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BUZZARDS BAY

CAGED MUSSEL PILOT BIOMONITORING STUDY

1987 - 1988

Prepared by

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FOREWORD

The Division of Water Pollution Control in 1987 proposed and received funding from the U.S. Environmental Protection Agency (EPA) to conduct a pilot biomonitoring study in Buzzards Bay using caged mussels (*Mytilus edulis*). The study is part of a national estuarine management program developed by the U.S. EPA Office of Marine and Estuarine Protection and Region I of the EPA for Buzzards Bay. The program was initiated to promote and develop coordinated efforts between federal, state, local authorities, research institutions and the public to identify, correct, and monitor environmental problems affecting this nation's estuaries.

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Patricia Austin, Lawrence Gil, Steve Halterman, Jay Loubris, Steve McGee, Cathy O'Riordan, crew and captain of the fishing boat Phalarope, and Gerry Szal for assisting in the collection and/or deployment of the mussels while maintaining a sense of humor in the face of (usually) adverse weather conditions;

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ABSTRACT

Buzzards Bay Caged Mussel Pilot Biomonitoring Study 1987 - 1988

A caged mussel pilot biomonitoring study was conducted in Clarks Cove, New Bedford/Dartmouth, Massachusetts from October 1987 to September 1988. Mussels were deployed at three stations for five consecutive, 60-day exposure periods. Mussel tissue was analyzed for the trace elements: As, Cd, Cr, Cu, Hg, Ni, Pb, Zn, as well as total and fecal coliform bacteria and polychlorinated biphenyls (PCBs), and percent lipid content before and after the exposure periods.

Trace element tissue concentration was extremely variable at all of the stations. Within station (replicate) variability was usually high and masked between station differences in trace element concentration for many of the deployments. However, significant differences were detected between baseline and one or more of the Clarks Cove Stations for tissue concentrations of arsenic, zinc, and lead for several of the exposure periods. None of the Clarks Cove Stations (A, B, or C) exhibited significant differences in trace element tissue concentration from each other, indicating bio-available trace element concentration was not spatially different in Clarks Cove.

Bacteria concentration in the mussel tissue was variable and showed no consistent pattern throughout the study. Based on these results this technique is not recommended for long-term monitoring of coliform densities in coastal areas.

PCB tissue concentration between baseline and Clarks Cove Stations showed a consistent pattern of low baseline values, highest concentration at Station A, next highest at Station B, and low at Station C, indicating that this method may be effective for monitoring PCB concentration in coastal areas.

Inter-laboratory calibration exercises performed between the Lawrence Experiment Station and the Division of Marine Fisheries, Cat Cove Laboratory showed large inter-laboratory differences in results from mussel tissue analyzed for trace element concentration from the Clarks Cove study sites. However, results from similar analyses of EPA prepared standard "mega mussel" samples showed good inter-laboratory agreement.

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INTRODUCTION

In 1987 the Massachusetts Division of Water Pollution Control (DWPC), Department of Environmental Protection (DEP) applied for and received funding from the U.S. Environmental Protection Agency (EPA) Buzzards Bay Project to conduct a pilot biomonitoring program in Clarks Cove, New Bedford, Massachusetts using caged mussels (Mytilus edulis). This study is one of several being conducted in Buzzards Bay for the EPA Buzzards Bay Project over the past two years. These research projects are diverse and address water quality issues identified as being priority concerns in Buzzards Bay, mainly; bacterial contamination, nutrient enrichment, and toxic contaminants in fish and shellfish. Information gathered during this study phase will be used by the Buzzards Bay Project staff to develop a Comprehensive Conservation and Management Plan (CCMP) for Buzzards Bay.

The CCMP will provide strategies for pollution abatement and prevention throughout the watershed of the bay. In addition, the CCMP will include recommendations for long-term monitoring to assess the effectiveness of the water quality clean-up and management techniques that are employed.

The goals of this project were primarily to address questions relating to water quality monitoring techniques. In general, the "pilot" portion of the study was to design and implement a simple biomonitoring technique that could be performed by local, state, and/or regional agencies that would enable detection of long-term spatial and temporal trends in contaminant concentrations. More specifically, the study was to provide information that could be used to assess trace element and bacterial contamination in the water column of Clarks Cove, an area that receives discharges from as many as nine (9) combined sewer overflows from the City of New Bedford and flows from seven (7) storm drains from Dartmouth and New Bedford watersheds. In addition, the DWPC saw this as an opportunity to expand its water quality monitoring capabilities by examining this methodology for use as a tool to assess trace element contamination in sea water. The Massachusetts state analytical laboratory, the Lawrence Experiment Station (LES), does not have a "Clean bench" facility that is necessary to directly measure the trace concentrations of heavy metals and metalloids present in sea water.

Historically, the basic goal of water quality monitoring programs was to collect chemical and physical data which was used to characterize the general water quality of an area (Perry et. a. 1987). The design of many monitoring programs today still reflect this often random data gathering "objective", despite the fact that the intent and expectations of monitoring programs have matured. Monitoring programs are now relied upon to provide sound information on which to base management decisions. According to Segar, et. al. (1987) most marine monitoring programs have been inefficient or ineffective in providing specific information that can be used by the manager. These researchers recommend the use of transplanted bioindicator organisms to monitor temporal changes of bio-available contaminants in an area. The test animals, suspended in the water column, ingest, filter and/or absorb what is biologically available to them, providing a time integrated measure of the abundance of specific bio-available contaminants.

Within approximately the past fifteen years, the use of indicator organisms to monitor coastal water quality has become widely accepted. These studies have used both transplanted (i.e., caged) or indigenous test animals. The most ideal organisms for these types of studies appears to be bivalves. Capuzzo et. al. (1987) attribute the use of shellfish for these types of studies, particularly in monitoring heavy metals, to their metals bioaccumulation ability, sensitivity to metals concentration gradients, and importance to large programs such as the National Shellfish Sanitation Program and the Mussel Watch Program. They also point out, however, that there is no standard methodology for collecting these data sets.

Farrington et. al. (1987) and Tripp and Farrington (1984) presented the following comprehensive list of reasons why bivalves are considered the most useful organisms for this approach:

1. Bivalves are widely distributed geographically. This characteristic minimizes the problems inherent in comparing data for markedly different species.

2. They are sedentary and are thus better than mobile species as integrators of chemical pollution in a given area.

3. They have reasonably high tolerances to many types of pollution, in comparison to fish and crustacea.

4. They concentrate many chemicals by factors of 10^2 to 10^5 compared to seawater in their habitat making trace constituent measurements easier to accomplish in their tissues than in seawater.

5. An assessment of biological availability of chemicals is obtained.

6. In comparison to fish and crustacea, bivalves exhibit low or undetectable activity of those enzyme systems which metabolize many xenobiotics such as aromatic hydrocarbons and PCBs. Thus, a more accurate assessment of the magnitude of xenobiotic contamination in the habitat of the bivalves can be made.

7. They have many relatively stable, local populations that are extensive enough to be sampled repeatedly, providing data on short and long-term temporal changes in the concentrations of pollution chemicals.

8. They survive under conditions of pollution that often severely reduce or eliminate other species.

9. They can be successfully transplanted and maintained on subtidal moorings or on intertidal shore areas where populations normally do not grow - thereby allowing expansion of areas to be investigated.

10. They are commercially valuable seafood species on a worldwide basis. Therefore, measurement of chemical contamination is of interest for public health considerations.

Another advantage of using mussels and oysters that is relative to this particular study is that these animals can integrate pollutant levels over space and time, an advantage over sampling seawater and sediment for pollution assessment that can provide only very short-term (via seawater) or long-term (via sediments) contaminant integration (Goldberg, 1986).

Specific advantages of using transplanted animals taken from a relatively unpolluted site and suspended in cages in the test area over sampling indigenous animals for contaminants are (de Kock and van het Groenewoud, 1985): 1) the animals are derived from a common stock, thereby reducing a potential source of variability when comparing geographical locations; 2) the period of exposure to the environment is known and can be controlled; 3) monitoring locations can be chosen, regardless of whether or not the animals occur there naturally.

The EPA conducted a study in 1982 to evaluate the use of caged mussels to monitor ocean disposal of municipal sewage sludge in the New York Bidge (Phelps et. al., 1982). The study concluded that the use of transplanted caged mussels as a biomonitoring tool in coastal waters was feasible. Some of the large scale national water quality monitoring programs employing bivalves include the EPA Mussel Watch Program, which was conducted at over 100 sites around the coast during 1976-1978, and the current National Status and Trends Mussel Watch Program being conducted by National Oceanic and Atmospheric Administration (NOAA) on 150 coastal sites. In the United Kingdom, mussel watch programs were conducted from 1977-1979 at over two hundred sites along the coastlines of England, Wales, Scotland, and Ireland.

There are also more localized bioaccumulation studies using indicator organisms designed to monitor a specific point source. For example, the EPA has required bioaccumulation assessment plans to be included in several recent NPDES permits. These plans call for the use of Mytylis edulis (blue mussel) and Crassostrea virginica (eastern oyster) to monitor survivability and contaminant bioaccumulation at sites within the zone of initial dilution of the sewage outfalls. Massachusetts sewage treatment facilities that are currently developing a plan or are already conducting bioaccumulation studies as part of their NPDES permit requirement include the Lynn Water and Sewer Commission, Swampscott Wastewater Treatment Plant, South Essex Sewerage District (SESD), and the Massachusetts Water Resources Authority (MWRA). The EPA provides a guidance document entitled, "Methods for Use of Caged Mussels for In Situ Biomonitoring of Marine Sewage Discharges" (1983) that they recommend for use when designing bioaccumulation studies for these permits. Also in Massachusetts, caged mussel studies conducted by the New England Aquarium (1986, 1988) have been included as part of environmental impact studies to aid in the design and siting of ocean outfalls for SESD and MWRA.

It is evident from the literature that this methodology has become widely used and accepted by researchers as well as environmental regulators. The Buzzards Bay Technical Advisory Committee (TAC) has recognized the importance of this technique in the development of a coastal monitoring program that would be capable of detecting water quality trends in space and time. The monitoring effort in Buzzards Bay requires efficient techniques that will enable scientists to characterize long-term temporal and spatial water quality changes that result from point and/or nonpoint pollution abatement strategies and/or deleterious activities that may occur within the watershed. Although biomonitoring guidance documents do exist (U.S. EPA, 1983), there still is no single, widely accepted

standard operating procedure for conducting these types of bioaccumulation studies. More over, there appears to be even less agreement on how to interpret the results. With these problems and the needs of DWPC and the Buzzards Bay Project in mind, this pilot study was designed to address the following objectives:

1. To evaluate the impact of urban point sources of contamination into Buzzards Bay by assessing concentrations of selected trace elements and coliform bacteria in the tissues of the blue mussel (*M. edulis*) that have been suspended in cages at three sites located along a transect originating in Clarks Cove, New Bedford.

2. To compare shell growth between stations in a percentage of the test animals.

3. To examine the feasibility of this type of bio-indicator study as a water quality monitoring technique for the Division of Water Pollution Control.

4. To conduct an inter-laboratory calibration exercise with the Division of Marine Fisheries to demonstrate the degree of variability between laboratories that may be encountered in a study of this kind.

This report also contains the results of mussel tissue PCB analysis, although this task was not included in the biomonitoring study funded by EPA. Results are reported and briefly discussed in this report mainly because the task was an integral part of this pilot study and the information it provides will be used by DWPC to assess the usefulness of this technique for monitoring PCB contamination in other coastal areas of Massachusetts.

METHODS AND DESCRIPTION OF STUDY SITE

Study Design

Arrays cages were deployed at three stations oriented along a north-south transect originating in Clarks Cove, New Bedford and extending approximately 5.6 km (3.5 mi) in a south-south easterly direction out into Buzzards Bay (Figure 1). Station A was located near the head of Clarks Cove. Station B was established at the mouth of the cove midway between the eastern and western shorelines. Station C was located in Buzzards Bay near Nun #4 LR approximately 1.7 miles northeast of Round Hill Point in Dartmouth. Water depth at Station A and B at low tide was approximately 5 meters and low tide depth at Station C was approximately 9 meters.

By establishing stations in a land to seaward direction a contamination gradient was expected to be observed, with highest levels of metals and bacteria predicted in tissues collected from Station A at the head of the cove nearest the urban sources (i.e., combined sewer overflows and storm drains), and lowest levels anticipated in tissues from reference Station C located over 1 mile (1.6 km) located away from land based pollution sources. (See pages 10 - 13 for a complete description of the study site.) Before establishing these station locations it was important to consider the influence of currents in the study area. Although little information has been published on the hydrodynamics of Clarks Cove, available research results supported the selection of a north-south transect on which to locate stations. In the main body of Buzzards Bay the currents are complex. Net displacement of a particle over a tidal cycle is about 102 km (EG&G for CDM). Signell (1987) characterized the circulation pattern in the bay as tidally dominated. Wind is also an important mechanism determining subtidal circulation especially in shallow embayments and estuaries. EG&G's survey described tidal currents in the New Bedford Clarks Cove area as running generally south to north-northeast into Clarks Cove on the flood tide and north to south-southwest on the ebb tide.

Each station was located in an area of soft bottom sediments indicating that deposition, rather than scouring was taking place. This also enabled sediments to be collected for analyses from each site and helped maintain similarity between stations. The cage assembly was anchored by one or two 8"x16" cinder blocks and attached to floating lobster buoys to mark their location. This design was identical to that used by the EPA, Environmental Research Laboratory (ERL) in Narragansett, RI for similar caged mussel biomonitoring studies they have conducted in New Bedford Harbor (Don Phelps and William Nelson, EPA, ERL, Narragansett, RI, personal communication). With this design, field personnel were able to set out and retrieve the cages from a boat rather than rely on scuba divers to access the cages. Each cage contained twenty-five (25) mussels (*Mytilus edulis*) with total shell lengths all between 5-7 cm. Figure 2 illustrates the design of the cage array for one station. For the growth study ten of the twenty-five animals in one cage of each replicate were marked with an individual number etched in the shell surface (methods employed for the growth study are described below). Each station was made up of four replicates. Each replicate consisted of 50 animals divided equally into two cages for a total of 200 animals per station. A typical exposure period, from deployment to collection lasted about sixty days with a new group of mussels set out each time.

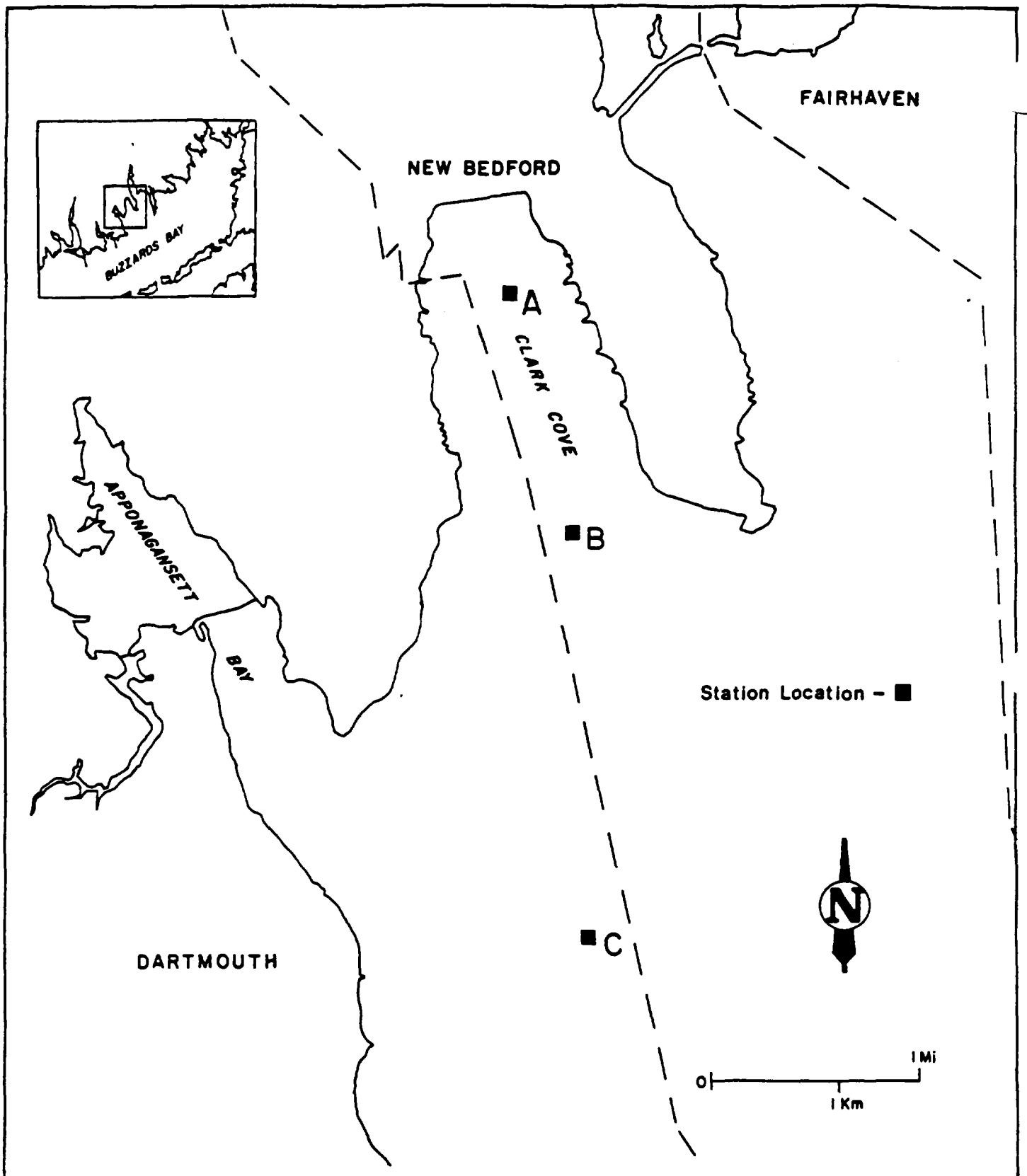
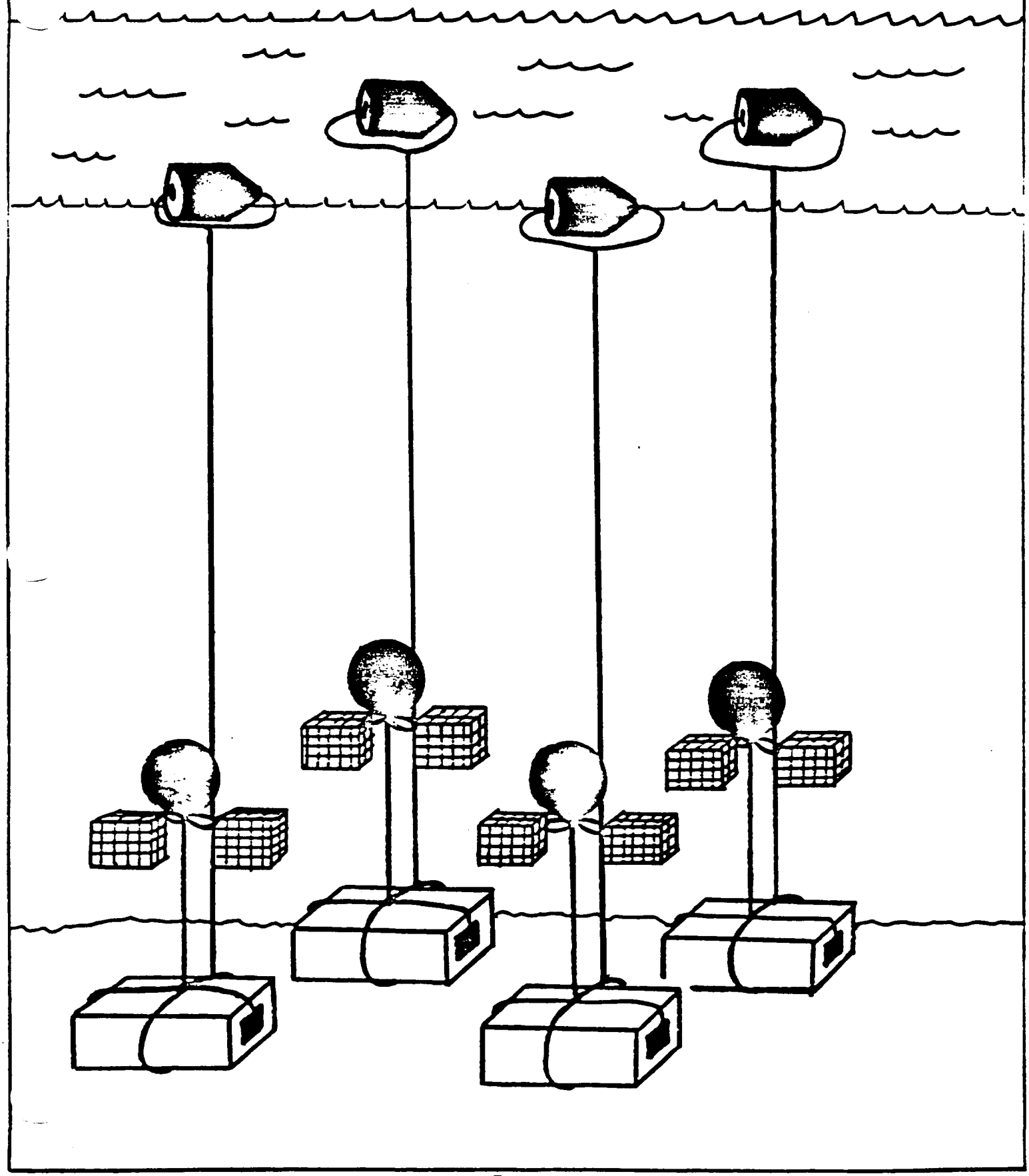


FIGURE 1 BUZZARDS BAY CAGED MUSSEL PILOT BIOMONITORING STUDY Oct.1987-Sept.1988

Clarks Cove Station Locations

FIGURE 2 BUZZARDS BAY CAGED MUSSEL PILOT BIOMONITORING STUDY Oct.1987-Sept.1988

Station Description: 4 Replicates per Station, 2 Cages per Replicate, 25 Mussels per Cage, 200 Mussels per Station



The EPA (1983) recommends a 30 day exposure time for metals bioaccumulation studies whereas de Koch and van het Groenewoud (1985) state that some metals may require over 150 days to bioaccumulate in mussels. After discussions with the Buzzards Bay Technical Advisory Committee and personnel from Woods Hole and EPA, ERL, Narragansett the 60 day exposure period was selected. This allowed for twice the EPA recommended exposure time. Longer periods were rejected to avoid or minimize the degree of fouling that may occur on the cages and to reduce cage loss due to wear and tear from extended periods of weathering. The one year study period that began in October thus allowed for five, 60 day exposure periods, or deployments, that occurred on the following dates:

First deployment - October 29, 1987 - January 13, 1988
Second deployment - January 13, 1988 - March 16, 1988
Third deployment - March 16, 1988 - May 11, 1988
Fourth deployment - May 11, 1988 - July 13, 1988
Fifth deployment - July 13, 1988 - September 21, 1988

Field and Laboratory Procedures

The same field procedures were followed for each deployment period. Blue mussels (*M. edulis*) were collected by hand by Division of Water Pollution Control (DWPC) personnel at low tide from a tidal creek near the town beach in Sandwich, MA. Immediately after collection, a subset of these animals were sent, on ice, to the Lawrence Experiment Station (LES) for baseline tissue analysis. These baseline samples consisted of the following: four replicates of 15 animals each were placed in labeled, sterile plastic bags for trace element tissue analysis (As, Cd, Cr, Cu, Hg, Ni, Pb, Zn). Twenty-five mussels were placed in a labeled sterile plastic bag for total and fecal coliform bacteria tissue analyses. Although not funded as part of this study, four replicates of 15 animals each were wrapped in aluminum foil and labeled for PCB and PAH analysis. The samples for the organics analysis were taken to the DWPC laboratory in Westborough and frozen for later analysis at the LES. The remaining mussels were transported in clean, plastic-lined coolers to the EPA Environmental Research Laboratory in Narragansett, RI. At this lab the animals were placed in flow-through seawater tables and left overnight. The following morning the mussels were sorted by size and 120 animals in the 5-6 cm range were selected for the growth study. These mussels were consecutively numbered from 1 to 120 using a dremel drill to etch the surface of the shell. The longest portion of the shell was measured to the nearest 0.1 mm using a Manostat (model 5921) caliper. The same individual performed all of the shell measurements with same caliper throughout the study. This technique was similar to that followed by personnel at the EPA, ERL, Narragansett, RI (William Nelson, EPA, Narragansett, RI, personal communication).

Twenty-five mussels were placed in each cage, which was appropriately labeled by station and replicate. One cage per replicate contained 10 numbered animals among the twenty-five. Lids were secured with small plastic tie wraps. Cages were secured to the trawler float with heavy duty tie wraps for easy removal. The cages were left in the flow through seawater tables overnight.

The following morning the mussel cages were transported in coolers to Clarks Cove, New Bedford. All stations were accessed by boat. At each station the cages from the previous deployment were retrieved and the new replicates were deployed. The replicates were spaced approximately 25 meters apart.

At each station water samples were collected with a van Dorn grab sampler 1 meter below the surface and 1 meter from the bottom.

These samples were collected to assess whether basic environmental conditions were similar at each site, as well as to make sure these conditions were suitable for mussel survival.Water samples to be analyzed for total solids, suspended solids, chlorides, and turbidity were collected in clean polypropylene containers. Samples to be analyzed for total phosphorus, orthophosphate, ammonia, and total Kjeldahl-nitrogen were collected in clean, acid rinsed bottles and acidified to pH 2.0 with 2 ml of 50 percent H_2SO_4. Samples for chlorophyll a analysis were collected in clean polypropylene containers. All samples were tagged for identification and stored on ice in coolers for transport to the LES laboratory.

Temperature, dissolved oxygen, salinity, and conductivity in the water column at each station were measured with a Hydrolab Surveyor II. Data and field observations pertaining to weather, sea conditions, and test animal and cage conditions after retrieval were recorded in a bound field notebook. On several occasions a YSI Model #33 SCT meter was used to measure temperature, conductivity and salinity; dissolved oxygen was measured according to the Winkler technique. (Refer to Appendix A for details of meter accuracy, and sample treatment methods.)

On September 21, 1988 one sediment grab was collected at each station with a petite ponar grab dredge (Karlisco International Corp., El Cajon, Ca 92002). Prior to sampling the dredge was rinsed in seawater to remove any residual sediment. The inside of the dredge was then rinsed with reagent grade acetone, followed by a rinse with reagent grade hexane, followed by a final rinse with seawater. All chemical rinse waste was collected and transported back to the laboratory for proper disposal. The dredge contents were emptied into a plastic tray and subsamples of the sediment were scooped into separate specially cleaned 16 oz. glass, screw-top, wide-mouth jars prepared for metals and organics. Care was taken to prevent the collection of sediments in direct contact with the tray and/or sides of the dredge. All samples were identified with tags and stored on ice in coolers for delivery to LES. See Appendix A for details of sample bottle preparation.

The sediments were analyzed at the LES for the following parameters: Trace elements (as total metals or metalloids): As, Cd, Cr, Cu, Hg, Ni, Pb, Zn; percent total volatile solids; PCBs and PAHs.

Appendix A presents the methodology employed at the LES for the analysis of the various water and sediment quality parameters.

After collection the cages were left unopen and placed on ice in coolers for transport back to the DWPC laboratory in Westborough. The following day the cages were opened and the numbered animals were measured and individual shell length was recorded. The number of animals that were dead were noted along with the degree of fouling on the cages and on the animals themselves. Dead animals were identified by empty shells or by a strong odor of decay. Fifteen mussels were randomly selected from each replicate group and were placed in sterile plastic bags identified for trace element analysis. Fifteen animals were wrapped in aluminum foil and labeled and stored in the freezer (at 4°C) for later PCB and PAH analysis, and the remaining mussels (depending on how many were lost due to

mortality) were placed in labeled sterile plastic bags for total and fecal coliform bacteria analysis. The samples for trace element and bacteria analysis were then immediately transported on ice to the LES.

Methods of tissue preparation and analysis for trace element and bacteria in shellfish employed at the LES are outlined in Appendix A.

Inter-Laboratory Calibration

An inter-laboratory calibration exercise was carried out between the Massachusetts Division of Marine Fisheries (DMF) and the Lawrence Experiment Station. The DMF proposed to analyze mussel tissue homogenate samples for trace elements: As, Cd, Cr, Cu, Hg, Ni, Pb, Zn at their Cat Cove Marine Laboratory in Salem, MA. A portion of the same tissue homogenate prepared by the LES for trace element analysis was frozen and stored at the laboratory for later delivery by DWPC personnel to the DMF. In September of 1988 the DMF notified DWPC that it would tissue homogenate samples from LES.

Ten samples were delivered to DMF on October 3, 1988. Appendix D contains a complete description of the DMF project plan and analytical procedures followed at the Cat Cove DMF laboratory.

On March 28, 1989 the LES and DMF were each given 3 replicate frozen samples of a standard mussel tissue homogenate ("mega mussel") prepared by the EPA. Both laboratories were requested to analyze the tissue homogenate for the same suite of eight heavy metals and metalloids using the same methodologies employed during the caged mussel study.

Description of Study Site

Clarks Cove is small, with a surface area of 5.18 km^2 (2 mi^2) and an average water depth of 5 meters at MLW. The drainage area for the cove is comparatively large with the majority (approximately 8.1 km^2 or 2,000 acres) lying within the City of New Bedford. The remaining watershed (approximately 2.4 km^2 or 500 acres) is located within the boundaries of the Town of Dartmouth. Almost 94 percent of the total New Bedford drainage area is served by combined sewers (CDM, 1983).

Along the shoreline of the cove there are nine combined sewer overflow (CSO) outfalls and seven storm drain pipes (Figure 3). Table 1 lists each CSO and its location and description.

CDM estimated that 961 million gallons of storm and untreated wastewater were discharged to Clarks Cove in 1983. Forty-three percent (or 413 million gallons) of this was from CSO discharges, 6 percent (58 million gallons) was from dry weather discharges and 51 percent was from storm water runoff. They estimated that CSO discharges occur on an average of 75 times a year and they come from two major active outlets at the head of the cove (CSO #003 and #004).

FIGURE 3 BUZZARDS BAY CAGED MUSSEL PILOT BIOMONITORING STUDY Oct.1987-Sept.1988

Clarks Cove Combined Sewer Overflow Outfalls (CDM, 1983) and Storm Drain Locations

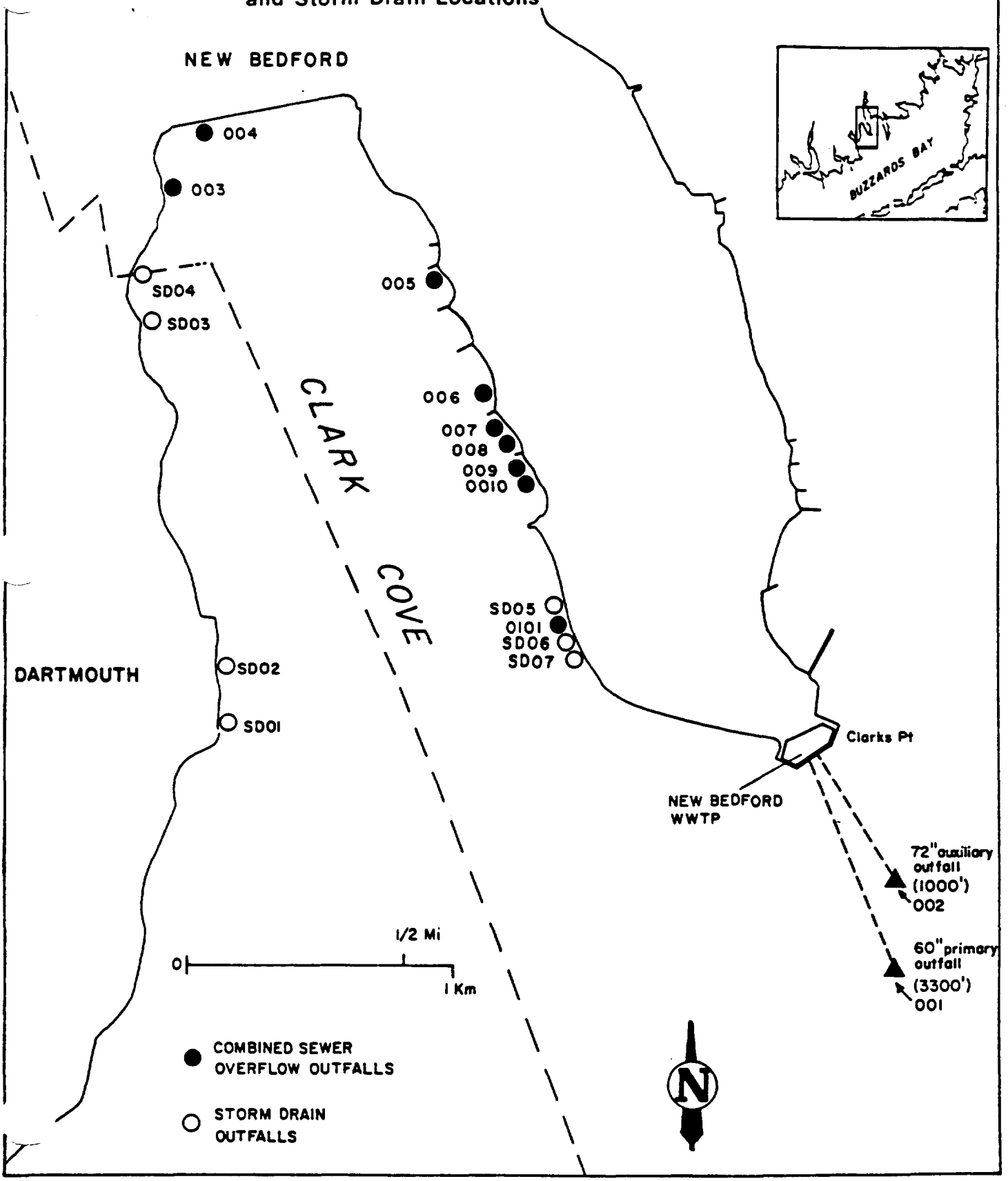


TABLE 1

CLARKS COVE COMBINED SEWER OVERFLOWS[1]

CSO OUTLET NUMBER	LOCATION	DIAMETER (Inches)
003*	Cove Road and Padanaram Ave.	54"
004	Hurrican Barrier Pumping Station	96" x 84"
005*	Dudley Street and West Rodney French Blvd. (W.R.F. Blvd.)	18"
006	Lucas Street and W.R.F. Blvd.	24"
007	Capitol Street and W.R.F. Blvd.	24"
008	Calamet Street and W.R.F. Blvd.	18"
009	Aquidneck St. and W.R.F. Blvd.	18"
010	Bellevue St. and W.R.F. Blvd.	12"
0101	Hudson St. and W.R.F. Blvd.	18"

* Contaminated by dry weather sanitary flow from storm drains connected to the outfall, as observed by CDM (1983).

[1] CDM Interim Summary Report on CSO Phase I, December 1983

The dry weather discharges occur as a result of structural or maintenance related problems of the existing sewer system. For example, the dry weather flow at CSO #004, estimated at over 0.16 MGD, is caused by a plugged dry weather connection. Historically, the highest coliform densities in Clarks Cove have been in the northern sector of the cove, presumably because of CSO dry weather discharges. The waterbody is classified as SA in accordance with the Massachusetts Water Quality Standards, but these standards are violated frequently. Clarks Cove receives heavy recreational use in the form of swimming, fishing, and boating. There are two public beaches and one private beach, and several boat ramps located around the cove. The cove is closed to commercial fishing and shellfishing. Beach closings are reportedly rare.

RESULTS

Water Quality

The physical and chemical water quality data collected during the study year are presented by station and date in Appendix B. Figures 4-6 illustrate the seasonal trend of temperature, dissolved oxygen, and salinity measured at one meter above the bottom at the three station locations. As shown, these parameters fluctuated similarly at each station throughout the survey year.

Salinity at all stations ranged between 27 - 32.2 parts per thousand during the year. Dissolved oxygen values ranged from a low of 5.0 mg/l measured at Station A in July to high of 12.8 mg/l measured at Station C in March. The July dissolved oxygen values exhibited the greatest between station differences (5.0 mg/l at Station A and 7.2 mg/l at Station C).

Temperature, salinity and dissolved oxygen concentrations were within ranges necessary for mussel growth and survival at all of the stations.

Nutrient concentrations measured at the stations during the study were low to moderate and fell within ranges reported in the Buzzards Bay water quality surveys (MDWPC, 1985, 1986a), with the exception of Station B during March. This station exhibited elevated suspended solids and turbidity as well as high total Kjeldahl-nitrogen, total phosphorus and orthophosphate concentrations in the bottom water column sample. It is possible that the sediments were disturbed during sampling and this contaminated the sample. Suspended solids and turbidity were otherwise low and within expected ranges. These parameters followed similar trends between stations throughout the year.

Sediment Quality

Table 2 presents the sediment trace element, PCB, PAH, and percent total volatile solids data for each station. All sediment samples were collected on September 21, 1988. A rigorous assessment of the sediment quality was beyond the scope of this study. Since the results cannot be normalized, and only one sediment grab per station was collected, an in-depth comparison and evaluation of sediment quality cannot be made from these data.

Station A sediments contained the highest concentrations of all trace elements, and organics measured, with the exception of nickel, which was slightly higher at Station C (6.5 mg/km versus 5.5 mg/km at Station A). Zinc and PCB 1254 concentrations were above category III dredge spoils criteria (MDWPC, 1983) at Station A. Arsenic was also elevated at this site. Station B and C sediments contained similar concentrations of most of the trace elements, and results were within ranges reported in the Buzzards Bay sediment survey (MDWPC, 1985-86). PCB 1254 concentration was higher at Station B (exceeded Category III criteria) than at Station C (Category II).

PAH concentrations were relatively low at all of the stations, but the greatest number of compounds (and concentrations) were found at Station A and the least at Station C.

Percent total volatile solids were similar at all stations.

FIGURE 4
CLARKS COVE TEMPERATURE
Stations A, B, and C

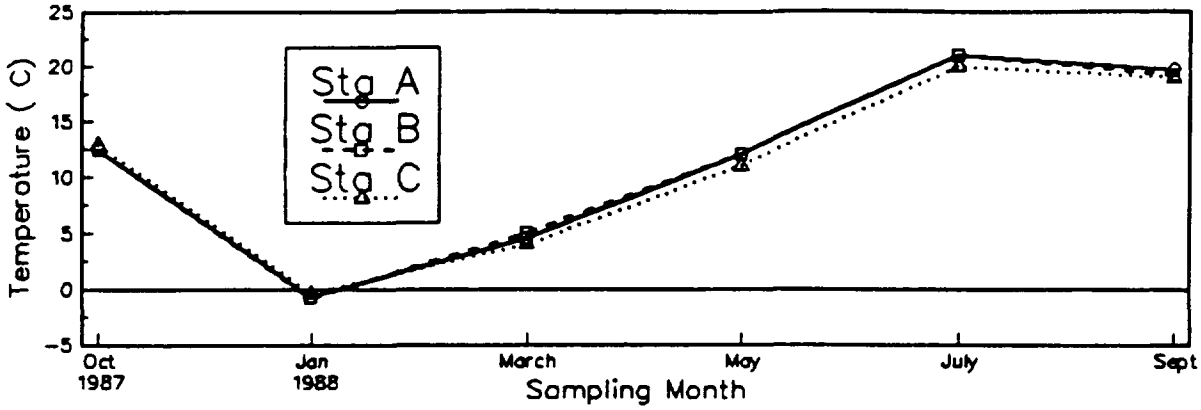


FIGURE 5
CLARKS COVE DISSOLVED OXYGEN
Stations A, B, and C

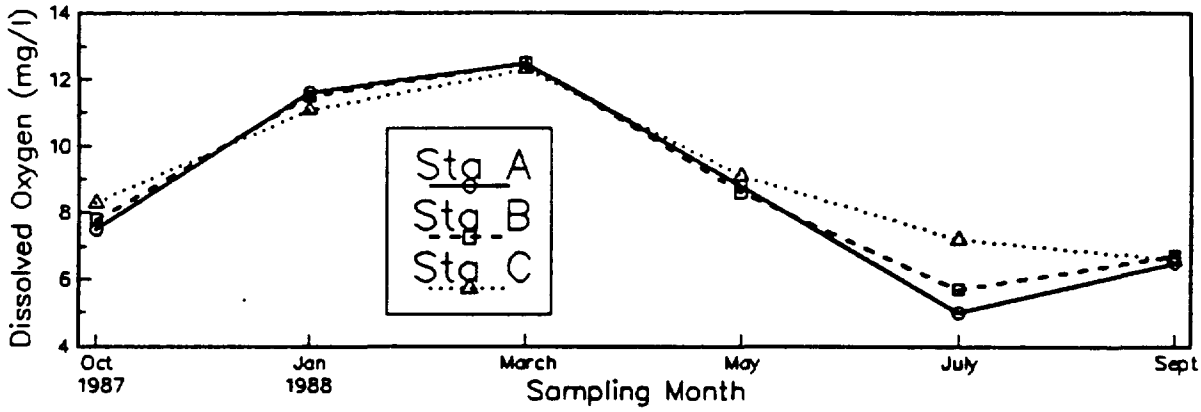


FIGURE 6
CLARKS COVE SALINITY
Stations A, B, and C

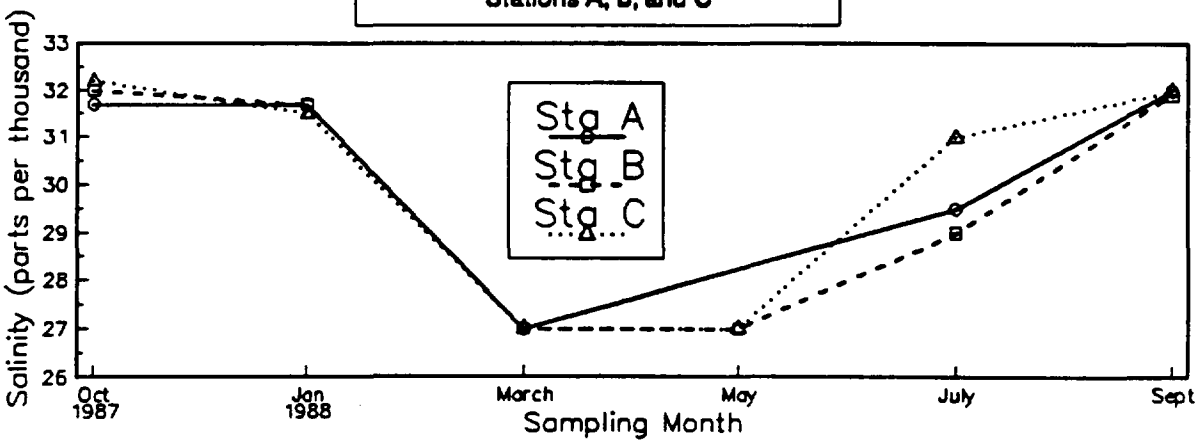


TABLE 2

CLARKS COVE

SEDIMENT DATA

TRACE ELEMENTS, POLYCHLORINATED BIPHENYLS, POLYCYCLIC AROMATIC HYDROCARBONS AND PERCENT TOTAL VOLATILE SOLIDS[1]

September 21, 1988

PARAMETER	STATIONS A	B	C	CATAGORY III DREDGE SPOILS CRITERIA[2]
Trace Elements (mg/kg dry weight):				
Arsenic	2.4	1.4	2.0	>20
Cadmium	6.5	<1.0	1.0	>10
Chromium	41	23	29	>300
Copper	60	60	24	>400
Mercury	0.335	0.170	0.105	>1.5
Nickel	5.5	2.5	6.5	>100
Lead	90	33	25	>200
Zinc	500	85	90	>400
PCB 1254 (μg/g)	2.3	1.3	0.91	>1.0
Percent Total Volatile Solids	5.9	5.4	6.7	-
PAH (μg/g dry weight)				
Benzo(a)anthracene	0.80	0.20	-	-
Benzo(a)pyrene	0.57	-	-	-
Bnezo(k)fluoranthene	0.96	-	-	-
Chrysene	0.56	0.13	-	-
Fluoranthene	1.10	0.41	0.20	-
Phenanthrene	0.55	0.18	0.10	-
Pyrene	1.10	0.32	0.20	-
Total PAHs reported by LES	5.64	1.24	0.50	

[1] See Appendix A, Table A-5 for methods of analysis and limits of detection.

[2] DWPC, 1983

Cage Loss and Mussel Mortality

Percent mortality that occurred at each station during each deployment is presented in Table 3. The number of cages (replicates) lost during each exposure period is also listed in this table. The percent mortality was calculated by dividing the total number of dead animals found at a station by 200 (the total number of animals deployed at each station) and multiplying by 100. Mortality was usually very low, generally only 0-4 animals per station were lost. However, during the last exposure period of 7/13-9/21 mortality was very high (25-53 percent). An extreme degree of fouling by barnacles and algae was observed on the cages and animals themselves from this period. Also, several small starfish were found in many of the cages. Cages collected from all other deployments exhibited very little fouling and no starfish were observed inside them.

Four cages were lost during two of the deployment periods. Other periods experienced only a loss of 1 or 2 cages. One replicate (C4) lost during the first deployment period was recovered on 9/21/88 at the same site after almost one year of exposure. Out of the original 50 animals, 27 survived from this group.

Shell Growth

Mean shell growth and standard deviation for animals at each station and for each deployment are shown in Figure 7. The average shell growth over a 60-day period of 120 mussels is highly variable as illustrated by the standard deviation bars (one S.D.) on the graph. This variability masks any statistical differences that may exist between Stations A, B, and C for any one deployment period. However, from the graph it appears that mean shell growth at these stations exhibit fairly similar trends during each period. The largest differences in shell growth are seen between exposure periods, although these are not statistically significant due to the large standard deviations. As expected, in general, the spring and early summer exposure groups show the largest increase in average shell growth, and the fall and winter periods produce the least amount of growth.

Tissue Bacteria Concentrations

Tissue total and fecal coliform bacteria concentrations are presented in Table 4. Tissue samples from the last deployment period were not analyzed for bacteria concentration due to high mortality resulting in an insufficient number of live mussels available for the analysis. It was felt that the bacteria analysis was the most expendable of the parameters, because tissue bacteria data obtained from the last four deployments were erratic and did not supply any more useful information for monitoring long-term trends in bacteria contamination than could be obtained from direct water column sampling techniques (see discussion section).

Baseline tissue bacteria concentrations were generally much higher than tissue concentrations measured in animals after exposure, indicating that the Sandwich, MA site may not be appropriate for collecting "clean" mussels if bacterial contamination is a concern. A large number of birds were often observed near the area where the mussels were collected.

TABLE 3

PERCENT MORTALITY[1] OF MUSSELS AND CAGE LOSS[2]

	STATION A		STATION B		STATION C	
DEPLOYMENT PERIOD	% MORTALITY	# OF LOST CAGES	% MORTALITY	# OF LOST CAGES	% MORTALITY	# OF LOST CAGES
10/28/87 - 1/13/88	3.3	1	2.0	1	4.0	2*
3/16/88 - 3/16/88	3.0	0	2.0	0	1.3	1
3/16/88 - 5/11/88	0.5	0	1.0	2	0	2
5/11/88 - 7/13/77	4.0	0	4.0	0	2.7	1
7/13/88 - 9/21/88	53.0	0	46.0	1	25.3	1

1 Number of animals dead/200 x 100 = % mortality

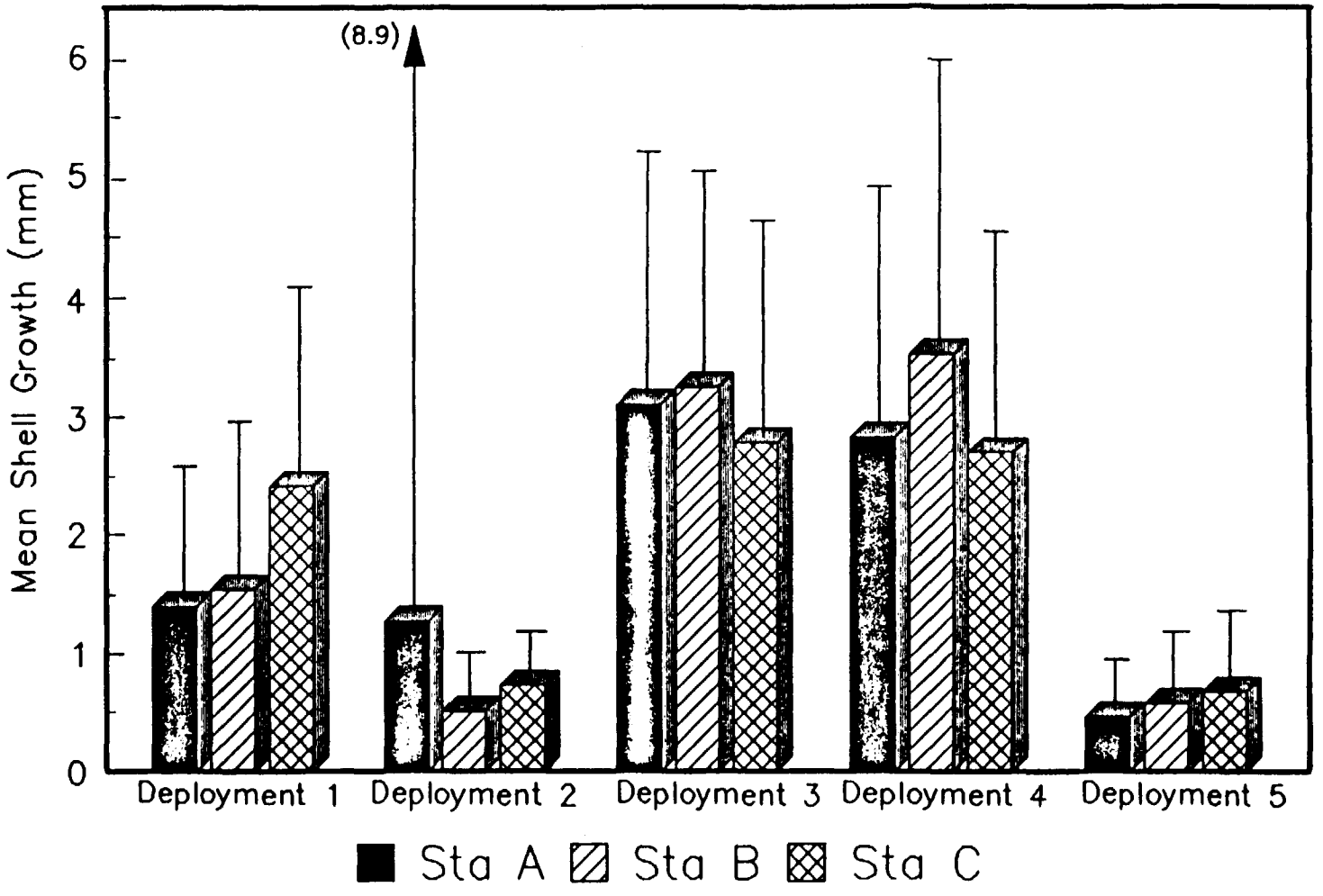
2 Total number of cages deployed during each deployment period = 12

* One of these cages lost during the 1st deployment period was recovered on 9/21/88.

FIGURE 7

MEAN MUSSEL SHELL GROWTH

By Station for Each Deployment



Error bars illustrate one standard deviation from the mean.

TABLE 4

MUSSEL TISSUE TOTAL AND FECAL COLIFORM DENSITIES - MPN per 100 ml

	BASELINE		STATION A		STATION B		STATION C	
	TOTAL	FECAL	TOTAL	FECAL	TOTAL	FECAL	TOTAL	FECAL
1st Deployment								
October 29, 1987 -	5,400	700	300	78	230	<20	430	40
January 13, 1988	-	-	490	78	230	<20	-	-
(79 days)	-	-	490	100	230	20	-	-
2nd Deployment								
January 13 -	1,300	20	790	45	1,300	130	1,300	140
March 16, 1988	-	-	1,300	230	230	20	430	20
(62 days)	-	-	2,400	270	490	40	1,100	130
	-	-	790	45	230	<20	-	-
3rd Deployment	2,400	330	490	<20	490	<20	330	<20
March 16 -	2,400	230	790	20	330	<20	490	20
May 11, 1988	1,300	170	330	<20	-	-	-	-
(57 days)	2,400	490	490	<20	-	-	-	-
4th Deployment	5,400	330	-	61	-	45	-	<20
May 11 -	9,200	490	-	20	-	130	-	40
July 13, 1988	5,400	490	-	<20	-	<20	-	<20
(64 days)	16,000	220	-	20	-	78	-	-
5th Deployment July 13 - September 21, 1988 (70 days)	SAMPLES NOT ANALYZED FOR BACTERIA							

In addition, bacteria concentrations between stations for each deployment did not exhibit a discernable pattern. It was expected that animals nearest the head of the cove would accumulate the highest bacteria concentrations. This was not the case. On several occasions, Station C, the reference site located out in Buzzards Bay, had the highest bacteria counts. In general, if total coliform was high (>1,000 colonies per 100 ml), fecal coliform was also elevated.

Tissue Trace Element Concentrations

Figures 8 through 15 illustrate the results from the tissue analysis for trace elements. Concentration is reported in mg/kg (wet weight) for mercury (Hg), chromium (Cr), cadmium (Cd), arsenic (As), lead (Pb), nickel (Ni), copper (Cu), and zinc (Zn).

Each graph illustrates the tissue concentration of one trace element over all of the deployment periods. The bars represent the mean tissue concentration of the metalloid of all the replicates for each station, grouped by deployment period. One standard deviation is depicted on the graph to illustrate the variability of the data about the mean. Appendix B contains the tissue trace element concentration data as reported by the LES. Results from each deployment were examined separately. Comparison of contaminant concentration throughout the year is not possible since a new set of animals was used for each 60 day deployment period. Inter-exposure period comparisons of this nature would only have been possible if all of the animals had been deployed at the beginning of the study and subsampled throughout the year.

Statistical analysis using the nonparametric Kruskal-Wallis test (Zar, 1984) was performed on the tissue trace element concentration data. Nonparametric statistics were chosen because the variances of the groups of data being compared were not homogeneous. Under these conditions this nonparametric ANOVA test is more powerful than the one-way ANOVA (Zar, 1984). The Kruskal-Wallis statistic tested the null hypothesis that trace element concentration in tissue from the baseline station and Stations A, B, and C were the same. (H_o: [metalloid] is the same at all stations.)

Appendix C contains sample statistical calculations. Table 5 presents a summary of the results of the nonparametric ANOVA tests.

A significant difference between mean trace element concentration was detected at the 95% confidence level for only 13 of the 35 groups of data tested. (During deployments four and five, detection limits of Cd, Cr and Pb were increased as a result of a change in laboratory procedure. As a consequence almost all values were reported as less than detection limits for these periods, thus limiting further analysis and comparison of these data sets.)

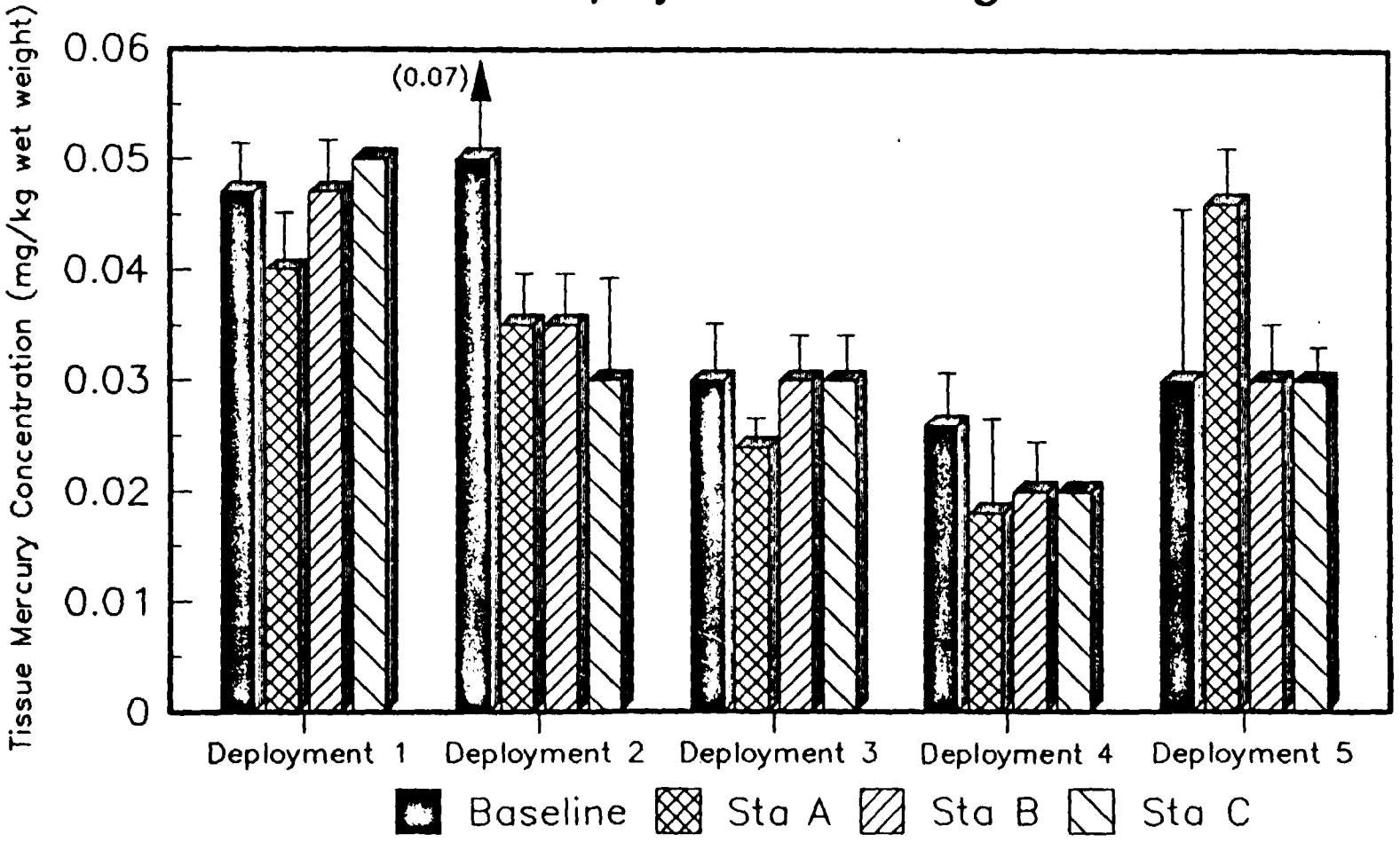
Since the Kruskal-Wallis multiple comparison test does not indicate where the significant differences occur in the data set, a nonparametric Tukey-type multiple comparison test was applied to locate where the differences existed (Zar, 1984) for these 13 data sets. (See Appendix C for sample calculations.) Table 6 summarizes the results of these calculations.

Due to high standard error values in several of the data sets only 8 of the 13 Tukey tests detected significant differences between the means.

FIGURE 8

MUSSEL TISSUE MERCURY CONCENTRATION

For Deployments 1 Through 5

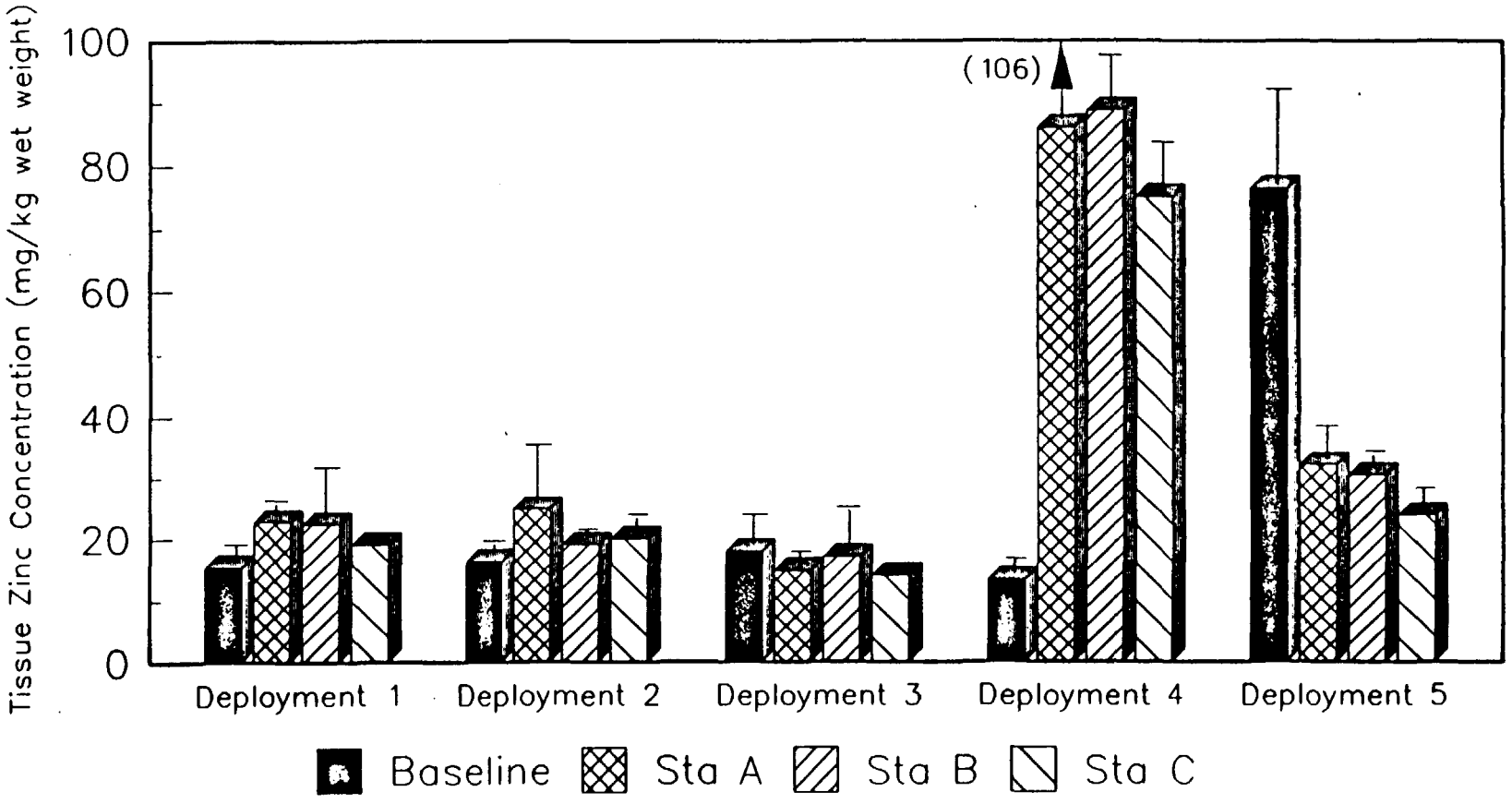


Bars represent mean mussel concentration of mercury for each station.
Lines above the bars illustrate one standard deviation from the mean.
Where no s.d. lines are indicated s.d.=0.

FIGURE 9

MUSSEL TISSUE ZINC CONCENTRATION

For Deployments 1 Through 5

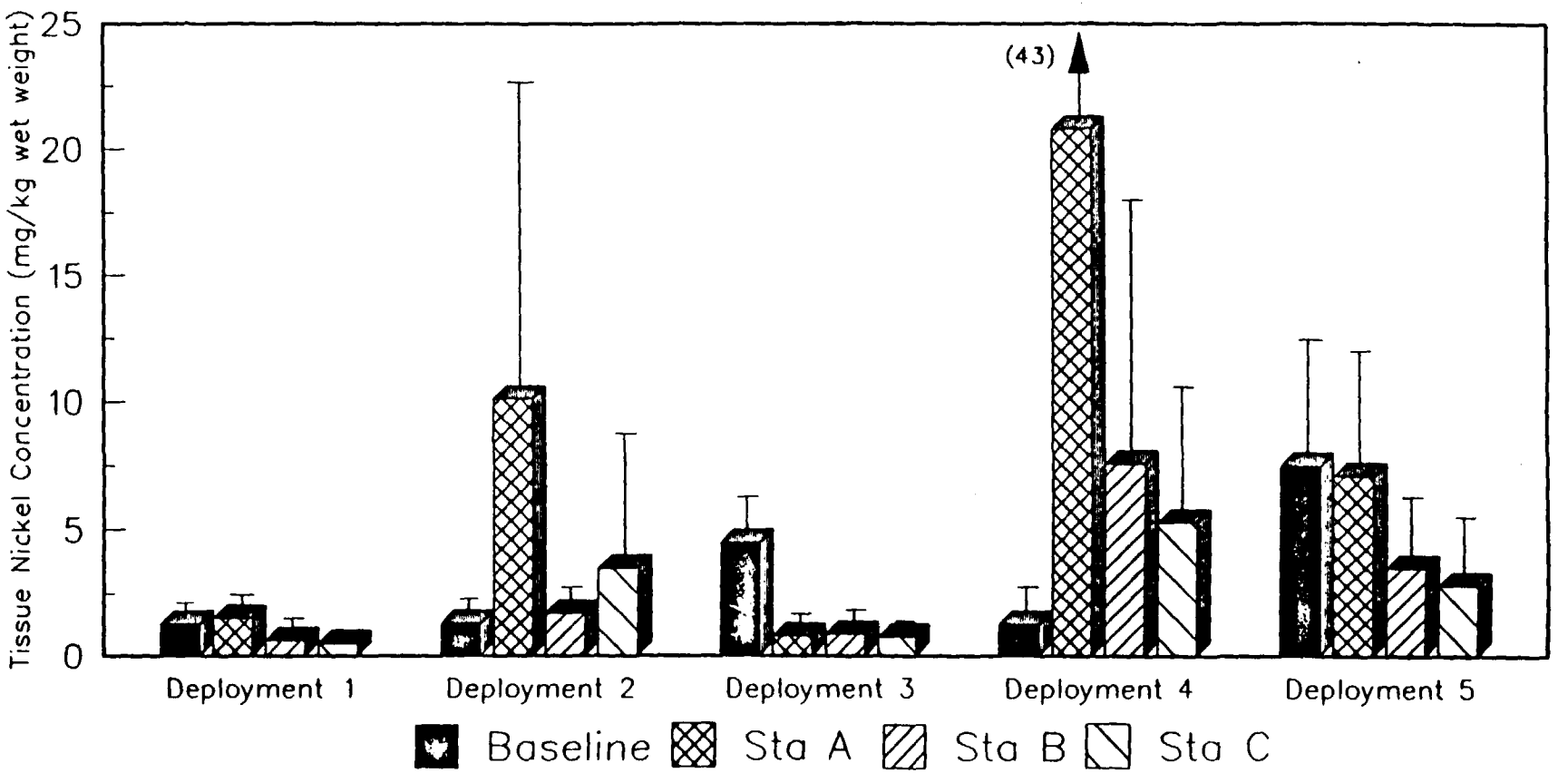


Bars represent mean mussel tissue concentration of zinc for each station. Lines above the bars illustrate one standard deviation from the mean. Where no s.d. lines are indicated s.d.=0.

FIGURE 10

MUSSEL TISSUE NICKEL CONCENTRATION

For Deployments 1 Through 5

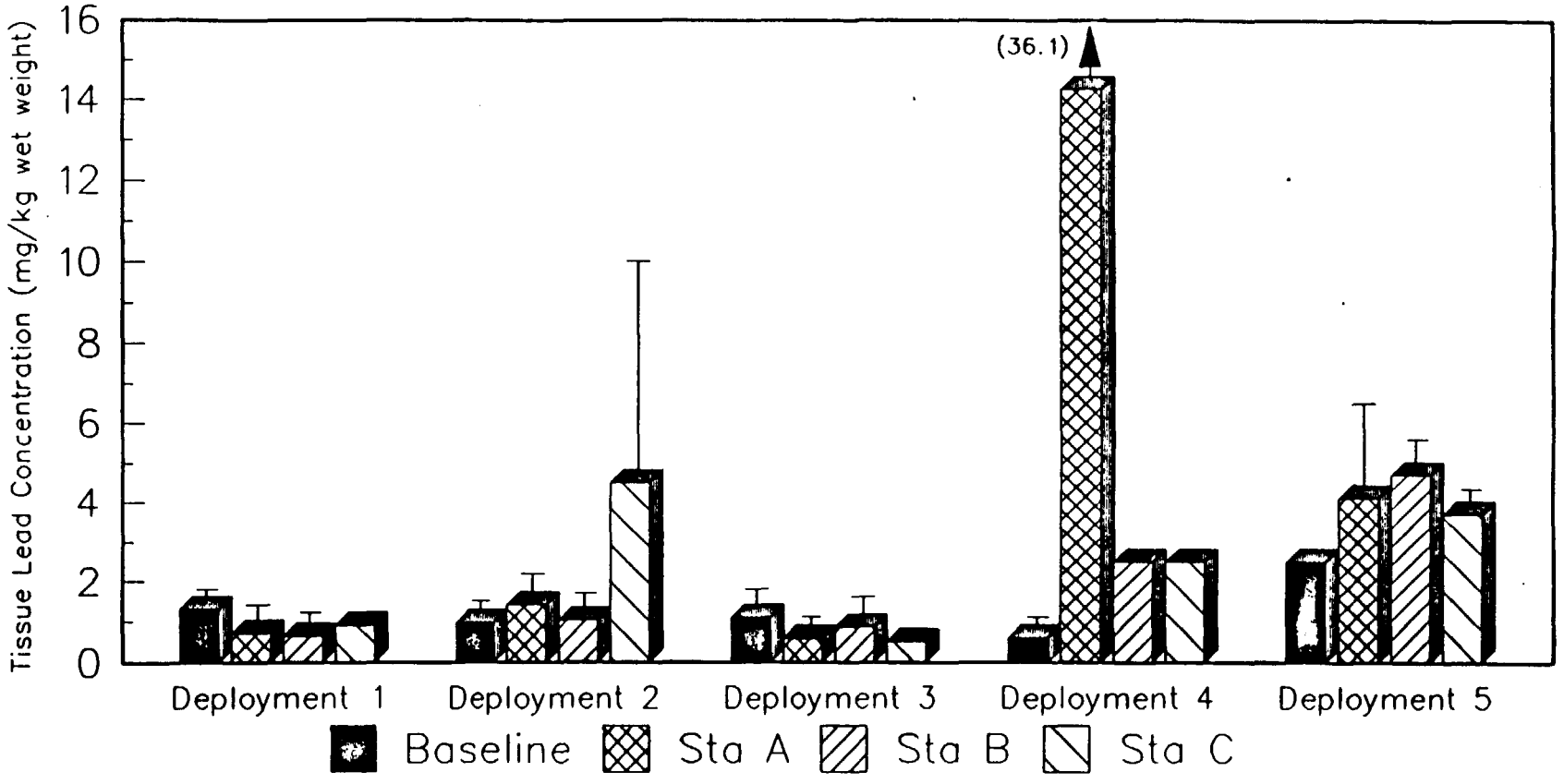


Bars represent mean mussel tissue concentration of nickel for each station. Lines above the bars illustrate one standard deviation from the mean. Where no s.d. lines are indicated s.d.=0.

FIGURE 11

MUSSEL TISSUE LEAD CONCENTRATION

For Deployments 1 Through 5

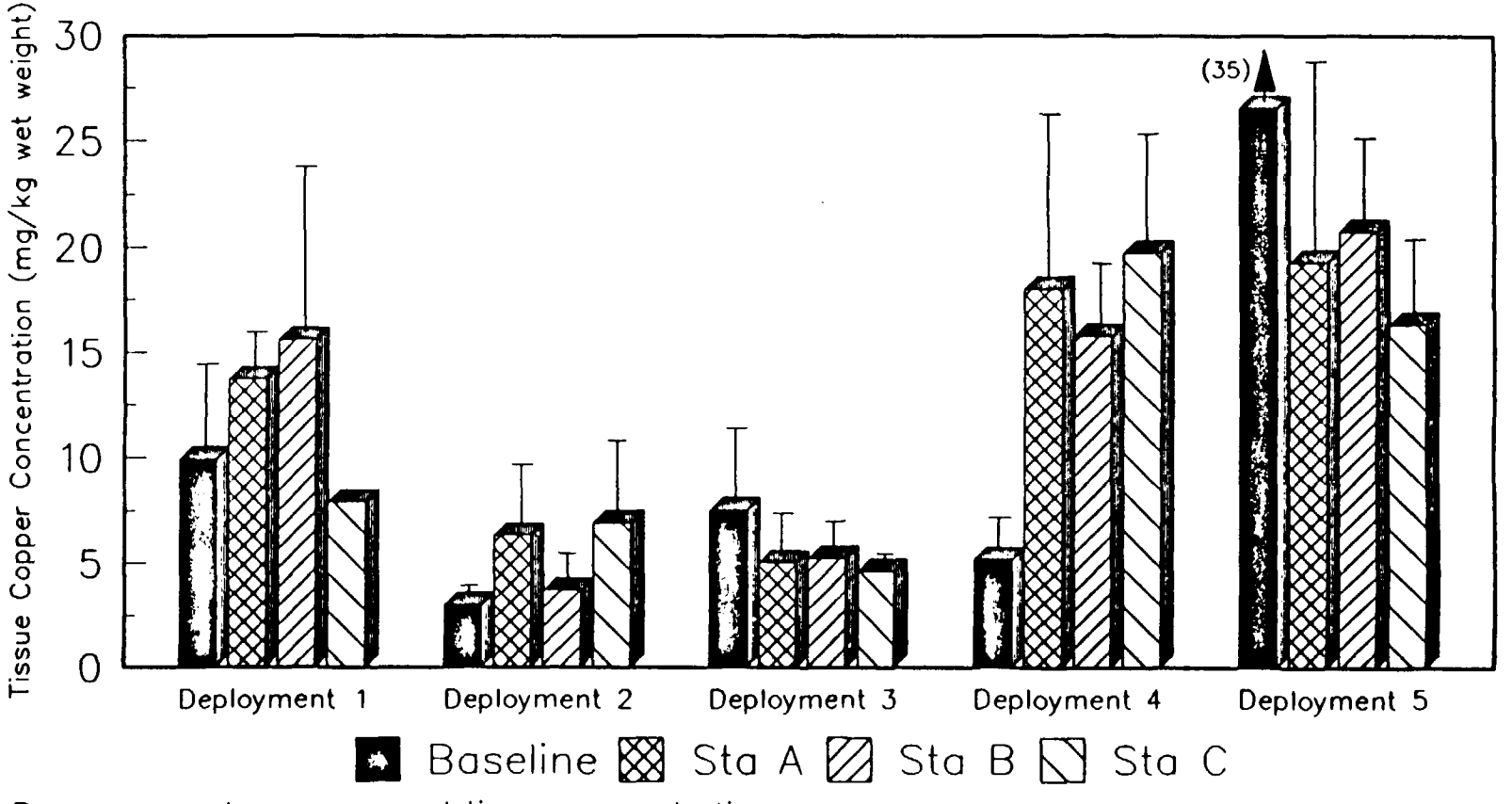


Bars represent mean mussel tissue concentration of lead for each station. Lines above the bars illustrate one standard deviation from the mean. Where no s.d. lines are indicated s.d.=0.

FIGURE 12

MUSSEL TISSUE COPPER CONCENTRATION

For Deployments 1 Through 5

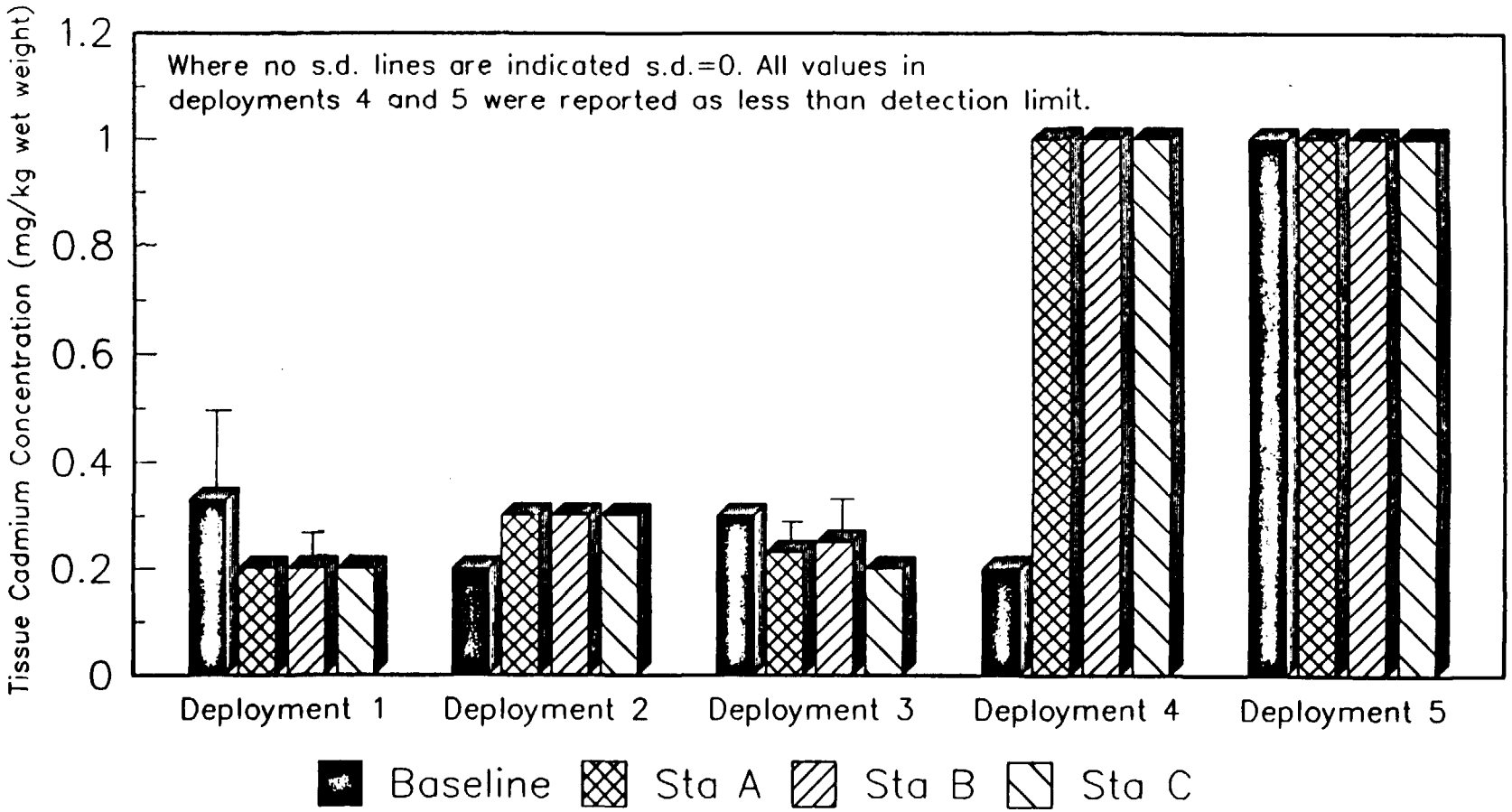


Bars represent mean mussel tissue concentration of copper for each station. Lines above the bars illustrate one standard deviation from the mean. Where no s.d. lines are indicated s.d.=0.

FIGURE 13

MUSSEL TISSUE CADMIUM CONCENTRATION

For Deployments 1 Through 5

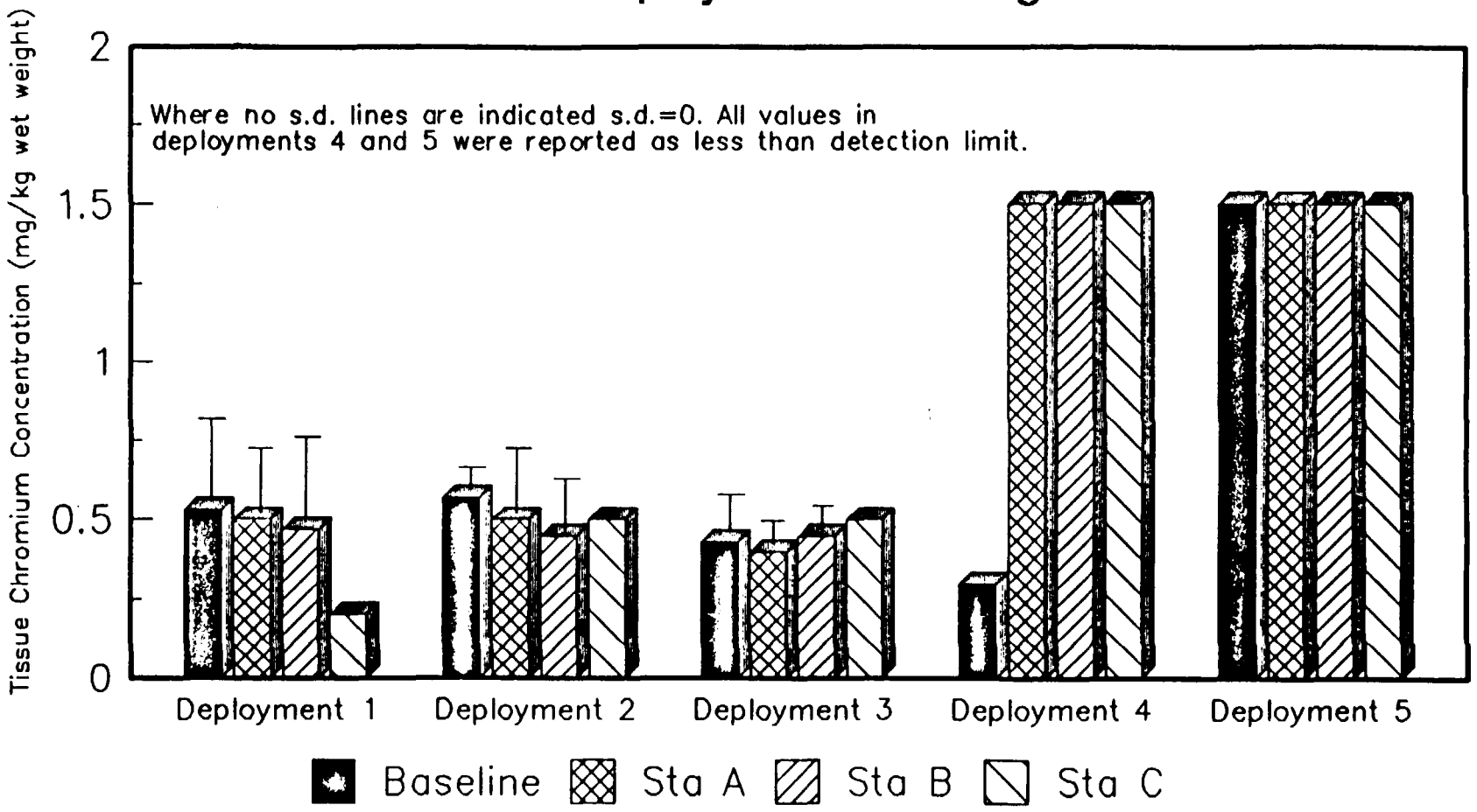


Bars represent mean mussel tissue concentration of cadmium for each station. Lines above bars illustrate one standard deviation from the mean.

FIGURE 14

MUSSEL TISSUE CHROMIUM CONCENTRATION

For Deployments 1 Through 5

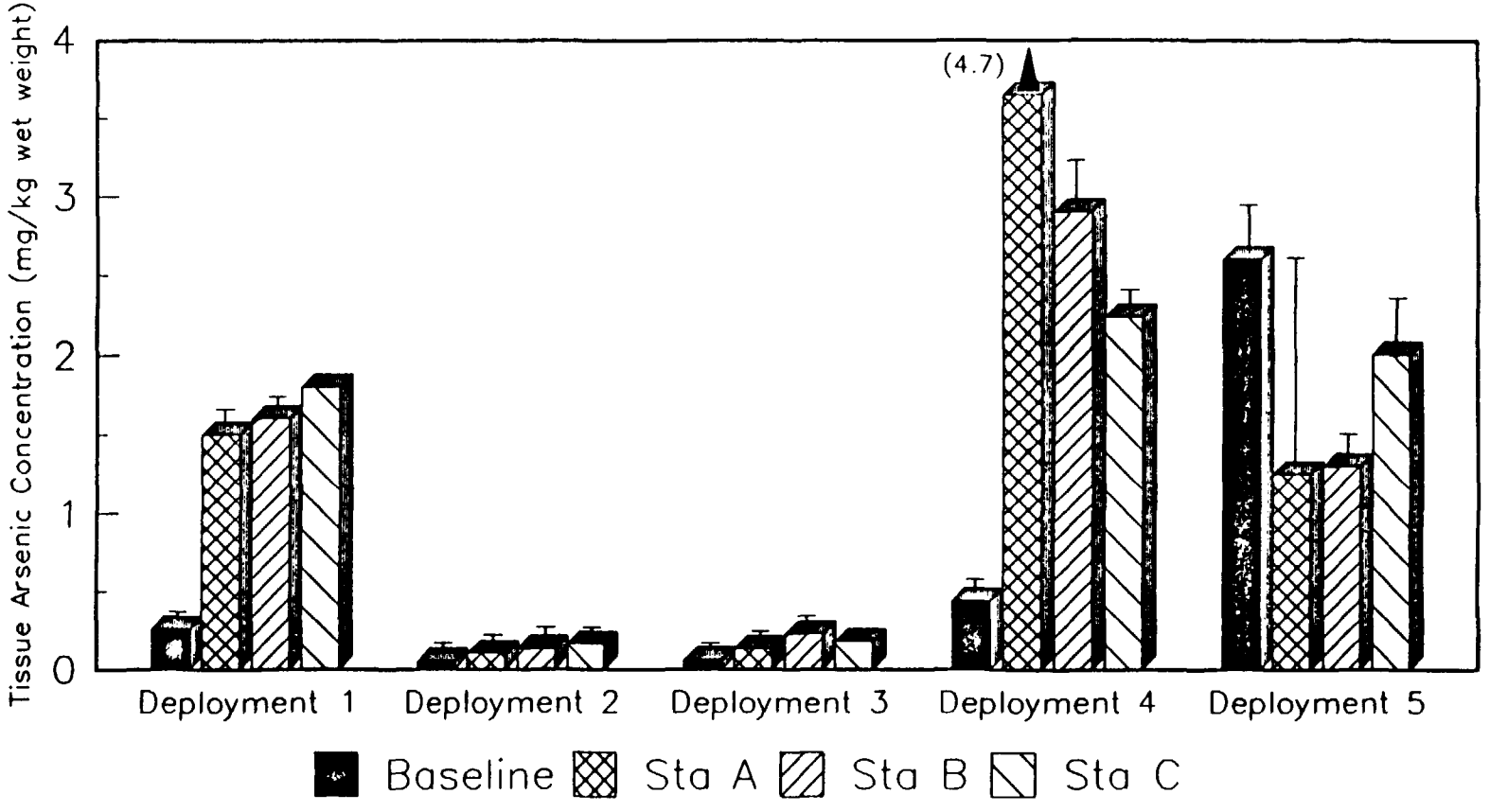


Bars represent mean mussel tissue concentration of chromium for each station. Lines above bars illustrate one standard deviation from the mean.

FIGURE 15

MUSSEL TISSUE ARSENIC CONCENTRATION

For Deployments 1 Through 5



Bars represent mean mussel tissue concentration of arsenic for each station. Lines above bars illustrate one standard deviation from the mean. Where no s.d. lines are indicated s.d.=0.

TABLE 5

SUMMARY OF KRUSKAL-WALLIS NONPARAMETRIC ANALYSIS OF VARIANCE

TRACE ELEMENT	DEPLOYMENT 1	DEPLOYMENT 2	DEPLOYMENT 3	DEPLOYMENT 4	DEPLOYMENT 5
Zinc	accept H_o	reject H_o	accept H_o	reject H_o	reject H_o
Mercury	accept H_o	accept H_o	accept H_o	accept H_o	accept H_o
Nickel	accept H_o	accept H_o	reject H_o	accept H_o	accept H_o
Cadmium	accept H_o	accept H_o	accept H_o	can not analyze	can not analyze
Chromium	accept H_o	accept H_o	accept H_o	can not analyze	can not analyze
Arsenic	reject H_o	reject H_o	reject H_o	reject H_o	reject H_o
Lead	reject H_o	accept H_o	reject H_o	can not analyze	accept H_o
Copper	reject H_o	accept H_o	accept H_o	reject H_o	accept H_o

Hypothesis being tested:

H_o: The mean trace element concentration of baseline = Station A = Station B = Station C

TABLE 6

SUMMARY OF TUKEY-TYPE NONPARAMETRIC MULTIPLE COMPARISON TEST

DATA SET		RESULTS
Zinc deployment	2:	Baseline different (lower) than Sta. A,B,C; but A,B,C same
Zinc deployment	4:	Baseline different (lower) than Sta. B; but all others same
Zinc deployment	5:	Baseline different (higher) than Sta. C; but all others same
Nickel deployment	3:	No significant differences detectable due to large standard error
Arsenic deployment	1:	No significant differences detectable due to large standard error
Arsenic deployment	2:	Baseline different (lower) than Sta. C, but all others same
Arsenic deployment	3:	Baseline different (lower) than Sta. B, but all others same
Arsenic deployment	4:	Baseline different (lower) than Sta. A, but all others same
Arsenic deployment	5:	Baseline different (higher) than Sta. A, but all others same
Lead deployment	1:	No significant differences detectable due to large standard error
Lead deployment	3:	Baseline different (higher) than Sta. C, but all others same
Copper deployment	1:	No significant differences detectable due to large standard error
Copper deployment	4:	No significant differences detectable due to large standard error

In every case, the significant differences in the means were due to the baseline mean tissue trace element concentration being different from one or more of the other stations (A, B, or C). Usually, but not always, baseline concentrations in these cases were lower.

In four out of five of the exposure periods arsenic baseline tissue concentrations were significantly different from either Station A, B, or C. In three of these data sets arsenic was lowest in the baseline samples. However, since neither Station A, B, or C were consistently highest (or lowest) throughout the study, spatial patterns of arsenic distribution in this area are not evident.

Baseline concentration of zinc for three out of five exposure periods was significantly different from Station A, B, or C. However, as with arsenic, consistent spatial patterns of distribution of zinc at these stations cannot be detected nor can further speculation as to what may be causing these differences be made.

For lead, the baseline concentration was significantly higher than Station C for one exposure period.

Tissue concentration of cadmium, chromium, mercury, nickel and copper were not significantly different for any of the exposure periods.

None of the test Stations (A, B, or C) exhibited significant differences in trace element tissue concentration indicating differences in bioaccumulation of these elements were not spatially significant for these stations. This suggests that trace element concentration available for uptake in the water column at these stations was not significantly different between Station A, B, or C.

PCB Tissue Concentrations

The results of the PCB analysis of tissue from deployments 1, 3, 4, and 5 are illustrated in Figure 16. Mean values of the data normalized with percent lipids are shown on the graph. Appendix B lists the PCB tissue concentrations as reported by LES. Only arochlor 1254 was detected in any of the tissue samples. Percent lipid concentration for each sample is also reported in Appendix B.

The lowest PCB concentrations were consistently measured in the baseline mussel tissue and the highest PCB concentrations were found in tissue from Station A. The next highest PCB concentrations were found at Station B and relatively low concentrations of PCB were usually detected in tissue from Station C.

Interlaboratory Calibration Exercise

The results from the interlaboratory calibration exercise between the Department of Environmental Protection's laboratory (LES) and the Division of Marine Fisherie's laboratory (DMF) at Cat Cove, Salem, MA are presented in Table 7.

FIGURE 16

Comparison of PCB Tissue Concentration

Normalized With % Lipids

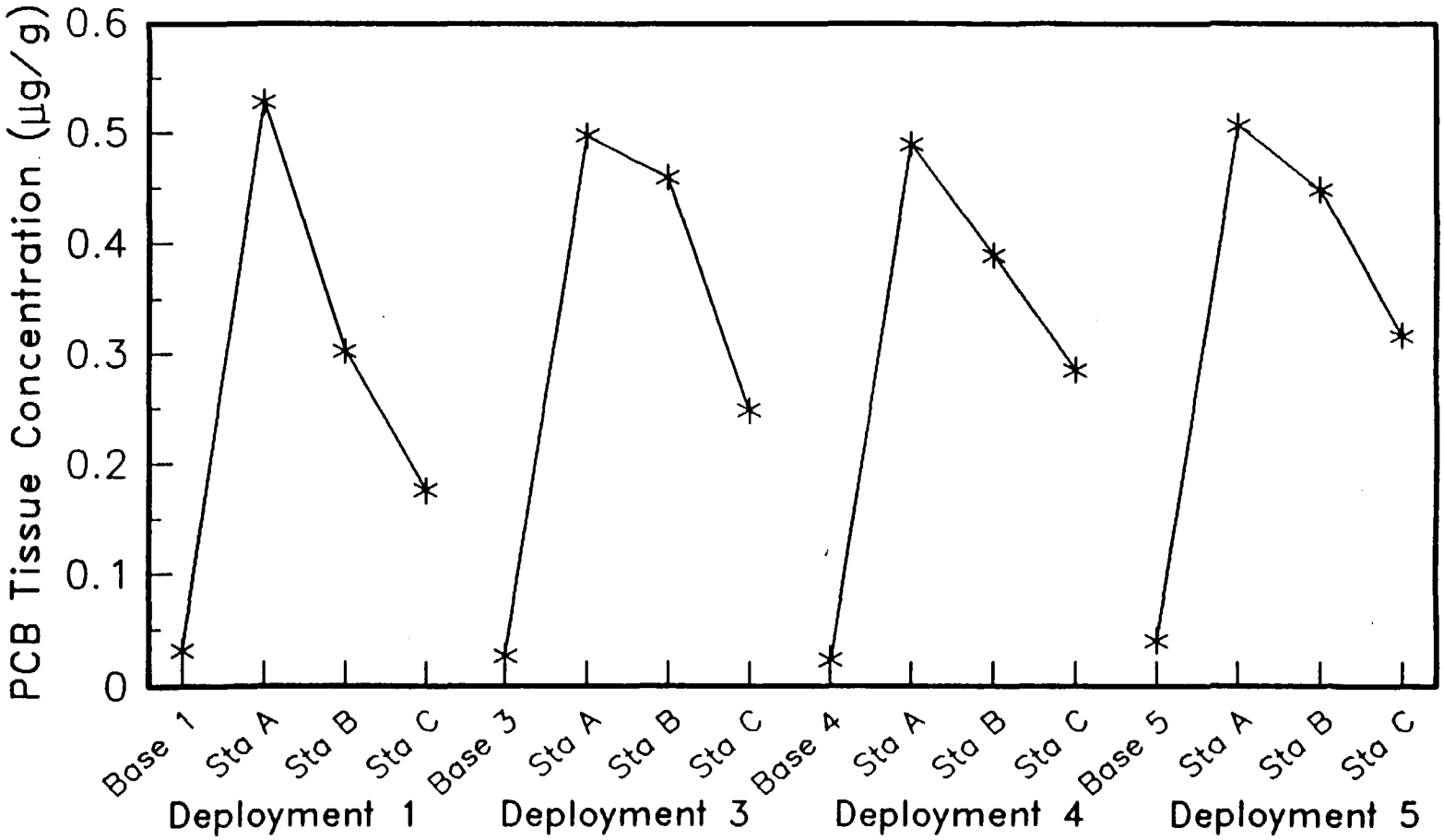


TABLE 7

INTERLABORATORY CALIBRATION RESULTS

TRACE ELEMENT CONCENTRATION (mg/kg wet weight)

SAMPLE	Cd	Cr	Cu	Hg	Ni	Pb	Zn
DEP/Repl	<1.0	<1.5	24	0.05	4.5	<2.5	70.0
DMF/M1709	0.24(SE=.003)	0.25(SE=.000)	5.11(SE=.125)	NQ*	0.89(SE=.003)	0.91(SE=.047)	15.3(SE=.376)
DEP/Rep3	<1.0	<1.5	24.0	0.02	8.5	<2.5	70.0
DMFM1710	0.211(SE=.004)	0.42(SE=.041)	4.71(SE=.047)	NQ	1.53(SE=.018)	0.88(SE=.047)	14.0(SE=.008)
DEP/C3	<1.0	<1.5	13.0	0.02	1.5	<2.5	70.0
DMF/M1711	0.182(SE=.003)	0.22)SE=.017)	2.66(SE=.026)	NQ	0.80(SE=.019)	0.74(SE=.175)	17.8(SE=.120)
DEP/B4	<1.0	<1.5	18.0	0.01	23.0	<2.5	90.0
DMF/M1712	0.21(SE=.006)	0.20(SE=.000)	3.55(SE=.090)	NQ	5.02(SE=.035)	1.02(SE=.017)	21.0(SE=.058)
DEP/C1	<1.0	<1.5	23.0	0.02	11.0	<2.5	85.0
DMF/M1713	0.18(SE=.003)	0.16(SE=.015)	4.39(SE=.029)	NQ	2.03(SE=.043)	0.83(SE=.003)	18.8(SE=.088)
DEP/B1	<1.0	<1.0	13.0	0.02	2.0	<2.5	85.0
DMF/M1714	0.172(SE=.003)	0.20(SE=.000)	2.00(SE=.042)	NQ	0.49(SE=.029)	0.69(SE=.231)	18.2(SE=.231)
DEP/C4	<1.0	<1.5	13.0	0.02	1.5	<2.5	70.0
DMF/M1715	0.17(SE=.000)	0.15(SE=.000)	2.68(SE=.019)	NQ	0.29(SE=.026)	0.39(SE=.058)	15.6(SE=.033)
DEP/A1	<1.0	<1.5	15.0	0.01	40.0	5.0	69.0
DMF/M1716	0.151(SE=.004)	0.15(SE=.000)	2.89(SE=.084)	NQ	8.54(SE=.038)	1.56(SE=.156)	19.8(SE=.088)
DEP/A2	<1.0	<1.5	14.0	<0.01	<1.5	<2.5	75.0
DMF/M1717	0.172(SE=.006)	0.18(SE=.017)	2.71(SE=.015)	NQ	0.28(SE=.015)	0.46(SE=.067)	14.4(SE=.153)
DEP/A4	<1.0	<1.5	30.0	0.03	40.0	47.0	85.0
DMF/1718	0.121(SE=.003)	0.15(SE=.000)	5.35(SE=.059)	NQ	8.62(SE=.039)	12.39(SE=.73)	21.7(SE=.285)

SE = Standard Error

*NQ = Not quantifiable for Hg 0.006 ppm < x <0.020 ppm

Methods used by Lawrence Experiment Station for the analysis of tissue samples for metals:

Hg - Cold vapor method using VGA76 hydride generator

As - EPA "Methods for Chemical Analysis of Water and Wastes," 1983. Section 206.3, gaseous hydride (FlameAA)

Cd, Cr, Cu, Ni, Pb, Zn - Flame Atomic Absorbtion Spectroscopy, Varian 1475, Standard Methods, 16th Ed. 303A.

Values for cadmium and chromium are not comparable because the detection limits of the LES analyses were much higher than the DMF's detection limits. The DMF reported "not quantifiable" concentrations of mercury with the values falling between 0.006 mg/kg and 0.020 mg/kg. This range is less than or near the detection limit reported by the LES for mercury analysis.

For copper, nickel, zinc and lead the values reported by the LES were approximately five times higher than that reported by the DMF for the same tissue homogenate samples.

On March 28, 1989 standard mussel tissue samples prepared by the EPA laboratory in Narragansett, RI were hand delivered to the Lawrence Experiment Station and the Division of Marine Fisheries laboratory at Cat Cove, Salem, MA. Results of each laboratory's analyses are presented in Table 8.

TABLE 8

INTERLABORATORY CALIBRATION RESULTS

U.S. EPA STANDARD "MEGA MUSSEL" TISSUE (mg/kg dry weight[1])

AGENCY	METAL					
	Cd	Cr	Cu	Ni	Pb	Zn
U.S. EPA, Narragansett, RI (Average tissue metals concentration)	2.08	2.15	12.8	6.84	9.11	135
Lawrence Experiment Station[2]	1.9	1.9	11.7	6.2	8.3	119
U.S. EPA range of values[3]	1.99-2.18	1.91-2.36	12.2-13.8	6.37-7.24	7.94-10.25	126-142
Division of Marine Fisheries[4]	2.17	1.98	11.7	-	8.06	90

[1] LES obtained dry weight of sample by drying homogenate for 2 days at 90°C and weighing entire sample.

[2] LES results are reported as an average of 2AA analyses (except for Pb - only enough sample for one analysis).

[3] U.S. EPA analyzed 50 samples to obtain range and average tissue metals concentration of the standard homogenate.

[4] Division of Marine Fisheries results were converted to dry weight by multiplying wet weight values by 6.83 (EPA's reported wet/dry weight ratio for the "mega mussel" homogenate).

DISCUSSION

Study Design

Studies that involve comparisons of selected variables over space and time ideally require that all environmental conditions that may affect test results be similar either through controlled laboratory conditions or, in field studies, as a function of study design. However, too much control placed on the experimental design may create an artificial situation which may obscure interpretation of the relationship of the data to actual field conditions. For this study it was important that the stations selected exhibit very similar measurable environmental conditions. The three stations chosen were oriented on a north-south transect from the head of shallow Clarks Cove to open water in Buzzards Bay; consequently depth was not the same at each location. (5 meters at Station A and B vs 9 meters at Station C.) Despite depth differences however, temperature, dissolved oxygen and salinity were essentially the same at each station supporting the assumption that all of the animals were most likely exposed to similar environmental conditions during each deployment period.

Growth (as measured by average shell length increases over the exposure period) and mortality were not significantly different between stations which also indicates that the environmental conditions necessary for mussel growth and survivorship at each site were the same.

If growth differences between sites were evident in this study, then differences in tissue trace element bioaccumulation between sites (if present) would be more difficult to interpret and could not necessarily be attributed solely to available contaminant concentrations in the water column.

Enseco, Inc. (1990) reported that mussels deployed near sewage treatment outfalls in Boston Harbor that survived appeared to be generally healthier than reference site organisms. Based on these findings, assumptions that more polluted sites would negatively affect the health (and growth) of test animals cannot be made.

Although not performed in this study other methods of growth or condition assessment may be more effective than simple shell length measurements. A practical method of determining a body condition index should be investigated and, if at all possible, applied in future caged mussel studies of this kind.

Mortality was usually very low except for the last exposure period where predation by starfish was suspected to have caused the 25-53 percent mortality observed in the cages. Although it is not known if starfish predation on bivalves occurs seasonally in Buzzards Bay it may be wise to avoid deploying the mussels in cages during this time of year in this particular area. For all but the last deployment, the low mussel mortality assured sufficient numbers of animals for tissue analysis. In addition, similar mussel growth, mortality and environmental conditions found at each station reduces sources of variation that may influence spatial differences in contaminant uptake by the mussels.

Coliform Contamination

The use of caged mussels to monitor coliform contamination over time and space was ineffective. Since the animals clear their gut in approximately 24-48 hours, any assumptions regarding temporal changes in bacteria concentration in the water column are limited to one day time periods. Furthermore, the potential for encountering variability within the stations is high due to the fact that the animals are filter feeders, and may each be filtering different volumes of water over a 24-hour periods and thus ingesting highly variable amounts of bacteria over this relatively short time. For this reason, monitoring of coliform bacteria to detect long-term changes in water column bacteria densities should not be performed via tissue concentrations.

Monitoring whole mussel tissue bacteria concentration is potentially a valid technique for making spatial comparisons of bacteria concentrations in the water column at discrete time periods. However, the method is much more labor intensive, results are highly variable, and it offers only slight advantage (i.e, from a temporally non-integrated grab sample of water versus a 24-48 hour time integrated tissue sample) over simple, direct water column bacteria sampling methods.

Trace Element Concentration

As is evident from the data, tissue trace element concentration was extremely variable, not only statistically between replicates at each station, but spatially and temporally as well. Due to variation within the data set significant differences in trace element concentrations, if they existed in the water column at any of these stations over time, were not usually detectable. The magnitude of trace element bioaccumulation in the mussel tissue was small in comparison to this variability. It is important to examine the major factors that may influence the variability of the data and its resulting usefulness.

The often large variances of the station replicates as well as the differences in average tissue trace element concentration between Stations A, B and C (spatial differences) and between baseline mussel tissue and Stations A, B, and C (temporal differences) may be the result of any one, or a combination of the following factors: 1) natural seasonal variability; 2) data bias or errors resulting from field study design and implementation; 3) data bias or errors resulting from laboratory procedures; 4) actual temporal or spatial differences in water column trace element concentrations.

Natural seasonal variation can account for as much as 15-60 percent of the variability in observed values (Capuzzo, et. al., 1987). Seasonal variation may be a result of the physiological state of the animals, environmental conditions, and metal speciation and bioavilability (Capuzzo, et. al., 1987). Seasonal variability would not influence the between station (spatial) differences of the data because comparisons of these results were made between mussel tissue from the same exposure period. As previously discussed, results indicated that these mussels were experiencing similar seasonal environmental and physiological conditions as measured by temperature, dissolved oxygen, salinity, chlorophyll a concentrations, and shell growth at each site.

Tissue trace element concentrations were not compared at each station over several exposure periods. With this study design, comparisons of this type would

be weak because discrete groups of animals were set out and measured each exposure time, rather than subsampled periodically from a large group that had been exposed for the entire study year. However, seasonal variability may have caused differences between baseline tissue concentrations and Stations A, B, and C since baseline animals were collected in Sandwich at the beginning of the exposure period approximately 60 days earlier than the animals they were compared to from Clarks Cove.

Percent lipids were not measured in the tissues homogenized for trace element analyses; except for growth, no other parameters were measured to assess the physiological condition of the mussels. Percent lipids were measured in tissue homogenate prepared for organics analysis (see Appendix B). Although not assessed during this study, spawning condition of the animals is known to be directly related to whole animal percent lipid concentration. Lipid concentration increases as animals prepare to spawn and drops sharply after spawning. Spawning reportedly leads to loss in tissue weight, increase in percent water and decline in condition indices. Prior to spawning lipid-rich gametes may contain higher concentrations of lipophilic organic contaminants and lower concentrations of heavy metals than somatic tissues. After spawning a drop in organic concentrations and an increase in metal concentrations may result (Robinson and Ryan, 1988). Therefore, to greatly enhance tissue data interpretation future caged mussel studies should include an assessment of the spawning condition of the animals. This should be made at the time of deployment, when baseline trace element tissue concentrations are measured, and when the animals are retrieved after the exposure period. Inferences about adverse impacts of toxic trace elements on the health of the mussel cannot be made, although this factor may have also been responsible for some variability of the data. Animals showed an average increase in shell length for each exposure period. Average shell increases were the largest during the third and fourth deployments (March 16 - May 11 and May 11 - June 16, respectively). No correlation between growth and trace element concentrations can be made. The goals of this study did not include an attempt to relate contamination concentration with indications of stress in the organism.

As previously discussed, the field study design attempted to equalize as many between station environmental variables as possible. The study design may benefit from including at least one more replicate at each station since the variances between the four replicates were often high. In addition, it has been suggested that not all of the animals receive equal exposure time bunched-up in the square cages. Flat cages that spread the animals into one-layer would allow all to have more of a chance to filter equal amounts of water. Cages of this design were not available for this study. To reduce the likelihood of this type of bias animals were selected randomly from the bunches in the cages when preparing the sample bags for the laboratory. Other studies performed with square cages did not report evidence of this type of bias (Robinson and Ryan, 1986, 1988 and Nelson, personal communication).

Besides the systematic or random variability introduced via seasonality and field study design, data variability introduced through laboratory procedures must be considered an important factor when interpreting the results. The Lawrence Experiment Station analyzes samples in "batches." QA/QC tests are performed on

a percentage of samples from each batch. The QA/QC results during this study were acceptable, suggesting that variation between stations and/or replicate samples was due to other factors (i.e., the effects of seasonality, or differences in contaminant concentrations).

Determination of dry weight concentrations of the trace element was not requested as part of this study. However, water content is extremely variable in these animals, not only seasonally but individually, and will definitely affect the calculation of the results. Ideally, dry weight should be determined separately for each sample homogenate prepared, rather than using an average dry weight of mussel tissue to normalize the data. Robinson and Ryan (1988) state that in transplant studies it is impossible to determine whether metal body burdens actually changed as a result of exposure if changes in tissue weights were not monitored. They report that changes in mussel tissue weight can be assessed by measurements of tissue dry weight, condition index and gonadal index. Future tissue biomonitoring studies should include a determination of tissue dry weight to reduce data variability.

Possible sources of data variability were discussed with LES personnel and they included procedures in sample preparation and analytical methodologies. Some of these sources can be minimized with the use of a more efficient method of tissue homogenation and/or via procedural modifications such as determining the dry weight of samples and using consistent sample sizes for analysis throughout the study.

Results of tissue metals concentration from this study and ranges of values reported for several other metals bioaccumulation studies are compared in Table 9. Arsenic concentration was not measured in the other studies listed here, so it is not included in this comparison. Mercury, cadmium, and chromium concentrations fall within the ranges reported by other researchers. Mercury concentration never approached the US Food and Drug Administration limit of 1 $\mu g/g$ wet weight. Cadmium and chromium were also very low, often below the detection limits of the analyses, and concentrations never fluctuated much from site to site, nor did they vary over exposure times. From this study, it appears that mercury, cadmium and chromium either require a longer exposure period to bioaccumulate in the mussel or there were low concentrations of bioavailable metal in the water column at these sites. de Kock and van het Groenewoud (1985) report that cadmium accumulation is a slow process requiring about 150 days to reach equilibrium values. These researchers were also unable to demonstrate differences in mercury concentration from several sites in 60 day transplant studies. Robinson and Ryan (1988) state that transplanting clean mussels to polluted sites to assess seawater contaminant levels is only successful when metals concentrations are high enough to result in appreciable bioaccumulation.

Maximum concentrations of lead, copper, nickel, and zinc greatly exceeded ranges reported from other studies (see Table 9). Station A tissue most often contained the highest metals concentrations; however as previously discussed, between station differences of tissue concentrations of these metals could not be detected due to large within station variances. In general, seasonal peaks in Cu, Ni, Pb and Zn tissue concentration occurred more in the late spring and early summer (also the period when the greatest shell length increases were measured).

Possible reasons for these extremely high values of Zn, Cu, Ni, and Pb includes laboratory sources of variability discussed previously as well as the natural

TABLE 9

TISSUE TRACE ELEMENT CONCENTRATION-COMPARISON TO OTHER STUDIES

(Ranges of values reported in mg/kg wet weight)

Metal	This Study	1976-1978[1] U.S. Mussel Watch (Mass. Sites)	1977-1979[2] U.K. Mussel Watch	1983 N.E. Aquarium	1983-1984[1] Feng, et al	DEP Salem Harbor Study
Hg	<0.01-0.07	Not measured	0.01-0.46	0.006-0.014	0.0182-0.0266	0.043-0.075
Cd	<0.2-0.5	0.14-0.53	0.12-16.7	0.18-0.27	0.126-0.3192	0.0-0.05
Pb	<0.5-47.0	0.49-4.5	<0.3-35.0	0.45-1.30	Not Measured	4.5-5.0
Cu	2.1-39.0	0.602-4.9	0.67-6.9	1.07-1.13	1.068-2.206	1.8-2.3
Zn	12.0-115.0	8.82-28.0	6.5-90.0	13.4-20.1	17.78-27.3	0.5-21.0
Ni	<0.05-40.0	0.056-0.266	Not measured	0.17-0.23	0.3094-0.7042	0.0-1.3
Cr	<0.2-0.7	Not measured	Not measured	0.14-0.17	0.2688-0.8064	2.8-4.0

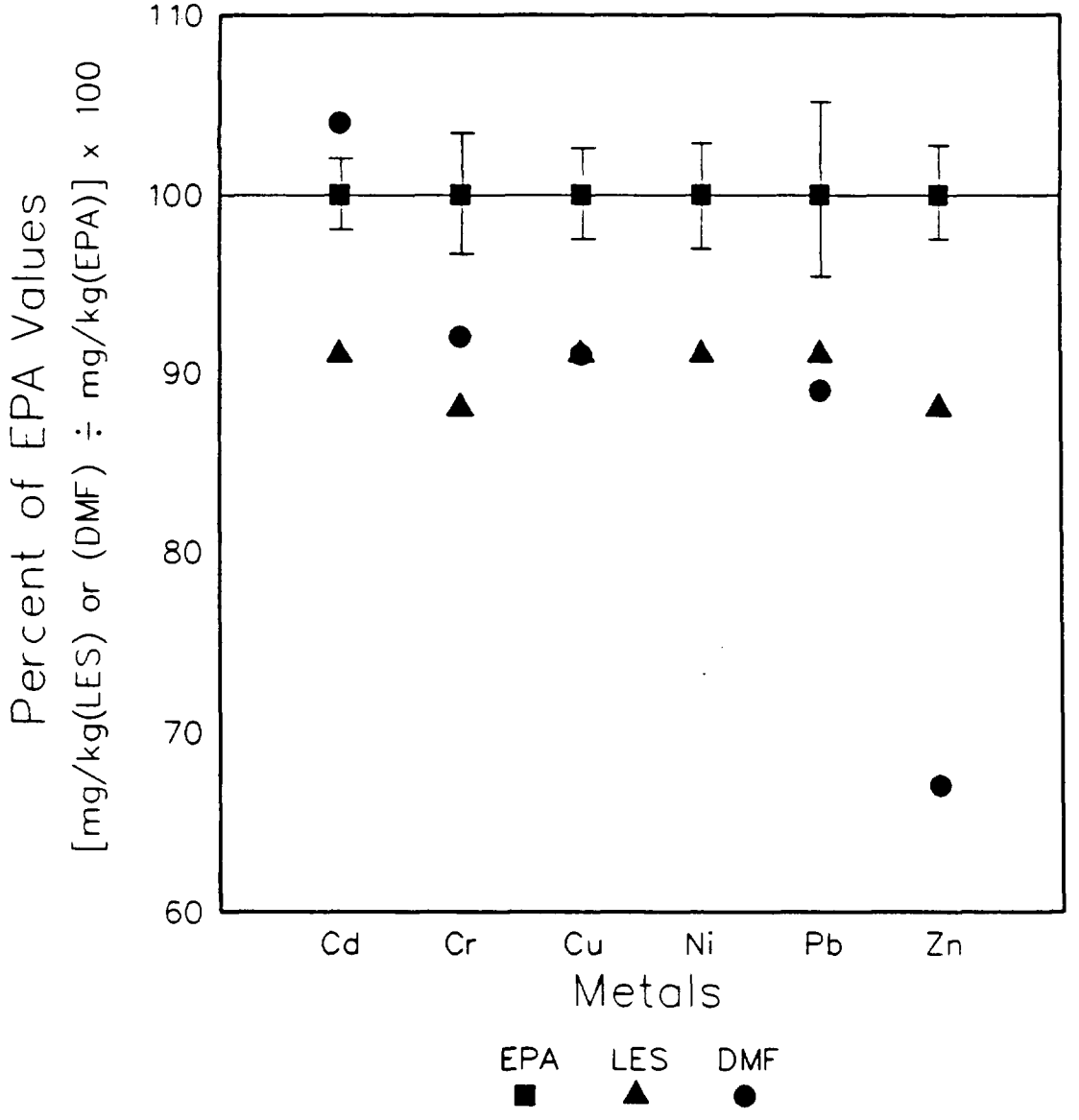
[1] As reported by Capuzzo, et al., 1987

[2] As reported by Farrington, et al., 1987

[3] Robinson and Ryan, 1988 (Results converted from dry weight to wet weight using tissue wet weight values as reported in 1988 study.)

FIGURE 17

Plot of Lawrence Experiment Station (LES) vs. Division of Marine Fisheries (DMF) Values as a Percent of EPA "Mega-Mussel" Values



Error bars indicate one standard deviation.

variability of actual metals concentration and varying rates of bioaccumulation and regulation of each metal by Mytilus edulis during different times of the year.

No range of arsenic tissue concentrations were available for comparison. Ranges of concentration for this element were not included in Table 9 for this reason. For arsenic the within station variances were usually lower than for other metals. Arsenic concentrations from July and September samples were significantly higher at all stations than at any other time of year.

Interlaboratory Calibration Exercise

The interlaboratory calibration exercise with the DMF yielded differences in tissue wet weight concentrations of copper, nickel, and zinc from aliquots of the same sample homogenate. Values of cadmium, chromium, and lead reported by DMF were lower than the detection limits established by LES in their analysis therefore these metals data were not comparable. DMF obtained unquantifiable concentrations of mercury (0.006 ppm<x<0.02ppm) which were also not comparable to the LES values.

The LES values of Cu, Ni, and Zn were on the order of 5-6 times higher than the DMF results. Discussions with the LES and DMF concluded that differences in their results could not be definitively explained. It is difficult to attribute the differences in results to the analytical equipment because samples were exposed to variations in handling before being extracted for analysis. It was not the goal of this exercise to isolate and test for variability in analytical equipment, otherwise complete sample preparation would have been performed at only one laboratory. Galloway, et. al. (1983) found that identical techniques in different laboratories do not necessarily give similar results. They report that homogenates of several matrices prepared by two different agencies and subjected to intercomparison exercises have consistently shown wide ranging results. For comparison of methodologies used by each laboratory please refer to Appendices A and D.

In an attempt to assess whether these differences were actually due to differences in laboratory techniques and not in the samples themselves, each laboratory was requested to analyze standard EPA mega mussel tissue homogenate. Results show concentrations obtained by the LES are within 9-12 percent of the values reported by EPA (Figure 17). It is interesting to note that the LES values are all slightly less than EPA average concentrations. The LES results for Pb and Cr fall within the range of values reported by EPA. Cd, Cu, Ni, and Zn values were only slightly less than the minimum EPA values. DMF results for Cd, Cr, and Pb fall within EPA's reported ranges. As illustrated in Figure 17, values for Cd, Cr, Cu, and Pb reported by DMF are within 4 - 11 percent of EPA averages. DMF did not report results for nickel. Zinc concentration obtained by DMF was comparatively low however, differing by more than 30 percent from the EPA average.

Considering that the EPA averages were based on a sample size of 50 and the LES and DMF values were derived from an average of two (or less) analysis, results for these trace element analyses appear to be in very good agreement among the laboratories. Both laboratories tended to have a bias toward lower values as compared to EPA results but the reasons for this trend are unknown.

Based on the results of the "mega mussel" interlaboratory calibration exercise, no evidence for why the results of the study mussel tissue interlaboratory analyses were so different between LES and DMF can be found.

Tissue Concentrations of PCBs and PAHs

This project only required that a portion of the animals from each exposure period be archived (frozen) for future organics analysis. However, since the organics laboratory at the LES was able to perform the analysis on many of the archived samples during the study period, the results are presented and briefly discussed as part of this report.

No PAHs were detected in the tissue samples from either Clarks Cove or Sandwich, MA. In contrast, Capuzzo, et. al. (1987) report mussel tissue collected from a variety of sites in New England, including Cape Cod, contained detectable levels of PAHs. Eisler (1987) however, found in general that PAHs show little tendency to biomagnify in food chains. He attributed this to the fact that most PAHs are rapidly metabolized. Specific reasons for PAHs not being detected in this study cannot be offered. An interlaboratory comparison between the LES and EPA, Narragansett or Woods Hole Oceanography Institute organics laboratories may provide some insight as to what is happening here.

PCB tissue concentrations were normalized by the percent lipid concentration of the sample to account for differences in PCB concentration created by differences in lipid content of the tissue. As seen from Figure 16 the results show a consistent pattern of higher PCB concentrations in the tissues from Station A to decreasing amounts in Station B and even lesser amounts in Station C. Not only are spatial differences evident, but differences can be seen between baseline and test site concentrations for each deployment period. From this consistent pattern it appears that 60 day exposure periods allow sufficient time for bioaccumulation of measurable amounts of PCBs in mussels. EPA recommends at least 30 days (U.S. EPA, 1983), although differences in PCB concentration of test animals have been detected after just 2 weeks of exposure in New Bedford Harbor (W. Nelson, personnel communication).

Based on the well documented PCB contamination in New Bedford Harbor it is not surprising that PCB concentrations at Station B as well as Station A, were relatively high. The area that encompasses both stations has been closed to bottom fishing and lobstering by the Department of Public Health due to PCB contamination. None of the tissues from this study contained PCBs in excess of the FDA action level of 2.0 $\mu g/g$. Concentrations ranged from <0.04-1.2 $\mu g/g$. This range falls within that observed in US Mussel Watch data from Cape Cod and Buzzards Bay. In New Bedford Harbor the range of PCB tissue concentration from Mussel Watch data was much higher (3.08-6.86 $\mu g/g$) (Capuzzo, et. al., 1987).

In this study the use of *Mytilus edulis* as a sentinel organism to monitor PCB contamination in the water column appears more successful than for monitoring metals contamination. The standard deviations of the station replicates were low and concentration averages followed an expected pattern for every deployment. Sediment PCBs also followed a similar, relative concentration gradient. Results appear to be more straightforward to interpret both spatially and temporally. Also by normalizing with percent lipids much of the variability that may have been introduced as a result of differences in reproductive condition of the animals was eliminated.

SUMMARY

The use of caged mussels for coastal biomonitoring proved to be a very feasible field technique from the standpoint of available resources at the Technical Services Branch of DWPC. Questions that remain should be addressed through increased communication with the analytical laboratory, continued interlaboratory calibration exercises, and modification of the study design. Based on the results and suggestions from other researchers, several modifications of the study design and analytical procedure are recommended: 1) trace elements that exhibited low bioconcentration should be eliminated from the study (Cd, Cr, and Hg); 2) tissue dry weight should be determined for each sample homogenate; 3) the sample should be thoroughly homogenized; 4) interlaboratory calibrations should continue with sample tissues from the study sites as well as with a standard tissue homogenate (EPA mega mussel); 5) increase focus on using this technique to monitor PCB contamination; 6) examine the effect of longer exposure periods by subsampling from a large group of transplanted mussels over a one year period; and 7) the method should not be used to monitor coliform bacteria contamination.

In most of Buzzards Bay, metals contamination is most likely not high enough to bioaccumulate to statistically significant amounts. If definitive bioaccumulation was not measured at Clarks Cove, other less impacted areas would be less expected to show significant bioaccumulation of tissue in trace element concentrations. From this study it is evident that actual differences, either spatial or temporal would have to be very large to be significant. However, this study as well as others indicate that temporal and spatial characterization of changes in PCB contamination are possible using caged mussels. Serious consideration should be given to using this technique as part of a LONG-TERM monitoring program in Buzzards Bay, especially in the New Bedford area.

It is important that biomonitoring studies such as this continue to be developed and performed by agencies responsible for water quality monitoring. Of the three basic methods used to assess pollutants in the coastal environment; water sampling, sediment sampling, and sampling of biota, the later has received the least attention by the Massachusetts Division of Water Pollution Control. The bioavailability of contaminants however, should be a major concern, not only because it can provide a means of determining time-integrated pollutant concentrations but because of the long-term implications to human health, and more important, the overall health of the ecosystem. Although water pollution standards today are based on measurements of water and sediment, a contaminant can only be considered a threat to the environment if it can be taken up by the biota.

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APPENDIX A

FIELD METHODOLOGY

AND

LAWRENCE EXPERIMENT STATION LABORATORY METHODOLOGY

TABLE A-1

COMMON SAMPLE TREATMENT METHODS

PARAMETER	SAMPLE VOLUME	SAMPLE CONTAINER[1]	IMMEDIATE SHIPBOARD PROCESSING & STORAGE
Dissolved Oxygen	300 ml (2)	G (1)	$MnSO_4$; KI: no sunlight/ or (4) "in situ."
Temperature	-	- (1)	In situ recorded to nearest 0.1°C/F or (3), (4), (5)
Specific Conductance	1 1 (2)	P/G (1)	"In situ" reading/or cool 4°C (3), (4)
Total Solids	1 1 (2)	P/G (1)	Cool 4°C
Suspended Solids	1 1 (2)	P/G (1)	Cool 4°C
Chloride	1 1 (2)	P/G (1)	Cool 4°C
Total Kjeldahl-Nitrogen	500 ml (2)	G (1)	H_2SO_4, pH $\leq$2.0, cool 4°C
Ammonia-Nitrogen	500 ml (2)	G (1)	H_2SO_4, pH $\leq$2.0, cool 4°C
Total Phosphorus	500 ml (2)	G (1)	H_2SO_4, pH $\leq$2.0, cool 4°C
Orthophosphate	500 ml (2)	G (1)	H_2SO_4, pH $\leq$2.0, cool 4°C
Turbidity	1 1 (2)	G (1)	Cool 4°C
Chlorophyll a/ Phytoplankton	200 ml	P/G (1)	Cool 4°C (5)

[1] G - Glass
P/G - Polypropylene or glass

(1) Required containers, preservation techniques, and holding time, per Table II 40 CFR Part 136.

(2) Massachusetts Division of Water Pollution Control, Technical Services Branch, Engineering Section, Standard Operating Procedures.

(3) Yellow Springs Instrument, Model 33-S-C-T meter and probe. Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio 45387.

(4) Hydrolab Surveyor II, Model SVR2-SU sonde unit, Model SVR2-DV Digital read out. Hydrolab Corp., P.O. Box 50116, Austin TX 78763.

(5) Massachusetts Division of Water Pollution Control, Technical Services Branch, Biomonitoring Program 1988, Standard Operating Procedures.

TABLE A-2

PARAMETER AND COLLECTION METHODS EMPLOYED AT SEDIMENT STATIONS

PARAMETER	SAMPLE VOLUME (Liters)	SAMPLE CONTAINER	IMMEDIATE FIELD PROCESSING & STORAGE
PCB 1016/1242 Sediment	2(25-100 g)	G/Aluminum Foil Septum	Cool to 4°C
PCB 1248 Sediment	2(25-100 g)	G/Aluminum Foil Septum	Cool to 4°C
PCB 1254 Sediment	2(25-100 g)	G/Aluminum Foil Septum	Cool to 4°C
PCB 1260 Sediment	2(25-100 g)	G/Aluminum Foil Septum	Cool to 4°C
PAHs Sediment	2(25-100 g)	G/Aluminum Foil Septum	Cool to 4°C
Metals Sediment	25-100 g	G/Teflon Septum or Plastic Wrap Septum	Cool to 4°C

G = Glass

TABLE A-3

SUMMARY OF RATED ACCURACY OF FIELD METERS AND UNIT OF MEASURE

PARAMETER	UNIT OF MEASURE	RATED ACCURACY	METER
Temperature	Degrees Centigrade	± 0.6°C	YSI Model 57 Dissolved Oxygen Meter
	Degrees Centigrade	± 0.6°C	YSI Model 33 SCT
	Degrees Centigrade	± 0.1°C	Hydrolab Surveyor II
Dissolved Oxygen	mg/l	± 3% of D.O. reading over entire temperature range of probe (-5 to + 45°C)	YSI Model 57 D.O. Meter
	mg/l	± 0.2 with temperature and salinity compensation	Hydrolab Surveyor II
Specific Conductivity	µmhos/cm	± 2.5% - ± 3.0%	YSI Model 33 SCT
	µmhos/cm	range from ± 0.015 to ± 1.5 with 25°C temperature compensation	Hydrolab Surveyor II
Salinity	Parts per thousand (°/oo)	± 0.7	Hydrolab Surveyor II
Depth	meters	± 1	Hydrolab Surveyor II

TABLE A-4

PARAMETERS AND ANALYTICAL METHODS FOR WATER SAMPLES

PARAMETER	METHOD	REPORTED AS	LIMITS OF DETECTION	REFERENCE	MAXIMUM HOLDING TIME
Dissolved Oxygen	Azide modification of Winkler method. 0.0375 N sodium thiosulfate titrant, 300 ml sample	mg/l D.O.	±0.05 mg/l	Standard Methods 15th ed. sec. 421B	8 hours
Turbidity	Nephelometric. Hach Turbidimeter. Model 2100A	Nephelometric Turbidity Units	-	Standard Methods 15th ed., sec. 214A	48 hours
Suspended Solids	Filtration through standard glass fiber filter paper. Residue dried at 103-105°C. Gravimetric	mg/l S.S.	10 mg/l	Standard Methods 16th ed., sec. 403, p269	48 hours
Total Solids	Evaporation to dryness at 103 - 105°C. Gravimetric	mg/l T.S.	5 mg/l	Standard Methods 16th ed., sec. 209C, p96	7 days
Total Kjeldahl-Nitrogen	Acid digestion using Technical BD-40 Block Digester. Colorimetric analysis (reaction of ammonia, sodium salicylate, sodium nitroprusside, and sodium hypochlorite in buffered alkaline medium) using Technicon Auto Analyzer II	mg/l TKN	0.05 mg/l	EPA 1979, p351.2	28 days
Ammonia-Nitrogen	Phenate method, automated. Colorimetric analysis using Technicon Auto Analyzer II	mg/l NH_3-N	0.02 mg/l	Standard Methods 15th ed., sec. 417F	28 days

TABLE A-4 (CONTINUED)

PARAMETER	METHOD	REPORTED AS	LIMITS OF DETECTION	REFERENCE	MAXIMUM HOLDING TIME
Total Phosphorus	Acid digestion using Technicon BD-40 Block Digester. Ascorbic acid reduction colorimetric method using Technicon Auto Analyzer II	mg/l P	0.02 mg/l	EPA 1979, p365.4	28 days
Chlorophyll a	Fluorometric	mg/m^3	-	MDWPC, 1988, p98	12 months
Conductivity	Wheatstone Bridge type meter. Yellow Springs Instrument conductivity bridge, Model 31	umhos/cm	-	Standard Methods 15th ed., sec. 205	28 days
Chloride	Argentometric (titration with silver nitrate)	mg/l Cl	0.5 mg/l	Standard Methods 15th ed., sec. 407A, p287	28 days
Temperature	"In situ" reading	C°/°F	± 0.05	Omega dial temp. thermometer models K-79-8, K-79-7. Omega Engineering Inc. Stamford, CT.	Analyze Immediately
Orthophosphorus	Ascorbic acid method	mg/l as P	0.01 mg/l	Standard Methods 15th ed., sec. 314A	48 hours
Depth	Hydrolab Surveyor II	meter	0.1 meters	Hydrolab Surveyor II, Model SVR2-SU Sonde Unit, Model SVR2-DV Digital readout	-

TABLE A-5

PARAMETERS AND ANALYTICAL METHODS FOR SEDIMENT SAMPLES

PARAMETER	METHOD	REPORTED AS	LIMITS OF DETECTION	REFERENCE	MAXIMUM HOLDING TIME
Metals Analysis					
Cadmium - Sediment	AA spectro air-acetylene flame (3)	mg/kg(d.w.)*	0.2	EPA Method 213.1	6 months
Total Chromium - Sediment	AA Spectro air-acetylene flame (3)	mg/kg (d.w.)*	0.2	EPA Method 218.1	6 months
Total Copper - Sediment	Atomic Absorption, direct (3)	mg/kg (d.w.)*	0.2	EPA Method 220.1	6 months
Total Lead - Sediment	Atomic Absorption, direct aspiration (3)	mg/kg (d.w.)*	0.5	EPA Method 239.1	6 months
Total Mercury - Sediment	Manual Cold Vapor technique	mg/kg (d.w.)*	0.0002	EPA Method 245.5	6 months
Total Nickel - Sediment	AA spectro air-acetylene flame (3)	mg/kg (d.w.)*	0.3	EPA Method 249.1	6 months
Total Zinc - Sediment	Atomic Absorption, direct aspiration (3)	mg/kg (d.w.)*	0.2	EPA Method 289.1	6 months
Arsenic - Sediment	AA graphite furnace	mg/kg (d.w.)*	<0.001	EPA Method 206.2	6 months
PAHs - Sediment	Gas chromatography/Mass spectrometry	μg/g (d.w.)*	(1)	EPA Method 3510 (2) EPA Method 8100 (2)	7 days to extraction, analysis.

TABLE A-5 (CONTINUED)

PARAMETER	METHOD	REPORTED AS	LIMITS OF DETECTION	REFERENCE	MAXIMUM HOLDING TIME
Polychlorinated Biphenyl Analysis					
PCB 1016/1242 - Sediment	Gas chromatography	µg/g	0.16	EPA Soxhlet Procedure (3)	7 days to extraction, 40 days to analysis.
PCB 1248	Gas chromatography	µg/g	0.084	EPA Soxhlet Procedure (3)	7 days to extraction, 40 days to analysis.
PCB 1254 - Sediment	Gas chromatography	µg/g	0.56	EPA Soxhlet Procedure (3)	7 days to extraction, 40 days to analysis.
PCB 1260 - Sediment	Gas chromatography	µg/g	0.17	EPA Soxhlet Procedure (3)	7 days to extraction, 40 days to analysis.

* Dry weight

(1) No standard available for quantitation. The Mass Spectrum obtained was compared to a Mass spectral database for identification.

(2) U.S. EPA 1984 40 CFR Part 136. Guidelines Establishing Test Procedure for the Analysis of Pollutants Under the Clean Water Act; Final Rule and Interim Final Rule and Proposed Rule.

(3) U.S. EPA. Environmental Monitoring and Support Laboratory. Interim Methods for the Sampling and Analysis of Priority Pollutants in Sediments and Fish Tissue. 1980 Oct. Cincinnati, OH.

TABLE A-6

PARAMETERS AND ANALYTICAL METHODS FOR TISSUE SAMPLES

PARAMETER	METHOD	REPORTED AS	LIMITS OF DETECTION	REFERENCE
Metals[1]				
Cadmium	A.A., direct aspiration	mg/kg (w.w.)[2]	0.2	Standard Methods 16th ed., 303A
Chromium	A.A., direct aspiration	mg/kg (w.w.)	0.3	Standard Methods 16th ed., 303A
Copper	A.A., direct aspiration	mg/kg (w.w.)	0.2	Standard Methods 16th ed., 303A
Lead	A.A., direct aspiration	mg/kg (w.w.)	0.5	Standard Methods 16th ed., 303A
Mercury	Cold Vapor Technique	mg/kg (w.w.)	0.002	Standard Methods 16th ed., 303A
Nickel	A.A., direct aspiration	mg/kg (w.w.)	0.4	Standard Methods 16th ed., 303A
Zinc	A.A., direct aspiration	mg/kg (w.w.)	0.2	Standard Methods 16th ed., 303A
Arsenic	A.A., gaseous hydride or graphite furnace	mg/kg (w.w.)	0.02	EPA, 1983
PAHs	Soxhlet extraction and Saponification process	μg/g (d.w.)[3]	-	Farrington and Medeiros, 1985.
PCB 1254	Pesticide Analytical Method 211.13f	μg/g (d.w.)	0.040	Federal Food and Drug Procedure. Pesticide Analytical Manual. January, 1988.

TABLE A-6 (CONTINUED)

PARAMETER	METHOD	REPORTED AS	LIMITS OF DETECTION	REFERENCE
Coliform				
Total	Multitube MPN	colonies/100 ml	-	APHA. 1970.[4]
Fecal	Multitube MPN	colonies/100 ml	-	APHA. 1970.[4]

[1] Tissue samples analyzed in accordance with "Wet Tissue Digestion for Metals Analysis by Atomic Absorbtion Spectroscopy (fish, clams, mussels, etc.)" LES - Standard Operating Procedures, updated 4/88.

[2] Wet Weight

[3] Dry Weight

[4] American Public Health Association, Inc. 1970. Recommended Procedures for the Examination of Sea Water and Shellfish, Fourth Ed. Section B. New York, NY.

TABLE A-7

METHOD FOR CHLOROPHYLL a ANALYSIS (MDWPC, 1988)

3.7.1 DEFINITION: Chlorophyll is a pigment found in plants that allows the organism to use radiant energy for converting carbon dioxide into organic compounds in a process called photosynthesis. Several types of chlorophylls exist and these and other pigments are used to characterize algae. One type, chlorophyll a, is measured for it is found in all algae. A knowledge of chlorophyll a concentrations provides qualitative and quantitative estimations of phytoplanktonic and periphytic biomasses for comparative assessments of geographical, spatial and temporal variations.

3.7.2 EQUIPMENT NEEDS

1. Fluorometer - either Turner 111 or the Turner Design 10-005-R field fluorometer is used. They must be equipped with blue lamp F4T5.

   Corning filter -5-60-excitation
   Corning filter - 2-64-emission
   Photomultiplier

2. Tissue grinder and tube - Thomas Tissue Grinder

3. Side arm vacuum flask and pump

4. Millipore filter holder

5. Glass fiber filter: Reeve angel, grade 934H, 2.1 cm

6. Centrifuge (Fisher Scientific Safety Centrifuge)

7. 15 ml graduated conical end centrifuge tubes with rubber stoppers

8. 90% aqueous acetone

9. 1 N HCL

10. Saturated magnesium solution in distilled water

11. Test tube racks

12. Borosilicate cuvettes - Turner 111 - 3" cuvettes
    Turner Design - 8" cuvettes

13. Aluminum foil

14. Test tube brushes - conical end

15. Parafilm

3.7.3 LOG-IN PROCEDURE

As samples are received they are logged in and assigned a number. The samples can be frozen for further analysis, or the filter ground up for analysis the following day.

3.7.4 SAMPLE PREPARATION

Samples are generally processed as soon as they come into the laboratory, unless there are extenuating circumstances, such as faulty equipment and/or time constraints. Samples not to be analyzed within 24 hours are frozen for future analysis.

The procedure for freezing samples follows:

1. Label a 2-inch Whatman petri dish with the sample number using an indelible pen.

2. Using tweezers, take a 2.1 cm Reeve Angel, grade 934AH, glass fiber filter and place it on the Millipore filtering flask screen. Do not touch the filter. Attach the glass tube to the filter flask with the metal clamp.

3. Shake the sample well.

4. Measure out 50 mls of sample or less. If an amount other than 50 mls is used it should be recorded in the chlorophyll data book.

5. Pour the measured sample into the filter tube and turn on the vacuum. The sample should pass quickly through the glass fiber filter; therefore more of the sample should be added. If the sample is not filtering through - either because too much sediment is present or the algal concentration is too high - then less than 50 mls can be filtered. A notation is made in the chlorophyll data book which lists the amount that was filtered.

6. Unclamp the filter holder and with tweezers transfer the filter to the previously marked petri dish.

7. Cover the petri dish and wrap it in aluminum foil to keep out the light. The petri dish with the glass fiber filter is then stored in the freezer.

8. Return the sample bottle to the refrigerator if algal counts or identifications are requested.

9. Rinse the graduated cylinder and filter holder in distilled water.

3.7.5 ANALYTICAL PROCEDURE

1. Follow steps 2-6 under "Sample Preparation."

2. Filter 50 ml (or less if necessary) of sample through a glass fiber filter under vacuum.

3. Push the filter to the bottom of tissue grinding tube.

4. Add about 3 ml of 90% acetone and 0.2 ml of the $MgCO_3$ solution.

5. Grind contents for 3 minutes.

6. The contents of the grinding tube are carefully washed into a 15 ml graduated centrifuge tube.

7. Bring the sample volume to 10 ml with 90% acetone.

8. Test tubes are wrapped with aluminum foil and stored in the refrigerator for 24 hours.

9. Test tubes are taken out of the refrigerator and put into the centrifuge.

10. Test tubes are then centrifuged for 20 minutes and the supernatant decanted immediately into stoppered test tubes.

11. Tubes are allowed to come to room temperature. The temperature is recorded and the samples are poured into a cuvette (3" for Turner 111 and 8" for Turner Design).

12. The Turner 111 requires a warm-up period of at least one-half hour, while the Turner Design 10-005-R does not require a warm-up period.

13. With Turner 111, use a blank of 90% aqueous solution of acetone to zero the instrument. Open the front door of the fluorometer and put in the cuvette containing the 90% acetone and close the door. Press the start switch. The dial should move back to 0; adjustments can be made with the calibration knob. This process should be repeated as often as necessary, i.e., if the blank is not staying on zero; but no alteration should be made until a series of samples is completed.

14. The Turner Design must also be zeroed to an acetone blank. The sample holder is located at the top of the Turner Design field fluorometer and should be recovered with the black cap after the sample is put in it.

15. Readings for both the Turner 111 and the Turner Design should be within 20-80% of the scale. This can be achieved by either reducing or increasing the opening to the lamp by moving the knob on the right front of the Turner 111 fluorometer. The sensitivity levels are 1x, 3x, 10x, and 30x. The sensitivity level must be recorded in the chlorophyll data book in addition to whether the high intensity or regular door was used. After the first reading, 2 drops of 2N HCl is added to the cuvette. A piece of parafilm is used to cover the cuvette which is then inverted four times to mix the sample thoroughly. The sample is re-read and the new value recorded.

16. The procedure for the Turner Design field fluorometer is basically the same as for the Turner 111. The sample is put into the cuvette holder and the manual switch used to go from one sensitivity level to the next without opening the door. A reading of between 20-80% is still required for accuracy. Readings are taken before and after acid is added to the sample. The level of sensitivity (1x, 3x, 6x, 10x, 31.6x) must be recorded in the chlorophyll data book, as well as whether the levels were set at 1 or 100.

Calculation of Chlorophyll Concentrations

Chlorophyll concentrations are determined by using the following formulas:

$$\text{chlorophyll } (\mu g/l) = Fs \frac{rs}{rs-1} (Rb-RA)$$

$$\text{pheophytin } (\mu g/l) = Fs \frac{rs}{rs-1} (rsRa-Rb)$$

where,
Fs = conversion factor for sensitivity level "s"
rs = before and after acidification ratio of sensitivity level "s"
Rb = fluorometer reading before acidification
Ra = fluorometer reading after acidification

A computer program is used to calculate the chlorophyll concentrations for samples run on the Turner Design fluorometer. This program requires the investigator to type in the sensitivity level and the difference between the before and after acidification values.

During the summer of 1986 personnel of the Technical Services Branch (TSB) conducted a laboratory experiment with a Turner Design Fluorometer in order to determine the effect of pheophytin b on freshwater chlorophyll a readings. Pheophytin b is the degradation product of chlorophyll b which is the primary pigment of green algae. The Turner

Design instrument measures the fluorescence of chlorophyll a as well as that of pheophytin a and b. Chlorophyll b is not read at the same frequency as chlorophyll a. The emission filter used at the TSB (Corning C/S 2-64) partially rejects pheophytin b (See: "References" - Turner Designs, 1981). It was found and recorded in various unpublished memoranda (See "References") that unless a sample had elevated counts of green algae the readings obtained prior to acidification and 90 seconds thereafter would give a reliable estimate of the concentration of chlorophyll a in an algal sample. In cases with elevated counts of green algae an annotation should be made alongside the chlorophyll a concentration stating that the concentration may reflect the presence of chlorophyll b and is probably lower than as recorded. As a result of this investigation, the TSB now present chlorophyll data as chlorophyll a in mg/m^3.

3.7.6 INSTRUMENT CALIBRATION

Fluorometers are calibrated using chlorophyll samples provided by the United States Environmental Protection Agency. Calibrations are performed at the start of every field season and redone if any changes are made to the fluorometer such as changing the light bulb.

Samples for chlorophyll analysis are periodically split with another laboratory or run on two separate fluorometers.

WET TISSUE DIGESTION FOR METALS ANALYSIS

BY ATOMIC ABSORPTION SPECTROSCOPY

AND/OR ICP EMMISSION SPECTROSCOPY

(FISH, CLAMS, MUSSELS, ETC.)

CHEMISTRY LAB SOP

UPDATED 04/13/88

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I. Sample Storage

a. Fish samples should be wrapped with plastic wrap and stored in sealed plastic bags.

b. Clam and Mussel samples should be stored in sealed plastic bags.

II. Sample Transport

a. Samples collected and brought to LES the same day should be transported in a cooler with ice.

b. Samples collected and stored for future delivery to LES should be placed in freezer. Samples should be removed from freezer and placed in a cooler with ice for delivery.

III. Sample Receipt and Recording

a. Samples received by the LES Chemical Lab personnel are immediately numbered on I.D. Tags and recorded into the Chemistry Lab Log Book.

b. Chain of Custody Samples must be accompanied with approved forms.

c. Samples are stored in freezer until they are readied for processing.

IV. Preparation of Glassware

a. All glassware is washed in micronox cleaning solution, rinsed with tap water, acid washed with 40% nitric acid solution, and rinsed 2x or 3x with deionized - distilled water.

V. Sample Preparation

a. Remove samples from freezer and thaw.

b. (1) Fish - The total fish fillet is diced into small sections on a nalgene cutting board using a stainless steel knife. Transfer the diced fish sections into a 40 oz. or small size glass blender top (depending on the amount of sample.)

(2) Shellfish - Scrub outside of shellfish with a stiff nylon bristled hand brush while rinsing under tap water. Shuck total clam or mussel sample collected into glass blender top.

c. Using a variable speed blender start homogenizing sample on low speed for 1 or 2 minute intervals (shut off blender between intervals to prevent overheating or burning out the blender motor).

d. Once blender blades start making a uniform contact with sample, use higher speeds for 1 or 2 minute intervals. Continue this procedure until sample is thoroughly homogenized.

e. With a teflon spatula transfer homogenized sample to plastic or glass container and seal. Label and number. Place in refrigerator or freeze samples for up to 6 months.

   Note: For some large fillets, it may be necessary to split sample into aliquots, homogenize separately, and recombined in a clean plastic container. Transfer to multi purpose plastic containers, label and number.

f. Rinse knife with deionized - distilled water and wipe clean with paper towels. Rinse cutting board with tap water, wash with 5% nitric acid solution, rinse 2x or 3x with deionized - distilled water, and wipe dry. Clean inside of glass blender and rotor blades with hard bristled nylon brush and hot tap water. Rinse with deionized - distilled water 2x or 3x. This cleaning procedure must be repeated after every sample.

VI. Sample Weighing

a. Label and tare 400 ml beaker on balance.

b. Weigh 10.0 gms of homogenized sample into beaker.

   Note: Teflon spatula used to transfer sample.

c. Cover beaker with watch glass.

d. Record weight to nearest 0.1 gm into Digestion workbook.

e. For every 10 samples or less a duplicate and spiked sample is weighed out.

   Note: The sample is spiked before digestion using Eppendorf pipets and stock 1000 ppm certified standards. Spiked concentrations are determined for each batch of samples.

VII. Digestion Procedure

a. Add 10 ml concentrated nitric acid to the beaker with sample.

   Note: Acid should be added under fume hood, safety glasses and gloves must be worn.

b. Cover with watch glass.

c. Place on steam bath and reflux for 2 hours.

d. Remove watch glass and evaporate to near dryness.

e. Add 10 ml concentrated nitric acid and 10 ml of 30% hydrogen peroxide (H_2C_2) to beaker.

f. Cover beaker with watch glass and reflux on steam bath for 2 hours.

g. Remove watch glass and evaporate to near dryness.

h. Add approximately 50 ml of 1% vol/vol hot nitric acid to beaker and let stand for 15-30 minutes on steam bath.

VIII. Sample Digest Filtration

a. Set up nessler tube (100 ml graduated) in rack with filter funnel and #42 Whatman filter paper (18.5 cm).

b. Wash down filter paper with deionized - distilled water. Discard washing from nessler tube. Rinse nessler tube with deionized - distilled water. Replace tube in rack with washed filter paper and funnel.

c. Remove beaker from steam bath. While decanting sample into funnel, wash sidewalls (inside) and bottom of beakers with deionized - distilled water (use a 500 ml side arm wash bottle).

d. Rinse beaker with two 10 ml aliquots of hot 1% nitric acid solution, and filter.

e. Rinse filter with deionized - distilled water.

f. Q.S. to 100 ml with deionized - distilled water.

g. Transfer digest to labeled sample container (125 ml rectangular H.D. polypropylene bottle).

- To ensure thorough mixing, pour digest back into nessler tube, and transfer back into sample bottle.

Note: High density polypropylene sample containers may become porous. Acid washing or acid soaking in some cases doesn't remove 100% of the contaminates adsorbed within the container. Therefore, it is recommended that once samples have been quantitated, reports have been checked and mailed, that the sample containers be discarded.

IX. Q.C. Samples

a. A reference standard, duplicate and spiked samples are processed through the digestion and filtration procedure for each set of 10 samples or less. One reagent blank is processed through the digestion and filtration procedure for every set of samples.

X. Safety Precautions

a. Lab safety practices must be strictly followed.

b. Protective glasses, gloves, and lab coats must be worn.

c. Fume hoods should be used whenever necessary.

d. Safety respirator with acid vapor removal cartridge should be worn.

XI. Glassware, Chemicals, Equipment and Supplies

a. Glassware

1. 400 ml beakers (heavy duty)
2. 50 ml graduated cylinders
3. Watch glasses
4. 100 ml graduated nessler tubes

b. Chemicals

1. Nitric acid
2. 1000 mg/L Standards for Atomic Absorption spectrophotometers (certified ACS grade)
3. 30% Hydrogen Peroxide (certified ACS grade)

c. Equipment

1. Nalgene filter funnels (10 cm diameter)
2. Teflon spatulas
3. 125 ml H.D. Polypropylene sample bottles
4. 4 oz. and 16 ox. polypropylene multipurpose containers with lids.
5. Repeater pipettors, or automatic dilutors. (500 ml base, 10 ml delivery)
6. 500 ml side arm wash bottle
7. Shellfish shucking knife
8. Stainless steel fillet knife
9. Nessler tube rack
10. Stiff bristled nylon brush (wooden handle)
11. Nalgene cutting board
12. Safety glasses
13. Safety gloves
14. Safety respirator with acid vapor removal cartridges
15. Mettler PE1600 Balance
16. Waring Blender #7012, Model 34BL97, 7 speed
17. Eberback 40 oz. glass blender with handle (#8442)
18. Eberback small size glass blender (#8470)

d. Supplies

1. Micronox cleaning solution
2. Plastic Bags (sealable)
3. Label tape
4. China marker
5. Lab coat
6. Paper towels

APPENDIX B

FIELD AND LABORATORY DATA

TABLE B-1

CLARKS COVE WATER QUALITY DATA
FIELD MEASUREMENTS

(Temperature, Dissolved Oxygen and Salinity)

STATION	DATE	TIME	DEPTH (m)	TEMPERATURE (°C)	D.O. (mg/l)	SALINITY (°/••)
A	10/29/87*	1140	0.3	12.6	7.8	31.6
			2.0	12.5	7.7	31.7
			4.0	12.5	7.5	31.7
B	10/29/87*	1300	0.3	12.5	8.3	32.0
			2.0	12.5	8.0	32.0
			4.0	12.5	7.9	32.0
			5.0	12.5	7.8	32.0
C	10/29/87*	1422	0.3	12.9	8.4	32.2
			2.0	12.9	8.1	32.2
			4.0	12.9	8.0	32.2
			6.0	12.9	8.4	32.1
			8.0	12.9	8.3	32.2
A	1/13/88*	-	1.0	-0.7	12.2	31.7
			3.0	-0.7	11.6	31.7
B	1/13/88*	-	1.0	-0.8	12.3	31.6
			3.0	-0.8	11.8	31.7
			5.0	-0.8	11.5	31.7
C	1/13/88*	-	1.0	-0.2	11.7	31.4
			3.0	-0.3	11.2	31.4
			5.0	-0.3	11.1	31.5
			7.0	-0.4	11.1	31.5

TABLE B-1 (CONTINUED)

STATION	DATE	TIME	DEPTH (m)	TEMPERATURE (°C)	D.O. (mg/l)	SALINITY (°/••)
A	3/16/88**	-	0.3	4.8	12.6	27.0
			5.0	4.5	12.5	27.0
B	3/16/88**	-	0.3	5.5	12.4	27.0
			4.5	5.0	12.5	27.0
C	3/16/88**	-	0.3	5.0	12.8	27.0
			8.0	4.0	12.3	27.0
A	5/11/88**	1217	1.0	12.0	9.1	-
			5.0	12.0	8.8	-
B	5/11/88**	1140	1.0	12.0	8.9	27.0
			5.0	12.0	8.6	27.0
C	5/11/88**	1055	1.0	12.0	9.1	27.0
			8.0	11.0	9.1	27.0
A	7/13/88*	1140	1.0	23.0	7.4	29.0
			3.5	21.0	5.0	29.5
B	7/13/88*	1112	1.0	23.0	8.4	30.9
			4.0	21.0	5.7	29.0
C	7/13/88*	1050	1.0	21.5	9.1	30.9
			8.0	20.0	7.2	31.0

TABLE B-1 (CONTINUED)

STATION	DATE	TIME	DEPTH (m)	TEMPERATURE (°C)	D.O. (mg/l)	SALINITY (°/••)
A	9/21/88*	1245	0.5	20.2	6.9	31.7
			2.0	19.7	7.0	31.8
			4.0	19.7	6.5	32.0
B	9/21/88*	1200	0.5	20.0	7.2	31.9
			2.0	19.4	6.9	31.8
			4.0	19.2	6.7	31.9
C	9/21/88*	1100	0.5	19.3	7.6	32.0
			2.0	19.1	7.2	32.0
			4.0	19.0	6.9	32.0
			6.0	19.0	6.6	32.0

* Hydrolab Surveyor II
** SCT Meter and Winkler D.O.

TABLE B-2

CLARKS COVE WATER QUALITY DATA

CHEMICAL PARAMETERS

Station Number:	A	A	B	B	C	C
Date:	10/29/87	10/29/87	10/29/87	10/29/87	10/29/87	10/29/87
Time (hrs):	11:49	11:55	13:00	13:10	14:30	14:40
Depth (meters):	1	6	1	6	1	8
Parameter						
Suspended Solids	17	12	6	17	7.5	5.0
Total Solids	35,000	36,000	34,000	34,000	35,000	34,000
Turbidity (NTU)	3.5	1.5	1.3	1.6	0.3	1.0
Total Kjeldahl-Nitrogen	0.90	0.60	0.56	0.74	0.56	1.0
Ammonia Nitrogen	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Total Phosphorus	0.09	0.09	0.09	0.10	0.09	0.10
Orthophosphate	0.06	0.06	0.05	0.05	0.04	0.04
Chloride	17,5000	17,500	17,500	17,500	17,500	17,500

Station Number:	A	A	B	B	C	C
Date:	1/13/88	1/13/88	1/13/88	1/13/88	1/13/88	1/13/88
Time (hrs):	-	-	-	-	-	-
Depth (meters):	1.0	3.0	1.0	5.0	1.0	8.0
Parameter						
Suspended Solids	6.0	5.0	8.5	5.0	4.5	2.5
Total Solids	34,000	35,000	34,000	34,000	34,000	36,000
Turbidity (NTU)	1.3	1.0	1.5	1.0	1.1	0.7
Total Kjeldahl-Nitrogen	1.6	1.6	1.5	1.4	0.76	1.3
Ammonia Nitrogen	<0.02	<0.02	<0.02	<0.02	<0.02	0.02
Total Phosphorus	0.12	0.12	0.12	0.12	0.07	0.12
Orthophosphate	0.01	0.04	0.01	<0.01	<0.01	<0.01
Chloride	16,500	16,500	17,000	16,500	16,500	16,500

TABLE B-2 (CONTINUED)

CHEMICAL PARAMETERS

Station Number:	A	A	B	B	C	C
Date:	3/16/88	3/16/88	3/16/88	3/16/88	3/16/88	3/16/88
Time (hrs):	-	-	-	-	-	-
Depth (meters):	1.0	5.0	1.0	4.5	1.0	8.0
Parameter						
Suspended Solids	4.0	7.5	3.0	140	1.5	1.0
Turbidity (NTU)	1.2	1.9	0.9	10	1.1	0.9
Total Kjeldahl-Nitrogen	0.54	0.52	0.94	2.5	0.92	0.78
Ammonia Nitrogen	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Total Phosphorus	0.10	0.11	0.10	0.49	0.10	0.10
Orthophosphate	0.04	0.04	0.04	0.27*	<0.01	0.02
Chloride	16,000	16,500	16,500	16,500	16,500	16,500

Station Number:	A	A	A	B	B	C	C
Date:	5/11/88	5/11/88	5/11/88	5/11/88	5/11/88	5/11/88	5/11/88
Time (hrs):	12:23	-	12:25	11:53	12:00	11:00	11:08
Depth (meters):	1.0	**	5.0	1.0	5.0	1.0	8.0
Parameter							
Suspended Solids	4.5	-	3.0	2.0	1.5	1.0	1.0
Total Solids	34,200	-	34,600	34,500	34,700	34,600	34,300
Turbidity (NTU)	1.0	-	0.6	0.5	0.5	0.7	0.6
Spec. Conductivity	48,100	-	48,300	48,000	48,300	47,900	48,200
Total Kjeldahl-Nitrogen	0.96	1.4	1.2	1.5	1.8	1.0	1.5
Ammonia Nitrogen	0.04	0.06	0.05	0.06	0.10	0.04	0.09
Total Phosphorus	0.12	0.11	0.16	0.10	0.13	0.11	0.16
Orthophosphate	0.05	0.05	0.04	0.03	0.08	0.04	0.04
Chloride	16,500	-	17,000	16,500	16,500	16,500	16,500
Chlorophyll a (mg/m^3)	1.34	-	1.34	3.81	3.42	0.56	1.57

TABLE B-2 (CONTINUED)

CHEMICAL PARAMETERS

Station Number:	A	A	B	B	C	C
Date:	7/13/88	7/13/88	7/13/88	7/13/88	7/13/88	7/13/88
Time (hrs):	-	-	-	-	-	-
Depth (meters):	1.0	5.0	1.0	5.0	1.0	8.0
Parameter						
Suspended Solids	2.0	6.0	1.0	5.5	3.5	50
Total Solids	37,000	37,000	37,000	36,000	36,000	36,000
Turbidity (NTU)	0.5	0.7	0.5	0.4	0.5	1.3
Spec. Conductivity	45,000	43,000	45,000	45,000	45,000	46,000
Total Kjeldahl-Nitrogen	1.7	2.1	2.1	1.8	1.6	1.7
Ammonia Nitrogen	<0.02	1.1	1.0	0.05	0.02	0.02
Total Phosphorus	0.11	0.19	0.25	0.12	0.06	0.19
Orthophosphate	0.06	0.09	0.11	0.06	0.03	0.11
Chloride	17,200	17,600	17,200	17,200	17,200	17,200
Chlorophyll a (mg/m^3)	2.88	1.98	3.24	3.36	2.52	1.98

Station Number:	A	A	B	B	C	C
Date:	9/21/88	9/21/88	9/21/88	9/21/88	9/21/88	9/21/88
Time (hrs):	12:46	12:50	12:00	12:05	11:00	11:10
Depth (meters):	1.0	3.5	1.0	3.5	1.0	6.0
Parameter						
Suspended Solids	14.0	12.0	10.0	14.0	12.0	7.5
Total Solids	35,000	36,000	35,500	37,000	35,200	36,200
Turbidity (NTU)	0.8	0.7	0.6	0.4	0.6	0.5
Spec. Conductivity	46,700	46,900	46,900	47,000	47,200	47,100
Total Kjeldahl-Nitrogen	1.50	1.30	0.98	1.50	0.90	0.60
Ammonia Nitrogen	<0.02	<0.02	<0.02	<0.02	0.02	0.04
Total Phosphorus	0.10	0.12	0.09	0.20	0.10	0.10
Orthophosphate	0.06	0.06	0.05	0.05	0.05	0.04
Chloride	17,500	17,500	17,500	17,500	17,500	17,500

* Sample filtered

** Split nutrient sample (duplicate) at Station A at 5 m

TABLE B-3

RESULTS OF TISSUE TRACE ELEMENT ANALYSIS
(mg/kg wet weight)

DEPLOYMENT-REPLICATE	CADMIUM				CHROMIUM				ARSENIC			
	BAS	A	B	C	BAS	A	B	C	BAS	A	B	C
1-1	0.2	0.2	<0.2	-	0.3	0.6	0.7	-	0.28	1.6	1.7	-
1-2	0.3	-	<0.2	-	0.6	-	0.4	-	0.26	-	1.6	-
1-3	0.5	0.2	-	0.2	0.7	0.3	-	<0.2	0.25	1.4	-	1.8
1-4	-	<0.2	0.3	<1.0*	-	0.6	0.3	<1.5*	-	1.5	1.6	2.1*
$\bar{x}$	0.33	0.2	0.2	-	0.53	0.50	0.47	-	0.26	1.5	1.6	-
s	0.15	0	0.06	-	0.21	0.17	0.21	-	0.02	0.1	0.06	-
2-1	0.2	0.3	0.3	0.3	0.6	0.7	0.6	0.5	0.05	0.10	0.11	0.12
2-2	0.2	0.3	0.3	0.3	0.5	0.5	<0.3	0.5	0.05	0.13	0.09	0.16
2-3	0.2	0.3	0.3	-	0.6	0.5	0.4	-	0.06	0.08	0.23	-
2-4	-	0.3	0.3	0.3	-	0.3	0.5	0.5	-	0.08	0.09	0.19
$\bar{x}$	0.2	0.3	0.3	0.3	0.57	0.50	0.45	0.50	0.05	0.10	0.13	0.16
s	0	0	0	0	0.06	0.16	0.13	0	0.006	0.02	0.07	0.04
3-1	0.3	0.2	0.3	0.2	0.5	0.5	0.4	0.5	0.07	0.16	0.24	0.18
3-2	0.3	0.2	0.2	0.2	0.4	0.3	0.5	0.5	0.05	0.12	0.22	0.18
3-3	0.3	0.2	-	-	0.3	0.4	-	0	0.06	0.12	-	-
3-4	0.3	0.3	-	-	0.5	0.4	-	-	0.04	0.13	-	-
$\bar{x}$	0.3	0.23	0.25	0.2	0.43	0.40	0.45	0.50	0.06	0.13	0.23	0.18
s	0	0.05	0.07	0	0.10	0.08	0.07	0	0.01	0.02	0.01	0
4-1	<0.2	<1.0	<1.0	<1.0	<0.3	<1.5	<1.5	<1.5	0.45	2.2	3.2	2.4
4-2	<0.2	<1.0	<1.0	-	<0.3	<1.5	<1.5	-	0.37	4.0	3.0	-
4-3	<0.2	<1.0	<1.0	<1.0	<0.3	<1.5	<1.5	<1.5	0.48	4.5	2.7	2.1
4-4	<0.2	<1.0	<1.0	<1.0	<0.3	<1.5	<1.5	<1.5	0.45	3.9	2.8	2.2
$\bar{x}$	<0.2	<1.0	<1.0	<1.0	<0.3	<1.5	<1.5	<1.5	0.44	3.65	2.9	2.23
s	0	0	0	0	0	0	0	0	0.05	1.0	0.22	0.15
5-1	<1.0	<1.0	-	<1.0	<1.5	<1.5	-	<1.5	2.6	1.3	-	1.7
5-2	<1.0	<1.0	<1.0	-	<1.5	<1.5	<1.5	-	2.4	1.4	1.2	-
5-3	<1.0	<1.0	<1.0	<1.0	<1.5	<1.5	<1.5	<1.5	2.4	1.1	1.5	2.2
5-4	<1.0	<1.0	<1.0	<1.0	<1.5	<1.5	<1.5	<1.5	2.9	1.2	1.2	2.1
$\bar{x}$	<1.0	<1.0	<1.0	<1.0	<1.5	<1.5	<1.5	<1.5	2.6	1.25	1.3	2.0
s	0	0	0	0	0	0	0	0	0.24	1.29	0.17	0.27

TABLE B-3 (CONTINUED)

DEPLOYMENT-REPLICATE	ZINC				MERCURY				NICKEL			
	BAS	A	B	C	BAS	A	B	C	BAS	A	B	C
1-1	17.0	21.0	20.0	-	0.04	0.03	0.04	-	1.7	1.3	<0.5	-
1-2	14.0	-	15.0	-	0.05	-	0.05	-	0.6	-	<0.5	-
1-3	15.0	24.0	-	19.0	0.05	0.04	-	0.05	1.5	1.0	-	0.5
1-4	-	23.0	32.0	24.0*	-	0.04	0.05	0.03*	-	2.2	0.8	8.0*
$\bar{x}$	15.3	22.7	22.3	-	0.047	0.04	0.047	-	1.27	1.5	0.6	-
s	1.53	1.5	8.7	-	0.006	0.006	0.006	-	0.59	0.63	0.17	-
2-1	17.0	34.0	20.0	22.0	0.07	0.04	0.04	0.02	1.3	2.7	1.9	0.4
2-2	15.0	18.0	18.0	19.0	0.04	0.03	0.04	0.03	0.8	4.7	1.6	9.1
2-3	17.0	26.0	20.0	-	0.03	0.03	0.03	-	1.8	29.0	2.3	-
2-4	-	21.0	18.0	19.0	-	0.04	0.03	0.04	-	3.9	1.0	1.0
$\bar{x}$	16.3	24.8	19.0	20.0	0.05	0.035	0.035	0.03	1.3	10.1	1.7	3.5
s	1.2	7.0	1.2	1.7	0.02	0.006	0.006	0.01	0.5	12.6	0.55	4.9
3-1	16.0	16.0	14.0	14.0	0.03	0.020	0.025	0.025	4.5	1.1	0.6	0.7
3-2	16.0	14.0	20.0	14.0	0.03	0.025	0.030	0.030	5.9	0.6	1.1	0.8
3-3	22.0	15.0	-	-	0.04	0.025	-	-	5.2	1.0	-	-
3-4	18.0	14.0	-	-	0.03	0.025	-	-	2.2	0.4	-	-
$\bar{x}$	18.0	14.8	17.0	14.0	0.030	0.024	0.030	0.030	4.5	0.78	0.85	0.75
s	2.8	0.96	4.2	0	0.005	0.003	0.004	0.004	1.6	0.33	0.35	0.07
4-1	14.0	69.0	85.0	85.0	0.025	0.01	0.02	0.02	2.3	40.0	2.0	11.0
4-2	12.0	75.0	80.0	-	0.025	<0.01	0.02	-	1.5	<1.5	2.5	-
4-3	14.0	115.0	100.0	70.0	0.035	0.02	0.02	0.02	0.7	1.5	3.0	3.5
4-4	13.0	85.0	90.0	70.0	0.020	0.03	0.01	0.02	0.5	40.0	23.0	1.5
$\bar{x}$	13.25	86.0	88.8	75.0	0.026	0.018	0.020	0.02	1.25	20.75	7.6	5.3
s	0.96	20.4	8.5	8.6	0.006	0.010	0.005	0	0.82	22.23	10.0	5.0
5-1	70.0	36.0	-	21.0	0.05	0.050	-	0.030	4.5	5.0	-	1.5
5-2	65.0	26.0	29.0	-	0.02	0.050	0.03	-	3.0	<1.5	1.5	-
5-3	70.0	35.0	31.0	25.0	0.02	0.040	0.03	0.030	8.5	12.0	7.0	2.0
5-4	100.0	31.0	31.0	25.0	0.02	0.045	0.04	0.035	14.0	10.0	2.0	5.0
$\bar{x}$	76.3	32.0	30.3	23.7	0.03	0.046	0.03	0.03	7.5	7.1	3.5	2.8
s	16.01	4.6	1.16	2.31	0.015	0.005	0.006	0.003	4.9	4.8	3.04	1.9

TABLE B-3 (CONTINUED)

DEPLOYMENT-REPLICATE	LEAD				COPPER			
	BAS	A	B	C	BAS	A	B	C
1-1	1.4	<0.5	<0.5	-	15.0	11.0	20.0	-
1-2	1.3	-	0.5	-	6.2	-	5.7	-
1-3	1.2	1.1	-	0.9	8.6	14.0	-	7.9
1-4	-	<0.5	0.9	4.5*	-	16.0	21.0	11.0*
$\bar{x}$	1.3	0.70	0.63	-	9.9	13.7	15.6	-
s	0.10	0.35	0.23	-	4.6	2.5	8.6	-
2-1	0.9	2.1	0.6	11.0	3.5	9.9	2.1	5.1
2-2	1.1	1.0	1.1	1.3	2.8	2.4	4.7	11.0
2-3	0.9	1.5	1.4	-	2.8	6.5	5.3	-
2-4	-	1.1	1.0	1.3	-	6.5	2.7	4.7
$\bar{x}$	0.97	1.4	1.03	4.5	3.0	6.3	3.7	6.9
s	0.12	0.5	0.33	5.6	0.4	3.1	1.5	3.5
3-1	1.0	0.6	0.7	<0.5	5.3	5.1	4.1	4.7
3-2	0.8	0.5	1.0	<0.5	5.6	6.6	6.3	4.4
3-3	1.4	0.5	-	-	14.0	4.0	-	-
3-4	1.0	0.7	-	-	5.2	4.1	-	-
$\bar{x}$	1.10	0.58	0.85	<0.5	7.5	5.0	5.2	4.6
s	0.25	0.10	0.21	0	4.3	1.2	1.6	0.21
4-1	0.7	5.0	<2.5	<2.5	5.8	15.0	13.0	23.0
4-2	0.6	<2.5	<2.5	-	7.6	14.0	15.0	-
4-3	0.6	<2.5	<2.5	<2.5	3.7	13.0	17.0	23.0
4-4	<0.5	47.0	<2.5	<2.5	3.5	30.0	18.0	13.0
$\bar{x}$	0.6	14.25	<2.5	<2.5	5.15	18.0	15.75	19.7
s	0.08	21.87	0	0	1.94	8.04	2.22	5.8
5-1	<2.5	5.5	-	3.5	24.0	17.0	-	12.0
5-2	<2.5	<2.5	5.0	-	19.0	9.0	20.0	-
5-3	<2.5	6.0	4.0	4.0	24.0	32.0	25.0	18.0
5-4	<2.5	<2.5	5.0	3.5	39.0	19.0	17.0	19.0
$\bar{x}$	<2.5	4.1	4.7	3.7	26.5	19.25	20.7	16.3
s	0	1.9	0.6	0.3	8.7	9.5	4.04	3.8

* Replicate lost during 1st deployment period and recovered on 9/21/88.

** Averages including less than values were calculated with maximum reported less than value.

TABLE B-4

MUSSEL TISSUE POLYCHLORINATED BIPHENYLS
(μg/g wet weight)

Aroclors 1254 and 1242

DEPLOYMENT-REPLICATE	BASELINE	STATION A	STATION B	STATION C
1-1	0.49	0.95	0.82	0.39
1-2	ND**	-	-	-
1-3	0.15	-	-	-
1-4	-	-	-	0.68***
$\bar{x}$	0.066	-	-	-
s	0.077	-	-	-
3-1	0.047	1.1	0.40	0.74
3-2	<0.040*	1.0	1.1	-
3-3	<0.040	0.81	1.0	-
3-4	-	1.2	-	0.62
$\bar{x}$	0.423	1.028	0.83	0.68
s	0.004	0.166	0.38	0.09
4-1	0.058	0.58	-	0.28
4-2	<0.040	0.78	0.81	-
4-3	0.040	0.58	0.68	0.29
4-4	ND	0.71	0.62	0.20
$\bar{x}$	0.035	0.66	0.70	0.26
s	0.025	0.10	0.097	0.05
5-1	0.041	0.61	-	0.45
5-2	<0.040	0.55	0.94	-
5-3	0.070	0.65	0.41	0.66
5-4	<0.040	-	0.43	0.53
$\bar{x}$	0.048	0.60	0.59	0.547
s	0.02	0.05	0.30	0.110

- Sample not analyzed
* Less than values averaged as reported number
** ND (none detected) values treated as 0 in calculation of average.
*** Tissue exposed from 10/27/87 to 9/21/88.

TABLE B-5

PERCENT LIPID CONCENTRATION IN MUSSEL TISSUE

DEPLOYMENT-REPLICATE	BASELINE	STATION A	B	C
1-1	2.6	1.8	2.2	2.7
1-2	1.7	-	-	-
1-3	1.9	-	-	-
1-4	-	-	-	1.4**
$\bar{x}$	2.1	1.8	2.2	2.7
3-1	1.4	2.7	0.87	3.0
3-2	1.4	3.0	2.2	-
3-3	2.8	1.4	2.4	-
3-4	-	1.8	-	2.5
$\bar{x}$	1.9	2.2	1.8	2.75
4-1	1.9	1.3	-	0.80
4-2	1.4	1.2	1.8	-
4-3	1.3	1.4	1.4	1.1
4-4	1.1	1.6	2.7	0.83
$\bar{x}$	1.4	1.38	2.0	0.91
5-1	1.8	1.3	-	1.5
5-2	1.1	1.2	1.9	-
5-3	1.0	1.1	0.98	*
5-4	1.3	-	1.0	1.6
$\bar{x}$	1.3	1.2	1.3	1.6

- Sample not analyzed
* Sample lost in analysis
** Tissue exposed from 10/27/87 - 9/21/88

APPENDIX C

SAMPLE STATISTICAL CALCULATIONS

KRUSKAL - WALLIS TEST FOR ANOVA

DEPLOYMENT #4

Arsenic

H_o: As concentration is the same in all groups
H_A: As concentration is not the same $\alpha = 0.05$

Arsenic

Base	A	B	C
0.45 (2.5)	2.2 (6.5)	3.2 (12)	2.4 (8)
0.37 (1)	4.0 (14)	3.0 (11)	-
0.45 (4)	4.5 (15)	2.7 (9)	2.1 (5)
0.45 (2.5)	3.9 (13)	2.8 (10)	2.2 (6.5)
$n_1 = 4$	$n_2 = 4$	$n_3 = 4$	$n_4 = 3$
$R_1 = 10$	$R_2 = 48.5$	$R_3 = 42$	$R_4 = 19.5$

$N = 4+4+4+3 = 15$

$$H = \frac{12}{N(N+1)} \sum^{K} \frac{R_i^2}{n_i} - 3(N+1)$$

$$= \frac{12}{15(16)} \left[\frac{10^2}{4} + \frac{48.5^2}{4} + \frac{42^2}{4} + \frac{19.5^2}{3} \right] - 3(16)$$

$$= \frac{12}{240} \left[25 + 588.06 + 441 + 126.75 \right] - 48$$

$$= 0.05 \left[1{,}180.81 \right] - 48$$

$$= 59.04 - 48$$

$$H = 11.04$$

number of groups of tied ranks = 2

$$\sum T = \sum (t_i^3 - t_i)$$

$$= (2^3 - 2) + (2^3 - 2)$$

$$12 = 6 + 6$$

$$C = 1 - \frac{\sum T}{N^3 - N}$$

$$C = 1 - \frac{12}{3{,}360}$$

$$C = 0.9964$$

$$H_C = \frac{H}{C} = \frac{11.04}{0.9964} = 11.0799$$

H_o 0.05,4,4,4,3 = 7.14

$\therefore$ reject H_o because $H_C > 7.14$

NON-PARAMETRIC MULTIPLE COMPARISONS

A Nonparametric Kruskal-Wallis test is applied to Deployment 4 Arsenic values and the null hypothesis (they are the same) is rejected. To determine where the significant differences occur use: Nonparametric Tukey-type multiple comparisons:

$\sum T = 12$ SE =

$$\sqrt{\left(\frac{N(N+1)}{12} - \frac{T}{12(N-1)}\right)\left(\frac{1}{n_A} + \frac{1}{n_B}\right)}$$

SE for n =4,4 =

$$\sqrt{\frac{15(16)}{12} - \frac{12}{12(14)}\left(\frac{1}{4} + \frac{1}{4}\right)} = \sqrt{9.9643} = 3.157$$

SE for n = 3,4 =

$$\sqrt{\frac{15(16)}{12} - \frac{12}{12(14)}\left(\frac{1}{3} + \frac{1}{4}\right)} = \sqrt{11.624} = 3.41$$

Samples ranked by mean ranks	(i)	1 Baseline	4 (C)	3 (B)	2 (A)
rank sums	(R_i)	10	19.5	42	48.5
sample sizes	(n_i)	4	3	4	4
mean ranks	$\overline{R}_i$	2.5	6.5	10.5	12.13

$$Q = \frac{\overline{R}_B - \overline{R}_A}{SE}$$

Comparison	Difference	SE	Q	$Q_{0.05,4}$	Conclusion
2 vs 1	12.13-2.5=9.63	3.157	3.050	2.639	Reject H_o: [As] different in A & Baseline
2 vs 4	12.13-6.5=5.63	3.41	1.651	2.639	Accept H_o: [As] same in A & C
2 vs 3	do not test				
3 vs 1	10.5-2.5=8.0	3.157	2.534	2.639	Accept H_o: [As] same in B & baseline
3 vs 1	do not test				
4 vs 1	6.5-2.5=4.0	3.41	1.173	2.639	Accept H_o: [As] same in Baseline & C

Overall conclusion:
Arsenic concentration is different between baseline and Station A but the same in all other comparisons

APPENDIX D

DIVISION OF MARINE FISHERIES

PROJECT PLAN AND LABORATORY METHODOLOGY

QUALITY ASSURANCE PROJECT PLAN

QUALITY CONTROL SECTION OF THE PILOT
MONITORING PROGRAM, DEPARTMENT OF ENVIRONMENTAL QUALITY
AND ENGINEERING, DIVISION OF WATER POLLUTION CONTROL

PREPARED BY
COMMONWEALTH OF MASSACHUSETTS,
DEPARTMENT OF FISHERIES, WILDLIFE, AND
ENVIRONMENTAL LAW ENFORCEMENT

FOR

U.S. ENVIRONMENTAL PROTECTION AGENCY
REGION 1
WATER MANAGEMENT DIVISION

MAY 28, 1987
(revised August 19, 1987)

APPROVALS:

W. Leigh Bridges — Aug. 20, 1987

Mr. W. Leigh Bridges, Principal Investigator — Date

Dr. Wendy Wiltse, Buzzards Bay Project Monitor — Date

Mr. Charles Porfert, Deputy Quality Assurance Officer — Date

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Copies sent to:

Wendy Wiltse (EPA)
Charles Porfert (EPA)
W. Leigh Bridges (DMF)
Jack P. Schwartz (DMF)
Nina M. Duston (DMF)
Chris Duerring (DEQE)

1. Project name:
   Quality Control section for DEQE Pilot Monitoring Program

2. Project requested by:
   U.S. EPA, Region 1

3. Date of request:
   April 15, 1987

4. Date of project initiation:
   to be determined by DEQE

5. Project Officer:
   Mr. Ronald Manfreconia

6. Project Monitor:
   Dr. Wendy Wiltse

7. Project description:

A. Objective and scope

The Division of Water Pollution Control (Department of Environmmental Quality and Engineering, Commonwealth of Massachusetts) is conducting a monitoring program involving the analysis of the blue mussel, Mytilis equlis, for trace quantities of arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), nickel (Ni), and zinc (Zn). As part of this study, Cat Cove Marine Laboratory (Division of Marine Fisheries, Department of Fisheries, Wildlife, and Environmental Law Enforcement) has the objective of providing quality control information on a subset of mussel samples in order to verify trace metal analyses on the larger data base and ensure consistency between sampling periods for the duration of the monitoring program.

B. Data Usage
(to be determined by DEQE.)

C. Design and Rationale
(to be determined by DEQE)

D. Monitoring parameters/frequency of collection

Mussels will be monitored for the eight aforementioned metals. Sampling will be conducted once every two months for one year.

E. Parameter Table

Table 1. Laboratory Analyses

Parameter	Matrix	Units	Method	Reference	Maximum holding time
As	M. edulis tissue	ug/g	acid digestion AA/hot vapor	EPA (1979) 206.5/206.3	6 months
Hg	"	"	acid digestion AA/cold vapor	Std. Methods 16th ed. 303f	"
Cd	"	"	acid digestion AA/flame	EPA (1979) 213.1	"
Cr	"	"	"	218.1	"
Cu	"	"	"	220.1	"
Pb	"	"	"	239.1	"
Ni	"	"	"	249.1	"
Zn	"	"	"	289.1	"

8. Project fiscal information

Table 2. Estimated Project Costs

Total # samples = 60

X

Total cost for analysis
@ $170.00/sample

Total Project Cost = $10,200.00

9. Schedule of Tasks and Projects
(to be determined by DEQE)

10. Project Organization and Responsibility

Mr. W. Leigh Bridges (Massachusetts Division of Marine Fisheries, Boston, MA 02202, telephone [617] 727-3194) will be the principal investigator for this project. He will be responsible to EPA for the timely completion of the project and will have overall responsibility for data interpretation as well as preparation and submission of reports to EPA.

Mr. Bridges will be assisted by Dr. Jack P. Schwartz (Division of Marine Fisheries, Cat Cove Marine Laboratory, Salem, MA 01970, telephone [617] 727-3958) as laboratory analysis leader. Dr. Schwartz will be responsible for the processing of all samples received from DEQE including quality control/quality assurance, analytical procedures, and data storage and analysis.

11. Data Quality Requirements and Assessments

Accuracy will be measured as percent recovery of an EPA standard reference material analyzed with each batch. Corrections will be made for background levels. Average laboratory recoveries will be maintained in the range of 80-120%. Unspiked blanks will accompany every batch as a further measure of accuracy.

Precision will be measured as the relative standard deviation of triplicate analyses performed upon 10% of the samples in each batch. Instrumental precision will be monitored through the use of triplicate readings on the transition metals digestate or through triplicate readings of calibration standards for arsenic and mercury. Should results vary by more than 10% readings will be repeated.

Completeness will be measured as the percentage of total samples received that were completely analyzed. We expect to achieve 100% completeness of all analyses.

12. Sampling and Analytical Procedures

Field Sampling

(to be determined by DEQE)

Analytical Procedures

Arsenic analyses will be performed according to U.S. EPA method 206.5/206.3. Mercury analyses will be performed according to Standard Methods (16th ed.) 303F for the Examination of Water and Wastewater. Analysis for cadmium, chromium, copper, lead, nickel, and zinc will be performed using EPA methods 213.1, 218.1, 220.1, 239.1, 249.1, and 289.1, respectively. All methods will compliment EPA methods in use by DEQE. Concentrations of arsenic will be determined by atomic absorption hot vapor technique. Mercury concentrations will be

determined by atomic absorption cold vapor technique. Transition metal concentrations will be determined by atomic absorption flame techniques. Analyses will be performed using a Perkin-Elmer Model 3030B atomic absorption spectrophotometer. Arsenic and mercury analyses will also use a Perkin-Elmer Model MHS 10 mercury hydride system. Samples will be compared to external standards suitable for the metal being analyzed.

13. Sample Custody Procedures.

Homogenized mussel samples will be shipped frozen in polyethylene bags by DEQE personnel accompanied by an analysis request form (figure 1). Laboratory personnel will take custody of all sample material which will be assigned laboratory tracking numbers (logged-in) and locked in freezers. Due to a lack of space there are no plans to archive samples. Any sample material remaining after the completion of all analyses will be made available to DEQE. Mussel samples that are not frozen upon delivery will not be taken into custody and returned to DEQE with the shipper.

14. Calibration Procedures and Preventive Maintenance

The atomic absorption spectrophotometer will be calibrated through the use of external standards (certified atomic absorption grade standards obtained from Fisher Scientific Company). Calibration of the instrument will occur at the beginning of every sampling run and will be checked every ten samples and the end of every sampling run. Routine maintenance performed at the time of a run will be noted in the laboratory notebook. The instrument is covered by a maintenance contract with Perkin-Elmer. Any breakdowns will be promptly repaired.

15. Documentation, Data Reduction, and Reporting

A. All raw data generated during laboratory analysis will be kept in a permanently bound notebook. A permanently bound notebook will be kept of all quality control tests conducted at the laboratory. Data printouts will be kept on file and available for inspection.

16. Data Validation

All data produced by the laboratory will be subject to a 100% check for errors in transcription and calculation by the Senior Chemist, Dr. Nina M. Duston, and the Laboratory Analysis Leader, Dr. Jack P. Schwartz. The Principal Investigator, Mr. W. Leigh Bridges, will look at all logbooks and notebooks to ensure that requirements are met. Data which do not meet the specified quality requirements will not be included in the report. Analytical reports will be signed by the Senior Chemist or Laboratory Analysis Leader before being released.

17. Performance and System Audits

Performance will be monitored through EPA Water Pollution Laboratory Performance Evaluation Studies which provides for routine intercalibration with U.S. EPA every six months.

18. Corrective Action

Meetings between all laboratory personnel and the Principal Investigator of the study will be held at the completion of each sample batch. Problems will be identified as the study progresses. When corrective action is required it will be taken immediately and noted in the appropriate laboratory notebook.

19. Reports

The reports generated during this study are as follows:

A. Quality assurance project plan, due May 29, 1987. This report will include the objectives, scope, methods, and products associated with this study.

B. At the completion of analyses of each sample batch a report will be forwarded to the Principal Investigator for transmittal to appropriate U.S. EPA and DEQE personnel. This report will be completed before the next batch of samples is received.

DIVISION OF MARINE FISHERIES

Laboratory Methodology

Wet tissue Digestion Procedure for Trace Metals Analysis

Chemicals

1. HNO_3 70.0 - 71.0% n Baker Instra-Analyzed Reagent for Trace Metal Analysis.

2. H_2O_2, 30% Baker Analyzed Reagent.

   a. Weigh approximately 10 grams of blended tissue sample in a preweighed or tared tall form beaker (200 ml). Record sample wet weight to nearest 0.01 grams.

   b. Add 10 ml concentrated HNO_3 to sample in the tall form beaker. Cover with a watch glass and let sit overnight (15 to 16 hours) in ventilated fume hood to cold digest.

   c. Place covered samples on a steam bath until almost all tissue is digested. At this time spike the appropriate quality control samples with a standard spike solution containing concentrations as listed below for the particular species being digested.

Analyte	Finfish ppm	Lobster ppm	Shellfish ppm
Pb	4.0	4.0	4.0
Zn	10.0	50.0	20.0
Cu	10.0	50.0	2.0
Cr	1.0	1.0	1.0
Cd	0.5	0.5	0.5

(All standard solutions made in 2% V/V HNO_3)

Use of these standard spike solutions will result in the enrichment values listed below for the final 50 mL volume of spiked digestate.

Analyte	Finfish ppm	Lobster ppm	Shellfish ppm
Cd	0.05	0.05	0.05
Cr	0.10	0.10	0.10
Cu	1.00	5.00	0.20
Pb	0.40	0.40	0.40
Zn	1.00	5.00	2.00

4. Reflux the samples for 2 hours.

5. Remove watch glass after 2 hours of refluxing and evaporate sample to near dryness.

6. Once all samples are evaporated to near dryness and are at room temperature, add 10 ml concentrated HNO_3 and 10 ml of 30% H_2O_2 to each sample. Cover beaker with watch glass and let sit overnight (15 to 16 hours).

7. Place covered samples on cold stream bath and slowly bring up to temperature. (Watch for violent reactions.) Reflux for 2 hours on steam bath.

8. Remove watch glass and evaporate to near dryness.

9. Add approximately 20 ml of a 2% v/v hot HNO_3 solution to beaker and let heat for 5 minutes on steam bath.

10. Remove beaker from steam bath, wipe off any moisture on the outside of beaker and filter the sample using a glass filter funnel with a reeve Angel 802 12.5 cm fluted filter paper or equivalent. Collect filtrate in 50 ml volumetric flask. Rinse beaker with two aliquots of 5-10 ml hot 2% v/v HNO_3 to remove as much yellow coloring as possible from the filter paper. Remove filter paper and rinse glass funnel with hot 2% HNO_3 taking care not to go over the 50 ml mark.

11. Q.S. to 50 ml with 2% v/v HNO_3 and transfer to sample containers.

12. Sample digestate is then analysed for metals on a Perkin Elmer AAS 3030B according to the manufacturer's specifications.

Mercury Digestion Method

Chemicals

1. HNO_3, 70.0-71.0%, "Baker Instra-Analyzed" Reagent for Trace Metal Analysis

2. H_2SO_4, 95.0-98.0%, "Baker Instra-Analyzed" Reagent for Trace Metal Analysis

3. $KMnO_4$, "Baker Instra-Analyzed" Reagent for Hg Determination

4. $K_2S_2O_8$, "Baker Instra-Analyzed" Reagent for Hg Determination

Solutions Needed

1. 5% Potassium permanganate solution: Dissolve 25 g $KMnO_4$ in deionized distilled water and dilute to 500 ml.

2. 5% Potassium persulfate solution: Dissolve 25 g $K_2S_2O_8$ in deionized distilled water and dilute to 500 ml

Procedure for Shellfish Tissue Digestion

1. Weigh approximately 2 grams of blended sample, to the nearest 0.1 mg, in a pre-weighed or tared 125 ml Erlenmeyer reaction flash.

2. Add 7.0 ml conc. H_2SO_4 and 3.0 ml HNO_3 to each flask and place in a 70°C water bath.

3. Remove samples to be spiked from water bath when a colored liquid with no visible tissue has formed. Spike appropriate Q.C. samples with 1.0 ml of 100 ng/ml Hg. This will yield 50 ng Hg enrichment in final sample (refer to Step 8). Return samples to water bath.

4. Samples should remain in the water bath for four (4) hours.

5. Remove samples from water bath. Allow to cool to room temperature. Add 5.0 mL deionized distilled water to the samples to cause precipitation of waxy digestion products and decrease the acidity of the sample solutions.

6. Filter samples through VWR grade 615 9 cm or equivalent filter paper into a stoppered glass 25.0 mL graduated cylinder to remove the waxy precipitate. Rinse the sample flask twice with small amount of 20% v/ HNO_3. Rinse filter paper with small amount of 20% HNO_3 taking care not to exceed 25.0 mL of liquid in cylinder.

7. Q.S. to 25.0 mL with 20% HNO_3. Stopper cylinder and shake well.

8. Using acid washed disposable 9 inch Pasteur pipets, divide sample into two equal portions and place in two clean 125 mL Erlenmeyer flasks. Rinse cylinder with two 2.5 mL portions of 20% HNO_3 solution, divide rinses equally between the two sample flasks.

9. Ice samples.

10. Add 10 mL $KMnO_4$ solution to each flask and let stand 15 minutes in ice bath.

11. Add 8 mL $K_2S_2O_8$ solution to each flask while still in the ice bath.

12. Add 0.5 - 1.5 g of $KMnO_4$ crystals as needed to keep the solutions purple. Remove from ice bath. Samples are left overnight to digest or are placed in a 70°C water bath for 2 to 4 hours. Please note, solutions must remain purple until analysis. Analysis must be with 24 hours.

Washing Procedure for All Labware Used for Metals Analysis

1. A 12 hour presoak is used if glassware has an organic/waxy film. The presoak solution is made from Terg-A-Zyme (as instructions indicate on the carton).

2. Wash with soap (Liquinox) and tap water, rinse well with tap water.

3. Rinse thoroughly with 1:1 HNO_3 followed by 1:1 H_2SO_4 (twice). A squeeze bottle is used to deliver the rinse.

4. Rinse thoroughly with deionized distilled water at least three times. The deionized distilled water should have a resistance of 2Mohm or higher.

5. Air dry or place in oven to dry.

6. Store clean labware in assigned areas, covering with parafilm or glass stoppers, whichever is appropriate.

Limits of detection and quantification (ug/g wet weight) for trace metals based on replicate blank determinations

Element	No. Blanks	No. Readings	Mean	Standard Deviation	Minimum Machine Reading to Detect	Minimum Machine Reading to Quantify	Limit of Detection	Limit of Quantification
Cd	29	39	-0.003	0.004	0.012	0.040	0.001	0.004
Cr	16	43	0.03	0.01	0.03	0.10	0.003	0.01
Cu	20	23	0.00	0.02	0.06	0.20	0.006	0.02
Hg	16	16	0.000	0.002	0.006	0.020	0.006	0.020
Pb	21	27	0.02	0.03	0.09	0.30	0.009	0.03
Zn	20	27	0.04	0.02	0.06	0.20	0.006	0.02

a. Minimum corrected machine reading required to detect element, 3X the Standard Deviation of the Blanks.
b. Minimum corrected machine reading required to quantify element, 10X the Standard Deviation of the Blanks.
c. Assumes 10g sample for 50ml digestate.
d. Assumes 1.0g sample digested.