University of New Hampshire

University of New Hampshire Scholars' Repository

PREP Reports & Publications

Institute for the Study of Earth, Oceans, and Space (EOS)

12-2002

Shellfish and Water Quality Monitoring Activities That Support the New Hampshire Estuaries Project

Stephen H. Jones
University of New Hampshire, Stephen.Jones@unh.edu

Richard Langan
Cooperative Institute for Coastal and Estuarine Environmental Technology, Richard.Langan@unh.edu

Follow this and additional works at: https://scholars.unh.edu/prep

Part of the Marine Biology Commons

Recommended Citation

Jones, Stephen H. and Langan, Richard, "Shellfish and Water Quality Monitoring Activities That Support the New Hampshire Estuaries Project" (2002). *PREP Reports & Publications*. 277.
https://scholars.unh.edu/prep/277

This Report is brought to you for free and open access by the Institute for the Study of Earth, Oceans, and Space (EOS) at University of New Hampshire Scholars' Repository. It has been accepted for inclusion in PREP Reports & Publications by an authorized administrator of University of New Hampshire Scholars' Repository. For more information, please contact Scholarly.Communication@unh.edu.

310.UNH.02

WQ-36

New Hampshire Estuaries Project
152 Court Street, Suite 1, Portsmouth, NH 03801
603/433-7187 • FAX 603/431-1438 • www.state.nh.us/nhep

SHELLFISH AND WATER QUALITY MONITORING ACTIVITIES THAT SUPPORT THE NEW HAMPSHIRE ESTUARIES PROJECT

A Final Report to

The New Hampshire Estuaries Project

Submitted by

Dr. Stephen Jones
Jackson Estuarine Laboratory

Dr. Richard Langan
The Cooperative Institute for Coastal and Estuarine Environmental Technology

University of New Hampshire
Durham, NH 03824

December, 2002

This report was funded in part by a grant from the Office of State Planning, New Hampshire Estuaries Project, as authorized by the U.S. Environmental Protection Agency pursuant to Section 320 of the Clean Water Act.

Table of Contents

Title Page 1
Table of Contents 2
List of Tables 3
List of Figures 3
Executive Summary 4
Project Goals and Objectives 4

INVESTIGATION OF NATURAL PURGING OF MICROBIAL CONTAMINANTS IN SOFTSHELL CLAMS IN HAMPTON/SEABROOK HARBOR, NH 5
- Introduction 5
- Methods 5
- Results and Discussion 5
- Conclusions and Recommendations 7

EXPOSURE OF NEW HAMPSHIRE SHELLFISH TO TOXIC CONTAMINANTS 8
Introduction 8
- Methods 8
- Results and Discussion 9
- Conclusions and Recommendations 9

RIBOTYPING OF *Escherichia coli* STRAINS TO TRACK SOURCES OF FECAL CONTAMINATION 10
Introduction 10
- Methods 10
- Results and Discussion 12
- Conclusions and Recommendations 13

RECOMS WATER QUALITY MONITORING PROGRAM 14
- Introduction 14
- Methods 14
- Results and Discussion 15
  - Salmon Falls River dissolved oxygen study 15

References 22
Appendices 23

List of Tables

Table I-1. Hampton Harbor clam purging study results for 3 storm events............... 6
Table IV-1. Deployment dates and water depths in the Salmon Falls River............... 14

List of Figures

Figure IV-1. Map of the Great Bay Estuary showing the locations of in-situ 18
monitoring stations

Figure IV-2. The EM 550 telemetry buoy used for sonde deployment in mid-Great Bay.. 18

Figure IV-3. A piling mounted deployment of a sonde and telemetry electronics. 19
The gray tube parallel to the piling holds the sonde. The telemetry radio (gray box), solar panel and multi-directional antenna are mounted on the top of the piling.

Figure IV-4. The online dialogue box that appears when "Real-time data" is selected..... 20
on the CICEET website. The station markers are links that allow selection of a sonde location to view the most recent data. There are also links to metadata and a Disclaimer warning the visitor that the data has not been reviewed for accuracy.

Figure IV-5. An example of the display template for real-time data on the CICEET 21
website.

Executive Summary

Four different studies were conducted to address important issues and data gaps in the NHEP Monitoring Program. Whereas the different studies had varying levels of success, the work collectively had advanced the level of understanding about the subjects addressed. For determining the time required for clams to eliminate bacteria from their tissue, more work is needed on more storm events. Data on toxic chemicals in shellfish sampled in 2000 from NH waters are still not available from the analytical labs. Ribotyping for identifying sources of fecal contamination in NH surface waters has been significantly advanced by the work associated with this project. Detailed results for related projects supported by this project can be found in reports for those projects. Finally, deployment of data sondes around the Great Bay Estuary has been useful for determining best methods and equipment to use to enable continuous real-time monitoring throughout deployment times. These results will provide unique data that can help to provide a better understanding of variations in water quality, especially dissolved oxygen.

Project Goals and Objectives

To meet the variety of monitoring goals delineated in the NHEP Monitoring Plan, different types of activities need to be conducted. Probably the most important issue included in the Monitoring Plan is water quality. Many current and past monitoring programs and research projects have addressed a variety of important water quality questions, and information for many areas of concern is adequate to be used as a basis for focusing management activities to deal with problems. However, there are still gaps in information that are crucial for establishing a comprehensive picture of the important water quality problems facing coastal New Hampshire. The following sections describe the details of four separate studies that focused on different water quality issues that have been identified as priority issues and for which information is needed. These include:

1.) the time required for softshell clams to purge microbial contaminants from their tissue following rainfall events;

2.) determination of the levels of bioexposure to toxic metals and organic compounds in softshell clams and oysters;

3.) ribotyping analysis of *Escherichia coli* strains for tracking sources of fecal-borne microbial contaminants;

4.) continuous monitoring of water quality (temperature, salinity, dissolved oxygen, oxygen saturation, depth, pH) at five stations in Great and Little bays.

Because there were four separate investigations that were part of this overall project, the report is organized to have four separate smaller reports in the order of the numbered Objectives that each include separate Methods, Results and Discussion, Conclusions and Recommendations sections.

I. INVESTIGATION OF NATURAL PURGING OF MICROBIAL CONTAMINANTS IN SOFTSHELL CLAMS IN HAMPTON/SEABROOK HARBOR, NH

Introduction

Shellfish harvesting in New Hampshire is often limited by elevated levels of fecal-borne bacterial contaminants. The target bacteria are various indicators, specifically fecal coliforms for shellfishing areas, that indicate the potential presence of human pathogens that can be associated with sewage and other sources of fecal contamination. One of the main mechanisms by which these bacteria reach surface waters is in association with runoff from storm events. Thus, bacterial concentrations in surface waters often increase following storm events, and where these levels exceed standards, shellfishing is closed down. The length of time that closures last in NH is 5 days. However, there is evidence from studies in other states that less time may be adequate for shellfish to purge bacteria from their tissue to lower levels as were present prior to the contamination event. It is this question that was the focus of this study.

Methods

Clam samples were collected from the two study sites, Middle Ground and Common Island, in Hampton/Seabrook Harbor before, during and following a significant rainfall event in September, 2001. Samples were collected at low tide the day before, the day of and three days after the rainfall event. They were transported in refrigerated containers to JEL, stored overnight in a refrigerator and included ≥20 clams from each site on each date. The twenty clams were separated into subsamples of ten individuals with equal size distributions, shucked, weighed, diluted, homogenized and analyzed for fecal coliforms and *Escherichia coli*. Two previous storm events had been studied during 2000, and the results are included for comparison.

Results and Discussion

The MPN estimates for fecal coliforms and *E. coli* in clam tissue during the 3 sample events are summarized in Table 1; the raw data for these events is summarized in Appendix I. The MPN estimates represent arithmetic averages and standard deviations for four measurements, the duplicate analyses of each of two 10-clam samples for each site. It appears from the data for this single storm that contamination of clams from the storm event may not have been fully purged 3 days after the storm. No samples were collected after the 3 days because another significant storm event occurred on the 4th day after the storm.

Table I-1. Hampton Harbor clam purging study results for 3 storm events.

1st storm: September 27, 2000

Fecal coliforms				***E. coli***			
Site	Timing	Average	Std. Dev.	Site	Timing	Average	Std. Dev.
CI	prior day	**550**	355	CI	prior day	**465**	470
	storm day	**280**	85		storm day	**220**	0
	2 days after	**1530**	1800		2 days after	**1530**	1800
	4 days after	**3000**	570		4 days after	**2500**	1270
MG	prior day	**600**	420	CI	prior day	**600**	420
	storm day	**250**	240		storm day	**190**	210
	2 days after	**260**	255		2 days after	**240**	280
	4 days after	**4100**	2690		4 days after	**3230**	3920

2nd storm: October 6, 2000

Fecal coliforms				***E. coli***			
Site	Timing	Average	Std. Dev.	Site	Timing	Average	Std. Dev.
CI	prior day	**160000**	214000	CI	prior day	**160000**	214000
	storm day	**6000**	0		storm day	**3100**	2400
	3 days after	**870**	750		2 days after	**870**	750
	4 days after	**600**	0		4 days after	**600**	0
MG	prior day	**250000**	99000	CI	prior day	**250000**	99000
	storm day	**14000**	11300		storm day	**14000**	11300
	3 days after	**2200**	1700		2 days after	**1600**	850
	4 days after	**2100**	710		4 days after	**2100**	710

3rd storm: September 21, 2001

Fecal coliforms				***E. coli***			
Site	Timing	Average	Std. Dev.	Site	Timing	Average	Std. Dev.
CI	prior day	**5400**	849	CI	prior day	**5400**	849
	storm day	**2100**	707		storm day	**2100**	707
	3 days after	**21000**	15556		3 days after	**21000**	15556
MG	prior day	**1000**	0	CI	prior day	**800**	283
	storm day	**1200**	283		storm day	**800**	283
	3 days after	**4387**	2527		3 days after	**3887**	3234

Conclusions and Recommendations

The results for the 2001 and 2000 storm events were expected to provide the NH Shellfish Program with information upon which they could more accurately base the closure time following rain events for harvesting clams in NH. According to the NH Shellfish Program 2001 Annual Report (Nash, 2002), the data were difficult to interpret because of high levels of bacterial contamination in clam tissue samples collected prior to two of the storm events. This made it impossible to determine the effect of what was expected to be the contamination associated with the storm event runoff, so any apparent purging of bacteria was difficult to discern. The conclusion of the Shellfish Program is that more sampling is needed to garner enough data to determine the kinetics of purging of bacteria from clams following a storm-related contamination event.

II. EXPOSURE OF NEW HAMPSHIRE SHELLFISH TO TOXIC CONTAMINANTS

Introduction

Shellfish growing areas are classified to protect humans from exposure to a variety of potentially harmful substances. The most frequent limiting factor is bacterial contamination levels, but toxic chemicals are also a concern. In coastal New Hampshire, cities and towns have had a rich history of industries that were also sources of toxic chemicals released into the estuarine waters. Wastewater treatment facilities (WWTF) and urban runoff have also been potentially significant sources of toxic chemicals. Most WWTFs and industries are closely regulated to limit discharges of toxic chemicals like a variety of trace metals and anthropogenic organic chemicals. Current documented significant sources of toxic chemicals include atmospheric deposition of mercury, urban runoff and resuspension of historically contaminated sediments. These chemicals can be taken up into shellfish tissue via filter feeding and accumulated over time to potentially toxic levels. Consumption of contaminated shellfish over time can also result in buildup of toxic chemicals in humans to harmful levels.

Since 1991, blue mussels in NH have been monitored for toxic chemicals as part of the Gulf of Maine Gulfwatch program. In 1998, NHDES expanded this program to include more sites and more frequent sampling in NH. Various studies and short-term monitoring programs over the past two decades have also provided useful information on shellfish contamination. However, long-term monitoring of shellfish species other than blue mussels is a significant gap because both clams and oysters harvested from NH waters are more commonly consumed by humans than blue mussels from NH. The purpose of this study was to initiate monitoring of oysters (*Crassostrea virginica* and softshell clams *(Mya arenaria*) from popular harvesting areas.

Methods

All sampling has occurred for this study. Oysters (*Crassostrea virginica*) were collected from the beds at Nannie Island in Great Bay and softshell clams *(Mya arenaria*) were collected from the Middle Ground and Yankee Co-op clam flats in the Hampton/Seabrook Harbor. The sample collection time coincided with mussel sample collections conducted as part of the NH Gulfwatch program. At each site, one shellfish bed was targeted and 25 individual shellfish were collected from four distinct areas within the bed. The four areas were mapped at the time of sampling. The four replicate shellfish samples were treated as separate samples. All samples were placed in plastic mesh cages inside of coolers containing frozen ice packs and transported immediately to the Jackson Estuarine Laboratory.

The shellfish samples were processed for toxic contaminant analyses. All external debris was removed from individual shellfish, then they were rinsed in clean water from the sample location water prior to shucking. Twenty individual shellfish of uniform size were chosen and dimensions (length, height, width) and wet weight of tissue were measured for each individual in the first three replicate samples shucked for metals analysis to determine condition index. Shellfish shucked for metals analysis involved use of acid-washed oyster or clam knives to open

the shells, then acid-washed plastic knives to quantitatively remove tissue and deposit it into acid-washed Mason jars. The jars were covered with Saran wrap and lids screwed on tight. For toxic organic chemical analysis, shellfish were shucked entirely using organic solvent cleaned metal knives, and the tissue deposited into solvent cleaned Mason jars. The jars were covered with solvent-rinsed aluminum foil and lids screwed on tight. All sample Mason jars were labeled and immediately frozen at –20°C. Label information included sample date, site and replicate number.

Results and Discussion

Tissue samples have been shipped (in early 2002) to the State of Maine Health and Environmental Testing Laboratory for inorganic (metal) contaminant analyses. Inorganic contaminants include silver, aluminum, cadmium, copper, nickel, lead and mercury. Tissue samples have also been shipped (in early 2002) frozen to the Environment Canada ECB Laboratory in Moncton, NB for organic contaminant analyses. Organic contaminants include 24 different PAHs (naphthalene up to benzo [g,h,I] perylene), 24 different PCB congeners, and 16 different chlorinated pesticides.

To date (December, 2002) we have not received any of the analytical results. We anticipate completion of these analyses in January, 2003 (metals) and March, 2003 (organics).

Data on toxic contaminant concentrations and condition index will be entered into spreadsheets. Arithmetic means and standard deviations will be determined for the four replicate samples from each site for comparisons between sites. PAH, PCB and chlorinated pesticides data will be reported as totals for each of these three contaminant types. Graphs of the data will be used to show spatial trends and for comparisons to Gulfwatch and Mussel Watch mussel data.

Conclusions and Recommendations

None, pending receipt of analytical results

III. RIBOTYPING OF *Escherichia coli* STRAINS TO TRACK SOURCES OF FECAL CONTAMINATION

Introduction

Surface waters are classified according to levels of bacterial contaminants to protect human health. The target bacteria are fecal-borne, and elevated levels are indicators of fecal contamination that may contain microbial pathogens. Swimming or consumption of shellfish from waters with elevated levels of fecal indicators is thus potentially harmful to humans. NH state agencies responsible for classifying surface waters have been effective at successfully protecting human health as well as in their efforts to reduce contamination levels by eliminating obvious sources. However, surface waters in coastal NH remain limited in some areas where there are no obvious sources. Efforts to improve water quality in these areas has been limited by the inability to differentiate between sources of the bacteria that contaminate waters using traditional enumeration methods.

Recently research has resulted in the development of methods that hold promise for identifying sources of bacteria in surface waters. These Microbial Source Tracking (MST) methods include a variety of approaches, including ribotyping. Ribotyping involves determining the DNA pattern that results from electrophoresis of digested DNA that is probed to detect only pieces that contain ribosomal RNA DNA genes. The patterns for water sample isolates are then compared to patterns for bacteria from known fecal sources, and the patterns for source species that match most closely to water sample patterns may be considered the most likely source species of the contaminant strain. The objective of this project was to increase the number of source species isolates from which ribopatterns could be added to a state database, and increase the number of isolates from water samples collected in the Varney Brook and Hampton Harbor watersheds.

Methods

The procedures used for ribotyping *E. coli* isolates for this study are based to a large extent on those of Parveen et al. (1998) and more detailed protocols developed and kindly provided by Dr. Peter Hartel of the University of Georgia. The *E. coli* isolates were stored in cryovials and thawed and re-cultured onto trypticase soya agar (TSA). Some of the isolates could not be re-cultured. Cultures on TSA were incubated overnight at room temperature (~20°C). Some of the resulting culture was transferred to duplicate cryovials containing fresh glycerol/DMSO cryo-protectant media for long-term storage at -80°C.

E. *coli* isolate cultures were used for DNA extraction. Extraction was performed using Puregene (Gentra) kits and the manufacturer's instructions. Briefly, 5 ml of overnight cultures was centrifuged at 10,000 rpm for 5 minutes to concentrate the cells from the liquid medium. 300 μl of lysis solution was added to the pelleted cells, mixed and incubated for 5 minutes at 80°C. 1.5 μl of Rnase solution was added then incubated at 37°C for 15-60 minutes. A protein precipitation solution was added, then the tube contents were mixed and centrifuged at 13,000 x

g. The supernatant was transferred into a clean tube. Isopropanol and ethanol were added to remove DNA, and a hydration solution was added to re-hydrate the DNA at 65°C for 1 h, then stored at 4°C.

The resulting DNA for each isolate was quantified by fluorometer (Turner TD700) using Hoesct's dye and calf thymus DNA at 100 μg/ml as a standard. DNA concentrations were recorded on the vials, in a lab notebook and in a computer database.

Restriction of the DNA was conducted using EcoRI (Sigma) and the manufacturer's instructions. Briefly, 2 μg of isolate DNA, 2 μl of the appropriate 1x buffer and 0.5 μl of EcoRI restriction enzyme were added to a 0.5 ml tube. Autoclaved diethylpyrocarbonate (DEPC; Sigma) water (0.1%) was added (~16 μl) to bring the total volume in the tube to 20 μl. The mixture was incubated overnight at 37°C. The next morning, 0.2 μl of EDTA was added to stop the reaction.

Restriction-digested DNA was separated by sub-marine gel electrophoresis (EC App. Corp.) in Tris-acetate-EDTA (TAE) buffer. Volumes (12 μl) of positive and negative control, isolate and standard samples were loaded into 0.8% (Nu-Seive 3:1) agarose gels. Denaturation, neutralization and Southern blotting were performed using a Vacugene XL (Amersham). When the transfer was complete the membrane was washed, placed on blotting paper then crosslinked (Spectrolinker XL1000).

A probe was made as follows. In a 2 ml tube, 20 μl of 16S 23S rRNA (Sigma), 2 μl of DEPC water, 2 μl of reverse transcriptase (Sigma), 8 μl of 5x buffer, 4 μl of dNTP (Roche) and 4 μl of hexanucleotide mix (Roche) were mixed together. The solution was incubated overnight at 42 °C.

Prehybridization was performed in an Isotemp (Fisher) hybridization oven at 42°C for 2 h, using 30 ml hybridization solution per membrane. The probe was denatured by boiling for 10 minutes and rapid cooling in an ethanol-ice bath. The probe was added to 30 ml pre-warmed hybridization solution and incubated for 30 minutes at 68°C. The original hybridization solution was poured off the membranes and the probe solution was added and incubated overnight at 42°C.

For probe detection, the membranes were then subject to a series of stringency washes. Blocking was done at room temperature for 60 minutes and the solution was poured off. Freshly prepared anti-DIG solution was added, incubated for 30 minutes at room temperature and poured off. Tween buffer was added and incubated for 15 minutes at room temperature. Detection buffer (Roche) was added and incubated for 2 minutes. The membranes were then placed into an acetate sheet and 20 drops of CDP-Star (Roche) was added and incubated at room temperature for 7 minutes.

Image Digitization, Optimization and Band Identification

Processed membranes were placed into the darkroom of an Epi Chem (UVP) chemiluminescence imager and the image was digitized with a 12-bit CCD camera. Each image was converted to 16-bit data, inverted and the display range set with LabWorks software (UVP).

The images were transferred into GelComparII (Applied-Maths) analytical software and the lanes for each gel were visually demarcated. The bands in lanes containing the standard were

labeled and entered into the memory for optimization of gel pattern images. Densiometry data were processed for band identification.

Statistical Analysis

Individual water sample isolate data were selected from the computer database for identification of source species. The entire New Hampshire library of isolate profiles for known source species was used for comparison with each unknown isolate, excluding those with <4 bands. Similarity indices between the unknown isolates and the known source isolates were determined by using Dice's coincidence index, using 2% for tolerance and optimization settings. More stringent tolerances (0.5-1.5%) were used to enable differentiation between profiles that initially yielded matches with the same % similarity coefficients using 2% tolerance. The source species profile with the best similarity coefficient at a more stringent tolerance was accepted as the source species. Cluster analysis was used to determine the relationships among isolates from the same sources and the same sites, as well as banding patterns that were identical for different isolates.

Results and Discussion

All of the bacterial isolates from the NHDES sampling programs thorough October 31, 2001, have been purified, confirmed as *E. coli*, and their DNA extracted. The samples are all from Varney Brook in the Bellamy River watershed or Hampton/Seabrook Harbor. All of these isolates were fully processed and ribotype patterns were determined. The completed data include all isolates from Hampton/Seabrook Harbor.

There have been 632 source speciesi solates from New Hampshire that have been processed using the "manual" ribotyping procedures, yielding a source species database consisting of 249 isolates. As water sample isolate patterns have been fully processed, their source species have been determined by comparison to patterns for known source species using the NH database and GelComparII computer software.

There were ~600 water sample isolates from Hampton/Seabrook Harbor that were processed and were confirmed as *E. coli*, yielding useful ribotype patterns for 390 isolates. The patterns have all been analyzed and putative source species identifications have been made. The overall results suggest that humans are the most significant type of source species in the Harbor, followed in significance by wildlife species. Livestock, pets and avian species were much less significant.

There were 316 water sample isolates from the Varney Brook watershed samples that were also processed and were confirmed as *E. coli*, yielding useful ribotype patterns for 192 isolates. The patterns have all been analyzed and putative source species identifications have been made. The overall results suggest that humans are the most frequently identified source species, followed by cows. According to source species types, livestock (cows, horses) were the most significant type of source species, followed by wildlife (raccoons, foxes, deer) then humans. Avian and pet species were much less significant.

Conclusions and Recommendations

The results of this and the different related projects have provided environmental managers with valuable insights about which potential sources of bacteria contaminants are most significant in two important coastal watersheds. However, uncertainties remain with the approach used because identifications of source species were based on matching at only 80-80% similarity. Improved accuracy is expected as JEL and NHDES gain experience with this new method.

The JEL laboratory purchased a RiboPrinter (in July, 2002), which integrates all lab steps in one machine and will eventually speed up processing time and reduce costs. The source species database has been expanded to >720 isolates with even more new patterns being added to it each day. Future ribotyping studies will take advantage of this equipment and streamlined approach to more rapidly and accurately identify source species.

IV. RECOMS WATER QUALITY MONITORING PROGRAM

Introduction

In 1995, The National Estuarine Research Reserve initiated the Systemwide Monitoring Program at 23 Reserves around the country. The program consisted of deploying two YSI 6000 datasondes that measure temperature, salinity, dissolved oxygen, oxygen saturation, depth, and pH continuously at half-hour intervals. An annual grant from the Great Bay National Estuarine Research Reserve to Dr. Richard Langan at the Jackson Estuarine Laboratory supports the field, lab and data management for two sondes and a meteorological station at the Jackson Estuarine Laboratory. In 1997, a project funded by the NOAA/UNH CICEET program supported technology enhancement and expansion of this program which included upgrade to the 6600 series sondes that can also measure turbidity and chlorophyll a fluorescence; radio telemetry for real-time data transmission, the addition of three new stations, and by August 2001, live data streaming on the internet. This program, called RECOMS (Remote Estuarine Contaminant Monitoring System) has equipped the two NERR sampling sites (mid-Great Bay and the Squamscott River) and added additional stations in the tidal portions of the Lamprey, Oyster and Bellamy rivers (Figure 1). The monitoring stations collect and transmit temporally intensive data on water column conditions at representative sites throughout the estuarine system, providing a comprehensive dataset on estuarine-wide water column conditions. The data generated by the RECOMS stations has intrinsic value for understanding short and long term changes water in water quality and is of tremendous value for biological studies, other NHEP monitoring activities and modeling efforts. While the technology upgrades of the NERR SWMP program was appropriate for CICEET funding, the program has a policy whereby it will not provide support for existing monitoring programs. This project is a part of the effort to maintain the sondes at the designated sites for longer term monitoring.

Methods

From April to early December 2002, YSI 6600 datasondes were deployed continuously at five sites in the Great Bay Estuary. The sites included the tidal portions of the Lamprey, Squamscott and Oyster Rivers, the UNH pier at Fort Point in Portsmouth Harbor and a reference site in the middle of Great Bay (Fig. 1). A sixth sonde was deployed in the upper tidal portion of the Salmon Falls River from July 17 to October 28 in order to obtain temporally intensive DO data for this location. The deployment method at each of the sites was determined by physical characteristics (e.g. depth), compatibility with existing uses (e.g. navigation), location of attachment structures for telemetry equipment and budgetary constraints. The mid-Great Bay sonde was deployed in a telemetered buoy (YSI model EM 550) (Fig. 2). The buoy deployment allowed for the greatest flexibility for placement, but required the deployment of a pressure sensor to record tidal stage because the buoy and sonde remained at the surface regardless of tidal stage. The Squamscott, Lamprey, and Portsmouth Harbor sondes were deployed in piling-mounted, vertical PVC pipes with the telemetry equipment attached to platforms fixed to the

pilings (Fig. 3). The pipes were perforated to allow adequate water flow. Due to shallow depths and narrow channels, the Oyster River and Salmon Falls River sondes needed to be deployed with the least amount of vertical expression above bottom. They were deployed horizontally in perforated PVC pipes with four weighted "legs" keep them above bottom. The Oyster River sonde was hard-wired to a radio telemetry station on shore (approximately 25 meters away).

Sondes at the Oyster River, Great Bay and Squamscott River were equipped with telemetry radios that transmitted data in real-time to the Jackson Estuarine Laboratory. VHF radios were tried at the Lamprey and Fort Point sites, however, communications could not be established. At the Salmon Falls site, it was logistically impossible to use radio equipment so the sonde was deployed solely in unattended operations (record only) mode.

LabView software was successfully used to read the binary data generated by YSI EcoWatch DOS software and script was written to create a new html file each time the sondes reported new data. Data can be obtained via the CICEET website (http://ciceet.unh.edu) by selecting "Great Bay Data" and selecting the "Real-Time Data" link under "In-Situ Instruments". A map image appears (Figure 4) and the most recent data is displayed by selecting the desired location (Figure 5).

All data collected has undergone a preliminary review and final QA/QC will be completed in January 2003. Electronic copies of the data will be provided to the NHEP Coastal Scientist, Philip Trowbridge.

Results and Discussion

Salmon Falls River dissolved oxygen study

In 1993, a study conducted by Paul Mitnik (Mitnik 1994) of the Maine DEP identified areas of hypoxia in the tidal portion of the Salmon Falls River. The most troublesome spot was a small deep area just below Hamilton House approximately 0.5 miles south of the last empoundment in South Berwick, Maine. In a three year study of water quality in the seven tidal rivers entering Great Bay conducted by Jones and Langan (1996), very high (70 µg/L) chlorophyll concentrations were measured in the tidal portion of the Salmon Falls River, however, monthly low tide measurements of dissolved oxygen over a three-year period did not indicate low D.O. conditions. The purpose of this study was to revisit areas that Mitnick (1994) identified as having low dissolved oxygen conditions to collect continuous dissolved oxygen data during the summer months. A moored YSI 6600 multiparameter sonde was used for five deployments beginning on July 17, 2002 at the location identified by Mitnick (1993). Deployments were made in the deep hole as well as in the surrounding shoal area. Deployment dates and water depths are presented in Table 1.

Date Deployed	Date Retrieved	Minimum Depth	Maximum Depth
July 17, 2002	August 5, 2002	0.63 m	3.38 m
August 5, 2002	August 21, 2002	1.14 m	3.93 m
August 21, 2002	September 6, 2002	0.04 m	2.26 m
September 6, 2002	October 1, 2002	0.60 m	3.50 m
October 1, 2002	October 28, 2002	0.83 m	3.75 m

Table IV-1. Deployment dates and water depths in the Salmon Falls River.

Data for oxygen saturation as well as graphical analysis of oxygen saturation relative to depth are presented in Figures 6 through 17. Several of the deployments were made with instruments equipped with a chlorophyll (fluorescence) probe and these data are also presented.

Despite passing pre-deployment calibrations, oxygen probe failure occurred during the 7/17/02 to 8/5/02 and the 8/05/02 to 8/21/02 deployments so all information about oxygen conditions during those time periods was lost (Figs. 6 and 8). The sonde was moved from its initial position to deeper water four days after deployment on 7/17/02 and a large spike in oxygen saturation was observed on 7/21/02. While moving the sonde appears to have caused the spike and subsequent upward drift of oxygen saturation, the probe was measuring saturation of 150% prior to the move (Fig. 7), so it is likely that the probe failed almost immediately after deployment. Similarly, during the 8/5/02 to 8/21/02 deployment, the probe appears to have failed within 24 hours of deployment (Fig. 8). Though there appears to be some functional recovery on 8/12 based on the relationship of tidal height and percent saturation, the saturation values are far out of range (Fig. 9). A fluorescence probe was attached to the sonde during the 8/5/02 deployment and while it sheds little light on oxygen conditions, it does illustrate that fluorescence peaks coincide with low tide (Fig. 10).

The oxygen probe appears to have functioned properly during the subsequent three deployments. From 8/21/02 to 9/5/02, oxygen saturation ranged from 60% to 160% (Fig. 11) and the variation appears to be driven by a combination of tide and time of day (Fig. 12). From 9/5/02 to 10/1/02 a similar range of saturation values were observed, though the lower saturation values were recorded after 9/21 where there appears to be some fouling related downward drift (Fig. 13). As with the prior deployment, there appears to be the same tidal and day-night influence on dissolved oxygen (Fig. 14).

During the final deployment from 10/1/02 to 10/28/02, dissolved oxygen saturation ranged between 80% and 120%, with two peaks in the 160% range on 10/14/02 and 10/15/02 (Fig. 15). The early part of the deployment shows the greatest daily and tidal variation, which seems to be somewhat dampened from 10/17 to the end of the deployment on 10/28 (Fig. 16). Fluorescence values observed for this deployment clearly show the relationship of tide to water column fluorescence (Fig. 17).

While no hypoxic conditions were observed during this study, probe failure during the first two deployments resulted in a loss of data for the most critical period of the summer (highest temperatures). Failure of the YSI oxygen probes occurred frequently during 2002, and replacement of all probes purchased prior to 2002 is warranted.

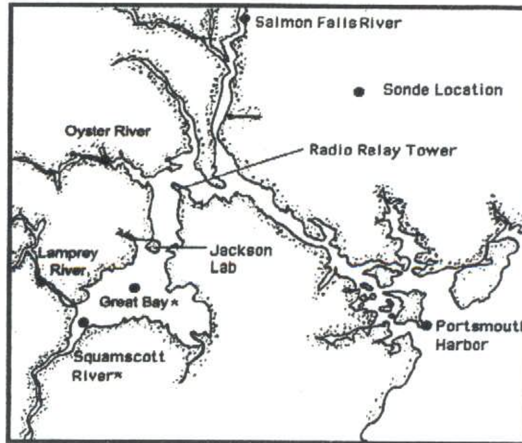


Figure IV-1. Map of the Great Bay Estuary showing the locations of in-situ monitoring stations

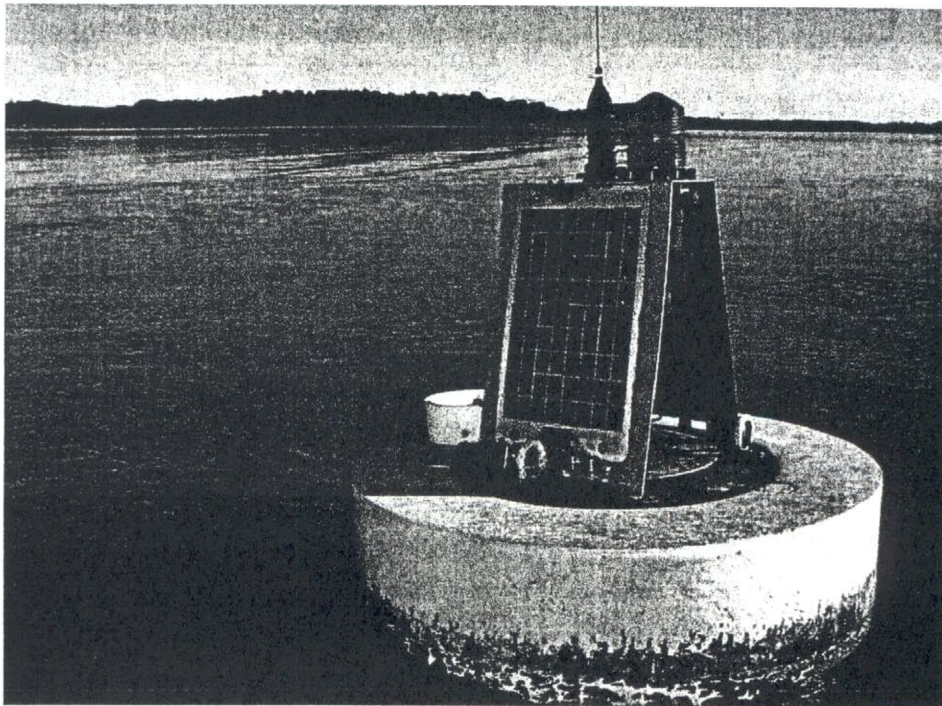


Figure IV-2. The EM 550 telemetry buoy used for sonde deployment in mid-Great Bay

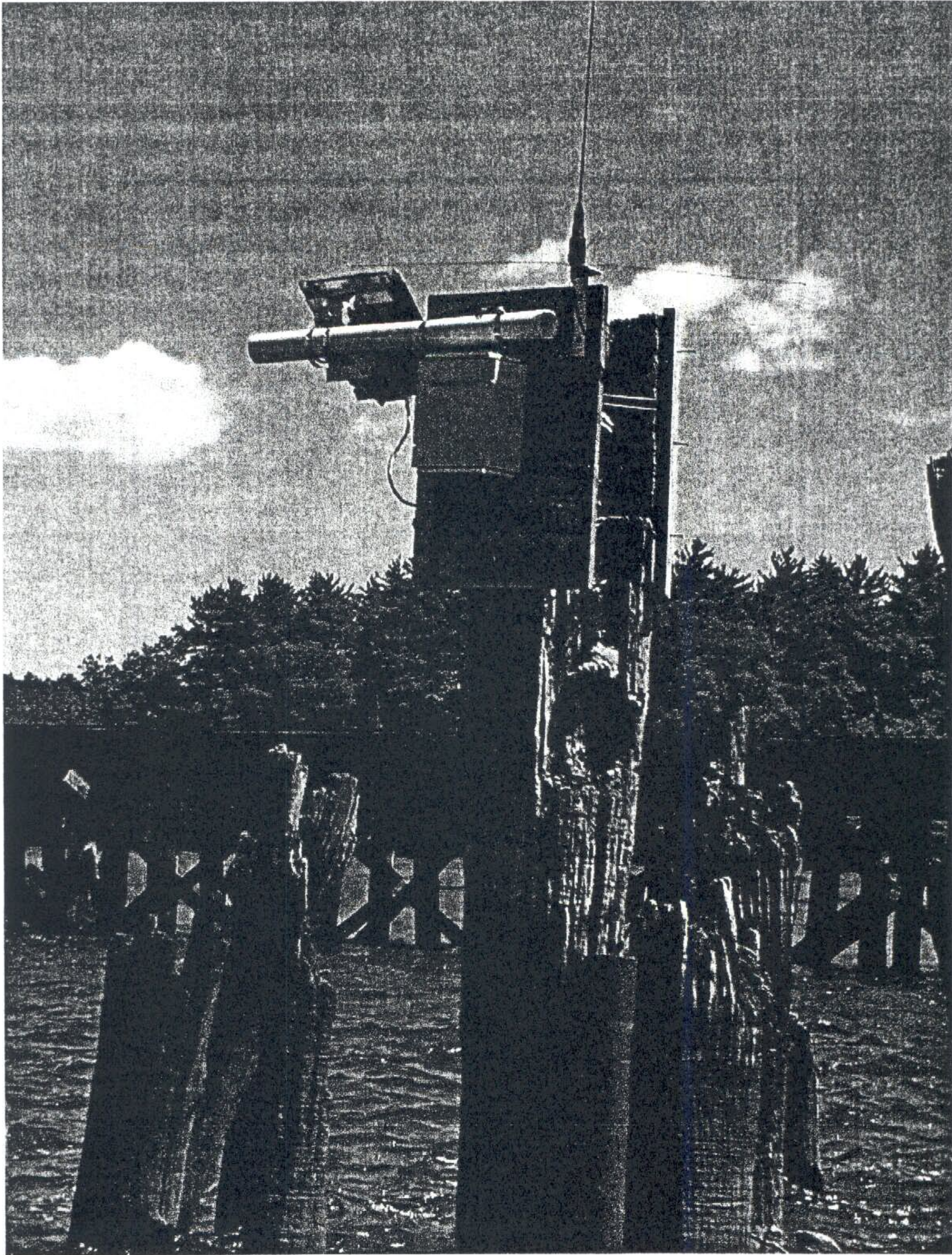
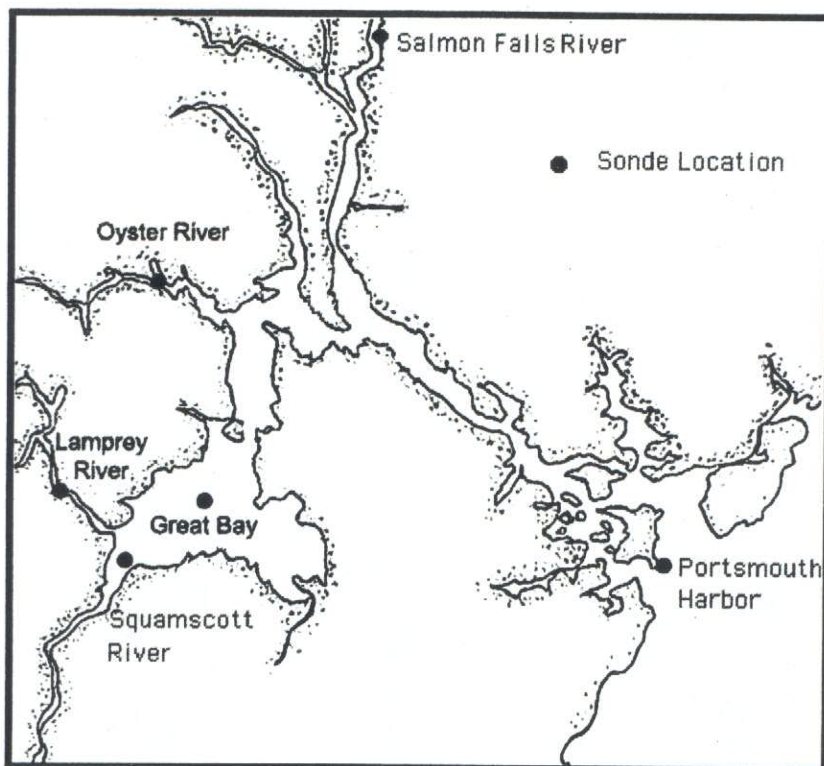


Figure IV-3. A piling mounted deployment of a sonde and telemetry electronics. The gray tube parallel to the piling holds the sonde. The telemetry radio (gray box), solar panel and multi-directional antenna are mounted on the top of the piling.

Great Bay monitoring data

Click on a buoy location below to see real time data*.
Metadata



*Disclaimer

Figure IV-4. The online dialogue box that appears when "Real-time data" is selected on the CICEET website. The station markers are links that allow selection of a sonde location to view the most recent data. There are also links to metadata and a Disclaimer warning the visitor that the data has not been reviewed for accuracy.

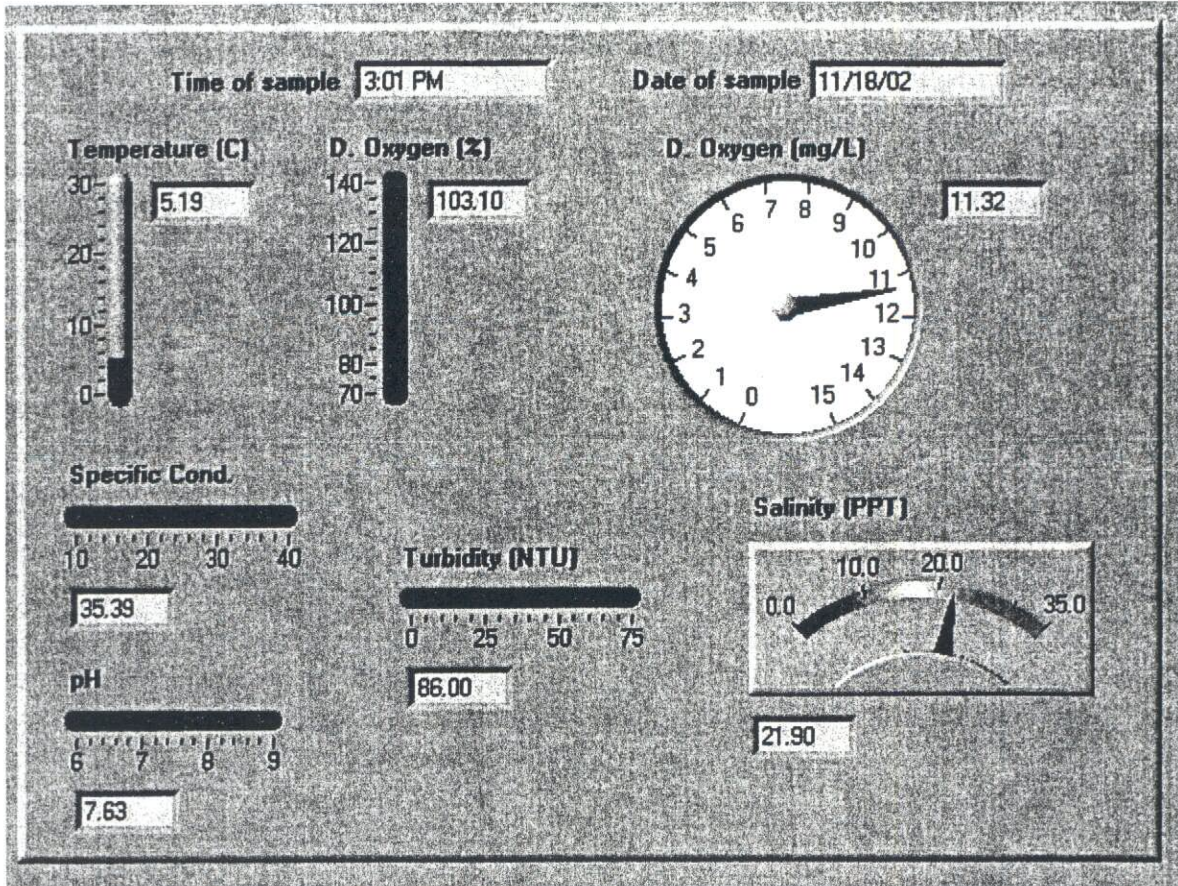


Figure IV-5. An example of the display template for real-time data on the CICEET website.

REFERENCES

Nash, C. 2002. New Hampshire Department of Environmental Services Shellfish Program: 2001 Annual Report. R-WD-02-3.. New Hampshire Department of Environmental Services, Concord, NH. 48 pp.

Appendix

INVESTIGATION OF NATURAL PURGING OF MICROBIAL CONTAMINANTS IN SOFTSHELL CLAMS IN HAMPTON/SEABROOK HARBOR, NH

Table A. Hampton Harbor clam purging study: 1st storm 24

Table B. Hampton Harbor clam purging study: 2nd storm ... 26

Table C. Hampton Harbor clam purging study: 3rd storm ... 28

Table A. Hampton Harbor clam purging study: 1st storm

Sample #	Target organisms	MPN reading	1st dilution	Closest table MPN	Table MPN per 100 g	MPN/100g	Standard deviation
Sample date	9/26/00 previous day						
CI #1	Fecal coliforms	530	1	530	80	800	
	E. coli	530	1	530	80	800	
CI #2	Fecal coliforms	510	1	510	30	300	
	E. coli	400	1	400	13	130	
CI ave.					Fecal coliforms	**550**	354
					E. coli	**465**	474
MG #1	Fecal coliforms	522	1	522	90	900	
	E. coli	522	1	522	90	900	
MG #2	Fecal coliforms	510	1	510	30	300	
	E. coli	510	1	510	30	300	
MG ave.					Fecal coliforms	**600**	424
					E. coli	**600**	424
Sample date	9/27/00 storm day						
Sample #	Target organisms	MPN reading	1st dilution	Closest table MPN	Table MPN per 100 g	MPN/100g	
CI #1	Fecal coliforms	420	1	420	22	220	
	E. coli	420	1	420	22	220	
CI #2	Fecal coliforms	410	0.5	410	17	340	
	E. coli	310	0.5	310	11	220	
CI ave.					Fecal coliforms	**280**	85
					E. coli	**220**	0
MG #1	Fecal coliforms	411	0.5	411	21	420	
	E. coli	410	0.5	410	17	340	
MG #2	Fecal coliforms	110	0.5	110	4	80	
	E. coli	100	0.5	100	2	40	
MG ave.					Fecal coliforms	**250**	240
					E. coli	**190**	212
Sample date	9/29/00 2 days after						
Sample #	Target organisms	MPN reading	1st dilution	Closest table MPN	Table MPN per 100 g	MPN/100g	
CI #1	Fecal coliforms	532	0.5	532	140	2800	
	E. coli	532	0.5	532	140	2800	
CI #2	Fecal coliforms	400	0.5	400	13	260	
	E. coli	400	0.5	400	13	260	
CI ave.					Fecal coliforms	**1530**	1796
					E. coli	**1530**	1796
MG #1	Fecal coliforms	420	0.5	420	22	440	
	E. coli	420	0.5	420	22	440	
MG #2	Fecal coliforms	200	0.5	200	4	80	
	E. coli	100	0.5	100	2	40	
MG ave.					Fecal coliforms	**260**	255
					E. coli	**240**	283

Table A. Hampton Harbor clam purging study: 1st storm

Page 2

Sample date	10/1/00 4 days after						
Sample #	Target organisms	MPN reading	1st dilution	Closest table MPN	Table MPN per 100 g	MPN/100 g	Standard deviation
CI #1	Fecal coliforms	540	0.5	540	130	2600	
	E. coli	530	0.5	530	80	1600	
CI #2	Fecal coliforms	541	0.5	541	170	3400	
	E. coli	541	0.5	541	170	3400	
CI ave.					Fecal coliforms	**3000**	566
					E. coli	**2500**	1273
MG #1	Fecal coliforms	531	0.5	531	110	2200	
	E. coli	500	0.5	500	23	460	
MG #2	Fecal coliforms	551	0.5	551	300	6000	
	E. coli	551	0.5	551	300	6000	
MG ave.					Fecal coliforms	**4100**	2687
					E. coli	**3230**	3917

Table B. Hampton Harbor clam purging study: 2nd storm

Sample date	10/5/00 previous day						
Sample #	Target organisms	MPN reading	1st dilution	Closest table MPN	Table MPN per 100 g	MPN/g sample	Standard deviation
CI #1	Fecal coliforms	5555	0.05	555	1600	3.20E+05	
	E. coli	5555	0.05	555	1600	3.20E+05	
CI #2	Fecal coliforms	553	0.5	553	900	1.80E+04	
	E. coli	553	0.5	553	900	1.80E+04	
CI ave.					Fecal coliforms	**169000**	213546
					E. coli	**169000**	213546
MG #1	Fecal coliforms	5553	0.05	553	900	1.80E+05	
	E. coli	5553	0.05	553	900	1.80E+05	
MG #2	Fecal coliforms	5555	0.05	555	1600	3.20E+05	
	E. coli	5555	0.05	555	1600	3.20E+05	
MG ave.					Fecal coliforms	**250000**	98995
					E. coli	**250000**	98995

Sample date	10/6/00 storm day						
Sample #	Target organisms	MPN reading	1st dilution	Closest table MPN	Table MPN per 100 g	MPN/g sample	
CI #1	Fecal coliforms	551	0.5	551	300	6.00E+03	
	E. coli	550	0.5	550	240	4.80E+03	
CI #2	Fecal coliforms	551	0.5	551	300	6.00E+03	
	E. coli	521	0.5	521	70	1.40E+03	
CI ave.					Fecal coliforms	**6000**	0
					E. coli	**3100**	2404
MG #1	Fecal coliforms	5531	0.05	531	110	2.20E+04	
	E. coli	5531	0.05	531	110	2.20E+04	
MG #2	Fecal coliforms	551	0.5	551	300	6.00E+03	
	E. coli	551	0.5	551	300	6.00E+03	
MG ave.					Fecal coliforms	**14000**	11314
					E. coli	**14000**	11314

Sample date	110/9/00	3 days after					
Sample #	Target organisms	MPN reading	1st dilution	Closest table MPN	Table MPN per 100 g	MPN/g sample	
CI #1	Fecal coliforms	410	0.5	410	17	3.40E+02	
	E. coli	410	0.5	410	17	3.40E+02	
CI #2	Fecal coliforms	521	0.5	521	70	1.40E+03	
	E. coli	521	0.5	521	70	1.40E+03	
CI ave.					Fecal coliforms	**870**	750
					E. coli	**870**	750
MG #1	Fecal coliforms	520	0.5	520	50	1.00E+03	
	E. coli	520	0.5	520	50	1.00E+03	
MG #2	Fecal coliforms	541	0.5	541	170	3.40E+03	
	E. coli	531	0.5	531	110	2.20E+03	
MG ave.					Fecal coliforms	**2200**	1697
					E. coli	**1600**	849

Table B. Hampton Harbor clam purging study: 2nd storm

Page 2

Sample date 10/10/00 4 days after

Sample #	Target organisms	MPN reading	1st dilution	Closest table MPN	Table MPN per 100 g	MPN/g sample	Standard deviation
CI #1	Fecal coliforms	510	0.5	510	30	6.00E+02	
	E. coli	510	0.5	510	30	6.00E+02	
CI #2	Fecal coliforms	510	0.5	510	30	6.00E+02	
	E. coli	510	0.5	510	30	6.00E+02	
CI ave.					Fecal coliforms	**600**	0
					E. coli	**600**	0
MG #1	Fecal coliforms	540	0.5	540	130	2.60E+03	
	E. coli	540	0.5	540	130	2.60E+03	
MG #2	Fecal coliforms	530	0.5	530	80	1.60E+03	
	E. coli	530	0.5	530	80	1.60E+03	
MG ave.					Fecal coliforms	**2100**	707
					E. coli	**2100**	707

Table C. Hampton Harbor clam purging study: 1st storm

Sample #	Target organisms	MPN reading	1st dilution	Closest table MPN	Table MPN per 100 g	MPN/g sample	Standard deviation
Sample date	**9/20/01** previous day						
CI #1	Fecal coliforms	5510	0.5	551	300	6.00E+03	
	E. coli	5510	0.5	551	300	6.00E+03	
CI #2	Fecal coliforms	5500	0.5	550	240	4.80E+03	
	E. coli	5500	0.5	550	240	4.80E+03	
CI ave.					Fecal coliforms	**5400**	849
					E. coli	**5400**	849
MG #1	Fecal coliforms	5200	0.5	520	50	1.00E+03	
	E. coli	5200	0.5	520	50	1.00E+03	
MG #2	Fecal coliforms	5200	0.5	520	50	1.00E+03	
	E. coli	5100	0.5	510	30	6.00E+02	
MG ave.					Fecal coliforms	**1000**	0
					E. coli	**800**	283
Sample date	**9/21/01** storm day						
CI #1	Fecal coliforms	5400	0.5	540	130	2.60E+03	
	E. coli	5400	0.5	540	130	2.60E+03	
CI #2	Fecal coliforms	5300	0.5	530	80	1.60E+03	
	E. coli	5300	0.5	530	80	1.60E+03	
CI ave.					Fecal coliforms	**2100**	707
					E. coli	**2100**	707
MG #1	Fecal coliforms	5210	0.5	521	70	1.40E+03	
	E. coli	5110	0.5	511	50	1.00E+03	
MG #2	Fecal coliforms	5200	0.5	520	50	1.00E+03	
	E. coli	5100	0.5	510	30	6.00E+02	
MG ave.					Fecal coliforms	**1200**	283
					E. coli	**800**	283
Sample date	**9/24/01** 3 days after						
CI #1	Fecal coliforms	552	0.5	552	500	1.00E+04	
	E. coli	551	0.5	551	300	6.00E+03	
CI #2	Fecal coliforms	554	0.5	554	1600	3.20E+04	
	E. coli	554	0.5	554	1600	3.20E+04	
CI ave.					Fecal coliforms	**21000**	15556
					E. coli	**19000**	18385
MG #1	Fecal coliforms	553	0.5	553*	900	1.80E+04	6174
	E. coli	553	0.5	553*	900	1.80E+04	6174
MG #2	Fecal coliforms	540	0.5	540	130	2.60E+03	
	E. coli	530	0.5	530	80	1.60E+03	
MG ave.					Fecal coliforms	**4387**	2527
					E. coli	**3887**	3234

*Sample dilution for MPNs used different diluent volume.