

## APPENDIX 1-3

Penobscot River Mercury Study

**Update to the Phase I Report**

Report to:  
Judge John Woodcock  
U.S. District Court (District of Maine)  
Bangor, Maine

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## **TABLE OF CONTENTS**

EXECUTIVE SUMMARY	vii
OVERALL DATA SUMMARY	xv
Riverine Sediments	xv
Wetlands	xvi
Long Sediment Cores	xvii
Mercury in Invertebrates	xviii
Mercury in Fish	xix
Mercury in Birds	xxi
Conclusions	xxiii
INTRODUCTION	1
METHODS	1
Field and analytical contractors	1
Sampling design	1
Analytical methods	2
Quality assurance /quality control program	2
Sampling methods	4
Riverine sediments	4
Long cores	11
Methyl mercury in wetlands	11
Mercury in biota	11
RESULTS AND DISCUSSION	22
Mercury in sediments	22
Mercury in riverine sediments	22
Mercury in long sediment cores	32
Mercury in wetland soils	37
Mercury in biota	45
Mercury in invertebrates	45
<i>Nereis</i> (Polychaete) worms	45
<i>Macoma</i> clams	47
Soft-shelled clams	49
European green crabs	51
Rock crabs	53

Mercury in fish	56
Tomcod	56
American eels	59
Banded killifish	63
Rainbow smelt	66
Winter flounder	69
Golden shiners	72
Mercury in birds	75
Double-crested cormorants	75
Black guillemots	78
Nelson's sharp-tailed sparrows	81
Song sparrows	84
Swamp sparrows	88
Red-winged blackbirds	92
Virginia rails	94
Other Passerines and shorebirds	96
Belted kingfishers	98
Osprey	102
Bald eagles	105
 CONCLUSIONS	 106
General Conclusions	106
Phase II of the Study	110
 REFERENCES	 112
 APPENDICES	
Appendix 1. Raw data for total Hg and TOC (total organic carbon) from Core D-01.	
Appendix 2. Raw data for total Hg and TOC (total organic carbon) from Core D-02.	
Appendix 3. Raw data for total Hg and TOC (total organic carbon) from Core SC-01.	
Appendix 4. Raw data for wetlands sampled in August 2007, including total and methyl mercury (THg and MeHg), and total organic carbon (TOC).	
Appendix 5. Total and methyl mercury (THg and MeHg), and total organic carbon (TOC) in the top 3 cm of estuarine sediments of Penobscot Bay and St. George River estuary.	



Appendix 6. Mean total Hg and methyl Hg (with sample sizes, standard deviations, and ranges) for *Nereis* worms sampled at sites in the Orrington-Bucksport (OB) and Estuary (ES) reaches, 2006, all sample times combined.

Appendix 7. Mean total Hg and methyl Hg (with sample sizes, standard deviations, and ranges) for *Nereis* worms captured in three different sample periods in 2006.

Appendix 8. Figure of mean total weights of *Nereis* worms by sample site.

Appendix 9. Figure of mean total mercury in *Nereis* worms by sample site, 2006

Appendix 10. Raw data for *Macoma* clams sampled in 2006 in OB and ES sampling reaches.

Appendix 11. Raw data for mercury in soft-shelled clams (*Mya arenaria*), 2006.

Appendix 12. Raw data for mercury in green crab (*Carcinus maenas*) sampled in 2006.

Appendix 13. Raw data for size, total mercury and methyl mercury in rock crabs, 2006.

Appendix 14. Sampling locations for small fish in sampling reaches OB and ES, 2006. The original site name, the geographic ID originally given by Normandeau field staff, fishing method, amended site name and geographic coordinates are given.

Appendix 15. Raw data for size, total mercury and methyl mercury in tomcod sampled in the Penobscot River and estuary in 2006. Note that sample locations have changed from those given in the Phase I report.

Appendix 16. Raw data on size, age, total mercury and methyl mercury in American eels sampled from the Penobscot River, 2007.

Appendix 17. Mercury levels in banded killifish (*Fundulus*) sampled in the lower Penobscot River and at one site in Penobscot Bay.

Appendix 18. Raw data for length, total mercury and methyl mercury in rainbow smelt capture at Penobscot River and Bay sites, 2006.

Appendix 19. Raw data for size, total mercury and methyl mercury in winter flounder, 2006.

Appendix 20. Size, total mercury concentrations and methyl mercury concentrations for golden shiner (*Notemigonus crysoleucas*) collected in the lower Penobscot River, 2006.

Appendix 21. Total mercury ( $\mu\text{g/g w.w.}$ ) in the eggs of double-crested cormorants sampled in the Penobscot River and estuary in 2007.

Appendix 22. Total mercury ( $\mu\text{g/g w.w.}$ ) in the eggs of black guillemots sampled in the Penobscot estuary in 2007.

Appendix 23. Total mercury concentrations ( $\mu\text{g/g w.w.}$ ) in the blood of Nelson's sharp-tailed sparrows in Mendall Marsh (Penobscot River) and reference areas in Maine.

Appendix 24. Mercury concentrations in the blood of song sparrows ( $\mu\text{g/g w.w.}$ ) sampled at various sites in 2007.

Appendix 25. Mercury concentrations in the blood and feathers of swamp sparrows ( $\mu\text{g/g w.w.}$ ) sampled at various sites in 2007.

Appendix 26. Raw data for mercury in red-winged blackbirds sampled in Mendall Marsh in 2007.

Appendix 27. Total mercury concentrations in the blood of Virginia rail ( $\mu\text{g/g w.w.}$ ) sampled at Mendall Marsh and a reference site (Scarborough Marsh) in 2007.

Appendix 28. Total mercury (THg) in the blood and feathers of passerine and shorebirds sampled at Mendall Marsh and other sites, 2007.

Appendix 29. Raw data for total mercury concentrations ( $\mu\text{g/g w.w.}$ ) in the blood of belted kingfisher chicks and adults and in adult feathers sampled at various sites in the Penobscot basin, 2007.

Appendix 30. Raw data for mercury in osprey (*Pandion haliaetus*) chick and adult blood and feathers.

Appendix 31. Mercury in blood and feathers of bald eagle (*Haliaeetus leucocephalus*) chicks.

Appendix 32. Mercury dissolved in water and on suspended particles in the Penobscot River and estuary. This material is reproduced from the Phase I report.

Appendix 33. Mercury in freshwater snails in the Penobscot River. This material is reproduced from the Phase I report.

Appendix 34. Mercury in periwinkles in the Penobscot estuary. This material is reproduced from the Phase I report.

Appendix 35. Mercury mussels in the Penobscot estuary. This material is reproduced from the Phase I report.

Appendix 36. Mercury in lobsters from the Penobscot estuary. This material is reproduced from the Phase I report.

Appendix 37. Mercury in mink and otter from the Penobscot system. This material is reproduced from the Phase I report.

## ***Executive Summary***

The following is an overview of our present understanding of mercury in the Penobscot ecosystem resulting from Phase I of the Penobscot River Mercury Study. Phase I of the study was shaped by two orders of the court. We were to determine:

1. “the extent of the existing harm to the Penobscot River and Bay south of the plant site”,
2. “the need for a remediation plan, if any”.

### **Extent of existing harm**

Phase I of the study has shown that the lower Penobscot River and Bay are contaminated with industrial mercury, and that mercury concentrations in some of the biota in these contaminated areas are high enough to be of concern for both the organisms themselves and for human consumption. Most of the mercury in the biota is methyl Hg, a very toxic form of mercury. Methyl mercury biomagnifies in food chains and we found that the biota with the highest mercury concentrations were at the top or near the top of food chains. Not all of the biota were at concentrations that experts considered to be toxic levels, but several species of wetlands song birds and shore birds were at levels considered to be toxic (Table i). Cormorants, guillemots, and eels, three aquatic species, were also considered to be at risk. Because of depleted fish stocks there is limited human consumption of finfish from the Penobscot; however we were able to sample eels and their concentrations exceeded agency guidelines. At some locations two species of shell fish, lobster and rock crab, approached or exceeded Maine DEP guidelines for human consumption. The geographic pattern of mercury concentrations in several species revealed higher concentrations at locations closer to the HoltraChem site, which is consistent with HoltraChem being the major source of mercury to the river (Table i). We repeated samplings of key organisms during year two of the study, and found similar concentrations and geographic patterns.

The reason that methyl Hg concentrations are high in the upper levels of the aquatic and wetland food chains is that methyl Hg concentrations in river sediments and in the riparian wetlands, which are closely connected to the river, are high in methyl Hg concentration. Methyl Hg is produced in the aquatic sediments and wetland soils by bacteria that convert inorganic mercury to the much more toxic methylated form. Our data have conclusively shown that in the Penobscot sediments and in the wetlands

there is a direct positive relationship between the concentration of inorganic (industrial) mercury in sediments and soils and the quantity of methyl Hg that is produced by the bacteria (Figure I, A&B). Therefore areas contaminated with high levels of inorganic Hg also have high levels of methyl Hg.

We also now know the geographic extent of inorganic mercury contamination of the ecosystem. The lower river is contaminated with industrial mercury from a point above the HoltraChem site southward and into Penobscot Bay (Figure ii). In the river, mercury concentrations in surface sediments are about 30 times higher than background concentrations. In deeply buried sediments of Southerly Cove near the HoltraChem site, mercury concentrations are least 1300 times higher than background concentrations. The high level of surface sediment contamination extends into the upper bay including Fort Point Cove and up the tidal reaches of the Orland River. In the deeply buried sediments of Fort Point Cove in the upper estuary, which have been deposited since the 1960's, maximum mercury concentrations are about 80 times higher than background concentrations, suggesting that that mercury contamination of the Penobscot River and Bay in the past was much greater than it is presently. The mercury in the most contaminated reaches of the river and upper bay has been distributed by the twice daily tidal mixing of the upper bay and lower river. As a result surface sediment mercury concentrations and wetland mercury concentrations are quite consistently high throughout the lower river and upper bay. South of Fort Point Cove mercury concentrations dissipate (Figure ii), but are still above background concentrations at our farthest southward sampling point, an east-west sampling transect offshore of Rockland.

Overall these distribution data are very solid and consistent. We sampled the river and bay sediments 6 times at 35 sites over a one year period and this geographic pattern described above was consistent on each occasion. We also re-sampled mercury concentrations of several key species during a second summer field season and both years found similar geographic patterns of methyl Hg concentration in the biota.

Our studies have shown that mercury methylation is faster in the surface sediments than in deeper sediments, and in the wetlands as compared to the aquatic sediments. This means that methyl Hg concentration in the surface sediments and wetland soils is the key in determining the supply of methyl Hg to the food chain. Knowing that high inorganic mercury concentrations are the primary factor stimulating the rate of methyl Hg production, where inorganic mercury concentrations are high, and knowing where methylation is occurring in the Penobscot system are the basis for the design of both active and passive remediation measures. If inorganic mercury concentrations could be reduced in the surface sediments and in the wetlands, either by active mitigation

measures or by natural attenuation, bacterial production of methyl Hg would slow and concentrations of methyl Hg in the food chain organisms and top predators would decline.

In the Phase I report we concluded that there was extensive harm to the river and bay south of the plant site as a result of mercury contamination. Analyses of additional data, which are presented in this update, support and extend this conclusion.

### **Need for a remediation plan**

To determine the need for a remediation plan, if any, we bounded our decision making process by establishing 4 criteria, which were used to evaluate our data. These criteria were based on:

1. Comparison of concentrations of mercury seen in the Penobscot system to available agency guidelines (NOAA, MDEP, and USEPA) for toxic effects on benthic organisms and for human consumption,
2. Evaluations of mercury concentrations in biota by toxicologists and by comparisons to the scientific literature on toxic effects,
3. Geographical patterns of the distribution of mercury within the Penobscot system, especially in relation to the HoltraChem plant site, and
4. Comparisons of mercury concentrations in the Penobscot to other known uncontaminated and contaminated sites.

We collected data that satisfied each of the above criteria. Some examples are listed below, with the criteria number following in bold text.

- Concentrations of Hg in the sediments of the lower Penobscot River and upper bay are higher than NOAA levels of concern for toxic effects on benthic biota. **1**
- Some lobsters, rock crab, tomcod, and eels, are at levels of methyl Hg that exceeded the Maine DEP criteria for protection of human health. **1**
- Mercury concentrations in species of songbirds and shore birds are high compared to levels of concern for possible toxic effects on the birds themselves.

Mercury in cormorant eggs in the upper estuary approached or exceeded levels thought to impair reproduction. Eels may also be at risk of toxicity. **2**

- Clear evidence of geographic patterns of mercury concentration with increasing levels of mercury at locations closer to the HoltraChem site was found for particles suspended in water, for river and bay sediments, and in wetlands adjoining the lower river and bay. **3**
- Mercury in many species of biota, including periwinkles, mussels, lobster, tomcod, cormorants, were found to be higher in the lower river and upper estuary than in the outer estuary, a pattern that was consistent with a large source of Hg in the lower river. **3**
- Hg in mussels was found to be high compared to other sites in Maine and to most other sites in the United States. Mercury concentrations in the blood of songbirds inhabiting Penobscot wetlands were very high compared to songbirds in reference areas in other parts of Maine. Hg levels in cormorant eggs were relatively high compared to other locations in Maine. **4**
- Mercury concentrations in sediments of the lower Penobscot River and upper Bay are approaching or higher than mercury concentrations at other contaminated sites. **4**

As a result of these evaluations we recommend to the court that the study proceed to Phase II with the overall goal being to determine if there are feasible and practical remedial measures that could be applied to the Penobscot River and Bay.

### **Elements of a remediation plan**

Phase II of the study is addressing our third charge from the court, which was:

“(3) the elements of, and schedule for, completion of such a remediation plan”

Although it was not the primary goal of Phase I of the study, this work did produce important data that are pertinent to Phase II of the study. These data were used to shape the Phase II proposal, and they are now being used to narrow the options we need to explore to remediate the system. Important lessons learned are:

- Methyl Hg concentrations in the Penobscot system are mainly determined by total Hg concentrations in surface sediments and wetland soils. Therefore, remediation measures that lower total Hg concentrations, or, that lower the availability of mercury to methylating bacteria in wetlands, and aquatic sediments, will reduce concentrations of methyl Hg in those sediments and wetlands and methyl Hg concentrations in biota that depend on sediment-based food chains.
- Now that the overriding importance of total Hg concentration is understood, it is imperative that any significant ongoing sources of mercury to the lower Penobscot River (from the HoltraChem site and elsewhere) be stopped. Failure to do this will indefinitely postpone the recovery of the system.
- Preliminary sediment coring data suggest that there are long term burial sites for mercury in the Penobscot system. So, if significant ongoing sources can be stopped, natural attenuation of the system will proceed.
- Mendall Marsh is an area of special concern. Mercury concentrations in songbirds and shorebirds living at Mendall Marsh are especially high compared other contaminated wetlands in the lower Penobscot. It is the largest contiguous area of marsh in the Penobscot system, it is an important breeding habitat for wetland birds, and it is a source of recruitment for inhabiting other Penobscot wetlands. Thus remediation of this site should be a high priority, if possible.
- We see two general approaches to remediation, neither of which is mutually exclusive. First, if we can demonstrate that the process of natural attenuation is occurring quickly enough that the high concentrations of mercury in surface sediments and wetlands are being buried at a reasonable rate; then allowing the system to recover without intervention may be the best possible solution. Second, if natural attenuation is not occurring quickly enough, or if there are particular parts of the system that are amenable to active remediation, and if there are practical and cost effective methods that could be used, then active remediation will be considered.



Biota group	Concentrations high compared to other areas?	Geographic patterns consistent with HoltraChem?	Levels of concern for human consumption?	Levels of concern for toxic effects?
Periwinkles	No	Yes	n/a	No
Freshwater Snails	No	No	n/a	No
Lobster	No	Yes	Yes	No
Mussels	Yes	Yes	No	No
Nereis worms	No	Yes	n/a	No
Soft-shelled clams	Yes	Yes	No	No
Macoma clams	?	Yes	n/a	No
Green crabs	?	Yes	n/a	No
Rock crabs	Yes	No	Yes	No
Tomcod	?	Yes	n/a	No
Eels	Yes	Yes	Yes	Yes
Killifish	Yes	Yes	n/a	No
Smelt	Yes	Yes	No	No
Flounder	Yes?	No	n/a	No
Golden shiners	Yes	?	n/a	No
Songbirds	Yes	?	n/a	Yes
Shorebirds	Yes	?	n/a	Yes
Cormorants	Yes	Yes	n/a	Yes
Guillemots	Yes	?	n/a	Yes
Kingfishers	No	No	n/a	No
Osprey	No	Yes	n/a	No
Bald eagles	No	?	n/a	No
Otters	No	No	n/a	No
Mink	No	No	n/a	No

Table i. Summary of conclusions regarding Hg levels in all species of biota that have been sampled to date. See text for specific groups for detailed discussion. n/a = not applicable. ? = not certain; information lacking.

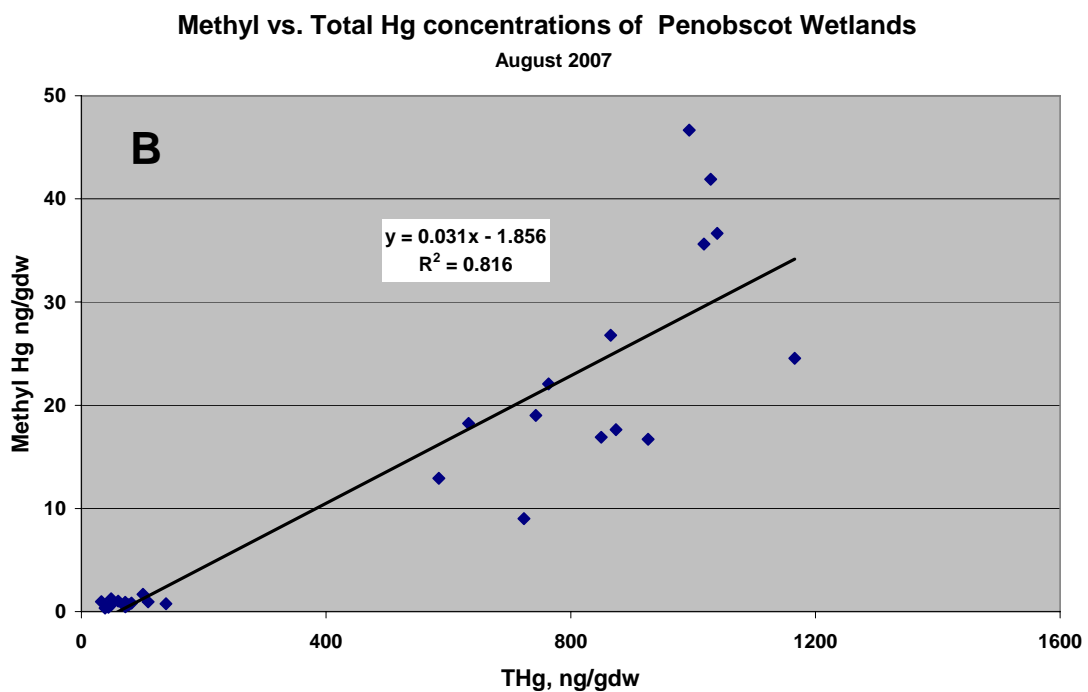
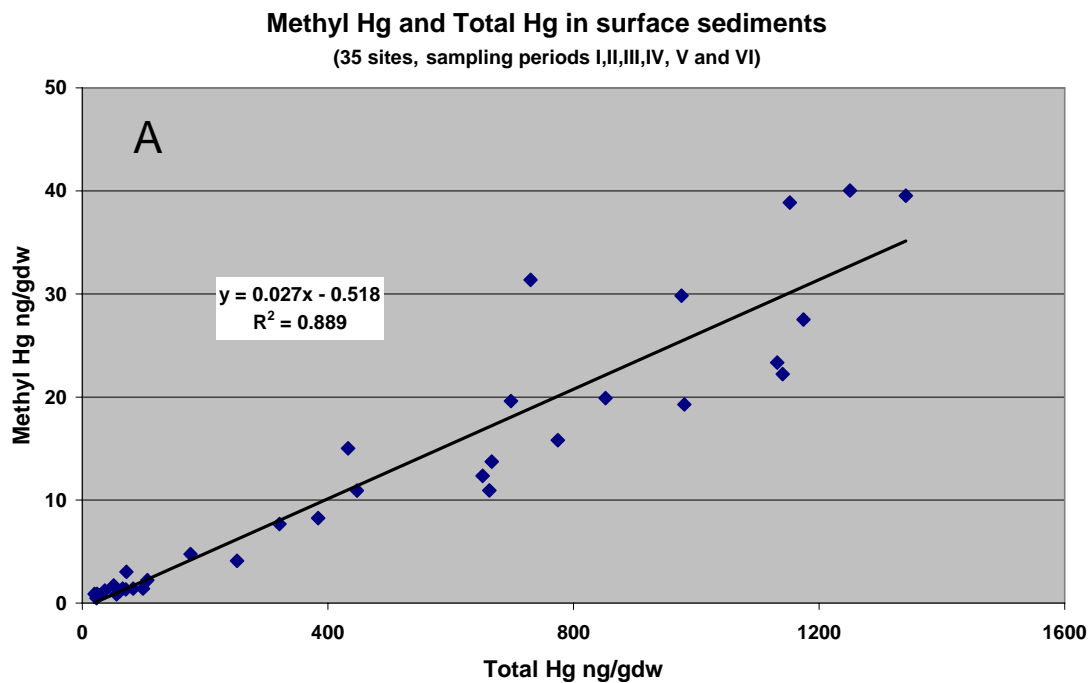


Figure i. Average concentrations of methyl Hg vs total Hg in surface riverine sediments (A) and wetland soils (B), showing that the concentrations of methyl Hg are dependent on the concentrations of total Hg.

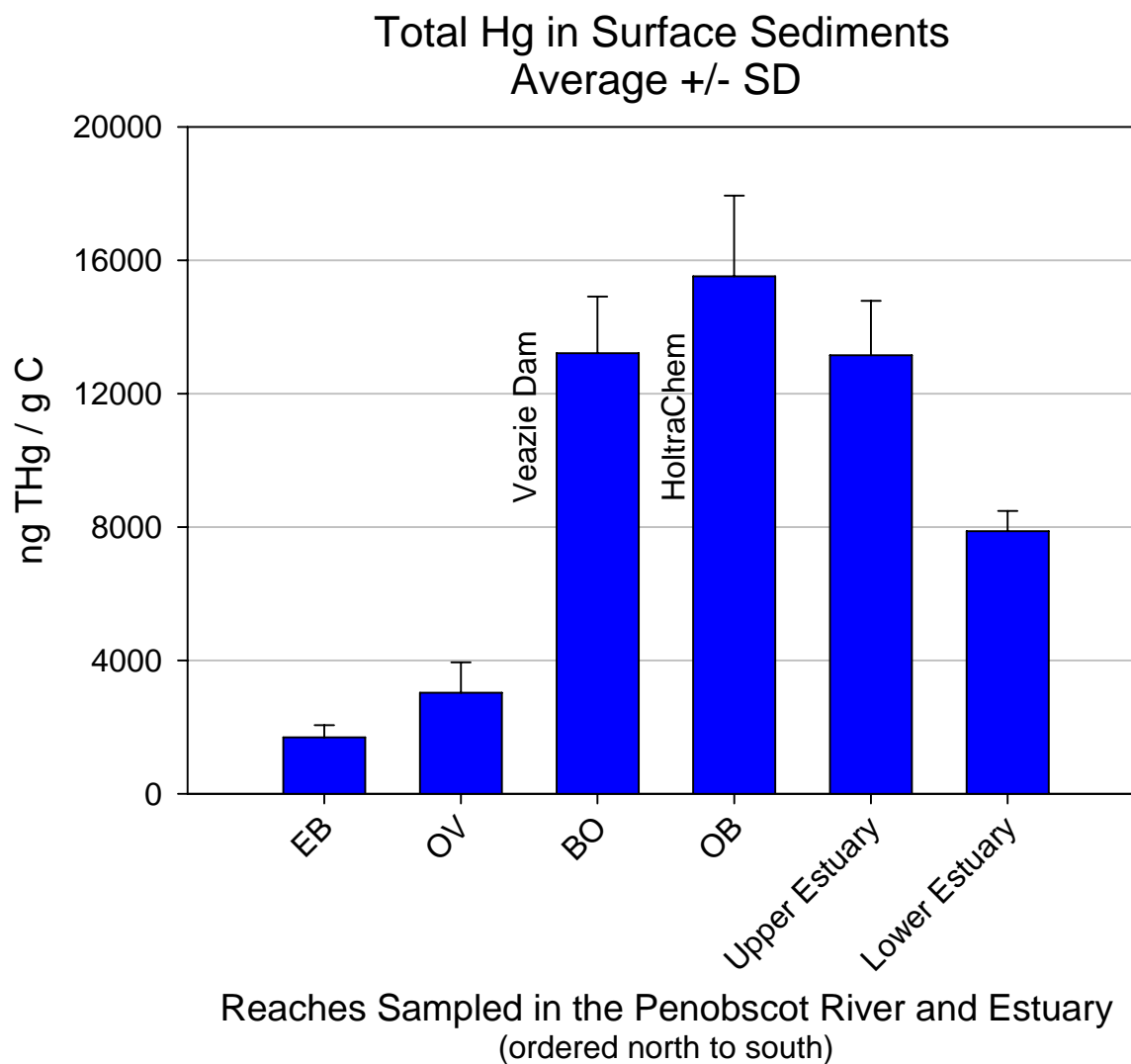


Figure ii. Concentrations of total Hg in surface sediments of the Penobscot River and Estuary. The East Branch of the Penobscot River (EB) is upstream of all known industrial point sources of Hg. The Old Town to Veazie reach (OV) is downstream of paper mills, which have used Hg in the past. High mercury concentrations in the Brewer to Orrington reach (BO) likely result from upstream/tidal movement of Hg. The Orrington to Bucksport reach (OB) extends to the mouth of the river. The upper estuary includes sampling sites north of Ft. Point in Penobscot Bay; the lower estuary includes sampling sites south of Ft. Point (see Figure 7).

## ***Overall Data Summary***

This report provides an update to the Phase I report for the Penobscot River Mercury Study (Bodaly et al. 2008). Additional data in the update include information on mercury (Hg) in sediments taken from the fifth and sixth sampling periods of 2007, on methyl Hg in wetlands in the lower river and upper estuary, and on Hg in cores taken from Southerly Cove, adjacent to the HoltraChem site, and from the upper Penobscot estuary. New Hg concentration data for a number of biota species are presented, including fish, shellfish, and birds. Some of the new biota data are for species that were sampled in 2006 and re-sampled in 2007 to confirm concentrations and geographic trends seen in 2006 (e.g. sparrows and cormorants). In order to have a document which contains all of the pertinent data from the Study to date, sections on mercury in water, mercury on suspended particles, and mercury in some species of biota (freshwater snails, periwinkles, mussels, lobster, mink and otter) that were part of the Phase I report are included here in appendices.

### ***Riverine sediments***

New data presented here on Hg concentrations in riverine sediments at our regular sampling stations extend the seasonal picture to spring and early summer. Data on Hg in sediments presented in the Phase I report for the first four sampling periods are re-presented in this report, individually for each sampling period, rather than as averages for all sampling periods. In the Phase I report, it was concluded that total Hg was high in the lower river and upper estuary of the Penobscot. New data for the fifth and sixth sampling periods confirms that concentrations of total Hg in riverine sediments were slightly elevated in the Old Town – Veazie reach compared to the East Branch. This increase was likely caused by mercury release from the paper mills upstream of the Veazie Dam. As in the previous summers sampling, there was large increase in total Hg concentration in the lower river where the HoltraChem facility is located. Concentrations decreased in the outer estuary as the mercury dispersed.

Rates<sup>1</sup> of microbial methyl Hg production and methyl concentrations in the sediments of the lower river sediments and upper estuary appear to be elevated by the presence of the high total Hg concentrations. As was found for the first four sampling periods (see Phase I report), we found a strong relationship between methyl Hg concentrations and total Hg concentrations when the river and estuary were sampled again in May and July of 2007 (Fig. 14). Methyl Hg production was found to be occurring mostly in the surface sediments to a depth of 3 cm below the sediment-water interface.

A corollary of these observations is that reducing total mercury concentrations in surface sediments, where methylation is particularly active, would reduce methyl Hg concentrations in sediments, and ultimately methyl Hg concentrations in the food web of the river.

## ***Wetlands***

We conducted a spatial survey of total and methyl Hg concentrations in riparian wetlands during the summer of 2007, to determine the geographic extent of mercury contamination in wetlands. We hypothesized that some of the Penobscot wetlands would be sites of high rates of methyl Hg production because of discoveries of high rates of methyl Hg production in wetlands at other estuarine locations. Total Hg concentrations were presented in the Phase I report and methyl Hg concentrations are presented in this update. Total Hg concentrations in riparian wetlands along the Penobscot River and estuary were found to be heavily contaminated from below WO5 at Brewer to the southern tip of Verona Island, including wetlands in the Orland River. Wetlands south of Verona Island were not significantly contaminated.

Methyl concentrations in the contaminated wetlands were found to be about twice as high as in the contaminated riverine sediments, confirming our hypothesis that the Penobscot wetlands are important sites of methyl Hg production. As in the riverine sediments, methyl Hg production in the contaminated wetlands was primarily stimulated by the elevated concentrations of total (inorganic) mercury in the wetlands soils. It was also found that methyl Hg production was further stimulated in several wetlands in a

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<sup>1</sup> The rate of methyl Hg production is defined as

$$R = k_i * [HgII]$$

where R is the rate of methyl Hg production (ng MeHg/g/sediment/day),  $k_i$  are the rate constants that affect the intensity of methyl Hg production (e.g. pH, DOC concentration, temperature, microbial activity), and [HgII] is the concentration of HgII available for uptake by the methylating bacteria.

transition zone between freshwater wetlands and salt marshes in the vicinity of Mendall Marsh. There are several other environmental factors, in addition to inorganic mercury concentration, that stimulate mercury methylation. Determining which of these factors is further enhancing methylation in the transition zone wetlands is an important topic of investigation for Phase II of the study. In addition to these studies, sampling of two freshwater wetlands, two transitional wetlands and two salt marshes over an annual cycle is underway to determine the seasonality of methyl Hg production in wetlands. All of this information is necessary to evaluate the possibility of active remediation for some of these wetlands where methyl mercury production and wildlife mercury concentrations have been found to be particularly high.

### ***Long Sediment Cores***

In 2007, a number of long sediment cores were taken from the lower Penobscot River and upper estuary to provide some preliminary results on the burial of Hg mercury in the lower Penobscot River and upper estuary. Sediment cores allow for a look back in time because at sites where the sediments are undisturbed by natural or human processes, deeper layers in cores represent older sediments. When mercury is buried permanently more than about 5 cm below the sediment-water interface it is below the surface zone of sediments where bacteria are most active and methyl mercury is produced. Thus it is permanently out of the ecosystem and is no longer contributing to the contamination problem – a process known as natural attenuation.

Three of the cores taken in 2007 have been analyzed for depth profiles total Hg concentrations. Two of the cores were taken from Fort Point Cove and one was taken from Southerly Cove, adjacent to the HoltraChem site. The highest concentrations of total Hg in all three cores were in quite deep layers, about 30 to 40 cm in depth. These peak concentrations were about 3 times as high as surface concentrations in the cores from Fort Point Cove, and about 20 times as high as surface concentrations in the core taken from Southerly Cove. In one of the cores, mercury concentration in the deepest sediment layers was at very low levels, representing background concentrations before the operation of HoltraChem or the presence of other significant sources of Hg in the watershed. These results indicate that concentrations of Hg in sediments were much higher in the past, probably during the early period of operation of HoltraChem, than they are now. The extremely high concentration of total Hg in the deepest layer of the core taken near the HoltraChem site is confirmatory of a HoltraChem source for Hg contamination in the lower Penobscot. The cores also showed generally decreasing levels near the surface over more recent times suggesting that the process of natural attenuation is ongoing, but at an as yet unknown rate.

One of the cores taken from Fort Point Cove has now been dated as a pilot test for the natural attenuation study that will begin in the summer of 2009. Two methods of radioisotopic dating were used (Pb-210 and Cs-137). Both methods were in close agreement and they demonstrated that the sediment layers between the sediment-water interface and 30cm depth have been undisturbed since about 1960. At this coring site, burial of mercury below the zone of mercury methylation has been continuous since about 1960. A more extensive program of sediment coring is needed to confirm these early results. This will be conducted in 2009 giving us more geographic coverage in the lower river and estuary to establish a rate of natural attenuation of Hg in the Penobscot system.

### ***Mercury in Invertebrates***

One of the most difficult aspects of the Penobscot study is that because of the transition from fresh to salt water in the Penobscot River/estuary, no species exist throughout the system, so geographical patterns are difficult to establish. We have attempted to minimize this difficulty by sampling species with wide distributions, by sampling species that feed in a similar way in fresh and salt water habitats, and by trying to relate concentrations of mercury in biota to concentrations of mercury in sediment and water. If Hg in a biota species reflected Hg in sediments, it would be expected that levels would be noticeably higher in the Brewer-Orrington, Orrington-Bucksport and upper estuary sampling stations, as compared to areas further upstream or downstream. If Hg in a biota species reflected Hg in suspended particulates, it would be expected that levels would be noticeably higher in the OB and upper estuary sampling stations, as compared to further upstream or downstream.

In 2006 and 2007, samples of many species of biota were taken to determine concentrations of Hg and the geographic distribution of those concentrations. Some of these species are reported on for the first time here, whereas some species were sampled in both 2006 and 2007, thus providing comparisons of concentrations in more than one year. The invertebrates reported on here include *Nereis* (Polychaete worms), two species of clams, and two species of crabs. Data on Hg in freshwater snails, periwinkles, mussels, and lobsters were included in the Phase I report, and are presented again in this report in the form of appendices. Table 3 provides a summary of patterns seen in various species of biota.

#### ***Nereis* worms**

Total Hg concentrations in *Nereis* worms sampled in intertidal sediments in 2006 were generally similar among sample sites in the Orrington-Bucksport sampling reach and

sites in the upper estuary. South of Verona Island, total Hg levels declined significantly. Percent methyl Hg found in *Nereis* worms did not vary significantly among reaches. Total Hg levels in *Nereis* in the OB (Orrington-Bucksport) reach of the Penobscot River and in upper Penobscot Bay were greater than *Nereis* sampled in an uncontaminated estuary on the southeast coast of England and a contaminated estuary on the north coast of the Netherlands, but lower than levels in *Nereis* reported for two other contaminated estuaries, one in England and one in Australia.

### **Soft-shelled clams**

Both total Hg and methyl Hg concentrations in soft-shelled clams were significantly greater north of Fort Point than further to the south. The proportion of the total Hg that was methyl Hg did not vary significantly by location. Concentrations in the Penobscot estuary were much higher than at both reference and contaminated sites in the St. Lawrence estuary in Quebec, Canada.

### **Macoma clams**

Total Hg in *Macoma* clams in the lower Penobscot River and upper Penobscot Bay in 2006 did not vary significantly by reach, site or period, however, both methyl Hg and % methyl Hg were significantly greater in the lower river as compared to the estuary.

### **Green crabs**

Green crabs had higher concentrations of both total Hg and methyl Hg in the upper Penobscot estuary compared to the lower estuary in 2006. The proportion of the Hg that was present as methyl Hg did not vary significantly over the sampling area.

### **Rock crabs**

Within the area sampled, total and methyl Hg in rock crabs did not vary with distance from HoltraChem. About ¼ of crabs sampled had methyl Hg concentrations greater than the Maine DEP action level of 200 ng/g w.w. Total Hg concentrations in rock crab in Penobscot Bay were as high or higher as a species with a similar diet (blue crabs) in NY, CT and FL.

## ***Mercury in Fish***

A number of fish species were sampled in 2006 and 2007 and the results of Hg analyses are presented here, as well as a re-presentation of data on Hg in tomcod, originally presented in the Phase I report. Table 3 provides a summary of patterns seen in various species of biota, including fish.



### **Tomcod**

Data on Hg in tomcod from 2006 were re-analyzed based on new information on sample locations. Hg in tomcod, as presented in the Phase I report, was found to be higher in the Brewer-Orrington reach, intermediate in the Orrington-Bucksport reach and lower in the upper estuary. A majority of fish caught in the Brewer-Orrington reach exceeded the Maine DEP action level for methyl Hg.

### **Eels**

Total Hg concentrations in the muscle of American eels were significantly greater in the Penobscot River reaches directly influenced by HoltraChem than in the upstream OV (Old Town-Veazie) reach. The majority of eels sampled in the lower Penobscot had muscle methyl Hg levels exceeding the 200 ng/g action level defined by the Maine Department of Environmental Protection. Hg in eels sampled from the lower Penobscot was higher than in other Maine rivers and in the St. Lawrence River estuary (Quebec, Canada) after adjusting for age. Also, Hg concentrations in eels in the lower Penobscot River exceeded levels reported in European eels in Italy, Bosnia, and France, except for notably larger eels sampled near Liverpool, England at a site historically contaminated by the chlor-alkali industry.

### **Killifish**

Killifish (*Fundulus*) were sampled in the two lower reaches of the Penobscot River (Brewer-Orrington and Orrington-Bucksport) with one sample collected in the estuary (ES). Total Hg levels were significantly greater in the OB reach, and all methyl Hg levels in that reach exceeded the Maine DEP methyl Hg action level of 200 ng/g w.w.

### **Smelt**

Rainbow smelt were collected from the OB reach in the Penobscot River and in Penobscot Bay (ES). Both total Hg and methyl Hg levels in smelt, adjusted for fish length, varied significantly among sites, and showed a general decline from HoltraChem to the southernmost sites in Penobscot Bay. Hg in smelt from the lower Penobscot River and upper estuary were higher than in Canadian lakes.

### **Flounder**

Winter flounder were sampled at a number of sites in Penobscot Bay (ES reach) in 2006. Total Hg concentrations in muscle varied significantly by site, after adjustment for fish length, and showed a decline with distance south from HoltraChem. All concentrations of total Hg in winter flounder from Penobscot Bay were significantly greater than found in winter flounder sampled from further east on the coast of Maine.

### **Golden Shiners**

Golden shiners were sampled in the two lowest reaches of the Penobscot River, Brewer-Orrington (BO) and Orrington-Bucksport (OB). Hg levels in this species were statistically equivalent from Brewer to Bucksport; the whole sampling area is contaminated with HoltraChem Hg. Methyl Hg levels exceeded the Maine DEP action level (200 ng MeHg/g muscle w.w.) in 65% of the samples analyzed. Hg was much higher than in golden shiners in Canadian lakes.

### ***Mercury in Birds***

Sampling of various bird species, including eagles, osprey, kingfishers, cormorants, seaducks and songbirds was carried out in 2007 to confirm and extend the observations made in 2006. Patterns seen in various groups of birds are summarized in Table 3.

### **Songbirds and shorebirds**

Hg in songbirds in the Mendall Marsh area was very high in 2007, as was observed in 2006. Species that had Hg in blood that exceeded the level of concern for toxic effects included swamp sparrow, song sparrow, Virginia rail, marsh wren, savannah sparrow, spotted sandpiper, red-winged blackbird, and Nelson's sharp-tailed sparrow. Hg in sharp-tailed sparrows was the highest of these species, as in 2006, at an average of 6.9  $\mu\text{g/g}$  w.w. in blood, compared to levels of concern of 1.2 – 3  $\mu\text{g/g}$ . This average concentration was similar to that found in 2006 (5.7  $\mu\text{g/g}$ ). Hg in Savannah sparrows and red-winged blackbirds sampled at Mendall Marsh was also high (3.2 and 3.8  $\mu\text{g/g}$  w.w. in blood, respectively). Hg in the blood of swamp and song sparrows was highest at Mendall Marsh (averages of 2.4 and 1.7  $\mu\text{g/g}$ , respectively), compared to other sites downstream of HoltraChem and to reference sites. In contrast, Hg in the Veery was very low at Mendall Marsh. This lower concentration in the Veery may have been because of a difference in the food chain of this species. To understand this we will propose to the court to study the wetland foodchains during the 2009 field season.

Some shore birds sampled from Mendall Marsh also had high concentrations of mercury in blood. Virginia rails averaged 1.83  $\mu\text{g/g}$  in blood at Mendall Marsh, as compared to 0.14  $\mu\text{g/g}$  at a reference area. Spotted sandpiper at Mendall were also high (3.3  $\mu\text{g/g}$  in blood; however, only one bird was sampled) whereas Wilson's snipe and killdeer were lower (0.88 and 0.56  $\mu\text{g/g}$ , respectively). These results confirm the finding, based on sampling in 2006, that Mendall Marsh, adjacent to the lower Penobscot River, is an area of concern regarding Hg in wildlife.

### **Cormorants**

Double-crested cormorant eggs were re-sampled in 2007 to compare levels to those in 2006 and to extend the geographic area of sampling. In 2006, there were higher concentrations at the site further up in the estuary compared to sites to the south in the outer estuary. The highest concentrations observed in 2006 approached levels of concern for toxic effects. In 2007, concentrations were similar to the pattern seen in 2006. Hg in cormorants at two of the three most northern sites in the Penobscot estuary were significantly greater than at other locations, while sites further south in the estuary were similar to each other and did not show a decline with distance. The highest average levels seen in 2007 (approximately 0.6 to 0.7  $\mu\text{g/g}$  w.w. in eggs) were less than those seen in 2006, but still approached levels of concern for toxic effects.

### **Guillemots**

Hg in black guillemots was noticeably higher than in cormorants, possibly reflecting their year-round residence in Penobscot Bay. All sampling sites in 2007 were in the outer Penobscot estuary, from near Islesboro Island and further to the south, out of the area of most severe contamination from HoltraChem. Concentrations of Hg in many eggs were higher than levels of concern for sublethal toxic effects.

### **Kingfisher**

Hg concentrations in the blood of belted kingfisher chicks in the area were low and did not reflect geographic extent of Hg contamination from HoltraChem. Kingfishers may be foraging predominately off the mainstem of the Penobscot River and this may account for the lack of reflection of Hg contamination in the river. Total Hg concentrations in chick blood did not exceed levels associated with toxicity.

### **Osprey**

Hg in osprey was determined for both chicks and adults using blood and feather samples collected from riverine and coastal sites in the Penobscot system and from similar habitats in southern Maine. Total Hg in chick feathers and blood declined significantly with distance south from HoltraChem. Hg in chick feathers ranged from an average of 2,690 ng/g fresh weight, in the OB reach downstream of HoltraChem, to an average of 970 ng/g w.w. in lower Penobscot bay. Hg in chick blood also declined significantly with distance south from HoltraChem, from over 100 ng/g w.w. in the lower Penobscot River to about 35 ng/g w.w. in lower Penobscot Bay. Mercury in adult blood and feathers was not consistent with total Hg in osprey chicks, because during the breeding season Hg in adult blood and feathers probably does not reflect exposure from breeding areas in Penobscot Bay. Hg in ospreys from the Penobscot were similar to or lower than those in southern Maine, when comparisons were done between equivalent

habitat types. Hg concentrations in osprey did not approach levels of concern for toxic effects.

### **Eagles**

Bald eagle chick blood and feather samples were collected from nests along the Penobscot River and Bay and along Maine's southern coast in 2007. Late winter snow storms caused high chick mortality in the lower Penobscot River and upper Penobscot Bay, virtually eliminating samples from those areas and greatly reducing the value of the data set for this study. In general, Hg concentrations in chick feathers were greatest at inland sites and lower in coastal areas, probably reflecting the reported shift in diet from predominantly fish at inland sites to predominantly birds and mammals in coastal and marine habitats.

The sampling of mammals was discontinued after 2006, and all data were presented in the Phase I report. Appendices of this report contain the mink and otter data from the Phase I report.

### ***Conclusions***

Most of the results presented in this report are confirmatory of those presented in the Phase I report and strengthen the conclusions presented in that report, that the lower Penobscot River and upper Penobscot estuary are significantly contaminated with Hg. The area of most significant contamination is from South Brewer, about 3 miles upstream of HoltraChem to Fort Point in Penobscot Bay, about 20 miles downstream of HoltraChem. Data presented confirms the high levels of Hg in songbirds and cormorants, in relation to levels of concern for toxic effects. Other biota species are also shown to have quite high levels of Hg in relation to reference areas or other contaminated sites, such as soft-shelled clams, eels, winter flounder, and rails. Specimens of rock crabs (about 1/4 of those sampled), eels (most), killifish (all), golden shiners (most) exceeded the Maine DEP action level for methyl Hg in biota. These conclusions are in addition to those presented in the Phase I report concerning Hg in mussels and songbirds. Some species of biota have individuals with concentrations of Hg that exceed levels of concern for toxic effects, such as a number of species of sparrows, guillemots, shorebirds, and to a lesser extent, cormorants. Levels of Hg seen in populations of American eels may also be causing sublethal toxic effects to some fish.

The data presented here also is in agreement with our conclusion of the Phase I report that the mercury contamination of the Penobscot is consistent with the release of mercury from HoltraChem. A long core taken adjacent to the HoltraChem site had a

very high concentration of Hg in the deepest layer - consistent with HoltraChem being the source of contamination. The geographic pattern of Hg concentrations in *Nereis* worms, soft-shelled clams, *Macoma* clams, green crabs, tomcod, killifish, eels, rainbow smelt, winter flounder, cormorants, songbirds, rails and osprey were consistent with HoltraChem as the dominant source of Hg in the Penobscot system. These data are in agreement with earlier data in the Phase I report for Hg in riverine sediments, Hg in estuarine sediments, and Hg in wetland soils, as well as Hg in periwinkles, mussels and lobsters.

On the other hand, some species of biota were not high in Hg or did not show geographic patterns of Hg concentrations that were consistent with HoltraChem being the dominant source of Hg to the lower Penobscot River. Such species included rock crabs, and wide-ranging bird species such as eagles and birds with localized foraging preferences, including kingfishers, that may reduce exposure to Hg. Included in this list are species for which data was presented in the Phase I report, including mink, otter, and freshwater snails. Although Hg in osprey declined with distance from HoltraChem, levels were not high compared to reference areas or to levels of concern for toxic effects. No samples were available for eagles in the zone of the river shown to be contaminated with Hg from HoltraChem. As was concluded in the Phase I report, wide-ranging bird and mammals species do not show patterns related to the location of the HoltraChem plant, perhaps due to foraging over relatively large distances, to feeding off the Penobscot main stem, to dietary changes unrelated to HoltraChem contamination, or in some cases because of the small sample sizes.

We conclude that the high methyl Hg concentrations found in the biota originate primarily from methyl Hg produced by bacteria active in the surface river sediments and in the riparian wetlands located along the lower river and upper estuary. Methyl Hg production appears to have been stimulated by the presence of the high concentrations of total Hg. Reducing these total Hg concentrations either by active remediation or by natural attenuation would improve the situation.

## INTRODUCTION

This update report is intended to provide additional results from sampling conducted under Phase I of the Penobscot River Mercury Study, and to compare these results to those presented in the Phase I report. The Phase I report, dated January 24, 2008, contained data on mercury in water, suspended particles, sediments, invertebrates, shellfish, fish, birds and mammals but some data were not available for inclusion in that report when it was written. Material on Hg in water, suspended particles, freshwater snails, periwinkles, mussels, lobsters, mink and otters original part of the Phase I report are included as appendices to this report to provide a more complete record of all Study results in one report. New information presented in this report includes data on Hg in riverine sediments that were not available for inclusion in the Phase I report, information on methyl Hg in wetland soils, and data on Hg in a number of species of invertebrates (including *Nereis* (Polychaete) worms, two species of clams, and two species crabs), fish (including tomcod, eels, killifish, rainbow smelt, winter flounder, two species of shiners and Atlantic silversides) and birds (including cormorants, guillemots, songbirds, shorebirds, kingfishers, osprey, and eagles). Sampling of songbirds and cormorants was repeated and expanded to include new sites and species while other bird species are reported on for the first time.

## METHODS

### *Field and analytical contractors*

As for work conducted in 2006, most aquatic sampling was carried out by Normandeau Associates Inc., under the direction of Marcia Bowen, Vice President. Sampling of birds was conducted by staff of BioDiversity Research Institute, under the direction of David Evers, Executive Director. Water, some biota tissues, and some sediments were analyzed by Battelle Marine Sciences Laboratory under the direction of Brenda Lasorsa, Senior Research Scientist. Some biota tissues and some sediments were analyzed by Flett Research Ltd. under the direction of Robert Flett, President. Stable isotope samples were analyzed at Trent University, Water Quality Centre (Holger Hintelmann).

### *Sampling design*

Sampling design for sediments in the fifth and sixth sampling periods was carried out under the original reach-based statistical design of Phase I of the study, outlined in

detail in the Phase I report. The original five sampling reaches and sampling sites were utilized. The sampling reaches are East Branch (EB), Old Town-Veazie (OV), Brewer-Orrington (BO), Orrington-Bucksport (OB), and Estuary (ES). These sampling reaches are shown in Figure 2, reprinted from the Phase I report. The locations of individual sampling sites in each sampling reach are shown in Figures 3-7. Some of the stations in each reach were chosen randomly and some were chosen to represent possible “hot-spots” of methyl Hg production (see Phase I report).

Sampling of other components of the study (such as fish and birds) was usually customized depending on the objectives of the sampling and/or the species of biota being sampled. If sampling locations corresponded to the original sampling sites, the same site designation was used (i.e. reach code and site number, such as OB-1). If sampling was in particular sampling reaches but not at original sampling sites, locations were given new, unique identifying codes following the sampling reach code, e.g. OB-5N-SN for killifish. If sampling was not entirely within the original reach designations, names were provided that were unique to the species or component being sampled. The geographic locations of all sampling locations not part of the original set of sites are provided in the Appendix of this report (or the Phase I report) for each component sampled.

### ***Analytical methods***

The same analytical methods for mercury concentrations were used for the samples reported here as were outlined previously (Kelly 2007), although see below and Kelly (2008) for specific notes related to the analyses of methyl Hg in sediments. Total Hg concentration was determined by EPA Method 7473 (using a Direct Mercury Analyzer). Methyl Hg concentration was determined for the first four sampling periods by EPA Method 1630 with solvent extraction and for the last two sampling periods by EPA Method 1630 with distillation. Data from the first four sampling periods was adjusted to provide comparability with later data obtained using the distillation method (see below).

### ***Quality assurance/quality control program***

A program of several quality assurance/quality control (QA/QC) procedures was put into place to ensure the integrity of the mercury concentration data presented in the report. The objectives of this program are threefold: 1. To ensure that the data produced by the two analytical labs employed by the project are comparable. 2. To ensure that the data produced by both laboratories are accurate. 3. To ensure that sampling procedures are not contaminating samples taken from the field.

These procedures include ultra-clean sampling techniques for water, analyses of standard materials by the analytical laboratories, field blanks, field and analytical duplicates, and inter-laboratory comparisons of various sample types among three internationally recognized laboratories. Detailed methods and results for QA/QC program are provided in two reports to the Study Panel, and a recent update (Kelly 2007, 2008, 2009).

To briefly summarize these QA/QC results, it was found that precision of total and methyl Hg analyses in water were well within the recommended EPA limit, that field blanks for total Hg in water were acceptable whereas there was concern over methyl Hg concentrations in blanks, that sample replication for both total and methyl Hg and for total suspended solids in water was good, that inter-laboratory comparisons for total and methyl Hg in water were acceptable, that analytical variability for total Hg in sediments was suitably low, that variation among field replicates and different laboratories was suitably low for total Hg in sediments, that analytical variability for both total and methyl Hg in biological tissues was suitably low, and that variation among laboratories was suitably low for both total and methyl Hg in tissues.

The only QA/QC question left unresolved from the first year of the Study concerned methods for the analysis of methyl Hg in riverine sediments. This was resolved (Kelly 2008) by a comparison of two commonly used methods (distillation and solvent extraction). The QA/QC program found that the analytical method we were originally using (solvent extraction) was not extracting all methyl Hg from Penobscot sediments. This under-estimation was occurring despite the fact that extraction of methyl Hg from standard materials was highly efficient as expected. This is the first known case of this occurring, and we have concluded that this extraction problem is peculiar to Penobscot sediments.

As a result of this finding, we now know that concentrations of methyl Hg in samples of Penobscot sediments taken in the first four sampling periods were being underestimated. To enable us to properly make adjustments for these underestimates, we undertook an extensive inter-comparison of the two methods. During sampling periods five and six 20% of the sediment samples were analysed using both the extraction distillation methods. We found that the extraction method was underestimating methyl Hg concentrations by a factor of 2.0 (Figure 1). In this update we are presenting the sediment methyl Hg data for all six sampling periods after adjusting (increasing) the concentrations for the first four sampling periods by a factor of 2.0. More details of these comparisons and are given in Kelly (2008). Going forward analyses of methyl Hg concentrations in sediments and wetland soils are being done by the distillation method.



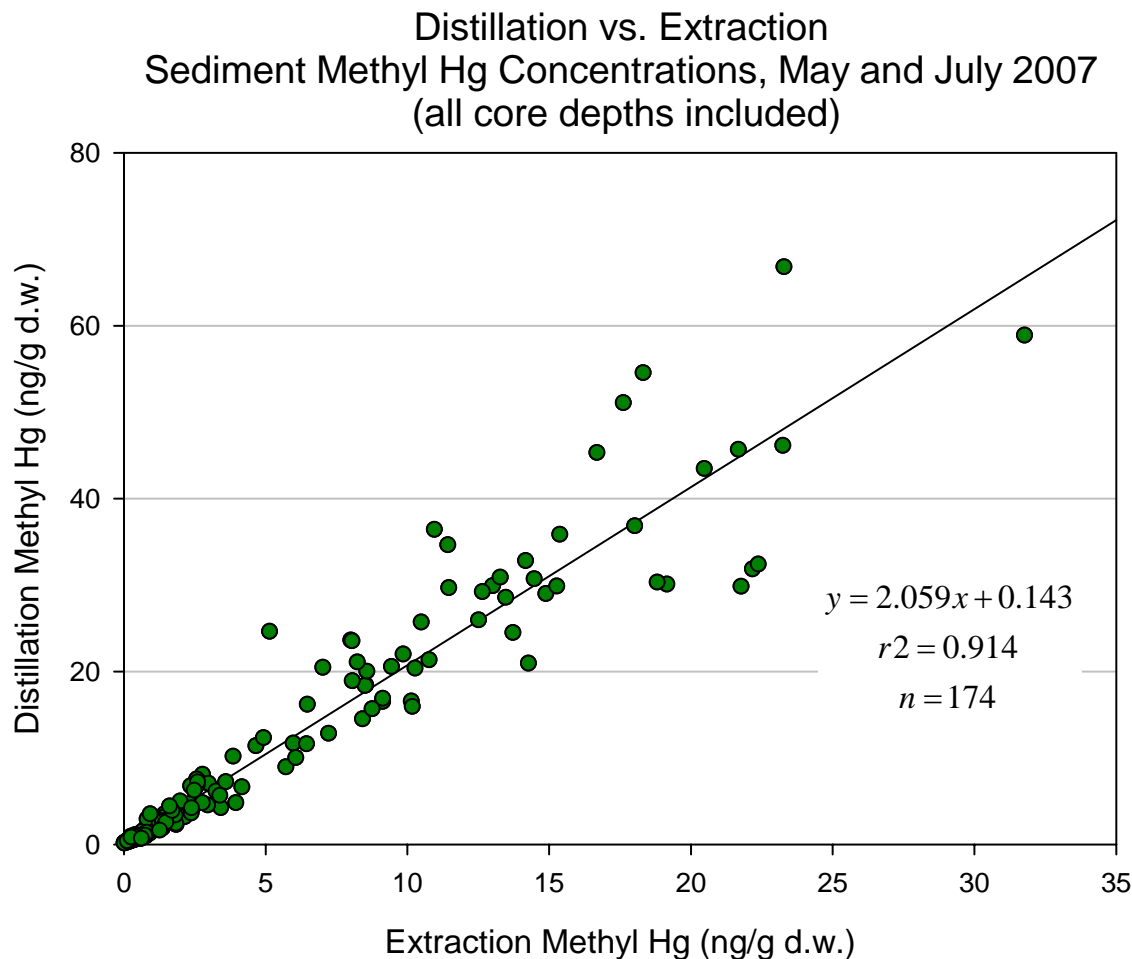


Figure 1. This graph illustrates the two-fold greater methyl Hg concentrations found in sediment using the distillation method as compared to the extraction method.

### ***Sampling methods for riverine sediments and wetland soils***

#### **Riverine sediments**

Methods used for the sampling of sediments at riverine sites in the Penobscot River and estuary were provided in the Phase I report. Briefly, the top 3 cm of sediments were sampled using a 3-inch piston corer at five sites in each of four sampling reaches in the river and at 15 sites in the estuary. Some cores were 3 cm deep; these samples were mixed and analyzed in whole. Some cores were 10 cm deep and were sliced at 1 cm intervals; concentrations for the top 3 cm were calculated as the mean of the top 3 slices. Locations of sampling stations are mapped in Figures 3-7.

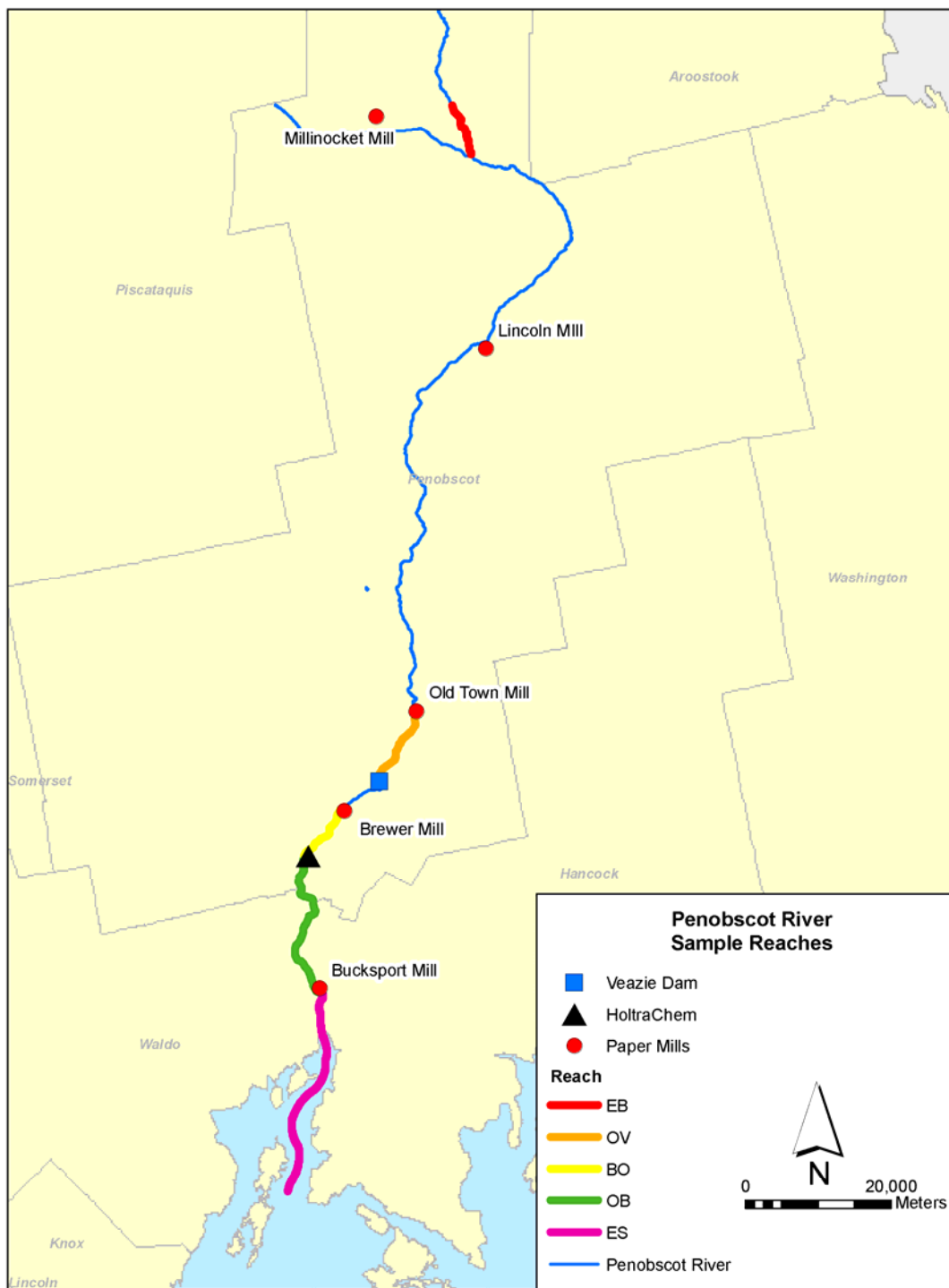


Figure 2. Map of the Penobscot River and estuary showing the locations of sampling reaches, active and inactive paper mills, the Veazie Dam, and the HoltraChem site at Orrington. The locations of sampling sites within each reach are shown in Figures 3 - 7. The river is tidal to a point located between the Brewer Mill and the Veazie Dam .



Figure 3. Location of East Branch sampling sites, 2006-2007.



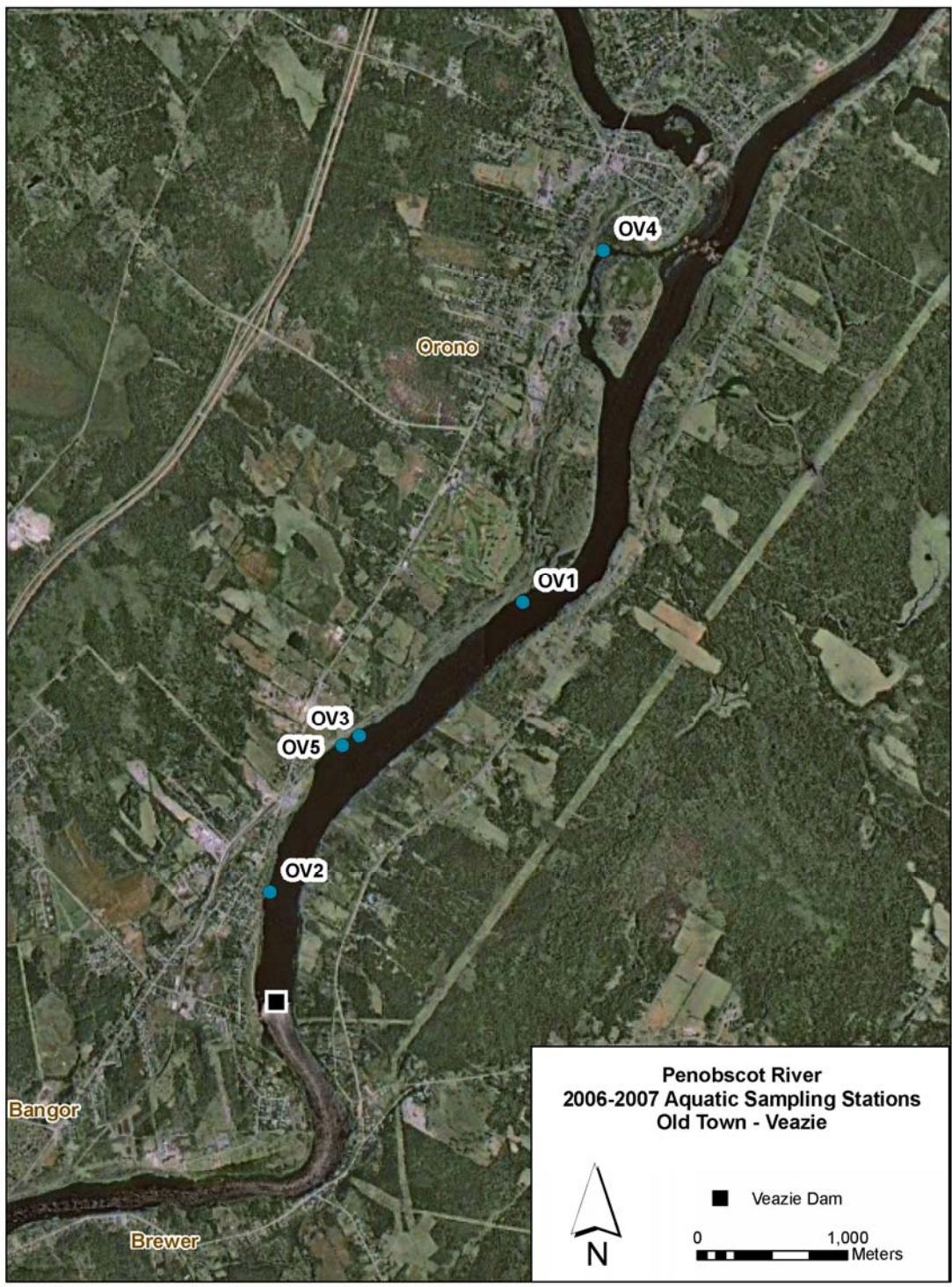


Figure 4. Location of Old Town – Veazie sampling sites, 2006-2007.



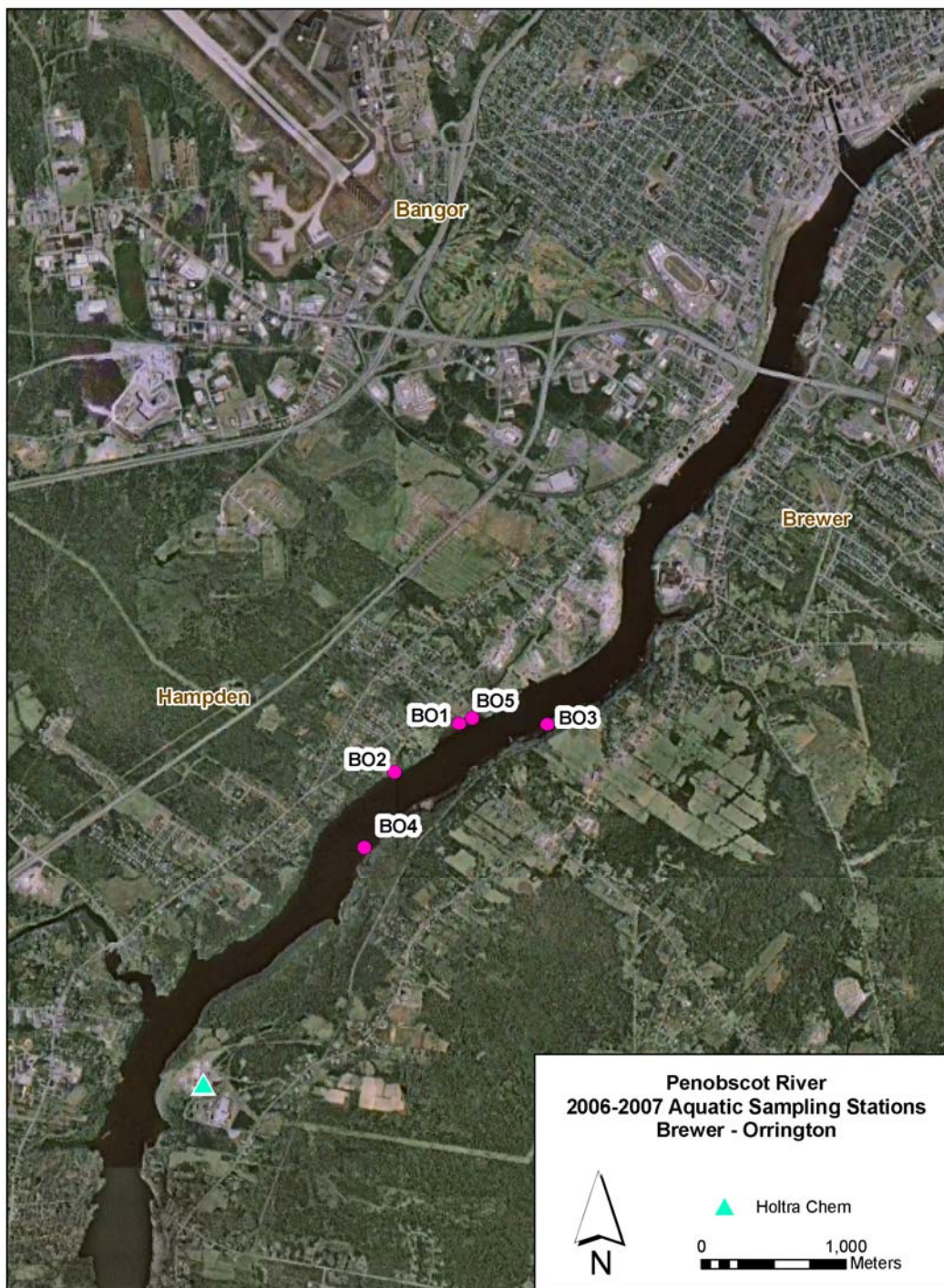


Figure 5. Location of Brewer – Orrington sampling sites, 2006-2007.



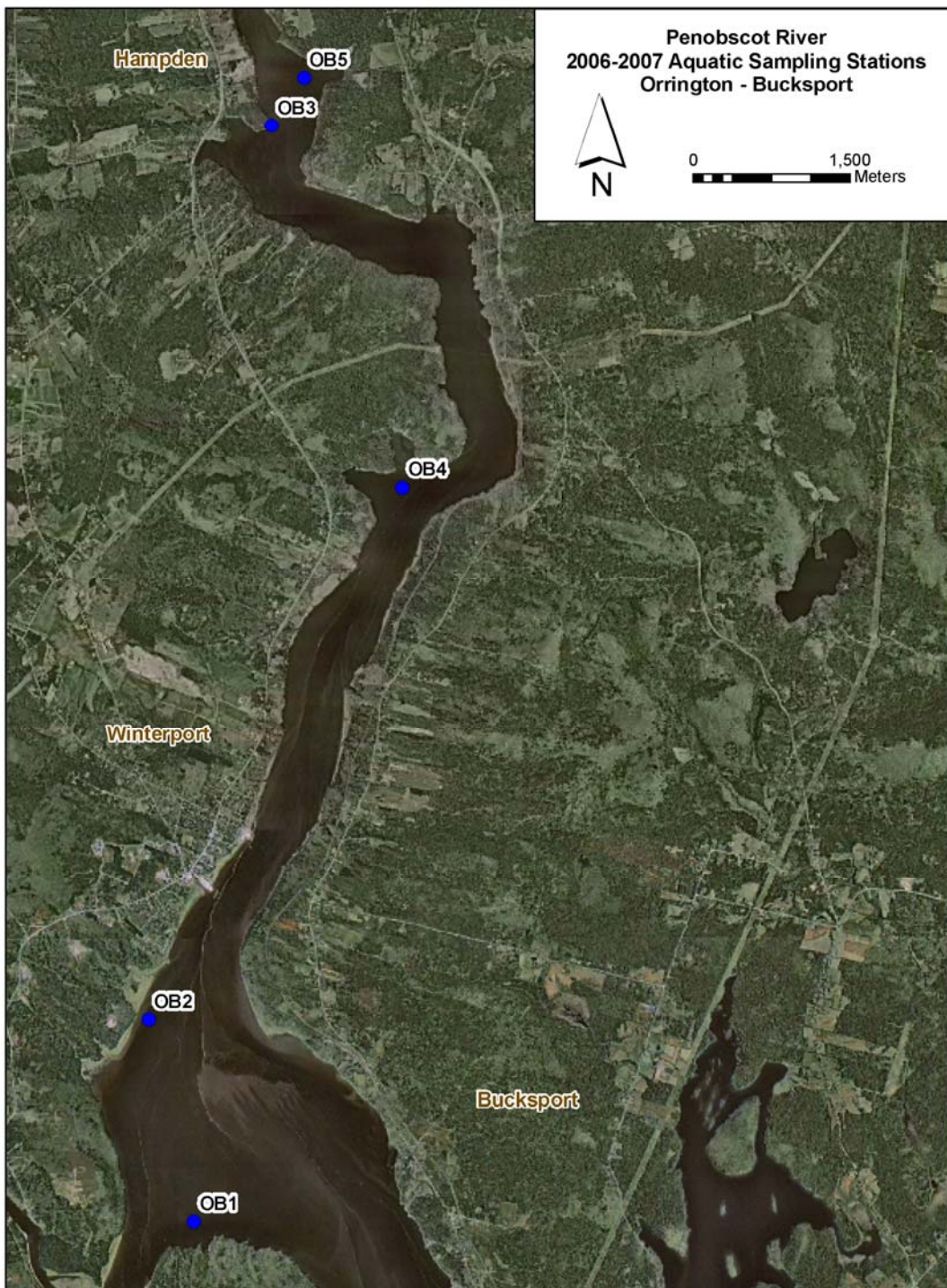


Figure 6. Location of Orrington – Bucksport sampling sites, 2006-2007.

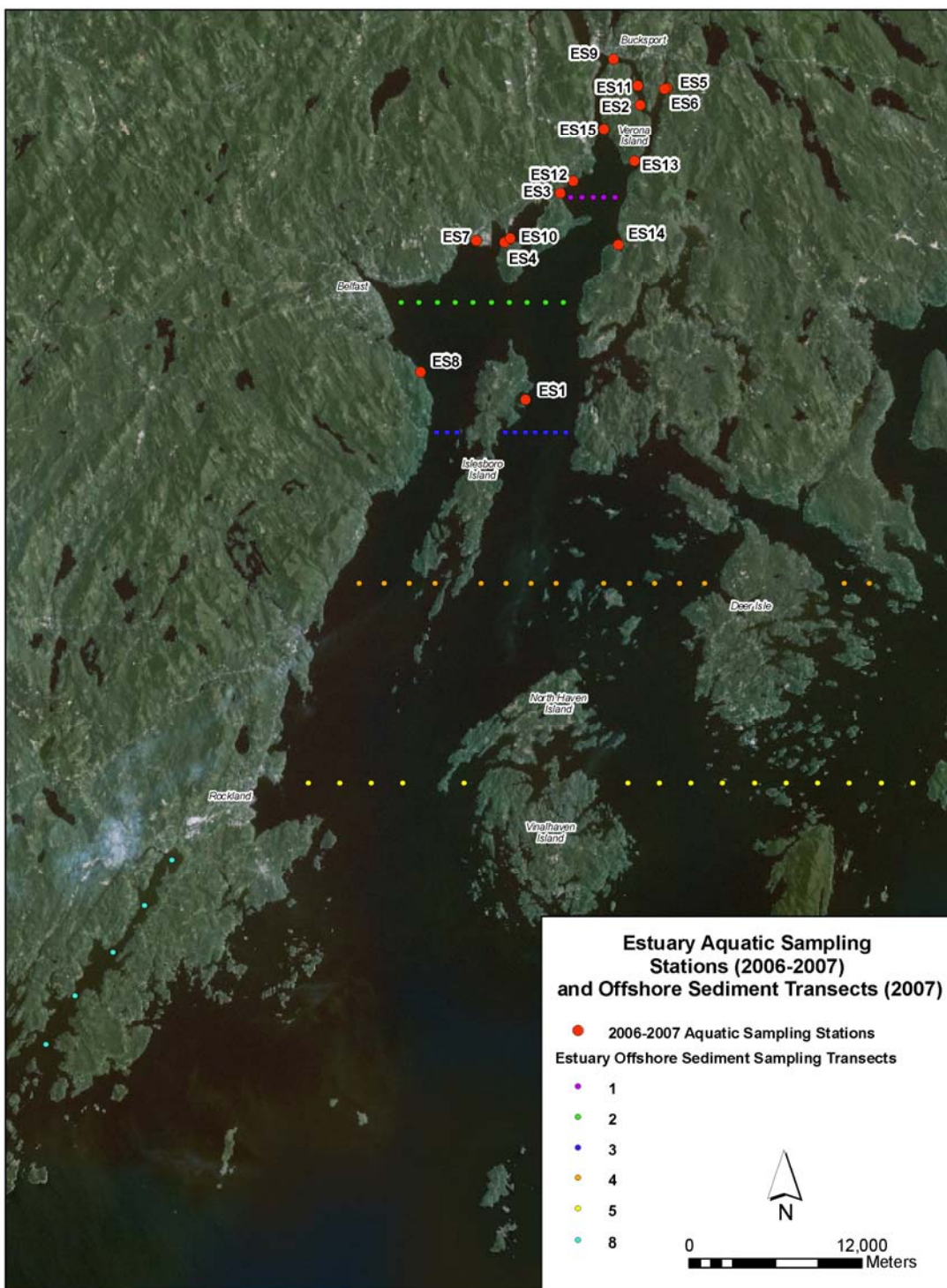


Figure 7. Location of Estuary (ES) aquatic sampling sites, 2006-2007. Also shown are the locations of sampling transects and sites in the estuary sampled for offshore surficial sediments in August 2007. Transects 1-5 are in the Penobscot estuary; Transect 8 is in the St. George estuary.

### **Long cores**

In 2007, a number of long cores (generally > 30 cm) were taken in the Penobscot River and estuary. Three of the cores have been analyzed for total Hg, total organic carbon and grain size. Two of the analyzed cores were taken in Fort Point Cove in the upper estuary and one was in Southerly Cove, adjacent to HoltraChem. Locations for the cores and raw data are shown in Appendices 1, 2, and 3. Cores were taken using a 4 inch piston corer. Layers were sliced in thicknesses of 1 - 3 cm and samples were separated for analysis of Hg, grain size distribution, and total organic carbon as for sediment cores given in the Phase I report.

### **Methyl mercury in wetlands**

Methods for the sampling of wetlands adjacent to the Penobscot River and estuary in August 2007 were provided in the Phase I report. Briefly, samples of the top 3 cm of sediment were taken from 27 wetlands, at four elevations within each wetland. The four elevations were: “intertidal” (intertidal zone below zone of vegetation), “low” (vegetated zone below the elevation of frequent high tides), “medium” (vegetated zone between the frequent high tide mark and the extreme high tide mark), and “high” (vegetated zone above the extreme high tide mark). Wetlands ranged geographically from Brewer in the north (upstream of HoltraChem) to the Bagaduce estuary in the south. Figure 18 shows the geographic locations of wetlands sampled. All sediment samples for methyl Hg determination were frozen in the field on dry ice within 1 minute of mixture of the sample in contact with the air. All samples from wetlands were analyzed for methyl Hg by the distillation method.

### ***Mercury in biota***

Table 1 provides a summary, for all invertebrates and fish, of the timing and locations of field collections, as well as a summary of statistical analyses performed for each species. Table 2 provides the same information for birds sampled.

### **Nereis worms**

*Nereis* (Polychaete) worms were collected in 2006 in the lowest reach of the Penobscot River, Orrington-Bucksport (OB, 34 samples), and throughout upper Penobscot Bay (ES, 300 samples), during Sample Periods I, II, and III. Worms were collected by hand from the intertidal zone at each station. In general, small, young worms were sampled. The mean dry weight of *Nereis* collected were generally below one gram, however, a few large worms were sampled south of Verona Island ranged from 1 – 4 grams dry weight. *Nereis* dry weight varied significantly among sample sites (ANOVA,  $p < 0.0005$ ,



$r^2 = 0.367$ ). *Nereis* weights were lowest in the OB reach, averaging (mean  $\pm$  SD)  $0.05 \pm 0.01$  to  $0.08 \pm 0.05$  g d.w., while weights from Penobscot Bay sample sites were greater and more variable, ranging from  $0.10 \pm 0.09$  g d.w. at ES09 at the north tip of Verona Island, to  $0.61 \pm 0.55$  g d.w. at ES01 on Islesboro (Appendix 6). Mercury levels were R-skewed and both total Hg and methyl Hg values were log transformed to achieve a normal distribution. All statistical tests used log transformed mercury data. Several covariates showed a meaningful influence on Hg levels. Total Hg in *Nereis* had a weak, but significant negative correlation with worm dry weight; methyl Hg did not have a substantial correlation with *Nereis* dry weight. Both total Hg and methyl Hg varied significantly with sample period, with the greatest *Nereis* mercury levels found in Sample Period I (Appendix 7).

### **Macoma clams**

During 2006 *Macoma* clams were sampled in two reaches, OB (52 samples from three sites) and ES (35 samples from two sites), and two sample periods (II and III) in 2006. Sample lengths were recorded on all *Macoma* used in this analysis. *Macoma* length did not vary by reach, but did vary significantly by site (ANOVA, Tukey pairwise); *Macoma* from OB2 were significantly longer from the other four sites, while those sampled from OB1 were shorter than all other sites. Sample period did not significantly influence *Macoma* length. All Hg concentrations were transformed to achieve the normal distribution needed for statistical analyses (total Hg, log transformation; methyl Hg, square root transformation). Total Hg concentrations were significantly correlated with *Macoma* length (linear regression,  $r^2 = 0.23$ ) while neither methyl Hg nor % methyl Hg varied with length. All analyses of *Macoma* THg levels were adjusted by length (ANCOVA). Sample period did not significantly influence Hg concentrations in *Macoma*.

### **Soft-shelled clams (*Mya arenaria*)**

Soft-shelled clams were dug from exposed mudflats in Penobscot Bay and at one site in the lower Penobscot River during Sample Periods I, II, and III in 2006. A total of 151 clams were collected. Maximum shell length (mm) was recorded for clams collected in Periods II and III. The total weight of all soft tissue was recorded prior to drying, homogenization, and sub-sampling for Hg analysis. Hg levels and clam lengths and weights were right-skewed; distribution was improved by log transformation and log transformed data was used in all statistical analyses.



### **Rock Crab (*Cancer irroratus*)**

Rock crabs were collected in September 2006 (Sample Periods II and III) from lobster traps pulled from Penobscot Bay, at sites from Fort Point in the north to North Islesboro in the south. Carapace width was recorded in the field; whole crabs were frozen for later analysis. Claw muscle tissue (n = 89) was analyzed for both total Hg and methyl Hg levels. Both total Hg and methyl Hg values were R-skewed. Log transformation improved the distribution, and therefore all statistical tests were run using log transformed Hg data. Carapace width was normally distributed. Neither total Hg nor methyl Hg levels showed statistically significant correlations with carapace width. Crab ages were estimated using carapace widths (Bigford 1979). Female crabs had significantly greater total Hg and methyl Hg levels than male crabs (two sample t-test, separate variance,  $p < 0.05$ ). Geographic comparisons were adjusted for this difference, using crab sex as a covariate.

### **Green Crab (*Carcinus maenas*)**

Green crabs were collected by hand in the intertidal zone in August and September, 2006 - Sample Periods I, II, and III. Collections were made at established ES sites in Penobscot Bay, from ES09, at the north tip of Verona Island, to ES01 on the East shore of North Islesboro. Green crabs were frozen whole and weighed in the laboratory immediately prior to analysis. Carapace widths were estimated using a power regression relating wet body mass to carapace width (Audet et al. 2008). Crab age class was estimated using length-at-age relationships reported in Berrill (1982). Whole crabs were homogenized and subsampled for total Hg and methyl Hg analyses. Hg concentrations are reported on a dry weight basis. Percent moisture varied widely in the whole crab homogenates. The distribution of total Hg, methyl Hg, and estimated carapace width were R-skewed, however, log transformation improved the distributions and all statistics were done using log transformed Hg and size data. ANCOVA compared Hg concentrations by site. Sample period and estimated carapace width were significant co-variables.

### **Tomcod (*Microgadus tomcod*)**

In the fall of 2006, tomcod were collected from the BO (n = 12) and OB (n = 48) reaches of the lower Penobscot River, and around Verona Island in the upper estuary of Penobscot Bay (ES, n = 100), during sample periods II and III. All samples were analyzed for total Hg (n = 160), and a subset were analyzed for methyl Hg (n = 14). Total Hg and fish length and weight were log transformed to create the normal distribution needed for the statistical analyses; raw methyl Hg data were normally distributed. Fish sampled from the Brewer-Orrington reach were significantly smaller than from the two reaches sampled to the south (ANOVA,  $p = 0.004$ ).

### **Banded killifish (*Fundulus diaphanus*)**

Killifish were primarily collected from the lower Penobscot River with one sample also collected from Penobscot Bay near Sears Island. All samples were collected during Sample Period III, late September – early October 2006. Fish were chilled on ice in the field, and frozen at -20°C at the lab. Thawed fish were weighed and measured prior to sampling muscle for Hg analysis. Neither fish length nor weight varied significantly by reach (ANOVA,  $p > 0.05$ ). Muscle total Hg ( $n = 36$ ) showed a weak but significant correlation with fish weight and length ( $r^2 \cong 0.2$ ). Methyl Hg in muscle ( $n = 11$ ) was strongly correlated with fish size ( $r^2 \cong 0.7$ ). Hg levels were adjusted for fish size using ANCOVA for statistical comparisons between reaches.

### **Rainbow smelt (*Osmerus mordax*)**

Rainbow smelt were collected from two reaches, Orrington-Bucksport (OB) and Penobscot Bay (ES). All samples were collected during Sample Period III, late September – early October 2006. Smelt were chilled on ice in the field, and frozen at -20°C at the lab. Thawed fish were weighed and measured prior to sampling muscle for Hg analysis. Total mercury (THg) was determined for 107 smelt samples, and methyl mercury (MeHg) was determined for a subset of 21 samples. Total mercury values were log transformed to meet the assumptions of a normal distribution; log THg values were used in all statistical tests. Methyl mercury values met the normality assumption and were not transformed for statistical analyses. Smelt length and weight values increased from north to south (ANOVA,  $r^2 = 0.53$  and  $r^2 = 0.46$ , respectively,  $P < 0.0005$ ); smelt length in the OB reach ranged between 26 and 46 mm, and in the southern ES sites length ranged between 83 and 90 mm. Total mercury had a significant, but meaningless correlation with smelt length (Linear regression,  $r^2 = 0.07$ ,  $P = 0.011$ ) and MeHg did not significantly correlate with smelt length. This absence of a linear correlation between mercury and fish length may be related to the apparently greater mercury exposure in the northern area where smaller fish were collected. Fish length was a significant covariate in analyses of geographic trends in smelt THg and MeHg levels.

### **Winter flounder (*Pleuronectes americanus*)**

Winter flounder were collected from eight intertidal sites in Penobscot Bay (ES,  $n = 83$ ). Sampled fish were chilled on ice in the field and later frozen at -20°C prior to sampling. Fish were thawed, and weighed and measured prior to the removal of a muscle sample for mercury analyses. All flounder were collected during Sample Period III, late September – early October 2006. Total Hg and methyl Hg data and the fish lengths and weights were log transformed to meet the normality assumptions needed for statistical tests; all statistical tests used log transformed data. All flounder sampled in Penobscot Bay were young fish under one year of age (Pentilla et al. 1989; mean length  $61.3 \pm$

14.5 mm, mean weight  $5.0 \pm 4.2$  g). The length and weight of flounder varied significantly among ES sites (ANOVA,  $p < 0.0005$ ) but there was no regional trend to the size variations. Both total Hg and methyl Hg levels were positively correlated with fish weight and length (total Hg - linear regression,  $p < 0.0005$ ,  $r^2 \cong 0.40$ ,  $n = 83$ ; methyl Hg - linear regression,  $p < 0.05$ ,  $r^2 \cong 0.40$ ,  $n = 15$ ).

Total Hg concentrations in flounder caught in Penobscot Bay were compared to those from flounder collected along Maine's Downeast Coast (Frenchman Bay and Schoodic Point; Kopec, in prep.). The Downeast winter flounder were collected in 2001, and were larger (mean length  $180 \pm 68$  mm, mean weight  $107 \pm 122$  g) than flounder sampled in Penobscot Bay. Downeast THg levels had a weak, positive correlation with winter flounder length (linear regression,  $p < 0.01$ ,  $r^2 = 0.24$ ). Total Hg values in the Downeast samples were increased by 25% for a more accurate comparison with the winter flounder samples collected from Penobscot Bay because the Downeast samples were analyzed on a whole body basis and the Penobscot Bay samples were analyzed for muscle only.

#### **Golden shiner (*Notemigonus crysoleucas*)**

Golden shiners were collected from two reaches in the lower Penobscot River, Brewer-Orrington (BO, 11 samples) and Orrington-Bucksport (OB, 9 samples). All samples were collected in Sample Period III (late September – early October). The fish were weighed and measured in the field, chilled on ice and frozen at  $-20^\circ\text{C}$  at the lab. Muscle was sub-sampled from thawed fish for Hg analyses. Fish length (mean  $\pm$  SD,  $112.8 \pm 21.1$ ) and weight ( $15.5 \pm 6.2$  g) did not vary between reaches. Hg levels had fairly normal distributions, given the small sample sizes, and so mercury values were not transformed for statistical analyses (residuals were normally distributed). Hg levels did not vary with fish length or weight (linear regression,  $P > 0.05$ ).

#### **American eel (*Anguilla rostrata*)**

Eels were sampled in July 2007 from multiple sites in the three lower reaches of the Penobscot River, OV ( $n = 24$ ), BO ( $n = 78$ ), and OB ( $n = 46$ ). Eels were captured using eel pots in the BO and OB reaches, and collected by electrofishing in the OV reach. All eels collected were in the yellow phase (external coloration indicating immature individuals). Total length and weight were recorded in the field, and muscle samples taken and frozen for total Hg and methyl Hg analyses. Otoliths (inner ear bones) were collected and read for age; 21% of the otoliths could not be read, and so were not aged. There was no significant difference in the length of eels that were aged versus those that could not be aged (two-sample t-test,  $p > 0.05$ ). However, there was a small, but significant difference in the mean total Hg concentration between the aged eels (geometric mean total Hg  $482$  ng/g w.w.) and the eels that could not be aged (geometric

mean total Hg 387 ng/g w.w.; two-sample t-test,  $p = 0.019$ , pooled variance). Data for total Hg, eel length and eel weight were right skewed; log transformation improved the distribution and all statistical analyses were run with log transformed data for these three variables. Data for methyl Hg were normally distributed.

### **Double-crested cormorant (*Phalacrocorax auritus*)**

Cormorant eggs were again sampled in 2007, following an initial sampling of eggs from five Penobscot Bay colonies in 2006. One to four viable eggs were collected from each nest, with the goal of collecting 12 eggs per colony. Egg development varied from stage 0 (no development) to stage 3 (advanced, body formed, some feathers present). Development stage did not influence mercury level (ANOVA,  $p = 0.902$ ). In 2007, eggs were collected between June 1<sup>st</sup> and July 1<sup>st</sup>, whereas the 2006 samples had been collected between July 13<sup>th</sup> and August 16<sup>th</sup>. The earlier 2007 collection dates were closer to the cormorant's late-spring arrival from the wintering grounds.

### **Black Guillemot (*Cepphus grille*)**

Guillemots were sampled in 2007 in outer Penobscot Bay, from Cape Rosier to ledges south of Islesboro, and east of Swan's Island. Blood was collected from nesting chicks at three sites in Penobscot Bay, from adults at two sites in the Bay and viable eggs were collected from six sites. Eggs and blood were analyzed for total Hg. Data on total Hg and bird measurements (weight and tail length) were normally distributed within age classes. Guillemot weight and tail length were significantly lower in juveniles sampled from Western Island, relative to Mouse and Pond Island (ANOVA,  $p < 0.0005$ ). Adult size did not vary significantly among sample sites.

### **Nelson's sharp-tailed sparrow (*Ammodramus caudacutus*)**

Adult and juvenile sparrows were collected using mist nets in late June and July at Mendall Marsh, in the OB reach of the Penobscot River ( $n = 81$ ), and at coastal marsh reference areas in Maine, south of Portland ( $n = 10$ ) and in Massachusetts ( $n = 1$ ). Standard measurements were taken, and blood and tail feather samples collected for mercury analyses. Hg concentrations in feathers were not normally distributed, and data were not improved by transformation so non-parametric tests were used to assess differences in total Hg in feathers among age classes and among collection sites. Blood Hg levels were normally distributed within age classes. In the Mendall Marsh birds, both blood and feather total Hg varied significantly with age class, while sex had no influence on Hg concentrations.

Table 2. Summary of the bird samples collected and an outline of the statistical analyses performed.  
 NS = not significant, Sig Var = significant variation, LOG = log transformation, SQRT = square root transformation.

BIRD SPECIES		tissue sample		SAMPLES COLLECTED by REACH and MONTH														2007 Total Hg					2007 Size									
				EB		Penobscot River North of Old Town			OV		BO	OB				ES				Southern Maine - Massachusetts			THg distribution	THg v. size	THg vs. Sample Date	THg v. Age Class	THg v. Site	Size distribution	Size vs. Site			
				2007		2007			2007		07	2006		2007				2006		2007										2007		
June	July	June	July	August	June	July	June	July	August	May	June	July	August	July	August	June	July	August	September	May	June	July	August									
Double-crested cormorant <i>Phalacrocorax auritus</i>	egg													39	1	86	19									LOG	NS	NS	—	Sig Var	LOG	NS
Black guillemot <i>Cepphus grille</i>	egg													5		5	2	3							RAW	NS	NS	—	NS	RAW	NS	
	AD blood																	12	4					RAW	NS	NS	Sig Var	NS	RAW	NS		
	JUV blood																		13					RAW	NS	NS	Sig Var	NS	RAW	Sig Var		
Nelson's sharp-tailed sparrow <i>Ammodramus caudacutus</i>	blood								10	8	3	78									5	5		RAW	NS	NS	Sig Var	Sig Var	RAW	NS		
	feathers								9	3	4	74												non-parametric	NS	NS	Sig Var	—	RAW	NS		
Song sparrow <i>Melospiza melodia</i>	blood			10					4	12	11	17				31	7							LOG	NS	NS	Sig Var	Sig Var	RAW	Sig Var*		
	feathers			10						4	11	17				32								LOG	NS	NS	Sig Var	Sig Var	RAW	Sig Var*		
Swamp sparrow <i>Melospiza georgiana</i>	blood			8	5				1	7	6	20	3			3								LOG	NS	NS	NS	Sig Var	RAW	NS		
	feathers			8	5						7	17	3			3								RAW	NS	NS	NS	NS	RAW	NS		
Red-winged blackbirds <i>Agelaius phoeniceus</i>	blood										3	32												LOG	NS	NS	NS	Sig Var	RAW	NS		
	feathers										3	32												LOG	NS	NS	Sig Var	NS	RAW	NS		
Virginia Rail <i>Rallus limicola</i>	blood										5	6	7								2			LOG	NS	Sig Var	NS	Sig Var	RAW	NS		
	feathers										5	6	7								2			LOG	Sig Var	NS	NS	Sig Var	RAW	NS		

Table 2. (continued)

BIRD SPECIES		tissue sample		SAMPLES COLLECTED by REACH and MONTH																2007 Total Hg					2007 Size							
				EB		Penobscot River North of Old Town			OV		BO	OB				ES				Southern Maine - Massachusetts				THg distribution	THg v. size	THg vs. Sample Date	THg v. Age Class	THg v. Site	Size distribution	Size vs. Site		
				2007		2007			2007		07	2006		2007		2006		2007		2007												
June	July	June	July	August	June	July	June	July	August	May	June	July	August	July	August	June	July	August	September	May	June	July	August									
Belted kingfisher <i>Ceryle haliaetus</i>	AD blood	2	2								3					2									LOG	NS	NS	Sig Var	-	-	-	-
	AD feathers	2	2								3					1									LOG	NS	NS	-	-	-	-	
	JUV blood		26				2																		LOG	NS	NS	Sig Var	Sig Var	RAW	Sig Var	
Osprey <i>Pandion haliaetus</i>	eggs										1			3		2	3				2	2			LOG	-	NS	-	NS	-	-	
	AD blood										2			3			7	1				3	1		LOG	-	-	Sig Var	NS	-	-	
	AD feathers										2						7	1				3	1		LOG	-	-	Sig Var	NS	-	-	
	JUV blood										7						14	5					23		LOG	NS	NS	Sig Var	Sig Var	RAW	Sig Var	
	JUV feathers										7						14	5					23		LOG	NS	NS	Sig Var	Sig Var	RAW	Sig Var	
Bald eagle <i>Haliaeetus leucocephalus</i>	JUV blood			7			5		1							6					6				-	-	-	-	-	-	-	
	JUV feather			7			5		1							6					3				-	-	-	-	-	-	-	



### **Song sparrow (*Melospiza melodia*)**

Song sparrows (n = 76) were mist-netted over a wide geographic area extending from Greenbush, on the Penobscot River north of Old Town, to Bald Island, in lower Penobscot Bay. Blood and tail feathers were collected at all sites except Bald Island, where blood alone was collected. Raw total Hg levels were non-normally distributed, but log transformation gave good distributions for parametric tests. The bird measurement data that were used were normally distributed. Sites were sampled between July 3<sup>rd</sup> and September 2<sup>nd</sup>, 2007, with most sites sampled between 27 July and 7 August. The majority of sites were sampled once during the summer, with the exception of Mendall Marsh, which was sampled over a 20 day period, and Winterport North, which was sampled over a two day period. Bird age class correlated significantly with Hg levels (ANOVA,  $p < 0.05$ ), and blood total Hg was adjusted for age in ANCOVAs testing variation among sites. Sex and sample date were not significant covariates. Within the hatch year chicks, weight and bill length varied significantly by site, with the smallest birds at Mendall Marsh and Holbrook Island, and the largest chicks at Greenbush, Smith Cove and Winterport North (ANOVA,  $p < 0.01$ ,  $r^2 \cong 0.43$ , Tukey HSD  $< 0.05$ ); chick tail length and wing chord did not vary by site.

### **Swamp sparrows (*Melospiza georgiana*)**

Swamp sparrows were sampled at Mendall Marsh (n = 27) between June 25 and July 25, 2007, at four additional upstream sites along the Penobscot River (n = 16), and at two sites in Penobscot Bay (n = 3) between July 30 and August 7, 2007. Blood and tail feathers were sampled for Hg analysis and standard measurements recorded while the birds were in brief captivity. When grouped by sample sites, log-transformed blood total Hg data and raw feather total Hg data were normally distributed; parametric statistical tests were used. All measurement data were normally distributed. Most bird measurements (tail length, weight, wing chord) did not vary by sample site (ANOVA,  $p > 0.05$ ). Bill length was initially found to be significantly greater at Greenbush relative to Mendall Marsh (ANOVA,  $p = 0.001$ , Tukey pairwise HSD  $< 0.05$ ), yet this difference was no longer significant when bird age was added as a covariate.

### **Red-winged blackbirds (*Agelaius phoeniceus*)**

Red-winged blackbirds were sampled in three areas of Mendall Marsh in 2007 (n=35). Blood and tail feathers were collected from both adults and chicks for Hg analyses, and standard measurements were recorded. Mercury data were right-skewed, and therefore were log-transformed to create a normal distribution.

### **Virginia Rail (*Rallus limicola*)**

Virginia rails were sampled in 2007 at three areas in Mendall Marsh (n = 18) and at a reference site in southern Maine, Scarborough Marsh (n = 2). Chicks were collected in the middle section of Mendall Marsh (n = 5). Rails were captured primarily using walk-in

traps with lead lines placed to funnel walking rails into the trap. Raw blood and feather total Hg concentrations were non-normally distributed and log-transformed total Hg levels were used for all statistical analyses. Bird tail length, weight and bill length were normally distributed, and used to define bird size in comparative analyses. Total Hg in blood was significantly correlated with sample date (linear regression,  $p = 0.017$ ,  $r^2 = 0.31$ ), and was a significant covariate in analyses comparing total Hg in blood among collection sites.

### **Belted Kingfisher (*Ceryle alcyon*)**

Kingfishers were sampled over a wide geographic range from the East Branch of the Penobscot River, north of Millinocket, to the Bagaduce River near Castine. Adults were netted near the nesting burrows and chicks were removed physically from the nest. Measurements were taken on chicks. Blood samples were taken from both chicks and adults, and feather samples were taken from adults. These were analyzed for total Hg. Data on chick weight and bill length were normally distributed and total Hg in chick blood was log-transformed for statistical tests.

### **Osprey (*Pandion haliaetus*)**

Osprey eggs, and blood and feather samples from chicks and adults, were sampled in 2007 from 12 nest sites in the Penobscot River and Penobscot Bay. Additional osprey samples were collected, for comparison, in Southern Maine from the Sheepscot River, Portland's Fore River, and offshore islands in Harpswell. Sample details are given in the table below.

Region	Site	Habitat	Nests	Adults	Chicks	Eggs	Chick replicates
Penobscot	Penobscot River, OB	River	3	2	5	1	1 x 3 replicates
	Upper Penobscot Bay	Coastal	6	4	10	3	1 x 2 replicates
	Lower Penobscot Bay	Marine	5	4	7	2	
Southern Maine	Sheepscot River	River	7	2	11	1	2 x 2 replicates
	Portland, Fore River	Coastal	4	2	7	2	
	Harpswell	Marine	4	0	3	1	

Sampling procedures were consistent throughout both regions. Samples of adults and chicks included whole blood and feather samples collected the same day. Total Hg was determined for whole blood, breast feather and egg samples. The dates that osprey were sampled varied significantly among certain habitat types. While sampling times for riverine and coastal populations were similar (7/06 – 7/26, 7/10 – 7/31, respectively), marine sites were sampled significantly later in the season (7/18 – 8/02). Within habitat types, sample dates did not vary significantly between regions. Numerous morphometrics of chicks and adults were collected. Among all chicks sampled, culmen length, tarsus length, bill width and chick weight were normally distributed and used as covariates in statistical tests of differences in mercury levels among sites samples. Both culmen length and chick weight were significantly correlated with sample date (linear regression,  $p < 0.01$ ,  $r^2 = 0.49$  and  $0.13$ , respectively). Culmen length may

provide a rough estimate of chick age. Chick weight varied significantly among habitats, when adjusted for sample date (ANCOVA,  $p < 0.05$ , Tukey pairwise,  $p < 0.05$ ). The weights of chicks sampled in coastal habitats (least square mean 1.47 g) were significantly greater than chick weights collected from marine habitats (least square mean 1.27g) but the weights of chicks sampled in riverine habitats were not significantly different from the other two habitats. Culmen length did not vary significantly among habitats sampled. Within the Penobscot region, chick bird lengths and weight did not vary significantly when grouped by habitat – river (OB), coastal and marine. Total Hg in the blood and feathers of chicks did not vary significantly in relation to body lengths or weight.

### **Bald eagle (*Haliaeetus leucocephalus*)**

Blood and breast feathers from bald eagle chicks were sampled in 2007 from nests in the Penobscot River Valley, Penobscot Bay, and along the South Coast of Maine. Chicks were sampled from nests just prior to fledging. In central Maine, late winter storms caused high chick mortality, and greatly reduced the number of samples collected from the lower Penobscot River (BO and OB) and upper Penobscot Bay (ES). Both blood and feather total Hg levels were normally distributed (Shapiro-Wilk,  $p > 0.05$ ) and were not transformed for analyses.

## **RESULTS AND DISCUSSION**

### ***Mercury in Sediments***

#### **Mercury in Riverine Sediments**

Figures 8-13 present total Hg concentrations, methyl Hg concentrations and % methyl Hg in the top 3 cm of river sediments at 35 sampling stations in the Penobscot River and estuary for each of the six sampling times. Stations are ordered from north to south and panels for each sampling time and the figures are ordered by time of year, not chronologically.

In the Phase I report, mean concentrations for the four sampling periods in 2006 were presented. It was concluded that mean concentrations of total Hg increased about threefold from the East Branch reference area to the Old Town – Veazie Dam reach, likely because of losses of mercury from the paper mills upstream of the Veazie Dam. Concentrations increased about tenfold in the Brewer – Orrington, Orrington – Bucksport, and upper estuary sampling reaches. Concentrations then decreased with distance from the river mouth to sampling stations beyond Vinalhaven Island in the outer estuary, at which point they did not differ from the neighbouring St. George estuary, where there is no known source of industrial mercury. The sampling conducted

in 2007 (Figures 8,9) showed patterns that were very similar to those found in 2006 (Figures 10-13).

The within site core-to-core variation of these data is typical of sediment mercury data, and is the reason why we sampled sites repeatedly to adequately characterize mercury concentrations. For example, for sample period II (Sept. 2006; Figure 11) both the total Hg and methyl Hg concentrations at sites OB 1 and OB 2 were very high. These concentrations were not seen for the other five sampling periods at these two stations, indicating that 2 “hotspots” of mercury had been sampled during period II.

The geographic pattern of methyl Hg concentrations was very similar to the geographic pattern seen for total Hg concentrations (Figures 8-13). There was a noticeable increase from East Branch to Old Town – Veazie reach and a much larger increase in the Brewer – Orrington, Orrington – Bucksport reaches. Concentrations then decreased with distance into the estuary. This was consistent at all sampling times. Methyl Hg concentrations were generally lower in the outer part of the estuary, especially at the most southerly five Estuary sites. Methyl Hg concentrations showed similar levels of variation at particular sites among sampling times as was evident for total Hg concentrations. This is because in the Penobscot system microbial production of methyl Hg is primarily controlled by concentrations of inorganic mercury (see discussions below).

Also plotted on Figures 8-13 is the percent of total Hg that is methyl Hg. Several other studies have concluded that the percent of total Hg that is methyl Hg is a good indicator of the intensity of bacterial methyl Hg production in sediments<sup>2</sup>. In the top 3 cm of the sediment cores, while some sites were higher than others, there was little overall geographic trend through out the river and upper estuary (Figures 8-13). This lack of trend suggests that the efficiency of methylation of inorganic mercury is quite constant, per unit of total Hg. Efficiencies were somewhat lower in the outer estuary. This may have been because sulfide concentrations were higher in the waters of the outer estuary. It is well known that high sulfide concentrations reduce the production of methyl Hg by binding the inorganic mercury making it unavailable for the mercury methylating bacteria.

We investigated at what depth in the sediments methyl mercury was being produced most rapidly. A subset of the cores was analysed for both total and methyl Hg concentrations for the depth intervals between 3-5 cm and 5-10 cm below the sediment-

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<sup>2</sup> The method used for determination of total Hg concentrations in sediments analyzes for all chemical forms of mercury in a sediment sample. In the Penobscot, about 3% of total Hg is methyl Hg. The remainder is inorganic mercury (usually HgII). Mercury methylating bacteria produce methyl Hg from HgII. Thus a sample with high % methyl mercury is indicative of a sample in which the methyl Hg producing bacteria have been very active.

water interface, as well as 0-3 cm (Figure 14). There was a positive relationship between total Hg concentration and methyl Hg concentration at all depth intervals. However the slope of the line in the 0-3 cm samples was about 1.6 times as steep as for the two deeper depth intervals (Figure 14). This difference in slope suggests that the production of methyl Hg in the surface sediments was more intense than in the deeper sediments. More intense mercury methylation in surface sediments is typical of methyl Hg production in aquatic sediments.

A strong linear relationship was seen for the 0-3 cm samples ( $R^2 = 0.77$  and  $0.81$  for the May and July 2007 sampling, Figure 14) indicating that the concentration of inorganic mercury is an important factor in controlling rates of methyl Hg production. The corollary of this observation is that reducing inorganic mercury concentrations in surface sediments, where methylation is particularly active, would reduce methyl Hg concentrations in sediments, and ultimately methyl Hg concentrations in the food web. This positive relationship between methyl Hg and total Hg concentrations was also observed for the first four sampling periods in 2006 (Phase I report). Thus we are consistently finding that if surface sediment inorganic Hg concentrations could be lowered in the Penobscot over a reasonable length of time either by natural attenuation or by active remediation the mercury concentrations of biota would also decline over time.

Percent of total Hg that was methyl Hg also showed a seasonal trend. On average the percentage was about 50% higher in August and early September (3.0 – 3.2 % of total Hg) as compared to other times of the year (2.2 – 2.6%) for stations in the river and upper estuary (not including the 5 stations in the southernmost estuary that consistently showed lower % methyl values, Figures 8-13). This indicates that methyl Hg production was higher in late summer than other times of the year. This is typical of northern systems, which appear to take extended periods of warm weather for rates of methylation to reach maximums (e.g. Ramlal et al. 1993) and is unlike Lavaca Bay, Texas where methyl Hg concentrations were highest in spring (Gill et al. 1999).

Comparisons between concentrations found in the sediments of the contaminated area of the Penobscot system and other contaminated and reference sites are found in the Phase I report, as is a comparison to levels thought to put sediment-dwelling animal life at risk.

Summary – Microbial production of methyl Hg is most rapid in the surface sediments of the BO, OB reaches and the upper part of the ES reach. The rate of methyl Hg production in these surface sediments is primarily stimulated by high concentrations of inorganic mercury. Lowering inorganic mercury concentrations in surface sediments either by active remediation or by natural attenuation, would improve the Hg contamination problem of the Penobscot system.

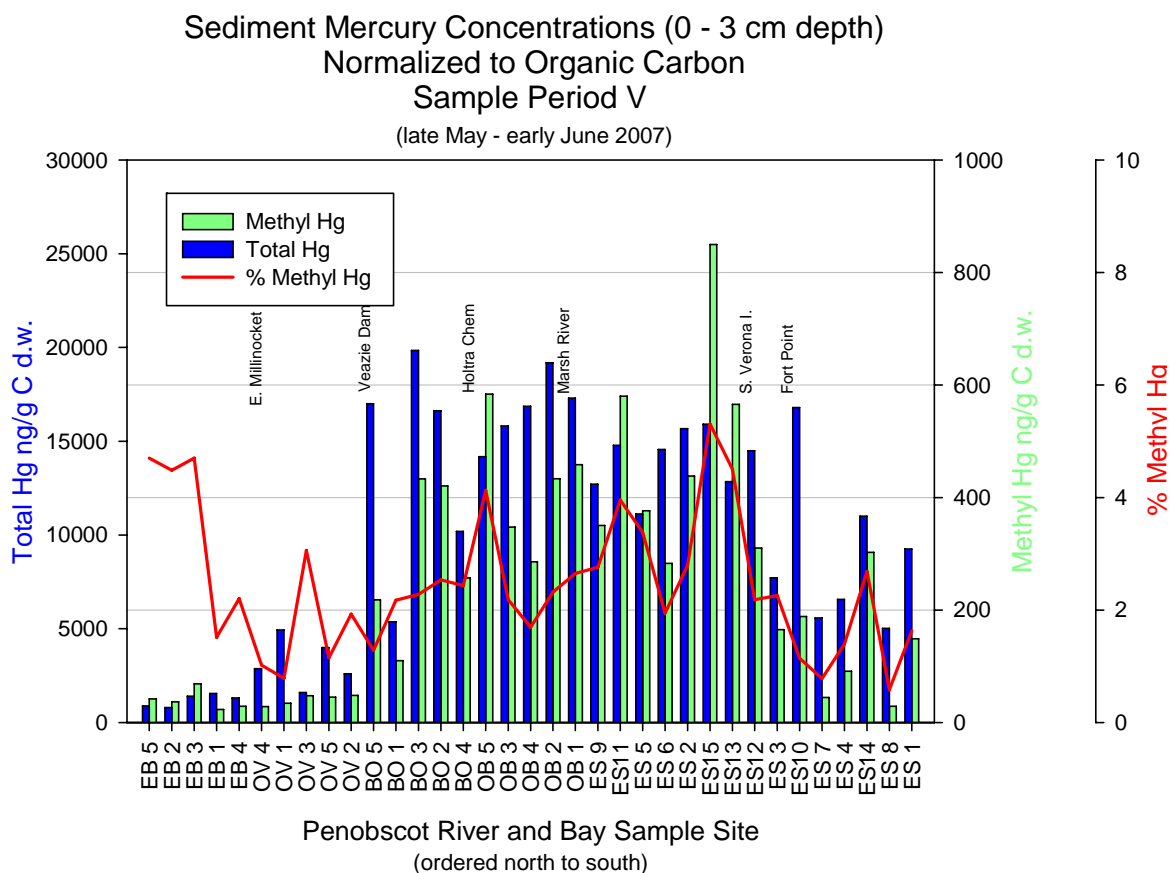


Figure 8. Total Hg concentrations, methyl Hg concentrations and % methyl Hg in the surface 3 cm of sediments in the Penobscot River and Estuary, Sampling Period V, May 2007. Total Hg concentrations were normalized to organic carbon. Sampling sites are ordered from north to south. The locations of sampling sites are shown in Figures 2-7.

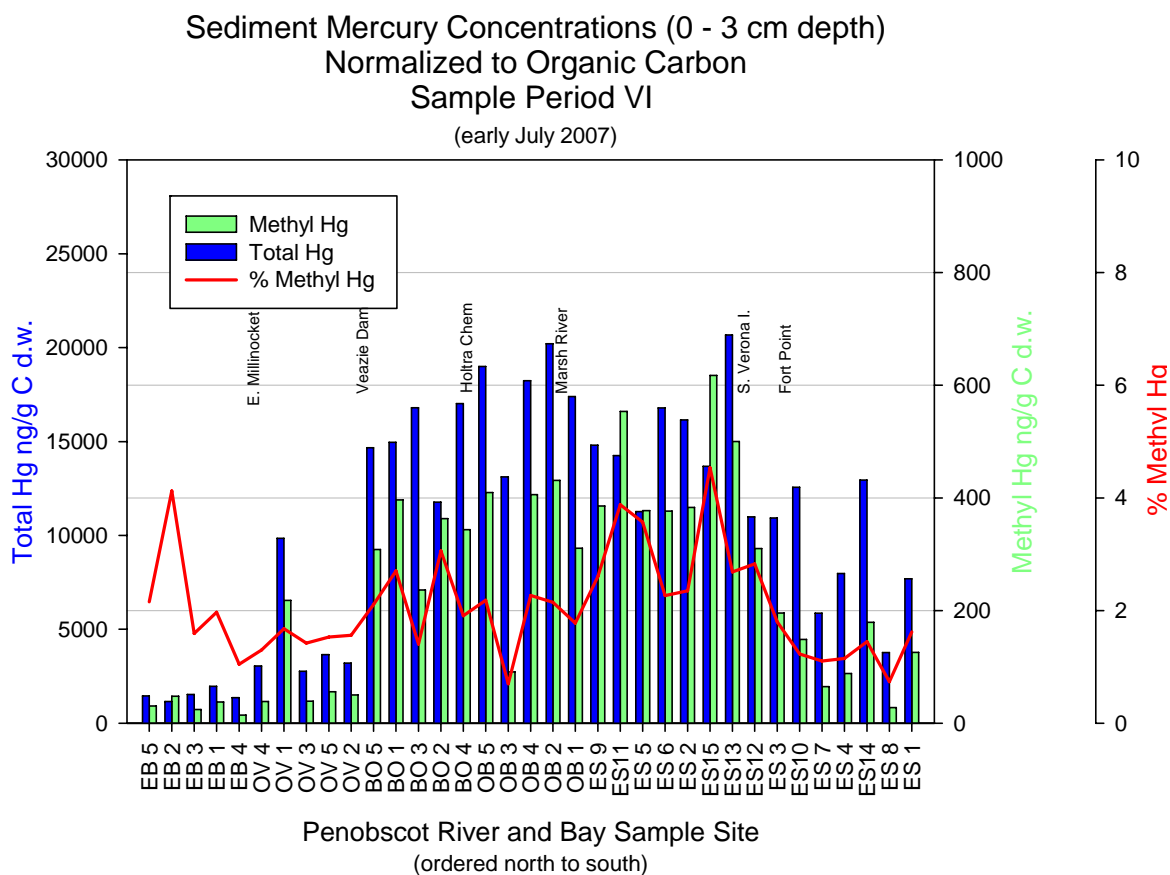


Figure 9. Total Hg concentrations, methyl Hg concentrations and % methyl Hg in the surface 3 cm of sediments in the Penobscot River and estuary, Sampling Period VI, July 2007. Total Hg concentrations were normalized to organic carbon. The location of sampling sites is shown in Figures 2-7.

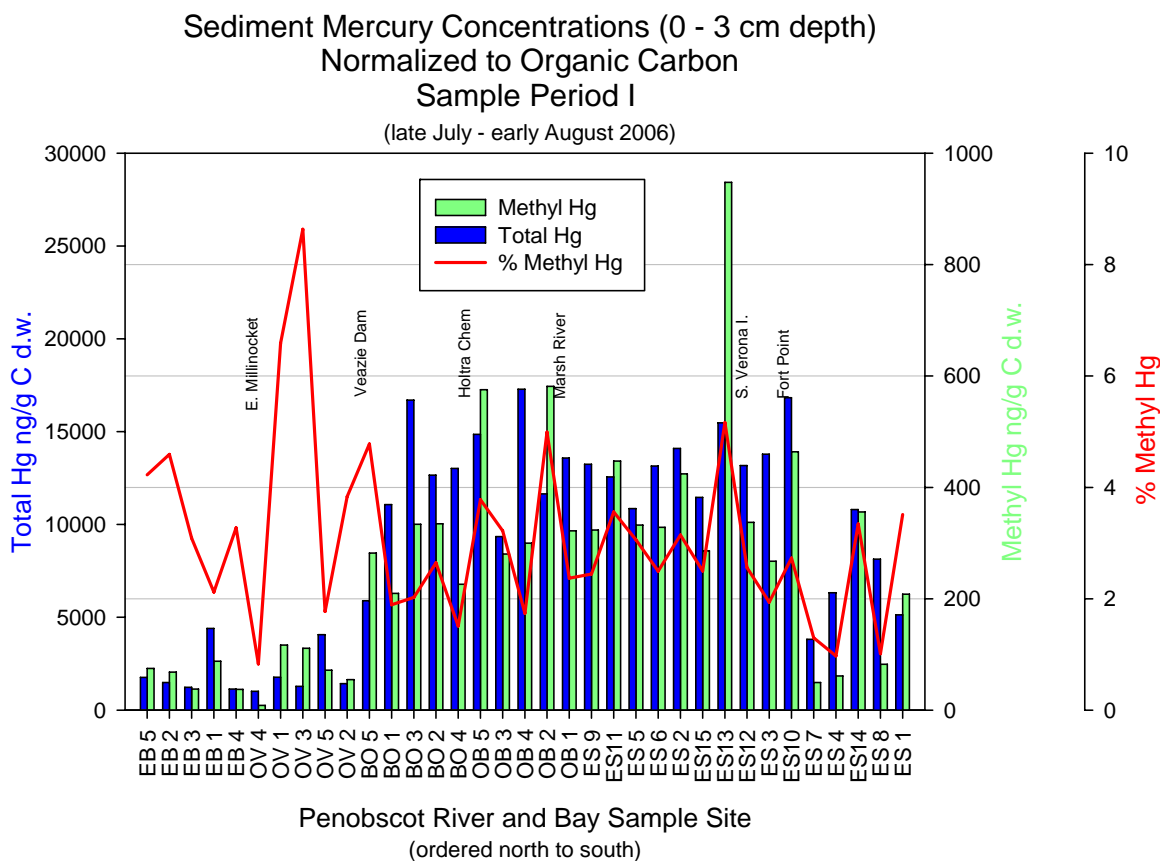


Figure 10. Total Hg concentrations, methyl Hg concentrations and % methyl Hg in the surface 3 cm of sediments in the Penobscot River and Estuary, Sampling Period I, August 2006. Total Hg concentrations were normalized to organic carbon. Sampling sites are ordered from north to south. The location of sampling sites is shown in Figures 2-7.



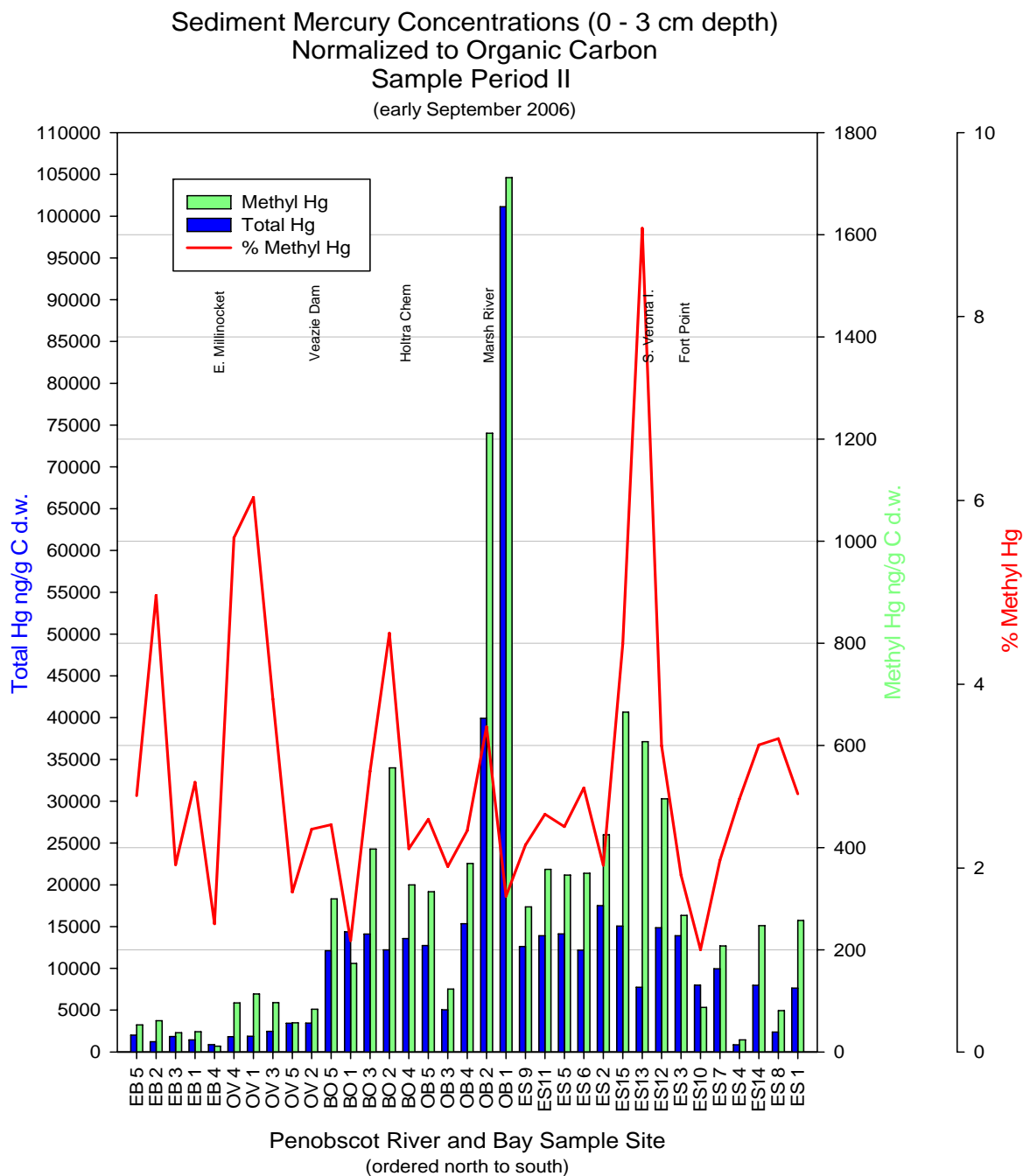


Figure 11. Total Hg concentrations, methyl Hg concentrations and % methyl Hg in the surface 3 cm of sediments in the Penobscot River and Estuary, Sampling Period II, early September 2006. Note difference in scale from other figures in this series. Total Hg concentrations were normalized to organic carbon. Sampling sites are ordered from north to south. The location of sampling sites is shown in Figures 2-7.

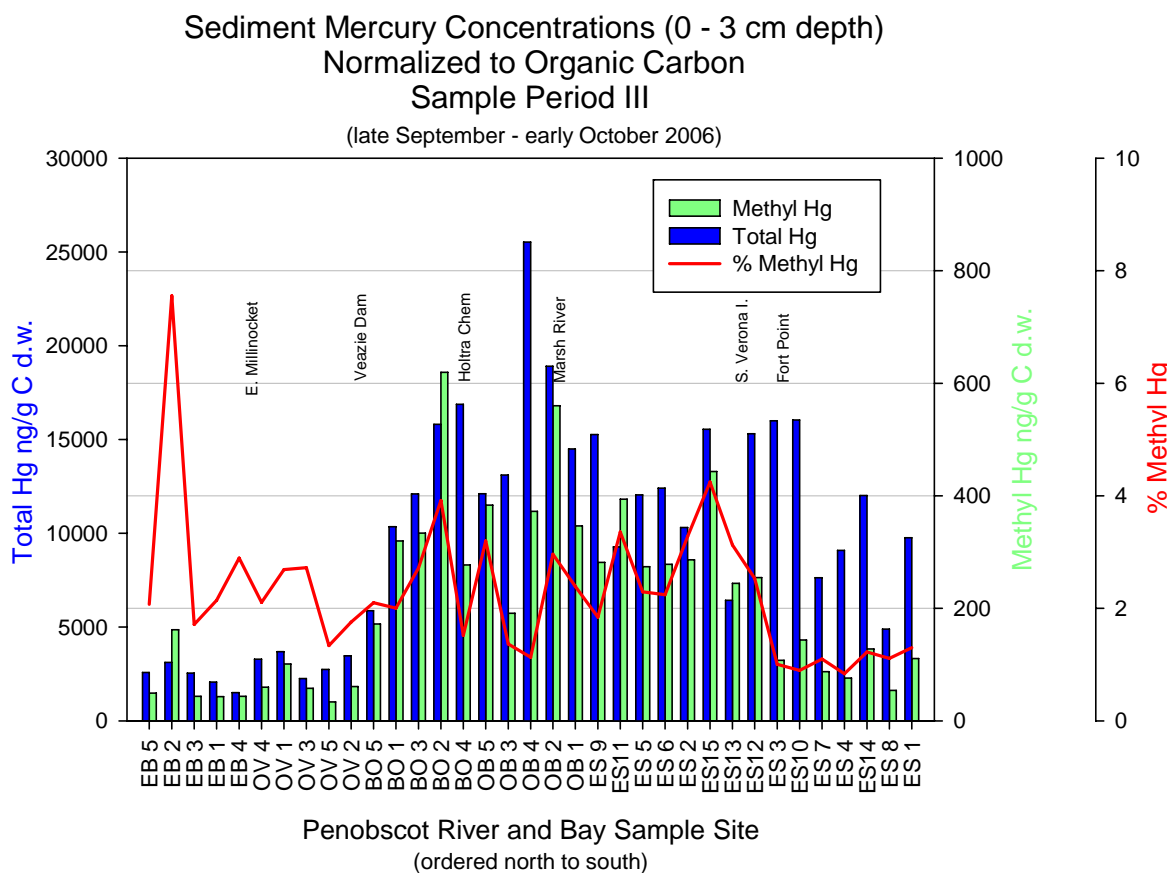


Figure 12. Total Hg concentrations, methyl Hg concentrations and % methyl Hg in the surface 3 cm of sediments in the Penobscot River and estuary, Sampling Period III, late September – early October 2006. Total Hg concentrations were normalized to organic carbon. Sampling sites are ordered from north to south. The location of sampling sites is shown in Figures 2-7.

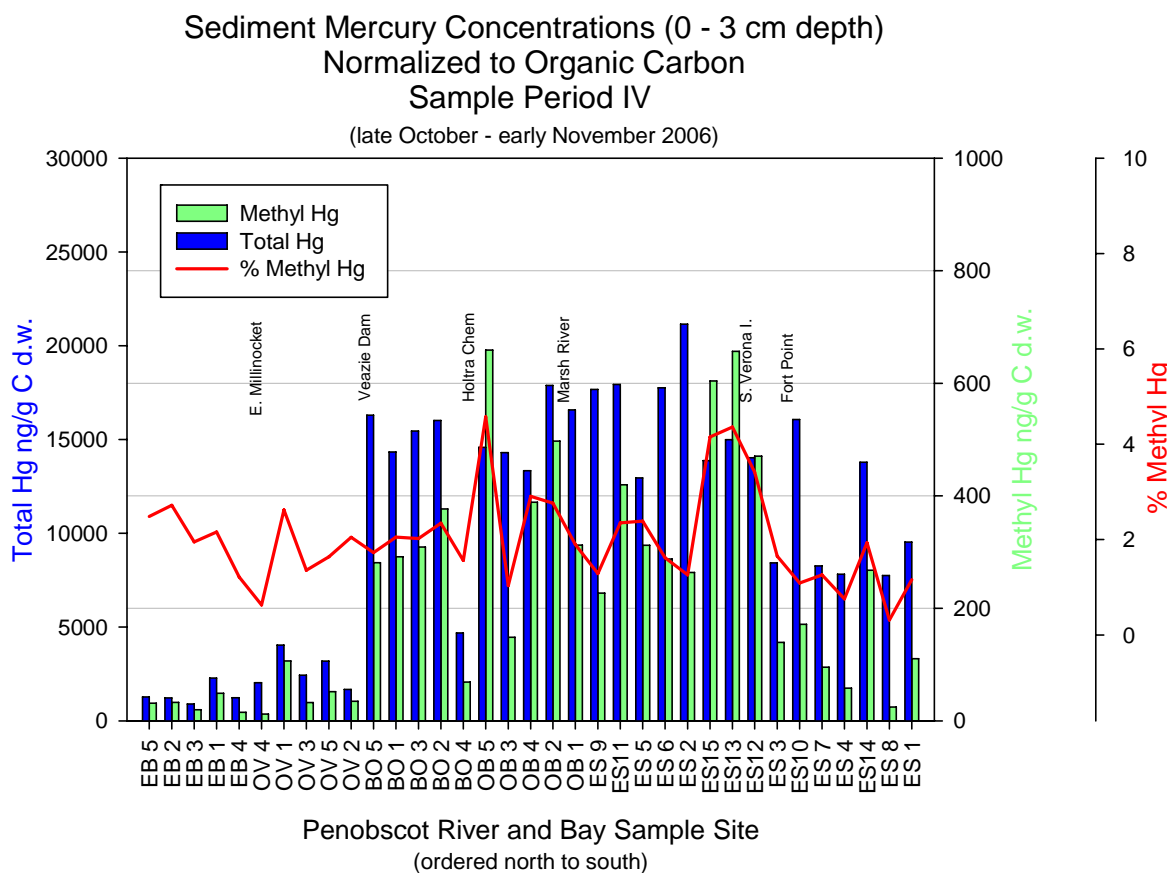


Figure 13. Total Hg concentrations, methyl Hg concentrations and % methyl Hg in the surface 3 cm of sediments in the Penobscot River and estuary, Sampling Period IV, late October 2006. Total Hg concentrations were normalized to organic carbon. Sampling sites are ordered from north to south. The location of sampling sites is shown in Figures 2-7.

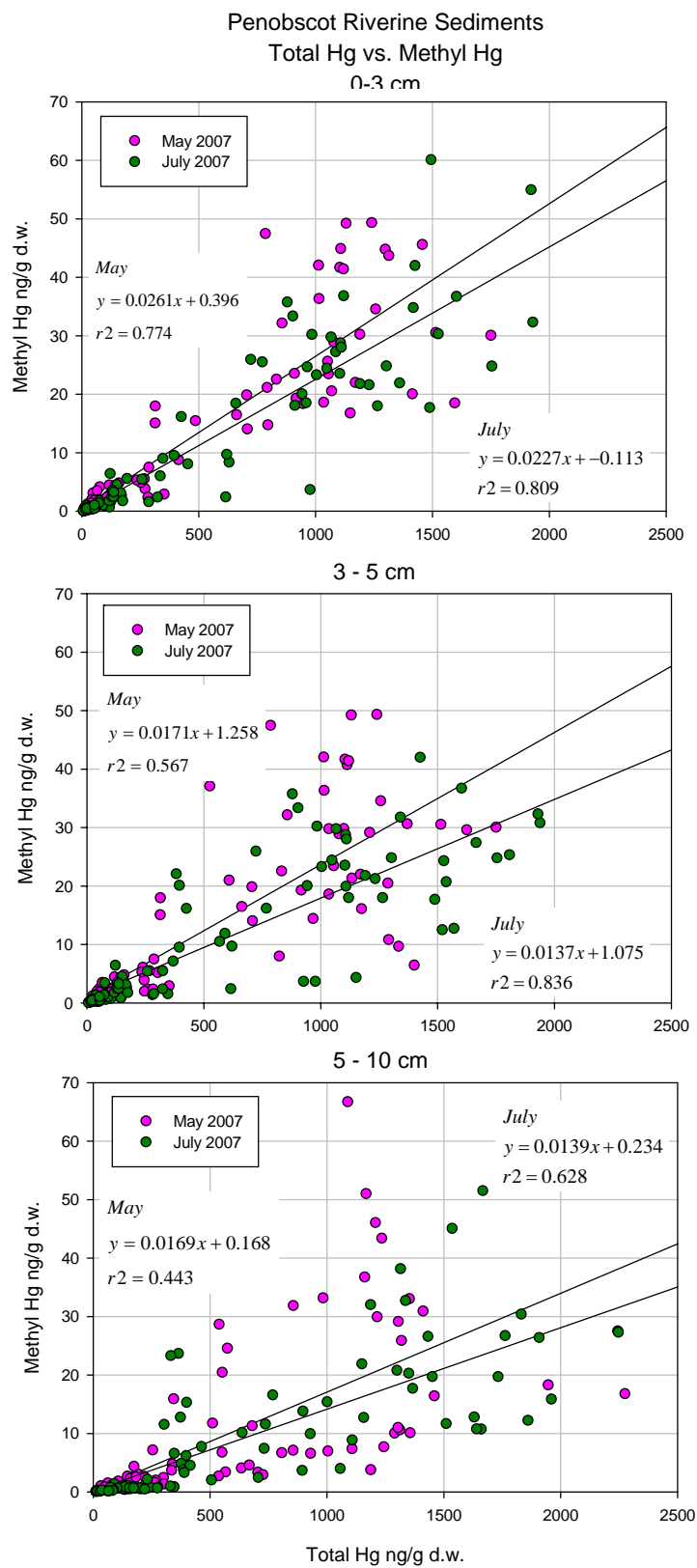


Figure 14. Relationships of methyl Hg to total Hg at three depths showing that methylation rates are greater in the sediments sampled at 0-3 cm

## **Mercury in Long Sediment Cores**

In 2007, long cores were taken from the sediments of the lower Penobscot River and upper estuary. The three cores taken were D-01 and D-02 (located in Fort Point Cove, in the upper Penobscot estuary, just to the north of Fort Point) and SC-01 (located in Southerly Cove, adjacent to the HoltraChem site). The total Hg concentrations with depth are shown in Figures 15, 16, and 17, and raw data are shown in Appendices 1, 2, and 3.

In Core D-01 (Figure 15, Appendix 1), concentrations of total Hg peaked at 1380 ng/g d.w. in the 27-30 cm deep core slice. Concentrations decreased rapidly to a depth of 20 cm below the sediment - water interface, and then more slowly with depth to the surface of the sediment. The very low concentrations (18 ng Hg/g d.w.) in the deepest layers probably represent regional background concentrations in the sediments before the operation of the HoltraChem plant, or the presence of other significant sources of anthropogenic Hg in the watershed.

Also plotted on Figure 15 are dates of sedimentation using Pb-210 and Cs-137 radioisotopes. The top 27-30 cm of core D-01, to the depth of maximum mercury concentration, appear to be undisturbed. In this part of the core, there is close agreement between the two dating methods on estimates of sedimentation rates (0.7 cm/yr Cs-137, 0.65 cm/yr Pb-210). Below the depth of maximum mercury concentration, sedimentation rates may be slower, or there may have been disturbance at this site around 1960. Therefore interpretation of the data below the peak in mercury concentrations requires data from additional cores.

Overall the dating and mercury concentration data from core D-01 suggest that there are sites with reasonably long deposition histories in the Penobscot system, and this bodes well for the success of the coming natural attenuation study. These initial core data will be extended in Phase II of the study using cores taken from wetlands, the lower river and from other sites in the estuary.

Dating is not yet completed for core D-02 (Figure 16). The shape of the core D-02 total mercury profile was very similar for core D-01. It peaked at a somewhat higher concentration of 2200 ng Hg/g d.w. at a depth of about 33 cm below the interface, suggesting a similar sedimentation rate. There was also a gradual decrease in mercury concentration in the top 25 cm of sediments; these concentrations were also somewhat higher than in core D-01. Below the peak, mercury concentrations decreased, but to only about 70 ng Hg/g d.w., suggesting that this core was not long enough to sample sediments deposited before the deposition of anthropogenic mercury.

Total Hg concentrations in the top 33 cm of core of SC-01 core (Figure 17), located in Southerly Cove adjacent to the HoltraChem site, were much higher than in the two Fort Point Cove cores. In core SC-01, concentrations increased progressively from 1,270 ng Hg/g d.w. near the sediment – water interface (at depths where the sediment appears to be undisturbed) to 2,100 ng Hg/g d.w. at a depth of 33 cm (Figure 15, Appendix 3). Total Hg concentration in the deepest core slice (36 – 41 cm) was very high (over 24,000 ng/g). This peak layer probably represents discharges from the HoltraChem site during an early period of its operation. The core was not deep enough to see a return to background concentrations in deeper, older layers.

Summary - Three long sediment cores have been analyzed for total Hg concentrations. For all three cores, concentrations peaked at depths of 30 -35 cm below the sediment-water interface, but the peak concentrations in the Southerly Cove core were much higher than in the Fort Point Cove cores. The shape of the two Fort Point Cove cores suggests that there are sites in the upper estuary that are depositional on the long term. The pattern of a deep peak, with lowering concentrations towards the surface of the sediments is indicative of an ongoing natural attenuation in the ecosystem. These conclusions are supported by the dating of one of the cores, which demonstrates that at that site sedimentation and burial of mercury has been continuous since at least about 1960.

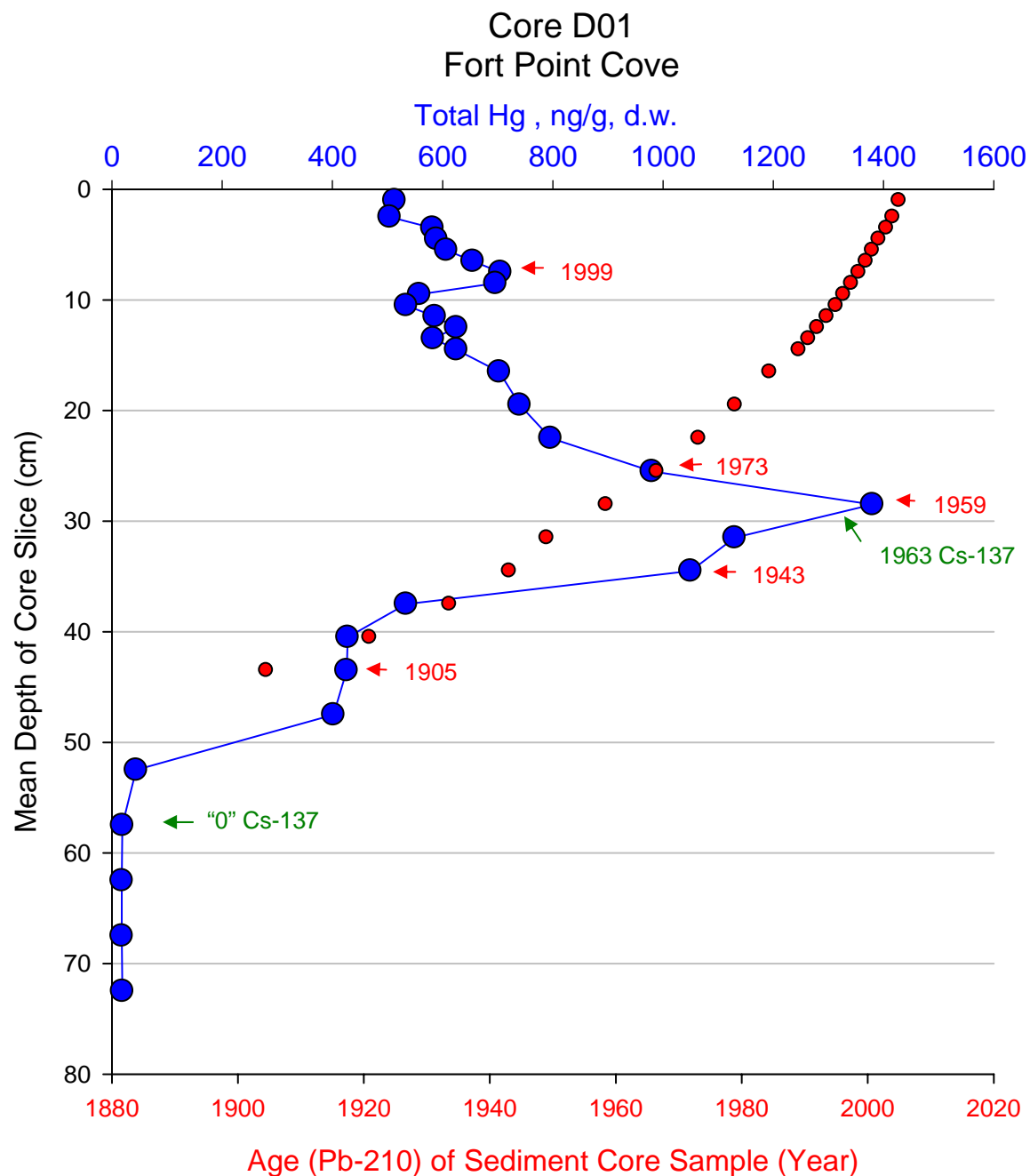


Figure 15. Total mercury concentrations, **Pb-210**, and **Cs-137** dating of sediment core (D-01) taken at Fort Point Cove, N44.48233 W68.8087. August 20, 2007. Raw data given in Appendix 1.

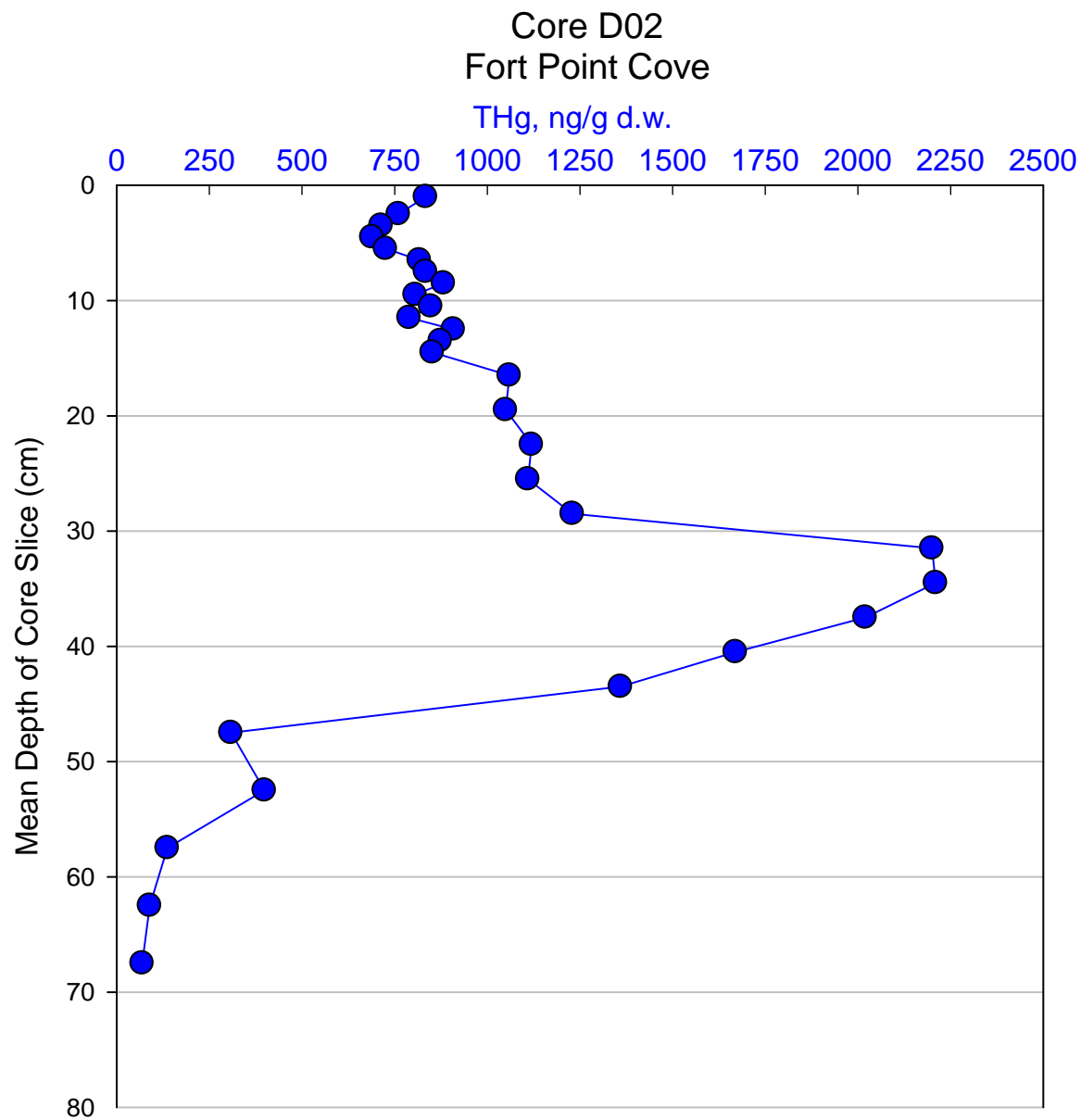


Figure 16. Total mercury concentrations in sediment core (D-02) taken at Fort Point Cove, (N44.48418, W68.81883), August 22, 2007. Raw data are given in Appendix 2.



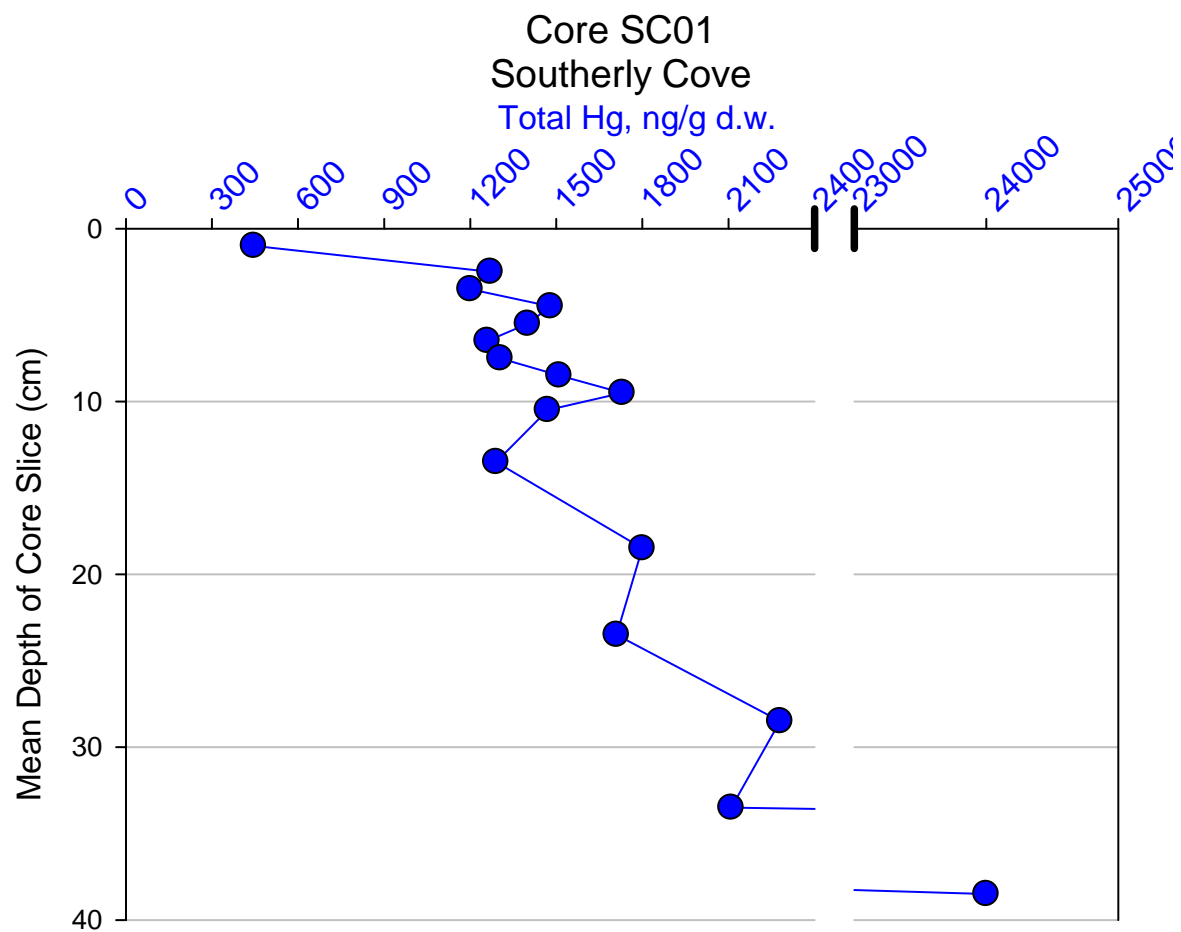


Figure 17. Total mercury concentrations in sediment core SC 01 taken at Southerly Cove (N44.73737, W68.82926), August 16, 2007. Raw data for this core are provided in Appendix 3.

## **Mercury in Wetland Soils**

As presented in the Phase I report, wetland soils were found to be most contaminated with Hg in the region from between Brewer to the South end of Verona Island (Figures 18 and 19; Appendix 4). One wetland sampled upstream of the Brewer (W05, Fig. 18) was not heavily contaminated with Hg. This wetland appears to be located beyond the most northerly point of Hg contamination from the HoltraChem plant. Wetlands in the Bagaduce Estuary and on the West and East sides of the Penobscot estuary (south of Verona Island) were also not heavily contaminated with Hg (Figure 18). The most contaminated wetland was W07, which is in Southerly Cove, adjacent to the HoltraChem site. Concentrations of total Hg in wetland soils in the contaminated zone ranged from about 18,000 to 45,000 ng/g C. This range is similar to that seen in riverine sediments at sites in the Brewer-Orrington reach, the Orrington-Bucksport reach and the upper Penobscot estuary (Phase I report).

We also determined methyl Hg concentrations in the soil samples from these wetlands. It is well known from other studies that wetlands are important sites of microbial methyl Hg production (St. Louis et al. 1994; Hall et al. 2008; Canario et al. 2007). The intensity of microbial production of methyl Hg is influenced by a number of environmental factors including the overall rate of microbial activity, as well as other environmental parameters such as pH, sulfide concentration and DOC concentration, which enhance or limit the proportion of the inorganic mercury that is bioavailable to the methylating bacteria (Winfrey and Rudd, 1990). In addition to all of these factors, the inorganic Hg concentration influences the amount of methyl Hg produced because it is the substrate of the methylation reaction. In the Penobscot we have found that the Hg concentration is the overriding factor (see discussion below).

Concentrations of methyl Hg in the contaminated wetland soils were on average much higher than in the contaminated riverine sediments (averaging 760 ng MeHg/ng org C in the wetlands as compared to 330 ng MeHg/ng org C the river sediment samples). During the August 2007 wetland survey, intertidal (unvegetated) sampling sites in front of wetlands were also sampled. When methyl Hg concentrations were plotted against total Hg concentrations the slope of the regression line at the intertidal sites was about half of the low elevation and high elevation wetlands sites, and also less than medium elevation wetlands sites (Figure 20, A-B). This also demonstrates the greater intensity of methylation in wetland soils as compared to intertidal (riverine) sediments. These observations confirm the importance of wetlands as sites of high rates of methyl Hg production.

As was the case for the riverine sediments (Figure 14), production of methyl Hg in riparian wetlands appears to be primarily controlled by the concentrations of total Hg, which is > 95% inorganic Hg. This was particularly so for the low elevation wetland

samples (Figure 20A) where the concentrations of methyl Hg were closely correlated with the concentrations of total Hg, and somewhat less so at the medium and high elevation sites that were sampled in these wetlands (Figure 20B). This controlling relationship of inorganic mercury concentration on methyl mercury production is also obvious when the wetland data are plotted in a north to south direction (Figures 19 and 21). MeHg concentrations were lowest in the wetlands with lowest total Hg concentrations (e.g. W05 above the zone of mercury contamination and the wetlands below the southern end of Verona Island), and highest in the zone where total Hg concentrations are also high (Figure 22).

An exception to above discussion is the W07 site, Southerly Cove, adjacent to HoltraChem, which had high total Hg but relatively low % methyl Hg (Figure 22). Several other studies have shown that % methyl mercury underestimates the rate of methyl Hg production in this circumstance. This is possibly because the inorganic mercury concentrations are high enough to be toxic to mercury methylating bacteria.

Within the Hg contaminated zone, the percentage of the total Hg that was methyl Hg varied noticeably depending on the location of the wetlands (Figure 22). Percent methyl Hg was highest (up to about 6%) in several transition wetlands between W13 (upstream of Mendall Marsh) and the mouth of the Marsh River – including several sites in Mendall Marsh. At this time we do not know the reason why mercury is methylated more efficiently in these wetlands than elsewhere. There are a number of environmental factors known to affect the rate of methyl mercury production (e.g. pH and sulfide concentration). Investigating which of these factors are important in these wetlands is part of Phase II of the study. However, these are quite high values for percent methyl Hg in wetland soils. In the Florida Everglades, Gilmour et al. (1998) found that average percent methyl Hg at various sites did not exceed 2%, although Canario et al. (2007) found up to 18% methyl Hg in wetlands in estuaries in Portugal.

Percent methyl Hg was lower in wetlands downstream of the mouth of the Marsh River, possibly because of reduced bioavailability of inorganic mercury to methylators due to higher sulfide concentrations in the estuary. It was also lower in the contaminated wetlands upstream of W13 possibly because of differences in pH, sulfate concentrations, or overall rates of microbial activity of the methylators.

The intensity of methyl Hg production per unit of inorganic mercury may be particularly high in the transition wetlands in the vicinity of the Mendall Marsh because in addition to the high inorganic Hg concentrations in these wetlands one of the other factors that is known to stimulate mercury methylation is likely also be optimal (e.g. pH, sulfide concentration, DOC quality). Understanding which of these factors (in addition to the high inorganic mercury concentrations) are stimulating rates of methyl mercury

production in the wetlands is a primary focus of Phase II. This understanding will be very useful for the design of active remedial measures.

This survey provided data on the concentrations of methyl Hg in riparian wetlands at one point in time only, albeit at a time (August) when methyl Hg concentrations are expected to be highest due to higher ambient temperatures. To provide a more dynamic, seasonal examination of methyl Hg dynamics in these important habitats, we are currently conducting bi-weekly sampling of two wetlands in the freshwater (lower % methyl Hg) zone, two wetlands in the transition (higher % methyl Hg) zone and two wetlands in the saline zone which has lower % methyl Hg. Also, a characterization of the vegetation in these zones is being carried out.

Summary – As with the riverine sediments, rates of production of methyl Hg in the wetland appear to be stimulated primarily by elevated concentrations of inorganic mercury in the soils where methylation occurs. It follows that methyl Hg concentrations in the wetland soils and in Penobscot biota could be lowered by reducing inorganic Hg concentrations either by the process of natural attenuation or by and active remediation measures designed to reduce the bioavailability of inorganic Hg to methylating bacteria.

Mercury methylation in both the wetlands and riverine sediments appear to be stimulated primarily by elevated inorganic mercury concentrations. The wetlands, including, Mendall Marsh, appear to have an even higher efficiency of methylation (per ng of inorganic Hg) than the riverine sediments. The reason for the higher efficiency of methylation, particularly in the transition wetlands, is likely related to environmental factors other than inorganic mercury concentration, which also stimulate methyl Hg production. This will be investigated in depth during Phase II of the study.

It is important to understand which environmental factors (in addition to inorganic mercury concentration) are stimulating methyl Hg production in the transition wetlands because this is where the highest methyl Hg concentrations are found and where song bird and shore bird methyl mercury concentrations are at toxic levels. Because of the relatively small area of these wetlands, if we can understand which factors are stimulating methyl Hg production, these wetlands may be candidates for active remediation, if a practical cost effective approach can be proven.

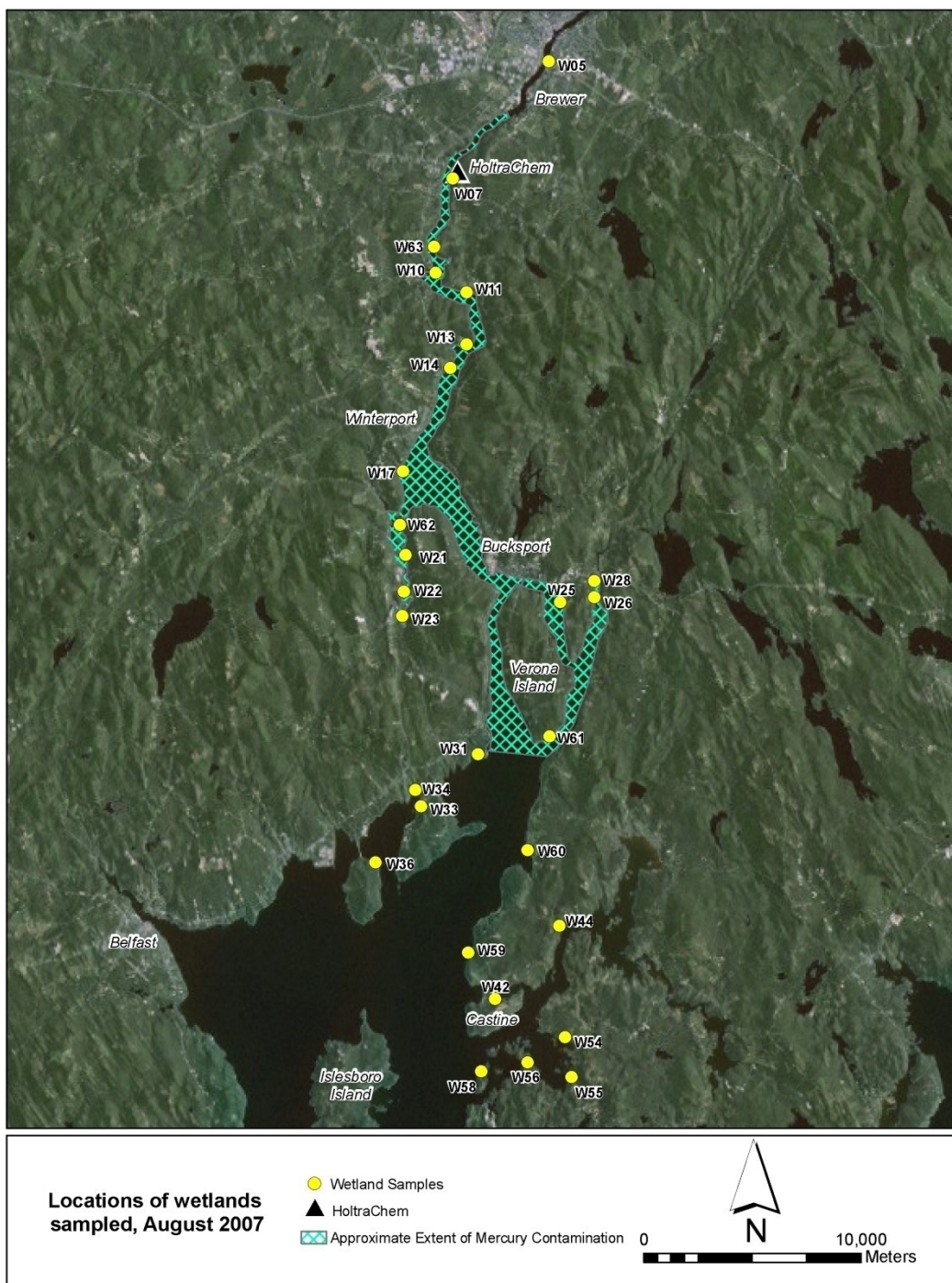


Figure 18. Map of wetland locations sampled for mercury in August 2007. Hatched area corresponds to the approximate extent of the most contaminated wetlands.

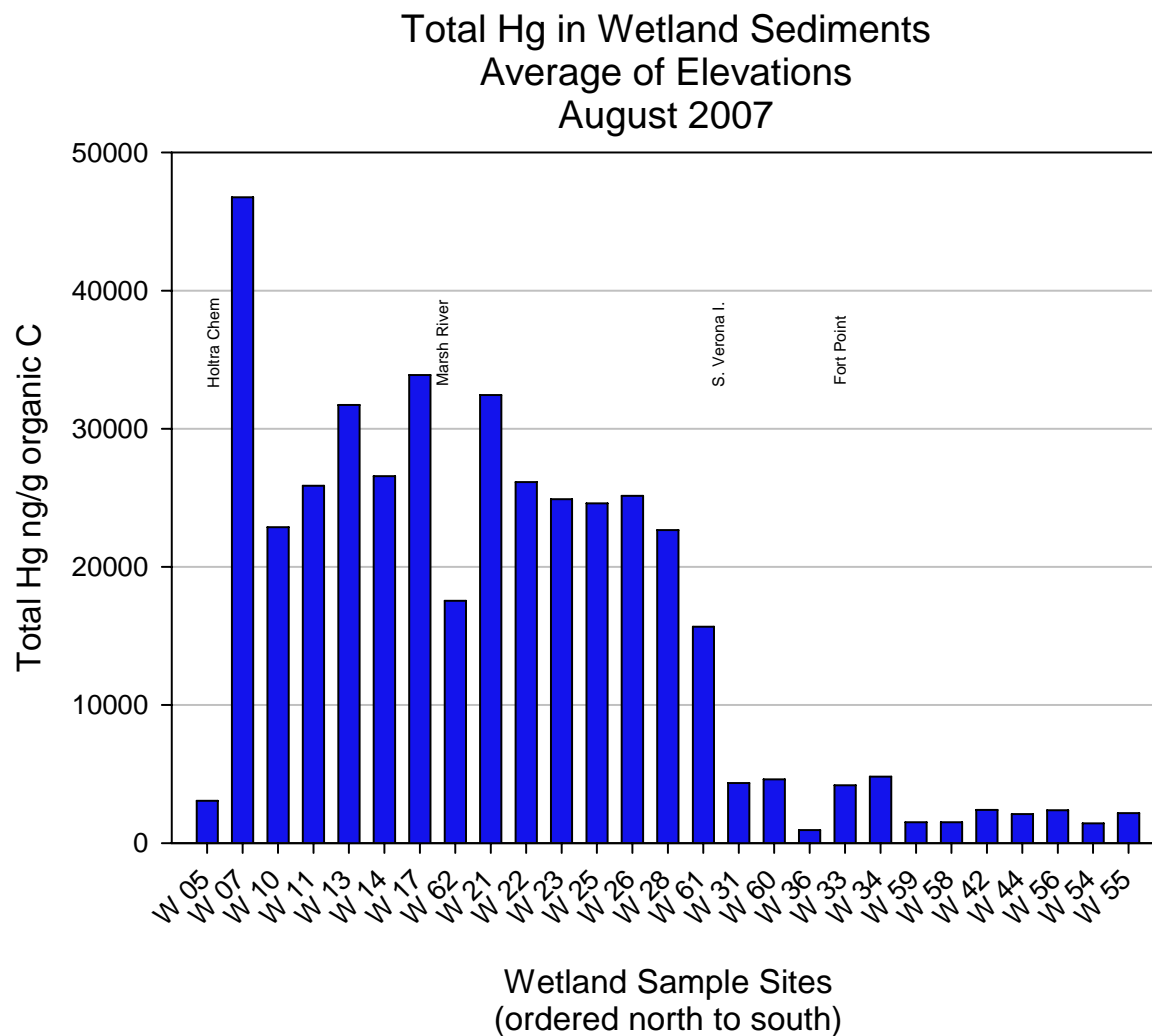


Figure 19. Concentrations of total Hg in soils of riparian wetlands in the lower Penobscot River and upper estuary. Sites are ordered from north to south. HoltraChem is adjacent to W 07 and Ft. Point lies south of W 31. Each wetland was sampled at four elevations, this graph presents the average total Hg concentration for all four elevations at each wetland. Raw data can be found in Appendix 4.

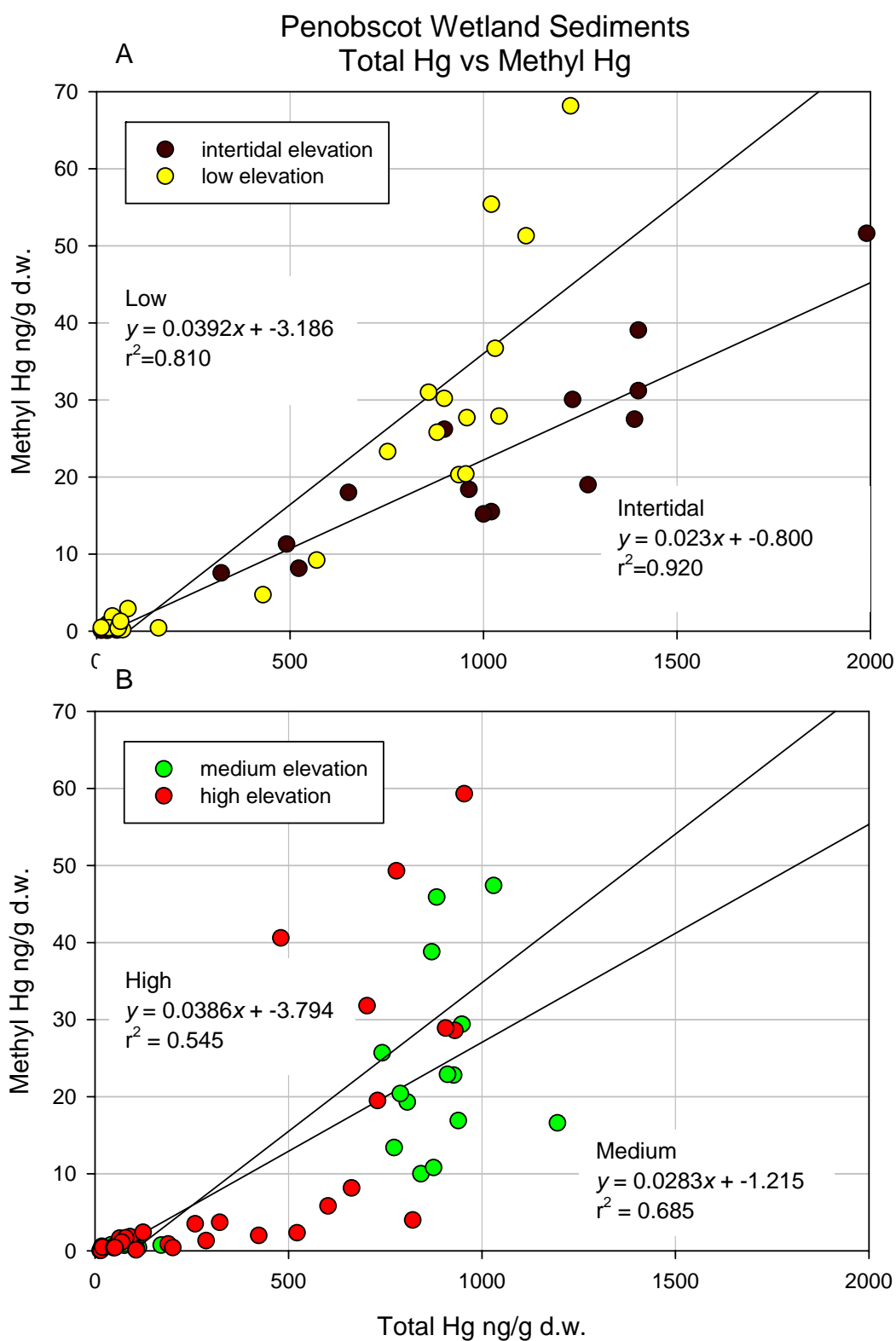


Figure 20. Relationships between methyl Hg and total Hg concentrations in wetland sediments at four different wetland elevations.

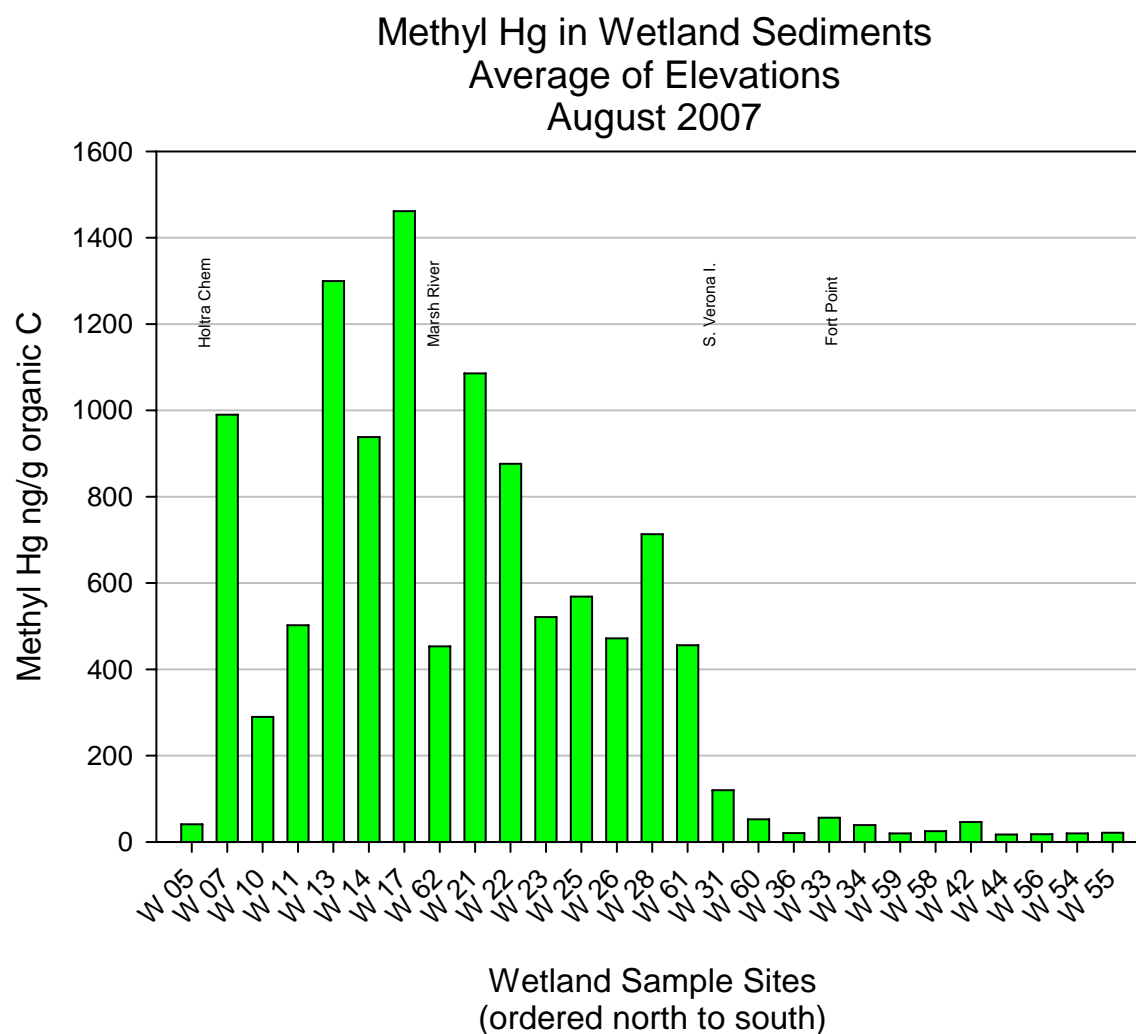


Figure 21. Concentrations of methyl Hg in soils of riparian wetlands in the lower Penobscot River and upper estuary. Sites are ordered from north to south. HoltraChem is adjacent to W 07 and Ft. Point lies south of W 31. Each wetland was sampled at four elevations, this graph presents the average methyl Hg concentration for all four elevations at each wetland. Raw data can be found in Appendix 4.



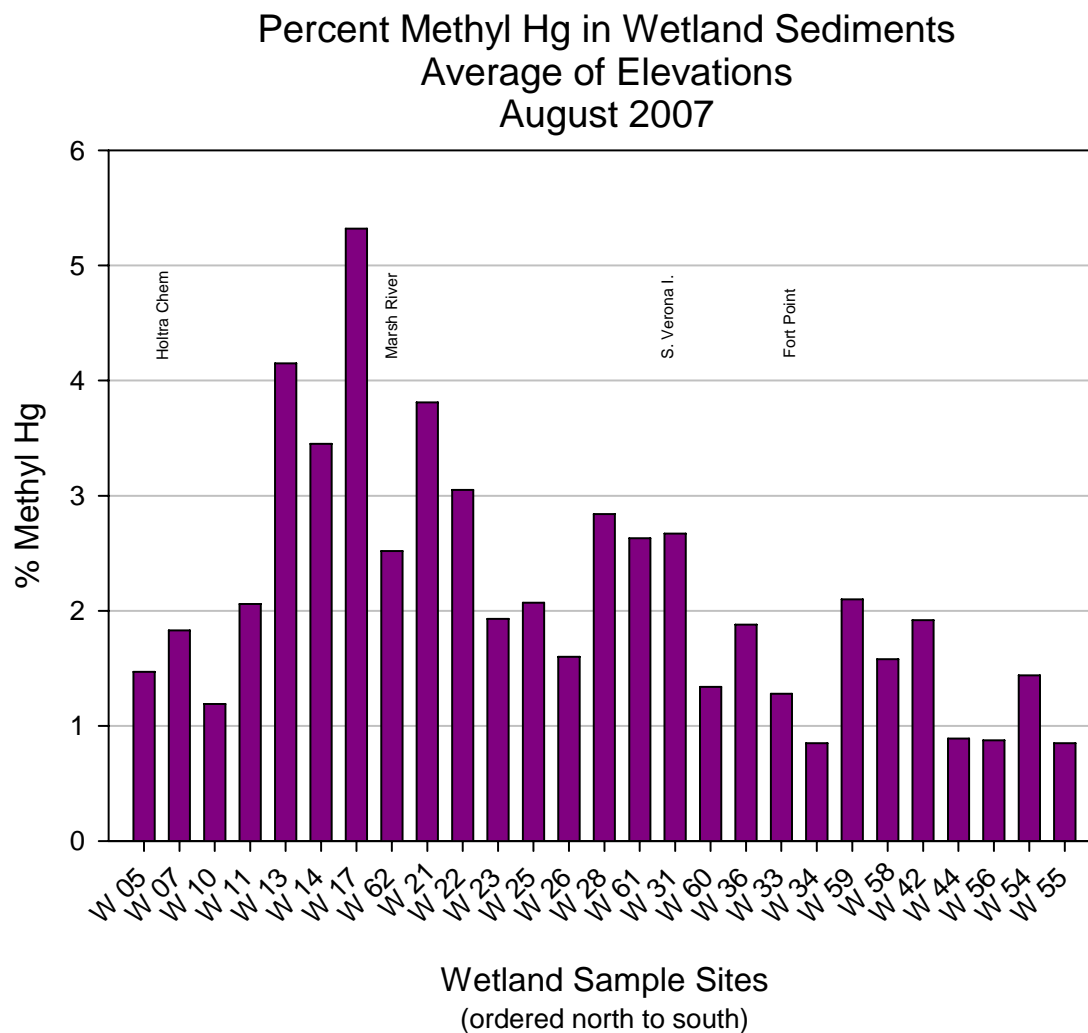


Figure 22. Percent methyl Hg in soils of riparian wetlands in the lower Penobscot River and upper estuary. Sites are ordered from north to south. HoltraChem is adjacent to W 07 and Ft. Point lies south of W 31. Each wetland was sampled at four elevations, this graph presents the average percent methyl Hg concentration for all four elevations at each wetland sampled.

## ***Mercury in Biota***

### ***Mercury in Invertebrates***

#### ***Nereis* (Polychaete worms)**

The sandworm, *Nereis virens*, inhabits the intertidal zone in estuarine and brackish waters along North Atlantic coastlines, and is harvested commercially in Maine for bait. They live in U-shaped borrows in the top 10 cm of sediment. They are classified as omnivores, yet are voracious predators on amphipods and polychaete worms (Wilson and Ruff 1988). Larger *Nereis* worms colonize lower intertidal areas (Miron and Desrosiers 1990), due to both competitive fitness and to greater tolerance of sediment sulfide levels. *Nereis* reproduce at about age 5, and die soon thereafter.

Hg concentrations in *Nereis* worms varied significantly among the sites sampled in 2006 in the OB and ES reaches and were generally lower in the outer estuary than the upper estuary (Figure 23). Total Hg levels were variable, but generally similar among the OB sample sites and sites in the upper estuary to the southern tip of Verona Island. Below Verona Island, total Hg levels declined significantly (ANOVA,  $p < 0.01$ , adjusted for *Nereis* weight and sample period, Tukey pairwise  $p < 0.05$ ) (Figure 23, Appendices 6-9). Total Hg levels were not significantly different between the two reaches sampled, OB and ES.

Methyl Hg concentrations varied significantly between reaches and among sites, with *Nereis* weight a significant covariate (Appendix 8). Methyl Hg levels were greatest in the OB reach, with the highest methyl Hg levels found at OB4 ( $214 \pm 128$  ng/g d.w.). Within the ES reach, methyl Hg concentrations were similar at most sites, although there were significantly lower levels found in *Nereis* at ES11, ES13, and ES04.

The % methyl Hg found in *Nereis* worms varied significantly among sites, but not between reaches. There was no clear geographic trend in % methyl Hg levels. Percent methyl Hg levels generally ranged from 15 – 30%, with some notable exceptions. The greatest level, 53 % methyl Hg, was found in *Nereis* sampled at OB4, in a marsh on the west side of the Penobscot River. In contrast, two sites in Penobscot Bay, ES11 and ES13, had % methyl Hg levels near 5%, and were among the four *Nereis* sites sampled with the greatest total Hg levels, above 350 ng/g d.w (Figure 23).

Total Hg levels in *Nereis* in the OB reach of the Penobscot River and in upper Penobscot Bay were greater than *Nereis* sampled in an uncontaminated estuary on the southeast coast of England and a contaminated estuary on the north coast of the Netherlands, but lower than levels in *Nereis* reported for two other contaminated

estuaries, one in England and one in Australia. In the Stour Estuary on the southeast

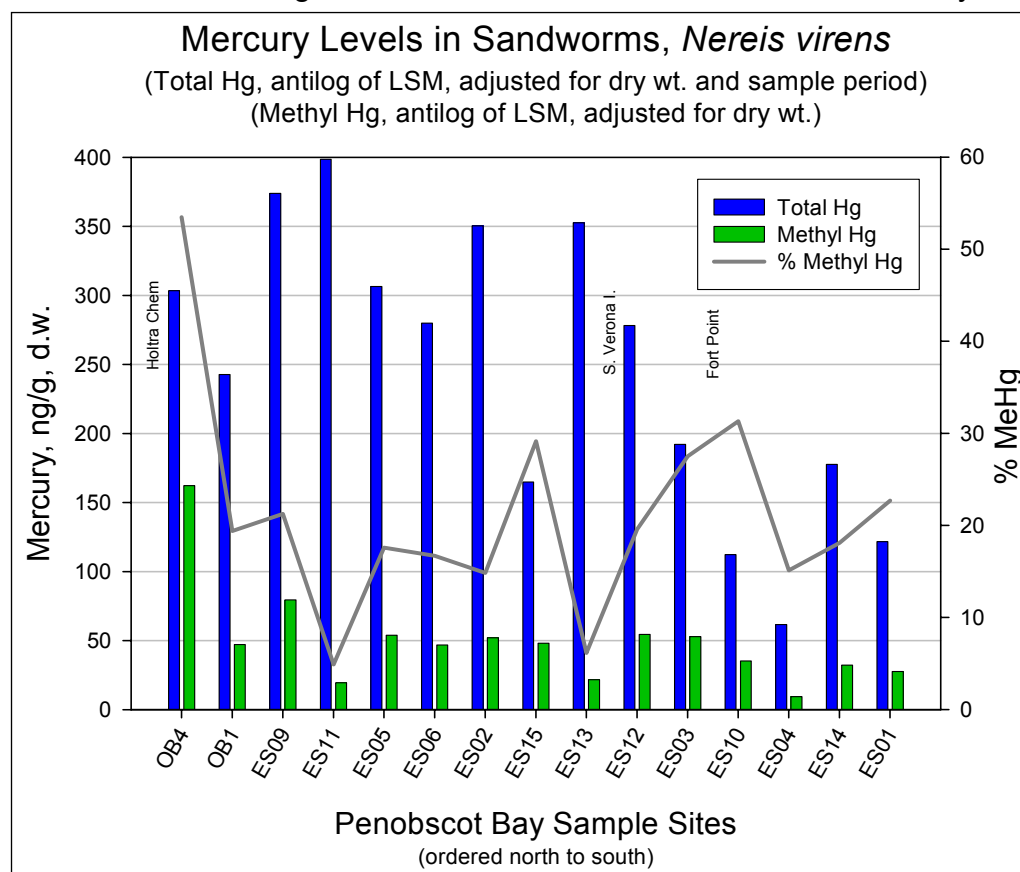


Figure 23. Mean total and methyl Hg concentrations and % methyl Hg in *Nereis* worms in Penobscot River and estuary, 2006. Total Hg levels were significantly different among certain sites sampled, but remained fairly constant to the southern tip of Verona Island (ES13), south of which levels dropped significantly. Percent methyl Hg was highly variable, ranging from 53% at OB4 to roughly 5% at ES11 and ES13.

et al. (1992) reported mean total Hg in Nereidae of 140 ng/g w.w. (calculated as 700 ng/g dry wt., assuming 80% moisture) in southwest Australia in an enclosed coastal bay contaminated with past effluent from a chlor-alkali plant. Wright and Mason (1999) reported mean total Hg of 740 ng/g d.w. in *N. diversicolor* in the Orwell Estuary, a contaminated urban estuary on the southeast coast of England. Mean *Nereis* THg in this study ranged from 321 – 569 (ng/g d.w.) in the OB reach and from 181 – 467 (ng/g, d.w.) in the upper portion of Penobscot Bay.

**Summary** – Total Hg *Nereis* worms was similar between the lower river and the upper estuary but decreased South of Verona Island. Percent methyl Hg did not vary significantly among reaches. Total Hg levels in *Nereis* in the lower Penobscot River

coast of England, mean total Hg in *Nereis* was 290 ng/g d.w. (Wright and Mason 1999). Baeyens et al. (1998) reported total Hg in *Nereis diversicolor* of 97.8 ng/g d.w. (52-164 ng/g d.w.) in samples collected from the estuary of the river Scheldt,

which drains a highly industrialized area of northern Europe. However, Francesconi

were greater than at one other uncontaminated and one contaminated site, but were less than at two other contaminated sites.

### **Macoma clams (*Macoma balthica*)**

*Macoma* clams live in nearshore estuarine waters from the intertidal zone to a depth of 40 meters. *Macoma* are sedentary, lying buried 5 to 20 cm in soft mud substrates. They are deposit feeders, using an incurrent siphon to vacuum detritus from the surface of the sediment. Most growth, to a maximum length of 20-22 mm, occurs by age 2, though this clam may live 6 – 10 years (Gilbert 1973).

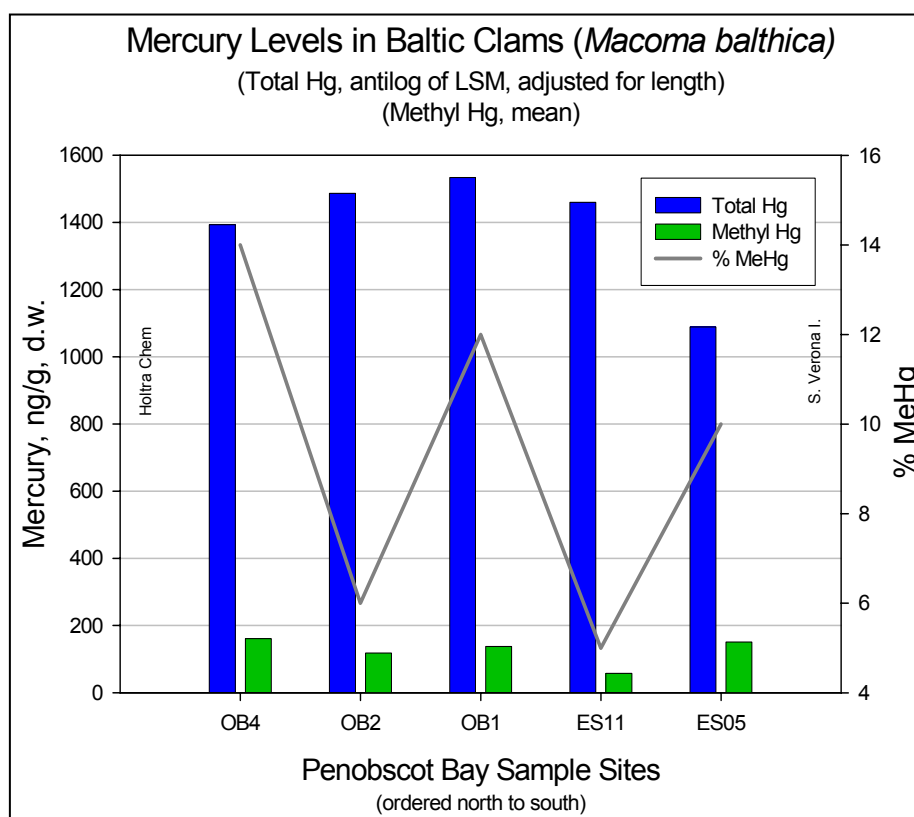


Figure 24. Mean total mercury and methyl mercury concentrations in *Macoma* clams sampled in 2006. Total Hg did not vary significantly by reach or site, after adjusting for *Macoma* length (ANCOVA,  $p > 0.05$ ), while methyl Hg levels, which were not correlated with length, were significantly greater in the OB reach (ANOVA,  $p < 0.01$ ).

Clams were sampled from the lower Penobscot River (OB) and the upper end of Penobscot Bay (ES11 and ES05) in 2006. Total Hg levels, adjusted for *Macoma* length, did not vary significantly by reach, site or period (ANCOVA). Raw data can be found in Appendix 10.

Methyl Hg concentrations, which did not vary with *Macoma* length, were found to vary significantly by both reach and by site (Figure 24). Methyl Hg levels in the OB reach were significantly greater

than in the ES reach (ANOVA), and all sites sampled had greater methyl Hg levels than found at ES11 (ANOVA,  $p < 0.0005$ , Tukey HSD,  $p < 0.05$ ). Similarly, mean % methyl Hg levels were significantly greater in the OB reach (11.2%) than the ES reach (5.6%)

(ANOVA,  $p = 0.003$ ). Among sites, % methyl Hg was significantly greater in *Macoma* sampled at OB4 and OB1 than at the other three sites sampled (ANOVA,  $p < 0.0005$ , Tukey HSD,  $p < 0.05$ ).

Total Hg in *Macoma balthica* sampled in the lower Penobscot River ( $1,654 \pm 835$  ng/g, d.w.) and upper estuary ( $1,554 \pm 1,045$  ng/g d.w.) were over seven times greater than levels reported in *M. balthica* (mean total Hg 214 ng/g d.w.) sampled from the Fraser River estuary near Vancouver, Canada (Thomas and Bendall-Young 1998). However, the Fraser River estuary historically had greater total Hg levels in *M. balthica* (890 ng/g, d.w.; McGreer 1979, cited in Thomas and Bendall-Young 1998) prior to reductions of municipal and industrial effluents. Notably greater total Hg levels (1,298 ng/g w.w.; calculated for comparison as 8,961 ng/g d.w., assuming moisture content of 84%, the % moisture level of our *Macoma* clams) were reported in *M. balthica* from a region of the Danish coast contaminated by a mercury-containing fungicide factory (Riisgård et al. 1985).

Summary - Total Hg in *Macoma* clams in the lower Penobscot River and upper Penobscot Bay in 2006 did not vary significantly by reach but methyl Hg was significantly greater in the lower river, closer to HoltraChem, as compared to the estuary.

### Soft-shelled clams (*Mya arenia*)

Soft-shelled clams are exceptionally sedentary, remaining at the same spot where they settled to the bottom as larvae. They live 10 to 20 years and feed by filtering phytoplankton, small zooplankton, benthic diatoms and suspended particulates from the water. Soft-shelled clams, also known as steamers, the clams that squirt water upwards from exposed mudflats, are harvested commercially in Maine when their shell length exceeds 50.8 mm (2 in.)

Soft-shelled clams were collected at 11 sites in Penobscot Bay and at one site in the Orrington-Bucksport reach of the Penobscot River, during Sample Periods I, II and III in

Sample Area	THg*	MeHg*
North of Ft. Point	562	328
South of Ft. Point	181	106

\* ng/g, d.w., geometric mean

2006. Sampled clams were significantly larger south of Fort Point (two-sample t-test, pooled variance,  $p \leq 0.001$ ; geometric mean shell length - north, 40.1 mm and south, 51.7 mm, geometric mean wet tissue weight

– north 1.7 g, south, 3.1 g). Size did not vary with sample period (ANOVA,  $p = 0.48$ ),

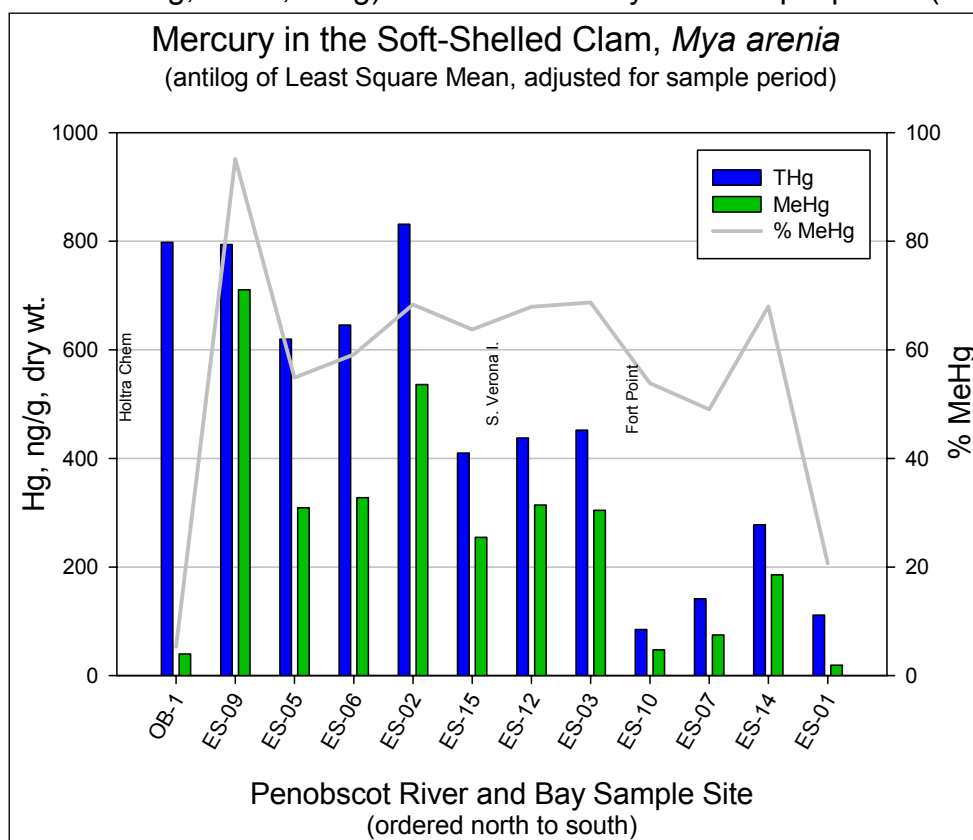


Figure 25. Mean total and methyl Hg concentrations and % methyl Hg in soft-shelled clams (*Mya*) by sample site, 2006. Raw data is in Appendix 11.

while total Hg declined significantly with sample period (ANOVA,  $p < 0.0001$ ) from a mean of 761 ng/g d.w. in sample period I (August) to 438 ng/g d.w. in period III (late September/early October).

Hg concentrations in soft-shelled clams were significantly greater north of Ft. Point than to the south (two-sample t-test, separate

variances,  $p = 0.001$ , log total Hg and log methyl Hg). Both total Hg and methyl Hg varied significantly by individual site, with sample period as a significant covariate (ANCOVA,  $p < 0.005$ ,  $r^2 = 0.897$  and  $0.752$ , respectively, Figure 25). Clam size did not significantly influence Hg concentrations levels once sample site was accounted for, nor was clam condition factor (weight/length) a significant covariate.

Percent methyl Hg varied significantly among sites (ANOVA,  $p < 0.003$ , adjusted for period), however there was no clear geographic pattern (Figure 25). Lowest % methyl Hg levels were found at OB-1 and ES-01, sites with a small sample size ( $n = 1$ ). The greatest % methyl Hg was at ES-09, on the north tip of Verona Island (Appendix 11).

Mercury levels in soft-shelled clams from the Penobscot Bay were notably greater than levels reported at both reference and contaminated sites in the St. Lawrence estuary and tributaries in Quebec, Canada. In the St. Lawrence estuary, total Hg concentrations in the whole soft-tissue of soft-shelled clams ranged from non-detected – 8 ng/g d.w., at reference sites and from 20 – 50 ng/g d.w., at sites contaminated by wastewater and commercial and recreational boating activity (Blaise et al. 2002, Gagne et al. 2006, Gagne et al. 2007). Total mercury levels in Penobscot Bay were over ten times greater than these levels. Note that 76% of the clams sampled in this study were smaller than the size legally harvested commercially.

Summary - Total Hg and methyl Hg concentrations in soft-shelled clams were significantly greater north of Fort Point, closer to HoltraChem, than further to the south. Concentrations in the Penobscot estuary were much higher than at both reference and contaminated sites in Canada.

### **European green crab (*Carcinus maenas*)**

Green crabs invaded Penobscot Bay in the 1930's and now compete with the commercially harvested rock and Jonah crabs native to the area. Green crabs are omnivores, eating a range of food from eggs, vegetation and carrion to small crustaceans. Animal matter increases in the diet as the crabs age, reaching 90% of the diet in larger crabs over 30 mm carapace width (Berrill 1982; Behrens Yamada 2001). Green crabs do not migrate in Maine, though they move to lower tidal levels with age.

Green crabs were collected in 2006 at eleven ES sites in Penobscot Bay, from ES09 on

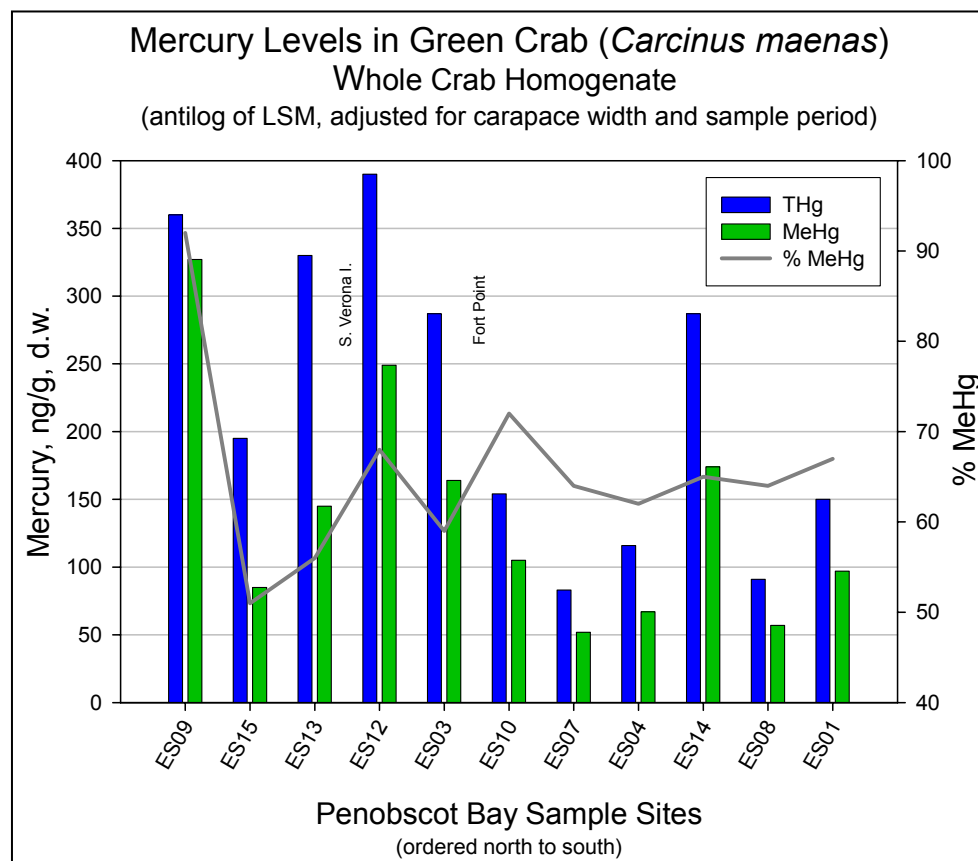


Figure 26. Mean total and methyl Hg concentrations and % methyl Hg in green crabs by sample site, 2006.

those collected at other sites (ANOVA). Green crab age was estimated from carapace width (Audet et al. 2008), and ranged from age 1 to ages 4 – 6 (crabs with carapace widths exceeding 46 mm).

Total Hg and methyl Hg levels in homogenized whole crabs varied significantly among sites, showing a general downward trend from north to south, notwithstanding inconsistencies at ES15 and ES14 (ANCOVA, adjusted for carapace width and sample

the north tip of Verona Island to the southernmost site, ES01 on the east shore of North Islesboro. All crabs were collected by hand at low tide. Green crabs varied widely in size (carapace width ranged from 13 – 81 mm), and those sampled at three sites in the southern half of the sample area (ES 07, 08, 01) were significantly smaller than



period,  $r^2 = 0.77$  and  $0.68$ , respectively; Figure 26, Appendix 12). Similarly, total Hg and methyl Hg concentrations in green crabs were greater at the five sites sampled north of Ft. Point compared to levels from the six sites south of Ft. Point (two-sample t-test). Percent methyl Hg levels did not vary significantly among sites (ANOVA). While not significant, % methyl Hg was greatest and least variable at ES09 ( $n = 5$ ,  $92 \pm 6\%$ , mean  $\pm$  SD).

Regional comparisons of Hg concentrations in green crab were complicated by the whole crab Hg values generated in this study. Coelho et al. (2008) report muscle total Hg levels from a contaminated lagoon in Portugal, ranging from 100 – 500 ng/g w.w. in one year old crabs and from 100 – 900 ng/g w.w. in crabs three years of age or older. These levels are notably greater than whole green crab total Hg concentrations in the Penobscot River, which on a wet weight basis were less than 250 ng/g. Total Hg concentrations in muscle would be expected to be significantly greater than whole body levels, but the exact ratio is unknown.

Summary - Green crabs had higher concentrations of both total Hg and methyl Hg in the upper Penobscot estuary as compared to the lower estuary in 2006.

### **Rock Crab (*Cancer irroratus*)**

Rock crab are non-migratory and carnivorous, foraging primarily on mussels, polychaete worms, sea urchins and other benthic prey (Bigford 1979). They are fished commercially using lobster traps or specially designed crab traps, with over 40% of landings in Maine coming from Penobscot and Blue Hill Bays. There is no minimum size limit, but most crabs harvested exceed a carapace width of 90 mm (Krause and Cowger 1980).

Rock crab were collected from 13 sites in central Penobscot Bay. Female crabs were significantly smaller than male crabs (mean carapace width, female =  $93 \pm 16$  mm, male =  $104 \pm 12$  mm; two-sample t-test  $p < 0.0005$ , pooled variance) (Appendix 13). Crab

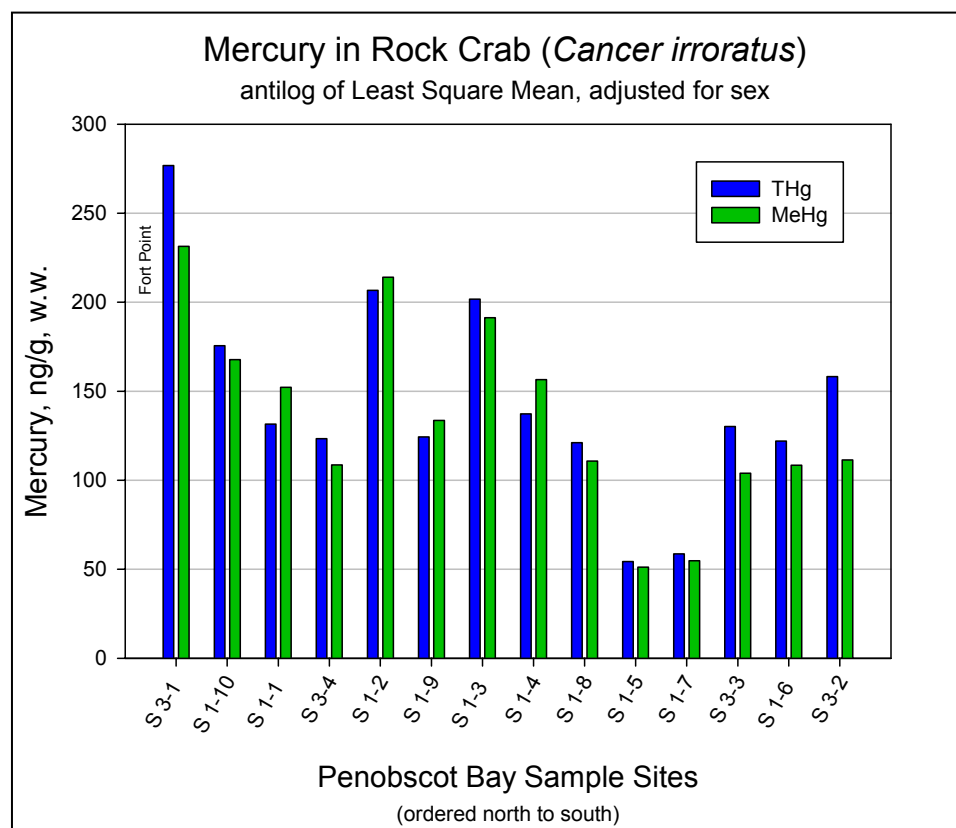


Figure 27. Mean mercury concentrations in the claw muscle of rock crab collected in Penobscot Bay in 2006

age was estimated using carapace width-at-age tables (Bigford, 1979) and females were found to be significantly older than the males (mean age 6.6 and 4.8 years, respectively; two-sample t-test,  $p < 0.0005$ , pooled variance). Adult male rock crabs grow faster than females (Krause 1972). Samples of muscle from the claws of crabs were analyzed for both total Hg and methyl Hg ( $n = 89$ ). Hg concentrations in crabs did not correlate with crab size (linear regression,  $p > 0.80$ ), yet mercury levels did vary significantly with sex. Females had significantly greater total Hg and methyl Hg concentrations in claw muscle than males. As noted above, females were older than the males, and therefore age may contribute to the greater Hg concentrations in females. However, in the combined data set,

age was estimated using carapace width-at-age tables (Bigford, 1979) and females were found to be significantly older than the males (mean age 6.6 and 4.8 years, respectively; two-sample t-test,  $p < 0.0005$ , pooled variance). Adult male rock crabs grow faster than females (Krause 1972). Samples of muscle from the claws of crabs were analyzed for both total Hg and

concentrations of total Hg did not vary significantly with crab age when adjusted for crab sex (ANCOVA, adjusted for sex,  $p > 0.05$ ).

Both total Hg and methyl Hg in rock crabs varied significantly among sample sites (ANCOVA, adjusted for crab sex,  $p = 0.003$ ; Tukey HSD found significant pairwise differences among several sites) (Figure 27, Appendix 13). While two of the four sites with elevated mercury levels were at the northern edge of the sample area, there was no overall geographic trend in Hg concentrations in crabs in the area sampled (Figures 27 and 28). Crabs at the two northern most sites near Wilson Point (S 3-1, S 1-10) and at two sites north of Islesboro (S 1-2, S 1-3) had greater concentrations of total Hg and methyl Hg than at one site northwest of Islesboro (S 1-5) (Figure 28). Hg concentrations were not significantly different at all other sites. Several possible covariates were tested, including carapace width, crab age, and sample period, but none were found to be significant. 24% of the crabs sampled (21 of 89) had methyl Hg levels exceeding the Maine methyl Hg action level of 200 ng methyl Hg/g muscle w.w.

Percent methyl Hg levels varied significantly among sites, but again no geographic trend was found within the area sampled (ANOVA,  $P < 0.05$ ) (Figure 27). Percent methyl Hg levels did not correlate with crab size (linear regression), or estimated age (ANOVA), but were significantly greater in female relative to male crabs ( $94.5 \pm 9.2\%$  and  $86.9 \pm 12.0\%$ , respectively; two-sample t-test, pooled variance,  $p = 0.04$ ).

Total Hg concentrations in rock crabs sampled in Penobscot Bay ( $204 \pm 193$  ng/g w.w.) were as high or higher than levels reported for blue crabs sampled in New York, Connecticut and Florida. Rock crabs share a similar diet with blue crabs (*Callinectes sapidus*) and their geographic range overlaps from New York to Virginia (Stehlik et al. 2004). Outside of Penobscot Bay, the greatest Hg concentrations in crab muscle were found in the New York – New Jersey Harbor, an area of known industrial contamination ( $170 \pm 120$  ng/g w.w.; NYSDEC 1996). Hg concentrations in crab muscle were lower in the Connecticut River ( $110 \pm 20$  ng/g, w.w.) and the nearby Quinnipiac River ( $60 \pm 10$  ng/g w.w.; Jop et al. 1997). Karouna-Renier et al. (2007) reported mean muscle total Hg levels of  $156 \pm 43$  (ng/g w.w.) in blue crabs collected from Pensacola Bay, Florida.

Summary - Hg in rock crabs did not vary with distance from HoltraChem. About  $\frac{1}{4}$  of crabs sampled had methyl Hg concentrations greater than the Maine DEP action level. Total Hg concentrations in rock crab in Penobscot Bay were as high or higher as a species with a similar diet from other sites.

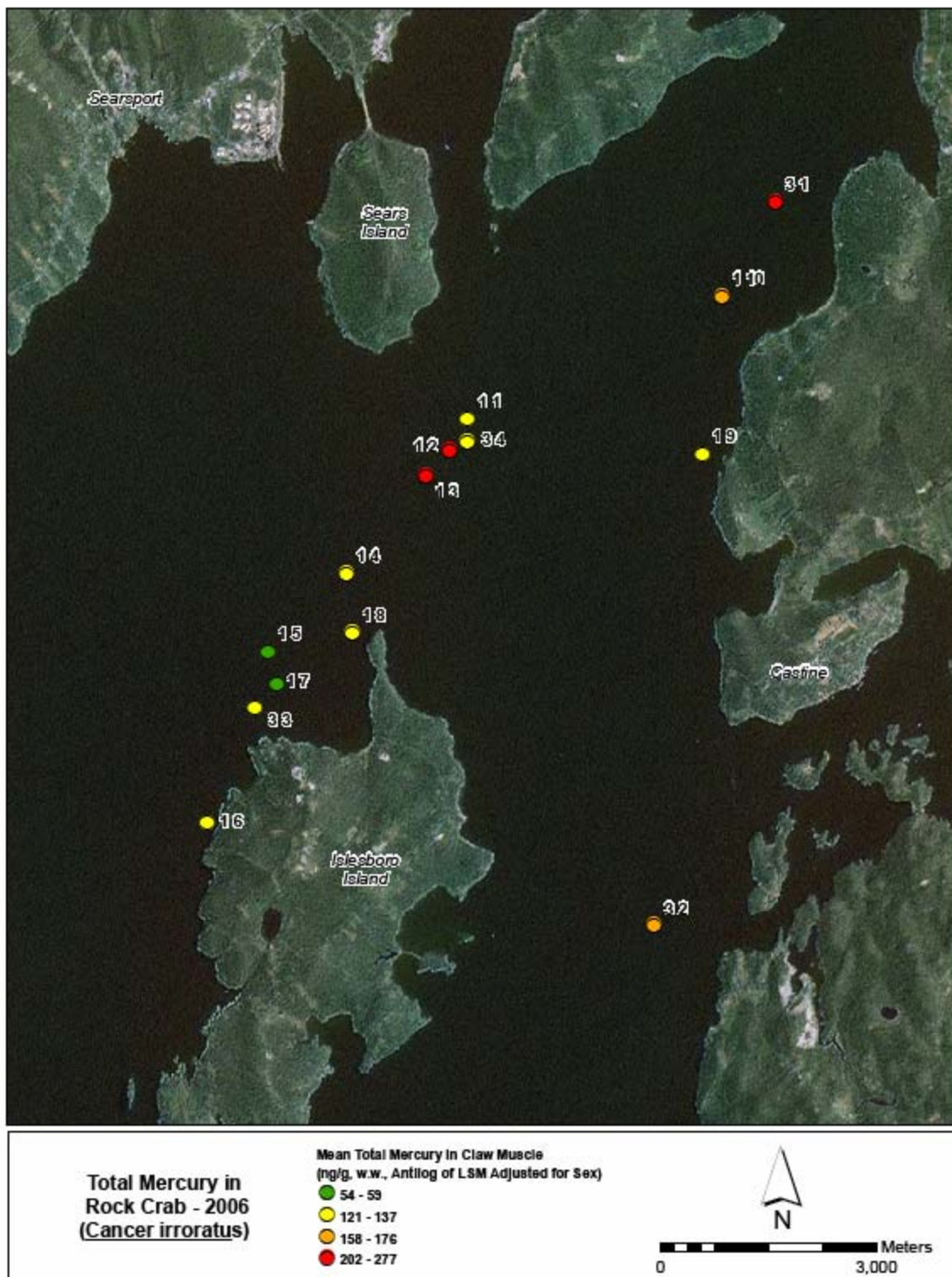


Figure 28. Map of total mercury concentrations in rock crab in the estuary of the Penobscot River, 2006.

## Mercury in Fish

### Tomcod (*Microgadus tomcod*)

Tomcod are a localized, inshore fish favoring stream mouths and estuaries. In winter they migrate upstream to freshwater for spawning, returning to estuarine water by February. Copepods are the primary food of juvenile tomcod, whereas the adult diet includes polychaete worms, amphipods, small decapods, and Crangon shrimp.

Tomcod were historically harvested by commercial fisheries. Today, sport fishermen catch them by hook and line in the fall (Collette and Klein-McPhee 2002).

It was concluded in the Phase I report that Hg in tomcod muscle was higher in the two reaches of the lower river than in the estuary, and that mean concentrations, adjusted for fish length, decreased with distance from the HoltraChem site. Data presented in the Phase I report has been updated in this report. It was found that the sampling

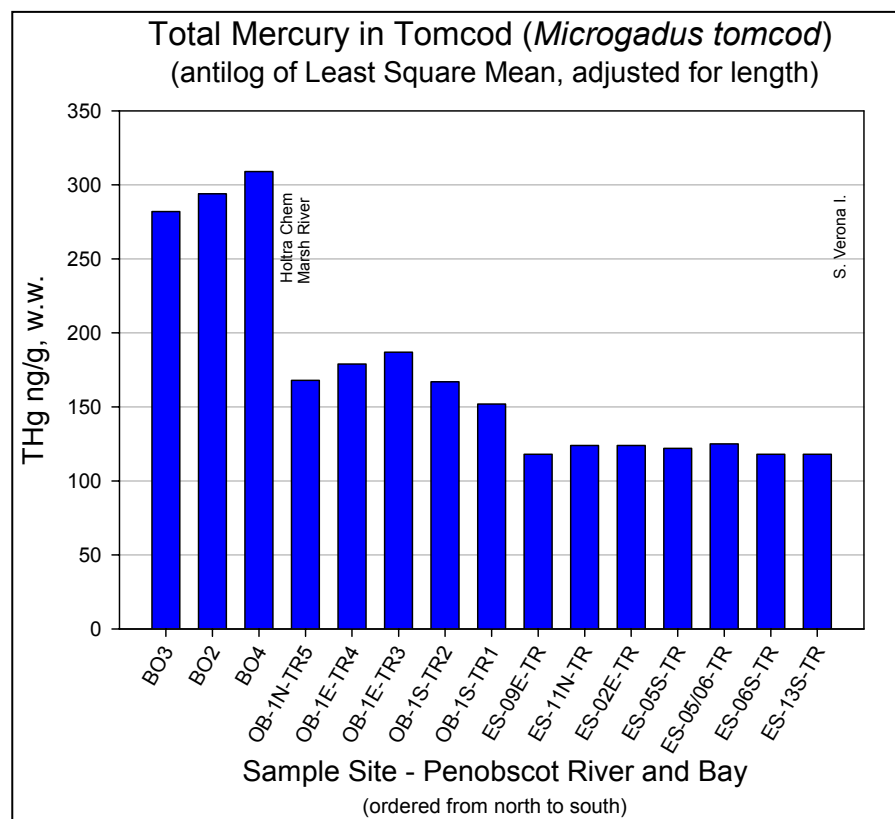


Figure 29. Mean total Hg concentrations in the muscle of tomcod sampled in the lower Penobscot River and upper estuary, September/October 2006. Sample sites have been revised from those presented in the Phase I report.

locations provided in the Phase I report were incorrect. In the Phase I report they were identified as being near to the primary (sediment) sampling stations but in the Orrington-Bucksport and Estuary sampling reaches, fish were caught at different locations than originally thought. The conclusions presented previously have not changed, however. Appendix 14 gives the locations at which tomcod were actually sampled, Figure 29 shows the relationship between Hg in tomcod and distance from HoltraChem, and

Figure 30 shows the geographic distribution of Hg in tomcod in the lower river and upper estuary. Raw data appears in Appendix 15.

Tomcod were collected in 2006 in the lower reaches of the Penobscot River and in the upper estuary of Penobscot Bay. Mercury concentrations in tomcod were significantly correlated with both length and weight (linear regression,  $p < 0.0005$ ). There was a significant north to south trend in total and methyl Hg concentrations in the three reaches sampled (ANCOVA,  $p < 0.0005$ ,  $r^2 = 0.44$ , adjusted for fish length). Hg was significantly greater in BO than in OB, and greater in OB than in ES (Tukey HSD;  $p < 0.0005$ ). Percent methyl Hg levels did not vary significantly among reaches.

Many tomcod caught in the Penobscot River and Bay had methyl Hg concentrations that exceeded 200 ng/g w.w., the Maine DEP action level for methyl Hg in fish muscle. Overall, the level of concern was exceeded in 36% of tomcod sampled. Estimated methyl Hg concentrations in tomcod muscle exceeded the Maine level of concern in 75% of the samples from the BO reach, 27% of the samples in the OB reach, and 9% of the samples in the upper region of Penobscot Bay (ES reach). We estimated the methyl Hg concentration in all samples ( $n = 160$ ), using a conservative estimate that methyl Hg comprised 95% of the total Hg in each fish.

Summary - Hg in tomcod, as was presented in the Phase I report, was found to be higher in the Brewer-Orrington reach, intermediate in the Orrington-Bucksport reach and lower in the upper estuary. Many tomcod exceeded the Maine DEP action level for methyl Hg, especially those caught in the Brewer-Orrington reach.



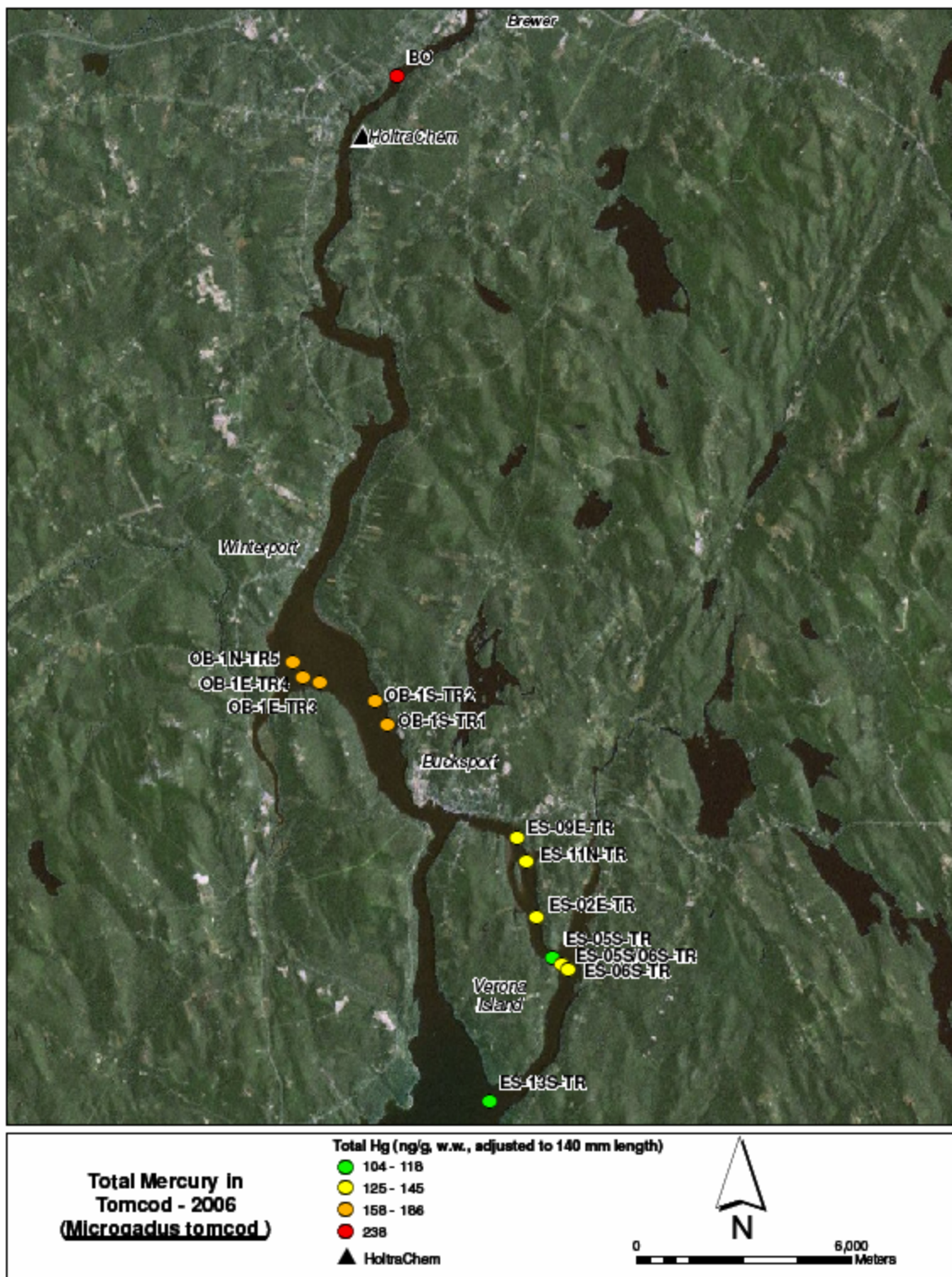


Figure 30. Map of total mercury in tomcod sampled in 2006.

### **American Eel (*Anquilla rostrata*)**

Eels are catadromous, living in fresh or brackish waters as sub-adult yellow eels until 5 to 20 years of age, when they mature to the silver phase and migrate to spawn and die in the Sargasso Sea in the mid-Atlantic. Females grow faster and mature to silvers at a later age than males. Yellow eels have strong site fidelity, with small home ranges and limited, if any, seasonal or annual movements (Oliviera 1999), and a broad diet ranging from scavenged food to polychaetes, crustaceans and bivalves. Only females reach lengths greater than 400 mm, large enough to add fish to their diet (Oliviera and McCleave 2000).

Eels, all in the non-migratory, yellow-phase, were collected in 2007 at 10 sites in the three lowest reaches of the Penobscot River, OV, BO, and OB. The average lengths of

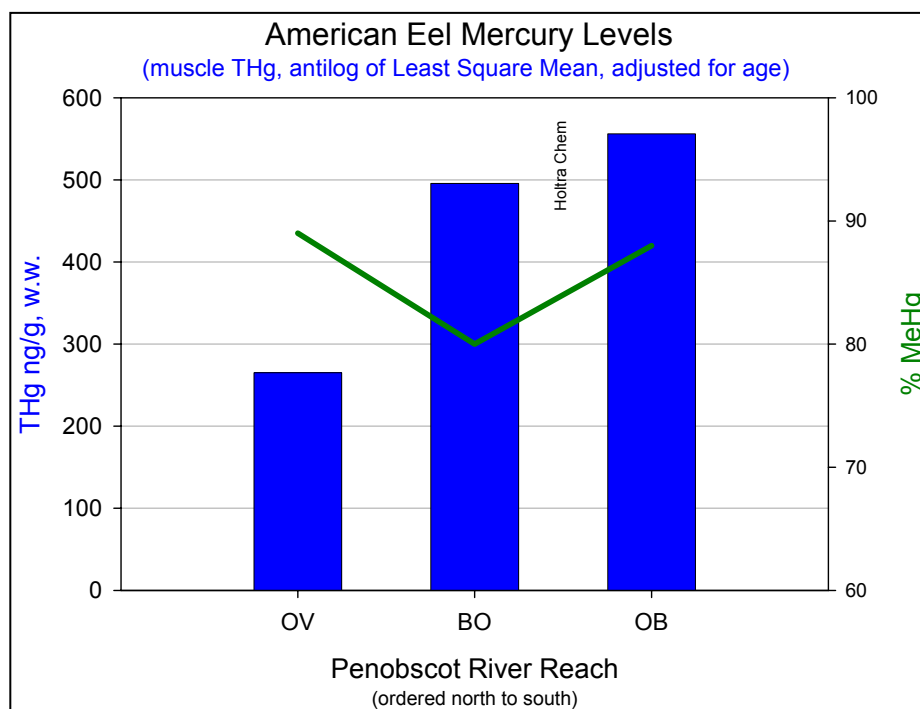


Figure 31. Mean total and methyl Hg concentrations in the muscle of American eels sampled in the Penobscot R., 2007.

significantly among reaches (Kruskal Wallace ANOVA,  $p = 0.057$ ). Eels captured in the Old Town-Veazie reach were on average one year older ( $8.7 \pm 3.9$  years) than eels captured in the Brewer-Orrington reach ( $7.8 \pm 2.4$  years) which were approximately one year older than eels captured in the Orrington-Bucksport reach ( $6.7 \pm 1.4$  years) (Appendix 16). A majority of the eels collected from the BO and OB reaches were sexually undifferentiated (72% and 70%, respectively), while over 80% of the eels from the OV reach had differentiated into males or females.

eels varied significantly by reach (ANOVA,  $p < 0.0001$ ). Eels collected from the OV reach were approximately 100 mm longer (mean length, 380 mm) and three times heavier (mean weight, 141 g) than eels collected downstream in the BO and OB reaches (Appendix 16). Despite the significant difference in eel size among the reaches sampled, age did not vary



Total Hg concentrations in eel muscle were significantly greater in the Penobscot River reaches directly influenced by HoltraChem (Figures 31 and 32). Eels collected from the BO and OB reaches in the lower Penobscot River had significantly greater total Hg concentrations in muscle than eels collected above the dam in the OV reach (ANCOVA, adjusted for age,  $p < 0.0001$ ,  $r^2 = 0.28$ , Tukey HSD  $p < 0.001$ ). While several additional variables, i.e. length, weight, and sex, were significant individual covariates, they were no longer significant when age was added to the equation; Hg in eels was therefore not adjusted for length, weight or sex. Raw total Hg concentrations in the BO and OB reaches (mean  $\pm$  SD,  $533 \pm 259$  and  $556 \pm 216$  ng/g w.w., respectively), were also greater than total Hg in the OV eels ( $350 \pm 191$  ng/g w.w.).

Methyl Hg levels, determined for a subset of the eel samples ( $n = 16$ ), did not vary significantly by reach (ANOVA,  $P = 0.56$ , no significant covariates). The small sample size limited the power of this test. The mean methyl Hg levels in the BO and OB reaches ( $563 \pm 345$  and  $591 \pm 173$  ng/g w.w., respectively) and in the OV reach ( $379 \pm 186$  ng/g w.w.), were greater than the mean total Hg levels for each reach. This inconsistent pattern was an artifact of the random subsample analyzed for MeHg, from each reach. Percent MeHg values (80 – 88%) also did not vary significantly among reaches (ANOVA,  $p = 0.26$ ) (Figure 31).

The majority of eels sampled in the lower Penobscot had muscle methyl Hg levels exceeding the 200 ng/g action level defined by the Maine Department of Environmental Protection. Methyl Hg concentrations were estimated for the full data set using the most conservative level of percent methyl Hg (80%) found in the subset actually analyzed for methyl Hg. Using this estimated methyl Hg concentration, 58% of the OV eels exceeded the Maine methyl Hg action level, and over 95% of the BO and OB eels exceeded this level.

The concentrations of methyl Hg seen in many of the eels sampled in the Brewer-Orrington and Orrington-Bucksport reaches of the Penobscot River exceed those that have been shown to cause reproductive impairment in fathead minnows. Hammerschmidt et al. (2002) demonstrated that methyl Hg at average whole body concentrations of 0.71 to 0.85  $\mu\text{g/g}$  w.w. (assuming 75% moisture content) were associated with reduced spawning success. Even lower whole body concentrations were shown to be associated with lower spawning success in fish that were switched from elevated to control concentrations of Hg in diet (Hammerschmidt et al. 2002). Drevnick and Sandheinrich (2003) showed effects on reproductive endocrinology of fathead minnows at similar concentrations of Hg. Sandheinrich (2007) summarized studies on the sublethal effects of methyl Hg on fish by noting that impacts on the

behavior of fathead minnows and other species have been observed at whole body concentrations of greater than 0.5 µg/g ww but that threshold concentrations are likely lower. Thus, it is possible that eels in the lower Penobscot River are suffering sublethal effects of mercury on their behavior and reproductive success.

Hg levels in migratory, silver-phase eels sampled in other Maine rivers and the St. Lawrence Estuary were similar to levels found in the notably younger and smaller yellow-phase eels collected from the Penobscot River. Hg concentrations in eels increase with age (Leaman 1999; Arleny et al. 2007). Leaman (1999) captured silver eels migrating from three small Maine rivers and associated upstream lakes, and reported muscle Hg levels in the range of 330 to 642 ng/g w.w., for eels with mean ages of 12 to 16 years. The mean age of Penobscot River eels ranged from 6.7 to 8.7 years. After adjusting for age, Hg in the younger Penobscot eels was clearly greater than in eels from the three nearby Maine rivers. Similarly, Hodson et al. (1994) report whole body Hg levels in silver eels captured in the St. Lawrence Estuary, migrating from upstream of Quebec City. Whole body mean Hg levels ranged from 50 to 946 ng/g w.w., for silver eels weighing between 300 and 3,000 g. Whole body eel mercury levels are reported to be 25% lower than eel muscle mercury levels (Leaman 1999). For comparison, the mean weight of Penobscot River eels was 40 to 42 grams, in the BO and OB reaches, respectively, and 141 grams in the OV reach.

Hg concentrations in eels in the lower Penobscot River exceed levels reported in European eels (*Anguilla anguilla*), except for notably larger eels sampled near Liverpool, England at a site historically contaminated by the chlor-alkali industry. Total Hg levels in the European eel ranged from 60 ng/g w.w. in the Tiber River in Italy and 160 ng/g in Bosnia and Herzegovina (Mancini et al. 2005, Has-Schön et al. 2008) to 310 ng/g in estuarine waters with industrial exposure along the coast of France (Arleny et al. 2007). The greatest mean levels were reported in eels sampled in the early 1990s in the Mersey Estuary near Liverpool, reaching 1,350 ng/g w.w. in eels exceeding 500 mm in length. The larger size of eels from the Mersey Estuary precludes a direct comparison with mercury levels in the smaller Penobscot River eels.

Summary - Hg concentrations in American eels were significantly greater in the Penobscot River reaches directly influenced by HoltraChem than in the upstream OV (Old Town-Veazie) reach. Most eels from the lower Penobscot exceeded the Maine DEP action level for methyl Hg. Hg in eels sampled from the lower Penobscot was higher than in most other sites in Maine, Canada and Europe.

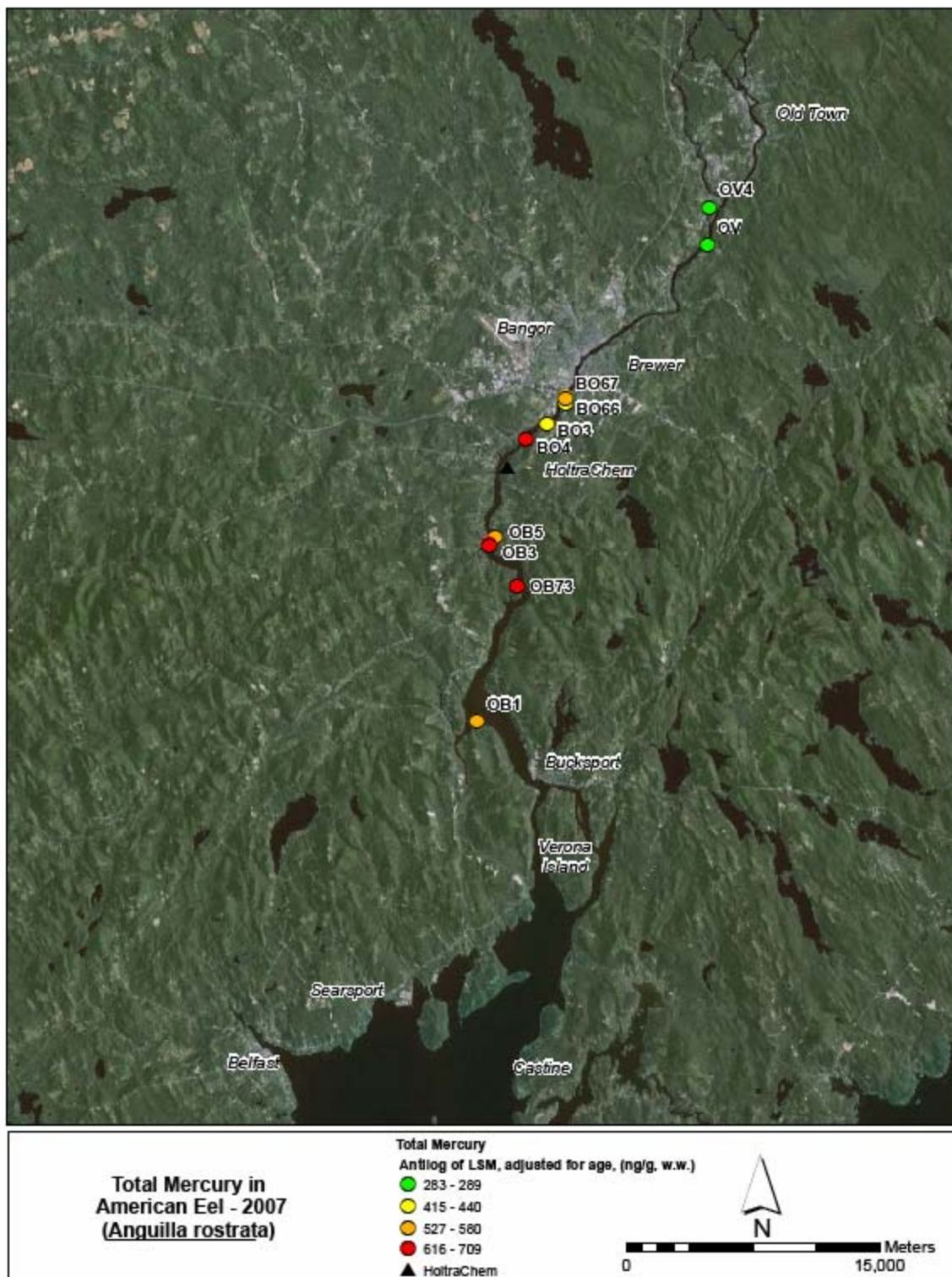


Figure 32. Map of total mercury concentrations in American eels, Penobscot River, 2007.

### **Banded killifish (*Fundulus diaphanus*)**

Banded killifish habitat ranges from shallow, brackish, coastal waters to freshwater lakes and streams. Their broad diet includes aquatic insects, small crustaceans and aquatic plants. The closely related mummichog (*F. heteroclitus*) is found primarily in salt and brackish coastal waters (Scarola 1987).

Killifish were sampled in the two lower reaches of the Penobscot River, Brewer-Orrington (BO) (26 fish collected at 5 sites), Orrington-Bucksport (OB) (9 fish collected at 5 sites), with one fish collected in the Estuary (ES). Significantly greater total Hg levels (ANCOVA, adjusted for fish length,  $p < 0.01$ ,  $n = 35$ ) were found in OB killifish collected south of the former Holtrachem facility (LS Mean, 333 ng total Hg/g w.w.), than in BO samples collected upstream of the site (LS Mean, 227 ng total Hg/g w.w.) (Figures 33 and 34). Within each reach, total Hg did not vary significantly by site. Methyl Hg levels did not vary significantly by reach (ANCOVA, adjusted for length,  $p > 0.05$ ,  $n = 11$ ); the power of the test may have been compromised by the small sample size. Methyl Hg concentrations in all killifish in the Orrington-Bucksport reach exceeded the Maine DEP mercury action level (200 ng methyl Hg/g w.w.), as did methyl Hg in three of the five samples in the Brewer-Orrington reach. As reported, % methyl Hg levels in killifish muscle averaged 104%, analytically impossible, but within the combined analytical errors for total Hg and methyl Hg analyses, and indicating a % methyl Hg in killifish greater than 95%.

Killifish total Hg concentrations in the lower Penobscot River were over ten times greater than reported in the closely related mummichog sampled in the lower Passaic River in the New York / New Jersey Harbor. Ianuzzi et al. (2004) report mean total Hg concentrations in whole mummichog of  $20 \pm 10$  ng/g w.w. This mean Hg level is notably lower than the mean total Hg concentrations in the lower Penobscot River of 230-330 ng/g w.w., even noting that the Passaic River whole fish Hg concentrations are approximately 25% lower than the muscle Hg concentrations reported for the Penobscot River.

Summary - Total Hg concentrations in killifish were higher in the Orrington – Bucksport reach compared to the Brewer – Orrington reach. All methyl Hg levels in the Orrington – Bucksport reach exceeded the Maine DEP methyl Hg action level of 200 ng/g w.w.

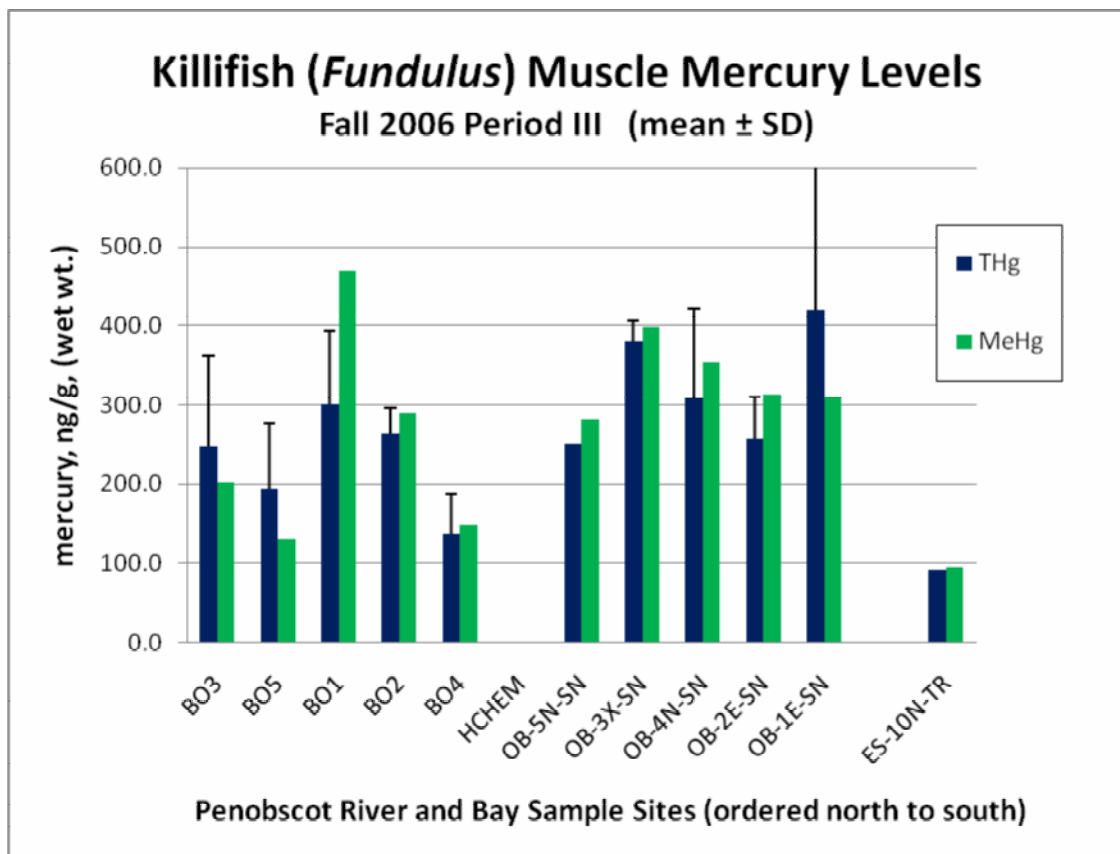


Figure 33. Total and methyl Hg concentrations (mean  $\pm$  standard deviation) in the muscle of killifish (*Fundulus*) in the BO (Brewer-Orrington), OB (Orrington-Bucksport) and ES (Estuary) sampling reaches of the Penobscot River and Penobscot Bay, 2006. Raw data can be found in Appendix 17.



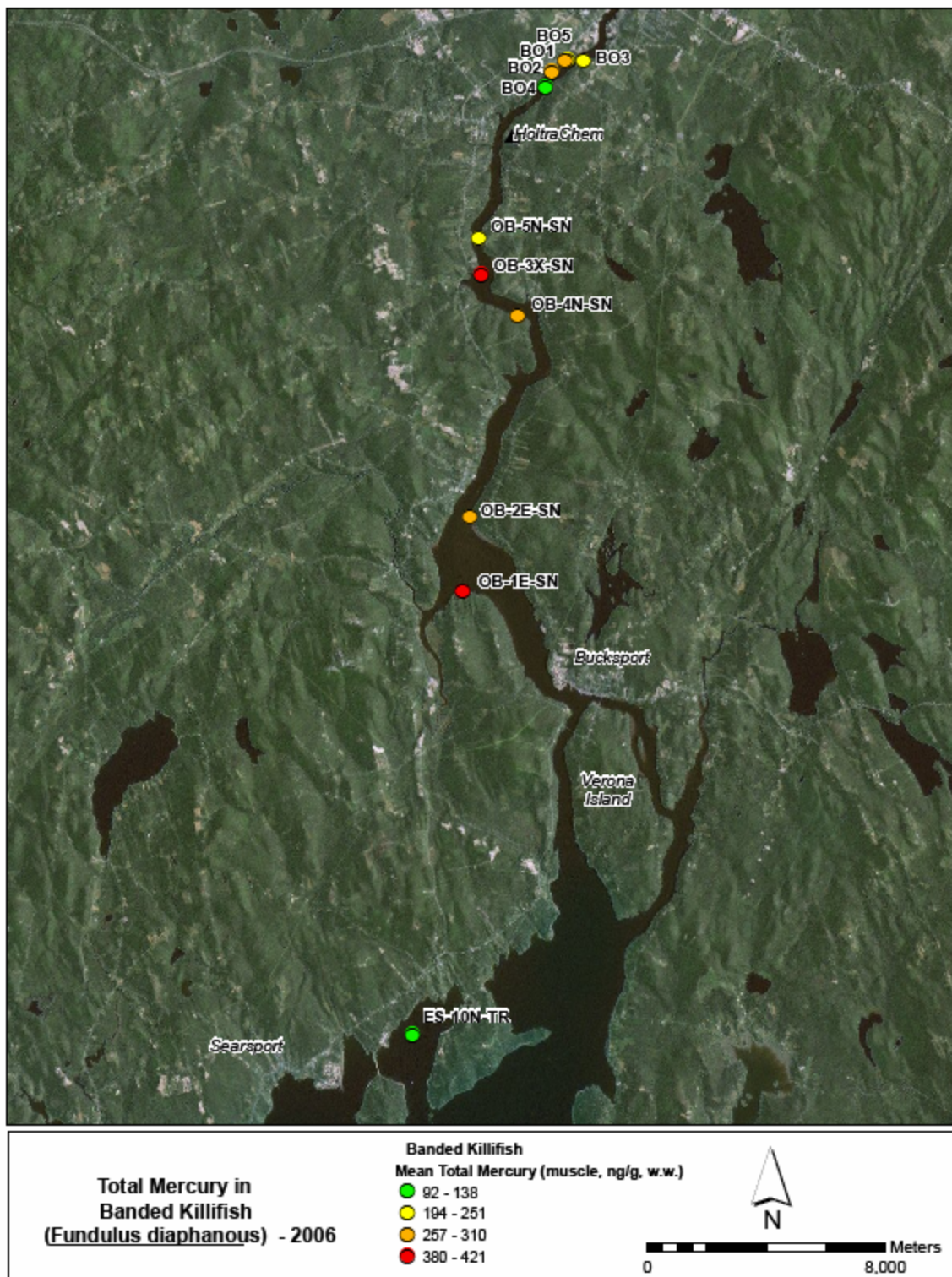


Figure 34. Map of total mercury concentrations in banded killifish sampled from the Penobscot River and estuary, 2006.

### **Rainbow smelt (*Osmerus mordax*)**

Rainbow smelt are anadromous, spawning in the spring in coastal freshwater streams and returning to nearshore saltwater by early May. Smelt are carnivores, feeding on shrimp, amphipods and marine worms when young, and small fish, including herring and shiners, as they mature. They are fished commercially, and there is a large spring sport fishery for this fish. Maine has no minimum length restrictions for rainbow smelt; mature, spawning fish are generally two to three years old, and 125 to 200 mm in length (Collette and Klein-McPhee 2002), roughly twice the mean length of fish sampled in this study.

Rainbow smelt were collected from the Orrington-Bucksport reach (OB, n = 7) and in Penobscot Bay (ES, n = 100). Smelt length and weight increased with distance south from Orrington through the sample sites in Penobscot Bay (ANOVA,  $r^2 = 0.53$  and  $r^2 = 0.46$ , respectively,  $P < 0.0005$ ). Raw data can be found in Appendix 18.

Mercury concentrations in smelt (adjusted for fish length) declined with distance south from the Holtrachem facility (Figures 35 and 36). Total Hg in smelt from OB were significantly greater than smelt sampled in Penobscot Bay at and below Verona Island. Lower but statistically equivalent levels were found west of Verona Island (Figure 34) (ANCOVA, log THg adjusted for smelt length,  $r^2 = 0.46$ ,  $P = <0.0005$ , Tukey pairwise,  $P < 0.05$ ). Smelt MeHg levels also varied significantly among sites, showing a general decline from Orrington to the southernmost sites in Penobscot Bay (ANCOVA, adjusted for length,  $r^2 = 0.96$ ,  $P = 0.035$ ). Percent methyl mercury levels varied widely (48 – 103%), averaged 94%, and did not vary significantly with total mercury levels, fish length or collection site. No smelt methyl Hg levels exceeded the Maine DEP Hg action level in fish muscle (200 ng/g w.w.).

THg levels in the lower Penobscot River ( $94 \pm 23$  ng/g w.w.) and upper section of Penobscot Bay north of Ft. Point ( $90 \pm 46$  ng/g w.w.) exceeded levels reported in smelt from freshwater Canadian lakes. Swanson et al. (2006) found that rainbow smelt from 25 lakes in Eastern Canada had a mean muscle total Hg level of 60 ng/g w.w. (standardized to a fish mass of eight grams; fish exceeding ~100 mm in length). Hg levels in larger specimens (length 160 – 220 mm) of the European smelt (*Osmerus eperlanus*) sampled in the Helsinki region of the Gulf of Finland (THg, 90 – 130 ng/g muscle, w.w.) were equal to or greater than levels in Penobscot Bay. However, the smelt sampled in the Penobscot River and Bay were notably smaller (mean smelt length, OB = 35 mm, ES north of Ft. Point = 67 mm, and ES south of Ft. Point = 89 mm), precluding a direct comparison of Hg in smelt from the two regions.

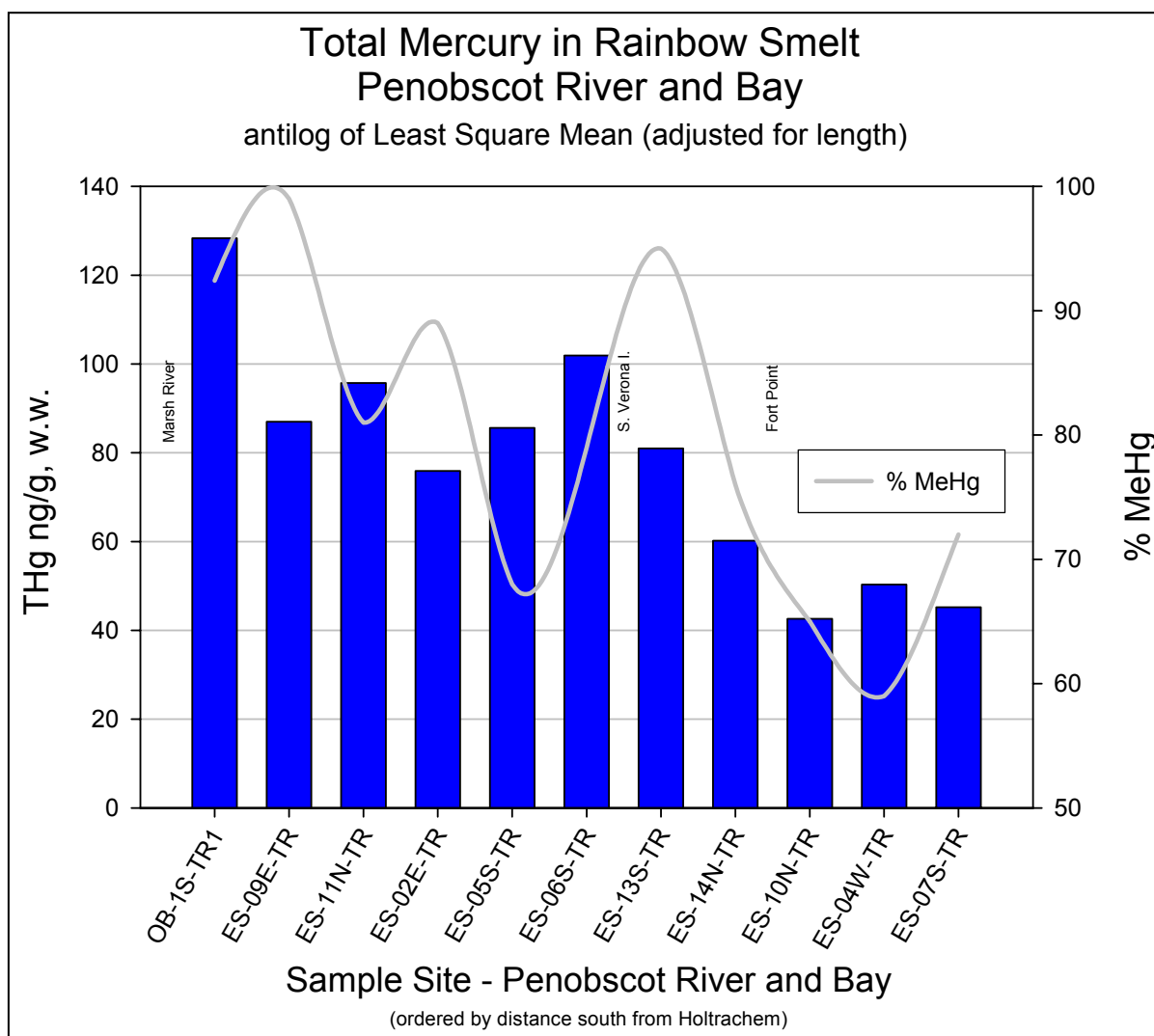


Figure 35. Mean total and methyl Hg concentrations in rainbow smelt in the lower Penobscot River and estuary, sampled in 2006. Concentrations generally decline with distance south from the Holtrachem facility. Note that methyl Hg was analyzed on a subset of the total Hg samples.

**Summary** - Total Hg and methyl Hg levels in smelt varied significantly among sites in the lower river and estuary, and showed a general decline from HoltraChem to the southernmost sites in Penobscot Bay. Hg in smelt from the lower Penobscot River and upper estuary were higher than in Canadian lakes.



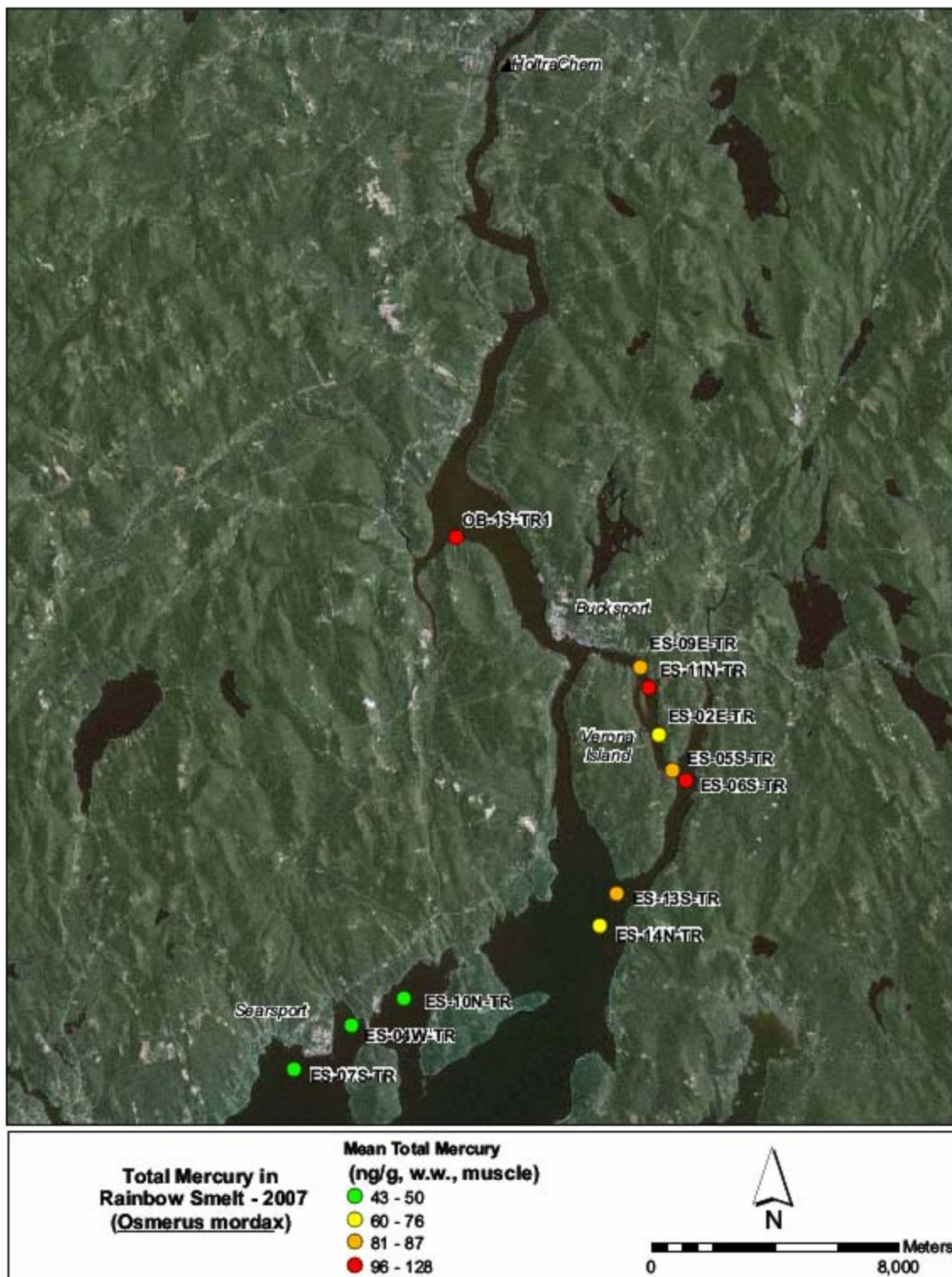


Figure 36. Map of total mercury concentrations in rainbow smelt, adjusted for fish size, in the lower Penobscot River and upper estuary, 2006.

**Winter flounder (*Pleuronectes americanus*)**

Winter flounder is an inshore fish with limited seasonal movements. They tolerate the brackish water of estuaries and river mouths and prefer a muddy sand substrate in which they can bury themselves. Their diet is mostly small invertebrates, including polychaete worms, anthozoans, clam siphons, and small crustaceans. They can live up to 15 years (Collette and Klein-McPhee 2002).

Winter flounder were collected from eight sites within Penobscot Bay (ES, n = 83) during Sample Period III in the fall of 2006. Raw data are located in Appendix 19.

Total Hg concentrations in the muscle of flounder varied significantly among sites (ANCOVA, adjusted for fish length,  $p < 0.0005$ ,  $r^2 = 0.7$ ) and there was a general decrease in mean concentrations from stations further north to those further south (Figures 37 and 38). The highest Hg concentrations were at ES13S-TR, off the southern tip of Verona Island and the lowest were at ES-04W-TR, located near Searsport. Mean total Hg concentrations in all samples of winter flounder from Penobscot Bay fish were significantly greater than Hg in winter flounder sampled at the Downeast sites (ANCOVA, adjusted for fish length,  $p < 0.00005$ ,  $r^2 = 0.74$ ).

Methyl mercury levels in winter flounder also varied significantly among Penobscot Bay sampling sites (ANCOVA, adjusted for fish length,  $p = 0.03$ ,  $r^2 = 0.9$ ), following the same geographic pattern found for total Hg (Appendix 19). Percent methyl Hg values were variable, though greatest at the northern-most site, ES15S-TR, and lowest at sites on the south and west side of Penobscot Bay (Figure 37).

Overall, total Hg levels in flounder muscle were fairly low (grand mean  $30.1 \pm 20.5$  ng/g w.w.) and no methyl Hg levels (grand mean  $31.3 \pm 26.3$  ng/g w.w.) exceeded the Maine DEP action level for methyl Hg of 200 ng/g. However, the fish sampled were less than a year old, averaging 61 mm in length, and too small to be caught as sportfish. As the minimum size of winter flounder caught as sportfish is 300 mm (12 inches), roughly a three year old fish, these findings are not indicative of the exposure to people eating winter flounder.

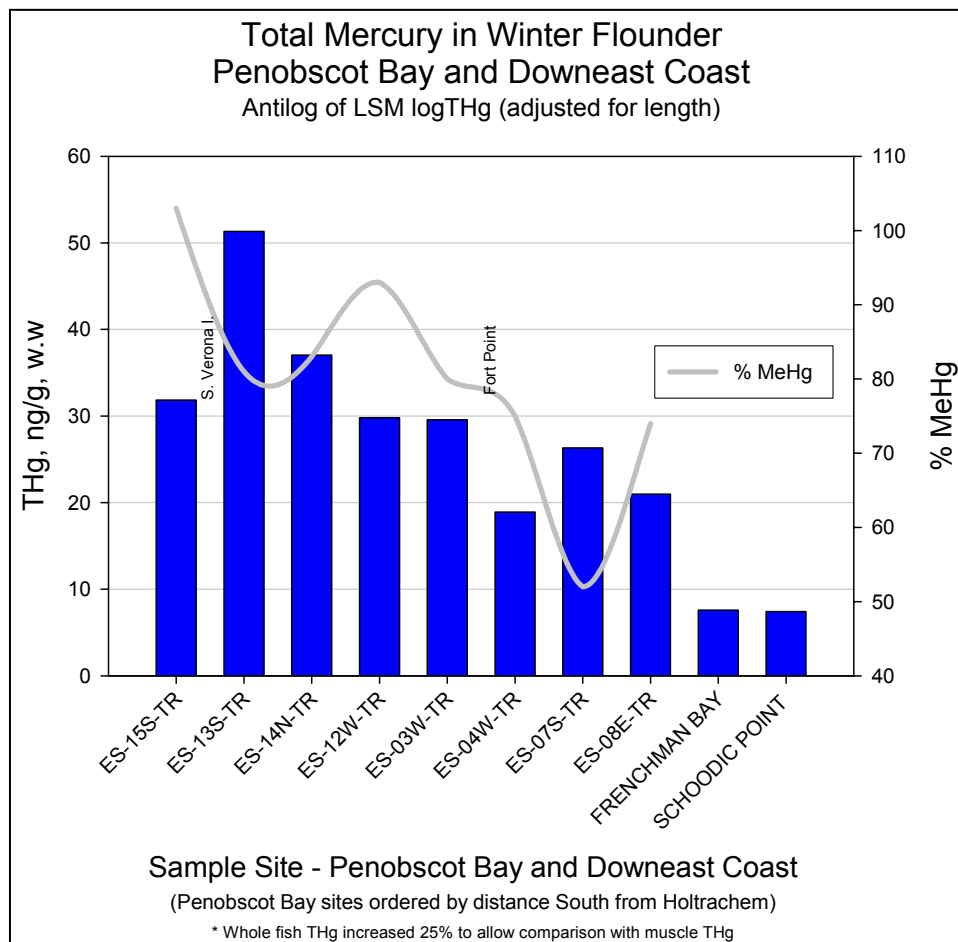


Figure 37. Mean total Hg concentrations and percent methyl Hg in winter flounder.

Mercury in Penobscot Bay winter flounder was notably greater than found in Long Island Sound ( $21 \pm 18$  ng methyl Hg/g muscle, Hammerschmidt and Fitzgerald 2006), despite the greater size (and presumably age) of the Long Island Sound flounder (mean sample length Long Island Sound, 236 mm; mean sample length Penobscot Bay, 61 mm).

Winter flounder sampled in Delaware Bay in 1975 had total Hg

levels ( $57 \pm 29$  ng total Hg/g muscle, Gerhardt 1977) equivalent to levels found in winter flounder at the most contaminated sites in Penobscot Bay. Again, the flounder sampled in Delaware Bay were notably larger (180 – 200 mm in length) than flounder sampled in Penobscot Bay. Given the accumulation, over time, of mercury in fish muscle, larger, older fish are expected to have greater mercury levels than smaller, younger fish.

**Summary** - Total Hg concentrations in the muscle of winter flounder sampled in 2006 in Penobscot Bay varied significantly by site but did not show a geographic decline with distance south from HoltraChem. All concentrations of total Hg in winter flounder from Penobscot Bay were significantly greater than found in winter flounder sampled from further east on the coast of Maine

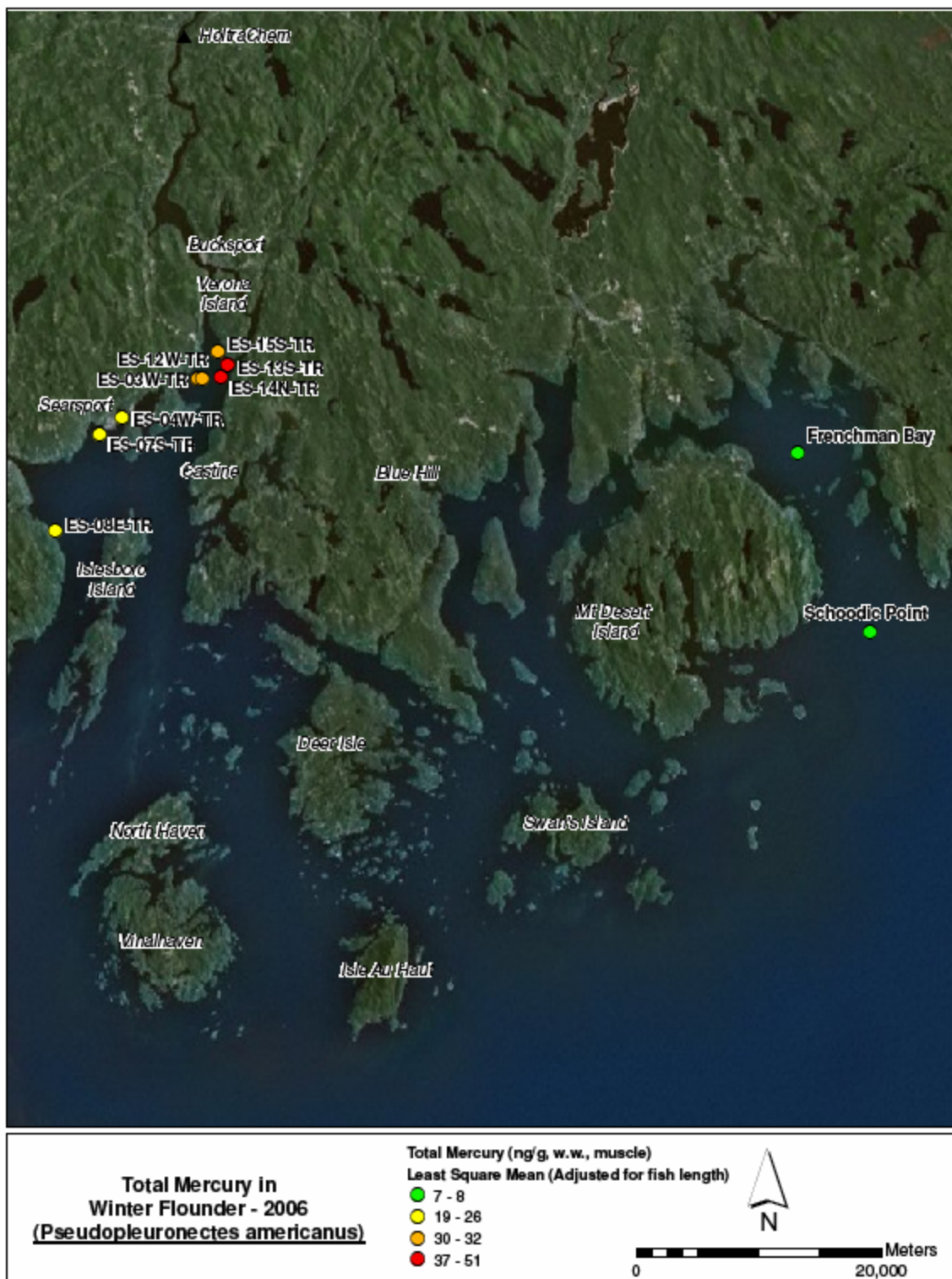


Figure 38. Map of total mercury concentrations in winter flounder sampled from the lower Penobscot River, Penobscot Bay, and from two sites on the eastern Maine coast.

**Golden shiner (*Notemigonus crysoleucas*)**

Golden shiners are freshwater minnows that prefer slow streams, ponds or lakes. They filter feed on phytoplankton and zooplankton, along with eating insect larvae, snails, clams, and (rarely) small fish (Scarola 1987).

Hg concentrations in golden shiners were similar from Brewer to Bucksport, spanning the two lower Penobscot River reaches that border the Holtrachem facility (Figures 39 and 40). Golden shiners had mean total Hg concentrations in muscle of  $222.6 \pm 52.4$  ng/g w.w. and mean methyl Hg levels of  $198.9 \pm 66.3$  ng/g w.w. Hg concentrations did not vary by reach or by site (ANOVA,  $p > 0.05$ ). Percent methyl Hg in golden shiner muscle averaged 86% (min-max, 63 – 104%), and also did not vary between reaches (ANOVA,  $p > 0.05$ ). Methyl Hg levels exceeded 200 ng/g w.w. (the Maine DEP action level for methyl Hg in fish muscle) in 65% of the golden shiner samples analyzed for methyl Hg.

Golden shiner Hg concentrations in the lower Penobscot River were over three times greater than reported in the same species collected from 25 Canadian lakes. Swanson et al. (2006) found a mean total Hg concentration in muscle of 340 ng/g d.w in golden shiner standardized to a mean weight of 8 grams. Assuming a moisture content of 80%, this Hg concentration translates into roughly 68 ng/g w.w.

Summary - Hg levels in golden shiners were statistically equivalent from Brewer to Bucksport, over the whole area contaminated with HoltraChem Hg. Methyl Hg levels exceeded the Maine DEP action level (200 ng MeHg/g muscle w.w.) in 65% of the samples analyzed. Hg in golden shiners in the Penobscot was much higher than in golden shiners from Canadian lakes.



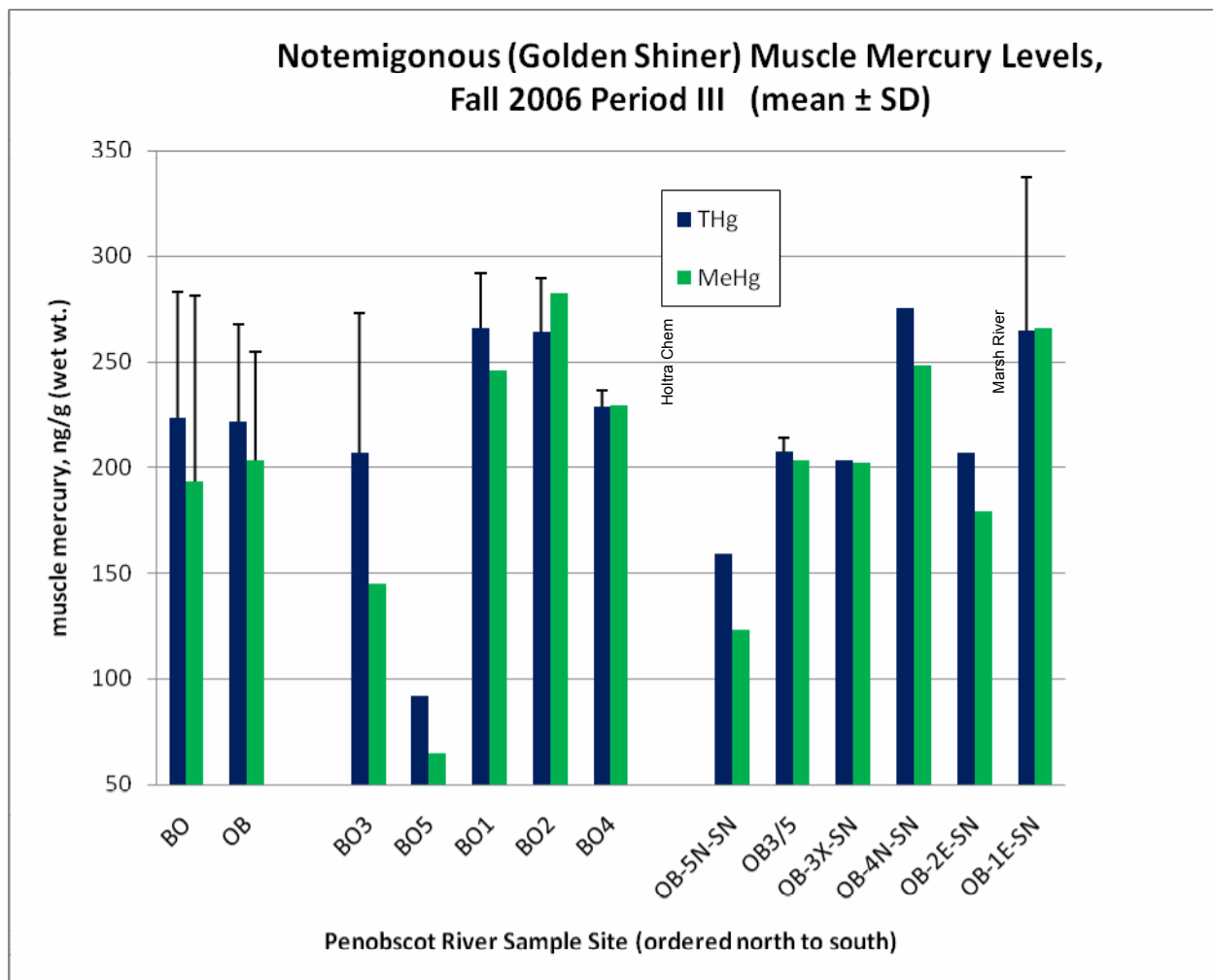


Figure 39. Mean total mercury and methyl Hg concentrations in the muscle of golden shiners from the lower reaches of the Penobscot River. Mercury did not vary significantly among individual sites or between the two reaches sampled (ANOVA,  $p > 0.05$ ). Raw data can be found in Appendix 20.

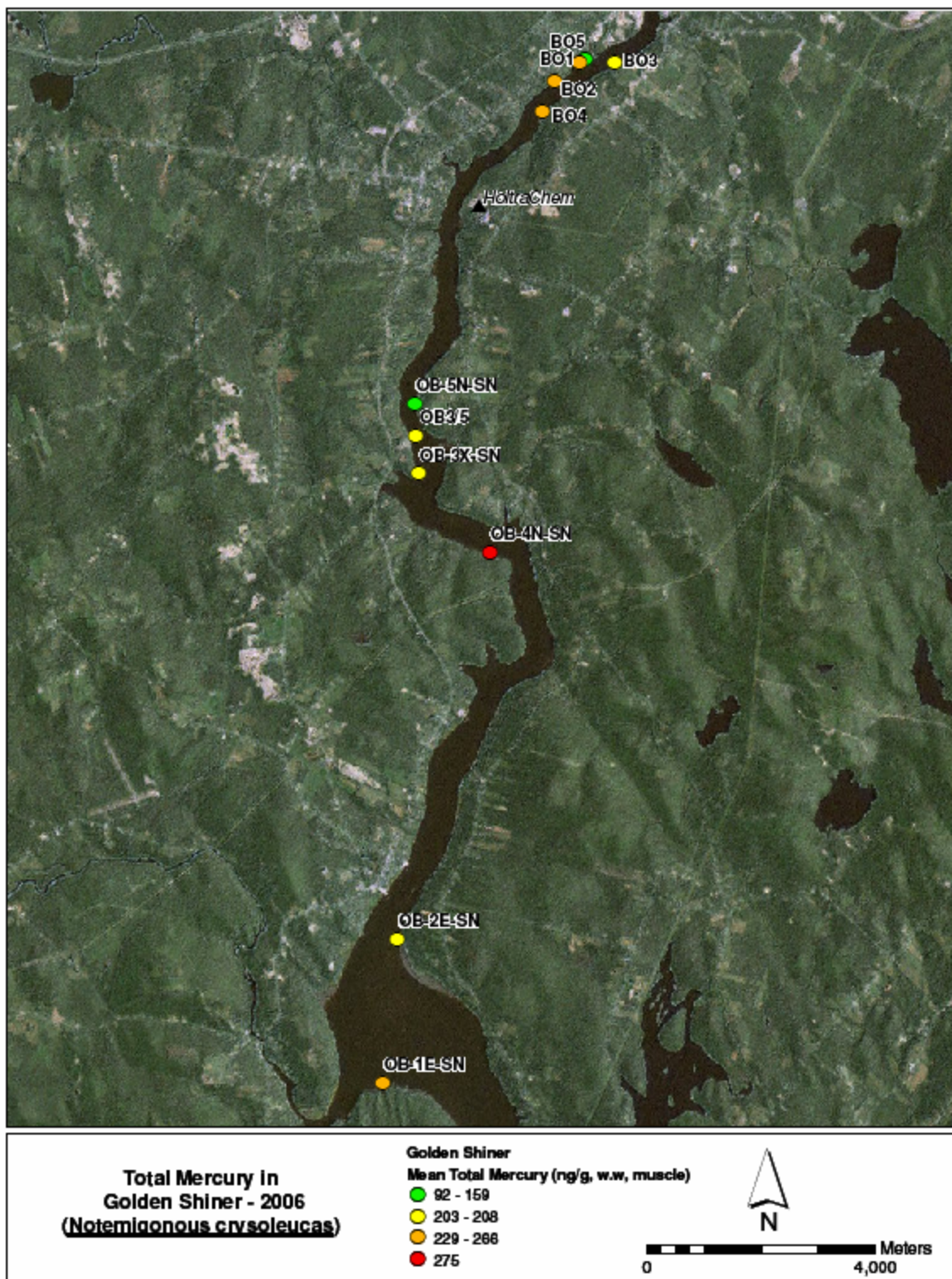


Figure 40. Map of total mercury concentrations in golden shiners from the Penobscot River, 2006.

## Mercury in Birds

### Double-crested cormorants (*Phalacrocorax auritus*)

Double-crested cormorants arrive at their summer breeding sites along the Maine coast in mid to late April. Mature cormorants show strong site fidelity, returning to the same

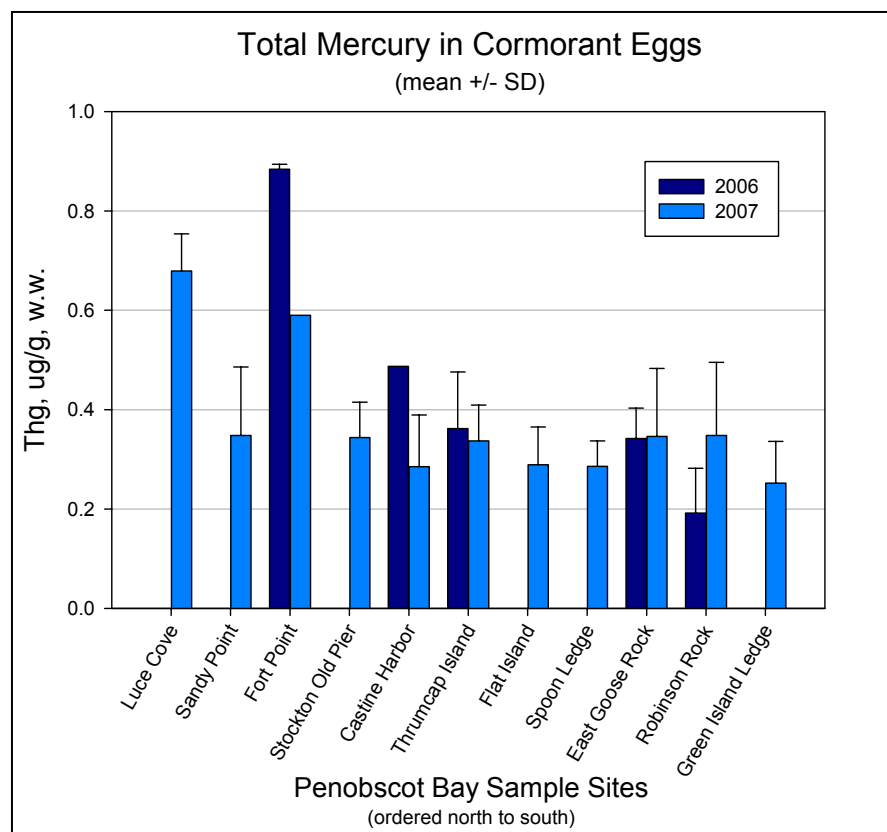


Figure 41. Mean total Hg concentrations in the eggs of double-crested cormorants in the lower Penobscot River

successful colonies year after year. Foraging range on the breeding grounds is typically within 10 km of the colony, but may extend over 60 km. Diet is primarily fish, and most prey species are slow-moving or schooling species with individuals less than 15 cm long. Prey include rainbow smelt, cunner, rock gunnel, eel, and sand shrimp. Cormorants usually raise one brood of five to seven, or more, eggs per season, but pairs may lay a second brood if the first is lost to predation. In late September or early October, cormorants migrate back to the southern United States or Gulf of Mexico (Hatch and Weseloh 1999).

Hg concentrations in the eggs of double-crested cormorants sampled in 2006 were presented in the Phase I report, and are shown again for comparison with 2007 data in Figures 41 and 42. Raw data can be found in Appendix 21. In 2006, there was a strong gradient from the most northern site which had the highest concentration to the most southerly site which had the lowest concentration. The highest concentrations approached those of concern for toxic effects. In 2007, a larger number of sites were sampled and a somewhat different pattern emerged (Figures 41 and 42). Hg in cormorants was, as in 2006, generally higher in the upper Penobscot estuary than in the



lower estuary; two of the three most northern sites in the Penobscot estuary were much higher than at other locations. However, sites south of Ft. Point did not show a trend towards lower levels with distance south, but rather were quite similar to each other. This geographic pattern is very similar to the pattern of contamination of mercury in wetlands in the upper and lower estuary. The highest average levels seen in 2007 were less than those seen in 2006.

This change in the geographic pattern of total Hg in the 2007 cormorant eggs may reflect the females' shorter residence time in Penobscot Bay prior to egg sampling that year. In 2007, eggs were sampled 24 to 70 days earlier than at the same sites in 2006. The later sampled 2006 eggs had greater total Hg levels in sites at the north end of Penobscot Bay and lesser total Hg levels from sites in the outer Bay, than found in eggs from the same sites in 2007.

The 2006 cormorant data may better reflect local mercury exposure, as the birds had been residents of Penobscot Bay for longer periods prior to sampling. Data for 2006 were presented in Appendix 14 of the Phase I report and data for 2007 are given in Appendix 21 of this report.

Total Hg in cormorant eggs from the upper Penobscot estuary exceeded levels reported for a colony in eastern England of 0.45  $\mu\text{g/g}$  w.w. (estimated from 2.63  $\mu\text{g/g}$  d.w., assuming 83% moisture, the mean % moisture in 2007 Penobscot Bay cormorant eggs,  $n = 104$ ) (Mason et al. 1997). This mean was for non-viable cormorant eggs and may be elevated above the mean for the general population because of that. Other comparisons, given in the Phase I report, to sites in Maine, Canada, Washington State, and San Francisco Bay also established that the levels seen in cormorant eggs in the upper part of the Penobscot estuary are quite high compared to other areas.

None of the 2007 Penobscot Bay cormorant eggs exceeded the reported lowest observed effect level for total Hg in bird eggs of 0.80  $\mu\text{g/g}$  w.w. (Heinz and Hoffman 2003). In 2006, 5% of the cormorant eggs exceeded the total Hg LOEL for bird eggs.

Summary - Cormorant eggs were re-sampled in 2007 to compare to levels seen in 2006 and to extend the geographic area sampled the previous year. In 2007, concentrations were similar to the pattern seen in 2006. Hg in eggs at two of the three most northern sites in the Penobscot estuary were significantly greater than at other locations, while sites further south in the estuary were similar to each other and did not show a decline with distance. The highest average levels seen in 2007 (approximately 0.6 to 0.7  $\mu\text{g/g}$  w.w. in eggs) were less than those seen in 2006, but still approached levels of concern for toxic effects.

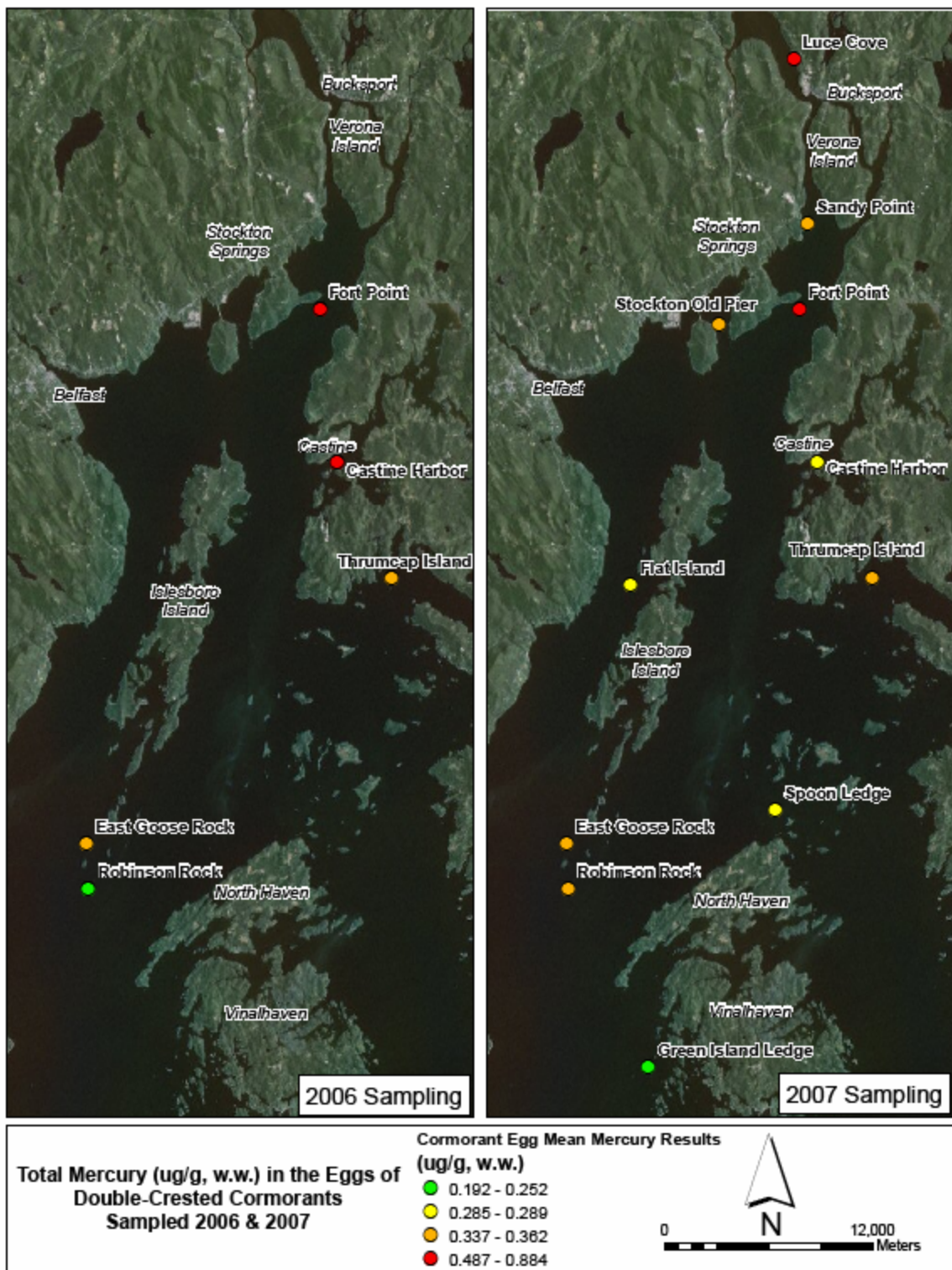


Figure 42. Average total Hg concentrations ( $\mu\text{g/g}$  w.w.) in the eggs of double-crested cormorants sampled in the Penobscot River and estuary in 2006 and 2007.

### **Black Guillemot (*Cepphus grille*)**

Black guillemots are year-round residents in Penobscot Bay, foraging in shallow, inshore waters near their breeding colony throughout the year. Guillemots are benthic foragers. Adult diet is primarily fish during the breeding season, including blennies, sea scorpions, herring, sandlance, cod and sculpins, supplemented with some invertebrates, especially amphipods and mysid shrimp. After their first year, they undergo two complete molts per year. The summer molt extends from mid July through October. Black guillemots have strong inter-annual site fidelity. Approximately 35% of juveniles return to their natal colony, and over 85% of breeding adults nest at the same colony each summer (Butler and Buckley 2002).

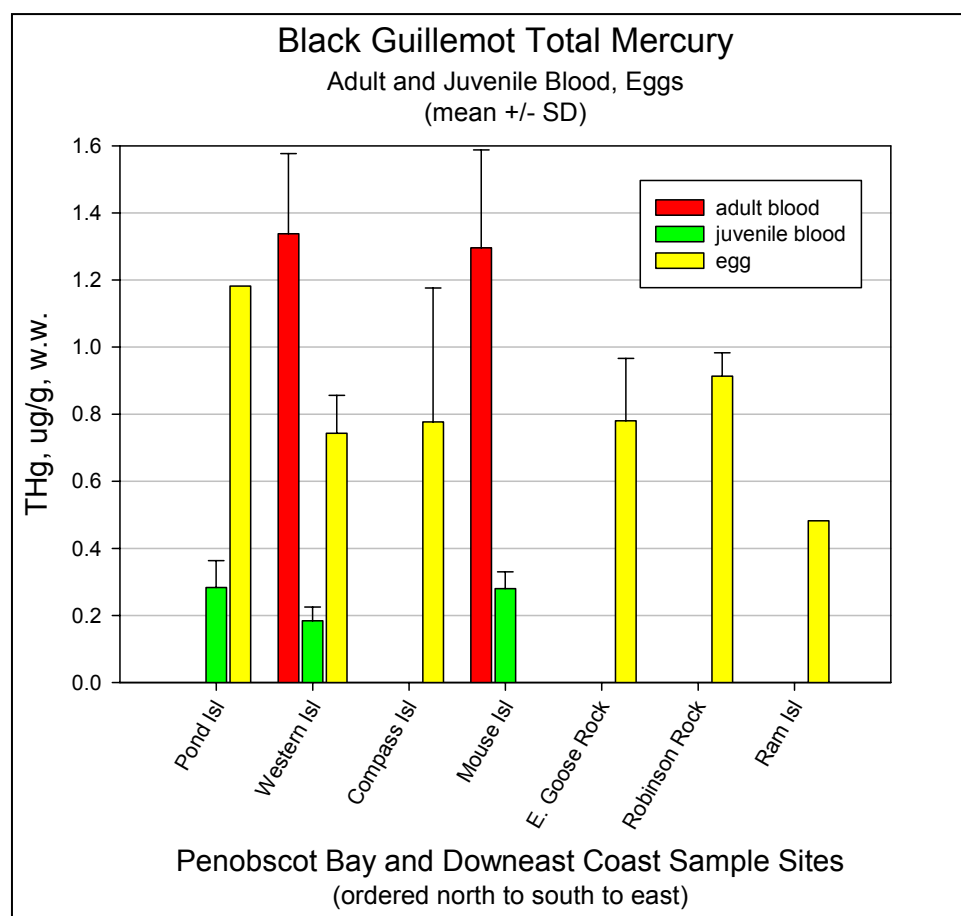


Figure 43. Mean total Hg in adult blood, juvenile blood and eggs of black guillemots sampled in the Penobscot estuary, 2007.

All sampling sites were in the outer Penobscot estuary, from near Islesboro Island and further to the south. Thus, all of the sites sampled for guillemots were outside of the area of the most severe contamination of HoltraChem Hg. One site (Ram Island) was well to the east of the estuary (Figure 44). Sample sizes were small, so these data should be treated with caution. Levels in guillemots were much higher than

in cormorants found in the outer Penobscot estuary (Figures 43 and 44), possibly reflecting the guillemots year-round residence in Penobscot Bay. Total Hg in blood was significantly greater in adults than in juveniles (two-sample t-test,  $p < 0.0005$ , separate

variance). Total Hg in adult blood did not vary significantly between sites (two-sample t-test,  $P = 0.78$ , pooled variance). Total Hg in juvenile blood also did not vary significantly among the three sites sampled (Figure 41; ANOVA,  $p = 0.07$ ). Neither sample date, bird weight nor tail length were significant covariates.

There was a suggestion of a geographic pattern to the total Hg concentrations in guillemot eggs, in that the highest site was one further north in the Penobscot estuary and the lowest site was the one site furthest away from the Penobscot estuary (Figure 44). The mean concentration at Pond Island (one of the most northern sites in Penobscot Bay) was  $1.18 \mu\text{g/g w.w.}$  as compared to the average at Ram Island (located east of Penobscot Bay) of  $0.48 \mu\text{g/g w.w.}$  (Figures 43 and 44, Appendix 22). The other four sites in outer Penobscot Bay had intermediate total Hg concentrations in eggs. Statistical tests of differences among sites were not possible due to small sample sizes.

Total Hg concentrations in the blood of guillemots were below levels found to have sub-lethal toxicity in birds (Evers et al. 2008). However, 60% of the eggs sampled, all from Penobscot Bay, exceeded levels associated with sub-lethal toxicity for total Hg (Heinz and Hoffman 2003).

Summary - Hg in black guillemots was noticeably higher than in cormorants, even though sampling in 2007 was outside the area of most severe contamination from HoltraChem. Concentrations of Hg in many guillemot eggs were higher than levels of concern for sublethal toxic effects.

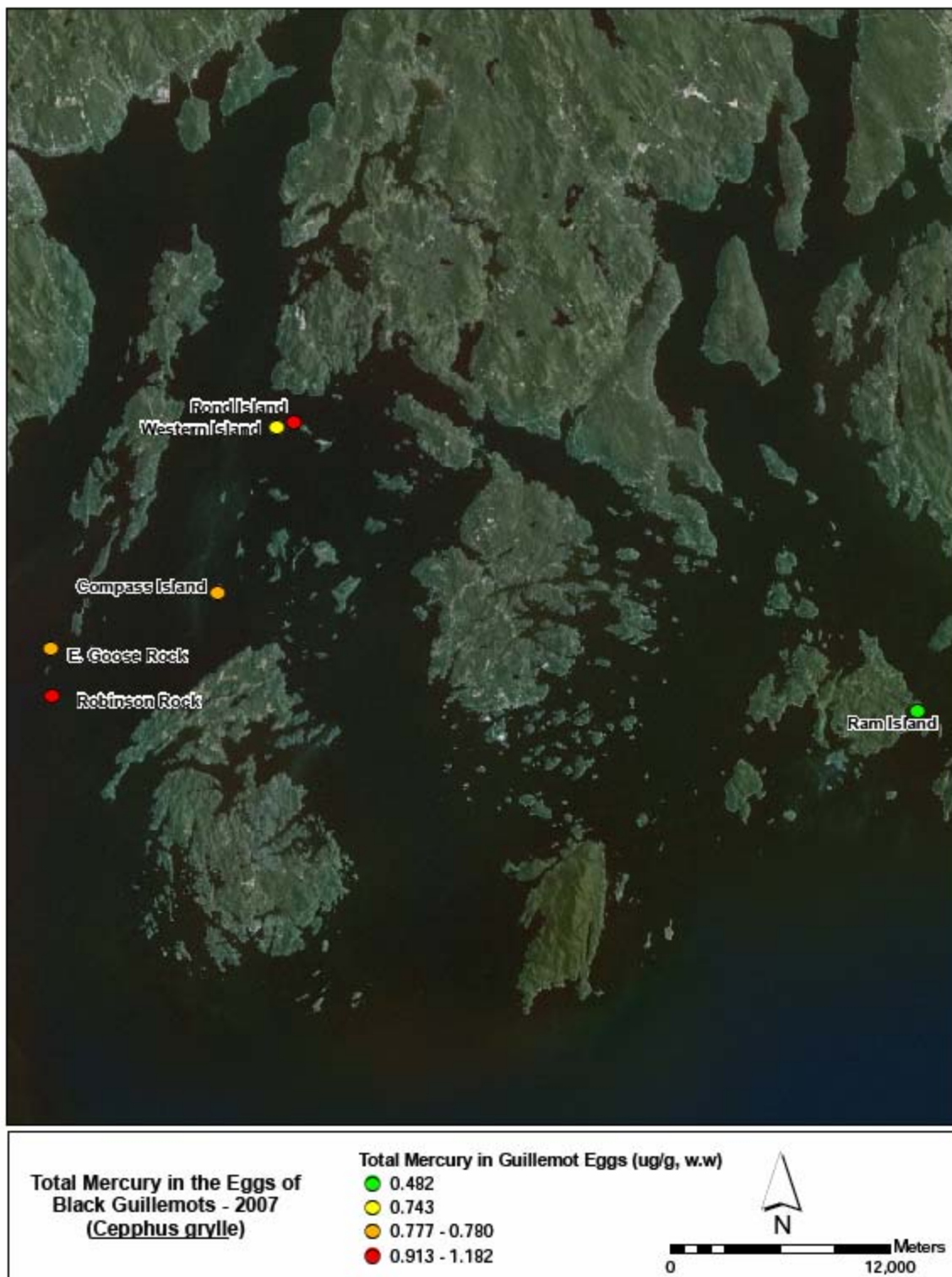


Figure 44. Average total Hg concentrations ( $\mu\text{g/g w.w.}$ ) in the eggs of black guillemots sampled in the Penobscot estuary in 2007.



### **Nelson's sharp-tailed sparrow (*Ammodramus caudacutus*)**

Sharp-tailed sparrows arrive in coastal Maine from mid May to early June from wintering sites along the southeast Atlantic and Gulf of Mexico coasts. This species is very localized - females occupy a small breeding home range, as small as 0.4 ha, while the non-territorial males have larger, overlapping home ranges of up to 4 ha. In the Penobscot watershed, they were found only at Mendall Marsh. Over 90% of the breeding season diet is insects and spiders, foraged at ground level among dense grasses, the edge of marsh pools, and patches of tidal wrack. Females generally forage close to the nest, feeding the chicks exclusively on invertebrates. This species undergoes two complete molts; the pre-alternate molt occurs in spring on the wintering grounds, although some report the prealternate molt is completed on the breeding

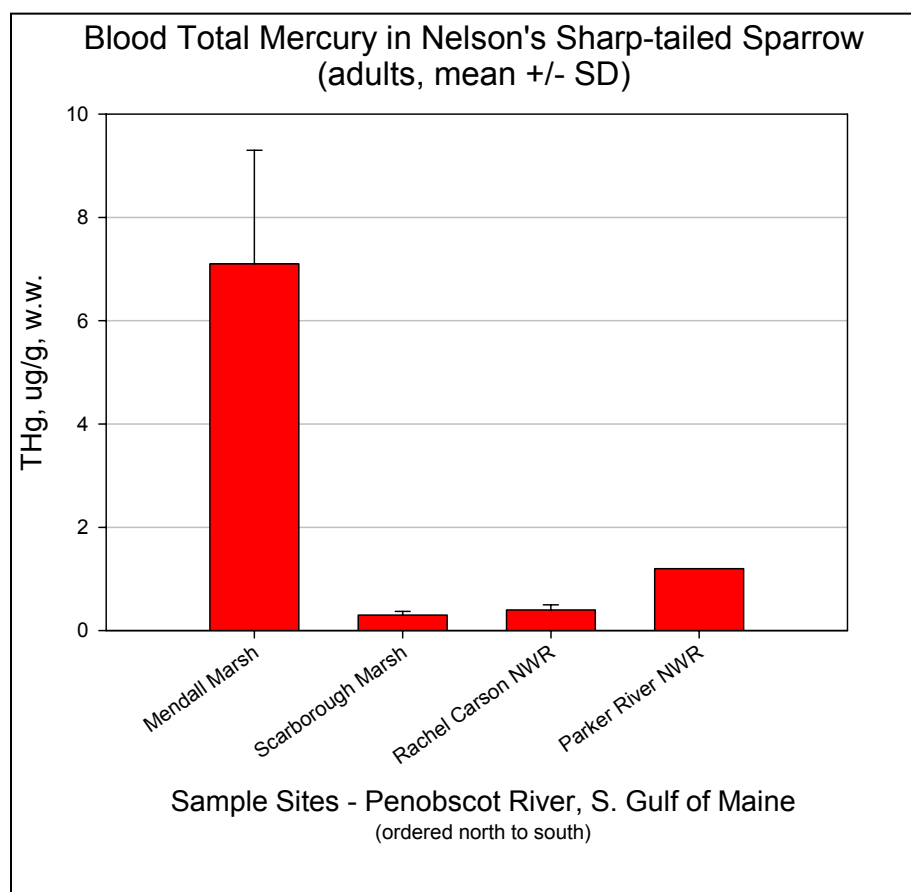


Figure 45. Mean total Hg in the blood of adult sharp-tailed sparrows, 2007.

Marsh. Hg concentrations in blood were seven or more times greater in Mendall Marsh sparrows relative to the three control sites (ANCOVA, adjusted for age,  $p < 0.0005$ ,  $r^2 = 0.52$ ; Figure 45). Within Mendall Marsh, blood Hg varied significantly by age. Chicks

grounds; tail feathers collected in July and early August are likely formed on the wintering grounds. The pre-basic molt occurs in late August to September on the breeding grounds. Migration south begins during September (Greenlaw and Rising 1994).

Adult Nelson's sharp-tailed sparrows were sampled at Mendall Marsh (n=82) and at three reference sites along the southern Gulf of Maine (n=10), and three chicks (HY, hatch year) were sampled at Mendall

(HY) had significantly lower total Hg concentrations (ANOVA,  $p = 0.003$ ,  $r^2 = 0.20$ ) than adult birds.

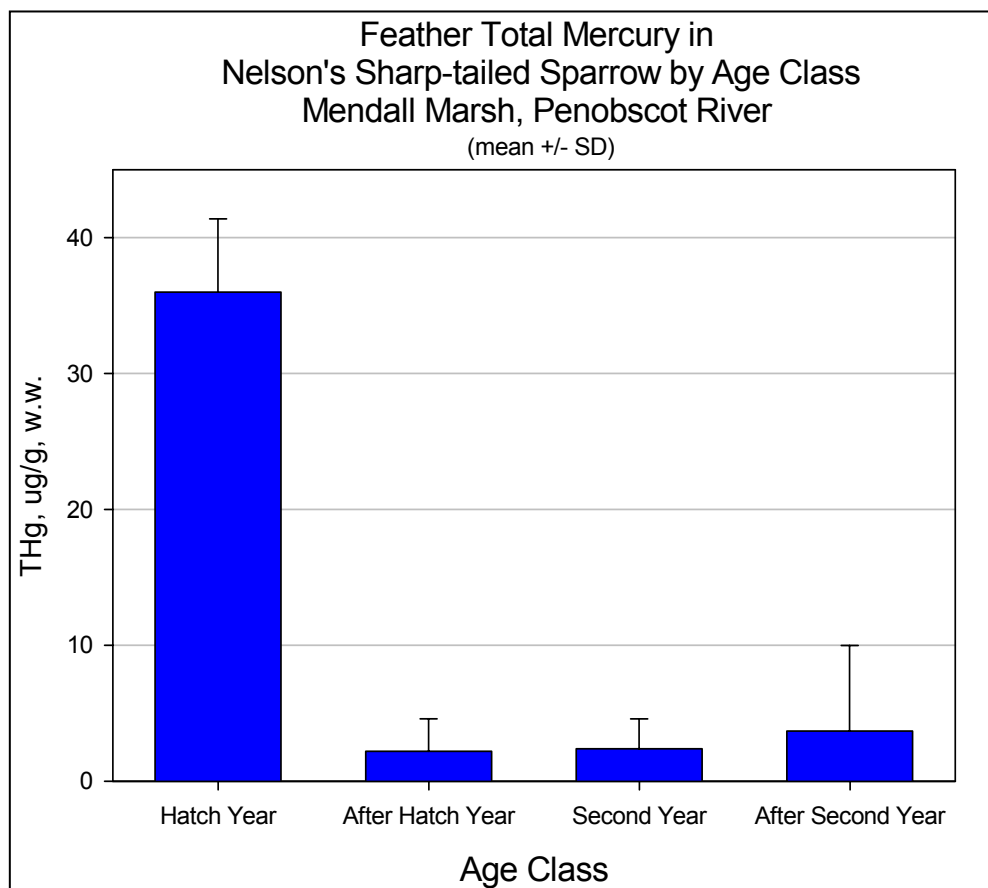


Figure 46. Mean total Hg in the feather of sharp-tailed sparrows by age class in Mendall Marsh, 2007

Feathers collected from the chicks had been formed while still in the nest, and reflect mercury exposure from the diet at the summer breeding grounds. Adult tail feathers sampled in midsummer had been formed during the spring molt, which in this species likely occurs on the wintering grounds, and so reflect exposure on the wintering grounds.

Total Hg concentrations in Nelson's sharp-tailed sparrows sampled at Mendall Marsh were consistent between 2006 and 2007. Mean adult blood THg levels in 2006, at Mendall and Prospect Marshes (the southern tip of Mendall Marsh) ranged from 4.2 to 5.7 ug/g w.w. ( $n = 21$ ), slightly lower, but within the range of blood mercury levels found in Mendall Marsh adults in 2007. Similarly, total Hg concentrations in feathers in 2006 from Mendall and Prospect Marshes averaged  $1.80 \pm 2.52$  (0.54-8.86) ug/g w.w. ( $n = 10$ ), within the range of total Hg concentrations in feathers from adults sampled in 2007.

Tail feathers were collected for total Hg analyses at Mendall Marsh only. Concentrations of total Hg in feathers were significantly greater in birds in their hatch year than in the three adult age classes (Kruskal-Wallis HSD test,  $p = 0.014$ ) (Figure 46). Feathers collected from

Total Hg concentrations in the blood of Nelson's sharp-tailed sparrows were an order of magnitude greater at Mendall Marsh than at any other site sampled within Maine, and exceeded levels associated with avian toxicity. Shriver et al. (2006) reported much lower total Hg in blood in this species, sampled at five marshes along the Maine coast, from Thomaston south to York ( $0.406 \pm 0.162$  ug/g w.w.) Total Hg concentrations in blood associated with sublethal toxicity in birds (1.1 to 3.0 ug/g, w.w.: Spaulding et al. 2000; Kenow et al. 2007; Evers et al. 2008) were greatly exceeded by the blood mercury levels found in Mendall Marsh for this species.

Summary - Hg in sharp-tailed sparrows was the highest of the songbirds. Concentrations in 2007 were similar to those found in 2006. Concentrations at Mendall Marsh were much higher than at reference sites in Maine and exceeded levels associated with toxicity.



### **Song sparrow (*Melospiza melodia*)**

A portion of coastal Maine's breeding population of song sparrows may overwinter in the area, but what percentage, if any, overwinters along the shores of Penobscot Bay is unknown, and it is unlikely that any overwinter north of the Bay along the Penobscot River. If migratory, song sparrows return to the Penobscot River and Bay during April, form mating pairs and set up territories of variable sizes, ranging from 400 – 500 m<sup>2</sup> in salt marshes to several thousand square meters in more inland sites. Diet varies with

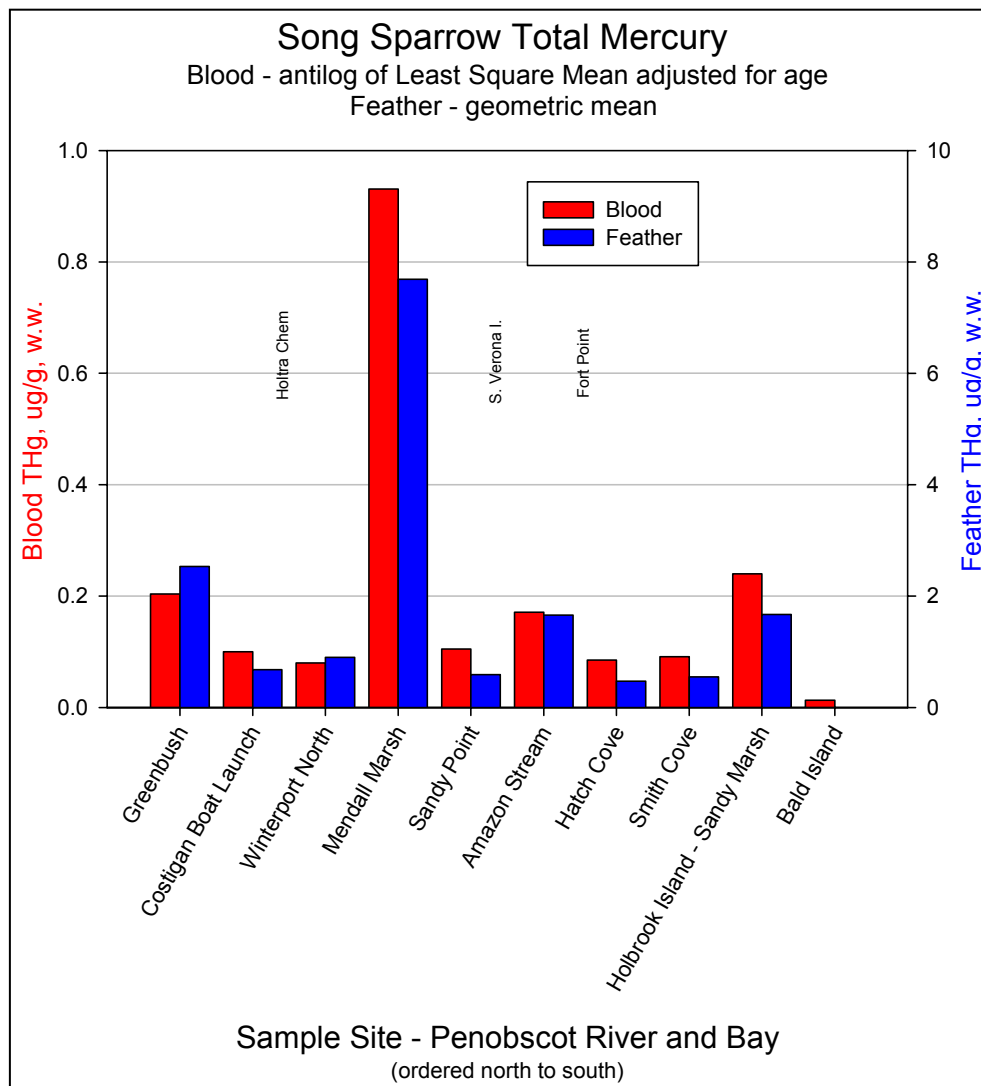


Figure 47. Mean total Hg concentrations in the blood and feathers of song sparrows collected from various sites along the Penobscot River, 2007. Sites are ordered from north to south.

habitat; in salt marshes the diet is primarily invertebrates, especially snails, small nereid worms, and insects. In other habitats, invertebrate prey increases in the summer, but remains below 40% of the diet. Song sparrows undergo one molt per year, completed on the summer breeding grounds. Molt timing varies with breeding status, age and habitat type. In migratory populations, available data indicates limited inter-annual site

fidelity, with a minority of song sparrows returning to the same breeding site in successive years (Arcese et al. 2002).

Song sparrows were sampled at Mendall Marsh as well as nine other sites in the Penobscot basin. Two of these nine sites (Costigan Boat Launch and Greenbush) were on the Penobscot, but upstream of the Veazie dam, and therefore outside of the aquatic influence of HoltraChem. Raw data for song sparrows is shown in Appendix 24. Mercury levels varied significantly by sample site, with by far the greatest levels found in birds sampled at Mendall Marsh (Figures 47 and 48). Total Hg in blood at Mendall Marsh was significantly greater than found at all other sites, and Bald Island song sparrows had significantly lower total Hg in blood than all sites except Costigan boat landing (ANCOVA, adjusted for age class,  $p < 0.005$ ,  $R^2 = 0.686$ ; Tukey pairwise HSD,  $p < 0.05$ ). Neither sample date, sex, bill length nor bird weight were significant covariates.

A correlation between blood and feather mercury levels may indicate the similarity of exposure levels between when the blood was sampled and when the feathers were formed. In hatch year song sparrow chicks, total Hg concentrations in blood and feathers were highly correlated (linear regression,  $p < 0.0005$ ,  $r^2 = 0.747$ ) while the same correlation was only moderate in adult song sparrows (linear regression,  $p < 0.0005$ ,  $r^2 = 0.506$ ). Hg concentrations in the blood of chicks indicate local exposure as they are fed only a local diet, and Hg in the feathers formed while in the nest directly correlate with blood levels. Adult feathers may have been formed the previous year – possibly at a different breeding site, as feathers were collected from song sparrows just prior to or during the molt. Hg concentrations in adult feathers may result from different Hg exposure levels at the time of feather formation than experienced by the bird at the time of blood collection. Despite this uncertainty, Hg concentrations in feathers varied significantly with site, and again the greatest levels were found at Mendall Marsh (ANCOVA,  $p < 0.0005$ ,  $r^2 = 0.547$ ). Neither age, sex, sample date, bill length, nor bird weight were significant covariates. Despite the highly elevated total Hg concentrations in song sparrows at Mendall Marsh, no geographic trend in song sparrow Hg levels was found immediately downstream from the HoltraChem site (Figure 48). Song sparrows sampled at Winterport North, 3.5 miles south of HoltraChem, did not have elevated concentrations of total Hg in blood.

Song sparrows were sampled previously in 2006, at Mendall Marsh in July and August, and at a small marsh south of Winterport in August. The 2006 and 2007 July blood samples from Mendall Marsh were not significantly different with mean values of  $1.93 \pm 0.96$  and  $1.7 \pm 1.4$   $\mu\text{g/g w.w.}$ , respectively. Total Hg concentrations in blood in August 2006 at Mendall Marsh were approximately 70% lower,  $0.55 \pm 0.57$   $\mu\text{g/g w.w.}$ , than reported for July collections in 2006 or 2007. The 2006 Winterport samples were collected five miles downstream of the 2007 Winterport North song sparrow sample site,

and had four times greater concentrations of total Hg in blood,  $0.35 \pm 0.29 \mu\text{g/g w.w.}$  in 2006 compared to  $0.09 \pm 0.08 \mu\text{g/g w.w.}$  in 2007.

Total Hg concentrations in the blood of song sparrows from Mendall Marsh were over five times greater than found in song sparrows sampled in Eastern Massachusetts between 2001 and 2003. Evers et al. (2005) reported total Hg in the blood of song sparrows of  $0.35 \pm 0.30 \mu\text{g/g}$  in adults and  $0.21 \pm 0.14 \mu\text{g/g w.w.}$  in juveniles. Rimmer et al. (2005) found notably lower levels in white-throated sparrows sampled in montaine forests at numerous sites throughout New England ( $0.062 \pm 0.026 \mu\text{g/g w.w.}$ ).

Mercury toxicity in passerines has not been specifically studied relative to total Hg concentrations in adult blood. The lowest observed effect levels for total Hg in the blood of birds range from  $1.1 \mu\text{g/g w.w.}$  in snowy egrets (Spaulding et al. 2000) to  $3.0 \mu\text{g/g w.w.}$  in common loons (Evers et al. 2008). Total Hg concentrations in the blood of song sparrows at Mendall Marsh exceeded the lower end of this range.

Summary - Hg in the blood of song sparrows was highest at Mendall Marsh compared to other sites downstream of HoltraChem and to reference sites. Hg in song sparrows from Mendall Marsh exceeded levels of concern for toxic effects.

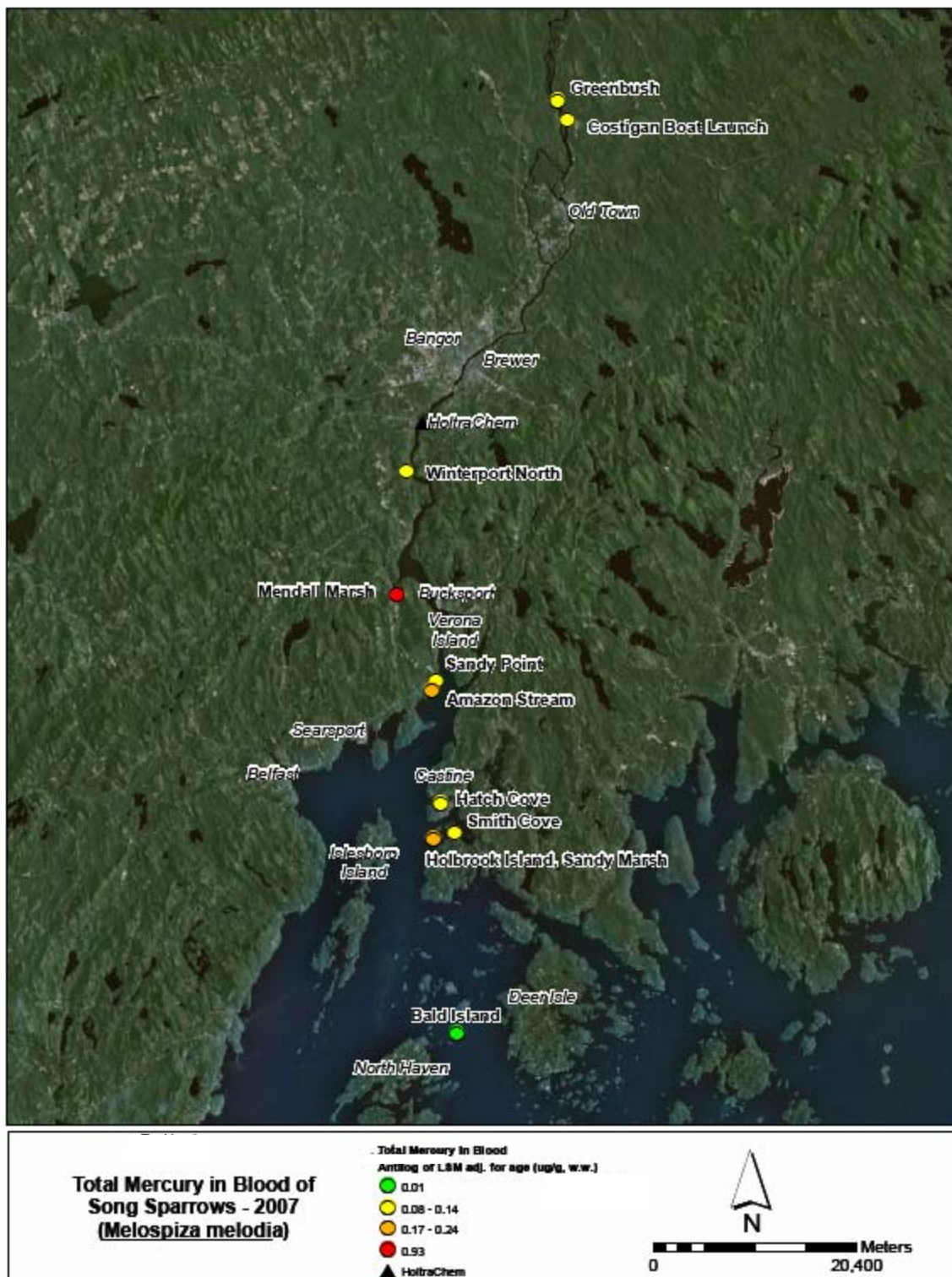


Figure 48. Mean total Hg ( $\mu\text{g/g}$  w.w.) concentrations in the blood of song sparrows, 2007.

### Swamp sparrows (*Melospiza georgiana*)

As for sharp-tailed and song sparrows, Hg in the blood of swamp sparrows was very high at Mendall Marsh but lower at other sites (Figure 49). The mean for Mendall Marsh was 2.4  $\mu\text{g/g}$ , whereas at other sites potentially impacted by HoltraChem, means ranged from 0.37 to 0.51  $\mu\text{g/g}$ , as compared to means of 0.18 to 0.49  $\mu\text{g/g}$  at two reference sites. Data for swamp sparrows from Mendall Marsh and other sites are found in Appendix 25.

Migratory swamp sparrows arrive at their summer breeding sites in New England from mid April (males) to early May (females). They prefer localized breeding habitats close to water. Marsh territories range in size from 0.03 to 0.61 ha (300 – 6100 m<sup>2</sup>).

Approximately half of breeding adults return to the same site used the previous year. 30% of males use the same breeding territory one year to the next, and another 20%

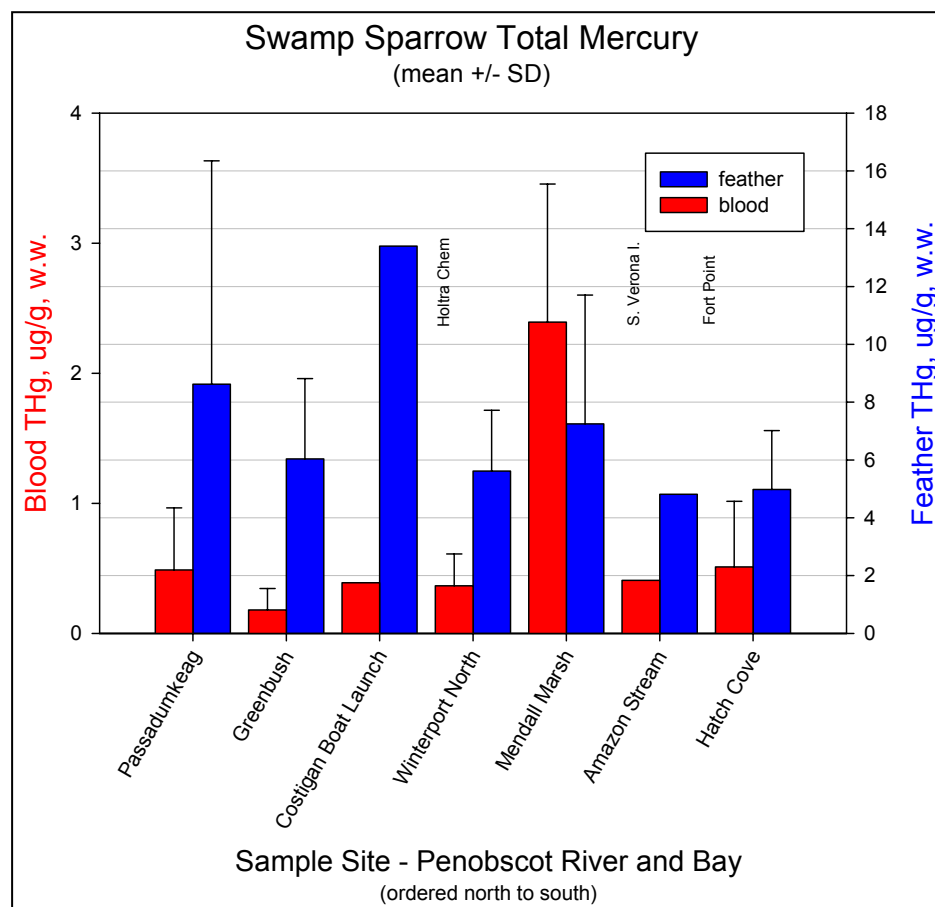


Figure 49. Mean total Hg concentrations in the blood and feathers of swamp sparrows.

return to the general area. Females are not known to return to the exact territory the following year, but 50% return to the same general area. Insects, primarily beetles, ants, grasshoppers, caterpillars and crickets, make up a large part of the swamp sparrow breeding diet. In the winter their diet switches to over 85% grains. They molt once annually, on the breeding grounds in August and early September, just prior to the fall migration (Mowbray

1997).

Total Hg concentrations in blood were five to twelve times higher in Mendall Marsh swamp sparrows than at all other sites. These differences were significant for all sites except those with a sample size of one (Figure 47; ANOVA,  $p < 0.0005$ , Tukey HSD,  $p < 0.05$ ). No geographic trend relative to the HoltraChem site was evident, other than the notably elevated blood levels at Mendall Marsh, downstream of the site (Figure 50). There were no significant covariates, including bird age. However, a two-sample t-test found a significantly lower total Hg level in blood in chicks (HY) compared to adult (AHY) swamp sparrows ( $p < 0.0005$ ). Since adults dominated the Mendall Marsh samples, while most birds sampled from other sites were chicks, we examined further whether this age distribution biased our findings. In the chick subset, total Hg in blood was greater at Mendall Marsh (geometric mean (GM)  $2.42 \mu\text{g/g w.w.}$ ) than at Greenbush (GM =  $0.136$ ; ANOVA,  $p = 0.03$ ,  $r^2 = 0.67$ , Tukey HSD  $< 0.05$ ), but not significantly different from the other sites sampled (GM range  $0.306$ - $0.408 \mu\text{g/g w.w.}$ ). For swamp sparrows of all ages, total Hg in feathers did not vary significantly among sites (ANOVA,  $p = 0.698$ ), and again there were no significant co-variates. In the subset of chicks, total Hg in feathers did vary significantly among sites (ANOVA,  $p = 0.044$ ,  $r^2 = 0.669$ ), although, perhaps due in part to very small samples sizes, no significant pairwise differences were present (Tukey HSD,  $p > 0.05$ ).

Several factors may explain why total Hg in blood was significantly greater at Mendall Marsh while total Hg in feathers was not significantly different. Since adult swamp sparrow feathers were collected by August 7, before the annual molt, the collected feathers were one year old, and reflected mercury exposure the previous breeding season, which had only a 50% chance of being the same site occupied by the bird during the year sampled. Total Hg in the blood of adults, which indicates exposure levels during, roughly, the preceding seven weeks, did not correlate with adult feather total Hg (linear regression,  $p = 0.466$ ). However, chick feathers are formed while the birds remain in the parents' territory eating a local diet, so mercury exposure should be similar for both blood and feathers. If chicks are sampled at approximately the same age, as was the case in this data set since there was no significant difference in chick size among sites (ANOVA,  $p > 0.05$ ), a significant correlation between total Hg in chick blood and total Hg in chick feathers is expected, but was not found (linear regression,  $p = 0.061$ ).

Swamp sparrows sampled in 2006 at Mendall Marsh ( $n = 4$ ) and at a marsh just south of Winterport ( $n = 4$ ) had concentrations of total Hg in blood in the range of 2007 levels, with some variation. The 2006 Mendall Marsh swamp sparrows, all chicks, had lower mean total Hg in blood ( $1.62 \pm 0.50 \mu\text{g/g w.w.}$ ) than found in the 2007 swamp sparrows ( $n = 24$ , mean total Hg in blood  $2.4 \mu\text{g/g w.w.}$ ), which were 90% adult birds. Age, and

the larger sample size in 2007, may explain the difference between years. The 2006 Winterport birds, an equal mix of adults and chicks, were sampled 5 miles downstream of the 2007 Winterport North samples (all chicks) and the 2006 samples had lower total Hg in blood ( $0.73 \pm 0.47 \mu\text{g/g w.w.}$ ) than was found in 2007.

No species specific study has reported mercury levels in swamp sparrows, but the total Hg found in blood in 2007 at Mendall Marsh was between three and ten times greater than total Hg in blood reported in two related species, Nelson's sharp-tailed sparrow and Saltmarsh sharp-tailed sparrow, sampled at six sites along the central and southern Maine coasts (mean total Hg in blood  $0.256 - 0.867 \mu\text{g/g w.w.}$ ; Shriver et al. 2006). Also, the Mendall Marsh swamp sparrows had total Hg concentrations in blood in the range of levels associated with sub-lethal toxicity in other bird species (Spaulding et al. 2000, Evers et al. 2008).

Summary - Hg in the blood of swamp sparrows was highest at Mendall Marsh compared to other sites downstream of HoltraChem and to reference sites. Levels in 2007 were similar to those found in 2006. Hg in the blood of swamp sparrows from Mendall Marsh exceeded levels of concern for toxic effects.



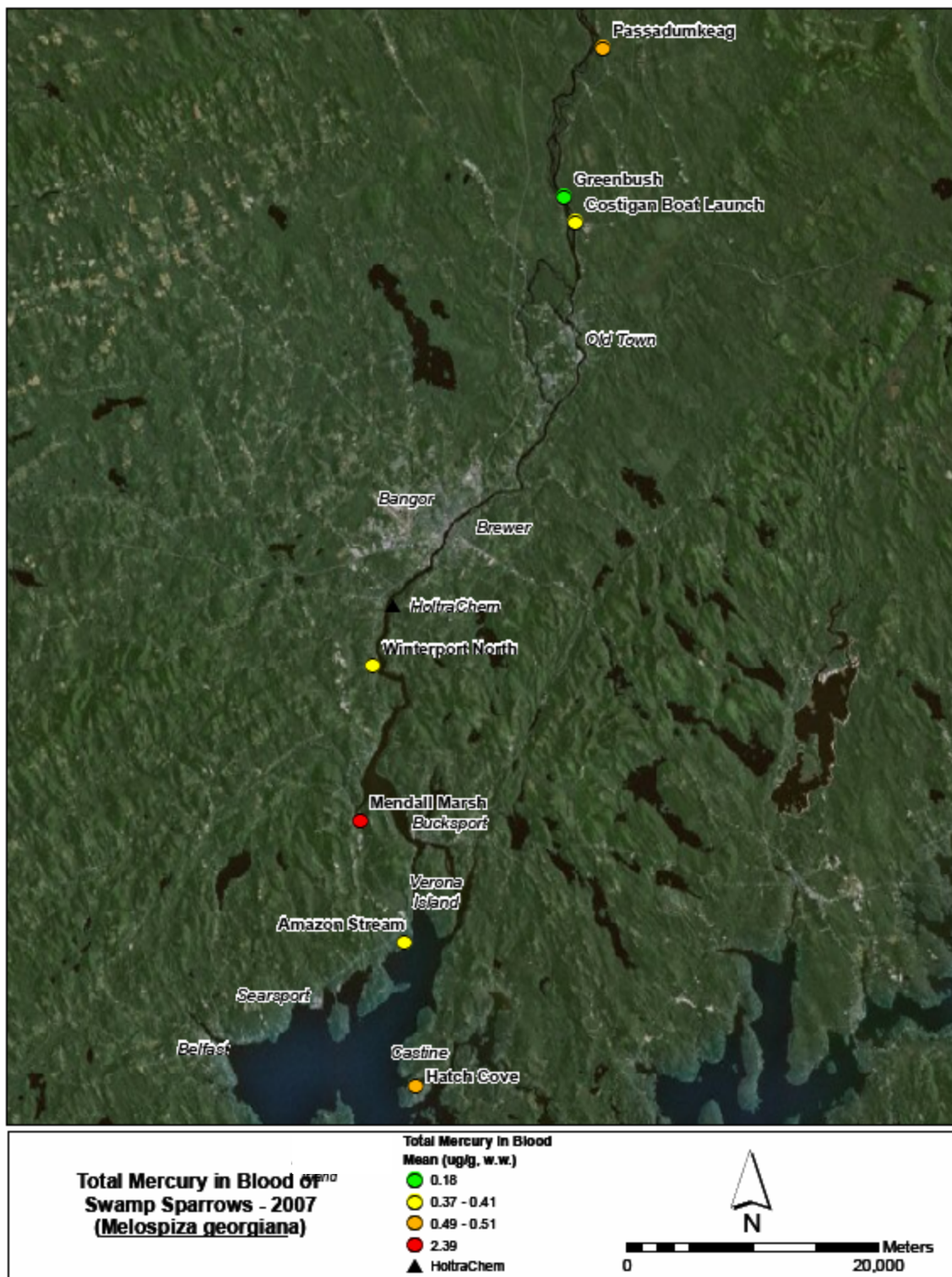


Figure 50. Mean total Hg ( $\mu\text{g/g w.w.}$ ) concentrations in the blood of swamp sparrows sampled in the summer of 2007.



### **Red-winged Blackbirds (*Agelaius phoeniceus*)**

Red-winged blackbirds likely return to breed in the Penobscot River valley by late April or early May, although some may overwinter along the coast. Males return first, establishing breeding territories used by up to 15 females, which in marsh habitat have a mean size of 1,625 m<sup>2</sup>. Males have strong inter-annual site fidelity; roughly 90% of breeding adult males return to the same or an overlapping territory each year. Females have a lower return rate of about 60%. In marshes, breeding blackbirds feed on insects almost exclusively. Nestlings are fed Odonata (dragonflies and damselflies), Lepidoptera (butterflies and moths), and Diptera (flies), which comprise 80 -100% of

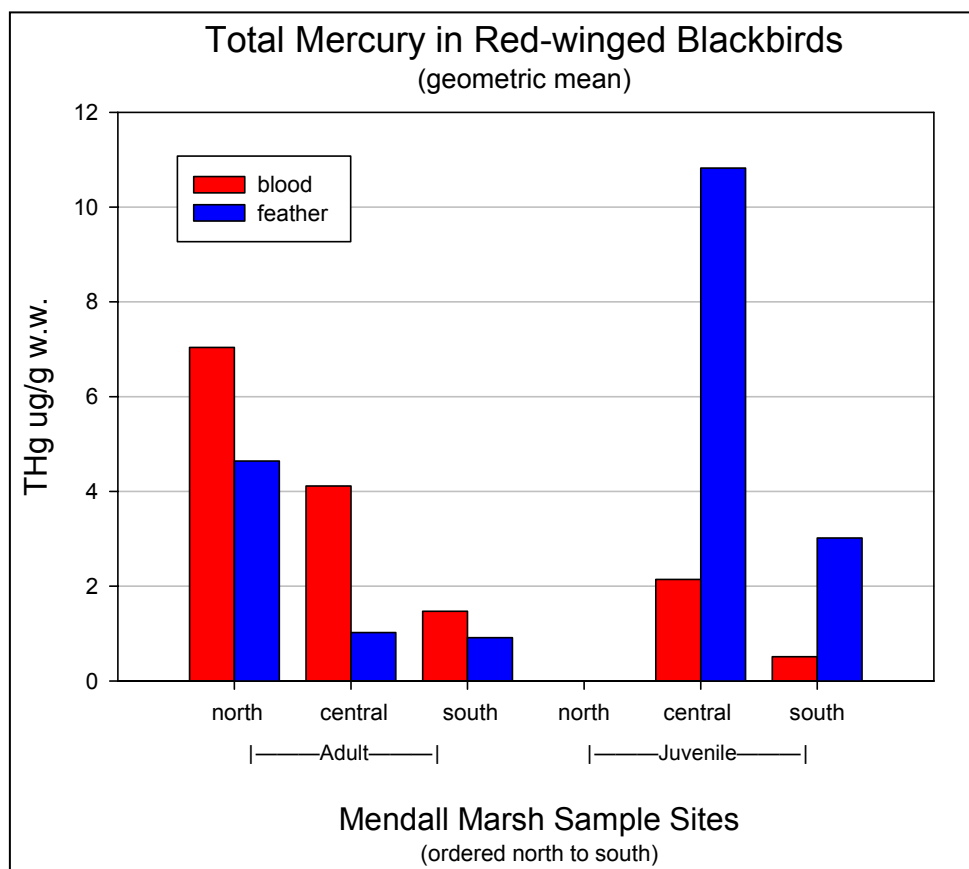


Figure 51. Mean total Hg concentrations in the blood and feathers of red-winged blackbirds, Mendall Marsh, 2007.

different in adult and juvenile (chick) blackbirds, and so were analyzed separately for differences in Hg among sample sites. Mercury concentrations in the blood of adult blackbirds were significantly lower in the south section of Mendall Marsh than in the central and north sections (ANOVA,  $p = 0.031$ , Tukey HSD,  $p < 0.05$ ) (Figure 51). Hg concentrations in adult feathers were highly variable, and not significantly different among sites (ANOVA,  $p > 0.05$ ). Numerous potential covariates were tested, but none were significant.

their diet. The species has one annual pre-basic molt from mid-July through September. Feathers collected in early to mid summer reflect mercury exposure on the previous year's breeding grounds (Yasukawa and Searcy 1995).

Red-winged blackbirds were sampled only in Mendall Marsh.

Mercury concentrations were significantly

In juveniles, Hg concentrations in blood were significantly greater in samples from the central section of Mendall Marsh, than in those sampled at the south end (ANOVA,  $p = 0.011$ ) (Figure 51; Appendix 26). Hg in juvenile feathers was also highly variable, and not significantly different between sample sites (ANOVA,  $p > 0.05$ ).

Many of the blackbirds sampled had blood or feather Hg concentrations exceeding levels associated with sub-lethal toxicity (Heinz 1979, Evers et al. 2008). Hg in blood exceeded  $3.0 \mu\text{g/g w.w.}$  in all adults sampled in the north part of Mendall Marsh, 70% of adults and 30% of the juveniles sampled in the central section of the marsh and 31% of the adults sampled in the south section. Mercury concentrations in feathers exceeded  $10 \mu\text{g/g f.w.}$  in 30% of the adults sampled in the north, 60% of the juveniles from the central region, and 8% of the adults in the south.

Mean Hg concentrations in red-winged blackbirds from Mendall Marsh were between five and 35 times greater than reported for the same species sampled in the Hackensack Meadowlands in New Jersey, an area of known industrial contamination. Tsipoura et al. (2008) reported Hg in blackbird blood of  $0.23 \mu\text{g/g w.w.}$  and feathers of  $0.83 \mu\text{g/g f.w.}$

Summary - Hg concentrations in red-winged blackbirds sampled at Mendall Marsh were high compared to a contaminated site in New Jersey and compared to levels of concern for toxicity.

### **Virginia Rail (*Rallus limicola*)**

Virginia rails breed during the warmer months in Maine's salt and freshwater wetlands, arriving by mid May from wintering grounds further south. Breeding pairs form territories of varying size and the distance between nests can range from 17 to 46 m, but foraging ranges may overlap. During the summer breeding season, rails feed primarily on animal prey (85-97% of diet), including aquatic invertebrates, beetles, snails, spiders and small fish. They undergo a complete molt from mid July to mid August, prior to their fall migration in late September, so tail feathers collected before the molt, barring mid-season loss and re-growth due to predation, were likely grown on the breeding grounds occupied the previous year (Conway 1995).

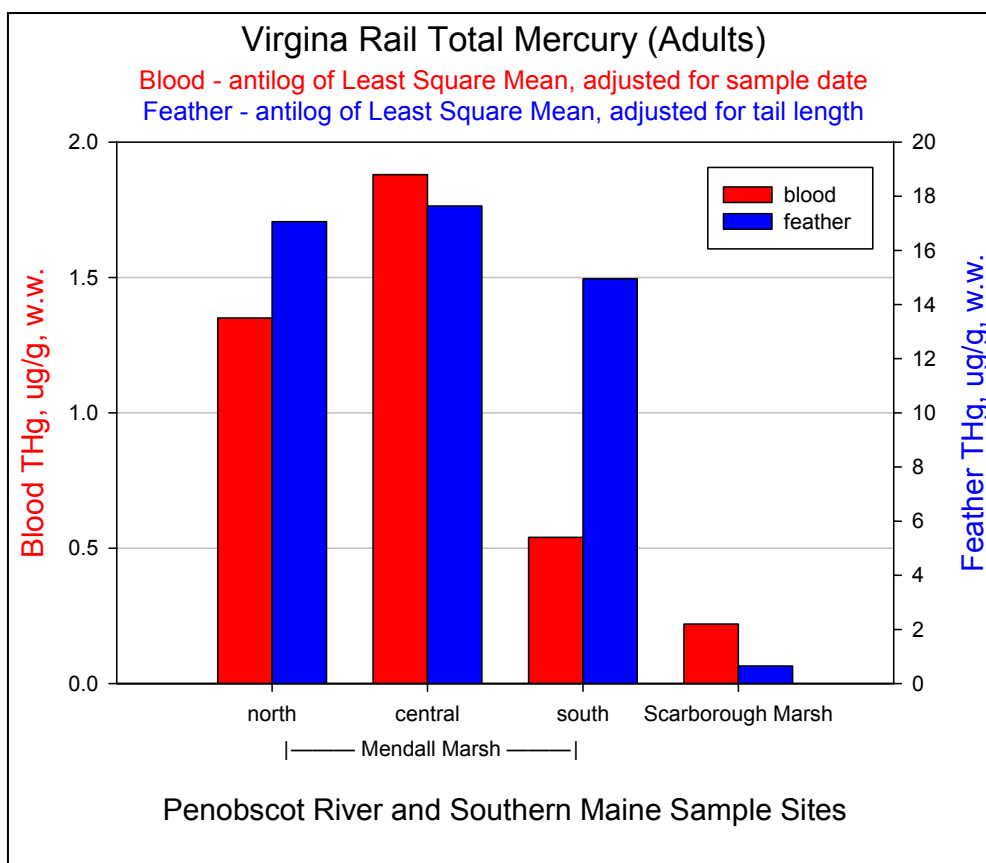


Figure 52. Mean total Hg concentrations in the blood and feathers of Virginia rail adults, 2007.

In adult Virginia rails, total Hg concentrations in blood were significantly greater in birds sampled in the central and northern sections of Mendall Marsh than at Scarborough Marsh, a reference site in southern Maine (ANCOVA,  $p < 0.05$ ,  $r^2 = 0.85$ , adjusted for sample date;

Tukey pairwise comparison,  $p < 0.05$ ) (Figure 52;

Appendix 27). While controlling for sample date, and so the amount of time birds had spent foraging on the breeding grounds prior to sampling, blood levels in adult rails sampled at the southern tip of Mendall Marsh were not significantly greater than those in Scarborough Marsh. Rail chicks were caught and sampled only at the central area of

Mendall Marsh, and found to have lesser blood total Hg levels (hatch year mean total Hg  $1.4 \pm 0.3 \mu\text{g/g w.w.}$ ), but not significantly so, than adults sampled at that site (after hatch year mean total Hg  $2.8 \pm 1.5 \mu\text{g/g w.w.}$ ; two-sample t-test,  $p = 0.06$ ).

Total Hg in adult feathers was significantly greater at all Mendall Marsh sites than in feathers collected at the Scarborough Marsh reference site (ANCOVA,  $p = 0.006$ ,  $r^2 = 0.74$ , adjusted for tail length, Tukey pairwise comparison  $p < 0.05$ ) (Figure 52). Chick feathers, again collected at central Mendall Marsh only, had notably greater feather total Hg concentrations (hatch year mean  $49.2 \pm 7.4 \mu\text{g/g w.w.}$ ) than in adults at the same site (after hatch year mean  $26.2 \pm 20.7 \mu\text{g/g w.w.}$ ), but given the high variance in total Hg in adult feathers, the difference was not significant (two-sample t-test,  $p = 0.14$ , separate variance).

Total Hg concentrations in feathers from adults sampled at Mendall Marsh averaged up to 2.5 times greater than levels associated with sub-lethal toxicity in birds ( $9.85 \mu\text{g/g}$ , f.w., Heinz 1979) and total Hg in chick feathers from Mendall Marsh was five times greater than the same toxicity threshold. Similarly, total Hg concentrations in blood at the central and northern Mendall marsh sites were two to three times greater than the blood toxicity threshold for birds of  $0.8 \mu\text{g/g w.w.}$  (Heinz and Hoffman 2003). Neither feather nor blood total Hg levels in Virginia rails sampled at Scarborough Marsh exceeded these bird toxicity thresholds.

Summary - Virginia rails from Mendall Marsh had much higher concentrations of Hg in blood than at a reference area in southern Maine. They were also high compared to levels of concern for toxic effects.

### Other Passerines and Shorebirds

Several other passerine birds and shorebirds were sampled in small numbers, precluding full statistical analyses of geographic trends in Hg levels, but the data reveals interesting, though preliminary, findings.

Hg concentrations in the blood of adult common yellowthroats (COYE) were an order of magnitude greater in birds sampled in Mendall Marsh ( $1.30 \pm 0.37 \mu\text{g/g w.w.}$ ) than in yellowthroats sampled further south along Penobscot Bay ( $< 0.07 \mu\text{g/g w.w.}$ ) (Figure 53,

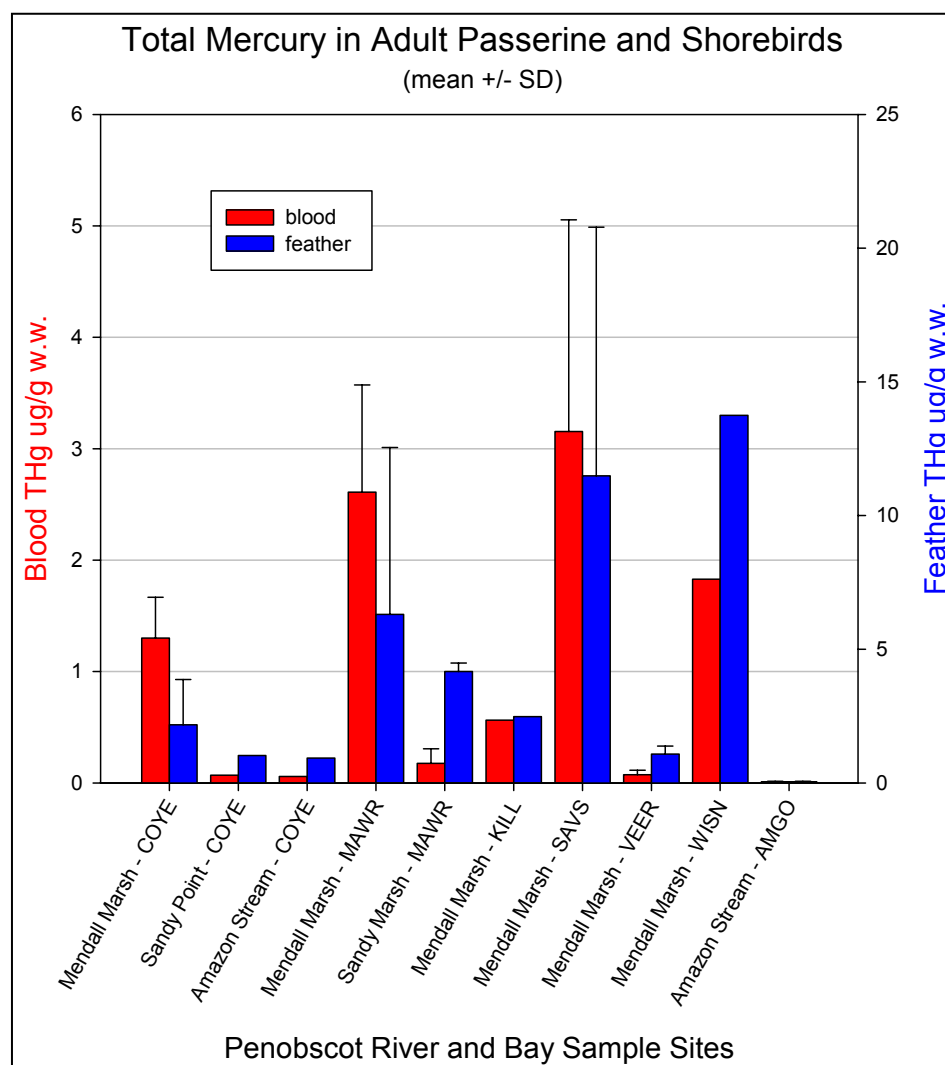


Figure 53. Total mercury concentrations in adult passerines and shorebird blood and feathers, 2007.

Appendix 28. Hg in feathers appeared to be somewhat greater in Mendall Marsh birds. Yellowthroats breed in the summer in Maine, forage primarily on insects and spiders, molt their primary feathers on the breeding grounds, but few return to a given breeding site in subsequent years. Several marsh wrens (MAWR) sampled in Mendall Marsh had mercury concentrations in their blood that

exceeded  $3.0 \mu\text{g/g w.w.}$  levels associated with

sub-lethal toxicity in birds (Evers et al. 2008) (Figure 53, Appendix 28). Overall, marsh wrens from Mendall Marsh had notably greater blood and feather mercury levels than those sampled in Sandy Marsh, in Penobscot Bay. Marsh wrens breed in Maine, eating

insects and spiders during the breeding season, and completely molt their feathers in late summer prior to migrating south.

Four adult bird species, killdeer (KILL), Savannah sparrows (SAVS), veery (VEER), and Wilson's snipe (WISN), were sampled only in Mendall Marsh, and showed a wide range of mercury concentrations which seemed linked to several aspects of their life histories (Figure 51, Appendix 28). The greatest overall Hg concentrations were found in the Savannah sparrows, which had mean blood concentrations of  $3.15 \pm 1.90 \mu\text{g/g w.w.}$  and mean feather concentrations of  $11.48 \pm 9.33 \mu\text{g/g w.w.}$  Many of the Savannah sparrow blood (47%) and feather (58%) samples exceeded levels associated with sub-lethal toxicity in birds (Heinz 1979; Evers 2008). These sparrows arrive in early April, feed on animal matter including insects, spiders, amphipods and small crustaceans during the summer breeding season, and have shown strong inter-annual site fidelity, most returning to breed within 50 m of last year's nest.

In contrast, the adult veerys had the overall lowest mercury concentrations of these Mendall Marsh birds (Figure 51, Appendix 28). Veerys arrive in Maine in May to begin breeding, have an omnivorous diet of insects and fruit and a very low return rate from one breeding season to the next.

American goldfinch (AMGO) were sampled in Amazon Stream, in upper Penobscot Bay, and found to have very low mean blood ( $0.003 \pm 0.001 \mu\text{g/g w.w.}$ ) and feather ( $0.05 \pm 0.02 \mu\text{g/g w.w.}$ ) total Hg concentrations (Figure 51, Appendix 28).

Summary - Hg in some other species were quite high in Mendall Marsh compared to other areas and levels of concern for toxic effects, whereas Hg in two species were very low.

### **Belted kingfisher (*Ceryle alcyon*)**

Belted kingfishers are likely seasonal migrants, arriving in mid April from winter ranges to the south. Kingfishers are known to feed within limited areas in relation to the location of their nest sites and 3 km is a conservative (upper limit) estimate of the possible distance that they will move to feed (Brooks and Davis 1987; Cornwell 1963; Davis 1982). They prefer foraging in clear, shallow water, especially stream riffles where increased visibility aids surface foraging. Small fish (<10 cm in length) are preferred food, especially sticklebacks or mummichogs, though a wide range of prey is possible, including molluscs, crustaceans, insects, amphibians, young birds and small mammals. In one study, increased water turbidity led to a prey switch from fish to crayfish. Their one annual molt begins on the breeding grounds in late June or July, is suspended during the fall migration, and completed on the wintering grounds by December. The 2<sup>nd</sup> secondary feather is molted on the wintering grounds, and so reflects mercury exposure on the wintering grounds. Adults rarely return to the same breeding site in subsequent years, although it is possible if nesting is successful, or if the birds overwinter in the area (Hamas 1994).

Total Hg in the blood of belted kingfisher chicks (juveniles) was sampled in 2007 at nine sites in the Penobscot drainage basin. Seven of the sites were considered to be reference sites because they were located on the Penobscot river but more than 3 km upstream of the area of contamination of Hg from HoltraChem or were located more than 3 km away from the mainstem of the Penobscot or Orland rivers. Two of the sites were considered to be impacted by Hg from HoltraChem (Mendall Marsh and Ferry Road).

Overall, total Hg concentrations in the blood of kingfisher chicks in the area were low, averaging 0.15 µg/g w.w. (Figures 54 and 55; Appendix 29). Mean total Hg concentrations in blood at impacted sites were significantly lower than at reference sites (mean total Hg at impacted sites = 0.07 µg/g; mean at reference sites = 0.18;  $p < 0.0001$ ; t-test assuming unequal variances). Total Hg concentrations in blood in chicks were significantly higher at Snow Rover, at the mouth of the East Branch of the Penobscot, and at the Rt. 43 Gravel Pit (ANOVA,  $p < 0.0005$ , Tukey HSD,  $p < 0.05$ ), both reference sites. Chick weight, bill length or sample date were not significant covariates. The lowest chick blood total Hg was at Ferry Road, adjacent to the HoltraChem site. Hg in chick blood therefore does not reflect the geographic pattern of contamination of HoltraChem Hg in the Penobscot River. Kingfishers may be foraging predominately off the mainstem of the Penobscot River and this may account for the lack of reflection of Hg contamination in the river. Total Hg concentrations in chick blood did not exceed levels associated with toxicity (Evers et al. 2008).

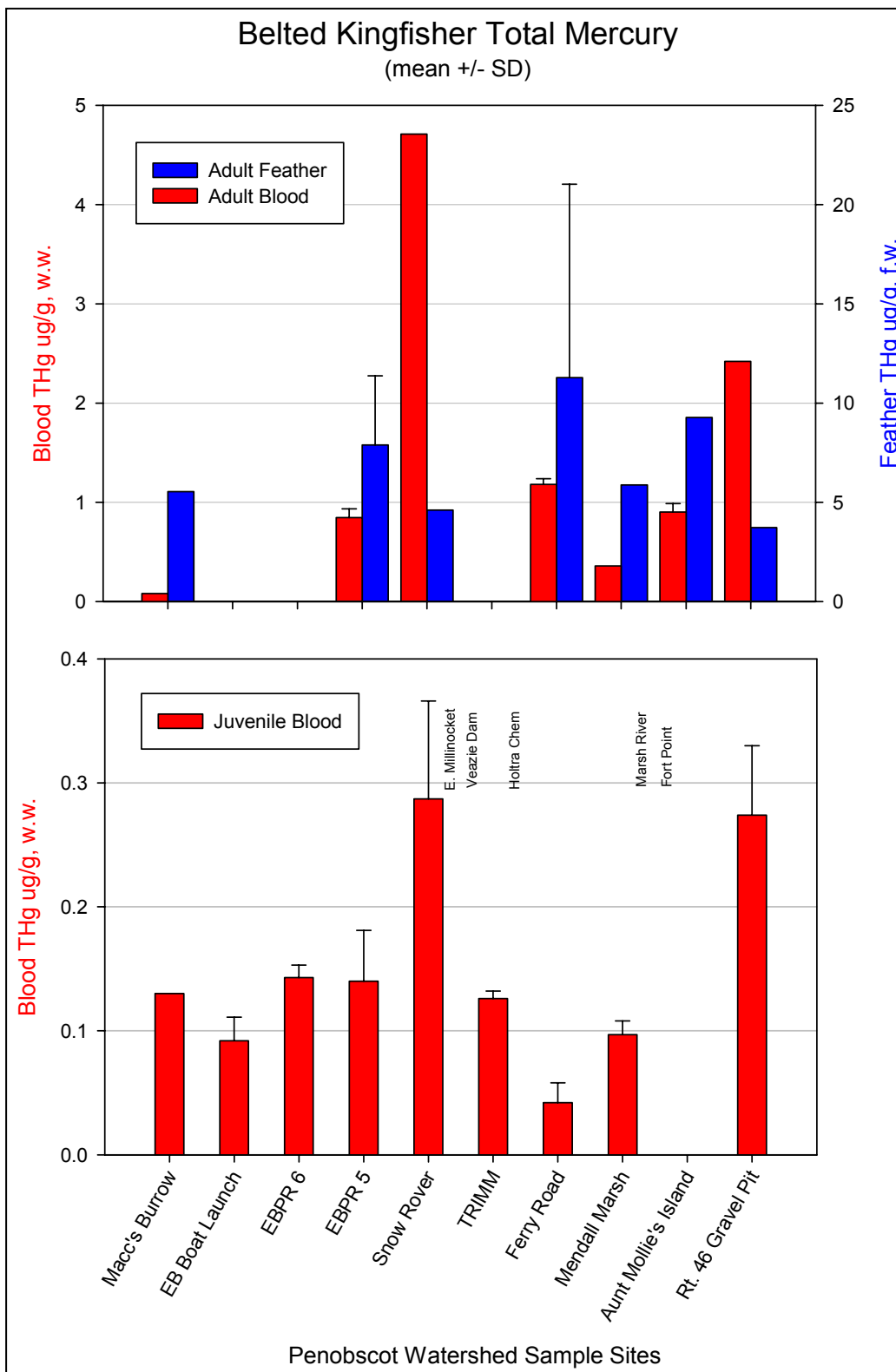


Figure 54. Total mercury concentrations in the blood and feathers of adult belted kingfishers, Penobscot watershed, 2007.



Adult belted kingfishers were sampled at seven sites ( $n = 10$ ). As for juveniles, Hg concentrations in the blood and feathers of adult kingfishers were highly variable and showed no geographic trend relative to the HoltraChem site (Figure 55, Appendix 29). Blood concentrations in adults were much higher than in chicks. The greatest total Hg concentrations in blood were found at Snow Rover, at the mouth of the East Branch of the Penobscot River ( $4.71 \mu\text{g/g w.w.}$ ), a pond site at the Rt. 43 Gravel Pit, five kilometers north of Orland ( $2.42 \mu\text{g/g w.w.}$ ), and at Ferry Road, adjacent to the HoltraChem site on the east bank of the Penobscot River ( $1.18 \mu\text{g/g w.w.}$ ). Total Hg in adults blood was not significantly different between reference and impacted sites ( $p=0.18$ ; t-test assuming unequal variance). Average blood concentrations of total Hg in adult kingfishers was  $1.53 \mu\text{g/g w.w.}$  ( $n=7$ , s.d. = 1.57, range  $0.079 - 4.71 \mu\text{g/g}$ ) at reference sites and  $0.91 \mu\text{g/g}$  ( $n=3$ , s.d. = 0.48, range  $0.359 - 1.22 \mu\text{g/g}$ ) at impacted sites. Total Hg in blood at Snow Rover exceeded levels associated with sub-lethal toxicity in birds (Evers et al. 2008). In adult feathers, Hg was not significantly different at impacted vs. reference sites ( $p = 0.25$ , t-test assuming unequal variance). The average feather concentration at impacted sites was  $9.5 \mu\text{g/g}$  ( $n=3$ , s.d. 7.56, range  $4.39 - 18.17$ ) as compared to an average of  $5.82 \mu\text{g/g}$  ( $n=7$ , s.d.=3.01, range  $1.86 - 10.35$ ) at reference sites. One individual, sampled at the Ferry Road site, near HoltraChem had very high levels of total Hg in feathers (over  $18 \mu\text{g/g w.w.}$ ) as compared to levels of concern. The sampled feathers (2<sup>nd</sup> secondary) had been molted 7 – 10 months earlier, most likely on the wintering grounds. Because adult kingfishers are season migrants to the Penobscot region, Hg in adult blood and feathers probably does not consistently reflect local exposure levels.

Summary - Hg concentrations in the blood of belted kingfisher chicks in the area were low and did not reflect contamination from HoltraChem. Total Hg concentrations in chick blood did not exceed levels associated with toxicity.

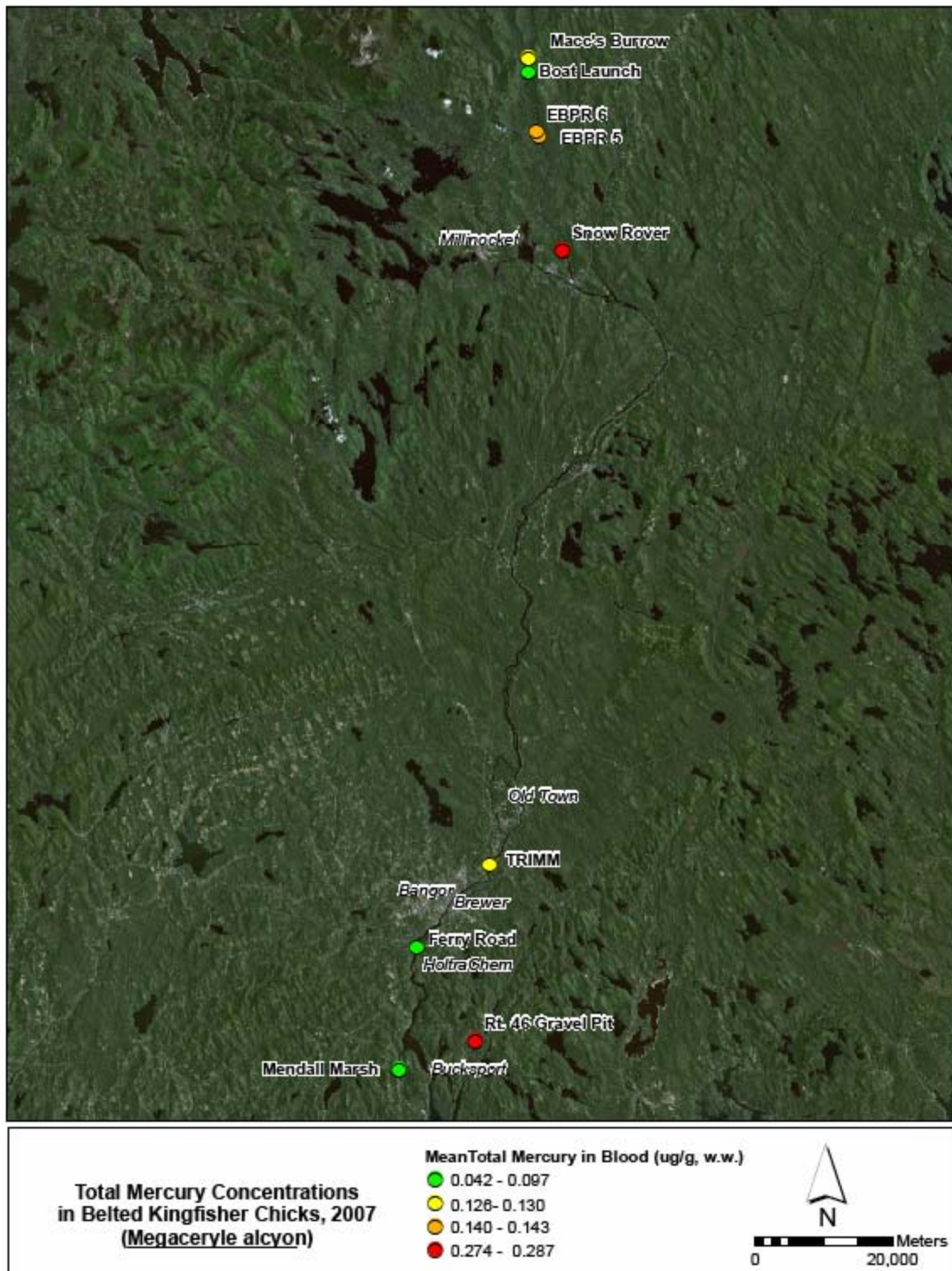


Figure 55. Total mercury concentrations in the blood of kingfisher chicks, Penobscot basin, 2007.

### **Osprey (*Pandion haliaetus*)**

Osprey blood and feather samples, collected from nests in the Penobscot region and in Southern Maine, were analyzed for total Hg levels. A summary of raw data is provided in Appendix 30. Within the Penobscot region, total Hg concentrations in the feathers of osprey chicks showed a strong, significant decline with distance south from Holtrachem

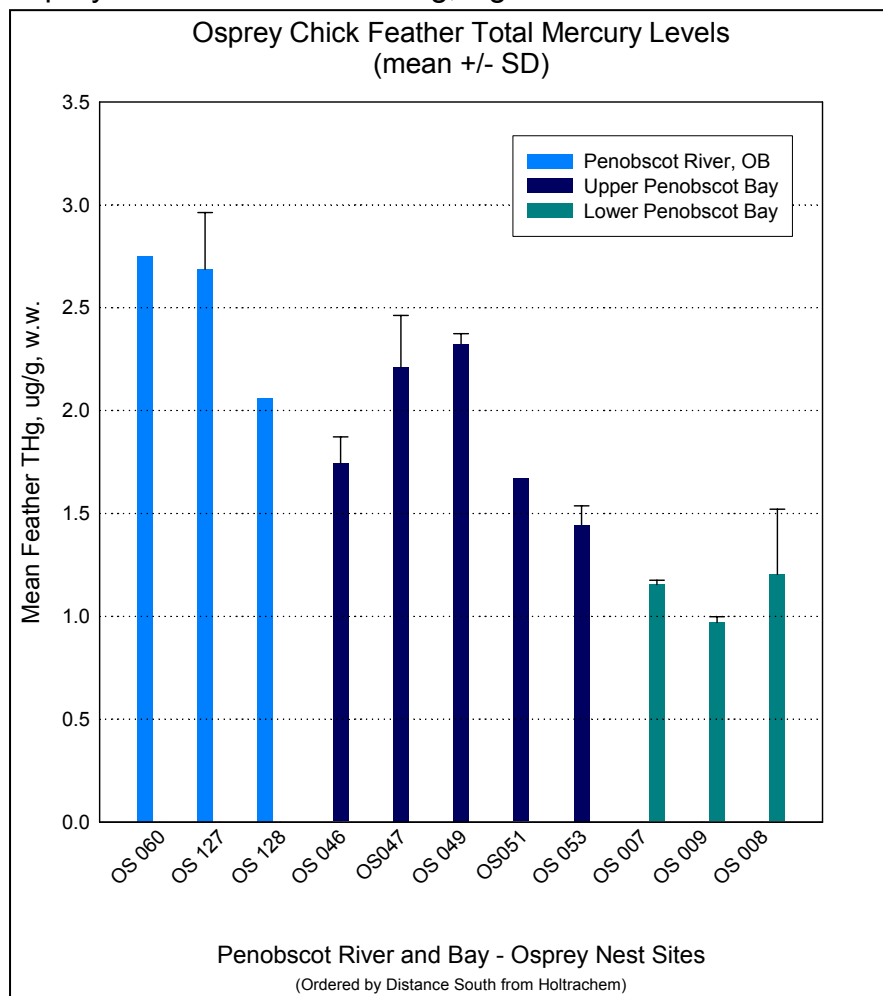


Figure 56. Total Hg concentrations in the feathers of osprey chicks collected in the Penobscot region, 2007.

in chicks reflects Hg transferred from the mother during egg formation and from local prey fish fed to the developing chicks.

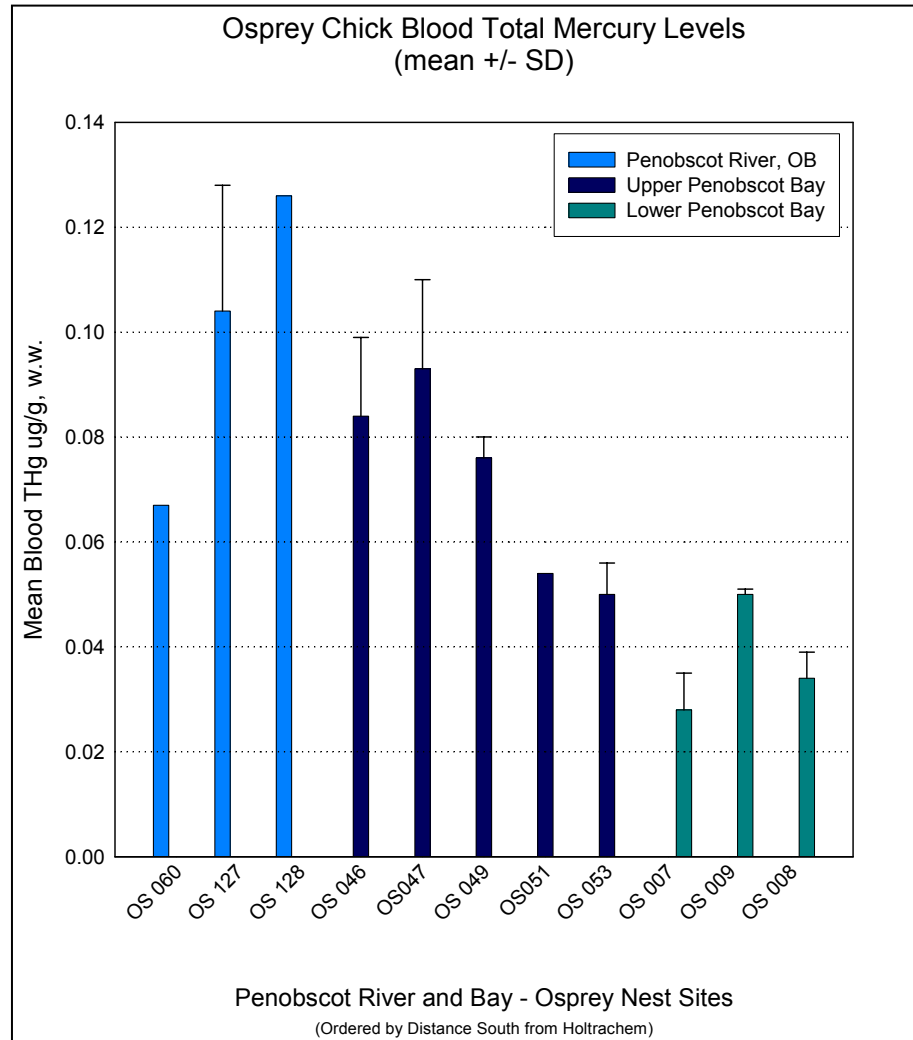
When osprey chicks were grouped by habitat within the Penobscot region, total Hg concentrations in chick feathers declined significantly from riverine, to coastal and then to marine habitat groups. Total Hg in chick blood, when grouped by habitat, were statistically equivalent between sites in the Penobscot River and the coastal sites in

(Figure 56); feather total Hg levels ranged from 2.687  $\mu\text{g/g}$  w.w., the average level in chicks from a nest in the Penobscot River (OB), to an average THg level of 0.970  $\mu\text{g/g}$  w.w. from a nest on North Haven Island in Lower Penobscot Bay. Total Hg concentrations in the blood of chicks also declined significantly with distance south from Holtrachem (Figure 57). Blood levels ranged from over 0.10  $\mu\text{g/g}$  w.w. in chicks nesting in the Penobscot River to roughly 0.035  $\mu\text{g/g}$  w.w. in chicks from

nests in Lower Penobscot Bay (Figure 57). Hg concentrations

Upper Penobscot Bay, and both were significantly greater than total Hg in chick blood collected from the marine sites in Lower Penobscot Bay.

Total Hg concentrations in adult osprey blood and feathers were highly variable. While the greatest concentration of total Hg in adult blood was found in an adult nesting in the



Penobscot River, 2.430  $\mu\text{g/g}$  w.w., and the lowest level was found in an adult sampled in Lower Penobscot Bay, 0.189  $\mu\text{g/g}$  w.w., adult blood levels did not correlate with distance from Holtrachem. Similarly, total Hg concentrations in the feathers of adults varied widely, from 58.900  $\mu\text{g/g}$  w.w. in an adult sampled in lower Penobscot Bay, to 1.770  $\mu\text{g/g}$  w.w. in an adult sampled in Upper Penobscot Bay. As for concentrations in blood, total Hg concentrations in the

Figure 57. Mean total Hg concentrations in the blood of osprey chicks collected in the Penobscot region, 2007.

feathers of osprey adults did not correlate with

distance from Holtrachem. Concentrations in adults may reflect variation in mercury exposure from the adult wintering grounds in Central and South America, and foraging areas along the migratory route more than exposure in the breeding grounds in Maine. Adult ospreys return to the Penobscot region to breed in late May and early April and leave the area in late August and September (Poole et al. 2002).

The results of regional comparisons of Hg in osprey chicks between the Penobscot area and Southern Maine were inconsistent. Comparisons were made within the previously defined habitat types, riverine, coastal and marine. In the riverine habitat type, the Sheepscot River sites had the greatest total Hg concentrations in the feathers of chicks (mean  $\pm$  SD,  $4.022 \pm 0.769$   $\mu\text{g/g w.w.}$ ) and blood ( $0.212 \pm 0.138$   $\mu\text{g/g w.w.}$ ) levels of any area sampled, and total Hg levels in Sheepscot chicks were significantly greater than levels in Penobscot River chicks (ANOVA,  $p < 0.05$ ). In the coastal habitat, chick blood and feather total Hg levels were statistically equivalent between sites in Portland's Fore River and Upper Penobscot Bay. Within the marine habitat, chicks sampled in the Harpswell area had significantly greater blood and feather total Hg concentrations than chicks sampled in Lower Penobscot Bay. Notably, total Hg levels in the Harpswell chicks (marine habitat) were not significantly different from levels in chicks from the Fore River in Portland (coastal habitat), but total Hg levels in both of those Southern Maine sites were significantly less than in chicks in the Sheepscot River.

Summary - Total Hg in chick feathers and blood declined significantly with distance south from HoltraChem. Hg in ospreys from the Penobscot were similar to or lower than those in southern Maine, when comparisons were done between equivalent habitat types. Hg concentrations in osprey did not approach levels of concern for toxic effects.

### **Bald eagle (*Haliaeetus leucocephalus*)**

Total Hg concentrations in the breast feathers of bald eagles varied widely among the

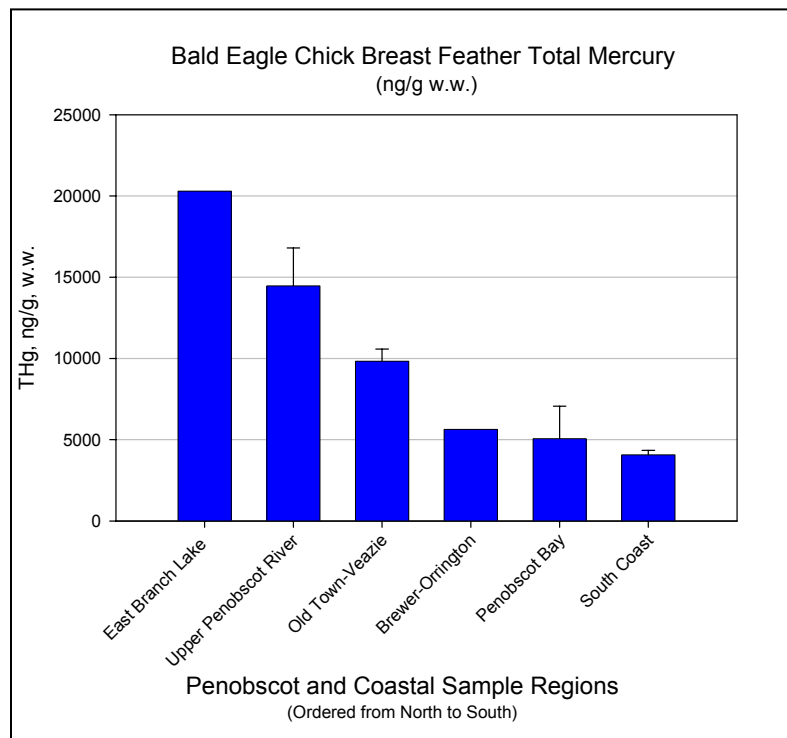


Figure 58. Mean total Hg concentrations in the feathers of bald eagle chicks sampled in the Penobscot region, 2007.

regions sampled, yet the lack of samples in the regions potentially impacted by the Holtrachem plant, and the shift in bald eagle diet between inland and coastal habitats limit the value of this data set. Figure 58 illustrates the wide range in concentrations of total Hg found in breast feathers in 2007. (A summary of raw data is provided in Appendix 31.) Total Hg levels were greatest at the more inland sites, and declined at sites further downstream with the lowest levels found from sites in

central Penobscot Bay and along the Southern Coast of Maine. This decline may reflect the reported shift from a

diet dominated by fish at inland sites to one dominated by birds and mammals at coastal sites (Buehler 2000).

## DISCUSSION AND CONCLUSIONS

### General conclusions

Taken together, the results presented in this report and in the Phase I report give a coherent and strengthened picture of the contamination of Hg in the Penobscot River and estuary. Results presented in this report are confirmatory of those presented in the Phase I report and strengthen the conclusions presented in that report, that is, the lower Penobscot River and upper Penobscot estuary is significantly contaminated with Hg. The upstream limit of the area of most severely contamination is now clearly shown to be South Brewer, about 5 km upstream of HoltraChem, somewhere between sampling site BO3 and wetland W05. The sediment sampling site BO3, located about 3.8 km (2.3 miles) upstream of HoltraChem was shown to be noticeable contaminated whereas the wetland W05, located about 6.5 km (4 miles) upstream of HoltraChem, was shown to be not significantly contaminated with HoltraChem Hg. Tidal movements have evidently pushed Hg from the HoltraChem site approximately 4-6 km upstream on the river. The downstream (southern) limit of the area most severely contaminated is more difficult to define because concentrations in sediments and biota generally show gradual declines from about the south end of Verona Island to much further south in the estuary. For example, total Hg in estuary sediments decline from Fort Point Cove (north of Fort Point) to much further south in the estuary (although concentrations normalized to organic carbon are unchanged in this same geographic area). Total Hg in wetland soils declined noticeably south of the south end of Verona Island, which is located north of Fort Point.

Many species of biota showed significantly lower concentrations of Hg in the upper estuary as compared to the lower river, or in the lower estuary as compared to the upper estuary, especially in invertebrates, shellfish and small fish and cormorants, which generally have limited mobility ranges and therefore are more likely to reflect concentrations of methyl Hg in their immediate environment. Patterns showing concentration declines in Hg in biota with distance from HoltraChem in the lower river and upper estuary, however, were not always consistent among different species. It does seem clear, however, that the area of most severe contamination ends at about Fort Point, located 33 km (20.5 miles) south of the HoltraChem site.

Data presented in this update report confirm the high levels of Hg in sediments and various species of biota that were reported in the Phase I report. Total Hg concentrations in riparian wetlands along the river and estuary were shown in the Phase I report to be significantly contaminated with Hg from near HoltraChem to the south end of Verona Island. Like the river sediments, methyl Hg concentrations in these wetlands

are mostly dependent on concentrations of total Hg, but also there is also a zone in the lower river where the % of total Hg that is methyl Hg is noticeably higher. This zone is in the transition between freshwater wetlands in the north and salt marshes in the south and may be related to sulfate concentrations or other environmental variables, which are known to affect rates of Hg methylation.

In the Phase I report, Hg in a number of species of biota were shown to be high in relation to reference sites, comparable to other known contaminated sites and/or high in relation to levels of concern for toxic effects. This update report confirms and extends these original observations. Table 3 provides a comparison, for all species of biota that have been sampled, of Hg concentrations seen in the Penobscot system compared to other areas, and the geographic pattern of the Penobscot biota. Table 3 also lists which species are of concern for human consumption and which species are at risk of toxicity.

High concentrations of Hg in songbirds in relation to reference areas and levels of concern, especially in sparrows inhabiting riparian wetlands, were confirmed and extended to other species. Cormorants were shown in the Phase I report to have concentrations of Hg that approach those of concern for toxic effects and this observation has been confirmed in this update report, and extended to black guillemots that were higher in Hg than cormorants. New data on biota species not included in the Phase I report are also shown to have quite high levels of Hg in relation to reference areas or other contaminated sites, such as soft-shelled clams, eels, winter flounder, and rails (Table 3).

The data presented here also strengthens the conclusion of the Phase I report that HoltraChem is likely the dominant source of the Hg contamination observed in the lower Penobscot river and upper estuary. A deep core taken adjacent to the HoltraChem site showed a very high concentration of Hg in the deepest layer, consistent with HoltraChem being the source of contamination. In addition, a number of species of biota showed geographic patterns that were consistent with HoltraChem being the dominant source of Hg to the lower Penobscot River. These species include Nereis worms, soft-shelled clams, Macoma clams, green crabs, tomcod, eels, rainbow smelt, cormorants (confirmed from Phase I report), songbirds, and rails (Table 3). However there may be other sources of mercury to the lower Penobscot, and this is a topic of investigation for Phase II of the project.

On the other hand, some species of biota were not high in Hg or did not show geographic patterns of Hg concentrations that were consistent with HoltraChem being the dominant source of Hg to the lower Penobscot River. Such species included rock crabs, killifish, golden shiners, and wide-ranging bird species such as kingfishers and



eagles (Table 3). Although Hg in osprey declined with distance from HoltraChem, levels were not high compared to reference areas or to levels of concern for toxic effects. As was concluded in the Phase I report, wide-ranging bird and mammals species do not

Biota group	Concentrations high compared to other areas?	Geographic patterns consistent with HoltraChem?	Levels of concern for human consumption?	Levels of concern for toxic effects?
Periwinkles	No	Yes	n/a	No
Freshwater Snails	No	No	n/a	No
Lobster	No	Yes	Yes	No
Mussels	Yes	Yes	No	No
Nereis worms	No	Yes	n/a	No
Soft-shelled clams	Yes	Yes	No	No
Macoma clams	?	Yes	n/a	No
Green crabs	?	Yes	n/a	No
Rock crabs	Yes	No	Yes	No
Tomcod	?	Yes	n/a	No
Eels	Yes	Yes	Yes	Yes
Killifish	Yes	Yes	n/a	No
Smelt	Yes	Yes	No	No
Flounder	Yes?	No	n/a	No
Golden shiners	Yes	?	n/a	No
Songbirds	Yes	?	n/a	Yes
Shorebirds	Yes	?	n/a	Yes
Cormorants	Yes	Yes	n/a	Yes
Guillemots	Yes	?	n/a	Yes
Kingfishers	No	No	n/a	No
Osprey	No	Yes	n/a	No
Bald eagles	No	?	n/a	No
Otters	No	No	n/a	No
Mink	No	No	n/a	No

Table 3. Summary of conclusions regarding Hg levels in all species of biota that have been sampled to date. See text for specific groups for detailed discussion. n/a = not applicable. ? = not certain; information lacking.

show patterns related to the location of the HoltraChem plant, perhaps due to foraging over relatively large distances or feeding off the Penobscot main stem.

### ***Phase II of the Study***

Phase II of the study will begin in the spring of 2009. It will be conducted under the guidance of the Phase II Study Plan, approved by the Court in 2008. The overall objectives of Phase II are to (1.) Determine if a process of natural attenuation can reduce concentrations of Hg in the contaminated area of the Penobscot system to acceptable levels within a reasonable timeframe, and (2.) Determine if active remediation measures could feasibly accelerate recovery in certain contaminated locations. Work will include the determination of rates of natural attenuation in the system, the determination of the dynamics and limiting factors of the production of methyl Hg, assessment of continued inputs of Hg from the HoltraChem site, initial assessments of the efficacy of active mitigation methods, and the monitoring of Hg in selected species of biota. A few of the aspects of field work as part of Phase II of the study were begun in the 2008 field season. A field visit was hosted for researchers from the University of Southern Mississippi and the University of New Orleans who will in 2009 carry out the field sampling of sediment cores in the Penobscot system to determine rates of natural attenuation of Hg contamination of the area. Also, a field visit was hosted for researchers from the Smithsonian Environmental Research Center and the University of Toronto who will carry out studies of mercury methylation. Preliminary field sampling by Dr. Ralph Turner of RT Geosciences related to work at the HoltraChem site on seepage and runoff was carried out. The first three months of intensive sampling of wetlands in the lower Penobscot River was completed, with sampling done every three weeks at two wetlands in each of three salinity zones in the lower river (freshwater, salt and transitional) to examine the seasonal dynamics in the wetlands in these three zones that have been shown to be different in their capacity to produce methyl Hg. The monitoring of Hg in aquatic and bird species, including lobster, mussels, small fish such as tomcod, winter flounder and rainbow smelt, cormorants and songbirds was begun with sampling of all of these species. New investigations into Hg in seals and bats were completed in the 2008 field season. Water sampling in support of modelling of mass balance fluxes of total and methyl Hg in the lower river was begun in 2008.

What lessons are apparent in the results of the Phase I of the Study for mercury remediation in the Penobscot system? First, methyl Hg concentrations in the Penobscot system are mainly determined by total Hg concentrations. Although there is a slight seasonality in methyl Hg concentrations in riverine sediments in the Penobscot,

these differences are relatively small compared to differences in total Hg concentrations. That is, seasonal differences in percent methyl Hg in riverine sediments are less than 2X higher during August and early September, whereas concentrations of total Hg are on the order of 10X higher in the contaminated zone. Differences in percent methyl Hg in wetland soils are somewhat more pronounced than the seasonal differences in percent methyl Hg in riverine sediments but are still dwarfed by differences in total Hg concentrations. Therefore, it would appear that reducing total Hg in, whether wetlands, riverine or estuarine sediments, will reduce concentrations of methyl Hg and methyl Hg concentrations in biota that depend on sediment-based food chains. One caveat to this statement is that we do not yet have data on the seasonality of methyl Hg concentrations in wetland soils in the Penobscot system. Even for those animal species that depend on suspended particles, reducing Hg in riverine and other sediments would probably reduce Hg in animals because suspended particles are probably mainly resuspended from bottom sediments by currents and tidal action. Therefore, if a practical method of reducing total Hg concentrations can be found for the sediments of the contaminated zone in the Penobscot, methyl Hg concentrations should be reduced proportionately in animal species.

The second lesson from Phase I results for remediation is that Mendall Marsh is an area of special concern. Hg concentrations in songbirds and shorebirds sampled at Mendall Marsh are very high compared to reference areas and to levels of concern for toxic effects. Also, concentrations in species such as song sparrows are high in Mendall even compared to other contaminated wetlands in the lower Penobscot. Why this is true is not obvious because concentrations of methyl Hg in the soils of Mendall Marsh are not higher than other contaminated wetlands in the lower Penobscot, for example W17, where Hg concentrations in birds are much lower. It may be that the large area of Mendall Marsh serves, along with relatively high concentrations in soils (and presumably in the food chain supporting birds), to promote high concentrations in birds. This may be because in small wetland systems that are contaminated with Hg from HoltraChem, birds are foraging part of the time in the contaminated wetlands but also part of the time in uncontaminated areas adjacent to the wetland. In the more extensive Mendall Marsh system, resident birds may be feeding within Mendall Marsh itself all or almost all of the time. Whatever the reason, special attention should be given to Mendall Marsh. Remedial activities that could reduce total Hg in the soils of Mendall Marsh would probably reduce Hg in song and shore birds. We will carry out studies on the food chain of this contaminated system and will be continuing to sample this and other wetland areas to examine the seasonality of methyl Hg production.

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Appendix 1. Raw data for total Hg, TOC (total organic carbon) from Core D-01. The core was taken from Fort Point Cove (44.48233 N; 68.80870W) on August 20, 2007.

Depth (cm)	TOC (%)	Total Hg ng/g w.w.	Total Hg ng/g d.w.	Total Hg ngHg/g TOC
0-2	0.99	119	513	51818
2-3	1.3	182	504	38769
3-4	1.3	197.5	582	44731
4-5	1.6	209	589	36813
5-6	1.4	211	607	43357
6-7	1.4	224	655	46786
7-8	1.6	233	705	44063
8-9	1.6	230	696	43500
9-10	1.4	208	558	39857
10-11	1.4	206	534	38143
11-12	1.3	237	586	45077
12-13	1.5	251	625	41667
13-14	1.4	236	583	41643
14-15	1.7	251	625	36765
15-18	1.7	271	703	41353
18-21	1.6	313	740	46250
21-24	1.9	325	796	41895
24-27	1.8	454	980	54444
27-30	2.1	617	1380	65714
30-33	2.0	537	1130	56500
33-36	2.4	474	1050	43750
36-39	2.9	248	534	18414
39-42	2.1	205	428	20381
42-45	2.1	204	426	20286
45-50	2.0	199.5	402	20075
50-55	1.2	23.9	44	3692
55-60	1.1	9.86	19	1736
60-65	1.0	9.58	18	1870
65-70	1.0	9.59	18	1830
70-75	1.2	10.3	19	1617

Appendix 2. Raw data for total Hg, TOC (total organic carbon) from Core D-02. The core was taken from Fort Point Cove (44.29051N; 68.49130W) on August 22, 2007.

Depth (cm)	TOC (%)	Total Hg ng/g w.w.	Total Hg ng/g d.w.	Total Hg ngHg /g TOC.
0-2	1.4	171	834	59571
2-3	1.4	203.5	760	54321
3-4	1.4	191	714	51000
4-5	1.5	189	689	45933
5-6	1.4	194	726	51857
6-7	1.7	222	817	48059
7-8	1.4	234	834	59571
8-9	1.6	240	882	55125
9-10	1.6	234	806	50375
10-11	1.6	246	848	53000
11-12	1.6	220	789.5	49344
12-13	1.5	267	909	60600
13-14	1.6	257	874	54625
14-15	1.8	268	852	47333
15-18	2.0	370	1060	53000
18-21	2.0	365	1050	52500
21-24	1.8	395.5	1120	62222
24-27	2.0	377	1110	55500
27-30	2.0	439	1230	61500
30-33	2.65	755	2200	83019
33-36	2.6	762	2210	85000
36-39	2.4	693	2020	84167
39-42	2.4	584	1670	69583
42-45	2.2	521	1360	61818
45-50	2.0	130.5	309	15450
50-55	1.6	171	399	24938
55-60	1.7	59.3	137	8059
60-65	1.3	40.2	89.5	6885
65-70	1.4	34.3	69.3	4950

Appendix 3. Raw data for total Hg, TOC (total organic carbon) from Core SC-01. The core was taken from Southerly Cove (44.73737N; 68.82926W) on August 16, 2007.

Depth (cm)	TOC (%)	Total Hg ng/g w.w.	Total Hg ng/g d.w.	Total Hg ngHg/g TOC
0-2	1.7	57.2	446	26235
2-3	1.9	192	1270	66842
3-4	2.4	213	1200	50000
4-5	2.4	270	1480	61667
5-6	2.5	252	1400	56000
6-7	2.5	246	1260	50400
7-8	2.6	258	1305	50192
8-9	2.6	287	1510	58077
9-10	2.7	358	1730	64074
10-11	2.4	315	1470	61250
11-16	3.2	364	1290	40312
16-21	3.9	553	1800	46154
21-26	3.6	597	1710	47500
26-31	4.7	798	2280	48511
31-36	4.9	1080	2110	43061
36-41	3.8	11090	24000	631578

Appendix 4. Raw data for wetlands sampled in August 2007, including total and methyl Hg (THg and MeHg) concentrations, and total organic carbon (TOC) concentrations. Locations of wetlands are given in Phase I report. All wetlands were sampled between August 17, 2007 and August 31, 2007. Explanation of elevations given in Methods and Phase I report.

Wetland	Elevation	THg ng/g w.w.	THg ng/g d.w.	TOC(%)	THg µg/g OC d.w.	MeHg ng/g w.w.	MeHg ng/g d.w.
W 05	Intertidal	26.0	51.5	1.4	3.7	0.37	0.72
W 05	Low	22.6	45.2	1.6	2.8	0.49	0.98
W 05	Medium	20.4	41.6	2.4	1.7	0.38	0.77
W 05	High	91.2	189	4.7	4.0	0.42	0.87
W 07	Intertidal	406	1990	2.6	76.5	10.5	51.6
W 07	Low	275	957	2.2	43.5	7.96	27.7
W 07	Medium	506	1195	2.7	44.3	7.05	16.6
W 07	High	312	522	2.3	22.7	1.39	2.33
W 10	Intertidal	357	1020	2.8	36.4	5.45	15.50
W 10	Low	232	430	2.5	17.2	2.54	4.72
W 10	Medium	416	842	3.7	22.8	4.94	10.0
W 10	High	269	602	4	15.1	2.60	5.82
W 11	Intertidal	430	1270	3.2	39.7	6.44	19.0
W 11	Low	292	569	2.7	21.1	4.73	9.21
W 11	Medium	347	927	3	30.9	8.52	22.8
W 11	High	286	730	6.2	11.8	7.62	19.5
W 13	Intertidal	420	1230	3.2	38.4	10.35	30.05
W 13	Low	329	1020	2.9	35.2	17.8	55.4
W 13	Medium	299	911	3.5	26.0	7.50	22.9
W 13	High	259	954	3.5	27.3	16.1	59.3
W 14	Intertidal	333	1000	3.5	28.6	5.05	15.2
W 14	Low	338	1110	3.2	34.7	15.6	51.3
W 14	Medium	429	1030	4	25.8	19.8	47.4
W 14	High	287	930	5.4	17.2	8.82	28.6
W 17	Intertidal	365	1400	2.4	58.3	10.25	39.05
W 17	Low	384.5	1225	2.9	42.2	21.40	68.15
W 17	Medium	307	870	3.2	27.2	13.7	38.8
W 17	High	105	480	6.2	7.7	8.88	40.6
W 21	Intertidal	398	1400	3.2	43.8	8.86	31.20
W 21	Low	310	1030	2.7	38.1	11.1	36.7
W 21	Medium	300	948	3	31.6	9.30	29.4
W 21	High	296	779.	4.8	16.2	18.7	49.3



Wetland	Elevation	THg ng/g w.w.	THg ng/g d.w.	TOC(%)	THg µg/g OC d.w.	MeHg ng/g w.w.	MeHg ng/g d.w.
W 22	Intertidal	325	899	2.8	32.1	9.48	26.2
W 22	Low	330	858	2.7	31.8	11.9	31.0
W 22	Medium	363	883	3.4	26.0	18.9	45.9
W 22	High	356	821	5.6	14.7	1.73	3.99
W 23	Intertidal	340	962	3	32.1	6.50	18.4
W 23	Low	355	898.5	3	30.0	11.9	30.2
W 23	Medium	390	875	3.6	24.3	4.80	10.8
W 23	High	215	663	5	13.3	2.65	8.16
W 25	Intertidal	260	491	1.5	32.7	5.97	11.30
W 25	Low	281.5	752	2.4	31.3	8.72	23.3
W 25	Medium	231	773	2.9	26.7	4.01	13.4
W 25	High	121	322	4.2	7.7	1.39	3.69
W 26	Intertidal	507	1390	3.4	40.9	10.03	27.50
W 26	Low	347	954	3.1	30.8	7.42	20.4
W 26	Medium	289	939	4.4	21.3	5.20	16.9
W 26	High	286	423	5.6	7.6	1.35	2.00
W 28	Intertidal	235.5	522.5	3.2	16.3	3.67	8.16
W 28	Low	438	1040	3.7	28.1	11.7	27.9
W 28	Medium	286	789	6.2	12.7	7.41	20.4
W 28	High	192	703	2.1	33.5	8.69	31.8
W 31	Intertidal	40.0	56.7	0.54	10.5	0.90	1.27
W 31	Low	26.95	40.95	1.1	3.7	1.29	1.96
W 31	Medium	13.4	17.6	0.92	1.9	0.44	0.58
W 31	High	13.8	15.5	1.2	1.3	0.06	0.06
W33	Intertidal	34.9	49.7	0.5	9.9	0.56	0.79
W33	Low	22.4	36.1	1.4	2.6	0.22	0.36
W33	Medium	29.35	62.8	4.7	1.3	0.61	1.30
W33	High	76.7	287	10	2.9	0.35	1.31
W34	Low	73.4	160	2.5	6.4	0.19	0.42
W34	Medium	41.3	171	3.2	5.3	0.18	0.74
W34	High	48.5	201	3.9	5.2	0.10	0.41

Wetland	Elevation	THg ng/g w.w.	THg ng/g d.w.	TOC(%)	THg µg/g OC d.w.	MeHg ng/g w.w.	MeHg ng/g d.w.
W36	Intertidal	20.6	36.7	2.9	1.3	0.23	0.42
W36	Low	25.4	81.1	7.2	1.1	0.92	2.92
W36	Medium	12.5	13.1	3.5	0.4	0.03	0.03
W36	High	19.3	63.7	6.6	1.0	0.49	1.63
W42	Intertidal	18	27.9	0.99	2.8	0.59	0.91
W42	Low	22.25	58.8	2.2	2.7	0.31	0.81
W42	Medium	25.6	75.3	2.9	2.6	0.24	0.71
W42	High	24.90	78.9	5.4	1.5	0.53	1.67
W44	Intertidal	18.4	27.3	0.88	3.1	0.07	0.09
W44	Low	16.55	31.6	1.7	1.9	0.23	0.44
W44	Medium	24.1	47.6	2.2	2.2	0.26	0.51
W44	High	24.1	48.1	3.9	1.2	0.20	0.39
W54	Intertidal	7.54	13.8	0.77	1.8	0.15	0.28
W54	Low	24.8	55.8	3	1.9	0.16	0.36
W54	Medium	16	54.1	4	1.4	0.23	0.79
W54	High	32.9	68.9	10	0.7	0.54	1.14
W55	Intertidal	10.5	12.3	0.35	3.5	0.10	0.12
W55	Low	19.6	62.2	2.8	2.2	0.40	1.27
W55	Medium	27.5	107	8	1.3	0.08	0.31
W55	High	45.4	106	6.8	1.6	0.05	0.12
W56	Intertidal	34.5	52.5	0.8	6.6	0.09	0.13
W56	Medium	15.55	105	4	2.6	0.04	0.27
W56	High	10.2	51.5	9	0.6	0.08	0.41
W58	Medium	12.4	77.2	6.5	1.2	0.15	0.95
W58	High	17	124	6.8	1.8	0.33	2.40
W59	Low	7.49	12.3	2.3	0.5	0.29	0.48
W59	Medium	46.7	112	4.4	2.5	0.17	0.41
W59	High	22.1	90.6	6.2	1.5	0.45	1.83
W60	Intertidal	33.25	45.3	0.45	10.1	0.43	0.58
W60	Low	33.25	67.8	2.3	2.9	0.10	0.20
W60	High	16.3	19.3	2.4	0.8	0.40	0.47

Wetland	Elevation	THg ng/g w.w.	THg ng/g d.w.	TOC(%)	THg µg/g OC d.w.	MeHg ng/g w.w.	MeHg ng/g d.w.
W61	Intertidal	302	651	2.9	22.4	8.35	18.0
W61	Low	273	880	4.5	19.6	7.99	25.8
W61	Medium	187	742	4.5	16.5	6.47	25.7
W61	High	74.9	259	6.2	4.2	1.01	3.50
W62	Intertidal	145	322	3	10.7	3.40	7.55
W62	Low	324	936	4.6	20.3	7.03	20.3
W62	Medium	224	807	5.1	15.8	5.37	19.30
W62	High	284	906	3.9	23.2	9.05	28.9

Appendix 5. Total and methyl Hg concentrations (THg and MeHg), and total organic carbon (TOC) concentrations, in the top 3 cm of estuarine sediments of Penobscot Bay and St. George River estuary. All samples taken between August 17, 2007 and August 27, 2007. Transects E-01 to E-05 are in Penobscot Bay and Transect E-08 is in the St. George River estuary.

Site	Location - latitude	Location - longitude	THg ng/g w.w.	THg ng/g d.w.	TOC (%)	THg µg/g OC d.w.	MeHg ng/g w.w.	MeHg ng/g d.w.
E01-01	44.48209	68.82766	115	606	1.4	43.3	5.51	29.0
E01-02	44.48220	68.81854	167	672	1.8	37.3	3.24	13.0
E01-03	44.48138	68.80859	140	447	1.3	34.4	2.10	6.69
E01-04	44.48163	68.79856	121	278	1.5	18.5	1.37	3.14
E01-05	44.48299	68.78791	305	631	1.4	45.1	5.17	10.7
E02-01	44.41602	68.97579	46.4	236	0.57	41.4	0.73	3.69
E02-03	44.41608	68.94488	49.5	211	0.6	35.2	0.55	2.35
E02-05	44.41589	68.91289	65.8	232	0.69	33.6	0.62	2.19
E02-06	44.41619	68.89892	47.6	192	0.66	29.1	0.61	2.48
E02-07	44.41618	68.89212	81.4	277	0.68	40.7	0.80	2.73
E02-08	44.41546	68.86632	65.3	268	0.8	33.5	0.80	3.29
E02-09	44.41582	68.85031	80	312	0.7	44.6	0.63	2.45
E02-10	44.41602	68.83488	83.5	321	0.7	45.9	0.91	3.48
E03-01	44.33470	68.9439	33.7	80.5	0.57	14.1	0.44	1.05
E03-02	44.33296	68.93748	28.9	91.1	0.78	11.7	0.35	1.12
E03-03	44.33516	68.92722	29.4	109	0.54	20.2	0.26	0.95
E03-04	44.33487	68.89513	49.9	213	0.48	44.4	0.43	1.84
E03-05	44.33497	68.87652	34.7	181	0.58	31.2	0.30	1.58
E03-06	44.33448	68.86796	44.1	160	0.58	27.6	0.38	1.40
E03-07	44.33425	68.85910	38.1	149	0.58	25.7	0.34	1.32
E03-08	44.33445	68.85007	56.7	154	0.57	27.0	0.35	0.95
E03-09	44.33425	68.84209	32.3	125	0.57	21.9	0.28	1.06
E03-10	44.33405	68.83304	35.4	115	0.54	21.3	0.22	0.73
E04-01	44.23965	69.00990	26.7	109	0.62	17.6	0.23	1.03
E04-02	44.23965	68.98928	22.3	82.9	0.72	11.5	0.02	0.09
E04-03	44.23954	68.96849	17.9	77.3	0.69	11.2	0.12	0.53
E04-04	44.23970	68.94678	21.1	109	0.86	12.7	0.12	0.60
E04-05	44.23981	68.90638	16	83.6	0.59	14.2	0.10	0.55

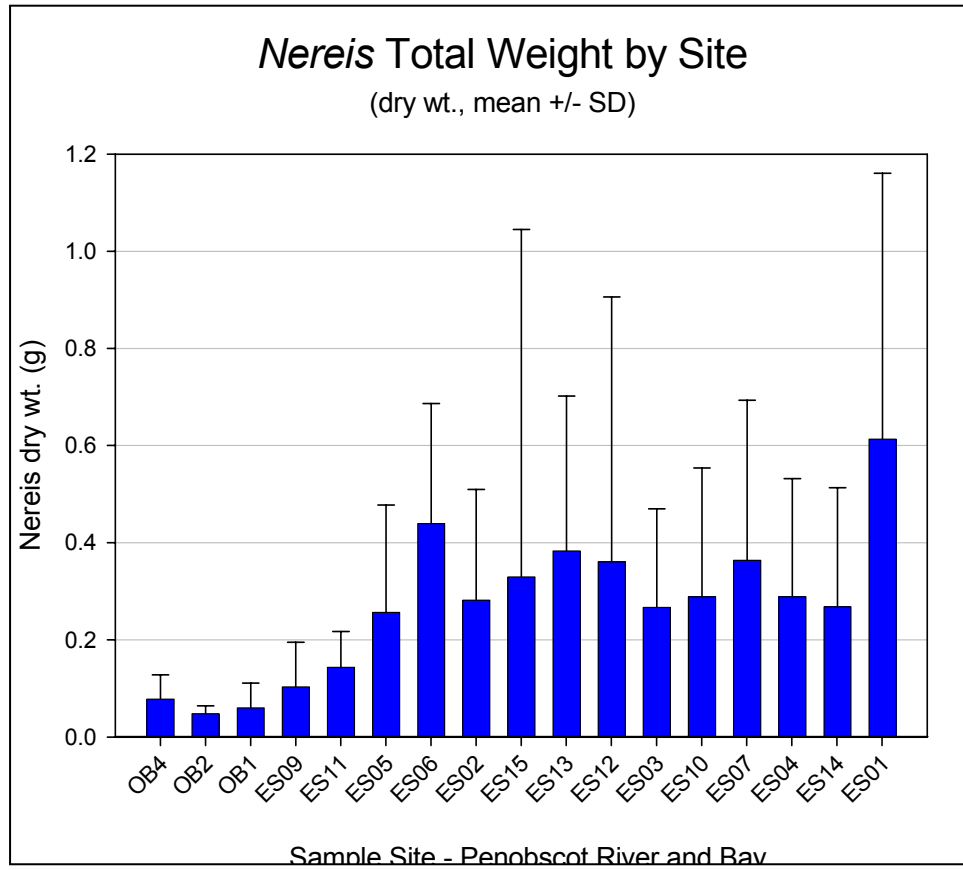
Site	Location - latitude	Location - longitude	THg ng/g w.w.	THg ng/g d.w.	TOC (%)	THg µg/g OC d.w.	MeHg ng/g w.w.	MeHg ng/g d.w.
E04-06	44.23991	68.88896	16.5	76.1	0.65	11.7	0.08	0.38
E04-07	44.24065	68.86294	19	95.1	0.66	14.4	0.12	0.59
E04-08	44.24070	68.8424	29.5	115	0.74	15.5	0.13	0.53
E04-09	44.23975	68.79867	21.1	90.6	0.5	18.1	0.16	0.66
E04-10	44.23895	68.77666	18.2	43.6	0.62	7.0	0.24	0.57
E04-11	44.23966	68.75572	11.2	98.9	0.47	21.0	0.18	1.59
E04-12	44.23903	68.73352	14	79	0.48	16.4	0.12	0.65
E04-13	44.23967	68.71136	12.4	82.3	0.49	16.8	0.15	0.98
E05-01	44.11487	69.05456	18.4	61.8	0.57	10.8	0.15	0.51
E05-02	44.11474	69.02993	16.4	60.3	0.72	8.4	0.17	0.62
E05-03	44.11441	68.99982	22.7	89.5	0.75	11.9	0.17	0.66
E05-04	44.11593	68.97286	11.5	50.6	1.1	4.6	0.09	0.41
E05-05	44.11391	68.92167	20.5	68.4	0.81	8.4	0.10	0.34
E05-06	44.11424	68.77984	17.9	63.7	0.64	10.0	0.10	0.35
E05-07	44.11404	68.75247	14	45.9	0.72	6.4	0.12	0.39
E05-08	44.11494	68.72430	8.88	14.2	3.5	0.4	0.06	0.09
E05-10	44.11748	68.67036	10.5	45.1	0.86	5.2	0.11	0.49
E05-11	44.11460	68.63982	6.84	40.6	0.77	5.3	0.16	0.96
E05-12	44.11406	68.61410	5.18	23.8	0.86	2.8	0.09	0.40
E05-13	44.11327	68.58661	7.97	35.5	0.8	4.4	0.10	0.42
E08-01	43.94913	69.28264	16.2	49.3	0.77	6.4	0.21	0.63
E08-02	43.9736	69.26436	16.2	52.9	0.77	6.9	0.16	0.54
E08-03	44.00802	69.22604	17.1	53.9	0.7	7.7	0.14	0.44
E08-04	44.03767	69.19842	19.5	55.3	1	5.5	0.16	0.46
E08-05	44.06709	69.18121	19.8	45.9	1.2	3.8	0.22	0.50

Appendix 6. Mean total Hg and methyl Hg concentrations (with sample sizes, standard deviations, and ranges) for *Nereis* worms sampled at sites in the Orrington-Bucksport (OB) and Estuary (ES) reaches, 2006, all sample times combined.

<i>Nereis</i> SAMPLE SITES (ordered north to south)	n	DRY WEIGHT (g) mean $\pm$ SD (min-max)	n	Total Hg (ng/g d.w.) mean $\pm$ SD (min-max)	n	Methyl Hg (ng/g d.w.) mean $\pm$ SD (min-max)	% MeHg mean $\pm$ SD (min-max)
OB4	3	0.078 $\pm$ 0.050 (0.042-0.135)	3	381 $\pm$ 148 (233-528)	3	214 $\pm$ 128 (119-359)	54 $\pm$ 13 (43-68)
OB2	3	0.048 $\pm$ 0.016 (0.034-0.066)	3	570 $\pm$ 270 (354-873)	1	31	4 (n = 1, THg = 873)
OB1	29	0.060 $\pm$ 0.051 (0.022-0.225)	29	321 $\pm$ 120 (90-479)	29	79 $\pm$ 49 (5-196)	23 $\pm$ 11 (3-51)
ES09	30	0.103 $\pm$ 0.092 (0.022-0.552)	20	467 $\pm$ 170 (279-974)	30	106 $\pm$ 67 (17-307)	30 $\pm$ 17 (7-63)
ES11	28	0.143 $\pm$ 0.074 (0.047-0.287)	28	440 $\pm$ 118 (253-602)	28	29 $\pm$ 22 (3-81)	7 $\pm$ 6 (1-25)
ES05	30	0.256 $\pm$ 0.221 (0.032-0.684)	30	342 $\pm$ 141 (168-607)	30	62 $\pm$ 35 (15-170)	21 $\pm$ 12 (6-56)
ES06	23	0.439 $\pm$ 0.247 (0.037-1.007)	23	260 $\pm$ 84 (139-473)	23	55 $\pm$ 53 (10-239)	20 $\pm$ 12 (5-51)
ES02	32	0.281 $\pm$ 0.228 (0.028-0.912)	32	352 $\pm$ 135 (170-654)	42	58 $\pm$ 30 (18-143)	20 $\pm$ 13 (4-57)
ES15	28	0.329 $\pm$ 0.715 (0.037-3.862)	28	181 $\pm$ 75 (73-400)	28	51 $\pm$ 19 (28-121)	32 $\pm$ 14 (13-58)
ES13	10	0.382 $\pm$ 0.319 (0.060-1.367)	10	382 $\pm$ 215 (218-906)	16	27 $\pm$ 18 (3-61)	5 $\pm$ 2 (0.7-7)
ES12	20	0.360 $\pm$ 0.545 (0.115-3.072)	20	264 $\pm$ 93 (139-431)	30	63 $\pm$ 46 (26-203)	29 $\pm$ 16 (6-58)
ES03	30	0.266 $\pm$ 0.203 (0.047-0.910)	30	201 $\pm$ 73 (92-429)	30	57 $\pm$ 26 (12-143)	30 $\pm$ 13 (9-59)
ES10	12	0.288 $\pm$ 0.265 (0.111-1.110)	12	106 $\pm$ 29 (50-139)	12	39 $\pm$ 18 (3-69)	39 $\pm$ 18 (2-63)
ES07	8	0.363 $\pm$ 0.329 (0.047-0.780)	0		8	29 $\pm$ 11 (17-50)	
ES04	3	0.288 $\pm$ 0.243 (0.056-0.540)	3	71 $\pm$ 27 (44-99)	3	16 $\pm$ 17 (4-35)	18 $\pm$ 15 (8-35)
ES14	30	0.267 $\pm$ 0.245 (0.043-1.089)	30	191 $\pm$ 66 (43-334)	30	45 $\pm$ 36 (5-135)	22 $\pm$ 14 (3-68)
ES01	22	0.612 $\pm$ 0.547 (0.179-2.347)	22	107 $\pm$ 29 (65-176)	22	25 $\pm$ 9 (10-49)	24 $\pm$ 9 (13-47)

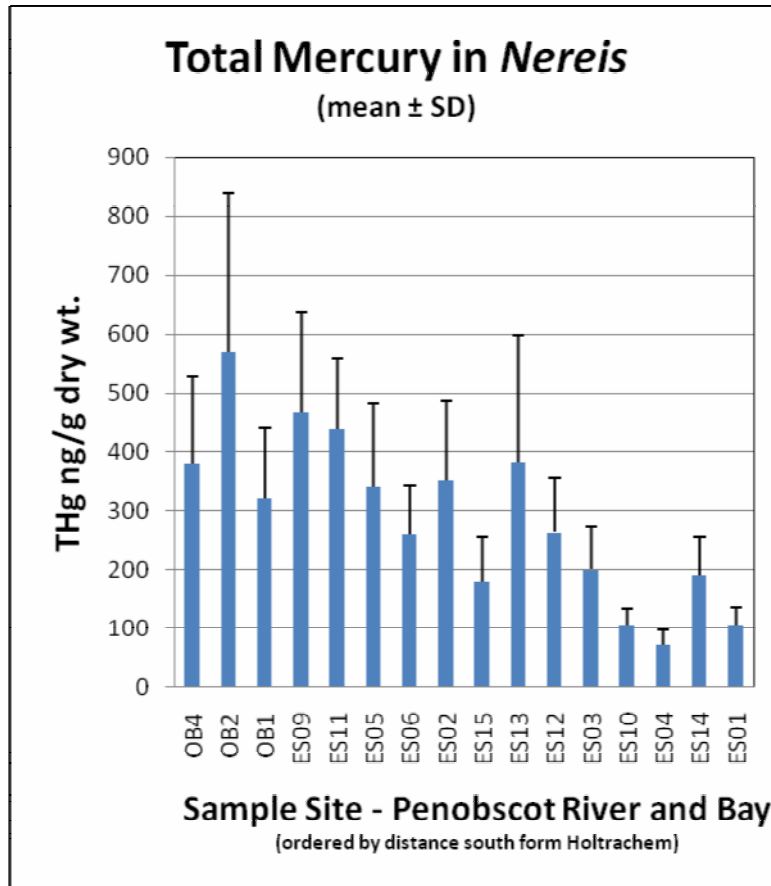
Appendix 7. Mean total Hg and methyl Hg concentrations (with sample sizes, standard deviations, and ranges) for *Nereis* worms captured in three different sample periods in 2006.

<i>Nereis</i> Sample Period	n	Total Hg (ng/g d.w.) mean $\pm$ SD (min-max)	n	Methyl Hg (ng/g d.w.) mean $\pm$ SD (min-max)
I	79	333 $\pm$ 173 (15-650)	105	72 $\pm$ 57 (4-307)
II	128	279 $\pm$ 141 (90-974)	128	48 $\pm$ 45 (3-359)
III	116	243 $\pm$ 143 (43-654)	132	53 $\pm$ 33 (9-170)



Appendix 8. Mean total weights of *Nereis* worms by sample site, 2006.





Appendix 9. Figure of mean total Hg in *Nereis* worms by sample site, 2006.

Appendix 10. Raw data for total and methyl Hg concentrations in *Macoma* clams sampled in 2006 in OB and ES sampling reaches.

<i>Macoma balthica</i> SAMPLE SITES (ordered north to south)	n	SHELL LENGTH (mm) mean $\pm$ SD (min-max)	Total Hg ng/g d.w. mean $\pm$ SD (min-max)	n	Methyl Hg ng/g d.w. mean $\pm$ SD (min-max)	% MeHg mean $\pm$ SD (min-max)
OB4	20	22.4 $\pm$ 3.8 (12-29)	1504 $\pm$ 743 (546-3570)	16	161 $\pm$ 49 (75-242)	13.7 $\pm$ 8.2 (3.2-32.6)
OB2	20	26.8 $\pm$ 3.7 (16-31)	2045 $\pm$ 904 (839-3890)	10	118 $\pm$ 62 (57-246)	5.1 $\pm$ 3.1 (1.9-11.2)
OB1	12	17.3 $\pm$ 4.6 (10-25)	1251 $\pm$ 609 (547-2260)	12	138 $\pm$ 75.2 (48-250)	13.0 $\pm$ 9.1 (2.6-34.0)
ES11	20	22.1 $\pm$ 5.1 (11-28)	1671 $\pm$ 1119 (460-4790)	20	58 $\pm$ 32 (9-163)	4.6 $\pm$ 4.1 (0.8-19.1)
ES05	15	23.5 $\pm$ 2.1 (20-27)	1397 $\pm$ 952 (456-3915)	5	151 $\pm$ 43 (118-224)	9.4 $\pm$ 5.7 (3.2-17.8)

Appendix 11. Raw data for total and methyl Hg concentrations in soft-shelled clams (*Mya arenaria*), 2006.

<i>Mya arenaria</i> SAMPLE SITES (ordered north to south)	n	LENGTH (mm) mean ± SD (min-max)	n	THg ng/g d.w. mean ± SD (min-max)	n	MeHg ng/g d.w. mean ± SD (min-max)	% MeHg mean ± SD (min-max)
ALL	139	44 ± 11 (23-79)	151	520 ± 261 (65-1,380)	150	329 ± 218 (25-1,270)	63 ± 20 (5-119)
OB-1			1	828	1	44	5
ES-09	1	57	1	824	1	784	95
ES-05	15	36 ± 9 (23-55)	17	646 ± 235 (312-1,380)	17	339 ± 85 (153-485)	55 ± 17 (20-82)
ES-06	22	41 ± 8 (26-60)	28	699 ± 235 (312-1,380)	28	430 ± 292 (112-1,270)	59 ± 25 (18-120)
ES-02	21	40 ± 14 (29-79)	21	846 ± 159 (548-1,190)	20	581 ± 207 (77-1,040)	68 ± 23 (11-100)
ES-15	19	40 ± 5 (33-54)	20	410 ± 43 (303-464)	20	260 ± 61 (89-331)	64 ± 15 (24-86)
ES-12	10	45 ± 16 (29-79)	10	426 ± 92 (257-566)	10	292 ± 90 (144-413)	68 ± 13 (42-88)
ES-03	18	46 ± 6 (35-57)	20	462 ± 112 (298-718)	20	323 ± 121 (133-588)	69 ± 16 (37-89)
ES-10	10	50 ± 7 (38-61)	10	81 ± 11 (65-99)	10	43 ± 11 (25-57)	54 ± 15 (31-74)
ES-07	2	39 ± 9 (32-45)	2	135 ± 5 (131-138)	2	66 ± 4 (63-69)	49 ± 1 (48-50)
ES-14	20	56 ± 10 (38-72)	20	280 ± 46 (144-360)	20	193 ± 61 (75-315)	68 ± 16 (52-110)
ES-01	1	40	1	126	1	26	21

Appendix 12. Raw data for total and methyl Hg in whole green crab (*Carcinus maenas*) sampled in 2006.

GREEN CRAB SAMPLE SITES (ordered north to south)	n	WEIGHT (g) mean $\pm$ SD (min-max)	estimated CARAPACE WIDTH (mm) mean $\pm$ SD (min-max)	estimated AGE (yr) mean $\pm$ SD (min-max)	Total Hg ng/g d.w. mean $\pm$ SD (min-max)	Methyl Hg ng/g d.w. mean $\pm$ SD (min-max)	% MeHg mean $\pm$ SD (min-max)
ALL	153	16 $\pm$ 21 (0.5-112)	36 $\pm$ 16 (13-81)	2.5 $\pm$ 1.0 (1 - 4-6)	216 $\pm$ 163 (50-857)	145 $\pm$ 128 (14-665)	66 $\pm$ 20 (19-108)
ES09	5	14 $\pm$ 7 (8-26)	39 $\pm$ 7 (32-49)	3.0 $\pm$ 0.7 (2 - 4-6)	406 $\pm$ 170 (300-707)	368 $\pm$ 132 (296-602)	92 $\pm$ 6 (85-101)
ES15	2	10 $\pm$ 12 (2-18)	32 $\pm$ 16 (21-44)	2.5 $\pm$ 0.7 (2-3)	207 $\pm$ 57 (166-247)	118 $\pm$ 111 (39-196)	51 $\pm$ 40 (24-79)
ES13	2	37 $\pm$ 41 (8-66)	50 $\pm$ 24 (33-68)	3.0 $\pm$ 1.4 (2 - 4-6)	442 $\pm$ 243 (270-613)	191 $\pm$ 75 (138-244)	56 $\pm$ 48 (23-90)
ES12	20	19 $\pm$ 22 (0.8-97)	39 $\pm$ 16 (15-77)	2.7 $\pm$ 1.1 (1 - 4-6)	409 $\pm$ 110 (260-728)	285 $\pm$ 135 (80-561)	68 $\pm$ 22 (23-107)
ES03	11	11 $\pm$ 7 (3-27)	35 $\pm$ 8 (24-50)	2.5 $\pm$ 0.7 (2 - 4-6)	314 $\pm$ 198 (143-857)	191 $\pm$ 146 (57-597)	59 $\pm$ 15 (37-79)
ES10	19	32 $\pm$ 32 (0.6-108)	46 $\pm$ 20 (14-80)	3.1 $\pm$ 1.1 (1 - 4-6)	187 $\pm$ 111 (86-513)	137 $\pm$ 94 (48-386)	72 $\pm$ 20 (39-108)
ES07	17	4 $\pm$ 4 (0.6-14)	24 $\pm$ 8 (13-40)	1.6 $\pm$ 0.6 (1-3)	73 $\pm$ 17 (54-118)	48 $\pm$ 21 (14-94)	64 $\pm$ 22 (26-106)
ES04	14	22 $\pm$ 21 (5-87)	43 $\pm$ 12 (27-74)	3.0 $\pm$ 0.8 (2 - 4-6)	136 $\pm$ 44 (81-221)	85 $\pm$ 40 (32-163)	62 $\pm$ 16 (19-81)
ES14	15	28 $\pm$ 29 (2-112)	46 $\pm$ 16 (21-81)	3.3 $\pm$ 0.8 (2 - 4-6)	356 $\pm$ 181 (183-759)	236 $\pm$ 154 (94-665)	65 $\pm$ 21 (38-96)
ES08	24	9 $\pm$ 17 (0.6-84)	29 $\pm$ 13 (14-73)	1.9 $\pm$ 0.9 (1 - 4-6)	89 $\pm$ 28 (50-175)	56 $\pm$ 21 (22-112)	64 $\pm$ 18 (27-94)
ES01	24	8 $\pm$ 8 (0.5-31)	29 $\pm$ 11 (13-52)	2.1 $\pm$ 0.9 (1 - 4-6)	163 $\pm$ 91 (67-426)	103 $\pm$ 54 (54-225)	67 $\pm$ 17 (29-91)

Appendix 13. Raw data for size, total and methyl Hg concentrations in rock crabs, 2006. Sample sites shown are the same as for lobsters collected in 2006 (See Phase I report).

Rock Crab SAMPLE SITES (ordered north to south)	SEX	n	CARAPACE WIDTH (g) mean $\pm$ SD (min-max)	Total Hg (ng/g, w.w.) mean $\pm$ SD (min-max)	THg (ng/g d.w.)	Methyl Hg (ng/g, w.w.) mean $\pm$ SD (min-max)	MeHg (ng/g d.w.)
ALL		89	103 $\pm$ 13 (75-136)	204 $\pm$ 193 (36-1340)	971	185 $\pm$ 194 (38-1400)	875
S 3 1	M	10	109 $\pm$ 8.2 (98-122)	273 $\pm$ 152 (111-172)	1,370	102 $\pm$ 56 (47-211)	502
S 1 10	M	9	114 $\pm$ 14 (94-136)	181 $\pm$ 131 (69-491)	904	172 $\pm$ 133 (65-492)	857
	F	1	135	507	2,140	494	2,090
S 1 1	M	4	97 $\pm$ 10 (82-107)	131 $\pm$ 50 (69-191)	583	126 $\pm$ 63 (62-212)	561
S 3 4	M	5	111 $\pm$ 10 (96-124)	111 $\pm$ 31 (77-159)	580	97 $\pm$ 27 (76-138)	508
S 1 2	M	5	95 $\pm$ 10 (85-106)	230 $\pm$ 152 (115-489)	985	196 $\pm$ 127 (101-140)	843
S 1 9	M	5	99 $\pm$ 12 (84-112)	150 $\pm$ 97 (46-258)	687	147 $\pm$ 94 (50-260)	672
	F	1	91	199	1,210	209	1270
S 1 3	M	7	94 $\pm$ 15 (77-120)	179 $\pm$ 121 (83-438)	725	165 $\pm$ 125 (68-435)	673
	F	1	97	1,340	5,790	1,400	6,060
S 1 4	M	3	92 $\pm$ 7 (86-99)	139 $\pm$ 53 (88-194)	685	114 $\pm$ 33 (79-143)	565
	F	6	86 $\pm$ 8 (76-97)	430 $\pm$ 284 (165-818)	2,448	405 $\pm$ 283 (152-816)	2,334
S 1 8	M	3	98 $\pm$ 5 (93-101)	108 $\pm$ 6 (105-115)	484	99 $\pm$ 7 (91-104)	438
	F	1	110	251	1,190	232	1,100
S 1 5	M	2	100 $\pm$ 4 (97-103)	103 $\pm$ 94 (36-169)	518	97 $\pm$ 83 (38-156)	492
	F	2	82 $\pm$ 10 (75-89)	74 $\pm$ 4 (71-77)	373	66 $\pm$ 4 (63-69)	332
S 1 7	F	1	93	133	524	122	481
S 3 3	M	7	111 $\pm$ 5 (102-115)	124 $\pm$ 59 (61-240)	611	102 $\pm$ 56 (47-211)	502
S 1 6	M	5	94 $\pm$ 3 (92-99)	113 $\pm$ 45 (60-184)	484	98 $\pm$ 30 (62-144)	420
S 3 2	M	10	112 $\pm$ 6 (105-122)	144 $\pm$ 45 (75-229)	702	105 $\pm$ 45 (56-180)	517
ES01		1	110	625	1,280	641	1,310

Appendix 14. Sampling locations for small fish in sampling reaches OB and ES, 2006. The original site name, the geographic ID originally given by Normandeau field staff, fishing method, amended site name and geographic coordinates are given.

2006 SITE NAME	2006 Normandeau lat/long ID	Fishing method	Amended fish site name (ordered N to S)	Latitude trawl N end	Longitude trawl N end
OB5	OB5 seine	Seine	OB-5N-SN	44 42 32.0	68 50 21.1
OB3	OB3 seine	Seine	OB-3X-SN	44 41 52.7	68 50 18.0
OB4	OB4 seine	Seine	OB-4N-SN	44 41 07.9	68 49 21.7
OB2	OB2 seine	Seine	OB-2E-SN	44 37 29.5	68 50 35.8
OB5	OB5 e	Trawl	OB-1N-TR5	44 36 30.6	68 51 00.1
OB4	OB4 e	Trawl	OB-1E-TR4	44 36 17.8	68 50 47.1
OB3	OB3 e	Trawl	OB-1E-TR3	44 36 12.8	68 50 25.6
OB1	OB1 seine	Seine	OB-1E-SN	44 36 08.4	68 50 47.5
OB2	OB2 s	Trawl	OB-1S-TR2	44 35 56.4	68 49 16.0
OB1	OB1 s	Trawl	OB-1S-TR1	44 35 34.0	68 49 00.6
ES09	es9 s	Trawl	ES-09E-TR	44 33 51.6	68 46 17.2
ES11	es11 s	Trawl	ES-11N-TR	44 33 30.7	68 46 05.2
ES02	es2 s	Trawl	ES-02E-TR	44 32 41.3	68 45 52.5
ES05	es5 s	Trawl	ES-05S-TR	44 32 04.7	68 45 32.4
ES5/6	es5/6	Trawl	ES-05S/06S-TR		
ES6	es5 e	Trawl	ES-06S-TR	44 31 53.3	68 45 12.4
ES15	es15 s	Trawl	ES-15S-TR	44 30 29.7	68 47 28.4
ES13	es-13 seine	Seine	ES-13X-SN	44 30 17.3	68 46 19.7
ES13	es13 s	Trawl	ES-13S-TR	44 29 54.5	68 46 52.9
ES14	es14 e	Trawl	ES-14N-TR	44 29 21.7	68 47 18.1
ES12	es12 s	Trawl	ES-12W-TR	44 29 18.2	68 48 27.2
ES03	es3 s	Trawl	ES-03W-TR	44 29 17.8	68 48 42.7
ES10	es10 s	Trawl	ES-10N-TR	44 28 05.4	68 52 04.2
ES04	es4 s	Trawl	ES-04W-TR	44 27 37.4	68 53 22.5
ES07	es7 s	Trawl	ES-07S-TR	44 26 52.0	68 54 46.3
ES08	es8 s	Trawl	ES-08E-TR	44 22 36.7	68 57 29.7
ES01	es1 e	Trawl	ES-01E-TR	44 21 41.0	68 51 33.6

Appendix 15. Raw data for size, total Hg and methyl Hg concentrations in tomcod muscle sampled in the Penobscot River and estuary in 2006. Note that sample locations have changed from those given in the Phase I report.

SAMPLE SITES (ordered north to south)	n	LENGTH (mm) mean $\pm$ SD (min-max)	WEIGHT (g) mean $\pm$ SD (min-max)	Total Hg (ng/g w.w.) mean $\pm$ SD (min-max)	n	Methyl Hg (ng/g w.w.) mean $\pm$ SD (min-max)	% MeHg mean $\pm$ SD (min-max)
BO3	2	112.5 $\pm$ 6.4 (108-117)	11.8 $\pm$ 1.6 (10.6-12.9)	249.5 $\pm$ 129.4 (158-341)	1	333	97.7
BO2	3	120.3 $\pm$ 8.4 (115-130)	13.6 $\pm$ 2.6 (10.9-16.1)	260.3 $\pm$ 51.4 (201-291)			
BO4	7	126.6 $\pm$ 6.3 (114-133)	16.8 $\pm$ 2.8 (11-19.3)	286.5 $\pm$ 45.0 (195-329)	1	242	124.1
OB-1N-TR5	10	154.3 $\pm$ 31.8 (105-204)	34.0 $\pm$ 21.8 (9.9-79.2)	196.7 $\pm$ 83.7 (81-372)	1	181	98.9
OB-1E-TR4	8	132 $\pm$ 25.3 (108-187)	21.2 $\pm$ 14.3 (9.3-54.5)	172.5 $\pm$ 39.7 (108-231)	1	199	101.5
OB-1E-TR3	10	157.1 $\pm$ 45.8 (109-256)	41.7 $\pm$ 42.5 (9.8-147.3)	217.1 $\pm$ 90.7 (130-416)	1	174	90.6
OB-1S-TR2	10	147.1 $\pm$ 26.6 (107-187)	29.0 $\pm$ 13.8 (10.7-54.5)	181.2 $\pm$ 57.0 (95-275)	1	312	113.5
OB-1S-TR1	10	141.7 $\pm$ 24.5 (121-185)	25.0 $\pm$ 13.9 (13.3-45.9)	160.7 $\pm$ 64.4 (103-334)	1	132	105.6
ES-09E-TR	19	137.4 $\pm$ 11.7 (117-165)	21.7 $\pm$ 6.0 (11.5-34.7)	121.4 $\pm$ 38.3 (66.5-226)	2	104 $\pm$ 12.7 (95-113)	95.9 $\pm$ 12.4 (87.1-104.6)
ES-11N-TR	17	142.9 $\pm$ 24.0 (108-199)	23.8 $\pm$ 11.8 (8.8-53.1)	137.0 $\pm$ 61.1 (56.2-296)	1	244.5	126
ES-02E-TR	17	151 $\pm$ 21.5 (109-198)	29.0 $\pm$ 11.0 (8.8-53.1)	147.7 $\pm$ 70.8 (46.8-288)	1	282	97.9
ES-05S-TR	5	129 $\pm$ 9.2 (121-140)	17.8 $\pm$ 4.1 (13.1-21.5)	114.3 $\pm$ 17.9 (92.2-141)	1	118	100
ES-05/06-TR	30	136.8 $\pm$ 14.9 (105-167)	22 $\pm$ 7.3 (10-40)	128.6 $\pm$ 40.7 (68.4-235)			
ES-06S-TR	5	144.2 $\pm$ 10.1 (137-161)	25.4 $\pm$ 6.5 (20.8-36.4)	133.2 $\pm$ 70.4 (78.1-256)	1	85	108.8
ES-13S-TR	7	131.6 $\pm$ 9.3 (118-145)	18.6 $\pm$ 5.1 (11.5-27.4)	113.3 $\pm$ 16.9 (85.4-139.5)	1	92	107.7

Appendix 16. Total and methyl Hg concentrations in the muscle of American eels sampled from the Penobscot River, 2007.

AMERICAN EEL SAMPLE SITES (ordered north to south)	n	LENGTH (mm) mean $\pm$ SD (min-max)	WEIGHT (g) mean $\pm$ SD (min-max)	Total Hg ng/g, w.w. mean $\pm$ SD (min-max)	n	Methyl Hg ng/g, w.w. mean $\pm$ SD (min-max)	n	AGE (yr) mean $\pm$ SD (min-max)	Latitude / Longitude (new sites only)
OV4	7	370 $\pm$ 127 (210-557)	142 $\pm$ 156 (13-433)	313 $\pm$ 207 (186-780)	1	210	6	8.2 $\pm$ 3.9 (4-14)	
OV	15	392 $\pm$ 99 (203-543)	139 $\pm$ 99 (15-341)	358 $\pm$ 185 (73-650)	1	349	10	9.3 $\pm$ 4.2 (4-16)	(sampled along entire reach)
OV5	2	422 $\pm$ 82 (364-480)	153 $\pm$ 100 (82-223)	427 $\pm$ 281 (228-626)	1	578	1	6	
BO4	18	246 $\pm$ 40 (180-335)	36 $\pm$ 22 (12-96)	621 $\pm$ 160 (391-974)	2	463 $\pm$ 55 (424-502)	14	6.1 $\pm$ 1.8 (4-11)	
BO67	20	274 $\pm$ 32 (204-340)	40 $\pm$ 13 (14-66)	633 $\pm$ 431 (193-2303)	2	1014 $\pm$ 504 (657-1370)	16	8.2 $\pm$ 2.7 (5-15)	N44 46.597 W68 46.876
BO66	20	292 $\pm$ 38 (254-430)	49 $\pm$ 23 (29-132)	466 $\pm$ 102 (275-679)	2	405 $\pm$ 167 (287-523)	16	8.9 $\pm$ 2.2 (6-13)	N44 46.401 W68 46.876
BO3	20	278 $\pm$ 31 (244-384)	43 $\pm$ 19 (29-111)	421 $\pm$ 128 (210-658)	2	371 $\pm$ 7 (366-376)	14	8.1 $\pm$ 1.8 (5-12)	
OB5	22	280 $\pm$ 30 (242-373)	38 $\pm$ 12 (24-77)	499 $\pm$ 239 (213-1130)	2	757 $\pm$ 146 (654-860)	18	6.5 $\pm$ 1.3 (5-10)	
OB3	10	310 $\pm$ 44 (239-380)	55 $\pm$ 22 (19-95)	654 $\pm$ 267 (342-1030)	1	544	9	7.3 $\pm$ 1.7 (5-9)	
OB73	4	262 $\pm$ 48 (193-298)	38 $\pm$ 19 (14-58)	595 $\pm$ 98 (489-706)	1	442	3	5.3 $\pm$ 0.6 (5-6)	N44 40.533 W68 49.052
OB1	10	269 $\pm$ 34 (229-338)	34 $\pm$ 14 (16-65)	567 $\pm$ 77 (491-691)	1	457	9	7.1 $\pm$ 1.3 (6-9)	



Appendix 17. Total and methyl Hg concentrations in banded killifish (*Fundulus*) sampled in the lower Penobscot River and at one site in Penobscot Bay. BO = Brewer – Orrington reach. OB = Orrington – Bucksport reach. ES = estuary.

BANDED KILLIFISH ( <i>Fundulus diaphanous</i> ) SAMPLE SITES (ordered north to south)	n	LENGTH (mm) mean $\pm$ SD (min-max)	WEIGHT (g) mean $\pm$ SD (min-max)	Total Hg (ng/g w.w.) mean $\pm$ SD (min-max)	n	Methyl Hg (ng/g w.w.) mean $\pm$ SD (min-max)	% MeHg mean $\pm$ SD (min-max)
BO3	5	80 $\pm$ 3 (75-84)	4.5 $\pm$ 0.7 (3.3-5.0)	248 $\pm$ 115 (144-434)	1	202	117
BO5	6	51 $\pm$ 5 (42-55)	0.9 $\pm$ 0.3 (0.4-1.3)	194 $\pm$ 84 (123-345)	1	130	106
BO1	5	88 $\pm$ 7 (78-94)	5.9 $\pm$ 1.5 (3.8-7.1)	300 $\pm$ 92 (232-438)	1	470	107
BO2	5	85 $\pm$ 3 (81-89)	5.2 $\pm$ 0.4 (4.7-5.6)	263 $\pm$ 33 (210-293)	1	289	99
BO4	5	60 $\pm$ 3 (57-64)	1.7 $\pm$ 0.2 (1.4-1.9)	138 $\pm$ 50 (78-206)	1	149	88
OB5	1	76	4.9	251	1	281	112
OB3	2	67 $\pm$ 17 (55-79)	3.8 $\pm$ 3.1 (1.6-6.0)	380 $\pm$ 25 (362-398)	1	398	110
OB4	2	64 $\pm$ 26 (45-82)	3.5 $\pm$ 3.8 (0.8-6.3)	310 $\pm$ 112 (230-389)	1	354	91
OB2	2	69 $\pm$ 15 (58-79)	4.3 $\pm$ 3.6 (1.8-6.8)	257 $\pm$ 54 (218-295)	1	313	106
OB1	2	82 $\pm$ 8 (76-88)	6.6 $\pm$ 2.1 (5.1-8.2)	421 $\pm$ 189 (287-554)	1	311	108

Appendix 18. Raw data for length, total and methyl Hg concentrations in rainbow smelt captured at Penobscot River and Bay sites, 2006.

RAINBOW SMELT SAMPLE SITES (ordered north to south)	n	LENGTH (mm) mean $\pm$ SD (min-max)	WEIGHT (g) mean $\pm$ SD (min-max)	n	Total Hg (ng/g w.w.) mean $\pm$ SD (min-max)	n	Methyl Hg (ng/g w.w.) mean $\pm$ SD (min-max)	% MeHg mean $\pm$ SD (min-max)
OB4	3		0.078 $\pm$ 0.050 (0.042-0.135)		381 $\pm$ 148 (233-528)	3	214 $\pm$ 128 (119-359)	54 $\pm$ 13 (43-68)
OB2	3		0.048 $\pm$ 0.016 (0.034-0.066)		570 $\pm$ 270 (354-873)	1	31	4 (n=1, THg=873)
OB1	29		0.060 $\pm$ 0.051 (0.022-0.225)		321 $\pm$ 120 (90-479)	29	79 $\pm$ 49 (5-196)	23 $\pm$ 11 (3-51)
OB-1S-TR1	7	35 $\pm$ 7 (26-46)	0.29 $\pm$ 0.18 (0.07-0.64)	7	94 $\pm$ 23 (69-138)	1	64	92
ES-09E-TR	10	64 $\pm$ 7 (51-75)	1.70 $\pm$ 0.66 (0.67-2.77)	10	83 $\pm$ 18 (51-119)	2	86 $\pm$ 0.7 (85-86)	102 $\pm$ 1.6 (101-104)
ES-11N-TR	10	60 $\pm$ 6 (47-71)	1.51 $\pm$ 0.56 (0.78-2.90)	10	85 $\pm$ 17 (62-116)	2	76 $\pm$ 8 (70-81)	81 $\pm$ 7 (76-87)
ES-02E-TR	10	73 $\pm$ 11 (54-92)	2.45 $\pm$ 1.12 (0.81-4.43)	10	80 $\pm$ 23 (52-125)	2	62 $\pm$ 18 (49-75)	89 $\pm$ 8 (83-95)
ES-05S-TR	6	65 $\pm$ 8 (55-76)	1.54 $\pm$ 0.70 (0.71-2.60)	10	106 $\pm$ 96 (43-373)	2	53 $\pm$ 30 (31-74)	68 $\pm$ 6 (64-73)
ES-06S-TR	5	61 $\pm$ 8 (47-66)	1.40 $\pm$ 0.53 (0.53-1.85)	10	125 $\pm$ 53 (68-245)	2	102 $\pm$ 54 (64-140)	79 $\pm$ 15 (68-90)
ES-13S-TR	9	62 $\pm$ 13 (47-93)	2.02 $\pm$ 1.60 (0.89-6.15)	10	75 $\pm$ 23 (41-126)	2	60 $\pm$ 7 (55-65)	95 $\pm$ 32 (73-117)
ES-14N-TR	9	88 $\pm$ 22 (51-111)	5.03 $\pm$ 2.95 (0.68-9.20)	10	75 $\pm$ 23 (43-118)	2	63 $\pm$ 31 (41-85)	76 $\pm$ 30 (54-97)
ES-10N-TR	10	91 $\pm$ 9 (76-103)	5.25 $\pm$ 5.12 (2.53-7.58)	10	54 $\pm$ 19 (34-95)	2	28 $\pm$ 11 (20-35)	65 $\pm$ 26 (47-83)
ES-07S-TR	8	83 $\pm$ 12 (64-100)	4.05 $\pm$ 1.74 (1.47-6.21)	10	51 $\pm$ 16 (34-80)	2	31 $\pm$ 8 (25-36)	72 $\pm$ 2 (71-74)
ES-04W-TR	10	90 $\pm$ 8 (82-105)	5.12 $\pm$ 1.39 (3.78-7.73)	10	64 $\pm$ 22 (41-101)	2	49 $\pm$ 23 (32-65)	59 $\pm$ 7 (54-64)

## Appendix 19. Raw data for size, total and methyl Hg in winter flounder, 2006.

WINTER FLOUNDER SAMPLE SITES (ordered north to south)	n	LENGTH (mm) mean $\pm$ SD (min-max)	WEIGHT (g) mean $\pm$ SD (min-max)	Total Hg (ng/g w.w.) mean $\pm$ SD (min-max)	n	Methyl Hg (ng/g w.w.) mean $\pm$ SD (min-max)	% MeHg mean $\pm$ SD (min-max)
Penobscot Bay							
ES15S-TR	10	52.7 $\pm$ 7.0 (45-66)	2.9 $\pm$ 1.2 (2-5)	26.1 $\pm$ 6.1 (20-36)	2	21.5 $\pm$ 0.7 (21-22)	103 $\pm$ 12 (95-112)
ES13S-TR	10	76.4 $\pm$ 15.7 (54-105)	8.9 $\pm$ 4.8 (3-18)	60.4 $\pm$ 40.2 (20-141)	2	81.5 $\pm$ 44.5 (50-113)	81 $\pm$ 0.8 (80-81)
ES14N-TR	18	59.9 $\pm$ 15.8 (39-95)	5.0 $\pm$ 4.5 (1-17)	35.9 $\pm$ 16.6 (20-75)	2	41.5 $\pm$ 10.6 (34-49)	83 $\pm$ 9 (76-88)
ES12W-TR	10	57.0 $\pm$ 7.0 (48-66)	3.4 $\pm$ 1.4 (2-6)	25.8 $\pm$ 3.3 (19-38)	2	24.5 $\pm$ 0.7 (24-25)	93 $\pm$ 4 (90-96)
ES03W-TR	10	69.3 $\pm$ 14.5 (57-105)	6.7 $\pm$ 4.7 (3-19)	29.3 $\pm$ 13.2 (18-64)	2	33.5 $\pm$ 14.8 (23-44)	80 $\pm$ 15 (69-90)
ES04W-TR	10	50.2 $\pm$ 7.1 (40-57)	2.4 $\pm$ 1.1 (1-4)	15.1 $\pm$ 3.4 (11-21)	2	11.0 $\pm$ 4.2 (8-14)	75 $\pm$ 18 (63-88)
ES07S-TR	9	65.7 $\pm$ 15.6 (53-105)	6.4 $\pm$ 6.0 (3-22)	24.7 $\pm$ 8.0 (16-40)	1	11	52
ES08E-TR	10	59.8 $\pm$ 9.1 (42-71)	4.4 $\pm$ 2.1 (1-8)	18.1 $\pm$ 2.6 (15-23)	2	14.5 $\pm$ 0.7 (14-15)	74 $\pm$ 13 (64-83)
Downeast Coast							
Frenchman Bay	15	162 $\pm$ 62.7 (60-270)	74.5 $\pm$ 74.7 (3-256)	11.06 $\pm$ 2.05 <sup>1</sup> (9-16)			
Schoodic Point	14	199 $\pm$ 69.2 (100-300)	141 $\pm$ 153.2 (13-461)	13.15 $\pm$ 6.1 <sup>1</sup> (8-25)			

<sup>1</sup>25% THg increase, whole fish to muscle

Appendix 20. Total Hg concentrations and methyl Hg concentrations for golden shiner (*Notemigonus crysoleucas*) collected in the lower Penobscot River, 2006.

GOLDEN SHINER ( <i>Notemigonus c.</i> ) SAMPLE SITES (ordered north to south)	n	LENGTH (mm) mean $\pm$ SD (min-max)	WEIGHT (g) mean $\pm$ SD (min-max)	Total Hg (ng/g w.w.) mean $\pm$ SD (min-max)	n	Methyl Hg (ng/g w.w.) mean $\pm$ SD (min-max)	% MeHg mean $\pm$ SD (min-max)
BO3	3	112 $\pm$ 8 (105-120)	14 $\pm$ 2 (11-15)	207 $\pm$ 66 (132-257)	1	145	63
BO5	1			92	1	65	71
BO1	2	129	19 $\pm$ 4 (16-22)	266 $\pm$ 26 (247-284)	1	246	99
BO2	2	122 $\pm$ 3 (120-124)	19 $\pm$ 1 (19-20)	264 $\pm$ 25 (246-282)	1	282	100
BO4	3	118 $\pm$ 26 (89-140)	16 $\pm$ 9 (6-24)	229 $\pm$ 8 (220-235)	1	229	104
OB5	1	114	15	159	1	123	77
OB3/5	2			208 $\pm$ 6 (203-212)	1	203	100
OB3	1	119	19	203	1	202	100
OB4	1	119	15	275	1	248	90
OB2	2	69 $\pm$ 18 (56-82)	5 $\pm$ 4 (2-7)	207 $\pm$ 0 (207-207)	1	179	86
OB1	2	125 $\pm$ 11 (117-133)	20 $\pm$ 6 (16-25)	265 $\pm$ 73 (213-316)	1	266	84

Appendix 21. Total Hg ( $\mu\text{g/g w.w.}$ ) in the eggs of double-crested cormorants sampled in the Penobscot River and estuary in 2007.

DOUBLE-CRESTED CORMORANT SAMPLE SITES (ordered north to south)	n	Total Hg ( $\mu\text{g/g w.w.}$ ) mean $\pm$ SD (min-max)	LATITUDE / LONGITUDE
Luce Cove	4	0.679 $\pm$ 0.075 (0.626-0.790)	44.59084 68.81402
Sandy Point	12	0.348 $\pm$ 0.138 (0.225-0.664)	44.5051 68.8039
Fort Point	1	0.59	44.46107 68.81004
Stockton Old Pier	2	0.344 $\pm$ 0.071 (0.294-0.394)	44.45329 68.86860
Castine Harbor	13	0.285 $\pm$ 0.104 (0.166-0.471)	44.38150 68.79784
Thrumcap	12	0.337 $\pm$ 0.072 (0.223-0.471)	44.32098 68.75818
Flat Island	12	0.289 $\pm$ 0.076 (0.188-0.483)	44.317725 68.932970
Spoon Ledge	7	0.286 $\pm$ 0.051 (0.230-0.349)	44.200737 68.828108
E. Goose Rock	13	0.346 $\pm$ 0.137 (0.161-0.677)	44.183447 68.979321
Robinson Rock	12	0.348 $\pm$ 0.147 (0.193-0.685)	44.160446 68.978167
Green Island	18	0.252 $\pm$ 0.084 (0.137-0.435)	44.0675 68.9205

Appendix 22. Total Hg ( $\mu\text{g/g}$  w.w.) in the blood and eggs of black guillemots sampled in the Penobscot estuary in 2007.

Black Guillemot SAMPLE SITES (ordered north to south)	AGE	n	TAIL LENGTH (mm) mean $\pm$ SD (min-max)	WEIGHT (g) mean $\pm$ SD (min-max)	BILL LENGTH (mm) mean $\pm$ SD (min-max)	BLOOD THg ( $\mu\text{g/g}$ , w.w.) mean $\pm$ SD (min-max)	EGG n	EGG THg ( $\text{ng/g}$ , w.w.) mean $\pm$ SD (min-max)	latitude longitude
Pond Island	HY	3	37.7 $\pm$ 4.6 (35-43)	346.7 $\pm$ 32.1 (310-370)	17.6 $\pm$ 12.8 (2.8-25.2)	0.283 $\pm$ 0.080 (0.222-0.373)	1	1.182	44.294737 68.812130
Western Island	AHY	12	49.5 $\pm$ 3.4 (45-53)	369.2 $\pm$ 11.6 (350-390)	29.8 $\pm$ 2.9 (24.4-32.2)	1.338 $\pm$ 0.239 (1.018-1.799)	2	0.743 $\pm$ 0.113 (0.663-0.823)	44.292452 68.823985
	HY	3	15.0 $\pm$ 0.0 (15-15)	196.7 $\pm$ 11.5 (190-210)	19.7 $\pm$ 0.2 (19.6-19.9)	0.184 $\pm$ 0.041 (0.143-0.224)			
Compass Island							2	0.777 $\pm$ 0.399 (0.495-1.059)	44.210983 68.864415
Mouse Island	AHY	4	47.0 $\pm$ 2.9 (44-50)	372.5 $\pm$ 29.9 (330-400)	30.5 $\pm$ 0.9 (29.2-31.0)	1.296 $\pm$ 0.292 (0.894-1.585)			44.198727 68.942209
	HY	7	36.7 $\pm$ 4.1 (31-42)	341.4 $\pm$ 16.8 (320-360)	24.5 $\pm$ 1.4 (23.0-26.9)	0.280 $\pm$ 0.05 (0.217-0.345)			
East Goose Rock							2	0.780 $\pm$ 0.186 (0.649-0.912)	44.183447 68.979321
Robinson Rock							2	0.913 $\pm$ 0.070 (0.864-0.963)	44.160446 -68.978167
Ram Island							1	0.482	44.151195 -68.386280

Appendix 23. Total Hg concentrations ( $\mu\text{g/g}$  w.w.) in the blood of Nelson's sharp-tailed sparrows in Mendall Marsh (Penobscot River) and reference areas in Maine. NWR = National Wildlife Refuge.

Nelson's Sharp-tailed sparrow SAMPLE SITES (ordered north to south)	Age Class	n	TAIL LENGTH (mm) mean $\pm$ SD (min-max)	WEIGHT (g) mean $\pm$ SD (min-max)	BLOOD THg ug/g, w.w. mean $\pm$ SD (min-max)	n	FEATHER THg ug/g, w.w. mean $\pm$ SD (min-max)	lat /lon (new sites only)
Mendall Marsh, Penobscot River	AHY <sup>1</sup>	78	47.9 $\pm$ 1.8 (44-51)	17.4 $\pm$ 1.3 (13.8-20.8)	7.1 $\pm$ 2.2 (0.3-13.5)	75	3.0 $\pm$ 4.6 (0.5-30.2)	
	HY <sup>2</sup>	3	41.0 $\pm$ 7.6 (34-49)	16.6 $\pm$ 0.8 (16.0-17.5)	2.4 $\pm$ 0.4 (2.0-2.8)	3	36.0 $\pm$ 5.4 (30.2-40.8)	
Scarborough Marsh, Southern Maine	AHY	5	46.8 $\pm$ 1.0 (46-48)	17.2 $\pm$ 0.9 (15.8-18.2)	0.3 $\pm$ 0.07 (0.2-0.4)			43.56687 -70.35990
Rachel Carson NWR, Southern Maine	AHY	4	45.0 $\pm$ 1.6 (43-47)	17.1 $\pm$ 1.4 (15.0-18.2)	0.4 $\pm$ 0.1 (0.3-0.5)			43.28255 -70.58126
Parker River NWR, Massachusetts	AHY	1	50	17.9	1.2			42.75551 -70.83244

<sup>1</sup> AHY = after hatch year, adult

<sup>2</sup> HY = hatch year, chick/juvenile

Appendix 24. Mercury concentrations in the blood of song sparrows ( $\mu\text{g/g}$  w.w.) sampled at various sites in 2007. Costigan Boat Launch and Greenbush are on the Penobscot River, upstream of Orono and are therefore outside of any direct aquatic influence of HoltraChem. Other sites are on the Penobscot River or estuary, downstream of HoltraChem.

Song sparrow SAMPLE SITES (ordered north to south)	n	TAIL LENGTH (mm) mean $\pm$ SD (min-max)	WEIGHT (g) mean $\pm$ SD (min-max)	BILL LENGTH (mm) mean $\pm$ SD (min-max)	BLOOD THg ug/g, w.w. mean $\pm$ SD (min-max)	FEATHER THg ug/g, w.w. mean $\pm$ SD (min-max)	latitude longitude
Greenbush	8	63.6 $\pm$ 3.3 (60-69)	21.9 $\pm$ 1.5 (20-24)	11.5 $\pm$ 1.1 (10-13)	0.137 $\pm$ 0.06 (0.07-0.26)	3.2 $\pm$ 1.8 (0.61-5.74)	45.03128 -68.65236
Costigan Boat Launch	2	66 $\pm$ 1.4 (65-67)	20.9 $\pm$ 1.1 (20-22)	12.2 $\pm$ 0.3 (12-12.4)	0.115 $\pm$ 0.07 (0.07-0.16)	0.71 $\pm$ 0.31 (0.49-0.93)	45.01339 -68.64085
Winterport North	17	63.2 $\pm$ 2.3 (59-68)	20.1 $\pm$ 1.1 (18.7-22.5)	11.8 $\pm$ 0.6 (10.8-12.8)	0.09 $\pm$ 0.08 (0.02-0.28)	1.35 $\pm$ 1.44 (0.11-5.76)	44.69685 -68.84542
Mendall Marsh	11	61.9 $\pm$ 4.8 (55-69)	19.5 $\pm$ 2.1 (15.6-22.5)	11.5 $\pm$ 1.2 (9.8-13.1)	1.7 $\pm$ 1.4 (0.02-3.97)	11.6 $\pm$ 7.6 (0.45-22.55)	various
Sandy Point	3	68 $\pm$ 3 (65-71)	20.9 $\pm$ 0.2 (20.8-21.1)	12.7 $\pm$ 0.3 (12.4-13.0)	0.21 $\pm$ 0.09 (0.13-0.30)	0.75 $\pm$ 0.60 (0.23-1.41)	44.50851 -68.80927
Amazon Stream	9	63.6 $\pm$ 3.8 (56-68)	20.5 $\pm$ 1.3 (18.6-22.5)	11.7 $\pm$ 1.2 (10.2-13.5)	0.18 $\pm$ 0.09 (0.06-0.30)	1.95 $\pm$ 1.2 (0.70-3.95)	44.49958 -68.81485
Hatch Cove	8	62.1 $\pm$ 3.0 (59-67)	21.7 $\pm$ 1.7 (19.8-24.5)	12.4 $\pm$ 0.5 (11.7-13.3)	0.11 $\pm$ 0.06 (0.04-0.18)	0.58 $\pm$ 0.37 (0.11-1.27)	44.39686 -68.80357
Smith Cove	5	62.2 $\pm$ 3.8 (58-68)	22.1 $\pm$ 1.03 (21.0-23.6)		0.18 $\pm$ 0.11 (0.04-0.31)	0.65 $\pm$ 0.38 (0.20-1.21)	44.37066 -68.78630
Holbrook Island, Sandy Marsh	6	63.9 $\pm$ 2.2 (60-65)	19.9 $\pm$ 1.2 (18.4-21.3)	11.8 $\pm$ 1.3 (10.2-13.7)	0.33 $\pm$ 0.29 (0.08-0.87)	1.92 $\pm$ 0.88 (0.47-2.93)	44.36585 -68.81246
Bald Island	7		22.1 $\pm$ 2.3 (19.5-25.5)		0.016 $\pm$ 0.012 (0.005-0.040)		44.18987 -68.78407



Appendix 25. Total Hg concentrations in the blood and feathers of swamp sparrows ( $\mu\text{g/g w.w.}$ ) sampled at various sites in 2007. Costigan Boat Launch and Greenbush are on the Penobscot River, upstream of Orono and are therefore outside of any direct aquatic influence of HoltraChem. Other sites are on the Penobscot River or estuary, downstream of HoltraChem.

SWAMP SPARROW SAMPLE SITES (ordered north to south)	n	TAIL LENGTH (mm) mean $\pm$ SD (range)	WEIGHT (g) mean $\pm$ SD (range)	BILL LENGTH (mm) mean $\pm$ SD (range)	BLOOD Total Hg ( $\mu\text{g/g w.w.}$ ) mean $\pm$ SD (range)	FEATHER Total Hg ( $\mu\text{g/g w.w.}$ ) mean $\pm$ SD (range)	LOCATION Latitude / Longitude
Passadumkeag	5	57.2 $\pm$ 2.2 (55-60)	16.0 $\pm$ 0.9 (14.9-17.3)	10.7 $\pm$ 0.7 (9.5-11.4)	0.49 $\pm$ 0.48 (0.10-1.31)	8.62 $\pm$ 7.73 (2.97-21.79)	45.13772 68.61208
Greenbush	7	55 $\pm$ 1.5 (53-57)	17.5 $\pm$ 1.6 (15.3-19.3)	9.3 $\pm$ 0.4 (9.0-9.5)	0.18 $\pm$ 0.17 (0.05-0.53)	6.03 $\pm$ 2.78 (1.77-9.11)	45.03128 68.65236
Costigan Boat Launch	1	59	17.2	10.2	0.39	13.4	45.01339 68.64085
Winterport North	3	58.7 (55-62)	16.5 $\pm$ 1.7 (14.9-18.2)	10.43 $\pm$ 0.42 (10.1-10.9)	0.37 $\pm$ 0.25 (0.14-0.62)	5.61 $\pm$ 2.11 (3.30-7.42)	44.69685 68.84542
Mendall Marsh	24	55.0 $\pm$ 2.7 (50-60)	16.9 $\pm$ 1.4 (13.4-19.1)	11.3 $\pm$ 0.5 (10.1-12.3)	2.39 $\pm$ 1.06 (0.76-4.64)	7.25 $\pm$ 4.45 (0.77-15.93)	various
Amazon Stream	1	60	15.6	10.5	0.41	4.81	44.49958 68.81485
Hatch Cove	2	55.0 $\pm$ 2.8 (53-57)	16.2 $\pm$ 0.6 (15.7-16.6)	10.7 $\pm$ 0.5 (10.3-11.0)	0.51 $\pm$ 0.50 (0.16-0.87)	4.98 $\pm$ 2.04 (3.54-6.42)	44.39686 68.80357

Appendix 26. Raw data for mercury concentrations in red-winged blackbirds sampled in Mendall Marsh in 2007. AHY = After hatch year, HY = Hatch year.

RED-WINGED BLACKBIRD SAMPLE SITES (ordered north to south)	AGE CLASS	n	TAIL LENGTH (mm) mean $\pm$ SD (min-max)	WEIGHT (g) mean $\pm$ SD (min-max)	BILL LENGTH (mm) mean $\pm$ SD (min-max)	BLOOD Total Hg ( $\mu\text{g/g}$ w.w.) mean $\pm$ SD (min-max)	FEATHER Total Hg ( $\mu\text{g/g}$ w.w.) mean $\pm$ SD (min-max)	LATITUDE / LONGITUDE
North Mendall Marsh	AHY	3	71.0 $\pm$ 7.6 (63-78)	34.9 $\pm$ 7.3 (26.4-39.4)	18.2 $\pm$ 0.3 (18.0-18.6)	7.9 $\pm$ 4.8 (4.7-13.4)	11.283 $\pm$ 16.0 (1.1-29.8)	44.5882 68.8577
Central Mendall Marsh	HY	7	71.4 $\pm$ 15.3 (40-82)	49.0 $\pm$ 8.1 (38.2-57.0)	18.7 $\pm$ 2.3 (15.8-22.0)	2.9 $\pm$ 1.9 (1.2-7.0)	15.0 $\pm$ 12.5 (2.6-34.1)	44.5814 68.8592
	AHY	10	79.6 $\pm$ 8.9 (68-91)	52.8 $\pm$ 11.7 (38.0-67.2)	20.9 $\pm$ 2.0 (18.0-23.2)	5.7 $\pm$ 3.9 (0.42-11.9)	1.5 $\pm$ 1.4 (0.16-4.9)	
South Mendall Marsh (Prospect)	HY	2	80.5 $\pm$ 6.4 (76-85)	54.7 $\pm$ 5.1 (51.1-58.3)	18.6 $\pm$ 0.07 (18.5-18.6)	0.51 $\pm$ 0.04 (0.49-0.54)	3.0 $\pm$ 0.3 (2.8-3.3)	44.5560 68.8587
	AHY	13	84.2 $\pm$ 6.0 (66-91)	60.1 $\pm$ 7.5 (37.3-66.5)	21.7 $\pm$ 1.6 (17.9-22.9)	2.4 $\pm$ 2.2 (0.19-6.5)	3.7 $\pm$ 10.6 (0.20-38.9)	

Appendix 27. Total mercury concentrations in the blood of Virginia rail ( $\mu\text{g/g}$  w.w.) sampled at Mendall Marsh and a reference site (Scarborough Marsh) in 2007.

Virginia Rail SAMPLE SITES (ordered north to south)	n	AGE CLASS	TAIL LENGTH (mm) mean $\pm$ SD (min-max)	WEIGHT (g) mean $\pm$ SD (min-max)	BILL LENGTH (mm) mean $\pm$ SD (min-max)	BLOOD THg ug/g, w.w. mean $\pm$ SD (min-max)	FEATHER THg ug/g, w.w. mean $\pm$ SD (min-max)	latitude longitude
Mendall Marsh North	4	AHY	41.8 $\pm$ 1.3 (40-43)	85.0 $\pm$ 10.2 (71.3-93.6)	39.7 $\pm$ 1.2 (38-41)	2.04 $\pm$ 1.52 (0.61-4.19)	17.4 $\pm$ 8.9 (11.8-30.9)	44 35.636 <sup>1</sup> -68 51.798
Mendall Marsh Central	6	AHY	41.8 $\pm$ 2.9 (38-45)	91.5 $\pm$ 11.7 (75.9-106.6)	39.0 $\pm$ 3.7 (32.9-44.0)	2.8 $\pm$ 1.5 (1.2-4.6)	26.2 $\pm$ 20.7 (1.8-58.7)	44 34.910 -68 51.625
	5	HY		69.2 $\pm$ 10.7 (53.3-83.3)	31.4 $\pm$ 3.6 (28.1-37.4)	1.39 $\pm$ 0.25 (1.07-1.66)	49.2 $\pm$ 7.4 (43.3-57.5)	
Mendall Marsh South	3	AHY	41.0 $\pm$ 3.6 (37-44)	101.2 $\pm$ 8.2 (91.7-106.2)	36.9 $\pm$ 4.5 (33.0-41.8)	0.44 $\pm$ 0.31 (0.15-0.76)	19.3 $\pm$ 22.4 (4.1-45.0)	44 33.350 -68 51.581
Scarborough Marsh	2	AHY	44.5 $\pm$ 3.5 (42-47)	100.6 $\pm$ 3.5 (98.1-103.1)	41.5 $\pm$ 2.1 (40.0-42.9)	0.14 $\pm$ 0.06 (0.09-0.18)	1.28 $\pm$ 0.67 (0.81-1.75)	44 34.579 -70 22.625

<sup>1</sup>In Mendall Marsh, lat/long is a central location of 2 - 5 sample sites

Appendix 28. Total Hg concentrations in the blood and feathers of passerine and shore birds sampled at Mendall Marsh and other sites, 2007.

PASSERINE and SHOREBIRD SPECIES	SAMPLE SITES (ordered north to south)	n	BLOOD Total Hg $\mu\text{g/g}$ , w.w. mean $\pm$ SD (min-max)	FEATHER Total Hg $\mu\text{g/g}$ , w.w. mean $\pm$ SD (min-max)	LATITUDE / LONGITUDE
Common yellowthroat	Mendall Marsh	3	$1.3 \pm 0.37$ (1.04-1.72)	$2.17 \pm 1.74$ (0.70-4.09)	various
	Sandy Point	1	0.07	1.03	44.50851 68.80927
	Amazon Stream	1	0.06	0.93	44.49958 68.81485
Marsh wren	Mendall Marsh	8	$2.31 \pm 0.96$ (1.53-4.33)	$6.30 \pm 6.24$ (1.19-20.3)	
	Holbrook Island, Sandy Marsh	2	$0.18 \pm 0.13$ (0.09-0.27)	$4.17 \pm 0.31$ (3.95-4.39)	44.36585 68.81246
Killdeer	Mendall Marsh	1	0.56	2.48	
Savannah Sparrow	Mendall Marsh	17	$3.15 \pm 1.90$ (0.31-6.40)	$11.48 \pm 9.30$ (0.33-34.45)	
Veery	Mendall Marsh	2	$0.07 \pm 0.04$ (0.05-0.10)	$1.08 \pm 0.30$ (0.87-1.29)	
Wilson's snipe	Mendall Marsh	1	1.83	13.75	
American goldfinch	Amazon Stream	2	$0.003 \pm 0.001$ (0.002-0.003)	$0.05 \pm 0.02$ (0.04-0.06)	

Appendix 29. Raw data for total Hg concentrations in the blood of belted kingfisher chicks and adults and in adult feathers sampled at various sites in the Penobscot basin, 2007. Age refers to adults (AHY = after hatch year) and chicks (HY = hatch year).

BELTED KINGFISHER SAMPLE SITES (ordered north to south)	AGE	n	WEIGHT (g) mean $\pm$ SD (min-max)	BILL LENGTH (mm) mean $\pm$ SD (min-max)	BLOOD Total Hg $\mu$ g/g, w.w. mean $\pm$ SD (min-max)	FEATHER Total Hg $\mu$ g/g, w.w. mean $\pm$ SD (min-max)	LATITUDE / LONGITUDE
Macc's Burrow E Branch Penobscot River	AHY	1			0.079	5.53	45.90397 68.61474
	HY	1	157.2	42.2	0.130		
Boat Launch E Branch Penobscot River	HY	7	158.7 $\pm$ 6.8 (151.2-168.5)	36.4 $\pm$ 1.4 (34.4-38.6)	0.092 $\pm$ 0.019 (0.068-0.120)		45.88661 68.61627
	HY	5	170.4 $\pm$ 11.1 (160.2-185.5)	39.5 $\pm$ 1.1 (37.9-40.8)	0.143 $\pm$ 0.010 (0.127-0.153)		45.80751 68.60043
EBPR 5 E Branch Penobscot River	AHY	2			0.847 $\pm$ 0.087 (0.785-0.908)	7.89 $\pm$ 3.48 (5.43-10.35)	45.80075 68.59634
	HY	7	163.2 $\pm$ 6.8 (153.5-174.2)	41.1 $\pm$ 1.3 (39.9-43.7)	0.140 $\pm$ 0.041 (0.102-0.204)		
Snow Rover E Branch Penobscot River	AHY	1			4.71	4.61	45.65132 68.55299
	HY	6	142.1 $\pm$ 8.5 (132.4-154.4)	41.6 $\pm$ 1.2 (39.2-42.6)	0.287 $\pm$ 0.079 (0.180-0.386)		
TRIMM Penobscot River Eddington	HY	2	154.6 $\pm$ 11.7 (146.4-162.9)	36.7 $\pm$ 0.4 (36.4-36.9)	0.126 $\pm$ 0.006 (0.121-0.130)		44.84380 68.69444
Ferry Road Penobscot River Orrington	AHY	2			1.180 $\pm$ 0.057 (1.140-1.220)	11.28 $\pm$ 9.74 (4.39-18.17)	44.73653 68.82908
	HY	7	164.5 $\pm$ 8.4 (153.9-178.5)	40.8 $\pm$ 2.0 (38.0-44.3)	0.042 $\pm$ 0.016 (0.024-0.072)		
Mendall Marsh	AHY	1			0.359	5.87	44.57509 68.86204
	HY	6	153.7 $\pm$ 11.4 (138.4-170.9)	40.2 $\pm$ 1.4 (38.0-42.0)	0.097 $\pm$ 0.011 (0.082-0.115)		
Aunt Mollie's Island	AHY	2			0.901 $\pm$ 0.086 (0.841-0.962)	9.27* *n = 1	44.44940 68.72515
Rt. 46 Gravel Pit	AHY	1			2.42	3.73	44.61392 68.72295
	HY	5	167.5 $\pm$ 14.8 (151.8-188.5)	38.9 $\pm$ 0.6 (37.8-39.4)	0.274 $\pm$ 0.056 (0.217-0.360)		

Appendix 30. Raw data for mercury concentrations in osprey (*Pandion haliaetus*) chick and adult blood and feathers.

OSPREY SAMPLE SITES (ordered north to south)		AGE	n	WEIGHT (g) mean ± SD (min-max)	CULMEN LENGTH (mm) mean ± SD (min-max)	BLOOD Total Hg µg/g, w.w. mean ± SD (min-max)	FEATHER Total Hg µg/g, w.w. mean ± SD (min-max)
REGION	SITE						
Penobscot	Penobscot River (OB)	CHICK	7	1392 ± 98 (1275-1525)	28.1 ± 1.4 (26.4-30.4)	0.11 ± 0.02 (0.07-0.13)	2.46 ± 0.35 (2.06-2.88)
	Upper Penobscot Bay	CHICK	12	1487 ± 175 (1225-1825)	27.3 ± 1.8 (24.2-30.5)	0.08 ± 0.02 (0.05-0.11)	1.92 ± 0.35 (1.38-2.44)
	Lower Penobscot Bay	CHICK	7	1425 ± 151 (1200-1600)	28.1 ± 2.5 (24.5-30.7)	0.04 ± 0.01 (0.02-0.05)	1.12 ± 0.21 (0.95-1.57)
Southern Maine	Sheepscot River	CHICK	13	1306 ± 205 (1000-1650)	27.2 ± 1.9 (23.6-30.1)	0.21 ± 0.14 (0.07-0.60)	4.02 ± 0.77 (2.26-5.42)
	Fore River, Portland	CHICK	3	1283 ± 306 (950-1550)	29.3 ± 2.4 (27.2-31.9)	0.11 ± 0.09 (0.06-0.21)	2.13 ± 0.28 (1.83-2.39)
	Harpowell	CHICK	6	1317 ± 175 (1100-1550)	27.2 ± 2.8 (24.5-32.7)	0.09 ± 0.02 (0.05-0.13)	2.17 ± 0.24 (1.85-2.41)
Penobscot	Penobscot River (OB)	ADULT	2	1725 ± 71 (1675-1775)	33.8 ± 0.6 (33.4-34.2)	1.93 ± 0.71 (1.43-2.43)	36.65 ± 11.81 (28.30-45.00)
	Upper Penobscot Bay	ADULT	4	1694 ± 307 (1250-1950)	34.1 ± 2.3 (31.7-37.0)	1.04 ± 0.20 (0.89-1.33)	17.04 ± 13.99 (1.77-33.70)
	Lower Penobscot Bay	ADULT	4	1658 ± 184 (1450-1800)	34.0 ± 1.5 (32.8-36.0)	1.00 ± 0.90 (0.19-2.21)	22.76 ± 24.28 (8.07-58.90)
Southern Maine	Sheepscot River	ADULT	2	1575 ± 318 (1350-1800)	29.9 ± 5.8 (25.8-34.0)	1.36 ± 0.02 (1.34-1.37)	17.00 <sup>1</sup> <sup>1</sup> n=1
	Fore River, Portland	ADULT	2	1550 ± 212 (1400-1700)	33.9 ± 0.8 (33.4-34.5)	1.15 ± 0.09 (1.09-1.22)	3.79 ± 1.70 (2.59-4.99)

Appendix 31. Mercury concentrations in the blood and feathers of bald eagle (*Haliaeetus leucocephalus*) chicks.

BALD EAGLE SAMPLE SITES (ordered north to south)	n	BLOOD Total Hg ( $\mu\text{g/g}$ , w.w.) mean $\pm$ SD (min-max)	n	FEATHER Total Hg ( $\mu\text{g/g}$ , w.w.) mean $\pm$ SD (min-max)
East Branch Lake	1	1.00	1	20.30
Upper Penobscot River (Mattawamkeag to Milford)	6	0.46 $\pm$ 0.13 (0.31-0.67)	6	14.46 $\pm$ 2.34 (11.10-17.80)
OV (Old Town - Veazie)	5	0.47 $\pm$ 0.13 (0.34-0.62)	5	9.83 $\pm$ 0.75 (8.54-10.50)
BO (Brewer - Orrington)	1	0.25	1	5.64
Penobscot Bay (Bagaduce R. to S. Islesboro)	6	0.20 $\pm$ 0.07 (0.10-0.29)	6	5.06 $\pm$ 2.01 (2.94-7.45)
Southern Maine Coast (Boothbay Harbor to Casco Bay)	6	0.20 $\pm$ 0.11 (0.07-0.37)	3	4.07 $\pm$ 0.27 (3.84-4.37)

Appendix 32. Mercury dissolved in water in the Penobscot River and estuary. This material is reproduced from the Phase I report.

Total Hg dissolved in water is shown for the five sampling reaches, averaged over all six sampling periods, in Figure 32-1. Average concentrations ranged from about 0.75 ng/L to about 2.6 ng/L. These concentrations are typical of unimpacted sites receiving no point sources of Hg and with low rates of atmospheric deposition. For example, St. Louis et al. (1994) found concentrations of 1.4 – 13.4 ng/L total Hg in streams in a remote area of northwestern Ontario where there is no industry and atmospheric deposition rates of mercury are at background levels. Dissolved total Hg was higher in the river and lower in the estuary, especially at outer estuary sites. In the river, concentrations of dissolved total Hg were slightly higher on average in the Old Town to Veazie reach of the river (Figure 32-1). This pattern was quite consistent over the six sampling times. Dissolved total Hg was lower in the estuary than in the river during all times of the year and was highest in the East Branch, Old Town to Veazie or Brewer to Orrington, with decreasing concentrations further downstream. Dissolved total Hg in the river showed no pattern in relation to the location of the HoltraChem site.

MeHg dissolved in water averaged about 0.02 to 0.31 ng/L and was also higher in the river, as compared to the estuary (Figure 32-2). Concentrations were generally higher in the Old Town to Veazie reach than other river reaches, as for dissolved total Hg. Also as for total Hg, concentrations seen are typical of unimpacted sites (e.g. St. Louis et al. 1994). This pattern was very consistent at different times of the year. Thus, dissolved MeHg showed no relationship to the HoltraChem site.

The observation of lower concentrations of dissolved Total Hg and MeHg in the lower river and upper estuary as compared to further upstream is consistent with other studies, which demonstrate that mercury tends to adsorb more to particles as the water becomes more brackish and salinity increases (Turner et al. 2001; 2002). The lower concentrations at the outer estuarine sites may also have been caused by dilution of river water by sea water.

The lower reaches of the Penobscot River carried much higher loads of suspended particles than the upper river reaches or the estuary (Figure 32-3). Concentrations of suspended particles are usually related to water turbulence. In the East Branch, total suspended solids (TSS) generally averaged about 2 mg/L, increasing to about 5 mg/L in the Old Town-Veazie and Brewer-Orrington reaches, and to 10 mg/L or higher in the river downstream of Orrington. In the estuary, average TSS values ranged from 2 to 25 mg/L. River flows apparently cause the suspension of significant amounts of fine particles in the river, especially downstream of Orrington. TSS was highest in the



Orrington to Bucksport reach in late October and July but was highest in the estuary in late May/early June.

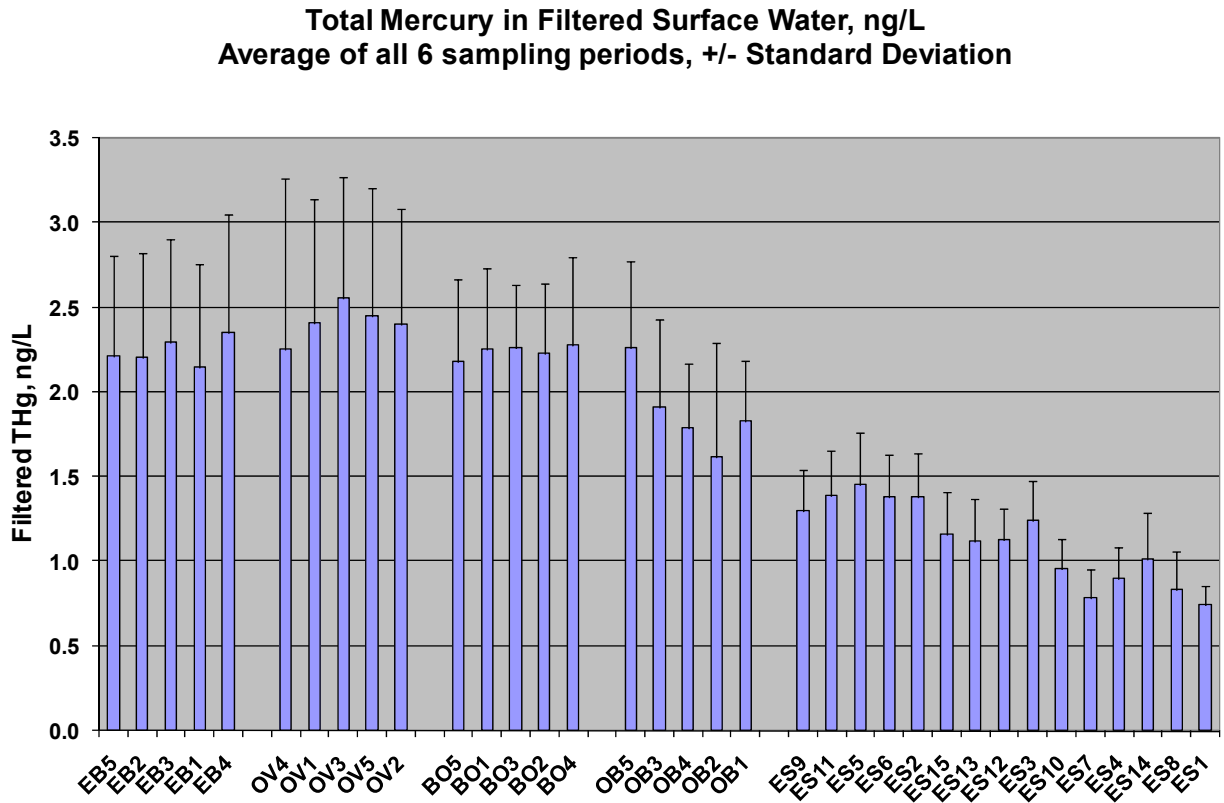


Figure 32-1. Mean concentrations (+/- 1 standard deviation) of total mercury (ng/L) in filtered water in the Penobscot River and estuary during the six sampling periods in 2006 and 2007. With the exception of some stations during Sampling period I, each site was sampled in duplicate during each sampling period, so most means are from 12 determinations at each site. EB=East Branch, OV=Old Town-Veazie, BO=Brewer-Orrington, OB=Orrington-Bucksport, ES=Estuary. Stations are plotted in geographic order from upstream to downstream (north to south); sites are mapped in Figures 2-7.

**Methyl Mercury in Filtered Surface Water, ng/L**  
**Average of all 6 sampling periods, +/- Standard Deviation**

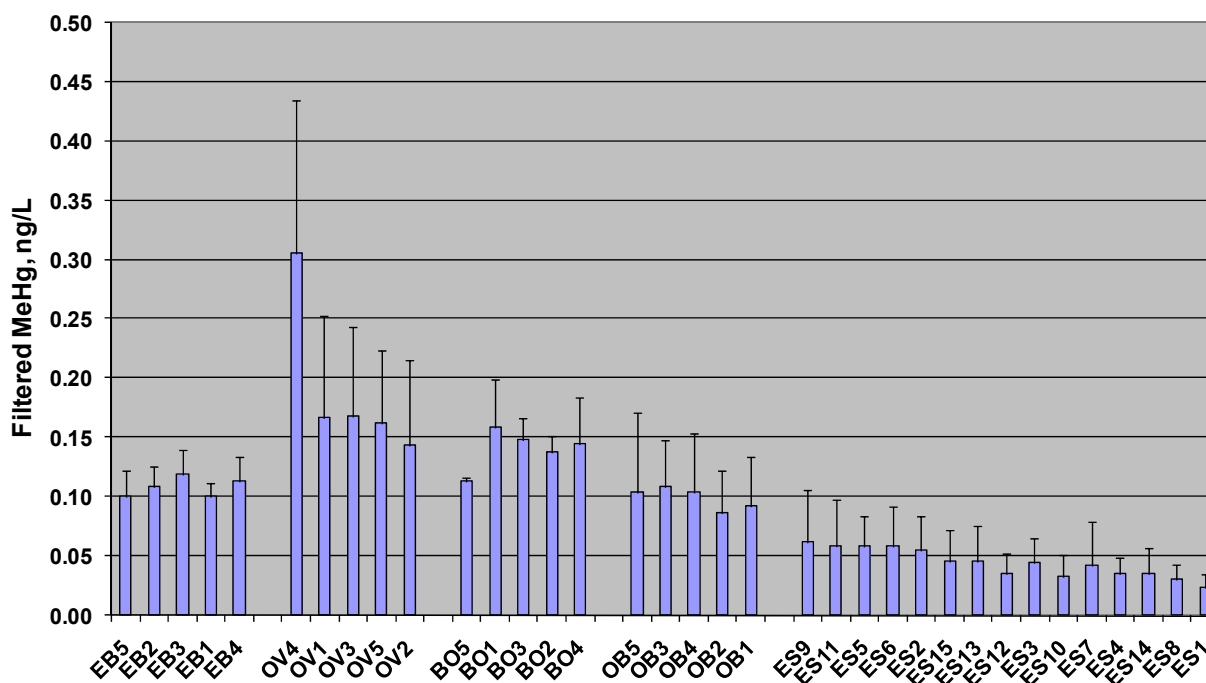


Figure 32-2. Mean concentrations (+/- 1 standard deviation) of methyl mercury (ng/L) in filtered water in the Penobscot River and estuary during the six sampling periods in 2006 and 2007. With the exception of some stations during Sampling period I, each site was sampled in duplicate during each sampling period, so most means are from 12 determinations at each site. EB=East Branch, OV=Old Town-Veazie, BO=Brewer-Orrington, OB=Orrington-Bucksport, ES=Estuary. Stations are plotted in geographic order from upstream to downstream (north to south); sites are mapped in Figures 2-7.

**Total Suspended Solids in Surface Water, mg/L**  
**Average of Periods IV, V and VI, +/- Standard Deviation**

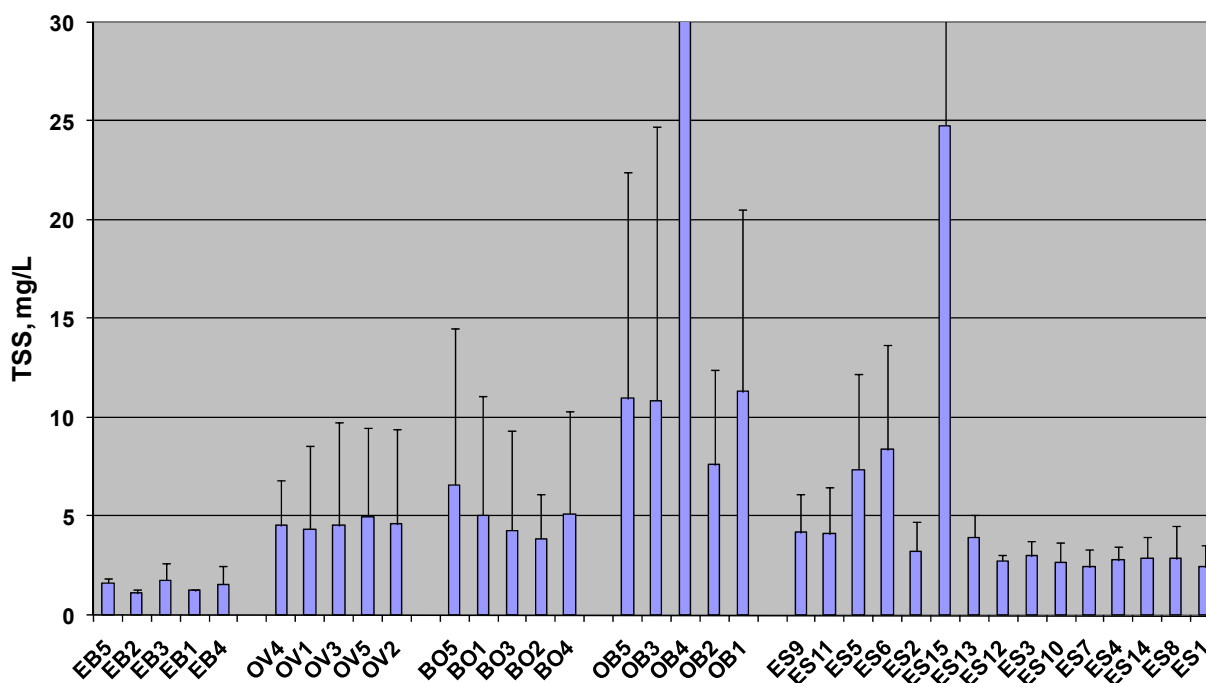


Figure 32-3. Mean concentrations ( $\pm 1$  standard deviation) of suspended sediments (mg/L) in surface water in the Penobscot River and estuary during Sampling periods IV, V, and VI, 2006 and 2007. All water samples were analyzed in duplicate in the laboratory and therefore each mean is from duplicate analyses taken at three different sampling times. EB=East Branch, OV=Old Town-Veazie, BO=Brewer-Orrington, OB=Orrington-Bucksport, ES=Estuary. Actual mean for OB4 was 107.0 and s.d. was 180.6. Standard deviation for ES15 was 26.1. Stations are plotted in geographic order from upstream to downstream (north to south); sites are mapped in Figures 2-7.

The concentration of total Hg on particles suspended in river and estuary water was relatively constant in the upper river (about  $0.3 \mu\text{g THg/g}$ ), but increased noticeably downstream of Orrington to about  $0.7 \mu\text{g/g}$ , and decreased with distance out into the estuary (Figure 32-4). This pattern was quite consistent among the three sampling periods (Late October, late May/early June and July). Concentrations were much less than were observed in the mercury contaminated Elbe River (Germany) (Wilken and Hintelmann 1991).

Because the total amount of suspended particles also increased in the lower river, the load of Hg carried by particles increased more than based only on concentration. TSS was typically 2 times as high in the reach downstream of HoltraChem (OB) as compared to upstream of HoltraChem (BO) and total Hg concentrations on particles were also typically twice as high. Therefore, the total load of total Hg on suspended averaged about 4 times as high downstream of HoltraChem.

**Total Mercury in Particulates in Surface Water, ug/g**  
**Average of Sampling Periods IV, V, and VI, +/- Standard Deviation**

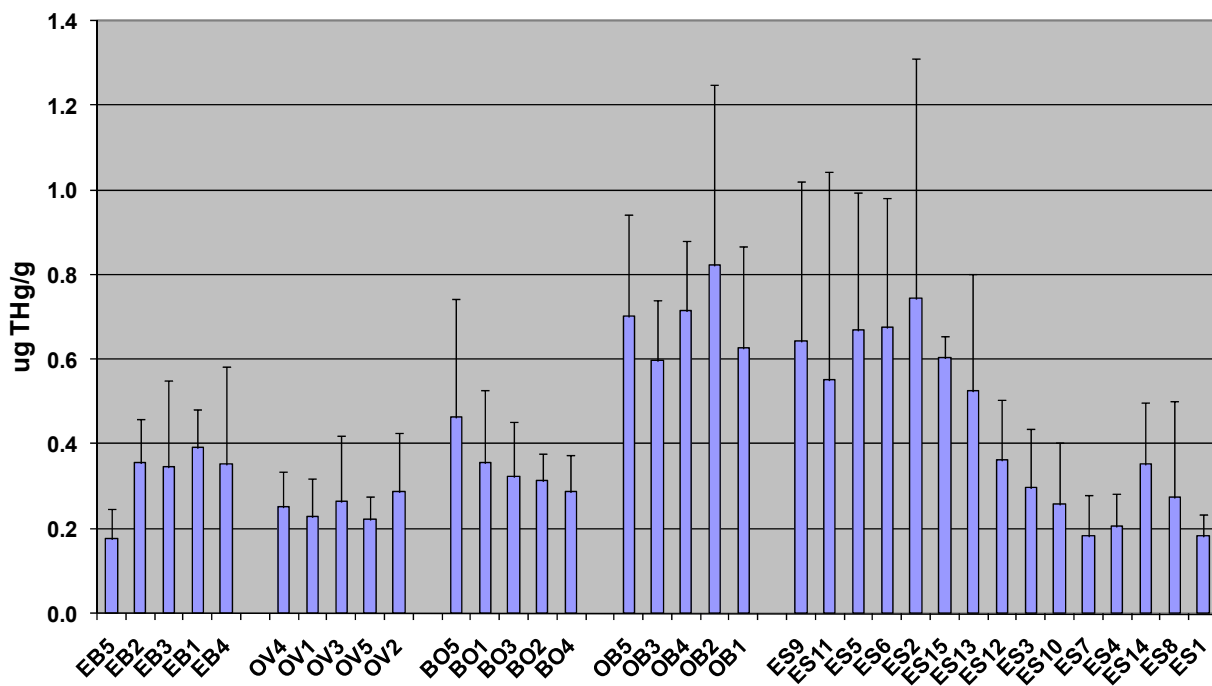


Figure 32-4. Mean concentrations (+/- 1 standard deviation) of total mercury ( $\mu\text{g/g}$ ) on suspended particles in the Penobscot River and estuary during sampling periods IV, V, and VI, 2006 and 2007. Means for each of the three sampling periods were calculated from field duplicate samples for total mercury in unfiltered filtered water and from laboratory duplicate determinations of suspended solids. EB=East Branch, OV=Old Town-Veazie, BO=Brewer-Orrington, OB=Orrington-Bucksport, ES=Estuary. Stations are plotted in geographic order from upstream to downstream (north to south); sites are mapped in Figures 2-7.

MeHg on particles in the Penobscot River and estuary averaged from 0.005 to 0.04  $\mu\text{g/g}$  and did not show noticeable or consistent differences over the study area (Figure 32-5). MeHg on particles did not show patterns related to the location of the HoltraChem site, although on average, it tended to be lower in the East Branch and the lower estuary (Figure 17).

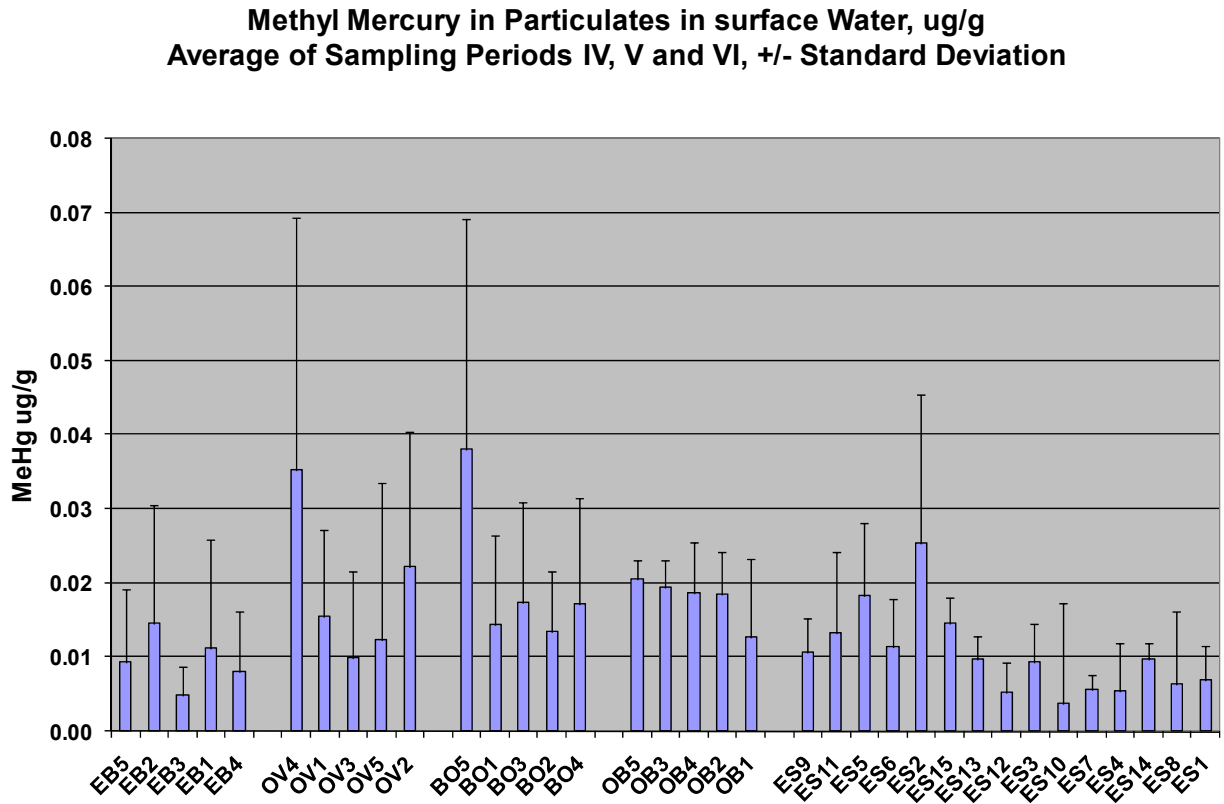


Figure 32-5. Mean concentrations ( $\pm 1$  standard deviation) of methyl mercury ( $\mu\text{g/g}$ ) on suspended particles in the Penobscot River and estuary during sampling periods IV, V, and VI, 2006 and 2007. Means for each of the three sampling periods were calculated from field duplicate samples for total mercury in unfiltered filtered water and from laboratory duplicate determinations of suspended solids. EB=East Branch, OV=Old Town-Veazie, BO=Brewer-Orrington, OB=Orrington-Bucksport, ES=Estuary. Stations are plotted in geographic order from upstream to downstream (north to south); sites are mapped in Figures 2-7.

Appendix 33. Mercury in freshwater snails in the Penobscot River. This material is reproduced from the Phase I report.

Lymnaed (freshwater) snails (*Lymnaea megasoma*) were found in all four river reaches, but not in the estuary. Total mercury in the soft tissues of snails varied among river reaches, but did not present a simple pattern, or a strong pattern related to the location of HoltraChem. Supporting data were given in Appendices 4 and 5 of the Phase I report. Because Hg in freshwater snails was found to be significantly higher during sampling period II, data for this period were analyzed and presented separately from the other three sampling periods in 2006. Patterns were consistent, however, between Sampling II and the other times (Figures 33-1 and 33-2). Hg was statistically significantly higher at the reference sites (EB) relative to the other three river reaches. These higher concentrations are probably related to site-specific environmental influences in the East Branch that are unrelated to Hg in the environment, such as pH, temperature or river productivity (that could influence snail growth rates and ages). Snails from the BO and OB reaches adjacent to the HoltraChem site had significantly higher total Hg levels than snails sampled in the OV reach, immediately upstream of any tidal influence. This is consistent with observations of higher Hg in suspended particles and sediments in the lower Penobscot River.

## Total Mercury in Lymnaed Snails Sample Periods I, III, IV (geometric mean +/- CI)

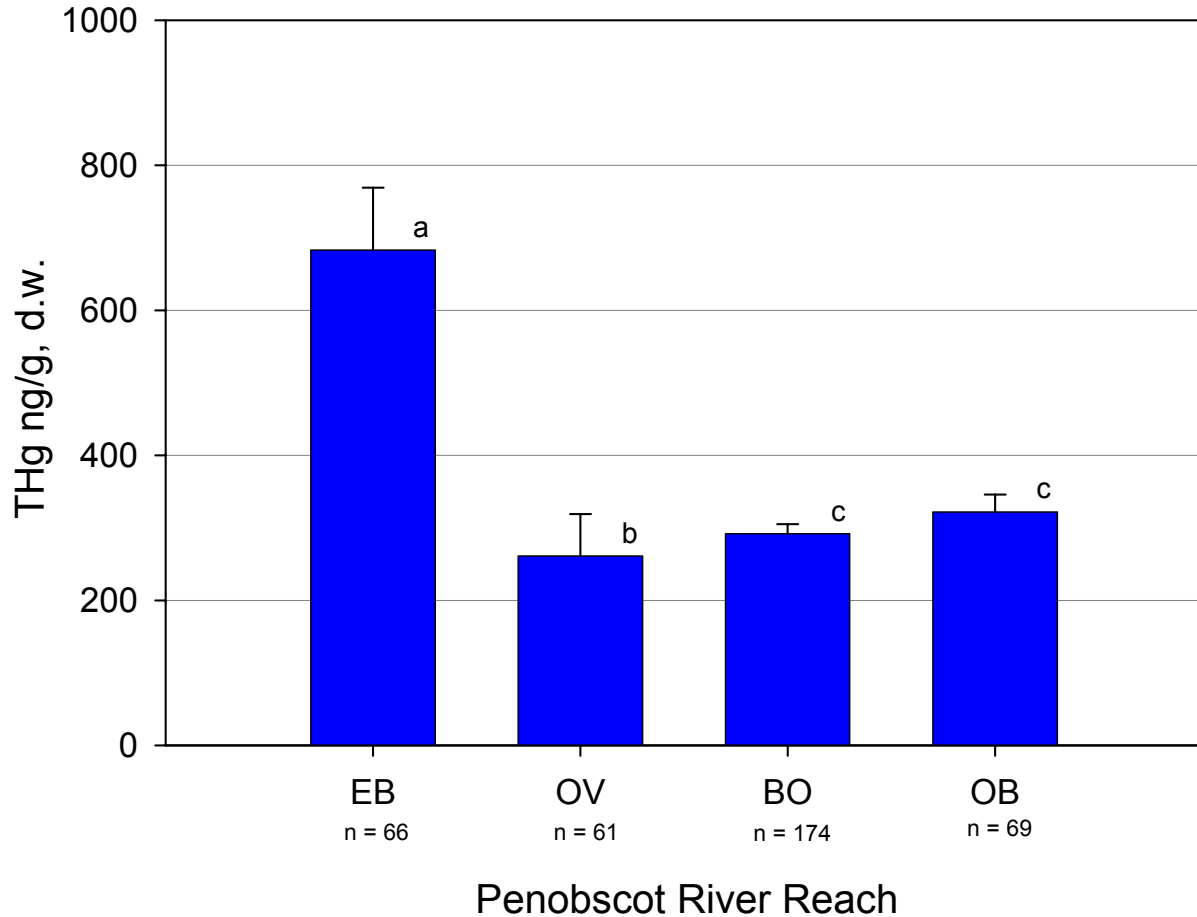


Figure 33-1. Geometric means (+/- 95% confidence intervals) of total mercury (ng/g d.w.) in freshwater snails in the Penobscot River, Sampling Periods I (late July/early August, 2006), III (late September/early October, 2006) and IV (late October/early November, 2006). Sample sizes are shown under each bar. Lower case letters above each bar indicate statistical differences or similarities (Tukey's Multiple Comparison Test); the same letter indicates that means are not statistically significantly different whereas different letters indicates significant differences.

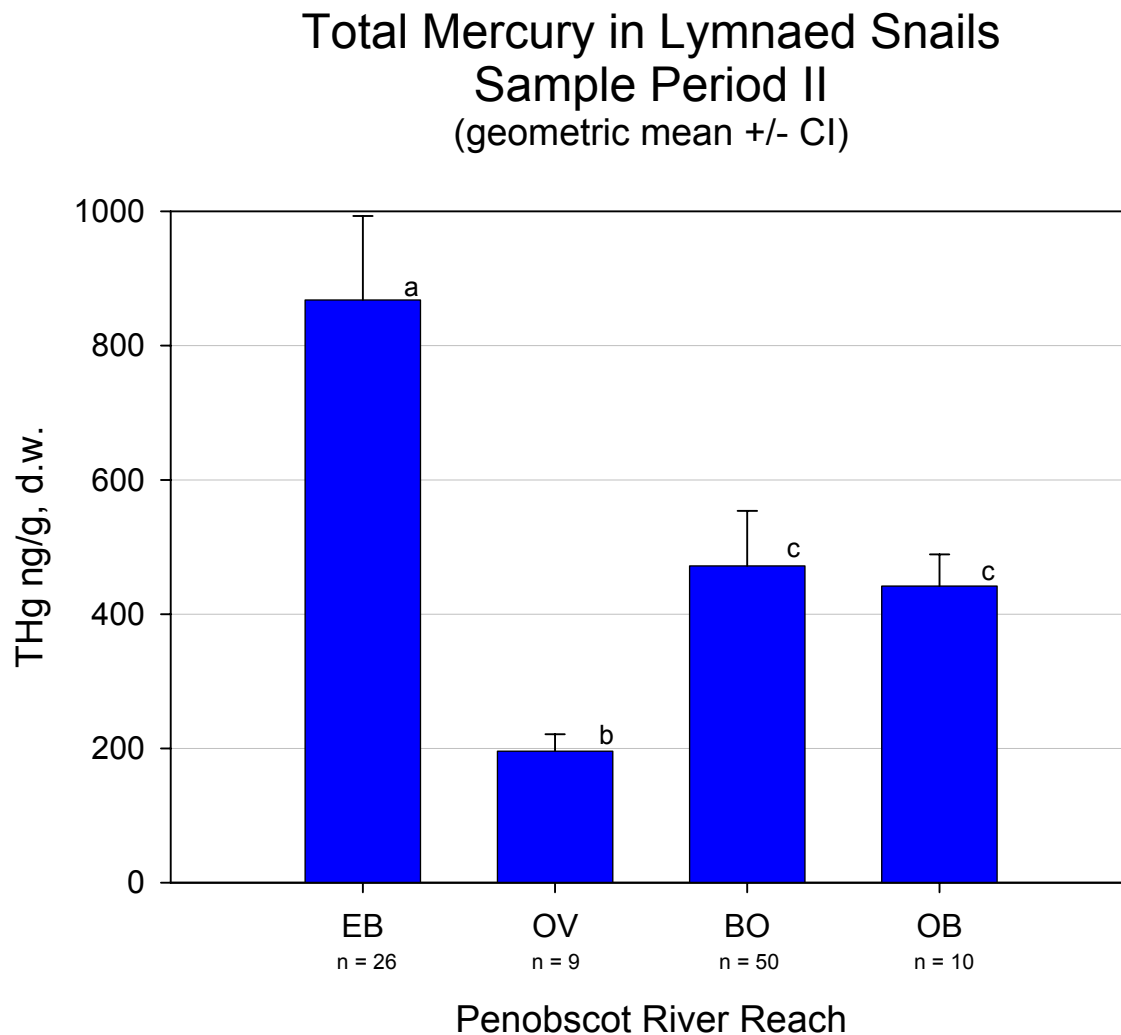


Figure 33-2. Geometric means (+/- 95% confidence intervals) of total mercury (ng/g d.w.) in freshwater snails in the Penobscot River, Sampling Period II (September, 2006). Sample sizes are shown under each bar. Lower case letters above each bar indicate statistical differences or similarities (Tukey's Multiple Comparison Test); the same letter indicates that means are not statistically significantly different whereas different letters indicates significant differences.



Appendix 34. Mercury in periwinkles in the Penobscot estuary. This material is reproduced from the Phase I report.

Periwinkles (*Littorina*) were found at almost all estuary sites, but not in the Penobscot River. Periwinkles were sampled at the regular aquatic sampling sites, in 2006. Total mercury in the soft tissues of periwinkles varied significantly among sites in the Penobscot estuary. Average concentrations ranged from 155 ng/g d.w. to 539 ng/g (Figure 34-1). Hg decreased with increasing distance from HoltraChem (Figure 34-2, Appendix 6 of the Phase I report). Table 34-1 shows the raw data by site. An analysis of variance showed that variation among sampling times was not statistically significant; data from different sampling times were therefore combined and considered together (Appendix 6 of the Phase I report). Most of the variation in Hg in periwinkles was explained by distance from the HoltraChem site, with lesser amounts being explained by snail weight and % moisture. Methyl mercury comprised, on average, 28% of the total mercury in periwinkles.

Concentrations of mercury in periwinkles in the Penobscot estuary were not high compared to other polluted sites but were higher than pristine sites. In a salt marsh polluted by a chlor-alkali facility in Georgia, Windom et al. (1976) found total mercury concentrations of 1,600 – 9,400 ng/g d.w., of which 3 – 10% was methyl mercury. In the polluted Limfjord, Denmark, Kiorbe et al. (1983) found about 10,000 ng/g d.w. THg in periwinkles. In Southampton Water (UK), Leatherland and Burton (1974) found 750 ng/g d.w. in periwinkles. Hg in periwinkles in the Penobscot was similar to those found in the Elbe estuary, Germany (about 400-800 ng/g d.w.) (Zauke 1977). The Elbe River is considered to have elevated concentrations in biota compared to pristine sites (Zauke 1977). Hg in periwinkles in other areas were generally lower than levels seen in the Penobscot (Severn estuary, UK: 300 ng/g, Tay Region, Scotland: 200 ng/g, Fjord of Kiel, Baltic Sea, Germany: 50-250 ng/g, Helgoland, North Sea, Germany: 250 ng/g (see Zauke 1977)).

Mercury Levels in Periwinkle Snails (*Littorina*), Penobscot Bay  
(mean  $\pm$  SD)

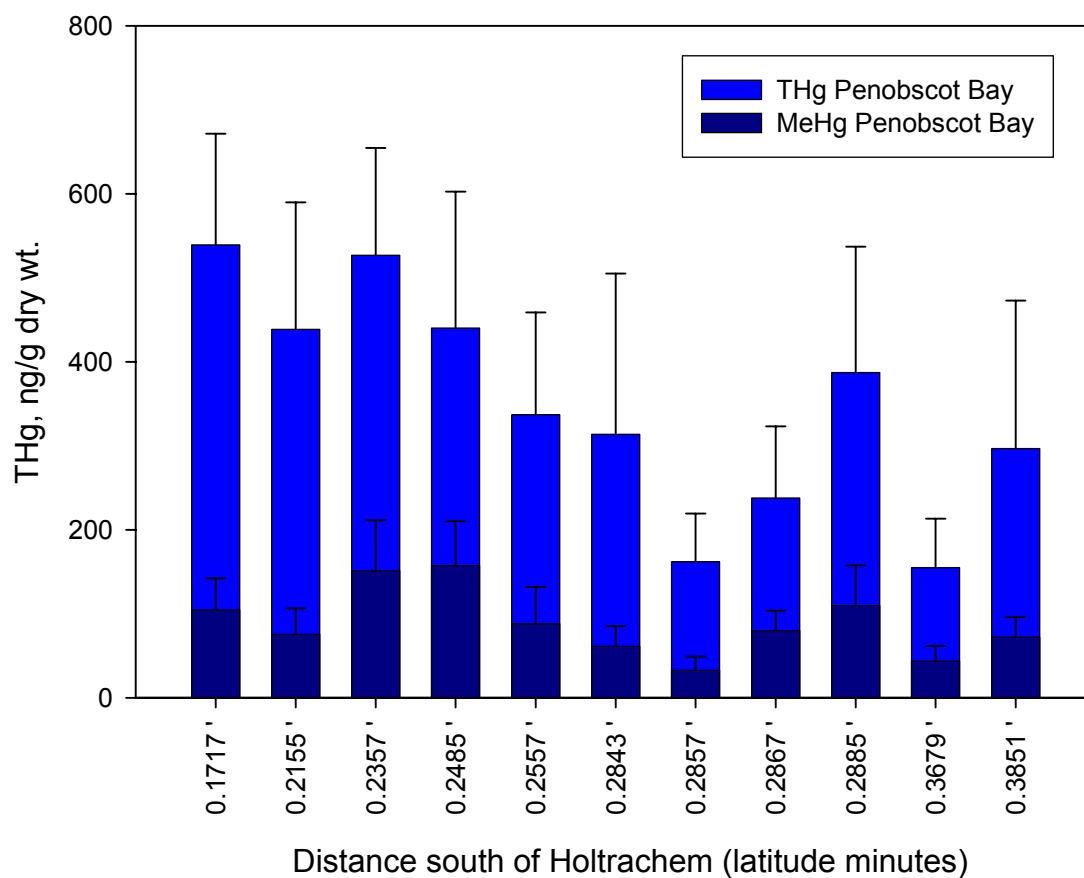


Figure 34-1. Mean total mercury concentrations ( $\pm$  1 s.d.) in periwinkles sampled from the Penobscot estuary in 2006. Site means are plotted in order of distance from the HoltraChem site. Also shown are mean methyl mercury concentrations ( $\pm$  1 s.d.) for each site.

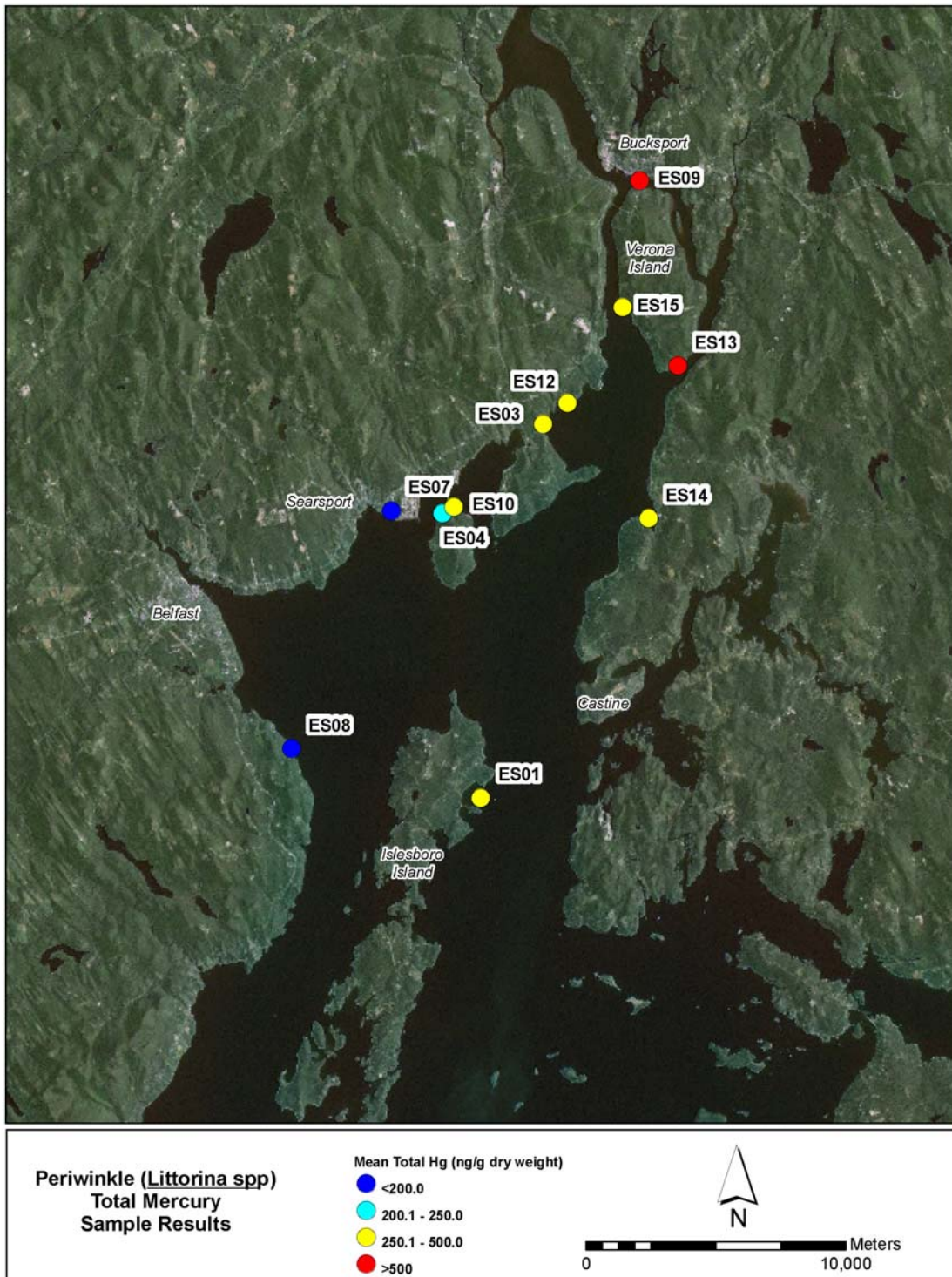


Figure 34-2. Map figure of average total mercury concentrations in periwinkles in the Penobscot estuary, sampled during four sampling periods in 2006.

Table 34-1. Mean total mercury concentrations in periwinkles sampled at 11 sites in the Penobscot estuary, 2006. Data from four sampling periods were combined. Also shown are the standard deviations, sample sizes (n), and range of total mercury concentrations.

Site	Mean (THg ng/g d.w.)	Standard Deviation	n	Range (THg ng/g d.w.)
ES-01	296.6	176.2	40	79-1150
ES-03	336.7	121.9	39	155-596
ES-04	237.9	85.2	49	116-519
ES-07	162.0	57.3	48	46-342
ES-08	154.8	58.3	40	61-376
ES-09	539.1	132.3	40	307-853
ES-10	310.1	191.2	50	70-902
ES-12	440.0	162.6	40	66-797
ES-13	526.7	127.9	40	289-860
ES-14	387.0	150.0	30	176-892
ES-15	438.5	151.3	50	135-790

Appendix 35. Mercury mussels in the Penobscot estuary. This material is reproduced from the Phase I report.

Mussels (*Mytilus*) were found at all sites in the estuary except those at the north and east site of Verona Island and those in the Orland River. Total mercury in the soft tissues of mussels showed a large amount of variation among sites, ranging from 146 to 1262 ng/g d.w. in early September and from 101 to 1279 ng/g d.w. in late September/early October (Table 35-1). Mercury concentrations in mussels were higher in the upper estuary (southern end of Verona Island and Fort Point Cove) and were lower at sites further south in the lower estuary (Searsport, Islesboro) (Figure 35-1). The rankings of sites were similar at both sampling times. Differences among sites were found to be statistically significant by analysis of variance and analysis of covariance on total mercury data (w.w.), whether animal size was used as a covariate or not (Appendices 7 and 8).

From early September to late September/early October, total mercury concentrations generally decreased (Table 11). However, the average concentration of MeHg in mussels stayed about the same over the same time period. Therefore, the proportion of the total mercury that was MeHg increased. The proportion of total Hg that was MeHg averaged 32% in the first sampling as compared to 43% in the second sampling. It would be expected that MeHg concentrations would be less changeable than total Hg concentrations based on the physiology of MeHg vs. inorganic mercury; MeHg is known to have longer turnover times in biotic tissues than inorganic Hg. The % MeHg did not show any geographic patterns in either sampling period, so the geographic patterns seen for total Hg were also present for MeHg.

Table 35-1. Mean concentrations of total mercury (ng/g d.w.) in mussels sampled in the Penobscot River estuary, 2006. N=10 for all means. Late September sampling period was September 7 – 11, 2006. Sept/Oct sampling period was September 27 – October 2, 2006. Sites listed in geographic order from North to South.

Site	Mean (Late Sept)	Standard Deviation (Late Sept)	Mean (Sept/Oct)	Standard Deviation (Sept/Oct)
ES15	857.6	217.8	513.3	145.1
ES13	1262.0	272.3	1278.9	309.2
ES12	884.0	202.5	850.2	172.1
ES3	985.1	209.4	431.0	87.9
ES10	174.7	96.1	134.2	22.1
ES7	146.0	35.4	101.3	15.5
ES4	231.9	75.1	181.9	51.9
ES14	834.4	325.2	803.4	247.6
ES8	172.1	43.0	170.9	58.3
ES1	304.4	126.2	269.0	68.4



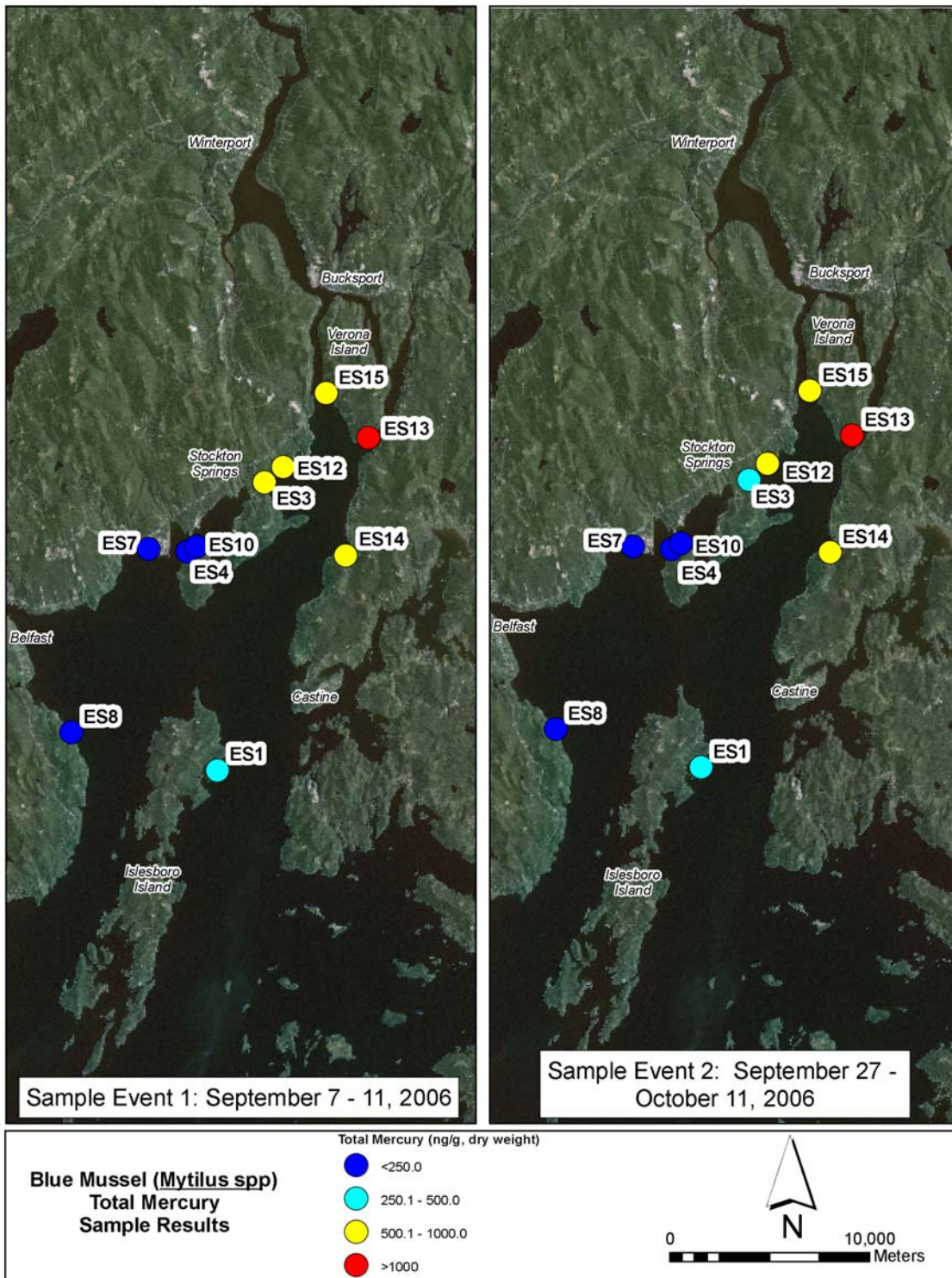


Figure 35-1. Average concentrations of total mercury (ng/g d.w.) in mussels in the Penobscot estuary, sampled in early September, 2006 (left map) and late September/early October, 2006 (right map).

Average total Hg concentrations in mussels at all sites in the Penobscot estuary in 2006 (both sampling times combined) ranged from about 150 to 1270 ng/g d.w., but were 685 – 1270 ng/g in the upper estuary. The concentrations determined in 2006 were usually similar to those reported in other recent studies for the Penobscot, for example by Livingston (2000), Mussel Watch ([www8.nos.noaa.gov](http://www8.nos.noaa.gov)), Gulf Watch ([www.gulfofmaine.org](http://www.gulfofmaine.org)), and Maine DEP ([www.maine.gov/dep](http://www.maine.gov/dep)). It does appear, however, that present-day levels are significantly lower than concentrations in the 1990's. At the Sears Island Mussel Watch site, mean concentrations were always higher than 300 ng/g d.w. from 1990 – 1997, compared to the present concentration of 120 ng/g. At the Pickering Island Mussel Watch site, concentrations were also noticeably higher in the early 1990's than at present ([www8.nos.noaa.gov](http://www8.nos.noaa.gov)). These data suggest that there may already have been some natural attenuation of Hg pollution in the Penobscot River and Estuary. The topic of rates of natural attenuation of Hg contamination of the Penobscot ecosystem will be addressed by several of the tasks that are being planned for Phase II of the study.

The concentrations of total Hg in mussels in the upper Penobscot estuary are high relative to other sites in the region. In 2006, mean concentrations in the upper estuary (both sampling times combined) ranged from 685 to 1270 ng/g d.w. In 2005, the median concentration for Maine from Mussel Watch data was 166 ng/g and the 85<sup>th</sup> percentile for Maine was 304 ng/g. For the Gulf Watch data, 36 of 38 sites had medians less than 790 ng/g. Maine DEP found means ranging from 76 to 518 ng/g d.w. at eight sites in Maine outside of the Penobscot estuary in 2001. None of the mussels sampled in the Penobscot estuary exceeded the most protective criteria for protection of human health for consumption of MeHg in biota (set by Maine DEP at 0.2 µg/g w.w. or approximately 1,000 ng/g d.w. and by the USEPA at 0.3 µg/g w.w. or approximately 1,500 ng/g d.w.).

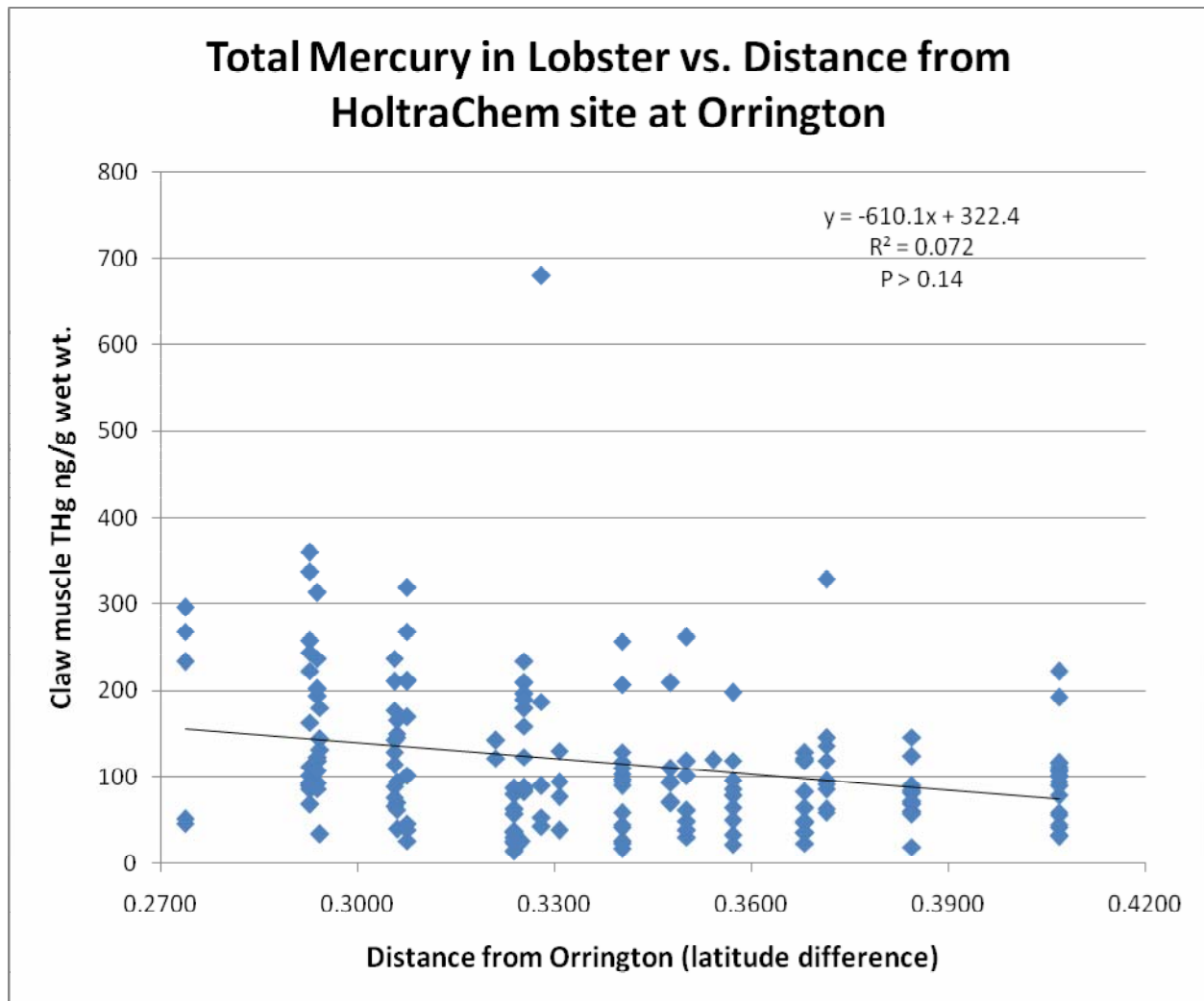


Appendix 36. Mercury in lobsters from the Penobscot estuary. This material is reproduced from the Phase I report.

Lobsters were sampled in the estuary from Fort Point to Islesboro Island. The average concentrations of total mercury in lobster claw muscle at various sites in the Penobscot estuary ranged from 46 to 211 ng/g w.w. (for samples greater than one individual) (Appendix 10 of the Phase I report). Average methyl mercury concentrations ranged from 39 to 176 ng/g w.w. and MeHg comprised, on average, about 76% of the total Hg in lobster claw muscle. A subsample of lobsters was analyzed for Hg and MeHg in tail muscle and tomalley (hepatopancreas) and it was found that 75% of the Hg in tail muscle was MeHg, similar to claw muscle. Concentrations of Hg in tail muscle were on average 53% higher than in claw muscle (n=8). Therefore, some of the lobsters sampled from the upper estuary exceeded the Maine DEP and USEPA concentrations of 200 and 300 ng/g w.w. that serve as criteria for the protection of human health due to consumption of MeHg in biota (Figure 36-1). At the eight upper estuary sites (see map Figure 36-2), of 67 lobster sampled, 25% exceeded the MDEP criterion of 200 ng/g w.w. MeHg and 6% exceeded the USEPA criterion of 300 ng/g. This was calculated from the mean of total Hg in claws and tails (from individual total Hg concentration in claws assuming tail muscle was 53% higher in total Hg) and that 75% of the total Hg in both tissues was methyl Hg.

There was an apparent relationship between Hg in lobster claw muscle and distance from the HoltraChem site, with mercury decreasing with distance from Orrington, although this relationship was not statistically significant (Figures 36-1 and 36-2).

Maine DEP found total mercury in lobster near Verona Island to average 120 ng/g w.w. in 1995, which is slightly lower than was seen in 2006 near the southern end of Verona Island. The levels of Hg seen in lobsters in the Penobscot estuary overlap with those from other Maine estuaries. Sowles (1997) summarized data for Hg in lobsters in Maine in 1995 and noted that means in lobster muscle (claw vs. tail muscle not specified) ranged from 82 to 208 ng/g w.w. at seven sites outside the Penobscot system.



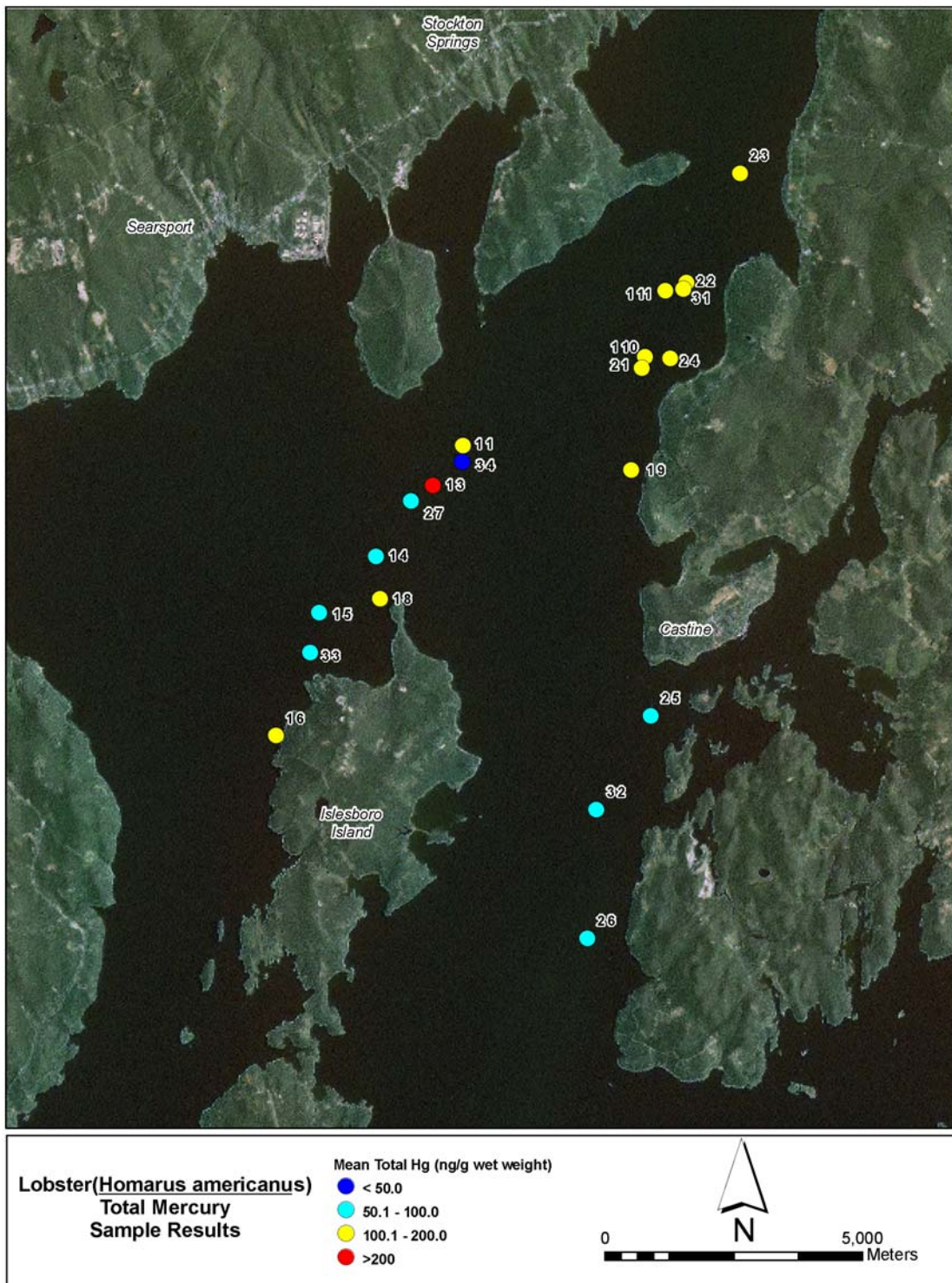


Figure 36-2. Total Hg concentrations in the claw muscle of lobster, Penobscot estuary, 2006.

Appendix 37. Mercury in mink and otter from the Penobscot system. This material is reproduced from the Phase I report.

Attempts were made to assess Hg concentrations in the tissues of both mink and river otter. We attempted to compare concentrations in animals from potentially contaminated to non-contaminated sites. Unfortunately, few animals were collected from contaminated sites, so no firm conclusions can be drawn from these data. Nevertheless, a description of the results follows.

Mink were sampled at one site potentially contaminated by Hg from HoltraChem (S. Branch, Marsh River) and four reference sites (East Branch Penobscot River, Alger Pond, Carley Brook and Pushaw Lake). Otters were sampled at two sites potentially contaminated by Hg from HoltraChem (Bagaduce River and Reeds Brook) and at six reference sites (East Branch Penobscot River, Carley Brook, Fields Pond, Jordan Brook, Pushaw Lake and Souadabscook Stream). Recent results from sampling of mercury in wetlands in the Bagaduce River estuary indicate, however, that this area may not be contaminated with Hg from HoltraChem, thus limiting the value of the comparisons involving those sites.

Mercury in the tissues of mink sampled in the vicinity of the Penobscot was usually higher at sites that were potentially contaminated by mercury from the Orrington HoltraChem site than at reference sites (Table 37-1). Average mercury concentrations in three of the four tissues analyzed (brain, fur, and muscle) were higher at potentially contaminated sites than at reference sites, although none of the comparisons of the data were statistically significant (Table 37-1). Sample sizes were small for potentially contaminated sites, limiting the power of statistical comparisons.

Table 37-1. Mean concentrations of total mercury ( $\mu\text{g/g}$  w.w.) found in the tissues of mink sampled in the vicinity of the Penobscot River in 2006. Sampling sites were classified as being potentially contaminated with mercury from the Orrington HoltraChem site or as reference sites by proximity to the lower Penobscot River and estuary. Also shown are sample sizes, ranges, standard deviations and the p value for one-tailed t-tests (assuming equal or unequal variances as appropriate after an F-test to compare sample variances) for statistical comparisons between potentially contaminated and reference sites.

Tissue	Site Classification	Average mercury (THg $\mu\text{g/g}$ w.w.)	Sample size	Range (THg $\mu\text{g/g}$ w.w.)	Sample Variance	P value compared to $\alpha=0.05$
Brain	Reference	0.46	17	0.16-1.15	0.07	P=0.23
	Contaminated	0.79	3	0.27-1.44	0.35	
Fur	Reference	20.38	17	11.4-33.3	52.3	P=0.28
	Contaminated	29.93	3	14.5-56.9	551.2	
Liver	Reference	2.93	15	0.58-18.4	19.21	P=0.39
	Contaminated	2.15	3	0.76-3.69	2.17	
Muscle	Reference	1.04	17	0.32-2.06	0.27	P=0.20
	Contaminated	1.66	3	0.76-2.77	1.05	

Concentrations of mercury in the tissues of mink at both reference and potentially contaminated sites in the Penobscot area were generally similar to other sites in North America. Comparisons to concentrations reported in the literature are shown in Table 37-2.

In mink, it is known that concentrations of total mercury in the brain higher than  $4.1 \mu\text{g/g}$  cause negative alterations to the brain's cholinergic system (Basu et al. 2006) and Sandheinrich (2007) suggested that brain concentrations exceeding  $5 \mu\text{g/g}$  will be associated with reduced reproductive success in mammals, but none of the individual otter sampled in this study approached these concentrations in their brains.

Sublethal and lethal effects are known in mink at concentrations of  $20\text{-}30 \mu\text{g/g}$  in liver (Halbrook et al. 1994; Mierle et al. 2000), but again, no animals sampled had concentrations this high. However, levels of greater than  $20 \mu\text{g/g}$  total mercury in fur have been associated with reduced survivorship (Halbrook et al. 1994; Mierle et al. 2000) and some animals had mercury concentrations in fur higher than  $20 \mu\text{g/g}$ . The mean concentration in fur at potentially contaminated sites was about  $30 \mu\text{g/g}$  and the

Table 37-2. Comparisons of concentrations of mercury observed in the tissues of mink in the Penobscot River Mercury Study with other areas in North America. All concentrations given as µg/g w.w.

Location	Sample size	Mean in muscle	Range in muscle	Mean in brain	Range in brain	Mean in liver	Range in liver	Mean in fur	Range in fur	Reference
Connecticut	8						1.1 - 8.5			Major and Carr 1991
Massachusetts	4						0.01 - 1.9			Major and Carr 1991
New York	60						0.25 - 7.66			Foley et al. 1988
Ohio	n/a					0.1				Lynch 1973
Ontario	94		0.01 - 4.1		0.3 - 0.7		0.01 - 7.5			Wren et al. 1987
Ontario	39-316			0.96		3.71		30.1		Fortin et al. 2001
Quebec	n/a	1.9		0.8		9.2				Desai-Greenway and Price 1976
Quebec	n/a	2.4	0.41 - 6.2			8.3	2.2 - 20.0			Langis et al. 1999
Saskatchewan	1					58.2				Wobeser and Swift 1976
Tennessee	1							104		Stevens et al. 1997
Wisconsin	39	1.3		0.5		2.1		7.6		Sheffy and St.Amant 1982
Maine	92			0.55	0.1 - 2.6	1.64	0.1 - 8.0	20.92	1.8 - 68.5	Yates et al. 2004
Penobscot Reference	15 - 17	1.0	0.3 - 2.1	0.46	0.2 - 1.2	2.9	0.6 - 18.4	20.4	11.4 - 33.3	Penobscot River Mercury Study
Penobscot Contaminated	3	1.7	0.8 - 2.8	0.79	0.3 - 1.4	2.2	0.8 - 3.7	29.9	14.5 - 56.9	Penobscot River Mercury Study

highest individual had about 57  $\mu\text{g/g}$  total mercury in fur. At reference sites, the mean concentration in fur was about 20  $\mu\text{g/g}$ , the highest concentration observed was 33  $\mu\text{g/g}$  and a number of individuals had concentrations above 20  $\mu\text{g/g}$ . Given the small number of animals sampled at sites that were potentially contaminated with mercury from the Orrington HoltraChem site, it may be worthwhile to attempt to perform more complete sampling in the future.

Average concentrations of mercury in the tissues of otter sampled in the vicinity of the Penobscot River were always lower at sites that were potentially contaminated by mercury from the Orrington HoltraChem site than at reference sites, although only one of the three statistical comparisons that were possible were significant (Table 37-3). Sample sizes were small for three of the four tissues at potentially contaminated sites.

Table 37-3. Mean concentrations of total mercury ( $\mu\text{g/g}$  w.w.) found in the tissues of river otter sampled in the vicinity of the Penobscot River in 2006. Sampling sites were classified as being potentially contaminated with mercury from the Orrington HoltraChem site or as reference sites by proximity to the lower Penobscot River and estuary. Also shown are sample sizes, ranges, standard deviations and the p value for one-tailed t-tests (assuming equal or unequal variances as appropriate after an F-test to compare sample variances) for statistical comparisons between potentially contaminated and reference sites.

Tissue	Site Classification	Average mercury (THg $\mu\text{g/g}$ w.w.)	Sample size	Range (THg $\mu\text{g/g}$ w.w.)	Sample Variance	P value compared to $\alpha=0.05$
Brain	Reference	0.50	11	0.38-0.64	0.006	P=0.18
	Contaminated	0.28	2	0.14-0.42	0.037	
Fur	Reference	23.18	10	17.0-31.9	35.7	P=0.003
	Contaminated	15.29	9	8.13-21.7	21.8	
Liver	Reference	1.90	11	1.10-3.00	0.34	P=0.52
	Contaminated	1.08	2	0.56-1.61	0.55	
Muscle	Reference	0.94	12	0.35-1.49	1.01	n/a
	Contaminated	0.91	1	n/a	n/a	

Concentrations of mercury in otter tissues from the Penobscot were generally similar to other sites in North America and Europe and were within the range seen at other sites in Maine. Comparisons to concentrations reported in the literature are given in Table 37-4.

In otter, it is known that concentrations of total mercury in fur that exceed 20  $\mu\text{g/g}$  are associated with reduced survivorship (Halbrook et al. 1994; Mierle et al. 2000). The mean concentration of mercury seen in otter fur at reference sites was about 23  $\mu\text{g/g}$ , with a number of animals having concentrations higher than 20  $\mu\text{g/g}$ . At contaminated sites, the mean concentration in otter fur was about 15  $\mu\text{g/g}$  and two individual animals had concentrations slightly above 20  $\mu\text{g/g}$ . Sandheinrich (2007) suggests that brain concentrations exceeding 5  $\mu\text{g/g}$  will be associated with reduced reproductive success in mammals; none of the individual otter sampled in this study approached this concentration in their brains.



Table 37-4. Comparisons of concentrations of mercury observed in the tissues of river otter in the Penobscot River Mercury Study with other areas in North America and Europe. All concentrations given as µg/g w.w.

Area	Sample sizes	Mean in muscle	Range in muscle	Mean in brain	Range in brain	Mean in liver	Range in liver	Mean in fur	Range in fur	Reference
Britain	7						0.2 - 4.3			Mason 1988
Denmark	69						0.03 - 12.4			Mason and Madsen 1992
Georgia	n/a	4.4c, 1.5i				7.5c		24.3c, 15.2i		Halbrook et al. 1994
Ireland	32						0.15 - 17.03			Mason and Sullivan 1993
Manitoba	38				0.04 - 9.5		1.3 - 21.7			Kucera 1983
Mass.	96					1.9	0.5 - 4.8			Organ 1989
New York	34						0.01 - 6.95			Foley et al. 1988
Nova Scotia	23				0.07 - 1.8c, 0.5 - 10.2i					Burgess et al. 2002
Ontario <sup>1</sup>	1	36		30		96		47		Wren 1985
Ontario	n/a	0.9				2.9				Wren et al. 1980
Ontario	84		0.1 - 4.3		0.2 - 7.2		0.2 - 17.4			Wren et al. 1986
Ontario	n/a						1.0 - 3.5			Wren and Stokes 1988
Ontario	130			2		6.7		13.8		Mierle et al. 2000
Vermont	21							13.58	4.91 - 46.5	BRI unpubl. Data
Wisconsin	49	1.4		0.7		3.3		6.5		Sheffy and St. Amant 1982
Maine	69			0.55	0.06 - 3.25	1.76	0.24 - 8.66	25.9	1.1 - 234	BRI unpubl. data
Penobscot (Reference)	10-12	0.9	n/a	0.5	0.38 - 0.64	1.9	1.10 - 3.00	23.2	17.0 - 31.9	Penobscot River Mercury Study
Penobscot (Contaminated)	1-9	0.9	0.35 - 1.49	0.28	0.14 - 0.42	1.1	0.56 - 1.61	15.3	8.1 - 21.7	Penobscot River Mercury Study

c= coastal, i=inland, 1 = single individual found near former chlor-alkali plant, apparently dead due to mercury exposure

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