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An In-Situ Assessment of Mercury Contamination in the Sudbury River,
Massachusetts, Using Bioaccumulation and Growth in Transplanted Freshwater
Mussels (*Elliptio complanata*)

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An In-Situ Assessment of Mercury Contamination in the Sudbury River, Massachusetts, Using Bioaccumulation and Growth in Transplanted Freshwater Mussels

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CONTENTS

	Page
Introduction.....	1
Site History.....	1
Previous Investigations of the Sudbury River	1
NOAA's Involvement - This Study.....	7
Use of Bivalves in Monitoring Programs.....	8
Objectives.....	11
Methods.....	11
Description of Study Area.....	11
Mussel Collection, Processing, Deployment, and Retrieval Procedures	12
Mid-Test Measurements	14
End-of-Test Measurements.....	17
Chemical Analyses.....	17
Temperature	18
Testing for Differences in Mean Temperature	19
Testing for Differences in Temperature Range	20
Statistical Analyses of Growth Parameters	24
Results	25
Sediment Chemistry and Conventional Analyses	28
Tissue Chemistry.....	29
Initial Tissue Mercury	30
Mid-Test Tissue Mercury.....	30
End-of-Test Tissue Mercury.....	34

CONTENTS, *cont.*

	Page
RESULTS, <i>cont.</i>	
Mussel Growth	35
Mid-test Observations	36
End-of-test Growth	36
Comparisons Between Tissue Mercury Concentrations and Growth	38
Ancillary Observations	38
Discussion	39
Extent of Mercury Bioaccumulation	39
Sources of Bioavailable Mercury	47
Effects of Mercury Exposure	50
Effects of Temperature	53
Reference Station and Collection Site Concerns	55
Limits of Data Interpretation and Future Work	55
Summary and Conclusions	56
References	58

FIGURES

	Page
1A	The Nyanza site and the Sudbury River drainage basin in Middlesex County, Massachusetts..... 2
1B	White Hall Reservoir Impoundment Reference (Station #1) and Wood Street River Reference (Station #2)..... 3
1C	Sediments collected from Reservoir 2, the first major depositional area downstream of the Nyanza site, contained the highest concentrations of mercury at 55 mg/kg..... 4
1D	Saxonville Dam (Station #5)5
1E	Sherman Street Bridge (Station #6), Fairhaven Bay (Station #7), and Thoreau Street Bridge (Station #8). 6
2A	Mussel rack arrangement used in study: five mussels per mesh bags, seven bags per rack. 15
2B	Individual mesh bags showing procedure used to distribute animals. All bags of a common number were filled before any of the next number 15
3	<i>Elliptio complanata</i> showing the length, height, and width measurements made at the beginning and end of the test. 16
4	Average daily temperature 21
5	Boxplots showing the center and spread of the distribution of weekly temperature ranges (C) by station. The line within the box is the median value; the height of the box is equal to the interquartile difference, or IQD (the difference between the 3rd quartile and the 1st quartile of the data); the "whiskers" extend to the minimum/ maximum values, or a distance 1.5xIQD from the center, whichever is less. Extreme values falling outside the whiskers are indicated by horizontal lines 23
6	Initial and end-of-test tissue concentrations of total-, inorganic-, and methylmercury (ng/g-dry station (\pm 2 standard errors [SE])). Station 6 data are presented for comparative purposes only (open bar); they were not included in the statistical analyses. * = end-of-test concentration significantly different than initial concentration. 32
7	Initial and end-of-test tissue content of total-, inorganic-, and methylmercury (ng/g-dry) by station. Station 6 data are presented for comparative purposes only (open bar); they were not included in the statistical analyses. * = end-of-test concentration significantly different than initial concentration. 33

FIGURES, cont.

Page

8	Mussel growth rates (based on whole-animal wet weights), changes in tissue weight, changes in whole-animal length, and changes in shell weight by station. Station 6 data are presented for comparative purposes only (open bar); they were not included in the statistical analyses. *** = stations that form a statistically similar group (based on non-parametric ANOVA, and multiple range tests).....	40
9	Percent water (± 2 SE measured) in mussel tissues by station at the end of the test.....	41
10	Percent water vs. growth rate. Stations with similar mussel growth metrics are grouped.	42
11	Regression relationships for mussel tissue concentrations of total, methyl-, and inorganic mercury and mussel growth rates based on changes in whole-animal wet weights. Station 6 (S6) was excluded from these regressions.	44
12	Regression relationships for mussel tissue concentrations of total-, methyl-, and inorganic mercury and changes in mussel tissue dry weight. Station 6 (S6) was excluded from these regressions	45
13	Regression relationships for mussel tissue concentrations of total, methyl-, and inorganic mercury and overall increases in mussel shell length. Station 6 (S6) was excluded from these regressions.....	46
14	Relationship between mussel growth rates and temperature in the Sudbury River	54

TABLES

	Page
1	Sediment sampling and mussel deployment locations. Stations were either impoundment (I) or river (R) conditions. Approximate distance from suspected mercury source is provided 12
2	Mean shell (length, width, height, mm), whole-animal wet weight (g), and tissue weight (g-wet) measurements (\pm standard deviation) by station for animals at T ₀ (n=105) 13
3	Summary of temperature conditions by station during study period. 19
4	Results of Newman-Keuls Multiple Range Test on weekly temperature ranges 22
5A	Mussel measurement at the start of the test (initial) and after 42 days' (mid-test) exposure in the Sudbury River. Mussels in bags 1 and 2 were used..... 26
5B	Mussel measurement after 84 days exposure (end of test) in the Sudbury River. Mussels in bags 3 through 7 were used 27
6	Results of selected trace element analyses and conventional parameters for sediments collected from Whitehall Reservoir and the Sudbury River 29
7	Tissue mercury concentrations (\pm SD) in mussels collected from Lake Massesecum at the start of the test, growth, and tissue mercury concentrations by station for mussels after 84 days exposure in the Sudbury River..... 31
8	Results of correlation analyses (r values) on selected parameters. Bold numbers = significant correlation ($r_{crit}=0.707$; 95% confidence level) 37
9	Ranges in response for the different growth metrics measured at the end of the study. 38
10	Significant correlation coefficients. 39

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Introduction

Site History

The Nyanza Chemical Waste Dump Site (Nyanza) is the former location of several textile dye production companies near the Sudbury River in Ashland, Massachusetts (MA; Figure 1A), approximately 35 km west of Boston. Mercury and chromium were used as catalysts in the production of textile dyes from 1917 to 1978. Approximately 2.3 metric tons of mercury were used per year from 1940 to 1970 [JBF Scientific Corporation (JBF) 1972] with approximately 45 to 57 metric tons of mercury released to the Sudbury River during this period (JBF 1973). From 1970 until the facility closed in 1978, wastes were treated on site and wastewater was discharged to Ashland's town sewer system. These changes in waste management practices reduced the amounts of mercury released to the Sudbury River to between 23 and 30 kg per year. Since dye production stopped in 1978, the property has been leased to various light industries and commercial companies. The Nyanza site was added to the National Priorities List and declared a Superfund site in 1982.

Land along the Sudbury River ranges from semi-rural to urban-suburban. There are several impoundments, including Mill Pond and the Saxonville Dam Impoundment, behind intact or partially collapsed dams built for milling operations during the early 1900s (NOAA 1993). Below the Saxonville Dam, the river is primarily depositional and meanders through an extensive floodplain. Figures 1B through 1E detail the pathway of the Sudbury River from its inception near Cedar Swamp to its confluence with the Assabet River to form the Concord River. These figures also illustrate the various dams and bays associated with the Sudbury River.

Previous Investigations of the Sudbury River

Numerous studies have been conducted since 1970 to assess mercury contamination in the Sudbury River (JBF 1971, 1972, 1973; MA Division of Fish and Wildlife (DFW) 1977; MA Department of Environmental Quality Engineering (DEQE) 1980, 1986; Maietta 1990; U.S. Fish and Wildlife Service (USFWS) 1990; NUS 1992). The most intensive and thorough sampling was conducted as part of the remedial investigation for Operable Unit III (the Sudbury River and wetlands next to the site) in 1989 and 1990 (c.f., NUS 1992). The Operable Unit III sampling plan emphasized depositional areas of the Sudbury River, such as those near stream confluences or inside river bends. Sediments collected from Reservoir 2 (Figure 1C), the first major depositional area downstream of the Nyanza site, contained the

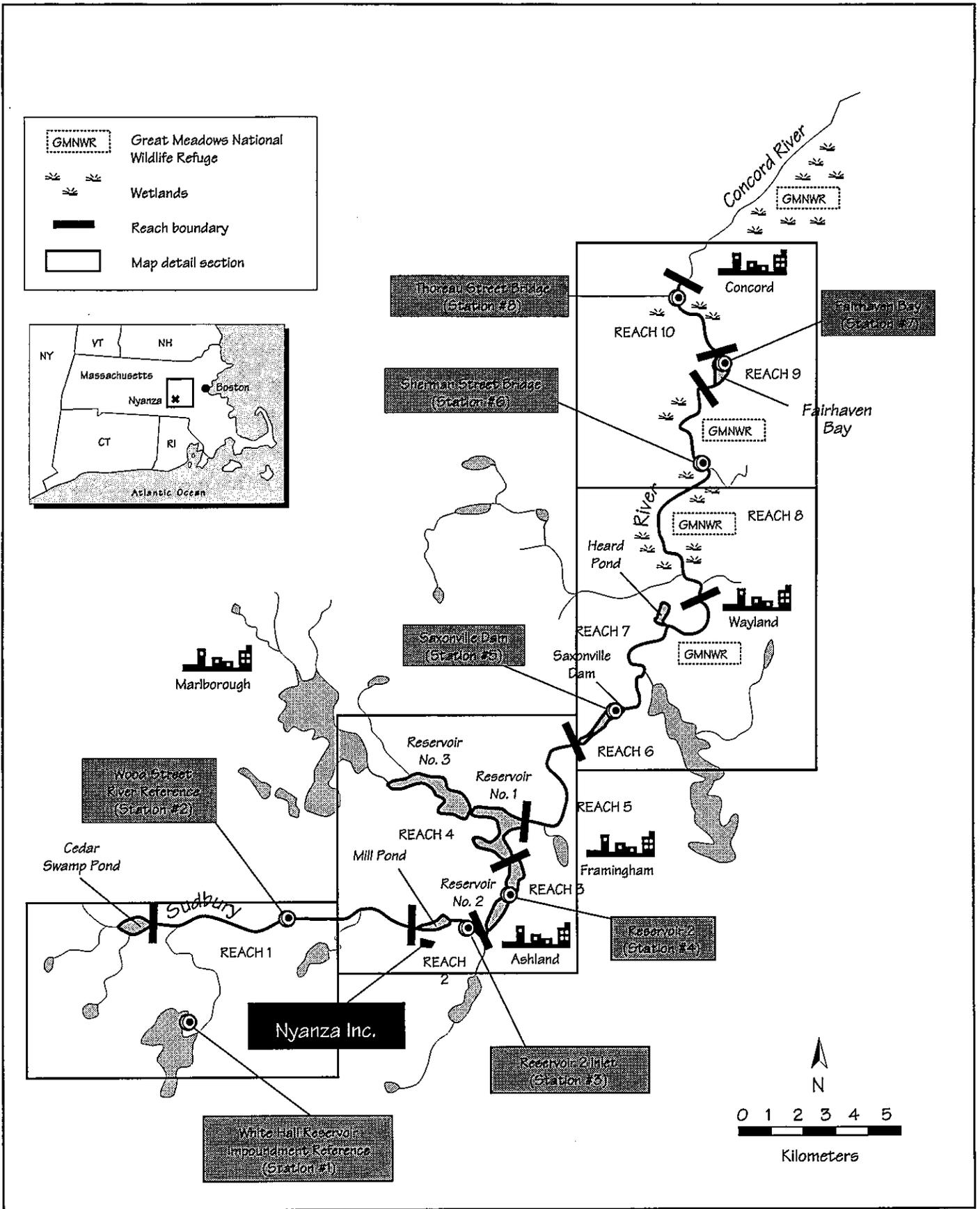


Figure 1A. The Nyanza site and the Sudbury River drainage basin in Middlesex County, Massachusetts.

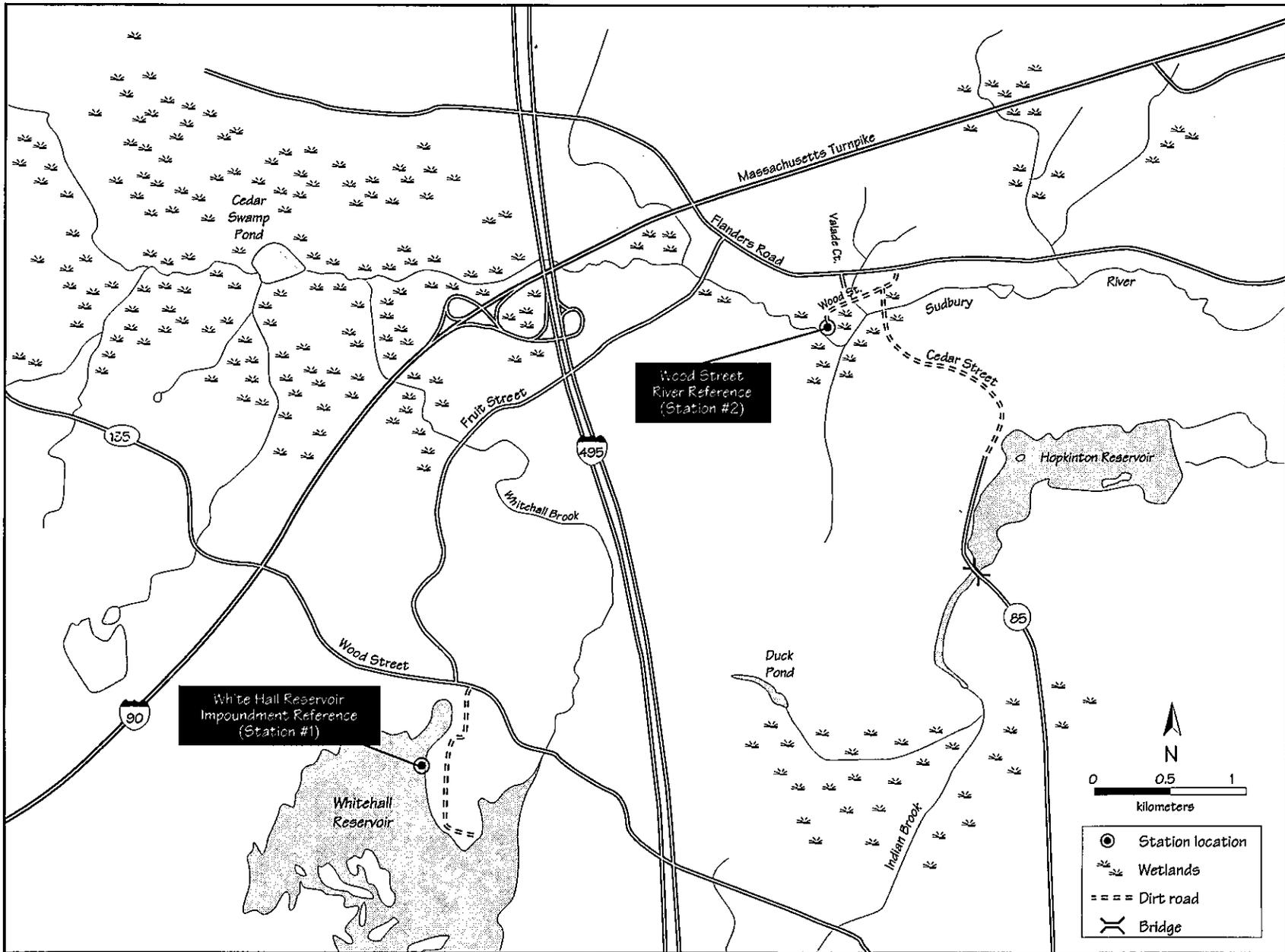


Figure 1B. White Hall Reservoir Impoundment Reference (Station #1) and Wood Street River Reference (Station #2).

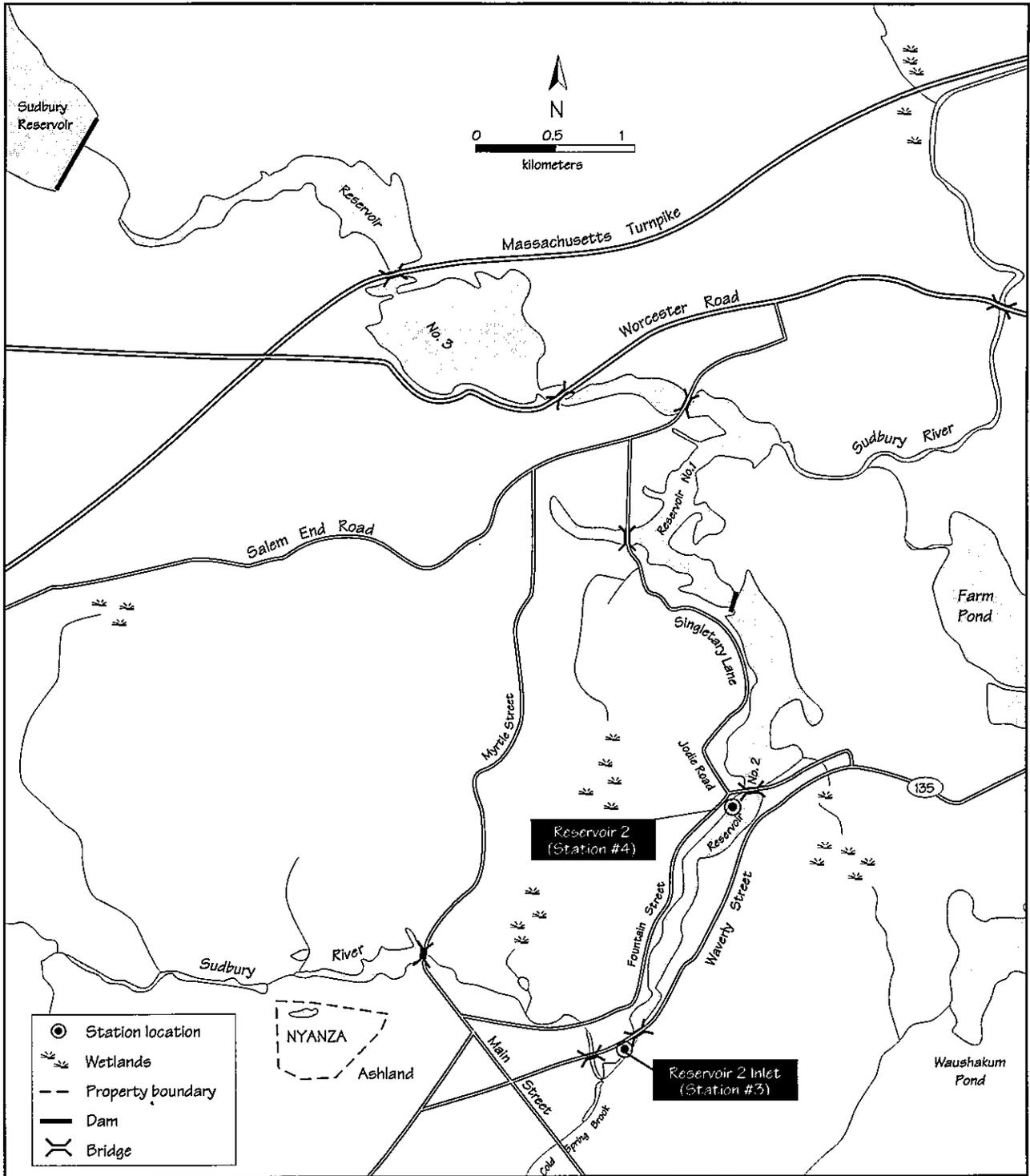


Figure 1C. Sediments collected from Reservoir 2, the first major depositional area downstream of the Nyanza site, contained the highest concentrations of mercury at 55 mg/kg.

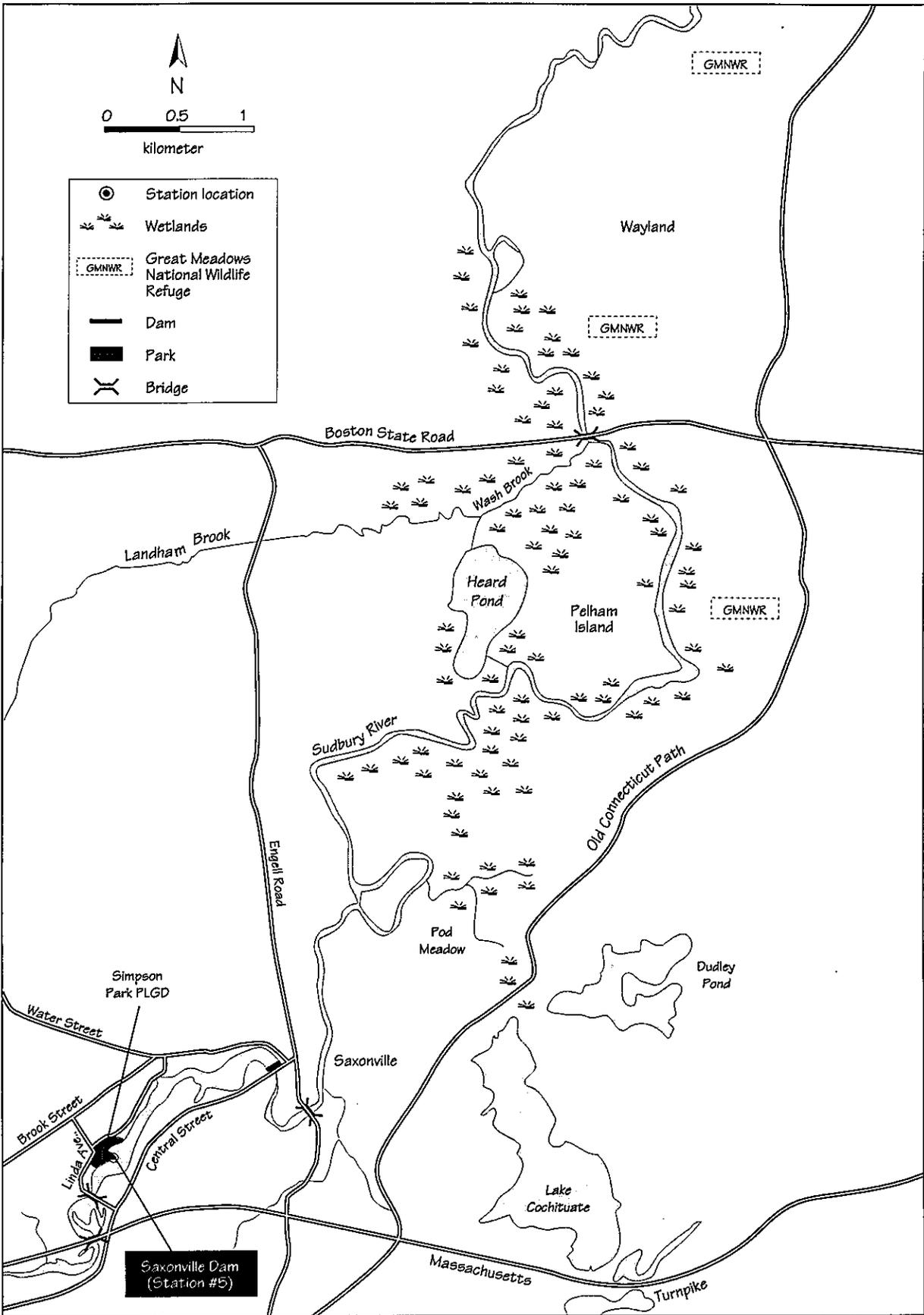


Figure 1D. Saxonville Dam (Station #5).

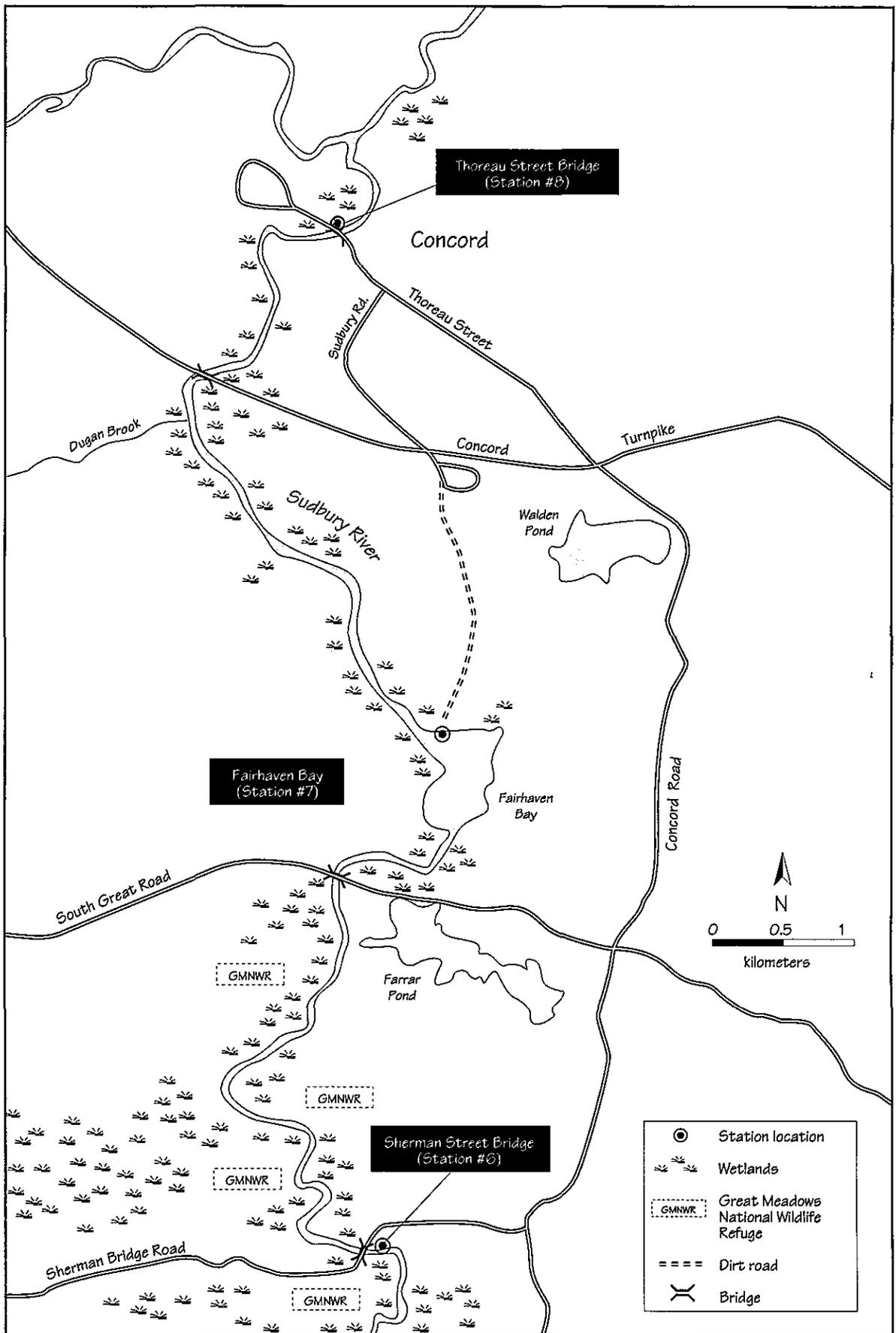


Figure 1E. Sherman Street Bridge (Station #6), Fairhaven Bay (Station #7), and Thoreau Street Bridge (Station #8).

highest concentrations of mercury at 55 mg/kg; sediments collected near the Concord River had mercury concentrations as high as 0.5 mg/kg. This latter concentration was approximately five times higher than observed in background sediments collected from Southville Pond, Sudbury Reservoir, and Reservoir 3, where mercury concentrations were all less than the detection limit of 0.1 mg/kg. Two background samples collected in the downstream section of Reach 1 had sediment mercury concentrations of 1.6 and 0.5 mg/kg (Figure 1A). These historical data suggest that mercury contamination extends throughout the Sudbury River.

Fish collected from the major reservoirs on the Sudbury River contained tissue concentrations of mercury as high as 12 mg/kg (MA DEQE 1980). Limited data are available regarding mercury in fish between 1971 and 1990. When the fish tissue data from 1971 (JBF 1972) are compared to 1990 data (NUS 1992) on a qualitative basis, it does not appear that there has been a substantial reduction in bioavailable mercury. In 1971, fish tissues contained approximately 10 mg/kg; in 1990, concentrations were detected as high as 8 mg/kg. Mercury was detected in 74 percent of the fish sampled between the site and Concord, Massachusetts 39 km away; a maximum concentration of 7.6 mg/kg was measured in fish collected from Reservoir 2 (NUS 1992). In Fairhaven Bay, approximately 33 km downstream of the Nyanza site, 93 percent of the fish sampled contained detectable concentrations of mercury with a maximum concentration of 3.2 mg/kg.

Since mercury appeared to be readily bioavailable within the Sudbury River system, the Massachusetts Department of Environmental Protection (MA DEP) and the U.S. Environmental Protection Agency (EPA) posted and maintained signs advising against consumption of fish from the river. Studies have been performed in the Sudbury River to evaluate mercury bioavailability and the geographical extent of the mercury contamination in biota. General trends have been established for the predominant form of mercury within sediments and the biological effects of exposure to mercury. However, additional data are necessary to specify the sources of mercury in sediments and biota, and to conclude whether environmental concentrations pose a substantial threat to aquatic resources.

NOAA's Involvement - This Study

To address these concerns and develop a scientifically defensible ecological risk assessment for the Sudbury River (Operable Unit-IV), EPA has elicited the help of other Federal agencies who have interests and concerns regarding natural resources and the improvement of impacted habitats. This study is one part of a larger, multi-agency program. Decisions

about the site will be based on the combined results from all of the studies. The findings presented in this report could be enhanced when supporting data are available.

Because habitats could be used for migration, spawning, and nursery activities, the lower reaches of the Sudbury River are of concern to NOAA, who acts for the U.S. Department of Commerce as a trustee for natural resources. Trust resources (e.g., anadromous fish) will have access to the Sudbury River as far upstream as the Saxonville Dam Impoundment (approximately 13.5 km from the Nyanza site) when proposed fish passage facilities on the Concord River become operational. Sections of the river above this dam provide habitat for the catadromous American eel. As part of EPA's joint effort, NOAA conducted a study to measure total- and methylmercury bioaccumulation and to estimate chronic effects on a resident bioindicator species. The freshwater mussel *Elliptio complanata* was selected to test effects from exposure to mercury-contaminated water, sediments, and food. Mussels were transplanted both to selected sites along the Sudbury River and a reference site in a distant reservoir. Our goal was to estimate mercury exposure and effects that could be used in EPA's quantitative ecological risk assessment. The information obtained in the mussel transplant study will also help NOAA assess potential impacts to trustee natural resources.

Use of Bivalves in Monitoring Programs

Resident and transplanted populations of both freshwater and marine bivalves have been used as biomonitors of environmental contamination for almost 30 years, although the use of marine bivalves like *Mytilus* spp. has been more extensive (Bedford et al. 1968; Godsil and Johnson 1968; Young et al. 1976; Eganhouse and Young 1978b; Phillips 1980; McMahon 1991). Monitoring resident bivalve populations for the accumulation of contaminants has been the most common form of biomonitoring, but the development of transplant methodologies has increased the use of caged animals and has facilitated synoptic measurements of bioaccumulation and bioeffects (Salazar and Salazar 1995). This in-situ approach combines the experimental control of laboratory studies with the environmental realism of field monitoring to assess site-specific contamination and effects. Freshwater and marine mussels are probably the most common bioindicators because they are ubiquitous, sedentary, and responsive to their environment on both micro- and macro-geographical scales (Green et al. 1985). Their hard shells make them easy to collect, handle, cage, and measure; their sedentary nature makes them excellent for transplant studies. Mussels can integrate and accumulate bioavailable contaminants at concentrations orders of magnitude above those found in other environmental media (e.g., water or sediment). Their soft tissues

can be analyzed to estimate contaminant uptake and exposure. Even though they can tolerate elevated contaminant concentrations, mussels respond to environmental perturbations by altering their physiology and metabolism. Growth is commonly used as a measure of effects because it provides an integration of many biological processes (Salazar and Salazar 1995).

E. complanata is a filter-feeding bivalve that is widely distributed in the streams of northeastern North America (Magnin and Stanczykowska 1971; Curry 1977; Heit et al. 1980). It is a long-lived, sedentary organism that comes into contact with both sediment and water during filtration activities (feeding and respiration), and it can accumulate trace metals, including mercury, and organic contaminants (Kauss and Hamdy 1991; Metcalfe-Smith et al. 1992). *E. complanata* has been used in a number of monitoring studies with both resident populations (Tessier et al. 1984; Creese et al. 1986; Hinch and Stephenson 1987; Servos et al. 1987; Russell and Gobas 1989; Metcalfe and Charlton 1990; Campbell and Evans 1991; Elder and Collins 1991; Metcalfe-Smith and Green 1992; Metcalfe-Smith et al. 1992) and transplanted animals (Curry 1977; Hinch and Green 1989; Day et al. 1990; Koenig and Metcalfe 1990; Kauss and Hamdy 1991; Langdon 1993). Freshwater bivalves are increasingly used as sentinels for trace metals, including mercury. The database associating bioaccumulation, bioeffects, and contamination in various environmental compartments such as water and sediment is making the results more useful in environmental assessments.

Bioaccumulation of contaminants by the freshwater mussel *E. complanata* has been used to evaluate several major waterways, including the Niagara, St. Clair, and St. Mary's rivers (Creese et al. 1986). In 1977, Curry (1977) proposed caged *E. complanata* as a practical approach for detecting organic trace contaminants in water after an exposure period of four to six weeks. Creese et al. (1986) presented a preliminary, standard, biomonitoring methodology for caged *E. complanata* based on their ability to accumulate environmental contaminants such as organochlorine compounds and heavy metals. Hinch and Green (1989) studied the effects of source and destination on growth and metal uptake in *E. complanata* reciprocally transplanted in Ontario lakes. Kauss and Hamdy (1991) used caged *E. complanata* to assess the availability of polynuclear aromatic hydrocarbons in sediment. Metcalfe-Smith et al. (1992) used two species of freshwater mussels (*E. complanata* and *Lampsilis radiata*) to evaluate the relationships between concentrations of metals in sediment and in mussel tissues. Metcalfe-Smith et al. (1992) also sought to determine whether mussels could provide useful information on the bioavailability of sediment-bound metals that is necessary to predict environmental effects. Metcalfe-Smith

(1994) found that *Elliptio complanata* demonstrated a broader response range to metal exposures (including mercury) than other species, suggesting that this species may be more sensitive to changes in pollution status.

Tessier et al. (1992) evaluated mercury bioaccumulation kinetics in *E. complanata* and suggested that this species concentrates mercury primarily from the water column (e.g., in the dissolved phase or as food particles). Others have found similar results with different bivalve species (Davies and Pirie 1978; Fowler et al. 1978; King and Davies 1987; Muncaster et al. 1990).

Investigators have shown that mercury is biologically available in marine, estuarine, and freshwater systems with availability partially dependent on the form of mercury present (Fowler et al. 1978; Riisgård and Hansen 1990). Mercury undergoes methylation and behaves differently than other "metals." Methylmercury, the form of particular concern, more closely resembles organic compounds than metals with respect to mobility, bioavailability, accumulation/depuration, and toxicity. Previous studies have shown a preferential accumulation of methylmercury over other forms of mercury (Fowler et al. 1978; Tessier et al. 1984; Mohlenberg and Riisgrd 1990; Metcalfe-Smith et al. 1992).

Relationships between the concentrations of mercury in water and in tissues have been demonstrated more consistently than those for sediment and tissue (Fowler et al. 1978; Tessier et al. 1994; Malley et al. in press). Although the concentrations of mercury measured in the water column are usually much lower than in bivalve tissues, the relationship between sediment and tissue mercury concentrations is equivocal, because sediment concentrations have been shown to be higher, lower, or the same as tissue concentrations (Bryan and Langston 1992; Metcalfe-Smith et al. 1992). Similarly, a number of studies have shown positive correlations between tissue burdens and sediment concentrations (Langston 1982 1986; Bryan and Langston 1992) while others have shown no relationship (Luoma 1977; Rubinstein et al. 1983; Lasorsa and Allen-Gil 1995).

Objectives

The primary objectives of the mussel transplant study conducted by NOAA were to:

- Demonstrate the extent of bioavailable mercury within the downstream reaches of the Sudbury River resulting from operations at the Nyanza site;
- Identify areas that could act as sources of mercury for transport downstream; and
- Determine the effect of mercury exposure on a resident species.

The data generated in this study can be used to identify areas that show significant mercury bioaccumulation and biological impacts as candidates for EPA remedial action.

Methods

Description of Study Area

Mussels (*E. complanata*) were transplanted to eight stations during this study: six stations in the river downstream of the Nyanza site, one reference station upstream (river reference) of the facility, and one reference station in White Hall Reservoir (reservoir reference; Figures 1A-1E). The White Hall Reservoir is connected to the Sudbury River by a small creek. EPA and other agencies participating in the investigation (NOAA, the National Biological Survey, the U.S. Geological Survey (USGS), the USFWS, and the Army Corps of Engineers) selected these reference stations and the impoundment stations. Each team member attempted to establish stations in the areas identified in the investigation.

We selected stations that represented a gradient of mercury contamination associated with sediments. Highest sediment mercury concentrations were expected at Station 3, approximately 2.5 km from the Nyanza site. Stations were located as far downstream as the Concord River, approximately 39 km from the site. Final station locations were situated near the shore (water depths 0.6 to 1.3 m) for easy access from the shoreline.

To compare mercury availability in free-flowing and impounded areas in the river, three stations plus one reference were located in impoundments (Stations 1, 4, 5, and 7) and three stations plus one reference were located in free-running segments of the Sudbury River (Stations 2, 3, 6, and 8). Stations 6 and 8 were located within wetland areas of the river in an attempt to assess availability of mercury where methylmercury production may be higher

(St. Louis et al. 1994). Station descriptions and distances from the suspected mercury source are provided in Table 1. Our Station 2 (Wood Street; river reference station) was situated upstream of the Cedar Street Bridge reference station used by other team members because of shallow water and high visibility of mussel racks near the Cedar Street Bridge.

Table 1. Sediment sampling and mussel deployment locations. Stations were either impoundment (I) or river (R) conditions. Approximate distance from suspected mercury source is provided.

Location	Stn.	Type	Distance
White Hall Reservoir (WHR)	1	I*	NA
Wood Street (WS)	2	R*	-4 km
Reservoir 2 Inlet (R2I)	3	R	2.5 km
Reservoir 2 (RES2)	4	I	4 km
Saxonville Impoundment (SXI)	5	I	13 km
Sherman Street Bridge (SSB)	6	R	26 km
Fairhaven Bay (FHB)	7	I	33 km
Thoreau Street Bridge (TSB)	8	R	39 km

NA = not available
 *: reference station (see text for station selection)

Mussel Collection, Processing, Deployment, and Retrieval Procedures

E. complanata was used as the test species because it is endemic to the Sudbury River and has a demonstrated ability to accumulate mercury in laboratory and field studies (Metcalf-Smith et al. 1992; Tessier et al. 1992). *E. complanata* were collected from Lake Massesecum, Bradford, New Hampshire on June 26, 1994, and deployed the next day. The USFWS suggested this lake be the source of uncontaminated mussels because it had no known contaminant point sources nor any resident endangered bivalve species. A large mussel population ensured minimal disturbance to the resident population. Large beds of mussels were found in shallow water (0.5 to 3 m) overlying a predominantly sand substrate. Species identification was confirmed by a USFWS bivalve expert. Scuba divers from the New England Aquarium Dive Club hand-picked approximately 1,500 individuals in the 50- to 70-mm length range. Mussels were sorted into groups of 1-mm increments by measuring shell length with vernier calipers; 840 mussels ranging from 57 to 63 mm in shell length were selected for the study because this was the minimum size range with the maximum number of mussels. Smaller mussels were targeted because they were expected to grow faster. Mussels were temporarily held in buckets filled with lake water. Fresh lake water was added to the buckets approximately every hour for six hours until all mussels were distributed among the mussel racks.

Each rack consisted of a square frame (made from three-quarter-inch plastic PVC pipe) to which seven mesh bags were attached, each containing five mussels (total 35 mussels per rack; Figure 2A). Tube-shaped, plastic mesh bags (four-inch diameter; 0.5-inch mesh size) were knotted at each end to prevent mussels from escaping. Mussels within the bags were separated from each other by constricting the mesh with plastic washers. A random-number table was used to distribute the 24 racks among the eight stations (three racks per station). A total of 105 mussels was deployed at each of the eight stations. Procedures described by Salazar and Salazar (1995) were used to ensure a statistically similar mussel size distribution among all racks. The mesh bags on each rack were numbered from 1 to 7. Starting with the smallest-sized class mussels, all bags of a given number were filled consecutively (e.g., all 24 racks had #1 bags filled before #2 bags). All mussels in each 1-mm size class were distributed before the next size class was used (Figure 2B).

Before placement in mesh bags, each mussel was measured for shell length, width, height (Figure 3), and whole-animal wet weight. Each shell measurement was made to the nearest 0.1 mm with vernier calipers; whole-animal wet weights were made to the nearest 0.01 g with a portable analytical balance. Mean values (\pm standard deviation [SD]) by rack and station measured at the start of the test are provided in Table 2. The field assessment procedures of Salazar and Salazar (1995) are based on shell length and whole-animal wet weight; the additional shell measurements were made in this study to provide background information on this species of freshwater mussels. At the start of the test, there were no statistical differences in mussel lengths or whole-animal wet weights among the individual racks, or the groups of three racks randomly selected for each station ($\alpha = 0.05$).

Table 2. Mean shell (length, width, height; mm), whole-animal wet weight (g), and tissue weight (g-wet) measurements (\pm SD) by station for animals at T_0 ($n = 105$).

Station	Length ¹	Width ¹	Height ¹	Whole-Animal Wet Weight	Tissue Weight ²
1-WHR	59.5 (1.55)	15.2 (1.21)	30.7 (1.64)	15.15 (2.56)	3.87 (0.54)
2-WS	59.5 (1.69)	15.1 (1.14)	30.6 (1.61)	15.18 (2.38)	3.88 (0.51)
3-R2I	59.5 (1.50)	15.4 (2.24)	30.7 (1.33)	15.10 (2.00)	3.86 (0.42)
4-RES2	59.5 (1.45)	15.0 (1.11)	30.4 (1.45)	15.07 (2.23)	3.85 (0.47)
5-SXI	59.5 (1.55)	15.3 (1.22)	30.8 (1.30)	15.09 (2.15)	3.86 (0.46)
6-S5B	59.6 (1.43)	15.2 (1.11)	31.3 (3.37)	15.23 (2.17)	3.89 (0.46)
7-FHB	59.6 (1.40)	15.2 (1.17)	30.5 (1.35)	15.12 (2.03)	3.87 (0.43)
8-TSB	59.6 (1.42)	15.1 (1.13)	31.0 (1.47)	15.11 (2.23)	3.87 (0.47)

¹ See Figure 3 for measurement information.
² Tissue weights calculated from regression equation based on a subsample of 30 individuals; see text for further details.

Mussels prepared for field-deployment were held in racks overnight in Lake Massesecum. Racks were retrieved the next morning and mesh bags containing mussels were placed in coolers containing only ice and newspaper. (Newspaper was used to separate mussels from the ice.) The mussels were not held in water during transportation. Mussels were moved from New Hampshire to Massachusetts by automobile. Deployment started at Station 8 and finished at Station 1. Mussels were out of water from 3 to 12 hours. Based on the current literature and discussions with Dr. Stansbery (Curator of Bivalve Mollusks, College of Biological Sciences, Ohio State University), this is the preferred method to transport *E. complanata* as it results in minimal stress for periods of up to 72 hours.

Before deployment at each station, mussel bags were removed from the cooler and attached to the PVC racks with an overhand knot and plastic cable ties. Each of three racks was tethered with a one-meter line to a cinder block and placed on the river bottom. All practical attempts were made to situate the caged mussels over soft (i.e., muddy) substrates; areas with large rocks or boulders were avoided.

Three sediment grabs were collected from each station for chemical analysis using a hand-held ponar. The grab was checked for integrity and completeness after sediment collection. Samples that contained rocks or other foreign material were discarded as were samples in grabs that did not completely close upon retrieval. On shore, the grab was released and sediments were deposited into a plastic tray. The top 5 cm of each sample was collected for analysis of selected chemicals and conventional parameters.

Mid-Test Measurements

Mid-test measurements were made after 42 days' exposure (August 8, 1994) to ensure that the mussel racks were undisturbed and not overly fouled; to determine whether the mussels were growing; to determine whether mussels were accumulating mercury in the soft tissues; and to obtain another datum point for rate of mercury accumulation.

Bags 1 and 2 were removed from each rack and all surviving mussels ($n \leq 10$) processed. Mussels were presumed missing (i.e., not dead) if their respective space in the bag was empty. Mussels were considered dead only if empty shells or gaping, unresponsive individuals were found. Mussels were held in tubs containing site water to help ensure that the internal shell chambers were completely filled prior to whole-mussel, wet-weight measurement. Whole-animal wet-weights, shell measurements (i.e., length, width, and height), and tissue wet-weights were determined for each animal. For each rack, tissues

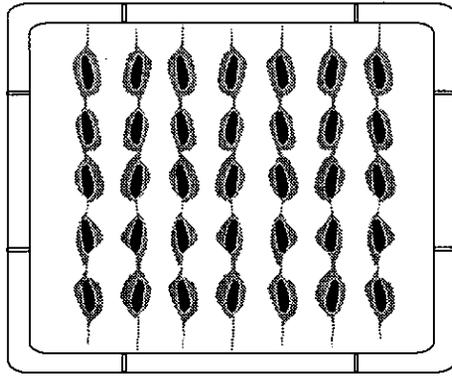


Figure 2A. Mussel rack arrangement used in study: five mussels per mesh bag, seven bags per rack.

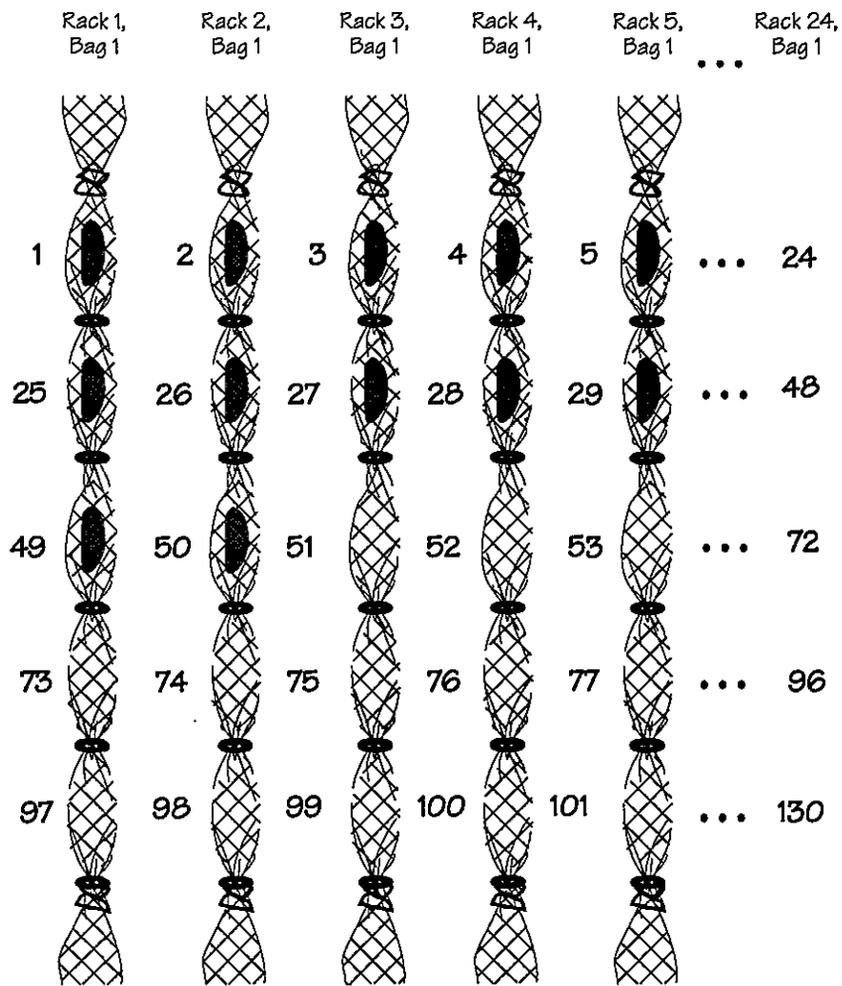


Figure 2B. Individual mesh bags showing procedure used to distribute animals. All bags of a common number were filled before any of the next number.

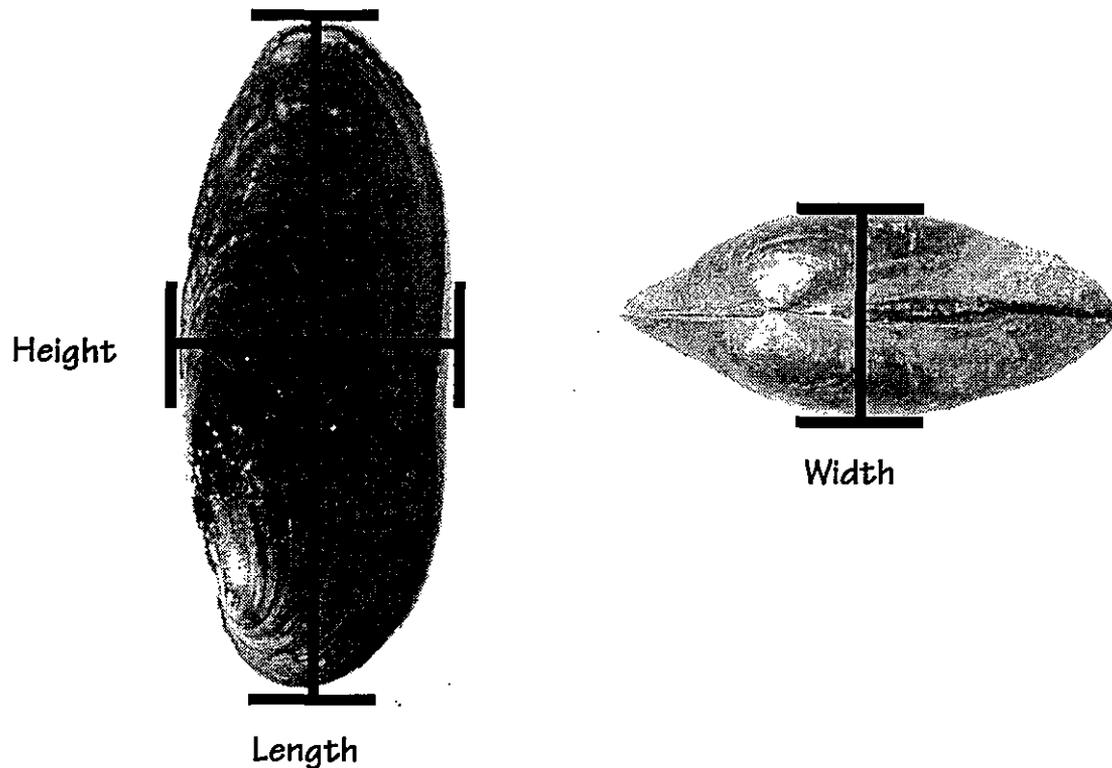


Figure 3. *Elliptio complanata* showing the length, height, and width measurements made at the beginning and end of the test.

from all mussels in Bags 1 and 2 ($n \leq 10$) were composited and chemically analyzed for total mercury. All equipment used during the shucking procedure was first decontaminated with a warm soap wash, then rinsed, acetone-rinsed, hexane-rinsed, air-dried, and wrapped in foil.

During the mid-test measurements, high mortality (>50 percent) was noted for animals transplanted at Station 6 (Sherman Street Bridge). Dissolved oxygen concentrations were suspected to be low at the original deployment location due to the high density of aquatic vegetation in the immediate area and the presence of a sulfur odor. The State of Massachusetts confirmed that episodes of low dissolved oxygen occurred during the summer in the area of the Sherman Street Bridge (Goldman Environmental Consultants, Inc. 1994). After retrieving Bags 1 and 2, the three racks were relocated approximately 50 m upstream in a less vegetated area.

End-of-Test Measurements

The mussel racks were retrieved on September 18, 1994, after 84 days' exposure. The mesh tubes containing mussels were removed from the arrays and placed in coolers containing crushed ice and newspaper; again using newspaper to separate the mussels from the ice. Mussels were measured according to methods previously described. Decontamination procedures were the same as at mid-test. Tissues from all surviving mussels for each rack (for all stations except Station 6: minimum of 19; maximum of 25 mussels) were pooled and frozen before chemical analysis. This procedure provided three replicates at all sites except Station 6 where only two replicates were available due to high mortality. A minimum of eight mussels were used in the composites for Station 6.

Chemical Analyses

The 24 sediment samples (three replicates for each of eight stations) collected during mussel deployment were analyzed for selected metals (antimony, arsenic, cadmium, chromium, lead, and mercury) and conventionals (total solids, total organic carbon, and grain size). The metals selected for analysis were the same as those analyzed by other agencies evaluating Sudbury River sediments. Antimony, arsenic, and lead were analyzed by graphite furnace atomic absorption spectrophotometry (GFA), cadmium and chromium were analyzed by inductively coupled argon plasma (ICP), and mercury by cold vapor atomic absorption (CVA). All metals analyses and grain-size determinations were conducted according to Puget Sound Estuary Program (PSEP) protocols (PSEP 1989). Total organic carbon (TOC) was analyzed according to the procedure provided in Plumb (1981) and total solids according to U.S. EPA Method 160.3 SM 2540 B (APHA/AWWA/WEF 1992).

Mussel tissues were analyzed for total mercury and methylmercury concentrations according to the methods provided in Bloom (1989, 1992) and Bloom and Fitzgerald (1988). Initial total and methylmercury concentrations in mussel tissues before deployment in the Sudbury River were estimated by measuring a subsample of 30 mussels (61.3 to 63.8 mm in length; ten mussels in each of three replicates) collected from Lake Massesecum. Mid-test mussels were analyzed only for total mercury; end-of-test analyses included both methyl- and inorganic mercury. Methylmercury was analyzed in 50- μ l aliquots of potassium hydroxide digest by aqueous-phase ethylation, isothermal gas-chromatograph separation, and cold vapor atomic fluorescence (CVAFS) detection (instrument detection limits of approximately 0.2 picograms). Total mercury was analyzed in 50- μ l aliquots of acid digest by SnCl_2 reduction, dual gold amalgamation, and CVAFS detection. Detection limits for total and

methylmercury were 0.0005 and 0.0002 $\mu\text{g/g}$, respectively. The dry fraction material of the samples was determined by drying an aliquot (approximately 5 grams) overnight at 105°C in aluminum drying pans. Inorganic mercury concentration was calculated as the difference between total and methylmercury:

$$\begin{aligned} \text{Inorganic mercury concentration (ng/g)} &= \text{total mercury concentration (ng/g)} - \\ &\text{methylmercury concentration (ng/g)} \end{aligned} \quad \text{Equation (1)}$$

The total and methylmercury content were determined for all tissue samples by the following equation:

$$\text{Content (ng)} = \text{Concentration (ng/g)} * \text{Animal Weight (g)} \quad \text{Equation (2)}$$

Inorganic mercury content was calculated as the difference between total- and methylmercury:

$$\begin{aligned} \text{Inorganic mercury content (ng)} &= \text{total mercury content (ng)} - \text{methylmercury} \\ &\text{content (ng)} \end{aligned} \quad \text{Equation (3)}$$

The content information can be used to determine whether growing mussels have accumulated mercury, since the overall mercury concentrations (ng/g-dry weight) may actually decrease in fast-growing individuals due to growth dilution. Salazar and Salazar (1995) and Riisgård and Hansen (1990) have shown that faster-growing, smaller animals take up more contaminants, even though tissue concentrations decrease. Therefore, mercury content provides data on net uptake or depuration and was used in this study to determine whether mussels transplanted in the Sudbury River for 84 days contained more mercury than they did at the onset of the study.

Temperature

Water temperature conditions at each station were recorded at 24-minute intervals (i.e., 60 observations per day) from June 26, 1994 to September 16, 1994 using one in-situ computerized data logger per station (HoboTemp, Onset Instruments). Data were downloaded from the logging devices using the instruments' data recovery software. Temperature data for Station 1 (reference station) were recorded only from June 26, 1994 to July 13, 1994, due to a malfunction in the temperature recording device. Data for this station was not included in the analysis.

Water temperature conditions were fairly similar with short- and long-term cycles during the first half of the study and declining temperatures after the first week of August. Minimum and maximum temperatures are summarized in Table 3. Apparent differences between upstream and downstream stations, and between river and impoundment stations were observed. These differences were investigated using statistical approaches to test two primary hypotheses:

1. Is the mean temperature different across stations, and
2. Is the range of temperatures different across stations?

Table 3. Summary of temperature conditions by station during study period.

Station	Date Range	Minimum—Maximum (C)
1	6/26 - 7/13/94	22.7 - 31.5
2	6/26 - 9/17/94	13.2 - 26.9
3	6/26 - 9/17/94	14.8 - 28.1
4	6/26 - 9/17/94	18.1 - 29.6
5	6/26 - 9/17/94	16.5 - 29.4
6	6/26 - 9/17/94	15.1 - 29.0
7	6/26 - 9/17/94	15.6 - 30.0
8	6/26 - 9/17/94	15.6 - 30.0

The water temperatures measured during the study are within the natural range for *E. complanata* in the northeastern United States (Stansbery personal communication 1994), and are not expected to be a significant factor for either bioaccumulation or growth in this species. However, these data were subjected to a statistical evaluation to investigate the presence of any trends. The two hypotheses are addressed separately in the following sections.

Testing for Differences in Mean Temperature

The temperature series at Stations 2-8 displayed similar patterns with daily and seasonal cycles, as well as both short and long-term trends. These series showed very strong *autocorrelations* (a measure of the dependence between observations of the same series). The standard analysis of mean differences (e.g., t-test) requires independent observations. Therefore, the data from these series required transformation and subsampling to produce an uncorrelated series which would adequately summarize the data.

The data sets were reduced to daily mean temperatures to reduce both the internal variability and autocorrelation of each temperature series (Figure 4). The series of daily means for all stations displayed very similar patterns. Each series of daily means (each of length 82) exhibited autocorrelation beyond 20 lags. Each of the seven stations which had sufficient temperature data was paired with every other station resulting in 21 station pairs. An independent sample of daily mean differences of the stream temperatures was associated with each of these pairs. The pattern of these differences indicate whether one station is consistently warmer than another; if the differences are not distinguishable from zero, then the two stations can be said to have similar daily mean temperatures. The test of the hypothesis for differences in mean temperature was accomplished by comparing each independent sample of differences between two stations to zero via a one-sample *t*-test (using a two-tailed α -level of 0.05). The results indicate that the mean temperatures were significantly different between most stations, with the exception of Stations 4 and 8, Stations 5 and 6, Stations 5 and 7, and Stations 5 and 8. The order of the mean temperatures was as follows:

Sta 2 (WS) < Sta 3 (R2I) < Sta 6 (SSB) < Sta 5 (SXI) < Sta 7 (FHB) < Sta 8 (TSB) < Sta 4
(RES2).

This temperature pattern is consistent with water residence times in a river system. Upstream stations (shorter water residence times) are cooler than the downstream stations, and stations in impoundments (i.e., Stations 4, 5, and 7) are generally warmer than faster flowing river stations.

Testing for Differences in Temperature Range

E. complanata is highly adaptable and can readily acclimate to changes in temperature; its natural habitat ranges from the Great Lakes area to the Gulf of Mexico and it therefore naturally experiences a wide temperature range (Stansbery personal communication 1994). To assess the effects of environmental conditions on growth in *E. complanata*, we evaluated temperature ranges over periods of one week. This time interval was selected because: seven days is a manageable time period, as opposed to comparisons based on an hourly or daily basis, it is expected to have some biological relevance, and it is a common interval used to measure changes in environmental conditions and growth in aquatic organisms.

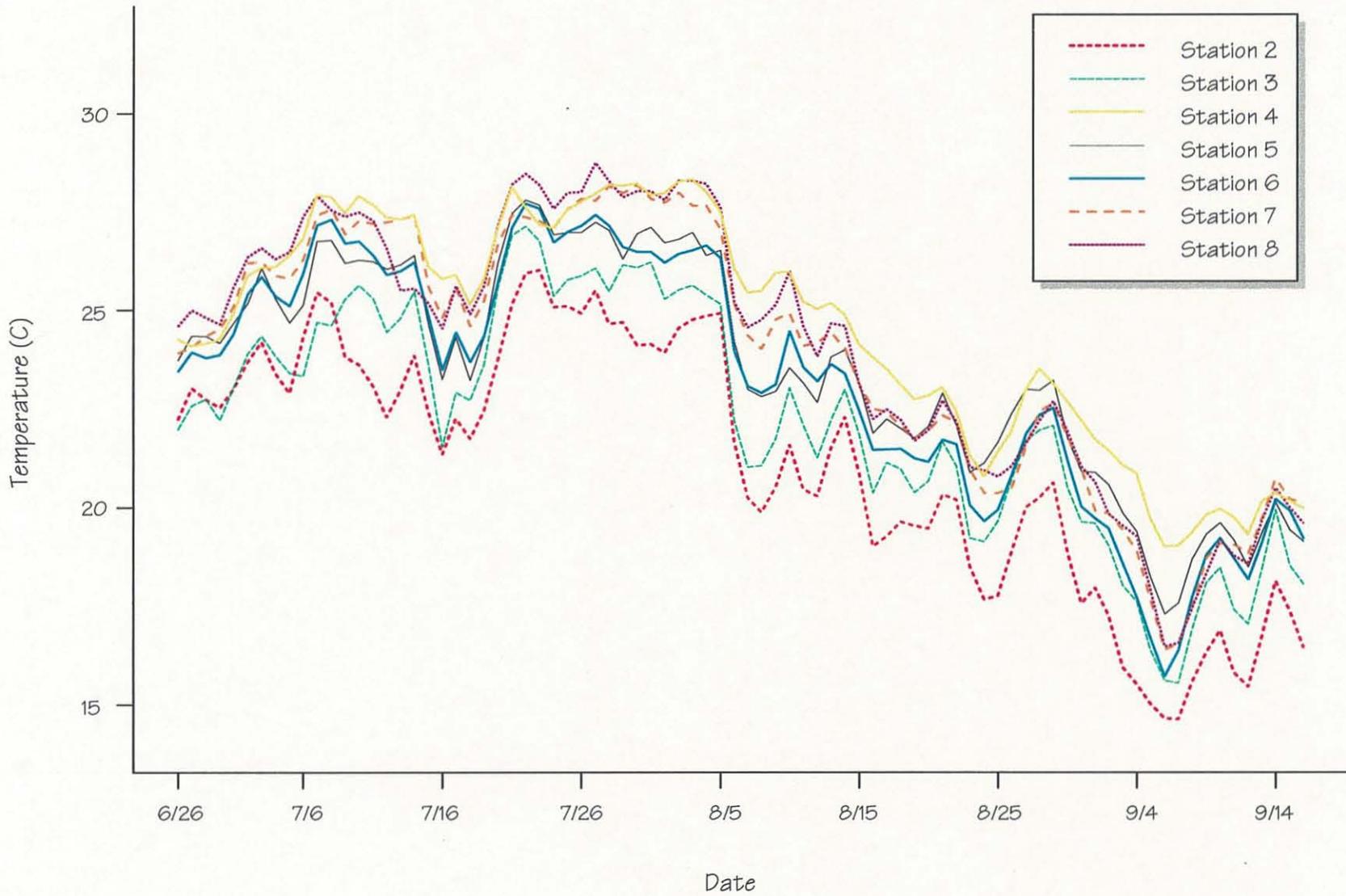


Figure 4. Average Daily Temperatures



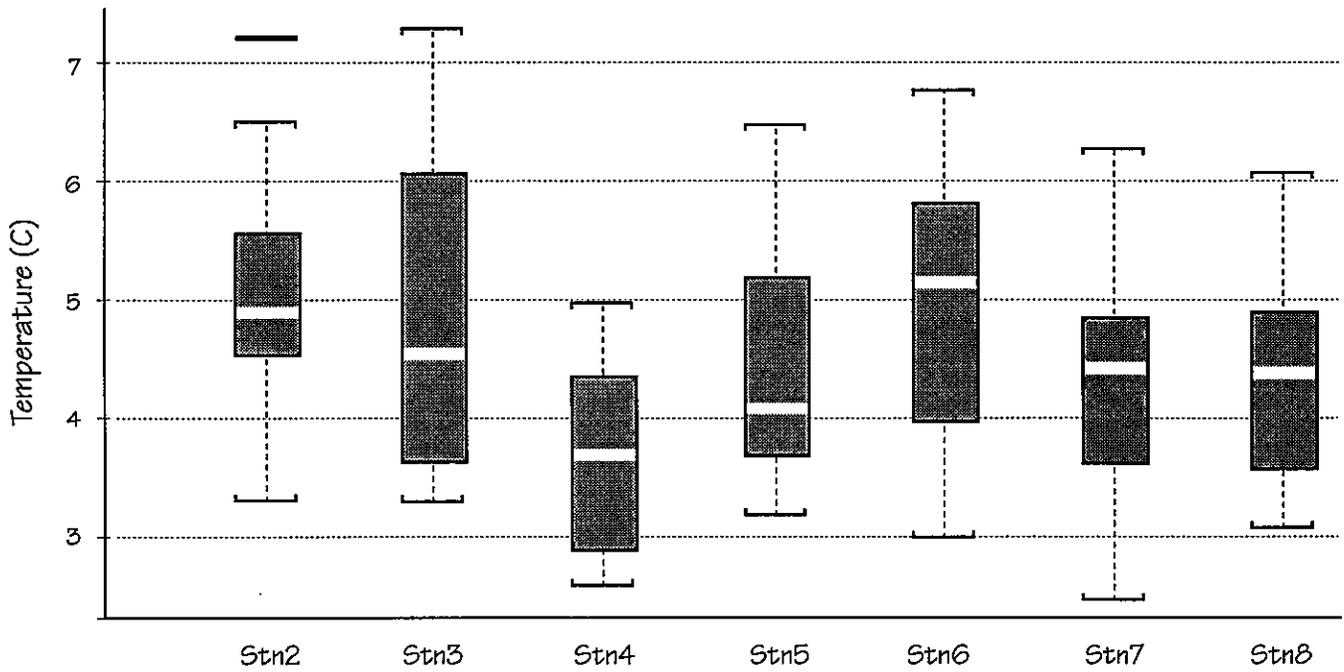


Figure 5. Boxplots showing the center and spread of the distribution of weekly temperature ranges (C) by station. The line within the box is the median value; the height of the box is equal to the interquartile difference, or IQD (the difference between the 3rd quartile and the 1st quartile of the data); the "whiskers" extend to the minimum/maximum values, or a distance 1.5xIQD from the center, whichever is less. Extreme values falling outside the whiskers are indicated by horizontal lines.

Statistical Analyses of Growth Parameters

Growth of individual mussels was measured in this study. Individuals were identified by rack position and measured at the beginning and end of the study. Growth can be estimated from a variety of mussel metrics. In this study, mussel growth was calculated from changes in whole-animal wet weight, shell length, shell weight, and tissue weight. Changes in whole-animal wet weight and length provide integrated measures of animal response. The error associated with the whole-animal wet-weight measurement is primarily due to air within the shell cavity that could add a low bias to the measurements. The error associated with the length measurement is uncertainty in locating the longest axis. The researchers minimized initial variability within and among stations by selecting mussels within a very narrow size range.

Changes in tissue weights can also be used as an estimate of mussel growth. Determining soft-body wet-weights is a destructive process. Thus, the initial tissue weight measurement could only be estimated from the following regression equation generated for the subsample of 30 individuals measured at the start of the test:

$$\text{Initial tissue weight (g-wet)} = 0.21 (\text{whole-animal wet-weight}) + 0.66 \quad \text{Equation (4)}$$

The error associated with using end-of-test tissue weight as an estimate of growth is primarily due to not knowing the exact tissue weight of the individuals before deployment.

Shell weight also provides a measure of animal growth. However, as with tissue weights, only end-of-test shell weights can be obtained because this is a destructive process. The following regression equation was generated from the subsample of 30 individuals measured at the start of the test and used to estimate initial shell weights for the transplanted individuals:

$$\text{Initial shell weight (g-wet)} = 0.44 (\text{whole-animal wet-weight}) + 0.21 \quad \text{Equation (5)}$$

All mussel metrics were recorded and analyzed; whole-animal wet weight, tissue weight, and shell weight were the metrics with the greatest potential for identifying stressed animals. To reduce variability attributable to water and facilitate comparisons with current literature, the dry-weight data were used throughout our analyses. Both dry- and wet-weight tissue mercury data are provided in this report. The wet- to dry-weight conversions were made using the percent-dry fraction data provided by the analytical laboratory. There

was a very high, significant correlation between wet and dry tissue weights ($r^2=0.97$, $\alpha=0.05$).

Mussels selected for deployment were between 57 and 63 mm in length. At the start of the test, mussels were sorted by length (to the nearest 0.1 mm), weighed (to the nearest 0.01 g), and distributed among the mesh bags. An analysis of variance (ANOVA; n = number of arrays) was used to ensure even distribution within arrays. No statistical difference was found in the distribution of mussels among the racks. Following the rack-by-rack analysis, the data for all mussels assigned to a station were pooled and re-analyzed on a station-by-station basis.

Growth rates (mg/wk) were calculated for individuals according to the following equation:

$$\text{Growth rate (mg/wk)} = \frac{\text{weight}_{(f)} - \text{weight}_{(i)}}{\text{number of weeks}} \quad \text{Equation (6)}$$

All data sets were analyzed for homogeneity in variances (Zar 1974) before conducting ANOVAs and Duncan's multiple range test (NW Analytical StatPak, Ver. 4.1) to determine differences among stations. If data did not meet the requirements for parametric analyses, the non-parametric equivalents were used (i.e., Kruskal-Wallis non-parametric ANOVA and Dunn's Multiple Comparison Test). At no station was there a significant difference among the three replicate racks. This allowed pooling of the data for each station and analyses on a station-by-station basis. A correlation analysis was run between selected variables to help identify trends and potential relationships. All statistical analyses were run at the 95-percent confidence level.

Results

Overall, the test was considered successful because all mussel racks were retrieved. Results for mid- and end-of-test mussel measurements are presented in Tables 5A and B. End-of-test survival ranged from 83 to 95 percent at all sites except Station 6, where it was 36 percent; growth rates for these mussels were significantly lower than other downstream sites. Mussels at Stations 7 and 8 had the greatest increases in tissue weight, shell length, and whole-animal wet weight. Survival at Stations 1 and 2 was 83 and 91 percent, respectively; animals at these stations had negative changes in whole-animal wet weight suggesting no growth (Table 5B). Since mussels at Stations 1 and 2 appeared to be in poor condition and the mercury concentrations were much higher than expected, they did not qualify as reference stations, and the planned comparisons could not be made. These data

Table 5A. Mussel measurements at the start of the test (initial) and after 42 days' (mid-test) exposure in the Sudbury River. Mussels in Bags 1 and 2 were used.

Station	Initial T ₀ (n=30)		Mid-test		Increase		%	N	%
	Mean	±SD	Mean	±SD	Mean	±SD	Moisture		Survival
Tissue Weights (g-wet)									
1-WHR	3.47	0.27	3.72	0.64	0.25	0.57	86.9	23	77%
2-W5	3.58	0.40	3.49	0.58	-0.09	0.46	87.6	29	97%
3-R2I	3.56	0.37	3.85	0.45	0.29	0.42	86.9	30	100%
4-RES2	3.55	0.37	4.84	0.48	1.29	0.43	84.9	22	73%
5-SXI	3.66	0.36	5.11	0.55	1.44	0.52	84.3	30	100%
6-SSB	3.62	0.26	4.25	0.67	0.63	0.77	87.2	13	43%
7-FHB	3.69	0.39	5.36	0.71	1.67	0.44	82.9	24	80%
8-TSB	3.62	0.33	5.70	0.89	2.08	0.66	82.5	27	90%
Whole-Animal Length (mm)									
1-WHR	57.8	1.10	57.8	1.10	0.0	0.37		23	77%
2-W5	57.9	0.83	57.9	0.89	-0.1	0.20		29	97%
3-R2I	57.8	0.70	57.9	0.74	0.0	0.25		30	100%
4-RES2	57.9	0.73	59.6	1.22	1.6	0.66		22	73%
5-SXI	58.1	0.72	59.3	1.08	1.2	0.70		30	100%
6-SSB	58.2	0.68	58.7	1.04	0.5	0.26		13	43%
7-FHB	58.0	0.66	59.0	1.08	1.0	0.94		24	80%
8-TSB	58.0	0.65	59.5	1.33	1.6	1.14		27	90%
Whole-Animal Wet-Weight (g)									
1-WHR	13.27	1.3	13.22	1.33	-0.05	0.41	-24	23	77%
2-W5	13.77	1.9	13.37	1.74	-0.40	0.34	-64	29	97%
3-R2I	13.68	1.7	13.68	1.69	0.00	0.30	0	30	100%
4-RES2	13.64	1.7	15.38	1.75	1.74	0.74	252	22	73%
5-SXI	14.16	1.7	15.69	2.03	1.53	0.78	255	30	100%
6-SSB	13.95	1.2	14.72	1.31	0.77	0.35	15	13	43%
7-FHB	14.28	1.8	16.14	1.98	1.86	0.96	281	24	80%
8-TSB	13.93	1.6	16.04	1.98	2.11	1.21	318	27	90%
Growth Rate (mg/wk) ¹									

Table 5B. Mussel measurements at the start of the test (initial) after 84 days' exposure (end of test) in the Sudbury River. Mussels in Bags 3 through 7 were used.

	Initial (n=75)		Exposed		Increase		%	N	%
	Mean	±SD	Mean	±SD	Mean	±SD	Moisture		Survival
Tissue Weights (g-wet)									
1-WHR	4.03	0.54	4.29	0.7	0.26	0.49	88.4	62	83%
2-WS	4.00	0.50	3.99	0.6	-0.01	0.49	87.1	68	91%
3-R2I	3.98	0.39	4.26	0.5	0.28	0.50	86.5	70	93%
4-RES2	3.98	0.46	5.79	0.8	1.81	0.61	85.4	71	95%
5-SXI	3.94	0.47	5.09	0.9	1.15	0.92	84.9	65	87%
6-SSB	4.00	0.48	5.33	0.7	1.33	0.68	84.3	27	36%
7-FHB	3.94	0.43	6.26	1.1	2.32	1.15	82.8	66	88%
8-TSB	3.97	0.49	6.87	1.2	2.90	1.15	81.3	65	87%
Whole-Animal Length (mm)									
1-WHR	60.2	1.2	60.5	0.3	0.2	0.29		62	83%
2-WS	60.1	1.6	60.2	0.1	0.1	0.26		68	91%
3-R2I	60.2	1.1	60.4	0.2	0.3	0.27		70	93%
4-RES2	60.1	1.2	62.0	1.9	1.9	1.14		71	95%
5-SXI	60.3	1.1	62.1	1.8	1.9	1.65		65	87%
6-SSB	60.2	1.2	60.6	0.4	0.3	0.64		27	36%
7-FHB	60.2	1.0	62.4	2.2	2.1	1.35		66	88%
8-TSB	60.1	1.2	62.8	2.7	2.6	1.76		65	87%
Whole-Animal Wet-Weight (g)									
1-WHR	15.90	2.56	15.62	2.43	-0.28	0.57			
2-WS	15.74	2.34	15.43	2.29	-0.31	0.49			
3-R2I	15.67	1.82	15.80	1.62	0.13	0.44			
4-RES2	15.64	2.16	17.84	2.16	2.20	1.28			
5-SXI	15.46	2.21	17.62	2.60	2.16	1.99			
6-SSB	15.74	2.26	16.25	2.37	0.51	1.75			
7-FHB	15.46	2.02	18.82	2.17	3.36	1.86			
8-TSB	15.59	2.29	19.26	2.40	3.67	1.51			
Growth Rate (mg/wk) ¹									
1-WHR							-21	62	83%
2-WS							-38	68	91%
3-R2I							23	70	93%
4-RES2							185	71	95%
5-SXI							198	65	87%
6-SSB							46	27	36%
7-FHB							270	66	88%
8-TSB							303	65	87%

¹Growth Rates based on changes in whole-animal wet-weight

will be included in the statistical analyses, but the results must be interpreted with caution. Although the data for Station 6 will be included for completeness, they were not included in statistical comparisons because of high mortality, low growth rates, and the station relocation at mid-test.

Sediment Chemistry and Conventional Analyses

Mean total mercury concentrations in sediments ranged from 0.07 at Station 7 (Fairhaven Bay) to 17.9 mg/kg-dry at Station 3 (Reservoir 2 Inlet). The second highest total mercury concentration, 5.4 mg/kg-dry, was measured in sediments from Station 5 (Saxonville Impoundment). Although the high measurement was over two orders of magnitude greater than the low measurement, sediments from six of the eight stations had total mercury concentrations ≤ 0.5 mg/kg (Table 6). Total-mercury concentrations in sediments collected from Reservoir 2 (Station 4) were very low. Elevated concentrations of chromium and lead were also detected in sediments, with the highest concentrations at Stations 3 (Reservoir 2 Inlet) and 5 (Saxonville Impoundment; Table 6).

Correlation analyses were run on the following metals measured in sediment: mercury, chromium, lead, and cadmium. These metals were selected for correlation analysis because measured concentrations in sediments exceeded concentrations known to produce adverse effects in aquatic organisms in other studies; mercury and chromium are the primary contaminants of concern associated with Nyanza. Results of the correlation analyses (Table 7) show a strong, significant association between mercury and chromium, with a correlation coefficient of 0.98 ($r_{0.05,(2),6}=0.707$). Mercury was not strongly associated with other metals, but there was a strong association between cadmium and lead ($r^2 = 0.91$). Both mercury and chromium concentrations were moderately associated with TOC concentration ($r^2 = 0.72$ and 0.77 , respectively). Lead and cadmium were weakly associated with TOC ($r^2 = 0.53$ and 0.5 , respectively).

The grain size analyses showed sediments varying in composition (Table 6). Although attempts were made to locate stations in similar substrate types, Stations 2, 4, 7 and 8 were predominantly sand (>80 percent sand) while the remaining stations were predominantly fines (61 to 80 percent silt and clay). TOC concentrations ranged from 1.62 to 11.7 percent (Table 6). The correlation analysis indicates a significant positive correlation between fines and TOC ($r^2 = 0.80$).

Table 6. Results of selected trace element analyses and conventional parameters for sediments collected from Whitehall Reservoir and the Sudbury River.

	1-WHR	2-WS	3-R21	4-RES2	5-SXI	6-SSB	7-FHB	8-TSB
Trace Elements (mg/kg-dry) ¹								
Mercury	0.17	0.11	17.9	0.17	5.4	0.5	0.07	0.36
Chromium	24.3	10.3	152.3	14	78	22.3	7.9	28
Lead	132.7	107	225	17.7	410	58.7	5.4	40
Antimony	U (0.4)	U (0.3)	1.4	U (0.2)	1.1	U (0.5)	U (0.2)	U (0.2)
Arsenic	5.9	3.7	12.2	3	11.9	8.1	9.2	10.7
Cadmium	U (0.8)	0.6	3.3	0.4	10	3.6	0.3	1
Physical Parameters (%) ²								
TOC	5.93	3.45	11.7	3.37	7.7	10	1.62	4.58
Total solids	22.87	43.97	18.57	49.43	19.28	18.98	65.98	38.79
Grain size								
Sand	17	82	30	80	38	34	90	85
Silt	70	14	58	16	46	42	6	10
Clay	10	3	10	2	14	22	4	5
U Undetected; concentration in parentheses equals the detection limit.								
¹ Concentrations were determined as the mean of three replicate samples.								
² Measurements were made on one sample only at each station, except for grain size at Station 6, determined as a mean of triplicate samples.								
Detection Limits (mg/kg)								
Cadmium	0.2							
Chromium	0.5							
Arsenic	0.1							
Lead	0.1							
Antimony	0.1							
Mercury	0.05							

Tissue Chemistry

All tissue chemistry data reported here (Table 7; Figures 6 and 7) represent the mean of three replicate samples and have been rounded to two significant digits. Although results in Table 7 are presented on both a wet- and dry-weight basis, only the dry-weight data are discussed in the text. The laboratory that performed the chemical analyses obtained a uniform homogenate. The laboratory conducted both duplicate analyses of the same digest, and duplicate digestions of a mussel composite. Their results indicate that the variance between replicates is similar to the variance associated with multiple extractions of a given sample (approximately 16 to 20 percent variability).

The laboratory quality assurance results provided for tissue analyses were within the specified control limits for this study. Analytical and injection replicate results for both total and methylmercury analyses indicated that results are within the acceptable limits of ± 35 relative percent difference (RPD). Samples were not affected by the total- or methylmercury detected in the method blanks, because all sample results were greater than five times the amount of contamination found in the corresponding method blank.

Standard Reference Materials (SRM) were analyzed for both total and methylmercury determinations on mussel tissue. All SRM percent recoveries for total mercury in the initial, middle, and final stages of the study fell within the control limits. The SRM percent recovery for methylmercury in the initial stage also fell within control limits. Methylmercury analysis was not conducted for samples collected in the middle stage. The SRM percent recovery for methylmercury in the final stage fell slightly below the control limits (86 percent vs. 92 percent).

Initial Tissue Mercury

Tissues of mussels collected from Lake Massesecum had mean total and methylmercury concentrations of 640 (± 103) and 140 (± 9.29) ng/g-dry, respectively (Table 7). These concentrations were much higher than expected for mussels collected from a relatively pristine lake. Methylmercury accounted for approximately 22 percent of the total mercury. Initial mean tissue total mercury content was 510 (± 125) ng; initial mean tissue methylmercury content was 110 (± 18.2) ng. Initial mean tissue inorganic mercury concentration was 500 ng/g-dry; initial mean tissue inorganic mercury content was 400 ng per mussel.

Mid-Test Tissue Mercury

After 42 days' exposure, mussels had mean tissue total mercury concentrations ranging from 330 to 930 ng/g-dry. The tissue total mercury concentrations decreased with distance from Nyanza (Table 7; Figure 6). Mean total mercury concentrations in mussel tissues at the reference stations (Stations 1 and 2) increased by approximately 110 to 140 ng/g-dry. Mean total mercury concentrations in tissues of mussels closest to Nyanza (Station 3) increased by about 290 ng/g-dry. Mean tissue total mercury concentrations decreased by 90 to 310 ng/g-dry for mussels at all other stations.

Table 7. Tissue mercury concentrations (\pm SD) in mussels collected from Lake Massesecum at the start of the test; growth and tissue mercury concentrations by station for mussels after 84 days' exposure in the Sudbury River.

Station	Growth Rate (mg/wk)	Total Hg (ng/g wet)	Methyl Hg (ng/g wet)	Total Hg (ng/g dry)	Methyl Hg (ng/g dry)	Inorganic Hg (ng/g dry)	Total Hg Content (ng)	Methyl Hg Content (ng)	Inorganic Content (ng)	% MeHg
Initial	-	120 (20)	25 (1.66)	640 (103)	140 (9.29)	500	510 (125)	110 (18.2)	400	22
Mid Test										
1-WHR	-24	99 (22.4)	-	750 (165)	-	-	370 (116)	-	-	-
2-WS	-64	96 (17.7)	-	780 (179)	-	-	330 (66.3)	-	-	-
3-R2I	0	120 (9.60)	-	930 (79.4)	-	-	470 (60.0)	-	-	-
4-RES2	252	84 (8.02)	-	550 (47.1)	-	-	400 (54.3)	-	-	-
5-SXI	255	85 (7.81)	-	550 (70.0)	-	-	440 (56.4)	-	-	-
6-SSB	15	67 (13.6)	-	520 (104)	-	-	310 (66.5)	-	-	-
7-FHB	281	63 (8.66)	-	370 (60.6)	-	-	330 (45.2)	-	-	-
8-TSB	318	58 (6.46)	-	330 (31.9)	-	-	320 (50.7)	-	-	-
End of Test										
1-WHR	-21	100 (5.43)	41 (4.19)	890 (85.5)	360 (46.7)	530	440 (70.4)	180 (28.5)	260	40
2-WS	-38	110 (17.3)	33 (5.41)	850 (71.9)	260 (44.3)	600	440 (90.5)	130 (28.0)	310	30
3-R2I	23	130 (5.53)	43 (3.89)	950 (33.3)	320 (29.6)	640	550 (73.1)	180 (25.2)	370	33
4-RES2	185	100 (26.3)	38 (2.08)	690 (228)	260 (24.8)	430	570 (140)	220 (33.4)	350	38
5-SXI	198	78 (5.40)	29 (6.47)	520 (56)	200 (49.8)	320	390 (64.5)	150 (31.0)	240	38
6-SSB	46	94 (26.6)	27 (3.96)	590 (127)	170 (42.8)	420	450 (108)	150 (25.5)	350	33
7-FHB	270	69 (8.95)	24 (2.44)	400 (51.1)	140 (11.0)	260	430 (92.5)	150 (31.2)	280	34
8-TSB	303	62 (5.96)	24 (0.81)	340 (35.7)	130 (3.5)	210	430 (59.5)	170 (20.1)	260	39
- = Not Measured or Not Applicable										

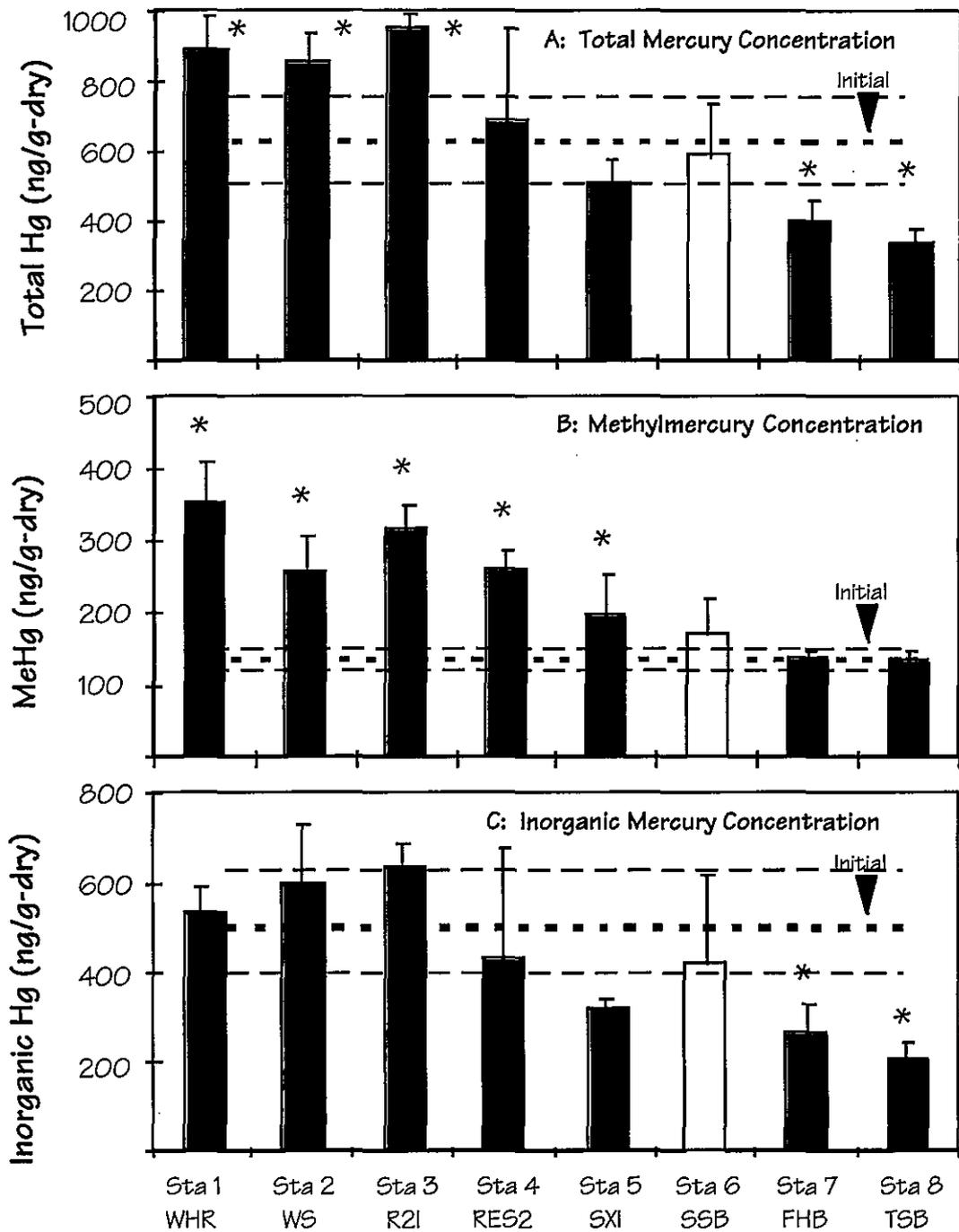


Figure 6. Initial and end-of-test tissue concentrations of total, methyl-, and inorganic mercury (ng/g-dry) by station ($\pm 2SE$). * indicates end-of-test tissue concentration significantly different than initial concentration. Station 6 data are presented for comparative purposes only (open bar); they were not included in the statistical analyses.

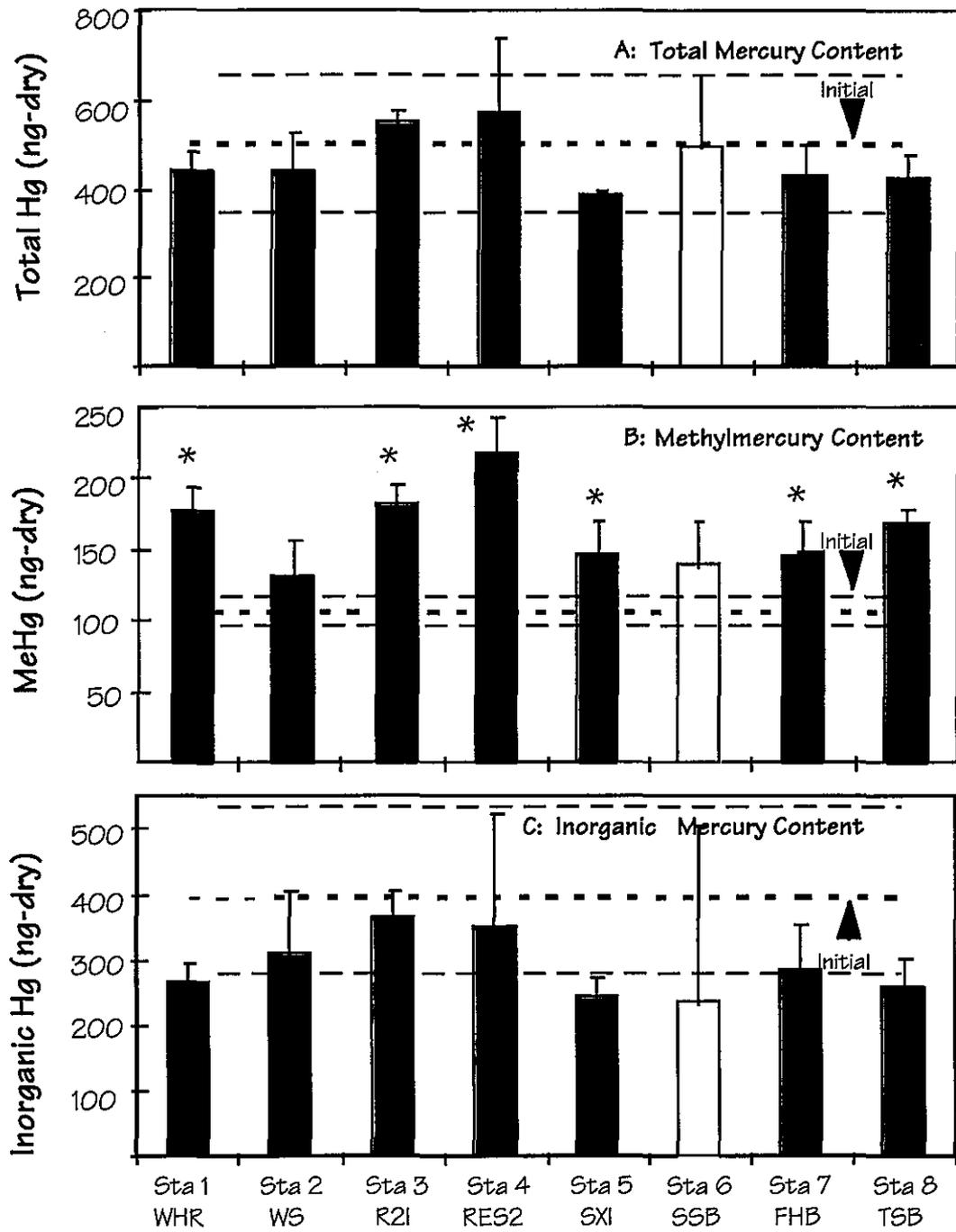


Figure 7. Initial and end-of-test tissue content of total, methyl, and inorganic mercury (ng-dry) by station (\pm 2SE). * indicates end-of-test tissue content significantly different than initial content. Station 6 data are presented for comparative purposes only (open bar); they were not included in the statistical analyses.

End-of-Test Tissue Mercury

The mean concentrations of total, inorganic, and methylmercury in mussel tissues decreased downstream (with distance away) from Nyanza (Figure 6). Compared to initial values, mean methylmercury content in mussel tissues increased at all stations, while mean inorganic content decreased and mean total content remained about the same (Figure 7).

Mean tissue total mercury concentrations ranged from 340 ng/g-dry at Station 8 to 950 ng/g-dry at Station 3. These concentrations were similar to mid-test tissue total mercury concentrations. The downstream gradient of decreasing tissue total mercury concentrations persisted through the end of the test (Figure 4). Mean tissue total mercury concentrations for mussels at the two reference stations (Stations 1 and 2) increased above the mid-test concentrations to final concentrations of 890 and 850 ng/g-dry, respectively. These concentrations were slightly lower than those measured in mussels deployed at Station 3, the station closest to Nyanza. Mean tissue total mercury concentrations were significantly higher at Stations 1, 2, and 3 at the end of the test than at the start; mean tissue total mercury concentrations at Stations 7 and 8 were significantly lower at the end of the test than at the start ($\alpha=0.05$). At the end of the test, the tissue total mercury concentrations for mussels at Stations 1, 2, and 3 (850 to 950 ng/g) were significantly higher than for mussels at Stations 7 and 8 (340 to 400 ng/g).

Mean tissue methylmercury concentrations ranged from 130 ng/g-dry to 360 ng/g-dry, with the lowest concentration measured in a mussel from Station 8 and the highest at Station 1. Tissue methylmercury concentrations generally paralleled those of total mercury (Figure 6): concentrations were significantly higher at Stations 1 through 4 at the end of test than at the start, and the tissue methylmercury concentrations for mussels at Stations 1 and 3 (320 to 360 ng/g) were significantly higher than measured in mussels at Stations 7 and 8 (130 to 140 ng/g).

The mean inorganic mercury concentration in tissues decreased for mussels at all stations downstream of Station 3 (Figure 6). Tissue concentrations of inorganic mercury for the reference mussels and Station 3 mussels were higher than the initial concentration, but this increase was not statistically significant. The tissue inorganic mercury concentrations at Stations 7 and 8 were significantly lower than at the start of the test.

The mean concentrations of total-, inorganic-, and methylmercury in tissues of mussels at Stations 1 and 2 (the reference stations) were not significantly different than the

concentrations measured in mussels transplanted at the station situated nearest Nyanza. The data suggest that mussels at Stations 1 and 2 were exposed to bioavailable mercury and thus may not be appropriate as reference animals.

The mean tissue content data indicate that there were no significant changes in the total amount of mercury within the mussel tissues (Table 7; Figure 7). Except for Stations 3 and 4, the total mercury content in all mussel tissues was slightly lower at the end of the test. Total mercury content increased slightly for mussels at Stations 3 and 4. Methylmercury content per mussel increased at all stations during the course of the test (Table 7; Figure 7). This increase was statistically significant for mussels at all stations except Stations 2 and 6, where the methylmercury content increased by 20 and 30 ng, respectively. For mussels at the other stations, the methylmercury content increased by 40 to 110 ng, with the greatest increase at Station 4. Mussels at all stations had inorganic mercury contents that were lower than at the start of the test, but these decreases were not statistically significant (Figure 7).

The proportion of methylmercury within the total mercury content of mussels increased at all stations during the test. Methylmercury accounted for 30 to 40 percent of the total mercury content in mussels at the end of the test, compared to an initial composition of 22 percent methylmercury (Table 7).

The correlation analysis for total mercury concentrations in sediment and tissues resulted in an r-value of 0.446 (Table 7). This relationship is not significant at the 95-percent confidence level ($r_{0.05,(2),6} = 0.707$; Zar 1974). Correlation analyses were also conducted on TOC-normalized sediment mercury concentrations, but normalizing did not raise the correlation. Since high total mercury concentrations were predicted for Station 4 sediments, and other investigators' results were up to two orders of magnitude higher, their chemistry data were used in a separate correlation analysis. This substitution did not increase the correlation. An r-value of 0.381 was obtained for sediment total mercury versus tissue methylmercury concentration (Table 8).

Mussel Growth

The best estimates of mussel growth in this study were final tissue weights and change in tissue weight, change in whole-animal wet weights, and change in shell length. Although changes in shell weight differed among stations, the ecological significance of these data are unclear (Figure 8; Table 5B). Apparent changes in shell width and height were within measurement error and were not useful metrics. The ranges in response measured among

animals at all test stations at the end of the study are presented in Table 9.

Mid-test Observations

Mid-test survival rates varied from 43 to 100 percent (Table 5A). The low survival for individuals transplanted to Station 6, in addition to observations of sulfur in the sediment and dense plant material, caused us to relocate Station 6 mussels mid-test.

Mussels at Stations 1 and 2 decreased slightly in whole-animal wet-weight; mid-test growth rates (based on changes in whole-animal wet weights) for these mussels were -24 and -62 mg/week, respectively. Mussels at Station 3 (near Reservoir 2 Inlet) did not grow. Mussels appeared to grow at all stations below Nyanza, although mussels at Station 6 had a very low growth rate (15 mg/week). Mussels from Station 8 had the highest growth rates at 318 mg/week. Table 5A presents mid-test growth measurements.

Changes in tissue weight and shell length were similar to the changes observed for whole-animal wet weights. There was little change for animals at Stations 1, 2, 3, and 6, while mussels at Stations 4, 5, 7, and 8 showed increases.

End-of-test Growth

In general, mussel growth increased from Station 1 to Station 8 as shown by increases in whole-animal wet weights, lengths, tissue weights, and shell weights (Figure 8). Based on changes in tissue weight, whole-animal wet-weight, and whole-animal shell length, Stations 1, 2, and 3 had very low or negative growth and form a statistical grouping. Stations 7 and 8 had the highest growth and also form a separate statistical grouping. It is difficult to include Stations 1 and 2 in these comparisons. Mussels at both the White Hall Reservoir and Station 2 had unexpectedly high tissue concentrations of mercury. Beginning with Station 4, mussel growth rates generally increased with distance away from the site, except for Station 6.

Considering all the metrics evaluated, mussels at Stations 1, 2, and 3 demonstrated little to no growth (Figure 8; Table 5B). Based on changes in whole-animal wet-weight, mussels at Stations 7 and 8 had the highest growth rates. Similarly, statistical analyses confirm that mussel growth metrics from Stations 1, 2, and 3 were similar (although only Stations 1 and 2 sometimes form the group), as they were at Stations 7 and 8 (Figure 8).

Figure 9 shows the change in percent water in mussel tissues during the test period. There is a decreasing gradient with distance from the White Hall Reservoir. End-of-test percent

Table 8. Results of correlation analyses (r-values) on selected parameters. Bold numbers = significant correlation (rcrit = 0.707; 95% confidence level).

A. Sediment Metal Concentrations and Conventional versus tissue mercury levels.

	Mercury	Chromium	Lead	Cadmium	TOC	% Sand	% Silt	% Clay
Mercury	1							
Chromium	0.981	1						
Lead	0.547	0.662	1					
Cadmium	0.393	0.527	0.913	1				
Total Organic Carbon	0.720	0.767	0.529	0.503	1			
% Sand	-0.447	-0.525	-0.569	-0.451	-0.797	1		
% Silt	0.182	0.559	0.582	0.405	0.740		1	
% Clay	0.182	0.267	0.383	0.528	0.767			1
Tissue[THg]	0.446							
Tissue[MeHg]	0.381							
Tissue-THg-Content	0.359							
Tissue-MeHg-Content	0.176							

B. Tissue mercury levels versus mussel metrics (Sta6 included).

	Concentration			Content				
	Tissue [THg]	Tissue [MeHg]	Tissue [InoHg]	Tissue THg	Tissue MeHg	Tissue InoHg	Tissue Weight	Percent Water
Tissue Weight	-0.924	-0.858	-0.910	-0.184	0.031	-0.299	1	0.965
Whole Animal Growth	-0.982	-0.754	-0.918	-0.232	0.149	-0.198	0.913	0.868
Whole Animal Length	-0.850	-0.684	-0.893	-0.249	0.207	-0.172	0.858	0.791
Shell Weight	-0.873	-0.746	-0.894	-0.176	0.183	-0.135	0.902	0.844

C. Tissue mercury levels versus mussel metrics (Sta6 excluded).

	Concentration			Content				
	Tissue [THg]	Tissue [MeHg]	Tissue [InoHg]	Tissue THg	Tissue MeHg	Tissue InoHg	Tissue Weight	Percent Water
Tissue Weight	-0.925	-0.881	-0.911	-0.195	0.050	-0.309	1	0.972
Whole Animal Growth	-0.947	-0.875	-0.946	-0.203	0.080	-0.340	0.949	0.945
Whole Animal Length	-0.941	-0.853	-0.948	-0.210	0.111	-0.368	0.923	0.907
Shell Weight	-0.931	-0.872	-0.924	-0.144	0.013	-0.274	0.941	0.925

Table 9. Ranges in response for the growth metrics measured at the end of the study.

Growth Metric	Lowest Value Observed	Highest Value Observed
Growth rate based on whole-animal wet weight	-21 mg/wk	303 mg/wk
Change in tissue weight (g-wet)	0 g	2.90 g
Change in whole-animal shell length	0.1 mm	2.6 mm
Change in empty shell weight	-0.25 g	2.01 g

water concentrations ranged from 81.8 to 88.4; initial concentration was 81.9 percent. Mussels at Stations 7 and 8 showed very little change in water concentration during the test. There was a negative relationship between percent water and growth rate, with the data falling into four distinct groups (Figure 10).

Comparisons Between Tissue Mercury Concentrations and Growth.

Correlation analyses (NW Analytical StatPak, Ver. 4.1) were conducted on various mussel growth metrics and mercury concentrations (Table 8). Station 6 was not included in these analyses because the station was considered an outlier. The significant correlation coefficients resulting from that analysis are shown in Table 10.

No significant correlations were found for tissue mercury content (on a per-animal basis) and mussel growth metrics. Station 6 data were excluded from these analyses because of the high mortality and the low growth rates observed for these mussels. Regression analyses (NW StatPak, Ver. 4.1) were conducted on the three forms of mercury and the three mussel growth metrics (Figures 11-13). Based on the r^2 values, for each mussel growth metric, the relationship between methylmercury is not as strong as for either total or inorganic mercury.

Ancillary Observations

Mussels and racks at reference Station 1 (White Hall Reservoir) looked clean and scrubbed. Unlike the other stations, there was very little algal fouling or growth of plant matter directly on the racks. Mussels deployed at Station 2 had very fragile shells and the plastic

Table 10. Significant correlation coefficients.

Comparison	r value
WAWW* growth vs. tissue total mercury concentration	-0.95
WAWW growth vs. tissue methylmercury concentration:	-0.88
WAWW growth vs. tissue inorganic mercury concentration	-0.95
EOT** tissue weight vs. tissue total mercury concentration	-0.93
EOT tissue weight vs. tissue methylmercury concentration	-0.88
EOT tissue weight vs. tissue inorganic mercury concentration	-0.91
EOT shell length vs. tissue total mercury concentration	-0.94
EOT shell length vs. tissue methylmercury concentration	-0.85
EOT shell length vs. tissue inorganic mercury concentration	0.95
EOT shell weight vs. tissue total mercury concentration	-0.93
EOT shell weight vs. tissue methylmercury concentration	-0.87
EOT shell weight vs. tissue inorganic mercury concentration:	-0.92
* whole-animal wet weight	
** end of test	
(Critical r-value $r_{0.05,(2),5}=0.755$; all correlations are significant at the 95-percent confidence level.)	

tag labels were dissolved. Mussels deployed at Station 4 (Reservoir 2) had an oily sheen and distinct odor of petroleum product. Mussels at Station 6 (Sherman Street Bridge) required relocation because of presumed low-oxygen conditions and poor mussel survival. Most of the dead mussels were in the smaller size classes. The surviving mussels were relocated to an area where dissolved oxygen concentrations were expected to be better. No other abnormalities were noted.

Discussion

Extent of Mercury Bioaccumulation

The primary goal of this study was to determine the geographic extent of bioavailable methylmercury within the reaches of the Sudbury River below Nyanza. Results based on evaluations of methylmercury concentration and content data suggest that methylmercury

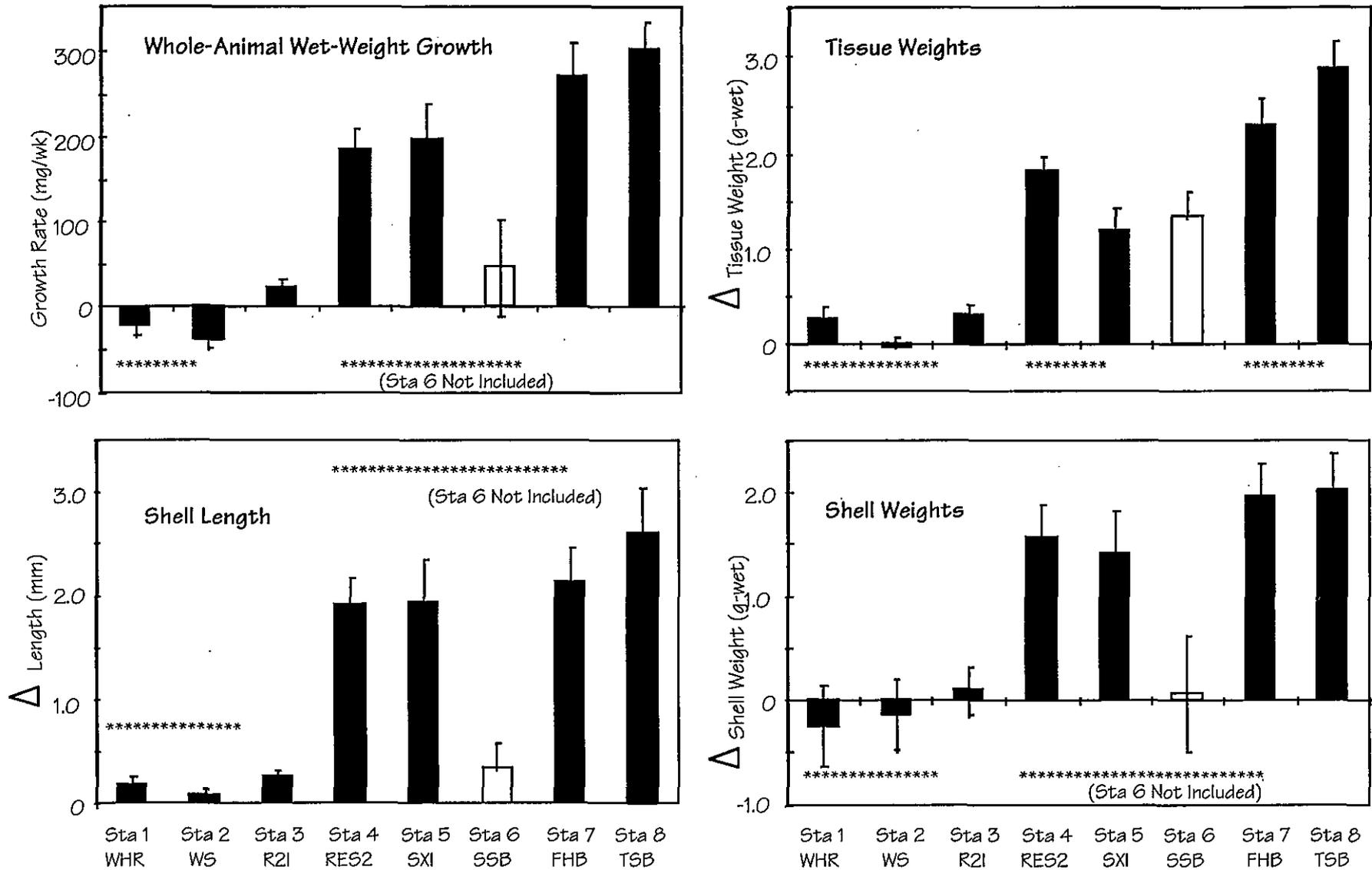


Figure 8. Mussel growth rates (based on whole-animal wet-weights), changes in tissue weight, shell length, and shell weight by station. *** indicates stations that form a statistically similar group (based on non-parametric ANOVA and multiple range tests). Station 6 data are presented for comparative purposes only (open bar); they were not included in statistics.

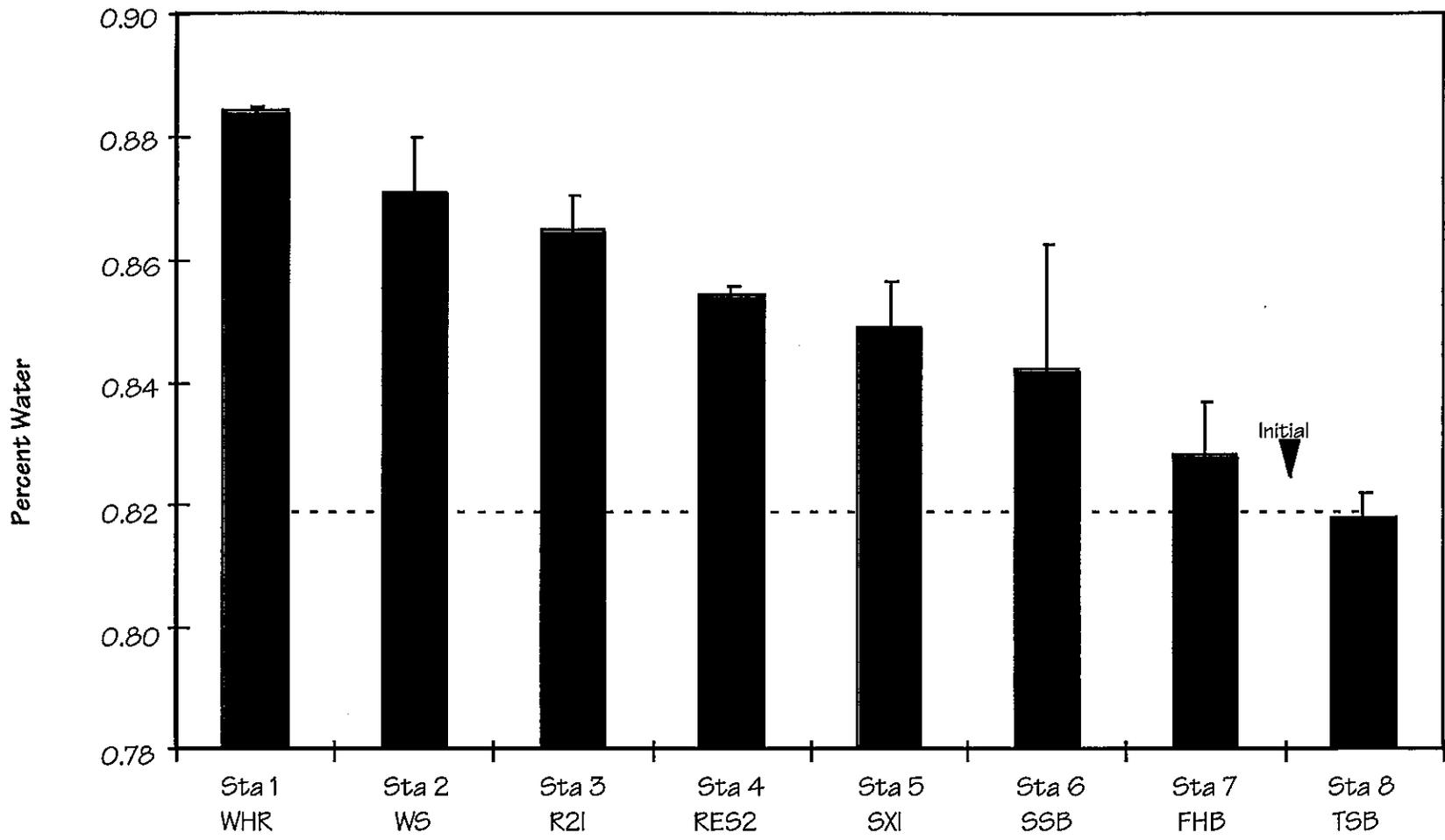


Figure 9. Percent water (+/- 2SE) measured in mussel tissues at the end of the study.

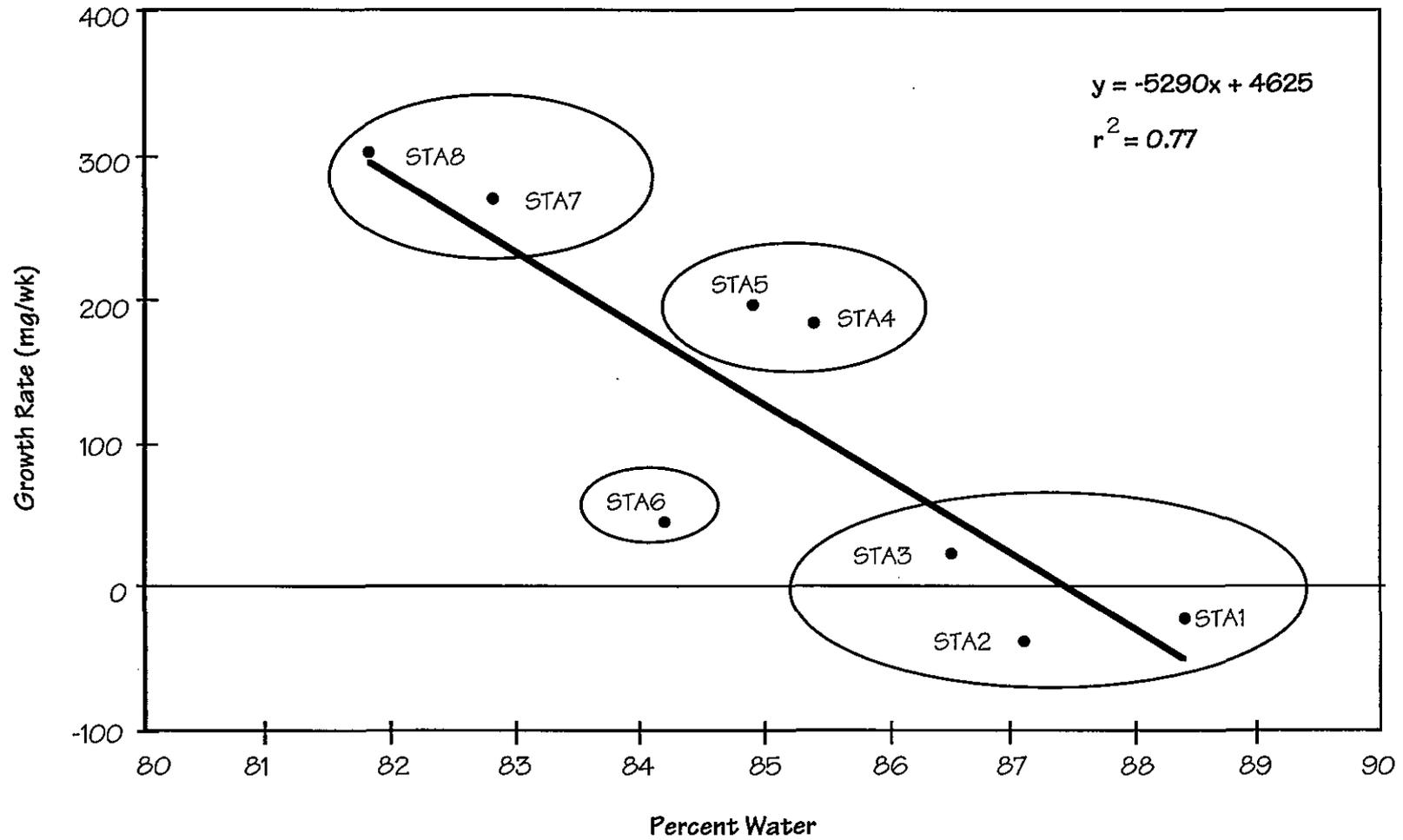


Figure 10. Percent water versus growth rate (based on whole-animal wet-wet). Stations with similar mussel growth metrics (e.g., growth rate, tissue weights, shell length, and shell weight) are grouped.

was biologically available to *E. complanata* in an area extending from the White Hall Reservoir to Thoreau Street Bridge.

The concentration data suggest that only mussels at Stations 1, 2, and 3 (stations where mussel growth was minimal) accumulated methylmercury, hence elevating their total mercury burdens. However, the *content* data, which normalizes the data for growth, strongly suggests that mussels at all stations actively increased their methylmercury burden. Total mercury burdens were maintained by a decrease in the inorganic mercury burden for mussels at all stations. Measured increases in methylmercury are attributed to accumulation from either water or sediments.

Study results are consistent with elevated concentrations of methylmercury in the water column (Coleman 1994), in sediments, and in fish tissues (NUS 1992), and with current advisories against the consumption of fish in this area (Maietta 1990). Concentrations of methylmercury increased during the study period in transplanted mussels at all stations except 7 and 8, which were closest to the Concord River and surrounded by wetlands. The lack of change in methylmercury concentration for mussels at these two stations could suggest an interpretation, based on concentration data, that methylmercury was only available in the non-wetland areas near Nyanza (Stations 3-5). However, the significant increases in methylmercury *content* for mussels at all stations except 2 and 6 suggest that methylmercury is bioavailable in all areas, including the wetlands, and is not being depurated by the mussels. Calculations of the amount of growth that would be necessary to account for the changes in measured tissue methylmercury concentrations demonstrate that growth dilution cannot completely explain increases in methylmercury. The results of this study emphasize the difficulty in interpreting data, and demonstrate the need to examine not only concentration, but also content per animal and growth effects. These results also reaffirm the importance of making synoptic measurements of bioaccumulation and growth as suggested previously by others (Depledge and Rainbow 1990).

Measuring both total and methylmercury helped us identify the corresponding decreases in inorganic mercury. Although we did not measure inorganic mercury directly, we have assumed that inorganic mercury is equivalent to the difference between total and methylmercury. Two methods were used to determine total mercury concentration in tissues at the end of the test: hot acid digestion and sum of species. Overall, the totals determined by acid digestion were about 10 percent higher than the totals determined by sum of species. Almost all of this difference was in the animals from Stations 1, 2, and 3; the analytical

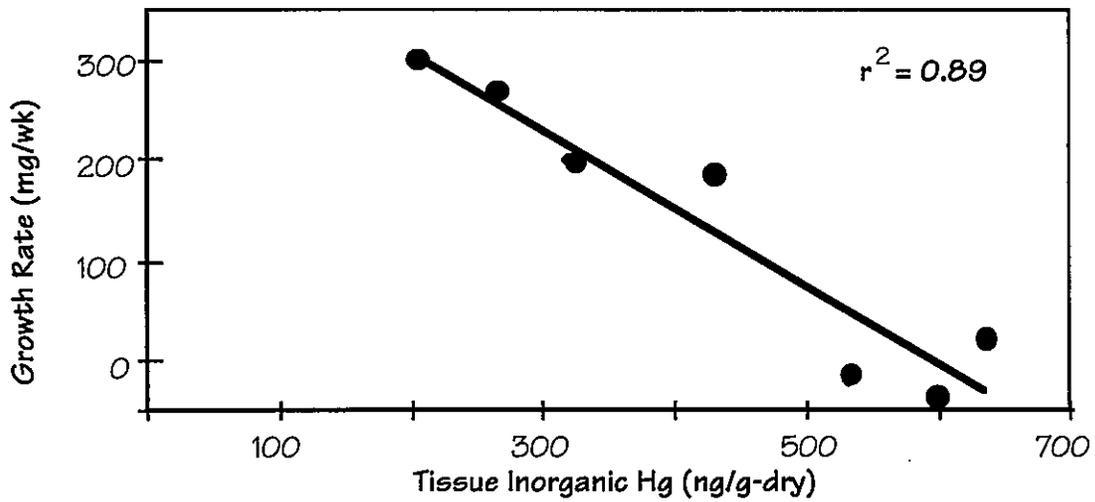
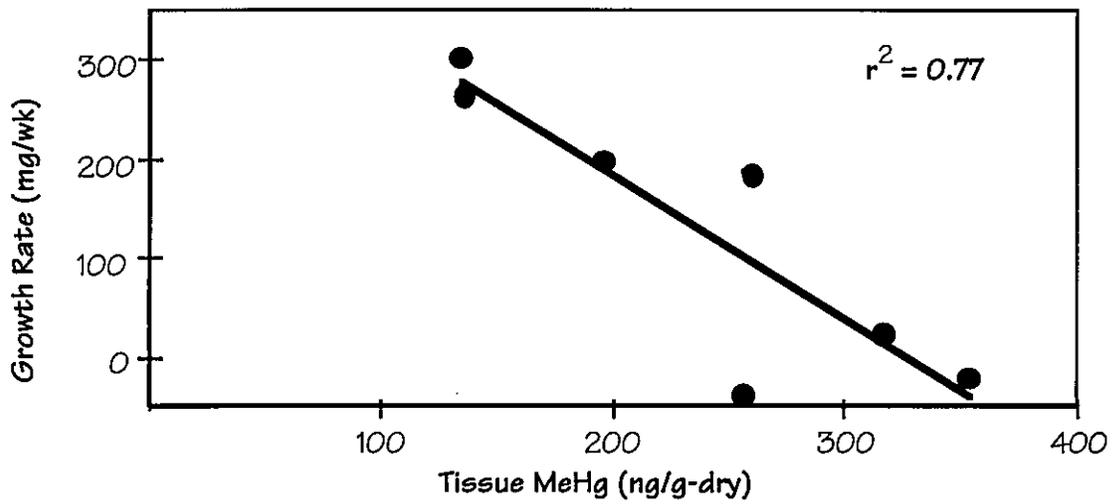
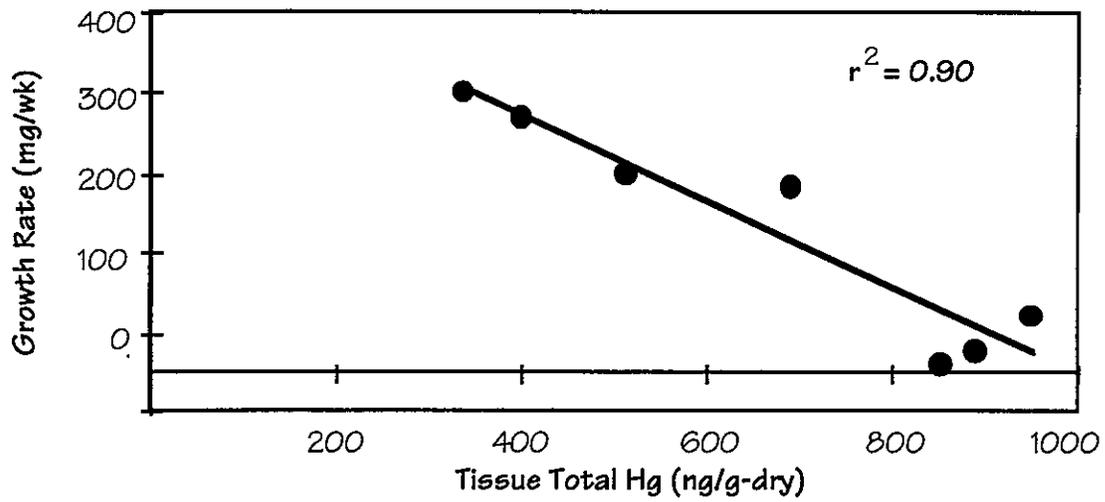


Figure 11. Regression relationships for mussel tissue concentrations of total, methyl-, and inorganic mercury and mussel growth rates (changes in whole-animal wet-weights). Station 6 was excluded from these analyses.

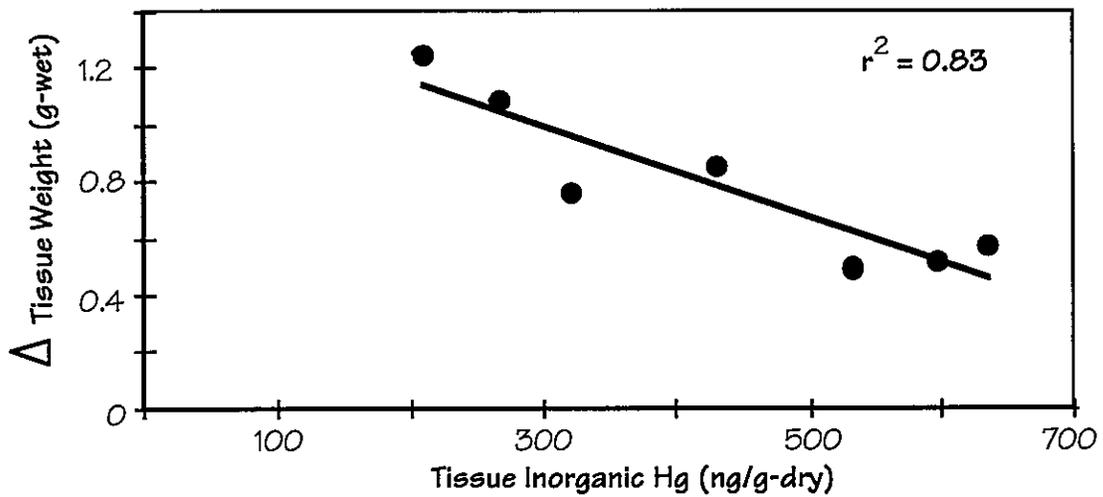
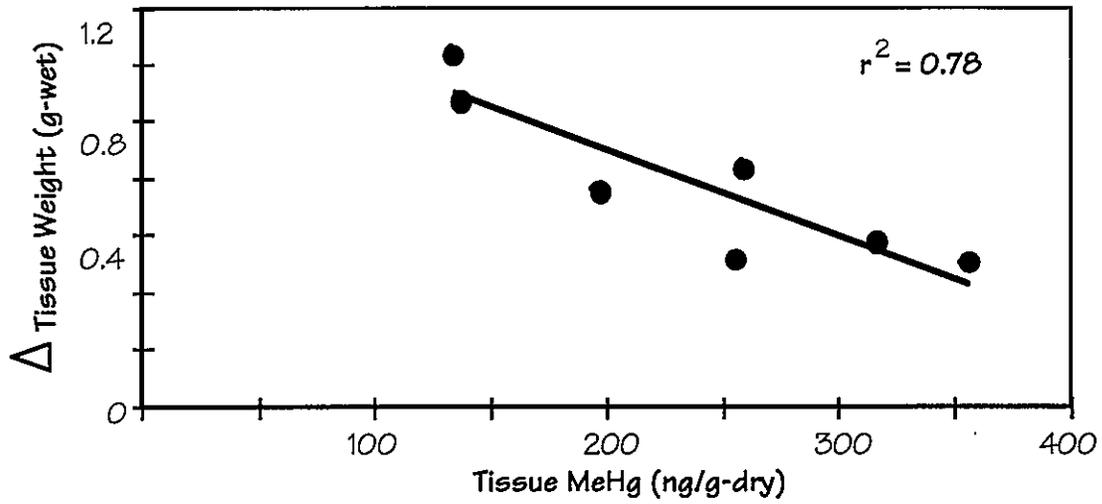
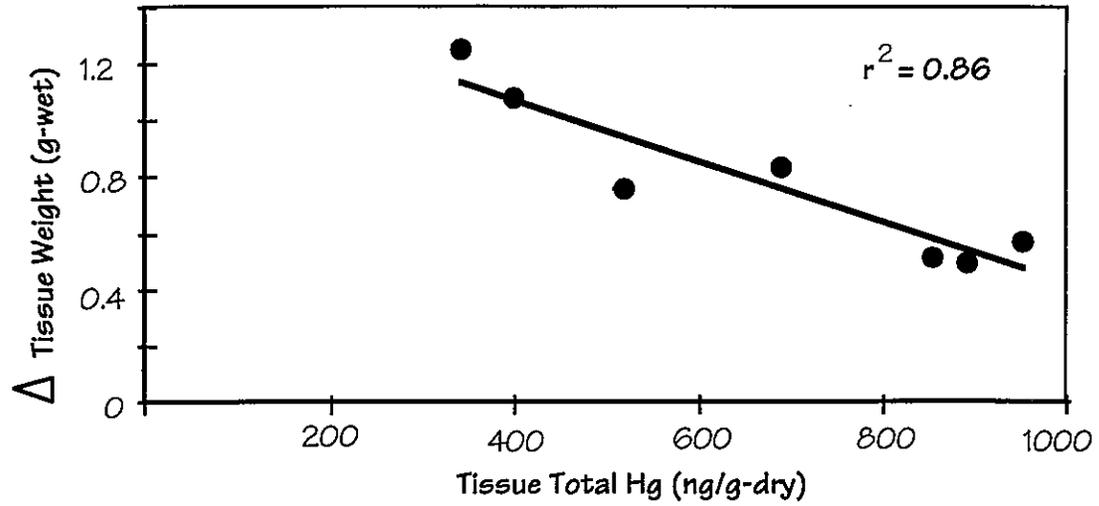


Figure 12. Regression relationships for mussel tissue concentrations of total, methyl-, and inorganic mercury and changes in mussel tissue dry weight. Station 6 was excluded from these analyses.

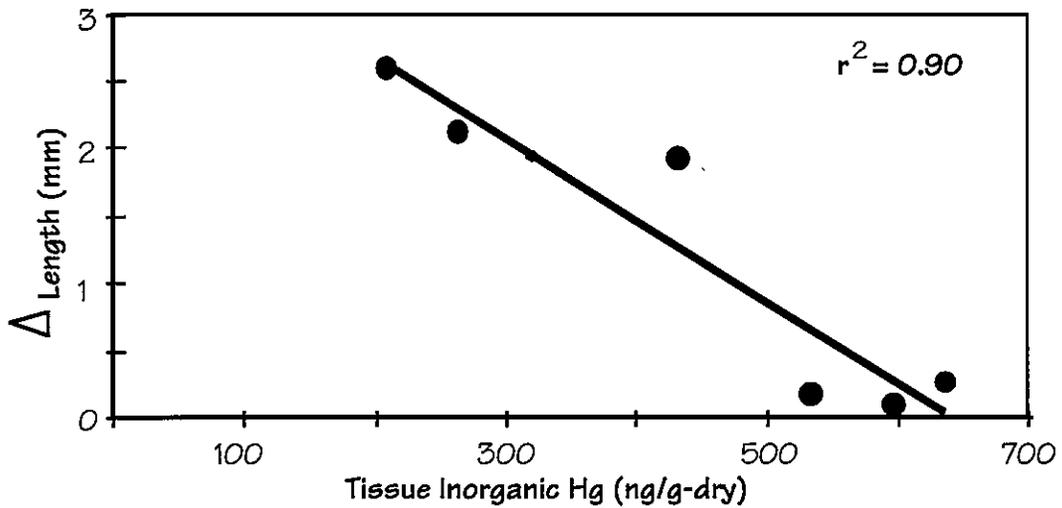
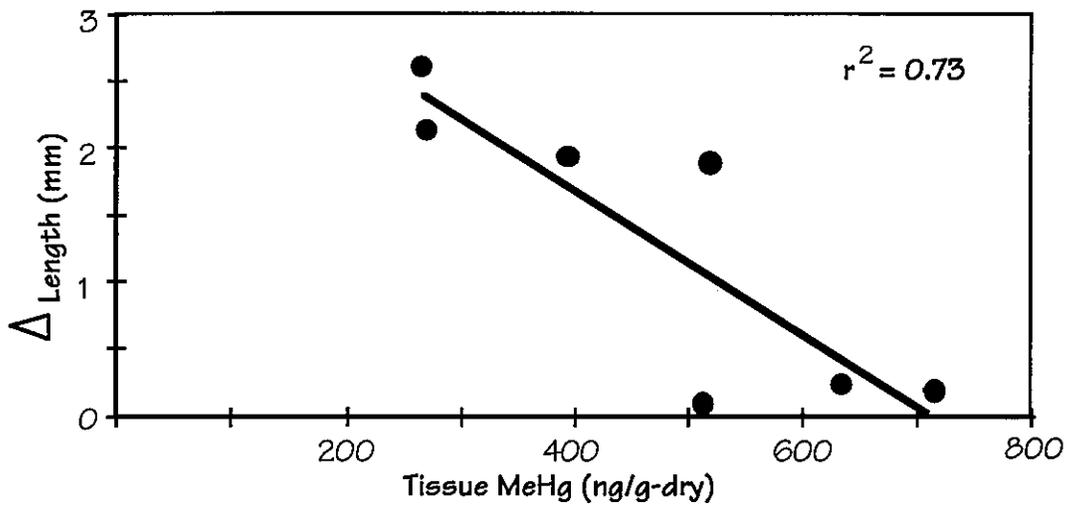
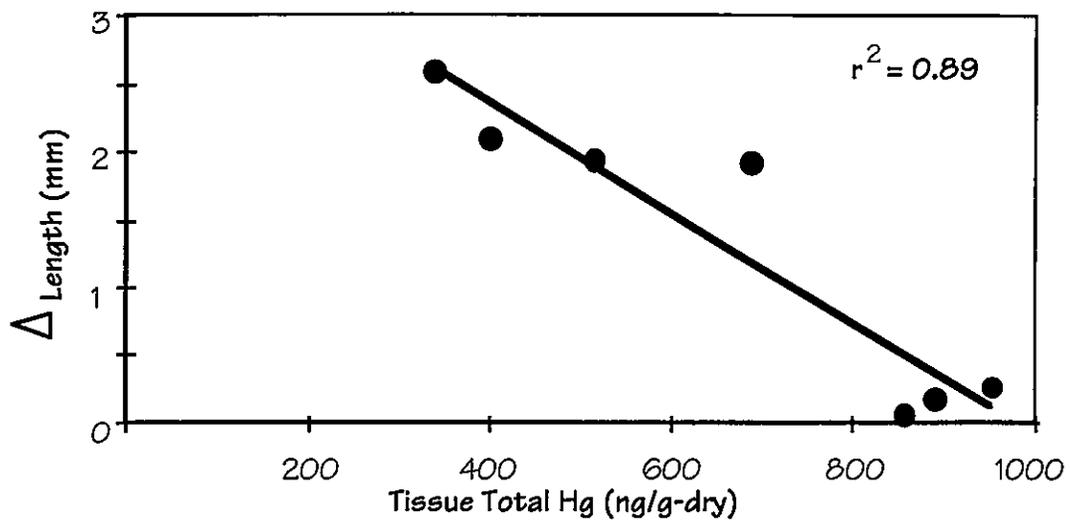


Figure 13. Regression relationships for mussel tissue concentrations of total, methyl-, and inorganic mercury and overall increases in mussel shell length. Station 6 was excluded from these analyses.

laboratory suggests other forms of organic mercury (e.g., ethylmercury) make up the difference.

The inorganic mercury content in mussels decreased at all Sudbury River stations. Except at Stations 3 and 4, this loss of inorganic mercury would most likely be significant with expected lower variances in the chemical data. The depuration of inorganic mercury was not unexpected, since several investigators have reported similar results (Cunningham and Tripp 1975; Fowler et al. 1978; Riisgård et al. 1985). In general, filter-feeding bivalves and other animals tend to accumulate methylmercury and release inorganic mercury. The tissue inorganic mercury concentration data suggest a trend similar to that seen for the total and methylmercury data—accumulation by animals closest to Nyanza and depuration by those farthest away. Decreases in inorganic mercury content on a per-mussel basis indicate that mussels at all stations depurated inorganic mercury. Examining only total mercury concentrations can be equally deceiving. The concentration data suggest accumulation by animals closest to Nyanza and depuration by those in the wetland area near the Concord River. However, the total mercury content data indicate that there were no statistically significant changes in total mercury content on a per animal basis, although a less variable dataset might have shown some statistical differences. However, the balance shifted: methylmercury increased while inorganic mercury decreased.

There is little evidence that mussels convert inorganic mercury to methylmercury; most of the evidence suggests that bivalves are incapable of methylation (Fowler et al. 1978; Bryan and Langston 1992). Thus the likeliest explanation for the increase noted in methylmercury concentration in mussel tissues during the field exposure is preferential accumulation of organic mercury. This has been demonstrated in several species of freshwater, estuarine, and marine bivalves (Fowler et al. 1978; Riisgård and Hansen 1990; Bryan and Langston 1992; Tessier et al. 1994; Malley et al. in press). Methylmercury was accumulated at all sites, but it is not clear whether all of this mercury originated at Nyanza.

Sources of Bioavailable Mercury

A second objective of our study was to identify areas near Nyanza that might act as sources of methylmercury for transport to downstream locations. We anticipated that sediments from Stations 3, 4, and 5 would have high total mercury concentrations. Sediment mercury concentrations were low at all stations except Station 3 (17.9 mg/kg) and Station 5 (5.4 mg/kg), which were about two orders of magnitude above concentrations

measured at Station 4 in Reservoir No. 2. Data presented by Wiener et al. (1994) suggest a total mercury concentration in sediments of about 18 mg/kg for Reservoir No. 2 compared to our value of 0.17 mg/kg. Our station was located nearshore, at the base of an embankment. Clean soil from the embankment is likely to have eroded into the local area of our station.

Total mercury in sediment did not correlate with total mercury in mussel tissue when all stations (except 6) were used. This correlation remained statistically insignificant even after normalizing for total organic carbon or using the sediment data from Wiener et al. (1994). However, tissue and sediment mercury concentration were significantly correlated when the reference stations were excluded from the analysis. Despite a low concentration of total mercury in sediment at Station 4, mussels at this site accumulated the most methylmercury content on a per-animal basis. The high methylmercury concentrations in tissue could be associated with a high methylmercury concentration in water, as measured in another concurrent study (Coleman 1994).

Sediment and water are both potential sources of mercury to mussels. Mussels most likely accumulated water-borne methylmercury by ingesting contaminated food or sediment particles. Tessier et al. (1992) indicate that *E. complanata* actively accumulates mercury from the water and found a significant relationship in laboratory exposures. Both laboratory and field studies have demonstrated the importance of food particles (King and Davies 1987; Bruner et al. 1994b). Metcalfe-Smith et al. (1992) found some relationships between mercury in sediment and accumulation by *E. complanata* and *Lampsilis radiata* in a field study, but the results were equivocal. In two studies in Southern California with *Mytilus californianus*, Eganhouse and Young (1978 a,b) found no relationship between tissue concentrations of mercury in caged mussels placed different distances away from the sediment, and concluded that the sediment was not a source of bioavailable mercury to the mussels. In other studies with natural populations (Eganhouse and Young 1976, 1978a), mercury was not available close to the source of contamination (a sewage outfall), but became more available with distance away from the source of mercury. Organic material associated with outfall material apparently made the mercury less available to the mussels (Eganhouse et al. 1978). Similar results have been reported for freshwater unionids in both laboratory and field exposures (Breteler et al. 1981; Bryan and Langston 1992).

In our study, it is not clear whether the immediate source was dissolved or particulate methylmercury, or both. Based on laboratory studies, Tessier et al. (1984) has shown that *E. complanata* filters particles that are predominantly in the 10 to 13 μ size range. These

small particles also tend to sequester the highest concentration of contaminants because of their relatively high surface-to-volume ratio (Boese et al. 1988). Mercury accumulation from sediments accounts for relatively high concentrations in deposit feeders in both freshwater and estuarine systems (Jernelov and Lann 1971, Kiorboe et al. 1980, Langston 1986, Bryan and Langston 1992). Particles may be the primary source of mercury to suspension feeders such as *Mytilus*, despite the high bioavailability of dissolved forms (King and Davies 1987). Even detritus and other sediment particles have been shown to be a significant food source for several species of marine and freshwater bivalves (Breteler et al. 1981; Williams 1981; King and Davies 1987; V.-Balogh 1988; Bruner et al. 1994 a,b). These questions regarding bioavailability and routes of exposure call for more site-specific studies such as this one to supplement laboratory studies that do not include many of the variables inherent in field studies. This is particularly true in light of the different bioaccumulation and depuration findings of laboratory versus field studies of mercury.

The origin of the methylmercury accumulated by mussels at Stations 6, 7, and 8 is unknown. Methylmercury could have formed upstream, within the river, or within nearby wetlands (St. Louis et al. 1994). Studies conducted by USGS (Coleman 1994) as part of this cooperative effort indicate that total and methylmercury concentrations in the water column correlate with proximity to wetlands. They question, however, whether the source of this methylmercury is upstream contamination. Concentrations of total and methylmercury measured by USGS in water collected near our Station 6 were approximately 8 to 10 ng/l and 0.21 to 0.75 ng/l, respectively. Additionally, Coleman (1994) suggests that aqueous mercury from Nyanza does not pass the Saxonville impoundment. Some of the lowest methylmercury concentrations (0.07 to 0.2 ng/l) were measured in water collected from the Sudbury River below the Saxonville impoundment—an area removed from wetlands.

The USGS stations did not extend beyond the Sherman Street Bridge (our Station 6), and USGS does not have data for the part of the Sudbury River near the Concord River (i.e., near our Stations 7 and 8). Methylmercury in this portion of the river may be entering from the wetlands (Coleman 1994), a possible source of methylmercury accumulated by our mussels at Stations 6, 7, and 8.

Recent studies have shown that some watersheds in northern latitudes have naturally high concentrations of both total and methylmercury, when compared to watersheds in more southern latitudes (St. Louis et al. 1994; Lasorsa and Allen-Gil 1995). Tissues of fish collected from Locust Pond, a presumed unaffected area in the northeast, contained total mercury concentrations of approximately 1.1 mg/kg-wet (Metcalf and Eddy 1994). In

addition, St. Louis et al. (1994) have shown that the methylmercury produced in lake sediments is often retained within lakes with little opportunity for transport or migration. These factors could account for the elevated mercury concentrations measured in the Lake Massesecum mussels. Since there is a similar temperature effect on methylmercury production in lake sediments and wetlands, mussels collected from Lake Massesecum could contain a maximum tissue burden. The Lake Massesecum mussels would accumulate more methylmercury when summer temperatures increased its bioavailability.

Effects of Mercury Exposure

Changes in tissue weight were the most informative growth metric for mussels in this study. We believe that the growth measured for mussels at Stations 7 and 8 was not an artifact of caging or reproductive tissue development. Mussel growth rates at these stations were greater than those reported for this size range by Kat (1982), but the conditions in the two studies are different. Increase in reproductive tissue is unlikely since none of the animals in our study contained glochidia (a larval stage retained by the female).

Even though we have been unable to identify the source of methylmercury, our data indicate that accumulation of methylmercury may significantly affect mussel growth. The statistical clusterings of three or four groups found for each of the mussel metrics suggest that areas closest to Nyanza are most impacted and may elicit adverse effects in exposed organisms. The impact of conditions in the midsection of the river is less certain. Perhaps more exposure time is required to evaluate this area. The biological effects of the methylmercury at the stations furthest downstream are unclear.

The regression relationships (Figures 11-13) between growth and tissue mercury concentrations suggest that the highest concentrations of mercury in mussel tissues were associated with, and may have caused, reductions in mussel growth. It cannot be stated conclusively that mercury caused all the observed growth effects because there could be other physical or chemical factors influencing mussel growth. It is unlikely that the transplant procedure of caging and relocating animals significantly stressed the mussels. The effects of caging could have been evaluated by deploying additional mussels at Lake Massesecum. We did not add this element to the study because of budgetary constraints and the additional time required to revisit the collection site at the end of the study. In addition, our previous work with marine mussels (Salazar and Salazar, in press) and the

evidence in the literature indicate that caging and transplanting do not add significant stress under proper handling conditions (Muncaster et al. 1990; Stansbery 1994).

The observed trend of increasing final tissue weights and decreasing tissue mercury concentrations with distance from Nyanza must be evaluated judiciously. These data could suggest that tissue concentrations of mercury are being diluted by the growth process. By analyzing the growth data concurrently with the tissue content data, it becomes apparent that mussels are accumulating methylmercury and effects are occurring that could be related to bioaccumulated mercury. Each growth metric (i.e., whole-animal wet weight, change in tissue weight, change in shell length) strongly correlated with all three forms of mercury evaluated. The highest tissue concentrations of total, inorganic, and methylmercury were associated with the lowest mussel growth rates. The literature suggests that methylmercury is the most toxic form (Bryan and Langston 1992) and that it is the most readily accumulated. In our study, the strongest relationships (based on r^2 values from the regression equations) were found with total and inorganic mercury. With only seven stations (Station 6 was omitted for reasons previously described) in one short study, we cannot conclude that methylmercury is less toxic, but this finding is of interest

Even though our data set is limited in size ($n=8$) and the reference stations were inappropriate, some trends are apparent with respect to tissue burdens of mercury and growth. Growth rates for animals that had tissue burdens above 800 ng/g total mercury-dry were at least ten times lower than growth rates for animals with less total mercury in their tissues. Animals with less than 800 ng/g total mercury-dry increased in tissue weight by as much as 75 percent. Animals with tissue burdens greater than 800 ng/g had no increase in tissue weight. These data are not sufficient to establish effect concentrations but they can be used as a starting point for comparative purposes. Our data also suggest that the tissue concentration of total mercury for mussels at Stations 7 and 8 did not adversely impact mussel growth. However, without comparable growth data from reference animals, it cannot be established that this is a no-effect tissue concentration, even though growth rates are comparable with literature values. The trends demonstrated in this study require further testing and confirmation.

Sediment mercury concentrations and mussel growth did not correlate when all stations (except 6) were used. However, when the five Sudbury River stations were used and the reference stations were excluded, mussel growth correlated significantly with sediment mercury concentrations.

There was no significant relationship between mercury content and any of the mussel growth metrics, and we did not expect to find one. The concentration of contaminants in mussel tissues appears to elicit toxic responses and not the per-animal content (Depledge and Rainbow 1990). While the total mercury content is informative for understanding accumulation and depuration processes, action levels and effects concentrations must be determined by using concentrations.

Several investigators have advocated moving toward criteria based on tissue burdens in addition to, or instead of, the concentration of contaminants in water or sediment (McKim and Schmeider 1991, Calabrese and Baldwin 1993). Niimi and Kissoon (1994) have suggested that whole-body concentrations of 1 to 5 mg total mercury/kg tissue could have chronic effects on adult fish and other aquatic organisms. Widdows and Donkin (1992) have pioneered using synoptic measurements of bioaccumulation and physiological responses (scope for growth) to predict tissue concentrations at which adverse effects are expected in mussels. This approach is gaining importance because of the applications to ecological risk assessments.

The final percent water concentration data provide another interesting correlation, although the environmental significance is unclear. Percent water concentration steadily decreases with distance from the White Hall Reservoir. Although there could be a relationship between percent water and mercury exposure, the decrease appears too regular to be associated with mercury alone and may be due to other physical or chemical factors associated with distance downstream. The similar percent water content between Station 8 and Lake Massesecum mussels suggests that the animals were of similar health. Mussels with the greatest deviation from the initial percent water concentration may have been under stress and less healthy. This interpretation is supported by the relationship between percent water and growth rate. The groupings shown in Figure 8 are similar to those obtained for tissue mercury concentration. Although the usefulness of percent water as an effects measurement endpoint requires further investigation, these data may indicate that mussels at Stations 7 and 8 were unaffected by exposure conditions.

Effects of Temperature

Although water temperature differences measured in the Sudbury River are expected given the nature of the system being tested, these differences are statistically significant.

However, separating statistical significance from ecological significance is important. What affect, if any, do the measured temperature differences have on the observed growth results? Temperatures and mussel growth rates are positively correlated ($R^2=0.73$; Figure 14). The R^2 value obtained for this regression is slightly smaller than the R^2 values obtained for the regression relationships for mussel tissue concentrations of mercury and growth parameters (Figures 11-13).

Unionid mussels, including *E. complanata*, are slow growing and can live for decades. Mean shell length increases with increasing age over the entire life span of these mussels, but as the mussels reach maturity, the increase in shell length declines with age. Tissue weight increases with increasing age over the entire life span of these organisms, sometimes increasing exponentially. Mussels in our study were estimated to be 9 years old based on an average shell length of approximately 60 mm. Mussels of this age grow about 5 mm per year and increase tissue weights by about 1 g (wet weight)/year (McMahon 1991). The 84-day exposure period used in this study represents a very small portion of their life and the overall effects of the slight differences in temperatures measured across stations on growth are expected to be minimal. The indirect effects of temperature on growth may be greater than the direct effects. For example, it is possible that food and nutrient availability was less in areas where temperatures were lower.

Downstream stations and stations in impoundments have slightly elevated temperatures from upstream stations—this temperature increase may result in a slight increase in tissue growth causing a dilution effect on the tissue concentration of total and methylmercury. This might explain the phenomenon of downstream stations having a lower tissue residue, even though the local mercury concentration in water or sediment may be higher at these stations through an accumulation of mercury from the entire watershed.

We are not able to determine the relative importance of temperature or mercury exposure on growth because of the lack of proper reference stations (see following section). We do have a good correlation between tissue mercury concentration and growth as well as a reasonably good correlation between temperature and growth. However, we can not determine how much different growth would have been in the absence of mercury because we don't have uncontaminated reference stations with the same temperature ranges.

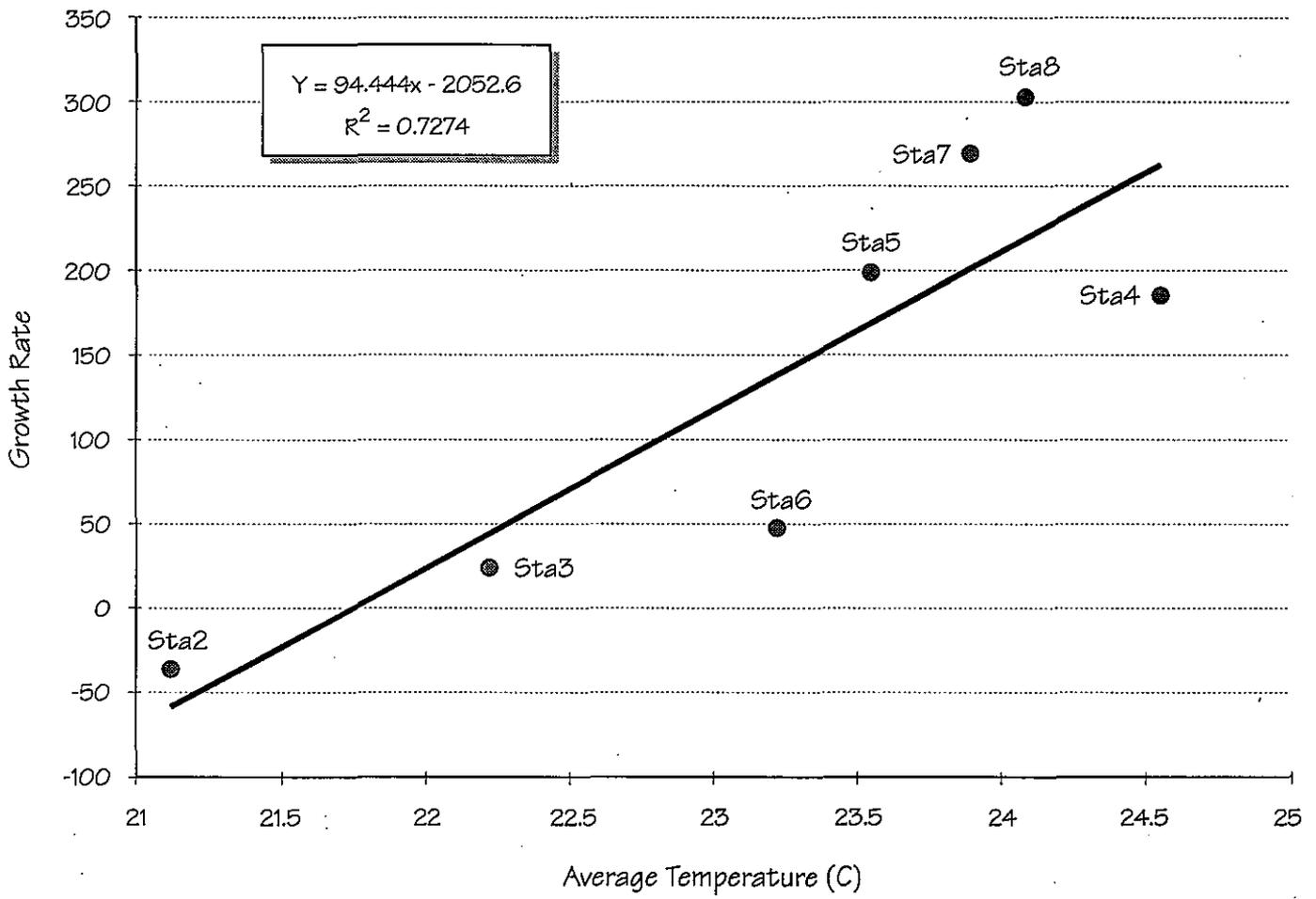


Figure 14. Relationship between mussel growth rates and temperature in the Sudbury River.

Reference Station and Collection Site Concerns

Stations 1 and 2 clearly do not represent uncontaminated "background" conditions, and mussel growth data from these stations cannot be used as reference data to compare with data from mussels transplanted to stations below Nyanza. The tissue mercury concentrations in mussels from the White Hall Reservoir were among the highest measured in our study, and a methylmercury source has been identified for this reservoir (Coleman 1994) that could account for these data. Mussels transplanted to Stations 1 and 2 had negative growth rates, confirming their inappropriateness as reference animals. Correlations that included these stations (sediment mercury and tissue mercury, sediment mercury and mussel growth) became significant when these reference stations were excluded.

The initial mercury concentrations from the Lake Massesecum mussels were higher than anticipated. Although the presence of mercury in tissues of mussels from Lake Massesecum may have affected animal health and their ability to accumulate mercury at the beginning of the test, mussels transplanted to some stations had significant increases in growth, and all mussels accumulated methylmercury on a content-per-animal basis (Figure 7). The effects on the mussel population in Lake Massesecum is unknown. They may have adapted and remained unaffected. Enhanced mercury tolerance by induction of metallothioneins has been demonstrated in *Mytilus* exposed to mercury (Roesijadi et al. 1982) and even increased mercury tolerance after exposure to copper, cadmium, and zinc (Roesijadi and Fellingham 1987).

Limits of Data Interpretation and Future Work

While methylmercury appears to be available to mussels throughout the Sudbury River, we cannot extrapolate the potential impact to higher trophic species. Given mercury's potential to biomagnify, exposure of lower trophic species to low environmental concentrations may cause higher trophic species to have unacceptably high levels. Fish throughout the Sudbury River have elevated tissue mercury concentrations (NUS 1992); concentrations measured in fish collected from the river between 1971 and 1991 are similar to those predicted to cause effects in other fish species (Niimi and Kissoon 1994). Recent observations indicate that the abundance of *E. complanata* is lower at Nyanza than in downstream areas (Wicklów 1995). It would be useful to compare methylmercury concentrations in resident mussels with the concentration findings in this report.

It is difficult to extrapolate the apparent effects on growth in *E. complanata* to other species or biological endpoints. More sensitive species or life stages (e.g., larval or juvenile stages) may experience greater effects as a result of mercury exposure in the Sudbury River. Further, the limited number of stations in this study do not provide a detailed picture of the availability of mercury throughout the Sudbury River. Variability within impoundments was not measured, nor the effect of depth. Another uncertainty is what influence wetland areas have on the availability of methylmercury. Adding one or two stations in the Great Meadows National Wildlife Refuge and one directly upstream away from the wetland could help to establish the distribution of methylmercury in the wetlands.

A more integrated program measuring exposure, dose, and bioeffects would help to explain some of our results. Using appropriate reference areas for a study of this type is extremely important because poor reference data limit both interpretation of the data and conclusions. While growth at upstream stations clearly differed from that of downstream stations, growth effects at Stations 7 and 8 cannot be determined because of the poor performance of reference animals. Although it appears that mussels at Stations 7 and 8 were unaffected, we do not know what the growth would have been under pristine conditions. Similarly, lack of suitable reference data for determining ambient methylmercury concentrations precludes definitive conclusions about the extent of Nyanza methylmercury contamination. Future work should begin by locating reference areas for data on growth and ambient methylmercury concentration. Reference areas should be unaffected by direct sources of contamination and should not have unusual water chemistry.

Summary and Conclusions

The objectives of this mussel transplant study were to determine how far downstream from the Nyanza site mercury was bioavailable, and whether adverse effects were associated with exposure to bioavailable mercury. We used sediment and tissue chemistry data to estimate mercury availability; various mussel growth metrics were used in the effects assessment. Study results indicate the following:

- Total mercury concentrations in the sediment were highest near Reservoir 2 and the Saxonville Impoundment.
- Total mercury concentrations in mussel tissues increased significantly at the two reference stations and at the station nearest the Nyanza site. Total mercury concentrations in mussel tissues decreased significantly at the two stations farthest

downstream, in Fairhaven Bay and the wetland area near the confluence of the Sudbury and the Assabett Rivers.

- Methylmercury concentrations in mussel tissues increased significantly at all stations except Stations 7 and 8, the two stations farthest downstream from the Nyanza site.
- Total mercury content in mussel tissues did not change significantly at any station during the study.
- Methylmercury content in mussel tissues was significantly higher for mussels at all stations except Station 1, a reference station.
- Mussel growth rates had a downstream trend: growth rates were lowest near the Nyanza site and increased with distance away from the site. Mussels at Stations 1, 2, and 3 had lower soft tissue weights. Changes in whole-animal wet-weights for these mussels yielded negative growth rates. Mussels at Stations 1, 2, and 3 also had elevated percent-water concentrations. The increase in soft tissue weights for mussels at Stations 7 and 8 were comparable to literature values. Changes in whole-animal wet-weights and soft tissue weights for mussels at Stations 4 and 5 were intermediate between those from the upstream and farthest downstream stations. Poor survival of mussels at Station 6 appeared to be related to environmental factors (i.e., dissolved oxygen) and not mercury exposure.
- Temperatures were statistically lower in upstream stations; impoundment stations were generally warmer than faster flowing river stations. Except for the extremes, there was no difference in the mean temperature ranges across stations. A positive correlation was found between average temperatures and growth rates.

Based on this information, we are able to conclude that methylmercury was bioavailable throughout our study area. The affects on mussel growth are correlated to, and likely associated with exposure to methylmercury. However, without supporting sediment and water chemistry data we can not definitively conclude that the measured effects are due only to mercury exposure. The presence of other unmeasured chemicals or environmental factors, such as food availability, may also have influenced mussel growth. The influence of temperature on mussel growth cannot be determined with the current study design. Uncontaminated reference stations with similar temperature ranges would be needed to clarify this issue. Differences in temperature measured across stations may have had more indirect effects, such as in limiting food and nutrient availability, than direct effects. The

data presented in this report should be interpreted in combination with data collected from the other studies in this collaborative effort.

The source of methylmercury that was accumulated by mussels throughout the study area is uncertain. It is likely that the Nyanza site is the primary source, particularly in the areas represented by Stations 3, 4 and 5. It is uncertain whether the source of methylmercury in the wetland area is due to the downstream transport of sediment-bound mercury or other more localized sources.

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