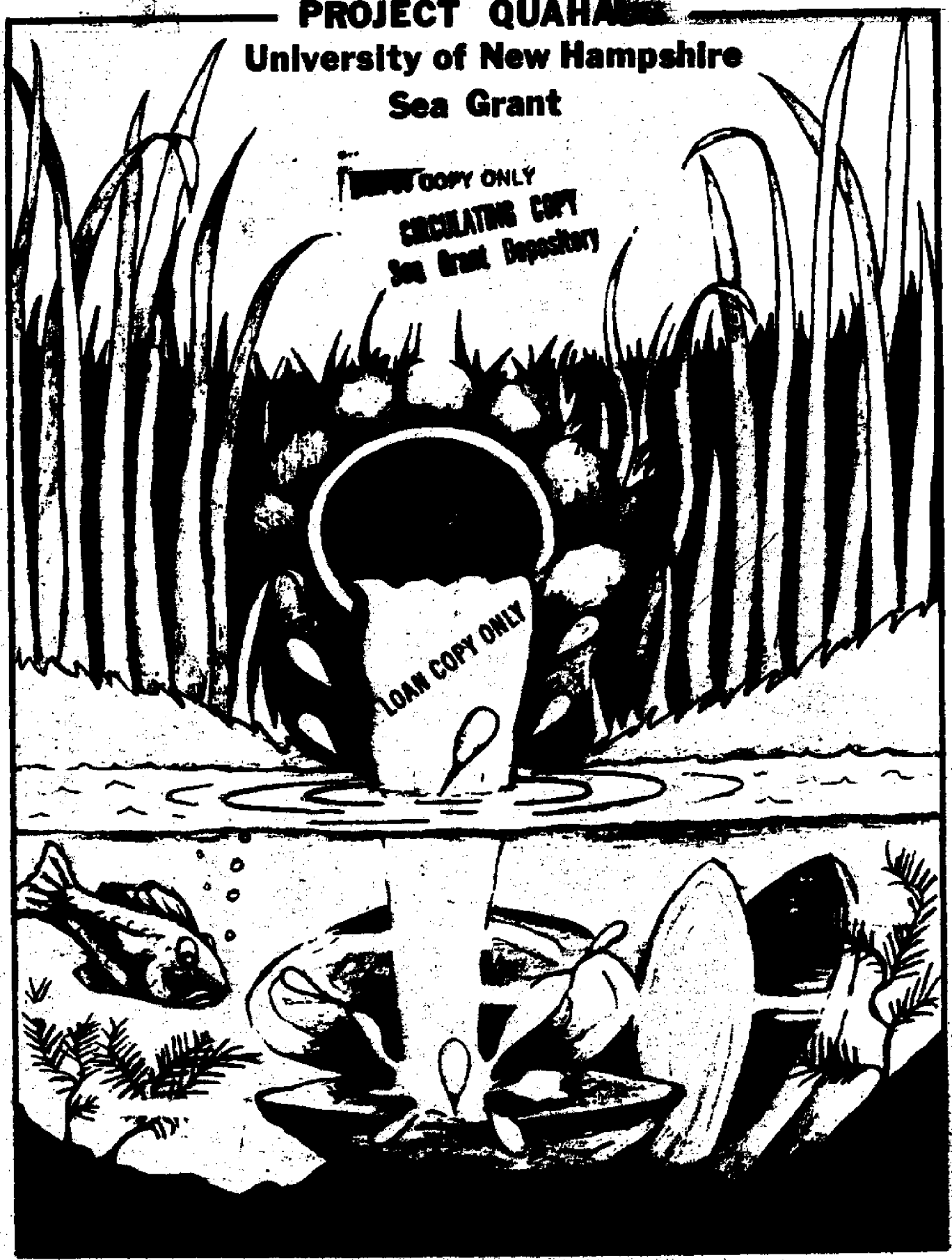
PROJECT QUAHAUG

University of New Hampshire Sea Grant



EVALUATION OF DEPURATION TECHNIQUES FOR CONTAMINATED SHELLFISH

PROJECT QUAHAUG

by
Susan Panas
Jeffrey Fisk
Elise Dessert
Steven Masse (group leader '85)
Thomas Plante (group leader '86)
Todd Walles (group leader '86)

Faculty Advisors
Dr. Nancy E. Kinner
Dr. Paul J. Ossenbruggen

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ABSTRACT

The hard shell clam (Mercenaria mercenaria) industry in the United States, based mainly in the east coast estuaries including Long Island's Great South Bay and Rhode Island's Naragansett Bay, has been greatly impacted by the increase in sewage discharge to these waters. The health risks associated with shellfish grown in sewage contaminated water are too great to market the quahaugs directly because they concentrate microorganisms which cause hepatitis, gastroenteritis and Vibrio parahemolyticus food poisoning. As a result, large portions of estuaries once available to the fishery are now closed. Shellfish are either not harvestable at all or are harvestable only after they have been relayed to less contaminated waters for lengthy in situ depuration. Artificial depuration in engineered facilities using ultraviolet disinfection has become an increasingly popular alternative and is currently being proposed or required for some areas. The goal of this project was to determine the best depuration technique in terms of reducing public health risks. The project had three major objectives:

[1] to determine the maximum contaminant level in quahaugs (M. mercenaria) grown in sewage impacted waters using fecal coliform bacteria as an indicator organism.

[2] to evaluate the effectiveness of artificial depuration compared to relaying in reducing the risk associated with ingestion of quahaugs grown in sewage contaminated water.

[3] to construct a risk assessment model based on data which determines the likelihood of the public ingesting

contaminated shellfish.

The project included the construction of quahaug contamination and depuration facilities, laboratory analysis and development of a risk assessment model. The work was conducted in cooperation with the Spinney Creek Oyster Company, Eliot, Maine.

The results indicate that the rate of contamination of the quahaugs was highly variable primarily as a function of the fecal coliform levels in the reactor and the water temperature. The maximum fecal coliform level ranged from 230 to 10,500 CFU per contaminated quahaug. A first order kinetic equation, $C = N \exp[-kt]$, was used to estimate the contamination level after depuration, where N equaled the initial contaminant level, k equaled the rate constant of depuration or relaying, and t equaled the depuration time. This equation was used to develop a risk assessment model to forecast the probability of using depuration to reduce fecal coliform contamination to 11 CFU per contaminated quahaug, the safe limit for public consumption. The rate constants, k, of depuration and relaying ranged from 0.055 to 0.066/hour and 0.074 to 0.075/hour, respectively. Both alternatives were proven to be feasible based upon their ability to lower fecal coliform levels in grossly contaminated quahaugs below 11 CFU/ quahuag in a period of 72 hours. Artificial depuration may be more advantageous because it can reduce wholesale costs and allow states lacking relaying areas a feasible alternative to their sewage impacted shellfish programs.

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INTRODUCTION

THE ECONOMIC IMPORTANCE OF THE SHELLFISH INDUSTRY

The shellfish industry is of economic importance to the Atlantic states, particularly the Northeast. In 1983 the United States shellfish landings were worth $299,000,000 (Table 1). The data in Table 1 also show the value of the shellfish industry in the Northeast and other Atlantic states.

Table 1: Shellfish Landings in U.S. in 1983

Shellfish	Total landings (million pounds of meat)	Value (million dollars)	State	Percentage of total landings
Surf Clam	55.9	24.9	NJ	43.6
			VA	32.7
			NY	4.3
Ocean quahaug	35.2	10.8	NJ	60.5
			MD	30.1
			RI	9.6
Hard Shell Clam	14.2	42.4	RI	40.8
			NJ,NY	33.1
			MD,VA,DE	8.4
			SC	12.6
Soft Shell Clam	8.5	17.6	ME	48.0
			MA	24.0
			MD	22.0
Oyster	50.2	67.3	LA,FL	55.0
			MD,VA,DE	22.0
			NJ,NY,MA	11.0
Scallop	32.4	136.0	MA	80.0

*Compiled from U.S. Department of Commerce (1984), Fisheries in the U.S. (1983).

It is an industry, however, that is threatened by pollution which has forced the closure of a significant number of shellfish beds in the United States representing millions of dollars in potential revenues lost. In the states of Maine, New York and Rhode Island alone, a reduction of at least 16% in the total harvestable areas represents a loss approaching $12,000,000 (Table 2).

Table 2: Shellfish Industry in the Northeast

State	Maine	Rhode Island	New York
Annual shellfish harvest (million dollars), 1984	12.0 (largely soft shell clam)	30.0 (largely hard shell clam)	25.0 (largely hard she clam)
Percent shellfish beds closed, 1984	20%	16%	19%
Potential harvest from closed areas (million dollars)	2.4	4.8	4.8

*Compiled from conversations with Maine DMR, Rhode Island DEM, and New York DEC personnel.

WATER QUALITY

Estuaries are habitats for many economically important shellfish such as *Mercenaria mercenaria* (quahaug or hard shell clam), *Mya arenaria* (soft shell clam), and *Crassostrea virginica* (American oyster).

The areas adjacent to estuaries have historically been focal points for the urban development which has placed demands on them. Many estuaries receive industrial wastes, municipal sewage, and agricultural and urban runoff.

Although a few shellfish area closures have resulted from industrial pollution, such as New Bedford Harbor in Massachusetts for PCB contamination, the major reason for the closures has been municipal sewage contamination (Hunt, 1980).

Municipal sewage contains a number of viral and bacterial species that become suspended in the water column when entering the estuary from sewage outfalls. Many of the organisms are pathogenic (disease causing). These suspended species may be consumed and accumulated by shellfish. The shellfish may then potentially transmit disease to man. The possibility of disease transmission is particularly high if the shellfish are consumed raw (Last, 1980).

Sewage treatment plants have been successful in reducing the amount of bacteria released into estuaries, but there is evidence that suggests that current practices in wastewater treatment, particularly disinfection, are inadequate. Keswick *et al.* (1980) found that the Norwalk virus, one of the two principal etiological agents for

shellfish-borne gastroenteritis, is extremely resistant to chlorination. Similarly, destruction of human rotavirus has been shown to require prolonged exposure to disinfectants (Rodgers et al., 1985).

ACCUMULATION OF BACTERIA AND VIRUSES IN SHELLFISH

M. mercenaria, M. arenaria, and C. virginica are filter feeders, that is, they siphon large amounts of sea water to extract nutrients for ingestion. The favored diet of these organisms is phytoplankton, but they may also ingest enteric bacteria and enteroviruses attached to suspended particles or phytoplankton. Unwanted debris is ejected from the organism as pseudofeces. The enteric bacteria and enteroviruses accumulate in the shellfish mainly in the viscera (digestive tract) and to a lesser degree in the siphon (Cabelli and Heffernan, 1970). The degree and rate of accumulation is a function of the organisms metabolic rate. The metabolic rate is a function of the temperature of the water, the season and the amount of phytoplankton present (Cabelli and Heffernan, 1970, 1971; Sea Grant Maine/NH, 1983).

EFFECT OF ACCUMULATION ON HUMANS

Accumulated pathogenic organisms in shellfish may transmit a number of diseases to humans when the shellfish are consumed. Prior to 1954 typhoid fever was the prevalent shellfish-borne malady associated with fecal contamination. Not one documented case of typhoid fever has occurred since 1954 because of the improvement in sanitation and monitoring practices and the reduction of the causative bacterium Salmonella typhi from the general population (Richards,

1985; Last, 1980).

Present day maladies are associated with enteroviruses. Outbreaks of acute gastroenteritis, hepatitis A, and Vibrio parahemolyticus food poisoning are generating concerns and debate over the effectiveness of present day standards (Richards, 1985; Hunt, 1980; Portnoy et al., 1975).

ESTABLISHMENT OF WATER QUALITY STANDARDS

The specific disease producing organisms present in sewage contaminated water and ingested by shellfish are not easily identified. The techniques for comprehensive bacteriological examination are complex and time consuming (Davis and Cornwell, 1985). Instead of trying to detect the presence of the pathogenic organisms directly, bacteriological tests of water and shellfish establish the degree of fecal contamination by demonstrating the presence of indicator organisms. An ideal indicator (1) occurs whenever the pathogens are present; (2) occurs only when there is a real danger of pathogens being present; (3) occurs in greater numbers than the pathogens to provide a safety margin; (4) survives in the environment as long as potential pathogens and (5) is easily detected with a high degree of reliability (Atlas, 1984).

Bacterial standards are based on the presence of indicator organisms, namely, the coliform group of bacteria. The level of contamination is expressed as total coliforms per 100 ml of water. The total coliform group consists of Gram-negative, lactose fermenting enteric rods which normally inhabit the intestinal tract of humans, other warm-blooded animals, and soils. Fecal coliforms are a subgroup

of the total coliforms. The fecal coliforms only grow in the guts of warm-blooded animals and are therefore better indicators of sewage contamination than the total coliform group.

MacConkey agar is a differential growth media for the selection and recovery of fecal coliforms. The colonies that grow on this media are those of Escherichia coli, the most frequently used indicator organism for assessing estuarine water quality. Positive tests for E. coli do not prove the presence of viable enteropathogenic organisms in sewage, but establish the possibility of their presence and the potential for shellfish-borne illness. This test has a built in safety factor for detecting potentially dangerous fecal contamination because E. coli are more numerous and easier to grow than the enteropathogens (Atlas, 1984).

The National Shellfish Sanitation Program (NSSP) was established in 1925, in response to a 1924-1925 oyster-borne typhoid epidemic (Richards, 1985). The conference initiated the National Shellfish Certification Program, through which water criteria for shellfish harvest areas were imposed for the first time. The criteria included the bacterial examination of 1 ml dilutions of estuarine water for the coliform group of bacteria. Refinements in this procedure led to development of the Most Probable Number (MPN) analysis for the total and fecal coliforms (Last, 1980; Richards, 1985).

Today shellfish growing and harvesting areas are classified based upon a two part survey, the sanitary survey and the bacteriological survey.

The sanitary survey is basically a point source detection. It provides an overall view of the sources, types, and volumes of pollutants entering the estuary.

The bacteriological survey is based upon MPN analysis for total coliforms of the shellfish and the growing area. Shellfish growing area standards (Last, 1980) are as follows:

1.) APPROVED-results of sanitary survey satisfactory, the median total coliform MPN of water is < 70/100 ml and the median fecal coliform level is < 17 CFU/100 ml (CFU = colony forming units).

2.) CONDITIONALLY APPROVED-median total coliform MPN of water is <70/100 ml and the median fecal coliform level is <17 CFU/100 ml, but reliability, effectiveness, or degree of sewage treatment must be improved.

3.) RESTRICTED-median total coliform MPN is >70/100 ml, but <700/100 ml and the median fecal coliform level is between 17 and 88 CFU/100 ml. Unsafe for direct marketing but may be used for relaying or depuration under supervision

4.) PROHIBITED-median total coliform MPN of water is >700/100 ml and the median fecal is >88 CFU/100 ml, evidence of raw sewage pollution, or area is too close to sewage outfalls and regardless of coliform MPN is unsafe for direct marketing.

These standards have been effective in significantly reducing the incident of shellfish-borne diseases attributable to pathogenic bacteria.

DEPURATION AND RELAYING

The Food and Drug Administration (FDA) allows shellfish to be harvested from marginally polluted waters provided that the shellfish are depurated in an engineered system or relayed from a restricted area to an approved open area, where they are allowed to depurate naturally. The concept of depuration is to provide clean pathogen-free water to the shellfish so that they may purge themselves of the contaminants during the process of filter feeding.

The concept of depuration is not new. It was recognized from research prior to 1896 that cleansing of sewage polluted oysters could be accomplished by placing them in "disgorging tanks" containing clean water, for a short time before they were shipped to consumers (Richards, 1985). More sophisticated tanks were developed by Fabre-Domegue in France in 1912. These used filtered artificial seawater (Fabre-Domegue, 1912). Many later engineered systems used chlorination as a means of water disinfection, but it was found that chlorine was an irritant that induced shellfish into a state of reduced metabolic activity (Last, 1980).

Today's depuration techniques involve the filtration of recirculated seawater with ultraviolet light disinfection of the water. The depuration of shellfish in these systems is performed at temperatures and salinities comparable to the area where the shellfish were harvested. Currently, the states of Maine, Massachusetts, New Jersey, South Carolina, Louisiana, Florida and Maryland have depuration facilities. New York had depuration facilities that were closed under pressure from the shellfish lobby.

Relaying may be regarded as in situ depuration. The process of relaying involves harvesting shellfish from a restricted area and transporting them to an approved site. After a specific period of time the shellfish are harvested for marketing.

Although the processes of depuration and relaying are labor intensive, they serve to broaden the market for shellfish production.

FAILURE OF SHELLFISH CERTIFICATION PROGRAM TO PREVENT OUTBREAKS

Many researchers feel that continued outbreaks of nonspecific gastroenteritis, hepatitis A and *Vibrio parahemolyticus* food poisoning from shellfish taken from approved areas are indicative of the inadequacy of current bacterial standards in detecting viral species (Gerba *et al.*, 1980; Hunt, 1980; Portnoy *et al.*, 1975; Richards, 1985; Sobsey *et al.*, 1980).

The study conducted by Gerba *et al.* (1980) found viral occurrence in approved shellfish areas 35% of the time. Portnoy *et. al.* (1980) found a high frequency of occurrence of the indigenous species *Vibrio parahemolyticus* in Louisiana water, with seasonal variability. More recent studies by Richards have found a frequent occurrence of the hepatitis A and Norwalk viruses in *C. virginica* (Richards, 1985).

Most studies investigating this phenomena suggest that more suitable indicators of viruses should be adopted that would be adjunct to, but not replacing current certification standards (Gerba *et al.*, 1980; Hunt, 1980; Portnoy *et al.*, 1975)

At first glance depuration of all shellfish would be an attractive alternative. In the United Kingdom, depuration of all shellfish is mandatory as a prerequisite of marketing. Even with these safeguards, however, outbreaks of gastroenteritis have still occurred (Richards, 1985). Some of the outbreaks were attributed to improper depuration techniques (FDA, 1983).

Many studies suggest that the fecal coliform standards currently used for routine testing of depurated shellfish are inadequate and that viral indicators such as Clostridium perfringens and Vibrio parahemolyticus should be used for quality control and determination of adequate depuration time (Bisson and Cabelli, 1979; Bisson and Cabelli, 1980; Cabelli, 1981; Emerson and Cabelli, 1982; Emerson and Cabelli, 1985; Watkins et al., 1976; Watkins and Cabelli, 1985)

PROJECT OBJECTIVES

There has been much research done in the area of depuration as a means of eliminating bacteria such as Escherchia coli and Salmonella (Cabelli and Heffernan, 1970; Hartland and Timoney, 1979; Janssen, 1983; Perkins et al., 1980; Timoney and Abston, 1984) and viral species such as Coxsackievirus and poliovirus (Landry et al., 1982) from artifically infected shellfish.

Research by Cabelli and Heffernan (1971) suggests that shellfish contaminated artificially with virus or bacteria may be inadequate in the assessment of the feasibility of depuration. They found marked differences in depuration efficiencies between quahaugs contaminated artificially with E. coli and those contaminated in natural waters (Heffernan and Cabelli, 1971). They theorized that there could be two

possibilities as to why there was a variation in response:

1.) The mechanics of harvesting from the natural environment are more detrimental to this aspect of the quahaugs physiology than their transfer from basketed storage in the laboratory.

2.) The nature of the source of the pollutant presented to the quahaug may be different.

   a.) Under artificial contamination organisms are taken from a pure culture, appropriately diluted and mixed into the water, and are presented to the quahaug in small particles containing one to a few cells.

   b.) Under natural contamination, quahaugs burrowed into the bottom sediments may ingest organisms contained in larger particles which are derived from sewage effluents. These particles, by virtue of their size, could be carried to locations within the quahaug, from which their elimination during depuration may be more difficult.

Previous research by Heffernan and Cabelli (1970) argued that the contamination period was not a significant factor.

Although there has been much research done in determining the efficiency of relaying (Cook and Ellander, 1986; Quayle and Barnard, 1976; Son and Fleet, 1980) and depuration (Cabelli and Heffernan, 1970, 1971; Heffernan and Cabelli, 1971) of shellfish contaminated in natural waters, no information could be found in the literature for a comparative risk assessment model between these two commercially accepted methods of pathogenic elimination. Based on this information the objectives for the project were:

[1.] Determination of the maximum contaminant level in quahaugs, M. mercenaria, grown in sewage contaminated water.

[2.] Evaluation of the effectiveness of depuration versus relaying in the removal of fecal coliforms from quahaugs grown in sewage contaminated water.

[3.] Development of a risk assessment model based upon our data that determines the probability of the public ingesting contaminated shellfish.

METHODS AND MATERIALS

EXPERIMENTAL ANIMALS

Hard shell clams (Mercenaria mercenaria) were obtained from Sandy Hook Seafood, Highlands, NJ. Following depuration, the quahaugs were initially transported to the University of New Hampshire Department of Civil Engineering research greenhouse, located at the Durham Wastewater Treatment Facility, Durham, NH. One and a half bushels of quahaugs (approx. 300 individuals) were used for Experiment 1. The quahaugs were gathered directly after depuration and transported in a closed container without ice for six hours. At the start of Experiment 2, one bushel of depurated quahaugs (approx. 200) was obtained from a refrigerated unit at Sandy Hook Seafood and transported in a closed container on ice for six hours. For Experiment 3 one bushel of quahaugs was obtained from a Sandy Hook Seafood refrigerated truck in Attleboro, MA and transported by car at 18 C (NOTE:"C" equals degrees Celsius) for 2.5 hours. Quahaugs used for all experiments were subject to a one half hour adjustment period at room temperature (18 C) before being introduced to the contamination environment. The quahaugs were placed gently into the reactors with sufficient spacing between them. No death occurred due to transportation. The quahaugs measured 7 to 9 cm in length with meat weights ranging from 40 to 90 g. The quahaugs were received depurated so they would be ready to feed allowing for careful monitoring of their contamination levels from an initial level of zero fecal coliforms per quahaug.

EXPERIMENTAL CONTAMINATION SYSTEM

Experimental quahaug uptake of contaminants was achieved by placing the quahaugs in a flowing water system in which the contamination level, temperature and salinity of the water were controlled. All contamination experiments were performed at the Department of Civil Engineering greenhouse. Air temperatures were maintained between 11 and 23 C in the greenhouse by using a thermostat controlled heater/exhaust fan airflow regime. Experiment 3 was initially affected by unseasonably warm air temperatures (>23 C) which created higher temperatures than the cooling fans could control. This isolated incident had no apparent effect except for prolonging the contamination rate of the quahaugs due to an algal bloom. Water temperatures fluctuated from 11 to 22 C (up to 25 C in Experiment 3), thus simulating mean operating season temperatures (April to October) for depuration facilities.

A four tank flow-through experimental system was constructed using 120 l polyethylene observation reactors (Figure 1). Initially, the reactors were filled with a pretreated wastewater mixture and the salinity was adjusted to 20 o/oo. Each reactor was bedded with approximately three inches of natural sediment obtained from the estuarine portion of the Oyster River. The sediment was dug with shovels at low tide, transported to the greenhouse in plastic totes, distributed evenly into each reactor and allowed to settle for several days. Flow rates were adjusted to achieve an 11 day retention time in the reactors. Pretreated wastewater (raw wastewater that was screened and degritted) was periodically pumped from the Durham WWTP grit chamber into a 95 l holding tank located in the greenhouse. From the

CONTAMINATION FACILITY

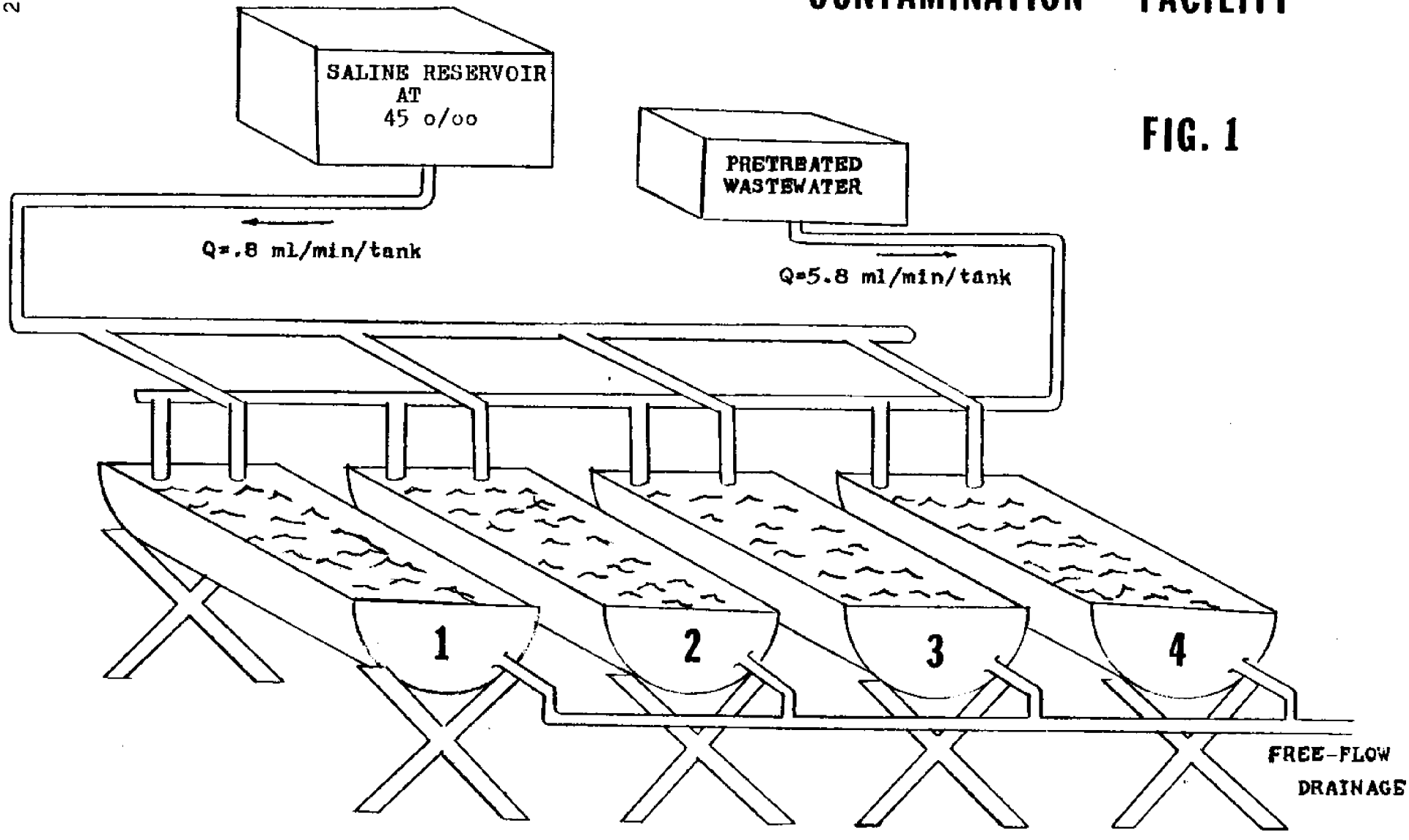


FIG. 1

holding tank the influent was pumped by peristaltic Masterflex (Barnant Co., Barrington, IL) pumps at a rate of 5.8-6.0 ml/min into each reactor using Tygon plastic tubing. Waste effluent was discharged into a separate holding tank and was periodically pumped back into the municipal treatment system.

Saline water was artificially prepared from Instant Ocean synthetic sea salts (Aquarium Systems, Inc. East Lake, Ohio). Near the completion of Experiment 3, Morton iodized table salt was substituted with no apparent effects. A 55 l saline reservoir tank was prepared at 45 o/oo salinity for each experiment. The saline water was pumped by peristaltic Masterflex pumps into each reactor using Tygon plastic tubing at a flow rate of 0.8ml/min to maintain a salinity of 20 o/oo in each reactor.

Coarse bubble aeration powered by 1/10 hp Gast compressors (Gast Mfg. Corp, Benton Harbor, MI) was used as an air supply to maintain aerobic conditions in the reactors. This aeration process also guaranteed a completely mixed environment which assured even levels of contamination throughout the tanks (Eighmy, 1986).

The bacterial suspension in the sewage impacted environment varied from a bacterial concentration between 17,000 to 34,000 colony forming units (CFU)/100 ml. Coliform levels plunged significantly at the start of Experiment 3 due to an algal bloom in the reactors initiated by unseasonably warm temperatures. This induced aerobic treatment of the contamination water, killing most of the coliforms. The coliform levels were revived by replacing 45 l of reactor broth with an equivalent amount of pretreated sewage. The quahaugs were

removed briefly while the reactor water was changed.

An algal culture was introduced to the reactor environment to stimulate feeding. One liter of aerobic wastewater containing unicellular algae was added to each contamination tank at the start of the experiments.

The environmental contamination system was carefully monitored three times daily during each experimental cycle, thus reducing the possibility of any malfunctioning of equipment and variability in the environmental parameters.

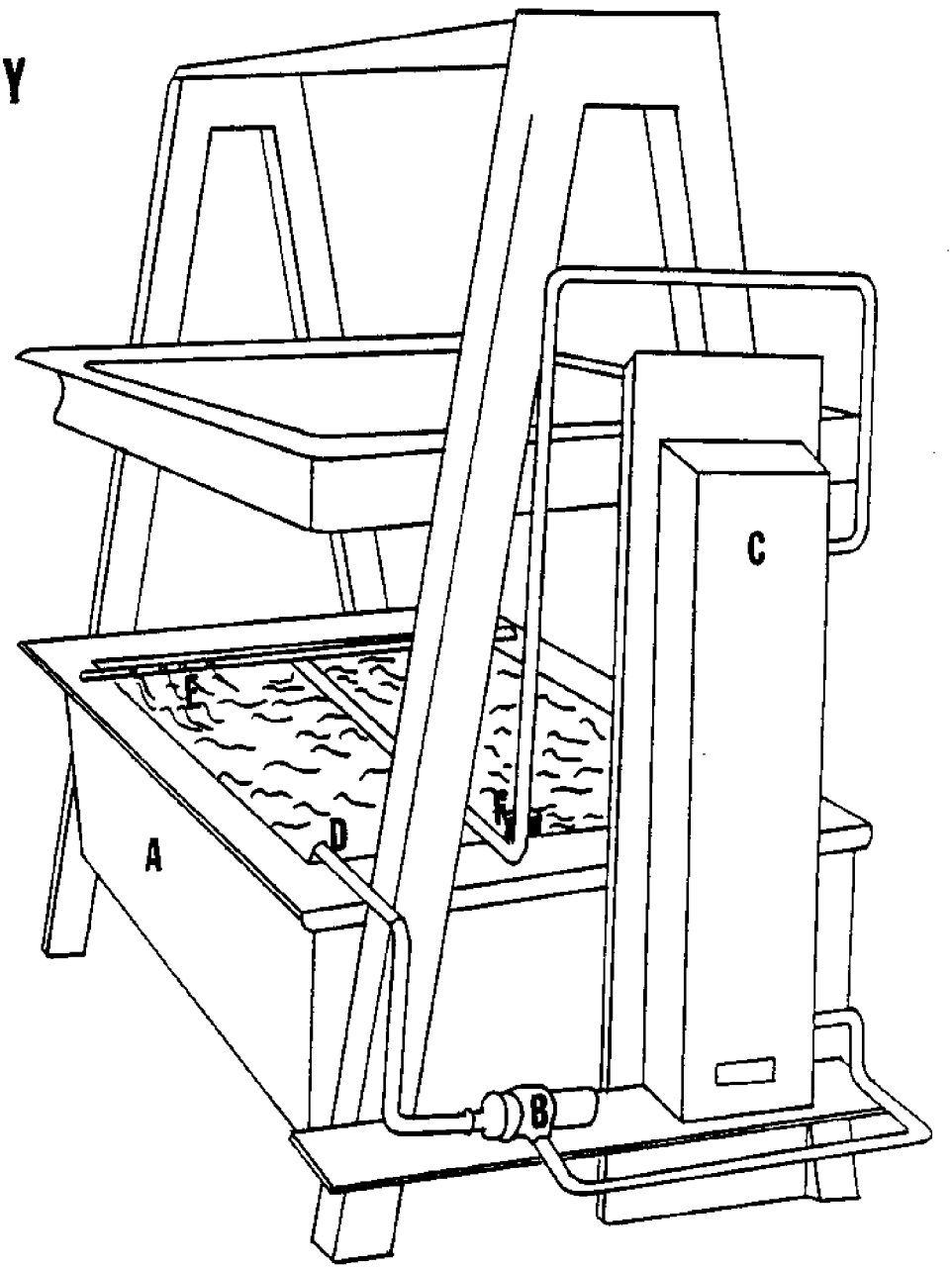
EXPERIMENTAL DEPURATION AND RELAYING SYSTEMS

The experiments were conducted at the University's Jackson Estuarine Laboratory located on Little Bay in Durham, N.H. This comparison study required two facilities that were constructed at Jackson Laboratory. These facilities are the depuration facility and the relaying simulation facility; both were constructed in an attempt to simulate the two techniques used in the decontamination of quahaugs (Figures 2 and 3).

The depuration system consisted of two 132 cm x 76 cm x 38 cm fiberglass tanks (Figure 2). These depuration tanks were part of a closed-loop system constructed with 1.9 cm PVC pipe. The system inlet was located on the bottom of the tank, in the corner diagonally across from the outlet. Sea water was circulated through an Aquafine ultraviolet sterilizer model SL-1 (Aquafine, Valencia, CA), which has a maximum flow capacity of 37.85 l/min. This device provides in excess of 99% reduction of all bacteria (Appendix D). A Little Giant

DEPURATION FACILITY

FIG. 2



A. DEPURATION TANK (132 cm x 76 cm x 38 cm)

B. LITTLE GIANT PUMP, MODEL 2-MD

C. AQUAFINE ULTRAVIOLET WATER STERILIZER, MODEL SL-1

D. INTAKE LINE, INLET ON BOTTOM OF ADJACENT CORNER

E. OUTLET (OXYGENATION TUBE)

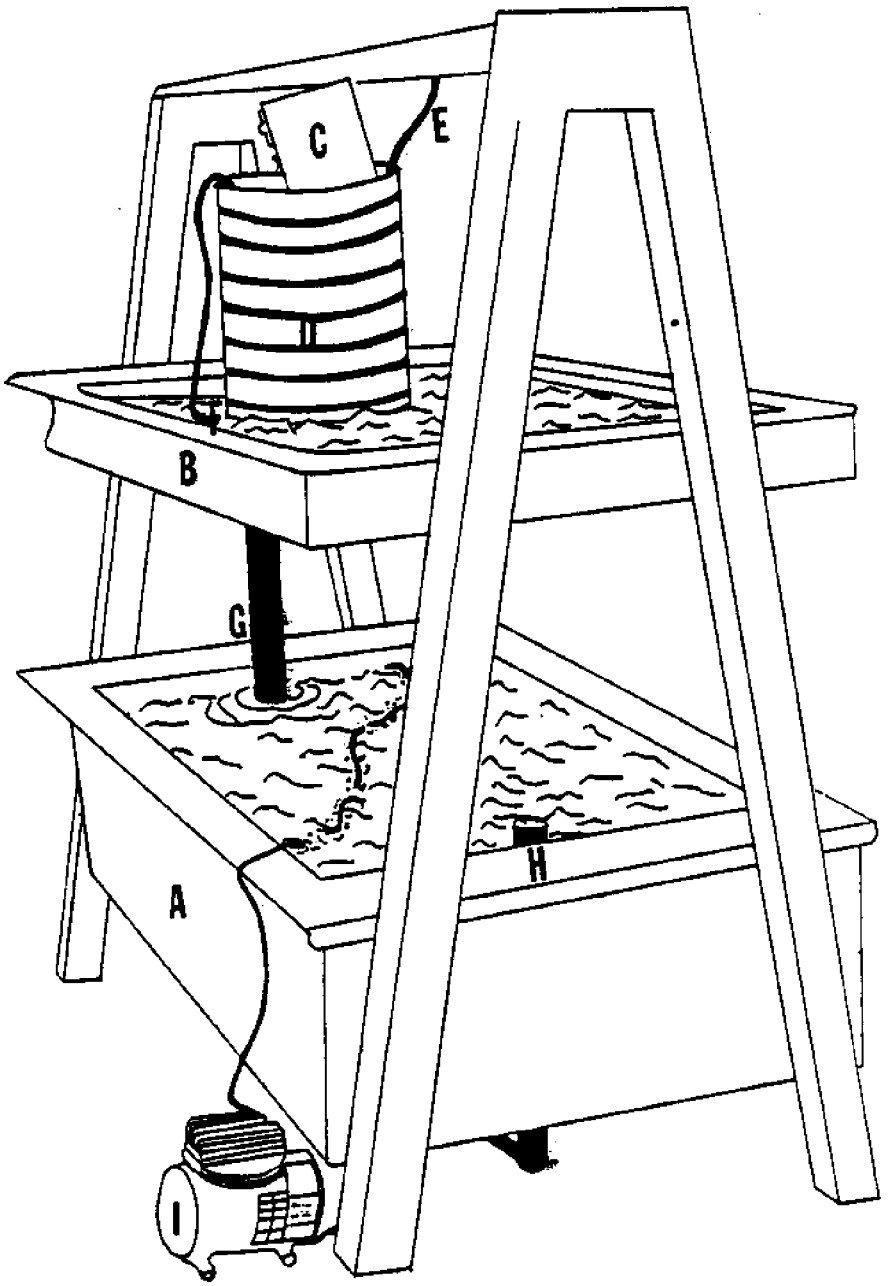
F. DRAIN

(Little Giant, Oklahoma City, OK) non-submersible chemical pump model 2-MD was used. This pump had the capacity to pump 3.78 l/min of water with a 2.1 m head. The outlet consisted of a capped section of 1.9 cm PVC pipe with 6.4 mm diameter holes, which broke more of the water's surface allowing additional aeration. An Ametek filter (Ametek,Sheboygan,WI) model PS-S1 was used when filling the depuration tanks; it was not part of the recycling system. This filter removed suspended matter down to 20 um from the sea water inflow used to fill the tanks before recirculation of the water began. The characteristics of the specific systems are outlined in Appendix C.

The relaying facility used a 132 cm x 76 cm x 9 cm fiberglass reservoir tank with a 7.6 cm drain (Figure 3). Located below the reservoir tank on an "A" frame stand was the 132 cm x 76 cm x 38 cm fiberglass relaying tank. An A.B. Braun Melsungen AG (Germany) electric water heater was used in conjunction with a 37.85 l cylindrical glass container to serve as a heat exchanger. Water entered this open-loop system through 6.4 mm inner diameter plastic tubing, 9.5 m of this tubing was coiled in the heat exchanger. An additional 0.5 m section brought the sea water from the heat exchanger into the upper reservoir tray. A 7.5 cm long section of 7.6 cm PVC pipe was inserted in the drain of the reservoir tank to maintain 7.5 cm of water in this tank. Two additional sections of this 7.6 cm PVC pipe were used. One section of pipe 38 cm long bridged the gap between the upper reservoir tank and the lower relaying tank. The second section of pipe 30.5 cm long was inserted in the drain of the relaying tank to maintain this tank's water level at 30.5 cm from the bottom of the relaying tank. The bottom of this relaying tank was

RELAYING FACILITY

FIG. 3



A. RELAYING TANK (132 cm x 76 cm x 9 cm)

B. RESERVOIR TANK (132 cm x 76 cm x 38 cm)

C. ELECTRIC WATER HEATER

D. HEAT EXCHANGER (COILED TUBING IN HOT WATER BATH)

E. INLET TO HEAT EXCHANGER

F. OUTLET FROM HEAT EXCHANGER INTO RESERVOIR

G. INLET FROM RESERVOIR TANK INTO RELAYING TANK

H. DRAIN

I. AIR COMPRESSOR

bedded with 8 cm of sterile sediment taken from a mud flat in Great Bay. (The sediment was sterilized by a 30 minute wet cycle in a Barnstead autoclave at 121 C, 10546 kg/sq.m). The sea water in the relaying tank was aerated by a punctured plastic tube spanning the length of the tank. The air was supplied through a 1/10 hp. Gast air compressor that ran continuously.

Jackson Estuarine Laboratory pumps sea water from Little Bay into six, 2650 l fiberglass tanks located on the third floor of the building. The intake is located 12 m below mean low water. The water is pumped through 10.2 cm diameter armored PVC pipe to the six loft tanks. These tanks are on a timed system and are filled intermittently. By storing 15,900 l of water, variations in temperature and salinity are dampened. This sea water is gravity-fed to the laboratories on the lower floors.

One day prior to starting depuration the two depuration tanks were filled with 0.23 cubic meters of water which was passed through a cartridge filter to remove particles $\geq$ 20 um. On average, the water from the loft tanks was 6 C and the salinity varied from 15 to 20 o/oo. At the start of depuration, the water temperature was 16 C. A half bushel of quahaugs, brought from the contamination facility at the Durham WWTP, was scrubbed by hand with a stiff brush. The half bushel of quahaugs was placed in plastic mesh trays. These trays of quahaugs were submerged in the depuration tanks. The pump was turned on and the flow of the circulating water was set at 3.78 l/min. The water was taken from the bottom of the depuration tank and pumped through the ultraviolet sterilizer to the aeration tube. From the aeration tube the recycled water was cascaded back into the tank in

the corner diagonally across from the intake.

At 24 hours, the two trays of quahaugs were moved into the second depuration tank which had been filled with water 48 hours before and was 16 C. Having a second tank of equivalent temperature was necessary to avoid any temperature shock to the quahaugs. The quahaugs were exposed to this second tank for a subsequent 24 hour period.

One day prior to exposing the half bushel of quahaugs to the relaying tank, the relaying and reservoir tanks were filled. The water came from the loft tanks on the third floor at an average temperature of 6 C and salinity ranged from 15 to 20 o/oo. The heat exchanger was started at this time. One half bushel of quahaugs was received from the contamination facility at the Durham WWTP. These quahaugs were put into the relaying tank and allowed to burrow in the sediment. The sea water flow through the open-loop system was started. The flow was set at 600 ml/min. After passing through the heat exchanger the water was elevated from 11 to 17 C. The water exiting the heat exchanger entered the reservoir tank which was also 17 C. Since this was a flow through system, as fast as water entered the reservoir tank it was displaced through the 7.6 cm drain tube into the relaying tank below. The retention time in this tank was 13 hours. The water exiting the relaying tank was displaced over the 30.5 cm high drain tube on the opposite end of the tank.

SAMPLE COLLECTION AND BACTERIAL ENUMERATION

A modification of the MacConkey Agar Pour Plate Technique for Elevated Temperature Coliform Determination allowed for effective and rapid sampling of large numbers of quahaugs for fecal coliform accumulation. The formula for the single strength media (Appendix B) included 44.5g of the media ingredients mixed in 1000ml of distilled water which was boiled in a covered volumetric flask for 10 minutes. The medium was held in a water bath at 55-60 C until use, a period not exceeding four hours.

Samples of six quahaugs were gathered at 0, 12, 24, 48 and 96 hours for each contamination experiment. The quahaugs were randomly chosen, labeled according to the tank in which they resided and placed in a bag. A 50 ml water sample was also taken from one reactor with a sterile plastic sampling bag. The water samples and quahaugs were then transported to the Environmental Engineering Laboratories in Parsons Hall at the University of New Hampshire and analyzed within one hour. Once at the laboratory, the quahaug's reactor number was recorded and then the quahaugs were scrubbed clean with running tap water. The quahaugs were weighed on a top loading Mettler K5 balance. At this point the quahaugs were ready for analysis.

Sampling in the depuration and relaying systems proceeded in a slightly different manner. Samples of 16 quahaugs were randomly selected, 8 each from the relaying tank and the depuration tank. This selection was done at 12, 24, and 48 hours. Once removed from the tanks, the quahaugs were bagged and labeled. Two 50 ml water samples were taken in plastic sample bags. A sample was taken from the outlet

of the ultraviolet sterilizer in the depuration facility to assure that the ultraviolet disinfection was effective. The second water sample came from the outlet of the heat exchanger on the relaying facility. The sixteen quahaugs and the water samples were then transported to the Environmental Engineering laboratory in Parsons Hall. At the lab the quahaugs were scrubbed clean under running tap water. The quahaugs from both the depuration and relaying facilities were weighed on a top loading Mettler K5 balance. At this point the quahaugs were ready for analysis.

The quahaug samples were shucked using a knife (flame sterilized with 95% ethyl alcohol). The visceral mass and siphon interior of the quahaug were carefully placed into an autoclaved glass blender (Hamilton Beach Scoville, Washington, NC). Each individual quahaug shell and muscle tissue was weighed. The samples were individually prepared for assay by homogenizing their contents in the blender for 90 seconds. In between shuckings the knife was washed and resterilized for further use. Six grams of the homogenate were then weighed out into an autoclaved 500 ml prescription bottle using the top loading Mettler balance. The contents were diluted to 60 ml with sterile phosphate-buffered saline (9 g/l NaCl and 1.25 ml buffer solution per 1000 ml distilled water) using sterile technique. A 60 ml portion of single strength medium was added to the bottle and the contents gently shaken. Then the bottle contents were distributed into six sterile petri dishes (100mm X 15 mm) for incubation. The medium was allowed to solidify and the inverted plates were incubated in an air incubator at 45.5±0.5 C for 24±2 hours. At this temperature the fecal coliform bacteria produced their characteristic pink/purple

subsurface colonies. Only the characteristic fecal coliform colonies >0.5mm in diameter were counted. Water samples were analyzed by pipetting 5 ml into a sterile prescription bottle and then following the previously described MacConkey agar procedure.

BACTERIAL ENUMERATION EXPERIMENT

One of the standard methods used for fecal coliform detection is the Membrane Filtration technique (Standard Methods, 1985). This technique was compared in the lab with the ETCP method (described above) using the water in the contamination reactors. This experiment determined if the results obtained from the ETCP method would be similar to those obtained through the use of the standard Membrane Filtration technique. For the Membrane Filtration technique, an appropriate sample size was chosen depending on the sewage strength and diluted up to 10 ml with sterile phosphate-buffered saline solution. A sterile 45 um membrane filter was placed on the filter apparatus using sterile forceps. The funnel was placed on the unit and the vacuum pump turned on. The sample was added to the funnel and filtered. The pump was then turned off and the filter was removed with sterile forceps. The filter was placed in a petri dish on a sterile pad containing 2 ml of m-FC medium (see Appendix B). The filter apparatus was rinsed with deionized water between samples. The dishes were incubated at 44.5±0.5 C in a water bath. Blue colonies were counted after 24±2 hours of incubation.

OTHER ANALYTICAL METHODS

Salinity , dissolved oxygen and temperature were monitored in the contamination tanks. Temperature was monitored using a VWR Scientific (Boston, MA) mercury-filled, Celsius thermometer marked in 0.1 C intervals. It was calibrated with an ASTM thermometer according to Standard Methods (1985).

Salinity was monitored by use of an AO Goldberg T/C refractometer (Model 10419, AO Scientific Instruments, Rochester, NY). This intrument is self-compensating for temperatures from 15 to 38 C.

Dissolved oxygen was measured using a YSI Model 51A Dissolved Oxygen meter with a membrane probe (Yellow Springs Instrument Co. Yellow Springs, Ohio) according to the procedure outlined in Standard Methods (1985).

Salinity and temperature in the depuration and relaying facilities were monitored by a Tempsal meter (Interocean Systems,Inc.,San Diego,CA).

Appendix A lists the ranges of these parameters monitored in the contamination system.

RESULTS

BACTERIAL ENUMERATION TECHNIQUES

Pretreated municipal wastewater samples were analyzed by the ETCP and Membrane Filtration methods and were found to yield similar results. The results of the comparison are shown in Table 3. Figure 4 shows the relationship between these data in graphical form.

TABLE 3:

TEST	MEMBRANE FILTRATION (CFU/100 ml)	ETCP (CFU/100 ml)
1	7250	7040
2	2967	3480
3	1400	1400
4	11250	12960

Comparison of Enumeration Techniques

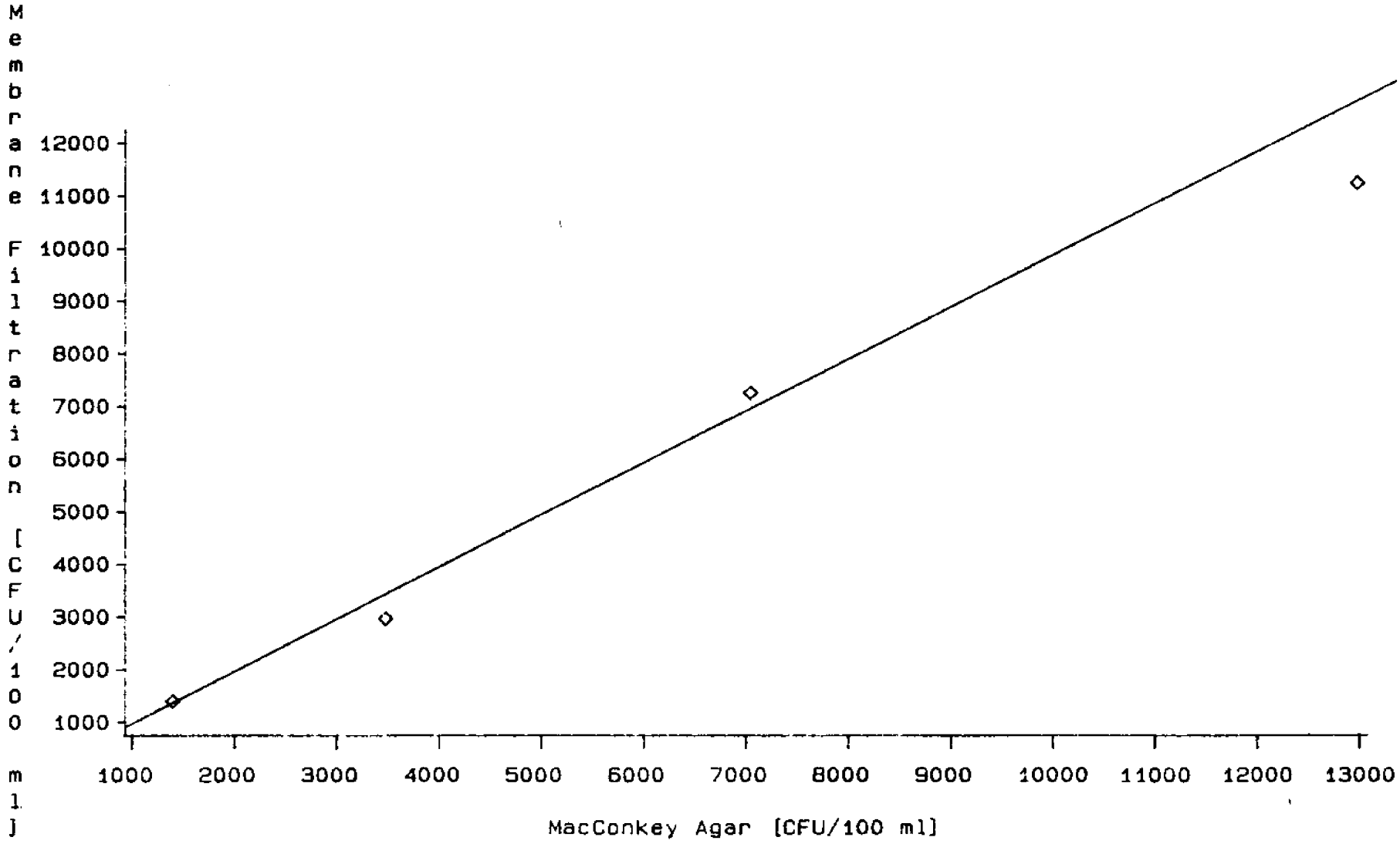


Figure 4

CONTAMINATION EXPERIMENTS

The quahaugs had an initial contamination level of zero or near zero CFU/quahaug. In Experiments 1 and 2, the initial contamination levels were 0 CFU/quahaug. In Exeriment 3 the initial coliform level was 324 CFU/quahaug. Maximum contamination levels obtained in the three experiments are shown in Table 4.

TABLE 4:

EXPERIMENT#	MAX. CONTAMINATION LEVEL (CFU/quahaug)	HOURS TO MAX. LEVEL
1	3092	96
2	4556	48
3	4939	12

DEPURATION AND RELAYING

The data collected during depuration was the mass of meat in each quahaug and the number of CFU per 6 g of sample at measured time intervals. Figure 5 shows the data for the depuration (5a and 5b) and relaying (5c and 5d) experiments. The model used to describe these results was $C = N \exp[-kt]$, where C was the final concentration of contaminants in each quahaug, N was the initial concentration , t was the time, and k was the rate constant for cleansing. For experiments 1 and 2, k equaled 0.0549 and 0.0661, respectively for depuration and k equaled 0.0736 and 0.0752 for relaying. These were obtained from a linear regression of time versus the natural log of C.

Depuration 1

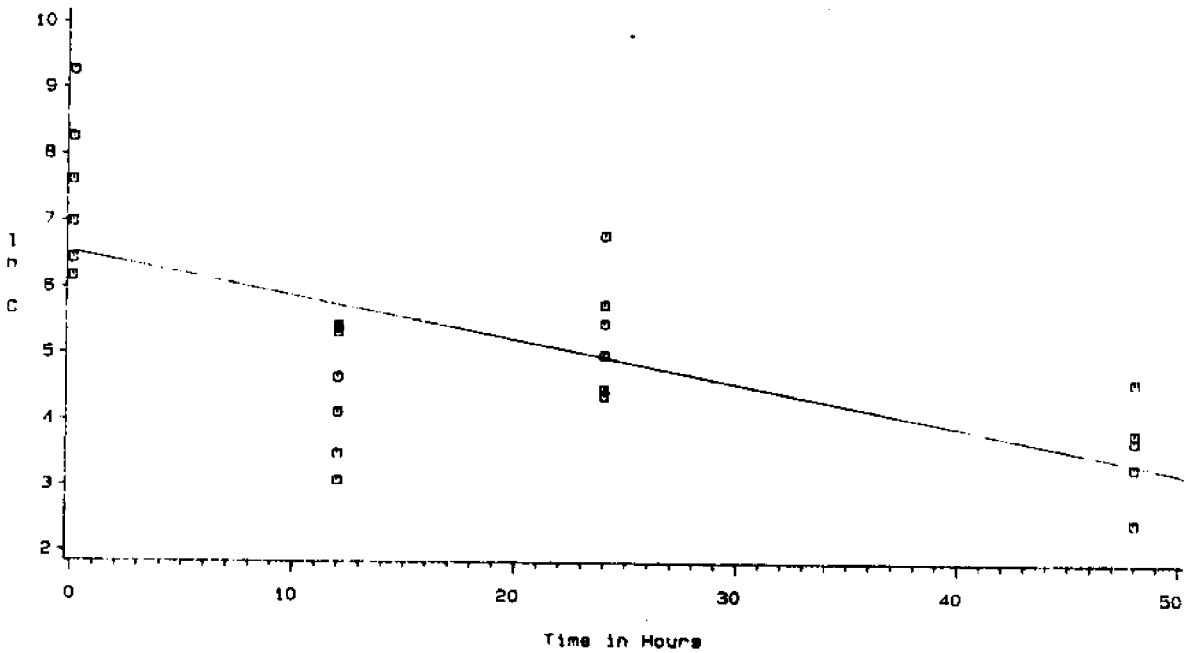


Figure 5A

Depuration 2

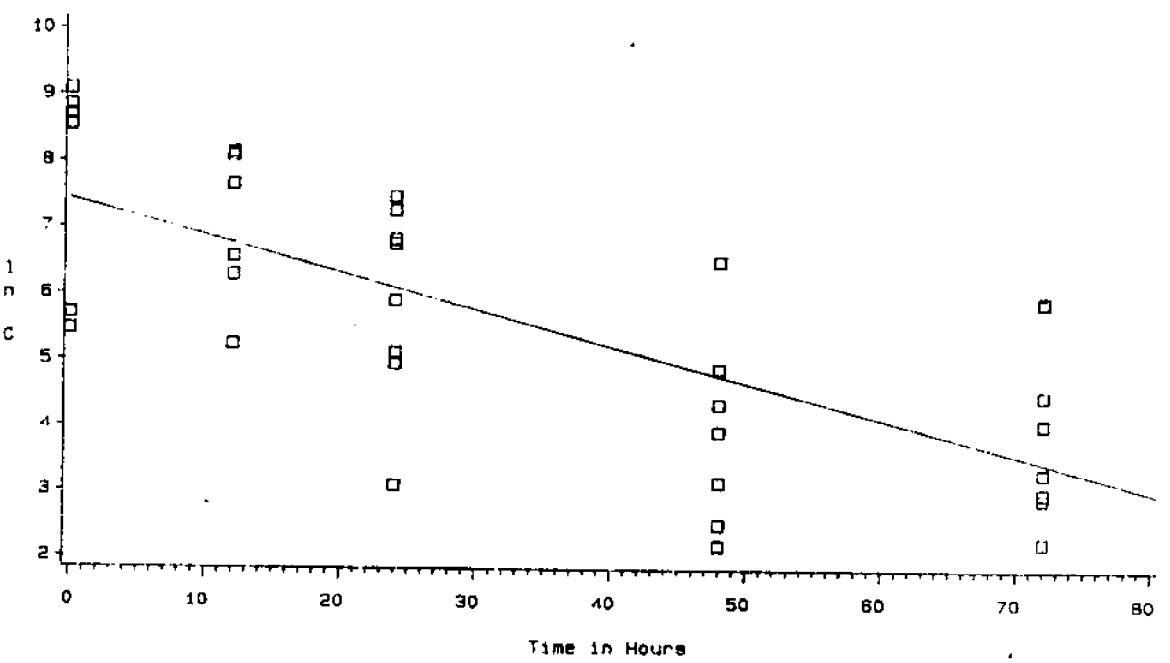


Figure 5B

Relaying 1

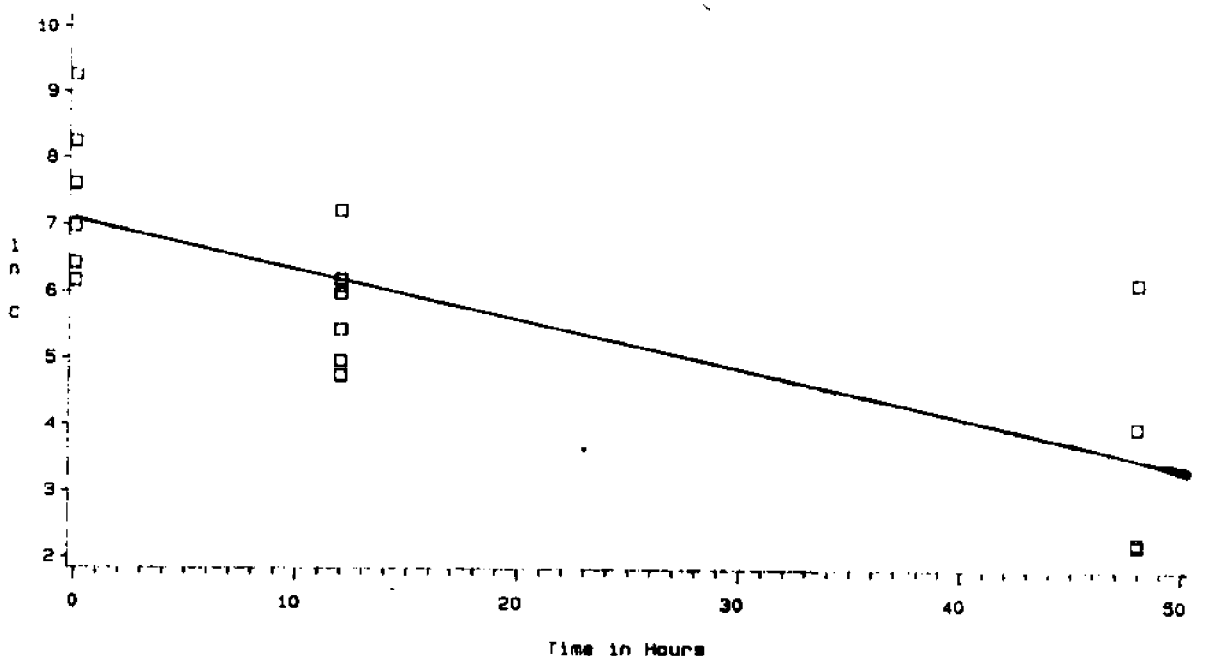


Figure 5c

Relaying 2

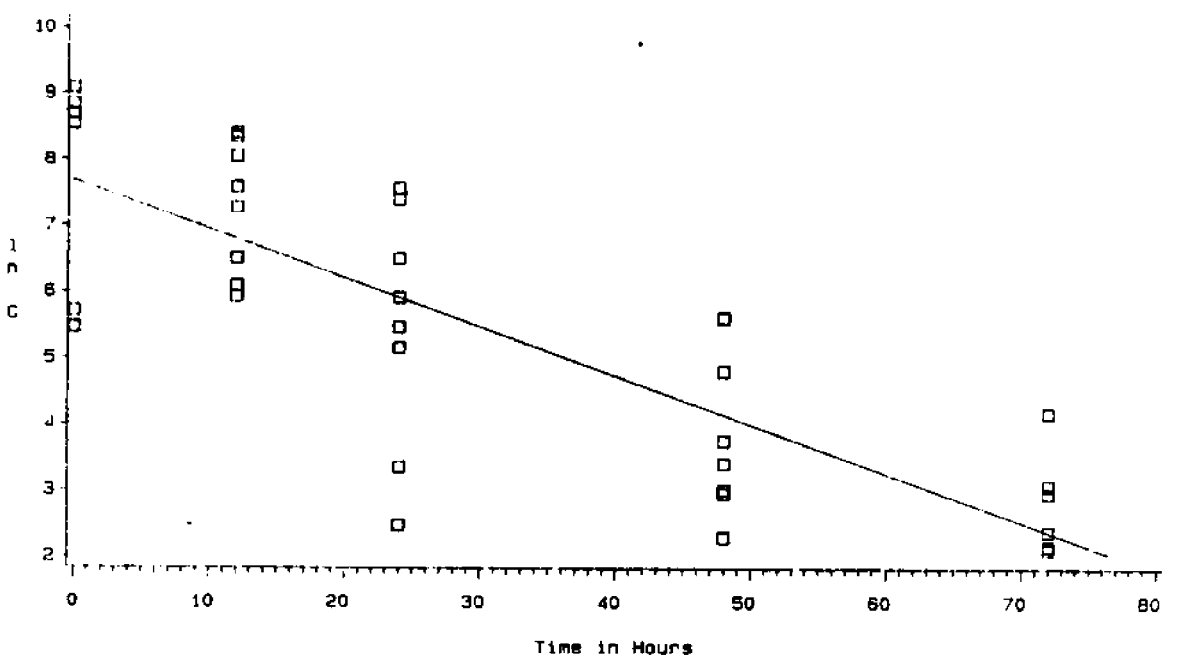


Figure 5d

DISCUSSION

BACTERIAL ENUMERATION TECHNIQUES

There are two standard methods for the enumeration of coliforms. These are the Most Probable Number (MPN) and the Membrane Filtration techniques (Hammer, 1975). The ETCP Method has also been used in the shellfish industry (Division of Marine Resources, Boothbay Harbor,ME).

The coliform group is defined as all aerobic and facultative anaerobic, non-spore forming, Gram negative rods that ferment lactose with gas production within 48 hours of incubation (Hammer, 1975). All three coliform detection methods (MPN, Membrane Filtration, and ETCP) are based on the lactose fermentation by the coliforms.

The MPN technique involves the transfer of a measured volume of sample into fermentation tubes containing lactose or lauryl tryptose broth. The tubes showing growth after 24 or 48 hours are used to inoculate culture tubes containing EC medium broth, which are incubated in a water bath at 44.5±0.2 C. Results of the test are expressed as the most probable number (MPN), since the count is based on statistical analysis of sets of tubes using serial dilutions (Hammer, 1975). A positive test using this method occurs when gas is produced due to the fermentation and is trapped in an inverted glass tube (Hammer, 1975).

In the Membrane Filtration method (Standard Methods, 1985), an appropriate sample size is chosen, depending on sample strength. The sample is diluted and filtered under a vacuum through a 45um membrane

filter. The filter paper is then placed on a pad containing 2 ml of m-FC medium (Appendix B). The plates are incubated at 44.5±0.5 C in a water bath. The coliforms ferment the lactose causing a decrease in pH which turns the rosinolic acid dye blue. The presence of blue colonies is a positive test. These colonies are counted after 24±2 hours of incubation.

The ETCP method used in depuration facilities involves the dilution of a 6 g sample of homogenized shellfish with phosphate-buffered saline to 60 ml in a sterile container. The contents are further diluted to 120 ml with MacConkey Agar and the container gently shaken. The container is then emptied into six petri dishes, covered, and allowed to cool until the solution hardens. The plates are then inverted and incubated for 24 hours at 45.5±0.5 C in an air incubator. Fecal coliform colonies are characteristically pink to red due to the decrease in pH. This is caused by the reaction of acids produced during fermentation with the bile salts in the medium resulting in the subsequent absorption of neutral red (Difco, 1977). Colonies greater than 0.5mm in diameter are counted after incubation.

The ETCP method was recommended to us by Samuel and Thomas Howell, of the Spinney Creek Oyster Co. depuration facility in Eliot, ME. This method has been used extensively in research conducted by Heffernan and Cabelli (Cabelli and Heffernan, 1969 and 1971, Heffernan and Cabelli, 1971), however, they did not perform a quantitative comparison of the ETCP method to standardized coliform enumeration tests.

McCall (1973) found that the ETCP method was an acceptable technique for detection of fecal coliforms in quahaugs. As a result, the Food and Drug Administration accepted the ETCP method and it has been used by depuration facility operators since 1973 (Santo Fufari, FDA, Northeast Technical Service Unit, Davisville, Rhode Island, 1986).

The comparison of the ETCP method to the Membrane Filtration technique conducted in this study supported the work of McCall (1973). Four water samples were tested and based on the results of this study and the data from the FDA, it was found that the ETCP method was adaquate for fecal coliform analysis. Therefore the ETCP method was chosen since it was more economical and less time consuming than either the MPN or Membrane Filtration techniques.

CONTAMINATION EXPERIMENTS

At the start of the contamination experiments (Fall 1985), the reactors were filled with a 1:1 mixture of 13 o/oo saline water and primary sewage. The tanks were aerated and the temperature maintained between 11 and 25 C. Quahaugs placed in these reactors did not feed and coliform counts obtained from these organisms were very low. Two factors contributed to the low coliform levels in the quahaugs.

With the 1:1 mixture of sewage and saline water, the dilution ratio was too high so very low coliform levels were detected in the reactor water (< 50 CFU/100ml). If the quahaugs were feeding at all, the low level in the water would account for the low levels detected in the quahaugs. In addition, the depurated quahaugs used for this experiment had come from water where the ambient salinity was 21±2

o/oo. They were placed in the contamination reactors where the salinity was 13 o/oo . Rapid changes in salinity have been shown to decrease metabolic and feeding rates (Heffernan and Cabelli, 1970). Also the contamination reactors did not have any phytoplankton in them during this experiment.The only source of nutrients in the reactors was the primary sewage, which did not contain their favored diet of phytoplankton (Wilbur, 1964).

For Experiments 1,2 and 3 conducted during the Winter and Spring of 1986, the salinity was maintained at 20±2 o/oo, the reactors were injected with a unicellular algae broth, and a much higher concentration of the sewage was added.

In Experiments 1,2 and 3 the quahaugs had an inital contamination level of zero or near zero CFU/quahaug. The clams contaminated to a maximum level within 96 hours in Experiment 1. In Experiments 2 and 3, the quahaugs attained a maximum level within 48 hours. The quahaugs were considered to be maximally contaminated when they reached a peak level of contamination. Maximum levels obtained in the three experiments were different. They were 3092, 4556 and 4939 CFU/quahaug for Experiment 1, 2 and 3, respectively. The results also indicated that after the quahaugs achieved a maximum level, the level of contamination fluctuated. There are several reasons why the variability in contamination level changed within an individual and with each experiment.

The variability in the maximum contaminant level between the experiments was probably a function of the different coliform levels in the reactors. The coliform concentration in the pretreated

wastewater varied. This is typical in municipal wastewater since the coliform level is a function of the environmental parameters and physiological condition of the human population (Hunt, 1980). This varying concentration meant changing levels of coliforms were available to the quahaugs in the reactor water.

Temperature seemed to affect coliform levels at the beginning of Experiment 3. During this experiment, the fecal coliform level in the water column dropped significantly (<200 CFU/100ml). High temperatures (25 C) occurred in the greenhouse causing an algal bloom in the reactors. At this point the contamination reactors began acting like aerobic wastewater treatment lagoons (Metcalf and Eddy, 1979). Once the algal bloom was reduced by harvesting and lowering the temperature, the fecal coliform level rapidly increased up to 34,000 CFU/100ml.

Sedimentation of the coliforms may also have affected the maximum coliform levels. The reactors were operating almost continuously for five months, so some of the coliforms in the overlying water probably adsorbed to heavier particles and settled, forming a coliform-rich layer on the top of the sediments. Since the quahaugs lived on or in this sediment, they may have been exposed to progressively higher in situ coliform levels during the course of the study.

The fluctuation in contamination within an individual quahaug over time may be a function of the feeding habits of the organisms. Quahaugs are suspension feeders that obtain their food by filtering microscopic particles of organic material from sea water. The organic particles may include phytoplankton, zooplankton, indigenous bacteria,

detritus and fecal coliforms and a variety of other enteric microorganisms. Fecal coliforms ingested may be destroyed by digestive enzymes and phagocytic cells. The others are rejected from the gut as feces. Some fecal coliforms enter the quahaug, but are not ingested. These coliforms may be rejected as pseudofeces or removed by mucus (Hartland and Timoney, 1979).

There are several factors that may affect the number of fecal collforms contained within individual quahaugs. Quahaugs will continue to feed until their stomachs are sufficiently distended which will cause them to stop the feeding process (Maine/NH Sea Grant, 1983). As a result, the fecal coliform concentration observed in a given animal is largely a function of when the animal is tested in relationship to this cycle of feeding. The main physiological factor that affects the individual variations in coliform concentration is the animal's size. If the quahaug is large it will have a larger stomach and hence it will have a greater opportunity to accumulate fecal coliforms. The ratio of fecal coliforms to other ingestable particulates is also an important consideration that will affect the accumulation among individual specimens (Cabelli and Heffernan, 1970).

The variability which results from individual feeding responses means that a maximum contamination is not a constant value. Since a different quahaug is assayed when the contamination level is checked, the numbers found will depend on the digestive stage of the quahaug. If they are actively feeding, the levels will reflect the accumulation rate of the quahaugs. If the quahaugs are not feeding and are only digesting and excreting feces, the coliform levels detected will be lower. This cyclic pattern of coliform contamination was observed in

our experiments.

DEPURATION AND RELAYING

There are two methods for reduction of fecal coliforms in quahaugs. One technique is relaying, or the transfering of quahaugs from restricted waters into clean waters. The other method is depuration where quahaugs are moved from restricted waters and placed into an engineered system of decontamination tanks with water purifying elements. Since quahaugs are filter feeders, they will cleanse themselves by natural feeding over time if the water source is free of contamination. Although the two decontamination methods are similar in theory, the relaying method has two possible drawbacks: reingestion of fecal coliforms and aquisition of viruses indigenous to the relaying area. Depuration does not have these drawbacks because ultraviolet light is used to destroy the pathogens in the water.

A first order reaction model was assumed to be representitive of the depuration and relaying processes. The model form is $C = N \exp[-kt]$ where t equals the depuration time, N is the initial concentration of fecal coliforms in the quahaug before depuration, C is the concentration measured in each quahaug after time t, and k is the rate constant. This model form has been used to explain the disinfection process in water and wastewater treatment. (Johnson, 1983)

The method of least squares was used to estimate the rate constant, k, for each experiment. The model, $C = N \exp[-kt]$, was transposed to a linear model by taking the natural log of each side of the equation. As a result, ln C was plotted versus the time of each

sampling (Figure 6). These graphs show that the first-order reaction model is a reasonable form. Standard linear regresion methods were used to estimate the parameter of the linear regression model. The estimates of k for Experiments 1 and 2 are 0.0549 and 0.0661 for depuration and 0.0736 and 0.0752 for relaying.

A similar rate constant was calculated using data from Heffernan and Cabelli, 1970). The value is k=0.0617 /hr. This is considered a crude estimate because there were only two points to estimate k. These points were the initial contamination at t=0 and the contamination at t=48. The contamination level at t = 48 hours was estimated to be 17 CFU/100g because the results reported could not be accurately measured below that concentration. ' In spite of those shortcomings, there is good agreement between Heffernan and Cabelli and the estimates from this study.

The data show a constant variation about the regression line. This variation has an important bearing on the risk assessment model that was developed. For risk assessment, k was assumed to be a constant and N to be a random variable. Unfortunately, destructive testing had to be used; therefore, it was impossible to trace the contamintion levels in an individual quahaug over time. If this had been possible, the contamination levels would have been monitored in each quahaug as a function of time and these results would have been used to determine the depuration rate constant. Since destructive testing was used, the best estimate of k was made by the method of least squares. In order to account for the variation in the process, the variable N was treated as a random variable. Figure 7 shows that the log-normal probability distribution is a good estimate of the

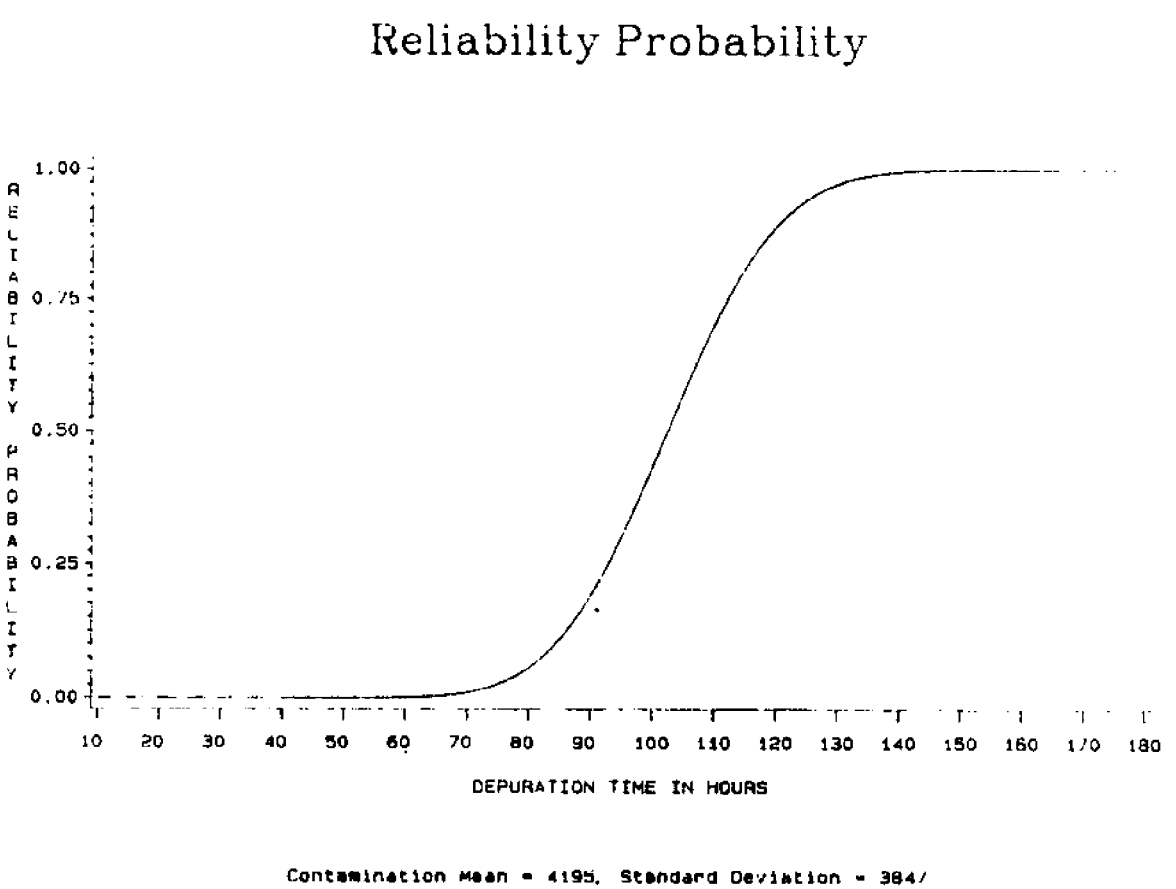


Figure 6a

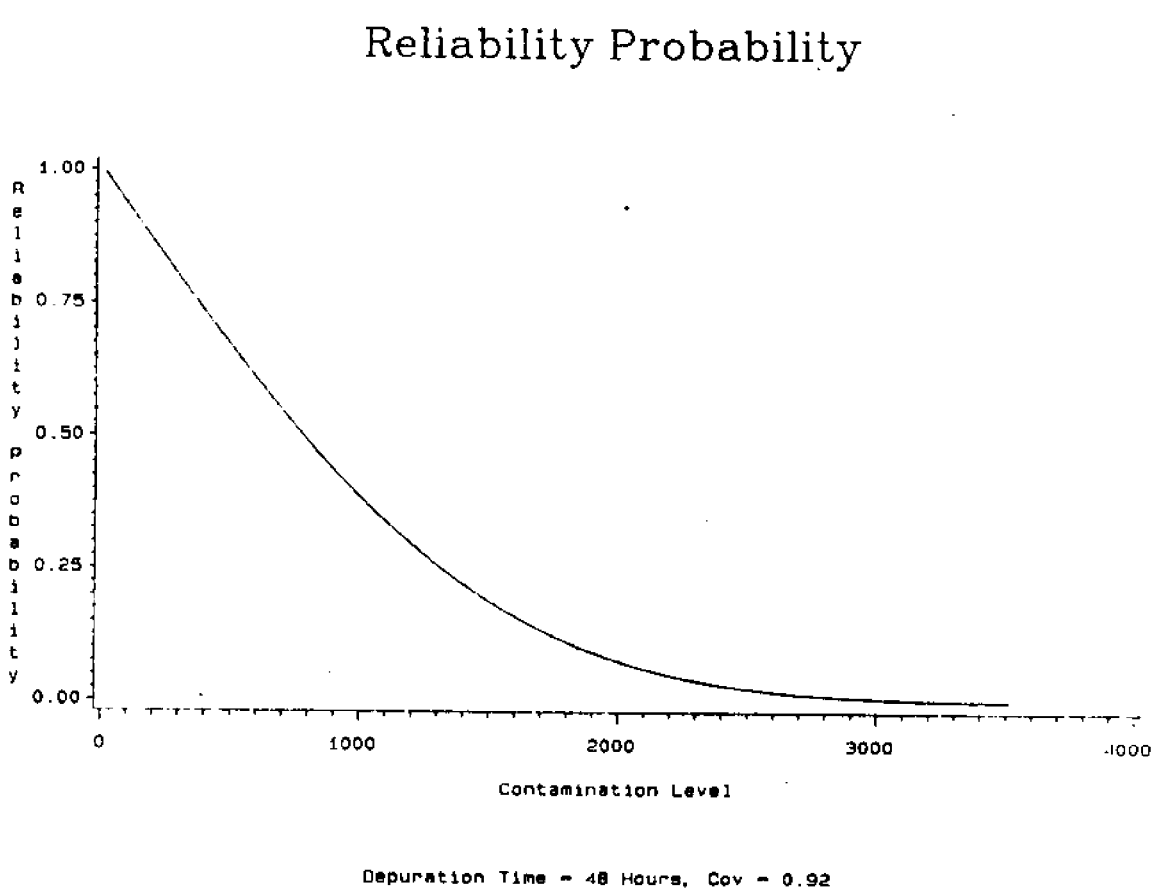


Figure 6b

distribution found in the histogram.

The variability observed in the decontamination data is a result of environmental parameters, such as temperature and salinity, and of individual feeding responses. (Refer to discussion of contamination experiments) The quahaugs which had just completed feeding in the contamination tanks may not have resumed feeding in the depuration or relaying tanks. In this case little contamination would be purged by depuration or relaying. As a result, a longer depuration time would be needed to assure a high probability that the consumer would not ingest a contaminated quahaug.

There were variations between the experiments which should also be considered. For the first experiment the clams were placed in large plastic bread racks which allowed the water to flow around them. It was found that the times for depuration and for relaying were basically the same. Before the second experiment commenced, the relaying tank was filled with sterile sediment. This allowed for the simulation of a natural relaying area where the clams could bury into the sediment. Again results showed that the time required for depuration and relaying was basically the same. It should be noted that at an actual relaying site the sediment would not be sterile. Depending on the relaying site this could have a minimal effect on the rate of cleansing if the sediments were clean or it could be an important factor in the time required for relaying if the sediments were contaminated. In the relaying states of Rhode Island and New York the latter is more prevalent.

Contamination Data Distribution

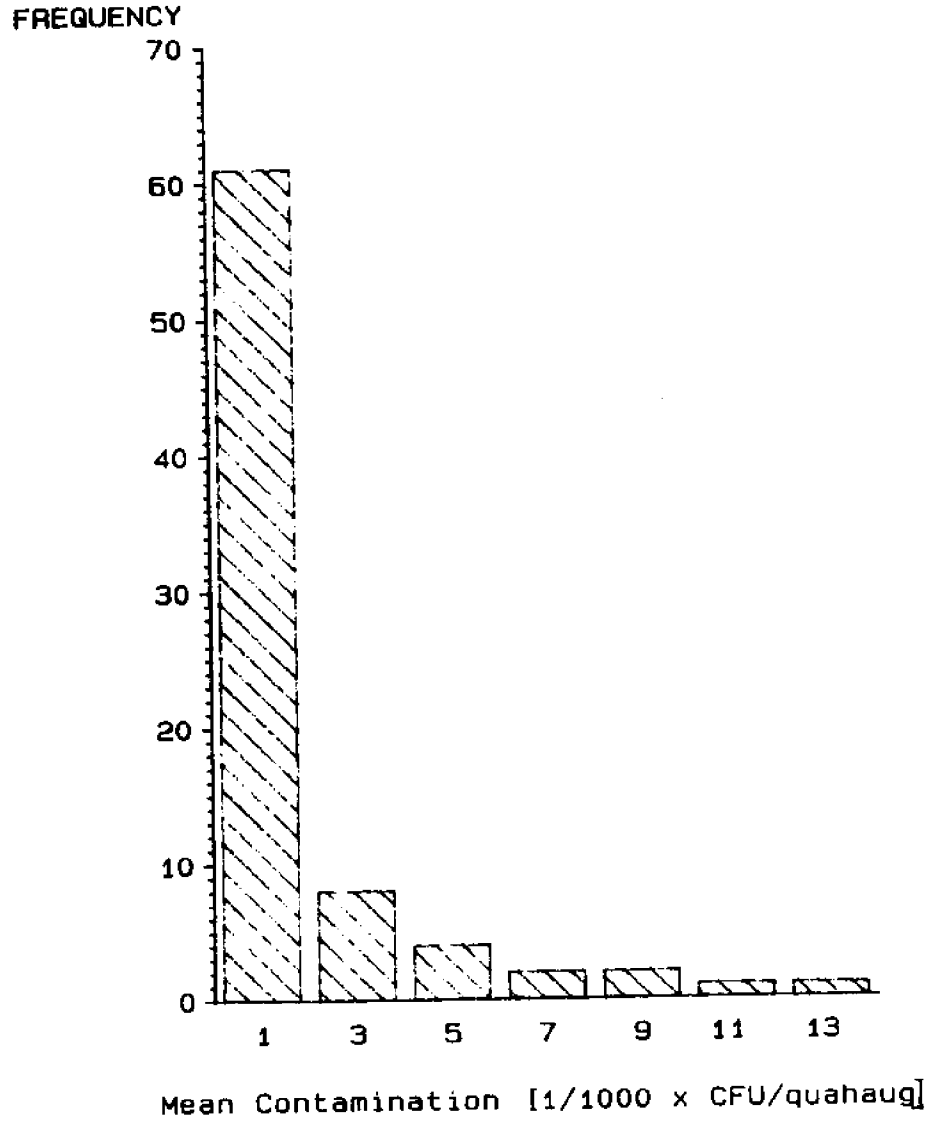


Figure 7

RISK ASSESSMENT

One of the objectives of this study was to assess the risk associated with ingesting a contaminated quahaug. For effective treatment, the contamination in a relayed or depurated quahaug, C, must be less than the assurance or compliance standard, S. That is:

$$S \geq C$$

Standards imposed by the FDA set the maximum level of contamination in a depurated quahaug at 17 CFU/100g. This was converted to coliforms per quahaug by multiplying S by the average mass of the quahaug meat, 65g.

$$S = 17 \text{ CFU}/100g * 65g = 11 \text{ CFU/quahaug}$$

If $S < C$, then inadequate treatment has been provided. Since $C = N \exp[-kt]$, the relationship becomes

$$S \geq N \exp[-kt]$$

It was assumed that N, the initial concentration of contamination, followed a log-normal probability distribution. This assumption was found to be valid using graphical techniques. (Figure 7) N is a function of the mean of the log-normal probability distribution, Log x, and the square of the variance of the log-normal probability distribution, SDx. Log x and SDx can be estimated as follows:

$$\text{Log } x = \ln x - \frac{(SDx)^2}{2} \quad \text{and}$$

$$(SDx)^2 = \left[1 + \frac{(S)^2}{(x)^2} \right] = \ln (1 + COV^2)$$

where x in the mean of the model, S is the acceptance level of

contamination, and COV, the coefficient of variation, is (S/x). The values of these constants are as follows: x = 4195 CFU/quahaug, S = 11 CFU/quahaug, COV = 0.92, Log x = 8.04, and SDx = 3846.8 CFU/quahaug. The probability of successfully decontaminating a quahaug is:

$$p = P\ [S \geq N \exp(-kt)]$$

This equation can be written as:

$$p = P\ [N \leq S \exp(kt)]$$

This investigation has shown the random variable N to be adequately described by a log-normal probability distribution. The log-normal distribution may be transposed to a normal distribution. The reliability probability is the cumulative normal probability with argument

$$B = [\ \ln S + kt - \text{Log } x] \ / \ SDx$$

With this relationship the reliability probability may be estimated for different depuration times t. The rate constant used for the risk assessment was 0.055 which was the most conservative of the values calculated. The result is shown in Figure 6a. The purpose of this analysis is to determine the reliability probability for the heavily contaminated quahaugs (4195 CFU/quahaug). In order to approach the probability of p = 1, a depuration time of 140 hours is needed.

To aid in decision making about viable harvest areas, Figure 6b was prepared. Here, the initial concentration, N, is assumed to be a variable and the coefficient of variation, COV, is a constant. The COV is only known for the case of grossly contaminated quahaugs; all

other cases were approximated using these observations. The probability of S occurring was plotted versus the contamination level in a quahaug at t=48 hours. Using this reliability model, it was determined that the probalility of depurating a grossly contamination quahaug (3840 CFU/quahaug) to the acceptance level in 48 hours is zero.

For a quahaug grown in restricted waters, 88 CFU/100 ml, the estimated fecal coliform count is 60 CFU/quahaug. Since quahaugs were not sampled from restricted waters this contamination level was estimated from the ratio

$$\frac{\text{contamination/quahaug}}{\text{88 CFU/100 ml (restricted)}} = \frac{\text{4195 CFU/quahaug}}{\text{6220 CFU/100 ml (prohibited)}}$$

where the values for prohibited waters are the averages of the contamination results and of the enumeration methods results. The time recommended for decontamination is 100 hours. At the required time of 48 hours the reliabibity is only 85%. This model may not be a good estimate for moderately contaminated quahaugs because the coefficient of variance was assumed to be a constant for all estimates of N. In order to confirm this assumption N should be measured for quahaugs grown in restricted waters. By applying this risk model to moderately contaminated quahaugs one assumes that the same coefficient of variation applies. The actual COV may be lower which would decrease the time required for depuration or relaying.

SUMMARY AND FUTURE RESEARCH

Reducing public health risks is an important and vital topic in today's society where high technology and development threaten the environment and its inhabitants. Shellfish such as quahaugs, concentrate pathogens when subjected to waters impacted by sewage discharge. The health risks associated with human consumption of contaminated quahaugs are too severe to risk direct marketing. The results of this project indicate that artificial depuration and relaying are successful techniques in reducing these public health risks. The major conclusions of this research are:

[1] The actual maximum contamination level of sewage impacted quahaugs is highly variable possibly due to fluctuations in environmental parameters such as temperature, salinity, wastewater coliform levels, and the feeding patterns among individual specimens.

[2] Depuration and relaying are both feasible methods of reducing fecal coliform levels in grossly contaminated quahaugs below the calculated standard of 11 CFU/quahaug in a period of 100 hours. It should be noted that from this study it was not possible to recommend one method over the other since the data showed no statistical difference between their cleansing efficiencies.

[3] The study proved that a first order disinfection model, $C = N \exp[-kt]$, is suitable for predicting depuration kinetics. Rate constants from the equation were consistent in comparison to previous work done by Heffernan and Cabelli (1970). The most conservative rate constant from the

reliability probability distribution was used to determine that 140 hours of depuration are required to cleanse the grossly contaminated quahaugs.

Further studies in the area of shellfish contamination and depuration techniques should lead to significant reductions in public health risks. Several aspects of quahaug culturing and analysis need to be investigated in order to truly understand and control the transmittance of pathogenic diseases by the quahaug. More quality control analyses need to be conducted comparing the various fecal coliform detection techniques (MPN, Membrane Filtration, and ETCP) in order to determine which is the most reliable. In addition, research needs to be conducted to determine if a better indicator organism can be found. Monitoring of individual quahaug specimens could yield data more closely related to the actual maximum contaminant levels. Accordingly more depuration time intervals and additional experimental trials could solidify the reliability probabilities and in turn reduce health risks.

APPENDIX A: ENVIRONMENTAL PARAMETERS AT THE CONTAMINATION FACILITY

PARAMETER	DESIRED VALUE	RANGE
Reactor water temperature	18 C	11-24 C
Reactor salinity	20 o/oo	18-24 o/oo
Influent sewage flowrates	5.8 ml/min	5.5-6.5 ml/min
Saline water flowrate	0.8 ml/min	0.8-1.4 ml/min
Dissolved oxygen levels	> 4.0 mg/l	> 4.0 mg/l
Fecal coliform levels in water column	> 10000 CFU/100 ml	17000-34000 CFU/100 ml

APPENDIX B: INGREDIENTS FOR MacCONKEY AGAR AND m-FC BROTH

MacConkey Agar Ingredients:	grams/liter of water
Peptone	17
Proteose Peptone	3
Lactose	10
Bile Salts #3	1.5
Sodium Chloride	5
Agar	13.5
Neutral Red	0.03
Crystal Violet	0.001

m-FC Broth Ingredients:	grams/liter
Tryptose	10
Proteose Peptone #3	5
Yeast Extract	3
Sodium Chloride	5
Lactose	12.5
Bile Salts #3	1.5
Aniline Blue (water blue)	0.1

APPENDIX C: DEPURATION FACILITY AND OPERATIONAL GUIDELINES

I. Facility

Materials: All Depuration tanks, lines, pumps, etc. conveying water for the depuration process shall be constructed of relatively smooth impervious material, non-toxic to quahaugs or humans, corrosion resistant, and easily cleanable material.

Capacity: The depuration tank shall have a capacity of at least eight cubic feet per bushel of quahaugs. This is important as quahaugs must have adaquate water volumes to filter feed. Additionally, there should be space to hose away all feces and psuedofeces.

Baskets: The quahaug holding baskets are meshed and do not exceed three inches in depth. This is to allow full circulation of water, otherwise quahaugs on top might contaminate those on the lower levels.

Outlet: Each depuration tank is provided with an outlet drain of sufficient size to adequately drain away all detritus, quahaug feces, pseudofeces and sand rapidly.

Ultraviolet Light: Each depuration plant shall maintain all ultraviolet light units in good working order. The effectiveness of ultraviolet light as a bacteriocidal agent is determined to a large extent by bulb intensity and cleanliness. Experience has demonstrated that if the water quality criteria, (Table 2), are not exceeded, the ultraviolet light unit can effectively reduce the coliform content of the depurating water to 1 CFU/100 ml.

Flow Rate: The flow rate for any depuration tank is at least 63 ml/sec of water per bushel of quahaugs. This assures that there will be sufficient nutrients presented to the quahaugs to cause them to feed actively.

II. Operation

Cleaning: All quahaugs are washed and culled prior to and after the depuration process.

Depurating: All quahaugs shall be submerged in depuration water meeting the criteria of Table 2 below, and for an elapsed time of not less than 48 hours. (Longer times than 48 hours may be required to meet the bacteriological criteria of less than 17 CFU/100 gms of quahaug.) All quahaug depuration tanks are drained at the end of the first 24 hour period, and at each subsequent 24 hour period or at the end of the depuration process. The quahaugs are washed with seawater to remove detritus and feces. Then the tanks are refilled if depuration is to continue for an additional 24 hour period.

Environmental Factors: There are many factors in Appendix D, such as temperature, turbidity, pH, salinity and dissolved oxygen which may influence the feeding activity of shellfish. Therefore, it is necessary that these environmental factors be favorably controlled to assure that depuration will take place. During the winter, in the interval when the ambient water temperature falls below 10 C, it has been shown that the quahaugs growing in polluted waters no longer contain significant fecal coliform indicator organisms. This doesn't imply the quahaugs are clean but that fecal coliforms cannot be detected or used as an indicator of contamination. The operating and monitoring procedures as well as the standards contained in Table 1 are extracted from SUPPLEMENT I TO PART IV OF THE NATIONAL SHELLFISH SANITATION MANUAL OF OPERATIONS. "INTERIM STANDARDS FOR THE DEPURATION OF THE NORTHERN QUAHAUG, Mercenaria mercenaria." Published by the U.S. Department of Health, Education, and Welfare, Bureau of Disease Prevention and Environmental Control.

APPENDIX D: SEA WATER QUALITY - INFLUENT TO DEPURATION TANKS

Temperature*	15 C < T < 20 C
Turbidity*	20 units (Jackson Turbidity Units)
pH	7 < PH < 8.4
Salinity*	=20% of harvest value, but not less than 22%
Oxygen	5.0 mg/l < oxygen < saturation
Metallic Ions & Compounds	Not exceeding values of normal sea water
	Pesticides, Detergents, Dye stuffs and Radioisotopes
	Not exceeding values which would comply with regulations of th Food and Drug Administration, DHEW.
Marine Toxins	Not present in quantities which would be concentrated by shell to a level exceeding 80 ug/100g for PSP or 20 mouse units per grams of meats for Ciguatera-like poison.
Coliform MPN/100 ml	Maximum: 1.0
Flow rate of water	63 ml/sec/bushel
Time	48 hours

*Items marked with an asterisk may be subject to geographical and local differences. Unless the differences are shown experimentally, the limits of Table 2 shall be adhered to.

SUPPLEMENT 1 TO PART #IV OF THE NATIONAL SHELLFISH SANITATION MANUAL OF OPERATIONS. "INTERIM STANDARDS FOR THE DEPURATION OF THE NORTHERN QUAHAUG,"Mercenaria mercenaria" U.S. Department of Health, and Welfare, Bureau of Disease Prevention and Environmental Control.

APPENDIX E: EXPERIMENTAL DATA - CONTAMINATION EXPERIMENTS

Contamination 1		Contamination 2		Contamination 3	
Time (hr)	Contam (CFU/ quahaug)	Time (hr)	Contam (CFU/ quahaug)	Time (hr)	Contam (CFU/ quahaug)
0	0	0	0	0	0
0	0	0	0	0	16
0	0	0	0	0	324
0	0	0	0	0	0
0	0	0	0	0	55
0	0	0	0	0	30
12	259	12	1447	12	4113
12	1057	12	860	12	9201
12	367	12	244	12	12469
12	312	12	326	12	2538
12	44	12	142	12	398
12	1290	12	73	12	917
24	0	24	1058	48	125
24	482	24	51	48	411
24	0	24	316	48	237
24	368	24	1002	48	32
24	317	24	3001	48	102
24	943	24	183	48	32
48	1117	48	6910	84	0
48	1250	48	236	84	0
48	3371	48	5960	84	31
48	2208	48	8811	84	194
48	3103	48	5113	84	192
48	828	48	303	84	103
72	916	112	340		
72	442	112	434		
72	789	112	2244		
72	4804	112	240		
72	1258	112	368		
72	378	112	6511		
96	10542				
96	477				
96	623				
96	3816				
96	2012				
96	1080				
average salinity [o/oo]	19		23		21
average water temperature [C]	19.5		19		18

APPENDIX E CONTINUED: EXPERIMENTAL DATA - DEPURATION AND RELAYING

Depuration 1		Depuration 2		Relaying 1		Relaying 2	
Time (hr)	Contam (CFU/ quahaug)	Time (hr)	Contam (CFU/ quahaug)	Time (hr)	Contam (CFU/ quahaug)	Time (hr)	Contam (CFU/ quahaug)
0	10541	0	6909	0	10542	0	6910
0	476	0	236	0	477	0	236
0	623	0	5960	0	623	0	5960
0	3815	0	8811	0	3816	0	8811
0	2011	0	5113	0	2012	0	5113
0	1080	0	303	0	1080	0	303
12	21	12	544	12	232	12	378
12	218	12	2111	12	448	12	4466
12	204	12	3429	12	144	12	3157
12	225	12	714	12	1362	12	1447
12	32	12	3295	12	491	12	672
12	104	12	191	12	400	12	443
12	61	24	22	12	115	12	1973
24	236	24	930	48	9	12	4234
24	79	24	1781	48	10	24	12
24	146	24	170	48	0	24	175
24	86	24	143	48	0	24	668
24	314	24	375	48	482	24	1630
24	889	24	874	48	55	24	372
48	41	24	1442	48	0	24	238
48	45	48	9	48	0	24	29
48	100	48	78			24	1945
48	11	48	78			48	10
48	27	48	24			48	31
		48	51			48	277
		48	681			48	43
		48	134			48	20
		48	12			48	19
		72	20			48	124
		72	384			72	11
		72	19			72	66
		72	374			72	19
		72	10			72	9
		72	59			72	22
		72	28			72	8
		72	91				

APPENDIX F: COMPUTER PROGRAMS EXAMPLE OF DEPURATION AND RELAYING

```
DATA;
  INFILE deplart;
  INPUT T X L S;
  C = X * 100. / 6.;
  W = L - S;
  CLAM = C * W / 100;
  Y = LOG ( CLAM );
  OUTPUT;
TITLE 'Depuration 1';
PROC GPLOT;
  PLOT Y * T/ CAXIS=RED CTEXT=BLUE;
  SYMBOL1 I=RL V=SQUARE C=BLUE;
  LABEL T='Time in Hours';
GOPTIONS  DEVICE=TEK4105 CTITLE=RED;
PROC GLM;
   MODEL  Y=T;
PROC PRINT;
   VAR T X C CLAM L S W Y;
```

Reliability Probability vs Contamination

```
DATA;
  DO X = 10 TO 3600.00 BY 500;
  S  = 11.05;
  K  = 0.055;
  T  = 48;
  SD = 3847;
  COV = SD / X ;
  A   = 1 + COV ** 2;
  DEL2 = LOG ( A ) ;
  DEL = SQRT ( DEL2 ) ;
  LAM =  LOG ( X ) - 0.5 * DEL2 ;
  BETA = ( LOG ( S ) + K * T - LAM ) / DEL ;
  PS =  PROBNORM( BETA ) ;
  OUTPUT;
  END;
PROC PRINT;
  VAR T PS S K X SD COV DEL LAM BETA;
GOPTIONS CTITLE = blue;
PROC GPLOT;
  PLOT PS * X / CA = BLUE CT = BLUE VAXIS = 0 TO 1 BY 0.25 ;
  SYMBOL1 I = SPLINE C = blue;
  LABEL  PS = ' Reliability Probability ';
  LABEL  X  = ' Contamination Level ';
TITLE ' Reliability Probability';
FOOTNOTE 'Depuration Time = 48 Hours, Cov = 0.92';
```

Reliability Probability vs Time

```
DATA;
  DO T = 10 TO 175 BY 5;
  S  = 11.05;
  K  = 0.055;
  X  = 4195 ;
  SD = 3847 ;
  COV = SD / X ;
  A   = 1 + COV ** 2;
  DEL2 = LOG ( A ) ;
  DEL = SQRT ( DEL2 ) ;
  LAM =  LOG ( X ) - 0.5 * DEL2 ;
  BETA = ( LOG ( S ) + K * T - LAM ) / DEL ;
  PS =  PROBNORM( BETA ) ;
  OUTPUT;
  END;
PROC PRINT;
  VAR T PS S K X SD COV DEL LAM BETA;
GOPTIONS CTITLE = blue;
PROC GPLOT;
  PLOT PS * T / CA = BLUE CT = BLUE VAXIS = 0 TO 1 BY 0.25 ;
  SYMBOL1 I = SPLINE C = blue;
  LABEL  PS = ' RELIABILITY PROBABILITY ';
  LABEL  T  = ' DEPURATION TIME IN HOURS ';
TITLE ' Reliability Probability';
FOOTNOTE 'Contamination Mean = 4195, Standard Deviation = 3847';
```

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