

11th ANNUAL SHELLFISH BIOLOGY SEMINAR

FEBRUARY 26-27, 1991



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**NATIONAL MARINE FISHERIES SERVICE
NORTHEAST FISHERIES CENTER
MILFORD LABORATORY
MILFORD, CONNECTICUT 06460**

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Program Agenda

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11th Shellfish Biology Seminar
February 26-27, 1991
Howard Johnson Lodge
1052 Boston Post Road
Milford, CT 06460

February 26, 1991

- 8:45 a.m. Introduction
Walter Blogoslawski
Conference Chairman
- 9:00-9:30 a.m. Effects of food availability and food quality on the growth of juvenile shellfish in suspended culture
R. Rheault, Spatco Co. Ltd., Narragansett, RI, and M. Rice, URI, S. Kingston, RI
- 9:30-10:00 a.m. Growth of hard clams in Long Island Sound: sorting out the determining factors
R. Goldberg, G.H. Wikfors, NMFS, Milford CT
- 10:00-10:25 a.m. Hard clam nutrition in the lab and in the field: What does one tell us about the other?
G.H. Wikfors, NMFS, Milford CT
- 10:25-10:45 a.m. Dissolved oxygen regimes and shellfish recruitment
B.L. Welsh, UCONN, Avery Pt. CT
- 10:45-11:00 a.m. BREAK - POSTERS
CHURCHILL ROOM
- 11:00-11:30 a.m. European winkles, Japanese oysters, and zebra mussels: perspectives on the introduction of exotic mollusks on the east coast of North America
J.T. Carlton, Williams College, Mystic Seaport, Mystic CT
- 11:30-12:00 a.m. The Asiatic clam, Corbicula fluminea, extends its North American distribution to the Connecticut River
D.E. Morgan, M. Keser, J.F. Foertch, J.M. Vozarik, Northeast Utilities Co, Waterford CT
- 12:00-1:00 p.m. LUNCH
DICKENS ROOM
- 1:00-1:30 p.m. Unexplained oyster mortalities in New England: 1989-1990
K. Rask, U. MASS, Barnstable MA

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Atmospheric Administration
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1:30-1:45 p.m.	Unexplained mortalities in juvenile hatchery-raised oysters	D. Relyea F.M. Flower & Sons Bayville NY
1:45-2:15 p.m.	Responses of oyster hemocytes to MSX parasites	S.E. Ford K.A. Ashton-Alcox Haskin Shellfish Research Laboratory Port Norris NJ
2:15-2:45 p.m.	Detection of human <u>Vibrio</u> pathogens in shellfish	J.G. Simonson R.J. Siebeling Louisiana State Univ. Baton Rouge LA
2:45-3:15 p.m.	Oyster depuration and relaying to remove indigenous and fecal-borne bacteria	S.H. Jones, R. Langan K.R. O'Neill, U. New Hampshire, Durham NH and T.L. Howell Spinney Creek Oyster Co. Eliot ME
3:15-3:30 p.m.	BREAK - POSTERS	CHURCHILL ROOM
3:30-3:50 p.m.	A planter for the even dispersal of hard clam seed	G. Rivara, R. Paulsen P. Murray Cornell Coop Extension Riverhead NY
3:50-4:15 p.m.	Characterization of an area of high and of low hard clam (<u>Mercenaria</u>) abundance in the eastern Great South Bay, NY	J. Kassner, T. Carrano Town of Brookhaven Medford, NY and R. Cerrato SUNY, Stony Brook NY
4:30-6:30 p.m.	NRAC MEETING	CHURCHILL ROOM
6:30-7:45 p.m.	DINNER	DICKENS ROOM
7:45-9:30 p.m.	JUVENILE OYSTER MORTALITY MEETING	(BY INVITATION)
1:30-2:00 p.m.	Performance of oysters from inbred Long Island Sound and Delaware Bay lines and their hybrids	S.E. Ford, H.H. Haskin Haskin Shellfish Res. Lab, Port Norris NJ

February 27, 1991 11TH Shellfish Biology Seminar

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|------------------|---|--|
| 9:00-9:30 a.m. | Docks, boats and shellfish:
are they compatible? | S.L. Macfarlane
Town of Orleans
Orleans MA |
| 9:30-10:00 a.m. | Recent developments in the
improving shellfisheries of
Canada's maritime provinces | C.L. MacKenzie, Jr.
NMFS
Highlands NJ |
| 10:00-10:15 a.m. | BREAK - POSTERS | CHURCHILL ROOM |
| 10:15-10:45 a.m. | Long Island bay scallop
reseeding efforts - life
after the brown tide | P. Wenczel
Long Island Green
Seal Committee,
Southold NY and
S. Tettelbach
Long Island University
Southampton NY |
| 10:45-11:15 a.m. | Survival of juvenile scallops
in eelgrass beds: the role
of spatial and size refuge
from predators | M. Bricelj
Z. Garcia-Esquivel
M. Strieb
SUNY, Stony Brook NY |
| 11:15-11:30 a.m. | The Northeastern Regional
Aquaculture Center: An
overview | H.S. Parker
Northeastern Regional
Aquaculture Center
Southeastern
Massachusetts Univ.
N. Dartmouth MA |
| 11:30-12:00 a.m. | Triploidy and growth rates
in <u>Crassostrea virginica</u> | G. Matthiessen
Ocean Pond Co.
Fishers Island NY
and J. Davis
Baywater Research
Bainbridge Island WA |
| 12:00-1:00 p.m. | LUNCH | DICKENS ROOM |
| 1:00-1:30 p.m. | Growth and survival of
selectively bred Long Island
and Delaware Bay oysters | D.E. Dittman
S.E. Ford
Haskin Shellfish Res.
Lab, Port Norris NJ |
| 1:30-2:00 p.m. | Performance of oysters from
inbred Long Island Sound and
Delaware Bay lines and their
hybrids | S.E. Ford, H.H. Haskin
Haskin Shellfish Res.
Lab, Port Norris NJ |

- 2:00-2:30 p.m. Culture of triploid oysters and Sp...
in Virginia
K. Kurkowski
VIMS, Gloucester Pt VA
and M. Luckenbach,
M. Castagna, VIMS,
Wachapreague VA
and H. Hidu,
Univ. ME, Walpole ME
- 2:30-3:00 p.m. BREAK - POSTER TAKE DOWN
CHURCHILL ROOM
- 3:00-3:30 p.m. Recent trends in oyster
recruitment of selected sites
in Long Island Sound
J.H. Volk
Connecticut Dept.
Agriculture
Milford CT
- 3:30-3:45 p.m. CONCLUDING REMARKS
W. Blogoslawski
Conference Chairman
- *POSTERS*
- February 25, 1991
- 7:00 p.m. Using flow cytometric techniques
to monitor pure-algal cultures
10:00 p.m. and natural particle
assemblages - going from the
sublime to the ridiculous?
S.E. Shumway
Dept. Marine Resources,
W. Boothbay Harbor, ME
and T.L. Cucci,
Bigelow Lab,
W. Boothbay Harbor ME
- Aquaculture systems for the
Bridgeport Regional Vocational
Aquaculture Center
J.J. Curtis
T.B. Cunningham
T.C. Visel
Bridgeport Regional
Vocational Aquaculture
Center, Bridgeport CT
- A preliminary study of toxin
uptake and retention by surf
clams on Georges Bank
S. Shumway, K. Geib,
J. Barter, S. Sherman
Caswell, Dept. Marine
Resources
W. Boothbay Harbor ME
- Influence of environmental
factors on genotoxicity of
petroleum and PCB compounds
on American oyster eggs
and embryos
S. Stiles
NMFS
Milford CT
- Morphological and biochemical
changes during metamorphosis
of bivalve larvae
S.M. Gallager
P. Alatalo
WHOI, Woods Hole MA

Effects of Food Availability and Food Quality on
the Growth of Juvenile Shellfish in Suspended Culture

Robert B. Rheault

Spatco, Ltd.
264 Foddering Farm Road
Narragansett, Rhode Island 02882

Michael A. Rice

Department of Fisheries and Aquaculture Science
University of Rhode Island
South Kingston, Rhode Island 02879

Shellfish growth studies were initiated in 1988 using predator proof cages suspended under floating docks in small boat marinas and a "clean water" control site. In the summer of 1990 the growth rates of three species of juvenile shellfish were monitored bi-weekly for 20 weeks: American oysters, Crassostrea virginica; hard-clams, Mercenaria; and bay scallops, Argopecten irradians.

To evaluate the relative influence of various environmental variables on the growth and survival of juvenile shellfish, three sites in Point Judith Pond in Narragansett, Rhode Island were selected along a gradient of food conditions and current speeds. Weekly measurements of temperature, salinity, total seston, particulate organic matter (POM), chlorophyll-a, phytoplankton species composition and fecal coliform were compared with semi-weekly growth rate determinations.

Instantaneous growth (as percent increase in volume per day) and survival were excellent at all sites. A strong seasonal component of growth obscured most significant correlations between growth and chlorophyll, POM or seston. High current speeds at the site with lowest food concentrations produced similar growth rates confirming the horizontal seston flux model proposed by Grizzle and Lutz.

Preliminary data describing the influence of suspended culture versus on bottom culture and separate work describing the bioaccumulation of heavy metals will be briefly discussed.

Growth of Hard Clams in Long Island Sound:
Sorting out the Determining Factors

Ronald Goldberg
Gary H. Wikfors

National Oceanic and Atmospheric Administration
National Marine Fisheries Service
Northeast Fisheries Center
Milford Laboratory
212 Rogers Avenue
Milford, Connecticut 06460

Growth of juvenile hard clams, Mercenaria mercenaria, was studied at three sites in Long Island Sound (LIS) by periodically measuring caged juvenile clams in 1987 and 1988 and by back-calculating growth from shell age-ring analysis of collected adults. Sites were chosen at Stonington, Milford, and Greenwich, Connecticut to represent changing environmental conditions along the east - west axis of LIS. At the Milford site, growth of caged juvenile clams up to about 23 mm was not significantly different ($P < 0.001$) at planting densities from 100/m² to 5,000/m². Clams at each site, however, grew at different rates and time periods specific to site and year. Temperature, dissolved oxygen, seawater current flow, and seston biochemical composition were measured at the sites. Principal components analysis (PCA) of 1987 data showed that temperature and the combination of potential food and water movement accounted for sufficient variability to distinguish the Stonington site from Greenwich and Milford. Growth of caged animals at Greenwich was inhibited in mid-summer of 1987 during a bloom of the dinoflagellate Prorocentrum; consequently, data from this period were not used in statistical analyses. Regression of growth data against environmental factors generated by PCA indicates a strong correlation between growth and the combined effects of temperature and food availability. Analysis of population age and growth data from the shell record revealed long-lived clams of up to 50 years and evidence of past recruitment trends. Age at length and the rate of growth appear to be influenced by population density and are site specific. This study provides insight into how environmental conditions in LIS affect the population dynamics of the hard clam.

Hard Clam Nutrition in the Lab and in the Field: What Does One Tell Us About the Other?

Gary H. Wikfors

National Oceanic and Atmospheric Administration
National Marine Fisheries Service
Northeast Fisheries Center
Milford Laboratory
212 Rogers Avenue
Milford, Connecticut 06460

Controlled experiments in which hard clams (Mercenaria mercenaria) are fed known ratios of cultured algae have allowed us to determine which algal strains best support the growth of individual animals. Similar experiments with other bivalve species -- and using non-algal food sources, ranging from micro-encapsulated diets to detrital particles -- have yielded reliable growth data as well. Further, if physical (size, shape, cell-wall type) and biochemical (gross biochemical composition, fatty acids, sterols, amino acids, and others) characteristics of the food source are measured or systematically varied, then the contributions of these characteristics to the recipe for a suitable food can be determined. Meanwhile, sitting on the bottom of the sea, clams and their kin are subject to widely-varying environmental conditions and a largely undefined menu that likely changes drastically in both quantity and quality over short and long time scales. Fluctuations in natural bivalve populations will, over time, integrate combined effects of all environmental variables upon the physiology of individual animals; nutrition is only one such variable.

Research efforts to understand the nutrition of bivalve mollusks have proceeded along both field and laboratory fronts. Lab studies have provided the most information on food quality; whereas, more recently, field studies have addressed food quantity. The challenge now facing bivalve nutrition research is to combine knowledge gained through both approaches into a coherent model of bivalve nutrition as a controlling factor in population biology. Multivariate statistics and mathematical modeling offer promising ways to do this, and will be the subject of this presentation.

Dissolved Oxygen Regimes and Shellfish Recruitment

Barbara L. Welsh

Marine Sciences Department
University of Connecticut
Avery Point
Groton, Connecticut 06340

This paper discusses the causal dynamics of seasonal hypoxia (low dissolved oxygen) and anoxia (no dissolved oxygen) in shallow and deep habitats of Long Island Sound, and the potential for impact on shellfish recruitment through several mechanisms. Hypoxia and anoxia occur periodically during most summers, but their spatial and temporal dynamics differ between unstratified (shallow) and stratified (deep) waters. As a result, their occurrences in shallow habitats may or may not be linked to those in deep habitats in any particular year. Hypoxia and anoxia can impact shellfish directly through lethal or debilitating physiological effects, either independently or in combination with other factors (synergistically). Hypoxic and anoxic conditions may also impact shellfish indirectly through physico-chemical mobilization of potentially harmful materials sequestered in sediments.

Coast have occurred for at least 50 years, since the 1950s. Concerns over the hyperinflation, potential success and spread of the Japanese oyster on the Atlantic coast focus on ecological and disease questions, although formal studies are few. The most recent accidental invasion, the zebra mussel in the Great Lakes basin, holds two messages for the Atlantic coast biologist. The first is that it will eventually spread into fresh (and possibly oligohaline) water drainage throughout the Eastern seaboard. Second, and perhaps more important for marine and estuarine fisheries biologists, is that the vector that brought the zebra mussel to North America--ballast water--continues to release, on a daily basis, large numbers of exotic mollusks into American coastal waters. Long Island Sound, Chesapeake Bay, and other major estuaries are playing biological roulette with ballast water invasions.

European Winkles, Japanese Oysters, and Zebra Mussels:
Perspectives on the Introduction of Exotic Mollusks
on the East Coast of North America

James T. Carlton

Maritime Studies Program
Williams College - Mystic Seaport
Mystic, Connecticut 06355

Exotic marine and estuarine mollusks have been continuously introduced to the east coast of North America for over 200 years. Inadvertent (accidental) introductions include shipworms, marsh snails, and nudibranchs (sea slugs). Intentional introductions include European oysters, Japanese oysters (not yet represented by established populations) and the European periwinkle. The latter invasion led to spectacular alterations to rocky shores, marshes, and mudflats from Newfoundland to New Jersey. Currently, the most debated intentional introduction focuses on the Japanese oyster: interest in importing this oyster has arisen almost every decade since the 1920s, with later workers frequently overlooking both earlier attempts and debates. Private (including secret) and government plantings of Japanese oysters on the Atlantic coast have occurred for at least 50 years, since the 1940s. Concerns over the importation, potential success and spread of the Japanese oyster on the Atlantic coast focus on ecological and disease questions, although formal studies are few. The most recent accidental invasion, the zebra mussel in the Great Lakes basin, holds two messages for the Atlantic coast biologist. The first is that it will eventually spread into fresh (and possibly oligohaline) water drainage throughout the Eastern seaboard. Second, and perhaps more important for marine and estuarine fisheries biologists, is that the vector that brought the zebra mussel to North America--ballast water --continues to release, on a daily basis, large numbers of exotic mollusks into American coastal waters. Long Island Sound, Chesapeake Bay, and other major estuaries are playing biological roulette with ballast water invasions.

The Asiatic Clam, *Corbicula fluminea*, Extends
its North American Distribution to the Connecticut River

Douglas E. Morgan

Milan Keser

James F. Foertch

Joseph M. Vozarik

Northeast Utilities Co.

Northeast Utilities Environmental Laboratory

P.O. Box 128

Waterford, Connecticut 06385

In May 1990, *Corbicula fluminea* (Asiatic clam) was found in service water piping at the Haddam Neck Plant (Connecticut Yankee-CY), a nuclear power station on the Connecticut River in Haddam, Connecticut. Sampling in October and November 1990 of the 1.7 km long CY discharge canal and a 16 km section of the Connecticut River from the mouth of the Salmon River to the Middletown Power Plant, Middletown, Connecticut, found Asiatic clams in densities of 15-117 clams/m², with shell lengths ranging from 1.9-12.2 mm. This report of *C. fluminea* in the Connecticut river documents the farthest northern distribution of this species along the east coast of North America. The successful establishment of this clam in the Connecticut River may cause problems to other raw freshwater users, and the invasive nature of this species should be of concern to freshwater users throughout New England.

Unexplained Oyster Mortalities in New England: 1989-1990

Karl Rask

University of Massachusetts at Amherst
Cape Cod Cooperative Extension
Deeds Probate Building
Barnstable, Massachusetts 02630

In mid-August 1989, growers from Maine to Long Island experienced similar mortalities in first-year oyster seed. Oysters grew well initially, then died suddenly with little advance warning. Only small seed under 25 mm died, with those in surface trays being hardest hit. Some shell deformity was seen (lower shell outgrowing the upper shell) as a characteristic of any mantle edge irritation, including MSX. Pathology reports revealed that the oysters were in poor condition or emaciated, but no traces of MSX or any other pathogen could be found. While leading shellfish pathologists were mystified, the problem appeared to be contagious, culminating in the loss of over 30 million seed in 1990. Various hypotheses have been presented, such as a nutritionally undesirable bloom of phytoplankton, and genetic differences in seed. To date, none have explained all of the observed symptoms, which were noted by some growers even before the 1989 season. It is hoped that discussion at the Milford meetings will improve the understanding of this problem.

Unexplained Mortalities in Juvenile Hatchery Raised Oysters

David Relyea

Frank M. Flower & Sons Inc.
P.O. Box 1436
Bayville, New York 11709

During the summer of 1990, Frank M. Flower and Sons experienced a significant die-off of 1/4"-1" juvenile oysters (C. virginica) in a grow-out raft system located at Mill Neck Creek, N.Y. These mortalities took place in July and August and ultimately resulted in the loss of 50% of the year's production. Examination of samples by Dr. Harold Haskin of Rutgers University and Dr. Frank Perkins of VIMS did not reveal any apparent disease organisms. Similar symptoms had been reported by oyster aquaculturists during the summer of 1989 to Karl Rask of the Massachusetts Cooperative Extension Office (University of Massachusetts). Dr. Steve Malinowski from The Clam Farm, Inc. of Fishers Island, New York, indicates that he has experienced a similar problem for the last three years. Preliminary work suggests that the problem may be linked to seed oysters from the Northeast.

Infected oysters and enriched. They are then treated in a washed seawater and incubated with hemocytes from uninfected oysters in a 24-well tissue culture plate. The percentage of phagocytosed and non-phagocytosed parasites is determined by fluorescence examination.

Our findings with the in vitro system parallel observations from tissue sections: live parasites are rarely (1-5%) phagocytosed. Failure of phagocytosis occurs regardless of whether hemocytes are taken from resistant or susceptible oysters. Killed parasites, however, are readily engulfed. The possibility that a substance toxic to *G. helveticus* is present in the serum of resistant oysters was examined by incubating parasites in sera of resistant and susceptible animals and then testing them for viability using trypan blue dye exclusion. No differences in viability were found associated with resistance or susceptibility. Experiments in which the parasites were treated to inhibit metabolism and to alter surface molecules indicate that the process by which the parasites evade detection is an active one and that it probably involves surface structure.

Responses of Oyster Hemocytes to MSX Parasites

Susan E. Ford
Kathryn A. Ashton-Alcox

Haskin Shellfish Research Laboratory
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Port Norris, New Jersey 08349

Hemocytes, the blood cells of oysters, are considered to be a primary line of defense against invading microorganisms because they are capable of recognizing and phagocytosing foreign material. Nevertheless, Haplosporidium nelsoni, the protozoan parasite causing MSX disease, is rarely found within hemocytes of oysters studied by tissue section. To further investigate the oyster hemocyte-H. nelsoni system, we have used in vitro experiments that allow us to verify quantitatively the observations from tissue sections and to understand why oyster hemocytes apparently fail to recognize and attack H. nelsoni plasmodia.

H. nelsoni plasmodia are collected from the hemolymph of heavily infected oysters and enriched. They are then treated in a desired manner and incubated with hemocytes from uninfected oysters in a 24-well tissue culture plate. The percentage of phagocytosed and non-phagocytosed parasites is determined by microscopic examination.

Our findings with the in vitro system parallel observations from tissue sections: live parasites are rarely (1-3%) phagocytosed. Failure of phagocytosis occurs regardless of whether hemocytes are taken from resistant or susceptible oysters. Killed parasites, however, are readily engulfed. The possibility that a substance toxic to H. nelsoni is present in the serum of resistant oysters was examined by incubating parasites in sera of resistant and susceptible animals and then testing them for viability using trypan blue dye exclusion. No differences in viability were found associated with resistance or susceptibility. Experiments in which the parasites were treated to inhibit metabolism and to alter surface molecules indicate that the process by which the parasites evade detection is an active one and that it probably involves surface structure.

Cell concentrations of approximately 10^8 colony forming units (CFU) per ml of enrichment broth. When AP broth was seeded with as few as 10^3 V. cholerae O1 cells, the membrane-ELISA detected the cells following 6 hours incubation, while 16 hour AP broth burdened with heterologous Vibrio species at concentrations as high as 10^8 cells per ml routinely produced negative reactions. The dipstick membrane was tested in the three-tube three-dilution MPN. AP broth inoculated with oyster meat homogenate was spiked with various concentrations of V. cholerae O1 cells. Discernible color reactions appeared on the dipstick membranes inserted into 6-hour AP enrichment broths inoculated with oyster meat seeded with as few as 100 cells per gram of meat. Vibrio cholerae O1 cells can be detected in seafood or environmental specimens within the time-frame of one 8-13 work day.

Detection of Human Vibrio Pathogens in Shellfish

Janet G. Simonson
Ronald J. Siebeling

Department of Microbiology
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Baton Rouge, Louisiana 70803

Halophilic Vibrio species are ubiquitous in the marine environment and, as a consequence, shellfish burdened with the 01 and non-01 serovars of V. cholerae, V. parahaemolyticus and V. vulnificus may deliver these pathogens to the human consumer--most especially when eaten raw such as the oyster. Regulations are not yet in place which specify levels and Vibrio species that will be acceptable for retail markets. In order not to delay entry of shellfish into the market place, specific, sensitive and rapid methodologies are needed to detect and enumerate these pathogens in shellfish.

In our investigations we examined two serological approaches to develop rapid detection methods. Initially, we exploited the finding that each Vibrio species expresses species-specific flagellar (H) antigens. Latex beads armed with monoclonal antibody (MAb) to H-antigens of V. vulnificus, V. cholerae, V. parahaemolyticus and V. anguillarum agglutinated the target species, exclusively, within 1 to 3 minutes by the slide test. These reagents, though specific, do not meet the time constraints to obtain results within a working day.

A second approach examined consisted of a prototype dipstick, fitted with a detection pad designed to exhibit a blue color when exposed to a specimen burdened with the target Vibrio. Affinity-purified polyclonal anti-Vibrio cholerae 01 Inaba immunoglobulin was covalently bound to polyvinylidene difluoride membrane squares. The antibody-coated membrane pads were tested for the ability to capture and immobilize V. cholerae 01 cells from alkaline-peptone (AP) broth enrichment cultures. MAb reactive with the 01 serovar-specific antigen A were used to detect the immobilized V. cholerae cells by an enzyme-linked immunosorbent assay (ELISA). The membrane-ELISA, prototype dipstick detected Inaba and Ogawa 01 serovars at cell concentrations of approximately 10^6 colony forming units (CFU) per ml of enrichment broth. When AP broth was seeded with as few as 10^2 V. cholerae 01 cells, the membrane-ELISA detected the cells following 6 hours incubation, while 18 hour AP broth burdened with heterologous Vibrio species at concentrations as high as 10^8 cells per ml routinely produced negative reactions. The dipstick membrane was tested in the three-tube three-dilution MPN. AP broth inoculated with oyster meat homogenate was spiked with various concentrations of V. cholerae 01 cells. Discernible color reactions appeared on the dipstick membranes inserted into 6-hour AP enrichment broths inoculated with oyster meat seeded with as few as 100 cells per gram of meat. Vibrio cholerae 01 cells can be detected in seafood or environmental specimens within the time-frame of one 8-hour work day.

Oyster Depuration and Relaying to Remove
Indigenous and Fecal-Borne Bacteria

Stephen H. Jones
Kathleen R. O'Neill
Richard Langan

University of New Hampshire
Jackson Estuarine Laboratory
Department of Natural Resources
Department of Microbiology
Durham, New Hampshire 03824

Thomas L. Howell
Spinney Creek Oyster Company
1 Howell Lane
Eliot, Maine 03903

The Great Bay/Piscataqua River Estuary in New Hampshire and Maine has an abundant oyster resource which is commercially harvested from restricted water on the Maine side and harvested from approved waters in New Hampshire by recreational shellfishermen. Spinney Creek Oyster Company (SCOC) operates a depuration facility and relay lagoons for the Maine oysters. Since Vibrio vulnificus was found in the estuary in 1989, we have studied the potential for depuration and/or relaying to promote elimination of this pathogen from shellfish. Oysters were collected from SCOC harvest lots before and after depuration or relaying. Oysters containing V. vulnificus were also harvested from the Piscataqua River and relayed to Great Bay. Weekly water and oyster samples were collected from the harvest and relay sites, and analyzed for the presence of total and fecal coliforms, Escherichia coli, V. vulnificus, and total vibrios. The data presented represent the comparative responses of the vibrios and the fecal indicator bacteria to depuration and relaying at the SCOC facility and relaying oysters to Great Bay.

Depuration and relaying at SCOC always resulted in drastic reductions in levels of fecal coliforms and E. coli in oysters. Total coliforms were depurated to a lesser extent and total vibrios did not respond to depuration or relaying. Depuration had an inconsistent effect on V. vulnificus and Vibrio parahaemolyticus. However, consistent decreases in V. vulnificus levels occurred in oysters relayed to lagoons in late August through October. Levels of fecal indicator bacteria in oysters relayed to Great Bay decreased compared to oysters at the Piscataqua River harvest site, yet remained relatively high. V. vulnificus, which had previously not been detected in Great Bay, was present in relayed oysters at lower levels than at the harvest site for the initial 4 weeks of relaying. Thereafter, V. vulnificus was not detected in relayed oysters, yet it was detected for 4 more weeks in oysters at the harvest site. These results demonstrate the differences in responses between indigenous, estuarine bacteria and fecal-borne contaminants in oysters.

A Planter for the Even Dispersal of Hard Clam Seed

Gregg Rivara
Ronald Paulsen
Patrick Murray

Cornell Cooperative Extension
Marine Education Center
39 Sound Avenue
Riverhead, New York 11901-1098

Currently, public and private mariculture facilities use state-of-the-art techniques for hatchery and nursery production of bivalve shellfish seed. Yet, for those that plant unprotected seed directly on the bottom for final growout, little or nothing is done to ensure that seed are evenly distributed. The literature confirms the existence of density dependent mortality, i.e., the more dense shellfish are planted the greater the chance they will be preyed upon. Present methods of seed planting create clumps of animals with empty spaces between; foraging predators locate these areas of high density and consume shellfish at a high rate. The present study modified an agricultural seed planter for use with hard clams, Mercenaria mercenaria. The planter chosen (Case IH 900 Cyclo Air[®]) uses low-pressure air to handle and deliver seed, lessening the chance of clam breakage.

The planter was mounted along with an engine to power the hydraulics onto a 28 foot boat. No attempt was made to plant the clams into the bottom, as Paulsen and Murray had previously determined that doing so does not increase survival over clams dropped onto the bottom. Clams were delivered under the boat's propeller wash to minimize swirling. Two size ranges of seed, (60,000 of 8-12 mm and 20,000 of 16-20 mm) were used; which were planted at densities of 10, 20 and 50 clams per square meter in Great South Bay, New York. Diver observations determined that actual clam density and distribution approximated target values. Divers also looked at breakage (less than 1%) and burrowing response (good). No attempt was made to determine subsequent survivorship of those clams planted. A commercial plant was made at the F.M. Flower and Sons Company in Oyster Bay, New York, where 256,000 of 10-15 mm clam seed were planted.

It is hoped that this planter will be used and copied where warranted to increase survival of planted hard clam populations. Mariculture firms could use the planter to plant evenly-dispersed high densities of clams on prepared beds, while public projects would more likely plant lower densities to enhance natural sets and avoid a harvesting "bonanza".

Partial funding for this project was provided by the New York State Urban Development Corporation. Trade names mentioned do not imply endorsement.

Characterization of an Area of High and of
Low Hard Clam (*Mercenaria mercenaria*) Abundance
in the Eastern Great South Bay, New York

Jeffrey Kassner
Thomas Carrano

Town of Brookhaven
Division of Environmental Protection
3233 Route 112
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Robert Cerrato

Marine Sciences Research Center
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Analysis of the distribution of hard clam (*Mercenaria mercenaria*) abundance in the eastern portion of the Great South Bay, New York, as determined by annual population censuses from 1987 to 1989, was used to identify nine distinct and stable regions of high hard clam abundance interspersed within regions of low clam abundance. To define the regional and local environmental parameters associated with hard clam abundance, side-scan sonar, sediment profile photography, sediment surface video photography, and depth profiling were undertaken within areas of high and low abundance regions. When data from the high abundance areas is pooled and compared to the low abundance areas, high abundance areas have a coarser sediment grain size, shallower RPD depth, greater sediment compactness, greater sediment surface roughness, a thinner flocculent layer, and shells and shell fragments in the sediment. In addition, high abundance areas are generally superimposed on relic oyster reefs. The transition from low to high abundance generally coincides with increased topographic relief and spatial changes in sediment type parameters.

A detailed characterization was undertaken for one of the high and low clam abundance regions. The high abundance area had a muddy-sand sediment which was relatively hard. Sediment surface relief was provided by the sediment, shells and worm tubes. Biogenic reworking was minor. The low abundance area had sandy-mud sediment which was soft, had a thick flocculent layer, and considerable bioturbation. Surface relief was minor and shells and worm tubes were absent. Topographically, the high abundance area was slightly elevated relative to the adjacent area while the low abundance area was slightly depressed.

Population data suggest that differential settlement and/or survival to age 1 and account for the difference in clam abundance between high and low abundance regions. This information, together with the environmental characterization, is being used to evaluate the spectrum of factors that may be contributing to the differential abundance of hard clams.

Docks, Boats and Shellfish: Are They Compatible?

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Communities in Massachusetts have been increasingly concerned about the proliferation of private docks and piers on inland waterways within the public tidelands and their effect on shellfish resources. Docks are generally licensed on an individual case-by-case basis where adverse effects to shellfish from one dock may be minimal, but cumulative effects of numerous docks in shallow and narrow embayments may be substantial. Potential problems discussed include the structures themselves and the use of the docks by recreational boats producing habitat loss due to pilings and pipes and unsuitable sediment conditions around the pilings. Other problems included shading of eelgrass by docks, destruction of eelgrass by boats, physical obstruction of traditional fishing areas, decreased setting and sediment alteration from prop wash, changes in circulation patterns, unknown toxicity of chemicals from pressure treated wood in estuarine environments, and unknown effects of toxic chemicals in bottom paint. Proof of these potential problems on site is nearly impossible to determine prior to licensing. Scientific literature as supporting documentation in court challenges is very limited. Communities have responded by prohibiting the transplant of shellfish, often cited as a mitigating measure, to an area outside the area affected by the dock, and have adopted criteria to determine the appropriateness of docks in certain shellfish habitats through the regulatory process. Arguments have been made that assessment should include historic data as well as present day productivity in determining shellfish habitat.

The Maritime Provinces have achieved their successes in producing shellfish by: (1) using wild seed (it is abundant and inexpensive to collect; little seed has been raised in hatcheries); (2) providing their industries with substantial government financial and technical support; (3) inviting shellfishermen to contribute to development plans; (4) sending biologists and lead fishermen to the U.S. and Europe to observe the best culture methods; and (5) having government personnel aggressively market their shellfish in Canada and the U.S. Thus, sales of oysters and mussels have been good.

Recent Developments in the Improving
Shellfisheries of Canada's Maritime Provinces

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Following a large decline from 1954 to 1972, production of oysters (C. virginica) on Prince Edward Island has since turned upward. Production in 1989, about 60,000 bushels worth \$3.8 million Canadian dollars (ex-island), was four times the total in 1954 and was two-thirds of the total in the best year, 1890. The province produced the increase by transplanting unfished oysters to good market beds and by spreading fossil shells it dredged from deposits on depleted oyster beds to collect spat. In 1989 and 1990, the Province used a new procedure, i.e., cultivating dormant shell beds to remove a deposit of silt. It was successful as the beds (60 acres) received good sets of spat. Such cultivating is perhaps one of the least expensive methods available for producing oysters. New Brunswick has been using cone-shaped spat collectors, about 2/3 meter in diameter and termed Chinese hats, to cultivate oysters successfully. In contrast to Prince Edward Island which has a mixed public and private oyster fishery, New Brunswick's is mainly private. Leaseholders remove the seed collected on the hats, spread them on their leases for growth, and then market them after they reach a length of three inches.

Since the early 1980s, the Maritime Provinces, led by Prince Edward Island and Nova Scotia, have been culturing mussels (Mytilus edulis). Mussel production has been rising rapidly. In 1989 production from Prince Edward Island was about 90,000 bushels worth \$4.3 million Canadian dollars.

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Survival of Long Island Bay Scallop Reseeding Efforts - Life After the Brown Tide

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Populations of the bay scallop, Argopecten irradians, in Long Island, New York, were decimated after extensive blooms of Aureococcus anophagefferens ("brown tide") occurred between 1985-87. Commercial landings fell from 279,000 lbs (worth \$1.26 million) in 1984 to just 250 lbs (worth \$2,375) in 1988. Large scale reseedling of hatchery-reared scallops in the Peconic Bays was initiated by the Long Island Green Seal Committee in 1986, and continued through 1989. Transplants during the first two years were thwarted by the persistence of the brown tide. Extensive monitoring of the Fall 1988 transplants revealed that survival of the 30-mm seed until the next spawning season ranged from 0-12% at six different sites. Nevertheless, the surviving broodstock is thought to have contributed significantly to the widespread scallop set which was observed in 1989. Increased commercial landings and a very heavy set in 1990 suggest that Long Island bay scallop populations are recovering.

Size-specific losses of scallops due to emigration and/or predation were determined by a) tethering, and b) free release of hatchery-reared scallops of two size classes (13 and 30 mm) within contrasting eelgrass habitats in Hallock Bay, Long Island. Major predators were identified by diver surveys and video recording. Mud crabs, Decapoda sp., were the numerically dominant predator in this system. The relevance of these studies in assessing seasonal predatory risk and the success of scallop reseedling efforts is discussed.

Survival of Juvenile Scallops in Eelgrass Beds: the Role of
Spatial and Size Refuge from Predators

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Post-settlement by scallops, Argopecten irradians, appear to gradually shift from a spatial to a size refuge from predation as they move from the eelgrass canopy to the bottom during their early life history. Prior studies conducted in our laboratory have shown that the scallops' ability to attach to elevated substrates is strongly size dependent between 5 and 20 mm in shell height. In order to determine the rate and size at which scallops relocate to the bottom under natural conditions, temporal patterns in attachment position on eelgrass blades were determined for natural postset (mean initial size > 5 mm) in two eastern Long Island bays throughout the summer and fall of 1990. Once scallops attained about 12 mm, the proportion of individuals attached above-bottom decreased markedly (from 100% to < 5%) over a five-week period, such that nearly 100% of the population was found on the bottom by early October, when scallops attained about 30 mm in shell height.

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The Northeastern Regional Aquaculture
Center: An Overview

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The Northeastern Regional Aquaculture Center (NRAC), headquartered at Southeastern Massachusetts University, is one of five Regional Aquaculture Centers established by the U.S. Congress. Funded by the U.S. Department of Agriculture at an annual level of approximately \$700,000, and representing 12 states and the District of Columbia, NRAC develops and sponsors cooperative regional research and extension projects in support of the aquaculture industry in the northeastern United States.

A Board of Directors representing the region's aquaculture industries, academic institutions, and government agencies provides overall direction and management of NRAC. Research and extension priorities are established by a Technical-Industry Advisory Council (TIAC) consisting of a Technical Committee and an Industry Committee. The Technical Committee includes key aquaculture researchers and extension agents in the region, while the Industry Committee represents principal commercial aquaculture interests in the Northeast. The TIAC is also responsible for annual reviews of progress of NRAC-supported projects.

NRAC is presently supporting nine regional projects in finfish nutrition, oyster genetics and reproductive biology, government regulations affecting aquaculture, finfish economics, and development of a regional aquaculture extension program. NRAC is currently developing five additional projects in the areas of water quality, aquaculture systems technology, and fish health. Total NRAC funding commitment to projects in progress or under development exceeds \$2.6 million. NRAC also publishes "Northeastern Aquaculture", a quarterly newsletter highlighting NRAC projects and other topics of interest to the northeastern aquaculture community.

Triploidy and Growth Rates in Crassostrea Virginia

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The growth rates of juvenile triploid and diploid C. virginica from the same parent stock were compared during their first season of growth at three locations. At all three sites, the growth rate of the triploid groups was found to be significantly greater than that for the diploids with respect to shell height, whole weight and total volume. At one of the sites where MSX (Haplosporidium nelsoni) is endemic - Cotuit Bay, Massachusetts - the survival rate was higher among the triploids than among the diploids even though MSX prevalence was lower in the latter group. These results are felt to be encouraging, although preliminary.

We have analyzed growth and mortality data, collected between 1977 and 1980, on oyster strains of different geographic origin. After up to seven generations of selective breeding for resistance to MSX disease, strains of Long Island origin grow faster and are significantly larger at a given age than oyster strains of Delaware Bay origin. The differences in growth are not strongly correlated with differences in mortality among these strains. These observations are consistent with genetic adaptation to maximize the energy available for growth and reproduction in a given thermal regime and may reflect the optimum balance of energy acquisition vs energy expenditure in a given population.

Growth and Survival of Selectively Bred Long
Island and Delaware Bay Oysters

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Local populations of species with large geographic ranges have often been shown to be physiologically differentiated. One example is the oyster, Crassostrea virginica, which shows genetically based latitudinal differences in reproductive cycles. Physiological differentiation of populations is often correlated with geographic temperature clines. Organisms living in higher latitude temperature regimes compensate by increasing their metabolic rate at a given low temperature relative to those organisms living at lower latitudes.

Strains of C. virginica from areas of contrasting thermal regimes (Long Island Sound and Delaware Bay) have been maintained in a single temperature regime by the Haskin Shellfish Laboratory for multiple generations. The situation is ideal for the separation of genetically based physiological variation from environmentally influenced differences (acclimatization). If genetically based physiological compensation has occurred, the population from farther north will grow faster in the common environment, even after several generations.

We have analyzed growth and mortality data, collected between 1977 and 1989, on oyster strains of different geographic origin. After up to seven generations of selective breeding for resistance to MSX disease, strains of Long Island origin grow faster and are significantly larger at a given age than oyster strains of Delaware Bay origin. The differences in growth are not strongly correlated with differences in mortality among these strains. These observations are consistent with genetic adaptation to maximize the energy available for growth and reproduction in a given thermal regime and may reflect the optimum balance of energy acquisition vs energy expenditure in a given population.

Performance of Oysters from Inbred Long Island Sound
and Delaware Bay Lines and Their Hybrids

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Physiological differences in eastern oyster, Crassostrea virginica, from different geographical regions of the United States have long been noted. Recently, we have documented genetically based differences in reproductive cycles of oysters from Long Island Sound and Delaware Bay. Differences in growth rates of oysters from the two locations also appear to be genetically based. Even after several generations of residence in Delaware Bay, Long Island strains consistently grow faster and to a larger size than Delaware Bay strains (see abstract on page 24 by D.E. Dittman and S.E. Ford).

Although they grow faster, oysters in the Long Island line currently maintained at the Rutgers laboratory are not as resistant to MSX disease as are Delaware Bay lines. In an effort to combine the traits of fast growth and good survival, we produced a cross between Delaware Bay and Long Island oysters in 1988. Representatives of the cross and the two "pure" lines were deployed on Cape Cod (2 locations), in Delaware Bay, and in Chesapeake Bay beginning in May 1989 and have been monitored since then for growth, survival, meat quality (condition index), and disease levels.

In the first year of testing (1989), during which MSX pressure was low in all locations, survival of the hybrid was slightly, but consistently better, at all locations compared to the pure lines. In 1990, under challenge by both MSX and Dermo diseases, survival of the hybrid was considerably better than that of the pure lines. Growth rates were site specific, but growth of the pure Delaware Bay line was consistently the least whereas that of the hybrid was generally equal to, or better than, that of the Long Island pure line. Preliminary data analysis, then, indicates that in this particular test, the Delaware Bay-Long Island hybrid performed better than either pure line.

Culture of Triploid Oysters in Virginia

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Polyploidy (mostly triploidy) has been important in the development of agricultural and aquacultural stocks. For oyster culture triploidy offers several potential advantages, including (1) enhanced growth rates, (2) expanded marketing period, and (3) some disease resistance. These issues have generally not been explored for Crassostrea virginica, in large measure because the production of triploids of this species has proven difficult. In 1990 we produced triploid C. virginica using cytochalasin B and modification of techniques developed for C. gigas. Survival after 2 days was 12%; percent triploidy, determined by cytofluorimetry, was 90%. Grow-out of triploids and equal-aged diploids in off-bottom culture was initiated in May 1990 in Chesapeake Bay and is ongoing. Monthly measures of shell height have been made to determine growth rates for triploids and diploids. Preliminary results indicate significantly greater growth rates for the triploid oysters. Though it is premature at this time to evaluate their robustness with respect to disease pressure, our results suggest that triploidy may be beneficial to the culture of C. virginica.

Recent Trends in Oyster Recruitment of Selected
Sites in Long Island Sound

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The seasonal monitoring for the recruitment of Crassostrea virginica in Connecticut waters of Long Island Sound provides useful information to resource managers in determining production potential and site selection for oyster propagation programs.

Shell bag spat collectors are used by the State Aquaculture Division to measure the duration, intensity and distribution of oyster recruitment to the benthos.

Data collected from 1981 through 1990 shows considerable variability from season to season but, overall, reflects successful oyster recruitment for the ten-year period. The management of the resources on State seed beds and private oyster ground leases has effectively elevated the Connecticut shellfish industry to a prominent position in the national market.

Using Flow Cytometric Techniques to Monitor
Pure-Algal Cultures and Natural Particle
Assemblages - Going from the Sublime to the Ridiculous?

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The introduction of flow cytometric techniques to marine science has enabled researchers to study feeding habits of various marine organisms. These studies were previously hampered by the lack of techniques to distinguish quantitatively between different particles of the same size. Flow cytometric techniques were first applied to feeding studies in marine organisms in 1985 using bivalve molluscs. These studies utilized pure algal cultures fed to animals in combination and we were able to estimate not only the clearance rate of individual cell types, but also their proportional occurrence in the pseudofaeces and faeces. More recently we have translated our laboratory studies into field studies and have focused on natural seston as a food source for filter-feeding organisms. Particles are analyzed for their fluorescing intensities as well as particle size (measured as volume and/or forward angle light scatter) and we have been able to monitor feeding rates as functions of both particle size and organic content. Data are presented for various animals fed on pure algal cultures as well as natural assemblages of particulates.

design to allow the staff to grow and maintain both fresh and saltwater species of finfish, as well as various types of shellfish, microalgae and sea vegetables. Several independent systems, from totally closed to totally opened, were developed to accomplish these goals and to provide water for fish and sea-fry incubators, water tables, student aquaria, algal growth, and both shellfish and finfish hatcheries. In addition, an ozone and ultraviolet, sterilization - deuration module is planned. These systems are constantly being redefined and modified as the planning and construction process proceeds. We have obtained invaluable help and assistance from many people in the aquaculture community.

Aquaculture Systems for the Bridgeport Regional Vocational
Aquaculture Center

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The Bridgeport Regional Vocational Aquaculture Center was established by Public Act #90-22 in January, 1989. The Center is a Vocational Agriculture High School Program focusing on marine trades and the science of aquaculture, providing both educational and vocational opportunities to the students while allowing them to pursue possible college preparation. It is patterned after other Vocational Agriculture Centers and draws students from the school districts of Bridgeport, Milford, Stratford, Shelton, Trumbull, Monroe, and Fairfield. The curricula of the Center follow the State of Connecticut approved Natural Resources and Aquaculture Curriculum. In the spring of 1990, the Connecticut Legislature allocated 7.5 million dollars to design and construct an aquaculture facility to be located near Captain's Cove in the Black Rock Harbor section of Bridgeport. A substantial portion of the building will accommodate an aquaculture lab of nearly 9,000 square feet of work area, which will include a classroom, storage facilities, a pathology area, an algal growing room, pumphouse, and a greenhouse. Much effort was needed to overcome some water supply problems, and at the same time maintain flexibility in design to allow the staff to grow and maintain both fresh and saltwater species of finfish, as well as various types of shellfish, microalgae and sea vegetables. Several independent systems, from totally closed to totally opened, were developed to accomplish these goals and to provide water for fish and sac-fry incubators, water tables, student aquaria, algal growth, and both shellfish and finfish hatcheries. In addition, an ozone and ultraviolet, sterilization - depuration module is planned. These systems are constantly being redefined and modified as the planning and construction process proceeds. We have obtained invaluable help and assistance from many people in the aquaculture community.

A Preliminary Study of Toxin Uptake and Retention by Surf Clams on Georges Bank

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Surf clams, Spisula solidissima, have been collected from several locations on Georges Bank and have exhibited high levels of paralytic shellfish toxins. These levels are far in excess of the 80 ug saxitoxin equivalents/100 g tissue used by public health officials to ensure public safety. Samples of whole animals and of individual tissues have been collected for toxin analysis. Some toxic individuals have been maintained in the laboratory for depuration studies.

Preliminary data are presented for the uptake and release of toxins by whole animals and by individual tissues.

Major types of chromosomal abnormalities were anaphase bridges and lagged chromosomes, multipolar spindles, polyploidy, aneuploidy and chromosomeally altered embryos with different numbers of chromosomes in different embryo sectors. Micronuclei and clumped, pyknotic and deteriorating nuclei were observed in moribund larvae. Effects observed at the early developmental stages increased in number and severity as development proceeded. At the highest concentration in some procedures, mortality was 100% by the larval stage. Overall, naphthalene and arctic (NCA) were more toxic than benzene. High temperature increased toxicity of naphthalene and arctic, probably by increasing solubility and uptake. Benzene and naphthalene together in high temperature-high salinity water were more toxic than in low temperature-low salinity water.

Results suggest that in an oil spill, or with chronic cumulative seepage of oil, toxicity of some major components of oil would be influenced by temperature and salinity of the seawater. Toxicity would also be influenced by prevailing levels of other environmental contaminants such as PCBs. Cytogenetic and viability data can be used in ecological models which include estimates of pollution-related mortality and reproductive failure of early-life stages.

Influence of Environmental Factors on Genotoxicity of Petroleum
and PCB Compounds on American Oyster Eggs and Embryos

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Cytogenetic, cytotoxic and embryo-toxic effects of petrochemicals and PCBs (polychlorinated biphenyls) on early reproductive stages of the American oyster, Crassostrea virginica, were determined individually and in combination. In addition, larval development was investigated as a measure of viability. Dose-related responses were measured for concentrations of 0.1, 10 and 100 mg/L of benzene, naphthalene and aroclor 1254 at two temperatures and salinities.

Major types of chromosomal abnormalities were anaphase bridges and laggard chromosomes, multipolar spindles, polyploidy, aneuploidy and chromosomally mosaic embryos with different numbers of chromosomes in different embryo sectors. Micronuclei and clumped, pycnotic and deteriorating nuclei were observed in moribund larvae. Effects observed at the early developmental stages increased in number and severity as development proceeded. At the highest concentration in some exposures, mortality was 100% by the larval stage. Overall, naphthalene and aroclor (PCB) were more toxic than benzene. High temperature increased toxicity of naphthalene and aroclor, probably by increasing solubility and uptake. Benzene and naphthalene together in high temperature-high salinity water were more toxic than in low temperature - low salinity water.

Results suggest that in an oil spill, or with chronic cumulative seepage of oil, toxicity of some major components of oil would be influenced by temperature and salinity of the seawater. Toxicity would also be influenced by prevailing levels of other environmental contaminants such as PCBs. Cytogenetic and viability data can be used in ecological models which include estimates of pollution-related mortality and reproductive failure of early-life stages.

Morphological and Biochemical Changes
During Metamorphosis of Bivalve Larvae

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Under laboratory, hatchery, and field conditions, metamorphosis of bivalve molluscs is often coincident with high mortality that continues through a critical period of four to eight weeks post-settlement. Bivalve larvae undergo a rapid transition from a free-swimming, planktonic form, to a benthic juvenile. Young juveniles possess rudimentary gill lamellae that aid respiration, but are virtually incapable of capturing particulate food.

Time-lapse video recordings of the morphological changes associated with metamorphosis of Crassostrea virginica, Mercenaria mercenaria, and Ostrea edulis show that there is a 50% decrease in retention efficiency for particular food during the first few weeks following metamorphosis. Algal supplements during this transition do little to suppress the loss of protein, lipid and carbohydrate in metamorphosing bivalves. This suggests that metabolism is fueled by stored energy reserves and, possibly, uptake of dissolved organic material.

Superimposed on the transition from feeding by the velum to particle capture by the gills, a biochemical transition occurs from lipid-protein in larvae to that of carbohydrate-protein in juveniles. Between 30 and 60 days following metamorphosis in Mercenaria, lipid decreases and carbohydrate increases by more than 100% of pre-metamorphic values.

Together, these results suggest a period of extreme stress for metamorphosing juvenile shellfish. It is not surprising that this stage is particularly susceptible to less than optimal environmental conditions, such as poor food quality or quantity, extreme temperatures, presence of pathogens, poor water quality, etc.

Future research should concentrate on documenting the morphological and physiological reasons for these transitions and the source of concomitant mortality.

ENVIRONMENTAL AUDITING

Aquaculture and Environmental Stewardship: Milford Shellfish Biology Seminar—1991

WALTER J. BLOGOSLAWSKI

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For the past 11 years the annual Shellfish Biology Seminar at Milford CT has provided a unique forum for aquaculture scientists and industry officials to exchange information about estuaries facing increased pollution problems, especially Long Island Sound and the Great South Bay. Because these two areas are so rich in productivity and diversity, fish and shellfish farmers utilize their waters, shellfish beds, and shore land for hatcheries and grow-out facilities. These individuals seek better management of the coastal estuarine environment and its resources, providing a working example of environmental stewardship. In aquaculture, good science is required to understand the complex variables and interaction of estuarine currents, tides, temperature, and cycles of reproduction. Aquaculturists are beginning to understand the

need for specific nutrients and how the wastes of one species can be utilized for enhanced production of another species.

Over the years, this meeting has formed an amalgam of both the aquaculture industry and research scientists where both groups foster mutual environmental concern. Science is able to focus on the theoretical aspects of pollutant damage, while the aquaculture industry is able to define the problem and need for assistance to eliminate pollutants from their crops—shellfish and finfish. Overfishing is not an issue at these meetings, as the group accepts the damage already done to wild resources and seeks new technologies to grow food sources under controlled and stable market conditions.

Therefore, it could be said that the seminar serves as a meeting ground where the theoretical knowledge of scientific study finds practical application in the industry and is fueled by the needs of that industry. This ideal blend of the two groups produces better management of the resource and a safer environment—the goal of stewardship.

Effects of Food Availability and Food Quality on the Growth of Juvenile Shellfish in Suspended Culture

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Shellfish growth studies were initiated in 1988 using predator-proof cages suspended under floating docks in small boat marinas and a "clean water" control site. In the summer of 1990, the growth rates of three species of juvenile shellfish were monitored biweekly for 20 weeks: American oysters, *Crassostrea virginica*; hard clams, *Mercenaria*; and bay scallops, *Argopecten irradians*.

To evaluate the relative influence of various environmental variables on the growth and survival of juvenile shellfish, three sites in Point Judith Pond in Narragansett, Rhode Island were selected along a gradient of food conditions and current speeds. Weekly measurements of temperature, salinity, total seston, particulate organic matter (POM), chlorophyll-a, phytoplankton species composition, and fecal coliform were compared with semiweekly growth-rate determinations.

Instantaneous growth (as percent increase in volume per day) and survival were excellent at all sites. A strong seasonal

component of growth obscured most significant correlations between growth and chlorophyll, POM, or seston. High current speeds at the site with lowest food concentrations produced similar growth rates confirming the horizontal seston flux model proposed by Grizzle and Lutz.

Preliminary data describing the influence of suspended culture versus on bottom culture and separate work describing the bioaccumulation of heavy metals will be briefly discussed.

Growth of Hard Clams in Long Island Sound: Sorting Out the Determining Factors

Ronald Goldberg and Gary H. Wikfors

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Growth of juvenile hard clams, *Mercenaria mercenaria*, was studied at three sites in Long Island Sound (LIS) by periodically measuring caged juvenile clams in 1987 and 1988 and by back-calculating growth from shell age-ring analysis of collected adults. Sites were chosen at Stonington, Milford, and Greenwich, Connecticut to represent changing environmental conditions along the east-west axis of LIS. At the Milford site, growth

of caged juvenile clams up to about 23 mm was not significantly different ($P < 0.001$) at planting densities from 100/m² to 5000/m². Clams at each site, however, grew at different rates and time periods specific to site and year. Temperature, dissolved oxygen, seawater current flow, and seston biochemical composition were measured at the sites. Principal components analysis (PCA) of 1987 data showed that temperature and the combination of potential food and water movement accounted for sufficient variability to distinguish the Stongington site from Greenwich and Milford. Growth of caged animals at Greenwich was inhibited in mid-summer of 1987 during a bloom of the dinoflagellate *Prorocentrum*; consequently, data from this period were not used in statistical analyses. Regression of growth data against environmental factors generated by PCA indicates a strong correlation between growth and the combined effects of temperature and food availability. Analysis of population age and growth data from the shell record revealed long-lived clams of up to 50 years and evidence of past recruitment trends. Age at length and the rate of growth appear to be influenced by population density and are site-specific. This study provides insight into how environmental conditions in LIS affect the population dynamics of the hard clam.

Hard Clam Nutrition in the Lab and in the Field: What Does One Tell Us About the Other?

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Controlled experiments in which hard clams (*Mercenaria mercenaria*) are fed known ratios of cultured algae have allowed us to determine which algal strains best support the growth of individual animals. Similar experiments with other bivalve species—and using nonalgal food sources, ranging from microencapsulated diets to detrital particles—have yielded reliable growth data as well. Further, if physical (size, shape, cell-wall type) and biochemical (gross biochemical composition, fatty acids, sterols, amino acids, and others) characteristics of the food source are measured or systematically varied, then the contributions of these characteristics to the recipe for a suitable food can be determined. Meanwhile, sitting on the bottom of the sea, clams and their kin are subject to widely varying environmental conditions and a largely undefined menu that likely changes drastically in both quantity and quality over short and long time scales. Fluctuations in natural bivalve populations will, over time, integrate combined effects of all environmental variables upon the physiology of individual animals; nutrition is only one such variable.

Research efforts to understand the nutrition of bivalve mollusks have proceeded along both field and laboratory fronts. Laboratory studies have provided the most information on food quality; whereas, more recently, field studies have addressed food quantity. The challenge now facing bivalve nutrition research is to combine knowledge gained through both approaches into a coherent model of bivalve nutrition as a controlling factor in population biology. Multivariate statistics and mathematical modeling offer promising ways to do this, and will be the subject of this presentation.

Dissolved Oxygen Regimes and Shellfish Recruitment

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This paper discusses the causal dynamics of seasonal hypoxia (low dissolved oxygen) and anoxia (no dissolved oxygen) in shallow and deep habitats of Long Island Sound, and the potential for impact on shellfish recruitment through several mechanisms. Hypoxia and anoxia occur periodically during most summers, but their spatial and temporal dynamics differ between unstratified (shallow) and stratified (deep) waters. As a result, their occurrences in shallow habitats may or may not be linked to those in deep habitats in any particular year. Hypoxia and anoxia can impact shellfish directly through lethal or debilitating physiological effects, either independently or in combination with other factors (synergistically). Hypoxic and anoxic conditions may also impact shellfish indirectly through physiochemical mobilization of potentially harmful materials sequestered in sediments.

European Winkles, Japanese Oysters, and Zebra Mussels: Perspectives on the Introduction of Exotic Mollusks on the East Coast of North America

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Exotic marine and estuarine mollusks have been continuously introduced to the east coast of North America for over 200 years. Inadvertent (accidental) introductions include shipworms, marsh snails, and nudibranchs (sea slugs). Intentional introductions include European oysters, Japanese oysters (not yet represented by established populations), and the European periwinkle. The latter invasion led to spectacular alterations to rocky shores, marshes, and mudflats from Newfoundland to New Jersey. Currently, the most debated intentional introduction focuses on the Japanese oyster: interest in importing this oyster has arisen almost every decade since the 1920s, with later workers frequently overlooking both earlier attempts and debates. Private (including secret) and government plantings of Japanese oysters on the Atlantic coast have occurred for at least 50 years, since the 1940s. Concerns over the importation, potential success, and spread of the Japanese oyster on the Atlantic coast focus on ecological and disease questions, although formal studies are few. The most recent accidental invasion, the zebra mussel in the Great Lakes basin, holds two messages for the Atlantic coast biologist. The first is that it will eventually spread into fresh (and possibly oligohaline) water drainages throughout the Eastern seaboard. Second, and perhaps more important for marine and estuarine fisheries biologists, is that the vector that brought the zebra mussel to North America—ballast water—continues to release, on a daily basis, large numbers of exotic mollusks into American coastal waters. Long Island Sound, Chesapeake Bay, and other major estuaries are playing biological roulette with ballast water invasions.

The Asiatic Clam, *Corbicula fluminea*, Extends Its North American Distribution to the Connecticut River

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In May 1990, *Corbicula fluminea* (Asiatic clam) was found in service water piping at the Haddam Neck Plant (Connecticut Yankee-CY), a nuclear power station on the Connecticut River in Haddam, Connecticut. Sampling in October and November 1990 of the 1.7 km long CY discharge canal and a 16 km section of the Connecticut River from the mouth of the Salmon River to the Middletown Power Plant, Middletown, Connecticut, found Asiatic clams in densities of 15–117 clams/m², with shell lengths ranging from 1.9–12.2 mm. This report of *C. fluminea* in the Connecticut River documents the farthest northern distribution of this species along the east coast of North America. The successful establishment of this clam in the Connecticut River may cause problems to other raw freshwater users, and the invasive nature of this species should be of concern to freshwater users throughout New England.

Unexplained Oyster Mortalities in New England: 1989–1990

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In mid-August 1989, growers from Maine to Long Island experienced similar mortalities in first-year oyster seed. Oysters grew well initially, then died suddenly with little advance warning. Only small seed under 25 mm died, with those in surface trays being hardest hit. Some shell deformity was seen (lower shell outgrowing the upper shell) as a characteristic of any mantle edge irritation, including MSX. Pathology reports revealed that the oysters were in poor condition or emaciated, but no traces of MSX or any other pathogen could be found. While leading shellfish pathologists were mystified, the problem appeared to be contagious, culminating in the loss of over 30 million seed in 1990. Various hypotheses have been presented, such as a nutritionally undesirable bloom of phytoplankton, and genetic differences in seed. To date, none have explained all of the observed symptoms, which were noted by some growers even before the 1989 season. It is hoped that discussion at the Milford meetings will improve the understanding of this problem.

Unexplained Mortalities in Juvenile Hatchery-Raised Oysters

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During the summer of 1990, Frank M. Flower & Sons experienced a significant die-off of ¼–1" juvenile oysters (*C. virginica*) in a grow-out raft system located at Mill Neck Creek, New York. These mortalities took place in July and August and ultimately

resulted in the loss of 50% of the year's production. Examination of samples by Dr. Harold Haskin of Rutgers University and Dr. Frank Perkins of VIMS did not reveal any apparent disease organisms. Similar symptoms had been reported by oyster aquaculturists during the summer of 1989 to Karl Rask of the Massachusetts Cooperative Extension Office (University of Massachusetts). Dr. Steve Malinowski from The Clam Farm, Inc. of Fishers Island, New York, indicates that he has experienced a similar problem for the last three years. Preliminary work suggests that the problem may be linked to seed oysters from the northeast.

Responses of Oyster Hemocytes to MSX Parasites

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Hemocytes, the blood cells of oysters, are considered to be a primary line of defense against invading microorganisms because they are capable of recognizing and phagocytosing foreign material. Nevertheless, *Haplosporidium nelsoni*, the protozoan parasite causing MSX disease, is rarely found within hemocytes of oysters studied by tissue section. To further investigate the oyster hemocyte *H. nelsoni* system, we have used in vitro experiments that allow us to verify quantitatively the observations from tissue sections and to understand why oyster hemocytes apparently fail to recognize and attack *H. nelsoni* plasmodia.

Haplosporidium nelsoni plasmodia are collected from the hemolymph of heavily infected oysters and enriched. They are then treated in a desired manner and incubated with hemocytes from uninfected oysters in a 24-well tissue culture plate. The percentage of phagocytosed and nonphagocytosed parasites is determined by microscopic examination.

Our findings with the in vitro system parallel observations from tissue sections: live parasites are rarely (1–3%) phagocytosed. Failure of phagocytosis occurs regardless of whether hemocytes are taken from resistant or susceptible oysters. Killed parasites, however, are readily engulfed. The possibility that a substance toxic to *H. nelsoni* is present in the serum of resistant oysters was examined by incubating parasites in sera of resistant and susceptible animals and then testing them for viability using trypan blue dye exclusion. No differences in viability were found associated with resistance or susceptibility. Experiments in which the parasites were treated to inhibit metabolism and to alter surface molecules indicate that the process by which the parasites evade detection is an active one and that it probably involves surface structure.

Detection of Human *Vibrio* Pathogens in Shellfish

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Halophilic *Vibrio* species are ubiquitous in the marine environment and, as a consequence, shellfish burdened with the O1 and non-O1 serovars of *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus* may deliver these pathogens to the human consumer—most

especially when eaten raw, such as the oyster. Regulations are not yet in place which specify levels and *Vibrio* species that will be acceptable for retail markets. In order not to delay entry of shellfish into the marketplace, specific, sensitive, and rapid methodologies are needed to detect and enumerate these pathogens in shellfish.

In our investigations we examined two serological approaches to develop rapid detection methods. Initially, we exploited the finding that each *Vibrio* species expresses species-specific flagellar (H) antigens. Latex beads armed with monoclonal antibody (MAb) to H-antigens of *V. vulnificus*, *V. cholerae*, *V. parahaemolyticus*, and *V. anguillarum* agglutinated the target species, exclusively, within 1–3 min by the slide test. These reagents, though specific, do not meet the time constraints to obtain results within a working day.

A second approach examined consisted of a prototype dipstick, fitted with a detection pad designed to exhibit a blue color when exposed to a specimen burdened with the target *Vibrio*. Affinity-purified polyclonal anti-*Vibrio cholerae* 01 Inaba immunoglobulin was covalently bound to polyvinylidene difluoride membrane squares. The antibody-coated membrane pads were tested for the ability to capture and immobilize *V. cholerae* 01 cells from alkaline-peptone (AP) broth enrichment cultures. MAb reactive with the 01 serovar-specific antigen A were used to detect the immobilized *V. cholerae* cells by an enzyme-linked immunosorbent assay (ELISA). The membrane-ELISA, prototype dipstick detected Inaba and Ogawa 01 serovars at cell concentrations of approximately 10^6 colony-forming units (CFU) per milliliter of enrichment broth. When AP broth was seeded with as few as 10^2 *V. cholerae* 01 cells, the membrane-ELISA detected the cells following 6 h incubation, while 18 h AP broth burdened with heterologous *Vibrio* species at concentrations as high as 10^8 cells per milliliter routinely produced negative reactions. The dipstick membrane was tested in the three-tube three-dilution MPN. AP broth inoculated with oyster meat homogenate was spiked with various concentrations of *V. cholerae* 01 cells. Discernible color reactions appeared on the dipstick membranes inserted into 6 h AP enrichment broths inoculated with oyster meat seeded with as few as 100 cells per gram of meat. *Vibrio cholerae* 01 cells can be detected in seafood or environmental specimens within the time-frame of one 8 h work day.

Oyster Depuration and Relaying to Remove Indigenous and Fecal-Borne Bacteria

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The Great Bay/Piscataqua River Estuary in New Hampshire and Maine has an abundant oyster resource which is commercially harvested from restricted water on the Maine side and harvested from approved waters in New Hampshire by recreational shellfisherman. Spinney Creek Oyster Company (SCOC) operates a depuration facility and relay lagoons for the Maine oysters. Since *Vibrio vulnificus* was found in the estuary in 1989, we have studied the potential for depuration and/or relaying to promote elimination of this pathogen from shellfish. Oysters

were collected from SCOC harvest lots before and after depuration or relaying. Oysters containing *V. vulnificus* were also harvested from the Piscataqua River and relayed to Great Bay. Weekly water and oyster samples were collected from the harvest and relay sites, and analyzed for the presence of total and fecal coliforms, *Escherichia coli*, *V. vulnificus*, and total vibrios. The data presented represent the comparative responses of the vibrios and the fecal indicator bacteria to depuration and relaying at the SCOC facility and relaying oysters to Great Bay.

Depuration and relaying at SCOC always resulted in drastic reductions in levels of fecal coliforms and *E. coli* in oysters. Total coliforms were depurated to a lesser extent and total vibrios did not respond to depuration or relaying. Depuration had an inconsistent effect on *V. vulnificus* and *Vibrio parahaemolyticus*. However, consistent decreases in *V. vulnificus* levels occurred in oysters relayed to lagoons in late August through October. Levels of fecal indicator bacteria in oysters relayed to Great Bay decreased compared to oysters at the Piscataqua River harvest site, yet remained relatively high. *Vibrio vulnificus*, which had previously not been detected in Great Bay, was present in relayed oysters at lower levels than at the harvest site for the initial 4 weeks of relaying. Thereafter, *V. vulnificus* was not detected in relayed oysters, yet it was detected for 4 more weeks in oysters at the harvest site. These results demonstrate the differences in responses between indigenous, estuarine bacteria and fecal-borne contaminants in oysters.

A Planter for the Even Dispersal of Hard Clam Seed

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Currently, public and private mariculture facilities use state-of-the-art techniques for hatchery and nursery production of bivalve shellfish seed. Yet, for those that plant unprotected seed directly on the bottom for final growout, little or nothing is done to ensure that seed are evenly distributed. The literature confirms the existence of density-dependent mortality (i.e., the more dense shellfish are planted, the greater the chance they will be preyed upon). Present methods of seed planting create clumps of animals with empty spaces between; foraging predators locate these areas of high density and consume shellfish at a high rate. The present study modified an agricultural seed planter for use with hard clams, *Mercenaria mercenaria*. The planter chosen (Case IH 900 Cyclo Air[®]) uses low-pressure air to handle and deliver seed, lessening the chance of clam breakage.

The planter was mounted along with an engine to power the hydraulics onto a 28-ft boat. No attempt was made to plant the clams into the bottom, as Paulsen and Murray had previously determined that doing so does not increase survival over clams dropped onto the bottom. Clams were delivered under the boat's propeller wash to minimize swirling. Two size ranges of seed, (60,000 of 8–12 mm and 20,000 of 16–20 mm) were used; which were planted at densities of 10, 20, and 50 clams per square meter in Great South Bay, New York. Diver observations determined that actual clam density and distribution approximated target values, except for the largest clams at the highest density. Divers also looked at breakage (less than 1%) and burrowing response (good). No attempt was made to determine subsequent survivorship of those clams planted. A commercial plant was made at the F.M. Flower & Sons Company in Oyster Bay, New York, where 256,000 of 10–15 mm clam seed were planted.

It is hoped that this planter will be used and copied where warranted to increase survival of planted hard clam populations. Mariculture firms could use the planter to plant evenly dispersed high densities of clams on prepared beds, while public projects would more likely plant lower densities to enhance natural sets and avoid a harvesting "bonanza."

Characterization of an Area of High and of Low Hard Clam (*Mercenaria mercenaria*) Abundance in the Eastern Great South Bay, New York

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Analysis of the distribution of hard clam (*Mercenaria mercenaria*) abundance in the eastern portion of the Great South Bay, New York, as determined by annual population censuses from 1987–1989, was used to identify nine distinct and stable regions of high hard clam abundance interspersed within regions of low clam abundance. To define the regional and local environmental parameters associated with hard clam abundance, side-scan sonar, sediment profile photography, sediment surface video photography, and depth profiling were undertaken within areas of high and low abundance regions. When data from the high abundance areas are pooled and compared to the low abundance areas, high abundance areas have a coarser sediment grain size, shallower RPD depth, greater sediment compactness, greater sediment surface roughness, a thinner flocculent layer, and shells and shell fragments in the sediment. In addition, high abundance areas are generally superimposed on relic oyster reefs. The transition from low to high abundance generally coincides with increased topographic relief and spatial changes in sediment type parameters.

A detailed characterization was undertaken for one of the high and low clam abundance regions. The high abundance area had a muddy-sand sediment which was relatively hard. Sediment surface relief was provided by the sediment, shells, and worm tubes. Biogenic reworking was minor. The low abundance area had sandy-mud sediment which was soft, had a thick flocculent layer, and considerable bioturbation. Surface relief was minor and shells and worm tubes were absent. Topographically, the high abundance area was slightly elevated relative to the adjacent area, while the low abundance area was slightly depressed.

Population data suggest that differential settlement and/or survival to age 1 can account for the difference in clam abundance between high and low abundance regions. This information, together with the environmental characterization, is being used to evaluate the spectrum of factors that may be contributing to the differential abundance of hard clams.

Docks, Boats, and Shellfish: Are They Compatible?

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Communities in Massachusetts have been increasingly concerned about the proliferation of private docks and piers on

inland waterways within the public tidelands and their effect on shellfish resources. Docks are generally licensed on an individual case-by-case basis, where adverse effects to shellfish from one dock may be minimal, but cumulative effects of numerous docks in shallow and narrow embayments may be substantial. Potential problems discussed include the structures themselves and the use of the docks by recreational boats producing habitat loss due to pilings and pipes and unsuitable sediment conditions around the pilings. Other problems included shading of eelgrass by docks, destruction of eelgrass by boats, physical obstruction of traditional fishing areas, decreased setting and sediment alteration from prop wash, changes in circulation patterns, unknown toxicity of chemicals from pressure-treated wood in estuarine environments, and unknown effects of toxic chemicals in bottom paint. Proof of these potential problems on site is nearly impossible to determine prior to licensing. Scientific literature as supporting documentation in court challenges is very limited. Communities have responded by prohibiting the transplant of shellfish, often cited as a mitigating measure, to an area outside the area affected by the dock, and have adopted criteria to determine the appropriateness of docks in certain shellfish habitats through the regulatory process. Arguments have been made that assessment should include historic data as well as present-day productivity in determining shellfish habitat.

Recent Developments in the Improving Shellfisheries of Canada's Maritime Provinces

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Following a large decline from 1954–1972, production of oysters (*C. virginica*) on Prince Edward Island has since turned upward. Production in 1989, about 60,000 bushels worth \$3.8 million Canadian dollars (ex-island), was four times the total in 1972 and was two thirds of the total in the best year, 1890. The province produced the increase by transplanting unfished oysters to good market beds and by spreading fossil shells it dredged from deposits on depleted oyster beds to collect spat. In 1989 and 1990, the Province used a new procedure (i.e., cultivating dormant shell beds to remove a deposit of silt). It was successful as the beds (60 acres) received good sets of spat. Such cultivating is perhaps one of the least expensive methods available for producing oysters. New Brunswick has been using cone-shaped spat collectors, about 2/3 m in diameter and termed Chinese hats, to cultivate oysters successfully. In contrast to Prince Edward Island, which has a mixed public and private oyster fishery, New Brunswick's is mainly private. Leaseholders remove the seed collected on the hats, spread them on their leases for growth, and then market them after they reach a length of three inches.

Since the early 1980s, the Maritime Provinces, led by Prince Edward Island and Nova Scotia, have been culturing mussels (*Mytilus edulis*). Mussel production has been rising rapidly. In 1989 production from Prince Edward Island was about 90,000 bushels, worth \$4.3 million Canadian dollars.

The Maritime Provinces have achieved their successes in producing shellfish by: (1) using wild seed (it is abundant and inexpensive to collect; little seed has been raised in hatcheries); (2) providing their industries with substantial government financial and technical support; (3) inviting shellfishermen to

contribute to development plans; (4) sending biologists and lead fishermen to the U.S. and Europe to observe the best culture methods; and (5) having government personnel aggressively market their shellfish in Canada and the U.S. Thus, sales of oysters and mussels have been good up to now.

Long Island Bay Scallop Reseeding Efforts: Life After the Brown Tide

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Populations of the bay scallop, *Argopecten irradians*, in Long Island, New York, were decimated after extensive blooms of *Aureococcus anophagefferens* ("brown tide") occurred between 1985–1987. Commercial landings fell from 279,000 lb (worth \$1.26 million) in 1984 to just 250 lb (worth \$2,375) in 1988. Large-scale reseedling of hatchery-reared scallops in the Peconic Bays was initiated by the Long Island Green Seal Committee in 1986, and continued through 1989. Transplants during the first two years were thwarted by the persistence of the brown tide. Extensive monitoring of the Fall 1988 transplants revealed that survival of the 30 mm seed until the next spawning season ranged from 0–12% at six different sites. Nevertheless, the surviving broodstock is thought to have contributed significantly to the widespread scallop set which was observed in 1989. An even heavier scallop set occurred in 1990.

Survival of Juvenile Scallops in Eelgrass Beds: The Role of Spatial and Size Refuge from Predators

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Postsettlement bay scallops, *Argopecten irradians*, appear to gradually shift from a spatial to a size refuge from predation as they move from the eelgrass canopy to the bottom during their early life history. Prior studies conducted in our laboratory have shown that the scallops' ability to attach to elevated substrates is strongly size-dependent between 5 and 20 mm in shell height. In order to determine the rate and size at which scallops relocate to the bottom under natural conditions, temporal patterns in attachment position on eelgrass blades were determined for natural postset (mean initial size > 5 mm) in two eastern Long Island bays throughout the summer and fall of 1990. Once scallops attained about 12 mm, the proportion of individuals attached above-bottom decreased markedly (from 100% to < 5%) over a 5-week period, such that nearly 100% of the population was found on the bottom by early October, when scallops attained about 30 mm in shell height.

Size-specific losses of scallops due to emigration and/or predation were determined by (a) tethering, and (b) free release of hatchery-reared scallops of two size classes (12 and 20 mm) within contrasting eelgrass habitats in Hallock Bay, Long Island. Major predators were identified by diver surveys and video recording. Mud crabs, *Dyspanopeus sayi*, were the numerically dominant predator in this system. The relevance of these stud-

ies in assessing seasonal predatory risk and the success of scallop reseedling efforts is discussed.

The Northeastern Regional Aquaculture Center: An Overview

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The Northeastern Regional Aquaculture Center (NRAC), headquartered at Southeastern Massachusetts University, is one of five Regional Aquaculture Centers established by the U.S. Congress. Funded by the U.S. Department of Agriculture at an annual level of approximately \$700,000, and representing 12 states and the District of Columbia, NRAC develops and sponsors cooperative regional research and extension projects in support of the aquaculture industry in the northeastern United States.

A Board of Directors representing the region's aquaculture industries, academic institutions, and government agencies provides overall direction and management of NRAC. Research and extension priorities are established by a Technical-Industry Advisory Council (TIAC), consisting of a Technical Committee and an Industry Committee. The Technical Committee includes key aquaculture researchers and extension agents in the region, while the Industry Committee represents principal commercial aquaculture interests in the northeast. The TIAC is also responsible for annual reviews of progress of NRAC-supported projects.

NRAC is presently supporting nine regional projects in finfish nutrition, oyster genetics and reproductive biology, government regulations affecting aquaculture, finfish economics, and development of a regional aquaculture extension program. NRAC is currently developing five additional projects in the areas of water quality, aquaculture systems technology, and fish health. Total NRAC funding commitment to projects in progress or under development exceeds \$2.6 million. NRAC also publishes "Northeastern Aquaculture," a quarterly newsletter highlighting NRAC projects and other topics of interest to the northeastern aquaculture community.

Triploidy and Growth Rates in *Crassostrea virginica*

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The growth rates of juvenile triploid and diploid *C. virginica* from the same parent stock were compared during their first season of growth at three locations. At all three sites, the growth rate of the triploid groups was found to be significantly greater than that for the diploids with respect to shell height, whole weight, and total volume. At one of the sites where MSX (*Haplosporidium nelsoni*) is endemic—Cotuit Bay, Massachusetts—the survival rate was higher among the triploids than among the diploids, even though MSX prevalence was lower in the latter group. These results are felt to be encouraging, although preliminary.

Growth and Survival of Selectively Bred Long Island and Delaware Bay Oysters

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Local populations of species with large geographic ranges have often been shown to be physiologically differentiated. One example is the oyster, *Crassostrea virginica*, which shows genetically based latitudinal differences in reproductive cycles. Physiological differentiation of populations is often correlated with geographic temperature clines. Organisms living in higher-latitude temperature regimes compensate by increasing their metabolic rate at a given low temperature relative to those organisms living at lower latitudes.

Strains of *C. virginica* from areas of contrasting thermal regimes (Long Island Sound and Delaware Bay) have been maintained in a single temperature regime by the Haskin Shellfish Laboratory for multiple generations. The situation is ideal for the separation of genetically based physiological variation from environmentally influenced differences (acclimatization). If genetically based physiological compensation has occurred, the population from farther north will grow faster in the common environment, even after several generations.

We have analyzed growth and mortality data, collected between 1977 and 1989, on oyster strains of different geographic origin. After up to seven generations of selective breeding for resistance to MSX disease, strains of Long Island origin grow faster and are significantly larger at a given age than oyster strains of Delaware Bay origin. The differences in growth are not strongly correlated with differences in mortality among these strains. These observations are consistent with genetic adaptation to maximize the energy available for growth and reproduction in a given thermal regime and may reflect the optimum balance of energy acquisition versus energy expenditure in a given population.

Performance of Oysters from Inbred Long Island Sound and Delaware Bay Lines and Their Hybrids

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Physiological differences in eastern oyster, *Crassostrea virginica*, from different geographical regions of the United States have long been noted. Recently, we have documented genetically based differences in reproductive cycles of oysters from Long Island Sound and Delaware Bay. Differences in growth rates of oysters from the two locations also appear to be genetically based. Even after several generations of residence in Delaware Bay, Long Island strains consistently grow faster and to a larger size than Delaware Bay strains (see abstract on this page by D.E. Dittman and S.E. Ford).

Although they grow faster, oysters in the Long Island line currently maintained at the Rutgers laboratory are not as resistant to MSX disease as are Delaware Bay lines. In an effort to combine the traits of fast growth and good survival, we produced a cross between Delaware Bay and Long Island oysters in 1988. Representatives of the cross and the two "pure" lines were deployed on Cape Cod (two locations), in Delaware Bay, and in Chesapeake Bay beginning in May 1989 and have been moni-

tored since then for growth, survival, meat quality (condition index), and disease levels.

In the first year of testing (1989), during which MSX pressure was low in all locations, survival of the hybrid was slightly, but consistently, better at all locations compared to the pure lines. In 1990, under challenge by both MSX and Dermo diseases, survival of the hybrid was considerably better than that of the pure lines. Growth rates were site-specific, but growth of the pure Delaware Bay line was consistently the least, whereas that of the hybrid was generally equal to, or better than, that of the Long Island pure line. Preliminary data analysis, then, indicates that in this particular test, the Delaware Bay-Long Island hybrid performed better than either pure line.

Culture of Triploid Oysters in Virginia

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Polyploidy (mostly triploidy) has been important in the development of agricultural and aquacultural stocks. For oyster culture, triploidy offers several potential advantages, including: (1) enhanced growth rates, (2) expanded marketing period, and (3) some disease resistance. These issues have generally not been explored for *Crassostrea virginica*, in large measure because the production of triploids of this species has proven difficult. In 1990 we produced triploid *C. virginica* using cytochalasin B and modification of techniques developed for *C. gigas*. Survival after 2 days was 12%; percent triploidy, determined by cytofluorimetry, was 90%. Grow-out of triploids and equal-aged diploids in off-bottom culture was initiated in May 1990 in Chesapeake Bay and is ongoing. Monthly measures of shell height have been made to determine growth rates for triploids and diploids. Preliminary results indicate significantly greater growth rates for the triploid oysters. Though it is premature at this time to evaluate their robustness with respect to disease pressure, our results suggest that triploidy may be beneficial to the culture of *C. virginica*.

Recent Trends in Oyster Recruitment of Selected Sites in Long Island Sound

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The seasonal monitoring for the recruitment of *Crassostrea virginica* in Connecticut waters of Long Island Sound provides useful information to resource managers in determining production potential and site selection for oyster propagation programs.

Shell bag spat collectors are used by the State Aquaculture Division to measure the duration, intensity, and distribution of oyster recruitment to the benthos.

Data collected from 1981 through 1990 shows considerable variability from season to season but, overall, reflects successful

oyster recruitment for the 10-year period. The management of the resources on state seed beds and private oyster ground leases has effectively elevated the Connecticut shellfish industry to a prominent position in the national market.

Using Flow Cytometric Techniques to Monitor Pure-Algal Cultures and Natural Particle Assemblages: Going from the Sublime to the Ridiculous?

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The introduction of flow cytometric techniques to marine science has enabled researchers to study feeding habits of various marine organisms. These studies were previously hampered by the lack of techniques to distinguish quantitatively between different particles of the same size. Flow cytometric techniques were first applied to feeding studies in marine organisms in 1985 using bivalve molluscs. These studies utilized pure algal cultures fed to animals in combination, and we were able to estimate not only the clearance rate of individual cell types, but also their proportional occurrence in the pseudofaeces and faeces. More recently, we have translated our laboratory studies into field studies and have focused on natural seston as a food source for filter-feeding organisms. Particles are analyzed for their fluorescing intensities as well as particle size (measured as volume and/or forward angle light scatter), and we have been able to monitor feeding rates as functions of both particle size and organic content. Data are presented for various animals fed on pure algal cultures as well as natural assemblages of particulates.

Aquaculture Systems for the Bridgeport Regional Vocational Aquaculture Center

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The Bridgeport Regional Vocational Aquaculture Center was established by Public Act 90-22 in January 1989. The Center is a Vocational Agriculture High School Program focusing on marine trades and the science of aquaculture, providing both educational and vocational opportunities to the students while allowing them to pursue possible college preparation. It is patterned after other Vocational Agriculture Centers and draws students from the school districts of Bridgeport, Milford, Stratford, Shelton, Trumbull, Monroe, and Fairfield. The curricula of the Center follow the State of Connecticut approved *Natural Resources and Aquaculture Curriculum*. In the spring of 1990, the Connecticut Legislature allocated \$7.5 million to design and construct an aquaculture facility to be located near Captain's Cove in the Black Rock Harbor section of Bridgeport. A substantial portion of the building will accommodate an aquaculture lab of nearly 9000 ft² of work area, which will include a classroom, storage facilities, a pathology area, an algal growing room, pumproom, and a greenhouse. Much effort was needed to overcome some water supply problems, and at the same time maintain flexibility in design to allow the staff to grow and

maintain both fresh and saltwater species of finfish, as well as various types of shellfish, microalgae, and sea vegetables. Several independent systems, from totally closed to totally opened, were developed to accomplish these goals and to provide water for fish and sac-fry incubators, water tables, student aquaria, algal growth, and both shellfish and finfish hatcheries. In addition, an ozone and ultraviolet, sterilization-depuration module is planned. These systems are constantly being redefined and modified as the planning and construction process proceeds. We have obtained invaluable help and assistance from many people in the aquaculture community.

A Preliminary Study of Toxin Uptake and Retention by Surf Clams on Georges Bank

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Surf clams, *Spisula solidissima*, have been collected from several locations on Georges Bank and have exhibited high levels of paralytic shellfish toxins. These levels are far in excess of the 80 µg saxitoxin equivalents/100 g tissue used by public health officials to ensure public safety. Samples of whole animals and of individual tissues have been collected for toxin analysis. Some toxic individuals have been maintained in the laboratory for depuration studies.

Preliminary data are presented for the uptake and release of toxins by whole animals and by individual tissues.

Influence of Environmental Factors on Genotoxicity of Petroleum and PCB Compounds on American Oyster Eggs and Embryos

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Cytogenetic, cytotoxic, and embryotoxic effects of petrochemicals and PCBs (polychlorinated biphenyls) on early reproductive stages of the American oyster, *Crassostrea virginica*, were determined individually and in combination. In addition, larval development was investigated as a measure of viability. Dose-related responses were measured for concentrations of 0.1, 10, and 100 mg/L of benzene, naphthalene, and aroclor 1254 at two temperatures and salinities.

Major types of chromosomal abnormalities were anaphase bridges and laggard chromosomes, multipolar spindles, polyploidy, aneuploidy, and chromosomally mosaic embryos with different numbers of chromosomes in different embryo sectors. Micronuclei and clumped, pycnotic, and deteriorating nuclei were observed in moribund larvae. Effects observed at the early developmental stages increased in number and severity as development proceeded. At the highest concentration in some exposures, mortality was 100% by the larval stage. Overall, naphthalene and aroclor (PCB) were more toxic than benzene. High temperatures increased toxicity of naphthalene and aroclor,

probably by increasing solubility and uptake. Benzene and naphthalene together in high temperature-high salinity water were more toxic than in low temperature-low salinity water.

Results suggest that in an oil spill, or with chronic cumulative seepage of oil, toxicity of some major components of oil would be influenced by temperature and salinity of the seawater. Toxicity would also be influenced by prevailing levels of other environmental contaminants such as PCBs. Cytogenetic and viability data can be used in ecological models which include estimates of pollution-related mortality of early-life stages.

Morphological and Biochemical Changes During Metamorphosis of Bivalve Larvae

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Under laboratory, hatchery, and field conditions, metamorphosis of bivalve molluscs is often coincident with high mortality that continues through a critical period of 4–8 weeks postsettlement. Bivalve larvae undergo a rapid transition from a free-swimming, planktonic form, to a benthic juvenile. Young juve-

niles possess rudimentary gill lamellae that aid respiration, but are virtually incapable of capturing particulate food.

Time-lapse video recordings of the morphological changes associated with metamorphosis of *Crassostrea virginica*, *Mercenaria mercenaria*, and *Ostrea edulis* show that there is a 50% decrease in retention efficiency for particular food during the first few weeks following metamorphosis. Algal supplements during this transition do little to suppress the loss of protein, lipid, and carbohydrate in metamorphosing bivalves. This suggests that metabolism is fueled by stored energy reserves and, possibly, uptake of dissolved organic material.

Superimposed on the transition from feeding by the velum to particle capture by the gills, a biochemical transition occurs from lipid-protein in larvae to that of carbohydrate-protein in juveniles. Between 30 and 60 days following metamorphosis in *Mercenaria*, lipid decreases and carbohydrate increases by more than 100% of premetamorphic values.

Together, these results suggest a period of extreme stress for metamorphosing juvenile shellfish. It is not surprising that this stage is particularly susceptible to less-than-optimal environmental conditions, such as poor food quality or quantity, extreme temperatures, presence of pathogens, poor water quality, etc.

Future research should concentrate on documenting the morphological and physiological reasons for these transitions and the source of concomitant mortality.