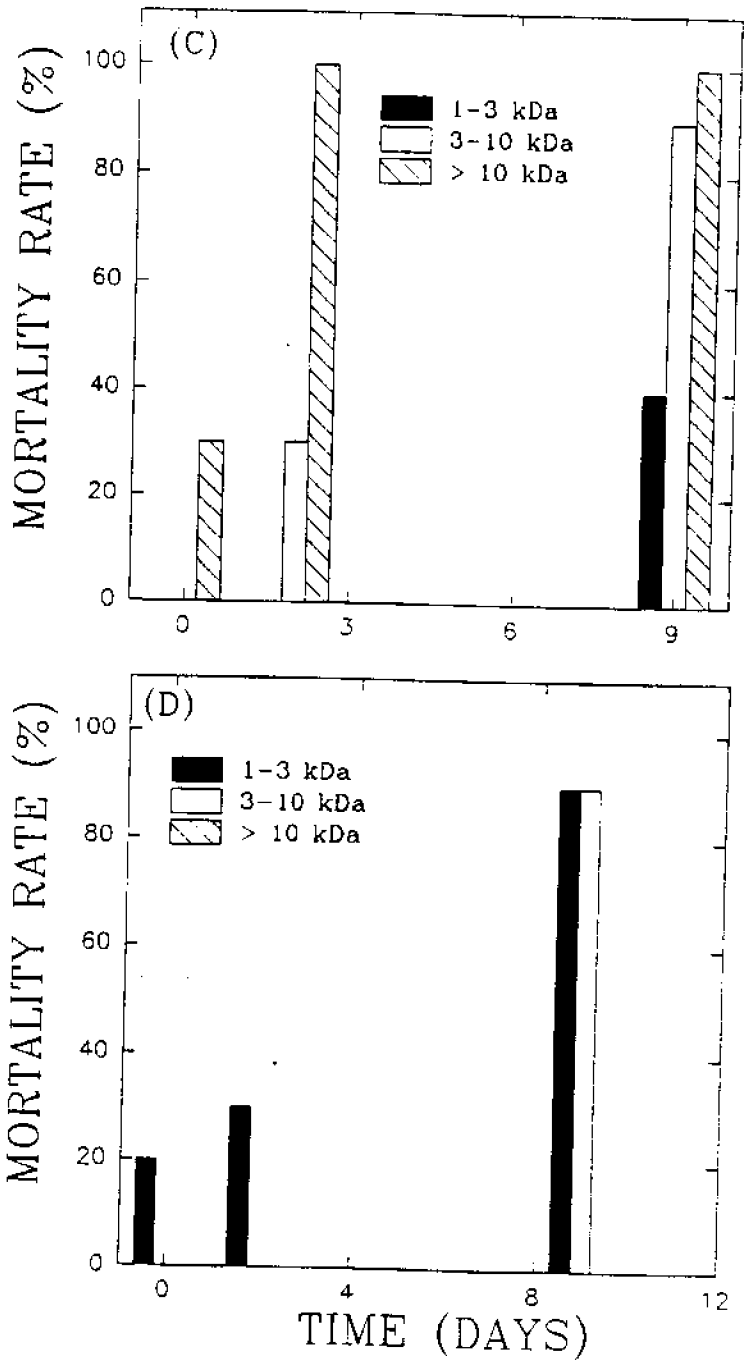

Fig. 2. Continued

research are to isolate the chemical component(s) produced by bacteria that have specificity for killing or repelling zebra mussels, to identify their chemical structure, and ultimately to incorporate these products into a coating for testing in natural environments.

ACKNOWLEDGMENTS

This research was supported in part by grants to Harvard University from Ontario Hydro; Ontario Ministry of the Environment; and the U.S. National Oceanic and Atmospheric Administration Sea Grant Program.

REFERENCES

Blumenkrantz, N., and G. Asboe-Hansen. 1973. New method for quantitative determination of uronic acids. *Anal. Biochem.* **54**:484-489.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**:248-254.

Daniels, L., R.S. Hanson, and J.A. Phillips. 1994. Chemical analysis. p.512-554. *In* P. Gerhardt, R.G.E. Murray, W.A. Wood, and N.R. Krieg, eds., *Methods for general and molecular bacteriology*, American Society for Microbiology, Washington DC.

Hebert, P.D.N., B.W. Muncaster, and G.L. Mackie. 1989. Ecological and genetic studies on *Dreissena polymorpha* (Pallas.): a new mollusc in the Great Lakes. *Can. J. Fish. Aquat. Sci.* **46**:1587-1591.

Hobbie, J.E., R.J. Daley, and S. Jasper. 1977. Use of Nuclepore filters for counting bacteria by fluorescence microscopy. *Appl. Environment. Microbiol.* **33**:1225-1228.

Holmstrom, C., D. Rittschof, and S. Kjelleberg. 1992. Inhibition of settlement by larvae of *Bamus amphitrite* and *Ciona intestinalis* by a surface-colonizing marine bacteria. *Appl. Environ. Microbiol.* **58**:2111-2115.

Leitch, E.G., and B. Sim. 1991. Methods for determining live-dead zebra mussels (*Dreissena polymorpha*). Report No. 91-92-K, Ontario Hydro., Canada.

Ludyanskiy, M.L., D. McDonald, and D. MacNeill. 1993. Impact of the zebra mussel, a bivalve invader. *Bioscience*. **43**:533-544.

Maki, J.S., and R. Mitchell. 1986. The function of lectins in interactions among marine bacteria, invertebrates, and algae. p 409-425. *In* D. Mirelman, ed., *Microbial lectins and agglutins: properties and biological activity*, John Wiley & Sons, Inc., New York.

Maki, J.S., and R. Mitchell. 1994. Effect of starvation stress on the heterotrophic bacterial flora of the zebra mussel *Dreissena polymorpha*. *In* Proc. 4th International Zebra Mussel Conference, Madison, Wisconsin.

Maki, J.S., D. Rittschof, M.-O. Samuelsson, U. Szewzyk, A.B. Yule, S. Kjellberg, J.D. Costlow, and R. Mitchell. 1990. Effect of marine bacteria and their exopolymers on the attachment of barnacle cypris larvae. *Bull. Mar. Sci.* **46**:499-511.

Master, R.W. 1965. An improved diphenylamine method for the estimation of deoxyribonucleic acid. *Nature (London)*. **206**:93.

Mitchell., R. 1984. Colonization by higher organisms. p. 189-200. *In* K.C. Marshall, ed., *Microbial adhesion and aggregation*, Dahlem Konferenzen, Berlin.

Mitchell, R., and J. Maki. 1993. Use of microorganisms for unconventional control treatments of zebra mussels. *In* Proc. 3rd International zebra mussel conference, Toronto, Canada.

Osborn, M.J. 1963. Studies on the Gram-negative cell wall. I. Evidence for the role of 2-keto-3-deoxyoctanate in the lipopolysaccharide of *Salmonella typhimurium*. *Proc. Nation. Acad. Sci.* **50**:499-506.

Ross, J. 1994. An aquatic invader is running amok in U.S. waterways. *Smithsonian*. February. 40-51.

Smith, H. 1977. Microbial surfaces in relation to pathogenicity. *Bacteriol. Rev.* **41**:475-500.

The Survival of Zebra Mussels (*Dreissena polymorpha*) and Asian Clams (*Corbicula fluminea*) Under Extreme Hypoxia

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ABSTRACT

Zebra mussels (*Dreissena polymorpha*) and Asian clams (*Corbicula fluminea*) were exposed to acute hypoxia (Po_2 < 3% of full air oxygen saturation) to determine anoxia tolerance and assess O_2 depletion as an efficacious, nonchemical, macrofouling control strategy. Adults of both species were acclimated to 5°, 15° or 25°C for 14 days prior to testing. After acclimation, paired samples of 30 individuals from each acclimation group were placed in separate 5 l air tight plastic chambers containing 4 l of dechlorinated tap water, leaving a 1 l gas head-space. Paired samples of both species were held in either aerated (control) or anaerobic (treatment) water, made anoxic by continuous gassing with N_2, and maintained at test temperatures of either 15°C or 25°C. Media O_2 concentrations and mortalities were recorded daily. Dead individuals were removed and their shell lengths (SL) measured to the nearest 0.1 mm. Container medium was replaced every 2-3 days with previously deoxygenated or oxygenated water at the appropriate test temperature. Negligible mortality (< 3%) was recorded in all aerated control samples of both species. Mean anoxia tolerance (MAT) for *D. polymorpha* was significantly ($P<0.05$) affected by acclimation temperature at both test temperatures. At 25°C, MAT was 53.1 h (S.D. = ±23.9), 61.1 h (±28.8) and 82.8 h (±43.1) for 5, 15 and 25°C acclimated mussels, respectively. At 15°C, corresponding values were 228.8 h (±132.3), 371.2 h (±178.0) and 428.0 h (±179.2). Higher anaerobic metabolic rates among 5°C acclimated mussels may have induced more rapid accumulation of toxic end-products leading to reduced survival. Temperature acclimation did not affect MAT in *C. fluminea*. At a test temperature of 25°C, MAT ranged from 250.3 h (±87.9) to 283.2 h (±88.4) across the three acclimation groups. At a test temperature of 15°C, MAT was 841.7 h (± 327.4) for the single acclimation temperature of 15°C tested. Body size did not significantly influence anoxia tolerance of *D. polymorpha* over the size range used (12-34 mm SL) although larger specimens of *C.fluminea* showed decreased tolerance at a test temperature of 25°C (10-30 mm SL). Both species were less tolerant of anoxia than most native North American freshwater bivalves, suggesting that exposure to anoxic conditions may be an

efficacious nonchemical control technology, particularly for *D. polymorpha* which was 2-7 times less tolerant than *C. fluminea* depending on test and acclimation temperature. Low anoxia tolerance may restrict the distribution of both species to well oxygenated rivers and the epilimnetic zone of lakes.

INTRODUCTION

Zebra mussels (*Dreissena polymorpha*) and Asian clams (*Corbicula fluminea*) are both major macrofouling species of municipal potable water, agricultural, industrial and power station raw water systems in North America (McMahon, 1983; Claudi and Mackie, 1993). *C. fluminea* is found in 36 of the contiguous states of the United States (Counts, 1986) as well as in Hawaii and Mexico (McMahon, 1991). Macrofouling by this species has been estimated to cost the power industry over one billion dollars annually (Isom, 1986). *D. polymorpha* was introduced to the United States from Europe in 1986 into the Detroit River - Lake St. Clair region of the Great Lakes (Mackie *et al.*, 1989) and has since spread rapidly throughout the Great Lakes, St. Lawrence River and the navigable inland waterways of the Mississippi Drainage (Zebra Mussel Information Clearinghouse, 1993). A projected cost of two billion dollars has been proposed for the control of *D. polymorpha* over the decade of the 1990's in the Great Lakes alone (Roberts, 1990) with this figure likely to rise exponentially as mussels continue to expand their range in North America.

A further implication of the spread of zebra mussels throughout North American freshwater drainages could be a dramatic increase in the use of molluscicides for mitigation and control of their macrofouling in once-through raw water systems, exacerbating the biocide load already carried by our continental river systems. The likelihood of stricter future regulation of molluscicide usage, will require development of readily implemented, cost-effective, environmentally neutral control technologies for freshwater macrofouling bivalves (*i.e.*, *D. polymorpha* and *C. fluminea*).

Both *D. polymorpha* (Mikheev, 1984; Mackie *et al.*, 1989) and *C. fluminea* (Fast, 1971; McMahon, 1983) have been reported to be intolerant of acute hypoxia (*i.e.*, low oxygen concentrations). Neither species can tolerate oxygen depleted waters below the thermocline of lakes and both species are excluded from chronically hypoxic waters (for reviews see McMahon, 1983, 1991 and Mackie *et al.*, 1989). Indeed, exposure to oxygen scavenging chemicals has been reported to be an effective means of mitigating *C. fluminea* macrofouling in raw water intake embayments (Smithson, 1986).

Such reports strongly suggest that exposure to anoxia may be an efficient, environmentally neutral means of controlling zebra mussel and Asian clam fouling in raw water systems. However, a search of the literature revealed no hard information on anoxia tolerance in *C. fluminea* and only a preliminary study on *D. polymorpha* in which the effects of temperature acclimation and accumulating anaerobic toxins were not accounted for (Mikheev, 1964). This study was undertaken to more fully detail the anoxia tolerance of both *D. polymorpha* and *C. fluminea* at different experimental and acclimation temperatures. The results are discussed in relation to both species' depth distributions and the efficacy of anoxia as a control strategy.

MATERIALS and METHODS

Specimens of *D. polymorpha* were collected from Black Rock Lock, on the Niagara River, at its inlet from Lake Erie in Buffalo, New York. Mussels were scraped from a guide wall on the upstream entrance to the lock. Specimens of *C. fluminea* were collected from the water discharge stream of the U.S. Army Corps of Engineers, Lewisville Aquatic Plant Research Facility which is situated directly below the dam of Lake Lewisville in Denton County, Texas. Water from the lake is gravity fed into a series of experimental ponds that discharge into a canal that empties into the Elm Fork of the Trinity River. Asian clams were collected from the discharge canal, 20 m downstream of the Aquatic Plant Research Facility.

Following collection, zebra mussels were transported overnight, wrapped in moistened paper toweling, in insulated containers with frozen refrigerant packs to keep mussels cool. Mussels arrived in good condition with little observable mortality and were immediately transferred to a 284 l refrigerated holding tank containing aerated, dechlorinated, City of Arlington tap water and maintained at 5°C without feeding until utilized in experiments. Asian clams were returned to laboratory immersed in water from the collection site within 3 h of collection. They were placed in a 284 l holding tank in continuously aerated, dechlorinated tap water maintained at a constant temperature of 15°C on a 12:12 h light-dark cycle without feeding prior to experimentation. Both species have been successfully held in our laboratory under these conditions for greater than six months without significant tissue biomass loss (Chase and McMahon, 1994; Cleland *et al.*, 1986).

All experiments were initiated within 40 days of collection. Prior to experimentation, samples of individuals from each species were acclimated to 5°, 15° or 25°C for 14 days in plastic tanks holding 17 l of continuously aerated tap water. Following acclimation, 30 adult individuals from each species acclimation group were placed in 9 cm diameter by 5 cm high glass crystallization dishes which were submersed in approximately 4 l of water held in an air-tight, 5 l plastic container (22 cm long X 22 cm wide X 12 cm high) leaving a 1 l gas head-space. Water in these containers was continuously bubbled with N_2 to deplete media of oxygen. The tolerance of each zebra mussel acclimation group to prolonged anoxia was tested at both 15°C and 25°C (± 0.5°) in a refrigerated incubator under constant darkness. When Asian clams were similarly tested at 25°C, results indicated that temperature acclimation did not affect survival of anoxia (see below). Therefore, only one acclimation treatment (15°C) was tested at the 15°C test temperature. Control treatments consisting of identical acclimation groups in media bubbled with air rather than N_2 were run concurrently.

Media oxygen saturation in test containers was measured daily with a polarographic silver-platinum oxygen electrode (YSI Model 53). The media was changed in

experimental and control containers every two to three days. In all cases, the replacement media was at the test temperature and either aerated (control) or depleted of oxygen by nitrogen bubbling (experimental) before being added to test containers.

The viability of all individuals was tested daily. The posterior mantle edges and siphons of all gaping zebra mussels were gently prodded with the tip of a blunted dissection needle. Individuals which failed to close valves under such stimulation were considered dead. All dead zebra mussels were gaped beyond the limits of normal valve activity. As Asian clams sometimes do not gape on death, viability testing required forcing the tip of a dissection needle several millimeters between the posterior valve margins in the region of the siphons. Living individuals resist needle entry strongly by tight clamping of their valves (McMahon *et al.*, 1992). In contrast, dead clams offer little resistance and fail to close their valves after needle removal. Such testing is not damaging to clams as indicated by high survival of control individuals. For both species, viability testing required removal of the container from the gas source for not more than 5 min and was carried out on submerged individuals to minimize exposure to oxygen. Viability testing continued until 100% mortality was achieved in all samples exposed to anoxia.

RESULTS

Throughout the experimental period at test temperatures of 15° and 25°C, daily measurements indicated that the Po_2 (partial pressure of oxygen) of chamber media bubbled with N_2 never exceeded 5 torr and was usually below 3 torr (*i.e.*, < 3% of full air O_2 saturation). Thus, test specimens were essentially exposed to anoxic conditions. Zebra mussels in aerated normoxic conditions (*i.e.*, full air saturation with oxygen) readily attached to the walls and floor of holding tanks at 15° and 25°C regardless of prior temperature acclimation experience. In contrast, zebra mussels held under anoxic conditions never produced byssal attachment threads at either test temperature. In addition, individuals which had attached to the shells of other mussels prior to experimentation routinely released from their byssal holdfasts well in advance of death.

Multiple factor ANOVA showed that temperature acclimation had a significant effect on anoxia tolerance in *D. polymorpha* at both test temperatures (Tables 1 and 2). Mean survival increased with increasing acclimation temperature in *D. polymorpha* with the mean tolerance of 5°C acclimated mussels being significantly lower than that of 25°C acclimated mussels (Tukey's Multiple Comparison, $P < 0.5$, Table 1, Fig. 1). Similarly, at 15°C, mean tolerance time of 5°C acclimated mussels was significantly lower than those of either 15° or 25°C acclimated specimens. In contrast, there was no difference in the mean anoxia tolerance time of temperature acclimated groups of

C. fluminea at a test temperature of 25°C (Table 3, Fig. 1). Therefore, at the subsequent test temperature of 15°C, anoxia tolerance was only determined for clams acclimated to the median temperature of 15°C (Table 3, Fig. 1).

Table 1
Multiple Factor ANOVA for testing for differences in mean survival time for individuals of *Dreissena polymorpha* acclimated to 5, 15 and 25°C and exposed to prolonged anoxia at 25°C.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square Error	F-Ratio	Probability
Covariate Shell Length	1414.82	1	1414.815	1.306	0.256
Acclimation Temperature	13637.96	2	6818.98	6.293	0.0028**
Residual	93185.49	86	1083.55		
Total	108773.58	89			

** Significant effect at $P \leq 0.05$

Mean survival times for different acclimation groups of *Dreissena polymorpha* exposed to prolonged anoxia at 25°C with Tukey Multiple Range Analysis for significant difference.

Acclimation Temp. (°C)	Mean Hours Survived	n	Standard Error of the Mean	Range	Signif. Diff. (P<0.05)
5	53.08	30	4.36	9.0 - 104.5	
15	61.13	30	5.26	9.0 - 129.0	
25	82.80	30	7.88	34.0 -156.0	

Specimens of *D. polymorpha* in all three acclimation groups appeared to be considerably less tolerant of anoxia than those of *C. fluminea* (Figs 1 and 2). At a test temperature of 15°C, mean anoxia tolerance ranged from 229 - 428 h, in 5°C and 25°C acclimated mussels, respectively. Mean tolerance of 15°C acclimated specimens of *C. fluminea* was 842 h, twice that of the highest levels recorded in zebra mussels (Fig. 1). At a test temperature of 25°C, mean tolerance times for zebra mussels

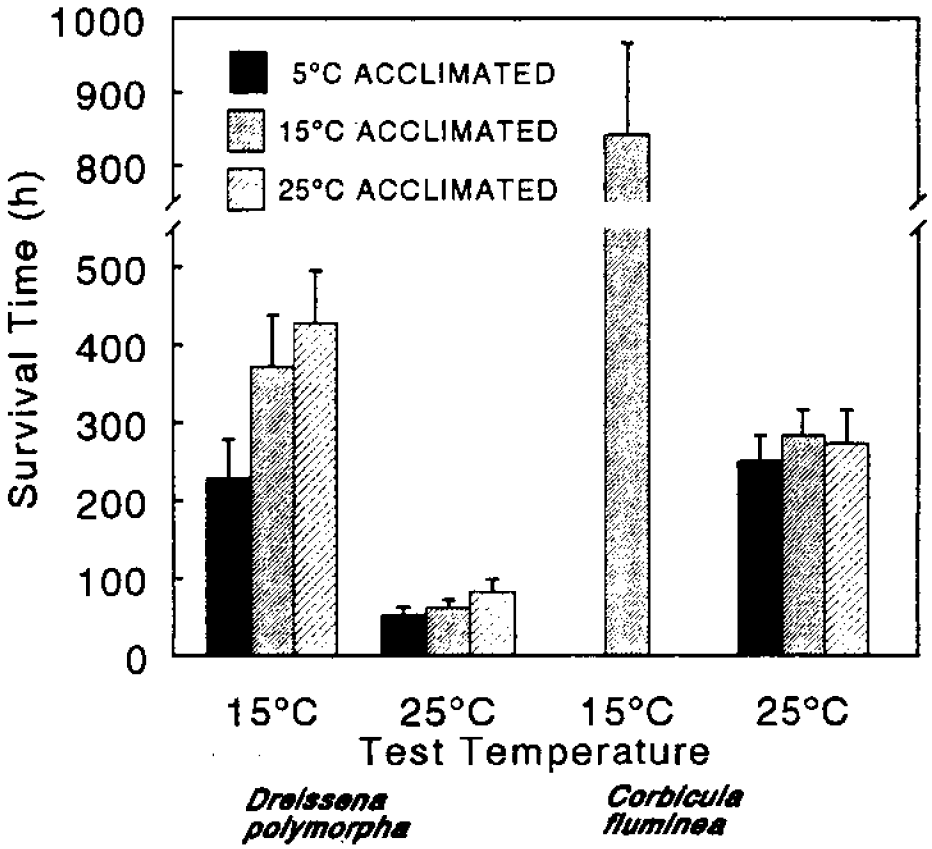

Figure 1. *The effects of test temperature and prior temperature acclimation on mean survival time in hours of zebra mussels* (Dreissena polymorpha) *and Asian clams* (Corbicula fluminea) *under anoxic stress (Po_2 < 5 torr) induced by nitrogen bubbling. Solid histograms are tolerance times of 5°C acclimated individuals, finely cross hatched histograms of 15°C acclimated individuals and widely cross hatched histograms of 25°C individuals at test temperatures of either 15° or 25°C (horizontal axis). Bars at tops of histograms indicate standard error of the mean.*

Table 2
Multiple Factor ANOVA for testing for differences in mean survival time for individuals of *Dreissena polymorpha* acclimated to 5, 15 and 25°C and exposed to prolonged anoxia at 15°C.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square Error	F-Ratio	Probability
Covariate Shell Length	150510.49	1	1505.49	586.5	0.0175**
Acclimation Temperature	576956.84	2	288478.42	11.241	<0.0001**
Residual	2207115.1	86	25664.13		
Total	2989472.0	89			

** Significant effect at $P \leq 0.05$

Mean survival times for different acclimation groups of *Dreissena polymorpha* exposed to prolonged anoxia at 15°C with Tukey Multiple Range Analysis for significant difference.

Acclimation Temp. (°C)	Mean Hours Survived	n	Standard Error of the Mean	Range	Signif. Diff. (P<0.05)
5	228.8	30	24.16	72 - 504	■
15	371.2	30	32.5	96 - 744	■
25	428.00	30	32.71	120 - 768	■

ranged from 53 - 83 h over 5° to 25°C acclimated individuals. Those for 5°- 25°C acclimated specimens of *C. fluminea* at 25°C ranged from 250 - 283 h, representing a 3.3 to 4.7 fold longer anoxia tolerance in Asian clams relative to zebra mussels (Fig. 1). Almost identical patterns of reduced anoxia tolerance in zebra mussels relative to Asian clams were observed when tolerance was recorded as LT_{50} values (*i.e.*, Time for

Table 3
Multiple Factor ANOVA for testing for differences in mean survival time for individuals of *Corbicula fluminea* acclimated to 5, 15 and 25°C and exposed to prolonged anoxia at 25°C.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square Error	F-Ratio	Probability
Covariate Shell Length	52557.64	1	52557.64	5.87	0.0175**
Acclimation Temperature	12667.03	2	6333.52	0.71	0.50
Residual	761219.68	85	8955.53		
Total	830468.68	88			

** Significant effect at $P \leq 0.05$

Mean survival times for different acclimation groups of *Corbicula fluminea* exposed to prolonged anoxia at 25°C with Tukey Multiple Range Analysis for significant difference.

Acclimation Temp. (°C)	Mean Hours Survived	n	Standard Error of the Mean	Range	Signif. Diff. (P<0.05)
5	250.31	29	16.32	105 - 369	
15	283.23	30	16.13	105 - 393	
25	273.017	30	20.65	81 - 405	

Mean survival times for specimens of *Corbicula fluminea* acclimated to 15°C and exposed to prolonged anoxia at 15°C.

Acclimation Temp. (°C)	Mean Hours Survived	n	Standard Error of the Mean	Range
15	841.66	29	60.79	240 - 1248

50% sample mortality, estimated by Probit Analysis [Bliss, 1936]) (Fig. 2A) or as SM_{100} values (*i.e.*, Time for actual 100% mortality of the sample) (Fig. 2B).

For specimens of *D. polymorpha*, Multifactor ANOVA revealed a significant correlation between anoxia tolerance and shell length at a test temperature of 15°C (Table 2), but not at a test temperature of 25°C (Table 1). Individual least squares linear regression analysis indicated that, at the 15°C test temperature, only 5°C acclimated individuals displayed a significant size effect with larger individuals having elevated tolerance times (n = 30, r = 0.51, F = 10.2, P = 0.0035). In contrast, correlation between SL and anoxia tolerance in *C. fluminea* was recorded only at a test temperature of 25°C (Table 3), in which larger clams from the 15° and 25°C acclimation groups showed significantly lower survival (15°C acclimated: n = 30, r = -0.45, F = 7.14, P = 0.012; 25°C acclimated: n = 30, r = -0.37, F = 4.41, P = 0.045).

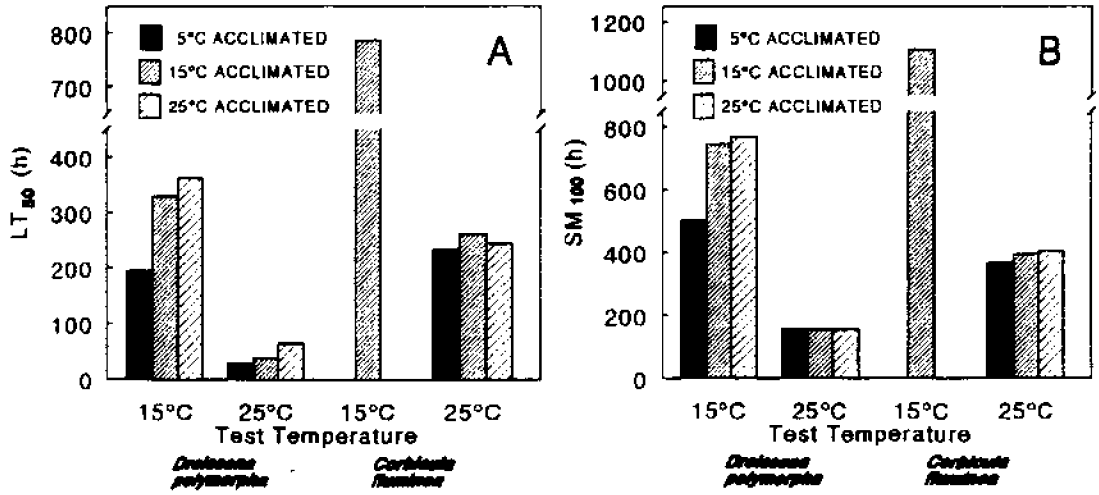

Figure 2. The effects of test temperature and prior temperature acclimation on survival time in hours expressed as LT_{50} and SM_{100} values for zebra mussels (Dreissena polymorpha) *and Asian clams* (Corbicula fluminea) *under anoxic stress (Po_2 < 5 torr) induced by nitrogen bubbling. Solid histograms are tolerance times of 5°C acclimated individuals, finely cross hatched histograms of 15°C acclimated individuals and widely cross hatched histograms of 25°C individuals at test temperatures of either 15° or 25°C (horizontal axis). A. LT_{50} values showing time to 50% sample mortality as estimated by probit analysis. B. SM_{100} values showing time required for actual 100% mortality of experimental samples.*

DISCUSSION

Specimens of *D. polymorpha* where much less tolerant of anoxia than those of *C. fluminea*. Mean tolerance time, LT_{50} and SM_{100} values for zebra mussels were 2-7 times lower than those of Asian clams. Among zebra mussels, those acclimated to 25°C showed the greatest anoxia tolerance at both test temperatures, surviving a mean of 3.45 days at 25°C and a mean of 17.8 days at 15°C. In contrast, Asian clam survival times did not appear to be influenced by temperature acclimation. The highest mean anoxia tolerance times for *C. fluminea* were 11.8 and 35.1 days among 15°C acclimated clams at test temperatures of 25°C and 15°C, respectively.

A previous, less extensive study of anoxia tolerance in *D. polymorpha* yielded results similar to ours. When mussels were enclosed in sealed containers allowing metabolic removal of oxygen, 100% mortality was observed among adult specimens within three days at a test temperature of 23-24°C, four days at 20-21°C and six days at 17-18°C (Mikheev, 1964). The somewhat elevated mortality rates reported by Mikheev (1964) may have been due to accumulation of anaerobic metabolic poisons in sealed containers as a consequence of not removing dead specimens. Exposure to relatively low concentrations of sulfide, an anaerobic bacterial decomposition end-product, has been shown to greatly reduce anoxia tolerance in the marine heterodont bivalves, *Macoma secta*, *Macoma nasuta* (Levitt and Arp, 1991), *Mulinia lateralis* (Shumway *et al.*, 1983), *Scorbicularia plana*, *Mya arenaria*, *Mytilus edulis* and *Cardium edule* (Theede *et al.*, 1969).

Mikheev (1964) also reported that smaller individuals of *D. polymorpha* were more susceptible to the lethal effects of prolonged anoxia than were adults, with 100% of individuals 1-4.9 mm in shell length (SL) succumbing to a 37 hour anoxic exposure at 22°C while 100% of individuals of 20-24.9 mm SL survived. In contrast, our data showed no size effects among any acclimation group at a test temperature of 25°C. At 15°C, only mussels acclimated to 5°C showed a significant size effect with smaller individuals appearing less tolerant of anoxia. As water was changed regularly in our experiments, the size effect reported by Mikheev (1964) may have been due to smaller mussels being more sensitive to accumulation of toxic end-products from anaerobically metabolizing living and decomposing dead mussels in sealed test containers, particularly as the mean anoxia survival time for smaller specimens in our open, N_2 bubbled media was at least twice that reported by Mikheev (1964).

No data has been previously published regarding the anoxia tolerance of *C. fluminea*. This species shows no capacity to regulate oxygen uptake rate with declining oxygen concentration (McMahon, 1979) and has been reported to be unable to tolerate reduced oxygen concentrations associated with discharge of treated sewage (Belanger, 1991) and hypolimnetic waters (Fast, 1971). The inability of Asian clams to survive for

more than 17 days suggests that exposure to anoxia or acute hypoxia during summer months could result in massive population decline.

While data for molluscs are sparse, both zebra mussels and Asian clams appear relatively intolerant of anoxia compared to other freshwater and marine molluscan species. Profundal freshwater sphaeriid clams can survive 4.5 to greater than 200 days of complete anoxia depending on season and temperature (Holopainen, 1987) and a freshwater unionid, *Anodonta cygena*, survives at least 7 days of anoxia at room temperature (Zs.-Nagy *et al.*, 1982). Specimens of the freshwater pond unionid mussel, *Ligumia subrostrata*, survived anoxia at 22-25°C for greater than 15 days (Dietz, 1974). The freshwater, prosobranch, ampullariid snail, *Pomacea lineata*, can survive approximately 40 days of anoxia at 25°C (Santos *et al.*, 1987). In contrast, most freshwater gastropods have anoxia tolerances similar to the range recorded in *D. polymorpha* and *C. fluminea*. The freshwater pulmonate pond snail, *Lymnaea stagnalis*, tolerated anoxia at 20°C for two days, but experienced a latent 100% mortality five days after return to normoxic conditions (Wijsman *et al.*, 1985). Among tested freshwater pulmonate snails, the majority of species tolerated 0.25 - 3 days of anoxia, with species in the Ancylidae (*i.e.*, freshwater pulmonate limpets) being most tolerant, surviving 4-11 days of anoxia (for a review see McMahon, 1993).

Marine bivalves appear to have a generally similar anoxia tolerance to *D. polymorpha*, but lower tolerance than that of *C. fluminea*. Specimens of *Mulinia lateralis* had LT_{50} values of approximately 11 days at 10°C, 8 days at 20°C and 1.8 days at 30°C (Shumway *et al.*, 1983), while *Macoma secta* and *M. nasuta* had LT_{50} values of 13 and 18 days, respectively at 14-16°C (Levitt and Arp, 1991). In contrast, the Atlantic oyster, *Crassostrea virginica*, which often encounters hypoxic waters in its estuarine habitats, survived over 28 days of anoxia at 10°C (Stickle, *et al.*, 1989) a tolerance similar to that of the mean of 35 days anoxia tolerated by *C. fluminea* at 15°C. Two anoxia tolerant marine bivalves, the mussel, *Mytilus galloprovincialis*, and the ark shell clam, *Scapharca inaequivalvis*, tolerated 15 and 20 days anoxia, respectively, at 20°C (de Zwann *et al.*, 1991). The anoxia tolerance of four species of marine bivalves at 10°C ranged from an LT_{50} of 500-600 h for the anoxia tolerant *Scorbicularia plana* and *Mya arenaria* to 810 h for *Mytilus edulis*, to a low of for 102 h for *Cardium edule* (Theede *et al.*, 1969).

Somewhat surprisingly, larger specimens of *C. fluminea* acclimated to 15° and 25°C were less tolerant of anoxia than smaller individuals at a test temperature of 25°C. The reason for the reduced anoxia tolerance of larger Asian clams remains unclear, however older individuals within a *C. fluminea* population may become senescent with reduced tissue glycogen stores, particularly during and after reproductive periods (Williams and McMahon, 1989). Reduction of tissue glycogen levels could make larger, senescent clams less tolerant of anoxia, as glycogen is one of the main sources

of anaerobic metabolic substrate in molluscs (de Zwaan, 1983). Reduction of anoxia tolerance in larger senescent clams may have occurred at 25°C because, at this elevated temperature, increased metabolic drive relative to specimens held at 15°C (McMahon, 1979) would lead to more rapid depletion of glycogen energy stores.

Our literature search revealed no information on the effects of temperature acclimation on anoxia tolerance in molluscs. Yet, our results indicated distinct temperature acclimation effects on tolerance in *D. polymorpha*. At both 15° and 25°C test temperatures, specimens of *D. polymorpha* acclimated to 5°C had a lower tolerance of prolonged anoxia than did individuals acclimated to 15°C and/or 25°C (Figs. 1 and 2, Tables 1 and 2), while acclimation temperature did not significantly affect mean anoxia tolerance time in specimens of *C. fluminea* (Figs. 1 and 2, Table 3). *D. polymorpha* has a typical pattern of metabolic acclimation in which cold acclimated individuals exhibit higher metabolic rates than do warm acclimated individuals (Alexander and McMahon, 1991). Thus, the reduced anoxia tolerance times of 5°C acclimated mussels may have been due to accelerated build up of toxic anaerobic end-products to lethal levels as a result of their elevated metabolic rates. In contrast, temperature acclimation did not affect anoxia tolerance in *C. fluminea*. Like *D. polymorpha*, *C. fluminea* has a typical pattern of metabolic temperature acclimation (McMahon, 1979). However, the greatly extended anoxia tolerance times of this species (11.8 days at 25°C and 35.1 days at 15°C) may have allowed previously acclimated specimens to fully re-acclimate to test temperatures, thus masking any effect of prior temperature acclimation on anoxia tolerance.

Zebra mussels in media bubbled with air rapidly made byssal holdfasts while those under anoxia never produced byssal threads. Furthermore, the majority of previously attached zebra mussels were observed to drop their byssal holdfasts well in advance of death. Similar inhibition of byssal holdfast production under anoxic conditions has been reported in the marine mussel, *Mytilus edulis* (Ravera, 1952).

CONCLUSIONS

Exposure to prolonged anoxia has been utilized to mitigate Asian clam fouling in the raw water intake embayments of a power plant. When the embayment was off-line, oxygen was scavenged from water above the clams by pumping sodium-meta-bisulfite ($Na_2S_2O_5$) and hydrogen sulfide (H_2S) to the embayment floor and leaving it undisturbed for 60-72 h at water temperatures above 21°C (Smithson, 1986). The rapid kill achieved by this method compared to death by anoxia alone documented in this paper was likely due to concomitant exposure to sulfide which is known to reduce anoxia tolerance in other species of molluscs (Theede *et al.*, 1969; Shumway *et al.*, 1983; Levitt and Arp, 1991). Our data indicate that similar embayment treatments

may result in more rapid mitigation of *D. polymorpha* macrofouling, as this species is much less anoxia tolerant than *C. fluminea*. However, because *D. polymorpha* fouling occurs on all hard surfaces within an embayment, and not just on the embayment floor as seen with *C. fluminea* fouling (Smithson, 1986), application procedures will require adequate distribution of oxygen scavenging chemicals throughout the embayment water column.

Exposure to anoxia has been previously suggested as an environmentally acceptable, nonchemical control methodology for *D. polymorpha* macrofouling (McMahon, 1990; Claudi and Mackie, 1993; Electric Power Research Institute, 1993). Oxygen depletion can be achieved within a raw water system by injecting sodium-meta-bisulfite and/or hydrogen sulfide immediately prior to valving it off. Alternatively, the system may be valved shut and the natural oxygen demand of mussels and other fouling organisms utilized to create anoxic conditions in static, stagnant water (Mikheev, 1964). At higher temperatures (> 20°C), our data suggest that 100% mortality could be achieved within 12 days of anoxia for *D. polymorpha* and within 25 days for *C. fluminea*. It is highly likely that utilization of hydrogen sulfide as an oxygen scavenger (Levitt and Arp, 1991; Shumway *et al.*, 1983) or the accumulation of naturally produced toxic anaerobic end-products and sulfide from dying mussels in an off-line static system (Mikheev, 1964) would significantly reduce the anoxic exposure required for 100% mitigation, particularly at higher temperatures (> 20°C). In contrast, the greatly extended anoxia tolerance of both *D. polymorpha* and *C. fluminea* at temperatures below 15°C is likely to render anoxic treatment unsuitable for bivalve macrofouling control under low temperature conditions at most facilities. Indeed, at 5°C, specimens of *D. polymorpha* survived up to 62 days anoxia while those of *C. fluminea* showed no mortality over a 12 week period (Matthews and McMahon, unpublished results).

Use of anoxia for mitigation of bivalve fouling will lower pH in treated raw water systems due to release of organic acids and hydrogen sulfide from decomposing mussels and other fouling organisms. Injection of sodium-meta-bisulfite or hydrogen sulfide as oxygen scavengers will further reduce pH, increasing the rate of corrosion in metallic piping (Claudi and Mackie, 1993). Further, static lay-up of systems may stimulate growth of sulfate reducing bacteria (*Desulfovibrio* and *Desulfomaculum*) which reduce sulfate (SO_4^{2-}) to sulfide (S^{2-}) ions that can attack cast iron, carbon and low alloy steels (Licina, 1988; Claudi and Mackie, 1993). Thus, application of anoxia as a mitigation treatment should not greatly exceed durations required to kill fouling bivalves. Indeed, where fouling infestations of Asian clams or zebra mussels have been allowed to achieve high densities, the potential for massive production of sulfide by decomposing bivalve bodies may preclude use of anoxia as an initial mitigation treatment. Periodic annual or biannual anoxia treatment should prevent excessive accumulation of a large fouling biomass, making mitigation of *D. polymorpha* or *C. fluminea* macrofouling both highly cost-effective and environmentally acceptable.

It has been reported that populations of *D. polymorpha* (Mackie *et al.*, 1989; McMahon, 1990) and *C. fluminea* (McMahon, 1983) do not normally extend below the thermocline (*i.e.*, zone of thermal discontinuity) into the hypolimnetic waters of lentic habitats which may become highly hypoxic during summer months due to thermal stratification. The results of this research suggest that the relatively poor tolerance of *D. polymorpha* and *C. fluminea* to prolonged anoxia acts to restrict both species to shallow, well-oxygenated, surface waters. Fast (1971) showed that artificial aeration allowed *C. fluminea* to invade the deeper hypolimnetic waters of a small lake from where they had been previously absent. Placement of intake structures below the thermocline of cooling water reservoirs has been utilized as a effective means of controlling raw water system fouling by these species (McMahon, 1990; Claudi and Mackie, 1993). Our data suggests that modification of existing intake structures to allow periodic drawing of anoxic water from below a source water's thermocline during summer months could serve to mitigate zebra mussel or Asian clam infestations. Periodic application of anoxic, subsurface water could minimize the microbially induced corrosion (M.I.C.) associated with anoxic static lay-up (Licina, 1988) by flushing anaerobically produced sulfides and organic acids into the discharge. Indeed, application of anoxic, subsurface water may cause fouling mussels to release from byssal holdfasts and be flushed from the system well in advance of death as observed in our experiments.

ACKNOWLEDGEMENTS

The authors express their appreciation to Thomas A. Ussery and Michael Clarke of the Center for Biological Macrofouling Research for providing technical assistance with data collection and to Gary L. Dye, Lockmaster of the U.S. Corps of Engineers, Black Rock Lock, in Buffalo, New York who supplied the zebra mussels. This study was conducted as part of the Zebra Mussel Research Program of the United States Army Corps of Engineers by the Center for Biological Macrofouling Research of The University of Texas of Arlington. Permission was granted by the Chief of Engineers to publish this information.

LITERATURE CITED

Alexander, J.E., Jr. and **McMahon, R.F.** 1991. Temperature and oxygen concentration limits for zebra mussels. Abstract of paper presented at *Zebra Mussels - Mitigation Options for Industries*, Sponsored by Ontario Hydro, 11-12 February, 1990, Toronto, Ontario.

Belanger, S.E. 1991. The effect of dissolved oxygen, sediment, and sewage treatment plant discharges upon growth, survival and density of Asiatic clams. *Hydrobiol.* 218: 113-126.

Bliss, C.I. 1936. The calculation of the time mortality curve. *Ann. Appl. Biol.* 24: 815-852.

Chase, R. and **McMahon, R.F.** 1994. Effects of starvation at different temperatures on dry tissue and dry shell weights in the zebra mussel, *Dreissena polymorpha* (Pallas). Abstract of paper presented at the *Fourth Annual Zebra Mussel Conference '94*, Sponsored by Great Lakes Sea Grant Network, held 7-10 March, 1994, Madison, Wisconsin.

Claudi, R. and **Mackie, G.L.** 1993. *Practical Manual for Zebra Mussel Monitoring and Control.* Lewis Publishers, CRC Press, Boca Raton, Florida. 227 pp.

Cleland, J.D., McMahon, R.F. and **Elick, G.** 1986. Physiological differences between two morphotypes of the Asian clam, *Corbicula. Am. Zool.* 26: 103A.

Counts, C.L., Jr. 1986. The zoogeography and history of the invasion of the United States by *Corbicula fluminea* (Bivalvia: Corbiculidae). *Am. Malacol. Bull.* Special Ed. # 2: 7-39.

Dietz, T.H. 1974. Body fluid composition and aerial oxygen consumption in the freshwater mussel, *Ligumia subrostrata* (Say): effects of dehydration and anoxic stress. *Biol. Bull.* 147: 560-572.

Electric Power Research Institute. 1993. *Zebra Mussel Monitoring and Control Guide.* EPRI TR-1017382, RP 3052-03, Electric Power Research Institute, Palo Alto, California. 582 pp.

Fast, A.W. 1971. The invasion and distribution of the Asiatic clam (*Corbicula manilensis*) in a southern California reservoir. *Bull. South. Calif. Acad. Sci.* 70: 91-98.

Holopainen, I.J. 1987. Seasonal variation of survival time in anoxic water and the glycogen content of *Sphaerium corneum* and *Pisidium amnicum* (Bivalvia: Pisidiiae). *Am. Malacol. Bull.* 5: 41-48.

Isom, B.G. 1986. Historical review of Asiatic clam (*Corbicula*) invasion and biofouling of waters and industries in the Americas. *Am. Malacol. Bull.* Special Ed. # 2: 1-5.

Levitt, J.M. and **Arp, A.J.** 1991. The effects of sulfide on the anaerobic metabolism of two congeneric species of mudflat clams. *Comp. Biochem. Physiol.* 98B: 339-347.

Licina, G.J. 1988. *Sourcebook for Microbiologically Influenced Corrosion in Nuclear Power Plants.* EPRI NP-5580, RP 2812-2, Electric Power Research Institute, Palo Alto, Calif. 96 pp.

Mackie, G.L. , **Gibbons, W.N.** , **Muncaster, B.W.** and **Gray, I.M.** 1989. *The Zebra Mussel, Dreissena polymorpha: A synthesis of European Experiences and a Preview for North America.* Water Resources Branch, Great Lakes Section, Ontario Ministry of the Environment, Toronto, Ontario. 76 pp. plus appendices.

McMahon, R.F. 1979. Response to temperature and hypoxia in the oxygen consumption of the introduced Asiatic freshwater clam, *Corbicula fluminea* (Müller). *Comp. Biochem. Physiol.* 63A: 383-388.

McMahon, R.F. 1983. Ecology of an invasive pest bivalve: *Corbicula.* Pages 505-561 *in*: W.D. Russell-Hunter (Ed.), *The Mollusca, Volume 6, Ecology.* Academic Press, Orlando, Florida.

McMahon, R.F. 1990. *The Zebra Mussel: U.S. Utility Implications.* EPRI GS-6995, RP 1689-24, Electric Power Research Institute, Palo Alto, Calif. 70 pp.

McMahon, R.F. 1991. Mollusca: Bivalvia. Pages 315-399 *in*: J.H. Thorp and A.P. Covich (Eds.), *Ecology and Classification of North American Freshwater Invertebrates.* Academic Press, Orlando, Florida.

McMahon, R.F., **Shipman, B.N.** and **Long, D.P.** 1992. Laboratory efficacies of nonoxidizing molluscicides on the zebra mussel (*Dreissena polymorpha*) and the Asian clam (*Corbicula fluminea*). Pages 575-597 *in*: T.F. Nalepa and D.W. Schloesser (Eds.), *Zebra Mussels: Biology, Impacts and Control.* Lewis Publishers, Boca Raton, Florida.

Mikheev, V.P. 1964. Mortality rate of *Dreissena* in anaerobic conditions. Pages 65-68 in: B.K. Shtegman (Ed.), *Biology and Control of Dreissena.* Israel Program for Scientific Translations, Ltd., IPST Cat. no. 1774, Jerusalem, Israel. Available from U.S. Depart. of Commerce, National Technical Information Service, Springfield, Virginia. ISBN 11 67-51396.

Ravera, O. 1952. Ricerche sul bisso e sulla sua secrezione. *Publicazioni della Stazione Zoologica di Napoli* 22: 1-11.

Roberts, L. 1990. Zebra mussel invasion threatens U.S. waters. *Science* 249: 1370-1372.

Santos, C.A., Penteado, C.H.S. and **Mendes, E.G.** 1987. The respiratory responses of an amphibious snail *Pomacea lineata* (Spix, 1827), to temperature and oxygen tension variations. *Comp. Biochem. Physiol.* 86A, 3: 409-415.

Schumway, S.E., Scott, T.M. and **Schick, J.M.** 1983. The effects of anoxia and hydrogen sulphide on survival, activity and metabolic rate in the coot clam, *Mulinia lateralis* (Say). *J. Exp. Mar. Biol. Ecol.* 71: 135-146.

Smithson, J.A. 1986. Development of a *Corbicula* control treatment at the Baldwin Power Station. *Am. Malacol. Bull.* Special Ed. # 2: 63-67.

Stickle, W.B., Kapper, M.A. , Liu, L. , Gnaiger, E. and **Wang, S.Y.** 1989. Metabolic adaptations of several species of crustaceans and molluscs to hypoxia: tolerance and microcalorimetric studies. *Biol. Bull.* 177: 303-312.

Theede, H. , Ponat, A. , Hiroki, K. and **Schlieper, C.** 1969. Studies on the resistance of marine bottom invertebrates to oxygen deficiency and hydrogen sulphide. *Mar. Biol.* (Berlin) 2: 325-337.

Wijsman, T.C.M. , van der Loot, H.C. and **Hoogland, H.P.** 1985. Anaerobic metabolism in the freshwater snail *Lymnaea stagnalis*: hemolymph as a reservoir of D-lactate and succinate. *Comp. Biochem. Physiol.* 81B: 889-895.

Williams, C.J. and **McMahon, R.F.** 1989. Annual variation in the tissue condition of the Asian freshwater bivalve, *Corbicula fluminea*, in terms of dry weight, ash weight, carbon and nitrogen biomass and its relationship to downstream dispersal. *Can. J. Zool.* 67: 82-90.

Zebra Mussel Information Clearinghouse. 1993. North American range of the zebra mussel. *Dreissena Polymorpha Information Review* 4(5): 6-7.

Zs.-Nagy, I., Holwerda, D.A., and **Zandee, D.I.** 1982. The cyotosomal energy production of molluscan tissues during anaerobiosis. *Basic Appl. Histochem.* 26 (Suppl.): 8-10.

de Zwaan, A. 1983. Carbohydrate catabolism in bivalves. Pages 137-175 *in*: P.W. Hochachka (Ed.), *The Mollusca, Volume 1, Metabolic Biochemistry and Molecular Biomechanics*. Academic Press, Orlando, Florida.

de Zwaan, A., Cortesi, P., van den Thillart, G., Roos, J. and **Storey, K.B.** 1991. Differential sensitivities to hypoxia of two anoxia-tolerant marine molluscs: a biochemical analysis. *Mar. Biol.* (Berlin) 111: 343-351.

Further Studies of Heat Tolerance in Zebra Mussels: Effects of Temperature Acclimation and Chronic Exposure to Lethal Temperatures

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ABSTRACT

Chronic (*i.e.*, long-term incipient) upper lethal temperatures were determined for zebra mussels (*Dreissena polymorpha*) acclimated to 5, 10, 15, 20, 25, or 30°C for at least 14 days. Subsamples (n = 25-33) from each acclimation group were exposed to constant temperatures of 31, 32, 33, 34, 35, 36, or 37°C (± 0.1°C). Mussels were brought from acclimation temperature to test temperature by increasing media temperature at a rate of 1°C/10 min. After reaching the test temperature, mortality was determined every 15 min for the first 2 hours, every 30 min for the next 3-5 h and every h for the next 17-21 h and every 3-4 h thereafter until all individuals had died. Multiple regression analysis indicated that acclimation temperature, exposure temperature, and mussel shell length (SL) were significantly correlated ($P < 0.00001$) with the natural log of thermal tolerance time. Tolerance time increased with increasing acclimation temperature and decreasing SL and decreased with increased exposure temperature. LT_{50} values (*i.e.*, estimated time for 50% sample mortality) ranged from < 1 h at 37°C regardless of acclimation temperature to 456 h in 25°C acclimated individuals at 31°C. Tolerance times were much greater than reported for northern European zebra mussels (100% kill within 3 h at 32°C compared to 54-399 h dependent on acclimation temperature). Multiple regression analysis indicated that the natural logarithm of either LT_{50}, LT_{100} or of SM_{100} (actual hours for 100% sample mortality) were significantly affected by both acclimation temperature and exposure temperature ($P < 0.00001$), increasing with increasing acclimation temperature and exponentially decreasing with increasing test temperature. Mathematical models are presented which allow prediction of the duration of exposure to lethal temperatures to induce mortality in zebra mussel infestations based on the temperature of thermal treatment, and the prior operating temperature experience of mussel infestations.

INTRODUCTION

Thermal treatment is an accepted nonchemical mitigation technology for control of raw water system macrofouling by both marine and freshwater bivalves (Electric Power Research Institute, 1984; Stock and Del La Parra, 1983) and has been utilized both in Europe and the United States to mitigate zebra mussel, *Dreissena polymorpha* (Pallas), fouling, especially in steam-electric power stations in which raw water system temperatures can be elevated to lethal levels by partial recirculation of heated discharge waters (Electric Power Research Institute, 1992; Claudi and Mackie 1993; Jenner and Janssen-Mommen, 1992; Mackie *et al.*, 1989).

For *D. polymorpha* and other macrofouling species, laboratory determinations of the upper lethal thermal limits on which thermal mitigation strategies are based have generally been determined as either "acute upper lethal temperatures" or as "chronic or incipient upper lethal temperatures." Acute upper lethal temperatures are measured as the temperature at which death occurs when water temperature is raised at a specific rate. The results of testing for acute upper lethal temperatures yield the mean lethal temperature, LT_{50} or LT_{100} values (*i.e.*, estimated temperature for 50% or near 100% sample mortality estimated by probit analysis [Bliss, 1936]) or SM_{100} values (*i.e.*, the actual recorded temperature of 100% sample mortality) (McMahon *et al.*, 1993; Stirling, 1982). Use of acute upper lethal temperature treatment to mitigate zebra mussel fouling would be most applicable in raw water systems where it is difficult to maintain lethal temperatures for extended periods. In these systems, it is more practical to increase water temperature to a level which induces an instantaneous or "acute" 100% mussel mortality followed by return to normal operating temperatures (McMahon *et al.*, 1993). As acute thermal treatment does not require precise, long-term regulation of elevated temperatures, it has been proposed for use in zebra mussel mitigation in raw water systems where operation above normal water temperatures for prolonged periods reduces efficiency and increases component wear, making chronic thermal treatment of zebra mussels economically unfeasible. Acute thermal mitigation may also be a particularly applicable for use in off-line components such as intake embayments heated by steam injection (Kovalak, 1993) or in various isolated sections or components of mussel fouled raw water systems warmed by steam injection or other means (Miller *et al.*, 1992).

In contrast to zebra mussel thermal mitigation strategies based on acute upper thermal limits, strategies based on "chronic or incipient upper thermal limits" involve continuous exposure of zebra mussel infestations to constant lethal temperatures for periods of long enough duration to achieve significant mortality. The laboratory studies on which chronic thermal mitigation treatments are based yield estimates of the period of time over which a sample of mussels can tolerate continuous exposure to specific upper lethal temperatures. This type of temperature tolerance testing involves

long-term holding of test individuals at a specific acclimation temperature, followed by instantaneous or near instantaneous transfer into a series of constantly maintained lethal test temperatures and recording survival times. The results of such testing are generally expressed as the mean time to death at a specific temperature, LT_{50} or LT_{100} values (*i.e.*, the estimated time required for induction of 50% or near 100% sample mortality estimated by probit analysis [Bliss, 1936]) or SM_{100} values (*i.e.*, the actual exposure time required to induce 100% sample mortality at a particular lethal temperature) (Iwanyzki and McCauley, 1992; Stirling, 1982; Stock and Del La Parra, 1983). Utilization of chronic thermal treatment for mitigation of zebra mussel infestations is most applicable in industrial and steam-electric power station raw water systems which generate heated discharge water and are designed to recirculate or backwash heated effluents into their intakes in order to maintain operating temperatures at relatively constant, elevated, lethal levels for prolonged periods.

Many industrial and power station raw water systems, particularly in the northern latitudes of North America have been designed for such recirculation of heated discharge water to prevent winter freezing of water or formation of frazzle ice within their raw water systems (Claudi and Mackie, 1993; Electric Power Research Institute, 1992; Neuhauser *et al.*, 1993) giving them the capability for chronic thermal mitigation of zebra mussel infestations. Chronic thermal mitigation of zebra mussel infestations is commonly employed in Europe (Jenner and Janssen-Mommen, 1992) and has begun to be utilized in North America (Neuhauser *et al.*, 1993).

The main advantage of chronic thermal mitigation strategies is that the water temperatures required for zebra mussel mitigation are generally lower (Claudi and Mackie, 1993; Electric Power Research Institute 1992; Iwanyzki and McCauley, 1992; Jenner and Janssen-Mommen, 1992; Neuhauser *et al.*, 1993) than those required for mitigation by acute thermal treatment (McMahon *et al.*, 1993). As the discharge temperatures of many industries and power stations are regulated by state and federal environmental agencies (Electric Power Research Institute, 1992; Neuhauser *et al.*, 1993), the higher discharge temperatures required for acute thermal mitigation treatment may not be permitted by these agencies, especially in systems such as power stations with once-through condenser water systems that discharge large volumes of heated water.

The acute upper lethal temperature of zebra mussels is affected by both their prior temperature experience (*e.g.*, the "acclimation" temperature or operating water temperature prior to thermal treatment) and the rate at which temperature rises to the acute temperature inducing instantaneous death. The temperature at which instantaneous death ensues increases with increased acclimation temperature and increased heating rate (McMahon *et al.*, 1993). Experimental data has been utilized to develop mathematical models predicting the acute upper lethal temperatures of zebra

mussels based on acclimation temperature and heating rate (McMahon *et al.*, 1993). These models allow development of acute thermal mitigation strategies for zebra mussel fouling on a "site specific" basis, predicting the temperature which must be exceeded for 100% mussel kill based on previous operating temperature and the rate at which system water temperature can be heated. Similarly, acclimation temperature and treatment temperature have been shown to effect the exposure time required to kill zebra mussels under chronic thermal mitigation treatment with required exposure time increasing with increased acclimation temperature and decreased treatment temperature (Jenner and Janssen-Mommen, 1992; Iwanyzki and McCauley, 1992; and citations therein). However, a mathematical model has yet to be developed which will allow prediction of the required exposure time for mitigation of a zebra mussel infestation based on its prior operating (*i.e.*, acclimation) temperature experience and the specific lethal treatment temperature applied to the system. Such models could be of great value in designing a site specific chronic thermal treatment strategy for mitigation of zebra mussel fouling, incorporating prior intake water temperatures, temperature of thermal treatment and maximum discharge temperatures permitted by regulatory agencies. Such a model would also assist a facility in the decision whether to utilize either "acute" or "chronic" treatment for thermal mitigation of a zebra mussel infestation.

This paper presents a laboratory study of the effects of both prior acclimation temperature and exposure temperature on the chronic upper thermal tolerance times of zebra mussels. The resulting data is utilized to develop a simple mathematical model predicting the required chronic exposure time required to induce death based on prior acclimation and exposure temperatures. This model can be utilized for development of site-specific chronic thermal mitigation strategies along with that previously developed for acute thermal treatment strategies (McMahon *et al.*, 1993), providing a comprehensive means for evaluating the efficacy of thermal treatment for zebra mussel fouling and the most appropriate thermal treatment strategy on a site-specific basis.

METHODS

Zebra mussels were collected from the vertical sides of a cement guide wall at the U.S. Army Corps of Engineers, Black Rock Navigation Lock on the Niagara River in Buffalo, New York. Immediately following collection, mussels were shipped overnight in insulated, cooled containers to the Center for Biological Macrofouling Research at The University of Texas at Arlington where they were maintained in a 200 l (75 gal) refrigerated 'Living Stream' holding tank at a constant temperature of 5°C (41°F) without feeding in dechlorinated City of Arlington tap water. All mussels were utilized in experiments within two months of collection. The metabolic rate of zebra mussels held at 5°C is so greatly depressed that significant reduction in dry

tissue mass cannot be detected within a 60 day holding period (Chase and McMahon, 1994). Thus, mussels utilized in the experiments were in good physiological condition.

Groups of greater than 30 mussels were removed from the holding tank and placed in 9 cm diameter by 5 cm high glass crystallization dishes covered with a 1 mm mesh nylon screen held in place with a rubber band, preventing mussel escape. The crystallization dishes were transferred into plastic holding tanks (22 cm deep x 21 cm wide x 31 cm long) containing 17 l (4.5 gal) of dechlorinated tap water. Holding tanks were held in refrigerated incubators in which five groups of mussels were acclimated to constant temperatures of 10°, 15°, 20°, 25°, or 30°C (50°, 59°, 68°, 77° or 86°F) (± 0.5°C) for a period of greater than 14 days prior to determination of chronic lethal temperature tolerance times. A sixth group of mussels acclimated to 5°C (41°F) were similarly held in a plastic aquarium placed in the 200 l, 5°C 'Living Stream' holding tank. Medium in acclimation tanks was replaced every three days with dechlorinated tap water preheated or precooled to the temperature of acclimation.

There was little, if any, mortality observed in mussels held at any of the acclimation temperatures. Only mussels which had made byssal attachment to the walls of the crystallization dishes or to the shell of other mussels during the acclimation period were utilized in determination of chronic thermal tolerance. Unattached mussels were removed from the dishes prior to experimentation. In dishes with more than 30 attached mussels, individuals were randomly culled to sample sizes of approximately 30 mussels just prior to experimentation. Sample size at any one acclimation/test temperature combination ranged from 26 to 33 (Table 1). Only byssally attached mussels were utilized in experiments because removal from the byssus has been demonstrated to reduce the tolerance of zebra mussels to stress, such as that induced by biocide exposure (McMahon *et al.*, 1992).

After temperature acclimation, groups of mussels in crystallization dishes for each of the six acclimation temperatures were submerged separately in 25 cm deep x 22 cm wide x 43 cm long insulated water baths containing 23 l of dechlorinated tap water constantly cooled by a Forma Scientific, Refrigerated Cold Finger (Model 2535). Water in the baths was circulated and initially held at 5°C by a Haake D1 Water Bath Temperature Regulator. One group of 5°C acclimated individuals was placed in each bath and the water temperature raised 1°C every 10 minutes. Dishes containing samples of the other acclimation groups (10°, 15°, 20°, 25° and 30°C) were placed in the bath at the point when rising bath temperature corresponded to their acclimation temperature, thus avoiding temperature shock to any test group. After all acclimation groups had been placed in water baths, bath temperature was increased at 1°C/10 min to final test temperatures of 31°, 32°, 33°, 34°, 35°, 36° or 37°C (87.8°, 89.6°, 91.4°, 93.2°, 95.0°, 96.8° and 98.6°F) where they were then held constant (± 0.1°C) by

temperature regulators. Starting times for raising water temperatures in the baths were staggered so that all baths reached their respective final test temperatures at the same time. Rapid water circulation by the regulators ensured uniform temperature and oxygenation throughout the baths.

Throughout thermal tolerance time determinations, bath water temperature was monitored with a fast responding micro-thermistor and a Model 43-DT, Yellow Springs Instrument Company Tele-Thermometer. Every three days throughout the course of the experiment samples of mussels were transferred to water baths containing fresh media at the appropriate test temperature to prevent media contamination with the mussel's metabolic end products.

The thermal tolerance times of mussels held at each test temperature were determined by periodically removing crystallization dishes, and observing the contained mussel samples for mortality as indicated by widely gaping valves. The viability of mussels with gaping valves was determined by gentle touching the tissues of the posterior mantle edge or siphons with the bristles of a fine brush. If this tactile stimulation did not elicit a valve closure response, the mantle edges and siphons were more vigorously probed with the hard, pointed end of the brush handle. If this more vigorous tactile stimulation still did not elicit valve closure, the individual was considered to be dead. A previous study had indicated that thermally stressed mussels which displayed widely gaping valves did not regain capacity to close their valves after 12 hours recovery at room temperature and thus, were considered dead (McMahon *et al.*, 1993). Dead mussels were removed from the crystallization chambers, their times of death recorded and their shell lengths (SL, the linear distance between the posterior margin of the shell and the anterior tip of the umbos) measured to the nearest 0.1 mm with dial calipers. The size range of all mussels utilized in the experiment was 11.0 - 35.4 mm with mean size being 20.2 mm (n = 1259, s.d = ± 3.3). After baths reached test temperatures, mortality in mussel samples was monitored every 15 min for the first 2 h, every 30 min for the next 3-5 h, every hour for the next 17-21 h and every 3-4 h thereafter, until all mussels in all acclimation groups at all test temperatures had died.

RESULTS

Mean times to death were determined for each acclimation group at each test temperature. The time required for 50% sample mortality (LT_{50}) and near 100% sample mortality (LT_{100}) were estimated from cumulative percent mortality values for each acclimation/test temperature sample by Probit Analysis (Bliss, 1936). In addition, the actual time required to achieve 100% sample mortality was recorded (Table 1). Mean thermal tolerance times, LT_{50} values and SM_{100} values increased exponentially with both increasing acclimation temperature and decreasing test

TABLE 1

Effect of Temperature Acclimation on Temperature Tolerance Times of *Dreissena polymorpha* on Exposure to Different Lethal Test Temperatures Expressed as Means, LT_{50} Values (Estimated time for 50% sample mortality), LT_{100} Values (Estimated time for 100% sample mortality) and SM_{100} Values (Actual time for 100% mortality)

Acc. Temp. (°C)	Test Temp. (°C)	n	Mean Hours to Death (±S.E.)	LT_{50} (°C)	LT_{100} (°C)	SM_{100}	Range
5	31	32	148.15 ±18.93	124.93	1601.391	399.70	8.7 - 399.7
	32	33	25.17 ±2.37	21.95.	01.44	54.50	4.5 - 54.5
	33	33	7.45 ±0.37	83	12.79	10.30	4.3 - 10.3
	34	29	2.74 ±0.21	1.87	5.88	5.50	1.5 - 5.5
	35	28	1.62 ±0.13	1.13	3.23	3.33	1.33 -3.33
	36	30	1.17 ±<0.0001	0.59	1.17	1.17	1.17 - 1.17
	37	26	1.0 ±<0.0001	0.50	1.00	1.00	1.17 - 1.17
10	31	30	292.58 ±55.40	203.62	5189.83	1040.21	15.67 -1040.16
	32	30	23.09 ±3.73	2.11	186.83	15.99	6.83 -115.99
	33	31	6.85 ±0.46	6.39	15.56	12.34	3.17 - 12.34
	34	29	1.66 ±0.18	1.77	8.05	5.50	1.0 - 5.50
	35	30	0.94 ±0.04	0.70	1.59	1.25	0.50 - 1.25
	36	30	0.47 ±0.02	0.39	0.09	0.50	0.25 - 0.50
	37	31	0.46 ±0.02	0.40	0.10	0.50	0.25 - 0.50
15	31	26	340.25 ±56.26	250.32	5423.98	1081.66	12.67 -1081.66
	32	30	20.84 ±2.46	0.55	87.95	7.99	7.83-67.99
	33	29	8.58 ±0.83	8.34	36.44	23.34	4.17 - 23.34
	34	30	2.91 ±0.21	2.43	7.50	5.00	1.25 - 5.00
	35	31	1.06 ±0.10	1.11	4.62	4.00	0.50 - 4.00
	36	30	0.52 ±0.01	0.44	0.73	0.75	0.50 - 0.75
	37	30	0.52 ±0.02	0.45	0.75	0.75	0.25 - 0.75
20	31	32	203.53 ±22.53	186.54	1308.46	471.70	38.7 - 471.7
	32	33	56.38 ±4.19	9.60	208.58	110.50	10.5 - 110.5
	33	33	14.48 ±1.17	11.81	44.23	30.30	4.3 - 30.3
	34	30	5.50 ±0.33	4.76	13.37	11.50	3.5 - 11.5
	35	30	3.26 ±0.07	1.76	3.23	3.33	1.33 -3.33
	36	30	1.17 <±0.0001	0.59	1.17	1.17	1.17 - 1.17
	37	30	1.0 <±0.0001	0.50	1.00	1.00	1.00 -1.00
25	31	31	527.10 ±50.17	456.16	3449.07	1129.42	68.16 - 1129.41
	32	28	72.90 ±10.01	0.54	654.25	75.99	18.50 - 275.99
	33	30	12.32 ±0.91	11.97	35.11	23.34	5.67 - 23.34
	34	28	5.88 ±0.50	5.55	17.06	12.17	3.50 - 12.17
	35	30	3.75 ±0.21	2.79	12.39	5.00	1.00 - 5.00
	36	31	1.04 ±0.06	0.79	2.16	1.50	0.50 - 1.50
	37	30	0.84 ±0.04	0.66	1.48	1.25	0.50 - 1.25
30	31	29	390.67 ±32.98	333.41	2384.84	769.99	32.17 - 769.99
	32	29	162.49 ±19.69	42.367	993.19	399.99	32.00 - 399.99
	33	28	73.43 ±5.82	.64	209.46	13.99	29.00 - 139.99
	34	29	14.77 ±1.13	12.17	72.58	23.17	2.50 - 23.17
	35	30	5.21 ±0.38	4.04	31.60	8.50	0.50 - 8.50
	36	30	1.82 ±0.14	1.49	5.17	3.00	0.50 - 3.00
	37	30	0.84 ±0.04	0.89	3.40	1.25	0.25 - 1.25

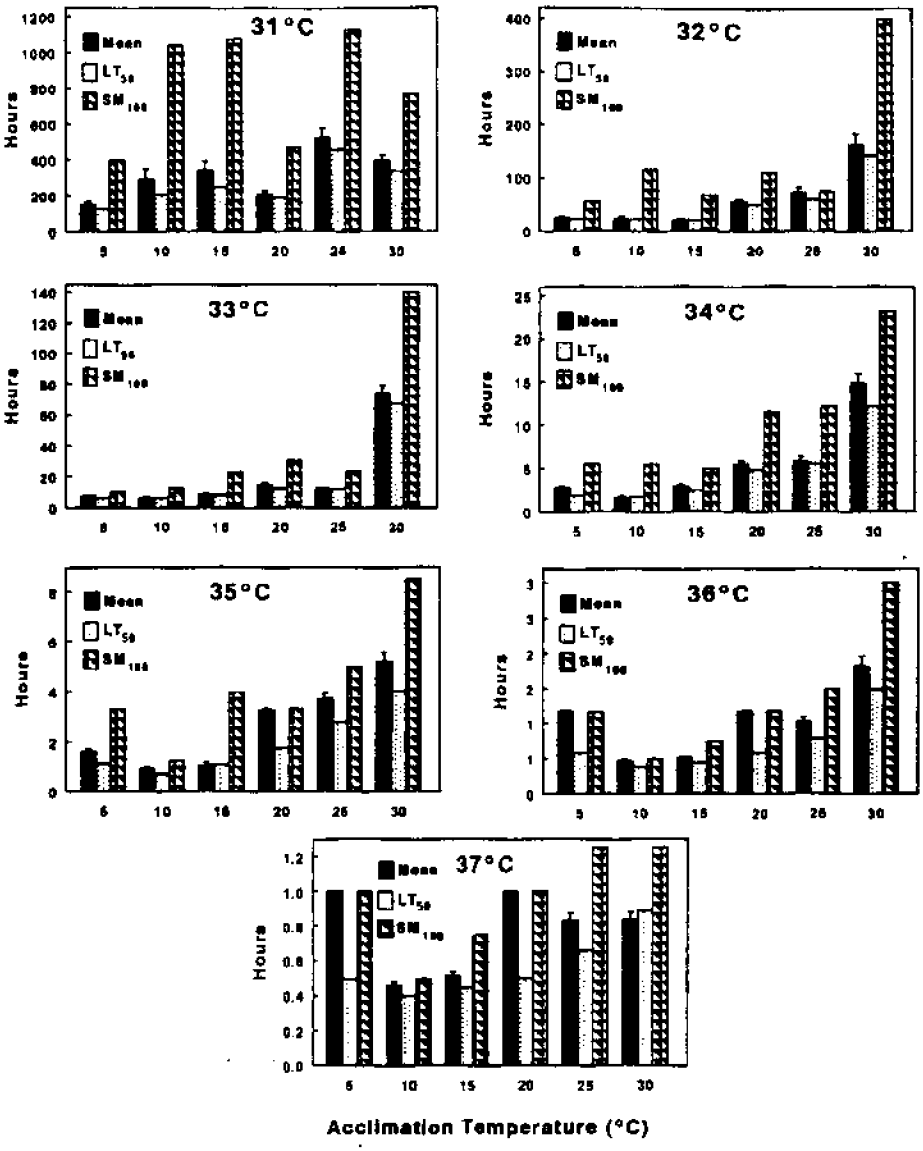

Figure 1. *Chronic (incipient) upper lethal thermal tolerance times in zebra mussels,* Dreissena polymorpha *from the Niagara River. Vertical axis in all graphs is tolerance of lethal temperatures measured in hours as mean tolerance times (solid histograms),* LT_{50} *values, the estimated time for 50% sample mortality (stippled histograms) and* SM_{100} *values, the actual time for 100% sample mortality (checkered histograms). The horizontal axis in all graphs is acclimation temperature in °C. Verical bars atop histograms are standard errors of the means. Tolerance times are presented for exposures to lethal temperatures of 31°, 32°, 33°, 34°, 35°, 36°, and 37°C.*

TABLE 2

Model Fitting Results and Coefficients for a Multiple Linear Regression Analysis Relating the Natural Logarithm of Time to Death (h) in specimens of *Dreissena polymorpha* (Dependent Variable) to Acclimation Temperature (°C), Test Temperature (°C) and Shell Length (mm) (Independent Variables)

Indpend. Variable	Coefficient	Standard Error	T-value	Probability
Constant	33.982	0.391	86.90	<0.00001**
Acclimation Temperature	0.0599	0.00255	22.73	<0.00001**
Test Temperature	-0.944	0.0106	-88.67	<0.00001**
Shell Length	-0.0517	0.00656	-7.89	<0.0001**

Source	Sum of Squares	Degrees of Freedom	Mean Square	F-Ratio	Probability
Model	4697.09	3	1565.7	2756.73	<0.00001**
Error	712.78	1255	0.657		
Total	5409.87	1258			

** Significant difference at $P \leq 0.05$.
Regression Coefficient (r) = 0.932

temperature (Fig. 1), thus, in all statistical testing the natural logarithm (ln) of tolerance time was utilized to linearize the data.

Least squares multiple linear regression analysis relating time to death in individual mussels as the dependent variable to acclimation temperature, test temperature and SL as independent variables indicated that all three variables significantly affected thermal tolerance ($n = 1259$, $r = 0.932$, $F = 2757$, $P < 0.00001$) (Table 2). The very high correlation coefficient (R^2) of this multiple regression indicated that the effects of these three variables accounted for 87% of all recorded variation in thermal tolerance times. Such a high level of correlation of tolerance time with treatment variables suggested that there was little or no effect of either sample holding chambers or water baths on tolerance

TABLE 3

Model Fitting Results and Coefficients for a Multiple Linear Regression Analysis Relating the Natural Logarithm of Estimated Time to Death of 50% of the Sample (LT_{50}) (Dependent Variable) in specimens of *Dreissena polymorpha* to Acclimation Temperature (°C) and Test Temperature (°C) (Independent Variables).

Indpend. Variable	Coefficient	Standard Error	T-value	Probability
Constant	35.831	1.480	23.91	<0.00001**
Acclimation Temperature	0.0578	0.0101	5.72	<0.00001**
Test Temperature	-1.018	0.0431	-23.61	<0.00001**

Source	Sum of Squares	Degrees of Freedom	Mean Square	F-Ratio	Probability
Model	184.42	2	92.21	249.96	<0.00001**
Error	12.19	39	0.0313		
Total	196.62	41			

** Significant difference at $P \leq 0.05$.
Regression Coefficient (r) = 0.967

times, allowing statistical analysis of the data to utilize individual mussels as the experimental unit.

Thermal tolerance times were found to increase significantly with increased acclimation temperature ($P < 0.0001$), and decrease significantly with increased test temperature ($P < 0.0001$) and increased individual SL ($P < 0.0001$) (Table 2). The correlation coefficients for these variables indicated that test temperature had the greatest effect on thermal tolerance time, with acclimation temperature and SL having relatively similar, but lesser effects over the tolerated acclimation temperature range (0-30°C) and typical SL range of North American zebra mussels (1-35 mm). Multiple least squares linear regressions also indicated that natural logarithms of LT_{50}, LT_{100} and SM_{100} values were similarly correlated to the independent variables of acclimation temperature and test temperature ($n = 42$, $r = 0.949$-0.969, $F = 185$-294, $P < 0.00001$) (Tables 3, 4, 5).

TABLE 4

Model Fitting Results and Coefficients for a Multiple Linear Regression Analysis Relating the Natural Logarithm of Estimated Time to Death of nearly 100% of the Sample (LT_{100}) (Dependent Variable) in specimens of *Dreissena polymorpha* to Acclimation Temperature (°C) and Test Temperature (°C) (Independent Variables).

Indpend. Variable	Coefficient	Standard Error	T-value	Probability
Constant	46.817	2.442	19.17	<0.00001**
Acclimation Temperature	0.0788	0.0167	4.73	<0.00001**
Test Temperature	-1.328	0.0712	-18.66	<0.00001**

Source	Sum of Squares	Degrees of Freedom	Mean Square	F-Ratio	Probability
Model	315.50	2	157.7	185.30	<0.00001**
Error	33.20	39	0.851		
Total	348.70	41			

** Significant difference at $P \leq 0.05$.
Regression Coefficient (r) = 0.949

Multiple linear regression equations allowing prediction of ln mean thermal tolerance time (h) based on acclimation temperature (°C), test temperature (°C) and shell length (mm) and prediction of ln LT_{50}, ln LT_{100} or ln SM_{100} values (h) based on acclimation and test temperatures are displayed in Table 6 along with corresponding regression parameters. When the mean thermal tolerance times predicted by these equations (mean thermal tolerance computed for a mussel with an SL of 15 mm) was graphically expressed against different treatment temperatures at different acclimation temperatures they indicated that thermal tolerance time exponentially declined with increasing treatment temperature and decreasing acclimation temperature (Fig. 2 A-D). Whether expressed as absolute tolerance times (Fig. 2 A), LT_{50} (Figure 2B), LT_{100} (Fig. 2C) or SM_{100} values (Figure 2D), mussel death at treatment temperatures of 37°C and above was nearly instantaneous, occurring within less than one hour regardless of prior acclimation temperature experience. Regression analysis (Fig. 2A) indicated that, below 37°C, thermal tolerance times increased exponentially with decreased test temperatures and were greatly effected

TABLE 5

Model Fitting Results and Coefficients for a Multiple Linear Regression Analysis Relating the Natural Logarithm of Actual Time to Death of 100% of the Sample (SM_{100}) (Dependent Variable) in specimens of *Dreissena polymorpha* to Acclimation Temperature (°C) and Test Temperature (°C) (Independent Variables).

Indpend. Variable	Coefficient	Standard Error	T-value	Probability
Constant	40.002	1.622	24.65	<0.00001**
Acclimation Temperature	0.0514	0.0111	4.63	<0.00001**
Test Temperature	-1.126	0.0473	-23.81	<0.00001**

Source	Sum of Squares	Degrees of Freedom	Mean Square	F-Ratio	Probability
Model	221.17	2	110.59	294.26	<0.00001**
Error	14.66	39	0.376		
Total	235.81	41			

** Significant difference at $P \leq 0.05$.
Regression Coefficient (r) = 0.969

by acclimation temperature. Thus, at 34°C, the estimated mean tolerance times for a 15 mm SL mussel ranged from 4 h in 5°C acclimated individuals to 17 h in 30°C acclimated individuals (Fig. 2A) while estimated SM_{100} values over the corresponding acclimation temperature range ranged from 5.6 to 26.1 h (Fig. 2D). At treatment temperatures below 34°C, the exponential relationship with treatment and acclimation temperatures greatly extended thermal tolerance times. At 31°C (the lowest temperature lethal to the mussels we tested), mean tolerance time in a 15 mm SL individual was estimated to be 69 h if acclimated to 5°C, rising to 293 h if acclimated to 30°C (Fig. 2A). The range of corresponding SM_{100} values at a 31°C treatment temperature increased to 211 h to 737 h over a 5°C to 30°C acclimation temperature range (Fig. 2D).

TABLE 6

Multiple Least Squares Linear Regression Equations Relating the Natural Logarithm of Thermal Tolerance Time (Hours) Expressed as either Mean Tolerance Times, LT_{50} (Estimated Time for 50% Sample Mortality), LT_{100} (Estimated Time for Near 100% Sample Mortality) or SM_{100} (Actual Time for 100% Sample Mortality) (Dependent Variable) to Acclimation Temperature (AT in °C), Test Temperature (TT in °C) and Specimen Shell Length (SL in mm) (Independent Variables).

$$\ln \text{Mean Hrs} = 33.982 + 0.0579(\text{AT in °C}) - 0.944(\text{TT in °C}) - 0.0517(\text{SL in mm})$$
$$n = 1260,\ F = 2756,\ r = 0.932,\ P < 0.00001^{**}$$

$$\ln LT_{50} \ln \text{Hrs} = 35.381 + 0.0578(\text{AT in °C}) - 1.018(\text{TT in °C})$$
$$n = 42,\ F = 295,\ r = 0.967,\ P < 0.00001^{**}$$

$$\ln LT_{100} \ln \text{Hrs} = 46.817 + 0.0788(\text{AT in °C}) - 1.328(\text{TT in °C})$$
$$n = 42,\ F = 185,\ r = 0.948,\ P < 0.00001^{**}$$

$$\ln SM_{100} \ln \text{Hrs} = 40.002 + 0.0514(\text{AT in °C}) - 1.126(\text{TT in °C})$$
$$n = 42,\ F = 294,\ r = 0.967,\ P < 0.00001^{**}$$

DISCUSSION

Jenner and Janssen-Mommen (1992) have shown that zebra mussels acclimated to ambient water temperatures in the Netherlands have a chronic upper temperature tolerance of less than 10 min at 36°C, increasing to 1.5 h at 33°C. Mean tolerance times of North American zebra mussels from Lakes Erie and St. Clair when exposed to 30°C varied between 4.74 days when specimens where acclimated to 2.5°C and 3.96 days when they were acclimated to 25°C (Iwanyzki and McCauley, 1992). At a treatment temperature of 33°C, these values declined to 0.22 h and 17.5 h in mussels acclimated to 2.5°C and 25°C, respectively, and further declined to 0.17 h and 0.65 h, respectively, when 2.5°C and 25°C acclimated mussels were exposed to 36°C (Iwanyzki and McCauley, 1992). The mean incipient temperature tolerance times recorded by Iwanyzki and McCauley (1992) for North American zebra mussels were higher than those reported for 100% mortality in zebra mussels from northern Europe (Jenner and Janssen-Mommen, 1992) even though Iwanyzki and McCauley (1992) reported mean values which underestimate the duration of exposure required to achieve 100% mortality. However, their values are similar to those quoted from other, unpublished sources (as cited in Iwanyzki and McCauley, 1992) for North American

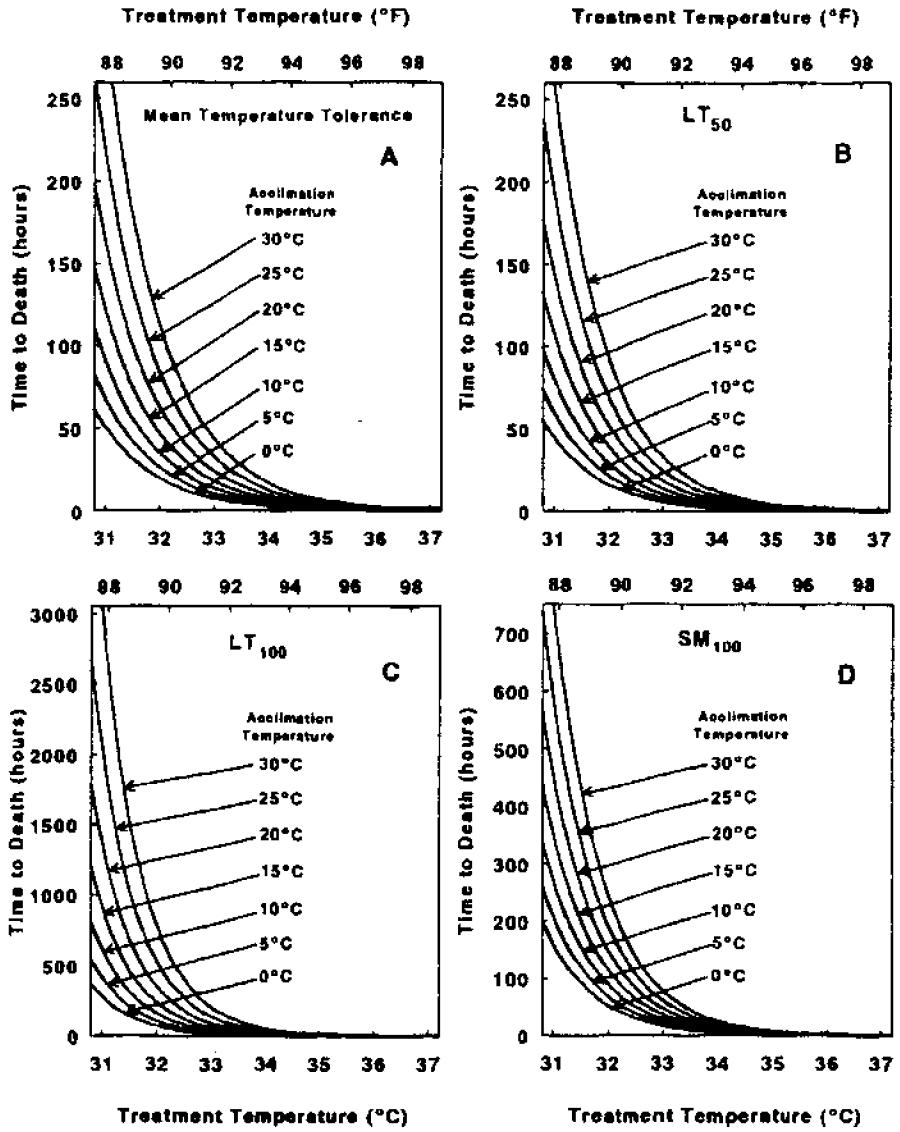

Figure 2. *Chronic (incipient) upper lethal thermal tolerance times in zebra mussels,* Dreissena polymorpha *from the Niagara River estimated from least squares multiple linear regression equations relating tolerance time to acclimation temperature and treatment temperature (Table 6). The vertical axis of all graphs is tolerance time in hours survived at lethal temperatures indicated on the horizontal axis in °C. Individual regression lines are for mussels acclimated to temperatures ranging from 0-30°C. Thermal tolerance times are expressed as (A) mean time to death, (B) LT_{50} values, estimated time for 50% sample mortality, (C) LT_{100} values, estimated time for near 100% sample mortality and (D) SM_{100} values, actual time of 100% sample mortality.*

zebra mussel populations from Lake Erie and the Rybinskoye Vodokhranifishche Reservoir in northwestern Russia.

Our values for chronic upper lethal temperature tolerance are also indicative of a much higher level of thermal tolerance in North American zebra mussels than reported for this species in northern Europe by Jenner and Janssen-Mommen (1992). They report that a 100% kill of zebra mussels can be achieved within a 1.5 h exposure to 33°C. Our regression equation for 100% sample mortality (*i.e.*, SM_{100}) predicts that a zebra mussel would tolerate 33°C for 17-80 h over an acclimation range of 0° to 30°C (Table 6, Fig. 2D), a tolerance 11 to 53 times greater than that reported for northern European mussels.
Regression equations relating thermal tolerance times to treatment temperature for North American zebra mussels from Lakes Erie and St. Clair indicate that on exposure to 33°C the mean thermal tolerance times of zebra mussels acclimated to 15°, 20° and 25°C would be 2.8, 6.8 and 10.2 h, respectively (Iwanyzki and McCauley, 1992). Corresponding values for a 15 mm SL zebra mussel estimated from our regression equation relating tolerance time to acclimation temperature, treatment temperature and SL (Table 6, Fig. 2A) would be 18.5 h, 24.8 h and 33.2 h, respectively, 6.6, 3.6 and 3.3 times greater than those previously reported. In addition, Iwanyzki and McCauley (1992) reported that 30°C was the lowest incipient upper lethal temperature inducing death in North American zebra mussels with tolerated exposures ranging from 64 h in 20°C acclimated individuals to 114 h in 2.5°C acclimated individuals. In contrast, we recorded minimal short-term mortality in mussels maintained at this temperature. Instead, we determined the chronic upper lethal temperature to be 31°C with mean tolerated exposure times ranging from 52-292 h depending on acclimation temperature 31°C (Fig. 1 and 2A).

The basis for the difference in thermal tolerance between these two groups of mussels from the Great Lakes is difficult to ascertain. However, Iwanyzki and McCauley (1992) did not let experimental specimens byssally attach prior to thermal tolerance determinations. McMahon *et al.* (1992) noted that lack of byssal attachment reduces the tolerance of zebra mussels to nonoxidizing biocides, thus, lack of byssal attachment by experimental individuals in previous determinations may have resulted in a reduction in recorded thermal tolerance values. Iwanyzki and McCauley (1992) also introduced test individuals directly from holding media at the acclimation temperature into media at the test temperature, perhaps subjecting them to a thermal shock which could have affected a reduction in subsequently recorded thermal tolerance times. In contrast, we increased tank temperature at a rate of 1°C/10 min from the acclimation temperature to the test temperature to avoid any thermal shock to test specimens that could be induced by an instantaneous temperature change. Such a slow increase in temperature from the acclimation to treatment temperature also better reflects the strategy with which chronic thermal treatment would be applied in industrial or steam-electric power facilities with temperature being relatively slowly increased from the normal operating (*i.e.*, acclimation) to the lethal treatment temperature through partial recirculation of heated effluents (Neuhauser *et al.*, 1993).

The thermal tolerance of *D. polymorpha* whether measured as incipient upper lethal temperature limit (this study) or acute upper lethal temperature (McMahon *et al.*, 1993) is lower than that of other common North American macrofouling bivalve species. At 32.2°C, 95% mortality was induced in specimens of the marine, macrofouling, blue mussel, *Mytilus edulis* L., within 23 h, with tolerance time decreasing to 0.23 h at 40.5°C (Stock and Del La Parra, 1993). In another study, maintenance of blue mussels at 35°C (95°F) for 1 h induced 56% sample mortality and, at 40°C, 100% sample mortality was induced within 0.33 h (Johnson *et al.*, 1983). The short-term upper thermal limit of the Atlantic or American oyster, *Crassostrea virginica* Gmelin, is 48.5°C (Sellers and Stanley, 1989) a value much higher than that of 38°C recorded in this study for *D. polymorpha*. The freshwater, macrofouling Asian clam, *Corbicula fluminea* (Müller) is also considerably more thermally tolerant than *D. polymorpha*. The instantaneous upper lethal temperature of Asian clams is approximately 44°C in individuals acclimated to 32°C with the lowest lethal temperature being 30°C in 5°C acclimated clams which survived exposure to this temperature for less than 4-7 h (Mattice, 1979). In *C. fluminea*, 36°C is the minimum long-term chronic upper lethal temperature (McMahon and Williams, 1986) while our study indicates that it is 31°C in *D. polymorpha*. The greatly elevated upper thermal limits of *C. fluminea* relative to *D. polymorpha* reflects the Asian clam's endemic distribution in tropical and subtropical areas of southeast Asia (Morton, 1979).

The reduced thermal tolerance of *D. polymorpha* relative to other North American biofouling species makes it particularly susceptible to chronic thermal mitigation treatment. Mitigation treatment with temperatures greater or equal to 34°C (93°F) could induce near 100% kills of zebra mussel infestations within 6-26 h depending on the prior acclimation/operating temperature experience of mussel infestations (Fig. 2D). Below treatment temperatures of 34°C, the exposure times required for near 100% mussel kills become too extended (17-80 h depending on acclimation temperature, Fig. 2D) to be economically applied in most industrial or electric generating facilities (Electric Power Research Institute, 1992; Claudi and Mackie, 1993; Neuhauser *et al.*, 1993). At treatment temperatures ranging between 34° to 37°C (93-99°F) times for 100% kills of zebra mussels are short enough (Fig. 2D) to be cost-effective, application temperatures are low enough to prevent major loss of production or excessive equipment wear and/or malfunction and discharge temperatures are likely to be low enough to meet the discharge temperature restrictions of state and/or national regulatory agencies (Electric Power Research Institute, 1992; Neuhauser *et al.*, 1993).

Use of acute thermal mitigation strategies for zebra mussels in which the temperature of a raw system is increased until the instantaneous upper lethal temperature of the zebra mussel is exceeded, while allowing for shorter durations of thermal treatment, requires subjecting raw water systems to higher water temperatures. Thus, at an acclimation temperature of 20°C (68°F) and a water heating rate of 1°C/5 min (a heating rate achievable by thermal backwashing in electric power stations, see Neuhauser *et al.*, 1993), a temperature of 42.3°C (108°F) would have to be achieved

to induce a 100% kill of zebra mussel infestations over a total water heating period of approximately 2 hours (based on SM_{100} values, McMahon *et al.*, 1993) while a chronic thermal mitigation strategy would produce 100% kills of zebra mussels within 15.6 - 0.53 h if applied at 34 - 37°C (93 - 99°F) (Fig. 2D). Thus, acute thermal mitigation of zebra mussel infestations in entire raw water systems may generate unacceptably high equipment operating and discharge water temperatures, making chronic thermal treatment a more applicable strategy for system-wide mitigation of zebra mussel fouling. In contrast, acute thermal treatments may be most efficacious for treatment of off-line components in which elevated water temperatures would be difficult to maintain for prolonged periods (*e.g.*, use of steam injection to increase to lethal limits the water temperature in mussel-fouled, off-line intake embayments, Kovalak, 1993) or for on-line treatment of individual components or sections of raw water systems (*e.g.*, treatment of the service water system only, treatment of only one of several units, or treatment of individual heat exchangers) whose high treatment discharge water temperatures could be tempered by mixing with untreated waters in the discharge channel.

The data presented here (Fig. 2 A-D) and in previous papers (Iwanyzki and McCauley, 1992; McMahon *et al.*, 1993) clearly demonstrate that previous temperature acclimation greatly affects both the acute and chronic thermal tolerance of zebra mussels. Thus, regardless of the thermal treatment strategy employed, higher water temperatures will be required to achieve 100% mitigation of zebra mussel infestations during summer months (Figs. 1 and 2, McMahon *et al.*, 1993). Thus, with either chronic or acute thermal treatment strategies, initiating treatments during periods when water temperatures are below maximal summer levels may significantly reduce both the amount of time required to apply the treatment and/or the temperature required to achieve 100% mitigation of mussel fouling. Also of importance is the fact that smaller zebra mussels have greater thermal tolerance times than larger mussels, thus infestations consisting primarily of smaller individuals, which is the usual case if a raw water system is subjected to annual or biannual mitigation treatments, will require higher and/or longer exposures to lethal temperatures to induce near 100% mussel kills.

CONCLUSIONS

The data presented strongly suggest that chronic thermal treatment can be a highly efficacious means of mitigating zebra mussel fouling in the raw water systems of facilities such as steam-electric power stations which produce heated effluents and are capable of partial recirculation of those effluents into intake structures. Chronic thermal treatment may be particularly applicable for mitigation of *D. polymorpha* fouling as this species appears to have the lowest level of thermal tolerance among common North American macrofouling bivalve species. Based on the model equations relating intake water temperature and treatment temperature to tolerance times in Table 6 and Fig. 2, efficacious chronic thermal mitigation of zebra mussel infestations resulting in 100% mitigation of fouling within less than a 24 h treatment period could

occur at treatment temperatures of 34°C (93°F) and above during summer months when intake water temperatures are equal to or greater than 20°C (68°F), and during winter months at 33°C (91°F) when water temperatures are 5°C (41°F) or below (Fig. 2D). Because the thermal tolerance time of zebra mussels exponentially decreases with increased treatment temperature (this study; Iwanyzki and McCauley, 1992; Jenner and Janssen-Mommen, 1992), the duration of application of a chronic thermal treatment will be greatly reduced with even small increases in treatment temperature (Fig. 2), reducing the productivity losses and equipment wear associated with system operation at above normal temperatures for prolonged periods.

This research has indicated that the chronic thermal tolerance levels of North American zebra mussels are at least an order of magnitude greater than those reported for this species in Northern Europe (Jenner and Janssen-Mommen, 1992). While available data are sparse and plagued by incongruent protocols for measurement of thermal tolerance (see Discussion above), our results do appear to suggest that North American populations of *D. polymorpha* may have been introduced to the Great Lakes from a zebra mussel population in the southern portion of this species' present European range where elevated ambient water temperatures may have selected for a more thermally tolerant physiological race than exists in the cooler freshwaters of northern Europe. Further evidence of a southern European origin for the race of zebra mussels introduced into the Great Lakes is the concurrent introduction of a second, dreissenid species *Dreissena bugensis* Andrusov (the "Quagga Mussel") (May and Marsden, 1992, Spidle *et al.*, 1994). *Dreissena polymorpha* is the only dreissenid species found in the freshwaters of northern Europe (Mackie *et al.*, 1989; Stańczykowska, 1977). In contrast, *D. bugensis* is restricted to the Southern Bug and Dnieper Rivers (Spidle *et al.*, 1994; Zhadin, 1952) which empty into the Dnieper Estuary on the northern shore of the Black Sea in the Ukraine. At the confluence of both rivers, is the Ukrainian city of Nikolayev, a major international shipping port, making the northern shore of the Black Sea, and particularly shipping ports, such as Nikolayev and the nearby city of Odessa, the likely source of the two dreissenid species introduced into North America either as veliger larvae transported in ballast water (Mackie *et al.*, 1989) or as adults attached to anchor chains (McMahon *et al.*, 1993).

The Black Sea and Southern Bug and Dnieper Rivers are at the most extreme southeastern and likely warmest portion of the distribution range for zebra mussels in Europe (Stańczykowska, 1977). Therefore, zebra mussels introduced to North America from this region could have been drawn from a genetically, thermally tolerant population relative to those found in the much cooler waters of northern Europe. In any case, the elevated thermal tolerance of North American zebra mussels demonstrated by this research and that of McMahon *et al.* (1993) strongly suggests that zebra mussels are likely to extend much further south into the freshwater drainage systems of the United States than has been previously estimated based on available information on the temperature tolerance of northern European zebra mussel populations (Electric Power Research Institute, 1992; McMahon, 1990, 1992; Strayer, 1991). Indeed, *D. polymorpha* has already been reported to have invaded the waters

of the lower Mississippi River as far south as New Orleans (Zebra Mussel Information Clearinghouse, 1993) where it appears to be successfully reproducing in areas with summer surface water temperatures approaching 30°C (T.H. Dietz, personal communication). The capacity of north American *D. polymorpha* to survive 30°C (this study; McMahon *et al.*, 1993) strongly suggests that this species will not be restricted by elevated ambient temperatures in its invasion of North American freshwater drainages in all but the warmest regions of the southwestern United States and Mexico (*Water Atlas of the United States*, 1973).

ACKNOWLEDGEMENTS

Gary L. Dye, Lockmaster of the U.S. Corps of Engineers, Black Rock Lock, in Buffalo, New York, collected and shipped mussels to the Center for Biological Macrofouling Research. This study was conducted as part of the Zebra Mussel Research Program of the United States Army, Corps of Engineers by the Center for Biological Macrofouling Research of the University of Texas at Arlington. Permission was granted by the Chief of Engineers to publish this information.

REFERENCES

Bliss, C.I. 1936. The calculation of the time-mortality curve. *Ann. Appl. Biol.* 24: 815-852.

Chase, R. and **McMahon, R.F.** 1994. Effects of starvation at different temperatures on dry tissue and dry shell weights in the zebra mussel, *Dreissena polymorpha* (Pallas). Abstract of paper presented at the *Fourth Annual Zebra Mussel Conference '94*, sponsored by the Great Lakes Sea Grant Network, 7-10 March, 1994, Madison, Wisconsin.

Claudi, R. and **Mackie, G.L.** 1993. *Practical Manual for Zebra Mussel Monitoring and Control.* Lewis Publishers, CRC Press, Boca Raton, Florida. 227 pp.

Electric Power Research Institute. 1984. *Condenser Macrofouling Control Technologies.* CS-3550, Electric Power Research Institute, Palo Alto, California.

Electric Power Research Institute. 1992. *Zebra Mussel Monitoring and Control Guide.* TR-101782, Electric Power Research Institute, Palo Alto, California. 582 pp.

Iwanyzki, S. and **R.W. McCauley**. 1992. Upper lethal temperatures of adult zebra mussels. Pages 667-673 *in*: T.F. Nalepa and D.W. Schloesser (eds.), *Zebra Mussels: Biology, Impacts, and Control*, Lewis Publishers, Boca Raton, Florida. ISBN 0-87371-696-5.

Jenner, H.A. and **J.P.M. Janssen-Mommen**. 1992. Monitoring and control of *Dreissena polymorpha* and other macrofouling bivalves in the Netherlands. Pages 537-554 *in*: T.F. Nalepa and D.W. Schloesser (eds.), *Zebra Mussels: Biology, Impacts, and Control*, Lewis Publishers, Boca Raton, Florida. ISBN 0-87371-696-5.

Johnson, G., J. Foertch, M. Keser and **B. Johnson**. 1983. Thermal backwash as a method of macrofouling control at Millstone Nuclear Power Station, Waterford, Connecticut, USA. Pages 25-1 - 25-15 *in*: I.A. Dias-Tous, M.J. Miller and Y.G. Mussalli (eds.), *Symposium on Condenser Macrofouling Control Technologies: The State of the Art.* CS-3343, Electric Power Research Institute, Palo Alto, California.

Kovalak, W.P. 1993. Living with the zebra mussel: Where have we been? Where are we going? Paper presented at a conference entitled *Zebra Mussels in Michigan: Implications for Industries and Municipalities*, sponsored by Michigan Sea Grant College Program and Michigan State University Extension, 11-12 January 1993, East Lansing, Michigan.

Mackie, G.L., W.N. Gibbons, B.W. Muncaster and **I.M. Gray**. 1989. *The Zebra Mussel*, Dreissena polymorpha: *A Synthesis of European Experiences and Review for North America*. Great Lakes Section, Water Resources Branch, Ontario Ministry of the Environment, London, Ontario, Canada, ISBN: 0-772905647-2. 76 pp.

Mattice, J.S. 1979. Interactions of *Corbicula* sp. with power plants. Pages 119-139 *in*: J.C. Britton (ed.), *Proceedings, First International Corbicula Symposium.* Texas Christian University Research Foundation, Fort Worth, Texas.

May, B. and **J. E. Marsden**. 1992. Genetic identification and implications of another invasive species of dreissenid mussel in the Great Lakes. *Can. J. Fish Aquat. Sci.* 49: 1501-1506.

McMahon, R.F. 1990. *The Zebra Mussel: U.S. Utility Implications*. GS-6995, Electric Power Research Institute, Palo Alto, California. 70 pp.

McMahon, R.F. 1992. The zebra mussel - the biological basis of its macrofouling and potential for distribution in North America. Reprint No. 342 of a paper presented at the annual meetings of the National Association of Corrosion Engineers, 26 April - 1 May 1992, Nashville, Tennessee. National Association of Corrosion Engineers, Houston, Texas. 14 pp.

McMahon, R.F. and **C.J. Williams**. 1986. Growth, life cycle, upper thermal limit and downstream colonization rates in a natural population of the freshwater bivalve mollusc, *Corbicula fluminea* (Müller) receiving thermal effluents. *Am. Malacol. Bull.* Special Edition No. 2: 231-239.

McMahon, R.F., B.N. Shipman and **D.P. Long**. 1992. Laboratory efficacies of nonoxidizing molluscicides on the zebra mussel (*Dreissena polymorpha*) and the Asian clam (*Corbicula fluminea*). Pages 575-598 *in*: T.F. Nalepa and D.W. Schloesser (eds.), *Zebra Mussels: Biology, Impacts, and Control*, Lewis Publishers, Boca Raton, Florida. ISBN 0-87371-696-5.

McMahon, R.F., T.A. Ussery, A.C. Miller and **B.S. Payne**. 1993. Thermal tolerance in zebra mussels (*Dreissena polymorpha*) relative to rate of temperature increase and acclimation temperature. Pages 4-98 - 4-118 *in*: J.L. Tsou and Y.G. Mussalli (eds.), *Proceedings: Third International Zebra Mussel Conference, 1993*, TR-102077, Electric Power Research Institute, Palo Alto, California.

McMahon, R.F., T.A. Ussery and **M. Clarke**. 1993. *Use of Emersion as a Zebra Mussel Control Method.* Contract Report EL-93-1, U.S. Army Corps of Engineers, Washington, D.C. 27 pp.

Miller, A.C., B.S. Payne, F. Neilson and **R.F. McMahon**. 1992. *Control Strategies for Zebra Mussel Infestations at Public Facilities*. Technical Report EL-92-25, U.S. Department of the Army, U.S. Army Corps of Engineers, Washington, D.C. 32 pp.

Morton, B. 1979. *Corbicula* in Asia. Pages 15-38 *in*: J.C. Britton (ed.), *Proceedings, First International Corbicula Symposium*. Texas Christian University Research Foundation, Fort Worth, Texas.

Neuhauser, E.F., J.J. Knowlton, D.P. Lewis, and **G.L. Mackie**. 1993. Thermal treatment to control zebra mussels at the Dunkirk Steam Station. Pages 4-71 - 4-94 *in*: J.L. Tsou and Y.G. Mussalli (eds.), *Proceedings: Third International Zebra Mussel Conference, 1993*, TR-102077, Electric Power Research Institute, Palo Alto, California.

Sellers, M.A. and **J.G. Stanley**. 1989. *Species Profiles: Life Histories and Environmental Requirements of Coastal Fishes and Invertebrates (North Atlantic). American Oyster*. FWS/OBS-82//11.23. U.S. Fish and Wildlife Service, Washington, D.C. and U.S. Army, Army Corps of Engineers, Vicksburg, Mississippi. 18 pp.

Spidle, A.P., J.E. Marsden and **B. May**. 1994. Identification of the Great Lakes quagga mussel as *Dreissena bugensis*, introduced from the Black Sea Drainage in Ukraine. Abstract of a paper presented at the *4th International Zebra Mussel Conference '94*, sponsored by the Great Lakes Sea Grant Network, 7-10 March 1994, Madison, Wisconsin.

Stańczykowska, A. 1977. Ecology of *Dreissena polymorpha* (Pall.) (Bivalvia) in Lakes. *Polskie Archiwum Hydrobiologii* 24: 461-530.

Stirling, H.P. 1982. The upper temperature tolerance of prosobranch gastropods of rocky shores at Hong Kong and Dar Es Salaam, Tanzania. *J. Exp. Mar. Biol. Ecol.* 63: 133-144.

Stock, J.N. and **R.A. Del La Parra**. 1983. Use of thermal backwash to control marine biofouling at San Onofre Nuclear Generating Station. Pages 15-1 - 15-14 *in*: I.A. Dias-Tous, M.J. Miller and Y.G. Mussalli (eds.), *Symposium on Condenser Macrofouling Control Technologies: The State of the Art*, CS-3343, Electric Power Research Institute, Palo Alto, California.

Strayer, D.L. 1991. Projected distribution of the zebra mussel, *Dreissena polymorpha*, in North America. *Can. J. Fish. Aquat. Sci.* 48(8): 1389-1395.

Water Atlas of the United States. 1973. Water Information Center, Inc., Port Washington, New York, ISBN 0-912394-030X. 189 pp.

Zhadin, V.I. 1952. *Mollusks of Fresh and Brackish Waters of the USSR*. Keys to the fauna of the USSR published by the Zoological Institute of the Academy of Sciences of the USSR, Moscow, Russia. No. 46.

Zebra Mussel Information Clearinghouse. 1993. North American range of the zebra mussel. *Dreissena Polymorpha Information Review* 4(5): 6-7.

Field Trials of Nontoxic Fouling-Release Coatings

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Abstract

Increasing emphasis must be placed on nontoxic control technologies for macrofouling organisms, particularly zebra mussels (*Dreissena polymorpha*), now rapidly proliferating through North American surface water networks. Barnacle and tubeworm fouling is significantly reduced by new low-surface-energy coatings that minimize biological adhesion strength, allowing "fouling release" with modest brushing or water spray pressures. These nontoxic coatings do not prevent static deposits of fouling layers, but rather function through a common mechanism by which most biofouling can be easily removed on the basis of controlled surface chemistry and physics. The "easy removal" process has been demonstrated by our research group and confirmed by others in environments as diverse as seawater, freshwater, blood, saliva, tissue culture, and food processing. Some of our zebra mussel-related field trials, testing selected nontoxic fouling-release coatings, are sponsored by Niagara Mohawk Power Corporation (NMPC). Panels of approximately 15 candidate coatings have been immersed at two NMPC sites in New York State (Dunkirk/Lake Erie and Oswego/Lake Ontario) since January 1992. Sea Grant supported concurrent fundamental studies of the interfacial biophysics and of initial veliger attachment at these and 4 additional sites. Coated steel, concrete, and other test substrata are included in the studies, as well as uncoated controls. Coating efficacy in prevention/release of zebra mussel fouling also has been monitored for 2 years by Lawler, Matusky & Skelly Engineers (Pearl River, NY). Results from the first 2 years of the 4-year NMPC study confirm our earlier finding that only coatings having pre-exposure critical surface tensions between 20 and 30 mN/m (relatively "low surface energy") remain effective in resisting and minimizing zebra mussel retention. Within the group of commercial coatings meeting this criterion, they are further differentiated by some exhibiting (1) release of lower molecular weight oils and (2) peculiar ratios of polar-to-apolar surface chemical functionalities. Detailed aspects of the dynamic surface compliance and wear resistance of such nontoxic fouling-release coatings are now under study in a project sponsored by the Office of Naval Research, in which seawater field trials of some of the same coatings in the NMPC project show comparable fouling-release capabilities.

Introduction and Background

Surface chemical and biophysical approaches to control of zebra mussel prevention and release include study of (a) the early interactions between organisms (veligers and adults) and different substrata, particularly the interface between the byssus disk and the substratum, (b) the protein-rich "conditioning" films that form on different substrata prior to veliger and adult attachment, and (c) the adhesives produced by zebra mussels to attach to all types of substrata.

It has been known for year that bioadhesives associated with marine organisms like tubeworms, barnacles, and mussels initially set under water and can sustain their adhesive bond strengths in the face of significant detachment forces, as well as long-term weathering. Since 1988, *Dreissena polymorpha* has been, after its earlier introduction into the Great Lakes from Eastern European sources, causing extreme difficulty to power plants, municipal water supplies, and marina facilities because of its tenacious adhesion to essentially all underwater solid substrata. Our laboratories and others have been analyzing zebra mussel discs, as well as bioadhesive proteins from other systems (e.g. Mussel Adhesive Protein, MAP, from the New England Blue Mussel, and proline-rich proteins isolated from human saliva)[1-4] to identify the bonding mechanisms for these natural products. Infrared spectroscopic, electron microscopic, and related test data show all these bioadhesive agents to be predominantly glycoproteinaceous substances that can spontaneously dehydrate and bond with various substrata during a surface-active displacement process. Glands in the zebra mussel foot apparatus secrete cement precursors that sequentially engulf bacterial/biofilm debris into the bulk byssal disc phase[5] and then "cure" to form tough adhesives at all the solid/liquid boundaries. Spontaneously predeposited "conditioning" films are the required "primer" coats for these bioadhesive substances to be effective in natural ecosystems.

The cements are generally DOPA-rich (dihydroxyphenylalanine) and glycoproteinaceous; the best characterized so far are from the common blue sea mussel.[2] Our comprehensive contact angle results suggest that display of a specific "surface polar fraction" of the chemical groups dictating the total surface free energy may be one of several physical-chemical requirements for underwater adhesion. Films formed from the repeating decameric fragment of MAP, and its smaller peptides, illustrated that exposure of H-bonding backbone sites probably also contributes to MAP's bioadhesiveness.[2,6] Waite and associates have found the zebra mussel adhesive precursors to require different solubilization protocols and to be more diverse than the blue mussel adhesive precursors.[1] Bonner and associates have shown by transmission electron microscopy that the precursor substances may reside in separate micron-size granules.[5] Work now in progress in Bonner's laboratory, using enzyme histochemical stains at the ultrastructural level to elucidate the segregation of

the precursor substances, will be correlated with light microscopic histology of the glandular products observed by our group and the related surface chemistry of these granules.

Since their first review of this topic in 1968[7], Baier and coworkers have increasingly focused on the identification, prediction, and control of the earliest events in the processes of biofouling; specifically, adhesion of living organisms to engineering materials in underwater environments. Prevention of biological fouling (and its numerous adverse consequences) does not require use of toxicants such as heavy metal compounds or chlorinated agents, according to results of these studies. Rather, physicochemical control of the original surface properties of biomaterials --- without compromise of their engineering strengths or design functions --- can induce the formation of "weak boundary layers" that allow prevailing shear/mechanical forces to shed thickening fouling deposits. Spontaneous spalling and re-entrainment of fouling mass allows consequent extended function of the biomaterials with no toxic risk to the surrounding environment. Materials with "critical surface tensions" between 20 and 30 mN/m are the ones least retentive to biofouling. A review presented in Marine Biodeterioration in 1984 explains these concepts.[8]

Additional work in a variety of test systems has since illustrated an apparently universal sequence of the earliest biofouling events. Spontaneous deposition of a primer film, named the "conditioning" film, precedes colonization of pioneer bacteria and their secretion of cellulosic slimes. The rates or speeds of these events are concentration dependent, and account for the variable, but previously obscure, "induction periods" where conventional engineering inspections fail to detect any fouling activity. It required the use of very sensitive, molecular-level biosurface analytical techniques to obtain the required "induction period" data under relevant, ambient flowing conditions.[9,10] Manifolds of inexpensive field flow cells were employed at a large number of field sites, including an Atlantic cruise on the USNS Lynch (T-AGOR-7) that demonstrated microfouling differences associated with differing oceanic regions.[11] These and other configurations of the flow cells[12-15] allowed assessment of thermal gradient influences and/or heat transfer measurements simultaneous with microfouling assays, as described in a 1988 update, Marine Biodeterioration.[16]

An important convergence of results was noted when materials of varying composition and controlled "critical surface tension" were employed to advance the understanding of bioadhesion in a variety of other biological environments, including blood, tissue, culture, animal and human implants, and the human oral cavity.[17-19] These and related data on fouling film formation in the wider variety of biological systems (pure protein, saliva, milk, seawater) suggest these fundamental and general bioadhesion principles:[20]

- Similar "conditioning" film compositions can still express surface properties that differ as a function of pre-exposure material substratum properties, and

- on a given substratum, the "conditioning" film changes as a function of time and varying mechanical forces.

- Subsequent biofouling deposits delaminate at differing locations or "planes of parting" (planes of failure), also as functions of pre-exposure material substrata surface properties (particularly their critical surface tensions), as well as functions of mechanical forces and exposure time.

- Increased shear forces during initial biofilm formation and subsequent maturation can significantly reduce the expressed surface energies and increase the packing (apparent densities) of the retained films.

When work by others explored how adjustment of each substratum material's critical surface tension correlates with retention of strengths of attached marine organisms,[21] Baier's earlier observations of a "zone" of minimum bioadhesion were confirmed. A *caveat* to keep in mind is that rate of fouling attachment is not a good predictor of strength of fouling adhesion.[22] Because most low-surface-energy materials also are hydrophobic, they become fouled more quickly than do higher-energy, usually hydrophilic materials. The strength of fouling adhesion, however, is sufficiently weak to allow easy detachment only from those few low-surface-energy materials (either hydrophobic, like methyl silicones, or hydrophilic, like dolphin skin[23]). The low-surface-energy materials do not allow maturation of the initially formed --- usually hydrophobic --- bonds to the high-strength levels characteristic of dipole-dipole or hydrogen-bonded systems. Thus, there is a significant limitation to the proper use of the phrase "fouling-release"!

In addition to ongoing basic research studies, we are now evaluating fouling-release coating systems that are designed to reduce the expense of maintenance, re-coating, and disposal, as well as to preserve worker health and environmental quality. Coatings are being evaluated in a study of (a) approximately 15 formulations for control of zebra mussel infestation of power plant intake tunnels and other structures; this is the NMPC-sponsored project, (b) 3 related systems, as well as solid polyethylene, for minimization of mussel blockage of trash racks (Gilbert/Commonwealth project), and (c) diverse formulations for control of marine biofouling of ships (ONR project). The methods recommended from our laboratory results for the routine evaluation of these coatings, and presentation of results of freshwater field testing, are the focus of the remainder of this paper.

Methods, Materials, and Field Sites

The analytical methods and apparatus used in these studies have been described in detail before.[24,25] Briefly, **comprehensive contact angle analysis**, as originally described by Young,[26] is an affordable, sensitive, and reliable method of monitoring the adhesive properties of any coating. A large body of data has been accumulated and a substantial literature exists correlating contact angle data with surface properties of many coating resins and polymer films.[27] Contact angle measurements characterize only the outermost 4-5 Angstroms of a sample, thus providing the most relevant and surface-specific analysis of the material as it is exposed to the real environment. The "critical surface tension" (gamma-c), derived from a graph of the contact angle data called the "Zisman plot", relates the surface tensions of a variety of diagnostic liquids to the cosines of the angles the liquid droplets make with a given coating surface. When nonsolvating and nonswelling wetting liquids are used, the intercept of the plot, defined as the critical surface tension, empirically and reproducibly characterizes the coating's real surface. Successful fouling-release materials have gamma-c's between 20 and 30 mN/m; the very best performance is for materials with gamma-c's between 20 and 27 mN/m. This is the required "first-level" descriptor for good v. poor fouling-release performance. Water contact angles, alone, are insufficient, and often misleading with regard to fouling performance (especially over the long term).

Internal reflection infrared (IR) spectroscopy[28] is a simple and powerful technique for learning the chemical composition of coatings and their transferrable residues. For thick-layer materials, the sampling depth of the technique ranges from approximately one micrometer (for nonabsorbing materials), to as little as 1000 Angstroms for absorbers. Superficial residues, as little as 10-15 Angstroms in thickness, are easily detected by re-analysis of the internal reflection test plate after removal of the primary coating sample. The method is nondestructive, so it can be applied as an early step in many combined test protocols. For fouling-release coatings, the amounts of silicone (or other materials) transferred to the test surface via pressure-contact is an excellent indicator of the presence of elutable or releasable components (e.g. oils) in the sample. Fouling-release coatings loaded with such oils may not be as environmentally acceptable as those that are free of such migratable components and may not perform as well as unloaded coatings in the long-term. The relative quantities of releasable ingredients provide "second-level" descriptors for discriminating among coatings having critical surface tensions between 20 and 27 mN/m. The values given in the data table here are "infrared absorbance units", and are directly proportional to mass.

A simple **brush shear test** also is suitable to evaluate the ease of fouling removal, especially when brush-based removal apparatus will be used in the field. This test is performed on small coupons removed from field test racks, and is a good indicator of

both the absolute and the relative performance of fouling-release coatings. Field evaluations include enumeration of retained zebra mussels after the panels are rinsed with a hose at 25 psi from a distance of approximately 6 inches. Other studies have utilized a "water jet" oral hygiene device for rinsing small rod samples.[29] The trash rack study utilizes a higher-pressure rinse underwater (2000 psi rating out of water). During the brush shear test, the biofouling and the brush are wet. The brush, alone (11g), is drawn 5 times down the length of the fouled test plate, and an observation is made as to whether any fouling has been removed. The process is repeated with increasing load (addition of 1, 3, 5, 10, 20, 30, 50, 100, 150, 200, and 250g), until all visible fouling has been removed. A value of " >250g additional load" is assigned for samples that are not clean by the end of the test; 300g is used for these samples to determine averages and standard deviations during the entire fouling period. Typical removal pressures (equivalent static pressure) range from 15 to 50psi for fouling-release coatings.

A **rotating brush abrasion test** is performed on pre-exposure coupons to evaluate their abilities to resist cleaning-related damage. We have found that routinely applied and accepted wear and abrasion tests (e.g. ASTM falling sand test) do not as well reveal the susceptibility to brush-induced wear of elastomeric fouling-release coatings. Average depth of wear is determined for coatings abraded by nylon brushes for up to 145,000 brush strokes. The brushes are loaded with a mass of approximately 270g. The test is directly relevant, in that it employs the brushing forces that must be applied during field cleaning and maintenance procedures. Determination of the worn coating's new operational critical surface tension is key to knowing how long the coating will maintain its fouling-release character after being abraded. [Comprehensive contact angle analyses of abraded samples are in progress; results are not included here.]

Field sites for the NMPC project are located at the Dunkirk Steam Station on Lake Erie and at the, generally, mussel-free Oswego Steam Station on Lake Ontario. The test panels have been in place since January 1992. The Oswego test racks will be transferred to a hydrostation on the Oswego River in Spring 1994 where zebra mussel infestation is reported to be moderate to severe. At Dunkirk, eight test racks (each carrying up to 5 steel and 5 concrete panels (8"x10"), as well as numerous 1"x3" coupons) are deployed through openings in the deck of a shoreline screenhouse. They are suspended from a support frame by stainless steel cables. The tops of the racks are approximately 5 feet below the water surface. Each rack includes one control steel and one control concrete panel. At the Oswego station, eight racks have been suspended from steel cable placed above and across a forebay, outdoors; the panels are at a depth of approximately 12 feet, at mean lake level. The test racks for the Dunkirk and Oswego trials were constructed by King Consulting, following a design found useful by Stone & Webster[30] and others in the past. The 8"x10" panels are

evaluated at regular intervals by Lawler, Matusky & Skelly Engineers (LMS Engineers) for zebra mussel infestation (6 evaluations in Year 1, 4 evaluations annually in Years 2-4). Our University at Buffalo team periodically retrieves sets of the small coupons for more extensive laboratory evaluation (3 sets retrieved in Year 1, 2 sets retrieved annually in Years 2-4).

Trash rack coatings are being examined at NMPC's hydrostation on Glenwood Lake, near Medina, NY. The trash racks are in normal-use function at the plant, having been coated and installed in early 1993. The racks are inspected by a diving team on a semi-annual basis for coating integrity, extent of fouling, and ease of cleaning (by both mechanical rubbing and hydroblasting), with underwater videotaping of the rack conditions and cleaning results.

Criteria for coatings to be included in these field trials are (a) absence of toxic components (i.e. heavy metals and/or biocides) and (b) critical surface tensions in the range between 20 and 30 mN/m. On occasion, coatings meeting the first criterion, but "borderline" on the second criterion, are evaluated when another advantage --- such as wear resistance --- is apparent. These "borderline" candidates have generally performed poorly in both freshwater and seawater fouling exposure trials. Table 1 presents the coatings being tested in the NMPC project. Controls at Dunkirk and Oswego include uncoated 8"x10" cold-rolled steel and concrete panels. Trash rack coatings include two methyl silicones and a material that was requested to be, and represented to be, a fluoropolymer by its supplier; upon analysis, the latter coating was found to be fluorine-deficient and turned out to be a polyvinylchloride-like material with a critical surface tension of 31.1 mN/m. A solid, black (filled) polyethylene trash rack (critical surface tension of approximately 32 mN/m) also is included in the study due to its reported durability, light weight, and claimed fouling resistance. The control for the study is a metal trash rack overcoated only with an anticorrosion primer.

Table 1
Coatings Under Evaluation for Zebra Mussel Control
(NMPC Project at Dunkirk and Oswego)

Type and Code #	Pre-Exposure Characteristics γc (mN/m)	% Polarity	Si-CH, residue*	Wear (.001")
Methyl Silicones				
1	24.9	16%	.028	<0.3
2	23.5	12%	.017	0.3
3	23.0	12%	.006	1.3
4	22.5	TBD	TBD	TBD
5 [no AC coat]	27.1	5%	.009	<0.3
6	21.3	12%	[mineral oil]	6.0
7	22.2	19%	[mineral oil]	9.0
8	21.9	8%	<.003	3.3
9	23.5	18%	.003	<0.3
10	21.6	14%	<.003	0.3
Epoxies				
sil.'91 [no AC]	24.4	30%	.027	<0.3
silicone '93	23.0	4%	<.003	<0.3
fluoro-	24.9	26%	**	<0.3
amine	32.4	44%	**	<0.3
Uncoated Steel	>30	HIGH	**	<0.3
Uncoated Concrete	>30	HIGH	**	<0.3

** values listed are infrared Absorbance units*
TBD: to be determined
AC: anticorrosion
*** silicone not a component*
Wear: average after 145,000 brush strokes

Results

During the first year of immersion of the coated trash racks at the Glenwood Lake site, it was clear that the silicone-based systems with the appropriate critical surface tensions remained intact and able to easily release the few attached zebra mussels with minimal mechanical force (handwiping or water spray), leaving no byssal structures behind. The zebra mussels attached (to the stage of complete flow occlusion) to the polyethylene rack could be removed by approximately 2000 psi water blasting, but only at the level of the mollusc shell; the byssal threads and bound bases were left behind on the trash rack. Zebra mussel attachment to the polyvinylchloride-like coating was equally tenacious. The polyvinyl-coated rack is, interestingly, at the center of the inflow structure, where water velocity and shear forces available for natural mussel detachment are the highest. Thus, natural shear-force differentials are not responsible for the differences in zebra mussel colonization of the 4 trash rack materials. The AC-coating-only control rack was fouled to the same extent and tenacity as the polyethylene and polyvinyl-coated racks. This field trial is continuing through 1994.

Results from brush shear tests of coupons retrieved from the Dunkirk and Oswego sites are summarized in Table 2, along with relative zebra mussel infestation grades for the larger steel and concrete panels at the Dunkirk site. Numbers of zebra mussels present at the Oswego site were not high enough to calculate statistically significant differences during the first two years of exposure, although qualitative patterns were the same as at the Dunkirk site. Statistical analyses of post-rinse zebra mussel retention on all panels have been completed by LMS Engineers and have been submitted for review by NMPC; these results will be published elsewhere. It is emphasized, here, that the brush shear test is an evaluation of the force required to remove all visible fouling, everything from slimes to macrofoulers. It is not surprising, then, that brush shear test results and relative zebra mussel infestation grades are not totally congruent for samples from the Dunkirk site. As a practical observation, the two candidate coatings that were supplied without anticorrosion undercoats in 1991 were self-eliminated from further evaluation by corrosion of their steel panels and coupons, which made evaluation of the extent and adhesion of biofouling nearly impossible.

Another practical observation is that, during the first two years of immersion, the silicone-based coatings containing exuding oils (as gauged by field observations, as well as by press-contact residue of methyl silicone in infrared spectroscopic analyses) had superior resistance to mussel colonization over non-oily and more abrasion-resistant silicone coatings. However, although mineral-oil-releasing silicone coatings produced good results in terms of resistance to zebra mussel infestation, the brush shear test results (removal of all visible fouling, Table 2) for the same materials do

not show any substantial benefit in comparison with the non-oily silicone coatings of the same initial critical surface tensions (Table 1). The mineral-oil-laden coatings do show, instead, an obvious penalty with regard to their susceptibility to abrasive wear when repetitively brushed at forces required to remove biofouling (Table 1).

Table 2
Post-Exposure Results: Brush Shear Test Data
and Relative Zebra Mussel Release

Coating Type and Code #	Mass Added to Brush to Remove all Visible Fouling (g)		Zebra Mussel Release (compared to controls)
	Dunkirk	Oswego	Dunkirk
Methyl Silicones			
1	80 ± 55	70 ± 90	excellent
2	60 ± 30	30 ± 10	excellent
3	5 ± 3	25 ± 20	excellent
4	40 ± 10	55 ± 30	excellent
5 [no AC coat]	>250	>250	fair
6	200 ± 55	100 ± 90	excellent
7	210 ± 50	160 ± 120	excellent
8	200 ± 70	110 ± 100	good
9	250 ± 45	85 ± 40	equal
10	220 ± 100	150 ± 95	fair
Epoxies			
sil.'91 [no AC]	>250	>250	poor
silicone '93	>250	150	-
fluoro-	250 ± 85	>250	worse
amine	>250	>250	worse
steel control	----	----	control
concrete control	----	----	control

brush shear test: 5 measurements per coating, per site (except sil.epoxy '93)
zebra mussel release: observation includes steel- and concrete-based samples; 2 steel and 2 concrete samples per coating at each site, 6 evaluations each (the first 4 surveys in 1992 are not included due to minimal zebra mussel attachment to any coating or control during that period)

The epoxy-based coatings immersed at Dunkirk and Oswego in January 1992 are very tough, abrasion-resistant materials, but field and lab results demonstrate that they are not good fouling-release materials. Two of the three epoxies immersed in 1992 had initial critical surface tensions in the "bioadhesive" zone, but were badly fouled within one year. The amine-epoxy had an initial critical surface tension of approximately 32 mN/m, and "failed" the fouling-release evaluations within one year. In terms of retained numbers of zebra mussels after rinsing with water from a hose at 25 psi, the fluoroepoxy and amine-epoxy remained more infested than the uncoated steel and concrete control panels.

A revised formulation of the silicone-epoxy was provided in 1993 (coupons only) and placed on the test racks at both sites; these samples were protected with an anticorrosion undercoat, but it is too early to tell if this will lead to longer term efficacy.

Figures 1, 2, and 3 present the brush shear test data for three of the silicone-based coatings. Figure 1 (coating #2) demonstrates good fouling-release at both sites throughout the test period. Figure 2 (coating #1) indicates improved performance after the first few months of exposure; perhaps the coating and the overlying proteinaceous conditioning film changes as a function of immersion time. Figure 3 (coating #9) shows the site-specific differences that were noted for several of the coatings [refer to Table 2].

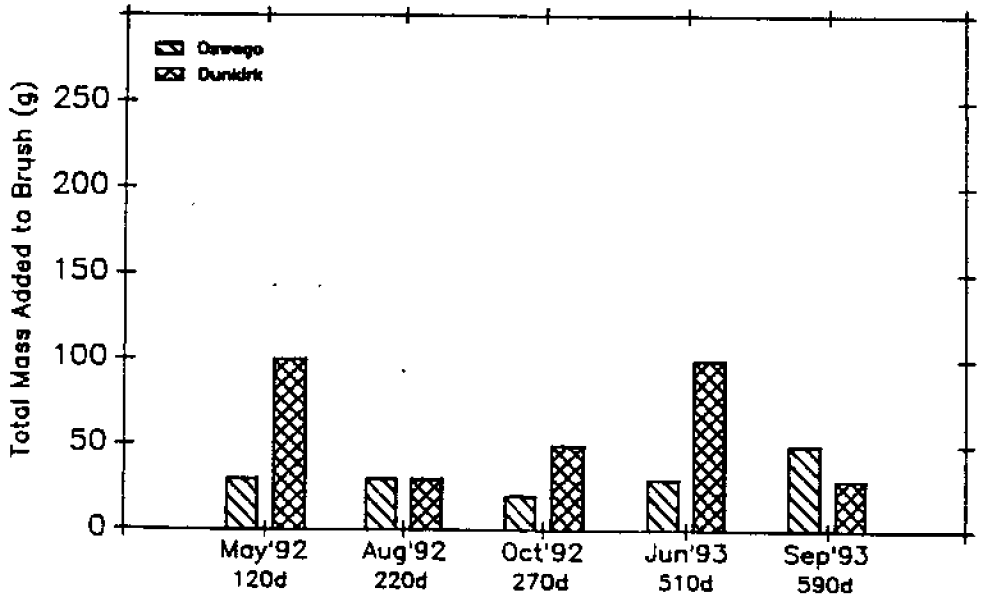

Figure 1 *Results of Brush Shear Test for Coating #2, a commercially available methyl silicone containing some exuding oils. Exposure period is shown on x-axis, mass added to 11-gram brush to remove all visible fouling is y-axis.*

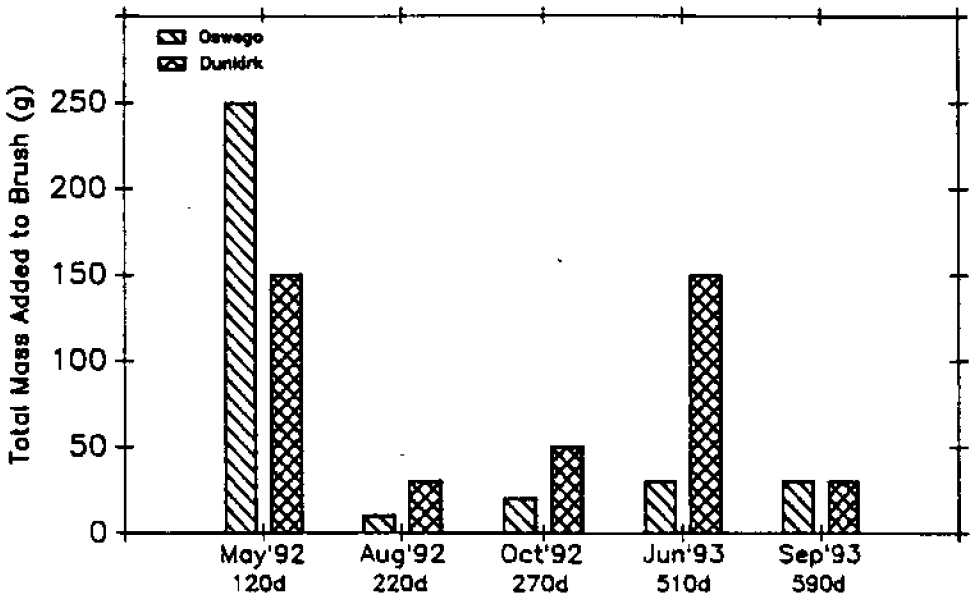

Figure 2 *Results of Brush Shear Test for Coating #1, another commercially available methyl silicone containing exuding oils. Note the improvement in fouling-release property between 120 and 220 days of exposure at both field sites.*

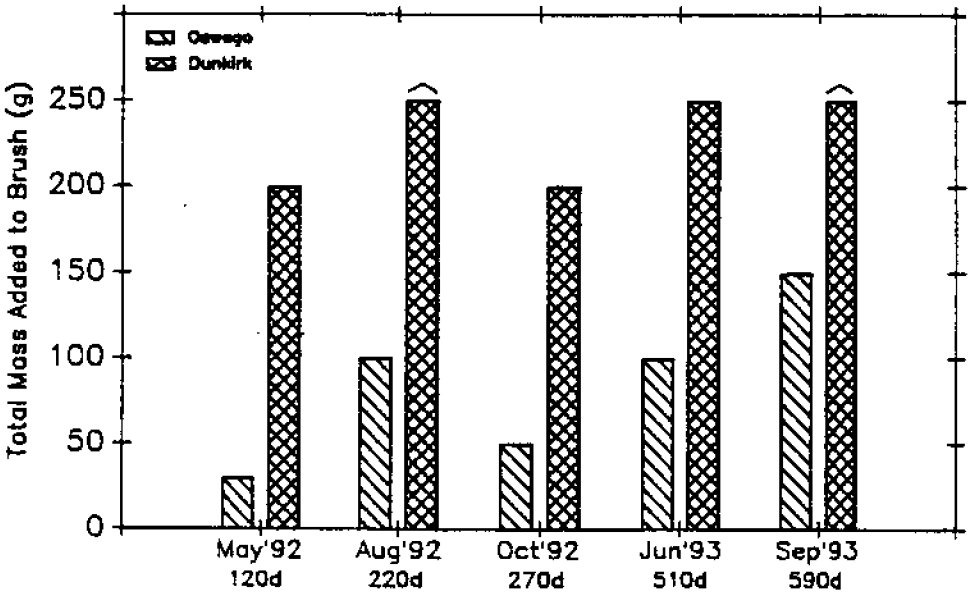

Figure 3 *Results of Brush Shear Test for Coating #9, a thin, hard methyl silicone coating in the development stage, with no apparent exuding oils. Arrows on top of two of the bars indicate that the 261-gram load (250g + 11g brush) was insufficient to remove all visible fouling from the coupon. Note the difference in fouling adhesion between the two test sites.*

Discussion

Most of the coatings and materials described here were selected for field testing because their initial critical surface tensions were between 20 and 30 mN/m, the range demonstrated by Baier and colleagues to minimize biological adhesion.[31] A few materials had critical surface tensions greater than 30 mN/m and, without exception, these have performed poorly in these field trials and elsewhere. Although not described here, seawater trials of coatings having critical surface tensions less than 20 mN/m also fail, again confirming prior findings --- biofouling of very-low-energy fluorocarbon materials is extensive and tenacious. Leitch, et al. presented similar findings from a 2-year study in freshwater.[32] As noted earlier, the critical surface tension criterion is thus considered to be a required "first-level" descriptor for good v. poor fouling-release performance. The reasons for failure of some coatings initially within the required critical surface tension range (e.g. the fluoroepoxy) are unknown, especially since Dexter showed consistent minimal fouling with variously fluorinated polymers meeting this same criterion.[33] Current experiments focus on interfacial mechanics and molecular dynamics as factors that may address these issues. Initial surface polarity may also play a major role; the two 1991-vintage epoxies with low critical surface tensions had very high polar fractions, considerably greater than any of the silicone-based coatings [see Table 1]. This may signal the presence of surface-bonding sites that could increasingly strengthen the biofouling-to-coating interfacial zone. The revised silicone-epoxy coating (immersed in 1993) has been exposed for too short a time to allow direct comparison with the others.

Although it is clear that silicone-based coatings that contain exuding oils are superior in resisting mussel colonization during the first two years of immersion at the Lake Erie and Lake Ontario sites, longer-term performance is yet to be determined. There is some evidence from field studies of similar coatings in seawater environments that, upon conclusion of active exudation, these coatings lose their advantage over other methyl-silicone-based materials. With regard to non-oily silicones, there is anecdotal evidence indicating that these coatings can improve their fouling-release performance with increasing immersion time. The improvement may be due to changes (e.g. hydrolysis) of the coating surfaces; our research group and others currently are pursuing this hypothesis in controlled, laboratory experiments. Preliminary evidence of such surface hydrolysis recently was presented at the annual meeting of the Society for Biomaterials.[34] We also are evaluating the hypothesis that lipid/oil uptake from attached biomass or from the environment may, with time, convert the oil-free silicones to easier-release oil-containing versions.

The results presented here for the field studies at Dunkirk and Oswego indicate the advantage of one main group of coatings over all others. The mechanical forces required to remove all visible fouling from these coatings are extremely low: less

than 100g normal force (16 psi equivalent static pressure) in the lab-based brush shear test and 25 psi in the field-based hose rinse test. The "second-best" group of coatings required an average of between 200 and 260g normal force in the brush shear test to remove all visible fouling, translating to an equivalent static pressure of only 40 psi or less. Considering that some of the "second best" coatings offer tangible benefits of increased wear resistance and the absence of exuding oils, perhaps they are not really out-performed by the first tier of coatings. Again, long-term immersion and fouling retention data are required before the most advantageous "mix" of pre-exposure properties can be determined.

Some of the coatings being evaluated in the current study are commercially available, others are still in the development stage. It is important to note, however, that the vintage of all of the coatings discussed here is late-1991 (NMPC project) and late 1992 (Gilbert/Commonwealth). Pre-exposure analyses of, reportedly, "the same" coatings in these and ONR-sponsored projects over the past 3 years reveal that manufacturers are making changes to their products. As a result, care must be taken in comparing different published reports of coating performance. It is not apparent that all changes result in improved performance. Quantitative surface analyses of pre-exposure samples are the best way to know what the material is before great time and expense are spent in the field.

Summary and Conclusions

Based on field trials of three major types --- immersed test panels of coated and control steel and concrete, coated and uncoated metal and plastic trash racks, and flow-controlled coupon analyses at 6 different freshwater sites --- it is demonstrated that methylsilicone-based coatings having critical surface tensions between 20 and 30 mN/m allow easy mechanical detachment of zebra mussel infestations over a 2-year period. The predominant mechanism in the attainment of this excellent result with nontoxic, nonpolluting coatings is the inability of the glycoprotein-based biological cements to develop secure bonds with low-energy, low-polarity surface layers.

Coatings which, in addition, contain elutable oils display an apparent further resistance to initial colonization by zebra mussels, but this result is obtained without benefit to the brush-removal forces required for coating cleaning, and with a penalty regarding coating abrasion resistance during such brushing. A brush shear stress between 15 and 50 psi is sufficient to completely remove biofouling from these panels and coupons (exposed to the full range of naturally complex, diverse, and variable field conditions) without loss of coating integrity. These results auger well for the eventual substitution of these nontoxic coatings, with a designed periodic cleaning cycle, for the currently deployed toxic coating systems or those that require adjunctive

use of chlorination to control zebra mussel infestations.

Future work must identify the optimal field lifetimes for these systems, including their anti-corrosion undercoats for steel structures. Future work must also address their entire life-cycle costs with regard to application requirements (e.g. substrata preparation, weather conditions), freedom from environmental or occupational hazards (e.g. volatile organic components, elutable components), and removal and re-coating charges (e.g. disposal of sand-blast residues, or needed primer layers). It is obvious that the intrinsic freedom from toxicity of the fouling-release topcoats, themselves, will minimize the costs and concerns in many of these areas.

Acknowledgments

Different aspects of the work reported here were supported by Niagara Mohawk Power Corporation, New York Sea Grant Institute Project R/EMS-2, and Office of Naval Research Grant N00014-89-J-3101. The long-term field tasks at Dunkirk and Oswego could not have been accomplished without the important contributions of Edward Neuhauser (NMPC/R&D), Robert Brombos (NMPC/Dunkirk), Thomas Coates (NMPC/Oswego), John Matousek (LMS Engineers), John Dembeck (LMS Engineers), and Raymond King (King Consulting). Thanks are due to James Knowlton and Rudolph Wahanik (Gilbert-Commonwealth, Inc) and Richard Tetrault (NMPC/Medina) for their guidance in the trash rack study. In addition, Thomas Bonner (SUNY/Brockport), Kevin Robison (NYSE&G/Somerset), Paul Sawyko (Rochester Gas & Electric), and Richard Tetrault are thanked for their participation in the flow cell field work.

References

1. Rzepecki LM and Waite JH (1993) Zebra Mussel Byssal Glue: An Unwelcome Bond Between Man and Mollusk?, Proceedings, 3rd International Zebra Mussel Symposium, Toronto, Canada.

2. Olivieri MP, Loomis RE, Meyer AE, and Baier RE (1990) Surface Characterization of Mussel Adhesive Protein Films, *J Adhesion Sci & Technol*, 4:197-204.

3. Nassar U, Meyer AE, Loomis PM, and Loomis RE (1993) A Comparative Physico-Chemical Study of Substrate Activated Peptide Domains from Human Salivary Proline-Rich Protein, in Symposium Notebook, Surfaces in Biomaterials '93, Surfaces in Biomaterials Foundation, Minneapolis MN, pp 197-201.

4. Olivieri MP and Baier RE (1994) Conformation of a Cell Attachment Peptide to a Model Surface, Transactions of the Society for Biomaterials, XVII:344.

5. Bonner TP and Rockhill RL (in press) Ultrastructure of the Byssus of the Zebra Mussel (*Dreissena polymorpha*, Mollusca: Bivalvia), *Trans Am Micr Soc*, 113:xx-xx.

6. Olivieri MP (1991) Surface Characterization of Selected Bioadhesives, doctoral dissertation, State University of New York at Buffalo.

7. Baier RE, Shafrin EG, and Zisman WA (1968), Adhesion: Mechanisms That Assist or Impede It, *Science*, 162:1360-1368.

8. Baier RE (1984) Initial Events in Microbial Film Formation, in Marine Biodeterioration, JD Costlow and RC Tipper (eds), Naval Institute Press, pp 57-62.

9. Baier RE, Meyer AE, DePalma VA, King RW, and Fornalik MS (1983) Surface Microfouling During the Induction Period, *J Heat Transfer*, 105:618-624.

10. Meyer AE, Baier RE, and King RW (1988) Initial Fouling of Nontoxic Coatings in Fresh, Brackish, and Sea Water, *Can J Chem Eng*, 66:55-62.

11. Meyer AE, Fornalik MS, Baier RE, King RW, Zambon JJ, Huber PS, Wattle BJ, and Gucinski H (1984) Microfouling Survey of Atlantic Ocean Waters, in Proceedings of the 6th International Congress on Marine Corrosion and Fouling, pp 605-621.

12. Baier RE and DePalma VA (1979) Flow Cell Method for Continuously Monitoring Deposits on Flow Surfaces, U.S. Patent Number 4,175,233.

13. Baier RE, King RW, and Meyer AE (1984) Thermal Chambered Flow Cell, U.S. Patent Number 4,466,277.

14. King RW, Meyer AE, Ziegler RC, and Baier RE (1982) New Flow Cell Technology for Assessing Primary Biofouling in Oceanic Heat Exchangers, in Proceedings of the 8th Ocean Energy Conference (June 1981), U.S. Department of Energy, DOE/Conf-810622-ESC, pp 431-436.

15. King RW, Baier RE, and Fornalik MS (1983) Improved Instrumentation for Oceanic Fouling Forecasts, in Oceans '83, MTS-IEEE Conference (August 1983), San Francisco.

16. Baier RE, Meyer AE, and King RW (1988) Improved Flow-Cell Techniques for Assessing Marine Microfouling and Corrosion, Chapter 32 in Marine Biodeterioration, Advanced Techniques Applicable to the Indian Ocean, MF Thompson, R Sarojini, and R Nahabhushanam (eds), Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, India.

17. Baier RE (1982) Conditioning Surfaces to Suit the Biomedical Environment: Recent Progress, *J Biomech Eng*, 104:257-271.

18. Baier RE (1983) Surface Chemical Factors Presaging Bioadhesive Events, *Ann NY Acad Sci*, 416:34-57.

19. Baier RE (1984) Surface Phenomena Associated with In Vivo Behavior of Biomaterials, in Contemporary Biomaterials: Material and Host Response, Clinical Applications, New Technology and Legal Aspects, JW Boretos and M Eden (eds), Noyes Publications, Park Ridge, NJ, pp 92-125.

20. Meyer AE (1990) Dynamics of "Conditioning" Film Formation on Biomaterials, monograph/doctoral dissertation, Lund University, Sweden.

21. Crisp DJ, Walker G, Young GA, and Yule AB (1985) Adhesion and Substrata Choice in Mussels and Barnacles, *J Coll and Interface Sci*, 104:40-50.

22. Rittle KH, Helmstetter CE, Meyer AE, and Baier RE (1990) *Escherichia coli* Retention on Solid Surfaces as Functions of Substratum Surface Energy and Cell Growth Phase, *Biofouling*, 2:121-130.

23. Baier RE, Gucinski H, Meenaghan MA, Wirth J, and Glantz P-O (1985) Biophysical Studies of Mucosal Surfaces, in Oral Interfacial Reactions of Bone, Soft Tissue and Saliva, P-O Glantz, SA Leach, and T Ericson (eds), IRL Press Limited, Oxford, pp 83-95.

24. Meyer AE and Baier RE (1992) Nontoxic Control of Zebra Mussels: Surface Chemistry/Physics of Biological Adhesion, in Proceedings, Electric Utility Zebra Mussel Control Technology Conference, EPRI Report TR-100434, pp 2/44 - 2/58.

25. Baier RE and Meyer AE (1992) Surface Analysis of Fouling-Resistant Marine Coatings, *Biofouling*, 6:165-180.

26. Young T (1805) An Essay on the Cohesion of Fluids, *Philos Trans Roy Soc Lond*, 95:65-87.

27. Zisman WA (1964) Relation of the Equilibrium Contact Angle to Liquid and Solid Constitution, in Contact Angle: Wettability and Adhesion, Adv Chem Ser 43, Amer Chem Soc, Washington, DC, pp 1-51.

28. Harrick NJ (1960) Surface Chemistry from Spectral Analysis of Totally Internal Reflected Radiation, *J Phys Chem*, 64:1110-1114.

29. Rittschof D, Clare AS, Gerhart DJ, Bonaventura J, Smith C, and Hadfield MG (1992) Rapid Field Assessment of Antifouling and Foul-Release Coatings, *Biofouling*, 6:181-192.

30. Mussalli YG (1989) Non-Toxic Foul-Release Coatings, Stone & Webster Final Report to Electric Power Research Institute, Research Project 1689-9.

31. Callow ME (1993) A Review of Fouling in Freshwaters, *Biofouling*, 7:313-327.

32. Leitch EG and Puzzuoli FV (1992) Evaluation of Coatings to Control Zebra Mussel Colonization: Preliminary Results 1990-1991, *J Protective Coatings & Linings*, 9(7):28-41.

33. Dexter SC (1979) Influence of Substratum Critical Surface Tension on Bacterial Adhesion -- in situ Studies, *J Coll and Interface Sci*, 70:346-354.

34. Marotta J, DePalma D, and Batich C (1994) Surface Changes in "Silicone" Elastomer Upon Exposure to Saline, Transactions of the Society for Biomaterials, XVII:148.

Developing Environmentally Sound Methods and Strategies to Control Zebra Mussels at Public Facilities

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Abstract

In response to the Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990 (Public Law 101-646) the U.S. Army Corps of Engineers initiated a program to develop methods and strategies to control zebra mussels at public facilities. Research is being facilitated by working groups that deal with the following structures: 1) locks and dams; 2) vessels and dredges; 3) power facilities; and 4) reservoirs, water intakes, gages, and pumping stations. Each working group consists of individuals with expertise in zebra mussel control, biology and ecology of zebra mussels, and design, construction, or operation of the facility of concern.

A central theme to the research is demonstration projects conducted at specific facilities along waterways. Findings from laboratory and field research are being further tested with these demonstrations. This paper describes three demonstration studies: 1) the use of water drawdown to kill zebra mussels at Black Rock Lock on the Niagara River, New York; 2) the use of a copper-containing epoxy coating to protect a tugboat used in the Great Lakes near Detroit, Michigan; and 3) installation of a chlorine injection system for zebra mussel control at hydropower plants along the Cumberland River, Tennessee.

Introduction

Zebra mussels (*Dreissena polymorpha*), first reported in North America in 1988 (1), have rapidly spread throughout waterways of the United States, mostly via commercial navigation traffic (2). It is likely that this species will negatively affect many facilities in the inland waterway system. The U.S. Army Corps of Engineers (USACE) maintains and operates 195 locks, 75 hydropower stations, 461 reservoirs, and 2,260 vessels and dredges. With the exception of those in brackish waters, many are susceptible to zebra mussel infestations.

In response to the Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990 (Public Law 101-646) the USACE initiated a program to develop methods and strategies to control zebra mussels at public facilities. "Public facilities" includes locks, dams, reservoirs, commercial dredges and vessels, as well as non-Corps structures such as intakes for power generation, potable water, and sewage treatment. Research was designed to develop new and evaluate existing zebra mussel control methods and strategies, and study the biology and ecology of this pest species. Studies are being conducted at the U.S. Army Engineer Waterways Experiment Station (WES) in Vicksburg, Mississippi, and the U.S. Army Construction Engineering Research Laboratories in Champaign, Illinois.

Laboratory studies are being conducted on the effects of reduced and elevated temperature and humidity on the duration of aerial exposure needed to kill zebra mussels. Attachment strength of mussels to different types of materials is being analyzed. Chemicals, coatings, and cleaning techniques are being evaluated. Field studies on environmental impacts to native biota, zebra mussel density, growth rates, and demography of newly established populations are under way.

Multidisciplinary Working Groups

In September 1991, the USACE held a planning meeting on zebra mussels in Fort Mitchell, KY. Over 50 scientists and engineers with experience in the design, operation, or maintenance of locks, dams, and reservoirs attended. The purpose was to identify facilities and structural components likely to be affected by zebra mussels. In addition, attendees prepared a preliminary list of strategies to deal with infestations. A summary of the meeting was prepared that provided the basis for further research (3).

Following that meeting, four working groups were formed to deal specifically with facilities of concern. The approach was based on programs developed by Ontario Hydro and the U.S. Coast Guard. Facilities subject to infestation and of concern to the USACE were placed into one of four categories:

1. Locks and dams;
2. Vessels and dredges;
3. Power facilities; and,
4. Reservoirs, water intakes, gages, and pumping stations.

Each working group consisted of individuals with expertise in zebra mussel control, biology and ecology of zebra mussels, and design, construction, or operation of the facility. A typical working group consisted of 20 members and had representation from academia, state agencies, municipalities, Canada, the U.S. Army Corps of Engineers, or other Federal agencies. Continuity among groups was ensured by having representation from WES at all meetings. Working group members were tasked with the same two objectives of the 1991 planning meeting: 1) identify components of the facility susceptible to infestation and, 2) devise environmentally sound strategies to control zebra mussels.

For example, working group members that dealt with navigation locks identified two particularly vulnerable components. These were gage wells used to house water-level sensing equipment, and raw water systems used for fire protection. The working group recommended the following to protect raw water systems:

* Ensure that the system is truly stagnant.
* Install screens over the entrance of intake pipes.
* Periodically treat with chemicals.
* Switch to chlorinated water if possible.
* Periodically drain and inject steam or hot water

The group recommended that gaging wells be inspected frequently by deploying inexpensive monitoring devices such as ceramic tile, concrete blocks, or PVC plates suspended from rope or cable that could be easily removed for examination. The interior of gaging wells could be treated with small amounts of chlorine. The pipe that enclosed meters or led to raw water could be treated with an anti-foulant coating.

Meetings that dealt with other facilities were structured in a similar manner. Group members with knowledge of each facility identified components of concern and recommended methods and strategies applicable to each. Recommendations included use of antifoulant paints and biocides, installation of screens, pigging (cleaning a pipe with a mechanical device), and treatment with desiccation, hot water, or steam.

Groups meet once per year, and individuals recommend methods and strategies for specific facilities. Recommendations are based on results of

government research as well as findings from the U.S. Coast Guard, Ontario Hydro, academic institutions under contract to WES, and others studying zebra mussels. Between 1991 and 1994, more than 275 individuals have participated in these working groups meetings (Table 1).

Demonstration Studies

An important component of the Corps' zebra mussel program are demonstration projects. These are designed to apply findings from previously conducted research to a specific facility. In addition to determining the efficacy of control strategies or methods, information on cost, environmental compatibility, and application methods is obtained. The following is a brief summary of these demonstrations.

Note: The contents of this paper are not to be used for advertising, publication, or promotional purposes. Citation of trade names does not constitute an official endorsement or approval of the use of such products.

Freezing to Kill Zebra Mussels at Black Rock Lock

Laboratory studies have shown that zebra mussels are relatively intolerant to aerial exposure at subfreezing temperatures (4). For example, the LT_{100} values of mussels exposed to air at -10.0°C, -7.5°C, and -5.0°C approximately equal 1.5, 2.0, and 5.0 hours. As temperature increases to approximately -1.5°C to 0.0°C and mussels are exposed in clusters instead of as individuals, survival time increases to >48 hours. Even in cold climates, during winter dewatering of a facility, it is possible that factors such as occasional warming of air, leaking water and ice formation, and clustering of mussels will increase tolerance times over those observed by McMahon et al. (4) for individual mussels in the laboratory.

In January 1994, studies were conducted during winter drawdown of Black Rock Lock, U.S. Army Engineer District, Buffalo, in Black Rock Canal (a side channel of the Niagara River in Buffalo, New York) to determine tolerance times and exposure conditions of zebra mussels. Constant recordings of air temperature were made at the top of the lock chamber during dewatering and at the bottom of the chamber after dewatering was complete. Zebra mussel samples were collected along a vertical transect down the lock chamber wall just as dewatering was nearly complete, and then approximately 18 hours later. Mussels were brought indoors, the shell length of each was measured, and gently probed (4) to determine if they were alive.

Near the end of the dewatering process, a cold front moved through the area and air temperatures dropped from near freezing to less than -10.0°C within 24 hours. This blast of sustained subfreezing air rapidly killed all mussels on the

chamber wall. Usually there was close agreement between mortality observed in this field demonstration and the laboratory studies.

This lock had also been dewatered for maintenance and inspection during January and February 1992. Air temperatures had remained moderate, with frequent warming above freezing during that dewatering. The Lockmaster reported a slower kill of mussels in previous years than was observed in 1993 (Gary Dye, personal communication). Nevertheless, samples taken from outside the chamber (i.e., at a location never exposed to air) and inside the chamber in January 1994 (i.e., exposed to air in early 1993 and again in early 1994) clearly indicated the general effectiveness of the first winter drawdown. Only settlers from the 1993 recruitment cohort occurred in the lock chamber, whereas the population outside the lock chamber consisted of three cohorts.

In summary, even very brief exposure (<24 hours) to air <3°C is an effective method of controlling zebra mussels. Sustained winter drawdown (several weeks) is sufficient to control mussels even if subfreezing temperatures are not consistently sustained.

Use of a Copper-Containing Epoxy Material to Protect a "Bay Class" Tug from Zebra Mussel Infestations

In March 1992, the U.S. Army Engineer District, Detroit protected the hull of a "bay class" tug, the *Tawas Bay*, from zebra mussel infestations. The tug is 45 feet long, with a beam of 13 feet and a draft of 7 feet. It is used as a vessel tender or in support of larger tugs for strike removal and repair operations in the Detroit and St. Clair Rivers, Michigan. In 1991 it was operated for 70 to 80 days.

The *Tawas Bay* was coated with EPCO-TEK 2000(tm) which is produced by the Hi-Tek Chemical Corporation, Hempstead, New York. The material was applied by Quality Marine Finishers, Mobile, Alabama. The coating is made by mixing epoxy with copper powder, wetting agents, and other materials to produce abrasion resistance and flexibility. The resultant paint is sprayable, hard, smooth, scratch-resistant, flexible, barnacle and mollusc repelling, and nontoxic. It also acts as a water-diffusion barrier. In addition to protecting the hull from zebra mussels, this coating will decrease friction in the water, thus increasing fuel efficiency.

Prior to application, the tug was completely sandblasted below the waterline to remove old paint and other foreign material. A high-profiled surface was produced by using 12/20 grit media. Immediately following sandblasting the surface was cleaned to remove all dust, since the antifoulant paint ultimately accentuates marks or unevenness (unfairness) in the substrate because of its enamel-like finish. After the surface was cleaned, a primer was applied. This was a low-viscosity 100% epoxy undercoat, with high "wicking" characteristics. As soon

as the primer became tacky, a single undercoat, which was a thick epoxy that gave an enamel-like finish, was applied to a thickness of 0.0006 inch.

The tug was then painted with five coats of the epoxy/copper coating. The first coat (0.001 to 0.002 inches) consisted of a thinner mixture (25% more than the usual amount of solvent was added). The second coat (0.005 to 0.007 inches thick) contained 15% additional solvent. Each new coat was applied within 30 minutes. The next coat was applied whenever the previous coat felt tacky. The final thickness was approximately 0.017 to 0.020 inches. The painter attempted to achieve a slightly higher thickness on the stern of the vessel to protect the vessel from abrasion caused by sediments stirred up by the propeller.

Development of a Zebra Mussel Control Strategy for Hydropower Plants

Personnel of the U.S. Army Engineer District, Nashville, developed a strategy for controlling zebra mussels at the District's power stations. Two aspects were central to the development of this strategy: 1) keeping the facilities operational regardless of zebra mussels and 2) implementing environmentally sound strategies that would meet the goals of the National Environmental Policy Act.

The Cheatham Project was the sixth of nine projects with hydropower built for the Nashville District. It was completed in 1960 as a navigation and hydropower project. The plant is a low-head design with three 20,000-horsepower vertical shaft kaplan (propeller-type) turbines with a design operating head of 22 feet. The generators are 3-phase, 60-cycle, 13.8-kv units rated at 13,333 kva and 60 rpm.

As is typical with hydropower generators and turbines of this design, excess heat is removed from turbine bearings, generator bearings, and the generator windings by raw water-cooled heat exchangers. The water for the heat exchangers is drawn from the river, circulated, and returned to the river. Much of the piping for these systems is embedded in concrete and has sharp bends. This dependence on raw river water for cooling the critical generator and turbine components makes Cheatham and other hydropower plants particularly vulnerable to the effects of zebra mussel infestations. Left unprotected, the Cheatham hydropower plant could be completely shut down due to fouling of the raw cooling water piping.

In the spring of 1992 the District developed a design for chlorine injection systems that would be installed at raw water systems at all USACE hydropower plants in the District. The principal system components are 1) a variable rate chemical injection pump that feeds the chlorine into the raw water system at its source; 2) storage tanks designed to hold a 30-day supply of chlorine; 3) an

analyzer that measures chlorine concentration in the discharge water and automatically signals a program logic controller to adjust the chemical feed pump to maintain the desired chlorine concentration, and 4) a control panel containing a strip chart recorder which continuously records chlorine concentration in the discharge water.

In the summer of 1992 a contract was let with Prominent Fluid Controls, Inc., of Pittsburgh, Pennsylvania, for the fabrication and installation of chlorine injection systems for the raw water piping systems for Cheatham and Barkley Power Plants. Installation of the systems has been completed, and they are currently undergoing final tests.

When veligers are detected at the plant, the chlorine injection systems will be activated. Initially, the system will be operated to produce continuous total residual chlorine at the raw water system discharge point of 0.5 ppm. However, personnel at Cheatham Power Plant will experiment with different concentration levels and injection intervals to determine a suitable protocol for their plant.

In addition to the technical aspects of zebra mussel infestation control, the environmental aspects must also be considered. At the same time the chlorine injection systems were being designed and installed, an Environmental Assessment was being conducted by personnel of the Nashville District and the Tennessee Valley Authority for all power generation facilities being operated by both organizations. During the process of conducting the Environmental Assessment it was determined that Cheatham Power Plant would be required to obtain a National Pollution Discharge Elimination System (NPDES) permit from the State of Tennessee to operate the chlorine injection system. The permit applications have been submitted and are now being processed. In the event that an infestation begins prior to approval of the NPDES permits, it will be necessary to request interim permission from the State of Tennessee to operate.

Having protected its most vulnerable component, the cooling raw water systems, Cheatham Power Plant is now prepared for zebra mussel infestations. However, attention must also be given to the many other system components that will be affected. Unlike the raw water systems, these components are physically accessible for the application of other control methods.

Zebra mussel infestations pose a major threat to all electric power production facilities. Facilities that rely on single-pass raw water cooling water systems are particularly at risk. However, with thorough study and planning, electric power production facilities can continue operations unaffected as zebra mussel infestations occur.

Summary

Environmentally sound control methods and strategies must be available for immediate use when zebra mussels are first detected at a facility. Applied

chemical, engineering, and biological studies are used to develop control methods. Those who design, operate, and maintain facilities are best able to develop strategies based on their own experience and research findings. As zebra mussels spread throughout the inland waterway system, control strategies will be tested at selected facilities. Success will be determined after evaluation of operational impacts, degree of control, and possible environmental effects. If successful, recommendations to other facility managers will be made. If strategies are not successful, working groups will reassess the problem and recommend new techniques.

No single control agent or method will eliminate zebra mussels from this Nation's waterways. Facility-specific methods and strategies are needed that reduce zebra mussels locally. Methods and strategies must be economical, easy to use, and must not harm native aquatic organisms.

Acknowledgments

Permission was granted by the U. S. Army Corps of Engineers to publish this information. Funds were provided through the Zebra Mussel Research Program of the U.S. Army Corps of Engineers.

Table 1
Attendance at working group meetings to develop strategies for zebra mussel control at public facilities. In 1994, two combined working groups meetings were held.

Meeting	1991	1992	1993	1994
Initial Strategy	53	--	--	--
Locks and Dams		25	24	23
Vessels and Dredges		18	19	
Power Facilities		27	26	23
Reservoirs, Intakes, Gages, and Pumping Stations		14	24	
Total Attendees	53	84	93	46

Literature Cited

1. Hebert, P.D.N., Muncaster, B.W., and Mackie, G.L. 1989. Ecological and genetic studies on *Dreissena polymorpha* (Pallas): a new mollusc in the Great Lakes. Canadian Journal of Fisheries and Aquatic Sciences 46:1587-1591.

2. Keevin, T.M., Yarbrough, R.E., and Miller, A.C. 1992. Long-distance dispersal of zebra mussels (*Dreissena polymorpha*) attached to hulls of commercial vessels. Journal of Freshwater Ecology 7(4):437.

3. Miller, A. C., Neilson, F., Payne, B. S., and McMahon, R.F. 1992. Control strategies for zebra mussel infestations at public facilities. Technical Report EL-92-25, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

4. McMahon, R.F., Ussery, T.A., and Clarke, M.C. 1993. Use of emersion as a zebra mussel control method. Contract Report EL-93-1, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

The Efficacy of Pulsed Electric Fields in Preventing Settlement of Zebra Mussel Veligers

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ABSTRACT

The three reference companies have joined in a technology development program focusing on the use of electric fields as a control mechanism for zebra mussel settlement in power plants or other facilities subject to infestation.

The device, dubbed the "Electric Trap" is intended to eliminate potential colonization by destroying or incapacitating zebra mussel larvae at the point of entry of water flow into a plant. The Electric Trap "killing field" concept is based upon the establishment of a strong pulsed electric field between metallic plates immersed in fresh or brackish water.

A field installation would consist of a series of thin metal plates coated with a low conductivity polymer, vertically positioned in a water duct or pipe, between which passes a rapidly alternating current of great intensity and with a short, sharp pulse at repetition rates of 400 Hz to 1 kHz. Any larvae present in the water will become polar upon application of the electric field, creating a dipole which induces a displacement current internal to the larvae.

It is believed that it will be possible to select pulse characteristics which will kill or incapacitate the zebra mussel larvae in its settling stage (in the magnitude of 150 microns) while leaving significantly smaller larvae and other smaller organisms unaffected. The process is expected to involve relatively low power consumption as the power is consumed only in charging the effective capacitance of the water-filled space between the metal plates. With such an approach, it is possible to utilize pulsed alternating current with a low duty cycle.

Very simple, but successful laboratory tests were conducted by Infrawave International during the summer of 1991, utilizing native Daphnia larvae. These

tests achieved combined mortality/immobilization results approaching 100%. Further laboratory tests will be undertaken by the technology development partnership during the summer of 1994 with field testing taking place as soon as practicable.

THE 1991 EXPERIMENTS

Introduction

Laboratory experiments were conducted in Norway and the United Kingdom by the principal investigator, Lars Ostlie, during the summer of 1991 in order to determine the effect that an AC electric field might have on freshwater macro-organisms. The use of electric fields was considered as a control measure for zebra mussels prior to the entry of water into a utility or industrial plant could represent a cost-effective and environmentally acceptable alternative to chemical methods of control.

In carrying out a literary search, it was found that the use of electric fields to control freshwater macro-organisms has generally not been widely investigated. One paper was of particular interest (M.Y. Kirpichenko 19) examining the relationship (using DC fields) between voltage and time parameters necessary to achieve Dreissena larvae mortality.

In going through this prior work, it became increasingly clear that there was a problem in using DC fields. The problem related to the fact that DC current tends to flow around the larvae and not through it. This was determined to be due to the fact that the shell (the outer surface) of the larvae has insulating properties and the voltage gradient across the larvae must accordingly be quite high in order to set up a fatal current inside the larvae. The substantial amounts of electrical energy required therefore rendered a DC field uneconomic and impractical.

Further investigative work was then undertaken regarding use of an AC field with the objective of inducing a current inside the larvae by rapidly changing the electric field across it and forcing an induced current inside it.

The Theory

The concept of the Electric Trap is based upon capacitor theory and the fact that freshwater has a relatively low conductivity and is very polar with a relative permittivity of approximately 75-80. Accordingly, it represents a strong dielectricum. In basic terms, the Electric Trap consists of two capacitor plates influencing the mass of freshwater between the plates.

When an alternating voltage is applied over the plates in the Electric Trap, an alternating electric field is in turn established in the water between the plates, causing a corresponding alternating polarisation of both the water mass and the liquid inside the larvae (consisting mostly of freshwater and some minerals), thus achieving the objective of setting up an alternating induced current inside the larvae.

Due to the fact that undistilled (natural state) freshwater is not an insulator, but rather somewhat conductive, the introduction of an insulating or semi-insulating coating on the plates will serve to limit the flow of electrons in the water. Consequently, the consumption of power can be significantly lower due to the fact that the only current flowing will be caused by the induced current.

The Experiment

The overall objective of the experiment was to prove the Electric Trap theory - that an induced electric field would either kill or immobilise larvae and that the control of this process can be demonstrated.

The experiments were carried out in Stavanger, Norway, under the direction of Lars Ostlie and with the assistance of the Rogalandsforskning organization and Ole B. Dahle who provided the necessary laboratory facilities and equipment.

In demonstrating the theory it was known from previous research that the depth of the polarised layer extending out from the electrode is only 0.1 - 0.3mm per 1kV in freshwater and that the purer the water the greater the depth of the polarised layer.

As was described in the patent, both plates were to be fully insulated with a coating carrying a high permittivity ($\in$) as represented in Fig. 1. This, it was assumed, would produce a polarisation layer over the coatings.

In Fig. 1 the 4 polarisation layers are shown in 2 pairs. The first pair depicts polarisation across electrode A and coating A, with the second layer of polarisation taking place across the coating A and the water. Similar pairs are depicted as water and coating (B) and electrode (B) on the opposite side (four polarisation).

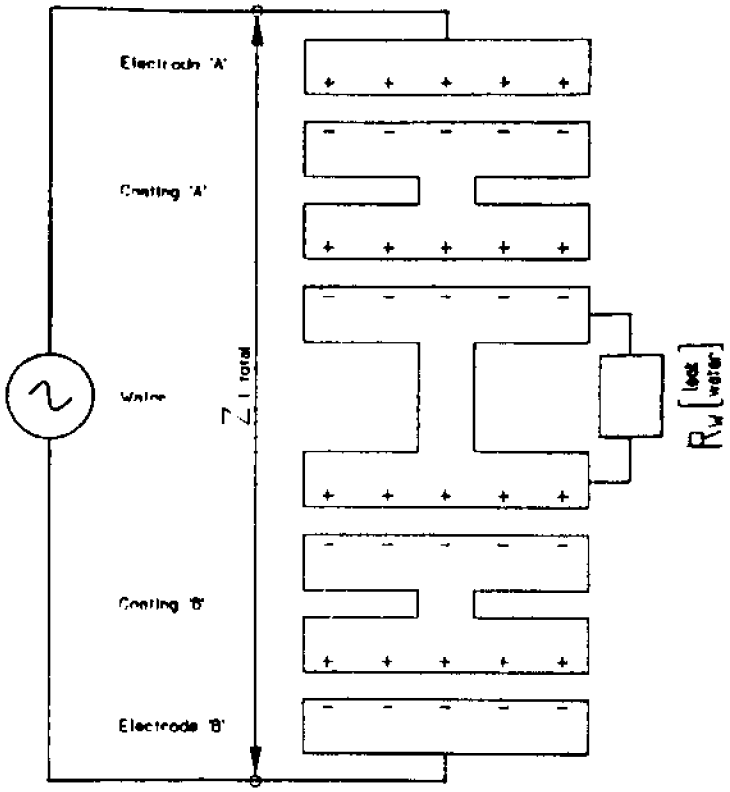

Fig 1. Illustrates the electric model using 2 insulated plates

Due to the conductive element of the water however, there will be a small leak current present that will serve to short circuit and break down the polarisation layer in the water. This leak current has a resistance which can be calculated (utilising non insulating plates) using the formula:

$$R = \frac{\rho \ell}{a} \quad \frac{\ell}{\sigma a}$$

where ρ is the specific resistivity of the water
σ is the conductivity of the water
ℓ is the distance between the plates
a is the area of the plates

given that: $\sigma \simeq 280\ \mu$mhos/cm
$\ell_1 =$ 6mm and $\ell_2 = 3$mm
$a = 100\text{cm}^2$

hence $R_1 = 21.4\ \Omega \qquad R_2 = 10.7\ \Omega$

One of the problems in this version of the experiment arose from the fact that the two fully insulated electrodes required very high voltages across the plates in order to permit a reasonable distance between the plates; this was due to the limitation in depth of the polarisation layer when using insulated electrodes. The minimum acceptable distance between the plates was defined as 3mm for this experiment.

Unfortunately, the range of hardware on hand was limited. In particular, the high voltage unit had a maximum voltage of only 4.8kV and a more powerful unit, enabling a greater depth of the polarisation layer extending out from the electrode, was not available.

The experiment was then repeated utilising two different coating approaches, i.e. with one coating which was semi-conductive and one that had a bare plate. In the latter case, the electrode was entirely exposed to the water and hence it was possible for electrons to freely flow across the surface.

These two types of experiment are depicted in Fig. 2 and Fig. 3. In Fig. 2 it can be observed that there is polarisation across the surface of the semi-conductive coating (C), which is, however in turn short circuited by the leak current due to the resistance in the coating. There is present, therefore, a combination of induced and ordinary flow current in coating (C).

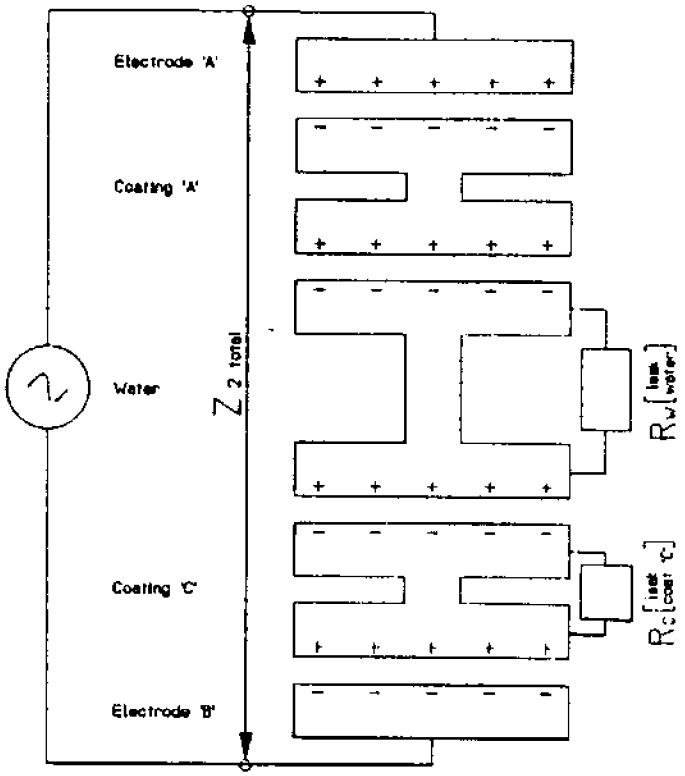

Fig 2. Illustrating the electric model device using one insulated and one semi-insulated plate

In the experiment described in Fig. 3, the polarisation takes place across one coating and the water. Consequently, the only current in the water is that driven by the electric field, setting up the polarisation layer across the coating and the bare electrode. the ΔV (voltage gradient between the bare and the coated electrode) is determined by the current through $R = [R_K + Rs]$ between the coating [A] and the bare [B] electrode and is equivalent to the induced current across the coating.

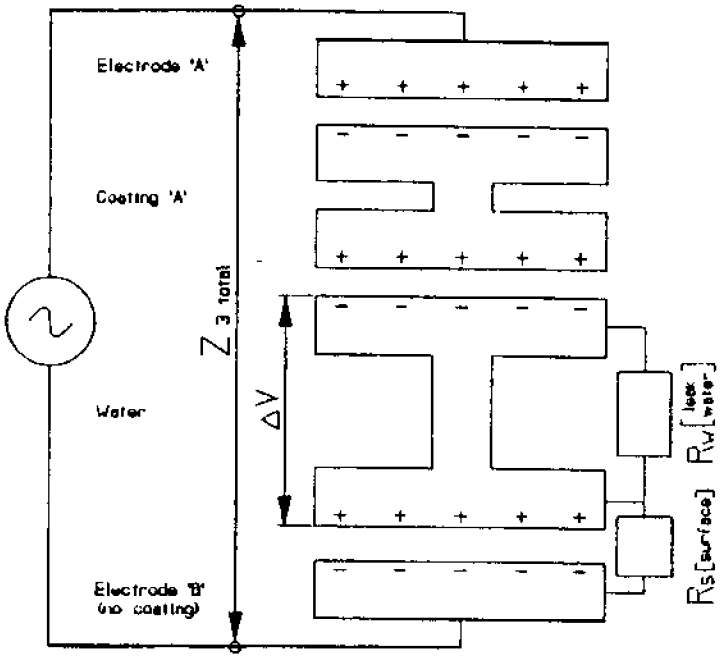

Fig 3. Illustrating the electric model device using one insulated plate and one bare plate

Physical Layout

The only device assembled specifically for the experiment was the "simplified" Electric Trap as shown in Fig. 4. All other equipment used could typically be found in a reasonably equipped laboratory, such as the universal meter, oscilloscope, cables, hand tools etc. The electrical hardware used was originally built for a different experiment, and was modified for this application.

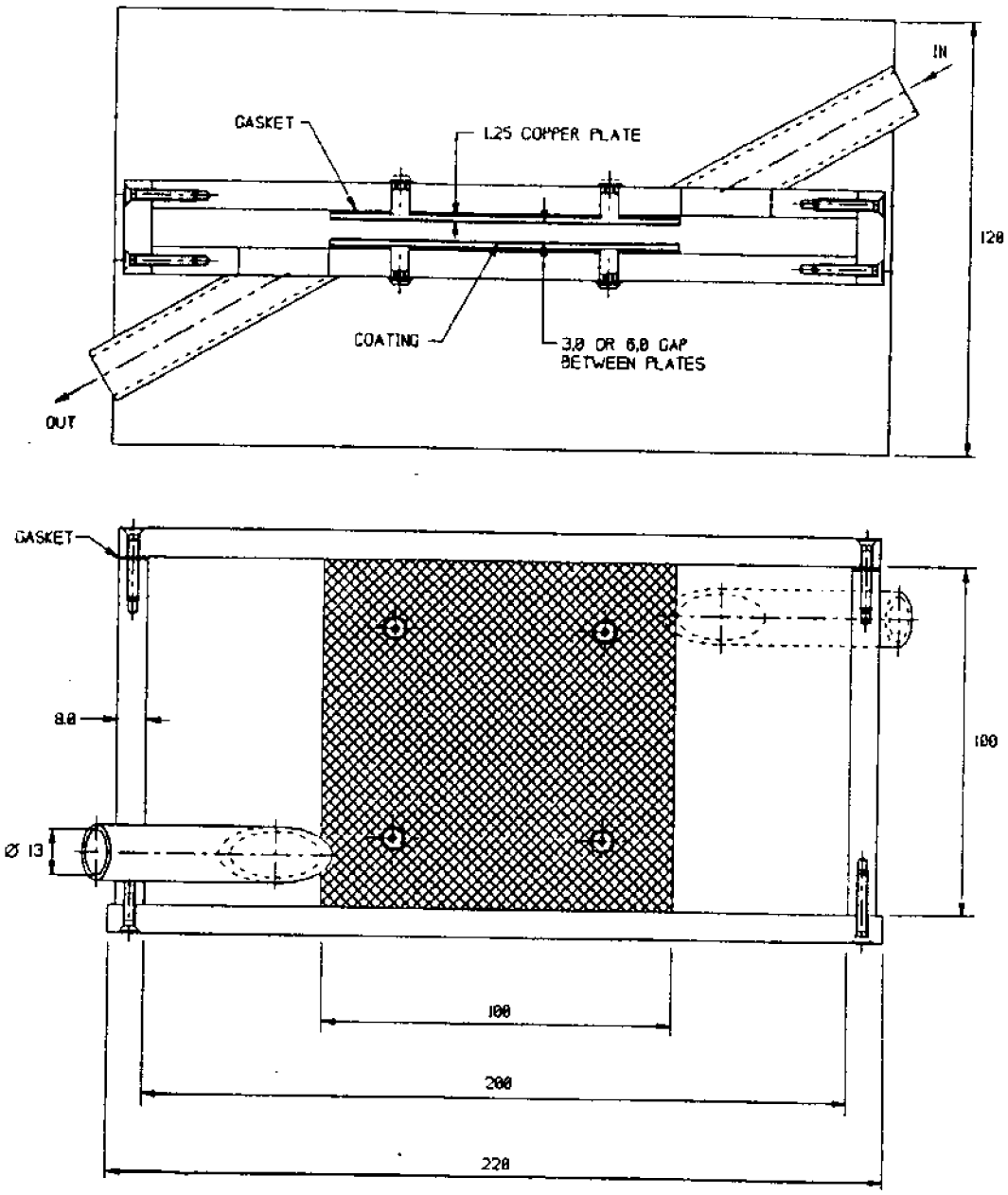

Fig 4. The layout of the simplified Electric Trap

As shown in Fig. 4, the Electric Trap was placed on a table, and connected to two water tanks. One tank (Tank 1) was situated approximately 1m above the table, and the other (Tank 2) was placed on the floor. The hosepipe on the outlet was bent upwards with it's highest point above the Electric Trap.

Tank 1 was filled with filtered (a plankton filter was used) water and released into the Electric Trap. It was ensured that there was no air in the Electric Trap.

By introducing a valve between Tank 1 and the Electric Trap the rate of flow could be controlled. The objective was to achieve a uniform flow distributed over the plates. As the trap was constructed of transparent material (polycarbonate), the flow over the plates could be observed by adding dye (ink) to the water and removing one of the plates. As expected, vortices or small whirlpools were seen to be present, especially at high flow rates.

After some experimentation, the flow valve was adjusted to release approximately 0.3ℓ/min in the case of a distance between the plates d_1 of 6mm, and 0.15ℓ/min when d_2 was 3mm. This produced a calculated flow rate over the plates of approximately 0.5m/min.

The Coatings

The coating used was a standard acrylic paint supplied by Jotun in Norway. Acrylic paint has a dielectric constant of approximately 5 - 5.5 at low frequencies and the resistivity is very high. Although changing in accordance with the various pigments used, generally the Volume Resistivity [Ωcm] is $> 10^{16}$. This produces a resistivity for a 100μm film of $> 10^{14}$ [Ωcm^2]. Accordingly, acrylic paint may be regarded as an ideal insulator.

In order to change the conductivity, graphite was added to the acrylic paint, and by experimenting with various levels of concentration of graphite, a resistance over the surface (100cm^2) of approx. 200-400Ω was achieved. The measuring method employed utilised the Electric Trap device, operating with one bare electrode and one electrode with a semi-conductive coating. By introducing a high conductivity water/salt solution between the plates, it was possible to measure the total resistance, the dominant part of which was the resistance across the coating.

The thickness of the coating was measured (after it had been dried in an oven for 3 hours) by using a micrometer. The thickness achieved was between 80-120μm. The plates were dipped in the pain, hung up and dried for approx. 30 minutes before they were baked in the oven. By adding solvent to the paint, the thickness could be adjusted.

Achieving a uniform thickness using this method proved difficult however,

especially around the edges and it was found necessary to brush additional paint along the edges of the insulating plate.

Biological Considerations

The Zebra Mussel (Dreissena Polymorpha) is the only bivalve that has a free swimming larval stage; most commonly the larvae of freshwater bivalves are parasitic in their larval stage. The Dreissena larvae is accordingly the only mussel larvae that has developed, spread and thrived in lakes and waters with very weak currents. These larvae are free swimming for the entire duration of the larval stage.

The other characteristic that is unique is that the physical size of the Dreissena larvae is larger than most other freshwater larvae, varying from 100-300 μm, dependent upon the stage. This compares to most other species which are smaller than 150μm (in their pre-settling parasitic stage).

It would have been preferable to carry out the test using Dreissena larvae, but such larvae are difficult to obtain in Norway as they are very rare (only in certain districts in the South-East of the country), are not allowed to be freely transported and are not commercially available.

Therefore, after consultations with Dr. Simon Cragg, a Marine Biologist specialising in freshwater bivalves, the experiments were carried out on a freshwater crustacean Daphnia (Subphylum Crustacea/Class Banchiapoda), which is commercially available (as fish food), and is easy to feed and sustain. The physical size of the daphnia is much larger than the Dreissena larvae - about 500 μm up to 1mm, but otherwise characteristics are very similar to Dreissena larvae. A sketch of the Daphnia is shown in Fig 5. The antennae are used as a swimming organ.

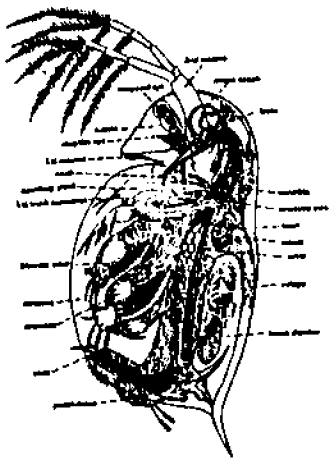

Fig 5. The Daphnia larva - Female of Daphnia Pulex

Approximately 20,000 larvae were bought from NIVA (Norsk Institutt for Vannforskning) and released in two batches of approximately 10,000 each, into two tanks filled with approximately 20 litres of filtered (100μm filter) but otherwise untreated water. These two tanks were later used interchangeably as Tank 1 as shown in Fig. 6.

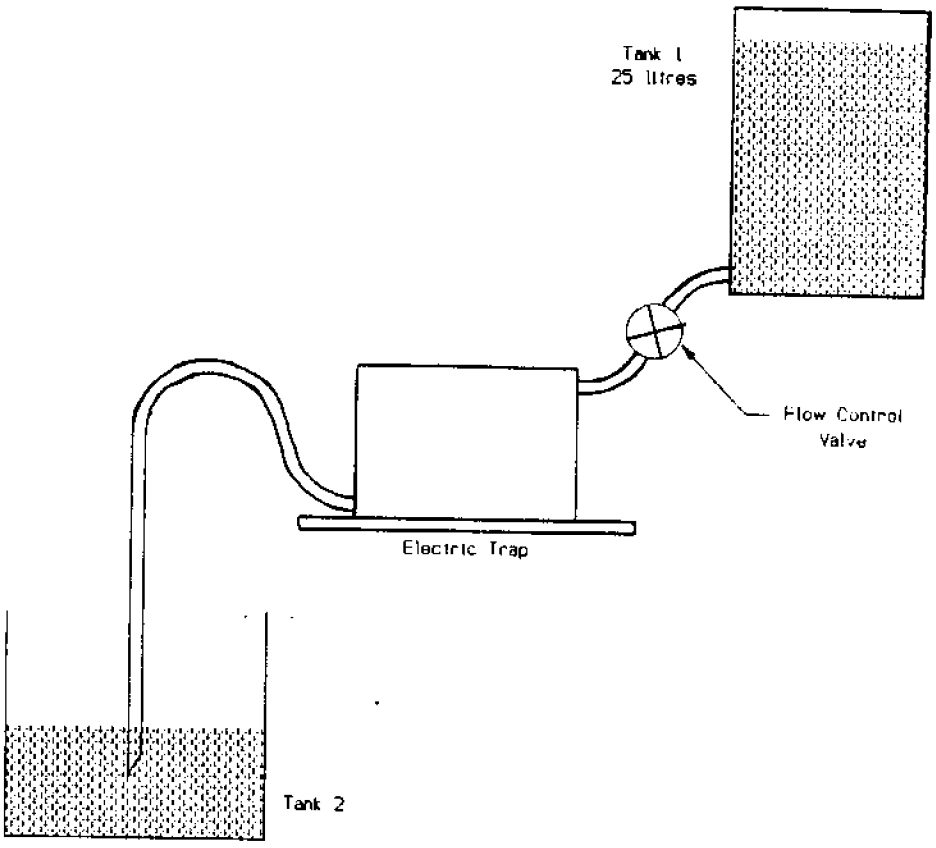

Fig 6. Sketch of the Set-Up

Please refer to Fig. 4 for an overall schematic of the Electric Trap device.

The Electric Trap was constructed of transparent polycarbonate, with machined butt joints, screwed together with adhesive, locked and sealed. On each side were set inlet and exhaust pipes allowing a flow of water from Tank 1, through the Trap itself and out to Tank 2. The bottom plate was formed as a foot to rest on the table. Two plates (the electrodes) were mounted on each side of the envelope container in the path of the water flow. The electrodes could easily be interchanged and by adding or deleting gaskets at the rear of the plate (between the plate and the housing) the distance between the plates could be varied to achieve the distances between the plates used in the experiment - 6mm and 3mm.

The Electronics and Instrumentation

As mentioned previously, the equipment available for the test could produce a maximum voltage of only 4.8kV. Somewhat higher voltage of 5-6kV would have been preferable, enabling a greater depth of the polarisation layer extending out from the electrode. The electrical hardware used is pictured in Figure 7.

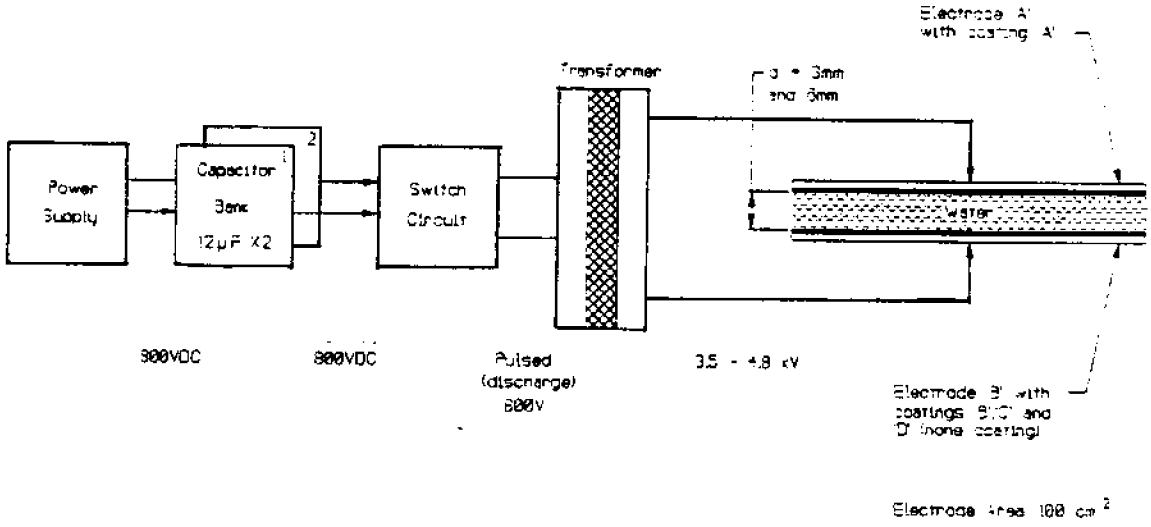

Fig 7. The Electrical Hardware used in the Experiment

The equipment included a power supply charging up two capacitor banks each of 12μF. Following charging, the capacitor banks were discharged through a transformer over the plates, alternating every other time with opposite polarity via a switching circuit (two thyristor circuits each consisting of two thyristors). After

the discharge, the whole circuit was short-circuited with the second thyristor through a resistor (approx. 1.85 mS between firing the two thyristors). This produced a wave form as represented in Fig. 8 across the plates in Experiment 3.

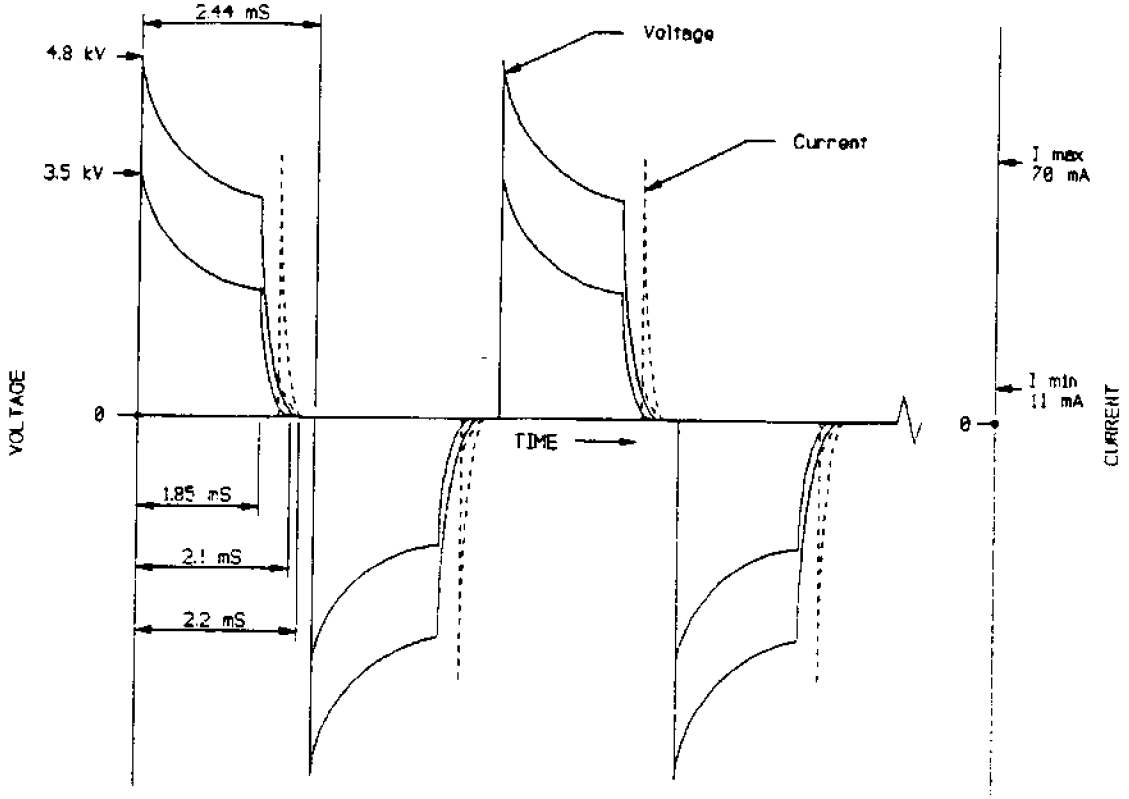

Fig. 8 Shows the Voltage and Current Curves

Originally the switch circuit produced 800V at 60Hz. In order to achieve high frequency together with high voltage, a change in the oscillator that triggered the thyristor in the switch circuit boosted the frequency output to around 400Hz. A transformer was then introduced on the output of the switch circuit, enabling a step up in voltage from 800V to 3.5-4.8kV. The transformer had two outputs on the secondary coil, one for 3.5kV and one for 4.8kV.

The Experiment Procedure

Certain set procedures were followed in all of the tests. First, prior to the start of the tests, through visual observation, it was determined that sufficient numbers of live larvae were present in Tank 1. Any larvae that were dead were prevented from entering the device due to the fact that the outlet in Tank 1 was positioned 50mm above the bottom of the tank. When dead, the larvae have a negative buoyancy and sink to the bottom.

Before commencing the test, the device was emptied of any water and larvae. Each test was conducted using 3 liters of water, producing a flow of 10 minutes in

duration for the 6mm spacing and 20 minutes when using spacing of 3mm between electrodes. After flow had stopped in the device, the water in Tank 2 was then filtered, using a 100 micron plankton filter. The larvae was collected in this fashion and placed into a glass containing approximately 100cm^3 of water, filtered through an 80 micron filter.

The larvae were then left to rest for 1-2 hours. During this period, they were visually observed to determine if they were alive. Following the rest period a sample of the larvae was transferred onto a disc, using an 8mm suction tube, and observed under microscope. Their condition was evaluated and classified according to the following three criteria:

A - Defined Dead	The shell was observed to be closed; the antennae were not moving and there was no sign of life
B - Immobilised	The shell was closed, but there was some discernible movement of the antennae or otherwise a sign of life
C - Alive	The shell was open or partly open and there was a clear movement of the antennae

The sample of larvae were counted and divided into the above three groups.

First Experiment

In the first experiment, the device was fitted with two insulated plates. The highest voltage attainable (4.8kV) and the narrowest spacing (3mm) between the plates, was used for each of the two samples taken.

The visual inspection following the resting period revealed a high degree of life in the glass container.

The two samplings were then examined under the microscope.

	Total	Group		
		A	B	C
Sampling 1.1	34	6-8	10-12	the rest
Sampling 1.2	48	8-10	12-15	the rest

In each of these samplings it was observed that approximately half of the animals

were still alive after transiting through the device.

Due to that fact that a high proportion were alive it was difficult to make a precise evaluation and count.

Second Experiment

In the second experiment, a configuration was used consisting of one insulated plate and one plate covered with a semi-insulating coating. There were four variables introduced in this experiment with two samplings taken for each set of variables.

The samplings were:

Sampling 2.1.1 and 2.1.2	6mm and 3.5kV
Sampling 2.2.1 and 2.2.2	6mm and 4.8kV
Sampling 2.3.1 and 2.3.2	3mm and 3.5kV
Sampling 2.4.1 and 2.4.2	3mm and 4.8kV

Following the rest period of each sampling, a visual inspection of the glass container clearly indicated that, unlike the first experiment, only a relatively small percentage were swimming and that most of the animals had accumulated at the bottom of the container.

The glass was then gently shaken and a random sampling of animals were carefully removed for investigation under a microscope.

	Total	Group				
		A	B	C	A/Total	A + B/Total
Sampling 2.1.1	24	14	6	4	58%	83%
Sampling 2.1.2	39	22	14	3	56%	92%
Sampling 2.2.1	42	29	10	3	69%	93%
Sampling 2.2.2	36	25	6	5	69%	86%
Sampling 2.3.1	56	44	8	4	78%	93%
Sampling 2.3.2	29	23	4	2	79%	93%
Sampling 2.4.1	34	25	4	5	74%	85%
Sampling 2.4.2	47	38	8	1	81%	98%

As can be seen from the above data, the number of defined dead (category A), generally increased as voltages were increased and spacing between plates was narrowed.

The relatively high number (5) of alive (category C) animals in sampling 2.4.1 may be an aberration.

Third Experiment

In this experiment the configuration consisted of one insulated plate and one bare plate.

During this experiment, approximately 2 dozen samplings were processed through the device, all of which, from visual inspection showed high percentages of category A.

Four samplings were taken for investigation under microscope and recording.

They were:

Sampling 3.1	6mm and 3.5kV
Sampling 3.2	6mm and 4.8kV
Sampling 3.3	3mm and 3.5kV
Sampling 3.4	3mm and 4.8kV

The test glass was inspected following the rest period after every sampling and it was observed that only a small number of live animals were left swimming.

The glass was then shaken and a random sample was removed for investigation under microscope.

	Total	Group				
		A	B	C	A/Total	A + B/Total
Sampling 3.1	64	49	11	4	76%	94%
Sampling 3.2	35	29	6	0	83%	100%
Sampling 3.3	41	36	4	1	88%	100%
Sampling 3.4	29	22	6	1	76%	97%

As can be seen, there was a somewhat higher defined dead and/or immobilised rate of occurrence as compared to the second experiment. There was also little difference between the samples in the third experiment.

The Results, Conclusion and Continuation

Certain observations, arising from the results of the three experiments, may be made:

In Experiment 1 using two insulated plates, the effect of the electric field was not fatal or debilitating as the relationship between Groups A, B and C is approximately equal.

However, the results of the second and third experiments clearly show the effect of the electric field on the larvae. Proportionally, the numbers of larvae classified in Group A in the second experiment were approximately 60-70% (A + B approx. 80-90%) and reached 70-80% in the third experiment, when the total of A + B was between 90-100%.

The conclusion drawn was that the alternating electric field as applied in the simplified Electric Trap produced a significant mortality or immobilizing effect on the Daphnia.

In subsequent experiments the following factors should be taken into consideration:

- The Daphnia larvae used in these experiments is a relatively large animal as compared to the Dreissena larvae. The larger the animal the more the effect of the electric field. Accordingly, in order for results to be directly transferable to the overall objective of the experiments, it would be preferable to use Dreissena larvae or an animal of similar size.

- The Daphnia larvae is almost transparent; in future tests, more emphasis should be placed upon establishing dead/immobilised/alive classifications by detecting movements and functions of inner organs.

- It is clear that coatings are important and more experimentation should be undertaken regarding various types of coatings, both on the insulating electrode and the semi-conductive electrode.

- In a process of "fine-tuning", the relationship between voltage, type of electrode, frequency, pulse wave and water flow rate should be further evaluated.

A Comparison of Metal Leachate Rate and Zebra Mussel Control Efficacy for Coatings and Materials

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ABSTRACT: Laboratory immersed test coupons of conventional antifouling coatings, metal pigmented coatings, thermal-sprayed metallic coatings, and metal substrates were evaluated for metal ion release rates over a 2-year period. Identical test coupons were evaluated for fouling over a 15-month period at Black Rock Lock, Buffalo, New York. This paper compares the efficacy of these materials and their release rates as a function of time. Other antifouling products, including capsaicin-based coatings and a biocide impregnated plastic, were evaluated at the field site. Control panels are heavily infested after 15-months while the majority of test materials continue to prevent zebra mussel attachment. Estimated minumum effective release rates for copper and zinc are determined.

INTRODUCTION

Background

Antifouling materials and coatings have been used to prevent fouling on marine exposed surfaces for centuries. The original antifoulants used to protect wooden hulled marine vessels were metal sheathing materials such as lead, copper and zinc.[1] The first documented test of copper sheathing was performed on the H.M.S. Alarm in 1758.[2] Antifouling coatings were invented by William Beale in 1625 and workable coatings have been availble since the 1860s.[2] A wide variety of materials have been used as active ingredients in antifouling coatings, including metals, metal oxides, metal salts, and organometallic compounds based on antimony, arsenic, copper, lead, mercury, tin, and zinc.[2,3] Most of these materials are no longer used because of their high human and aquatic toxicity. Noteworthy among the restricted materials are the organotin compounds, especially tributyltin (TBT). TBT-based antifoulants were popular until recently when prohibitions and restrictions were enacted in the U.S., Canada, Europe, Japan, Australia, and New Zealand.[3] The primary biocides now used in commercial antifoulants are copper and cuprous oxide. Numerous registered biocides may be used to reinforce the copper compounds.[3] Zinc oxide is also sometimes used to augment the copper compounds. The essential mechanism of antifouling coatings is the leaching of biocide into the water.

Aquatic Toxicity of Metal Ions

The toxicity of various metal ions to the zebra mussel has been reported.[4] Table 1 shows the mortality rates of selected metal ions over a 24 h period as determined by Dudnikov and Mikheev. Copper ions are more lethal to the zebra mussel than other metal ions. Similarly, Lukanin measured the relative toxicity of copper sulfate solutions of various concentrations as a function of temperature, showing that 25% mortality was achieved in 5 h for a 33 mg/l solution at 22.5 degrees C.[5] Copper-based antifoulants and, to a lesser degree, zinc-based materials should act to repel the settlement and growth of zebra mussels in infested waters.

Copper leach rates for antifoulant coatings of 1-2 ug/cm^2/day are reported to be adequate to repel sensitive marine organisms such as mussels.[2] As a first approximation this range would seem to apply to the zebra mussel. No comparable literature references to effective leach rates for zinc have been identified. However, proposed U.S. Environmental Protection Agency criteria exist for the protection of fresh water aquatic life.[6] Equations 1 and 2 may be used to calculate the recommended maximum allowable concentrations for a 24 h period for copper and zinc respectively.

Eq. 1 $$e^{[0.65 \ln (\text{hardness}) - 1.94]}$$

Eq. 2 $e^{[0.67 \ln (\text{hardness}) + 0.67]}$

For a given water hardness it is possible to estimate the relative toxicity of copper versus zinc. The water hardness of the Niagara River near Black Rock Lock, a COE facility infested with zebra mussels, averages around 92 mg/l. Maximum allowable chronic concentrations for copper and zinc at this water hardness are 2.7 and 40.4 ug/l respectively. For copper in salt water the recommended concentration is only 0.79 ug/l. Although recommended salt water criteria for zinc do not exist, the lowest reported concentration to cause chronic effects is 141 ug/l. Interestingly enough, zinc is more toxic to fresh water organisms than salt water organisms and the converse is true for copper. Zinc would appear to be better suited for use as a fresh water biocide than as a marine biocide.

Types of Antifouling Coatings

There are four principal types of antifoulants which are used today, (1) conventional, soluble matrix, (2) advanced, insoluble matrix, (3) self-polishing, and (4) polishing/ablative.[3] The self-polishing coatings are TBT-based and are not being considered for use by the Corps of Engineers (COE).

Conventional, Soluble Matrix Antifoulants. Soluble matrix-type antifoulants contain water soluble gum rosin. As the rosin dissolves, biocide is released into the water. Typical products are effective for only 12 to 15 months in marine applications. The biocide release rate is nearly constant over time.

Advanced, Insoluble Matrix Antifoulants. Insoluble matrix antifoulants use more durable binders than the soluble matrix materials and greater film thicknesses are possible. These products are highly loaded with either cuprous oxide, copper, or cuprous oxide and organotin. The coatings are formulated above the critical pigment volume concentration such that the biocide particles are in direct contact with each other. As one particle dissolves another is exposed. Insoluble matrix antifoulants last from 18 to 30 months in marine environments. The biocide leach rate decays exponentially with time until the coating is no longer effective.

Polishing/Ablative Antifoulants. The ablative-type antifoulants utilize water soluble and/or hydrolyzable binders which erode as water moves past the coating. After stabilizing, the release rate remains fairly constant until the coating is exhausted. Effective coating life is about 36 months in seawater.

Factors Affecting Antifoulant Biocide Release Rate

Water temperature and chemistry effect the leach rate of antifoulants.[2] Leach rate is reduced by 20% for each 0.1 increase in pH above 8.4. Leach rate increases 25% for each

0.1 reduction below pH 8.0. Sea water has a typical pH range of 8.0 to 8.4. The pH of natural fresh waters is usually below this range. The Niagara River at Black Rock Lock has a pH of about 7.5. Leach rates for conventional antifoulants should be roughly 2 times higher at pH 7.5 than pH 8.0. However, pH is not the only water chemistry variable affecting leach rate. Leach rate increases in direct proportion to the square of the salinity. Presumably this is because of the high solubility of copper (II) chloride. Leach rate decreases about 5% for each 1 degree C drop in temperature. Marine fouling is most severe in warm waters. The zebra mussel is a relatively cold water macrofouler and, as such, antifoulants used to control the animal will be used primarily in cooler waters where they will have somewhat reduced leaching rates. Water velocity may sometimes affect the release rate of ablative antifoulants. If velocities are too low the hydrolyzed resin may not be ablated.

Other Antifoulant Control Techniques

Similar to the metal sheathing of wooden ships, metal substrates may be employed as either a protective cladding or a construction material. Copper, zinc, and their alloys could potentially be used in this way. Alternatively, pure metallic and alloy coatings can be applied by thermal spray. Metal coatings, such as galvanizing, can also be applied by hot-dip or electrocoat processes. Surfaces can also be coated with conventional inorganic and organic zinc-rich coatings. These methods are all somewhat unconventional and have not been used to a great extent to prevent macrofouling.

COE ANTIFOULANT TEST PROGRAM

Scope

A wide variety of facilities are potentially impacted by the zebra mussel. Within the COE these facilities include navigation, flood control, hydroelectric, and recreational projects. Of chief concern is the operational status of these facilities. Of secondary concern are problems associated with visiblity for inspection of structural components and accelerated degradation of coatings and metallic substrates.

Coatings and Materials

In 1992 the COE initiated an evaluation of antifouling coatings and construction materials. The program consists of field and laboratory evaluations of selected materials. Black Rock Lock on the Niagara River, Buffalo, New York, is the field test site. The lock, operated by the COE's Buffalo District, has been heavily infested for 3 to 4 years. The field exposed coupons have been evaluated periodically for zerba mussel attachment. The laboratory test coupons, exposed in fresh municipal water, are periodically analysed for the rate of leaching of zinc and copper. Some of the test materials exposed at Black Rock have not been evaluated in the laboratory, however their repellent properties are of interest and are reported. Table 2 lists the antifoulant coatings and materials being tested at Black Rock and indicates whether companion coupons were analyzed in the laboratory

for rate of leaching.

Field Test

Triplicate 3 x 6 in test coupons were placed in test at Black Rock Lock in September 1992, with the exception of the pepper coatings, which were introduced in May 1993 (coatings 1 and 2) and August 1993 (coating 3). Most of the coating materials were applied to both steel and concrete test specimens. Where necessary and appropriate, standard COE vinyl or epoxy protective coating sytems were applied prior to the test coatings. Test coupons are secured in a fixture which is located on a concrete wall just upstream of the lock chamber. The test coupons are arrayed in the horizontal plane approximately 6 in from the concrete wall, at a mean depth of approximately 5 ft. Each test coupon is electrically isolated.

Laboratory Leachate Analysis

Selected test coatings and materials were evaluated in the laboratory for rate of release of zinc and copper as a function of immersion time. Test panels were completely immersed and isolated from each other and any conductive materials. The immersion tank is supplied with cold municipal water (13-18 degrees C) with an constant rate of exchange to prevent stagnation. Test coupons were periodically removed from the immersion tank and evaluated for rate of leaching under static conditions. Leaching was determined by completely immersing individual coupons in a measured volume of deionized water (20-25 degrees C) for 24 h. Samples were analyzed for metal ion concentration by atomic absorption. Leach rates were calculated in units of micrograms/cm^2/day.

Field Test Results

Test coupons were first inspected for the presence of adult and settled mussels in December 1992 (3 months). Subsequent inspections were conducted in May 1993 (8 months), August 1993 (11 months), and Dec 1993 (15 months). Mussel colonization rates and shell sizes (adult or non-adult) were noted at each inspection interval. No settled juvenile mussels were observed after 3 months, however some adults were observed on a few of the test materials as well as the test fixture. These adults most likely relocated from adjacent surfaces using their foot for mobility. In some cases there was evidence that adult mussels had attached themselves, probably with temporary threads, and subsequently detached. The 8 month inspection showed the first evidence of juvenile settlement. Table 3 indicates the antifoulant performance at the 15 month mark (7 months for pepper coatings 1 and 2 and 4 months for pepper coating 3) in terms of mussel densities.

Leachate Results

The rate of leaching of zinc and copper were periodically determined for selected coatings and materials. Figure 1 shows the range of leaching rates determined for each copper containing test material. Figure 2 shows the range for each zinc containing

material. Figures 3 (copper sulfate containing coatings), 4 (cuprous oxide containing coatings), 5 (metallic copper containing coatings), 6 (copper and copper alloy sheet materials), and 7 (zinc containing materials), present the metal leach rates versus time. Best fit curves have been applied to the data to more clearly show the long-term trend in leach rates.

DISCUSSION OF RESULTS

Pepper Coatings. A series of pepper coatings were evaluated in the field test. Anectdotal reports indicate capsaicin containing coatings are effective at preventing or reducing marine fouling.[7,8] Capsaicin, a non-toxic irritant, was dispersed as a powder in coating 1, dusted onto the surface of applied coating 2, and incorporated as an oil in coating 3. The pepper coatings exposed at Black Rock Lock have not been effective at preventing the settlement of juvenile zebra mussels. The results are not suprising in that capsaicin is practically insoluble in cold water.[9] Capsaicin may in fact be effective against organisms that are in more direct contact with the substrate. However, it is unlikely that zebra mussels can sense the presence of the irritant when attached by byssal threads. It may be possible to associate the capsaicin with some water soluble species such as rosin and thereby facilitate its release into the water column. The use of capsaicin or other low-toxicity irritant remains an intriguing possibility.

Biocide Impregnated UHMWPE. Ultra high molecular weight polyethylene impregnated with an unspecified registered biocide was evaluated at the field site. Although this material accumulated fewer mussels than the polyethylene control, fouling was still quite heavy. The utility of this product is questionable.

Brass, Bronze, Copper and Tin Thermal-Sprayed Coatings. Brass, bronze, copper, and tin coatings were applied by wire arc-spray to concrete test panels. These metals should not be applied directly to ferrous metal surfaces because corrosion of the substrate will occur. Each of the coating materials with the exception of tin was completely effective against both juvenile and adult mussels. Leach rates were not determined for these coatings, however rates similar to those determined for the copper and brass sheet materials are probable. The poor performance of the tin coating is not surprising in that tin has a relatively low aquatic toxicity, especially as compared to TBT, copper, and zinc.

Metal Substrates. Copper and brass sheet materials were completely effective against the zebra mussel over the 15-month test period. Aluminum-bronze had a low colonization rate. From Figure 6 the downward trend in copper leach rate is fairly evident for each of these materials. The final data point for brass is probably aberrant and thus copper leach rates for the 3 materials follow the trend; copper > aluminum-bronze > brass. Brass also has a fairly steady zinc release of about 2 ug/cm^2/day, which probably

reinforces the materials' efficacy. The copper leach rate for these materials progressively decreases with time. A direct time dependent relationship between field and laboratory exposed materials may not exist. In other words, the leach rates after 15-months of field and laboratory exposure are probably not the same. However, the trends and relative leach rates are probably reliable. The leaching data would seem to suggest that aluminum-bronze and copper sheet materials will eventually have copper leach rates too low to be effective. The decrease in leach rates are probably caused by the accumulation of insoluble corrosion products on the surface of the test materials. If this is the case, then periodic rejuvination of these surfaces by means of light abrasion would be possible.

Water-borne Acrylic Coatings Containing Copper Sulfate. Experimental water-borne acrylic coatings were received from a vendor. These products contain copper in the form of copper sulfate. The coatings were formulated with the intent of providing different release rates for each product. Copper leaching was quite low with little or no difference between the products. Very high colonization rates were measured for each of the field exposed coatings.

Ablative, and Soluble and Insoluble Matrix Antifouling Coatings. Both ablative coatings in this study prevented settlement and attachment of zebra mussels. Tin-free ablative had a copper leach rate well below the expected effective range of 1-2 $ug/cm^2/day$. This may be due to the stagnant conditions under which the leach tests are conducted. Ablative coatings require some minimum level of water flow to erode the hydrolyzed paint resin. The erosion process is responsible for the introduction of the metal ion species into the water column. The test panels at Black Rock Lock are subjected to periodic flow conditions primarily from vessels moving through the lock. Actual leach rates for the tin-free ablative are almost certainly higher than observed in the laboratory. The copper-zinc ablative coating had leach rates of about 1 $ug/cm^2/day$ for both zinc and copper. The higher copper leach rate observed for this ablative coating is probably due to the addition of a small amount of water soluble wood rosin. Ablative coatings sometimes are formulated in this way to prevent marine fouling of vessels that experience lengthy anchorages. Actual leach rates for this coating may also be higher than measured in the laboratory.

MIL-P-15931,a vinyl resin, soluble matrix-type antifoulant was also completely effective for the first 15-months of exposure. A nearly constant leach rate of about 1.5 $ug/cm^2/day$ was observed. This coating contains a large amount of water soluble rosin and a small amount of vinyl resin which strengthens the coating and allows greater film thicknesses to be applied. The service-life of soluble matrix coatings is a function of film thickness as well as water chemistry.

Insoluble matrix coating 1, a copper pigmented epoxy coating, was only slightly fouled

after 15 months. Leach rates were fairly stable, averaging about 2 ug/cm^2/day. Insoluble matrix coating 2, a modified isophthalate-polyester coating pigmented with copper powder, did not perform as well, exhibiting moderate fouling on steel coupons and heavy fouling on concrete coupons. This coating contains somewhat less copper pigment in the dry film than coating 1. Its copper release rate shows a strong downward trend moving from 2 to 0.5 ug/cm^2/day.

Zinc Containing Coatings. The thermal-sprayed zinc coating, the water-borne inorganic zinc coating, and galvanizing all exhibited relatively low levels of mussel attachment at 15-months. Zinc leach rates were approximately 6, 3, and 5 ug/cm^2/day, respectively, at 600 days of laboratory exposure. The zinc materials serve a secondary function as corrosion protection on steel substrates. Even at modest levels of colonization, zinc coatings would offer a significant advantage in terms of cost and simplicity over the other antifoulants. Zinc coatings marketed for corrosion protection do not require registration under the Federal Insecticide, Fungicide, and Rodenticide Act.

Conclusions

Coatings containing capsaicin, a non-toxic irritant, did not prevent the settlement and growth of zebra mussels at Black Rock Lock. A plastic material impregnated with an unspecified biocide and thermal-sprayed tin were not effective.

Products containing copper and zinc were generally effective with the exception of a series of water-borne coatings containing copper sulfate and an insoluble matrix coating pigmented with copper dust. The ineffective copper coatings had terminal leach rates for copper that were significantly lower than those observed for the effective products. The failed insoluble matrix product exhibited a steep decline in copper leach rate to values below the expected effective range. The leaching test was able to predict the drop off in performance of this material.

Thermal-sprayed brass, bronze, and copper coatings were all effective deterrents for the duration of the field test. Copper and brass sheet materials were also completely effective and aluminum-bronze alloy was only slightly fouled. Laboratory leach rates for the sheet materials declined with time to a level below the expected effective range for copper suggesting that their efficacy may be short-lived.

Two ablative antifoulants were effective in field tests. The laboratory copper leach rate for one of these products was significantly and consistently below the expected effective range, suggesting that the static conditions of the leachate analysis does not adequately model the actual leaching in the field for some ablative coatings.

Navy specification MIL-P-15931, a soluble matrix coating, was completely effective and exhibited a fairly steady leach rate within the expected effective range.

Brass and the copper-zinc ablative coating each had significant but relatively low leach rates of both copper and zinc, suggesting that copper and zinc may reinforce each other in some way. A study of the toxicity to zebra mussels of various concentrations of copper and zinc together would be valuable.

Zinc thermal spray, galvanizing, and water-borne inorganic zinc coatings all had low levels of zebra mussel colonization after 15 months at Black Rock Lock. Observed zinc leach rates for these materials suggest a minimum effective rate of about 4 ug/cm^2/day.

The estimated minimum effective release rate for copper is between 0.5 and 1.0 ug/cm^2/day.

REFERENCES

1. A. Holder and A.M. Duckworth, "Laboratory evaluation of a cuprous oxide ablative coating on laboratory-reared barnacles," JPCL, 8(11), 21 (1991).

2. C.H. Hare, "Anti-fouling coatings," JPCL, 10(2), 83 (1993).

3. E.B. Kjaer, "Bioactive materials for antifouling coatings," Progress in Organic Coatings, 20(3-4), 339 (1992).

4. V.F. Dudnikov and V.P. Mikheev, "The effect of certain metal ions on dreissena," in Biology and Control of Dreissena: A Collection of Papers, B.K. Shtegman, Ed., Moscow: Institute of the Biology of Inland Waters (Washington, DC: Federal Clearinghouse for Scientific and Technical Information, TT-67-51396), pp 60-64.

5. V.S. Lukanin, "Survival rate of adult dreissena in copper sulfate solutions of different concentration and temperature," in Biology and Control of Dreissena: A Collection of Papers, B.K. Shtegman, Ed., Moscow: Institute of the Biology of Inland Waters (Washington, DC: Federal Clearinghouse for Scientific and Technical Information, TT-67-51396), pp 69-70.

6. Priority Toxic Pollutants: Health Impacts and Allowable Limits; M. Sittig, Ed.; Noyes, Park Ridge, 1980, pp 163-166, 366-368.

7. "The habanero of the harbor," Los Angeles Times, Jul 1, 1993.

8. "Peppery paint can chase barnacles," The Middletown Journal, May 30, 1993.

9. The Merck Index; M. Windholz, Ed.; Merck, Rahway, 1976, p 224.

Table 1. Dreissena mortality due to exposure to metal ions for 24 hours at room temperature.

Metal	Concentration (mg/l)	Average of killed specimens (%)
Copper	5	100.0
Silver	5	71.5
Mercury	5	57.2
Zinc	5	4.8
Lead	5	0

Table 2. Test coatings and materials under study by the COE at Black Rock Lock.

Coating/Material Only	Active Biocide	Field Test
Tin free ablative	cuprous oxide	
Copper-zinc ablative	cuprous oxide/zinc oxide	
MIL-P-15931 (soluble matrix)	cuprous oxide	
Insoluble matrix 1	copper	
Insoluble matrix 2	copper	
Water-borne acrylic 1	copper sulfate	
Water-borne acrylic 2	copper sulfate	
Water-borne acrylic 3	copper sulfate	
Water-borne inorganic zinc	zinc	
Pepper 1	capsaicin	X
Pepper 2	capsaicin	X
Pepper 3	capsaicin	X
Zinc thermal-sprayed coating	zinc	
Naval brass thermal-sprayed coating	copper/zinc	X
Copper thermal-sprayed coating	copper	X
Bronze thermal-sprayed coating	copper/zinc	X
Tin thermal-sprayed coating	tin	X
Galvanized steel	zinc	
Copper sheet	copper	
Brass sheet	copper/zinc/lead	
Aluminum-bronze sheet	copper/aluminum	
UHMWPE, biocide impregnated	N/A	X
polypropylene	control	X

Table 3. Performance of test coatings and materials at Black Rock Lock.

Coating/Material	Zebra Mussel Density (number/m^2)
Tin free ablative	0 / 14*
Copper-zinc ablative	0 / 0
MIL-P-15931 (soluble matrix)	0 / 0
Insoluble matrix 1	72 / 0
Insoluble matrix 2	1200 / 6000
Water-borne acrylic 1	-- / 13,000
Water-borne acrylic 2	-- / 13,000
Water-borne acrylic 3	13,000 / 13,000
Water-borne inorganic zinc	0 / 86
Pepper 1	1500 / --
Pepper 2	6500 / --
Pepper 3	2200 / --
Zinc thermal-sprayed coating	170 / 0
Naval brass thermal-sprayed coating	-- / 0
Copper thermal-sprayed coating	-- / 0
Bronze thermal-sprayed coating	-- / 0
Tin thermal-sprayed coating	-- / 7500
Galvanized steel	258
Copper sheet	0
Brass sheet	0
Aluminum-bronze sheet	271
UHMWPE, biocide impregnated	4990
polypropylene	7320

* First number is density on coated steel and second is on coated concrete.

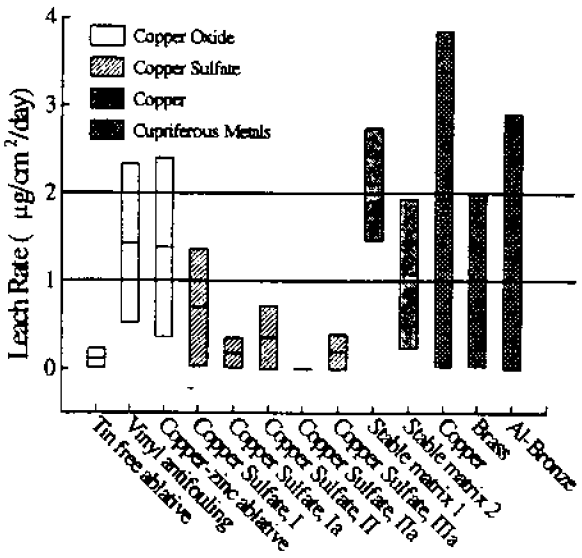

Figure 1: Range of leach rates for copper-containing test materials.

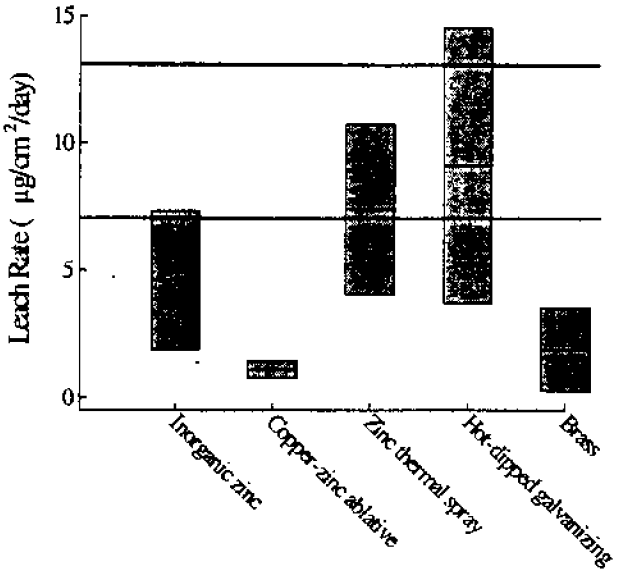

Figure 2: Range of leach rates for zinc-containing test materials.

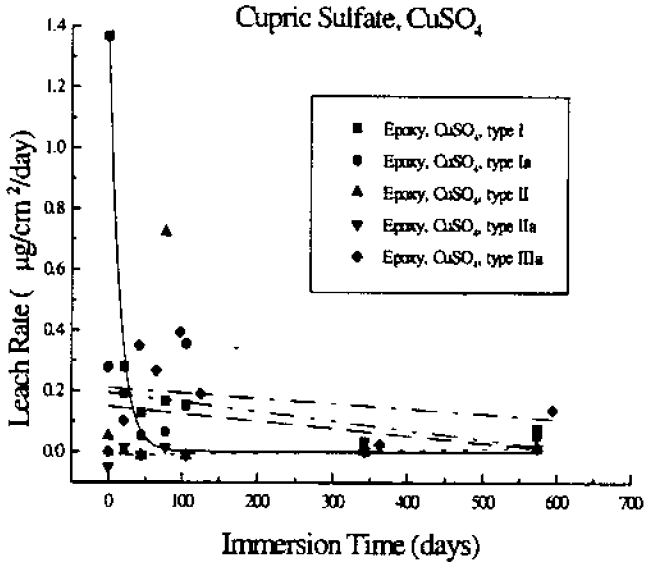

Figure 3: Leach rates for copper sulfate containing coatings.

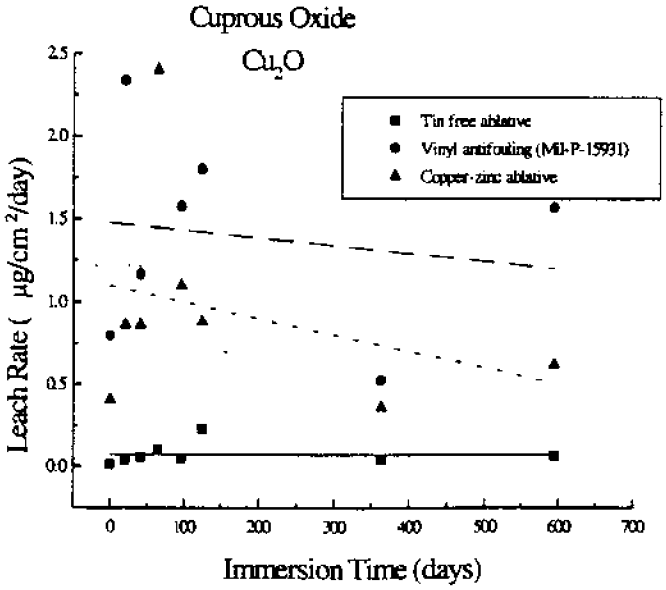

Figure 4: Leach rates for cuprous oxide containing coatings.

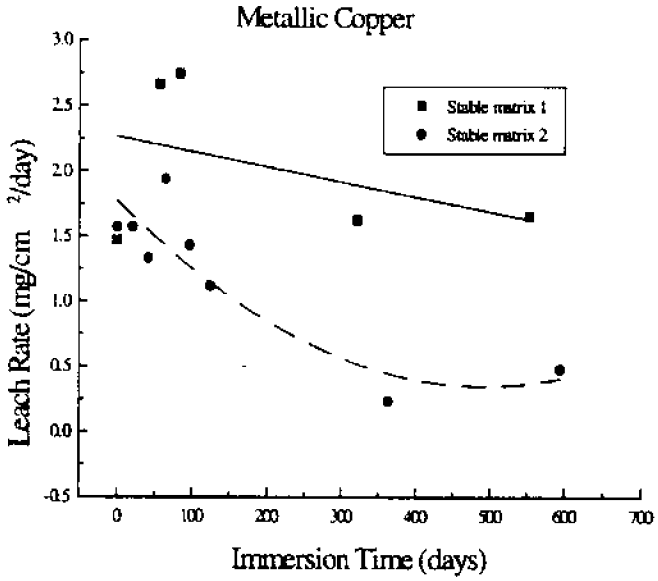

Figure 5: Leach rates for metallic copper containing coatings.

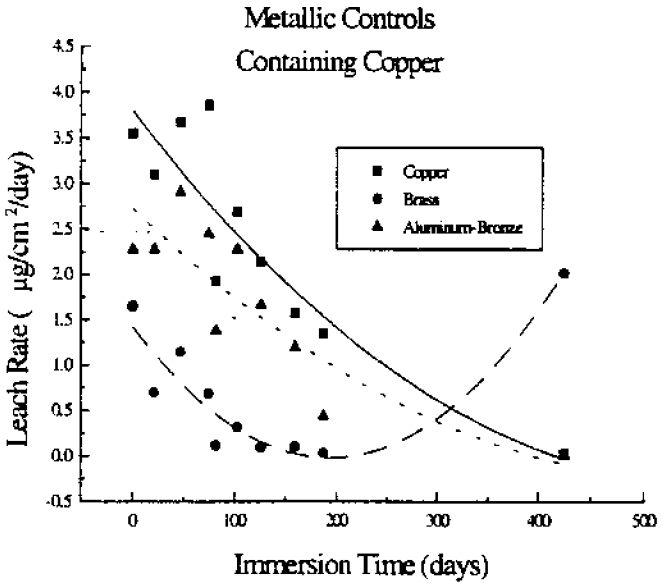

Figure 6: Leach rates for copper and copper alloy sheet materials.

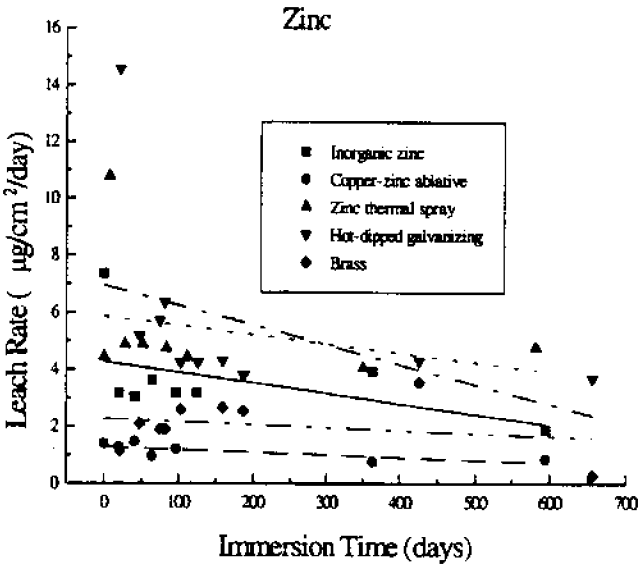

Figure 7: Leach rates for all zinc-containing materials.

Application of Cost Effective Electric Fields to Deter Attachment of the Zebra Mussel to Structures

A. Garry Smythe, John F. Doyle and Theresa A. Reed (Acres International Corporation) and Paul M. Sawyko (Rochester Gas & Electric Corporation)

ABSTRACT

A multiyear study was conducted to determine if electric fields could be used to eliminate or reduce attachment of zebra mussels (Dreissena polymorpha) to submerged structures. If settling-stage veliger attachment can be inhibited while under the influence of and for a short time after exposure to a sufficiently intense electric field, water flow would carry the veliger past a structure. Presently, chemical application is the most widely used method to control the mussel. However, due to environmental concerns and/or permit restrictions, some facility components such as trashracks cannot be protected by chemicals. Utilizing electric fields to prevent attachment could be a cost effective, proactive, non-chemical control alternative that could be applicable to other facility components as well.

Field studies to test the effects of electric fields on post-veliger settlement were conducted from 1991 through 1993 at the Rochester Gas & Electric (RG&E) Russell Station. Test apparatus included plate arrays of either steel or plexiglass, which were placed in test flumes. Untreated Lake Ontario water drawn from RG&E's Russell Station circulating water forebay was continuously pumped through the flumes. Direct current (DC) and alternating current (AC) were tested at field strengths that ranged from less than 1 volt/cm to 39 volts/cm. Not all array types were tested with all currents and voltages. All electrical apparatus were standard equipment routinely used to generate small scale AC or DC voltages, except for a capacitive discharge unit which was an "off the shelf" field generator intended to be used as a large scale, riverine fish deterrence device. Although none of the generated fields, as tested, were 100 percent effective, the DC capacitive discharge field had the most promising results.

INTRODUCTION

Water intake structures provide excellent zebra mussel (Dreissena polymorpha) habitat since the continuous flow of water maintains food and oxygen supplies while the pipe can protect them from scour and predators. Once in a facility, zebra mussels may colonize any surface within the distribution system. Some of the impacts associated with colonization of raw water systems are loss of intake head as well as fouling of valves, monitor probes, filters, and traveling screens.

The mussel may also colonize other areas such as the service water and fire protection systems. Clumps of mussels (druses) can clog condenser/heat exchanger tubes which would lead to possible system overheating, loss of revenue, or safety hazards. Attached mussels can potentially increase microbially induced corrosion (MIC) on steel and cast iron surfaces.

Little effect on plant operation is observed on larger structures such as intakes during the early years of colonization. Critical fouling can occur much more rapidly in small diameter piping. Once the mussels are well established on trashracks or screens, major operational impacts such as lost head, cooling capability, trashrack failure, and plant outage may occur. Chemical treatments are not suitable at the trashracks since Federal and state resource agencies will not permit chemical injection upstream of an intake pipe. Also, mechanical cleaning is not suitable for all intakes, so a proactive, non-chemical control method needs to be identified to protect trashracks.

In the former USSR, electric fields have been applied to control zebra mussels[1]. The use of electrostatic filters in the cross section of pipes was favored. The mussel larvae were killed by exposure (0.02 to 0.10 seconds) to a high voltage (generated field of 225 to 400 volts/cm). The mortality rate was dependent on whether veligers had their valves opened or closed. Generally, the higher the water temperature the more rapidly the mussels were affected. Voltages were applied at water intakes, on gates, and on the screenhouse walls. The use of high intensity fields for short exposure rates killed the veligers. This technique, however, proved to be technically and economically impractical under most industrial conditions due to the high power requirements. In other experiments Russian scientists applied low voltage fields (7 to 8 volts/cm) over long exposure periods (27 to 31 hours) at summer water temperatures. The low voltage appeared to be effective under these conditions. Unfortunately, in flowing water situations, such as those found at trashracks and most other places in a power station's raw water system, veligers drift so quickly that long exposure rates are not possible.

Ontario Hydro conducted studies on high voltage electric fields applied to low volume systems in 1990[2]. They concluded that electric fields could immobilize zebra mussels, but that lengthy exposure times reduce the value of the method. Wisconsin Electric

Power Company and the University of Wisconsin Center for Great Lakes Studies are continuing this electric field experimentation for use in low volume water systems but are not considering any application for trashracks[3].

New York State Electric and Gas Corporation conducted limited testing of high intensity electric barriers at their Kintigh Station on Lake Ontario[4]. The objective of the program was to kill zebra mussel veligers, however, exposures of 200 to 400 volts/cm for 0.1 second were ineffective. At 600 volts/cm for 0.1 second, a 30 percent mortality was obtained. Based on calculations, the power requirement to effectively operate high intensity electric barriers at the station made this system impractical.

Cathodic protection against structural damage through oxidation is in place at some power plants. However, casual observation has indicated that this protection does not entirely preclude the salt water blue mussel (Mytilus edulis) from attachment to the protected structure[5]. Studies to determine the effectiveness of cathodic protection in preventing zebra mussel attachment to metal and concrete surfaces are currently being investigated[6]. Continuous, low intensity electric fields are used by utilities to exclude fish from discharge areas, but their effectiveness on zebra mussels had not been evaluated as of 1991.

APPROACH

General

In most zebra mussel control strategies that are proposed or in use, the objective has been to cause mussel mortality. An alternative approach would be to delay settlement until veligers are carried beyond a structure by the water flow. The objective of this study is to determine whether electric fields can be used. If the mussel veligers' settling behavior can be delayed for a short period during or after contact with an electric field, and the conduit water flow can carry the mussel past a structure, it would not be necessary to kill the veliger.

In July 1991, testing to investigate the efficacy of this approach commenced at the Rochester Gas & Electric (RG&E) Russell Station. Low current and low voltage (AC, DC, or pulse DC) fields were tested. Electric field intensities were selected generally based on economic considerations (i.e., approximate projected operational cost to generate full-scale electric fields). During the first year of the study other factors including the potential impact on fish, safety of personnel using the test apparatus, and the authors' preliminary laboratory tests were weighed more heavily in selecting field strengths to be tested.

In 1992, higher AC voltages were tested. The objective was to determine the level of voltage that would be required to control zebra mussel attachment. This program was initially designed to apply a voltage at the upper limit relative to full-scale operational cost versus a reactive mechanical cleaning approach.

In 1993, the study objective was to continue the AC evaluation that was underway in 1992 (at field strengths of approximately 39 volts/cm) and to conduct a further evaluation of DC fields originally conducted in 1991. The further evaluation of DC fields came as a result of some recent studies conducted on the use of cathodic protection and pulse-power methods to control zebra mussels in Scandinavia, the United Kingdom (UK), and private and defense-related sectors in the U.S[10,11].

Apparatus Design

Over the three-year study the test apparatus was designed to minimize construction costs and maximize use of existing equipment and facilities. It consisted of two aluminum head tanks, four fiberglass flumes, and an aluminum discharge tank (Figure 1). The apparatus was housed in RG&E's environmental trailer located at the Russell Steam Station in Rochester, New York.

Raw Lake Ontario water was pumped from the Russell Station circulating water forebay to the environmental trailer located approximately 200 ft away. To make use of the existing pumping equipment, the pipes were oversized to reduce the pressure losses along the pipe route. Raw lake water entered Tank No. 1. Baffles were placed in Tank No. 1 to reduce the turbulence before the water entered Tank No. 2. An overflow was provided in Tank No. 2 as a precautionary measure should the inflow exceed the tank capacity. The water exited Tank No. 2 into the four flumes. The flumes discharged into Tank No. 3, and a drain on the bottom of this tank routed the raw water to the plant discharge tunnel.

Configuration Types

Three electrical configurations were used (Figure 2). Two configurations used arrays of steel plates simulating standard trashracks, and the third type consisted of two-piece arrays constructed of plexiglass plate.

Configuration Type 1 consisted of an upstream rod electrode and a downstream array of steel plates. The array consisted of five electrically common individual steel plates bolted together to form an electrode. The individual steel plates were constructed of ¼-inch thick steel, 7 inches high by 6 inches wide. Five-eighths inch long steel spacers were placed between the plates to hold them apart. Due to the electrode positions in Configuration Type 1, the field was parallel to the water flow with the greatest field

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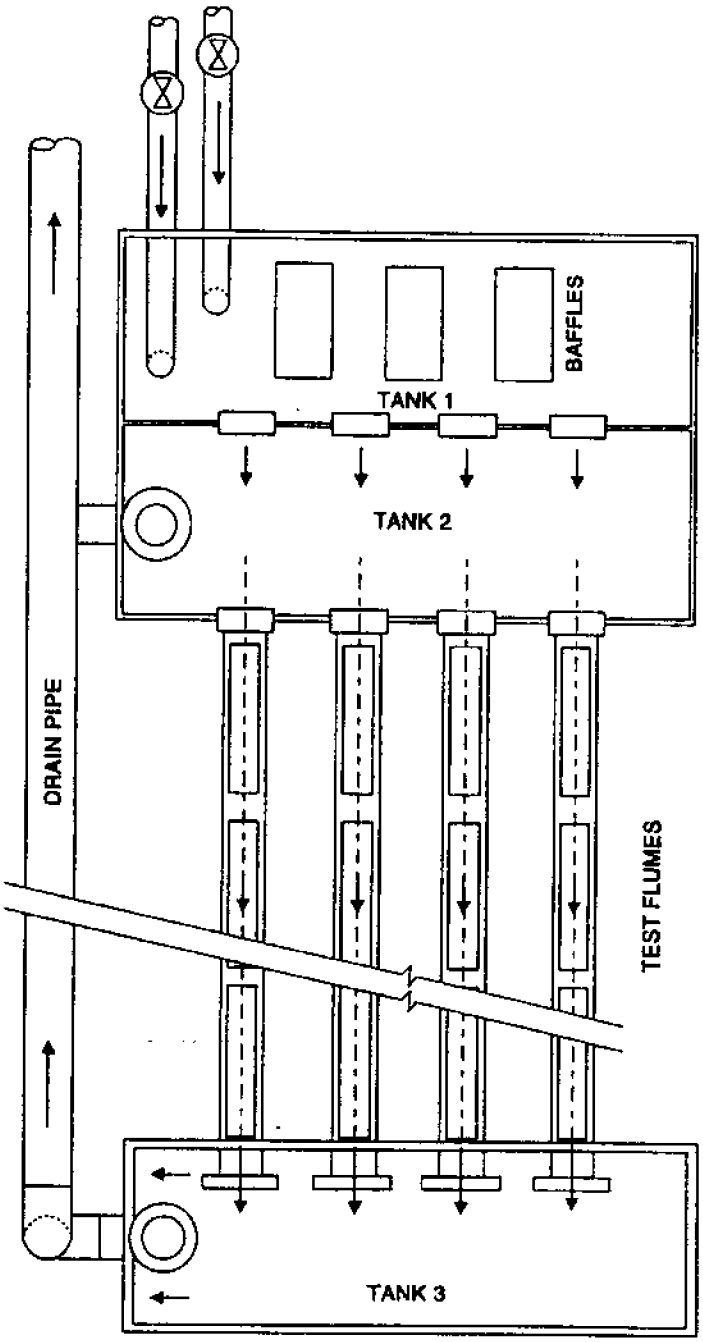
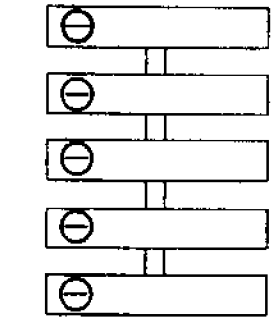


ZEBRA MUSSEL ELECTRIC FIELD TEST APPARATUS

Figure 1

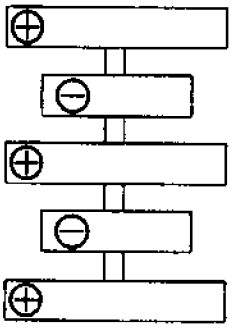


FLOW

STEEL BOLT

ELECTRICALLY CONTINUOUS
STEEL PLATE ARRAY

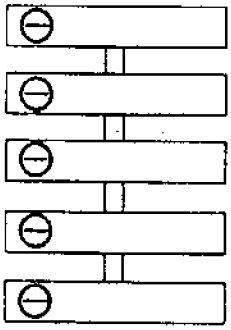
TYPE 1 CONFIGURATION



FLOW

ELECTRICALLY ISOLATED
STEEL PLATE ARRAY

TYPE 2 CONFIGURATION



FLOW

STEEL BOLT

NONCONDUCTIVE
PLEXIGLASS
PLATE ARRAY

ELECTRICALLY CONTINUOUS
STEEL PLATE ARRAY

TYPE 3 CONFIGURATION

ZEBRA MUSSEL ELECTRIC FIELD TEST CONFIGURATIONS

Figure 2

strength near the trailing edge of the upstream electrode and the leading edge of the downstream array. The weakest volt/cm was equidistant between poles.

Configuration Type 2 consisted of an array of five electrically isolated steel plates. The rack array was constructed of the same steel plates used in Configuration Type 1, but only three were used in alternating positions. The second and fourth plates were replaced with a smaller plate each measuring ⅛-inch thick by 7 inches high by 2 inches wide. Electrically nonconductive spacers were used between the plates to hold them apart. This configuration provided electrically isolated rack bars that functioned as separate electrodes. The electric field was generated perpendicular to the water flow with the lines of equipotential voltage generated parallel to the flow. The field was strongest where electrodes directly opposed each other. A much weaker field extended to the upstream and downstream edges of the three larger plates, unlike the Type 1 configurations where there was an electric field between the plates.

Configuration Type 3 consisted of an array of two pieces of 6-inch by 3-inch by ⅛-inch plexiglass plate placed between the upstream and downstream electrodes of a Type 1 configuration. The plexiglass arrays were electrically nonconductive. As the plexiglass arrays in Type 3 configurations were completely within the poles of a Type 1 configuration, they also were within a generated electric field. As the plexiglass plates were nonconductive and oriented so as to interface little with the electric field, it was assumed they would not affect the result for the Type 1 test with which they were associated.

Power Supplies

The five power supplies used for these experiments to provide the necessary voltages for the establishment of the electric fields were:

Power Supply	Voltage Range	AMP Range	Output Type	Year Utilized
No. 1	0 - 30 volts	0 - 3A	DC	1991
No. 2	0 - 125 volts	0 - 2A	AC	1991
No. 3	0 - 20 volts	0 - 10A	AC	1991
No. 4	250 volts (RMS)	500 MA	AC	1992, 1993
No. 5	400 volts (peak)	–	DC - capacitive discharge	1993

The 120 hertz pulsed DC, utilized in 1991, was developed by connecting a full wave rectifier to the output of Power Supply No. 2. Power Supply No. 5 was developed

through commercial electrofishing industry research and is available "off the shelf". The electrical system was set up much like a standard cathodic protection array where DC voltages were applied to Configuration Type 1.

All the above power supplies were equipped with an adjustable output voltage control to suit various test conditions. The voltage supplied by Power Supplies Nos. 4 and 5 were not varied during the 1992 and 1993 studies, however.

The electric field intensity was set by placing two test electrodes a fixed distance apart midway between the electrodes. The test electrodes were then connected to a high input impedance digital voltmeter. The output of the power supply was adjusted to obtain the desired electric field intensity (volts/cm). Field strength was measured approximately mid-way between positive and negative electrodes with voltmeter probes spaced 2.5 cm or less apart or oscilloscope probes approximately 7.5 cm apart. Excitation voltages were determined by measuring the voltage applied to the electrodes.

Variables Tested

Several electrical variables were tested to determine effects on mussel attachment. The variables included:

- Voltage intensity applied to the arrays (measured as excitation voltage and volts/cm);
- Electric current type (AC, DC, or pulse DC); and
- Electrode configuration (fields generated parallel or perpendicular to flow, i.e., Type 1 or Type 2).

Exposure time or the time an organism is in contact with a chemical or stimulus (in this study an electric field) is a major factor in all research studies where a biological reaction is to be measured. In the electric field studies, the exposure time was quite short and held relatively constant. Exposure time varied slightly as the pumped flow varied due to fluctuating head levels in the Russell Station forebay. There was also a probable, minor variation in exposure time between the Type 1 and Type 2 configurations that resulted from differences in effective field strengths with range. Exposure time values were arrived at by dividing the electric field distance (ranging from 0.2 to 0.5 ft) by the mean flow velocity (0.65 fsp). The estimated exposure times varied between approximately 0.3 and 0.8 seconds for the tests conducted.

The test voltage selection was based on biological and economic considerations and Acres preliminary laboratory tests. The excitation voltages in 1991 were selected to assure other aquatic life (specifically fish) would not be adversely affected nor would the test present an electrical hazard to personnel operating the test equipment. To be

economically feasible, the extrapolated energy cost for a full-sized electric field system should not exceed those costs associated with the protection of a similar-sized system against frazil ice formation. The extrapolated energy costs associated with applied test voltages did not approach that for frazil ice protection for the tests conducted in 1991. The voltages and field strengths used in 1992-1993 were of greater magnitude and would probably be a problem to fish which ventured too close to such a field. Costs to operate were expected to be acceptable but marginal for the AC, while the capacitive discharge DC unit was expected to be more economical.

Acres' preliminary uncontrolled laboratory tests, carried out previous to this reported study, indicated that low voltage, low intensity, AC fields induced a transitory response in adult mussels. Similar DC tests were inconclusive but indicated excitation voltages of 9 VDC or less caused effects ranging from death to no effect at all.

1991 Study

A total of 15 Type 1 and Type 2 configurations were exposed to zebra mussel post veligers in four test periods (Table 1). During the test periods, configurations were treated as control configurations or test configurations (one control/period and two or three tests per period). Control configurations were exposed only to a source of flowing water infested with veligers and post-veligers. The test configurations were exposed to the same source of flowing water and to electric fields of various intensities generated using alternating current (AC), direct current (DC), or pulse DC. Each test was conducted in a separate flume. Each flume was provided with an underflow slide gate or weir that was used to regulate the water flow through each flume. The applied voltages varied in magnitude within and among test periods. Configuration Type 1 was used exclusively in Test Period 1, while Configuration Types 1 and 2 were used in Periods 2 through 4. Configuration Type 1 was used as the control configuration in all four test periods.

Stainless ¼-inch bolts were deployed upstream of Type 2 configurations and all control configurations to create water flow characteristics similar to Type 1 test configurations. However, since these dummy electrodes probably had little, if any, effect on zebra mussel settlement rates, these electrodes were not deployed in 1992 or 1993.

1992 Study

Based on 1991 results, higher voltage fields were tested in 1992. The economic feasibility associated with projected power costs for full-scale operation to generate the 1992 "higher voltage" study was determined upfront. The costs, though higher, were still reasonable. Applying 250 volts AC across the electrodes of a Type 1 configuration resulted in the generation of a field of 39 volts measured over one centimeter between

TABLE 1

SUMMARY OF ELECTRICAL TREATMENTS FOR ELECTRIC FIELD TESTS

Test Period	Electrical Treatment	Excitation Voltage	Year Tested
1	Control[1] AC: 6.33 volts/cm[1] DC: 1.0 volts/cm[1]	17.9 19.1	1991
2	Control[1] AC: 8.0 volts/cm[2] AC: 2.0 volts/cm[1] DC: 1.6 volts/cm[1]	9.0 36.5 31.8	1991
3	Control[1] AC: 17.0 volts/cm[2] Pulse: DC: 1.6 volts/cm[1] DC: 1.7 volts/cm[1]	18.6 32.1 31.8	1991
4	Control[1] AC: 17.0 volts/cm[2] Pulse: DC: 1.0 volts/cm[1] DC: 1.0 volts/cm[1]	18.8 19.1 19.4	1991
1 and 2	3 Controls[1] AC: 39.0 volts/cm[1]	250	1992
1	4 Controls[1] AC: 39.0 volts/cm[1] DC: capacitative discharge[1]	250 400	1993
2	6 Controls[1] 6 Controls[3] AC: 39.0 volts/cm[1 and 3] DC: capacitative discharge[1 and 3]	250 400	1993

(1) Configuration Type 1
(2) Configuration Type 2
(3) Configuration Type 3

the configuration electrodes. Type 2 configurations were not utilized in 1992 because they were economically less feasible. Also, DC voltages were not applied due to the lack of equipment necessary to generate the higher DC field intensities.

The test apparatus that was used in 1991 was modified for 1992 tests. The modifications included new power supply equipment to provide the increased AC voltage, implementation of more safety precautions to restrict the study area to authorized personnel, deployment of two Type 1 configurations per flume to compare settlement rates within as well as across flumes, and a change in the flume weirs from a bottom gate spill to an overflow spill to prevent the buildup of flotsam.

A total of eight Type 1 configurations were exposed to zebra mussel post-veliger settlement in two test periods (Table 1). Only two flumes, one "test" and one "control", were used during each period. In a "test" flume, one configuration was electrically treated while the other was an "in line" control. The "control" flume contained two configurations, neither one electrified. A configuration was deployed in a third flume and was examined biweekly using a hand-held microscope, so initial zebra mussel settlement could be determined without disturbing the test or control configurations. This configuration was not analyzed for experimental results.

1993 Study

In 1993 the AC voltage which was first tested in 1992 (at 39 volts/cm) was retested using the same approach and test apparatus. A DC capacitive discharge (pulse-power) voltage was also tested. The DC pulse generator had a pulse rate of 10 pulses/second, a duty cycle of 60 milliseconds, and a field strength of 26.2 volts/cm (peak). Post-veligers would only be exposed to an estimated four-to-five pulses during transit between the electrodes. As a result of capacitor discharge, as influenced by the conductivity of the water, there was an exponential drop in the field strength over time.

In 1993, data from two test periods were collected. In Test Period 1, six Type 1 configurations were exposed to zebra mussel post-veligers (Table 1). Two test flumes and one control flume were used. Due to delays in obtaining the necessary equipment for the DC test, the two Type 1 configurations deployed in the DC test flume were exposed to the settlement of zebra mussel post-veligers during the last two weeks of Test Period 1. Eight Type 1 configurations and eight Type 3 configurations were exposed to zebra mussel post-veligers during Test Period 2. Two test flumes and two control flumes were used. Type 3 configurations were deployed immediately upstream of all Type 1 configuration arrays.

RESULTS

Effects of Electrical Current on Zebra Mussel Settlement

Densities of settled post-veligers were quite low in all four tests conducted in 1991. This may be attributed either to delays in initiation of testing or to overall low densities over the entire season. The highest settlement densities were in Tests 1 and 2, which ranged from 776 to 8,302/m^2. Densities in Tests 3 and 4 ranged from 14 to 104/m^2.

Although the experiment was designed to equally apportion the incoming population of larvae among the four test flumes, it was uncertain whether consistent distribution patterns existed. Veliger densities probably varied among flumes and over time based on limited veliger sampling conducted by RG&E and Acres. Since observations indicated that it was probable that veligers were not evenly distributed among test and control flumes, no statistical analysis was conducted on the 1991 results.

Even though 1992 testing encompassed the zebra mussel settlement season, very low densities of settled mussels were observed (0 to 41/m^2). This was probably due to the reported poor settlement throughout the lake in 1992. To address the uneven veliger distribution problems encountered during the 1991 study, a new treatment/control scheme was employed where two Type 1 configurations (upstream and downstream) were placed in each flume. However, due to the extremely low numbers of settled veligers, no statistical analysis was performed.

In 1993 veliger densities, though higher than in 1992, were considered to be low. The DC flume in Test Period 1 was started approximately one month after the control and AC flumes were set up and in place for only 13 days, missing the peak settlement period. Test Period 1 had higher densities (ranging from 24 to 2,047/m^2) than Test Period 2 (ranging from 6 to 53/m^2).

Each flume contained two Type 1 configurations (upstream and downstream). Paired T-tests were conducted on both configurations' steel plate arrays in the control flumes to determine if significant differences in veliger settlement were present. No significant differences ($P > 0.05$) were found in any control flumes during Test Periods 1 and 2. Configuration position did not influence zebra mussel settlement.

Paired T-tests were conducted to determine if any differences in the number of mussels counted on upstream and downstream arrays in AC and DC test flumes were significant. The only significant difference ($P < 0.05$) found was in the DC flume in Test Period 2 where 88 percent more veligers settled on the upstream array than on the downstream array.

To determine if AC and DC currents had any residual or stun effect on settling post-veligers, the number of settled individuals counted on the upstream half of each array was compared to the number counted on the downstream half. Significant differences were observed for the number of mussels found on the downstream DC array and on the downstream AC array during Test Period 1. In the DC array, the downstream half contained 83 percent more mussels than the upstream half. In the AC array, the downstream half contained 35 percent more mussels than the upstream half. During array removal, field scientists noticed that fewer mussels settled on flume walls within the electric field. During Test Period 2, plexiglass plates (Type 3 configurations) were added to observe any differences. However, the settlement season was ending and extremely low numbers of settled mussels were found, so no analysis was performed.

Both Type 1 configurations in the AC test flume (Test Period 3) were left in place until February 1994 in an effort to examine winter settlement by post-veligers or translocators. Upon retrieval, no settlement was observed within any flume. No settlement was observed after examination of 10 percent of the "in line" control array and 10 percent of the AC test array, so laboratory analysis was discontinued.

Electric Field Generation and Effects on Equipment

Both alternating current (AC) and direct current (DC) electric fields were used in experiments in 1991 and 1993. Both types of fields were studied primarily to investigate the relative effectiveness of each to deter mussel attachment. A secondary objective was to investigate significant advantages that favor the use of one type of field over the other (i.e., if both work equally in deterrence, is there an advantage in application).

Some differences in the behavior of the electrodes were predicted and experimentally observed. Specifically, alternating current (AC) electric fields caused no significant physical changes in the upstream stainless steel electrodes nor the array of steel plates (downstream electrode) for Type 1 configurations, or for individual plates of Type 2 configurations. In the Type 1 control and AC test configurations, the stainless steel upstream electrode showed no sign of deterioration, and the downstream steel plate electrodes showed only minor deterioration. However, direct current (DC, pulsed, and continuous), caused significant erosion of the upstream stainless steel bolt (anode) and significant plating of primarily calcium carbonate on the leading edge of the downstream steel plate array (cathode). This oxidation of the anode and plating on the cathode was expected, but the rate of deterioration of the anode was much faster than expected. In the 1991 test, within three weeks the anode almost completely oxidized into a red colored gelatinous cylinder of mainly iron oxide. This oxidation of the anode could have been eliminated by using a nonreactive conductive material as the anode, but because of the relatively short duration of the experiment and budgetary constraints, stainless steel was used. In 1993, a platinum clad anode, typically placed in cathodic protection systems,

was used and oxidation of the anode did not occur. However, the plating on the cathode (i.e., racks) was even further accelerated, relative to 1991, presumably due to the increase in voltages used in the 1993 study.

The calcium carbonate plating on the Type 1 steel plate array was also expected, but not at the deposition rate observed. Cathodic protection of trashracks provided by the use of DC would be an added benefit if the electric field could be shown to effectively influence the attachment behavior of the zebra mussels without overplating the trashracks during the mussel season. However, the spaces between the steel plates was almost completely occluded after four months of exposure to the capacitive discharge field in 1993. This may create a greater reduction in flow than might be expected due to zebra mussel attachment in the same time interval.

At the conclusion of the 1993 study, the Type 1 DC test configuration was left in the flume to determine if the calcium carbonate would erode or redissolve in the water when the generation of the DC field was discontinued. After approximately three months exposed to the flow of water alone, no significant change was noted in the calcium carbonate buildup.

CONCLUSIONS AND RECOMMENDATIONS

The overall results of the study indicated that pulse-power DC is a better approach than the tested AC method. As tested, however, neither approach was 100 percent effective in preventing attachment of post-veliger mussels to a treated surface.

In general, the results of the 1991 and 1992 tests were inconclusive and the fields, as tested, were not providing 100 percent protection. Analysis of data collected in 1993 provided more detailed insight into the effects of electric fields.

In the capacitive discharge DC tests, the overall settlement reduction on a test array, when compared to a corresponding control array, was inconsistent between test periods. In one of the two tests no significant effect was observed, while in the other a greater than 85 percent overall reduction ($P < 0.05$) in settlement on the test array was observed. The inconsistency in the observed effect between periods may have been related to variability in veliger settlement densities and/or temperature variation between test periods. However, the study was not designed to test for the effects of these factors.

In the AC tests, there were no significant differences between settlement on the treatment and control racks in either test period.

Data suggest that the AC and DC fields may elicit a short duration stunning effect on the post-veligers. Significant reductions in settlement densities between the upstream to

downstream test array halves (35 percent and 83 percent, respectively) were found in both the AC and DC tests conducted in Period 1. There were, however, no significant reductions in settlement for any control array halves.

In summary, the AC fields, generated by an experimental system, provided only one instance where a significant reduction in veliger density was observed. Experimental AC systems, as tested, could probably be enhanced to provide acceptable settlement reductions, and then developed for commercial applications. The costs to operate such an AC system would probably be significantly higher than for a comparable DC system. The DC pulse-power or capacitive discharge system, as tested, demonstrated significant reductions (> 80 percent) in settlement in two of the four tests. Although promising, this technology would also need refinement prior to full-scale application. The manufacturer of the tested commercial DC system can modify the existing fish guidance system to increase the repetition rate and/or the magnitude of the DC pulse, while reducing the duty cycle to less than 60 milliseconds for use as a zebra mussel control device. This should enhance the stun effect on post-veligers while maintaining acceptable operational costs. Calcium buildup on the leading edge of the downstream electrode will have to be addressed, but it should not be an insurmountable or cost-prohibitive problem.

ACKNOWLEDGEMENTS

The authors would like to thank RG&E's environmental staff for providing much of the information on larval densities from each flume in 1991, for weekly monitoring of the system during 1992 and 1993, and to the RG&E chemistry laboratory for providing the chemical analysis of the deposits on the test anodes and cathodes.

REFERENCES

(1) Mackie, G.L., W.N. Gibbons, B.W. Muncaster and I.M. Gray. 1989. The zebra mussel, Dreissena polymorpha: A synthesis of European experiences and a preview for North America Water Resources Branch, Great Lakes Section.

(2) Claudi, R. and P.M. Wianko. 1991. Summary of Ontario Hydro's 1990-1991 Zebra Mussel Research Program. Presented at the Electric Utility Zebra Mussel Control Technology Conference, Itasco (Chicago), Illinois, October 22-23, 1991.

(3) Michaud, D. 1991. Personal communication. WI Electric Power Company, Milwaukee, WI.

(4) Tuttle, L.R., NYSEG. 1990. Electric barriers for veligers. IN: Dreissena polymorpha Information Review. C. O'Neill, ed. New York Zebra Mussel Information Clearinghouse, New York Seas Grant Extension. Volume 1, Number 2, December 1990.

(5) Gross, C. 1991. Personal communication. Long Island Lighting Company, Melville, NY.

(6) Serli, N., R. Claudi, D. Lewis and R. Pawson. 1994. Use of cathodic protection for control of zebra mussel biofouling on steel and concrete surfaces. Presented at the Fourth International Zebra Mussel Conference, Madison, Wisconsin, February 1994.

(7) Reynolds, J.B. 1993. Electrofishing. IN: Fisheries Techniques. Nielsen, L.A. and D.L. Johnson, eds. American Fisheries Society, Southern Printing Company, Inc., Blacksburg, VA. pp. 147-163.

(8) FAO. 1967. Fishing with electricity: Its application to biology and management. R. Vibert, ed. Whitefriars Press Ltd., London. 267 pp.

(9) Acres International Corporation. 1992. The study of the effect of low intensity electric fields on zebra mussel attachment.

(10) Electric Power Research Institute. 1993. Final report on electrical methods for the control of zebra mussels.

(11) Nelson, J.R., L.S. Ostlie and R.J. Whiley. 1994. The efficacy of pulsed electric fields in preventing settlement of zebra mussel veligers. Presented at the Fourth International Zebra Mussel Conference, Madison, Wisconsin, February 1994.

Comparative Study of the Desiccation Resistance of Zebra Mussels (*Dreissena polymorpha*) and Quagga Mussels (*Dreissena bugensis*)

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ABSTRACT

The emersion tolerance of zebra mussels (*Dreissena polymorpha*) and quagga mussels (*Dreissena bugensis*) were comparatively studied. Mussels (n = 60) were emersed at 15°C under relative humidities (R.H.) of < 5%, 33%, 53%, 75% and > 95 %. LT_{50} (*i.e.*, time for 50% sample mortality estimated by probit analysis) in *D. polymorpha* ranged from 67.7 h in < 5% R.H. to 266.2 h in > 95% R.H. and SM_{100} values (*i.e.*, time required for actual 100% sample mortality) from 120 h to 537 h. LT_{50} and SM_{100} value ranges for *D. bugensis* were 80.4 h to 238.2 h and 120 h to 312 h, respectively. Total water lost during emersion (TWL, total water is corporal plus extracorporal water) was positively correlated with shell length (SL) and hours of emersion. While affected by R.H., TWL did not differ between species. When TWL was expressed as a percent of total water (PTW), correlation with SL was lost while that with emersion time was retained. As with TWL, PTW lost was affected by R.H., but did not vary between species. At equivalent SL, total water (TW) content was reduced in *D. bugensis* relative to *D. polymorpha*, due to its reduced internal shell volume. Mean percent of TW lost just prior to death in both species ranged from 45-60% at 5% - 53% R.H. suggesting that a 45%-60% loss of TW was lethal. In contrast, mean percent of TW lost just prior to death was reduced in *D. polymorpha* at > 95% R.H. and in *D. bugensis* at 75% and > 95% R.H., suggesting that, at higher R.H., death did not result from desiccation, but from accumulation of toxic anaerobic end-products. Emersion tolerance measured as SM_{100} values was reduced in *D. bugensis* compared to *D. polymorpha* at 53% - > 95% R.H. The reduced SM_{100} values of *D.bugensis* and very low fraction of TW lost by this species just prior to death at 75% and > 95% R.H. relative to *D. polymorpha* reflect its general restriction to greater depths where probability of emersion is low. Overall, emersion tolerance in both species is reduced compared to native North American bivalves. Recommended emersion times for mitigation of *D. polymorpha* fouling will also be effective for *D. bugensis*.

INTRODUCTION

The zebra mussel, *Dreissena polymorpha*, was introduced to North America in 1986. It originally became established near the Lake St. Clair - Detroit River region. It is believed that mussel larvae were released into the Great Lakes with the ballast water discharge or as adults escaping anchor chains of ships arriving from Europe (Hebert, *et. al.*, 1989; Mackie, *et. al.*, 1989, McMahon, *et. al.*, 1993). Since its original introduction, mussels have spread throughout Lakes Erie, Ontario, Michigan, Oneida, the Finger Lakes, the Erie-Barge Canal, and the St. Lawrence, Hudson, Oswego, Illinois, Mississippi, lower Ohio, lower Tennessee, lower Arkansas and lower Cumberland Rivers (Zebra Mussel Information Clearinghouse, 1993). Zebra mussels are now spreading rapidly throughout North American inland waterways via dispersal of adults attached to commercial barge hulls and downstream transport of their planktonic veliger larvae (McMahon, 1992). Reproductive zebra mussel populations are now reported to extend downstream in the lower Mississippi River as far as New Orleans (Zebra Mussel Information Clearinghouse, 1993).

Also apparently introduced to the Great Lakes concurrently with *D. polymorpha* was a second species of *Dreissena* originally called the "quagga mussel" (May and Marsden, 1992). This second North American dreissenid species was recently identified as the southeastern European species, *Dreissena bugensis* (University of Wisconsin Sea Grant Institute, 1993). Specimens of *D. bugensis* have more laterally compressed shell valves than those of *D. polymorpha* and lack the latter species' distinctively flattened ventral shell margins. *D. bugensis* is now found throughout Lakes Erie and Ontario, the St. Lawrence River and the western portion of the Erie Barge Canal in upstate New York (Dermott, 1993), but, unlike *D. polymorpha*, has yet to invade Mississippi drainages (Zebra Mussel Information Clearinghouse, 1993). *Dreissena bugensis* is sympatrically distributed with *D. polymorpha* over its present North American range, with the proportion of the two species in dreissenid populations varying greatly on both micro- and macrogeographic scales (Dermott, 1993).

Adults of both *D. polymorpha* and *D. bugensis* attach to natural hard substrata such as rocks, wood and macrophytic plants and to man-made structures constructed of concrete, metal piping, steel, nylon, fiberglass and wood. Attachment is by a holdfast of proteinaceous byssal threads produced from a gland just posterior to the foot (Mackie *et al.*, 1989; McMahon, 1990). In both species, individuals byssally attach to the shells of other mussels, forming encrusting mats many shells thick (10 - 30 cm) (McMahon, 1990). When such thick encrustations of mussels form on man-made structures or within raw water systems, they negatively impact their operation and efficiency. Because of its capacity for macrofouling, *D. polymorpha* (zebra mussel) has already had major detrimental impacts on recreational boating and commercial

shipping as well as on raw water using industries, potable water treatment plants and electric power stations in North America (Roberts, 1990; Claudi and Mackie, 1993).

Presently, the main control technologies for zebra mussel macrofouling center on molluscicides such as chlorine, bromine, ozone, aromatic hydrocarbon compounds and quaternary ammonium compounds (Electric Power Research Institute, 1992). However, federal and state regulations for use of molluscicides in controlling macrofouling by dreissenids and other freshwater macrofouling bivalves (*i.e.*, *Corbicula fluminea*, the Asian clam) are likely to become increasingly restrictive. With the specter of nearly every raw water using facility on the major waterways of the Mississippi Drainage applying molluscicides to control zebra mussel fouling, and presently increasing mussel infestations of raw water facilities on the Great Lakes, molluscicide usage will be highly regulated in order to prevent water quality degradation and maintain drinking water standards (McMahon *et al.*, 1993). Therefore, in order to maintain the quality of North American waterways there is likely to be an increasingly greater priority placed on the development of reliable, cost-effective, environmentally acceptable, nonchemical zebra mussel macrofouling control technologies.

Among nonchemical dreissenid mitigation strategies, dewatering of infested structures to expose mussels to lethal levels of desiccation appears to be a readily applied, efficacious and environmentally neutral technology worthy of further attention (Claudi and Mackie, 1993; Electric Power Research Institute, 1993; McMahon, *et al.*, 1993). Dewatering could be a particularly efficacious means of mussel control in raw water systems such as navigation locks and water intake structures that are designed to be periodically dewatered for maintenance. McMahon *et al.* (1993) have shown that zebra mussels are much less tolerant of emersion than are the majority of native North American bivalves. Mathematical models predicting the duration of emersion required for mitigation of *D. polymorpha* infestations relative to exposure air temperature and relative humidity have been developed (McMahon *et al.*, 1993). In contrast, there is no available information on the desiccation tolerance of *D. bugensis*. In raw water systems drawing from the Great Lakes, *D. bugensis* and *D. polymorpha* can form mixed macrofouling populations (Dermott, 1993). Thus, if the levels of desiccation lethal to *D. polymorpha* are also lethal to *D. bugensis*, similar durations of dewatering and emersion could be utilized to mitigate fouling by pure populations of either species or mixed populations of both species.

Dreissena bugensis has been reported to be a deeper water species than *D. polymorpha* (Dermott, 1993). Restriction to deeper water, suggests that *D. bugensis* may be less tolerant of emersion than *D. polymorpha* whose populations extend nearly to the surface (Mackie *et al.*, 1989) where they can be emersed by natural water level variation. This paper presents results of a study comparing the desiccation tolerance

of *D. bugensis* and *D. polymorpha*, allowing analysis of the potential for dewatering to be an effective, nonchemical mitigation technology for both species. The results are also utilized to relate desiccation tolerance to differences in the depth distributions of the two species.

METHODS

Specimens of *D. polymorpha* were collected from the intake of a power station drawing water from Lake Erie near Cleveland, Ohio. Specimens of *D. bugensis* were taken from the western basin of Lake Erie near the City of Monroe, Michigan. They were shipped overnight emersed on moist paper toweling in insulated, cooled containers to the University of Texas at Arlington. On arrival, they were maintained in 284 l of continually aerated, dechlorinated, City of Arlington tap water in a refrigerated 'Living Stream' holding tank at a constant water temperature of 5°C ±0.5°C without feeding for no more than 30 days before utilization in experiments. Specimens of *D. polymorpha* held under these conditions show no significant reduction in tissue mass or loss of condition over the short 30 day pre-experimental holding period (Chase and McMahon, 1994).

Prior to determination of emersion tolerance, adult individuals of both species were randomly selected (mean shell length for *D. polymorpha* = 20.2 mm, range = 15-27 mm, n = 300; mean shell length for *D. bugensis* = 21.8 mm, range = 14-30 mm, n = 296), individually marked by painting an identifying number on the shell, wet weighed to the nearest 0.1 mg, and reimmersed for 24 h. After 24 h reimmersion, separate samples of 60 mussels of both species were emersed in desiccation chambers under five different relative humidity conditions. Specific relative humidity (R.H.) levels were maintained in the desiccators over saturated salt solutions. Mussels were held above these solutions on stages constructed of 0.5 cm wire mesh covered with a 1 mm nylon mesh. Tested R.H. levels were: < 5% R.H. maintained over silica gel desiccant; 33% R.H. over a saturated solution of magnesium chloride ($MgCl_2 \cdot 6H_2O$); 53% R.H. over a saturated solution of magnesium nitrate ($Mg(NO_3)_2 \cdot 6H_2O$); 75% R.H. over a saturated solution of sodium chloride (NaCl); and > 95% R.H. over distilled water (after Byrne *et. al.*, 1988). Desiccation chamber temperature was held at a constant 15°C ±0.2°C in a refrigerated incubator.

Periodically, subsamples of six individuals of either species were removed from each desiccator, reweighed and their viability tested by rehydration in dechlorinated tap water for 12 h at room temperature (22-24°C). After rehydration, viability was tested by touching mussels in the vicinity of the siphons and posterior mantle tissues with the tip of a small paint brush. If this stimulation did not elicit an immediate valve closure response, the individual was considered to be dead. Subsampling frequency was designed to encompass a total duration of emersion over which subsamples exhibited

100% survival through to 100% mortality. Lethal emersion times were estimated as LT_{50} values (estimated time for 50% sample mortality) determined by probit analysis (Bliss, 1936) and SM_{100} values recorded as the actual time to the first observation of 100% subsample mortality.

At the end of the recovery period, all sampled individuals were oven dried to constant weight at 90°C. Subtraction of specimen dry weight from initial wet weight yielded the total water weight (TW, total water weight = corporal + extracorporal water weight [*i.e.*, body + mantle cavity water weight]). Subtraction of the wet weight after emersion from the initial, pre-emersion wet weight yielded the weight of water lost during the emersion period. Water loss was also expressed as the fraction of total pre-emersion water weight in fully hydrated individuals (after Byrne *et. al.*, 1988). Computation of mean fraction of TW lost values for subsamples of individuals removed at different periods over the course of emersion allowed estimation of cumulative water loss over tolerated emersion periods. In all cases, water loss values where only computed for individuals surviving a particular duration of emersion. In all statistical analyses, statements of significance are at an error level of $\alpha \leq 0.05$.

RESULTS

Multiple Least Squares Linear Regression Analyses of the natural logarithm (ln) of the weight of water lost versus ln time of emersion, shell length and R.H. as independent variables revealed that these independent variables explained 86% of all variation in weight of water lost in *D. polymorpha* and 84% in *D. bugensis*. Such high levels of correlation indicated little or no desiccation chamber effect on water loss rate in either species, allowing subsequent statistical analyses to utilize individual mussels as the experimental unit. For emersed specimens of *D. polymorpha* and *D. bugensis*, water loss rates were clearly correlated with R.H. Multiple Factor ANOVA indicated that the natural logarithm transformed weight of water lost during emersion increased with the covariants of size measured as shell length (SL) and the natural logarithm of hours of emersion ($P < 0.00001$). In contrast, there was no detectable difference in the weight of water lost between the two species ($P = 0.796$). Lack of difference in water weight lost occurred in spite of a reduced pre-emersion total water weight in specimens of *D. bugensis* relative to those of *D. polymorpha* of equivalent SL (Fig. 1) ($P < 0.00001$ as revealed by ANCOVA analysis of ln total water weight with SL as the covariant [$P < 0.0001$]). In contrast, water loss decreased significantly with increasing R.H. being insignificantly different between the 5% and 33% R.H. groups and the 53% and 75% R.H. groups, but significantly different between these two groups and mussels exposed to 95% R.H. (Table 1). Similar results were recorded for water loss expressed as a percent of TW (PTW) excepting a lack of correlation between SL and PTW lost (Table 2). These results indicate that while smaller individuals lost smaller actual volumes of water than did larger individuals, relative

TABLE 1

Multiple Factor ANOVA for testing for differences in mean in cumulative total water weight lost in specimens of *Dreissena polymorpha* (zebra mussel) and *Dreissena bugensis* (quagga mussel) emersed at 15°C under different relative humidities.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square Error	F-Ratio	Probability
Covariate Shell Length	28.98	1	28.98	28.77	<0.00001**
Covariate In Hours Emersed	123.95	1	123.95	123.06	<0.00001**
Species	0.089	1	0.089	0.089	0.769
Relative Humidity	190.06	4	47.51	47.17	<0.00001**
Residual	279.00	277	1.01		
Total	516.52	284			

** Significant effect at $P \leq 0.05$

Student Newman-Keuls Tests of differences in mean in cumulative total water weight lost between different relative humidity treatments for specimens of *Dreissena polymorpha* and *Dreissena bugensis* emersed at 15°C.

Percent Relative Humidity	Mean	n	Signif. Diff. (P<0.05)
< 5%	-1.102	46	
33%	-1.448	48	
53%	-2.002	62	
75%	-2.308	69	
> 95%	-3.855	60	

water loss expressed as a percentage of total available water was independent of size over the experimental SL range of 14-30 mm.

Multiple Factor ANOVA testing indicated that the mean PTW lost just prior to death (*i.e.*, mean percent of TW lost of individuals in the sample just preceding the sample in which greater than 60% mortality was recorded) was significantly different under different R.H. treatments ($P < 0.0001$) and between the two species ($P < 0.0151$), but

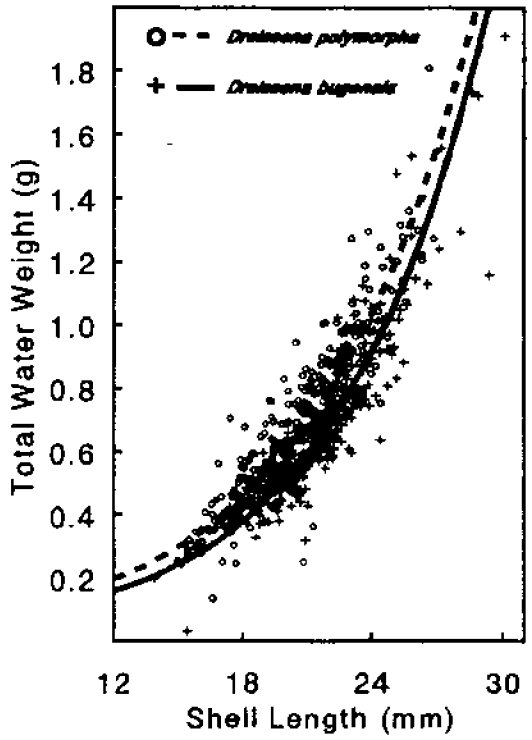

Fig. 1. *The relationship between total water weight (TW = corporal + extracorporal mantle cavity water weight in grams) (vertical axis) and shell length (SL in mm) (horizontal axis) of adult specimens of* Dreissena bugensis *(crosses) and* Dreissena polymorpha *(open circles). The dashed line represents the best fit of a least squares regression relating the natural logarithm of total water weight to shell length in* D. bugensis *as follows:*

$$\ln TW\ (g) = -3.6023 + 0.1463\ (SL\ in\ mm)$$
$$(n = 296,\ r = 0.867,\ F = 894,\ P < 0.00001).$$

The solid line represents the best fit of a similar regression for D. polymorpha *as follows:*

$$\ln TW\ (g) = -3.2800 + 0.1379\ (SL\ in\ mm)$$
$$(n = 300,\ r = 0.896,\ F = 1219,\ P < 0.00001).$$

TABLE 2
Multiple Factor ANOVA for testing for differences in the mean in cumulative percent of total water lost in specimens of *Dreissena polymorpha* (zebra mussel) and *Dreissena bugensis* (quagga mussel) emersed at 15°C under different relative humidities.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square Error	F-Ratio	Probability
Covariate Shell Length	0.0038	1	0.0038	0.004	0.951
Covariate In Hours Emersed	125.04	1	125.04	127.31	<0.00001**
Species	0.462	1	0.462	0.47	0.501
Relative Humidity	195.39	4	48.85	49.73	0.00001**
Residual	272.08	277	0.98		
Total	493.80	284			

** Significant effect at $P \leq 0.05$

Student Newman-Keuls Tests of differences in mean in cumulative % total water lost between different relative humidity treatments for specimens of *Dreissena polymorpha* and *Dreissena bugensis* emersed at 15°C.

Percent Relative Humidity	Mean	n	Signif. Diff. (P<0.05)
< 5%	3.858	46	
33%	3.491	48	
53%	2.979	62	
75%	2.653	48	
> 95%	1.064	46	

TABLE 3
Multiple Factor ANOVA for testing for differences in mean cumulative percent of total water lost just prior to death under different relative humidities in specimens of *Dreissena polymorpha* (zebra mussel) and *Dreissena bugensis* (quagga mussel) emersed at 15°C.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square Error	F-Ratio	Probability
Covariate Shell Length	41.38	1	41.377	0.217	0.649
Species	1223.99	1	1223.99	6.418	0.0151**
Relative Humidity	12030.94	4	682.19	3007.73	<0.00001**
Interaction	2728.76	4	682.19	3.577	0.0134**
Residual	8009.74	42	190.71		
Total	24564.03	52			

** Significant effect at $P \leq 0.05$

Student Newman-Keuls Test of differences in cumulative % total water lost just prior to death under different relative humidities for specimens of *Dreissena polymorpha* and *Dreissena bugensis* emersed at 15°C.

Percent Relative Humidity	Mean	n	Signif. Diff. (P<0.05)
33%	54.76	9	
< 5%	53.42	11	
53%	50.29	11	
75%	38.36	10	
> 95%	15.54	12	

was not correlated with SL ($P > 0.649$) (Table 3). Percent of TW lost just prior to death was least at > 95% R.H., and insignificantly different between 53% & 75% R.H. treated mussels and < 5% & 33% R.H. treated mussels. The percent of TW lost just prior to death values recorded in these 53 & 75% R.H. and < 5% & 33% R.H. groups were significantly different from each other and both were different from that recorded at > 95% R.H. (Table 3).

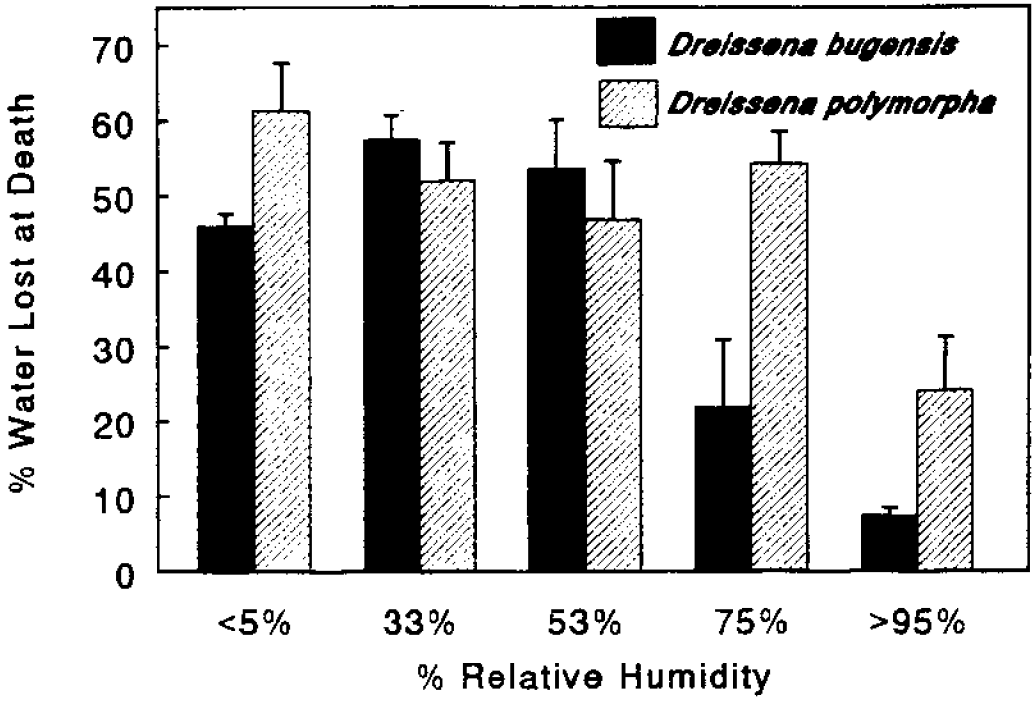

Fig. 2. *Mean percent of total water (corporal + extracorporal mantle cavity water) lost just prior to death (vertical axis) by specimens of* Dreissena bugensis *(solid histograms) and* Dreissena polymorpha *(cross hatched histograms) at various relative humidities (horizontal axis). Vertical bars above histograms represent standard errors of the means.*

When mean PTW lost just prior to death values were compared across species, values were similar for *D. polymorpha* and *D. bugensis* at relative humidities of < 5% through 53% at approximately 45% to 60% of total water. In *D. bugensis*, PTW lost just prior to death declined to < 30% at 75% R.H. and <

10% at < 5% R.H. (Fig. 2) In contrast, PTW lost just prior to death at 33% R.H. in *D. polymorpha* remained similar to that recorded at lower R.H. and declined to < 30% of total water only in the > 95% R.H. treatment. Thus, at 75% and > 95% R.H., PTW lost just prior to death was 2-3 times greater in *D. polymorpha* than in *D. bugensis* (Fig. 2).

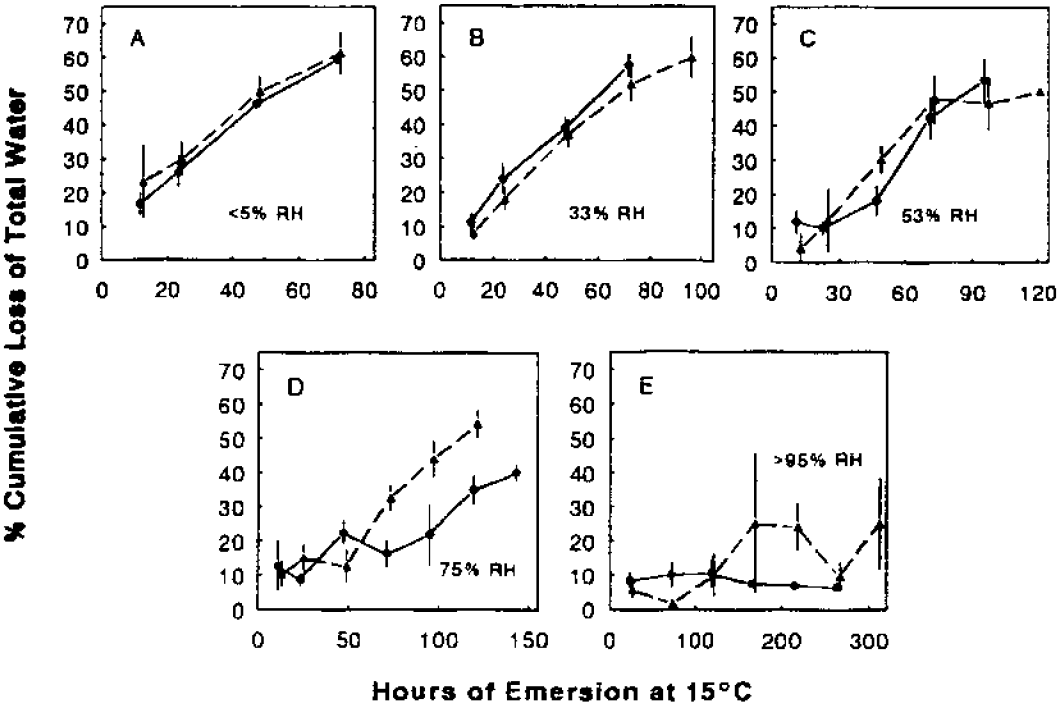

Fig. 3. *Mean cumulative percent of total water (corporal + extracorporal mantle cavity water) lost during emersion (vertical axis) by specimens of* Dreissena bugensis *(solid triangles and dashed lines) and* Dreissena polymorpha *(solid circles and solid lines) during emersion at 15°C in relative humidities of (A) < 5%, (B) 33%, (C) 53%, (D) 75% and (E) > 95% . Vertical bars about means represent standard errors of the means.*

The reduced PTW lost just prior to death in specimens of *D. bugensis* under 75% and > 95% R.H. was also reflected in the cumulative water loss rates of emersed specimens of this species. At < 5%, 33% and 53% R.H., cumulative PTW lost was essentially similar between the two species over their tolerated

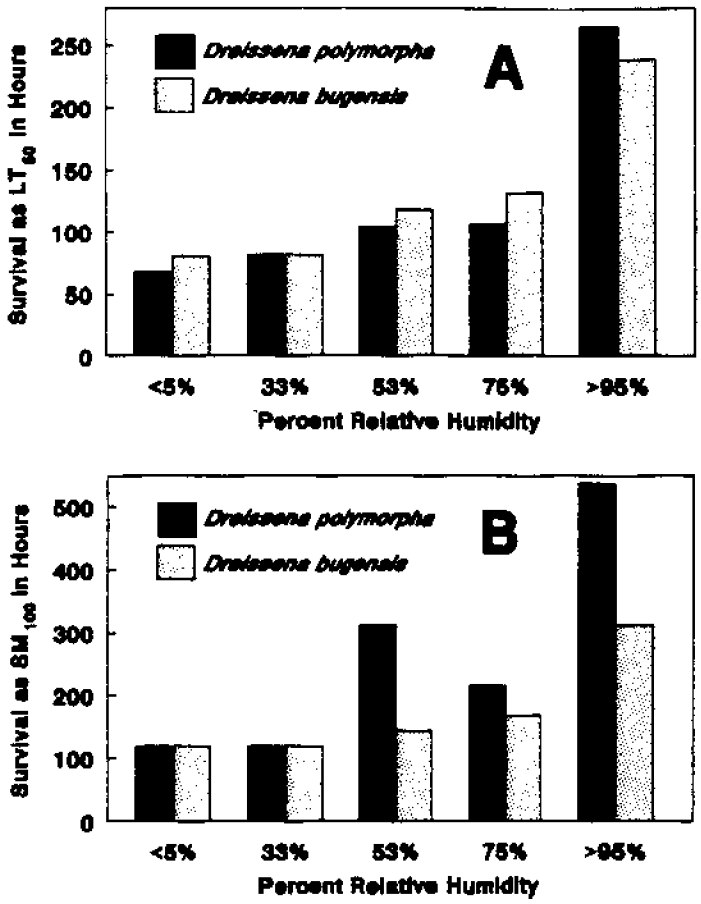

Fig. 4. *Emersion tolerance in* Dreissena bugensis *and* Dreissena polymorpha *at 15°C and various relative humidities (horizontal axis). The vertical axis is emersion tolerance time in hours expressed either as (A) LT_{50} (time for 50% sample mortality estimated by probit analysis) or (B) SM_{100} (actual time for 100% sample mortality). In both figures, stippled histograms represent emersion tolerance values in* D. bugensis *and solid histograms, emersion tolerance times in* D. polymorpha.

emersion periods (Figs. 3A-C), however, at 75% and 95% R.H., cumulative PTW lost did not increase as rapidly in *D. bugensis* with duration of emersion as it did in specimens of *D. polymorpha* leading to the reduced PTW lost just prior to death values recorded for *D. bugensis* at these two elevated R.H. treatments (Figs. 3D and E).

Measured as LT_{50} values (estimated time for 50% sample mortality, Bliss 1936), desiccation tolerance was essentially similar in specimens of *D. polymorpha* and *D. bugensis* across all R.H. treatments (Fig. 4A) in spite of lower PTW lost just prior to death values recorded for *D. bugensis* (Fig. 2). In contrast, times to actual 100% sample mortality (SM_{100}) were 25% to nearly 100% greater in *D. polymorpha* at the higher R.H. treatments of 53%, 75% and > 95% (Fig. 4B).

DISCUSSION

Until very recently, dewatering and exposure to lethal desiccation have had no experimental attention as a potential dreissenid macrofouling mitigation strategy. The only previously available information on desiccation tolerance in zebra mussels came indirectly from a study of their capacity to buffer the hemolymph (*i.e.*, blood) pH while emersed. Mussels emersed at room temperature (20-22°C) without controlled R.H. in this prior study survived emersion no longer than four days (Alyakrinskaya, 1978).

McMahon *et al.* (1993) described experiments designed to determine the effects of R.H and temperature on emersion tolerance in *D. polymorpha*. Multiple Linear Regression models were developed which could be utilized to estimate LT_{50} and SM_{100} times based on emersion temperature and R.H. conditions. Results showed that emersion tolerance increased exponentially with declining temperature and increased linearly with increased R.H.. At 25°C, R.H. had little effect on lethal emersion times in *D. polymorpha* with 4-5 days required for 100% mortality, a level of emersion tolerance similar to that reported by Alyakrinskaya (1978). However, at temperatures below 25°C, relative humidity became an increasingly important factor such that emersion durations at temperatures below 15°C, particularly at higher R.H., were extended beyond 10 days, rendering the recommended practice of holding of recreational boats out of water for 2-5 days to kill attached zebra mussels prior to transportation between water bodies (Ontario Ministry of Natural Resources, 1990, 1991a, 1991b) unlikely to be effective except during the warmest summer months. For similar reasons, it was suggested that dewatering of mussel infested structures for mitigation of zebra mussel fouling was likely to be most efficacious at ambient air temperatures above 20°C where emersion tolerance times were relatively short (< 5 days) regardless of R.H. (McMahon, *et. al.*, 1993).

Our present results indicate that, at 15°C, the emersion tolerance of *D. bugensis* expressed as LT_{50} or SM_{100} values was either equivalent to or less than that of *D. polymorpha* over a relative humidity range of < 5% to > 95%. The

equivalency of emersion tolerance between *D. bugensis* and *D. polymorpha* strongly suggests that the emersion times reported to be effective in mitigating infestations of *D. polymorpha* (McMahon *et al.*, 1993) will also be efficacious in mitigating *D. bugensis* macrofouling.

Our data suggest that both quagga and zebra mussels are relatively intolerant of prolonged emersion relative to other freshwater bivalve species. At 15°C between < 5% and 75% R.H., Asian clams (*Corbicula fluminea*) can tolerate emersion more than twice as long as *D. polymorpha* or *D. bugensis* (Dreissenid SM_{100} range = 115-530 h, Fig. 4B) (Byrne *et. al.*, 1988). Both freshwater unionid and sphaeriid bivalves appear to be much more tolerant of emersion than *D. polymorpha* or *D. bugensis*. Riverine and pond unionids and sphaeriids can survive many months emersion at high temperatures when exposed to air by receding water levels during droughts and dry seasons (for a review see McMahon, 1991) while zebra and quagga mussels can survive a maximum of only 22 days in water saturated air (> 95% R.H.) at a relatively low temperature of 15°C (Fig. 4B).

The very reduced emersion tolerance of dreissenids relative to unionid and sphaeriid bivalves suggests that they, like the emersion intolerant *C. fluminea*, are only recent invaders of freshwaters (McMahon, 1991). The frequency and duration of emersion experienced by intertidal bivalves is much shorter and more predictable than that experienced by freshwater bivalves, thus, marine species are generally less tolerant of emersion than freshwater bivalves (McMahon, 1991). Neither zebra mussels or Asian clams appear to have had a long enough evolutionary history in freshwater to have fully evolved the high levels of emersion tolerance characteristic of most freshwater unionid and sphaeriid bivalve species whose evolutionary histories in freshwater extend from the Triassic and Cretaceous periods, respectively (McMahon, 1991).

At 15°C, PTW loss prior to death in zebra mussels ranged from 45% to 60% over < 5%-53% R.H. in both *D. polymorpha* and *D. bugensis*. This range of tolerated water loss was similar to that of specimens of *C. fluminea* emersed under similar conditions (≈ 50-80%) (Byrne, *et. al.*, 1988) and falls within that reported for three species of freshwater unionids more tolerant of emersion than zebra mussels (Holland, 1991). The similarity of PTW loss prior to death values for zebra mussels emersed in < 5%-53% R.H. to that reported for other freshwater bivalve species suggests that mussels emersed under these conditions died as a result of lethal tissue desiccation. At 75% R.H., the PTW lost prior just to death in *D. polymorpha* remained above 50% suggesting that death also resulted from lethal tissue desiccation at this relatively high R.H. In contrast, PTW loss just prior to death was only 22% at 75% R.H. in *D.*

bugensis (Fig. 2). At > 95% R.H., PTW lost just prior to death in both *D. polymorpha* (23% of TW) and *D. bugensis* (8% of TW) was considerably below that recorded at relative humidities of 53% or less (Fig. 2). The occurrence of mortality at elevated R.H. at lower than maximally tolerated levels of desiccation suggests that death of emersed specimens of *D. polymorpha* at > 95% R.H. and *D. bugensis* at 75% and > 95% R.H. was not due to lethal tissue desiccation. Rather, some other emersion induced stress such as accumulation of toxic anaerobic metabolites, disruption of hemolymph acid-base balance, ammonia toxicity or exhaustion of organic energy stores must have been the cause of death (for reviews see McMahon, 1991; Byrne and McMahon, 1994).

When emersed at high relative humidities, shallow water, emersion tolerant freshwater bivalves including unionids (Holland, 1991; Byrne and McMahon, 1994) and *C. fluminea* (Byrne *et al.*, 1990) and marine intertidal bivalves (McMahon, 1988) periodically gape the valves allowing uptake of oxygen across the exposed epithelial surfaces of mucus-fused mantle edges. Such periodic aerial gas exchange behavior allows maintenance of an aerobic catabolism, but results in elevated water loss rates. In contrast, deeper water bivalves, not adapted to periodic emersion, keep the valves tightly closed in air and catabolize anaerobically. While this behavior greatly reduces water loss, it results in the rapid accumulation of toxic anaerobic metabolic end-products to lethal levels. Thus, even though emersed deep water bivalve species often lose water at a slower rate than do shallow water species, they are generally less tolerant of aerial exposure than shallow water species because of their dependence on anaerobic rather than aerobic catabolism. (McMahon, 1988; Byrne and McMahon, 1994 and references therein).

While emersed at high relative humidities, specimens of *D. polymorpha* were observed to periodically gape the valves and expose mantle tissues allowing aerial gas exchange. In contrast, specimens of *D. bugensis* gaped only when approaching death. Suppression of mantle edge exposure behavior in *D. bugensis* in high relative humidities may have lead to the reduced water loss rates recorded at 75% and > 95% R.H. relative to that of *D. polymorpha* (Fig. 3D and E) and could have accounted for the very reduced PTW lost just prior to death at 75% and > 95% R.H. (22% and 8% of total water, respectively, Fig. 2), particularly if maintenance of shut valves caused rapid accumulation of toxic anaerobic end-products to lethal levels.

In Lake Erie, the proportion of individuals of *D. bugensis* in mixed zebra/quagga mussel populations changes with increasing depth. They averaged 1-50% of mussels in shallow, near-shore waters and increased in

numbers with increasing depth up to 20 m depth below which they made up 100% of the population with living specimens being found to depths of 63 m (Dermott, 1993). In contrast, *D. polymorpha* populations generally reach maximum densities between depths of 1-16 m and do not extend below the thermocline (Mackie *et al.*, 1989). Thus, *D. polymorpha* populations can be periodically emersed by natural changes in water level while the more deeply distributed *D. bugensis* would rarely experience emersion. This difference in depth distribution also seems to be reflected in their emersion tolerance. Zebra mussels have greater emersion tolerance, reflected in elevated SM_{100} values, at high R.H. (Fig. 4B). In addition, specimens of *D. polymorpha* emersed at high R.H. display periodic mantle edge exposure behavior for aerial gas exchange and correspondingly elevated water loss rates characteristic of shallow-water, emersion-tolerant freshwater bivalve species. In contrast, specimens of *D. bugensis* emersed in high R.H. maintain closed valves likely to result in anaerobic catabolism leading to rapid accumulation of anaerobic end-products to lethal levels typical of emersion intolerant deep water bivalve species.

ACKNOWLEDGEMENTS

Dr. Milton A. Matthews, Michael Clarke, Michael R. Hernandez, Terry L. Cleland and Craig A. Burnside of the Center for Biological Macrofouling Research provided editorial assistance with the preparation of the manuscript. This study was conducted as part of the Zebra Mussel Research Program of the United States Army Corps of Engineers by the Center for Biological Macrofouling Research of The University of Texas at Arlington. Permission was granted by the Chief of Engineers to publish this information.

REFERENCES

Alyakrinskaya, I.O. 1978. Biochemical adaptations to drying conditions in bivalves in the Kurski Zaliv Baltic Sea USSR. *Zool. Zh.* 57: 136-138.

Bliss, C.I. 1936. The calculation of the time-mortality curve. *Ann. Appl. Biol.* 24: 815-852.

Byrne, R.A. and **R.F. McMahon.** 1994. Behavioral, respiratory, acid-base, osmotic and ionic responses to prolonged emersion in fresh water bivalves. *Am. Zool.*, 34: 000-000. In press.

Byrne, R.A., R.F. McMahon and **T.H. Dietz.** 1988. Temperature and relative humidity effects on aerial exposure tolerance in the freshwater bivalve, *Corbicula fluminea*. *Biol. Bull.* (Woods Hole, Mass.) 175: 253-260.

Byrne, R.A., R.F. McMahon and **T.H. Dietz.** 1990. Behavioral and metabolic responses to emersion and subsequent reimmersion in the freshwater bivalve *Corbicula fluminea*. *Biol. Bull.* (Woods Hole, Mass.) 178: 251-259.

Chase, R. and **McMahon, R.F.** 1994. Effects of starvation at different temperatures on dry tissue and dry shell weights in the zebra mussel, *Dreissena polymorpha* (Pallas). Abstract of paper presented at the *Fourth Annual Zebra Mussel Conference '94*, Sponsored by Great Lakes Sea Grant Network, held 7-10 March, 1994, Madison, Wisconsin.

Claudi, R. and **Mackie, G.L.** 1993. *Practical Manual for Zebra Mussel Monitoring and Control.* Lewis Publishers, CRC Press, Boca Raton, Florida. 227 pp.

Dermott, R.M. 1993. Distribution and ecological impact of "quagga" mussels in the lower Great Lakes. Pages 2-1 - 2-21 *in*: J.L. Tsou and Y.G. Mussalli, *Proceedings: Third International Zebra Mussel Conference*, TR-102077, RP 2504-13, Electric Power Research Institute, Palo Alto, California.

Electric Power Research Institute. 1992. *Zebra Mussel Monitoring and Control Guide.* EPRI TR-101782, RP-3052-03, Electric Power Research Institute, Palo Alto, California. 582 pp.

Hebert, P.D.N., B.W. Muncaster and **G.L. Mackie.** 1989. Ecological and genetic studies on *Dreissena polymorpha* (Pallas): a new mollusc in the Great Lakes. *Can. J. Fish Aquat. Sci.* 46: 1587-1591.

Holland, D.F. 1991. Prolonged emersion tolerance in freshwater mussels (Bivalvia: Unionidae): interspecific comparison of behavioral strategies and water loss rates. *Masters thesis*, The University of Texas at Arlington. 120 pp.

Mackie, G.L., W.N. Gibbons, B.W. Muncaster and **I.M. Gray**. 1989. *The Zebra Mussel*, Dreissena polymorpha: *A Synthesis of European Experiences and Review for North America*. ISBN: 0-772905647-2, Great Lakes Section, Water Resources Branch, Ontario Ministry of the Environment, London, Ontario, Canada. 76 pp.

May, B. and **J. E. Marsden**. 1992. Genetic identification and implications of another invasive species of dreissenid mussel in the Great Lakes. *Can. J. Fish Aquat. Sci.* 49: 1501-1506.

McMahon, R.F. 1988. Respiratory responses to periodic emersion in intertidal molluscs. *Am. Zool.* 28: 97-114.

McMahon, R.F. 1990. *The Zebra Mussel: U.S. Utility Implications*. EPRI GS-6995, Electric Power Research Institute, Palo Alto, California. 70 pp.

McMahon, R.F. 1991. Mollusca: Bivalvia. Pages 315-393 *in*: J. H. Thorp and A.P. Covich (eds.), *Ecology and Classification of North American Freshwater Invertebrates*. ISBN: 0-12-690645-9, Academic Press, Inc., New York,

McMahon, R.F. 1992. The zebra mussel - the biological basis of its macrofouling and potential for distribution in North America. Reprint No. 342 of a paper presented at the annual meetings of the National Association of Corrosion Engineers, 26 April - 1 May 1992, Nashville, Tennessee. National Association of Corrosion Engineers, Houston, Texas. 14 pp.

McMahon, R.F., T.A. Ussery and **M. Clarke**. 1993. *Use of Emersion as a Zebra Mussel Control Method*. Contract Report EL-93-1, U.S. Army Corps of Engineers, Washington, D.C. 27 pp.

Ontario Ministry of Natural Resources. 1990. *Guideline on Zebra Mussels*. Ontario Ministry for Natural Resources, London, Ontario, Canada. 29 May, 1990, 4 pp.

Ontario Ministry of Natural Resources. 1991a. *Zebra Mussels - Boater's Guide*. ISBN: 0-7729-8708-4, Ontario Ministry for Natural Resources, London, Ontario, Canada. 16 pp.

Ontario Ministry of Natural Resources. 1991b. *Zebra Mussels.* Fact Sheet, November, 1991, Ontario Ministry for Natural Resources, London, Ontario, Canada. 2 pp.

Roberts, L. 1990. Zebra Mussel invasion threatens U.S. waters. *Science* 249: 1370-1372.

University of Wisconsin Sea Grant Institute. 1993. Quagga quandary solved. *Zebra Mussel Update* #19: 3.

Zebra Mussel Information Clearinghouse. 1993. North American range of the zebra mussel. *Dreissena Polymorpha Information Review* 4(5): 6-7.

Zebra Mussel Control Using Thermal Treatment for Electric Utility Stations

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ABSTRACT

When electric utilities and other water users on the Great Lakes were confronted with operating problems due to zebra mussels, Commonwealth Edison Company (CECo) established a task force to develop a plan to counteract the threat at the CECo electric generating stations. A monitoring program was initiated at the stations and an evaluation of control options was started. At State Line Station, located on the southern tip of Lake Michigan, CECo experimented with thermal treatment of the circulating water and service water systems. The station design allows recirculation of the cooling water with minimal modifications. The trial at State Line Station proved successful in controlling the zebra mussels with minimal impact on operations. Based on the successful trial and the task force's assessment of other control options, CECo determined that, for their coal-fired generating stations, thermal treatment was the most cost-effective approach, with the least impact on station operation and the environment.

Of the 10 coal-fired generating stations operated by CECo, 8 have been selected for modifications. The other 2 stations have not yet been affected by zebra mussels. Before performing detailed design, a study was performed for each station to evaluate the operation of the equipment at elevated temperature and to determine the operating limits needed at the target treatment temperature of 95° F. Conceptual designs for the modifications were developed, and the most cost-effective arrangement was selected for detailed design.

Case studies of the modifications being constructed at several stations are presented. The modifications to the circulating water systems are described. Initial results of the treatment are reviewed.

INTRODUCTION

Commonwealth Edison Company (CECo) has found a simple approach to control zebra mussels at the utility's stations: *heat*. Thermal treatment is accomplished by recirculating the condenser discharge to raise the circulating water (CW) and service water (SW) temperature to 95° F. The effectiveness of thermal treatment was demonstrated at CECo's State Line Station in 1990 and 1991. At the State Line Station, the plant arrangement (see Figure 1) allows recirculation of the heated discharge back to the crib house and CW and SW pumps by opening the division wall and north and south recirculation gates. Thermal treatment took 10 hours to raise the water temperature and an additional 6 hours to maintain the 95° F treatment temperature. A diver's exam showed 100% zebra mussel mortality.

In parallel with the State Line demonstration, a CECo task force assessed treatment options for all their stations. The task force determined that thermal treatment was the least-cost control approach considering construction costs and long-term operating and maintenance expenditures. Compared to other alternatives, thermal treatment minimizes unit deratings and outages and minimizes impact to the environment. Based on the task force's assessment and the successful treatment at State Line Station, thermal treatment was selected as the preferred, least-cost method to control zebra mussels at their fossil-fueled stations.

This paper discusses the steps Commonwealth Edison Company took to develop a control strategy and why thermal treatment was selected as the preferred approach for their stations. The paper also provides examples of the station modifications and the results of thermal treatments performed to date.

BACKGROUND

Commonwealth Edison Company provides electric service to the northern one-fifth of Illinois, a service territory covering 11,500 square miles. CECo has over three million customers and eight million residents in their territory. CECo owns and operates 10 fossil-fueled and 6 nuclear stations. These 16 stations have a combined net generating capacity of 22,522 megawatts. Three stations (two fossil and one nuclear) are located next to Lake Michigan and get their cooling water from the lake. The other stations are located inland from Lake Michigan and obtain cooling water from either (1) a river or canal or (2) a man-made cooling lake. Zebra mussels have been sighted at all but one of the stations. Figure 2 shows Commonwealth Edison service territory and their fossil stations in Illinois.

Zebra mussel colonies can affect the generating station operation by clogging the vital circulating water and service water systems. The mussels can attach to power plant equipment including water pipes, crib houses, tunnels, traveling screens, bar grills, pumps,

heat exchangers, and condenser tubes. The mussel colonies can reduce or cut flow to critical plant equipment causing unit deratings or unplanned outages. In addition, the operability of the fire water system can be threatened. To respond to this threat to the operation of their stations, CECo established a task force to evaluate control alternatives and to develop a system-wide zebra mussel control strategy.

DEVELOPING A TREATMENT STRATEGY

In 1991, CECo established a zebra mussel task force comprised of representatives from the generating stations, engineering and support services, and environmental services. The task force was charged with reviewing treatment options and with developing a recommended treatment plan for all stations.

The task force approached the problem in the following steps:

- First, the task force researched zebra mussels to understand their life cycle and options for controlling them. The task force reviewed available literature, information from other utilities, and reports from EPRI (Electric Power and Research Institute), and attended seminars and conducted tests at the stations.

- Next, each control option was evaluated, including an assessment of the effectiveness, impact on station operation, and impact on the environment. For each control method, budgetary estimates of capital and operating and maintenance costs were developed.

- Finally, after weighing these factors, a control strategy was selected. Priorities for implementing the modifications were established based on results of monitoring activities at the stations and the task force's determination of the stations most susceptible to the zebra mussel threat.

There are several treatment methods that have been tried to control zebra mussels. The task force evaluated three proven methods: mechanical cleaning, chemical treatment, and thermal treatment. A brief discussion of each alternative and CECo's assessment of each approach follows.

Mechanical Cleaning

Cleaning methods typically include manual labor and hand tools. High-pressure water jets and mechanical cutting devices have also been used to dislodge the tightly adhered mussels from large surface areas such as found in cribhouses and circulating water tunnels. Cleaning heat exchangers, coolers, strainers, and other equipment includes disassembly, manual cleaning, and reassembly of the equipment.

The advantage of mechanical cleaning is its low capital cost. The disadvantages are that (1) it is labor intensive and must be repeated periodically as the zebra mussel colonies are reestablished and (2) station outage time or unit deratings are required to clean cribhouses and the circulating water and service water systems.

Chemical Treatment

Chemical treatment involves use of oxidizing chemicals, primarily chlorine or bromine, or nonoxidizing chemicals and molluscides. Chlorination is a common method that has been used for water treatment for many years by industry and municipalities. Chlorination includes the injection of chlorine, usually in the form of hypochlorite, and then dechlorination before the water is returned to the waterway in order to meet EPA limits on residual chlorine. In addition, the EPA requires that the discharge be analyzed and monitored to demonstrate that the system is working properly and that the EPA limits are met. Researchers continue to examine the potential health effects of chlorine and other chemicals used for control of zebra mussels. CECo's experience with the EPA and other regulatory bodies has been to reduce the amount of chemicals used at its stations and to closely monitor the station discharge. EPA regulations place additional burdens on the utility staff to keep records and file reports on chemical usage.

Advantages of chemical treatment are that (1) modifications can be constructed without interruption of operations and (2) treatment can be performed without limiting operations. Disadvantages of this approach are (1) the cost (which includes the capital cost for initial installation, the daily cost of chemicals to chlorinate and dechlorinate, and other operating and maintenance expenses); (2) a greater potential environmental impact; and (3) the high probability of future, more stringent, limits on use of chemicals.

Thermal Treatment

Thermal treatment uses heated water to control the zebra mussels. Experience has shown that the mussels are killed when exposed to a water temperature of 95° F for approximately 4 hours. Thermal treatment for circulating and service water systems at electric generating stations is accomplished by recirculating the heated condenser discharge back to the circulating water intake. With each pass of the recirculated water through the condenser, additional heat is provided by the steam turning the turbine, thereby raising the temperature of the cooling water. The time needed to raise the temperature to 95° F depends on the starting temperature of the cooling water, the amount of thermal losses, and the volume of water to be heated. These factors vary with station arrangement and environmental conditions, such as lake temperature, at the time of treatment. After initial treatment, periodic thermal treatment is performed to control reestablishment of the

mussels. Other methods for supplying the heated water, such as electric heaters, can be used to treat remote systems such as makeup pumps for cooling lakes and fire pumps.

Advantages of this approach include (1) the minimal environmental impact, (2) the low operating and maintenance cost, and (3) the minimal impact on operations. Disadvantages are (1) the high capital cost and (2) that some outage time may be needed to construct the modifications.

Selection of Preferred Treatment Method

The task force eliminated mechanical cleaning from consideration because of the disadvantages mentioned earlier. For the remaining approaches, chemical and thermal treatment, the task force developed estimates of capital, operating, and maintenance costs for several stations. The estimates, summarized in Tables 1 and 2, showed that thermal treatment is the least-cost option. The task force concluded that thermal treatment (1) was an effective method to control zebra mussels, (2) has little impact on station operation, and (3) has minimal impact on the environment.

Based on their assessment and the successful treatment at the State Line Station, thermal treatment was selected by the task force as the preferred, least-cost control approach, to control zebra mussels at their fossil-fueled stations.

MODIFICATION DESIGN

CECo contracted Sargent & Lundy to assist in developing the conceptual design and then in preparing the detailed design for the modifications for thermal treatment at their fossil stations. The design process included the following steps:

- Assess station operation at the 95° F treatment temperature.
- Develop alternate arrangements considering the station configuration and operating parameters.
- Prepare construction cost estimates for the most promising alternatives.
- Select the arrangement for detailed design.
- Prepare detailed design documents for construction.

The operation assessment was an important aspect of the design to determine any operating restrictions required during the treatment period with the cooling water at 95° F. Critical parameters that were evaluated for each station included turbine back pressure,

maximum service water temperature for operation of equipment such as the turbine lube oil coolers, and permit limits on the discharge water temperature.

Thermal treatment involves recirculation of the large flows from the circulating and service water systems. To reduce the cost of the modifications, they were designed for reduced flow, one half of the maximum flow. This stipulation meant that treatment could be performed on weekends or at night when unit loads can be less than full load. Operating at lower than full load also helps keep the discharge temperature within permit limits.

The existing plant facilities were considered in developing the arrangement for the modifications. Where possible, existing and abandoned piping and structures were incorporated into the design to reduce the cost.

The modification details were designed to minimize the outage time required for installation. In addition, the construction schedule was coordinated with the existing outage schedule. This arrangement ensured that station availability was not affected.

CASE STUDIES

The following case studies illustrate the modifications being constructed at several CECo stations. As these examples show, the modifications vary significantly depending on the plant arrangement.

Waukegan Station

Waukegan Station has three operating units with total net generating capacity of 625 MW. It is located in northern Illinois near the Wisconsin border. Cooling water is provided from Lake Michigan via an open channel, and the discharge is returned to the lake in a parallel channel separated from the intake by a sheet piling divider wall.

Figure 3 illustrates the modifications, which included a new gate structure across the discharge channel with eight 10-foot-wide gates to block the flow. A new gate structure, with three 10-foot 6-inch-wide gates, was added on the divider wall; these gates and the three existing 8-foot-wide gates are used to recirculate the cooling water.

The modifications were designed based on the station operating at low load on a weekend. The gates were sized for circulating water flow of 323,000 gpm, one half of the maximum circulating water and service water flow.

The initial thermal treatment was performed on a Saturday in October 1993 when Lake Michigan temperature was 56° F. The divider wall gates were opened and the discharge

gates were closed. In less than 8 hours the water temperature was raised to the 95° F treatment temperature. Because of the long path that the recirculated water travels before reaching the pump structures CECo was concerned about thermal stratification. CECo feared that the lower areas of the structures and equipment might not see the heated water, reducing the effectiveness of the treatment. To monitor the temperature profile, station personnel took temperature readings across the height and width of the pump structures and verified that the desired treatment temperature was achieved.

During treatment station personnel closely monitored operation of critical plant equipment such as the turbine generator and lube oil coolers. One concern arose due to differential thermal growth between the turbine low-pressure casing and the turbine shaft which reduced critical clearance dimensions. To avoid this concern in the future, CECo plans on reducing unit load during future treatments to reduce the rate of temperature increase and the differential movements.

After treatment, the remains of the zebra mussels from the structures, equipment, and piping were found in the tubes of heat exchangers and the condensers. Ten cubic yards of mussels were removed. Since this was the initial treatment, the mussels had grown for several years to a size large enough to plug the 1/2-inch to 3/4-inch diameter tubes. Future treatments are planned at least twice a year to kill the mussels when they are small enough to pass through the plant equipment.

Joliet Station 9 & 29

There are two stations at Joliet: Station 29 on the north side of the Des Plaines River and Station 9 on the south side. Joliet is located 55 miles southwest of downtown Chicago. Both stations get cooling water from the Des Plaines River.

Joliet Station 29 has two operating units with a net generating capacity of 1025 MW. The recirculation system at Joliet was designed for maximum circulating and service water flow. The turbines at Joliet have restrictive limits on turbine back pressure when operating with 95° F cooling water, limiting operations at reduced load. This restriction means that treatment cannot be performed with reduced flows as at the other stations. Therefore, the recirculation system was designed for the full circulating water flow of 920,000 gpm.

Figure 4 illustrates the modifications being constructed at Joliet Station 29. A 70-foot long concrete tunnel with two 14-foot-wide by 10-foot 6-inch-high sections connects the discharge flume to the intake. Two gates are provided at the entrance to the recirculation tunnel and four 11-foot-wide gates are provided in the discharge flume to block the flow. These isolation gates will regulate the amount of water recirculated to the intake.

At Joliet Station 9, only Unit 6 is operating with a net generating capacity of 302 MW. Unit 5 is retired. The cost of the modifications at this station was minimized by incorporating the abandoned Unit 5 circulating water pipe and pumphouse into the recirculation flow path. The modifications were designed for 168,000 gpm, one half the maximum flow.

Figure 5 shows the modifications at Joliet Station 9. The 11-foot diameter Unit 6 circulating water discharge pipe was connected to the 7-foot diameter Unit 5 supply pipe to recirculate the discharge water to the Unit 5 crib house. A sheet piling wall and gate structure were provided in front of the Unit 5 & 6 cribhouses to isolate the forebay from the river and to complete the recirculation path.

A 12-foot square roller gate was added at the end of the discharge pipe to block the discharge and initiate the recirculation flow.

Will County Station

Will County Station has four operating units with a total net generating capacity of 1092 MW. It is located 40 miles southwest of Chicago. Cooling water comes from the Chicago Sanitary and Ship Canal. Each unit has a pumphouse that supplies water from the canal to the station. The discharge goes to a common tunnel serving all four units which returns the heated water to the canal, 1500 feet from the intake.

Because of the plant configuration, the supply and discharge could not be economically connected. Instead, the thermal treatment scheme established the recirculation circuit by interconnecting the circulating water systems for two units. This arrangement takes advantage of the existing piping configuration designed to allow back washing the condensers. Figure 6 illustrates the recirculation paths for the paired Units 1 & 2; the arrangement for Units 3 & 4 is similar. The Unit 1 & 2 scheme includes the following features:

- Connection of the circulating water pipes for the two units near the condenser with isolation and crosstie valves.
- Connection of the adjacent circulating water pumphouses by cutting openings in the divider walls. Gates were added to the new openings.
- Use of existing bulkhead gates at the pumphouse inlet to isolate the pumphouse from the canal.

These modifications provide a closed circuit for the cooling water. Thermal treatment will be performed with one unit operating at reduced load and the other unit not operating.

Cooling water will be supplied from one of the operating unit's circulating water pumps (75,000 gpm) pumping water through the condenser. The valve in the discharge pipe at the condenser outlet is throttled and most of the condenser discharge flows through the new crosstie pipe then through the condenser of the nonoperating unit. The water is then returned to the crib house through the circulating water supply pipe (flow opposite to normal operation), through the pumps of the nonoperating unit. Finally the water flows through the openings in the divider walls to the pumphouse with the operating CW pumps, completing the recirculation path.

The modifications for Units 3 & 4 are similar except that the units are larger and the recirculation flows are greater, making the modifications larger in size.

SUMMARY

These examples illustrate the modifications being constructed at the CECo stations to accomplish thermal treatment. The concept of recirculating the condenser cooling water is the same at all stations; however, the different plant arrangements and other station limitations called for unique and innovative designs.

Table 3 summarizes the status and total cost of the thermal treatment modifications at the CECo fossil stations. By spring 1994, CECo will be using thermal treatment at six stations; the thermal treatment system will be operational at two other stations by the end of 1994. At the remaining two stations, CECo is presently monitoring for zebra mussel infestation.

Commonwealth Edison Company has been proactive in responding to the zebra mussel threat to their operating stations. Thermal treatment, using waste heat from the station, (1) is an effective control method, (2) is the least-cost control option, and (3) has minimal effect on the environment. For Commonwealth Edison, thermal treatment has proved to be the most effective means to control zebra mussels at their fossil stations.

Table 1

Estimated Capital Costs
Zebra Mussel Treatment Options

Station	Chemical Treatment	Thermal Treatment
Crawford	$2,400,000	$1,800,000
Fisk	$900,000	$500,000
Waukegan	$2,600,000	$1,100,000
Will County	$3,400,000	$3,500,000

Note: Costs based on projections from estimate for State Line Station.

Table 2

Estimated Operating and Maintenance Costs
Zebra Mussel Control Options

Control Approach	Cost per Treatment per Station
Mechanical Cleaning	$500,000
Chemical Treatment	$100,000
Thermal Treatment	$6,000

Table 3

Capital Budget and Completion Dates
Zebra Mussel Control Modifications

Station	Capital Budget Note 1	Scheduled Completion Date
Collins	$2,000,000	October 1994
Crawford	$1,500,000	March 1994
Fisk	$400,000	November 1994
Joliet 9	$1,100,000	March 1994
Joliet 29	$2,000,000	March 1994
State Line	$0	Note 2
Waukegan	$1,100,000	January 1993
Will County	$3,500,000	May 1994

Note 1: Budget based on preliminary design for each station.

Note 2: Recirculation capability was included in original design of station.

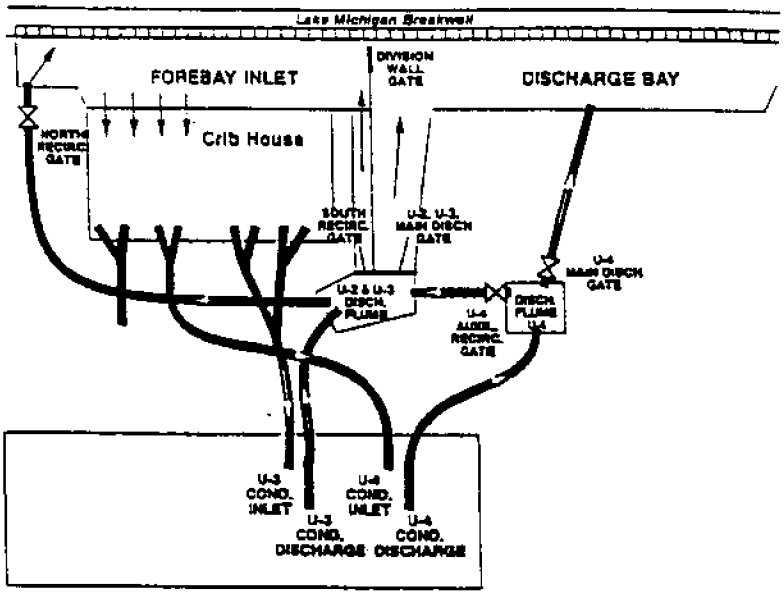

Figure 1—State Line Station Circulating Water Arrangement

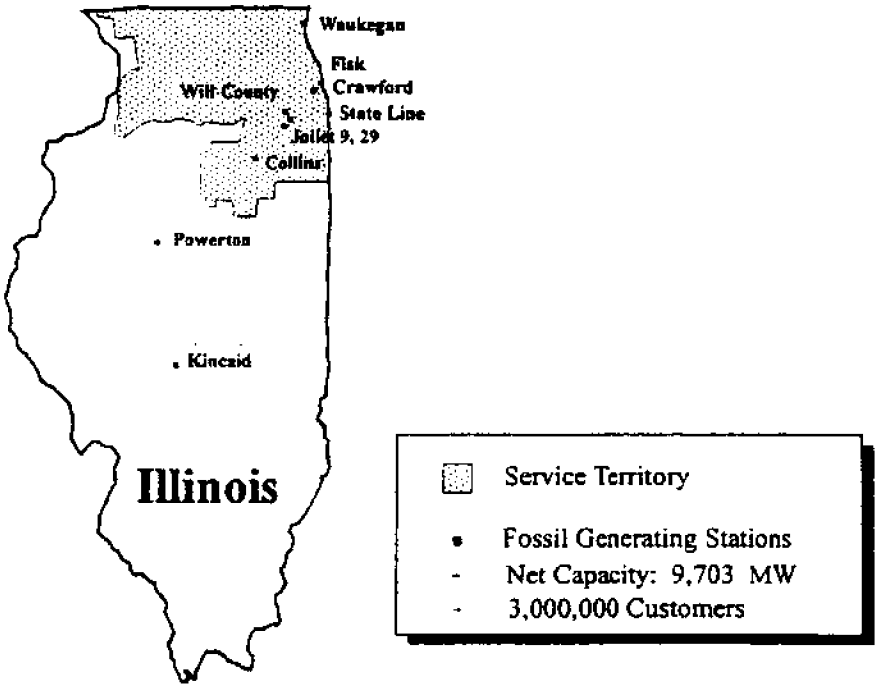

Figure 2—Commonwealth Edison Service Territory and Fossil Station Locations

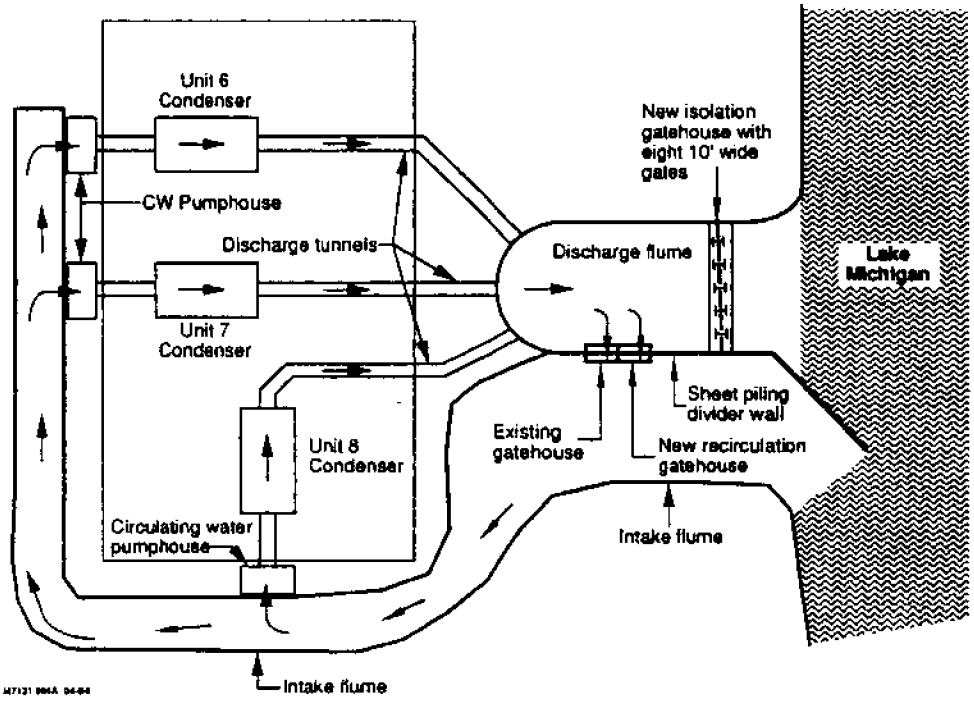

Figure 3—Waukegan Station—Modifications to Circulating Water System

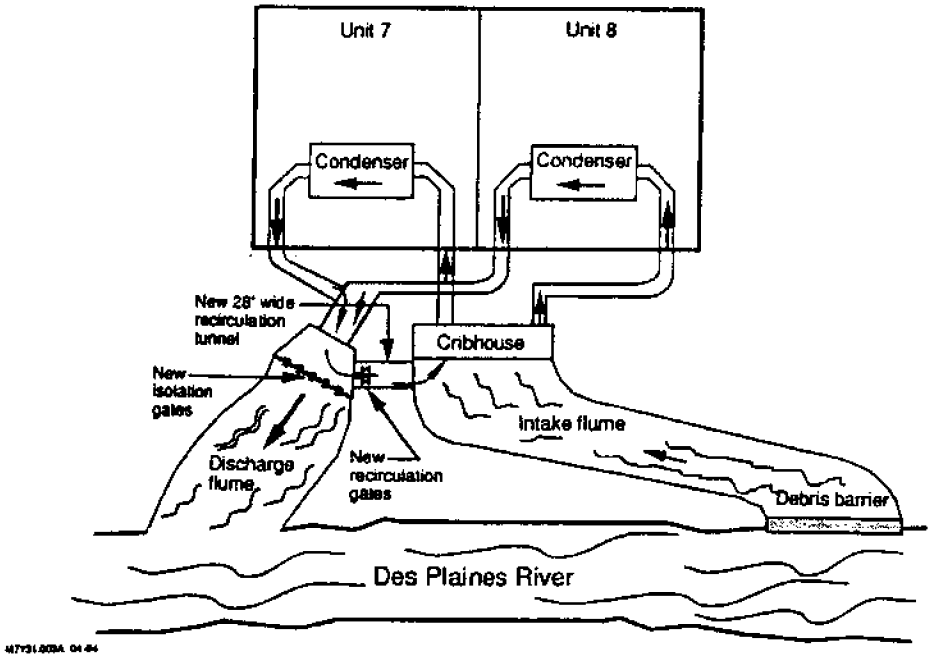

Figure 4—Joliet Station 29—Modifications to Circulating Water System

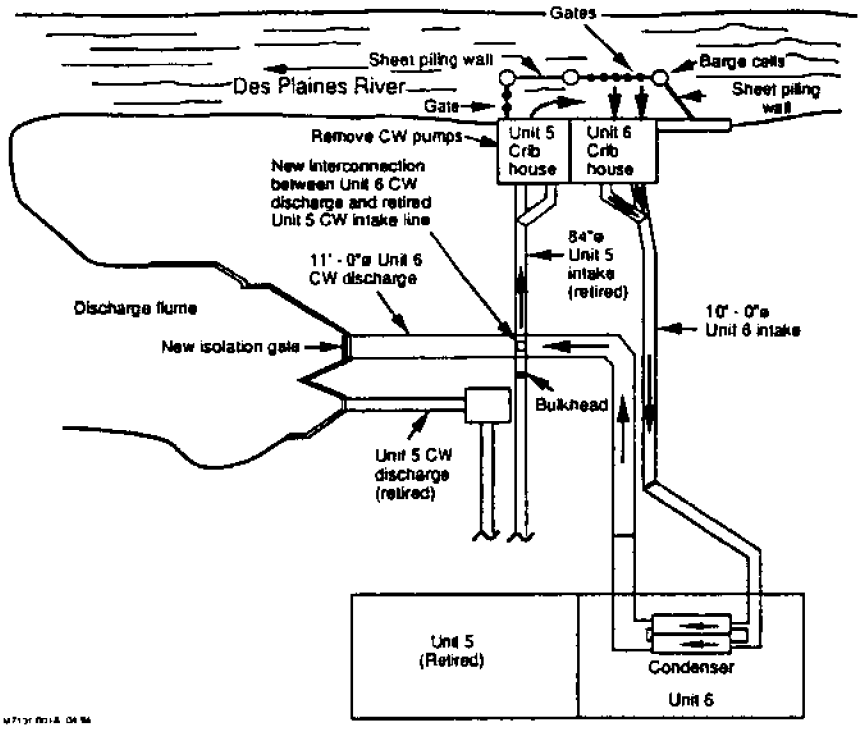

Figure 5—Joliet Station 9—Modifications to Circulating Water System

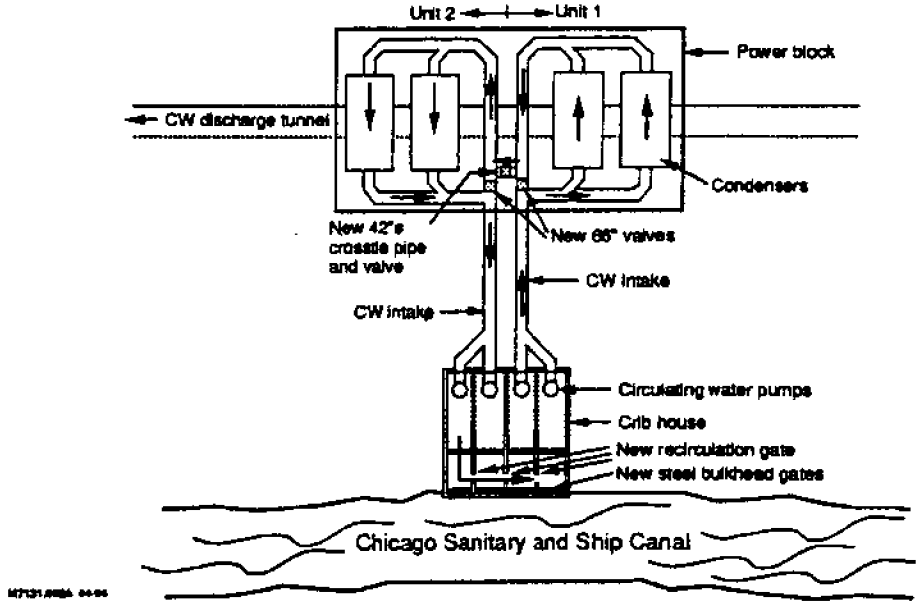

Figure 6—Will County Station Units 1 & 2— Modifications to Circulating Water System

4th International Zebra Mussel Conference '94 Proceedings

POPULATION DYNAMICS

Annual and Seasonal Variations in Veliger Density in the Upper Niagara River

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Abstract

This summary will review and compare 1993 veliger density data with those that were collected in 1991 and 1992. The objective of this analysis is to illustrate the vast differences in veliger density trends between these years. Four locations along the upper Niagara River were sampled weekly from June 1991 through December 1993.

Generally, veliger density fluctuated in magnitude and spawning duration throughout the three year period. For this analysis, spawning season is defined to be when veliger density exceeded 5,000/m^3. Spawning season occurred early in 1991 and was brief, starting in July and concluding in early September. The 1991 season was characterized by relatively high densities occurring over a short period of time.

The spawning seasons in 1992 and 1993 were much longer than 1991. In 1992, spawning was observed by mid-July and concluded in November. Two peaks of 20,000/m^3 were observed, one in August, the other in September 1992. Between December 1992 and March 1993, viable veligers were observed from the water column where temperature was less than 0.5°C. The spawning season of 1993 did not occur until September. The season was characterized by moderate to high densities with a single high peak density of 57,000/m^3. The 1993 spawning season was relatively late in the year compared to the 1992 and 1991 seasons.

This paper will incorporate the three year data set and will summarize inter-annual variations of veliger density in the upper Niagara River. Other environmental parameters such as water temperature and storm events, were monitored and will be included in the summary.

Introduction

Zebra mussel populations have become established and are a permanent component of the ecology of the Great Lakes benthic community. Since 1991, AquaTech *Environmental*, Inc. has been monitoring veliger density and documenting seasonality of veligers in Lake Erie and Niagara River. These are minor goals of a greater monitoring program that includes, but is not limited to, documentation of settling rate, growth rate and assessment of treatment effectiveness. Changes in veliger density and seasonality are basic to our understanding of zebra mussels. Analyzing veliger density and seasonality, both functions of adult mussel density, can supply insight into the degree of zebra mussel infestation. This study is a compilation of data that summarizes veliger seasonality, tracks variation and documents differences in veliger density trends from 1991 to 1994. Information generated from this work may help researchers comprehend invasion processes in similar habitats.

Materials and Methods

A total of eleven sites along the upper Niagara River were sampled weekly from June 1991 to February 1994 (Figure 1). Sampling was interrupted during the winters of 1992 and 1993 due to ice cover limitations. Five sample locations were used to summarize data for the purpose of this report. These locations allowed samples to be drawn from the open water column, in contrast to taken from within an industrial facility.

Zebra mussel veligers were collected by filtering 500-L of water through a 64-µm mesh plankton net using a 0.5 hp centrifugal pump. Flow rate was calculated before each collection by recording the length of time to fill a 20-L bucket. It was possible to determine the length of time to sample 500-L by extrapolation. Water temperatures were recorded using a Digi-Sense thermocouple thermometer during the collection of water samples. After filtration, samples were concentrated, placed in Nalgene jars and transported to the laboratory for analysis. All samples were analyzed within an hour of collection.

Water samples were analyzed to determine veliger densities. Veliger densities were determined by counting the entire sample if densities were relatively low ($<100/m^3$), or by subsampling if densities were particularly high ($>100/m^3$). A plankton splitter was used to subsample using standard zooplankton laboratory techniques. Samples were analyzed using a Wild M5 stereoscope at 25X, 50X, and 100 X magnification. Crossed polarization microscopy was used when appropriate to aid in the enumeration process.

Results

Generally, veliger density fluctuated in magnitude and spawning duration throughout the three year period (Figures 2 - 6). For this analysis, spawning season is defined as the dates when veliger density exceeded approximately 5,000/m^3. Spawning season occurred early in 1991 and was brief, starting in late July and concluding in early September. Veligers remained in low densities throughout October and were absent by mid-October, 1991. The 1991 season was characterized by relatively high densities occurring over a short period of time.

The spawning seasons in 1992 and 1993 were much longer than 1991 (Figure 7 and Table 1). In 1992, spawning was observed by mid-July and concluded in early November. Veligers remained in densities of less than 3,000/m^3 through December 1992. Two peaks of 20,000/m^3 were observed, one in August, the other in September 1992. Between December 1992 and March 1993, viable veligers were observed in the water column in extremely low densities. The lowest temperature that veligers were observed was 0.5°C, occurring in March 1993. Initiation of the 1993 spawning season did not occur until early September. The season was characterized by moderate to high densities with a single high peak of 57,000/m^3 on 10/7/93. The 1993 spawning season was relatively late in the year compared to the 1992 and 1991 seasons. Densities decreased to less than 5,000/m^3 by late November, 1993 and continued to decline to densities of less than 200/m^3 by February 1994. Viable veligers, in extremely low densities, were observed in January 1994 when water temperature was 0.2°C.

1993 peak veliger density was higher than the 1992 peak density of 24,192/m^3, but less than the 185,000/m^3 found in 1991. More veligers were present in the water column during the 1993 and 1992 seasons because spawning occurred over a longer period of time than in the 1991 season.

Discussion

Veligers first appeared at densities greater than 100/m^3 by the end of June, 1993. The first influx of veligers occurred when Niagara River temperatures reached and remained at approximately 19°C. This temperature was higher than previous years (16 - 18 °C for 4 - 5 weeks in 1992 and 22 °C for several weeks in 1991), indicating that more than water temperature alone is responsible for triggering zebra mussel spawning. Other studies (Walz 1978, Garton and Haag 1992) have found that additional factors are necessary to initiate *Dreissena* spawning. Increased water temperature, phytoplankton abundance, and water currents are important for dreissenid spawning to occur (Galperina,

1978 and Sprung 1989). Also, the presence of sperm from a few individuals encourages synchronous mass spawning within local populations (Haag and Garton, 1992). Niagara River water temperatures were consistently lower in 1992 than in 1991 until early September. From September on, water temperatures were almost identical to those monitored in 1991 and 1993 (Figure 8). Veligers appeared in samples approximately the same in 1991 and 1992 (7/9 - 7/16), however, in 1992, initial densities were higher.

Veliger density tended to be higher at sample sites close to Lake Erie than at sites further downstream in the Niagara River. This is probable due to the close proximity to Lake Erie where the largest local mussel population exists.

Wind speed and direction were recorded on each sampling date during the 1992 season. Recent research has suggested that high winds and wave action may serve to suspend veligers which were recently settled on the bottom. This phenomenon leads to slightly higher veliger densities in the Niagara River following storm events. Data collected during 1992 suggests a trend following periods of high winds. During these periods, an increase in veliger density was occasionally observed. Other studies indicate that some veligers remain alive in cold water and may become resuspended (Apstein 1896 - cited in Walz, 1978). Veligers observed in January - March 1993, and January - February 1994 were undoubtedly resuspended.

Year	Spawning Season	Duration	Magnitude
1991	Early	Short	High
1992	Early	Long	Low
1993	Late	medium	High

Table 1. Relative differences regarding veliger spawning seasons from 1991 to 1993.

Literature Cited

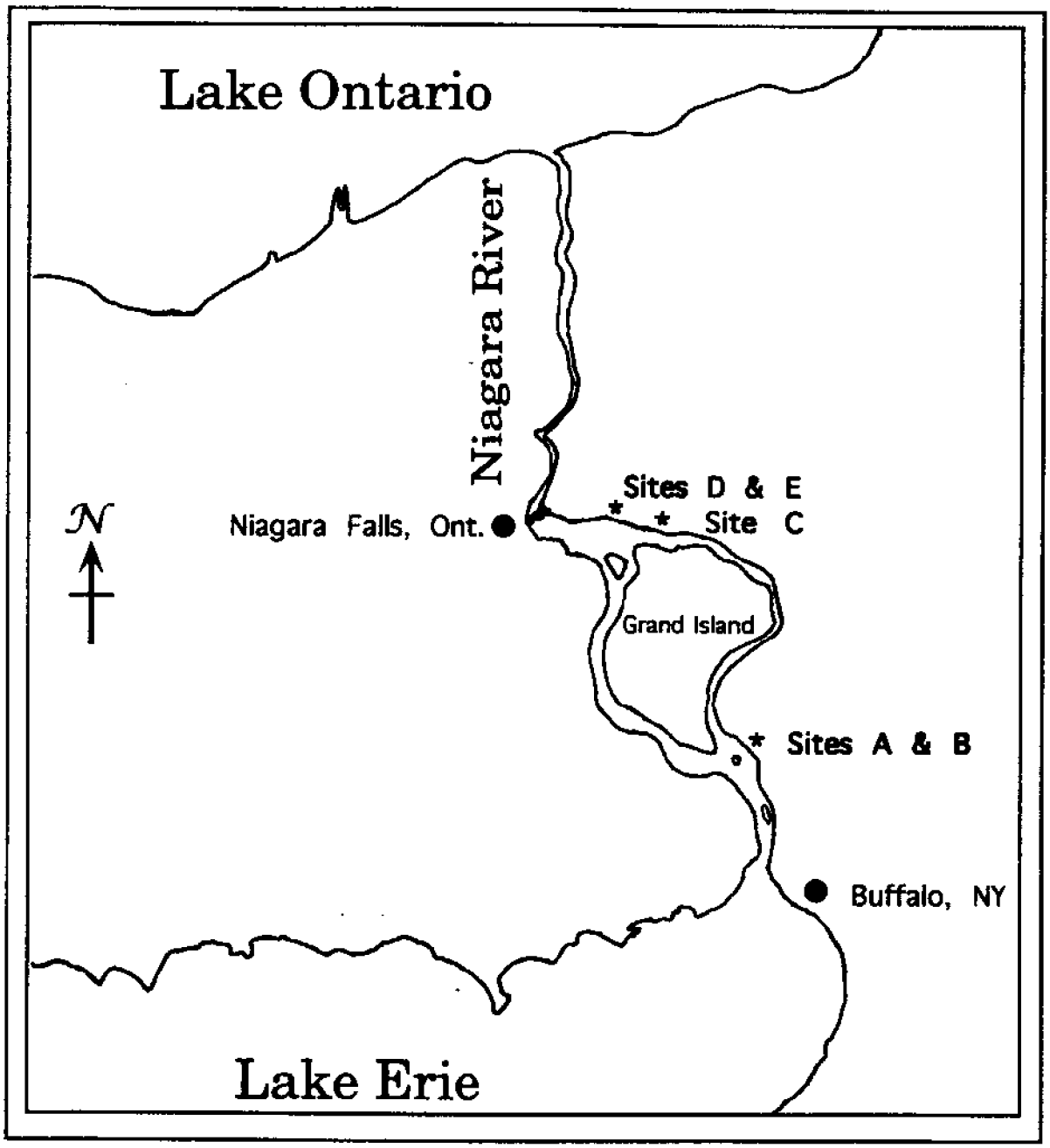
Galperina, G. 1978. The Relationship of Spawning of Some North Caspian Bivalves to the Distribution of Their Larvae in the Plankton. Malacol. Rev. 11:108-109.

Garton, D. and W. Haag. 1992. Seasonal Reproductive Cycles and Settlement Patterns of *Dreissena polymorpha* in Western Lake Erie. Zebra Mussels: Biology, Impacts and Control. Lewis Publishers.

Haag, W. and D. Garton. 1992. Synchronous Spawning in a Recently Established Population of the Zebra Mussel, *Dreissena polymorpha*, in Western Lake Erie, USA. Hydrobiol 234:103-110.

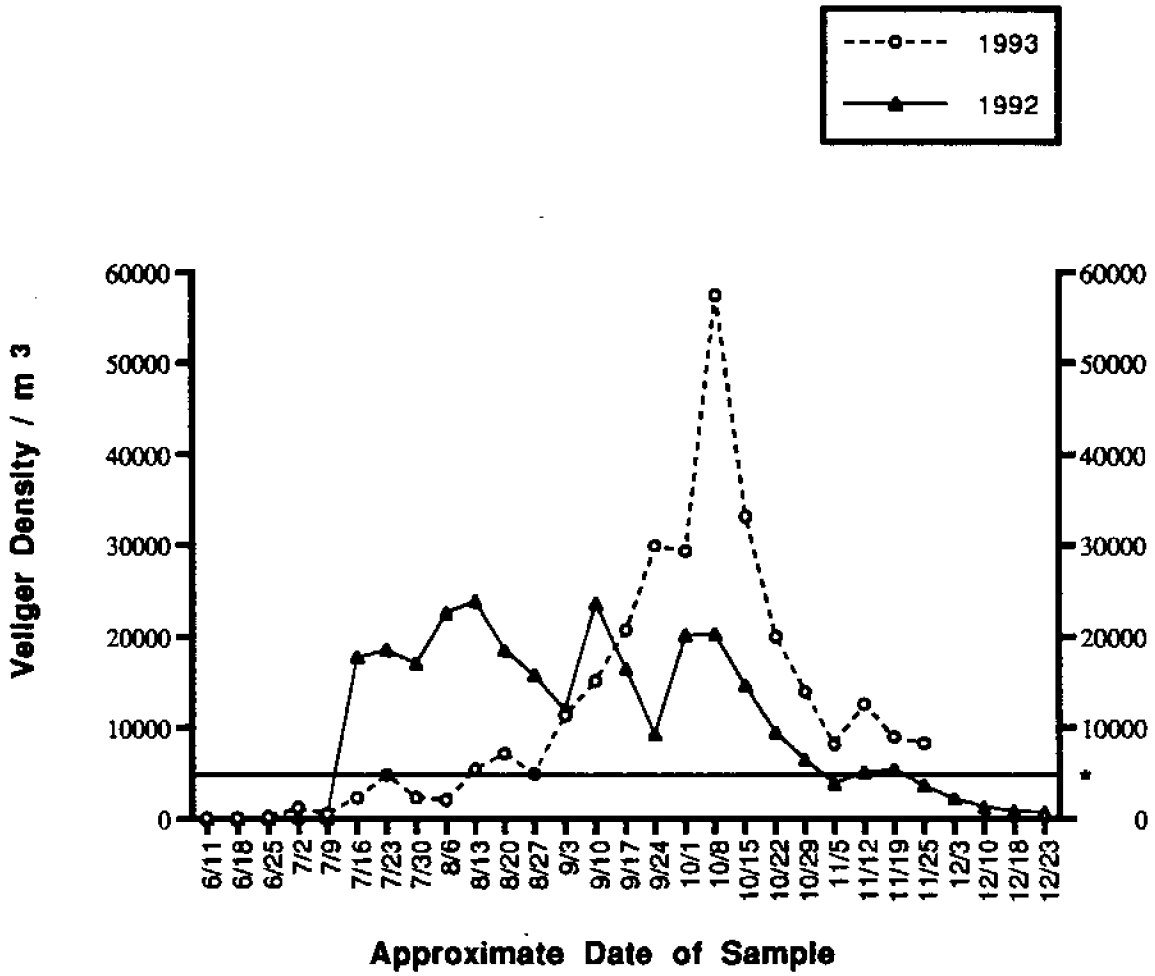
Sprung, M. 1989. Field and Laboratory Observations of *Dreissena polymorpha* Larvae: Abundance, Growth, Mortality and Food Demands. Arch. Hydrobiol. 115:537-561.

Walz, N. 1978. The Energy Balance of the Freshwater Mussel, *Dreissena polymorpha*, in Laboratory Experimetns and Lake Constance. II Reproduction. Arch Hydrobiol., Suppl. 55:106-119.



Location of sample sites along the Niagara River.

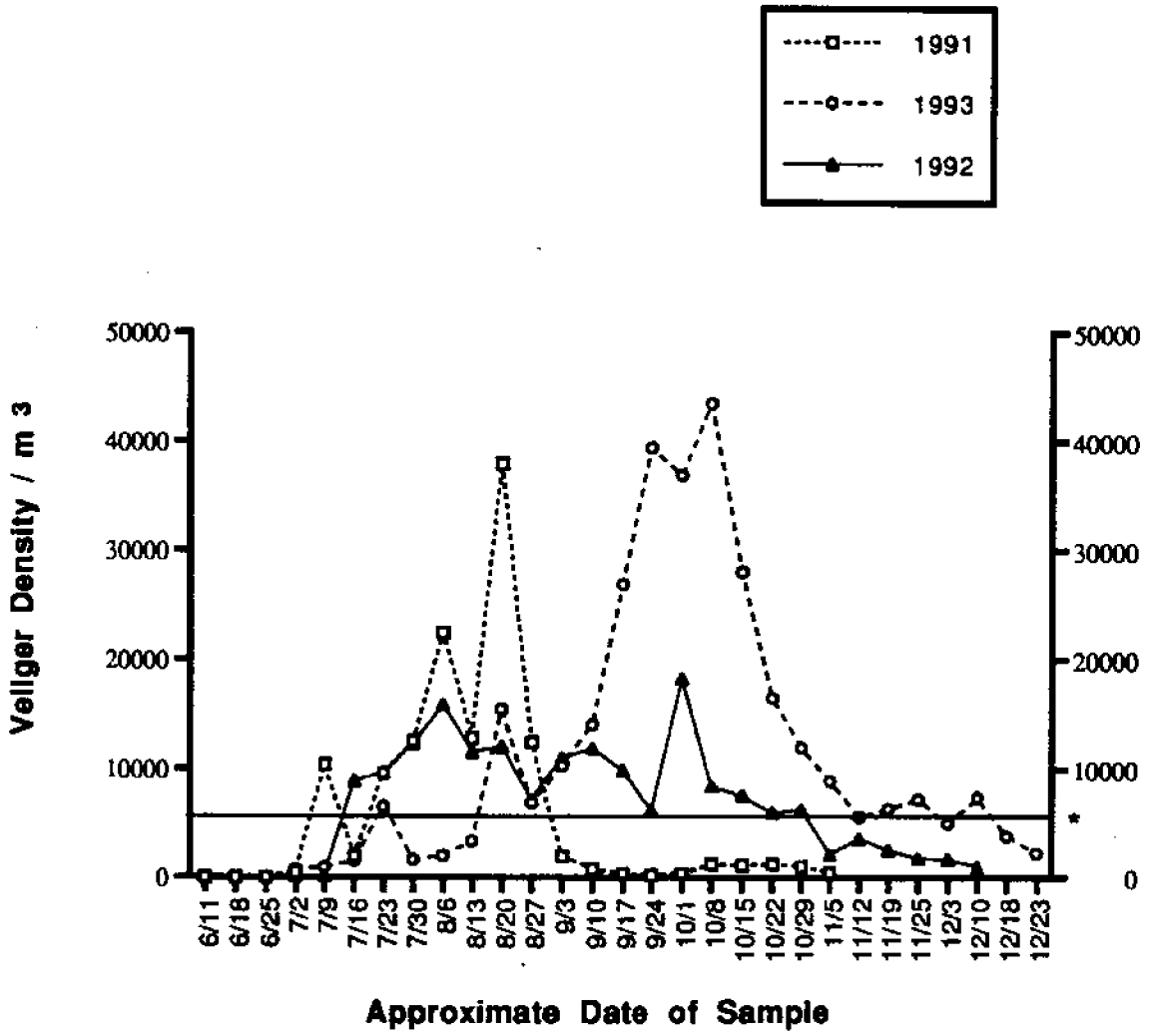
Figure 1



* Represents 5,000 veligers/m^3, the spawning season

Yearly Comparison of Veliger Densities at Site A

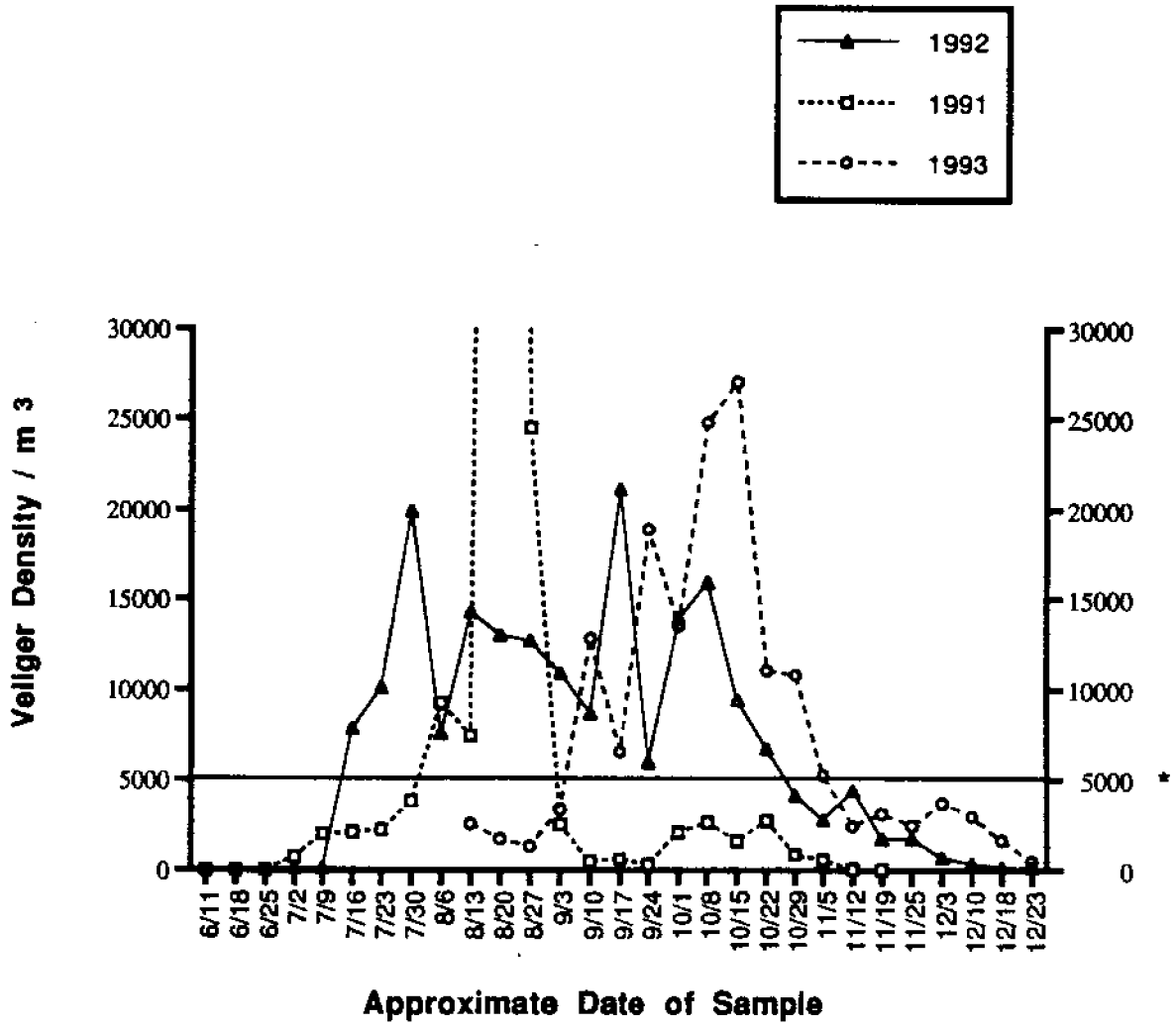
Figure 2



* Represents 5,000 veligers/m^3, the spawning season

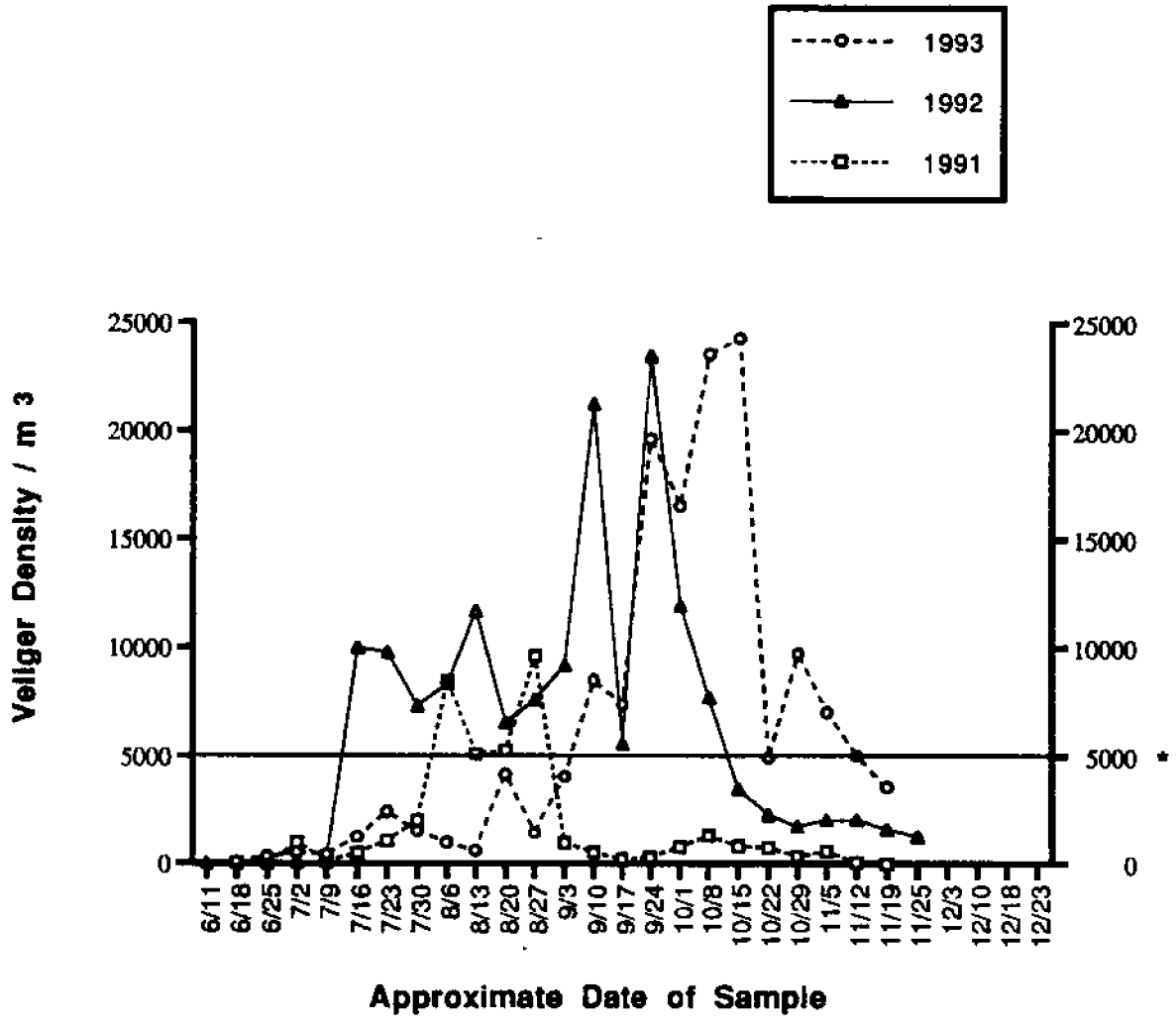
Yearly Comparison of Veliger Density at Site B

Figure 3



Yearly Comparison of Veliger Density at Site C

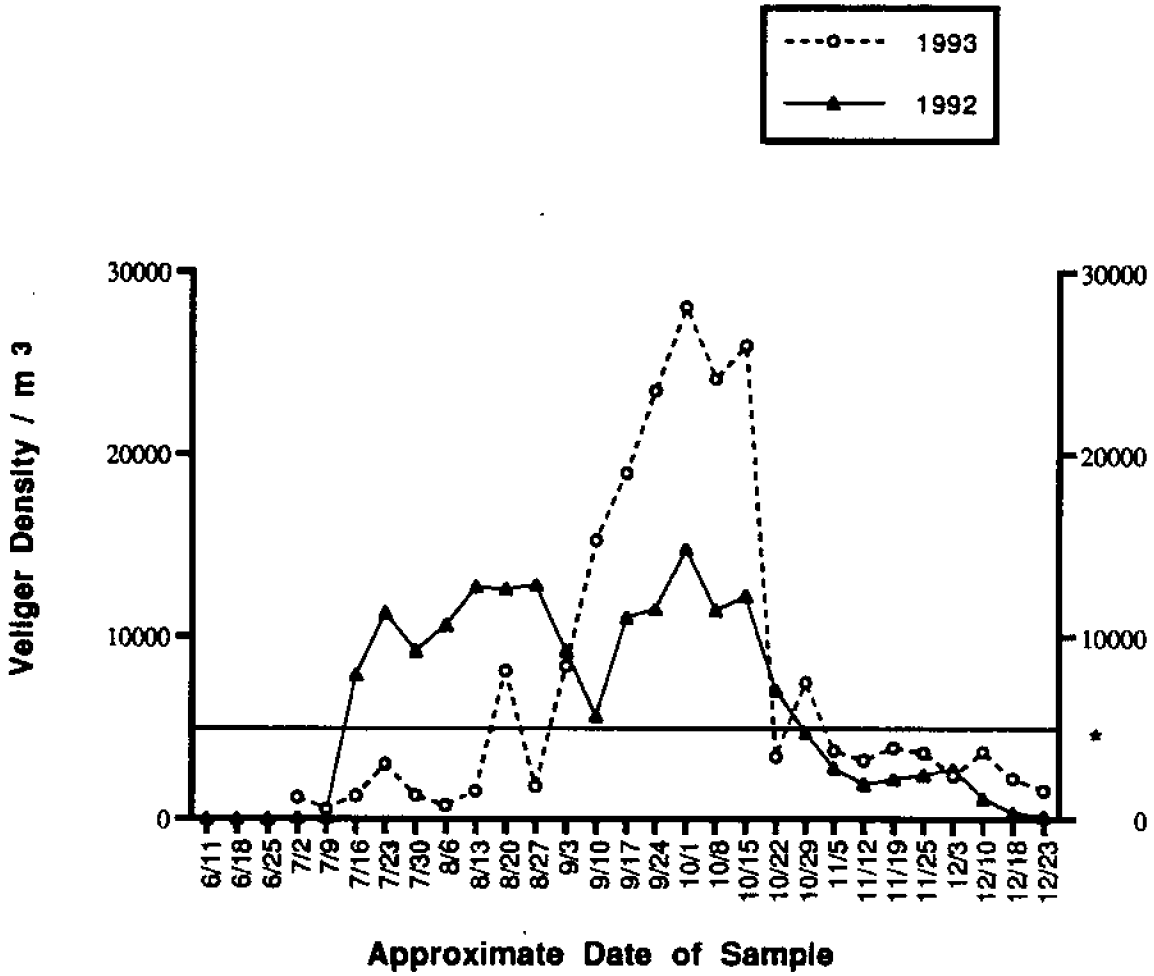
Figure 4



* Represents 5,000 veligers/m^3, the spawning season

Yearly Comparison of Veliger Density at Site D

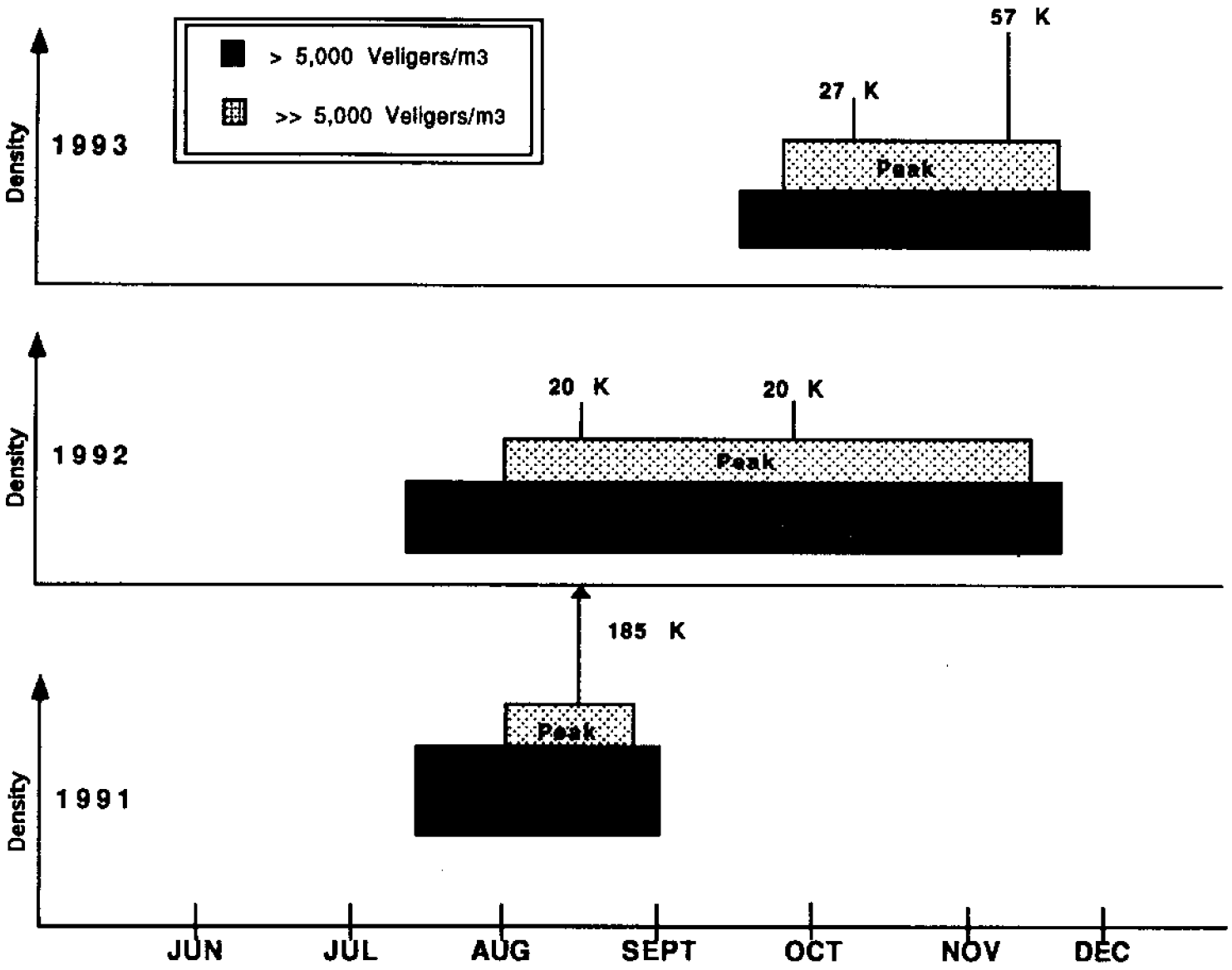
Figure 5



* Represents 5,000 veligers/m^3, the spawning season

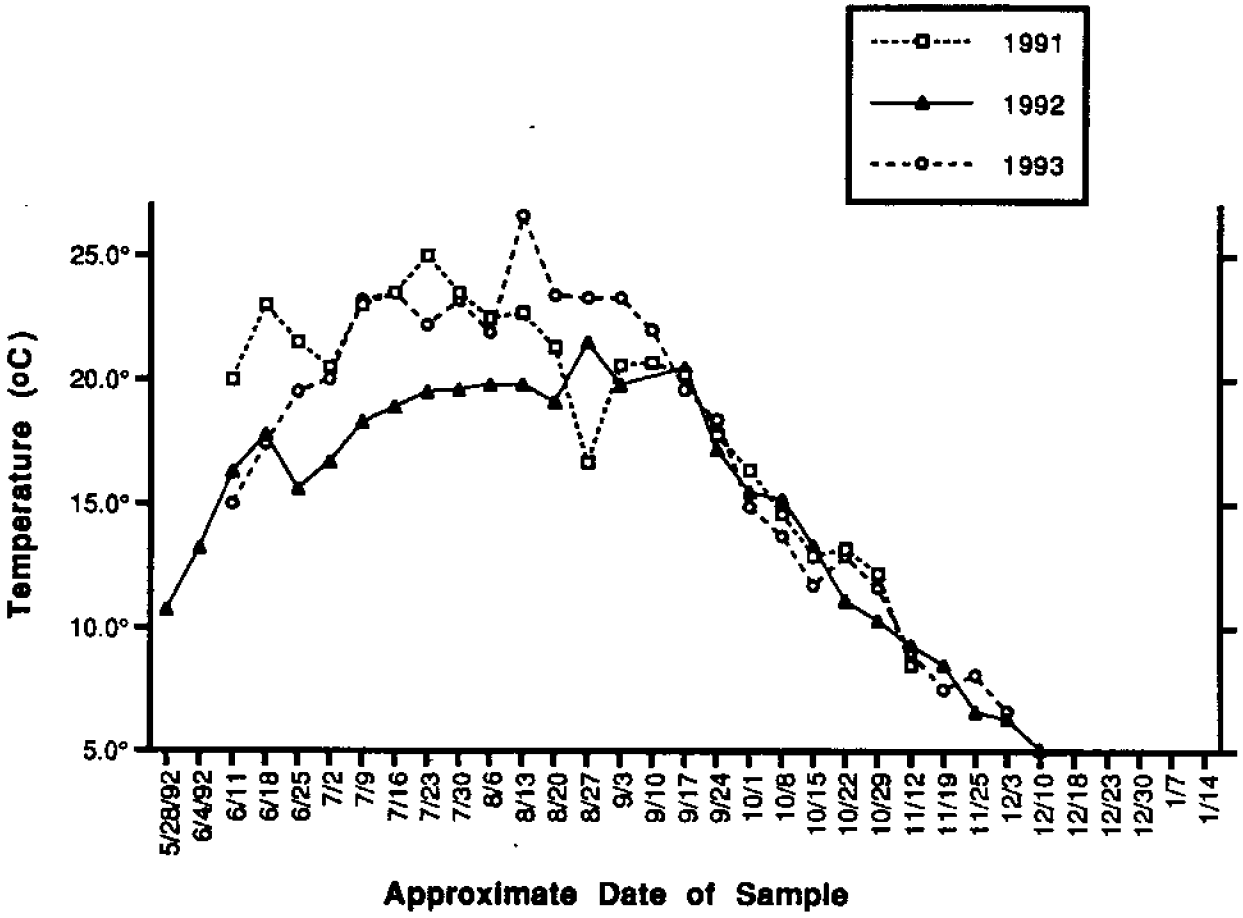
Yearly Comparison of Veliger Density at Site E

Figure 6



Relative differences regarding veliger spawning seasons from 1991 to 1993.

Figure 7



Yearly Comparison of Water Temperatures at Site A

Figure 8

Relative Abundance of Zebra Mussels (*Dreissena polymorpha*) and Quagga Mussels (*Dreissena bugensis*) in Eastern Lake Erie

by

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The discovery of a second dreissenid in the Great Lakes has created a great deal of interest in comparative research between the two species. Recent studies in the Great Lakes suggest that the quagga mussel may become more abundant than the zebra mussel in certain habitats. This phenomenon appears to occur more frequently in deep water locations.

A preliminary field study was performed in the eastern basin of Lake Erie near Buffalo, New York, on Seneca Shoals, to compare relative abundance of zebra and quagga mussels. Seneca Shoals is a shallow reef site, approximately 5.5 meters deep, characterized by rocky substrate. Collections were made using a benthic sled towed along a transect. Mussels were differentiated and quantified to determine the relative abundance of the two dreissenids.

This study suggests that quagga mussels have become a prominent member of the benthic community and, in fact, are more abundant than *D. polymorpha*. At one time this shallow area was dominated by *D. polymorpha*, however, over the last several seasons quagga mussels have become more abundant. Preliminary data suggest that 61% of the dreissenid mussel population is composed of quagga mussels. The remaining 39% of the dreissenid population is composed of zebra mussels. Complete summarization of the study, including size frequency of the two dreissenids, will be reported.

Introduction

The existence of a second species of *Dreissena* in the Great Lakes has been documented by a number researchers (Ludyanskiy 1993, May and Marsden 1992). This species, commonly referred to as the quagga mussel (*Dreissena bugensis*), arrived in the Great Lakes in 1989, approximately the same time as the zebra mussel (*Dreissena polymorpha*). The distribution of *D. bugensis* has differed significantly from *D. polymorpha* with respect to geography, water depth, and substrate composition (May and Marsden 1992).

Relative abundance of *D. polymorpha* and *D. bugensis* has also been quite variable (May and Marsden 1992). In Lake Erie, the trend appears to point toward *D. bugensis* overtaking *D. polymorpha* with respect to population density in certain habitats. Evidence of *D. bugensis* representing 100 percent of the *Dreissena* population in water depths of >20m suggests a possible habitat preference which differs from that of *D. polymorpha*. To date there has been some question as to whether *D. bugensis* would overtake *D. polymorpha* in relative abundance. To address this issue, **AquaTech** *Environmental*, Inc. collected and enumerated mussels from a shallow reef (Seneca Shoals) in the eastern basin Lake Erie.

Methods

To compare relative abundance of *D. polymorpha* and *D. bugensis*, adult mussels were collected from a shallow reef (Seneca Shoals) located in the eastern basin of Lake Erie near Buffalo, New York (Figure 1). The reef is characterized by shale and pitted limestone cobble substrate. It is bordered by a relatively steep drop-off on the east, north, and south sides with a gradual drop-off to the west. Surrounding water depths average 12m-13m where as the shallowest area is approximately 3 meters.

Collections were made on October 26, 1993 in 5.5 meters depth using a benthic sled towed on an East-West transect. Mussels were then retrieved and preserved in 90% ethanol. The samples were enumerated by species and subsamples were randomly selected for size frequency distribution analysis. Each mussel was measured to the nearest tenth of a millimeter using calipers. A size distribution histogram was generated using 5.0mm as the size class unit.

Results

The total number of mussels collected on Seneca Shoals was 3,508. The relative abundance of *D. polymorpha* was 1,360 and *D. bugensis* 2,148. Therefore, *D. polymorpha* represented 39 percent of the population sampled while D. *bugensis* represented 61 percent (Figure 2). Mussels ranged in size from 2.5mm to 25.8mm. *Dreissena bugensis* were more abundant than *Dreissena polymorpha* in the small to medium size classes (Figure 3). Relative abundance was nearly equal in the larger size

classes and variable in the very small size classes.

Discussion

The results of this preliminary study indicate that *D. bugensis* has become more abundant than *D. polymorpha* on this relatively shallow reef. Previous work revealed that quagga mussels only represented from one to fifty percent of the mussels found in shallow waters (<15m) in 1992. At that time, predominance of *D. bugensis* was observed only at 20m or greater.

The predominance of *Dreissena bugensis* in the medium size classes indicates a strong recruitment of new quagga mussels during the 1993 spawning season. This is in contrast to the larger size classes where there was little difference in relative abundance between the two species. Data from this study suggests a possible trend in the population dynamics of the two *Dreissenid* species. It appears as though *D. bugensis* may begin to dominate more than just the deep waters of its range. At this time, it would is not clear exactly how competition for available food resources and substrate will affect the relative abundance of either species. However, future collections and enumerations of adult mussels should include relative abundance analyses of each species. Such efforts will aid in the documentation of the population dynamics of both species.

Literature Cited

Ludyanskiy, M.L. 1993. Recent Introductions of *Dreissena* and other Forms into North America - the Caspian Sea/Black Sea Connection. In Zebra Mussels:biology, impacts, and control. CRC Press 699-704.

May, B. and J.E. Marsden. 1992. Genetic identification and implications of a second invasion species of dreissenid mussel in the Great Lakes. Can. J. Fish. Aquat. Sci. 49:1501-1506.

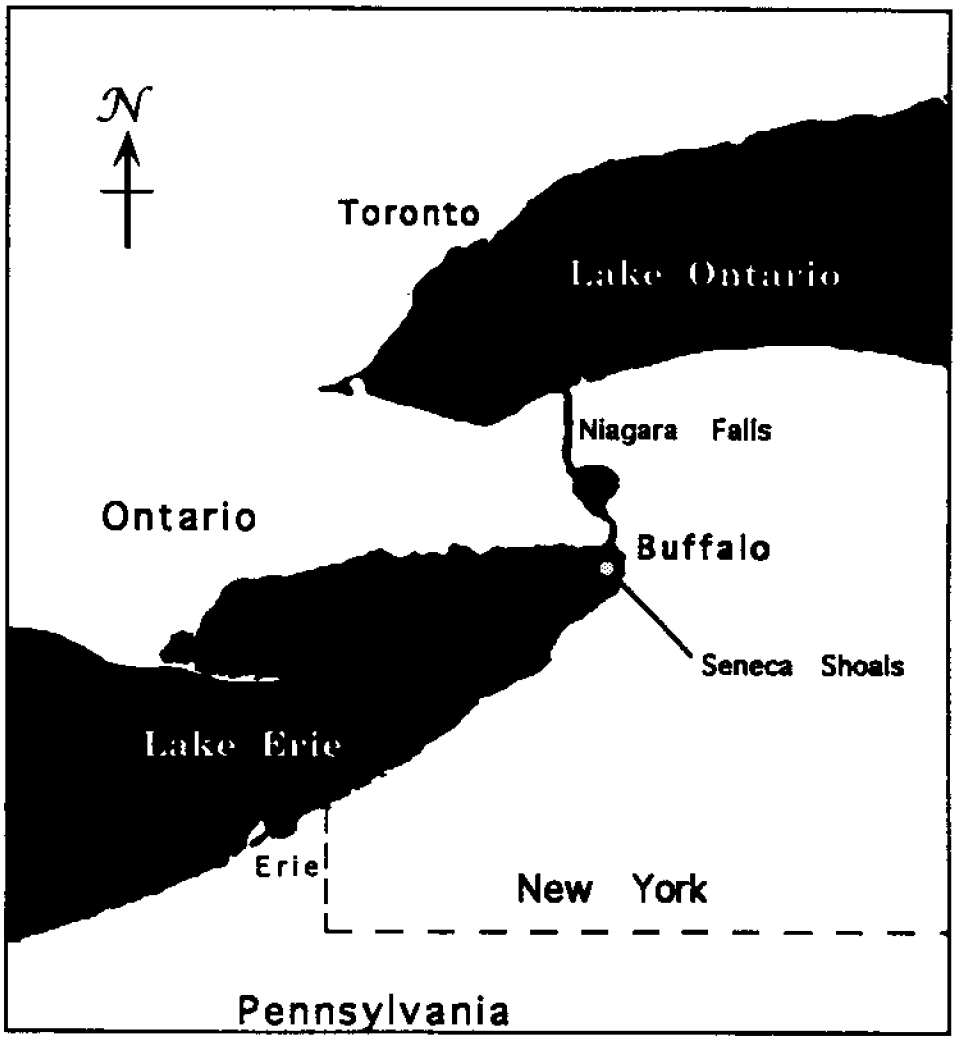

Figure 1. Collection site of zebra mussels used to determine relative abundance.

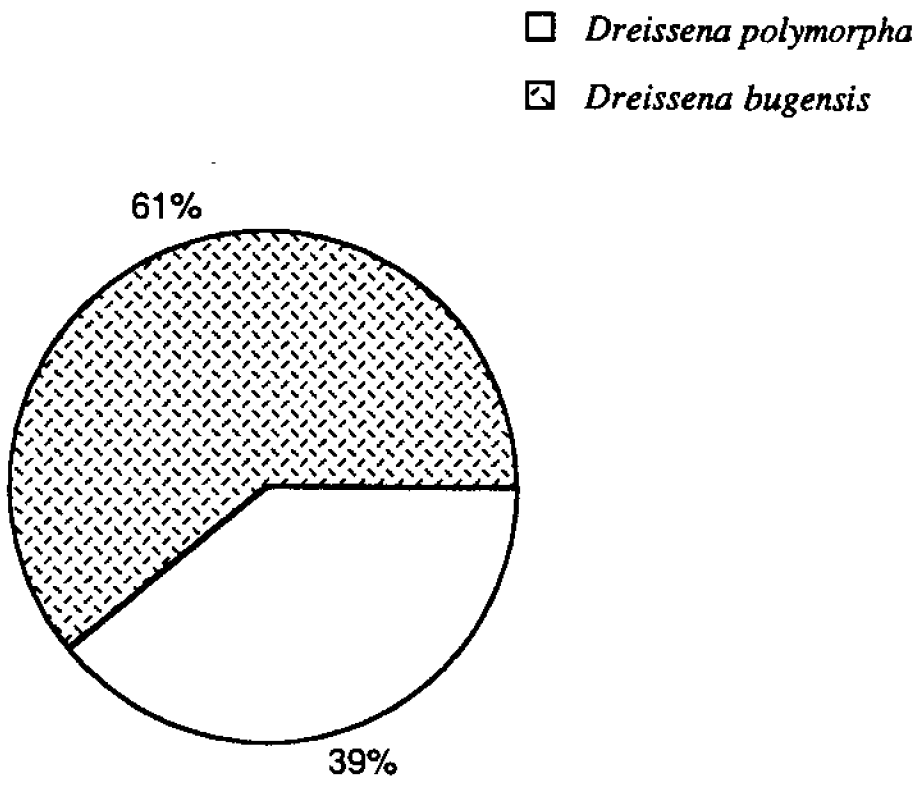

Figure 2. Relative abundance of Dreissena polymorpha vs Dreissena bugensis collected at Seneca Shoals (Lake Erie).

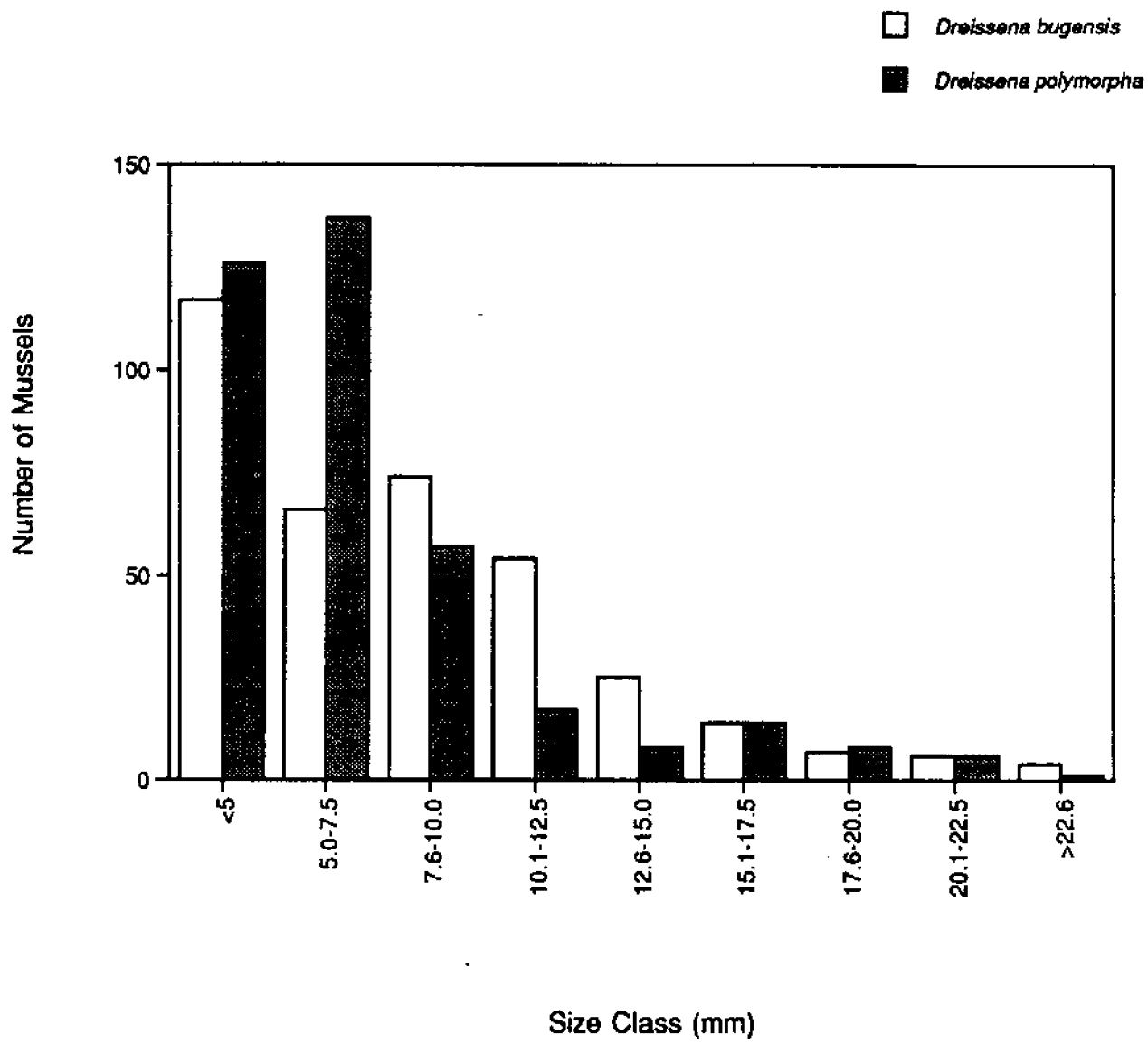

Figure 3. Size frequency distribution of mussels collected at Seneca Shoals.

Dreissena Settlement On Natural And Anthropogenic Substrates In The Bay of Green Bay (Lake Michigan)

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Abstract

Dreissena settlement on natural and anthropogenic substrates in the southern bay of Green Bay (Lake Michigan) was studied in 1993. Minimum depth from surface for colonization on emergent vegetation was 40 cm. in sun and 25 cm. in shade. Minimum depth for colonization on dolomitic limestone was 50 cm.; heavy colonization was evident at less than 1.5 meters. Lead, Teflon, and copper plates had, after 3 months immersion, maximum densities of 82,000/sq.m., 70,000/sq.m., and 51,000/sq.m. respectively.

Analysis of settlement patterns from a sample in early stages of colonization showed 17 of 17 clusters possessed 1 or 2 individuals from the largest-sized cohort, surrounded by mussels from smaller cohorts. Nearest-neighbor analysis between clusters and isolated individuals showed a random pattern of settlement (R=1.31); within clusters, zero distance was measured between neighbors (R=0). This settlement pattern suggests two types of veligers: free-settling and communal-settling.

A. Introduction

During the months of July through October, 1993, an irruption of Dreissena polymorpha (hereafter referred to simply as Dreissena) occurred in the waters of Green Bay (Lake Michigan), with as many as 400,000 mussels settling per square meter (Hieb, pers. comm.). The co-authors conducted a study of Dreissena settlement patterns on both anthropogenic and natural substrates to determine which substances, if any, might preclude settlement, and the degree to which native shallow-water communities were vulnerable to mussel invasion. A total of 17 materials, including lead, Teflon, copper, concrete, glass, fiberglass, ABS plastic, tin, styrofoam, zinc, Parkercoat 2000, latex paint, latex paint with added cayenne pepper, and four commercial copper- and/or tin-based anti-biofouling paints, were tested for Dreissena colonization. Near-shore shallows in natural areas were sampled to determine the current status of mussel colonization on biotic and abiotic substrates. The chance discovery of a random artifact in the early stages of colonization allowed the application of statistical analysis to Dreissena settling behavior.

B. Methodology

Seventeen anthropogenic substances were selected and acquired for testing as substrates for Dreissena settlement. Plates were fabricated either directly from the substances (e.g., Teflon, concrete, etc.) or from steel, onto which the substances were coated (e.g., lead, copper, zinc, paints, etc.); each individual plate was 0.9 sq. dm. in size. Duplicate racks approximately two meters in height, one consisting of 16, the other of 17, plates placed horizontally on a vertical axis at 10 cm. intervals, were constructed, and installed in late July. The 17-plate rack was submerged at a depth of approximately three-to-four meters at a U.S. Coast Guard lighthouse facility in the main channel leading into the port of Green Bay, and was recovered in late October. The 16-plate rack was installed at a depth of two-to-three meters at the Wisconsin Public Service Pulliam coal-fired, electricity-generating plant adjacent to the mouth of the Fox River in the city of Green Bay, and removed in mid-October.

Natural shallow-water substrates were surveyed in mid-August. Lacustrine Sagittaria, Typha, Sparganium, and Nymphaea were the emergent plant genera sampled. Minimum depth to initial colonization in both sun and shade was measured. Naturally-occurring dolomitic limestone formations at Bay Shore Co. Park, Brown Co., WI, were likewise studied for minimum depth to initial colonization.

Nearest-neighbor analysis was applied to Dreissena colonies on a weathered fragment of concrete (a random artifact discovered in a natural habitat). Distances between colonies and solitary individuals were mapped and recorded for each face. All colonies were removed from the surfaces; each individual mussel was measured to the nearest mm. to determine size cohorts.

C. Results

Observed depths of initial colonization on Sagittaria began at 40 cm. below the surface in 55 cm. of water (in full sun). Sagittaria from shaded environments in the center of the plant colonies exhibited colonization at 25-27 cm. below the water's surface. These particular specimens were less populated by mussels than those in full sun; however, colonization began at a lesser depth. Colonization of Typha (in the shaded center-bed habitat) began at 24-25 cm. below the surface. Sparganium yielded similar results to those of Typha. Settlement on Nymphea leaf petioles began at a slightly greater depth than that of Sagittaria, ranging from 41 to 45 cm. below the water surface.

The first occurrence of mussel settlement on naturally-occurring dolomitic limestone was recorded at a depth of less than one meter. Small colonies of Dreissena were found 50 cm. beneath

the surface. Heavy colonization began to appear in less than 1.5 m. of water. Substantially lower quantities of Dreissena were located on the top, as opposed to the sides, of the limestone. There appeared to be no significant difference between colonization of shore- and wave-sides of the stones. The mussels revealed a marked preference for filamentous-algae-covered-limestone rather than for bare rock.

The central focus of study for anthropogenic substrates was based upon three materials: lead, Teflon, and copper, which were not damaged or abraded, and so had significant colonization. After three months immersion, the plates at the U.S. Coast Guard lighthouse site yielded maximum densities of 82,000/sq. meter, 70,000/sq. meter, and 51,000/sq. meter, respectively, with a full range of size cohorts present on each plate (Figures 1-2). The plates at the Pulliam facility showed insignificant colonization, probably due to the polluted nature of the environment, as indicated by the large numbers of midge (Diptera: Chironomidae) larvae present on the plates, and the injection of sodium hypochlorate as an anti-Dreissena protocol at the intake where the study was undertaken.

Distances between the 17 clusters and 9 solitary individuals of Dreissena observed on the fragment of concrete were measured to the nearest mm., and nearest-neighbor analysis (Yeates, 1974) was applied, the results of which, R=1.31, revealed a random pattern of settlement of colonies and singletons.

D. Discussion

Conventional wisdom suggests that Dreissena begin to settle at 1-to-3 meters below the surface due to their negative phototropism. However, the co-authors' field research indicates colonization at a significantly lesser depth--settlement on emergent vegetation was observed at 0.4 m. in direct sunlight. A preference for reduced light intensity, however, was demonstrated by colonization closer yet to the surface (0.25-0.2 m.) under shaded conditions.

The application of nearest-neighbor analysis revealed a suprising pattern of settlement. The 17 colonies and 9 singletons showed a random pattern of distribution (R=1.31); however, the tightly-clustered nature of individuals within each colony was not consistent with the random pattern of overall colony distribution, raising the possibility of an unknown factor in Dreissena settlement.

Each colony consisted of one or two individuals from the largest-sized cohorts in the cluster, surrounded by smaller-sized cohorts (Figures 3-6). Our research team hypothesizes that the larger individuals represent singleton veligers that have settled randomly. As they grow, these individuals release an unknown biochemical factor that acts as an attractant for further veliger settlement, the origin of the smaller-sized cohorts within the colonies. This hypothesis of a biochemical attractant is consistent in part with that of Hebert et. al. (1991) and would explain the random vs. clustered dichotomy. It, however, also raises the additional question of the existence of two types of veligers, free-settling and communal-settling.

Since the singletons were generally of the smaller-sized cohorts (2-to-4 mm.), they apparently had settled after the largest-sized colonial individuals. Their cohort siblings were attracted into communal groupings surrounding the core colonial individuals; the singletons were not. This demonstrates that each cohort could be separated into two groups, solitary and communal settlers. This dichotomous pattern was also observed in mussels that had colonized Sagittaria stems.

Contrary to the 0 per sq. m. findings of Kilgour & Mackie (1993), Dreissena colonization density of 51,000 per sq. m. was observed on copper (in our study, steel electro-plated with elemental copper), raising further questions as to whether or not it is an effective deterrent to settlement. Kraak et. al. (1992) concluded that Dreissena is capable of regulating the body concentration of copper at low concentrations in water, suggesting a mechanism for copper tolerance, though Dudnikov & Mikhhev (1964) found that dissolved copper was effective as a control measure. At this juncture, the question over copper's effectiveness as a control measure seems unresolved.

Teflon also proved ineffective in controlling Dreissena colonization, although individual mussels appeared to be less securely anchored than on other substances. None of the other substances tested proved effective in inhibiting Dreissena; however, our study was incomplete due to mechanical damage and abrasion to a number of our test plates, particularly those coated with four commercial copper- and tin-based anti-biofouling paints. That part of our research will be repeated in 1994, in conjunction with additional field studies.

E. Conclusion

The co-authors' initial studies have led to a number of suprising, to us, at least, discoveries. First, Dreissena polymorpha colonizes at much shallower depths than we had expected. Second, Dreissena does not appear to be inhibited by conventional anti-biofouling substances. Third--and most interesting, Dreissena appears, admittedly from a very small sample, to have two types of veligers, free-settling and communal-settling. If this is accurate, why would Dreissena have two such classes of veligers?

Our hypothesis: a survival strategem for the species. The communal groupings are competitive for space and resources, so what is the advantage to grouping tightly? Our hypothesis: protection from predation. If communal grouping is advantageous in protecting the individual from predation, then what is the advantage in having singletons? Hypothesis: the species' most rapid colonization of available habitat is more important in survival than any individual's vulnerability to predation (each female lays ca. 40,000 eggs per season, so there are plenty of replacements for losses to predation).

If there is indeed a biochemical factor involved in Dreissena settling behavior, its identification and synthesis might be useful in localized management of zebra mussels. Attracting veligers to a lethal "trap" or other control measure might reduce the need for chlorination in municipal or industrial piping systems. However, the (unknown) percentage of free-settling veligers from Dreissena spawnings would still need control measures, so the biochemical approach might very well be of little potential in dealing with this irruptive adventive species. More basic research on anthropogenic substrates and natural controls such as predation, competition, etc., is required before we may begin to understand and implement less expensive and more environmentally-sound control approaches than are currently extant.

F. Acknowledgements

Without whose generosity our project could not have been undertaken:

--Anthony Tarnowski, Plating Engineering Company of West Allis, WI.
--Peterson Builders of Sturgeon Bay, WI.
--Clarence Hense, Midland Plastics of Brookfield, WI.

Without whose assistance our project would have been difficult to complete:

--UW-Sea Grant Institute, especial thanks to Jeff Hieb, Cliff Craft, and Allen Miller.
--Wisconsin Public Service Corporation of Green Bay, WI.
--Dave Stillman, Peterson Sheet Metal of Marinette, WI.
--Richard Nerenhausen, Oconto City Monument, Oconto, WI.

G. Literature Cited

Dudnikov, V.F. and V.P. Mikheev. 1964. "The effect of certain metal ions on Dreissena." In: Shtegman, B.K. (ed.). Biology And Control Of Dreissena. Trudy Instituta Biologii Vnutrennikh Vod, Akademia Nauk SSR 7: 1-145.

Hebert, Paul D.N. et. al. 1991. "Demography and ecological impacts of the invading mollusc Dreissena polymorpha." Can. J. Zool. 69: 405-409.

Kilgour, Bruce W. and G.L. Mackie. 1993. "Colonization of different construction materials by the Zebra Mussel (Dreissena polymorpha)." In: Nalepa, Thomas F. and D.W. Schloesser (eds.). 1993. Zebra Mussels: Biology, Impacts And Control. Boca Raton, FL: Lewis Pub.

Kraak, M.H.S. et. al. 1992. "Chronic ecotoxicity of copper and cadmium to the Zebra Mussel Dreissena polymorpha." Archives Of Environmental Contamination And Toxicology. 23(3): 363-369.

MacIsaac, Hugh J. and W. Gary Sprules. 1991. "Ingestion of small-bodied zooplankton by Zebra mussels (Dreissena polymorpha): Can cannibalism on larvae influence population dynamics?" Can. J. Fish. Aquat. Sci. 49: 1501-1505.

Yeates, Maurice. 1974. An Introduction To Quantitative Analysis In Human Geography. New York: McGraw-Hill.

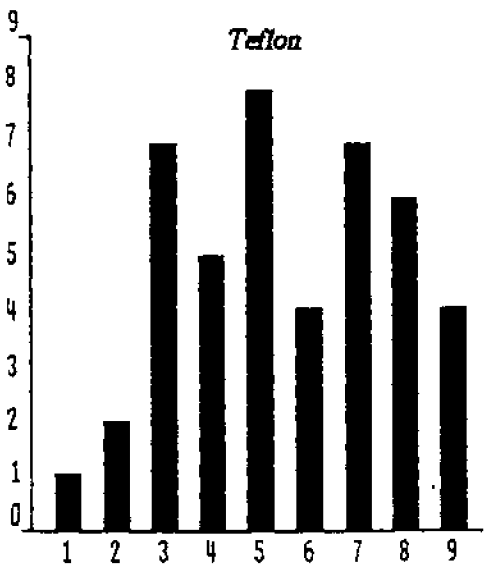

Figure 1

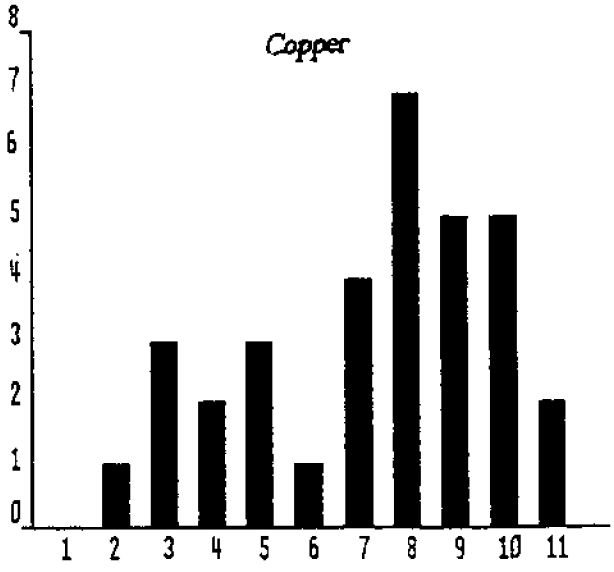

Figure 2

Size In MM (X-Axis) x Frequency (Y-Axis)

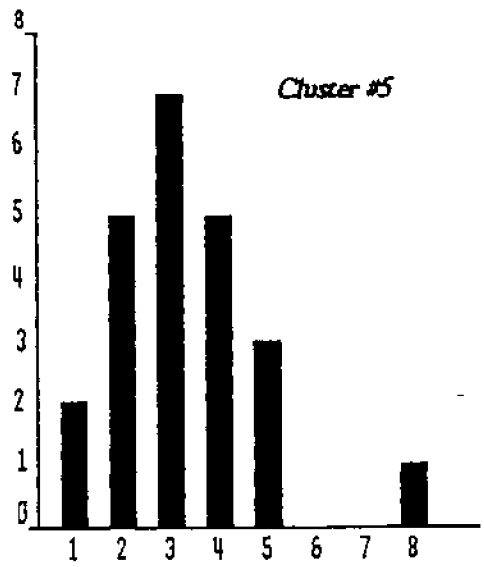

Figure 3

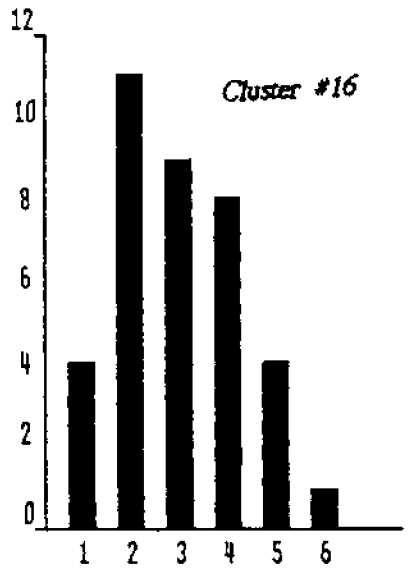

Figure 4

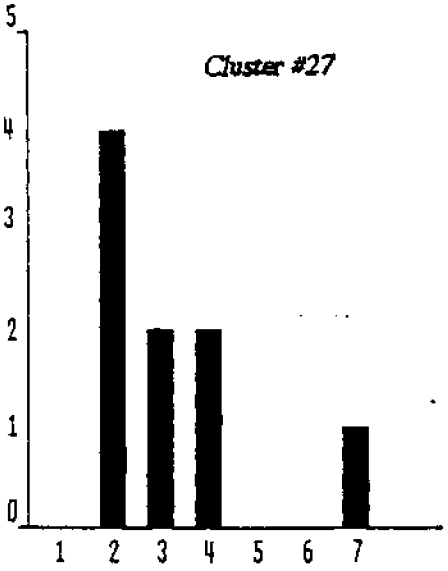

Figure 5

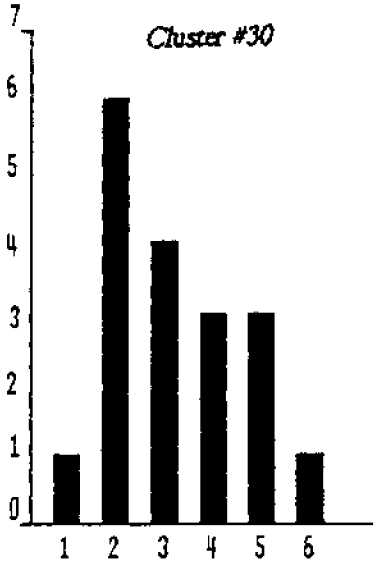

Figure 6

Size In MM (X-Axis) x Frequency (Y-Axis)

The Role of Zebra Mussels in Lake Ecosystems

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Belorussian State University

Abstract

These investigations have been carried out at Lukomskoe Lake in the Republic of Belarus. This lake has an area of 34.7 km^2, maximum depth - 11.5 m, volume - 243 million m^3. Since 1969 this lake has served as a cooling water reservoir for the Lukoml power station with a capacity of 2400 MW.

Zebra mussels first appeared in the reservoir in the late 1960s. By 1975 the zebra mussel biomass had reached levels as great as 490 g/m^2 in terms of the total area of the lake. After an initial outburst in density the quantity of zebra mussels declined, and in the last 15 years the population stabilized at a level of 150-180 g/m^2.

According to data obtained from laboratory experiments, the filtration rate of zebra mussels at a temperature of 20°C amounts to an average of 38 ml/hr for a 1 g zebra mussel. Our estimates were identical with filtration estimates made by other authors (Table 1).

Taking an average of our filtration estimate and that of other authors (Table 1) a zebra mussel with a mass of 1 g filters about 40 ml of water per hour at a temperature of 20°C. This value could be used for balance calculations and for determining the role of zebra mussels in the ecological system of a water reservoir.

The relationship between zebra mussel mass and filtration rate is described by the following equation:

$$F = 58.3 \cdot W^{0.96}$$

where F is the filtration rate (ml/h) and W is the mollusc mass (g).

For analyzing changes in the trophic structure of zoobenthos a classification has been used as developed by E. I. Izvekova (1975). Prior to the appearance of zebra mussels in Lukomskoe Lake, detritophages-collectors and phytodetritophages-filterers dominated the zoobenthic community (Table 2).

Following the massive infestation of the zebra mussel, the proportion of native filterers decreased eight-fold, while the role of detritophages-collectors has grown considerably in importance. This is due to the expansion of their food supply, which is composed of organic matter accumulated on the bottom due to feeding activity by zebra mussels. The quantity of predators, omnivorous collectors and grabbers, has

also increased somewhat. Analysis of the zoobenthic community demonstrated that, following the appearance of zebra mussels in the lake, the trophic structure of the zoobenthic community has been characterized by an extremely high dominance of one group, namely filterers (which are dominated by zebra mussels). This group amounts to 95% of the total biomass of benthic invertebrates.

Prior to the advent of zebra mussels at Lukomskoe Lake, benthic filterers would have been capable of filtering the total lake volume over a period of 15 years, and planktonic filterers would have filtered a volume of water equal to the cubic capacity of the lake over a period of 5 days (Fig. 1). Following the propagation of zebra mussels in the lake, the quantity of planktonic invertebrates declined, and the period required by the zooplankton to filter the total lake volume increased to 17 days. By 1975 the filtration capacity of the zoobenthos had increased 320-times, due to the presence of zebra mussels. At that time bottom invertebrates were capable of filtering the total volume of the lake in 17 days. At present, this period is equal to a month and a half (due to a decline in zebra mussel abundance).

Suspended material consumed by zebra mussels is now settling on the bottom. This material is partially used as food by bottom organisms and is partially conserved in mollusc shells, thus leaving the pelagic cycle. As a result, very serious changes have been observed in Lukomskoe Lake since the vigorous development of zebra mussels in it, and these changes have affected all links in the ecosystem (Fig. 2, 3). Water transparency in summer has increased two-fold, and the seston concentration has decreased three-fold. The quantity of dissolved organic matter has also decreased. The increased water clarity has resulted in an increased lake area covered by macrophytes (from 6 to 30% of the total area) due to an increase in the depth at which macrophytes can grow (from 2.5 to 5 m). Subsequent to the development of zebra mussels in the lake, the biomass of phytoplankton and zooplankton has declined considerably, while the quantity of zoobenthic organisms has greatly increased. The productivity of the fishery has doubled, and the catch composition is now characterized by an increase in benthophagous fishes adapted to feeding on zebra mussels.

Following the advent of zebra mussels to Lukomskoe Lake, macrophyte production has increased by a factor of 3.3, while the primary production of phytoplankton has been reduced more than four times (Table 3).

Total primary production has decreased by a factor of 3.3 and the portion of primary production produced by phytoplankton now amounts to 90% (prior to the advent of zebra mussels it was 98%). The total production of planktonic and benthic nonpredatory animals has declined from 95 kcal/m^2 to 44 kcal/m^2, and the role of bottom-dwelling invertebrates has increased from 3 to 77%. Production of nonpredatory invertebrates constituted 5.5% of the primary production (prior to the advent of zebra mussels it was 3.7%). Fish catches have increased by a factor of two and amounted to 18% of the total production of herbivorous zooplankton and zoobenthos (prior to the advent of zebra mussels it was 4%) or to 1% of the primary

production. As a result of the changes in the Lukomskoe Lake ecosystem subsequent to the advent of zebra mussels, the usage of the primary production by subsequent trophic levels has been appreciably increased.

References

Izvekova E.I., 1975. The nutrition and feeding links of mass species of Chironomid Larvae in the Uchinski reservoir. Avtoreferat kand.dissertacii. Moskow, 23 pp.

Karatayev A. Yu. and L. Ye. Burlakova, 1992. Changes in trophic structure of makrozoobenthos of an eutrophic lake, after invasion of Dreissena polymorpha: Inst. of Biol. of Inland Waters, Inform. Bulletin. N 93:67-71.

Kondratiev G.P., 1962. About some peculiarities of filtration of Dreissena polymorpha Pallas: Trudy Sarat. otd. Gos. Naychno- issled. Inst. Organ. Rybn. Khoz., T. 7:280-283.

Lvova A.A., 1977. Ecology of Dreissena polymoprha Pallas of the Uchinski reservoir: Avtoreferat kand.dissertacii. Moscow, MGU, 22 pp.

Stanczykowska A., 1964. The filtration capacity of populations of Dreissena polymorpha Pallas in different lakes, as a factor affecting circulation of matter in the lake: Ecol.pol., Ser. B. 14:265-270.

Figure Captions

Fig. 1. The dynamics of the filtration capacity of the benthos and planktonic invertebrates in Lukomskoe Lake. Y axis: the period of time required for benthic (1) or planktonic (2) filterers to filter a volume of water equal to the cubic capacity of the lake in summer.

Fig. 2. Long-term changes in water transparency, seston concentration and phytoplankton biomass in Lukomskoe Lake.

Fig. 3. Long-term changes in macrophyte abundance (given in percentage of the total lake area), biomass of zooplankton and zoobenthos in Lukomskoe Lake.

Figure 1

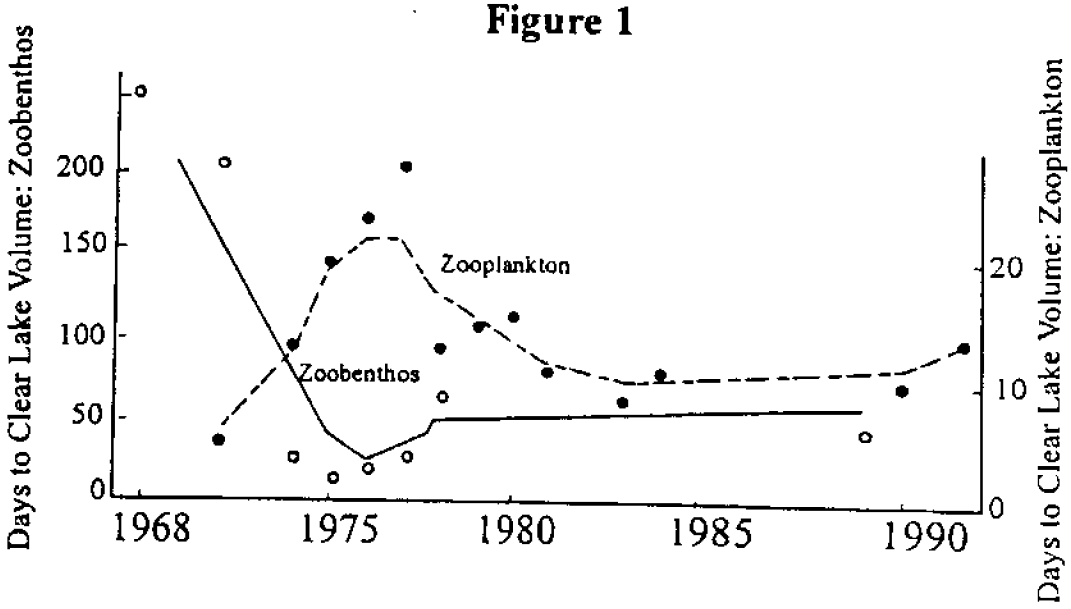
Days to Clear Lake Volume: Zoobenthos
200
150
100
50
0
Zooplankton
Zoobenthos
1968
1975
1980
1985
1990
20
10
0
Days to Clear Lake Volume: Zooplankton

Figure 2

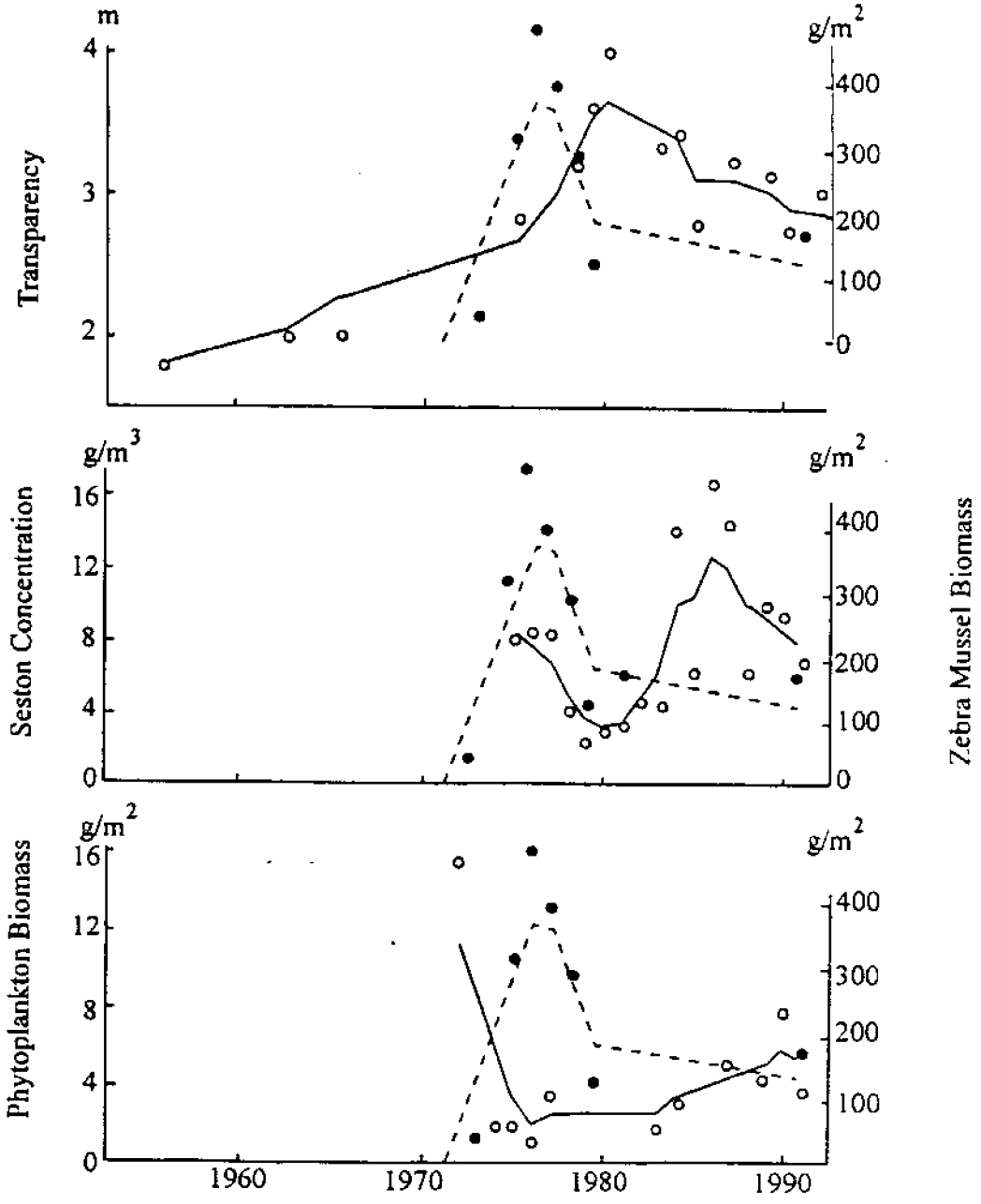
Transparency
m
4
3
2
g/m2
400
300
200
100
0
Seston Concentration
g/m3
16
12
8
4
0
g/m2
400
300
200
100
0
Phytoplankton Biomass
g/m2
16
12
8
4
0
g/m2
400
300
200
100
1960
1970
1980
1990
Zebra Mussel Biomass

Figure 3

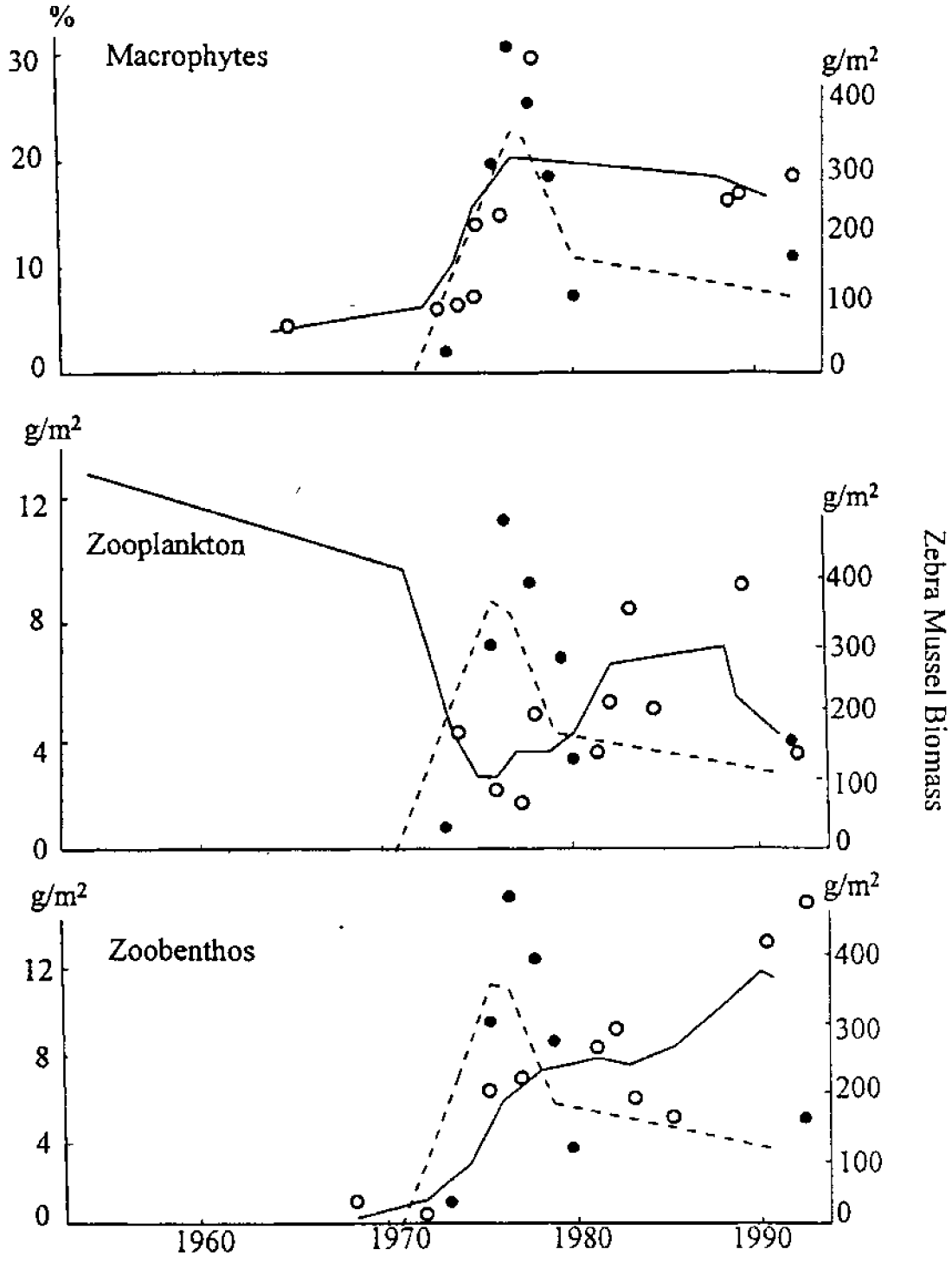
%
Macrophytes
30
20
10
0
g/m²
400
300
200
100
0
g/m²
Zooplankton
12
8
4
0
g/m²
400
300
200
100
0
g/m²
Zoobenthos
12
8
4
0
g/m²
400
300
200
100
0
1960
1970
1980
1990
Zebra Mussel Biomass

Table 1

The filtration rate of Zebra Mussel (F)
in different water bodies

Water body	F, ml/hr·g	Authors
Saratovskoe reservoir	43	Kondratiev, 1962
Uchinski reservoir	40	Lvova, 1977
Polish lakes	35	Stanczykowska, 1968
Lake Naroch	38	Our data

Table 2

Trophic structure of zoobenthos of the Lukomskoe Lake (biomass, % from total biomass) (Karatayev, Burlakova, 1992)

Trophic group	Prior to the appearance of Zebra Mussel 1968-69 years	Following the appearance (1977-1978 years)	
		without consideration for Zebra M	with consideration for Zebra M
"Phytodetritophages filtrators + collectors"	2.0	3.0	0.2
"Phytodetritophages filtrators"	40.9	5.3	94.8
"Detritophages collectors"	45.2	69.7	3.8
"Detritophages swallowers"	7.9	10.9	0.6
"Omnivorous collectors + grabbers"	0.4	3.2	0.2
"Predators-active grabbers"	3.6	7.9	0.4

Table 3

The elements of bionthic balance of the Lukomskoe Lake (biomass (B) and production (P) are given in kcal/m^2)

Trophic level	P/BZ	Prior to the appearance of Zebra Mussel		1978 y.	
		B	P	B	P
Primary production					
Phytoplankton	50.0	50.9	2544.5	12.5	624.5
Macrophytes	1.25	40.9	51.1	132.8	166.0
T o t a l :	-	91.8	2595.6	145.3	790.5
Zooplankton filtrators					
Crustacea	14.5	6.37	92.4	0.70	10.2
Rotatoria	46.0	0.03	1.4	0.10	4.6
T o t a l :	-	6.40	93.8	0.80	14.8
Zoobenthos nonpredatory	3.8	0.38	1.4	3.22	12.2
Zebra Mussel	0.6	-	-	27.80	16.7
Zooplankton + Zoobenthos nonpredatory	-	6.78	95.2	31.82	43.7
Zooplankton predatory					
Crustacea	9.6	1.22	11.7	0.18	1.7
Rotatoria	73.3	0.02	1.5	0.05	3.7
T o t a l :	-	1.24	13.2	0.23	5.4
Zoobenthos predatory	3.5	0.02	0.1	0.28	1.0
Zooplankton + Zoobenthos predatory	-	1.26	13.3	0.51	6.4
Fish					
nonpredators	0.4	8.75	3.5	17.00	6.8
predators	0.4	1.25	0.5	3.00	1.2

An Approach to Identify Potential Zebra Mussel Colonization in Large Water Bodies Using Best Available Data and a Geographic Information System

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The introduction of non-native species to eastern North America is a continuing concern to industries, coastal communities and state and federal resource managers. One of the first questions asked was "What areas are likely to be impacted?"

Using southern Green Bay as a test site, the study: 1) evaluated the usefulness of existing broad-based geographic data to identifying areas in the Great Lakes likely to be colonized by zebra mussels; and 2) examined the integration of large area data sets from diverse sources.

An analytical model combining water temperature during the spawning season, water depth and available substrate identifies areas of probable colonization. Relating colonization probability with river and population data resulted in the identification areas likely to be of concern. The result of this assessment is a first-order evaluation of areas likely to be infested by zebra mussels. The integration of existing data in an analytical model suggests that assessments of this nature can be accomplished in a timely manner, and that the course nature of the results will identify areas where monitoring or more detailed investigation may be necessary.

The introduction of non-native species to eastern North America is a continuing concern to industries, coastal communities and state and federal resource managers. Since the 1800s over 136 different species have been introduced into the Great Lakes basin including 61 plants, 24 fish, 24 algae, 9 mollusks, and 7 oligochaets (Mills et al.). None however, has received the notoriety of the now nationally infamous zebra mussel (*Dreissena polymorpha*). As the public gained knowledge of the devastating impacts occurring at Detroit Edison and the Monroe Michigan water utility in the fall of 1989, one of the first questions was "What areas are likely to be impacted?"

Research by Ramcharan, Padilla, and Dodson (Ramcharan), and Neary and Leach (Neary), has developed models to estimate where zebra mussels are likely to occur in inland water bodies. The question remains as to whether or not a reasonable prediction can be quickly made for large bodies of water, like the Great Lakes and associated bays, or the upper reaches of the Chesapeake Bay, where detailed uniform data may not be available, or where data locations may be widely scattered.

Zebra mussels are not liable to cover the entirety of large lakes or bays, but are more likely to be randomly spread based on suitable substrate and water temperatures, depths and chemistry. Neither is all colonization likely to be troublesome. Areas adjacent to urban water users are a concern because of possible disruption of services. Areas in the vicinity of boat landings and rivers pose a threat of movement of zebra mussels to inland waters.

Using southern Green Bay as a test site, the study had two objectives, the first focused on the zebra mussel issue -- *to evaluate the usefulness of existing broad-based geographic data in identifying areas in the Great Lakes likely to be colonized by zebra mussels*. The second objective was focused on the broader more technical issue of *the ability to integrate large area data sets from diverse sources.*

APPROACH: The logic behind the analytical model was to first evaluate physical parameters, water temperature during the spawning season, water depth, and available substrate to identify areas of *probable colonization*. The availability of calcium and the pH level of the water, both factors that contribute to the suitability of sites for colonization are adequate and relatively uniform in lower Green Bay where the model was tested, 40 mg/l and 7.5 respectively. Areas where colonization could be a *problem* were evaluated as areas that could lead to inland transport, and areas of possible disruption of urban services. Relating *colonization probability* with *problem areas* resulted in the identification *areas likely to be of concern*. The result of this assessment is a first-order evaluation of areas where monitoring or more detailed investigation may be necessary.

METHODOLOGY: *Surface water temperature.* Data collected since 1990 through the University of Wisconsin Sea Grant's Zebra Mussel Watch, a harbor and water intake monitoring program, indicated that juvenile mussels, called veligers, can be detected in Lake Michigan when water temperatures surpass 10°C. Reasoning that the longer the water exceeded 10°C, the higher the probability was for colonization. During the warm summer of 1993, several hatches were suspected in southern Lake Michigan. The water warmed early and stayed warm well into October and veligers were detected into early November, indicating two or more hatches.

Surface water temperature data was obtained electronically from AVHRR images through the National Oceanic and Atmospheric Agency's (NOAA) CoastWatch program at the Great Lakes Environmental Research Laboratory (GLERL) in Ann Arbor. Two problems arose in attempting to acquire the data. First of all, 1992 images were downloaded from the NOAA National Geophysical Data Center in Boulder, through GLERL, to computers at the Land Information and Computer Graphics Facility at UW-Madison. Since the intent was to combine these data with other formats, the images had to be processed to an ARC/INFO format. Learning how to accomplish this transition proved to be a time consuming process (Ignacio).

Secondly, satellite sensors can only record what can be seen and due to cloud cover images displaying the entire lower Green Bay were few and far between. While weekly measurements were desired, it was necessary to settle for one image a month, for the months of May through September. The infrequent usable images minimized the value of relative temperature. By May 9 most of the Bay was above the 10°C threshold and by June 9 all of the water in the lower Bay exceeded the threshold and remained above through the September 8 image.

Bathymetry and Substrate Bathymetric data was also obtained from the NOAA Geophysical Data Center by purchasing a tape containing the data. In addition to information on water depths, this data base contains other data typically found on nautical charts. Of particular interest

Temperature in °C
5=high probability
0=low probability

range	value
below 10°	0
10° or above	5

Temperature Duration -- in months above 10°C
5=high probability
0=low probability
(May 9, Jun 9, Aug 10, Sep 8)

range	value
2 months	3
3 months	4
4 months+	5

Table 1. Relative Values - Water Temperature

in this application are data on reefs, bottom structures and sunken vessels. From this data file, depth and substrate information was obtained. Additional substrate samples were obtained from UW-Green Bay (Kraft) and added to the file.

Depth of Water -- in feet
5=high probability
0=low probability

range ft	value
0-5	4.5
6-10	5
11-15	4.5
16-20	3.5
21-25	3.5
26-30	3
31-35	2.5
36-40	2
41+	1

Table 2. Relative Values - Water Depth

Analysis for Areas of Probable Colonization The analysis consists of assigning values to the data based on professional judgement. This is the point of interaction between the acquired data and decision makers. In this study, judgements were made based on four years experience with zebra mussels in Wisconsin and the Great Lakes, and many articles and books on the subject. If the approach were used elsewhere, a group of professionals could be brought in to provide the judgements and values used in analyzing the data.

In this case, each factor, e.g. water temperature, depth and substrate, was considered to be equally important in zebra mussel colonization, therefore the range of values used was constant, 5 = high probability and 0 = low probability. Judgements could also rate one factor more important than another and assign value ranges accordingly, e.g. temperature 0 to 5, depth 0 to 10.

Values Data were assigned relative values based on probability of mussel colonization. Surface water temperature was assigned a yes/no value for temperature, and a range of values for the duration above 10°C (Table 1).

Depth of water was assigned a range of values based on probability of finding mussels at a given depth (Table 2). Substrate similarly were assigned a range of values (Table 3). Substrate data are point data and therefore points were extrapolated to areas. The coarse nature of the data and the general conclusion sought to make the extrapolation reasonable.

Combining the valued data with the assigned weightings resulted in a map of probability of zebra mussel colonization.

Substrate Availability
5=high probability
0=low probability

type	value
silt	0
unknown	1
plants	2
sand/gravel	3.5
rocks	5
pilings	5

Table 3. Relative Values - Substrate

The next question was to determine where in lower Green Bay colonization would likely to be a problem. For this study the "problem" was seen as; 1) zebra mussels adhering to water intake pipes and reducing the flow through the pipes, 2) mussels adhering to other structures in the water, like boats and docks, 3) dead, sharp-shelled mussels littering the shoreline, and 4) mussels being transported from the Bay to inland rivers and lakes. All four concerns are directly related to the density of the shoreside population. Higher densities means more shore users and boaters, and more industry. The probability of transportation of mussels inland increases in rivers where anglers and boaters move from the infested Bay up the river to a launch site, unknowingly carrying zebra mussels on the boat, or in a live well or bait bucket. Data of the area population and the location of the major rivers were therefore important to the analysis. No attempt was made to model possible impact on the natural environment, because the impact is poorly understood at this time.

Population Densities per sq km
5=high probability 0=low probability

distance	value
6 - 7	1
7.1 - 11	2
11.1 - 13	3
13.1 - 23	4
23.1 - 242	5
242+	5

Table 5. Relative Values - Population

Proximity to Major Rivers
5=high probability
0=low probability]
(Fox, Oconto, Pestigo, Pensaukee, Little Saumico, Sturgeon Bay)

distance in km.	value
0-5	5
6-10	4
11-15	3
16-20	2
21+	1

Table 4. Relative Values - Rivers

Population Data on the area population was obtained from 1990 TIGER Census files for municipalities and townships. The population densities were then projected over the water surface to enable the analysis to combine population with other information on the lower Bay.

Rivers Proximity to major rivers was projected from data obtained from digital line graphs, a part of the TIGER files.

Analysis of Areas Where Colonization May Be a Problem The analysis consist of identifying areas that are near urban expanses and river mouths as those most likely to have a problem with zebra mussel colonization. The probability diminishes with increasing distance away from these features. In the case of river mouths a proximity program was run to establish buffer zones of 5, 10, 15 and 20 kilometers.

Values The two factors, population and river proximity were weighted as described above, in this case as the probability of the data describing a potential problem area if mussels were to colonize that area. Distance from a river is assigned a range of values as shown in Table 4, and Table 5 displays the range of values assigned to population density.

The result of this analysis is shown as areas where colonization may be a problem.

Analysis of Areas of Concern Continuing the logical progression, knowing the areas likely to be colonized based on physical parameters, and areas likely to be a problem, based on cultural factors, the final analysis sought to identify potential *problem areas likely to be colonized*, the combination of the two subsets.

CONCLUSIONS

The study set out to evaluate broad-based data sets for use in identifying areas likely to be colonized by zebra mussels. Data on bathymetry, substrate, surface temperatures, population, and natural and cultural features were used in the process. The ability to use data from a variety of sources depended on the metadata provided. The metadata file with NOAA's bathymetric data was particularly excellent.

The information obtained on surface temperature of the lower Bay was disappointing. Longitudinal data over the entire spawning season, May to October, was desired to make some judgement about multiple spawning in certain areas v. single spawning in others. Due to cloud cover the information was just not available. The potential to acquire data over broad areas via AVHRR is high, but current sensors are designed to look at clouds for weather prediction. Viewing the earth's surface in the Great Lakes region may need to wait for sensors that penetrate cloud cover. No attempt was made in this study to explore other sensors or sources since the purpose was to evaluate readily available data sources.

Applying the data to identify areas of concern in zebra mussel infestation was the judgement of the authors, with the help of colleagues. More important than the values applied is the process used to insert professional judgements. First of all, a judgement was made on what factors determine the likelihood of zebra mussel colonization. The physical parameters considered were: calcium, alkalinity, water temperature, depth, and substrate. Calcium and alkalinity were found to be relatively uniform in the lower bay and above threshold levels. Data were sought to define the other three factors. Population and the presence of rivers were determined to be cultural factors that would influence whether or not colonization would become a problem. Data were again sought to describe these factors.

The second level of professional judgement is the assignment of relative values to the data. How much more likely is zebra mussel colonization in eight feet of water v. forty feet of water? In this case, the judgement was five times more likely. Similarly, judgements were made that zebra mussel colonies near a river mouth were five times more likely to be a problem than a colony situated twenty kilometers away.

A third level of judgement was the relative importance of the various factors. Is depth a more important factor than water temperature? Or, are rivers more or less important than urbanization? No judgement was made on relative importance in this study. Doing so is possible if there is adequate knowledge on which to base the judgement.

The study demonstrates three points. One, geographic information systems can be used to obtain a quick first order identification of areas where zebra mussels are likely to colonize in large lakes or bays. The results can be applied to establish monitoring locations or to decide which areas need further study. Such analyses are useful in reducing initial costs and increasing effectiveness. Two, data sets covering large geographies and from various sources can be successfully integrated if the data is properly described in a metadata file. Three, geographic information systems can integrate professional judgement in the decision-making process by adding value judgements to data, and the relative importance of the data to the decision being made.

REFERENCES

Ignacio, J. A. F. 1992. Using Sea Surface Temperature (SST) Images as GIS Layers to Aid Environmental Monitoring and Management of the Great Lakes. Unpublished.

Mills, E.L., J.H. Leach, C.L. Secor and J.T. Carlton. 1993. *What's Next? The Prediction and Management of Exotic Species in the Great Lakes.* A Report of the 1991 Workshop, Great Lakes Fishery Commission.

Neary, B.P., 1992. Mapping Ontario's Zebra Mussel Threat. In *GIS World*, 5(1): 54-57.

Neary, B.P. and J. H. Leach, 1991. Mapping the Potential Spread of the Zebra Mussel (*Dreissena polymorpha*) in Ontario. Ontario Ministry of the Environment.

Ramcharan, C.W., D.K. Padilla and S.I. Dodson, 1992. Multivarate Model for Predicting Population Fluctuations of *Dreissena polymorpha* in North American Lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, 49(1):150-158.

The Introduction and Spread of the Zebra Mussel in North America

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Abstract

The zebra mussel, *Dreissena polymorpha*, is believed to have arrived in North America as a freshwater ballast stowaway in commercial vessels from Europe sometime around 1986. The mussel was first discovered in the Great Lakes in Lake St. Clair in June 1988. As a result of natural and human-influenced dispersal vectors, the intervening years have witnessed the intra- and interbasin transport of the zebra mussel throughout much of North America.

The mussel's first passage beyond the Great Lakes Basin was in 1991 when the mussel crossed New York State through the Erie Canal and Mohawk River into the Hudson River. By the end of 1993, zebra mussel-infested U.S. waters included: all of the U.S. shores of Lakes St. Clair, Erie, and Ontario; most of the Lake Michigan and Huron shores; Duluth-Superior Harbor in western Lake Superior; all or parts of the Arkansas, Calumet, Chicago, Cumberland, Des Plaines, Detroit, Genesee, Hudson, Illinois, Mississippi, Mohawk, Niagara, Ohio, Oswego, St. Lawrence, Susquehanna, and Tennessee Rivers; the Erie Canal; the St. Lawrence Seaway; three of the Finger Lakes; Saginaw Bay; and, Lake Champlain.

The expansion of the range of the zebra mussel in Canada, although initially rapid, has not been as dramatic as that in the United States. Since being discovered in Lake St. Clair in 1988, the mussels have spread throughout Lakes St. Clair, Erie, and Ontario; eastern Lake Huron; the St. Lawrence River; the Welland Canal; Georgian Bay; the Kawartha Lakes (interconnected to the Great Lakes); the Muskoka Lakes (overland transport); the Rideau Canal system; and, portions of the Ottawa River. To date, all of the Canadian zebra mussel sightings have been within the Great Lakes-St. Lawrence drainage basin.

Based upon atmospheric and surface water temperature data, it has been suggested that the mussels may ultimately infest most environmentally hospitable fresh surface waters of North America, south of central Canada, north of the Florida Panhandle, and from the Atlantic to the Pacific.

The Ballast Water Stowaway

The zebra mussel, *Dreissena polymorpha*, is native to the drainage basins of the Black, Caspian and Aral Seas in Eastern Europe and Western Asia. As commerce increased between Eastern, Central, and Western Europe, the mussel moved west, resulting in it's introduction into several Central and Western European freshwater systems during the late 1700s *(1790 - River Danube, Hungary; 1824 - London, England; 1826 - Rotterdam, The Netherlands; 1830 - Hamburg, Germany; 1840 - Copenhagen, Denmark; 1835 - River Rhine, Germany; 1844-1867 - various river systems, France)*. By the late-1800s, the zebra mussel was found throughout Central and Western European inland waterways. By the 1940s, the mussel made its way into water of Scandinavia, and is currently undergoing a rapid range expansion throughout the waters of the former Soviet Union.

The actual pathway of the mussel's introduction into North America is unknown, but experts believe that it arrived here as a stowaway in cargo vessels originating from European freshwater ports. Although zebra mussels are capable of attaching to ships' hulls, their transoceanic transport in this manner is unlikely since the mussels cannot survive the salinity of open ocean saltwater for the time required for such a crossing. More likely, the mussels arrived in North America as an internal stowaway. Cargo ships carry significant amounts of ballast water to stabilize the vessels during transoceanic crossings; the less cargo on board, the more ballast water is carried in ballast tanks. When ballast tanks are filled, many forms of aquatic life in the source water are drawn into the tanks (one study found some 240 different plant and animal life forms which may be carried in ballast water tanks). Once in ballast tanks, these organisms can be transported to other areas and subsequently be discharged into the waters of foreign ports. If the receiving waters are of the appropriate type (salt for saltwater ballast, fresh for freshwater ballast) and quality, there is a possibility that some of the ballast "exotics" may survive and establish new, breeding populations. This is believed to have been how the zebra mussel reached North America. It is also believed that more than 30 other exotic aquatic organisms, such as the ruffe and the spiny water flea, likely were introduced into the Great Lakes via ballast water dumping, as well. The problem is not limited to the Great Lakes, however, but is of great concern worldwide in both fresh- and saltwater ports.

Why, if the zebra mussel was in Europe for hundreds of years, did it take so long to reach North America? While this is the first time that the mussel *is known* to have colonized North American waters, it is possible that small populations may have been dropped off in the past, but in numbers too small to become established as breeding colonies, or in areas where they could not survive changing environmental factors. Serendipity may have brought a number of factors together, resulting in the current situation. Although zebra mussels have been distributed throughout most of Eastern, Central and Western Europe for hundreds of years, populations were substantially reduced due to the pollution of European waterways as a result of the industrial revolution and runoff from World War I. Post-World War II efforts to clean up those waters inadvertently resulted in a

reemergence of the zebra mussel in the years after the mid-1950s.

On the receiving end of the equation, prior to the 1900s, most shipping to North America carried solid ballast (instead of water) which is less likely to be a major vector of aquatic organisms. There was also less shipping than today. Although water had become the predominant ballast material by the early-1900s, transoceanic crossings still took two or more weeks, during which time ballast water could become a very inhospitable place for aquatic organisms to live, reducing the possibility of live aquatic organisms being discharged into North American waters. With the advent of new, faster ships after World War II, more exotics could conceivably survive the crossing.

Following North America's own industrial revolution, the quality of many of our waters was degraded to the extent that survival of new aquatic organisms became questionable in many harbors. These waters have been substantially cleaned up since the mid-60s, making them a much more viable habitat for new aquatic introductions.

Three pieces of the puzzle were now in place: cleaner European waters to provide ballast water immigrants, faster ships carrying freshwater ballast to serve as the transmittal vectors, and cleaner North American waters to receive the immigrants. The final piece of the puzzle was supplied by the completion of the St. Lawrence Seaway in the late-1950s, which provided the pathway for European-origin vessels (possibly carrying a number of exotics) to enter the Great Lakes. It is also possible that the increase in Eastern European shipping during the Soviet grain sales years of the late-70s and early-80s may have contributed to the number of gallons of European freshwater being purged from ballast tanks into the Great Lakes as those vessels took on cargo.

Mechanisms of Zebra Mussel Dispersal in North American Waters

Natural dispersal mechanisms for zebra mussels include swimming (during the first weeks of its life, a zebra mussel veliger is an actively swimming organism), water currents (both flowing streams and rivers and wind-driven waves), and attachment to other organisms (such as crayfish). Also suspected as a potential vector, but never quantified, is the attachment of mussels to feathers, feet, and legs of waterfowl and shorebirds, as well as transport on the fur of aquatic mammals.

Biologists believe, however, that interbasin transport of the zebra mussel throughout North America from infested waters of the Great Lakes and contiguous river systems into uninfested inland fresh surface waters will be very strongly enhanced by human influenced dispersal vectors. It is believed that the mussels will ultimately infest most susceptible (environmentally hospitable) waters of North America south of central Canada and north of the Florida Panhandle, and from Maine west to the California coast, a prediction which seems to be borne out by the rapidity with which the mussel has moved from the Great Lakes into inland river systems such as the Illinois, Susquehanna, Mississippi, Ohio, Tennessee, Arkansas, Ohio, and Hudson Rivers.

Major human-influenced dispersal vectors are interlake and interbasin transport of veligers in ship and barge ballast (the presence of zebra mussels in Duluth-Superior

Harbor in western Lake Superior is believed to be evidence of such commercial shipping transport) and juvenile and adult mussel attachment to ship, barge and recreational boat hulls. Other human influenced dispersal vectors include transport of juveniles and adults on or in recreational boats sailed or trailered from infested to uninfested waterbodies (zebra mussels can withstand desiccation for several days, depending upon relative humidity and temperature, thus allowing some transport while out of the water; mussels have been found on aquatic vegetation snagged on the props and trailers of boats being moved over land between water bodies); transport of veligers by commercial bait transport and fish stocking operations; in anglers' bait bucket water, and in recreational boat engine cooling water; in water flowing through navigation and irrigation canals (the Erie Canal provided an expressway across New York State from Lake Erie to the Hudson River); on or in equipment such as work barges, dredges, etc.; and by aquaria releases (this has been confirmed at a reservoir in the Province of Ontario).

The Zebra Mussel's Range Expansion in North America

The zebra mussel was first discovered in the Great Lakes Basin in routine samples taken from the bottom of Lake St. Clair in June 1988 (no one at that time was looking specifically for zebra mussels). Judging from the shell size of those mussels, it was theorized that the mussels had been introduced into the lake sometime in 1986. The first confirmed sighting in the western basin of Lake Erie was at South Bass Island *(Put-in-Bay, OH)* in October 1988.

1989

By early-summer 1989, extensive colonies of 30,000 to 40,000 individuals per square meter were reported on shoals in the shallow, nutrient rich, western basin of Lake Erie by the Ontario Ministry of Natural Resources. By the end of 1989, specimens were found in electric generating, public water treatment and industrial facility water systems in the Detroit River below Lake St. Clair, and on beaches and in power, water treatment and industrial facilities along most of the north and south shores of Lake Erie By the end of that year, zebra mussels could be found throughout the entire Lake Erie shoreline *(Monroe, MI; Ashtabula, Cleveland, Sandusky, and Toledo, OH; Port Stanley and Nanticoke, ONT; Erie, PA; Dunkirk and Buffalo, NY).*

Adult mussels were first reported in Lake Ontario in Port Weller, ONT, at the mouth of the Welland Canal in November 1989 and on a navigation buoy approximately four miles off the Niagara Bar in December 1989. Adult mussels were also found attached to a barge and an icebreaker at a shipyard in Sturgeon Bay, WI. No mussels were found anywhere else in the vicinity; those found on the vessels were believed to have been transported there by the vessels (both had spent time in Lake Erie).

1990

1990 witnessed the mussel's rapid range expansion throughout the entire Great

Lakes basin, the Niagara River and the Welland Shipping Canal; the western and southern shores of Lake Ontario *(Rochester and Oswego, NY; St. Catherines, Hamilton and Toronto, ONT)*; the Erie Canal from Buffalo, NY, on Lake Erie east to a point about one third of the way across the state; the northeastern end of Lake Ontario, the upper St. Lawrence River, portions of the St. Lawrence Seaway *(Picton, Kingston, and Cornwall, ONT; Snell and Eisenhower Locks)*, the lower St. Lawrence River *(Ile D'Orleans, QUE)*; virtually all of Lake St. Clair; southern and eastern Lake Huron *(Port Huron, MI; Goderich and Port Elgin, ONT)*; Saginaw Bay and Thunder Bay *(Alpena, MI)* on western Lake Huron; the southeastern and southern shores of Lake Michigan *(Grand Haven, Holland, and Muskegon, MI; East Chicago and Gary, IN)*; western Lake Michigan *(Kenosha and Sheboygan, WI)*, and the western end of Lake Superior in Duluth-Superior Harbor *(Duluth, MN; Superior, WI)*.

1991

By February 1992, the zebra mussel had followed the Erie Canal to its confluence with the Mohawk River and all of the Mohawk River east to its confluence with the Hudson River *(Herkimer, Amsterdam, and Albany, NY)*, marking the mussel's first passage beyond the Great Lakes Basin. The mussel then followed the Hudson River south *(Catskill, NY)*, to the lower reaches of the estuary into waters at the fringe of the salt wedge *(Poughkeepsie, Newburgh, NY)*.

During this same time span, the mussel had expanded its Great Lakes range to include most of the St. Lawrence River *(Cape Vincent and Alexandria Bay, NY)*; all of the western, southern and eastern shores of Lake Ontario and some locations on that lake's northern shore; most of Saginaw Bay *(Bay City, MI)*; isolated locations on Lake Huron's eastern shore *(Georgian Bay and the North Channel)*; all of the southern half of Lake Michigan *(Manistee, Grand Haven and South Haven, MI; Chicago, IL; Racine, Manitowoc, Green Bay, Milwaukee, WI)*; and the northeastern shore of Lake Michigan *(Charlevoix, MI)*. Multiple sightings were reported on the Chicago, Des Plaines, and Calumet Rivers and the Chicago Sanitary and Shipping Canal, all in Illinois.

The first sightings in Canadian inland waters were in Balsam, Big Bald, and Rice Lakes, in the Kawartha Lakes chain, and in Lake Simcoe, which are connected to the Great Lakes via the Trent-Severn canal system.

Sightings were confirmed in three of New York's Finger Lakes, *(Seneca and Cayuga, most likely due to canal connections to Lake Ontario or by overland transport by trailered boats, and Conesus, most likely by trailered boats)*.

An additional non-Great Lakes Basin was in the headwaters of the Susquehanna River near Johnson City, NY, opening a route for the mussels to travel into Pennsylvania and the Chesapeake basin (but no sightings emerged from the lower reaches of the river).

1992

By July 1992, the mussel had slipped the confines of the Great Lakes Basin, this

time in Illinois, traveling from the Chicago Sanitary and Shipping Canal into the Illinois River, and had reached that river's confluence with the Mississippi River at Alton, IL. The mussel then proceeded upstream in the Mississippi (most likely by means of commercial barge traffic) into southern Minnesota and downstream to the confluence of the Mississippi and the Ohio River. From there, the mussels were transported up the Ohio to the area of the Indiana, Ohio, Kentucky junction The mussel entered the Tennessee River via the Ohio and moved rapidly across the western tip of Kentucky, into and across western Tennessee, across the northern most reaches of Alabama, and back into Tennessee. Sightings were also being reported in the Cumberland River from the Tennessee River to Nashville.

By the end of 1992, the mussel's range had expanded up the Mississippi to St. Paul, MN, and downstream to Vicksburg, MS. Sightings were confirmed upstream in the Ohio River to a point near Hannibal, OH, and the mussel had added two more rivers to its range, Kanawha River, near Winfield, WV, and the Arkansas, with sightings in Little Rock, AR, and western Arkansas.

The mussel continued its spread around the Great Lakes, as well, turning up along more of the northwestern shore of Lake Ontario, the east and west central shores of Lake Michigan, Sault Ste. Marie, and eastern Lake Superior *(Gargantua, ONT)*.

1993

By September 1993, a sighting of mussels in White Star Park Quarry (Gibsonburg, OH) appeared to be strong evidence of transmittal by recreational boats or anglers, since the quarry lake has no direct water connections to any infested waters.

The mussel had also shown up in the Vermont waters of southeastern Lake Champlain. A direct canal connection to the Hudson River is the suspected transmittal vector in this case.

The mussel continued its Great Lakes Basin range expansion along the north shore of Lake Ontario, the eastern shore of Lake Huron, and Georgian Bay. It also colonized the Rideau Canal system which joins the Ottawa River with the eastern end of Lake Ontario. To date, all Canadian sightings of the mussel have been within the Great Lakes-St. Lawrence Drainage Basin.

The mussel had filled in much of its range throughout the entire length of the Illinois River; in the Upper Mississippi from Dubuque to St. Paul; the Lower Mississippi in a number of discrete locations as far downstream as Lettsworth, Baton Rouge, and New Orleans; the Atchafalaya River near New Orleans (Berwick Bayou, LA); the Tennessee River upstream past Chattanooga to Lenoir City; and the Arkansas River as far upstream (west) as Cowlington and Gore, OK.

Case Study: The Saga of Barge EMT-211B[1]

A look at a real-life example of human-influenced dispersal can help to shed light on why the zebra mussel has spread so dramatically in North America. In April 1992, barge EMT-211B was dry-docked in Hartford, Illinois. More than 1,000 live zebra mussels were found on the barge's hull. Randomly distributed clumps of mussels were attached to weld seams on the sides and bottom of the hull. Clump sizes along the side bumper plate ranged from 8 to 34 individuals; clumps from the underside of the hull contained 66 to 96 individual mussels.

The mean shell length of 100 randomly chosen zebra mussels from several clumps on the hull was measured to the nearest 0.1 mm with a dial caliper. The mean shell length was 17.3 mm (standard deviation - 1.6); minimum and maximum lengths were 11.6 and 21.1 mm, respectively. McMahon reported zebra mussel growth rates of 1.0 to 1.6 cm/year in slow and fast growing populations, respectively.[2] Based on such growth rates, it was estimated that the mussels on the barge were approximately one year old. Working on the assumption of mussel age, and using information provided by the barge's owner, on the barge's movement, it was found that the barge was in the Illinois River between February and May of 1991. It is likely that mussel attachment took place in that river during the early spring or summer of 1991.

If we assume that the mussels attached to the barge hull in or around Spring Valley, IL, in May 1991, the mussels could have been transported an approximate total distance of 15,884 km before the barge was dry-docked. Areas transmitted by the barge during that time period included sections of the upper Mississippi River (as far north as Winona, MN), the lower Mississippi (to Louisiana and Mississippi), and throughout the Illinois River. As there was a potential for mussels to be dislodged from the hull during barge transit, locking and fleeting, and since mussels on the hull could potentially been spawning during the latter part of their journey, the potential for long-distance transport and dispersal of zebra mussels by commercial barge traffic can be seen to be considerable.

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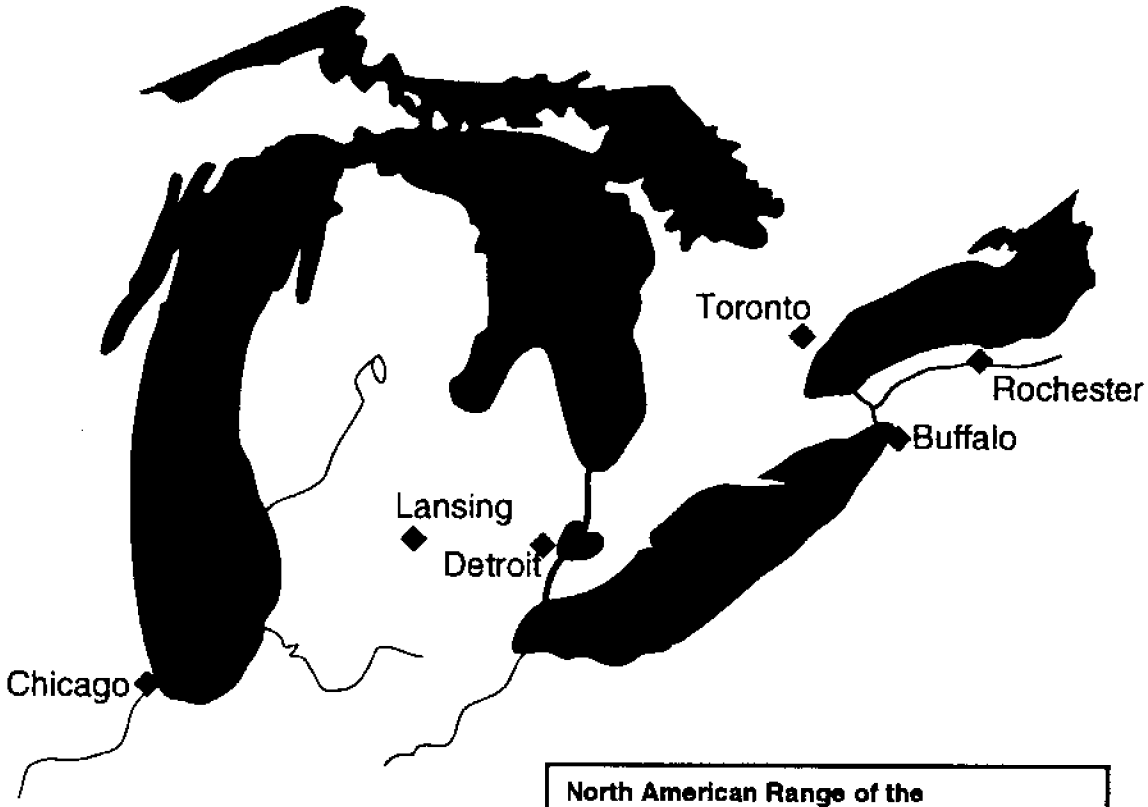
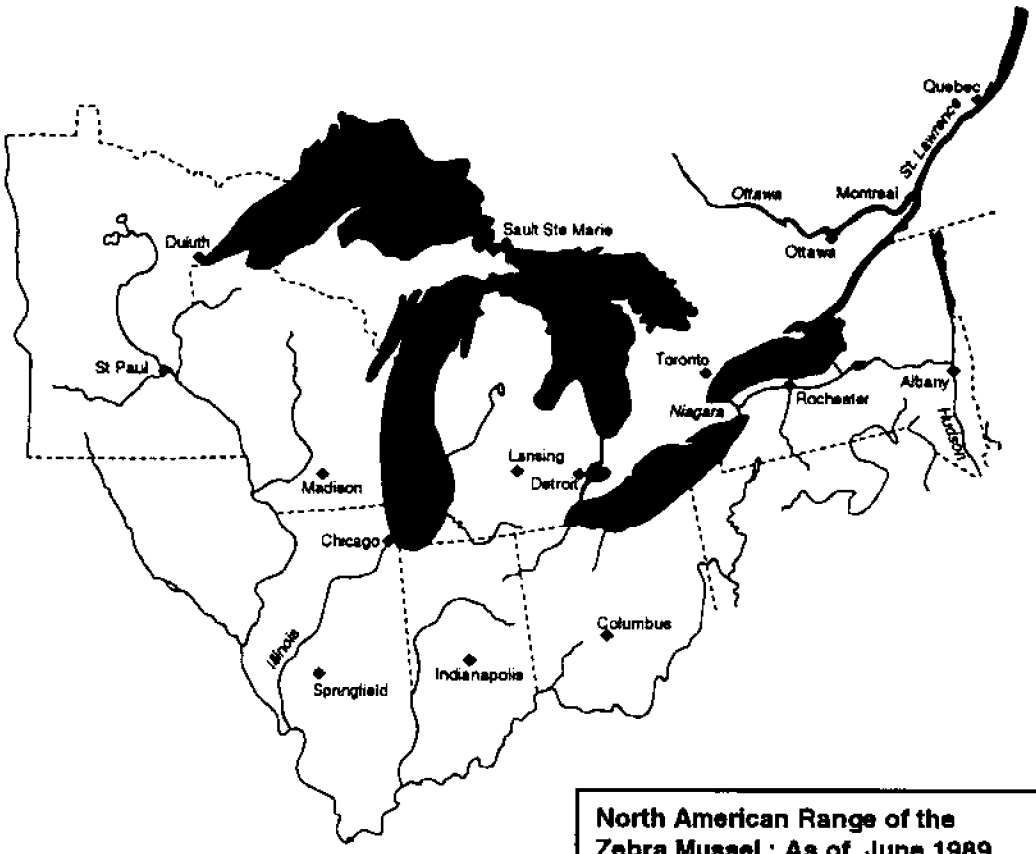
1 Source: Keevin, T., R. Yarbrough, and A. Miller. 1990. U.S. Army Corps of Engineers. St. Louis District and Waterways Experiment Station.

2 McMahon, R.F. 1990. *The Zebra Mussel: U.S. Utility Implications*. EPRI GS-6995, Electric Power Research Institute, Palo Alto, CA, pp. 1-70.



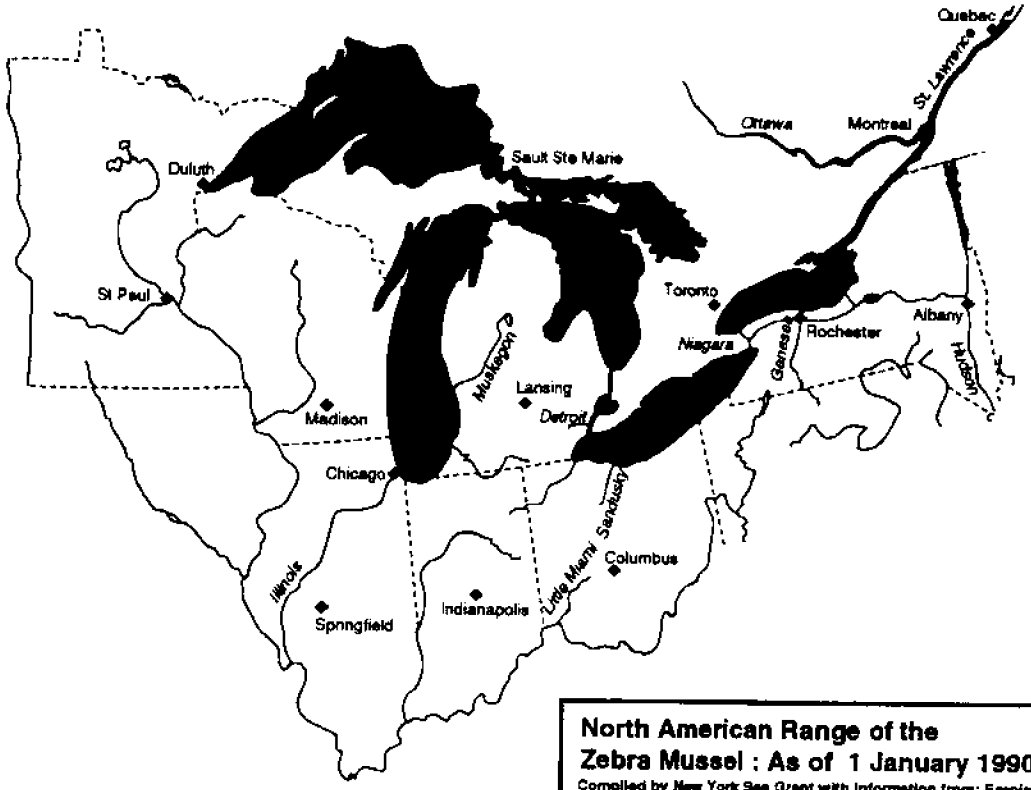
North American Range of the Zebra Mussel : As of June 1988

Compiled by New York Sea Grant with information from: Empire State Electric Energy Research Corporation, Fisheries and Oceans Canada, Great Lakes Sea Grant Network, Illinois Natural History Survey, Ontario Hydro, Ontario Ministry of Natural Resources, Tennessee Valley Authority, US Army Corps of Engineers, US Fish & Wildlife Service, and utilities and others throughout North America.



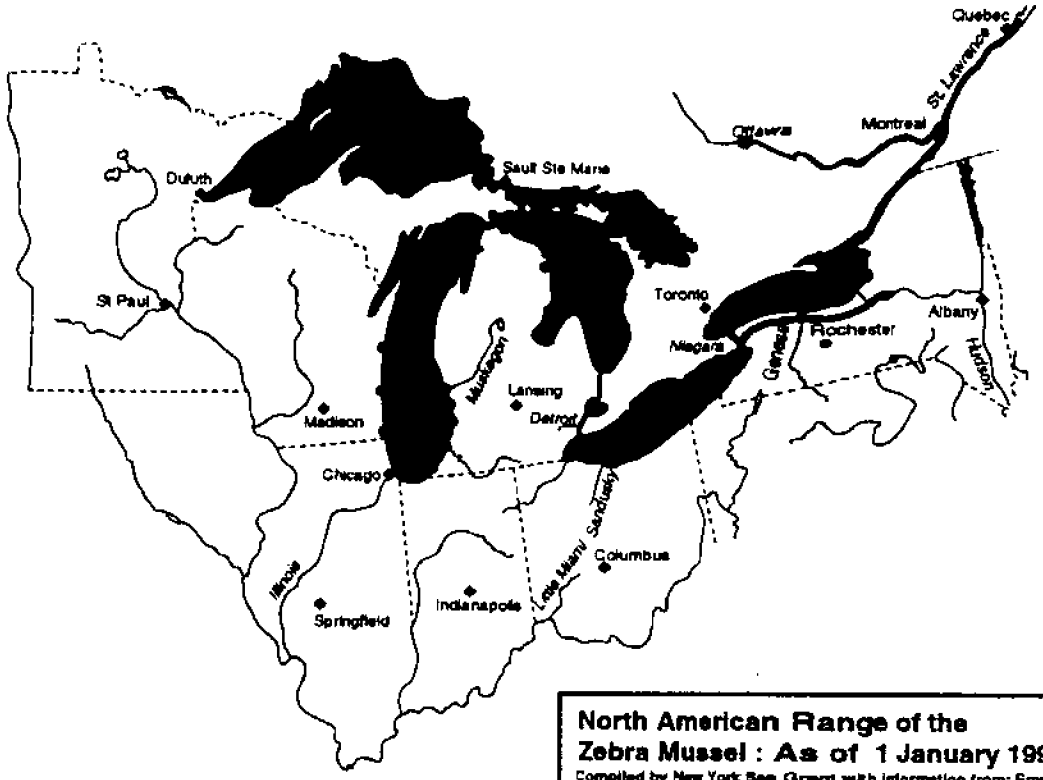
North American Range of the Zebra Mussel : As of June 1989

Compiled by New York Sea Grant with information from: Empire State Electric Energy Research Corporation, Fisheries and Oceans Canada, Great Lakes Sea Grant Network, Illinois Natural History Survey, Ontario Hydro, Ontario Ministry of Natural Resources, Tennessee Valley Authority, US Army Corps of Engineers, US Fish & Wildlife Service, and utilities and others throughout North America.



North American Range of the Zebra Mussel : As of 1 January 1990

Compiled by New York Sea Grant with information from: Empire State Electric Energy Research Corporation, Fisheries and Oceans Canada, Great Lakes Sea Grant Network, Illinois Natural History Survey, Ontario Hydro, Ontario Ministry of Natural Resources, Tennessee Valley Authority, US Army Corps of Engineers, US Fish & Wildlife Service, and utilities and others throughout North America.

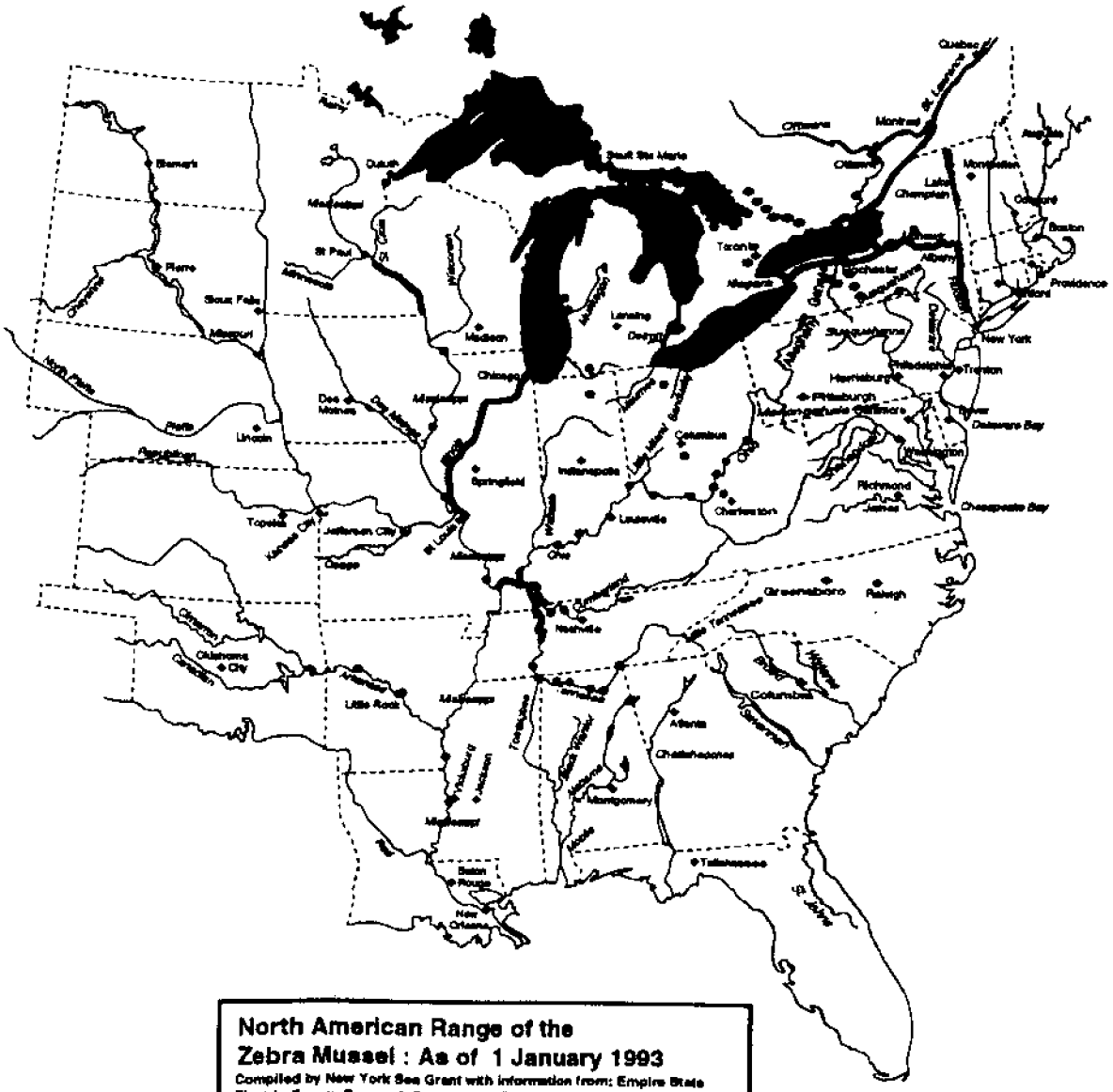


North American Range of the Zebra Mussel : As of 1 January 1991

Compiled by New York Sea Grant with information from: Empire State Electric Energy Research Corporation, Fisheries and Oceans Canada, Great Lakes Sea Grant Network, Illinois Natural History Survey, Ontario Hydro, Ontario Ministry of Natural Resources, Tennessee Valley Authority, US Army Corps of Engineers, US Fish & Wildlife Service, and utilities and others throughout North America.

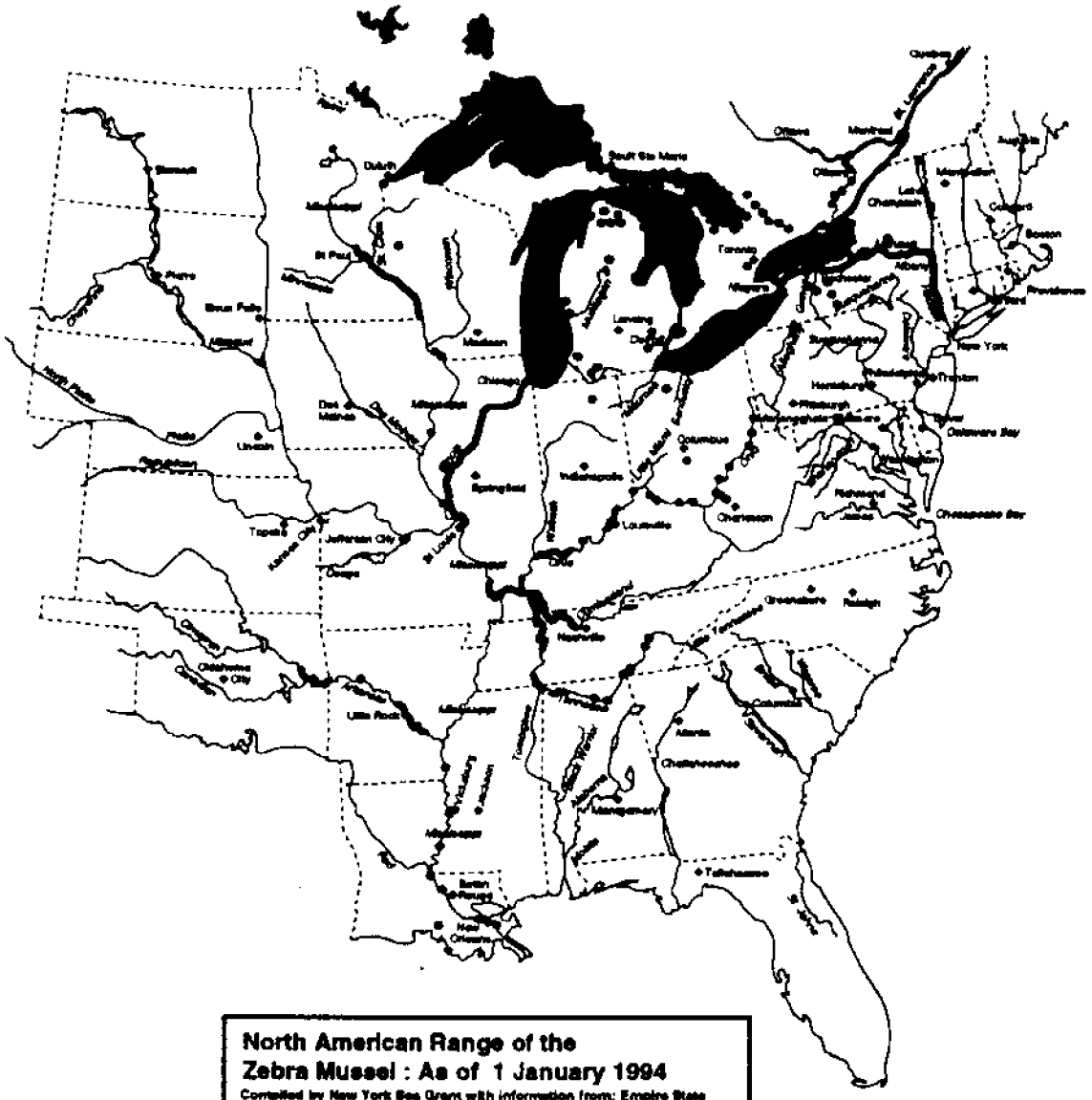
North American Range of the Zebra Mussel : As of 1 January 1992

Compiled by New York Sea Grant with information from: Empire State Electric Energy Research Corporation, Fisheries and Oceans Canada, Great Lakes Sea Grant Network, Illinois Natural History Survey, Ontario Hydro, Ontario Ministry of Natural Resources, Tennessee Valley Authority, US Army Corps of Engineers, US Fish & Wildlife Service, and utilities and others throughout North America.



North American Range of the Zebra Mussel : As of 1 January 1993

Compiled by New York Sea Grant with information from: Empire State Electric Energy Research Corporation, Fisheries and Oceans Canada, Great Lakes Sea Grant Network, Illinois Natural History Survey, Ontario Hydro, Ontario Ministry of Natural Resources, Tennessee Valley Authority, US Army Corps of Engineers, US Fish & Wildlife Service, and utilities and others throughout North America.



North American Range of the Zebra Mussel : As of 1 January 1994

Compiled by New York Sea Grant with information from: Empire State Electric Energy Research Corporation, Fisheries and Oceans Canada, Great Lakes Sea Grant Network, Illinois Natural History Survey, Ontario Hydro, Ontario Ministry of Natural Resources, Tennessee Valley Authority, US Army Corps of Engineers, US Fish & Wildlife Service, and utilities and others throughout North America.

Observations on Zebra Mussel Colonization in the Lower Ohio and Tennessee Rivers

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ABSTRACT

Zebra mussels were found in the lower Ohio and Tennessee Rivers for the first time in the fall of 1991. By October 1993 only a few additional individuals had been found in the Tennessee River other than inside locks where we assumed that the mussels had washed from barges or other vessels and attached to the lock structure. No veligers have been found in the plankton of the Tennessee River nor have small juveniles been found on submerged structures. This suggests to us that reproduction is not occurring in the Tennessee River. However, high densities of veligers, juveniles and adults have been observed in the Ohio River at numerous sites including locations near the confluence of the Tennessee River. Several populations of zebra mussels in the Ohio River were sampled, and densities, time of settling of juveniles, and growth rates were estimated. Even though veligers were present in the plankton as early as March in the Ohio River, it appears that few if any successfully settled before July, and the majority settled after July. Size distribution in the populations suggests that zebra mussels reach an average of 20 mm (16-24 mm) during their first full year of growth in the Ohio River. We suspect that the failure of zebra mussels to successfully establish populations in the Tennessee River is related to differences in water quality, perhaps dissolved calcium concentration which averages 16-20 mg/l in the Tennessee River and about 40 mg/l in the lower Ohio River.

INTRODUCTION

Since the invasion of the zebra mussel, *Dreissena polymorpha*, into the Great Lakes in the mid 1980's (Herbert, et al., 1989), millions of dollars have been spent on research directed at understanding the biology of the mussel and on controlling zebra mussels in water systems. The zebra mussel is rapidly becoming one of the best known of all the mollusks, yet there are many unanswered questions regarding its ability to colonize the inland rivers of the middle and southern United States. Studies have begun to document the effects of zebra mussels on native unionid clams (Haag, et al., 1991, 1993; Hebert et al., 1991; Hunter and Bailey, 1992; Mackie, 1991) and water quality (Reeders and bij de Vaate, 1990). Much of the research activity has been focused in the Great Lakes area. However, now that zebra mussels have invaded the Tennessee and Ohio Rivers within the past two years, studies have begun to evaluate their success and impact on these rivers. The impact of exotic species is often dramatic and usually unpredictable (Vermeij, 1991).

The environments of the lower Tennessee and Ohio Rivers differ considerably, although each has an extensive native unionid clam fauna. The lower Tennessee River consists of a series of large reservoirs formed by moderately high dams and which flood extensive areas of the lower flood plain. The Ohio River is regulated by relatively low dams which maintain the river within its original banks. Sediment consisting of fine silt has accumulated in the downstream reaches of the Tennessee River reservoirs while much of the Ohio River bottom still consists of sand and gravel. The water quality differs considerably. The Tennessee River is relatively soft with a dissolved calcium concentration between 16-20 mg/l while the Ohio River is moderately hard with a calcium concentration of around 40 mg/l. These differences provide a unique opportunity to examine factors that contribute to the success of the zebra mussel in riverine systems and its impact on native unionid clams.

The first zebra mussel found in Kentucky Lake was found attached to a native threeridge mussel (*Amblema plicata*) by a commercial musseler on September 10, 1991, about 8 miles upstream from Kentucky Dam (TVA, 1991). Since that time a few more have been found on native mussels in the Camden, Tennessee, area, and in July 1992 a commercial musseler pulled up a cluster of 7 adult zebra mussels which had been attached to a submerged stump near the west shore of Kentucky Lake at mile point 1.0 of the secondary channel (Tennessee River mile 23.8). The first zebra mussels found in the lower Ohio River were found by TVA divers during a unionid mussel study conducted October 1-3, 1991, at about mile 964.5, downstream from Dam 53 (personal communication: Barry Payne, Waterways Experiment Station). In the fall of 1992, the locks at Kentucky Dam on the Tennessee River and Cheatham Dam on the Cumberland River were dewatered for maintenance. Many attached zebra mussels were found in both locks (information from Cheatham Lock from Richard Tippit, Nashville District Corps of Engineers). These probably came from barges and other vessels traveling from areas where zebra mussels are successfully reproducing.

Zebra mussels are being found regularly by commercial musselers in the Ohio River and in Barkley Lake and the lower Cumberland River downstream from Barkley Dam, but only a few have been found downstream from Kentucky Dam in the lower Tennessee River.

In an ongoing project at Murray State University, zebra mussels have been held in aquaria with Tennessee River water with a dissolved calcium concentration of between 16-20 mg/l and fed a diet of dried *Chlorella* and low-fat rabbit food. Although mortality was less than 1%, growth was less than 1 mm in 8 months (Leek and Sickel, 1994). Under similar conditions other investigators have been able to achieve growth of 1 - 2 mm/month (Nichols, 1993). However, the water used by other investigators had calcium concentrations much higher than that of the water from the Tennessee River. Nichols (1993) used water with 125 mg/l calcium, and Sprung (1987) used reconstituted river water with a calcium concentration of 58 mg/l. Sprung (1987) reported reduced survival of developing zebra mussel larvae in water with less than 40 mg/l calcium. Ramcharan et al. (1992) examined data from 278 European lakes and found that zebra mussels were absent from lakes with calcium less than 28.3 mg/l and pH below 7.3. These observations led us to suspect that the water quality of the Tennessee River is not conducive for zebra mussel reproduction and development. However, there has not been sufficient research on zebra mussels in the Tennessee River to draw any definitive conclusions about growth and reproduction. The present paper reports on our observations of zebra mussel colonization in the lower Ohio and Tennessee Rivers.

METHODS

In conjunction with a number of surveys of native unionid clams being conducted for industries on the Ohio and Tennessee Rivers, it has been possible to observe the colonization of zebra mussels in these rivers. Figure 1 shows a map of the lower Ohio, Tennessee and Cumberland Rivers with various locations at which surveys were conducted or where other investigators have reported finding zebra mussels. In each case of our study, sampling was accomplished by divers collecting unionid clams either along transects consisting of anchored lines or within m^2 quadrats placed along transect lines. Shells of unionid clams and rocks were examined for the presence of attached zebra mussels which, if found, were removed and measured. A stereo microscope was used to look for the presence of recently settled juveniles or post-veligers. To check for veligers in the plankton, samples were collected with a 0.63 µm mesh plankton net. No attempt was made to quantify densities of zebra mussels.

We attempted to sample the same populations in the Ohio River at intervals so that growth could be more accurately estimated. However, barge activity on one occasion and high water on another prevented sampling the same locations. All zebra

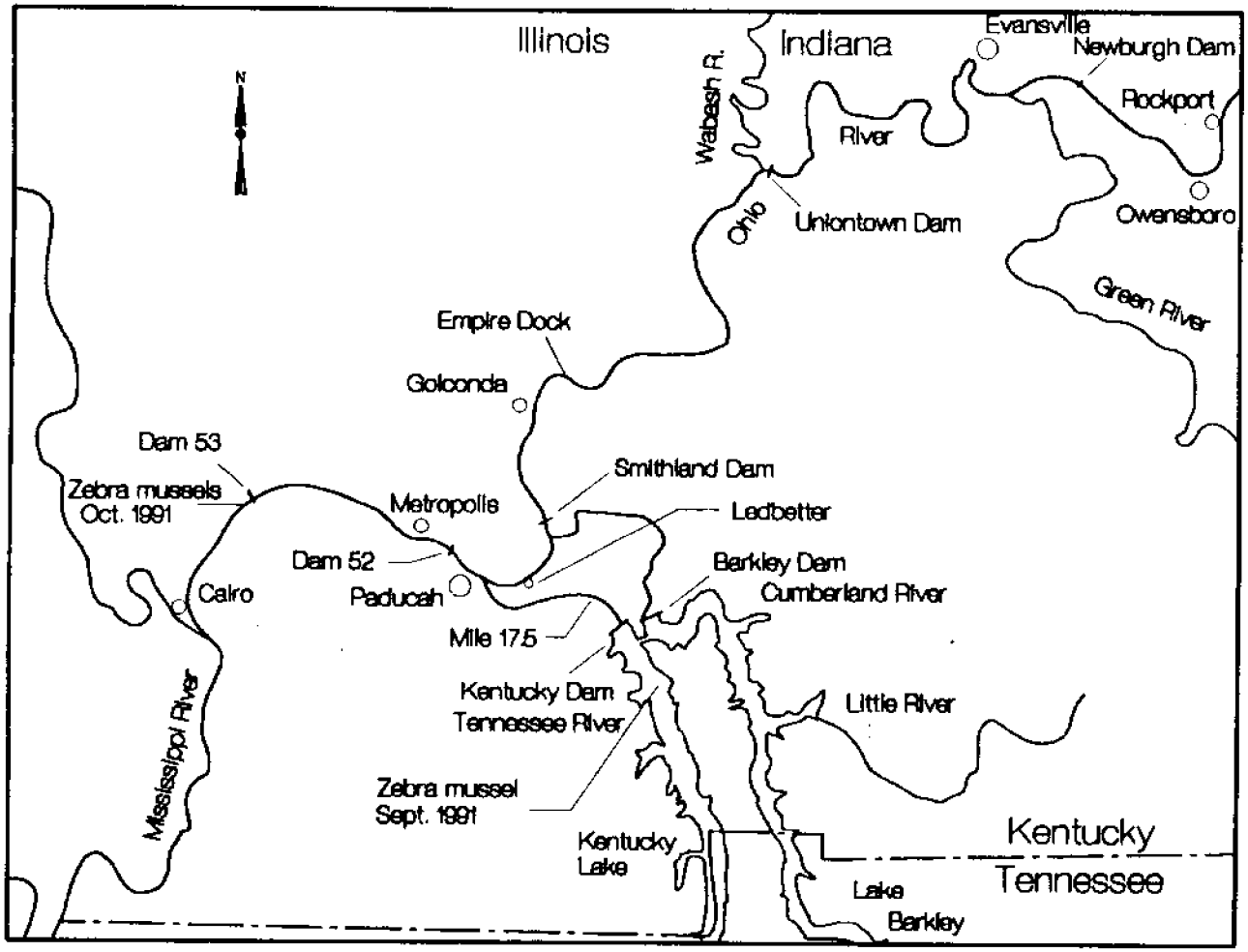

Fig. 1. Map showing Ohio, Tennessee, Cumberland, and Mississippi Rivers with locations of first zebra mussel finds in the Tennessee and lower Ohio Rivers and locations of collection sites.

mussels collected from each site were measured for shell length to the nearest 0.1 mm. Histograms of shell length at 1 mm intervals were plotted and used to estimate growth and time of settling of juveniles or post-veligers.

RESULTS AND DISCUSSION

One site in the lower Tennessee River downstream from Kentucky Dam was surveyed between river miles 17.4 and 17.7 from November 21 to December 6, 1993. Kentucky Dam is located at Tennessee River mile 22.4. In that survey, an area of approximately 1560 m^2 was searched and 2733 unionid clams representing 22 species were examined. Two of the unionid clams had 1 zebra mussel each attached to their shells. Earlier in the summer, from June 5-15, TVA biologists transplanted unionid clams from an area of 512 m^2 located just downstream from Kentucky Dam. At that site they found 2 zebra mussels attached to rocks (personal communication: John Jenkinson, Tennessee Valley Authority). This area of the river encounters heavy barge traffic, and these zebra mussels may have fallen from barges as they apparently have within the lock at Kentucky Dam. Only a few additional occurrences of zebra mussels attached to unionid clams in the Tennessee River have been reported by commercial musselers and mussel buyers. These people see large numbers of native unionid clams and would know if zebra mussels were increasing in the Tennessee River. All indications suggest that they are not increasing at this time.

Table 1 presents the locations and dates of unionid clam surveys in the lower Ohio River that are included in Fig. 1. The survey conducted on August 1, 1991 at Metropolis revealed no zebra mussels in an area of 925 m^2 in which 41 unionid clams were found. On June 6, 1992 another survey nearby at Paducah again revealed no zebra mussels. In the survey at Ledbetter on September 18-19, 1992, 410 individuals in 18 species of unionid clams were examined, and 2 zebra mussels were found. In 1993, 5 surveys were conducted in the lower Ohio River, and zebra mussels were abundant both on native unionid clams and on rocks. At Ohio River mile 785.0, downstream from the confluence of the Green River, 945 unionid clams in 20 species were found, but only 15 zebra mussels were collected on August 17-19, 1993. These were not measured and are not included in Fig. 2. One other survey conducted in 1993 (06/26-27/93) that is not included in Table 1 or Fig. 1 was located at Ohio River mile 690.8 upstream from Derby, Indiana. In that survey, two 720 m transects extending from the Kentucky to the Indiana shore were searched. The river bottom consisted mostly of sand and silty-sand with only sparse patches of gravel. A small bed of unionid clams was found near the Kentucky shore. No zebra mussels were found. It is assumed that the lack of stable substrate across most of the width of the river prevented the establishment of both unionid clam populations and zebra mussels. However, another possibility is that this area of the river has not been invaded by zebra mussels as of that date.

Table 1. Location of sample sites in the lower Ohio River and total area surveyed from which unionid clams were collected during initial zebra mussel colonization.

Collection Date	Ohio R. Mile	Area	Geographic Reference Point
09/01/91	942.6	925 m^2	Metropolis, Illinois
06/24/92	935.3	488 m^2	Paducah, Kentucky
09/18-19/92	929.9	1200 m^2	Ledbetter, Kentucky
02/14/93	896.0	230 m^2	Empire Dock, Illinois shore
07/30/93	896.7	n/d	Downstream from Empire Dock
08/17-19/93	785.0	575 m^2	Green River confluence
10/23/93	746.0	50 m^2	Rockport, Indiana
02/19/94	902.1	n/d	Golconda, Illinois

The other 4 sites indicated in Table 1, Empire Dock, downstream from Empire Dock, Rockport, and Golconda, each had high densities of zebra mussels attached to native unionid clams, snails, and rocks and larger gravel. Fig. 2 presents the frequency distribution histograms of shell length for the 4 sites. The sample from Empire dock collected 2/14/93 shows mostly young zebra mussels with an apparent bimodal distribution with mean lengths around 7 and 12 mm. We assume that this group of mussels ranging in length from 4 to 14 mm represents the recruitment from the previous summer of 1992. The lack of many large individuals indicates either that this is a recent population or that individuals don't live much beyond a year and a half. The wide range of sizes suggests an extended spawning period. The bimodal distribution suggests the occurrence of 2 peaks in gamete release during the spawning period. Plankton samples collected in March, June, July and October revealed large numbers of veligers indicating that some spawning is occurring throughout much of the year. No attempt was made to quantify the density of veligers in the plankton.

Comparing the 2/14/93 and 2/19/94 samples, we see that the 1992 cohort that measured 4-14 mm in length on 2/14/93 has increased in size to 20-29 mm in one year. The young-of-the-year recruits for 1993 appear as the group in the size range of 2-11 mm on 2/19/94. The larger size class, 20-29 mm, on 2/19/94 shows that zebra mussels do live beyond a year and a half, and suggests that the population was first established during the summer of 1992. The length range of 4-14 mm with bimodal peaks at 7 and 13 mm in February 1993 for the 1992 recruits compared to a range of 2-11 mm with peaks at 6 and 10 mm for the 1993 recruits indicate a slower growth rate for the younger mussels in 1993-94 than in 1992-93. One reason for this could be the presence of a high density of large mussels in 1993 that was absent in 1992. Also, the presence of fewer young mussels compared to the older mussels may suggest that the population could reach its maximum density in only a few years as older individuals inhibit settlement and growth of the young.

The July 1993 sample taken downstream from Empire Dock shows mean lengths around 19 and 23 mm, assuming a bimodal distribution. Of major interest is the lack

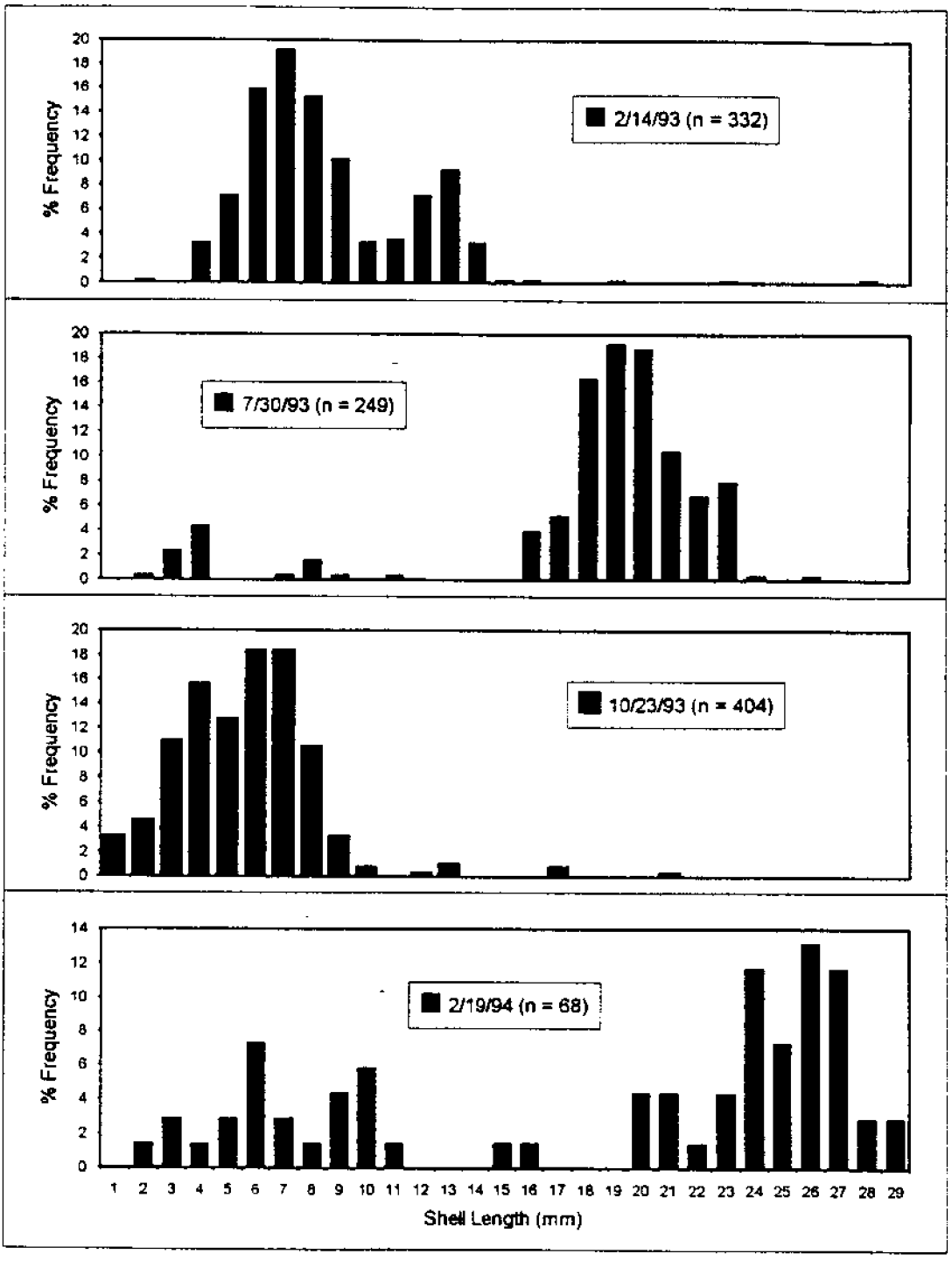

Fig. 2. Length frequency distribution of zebra mussels collected from the lower Ohio River. Collection date and number of individuals are indicated in legends.

of recently settled juveniles in July. Zebra mussel veligers had been abundant in the plankton since March, but few successfully settled before the end of July. The October sample from Rockport, Indiana, shows a large number of recently settled juveniles ranging from 1 - 9 mm in length. Rockport is 150 river miles upstream from Empire Dock, and based on the absence of a second year cohort it appears that the population has just been established. Based on the July sample, we estimate that zebra mussels grow to a length of 16 to 24 mm in the first full year of growth.

Clearly, more quantitative samples need to be collected from established populations in the Ohio River and at more frequent intervals before definitive statements about growth rates and densities can be made. Also, more sites in the Tennessee River need to be examined along with studies on the effects of water quality on zebra mussel reproduction and development before the success or failure of zebra mussel colonization in the Tennessee can be determined.

CONCLUSIONS

Zebra mussels have been introduced into both the Tennessee and Ohio Rivers, probably in 1990 or 1991. Dense, reproducing populations have developed in the lower Ohio River. Veligers occur in the plankton beginning as early as March and extending into winter. Population growth rates in some areas have been rapid, and individuals reach a length of from 16 to 24 mm in their first year. In dense populations during the second year of recruitment, new recruits are retarded in growth by the larger individuals. Zebra mussels are attaching to native unionid clams and snails as well as to other submerged substrates including barge hulls. Although sufficient opportunities exist for zebra mussels to be transported into the Tennessee River, and large numbers of adults occur within the locks, no reproducing populations have been found, nor have veligers been detected in the Tennessee River plankton. This suggests that something is preventing successful reproduction and development in the Tennessee River.

ACKNOWLEDGMENTS

Support for this project was provided in part by the Murray State University Committee on Institutional Studies and Research and the Center for Reservoir Research. Assisting in the collection of zebra mussels from the Ohio River were D. Craig Fortenbery and Donald Fortenbery of Mainstream Commercial Divers.

REFERENCES

Haag, W.R., D.J. Berg, and D.W. Garton. 1991. *Dreissena polymorpha* colonies encrusting native unionid bivalves produces species-specific and sex-specific effects. J. Shellfish Res. 10:259.

Haag, W.R., D.J. Berg, D.W. Garton, and J.L. Farris. 1993. Reduced survival and fitness in native bivalves in response to fouling by the introduced zebra mussel (*Dreissena polymorpha*) in western Lake Erie. Can. J. Fish. Aquat. Sci. 50:1-8.

Hebert, P.D.N., B.W. Muncaster and G.L. Mackie. 1989. Ecological and genetic studies on *Dreissena polymorpha* (Pallas): a new mollusc in the Great Lakes. Can. J. Fish. Aquat. Sci., 46:1587-1591.

Hebert, P.D.N., C.C. Wilson, M.H. Murdoch and R. Lazar. 1991. Demography and ecological impacts of the invading mollusc *Dreissena polymorpha*. Can. J. Zoology 69:405-409.

Hunter, R.D. and J.F. Bailey. 1992. *Dreissena polymorpha* (zebra mussel): Colonization of soft substrata and some effects on unionid bivalves. The Nautilus 106(2):60-67.

Leek, Denise A. and James B. Sickel. 1994. Growth of zebra mussels in Tennessee River water with and without added calcium. Abstract: Bulletin of the North American Benthological Society, 11(1):192.

Mackie, G.L. 1991. Biology of the exotic zebra mussel, *Dreissena polymorpha*, relative to native bivalves and its potential impact in Lake St. Clair. Hydrobiologia 219:251-268.

Nichols, S. Jerrine. 1993. Maintenance of the zebra mussel (*Dreissena polymorpha*) under laboratory conditions. *In:* Zebra Mussels: Biology, Impacts and Control. Thomas F. Nalepa and Donald W. Schloesser (editors). Lewis Publishers, Boca Raton . 810 pp.

Ramcharan, C.W., D.K. Padilla and S.I. Dodson. 1992. Models to predict potential occurrence and density of the zebra mussel, *Dreissena polymorpha*. Canadian Journal of Fisheries and Aquatic Sciences, 49(12):2611-2620.

Reeders, H.H, and A. bij de Vaate. 1990. Zebra mussels (*Dreissena polymorpha*): a new perspective for water quality management. Hydrobiologia 200/201:437-450.

Sprung, Martin. 1987. Ecological requirements of developing *Dreissena polymorpha* eggs. Arch. Hydrobiol. Suppl. 79:69-86.

TVA. 1991. Zebra Mussel Found in Tennessee River. News Release from Tennessee Valley Authority, Knoxville, Tennessee. September 18, 1991.

Vermeij, Geerat J. 1991. When biotas meet: understanding biotic interchange. Science 253:1099-1104.

Predicting Success of Riverine Populations of Zebra Mussels (*Dreissena polymorpha*) - Early Colonization and Microhabitat Distribution in the Ohio River.

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Abstract

We monitored 100 km of the Ohio River around Louisville, Kentucky from 1991-1994 for larval and settled zebra mussels, *Dreissena polymorpha*. Veligers, appearing first in May 1992, occurred at low densities (0.02-0.1/L), peaked in late summer to early autumn, and disappeared by November each year. Juveniles (post-veligers) were initially found in August 1993, occurred through October, and settled at ≤ 7 mussels/m^2/day in early September 1993. Benthic mussels were first detected in August 1993 at all sampled rocky sites at densities of 1-340 mussels/m^2 (shell lengths = 1-25 mm). Based on adult sizes and growth rates, juveniles must have first settled around September 1992. Mussels primarily occupied cobble, were typically attached to lower and side surfaces, and were randomly dispersed on these areas. Males and females were equally abundant and similar in size. Compared to lakes, primary factors likely to limit riverine populations of *Dreissena polymorpha* are lower primary production and greater siltation; favorable riverine factors, such as abundant oxygen and absence of thermoclines, should increase depth and latitudinal distribution of mussels. Aside from their probable negative impacts on native mussels and herbivorous zooplankton, we predict a net positive ecological effect from *D. polymorpha* on total riverine biomass and, possibly, species diversity.

Introduction

Zebra mussels, *Dreissena polymorpha* (Pallas), have spread rapidly into several major river systems in southeastern Canada and the eastern and midwestern portions of the United States since their successful invasion of the Great Lakes from Europe in the mid-1980s (Hebert et al. 1989, Mackie at el. 1989). The thermal and osmotic requirements of this exotic species should eventually allow them to disperse through much of Canada and the United States (Strayer 1991); and because of their realized and potential economic and ecological impacts, they pose serious threats to both industry and natural ecosystems (Mackie et al. 1989; Hebert et al. 1991; Mackie 1991; Hunter and Bailey 1992; MacIssac et al. 1992; Griffiths 1993).

Data on the dispersal and impacts of *Dreissena* spp. in North America and Europe have focused predominantly on lake populations, thereby providing little basis for predicting the likely success of zebra mussels in lotic environments (Strayer 1991). Only recently have ecologists examined the potential responses of dreissenids to lotic conditions (e.g., Alexander et al. 1994; Mellina and Rasmussen 1994). Although large rivers are sometimes described incorrectly as little more than slowly-flowing lakes, there are in fact major differences between lakes and large rivers in their physical, chemical, and biological characteristics (Ryder and Pesendorfer 1989; Thorp et al. 1994). Consequently, the population size, distribution, and ecological impacts of dreissenids may be correspondingly different in lentic and lotic ecosystems.

We describe here the results of a four year study (1991-1994) of the early colonization and microhabitat distribution patterns of *D. polymorpha* in a middle portion of the Ohio River near Louisville, Kentucky. Included are data on the densities of planktonic veligers, newly-settled juveniles (<1 mm long), and benthic mussels (≥ 1 mm long) as well as information on the microhabitat distribution, dispersion, and sex ratios of benthic mussels. We compare lake and river characteristics and predict responses of *D. polymorpha* to riverine conditions.

Materials and Methods

Sample Sites

The Ohio River is the second largest river in the United States, based on annual average discharge (3273 $m^3 \cdot s^{-1}$ for a 63 year period). Flow generally peaks in late winter through spring and reaches minimum levels in late summer through early fall. During our study, we recorded mid-channel surface current velocities of 0.2-1.7 $m \cdot s^{-1}$; velocities near shore ranged from 0-0.4 $m \cdot s^{-1}$ but were typically ≤ 0.12 $m \cdot s^{-1}$. Near Louisville, water temperatures ranged from 4-26°C during our study. Turbidity varied in a reasonably predictable pattern, with a average maximum of 40 NTU (nephelometric turbidity units) (or more, depending on the year) in early spring and a minimum value of 5-10 NTU in

summer through early fall.

The Ohio is a constricted-channel river around and upstream of Louisville and is approximately 800 m wide at Louisville. The river bottom is generally muddy near shore and gravelly to sandy in deeper mid-channel areas; large patches of limestone cobble and boulders are common in shallow water from Louisville upstream to Pittsburgh but disappear within 100 km downstream from Louisville as the Ohio becomes a floodplain river. However, rock has been added at numerous sites along the entire river for erosion control. Woody debris is also common, particularly away from the city near undeveloped mainland and island shores. All these hard substrates are important for settling juvenile zebra mussels. Vascular macrophytes, which are ephemeral and structurally less suitable for long term colonization by *D. polymorpha*, occur in patches along many parts of the river.

The initial year of sampling (1991) spanned over 350 km of the Ohio from the lowhead Cannelton Dam near Cloverport, KY upstream to Cincinnati, OH. Results of this zooplankton study are reported elsewhere (Thorp et al. 1994). Because no larval or settled benthic dreissenids were detected during the 12 monthly sampling dates of that study, the present paper concerns samples collected in 1992-1994 from a 100 km stretch of the Ohio River (Fig. 1) centered at the lowhead McAlpine Locks and Dam (MLD) in downtown Louisville (38°16'N, 85°47'W).

Veliger Monitoring

At 10-12 day intervals, 4 water samples were taken 11.5 km upstream of the McAlpine Dam (CPR in 1992-93) and 3.1 km below this navigation dam (NAR, in 1993 only) (Fig. 1). Two 50-L samples were pumped from 0.5-1 m depth near the river bank and filtered through a 63 µm mesh net; a third 50-L sample was pumped from the surface (0.5-1 m) in mid-channel; and a fourth sample was collected with a vertical plankton tow (63 µm mesh) from the bottom to the surface at mid-channel. All water samples were stored on ice, and the veligers were counted live in the laboratory within 24 h with a stereomicroscope (20 x) fitted with two parallel, horizontally-aligned polarizing filters (one above and one below the sample). The cross-polarized light produced by shifting the orientation of one filter by 90° allowed rapid and accurate counting of veligers, which appear as dark crosses on light shells; this effect is generated because of the birefringence of the calcareous shell under cross-polarized light (L. E. Johnson, and S. J. Nichols, pers. comm.). In this manner, zebra mussel veligers are easily distinguished from other zooplankton, detritus, algae, and sand grains. Veligers of the Asiatic clam, *Corbicula fluminea*, also have a birefringent pattern; so it was necessary to look for their shell striations under higher magnification to distinguish them from zebra mussels (S. J. Nichols, unpublished manuscript). Less than 1 % of all larvae collected, however, were *C. fluminea* in 1993.

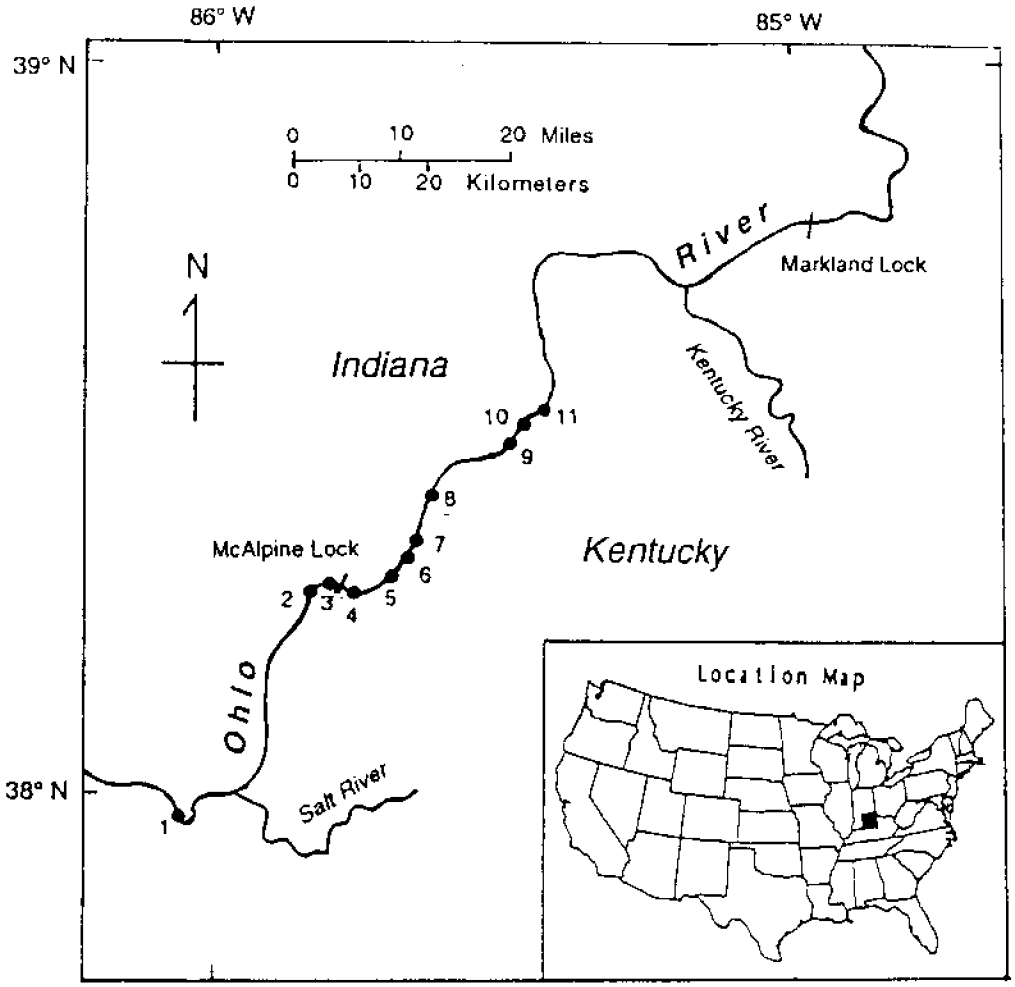

Figure 1. Location of all sampling sites in the Ohio River: (1) RHA: Rock Haven ramp (KY); (2) FRC: Falling Run Creek (IN); (3) NAR: New Albany ramp (IN); (4) FOW: Falls of the Ohio, below weir; (5) LWZ: Louisville Water Co. intake (KY); (6) JVR: Jeffersonville ramp (IN); (7) CPR: Cox Park ramp (KY); (8) LWP: Louisville Water Co. intake (KY); (9) EMI: across from 18 mile island (IN); (10) WES: upstream of Westport ramp (KY); (11) PAT: Patton's creek (KY). Downtown Louisville is between sites 3 and 4.

Juvenile Monitoring

During May through October 1993, we placed nylon mesh (bridal veil) samplers in the river to quantify the settling rate of juveniles (post-veligers). Sample units consisted of 0.1 m^2 (2.5 cm wide x 40 cm long) sections of nylon screen mesh, which were wadded into a loose ball and placed inside a larger container (vexar, 1.5 cm mesh opening). Samplers were set in the Ohio River at 10.1 km above the McAlpine Dam (LWZ) and

within a flowing water mesocosm at the Louisville Water Company's Payne water treatment station (LWP, Fig. 1). This mesocosm consists of 40 flow through channels (10 x 10 cm x 3.05 m long) which receive raw water directly from the nearby (1 km) Ohio River. We collected the veil every two weeks, alternating weekly between two samplers at each site. We searched for juveniles using a stereomicroscope and cross-polarized light.

Density, Size and Sex Ratio of Benthic Mussels

In conjunction with various projects on the Ohio River in 1991-92, we regularly examined hard substrates along the shoreline at several sites near Louisville for benthic zebra mussels; however, no *D. polymorpha* were found. In August 1993, we observed our first benthic mussels on natural substrate (at EMI, Fig. 1). After an initial survey by scuba diving, we confined our final quantitative sampling to 0.25-1 m depths where dreissenids were clustered (a few were retrieved from rocks and snags as deep as 4 m). During October and November 1993, ten sites along a 100-km stretch of the Ohio River, centered around Louisville, were examined for benthic mussels. At six sites (RHA, FRC, NAR, EMI, WES, PAT; Fig. 1), adult densities were determined with a 1 m^2 square, PVC pipe quadrat placed over random substrate areas. Five to twenty samples were collected at each site. Shell lengths (SL) of all individuals were measured to the nearest 0.01 mm. At four other sites (FOW, JVR, CPR, LWP), no quadrats were used; however, we collected and measured all zebra mussels during an intensive search of approximately 5-10 m^2 of substrate at each site. After pooling data from all 10 sites, we generated a frequency curve of the proportion of benthic mussels in each size class.

In June 1994, we collected 100 mature (9-23 mm) *D. polymorpha* from CPR (Fig. 1). Each mussel was dissected and gonadal tissue was observed under a compound microscope (400 X) to determine the sex of each animal. We tested the observed proportions of males and females against a 1:1 female:male pattern for all 100 animals and for each of three different size classes (9-14 mm, 15-19 mm, and 20-23 mm). In addition, we compared the mean size of mature males to mature females.

Microhabitat Distribution of Benthic Mussels

In November 1993, 10 rocks (linear dimensions approximately 10-20 cm) were collected at 0.25 - 1 m in depth from a site 11.5 km upstream of McAlpine Dam (CPR; Fig. 1). The distance (in mm) to their nearest neighboring mussel was measured for each zebra mussel on all rocks. This plotless technique evaluated microhabitat dispersion, using a method of testing for reciprocal pairs of nearest neighbors (Pielou 1977). In a randomly-dispersed population, 62.15% of the individuals are in reciprocal pairs, where each member of the pair is the other's closest neighbor; this percentage is the expected frequency used for comparing observed results with a chi-squared goodness-of-fit test

(Sokal and Rohlf 1981). A significant deviation from the expected value indicates either a clumped or uniform distribution.

In November 1993, we also collected mussels from 35 flat rocks (approximately 10-20 cm maximum dimension) lying horizontally in the river (but not buried) at a site 49.5 km below the dam (RHA; Fig. 1). Numbers of mussels on the upper and lower surfaces of each rock were tallied. The Wilcoxon matched-pairs signed-ranks test (Siegel 1956) was used to test whether zebra mussels were congregated on either lower or upper surfaces.

Results

Veliger Monitoring

Despite extensive sampling in 1991, we detected no dreissenid veligers until May 1992. Veligers were collected from July to November in 1992 and from July through October of 1993 (Fig. 2). No clear difference existed in the densities collected at different sites (from the bank, channel, or vertical tow, at both CPR and NAR), thus, we calculated mean veliger densities (numbers of veligers per liter) from all eight samples taken from the two sites each sampling date. In both years densities of veligers remained consistently low and peaked at an average of about 0.1 veligers/L. Although a clear larval-production pattern is not yet evident, data shown in Fig. 2 suggest a bimodal pattern with a weak mode in mid-July of both years followed by a prominent peak in late summer to early fall.

Juvenile Monitoring

Juveniles were not detected in 1992, although the size of benthic mussels collected in the late summer of 1993 suggests that a small amount of settling must have occurred in late 1992. The settling rate of juveniles in 1993 was unimodal, with peak numbers in August to September (Fig. 2). The peak in settled juveniles coincided with the second peak in veligers observed that year. Peak densities (3-7 juveniles settling/m^2/day) occurred throughout September.

Density, Size and Sex Ratio of Benthic Mussels

We collected zebra mussels from cobble, boulders, snags, and macrophytes in the Ohio River during 1993, but the overwhelming majority were on the rocks. Densities of mussels at the six principal sample sites were quite variable in 1993; the averages at the six sites ranged from 1 to 340 mussels/m^2 (highest value at the most downstream site, RHA). A preliminary survey in June 1994 indicated that the average density of mussels at one site (CPR) was 1,342 mussels/m^2 (based on eight 0.25 m^2 quadrat samples). Because all mussels collected in quadrats were attached to rocks, the densities recorded were affected

by the number of suitably-sized rocks in a quadrat. Although *D. polymorpha* were found on substrates as small as a snail (some pleurocerid snails held more than five mussels), most dreissenids inhabited rocks ≥ 5-10 cm in maximum linear dimension.

Settled mussels ranged in size from 1-25 mm SL; percentages of all individuals of different sizes are shown in Fig. 3. To distinguish young-of-the year (YOY) zebra mussels (i.e., those that settled in 1993) from those that had settled the previous year, we measured all zebra mussels that had colonized either our post-veliger, benthic samplers set out from May to November 1993 (at LWZ) or the flowing water stream mesocosm (at LWP), which began operating in May 1993. Estimates of the maximum size of YOY were based on monthly measurements of shell lengths of several hundred small zebra mussels that had colonized the sides of the mesocosm channels at LWP and benthic samplers at LWZ from September to November 1993; thus, these animals could not have been older

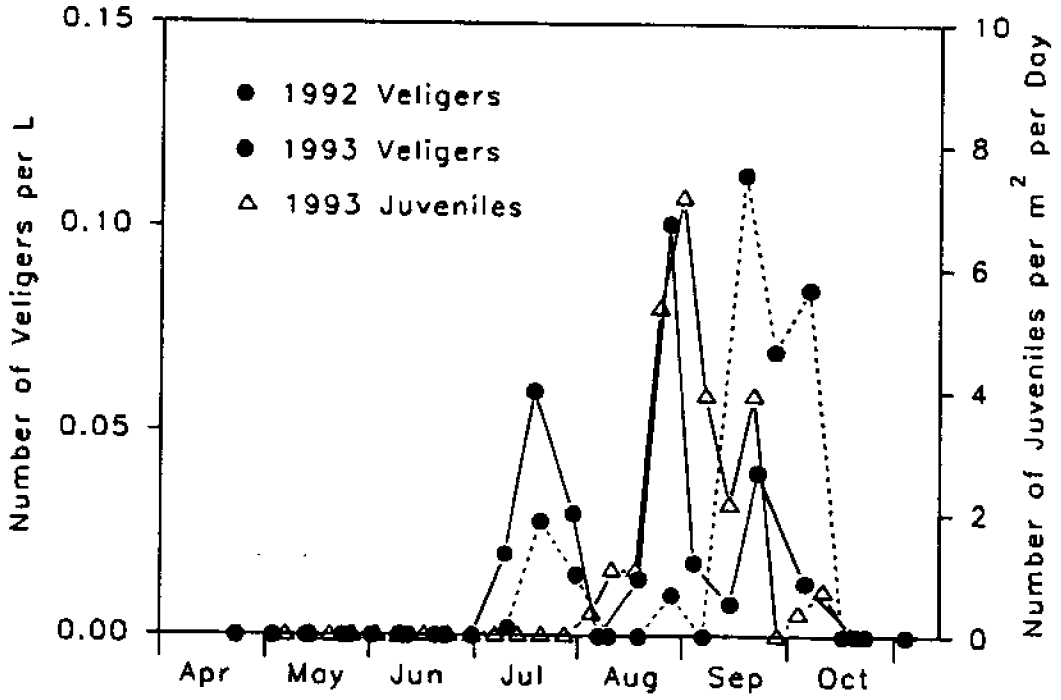

Figure 2. Zebra mussel veliger densities (at 3 and 7) and post veliger settlement (at sites 5 - 8).

than a few months at the time of our autumn survey. Based on these data, we estimated that the maximum shell length that could be attained by a YOY that settled in the 1993 was 10 mm by November. By applying this estimate to the size class data for Ohio River zebra mussels (Fig. 3), we calculated that a maximum of 9.4% of the *in situ* populations,

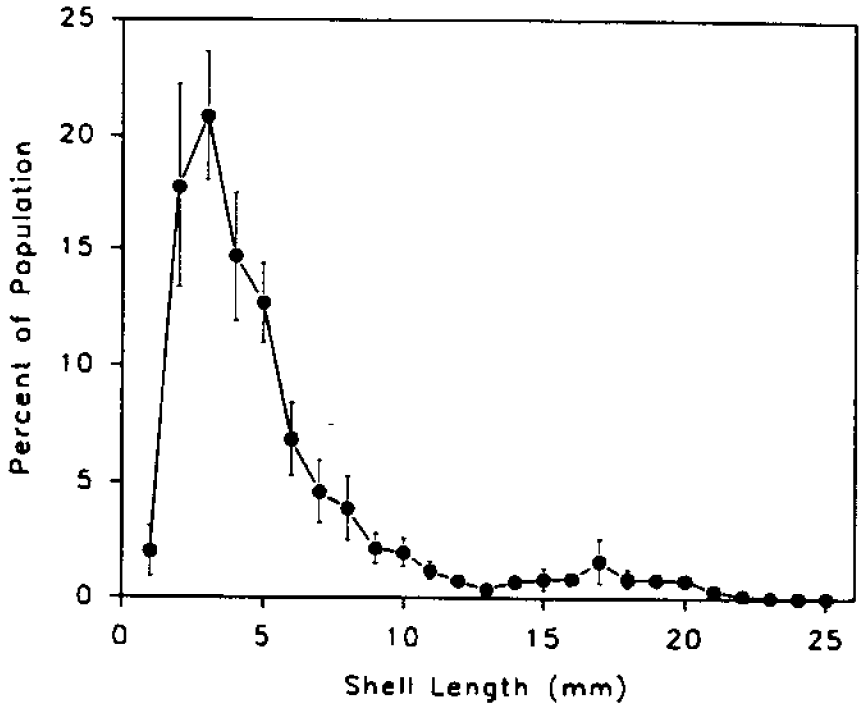

Figure 3. Proportion of Zebra mussel adults in each mm size class; data pooled from 10 sites located around Louisville, KY.

on average for the ten sites, were older than a YOY (i.e., they settled in August - October 1992 and were at least 11 mm long). Of the 100 mature mussels (9-23 mm long) that were sexed, 50 were males and 50 were females ($X^2 = 0$, $p > 0.9$). The percentage of females in each of three size classes (9-14 mm shell length, 15-19 mm, and 20-23 mm) was 49 %, 50 % and 54 % females, respectively. This strongly suggests a 1:1 sex ratio, regardless of size. The average size of the mature males was 15.44 mm ± 0.43 (S.E.), and the average size of females was 15.77 mm ± 0.45 (S.E.). This difference was not statistically significant ($t = 0.53$, $p > 0.5$).

Microhabitat Distribution of Benthic Mussels

Most zebra mussels occupied the sides and exposed undersurface of rocks (Fig. 4). In fact, 34 of the 35 flat rocks we examined had greater numbers of zebra mussels attached to the lower surface ($x = 7.46$, S.E. = 1.25) than to the upper surface ($x = 0.57$, S.E. = 0.18) (Wilcoxon matched-pairs signed-ranks test, $Z = -4.98$, $p < 0.001$).

A quantitative analysis of the early colonization dispersion pattern of zebra mussels on individual rocks showed it to be random, rather than uniform or clumped, within the

area they occupied. The mean distance to their nearest neighbor was 19.5 mm ± 15.0 (S.D.) for the small mussels (SL ≤ 10 mm) on the 10 rocks examined (Fig. 4). Out of 171 small zebra mussels collected, 107 (62.69%) were in reciprocal pairs (i.e., each member of a pair was each other's nearest neighbor). The expected percentage of reciprocal pairs is 62.15 % in a randomly-dispersed population. Therefore, because our results do not differ from theoretical predictions ($X^2 = 0.0129$, df = 1, $p > 0.9$), we cannot reject the null hypothesis of a random dispersion of zebra mussels on the sides and undersurface of

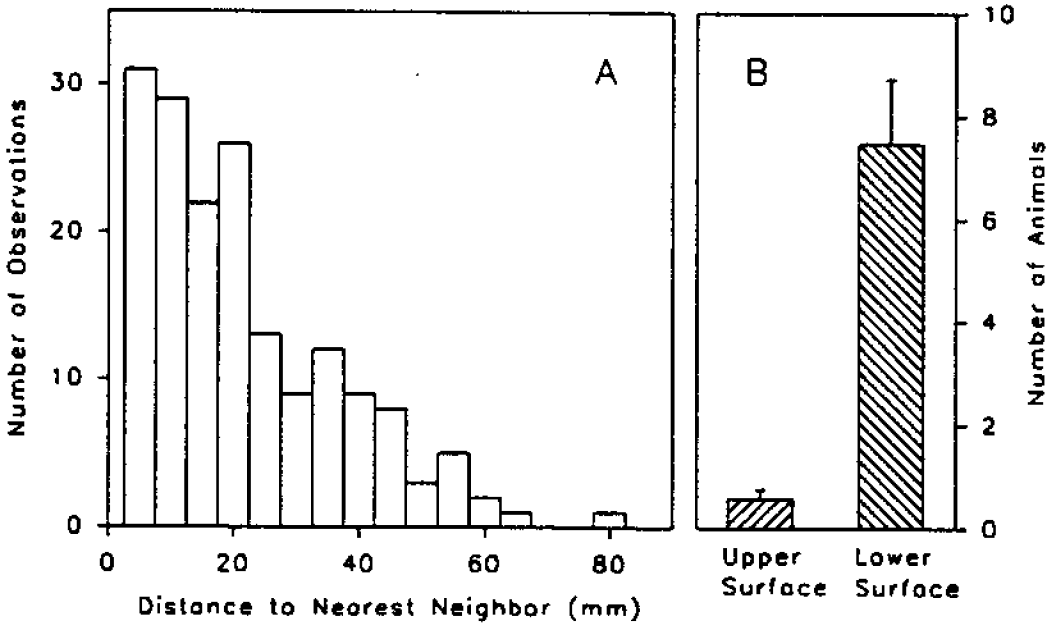

Figure 4. Small scale distribution patterns of zebra mussels on rocky habitats: the relative distances of nearest neighbors (A) and the proportion of zebra mussels on the upper and lower surfaces of rocks (B).

individual cobbles. Initial observations of mussels in 1994 suggest that the dispersion pattern becomes more clumped at higher densities.

Discussion

Even though streams and rivers are the primary natural habitats of most freshwater bivalve molluscs in Europe and North America (see McMahon 1991), aquatic ecologists

studying *D. polymorpha* have focused their research efforts on lake populations. Factors contributing to this research trend in North America include a general scarcity of ecologists working in navigable rivers and a relatively poor funding history for zebra mussel research in habitats outside the Great Lakes - the initial point of colonization by zebra mussels. If large rivers were not much more than slowly-flowing lakes, this research emphasis would scarcely matter; but given the erroneous nature of this portrayal (Ryder and Pesendorfer 1989; Thorp et al. 1994), it is crucial that predictions on effects of dreissenid molluscs in rivers be founded on data from these lotic ecosystems.

Features likely to influence the relative ecological success and impacts of dreissenids in North American river and lake habitats are described in Table 1. In the remainder of this paper, we interpret our field data in relation to some of these characteristics, compare our results with data obtained from other rivers as well as lakes, and provide preliminary estimates of the ultimate population size and ecological impact of *D. polymorpha* in the Ohio and other rivers.

Population Density

Zebra mussels escaped the confines of the Great Lakes and associated rivers during the late 1980s, entering both the Hudson River and the Mississippi River system (e.g., Whitney et al. 1993). *Dreissena polymorpha* were found on some living and dead unionid mussels and a few water intake pipes and boat hulls in the lower Ohio River as well as on most navigation dams in the Ohio from above Cairo, IL to near Pittsburgh, PA as early as 1991. By the end of 1993, the St. Lawrence, Hudson, Illinois, upper and lower Mississippi, Arkansas, Tennessee, Cumberland, and Ohio Rivers had adult populations of zebra mussels (O'Neill 1993). The pattern and rapidity of dispersal suggests that barge traffic was a primary vector for invasion.

We collected *D. polymorpha* veligers from the Ohio River in the vicinity of Louisville, KY, as early as May 1992. These larvae were consistently present throughout that summer and the next, but their densities were low (0.02-0.1/L) relative to maximum numbers of 50/L found in the lower Ohio below Paducah, KY (J. Jenkinson, pers. comm.), an area with a longer colonization history. Veliger densities in Lake Erie were also low during early colonization (< 3/L in 1989, Riessen et al. 1993) but rose rapidly thereafter (maximum values in 1990 of 137/L (Riessen et al. 1993); and 360/L (Fraleigh et al. 1993), for the eastern and western basins of Lake Erie, respectively). Veliger production in 1992 and 1993 in the middle portion of the Ohio River near Louisville seemed to follow a bimodal pattern with a weak mode in July followed by a much more prominent peak in late summer to early fall. Both bimodal and unimodal patterns of veliger production have been reported in Lake Erie, depending upon year and site (e.g., Garton and Haag 1993). Juveniles probably settled in late 1992, though we did not collect any until midsummer 1993, when settling rates reached approximately 7 animals/m^2/day. This maximum rate is substantially lower than the estimated peak settling rates in Lake Erie of 10 to1,885

juveniles/m^2/day for 1989 (Garton and Haag 1993) and 360/m^2/day for 1990 (Fraleigh et al. 1993). Garton and Haag (1993) estimated that 30,000 mussels per m^2 settled during the reproductive season of 1989, but only 17,000/^{2} settled in 1990.

Cobble, boulders, and wood snags were extensively sampled above and below Louisville through early August 1992 without any evidence of zebra mussels ≥ 1 mm long. However, the size of benthic zebra mussels first collected in August 1993 suggests that some settling must have occurred late in the previous reproductive year. Benthic zebra mussels were observed at every rock bed sampled in autumn 1993 along a 100 km stretch of the Ohio near Louisville, with densities ranging from 1-340 mussels/m^2. Based on size distributions and length-weight ratios, we estimate that the average biomass at peak densities (at RHA, Fig. 1) was 0.64 $g \cdot m^{-2}$ (shell-free dry biomass).

We expected the density of zebra mussels in the main stem of the Ohio River near Louisville to increase in the summer of 1994 by 1-2 orders of magnitude over 1993 values (resulting in average densities in shallow water rock beds of 2,000 to 5,000 mussels/m^2) for two reasons. First, benthic zebra mussel populations in the Ohio appear to have increased ten-fold between 1992 and 1993 (based on estimates from our size-class data). Second, similar trends have been detected in the Hudson River where numbers by the second to third year of dreissenid colonization reached upwards to 10-30,000 mussels/m^2 (D. Strayer, pers. comm.) with average densities of roughly 3,000/m^2 of rock bottom (J. Rasmussen, pers. comm.). Although we have not thoroughly surveyed the Ohio River population near Louisville in 1994, preliminary samples taken at one site (CPR) in June 1994 showed a four-fold increase in maximum density over the maximum observed in the vicinity in 1993. It is harder to predict the ultimate biomass of zebra mussel populations without knowing the thresholds of food and space competition; however, we expect the average individual biomass to increase at a slower rate than population density and then to decline as intraspecific competition intensifies.

Estimating maximum achievable population densities in rivers by comparing them with lakes is difficult at this time because of confounding factors, including the fact that we have tracked riverine populations from zero, whereas the oldest populations in the Great Lakes were not systematically observed during the initial few years of the invasion. Nonetheless, we hypothesize that the average density of *D. polymorpha* in rivers will be lower in general than those of lakes of comparable water quality because of the net effect of several positive and negative habitat characteristics that we expect to influence dreissenids (listed in Table 1 and summarized below).

Factors potentially limiting the success of *D. polymorpha* populations in rivers will usually include lower system primary productivity, poorer food quality, feeding and respiratory hinderance from sediment movement (Alexander et al. 1994), siltation problems, inadequate supply of hard substrates in floodplain rivers, fluctuations in river stage, and possible interference with settling at high current velocities (Table 1). Of these, the primary factors are likely to be ecosystem primary productivity, siltation/burial, and substrate limitations. Phytoplankton are typically less abundant in rivers than lakes (see

Table 6 in Ryder and Pesendorfer 1989), suggesting that food will be more limiting in rivers at comparable mussel densities. Mellina and Rasmussen (1994) indicated that densities of dreissenid mussels declined significantly in rivers as substrate size diminished. Within the Ohio River, hard substrates should quickly be limiting in the lower, floodplain portion of the river; the same will likely be true for most of the Mississippi River. However, substrate availability should not be limiting in the upper two-thirds of the Ohio for a few years, if ever, because that constricted-channel portion of the river has abundant rock beds.

Habitat features of rivers that should enhance population growth over that observed in lakes will normally include current-renewed food (albeit at lower total river phytoplankton production), little chemical or thermal stratification, higher oxygen levels throughout the water column, typically less ice cover and scouring, and possibly more optimal temperature patterns (Table 1). Many of these factors will influence within-habitat (e.g., depth ranges) and between-habitat distribution (e.g., latitudinal range) to an equal or greater extent than they affect population density. For example, *D. polymorpha* populations in rivers should colonize farther south in the United States than those in lakes because of lower summer temperature maxima in rivers, compared to lake epilimnia (where mussel food resources predominate). In addition, mussels can probably disperse geographically at a faster rate in rivers than in lakes.

Habitat Distribution and Dispersion

Almost all adult mussels collected in the lower Ohio during 1993 were on rocks, but a few individuals were taken from snags and aquatic weeds, especially in floodplain areas lacking abundant rocks. Individuals collected in the Ohio River from the leaf blades of vascular macrophytes (e.g., *Vallisneria*) were uniformly small (< 2 mm), probably because the mussels lacked adequate growth time between emergence and die-back of the above-sediment portion of the plant. An explanation for the scarcity of zebra mussels on snags is less evident but may relate to substrate preference, as the density of *D. polymorpha* on snags tended to rise with decreasing numbers of rocks. Two other plausible reasons are that snags are not retained long in rivers (relative to the mussel life span), and they are more subject to exposure because most are in shallow water and extend on to the bank. We observed no zebra mussels on soft substrates in the Ohio River, and they also appear to be absent from mud in the St. Lawrence and Hudson Rivers (J. Rasmussen, pers. comm.), except possibly where hard objects exist in the mud .

The vertical distribution range of *D. polymorpha* in rivers should proportionately exceed that of lakes because rivers are generally well-mixed and lack thermoclines. Therefore, a thermal, chemical, and physical barrier is absent which could otherwise impede movement of phytoplankton and lead to inhospitable oxygen and thermal conditions. Deep water habitats may have some disadvantages, however, because of their typically higher current velocities. During scuba diving, one of us (JHT) found zebra

mussels as deep as 4 m (low light hindered a deeper survey), and we suspect they occurred in the deepest part of the river (6-8 m at that site); however, densities appeared to be lower past 1-2 m, so quantitative sampling was restricted to shallow water.

Unlike Lake Erie, where zebra mussels are abundant on the tops and sides of rocks (J. Thorp, pers. observ.), the overwhelming majority of *D. polymorpha* in the Ohio River occupied the undersurface and sides of rocks. Rocks partially buried in the sediment (such as we observed while diving below 3 m in the Ohio) had few if any zebra mussels; no *D. polymorpha* could, we presume, live on the underside of those rocks. Zebra mussels in shallow waters of the Hudson River are also found primarily on the undersurface of stones; those in deep water were frequently observed on the upper surface of rocks (D. Strayer, pers. comm.).

Why is the microhabitat distribution of *D. polymorpha* different in Lake Erie and these two rivers? We hypothesize that several distinct disadvantages accrue to mussels living on the top side of a river rock. In comparison to the undersurface of a rock, the upper surface should be exposed to higher siltation, greater growth of filamentous algae on the rock and mussel shell (which may interfere with feeding), and more predation from molluscivorous fish. Lake *D. polymorpha* populations are not subjected to the high siltation and possible smothering which face river populations. Food can easily reach mussels attached to the undersurface of rocks in rivers because of persistent water currents. Living under rocks could be disadvantageous if predation from crayfish or small benthic fish exceeded that potentially occurring on the top surface. Predator avoidance might be another reason for selecting a rock over snag, as the latter would generally provide lesser protection all around its outer surface than afforded by the underside of a stone. The occurrence of *D. polymorpha* on the upper surface of stones in Lake Erie and deep water areas of the Hudson and Ohio Rivers may reflect either intense competition for space, partial burial of most available hard surfaces, or conditions different from those typical of shallow water in rivers.

We initially suspected that zebra mussels would be uniformly dispersed on rocks, because of qualitative observations of the distribution of large zebra mussels (SL $\geq$ 20 mm) on rip-rap stones below Paducah, KY in the floodplain portion of the Ohio. A uniform dispersion should also improve average filtration efficiency for the occupants of a stone. A quantitative analysis of 171 zebra mussels on 10 rocks near Louisville, however, clearly indicated a random dispersion within the microhabitat selected by the mussels. In another study (E. Marsden, unpublished manuscript), a clumped dispersion pattern was observed. It is not apparent at present whether dispersion is influenced by mean body size and density of *Dreissena*, as both factors could influence potential intraspecific interactions through exploitative or interference competition.

Ecological Impacts in Rivers

Dreissena polymorpha could have several significant positive and negative ecological impacts on riverine ecosystems. On the negative side, evidence accumulating in both the Great Lakes (Hebert et al. 1991; Mackie 1991; Hunter and Bailey 1992) and large rivers (Whitney et al. 1993) suggests that *Dreissena* eliminates native populations of unionid mussels. This problem may be especially acute in the highly-diverse Tennessee and Ohio River valleys where many species of unionid mussels are currently on the state and federal lists of threatened and endangered species. Refuges for native mussels may be limited to non-navigable rivers or possibly to more ephemeral headwater streams. Ecologists have also concluded that zebra mussels significantly depressed phytoplankton density in the Hudson River (D. Strayer, pers. comm.), which will directly affect not only *D. polymorpha*'s future population size but also those of other planktivores. Preliminary experiments we conducted in outdoor tanks using Ohio River water and plankton seem to support this finding. These tank experiments also showed that zebra mussels could reduce suspended sediment levels (J. Thorp, unpublished data); however, we are less confident that mussel densities will ever be sufficient to reduce significantly the heavy inorganic turbidity of the Ohio. On the positive side, *D. polymorpha* should enhance benthic habitat heterogeneity (providing refuges for small invertebrates), shorten nutrient spiraling lengths (through better retention of organic matter), and increase total ecosystem secondary production by extracting organic matter from the plankton (some of which would otherwise be exported) and adding it to the benthos, thus providing food for predators (molluscivores), scavengers (e.g., amphipods feed heavily on dead mussels in the lab), and detritivores (consumption of zebra mussel feces and pseudofeces). Positive benefits of the shift of energy from pelagic to benthic food webs have been noted previously in lakes (Hebert et al. 1991; Mackie 1991; Hunter and Bailey 1992; MacIssac et al. 1992; Griffiths 1993). Therefore, aside from their negative effects on native unionids and potential competition with herbivorous zooplankton, we predict a net positive ecological effect in rivers from the zebra mussel invasion, as judged by increases in ecosystem biomass and, possibly, benthic species diversity.

Acknowledgments

The authors thank Dr. G. A. Cobbs for statistical and editing assistance and Drs. K. H. Haag and J. Jack for advice and field help. Dr. V. Wiedeman helped construct several figures. We thank Dr. D. Strayer for comments on an earlier draft of this manuscript. This research was supported by contracts from the Louisville Water Company, Cincinnati Gas and Electric Company, Public Services Indiana, Kenton County Water Company, American Water Works Association Research Foundation (733-91) and U.S. Environmental Protection Agency, ERL - Duluth (CR820263-01-0).

Table 1. Hypothesized differences between lakes and large rivers that could influence population sizes and ecological impacts of *Dreissena*.

Factor	**Characteristics in Rivers and Lakes**	**Possible Influences on Riverine *Dreissena***
Erosion/siltation	Greater in rivers and seasonally fluctuating	Negative; limit to distribution and habitats; increased energetic costs from avoiding burial
Turbidity/sediment transport	Primarily from phytoplankton in lakes (except some reservoirs) but greater and abiotic in rivers during high flow, with some biotic in low flow warm periods	Negative from suspended sediment; interferes with feeding and increases energetic costs
Permanent hard substrates	Variable and habitat-dependent; limited to snags and artificial surfaces in floodplain rivers; more abundant in constricted channel rivers	More limiting to population sizes in floodplain rivers, except where rocks are more abundant than in lakes
Ice formation/scouring	Less common in rivers of same latitude	Positive; upper depth less likely to be set by ice scour in rivers
Water depth fluctuations	Greater and more rapid in rivers but differences decrease somewhat in dammed rivers	Shallow water distribution limited by fluctuations in river stage; seasonal flooding could strand mussels (especially veligers and early juveniles)

Table 1 cont.

Factor	Characteristics in Rivers and Lakes	Possible Influences on Riverine *Dreissena*
Directional water currents	Present consistently only in rivers	Net positive because of access to food and oxygen, waste disposal, and dispersal of young downstream, but negative because of difficulty moving upstream
Water circulation patterns	Mostly wind-driven in lakes but gravity flow in rivers; vertical circulation poor in lakes but excellent in rivers; helical flow in rivers	Improved access to food and oxygen in deeper waters; more stable thermal patterns
Temperature/depth Profille	Thermoclines occur in lakes but rivers are well-mixed circulation poor in lakes but excellent in rivers; helical flow in rivers	Positive; metabolic rate more more constant with depth; reduced oxygen or thermal stress
Temperature extremes and variability	Depth/time dependence; lesser diurnal changes over entire water column in rivers than lakes of comparative depths; diurnal variations greater in lakes than rivers for shallow water but lesser diurnal and annual changes in deep waters of lakes	Minor effects from differences in diurnal thermal changes but the seasonally less extreme temperatures in colonizable portions of rivers should allow greater latitudinal range expansion
Chemical stratification	Pronounced in lakes; rare in rivers	Positive; reduced chances of oxygen or pH stress in deep waters of rivers

Table 1 cont.

Factor	Characteristics in Rivers and Lakes	Possible Influences on Riverine *Dreissena*
Oxygen content and variability	Higher (average over water column) in rivers with lesser diurnal fluctuations	Positive (reduced oxygen stress averaged over water column)
Energy sources and amounts	Increased dependence on allochthonous sources in rivers; density of phytoplankton habitat-dependent; food more depth-depentent in lakes	Food quality declines as dependence on dead organic matter rises; growth rates variable depending on system productivity but probably lower in rivers; suspended sediment hinders general access to food in rivers; food more available to mussels in deep water in rivers compared to lakes
Space competition	Comparable in type and numbers	Neutral; space competition is mostly intraspecific in both systems because of low numbers of sedentary species requiring hard substrates
Food competition	Bivalve competitors more numerous in rivers but other planktivores more abundant in lakes	Probably neutral; interference competition should eliminate food competition from unionid mussels; other planktivores should be either less efficient or not sufficiently abundant to cause problems

Table 1 cont.

Factor	Characteristics in Rivers and Lakes	Possible Influences on Riverine *Dreissena*
Predation	Planktivorous fish and invertebrates relatively more common in lakes; unknown differences in relative abundance of fish able to eat adult *Dreissena*; diving waterfowl probably more abundant in lakes	Probably relatively positive in rivers for veligers because of fewer planktivores but unknown impacts of adults relative to lakes; predatory regulation of mussel populations is unlikely in either ecosystem

References

Alexander, J. E., Jr., J. H. Thorp, and R. D. Fell. 1994. Turbidity and temperature effects on oxygen consumption in zebra mussels (*Dreissena polymorpha*). Can. J. Fish. Aquat. Sci. 51:179-184.

Fraleigh, P. C., P. L. Klerks, G. Gubanich, G. Matisoff, and R. C. Stevenson. 1993. Abundance and settling of zebra mussel (*Dreissena polymorpha*) veligers in western and central Lake Erie, p. 129-142. *In:* T. F. Nalepa and D. W. Schloesser [Eds.], Zebra mussels: biology, impacts, and control. Lewis Publishers, Boca Raton, Florida.

Garton, D. W. and W. R. Haag. 1993. Seasonal reproductive cycles and settlement patterns of *Dreissena polymorpha* in western Lake Erie, p. 111-128. *In:* T. F. Nalepa and D. W. Schloesser [Eds.], Zebra mussels: biology, impacts, and control. Lewis Publishers, Boca Raton, Florida.

Griffiths, R. W. 1993. Effects of zebra mussels (*Dreissena polymorpha*) on the benthic fauna of Lake St. Clair, p. 415-437. *In*: T. F. Nalepa and D. W. Schloesser [Eds.], Zebra mussels: biology, impacts, and control. Lewis Publishers, Boca Raton, Florida.

Hebert, P. D. N., B. W. Muncaster, and G. L. Mackie. 1989. Ecological and genetic studies on *Dreissena polymorpha* (Pallas): a new mollusc in the Great Lakes. Can. J. Fish. Aquat. Sci. 46:1587-1591.

Hebert, P. D. N., C. C. Wilson, M. H. Murdoch, and R. Lazar. 1991. Demography and ecological impacts of the invading mollusc *Dreissena polymorpha*. Can. J. Zool. 69:405-409.

Hunter, R. D. and J. F. Bailey. 1992. *Dreissena polymorpha* (zebra mussel): colonization of soft substrata and some effects on unionid bivalves. Nautilus 106:60-67.

MacIsaac, H. J., W. G. Sprules, O. E. Johannsson, and J. H. Leach. 1992. Filtering impacts of larval and sessile zebra mussels (*Dreissena polymorpha*) in western Lake Erie. Oecologia 92:30-39.

Mackie, G. L., W. N. Gibbons, B. W. Muncaster and I. M. Gray. 1989. The zebra mussel, *Dreissena polymorpha*: a synthesis of European experiences and a preview for North America. Ontario Ministry of Natural Resources, Water Resources Branch, Great Lakes Section. Queen's Printer, Toronto, Ontario.

Mackie, G. L. 1991. Biology of the exotic zebra mussel, *Dreissena polymorpha*, in

relation to native bivalves and its potential impact in Lake St. Clair. Hydrobiologia 219:251-268.

McMahon, R. F. 1991. Mollusca: Bivalvia, p. 315-399. *In:* J. H. Thorp and A. P. Covich [Eds.], Ecology and classification of North American freshwater invertebrates. Academic Press, San Diego.

Mellina, E. and J. B. Rasmussen. 1994. Patterns in the distribution and abundance of zebra mussels (*Dreissena polymorpha*) in rivers and lakes in relation to substrate and other physico-chemical factors. Can. J. Fish. Aquat. Sci. 51(5):(In Press).

O'Neill, C. R., Jr. [Ed.]. 1993. *Dreissena polymorpha* information review. Volume 4, No. 5, Zebra Mussel Clearinghouse, Brockport, NY.

Pielou, E. C. 1977. Mathematical Ecology (2nd Ed.). John Wiley and Sons, Inc. NY.

Riessen, H. P., T. A. Ferro, and R. A. Kamman. 1993. Distribution of zebra mussel (*Dreissena polymorpha*) veligers in Lake Erie during the first year of colonization, p. 143-152. *In:* T. F. Naiepa and D. W. Schloesser [Eds.], Zebra mussels: biology, impacts, and control. Lewis Publishers, CRC Press, Inc., Boca Raton, Florida.

Ryder, R. A. and J. Pesendorfer. 1989. Large rivers are more than flowing lakes: a comparative review, p. 65-85. *In:* D. P. Dodge [Ed.], Proceedings of the international large river symposium (LARS). Can. Spec. Public. Fish. Aquat. Sci. 106.

Siegel, S. 1956. Nonparametric statistics for the behavioral sciences. McGraw-Hill, NY.

Sokal, R. R. and F. J. Rohlf. 1981. Biometry. 2nd Ed. W. H. Freeman and Co. San Francisco.

Strayer, D. L. 1991. Projected distribution of the zebra mussel, *Dreissena polymorpha*, in North America. Can. J. Fish. Aquat. Sci. 48:1389-1395.

Thorp, J. H., A. R. Black, K. H. Haag, and J. D. Wehr. 1994. Zooplankton assemblages in the Ohio River: seasonal, tributary, and navigation dam effects. Can. J. Fish. Aquat. Sci. (In Press).

Whitney, S. D., K. D. Blodgett, and R. E. Sparks. 1993. Zebra mussels and native unionids in the Midwest: an update. Illinois River, p. 8-9. *In:* T. D'Erchia [Ed.], River Almanac. National Biological Survey, Environmental Management Technology Center, Onalaska, WI.

4th International Zebra Mussel Conference '94 Proceedings

GENERAL BIOLOGY

Identification of Larval and Postlarval Zebra Mussels and Co-occurring Bivalves in Freshwater and Estuarine Habitats using Shell Morphology

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Abstract

Studies on the spread, recruitment, ecological impact, or control of nuisance bivalves require reliable species identification, especially of larval and postlarval stages. We have used scanning electron microscopy (SEM) techniques to discriminate among the larval and postlarval stages of the zebra mussel (Dreissena polymorpha) and other potentially co-occurring bivalves from freshwater and estuarine habitats, such as the Asiatic clam (Corbicula fluminea), the dark false mussel (Mytilopsis leucophaeata), and the wedge clam (Rangia cuneata). Detailed SEM micrograph sequences of larval and postlarval stages illustrate differences in hinge morphology and shell morphometery that appear to be species-specific. For example, larvae of D. polymorpha can be distinguished from the other species above based on their prominent hinge teeth. Postlarvae of D. polymorpha do not appear to have lateral or cardinal teeth and they develop an elongated shell directed in a postero-ventral direction with a relatively straight anterior margin and a somewhat square posterior margin. The above diagnostic features allow discrimination among the crucial, early life stages of bivalve species collected in plankton or benthic samples.

Introduction

The continuing, rapid advance of the zebra mussel, Dreissena polymorpha, throughout the inland freshwaters of North America and towards the rivers and estuarine environments of the heavily populated and industrial mid-Atlantic region is of great concern. Based on the distribution of D. polymorpha in Europe, Strayer (1991) and Strayer & Smith (1992) predicted that D. polymorpha will soon become widespread and abundant throughout much of the fresh and brackish waters of North America. Similar rapid invasions of North American freshwaters and estuaries have been experienced with the introduction of the biofouling Asiatic clam, Corbicula fluminea (McMahon 1982, Counts 1986) and the Asian clam, Potamocorbula amurensis (Carlton et al. 1990). Recently, Walton (1993) confirmed the spread of D. polymorpha into the oligohaline (i.e., salinity ≤ 6 ppt) reach of the Hudson River estuary. Thus, it is likely that this prolific and highly dispersive mussel will also expand down the Delaware and Susquehanna Rivers toward Delaware Bay and Chesapeake Bay, respectively.

In the course of this range expansion, zebra mussel populations will likely overlap with several other regional bivalve species that tolerate similar levels of salinity (Table 1) and that produce planktonic larvae. Indeed, beyond its co-occurence in freshwaters with populations of the quagga mussel, Dreissena bugensis, and the Asiatic clam, C. fluminea, the zebra mussel now overlaps with one brackish water species, the dark false mussel Mytilopsis leucophaeata (Walton 1993), and may soon co-exist with a second, the wedge clam Rangia cuneata. As a result, the continued effort to monitor and control the zebra mussel, particularly at the larval and postlarval life stage, will become more problematic since these important life stages can be difficult to distinguish in plankton and benthic samples (e.g., Lutz 1985).

While a limited number of identification guides for early life stages of D. polymorpha do exist (Mackie et al. 1989, Hopkins & Leach 1992, Conn et al. 1993, Nichols & Black 1994) it is difficult to unambiguously identify and discriminate larval and early postlarval specimens of this species from those of other bivalves that co-exist in freshwater and oligohaline environments. This is particularly true at the early (straight-hinge) larval stage. In this study we present data on shell morphology, derived from scanning electron and light micrograph sequences of larvae and postlarvae, that allows discrimination among Dreissena spp., C. fluminea, M. leucophaeata, and R. cuneata.

Materials and Methods

Larvae and postlarvae of Dreissena spp.were collected from the St. Lawrence River between Massena, New York, and Cornwall, Ontario. Larvae were collected in plankton nets and postlarvae were scraped from adult shells and other hard substrates (Conn et al. 1993). The collection area was populated with reproductively active zebra mussels, Dreissena polymorpha. However, because there was a small, reproducing population of the quagga mussel, Dreissena bugensis, upstream from the collection sites, there is a remote possibility that D. bugensis was included in our samples. For the purposes of this study, we will refer to these specimens as Dreissena spp. and will assume that both D. polymorpha and D. bugensis can be distinguished from the other study species based on the characters described herein.

Adult C. fluminea and M. leucophaeata were collected from fresh and brackish water habitats, respectively, in Maryland (Kennedy et al. 1991, Conn et al. 1993) and adult R.cuneata were collected from a brackish water habitat in the Cohansey River, New Jersey (approx. 39° 22' N; 75° 22' W). Larval C. fluminea were released at the pediveliger stage from adult animals held in the laboratory and postlarvae were collected from field locations (Kennedy et al. 1991). Adult M. leucophaeata and R.cuneata were spawned in the laboratory and the resultant larvae and postlarvae were reared following the procedures of Loosanoff & Davis (1963).

Specimens were preserved in 95% ethanol (Lutz et al. 1982, Kennedy et al. 1991) and were later rinsed in deionized water and immersed in a 5.25% solution of sodium hypochlorite for about 10 - 30 min to remove soft tissues and to disarticulate shell valves (after Rees 1950). Valves were rinsed in deionized water, dehydrated in ethanol (95 and 100%), air dried, and mounted on 3M-850 or double-sided tape. Specimens were coated under vacuum with approximately 600 Å of gold-palladium (Fuller & Lutz 1989) and photographed with an Amray 1830I or an ETEC Autoscan scanning electron microscope (SEM). Consistent alignment of specimen valves and machine calibration was performed according to Fuller et al. (1989) to facilitate accurate shell shape and dimensional analyses. The outlines of photographed shell valves were digitized using Morphosys software (Exeter, Inc.), were converted to average eigenshapes, and were plotted using Lpshape (Lohmann & Schweiter 1990).

Results and Discussion

Comparison of shell shapes and hinge structures provide diagnostic, species-specific features that can be used to identify both larval and postlarval specimens collected in plankton and benthic samples. Larval shells of Dreissena spp., M. leucophaeata, and R.cuneata are similar in size and shape (Fig. 1). In contrast, the larvae of C. fluminea, which are released as pediveligers, have large, D-shaped valves (Fig. 1). Further, a large portion of the external shell surface (corresponding to the prodissoconch II region) of Dreissena spp., M. leucophaeata, and R.cuneata consists of numerous commarginal growth ridges, whereas most of the shell surface (corresponding to the prodissoconch I region) of C. fluminea is smooth to irregular in sculpture, with a few commarginal ridges confined to the periphery of the shell (Kennedy et al. 1991). The larval hinge structure of Dreissena spp. is distinct from the other species in that it has numerous, small denticles, as opposed to the relatively featureless hinge region of M. leucophaeata, R.cuneata, and C. fluminea (Fig. 2).

After metamorphosis to the benthic life stage, differences in the shape of shell valves becomes more marked. In both Dreissena spp.and M. leucophaeata shell growth proceeds mainly along the postero-ventral margin, resulting in an elongation primarily in shell height (the maximal dimension from the umbo to the ventral margin) (Fig. 3). The shape of Dreissena spp. differs from that of M. leucophaeata in that the anterior margin is much straighter and the posterior margin is more square than M. leucophaeata. By comparison, shell growth in R.cuneata and C. fluminea proceeds mainly along the posterior-anterior axis, resulting in an elongation in shell length relative to height. The external surface of C. fluminea is distinct from the other species in that it has strong, widely spaced commarginal ridges as opposed to the fine ridges of Dreissena spp., M. leucophaeata, and R.cuneata. The hinge structure of Dreissena spp.and M. leucophaeata is similar in that both possess prominent anterior beaks with no evidence of hinge teeth (Fig.

4). In contrast, neither R.cuneata or C. fluminea have a prominent beak and both have relatively large cardinal and lateral teeth (Fig. 4). A difference in the hinge dentition of R.cuneata and C. fluminea is established in valves about 2 mm long; here, R.cuneata has two cardinal teeth (anterior and central), whereas C. fluminea has three (anterior, central, posterior).

Acknowledgements

New Jersey Agricultural Experiment Station publication H-32407-1-94 and Institute of Marine and Coastal Sciences, Rutgers University, contribution 94-20. Support provided by New Jersey State funds, NOAA Sea Grant R/E-45ZM to RAL and BSB, Rutgers University, and the Fisheries and Aquaculture Technology Extension Center, Rutgers University.

Literature Cited

Castagna, M., Chanley, P. 1973. Salinity tolerance of some marine bivalves from inshore and estuarine environments in Virginia waters on the western mid-Atlantic coast. Malacologia 12: 47-96.

Carlton, J. T., Thompson, J. K., Schemel, L. E., Nichols, F. H. 1990. Remarkable invasion of San Francisco Bay (California, USA) by the Asian clam Potamocorbula amurensis. I. Introduction and dispersal. Mar. Ecol. Prog. Ser. 66: 81-94.

Conn, D. B., Lutz, R. A., Hu, Y-P., Kennedy, V. S. 1993. Guide to the identification of larval and postlarval stages of zebra mussels Dreissena spp. and the dark false mussel Mytilopsis leucophaeata. New York Sea Grant Institute. 22 p.

Counts, C. L., III 1986. The zoogeography and history of the invasion of the United States by Corbicula fluminea (Bivalvia: Corbiculidae). American Malacological Bulletin, Special Edition 2: 7-39.

Filice, F. P. 1958. Invertebrates from the estuarine portion of San Francisco Bay and some factors influencing their distribution. Wasmann J. Biol. 16: 159-211.

Fritz, L. W., Ragone, L. M., Lutz, R. A. 1990. Microstructure of the outer shell layer of Rangia cuneata (Sowerby, 1831) from the Delaware River: Applications in studies of population dynamics. J. Shellfish. Res. 9: 205-213.

Fuller, S. C., Lutz, R. A. 1989. Shell morphology of larval and post-larval mytilids from the North-western Atlantic. J. mar. biol. Ass. U. K. 69: 181-218.

Fuller, S. C., Lutz, R. A., Pooley, A. 1989. Procedures for accurate documentation of shapes and dimensions of larval bivalve shells with scanning electron microscopy. Trans. Am. Microsc. Soc. 108(1): 58-63.

Hopkins, G. J., Leach, J. H. 1992. A photographic guide to the identification of larval stages of the zebra mussel (Dreissena polymorpha). In: Nalepa, T. F. and Schloesser, D. W. (eds.) Zebra Mussels: Biology, Impacts, and Control. Lewis Publishers, Inc. (CRC Press), Boca Raton, Fl., p. 761-772.

Kennedy, V. S., Fuller, S. C., Lutz, R. A. 1991. Shell and hinge development of young Corbicula fluminea (Muller) (Bivalvia: Corbiculoidea). American Malacological Bulletin 8(2): 107-111.

Loosanoff, V. L., Davis, H. C. 1963. Rearing of bivalve molluscs. Adv. Mar. Biol., 1: 1-136.

Lohmann, G. P., Schweiter, P. N. 1990. On eigenshape analysis. In: Rohlf, F. J. and Bookstein, F. L. (eds.) Proceedings of the Michigan Morphometrics Conference. Special Publication 2, Univ. Mich. Mus. Zool. p. 147-165.

Lutz, R. A., 1985. Identification of bivalve larvae and postlarvae: A review of recent advances. Am. Malacol. Union Inc. Bull., Spec. Ed. 1, p. 59-78.

Lutz, R. A., Goodsell, J., Castagna, M. , Chapman, S., Newell, C., Hidu, H. , Mann, R., Jablonski, D., Kennedy, V., Siddal, S., Goldberg, Beattie, R. H., Falmagne, C., Chestnut, A., Partridge, A. 1982. Preliminary observations on the usefulness of hinge structures for identification of bivalve larvae. J. Shellfish Res., 2:65-70.

Mackie, G. L. 1992. Biology of the zebra mussel (Dreissena polymorpha) and observations of mussel colonization on unionid bivalves in Lake St. Clair of the Great Lakes. In: Nalepa, T. F. and Schloesser, D. W. (eds.) Zebra Mussels: Biology, Impacts, and Control. Lewis Publishers, Inc. (CRC Press), Boca Raton, Fl., p. 153-165.

McMahon, R., F. 1982. The occurrence and spread of the introduced asiatic freshwater clam, Corbicula fluminea (Muller), in North America: 1924-1982. The Nautilus 96(4): 134-141.

Nichols, S. J., Black, M. G. 1994. Identification of larvae: The zebra mussel (Dreissena polymorpha), the quagga mussel (Dreissena rostriformis bugensis), and the Asian clam (Corbicula fluminea). Can. J. Zool. v72 In press.

Rees, C. B. 1950. The identification and classification of lamellibranch larvae. Hull Bull. Mar. Ecol., 3: 73-104.

Strayer, D. L. 1991. The projected distribution of the zebra mussel, Dreissena polymorpha, in North America. Can. J. Fish. Aquat. Sci. 48: 1389-1395

Strayer, D. L., Smith. L. C. 1992. Distribution of the zebra mussel (Dreissena polymorpha) in estuaries and brackish waters. In: Nalepa, T. F. and Schloesser, D. W. (eds.) Zebra Mussels: Biology, Impacts, and Control. Lewis Publishers, Inc. (CRC Press), Boca Raton, Fl., p. 715-728.

Walton, W. C. 1993. Invasion of the Hudson River estuary (N.Y., U.S.A.) by the zebra mussel, Dreissena polymorpha. Masters Thesis, Rutgers University, New Brunswick. 117 pp.

Table 1. Tolerance to estuarine salinity of Dreissena polymorpha and other bivalves producing planktonic larvae in freshwater and oligohaline habitats. Salinity given as parts per thousand (ppt).

Species	ppt	Source
Dreissena polymorpha	0 - 6	Strayer & Smith 1992 Walton 1993
D. bugensis	undetermined	
Corbicula fluminea	0 - 5	Filice 1958 Kennedy et al. 1991
Myrilopsis leucophaeata	0 - 30	Castagna & Chanley 1973
Rangia cuneata	1 - 18	Castagna & Chanley 1973 Fritz et al. 1990

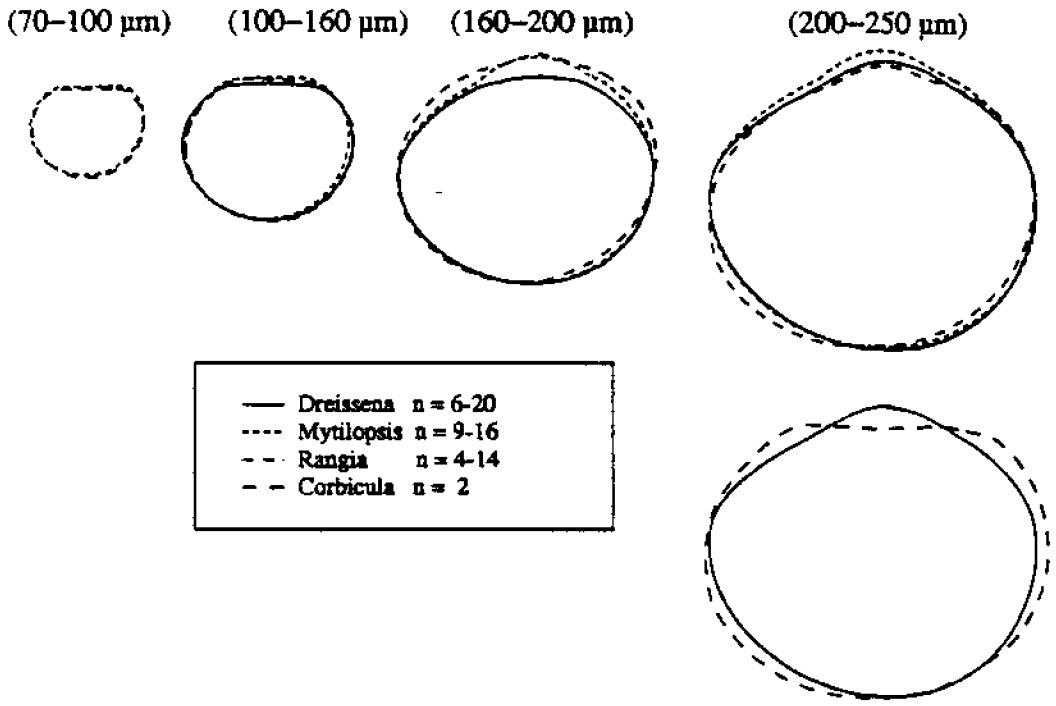

Fig. 1. Average larval shell shapes of Dreissena spp., Mytilopsis leucophaeata, Rangia cuneata, and Corbicula fluminea. Shapes taken from disarticulated right valves. Valves of Dreissena spp. < 100 µm were not available for study. Note the large, D-shaped C. fluminea valve (dashed line, bottom panel).

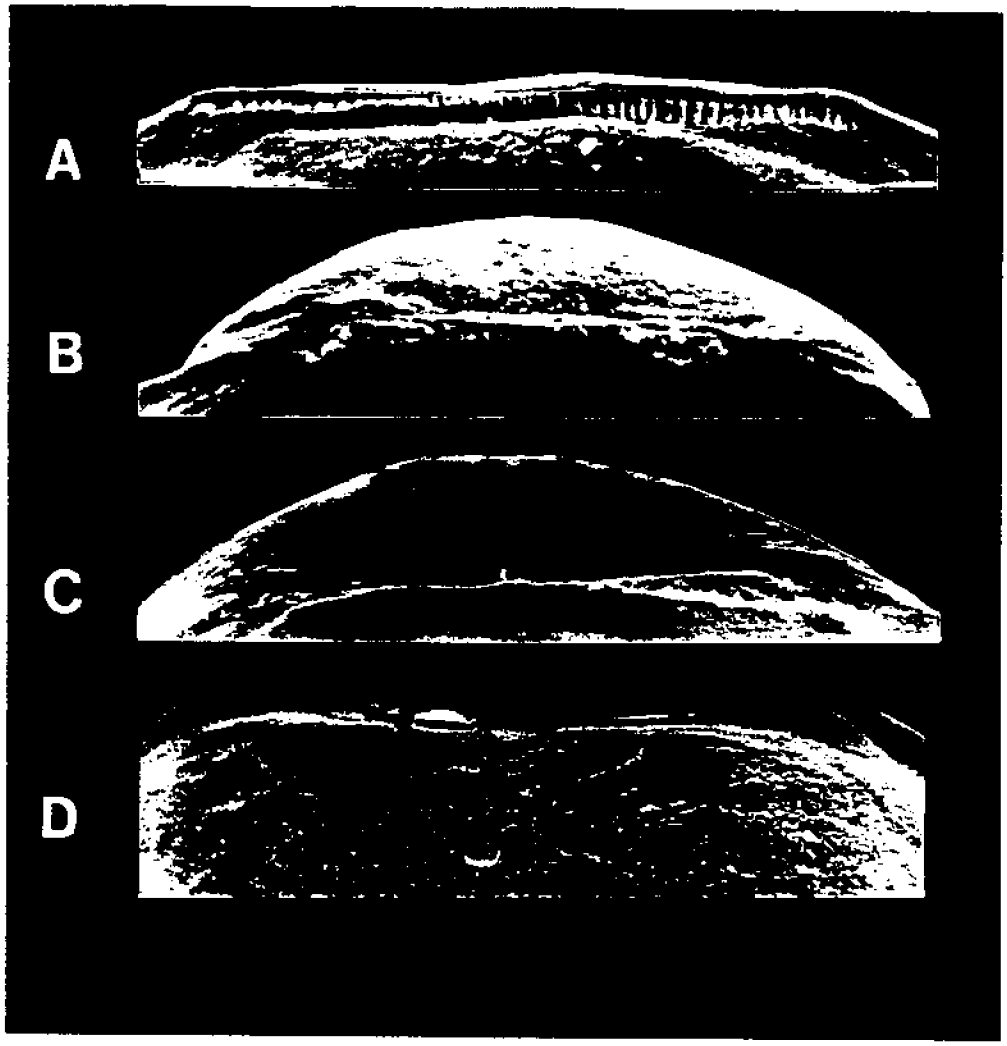

Fig. 2. Larval hinge structure of Dreissena spp. (A), Mytilopsis leucophaeata (B), Rangia cuneata (C), and Corbicula fluminea (D). Shells are right valves from specimens 180 - 230 µm shell length. Note dentition on Dreissena spp. valve.

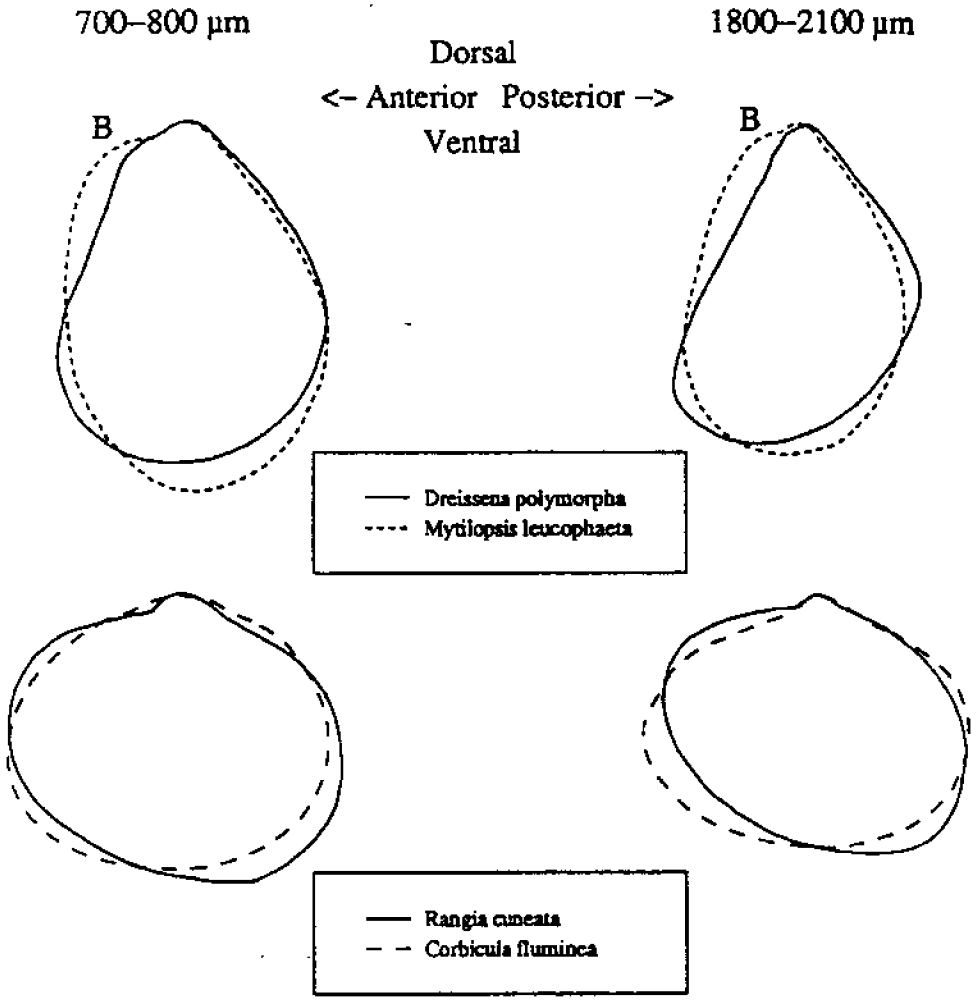

Fig. 3. Shell shapes of postlarval Dreissena spp., Mytilopsis leucophaeata, Rangia cuneata, and Corbicula fluminea. Shapes taken from disarticulated right valves. Valves oriented with hinge lines overlapping and horizontal. Beak region of mussel valves indicated with "B".

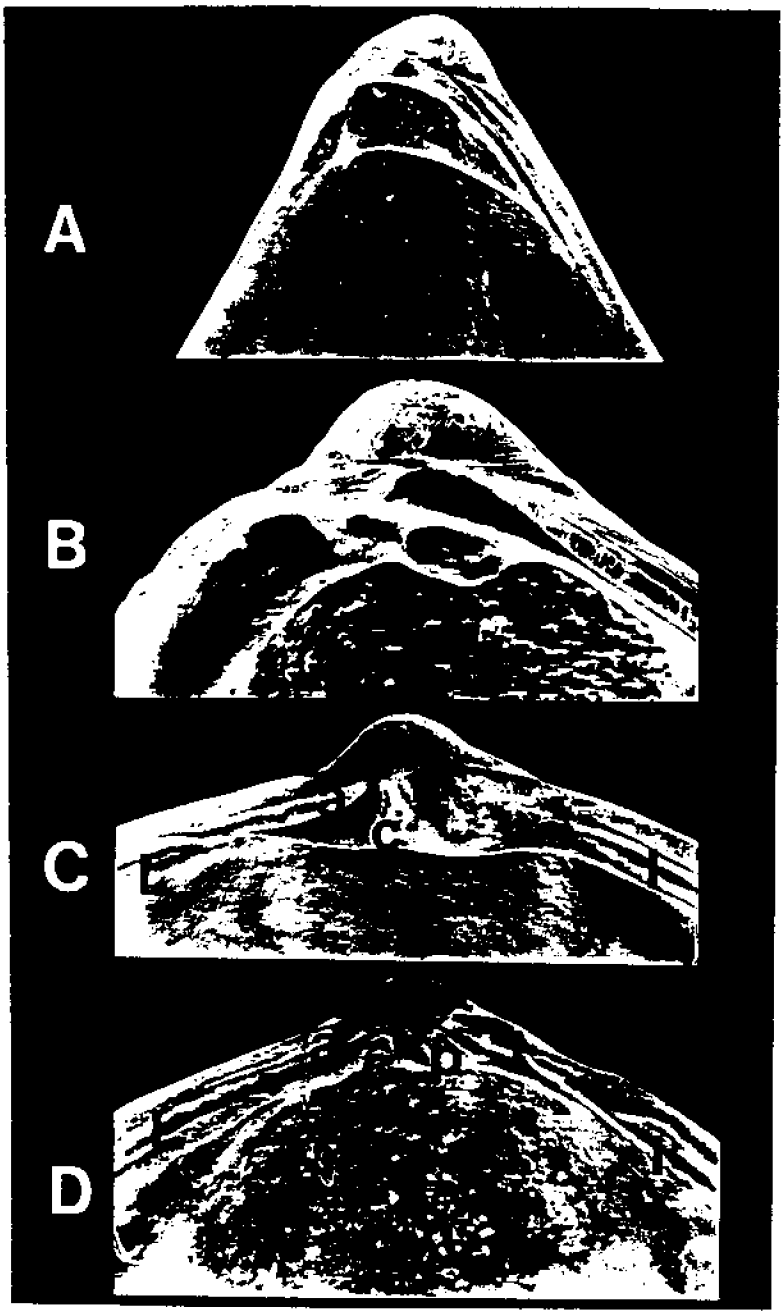

Fig. 4. Postlarval hinge structure (right valves) of Dreissena spp. (A), Mytilopsis leucophaeata (B), Rangia cuneata (C), and Corbicula fluminea (D). Presence of cardinal (a = anterior, c= central, p = posterior) and lateral (l) teeth indicated.

Potential for predator-mediated biological control of the zebra mussel in the Hudson River estuary

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Abstract

Predation controls community structure in aquatic systems. The success of introduced species like the zebra mussel, *Dreissena polymorpha*, is due in part to ineffective predators in the newly colonized systems. We quantified distribution, abundance, and mortality rates of a *D. polymorpha* population in the middle portion of the Hudson River Estuary, New York. Rocks were collected along a depth gradient in the field, and were sampled for density and size structure of the resident mussels. Predator exclusion experiments with rocks harboring a known number of *D. polymorpha* were used to estimate natural mortality. In addition, we conducted manipulative field experiments to test the effectiveness of the blue crab, *Callinectes sapidus*, at consuming zebra mussels by presenting similar rocks to crabs in field enclosures. Field sampling in the months of June, July and August indicated a dense (~30,000 m^{-2}) population composed of a single cohort of 1+ year class mussels. Mussel density increased dramatically with depth less than two meters below the spring-low-tide mark. In cage experiments, *C. sapidus* caused mortality rates an order of magnitude higher than those measured for the local predator guild, which was primarily composed of finfish. Consumption of zebra mussels by pumpkinseed, *Lepomis gibbosus*, was observed in the field on several occasions. Although blue crabs have the potential to limit zebra mussel abundance, at the present this is unlikely in the Hudson River Estuary due to the low natural abundance of the predator within the system. However, high predation rates of *D. polymorpha* by *C. sapidus* in this estuarine habitat indicate that the blue crab is capable of regulating the zebra mussel in habitats where the blue crab is abundant.

INTRODUCTION

In aquatic systems, predation regulates community structure and the dynamics of benthic species (Peterson 1979, Paine 1980). Predator-prey interactions in these systems are particularly complex and may be relatively stable because they are dominated by guilds of generalist predators capable of switching among diverse prey (Peterson 1979, Hines et al. 1990). Such generalist predators are not coupled to their benthic prey, and therefore are capable of controlling the dynamics of these species without being dependent upon any single species for their own persistence. Such features might characterize the predator-prey interactions between the zebra mussel, *Dreissena polymorpha*, and natural predators such as the blue crab, *Callinectes sapidus*, and finfish species, and thereby provide the requisite conditions for predator-mediated control of *D. polymorpha* population dynamics.

The zebra mussel initially invaded the Great Lakes in 1985, with the first reported sighting in Lake St. Clair in 1988 (Hebert et al. 1989). *D. polymorpha* rapidly colonized western Lake Erie, and now occurs in all the Great Lakes. The species was first discovered in the Hudson River in 1991, and has since expanded to its salinity limit (3-6 ppt) near Haverstraw, New York (Strayer et al. 1993). The rapid colonization has been facilitated by its high fecundity, a free-swimming larval stage, a fairly broad tolerance of environmental conditions in temperate habitats, and the apparent lack of competitors and predators (Hebert et al. 1991, Lemma 1991, MacIsaac et al. 1991, Strayer 1991). As a consequence, *D. polymorpha* occurs at densities exceeding 700,000 m^{-2}, and has thereby become a major and costly nuisance (Cooley 1991, Griffiths et al. 1991). Moreover, due to its salinity tolerance, the zebra mussel is expected to colonize and expand into most North American waters, including the low salinity portions of estuaries such as Chesapeake Bay (Bij de Vaate 1991, Strayer 1991, Strayer & Smith 1992). Thus, the potential exists for *D. polymorpha* to become a serious pest throughout North American waters, unless measures are discovered which effectively eradicate or regulate the zebra mussel in its distribution and abundance.

In this investigation we quantified natural mortality rates of *D. polymorpha* in the field, and tested the hypothesis that predation by the blue crab, *C. sapidus*, may serve as an effective biological control of the zebra mussel in the Hudson River estuary and potentially in other North American estuaries. The blue crab, *C. sapidus*, is a large (males up to 227 mm carapace width (CW)) epibenthic omnivore occurring in various habitats along the Northwest Atlantic Ocean, Gulf of Mexico and Caribbean Sea (Williams 1984). Blue crabs serve as both prey and consumers, and are abundant and actively foraging from late spring through autumn in Chesapeake Bay (Hines et al. 1987, 1990). The diet of Chesapeake Bay blue crabs consists of bivalves, crabs (both blue crabs and xanthids), fish and polychaetes, and to a lesser extent amphipods and isopods (Hines et al. 1990, Mansour & Lipcius 1991). Blue crab ecology in the Hudson River has not been well studied and consequently the abundance and range of the animal within the system is not known. Previous research has shown that *C. sapidus* is common in the freshwater and low salinity regions of the estuary in some years (Stein & Wilson 1991).

We conducted a series of sampling and field experiments in Hudson River freshwater habitats to determine limitations imposed by finfish and the blue crab upon zebra mussel abundance and distribution. Further trials compared the effectiveness in controlling zebra mussel abundance of the blue crab and the local predator guild (primarily finfish species). The specific objectives of the investigation included (1) a description of *D. polymorpha* abundance and distribution at the field site, (2) measurement of natural mortality of *D. polymorpha* and identification of likely finfish predators, and (3) testing the feasibility of biological control of *D. polymorpha* by *C. sapidus* and vertebrate species in the Hudson River and other North American estuaries.

STUDY AREA

Field experiments and sampling were conducted on the eastern shore of the Hudson River in the Tivoli Bays Region of the Hudson River National Estuarine Research Reserve, in New York State (Fig. 1). The tidal freshwater habitat was approximately 180 km north of the mouth of the estuary. In this region the benthic environment of the Hudson was characterized by large stones and cobbles covering a steeply sloping bottom that reached over 20 m depths in some areas. Underwater visibility was poor (< 3 meters) due to the heavy sediment load in the river.

METHODS

Rocks were sampled from the Hudson River during June, July and August, 1993 using SCUBA to examine the density and size structure of the zebra mussel. Tweezers were used to remove individuals from randomly-placed 16 cm^2 grids on the rocks. Mussels were counted and their shell lengths measured using Vernier calipers. We also conducted a series of five underwater transects to characterize the depth distribution of *D. polymorpha* . Four random rock samples were collected using SCUBA along depth profiles to determine density, while a circular grid was used to estimate percent coverage. Samples were collected at increasing depth profiles until consecutive runs generate 100% coverage.

The second component of the study involved manipulative field experiments. We first measured mortality rates of *D. polymorpha* due to predation. Rocks with attached mussels were collected from the river and maintained in aquaria to insure healthy test animals. These same stones were reintroduced into field enclosures with only 100 mussels remaining on each stone. Cages were constructed of 2.5 cm non-galvanized steel mesh, and covered 1 m^2 of substrate. Control treatments comprised fully-enclosed cages protecting one rock with 100 pre-counted mussels. Whereas, experimental cages were topless and open to predation. There were eight replicates of each treatment, lasting 14 days after which the rocks were removed from the cages and the surviving mussels enumerated. Differences in proportional mortality of *D. polymorpha* between the two experimental treatments were analyzed using ANOVA models with angularly transformed proportional mortality as the dependent variable and caged treatment as a fixed factor. Data were examined for normality and tested for homogeneity of variances with an F-max test.

The final experiment utilized the same field enclosures and another set of pre-counted mussels. In this trial all cages were closed and male blue crabs were introduced as predators. Six cages contained small crabs (60-80 mm CW), six cages contained large crabs (110-130 mm CW), and six cages contained only rocks with pre-counted mussels. After 72 h, crabs were removed and surviving mussels enumerated. Differences in proportional mortality of *D. polymorpha* between the three treatments were analyzed using ANOVA models with angularly transformed proportional mortality as the dependent variable and caged treatment as a fixed factor. Data were examined for normality and tested for homogeneity of variances with an F-max test.

RESULTS

Samples collected along depth transects beginning at the spring low tide mark indicated a significant effect of depth (Table 1), and a correlation of density with increasing depth (Fig. 2). A Scheffe's test of the mean densities indicated that abundance at the shallowest transect (0.26 m) was significantly less than the four deeper transects (Crit. value = 1.329). Densities of *D. polymorpha* in the Tivoli samples averaged 30,000 m^{-2}. Size frequency distributions (Fig. 3) revealed a single cohort with no individuals exceeding 20 mm shell length. Mean shell length increased 24% over the three month period covered by the study.

Mean zebra mussel mortality in the first manipulative experiment was significantly greater (Table 2) in the experimental treatments (Fig. 4). Predators consumed a mean of 24% of the attached *D. polymorpha* in the open cages. Mussels in the cage controls suffered less than 10% mortality over the two-week period.

The introduction of male blue crabs produced higher mortality rates in the second field experiment. Large blue crabs consumed nearly 50% of the prey in the 72 h trials (Fig. 5). Although the effect of crabs was highly significant (Table 3), mussel mortalities did not differ significantly between large and small crab treatments (Scheffe's test, Crit. value = .169).

DISCUSSION

Size-frequency distributions of *D. polymorpha* in the Hudson River indicated that the population was composed a single cohort spawned the previous year (Jenner and Janssen-Mommen, 1993). Given the planktonic larval stage of the mussel, the likely parental population was several kilometers upriver of the Tivoli Bays site. The depth distribution of increasing density with depth was consistent with the hypothesis that physical factors (e.g., desiccation, ice scour) rather than predation, restrict the upper limit of the vertical abundance of *D. polymorpha* in the Hudson River estuary.

D. polymorpha in European lakes and large rivers occurs at densities near 3000 mussels m^{-2} (Bij deVaate 1991). Yet in North America, the densities are larger by one or two orders of magnitude. The densities reported here (~30,000 mussels m^{-2}) are well within the ranges observed in North American waters (Dermott & Munawar 1994). Success of the zebra mussel in North America can be attributed at least in part to the lack of effective natural predators. In Europe, mussels are preyed upon by eels (de Nie 1982), fish (Daoulas & Economidis 1984), and ducks (Draulans 1984). These consumers have likely kept zebra mussel populations in check, and thereby prevented them from becoming an ecological nuisance.

A lack of strong predation pressure was evident in our field experiment using predator-exclusion cages. Subtracting control mortalities from those in the experimental treatments resulted in 14% mortality over two weeks. Mortality observed in the controls of both field experiments (approximately 10%) was attributed to handling-related mortality associated with the transport and manipulation of mussels in and out of the field, and was similar over the two experimental time periods (3 and 14-day trials). The only natural predation observed was by several pumpkinseed fish, *Lepomis gibbosus*, over three separate dives. Pumpkinseed have well-developed molariform teeth capable of cracking mollusk shells (French 1993).

Recently it has been suggested that the blue crab might be the kind of voracious predator that could control the explosive growth of the zebra mussel. The blue crab inhabits estuarine systems through the freshwater reaches (DeFur et al. 1987), and is capable of controlling the dynamics of bivalves (Lipcius & Hines 1986). The results of our crab predation experiment provided support for the hypothesis that blue crabs can be more effective in reducing zebra mussel abundance than local finfish or invertebrate predators. *D. polymorpha* mortality rates caused by *C. sapidus* were nearly twice those caused by the local predator guild in only 20% of the time.

Unfortunately, blue crab densities in the Hudson River system are relatively low, varying low to moderate densities capable of supporting a small commercial fishery, but too low to regulate *D. polymorpha* . In our study no crabs were caught in several baited traps and local fishermen indicated that there were few blue crabs in the middle portion of the Hudson River that summer. Our experimental crab densities (1 m^{-2}) are comparable to those observed in Chesapeake Bay (Orth and van Montfrans 1987).

Figure 1. Map of the Hudson River estuary showing the location of the Tivoi Bays National Estuarine Research Reserve.

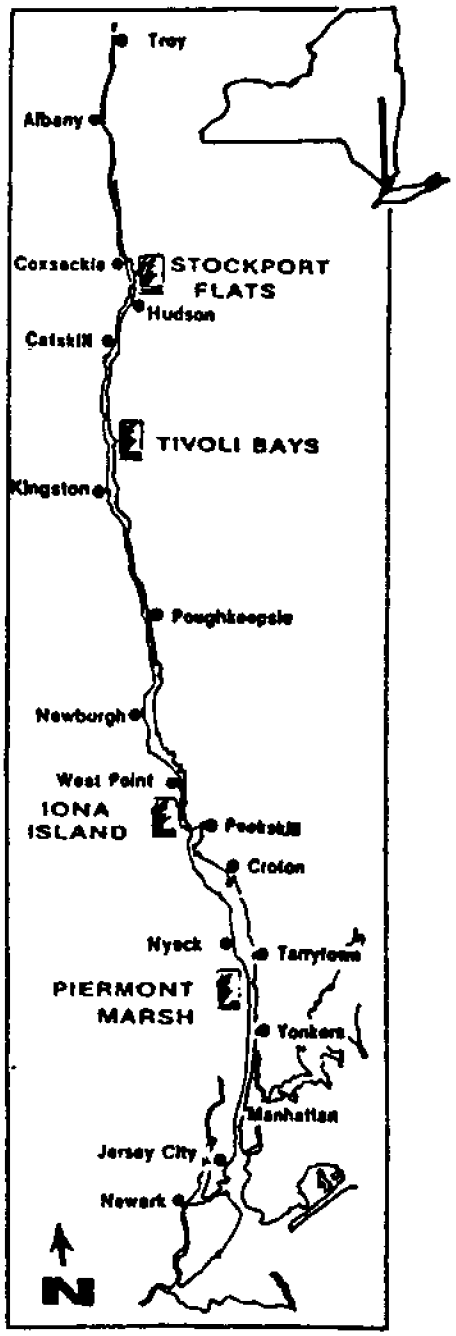


Table 1. ANOVA table for mean mussel density along five depth transects in the Tivoli Bays region of the Hudson River.

	DF	Sum of Squares	Mean Square	F-Value	P-Value
depth	4	16.046	4.011	13.879	<.0001
Residual	15	4.335	.289		

Figure 2. Depth distribution of mean zebra mussel density (± 1 std. error) in the Tivoli Bays region of the Hudson River.

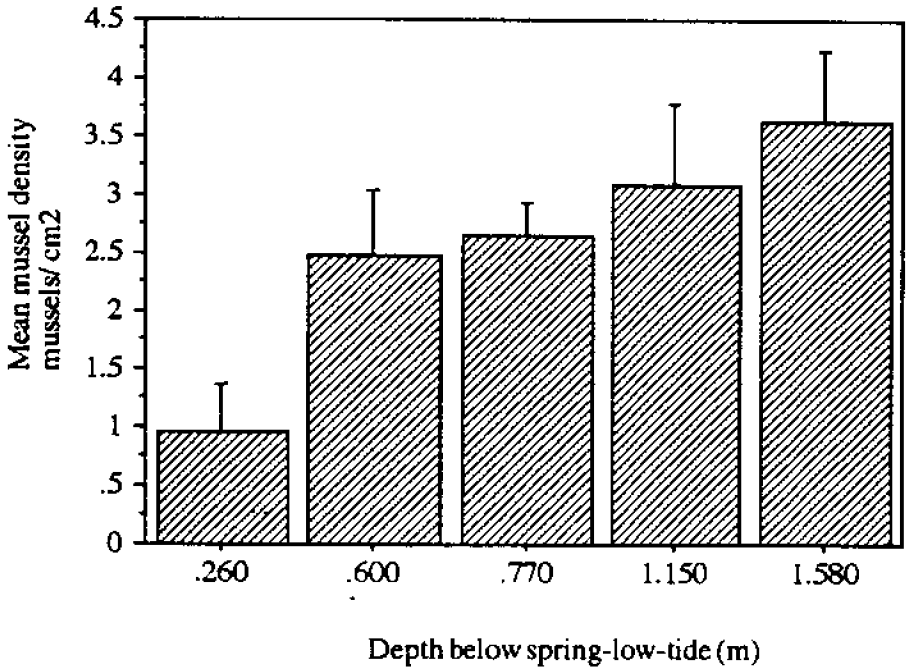


Figure 3. Size frequency distributions of the zebra mussel in the Tivoli Bays region of the Hudson River.

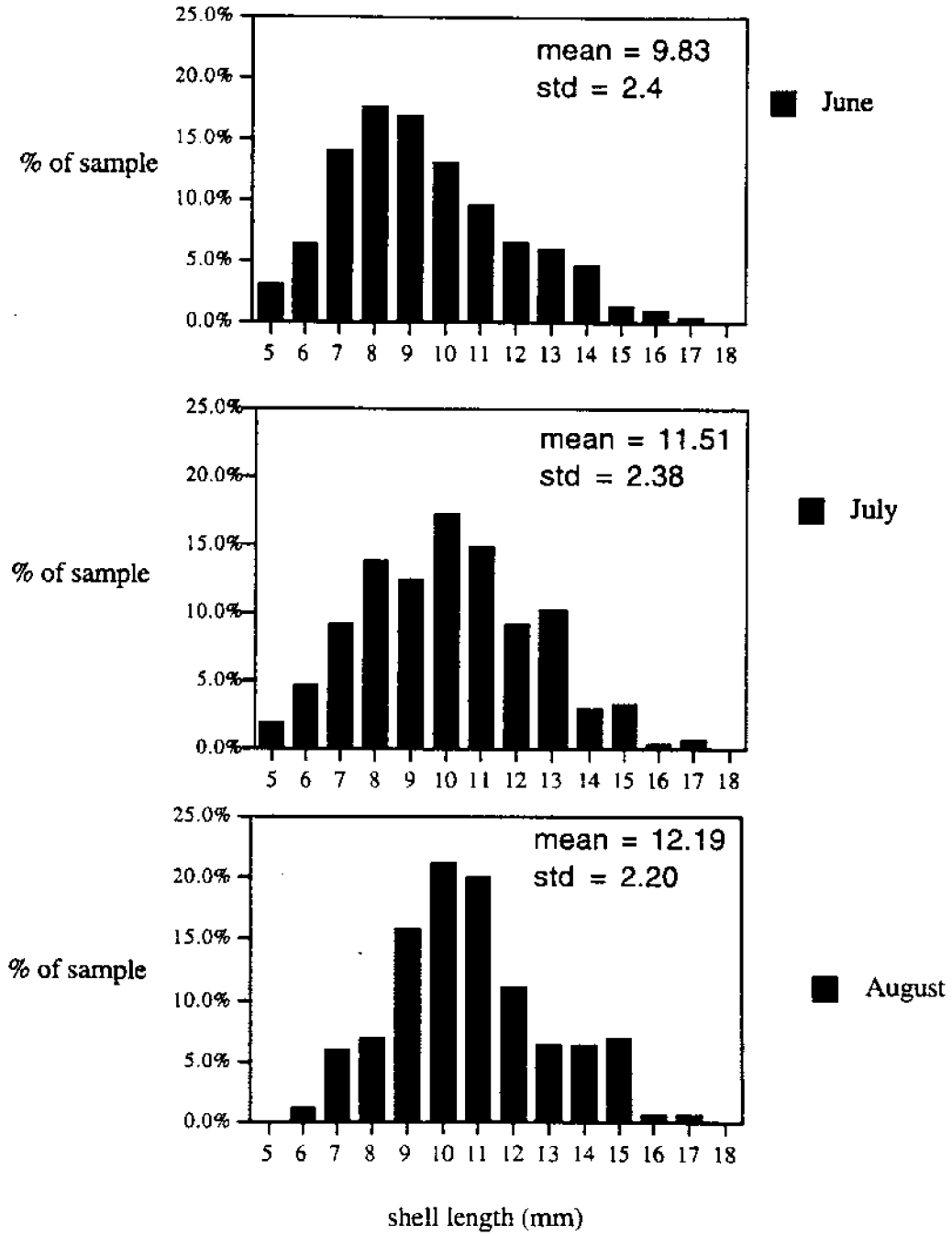


Table 2. ANOVA table for mean proportional mortality of zebra mussels outplanted at 100 mussels per rock in open and closed cage treatments in the Tivoli Bays region of the Hudson River.

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Treatment	1	.087	.087	13.425	.0026
Residual	14	.091	.006		

Figure 4. Mean proportional mortality of zebra mussels outplanted at 100 mussels per rock in open and closed cage treatments in the Tivoli Bays region of the Hudson River.

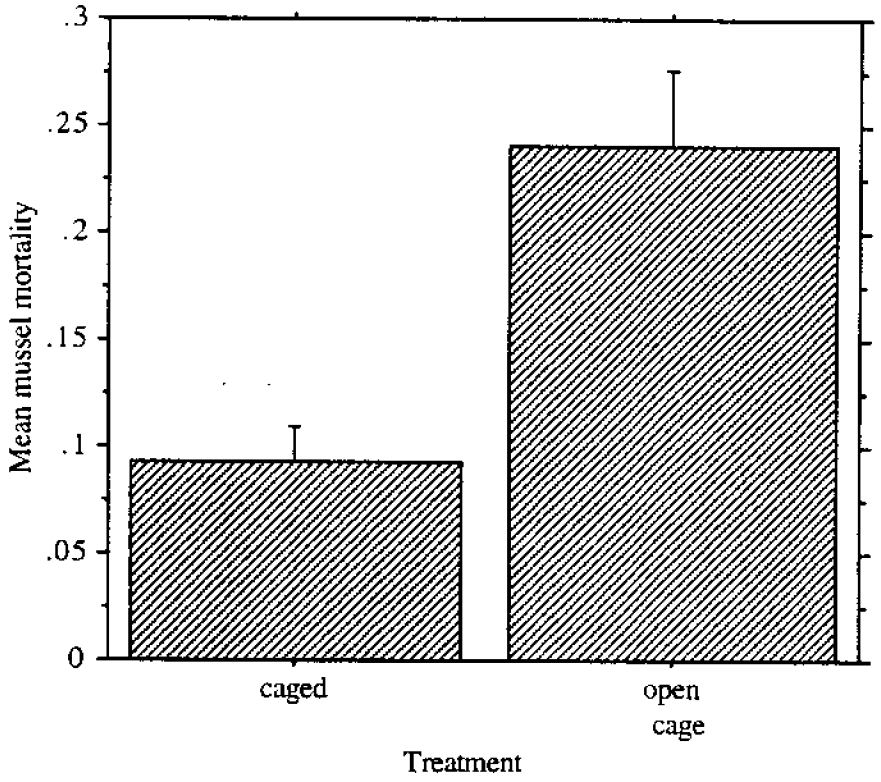
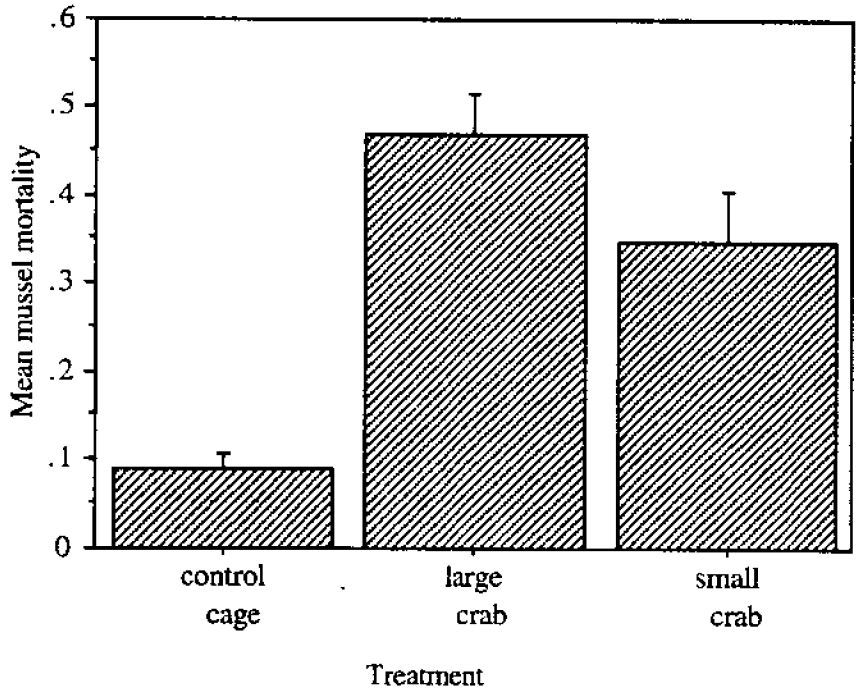


Table 3. ANOVA table for mean proportional mortality of zebra mussels outplamted at 100 mussels per rock in large and small crab treatments in the Tivoli Bays region of the Hudson River.

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Treatment	2	.449	.224	19.205	<.000
Residual	15	.175	.012		

Figure 5. Mean proporitonal mortality of zebra mussels (± 1 std. error) outplanted at 100 mussels per rock in control, large, and small crab treatments in the Tivoli Bays region of the Hudson River.



In contrast, *C. sapidus* was common in the middle region of the Hudson River in the summer of 1992. During this time a major decline in the abundance of adult zebra mussels was observed near Catskill, New York (D.P. Molloy, pers. comm.). The initial densities of *D. polymorpha* were lower than those recorded in our study.

In conclusion, *D. polymorpha* is unlikely to be regulated by the local predator guild in the Hudson River, unless the predator abundance increases significantly. In particular, the blue crab is capable of controlling zebra mussel abundance if the predator abundance increases to levels approximating 0.01 -1.0 m^{-2}, depending on crab size. Such densities are common in other estuaries such as Chesapeake Bay, and indicate the likelihood that the zebra mussel will be regulated in estuaries near the southern limit of the range of the zebra mussel.

ACKNOWLEDGMENTS

We thank the Hudson River Foundation, the Commonwealth of Virginia, and the New York State Museum for research support. Special thanks are given to Dan Molloy, John Waldman, Chuck Nieder, Dave Strayer and Betsy Blair for field support. This research was conducted while the senior author was supported by the Tibor Polgar Fellowship from the Hudson River Foundation, and a research assistantship from the Caribbean Marine Research Institute.

REFERENCES

Bij de Vaate, A. 1991. Distribution and aspects of population dynamics of the zebra mussel, *Dreissena polymorpha* (Pallas, 1771), in the lake Ijsselmeer area (The Netherlands). Oecologia 86: 40-50.

Cooley, J. M. 1991. Editorial, Zebra Mussels. J. Great Lakes Res. 17: 1-2.

Daoulas, C. C. and P. S. Economidis. 1984. The feeding of *Rutilus rubilio* (Bonaparte) (Pisces, Cyprinidae) in Lake Trichonis, Greece. Cybium 8: 29-38.

DeFur, P. L., D. Nusbaumer, and R. J. Lewis. 1987. Physiological aspects of molting in blue crabs from the tidal fresh-water Potomac River, Virginia. J. Crust. Biol. 8: 12-19.

Dermott, R., and M. Munawar. 1994. Invasion of Lake Erie offshore sediments by *Dreissena*, and its ecological implications. Can. J. Fish. Aquat. Sci. 50: 2298-2304.

deNie, H. W. 1982. A note on the significance of larger bivalve molluscs (*Anodonta* spp. and *Dreissena* sp.) in the food of the eel (*Anguilla anguilla*) in Tjeukemeer. Hydrobiologia 95: 307-310.

Draulans, D. 1984. Sub-optimal mussel selection by tufted ducks *Aythya fuligula*: test of a hypothesis. Anim. Behav. 32: 1192-1196.

French, J.R.P. 1993. How well can fishes prey on zebra mussels in eastern North America? Fisheries 18: 13-19.

Griffiths, R. W., D. W. Schlosser, J. H. Leach, and W. P. Kovalak. 1991. Distribution and dispersal of the zebra mussel (*Dreissena polymorpha*) in the Great Lakes region. Can. J. Fish. Aquat. Sci. 48: 1381-1388.

Hebert, P. D. N., B. W. Muncaster and G. L. Mackie. 1989. Ecological and genetic studies on *Dreissena polymorpha* (Pallas): a new mollusc in the Great Lakes. Can. J. Fish. Aquat. Sci. 46: 1587-1591.

Hebert, P. D. N., C. C. Wilson, M. H. Murdoch, and R. Lazar. 1991. Demography and ecological impacts of the invading mollusc *Dreissena polymorpha*. Can. J. Zool. 69: 405-409.

Hines, A. H., A. M. Haddon, and L. A. Weichert. 1990. Guild structure and foraging impact of blue crabs and epibenthic fish in a subestuary of Chesapeake Bay. Mar. Ecol. Prog. Ser. 67: 105-126.

Hines, A. H., R. N. Lipcius and A. M. Haddon, A. M. 1987. Population dynamics and habitat partitioning by size, sex, and molt stage of blue crabs (*Callinectes sapidus*) in a subestuary of Chesapeake Bay. Mar. Ecol. Prog. Ser. 36: 55-64.

Lemma, A., L. Wolde-Yohannes, P. C. Fraleigh, P. L. Klerks, and H. H. Lee. 1991. Endod is lethal to zebra mussels and inhibits their attachment. J. Shellfish Res. 10: 361-366.

Lipcius, R. N. and A. H. Hines. 1986. Variable functional responses of a marine predator in dissimilar homogeneous microhabitats. Ecology 67: 1361-1371.

MacIsaac, H. J., W. G. Sprules, and J. H. Leach. 1991. Ingestion of small-bodied zooplankton by zebra mussels (*Dreissena polymorpha*): can cannibalism on larvae influence population dynamics? Can. J. Fish. Aquat. Sci. 48: 2051-2060.

Mackie, G.L. 1991. Biology of the exotic zebra mussel, *Dreissena polymorpha*, in relation to native bivalves and its potential impact in Lake St. Clair. Hydrobiologia 219: 251-268.

Mansour, R. A. and R. N. Lipcius. 1991. Density-dependent foraging and mutual interference in blue crabs preying upon infaunal clams. Mar. Ecol. Prog. Ser. 72: 239-246.

Orth, R. J. and J. van Montfrans. 1987. Utilization of a seagrass meadow and tidal marsh creek by blue crabs *Callinectes sapidus*. I. Seasonal and annual variations in abundance with emphasis on post-settlement juveniles. Mar. Ecol. Prog. Ser. 41: 283-294.

Paine, R. 1980. Food webs: linkage, interaction strength and community infrastructure. J. Anim. Ecol. 49: 667-685.

Peterson, C. H. 1979. Predation, competitive exclusion, and diversity in the soft-sediment communities of estuaries and lagoons. in R.J.Livingston, editor. Ecological processes in coastal and marine systems. Plenum Publishing Corporation, New York, New York, USA.

Stein, J.J. and K.A. Wilson. 1992. Blue crab, *Callinectes sapidus*, distribution and habitat utilization in the lower Hudson River and tributaries. Pages VI-1-24 in J.R. Waldman and E.A. Blair, editors. Final reports of the Tibor T. Polgar Fellowship Program, 1991. Hudson River Foundation, New York.

Strayer, D.L. 1991. Projected distribution of the zebra mussel, *Dreissena polymorpha*, in North America. Can. J. Fish. Aquat. Sci. 48: 1389-1395.

Strayer, D.L., and L.C. Smith. 1993. The distribution of *Dreissena polymorpha* in estuaries and brackish waters. in T.F. Nalepa and D.W. Schlosser, editors. Zebra Mussels: Biology, Impact, and Control. CRC Press, Boca Raton, Florida.

Williams, A. B. 1984. Shrimps, lobsters, and crabs of the Atlantic coast of the eastern United States, Maine to Florida. Smithsonian Institution Press.

Effects of Starvation at Different Temperatures on Dry Tissue and Dry Shell Weights in the Zebra Mussel, *Dreissena polymorpha* (Pallas)

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ABSTRACT

Starvation effects on dry tissue and shell biomass were investigated in the zebra mussel, *Dreissena polymorpha* at 5°, 15° and 25°C. Subsamples of 30 individuals were examined daily for mortality. A second group was periodically randomly subsampled for dry tissue and shell weights. At 25°C, 100% mortality occurred after 166 days. No mortality occurred at 5°C or 15°C after 229 days. Dry shell weight (DSW) was constant in mussels starved at 25°C or 5°C, but increased significantly ($P < 0.05$) at 15°C, likely due to deposition of shell nacre in shells not increasing in length. Dry tissue weight (DTW) in starving mussels was significantly correlated with both shell length (SL) and days of starvation ($P < 0.00001$) and was significantly lower at higher test temperatures. Pre-starvation DTW of a 20 mm SL individual was 19.7 mg. DTW declined to 4.66 mg after 132 days at 25°C, and to 9.95 mg and 15.9 mg after 229 days at 15°C and 5°C, respectively, corresponding to tissue biomass reductions of 73.8%, 46.9% and 22.9%. DTW loss rates of 0.102, 0.043, and 0.021 mg/day were recorded for standard mussels starving at 25°, 15° and 5°C, respectively. As estimated from DTW loss rates, O_2 consumption rates in starving standard mussels were 0.196 µl $O_2 \cdot mg^{-1} \cdot h^{-1}$ at 25°C, 0.0837 µl $O_2 \cdot mg^{-1} \cdot h^{-1}$ at 15°C and 0.0399 µl $O_2 \cdot mg^{-1} \cdot h^{-1}$ at 5°C, approximately 22.1%, 13.5% and 8.5%, respectively, of O_2 uptake rates recorded directly in nonstarved individuals. Mussels held at 25°C lost 73.8% of body mass just prior to death suggesting that an ≈ 75% DTW loss is lethal and indicating that 365 days of starvation will be required for 100% mortality of mussels at 15°C and 747 days at 5°C. Extensive starvation tolerance in *D. polymorpha* is associated with ability to greatly reduce metabolic demand. As this capacity is maximized at low temperatures, mussels may survive winter months with minimal energy store reduction. Extensive tolerance, makes starvation impractical for mitigation of *D. polymorpha* fouling.

INTRODUCTION

The "zebra mussel", *Dreissena polymorpha*, has been a major macrofouling aquatic pest bivalve in Europe for over 200 years. It was introduced to North America in 1986, originally being released near the Lake St. Clair - Detroit River region. It is believed that larvae were released with ballast water or that adults escaped anchor chains on ships arriving from Europe (Hebert *et al.*, 1989; Mackie *et al.*, 1989; McMahon *et al.*, 1993). Since its introduction, mussels have spread throughout Lakes Erie, Ontario, Michigan, Oneida, the Finger Lakes, and the Erie-Barge Canal, and the St. Lawrence, Hudson, Oswego, Illinois, Mississippi, Lower Ohio, Lower Tennessee, Lower Arkansas and Lower Cumberland Rivers (Zebra Mussel Information Clearinghouse, 1993). Zebra mussels are spreading rapidly throughout U.S. inland waterways through dispersal of adults attached to barge hulls by byssal thread holdfasts and downstream transport of planktonic veliger larvae (McMahon, 1992). Isolated populations now extend downstream in the Lower Mississippi River to New Orleans, Louisiana (Zebra Mussel Information Clearinghouse, 1993).

Mussels utilize proteinaceous byssal threads to attach to natural hard substrata such as rocks, wood and macrophytic plants and man-made structures such as metal piping, steel, nylon, fiberglass and wood where they can form encrusting mats many shells thick (4 - 12 in) (McMahon, 1990). Because of its capacity for macrofouling, the zebra mussel has had major detrimental impacts on recreational boating and commercial shipping as well as on raw water using industries, potable water treatment plants and electric power stations which draw from water sources infested with mussels. Presently, the main control technologies for zebra mussel macrofouling center on molluscicides such as chlorine, chlorine dioxide, bromine, ozone, aromatic hydrocarbon compounds and quaternary ammonium compounds (McMahon, 1990). However, federal and state regulations for use of molluscicides are likely to become increasingly restrictive in the future. With the advent of nearly every raw water using facility on the major waterways of the Mississippi Drainage having to apply molluscicides to control zebra mussel fouling, use of molluscicides is likely to become even further restricted in order to prevent water quality degradation and maintain drinking water standards (McMahon *et al.*, 1993). Therefore, there has been a high priority placed on the development of reliable, cost-effective, environmentally acceptable, nonchemical means of zebra mussel macrofouling control.

Phytoplankton food resources can vary during the year exposing mussels to prolonged periods of starvation in the winter when algal concentrations are minimal and in the summer when inedible dinoflagellates dominate the plankton or during the algal productivity collapse following the first plankton bloom (Sprung and Borcherding, 1991). A search of the available literature revealed that while there have been several investigations of loss of gonadal and digestive tissues during starvation in zebra

mussels, there have been few published studies on the effects of prolonged starvation (Sprung and Borcherding, 1991; Bielefeld, 1991). Loss in wet and dry weight during starvation in the intertidal snail, *Morula granulata* (Duclos), was mainly due to utilization of body organic constituents during extended periods without feeding to provide energy to maintain metabolic functions (Devi *et al.*, 1985). Similar tolerance of extensive loss of body tissue has been reported for the freshwater pulmonate snail, *Planorbella trivolvis* (Russell-Hunter and Eversole, 1976). With the exception of that for the freshwater unionid bivalve, *Lamellidens marginalis* (Masthanamma *et al.*, 1984), there have been no studies of overall body tissue weight loss in *D. polymorpha* or any other marine or freshwater bivalve species nor have temperature effects on starvation rates been investigated. In order to add to the data base on starvation effects in zebra mussels and bivalves in general, this study was designed to examine the effects of temperature on whole body dry tissue mass and shell mass in zebra mussels subjected to prolonged starvation over a wide temperature range.

MATERIALS AND METHODS

Specimens of *D. polymorpha* were collected from the guide wall of the U.S. Corps of Engineers, Black Rock Navigation Lock on the Niagara River just downstream from Lake Erie, Buffalo, New York. Mussels were flown overnight to Texas emersed on moist paper toweling in cooled insulated containers. On arrival they were maintained at 5°C in a 284 l refrigerated 'Living Stream' holding tank for ≈ 60 days until utilized in the experiment. As will be detailed in the Results section of this paper, holding mussels at 5°C prior to experimentation greatly suppresses metabolic rate, making tissue loss negligible over the 60 day pre-experimental holding period.

A sample of mussels was removed from the 5°C holding tank on June 15, 1993 and 720 individuals (shell length 15 mm - 26 mm) were carefully separated from byssally bound mussel clusters using a scalpel to cut threads. Separated individuals were rinsed free of silt and organic debris accumulated on the shells by placing them in a sieve (opening 4.0 mm) and running dechlorinated tap water over them at holding room temperature (15°C). Three subsamples of 210 animals each were selected at random from the cleaned and separated mussels. They were placed in three 13 l plastic holding tanks that were filled with dechlorinated, City of Arlington tap water. The tanks were held at a constant temperature in either an incubator at 25°C, a cold room at 15°C or a refrigerated water bath at 5°C. These subsamples were utilized to determine shell and tissue biomass variation over the course of starvation. A further three subsamples of 30 mussels each were placed in 9 cm diameter by 5 cm high glass crystallization dishes covered with a 1 mm mesh nylon screen to prevent mussel escape. One of these smaller subsamples was placed in each of the 13 l experimental holding tanks and was assessed daily for survivorship. All mussels were maintained without food at the three holding temperatures for the duration of the experiment.

Tanks were inspected daily. Any dead mussels (from either subsample) were immediately removed to prevent contamination. Tanks were held in low light conditions to slow bacterial and algal growth. Water in each tank was aerated and changed three times weekly. Water temperature was taken daily using a standard mercury thermometer (± .1°C) and adjusted as needed.

A control sample of 10 mussels was removed from the larger subsample of 210 individuals from each tank on day zero. Initially, subsequent samples of 10 mussels were collected twice a week from mussels held at 25°C, once a week from those held at 15°C and once every two weeks from those held at 5°C. As the experiment progressed and it became clear that mussels tolerated starvation for much more extended periods than initially predicted, the duration between samples was extended for up to 30 days in order to allow determination of tissue loss throughout the tolerated period of starvation.

A number of parameters were determined for sampled mussels. These included shell length (SL) measured with a dial caliper to the nearest 0.1 mm, and dry tissue and dry shell weight measured to the nearest 0.0001 g. To determine dry tissue (DTW) and dry shell weights (DSW), sampled specimens were frozen for approximately 24 h. Freezing allowed tissues to be more completely excised from the shells with a scalpel than could be achieved with wet tissues at room temperatures. Excised tissues were defrosted on paper towels allowing absorption of extracorporal mantel cavity water. Separated wet tissues and shells were dried to constant weight for 24-26 h at 65°C.

Mortality was determined daily in the smaller subsamples (n = 30) of mussels. The glass crystallization dishes containing these samples were removed from the holding tanks and the nylon screen removed. Mussels stayed submerged in the media remaining within the crystalization dishes while their viability was determined. All mussels widely gaping the valves and unresponsive to strong stimulation of exposed siphon and mantle tissues with the tip of a blunted dissection needle (*i.e.*, did not close the valves) were considered dead and removed from the sample.

RESULTS

A 100% sample mortality was achieved in mussels at 25°C after 166 days of starvation. In contrast, no mortality had occurred in mussels starved at either 5°C or 15°C at the time this paper was written after 229 days of starvation. Estimated by probit analysis (Bliss, 1936), the LT_{50} value (estimated time for 50% sample mortality) for the 25°C mussel sample was 118 days and the LT_{100} (estimated time for near 100% sample mortality), 143 days.

Table 1
Multiple factor analysis of variance testing the significance of holding temperature (5°, 15° or 25°C) on the natural logarithm of dry tissue weight in starving specimens of *Dreissena polymorpha* with days of starvation and shell length as covariants.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square Error	F-Ratio	Probability
Covariant Days Starvation	17.48	1	17.48	107.32	<0.00001*
Covariant Shell Length	90.96	1	90.96	558.38	<0.00001*
Main Effect Holding Temperature	16.43	2	8.23	50.42	<0.00001*
Residual	75.23	462	0.162898		
Total	196.12	466			

* Indicates a significant correlation or effect at $P \leq 0.05$.

Student Newman-Keuls Multiple Range Analysis of the differences between the mean natural logarithm of dry tissue weights of specimens of *Dreissena polymorpha* subjected to prolonged starvation at a test temperature of 5°, 15° or 25°C

Test Temperature	n	Mean in Dry Tissue Weight (mg)	Significant Diff. at P < 0.05
5°C	117	-4.3812	▮
15°C	160	-4.1171	▮
25°C	190	-3.8784	▮

Multiple Least Squares Linear Regression analysis indicated that 61% of the total variation in the natural logarithm (ln) of DTW was explained by correlation with days of starvation, test temperature and individual shell length. An extremely high correlation of ln DTW with test temperature ($P < 0.00001$) suggested little or no tank effects on DTW during starvation allowing subsequent statistical analyses to utilize individual mussels as the experimental unit.

Multiple Factor Analysis of Variance was carried out on the mean natural logarithmically transformed values of DTW among temperature treatments with SL and days of starvation as covariants. The analysis indicated a strong positive correlation of ln DTW with SL and strong negative correlation with days of starvation ($P < 0.00001$) (Table 1). Holding temperature significantly affected DTW ($P < 0.00001$). A Student Newman-Keuls Multiple Range Test revealed that mussels held at the three test temperatures all had mean dry tissue weights that were significantly ($P < 0.05$) different from those held in the other temperature treatments with mussels held at 25°C having the lowest DTW and those held at 5°C, the highest (Table 1).

A similar Multiple Factor Analysis of Variance revealed no significant effects of holding temperature on the ln DSW of starving mussels ($P = 0.236$) and no correlation with days of starvation ($P = 0.310$) while, as expected, there remained a strong correlation of ln DSW with SL ($F = 648$, $P < 0.00001$).

For each sequential subsample of living mussels, a least squares semi-logarithmic linear regression of ln DTW or ln DSW versus SL was determined. The vast majority of these regressions were significant ($P < 0.05$) for all samples among all test temperature groups. In a few cases, limited size ranges of samples lead to insignificant regressions, $P > 0.05$. The average SL of all individuals in all samples from all three test temperatures was approximately 20 mm. The regression of ln DTW versus SL for individuals sampled on day zero from the three test temperature control groups (g DTW = -6.837 + 0.145 (mm SL): $n = 30$, $r = 0.819$, $F = 57.2$, $P = <0.00001$) was used to estimate DTW of a mussel with an average SL of 20 mm as 19.7 mg.

The individual SL versus ln DTW or ln DSW regressions for each sample were then utilized to estimate the DSW and DTW for an average size, standard 20 mm SL mussel. The estimated DSW or DTW of a standard mussel determined from each sample regression was then fitted to a least squares linear regression as the dependent variable versus time in days of starvation as the independent variable (Table 2). These regressions revealed that there was no significant shell weight loss ($P <0.05$) among zebra mussels held at 5°C after 229 days of starvation or at 25°C over 132 days of starvation. However, individuals held at 15°C displayed a significant trend ($P < 0.05$) toward increasing DSW over the 229 day period of starvation (Fig. 1, Table 2).

Table 2
Parameters of least squares linear regression equations relating the estimated milligrams dry tissue weight (DTW) or dry shell weight (DSW) of a standard, 20 mm long specimen of *Dreissena polymorpha* (dependent variables) to days of starvation at test temperatures of 5°, 15° or 25°C.

Temp.	DTW/ DSW(g)	a (Intercept)	b (Slope)	n	r	F	P
5°C	DTW	20.69	-0.0207	12	-0.603	5.74	<0.05*
15°C	DTW	18.79	-0.0386	16	-0.748	17.77	<0.0025*
25°C	DTW	18.22	-0.1028	19	-0.876	55.96	<0.0001*
5°C	DSW	417.88	-0.6310	12	-0.348	1.38	>0.25
15°C	DSW	316.12	.1485	16	0.561	6.41	<0.05*
25°C	DSW	320.98	.0629	19	.156	.423	>0.5

* Indicates Significant Correlation at $P \leq 0.05$.

Table 3
Dry tissue weight (DTW) loss rates in a starving, standard 20 mm shell length specimen of *Dreissena polymorpha* converted to oxygen consumption rates by assuming a value of 911 µl O_2 required to aerobically oxidize one milligram of molluscan dry tissue.

Temp °C	Days Starved	mg DTW Lost	Vol. O_2 to Oxidize Lost Tissue	$\frac{\mu l\ O_2}{(mg \cdot Day)}$	$\frac{\mu l\ O_2}{(mg \cdot hr)}$	Percent of Nonstarved O^2 Uptake Rate
5	229	4.75	4327.3	0.959	0.0399	8.5%
15	229	9.95	9064.5	2.009	0.0837	13.5%
25	132	13.46	12262.1	4.715	0.196	22.1%

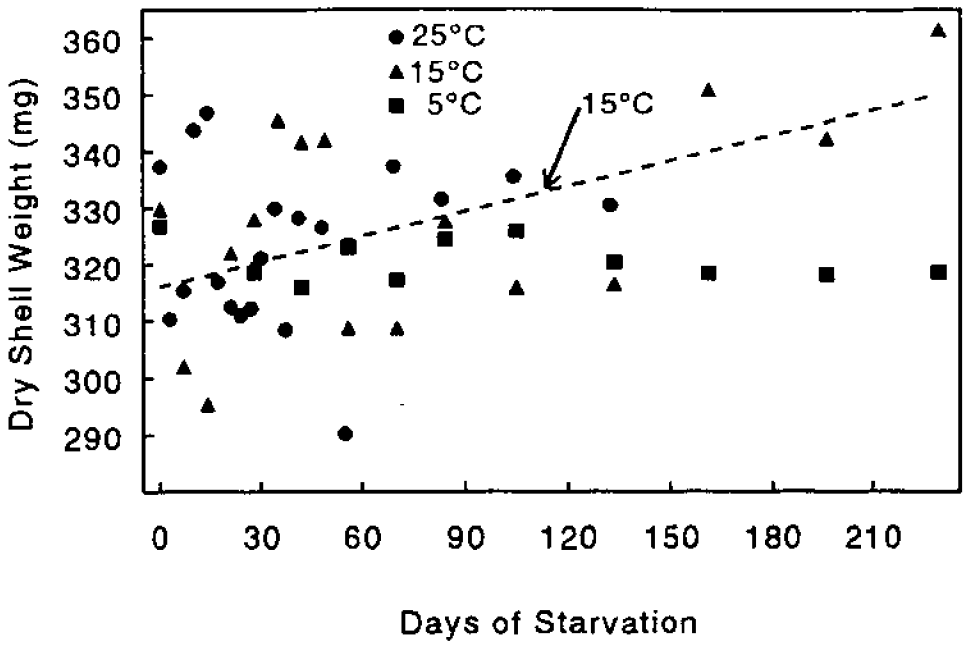

Figure 1. *Dry shell weight (DSW in mg, vertical axis) versus days of starvation (horizontal axis) in an averaged sized zebra mussel* (Dreissena polymorpha) *(shell length = 20 mm). DSW values were estimated from regressions of ln DSW versus SL computed for samples of starving mussels taken sequentially over the period of starvation. Circles are the DSW of mussels held at 25°C, triangles, of mussels held at 15°C and squares, of mussels held at 5°C. The dashed line represents a significant linear regression relating DSW to days of starvation at 15°C. No significant correlation was recorded at 5° or 25°C. Regression parameters are recorded in Table 2.*

In contrast, regressions relating the DTW of a standard 20 mm SL mussel to days of starvation indicated a significant reduction in dry tissue weight ($P < 0.05$) at all three test temperatures. The slopes of these regressions indicated that mussels starving at 25°C lost tissue mass 2.7 times faster than did mussels at 15°C and 5 times faster than did mussels at 5°C. DTW loss at 15°C was 1.9 times greater than that of mussels at 5°C (Fig. 2, Table 2).

The regressions of DTW versus days of starvation (Table 2) were utilized to estimate the dry tissue mass lost over the period of starvation. After 132 days, mussels at 25°C lost 73.8% of dry tissue body mass just prior to death. For mussels held 229 days at

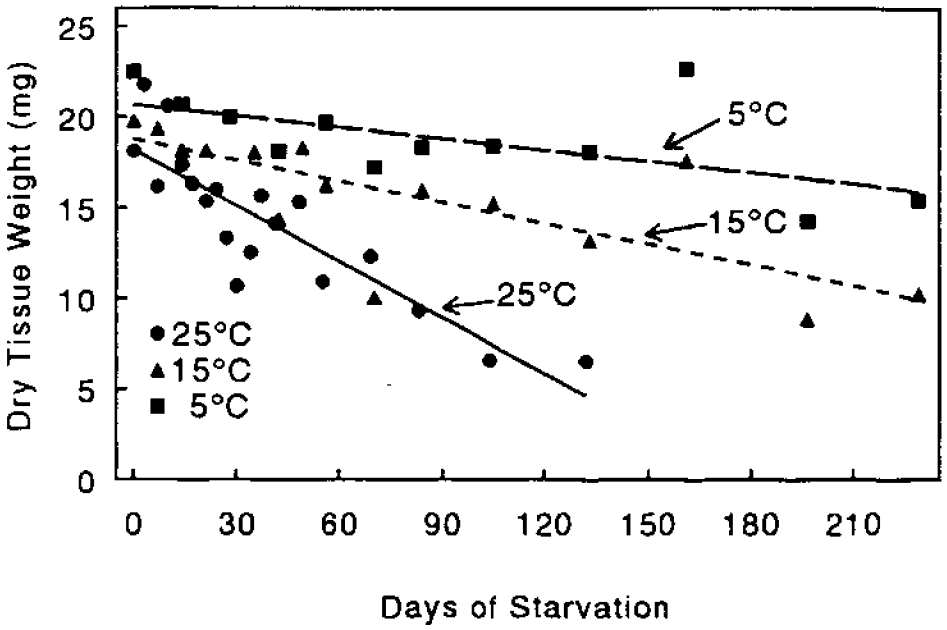

Figure 2. *Dry tissue weight (DTW in mg, vertical axis) versus days of starvation (horizontal axis) in an averaged sized zebra mussel* (Dreissena polymorpha) *(shell length = 20 mm). DTW values were estimated from regressions of ln DTW versus SL computed for samples of starving mussels taken sequentially over the period of starvation. Circles are the DTW of mussels held at 25°C, triangles, of mussels held at 15°C and squares, of mussels held at 5°C. The solid line represents a significant linear regression relating DTW to days of starvation in mussels held at 25°C, the finely dashed line, held at 15°C and the coarsely dashed line, held at 5°C. Regression parameters are recorded in Table 2.*

5°C and 15°C, dry tissue mass loss was 22.9% and 46.9%, respectively, and had not reached lethal levels at the time this paper was written. Based on an estimate that zebra mussel tissue is approximately 50% protein and 50% carbohydrate, 911 µl O_2 would be required for complete aerobic oxidation of one milligram of dry mussel flesh (Hill and Wyse, 1989). Utilizing this estimate, the oxygen uptake rate of an average 20 mm SL, 19.7 mg DTW mussel starving for 132 days at 25°C was estimated to be 3.86 µl $O_2 \cdot animal^{-1} \cdot h^{-1}$ or 0.196 µl $O_2 \cdot mg\ DTW^{-1} \cdot h^{-1}$ (Table 3). This oxygen uptake rate during starvation was approximately 22.1% that of a fed 20 mm SL mussel at 25°C (J.E. Alexander and R.F. McMahon, unpublished results). Similar values for zebra mussels starving for 229 days at 15°C and 5°C were estimated to be 1.64 µl

$O_2 \cdot animal^{-1} \cdot h^{-1}$ or 0.0837 µl $O_2 \cdot mg^{-1} \cdot h^{-1}$ and 0.786 µl $O_2 \cdot animal^{-1} \cdot h^{-1}$ or 0.0399 µl $O_2 \cdot mg^{-1} \cdot h^{-1}$, respectively, or 13.5% and 8.5% of the oxygen consumption rates of similarly sized fed mussels (Alexander and McMahon, unpublished results) (Table 3).

DISCUSSION

Zebra mussels appear to be extremely tolerant of starvation, with a mean tolerance time of 125.9 days (S.D. = ±29.6) at 25°C corresponding to an LT_{50} of 118 days and an LT_{100} of 143 days. Mussels held at 5° and 15°C have survived greater than 229 days without mortality. At 25°C, mussels tolerated a loss of approximately 75% of their dry tissue biomass before dying. Under conditions close to that of over-wintering, a 50% reduction in tissue biomass of the freshwater snail, *Planorbella trivolvis*, was observed to occur over 132 days of starvation with only a 10% mortality (Russell-Hunter and Eversole, 1976). The freshwater bivalve *Corbicula fluminea* and the unidentified dark species of *Corbicula* (*Corbicula* sp.) survived 154 days of starvation at room temperature (22-24°C) under holding conditions similar to those that we used for zebra mussels (Cleland *et al.*, 1986). Specimens of both species of *Corbicula* tolerated an approximately 75% loss of their dry tissue biomass (Cleland and McMahon, unpublished results), data very similar to that reported here for *D. polymorpha*. If approximately 75% of dry tissue weight loss is required to induce death in starving zebra mussels, the regression equations relating the decrease in DTW of a standard 20 mm SL zebra mussel during prolonged starvation (Table 2) can be utilized to estimate that mussels starving at 5°C will require 750 days to attain a lethal 75% tissue loss while those at 15°C will require 365 days. When this method of estimating lethality was applied to mussels held at 25°C, the resulting value of 133 days was extremely close to the actual recorded mean time to death of 125.9 days.

The effects of prolonged starvation on shell biomass in *D. polymorpha* were mixed. Shell weight did not change significantly over the entire tolerated 132 day period of starvation in mussels held at 25°C or in mussels starved at 5°C for 229 days. In contrast, a small, but significant increase in DSW was recorded in mussels starved for 229 days at 15°C (Table 2, Figure 2). There is a paucity of published results on the effects of starvation on shell biomass in molluscs. A significant loss in dry shell weight was observed after 130 days starvation in the freshwater unionid mussel, *Lamellidens marginalis* (Masthanamma *et al.*, 1984). In this species, it was suggested that organic (primarily proteinaceous) shell components were being reabsorbed to maintain tissue metabolic demands no longer supported by assimilation of organic nutrients across the gut wall (Masthanamma *et al.*, 1984). The increase in shell biomass in starving mussels at 15°C may be a result of starvation induced inhibition of secretion of new shell material at the edge of the shell thus, slowing or preventing increase in shell length while continued mantle secretion of new nacre to the inside of the shell allowed thickening of the shell and a corresponding increase in shell biomass.

McMahon and Whitehead (1987) demonstrated a similar increase in shell thickness and biomass in naturally slow growing populations (due to reduced food availability) of the European freshwater, pulmonate, limpet snail, *Ancylus fluviatilis*. At 25°C, mussels may not have survived long enough to secrete detectable amounts of new nacre to the inside surface of the shell. At 5°C, significant amounts of new nacre may not have been secreted by the end of the 229 day starvation period due to the greatly reduced metabolic rate at this temperature (Table 3).

Starving mussels displayed an approximate 75% loss of DTW over the tolerated 132 day period of starvation at 25°C, suggesting that they were extensively metabolizing tissue organic energy stores. Similarly, tissue losses of 22.9% and 46.9% were recorded in mussels starving at 5° and 15°C, respectively. The estimated 4.5, 7.4 and 11.8 fold reductions in oxygen uptake rates in starving zebra mussels at 25°, 15° and 5°C, respectively (Table 3), suggest that, during starvation, metabolic rates are suppressed to conserve energy stores and prolong the tolerated period of starvation. A similar 4 fold inhibition of O_2 uptake rate has been reported for starving marine blue mussels, *Mytilus edulis* L. (Bayne, 1973). However, other mollusc species, including *L. marginalis* (Masthanamma *et al.*, 1984) and the marine gastropod, *Thais lamellosa* (Stickle and Duerr, 1970) either maintain a constant metabolic rate at pre-starvation levels or display an increased metabolic rate during starvation. Clearly, lack of capacity to suppress metabolic demand during periods of low or no food availability would act to greatly reduce the tolerated period of starvation. The apparent capacity for zebra mussels and blue mussels to greatly suppress metabolic demands when starved, thus, appears to be an evolved adaption that allows survival of extended periods of low food availability by reducing the rate of organic energy store utilization.

Of interest, is the apparent capacity of *D. polymorpha* to sustain increasingly greater reductions in metabolic rate at lower ambient temperatures. Thus, starving mussels held at 5°C were able to reduce estimated metabolic demand by a 2.6 fold greater extent than could individuals held at 25°C (Table 3). The extremely, low metabolic and tissue biomass loss rates of starving zebra mussels at low temperatures (≤ 5°C) are almost certainly an adaptation that allows this species to survive long winter periods without feeding while still retaining the vast majority of its cellular organic energy stores for support of reproductive effort as temperatures rise above the spawning threshold in spring. The very reduced metabolic rates and tissue biomass loss rates of mussels starving at 5°C also strongly suggest that storage of zebra mussels for periods of up to six months at 5°C in well aerated water without feeding will have minimal impact on physiological condition when mussels are later used in experimentation at higher temperatures (McMahon *et al.*, 1992).

The apparent ability of zebra mussels to suppress metabolic rate, allowing tolerance of greatly extended periods of starvation even at relatively high ambient water temperatures, makes mitigation of zebra mussel macrofouling by filtration or other means of removal of the mussel's planktonic algal food sources from intake water appear generally impractical. Mitigation by starvation would have to involve utilization of alternative sources of water such as well water or treated, filtered tap water in an infested raw water system for extremely long periods. The algal and bacterial food of zebra mussels is far too small (< 10 μm in diameter) to be efficiently mechanically filtered from intake water. However, if a zebra mussel infested raw water system such as a fire protection system was switched to an alternative tap or well water source for make-up water, the long period required for mortality by starving mussels may be of some advantage. The prolonged period over which starving mussels would die could prevent sudden downstream fouling of small diameter components by massive release of dead mussel shells within the system as could occur with more rapidly acting mitigation technologies (*i.e.*, thermal or molluscicide treatments). Mortality over a long period in such systems could allow relatively easy removal of shells by periodic system flushing.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Milton Matthews, Michael Clarke and Thomas Ussery of The Center for Biological Macrofouling Research for their technical assistance during the course of the experiments. Gary L. Dye, Lockmaster of Black Rock Lock, Buffalo, NY, collected and shipped to us the zebra mussels utilized in this experiment. This study was primarily supported by a grant to R. Chase from the Ronald E. McNair Post-Baccalaureate Achievement Program managed through The University of Texas at Arlington. It was also conducted as part of the Zebra Mussel Research Program of the United States Army Corps of Engineers by the Center for Biological Macrofouling Research at the University of Texas at Arlington. Permission was granted by the Chief of Engineers to publish this information.

LITERATURE CITED

Bayne, B. 1973. Aspects of the metabolism of *Mytilus edulis* during starvation. *Netherl. J. Sea Res.* 7: 399-410.

Bielefeld, U. 1991. Histological observation of gonads and digestive gland in starving *Dreissena polymorpha* (Bivalvia). *Malacologia* 33(1-2): 31-42.

Bliss, C.I. 1936. The calculation of the time mortality curve. *Ann. Appl. Biol.* 24: 815-852.

Cleland, J.D., R.F. McMahon and **G. Elick**. 1986. Physiological differences between two morphotypes of the Asian clam, *Corbicula*. *Am. Zool.* 26: 103A.

Devi, V.U., U.P. Rao and **D.G.V. Prasada Rao**. 1985. Body component indices of an intertidal gastropod *Morula granulata* (Duclos) subjected to starvation. *Indian J. Mar. Sci.* 14: 44-45.

Hebert, P.D.N., B.W. Muncaster and **G.L. Mackie**. 1989. Ecological and genetic studies on *Dreissena polymorpha* (Pallas): a new mollusc in the Great Lakes. *Can. J. Fish. Aquat. Sci.* 46: 1587-1591.

Hill, R.W. and **G.A. Wyse**. 1989. *Animal Physiology* (Second Edition). Harper and Row, New York, New York. 656 pp.

Mackie, G.L., W.N. Gibbons, B.W. Muncaster and **I.M. Gray**. 1989. *The Zebra Mussel,* Dreissena polymorpha*: A synthesis of European Experiences and a Preview for North America.* Water Resources Branch, Great Lakes Section, Ontario Ministry of the Environment, Toronto, Ontario. 76 pp. plus appendices.

Masthanamma, P., K.R. Purushotham, and **R. Ramamurthi**. 1984. Metabolism of *Lamellidens marginalis* (Lamarck) in relation to starvation stress. I. Mortality, body weight, and oxygen consumption. *Indian J. Comp. Anim. Physiol.* 2: 60-63.

McMahon, R.F. 1990. *The Zebra Mussel: U.S. Utility Implications*. GS-6995, Research Project 1689-24, Electric Power Research Institute, Palo Alto, California. 70 pp.

McMahon, R.F. 1991. Mollusca: Bivalvia. Pages 315-399 *in*: J.H.Thorp and A.P. Covich (Eds.), *Ecology and Classification of North American Freshwater Invertebrates*. Academic Press, Inc., New York.

McMahon, R.F. 1992. The zebra mussel - the biological basis of its macrofouling and potential for distribution in North America. Reprint # 342 from *Corrosion 92* (Proceedings of the 1992 Annual Meeting of the National Association of Corrosion Engineers), National Association of Corrosion Engineers, Houston, Texas. 14 pp.

McMahon, R.F. and **B.E. Whitehead**. 1987. Environmental induction of shell morphometric variation in the European stream limpet, *Ancylus fluviatilis* (Müller) (Mollusca: Pulmonata). *Am. Malacol. Bull.* 5: 105-124.

McMahon, R.F., B.N. Shipman and **D.P. Long**. 1992. Laboratory efficacies of nonoxidizing molluscicides on the zebra mussel (*Dreissena polymorpha*) and the Asian clam (*Corbicula fluminea*). Pages 575-597 *in*: T.F. Nalepa and D.W. Schloesser (Eds.), *Zebra Mussels: Biology, Impacts and Control*. Lewis Publishers, Boca Raton, Florida.

McMahon, R.F., T.A. Ussery and **M. Clarke**. 1993. *Use of Emersion as a Zebra Mussel Control Method*. Contract report EL-93-1, U.S. Corps of Engineers, Waterways Experiment Station, Vicksburg, Mississippi. 27 pp.

Russell-Hunter, W.D. and **A.G. Eversole**. 1976. Evidence for tissue degrowth in starved freshwater pulmonate snails (*Helisoma trivolvis*) from tissue, carbon, and nitrogen analysis. *Comp. Biochem. Physiol.* 54A: 447-453.

Sprung, M. and **J. Borcherding**. 1991. Physiological and morphometric changes in *Dreissena polymorpha* (Mollusca: Bivalvia) during a starvation period. *Malacologia* 33(1-2): 179-191.

Stickle, W.B. and **F.G. Duerr**. 1970. The effects of starvation on the respiration and major nutrient stores of *Thais lamellosa*. *Comp. Biochem. Physiol.* 33: 689-695.

Zebra Mussel Information Clearing House. 1993. North American range of the zebra mussel as of 15 December 1993. *Dreissena Polymorpha Information Review* 4(2): 8-9.

Invading the Invaders: Infestation of Zebra Mussels by Native Parasites in the St. Lawrence River

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ABSTRACT: Between June and August, 1993, adult *Dreissena polymorpha* and *Dreissena bugensis* were collected from well-established populations at two sites along the international section of the St. Lawrence River. The mussels were taken to the laboratory for exhaustive examination for parasites by gross pathological and histological techniques. Seventy percent of the *D. polymorpha* at the site near Massena, New York were infested by the naidid oligochaete annelid, *Chaetogaster limnaei*, and 61% were infested by various nematode species. Both nematodes and *C. limnaei* also occurred in *D. bugensis* at the Cape Vincent, New York site. Other parasites that were found in smaller numbers (5% of mussels or less) were a hydracarinid mite larva, larvae of the chironomid midge *Paratanytarsus* sp., the naidid oligochaete *Ophidonais serpentina* and other unidentified naidids. Each of these was found only at the site near Massena. The only parasites that showed any evidence of pathology were *C. limnaei*, 1 of which was feeding on oocytes in the ovary of a female *D. polymorpha*. All other parasites were found only in the mantle cavity. Temporal patterns of prevalence and intensity showed a maximum for *C. limnaei* on 13 July and for nematodes on 3 August. This study demonstrated that some native North American animals normally associated symbiotically with native molluscs have adapted quickly to zebra mussels and quagga mussels as new hosts.

Introduction

Parasitic animals, including various worms and arthropods, are common associates of virtually all molluscs, including zebra mussels, *Dreissena polymorpha* (Davids and Kraak, 1993). However, these parasitize only adult mussels. Thus, because only veligers were brought to North America from Europe, none of the mussels' parasites were introduced with them. The resulting introduced mussel populations have been able to live free from parasites. Many potential native parasites may be pathogenic and exert some level of natural biological control, whereas others that parasitize native wildlife might become more widespread by using zebra mussels as intermediate hosts. The present study was conducted to determine the extent to which native North American parasitic animals have been successful in utilizing zebra mussels as hosts in two localities on the St. Lawrence River where the mussel colonies are well established.

Materials and Methods

Between 29 June and 3 August 1993 six weekly collections of zebra mussels, *D. polymorpha* were made by scraping adults from submerged steel support columns near the St. Lawrence River Seaway's Snell Lock between Massena, New York and Cornwall, Ontario. This dense population had been well established for 3 years prior to this study. No quagga mussels, *D. bugensis*, occurred at this site. Mussels were collected using a hand-held scraping basket extended to a depth of 1.5 m.

On 9 July and 30 July zebra mussels, *D. polymorpha*, and quagga mussels, *Dreissena bugensis*, were collected from unionids, rocks, logs and other substrates dredged from a depth of 10-12 m in the St. Lawrence River at Cape Vincent, New York. These populations, near where the St. Lawrence flows from Lake Ontario, had been well established for 2 years prior to this study.

Upon tranfer to the laboratory, all organs of the mantle cavity and visceral mass of each mussel were examined carefully under a dissecting microscope for the presence of parasites. After counting the parasites and noting their location, representative specimens were fixed in situ for examination of potential histopathology. Others were removed from the host and photographed alive or fixed for staining.

Results

Snell Lock, Massena collections

Of 151 *D. polymorpha* examined from Massena, 108 (72%) harbored at least one worm. By far the most common were naidid oligochaete annelids, *Chaetogaster limnaei*, which occurred in 76 (50%) of the mussels. The mean number of *C. limnaei*

per infested mussel was 3.20 (range = 1-18). More males (56%) were infested than females (43%), but females harbored a higher number of worms per host (3.19) than did males (2.38). The vast majority of *C. limnaei* inhabited the mantle cavity of their hosts, frequently occurring between the gill lamellae. Gross and histological examination showed only slight evidence of pathology in this location. However, one *C. limnaei* was found inside the ovary of a mussel and had caused appreciable damage by feeding on the host's oocytes and gonadal tissues.

The next most commonly encountered worms were juvenile and adult nematodes. At least four species were encountered; these were not identified, but one belonged to the genus *Mononchus* and another was a dorylaimid. Sixty-one (40%) of the mussels harbored nematodes. The mean number per infested mussel was 2.84 (range = 1-10). Unlike the situation with *C. limnaei*, fewer males (33%) were infested than females (44%), but males and females harbored a similar number of nematodes per infested host (2.71 and 2.81, respectively).

Figure 1 shows temporal trends in the prevalence (i.e., percentage of mussels infested) of *C. limnaei* and nematodes throughout the study period, whereas Figure 2 shows temporal trends in the intensity of infections (i.e., mean number of worms per infested mussel).

Low numbers of mussels harbored other parasites. Eight (5%) of the mussels harbored naidid oligochaetes other than *C. limnaei*. Two of these were *Ophidonais serpentina*, but the others were identified only to the family level. Only one mussel harbored a hydracarinid (water mite) larva in the mantle cavity, but many mussels had hydracarinid egg masses glued to their shells. Larvae of the chironomid fly, *Paratanytarsus* sp., were found in the mantle cavities of two mussels. No mussels were found to be naturally infested by echinostomatid metacercariae or other trematodes.

Cape Vincent collection

Of 30 *D. polymorpha* examined from Cape Vincent (none (0%) harbored *C. limnaei*, but 8 (27%) were infested by nematodes. Of 15 *D. bugensis* examined from Cape Vincent, 1 (7%) harbored a single *C. limnaei* and 3 (20%) were infested by nematodes. No other parasites were found at this site.

Discussion

Chaetogaster limnaei is known to be a commensal or parasite in several North American and European species of aquatic snails and bivalves (Barbour, 1977; Gale, 1973). However, this is the first report of its occurrence in *Dreissena* on either continent. It is also the first report of the ingestion of host gametes by this species. Because this organism appeared to be commensalistic in most of the mussels, it cannot be expected to inflict high mortality. However, some reduction of the mussels'

Figure 1

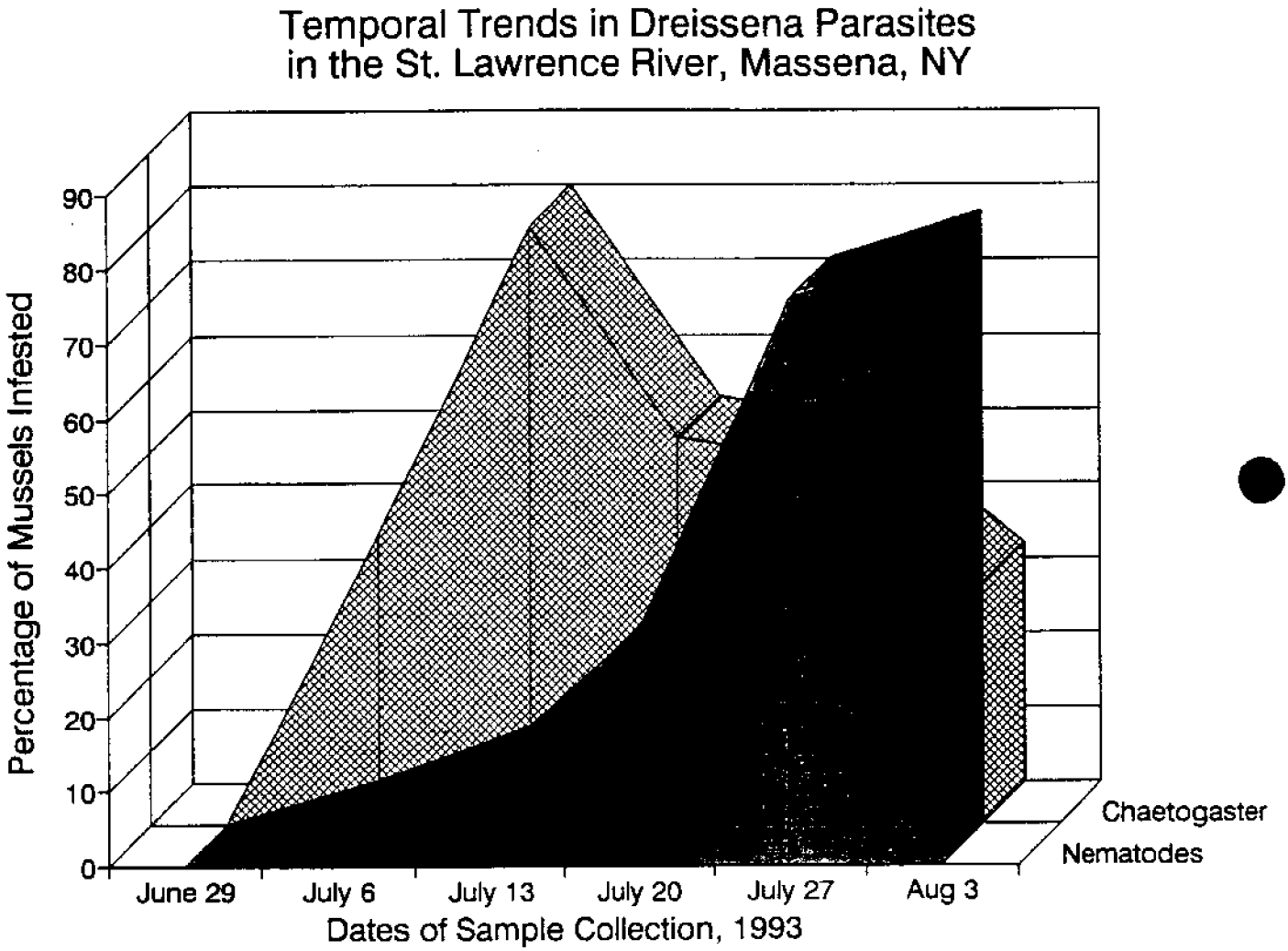
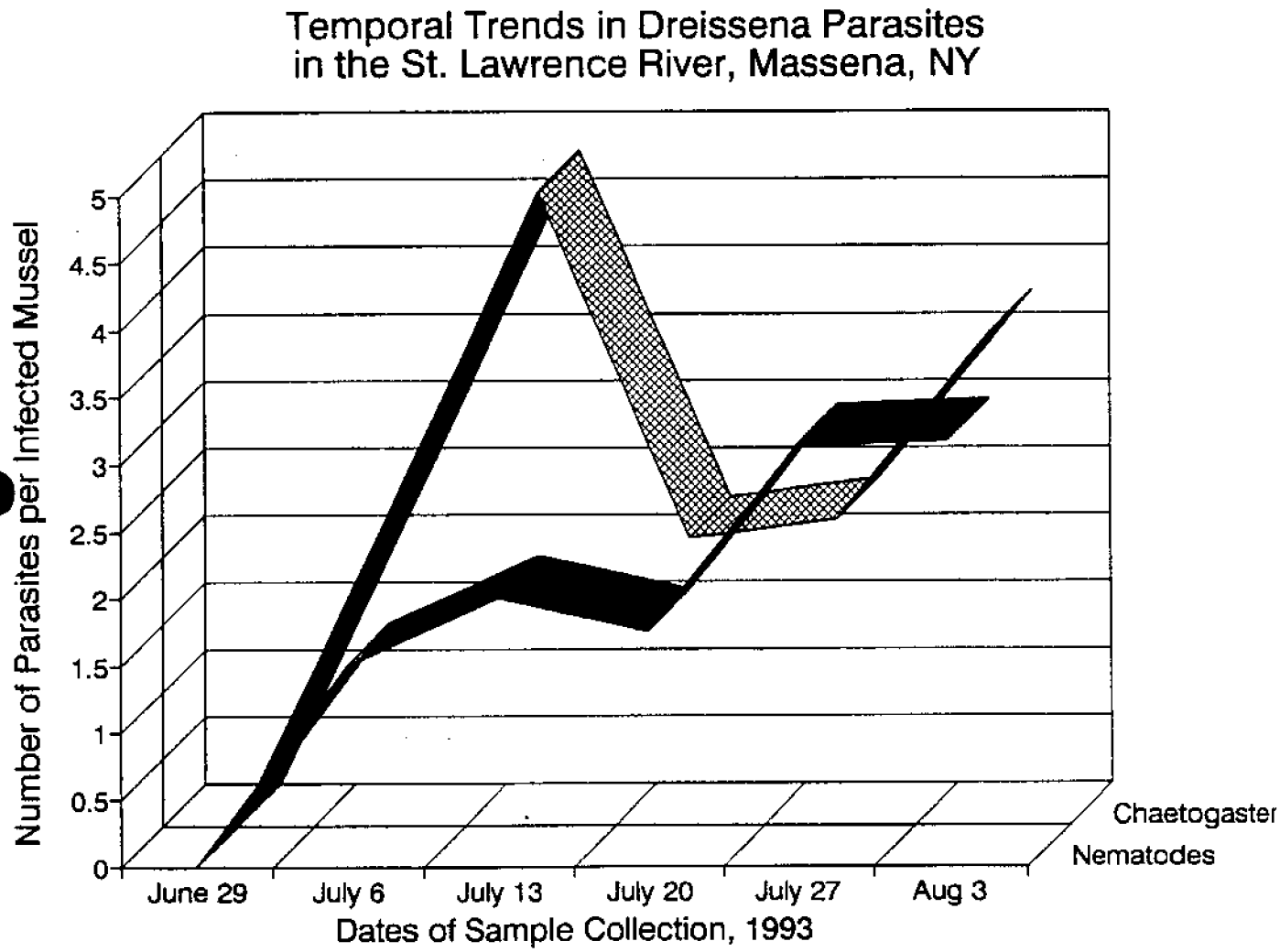

Temporal Trends in Dreissena Parasites
in the St. Lawrence River, Massena, NY
Percentage of Mussels Infested
90
80
70
60
50
40
30
20
10
0
June 29
July 6
July 13
July 20
July 27
Aug 3
Dates of Sample Collection, 1993
Chaetogaster
Nematodes

Figure 2


Temporal Trends in Dreissena Parasites
in the St. Lawrence River, Massena, NY
Number of Parasites per Infected Mussel
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
June 29
July 6
July 13
July 20
July 27
Aug 3
Dates of Sample Collection, 1993
Chaetogaster
Nematodes

reproductive potential could result from damage to the gonads. Further work is needed to ascertain the degree to which this occurs. Both prevalence (Fig. 1) and intensity (Fig. 2) of *C. limnaei* infestation peaked on 13 July, and overall prevalence and intensity patterns for this species were positively correlated ($r = 0.8252$, $P = 0.0432$). Future studies should examine the relationship between these population patterns with those of other native molluscs that serve as hosts for *C. limnaei*.

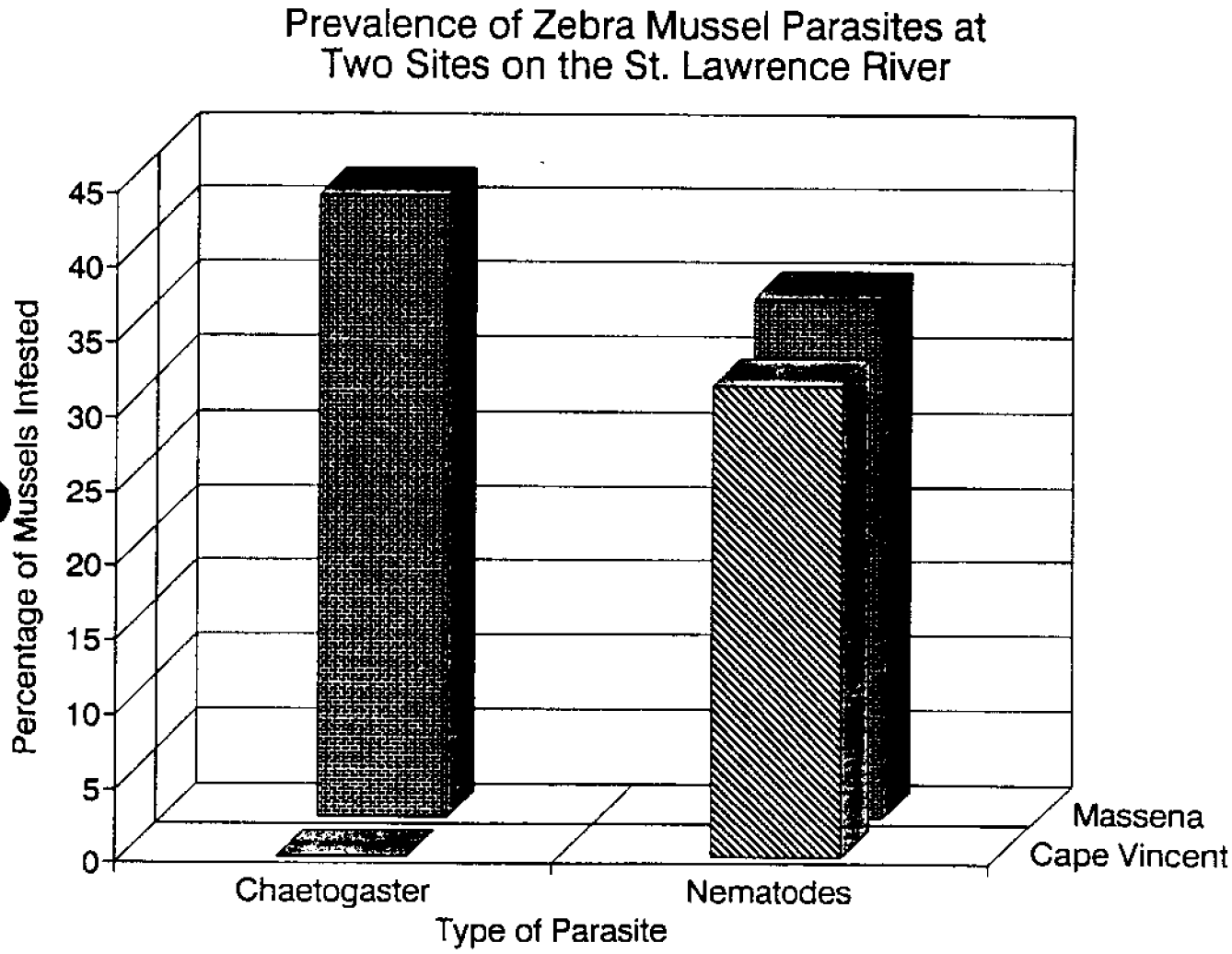
Both prevalence (Fig. 1) and intensity (Fig. 2) of infestation by nematodes rose steadily through the study period, reaching a maximum on 3 August, the last collection day. Because these sample included several nematode species, these data reveal nothing definitive about the population patterns of any particular nematode. Further studies in which species identities are known should be a priority in the future. Nematodes are ubiquitous in virtually all habitats around the world. Many are free living (Poinar, 1991), but many others are parasitic or commensalistic in nearly all species of animals and many plants. It is not known which of these categories includes the species observed in the present study. Most of those seen appeared not to be pathogenic to the mussel. However, because of their high prevalence and intensity of infestation in the mussels, further research on them is warranted.

Results from Cape Vincent are of limited value inasmuch as the sample size was small. However, they do demonstrate that *D. bugensis*, which did not occur at Massena, also can serve as a host for both nematodes and *C. limnaei*. A comparison of infestations of *D. polymorpha* at the 2 sites (Fig. 3) showed that there is a highly significant difference between the 2 sites with respect to *C. limnaei* infestation (Chi-square = 17.766; $P < 0.0001$). However, there is no significant difference with respect to nematode infestation (Chi-square = 0.3042; $P = 0.5813$). These results should be interpreted with extreme caution, however, because the 2 sites differed in depth, substrate type, collection method and many other parameters. Furthermore, there was not discrimination among the several nematode species present, and it is not known whether the species composition between the 2 sites differed. The results do suggest, however, that further studies should explore differences in parasite/host patterns among various localities and habitats.

Larvae of the midge, *Paratanytarsus* sp., have been reported as commensals in both *D. polymorpha* and *D. bugensis* in the St. Lawrence River at points downstream from the present study (Ricciardi, 1994). Chironomid larvae are among the most common benthic animals in freshwater systems around the world, but most species are free living (Coffman and Ferrington, 1984). Hydracarinids are known to be common parasites of native unionids (Smith and Cook, 1991), and the presence of their egg cases on the shells of living dreissenids in the present study suggests a potential for transmission to the dreissenids. The reasons for their failure to infest dreissenids in the present study are not clear.

Conn and Conn (1993) reported experimental infestation of zebra mussels by the metacarcarial stages of a native echinostomatid fluke. In their report, only 5% of the experimentally exposed mussels became infested. The absence of echinostomatids from

Figure 3


Prevalence of Zebra Mussel Parasites at
Two Sites on the St. Lawrence River
Percentage of Mussels Infested
45
40
35
30
25
20
15
10
5
0
Chaetogaster
Nematodes
Type of Parasite
Massena
Cape Vincent

any of the mussels in the present study, combined with the fact that echinostomatids have never been reported from *Dreissena* spp. in Europe, might suggest that such infestations are rare in natural populations. However, other trematode groups (in their sporocyst and redial stages) have been reported from European dreissenid populations (Chernogorenko, 1970) and are native parasites of North American molluscs (Schell, 1985), but were absent in the present study. Thus, it may be that establishment of trematode parasites in zebra mussels is likely to occur eventually in some areas of North America. Therefore, continued surveillance is warranted.

In summary, this study demonstrated that some native North American animals normally associated symbiotically with native molluscs have adapted quickly to zebra mussels. This basic finding may have important implications for biological control and/or for changes in the epizootiology of parasites native to North America.

Acknowledgments

We are grateful to Jerry LeTendre and Steve Cluett of the New York State Department of Environmental Conservation for assisting in the field work at Cape Vincent and for providing the research vessel. We are grateful to Stephen Hung and Thomas Lavigne of the Seaway Development Corporation, U. S. Department of Transportation for providing access to the Snell Lock study site. This study was funded by grants from the Pew Charitable Trusts and North American Scientific Enterprises (NASCENT), with additional funding from St. Lawrence University and The University of the South.

Literature Cited

Barbour, M. T. 1977. *Chaetogaster limnaei limnaei* (Oligochaeta: Naididae) inhabiting the mantle cavity of the pill clam *Sphaerium*. *Transactions of the American Microscopical Society* 96: 141-142.

Chernogorenko, M. I. 1970. Patternas in the establishment of a trematode fauna parasitic on benthic vertebrates in the reservoirs of the Dnepr Series. *Hydrobiological Journal* 6: 46-49.

Coffman, W. P., and L. C. Ferrington, Jr. 1984. Chironomidae. *In*: Merritt, R. W., and K. W. Cummins, editors. *An Introduction to the Aquatic Insects*, 2nd edition. Kendall/Hunt, Dubuque. pp. 551-652.

Conn, D. B., and D. A. Conn. 1993. Parasitism, predation and other biotic associations between dreissenid mussels and native animals in the St. Lawrence River. In: Tsou, J.L., editor, *Proceedings: Third International Zebra Mussel Conference*. Electric Power Research Institute, Palo Alto, California. pp. 223-234.

Davids, C., and M. H. S. Kraak. 1993. Trematode parasites of the zebra mussel (*Dreissena polymorpha*). In: T. F. Nalepa and D. W. Schlosser (editors), *Zebra Mussels: Biology, Impacts, and Control*. Lewis Publ., Boca Raton, FL. pp. 749-759.

Gale, W. F. 1973. Predation and parasitism as factors affecting *Sphaerium transversum* (Say) populations in Pool 19, Mississippi River. *Research in Population Ecology* 14: 169-187.

Poinar, G. O., Jr. 1991. Nematoda and Nematomorpha. *In*: Thorp, J. H., and A. P. Covich, editors. *Ecology and Classification of North American Freshwater Invertebrates*. Academic Press, San Diego. pp. 249-283.

Ricciardi, A. 1994. Occurrence of chironomid larvae (*Paratanytarsus* sp.) as commensals of dreissenid mussels (*Dreissena polymorpha* and *D. bugensis*). *Canadian Journal of Zoology*. In press.

Schell, S. C. 1985. *Handbook of Trematodes of North America North of Mexico*. University Press of Idaho, Moscow. 263 pp.

Smith, I. M., and D. R. Cook. 1991. Water mites. *In*: Thorp, J. H., and A. P. Covich, editors. *Ecology and Classification of North American Freshwater Invertebrates*. Academic Press, San Diego. pp. 523-592.

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The Zebra Mussel Invasion: A Marine Ecological Perspective

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Abstract

The Zebra mussel *Dreissena polymorpha* resembles a marine invertebrate, in its mode of reproduction and dispersal, in its ability to exert strong controls on phytoplankton in the water column, and in its strong keystone effects on benthic communities. I discuss some marine ecological perspectives that might enhance the study of the profound ecological and evolutionary effects of Zebra mussels. (1) Systematics and Genetics. Studies of marine invertebrates reveal several sibling species complexes, and these species often are heterogeneous in reproductive ecology, uptake of toxic substances, and morphology. Such heterogeneity may be important, both in the species that invaded from European waters and in the possible speciation that may be occurring in North America. We also should expect rapid evolution of life histories, morphology, and resistance to toxic substances. (2) Larval Transport and Recruitment. Planktonic larval dispersal must be studied in the context of hydrodynamics. Water flushing and coastal processes may strongly influence larval recruitment and dispersal patterns, and both recruitment and physical flow structure must be studied to understand larval movements. (3) Community Interactions. Zebra mussels resemble marine mussels in their ability to smother other epibenthos and the formation of dense mussel beds. Manipulative experiments will be required to understand competitive interactions. Predators may exert strong effects on Zebra mussel beds. (4) Trophic Dynamics. Owing to their clearance rates and high density, Zebra mussels strongly affect the water column and may transfer large amounts of organic particulates to the benthos. Purposeful introductions of Zebra mussels will aid in our understanding of all of these processes.

Introduction

The widespread invasion of North American fresh waters by the Zebra mussel, *Dreissena polymorpha*, and by the Quagga mussel *Dreissena bugensis* (?) will probably be among the best understood ecological changes ever followed, if only because of the rapid identification and location of the first appearance in the Great Lakes of North America (Lake St. Clair, 1988, but the first appearance was probably in 1986 — Nalepa and Schloesser 1993), the intense study of the spread, and owing to the need for society to evaluate the obvious negative impacts on a variety of structures in waterways and the great impact upon the ecology of many freshwater lakes, rivers, and streams.

Other invertebrates, such as the Asiatic clam *Corbicula fluminea*, have invaded our fresh waters before. But the Zebra mussel's ecological success, which is largely explained by its planktotrophic larva and by its versatility, owing to generalized substrate preference and mode of attachment by byssal threads, has upped the ecological ante, because it can spread so much more rapidly and it can effectively cover and smother so much more bottom than any previous invaders.

The Zebra mussel's dispersal mode and habits are much like those of marine epibenthic species, such as the blue mussel *Mytilus edulis*. This raises the question: Is a marine ecological and evolutionary perspective useful in understanding the potential ecological impacts that the Zebra mussel will have on fresh water communities and the potential evolutionary changes that might occur as this prolific species spreads throughout North America? It is my intent to sketch some possible benefits that might emerge from marine ecological and evolutionary perspectives, though I doubt that they will point to ideas that have completely failed to already touch the large and active community of Zebra mussel research.

Systematics and Evolution

Marine species, particularly those in the intertidal, are thought to have broad geographic ranges, across oceans and continents. Similar thinking exists for many fresh water species, such as the supposedly cosmopolitan oligochaete *Limnodrilus hoffmeisteri*. In recent years, several broad-ranging marine species have been found to consist of a series of sibling species, distinguishable by larval and allozyme characters (Grassle and Grassle 1976, Varvio et al., 1988, Koehn 1991). The mussel genus *Mytilus* was thought to consist of *M. galloprovincialis* within the Mediterranean and Iberian coasts and the much more broadly ranging *M. edulis* (see Seed 1978). Allozyme markers suggest however, that a third species, *M. trossulus*, occurs in northern Atlantic and Pacific latitudes, and that *M. galloprovincialis* may well occur on the California coast. Sibling species of the polychaete *Capitella capitata* complex have quite different larvae and potentials for

population increase and spread (see Ecklebarger and Grassle 1987). In fresh water, Christian Sturmbauer (unpublished) has recently discovered differences between supposed *Limnodrilus hoffmeisteri* populations between Austrian and New York waters that almost certainly signify species differences.

Such differences are also likely to be found for the wide ranging Zebra mussel. Recently, a second closely related mussel has been found, which has been dubbed the Quagga mussel. Other Eurasian species of *Dreissena* exist and it is possible that more have invaded North America. Careful DNA and allozyme surveys may also identify other sibling species within the North American Zebra mussel complex itself. If the *Capitella* story (Ecklebarger and Grassle 1987) can be generalized, then a careful study will be essential, since sibling species differ in larval morphology, dispersal, potential for population increase, and even in responses to physiological factors and toxic substances.

We can divide the Zebra mussel invasion into a period of spread of the geographic range, now underway, and a period of rapid population increase, with subsequent fluctuation and gene exchange with adjacent populations. Studies using allozyme markers demonstrate local geographic heterogeneity among the Great Lakes and three allelic variants appear to be unique to the Great lake populations (Boileau and Hebert 1993). Combined with other markers, such as mitochondrial RFLPs and direct DNA sequences, it may be possible to identify the source populations and the character of spread. New populations maybe established from small numbers of founding individuals, and this may influence the genetic differentiation that has been observed.

As the Zebra mussel spreads over a vast number of freshwater bodies of varying temperature, chemistry, presence of human—introduced toxic substances, and gamete and larval flushing rates, strong natural selection may cause rapid genetic divergence in a variety of ecologically important characters (see Levinton 1980). Lake St. Clair populations do not differ significantly from European populations in heterozygosity (Boileau and Hebert 1993), so we do not expect that genetic variation was seriously depleted even at the time of the initial invasion.

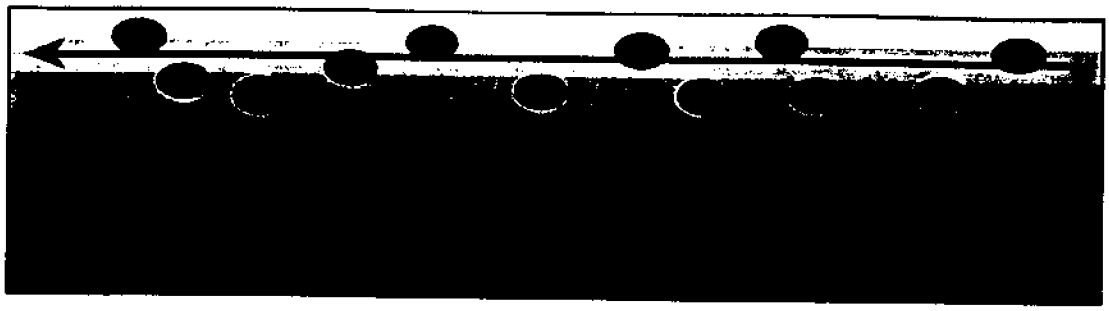
The following list of types of changes is incomplete, but gives an idea for areas of study:

(1) **Shell thickness, shape, and growth rate in response to predation.** As Zebra mussels have spread over a wide variety of habitats, they must be encountering some areas with potential predators, such as molluscivorous fishes and crustacea. This suggests a potential for rapid evolution of shell characters. When the invading marine green crab *Carcinus maenas* invaded the coastal waters of Maine, populations of the periwinkle *Littorina saxatilis* were exposed in some bays to intense predation, and the rapid evolution of increase shell thickness and lower spired shells ensued (Seeley 1986). The

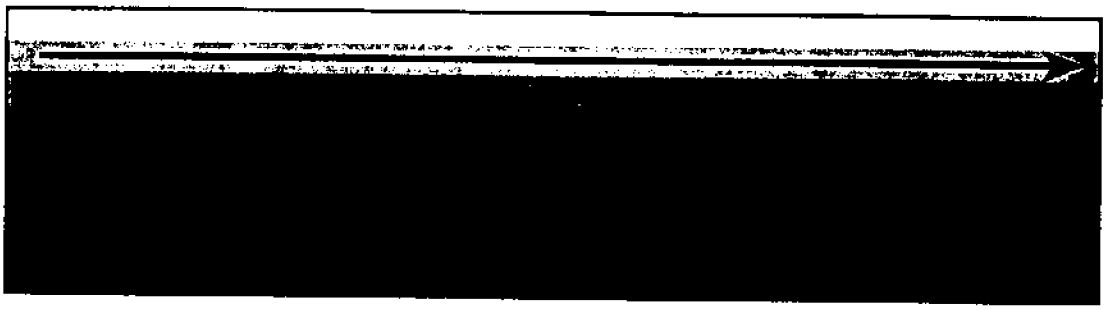
blue crab *Callinectes sapidus* may be an important predator on part of the Zebra mussel population that has invaded the Hudson River and may exert similar evolutionary pressures. Molluscivorous fish may be important in more interior North American freshwater bodies.

(2) **Larval behavior in response to water flushing.** Estuarine species with planktotrophic larvae have varying adaptations to the natural exchange and flushing of estuarine water into the adjacent coastal regions. While some species leave the estuary as larvae and return as recruiting settlers (e.g, Morgan 1990), others have extensive adaptations to remain in the estuarine system (Fig. 1). Zebra mussel populations live along the generally coastally associated hard substrata of lakes such as Lake Erie, but they also have colonized rivers such as the Hudson River, which have strong flushing rates. Zooplankton abundance in the Hudson River is inversely correlated with river flow, which suggests that flushing is a major factor in determining zooplankton densities (Pace et al. 1992). If there were genetic variability for larval development rate and behavior, one might expect that rivers with high flushing rates would select for more benthic larvae, perhaps with higher development rates, and maybe even a stronger dependence upon yolk to fuel larval development. It would be very useful to select a range of fresh water bodies with varying flushing rates to examine differentiation of larval characters. Clearly, the evolution of reduced dispersal would have important implications on population dynamics and exchange with distant populations.

(3) **Life history responses to varying sources of mortality.** It is likely that *D. polymorpha* populations will spread over lakes with widely differing sources of mortality. Some lakes will have abundant larval predators, whereas others may have predators that prey preferentially on adults. Strong predation on adults will cause natural selection for early reproduction and perhaps increased investment in reproduction to the expense of somatic growth. In some cases, larval recruitment may be quite variable, selecting for a "bet—hedging" life history adaptation of repeated reproduction, as opposed to concentrated devotion of resources in earlier life. Many marine bryozoa colonize seaweeds and die when the fronds senesce in the winter. If mussels colonize erect algae in lakes, they too may experience early mortality, as the algae senesce and the mussels fall into the mud (I thank Ladd Johnson for this suggestion). This cycle might select for rapid reproduction, and would be analogous to the early reproduction observed for Pacific coast populations of *Mytilus edulis*, that are subject to early mortality owing to storms and predation (Suchanek 1985).



FLOODING TIDE



EBBING TIDE

1. In bodies of water with considerable flow, flushing rates are an important factor in larval recruitment success. In tidal estuaries, larvae of several species are known to stay near the bottom during the ebbing tide, and rise in the water column during the flood. The net effect of this behavior is to transport larvae up the estuary.

(4) **Adaptation to varying concentrations of toxic substances.** As in many marine coastal waters, fresh water environments may have high concentrations of toxic substances in sediments, sediment pore waters, and in the overlying water column. Levinton (1980) suggested that this state of affairs might promote strong natural selection for resistance to toxins if there was genetic variation for resistance. Such resistance evolves commonly among insect populations that are exposed to insecticides and by bacteria that are exposed to drugs in hospitals. The oligochaete *Limnodrilus hoffmeisteri* evolved resistance to cadmium in only a few generations, and there is extensive genetic variation for resistance within populations living in relatively clean sediments (Klerks and Levinton 1989). The evolution of resistance to toxins is now widely recognized as a major feature of invertebrate populations in aquatic habitats (Klerks and Weis 1987, Klerks and Levinton 1989). Since *Dreissena polymorpha* has spread over many habitats, it is likely that it has been exposed to a wide variety of toxic substances. Once the problems of larval rearing are completely solved (see Vanderploeg et al. 1994) it will be possible to do genetic studies on resistance. Indeed, if these studies are done soon, then a remarkable record of rapid evolution may be acquired.

(5) **Temperature adaptations**. Because of the widespread latitudinal range already achieved, *D. polymorpha* lives in a series of water bodies of widely differing water temperatures. Studies of marine poikilotherms demonstrates extensive latitudinal differentiation in response to temperature. Some of this response involves adjustment of energy budgets, which lead to genetic differentiation of metabolic strategies for living in different thermal regimes (Levinton 1983, Lonsdale and Levinton 1989). Additionally, colder water populations may have accelerated life cycles, relative to warmer water populations, in order to reproduce in a shorter season, with a presumably shorter phytoplankton bloom that might nourish *D. polymorpha*'s planktotrophic larva. Latitudinal differentiation has been revealed in the past by common garden experiments, preferably with offspring of populations form different latitudes that have been raised under common conditions (Levinton and Monahan 1983).

Larval Transport and Recruitment

The Zebra mussel is a rather typical species, when compared with marine hard substratum species having planktonic larvae. Sexes are separate, eggs and sperm are spawned freely into the water column, and a planktotrophic larva resides in the plankton for a period estimated between 5 days and 5 weeks, depending upon various accounts (Sprung 1993). Larval food is not understood terribly well, but the apparent narrow range of 1—4 microns may limit feeding to bacteria and smaller green algae and cyanobacteria. The Hudson River may be ideal for Zebra mussel larval survival, owing to its rich bacterial production (Findlay et al. 1991). A number of predators have been identified, including various fish larvae (Wiktor 1958 — cited in Sprung 1993, Kornobis 1977) and Mesocyclops (Karabin 1978). Larval settling substratum preference is minimal, although bare sand and mud appears to be avoided, hard substrata are preferred, and there may be a preference for adult shells (Hebert et al. 1989).It would be of great interest to know whether *D. polymorpha* larvae employ byssal threads, either as parachutes to delay sinking or to use as drag anchors to promote settlement on the substratum. *Mytilus edulis* larvae have been described as two—phase settlers. The primary phase involves settlement and metamorphosis on filamentous algae, whereas a secondary movement is believed to occur to the location of larger mussel beds (Bayne 1964, 1976). This notion is now controversial (Kautsky 1982, Fell and Balsamo 1985), but it is of interest, since larval settlement of *D. polymorpha* may sometimes be focused on algae. Currents might redisperse recently metamorphosed larvae.

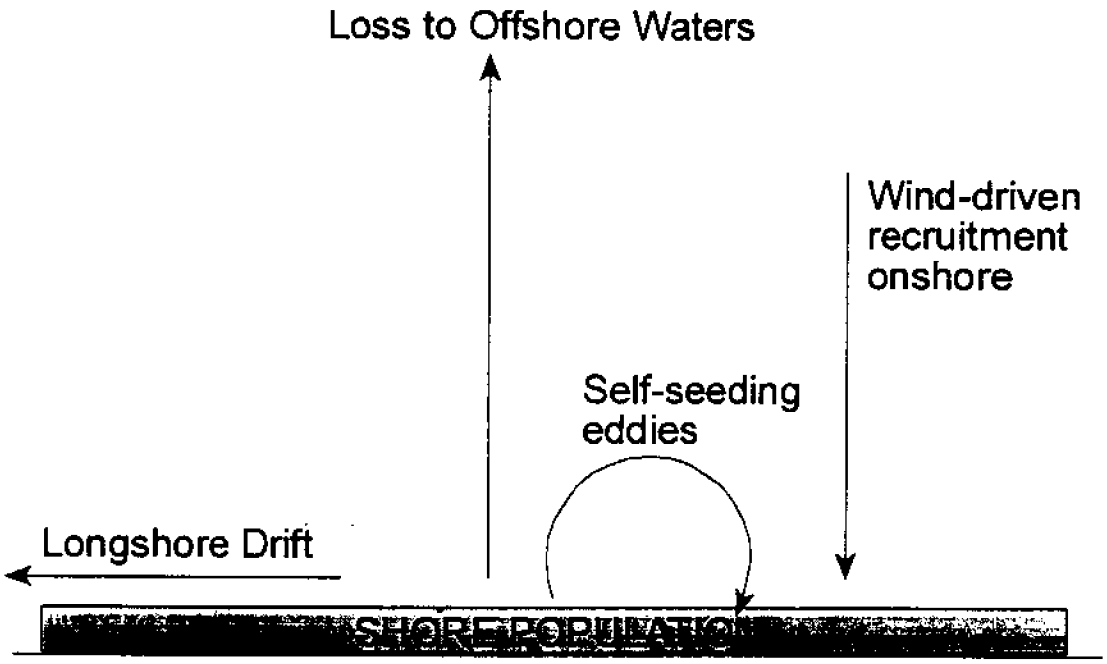
Because *D. polymorpha* is a free—spawner, water movement and turbulence may play an important role in fertilization success and population growth. In recent years, marine free spawning species have been investigated intensively and turbulence has proven to be a

major factor that reduces fertilization success (Denny and Shibata 1989, Levitan et al. 1992). Free spawning species also suffer the danger of gamete wastage owing to asynchronous spawning. Lunar and solar cycles are employed extensively by marine species to enable gamete union, and this reaches a peak in coral reefs, where mass spawning events, keyed into lunar cycles, involve many species simultaneously (Harrison et al. 1984). These events have been proven to greatly enhance fertilization success. In some cases, such as the crown—of—thorns starfish *Acanthaster planci* fertilization may be quite efficient, even at great distances (see Oliver and Babcock 1992). It would be of great interest to study such cycles in the Zebra mussel, given its trans—Atlantic migration and subsequent rapid spread throughout eastern North America.

In considering the potential for population growth and spread one must consider (a) factors that control extension of the geographic range and (b) maintenance and replenishment of a population, once it has been established in a region. Many marine species have moved across an ocean to a new area and spread subsequently along a coast. Although there are many species of fiddler crabs (genus *Uca*, family Ocypodidae, Crustacea) in the American tropics, a single species lives in the eastern Atlantic, with morphologically relatively homogeneous populations from west Africa to Portugal (Crane 1975). It seems unlikely that humans aided the transport, but the regional homogeneity suggests a fairly recent rapid spread. Several marine species probably were brought westward to the N. American Atlantic coast in the 19th century and have spread rapidly southward along the coast. The periwinkle *Littorina littorea* spread in all probability from Nova Scotia southward, reaching the middle Atlantic States. If we make the rough estimate that 1500 km were traversed in ca. 100 years, then we can readily see that the 15 km per year movement combined with a ca. 3 week larval life requires a current transport of ca. 1 cm per second, which is rather slow. This rough calculation suggests that range extension by planktonic larvae is very rapid and must be limited only by isolating current systems and perhaps ultimate temperature limitations. For example, *Mytilus edulis* larvae move southward and settle south of Cape Hatteras, but they usually fail to survive the high summer temperatures. Current systems may contribute to isolating provincial biotas north and south of Point Conception, California, which would bring a range extension along the western U.S. coast to a halt. Zebra mussels may extend their range along some long coastlines of the Great Lakes, and it would be of great interest to learn if the rate of range extension is as fast as current—aided larval dispersal can carry them.

Maintenance and replenishment of a population requires acknowledge of larval behavior, current transport, mortality, and larval life span. Mussels may be able to produce thousands of eggs, but population replenishment depends strongly upon fertilization efficiency, flushing of larvae from the system, predation on larvae, and mechanisms of successful larval recruitment to suitable adult settling sites (see Butman 1987). On the simplest level, larval replenishment of populations can be visualized as a competition

between larval production and flushing from the system. For example, Gaines and Bertness (1992) have found that larval recruitment of barnacles to settling panels is strongly related inversely to the degree of water flushing rates from a coastal marine bay, which is in turn a strong function of regional rainfall. Many estuarine species with planktonic larvae face the danger of loss to the open sea, which competes with an opposing danger of high predation rates within estuaries. Larvae of some oyster sand crabs modulate their behavior to reduce flushing from the estuary, especially by staying near the bottom during the ebbing tide (Fig. 1). This behavior can actually concentrate larvae further and further upstream in an estuary (Cronin 1982). On the other hand, larvae of species such as the blue crab *Callinectes sapidus*, move into coastal waters, and later recruit into the estuary (e.g., McConaugha 1988). Coastal waters often have lower predation rates, and crustacean larvae that are retained within estuaries often bear spines (Morgan 1990).



2. Some types of planktonic larval loss and gain with respect to a shoreline population.

In order to understand the dynamics of Zebra mussels it is crucial to not only know the larval life, behavior, and feeding biology, but we must also measure the hydrodynamic structure of the regional waters. Estuarine flow for example, may cause rapid evolution of Zebra mussel larval life span and behavior, in order to survive flushing from estuaries such as Chesapeake Bay and the Hudson River (Strayer 1991, Strayer and Smith 1993). In some cases, a population may be self—seeding (Fig. 2). Sammarco (1988) examined

larval recruitment dynamics on a coral patch reef by placing settling plates at increasing distances from the reef and by placing an array of current meters in surrounding waters. The current meter array allowed the development of a water circulation model, which predicted concentrated recruitment in two areas of the patch reef. Actual settling data conformed remarkably well to the prediction. Furthermore, despite a general regional flow, settlement seemed to increase rapidly as one approached the patch reef, suggesting that most settling larvae originated from the reef itself, and not some remote location. In order to understand Zebra mussel larval dispersal, such studies combining recruitment onto a suitable substratum (e.g., ropes or scrubbing pads) and hydrodynamic measurements are essential. Settling and recruitment may also be strongly modulated by weather change, particularly storm events. Recent studies of recruitment by blue crab larvae demonstrate simultaneous recruitment in localities that are hundreds of kilometers apart, which can only be explained effectively by regional storm systems. It would be quite useful to develop a large scale research program studying daily recruitment of Zebra mussel settlers along a shoreline or island such as are found in Lake Erie.

Community Interactions

Zebra mussel beds can be likened to marine mussel clumps, such as those of the western Pacific *Crenimytilus greyani* that live on isolated hard bits of substrata, or the more continuous beds of *Mytilus* that are found on sandy substrata on protected shores of the eastern U.S. Continuous beds of Zebra mussels are not so different from shallow subtidal beds of *Mytilus edulis*. With modest flows, mussel beds deplete the bottom waters of phytoplankton within a boundary layer, which is replenished by turbulent diffusion from above (e.g., Frechette et al., 1989).

Like *M. edulis*, Zebra mussels live a bed comprised of several layers, and mussels on the bottom experience reduced food supply, dissolved oxygen, and suffer exposure to deposition of pseudofeces and feces (Harger 1968, 1972, Call et al. 1994). Mobility of mussels is therefore an important source of intraspecific competition, as mussels climb to the top of the heap. The interference of upper mussels with the feeding and respiratory activities of lower layers of mussels will probably set an upper limit to the thickness of mussel beds. Manipulative experiments can be performed that alter the thickness of the beds, in order to estimate the optimal thickness that promotes maximum production, and the threshold at which population and somatic growth is severely limited.

Zebra mussels are mobile and therefore can smother competing epibenthos. While the life position of Zebra mussels on many aquatic species has been noted, it would be useful to perform the sort of manipulative experiments that have been done in marine habitats (see Levinton 1982) to quantify the effects of Zebra mussels on species to which they attach. This type of experiment can be done by small scale manipulations, but, as I discuss at the

end of this paper, larger scale introductions would be essential to understand population level effects. For example, zebra mussels attach to unionid bivalves, and there are likely negative effects to be expected (e.g., Ricciardi 1994). However, with the development of large mussel beds, hydrodynamic conditions around the bed may change the conditions, including the bottom substratum that might be filled with shells, in ways that might even enhance some benthos, including other bivalves. Marine mussel beds support scores of interstitial and attached invertebrate species, and oyster beds are important sites for recruitment of a variety of epibenthic species. Zebra mussel beds could conceivably increase local benthic biodiversity.

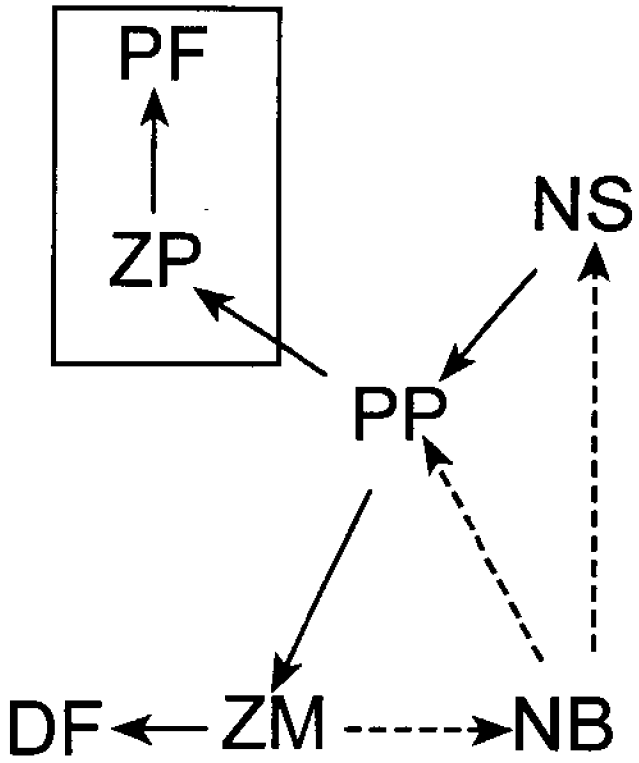
In marine mussel beds, predators are crucial in regulating abundance of the competitively dominant mussels. Seed (1969) and Paine (1974) have demonstrated the importance of asteroid starfish and gastropods in regulating the relative abundance and microhabitat occupation by mussels. While Seed's work was done by careful observation, Paine took an experimental approach by removing starfish and observing the increase in mussel abundance and spread of the mussel bed down intertidal rocky shores, into a zone normally subjected to intense predation. A similar approach needs to be taken to studying the Zebra mussel, which may be consumed extensively by molluscivorous fish and crustacea. Caging of mussels from predators is a readily applicable approach (Hamilton et al. 1994). An especially promising location for such study is the Hudson River, where Zebra mussels have invaded and the blue crab *Callinectes sapidus* can be abundant in the fresh water reaches. It is not clear however whether the blue crab feeds very much when migrating up into the fresh water part of the estuary. It is certain that the blue crab will eat Zebra mussels, but field studies must be done to assess the potential impact in natural habitats. It may well be that crabs and fishes may play important keystone roles in regulating Zebra mussel beds.

Zebra mussel populations may also greatly increase the abundance of certain carnivores, either by providing an increased food source and increasing population size of relatively sluggish predators, or by altering the migration routes of highly mobile carnivores, such as fishes and birds. Diving ducks feed extensively on Zebra mussels in Lake Erie, and exclosure experiments demonstrate an impact on the mussel population with respect to biomass, but not numbers (Hamilton et al. 1994).

Trophic Dynamics

Mussels are algal grazers and therefore can be considered as part of a large—scale interactive system between nutrients, algae, and other grazers (Fig. 3), as modulated by water column mixing and lateral water transport. Strayer and Smith (1993) calculated that a well-developed Zebra mussel population will filter 5—30 percent of the entire fresh water part of the Hudson River estuary per day. In 1993, the phytoplankton bloom of the

Hudson River failed to materialize (J. Cole and N. Caraco, personal communication), which may be explained by the Zebra mussel invasion and population burst a couple of years before. Lake Erie is a classic example of a eutrophic lake swept clear by Zebra mussels.



3. Scheme of presumed interactions in a simple system where Zebra mussels (ZM) have colonized. The zooplankton (ZP) - planktivorous fish (PF) compartment may be endangered owing to Zebra mussel consumption of phytoplankton. Deposition of mussel feces and pseudofeces might enhance the deposit feeding benthos (DF). Zebra mussel excretion might cause release of near bottom dissolved nutrients (NB), which may be mixed to the water column nutrients in the surface water (NS).

In recent years, several estuaries have been shown to be strongly influenced by bivalve grazers, particularly if the estuary is shallow, if there is relatively little lateral mixing, and if the bivalve population is dense. In shallow estuaries, phytoplankton is directly accessible to the benthos, either by deposition of ungrazed phytoplankton or by direct grazing of benthic bivalves (Cloern 1982, Officer et al. 1982, Cohen et al. 1984, Thompson and Nichols 1988, Koseff et al. 1993, Kamermans 1994). Most interesting is

the case of surf diatom blooms, which prevail off sandy coasts of Oregon and South Africa (Lewin et al. 1975). Here, offshore bars trap a cell of turbulent water and excretion by bivalves and other benthos feeds nitrogen into the water column, which stimulates phytoplankton production. This makes for very strong benthic pelagic coupling. It is likely that such strong coupling exists in shallow estuaries such as San Francisco Bay and Naragansett Bay.

Dense populations of bivalves in shallow estuaries exert major influences on control of phytoplankton, transport of nutrients to bottom sediments, and nutrient cycling back to the water column (references in Dame 1993). We should expect such strong interactive systems in the case of Zebra mussels as well (Fig. 3). In fresh waters the excretion of phosphorus may be more important as a limiting nutrient, and, unfortunately, we know very little about P excretion and turnover by mussels. Phosphorus kinetics tend to involve more rapid movement than nitrogen, and the bivalve stimulation of primary production may be not so easy to predict. Mussels, oysters and other bivalves often reject as pseudofeces large fractions of particles taken into the mantle cavity. Digestible cells, including diatoms, are ingested and assimilated with high efficiency. Fecal material and pseudofecal material is deposited on the bottom, and the organic fraction of this material may subsidize the growth of benthic deposit feeders. The process of particle processing, digestion and assimilation may cause strong partitioning of nutrient elements, which may, in turn, affect the phytoplankton. Deposition of silica tests on the bottom, despite return of P and N via excretion, might cause a reduction of silica—limited phytoplankton such as diatoms and favor green algae and cyanobacteria. Zebra mussel elemental partitioning is therefore a crucial area for future research, if we are to make a reasonable model of nutrient dynamics and benthic pelagic coupling.

An historical scenario proposed by Newell (1988) for oysters in Chesapeake Bay gives us a remarkable reverse perspective of the possible benthic-pelagic coupling between Zebra mussels and the water column. Since the 19th century, the eastern oyster *Crassostrea virginica* has been exploited by fishing, to the point that current populations are only a few percent of those that probably once existed. Newell's calculations suggest that former large populations of oysters were capable of filtering large proportions of Chesapeake Bay waters. This may have had a negative impact on the phytoplankton, which may have in turn made the oysters strong competitors with zooplankton such as copepods. The hypothesized clearer water may have been beneficial for surface-attached vegetation, and deposits of pseudofeces and feces may have subsidized deposit feeding benthos. Even the obnoxious sea nettles that currently dominate the Bay may have been absent, when oysters clarified the water column of phytoplankton and deprived the nettles of their probably important copepod prey.

Although the players would be different (substitute cladocera for copepods and planktivorous fish for sea nettles), these interactions give us strong insights as to the role of Zebra mussels in controlling the water column and in promoting benthic-pelagic coupling with the benthos. Recently, Padilla et al. (1994) have used a model of interactions in Green Bay, Wisconsin, which predicts the impact of Zebra mussels on areas of differing nutrient input.

A Heterodox Plea for Experimental Invasions

At present, many of the tools in the marine ecologist's gear bag can be applied directly to Zebra mussels. However, as studies of marine invasions attest, we often cannot understand ecological impacts very well, if only because the invaded habitats are not understood completely (indeed, we tend to study them carefully after we see the impacts of invading species, thus confounding the invasion with peculiarities of individual water bodies). In some cases it is possible to remove the invader, especially in cases where the invading species' effects are ecologically localized, as in strong effects of a grazer on algae. This is not so true for the Zebra mussel, whose effects on nutrient cycling, for example, are of a larger spatial scale. It would be very difficult indeed to remove all of the Zebra mussels from a lake or river, but it sure would be easier to add some!

Ironically, fresh water studies are far more instructive than marine work in planning for further study of Zebra mussel impacts. The Experimental Lakes Program (e.g., Levine and Schindler 1989), among others has demonstrated that controlled manipulations of fresh water bodies often yield far more insight than static observations. The manipulative experiments of predators in artificial ponds (Wilbur 1984) and in smaller lakes (e.g., Hairston et al. 1990) have demonstrated the strong linkages between life histories, predation and competition in fresh water bodies. Manipulations of nutrients also allow models of trophic dynamics to be tested with far more powerful analytic capabilities than might be available using natural, and often poorly controlled, variability. Therefore, I would strongly recommend immediate plans for introductions of Zebra mussels into lake and stream systems that had been studied well previously. While this seems somewhat drastic, perhaps an emphasis upon fresh water bodies that are bound to be victims of the invasion anyway should be selected for sacrifice. Boundless numbers of observations after the fact pale in the light of the results of a single well planned experiment.

References

Bayne, B.L. 1964. Primary and secondary settlement in Mytilus edulis L. (Mollusca). J. Anim. Ecol. 33: 513-523.

Bayne, B.L. 1976. The biology of mussel larvae. IN: B.L. Bayne, ed., *Marine Mussels:*

Their Ecology and Physiology. Cambridge Univ. Press, Cambridge, pp. 81-120.

Boileau, MG, Hebert, PDN, 1993. Genetics of the Zebra mussel (*Dreissena polymorpha*) in populations from the Great Lakes region and Europe. IN: Nalepa, T.F., Schloesser, D.W., *Zebra mussels: Biology, Impact, and Control.* Lewis Press, Boca Raton, Florida USA. pp. 227-238.

Butman, C.A. 1987. Larval settlement of soft-sediment invertebrates: the spatial scales explained by active habitat selection and the emerging role of hydrodynamic proceses. *Oceanogr. Mar. Biol. Ann. Rev.* 25: 113-165.

Call, C.A., Tuchman, N.C., Marsden, J.E. 1994. Vertical migration and mortality in dense Zebra Mussel colonies as a response to changes in interstitial water quality. 4th Intl. Zebra Mussel Conference, Madison, Wisconsin, abstract.

Cloern, J.E. 1982. Does the benthos control phytoplankton biomass in South san Francisco Bay? Mar. Ecol. Progr. Ser. 9: 191-202.

Cohen, R.R.H., Dresler, P.V., Philips, E.J.P., Cory, R.L. 1984. The effect of the asiatic clam *Corbicula fluminea* on phytoplankton of the Potomac River, Maryland. *Limnol. Oceanogr.* 29: 170-180.

Crane, J. 1975. *Fiddler Crabs of the World: Ocypodidae, Genus Uca.* Princeton Univ. Press, Princeton, New Jersey.

Cronin, T.W. 1982. The estuarine retention of larvae of the crab *Rhithropanopeus harrisi. Estuar. and Coast. Shelf Sci.* 15: 207-220.

Dame, R.F. 1993. Th erole of bivalve filter feeder material fluxes in estuarine ecosystems. In, Dame, R.F., ed., *Bivalve Filter Feeders in Estuarine and Coastal Ecosystem Processes*. Springer-Verlag, Berlin.

Denny, M.W., and M.F. Shibata. 1989. Consequences of surf-zone turbulence for settlement and external fertilization. *Am. Nat.* 134: 859-889.

Eckelbarger, K.J. and J.P. Grassle. 1987. Interspecific variation in genetical spine, sperm, and larval morphology in six sibling species of *Capitella. Bull. Biol. Soc. Washington*, 7: 62-76.

Fell, P.E., Balsamo, A.M. 1985. Recruitment of *Mytilus edulis* L. in the Thames Estuary, with evidence for differences in the time of maximal settling along the Connecticut shore.

Estuaries 8: 68-75.

Findlay, S., Pace, M.L., Lints, D., Cole, J.J., Caraco, N.F., Peierls, B. 1991. Weak coupling of bacterial and algal production in a heterotrophic ecosystem: The Hudson River estuary. *Limnol. Oceanogr.* 36: 268-278.

Fréchette, M., C.A. Butman and W.R. Geyer. 1989. The importance of boundary-layer flows in supplying phytoplankton to the benthic suspension feeder, *Mytilus edulis* L. *Limnol. Oceanogr.* 34: 19-36

Gaines, S.D., Bertness, M.D. 1992. Dispersal of juveniles and variable recruitment in sessile marine species. *Nature* 360: 579-580.

Grassle, J.P., and J.F. Grassle. 1976. Sibling species in the marine pollution indicator, *Capitella capitata*, (Polychaeta). *Science* 192: 567-569.

Hamilton, D.C., Ankney, C.D., Bailey, R.C. 1994. Predation of zebra mussels by diving ducks: An exclosure study. *Ecology* 75: 521-531.

Harger, J. R. 1968. The role of behavioral traits in infuencing the distribution of two species of sea mussel, *Mytilus edulis* and *Mytilus californianus*. *Veliger* 11: 45-49.

Harger, J. R. 1972. Competitive coexistence: maintenance of interacting associations of the sea mussels *Mytilus edulis* and *Mytilus californianus*. Veliger 14:387-410.

Hairston, N.G., Jr. , Dillon, T.A., de Stasio, B.T., Jr. 1990. A field test for the causes of diapause in a freshwater copepod. *Ecology* 71: 2218-2223.

Harrison, P., R. Babcock, G.D. Bull, J. Oliver, C. Wallace, and B. Willis. 1984. Mass spawning in tropical reef corals. *Science* 223: 1186-1189.

Hebert, PDN, Muncaster, P.W., and Mackie, G.L. 1989. Ecological and genetic studies on *Dreissena polymorpha* (Pallas): A new mollusc in the Great Lakes. *Can. J. Fish. Aquat. Sci.* 46: 1587-1591.

Karabin, A. 1978. The pressure of pelagic predators of the genus *Mesocyclops* (Copepoda, Crustacea) on small zooplankton. *Ekol. Pol.* 26: 241-257.

Kautsky, N. 1982. Quantitative studies on gonad cycle, fecundity, reproductive output and recruitment in a Baltic *Mytilus edulis* population. *Mar. Biol.* 68: 143-160.

Klerks, P.L., and J.S. Levinton. 1989. Rapid evolution of resistance to extreme metal pollution in a benthic oligochaete. *Biol. Bull.* 176:135-141.

Klerks, P.L., Weis, J.S. 1987. Genetic adaptation to heavy metals in aquatic organisms: a review. Environmental Pollution 45: 173-205

Koehn, R.K. 1991. The genetics and taxonomy of species in the genus *Mytilus*. *Aquaculture* 94: 125-146.

Kornobis, S. 1977. Ecology of *Dreissena polymorpha* (Pall.) (Dreissenidae, Bivalvia) in lakes receiving heated water discharges. *Pol. Arch. Hydrobiol.* 24: 531-546.

Koseff, J.R., Holen, J.K., Monismith, S.G., Cloern, J.E. 1993. Coupled effects of vertical mixing and benthic grazing on phytoplankton populations in shallow, turbid estuaries. *J.Mar. Res.* 51: 843-868.

Levinton, J.S. 1980. Genetic divergence in estuaries. In Kennedy, V.S., ed., *Estuarine Perspectives*. Academic Press, New York.

Levinton, J.S. 1982. Marine Ecology. Prentice-Hall, Englewood Cliffs, N.J.

Levinton, J.S. 1983. The latitudinal compensation hypothesis: Data and a model based upon energy budgets. I. Interspecific comparison of *Ophryotrocha* (Polychaeta, Dorvilleidae). *Biol. Bull. Woods Hole* 165:686-698.

Levinton, J.S. and R. Monahan. 1983. The latitudinal compensation hypothesis: Data and a model based upon energy budgets. II. Intraspecific comparison among subspecies of *Ophryotrocha puerilis* (Polychaeta, Dorvilleidae). *Biol. Bull. Woods Hole* 165:699-707.

Levine, S.N., Schindler, D.W. 1989. Phosphorus, nitrogen, and carbon dynamics of experimental Lake 303 during recovery from eutrophication. Can. J. Fish. Aq. Sci. 46: 2-10.

Levitan, D.R., M.A. Sewall, and F-S Chia. 1992. How distribution and abundance influence fertilization success in the sea urchin *Strongylocentrotus franciscanus*. *Ecology* 73: 248-254.

Lewin, J.C., T. Hruby, and D. Mackas. 1975. Blooms of surf-zone diatoms along the coast of the Olympic Peninsula, Washington: V. Environmental conditions associated with blooms (1971 and 1972). *Estuar. Coast. Mar. Sci.* 3:229-242.

Lonsdale, D.J., and J.S. Levinton. 1989. Energy budget differentiation among latitudinally separated *Scottolana canadensis* (Copepoda: Harpacticoida). *Limnol. Oceanogr.* 34:324-331.

McConaugha, J.R. 1988. Export and reinvasion of larvae as regulators of estuarine decapod populations. American Fisheries Society Symposium 3, pp. 90-103.

Morgan, S.P. 1990. Impact of planktivorous fishes on the dispersal, hatching and morphology of estuarine crab larvae. Ecology 71: 1639-1652.

Nalepa, T.F. and D.W. Schloesser. 1993. *Zebra mussels: Biology, Impact, and Control.* Lewis Press, Boca Raton, Florida USA.

Newell, R.I.E. 1988. Ecological changes in Chesapeake Bay: Are they the result of overharvesting the American oyster, *Crassostrea virginica.* In Understanding the Estuary: Advances in Chesapeake Bay Research. Proc. of a Conference, Baltimore, Maryland. Chesapeake Research Consortium, pp. 536-546.

Officer, C.B., Smayda, T.J., Mann, R. 1982. Benthic filter feeding: A natural eutrophication control. Mar Ecol. Progr. Ser. 9: 203-210.

Oliver, J. and R. Babcock. 1992. Aspects of the fertilization ecology of broadcast spawning corals: Sperm dilution effects and in situ measurements of fertilization. *Biol. Bull.* (Woods Hole) 183: 409-417.

Pace, M.L., Findlay, S.E.G., Lints, D. 1992. Zooplankton in advective environments: the Hudson River community and a comparative analysis. Can. J. Fish. Aq. Sci. 49: 1060-1069.

Padilla, D.K., Adolph, S.C., Cottinham, K.L., and Schneider, D.W. 1994. Predicting the consequences of a benthic invader on a pelagic food web: Effects of a nutrient gradient. Unpublished manuscript.

Paine, R.T. 1974. Intertidal community structure. Experimental studies on the relationship between a dominant competitor and its principal predator. *Oecologia* 15: 93-120.

Ricciardi, A. 1994. Infestation and impacts of *Dreissena* on native unionids in the upper St. Lawrence River. 4th Intl. Zebra Mussel Conference, Madison, Wisconsin, abstract.

Sammarco, P.W. 1988. Localized dispersal and recruitment in Great Barrier Reef corals:

The Helix experiment. *Science*, v. 239, pp. 1422-1424.

Seed, R. 1969. The ecology of *Mytilus edulis* L. (Lamellibranchiata) on exposed rocky shores. II. Growth and mortality. Oecologia 3: 317-350.

Seed, R. 1978. Systematics and evolution of Mytilus galloprovincialis LmK. In Marine Organisms: Genetics, Ecology and Evolution. ed. B. Battaglia and J.L. Beardmore, pp. 447-468. New York, Plenum Press.

Seeley, R.H. 1986. Intense natural selection caused a rapid morphological transition in a living marine snail. *Proc. Nat. Acad. Sci. USA* 83: 6897-6901.

Sprung, M. 1989. Field and laboratory observations of *Dreissena polymorpha* larvae: Abundance, growth, mortality, and food demands. *Arch. Hydrobiol.* 115: 547-561.

Sprung, M. 1993. The other life: An account of present knowlege of the larval phase of *Dreissena polymorphia.* In Nalepa, T.F., Schloesser, D.W., *Zebra Mussels: Biology, Impacts, and Control.* Lewis Publishers, Boca Raton, pp. 39-53.

Strayer, DL 1991 Projected distribution of the Zebra mussel, *Dreissena polymorpha*, in North America. *Can. J. Fish. Aquat. Sci.* 48: 1389-1395.

Strayer, D.L., Smith, L.C. 1993. Distribution of the zebra mussel (*Dreissena polymorpha*) in estuaries and brackish waters. In: Malepa, T.F., Schloesser, D.W., *Zebra Mussels: Biology, Impacts, and Control.* Lewis Publishers, Boca Raton, pp. 715-727.

Suchanek, T.H. 1985. Mussels and their role in structuring rocky shore communities. In Moore, P.G., Seed, R., eds., *The Ecology of Rocky Shores.* Hodder and Stoughton, London, pp. 70-96.

Vanderploeg, H.A., Liebig, J.R., Gluck, A.A., Goulden, C.E. 1994. From egg to settling stage: The culture and nutritional requirements of *Dreissena* larvae. 4th Intl. Zebra Mussel Conference, Madison, Wisconsin, abstract.

Varvio, S.-L., Koehn, R.K., Väinölä, R. (1988) Evolutionary genetics of the *Mytilus edulis* complex in the North Atlantic region. *Mar. Biol.* 98: 51-60.

Wilbur, H.M. 1984. Complex life cycles and community organization in amphibians. IN Price, P.W., Slobodchikoff, C.N., Gaud, W.S., eds., *A New Ecology: Novel Approaches to Interactive Systems,* pp. 195-224.

A Method for the Isolation of Total Genomic DNA from the Zebra Mussel, Dreissena polymorpha

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Introduced species, such as the zebra mussel (Dreissena polymorpha), provide favorable opportunities for the study of the evolutionary and population genetic consequences of the entry of a species into a previously uncolonized region. Zebra mussels provide an especially favorable circumstance because of the recency of their introduction. As well as providing interesting data on the genetic structure of a colonizing species, the identification and characterization of the distribution of genetic markers in the zebra mussel may also provide information on the sources and routes of dispersal that may be useful in the control and management of the species. As the first step in a study of the distribution of genetic variability in the zebra mussel, we have developed a method for the extraction and isolation of their total genomic DNA. We have obtained satisfactory results with a variation of the phenol/chloroform method using the entirety of the soft parts of the mussel. Cells are disrupted and proteins digested by an overnight incubation in 0.2% SDS with proteinase K in Tris-NaCl-EDTA buffer. The supernatant of this digestion is extracted once with 1:1 phenol:chloroform and once with chloroform. Nucleic acids are precipitated with cold ethanol, pelleted, dried and rehydrated in Tris-EDTA. This method produces an average of 1-4 milligrams nucleic acid per gram wet weight of mussel tissue, with a ratio of A_{260}/A_{280} of 1.8-2.0. We have used it successfully with fresh mussels, mussels frozen and stored at -80 C, and with mussels preserved in 95% ethanol and stored at room temperature.

Introduced species, such as the zebra mussel (Dreissena polymorpha), provide favorable opportunities for the study of the evolutionary and population genetic consequences of the entry of a species into a previously uncolonized region (Baker and Stebbins, 1965; Parsons, 1983). Zebra mussels provide an especially favorable circumstance because of the recency of their introduction into, and spread within, North America (Griffiths et al, 1991; Ludyanskiy et al., 1993). As well as providing interesting data on the genetic structure of a colonizing species, the identification and characterization of the distribution of genetic markers in the zebra mussel, by providing information on the sources and routes of dispersal, may also be useful in the control and management of the species. Aquatic invertebrates often present difficulties in obtaining DNA for study (Palumbi et al., 1991) and thus as the first step in a study of the distribution of genetic variability in the zebra mussel, we have developed a method for the extraction and isolation of their total genomic DNA.

Materials and Methods

Zebra mussels collected from intake pipes just north of the mouth of the Root River were obtained from the Racine, Wisconsin, Water & Wastewater Utility. Mussels were frozen by being placed in a -80 C freezer, preserved in 95% ethanol, or maintained in an aquarium at room temperature. Ethanol preserved mussels all had their shells cracked slightly to ensure that alcohol could rapidly reach the tissues, and were then stored at room temperature. Initial extractions were performed on these mussels, using as a guide the protocols of Palumbi et al. (1991), prior experience with vertebrate DNA extraction, and the advice of colleagues. After a successful protocol had been developed, further tests of the protocol were performed using mussels obtained from the Illinois Natural History Survey's Lake Michigan Biological Station in Zion, Illinois. Mussels from this source had been collected from various sites in northeastern Illinois and maintained in a lake water tank. It is possible that mussels from Green Bay, Wisconsin maintained elsewhwere in the same large lake water tank could have become mixed with the Illinois mussels. Zion mussels were preserved in the same way as Racine mussels, except that live mussels were maintained at 4 C in lake water.

The success of extraction was assessed quantitatively in two ways. First, the yield, expressed in milligrams per gram wet weight of mussel tissue, was determined from the voulume and concentration of the final extract as determined by UV spectrophotometry (Aquadro et al., 1992). Second, the ratio of absorbance of the extract at 260 nm (the wavelength of peak absorption by nucleic acids) to its absorbance at 280 nm was calculated. This ratio should be around 1.8; lower ratios indicate protein contamination (Hillis et al, 1990). Statistical analyses follow Sokal and Rohlf (1981). Extracts were also visually inspected for the presence of long-

stranded spoolable DNA at the ethanol precipitation stage of the extraction protocol (step 11, below). Most extracts were also subjected to agarose gel electrophoresis to determine the distribution of fragment size.

EXTRACTION PROTOCOL FOR ZEBRA MUSSELS

1) Weigh and tare a 1.5-2.0 ml micro-centrifuge tube. If using fresh mussels, this weight must include the weight of the lysis buffer and proteinase K.

2) Dissect out a zebra mussel:

a) If using a live mussel, pry the shell open and place the whole individual (including shell) in the tube.

b) If using a frozen mussel, remove the shell and place the entire frozen ellipsoid block of soft parts in the tube.

c) If using a mussel in ethanol, remove the shell, and rinse the mussel in distilled water.

3) Weigh the tube with mussel to get the wet weight of the individual by subtraction. If using live mussels, the wet weight must be taken after the digestion when the shell can be removed from the tube.

4) Add 500 ul of lysis buffer (100mM Tris, 5mM EDTA, 0.2% SDS, 200mM NaCl) to the tube.

5) Add proteinase K to the tube to a concentration of 100 ug/ml.

6) Incubate the sample at 55 C with gentle inversion in a rotator overnight. The next day, the soft parts should be digested down to a greenish-brown solution.

7) Spin sample for 5 minutes at 14,000 rpm (Eppendorf 5415C desktop microcentrifuge, 16,000 RCF) and transfer supernatant to a clean tube.

8) Add one volume of 1:1 phenol:chloroform and vortex until well mixed. Spin sample for 5 minutes at 10,000 rpm (Eppendorf 5415C desktop microcentrifuge, 8,160 RCF) and transfer aqueous layer to a clean tube.

9) Add one volume of chloroform and vortex until well mixed. Spin sample for 5 minutes at 10,000 rpm and transfer aqueous layer to a clean tube.

10) Add 1/20 volume of 5M NaCl to the sample and mix well.

11) Add 2 volumes of ice-cold 95% ethanol to the sample and mix very well. DNA should become visible.

12) Place the sample in a -20 C freezer anywhere from one hour to overnight to precipitate the DNA. The DNA should appear as white, fluffy strands which float.

13) Spin the sample for 10 minutes at 14,000x to pellet the DNA.

14) Carefully pour off the ethanol and air dry the pellet.

15) Resuspend the pellet in 100 ul TE (10mM Tris, 1mM EDTA) and store at either -20 C or -80 C.

Results and Discussion

Table 1 gives the results of extractions on Racine mussels preserved in 95% ethanol or frozen at -80 C. There are no differences in yield or ratio between frozen and ethanol preserved mussels.

Table 1. Results of extractions from frozen and ethanol preserved zebra mussels ($n_{froz}=n_{EtOH}=10$) from Racine, Wisconsin. The figures given are the mean ± standard error of the mean followed by (range).

	Frozen	Ethanol
Yield[1] (mg/g)	1.48 ± .054 (1.24-1.77)	1.44 ± .079 (1.04-1.74)
Ratio[2] (A_{260}/A_{280})	1.84 ± .044 (1.53-1.97)	1.84 ± .054 (1.59-2.08)

[1] Yield is expressed as milligrams of nucleic acid per gram wet weight of mussel tissue. The variances are homoscedastic (F_s=2.12, df=[9,9], ns) and the difference in means is nonsignificant (F_s=.140, df=[1,18]).

[2] Ratio is the ratio of absorbance at 260 nm to the absorbance at 280 nm. The variances are homoscedastic (F_s=1.51, df=[9,9], ns) and the difference in means is nonsignificant (F_s=.010, df=[1,18]).

All of the samples tabulated in Table 1 produced long-stranded, spoolable DNA upon addition of ice-cold ethanol. Five of the frozen and three of the ethanol preserved samples also yielded shorter stranded DNA when the precipitation was allowed to proceed longer. Agarose gel electrophoresis of several samples along with size markers showed maximum strand size to be good, with long stranded DNA (>14 kb) except for one frozen mussel, which had longest strands of about 6 kb. Short stranded DNA can be eliminated by using a very brief ethanol precipitation, or by precipitating at room temperature, both of which methods will preferentially precipitate the long-stranded fraction. (i.e. modifying steps 11 and/or 12). Strand size can also be enhanced by recovering the long-stranded DNA by spooling rather than pelleting (substituting for step 13). No obvious differences were observed between extracts from mussels frozen and stored for 3 months at -80 C versus mussels frozen and stored for 8 months at -80 C, although the one sample with subpar strand size was an 8 month-stored mussel.

Although preliminray extractions and protocol development had included fresh mussels from Racine, by the time a final protocol had been arrived at no live mussels remained for quantitative testing of the protocol. For this purpose, fresh mussels were obtained from the Illinois Natural History Survey. When preliminary results from a first sample of 10 mussels indicated that the yield of the fresh mussels was lower than

Table 2. Results of extractions from two samples of fresh zebra mussels (n=10 for both) from Zion, Illinois. The figures given are the mean ± standard error of the mean followed by (range).

	Sample 1	Sample 2
Yield[1] (mg/g)	.934 ± .079 (.554-1.34)	1.08 ± .036 (.877-1.26)
Ratio[2] (A_{260}/A_{280})	1.82 ± .029 (1.67-1.96)	1.82 ± .042 (1.61-1.99)

[1] Yield is expressed as milligrams of nucleic acid per gram wet weight of mussel tissue. The variances are heteroscedastic (F_s=4.75, df=[9,9], .05>p>.02); the difference in means is nonsignificant by an approximate t-test for heteroscedastic samples (t'_s=1.08, df=9).

[2] Ratio is the ratio of absorbance at 260 nm to the absorbance at 280 nm. The variances are homoscedastic (F_s=2.04, df=[9,9], ns) and the difference in means is nonsignificant (F_s=.000, df=[1,18]).

that of the frozen or ethanol preserved mussels from Racine, a second sample of fresh mussels from Zion was extracted. The results of these two samples are given in Table 2. There are no differences in yield or ratio between the two samples, but the yield does seem lower than that of the Racine mussels (ca. 1 mg/g vs. 1.4 mg/g). Because of the heterogeneous nature of the mussels extracted in the two series of extractions reported in Tables 1 and 2 (Racine vs. Zion, and preserved in mid-summer vs. overwintered in captivity), we could not know whether fresh mussels had a genuinely lower yield, or if one of the other differences between the two series was reponsible. We thus decided to perform a third series of extractions involving just Zion mussels treated in all 3 ways: frozen, ethanol preserved, and fresh. The results of these extractions are shown in Table 3.

Table 3. Results of extractions from frozen, ethanol preserved, and fresh zebra mussels ($n_{froz}=n_{EtOH}=n_{fresh}=4$) from Zion, Illinois. The figures given are the mean ± standard error of the mean followed by (range).

	Frozen	Ethanol	Fresh
Yield[1] (mg/g)	3.85 ± .539 (2.84-4.97)	2.95 ± .569 (1.38-4.09)	3.21 ± .990 (1.92-6.16)
Ratio[2] (A_{260}/A_{280})	2.04 ± .020 (1.99-2.08)	2.01 ± .017 (1.97-2.04)	2.02 ± .038 (1.95-2.11)

[1] Yield is expressed as milligrams of nucleic acid per gram wet weight of mussel tissue. The variances are homoscedastic ($F_{max}=3.38$, a=3, df=3, ns) and the differences among means are nonsignificant ($F_s=.383$, df=[1,18]).

[2] Ratio is the ratio of absorbance at 260 nm to the absorbance at 280 nm. The variances are homoscedastic ($F_{max}=5.02$, a=3, df=3, ns) and the differences among means are nonsignificant ($F_s=.303$, df=[1,18]).

There are no significant differences among treatments in either yield or ratio. We thus conclude that method of preservation does not affect these measures. All of these extracts were run on agarose gels, and all showed long-stranded (>14 kb) DNA. None of the ethanol preserved, 2 of the fresh, and all of the frozen mussels also showed some shorter stranded DNA. Ethanol and fresh mussels also had less RNA. It is notable however, that yields and ratios were higher in these samples than in the two prior series of extracts, and also that they were more variable. Especially notable is the difference in yield between the first two samples of fresh mussels from Zion

(Table 2) and the fresh sample in Table 3 (ca. 1 mg/g vs. 3 mg/g). These results suggest that although yield and ratio are consistent across methods of preservation within a series of extractions, there are differences across series which are currently uncontrolled for in the protocol. This "batch effect" might be due to some subtle variation in technique which affects all mussels within a batch done at one time equally, regardless of method of preservation, but that is not replicated across batches done at different times.

Regardless of the source of this variation, average yields across batches (ca. 1-4 mg/g) are comparable to what we have obtained using a version of the protocol on mammalian soft tissue (ca. 1-5 mg/g), and what others have obtained (1.5 mg/g, Seutin et al., 1991; .2-.4 mg/g, Aquadro et al., 1992; both of these authors added an RNAse step).

Several variations in the protocol have been tried which might be useful in particular situations. These variations include:

-- Live mussels may be flash-frozen in liquid nitrogen prior to extraction. When frozen in this way, whole mussels, including adductor muscles, can be easily separated from the shell, so that the shells need not be included in the lysis buffer. The removal of the mussel from the shell also facilitates the next variation.

-- Mussels may be minced or ground in liquid N_2 prior to addition of lysis buffer, which facilitates SDS and proteinase K reaching all the cells more rapidly.

-- Lysis and proteinase K digestion can be carried out for only 3-5 hours. This results in a less complete digestion, but allows the extraction to be completed in one day. Mincing or grinding is helpful when using a short digestion.

-- After overnight digestion, samples may be stored over chloroform at 4C for 2-5 days. This can increase the the purity of the samples.

-- After organic extraction, samples may be heated to 65C, cooled, and the DNA precipitated by adding 8M LiCl to a concentration of 4M. This may increase yield (Palumbi et al., 1991).

-- As mentioned earlier, precipitation may be carried out at higher temperatures or for shorter periods of time if it is desired to enhance the long-stranded fraction and impede the precipitation of shorter-stranded fraction.

-- For techniques in which RNA is undesirable (such as DNA-DNA hybridization), RNA may be removed with a DNAse-free RNAse.

Conclusion

The protocol outlined here can be used to successfully extract total genomic DNA from frozen, ethanol preserved, or fresh zebra mussels. Because of the danger of accidental release of live mussels transported for research purposes, we recommend that, in general, fresh mussels not be used in genetic studies. Although we do not have definitive evidence, agarose gel electrophoresis results provide some grounds for believing that mussels preserved in ethanol yield longer-stranded DNA with less RNA contamination than do frozen mussel. Although ethanol preservation does have the disadvantage of not preserving proteins, we tentatively recommend this mode of preservation.

Acknowledgments

We are grateful to Herb Schmidt of the Racine Water & Wastewater Utility and J. Ellen Marsden of the Illinois Natural History Survey for providing mussels, and to T. Quayle and R. Gundersen for providing access to equipment and technical advice.

Literature Cited

Aquadro, C.F., W.A. Noon, and D.J. Begun. 1992. RFLP analysis using heterologous probes. pp. 115-157 in A.R. Hoelzel, ed. Molecular Genetic Analysis of Populations: a Practical Approach. Oxford University Press, Oxford.

Baker, H.G. and G.L. Stebbins. 1965. The Genetics of Colonizing Species. Academic Press, New York.

Griffiths, R.W., D.W. Schloesser, J.H. Leach and W.P. Kovalak. 1991. Distribution and dispersal of the zebra mussel (Dreissena polymorpha) in the Great Lakes region. Can. J. Fish. Aquat. Sci. 48:1381-1388.

Hillis, D.A., A. Larson, S.K. Davis and E.A. Zimmer. 1990. Nucleic acids III: sequencing. pp. 318-370 in D.A. Hillis and C. Moritz, eds. Molecular Systematics. Sinauer Associates, Sunderland, Mass.

Ludyanskiy, M.L., D. McDonald and D. MacNeil. 1993. Impact of the zebra mussel, a bivalve invader. Bioscience 43:533-544

Palumbi, S., A. Martin, S. Romano, W.O. McMillan, L. Stice and G. Grabowski. 1991. The Simple Fool's Guide to PCR. University of Hawaii, Honolulu.

Parsons, P.A. 1983. The Evolutionary Biology of Colonizing Species. Cambridge University Press, Cambridge.

Seutin, G., B.N. White and P.T. Boag. 1991. Preservation of avian blood and tissue samples for DNA analyses. Can. J. Zool. 69:82-90.

Sokal, R.R. and F.J. Rohlf. 1981. Biometry. W.H. Freeman, San Francisco.

Settlings and Growth of *D. polymorpha* in the Raw Water Circuits of the Cattenom Nuclear Power Plant (Moselle, France)

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ABSTRACT

The evolution in density of veligers, settling of young animals and growth of *D. polymorpha* were studied over a 3-year period from 1991 to 1993, in the raw water circuits of the Cattenom nuclear power plant. During the same period, an analysis was conducted of certain physico-chemical parameters of the circulating water (temperature, dissolved oxygen, suspended solids, chlorophyll).

Veliger concentrations in the water vary greatly between May and the end of September. Two peaks can nonetheless be observed. The relative importance of the two peaks may vary from one year to the next.

Settling of zebra mussels on PVC collectors immersed in the flow of raw water begins as early as mid-June and occurs around every two weeks. The growth rate of young animals settling during the summer is very rapid. Maximum rates of 1.8 mm per week were observed, though the average growth rate for young animals during this period is 1.2 mm per week. The first cohort of settled young animals can reach a size of 15 mm by the end of summer. This cohort is therefore capable of reproduction the following year.

The growth can be correctly described with a simple model implemented to calculate shell increment on the long term. It is based on three functions representing the influence of size, temperature and food availability (plankton chlorophyll a). Coefficients have been adjusted with the shell growth data collected on caged samples or settled mussels on PVC plates.

Introduction

In France, zebra mussels are building up in the raw water circuits of the thermal power plants located in the Moselle, Rhône and Seine river valleys. Their larvae settle in summer of every year on the walls of the basins and galleries.

The EDF-operated Cattenom nuclear power plant consists in four 1300-MW PWR units and is located in the French part of the Moselle River valley. Each unit is equipped with a natural-draft cooling tower. Water is pumped from the Moselle at a rate of 2.2 m3/s for each unit. This water first cools the systems in the conventional and nuclear auxiliaries, then serves as makeup for the main cooling system. Under normal operating conditions, the blowdown waters are discharged into a large artificial reservoir - Mirgenbach - in which residence time is approximately ten days, before being released into the Moselle River downstream. Given evaporation in the cooling towers, the flow restored to the river is approximately 1.35 m3/s per unit.

Since 1989, the EDF Cattenom plant has suffered from problems of mussel invasion in a part of the cooling water circuit located upline of the heat exchangers on both nuclear and conventional auxiliary cooling systems. To eliminate the risk of total clogging of the exchangers, it was decided to clean the system mechanically every year, and gradually to install debris filters to protect the exchangers. The cleaning operation, performed by divers, takes about four months per year (from November to February).

In 1991 and 1992, experimental trials were carried out on site with a view to testing different anti-fouling treatments as possible alternatives to the annual cleaning: thermal shock, high-dosage chlorination, chlorine dioxide, potassium chloride and an organic compound. [1]

For most of these alternatives, the environmental acceptability and the cost depend greatly on the biological specificity of the local mussel population. Precise data on larval settlement periods and on growth rates of young mussels is needed for determination of the most appropriate frequency for cleaning the walls, or the choice of chemical treatments.

This was the objective of a three-year biological study (1991-1993) of the *Dreissena* population in the Moselle at Cattenom. The study was conducted jointly by the Laboratoire de Démoécologie of the University of Metz and the EDF Environment Branch, part of the Research and Development Division.

This paper will only present the results on the mussel population invading the Cattenom power plant circuits, though we have collected the same type of data on a Moselle River station and on Mirgenbach Lake.

Materials and methods

Water quality.

The environment team of the Cattenom plant continuously monitors the temperature, pH, dissolved oxygen and conductivity of the Moselle water. In 1992, between May and September, we measured additional parameters: Total Suspended Solids, nitrogen (NH_4-NO_2-NO_3), phosphorus (PO_4) and silica. The University of Metz also monitors Moselle River water quality as part of the "Cattenom Ecological Survey Program".

Zooplankton monitoring.

Moselle water was pumped into the chamber of the filtering screen at a point where the cooling water is continuously renewed, 3 meters above the bottom. Mussel larvae abundance was monitored between April/May and September/October. A daily sample of 60 liters of water was filtered on 80 mm meshsize plankton silk and concentrated to 400 ml. Veligers and other zooplankton were counted on 4 replicates of 5-ml sub-samples using a binocular microscope.

Phytoplankton biomass.

It was estimated by chlorophyll a measurements on 250- to 1000-ml water samples pumped at the same place. 90%-acetone extracts were analysed following the Lorenzen spectrophotometric method, modified to eliminate the acidification process [2].

Larval settlement.

Artificial substrates composed of PVC plates were used to quantify larval settlement. One or two collectors containing 8 rectangular plates (20 x 40 cm) were placed in the filtering screen chamber 3 meters above the bottom. The collectors were lowered by rope, guided by a rail fixed to a ladder. At this particular point, water velocity stays in the range between 10 cm/s to 50 cm/s, depending on the number of cooling water pumps in operation.
Every one or two weeks, one plate was removed and the settled material detached with a small brush, to avoid breaking small shells. Sub-samples of the shells measuring less than 5 mm in length were examined under a binocular microscope coupled to a video system; larger specimens were directly measured with slide calipers.

Growth measurement.

In 1991 and 1992, calibrated mussel batches were placed in 3-liter PVC cylinders with 10 circular openings covered with plastic netting of 1- or 4-mm meshsize. These cages were placed at the base of the PVC plate collector. The mussels had been collected on the walls of a lock on the Moselle, close to Cattenom. Shell length of each set of mussels was regularly measured.

In 1992 and 1993, growth of newly-settled mussels was monitored by precise measurement of shell length on the PVC plates. To identify the cohorts on size-frequency histograms, we adopted the Bhattacharya method [3].

All the basic data for these studies can be found in the reports drawn up by the University of Metz for EDF [4], [5], [6].

Profile of water quality in the Moselle River

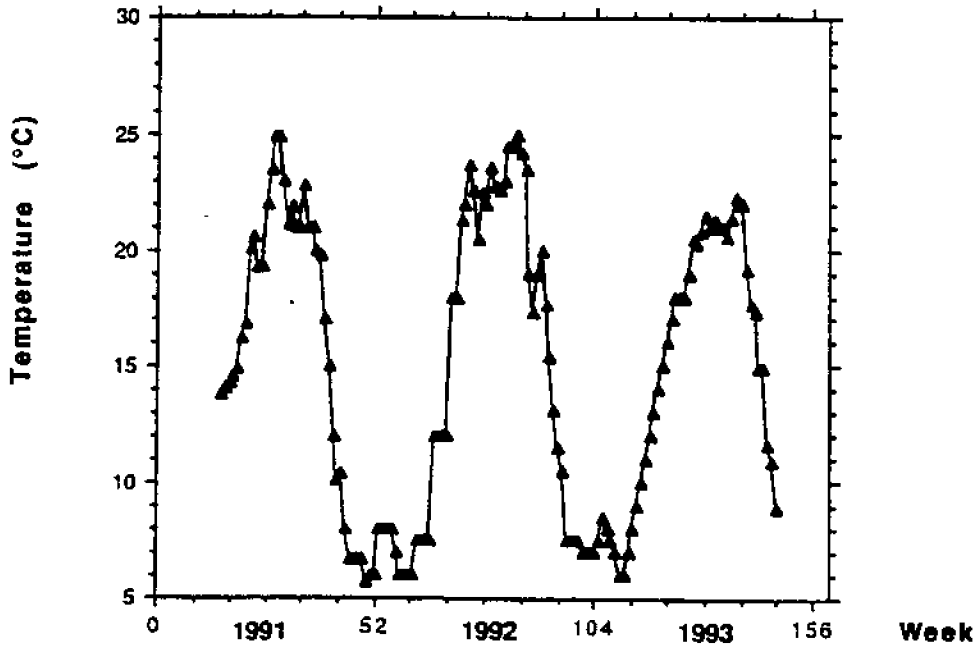
The quality of the water in this stretch of the Moselle is poor, primarily because of industrial and domestic pollution. Dissolved solids reach very high levels downstream of industrial discharges of chlorides (conductivity in the range of 1500 to 1800 mS/cm).

During the summer, and especially in the past few years marked by severe droughts, low flow rates have resulted in increased ammonia (0.2 to 1.0 ppm), nitrites (0.3 to 1.0 ppm) and orthophosphates (0.5 to 2.0 ppm).

The thermal regime is characterized in spring and summer by water temperatures above 20° C, with long periods of time in the 23° to 26° C range in 1991 and 1992 (**Figure 1**).

Phytoplankton is abundant in this nutrient-rich water: chlorophyll a: 10 to 120 mg/l from May to August (**Figure 2**). In spring and summer, phytoplankton contributes significantly to the suspended matter found in the river (10 to 85 ppm).

In the Moselle, the phytoplankton and the macrophytes phytosynthesis-respiration process exercise an influence over the dissolved oxygen balance producing day-nigth variations, but the DO does not lower under 4.0 ppm during the summer months.



Figures 1. Moselle water temperature recorded on the period 1991-1993 (weekly mean data).

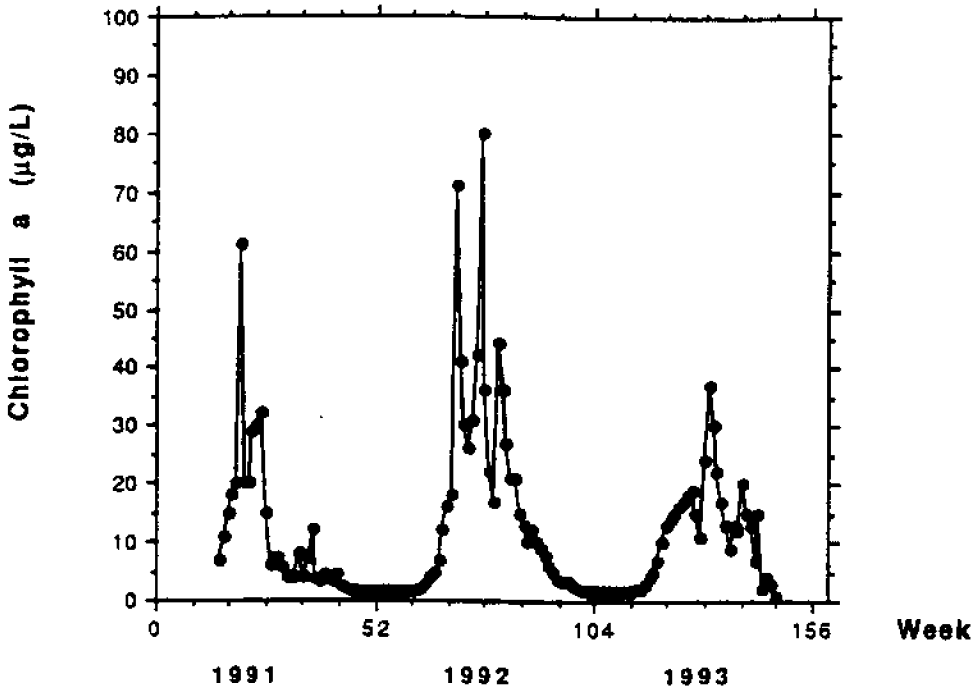


Figure 2: Chlorophyll a data on Moselle water on the period 1991-1993. Measurements have been made on samples from the cooling circuits of the Cattenom nuclear power plant from April-May to September-October (weekly mean data). The winter data have been completed by interpolation with a minimum winter value of 1.5 µg/L.

Biological data on *Dreissena polymorpha* larvae in the Moselle at Cattenom

The three-year set of data is summarized in **Table 1**. The first veligers were recorded in April 1991, with water temperature at 13.8° C. In 1992 and 1993, the sampling program was started too late in the season for detection of the appearance of veligers in the plankton. In the Belgian part of the Meuse River, Gillet and Micha [7] also found the first veligers at 13.0° C in 1984, but they assume that no precise water temperature threshold triggers *Dreissena* reproduction. In the lower Rhine, mussel larvae appear at 13° C (Jantz and Neumann [8]. Testard [9] also found that *Dreissena* reproduction in the Seine (around Paris) is restricted to a temperature range between 13° and 15° C. Some veligers, in low densities (2/L), were found as late as October 26 in 1993.

Though the density fluctuates from June to August over the three years, two major abundance peaks were found for larval density:

1991: end of June and mid-August (20 to 65 veligers per liter),
1992: mid-June and mid-July, with similar densities (190 to 220 veligers per liter),
1993: beginning of July and end of August (80 to 450 veligers per liter).

All veliger peaks were found in the temperature range of 21° to 24.7° C, which is slightly above the optimum temperature range generally quoted for free-swimming planktonic stages [10]. With the exception of this observation, no clear relationship was found between veliger density and temperature, water chemistry or chlorophyll a.

Settlements

Prior to the first mussel larvae settlement from April to June, other organisms colonize the PVC plates. Among them, Chironomid larvae, Tubificids and Hydroids (*Cordylophora*) play a major role. None of them seems to have an adverse effect on post-veliger settlement. *Cordylophora* tubes are frequently used as substrate by mussel larvae and juveniles. The whitish spots indicating sponges on the circuit walls seem to be the only areas exempt of *Dreissena*.. Our settlement data is summarized in **Table 1.**

Larval settlement is also subject to significant fluctuations during the summer, though there are 1 or 2 major peaks in the first week of July and August. In 1992 and 1993, settlement recorded in July (5,600 and 6,300 per m^2) was lower than that found in August (respective maximum of 68,800 and 12,900 per m^2).

These major fluctuations explain the formation of two major cohorts observed in most of the mussel samples collected on the circuit walls and on a plate left on site from June 1992 to May 1993. Over the period as a whole, maximum settlement was recorded in a water temperature range of 20° to 25° C.

		Véligers			Settlements		
		Dates	Nb/L	T° (C)	Dates	Ind/m²	T (°C)
1991	First record	04/10	0.3	13.8	06/04	25	20.6
	Main Peak	07/02 08/13	65 21	23.5 21.1	06/18 - 07/02	8300	19.3-23.5
1992	First record	<05/13	5	18.6	06/24	425	22.2
	Main Peak	06/16 07/20	190 219	23.3 24.7	06/24 - 07/02 08/05 - 08/19	5600 68800	22.2-24.0 21.1-24.9
1993	First record	no data	—	—	06/17	1500	20.3
	Main Peak	06/20 - 07/12 08/23	80-97 452	21.3-21.5 22.1	06/29 - 07/12 08/09 - 09/07	6300 12900	21.3-21.5 17.9-22.3

Table 1 : Summary of data collected during three years on the plankton stages (veligers) and early settled zebra mussels in the cooling circuit at the Cattenom Nuclear Power Plant, on the Moselle.
Ind/m² : maximum density on PVC plates
T (°C) : water temperature or water temperature range over the period.

	June	July	August
1991	26.5	5.9	5.0
1992	47.3	29.2	17.5
1993	17.3	26.5	13.3

Table 2 : Chlorophyll a content (µg/L) of the Moselle water during the summer over the 1991-1993 period (monthly means).

Average monthly concentrations of chlorophyll a (mg/l) were lower in July and August 1991 than in the two subsequent years. The absence of a second high settlement peak in 1991 corresponds to the low chlorophyll a values (**Table 2**).

Growth measurements

The data on shell length and shell length increments measured on mussel samples in the cages and on mussel samples taken from larval settlements on the PVC plates and basic water quality parameters are presented in the **Table 3**.

For the first cohort settled on PVC plates in June 1992, mean growth of 1.2 mm per week was recorded from June to September (maximum 1.75). Over the three months between mid-June and mid-September, the first cohort reached a size of 15.1 mm. In 1993, mean weekly growth from the end of June to the end of September was 0.9 mm (maximum 1.27). Measured shell increments on caged mussels seem lower than those found on the PVC plate specimens. It is most probable that clogging of the cage openings by mussel feces and external fouling reduced exchanges of water, and therefore of food and dissolved oxygen, with the inside of the cages.

Similar 15-mm growth during the first growing season was recorded on the lower part of the Rhine by Jantz and Neumann [8]. Growth recorded on the upper-Rhine population is, however, far less. From a bibliographical review on the growth of *Dreissena p.* populations, Testard [9] summarized the data on the size range of this species in european rivers and lakes:

End of first summer (stage 0+) : 4 - 5 mm (monthly increment 1 - 2 mm)
End of second summer (1+) : 10 mm (sexual maturity)
End of third summer (2+) : 20 - 22 mm
End of fourth summer (3+) : 20 - 28 mm

In the River Rhone, the shell length of the mussels settled in June reaches 8-10 mm at the end of the growing season in October with a mean growth increment of 2 mm per month [11].

Experiment	Period	SL (mm)	SLI (mm/wk)	Temp (°C)	Chla (µg/L)
caged mussels	91: 04/10-05/02	11.8	0.43	13.8-14.6	6.7-18.0
caged mussels	91: 05/03-05/16	13.1	0.30	14.6-16.2	18.0-61.1
caged mussels	91: 05/17-06/04	13.7	0.40	16.2-20.6	7.6-61.1
caged mussels	91: 06/05-06/18	14.8	0.35	19.3-20.6	7.6-32.4
caged mussels	91: 06/19-07/02	15.6	0.35	19.3-23.5	5.9-32.4
caged mussels	91: 07/03-07/16	16.3	0.40	23.5-24.9	5.9-7.1
caged mussels	91: 07/17-08/13	17.1	0.225	21.1-24.9	4.0-7.1
caged mussels	91: 06/04-06/17	5.2	0.6	19.3-20.6	7.6-32.4
caged mussels	91: 06/18-07/02	6.4	0.95	19.3-23.5	5.9-32.4
caged mussels	91: 07/03-08/13	8.3	0.73	21.1-24.9	4.0-7.1
caged mussels	91: 08/27-09/09	12.3	0.35	21.0-22.8	4.1-11.6
caged mussels	91: 09/10-09/24	13.0	0.40	19.8-21.0	3.2-11.6
caged mussels	92: 06/03-07/08	9.30	0.82	20.5-23.6	22.0-79.6
caged mussels	92: 07/08-08/06	13.4	0.43	22.6-25.0	16.8-43.8
caged mussels	92: 08/06-09/03	15.1	0.20	19.0-25.0	10.3-20.8
PVC plates	92: 06/24-07/02	1.0	0.9	22.2-24.0	10.7-42.7
PVC plates	92: 07/03-07/09	1.9	1.00	22.1-23.7	7.7-19.9
PVC plates	92: 07/10-07/23	2.9	1.75	22.3-25	18.7-59.4
PVC Plates	92: 07/24-08/05	6.4	1.35	24.2-25.7	12.0-30.4
PVC Plates	92: 08/06-08/19	9.1	1.25	21.1-26.0	10.1-23.3
PVC Plates	92: 08/20-09/02	11.6	1.0	18.4-23.7	7.5-21.7
PVC Plates	92: 09/03-09/16	13.6	0.75	14.1-19.5	5.0-21.7
PVC Plates	92: 07/09-07/23	0.5	1.75	24.2-25.7	18.7-59.4
PVC Plates	92: 07/24-08/05	4.0	0.8	24.2-25.7	12.0-30.4
PVC Plates	92: 08/06-08/19	5.6	1.0	21.1-26.0	10.1-23.3
PVC Plates	92: 08/20-09/02	7.6	1.25	18.4-23.7	7.5-21.7
PVC Plates	92: 09/03-09/16	10.1	1.25	14.1-19.5	5.0-21.7
PVC Plates	92: 07/23-08/05	0.5	1.1	24.2-25.7	12.0-30.4
PVC Plates	92: 08/06-08/19	2.7	1.25	21.1-26.0	10.1-23.3
PVC Plates	92: 08/20-09/02	5.2	1.5	18.4-23.7	7.5-21.7
PVC Plates	92: 09/03-09/16	8.2	1.0	14.1-19.5	5.0-21.7
PVC Plates	93: 06/28-07/12	1.3	0.62	21.3-21.5	55.5-66.2
PVC Plates	93: 07/13-07/26	2.54	1.15	20.6-21.3	55.5-41.7
PVC Plates	93: 07/27-08/23	4.84	1.27	20.6-22.3	24.4-41.7
PVC Plates	93: 08/24-09/06	9.91	1.15	17.9-22.1	30.7-38.7
PVC Plates	93: 09/07-09/21	12.20	0.48	17.3-17.7	24.1-38.9
PVC Plates	93: 09/22-10/07	13.15	0.52	14.9-17.3	21.3-38.9

SL: Shell lengt (initial size) SLI: Shell length Weekly Increment
Temp: Avera Temperature Chla: Chlorophyll a

Table 3: Measurements of shell length increments and water quality data (temperature and plankton chlorophyll a ranges) performed during the 3 year study at Cattenom.

Our data implies a more rapid growth, at least during the first season. A similar growth of 15 mm within the first growing season, was found in the lower part of the Rhine by Jantz and Neumann [8] when the growth of an upper Rhine population does not exceed 4 mm. Sprung [12] compared the growth of *Dreissena* in two lakes in the Cologne area in Germany: in one with high seston content, the population reaches 13 mm by the end of the settlement year. In the other, with a low seston content, the median shell length was 2.3 mm.

The Cattenom circuits population can be placed in the fastest growing european populations

Modelling shell growth

In Molluscs, growth is mainly dependent on one biological factor: individual size, and two environmental factors: water temperature and food availability. The model we have developed to estimate shell growth on the *Dreissena* population in the Cattenom circuits is based on these three factors.

For every one-week time step, shell length (L_t) is calculated as follows:

L_t (mm) = L_0(mm) e^r, L_t is the final shell length L_t is the initial shell length.

The model is based on calculation of the exponential growth rate (r) over a 1-week period, as dependent on three functions:

$$r\ (week^{-1}) = r_{max} * G * F$$

r_{max} is the maximum value of r with no thermal or food limitation,
G is the thermal limiting function, varying in a range between 0 and 1,
F is the nutritional limiting function, varying in a range between 0 and 1.

Over a weekly period: $r_{max} = \ln ((L_0 + SLI) / L_0)$, SLI is the weekly shell length increment.

The size factor.

Filtration rate, ingestion rate and energy metabolism balance are size-dependent: the individual growth rate decreases as the size increases. This is a basic assumption of all invertebrate-growth models illustrated by the Von Bertalanffy law. In a test tank fed by Rhine water, Jantz and Neumann [8] measured the shell length increment on mussel batches between December 1989 and June 1990. For the growing period (3.5 months from mid-March to the end of June), they obtained data which follows an exponential regression equation.

$$SLI\ (mm) = 10.3\ e^{(-0.071\ L_o)}\ \text{or}: SLI(mm/week) = 0.74\ e^{(-0.071\ L_o)},\ L_o \text{ in mm}$$

Thus the maximum weekly increment over the period is 0.74 mm.

The same regression from the data in **Table 3**, gives the following equation (see **Figure 3**):

$$SLI(mm/week) = 1.554\ e^{(-0.0863\ L_o)} \qquad L_o \text{ in mm}, r^2 = 0.59$$

The relatively poor correlation coefficient is probably due to the effect of the other two factors, temperature and food, and to the heterogeneity of samples (caged mussels and those on collectors). The key point to remember is that the exponential coefficient is quite similar to that found in the shell growth experiment on the Rhine.

Including the size limiting factor, r_{max} is calculated as follows:

$$r_{max} = \ln ((L_o + a\ .\ e\ (b.L_o)) / L_o)$$

The thermal factor.

In European waters, all current literature agrees on the absence of growth during the winter period, the only significant growth being observed in the spring and summer period. The winter temperature threshold for triggering growth is shown in the range of 5° to 10°C. The optimum temperature for growth is 15° C, and growth is, according to Waltz [13] greatly limited at temperatures exceeding 20° C.

Our data clearly shows that the period of maximum growth is the summer, in the Moselle [7] as in the Meuse or in lakes [12], when water temperature reaches its maximum annual values in the range of 20° to 25° C.

If we assume that the thermal optimum is close to 22° C, we must also take into account the fact that a sharp decrease in growth most probably occurs between 25° and 30° C. The thermal function in our model was selected to take these basic factors into account (**Figure 4**):

$$G(T) = e^{(z.(T-Topt))} . ((Tmax-T)/(Tmax-Topt))^{z(Tmax-Topt)}$$

G(Topt) = 1, G(Tmax) = 0, Topt and Tmax in °C, z: constant

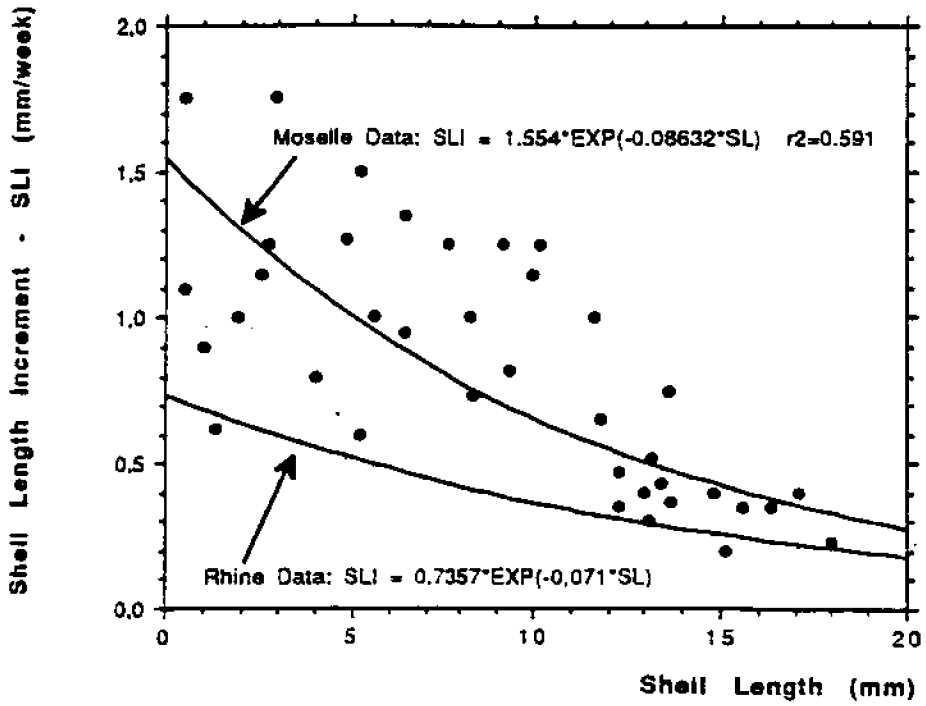


Figure 3: The size limitation function, the shell length is L_O Regression on Moselle data and comparison with the Rhine Data [8].

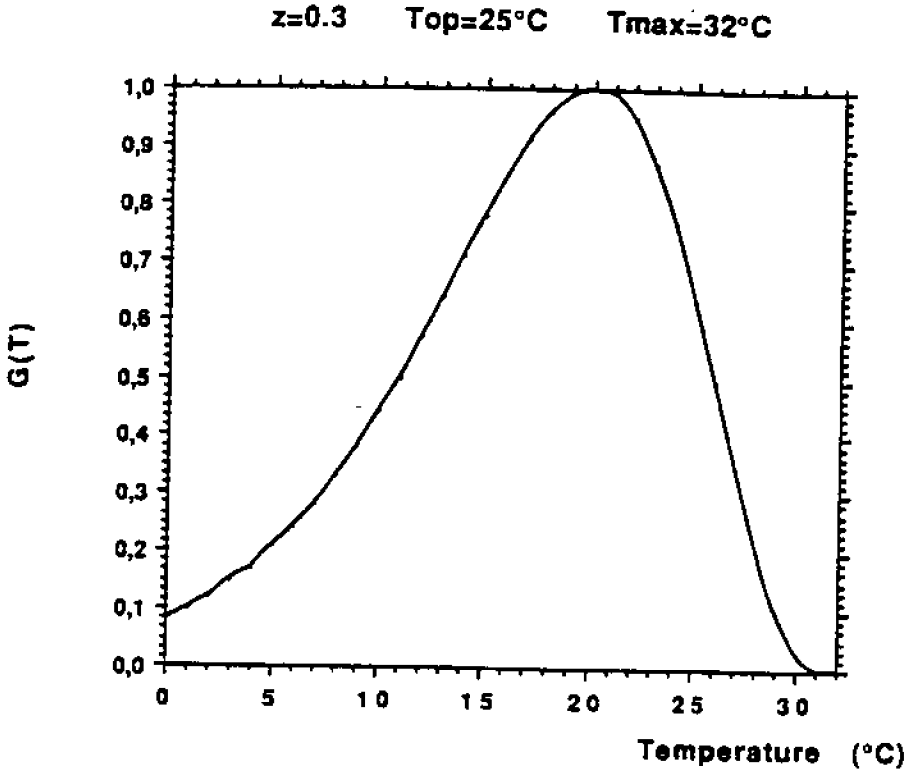


Figure 4: The thermal limitation function G(T)

The food factor.

Dreissena polymorpha feeds on a great variety of suspended particles in the water, primarily algae or organic detritus, though it is widely recognized that phytoplankton constitutes its prime food source in terms of quality. For this reason, we used planktonic chlorophyll a data to build the F function in accordance with a Michaelis-Menten relationship:

$$F(c) = \frac{C}{Kc + C}$$

C is the chlorophyll a concentration (mg/l)

Kc is the half-saturation constant: F(Kc)=0.5.

The resulting curve is shown in **Figure 5.**

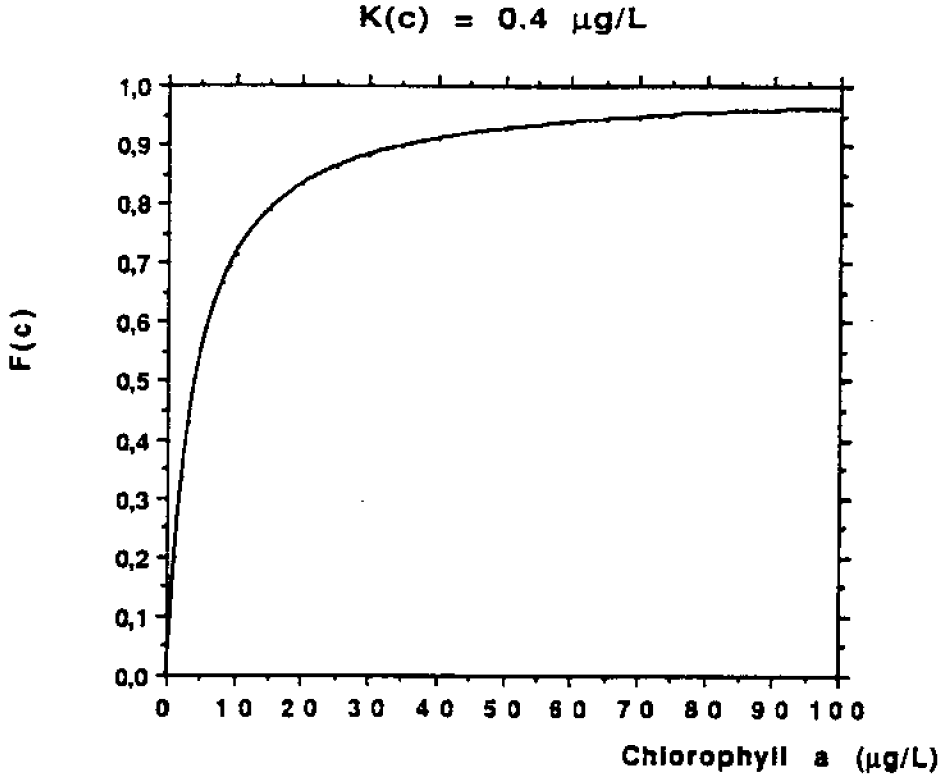


Figure 5: The food limitation function F(c).

Calibration of the coefficients.

For the purposes of calibration, we used the data presented in **Table 3.**

We used the model to test the coefficients within the following range of values:

a = 1.5 to 2.5 mm , b = -0.070 to -0.094 week^{-1}
Topt = 15 to 25 °C, Tmax= 25° to 32° C, z= 0.1 to 1.0
Kc =1 to 10 µg/L

Initially, the best coincidence between calculations and measurements for the thermal and food functions was obtained with the following coefficients:

Topt= 22°C, Tmax = 32°C, z= 0.3

Kc = 4 µg/L

It was found that coefficient a must be superior to 2 to reproduce weekly increments corresponding to the data, due to the thermal and nutritional limiting factors; a value of a = 2.5 was adopted.

At this point, it was necessary to determine coefficient b, for which an estimation is given by the regression from our data (b=-0.086). We found that the results are extremely sensitive to this coefficient, which obviously influences the largest-sized classes.

Figure 6 presents a comparison of calculations and measurements for three values of b. Using the data on mussels on the PVC plates , the model shows that the coefficient must be the lowest of these three values for good representation of the growth of the largest-sized classes; a discrepancy then appears, however, for the small sizes.

For the caged mussels, real data is by default different from model calculations, which we interpret as the result of growth-limitation due to gradual fouling of the cages.

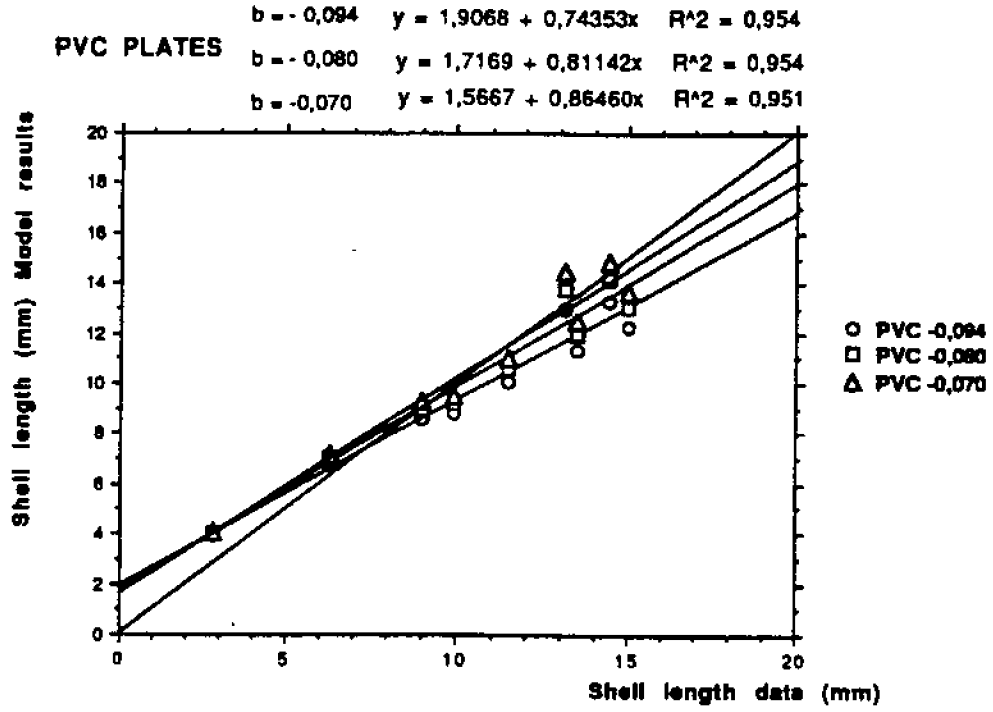
Shell length modelling from 1991 to 1993

During this period, we built up a data file of mean weekly temperatures recorded on site, chlorophyll a data collected in our measurement surveys, and data collected from other sources. In the absence of data on chlorophyll in winter, we set this value at a minimum of 1.5 mg/l for this period.

The **Figure 7** presents the model results with three coefficient b values for the five cohorts corresponding to the main larval settlement peaks during the 1991-1993 period. Initial shell length is taken as equal to 1 mm for each cohort.

The results synthesized in **Table 4** show that coefficient b must be in the range of -0.07 to -0.08 to find growth on the order of 15 mm during the first summer.

Figure 6: Comparison of measurements of shell length and calculation of the shell length with the growth model for 3 values of b coefficient. The data set is that carried out on three cohorts: 91 April, 92 June and 93 June.



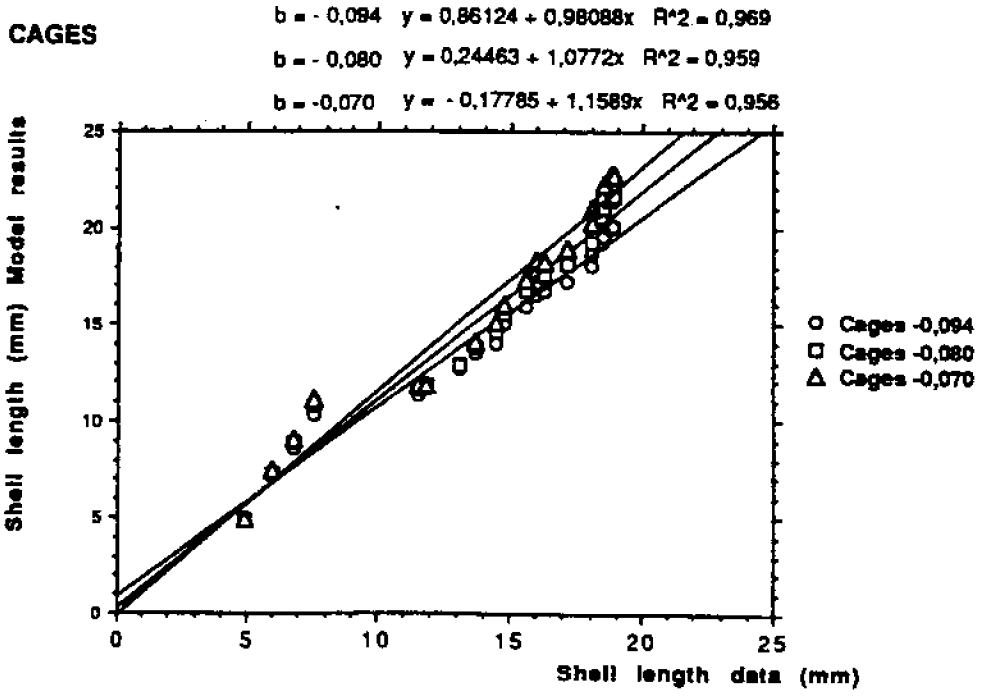
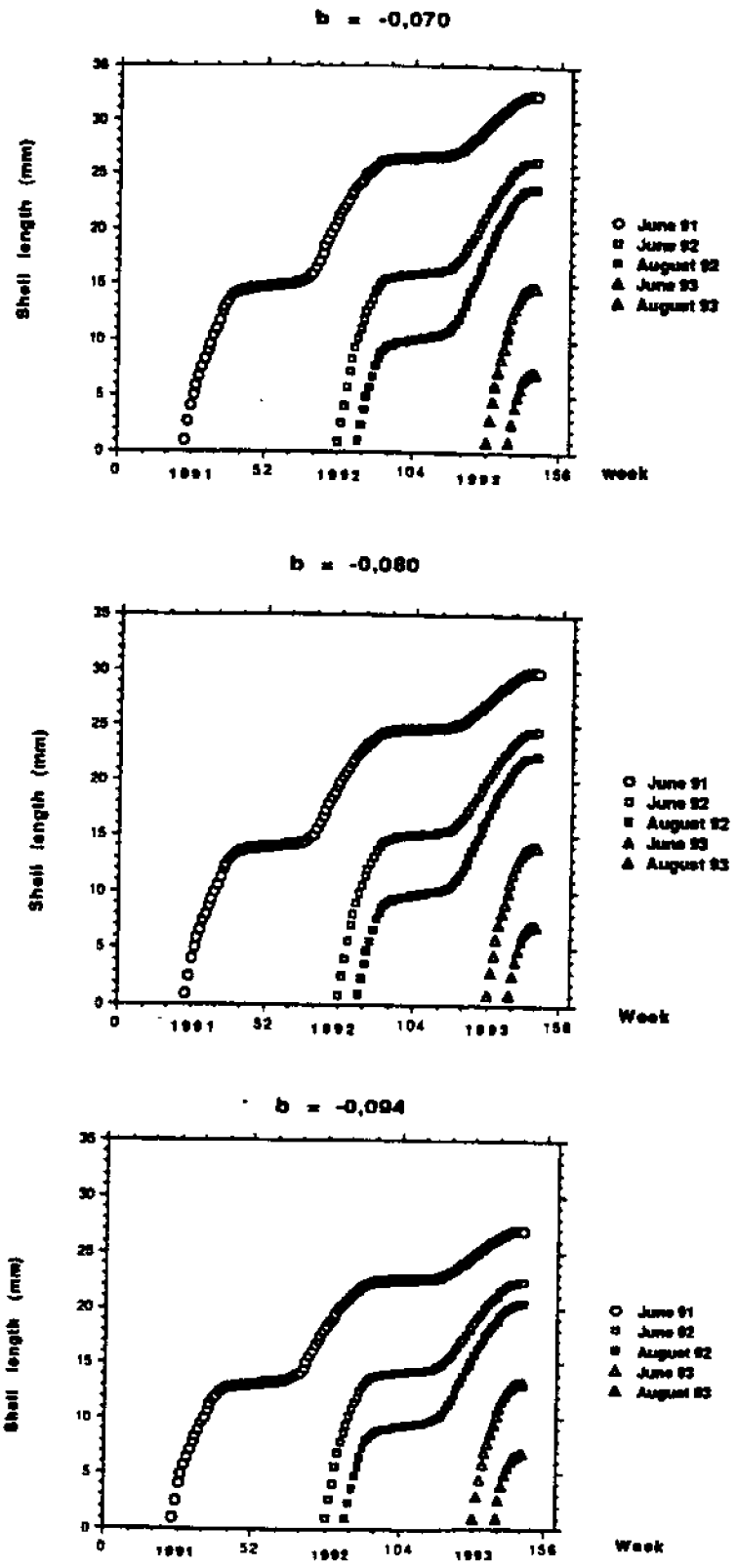


Figure 7: Shell growth modelling of the 5 major cohorts of *Dreissena polymorpha.* recorded into the Cattenom cooling circuits from 1991 to 1993 with 3 b coefficients.



b	1991			1992			1993		
	Period	week	SL (mm)	Period	week	SL (mm)	Period	week	SL (mm)
-0,07	June 91	24	1.00	June 92	78	1.00	June 93	130	1.00
	October91	44	14.35	October 92	96	15.54	October 93	148	14.80
	April 92	66	15.28	April 93	118	16.69	August 93	138	1.00
	October 92	96	26.31	October 93	148	26.25	October 93	148	7.22
	April 93	118	26.87	August 92	85	1.00			
	October 93	148	32.33	October 92	96	9.54			
				April 93	118	11.21			
				October 93	148	23.71			
-0,08	June 91	24	1.00	June 92	78	1.00	June 93	130	1.00
	October 91	44	13.7	October 92	96	14.76	October 93	148	14.08
	April 92	66	14.52	April 93	118	15.81	August 93	138	1.00
	October 92	96	24.45	October 93	148	24.40	October 93	148	7.04
	April 93	118	24.96	August 92	85	1.00			
	October 93	148	29.81	October 92	96	9,22			
				April 93	118	10.78			
				October 93	148	22.14			
-0,1	June 91	24	1.00	June 92	78	1.00	June 93	130	1.00
	October 91	44	12.52	October 92	96	13.46	October 93	148	12.88
	April 92	66	13,25	April 93	118	14.34	August 93	138	1.00
	October 92	96	21.53	October 93	148	21.49	October 93	148	6.71
	April 93	118	21.94	August 92	85	1.00			
	October 93	148	25.91	October 92	96	8,66			
				April 93	118	10.02			
				October 93	148	19.62			

Table 4: Modelling the shell length (SL), with three b coefficients in the range of -0.07 to -0.1 for the five cohorts: 1991 June, 1992 June, 1992 August, 1993 June and 1993 August.

Conclusions

The data collected during our three years of measurements and the growth model we have used now provide us with a "biological profile" of the zebra mussel population which is infesting certain circuits of the Cattenom plant.

Larvae are present in Moselle plankton between April (13° C) and the end of October (10° C). Their density is nonetheless at its greatest between June (June 16 in 1992) and August (August 23 in 1993), in a range of temperatures between 21° C and 25° C, with two abundance peaks. Annual maxima are recorded at between 65 individuals per liter and 452 individuals per liter.

Post-veligers settle between the beginning of June (June 6 in 1991) and mid-September (September 7 in 1993), in a temperature range between 20.6° and 17.9° C.

One or two settlement peaks were also observed:

- June 18 (20.6° C) to July 12 (24° C)
- August ((25° C) to September 7 (17.9° C)

The maximum densities recorded on the artificial substrates are between 8,300 individuals per m2 and 68,800 per m2.

A simple mathematical model was implemented to calculate shell increments in the long term. It is based on three functions representing the influence of size, temperature and food availability (planktonic chlorophyll a).

An adjustment of the coefficients enables taking into account the growth of batches settled on the PVC plates.

While the model overestimates the growth of small-size classes, it does constitute a simple means of estimating the growth of zebra mussels on the walls of the circuits needing protection. It is clear that the coefficients determined in this study can most probably not be extrapolated to other populations, nor even to the populations in the Moselle. The conditions in the power plant circuits are indeed close to the optimum in relation to water velocity (10-50 cm/s).

Acknowledgements

The authors sincerely thank the personnel of the Cattenom nuclear power plant, and in particular, Mr. Cisamolo and Mr. Carasco, for their interest in this study and for their assistance to the teams working on the site. Much of the data collection and processing was performed by Mr. Schmitt and the DESS students at the Universities of Nancy and Paris.

Références.

[1] Khalanski M. 1993. Testing of five methods for the control of zebra mussels in cooling circuits of power plants located on the Moselle river. Third International Zebra Mussel Conference. EPRI report TR-102077.

[2] Khalanski M. 1984. Evaluation de la biomasse phytoplanctonique des eaux courantes par le dosage de chlorophylle. Vehr. Int. Verein. Limnol. 22 : 2010-2019.

[3] Bhattachary C.G. 1967. A simple method of resolution of distribution into gaussian components. Biometrics. tome 23: 115-135.

[4] Moreteau J-C et Marino O. 1992. Analyse démographique de *Dreissena polymorpha* en Moselle à Cattenom. Rapport Université de Metz pour EDF/DER.89 p.

[5] Schmitt M. 1992. Etude de la biologie et de la moule zébrée en Moselle, croissance et reproduction. Rapport Université de Metz pour EDF/DER.80 p.

[6] Moreteau J-C. 1993. Biologie de la moule zébrée *Dreissena polymorpha* Pallas, en Moselle. Etude menée au CNPE de Cattenom. Rapport Université de Metz pour EDF/DER. 24 p.

[7] Gillet A. et Micha J-C. Etude de la biologie d'un mollusque bivalve *Dreissena polymorpha* P. en vue de son élimination dans le circuit "eau brute" de la centrale nucléaire de Tihange. Trib. Cebedeau. N°504, 38 : 3-28.

[8] Jantz B. and Neumann D. 1992. Shell growth and aspects of the population dynamics of *Dreissena polymorpha* in the river Rhine. Limnologie aktuell - vol.4 - Neumann/Jenner (Eds): The Zebra Mussel *Dreissena polymorpha*. Gustav Fischer Verlag : 49-66.

[9] Testard P. 1990. Elements d'écologie du lamellibranche invasif *Dreissena polymorpha* Pallas. Etude de la dispersion des larves en région parisienne et de leur fixation. Réponse à la désoxygénation de l'eau. Thèse Dc ès sciences. Univ. Paris 6: 358 p.

[10] McMahon R.F. 1990. The zebra mussel: US Utilities implications.EPRI report GS-6995: 41 p.

[11] Garric J. et Migeon B. 1983. Etude du cycle de reproduction de Dreissena polymorpha sur le Rhône en aval de Lyon. Rapport EDF/DER. HE 31/83-22: 47 p.

[12] Sprung M. 1992. Observations on shell hrowth and mortality of Dreissena polymorpha in lakes. Limnologie aktuell - vol.4 - Neumann/Jenner (Eds): The Zebra Mussel *Dreissena polymorpha*. Gustav Fischer Verlag : 19-28.

[13] Waltz N. 1978. Growth rates of *Dreissena polymorpha* under laboratory and field conditions.

The Impact of Zebra Mussels on Benthic Algal Communities in Saginaw Bay, Lake Huron.

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Abstract

The rapid proliferation of zebra mussels (*Dreissena polymorpha*) in Saginaw Bay, Lake Huron and their associated filtering activities was predicted to decrease densities of phytoplankton and increase light penetration which should affect the lake's periphyton. Quantitative periphyton samples were collected from natural substrata by SCUBA throughout the photic zone monthly through the growing seasons of 1991 (pre-*Dreissena* colonization) and 1992 (post-*Dreissena* colonization). Productivity rates were measured using carbon-14 in sealed acrylic chambers. Our data suggest that after *Dreissena* invaded, light penetration, productivity, benthic algal biomass and chlorophyll concentrations increased. This produced a shift from benthic diatoms to a flora dominated by filamentous green algae (Zygnematales).

INTRODUCTION

Following the recent invasion of *Dreissena polymorpha* (the zebra mussel) into the Great Lakes (Herbert *et al* 1989) populations have spread quickly and have reached high densities in hospitable aquatic habitats (Garton and Haag 1990). Habitats appearing to be conducive to zebra mussel colonization and growth are those with suitable firm substrata such as rock, wood, unionid clam shells and consolidated sand (Kachalova and Sloka 1964, Kerney and Morton 1970, Lewandowski 1976 and Wiktor 1963) and with ample supplies of food (plankton). Many areas in the Great Lakes meet the substrata requirements for zebra mussels with an abundance of exposed sedimentary bed rock in the lake basins. High densities of plankton that seem essential for the explosion of zebra mussel populations (Deksbakh 1935 and Zhadin 1952) are achieved in two ways. Shallow nutrient-rich lakes or portions of lakes such as the Western Basin of Lake Erie (Nicholls 1981), Saginaw Bay, Lake Huron (Lowe 1975 and Moll *et al* 1980) or Green Bay, Lake Michigan (Holland and Claflin 1975) are notorious for their rich and productive plankton communities. Such areas are expected to support high densities of *Dreissena polymorpha* . A second way that *Dreissena polymorpha* can increase its food supply rate is through the colonization of intake pipes where flowing water increases the supply rate of plankton.

In 1989 *Dreissena polymorpha* densities in the Western Great Lakes were among the highest ever reported for this species and exceeded levels observed in most European lakes (Garton and Haag 1990). Maximum densities of >700,000 individuals/m^2 were observed at Detroit Edison's Monroe Power Plant in August 1989 (Kovalak *et al* 1990). By mid-summer, 1990 estimates of *Dreissena polymorpha* densities in the Western Great Lakes ranged from 13,000/m^2 on the soft bottom of Lake St. Clair where mussels use unionid clams as a colonization epicenter (Hunter and Bailey 1990) to 30,000/m^2 in the Western Basin of Lake Erie (Garton and Haag 1990). In 1991, as the wave of *Dreissena polymorpha* propagules spread out from Lakes Erie and St Clair. Saginaw Bay in Lake Huron experienced the initial colonists of this wave late in 1991 and numbers were expected to continue to increase rapidly in the following years.

Dreissena polymorpha is a filter feeder and can pass relatively large sestonic particles into its incurrent siphon, including its own veliger larvae (MacIsaack and Spurles 1990). Individual mussels have a filtering capacity of from 10 to 200 ml/hr./day (Sprung and Rose 1988 and Stanczykowska *et al* 1976). Rates may vary by an order of magnitude over a 24 hour period (Benedens and Hinz 1980) and are also a function of both water temperature and mussel size (Hinz and Scheil 1972). *Dreissena polymorpha* exhibits feeding selectivity on the basis of size and shape of the sestonic particles (Morton 1969) as well as their chemical nature (Ten Winkel and Davids 1982). Selection is carried out primarily by ciliary resorting currents on the labial palps (Morton 1969). Ten Winkel and Davids (1982), found that selection was positive for particles 15-40 µm but that algae with gelatinous layers within the appropriate size range were rejected. Rejected particles are removed from the body compacted, capsulated in mucus and ejected back out the incurrent siphon as pseudofeces. In either instance, passage into the gut or rejection in pseudofeces, phytoplankton and other sestonic particles are removed from suspension in the lake and deposited in the benthos. With densities of several thousand individuals/m^2 the filtering activity of *Dreissena polymorpha* can drastically alter the quantity and quality of lake seston. In European studies *Dreissena polymorpha* has been observed to have a marked

positive impact on water clarity (Stanczykowska *et al* 1976) and populations have even been introduced into eutrophic lakes to reduce phytoplankton densities and essentially mediate "oligotrophication" of plankton-dense habitats. In Lake Erie water transparency has increased dramatically following zebra mussel invasion. In 1988 maximum secchi disk depth was 3.4 m whereas the maximum 1989 secchi disk depth was 6.5 m (Culver *et al* 1990). This increase in water clarity is positively correlated with increases in *Dreissena polymorpha* densities.

Although much of the data on rapidly changing Lake Erie is anecdotal, the rapid increase in transparency was a common observation among many long time fishermen on the lake. Over the course of our 1990 field season in the Catawba Island area of Lake Erie (Lowe et al 1990) we observed an increase in underwater visibility from a few decimeters in April to about five meters in October. Such changes are contradictory to past observations on Lake Erie prior to Dreissena polymorpha invasion (Holland, 1993).

Greater water column transparency should result in increased depth of light penetration with a resultant increase in the area of the sunlit eulittoral zone. This phenomenon should favor the growth of macrophytes and benthic algae. In addition the rain of fecal and pseudofecal pellets onto the lake bottom should enhance nutrient supplies to benthic algae. With a reduction of phytoplankton densities the relative importance of benthic algal production to lacustrine food webs should increase (Lowe *et al* 1990). Although the majority of Great Lakes' productivity is pelagic, lake littoral zones are areas of intense surface-associated primary productivity, often contributing a significant portion of a lake's fixed carbon (Wetzel 1983) and providing critical habitat space for many invertebrate and vertebrate species. Some of the ramifications of this hypothesized spatial shift in algal biomass from pelagic to benthic may already be appearing in Lake Erie. Increases in such benthic organisms as *Gammarus* and gastropods have been observed (Dermott *et al* 1990 and Lowe personal observation). Many of the changes in the relationship between *Dreissena polymorpha*, phytoplankton and benthic algae were documented as secondary observations or anecdotal information gleaned from on-going research not focused specifically on this phenomenon (but see Monoca *et al* in press). Thus, records on benthic algal communities and levels of benthic productivity in Lake Erie prior to *Dreissena polymorpha* invasion are poor or nonexistent.

Saginaw Bay in Lake Huron is similar in many ways to Lake Erie. It is relatively shallow, receives nutrient loading from streams in agriculturally dominated basins and supports relatively high phytoplankton densities with associated low water transparency (Moll *et al* 1980). Prior to the summer 1991 populations of *Dreissena polymorpha* were essentially absent in Saginaw Bay but were anticipated to invade and spread rapidly. These circumstances provided us with the opportunity to examine the structure and function of the benthic algal community of Saginaw Bay before and after zebra mussel-mediated changes in water clarity.

The objectives of this investigation were to determine the impact of increased densities of *Dreissena polymorpha* on benthic algal (periphyton) community structure and carbon fixation rates. We hypothesize that increased zebra mussel densities would lead to increased water clarity resulting in increased benthic algal density and carbon fixation rates.

MATERIALS AND METHODS

Study Site- Saginaw Bay, a large embayment of Lake Huron (figure 1), is a relatively shallow basin with high nutrient and phytoplankton concentrations (Vollenweider *et al.* 1974, Smith *et al.* 1977, Stoermer *et al.* 1982, and Kreis *et al.* 1985). Bedrock or large

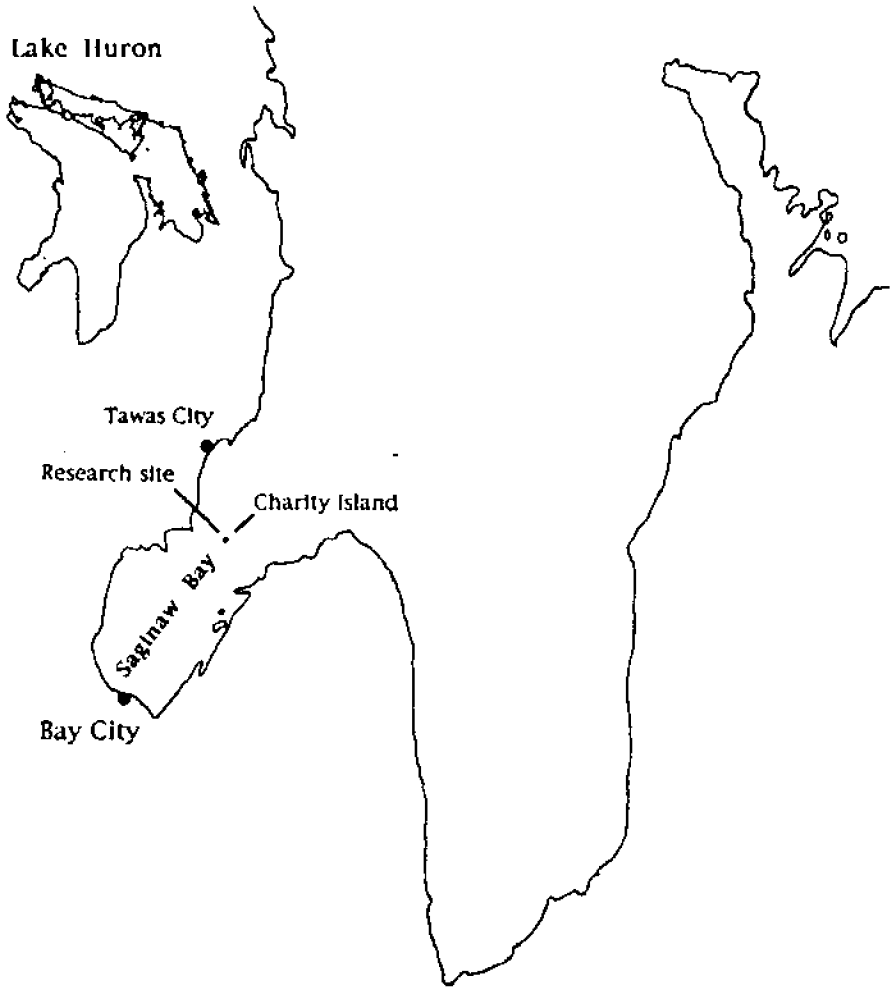

Figure 1. A map of Lake Huron with an enlargement of Saginaw Bay. The research site is approximately 1.5 km north-west of Charity Island.

cobble which composes most of the benthic substrata is ideal for zebra mussel colonization. Our study site was located near the center of the bay, approximately 2km north of Charity Island (N 44.03.10. W 83.26.31). This off shore location minimized the effects of human disturbances and storm runoff on the benthos. The site also afforded us a relatively wide range of depths (1.5 to 7m) within the biologically significant portion of the littoral zone. This range of depth is representative of most of Saginaw Bay. Sampling efforts were concentrated mainly at 2.5m (range = 2.2-3.1m) and 5.5m (range = 5.0-6.0m).

Zebra Mussel survey- Zebra mussel densities during 1991 and 1992 were measured at least every month throughout the growing season by SCUBA divers. In 1991 divers placed 5 quadrats (0.25 m^2) randomly over bedrock or cobble 5 times on each sampling trip. Zebra mussel densities were recorded. Divers also collected 7 to 10 rocks randomly along a transect. Attached zebra mussels longer than 1.0mm were enumerated on each rock and the exposed surface area of the rock was calculated so that zebra mussel densities could

be determined. Both survey techniques were done at 2.5m and 5.5m in 1991. In 1992, zebra mussels were assayed only by collecting rocks at both depths.

Benthic Productivity- In 1991 (during June, July, and August), and in 1992 (during June, July August, and October) three rocks were gathered randomly by divers and each was immediately placed into separate, clear, acrylic, incubation chambers (2.0L) similar in function to those described in Loeb (1981). Each chamber was placed at the depth where the rocks were collected (either 2.5m or 5.5m) and injected with 20 µci of labeled carbonate (^{14}C) and mixed with the attached mixing bulbs (figure 2). The chambers were left on the bottom to incubate for 3.5-4 hours and then brought to the surface. At the time these chambers were collected, an additional rock was placed in another chamber, injected with labeled carbonate and mixed as described above for each depth, and immediately brought to the surface with the rest of the chambers. These extra rocks were used as a "time zero" control for carbon uptake. The rocks were taken out of the chambers, rinsed and stored on ice in a dark cooler. Back on shore, each rock was scraped clean of periphyton (and zebra mussels, if present) and the samples were placed in a blender and homogenized for 15 s. One ml of the resultant slurry was placed into a scintillation vial in which 1ml of 0.05N HCl was added to volitolize any labeled carbonate that was not organically bound. Each vial had 10 ml of scintillation cocktail added. The samples were analysed on a Packard Tri-Carb 460 scintillation counter and corrected for quench rates. Data were log-transformed (base 10) and a t-test was applied to comparable dates from each year to compare net carbon uptake trends in 1991 and 1992.

Chlorophyll analysis- Divers collected 6-10 rocks from 3 depths in the 1991 field season (1.5m, 2.5m, and 5.5m) and from 2 depths from the 1992 field season (2.5m and 5.5m). Rocks of an appropriate size (7-20cm total length) at the specified depths were gathered at random along a transect and each carefully placed into Zip Lock freezer bags to prevent the loss of loosely attached periphyton. These bags were then brought to the surface and stored in coolers. Within 24h of collection, the rocks were scrapped clean of periphyton and zebra mussels (if present). The scraped material was then blended for 15 s. A subsample of this slurry was filtered onto a glass fiber filter (pore size <4.5um) and frozen. Another subsample was collected and preserved with formalin (3.0%). This portion was later analyzed for algal composition (see community structure section). Chlorophyll concentrations were determined with the tricolometric method (Jeffrey and Humphrey 1975) using a Perkin Elmer Lamba-6 UV-Vis spectrophotometer. A t-test on the log-transformed (base 10) data was conducted on each depth from 1991 and 1992 to determine annual differences in pigment concentration over different depths.

Community structure- A subsample of each periphyton slurry was collected and preserved in formalin (3.0%). At least 3 samples were examined from each depth using a Palmer-Maloney counting cell and a Baush and Lomb microscope at 430x. At least 300 cells were counted and identified to species from there samples when possible using Prescott (1952), and Taft and Taft (1971). Diatom slides were prepared by oxidizing part of the subsamples with 30% H_2O_2 and $K_2Cr_2O_7$. After diluting and decanting the solution several times, the samples were placed on a cover slip and dried. The cover slips were then mounted on slides with Naphrax mounting medium (R. I.=1.7). At least 300 diatom valves were identified and counted (at 1000x) from each sample. The information from the cleaned diatom counts was used to help identify "live" diatoms from the Palmer-Maloney cell counts. Only whole valves were counted. Biovolumes were calculated by

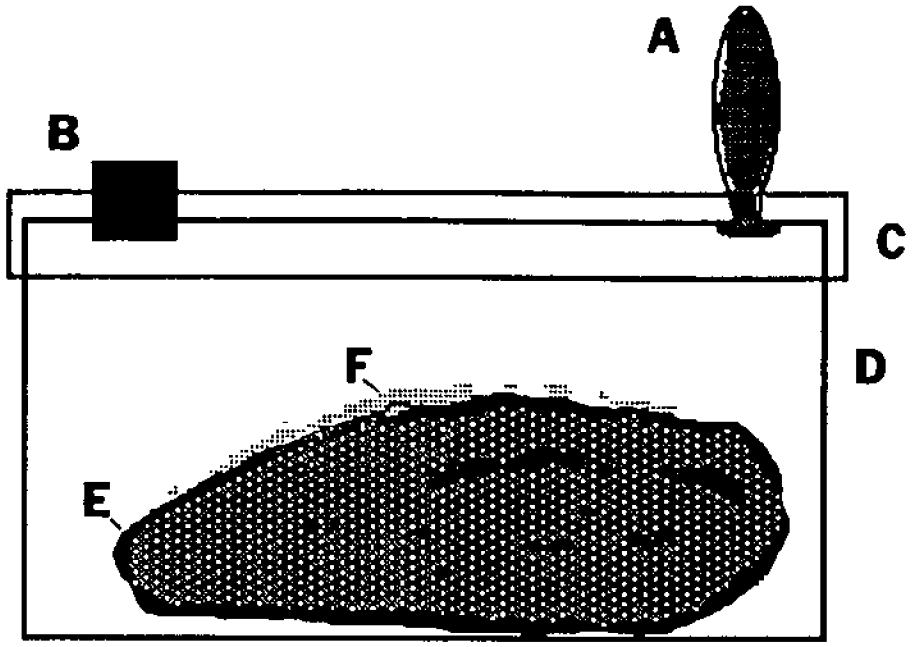

Figure 2. Productivity chamber used for determining carbon-fixation rates. A) mixing bulb, B) syringe port, C) acrylic lid, D) acrylic chamber, E) rock, F) periphyton.

using the average dimensions (taken from measurements of at least 10 cells for each taxon) and applying them to standard geometric forms which approximated the shape of each taxon. A t-test was performed on log-transformed (base 10) data collected at the same depths and nearly the same dates in 1991 and 1992 to help determine differences in density patterns.

RESULTS

Zebra mussel survey- No zebra mussels were seen until July,1993 (figure 3) when low densities were estimated from both the quadrats and rock samples with the quadrat technique giving a higher estimate. Zebra mussel densities had increased greatly by late August 1991. The rock gathering technique yielded densities of 17,000/m^2 while the quadrant technique yielded densities of 206/m^2. In October, 1991 densities on gathered rocks were 47,000/m^2. The quadrat method was not performed due to storm conditions.

During the 1992 sampling season the zebra mussel densities generally continued to increase and reached a peak of 115,000/m^2. The quadrat method was not employed that season and all densities are from collecting rocks.

Community structure- The most striking difference between the 1991 and 1992 seasons was a substantial increase in benthic algal biovolume in 1992. This increase was most notable at 2.5m where the average biovolume in late summer of 1992 was almost 6 times greater than in 1991 (figure 4). This relationship was significant ($p < 0.0005$). Algal

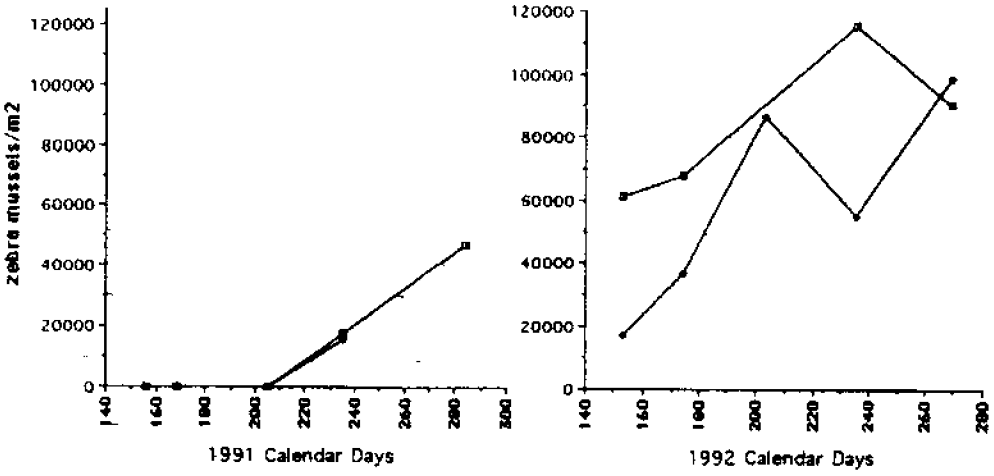

Figure 3. Zebra mussel densities plotted against time for 1991 and 1992. Densities were determined by randomly sampling rocks in the area. The open squares represent zebra mussel densities at 2.5m and the closed diamonds represent zebra mussel densities at 5.5m.

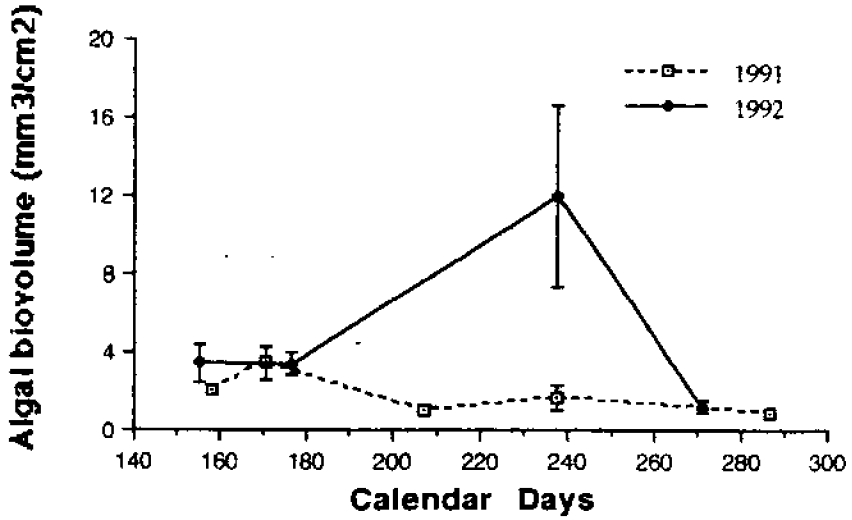

Figure 4. Total benthic algal biovolume (mm^3/cm^2)plotted against time in Saginaw Bay, Lake Huron at 2.5m. The open squares and dashed lines represent algal biovolume in 1991 (low zebra mussel densities). The closed diamonds and solid line represent algal biovolume in 1992 (high zebra mussel densities). The error bars represent standard error.

biovolume was significantly ($p < 0.005$) greater in 1991 compared to 1992 in early June at 5.5m. In 1992, benthic algal biovolume increased throughout the sampling season until a sharp decline in late September. By late August benthic algal biovolume was significantly greater ($p < 0.0001$) in 1992 compared with samples near the same time in 1991 (figure 5). In 1991 the peak biovolume for both depths was reached in June and tended to decline over the rest of the sampling period. In 1992, following zebra mussel colonization the area (as well as the rest of the bay) the June samples were some of the lowest biovolume averages found for the season (figure 5).

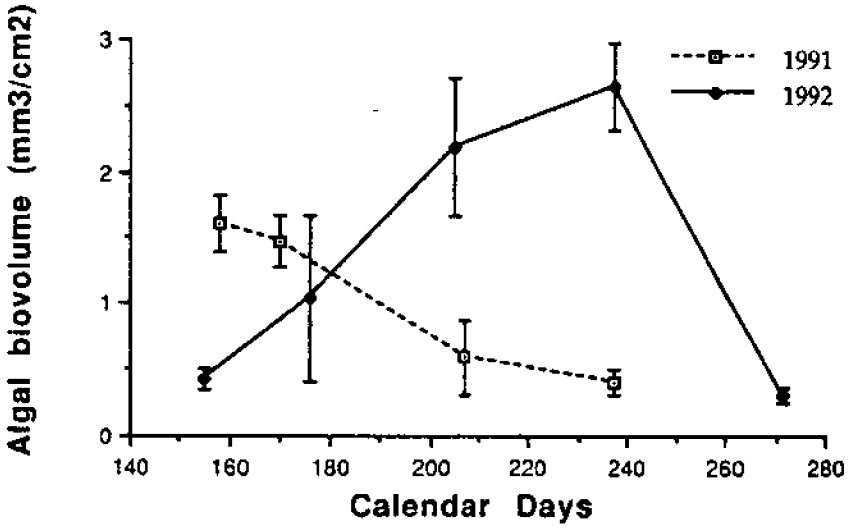

Figure 5. Total benthic algal biovolume plotted against time in Saginaw Bay, Lake Huron at 5.5m. The open squares and dashed lines represent algal biovolume in 1991 (low zebra mussel densities). The closed diamonds and solid line represent algal biovolume in 1992 (high zebra mussel densities). The error bars represent standard error.

The benthic algal flora in 1991 was dominated by diatoms that were mainly responsible for the high biovolumes in June. Diatom biovolumes were significantly higher in 1991 when compared to similar readings during 1992 at 2.5m for late June ($p < 0.005$), August ($p < 0.05$), and late September-early October ($p < 0.05$). At 5.5m, diatom biovolume was significantly higher in 1991 for comparable readings during early June ($p < 0.01$), late June ($p < 0.05$), and July ($p < 0.01$). Although diatoms were important in both depths, they were relatively more important at greater depths (figures 6 and 7). In 1992, green algae became dominant throughout the sampling season except for early June. Green algal biovolume at 2.5m was significantly greater ($p < 0.005$) in August of 1992 than during August of 1991. At 5.5m, 1991 had significantly more ($p < 0.05$) green algal biovolume than 1992 in early June. Green algal biovolume from 5.5m for 1992 was greater than 1991 for July ($p < 0.005$) and August ($p < 0.000005$). The green filamentous algae *Mougeotia* and *Spirogyra* were responsible for the large peak in biovolume during July and August in 1992 (figures 6 and 7). The biovolume of diatoms decreased from their 1991 levels in 1992. Blue-green algae were not an important component of this system.

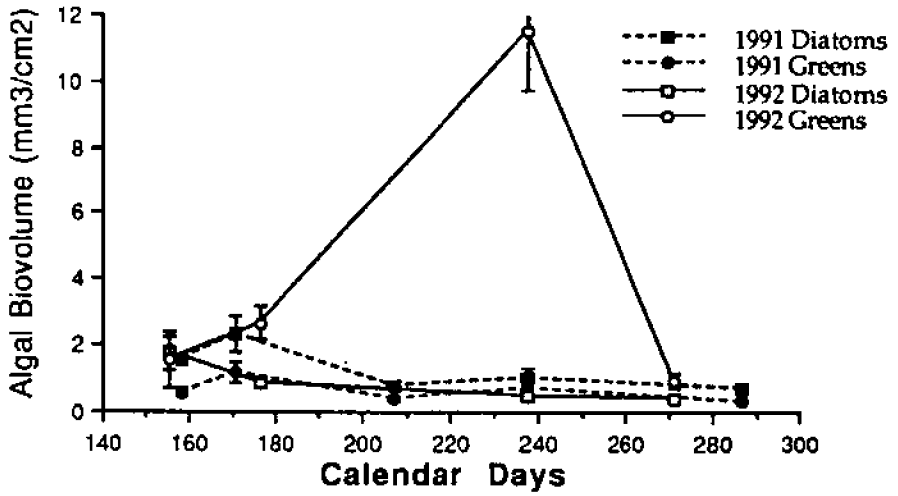

Figure 6. Benthic biovolume of diatoms (squares) and green algae (circles) plotted against time in Saginaw Bay, Lake Huron at 2.5m. The closed symbols and dashed line represents algal biovolumes in 1991 (low zebra mussel densities). The open symbols and solid line represents algal biovolumes in 1992 (high zebra mussel densities). The error bars represent standard error.

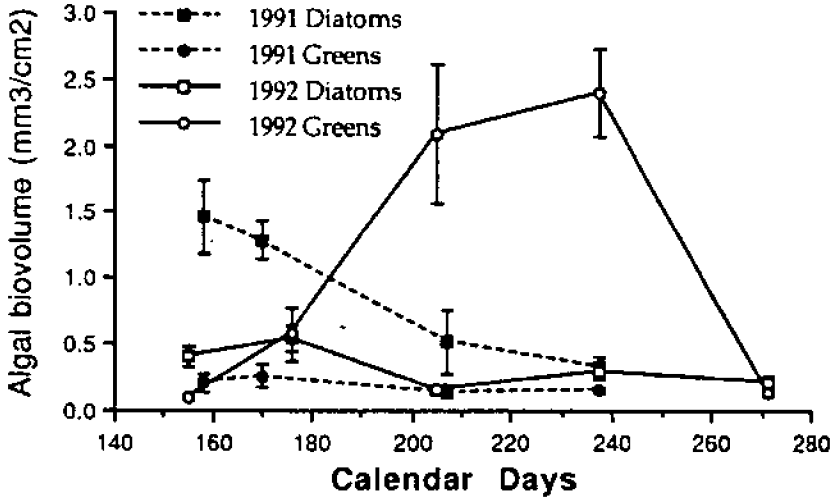

Figure 7. Benthic biovolume of diatoms (squares) and green algae (circles) plotted against time in Saginaw Bay, Lake Huron at a depth of 5.5m. The closed symbols and dashed line represents algal biovolumes in 1991 (low zebra mussel densities). The open symbols and solid line represents algal biovolumes in 1992 (high zebra mussel densities). The error bars represent standard error.

Chlorophyll- The chlorophyll-a concentrations (figures 8 and 9) generally follow the trends of total algal biovolume (figures 4 and 5). However, there were often not significant differences between depths from the same date. In early June the chlorophyll-a concentrations for 1991 and 1992 were not significantly different in the shallow depth but 1991 was significantly higher ($p < 0.001$) at 5.0-6.0m. In mid-late June, for both shallow and deep depths 1991 values were significantly higher ($p < 0.001$ and $p < 0.0005$ respectively). In late July, the deep sites for 1992 showed an increase in chlorophyll-a while 1991 values decreased (due to missing values, the shallow depths were not comparable). Deep chlorophyll values for July of 1992 became significantly greater than those of July 1991 ($p < 0.005$). The shallow depths for 1992 were significantly greater ($p < 0.05$) while the deep samples were not significantly different in late August chlorophyll-a samples. The shallow samples for 1991 were significantly greater ($p < 0.005$) while deep samples for this time in 1991 were not collected for late September to early October.

Productivity- In 1991 the productivity of the benthic community was measured in June, July, and August and only at deep sites. In 1992 benthic productivity was measured from early June to late September at the deep site and early to late June at the shallow site. Net carbon uptake was higher on average (around twice as much) in 1992 compared to similar dates in 1991 (figure 10). However only in late July of 1992 were the values significantly greater ($p < 0.05$). When comparable, productivity also had a higher average in the shallow site compared to the deeper site in 1992.

DISCUSSION

Zebra mussel densities- Although we are treating 1991 as a pre-*Dreissena* invasion period, significant zebra mussel population started to develop in August at our site (figure 3) and throughout the bay (Nalepa, personal communication). In July 1991, when zebra mussel densities were very low, the quadrat method seemed to give a higher estimate of density. This was probably due to the quadrat method's ability to sample a greater area (and hence, more likely to include rare druses of zebra mussels) than the rock collecting method. When zebra mussel densities increased, the rock gathering method gave much higher estimates, a function of zebra mussels preferentially colonizing the underside of rocks. The quadrat method will therefore underestimate densities in cobble substrates unless the underside of each rock in the quadrat is examined. Thus, even though sizable densities of zebra mussels were developing at the end of the 1991 sampling season, the rock surfaces facing up remained essentially clear of zebra mussels. Therefore, there was little direct competition for substrate space between zebra mussels and periphyton during this time. There is some evidence to suggest that zebra mussels may have had an impact on the plankton during the fall (Napala 1994). Therefore the periphyton may have also been affected indirectly but no data exist for Saginaw Bay periphyton at this time.

In 1992 the zebra mussels were dense enough to conceivably have an impact on the spring plankton blooms. Nalepa (1994) reports chlorophyll-a levels in the plankton to be 4 times lower than the ten year average for the bay. Also in 1992, the zebra mussels have colonized the upper surfaces of the rocks thereby changing the colonizing substrata for periphyton.

Community Structure- As the zebra mussels invaded Saginaw bay, the periphyton biovolume greatly increased and switched from a diatom dominated community to a community dominated by green algae. This was mainly due to an increase in filamentous

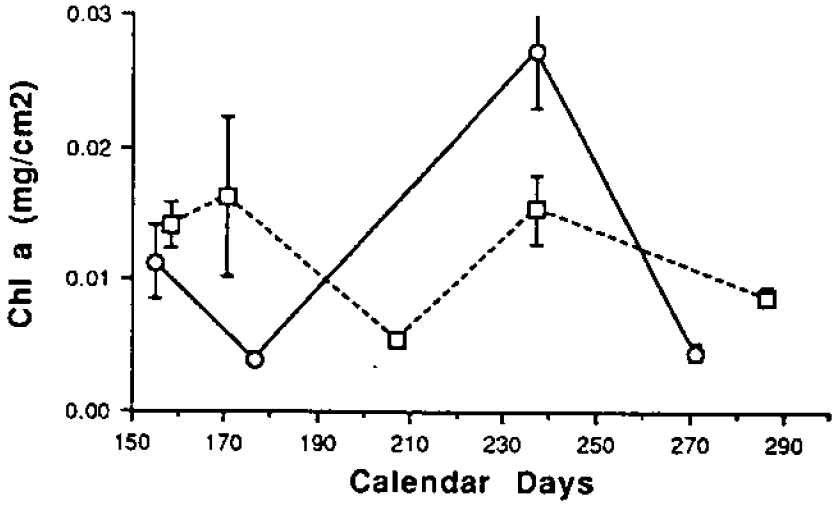

Figure 8. Concentrations of benthic chlorophyll-a at 2.5m for 1991(low zebra mussel densities, dashed line) and 1992 (high zebra mussel densities, solid line) plotted against time in Saginaw Bay, Lake Huron. Error bars represent standard error.

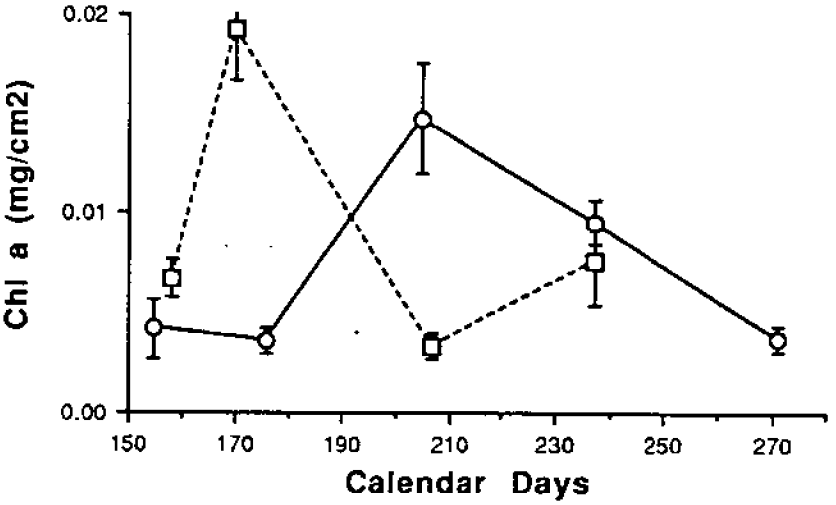

Figure 9. Concentrations of benthic chlorophyll-a at 5.5m in 1991(low zebra mussel densities, dashed line) and 1992 (high zebra mussel densities, solid line) plotted against time in Saginaw Bay, Lake Huron. Error bars represent standard error.

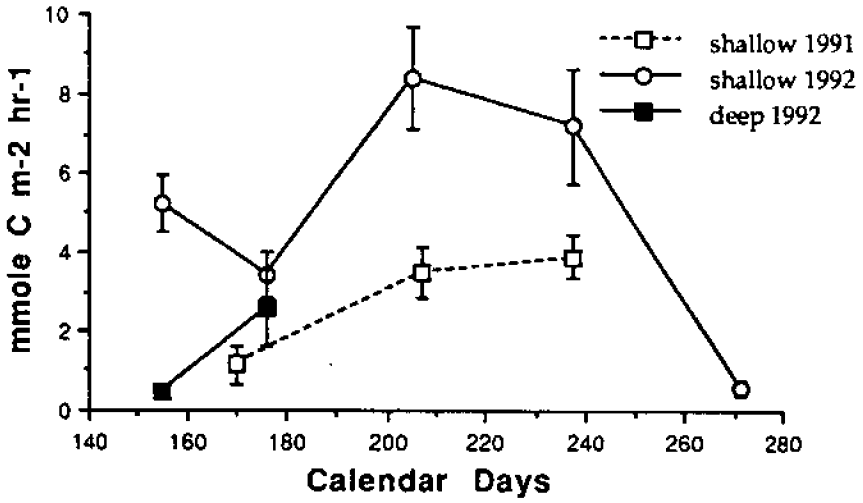

Figure 10. Benthic productivity of Saginaw Bay (mmole C m^{-2} hr^{-1}) plotted against time. Open symbols represent shallow depths (2.2-3.2m) and closed symbols represent deep depths (5.0-6.0m). The years1991 (low zebra mussel densities, dashed line) and 1992 (high zebra mussel densities, solid line) are shown. Error bars represent standard error.

green algae (mostly *Spirogyra* sp. and *Mougeotia* sp.). Although we have no direct evidence, this was probably caused by an increase in the amount of light received by the periphyton due to zebra mussel predation on phytoplankton. Filamentous green algae have been shown to quickly react to increased in light by dramatic increases in their biomass (Stokes 1984, Shortreed and Stockner 1983, and Sheath et al. 1986).

Nutrients are also likely to be more plentiful to the periphyton after zebra mussel colonization for several reasons. With the decrease in phytoplankton there will be less competition for nutrients for the periphyton. Also the deposition and decomposition of both the feces and psudo-feces could be a rich new source of nutrients for the periphyton.

The natural rock substrata had also changed. Since nearly all the available rock was covered with zebra mussels in 1992, the benthic colonizing surface has become much more complex and the available colonizing area for periphyton has increased many times. The feces and psudo-feces and other detritus can now collect between the mussel shells rather than being washed into deeper zones by the waves. These rich organic pockets between the zebra mussel shells may be more suited for an epipelic community rather than an epilithic community. This may have contributed to the changes observed in the periphyton community.

The period of peak periphyton biovolume shifted after the zebra mussel invasion from early June in 1991 to late August in 1992. Much of the filamentous green algae became detached late in the summer and washed up along the shores in large masses which closed beaches near Bay City.

Chlorophyll- The 1992 Chlorophyll-a concentrations were higher than the 1991 levels. This is another indicator of the greater periphyton biomass that appeared after the zebra mussel invasion. In general, the trends seen in chlorophyll concentrations follow closely

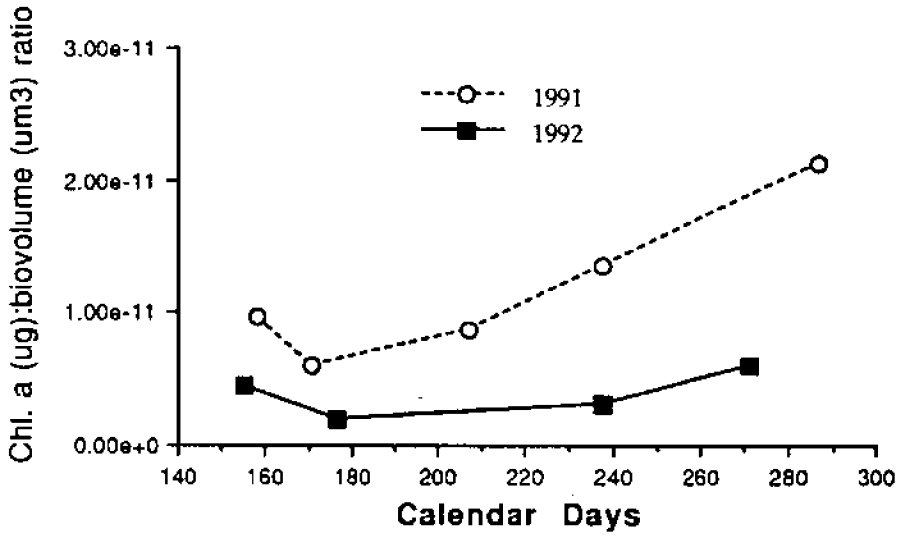

Figure 11. The raito of benthic chlorophyll (μg): benthic algal biovolume (μm³) Saginaw Bay at 2.5m plotted against time. Open symbols and dashed line represent data from 1991 (low zebra mussel densities) while closed symbols and a solid line represent 1992 (high zebra mussel densities).

the changes in periphyton biovolume through time. This relationship is not as clear when comparing benthic chlorophyll-a concentrations and periphyton biovolumes across depth. There is often significantly greater biovolume at 2.5m compared to 5.5m (as expected, due to the higher light levels), but this trend is often not followed by the corresponding chlorophyll-a levels. This is because algae can adjust their chlorophyll levels to compensate for lower light but do not normally change their cell volume. The ratio of average chlorophyll-a concentrations to average benthic algal biovolume for the same samples is shown in figures 11 and 12. Note that at 2.5, the chlorophyll : biovolume ratio is much lower in 1992. This may indicate that the total benthic algal biovolume in 1992 was not light limited while in 1991 it may have been (figure 11). For the deeper samples, there is no clear pattern (figure 12). This suggests that while samples in 1992 showed significantly more biovolume, they may have been still light limited.

Productivity- The productivity of the benthic community increased after the establishment of zebra mussels. Again this was probably due to the increase in light and algal biomass. We did not attempt to calculate respiration rates of the community using a "dark bottle" procedure because of the confusion over its interpretation. However this increase in carbon assimilation is in agreement with increases found in benthic algal biovolume and chlorophyll concentrations.

Conclusion- Our data suggest that as the zebra mussels invaded Saginaw Bay, periphyton biomass, chlorophyll a concentrations, light penetration, and net periphyton productivity all increased. This supports our hypothesis that phytoplankton is in competition for resources (i.e., light and nutrients). Light energy that would have normally gone into phytoplankton production is now being used by the underlying periphyton.

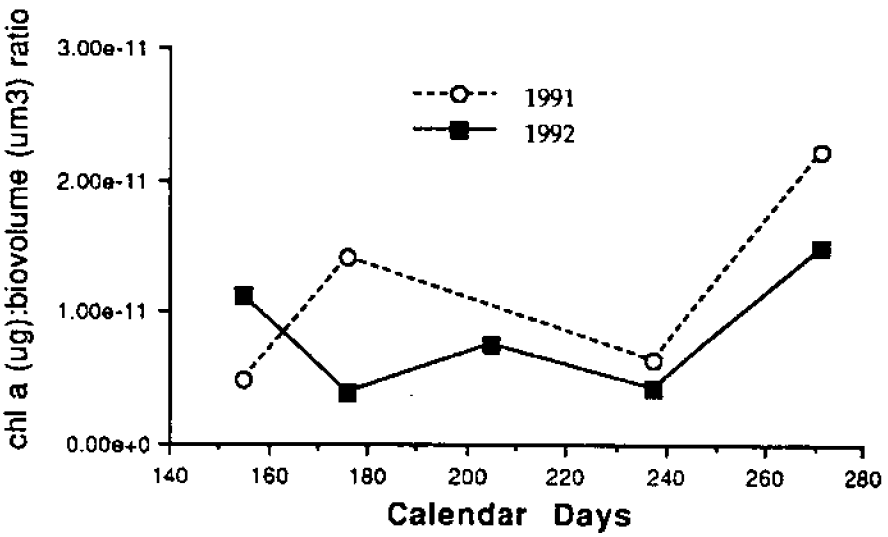

Figure 12. The raito of benthic chlorophyll (µg): benthic algal biovolume (µm^3) Saginaw Bay at 5.5m plotted against time. Open symbols and dashed line represent data from 1991 (low zebra mussel densities) while closed symbols and a solid line represent 1992 (high zebra mussel densities).

Although we have not yet quantified our observations, there seems to be a corresponding increase in the abundance of organisms that utilize benthic algae (*Gamerous* sp., crayfish, and sculpins). This may be a fruitful area for future research as many organisms from higher trophic levels may take years to respond to this increase in benthic food resources. Saginaw Bay is not currently at equilibrium and many more changes are expected as the entire system adjusts to changes generated by *Dreissena polymorpha*.

ACKNOWLEDGMENTS

This research was supported with grants from the Cooperative Institute for Limnology and Ecosystems Research and Ohio Sea Grant. Ship time was provided in part by the USEPA. Laboratory facilities were provided by the University of Michigan Biological Station, Great Lakes Environmental Research Lab and Bowling Green State University. Jerry Yotkois, Steve Francoeur, Randy Littoral, Joanne Roers, Mike Roehrs and Vern Bingman assisted with SCUBA. Dave Szymanski and Donna Nicholson assisted with counting algal samples. Tom Nalepa and Tom Bridgman provided technical assistance. Dennis Geniac provided a boat and knowledge of the bay. Jennifer Greenwood proofread sections of this manuscript.

LITERATURE CITED

Benedens, H. G. and W. Hinz 1980. Zur tagesperiodizitat der filtrationsleistung von *Dreissena polymorpha* und *Sphaerium corneum* (Bivalvia). Hydrobiol. 69(1-2): 45-48.

Culver, D. A., R. Pontius and L. Wu 1990. Interactions between zebra mussels (*Dreissena polymorpha*) and pelagic communities. Proceedings of the International Zebra Mussel Research Conference, Ohio Sea Grant, The Ohio State University, Columbus, OH. p. 12.

Deksbakh, N. K. 1935. The distribution of *Dreissena polymorpha* Pall. (Molluska) in European part of USSR. Byull. Mosk. Oshch. Ispyt. Prir., Otd. Biol. 44: 153-164.

Dermott, R., J. Leslie, J. Fitzsimmons, and V. Cairns 1990. Impact of *Dreissena* on fisheries potential in Lakes St. Clair and Erie. Proceedings of the International Zebra Mussel Research Conference, Ohio Sea Grant, The Ohio State University, Columbus, OH. p. 13.

Garton, D. W. and W. R. Haag 1990. Seasonal patterns of reproduction and larval abundance of *Dreissena* in Western Lake Erie: what a difference a year makes. Proceedings of the International Zebra Mussel Research Conference, Ohio Sea Grant, The Ohio State University, Columbus, OH. p. 7.

Hebert, P. D. N., B. W. Muncaster and G. L. Mackie 1989. Ecological and genetic studies on *Dreissena polymorpha* (Pallas): a new mollusk in the Great Lakes. Can. J. Fish. Aquat. Sci. 46: 1587-1591.

Hinz W. and H. G. Scheil 1972. Zur filtrationsleistung von *Dreissena, Sphaerium* und *Pisidium* (Eulamellibranchiata). Oecol. 11: 45-54.

Holland R. E. and L. W. Claflin 1975. Horizontal distribution of planktonic diatoms in Green Bay, mid-July 1970. Limnol. Oceanogr. 20(3): 365-378.

Holland R.E. 1993. Changes in planktonic diatoms and water transparency in Hatchery Bay, Bass Island Area, western Lake Erie since the establishment of the Zebra mussel. J. Great Lakes Res. 19(3):617-624.

Hunter, R. D. and J. F. Bailey 1990. Colonization of soft substrata by zebra mussels: Role of and cost to native bivalves. Proceedings of the International Zebra Mussel Research Conference, Ohio Sea Grant, The Ohio State University, Columbus, OH. p. 10.

Kachalova, O. L. and N. A.Sloka 1964. *Dreissena polymorpha* (Pallas) in the Daugava (Western Dvina) river basina. In: Shtegman, B. K. (ed.). Biology and control of *Dreissena*. Trudy Instituta Biologii Vnutrennikh Vod. Akademia Nauk SSSR 7: 1-145.

Kerney, M. P. and M. S. Morton 1970. The distribution of *Dreissena polymorpha* (Pallas) in Britain. J. Conchol. 27: 97-100.

Kovalak, W. P., C. D. Longton and R. D. Smithee 1990. Zebra mussel infestation at Monroe power plant: changes during 1990. Proceedings of the International Zebra Mussel Research Conference, Ohio Sea Grant, The Ohio State University, Columbus, OH. p. 16.

Kreis, R.G. Jr., E.F. Stoermer and T.B. Ladewski 1985. Phytoplankton species composition, abundance, and distribution in southern Lake Huron, 1980; including a

comparative analysis conditions in 1974 prior to nutrient loading reductions. Special report no. 107. EPA-Great Lakes Research Division. Ann Arbor, MI.

Lewandowski, K. 1976. Unionidae as a substratum for *Dreissena polymorpha* Pall. Polskie Archiwum Hydrobiologii 23(3): 409-420.

Loeb, S.L. 1981. An in situ method for measuring the primary productivity and standing crop of the epilithic periphyton community in lentic systems. Limnol. Oceanogr. 26:392-399.

Lowe, R. L. 1975. Phytoplankton in Michigans nearshore waters of Lake Huron and Lake Superior. Mich. Dept. Nat. Res., Tech. Rept.

Lowe, R. L., L. A. Reich and J. C. Sferra 1990. Algal herbivory by the zebra mussel: fate of algae in feces and pseudofeces. Proceedings of the International Zebra Mussel Research Conference, Ohio Sea Grant, The Ohio State University, Columbus, OH. p. 11-12.

MacIsaac, H. J. and W. G. Spurles 1990. Direct suppression of Lake Erie zooplankton by zebra mussels (*Dreissena polymorpha*). Proceedings of the International Zebra Mussel Research Conference, Ohio Sea Grant, The Ohio State University, Columbus, OH. p. 13.

Moll, R. A., C. O. Davis and C. L. Schelske 1980. Phytoplankton productivity and standing crop in the vicinity of the Lake Hron-Saginaw Bay front. J. Great Lakes Res. 6(3): 232-246.

Monaco, M.E., R.C. Lorenz and C.E. Herdendorf *in press*. The Areal and Vertical Distribution of *Cladophora glomerata* in Western Lake Erie and its Interaction with the Zebra Mussel (*Dreissena polymorpha*). Technical report. Ohio State University's Lake Erie Program.

Morton, B. S. 1969. Studies on the biology of *Dreissena polymorpha* Pall. 1. General anatomy and morphology. Proc. Malac. Soc. Lond., 38: 301-321.

Nalepa, T.F. 1994. Trends in zebra mussel populations in Saginaw Bay and subsequent changes in water quality, 1991-1993. Abstract. Forth International Zebra Mussel Conference. Madison, WI.

Nicholls, K. H. 1981. Recent changes in the phytoplankton of Lakes Erie and Ontario, pp. 41-84. *In*: Cap, R. K. and Frederick, V. R., Jr. (eds.). Proc. Conf. on Changes in the Biota of Lakes Erie and Ontario. Bull. Buffalo Soc. Nat. Sci. 25(4): 1-120.

Prescott, G.W. 1952. Algae of the Western Great Lakes Area. Otto Koeltz Science Publishers. Koenigstein. Germany.

Sheath, R.G., J.M. Burkholder, M.O. Morison, A.D. Steinman, and K.L. VanAlstyne 1986. Effect of tree canopy removal by gypsy month larvae on the macroalgae of a Rhode Island headwater stream. J. Phycol. 22:567-570.

Shortreed, K.S. and J.G. Stocker 1983. Periphyton biomass and species composition in a coastal rainforest stream in British Columbia: effects of environmental changes caused by logging. Can J. Fish. Aquat. Sci. 40:1887-1895.

Smith, V.E., K.W. Lee, J.C. Filkens, K.W. Hartwell, K.R. Rygwelski and J.M. Townsend 1977. Survey of Chemical Factors in Saginaw Bay (Lake Huron). USEPA, Office of Res. and Devel., Duluth, MN.

Sprung, M and U. Rose 1988. Influence of food size and food quality on the feeding of the mussel *Dreissena polymorpha*. Oecol. 77: 526-532.

Stanczykowska,A. , W. Lawacz, J. Mattice and K. Lewandowski 1976. Bivalves as a factor affecting circulation of matter in Lake Mikolajskie. Limnologica. 10(2): 347-352.

Stoermer, E.F., T.B. Ladewski and L. Frey 1982. Effects of Phosphorus Loading on Phytoplankton Distribution and Certain Aspects of Cytology in Saginaw Bay, Lake Huron. USEPA , Office of Res. and Devel., Duluth MN.

Stokes, P.M. 1984. pH-related changes in attached algal communities of softwater lakes. In Hendrey, G.R.(ed.) Early biotic responses to advancing lake acidification. Butterworth Publishers. Boston. pp.43-60.

Taft, C.E. and C.W. Taft 1971. The Algae of Western Lake Erie. Bulletin of Ohio Biological Survey. 4(1). College of Biological Sciences, Ohio State University. Columbus, OH.

Ten Winkel, E. H. and C. Davids 1982. Food selection by *Dreissena polymorpha* Pallas (Mollusca: Bivalvia). Freshw. Biol. 12: 553-558.

Vollenweider, R.A., M. Munawar and P. Stadelmann, 1974. A comparative review of phytoplankton and primary production in the Laurentian Great Lakes. J. Fish. Res. Board Can. 31:739-762.

Wetzel, R.G.(ed.) 1983. Periphyton of Freshwater Ecosystems. Dr W. Junk Publishers. Boston, MA.

Wiktor, J. 1963. Research on the ecology of *Dreissena polymorpha* (Pallas) in the Szczecin lagoon (Zawlew Szczecinski). Ecologia Polska-Seria A. 11: 275-280.

Zhadin, V. 1952. Mollusks of the fresh and brackish waters of the Soviet Union. English translation of article in Moskova Izdat. Zool. Inst. Akad. Nauk. USSR. In: Izd. Zoologicheskogo Instit. An SSSR Vol. 46.

Dreissena in Cooling Ponds of Power Plants in Ukraine: Structure-Functional Aspects

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Investigations of *Dreissena* in cooling ponds of Nuclear Power Plants (NPP) and Thermal Power Plants (TPP) are influenced by several unique features of these facilities. The peculiar thermal regime influences processes of *Dreissena* reproduction and metabolism. The presence of abundant solid substrate creates conditions for *Dreissena's* abundant development.

Power plant cooling ponds may serve several important purposes:

(1) The invasion of *Dreissena* into these newly-created artificial water bodies can serve as a model of zoogeographical processes in regions where it is a typical component of the hydrofauna;

(2) At the northern edge of *Dreissena* distribution cooling ponds can be refugia and sources of secondary invasion to nearby water bodies;

(3) In the cooling ponds the double role of *Dreissena* (in water purification and biofouling) is displayed very significantly.

The invasion of *Dreissena polymorpha* to new geographic regions must be considered in a broader sense than simply the appearance of a new species. Completely new periphytic and benthic communities have appeared in fresh waters with the appearance of *Dreissena* some centures ago in Europe and 6-8 years ago in North America. This community is similar to ecological morphotypes found in marine systems. So we must examine *Dreissena* in connection with the peculiarities of communities that it forms and dominates.

According to our research there are more than 100 species and forms of invertebrates in *Dreissena* communities. The structure-functional indices of *Dreissena* communities vary widely: from 1,600 to 1,230,000 ind./m^2 (number) with a *Dreissena* percentage from 1.5 to 99%; 0.3-20,000 g/m^2 (biomass) with *Dreissena* comprising 90-97% of the total biomass; 215.8-4900.0 KJ/m^2 (energetic equivalent of biomass); 2.1-29.7 KJ/$m^2 \cdot h$ (respiration).

Dreissena can create dense colonies. These colony types (brushes, druses, aggregates of druses) are defined by environmental conditions (Protasov, Afanasyev,

1984). The spatial structure, biomass and species diversity were taken as a basis for a *Dreissena* community classification system (Protasov, Afanasyev, 1990). Six types of communities were distinguished.

A juvenile type of community is characteristic for communities of temporary habitats where *Dreissena* is dominant and its biomass is about 10 g/m^2. The non-aggregated dominant community type develops from the juvenile type; molluscs have no ties between them, and the typical biomass is about 100 g/m^2. The type of community that forms in extreme conditions (e.g. temperature, flow, etc.) is horologicaly closed and *Dreissena* is only rarely present. The primary aggregated-dominant community type is based on such spatial structures as "brushes" and druses of mussels. This type is usually present in mean temperature zones of cooling ponds in lentic conditions. The observed biomass is usually about 1000 g/m^2. The secondary aggregated-dominant community type is composed of druse aggregates. This type is usually present in the intake canals of power plants, in which the biomass is several dozens of Kg per m^2. It should be distinguished as a subdominant type, where another species can also be subdominant (Spongia, Bryozoa). In every type a 10-fold increase of biomass takes place. Thus for reaching a biomass of about 100 Kg/m^2 it is necessary to form a new spatial structure.

In cooling ponds hydrodynamic peculiarities and thermal regimes create conditions for the formations of various *Dreissena* communities. Thus, in the cooling pond intake canal of the Chernobyl NPP the most stable was the secondary aggregated-dominant community type. Thirty-six species of invertebrates were found there with densities reaching 1.2 million ind./m^2, the biomass was about 16.7$\pm$3.5 Kg/m^2, species diversity was 2.4 bit/ind. and 0.03 bit/g. In *Dreissena polymorpha* communities of the primary aggregated-dominant community type -- which were located in the mean temperature zone (below 25°C) on solid substrata -- 28 species of invertebrates were found. The density was approximately 180,000 ind/m^2, biomass was 8.9$\pm$2.3 Kg/m^2, species diversity was 2.1 bit/ind. and 0.1 bit/g. In the zones which were situated near the discharge of heated waters some communities were observed with species typical of high temperature conditions: *Dreissena polymorpha* + *Pristina aequiseta*, *D. polymorpha* + *Aeolosoma hemprichi*. The biomass in these zones can reach the value of 1 Kg/m^2, but these communities are not stable and can be transformed into communities of extreme conditions with a biomass of several hundred g/m^2.

In cooling pond ecosystems the functional importance of *Dreissena* could be determined in different aspects: transformation of organic matter, accumulation of various elements (e.g. heavy metals), participation in partitioning of nutrients between filterers and the detritus food chain, alteration of spatial structure of habitat for other organisms, biohindrance in water treatment (Walz, 1973; Stanczykowska, 1977). The calculation of functional indices for *Dreissena* communities is grounded on the metabolic oxygen data for communities studied in the field using chambers designed by Protasov A.A. et al. (1987) and the relationship between the rate of *Dreissena*

oxygen consumption (R: mg O_2/h) and body weight (W: g fresh weight with shell) calculated by field and experimental data:

$R=0.035W^{0.79}$ (under 20 °C)

$R=0.096W^{0.84}$ (under 25-30 °C).

The dependence of *Dreissena* caloric composition (Q: cal.) upon mass (W: g fresh weight with shell) was approximated by the equation:

$Q=0.26W^{-0.27}$ (Sinitsina, 1989).

Our study showed that the highest level of energetic parameters were recorded for *Dreissena* communities of the secondary aggregated dominant type (2236.2-4926.0 KJ/m^2 (B), and 21.1-29.7 KJ/$m^2 \cdot h$ (R), which were located at water intake sections of cooling ponds. Perennial and seasonal dynamics have shown high stability of these indices. Under increasing water temperature and long-duration periods of high temperature a decrease of these parameters was observed. For example, in 1984-1985 in the cooling pond of the Southern Ukrainian NPP (during this period the pond was under a moderate thermal load, water temperature did not exceed 30 °C at the discharge site) *Dreissena* dominated practically all habitats. Respiration of *Dreissena* communities was rather high: 10.6-12.2 KJ/$m^2 \cdot h$ (1985). In the next year, when the water temperature rose to 37 °C, the elimination of *Dreissena* was observed up to a depth of 7 m and respiration decreased to 0.04 KJ/$m^2 \cdot h$ (energetic equivalent of biomass was 451.7 KJ/m^2).

At temperature ranges from 11 °C (spring and autumn) to 33 °C (summer) the intensity of metabolism of the *Dreissena* community varied from 0.0030 - 0.0094 h^{-1}. The increasing amount of energy dissipated via respiration per unit of energy in the community could be explained by different rates of these parameters under different temperature regimes. An increasing R/B ratio indicates an increased amount of energy needed to support the biomass of a community.

The role of *Dreissena* in the bioaccumulation of certain chemical elements is significant. At Kuchurgansk cooling pond of Moldova TPP the population of *Dreissena* contained 107 mg/g Mn, 130 mg/g Al, 32 mkg/g Ti, 22 mkg/g Ni, 0.7 mkg/g V (Toderash, Zubkova, 1989). Calcium is an essential chemical element for shell formation. In the summer season when significant production of *Dreissena* occurred they utilized more than ten tons of calcium in the cooling pond of the Krivoy Rog TPP.

Filtration activity of *Dreissena* was calculated from respiration parameters, assuming that 910 ml of water were filtered per 1 mg of consumed oxygen (Alimov, 1981). In the periphyton of cooling ponds of Chernobyl NPP (1) and Krivoy Rog

TPP (2) *Dreissena* filtered 817,000 - 1,410,000 m^3 and 561,000 - 2,260,000 m^3 of water per day respectively, an amount equal to approximately 1-6% of the total volume of the cooling ponds. In the benthos of these ponds rates of filtration by *Dreissena* were higher: 6,720,000 (1) and 4,350,000 - 6,340,000 (2) m^3 per day or from 7.2 to 11% of the total cooling pond volume. Assuming suspended matter concentrations of 15 mg/l, *Dreissena* were responsible for the sedimentation of an average of 19 tons/day (periphyton) and 80 tons/day (benthos) of suspended matter. This affected the water quality significantly.

The estimated flow of energy through *Dreissena* communities (for example, in cooling ponds of Chernobyl NPP) calculated with K2=0.2 (Alimov, 1987) was 12.2-81.4 $KJ/m^2 \cdot h$ at the zone of minimal heating effect. The largest portion of energy flow is involved in transformation by *Dreissena* communities in the zone of minimal heating at depths 1.5-3.5 m (86-98%). Average energy flow through *Dreissena* communities in cooling ponds of Chernobyl NPP, considering total flooded area, was 49.6×10^6 - 130.0×10^6 KJ/day.

Dreissena as a habitat creating factor are important for benthic and periphytic organisms. Spatially complex "settlements" of *Dreissena* create favorable microhabitats for small organisms. Thus, the abundance of Oligochaeta in Chernobyl NPP cooling ponds has increased from 17,000 to 39,000 ind./m^2. Significant correlations were observed between the biomass of *Dreissena* and the density of Oligochaeta.

Creation of various manmade structures can promote increases in *Dreissena* abundance and increase the potential destructive role of this organism in ecosystems.

Comparative analyses of periphyton on the concrete facing of a water body dam and a stone dam in the cooling ponds of the Chernobyl NPP and the Krivoy Rog TPP have shown that structure-functional indices rose 5-11 times in 1 m^2 of heterogeneous habitat. *Dreissena* on the stone dam filter about 1.3 m^3 of water per hour; on the concrete they filter about 0.3 m^3 per hour.

The presence of canals (intake and discharge) is a construction peculiar to cooling ponds. Lotic conditions are optimal for communities dominated by filtering organisms. Thus, in the cooling pond of Chernobyl NPP the total biomass of *Dreissena* has reached 750 tons.

Intensive development of *Dreissena* on sections of intake canals of power plant cooling ponds creates a precondition for the appearance of biological disturbances of water supply. Our investigations have shown that the main disturbances were connected with *Dreissena* located along the intake zones of cooling ponds.

Thus, the structure-functional approach for investigations of *Dreissena* communities will permit a better estimation of the diverse role of *Dreissena* in different ecosystems.

Alimov A.F. The functional ecology of freshwater Bivalves. Leningrad: Nauka publ. -1981.-248 p. (in Russian)

Alimov A.F. A study on the biotic balance of ecosystems of fresh water bodies in the USSR // Hydrobiol.journ. -1987. 23, N6.-p.3-9. (in Russian)

Protasov A.A., Afanasyev S.A. Spatial types of *Dreissena* settlements in the cooling pond of Chernobyl Nuclear Power Plant // Journ. of common biology. -1984, 45, N2. -p.272-277. (in Russian)

Protasov A.A., Sinitsina O.O., Kolomiets A.V. The method for exploring oxygen metabolism of periphytic communities on underwater substrata // Bull. of Inlandwater Institute (Borok). -1987, N76. -p.62-65. (in Russian)

Protasov A.A., Afanasyev S.A. Principal types of *Dreissena* communities in periphyton // Hydrobiol. journ. -1990. 26, N4. -p.15-22. (in Russian)

Sinitsina O.O. Peculiarities of production and decomposition characteristics of periphyton communities in cooling water bodies // Proceed. IX sci. conf. in Inlandwater Inst. (Borok), 1989. -p.6. (in Russian)

Stanczykowska A. Ecology of *Dreissena polymorpha* (Pall). (Bivalvia) in lakes // Pol. Arch. Hydrobiol. -1977. -24. -p.461-630

Toderash I.K., Zubkova E.I. The role of population of model species in migration of chemical elements in freshwater ecosystems // The species and its areal productivity. -Vilnius. -1989. -p.266-268. (in Russian)

Walz N. Studies on the biology of *Dreissena polymorpha* in Lake Constance // Arch. Hydrobiol. Suppl. -1973. -42. -p.452-482.

Sediment Toxicity and Bioaccumulation of Toxicants in the Zebra Mussel, *Dreissena polymorpha* at Times Beach, New York.

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ABSTRACT

The goal of this study, conducted at the Times Beach Confined Disposal Facility (CDF), was to determine if the zebra mussel can effectively bioaccumuate PAHs in a CDF. The concern being; is there the potential of zebra mussel induced PAH bioaccumulation along the food web? To answer this question two issues were addressed; 1) water and sediment quality evaluations at the CDF through both analytical techniques and toxicity tests and 2) an *in-situ* bioaccumulation studys to determine potential bioaccumulation of polycyclic aromatic hydrocarbons (PAHs). Analytical evaluation of the sediment indicated elevated concentrations of total PAHs, including a hot spot of 480 ppm. Biological toxicity of the sediment was evaluated by the 10 day *Daphnia magna* whole sediment toxicity test, resulting in significant impairment of both survival and reproduction. Chemical evaluation of the water samples showed no measurable concentrations of total PAHs at detection limits of 0.010 mg/L. These findings were supported by the 48-hour *Daphnia magna* survival elutriate toxicity test, where toxicity was apparent but not adequate to generate an LC50 in any sample. In the Fall of 1993 the 30-day *in-situ* study was conducted to determine zebra mussel bioaccumulation potential. The recollected tissues showed over a ten fold increase in total PAH concentrations compared to those collected at Day 0. The collected tissues from mussels placed both in the water column and the sediment showed that zebra mussels do bioaccumulate PAHs.

INTRODUCTION

Objectives

The spread of the zebra mussel, *Dreissena polymorpha,* in North America raises many potential environmental concerns. Among these, the potential for contaminant bioaccumulation demands study. Zebra mussels have a relatively high bioaccumulation potential, this aspect combined with their high filtration rates, could prove to be an environmental hazard, as zebra mussels build up large populations in contaminated areas (Fisher et al. 1993; Reeders and Bij de Vaate 1992). Assuming zebra mussels can accumulate and concentrate these contaminants, bioaccumulation and biomagnification of contaminants may occur through the food web. In addition if the mussels are in a location where they must be removed periodically, zebra mussel waste may be contaminated.

This study is part of a project being conducted by the U. S. Army Corps of Engineers Zebra Mussel Research Program. The Zebra Mussel Research Program deals with many aspects involving zebra mussels; however, this phase concerns contaminant issues. In particular contaminant bioaccumulation , resulting in environmental concerns through the food web or in mussel disposal. A combination of toxicity tests and chemical evaluations were performed to determine mussel placement and as a predictive measure to determine ecosystem stress (Swartz et al. 1990; Burton et al. 1987; ASTM 1991b). Zebra mussels were then used as bioindicators to determine total polycyclic aromatic hydrocarbon (PAH) bioaccumulation potential in a location of known contamination. If zebra mussels survive in the system, can they effectively accumulate contaminants.

The objectives of this study were (1) site characterization which was accomplished by a combination of toxicity tests and chemical evaluations and (2) determination of zebra mussel PAH bioaccumulation potential in a CDF.

Background - Site Location

The Times Beach Confined Disposal Facility (CDF) in Buffalo, New York was the chosen site location, as it is an area of documented contamination (Marquenie et al. 1990 and Stafford et al. 1991). The CDF lies close to the mouth of the Buffalo River and is separated by a dike from Lake Erie. The 186,000 m^2 site received dredged sediment from the Buffalo River. The dredged sediment consisted primarily of contaminants from industries located along the waterway. The industries present were an oil refinery, two steel plants, an aniline dye chemical plant and milling facilities. The dredging operations occurred form 1972-1976. During this time the CDF was colonized by plants and birds and provided a habitat for over 22 species of resident and migratory birds. On the request of the Buffalo

Ornithological Society, through the city of Buffalo, the site became a designated nature preserve and was left partially filled (Stafford et al. 1991). The elevation of dredged material decreased from around the disposal pipe towards the dike constructed around the site, resulting in upland, wetland and aquatic areas. In this study we dealt only with the aquatic area. this was an ideal location as we were provided with an area of known contamination, in which we could place zebra mussels for biomonitoring. Environmental concerns at this facility include the potential of contaminant leakage into the water column, acute and chronic toxicity to organisms inhabiting the site and the bioaccumulation of contaminants from the sediment through the food web.

METHODS

SITE CHARACTERIZATION

Sediment Collection and Storage

In June 1993 the Times Beach CDF was partitioned into 16 sites. At that time water and sediment for analysis were collected at each of the 16 sites. Site determination for the toxicity tests was done by grouping four sites into a plot making four plots. Water for use in the sediment toxicity tests was collected in the middle of each plot. Sediment was collected at each site. This sediment was later made into plot sediment composites for use in the toxicity tests.

Nearby Grand Island was the reference site for the sediment toxicity tests. This site was picked in accordance with the requirements set by the USEPA/USACE (1990). The reference point approach was used, as Grand Island sediment was collected from a single reference sediment sampling point. The justification for using Grand Island as a reference area is that sediment has a similar grain size and is environmentally similar to the Times Beach site without the addition of dredged material and has been found to be relatively uncontaminated (Stafford et al 1991).

All water for toxicity tests was collected in 20-liter plastic containers, on ice, and sent overnight to the laboratory. Upon arrival the containers were stored 4 ^{0}C until the toxicity tests were performed. Sediment was collected with an Ekman grab sampler at all sites. This sampler is a precise sampler which is efficient in less compacted, fine grained sediments (Downing 1984), such as the dredged material observed at Times Beach. Immediately after collection, sediment for toxicity testing was placed in labeled doubled plastic 3.8 L ziploc bags. The sediment was utilized within 6 weeks of sediment collection.

Culturing of Test Organisms

All sediment tests were performed with the cladoceran, *Daphnia magna*, which were cultured in aged tap water. The *Daphnia* culture were fed every other

day with a 6 mg/L tri-algal mixture of *Ankistrodesmus falcatus, Chlamydomonas reinhardi,* and *Chlorella vulgaris.* The tri-algal mixture was kept refrigerated and thawed to room temperature before feeding. On between-feeding days the culture media was stirred. Individuals were placed in fresh media twice a week. The *Daphnia* were maintained at 25 +/- 1 ^{0} C, with photoperiod as 24 hours of standard florescent lights (USEPA 1991). Three weeks prior to the start of a test, individual cultures were started with young from adults less than one week of age, having a 3rd or higher brood (Biesinger et al 1987). Each individual was placed in 150 ml of culture water in a 250 ml beaker. Young picked for the testing had to come from the above organisms. In addition, young used for testing had to meet standards set forth by (ASTM 1991a). For all transfers in both culturing and testing the organisms were transferred by a fire polished pipet of 6 mm bore.

Elutriate Toxicity Tests

The sediment and water samples from each plot were stored at 4^{0}C after the June 1993 collection, until the sediment toxicity tests began in July 1993. The elutriate was prepared with a 1: 4 ratio of sediment to site water as described in the (USEPA /USACE 1990; Plumb 1981). The elutriate tests were performed in the controlled environment of a culture room where the temperature stayed at 20 +/- 1 ^{0}C as recommended (ASTM 1991a; USEPA 1991; Biesinger et al. 1987). The photoperiod for the elutriate tests was 24 hours of light. This followed the recommendation that *D. magna* receive a minimum of 16 hours of light each day (USEPA 1991). At each station 100%, 50%, 10% elutriate concentrations, and a 0% concentration of site water taken at each individual station, were tested. There was also an additional control consisting of the *Daphnia* culture water. Five replicates for each concentration, 10 organisms per replicate, comprising a total of 50 organisms exposed to each concentration. The tests lasted 48 hours and organisms were checked at times 0, 4, 24 and 48 hrs. Temperature, pH and dissolved oxygen were measured at times 0, 24 and 48 hrs while conductivity, alkalinity and hardness readings were collected at 0 and 48 hrs.

Whole Sediment Toxicity Tests

The whole sediment toxicity tests were performed using the guidelines set by Nebeker et al. (1984) for the *D. magna* life cycle test. The test consisted of exposing 5-day old *Daphnia* for 10 days, through maturation and the release of three broods. The tests were conducted in 2-liter beakers, each containing 200 ml of sediment and 800 ml of site water. The sediment level was determined by volumetric displacement. There were five replicates performed for each sample. Each replicate contained 20 organisms for a total of 100 organisms exposed per sample. Organisms were fed every other day 6 mg/L of the tri-algal mixture used in

culturing. The beakers were kept lightly aerated by a glass tipped airline placed approximately 4 cm below the water surface. Each day temperature and DO readings were recorded. On day 0 and day 10 temperature, DO, pH, conductivity, alkalinity and hardness were recorded. At the end of the tests the organisms were counted. Water and fine suspended solids were poured through a 0.5 mm mesh screen, rinsed and placed in clean water to enable counting.

Contaminant Concentration of PAHs

The water and sediment samples were analyzed for PAHs at the Environmental Chemistry Branch (ECB) at the Waterways Experiment Station (WES). Water samples were analyzed for PAHs by the USEPA method 610 (GC-flame ionization detector (FID)). Sediments were analyzed by USEPA Method 8100 (GC-FID).

BIOACCUMULATION POTENTIAL

In-situ Study

The aquatic area of the Times Beach facility was divided into 16 grids forming 16 sites. Upon determination the individual sites were marked with a float. A 1.3-cm wide nylon rope was used to tie the float to 15-cm concrete block for anchorage. The rope length was the depth of the water plus an additional 5 cm to allow for water fluctuations.

In October 1993 mussels used in the bioaccumulation study were collected from the nearby Black Rock Lock Channel in Buffalo, NY. The average size mussels was ~ 1.6-cm. The mussels were collected by using a scraping device to scrap clumps of mussels from the walls of the Black Rock Lock. This device consisted of a long metal pole ~ 3-m which was attached to a rectangular metal box. The box had a screen on the bottom to collect the mussels and allow water to drain. The top of the box was used as a scraper. Immediately after collection, mussels were placed between layers of wet paper towels and placed in a 170-L cooler. The holding apparatus for the mussels were plastic cages with vents allowing water flow. The cages were filled halfway with ~ 1300 gms of mussels. The packaged mussel cages were placed in the Black Rock Lock water until the 15-min transport by van to the Times Beach CDF.

At the selected site, the hot spot TB10, enough mussels for chemical analysis were placed. Chemical evaluations were performed on sediment mussels (Lower) and water column (Upper) mussels . Additional samples were also randomly placed for QA/QC analyses. Sediment level (Lower) mussel cages were placed on a cord that was tied from the float to the sediment with enough extra cord to allow for water level fluctuations. Water column (Upper) mussel cages were tied

to the cord ~ 45-cm below the float.

On October 6, 1993, day zero of the bioaccumulation study, four groups of randomly selected mussels were placed in iced coolers and sent overnight to the WES. There, the mussels were kept in the freezer until they were prepared for analytical analysis. After ~ 30 days in the field, all mussels placed in the field were recollected for analysis. The procedures for shipping the recollected mussels to WES were the same as the above for the base line mussel analysis.

Contaminant Concentrations of PAHs

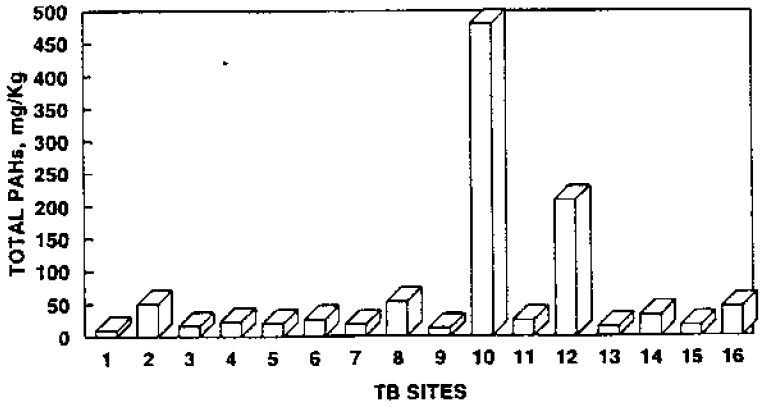
Tissues were manually removed from the mussel shells, homogenized , and sent to ECB for analysis. At the laboratory they were analyzed for PAHs by USEPA Method 8100 (GC-FID).

Table 1: Results of 10-Day Whole Sediment *Daphnia magna* Toxicity Test. (Range in Parentheses)

Site	% Survival	Mean Reproduction
Reference	**82 (80-90)**	**82.8 (73-113)**
TBP-1	**6 (0-15)***	**3.4 (0-10)***
TBP-2	**7 (0-15)***	**9.0 (0-19)***
TBP-3	**1 (0-5)***	**2.8 (0-9)***
TBP-4	**7 (0-20)***	**1.6 (0-4)***

***indicates significant difference from the control**

Figure 1: Total PAHs (mg/Kg) Found in Sediment, Times Beach, Buffalo, NY.



RESULTS

SITE CHARACTERIZATION

The toxicity tests showed no substantial mortality for *D. magna* exposed to all elutriate concentrations, no LC50 determinations could be calculated due to the minimal mortality. The 10-day whole sediment toxicity tests, however, showed significant impairment in survival and reproduction. Percent survival from the reference station (Grand Island) was high (82%) but was reduced (1-7%) at Plots P1 to P4. Reproductive impairment followed the same overt trend as survivorship. The mean reproduction at the reference site was high (82.8 neonates), with a significant reduction at plots P1 through P4 (1.6 to 9.0 neonates). (Table 1)

Water samples at all sites had total PAH concentrations below the detection limit of 0.010 mg/L. In the sediment, however, PAH concentrations were found above the detection limits. (Figure 1). Total PAHs ranged from 9.1 mg/Kg to the hot spot at site TB10 which had a total PAH concentration of 480 mg/Kg. This was the site used for mussel placement in the bioaccumulation study.

Table 2: Concentrations of Selected PAHs in Samples From Site TB10 at Times Beach, Buffalo, NY.

PAH	Water mg/Kg	Upper Sediment mg/Kg	Lower Tissue mg/Kg	Tissue mg/Kg
Flouranthene	**<0.010**	**82**	**2.49**	**1.84**
Pyrene	**<0.010**	**57**	**2.29**	**1.59**
Chrysene	**<0.010**	**31**	**1.33**	**1.29**
Benzo(a)Anthracene	**<0.010**	**28**	**1.06**	**.92**
Phenanthrene	**<0.010**	**130**	**1.27**	**<0.66**

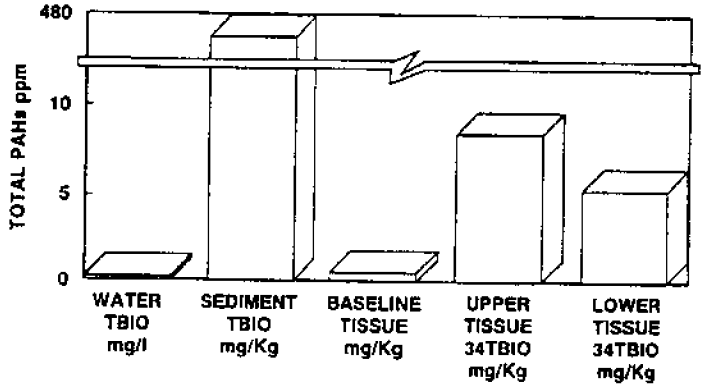
BIOACCUMULATION POTENTIAL

The mussels survived 30 days in the confined disposal facility and some PAHs accumulated in the mussel tissue over that time period. The following PAHs were analyzed for; naphthalene, acenapthene, phenanthrene, acenaphthylene, flouene, anthracene, flouranthene, chrysene, benzo (b) flouranthene, pyrene, benzo (a) anhracene, benzo (k) flouranthene, benzo (a) pyrene, dibenzo (a,h) anthracene, 2-methylnapthalene, indeno (1,2,3-c,d) pyrene and benzo (g,h,i) perylene. The PAHs

found in both the sediment and mussel tissue were phenanthrene, flouranthene, pyrene , chrysene and benzo (a) anthracene. (Table 2). PAHs found in the sediment but not in mussel tissues were flouranthene, anthracene and acenaphlene. They were present in the sediment at 55, 54 and 43 mg/Kg, respectively. The were no cases were a PAH was present in the tissues but not in the sediment. The remaining PAHs were all found to be below detection limits both in the sediment and tissue, including benzo (a) pyrene, which has been found to accumulate well in aquatic animal tissues (Neff, 1979).

Accumulation of total PAHs were significant in the tissues , the water column placed mussels (Upper) had a tissue concentration of 8.44 mg/Kg and the (Lower) sediment placed mussels accumulated 5.63 mg/Kg. This was compared to the baseline tissue analysis of <0.50 mg/Kg. (Figure 2)

Figure 2: Comparison of Total PAHs in Different Samples From Times Beach, Buffalo, NY.



DISCUSSION

SITE CHARACTERIZATION

The bioavailability of contaminants in the Times Beach CDF may be of environmental concern. The combination of the elutriate and solid phase tests (Nebeker et al. 1984) indicate that, while the sediment is toxic it does not seem to be releasing dissolved materials into the overlying water column. The elutriate (water phase) test had no significant toxicity, while the whole sediment test showed significant survival and reproductive impairment. The predictive toxicity tests were confirmed by analysis of the sediment and water for PAH concentrations. The water PAH concentrations were below detection limits and elevated PAH concentrations were seen in the sediment. The combination of both toxicity tests and chemical analysis indicated that contaminants are present in the system and bioavailable to a greater degree in the sediment than in the water column. This corresponds to previous site characterization studies where PAH concentrations were generally

found to be greater in the sediment by a factor of 1000 or more than in the water column (Neff 1979) .

BIOACCUMULATION POTENTIAL

Several PAHs accumulated in the mussel tissue. The PAHs may have been taken up by filtration of the water column, or the accumulation might have been from the sediment as seen with the mussel *Mytilus* (Dame and Dankers 1988; Pruell et al. 1986; Doherty 1990). It may be a collective accumulation from both the water and the sediment. The possibility of uptake from food (Clark and Finley 1975) should also be considered.

The zebra mussels did not accumulate levels of PAH sufficient to create an environmental hazard if disposed (Tatem 1994). However significant accumulation has occurred. (Figure 2) Thereby raising the question of potential bioaccumulation effects in the upper trophic levels of the food web. This could occur when the mussels are ingested by fish or waterfowl present in the system, or by the recycling of mussel pseudofaeces. Support for this theory is the work by Reeders and Bij de Vaate (1992) who found that once the zebra mussel accumulates PAHs, its pseudofaeces become contaminated to a higher degree than the suspended matter. This event makes the contaminant more available for transfer through the food web, although PAHs generally are not biomagnified through the food web (Biddinger and Gloss 1984), the potential pathway for a zebra mussel induced PAH bioaccumulation is of concern.

LITERATURE CITED

ASTM. 1991a. Standard guide for conducting renewal life-cycle toxicity tests with *Daphnia magna*. E 1193-87. Annual Book of ASTM Standards, Vol. 11.04. Amer. Soc. for Testing and Materials, Philadelphia, PA.

ASTM. 1991b. Standard guide for collection,storage , characterization and manipulation of sediments for toxicological testing; E 1391-90. Annual Book of ASTM Standards, Vol. 11.04. Amer. Soc. for Testing and Materials: Philadelphia, PA.

Biddinger G.R. and S.P. Gloss. 1984. The importance of trophic transfer in the bioaccumulation of chemical contaminants in aquatic systems. Residue Rev. 91:103-146.

Biesinger, K.E., L.R. Williams and W.H. Van der Schalie. 1987. Procedures for conducting *Daphnia magna* bioassay. EPA/600/8-87/011. Environmental Monitoring Systems Laboratory, Office of research and Development, U.S.

Environmental Protection Agency, Las Vegas, NV

Burton, G. A. Jr.,J.M. Lazorchak, W.T. Waller and G.R. Lanza. 1987. Arsenic toxicity changes in the presence of sediment. Bull Environ. Contam. Toxicol. 38:491-99

Clark, R.C. and J.S. Finely. 1975. Uptake and loss of petroleum hydrocarbons by the mussel, *Mytilus edulis* and *Macoma balactica* (L.). Ophelia 25:49-57.

Dame, R.F. and N. Dankers. 1988. Uptake and release of materials by a Wadden Sea mussel bed. J. Exp. Mar. Biol. Ecol. 108:207-216.

Doherty, F.G. 1990. The asiatic clam , *Corbicula* sp.. as a biological indicator in freshwater environments. Environ. Monitor. Assess. 15:398-401.

Downing, J.A. 1984. Sampling of benthos of standing waters. In J.A. Downingnad F.H. Rigler, eds., A Manual on the Methods for the Assessment of Secondary Productivity in Fresh Waters, 2nd ed. IBP Handbook 12. Blackwell Scientific Publications, Boston, Ma, pp. 87-130.

Fisher S.W., D.C. Gossiaux, K.A. Bruner and P.F. Landrum. 1993. Investigation of the toxokinetics of hydrophobic contaminants in the zebra mussel. Pgs. 465-490 in T.M. Nalepa and D.M. Schloesseer, editors. Zebra mussels biology, impacts and control. Lewis Publishers, Ann Arbor, MI, USA.

Marquenie, Johannes, J.W. Simmers, R.G. Rhett and D.L. Brandon. 1990. Distributions of PCB and pesticide contaminants in the vicinity of the Times Beach confined disposal facility , Buffalo, New York. Department of the Army, U.S. Army Corp of Engineers. Miscellaneous Paper EL-90-24.

Nebeker A.V., M. A. Cairns, J.H. Gakstatter, K.W. Malueg, G. S. Schytema and D. F. Krawczyk. 1984. Biological methods for determining toxicity of contaminated freshwater sediment to invertebrates. Environ. Toxicol. and Chem. 3:617-630.

Neff J.M., 1979. Polycyclic aromatic hydrocarbons in the aquatic environment. Sources, fates and biological effects. London: Applied Science.

Plumb, Russel H.Jr., Procedures for handling and chemical analysis of sediment and water samples. 1981. Technical Report EPA/CE-81-1

Pruell, R.J., J.L. Lake, W.R. Davis, and J.G. Quin. 1986. Uptake and depuration of organic contaminants by blue mussels (*Mytilus edulis*) exposed to environmentally

contaminated sediment. Mar. Biol. 91:497-507.

Reeders H. H., and A. Bij de Vaate. 1992. Bioprocessing of polluted suspended matter from the water column by the zebra mussel (*Dreissena polymorpha* Pallas). Hydrobiologia 239:53-63.

Stafford, Elizabeth A., Simmers J.W., R.G. Rhett and C.P. Brown. 1991. Interim Report: Collation and Interpretation of DATA for Times Beach Confined disposal facility. Department of the Army. US Corps of Engineers. Miscellaneous Paper D-91-17.

Swartz, R. C., D.W. Schults, T.H. DeWitt, G.R. Ditsworth, J.O. Lamberson, 1990. Toxicity of flouranthene in sediment to marine amphipods: a test of the equilibrium partitioning approach to sediment quality criteria. Environ. Toxicol. Chem. 9:1071-80.

Tatem, H.E., 1994. Contaminants and the zebra mussel *Dreissena polymorpha:* review of recent data in relation to disposal practices. Abstract : 4th International Zebra Mussel Conference. Madison WI.

USEPA/ USACE. 1990. Draft ecological evaluation of proposed discharge of dredged material into ocean waters. Office of Marine and Estuarine Protection. U.S. Environmental Protection Agency : Washington, DC, EPA-503-8-90/002.

USEPA. 1991. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. Office of Research and Development Washington DC, EPA 600 4-90 027.

Measures of Allometric Growth: The Relationship of Shell Length, Shell Height, and Volume to Ash-Free Dry Weight in the Zebra Mussel, *Dreissena polymorpha* Pallas and the Quagga Mussel, *Dreissena bugensis* Andrusov

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Abstract

The relationships of shell length, shell height, and volume to ash-free dry weight (AFDW) were each evaluated independently for both *Dreissena polymorpha* Pallas and *D. bugensis* Andrusov. Measurements were made on adult mussels collected from three Great Lakes sites. Shell length and shell height were each good predictors of AFDW for both *D. polymorpha* (length, r^2=0.971, N=99; height, r^2=0.963, N=99) and *D. bugensis* (length, r^2=0.962, N=61; height, r^2=0.970, N=61). Volume measurements did not provide as good an estimate of AFDW as did shell length and shell height. However, more of the variation in volume was explained by the relationship with AFDW for *D. bugensis* (r^2=0.784, N=54) than for *D. polymorpha* (r^2=0.470, N=42). Volume does not appear to be as accurate a predictor of AFDW as shell length or shell height, but may be a useful field measure for biomass estimates of *D. bugensis*. Other studies of both marine and freshwater bivalves have demonstrated that seasonal and population variations can influence measures commonly used to estimate biomass. Investigators must first establish the relationship of shell length or shell height to AFDW in their study before using either dimension to estimate biomass in a population.

INTRODUCTION

The introduction of the zebra mussel, *Dreissena polymorpha* Pallas, and the quagga mussel, *Dreissena bugensis* Andrusov, into the Great Lakes has prompted numerous scientific investigations, many of which have involved monitoring the growth of the mussels (e.g. Dermott *et al.*, 1993; Mackie, 1993). The measure of growth often used in many of these studies has been shell length. Shell length as well as shell height are commonly used estimators of growth in both freshwater (e.g. Bailey and Green, 1988; Garton and Haag, 1991; Smit *et al.*, 1992; Sprung, 1992) and marine bivalves (e.g. Incze *et al.*, 1980; Rodhouse *et al.*, 1984; Harvey and Vincent, 1990; Fritz, 1991). Both measures can be useful to the investigator in that they provide a nondestructive means of monitoring growth in bivalve populations. However, it has been demonstrated that shell length in bivalves may not always be an accurate reflection of the biomass of an organism (Seed,1968; Winberg and Duncan, 1971; Hilbish, 1986; Seed and Richardson, 1990; Jantz and Neumann, 1992). Various factors such as reproductive state of the animal (Hibbert, 1977; Griffiths and King, 1979; Harvey and Vincent, 1990; Smit and VanHeel, 1992), population density (Seed, 1968; Hunter and Bailey, 1992) and habitat (Brown *et al.*, 1976; Rodhouse *et al.*, 1984; Jantz and Neumann, 1992) have been demonstrated to independently influence the rate of increase in tissue weights and in shell dimensions (length, height, width). Measurements of volume, as determined by a number of different methods have also been used to estimate biomass in bivalves (eg. Seed, 1968; Fritz, 1991; Smit and Van Heel, 1992).

Previous studies of *D. polymorpha* have examined the relationships between shell length and dry tissue weight (Stanczykowska, 1977; Walz, 1978; Haag and Garton, 1992), shell length and ash-free dry weight (Bij de Vaate *et al.*, 1992; Smit and Van Heel, 1992), and volume displacement and ash-free dry weight (Smit and Van Heel, 1992). However, no other study has determined these relationships for the quagga mussel, *D. bugensis*.

In this study, shell lengths, shell heights, volume displacement and ash-free dry weights were measured for adults of *D. polymorpha* and *D. bugensis* collected from three Great Lakes sites. The relationships of shell length, shell height, and volume displacement to ash-free dry weight were individually evaluated for each species in order to assess the appropriateness of each parameter as an estimator of growth for zebra and quagga mussels.

METHODS

Adult *Dreissena polymorpha* were collected from three Great Lakes locations, Lake Erie sites at Sterling State Park, Monroe, MI, USA (August 1992 and June 1993) and Presque Isle, PA, USA (June 1993), and a Lake Michigan site at Diversey Harbor, Chicago, IL, USA (October 1992). *Dreissena bugensis* were also collected from the Lake Erie site at Presque Isle. All mussels were maintained in the laboratory at 10°C with a 12D:12L photoperiod in aerated 2.5 gallon aquaria with 0.45 µm filtered lake water. Lake water was obtained from Lake Evergreen, Bloomington, IL or Lake Michigan at the Lake Michigan Biological Station, Zion, IL. Mussels were fed every 1-2 days a mixed diet of dried *Chlorella vulgaris* (Nature's Herbs Better *Chlorella*) resuspended in 0.45 µm filtered lake water and live *Chlorella vulgaris* (UTEX- The Culture Collection of Algae at the University

of Texas at Austin). Ammonia levels in the aquaria were routinely monitored. The aquaria were cleaned weekly and the lake water was replaced.
The shell lengths and shell heights of 99 *D. polymorpha* and 61 *D. bugensis* were measured to the nearest 0.01 mm using a dial caliper. Shell length was recorded as the maximum dimension along the anterior (at the umbo) - posterior axis of the shell. The shell height was measured as the maximum dimension along the dorsal - ventral axis (see Fig. 1). Measurements of shell length and shell height were made on the same valve for a given mussel. Volume displacement was documented for 42 of the 99 *D. polymorpha* and 54 of the 61 *D. bugensis*. Volume measurements were made to the nearest 0.1 ml by recording the displacement caused by placing individual mussels into a graduate cylinder containing lake water (Smit and Van Heel, 1992). Ash-free dry weights were determined for individual mussels by first drying them for 48 hours to a constant weight at 65°C in a drying oven and then combusting them for 12 hours at 500°C. The dry weights and ash weights of individual mussels were measured to the nearest 0.01 mg using a Mettler microbalance. The ash-free dry weight (AFDW) was then calculated by subtracting the ash content from the dry weight. Some mussels were preserved in 10% buffered formalin and then measured at a later date. Preserved mussels were rinsed first in distilled water before drying.

All variables were logarithmically transformed. The relationships of shell length, shell height and volume displacement to ash-free dry weight were each independently evaluated using regression analysis for *D. polymorpha* and *D. bugensis* separately. The analysis of variance procedure was completed to test whether the slope of the regression lines were significantly different from zero (Zar, 1984). Statistical analyses were completed using the Statistical Package for Social Sciences-PC version (Norusis, 1990).

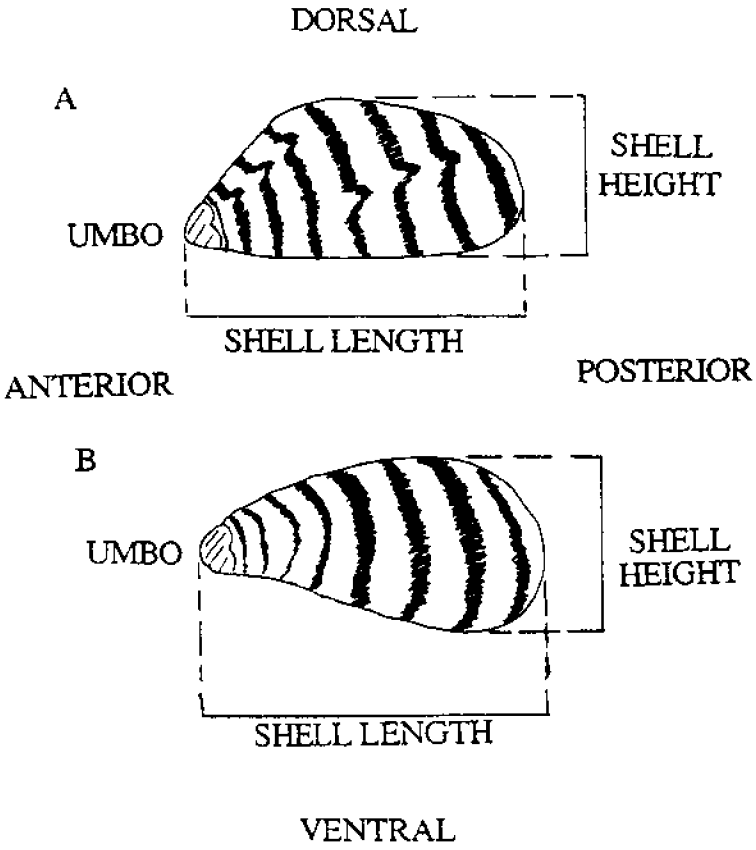

Figure 1: Shell length was measured as the maximum dimension between the umbo and the posterior end of the shell. Shell height was measured as the maximum dimension between the dorsal and ventral sides of the shell. (A) D. polymorpha, (B) D. bugensis

RESULTS

Shell length (Fig. 2) and shell height (Fig. 3) were each strongly correlated with ash-free dry weight (AFDW) for both *D. polymorpha* (length, r^2=0.97, N=99; height, r^2=0.96, N=99) and *D. bugensis* (length, r^2=0.96, N=61; height, r^2=0.97, N=61). Shell lengths measured for *D. polymorpha* ranged in size from a maximum length of 26.08 mm to a minimum length of 1.75 mm ($\bar{X}$=11.70 mm, S.D.±7.36 mm, N=99). The shell lengths of *D. bugensis* ranged in size from the largest at 25.92 mm to the smallest of 2.24 mm ($\bar{X}$=14.34 mm, S.D.±6.49 mm, N=61).The largest shell height documented for *D. polymorpha* was 13.01 mm and the smallest height was 1.10 mm, ($\bar{X}$=5.86 mm, S.D.±3.61 mm, N=99). The maximum shell height of *D. bugensis* measured was 14.18 mm and the minimum height measured was 1.30 mm ($\bar{X}$=3.57 mm, S.D.±3.57 mm, N=61).

Volume displacement was not as good an predictor of AFDW for either species (Fig. 4). However, volume displacement was more strongly correlated with AFDW for *D. bugensis* (r^2=0.78, N=54) than for *D. bugensis* (r^2=0.47, N=42).

The slopes of the lines of *D. polymorpha* and *D. bugensis* appear to be similar for for the relationships of AFDW to shell length (Fig. 2) and of AFDW to shell height (Fig. 3). A small sample t-test will be conducted to determine if the slopes of the lines for the two species are significantly different for the relationships of AFDW to shell length (Fig. 2), for the relationships of AFDW to shell height (Fig. 3), and for the relationships of AFDW to volume displacement (Fig. 4).

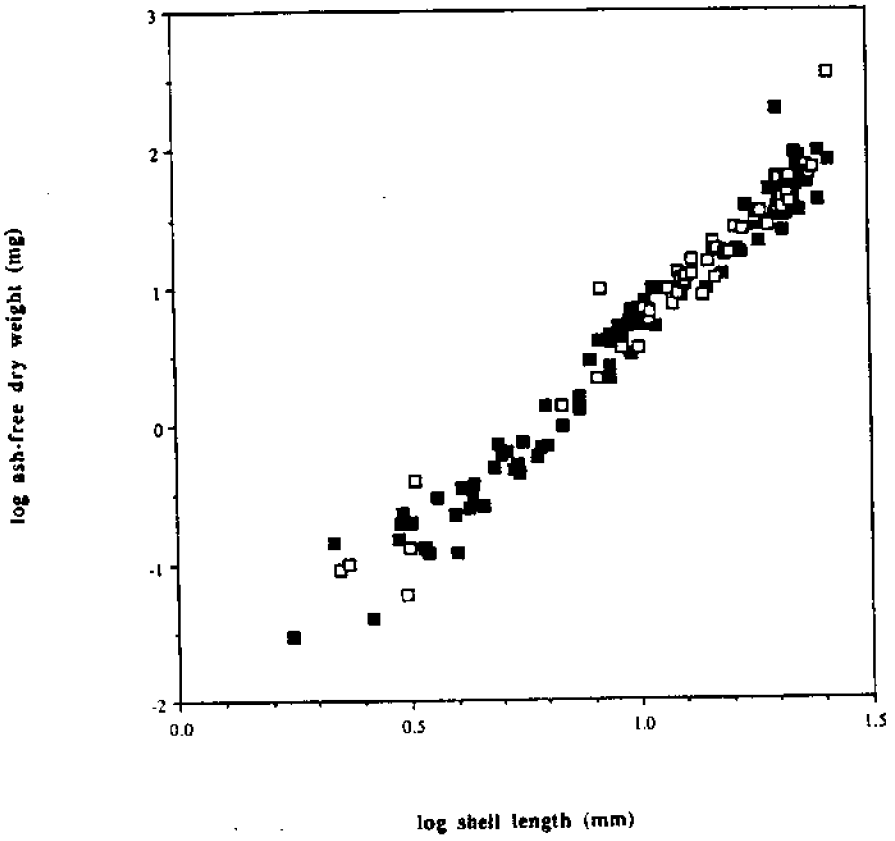

Figure 2: Shell length is highly correlated with ash-free dry weight in both D. polymorpha ((■) logy=3.08logx-2.39, r^2=0.97, N=99, F=3234.98, p<0.0005) and D. bugensis ((❑) logy=3.00logx-2.26, r^2=0.96, N=61, F=1500.28, p<0.0005).

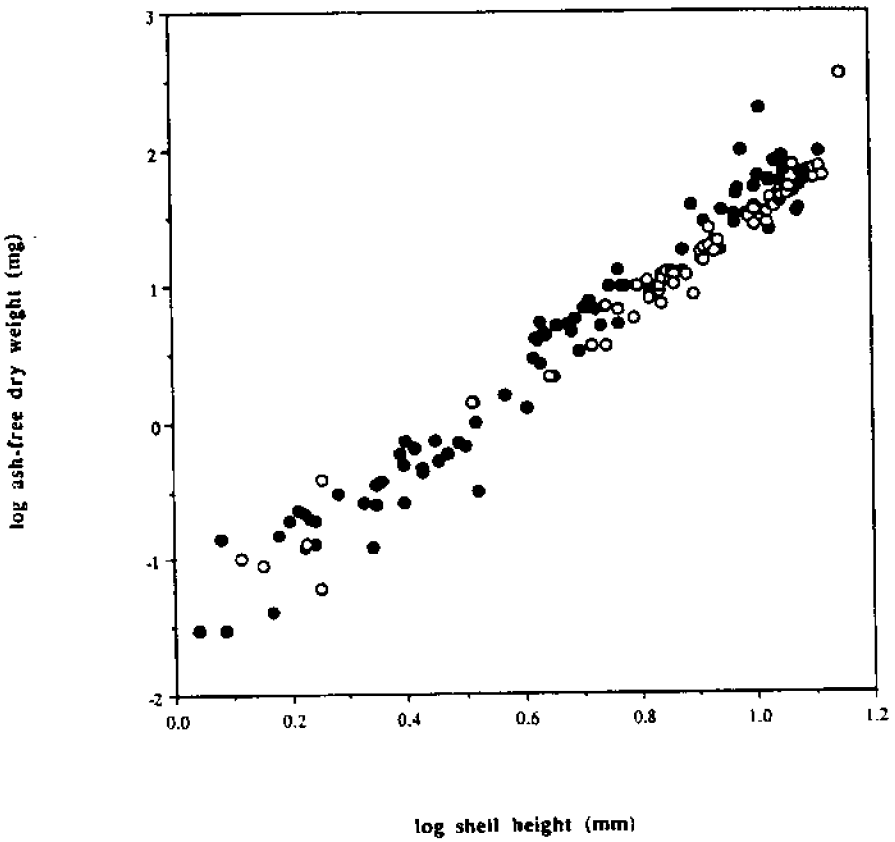

Figure 3: A strong relationship was found between shell height and ash-free dry weight for both D. polymorpha ((●) logy=3.21logx-1.57, r^2=0.96, N=99, F=2494.81, p<0.005) and D. bugensis ((○) logy=3.05logx-1.54, r^2=0.97, N=61, F=1875.4, p<0.005).

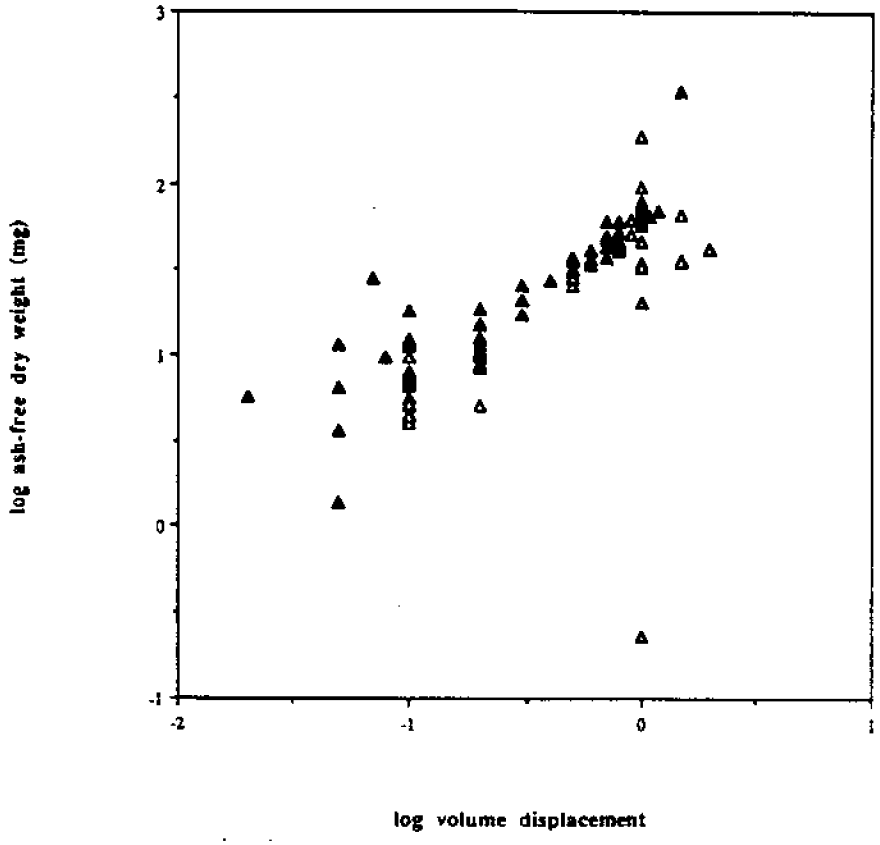

Figure 4: Volume displacement is more strongly related to ash-free dry weight in D. bugensis ((Δ) logy=0.81logx+1.78, r^2=0.78, N=54, F=188.48, p<0.005) than D. polymorpha ((▲) logy=0.80logx+1.59, r^2=0.47, N=42, F=35.45, p<0.005).

DISCUSSION

Shell length, wet weight, dry weight and volume displacement represent some of the measurements that have been commonly used to assess growth, or increases in biomass, in zebra mussels (Kornobis, 1977; Walz, 1978; Haag and Garton, 1992; Smit *et al.*, 1992; Smit and Van Heel, 1992). These measures estimate the organic content, or the biomass of the mussels. A more accurate method to determine organic content is to measure the ash-free dry weight (AFDW) (Paine, 1964). However, it is not always feasible to obtain AFDWS. AFDW measurements require the destruction of the animal which is not a suitable methodology for monitoring the long term growth of a group of individuals. In these studies, a nondestructive measure of growth, such as shell length, shell height or volume, is preferred.

Shell length and shell height were each good predictors of ash-free dry weight for both *D. polymorpha* and *D. bugensis* in our study. Other studies with *D. polymorpha* have also demonstrated a strong relationship between shell length and AFDW (Bij de Vaate *et al.*, 1992; Smit and VanHeel, 1992; Dorgello and Krack, 1993). Moreover, the relationship of shell length to AFDW has been documented for numerous marine bivalves, including *Mytilus edulis* (L.) (eg. Dare and Edwards, 1975; Rodhouse *et al.*, 1984), *Macoma baltica* (eg. Harvey and Vincent, 1990), and *Mercenaria mercenaria* (eg. Hibbert, 1977). Our data support the results of field studies which only measured shell length to estimate growth in zebra mussels (e.g. Smit *et al.*, 1992; Sprung, 1992).

Volume measurements did not provide as good an estimate of biomass as did shell length and shell height in our study. However, more of the variation in volume was explained by the relationship with weight for *D. bugensis* than for *D. polymorpha*. Volume was not as accurate a predictor of weight as shell length and shell height in our study, but may prove to be a useful field measure for *D. bugensis*. The relationship of volume to AFDW may be improved with a more accurate technique to measure volume. Volume displacement is difficult to measure for small mussels due to the little measurable change in displacement. We were interested in using a measure that was nondestructive and that could be easily completed in the field. Measures of volume displacement of small mussels may be improved by pooling similar sized mussels and measuring their total displacement as a group.

Shell length and shell height have been demonstrated to be important tools in estimating the growth of many freshwater and marine bivalves (e.g. Rodhouse *et al.*, 1984; Bailey and Green, 1988; Harvey and Vincent, 1990; Garton and Haag, 1991; Smit *et al.*, 1992; Sprung, 1992). However, these nondestructive measures (ie. shell length and shell height) may not always be good predictors of biomass. Other studies have demonstrated that environmental parameters, population density and reproductive state can influence measures used to estimate growth (Seed,1968; Brown *et al.*, 1976; Hibbert, 1977; Griffiths and King, 1979; Rodhouse *et al.*, 1984; Hilbish, 1986; Harvey and Vincent, 1990; Jantz and Neumann, 1992; Smit and VanHeel, 1992). In some cases, more energy may be go towards tissue growth than shell growth. Due to the potential influence of local environmental or population conditions, investigators must first establish the relationship of shell length or shell height to tissue weight in their study before using either dimension to estimate growth in a population.

ACKNOWLEDGEMENT

The authors wish to thank J.E. Marsden and her staff at the Lake Michigan Biological Station of the IL Natural History Survey for collecting *Dreissena polymorpha* from the Lake Michigan site at Diversey Harbor. We also wish to express our appreciation to S.J. Nichols of the U.S. Fish and Wildlife Lab at Ann Arbor, MI who provided *D. polymorpha* and *D. bugensis* from the Lake Erie site at Presque Isle. We are grateful for the fruitful discussions of bivalve morphometrics with S.C. Fuller. This study was completed by Tamara K. Ross in partial fulfillment of the requirements for Senior Honors at Illinois Wesleyan University. The research was supported in part by grant (ZM/3) to Gail M. Lima from the Illinois-Indiana Sea Grant College Program of the National Oceanic and Atmospheric Administration.

LITERATURE CITED

Bailey, R. C. and R. H. Green, 1988. Within-basin variation in the shell morphology and growth rate of a freshwater mussel. *Can. J. Zool.* 66:1704-1708.

Bij de Vaate, A., M. Greijdanus-Klaas and H. Smit, 1992. Densities and biomass of zebra mussels in the Dutch part of the Lower Rhine. In: *The Zebra Mussel Dreissena polymorpha*. (eds. D. Neumann and H.A. Jenner), *Limnologie aktuell*, Gustav Fischer Verlag, Stuttgart, Germany, 4:79-86.

Brown, R.A., R. Seed and R.J.O'Connor, 1976. A comparison of relative growth in *Cerastoderma (=Cardium) edule, Modiolus modiolus,* and *Mytilus edulis* (Mollusca: Bivalvia). *J. Zool. Lond.* 179:297-315.

Dare, P.J. and D.B. Edwards, 1975. Seasonal changes in flesh weight and biochemical composition of mussels (*Mytilus edulis* L.) in the Conwy Estuary, North Wales. *J. Exp. Mar. Biol. Ecol.* 18:89-97.

Dermott, R., J. Mitchell, I. Murray and E. Fear, 1993. Biomass and production of zebra mussels (*Dreissena polymorpha*) in shallow waters of northeastern Lake Erie. In: *Zebra Mussels Biology, Impacts, and Control.* (eds. T.F. Nalepa and D.W. Schloesser), Lewis Publishers, Ann Arbor, MI, pp. 399-413.

Dorgello, J. and M.H.S. Kraak, 1993. Seasonal variation in tissue dry biomass and its relative ash and organic carbon and nitrogen content in the freshwater mussel *Dreissena polymorpha* (Pallas). *Arch. Hydrobiol.* 127:409-421.

Fritz, L.W., 1991. Seasonal condition change, morphometrics, growth and sex ratio of the ocean quahog, *Artica islandica* (Linneaus, 1767) off New Jersey, U.S.A. *J. Shellfish Research 10:79-99.*

Garton, D.W. and W.R. Haag, 1991. Heterozygosity, shell length and metabolism in the European mussel, *Dreissena polymorpha,* from a recently established population in Lake Erie. *Comp. Biochem. Physiol.* 99A:5-48.

Griffiths, C.L. and J.A. King, 1979. Energy expended on growth and gonad output in the ribbed mussel *Aulacomya ater. Mar. Biol.* 53:217-222.

Haag, W. R. and D. W. Garton, 1992. Synchronous spawning in a recently established population of the zebra mussel, *Dreissena polymorpha*, in western Lake Erie, USA. *Hydrobiologia* 234:103-110.

Harvey, M. and B. Vincent, 1990. Density, size distribution, energy allocation and seasonal variations in shell and soft tissue growth at two tidal level of a *Macoma balthica* (L.) population. *J. Exp. Mar. Biol. Ecol.* 142:151-168.

Hibbert, C.J. 1977. Growth and survivorship in a tidal-flat population of the bivalve *Mercenaria mercenaria* from Southampton water. *Mar. Biol.* 44:71-76.

Hilbish, T.J., 1986. Growth trajectories of shell and soft tissue in bivalves: Seasonal variation in *Mytilus edulis* L. *J. Exp. Mar. Biol. Ecol.* 96:103-113.

Hunter, R.D. and J.F. Bailey, 1992. *Dreissena polymorpha* (Zebra Mussel): Colonization of soft substrata and some effects on unionid bivalves. *The Nautilus*. 106:60-67.

Incze L.S., R.A. Lutz and L. Watling, 1980. Relationships between effects of environmental temperature and seston on growth and mortality of *Mytilus edulis* in a temperate northern estuary. *Mar. Biol.* 57:147-156.

Jantz, B. and D. Neumann, 1992. Shell growth and aspects of the population dynamics of *Dreissena polymorpha* in the River Rhine. In: *Limnologie aktuell*, vol.4, (eds. D. Neumann and H.A. Jenner), Gustav Fischer Verlag, Stuttgart, Germany, pp.50-66.

Kornobis, S., 1977. Ecology of *Dreissena polymorpha* (Pall.) (Dreissenidae, Bivalvia) in lakes receiving heated water discharges. *Pol. Arch. Hydrobiol.* 24:531-545.

Mackie, G.L., 1993. Biology of the zebra mussel (*Dreissena polymorpha*) and observations of mussel colonization on unionid bivalves in Lake St. Clair of the Great Lakes. In: *Zebra Mussels Biology, Impacts, and Control.* (eds. T.F. Nalepa and D.W. Schloesser), Lewis Publishers, Ann Arbor, MI, pp. 153-173.

Norusis, M. J., 1990. *SPSS Base System User's Guide.* SPSS, Inc., Chicago, 520 pp.

Paine, R.T., 1964. Ash and calorie determinations of sponge and opisthobranch tissues. *Ecology*. 45:384-397.

Rodhouse, P.G., C.M. Roden, G.M. Burnell, M.P. Hensey, T. McMahon, B. Ottway and T.H. Ryan, 1984. Food resources, gametogenesis and growth of *Mytilus edulis* on the shore and in suspended culture: Killary Harbour, Ireland. *J. Mar. Biol. Assoc. U.K.* 64:513-529.

Seed, R. 1968. Factors influencing shell shape in the mussel *Mytilus edulis*. *J. Mar. Biol. Assoc. U.K.* 48:561-584.

Seed, R. and C.A. Richardson, 1990. *Mytilus* growth and its environmental responsiveness. In: *Neurobiology of Mytilus edulis*, (ed. G.B. Stefano), Manchester University Press, NY, pp.1-37.

Smit, H., A. Bij de Vaate, and A. Fioole. 1992. Shell growth of the zebra mussel (*Dreissena polymorpha* (Pallas)) in relation to selected physiochemical parameters in the Lower Rhine and some associated lakes. *Arch. Hydrobiol.* 124:257-280.

Smit, H. and E.D. VanHeel, 1992. Methodical aspects of a simple allometric biomass determination of *Dreissena polymorpha* aggregations. In: *Limnologie aktuell*, vol.4, (eds. D. Neumann and H.A. Jenner), Gustav Fischer Verlag, Stuttgart, Germany, pp. 79-86.

Sprung, M. 1992. Observations on shell growth and mortality of *Dreissena polymorpha* in lakes. In: *The Zebra Mussel Dreissena polymorpha.* (eds. D. Neumann and H.A. Jenner), *Limnologie aktuell*, Gustav Fischer Verlag, Stuttgart, Germany, 4:19-28.

Stanczykowska, A., 1977. Ecology of *Dreissena polymorpha* (Pall.) (Bivalvia) in lakes. *Pol. Arch. Hydrobiol.* 24:461-530.

Walz, N., 1978. The energy balance of the freshwater mussel *Dreissena polymorpha* Pallas in laboratory experiments and in Lake Constance IV. Growth in Lake Constance. *Arch. Hydrobiol./Suppl.* 55:142-156.

Winberg, G.G. and A. Duncan, 1971. *Methods for the Estimation of Production of Aquatic Animals.* Academic Press, NY, 175 pp.

Zar, J.H., 1984. *Biostatistical Analysis.* second edition, Prentice-Hall, Inc., Englewood Cliffs, NJ, 718 pp.

The Use of *Dreissena polymorpha* (The Zebra Mussel) as a Biofilter of Municipal Wastewater with Special Reference to Bioaccumulation of Heavy Metals

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Abstract

This research investigated the use of the zebra mussel (*Dreissena polymorpha*) as a biofilter of municipal sewage. Three experiments were conducted, each lasting seven days. Approximately 2500 mussels were placed in a flow-through tank while various sewage streams were applied. The sewage streams examined were raw sewage, primary effluent and secondary effluent. The sewage was monitored for removal of suspended solids, chemical oxygen demand and total phosphate. The greatest removal was experienced when diluted raw sewage and secondary effluent were applied to the mussels. The waste parameter that exhibited the most significant removal during most of the experiments was phosphate. The zebra mussel was responsible for removing 17% of the phosphate entering the tank when diluted raw sewage was applied and 15% when diluted secondary effluent was applied. The effects of temperature were negligible. The high removal experienced when using diluted secondary effluent makes the zebra mussel an excellent candidate to act as an effluent polisher.

The bioaccumulation of six heavy metals by the zebra mussel was also investigated. In all cases the concentration of heavy metals in the mussel increased after exposure to municipal sewage for one week. Chromium exhibited the greatest change in tissue concentration, increasing by over two orders of magnitude when applied to primary effluent and by more than a factor of four when applied to secondary effluent. Iron also showed very large increases.

This research concludes that the zebra mussel is an effective biofilter of municipal sewage. Further study is warranted to address development of a more efficient zebra mussel chamber that could be incorporated into the activated sludge treatment process.

INTRODUCTION

The zebra mussel (*Dreissena polymorpha*, Pallas 1771) is a small clam-like mollusk that grows to approximately one inch. It is indigenous to the Caspian Sea and most of Europe. In 1986 the mussel was inadvertently introduced into Lake St. Clair as a european freighter exchanged ballast water (Herbert et al. 1989). The warm, plankton-rich water of Lake St. Clair provided excellent breeding grounds and the zebra mussel soon proliferated downstream into Lake Erie. By 1990 the mussel had advanced into all five Great Lakes and by 1993 they could be found from Canada in the north to Louisiana in the south and from Oklahoma in the west to New York in the east.

The zebra mussel has affected surface waters in many ways. One is by securely attaching itself to nearly any hard surface including rocks, sea walls, boat hulls, navigation buoys and industrial/municipal water intake pipes. The mussels have shown preference for water intake pipes due to the large flow of water (and food) passing them continuously. Consequently, large mats of mussels can develop (up to 700,000 per m^2 (Kovalak et al. 1990)), effectively choking off the flow needed by the municipality or power plant. Removal efforts have cost boat owners, powerplants and municipalities considerable money and man-power. Finally, as mussels filter lake water to obtain food, they concentrate and settle out suspended particles from the water column -- a process often resulting in significant water clarification.

In recognition of the zebra mussel's ability to filter suspended solids from the water column, a set of preliminary experiments was designed to examine the potential utility of the organism within the context of municipal wastewater treatment. Additionally, the mussel's ability to remove heavy metals from wastewater through bioaccumulation was also investigated. The potential value of such an application is significant, providing practical beneficial use from what is commonly considered a nuisance species. More specifically, design and implementation of flow-through zebra mussel chambers could replace or reduce the number of primary and secondary clarifiers required. Utilization of the zebra mussel for enhanced removal of suspended matter could reduce chemical coagulants, thereby minimizing generation of chemical sludge. Furthermore, mussels applied to treated effluents could act as effluent polishers capable of removing very small particles (0.7 to 1.0 μm (Jorgensen et al., 1984; Sprung and Rose, 1988)). Mussels could also prove to be an effective method of removing heavy metals and persistent organic compounds from a wastestream. Finally, during wet weather the magnitude of combined sewer overflows (CSO) could be reduced by increasing flow through existing clarifiers retrofitted to house a population of zebra mussels. Anticipated decreased solids removal due to higher flows and lower detention times could be offset by the zebra mussel's filtering and settling ability.

PRELIMINARY INVESTIGATION

Previous studies have shown that the zebra mussel is intolerant of ammonia

concentrations above 2 mg/L (Nichols, 1990), a threshold typically exceeded in municipal sewage. Consequently, if the zebra mussel is to be used in the activated sludge treatment process, sewage must be diluted to reduce the toxic effects of ammonia. As a result, a study was conducted to determine zebra mussel viability in varying concentrations of municipal sewage. Populations of mussels exhibiting survival after 7-days of exposure to a given test ammonia concentration in diluted sewage were considered to be indicative of an acceptable background ammonia environment.

Five concentrations of sewage were scheduled for testing together with a control using distilled water. Zebra mussels were exposed to five incremental test concentrations of ammonia as prepared from dilution of fresh sewage (secondary effluent). The five ammonia conditions ranged from a minimum of 1 mg/L to 10 mg/L. Twenty-two mussels were exposed to each concentration in a tank maintained at 11°C. To insure temporal consistency in the ammonia concentration within each tank over the entire 7-day experiment, appropriate dilutions of fresh sewage were provided daily. Dissolved oxygen and ammonia concentrations in each tank were also recorded daily. Individual mussels were examined daily to distinguish percent mortality in each test ammonia concentration.

Results from this preliminary experiment are presented in Figure 1. Examination of the data indicates little mortality in all of the ammonia concentrations except that corresponding to full strength sewage. This clearly demonstrates that the majority of mussels can survive in sewage with an ammonia concentration less than 5 mg/L for at least a 7-day experimental period.

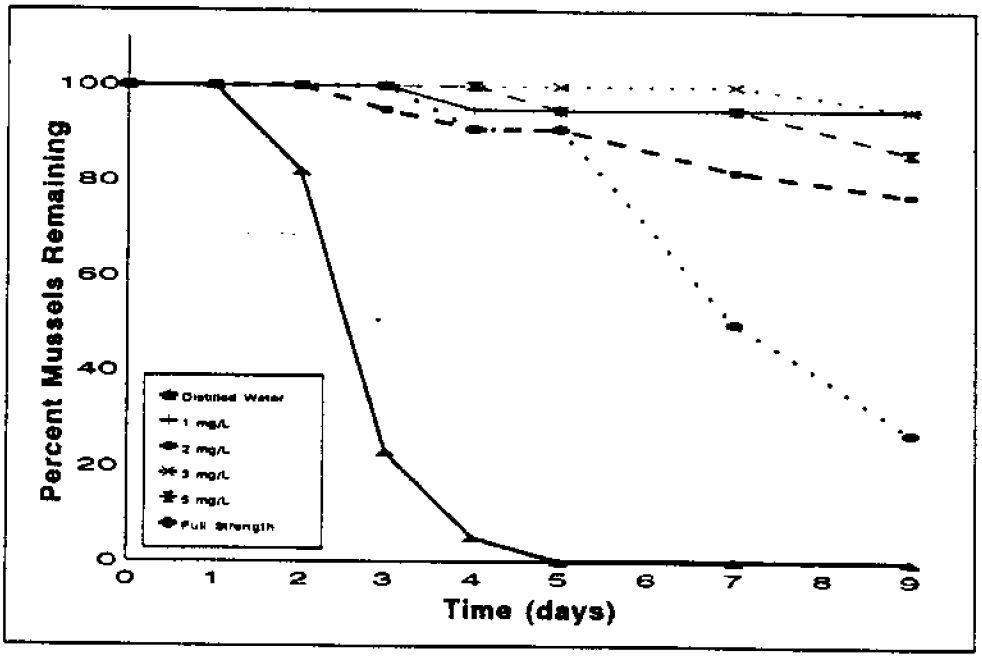

Figure 1: Zebra mussel mortality as a function of time and ammonia concentration.

MAIN INVESTIGATION

Three independent experiments were performed to evaluate the zebra mussel's ability to remove pollutants from municipal sewage. The first experiment involved exposing a population of zebra mussels to raw sewage influent, while the other two focused on exposure to primary effluent and secondary effluent, respectively. In each case the sewage streams were diluted with water to attenuate the toxic effects of ammonia. Sewage was initially passed through a dilution/mixing tank (Figure 2) and then into an experimental tank divided into two separate flow-through chambers, one containing approximately 2500 mussels and the other void of mussels (the control). The flow through each of these 5 gallon chambers was approximately 360 gallons per day. The detention time in each tank was 20 minutes. Concentrations of total suspended solids, volatile suspended solids, chemical oxygen demand and phosphate were measured in the flow stream entering and leaving the control and zebra mussel chambers. The difference in concentration leaving the two chambers was considered representative of removal attributed to the zebra mussel. The results of the three experiments are summarized in Table 1.

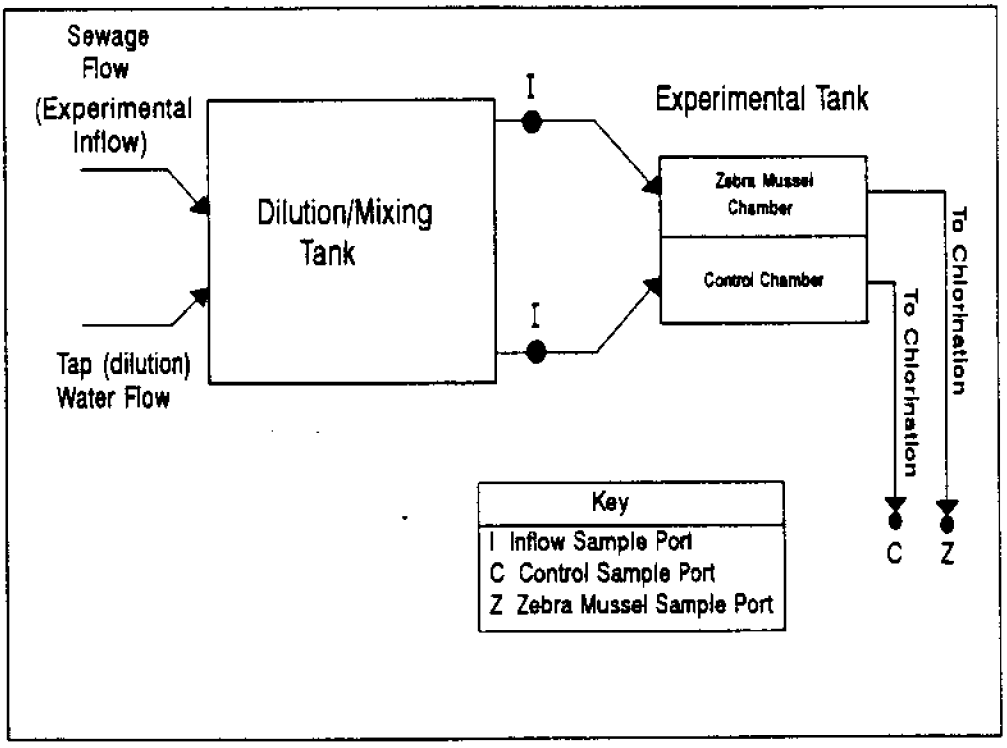

Figure 2: Flow Diagram of Experimental Setup

	Average Removal Attributed to the Zebra Mussel			
	Chemical Oxygen Demand	Total Suspended Solids	Volatile Suspended Solids	Total Phosphate
Raw Sewage	7%	14%	13%	17%
Primary Effluent	7%	5%	4%	9%
Secondary Effluent	3%	16%	17%	15%

Table 1: Summary of removal values.

Removal rates were highest when raw sewage and secondary effluent were applied to the mussel (as opposed to primary effluent). This variability in removal can be partially explained by examining the mussel's filtering process. A zebra mussel will filter approximately one liter of water per day (Stanczykowska et al., 1976). This filtration rate will be decreased at high and low turbidities (Morton, 1971; Benedens and Hinz, 1980; Lewandowski, 1983). Furthermore, filtration rate is increased by the presence of certain size particles and particular types of particles. It is possible that the primary clarifier reduces the concentration of a preferred class or size of particle. This would explain the reduction in removal when primary effluent is applied.

Of the sewage parameters observed, phosphate removal was highest. Because mussels are relatively-efficient in filtering particulate matter from the water column, they also exhibit high removal of particle-bound chemical constituents. Much of the phosphate associated with sanitary wastewater is adsorbed onto the surface of solids, and thus the comparatively high removal of phosphates by the zebra mussel is to be expected. Removal of both total and volatile suspended solids was modest, while the removal of COD was poorest. Chemical oxygen demand represents both soluble and insoluble constituents. The zebra mussel is incapable of removing soluble compounds, and thus the relatively low removal of COD is not unexpected.

METALS INVESTIGATION

Before and after each of the three experiments, the softbody of the zebra mussel was analyzed for concentrations of six heavy metals: cadmium (Cd), nickel (Ni), iron (Fe), chromium (Cr), zinc (Zn) and lead (Pb). Unfortunately, sample tissues of zebra mussels exposed to raw sewage were damaged during the digestion procedure;

therefore, metals data were limited to the experiments using primary effluent and secondary effluent.

Tables 2 and 3 summarize before and after metals concentrations in the softbody of mussels. The concentration of each metal increased in the softbody of the zebra mussel during each of these independent experiments. Chromium exhibited the most significant change, increasing by over 2 orders of magnitude during the experiment using primary effluent and quadrupled when secondary effluent was used. Iron concentrations also exhibited a dramatic effect, increasing by over 5 times during the experiment using primary effluent. For all but two of the metals (Cd, Pb), the increase in concentration was less when the mussels were applied to secondary effluent as opposed to primary effluent. This result is consistent with lower metal concentrations in secondary vs. primary effluent. Consequently, the mussel will likely accumulate less when applied to secondary effluent. It can be hypothesized that the accumulation would have been even greater in samples associated with raw sewage because metals concentrations are generally higher than in primary or secondary effluent.

It should be noted that the concentration of the six heavy metals varied in the wastewater. The bioaccumulation results presented here do not necessarily imply that the mussel has an affinity for one metal over another. A large bioaccumulation could simply mean there was more of a particular metal present.

Metal	Concentration (mg/kg)		Concentration Increase (mg/kg)
	Before	After	
Cd	1.485	1.514	+0.029
Ni	0.279	0.600	+0.321
Fe	24.257	157.018	+132.76
Cr	0.123	18.121	+18.00
Zn	18.251	28.074	+9.82
Pb	2.199	3.056	+0.857

Table 2: Increase in metals concentration in mussel before and after experiment using primary effluent as experimental inflow.

Metal	Concentration (mg/kg)		Concentration Increase (mg/kg)
	Before	After	
Cd	0.967	1.702	+0.735
Ni	0.552	0.544	+0.002
Fe	24.751	54.382	+30.63
Cr	0.128	0.664	+0.536
Zn	17.744	18.584	+0.84
Pb	1.627	3.817	+2.19

Table 3: Increase in metals concentration in mussel before and after experiment using secondary effluent as experimental inflow.

CONCLUSIONS

While the potential application of the zebra mussel for enhanced treatment of municipal wastewater is constrained by ammonia toxicity and the need for initial dilution to acceptable ammonia concentrations, there may be other treatment systems for which the mussel may serve a more direct beneficial purpose. Such an application pertains to fixed bed reactors. Fixed-bed reactors, like trickling filters, have large retention times which facilitate the nitrification of ammonia. Given reduced ammonia concentrations and the mussel's ability to filter very small particles (0.7 μm to 450 μm), the zebra mussel may be an ideal candidate for effluent polishing. Other potential applications include oxidation ponds, food processing wastestreams, steel industry and wood processing industries.

REFERENCES

Benedens, H.G. and W. Hinz. (1980). Daytime periodicity of the filtration performance of *Dreissena polymorpha* and *Sphaerium corneum* (Bivalvia). *Hydrobiologia*. 69: 45-48.

Herbert, P.D.N., B.W. Muncaster and G.L. Mackie. (1989). Ecological and genetic studies on *Dreissena polymorpha* (Pallas): a new mollusc in the Great Lakes. *Can. J. Fish. Aq. Sci.* :in press.

Jorgensen, C.B., T. Kiorboe, F. Mohlenberg and H.U. Riidgard. (1984). Ciliary and mucus net filter feeding with special reference to fluid mechanical characters.

Mar. Ecol. Progr. Ser. 15: 283-292.

Kovalak, W.P., G.D. Longton and R.D. Smithee. (1990). Infestation of Monroe Power Plant by the zebra mussel (*Dreissena polymorpha*). Proceedings of American Power Conference. 52: 998-1000.

Lewandowski, K. (1983). Formation of annuli of shells of young *Dreissena polymorpha*. *Polskie Archiwum Hydrobiologii*. 30(4): 343-352.

Morton, B.S. (1971). Studies on the biology of *Dreissena polymorpha* Pall. 5. Some aspects of filter-feeding and the effect of micro-organisms upon the rate of filtration. *Proceedings of the Malacological Society of London*. 39: 289-301.

Nichols, Susan Jerine. (1990). Laboratory culture of *Dreissena polymorpha*. Zebra Mussel Information Clearinghouse, Brockport, N.Y.

Sprung, M. and U. Rose. (1988). Influence of food size and quantity on the feeding of the mussel *Dreissena polymorpha*. *Oecologia* 77: 526-532.

Stanczykowska, A., W. Lawacz, J. Mattice and K. Lewandowski. (1976). Bivalves as a factor affecting circulation of matter in Lake Mikolajskie (Poland). *Limnologica*. 10(2): 347-352.

4th International Zebra Mussel Conference '94 Proceedings

SOCIO-ECONOMIC ISSUES

Zebra Mussel Awareness and Boat Use Patterns Among Boaters Using Three "High Risk" Connecticut Lakes

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Abstract

Three lakes in western Connecticut, all part of the Housatonic River drainage basin, are considered "high risks" for invasion by zebra mussels, based on water chemistry data and popularity among boaters and fishermen. A survey was conducted to assess the level of awareness of zebra mussels by users of these lakes, and to examine transient boat usage patterns.

During the summer 1993, 325 interviews were conducted with boaters using seven boat ramps on Candlewood Lake, Lake Lillinonah and Lake Zoar. At the conclusion of the interview, boaters were given a zebra mussel alert card, listing telephone numbers of key Sea Grant contacts in the Northeast, and an information card on boat cleaning to minimize overland transportation of aquatic nuisance species.

Fishermen (95.4%) had the greatest awareness of zebra mussels; many (75.9%) also knew that their boats and fishing activities could be a means for spreading the mussels. Far fewer pleasure boaters (69.2%) knew of the mussels or that boats were a potential dispersal mechanism (30.9%). Jet ski operators (44.7%) had little or no knowledge about the mussels or their transport (19.2%).

The majority of fishermen interviewed were not using live aquatic baits. Most had some type of live well on board, and a small number planned to bring fish home in lake water. "Drying out" periods between boat uses averaged eight days, with fishing boats averaging seven days and jet skis, four days. In contrast, boaters were more optimistic as to when the boat would be used next, averaging two days. With few exceptions, boats were kept on trailers at home.

Boats that had been or were expected to be used on another waterbody on the same day were relatively few in number and predominantly jet skis. Multiple daily uses occurred between the three lakes only. Most of the boats had been previously used on the lake of the interview location, or on one of the other two study lakes. About 5% of the boats had been previously used out-of-state, the majority in New York. Some of the New York boats had been used a day or two before, but only one of the four waterbodies named (Hudson River) has confirmed zebra mussel populations. Despite the small number of non-resident interviews, 62 fishing derby permits for these lakes were issued to non-residents, emphasizing the amount of interstate boat traffic.

Introduction

Like most of the continental United States, Connecticut faces the strong possibility of an invasion by non-indigenous freshwater mollusks, *Dreissena polymorpha* and *D. bugensis*, known collectively as zebra mussels. The mussels' ability to adapt physiologically, coupled with inadvertent human dispersal, predispose these species to become widely distributed in North America, with potentially serious economic and ecological consequences (Ludyanskiy *et. al.*, 1993). In particular, the mussels create tremendous and costly problems for users of raw fresh water, particularly power and water utilities, industries, lakeside and riverside residents, fishermen and boaters. Estimates by the U.S. Fish and Wildlife Service put the invasion pricetag at $5 billion by the year 2000 in the Great Lakes region alone.

Two physiological aspects of these mussels--a planktonic larval stage and the capability to produce strong elastic threads (byssal threads) for attaching to firm surfaces--characterize a biofouling organism of a magnitude never before experienced in North American fresh waters, overshadowing even the fouling problems caused by the non-native freshwater Asiatic clam, *Corbicula fluminea*. As noted by Ludyanskiy *et. al.* (1993), it has become rapidly apparent that zebra mussels are capitalizing on an open niche in North American freshwater ecosystems (hard substrates).

Since the initial discovery of zebra mussels in 1988, and the confirmation of a second species in 1992, the range of the mussels has expanded rapidly. Reviewing all potential dispersal methods, scientists predicted that transient boat activity between waterbodies and states would be the primary overland transport mechanism, carrying mussels or mussel larvae in live wells or bait buckets, on boat hulls and among aquatic weeds caught on boat propellers, ropes, and trailers. It has become apparent that the mussels are spreading more rapidly throughout the major riverine systems, aided by currents, and large boat and barge traffic, than they are spreading overland. The spread to inland lakes continues, but at a slower rate than expected.

For Connecticut, with the exception of the Connecticut River, which does support interstate barge traffic, the most probable method of introduction of the mussels will be via transient fishing or pleasure boats. Fishermen, in particular, are prone to move their boats around from waterbody to waterbody and from state to state, following tournaments and good fishing opportunities. Boater education will play an important role in slowing the spread of zebra mussels to inland lakes and rivers not traveled by commercial traffic. The more precautions these individuals take, the slower the overland spread of the mussels will be.

Once introduced to a waterbody, there is no guarantee that the mussels will survive and reproduce. Even if they do become established, the population size is dependent on how hospitable the environment is to them. Not every lake will support zebra mussels to the extent of causing major problems. Zebra mussels have certain environmental constraints. In particular, calcium ion content, pH and water temperature are critical, as well as adequate supplies of plankton for food. As knowledge about the

species of *Dreissena* currently inhabiting the United States and Canada increases, and as the species adapt physiologically, these environmental parameters are changing, evolving, and expanding. Areas originally perceived as "very low probability" may indeed eventually support mussel populations.

Neary and Leach (1992) used calcium ion concentrations and pH to predict which Ontario lakes might be suitable habitats for zebra mussels, using three categories. Survival was "unlikely" if pH was less than 7.4 and calcium ion concentration less than 12 mg/L. Survival was "possible" with calcium ranges between 12 and 20 mg/L and pH >7.4. Survival was "probable", with waters with calcium concentrations greater than 20 mg/L. Murray *et. al.* (1993) adopted this scheme to classify Connecticut's fresh waters into zones of potential zebra mussel threat, using water chemistry data and focusing on the calcium ion concentration of surface waters. The Housatonic River drainage system in western Connecticut, which runs along a marble valley, is considered to be the primary "high risk" area. The risk diminishes eastward across the state, as the waters become softer, albeit not uniformly.

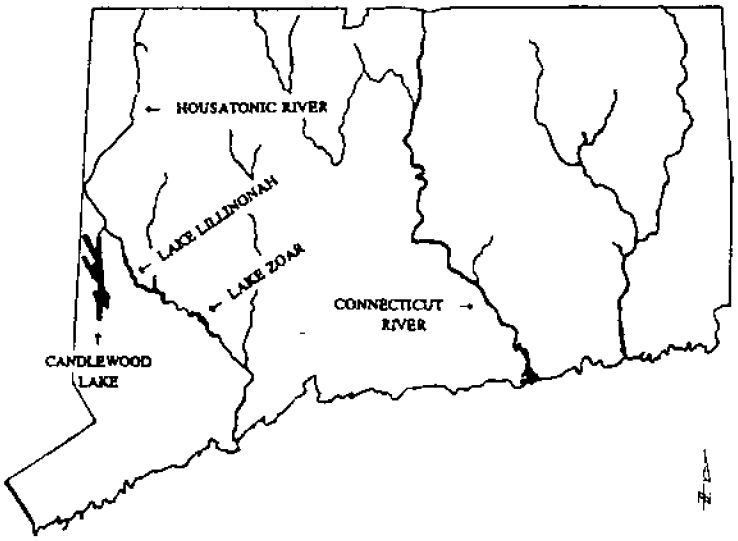
The report also notes that the Connecticut River may serve as the easternmost boundary for mussels in the state, with calcium ion concentrations of 10-12 mg/L. However, the Connecticut River currently supports a thriving introduced population of the Asiatic clam, *Corbicula fluminea*, which also requires calcium for shell formation. Some scientists believe that wherever *Corbicula* or other species of freshwater mollusks are found, zebra mussels could also survive (McMahon, pers. comm., 1992).

Boater Survey

Environmental suitability is not the only measure of the risk an inland waterbody faces from zebra mussels. Another measure is the likelihood of introduction: naturally via currents, or human-induced, such as by transient boat activity. To begin to assess the contribution of boat traffic to "high risk" waterbodies, the Connecticut Sea Grant Marine Advisory Program hired an intern during the summer of 1993 to conduct interviews with fishermen and boaters using the ramps at three popular lakes within the Housatonic River drainage system--Candlewood Lake, Lake Lillinonah and Lake Zoar (Figure 1). Candlewood Lake, the largest lake in Connecticut, is a man-made lake, drawing water from the Housatonic River via an aqueduct. The latter two lakes are part of the Housatonic River proper, with boundaries formed by hydroelectric dams. All three lakes have calcium ion concentrations (mg/l) greater than 17 and pH levels around 7.5.

Survey questions were developed with the assistance of Dr. Ladd Johnson (Research Associate, Williams College). Together with Dr. James Carlton (Director, Maritime Studies Program, Williams College) and Dr. Paul Marangelo (Research Associate, Williams College), Johnson has conducted extensive studies of the role transient boating activity plays in spreading zebra mussels to inland lakes in Michigan (Johnson and Carlton, 1993; Marangelo *et. al.*, 1994).

Figure 1. Location of three target lakes in western Connecticut.



The purpose of the interviews was to determine the level of awareness boaters using these lakes had of zebra mussels, and to determine their boat usage patterns--how frequently the boats were used, where and when they were last used, and where and when they would next be used. In addition, those individuals identified as fishermen were asked questions regarding the source of any live bait used, and in what manner would any kept fish be brought home. The results of these interviews help clarify the risks of inadvertent mussel introduction by boats to these waters and determine the effectiveness of the on-going public outreach and education programs in Connecticut and other states in terms of reaching one of the primary target audiences.

An undergraduate student, Eileen Rohmer, conducted interviews at seven boat ramps on the three lakes between mid-June and mid-August, primarily on evenings and weekends (Figure 1). Boaters were interviewed as they arrived, departed or refueled. In addition to surveying the ramp users, Rohmer also passed out zebra mussel alert cards and information on how boaters can minimize the possibility of transporting zebra mussels from infested waters to uninfested waters.

Results

During the two month survey period, Rohmer conducted 325 interviews at seven ramps on three lakes--Candlewood (139), Lillinonah (123) and Zoar (63). Survey participants were about evenly divided between arriving and departing the lake, with a small number, predominantly personal watercraft (jet skis) refueling . The majority of boaters interviewed had motor boats, distinguished from obvious fishing

boats, sailboats, personal watercraft or other watercraft (party barge) (Table 1). When participants were asked about their primary activity that day, the resulting breakdown was slightly different (Table 2). The majority of boats were being used solely for pleasure. If a respondent indicated both fishing and pleasure, the response was coded as "fishing." A very small number of boats were out for test rides for repairs or potential customers.

Table 1. Breakdown of interviews by boat category. N = 325.

Boat Category	No.
Fishing Boat	87
Motor Boat	187
Pers. Watercraft (jet skis)	47
Sailboat	3
Other (party barge)	1

Table 2. Breakdown of interviews by primary boating activity.

Primary Activity	No.
Fishing	96
Pleasure	225
Test Purchase	3
Test Repair	1

Average boat length was 18 feet, with a range of 13 to 30 feet. The average number of hours spent (or expected to be spent) on the water was four hours, with a range of one to ten hours. Personal watercraft (jet skis) were used on the water an average of two hours at a time, but often were used more than once, and sometimes on more than one lake, on the same day (Table 3).

Zebra Mussel Awareness

Survey participants were asked two questions about zebra mussels:
1. "Have you heard of zebra mussels?" and 2. "Do you know that boats are one

Table 3. Breakdown of mean hours on water by boat category, with range in parentheses.

Boat Category	Mean Hours on Water
Total Interviewed	4 (1-10)
Fishing Boats	4 (2-9)
Motor Boats	4 (1-10)
Jet Skis	2 (2-3)

possible way mussels can be spread from waterbody to waterbody?" (This second question was actually posed by Rohmer as: "Do you think your craft could spread zebra mussels?")

The overall results indicated that 73% of those interviewed had heard of zebra mussels and 41% thought their boat was a potential mechanism for dispersal of the mussels. Table 4 shows the breakdown of responses to the two questions, by boat category, which was done to see if any boater category had a greater awareness of zebra mussels than the others. (These calculations use the 87 boats identified as fishing boats, rather than the 96 individuals who indicated their primary activity was fishing.)

Table 4. Breakdown of responses by boat type to two questions posed: (1) "Have you heard of zebra mussels?" and (2) "Do you think your craft could spread zebra mussels from one waterbody to another?" Number of participants: 325

RESPONSE	YES / YES	YES / NO	NO / NO
Fishing Boats	66 (75.9%)	17 (19.5%)	4 (4.6%)
Motor Boats	58 (31.0%)	72 (38.5%)	57 (30.5%)
Sailboats	1 (33.3%)	2 (66.7%)	---
Pers. Watercraft*	9 (19.2%)	12 (25.5%)	26 (55.3%)
Other**	---	1 (100%)	---

***jet skis**
**** party barge**

The results show that 95.4% of the individuals identified with fishing boats were aware of the mussels and 75.9% also knew about the potential for transport by boats. Less than 5% of the fishermen interviewed knew nothing about zebra mussels. Of those individuals identified with motor boats, 69.5% had heard of zebra mussels, but only 31.0% knew that boats are a possible means of dispersal. A similar percentage of boaters (30.5%) knew nothing about zebra mussels. With regard to the personal watercraft (jet ski) operators, 44.7% had heard of zebra mussels, but less than 20% knew that boats were a dispersal mechanism. More than half of those interviewed knew nothing about zebra mussels.

Table 5 shows that by summarizing the "yes" responses to the two questions, it is readily apparent that public outreach and education efforts targeting anglers has been more effective or thorough than those targeting pleasure boaters. These responses indicate a need to reach out to all Connecticut boaters and operators of personal watercraft such as jet skis, to ensure that they too get the message about zebra mussels and other nuisance aquatic species.

Table 5. A summary of the "yes" responses to two questions posed about zebra mussels.

Summary of "YES" Responses	Aware of zebra mussels	Aware of possible boat transport
Fishing Boats	**83 (95.4%)**	**66 (75.9%)**
Other Craft	**155 (65.1%)**	**68 (28.6%)**
Motor Boats	**130 (69.5%)**	**57 (30.5%)**
Sailboats	**3 (100%)**	**---**
Jet Skis	**21 (44.7%)**	**9 (19.2%)**
Party Barge	**1 (100%)**	**---**

Respondents were asked at the end of the interview if they had been previously interviewed by Rohmer. Fourteen (4.3%) were repeat interviews. Looking at the data sheets indicating a repeat interview and examining the responses to the first two questions about zebra mussels, only two respondents indicated that they had never heard of zebra mussels or did not know that boats could transport the mussels around, information they should have received from Rohmer during the first interview. Since the interviews were anonymous, it was not possible to match up a repeat interview with a first interview, to see if the answers to the first two questions changed.

Fishing

The responses to these questions were based on the interviewees indicating that their primary activity was fishing (96), rather than the number of boats identified by the interviewer as an obvious fishing boat (87).

Because of the potential for transporting zebra mussel veligers in live wells, bait buckets or bait water, specific questions were addressed to those 96 individuals that had been fishing or planned to do so. When asked whether or not live bait--specifically minnows or crayfish--had been used, 12 anglers (14.6%) were using minnows and two were using crayfish. The rest either were using artificial baits or other non-aquatic type of bait, or the information was unavailable. When asked about the source of the live baits, nine said the minnows came from local bait shops, two brought them in from out-of-state and one response was missing. For the crayfish, one lot came from a local bait shop and the others were caught locally by the individuals themselves. In other words, 71.4% was purchased at a local bait shop, and 14.3% was brought in from out-of-state.

The anglers were asked: if they caught fish that day, if they were planning to bring fish home with them. Only 14 said yes, the remainder either did not plan to keep any fish or did not know (because they were just launching). Of the 14 who said yes, nine planned to take the fish home in lake water, while five would not. Of those that did not know if they would keep any fish, five would bring any fish home in lake water and the remainder either would not (five) or again, did not know (seven).

Individuals associated with any boat other than a personal watercraft were asked if their boat had a storage compartment for fish, and 94 of the 279 eligible respondents indicated that their boat had some storage compartment. Unfortunately, the question was not worded as well as it could have been, because there is no way of knowing if the storage compartments were all built in, as the question intended, or if some of the responses included portable bait buckets or aerated systems.

Boat Usage Patterns

One of the keys to incidental transport of zebra mussels and/or veligers from waterbody to waterbody by boat is the time period between uses--does a boat move from lake to lake one day to the next, or is there generally a "drying out" period between uses? A series of questions were asked to determine when and where a boat was last used, where and how the boat is typically stored, and when and where the boat is expected to be used next. It is commonly perceived that fishermen following tournaments may fish, for example, in the Hudson River one day and Candlewood Lake the next day. The responses to these questions provide a clearer picture of boat use practices, although they may not be entirely representative, since Rohmer did not interview fishing tournament participants specifically.

Table 6 shows the breakdown of responses by identified boat type to the questions "When was the last time boat was used?" in days and "When is the next time the boat is expected to be used?" in days. In the case where the responses was the same day as the interview, the number of days was indicated as "0".

Most people (313) had some idea of the last time their boat was used and on average, it was eight days prior to the interview, but ranged from earlier that same day to 45 days before. Fishing trips tended to run about seven days apart, while pleasure trips ten days apart, on average. The jet skis were used more frequently, in action four days prior to the interview on average. Four individuals had not used their boat since the 1992 boating season, the interview date being their first time out during 1993.

Far fewer people (50) had some idea of when they planned to use their boat next. On average, it was in about two days, with a range of same day to seven days hence. Breaking the responses out by boat type, fishing and motor boats were expected to be used generally within three days, while jet skis were expected to be used the next day.

The difference between the average number of days since the last use and the expected average time span before the next use is six days. It appears that people are more optimistic about how frequently they will use their boats than they actually do. If the responses in June and August are compared, the average number of days since the boat was last used remains at eight days for both months. However, the response for the next planned use averaged four days in June and two days in August. This allows for some "drying out" time, particularly since 320 of the 325 boaters interviewed keep their boats on trailers at home.

Five of those interviewed had used their boat or personal watercraft previously on the day of the interview. Three boats (two motor, one fishing) had come to Lillinonah from Candlewood and two jet skis had been moved from Lillinonah to Zoar on the same day.

When asked if they planned to use their boat on a different lake on the same day, 287 said "no," 28 did not know and nine said "yes." All of the multiple uses were to one of the three lakes where the interviews were being conducted--Candlewood, Lillinonah and Zoar. There appears to be a lot of movement between the three lakes, which are relatively close to one another. This observation was confirmed by the responses to the following question.

A breakdown of the 315 responses to the question "Where was the boat last used?" indicated that 85.4% of the boats had been previously used on one of the three target lakes, and that 62.8% were the same lake as where the interview was being conducted. Similarly, when asked "Where will the boat be used next?", of the 111 responses, 91% were for one of the three target lakes and 78.2% were expected to used at the same lake that the interview was being conducted.

Fifteen individuals (4.8%) had used their boat last on another Connecticut lake or pond, seventeen (5.4%) had been previously used out of state (Northeast), and fourteen (4.4%) had last been used on Long Island Sound.

Since to date, no zebra mussels have been found in either Massachusetts or New Hampshire, only the New York boat trips were used in the following calculation, particularly since eight of the boats had last been used in the Hudson River, which does have zebra mussels. The other three waterbodies in New York were Lake Carmel, Lake George and Peach Lake. No zebra mussels have been found in any of these lakes to date (O'Neill, pers. comm., 1993).

Table 6. Breakdown of responses to questions "When did you last use your boat and when do you plan to use it next?" Mean number of days given, with range in days in parentheses. The response "0" indicates planned use on same day as interview.

Boat Category	Last Boat Use (Days)	Estimated Next Use (Days)
TOTAL	8 (0 - 45) [N = 313]	2 (0 - 7) [N = 50]
Fishing Boats	7 (0 - 30) [N = 83]	3 (1 - 7) [N = 13]
Motor Boats	10 (0 - 45) [N = 179]	3 (0 - 7) [N = 20]
Sailboats	22 (6 - 30) [N = 3]	---
Jet skis	4 (0 - 14) [N = 47]	1 (0 - 2) [N = 17]

No. of individuals that did not know next use: 276
No. of individuals who last used boat in 1992: 4

While only a few boaters interviewed (5.2%) came from out-of-state, their responses indicate that only a day or a few days may pass before a boat is trailered to Connecticut, increasing the risk of zebra mussel survival, and therefore, introduction, if appropriate precautions are not taken. While the Connecticut Department of Environmental Protection (DEP) does not keep track of the number of out-of-state boats launched at Connecticut state ramps, it does keep records of fishing derby permits that are issued (CT DEP, 1993). During 1993, 166 permits were issued for these three lakes, 99 for Candlewood Lake alone. Of these permits, 62 were issued to individuals living in states other than Connecticut.

Conclusions

Based on the results of 325 interviews during 1993, fishermen in general are more knowledgeable about zebra mussels and the potential role boats play in dispersing the mussels, while pleasure boat and jet ski operators need more directed educational programs on zebra mussels.

The majority of fishermen interviewed were not using live aquatic baits, while those that were using minnows or crayfish had a variety of sources for them. The bait (minnows) that are sold in Connecticut bait shops come from two distributors in Massachusetts, who receive their supplies from baitfish farms in Arkansas. Based on the way these fish are raised in spring-fed ponds or well water, they can be considered free of the risk of zebra mussel contamination (Hyatt, pers. comm., 1993). Most boats identified as fishing boats had some sort of live well, although whether these "wells" were part of the boat or portable remains unclear.

The interview results combined with the number of fishing derby permits issued by the Connecticut DEP for these lakes confirm that there is a significant amount of interstate boat traffic, some coming from areas with zebra mussels and some involving no "drying out" period between boat uses. On average, however, boats tend to have a drying time of about eight days, and most are kept on trailers at home. There is also a lot of interlake movement, sometimes on the same day.

More comprehensive studies have been conducted in Michigan (Johnson and Carlton, 1993; Marangelo *et. al.*, 1994), documenting not only boat use patterns, but also examining exiting trailered boats for signs of mussels. Mussel monitoring programs were also initiated on a series of inland Michigan lakes. The researchers concluded that trailered boats are indeed viable dispersal mechanisms for all life stages of zebra mussels, and that precautionary measures should be taken by boat owners to minimize the possibility of contributing to the rapid spread of the mussels throughout the continental United States, particularly to inland lakes and waterways.

References

CT Department of Environmental Protection. 1993. Summary of Fishing Derbys.

Hyatt, W. 1993. (CT DEP). Personal communication.

Johnson, L. and J. Carlton. 1993. Dispersal of the Zebra Mussel *Dreissena polymorpha:* The Potential Role of Transient Boating Activities as a Vector of Overland Spread. IN: Proc. Third Intl. Zebra Mussel Conf. 23-26 Feb. Toronto, Ontario.

Ludyanskiy, M., D. McDonald, and D. MacNeill. 1993. Impact of the Zebra Mussel, a Bivalve Invader. *BioScience* Vol 43(8), pp. 533-544.

Marangelo, P., Carlton, J. and L. Johnson. 1994. The Spread of Zebra Mussels to Inland Waters: Advances and Lessons from Michigan. IN: Proc. Fourth Intl. Zebra Mussel Conf. 7-10 Mar., Madison, WI.

McMahon, R. 1992. Personal communication.

Murray, T., P. Rich and E. Jokinen. 1993. Invasion Potential of the Zebra Mussel, *Dreissena polymorpha* (Pallas), in Connecticut: Predictions from Water Chemistry Data. Connecticut Institute of Water Resources Special Report No. 36. 33pp.

Neary, B. and J. Leach. 1992. Mapping the Potential Spread of the Zebra Mussel *(Dreissena polymorpha)* in Ontario. Ca. J. Fish. Aquat. Sci. 49:406-415.

O'Neill, C. 1993. Personal communication.

The Evaluation of Impacts of Colonization of Zebra Mussels on the Recreational Demand in Lake Erie

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Abstract: The colonization of zebra mussels has a significant influence on the industrial system along the Lake Erie. But impacts of infestation of zebra mussels on the other industries, such as the recreational industry, have not been well evaluated. This study applies the framework of demand analysis for non-market goods to valuing the potential influence of colonization of zebra mussels on the demand for recreational goods under the situation of heterogenous consumers. The empirical results suggest that the failure of recognition of heterogenous consumers of the environmental goods is one of the major biased errors in practical evaluation, which might underestimate the impacts of the presence of zebra mussels on the recreational demand. Since the positive impacts of zebra mussels on the recreational demand in Lake Erie is quite large in this study, it might be possible to have reduced negative impacts of zebra mussels on the recreational demand. As a result, the combined impacts may not be significant in Lake Erie in the present.

I. Introduction

It is known that zebra mussels are a threat to water treatment plants, industries, and power utilities because they restrict the flow of water into plants and pluming facilities by fouling intake structures and piping [5, 14, 16]. The estimated costs from the U.S. Fish and Wildlife service assess the potential economic impacts of the zebra mussel alone at $5 billion over the next 10 years [9].

Beside the power generation, natural area, nature species appreciation, manufacturing, navigation, commercial fishing and public water supplies, the recreational industries, such as recreational fishing, tourism, beach use, etc., might be influenced by the infestation of zebra mussels [5]. Economic and environmental problems associated with the colonization of zebra mussels in Lake Erie, Ohio, create a need to investigate the effects of the infestation of zebra mussels on the recreational activities.

The overlapping literatures of water resources, recreation, biology, and environmental economics stress the need for values to attach to largely non-market interactions of species and affected environment and the associated impacts on the recreational economy, such as recreational boating, fishing, and swimming [1, 6, 8, 10]. But so far, little or no research has been carried out to investigate the potential economic impacts of the heavy fouling of zebra mussels in Lake Erie on the recreational industries. Much efforts have gone into the treatment and control of infestation of zebra mussels in industrial and commercial sectors.

The objectives of this paper are (a) to identify the key feature of consumers of non-market environmental goods, (b) to contrast the direct and indirect methods of estimating the demand for recreational boating under the impacts of zebra mussels, and (c) to analyze the possible recreational quality changes response to the colonization of zebra mussels and possible effects on the recreational attractiveness of Lake Erie.

II. The Model

When the zebra mussel was first discovered in Lake St. Clair of the Laurentian Great Lakes in June 1988, few people realized the potential impacts of the invasion of this kind of species on the economy along Lake Erie. Right now numerous populations of zebra mussels have been well established in all of the Great Lakes and the Lake Erie environment has experienced numerous changes over the past several years. As a result, recreational industries along Lake Erie have been impacted by zebra mussels [13].

Personal category --- A paradox to the standard demand theory

The standard demand analysis for the non-market goods is based on an implicit assumption that consumers are homogenous with respect to the knowledge about the change in quality of environment. In other words, each consumer has the same information or knowledge about the quality of recreational site (non-market goods). Based on this assumption, the utility maximization problem yields an identical demand function across individuals and this Marshallian demand function can correctly specify the preference of consumers. With incorporation of attitude variables [1], the impacts of the attitude arguments might shift the demand curve downward or upward. This direct approach is often used to evaluate the impacts of the change in environmental quality. Based on the personal category, each individual exposes his satisfaction level about the presence of zebra mussels. Therefore, there must be a positive correlation between the quantity consumed (**TRP**) and the level of satisfaction about the environmental quality with the presence of zebra mussels (**ZEB**).

Table 1. The Pearson correlation coefficients

	TAP	CST	ICM	AGE	SEX	ZEB
TAP	1.000					
	0.000					
CST	-0.259	1.000				
	0.002	0.000				
ICM	0.125	0.052	1.000			
	0.160	0.557	0.000			
AGE	-0.028	-0.019	-0.119	1.000		
	0.739	0.826	0.180	0.000		
SEX	0.006	-0.086	0.040	0.290	1.000	
	0.939	0.314	0.653	0.735	0.000	
ZEB	-0.055	-0.109	0.021	-0.142	-0.143	1.000
	0.516	0.200	0.813	0.094	0.092	0.000

[1] The attitude variable is based on the personal evaluating category. The key feature of the direct approach is to ask each individual to expose his overall evaluation about the subject, e.g. the change in environmental quality with the presence of zebra mussels in terms of his value system.

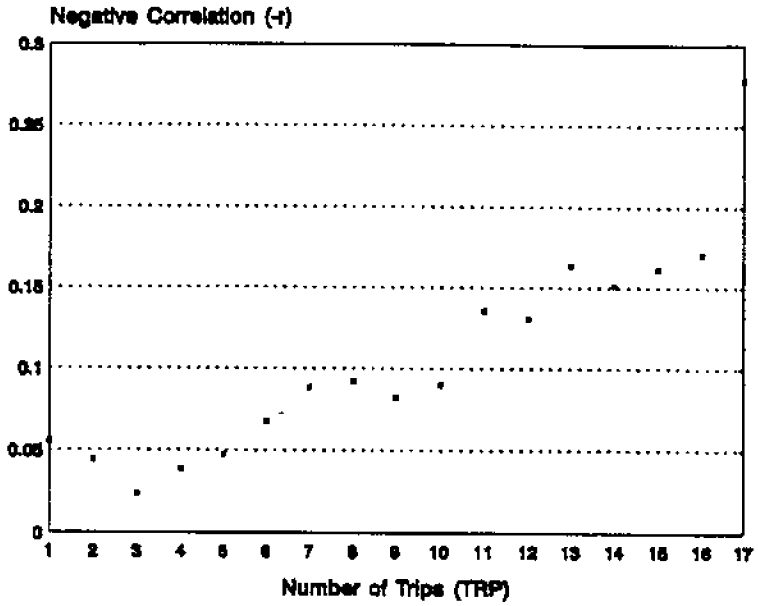

Figure 1 Negative relationship between the satisfaction level and the number of trips

Nevertheless, if the consumers are heterogenous with respect to the information or knowledge about the change in environmental quality caused by zebra mussels, the taste of consumers can not be appropriately evaluated in the standard demand framework by simply pooling data from these consumers. When we value the change of environmental quality, the consumers we are dealing with are typically the heterogenous consumers. In this case, the evaluation of the demand function might be misleading if the consumer are asked to give an overall assessment of the satisfaction about the presence of zebra mussels. For example, in this study, there are 466 respondents, among whom, 11.9% didn't know the presence of the zebra mussels in Lake Erie. In the present research, there appears a negative relationship between the level of recreational satisfaction about the presence of zebra mussels and the number of trips (see Table 1). This implies that the less satisfactory the consumer is about the presence of zebra mussels, the more times the consumer travels to Lake Erie for boating. This seems a paradox to the standard demand theory. Figure 1 shows that with the increase of the number of boating in Lake Erie, the negative relation becomes stronger and stronger.

The reason for this contradiction is that the respondents have very different information about the presence of zebra mussels and the associated impacts. From the survey data, it can be observed that the more trips a person made in 1991, the lower

his satisfaction level was over the presence of the colonization of zebra mussels in the Lake Erie.

This kind of behavior seems irrational. If one takes a close look at the motives behind this sort of behavior, it is not difficult to recognize that the more trips a boater makes, the more knowledge of the influence of zebra mussels he obtains [2]. As a result, the more trips make people tend to lower satisfaction level about the presence of zebra mussels. So the direct approach, which attempts to differentiate the overall satisfaction levels about the impacts of zebra mussels on the recreational boating, is obviously inappropriate for our purposes of evaluation.

An alternative approach has been widely employed in the demand analysis for evaluating the changes in environmental quality. Particularly, a "suggestive" category is empirically employed, which is identified as a set of major characteristics well describing the impacts of quality change of environment caused by the presence of zebra mussels **[1, 6, 8, 10, 15]**.

Social preference

In order to reduce the biasedness of estimation from the heterogenous consumers, we apply the indirect approach to exploit the impacts of zebra mussels. The basic idea of the indirect method is to identify several important features which can characterize the impacts of infestation of zebra mussels on the environmental conditions for boating. Then the marginal changes of demand by these characteristics can be evaluated and the corresponding welfare changes can be also computed. This approach tends to represent the 'social' preference instead of the 'personal' preference.

Let us take a look at how the social evaluating category is defined in this study [3]. Zebra mussel veligers first invaded the eastern end of Lake Erie during late summer 1989. They have changed the environmental quality positively and negatively. Researchers as well as boaters have noted greatly increased water clarity (WAT) in Lake Erie between 1989 and 1991. A significant part of this change in clarity has been

[2] If an individual takes many trips to the recreational site, his attitude about the impacts of zebra mussels might be formed as both an "average" over the season and an "accumulation" through the whole season. So it is different from the 'pure' average attitude Shaw mentioned in his paper **[14]**.

[3] The social evaluating category is concerned with the global, overall, and long-run characteristics rather than the local and short-run characteristics in the personal evaluating category.

attributed to the filtering activities of zebra mussels [13]. This is a positive influence of zebra mussels on the environmental conditions.

On the other hand, negative influences come from all kinds of sectors. The threat to the recreational attractiveness by the zebra mussel was sudden and serious. High mussel densities and rapid growth rates caused extensive environmental problems.

First, beaches are affected by zebra mussels. By autumn of 1989, extensive deposits of zebra mussel shells could be seen on many Lake Erie beaches. The extent of these deposits varied with successive periods of high wave activity. Sharp-edged shells accumulating along swimming beaches could be a hazard to unprotected feet. This effect causes a change in beach environmental conditions, for example, beach contaminants and pollutants caused zebra mussels (**POL**), beach pollution (**BEA**), and beach congestion (**CON**) with the presence of zebra mussels.

Table 2. The Pearson correlation coefficients

	TRP	CST	ICM	AGE	SEX	WAT	BEA	CON	ART	POL
TRP	1.00									
	0.00									
CST	-0.26	1.00								
	.002	0.00								
ICM	0.13	0.05	1.00							
	0.16	0.56	0.00							
AGE	-0.03	-0.02	-0.12	1.00						
	0.74	0.83	0.18	0.00						
SEX	0.01	-0.09	0.04	0.29	1.00					
	0.94	0.31	0.65	0.74	0.00					
WAT	0.19	-0.08	0.02	0.14	-0.04	1.00				
	0.02	0.36	0.87	0.11	0.65	0.00				
BEA	0.06	-0.05	-0.02	0.06	0.05	0.16	1.00			
	0.52	0.55	0.80	0.50	0.53	0.06	0.00			
CON	0.09	0.02	0.05	-0.08	0.11	-0.08	0.22	1.00		
	0.27	0.86	0.55	0.38	0.20	0.35	0.01	0.00		
ART	-0.01	0.08	0.15	0.06	0.04	0.10	0.05	-0.11	1.00	
	0.89	0.34	0.09	0.50	0.62	0.25	0.59	0.19	0.00	
POL	0.05	-0.01	0.04	0.04	-0.11	0.09	0.30	0.18	0.01	1.00
	0.55	0.91	0.65	0.60	0.19	0.29	.001	0.03	0.87	0.00

Biologists were also concerned about zebra mussel colonies covering rock and artificial reefs (ART). The zebra mussel has heavily encrusted fish spawning reefs and created other environmental and economic problems. Most rocky areas in Lake Erie are almost completely covered with mussels several inches deep. Such poor environmental conditions could potentially hinder normal egg development of reef-spawning fish (walleye, white bass, and smallmounth bass). Off shore reef areas in the western basin of Lake Erie are utilized by walleye (Stizostedion Vitreum), lake whitefish (Coregouus clupeaformis), white bass (Morone chrysops), and other species for spawning. The principal reef areas are bedrock which is sedimentary in origin. The bottom surface adjacent to rocky outcrops consists of unconsolidated sediments including boulders, cobbles, pebbles, sand, and med. The bedrock and gravel components of the reef areas were quickly colonized by zebra mussels, which caused concern among scientists that reproduction of walleye and possibly other species could be affected. The encroachment of zebra mussels to the rock and artificial reefs causes the problems for the boating activities.

We specify these five important impact characteristics of zebra mussels on the environmental quality. Table 2 shows the Pearson correlation for these variables. Basically, the TC approach estimates statistically a demand equation, using trip of visits as a measure of quantity and recreationist's travel costs (or distance) as measure of price (CST). The demand equation is often specified to include variables reflecting recreationists' income, and other socioeconomic variables. The model is based on the rational behavior, that is, the boater chooses the quantity to maximize his utility. The weak complementary condition also holds for this model.

The model is specified as follows:

$$TRP_i = f(CST_i, ICM_i, AGE_i, SEX_i, WAT_i, BEA_i, CON_i, ART_i, POL_i)$$

where CST_i is the round-trip variable and the demographic variables here are identified by respondent age (AGE_i), sex (SEX_i), and the respondent's income level (ICM_i).

Welfare analysis

Figure 2 gives a simple picture of the welfare analysis, which is the basis for the welfare evaluation. Consumer's surplus in this single equation case is computed as the area under the demand curve and above the cost curve. In practice, consumer's surplus is computed on the basis of mean values of independent variables from the demand equation.

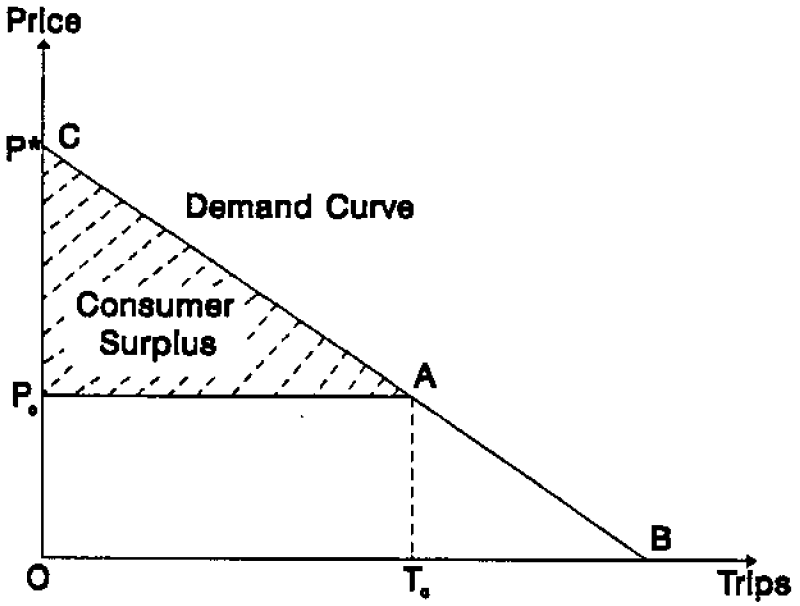

Figure 2. Consumers' surplus (CS) from a demand function for the recreational boating in Lake Erie

III. EMPIRICAL ESTIMATION AND RESULTS

Data considerations:

This analysis is based on the 1992 cross-sectional mail survey data in Lorain county, Ohio. About 4000 questionnaires were distributed in the recreational sites in Lorain country. The return rate was about 20%. About 140 observations, which have complete answers for the present model, were used in the estimation. The travel costs were accounted by a full scale [4], which includes all expenditures on-site and at home. The expenditure at home includes the costs of groceries and beverages, gas and oil for transportation, boating supplies and repairs, gas and oil for boat, fishing equipment, gear, bait, and other recreational equipment. The on-site expenditure includes not only the above expenditure at sites but also the costs of overnight loading, restaurants,

[4] In the preexisting literature, the travel costs are chiefly estimated by the 'pure' travel costs. Some of the major expenditures associated with the trips to recreational sites for boating are excluded. This kind of accounting system might cause the difficulties to analyze the 'indirect' welfare change from the consumption of the non-market environmental goods.

launching fees, and shopping at sites. The average full travel-cost was estimated as

Table 3. Variable definition for the demand function

Variable Name*	Description
TRP	Number of trips to Lake Erie for boating;
AGE	Household age;
ICM	Household income;
WAT	Water clarity;
BEA	Beach pollution;
CON	Beach congestion;
ART	Artificial reefs;
POL	Contaminants and pollutants;

* Three levels are assigned for last five variables. 1 is chosen if satisfaction has decreased, 2 if satisfaction has stayed the same, and 3 if satisfaction has increased.

Table 4. Estimate of the parameters for the demand function

Variable Name	Estimate	Standard Error
Intercept	-16.5701	29.31
CST	-0.0313	0.01
AGE	-0.0932	0.20
SEX	5.0821	19.12
WAT	11.2641	5.22
BEA	-1.0140	3.79
CON	4.4132	4.83
ART	0.0562	4.07
POL	0.8831	3.20
ICM	0.0014	0.001

$8.25 per mile. On the other hand, the pure travel cost is less than $1.00 per mile [5]. Table 3 shows the definition for all variables in the demand function.

Estimation process:

The empirical model was estimated using the OLS technique process by SAS on the IBM 3090/600J mainframe. Using the linear demand model, it is possible for us to predict how the total number of trips will change as a result of the environmental quality change.

Results of empirical estimation

The estimated results are represented in Table 4. Substituting the mean values for the explanatory variables except ICM and CST, we have the simplified demand function:

$$TRP = 23.850 - 0.031\ CST + 0.014\ ICM$$

This demand function can be further simplified as:

$$TRP = 30.6327 - 0.031\ CST$$

For mean values of independent variables, consumer's surplus is computed as $386.85. This value is obtained under the full scale expenditures at home and on site associated with a round-trip for boating.

Table 5. Two projected satisfaction levels

Variable Name	Original Level	Higher Level	Lower Level
WAT	2.79	2.98	1.81
BEA	2.09	2.82	1.27
CON	1.94	2.83	1.11
ART	2.49	2.94	1.55
POL	1.91	2.66	1.26

Results such as presented here can be used for policy analysis by measuring the changes in consumer's surplus induced by changes in the environmental quality caused

[5] Most of literature applied the 'pure' travel costs, which is only concerned with the on-site and travelling costs. The 'pure' travel costs are typically less than $1.00 [2,12].

by zebra mussels. Two possible scenarios were considered for the welfare changes: i.e. the higher and lower satisfaction levels about the environmental quality with the presence of zebra mussels. With the higher level, the satisfaction level is projected to increase by a level for all boaters. At the lower level, the satisfaction decreases to the lower level (see Table 5). Table 6 provides a marginal analysis for the projected scenarios. Each level is compared with the bench mark CS. The results indicate that the changes in WAT and CON have significant impacts on the consumers' surplus. On the other hand, the changes in ART don't show a significant impacts on the demand for boating.

Table 6. Marginal Consumer surplus and net consumer surplus

Variable Name	Higher Level		Lower Level	
	CS	Net CS	CS	Net CS
WAT	$432.06	$35.21	$218.21	-$178.64
BEA	$384.87	-$11.98	$410.35	$13.50
CON	$460.20	$63.34	$337.59	-$59.26
ART	$397.26	$0.41	$395.99	-$0.86
POL	$407.48	$10.63	$387.45	-$9.40

IV. CONCLUDING COMMENTS

An attempt is made here to represent a case study of environmental impacts analysis of the colonization of zebra mussels in Lake Erie. The paper uses existing data to provide some interesting empirical estimates of the values of recreational boating in Lake Erie.

The robust finding is that since the knowledge or information about the impacts of zebra mussels is not homogeneous across the consumers, the direct estimate might be biased or misleading. We must be careful at this point to avoid a biased evaluation for the heterogenous consumers. Even though there might exist a positive relationship between the overall satisfaction level and environmental quantity, it might be the case where the welfare may be underestimated. Empirically, it often appears that some measures of environmental goods cannot be decomposited into a set of major characteristics and we still want to use the attitude variables. In this case, the underestimation or overestimation could happen.

Three empirical conclusions can be drawn from this paper of the argument. First, the direct analysis suggests a very important fact that with the increase of knowledge of impacts of zebra mussels on the recreational boating, the consumer's surplus could be reduced and the number of trips to Lake Erie might be decreasing. Second, since the positive impacts of the zebra mussels on the recreational demand is quite large, the combined impacts on the recreational demand may not be significant in Lake Erie right now. Third, differentiating the recreational resources demanders with respect to information or knowledge of environmental quality so that the appropriate benefit measures may be applied, will increase the accuracy. Finally, one of the advantages of using the full-scale expenditure is that it is possible for us to evaluate the total consumers' surplus from sub-consumptions of non-market goods, which is associated with the expenditures at home and on-site. It would be beneficial to policymakers if the impacts could be decomposited into the direct impacts and indirect impacts.

A number of improvements could be made using a more realistic set of characteristics. The method discussed here represents a simple, straightforward way of evaluating the impacts of colonization of zebra mussels on the recreational demand. Many of the ideas and integration are still inchoate and not expressed in an entirely satisfactory manner. But this is less important than getting ideas into the open where they can be discussed and debated.

REFERENCES

[1] Bell, Frederick W., and Vernon R. Leeworthy. "Recreational Demand by Tourists for Saltwater Beach Days." *J. Environ. Econ. Manage.*18(1990):189-205.

[2] Brown, William G, and Farid Nawas. "Impacts of Aggregation on the Estimation of Outdoor Recreation Demand Functions." *Amer. J. Agr. Econ.* 55(May, 1973):246-49.

[3] Kealy, Mary Jo, and Richard C. Bishop. "Theoretical and Empirical Specifications Issues in Travel Cost Demand Studies." *Amer. J. Agr. Econ.* 68(1986): 660-667.

[4] Kealy, Mary Jo, Mark Montgomery, and John F. Povidio "Reliability and Predictive of Contingent Values: Does the Nature of the Good Matter?" *J. Environ. Econ. Manage.* 19(1990): 244-63.

[5] Kelch, David O. "Boaters --- Slow the Spread of Zebra Mussels, and Pretect Your Boat too." OHSU-FS-045, Ohio Sea Grant.

[6] Kling, Catherine L. "Comparing Welfare Estimate of Environmental Quality Changes from Recreation Demand Models." *J. Environ. Econ. manage.* 15(1988):331-340.

[7] Loomis, John. "Economic Losses to Recreational Fisheries due to Small-head Hydro-power Development: A Case Study of the Henry's Fork in Idoho." *J. Environ. Econ. Manage.* 22(1986):85-94.

[8] Macgregor, R.D., Fred J. Hitzhasen, and Judith A. Maxwell."Targeting soil erosion control with off-site damage estimates: The case of recreational boating." Unpublished working paper (February, 1990).

[9] Michigan Department of Natural Resources. "State of the Great Lakes: 1993 Annual Report." Office of the Great Lakes.

[10] Morey, Edward R., W. Douglas Shaw, and Robert D. Rowe. " A Discrete-Choice Model of Recreational Participation, Site Choice, and Activity Valuation When Complete Trip Data Are Not Available." *J. Environ. Econ. Manage.* 20(1991):181-201.

[11] Parsons, george R., and mary Jo Kealy. "Randomly Drawn Opportunity Sets in a Random Utility Model of Lake Recreation." *Land Econ.* 68(february, 1992):93-106.

[12] Smith, V. Kerry. "Selection and Recreation Demand." *Amer. J. Agr. Econ.* 70(February, 1988):29-36.

[13] Snyder, Fred.L, David W. Garton, and Maran Brainard. "Zebra Mussels in the Great Lakes: The Invasion and Its Implications." OHSU-FS-045, Ohio Sea Grant.

[14] Stone & Webster Environmental Technology & Services. *Proceedings: Third International Zebra Mussel Conference,1993*, Boston, Massachusetts.

[15] Vizayakumar, K., and P.K.J. Mchapatra "Environmental Impact Analysis of a Coalfield." *J. Environ. Econ. Manage.* 34(1992): 79-103.

[16] Thomas F. Nalepa, and Donald W. Schloesser (ed) *Zebra mussels: Biology, Impacts, and Control*, 1992.

Students Against Zebra Mussels

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Abstract:

Students from 250+ high schools throughout the Midwest are hot on the trail of the latest exotic invader of rivers and lakes. These students are participating in a Zebra Mussel Watch, a program coordinated by the Illinois Natural History Survey and the Water Resources Center at the University of Illinois, in cooperation with the Rivers Curriculum Project. Students place a monitoring device at a local testing site. From early spring through late fall, students visit their site and inspect the samplers for Zebra Mussels.

Besides the actual observation undertaken by students, a case study has been developed for in-depth study to accompany the monitoring. Students first learn about other examples of exotic species and their impacts, then zero in on zebra mussels for a more comprehensive study. They use maps to plot the spread of the mussel and play a decision-making game in which students assume various roles in a hypothetical town that has developed a zebra mussel problem. Finally, they develop and implement an action plan.

Students from 250+ high schools throughout the Midwest are hot on the trail of the latest exotic invader of our rivers and lakes. Students from the Rivers Project are armed with the latest devices for detecting the little monsters and have a place to send the information once they have been spotted. High schools in both Illinois and Minnesota have encountered live Zebra Mussel during the Fall of 1992. Due to the Flood of 93' the Illinois, Ohio, and Mississippi Rivers are now infested with them. If the Zebra Mussel follows its past history, they will be doing their passive little tricks of plugging every water pipe along those rivers and doing it soon.

At Meredosia, Illinois, students from the local Rivers Project high school and their teacher, Janet Franklin, load up in a van each week and travel a short distance to the Central Illinois Public service (CIPS) plant where they check for Zebra Mussels. Two students at the high school, Leslie Kilver and Michelle Smith, needed a river-related project and chose to study Zebra Mussels. Little did they know, they would stay with this project for three years and become actively involved with CIPS. They observed first hand, no Zebra Mussels at CIPS in 1992, and today, in March 1994, there are literally thousands and a real possibility exists that parts of the plant will be closed down to clean out in-take valves clogged by the mussels. Meredosia-Chambersburg High School is one of the many Illinois Rivers Project schools that have teamed up with industry or utilities to become involved in this special project.

The Zebra Mussel Watch is coordinated by the Illinois Natural History Survey(INHS) and the Water Resources Center at the University of Illinois. The Illinois-Indiana Sea Grant Program awarded a $2000 grant to the Illinois Rivers Project to construct Zebra Mussel monitoring devices for each school in the Project. Zebra Mussels are most active during the spring to fall months, which corresponds only somewhat with the school calendar. Therefore, high schools have had to work around the Zebra Mussel's mating and growth season.

Zebra Mussel monitoring works in the following manner: a high school chooses a site(s) in which to place the monitoring devices. Each sampler comes with a set of directions telling how to anchor the device. The Project has found that there is a real attrition, as they seem to disappear - naturally or otherwise. The best way to solve this problem has been for teachers to contact a company, a power or water treatment plant and have the samplers placed on their property. With this cooperation the devices don't disappear and during the summer months when school is not in session, plant officials can check for Zebra Mussels on a biweekly schedule. A great partnership develops between the two groups and students have also educated a few companies through this relationship.

Schools finding Zebra Mussels call Doug Blodgett, (INHS) coordinator of the Zebra Watch, at the Havana, Illinois LTRMP Field Station. Identified Zebra Mussels are placed in a jar of sugar/formalin solution and sent to Doug. A sampler covered with the young veligers, is to be packed and also mailed. Schools use the Project's telecommunications system, SOILED NET, to send their mussel sightings to INHS and the Rivers Project office.

Zebra Mussels seem to present a dismal picture, but research is being done to develop ways to control the invasion. Four methods, biological, chemical, physical, and mechanical controls look promising according to research scientists. Most likely a combination of these methods will help to solve the problem.

The basic goal of the Rivers Project is to educate schools about the importance of water quality. In keeping with this theme, the Rivers Project has developed a one month-long unit on the Zebra Mussel. The case study is based on an issue analysis model developed by Harold Hungerford at Southern Illinois University, Carbondale. In it, four goals are addressed:

1. Introduction to exotic species and their impacts. Topics include well known examples of biological invaders and supplemental reading from popular science periodicals.
2. Detailed consideration of the Zebra Mussel, including the life cycle, feeding habits, economic and ecological effects, and control and preventive measures.
3. Classroom activities, involving plotting the spread of Zebra Mussels, a decision making simulation game, and identification of probable ecological inter-relationships associated with the mussel.
4. Data collection activities including surveying, interviewing, and conducting an action plan to education the local community about Zebra Mussels and other exotic species.

In conjunction with the case study, a one day training session is planned. Teachers will be instructed by Rivers Project teachers who have piloted the case study. These training sessions will be open to high school science and social studies teachers, plus interested junior high school teachers.

If interested in the Zebra Mussel Watch, the case study, or the monitoring devices, contact:

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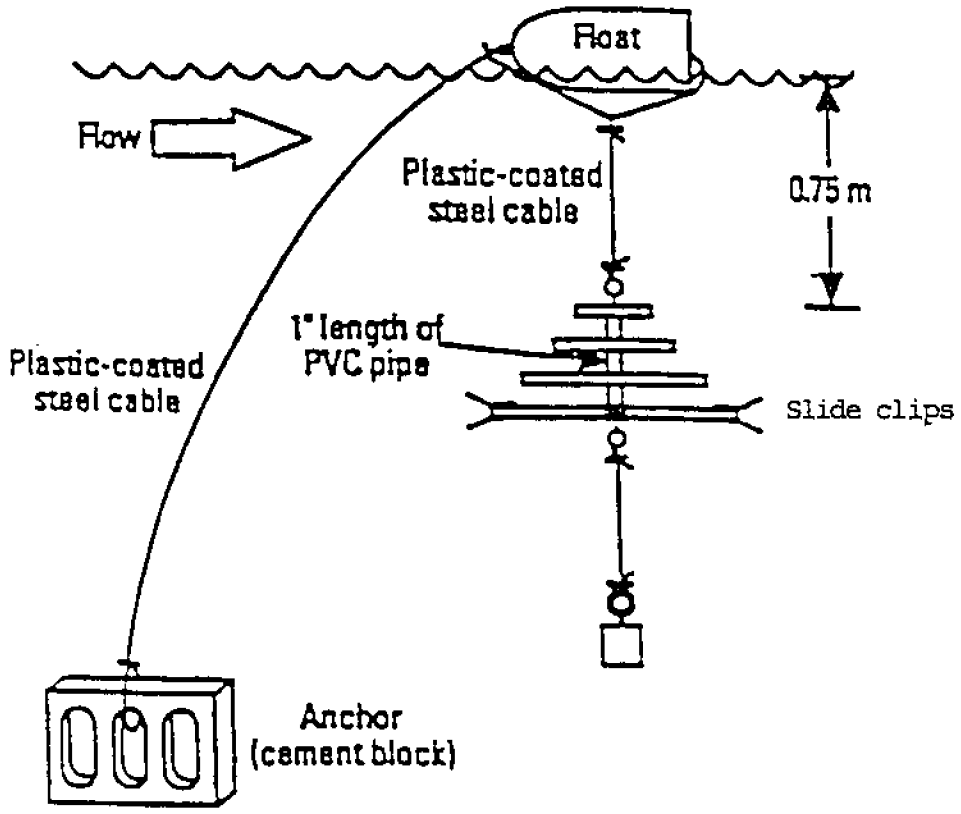
FAX: 618-692-3359
Email rivers@daisy.siue.edu

Zebra Mussel Monitoring Devices



The sampler is basically an artificial substrate, similar to a Hester-Dendy sampler, made of PVC plates. Zebra Mussels and larvae (called veligers) settle on the PVC plates and on the three glass microscope slides that are clipped to the bottom of the PVC plate.

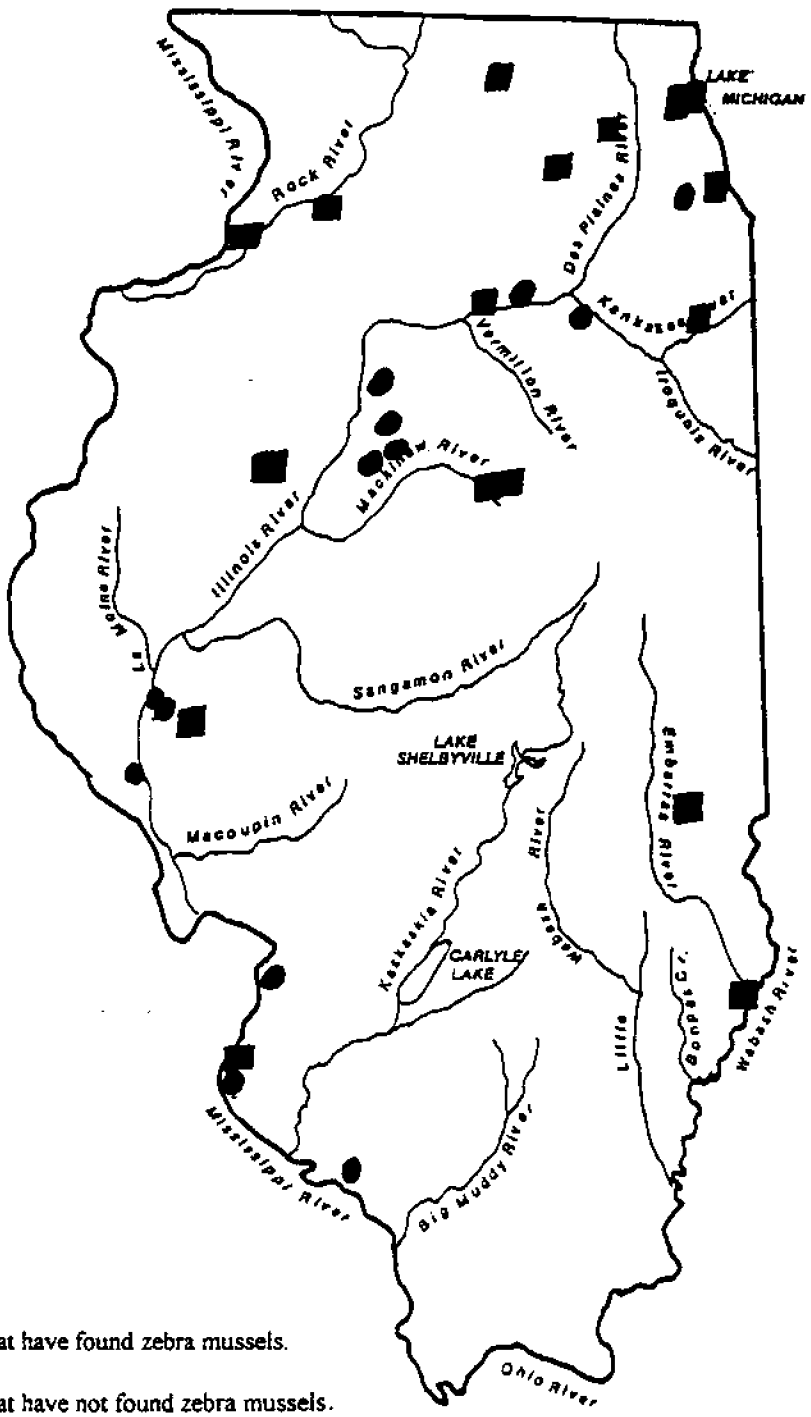
Every two or three weeks, the students revisit the site and inspect the PVC plates for zebra mussels, which are usually very small and may appear only as bumps. Some classes bring along a dissecting microscope to aid in on site identification of mussels. The students also remove the glass slides from the bottom PVC plate and take them back to school, where they use a microscope to examine them for small mussels and veligers. New glass slides are put in place, and the device is lowered back into the water.

The samplers have provided an unexpected, but welcome, additional learning opportunity. At certain times of the year, they may be covered with other organisms. Mayflies, diving beetles, and caddis flies have also been found as have ***Hydra*** *and* ***Planaria****. Students can take these specimens back to lab for identification.*

Zebra Mussel Sample Sheet

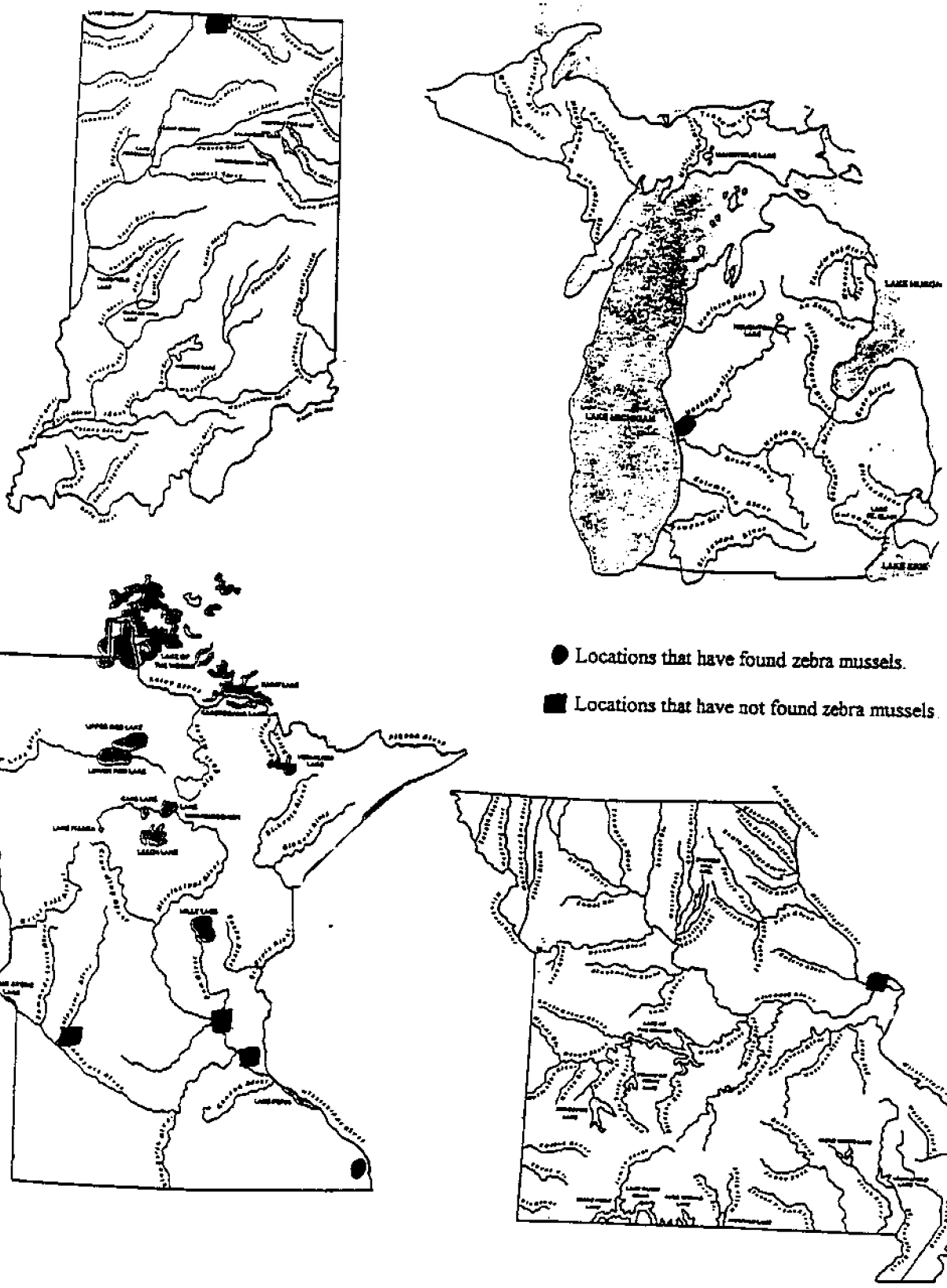
School Name	Joliet Catholic Academy
Address	1200 North Larkin, Joliet, IL 60435
Instructor	Ms. Amy Bokal
River/ Stream Name	Des Plaines River
Nearest Highway/Road/Railway Bridge	Brandon Road
Upstream or Downstream from Bridge	downstream
Miles from Bridge	1.5 mi
Nearest Town	Rockdale
County	Will
Date Deployed	Spring 1993
Sampler Depth (m)	1.0m
Water Depth(m)	3.5m
Date Retrieved	October 14, 1993
Sampler Depth (m)	1.0m
Water Depth(m)	3.5m

Plate #	Surface	Total #	Length in millimeters
1	upper	18	4,5,5,6,5,5,4,6,6,6,3,3,4,4,5,4,7,5
1	lower	8	6,4,3,2,4,3,2,2
2	upper	14	4,4,3,3,5,5,5,5,4,4,6,4,4,2
2	lower	30	3,4,3,4,4,4,5,5,4,5,5,4,4,4,5,6,4,3,3,3,3,3,4,5,4,4,4,2,5,4
3	upper	6	3,2,5,6,7,5
3	lower	5	3,6,5,4,8
4	upper	2	5,4
4	lower	4	5,6,5,4



● Locations that have found zebra mussels.

■ Locations that have not found zebra mussels.


Locations that have found zebra mussels.
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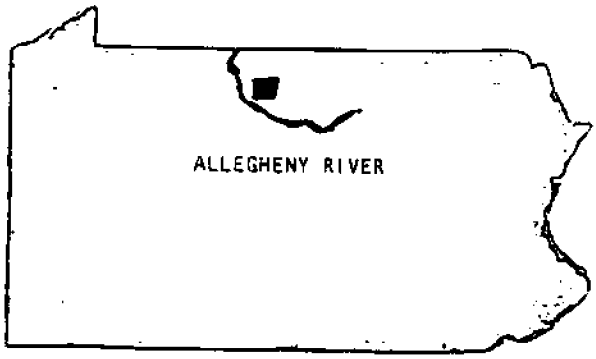

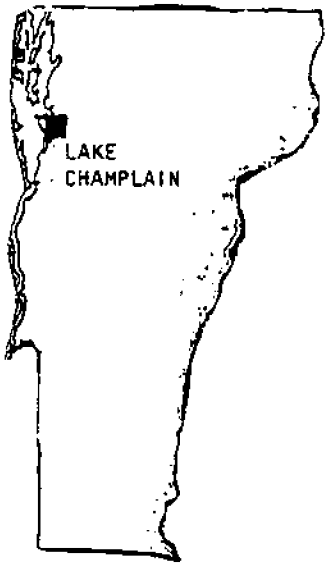

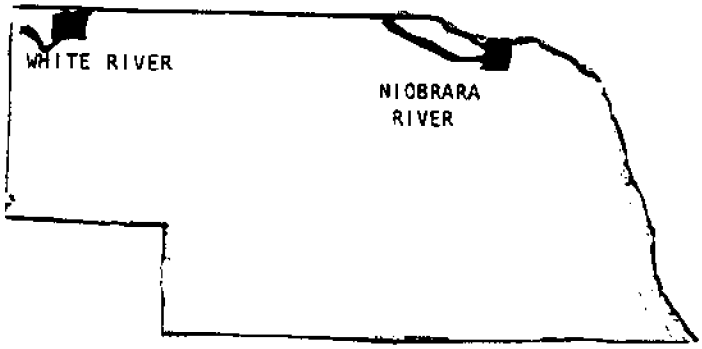

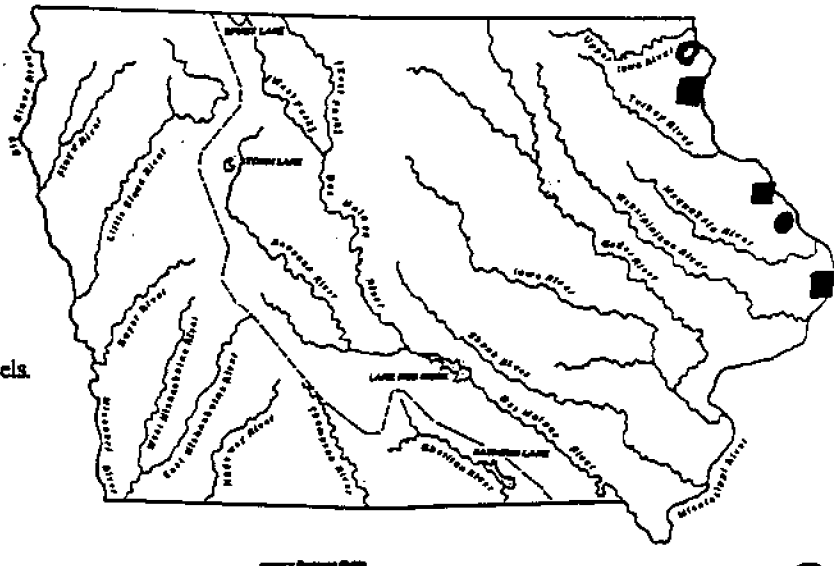

● Locations that have found zebra mussels.

■ Locations that have not found zebra mussels.

"DRAFT"

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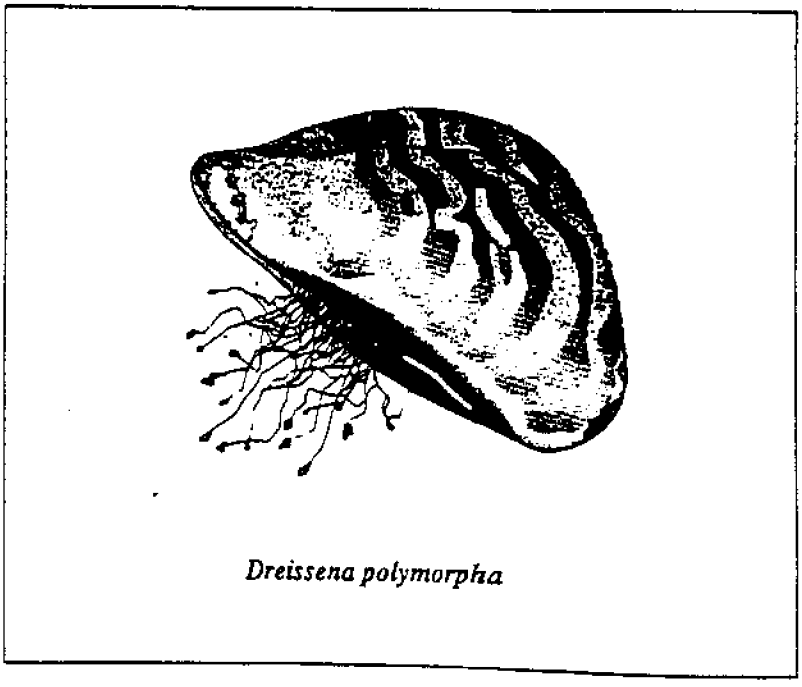
ALIEN INVADERS

A ZEBRA MUSSEL ISSUE INVESTIGATION



Dreissena polymorpha

Rivers Curriculum Project

Southern Illinois University at Edwardsville

APPENDICES

APPENDIX A: CONFERENCE AGENDA

Monday Morning, March 7, 1994

*8:00 a.m. - 12:00 noon *Registration*

Monday Afternoon, March 7, 1994

Plenary Session

1:00 p.m.
* **Conference Overview**
A.H. Miller, *Great Lakes Sea Grant Network, Conference Coordinator*

1:15 p.m.
* **The Invasion Kaleidoscope: Setting the Zebra Mussel Into Patterns of Global Introductions**
J. Carlton, *Williams College - Mystic Seaport*

1:45 p.m.
* **The Zebra Mussel as One of Many: The Nationwide Toll of Harmful Non-Indigenous Species**
P.N. Windle, *U.S. Congress, Office of Technology Assessment*

2:15 p.m.
* **Biological Invasions in the Laurentian Great Lakes and Hudson River Ecosystems: Historical Analysis and Assessment**
E. Mills et al., *Cornell University Biological Field Station*

2:45 - 3:00 p.m.
BREAK

3:00 p.m.
* **The Introduction and Spread of the Zebra Mussel in North America**
C. O'Neill, *New York Sea Grant* and A. Dextrase, *Ontario Ministry of Natural Resources*

3:20 p.m.
* **The Zebra Mussel Invasion - Is A Marine Ecological Perspective Useful?**
J. Levinton, *State University of New York*

3:40 p.m.
* **Implementation of the Non-Indigenous Aquatic Species Nuisance Prevention and Control Act**
G. Edwards, *U.S. Fish and Wildlife Service*

4:00 p.m.
* **Students Against Zebra Mussels**
R. Williams and C. Bidlack, *Southern Illinois University*

4:30 - 8:30 p.m. EXHIBITS OPEN

5:30 - 7:30 p.m. EXHIBITORS' RECEPTION

Tuesday Morning, March 8, 1994

7:00 - 8:15 a.m. Continental Breakfast

Concurrent Session One

BIOLOGY: GENERAL

8:15 a.m.
* **Zebra Mussel Feeding Behavior in Natural Seston: Implications for Phytoplankton Dynamics in Aquatic Systems**
M.J. Horgan et al., *Cornell University Biological Field Station*

8:35 a.m.
* **The Biomechanics of Filter Feeding in Zebra Mussels**
J.D. Ackerman, *JDA Associates and University of Toronto*

8:55 a.m.
* **Tissue Solute Regulation: Transport and Metabolism of Organic and Inorganic Solutes by *Dreissena polymorpha***
R.L. Preston, *Illinois State University*

9:15 a.m.
* **A Comparison of Shell Morphology of Zebra Mussels and Co-occurring Bivalve Larvae: Potential Use for Identification**
B. Baldwin et al., *Rutgers University*

9:35 a.m.
* **Does Temporal Scale Matter When Measuring Settlement?**
S. Doka and G.L. Mackie, *University of Guelph*

9:55 a.m.
* **Production and Biomass Allocation in *Dreissena polymorpha* in the Lower Great Lakes, Canada**
M.E. Chase and R.C. Bailey, *University of Western Ontario*

CONTROL: CHEMICAL

8:15 a.m.
* **Current Status of Zebra Mussel Control Measures in Ontario**
B. Chauhan and S. Pengilley, *Ontario Ministry of Environment and Energy*

8:35 a.m.
* **Molluscicidal Efficacy of Non-oxidizing Antimicrobial Agents to Control Adult Zebra Mussels**
J.C. Petrille et al., *Betz Water Management Group*

8:55 a.m.
* **Toxicity of Monochloramine to Zebra Mussels**
M. Balcer et al., *University of Wisconsin-Superior*

9:15 a.m.
* **Evaluation of Copper Ions and Aluminum Floc for Preventing Settlement of Zebra Mussels**
W.J. Blume et al., *MacroTech Inc.*

9:35 a.m.
* **TD 2335: Laboratory and Field Efficacy Studies for Control of Zebra Mussels in Electric Power Plants**
V.J. Piccirillo et al., *NPC Inc.*

9:55 a.m.
* **Relative Toxicity of Molluscicides to Different Developmental Stages of Quagga and Zebra Mussels**
H. Dabrowska et al., *Ohio State University*

10:15 - 10:30 a.m. BREAK

Tuesday Morning, March 8, 1994

Concurrent Session Two

BIOLOGY: GENERAL

10:30 a.m.
* **From Egg to Settling Stage: The Culture and Nutritional Requirements of *Dreissena* Larvae**
H.A. Vanderploeg et al., *NOAA-GLERL*

10:50 a.m.
* **Particle Sizes and Settling Velocities of *Dreissena polymorpha* Biodeposits**
D.M. Dean and G.L. Mackie, *University of Guelph*

11:10 a.m.
* **Vertical Migration and Mortality in Dense Zebra Mussel Colonies as a Response to Changes in Interstitial Water Quality**
C.A. Call, *Loyola University of Chicago*

11:35 a.m.
* **Settling and Growth of *D. polymorpha* in the Raw Water Circuits of the Cattenom Nuclear Power Plant (Moselle, France)**
J. C. Moreteau and M. Khalanski, *Université de Metz*

11:50 a.m.
* **Year-to-Year Variation in Condition and Reproductive Pattern of Zebra Mussels**
S.Y. Wang et al., *University of Southern Mississippi*

CONTROL: CHEMICAL

10:30 a.m.
* **Carbon Dioxide as a Narcotizing Pre-Treatment for Chemical Control of *Dreissena polymorpha***
W.J. Elzinga and T. S. Butzlaff, *Environmental Science and Engineering Inc.*

10:50 a.m.
* **Optimizing the Use of Hypochlorite in Zebra Mussel Control**
D.W. Evans and B.F. Sim, *Ontario Hydro Technologies*

11:10 a.m.
* **Enhanced Toxicity of Zebra Mussel Control Chemicals in Pure Water**
J.L. Ram et al., *Wayne State University*

11:30 a.m.
* **The Impact of Zebra Mussels and Their Control Measures on the Corrosion of Cooling Water System Materials**
C.T. Gauthier et al., *Ontario Hydro Nuclear*

11:50 a.m.
* **Control of Zebra Mussels in Hatcheries Using Aquaculture Chemicals**
D.L. Waller et al., *National Fisheries Research Center*

12:10 - 2:00 p.m. LUNCH & EXHIBITS OPEN

Tuesday Afternoon, March 8, 1994

Concurrent SessionThree

BIOLOGY: PHYSIOLOGY

2:00 p.m.
* **Physiological Energetics of *Dreissena polymorpha* at High Temperatures**
D.W. Aldridge et al., *North Carolina A&T State University*

2:20 p.m.
* **Comparative Study of the Desiccation Resistance of Zebra Mussels and Quagga Mussels**
T.A. Ussery and R.F. McMahon, *The University of Texas at Arlington*

2:40 p.m.
* **Potassium Transport in the Freshwater Bivalve *Dreissena polymorpha***
S.J. Wilcox and T.H. Dietz, *Louisiana State University*

3:00 p.m.
* **Growth and Reproductive Output at Different Environmental Conditions: Does Food Quality Matter?**
A.M. Stoeckmann and D.W. Garton, *Ohio State University*

3:20 p.m.
* **The Effects of Calcium and Alkalinity on the Reproductive Success of Adult Zebra Mussels**
S. Hincks and G.L. Mackie, *University of Guelph*

3:40 p.m.
* **Temperature/Salinity Tolerance in Larvae of Zebra Mussels, *Dreissena polymorpha*, and its Potential Impact in Northern Chesapeake Bay**
D.A. Wright et al., *University of Maryland*

CONTROL: CHEMICAL AND ELECTRICAL

2:00 p.m.
* **Effects of MEXEL 432 on the Settling, Detachment and Mortality of Adult Zebra Mussels**
L. Giamberini et al., *Université de Metz*

2:20 p.m.
* **Development of Zebra Mussel Control Programs Utilizing Chlorine, Acti-brom and Bromine**
P.M. Sawyko, *Rochester Gas and Electric Corporation*

2:40 p.m.
* **Application of Cost Effective Electric Fields to Deter Attachment of Zebra Mussel to Structures**
A.G. Smythe et al., *Acres International Corporation*

3:00 p.m.
* **Use of Cathodic Protection for Control of Zebra Mussel Biofouling on Steel and Concrete Surfaces**
N. Serli et al., *Hydro Quebec*

3:20 p.m.
* **The Efficacy of Pulsed Electric Fields in Preventing Settlement of Zebra Mussel Veligers**
J.R. Nelson et al., *The Seasonic Group Inc.*

3:40 p.m.
* **Study of Effects of Electric Currents on Zebra Mussel Veligers**
R.E. Baddour et al., *The University of Western Ontario*

4:00 - 4:30 p.m. BREAK

"A TASTE OF WISCONSIN"

4:30 - 6:00 p.m. Buses depart from Holiday Inn for the UW Memorial Union
Dinner on own - State Street

7:30 - 9:30 p.m. Dessert Buffet with Entertainment - UW Memorial Union
"The Torpedoes" - A lively band specializing in dance music from the 1950's and 1960's
"Randy Sabien" - Jazz Quartet featuring jazz violinist, Randy Sabien

9:30 p.m. **Buses depart from UW Memorial Union to hotels**

Wednesday Morning, March 9, 1994

7:00 - 8:15 a.m. Continental Breakfast

Concurrent Session Four

BIOLOGY: RIVERS AND INLAND LAKES

8:15 a.m.
* **Ecological Constraints on Dreissenid Mussels in Lotic Versus Lentic Ecosystems**
J.H. Thorp and J.E. Alexander, Jr., *University of Louisville*

8:35 a.m.
* **Growth and Survivorship of Zebra Mussels and Quagga Mussels in an Ohio River Mesocosm**
J.E. Alexander, Jr. and J.H. Thorp, *University of Louisville*

8:55 a.m.
* **Energetics of Zebra Mussels Under Various Food and Sediment Regimes: Implications for Large River Systems**
S.P. Madon et al., *Illinois Natural History Survey*

9:15 a.m.
* **Settlement, Growth Rate, and Habitat Colonization of Zebra Mussels in the Upper Mississippi River**
W.G. Cope and T.J. Naimo, *National Biological Survey*

9:35 a.m.
* **The Spread of Zebra Mussels (*Dreissena polymorpha*) Through the Inland Waterway System, 1993**
A.C. Miller et al., *U.S. Army Engineers Waterways Experiment Station*

9:55 a.m.
* **Saint Croix River Zebra Mussel Response Plan - 1993**
P. Burke, *U.S. Fish & Wildlife Service*

CONTROL: NON-CHEMICAL

8:15 a.m.
* **Further Studies of Heat Tolerance in Zebra Mussels: Effects of Chronic Lethal Temperatures and Geographical Distribution on Survival Time**
R.F. McMahon et al., *The University of Texas at Arlington*

8:35 a.m.
* **Acoustic Energy for Zebra Mussel Control**
J.J. Kowalewski et al., *Ontario Hydro Technologies*

8:55 a.m.
* **Cavitation Control of the Zebra Mussels**
D.M. Donskoy and M.L Ludyanskiy, *Stevens Institute of Technology*

9:15 a.m.
* **Mechanical Filtration as a Control Measure for Zebra Mussels - Phase 2**
R. Koopmans and R.W. Hughes, *Ontario Hydro Technologies*

9:35 a.m.
* **Operational Comparison of Two Types of Small-Pore Mechanical Filters for the Removal of Zebra Mussels**
B.W. Rigden, *Ontario Hydro*

9:55 a.m.
* **New Infiltration Intake System for Zebra Mussel Control and Larval Exclusion**
Y. Mussalli et al., *Stone & Webster Engineering Corp.*

10:15 - 10:30 a.m. BREAK

Wednesday Morning, March 9, 1994

Concurrent Session Five

BIOLOGY: RIVERS AND INLAND LAKES

10:30 a.m.
* **Zebra Mussels in the Illinois River and Implications for Native Molluska in the Mississippi Basin**
K.D. Blodgett et al., *Illinois Natural History Survey*

10:50 a.m.
* **Impact of Fouling by *Dreissena polymorpha* on Unionids of Lake Wawasee, Indiana**
D.W. Garton, *Indiana University-Kokomo*

11:10 a.m.
* **Infestation and Impacts of *Dreissena* on Native Unionids in the Upper St.Lawrence River**
A. Ricciardi, *McGill University*

11:30 a.m.
* **The Potential Overland Dispersal of Zebra Mussels by Waterfowl**
L. Johnson et al., *Williams College - Mystic Seaport*

11:50 a.m.
* **The Spread of Zebra Mussels to Inland Waters: Advances and Lessons fromMichigan**
P. Marangelo et al., *Williams College - Mystic Seaport*

CONTROL: NON-CHEMICAL

10:30 a.m.
* **Influence of Wide-Range Ultraviolet Radiation Upon Behavior and Mortality of *Dreissena polymorpha***
L. Chalker-Scott et al., *State University of New York at Buffalo*

10:50 a.m.
* **Use of Ultraviolet Radiation for Zebra Mussel Control**
D. Lewis and G.E. Whitby, *Aquatic Sciences Inc.*

11:10 a.m.
* **Underwater Cleaning Technique for Removal of Biofouling**
B.R. Hobbs and A.C. Gross, *Seaward Marine Services Inc.*

11:30 a.m.
* **The Survival of Zebra Mussels (*Dreissena polymorpha*) and Asian Clams (*Corbicula fluminea*) Exposed to Extreme Hypoxia**
M.A. Matthews and R.F. McMahon, *The University of Texas at Arlington*

11:50 a.m.
* **Discussion of Siphon Intake for Control of Zebra Mussels**
T.J. Chang, *Ohio University*

12:10 - 2:00 p.m. LUNCH

1:00 - 2:00 p.m. POSTER SESSION OPENS

Wednesday Afternoon, March 9, 1994

Poster Session

1:00 - 2:00 P.M. & 4:00 - 7:00 P.M.

Poster #1
* **Robotics Applications in the Aquatic Environment: Industrial Biofouling**
Bill Sherwood, *Aquatic Sciences Inc.*

Poster #2
* **Modification of an Optical Plankton Counter for Enumerating Veligers: Preliminary Results**
B.F. Sim et al., *Ontario Hydro Technologies*

Poster #3
* **Automatic Zebra Mussel Control System at Casco Co., Port Colborne, Ont.**
John Hibberd, *Aquatic Sciences Inc.*

Poster #4
* **The Implications of Patented Technology for Zebra Mussel Control and Eradication**
T.D. Paulius, *Lockwood, Alex, Fitzgibbon & Cummings*

Poster #5
* **Chlorination/Dechlorination Equipment Specification and Control System Design for Zebra Mussel Suppression**
G. deBruyn, *Prominent Fluid Controls, Inc.*

Poster #6
* **Chlorine Dioxide - A Molluscicidal Agent for Adult Zebra Mussel Eradication**
L. Rusnak, *Drew Industrial*

Poster #7
* **Candidate Antioxidants for Preventing Zebra Mussel Attachment Toxicity to Fish**
W.G. Cope and M.R. McPeak, *National Fisheries Research Center*

Poster #8
* **Levels of Copper in Indigenous and Transplanted *Dreissena polymorpha* in Relation to Water Concentration and Body Weight**
J. Mersch and J.C. Pihan, *Université de Metz*

Poster #9
* **Biodeposition and Accumulation of Polychlorinated Biphenyls and Cadmium by Zebra Mussel *(Dreissena polymorpha)* in Western Lake Erie**
E. Dobson and G.L. Mackie, *University of Guelph*

Poster #10
* **Contaminants and the Zebra Mussel *Dreissena polymorpha*: Review of Recent Data in Relation to Disposal Practices**
H.E. Tatem, *U.S. Army Engineer Waterways Experiment Station*

Poster #11
* **Use of Low Levels of Electric Current (A-C) for Controlling Zebra Mussels**
C. Fears et al., *Delta Applied Technology, Inc.*

Poster #12
* **Resistance to Zebra Mussel Attachment Using Copper Alloys**
D. Maxson, *Wheelabrator Engineered Systems Inc.*

Poster #13
* **Comparison of Byssal Attachment Strength of *Dreissena polymorpha* Under Natural and Laboratory Conditions**
B.S. Payne et al., *U.S. Army Engineers Waterways Experiment Station*

Poster #14
* **Fluid Dynamic Influences on the Recruitment of Zebra Mussels**
J.D. Ackerman, *JDA Associates and University of Toronto* and B.S. Sim, *Ontario Hydro Technologies*

Poster #15
* **Potential for Biological Control of the Zebra Mussel, *Dreissena polymorpha*, by the Blue Crab, *Callinectes sapidus***
L.C. Boles and R.N. Lipcius, *Virginia Institute of Marine Science*

Poster #16
* **Prey Preference of Redear Sunfish on Zebra Mussels and Gastropods in Experimental Aquaria**
J.R.P. French III, *National Biological Survey* and M.N. Morgan, *Michigan State University*

Poster #17
* **Identification of the Great Lakes Quagga Mussel as *Dreissena bugensis*, Introduced From the Black Sea Drainage in Ukraine**
A.P. Spidle et al., *Cornell University*

Poster #18
* **Relative Abundance of Zebra Mussels (*Dreissena polymorpha*) and Quagga Mussels (Dreissena bugensis) in Eastern Lake Erie**
D.J. Adrian et al., *AquaTech Environmental, Inc.*

Poster #19
* **Monitoring Zebra Mussel Young Stages Near Hydroelectric Dams and Power Stations**
B. Jacquaz et al., *Laboratoires SAB, Inc.*

Poster #20
* **Dreissenid Population Dynamics in the St. Lawrence River: Perspectives for Engaging Control Actions in Industries**
J. Dion and Y. Richer, *Subdev Canada, Inc.*

Poster #21
* **Annual and Seasonal Variations in Veliger Density in the Upper Niagara River**
D.J. Adrian et al., *AquaTech Environmental, Inc.*

Poster #22
* **Zebra Mussel Colonization of Saginaw Bay Coastal Emergent Marshes**
T.M. Burton et al., *Michigan State University*

Poster #23
* **Zebra Mussel Population Dynamics in Indiana Waters of Lake Michigan**
T.E. Lauer et al., *Purdue University*

Poster #24
* **Zebra Mussel Watch: Distribution and Abundance of Zebra Mussels in Wisconsin**
J.J. Hieb et al., *University of Wisconsin-Madison*

Poster #25
* ***Dreissena* Settlement on Natural and Anthropogenic Substrates in the Southern Bay of Green Bay, Lake Michigan**
H. Hanson and T. Mocco, *Oconto High School*

Poster #26
* **Distribution and Abundance of Zebra Mussels in the Duluth-Superior Harbor of Lake Superior**
M. Balcer, *University of Wisconsin-Superior*

Poster #27
* **Zebra Mussel Dispersal in the St. Joseph River Basin (Indiana-Michigan): Lakes as Sources for Downstream Dispersal**
T.G. Horvath et al., *University of Notre Dame*

Poster #28
* **Early Colonization Patterns of the Ohio River by Zebra Mussels**
J.H. Thorp and J.E. Alexander, Jr., *University of Louisville*

Poster #29
* **Eye on the Zebra Mussel: Settlement and Growth in the Ohio River**
J. Stice, *Dayton Power & Light Company*

Poster #30
* **Observations on Zebra Mussel Colonization in the Lower Ohio and Tennessee Rivers**
J.B. Sickel and D.A. Leek, *Murray State University*

Poster #31
* **Impact of Zebra Mussels on Inland Shipping**
J.H. Boy et al., *U.S. Army Corps of Engineers*

Poster #32
* **Effect of the Zebra Mussel on Recreational Participation at Lake Erie**
L.J. Hushak and J. Vilaplana, *Ohio State University*

Poster #33
* **Evaluation of the Economic Impacts of Zebra Mussels on the Recreational Demand in Lake Erie**
J-F Sun, *Ohio State University*

Poster #34
* **Encouraging the Public to Help Slow The Spread of Zebra Mussels**
B. MacKay, *Ontario Federation of Anglers and Hunters*

Poster #35
* **Zebra Mussel Awareness and Boat Use Patterns Among Boaters Using Three "High Risk" Connecticut Lakes**
N.C. Balcom, *University of Connecticut* and E.M. Rohmer, *Oberlin College*

Poster #36
* **Minnesota State Actions to Prevent Dispersal of Zebra Mussels and Other Aquatic Nuisance Species**
J. Rendall, *Minnesota Department of Natural Resources*

Poster #37
* **Students Against Zebra Mussels**
C. Bidlack and R. Williams, *Southern Illinois University*

Poster #38
* **Are Unionid Translocations a Viable Mitigation Technique? The Wolf River Experience, Shawano, WI, August 1992 and August 1993**
M.E. Havlik, *Malacological Consultants*

Poster #39
* **Mussel Community Structure in the Upper St. Croix River Prior to Zebra Mussel Infestation**
D.J. Hornbach et al., *Macalester College*

Poster #40
* **Water Flow Around *Dreissena polymorpha* Encrusted *Anodonta grandis* in a Lake System**
M.E. Benbow et al., *University of Dayton*

Poster #41
* **Observation of Freshwater Sponges on Zebra Mussels in Lake Erie**
F.L. Snyder and D.O. Kelch, *Ohio State University Extension Sea Grant Program*

Poster #42
* **An Approach to Identify Potential Zebra Mussel Colonization in Large Water Bodies Using Best Available Data and Geographic Information Systems**
A. Ignacio and A.H. Miller, *University of Wisconsin-Madison*

Poster #43
* **Identifying Potential Zebra Mussel Habitat in Maryland**
J. Chaillou, *Versar, Inc.* and J. Christmas, *Maryland Department of Natural Resources*

Poster #44
* **Modeling the Spread of Exotic, Invading Species Across a Landscape Using a Geographic Information System**
L.A. Buchan and D.K. Padilla, *University of Wisconsin-Madison*

Poster #45
* **Eurasian Watermilfoil Spread Across U.S. and Wisconsin**
S. Engel, *Wisconsin Department of Natural Resources*

Poster #46
* **Boom and Bust: Invasion of a Hardwater, Eutrophic Lake by the Introduced Crayfish *Orconectes rusticus***
H. Pearson, *Silver Lake College*

Poster #47
* **Ruffe, *Gymnocephalus cernuus*: Impacts on Native Fauna and Control Techniques**
T.D. Bills et al., *National Biological Survey*

Poster #48
* **The Influence of Zebra Mussels on Sport Fisheries in a Thermally-Stratified Reservoir**
N-Y Yu and D.A. Culver, *Ohio State University*

Poster #49
* **Interactive Effects of Zebra Mussels, Young Bluegills, and Water Retention Time on Experimental Food Webs: A Mesocosm Experiment**
W.B. Richardson and L.A. Bartsch, *National Biological Survey*

Poster #50
* **Ecosystem-level Consequences of the Zebra Mussel Invasion of North America: A Modeling Approach**
J.R. Duncan, *University of Tennessee*

Poster #51
* **Influence of Seston Quality on Zebra Mussel Energy Stores**
W.A. Brence et al., *University of Cincinnati*

Poster #52
* **Measures of Allometric Growth in the Zebra Mussel, *Dreissena polymorpha* and the Quagga Mussel, *Dreissena (rostriformis) bugensis***
T.K. Ross and G.M. Lima, *Illinois Wesleyan University*

Poster #53
* **Effects of Starvation at Different Temperatures on Dry Tissue and Dry Shell Weights in the Zebra Mussel, *Dreissena polymorpha* (Pallas)**
R.A. Chase and R.F. McMahon, *The University of Texas at Arlington*

Poster #54
* **^{31}P Nuclear Magnetic Resonance (NMR) Phospholipid Profiles of Healthy and Compromised Zebra Mussels**
L.G. Bardygula-Nonn, *University of Wisconsin-Milwaukee* and T. Glonek, *Midwestern University*

Poster #55
* **Laboratory Maintenance of the Zebra Mussel, *Dreissena polymorpha*: Effects on Ion Balance**
J.I. Scheide et al., *Central Michigan University*

Poster #56
* **Magnesium and Potassium Requirement by the Freshwater Zebra Mussel, *Dreissena polymorpha***
T.H. Dietz et al., *Louisiana State University*

Poster #57
* **Chemical Regulation of Zebra Mussel Siphon and Mantle Contractility**
F.F. Baidoun and J.L. Ram, *Wayne State University*

Poster #58
* **Species-Specific Sperm Attraction in Zebra Mussels and Quagga Mussels**
R.L. Miller et al., *Temple University*

Poster #59
* **Cytochemistry of the Foot of *Dreissena polymorpha* (Mollusca: Bivalvia)**
T.P. Bonner and R.L. Rockhill, *SUNY College at Brockport*

Poster #60
* **Mitochondrial DNA Analysis of Round Goby and Tubenose Goby in the Great Lakes**
J. Dougherty, *Wayne State University*

Poster #61
* **A Method for the Isolation of Total Genomic DNA from the Zebra Mussel, *Dreissena polymorpha***
R.L. Minton and G.C. Mayer, *University of Wisconsin-Parkside*

Poster #62
* **Genotype-Dependent Fecundity at the PGI Locus in *Dreissena polymorpha***
S.L. Kendall-Eagleson and D.W. Garton, *Indiana University Kokomo*

Poster #63
* ***Dreissena* as a Bioindicator of Radioactive Contamination in Freshwater Ecosystems**
I.V. Parkov, *Kiev Institute of Hydrobiology*

Poster #64
* ***Dreissena* in Cooling Ponds of Power Plants in Ukraine: Structure-Functional Aspect**
A.A. Protasov and O.O. Sinicina, *Kiev Institute of Hydrobiology*

Poster #65
* **The Role of Zebra Mussels in Lake Ecosystems**
A. Karatayev, *Belorussian State University*

Poster #66
* **Basic Control Methods for Water-Development Works Fouled with *Dreissena genus* Mollusks**
L.V. Shevtsova, *Kiev Institute of Hydrobiology*

Poster #67
* **The Chemical Control of *Dreissena* Mollusks by Chlorination**
I.A. Grigorovich, L.V. Shevtsova and K.N. Tsaplina, *Kiev Institute of Hydrobiology*

Wednesday Afternoon, March 9, 1994

Concurrent Session Six

BIOLOGY: *DREISSENA BUGENSIS* ("QUAGGA MUSSELS")

2:00 p.m.
* **Identification of the Quagga Mussel as *Dreissena bugensis* Andrusov**
G. Rosenberg and M. Ludyanskiy, *Academy of Natural Sciences of Philadelphia*

2:20 p.m.
* **The Ecology of *Dreissena bugensis* and *D. polymorpha* in the Dnieper River Drainage System**
M. Ludyanskiy et al., *Marine Biocontrol Corporation*

2:40 p.m.
* **Comparative Growth Rates of Zebra Mussels and Quagga Mussels**
H.J. MacIsaac, *University of Windsor*

3:00 p.m.
* **Functional Differences Between the Quagga Mussel, *Dreissena bugensis*, and the Zebra Mussel, *Dreissena polymorpha*, to Stress from Temperature and Salinity Changes**
A.P. Spidle and E.L. Mills, *Cornell University*

3:20 p.m.
* **Abundance and Distribution of Dreissenid Mussels at Nanticoke, Lake Erie: Effects of Depth and Temperature**
J. Mitchell et al., *University of Western Ontario*

3:40 p.m.
* **A Comparison of Zebra and Quagga Mussel Reproduction, Abundance, and Growth**
W.T. Claxton, *University of Guelph*

CONTROL: BIOLOGICAL

2:00 p.m.
* **Biological Control of Zebra Mussels by Indigenous Bacteria and Their Products**
J-D. Gu et al., *Harvard University*

2:20 p.m.
* **Effect of Starvation Stress on the Heterotrophic Bacterial Flora of the Zebra Mussel, *Dreissena polymorpha***
J.S. Maki and R. Mitchell, *Marquette University*

2:40 p.m.
* **The Effect of Molluscicidal Strains of *Bacillus brevis*/SS86-4 on *Dreissena polymorpha***
S. Singer and E.E Genovese, *Western Illinois University*

3:00 p.m.
* **"Birth Control" in Zebra Mussels: High Affinity, Sex-Specific, and Long-Lasting Effects of Serotonergic Ligands**
P.P. Fong et al., *Wayne State University*

3:20 p.m.
* **Invading the Invaders: Infestation of Zebra Mussels by Native Parasites in the St. Lawrence River**
D.B. Conn et al., *The University of the South*

3:40 p.m.
* **Survey of Endosymbionts Present in Russian Zebra Mussels**
D.P. Molloy et al., *New York State Museum*

4:00 - 7:00 p.m. POSTER SESSION RESUMES

5:30 - 7:00 p.m. POSTER SESSION RECEPTION & CASH BAR

8:00 p.m. ALL CANADA NIGHT
Sponsored by Ontario Hydro
(Please come "dressed as a Canadian, eh?")

Thursday Morning, March 10, 1994

7:00 -8:15 a.m.Continental Breakfast

Concurrent Session Seven

BIOLOGY: ECOLOGICAL IMPACTS

8:15 a.m.
* **Trends in Zebra Mussel Populations in Saginaw Bay and Subsequent Changes in Water Quality, 1991-1993**
T.F. Nalepa, *NOAA-GLERL*

8:35 a.m.
* **Do Zebra Mussels (*Dreissena polymorpha*) Affect Algal-Bacterial Coupling in Saginaw Bay?**
R.T. Heath and S-J Hwang, *Kent State University*

8:55 a.m.
* **Post-*Dreissena* Planktonic Diatoms in Hatchery Bay, Western Lake Erie, and Saginaw Bay, Lake Huron**
R.E. Holland (Beeton), *The University of Michigan*

9:15 a.m.
* **The Impact of Zebra Mussels on the Benthic Algal Communities in Saginaw Bay, Lake Huron**
R.W. Pillsbury and R.L. Lowe, *Bowling Green State University*

9:35 a.m.
* **Zebra Mussel-Mediated Benthic Algal Blooms in Saginaw Bay, Lake Huron: Are There Limits?**
R.L. Lowe and R.W. Pillsbury, *Bowling Green State University*

9:55 a.m.
* **Effects of Zebra Mussel Density on the Growth of Juvenile Fathead Minnow *Pimephales promelas***
C.A. Jennings, *National Biological Survey*

CONTROL: CASE STUDIES

8:15 a.m.
* **Eliminating Zebra Mussels at a Major Steel Mill: A Case Study**
R.L. Strand, *Stranco Inc.*

8:35 a.m.
* **The Ontario Hydro Final Strategy for Zebra Mussel Control**
P.M. Wiancko and R. Claudi, *Ontario Hydro*

8:55 a.m.
* **Cationic Surfactants for Zebra Mussel Control in Complex Intake/Makeup System - WEPCO Pleasant Prairie**
R.R. Goldmann et al., *Wisconsin Electric Power Company*

9:15 a.m.
* **Niagara Mohawk Research Efforts for Monitoring, Assessment and Control of Zebra Mussels in Cooling and Fire Water Systems**
E. Neuhauser et al., *Niagara Mohawk Power Company*

9:35 a.m.
* **Case History of a Working Zebra Mussel Chlorination System**
B. Polewski et al., *Ontario Hydro*

9:55 a.m.
* **Zebra Mussel Control Using Thermal Treatment of Circulating Water Systems of Electric Utility Stations**
S.L. Wahlert et al., *Sargent & Lundy Engineers*

10:15 - 10:30 a.m. BREAK

Thursday Morning, March 10, 1994

Concurrent Session Eight

BIOLOGY: ECOLOGICAL IMPACTS

10:30 a.m.
* **Response of Submersed Macrophytes to Increased Water Clarity During Colonization by Zebra Mussels in Saginaw Bay, Lake Huron**
J.P. Skubinna et al., *Michigan State University*

10:50 a.m.
* **Dreissenid Mussels in the Diet of Several Species of Ducks at Long Point, Lake Erie**
R.W. Knapton, *Long Point Waterfowl and Wetland Research Fund*

11:10 a.m.
* **Effect of Zebra Mussels on the Early Succession of Periphyton and Macrophytes**
P. Gerovac and J.L. Kaster, *University of Wisconsin-Milwaukee*

11:30 a.m.
* **Impact of Zebra Mussels on the Gastropod Fauna of Lake Michigan**
D.W. Schneider et al., *Illinois Natural History Survey*

11:50 a.m.
* **An Ecosystem Approach to Examining the Effects of Zebra Mussels on Lake Erie Pelagic Function**
R.A. Pontius and D.A. Culver, *Ohio State University*

CONTROL: CASE STUDIES

10:30 a.m.
* **Development of Zebra Mussel Control Strategies for a Coalition of Vermont Water Suppliers on Lake Champlain**
C.L. Lange et al., *Acres International Corporation*

10:50 a.m.
* **The Successful Use of Potassium Permanganate for Zebra Mussel Control in a Water Supply Intake: A Case Study**
J.A. DeKam, *Bay Metro Water Treatment Plant*

11:10 a.m.
* **The First Non-oxidizing Molluscicide Treatment in Canada**
R.C. Matthews and D.A. Comand, *Calgon Canada Inc.*

11:30 a.m.
* **A Cost-Effective Alternative to Continuous Chemical Feed Systems for Control of Zebra Mussels**
N. Kothari, *Manitowoc Public Utilities*

11:50 a.m.
* **Evaluation of a Raw Water System Specifically Designed for Mussel Control at Monroe, Michigan**
W.L. LePage, *City of Monroe, Michigan*

12:10 - 1:30 p.m. LUNCH

Thursday Afternoon, March 10, 1994

Concurrent Session Nine

BIOLOGY: ECOLOGICAL IMPACTS

1:30 p.m.
* **The Importance of Size-Frequency Distributions for Predicting Zebra Mussel Impact**
B.L. Young et al., *Daemen College*

1:50 p.m.
* **Zebra Mussel (*Dreissena polymorpha*) Impact on the Western Lake Erie Ecosystem: a Bioenergetics Approach**
C.P. Madenjian, *National Biological Survey*

2:10 p.m.
* **Uncertainty in Zebra Mussel Growth Estimates: Implications for Incorporating Zebra Mussels into Food Web Models**
N. Idrisi and D. Stewart, *State University of New York*

2:30 p.m.
* **Impact of Zebra Mussels on Lake Erie Pelagic Food Webs**
D.A. Culver and R.A. Pontius, *Ohio State University*

CONTROL: COATINGS AND ATTACHMENT

1:30 p.m.
* **Zebra Mussel Facilitated Corrosion at Black Rock Lock**
E.G. Segan et al., *U.S. Army Construction Engineering Research Laboratories*

1:50 p.m.
* **Coatings Which Prevent Zebra Mussel Attachment**
E.G. Leitch and F.S. Spencer, *Ontario Hydro Technologies*

2:10 p.m.
* **Testing of Anti-Zebra Mussel Coatings in TVA Service Area Waters**
M.L. Rollins and J.T. Johnson, *Tennessee Valley Authority*

2:30 p.m.
* **Field Trials of Non-Toxic Fouling-Release Coatings**
A.E. Meyer et al., *State University of New York*

2:50 - 3:10 p.m. BREAK

Thursday Afternoon, March 10, 1994

Concurrent Session Ten

BIOLOGY: CONTAMINANT BIOACCUMULATION

3:10 p.m.
* **Trophic Transfer of PCBs from Zebra Mussel Feces to the Benthic Invertebrate, *Gammarus fasciatus***
K.A. Bruner et al., *Ohio State University*

3:30 p.m.
* **The Use of *Dreissena polymorpha* as a Biofilter of Municipal Wastewater and Resulting Metal Bioaccumulation**
J.P. Selegean and T.M. Heidtke, *Wayne State University*

3:50 p.m.
* **Contaminants in Zebra Mussel Size Classes and a Comparison of Whole Mussel, Tissue, and Shell Concentrations**
R.G. Kreis et al., *U.S. Environmental Protection Agency*

4:10 p.m.
* **Sediment Toxicity and Bioaccumulation in the Zebra Mussel, *Dreissena polymorpha*, at Times Beach, New York**
J.M. Roper et al., *Virginia Tech*

CONTROL: COATINGS AND ATTACHMENT

3:10 p.m.
* **Use of Non-Fouling Coatings for Mussel Control**
A.C. Gross, *Long Island Lighting Company*

3:30 p.m.
* **A Comparison of Metal Leachate Rate and Zebra Mussel Control Efficacy for Coatings and Materials**
T.D. Race and M.A. Kelly, *U.S. Army Construction Engineering Research Laboratories*

3:50 p.m.
* **The Use of Copper and Copper-Nickel Alloys in the Prevention of Zebra Mussel Fouling**
C.M. Cottrell et al., *University of Toronto*

4:10 p.m.
* **Mechanisms of Zebra Mussel (*Dreissena polymorpha* and Quagga) Detachment and its Relation to Attachment Strength**
J.M. Dormon et al., *University of Toronto*

APPENDIX B:
LIST OF ATTENDEES

ABBOTT ROSS	UNDERWATER TECHNICAL SERVICES	302 S RIVER PARK DRIVE GUTTENBERG IA 52052
ACKERMAN JOSEF D.	UNIVERSITY OF TORONTO MECHANICAL ENGINEERING	5 KINGS COLLEGE ROAD TORONTO ONTARIO M5S 1A4
ADAMS CHARLIE	ENTERGY OPERATIONS	ROUTE 2 BOX 317G RUSSELLVILLE AR 72801
ADDY HENRY	NORTHERN STATES POWER	1717 WAKONADE DR. E. WELCH MN 55089
ADRIAN DAVID	AQUA TECH ENVIRONMENTAL	PO BOX 402 CLARENCE NY 14031-0402
ADRIAN GERALD	ELF ATOCHEM NORTH AMERICA INC	3 PARKWAY, RM 619 PHILADELPHIA PA 19102
AHLSTEDT STEVEN	TENNESSEE VALLEY AUTHORITY AQUATIC BIOLOGY DEPARTMENT	HIGHWAY 441 NORRIS TN 37828-2017
ALBISHARAT PHILLIP	ONTARIO HYDRO (BOG)	BOX 3000 TIVERTON ONTARIO N0G 2T0
ALDRIDGE DAVID	NORTH CAROLINA A & T	DEPT. OF BIOLOGY, BARNES HALL GREENSBORO NC 27411
ALEXANDER, JR. J. E.	UNIVERSITY OF LOUISVILLE WATER RESOURCES LABORATORY	LOUISVILLE KY 40292
AMEND JOHN R.	MALCOLM PIRNIE, INC.	1000 PITTSFORD-VICTOR ROAD PITTSFORD NY 14534
AMUNDSON DANIEL	US FISH & WILDLIFE SERVICE	51 EAST 4TH STREET WINONA MN 55987
ANDERSEN TONY	NATIONAL PARK SERVICE	PO BOX 708 ST. CROIX FALLS WI 54024
ANDERSON JIM	DREW INDUSTRIAL	1002 STONY BROOK DRIVE O'FALLON IL 62269
ANTHONY JERRY	WESTINGHOUSE OCEANIC	PO BOX 1488, MS 9230 ANNAPOLIS MD 21401
ARNOLD KEITH	BULLDOG BOILER RENTALS	5385 E. OUTER DRIVE DETROIT MI 48234
ASBILL T. C.		C/O RT. 1, BOX 2611 TIGER GA 30576
BABCOCK-JACKSON LISA	OHIO STATE UNIVERSITY	BT2 BLDG, 1735 NEIL AVE COLUMBUS OH 43210
BABINEC JOHN	WISCONSIN ELECTRIC POWER	333 E. EVERETT ST, A532 MILWAUKEE WI 53203
BADDOUR R. E.	UNIVERSITY OF WESTERN ONTARIO DEPARTMENT OF CIVIL ENGINEERING	LONDON ONTARIO N6A 5B9

BAKER PATRICK	MACALESTER COLLEGE DEPT. OF BIOLOGY	1600 GRAND AVE. ST PAUL MN 55105
BAKER SHIRLEY	MACALESTER COLLEGE DEPT. OF BIOLOGY	1600 GRAND AVE. ST PAUL MN 55105
BAKER BEVERLY	TENNESSEE SHELL COMPANY	PO BOX 647 CAMDEN TN 38320
BAKER PEGGY	TENNESSEE SHELL COMPANY	PO BOX 647 CAMDEN TN 38320
BALCER MARY	UNIVERSITY OF WISCONSIN-SUPERIOR CLES	1800 GRAND AVENUE BARSTOW BLDG 4 SUPERIOR WI 54880-2898
BANK LORRAINE	LTV STEEL - IHW	3001 DICKEY ROAD EAST CHICAGO IN 46312
BANKO MIKE	DUQUESNE LIGHT CO.	PO BOX 4, BV-ERF SHIPPINGPORT PA 15077
BARDYGULA-NONN LIDIA	UNIVERSITY OF WISCONSIN-MILWAUKEE CENTER FOR GREAT LAKES STUDIES	600 E. GREENFIELD MILWAUKEE WI 53204
BEATTY STEVE	US ARMY CORPS OF ENGINEERS	PO BOX 59 LOUISVILLE KY 40201
BEETON ALFRED M	NOAA/GLERL	2205 COMMONWEALTH BLVD ANN ARBOR MI 48105-1593
BENSON AMY J	US FISH & WILDLIFE SERVICE NATIONAL FISHERIES RESOURCES CENTER	7920 NW 71ST STREET GAINSVILLE FL 32606
BENSON LARRY	WISCONSIN DNR	WW/2, PO BOX 7921 MADISON WI 53707-7921
BERGER BRYAN	IMO INDUSTRIES	PO BOX 38 KIMBERLY WI 54136-0038
BERUBE ROGER	HYDRO QUEBEC	1010 EST STE CATHERINE 5IEME ETAGE MONTREAL QUEBEC H2L 2G3
BETBEZE ARNOLD	TENNESSEE VALLEY AUTHORITY	1101 MARKET ST CHATTANOOGA TN 37402-2801
BIALECKI DENNIS	CRANE ENGINEERING SALES	PO BOX 38 KIMBERLY WI 54136-0038
BIDLACK CINDY	SOUTHERN ILLINOIS UNIVERSITY ILLINOIS RIVER PROJECT	BOX 2222 EDWARDSVILLE IL 62026-2222
BIERMAN VICTOR	LIMNOTECH, INC.	20780 S GATEHOUSE DRIVE SOUTH BEND IN 46637
BILLS TERRY	US FISH & WILDLIFE SERVICE NATIONAL FISHERIES RESEARCH CENTER	2630 ZANTA REED ROAD LA CROSSE WI 54601
BIRD TONY	SEAWARD MARINE SERVICES	5409 BEAMON RD NORFOLK VA 23513

BIRDWELL TOM	CMP COATINGS, INC.	1610 ENGINEERS ROAD BELLE CHASSE LA 70037
BLODGETT DOUG	ILLINOIS NATURAL HISTORY SURVEY LONG TERM RESOURCE MONITORING PROGRAM	704 N SCHRADER AVENUE HAVANA IL 62644
BLOSCO JOHN	OHIO EDISON COMPANY	ROUTE 7, PO BOX 176 STRATTON OH 43961
BOERSMA ED	COOK SCREEN TECHNOLOGIES	1282 GLENDALE MILFORD RD CINCINNATI OH 45215
BOLES LARRY	VIRGINIA INSTITUTE OF MARINE SCIENCE	PO BOX 1346 GLOUCESTER POINT VA 23062-1346
BOLTE JIM	COMMONWEALTH EDISON 1 FIRST NATIONAL PLAZA	10 S DEARBORN, 35 FFW CHICAGO IL 60603
BOLTZ SUZANNE	EA ENGINEERING, SCIENCE & TECHNOLOGY	11019 MCCORMICK RD HUNT VALLEY MD 21031
BONNER THOMAS	SUNY COLLEGE/BROCKPORT DEPARTMENT OF BIOLOGICAL SCIENCES	012H COOPER HALL BROCKPORT NY 14420
BORDELEAU LUC	LABATT BREWERIES OF CANADA	150 SIMCOE STREET PO BOX 5050 LONDON ONTARIO N6A 4M3
BOYLE JIM	U.S. ARMY CORPS OF ENGINEERS	1776 NIAGARA ST BUFFALO NY 14207
BRADY VAL	MICHIGAN STATE UNIVERSITY	203 NATURAL SCIENCE BLDG, MSU EAST LANSING MI 48824
BRENCE WILLIAM ALEXANDER	UNIVERSITY OF CINCINNATI DEPARTMENT OF BIOLOGICAL SCIENCES	821 REIVESCHL, ML 0006 CINCINNATI OH 45221-0006
BREYLEY LOU	BUCKMAN LABORATORIES INC.	1256 N. MCLEAN BLVD. MEMPHIS TN 38108
BROCKHURST JACK	JVB DEVELOPMENT INC	94 S. LANE ANGOLA NY 14006
BRODIE GREG	TENNESSEE VALLEY AUTHORITY	1101 MARKET ST .WRBA-C CHATTANOOGA TN 37402
BROOKS GARY E.	EXXON CHEMICAL COMPANY	5909 N.W. EXPRESSWAY, ST. 238 OKLAHOMA CITY OK 73132
BROPHY JIM	AMIAD FILTRATION SYSTEMS	14141 COVELLO STREET, 7C VAN NUYS CA 91405
BRUENDERMAN SUE	VIRGINIA GAME & INLAND FISHERIES	2206 S. MAIN STREET, SUITE C BLACKSBURG VA 24060
BUCHAN LUCY	UNIVERSITY OF WISCONSIN-MADISON	361 BIRGE HALL, UW–MADISON MADISON WI 53706
BUCHANAN ALAN		1110 S. COLLEGE AVE. COLUMBIA MO 65201

BULERA STACEY	SAVE THE ST. CROIX	16777 16TH ST. S. LAKE ST. CROIX BEACH MN 55043
BULERA TOM	SAVE THE ST. CROIX	16777 16TH STREET LAKELAND MN 55043
BURKE PAUL	US FISH & WILDLIFE SERVICE	4101 E. 80TH ST. BLOOMINGTON MN 55425-1665
BURKES MARSHALL	VERONA AREA HIGH SCHOOL	700 N. MAIN ST. VERONA WI 53593
BURKY ALBERT	UNIVERSITY OF DAYTON DEPARTMENT OF BIOLOGY	DAYTON OH 45469-2320
BURLEY CURT	US FISH & WILDLIFE SERVICE	9317 HIGHWAY 99, SUITE I VANCOUVER WA 98665
BURTON TOM	MICHIGAN STATE UNIVERSITY DEPT OF ZOOLOGY	EAST LANSING MI 48824
BURTON LT JON	US COAST GUARD MARINE ENVIRONMENTAL PROTECTION DIV.	2100 SECOND STREET SW WASHINGTON DC 20593-0001
BUST DON		N1496 US-41 MENOMINEE MI 49858
BUTZLAFF TODD	ENVIRONMENTAL SCIENCE & ENGINEERING INC. SENIOR LAB TECHNICIAN	11665 LILBURN PARK ROAD ST. LOUIS MO 63146
CALL CHRISTOPHER	LOYOLA UNIVERSITY	6525 N. SHERIDAN RD. CHICAGO IL 60626
CARMICHAEL NANCY		PO BOX 359 SHARON WI 53585
CARROLL JOHN B	BETZ INTERNATIONAL	403 W LINCOLN HWY. SUITE 107 EXTON PA 19341
CASEY KEVIN	CMP COATINGS INC.	1610 ENGINEERS ROAD BELLE CHASSE LA 70037
CASPER ANDREW	UNIVERSITY OF LOUISVILLE DEPT. OF BIOLOGY	U. OF LOUISVILLE LOUISVILLE KY 40292
CAVALCOLI MONA	R & D ENGINEERING & LAND SURVEYING	600 R & D CENTRE 268 MAIN STREET BUFFALO NY 14202
CHAILLOU JANIS	VERSAR, INC	9200 RUMSEY RD COLUMBIA MD 21045
CHALKER-SCOTT LINDA	SUNY COLLEGE AT BUFFALO ASSISTANT PROFESSOR	1300 ELMWOOD AVENUE BUFFALO NY 14222-1095
CHANG TIAO	OHIO UNIVERSITY DEPARTMENT OF CIVIL ENGINEERING	ATHENS OH 45701

CHANLEY WILLIAM	**PSI ENERGY** GALLAGHER GENERATING STATION	**PO BOX 409** NEW ALBANY IN 47150
CHARLTON MURRAY N	CANADA CENTER FOR INLAND WATERS	867 LAKESHORE ROAD PO BOX 5050 BURLINGTON ONTARIO L7R 4A6
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CHASE RICKI	UNIVERSITY OF TEXAS-ARLINGTON CTR. FOR BIOL. MACROFOULING RESEARCH	BOX 19498 ARLINGTON TX 76019-9498
CHAUHAN BAK	MINISTRIES OF ENVIRONMENT & ENERGY	135 ST CLAIR AVENUE TORONTO ONT M4V 1P5
CHEESMAN ROY	ENVIREX INC	1901 S PRAIRIE AVENUE PO BOX 1604 WAUKESHA WI 53187-1604
CHOTKOWSKI MIKE	UNIVERSITY OF WISCONSIN-MADISON	361 BIRGE HALL MADISON WI 53706
CHRISTIANSEN DON	CHRISTIANSEN CONSULTING	PO BOX 2563 CARLSBAD CA 42018
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CLINE SHELLY	AWWA RESEARCH FOUNDATION	6666 W. QUINCY AVENUE DENVER CO 80235
CLUM DAVE	FIRST THERMAL SYSTEMS INC	PO BOX 4756 CHATTANOOGA TN 37405
COBB JOHN	ENVIRONMENTAL DYNAMICS	PO BOX 359 SHARON WI 53585
COLE JERRY	SPARTON ELECTRONICS	2400 E. GANSON STREET JACKSON MI 49202
COMAND DEAN A	STELCO INC.	WILCOX STREET, PO BOX 2030 HAMILTON ONTARIO L8N 3T1
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COOK KIM	S.P. KINNEY ENGINEERS, INC.	143 FIRST AVENUE CARNEGIE PA 15106
COOK LEE	WHEELABRATOR ENGINEERS	7575 CORNELL RD. CINCINNATI OH 45242
COON THOMAS G	MICHIGAN STATE UNIVERSITY DEPARTMENT OF FISHERIES & WILDLIFE	EAST LANSING MI 48824

COPE W GREGORY	US FISH & WILDLIFE SERVICE NATIONAL FISHERIES RESEARCH CENTER	PO BOX 818 LA CROSSE WI 54602-0818
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COSCARELLI MARK	MICHIGAN DNR OFFICE OF GREAT LAKES	PO BOX 30028 LANSING MI 48909-0028
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COTTRELL CATHY	UNIVERSITY OF TORONTO DEPARTMENT OF MECHANICAL ENGINEERING	5 KINGS COLLEGE ROAD TORONTO ONT M5S 1A5
COWAN JOE	MOLINE WATER DEPARTMENT	619 16TH ST MOLINE IL 61265
COYLE JEFF	CENTRAL ILLINOIS PUBLIC SERVICE	800 S. WASHINGTON MEREDOSIA IL 62650
CROWL LOWELL	WATER PROCESS SYSTEMS INC	7925 E 40TH STREET TULSA OK 74145
CULVER DAVID A	OHIO STATE UNIVERSITY DEPARTMENT OF ZOOLOGY	1735 NEIL AVENUE COLUMBUS OH 43210
CURIE BOB	STRANCO, INC.	595 INDUSTRIAL DRIVE PO BOX 389 BRADLEY IL 60915-0389
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zDELONG MIKE	WINONA STATE UNIVERSITY BIOLOGY DEPARTMENT	WINONA MN 55987
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DION JEROME	SUBDEV CANADA INC. BIOLOGIST	1841-B LAVOISIER STE JULIE QUE J3E 1Y6
DOBSON EVAN	UNIVERSITY OF GUELPH DEPARTMENT OF ZOOLOGY	GUELPH ONTARIO N1G 2W1
DOKA SUSAN	UNIVERSITY OF GUELPH DEPARTMENT OF ZOOLOGY	GUELPH ONTARIO N1G 2W1
DOLL BARBARA A.	NORTH CAROLINA STATE UNIVERSITY SEA GRANT COASTAL WATER QUALITY SPEC.	PO BOX 8208 RALEIGH NC 27605-8208
DONELL JOE	PECO ENERGY COMPANY	RD #1, PO BOX 208 DELTA PA 17314
DONNER STEPHEN P.	CONSUMERS POWER COMPANY	1945 W. PARNALL ROAD, MS P13 107 JACKSON MI 49201
DORMON JANE	UNIVERSITY OF TORONTO DEPT. OF MECHANICAL ENGINEERING	5 KINGS COLLEGE ROAD TORONTO ONTARIO M5R 2R6
DORNEANU ANE MARIE	ONTARIO HYDRO	5775 YONGE STREET N10-A3 NORTH YORK ONT M2M 4J7
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DUCHNIAK DAN	OAK CREEK WATER & SEWER	PO BOX 94 OAK CREEK WI 53154
DUNSTAN PAMELA	PECO ENERGY COMPANY	965 CHESTERBROOK BLVD WAYNE PA 19087
DYE GARY L.	US ARMY CORPS OF ENGINEERS	1776 NIAGARA STREET BUFFALO NY 14807
EADES RICHARD T.	DEPARTMENT OF GAME & INLAND FISHERIES	6530 INDIAN RIVER ROAD VIRGINIA BEACH VA 23464
EBERT THOMAS	BPCI-CHEMLINK	10717 BENTLEY PASS LANE CINCINNATI OH 45140
ELZINGA WILLIAM J.	ENVIRONMENTAL SCIENCE & ENGINEERING INC.	11665 LILBORN PARK ROAD ST LOUIS MO 63146-3535
ESTES TONY	ELF ATOCHEM NORTH AMERICA INC.	RT 3 BOX 259, RM 619 BLOOMFIELD IN 47424
EVANS DAVE	ONTARIO HYDRO TECHNOLOGIES CHEMIST	800 KIPLING AVENUE TORONTO ONTARIO M8Z 5S4

EWINS PETER	CANADIAN WILDLIFE SERVICE	PO BOX 5050 BURLINGTON ONTARIO L7R 4A6
FAHLSING RAY	MICHIGAN DNR	PO BOX 30257 LANSING MI 48909
FALK JAMES M.	UNIVERSITY OF DELAWARE COLLEGE OF MARINE STUDIES	700 PILLOTOWN ROAD LEWES DE 19958-1298
FARMA TONY	PHILADELPHIA WATER DEPT.	1500 E. HUNTING PARK AVE. PHILADELPHIA PA 19124
FEARS CLOIS D.	DELTA APPLIED TECHNOLOGY, INC.	100 SANDUNE COURT, SUITE B PITTSBURGH PA 15239
FEERS WILLIAM	THE HILL SCHOOL	917 EAST HIGH STREET POTTSLAWN PA 19464
FEIST TIM	LIMNO-TECH, INC.	2395 HURON PARKWAY ANN ARBOR MI 48104
FERRO THOMAS	AQUATECH ENVIRONMENTAL, INC. SENIOR SCIENTIST	PO BOX 402 CLARENCE NY 14031-0402
FICEK KEN	CARUS CHEMICAL COMPANY	1001 BOYCE MEMORIAL DRIVE OTTAWA IL 61350
FLANNERY JIM	CHEMICAL CONTROL SYSTEMS	420 EAST OHIO, SUITE 29G CHICAGO IL 60611
FLEURENT GINETTE	HYDRO-QUEBEC	1010 STE CATHERINE ST., EAST 6F1 MONTREAL QUEBEC H2L 2G3
FLOOD TERRY J.	ASSISTANT ENGINEER	1401 FRONT STREET TOLEDO OH 43605
FOBES RONALD L.	CONSUMERS POWER COMPANY ENVIRONMENTAL DEPARTMENT	1945 W. PARNALL ROAD JACKSON MI 49201
FONG PETER	WAYNE STATE UNIVERSITY DEPARTMENT OF PHYSIOLOGY	DETROIT MI 48201
FORESTER JOHN		230 PARKER COLISEUM REURG CA 70808
FOWLES MIKE	US ARMY CORPS OF ENGINEERS	RR #1, BOX 326 EXPORT PA 15632
FRANCIS PATRICK	OAK CREEK WATER & SEWER	PO BOX 94 OAK CREEK WI 53154
FRENCH JOHN	US FISH & WILDLIFE SERVICE	1451 GREEN ROAD ANN ARBOR MI 48105
GANT MICHAEL E	CITY OF ST. LOUIS WATER DIVISION	10450 RIVERVIEW DRIVE ST LOUIS MO 63137
GARRETT BILL	ALABAMA POWER COMPANY	PO BOX 2641 BIRMINGHAM AL 35291-2641
GARTON DAVID W.	INDIANA UNIVERSITY-KOKOMO DEPT. OF BIOLOGICAL & PHYSICAL SCIENCE	2300 S. WASHINGTON STREET KOKOMO IN 46904

GAULKE ALAN E.	AMERICAN ELECTRIC POWER SERVICE CORP.	PO BOX 16631 COLUMBUS OH 43216-6631
GAUTHIER CLAUDE	ONTARIO HYDRO	700 UNIVERSITY AVENUE A8-G8 TORONTO ONTARIO M5G 2C6
GEE RALPH	HYDRITE CHEMICAL COMPANY	PO BOX 2763 OSHKOSH WI 54903
GENOVESE ERIN	WESTERN ILLINOIS UNIVERSITY	DEPT. OF BIOLOGICAL SCIENCES MACOMB IL 61455
GERBER BURT	BORDEN	630 GLENDALE-MILFORD RD CINCINNATI OH 45215
GERMER MARK	RIO LINDA	413 BRETON LAFAYETTE LA 70508
GEROVAC PAUL	UNIVERSITY OF WISCONSIN-MILWAUKEE CENTER FOR GREAT LAKES STUDIES	600 E GREENFIELD AVE MILWAUKEE WI 53204-2944
GIAMBERINI LAURE	C.R.E.U.M. BP 4116	RUE DES RECOLLETS, CEDEX.01 METZ 57040 FRANCE
GILL PATRICK H	CALGON CORP. WATER MANAGEMENT DIVISION	PO BOX 1346 PITTSBURGH PA 15230-1346
GINGERICH ANGELA	OHIO STATE UNIVERSITY	1735 NEIL AVE. COLUMBUS OH 43210-1293
GLASER GUS	WISCONSIN DNR	PO BOX 10448 GREEN BAY WI 54307-0448
GLONEK THOMAS	MIDWESTERN UNIVERSITY MR LABORATORY	5200 S. ELLIS AVE. CHICAGO IL 60615
GOBLE BEN	FERRO CORPORATION	1301 N. FLORA ST. PLYMOUTH IN 46563
GOINDI S.	ONTARIO HYDRO	PO BOX 4000 TIVERTON ONTARIO N0G 2T0
GORDON GREG	ONTARIO HYDRO/DARLINGTON NGS OPERATIONS	PO BOX 4000 BOWMANVILLE ONTARIO L1C 3Z8
GOULET BLAKE	AQUATIC SCIENCES, INC.	45 HANNOVER, STE. 1, PO BOX 2205B ST. CATHARINES ONTARIO L2M 6P6
GRACH JOHN	CANADIAN COPPER AND BRASS	10 GATEWAY, STE. 375 DON MILLS ONTARIO M3C 3A1
GRIGOROVICH IGOR	INSTITUTE OF HYDROBIOLOGY	12, GEROYEV STALINGRADA PROSP. KIEV UKRAINE
GROSS SHARON	US FISH & WILDLIFE SERVICE	4401 N FAIRFAX DRIVE RM. 840 ARLINGTON VA 22203
GUILL GEORGE N	GEORGIA POWER COMPANY ENVIRONMENTAL LABORATORY	5131 MANER ROAD SMYRNA GA 30080

GUNDERSON JEFF	UNIVERSITY OF MINNESOTA/DULUTH MINNESOTA SEA GRANT EXTENSION	208 WASHBURN HALL DULUTH MN 55812-2420
HAGEMAN JOHN	OHIO STATE UNIVERSITY F.T. STONE LABORATORY	PO BOX 119 PUT-IN-BAY OH 43456-0119
HALE LARRY	DREW CHEMICAL	18 W 100-22ND STREET, SUITE 110 OAKBROOK TERRACE IL 60181
HALL JAMES J.	DUKE POWER COMPANY TRAINING & TECHNOLOGY CENTER	13339 HAGERS FERRY ROAD HUNTERSVILLE NC 28078-7929
HANSON HEATHER	OCONTO HIGH SCHOOL	1717 SUPERIOR AVENUE OCONTO WI 54153
HANSON ELIZABETH	UNIVERSITY OF WISCONSIN SEA GRANT ADVISORY SERVICES	1800 UNIVERSITY AVENUE MADISON WISCONSIN 53706
HARDING JULIANA		5400-L MONTGANERY SQUARE DR KETTERING OH 45440
HARILDSTAD KEITH	INTERSTATE POWER COMPANY	1000 MAIN STREET, PO BOX 769 DUBUQUE IA 52004-0769
HARMELING DAVE	WP&L/EDGEWATER STATION ASSISTANT GENERATING STATION MANAGER	PO BOX 356 SHEBOYGAN WI 53082-0356
HARTWICK TOM	US STEEL	1 N. BROADWAY GARY IN 46402
HAUSLER MARK	UW-LA CROSSE	PO BOX 360 NORTH LIBERTY IA 52317
HAVLIK MARIAN	MALACOLOGICAL CONSULTANTS	1603 MISSISSIPPI STREET LA CROSSE WI 54601-4969
HAY ROBERT	WISCONSIN DNR	PO BOX 7921 MADISON WI 53707
HEATH ROBERT T.	KENT STATE UNIVERSITY DEPARTMENT OF BIOLOGICAL SCIENCE	KENT OH 44242-0001
HEIL DAVID	CAPITAL CONTROLS COMPANY INC.	3000 ADVANCE LANE, PO BOX 211 COLMAR PA 18915-0211
HEJKA TED	ANN ARBOR WATER TREATMENT	919 SUNSET ANN ARBOR MI 48103
HELTON BARNEY	TENNESSEE VALLEY AUTHORITY	BR2A 1101 MARKET STREET CHATTANOOGA TN 37402-2801
HERMAN LAURA	WISCONSIN DNR	107 SUTLIFF AVENUE, PO BOX 818 RHINELANDER WI 54501-0818
HERMILLER MARC	NALCO CHEMICAL COMPANY	1 NALCO CENTER NAPERVILLE IL 60563
HESTER WILBURN L.	COMMONWEALTH EDISON COMPANY	1400 OPUS PLACE, SUITE 800 DOWNERS GROVE IL 60515

HILGENDORF MARAN	OHIO SEA GRANT	1314 KINNEAR RD. COLUMBUS OH 43212-1194
HINCKS SHERI	UNIVERSITY OF GUELPH DEPARTMENT OF ZOOLOGY	GUELPH ONTARIO N1G 2W1
HOBBS BRIAN	SEAWARD MARINE SERVICES	5409 BEAMON ROAD NORFOLK VA 23513
HOLBROOK DOUG	DAYTON POWER & LIGHT	PO BOX 147 MANCHESTER OH 45679
HOLLAND RUTH	UNIVERSITY OF MICHIGAN	A055 UNIV OF MICHIGAN ANN ARBOR MI 48109-2143
HOLT LEONARD	HARZA CONSULTING	233 S. WACKER DRIVE CHICAGO IL 60606-6392
HOOSE JOHN	UCISCO	222 PENNBRIGHT, SUITE 300 HOUSTON TX 77090
HOPPER BOB	HOPPER-STEPHEN HATCHERIES	5205 HWY. 31 SOUTH LONOKE AR 72086
HORNBACH DANIEL J.	MALCALESTER COLLEGE DEPARTMENT OF BIOLOGY	1600 GRAND AVENUE ST. PAUL MN 55105
HORVATH THOMAS	UNIVERSITY OF NOTRE DAME DEPARTMENT OF BIOLOGICAL SCIENCES	NOTRE DAME IN 64556
HUGHES LORAS C.	ALCOA	BOX 3567 DAVENPORT IA 52808
HUGHES ROBIN	ONTARIO HYDRO CIVIL RESEARCH	800 KIPLING AVENUE TORONTO ONTARIO M8Z 5S4
HUSHAK LEROY	OHIO STATE UNIVERSITY SEA GRANT ADVISORY SERVICES LEADER	2120 FYFFE ROAD 232 AGRICULTURAL ADMIN. COLUMBUS OH 43210-1010
ISBER PHIL	MYCO INDUSTRIES INC.	11 HERRIOT STREET PALGRAVE ONTARIO L0N 1P0
JACKLIN ROGER R.	DEARBORN CHEMICAL COMPANY LTD.	3451 ERINDALE STN ROAD PO BOX 3060 MISSISSAUGA ONTARIO L5A 3T5
JACQUAZ BERNADETTE	LABORATOIRES SAB INC.	7869 SAINT-DENIS MONTREAL QUEBEC H2R 2E9
JAIME BRUCE	NORTHWESTERN UNIVERSITY	2020 RIDGE AVE. EVANSTON IL 60208
JAMES WILLIAM	USAE EAU GALLE LIMNOLOGY	BOX 237 SPRING VALLEY WI 54767
JENKINSON JOHN	TENNESSEE VALLEY AUTHORITY	HB 2C 1101 MARKET STREET CHATTANOOGA TN 37402
JENNER H. A.	NV KEMA	LITRECHTSEWEG 310 ARNHETT 6812 AR THE NETHERLANDS

JENNINGS SUE	ST CROIX NATIONAL SCENIC RIVERWAY	PO BOX 708 ST CROIX FALLS WI 54024
JENNINGS CECIL A.	USFWLS	PO BOX 818 LA CROSSE WI 54602-0818
JENSEN DOUG	MINNESOTA SEA GRANT	2305 E. 5TH STREET DULUTH MN 55812
JOHNS CAROLYN	ST. LAWRENCE UNIVERSITY ENVIRONMENTAL STUDIES PROGRAM	CANTON NY 13617
JOHNSON ROGER	APPLETON WATER DEPT.	125 N. WALNUT STREET APPLETON WI 54911-4692
JOHNSON LADD	MYSTIC SEAPORT MUSEUM	UNIVERSITY OF CALIFORNIA SANTA BARBARA CA 93106
JOHNSON J KENT	UNIVERSITY OF IOWA	PO BOX 360 NORTH LIBERTY IA 53217
JOHNSON BRIAN	WALPOLE ISLE FIRST NATION	RR #3 WALLACEBURG ONTARIO N8A 4K9
JONES RICHARD	FORT HOWARD CORPORATION	PO BOX 19130 GREEN BAY WI 54307-9130
JORDAN ALAN	UNIVERSITY OF BUFFALO CENTER FOR BIO. SURFACE RESEARCH	6448 BOSTON RIDGE ROAD ORCHARD PARK NY 14127
KADINGER DAVID	KADINGER MARINE SERVICE INC.	401 E. GREENFIELD AVE. MILWAUKEE WI 53204
KAJOASZ RALPH	CHEMLINK	4022 BOOT ROAD DOWNINGTOWN PA 19335
KANDT DEAN	ASCI CORP LARGE LAKES RESEARCH STATION	9311 GROH ROAD GROSSE ILE MI 48138
KARSKI WES	ONTARIO HYDRO	BNGS "A" B06, PO BOX 3000 TIVERTON ONTARIO N0G 2T0
KENDALL-EAGLESON SHERIE	INDIANA UNIVERSITY	1715 OVERLOOK RD. MARION IN 46952
KENIRY TAMMY	ILLINOIS NATURAL HISTORY SURVEY LAKE MICHIGAN BIOLOGICAL STATION	400 17TH STREET ZION IL 60099
KENNEDY JASON	MALCOLM-PIRNIE INC.	1000 PITTSFORD VICTOR ROAD PITTSFORD NY 14534
KENNEDY VICTOR S.	UNIVERISTY OF MARYLAND HORN POINT ENVIRONMENTAL LAB	PO BOX 775 CAMBRIDGE MD 21613-0775
KENT JIM	DEPARTMENT OF FISHERIES & OCEANS	200 KENT STREET STN 1414 OTTAWA ONTARIO K1A 0E6
KERLEY BERNIE L.	TENNESSEE VALLEY AUTHORITY AQUATIC BIOLOGY DEPARTMENT	NORRIS TN 37828

KHALANSKI MICHEL	ELECTRICITE DE FRANCE DEPT ENVIRONNEMENT BP 49	6 QUAI WATIER CHATOU CEDEX 78401 FRANCE
KING JUSTIN	NEBRASKA PUBLIC POWER DISTRICT ENVIRONMENTAL SPECIALIST	PO BOX 499 COLUMBUS NE 68602-0499
KINNEY BABE	S P KINNEY ENGINEERS, INC.	143 FIRST AVENUE CARNEGIE PA 15106
KITCHEL HELEN	ILLINOIS NATURAL HISTORY SURVEY	607 E. PEABODY CHAMPAIGN IL 61820
KITCHELL JIM	CENTER FOR LIMNOLOGY	UW-MADISON MADISON WI 53706
KLIEGMAN JON	RIO LINDA CHEMICAL	PO BOX 345 WAYNE NJ 67470
KLINGER-KINGSLEY SHARON	WI POWER & LIGHT COMPANY	222 W. WASHINGTON AVENUE MADISON WI 53703-2793
KLUNDT STEVE	PURE-GRADE, INC.	215 W. PALM AVENUE, STE. 202 BURBANK CA 91502
KNOWLTON JAMES J.	GILBERT COMMONWEALTH INC. PROJECT MANAGER	PO BOX 1498 READING PA 19603-1498
KOEPLIN-GALL SANDY	NALCO CHEMICAL COMPANY MARKET DEVELOPMENT MANAGER	1 NALCO CENTER NAPERVILLE IL 60563-1198
KOOPMANS ROBERT	ONTARIO HYDRO TECHNOLOGIES SENIOR ENGINEER	800 KIPLING AVENUE TORONTO ONTARIO M8Z 5S4
KOTHARI NILAKSH J.	MANITOWOC PUBLIC UTILITIES	1303 S. 8TH STREET, PO BOX 278 MANITOWOC WI 54220-0278
KOWALEWSKI JOHN	ONTARIO HYDRO/MECHANICAL RESEARCH KR278	800 KIPLING AVENUE TORONTO ONTARIO M5G 1X6
KRAFT CLIFFORD	UNIVERSITY OF WISCONSIN SEA GRANT ADVISORY SERVICES	ES 105 UW- GREEN BAY GREEN BAY WI 54311-7001
KRACIUA RANDY	CORPS OF ENGINEERS	PO BOX 2004 ROCK ISLAND IL 61204-2004
KRASIN KENT	CRANE ENGINEERING SALES	PO BOX 38 KIMBERLY WI 54136-0038
KREIS RUSSELL G.	US ENVIRONMENTAL PROTECTION AGENCY	9311 GROH ROAD GROSSE ILE MI 48138
KREUSER RICHARD	R.T. KREUSER & ASSOCIATES	PO BOX 86622 BATON ROUGE LA 70879-6622
LACY JAMES	BETZ INDUSTRIAL	1 QUALITY WAY TREVOSE PA 19053
LAMMERS MARY	UNDERWATER TECH. SERVICES	302 S. RIVER PARK DRIVE GUTTENBERG IA 52052

LANE TONY	MYCO INDUSTRIES INC .	11 HERRIOT STREET PALGRAVE ONTARIO L0N 1P0
LANGE CAMERON L.	ACRES INTERNATIONAL CORP SENIOR ENVIRONMENTAL SCIENTIST	140 JOHN JAMES AUDUBON PKWY. AMHERST NY 14228-1180
LANGELLIER ROBERT A.	SUPERVISOR ENVIRONMENTAL SERVICES	76 S. MAIN STREET AKRON OH 44308
LAPCZYNSKI TOM	ONTARIO HYDRO	800 HYDRO RD. PORT CREDIT ONTARIO L5G 4M1
LARSON BRIAN	WISCONSIN POWER & LIGHT	PO BOX 0175 CASSVILLE WI 53806-0175
LATSHAW CHERYL	DREW INDUSTRIAL DIVISION	3155 FIBERGLASS RD. KANSAS CITY KS 66115
LATTA LARRY	AMERICAN ELECTRIC POWER	1 RIVERSIDE PLAZA COLUMBUS OH 43215-2355
LAUER THOMAS	PURDUE UNIVERSITY	1200 FOREST PRODUCTS BUILDING WEST LAFAYETTE IN 47907
LAVALLEE MATTHIEU	HYDRO-QUEBEC	855 E. ST. CATHERINE ST. MONTREAL QUEBEC H2L 4P5
LAW BRUCE	RONNINGEN-PETTER	9151 SHAVER ROAD, PO BOX 188 PORTAGE MI 49801-0188
Le PAGE WILFRED LAURIER	MONROE WATER WORKS SUPERINTENDENT WATER TREATMENT	915 E. FRONT STREET MONROE MI 48161
LEAK J. R.	FIRST THERMAL SYSTEMS INC.	PO BOX 4756 CHATTANOOGA TN 37405
LEASURE ROBERT	LEASURE SHELL CO. INC.	2612 JACKSON BRADFORD AR 72020
LEE EDWIN	GRACE DEARBORN	300 GENESEE ST. LAKE ZURICH IL 60047
LEEK DENISE	MURRAY STATE UNIVERSITY	CENTER FOR RESERVOID RESEARCH MURRAY KY 42071
LEMAY BRIAN	FACTORY MUTUAL ENGINEERING ASSN.	5650 YONGE STREET SUITE 1404 NORTH YORK ONTARIO M2M 4G3
LESTER MARTY	FERRO CORPORATION	1301 N. FLORA ST. PLYMOUTH IN 46563
LEWIS B. DON	AQUATIC SCIENCES INC.	45 HANNOVER DRIVE PO BOX 2205 STN ST. CATHARINES ONTARIO L2M 6P6
LEWIS RANDY	PSI ENERGY ENVIRONMENTAL PROGRAMS	1000 E. MAIN STREET PLAINFIELD IN 46168
LIMA GAIL M.	ILLINOIS WESLEYAN UNIVERSITY BIOLOGY DEPARTMENT	PO BOX 2900 BLOOMINGTON IL 61702-2900

LINDAHL BOB	MINNESOTA POWER	PO BOX 128 COHASSET MN 55721-0128
LIPPINCOTT BRUCE L.	LAWLOR MATUSKY & SKELLY	ONE PIERCE PLACE, SUITE 500 E ITASCA IL 60143
LITHERLAND P. M.	US COAST GUARD NAVAL ENGINEERING	1240 E. 9TH STREET CLEVELAND OH 44199
LOCATI BRENDA	MINNESOTA SEA GRANT	208 WASHBURN HALL, 2305 E. 5TH DULUTH MN 55812
LOMAQUAHU EMERSON S.	AWWA RESEARCH FOUNDATION	6666 W. QUINCY AVENUE DENVER CO 80235
LONG JIM	MUSCATINE POWER & WATER	3205 CEDAR STREET, PO BOX 899 MUSCATINE IA 52761-0899
LORD JEFF	INTERNATIONAL PROTECTIVE COATINGS	400 SOUTH 13TH STREET LOUISVILLE KY 47130
LOWE REX	BOWLING GREEN STATE UNIVERSITY DEPARTMENT OF BIOLOGICAL SCIENCES	BOWLING GREEN OH 43403
LOWTHER DAVE	ONTARIO HYDRO	700 UNIVERSITY AVENUE A7-A8 TORONTO ONTARIO M5G 1X6
LUBBEN DOUG	UNIVERSITY OF IOWA	209 AMRF IOWA CITY IA 52242
LUBNER JAMES	UNIVERSITY OF WISCONSIN SEA GRANT ADVISORY SERVICES	600 E. GREENFIELD AVE. MILWAUKEE WI 53204-2944
LYONS SID	PROCTOR & GAMBLE ANALYTICAL MANAGER	ROUTE 87, PO BOX 32 MEHOOPANY PA 18629
MacISAAC HUGH J.	UNIVERSITY OF WINDSOR BIOLOGICAL SCIENCE	RM 316 BIOLOGY 2601 UNION STREET BLDG WINDSOR ONTARIO N9B 3P4
MacKAY BETH	ONTARIO FEDERATION ANGLERS & HUNTERS	BOX 280 PETERBOROUGH ONTARIO K9J 6Y5
MacKINNON WENDELL	METROPOLITAN WORKS DEPT.	55 JOHN ST., 18TH FL., METRO HALL TORONTO ONTARIO M5V 3C6
MacKAY IAN	FOCAL TECHNOLOGIES, INC	40 THORNHILL DR, UNIT 7 DARTMOUTH NOVA SCOTIA B3B 1S1
MACKELBURG LAURI	ASCI CORPORATION	9311 GROH RD. GROSSE ILE MI 48138
MACKIE GERRY	UNIVERSITY OF GUELPH DEPARTMENT OF ZOOLOGY	GUELPH ONTARIO N1H 7B2
MADENJIAN CHARLES	NATIONAL BIOLOGICAL SURVEY	6100 COLUMBUS AVE SANDUSKY OH 44870
MADON SHAROOK	ILLINOIS NATURAL HISTORY	PO BOX 590 HAVANA IL 62644

MAKAREWICZ JOSEPH	SUNY COLLEGE AT BROCKPORT DEPARTMENT OF BIOLOGICAL SCIENCES	113 LENNON HALL BROCKPORT NY 14420-2928
MAKI JIM	MARQUETTE UNIVERSITY DEPARTMENT OF BIOLOGY	WEHR LIFE SCIENCES BLDG. MILWAUKEE WI 53233
MALIK NASIR	ELF ATOCHEM	300 MADISON AVE TOLEDO OH 43652
MALLEN ERIC	INDIANA MICHIGAN POWER COMPANY COOK NUCLEAR PLANT	ONE COOK PLACE BRIDGEMAN MI 49016
MALONE JIM	J. M. MALONE & SON	PO BOX 158 LONOKI AR 72086
MANICCIA-BOZZO VERA	GRACE DEARBORN INC.	BOX 3060 STATION A MISSISSAUGA ONT L5A 3T5
MARANGELO PAUL	WILLIAMS COLLEGE	1859 SHIRLEY LANE C5 ANN ARBOR MI 48105
MARCUS ROY	COMMONWEALTH EDISON	1411 OPUS PLACE, SUITE 360 DOWNERS GROVE IL 60515
MARSDEN J ELLEN	ILLINOIS NATURAL HISTORY SURVEY LAKE MICHIGAN BIOLOGICAL STATION	400 17TH STREET PO BOX 634 ZION IL 60069-0634
MARTIN RON	WISCONSIN DNR WATER RESOURCES	101 S. WEBSTER ST., 2ND FLOOR MADISON WI 53703
MATHWIG JOHN	COLLEGE OF LAKE COUNTY	19351 W. WASHINGTON STREET GRAYSLAKE IL 60030
MATTHEWS R. CRAIG	CALGON CANADA INC. PRODUCT SPECIALIST	27 FINLEY ROAD BRAMPTON ONTARIO L6T 1B2
MATTHEWS MILTON	UNIV OF TEXAS AT ARLINGTON DEPT. OF BIOLOGY	CNTR. FOR BIOL. MACRO. RES. BOX 19498 ARLINGTON TX 76019
MATTHEWS JOHN	WALLACE & TIERNAN CANADA INC.	925 WARDEN AVENUE SCARBOROUGH ONT M1L 4C5
MATYS GLENN	BETZ INDUSTRIAL	3026 SOLANDT ROAD KANATA ONTARIO K2K 2A5
MAXSON RICHARD C	WHEELABRATOR ENGINEERED SYSTEM	PO BOX 64118 ST PAUL MN 55164-4118
MAYER GREGORY	UNIVERSITY OF WISCONSIN-PARKSIDE	DEPARTMENT OF BIO. SCIENCES KENOSHA WI 53141-2000
McALLISTER DON	WEPCO	231 W. MICHIGAN, RM. A532 PO BOX 2046 MILWAUKEE WI 53201-2046
McCLANE M. BRENT	ECOLOGICAL SPECIALISTS INC.	95 ALSANA COURT ST PETERS MO 63376
McCLELLAN ERIC	RMC	1921 RIVER RD., PO BOX 10 DRUMORE PA 17518

McCLINTOCK KEITH	BRAND UNDERWATER DIVISION	7120 HARBERT RD SAWYER MI 49125
McDONALD ELLEN	OHIO STATE UNIVERSITY	2070 NEIL AVE. RM. 470 COLUMBUS OH 43210
McGOURTY MICHAEL J	EMARK MARINE SERVICES CORP GENERAL MANAGER	S3566 BENZING ROAD ORCHARD PARK NY 14127
McGRAW BILL	PROMINENT FLUID CONTROLS	136 INDUSTRY DRIVE PITTSBURGH PA 15275-1014
McKENNA GERRY	ONTARIO HYDRO	700 UNIVERSITY AVENUE, A6 F1 TORONTO ONTARIO M5G 1X6
McLAREN JIM	BEAK CONSULTANTS INC.	12072 MAIN ROAD AKRON NY 14001
McLEAN MIKE	UNIVERSITY OF MINNESOTA MINNESOTA SEA GRANT COMMUNICATIONS	1518 CLEVELAND AVENUE N. SUITE 302 ST PAUL MN 55108
McMAHON ROBERT	UNIVERSITY OF TEXAS-ARLINGTON CENTER BIOLOGICAL MACROFOULING RESEARCH	PO BOX 19498 ARLINGTON TX 76019-9498
McNULTY TED		100 MAIN ST., STE 200, PO BOX 8023 LITTLE ROCK AR 72201
MERSCH JACQUES	UNIVERSITÉ DE METZ	CREUM BP 4116 57040 METZ CEDEX FRANCE
METCALF BILL	NSP FRENCH ISLAND PLANT	200 S. BAINBRIDGE STREET LACROSSE WI 54603
MEYER ANNE	INDUSTRY/UNIVERSITY CTR. FOR BIOSURFACES PRIN. RESEARCH SCIENTIST, SUNY	110 PARKER HALL BUFFALO NY 14214-3007
MEYERS LEE	WISCONSIN DNR	1125 N. MILITARY AVENUE PO BOX 10448 GREEN BAY WI 54307-0448
MICHAUD DAVE	WEPCO	333 W. EVERETT, PO BOX 2046 MILWAUKEE WI 53201-2046
MIHAL ANDREW	CENTRAL MICHIGAN UNIVERSITY	BIOLOGY DEPARTMENT MT. PLEASANT MI 48859
MILLER ALLEN H.	UNIVERSITY OF WISCSONSIN SEA GRANT ADVISORY SERVICES	1800 UNIVERSITY AVE MADISON WI 53705-4094
MILLER ANDREW C.	US ARMY CORP OF ENGINEERS WATERWAYS EXPERIMENT STA./CEWES-ER-A	3909 HALLS FERRY ROAD VICKSBURG MS 39180-6199
MILLER MICHAEL	UNIVERSITY OF CINCINNATI DEPT. OF BIOLOGICAL SCIENCES	CINCINNATI OH 45221-0006
MILLIS JIM	BIO-TECHNICAL RESOURCES	1035 SOUTH 7TH ST MANITOWOC WI 54220

MILNE WILLIAM A.	ALEX MILNE ASSOCIATES LTD.	376 ORENDA ROAD E BRAMPTON ONTARIO L6T 1G1
MINTON RUSSELL	UNIVERSITY OF WISCONSIN-PARKSIDE DEPARTMENT OF BIOLOGY	900 WOOD ROAD KENOSHA WI 53141-2000
MISKOKOMON DAN	WALPOLE ISLE FIRST NATION	RR #3 WALLACEBURG ONTARIO N8A 4K9
MITCHELL JEREMY	THE UNIVERSITY OF WESTERN ONTARIO DEPARTMENT OF ZOOLOGY	LONDON ONTARIO N6A 5B7
MITMAN RICHARD	CAPITAL CONTROLS COMPANY INC.	3000 ADVANCE LANE PO BOX 211 COLMAR PA 18915-0211
MOCCO TARA	OCONTO HIGH SCHOOL	1717 SUPERIOR AVE OCONTO WI 54153
MOLDEN CRAIG	MIDWESTERN UNIVERSITY MR LABORATORY	5200 S ELLIS AVE. CHICAGO IL 60615
MONTGOMERY ART		1059 N 68TH STREET WAUWATOSA WI 53213
MONTZ GARY R.	MINNESOTA DNR	500 LAFAYETTE ROAD PO BOX 21 ST PAUL MN 55155-0021
MOWBRAY MIKE	BETZ LABORATORIES	9340 CEDAR FOREST RD EDEN PRAIRIE MN 55347
MUELLER BRIAN S.	ENVIRONMENTAL SCIENCE & ENGINEERING INC.	11665 LILBURN PARK ROAD ST LOUIS MO 63146-3535
MUELLER KENNETH	NORTHERN STATES POWER COMPANY	414 NICOLLET MALL MINNEAPOLIS MN 55401
MULLER MANFRED	TAPROGGE GESELLSCHAFT INC.	SCHLIEMANNSTR. 2-14 58300 WETTER GERMANY
NALEPA THOMAS F.	NOAA/GLERL	2205 COMMONWEALTH AVENUE ANN ARBOR MI 48105
NEIMEYER TERRY	KCI TECHNOLOGIES	10 NORTH PARK DRIVE HUNT VALLEY MD 21030-1888
NEVES RICHARD J.	VIRGINIA POLYTECHNIC INSTITUTE DEPT. OF FISHERIES & WILDLIFE SCIENCES	106B CHEATHAM HALL BLACKSBURG VA 24061-0321
NGO TU DANG	HYDRO-QUEBEC	680 SHERBROOK W, 18 FL MONTREAL QUE H3C 4T8
O'HALLORAN TERRY	NATIONAL PARK SERVICE	PO BOX 708 ST CROIX FALLS WI 54024
O'NEILL, JR. CHARLES R.	SUNY COLLEGE/BROCKPORT ZM CLEARINGHOUSE COORDINATOR	250 HARTWELL HALL BROCKPORT NY 14420-2928
OLLECH JOHN	BUCKMAN LABORATORIES INC.	1256 N. MCLEAN BLVD. MEMPHIS TN 38108

ORR DAN	NORTHERN STATES POWER CO.	414 NICOLLET MALL MINNEAPOLIS MN 55401
OSTLIE LARS	INFRAWAVE INTERNATIONAL	43 SOUTH ST., MAYFAIR LONDON W1Y-5PD ENGLAND
OVERMYER GARY		641A W. 100 N. VALPARIASO IN 46383
PALLO STEVE	ILLINOIS POWER COMPANY	PO BOX 678, T-33 CLINTON IL 61727
PARE SANDRA	US FISH & WILDLIFE SERVICE LOWER GREAT LAKES FISHERY RESOURCES	405 N. FRENCH ROAD AMHERST NY 14228
PARKINSON DUANE	DREW INDUSTRIAL DIVISION	1 MID RIVERS MALL DR., SUITE 256 ST. PETERS MO 63376
PATHY DIANE A.		1166 BAY STREET, APT. 1304 TORONTO ONTARIO M5S 2X8
PAULIUS TOM	LOCKWOOD, ALEX, FITZGIBBONS & CUMMINGS	3 1ST NATIONAL PLAZA, SUITE 1700 CHICAGO IL 60602
PAYNE BARRY S.	US ARMY CORP OF ENGINEERS WATERWAYS EXPERIMENT STATION	3909 HALLS FERRY ROAD VICKSBURG MS 39180-6199
PEARSON BRIAN	OCONTO SCHOOL DISTRICT	1717 SUPERIOR AVE. OCONTO WI 54153
PEARSON HANS	SILVER LAKE COLLEGE DEPARTMENT OF BIOLOGY	2406 S. ALVERNO ROAD MANITOWOC WI 54220-9319
PEDERSON TODD	PURDUE U. FORESTRY & NATURAL RESOURCES ILLINOIS/INDIANA SEA GRANT	1200 FOREST PRODUCTS BLDG WEST LAFAYETTE IN 47907-1200
PERRY BILL	UNIVERSITY OF NOTRE DAME DEPARTMENT OF BIOLOGICAL SCIENCES	NOTRE DAME IN 46556
PETRILLE JOSEPH	BETZ WATER MANAGEMENT GROUP	4636 SOMERTON RD TREVOSE PA 19053
PICCIRILLO VINCENT J.	NPC, INC. DIRECTOR OF TOXICOLOGY	22636 GLENN DRIVE, SUITE 304 STERLING VA 20164
PICKENS DEBRA	CARUS CHEMICAL COMPANY	1081 BOYCE MEMORIAL OTTAWA IL 61350
PICKLES STANLEY	ONTARIO HYDRO/BRUCE GS "B"	PO BOX 4000 TIVERTON ONTARIO N0G 2T0
PILLSBURY ROBERT	BOWLING GREEN STATE UNIVERSITY BIOLOGY DEPARTMENT	BOWLING GREEN OH 43403
PISTIS CHARLES	MICHIGAN SEA GRANT DISTRICT EXTENSION AGENT	333 CLINTON STREET GRAND HAVEN MI 49417-1492
PLAYFORD GARY	PORTSMOUTH USA, INC.	PO BOX 360 NORTH LIBERTY IA 52317

POLIZOTTO KIM	POTASH CORP. OF SASKATCHEWAN	1339 BLUEBIRD DRIVE GREENFIELD IN 46140
POMEROY JIM	RONNINGEN-PETTER A DOVER RESOURCES COMPANY	9151 SHAVER ROAD, PO BOX 188 PORTAGE MI 49081-0188
PONTIUS RUTH A.	OHIO STATE UNIVERSITY DEPARTMENT OF ZOOLOGY	1735 NEIL AVENUE COLUMBUS OH 43210
POSTLEWAITE MATT	SANITARY ENGINEER	1012 WATER STREET MEADVILLE PA 16335
PRESTON ROBERT L.	ILLINOIS STATE UNIVERSITY DEPARTMENT OF BIOLOGICAL SCIENCES	NORMAL IL 61761-4120
PUTZ FRANK	UNION ELECTRIC COMPANY BIOLOGIST ENVIRONMENTAL SERVICES	PO BOX 149, MC 602 ST. LOUIS MO 63166-0149
QUICK MIKE	ENVIREX INC.	1901 S. PRAIRIE AVE. WAUKESHA WI 53186
RACE TIM	US ARMY CERL	PO BOX 9005 CHAMPAIGN IL 61826-9005
RAM JEFFREY L.	WAYNE STATE UNIVERSITY DEPARTMENT OF PHYSIOLOGY	DETROIT MI 48201
RAMSEY DEAN	R & D ENGINEERING	268 MAIN STREET, 6TH FLOOR BUFFALO NY 14202
RAU DAN	LAKE SUPERIOR RESEARCH	1800 GRAND AVENUE SUPERIOR WI 54880
RENDALL WILLIAM	MINNESOTA DNR EXOTIC SPECIES COORDINATOR	500 LAFAYETTE RD. ST PAUL MN 55155-4020
RENS DON	NORTHERN STATES POWER COMPANY	414 NICOLLET MALL RS7 MINNEAPOLIS MN 55401
RICCIARDI ANTHONY	McGILL UNIVERSITY DEPARTMENT OF BIOLOGY	MONTREAL QUEBEC H3A 1B1
RICHER YVES	SUBDEV CANADA INC.	1841 B LAVOISIER STE. JOLIE QUEBEC J3E 1Y6
RIGDEN BERNARD	ONTARIO HYDRO	138 INVERLYN CRES. S KINCARDINE ONTARIO N2Z 1K9
RIGSBY ANGELA	OHIO STATE UNIVERSITY	1735 NEIL AVE. COLUMBUS OH 43210-1293
RIOPEL ALAIN	MINISTERE de L'ENVIR.	930 CHEMIN STE. FOY 2E ETAGE QUEBEC QUEBEC G1S 2L4
RITSIC RATKO	CARTOGRAPHY FOR SCIENCE	3558 NORTH MURRAY AVE. MILWAUKEE WI 53211
ROBINSON LEE	CORPS OF ENGINEERS, NASHVILLE	PO BOX 1070 NASHVILLE TN 37202-1070

ROCKHILL REBECCA	STATE UNIV. OF NEW YORK AT BROCKPORT	15 SHERWOOD DRIVE BROCKPORT NY 14420
ROGERS BARDEN	SUBCON INC.	1910 WEST SUMTER FLORENCE SC 29502
ROLLINS MARTHA A.	TENNESSEE VALLEY AUTHORITY	1101 MARKET STREET MR 3A CHATTANOOGA TN 37402-2801
ROPER WILLIAM E.	US ARMY CORP OF ENGINEERS	20 MASSACHUSETTS AVENUE NW WASHINGTON DC 20314
ROPER JEANNIE	VIRGINIA TECH/US ARMY CORP OF ENGINEERS	2006 DERRING HALL BLACKSBURG VA 24061
ROSENBERG GARY	THE ACADEMY OF NATURAL SCIENCES MALACOLOGY DEPARTMENT	1900 BENJAMIN FRANKLIN PKWY PHILADELPHIA PA 19103-1195
ROSENMAN RICHARD	US DEPARTMENT OF STATE	2201 C ST. NW, ROOM 5806 WASHINGTON DC 20520
ROSS TAMARA	IOWA STATE UNIVERSITY DEPT. OF ZOOLOGY AND GENETICS	SCI II AMES IA 50011
ROZIC JOHN	NORTH COAST LABS, INC.	711 ADAMS STREET TOLEDO OH 43624
RUSZNAK LINDA	ASHLAND OIL INC.	PO BOX 391 ASHLAND KY 41114
RYAN EAMONN	DOFASCO INC. UTILITIES DEPARTMENT	PO BOX 2460 ONTARIO L8N 3J5
SABLE DANIEL	UNIVERSITY OF WISCONSIN-WHITEWATER BIOLOGY DEPARTMENT	WHITEWATER WI 53190
SALE JEFF	ATOCHEM	8225 COYLE LANE CROSS PLAINS WI 53528
SANDBERG GARY	ELF ATOCHEM NORTH AMERICA INC.	2000 MARKET STREET PHILADELPHIA PA 19103-1195
SANDERS LARRY	USAE WATERWAYS STATION	3909 HALLS FERRY RD VICKSBURG VS 39180-6199
SAWCHUK DAVE	POLYSAR RUBBER CORP.	1265 VIDAL ST. S. SARNIA ONTARIO N7T 7M2
SAWYKO PAUL M.	ROCHESTER GAS & ELECTRIC CORP.	89 EAST AVENUE ROCHESTER NY 14649-0001
SCHEIDE JOHN I.	CENTRAL MICHIGAN UNIVERSITY DEPARTMENT OF BIOLOGY	MT PLEASANT MI 48859
SCHMIDT BRIAN	UNION ELECTRIC COMPANY	1530 SINGLETON AVENUE MAILCODE 613 ST LOUIS MO 63103
SCHNEIDER DANIEL	ILLINOIS NATURAL HISTORY SURVEY	607 E. PEABODY DRIVE CHAMPAIGN IL 61820

SCHNELLE ROBERT C	CINCINNATI GAS & ELECTRIC COMPANY	PO BOX 960, ROOM 522-A CINCINNATI OH 45201-0960
SCHUELLER MIKE	UNIVERSITY OF IOWA	102 OAKDALE CAMPUS, H101 OH IOWA CITY IA 52242-5002
SCHWARTZ JOHN	MICHIGAN STATE UNIVERSITY SEA GRANT EXTENSION PROGRAM LEADER	334 NATURAL RESOURCES BLDG. EAST LANSING MI 48824-1222
SEARLE GREG	WISCONSIN DNR WW-2	PO BOX 7921 MADISON WI 53707
SEGADA REGE	BETZ INDUSTRIAL	4636 SOMERTON ROAD TREVOSE PA 19053
SELEGEAN JAMES	WAYNE STATE UNIVERSITY DEPT. OF CIVIL & ENVIR. ENGINEERING	DETROIT MI 48082
SERLI NADIA	HYDRO-QUEBEC	500 SHERBROOKE OUEST 10IEME ETAGE MONTREAL QUEBEC H3A 3C5
SEXTON DAN	CRANE ENGINEERING SALES	9151 SHAVER RD PORTAGE MI 49081
SFERRAZZA CARMEN	AQUATIC SCIENCES INC	45 HANNOVER DRIVE PO BOX 2205 STN B ST CATHARINES ONTARIO L2M 6P6
SHERWOOD BILL	AQUATIC SCIENCES INC.	45 HANNOVER DRIVE PO BOX 2205 STN B ST CATHARINES ONTARIO L2M 6P6
SHIELDS DAVID F	WISCONSIN PUBLIC SERVICE CORP.	N490 HWY 42 KEWAUNEE WI 54216-9510
SHIMUNEK JIM	PETRA MAG	7440 N. ALGER ALMA MI 48801
SHULER SCOTT	BALL STATE UNIVERSITY BIOLOGY DEPARTMENT	MUNCIE IN 47907
SICKEL JIM	MURRAY STATE UNIVERSITY BIOLOGY DEPARTMENT	MURRAY KY 42071
SIM BLAIR	ONTARIO HYDRO TECHNOLOGIES	800 KIPLING AVENUE TORONTO ONTARIO M8Z 5S4
SINGER ANITA	WESTERN ILLINOIS UNIVERSITY DEPARTMENT OF BIOLOGICAL SCIENCES	MACOMB IL 61455
SINGER SAM	WESTERN ILLINOIS UNIVERSITY DEPARTMENT OF BIOLOGICAL SCIENCES	MACOMB IL 61455
SKUBINNA JOHN P	MICHIGAN STATE UNIVERSITY DEPARTMENT FISHERIES & WILDLIFE	EAST LANSING MI 48824
SLOSNERICK STEVE	TOLEDO EDISON COMPANY	5501 NORTH STATE ROUTE 2 OAK HARBOR OH 43449

SMITH SCOTT	VERONA AREA HIGH SCHOOL	700 N. MAIN ST VERONA WI 53593
SMITH CRAIG	WATERWAYS EXPERIMENT STATION	3909 HALLS FERRY RD VICKSBURG MS 39180
SMITHEE RICK	DETROIT EDISON CO.	6100 W. WARREN AVE DETROIT MI 48210
SMYTHE A. GARRY	ACRES INTERNATIONAL CORP	140 JOHN JAMES AUDUBON PKWY. AMHERST NY 14228-1180
SNYDER FRED L.	OHIO STATE UNIVERSITY EXTENSION OHIO SEA GRANT EXTENSION	CAMP PERRY, BLDG 3, ROOM 12 PORT CLINTON OH 43452
SPENCER FRED S.	ONTARIO HYDRO RESEARCH DIVISION	800 KIPLING AVENUE TORONTO ONTARIO M8Z 5S4
SPIDLE ADRIAN	CORNELL UNIVERSITY	FERNOW HALL ITHACA NY 14853
ST MARTIN BILL	WW OPERATION SERVICES	PO BOX 241 MENOMINEE MI 49858
STACEY ERIC	PURE-GRADE INC. DIRECTOR OF MARKETING	215 W., PALM AVENUE, SUITE 202 BURBANK CA 91502
STAFFORD LINDA	US ARMY CORP OF ENGINEERS PITTSBURG DISTRICT/CEORP-PD-R	1000 LIBERTY AVENUE RM. 1828, FEDERAL BLDG. PITTSBURGH PA 15222-4186
STEIN HENRY	ELECTRIC LIGHT & POWER	909 BERNARD DRIVE BUFFALO GROVE IL 60089
STEWART STEPHEN R.	MICHIGAN SEA GRANT DISTRICT EXTENSION AGENT	21885 DUNHAM ROAD CLINTON TOWNSHIP MI 48036
STEWART SONNY	TENNESSEE VALLEY AUTHORITY	PO BOX 1010 MUSCLE SHOALS AL 35660
STICE JAMES	DAYTON POWER & LIGHT COMPANY	PO BOX 468 ABERDEEN OH 45101-0468
STOECKEL JAMES	ILLINOIS NATURAL HISTORY	PO BOX 590 HAVANA IL 62644
STOECKMAN ANN	OHIO STATE UNIVERSITY	1735 NEIL AVE COLUMBUS OH 43210-1293
STOMA KEITH	GULF STATES UTILITIES COMPANY RIVERBEND NUCLEAR PLANT	PO BOX 220 ST. FRANCISVILLE LA 70775-0220
STOUT GLENN	UNIVERSITY OF ILLINOIS ILL./IND. SEA GRANT COLLEGE PROGRAM	205 N. MATHEWS AVENUE RM 2535 URBANA IL 61801
SUMMERS BRENT	UNIVERSITY OF LOUISVILLE BIOLOGY DEPARTMENT	LOUISVILLE KY 40292
SURRELL JEFFREY	NATIONAL ZEBRA MUSSEL ALLIANCE	2361 JEFFERSON DAVIS HWY. ARLINGTON VA 22202

SWORD LINDSAY	WALPOLE ISLE FIRST NATION	RR #3 WALLACEBURG ONTARIO N8A 4K9
TATEM HENRY	USA CORPS OF ENGINEERS	3909 HALLS FERRY RD VICKSBURG MS 39180-6199
TAYLOR ROBIN	OHIO SEA GRANT	1314 KINNEAR RD COLUMBUS OH 43212-1194
TESSIER CATHERINE	MCDONOLD COLLEGE, MCGILL UNIVERSITY WILDLIFE RESOURCES	21111 LAKESHORE ROAD ST. ANNE DE BELLEVUE QUEBEC H9X 3V9
THERIOT EDWIN A.	US ARMY CORP OF ENGINEERS WATERWAYS EXPERIMENT STATION	3909 HALLS FERRY ROAD VICKSBURG MS 39180-6199
THIEL JOHN C.	DAIRYLAND POWER COOPERATIVE	3200 EAST AVENUE S, PO BOX 817 LA CROSSE WI 54602-0817
THIEL PAM	US FISH & WILDLIFE SERVICE WINONA FISHERY RESOURCES OFFICE	51 E 4TH STREET ROOM 101 WINONA MN 55987
THIRY ROBERT	TRITON MARINE ENTERPRISES	1203 5TH STREET ALGOMA WI 54201
THORP JAMES H.	UNIVERSITY OF LOUISVILLE	LOUISVILLE KY 40292
THORPE DAN	WATER RESOURCES LABORATORY DAIRYLAND POWER COOPERATIVE	3200 E. AVENUE S. LA CROSSE WI 54602
TIPPIT RICHARD	US ARMY CORP OF ENGINEERS EP-E NASHVILLE DISTRICT/COERN-OR-R	PO BOX 1070 NASHVILLE TN 37202-1070
TIZZANO DONNA	CLEVELAND ELECTRIC ILLUM.	10 CENTER RD. E240 PERRY OH 44081
TORRES VINCE	JOHN DEERE DUBUQUE WORKS	PO BOX 538 DUBUQUE IA 52004-0583
TREANOR PAUL	SARGENT & LUNDY	55 EAST MONROE CHICAGO IL 60603
TSOU JOHN L.	EPRI PROJECT MANAGER	3412 HILLVIEW AVENUE PO BOX 10412 PALO ALTO CA 94303-0412
TUCHMAN NANCY	LOYOLA UNIVERSITY DEPARTMENT OF BIOLOGY	6525 N SHERIDAN ROAD CHICAGO IL 60626
TUTTLE, JR. L. RAY	NY STATE ELECTRIC & GAS CORP.	4500 VESTAL PKWY E., PO BOX 3607 BINGHAMTON NY 13902-3607
URBANOWSKI GREG	CARUS CHEMICAL COMPANY TECHNICAL SERVICE REPRESENTATIVE	1001 BOYCE MEMORIAL DRIVE OTTAWA IL 61350
VANDERPLOEG HENRY	GREAT LAKES ENV. RESEARCH	2205 COMMONWEALTH ANN ARBOR MI 48105
VATCHA NEVILLE	DMX INDUSTRIAL INC.	6540 MARTIN LUTHER KING ST. LOUIS MO 63133

VEECH BYRON	ILLINOIS POWER COMPANY	709 N. MORRISON COLLINSVILLE IL 62234
VERES DARRELL	WASHINGTON MILLS	PO BOX 1002 NIAGARA FALLS ONTARIO L2E 6V9
VORPAHL DWIGHT	COMMONWEALTH EDISON CO	101 SHILOH BLVD ZION IL 60039
WAHLERT STEVE	SARGENT & LUNDY ENGINEERS SENIOR STRUCTURAL PROJECT ENGINEER	55 EAST MONROE CHICAGO IL 60603
WALCH MARIANNE	NAVAL SURFACE WARFARE CENTER	10901 NEW HAMPSHIRE AVE SILVER SPRING MD 20903
WALKER MICHAEL	CHEMICAL CONTROL SYSTEMS	420 EAST OHIO, SUITE 29G CHICAGO IL 60611
WALLER DIANE L.	US FISH AND WILDLIFE SERVICE NATIONAL FISHERIES RESEARCH CENTER	PO BOX 818 LA CROSSE WI 54602-0818
WALTER MARK	MARTIN MARIETTA UTILITY SYSTEMS	PO BOX 1410 BLDG C-743-T-14 PADUCAH KY 42001-1410
WANG SHIAO	UNIVERSITY OF SOUTHERN MISSISSIPPI DEPARTMENT OF BIOLOGICAL SCIENCES	HATTIESBURG MS 39406-5018
WARDIUS KEN	MILWAUKEE METRO SEWERAGE DISTRICT CENTRAL LABORATORY	250 W. SEEBOTH STREET MILWAUKEE WI 53204-1446
WARNER BOB	TRI-STATE COMMER DIVING	PO BOX 1213 SOUTHGATE MI 48195
WAY CARL	WATERWAYS EXPERIMENT STATION	ER-A, 3909 HALLS FERRY RD VICKSBURG MS 39180
WEED ROBERT		700 E MAPLE RD STE 201 BIRMINGHAM MI 48009
WELKE KURT	WISCONSIN DNR	111 WEST DUNN ST PRAIRIE DU CHIEN WI 53821
WERNER ROBERT	BETZ LAB WATER MANAGEMENT	1205 CANTERBURY DRIVE STEVENS POINT WI 54481-6520
WHILEY BOB	GEC - MARCONI	ELETTRA AVENUE WATERLOOVILLE, HANTS POR77X5 ENGLAND
WHITBY G. ELLIOTT	FISCHER & PORTER (CANADA) LTD	134 NORFINCH DRIVE DOWNSVIEW ONTARIO M3N 1X7
WHITEKETTLE KURT	BETZ WATER MANAGEMENT GROUP	1 QUALITY WAY TREVOSE PA 19053
WHITMORE SHARON	US FISH & WILDLIFE	420 S GARFIELD AVE, SUITE 400 PIERRE SD 57501
WIANCKO PAUL	ONTARIO HYDRO/ENVIRONMENTAL PROTECTION	700 UNIVERSITY AVENUE A7-A4 TORONTO ONTARIO M5G 1X6

WILCOX SHAWN	LOUISIANA STATE UNIVERSITY	202 LIFE SCIENCE BLDG BATON ROUGE LA 70803
WILLIAMS ROBERT	PROJECT DIRECTOR SOUTHERN ILLINOIS UNIVERSITY	RIVERS CURRICULUM PROJECT BOX 2222 EDWARDSVILLE IL 62025
WILLIAMSON DWIGHT	MANITOBA DEPARTMENT OF ENVIRONMENT	BLDG 2 139 TUXEDO AVENUE WINNIPEG MANITOBA R3N 0H6
WILLIS RICK	VIRGINIA POWER	104 WILDEN DRIVE ASHLAND VA 23005-5908
WINDLE PHYLLIS	OFFICE OF TECHNOLOGY ASSESSMENT SENIOR ASSOCIATE	US CONGRESS WASHINGTON DC 20510
WITTIG DAVE	GREEN BAY WATER UTILITY	PO BOX 1210 GREEN BAY WI 54305
WOJTOWICZ GARY	NORTHWESTERN UNIVERSITY	2020 RIDGE AVENUE, RM. 200 EVANSTON IL 60208
WOOD CLIFF	TIMBER MILL RESEARCH	10607 SEALORD CT. INDIANAPOLIS IN 46230-9007
WOOMER NEIL	TENNESSEE VALLEY AUTHORITY	1101 MARKET STREET, HB 2C-C CHATTANOOGA TN 37402
WRIGHT BEN	ELF ATOCHEM	8777 PURDUE ROAD INDIANAPOLIS IN 46268
WRIGHT DAVID	UNIVERSITY OF MARYLAND SYSTEM	PO BOX 38 SOLOMONS MA 20688
YAFFE STANLEY S.	INDIANAPOLIS POWER & LIGHT COMPANY	PO BOX 1595 INDIANAPOLIS IN 46206
YAGER TIM	US ARMY CORPS OF ENGINEERS	190 5TH ST. E ST PAUL MN 55101
YESS SCOTT	US FISH &WILDLIFE SERVICE	51 E. 4TH STREET, ROOM 100 WINONA MN 55987
YOUNG BRENDA	DAEMEN COLLEGE DEPT. OF NATURAL SCIENCE	4380 MAIN STREET AMHERST NY 14226
YOUNT J. DAVID	US ENVIRONMENTAL PROTECTION AGENCY ENVIRONMENTAL RESEARCH LABORATORY	6201 CONGDON BLVD. DULUTH MN 55804
YU NENG	OHIO STATE UNIVERSITY	1735 NEIL AVE. COLUMBUS OH 43210-1293
ZHANG XIAOSONG	OHIO STATE UNIVERSITY	103 B&Z, 1735 NEIL AVE COLUMBUS OH 43210
ZIMMERMANN ARTHUR	NORTHERN STATES POWER	801 E. 5TH STREET RED WING MN 55066