EVALUATION OF GULFWATCH 1995:

FIFTH YEAR OF THE
GULF OF MAINE ENVIRONMENTAL MONITORING PLAN

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INTRODUCTION

Rational

The Gulf of Maine extends from Cape Sable, Nova Scotia, through New Brunswick, Maine, and New Hampshire to Cape Cod, Massachusetts and includes the Bay of Fundy and Georges Bank. The combined primary productivity of seaweeds, salt marsh grasses, and phytoplankton make it one of the worlds most productive system that supports a vast array of animal species, including many species of invertebrates, fish, seabirds, and marine mammals, some of great commercial importance. Commercial fisheries and aquaculture are its principal income generating enterprises, although tourism is very important source of income to many small coastal communities. As coastal populations around the Gulf and its watersheds have increased, agricultural lands have been converted to industrial and residential developments. Such changes in land use and increases in population have contributed to the deteriorating quality of sections of the coastal environment (GMCME, 1992; Dow and Braasch, 1996). Inputs from non-point source and point source pollution are a significant threat to the near shore environment of the Gulf (GMCME, 1992; Dow and Braasch, 1996). Growth in industrial activity during the 20th century has resulted in a rapid increase in inputs from chemicals, either mobilized or synthesized by man, into the estuarine and coastal environments. Many of these chemicals are bioaccumulated to concentrations significantly above ambient levels. Furthermore, some of these environmental contaminants may also be present at toxic concentrations, and thus induce adverse biological effects.

In order to protect water quality and commercial uses in the Gulf of Maine, the *Agreement on the Conservation of the Marine Environment of the Gulf of Maine* was signed in December, 1989 by the premiers of Nova Scotia and New Brunswick, and the governors of Maine, New Hampshire and Massachusetts, establishing the Gulf of Maine Council on the Marine Environment. The overarching mission of this council is to maintain and enhance the Gulfs' marine ecosystem, its natural resources and environmental quality.

To help meet the council's mission statement the Gulf of Maine Monitoring Committee was formed and charged with the development of the Gulf of Maine Environmental Monitoring Plan. The monitoring Plan is based on a mission statement provided by the council:

It is the mission of the Gulf of Maine Environmental Quality Monitoring Program to provide environmental resource managers with information to support sustainable use of

the Gulf and allow assessment and management of risk to public and environmental health from current and potential threats.

Three monitoring goals were established to meet the mission statement:

(1) To provide information on the status, trends, and sources of risk to the marine environment in the Gulf of Maine;

(2) To provide information on the status, trends, and sources of marine based human health risks in the Gulf of Maine; and

(3) To provide appropriate and timely information to environmental and resource managers that will allow both efficient and effective management action and evaluation of such action.

In support of the mission and to meet the desired goals a project named Gulfwatch was established, to measure chemical contamination Gulfwide.

Gulfwatch Objectives

Gulfwatch is presently a program in which the blue mussel, *Mytilus edulis*, is used as an indicator for habitat exposure to organic and inorganic contaminants. Bivalves, such as *M. edulis*, have been successfully used as indicator organisms in environmental monitoring programs throughout the world (see NAS, 1980; NOAA, 1991; and Widdows and Donkin, 1992) to identify variation in chemical contaminants between sites, and contribute to the understanding of trends in coastal contamination (NOAA, 1991; O'Connor, 1992; O'Connor and Beliaeff, 1995; Widdows et al., 1995). The blue mussel was selected as the indicator organism for the Gulfwatch program for the following reasons:

(1) mussels are abundant within and across each of the 5 jurisdictions of the Gulf Program and they are easy to collect and process;

(2) much is known about mussel biology and physiology;

(3) mussels are a commercially important food source and therefore a measurement of the extent of chemical contamination is of public health concern;

(4) mussels are sedentary, thereby eliminating the complications in interpretation of results introduced by mobile species;

(5) mussels are suspension-feeders that pump large volumes of water and concentrate many chemicals in their tissues; therefore the presence of trace contamination is easier to document; and the measurement of chemicals in bivalve tissue provides an assessment of biologically available

contamination that is not always apparent from measurement of contamination in environmental compartments (water, sediment, and suspended particles).

Gulfwatch has taken two approaches to using marine mussels as bioindicators of anthropogenic contamination. During the first two years of the program (1991 - 1992), both transplanted and native mussels sampled from areas adjacent to the transplant sites were analyzed for organic and inorganic contaminants (GMCME, 1992). Transplanted mussels were initially collected from relatively pristine sites in each jurisdiction, moved to sites selected for monitoring, and held there for approximately 60 days. Because of the logistics and the analytical costs, only two sites per jurisdiction could be monitored each year using this transplant technique. However, transplant experiments provided an assessment of the short-term exposure (on the order of weeks to months) to bioavailable contaminants throughout the region. In 1993 and 1994, only indigenous mussels were sampled, although a greater number of sites were monitored compared to the years when mussels were transplanted (GMCME, 1996a, 1996b). Sampling of native mussels provided an assessment of long-term exposure to bioavailable contaminants (on the order of months to a year). The 1995 sampling year followed the protocol for 1991 and 1992, sampling transplanted and indigenous mussels from two sites in each jurisdiction.

In addition to documenting the level of contaminants in mussel tissue, biological variables, including, shell growth and condition index, were measured as a means to determine the response of organisms to stress under different concentrations of contaminant burden. Growth is often one of the most sensitive measures of a contaminants' effect on an organism (Sheehan, 1984; Sheehan et al., 1984; Howells et al., 1990). Shell growth has often been used as a measure of environmental quality and pollution effects as the rate of growth is a fundamental measure of physiological fitness / performance (Widdows and Donkin, 1992; Salazar and Salazar, 1995) and therefore, is a direct, integrative measure of the impairment of the organisms physiology. Condition index (CI) was used as an indicator of the physiological status of the mussels. It relates the tissue wet weight to shell volume and is a measure traditionally used by shellfishery biologists (Widdows, 1985). Because gonadal weight is a significant contributor to total body weight just prior to spawning, CI also reflects differences in the reproductive state of the sampled mussels. Since gonadal material tends to have low concentrations of metals (LaTouche and Mix, 1981), tissue metal concentrations may be reduced in mussels having a high CI due to ripened gonads. Organic contaminants, however, would tend to partition into both somatic and gonadal lipids, and may be less impacted by changes in CI that are due to the presence of ripe gametes. Since variable amounts of ripe gametes may be found in some mussel populations even in late fall (Kimball, 1994), the relationship between CI and contaminant concentrations must be carefully considered.

The objective of the first two years (1991 and 1992) of the Gulfwatch program was to evaluate the feasibility of the project and the level of cooperation required through collecting comparative data from different locations in the Gulf of Maine. The sites that were selected fell into two categories; test sites that were suspected or known to be contaminated and reference sites that were free of any known contaminant source. After the success of the pilot studies in 1991 and 1992, it was recognized that there should be a broader or Gulf-wide orientation of the mussel watch in addition to known contaminated and reference sites within each jurisdiction. As such, a three year cycle was initiated in 1993. In 1993 and 1994 the sample design was expanded as described above. Native mussels were sampled in as many as 7 new locations within each jurisdiction (state or province), where feasible, to increase the geographic coverage. However, one location in each jurisdiction was chosen as a baseline station, to be resampled every year. This approach increased the chance of locating unforseen environmental contamination. Transplant experiments were again conducted at two sites in each jurisdiction in 1995. This three-year cycle, with transplants being conducted at two sites during one year and indigenous mussels alone being sampled at 2-7 sites per jurisdiction during the other two years, will be repeated for the remaining years of the Gulfwatch Program. This sampling design will allow the Program Investigators to assess both short-term and long-term contaminant exposures.

METHODS

The 1995 Gulf of Maine mussel survey is the third year of the nine year sampling design to extend the geographical coverage of the Gulf, yet maintain baseline stations for analysis of temporal trends. The 1995 approach included two sets of caged (C) mussels, and native, or indigenous, mussels (N) from areas adjacent to the transplant site in each jurisdiction. The caged mussels served to address short term contaminant uptake and biological responses in mussels at stations of special concern.

1995 Sampling Locations

The stations sampled in 1995 are shown in Figure 1 and Table 1. There were 3 sites in Massachusetts, 2 in New Hampshire, 4 in Maine, 3 in New Brunswick and 2 in Nova Scotia. Five sites were retained from previous years to enable trend analysis; Sandwich, MA, Little Harbor, NH, Clarke Cove, ME, Kennebec River, ME and Hospital Island, NB.

Field Procedures

Details regarding the mussel collection, measurement, and sample preparation are published in Sowles and Crawford (1994). Additional monitoring at the transplant stations included: continuous temperature recording (Hobo Temp), and measurements of salinity, turbidity, and nutrient and chlorophyll levels which were made at the time of cage deployment and retrieval. These data are not included in this report.

The mussels collected were intended to be *Mytilus edulis*. However, a similar species of *Mytilus*, *Mytilus trossulus* was identified in some of the Bay of Fundy samples (GMCME, 1996a). This species has a slower growth rate than *M. edulis* and attains a maximum size of approximately 50-60 mm compared to 70 - 80 mm for the blue mussel (Bayne, 1976). These physiological differences result in species-specific differences in shell allometric growth. Although an inter-species allometric gradient is present at all sites inhabited by both species, *M. trossulus* can often be distinguished from *M. edulis* by its higher shell length:height ratio (Lobel et al., 1990; Freeman et al., 1992). A more precise distinction between the two species can be made using canonical discriminant analysis (Mucklow, 1996).

All field sampling was conducted between August 15, 1995 and November 13, 1995. Cages were deployed between August 15th and September 14th and retrieved between October 13th and November 13th (see Table 15). Cages were deployed at MEDM on the 14th of October, approximately one month later than at MEKN (August 15th, 1995). Originally cages were deployed at both sites in August but the cages deployed at MEDM were lost. As such, a second cage deployment was initiated at MEDM on October 14th, 1995. At the time of cage retrieval, indigenous mussels were collected from areas adjacent to the transplant sites. Collection times were set to avoid collecting during or shortly after periods when storm water runoff and wave resuspension of bottom sediment result in unusual uptake and accumulation of sediment in the mussel gut. Presence of sediment in the mussels was suspected to be the cause of the elevated concentrations of some metals (iron, aluminum and associated metals) (Lobel et al., 1991;

TABLE 1. Gulf of Maine Gulfwatch study site locations sampled in 1995

CODE	DATE	SAMPLE [1]	LOCATION	LATITUDE	LONGITUDE
MASN	August 15	P	Sandwich, MA	41°45.73'N	70°28.38'W
MAIH	October 17	N,C	Boston, Inner Harbor, MA	41°21.53'N	71°02.94'W
MAPR	October 13	N,C	Pines River, MA	42°25.87'N	70°58.76'W
NHHS	August 27	P	Hampton / Seabrook Harbor, NH	42°53.50'N	70°49.00'W
NHLH	October 28	N,C	Little Harbor, NH	43°02.00'N	70°43.0'W
MECC	October 29	N,C	Clarkes Cove, ME	43°04.00'N	70°43.40'W
MEBC	August 15	P	Broad Cove, ME	43°45.95'N	70°10.75'W
MEDM	November 1	N,C	Damariscotta, ME	43°56.30'N	69°34.90'W
MEKN	November 13	N,C	Kennebec River, ME	43°47.5'N	69°47.6'W
NBHI	October 31	N,C	Hospital Island, NB	45°07.42'N	67°00.53'W
NBNR	August 22	P	Niger Reef, NB	43°51.35'N	69°35.41'W
NBMI	October 30	N,C	Manawagonish Island, NB	45°13.0'N	66°06.0'W
NSCW	November 7	N	Cornwallis, NS	44°65.70'N	65°66.77'W
NSBE	November 7	N	Belliveaus Cove, NS	44°24.15'N	66°02.45'W

[1] Sample: P = indicates subsample of mussels used in the cage experiment (preset)

N = site where collections of indigenous mussels were made

C = site where cage experiment was conducted

Robinson et al., 1993) in previous reports (GMCME, 1994, 1996a, 1996b).

Since mussel growth and mussel body burdens are affected by many different variables each site must meet certain minimum criteria to control variability:

(1) Whenever possible, the mussels were collected from subtidal areas at each site. Mussel growth is known to be negatively affected by the duration of aerial exposure (Phillips, 1976). In the Nova Scotian sites, however, mussels were collected in the low intertidal zone because of the extreme tidal range in the Bay of Fundy. The reader is referred to our manual for more detail (Sowles and Crawford, 1994).

(2) Stations should be adjacent to the mainland to reflect anthropogenic contaminant inputs. Water quality varies from offshore to near shore due to upwelling, terrigenous sources, and current.

(3) Natural indigenous subtidal mussels that are collected must be 50-60 mm shell length. Collecting mussels of a uniform size minimizes any differences associated with scaling effects (e.g. surface to volume ratios, and to some degree metabolic rates).

Caged Mussels

Stock for transplanted caged mussels were collected from a designated preset site (P) (Table 1) within each jurisdiction. Most mussels selected were between 40-50 mm in length. Shell length was determined in the field using a plastic ruler or wooden field gauge. Note that a smaller size class (40 -50 mm) was chosen for the transplants compared to the indigenous mussel collections (50 - 60 mm) in order to ensure that an adequate amount of growth could be realized during the transplant period. At the time of collection, the mussels were cleaned of all sediment, epibiota and other accretions in clean seawater from the collection site, then placed in clean glass containers, and transported to the lab in coolers. In the laboratory they were maintained in running ambient unfiltered sea water or were refrigerated until deployment.

Four cages, each containing 50 mussels, were deployed at each site (n=200 / site). The cages were 23 mm x 23 mm x 23 mm polypropylene moulded baskets A subset of the mussels (15 of the 50) in each basket were sorted, numbered using a high speed engraving tool (Dremel type) and measured for length to the nearest 0.1 mm with vernier calipers. These measurements were used later to determine growth over the deployment period. For details on the deployment design and procedures see Sowles and Crawford (1994).

Transplanted mussels were deployed for approximately 60 days to ensure adequate time for tissue contaminants to reach steady state and provided comparable exposure periods between sites.

Indigenous mussels

At the time of cage retrieval, indigenous mussels were collected from 4 discrete areas within a segment of the shoreline that is representative of local water quality. Using a wooden gauge or a ruler, 45-50 mussels of 50-60 mm shell length were collected. The mussels were cleaned of all sediment, epibiota, and other accretions in clean sea water from the collection site, placed in clean glass containers, then transported to the lab in coolers.

Laboratory Procedures

In the laboratory, individual mussel lengths, widths and heights (as defined by Seed, 1968) were determined to the nearest 0.1 mm using vernier calipers. Using plastic or stainless steel wedges, mussels were shucked directly into appropriately prepared containers for metal and organic analysis, respectively (for details see Sowles and Crawford, 1994). Composite samples (20 mussels/composite; 4 composites/station) were capped, labeled and stored in a freezer at -15°C.

While a number of condition indices have been proposed over the years (Seed, 1968), the Gulfwatch Condition Index (CI) has been defined as:

$$CI = \text{tissue wet weight (mg)} / \text{length (mm)} * \text{width (mm)} * \text{height (mm)}$$

CI was determined in the laboratory only on preset mussels and on mussels collected at the time of cage retrieval. This includes both caged and indigenous mussels at the test and reference sites. CI was determined for between 30 and 40 mussels, depending on the jurisdiction.

Analytical Procedures

Analytical procedures used followed those reported for the previous years (GMCME, 1994, 1996a, 1996b). Table 2 contains a summary of the metal and organic compounds measured.

Metals

Inorganic contaminants were analyzed at the State of Maine Health and Environmental Testing

TABLE 2. Inorganic and Organic contaminants analyzed in mussel tissues from the Gulf of Maine in 1995.

INORGANIC CONTAMINANTS

Metals
Ag, Al, Cd, Cr, Cu, Fe, Hg, Ni, Pb, Zn

ORGANIC CONTAMINANTS

Aromatic Hydrocarbons
Naphthalene
1-Methylnaphthalene
2-Methynaphthalene
Biphenyl
2,6-Dimethylnaphthalene
Acenaphthylene
Acenaphthene
2,3,5-Trimethylnaphthalene
Fluorene
Phenanthrene
Anthracene
1-Methylphenanthrene
Flouranthene
Pyrene
Benzo [a] anthracene
Chrysene
Benzo [b] flouranthrene
Benzo [k] flouranthrene
Benzo [a] pyrene
Benzo [e] pyrene
Perylene
Indeno [1,2,3-cd] pyrene
Dibenzo [a,h] anthracene
Benzo [g,h,i] perylene

Chlorinated Pesticides
Hexachlorobenzene (HCB)
gamma-Benzenehexachloride (BHC)
Heptachlor
Heptachlor epoxide
Aldrin
Mirex
cis-Chlordane
trans-Nonachlor
Dieldrin
Alpha-Endosulfan
beta-Endosulfan

DDT and Homologues

2,4'-DDE	4,4'-DDE
2,4'-DDD	4,4'-DDD
2,4'-DDT	4,4'-DDT

PCB Congeners

PCB 8, PCB 18, PCB 28, PCB 29, PCB 44,
PCB 50, PCB 52, PCB 66, PCB 77,
PCB 87, PCB 101, PCB 105, PCB 118,
PCB 126, PCB 128, PCB 138, PCB 153,
PCB 169, PCB 170, PCB 180, PCB 187,
PCB 195, PCB 206, PCB 209

Laboratory (Augusta, ME). Analyses for mercury were done on a subsample of 1 to 2 g of wet tissue and measured by cold vapor atomic absorption on a Perkin Elmer Model 503 atomic absorption spectrometer. Analyses for all other metals were conducted on 5 to 10 g of wet tissue dried at 100 °C. Zinc and iron were measured by flame atomic absorption using a Perkin Elmer Model 1100 atomic absorption spectrometer. All remaining metals (Ag, Al, Cd, Cr, Cu,Ni and Pb) were run using Zeeman background corrected graphite furnace atomic absorption on a Varian Spectra AA 400. The analyte detection limit for the metals in µg / g dry weight are as follows; Ag, 0.02; Al, 3.0; Cd, 0.2; Cr, 0.3; Cu, 0.6; Fe, 6.0, Hg, 0.1, Ni, 1.2, Pb, 0.6; and Zn, 1.5.

Organic Contaminants

Organic contaminants in mussel samples were analyzed at the Environment Canada Environmental Protection Laboratory in Dartmouth, N.S (Table 2). The analyte detection limit for aromatic hydrocarbons was 10 ng/g (20-30 ng/g for some lower molecular weight aromatics) and
< 2 ng/g for PCB congeners. Eighteen of the PCB congeners identified and quantified correspond to congeners analyzed by the National Oceanographic and Atmospheric Administration's (NOAA) National Status and Trends (NS&T) Program designated congeners. Other organic compounds selected for analysis are also consistent, for the most part, with NOAA National Status and Trends mussel monitoring (NOAA, 1989).

The analyses of mussel tissue samples follow the diagram shown in Figure 2 and are summarized below. A description of the full analytical protocol and accompanying performance based QA/QC procedures are found in Appendix A and Appendix B.

Tissue samples were extracted by homogenization with an organic solvent and a drying agent. Solvent extracts were obtained by vacuum filtration, and biomatrix interferences were separated from target analytes in extracts by size exclusion chromatography. Purified extracts were subjected to silica gel liquid chromatography which provided a non-polar PCB/chlorinated pesticides fraction and a polar chlorinated pesticide fraction. PCBs and pesticides were analyzed by High Resolution Dual Column Gas Chromatography/Electron Capture Detection (HRGC/ECD). Following PCB and pesticide analysis, the two fractions were combined and the resulting extract was analyzed for aromatic hydrocarbons by High Resolution Gas Chromatography/Masspectrometry (HRGC/MS).

Composite mussel sample
(blended with SS. blender)

Subsample (1g) for
dry weight
determination

+ NaSO$_4$
+ MeCl$_2$

Homogenate extraction
(10-15 g)

Vacuum filter concentrate to 10 ml

Remove 1 ml for lipid determination

Discard solid
material

Size exclusion
chromatography cleanup
(AS 2000 GPC)

Hexane
exchange

Silica gel column
fractionation

100% Hexane

50% Hexane
50% MeCl$_2$

Apolar fraction
PCB / CH pesticides

Polar fraction
CH pesticides

HRGC - ECD
Analysis

HRGC - ECD
Analysis

Combined fraction
Aromatic Hydrocarbons
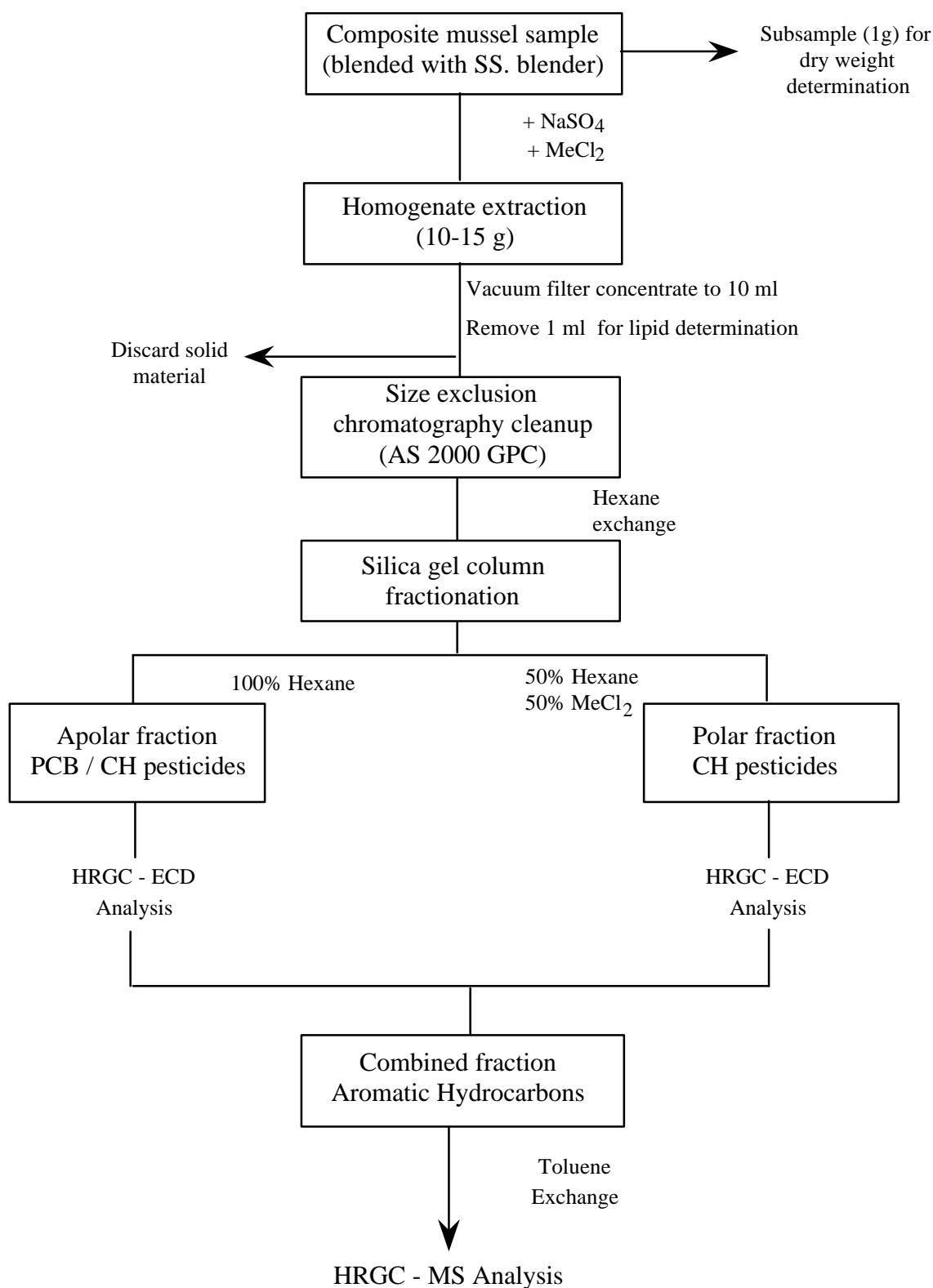
Toluene
Exchange

HRGC - MS Analysis

FIGURE 2. Analytical flow chart for organic analyte determination at the Environment
Canada Laboratory in 19945 HRGC-MS, high resolution gas chromatography
/massspectrometry; HRGC-ECD, high resolution dual column gas
chromatography/electron capture detection; GPC, Gel permeation
chromatography; SS., Stainless steel.

Quality Assurances / Quality Control

Standard laboratory procedures for metals incorporated method blanks, spike matrix samples, duplicate samples, surrogate addition and standard oyster tissue (SRM 1566A). The method blanks were inserted: three at the beginning of the run, one at the end, and six at various intervals during the run. Duplicate samples and matrix spike recoveries were conducted on 15% of the samples.

The Dartmouth laboratory participated in the NIST Status and Trends Intercomparison Marine Sediment Exercise IV and Bivalve Homogenate Exercise V. Internal quality control and method performance specifications are described in the Environment Canada Shellfish Surveillance Protocol (Appendix B). The protocol includes mandatory QC measures with every sample batch including method blanks, spike matrix samples, duplicate samples, surrogate addition, and certified reference materials (SRM, 1974a). The protocol specifies the performance criteria relevant to method accuracy, precision, and detection limits and data reporting requirements for the analysis of organic contaminants in shellfish samples.

Statistical Methods

All metal data was $\log_{10}$ transformed to correct for heterogeneity of variances. In several cases where there were non detectable (ND) data values. If all 4 replicates from a given site showed ND concentrations that the contaminant level was recorded as ND but if at least one of the replicates was greater than the detection limit then the other replicates were recorded as 1/2 the detection limit. Arithmetic means were used to summarize the results of replicate samples and are used in all subsequent tables and figures. In addition, geometric means were calculated for each metal for comparison with other data sets (O'Connor, 1992). The standard deviation (s) around the geometric mean (s_g) was calculated as:

$$s_g = \text{antilog } (s_l) = 10^{s_l}$$

where s_l = the standard deviation around the mean of the $\log_{10}$ transformed data (Snedecor and Cochran, 1967).

Total PAH (ΣPAH_{24}), total PCB (ΣPCB_{24}) and total pesticides ($\Sigma PEST_{17}$) values were created from the sum of all individual compounds or congeners with values greater than the detection limit for the compound. Total DDT (ΣDDT_6) is the sum of o,p'-DDT and p,p'-DDT and homologues (o,p'-DDE, p,p'-DDE, o,p'-DDD and p,p'-DDD). Organic variables, in which

all replicate measurements were below the detection limit, were treated as zero. All data were $\log_{10}(x+1)$ transformed to correct for nonnormality. Arithmetic means were used to summarize the results of replicate samples and are used in all subsequent tables and figures. In addition, geometric means were calculated for regional comparison. The standard deviation around the geometric means were calculated as described above.

Contaminant concentration data were analyzed so as to address the following questions on indigenous and caged mussels;

Indigenous Mussels:

(1) Does the level of contamination in indigenous mussels vary within jurisdictions?

Caged Mussels:

There was no reciprocal transplant done between preset sites and test sites. As such, it is difficult to separate the effects of mussel stock, site (polluted / unpolluted), and handling (caged / uncaged) on the mussel contaminant tissue concentrations. In an attempt to alleviate the problem the data was analyzed using the following pairwise comparisons.

(1) Are contaminant concentrations in mussels at preset sites different from native mussels at transplant sites? Test: preset vs. indigenous
(2) Do contaminant concentrations change (up or down) in the preset mussels with caging for 60 days at designated transplant sites within each jurisdiction? Test: preset vs. caged
(3) Do the contaminant concentrations in caged mussels differ from indigenous mussels at the transplant site? Test: caged vs. indigenous

All data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey-Kramer multiple comparison test of means. A probability of ≤ 0.05 was chosen as the level of significance.

RESULTS AND DISCUSSION

FIELD OPERATIONS AND LOGISTICS

Field collection proceeded as planned in all jurisdictions with the exception of Nova Scotia's. In Nova Scotia the experimental treatments (cages) were lost. All samples were transported successfully to the appropriate analytical labs in Augusta, ME (metals) and the Bedford Institute of Oceanography Environment Canada Laboratory in Dartmouth, N.S. (organic contaminants). However, one sample for organic analysis (Boston Inner Harbor, MA; MAIH-N) was not processed and no indigenous mussels were collected at NBMI.

CONTAMINANTS

METAL CONTAMINANTS

Table 3 contains the metal concentrations (arithmetic mean ± SD, µg / g dry weight) for caged (C) and indigenous (N) mussels from all sites sampled in 1995. Metal concentrations for each of the composite samples (n=4) are provided in Appendix C. Overall metal concentrations for indigenous mussels are given as geometric means (Table 3) to compare with NOAA (O'Connor, 1992) National Status and Trends program (NS & T) concentrations for Gulf of Maine sites (Table 4). All geometric means, except Ag, and Cu, were greater in Gulfwatch samples than in NOAA, NS&T samples. Moreover, the levels of Cd and Hg were greater than the calculated "high value" (geometric mean plus one standard deviation) for NOAA mussels. Similar results were observed in previous reports (see GMCME, 1994, 1996a, 1996b). This is not surprising given that half of the Gulfwatch stations were chosen as potentially contaminated areas, whereas the NS & T stations were essentially reference stations that were chosen to avoid acute human activity or known sources of contamination.

A. Spatial variation in metal concentrations: comparison of indigenous mussels

Figures 3 to 6 show the concentration of the metals measured in the tissue of *M. edulis* at the 1995 sampling stations presented from south to north. In addition, the mean tissue metal concentrations at each of the Gulfwatch sites are compared to two "benchmark" values for each

TABLE 3. Tissue metal concentrations (mean ± SD) for Gulfwatch mussels in 1995 (µg/g dry weight).
The geometric mean of all indigenous mussel data is given below. n = 4 replicates per sample.

STATION	Ag	Cd	Cr	Cu	Fe	Hg	Ni	Pb	Zn	Al
MASN-P	1.04±0.40	1.08±0.10	1.75±0.31	6.9 ± 0.7	245 ± 6	0.30 ± 0.03	0.88 ± 0.13	2.7 ± 0.3	98 ± 6	110 ± 14
MAIH-C	0.58±0.07	1.45±0.17	2.83±0.28	11.3 ± 1.3	315 ± 58	0.43 ± 0.06	1.08 ± 0.22	6.1 ± 0.7	158 ± 10	155 ± 42
MAIH-N	0.12±0.05	1.68±0.17	2.63±0.17	12.6 ± 2.6	345 ± 57	0.36 ± 0.05	1.10 ± 0.15	16.3 ± 3.4	175 ± 31	185 ± 19
MAPR-C	0.52±0.08	1.20±0.08	3.80±0.47	9.1 ± 0.3	448 ± 39	0.41 ± 0.05	1.35 ± 0.13	6.7 ± 0.4	110 ± 8	300 ± 23
MAPR-N	0.11±0.03	163±0.10	3.3±0.21	8.2 ± 0.5	305 ± 27	0.34 ± 0.01	0.95 ± 0.06	7.2 ± 0.5	110 ± 20	155 ± 19
NHHS-P	0.05±0.01	1.65±0.21	2.00±0.43	8.6 ± 0.8	363 ± 59	0.38 ± 0.04	1.33 ± 0.22	2.7 ± 0.3	143 ± 17	218 ± 43
NHLH-C	0.09±0.05	1.58±0.17	2.48±0.31	8.6 ± 0.6	460 ±3 7	0.50 ± 0.04	1.58± 0.05	3.0 ±0.3	140 ± 8	360 ± 28
NHLH-N	0.05±0.02	2.23±0.26	2.70±0.38	8.8 ± 0.9	510 ± 122	0.69 ± 0.10	1.73 ± 0.10	6.5 ± 1.2	155 ± 17	350 ± 56
MECC-C	0.10±0.05	1.70±0.16	2.70±0.68	8.3 ± 1.0	443 ± 39	0.50 ± 0.05	1.48 ± 0.17	3.9 ± 0.2	153 ± 13	260 ± 68
MECC-N	0.12±0.05	1.80±0.08	3.33±0.82	9.9 ± 1.4	535 ± 39	0.56 ± 0.13	1.65 ± 0.17	6.1 ± 0.7	135 ± 10	345 ± 26
MEBC-P	0.08±0.02	1.35±0.06	1.28±0.33	6.8 ± 0.2	255 ± 24	0.37 ± 0.02	1.30 ± 0.08	1.7 ± 0.2	98 ± 10	160 ± 23
MEDM-C	0.09±0.04	1.30±0.00	1.23±0.26	8.0 ± 0.6	280 ± 37	0.43 ± 0.15	1.40 ± 0.18	1.9 ± 0.2	87 ± 5	195 ± 41
MEDM-N	0.02±0.03	2.05±0.13	1.78±0.22	7.0 ± 1.2	445 ± 31	0.55 ± 0.11	1.50 ± 0.00	2.8 ± 0.3	75 ± 6	320 ± 14
MEKN-C	0.04±0.02	1.43±0.05	0.92±0.57	7.3 ± 0.3	180 ± 8	0.43 ± 0.10	1.13 ± 0.10	1.2 ± 0.2	81 ± 4	90 ± 8
MEKN-N	0.07±0.04	1.90±0.28	1.53±0.34	7.4 ± 1.3	225 ± 31	0.53 ± 0.11	1.08 ± 0.15	1.6 ± 0.4	79 ± 13	103 ± 10
NBHI-N	0.13±0.04	1.09±0.11	1.48±0.48	6.6 ± 1.3	240 ± 27	0.27 ± 0.04	0.92 ± 0.09	1.2 ± 0.1	71 ± 12	178 ± 32
NBHI-C	0.11±0.08	1.18±0.13	1.63±0.33	4.7 ± 0.5	265 ± 157	0.22 ± 0.05	0.76 ± 0.30	0.6 ± 0.4	67 ± 16	238 ± 155
NBNR-P	0.13±0.04	1.75±0.06	1.80±0.22	6.9 ± 0.2	560 ± 107	0.42 ± 0.04	1.70 ± 0.25	3.0 ± 1.8	97 ± 17	410 ± 74
NBMI-N	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data
NBMI-C	0.08±0.01	1.40±0.10	1.90±0.70	5.0± 0.5	465 ± 178	0.30 ± 0.09	1.19 ± 0.38	1.2 ± 0.6	95 ± 26	333 ± 196
NSBE-N	0.05±0.04	1.25±0.13	1.23±0.10	7.0 ± 0.6	413 ± 38	0.24 ± 0.01	1.12 ± 0.16	2.2 ± 0.1	56 ± 35	188 ± 59
NSCW-N	0.11±0.07	158±0.05	1.35±0.13	8.3 ± 1.5	353 ± 78	0.32 ± 0.02	1.48 ± 0.10	2.2 ± 0.3	86 ± 8	295 ± 31
Geometric mean (SD)	0.09 (2.8)	1.6 (1.3)	1.9 (1.5)	7.9 (1.2)	349 (1)	0.39 (1.4)	1.3 (1.3)	3.2 (2.1)	97 (1.6)	210 (1)

TABLE 4 . NOAA, National Status and Trends Mussel Watch summary statistics
the Gulf of Maine mussel samples collected in 1990, μg/g dry weight). n
= 13 sites (O'Connor, 1992).

	Ag	Al	Cd	Cr	Cu	Fe	Hg	Ni	Pb	Zn
Geometric mean	0.22	203	1.10	1.39	10.3	312	0.13	1.18	2.97	92
"high value"*	0.51	387	1.52	2.78	11.6	482	0.31	1.72	6.75	113

 * Logarithmic mean (geometric) plus one standard deviation (O'Connor, '92)

TABLE 5. Summary statistics for mussels collected at twenty-three Maine reference
stations (μg/g dry weight) (Sowles, 1993). ME-RM = Arithmetic, reference,
mean; ME-HV = Maine high value = Arithmetic mean plus three times the
standard deviation.

	Ag	Al	Cd	Cr	Cu	Fe	Hg	Ni	Pb	Zn
ME-RM	0.12	-	1.75	1.53	6.9	-	0.12	1.80	2.60	89
SD	0.09	-	0.46	0.66	1.28	-	0.12	0.38	1.13	15.5
ME-HV	0.40	-	3.14	3.51	10.7	-	0.48	2.90	6.00	136

TABLE 6. Analysis of tissue metal concentrations (arithmetic mean ± SD, µg/g dry wt.), by jurisdiction, for indigenous mussels at the Gulf of Maine stations, 1995. n = 4 replicates per sample. Same letter indicated no significant difference (P>0.05).

STATION	Ag	Cd	Cr	Cu	Fe	Hg	Ni	Pb	Zn	Al
MASN-P	1.04±0.40B	1.08±0.10A	1.75±0.31A	6.9±0.74A	245±6A	0.30±0.03A	0.88±0.13A	2.7±0.34A	98±6A	110±14A
MAIH-N	0.12±0.05A	1.68±0.17B	2.63±0.17B	12.6±2.56B	345±57B	0.36±0.05A	1.10±0.15A	16.3±3.40B	175±31B	185±19B
MAPR-N	0.11±0.03A	1.63±0.10B	3.13±0.21B	8.2±0.53A	305±27AB	0.34±0.01A	0.95±0.06A	7.15±0.45C	110±20A	155±19B
NHHS-P	0.05±0.01A	1.65±0.21A	2.00±0.43A	8.6±0.79A	363±59A	0.38±0.04A	1.33±0.22A	2.7±0.34A	143±17A	218±43A
NHLH-N	0.05±0.02A	2.23±0.26B	2.7±.38AB	8.8±0.92A	510±122B	0.69±0.10B	1.73±0.10B	6.5±1.15B	155±17A	350±56B
MECC-N	0.12±0.05B	1.80±0.08A	3.33±0.82B	9.9±1.41A	535±39B	0.56±0.13B	1.65±.17AB	6.1±0.68B	135±10A	345±26B
MEBC-P	0.08±0.02B	1.35±0.06A	1.28±0.33A	6.8±0.22A	255±24A	0.37±0.02A	1.30±0.08A	1.7±0.24A	98±10B	160±23B
MEDM-N	0.02±0.03A	2.05±0.13B	1.78±0.22A	6.9±1.22A	445±31B	0.55±0.11B	1.50±0.00A	2.8±0.33B	75±6A	320±14C
MEKN-N	0.07±0.04B	1.90±0.28B	1.53±0.34A	7.4±1.27A	225±31A	0.53±0.11B	1.08±0.15B	1.6±0.40A	79±13B	103±10A
NBHI-N	0.13±0.04A	1.09±0.11A	1.48±0.48A	6.6±0.72A	240±27A	0.27±0.04A	0.92±0.09A	1.2±0.13A	71±12A	178±32A
NBNR-P	0.13±0.04A	1.75±0.06B	1.80±0.22A	6.9±0.22A	560±107B	0.42±0.04B	1.70±0.25B	2.9±1.77B	97±17B	410±74B
NBMI-N	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data
NSBE-N	0.05±0.04A	1.25±0.13A	1.23±0.10A	7.00±0.55A	413±38A	0.24±0.01A	1.12±0.16A	2.18±0.10A	56±35A	188±59A
NSCW-N	0.11±0.07A	1.58±0.05B	1.35±0.13A	8.25±1.45A	353±78A	0.32±0.02B	1.48±0.10B	2.18±0.33A	86±8A	295±31B

metal previously reported by Sowles (1993) from 23 Maine reference sites: (1) the arithmetic mean for each metal concentration (Maine Reference Mean or ME-RM); and (2) the arithmetic mean plus three standard deviations (Maine High Value or ME-HV; referred to by Sowles as the "anomalous value"). These Maine reference stations are located in areas where anthropogenic contamination should be low. Maine Reference concentrations should therefore be lower than that observed at several of the Gulfwatch stations.

Table 6 shows the metal concentrations for indigenous mussels only. Sites were grouped by jurisdiction and ANOVA and Tukey Kramer tests were employed to examine differences among sites within a jurisdiction in 1995. Differences among all sites (14 stations throughout 5 jurisdictions) were not examined statistically. MECC is discussed as being a New Hampshire site because it is located in the Great Bay / Piscatagua River watershed, and therefore most comparable to other sites in New Hampshire.

Silver (Ag)

Elevated silver exposure concentrations have been shown to coincide with regions receiving municipal sewage (Sanudo-Wlhelmy and Flegal, 1992; Bucholzten ten Brink et al., 1996). Mussel tissue concentrations of Ag were the the lowest at MEDM (0.02 ± 0.03 µg / g dry weight) and the highest at MASN (1.04 ± 0.40 µg / g dry weight) (Table 3). As in previous reports (see GMCME, 1994, 1996a, 1996b) the concentration of Ag in mussel tissue increases in concentration from north to south (Figure 3). Ag concentrations at MASN were significantly higher than all other sites in 1995 and exceed the Maine high value (ME-HV) of 0.40 µg / g dry weight for the Maine reference stations. This exceptionally high silver concentration at MASN was also observed in the Gulfwatch 1993 and 1994 samples, but not in the 1992 samples (GMCME, 1994). However, even the mean silver concentrations measured in the 1992 samples (0.44 ± 0.13 µg / g dry weight) were higher than values obtained for the other Gulfwatch stations in 1995. These high Ag concentrations are unusual since there are no POTW outfalls or industrial effluent in the area.

All other sites examined in 1995 were below the Maine reference mean of 0.12 µg / g dry weight. Analysis of mussel tissue burdens within jurisdictions (Table 6) showed that for Massachusetts, other than MASN, there was no significant difference among sites (MAIH and MAPR). In addition, there was no significant difference between sites in Nova Scotia and New Brunswick. In New Hampshire MECC was significantly higher than NHHS and NHLH. In Maine, MEDM was significantly lower than MEKN and MEBC.

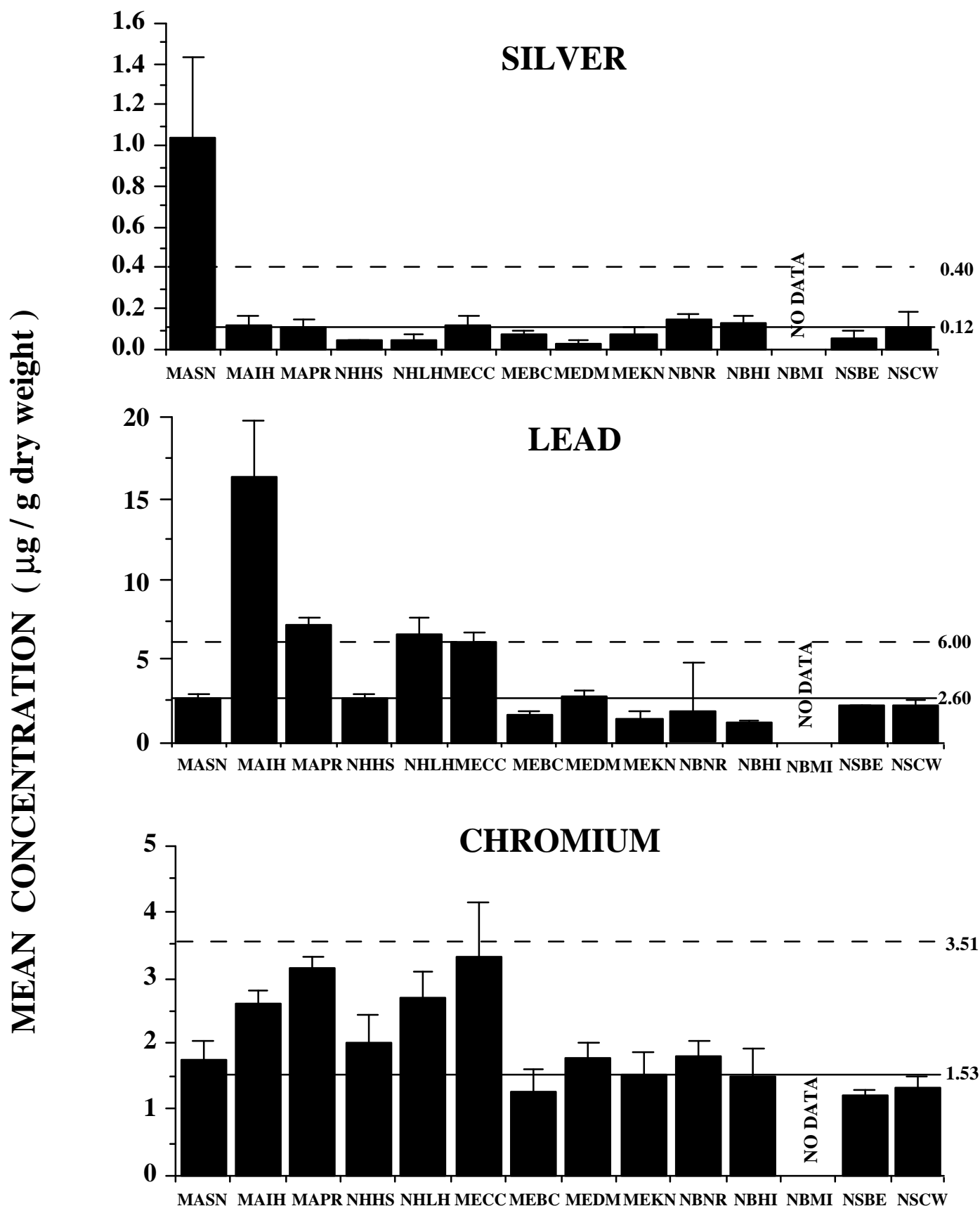Fig. 3 Distribution of silver, lead, and chromium tissue concentrations (arithmetic mean ± SD, μ g/g dry weight) in indigenous mussels at the Gulf of Maine stations in 1995. The Reference mean (ME-RM, straight line) and the High Value, dashed line) from the Maine reference data (Sowles, 1993) are shown for

Lead (Pb)

The concentration of lead ranged from a value of 1.15 ± 0.13 µg / g dry weight (NBHI) to 16.3±3.4 µg / g dry weight (MAIH) (Table 3, Figure 3). The highest lead concentrations were recorded at MAIH, MAPR, NHLH and MECC. Of these MAPR, NHLH and MECC all exceed the Maine reference concentrations (ME-RM) of 2.6 ± 1.1 µg / g dry weight but only MAIH exceeded the ME-HV (6.00 µg / g dry weight) with a value of 16.3 ± 3.4 µg / g dry weight. MAIH was significantly higher than all other sites. This site is located in Boston's Inner Harbor, and receives inputs from over a dozen combined sewer overflows which channel street runoff (and sewage) into the harbor during periods of high rainfall. In addition, the Inner Harbor receives inputs from the Charles, Mystic and Chelsea rivers, and most likely receives POTW effluent from the Massachusetts Water Resources Authority's outfall pipes (370 MGD) which currently discharge at the mouth of Boston Harbor, 7 km to the east. The close proximity to the Portsmouth Naval Shipyard may account for the elevated lead concentrations in mussels at both New Hampshire sites (NHLH and MECC). The Jamaica landfill and defense reutilization and Marketing Office on Seavy Island are sites of known sources of lead contamination to Portsmouth Harbor where waste plating sludge and lead batteries, respectively were disposed and stored.

Analysis of the concentrations of Pb in mussel tissue within each jurisdiction (Table 6) showed that the level of Pb varied. There were significant differences between sites within Massachusetts, New Hampshire, Maine and New Brunswick, while only Nova Scotia showed consistent Pb concentrations between sites. There is a trend for higher concentrations in population centres such as in Massachusetts and New Hampshire.

Chromium (Cr)

The concentration of chromium exceeded the ME-RM (1.53 ± 0.66 µg / g dry weight) in Massachusetts, New Hampshire, and in some Maine and New Brunswick sites, although not the ME-HV (3.51 µg / g dry weight). The the lowest concentration was at NSBE (1.23 ± 0.10 µg / g dry weight) and the highest at MECC (3.32 ± 0.82 µg / g dry weight) (Table 3, Figure 3). Elevated concentrations at MECC and NHLH probably reflect historical tanning industry discharges (Capuzzo et al., 1973; Jones et al., 1992).

Analysis of the mussel tissue concentrations of Cr within each jurisdiction (Table 6) revealed that there were no significant differences between sites in Nova Scotia, or New Brunswick or among sites in Maine in 1995. However, within Massachusetts MAPR and MAIH were significantly higher than MASN. In New Hampshire, NHHS was significantly lower than NHLH and MECC.

Zinc (Zn)

Zinc concentrations generally reflect human activity associated with tire wear, galvanized materials and industrial discharges. All sites in Massachusetts, New Hampshire, southern Maine (MECC and MEBC), and NBNR in New Brunswick had concentrations greater than the ME-RM (89 ± 16 µg / g dry weight) with MAIH, NHHS and NHLH having concentrations greater than the ME-HV (136 µg / g dry weight) (Table 3, Figure 4). The lowest concentration of Zn measured was at NSBE (56±35 µg / g dry weight) and the highest was at MAIH (175±31 µg / g dry weight).

Analysis of the mussel tissue concentrations of Zn within each jurisdiction revealed that only New Hampshire and Nova Scotia had consistent concentrations of Zn among sites (Table 6). In Massachusetts, the Zn concentration at MAIH was significantly higher than MASN and MAPR; in Maine MEDM was significantly lower than all other sites; and in New Brunswick NBNR was significantly higher than NBHI.

Nickel (Ni)

The concentration of nickel ranged from a value of 0.88 ± 0.13 µg / g dry weight at MASN to 1.73 ± 0.10 µg / g dry weight at NHLH (Table 3, Figure 4). All concentrations, however, were lower than the ME-RM of 1.8 ± 0.4 µg / g dry weight.

Analysis of the mussel tissue concentrations of Ni within each jurisdiction (Table 6) revealed that the level of Ni varied greatly within jurisdictions. Only in Massachusetts, was the level of Ni consistent among sites. In New Hampshire, NHHS was significantly lower than NHLH and MECC; in Maine, MEKN was significantly lower than the other sites. In Nova Scotia, NSBE significantly higher than NSCW and in New Brunswick, NBNR significantly higher than NBHI.

Mercury (Hg)

The concentration of mercury in mussel tissue ranged from a value of 0.24 ± 0.01 µg / g dry weight at NSBE to 0.69 ± 0.10 µg/g dry weight at NHLH (Table 3, Figure 4). Mercury exceeded the ME-RM of 0.12 ± 0.12 µg / g dry weight at all sites. NHLH, MECC, MEDM and MEKN
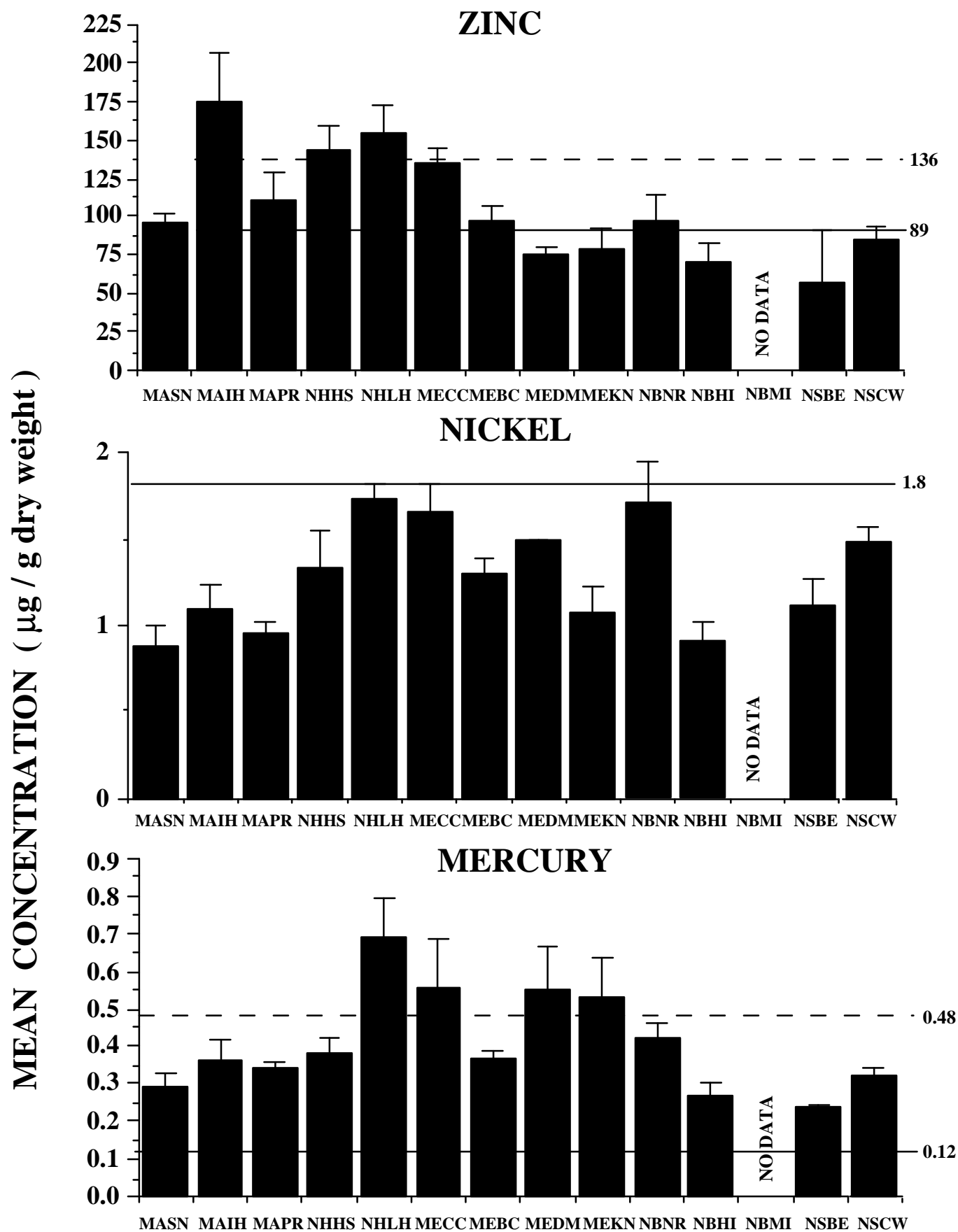
ZINC

NICKEL

MERCURY

Fig. 4 Distribution of zinc, nickel, and mercury tissue concentrations (arithmetic mean ± SD, μ g/g dry weight) in indigenous mussels at the Gulf of Maine stations in

exceed the ME-HV of 0.48 µg / g dry weight. NHLH and MECC lie downstream from known historical mercury sources that are suspected to be related to the Portsmouth Naval Shipyard (NCCOSC, 1994).

Analysis of the mussel tissue concentrations of Hg from sites within each jurisdiction (Table 6) showed that the level of Hg varied in all jurisdictions with the exception of Massachusetts. In New Hampshire, NHLH and MECC were significantly higher than NHHS; in Maine, MEBC was significantly lower than MEKN and MEDM; in New Brunswick, NBNR was significantly higher than NBHI; and in Nova Scotia, NSBE was significantly higher than NSCW.

Cadmium (Cd)

Cadmium is widely used in industry for batteries, plating, stabilizers and as a neutron absorber in nuclear reactors. The concentration of cadmium in mussel tissue ranged from 1.08 ± 0.10 µg / g dry weight at MASN to 2.23 ± 0.26 µg / g dry weight at NHLH (Table 3, Figure 5). All values were below the ME-RM of 1.75 ± 0.46 µg / g dry weight with the exception of NHLH, MEDM, MEKN, and NBNR. No values exceeded the ME-HV (3.14 µg / g dry weight).

Within the jurisdictions the concentration of Cd varied. There were significant differences among sites in each jurisdiction (Table 6). In general, however, the sites chosen a priori as the preset were significantly lower in each jurisdiction except for New Brunswick where NBNR was significantly higher than NBHI.

Copper (Cu)

The level of copper in mussel tissue ranged from 6.55 ± 0.72 µg / g dry weight at NBIH to 12.55 ± 2.56 µg / g dry weight at MAIH (Table 3, Figure 5). The Cu concentration at MAIH was significantly higher than all other sites. All values exceeded the ME-RM (6.9 ± 1.6 µg / g dry weight) with the exception of MEBC, and NBHI. Only MAIH, exceeded the ME-HV (10.9 µg / g dry weight).

Analysis of the mussel tissue level of Cu within each jurisdiction showed that the level of Cu was fairly consistent (Table 6). There were no significant differences among sites in New Hampshire, and Maine, or between sites in New Brunswick and Nova Scotia. However, in Massachusetts, MAIH was significantly higher than all other sites.
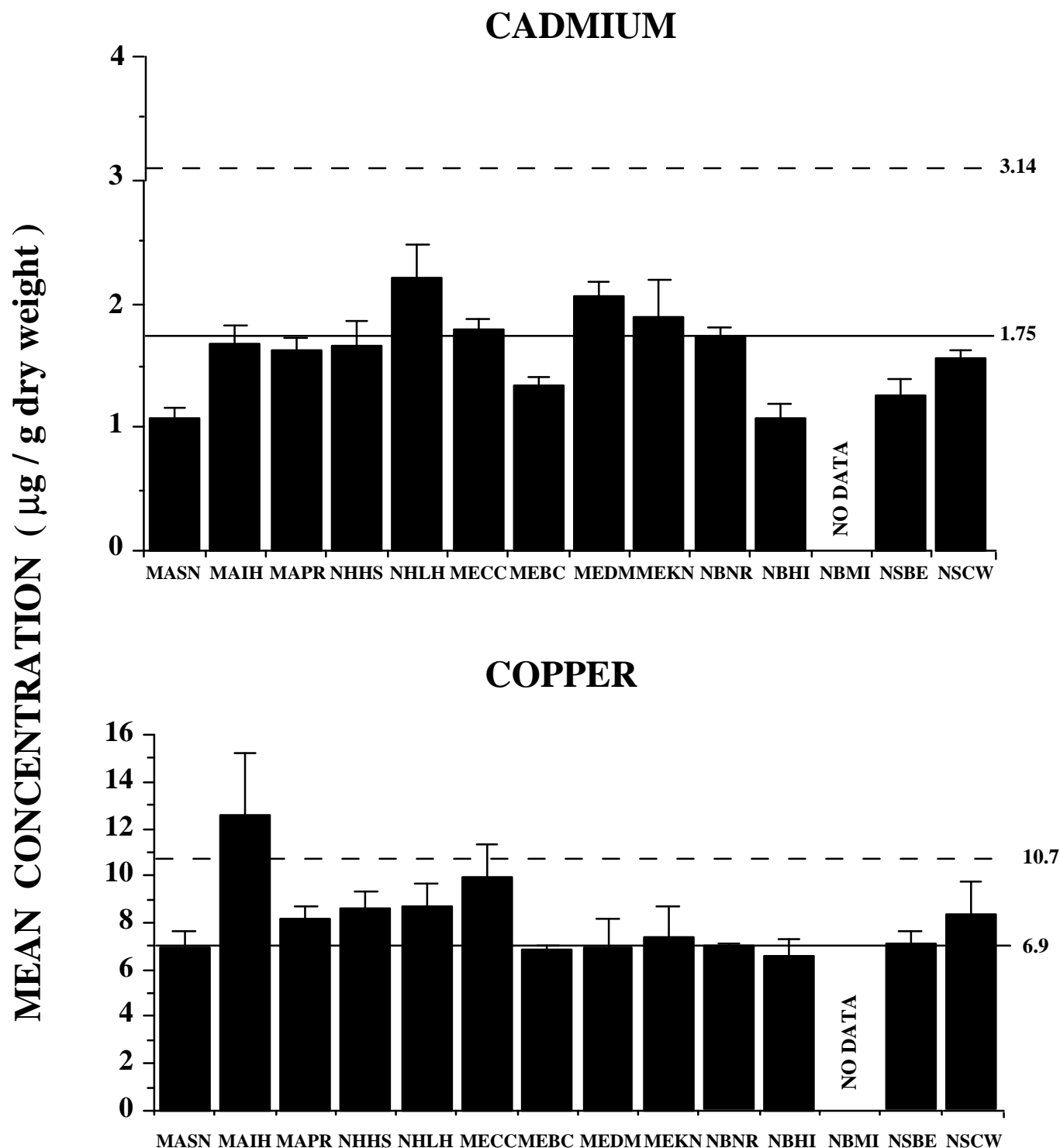
CADMIUM

COPPER

Fig. 5 Distribution of cadmium, and copper tissue concentrations (arithmetic mean ± SD, μ g/g dry weight) in indigenous mussels at the Gulf of Maine stations in 1995. The Reference Mean (ME-RM, straight line) and the High Value (ME-HV, dashed line) from the Maine reference data (Sowles, 1993) are shown for comparison.

Iron (Fe) and Aluminum (Al)

The concentration of iron in mussel tissue ranged from 255 ± 31 µg / g dry weight at MEKN to 560 ± 107 µg / g dry weight at NBNR (Table 3, Figure 6). There were no reference values for Fe from Maine stations with which to compare our data. Analysis of the mussel tissue concentrations of Fe within jurisdictions (Table 6) showed that there were no significant differences between sites in Nova Scotia but there were significant differences among sites in Massachusetts, New Hampshire, and Maine and between sites in New Brunswick.

The concentration of aluminum in mussel tissue ranged from 103 ± 10 µg / g dry weight at MEKN to 410 ± 74 µg / g dry weight at NBNR (Table 3, Figure 6). There were no reference values for Al from Maine stations with which to compare our data, but comparisons could be made to NS&T values. Analysis of the level of Al in mussel tissue within jurisdictions showed that the level of Al was not consistent in any jurisdiction. In Massachusetts, MASN was significantly lower than MAIH and MAPR; in New Hampshire, NHHS was significantly lower than NHLH and MECC; and in Maine, New Brunswick, and in Nova Scotia there were significant differences between all sites.

IRON

MEAN CONCENTRATION (µg / g dry weight)

700
600
500
400
300
200
100
0

57

38

MASN MAIH MAPR NHHS NHLH MECC MEBC MEDM MEKN NBNR NBHI NBMI NSBE NSCW

ALUMINUM

500
400
300
200
100
0

436

291

MASN MAIH MAPR NHHS NHLH MECC MEBC MEDM MEKN NBNR NBHI NBMI NSBE NSCW
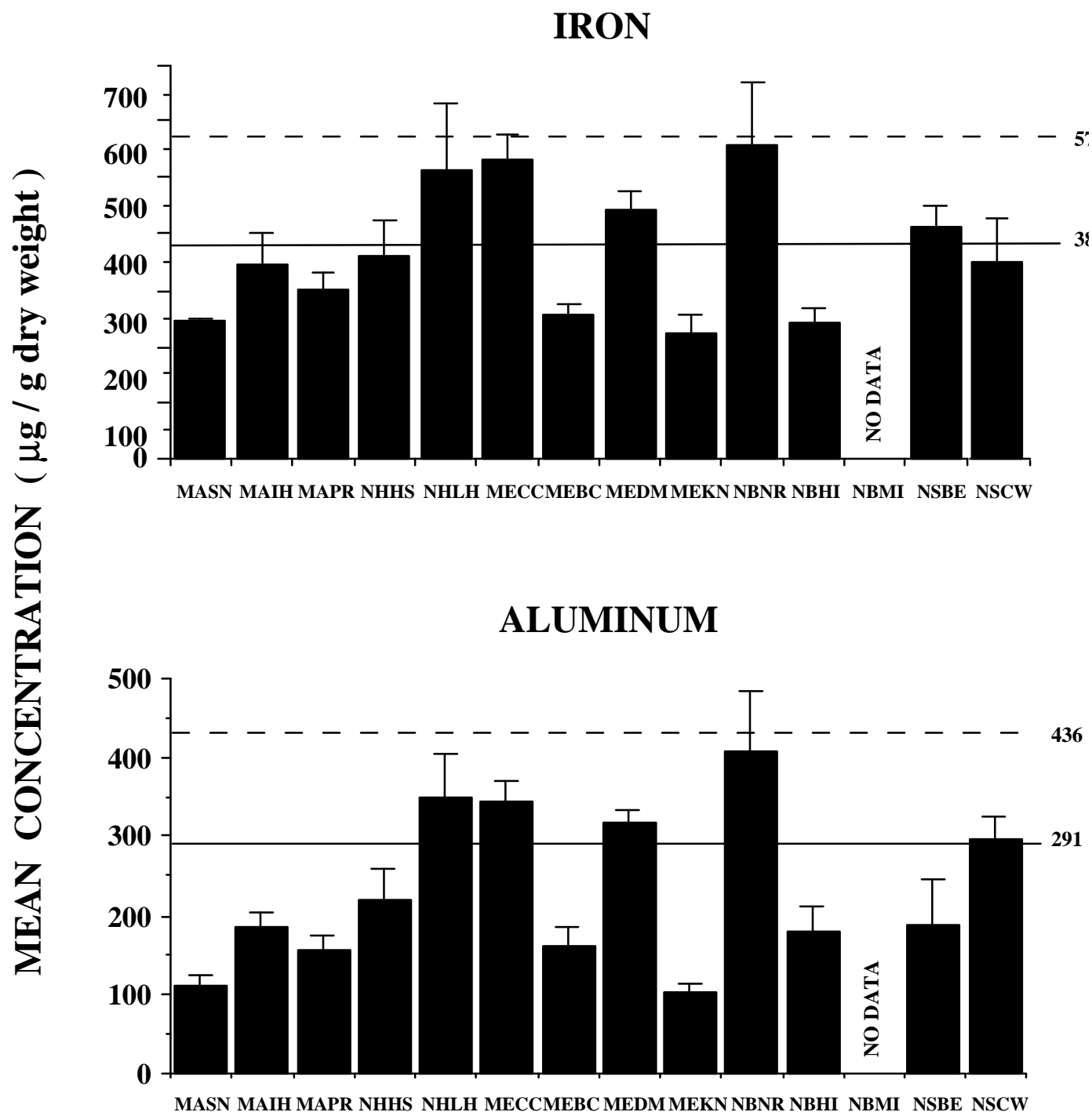
Fig. 6 Distribution of Iron, and aluminum tissue concentrations (arithmetic mean ± S
µ g/g dry weight) in indigenous mussels at the Gulf of Maine stations in 1995.
The mean (straight line) and the high value (mean plus one standard deviation,
dashed line) from the NS&T Gulf of Maine data are shown for comparison.

B. Short term exposure: transplant experiment

Figures 7 through 10 show the concentrations of the various metals from south to north at the preset and caged stations. Results of the ANOVA on metal tissue concentrations in caged versus preset mussels, preset versus indigenous mussels and indigenous versus caged mussels are shown in Table 7. As with previous analysis, MECC is considered a New Hampshire site.

Massachusetts

The concentration of metals in the caged mussels was significantly different from the preset mussels after 60 days deployment in all but 4 comparisons [Fe, Al and Ni (MAIH) and Cd (MAPR) (Table 7)]. In 14 out of the 16 significant observations the concentration of metals in the caged mussels had significantly increased over that of the preset tissue concentrations (C>P), indicating bioaccumulation of the metals. In 2 out of the 16 significant observations the caged mussels were less than the preset (C<P). In the 2 cases where C<P (Ag for both sites), the preset (MASN) had significantly higher concentrations of Ag than the indigenous mussels at the transplant destinations (MAIH and MAPR). This suggests depuration of the metals from preset mussels after deployment. Otherwise, metal concentrations in preset mussels are equal to or less than in the concentrations in the indigenous mussels at the transplant site.

New Hampshire

For the majority of metals there was no change in the mussel tissue burden after 60 days of deployment (C=P) (Table 7). The exceptions were, Fe (NHLH), Al (NHLH), Pb (MECC) and Hg (NHLH and MECC), where the concentrations in the caged mussels had significantly increased in comparison to preset tissue concentrations (C>P). For all cases where the level of metals increased after deployment, the level of metals in the preset mussels was significantly less than the indigenous mussels at the transplant site. In no cases were metal concentrations in preset mussels greater than concentrations in indigenous mussels at the transplant site.

Maine

With the exception of Cu, there was no significant difference in the metal tissue burden between caged and preset mussels after 60 days of deployment at MEDM (Table 7). The level of Cu was significantly higher in caged mussels. Four out of the 10 metals examined in caged mussels at MEKN had tissue concentrations that were significantly less than the preset (C<P). For Al, the tissue level in the preset was significantly greater than the indigenous mussels at the transplant site. For the metals Fe, Zn and Pb the level in the tissues of the preset mussels was higher than the indigenous, but not significantly. There was no significant change in metals Hg, Cu, Cr and Ag after deployment at MEKN (C=P).

New Brunswick

Analysis of the mussel tissue burdens in caged and preset mussels revealed one of two responses, either no significant difference between caged and preset (C=P) or the caged mussels had significantly lower tissue burdens than the preset mussels (C<P) after 60 days deployment (Table 7).

Unfortunately, only NBHI had samples of indigenous mussels for comparison. In all but one metal (Cu), in instances where the mussel tissue concentration of caged mussels was significantly less than the tissue concentration of preset mussels (C<P), the tissue concentration of the preset mussels was significantly greater than the indigenous mussels at the transplant site. For Cu, the tissue concentration in the preset mussels was not significantly different from the indigenous population at the transplant site.

TABLE 7. Summary of analysis of transplantation data; TEST, (1) C vs P: tissue metal concentrations in caged (C) versus preset (P) mussels; (2) P vs N: Preset mussels versus indigenous mussels (N); and (3) C vs N: Caged versus indigenous mussels. NS, indicates no significant difference between treatments. < / > indicates a significant difference in tissue metal concentrations between treatments and the direction.

| | | MASSACHUSETTS | | NEW HAMPSHIRE | | MAINE | | NEW BRUNSWICK | |
| | | Preset : MASN | | Preset : NHHS | | Preset : MEBC | | Preset : NBNR | |
METAL	TEST	MAIH	MAPR	NHLH	MECC	MEDM	MEKN	NBHI	NBMI
Ag	CvsP	C<P	C<P	NS	NS	NS	NS	NS	NS
	P vs N	P > N	P > N	NS	P < N	P > N	NS	NS	
	C vs N	C > N	C > N	NS	NS	C > N	NS	NS	
Cd	C vs P	C > P	NS	NS	NS	NS	NS	C < P	C < P
	P vs N	P < N	P < N	P < N	NS	P < N	P < N	P > N	
	C vs N	NS	C < N	C < N	NS	C < N	C < N	NS	
Cr	CvsP	C>P	C>P	NS	NS	NS	NS	NS	NS
	P vs N	P < N	P < N	NS	P < N	NS	NS	NS	
	C vs N	NS	C > N	NS	NS	C < N	NS	NS	
Cu	C vs P	C > P	C > P	NS	NS	C > P	NS	C < P	C < P
	P vs N	P < N	NS	NS	NS	NS	NS	NS	
	C vs N	NS	C > N	NS	NS	NS	NS	C < N	
Fe	C vs P	NS	C > P	C > P	NS	NS	C < P	C < P	NS
	P vs N	P < N	NS	P < N	P < N	P < N	NS	P > N	
	C vs N	NS	C > N	NS	C < N	C < N	C < N	NS	

TABLE 7. (Continued from pg. 33)

| | | MASSACHUSETTS | | NEW HAMPSHIRE | | MAINE | | NEW BRUNSWICK | |
| | | Preset : MASN | | Preset : NHHS | | Preset : MEBC | | Preset : NBNR | |
METAL	TEST	MAIH	MAPR	NHLH	MECC	MEDM	MEKN	NBHI	NBMI
Hg	C vs P	C > P	C > P	C > P	C > P	NS	NS	C < P	NS
	P vs N	NS	NS	P < N	P < N	P < N	P < N	P > N	
	C vs N	NS	C > N	C < N	NS	NS	NS	NS	
Ni	C vs P	NS	C > P	NS	NS	NS	NS	C < P	NS
	P vs N	NS	NS	P < N	NS	NS	P > N	P > N	
	C vs N	NS	C > N	C < N	NS	NS	NS	NS	
Pb	C vs P	C > P	C > P	NS	C > P	NS	C < P	C < P	NS
	P vs N	P < N	P < N	P < N	P < N	P < N	NS	P > N	
	C vs N	C < N	NS	C < N	C < N	C < N	NS	C < N	
Zn	C vs P	C > P	C > P	NS	NS	NS	C<P	NS	NS
	P vs N	P < N	NS	NS	NS	P > N	NS	P > N	
	C vs N	NS	NS	NS	NS	C > N	NS	NS	
Al	C vs P	NS	C > P	C > P	NS	NS	C < P	NS	NS
	P vs N	P < N	P < N	P < N	P < N	P < N	P > N	P > N	
	C vs N	NS	C > N	NS	NS	C < N	NS	NS	

Fig. 7. Distribution of silver, lead, and chromium tissue concentrations (arithmetic mean ± SD, μ g/g dry weight) in caged (gray) and preset (black) mussels at the Gulf of Maine stations, 1995. *, indicates a significant difference between caged and preset mussel tissue concentrations (p<0.05).
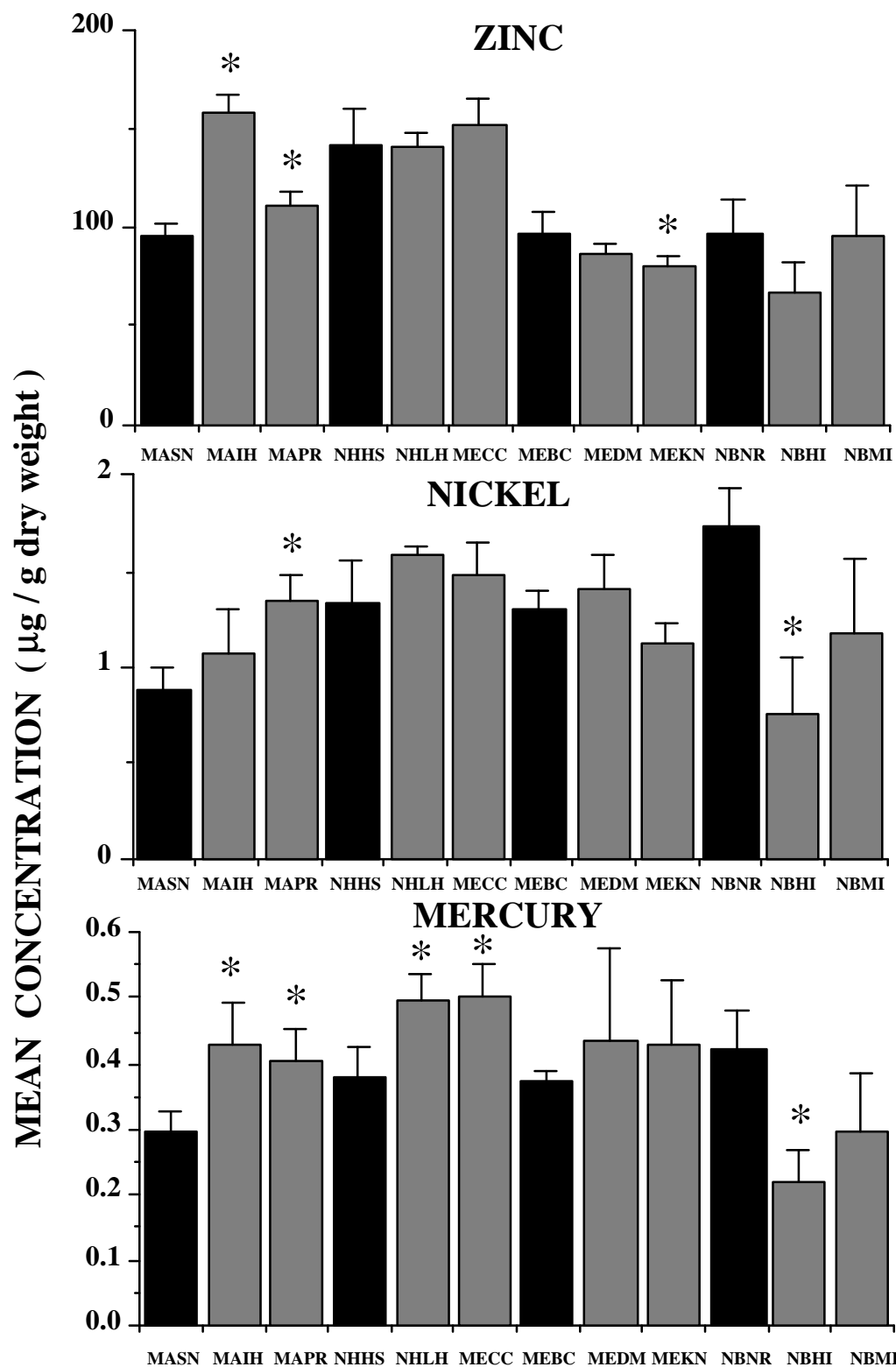
Fig. 8. Distribution of zinc, nickel, and mercury tissue concentrations (arithmetic mean ± SD, µg/g dry weight) in caged (gray) and preset (black) mussels at the Gulf of Maine stations, 1995. *, indicates a significant difference between caged and preset mussels (p<0.05).

CADMIUM

COPPER

Fig. 9. Distribution of cadmium, and copper tissue concentrations (arithmetic mean ± SD, μ g/g dry weight) in caged (gray) and preset (black) mussels at the Gulf of Maine stations, 1995. *, indicates a significant difference between caged and preset mussels (p<0.05)
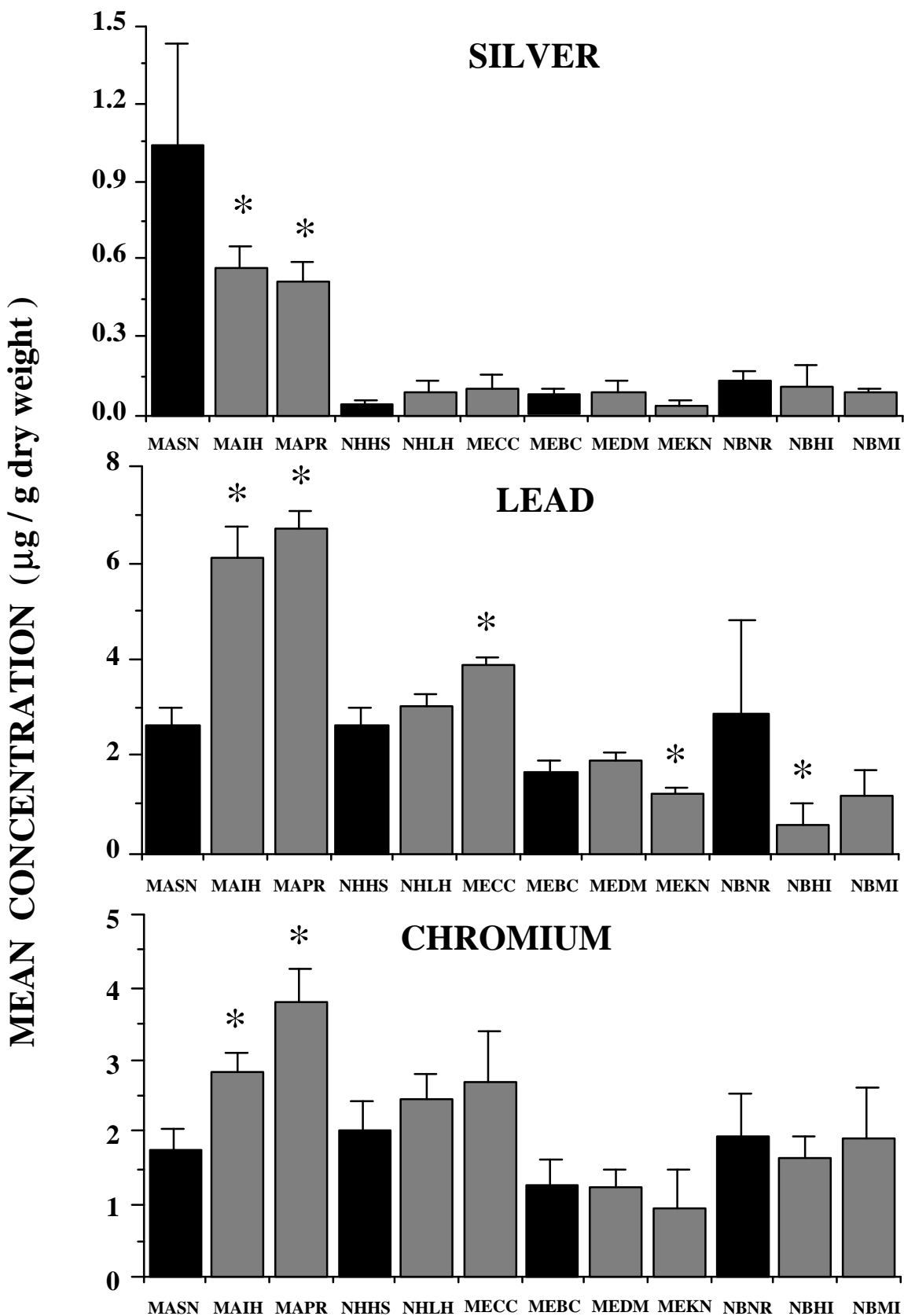
Fig. 10. Distribution of Iron, and aluminum tissue concentrations (arithmetic mean ± SD, μ g/g dry weight) in caged (gray) and preset (black) mussels at the Gulf of Maine stations, 1995. *, indicates a significant difference between caged and preset mussels (p<0.05).

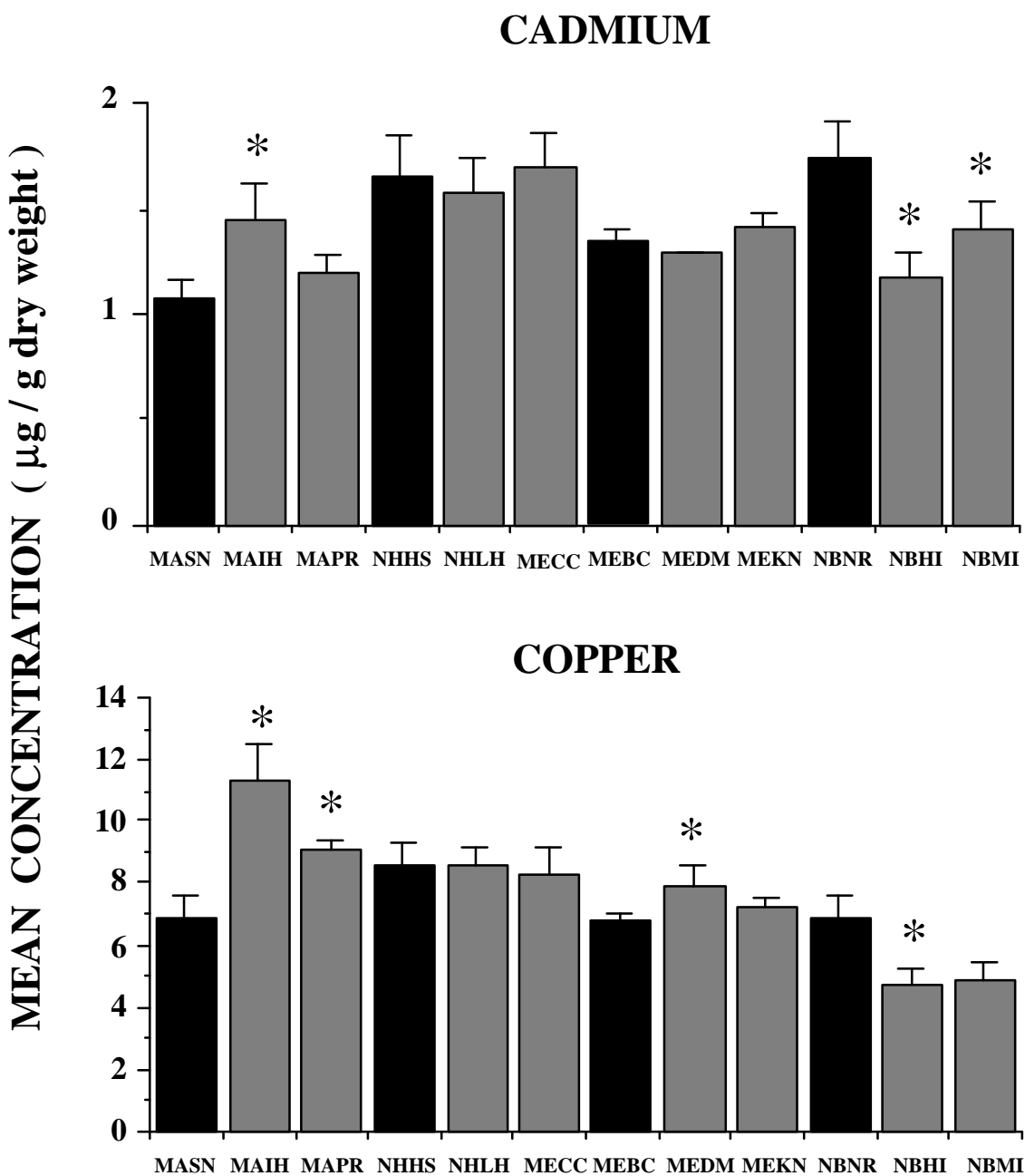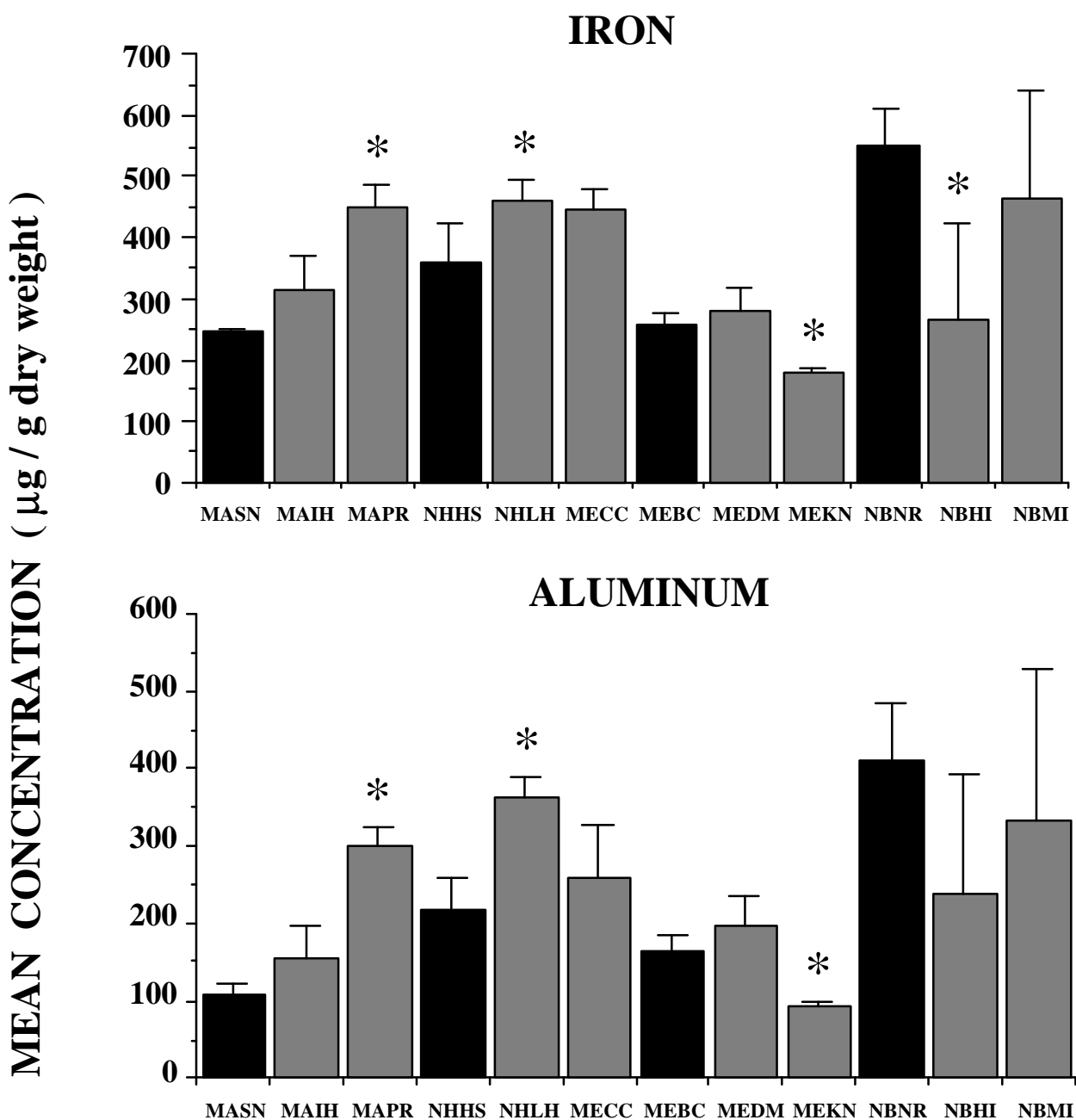**Summary of Metal Concentration changes during transplants**

As indicated above for each of the jurisdictions separately, tissue concentrations of at least one (and quite often several) metals increased at every transplant site except NBHI and MEKN. Silver was the only metal that did not bioaccumulate at any of the sites. Bioaccumulation of the other metals was most striking at the Massachusetts sites, particularly at MAPR. The degree of bioaccumulation tended to taper off along the south-to-north transect (Cd, Cr, Cu, Hg, Pb and Zn bioaccumulated at MAIH; Al, Cr, Cu, Fe, Hg, Ni, Pb and Zn at MAPR; Al, Fe and Hg at NHLH; Hg and Pb at MECC; and Cu at MEDM). These bioaccumulated metals are clearly of concern, and should spur additional investigations as to their source, temporal availability and geographical extent. At those sites where Al and Fe bioaccumulated along with other "heavy" metals, contamination by gut sediments may have contributed to the overall metal body burden (Robinson et al., 1993). This possibility should be tested for in the future by either analyzing these local sediments or else (more directly) by depurating a set of exposed mussels in clean sea water 48 h prior to dissection and metal analysis.

At four of the transplant sites, a reduction in some metal concentrations (depuration) was observed in the transplanted mussels (compared to the preset mussels). In both Massachusetts sites (MAPR and MAIH), Ag concentrations declined in the transplanted mussels, but did not reach the much lower concentrations found in indigenous mussels collected from sites adjacent to the transplant sites. It should be noted that the Massachusetts preset mussels (collected from MASN) had exceptionally high Ag concentrations; this order of magnitude elevation was not observed at any of the other Gulfwatch sites. This clearly points out that the MASN mussels were considerably contaminated with Ag (as discussed previously).

A greater degree of depuration was measured at MEKN (Al, Fe,Pb and Zn) and NBHI (Cd, Cu, Fe, Hg, Ni and Pb). This depuration indicates that the transplant sites had a far lower contaminant bioavailability than the site used to collect preset mussels, at least for the contaminants that exhibited depuration. At the MEKN site for example, mussels depurated several metals, yet simultaneously showed an increase in Cd and in organic contaminants (PAH, PCB, DDT, and pesticides, as discussed later). Thus, MEKN may be considered "cleaner" than MEBC with respect to Al, Fe, Pb and Zn), yet more contaminated than MEBC with respect to Cd and organic contaminants. However, the possibility that the preset mussels that were compared to the MEKN and NBHI transplants may have had artificially high tissue concentrations due to contamination by gut contents cannot be ruled out at this time. A more in-depth examination of the various factors which may have been responsible for these observations is justified.

Steady state tissue metal concentrations

Although changes were observed in metal concentrations during the transplant period (comparison of preset and 60-d transplants), the question arises as to whether the caged mussels had been exposed long enough to reach a steady state with their new environment. The time needed for an organism to reach a new steady state differs dramatically from contaminant to contaminant (Roesijadi et al., 1984; EPA, 1989; Peven et al., 1996). Most organic contaminants generally reach steady state much quicker than metals (de Kock, 1983; Nelson et al., 1995). Different metals, on the other hand, exhibit marked differences in the time needed to reach steady state (Schulz-Blades, 1974; Roesijadi et al., 1984).

At first examination of the Gulfwatch data, it appears that steady state conditions may have been reached for metals within the 60 day transplant period. Several metals that were accumulated by the transplant mussels attained similar levels as were measured in the indigenous mussels sampled adjacent to the transplant sites (Cu at MEDM; Al and Fe at NHLH; Hg at MECC; Cd, Cr, Cu, Hg and Zn at MAIH; Pb and Zn at MAPR). Even more remarkably, concentrations of Al, Cr, Cu, Fe, Hg, and Ni in the mussels transplanted to MAPR were significantly higher than the concentrations of these same metals in adjacent native metals, indicating that the transplanted mussels may have overshot the concentrations that would normally be present in mussels from that site. There were only a few instances where metals were accumulated by the transplanted mussels but tissue concentrations failed to attain the concentrations found in indigenous mussels (Cd at MEKN; Hg at NHLH; and Pb at both MECC and MAIH).

The pattern of metal depuration observed at MEKN and NBHI also appears to indicate that the transplanted mussels at these two sites reached a new steady state. In all cases, those metals that depurated either fell to concentrations that were similar to the indigenous mussels (Al, Ni, Pb, and ZN at MEKN; Cd, Fe, Hg, and Ni at NBHI) or were actually lower than the native mussels sampled adjacent to the transplant site (Fe at MEKN; Cu and Pb at NBHI).

This apparent attainment of steady state is perplexing in light of other published transplant studies as well as on theoretical grounds. For example, Schulz-Blades (1974) reported that more than 230 d is required for *M. edulis* to reach a new steady state for Pb. In contrast, Cu and Ag may attain steady state conditions within 8 weeks, yet Zn took 24 weeks under field conditions (Roesijadi et al., 1984). Cadmium failed to reach steady state conditions in *M. edulis* following a 60 d transplant (de Kock, 1983). In a related species, *Guekensia* (= *Modiolus*) *demissus*, transplanted for 28 d, Fe, Cr, Cu and Mn concentrations overshoot the levels measured in indigenous mussels, Zn and Ni attained roughly the same concentrations, and Cd and Pb concentrations remained far below the concentrations of native mussels (Nelson et al., 1995). These results indicate a similar pattern of variability as observed in the

present study.

Toxicokinetic calculations indicate that metals should not reach steady state within a 60 d period, whereas many organic contaminants should. According to toxicokinetic principles, steady state is never actually reached, but is asymptotically approached over time. Neither exposure concentration or uptake rate effect the time to a practical steady state (e.g., the time to reach 99.99% of steady state; Barron et al., 1990). The depuration (or elimination) rate constant, on the other hand, primarily determines the time needed to reach steady state:

$$f_{SS} = 1 - e^{-kt} \qquad (1)$$

where f_{SS} = fraction of steady state; t = time and k = depuration rate constant (Barron et al., 1990). Using equation (1), the time needed to reach various fractions of steady state (99.99% and 80%), as well as the fraction of steady state achieved in 60 d can be calculated. Unfortunately, only a few published studies contain the data needed to conduct these calculations. Results indicate that mussels transplanted to Gulfwatch sites for 60 days would achieve metal concentrations that are quite far from steady state, whereas PCB concentrations (and other hydrophobic organic contaminants) would be at steady state (Table 8). Estimates vary for each metal, ranging from 2 to 70% attainment of steady state for each metal. Estimates also vary for the same metal, depending on the findings from different laboratory studies. For Cd, for example, three estimates of the fraction of steady state attained in 60 d vary from 2%, to 19-35%, and finally to 48% (Table 8).

Differences in estimated times to reach steady state notwithstanding, it is clear that, based on theoretical grounds, metal concentrations should not have reached steady state conditions for the Gulfwatch 60 day transplant mussels. Yet, as previously discussed, a variety of metals attained similar or even higher concentrations than indigenous mussels collected adjacent to the transplant cages. There are several possible explanations that would account for this discrepancy: adaptation, acclimation, slight differences in environmental exposure, and cage effects. It is reasonable to assume that local mussel populations have adapted (i.e. genetic process by which a mussel population becomes fitted to its environment). Therefore, each of the mussel populations sampled has its own genetic makeup. Since adaptation is an evolutionary process (i.e. acts through natural selection), it would be impossible for caged mussels to truly adapt to their new environment during the 60 day transplant experiment (see discussion of acclimation that follows). The indigenous mussel populations, on the other hand, have had time to adapt to the general conditions in their local embayment. These local populations may have adapted to their local levels of metal

TABLE 8. Calculated times to steady state for a variety of metals and for two PCB congeners, based on Equation (1) and published depuration rate constants (k) or on depuration rate constants calculated from published half lives.

Contaminant	k (d-1)	Time$_{99.99\%}$ (d)	Time $_{80\%}$ (d)	f$_{ss}$ - 60d	Reference
Ag	0.019	485	85	68 %	Wang et al., 1996
Cd	0.011	837	146	48 %	Wang et al., 1996
Cd	0.004 - 0.007*	>1000	223 - 447	19 - 35 %	Borchardt, 1983
Cd	0.0003+	>1000	>1000	2 %	Thomann etal., 95
Hg (methyl)	0.0003+	>1000	>1000	2 %	Thomann etal., 95
Ni	0.019+	485	85	68 %	Thomann etal., 95
Pb	0.013+	708	124	54 %	Thomann etal., 95
Zn	0.020	460	80	70 %	Wang et al., 1996
PCB (#31)	0.202	46	8	100 %	Gilek et al., 1996
PCB (#153)	0.082	113	20	99 %	Gilek et al., 1996

*, determined from half-lives; T1/2 = 0.693 / k

+ "effective depuration rate constant

contamination, by either restricting metal adsorption, by increasing their capacity to sequester metals internally, or by increasing the rate at which they eliminate metals from their bodies. Since, in several cases, the caged mussels bioaccumulated similar or more metal than the corresponding indigenous mussels, it could be hypothesized that the indigenous mussels have, through natural selection, developed mechanisms to either restrict their metal input or accelerate their metal output.

While this explanation is appealing, there is little documentation in the literature to support the hypothesis that mussels can adapt to metal contaminant. Adaption to Cd, Ni and Co was clearly demonstrated for the oligochaete *Limnodrilus hoffmeisteri* sampled from Foundry Cove, NY (Klerks and Levinton, 1989). In this case, the oligochaete developed mechanisms to increase the sequestration capacity of metals (e.g. by increasing its titre of metallothionein). This type of adaptation would not explain the results observed in the Gulfwatch study since this would lead to elevated metal concentrations in indigenous mussels compared to the transplants. Additional field work would be needed to determine whether the indigenous mussels at the Gulfwatch stations have adapted to metal contaminants by modifying their uptake and/or depuration mechanisms. If it turns out that local populations of mussels are adapted to their contaminant exposures, reducing their body burdens over that which would be attained by transplanted mussels, it would be difficult to use metal concentrations in indigenous mussels to identify contaminated sites. This would have serious implications for such both national and regional "mussel watch" programs.

The second possibility is that the indigenous mussels have simply acclimated to their local conditions (physiological and biochemical changes within an organism by which it becomes better fitted to its environment). Unlike adaptation, acclimation (acclimatization) is a phenotypic response, lacking a genetic component. Nevertheless, if the indigenous mussels have acclimated to elevated metal contamination, and if caged mussels have not had sufficient time to acclimate, then the outcome would of course depend on whether the mussels acclimated by decreasing their absorption mechanisms, increasing their elimination mechanisms, or increasing their sequestration capacity. In order to explain the results obtained by Gulfwatch, we would have to hypothesis that the indigenous mussels acclimated by increasing their ability to depurate metals.

Slight differences in the physical environment surrounding the caged mussels compared to that of the indigenous mussels may also have contributed to the differences between the two. While indigenous mussels "adjacent" to the caged mussels were sampled, the environments could be quite different. Indigenous mussels were in intimate contact with the substrate, whereas the transplanted mussels were suspended midwater in their cages. Current patterns, suspended sediment loading, disturbance, shading and the subtle chemical differences may all play a role in the mussels response to contaminants. These environmental differences cannot be quantified, but

the potential for modifying the mussels bioaccumulation patterns should be kept in mind when attempting to interpret the Gulfwatch data.

Lastly, cage effects were not examined in this experiment. The experimental design did not include a control in which indigenous mussels were caged alongside transplanted mussels to see if handling and caging could effect bioaccumulation. This would have increased the cost of contaminant analysis by a third, and so was considered too costly to incorporate into the study design. Handling/caging would probably increase the stress of the caged mussels. This could lead to changes in metal uptake and elimination rates, which could then effect the observed toxicity. It seems unlikely, however, that cage effects could explain the greater bioaccumulation of some metals by the caged mussels compared to indigenous mussels. Sequestration requires internal ligands to build up the metals as they have entered. Mussels stressed by handling/caging would probably have a diminished capacity to synthesize these internal ligands, thereby reducing their bioaccumulation capacity not increasing it.

In summary, the use of caged mussels clearly provides a short term assessment of metal bioavailability, whereas indigenous mussels should provide a longer term assessment (depending on the time it takes to reach steady state conditions). While transplanted mussels, on theoretical grounds, should not have attained a new steady state condition in only 60 days, in several instances the caged mussels reached similar or even higher concentrations of metals than indigenous mussels collected at adjacent transplant sites. While differences in local environmental conditions and even cage effects may have contributed to this difference in bioaccumulation, it is also likely that adaptation or acclimation of the local native mussels may be a significant factor.

ORGANIC CONTAMINANTS

The total concentration of polynuclear aromatic hydrocarbons (ΣPAH_{24}), polychlorinated biphenyl (ΣPCB_{24}) and organochlorine pesticides ($\Sigma PEST_{17}$) measured in mussel tissue samples of caged and indigenous mussels are presented in Table 9. Individual analyte concentrations of each compound class are provided in appendices D, E and F.

A. Spatial variation in organic contaminants: comparison of indigenous mussels

Figures 11 and 12 show the concentration of ΣPAH_{24} (Figure 11), ΣPCB_{24} (Figure 11), and ΣDDT_6 (Figure 12) and $\Sigma PEST_{17}$ (Figure 12) measured in tissue of *M. edulis* in the 1995 sampling stations are presented from south to north. Concentrations of contaminants were plotted on a log scale and the geometric mean ± 1 SD has been added for comparison purposes. Concentrations above the geometric mean + 1 SD are considered high. Table 10 contains a summary of the geometric means for each jurisdiction as well as an overall Gulf of Maine estimate. Geometric means of the ΣPAH_{24} concentrations range from non-detectable, ND, in New Brunswick and Nova Scotia, to 82 ng / g dry weight in Massachusetts and New Hampshire. MAPR, NHHS, NHLH, MECC, and MEKN all exceed the geometric mean + 1 SD (Figure 11). The geometric mean of ΣPCB_{24} ranges from ND, in New Brunswick and Nova Scotia, to 70 ng / g dry weight in Massachusetts. MASN, MAPR, NHHS, MECC and MEKN all exceed the geometric mean + 1 SD (Figure 11). The geometric mean of ΣDDT_6 ranged from 1.3 ng / g dry weight in Nova Scotia to 23 ng / g dry weight in Massachusetts. MASN, MAPR, NHHS, MECC, MEDM and MEKN all exceed the geometric mean + 1SD (Figure 12). Four sites examined in 1995 (MAPR, NHHS, MECC and MEKN) exceed the geometric mean + 1SD in each of ΣPAH_{24}, ΣPCB_{24} and ΣDDT_6.

In 1995, as in previous years, there is a general southward trend toward higher organic contaminant concentrations. This north-to-south increase in contaminant concentrations can be attributed to the increasing population density and industrialization. This trend is most evident in the ΣPCB_{24} and ΣDDT_6 data sets (Figure 11 and 12) which probably reflects the historical use and deposition of these contaminants in sediments.

Table 11 shows the organic contaminant concentrations for indigenous mussels only. Sites were grouped by jurisdiction and ANOVA and Tukey Kramer tests were employed to examine differences among sites within a jurisdiction.

TABLE 9. Summary of tissue organic contaminant concentrations (arithmetic mean ± SD, ng/g dry wt.) for caged (C) and indigenous (N) mussels at Gulf of Maine, 1995 stations. n = 4 replicates per sample. ND, nondetect

LOCATION	ΣPAH_{24}	ΣPCB_{24}	ΣDDT_6	Other Pesticides	$\Sigma PEST_{17}$
MASN-P	17.5 ± 11.71	36.75 ± 7.63	22.35 ± 5.08	4.40 ± 1.97	26.75 ± 6.55
MAIH-C	1576.75±528.46	361.25 ± 10.47	46.55 ± 10.45	15.20 ± 1.76	61.75 ± 11.96
MAIH-N	no data	no data	no data	no data	no data
MAPR-C	571.75 ± 61.27	157.00 ± 13.90	27.20 ± 1.67	9.55 ± 1.28	36.75 ± 2.75
MAPR-N	406.25 ± 32.51	130.50 ± 18.27	21.93 ± 1.20	6.95 ± 0.76	28.88 ± 1.93
NHHS-P	60.75 ± 28.24	16.83 ± 7.06	10.83 ± 1.93	1.93 ± 0.15	12.75 ± 1.89
NHLH-C	52.38 ± 23.2	12.88 ± 2.02	13.85 ± 0.60	ND	13.50 ± 0.58
NHLH-N	64.75 ± 18.99	7.78 ± 4.83	10.25 ± 2.27	0.18 ± 0.24	10.40 ± 2.45
MECC-C	117.25 ± 14.43	17.25 ± 2.63	8.73 ± 1.35	3.53 ± 0.17	12.25 ± 1.50
MECC-N	157.75 ± 38.75	35.35 ± 10.24	13.75 ± 0.96	ND	13.75 ± 0.96
MEBC-P	5.50 ± 6.40	3.85 ± 4.57	6.76 ± 0.68	ND	6.78 ± 0.70
MEDM-C	10.75 ± 8.58	6.20 ± 0.78	9.33 ± 0.54	ND	9.33 ± 0.54
MEDM-N	3.14 ± 5.37	0.88 ± 1.01	11.05 ± 1.65	1.33 ± 1.54	12.38 ± 1.75
MEKN-C	122.00 ± 62.70	35.75 ± 23.68	9.58 ± 0.75	1.45 ± 1.00	11.03 ± 1.59
MEKN-N	64.00 ± 25.60	24.50 ± 7.19	13.05 ± 0.49	4.45 ± 0.61	17.50 ± 1.00
NBNR-P	ND	ND	3.78 ± 0.99	0.55 ± 1.10	4.33 ± 1.68
NBHI-C	ND	ND	3.85 ± 0.13	ND	3.85 ± 1.30
NBHI-N	ND	ND	5.35 ± 1.63	ND	3.86 ± 0.59
NBMI-C	ND	2.45 ± 1.37	3.85 ± 0.57	ND	5.35 ± 1.63
NBMI-N	no data	no data	no data	no data	no data
NSBE-N	ND	ND	0.55 ± 1.10	ND	0.55 ± 1.10
NSCW-N	ND	ND	0.50 ± 1.00	ND	0.50 ± 1.00

TABLE 10. Geometric mean (±SD) of tissue organic contaminants for indigenous mussels within each jurisdiction and for all the Gulf of Maine, 1995 stations. ND, nondetect.

JURISDICTION	ΣPAH_{24}	ΣPCB_{24}	ΣDDT_6	OTHER PESTICIDES	$\Sigma PEST_{17}$
Massachusetts	82 ± 5.8[1]	70 ± 2.0	23 ± 1.1	6.4 ± 1.4	28 ± 1.2
New Hampshire	82 ± 1.8	17 ± 2.1	12 ± 1.2	1.5 ± 1.7	13 ± 1.2
Maine	9.0 ± 5.9	4.9 ± 4.1	11 ± 1.3	2.2 ± 2.3	12 ± 1.5
New Brunswick	ND	ND	4.8 ± 1.2	1.2 ± 1.5	5.0 ± 1.3
Nova Scotia	ND	ND	1.3 ± 1.7	ND	1.3 ± 1.7
Gulf of Maine[2]	11 ± 9.3	6.1 ± 5.8	7.8 ± 2.6	1.9 ± 2.2	8.6 ± 2.8

[1] Geometric means (±SD)
[2] All sites
ND, nondetectable

TABLE 11. Analysis of tissue organic contaminant concentrations (arithmetic mean ± SD, ng/g dry wt.), by jurisdiction, for indigenous mussels at the Gulf of Maine stations, 1995. Same letter indicates no significant difference (P>0.05). ND, nondetect.

LOCATION	ΣPAH_{24}	ΣPCB_{24}	ΣDDT_6	OTHER PESTICIDES	$\Sigma PEST_{17}$
MASN-P	17.5 ± 11.71A	36.75 ±7.63A	22.35 ± 5.08A	4.40 ± 1.97A	26.75 ± 6.55A
MAIH-N	no data	no data	no data	no data	no data
MAPR-N	406.25±32.51B	130.50 ± 18.27B	21.93 ± 1.20A	6.95 ± 0.76A	28.88 ± 1.93A
NHHS-P	60.75±28.24A	16.83 ± 7.06AB	10.83 ± 1.93A	1.93 ± 0.15B	12.75 ± 1.89A
NHLH-N	64.75±18.99A	7.78 ± 4.83A	10.25 ± 2.27A	0.18 ± 0.24A	10.40 ± 2.45A
MECC-N	157.75±38.75B	35.35 ± 10.24B	13.75 ± 0.96A	ND	13.75 ± 0.96A
MEBC-P	5.50 ± 6.40A	3.85 ± 4.57A	6.76 ±0.68A	ND	6.78 ± 0.70A
MEDM-N	3.14 ± 5.37A	0.88 ± 1.01A	11.05 ± 1.65B	1.33 ± 1.54A	12.38 ± 1.75B
MEKN-N	64.00±25.60B	24.50 ± 7.19B	13.05 ± 0.49B	4.45 ± 0.61B	17.50 ± 1.00C
NBNR-P	ND	ND	3.78 ± 0.99A	0.55 ± 1.10A	4.33 ± 1.68A
NBHI-N	ND	ND	3.86 ± 0.59A	ND	3.86 ± 0.59A
NBMI-N	no data	no data	no data	no data	no data
NSBE-N	ND	ND	0.55 ± 1.10A	ND	0.55 ± 1.10A
NSCW-N	ND	ND	0.50 ± 1.00A	ND	0.50 ± 1.00A

Polyaromatic Hydrocarbons

The concentration of ΣPAH_{24} in indigenous mussels ranged from ND to 406 ± 33 ng / g dry weight at MAPR (Table 11, Figure 11). It is suspected that the concentration of ΣPAH_{24} may be much higher at MAIH, as the concentration in caged mussels was 1577 ± 528, however, there were no corresponding data for indigenous mussels at this site.

Some mean concentrations of ΣPAH_{24} were as high as those reported from areas influenced by oil spills and municipal sewage outfall (148 ng / g in Rainio et al., 1986; 63-1060 ng / g in Kveseth et al., 1982), but not as high as in industrialized areas affected by coking operations in Sydney Harbor NS (1400-16000 ng / g, in Environment Canada, 1986) or smelting operations in Saudafijord, Norway (5111 - 225163 ng / g in Bjorseth et al., 1979).

The highest mean concentration of ΣPAH_{24} was measured at MAPR (406 ± 33 ng / g dry weight). This value is high in comparison to other sites in the 1995 Gulfwatch program, however, it is lower than reported elsewhere in Boston Harbor (Dorchester Bay, 1865 ng / g; Deer Island, 2226 ng / g, in NOAA, 1989) and in Boston Harbor local areas (Hingham Bay, 744 ng / g in NOAA, 1989). High concentrations were also observed at MEKN (64 ± 14 ng / g dry weight) and the New Hampshire sites; MECC (158 ± 39 ng / g dry weight), NHHS (61 ± 28 ng / g dry weight) and NHLH (65 ± 19 ng / g dry weight). No PAH's were detected in New Brunswick or Nova Scotia samples in 1995.

There were significant differences in ΣPAH_{24} within all jurisdictions (Table 11) for which there was detectable concentrations. In Massachusetts, MAPR was higher than MASN, in New Hampshire MECC was significantly higher than NHHS and NHLH, and in Maine MEKN was significantly higher than MEBC and MEDM.

Polychlorinated Biphenyl

Mean ΣPCB_{24} concentrations in indigenous mussels ranged from ND to 131 ± 18 ng / g dry weight at MAPR (Table 11, Figure 11). As suggested above for ΣPAH_{24}, there is evidence that the concentration may be higher at MAIH as the caged sample contained 361 ± 10 ng / g dry weight ΣPCB_{24}.

There were significant differences in ΣPCB_{24} within all jurisdictions (Table 11) for which there was detectable concentrations. In Massachusetts MAPR was significantly higher than MASN, in New Hampshire MECC was significantly higher than NHLH but not NHHS, and in
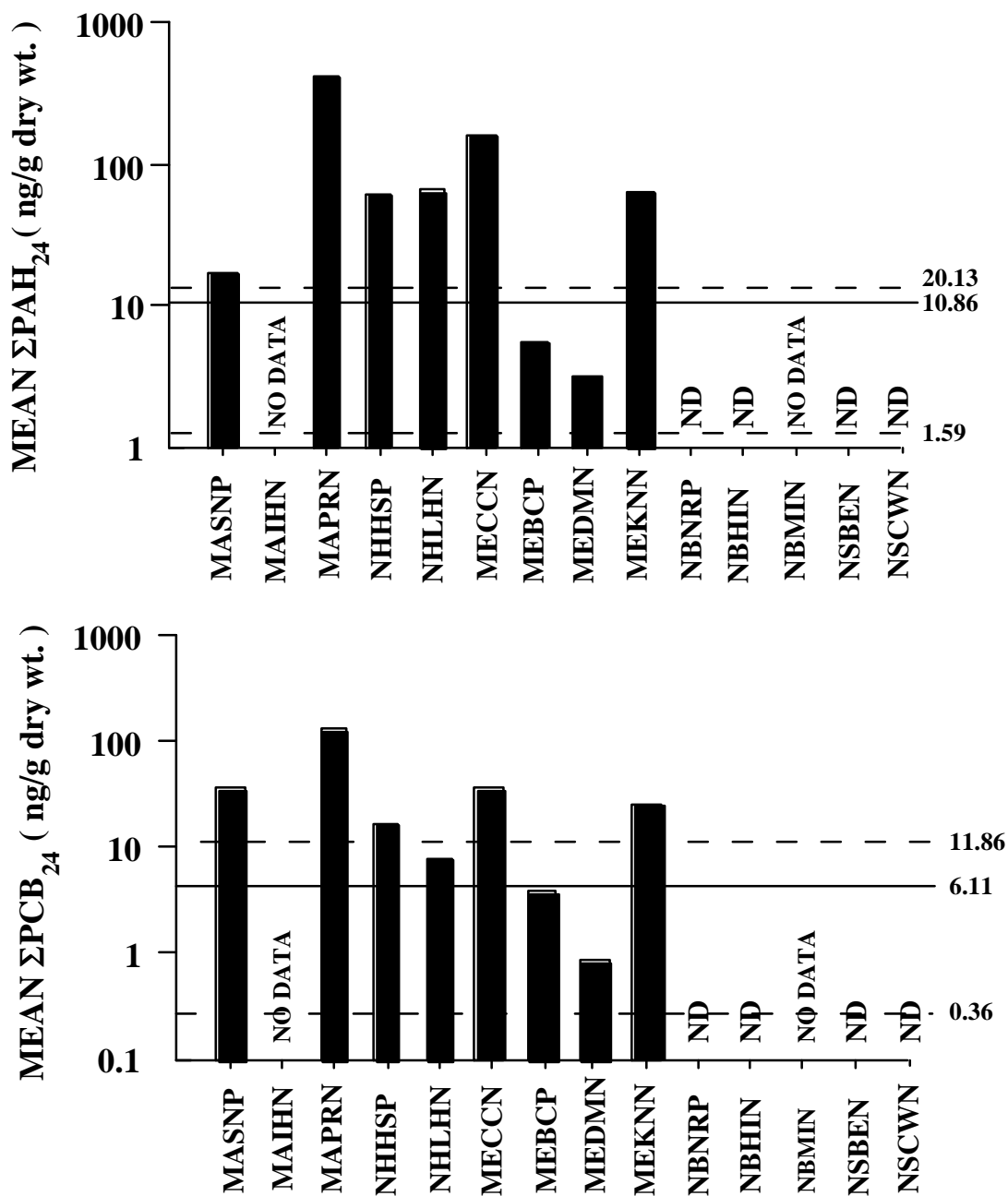
Fig. 11. Log distribution of mean ΣPAH_{24} and ΣPCB_{24} tissue concentrations (arithmetic mean: ng/g dry weight) in indigenous mussels at the Gulf of Maine Stations, 1995. Geometric mean (straight line) one standard deviation (dashed line) of all Gulf of Maine stations, 1995. ND, non detect.

Maine, MEKN was significantly higher than MEBC and MEDM.

Pesticides

The concentration of $\Sigma PEST_{17}$ in indigenous mussels ranged from 0.5 ± 1.0 ng / g dry weight at NSCW to 29 ± 2 ng / g dry weight at MAPR (Table 11, Figure 12). In 1995 as in previous reports (GMCME, 1994, 1996a, 1996b), ΣDDT_6 and its degenerative metabolites were the main contributors to total detectable pesticides. The range of ΣDDT_6 in native mussels was 0.5 ± 1.0 ng / g dry weight at NSCW to 22 ± 5 ng / g dry weight at MASN.

Analysis of each jurisdiction (Table 11) showed no significant difference in $\Sigma PEST_{17}$ among sites in Massachusetts, New Hampshire, New Brunswick and Nova Scotia. In Maine, however, there were significant differences among all sites. The lowest was at MEBC (6.8 ± 0.7 ng / g dry weight) and the highest concentration of $\Sigma PEST_{17}$ was at MEKN (17.5 ± 1.0 ng / g dry weight).
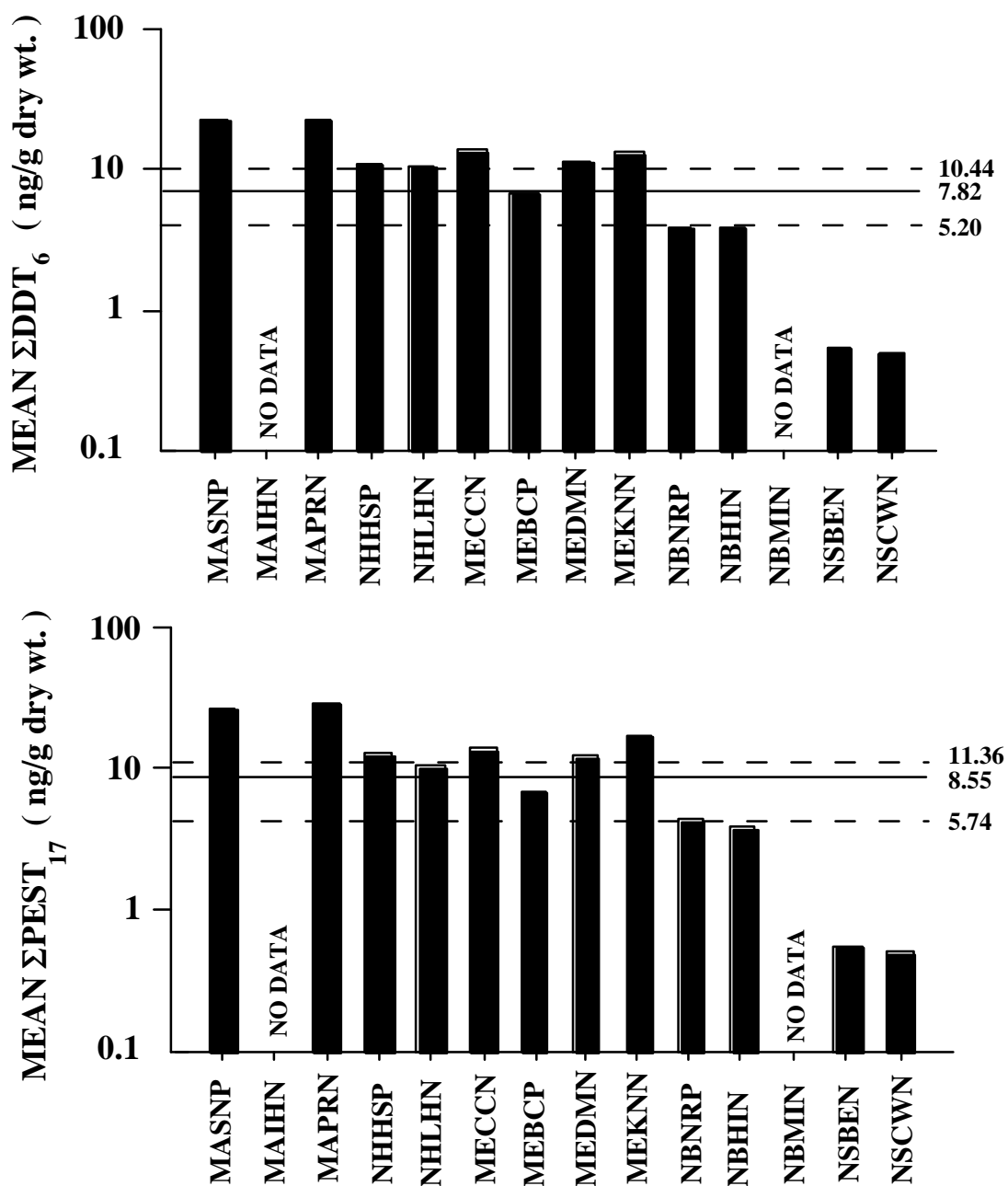
Fig. 12. Log distribution of ΣDDT_6 and $\Sigma PEST_{17}$ tissue concentrations (arithmetic mean: ng/g dry weight) in indigenous mussels at the Gulf of Maine Stations, 1995. Geometric mean (straight line) one standard deviation (dashed line) of all Gulf of Maine stations, 1995.

B. Short term exposure: transplant experiment

Figure 13 and 14 shows the concentrations of organic contaminants from south to north at the preset and caged stations. Results of the ANOVA on organic concentrations in caged versus preset are shown in Table 12. No data are presented for Nova Scotia as no cages were retrieved in that jurisdiction.

Massachusetts

The level of all organic contaminants increased significantly in caged mussels after deployment for 60 days at MAIH (Table 12). No indigenous mussel contaminant data were available for mussels at MAIH. As such, it is unknown whether indigenous mussel organic contaminant concentrations at this site were higher than those in preset mussels prior to transplantation; although one would suspect that this is the case by the elevated concentrations in the caged samples.

The concentrations of ΣPAH_{24}, ΣPCB_{24} and other pesticides in mussels caged at MAPR increased significantly over the 60 day deployment period, suggesting bioaccumulation. In all cases (although not significant for the other pesticides) the preset contaminant concentrations were less than that for the indigenous mussels. For ΣDDT_6 and total pesticides there was no significant change after 60 days of deployment, in both cases there was no significant difference between caged and preset concentration prior to the transplant.

New Hampshire

There were no changes in the mean concentrations of ΣPCB_{24}, ΣPAH_{24}, ΣDDT_6 and $\Sigma PEST_{17}$ after 60 days of deployment at NHLH (Table 11). In these cases there were no significant differences between the preset and the indigenous mussel concentrations. For other pesticides the concentration in the caged mussels were significantly lower than in the preset mussels. In this case, the concentration for the preset mussels was greater than that for the indigenous, suggesting that biodepuration of pesticides may have occurred during the deployment period.

At MECC there was no change in mean concentration of ΣPCB_{24}, ΣPAH_{24}, ΣDDT_6 and $\Sigma PEST_{17}$ after 60 days deployment. The concentration of other pesticides, however, increased significantly, from 1.9 to 3.5 ng / g dry weight, despite significantly lower concentrations of other pesticides (ND) recorded in indigenous mussels at this site.

Maine

Mussels caged at MEKN had significantly higher concentrations of all organic contaminants, ΣPCB_{24}, ΣPAH_{24}, ΣDDT_6, other pesticides and $\Sigma PEST_{17}$ after deployment for 60 days (Table 13). In all cases, the level of these contaminants in the preset was significantly lower than in the indigenous mussels. At MEDM, for tests in which the preset was not significantly different from the indigenous, there was no change in the caged mussels concentration (ΣPCB_{24}, ΣPAH_{24}, and other pesticides). However, when the preset concentration was less than that in the indigenous mussels (ΣDDT_6 and $\Sigma PEST_{17}$), the concentrations in the tissues of caged mussels significantly increased.

New Brunswick

There was no change in the concentration of all contaminants in caged mussels deployed at NBHI (Table 13). This result is not surprising considering that there were no detectable concentrations at either the preset or indigenous mussels at NBHI. Similar results occurred with mussels deployed at NBMI, with the exception of other pesticides and ΣPCB_{24}, which were significantly higher in preset mussels than in caged mussels.

TABLE 12. Summary of analysis of transplantation data; TEST, (1) tissue organic contaminant concentrations in caged (C) versus preset (P) mussels; (2) Preset mussels versus indigenous mussels (N); and (3) Caged versus indigenous mussels. NS indicates no significant difference between treatments. < / > indicates a significant difference in tissue metal concentrations between treatments and the direction. - indicates no test was possible due to missing data. $\sum OP_{11}$, other pesticides.

| ORGANIC	TEST	MASSACHUSETTS Preset : MASN		NEW HAMPSHIRE Preset : NHHS		MAINE Preset : MEBC		NEW BRUNSWICK Preset : NBNR	
		MAIH	MAPR	NHLH	MECC	MEDM	MEKN	NBHI	NBMI
$\sum PAH_{24}$	C vs P	C > P	C > P	NS	NS	NS	C > P	NS	NS
	P vs N		P < N	NS	P < N	NS	P < N	NS	
	C vs N		C > N	NS	NS	NS	NS	NS	
$\sum PCB_{24}$	C vs P	C > P	C > P	NS	NS	NS	C > P	NS	C < P
	P vs N		P < N	NS	NS	NS	P < N	NS	
	C vs N		NS	NS	C < N	C > N	NS	NS	
$\sum DDT_{6}$	C vs P	C > P	NS	NS	NS	C > P	C > P	NS	NS
	P vs N		NS	NS	NS	P > N	P < N	NS	
	C vs N		C > N	C > N	C < N	NS	C < N	NS	
$\sum OP_{11}$	C vs P	C > P	C > P	C < P	C > P	NS	C > P	NS	C < P
	P vs N		NS	P > N	P > N	NS	P < N	NS	
	C vs N		C > N	NS	C > N	NS	C < N	NS	
$\sum PEST_{17}$	C vs P	C > P	NS	NS	NS	C > P	C > P	NS	NS
	P vs N		NS	NS	NS	P < N	P < N	NS	
	C vs N		C > N	NS	NS	C < N	C < N	NS	
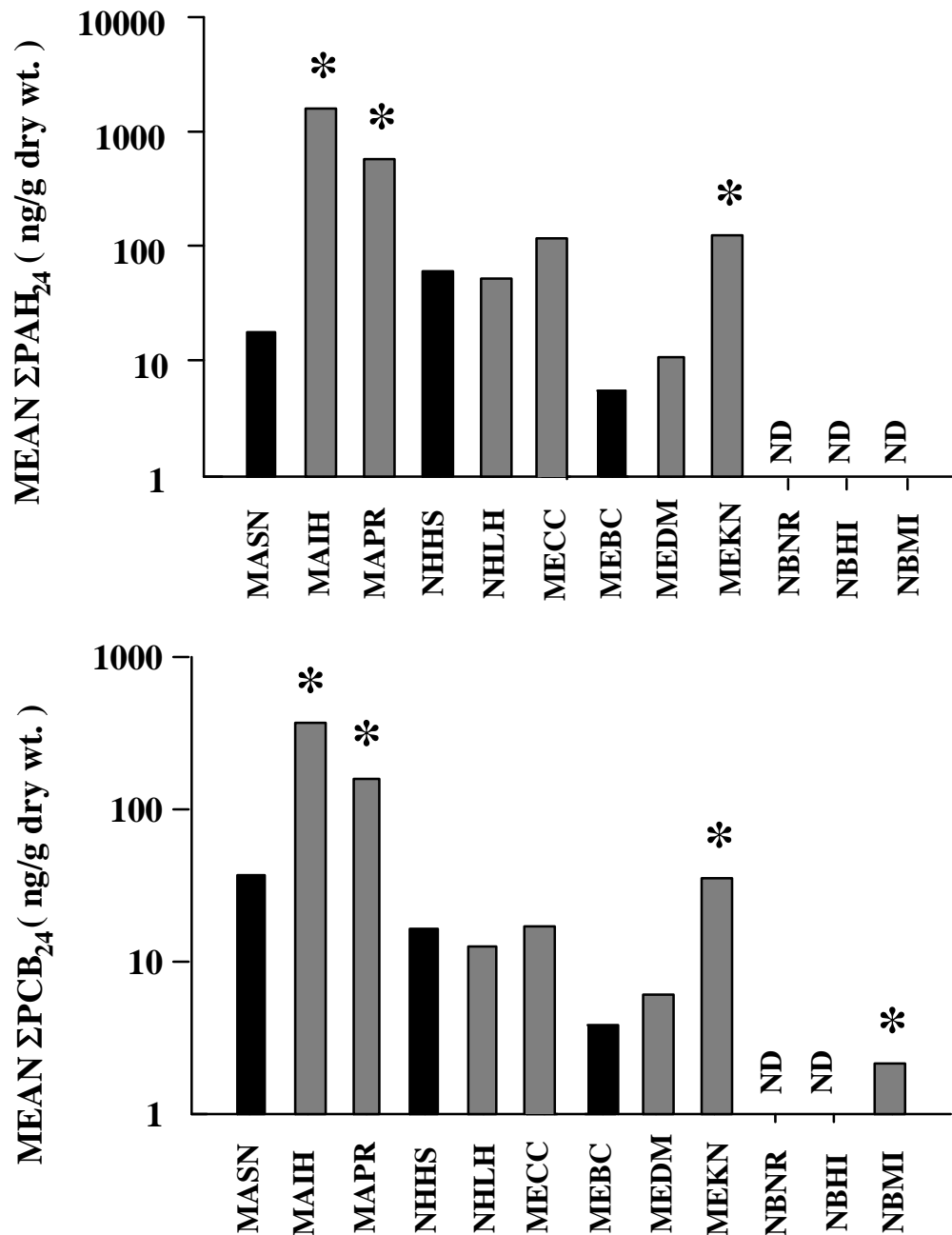
Fig. 13. Log distribution of ΣPAH$_{24}$ and ΣPCB$_{24}$ tissue concentrations (arithmetic mean: ng/g dry weight) in caged (gray) and preset (black) mussels at the Gulf of Maine Stations, 1995. ND, non detect. *, indicates a significant difference between caged and preset mussels ($p < 0.05$).

Fig. 14. Log distribution of ΣDDT_6 and $\Sigma PEST_{17}$ tissue concentrations (arithmetic mean: ng/g dry weight) in caged (gray) and preset (black) mussels at the Gulf of Maine Stations, 1995. *, indicates a significant difference between caged and preset mussels (p<0.05).

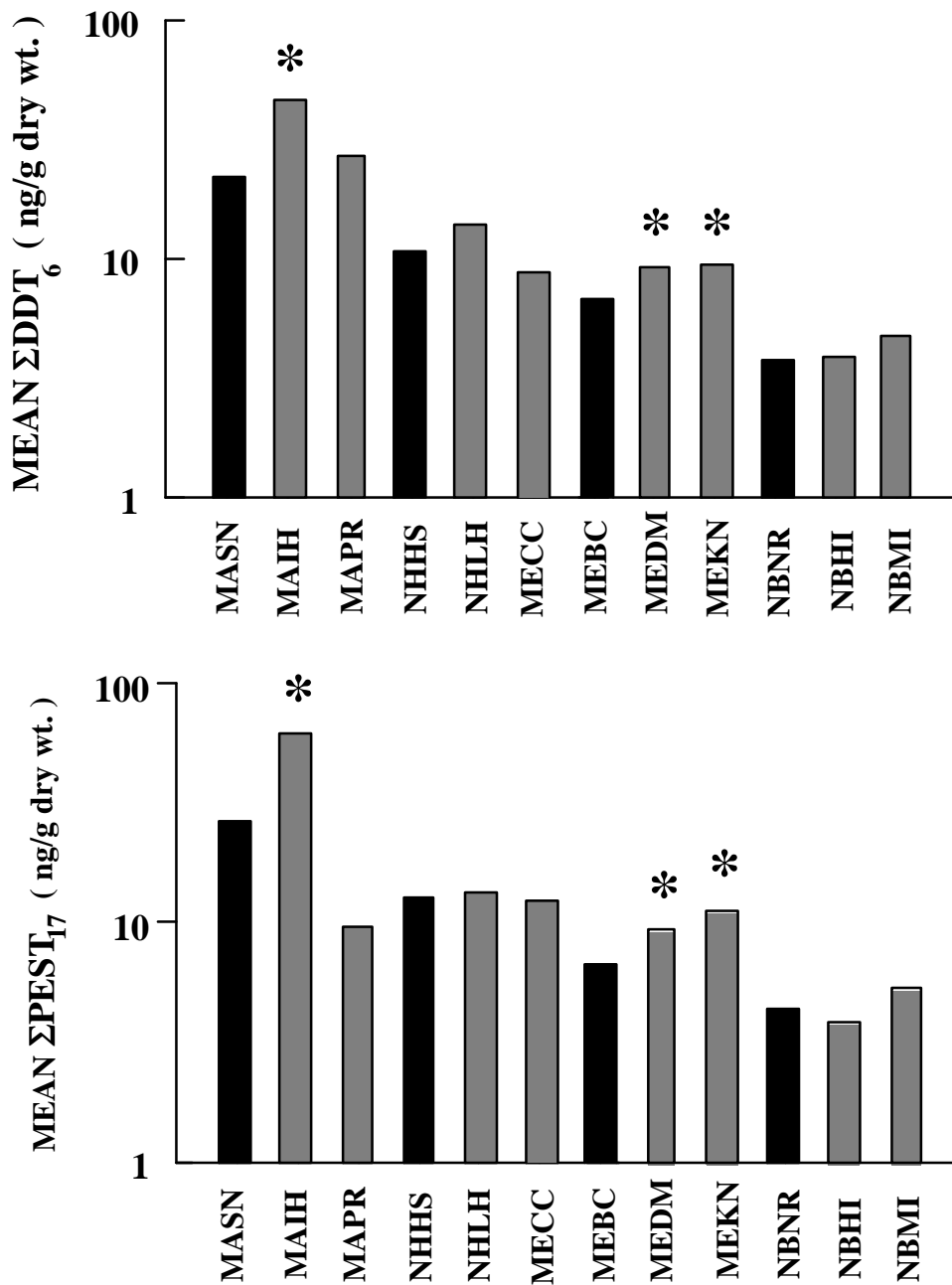**Summary of organic concentration changes during transplants**

As indicated in the discussion of each jurisdiction above, organic contaminants bioaccumulated at five of the seven Gulfwatch stations during the 60 d transplant experiment. No accumulation of any organic contaminant was observed at NBHI or NHLH (depuration of organochlorine pesticides was actually observed at this latter site).

Unlike the pattern observed for metal accumulation, a south-to-north gradient of decreasing organic contaminant concentrations was not apparent, since transplanted mussels at one of the Maine sites, MEKN, accumulated appreciable organic contaminants. All five groups of organic contaminants (ΣPAH_{24}, ΣPCB_{24}, ΣDDT_6, total organochlorine pesticides and $\Sigma PEST_{17}$) were accumulated at both MEKN and MAIH. The next most contaminated site was the Massachusetts site MAPR, where ΣPAH_{24}, ΣPCB_{24} and total organochlorine pesticides were all accumulated. Finally, ΣDDT_6 and $\Sigma PEST_{17}$ were bioaccumulated at MEDM, and organochlorine pesticides were bioaccumulated at MECC. Since depuration of organochlorine pesticides was observed at NHLH, organochlorine contamination at the preset site (NHHS) is suspected.

Steady state tissue organic concentrations

Unlike metals, there is every reason to expect, based on theoretical grounds, that organic contaminants would reach a new steady state within the 60 d transplantation period. Toxicokinetic calculations (Table 8) show that mussels can reach steady state for a range of PCB congeners within 60 days. It is expected that this would also be true for a variety of similar hydrophobic organic compounds. A U.S. EPA study (EPA, 1989) has substantiated this premise; steady state was either attained or was approached for a variety of organic contaminants in a number of marine invertebrates. Since this EPA study focused on deposit feeders, however, *M. edulis* was not among the species examined.

Several published transplant studies have indicated that organic contaminant bioaccumulation in transplanted *M. edulis* rapidly reaches the same concentrations as found in locally collected mussels. Nelson et al. (1995), for example, reported that PCB concentrations in *M. edulis* plateau within 28 days of exposure in the field. Similarly, de Kock (1983) found that PCBs reached an apparent steady state in mussels within 2 - 3 months of being transplanted to a contaminated site. Peven et al. (1996) investigated a wider range of organic contaminants. Their study showed that caged mussels apparently reached steady states for ΣPCB_{20} and ΣDDT_6 within 95 d, although concentrations were just below an apparent steady state at 60 d. For PAHs, mussels initially overshot the concentrations measured in indigenous mussels in the first 30 d of exposure, but came down to native mussel concentrations after 95 d. As previously discussed for metals, the fact that transplanted mussels attain similar concentrations

of contaminants as found in indigenous mussels is not proof that these mussels had indeed reached steady state. Additional sampling, at various times after 60 d sampling, would be needed in order to confirm that the mussels had actually reached asymptotic concentrations.

In the present study, ΣPCB_{24} concentrations in the transplanted mussels reached similar concentrations as indigenous mussels at both of the sites where PCBs were accumulated (MAPR and MEKN). ΣPAH_{24} concentrations at MAPR overshoot the concentrations observed for native mussels, whereas concentrations at MEKN mimicked that of the indigenous population. Whether the pesticides achieved a steady state at each of the sites where they were bioaccumulated is less clear. At MEKN, concentrations of ΣDDT_6, total organochlorine pesticides and $\Sigma Pest_{17}$ did not reach as high as the indigenous mussels. At MEDM, ΣDDT_6 concentrations were similar in the transplants and the indigenous mussels, yet $\Sigma Pest_{17}$ did not bioaccumulate as much in the transplants as in the native population. On the other hand, total organochlorine pesticides were accumulated at both MAPR and MECC, and overshot the concentrations measured in the indigenous mussels in both instances. The overshooting of the transplant mussel compared to the indigenous mussel concentrations (ΣPAH_{24} at MAPR and total organochlorine pesticides at MAPR and MECC) may indicate that the mussels at these two sites have acclimated or adapted to organic contamination. Further field work would be needed to substantiate this hypothesis.

ACCEPTABLE LEVELS AND STANDARDS OF MUSSEL CONTAMINATION

Limited information is available on human health effects of consumption of shellfish. Published tolerance or action levels for PAHs in commercial marine species are not available in Canada or in the United States. In marine areas where PAH contamination may be a human health concern, closure of commercial fisheries as a result of high contamination levels has been dealt with on a case by case basis. In general, most concentrations reported in the literature are on a wet weight basis in contrast to Gulfwatch dry weight values. To facilitate general comparisons with Gulfwatch values, an average moisture content of 85% has been applied to wet weight health values to derive dry weight equivalents. All reported organic concentrations are within acceptable concentrations for those compounds that have established FDA Action Limits in fish and shellfish. PCB concentrations found in Gulfwatch mussels (Appendix E) are less than the action level of 13 ppm dry weight or 2 ppm wet weight (USFDA, 1990; CSSP, 1992). MAPR had the highest concentrations of PCBs in mussels during the 1995 survey of 0.2 $\pm$ 0.01 ppm dry weight. Action level for the pesticides dieldrin, aldrin, chlordane, heptachlor and heptachlor epoxide is 2.0 ppm dry weight or 0.3 ppm wet weight (USFDA, 1990). All of these pesticides were below detection concentrations in the 1995 mussel survey. The total DDT concentrations found are several orders-of-magnitude below the action level of 33 ppm dry weight or 5

ppm wet weight (USFDA,1990; CSSP, 1992). Sandwich, MA had the highest level in 1995 of 0.02 ± 0.01 ppm dry weight. Canadian limits for agricultural chemicals exclusive of DDT are 0.67 ppm dry weight or 0.1 ppm wet weight.

Admissible levels of methyl mercury, expressed as mercury, are less than 6.7 ppm dry weight or 1 ppm wet weight in the United States (USFDA, 1990) and less than 3.3 ppm dry weight or 0.5 ppm wet weight in Canada (CSSP, 1992). The highest concentration of mercury found in the 1995 Gulfwatch Project was 0.69 ± 0.10 ppm dry weight, at Little Harbor, New Hampshire, which was well below both federal action concentrations.

Recently, a series of FDA "Guidance Documents" (USFDA, 1993) for cadmium, chromium, lead and nickel has been released in the United States to complement the FDA Mercury Action Level. These "alert" levels, however, are guidelines and by themselves do not warrant the issuance of health advisories. In Table 12, guidance concentrations are reported on both wet weight and dry weight basis and are compared to the highest observed concentration in any single replicate analyzed in the 1995 Gulfwatch Project. With the exception of lead, which is represented by high concentrations in all replicates from Boston, Inner Harbor, MA (station MAIH), no other metal approaches the guidelines. The average lead concentration at MAIH was 16 ± 3.0 µg / g dry weight, above the guideline of 11.5 µg / g dry weight. It would, therefore, be prudent to resample Boston, Inner Harbor, MA in the near future, although no human health risk is posed since the area is closed to harvest.

Table 13. A comparison of United States Food and Drug Administration guidelines for various metals with the Gulfwatch results.

Metal	Guideline (Wet weight)	Guideline (dry weight)	Highest Observed 1995 Gulfwatch value (dry weight)	Location
Cadmium	3.7 µg/g	25 µg/g	2.2 µg/g	Little Harbor, NH
Chromium	13 µg/g	87 µg/g	3.3 µg/g	Clarke Cove, ME
Lead	1.7 µg/g	11.5 µg/g	16.3 µg/g	Boston Inner Harbor, MA
Nickel	80 µg/g	533 µg/g	1.7 µg/g	Little Harbor, NH

The U.S. EPA has promulgated a series of "screening values" for three metals (Cd, Hg, Se), 11 organochlorine compounds, one chlorophenoxy herbicide, total PCBs and dioxins/dibenzofurans (EPA, 1993) which were derived using human health risk assessment procedures. The promulgated values are based on several exposure assumptions (70 kg man, an average consumption rate of 6.5 g/day), and either the most current Reference Dose (RfD) values for non-carcinogens or the most recent Slope Factor (SF) plus an acceptable lifetime cancer risk of 1 x 10-5 for the carcinogenic compounds listed. Exceedances of any of the screening values is meant to trigger a more in-depth assessment of actual human health risk. Applying these screening values to the Gulfwatch data provides yet another index of possible human health concern.

Mean concentrations of Cd, Hg and ΣDDT_6 at all 1995 Gulfwatch stations are well below the EPA Screening Values (EPA, 1993). The Screening Value for the ΣPCB_{24} is exceedingly low (0.01 µg / g wet weight or approximately 0.07 µg / g dry weight; EPA, 1993). Mean ΣPCB_{24} concentration at two 1994 Gulfwatch sites (MAPR and MAIH) exceeded this value. Individual composites of caged and indigenous mussels from MAPR were as much as 243 times higher than the EPA screening Value. Individual composites of caged and indigenous mussels from MAIH were as much as 527 times higher than the EPA screening Value. These stations should therefore be examined in much more detail in order to adequately assess the potential human health risk to PCBs.

GROWTH AND CONDITION INDEX

Shell morphology and condition index: indigenous mussels

Table 14 contains a summary of the morphological measurements [length (mm), height (mm), width (mm), wet weight (g) and condition index (CI)] for indigenous mussels collected at each site. The field protocol recommended the collection of mussels within the length range of 50 - 60 mm. The gulfwide mean length (±SD) at the 13 sites was 52.9 ± 3.7 mm (Table 14, Figure 15). For the majority of sites, the mean length of mussels collected fell within the range of 50 - 60 mm. ANOVA on the length of mussels collected among sites was significant (p<0.05) suggesting that there were significant differences in length. This significant difference appears to be attributed to sites MASN, NHHS and MEBC which were all below the overall gulfwide mean with lengths of 45.6 ± 2.9, 48.0 ± 4.4 and 46.0 ± 3.6 mm respectively. These sites were designated preset sites and as such the mean lengths were chosen a priori to be smaller (40 - 50 mm) to allow for maximum possible growth during the caging experiment while still allowing sufficient tissue for analysis. Mean lengths of preset mussels were within the shell length range to ensure adequate growth during transplantation, thus it may be expected that the lengths of mussels at these sites would be significantly lower. If these sites are removed from the analysis the ANOVA was still significant (P<0.05). Mussels at NBNR were significantly larger than at all other sites except MEDM and NBHI. Sample size at NBNR was low in comparison to other sites due to low population density. The larger mussels collected is thus a reflection of the limited size range available.

Condition indices (CI) of indigenous mussels collected in 1995 are shown in Table 14 and Figure 16. The average CI (±SD) for all sites throughout the Gulf of Maine was 0.171 ± 0.018. ANOVA on the mean CI of all indigenous mussels was significant (p<0.05). The CI of mussels at MEKN was significantly higher than all other sites with a value of 0.209 ± 0.029. The lowest CI was at NSBE, with a value of 0.154 ± 0.029. There was no consistent trend in CI within a jurisdiction. In most jurisdictions the CI varied. Only in New Hampshire were the CIs of all sites (NHHS, NHLH and MECC) consistently below the Gulf-wide mean.

Analysis of covariance (ANCOVA) on wet weight, using length, height and width as covariates was performed among sites within each jurisdiction to determine the cause of the differences in CI. ANCOVA revealed that for sites in two jurisdictions (Maine and Nova Scotia) length, width, and height were all significant covariates. Length and width were significant covariates for sites in New Hampshire and New Brunswick and only length was a significant

TABLE 14. Morphometric characteristics (mean ± SD) of indigenous mussels collected at Gulf of Maine, 1995 stations and ANOVA of measurements by jurisdiction. Same letter indicates no significant difference among sites within each jurisdiction.   Overall mean for all stations given below. Wet wt. (adj) = wet weight (g) adjusted for significant covariates (ANCOVA, p<0.05).

| STATION	N	LENGTH (mm)	HEIGHT (mm)	WIDTH (mm)	WET WGHT. (g)	WET WT. (ADJ)	CONDITION INDEX (CI)
MASN	30	45.6 (2.9)A	23.6 (2.3)A	18.1 (2.1)A	3.41 (0.95)	4.63 (1.61)A	0.180 (.070)A
MAPR	30	54.3 (2.7)B	28.1 (2.0)B	23.2 (1.7)B	5.54 (1.16)	4.74 1.22)AB	0.156(0.024)A
MAIH	30	54.0 (2.9)B	29.4 (2.4)B	22.8 (2.2)B	6.31 (1.51)	5.49 (0.90)B	0.174(0.003)A
NHHS	40	48.0 (4.4)A	26.0 (3.5)A	19.3 (3.2)A	3.71 (1.14)	5.04 (2.05)A	0.144(0.017)A
NHLH	30	54.9 (2.8)B	29.6 (1.5)B	22.4 (1.6)B	5.86 (1.07)	4.70 (0.75)A	0.159(0.017)B
MECC	30	54.1 (3.2)B	29.1 (1.4)B	21.2 (1.8)B	5.31 (1.01)	4.69 (0.83)A	0.158(0.018)B
MEBC	40	46.0 (3.6)A	25.1 (2.0)A	17.5 (2.2)A	3.27 (0.78)	4.64 (1.44)A	0.160(0.018)A
MEKN	40	53.9 (2.8)B	28.1 (2.2)B	21.8 (1.6)B	7.03 (1.41)	6.17 (1.16)B	0.209(0.029)B
MEDM	40	55.2 (2.7)B	29.1 (1.4)B	22.9 (1.9)B	6.13 (1.22)	4.88 (0.85)A	0.164(0.028)A
NBNR	30	57.0 (6.1)A	27.7 (3.4)A	24.0 (2.9)A	6.57 (2.17)	6.14 (1.96)A	0.172(0.039)A
NBHI	40	55.4 (7.0)A	28.4 (3.8)A	23.2 (5.2)A	7.18 (3.27)	7.57 (3.53)A	0.187(0.032)B
NBMI		no data	no data	no data	no data	no data	no data
NSBE	30	54.1 (2.4)A	28.3 (1.7)A	19.8 (1.6)A	4.66 (1.05)	4.94 (1.16)A	0.154(0.029)A
NSCW	30	54.8 (2.6)B	28.9 (2.1)B	22.3 (1.8)B	6.77 (1.48)	6.38 (1.35)B	0.191(0.030)B
mean (SD)		52.9 (3.7)	27.8 (1.8)	21.4 (2.1)	5.5 (1.4)		0.171 (0.02)
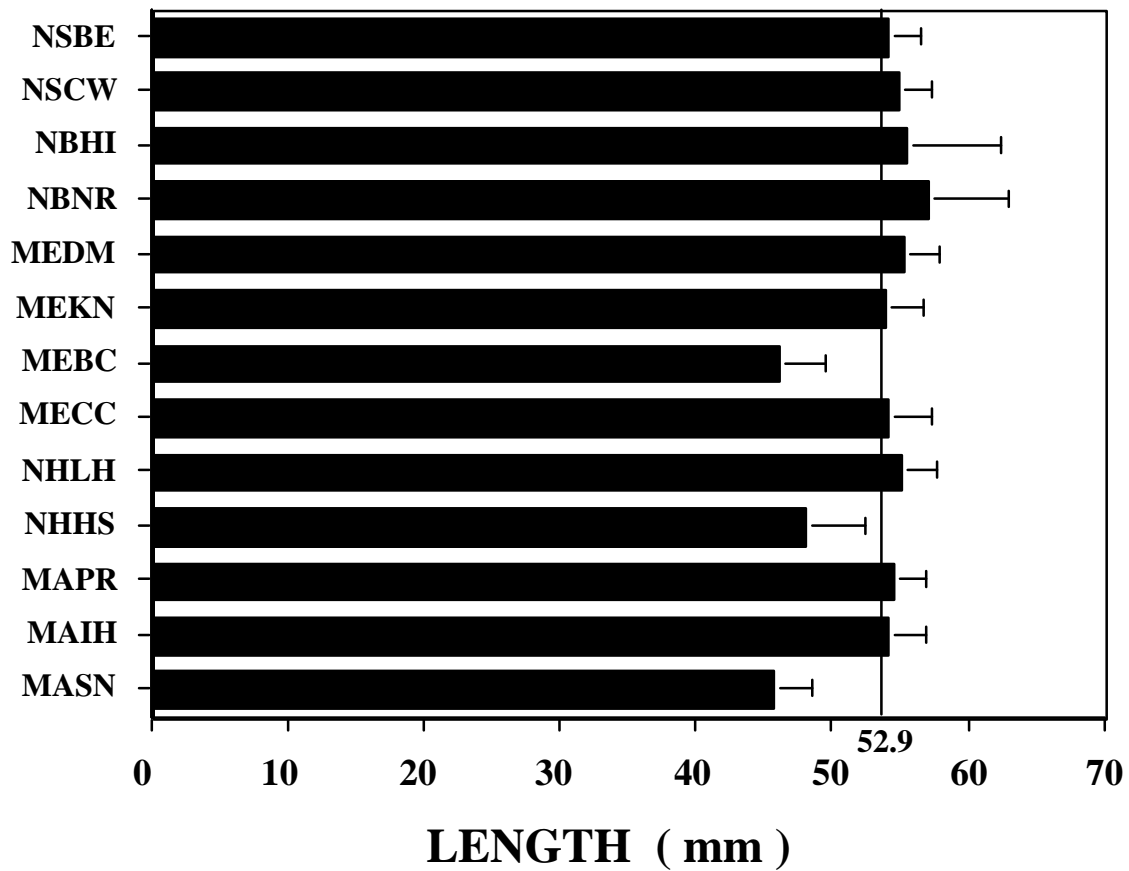
Figure 15. Mean length (± SD) of indigenous mussels collected at the Gulf of Maine stations, 1995, organized clockwise from south to north. Mean length of mussels from all sites indicated by the straight line.
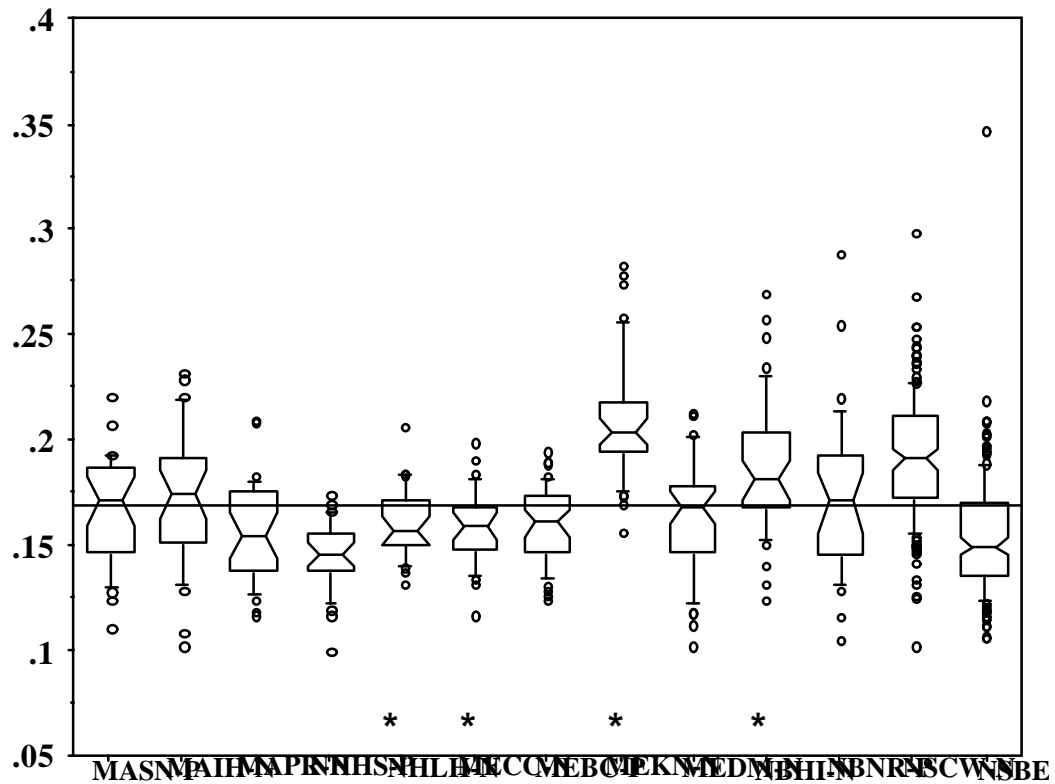
Figure 16. Notched box-and-whisker plots of condition index (CI) for indigenous
from the Gulf of Maine stations, 1995. The notched sections of the box
correspond to the 95% confidence interval about the median. The box gives
range of the middle 50% of the values. The whiskers indicate the range of
condition indicies, except for outliers (beyond1.5 times the box height) which
are plotted as individual points. *, significant difference between preset and
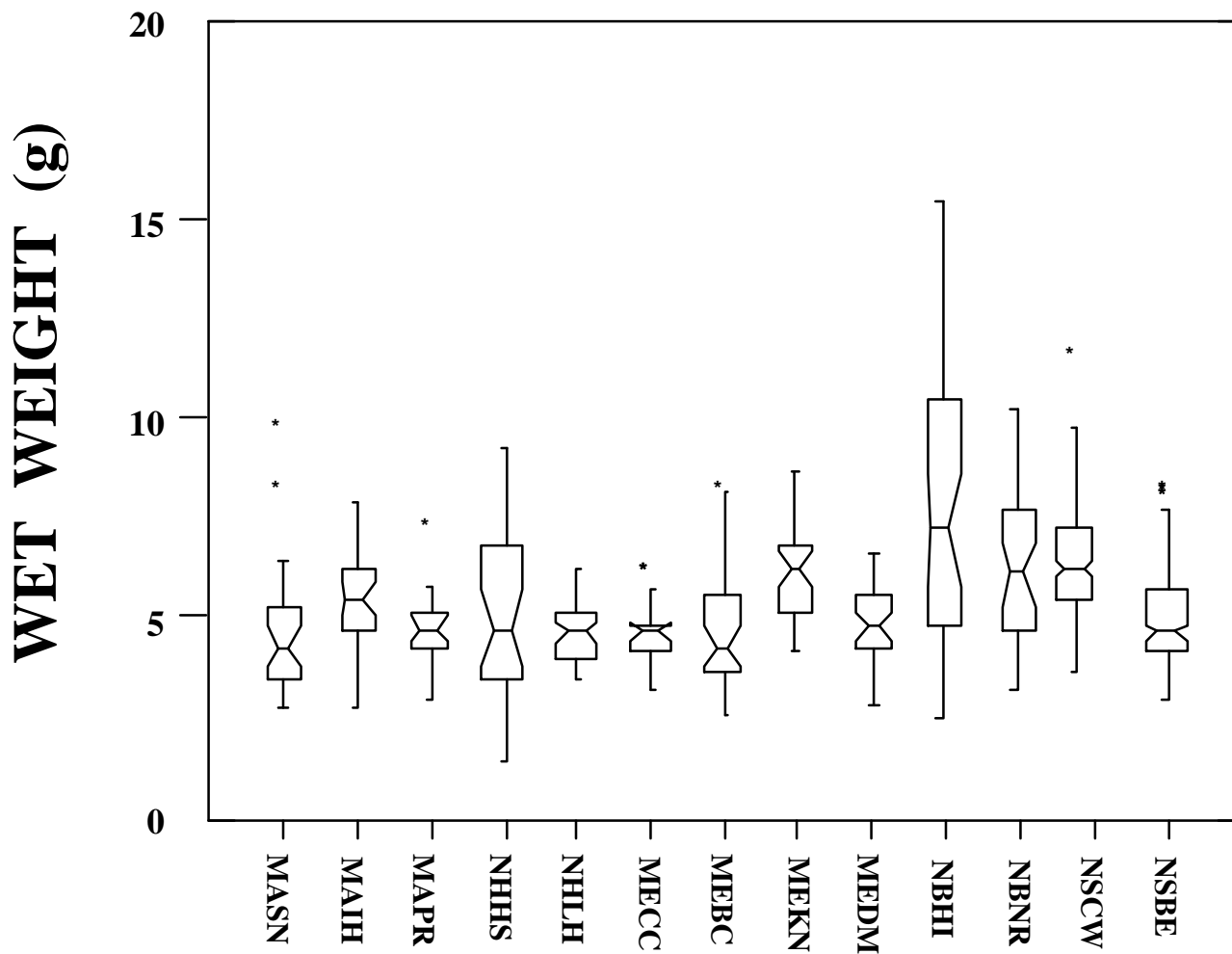indigenous mussels (p<0.05).

Figure 17. Notched box-and-whisker plots of wet weight adjusted for length, width and height of indigenous mussels from the Gulf of Maine stations, 1995. Symbols and explanation as in Figure 16.

covariate for sites in Massachusetts. As a result, the wet weight among sites within each jurisdiction was adjusted for the covariates and then analyzed by ANOVA and Tukey Kramer test. Figure 17 and Table 14 show the adjusted mean weights for stations sampled in 1995. There was a significant relationship between adjusted wet weight and the CI at a given site ($p<0.05$).

Shell growth and condition Index: caged mussels

The sites, dates of cage deployment, retrieval and duration are shown in Table 15. From the 40 cages that were deployed, only 32 were retrieved. Cages deployed at the Nova Scotia sites (NSBE and NSCW) were not recovered.

Shell growth

Table 16 contains information on the morphological measurements [length (mm), height (mm), width (mm), wet weight (g) and condition index (CI)] recorded for mussels from the 8 transplant sites. Figure 18 plots the mean length (±SD) of mussels at deployment and retrieval at each site. For all sites at which caged mussels were recovered, the mean shell length had increased significantly, based on paired t-tests of deployment and retrieval lengths of marked mussels ($p<0.05$). The largest change in length was 6.2 mm at MEKN and the lowest was 2.1 mm at MAIH. Due to the different deployment periods, growth was standardized by dividing by the number of days to obtain a measure of growth rate in millimetres per day. Growth rate of the mussels at each of the eight transplant stations are shown in Table 16 and Figure 19. Statistical analysis on growth rates was conducted on each jurisdiction separately, because a different stock / preset was used in each jurisdiction. In Massachusetts growth of preset mussels from MASN was significantly higher at MAPR than MAIH (ANOVA, $p<0.05$). The total concentrations of metals and organic contaminants was much lower in indigenous mussels at MAPR than at MAIH (Table 3 and Table 9). In addition, caged mussels at MAPR were suspended from a Wharf. The current in this area was high and much of the bottom was consequently scoured bare (J. Pederson and W. Robinson, pers. comm.). Higher flow rates have been shown to enhance growth in other bivalves (Walne, 1978, for *Ostrea edulis* and *Crassostrea gigas*; Smit et al., 1992, for *Dreissena polymorpha*).

In New Hampshire, preset mussels from NHHS transplanted to NHLH had a significantly higher growth rate than those transplanted to MECC (ANOVA, $p<0.05$). The total metal

TABLE 15. Gulfwatch cage deployment and retrieval information, 1995.

STATION	CATEGORY	DEPLOY DATE	RETRIEVAL DATE	DAYS DEPLOYED	# CAGES RETRIEVED
MASN	PRESET				
MAIH	TEST	08 / 15	10 / 17	63	4
MAPR	TEST	08 / 15	10 / 13	59	4
NHHS	PRESET				
NHLH	TEST	08 / 28	10 / 28	62	4
MECC	TEST	08 / 29	10 / 29	62	4
MEBC	PRESET				
MEKN	TEST	08 / 15	11 / 01	78	4
MEDM	TEST	09 / 14	11 / 13	60	4
NBNR	PRESET				
NBHI	TEST	08 / 23	10 / 31	69	4
NBMI	TEST	08 / 23	10 / 30	68	4
NSCW	TEST				0
NSBE	TEST				0

TABLE 16. Morphometric (mean ± SD) comparison of caged mussels before and after deployment at Gulf of Maine stations, 1995.

Variable	Stage	MAIH	MAPR	NHLH	MECC	MEKN	MEDM	NBHI	NBMI
Length (mm)	Deployn	44.9 (2.5) 60	47.3 (2.9) 60	46.4 (2.9) 60	46.0 (3.4) 60	45.1 (3.4) 47	45.4 (3.0) 57	47.8(4.4) 60	45.9(3.1) 60
	Retrieven	47.0 (3.2) 49	50.7 (2.8) 58	49.3 52	48.0 59	51.3 (3.9) 49	49.0 (2.9) 57	51.1 (4.5) 52	48.9 (3.3) 53
Height (mm)	Deploy	23.7 (1.5)	24.9 (1.5)	25.5 (1.8)	25.6 (2.1)			24.0 (2.5)	23.7 (2.1)
	Retrieve	24.4 (1.6)	26.0 (2.1)	27.7 (2.2)	27.8 (2.3)	27.7 (2.1)	26.1 (1.8)	25.6 (2.9)	24.5 (2.2)
Width (mm)	Deploy	18.0 (1.6)	19.4 (2.0)	18.5 (2.0)	18.4 (2.0)			21.2 (2.6)	20.6 (2.2)
	Retrieve	19.5 (1.7)	21.1 (1.7)	19.7 (1.6)	19.3 (1.9)	19.0 (1.9)	18.9 (1.9)	23.1 (2.2)	21.8 (2.1)
Tissue wt. (g)	Retrieven	3.24 (0.72) 49	4.87 (1.03) 58	3.99 (0.83) 52	3.87 (1.86) 58	4.97 (1.90) 47 57	3.84 (0.83) 52	7.65 (2.26) 53	5.15(1.17)
Condition Index(CI)	Retrieven	0.145 (0.03) 49	0.175 (0.03) 58	0.149 (0.03) 51	0.149 (0.08) 58	0.184 (0.02) 47	0.16 (0.02) 57	0.250(0.03) 52	0.20(0.03) 53
Duration (days)		63	59	62	62	78	60	69	68
Growth Rate (mm.d^{-1})		0.033 (0.04)	0.060 (0.05)	0.045 (0.03)	0.033 (0.03)	0.080 (0.04)	0.061 (0.04)	0.05(0.03)	0.04(0.03)

Condition index = wet tissue weight (mg) / Length (mm) * Width (mm) * Height (mm)
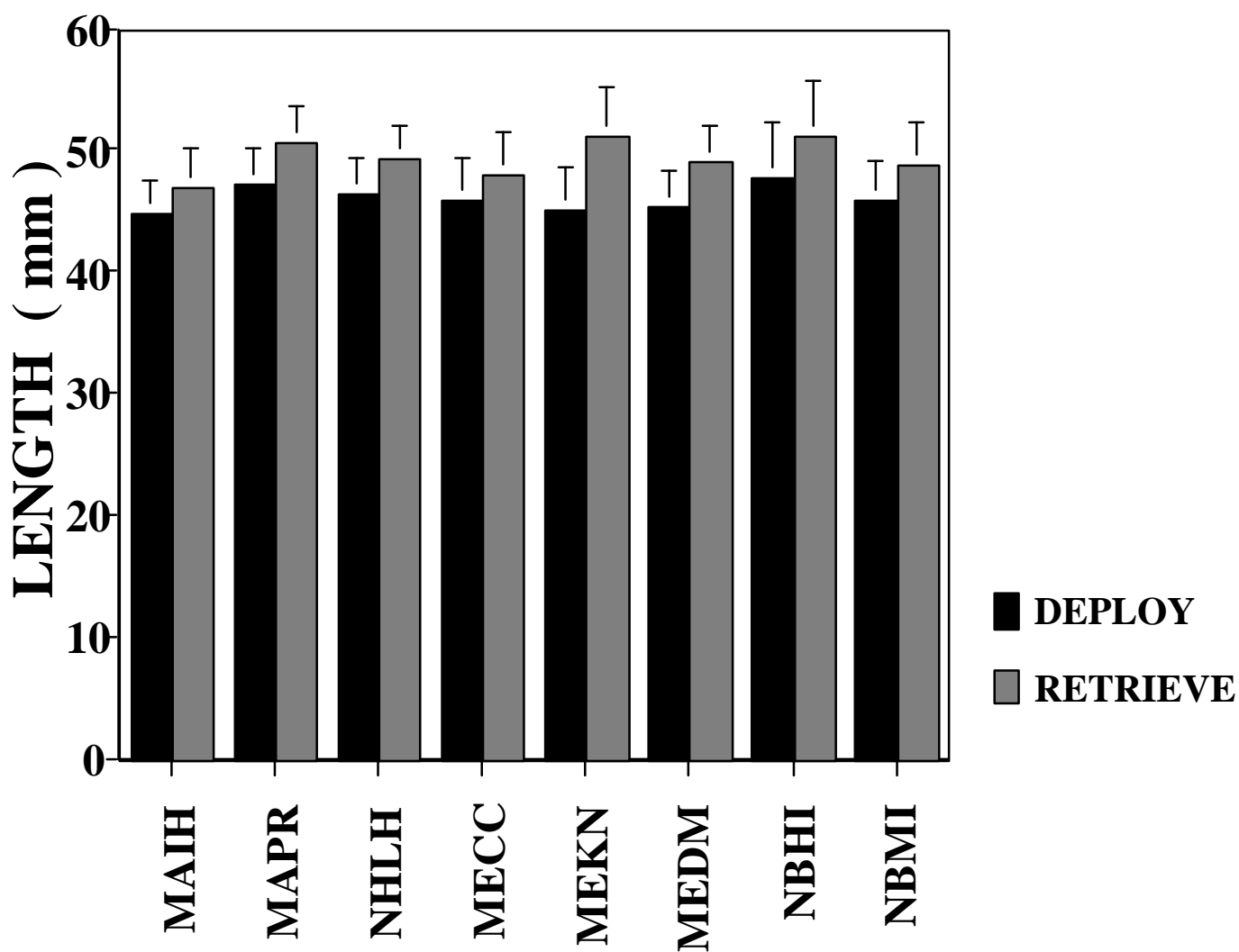Growth rate = retrieval length - deployment length / deployment days

Figure 18. Length (mean ± SD, mm) of marked mussels at deployment and retrieval in the Gulf of Maine, 1995.
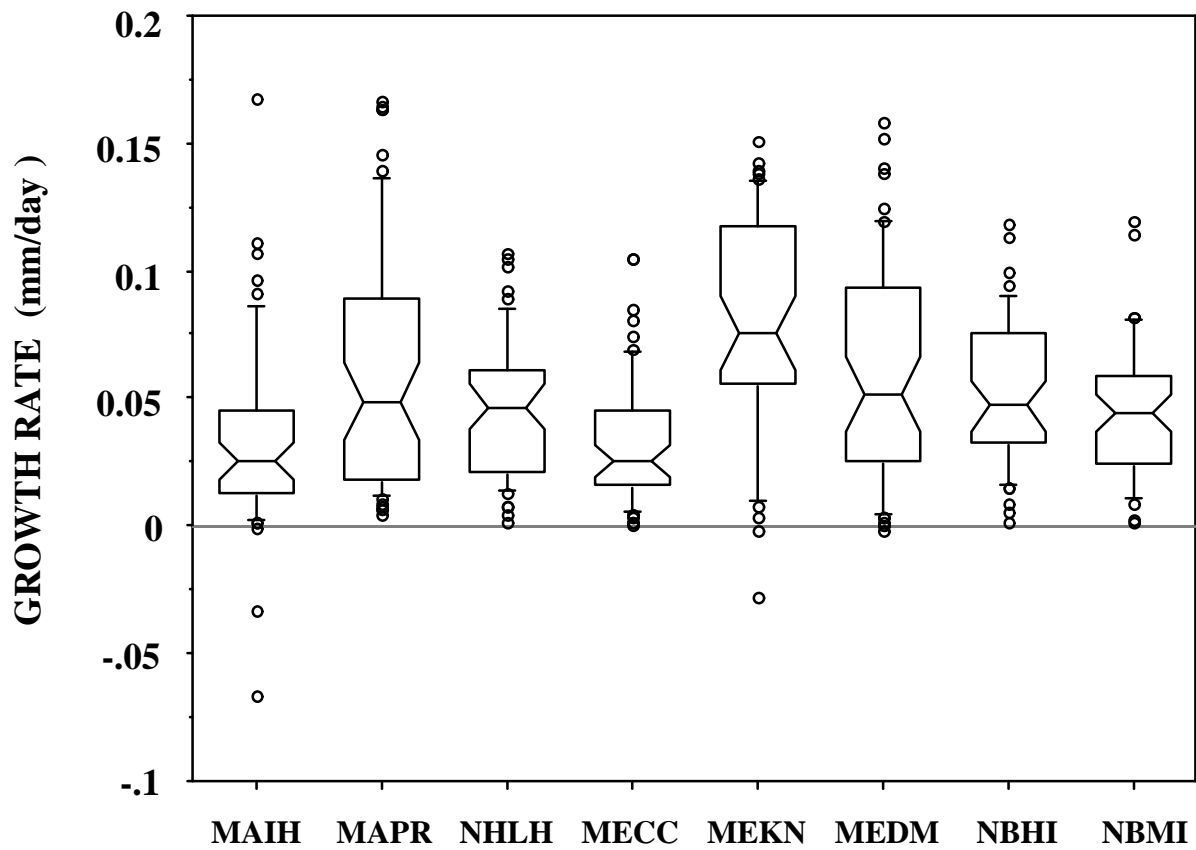
Figure 19. Notched box-and-whisker plots of growth rate (mm/day) of caged
           mussels from the Gulf of Maine stations, 1995.Symbols and
           explanation as in Figure 16.

concentrations in indigenous mussels at NHLH and MECC were similar (Table 3), however, the total organic burden of mussels at MECC was more than twice that of mussels at NHLH (Table 9).

In Maine, preset mussels from MEBC transplanted to MEKN had a significantly higher growth rate than those transplanted to MEDM (ANOVA, p<0.05). The total metal (Table 3) and total organic (Table 9) concentrations in indigenous mussels at MEKN were half that in mussels from MEDM.

In New Brunswick there was no significant difference (ANOVA, p>0.05) in the growth rate of preset mussels from NBNR transplanted to NBHI and NBMI. Unfortunately there were no indigenous samples taken at NBMI in 1995 to compare the total concentrations of metal and organic contaminants.

Condition index (CI)

Figure 20 shows the CI of preset (P) and caged mussels (C) within each jurisdiction. Statistical analysis on the data examined (1) whether the CI of the caged mussels was different from the preset mussels, and (2) was there was any difference in preset mussels and the indigenous mussels at the transplant site that may suggest that the mussels were of poorer quality at the transplant site. Results of this analysis are in Figure 16 (preset vs indigenous) and Figure 20 (caged vs preset).

In Massachusetts, the condition index of mussels transplanted to MAPR was not statistically different from the preset (p>0.05). Mussels transplanted to MAIH, however, had a significantly lower CI (p<0.05) than the preset mussels, suggesting a decrease in condition after transplant despite analysis indicating that there was no significant difference between the CI of preset and indigenous mussels at each site. In New Hampshire there was no significant difference in the CI of the caged and the preset mussels (p>0.05). In Maine there was no significant difference in the CI of mussels transplanted to MEDM (P>0.05), however, there was an increase in CI of mussels at MEKN from the preset level (P<0.05). Indigenous mussels at MEKN had a significantly higher CI that the preset site. In New Brunswick the CI of the caged mussels increased significantly over the preset mussels (P<0.05), despite analysis that revealed there was no significant difference between the preset and indigenous mussels at each site. The CIs of the caged mussels in New Brunswick were the highest of all sites examined. The elevated CIs at the New Brunswick sites could be the result of the caging treatment. Cages are suspended in the water column, thus the mussels have more access to food. In addition, the strong tidal forces in New Brunswick, result in complete turnover of the water column, thus providing more food for better growth. Although the CI was high in transplanted mussels at both New Brunswick sites, shell growth was low. This may suggest differential allocation of resources between somatic (body) tissue and shell growth.
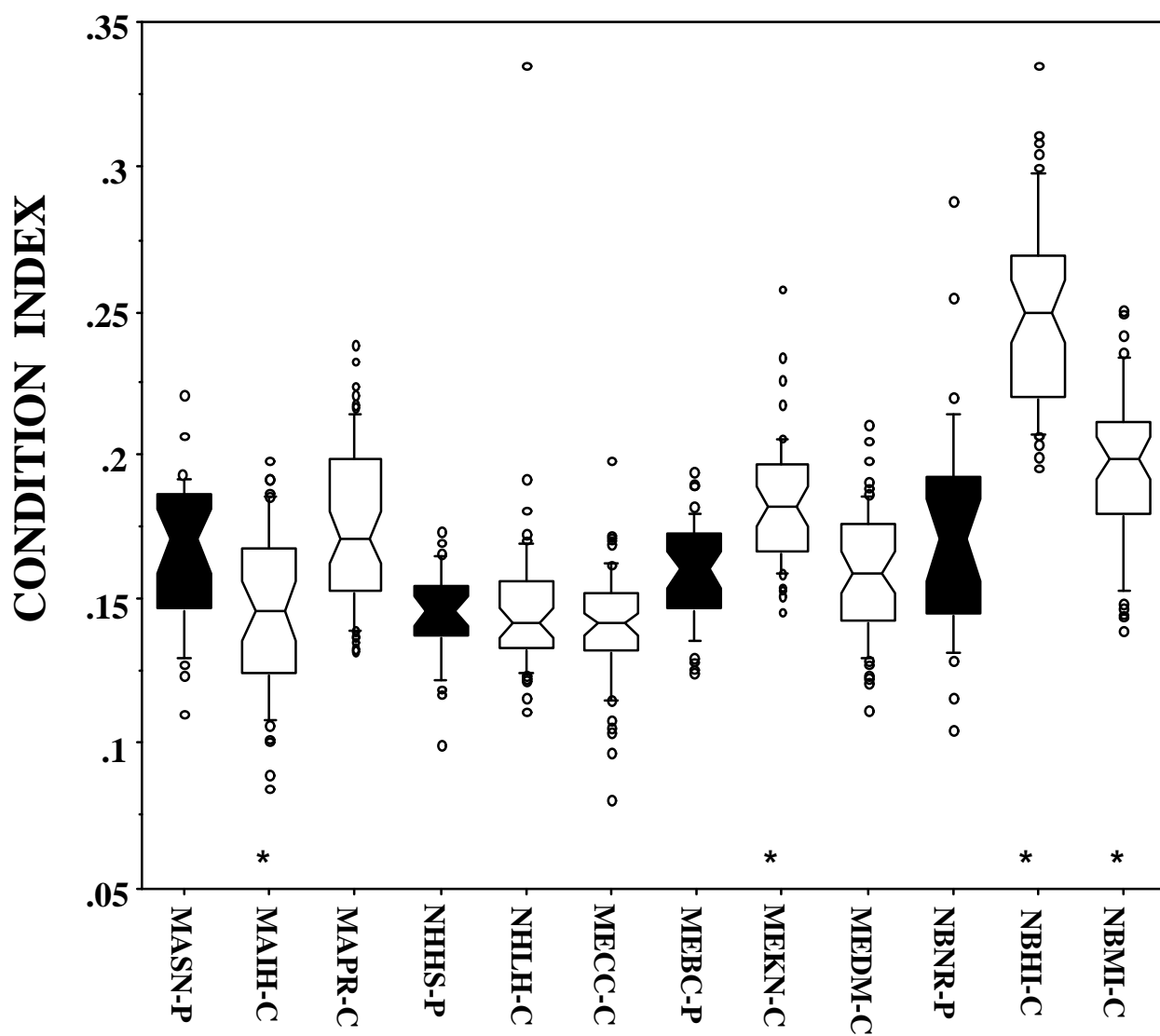
Figure 20. Notched box-and-whisker plots of condition index of caged (C, in white) and prese (P, in black) mussels in the Gulf of Maine, 1995, at retrieval. Symbols and explanation as in Fig. 16. *, significant difference between caged and preset mussels.

CONCLUSIONS

The field season of 1995 represented the final year of the first three year rotation of the overall long term plan in the Gulfwatch program. Beginning in 1996, sampling will begin a second three year rotation of the current sites as part of the remaining 6 years of the program. Such a sampling design will ensure that there will be sufficient spatial and temporal replication of sites to allow for analysis at the end of the study in the year 2001. As part of the three year plan, the monitoring of indigenous mussels at prescribed sites was accompanied by experimental transplants within each jurisdiction to examine mussel responses to short term exposure to contaminant concentrations. Similar activities are planned for 1998, the next third year of the plan. The results build on observations made in the pilot stages of the program in providing information on present and recent contamination conditions at sites.

Monitoring of indigenous mussels revealed that the sites with the highest concentration of metal and / or organic contaminants (MAIH, MAPR, NHLH, MECC) were generally sites with high human population densities and known sources of contaminant input. MAIH is located in Boston's inner harbor, MA where the primary sources of contamination are the numerous CSO's (combined sewer overflow) in the inner harbor, the Deer Island POTW outfall at the mouth of Boston Harbor, the Nut Island POTW outfall located in Quincy Bay and contaminant loadings from Charles River, Mystic River and Chelsea Creek. MAPR is located near Pines River, MA, an area impacted by non-point source runoff, from a recently capped dump site where incinerator ash has been dumped for over 20 years and by contaminant loadings from the Saugus River whose mouth is approximately 1 mile to the north. The close proximity to the Portsmouth Naval Shipyard may account for the elevated lead concentrations in mussels at both New Hampshire sites (NHLH and MECC). In addition, NHLH and MECC lie downstream from known historical mercury sources that are suspected to be related to the Portsmouth Naval Shipyard (NCCOSC, 1994) and other industrial sources. There are also eight POTWs that discharge to the tidal portions of the upstream estuary and two CSOs that discharge directly into the Portsmouth Harbor.

Analysis of the transplant experiment showed active bioaccumulation and depuration of metals and organic contaminants in the mussel tissue. In general, the concentration of contaminants (metal and organic) would increase or decrease in a predictable fashion in response to the concentration of contaminants in the indigenous mussels at the transplant site. For example, if the preset mussels had a lower level of PCB than indigenous mussels at the transplant site then, at the end of 60 days, the caged mussels would typically have significantly increased concentrations of PCB in their tissues. Such results confirm that short term water column contaminant conditions are indicative of long term conditions, which are reflected by contaminant concentrations in indigenous mussels.

The biological response (growth rate and CI) of transplanted (caged) mussels within a jurisdiction was strongly correlated with the total level of metal and organic contaminants present in indigenous mussels growing at the same site. The assumption is that bioresponse is related to exposure to contamination as measured in indigenous mussels that have been continuously exposed. The growth rate and CI of transplanted mussels was the highest at the site with the lowest concentration of total metal and organic contaminants in indigenous mussels. Gulfwide, the site with the highest growth and highest CI was MEKN. This site had the lowest concentration of total metals, although the concentration of organic contaminants, particularly PAH, was quite high. The site with the lowest growth rate and lowest CI of transplant mussels was MAIH. This site had high concentrations of total metals and the highest concentration of organic contaminants. The observation of a high concentration of organic contaminants in indigenous mussels at MAIH is based on the results from the caged mussels, as the organic contaminant concentration in indigenous mussels at this site was not analyzed. There is a significant relationship between the Log_{10} concentration of organic contaminants in indigenous mussels and caged mussels after deployment for 60 days (Figure 21). The estimated concentration of organic contaminants in indigenous mussels at MAIH, using this relationship is 1792 ng / g dry weight). Low growth rates and CIs were also observed in transplant mussels at MECC, a site with high concentrations of metal and organic contaminants. Interestingly, at MAPR, a site with a high concentration of metal and organic contaminants, mussels had very high growth rates and CI. Such a response may be the result of many different influences, including the following two examples. Mussels that were transplanted came from stock "preset" sites within each jurisdiction. As such, it is possible that responses within a jurisdiction may be a reflection of the different genetic makeup of the preset population and its response to varying concentrations of contaminants. Alternatively, the high growth rate and CI at MAPR may reflect the role of additional, environmental factors in determining the growth rate and CI of mussels at a given site. MAPR is located in high current area, which may result in enhanced food conditions for growth. The role of additional environmental factors will be taken into account for the report on the five year review of the Gulfwatch program. The results of that report should enable us to to gain insight into the factors important in predicting chemical accumulation in *M. edulis*.

Coastal monitoring programs such as Gulfwatch provide a valuable measure of the current state of the coastal environment, for identifying future problems which may be prevented by early action, for determining trends in contamination over space and time, and for identifying potential sources of contamination. Gulfwatch results provide a geographically comprehensive, region specific perspective on relative contaminant concentrations in both contaminated and pristine areas. As such, it is an unique and invaluable basis for making management decisions on issues relating to toxic contaminants. Continuation of the Gulfwatch program according to the ten year plan will provide the

temporal perspective necessary to determine trends and impacts of remediation efforts.
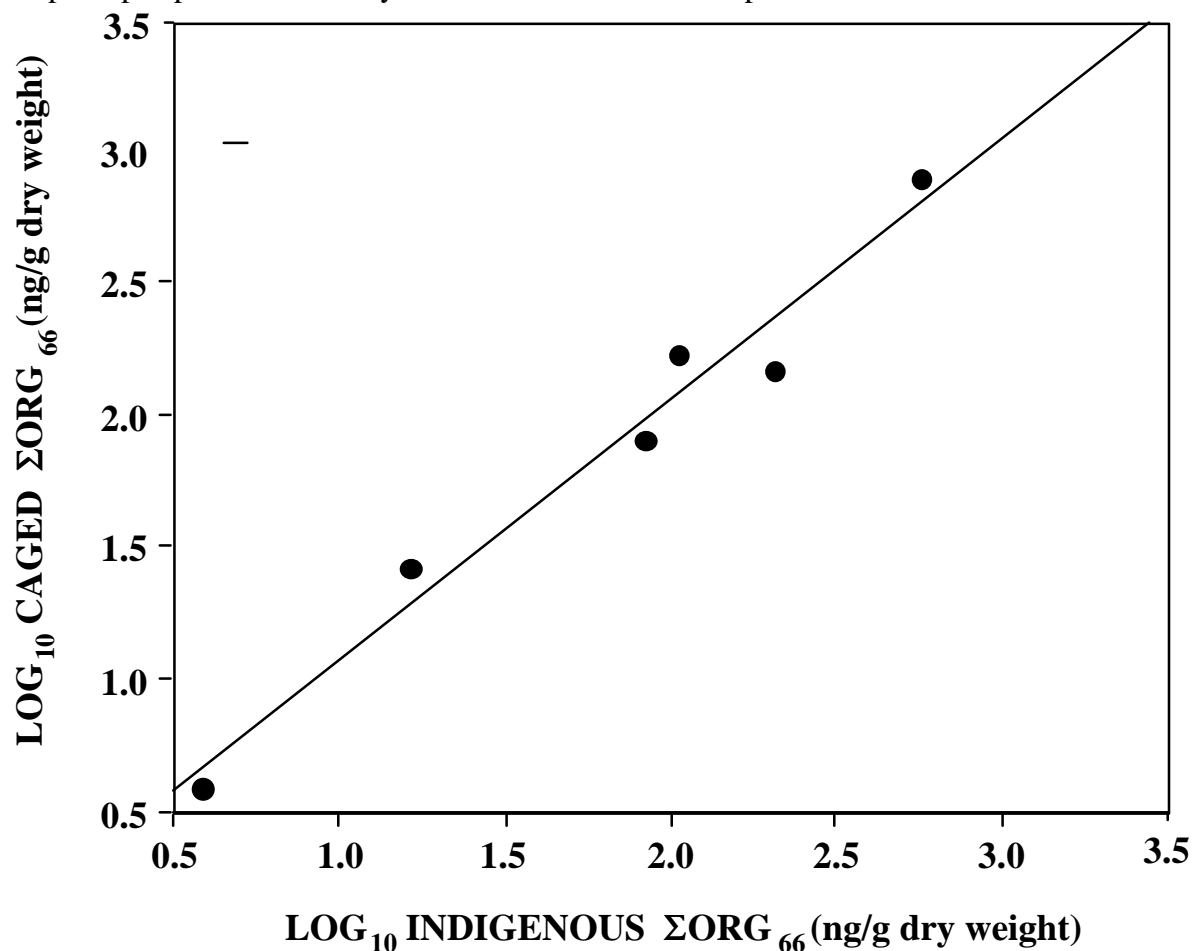
Figure 21. Regression of the Log10 of the ΣORG_{66} (ng/g dry weight) for indigenous mussels and the Log10 of the ΣORG_{66}(ng/g dry weight) for the caged mussels. n=6 sites. Log10(ΣORG_{66}caged) = 0.991 (log10ΣORG_{66} indigenous) + 0.077; $r^2 = 0.968$; $p < 0.001$.

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APPENDIX A. METHODS FOR MUSSEL EXTRACTION AND ANALYSIS OF ORGANIC CONTAMINANTS

Compounds for organic analysis were selected to be consistent with NOAA status and trends mussel monitoring. All samples were analyzed at the environment Canada Atlantic Regional Laboratory in Dartmouth, N.S.

Analytical Methods

Modifications to the methods for organic contaminants have been made since the 1991 Gulf of Maine Mussel Watch Effort (GMCME 1992a and b). The major changes include: (1) lowering target analyte detection limits to 10 ng/g for most aromatic hydrocarbons (20-30 ng/g for some of the lower molecular weight aromatic); (2) the addition of 17 chlorinated pesticides to the variable list including alpha and beta endosulfan; (3) identification and quantification of PCB by congener analysis which include 18 NOAA designated congeners and 6 other congeners including some coplanar PCBs. The specific compounds and their detection limits are listed in Tables A1 and A2.

Some modifications were made in the analysis of the 1993 samples in order to improve the analytical quality control. These include the addition of two PCB recovery surrogates (CB-103 and CB-198) and an organochlorine pesticide recovery surrogate (y-chlordene) to sample homogenates prior to extraction. The PCB and pesticide surrogates replace 3,4,5-trichlorobiphenyl-d5 which was used previously to assess method performance of both PCBs and pesticides.

Methods Description
Sample preparation and extraction

Composite samples of shucked mussels meats from various coastal locations were provided to the laboratory in solvent cleaned glass sample jars and stored at -20 °C until samples were processed.

Prior to analyzing mussel tissue samples, the laboratory verified that all glassware, chemical reagents, and solvents used in the analysis of tissue samples were free of contamination which could interfere with the identification and quantification of target analytes.

A frozen composite sample (5-200 g) was thawed and homogenized in a Waring blender at high speed for 3 minutes (for details on sample homogenization refer to Shrimpton, 1988). Ten grams of homogenate were transferred to a 300 ml Berzelius beaker and 50 ml of methyl chloride

added. Two grams of homogenate were put aside for dry weight determination.

One hundred microliters of surrogate solution containing five deuterated aromatic hydrocarbon recovery standards (Table A1) and 100 µl of surrogate solution containing one pesticide and two PCB congener recovery standards (Table A2) were added to the homogenate. Anhydrous sodium sulphate (7 g/g of homogenate) was added and the contents blended for 2 minutes at high speed with a Polytron tissumizer. The solvent was decanted, saved and the solid material extracted twice more with 50 ml methylene chloride. The combined solvent extract along with the solid material from the last extraction step were vacuum filtered with rinsings through a Whatman GF/C glass microfiber filter. The filtered solvent was concentrated to 10 ml an a 1 ml aliquot removed for lipid determination. The remaining extract was concentrated to about 3 ml, transferred to a 10 ml glass syringe, and forced through a 0.5 µm Milex SR filter unit into a 15 ml ABC AS2000 System GPC-autovap loading tube. The final volume of extract was made exactly to 8.0 ml with methylene chloride.

TABLE A1. Aromatic hydrocarbons.

Compound	Quantitation ion	Confirmatory ion	Spike * concentration (ng/g)
Naphthalene	128	127[15]	40
2-Methylnaphthalene	142	141[90]	"
1-methylnaphthalene	142	141[90]	"
Biphenyl	154	152[35]	"
2,6-Dimethylnaphthalene	156	155[30]	"
Acenaphthylene	152	151[20]	"
Acenaphthene	153	154[90]	"
2,3,5-trimethylnaphthalene	170	169[90]	"
Fluorene	166	165[90]	"
Phenanthrene	178	176[20]	"
Anthracene	178	176[20]	"
1-Methylphenanthrene	192	191[50]	"
Fluoranthene	202	200[20]	"
Pyrene	202	200[20]	"
Benzo(a)anthracene	228	226[20]	"
Chrysene	228	226[20]	"
Benzo(b)fluoranthene	252	250[20]	"
Benzo(k)fluoranthene	252	250[20]	"
Benzo(e)pyrene	252	250[20]	"
Benzo(a)pyrene	252	250[20]	"
Perylene	252	250[25]	"
Indeno(123cd)pyrene	276	277[25]	"
Dibenzo(ah)anthracene	278	279[25]	"
Benzo(ghi)perylene	276	277[25]	"

* Spike Matrix samples

[ ] % % of base peak

TABLE A1. Cont...

SURROGATES :   Amount *
                                                                     (ng)

Naphthalene-d8	136	137	120
Acenaphthene-d10	164	162	120
Chrysene-d12	240	241	60
Benzo(a)pyrene-d12	264	265	60
Benzo(ghi)perylene-d12	288	289	60

* Added to sample homogenate

Compound Concentration*	Quantitation ion	Confirmatory ion	(pg/µl)

INTERNAL STANDARDS

Fluorene-d10	176	174	350
Pyrene-d12	212	210	350
Perylene-d12	264	260	350

* In calibration curve

TABLE A2. Polychlorinated biphenyls.

IUPAC	Congener	Spike concentration * (ng/g)
8/5	2,4'-dichloro	20
18/15	2,2',5-trichloro	"
28/31	2,4,4'-trichloro	"
29	2,4,5-trichloro	"
44	2,2',3,5-tetrachloro	"
50	2,2',4,6-tetrachloro	"
52	2,2',5,5'-tetrachloro	"
66/95	2,3',4,4',-tetrachloro	"
77/110	3,3',4,4',-tetrachloro	"
87	2,2',3,4,5-pentachloro	"
101/90	2',2,4,5,5',-pentachloro	"
104	2,2',4,6,6',-pentachloro	"
105	2,3,3',4,4',-pentachloro	"
118	2,3',4,4',5-pentachloro	"
126/178	3,3',4,4',5-pentachloro	"
128	2,2',3,3',4,4'-hexachloro	"
138/163/164	2,2',3,4,4',5'-hexachloro	"
153/132	2,2',4,4',5,5',-hexachloro	"
169	2,2',4,4',5,6'-hexachloro	"
170/190	2,2',3,3',4,4',5-heptachloro	"
180	2,2',3,4,4',5,5'.-heptachloro	"
187	2,2',3,4',5,5',6-heptachloro	"
188	2,2',3,4',5,6,6',-heptachloro	"
195/208	2,2',3,3',4,4',5,6-octachloro	"
200	2,2',3,3',4,5',6,6'-octachloro "	
206	2,2',3,3',4,4',5,5',6-octachloro	"
209	decachloro	"

* Spike matrix samples
/coeluting congengers

TABLE A2 Cont....

SURROGATE :	Amount * (ng)
CB-103	25
CB-198	25

* In all samples

INTERNAL STANDARDS :	Concentration * (pg/µl)
4,4'-dibromooctafluorobiphenyl	10
octachloronaphthalene (ref time only)	10

* In calibration curve

Extract Clean-up and Fractionation
<u>**Gel permeation chromatography**</u>

Lipids, elemental sulphur, and other larger molecular-size compounds derived from the biomatrix were removed by gel permeation using an Autovap AS2000 GPC Sample Processing System (Analytical Bio-Chemistry Laboratories). The system included a low-pressure GPC column packed with methylene chloride preswollen SX-3 envirobeads and was run in dual GPC/auto-evaporation mode with an end-of-run hexane solvent exchange. A sample matrix effect helped reduce analyte losses during the evaporation stage; and a keeper, therefore, was not used.

<u>**Silica column chromatography**</u>

PCB congeners and apolar pesticides were fractionated from more polar pesticides on 1 cm X 10 cm silica gel columns. Columns were prepared by sandwiching a pentane slurry of 7% deactivated silica gel (Davidson 923, mesh 100/200) between two 1 cm layers of anhydrous sodium sulphate.

A column was pre-rinsed with 30 ml of pentane, and 1 ml of concentrated sample extract in pentane was placed on top of the column bed. Twenty millilitres of pentane was passed through the column which eluted most PCB congeners and apolar pesticides. This was followed by 20 ml of pentane/methylene chloride (1:1) which eluted the more polar pesticides.

Each fraction was collected separately and concentrated to 0.5 ml. Ten microliters of PCB/pesticide internal standard solution (Tables A2 and A3) were added to each fraction prior to analysis by high-resolution gas chromatography-ECD.

After completion of the PCB/pesticide GC-ECD analysis, the two fractions were combined and concentrated to 0.5 ml in toluene. Ten microliters of internal standard solution containing five deuterated aromatic compounds (Table A1) were added to the extract and reanalyzed by GC-MS for aromatic hydrocarbons.

TABLE A3. Pesticides.

Compound	Concentration * (ng/g)
Hexachlorobenzene	20
Heptachlor	"
Aldrin	"
4,4'-DDE	"
Mirex	"
Lindane	"
Heptachlor Epoxide	"
cis-Chlordane	"
trans-Nonachlor	"
Dieldrin	"
2,4'-DDE	"
2,4'-DDD	"
4,4'-DDD	"
2,4'-DDT	"
4,4'-DDT	"
alpha-Endosulfan	"
beta-endosulfan	"

* Spike matrix samples

SURROGATES :	Amount * (ng)
y-chlordene	40

* Added to sample homogenate

INTERNAL STANDARDS :	Concentration * (pg/µl)
4,4'-dibromooctafluorobiphenyl	10
Octachloronaphthalene (ref time only)	10

* In calibration curve

Instrumental Analysis

Polychlorinated biphenyls and pesticides

      PCB congeners and pesticides in mussel tissue extracts were analyzed by high resolution gas chromatography-electron capture detection. A four-point calibration curve was constructed covering the concentration range 2 to 500 pg/µl for theses analyses.

      Apolar PCB congeners and pesticides contained in fraction one and the more polar compounds contained in fraction two were analyzed on two different fused silica capillary columns. Column 1 contained a 5% phenylmethyl polysiloxane stationary phase while column 2 contained a 50% phenylmethyl polysiloxane stationary phase. PCB congeners analyzed on column 1 were identified and quantified individually or as co-eluting pair (Table A2). Pesticides detected on column 1 were confirmed on Column 2. PCB congeners and pesticides which co-eluted on Column 1 were resolved on Column 2 for identification and quantification.

Gas chromatography-electron capture detector operating conditions

Gas chromatograph :	HP 5890 Series 11
Column 1 :	DB-5, 30 m x 0.20 mm fused silica, 0.33 µ film
Injection :	Splitless
Temperature program:	90 °C for 0 minutes, to
	175 °C at 10 °C/minute, to
	280 °C at 2.5 °C/minute, and
	hold for 5 minutes
Carrier gas :	Helium

Secondary analysis

Gas chromatograph :	HP 5880
Column 2 :	HP-17, 25 m x 0.32 fused silica, 0.26 µ film
Injection :	Splitless
Temperature program :	As above
Carrier gas :	Helium

Polyaromatic Hydrocarbons

Analysis of aromatic hydrocarbons was conducted by high-resolution capillary gas chromatography and low-resolution quadruple mass spectrometry in selective ion mode. A five-point calibration curve was constructed for analysis covering the concentration range 10 to 100 pg/µl.

Gas chromatograph and mass spectrometer operating conditions

Gas chromatograph :	HP 5890 Series 11
Column :	DB-5, 30 m x 0.25 mm fused silica, 0.25 µ film or equivalent
Injection :	Cool on column
Temperature program :	70 °C for 1 minute, to
	250 °C at 10 °C/minute, to
	290 °C at 20 °C/minute, and
	hold 12 minutes
Carrier gas :	Helium,
Mass spectrometer :	HP 5971A MSD
Ionization mode :	Electron impact 70 ev
Ion dwell time :	150-250 msec (optimized for maximum sensitivity)
Scan speed :	1 cps

REFERENCES

GMCME (Gulf of Maine Council on the Marine Environment), 1992a. Evaluation of Gulfwatch 1991 pilot project of the Gulf of Maine Environmental Monitoring Plan. October. 39 p + Appendices A-D.

GMCME (Gulf of Maine Council on the Marine Environment), 1992b. Gulfwatch Project, standard procedures for field sampling, measurement and sample preparation. Gulfwatch Piolet project period 1991-1992. 11p.

Shrimpton J., 1988. Contaminants Control-Toxic Chemicals Program Technical Resources Manual, 1988. Environ. Can., Conservation and Protection, Pacific and Yukon Region. p. 14-17.

APPENDIX B. QUALITY ASSURANCE / QUALITY CONTROL PROTOCOL
1.0 INTRODUCTION

The quality assurance provisions of this performance-based standard are intended as a guide for the generation of acceptable analytical data for use in Canadian shellfish contaminants monitoring. The standard permits flexibility in the selection of an analytical method for the generation of chemical data, provided the laboratory institutes the quality control measures identified and the method can attain the minimum performance stated.

2.0 SPECIFIED VARIABLES

Essential target analytes required for reporting are listed in the following tables:
- Polyaromatic Hydrocarbons (Table A1 and Appendix D)
- PCB Congeners and Chlorinated Pesticides (Tables A2 and A3 and Appendices E and F)
- Metals (Appendix C)

3.0 EXTERNAL CHECK SAMPLE PROGRAM PARTICIPATION

A laboratory providing analytical data for use in shellfish contaminants monitoring is required to demonstrate proficiency in contaminant analysis through yearly participation in a reference interlaboratory check sample program if available. Exercise results are provided to the regional project coordinator for review. Deficiencies in check sample performance must be discussed with the project coordinator and corrective action taken where necessary.

The check sample program must be relevant to the analysis of organic and inorganic contaminants at trace concentrations in marine shellfish matrices. The National Institute of Standards and Technology (NIST) (Gaithersburg, Md.) conducts a QA intercomparison exercise program for both government and private laboratories engaged in the measurement of organic and inorganic contaminants in marie sediment, fish and shellfish samples.

4.0 INTRALABORATORY QUALITY CONTROL: INSTRUMENT OPERATING REQUIREMENTS AND PERFORMANCE CRITERIA

4.1 Aromatic Hydrocarbon Analysis

Gas chromatography-mass spectrometry : Aromatic hydrocarbons in mussel tissue extracts are analyzed on a GC-mass spectrometer in selective ion mode. The gas chromatograph must be capable of ramp temperature programming up to 290 °C and accommodating a 25 m or

longer DB-5 capillary column or equivalent. It is recommended that on-column injection is used in order to avoid mass discrimination of higher molecular weight aromatic hydrocarbons which can occur with flash vaporization injection. It also is advisable that deactivated retention gaps are used and routinely replaced in order to maintain column performance.

4.1.1 Initial Set-up

MS Tuning : The mass spectrometer is tuned to standard specifications with perfluorotributylamine (PFTBA). Periodic retuning after initial set-up should be performed to ensure MS calibration consistency. Recalibration of the calibration curve is necessary after each retuning.
Following PFTBA tuning, it may be necessary to manually tune the mass spectrometer, maximizing sensitivity in the low- to mid-mass range (e.g., maximizing the absolute abundance of mass 264) in order to achieve analyte target detection.
Calibration curve : A five-point calibration curve is constructed for every target and surrogate analyte. The concentration range covered is 10 to 1000 pg/µl. The curve should not be forced through the origin. Linearity is verified when the relative standard deviation of response factors for each analyte is less than 30%.
Detection limits : Laboratories must verify that the method and instruments achieve target method detection limits of 30 ng/g (dry weight) or lower for low molecular weight aromatic hydrocarbons (two-ring compounds) and 10 ng/g or lower for higher molecular weight aromatic compounds.
Analyte Identification : Positive identification is assumed when relative to an internal standard, the analyte retention time is within ±0.05 minutes of the corresponding standard retention time; the ratio of quantitation ion and confirmatory ion (Table A1) is within ±20% of the calculated theoretical value except when the abundance of the confirmation ion is too low to permit detection; and the peak maxima for quantification and qualifier ions coincide within 3 seconds. Identified analytes which fail to meet these criteria should be flagged.
Quantitation : An internal standard method is recommended for the quantification of sample data. A minimum of three internal standards should be spiked into sample extracts prior to GC-MS analysis. Suggested internal standards and spiking concentrations are given in Table A1.
Analyses are conducted within the range of the standard calibration curve. Sample extracts with concentrations of analytes greater than the highest calibration standard must be diluted to bring analyte concentrations within the calibration range.

4.1.2 Method Performance Test

Prior to processing any samples, a laboratory must demonstrate that its method and instrument operating conditions will provide acceptable recoveries of surrogate and target analytes. Three replicate uncontaminated tissue homogenate samples are spiked with surrogates and target analytes and analyzed by the full procedure. Recovery of target analytes and surrogates must meet the performance criteria stated in Section 4.1.2 under "Matrix Spike Sample" and "Surrogate Spikes". Method precision (RSD) for each analyte should be greater than 25%.

4.1.3 Daily Performance Checks

Calibration Curve Check : At least one calibration standard is run prior to each batch analysis. The calculated amount for each analyte must be within ±15% on average and not exceed ±25% for any one analyte.

Chromatographic Column Performance : Chromatographic resolution is verified on a daily basis. Adequate resolution is demonstrated if for the highest peak there is no more than 1% valley between the phenanthrene/anthracene peaks and less than a 25% valley between the Benzo(a)anthracene / chrysene peaks. If these performance criteria are not met, column resolution must be restored before any further sample analyses can proceed.

4.1.4 Batch Analysis

A laboratory is required to analyze tissue samples for organic contaminants in batches of no more than 15 samples including quality control samples. The following quality control measures are required for each batch of sample analyses:

Method Blank (1) : The method blank must be free of contamination at or above the method detection limit. If contamination is greater than the MDL a correction may be made by subtracting the average amount in the blanks from the amount in samples when the blank contamination can be shown to be constant over a number of batch runs. If blank contamination is greater than two times the MDL, corrective action must be taken to eliminate the source of contamination.

Duplicate Samples (1 set) : The relative percent difference between the analytical results for duplicate samples should be no more than 25% for measured values greater than five times the MDL.

The percent difference is calculated by dividing the absolute difference of the duplicate values by their average value.

<u>Spiked Matrix Sample (1)</u> : A matrix spike is prepared for all the analytes of interest (Table 1A). If possible, spike matrix concentrations should be in the same proximity as sample concentrations. Otherwise, the addition of 75 ng of each analyte to matrix homogenate is usually sufficient. Spike recoveries must fall into the range of 40-120% for 80% of analytes. If more than 20% of recoveries are outside the range, instrumental response, sample chromatographs and surrogate recoveries for each sample in the batch are checked to ensure that batch analysis is in control. Failing performance criteria will result in individual samples or the entire batch being reanalyzed.

<u>Standard Reference Material</u> : Ideally, one mussel tissue SRM should be included with each batch of sample analyses. Availability and cost of bivalve reference material, however, may preclude a SRM in every batch. As a minimum at least one SRMs is analyzed with every two or three batches. For projects with higher numbers of samples, a SRM is run at the beginning, mid-point, and end of the analytical project. Marine bivalve certified reference material (SRM 1974) can be obtained from NIST (United States Department of Commerce, Gaithersberg Md.).

On average, laboratory results (corrected for surrogate recoveries) should be within ±30% of the certified value's confidence range for all analytes and may not exceed ±35% for more than 30% of individual analytes.

<u>Surrogate spikes</u> : Deuterated surrogate analytes (Table A1), representative of each aromatic hydrocarbon group of the same ring number, are spiked into each sample homogenate and method blank prior to extraction. Surrogate recoveries must be in the acceptable range of 30-150%. Samples with surrogate recoveries outside the range are reanalyzed.

4.2 PCB CONGENER AND CHLORINATED PESTICIDES ANALYSIS

The following QC and performance standards are intended for the analysis of PCB congeners and chlorinated pesticides by high-resolution gas chromatography and electron capture detection. Requirements for the gas chromatograph are the same as described in section 4.2. If flash vaporization injection is used, care should be exercised in selecting the injection port temperature in order to minimize degradation of thermally labile compounds such as 4,4'-DDT.

Gas chromatographic analysis is performed on a 30 m or longer 5% phenylmethyl polysiloxane column (DB-5 or equivalent).

It is highly recommended that a laboratory include in its analytical method provision for the absorptive column fractionation of apolar PCB congeners and chlorinated pesticides from more polar pesticides. The laboratory must verify the PCB and pesticide column elution pattern for every new batch of absorptive material used. Correction of analytical results based on the distribution of some analytes in the two fractions may be required.

4.2.1 Initial Set-up

 Calibration Curve : For every target analyte a five point calibration curve is constructed which covers the concentration range 2 to 500 pg/µl. Calibration curve lineally is verified when the relative standard deviation of response factors for each analyte is less than 30%.

Detection Limit : The laboratory must verify that methods and instrument operating conditions can achieve target method detection limits of 2 ng/g for individual PCB congeners and chlorinated pesticides.

 Chromatographic Column Performance : Chromatograms of standard analytes are checked regularly to ensure that analyte peak shape, resolution, and sensitivity have not degraded with time.

 Analyte Identification : PCBs in mussel tissue are analyzed as selected congeners (Table A2). Co-eluting congeners are identified in data reports.

Analysis with a second capillary column possessing a stationary phase different from DB-5 type columns such as 50% phenylmethyl polysiloxane (DB-17 or equivalent) is required in order to resolve and accurately identify and quantify PCB congeners and pesticides which co-elute on DB-5 type columns.

The positive identification of a PCB congener or pesticide is assumed when relative to an internal standard, the analyte retention time is within ±0.05 minutes of the corresponding standard retention time.

 Quantitation : An internal standard method is recommended for the quantitation of sample data. Suggested internal standards and concentrations for the GC-ECD analysis of PCB congeners are given in Tables A2 and A3.

4.2.2 Method Performance Test

The same as per section 4.1.2.

4.2.3 Batch Analysis

Laboratories are required to analyze tissue samples in batches of 15 individuals or less. The following quality control measures are required with each batch:

 Method Blank (1) : See section 4.1.4.

 Duplicate Samples (1 set) : Performance criteria as in Section 4.1.4.

 Spiked Matrix Sample (1) : A matrix spike is analyzed for all PCB and pesticide target analytes (Table 3A). Performance criteria are the same as in Section 4.1.4.

<u>Standard Reference Material</u> : See Section 4.1.4

<u>Surrogate Spikes</u> : PCB congener and pesticide surrogates (Table A2) are spiked into each sample homogenate prior to extraction. Performance criteria as in Section 4.1.4.

4.4 REPORTING

Analytical results are reported individually on a dry weight basis. All surrogate recoveries and results of duplicate analysis must be reported with the relevant sample data. Data corrected for surrogate recoveries (done in agreement with the project coordinator) must be identified as such. The results of check sample exercises are reported and discussed with project coordinators. The results of all performance tests, matrix spike samples, and surrogate spiked method blanks are tabulated and provided at the request of the project coordinator.

A laboratory should be prepared to provide a copy of the analytical method including handling, storage, and any modifications required to accommodate problems encountered (example matrix interferences). A laboratory also should maintain on file all relevant sample, standard, and blank chromatographic and related QC data as well as tables of all calibration standard and surrogate solution concentrations for possible future examination.