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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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 form 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 then 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.

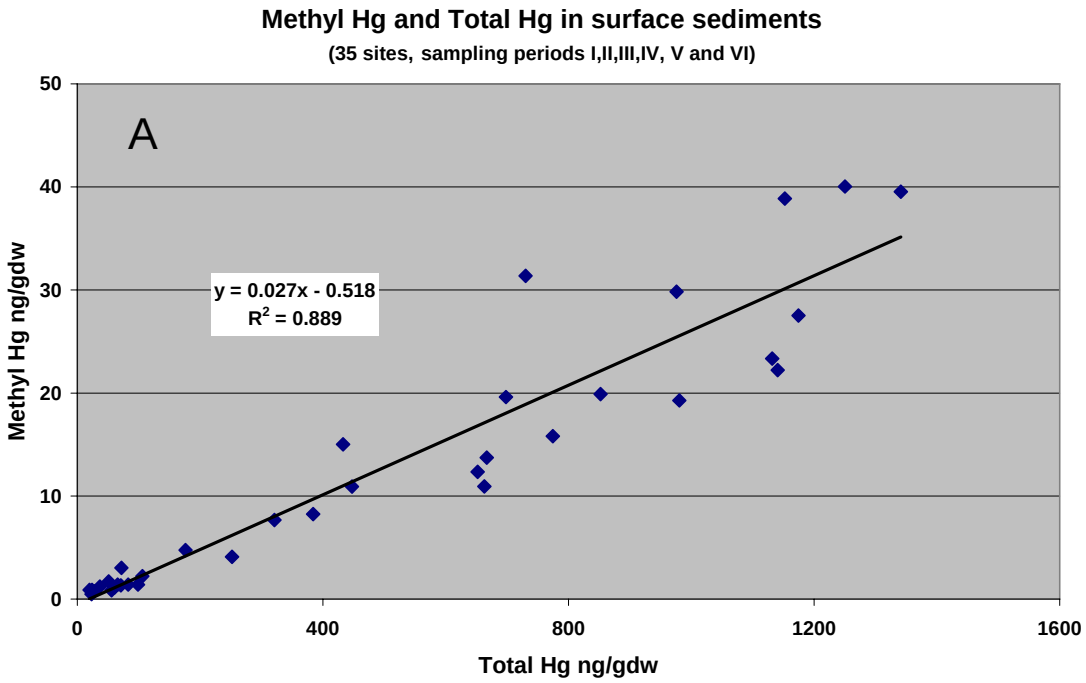
- Preliminary sediment coring data suggest that there are long term burial sites for mercury in the Penobscot system. So, if significant ongoing sources can be stopped, natural attenuation of the system will proceed.

- 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.



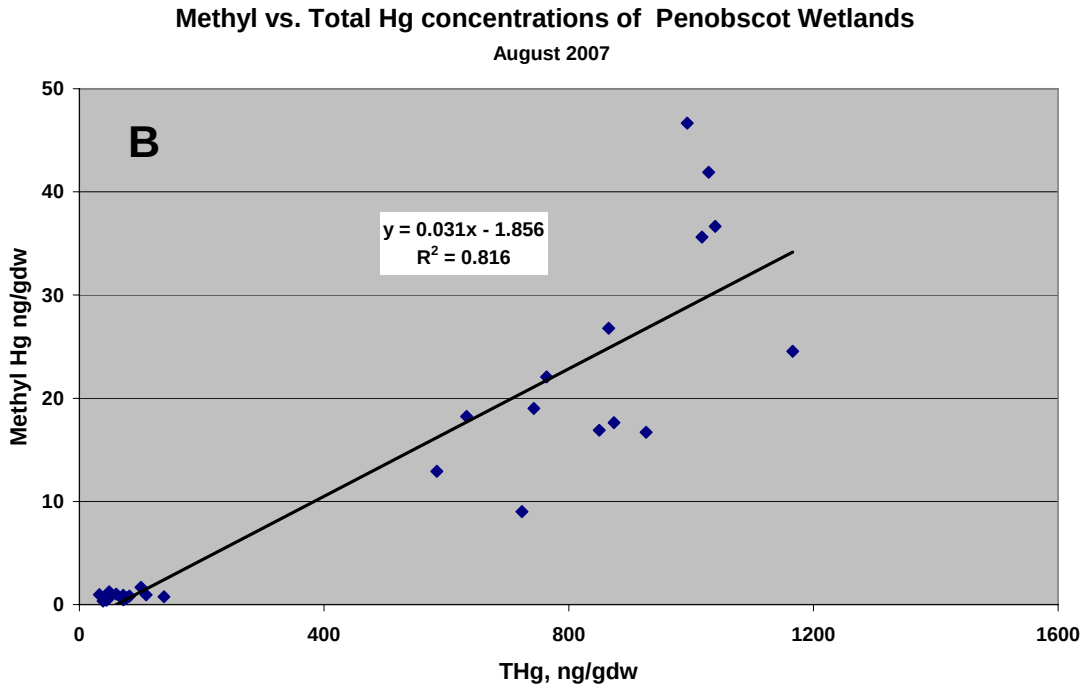


Figure i. Average concentrations of methyl Hg vs total Hg in surface riverine sediments (A) and wetland soils (B), showing that the concentrations of methyl Hg are dependent on the concentrations of total Hg.

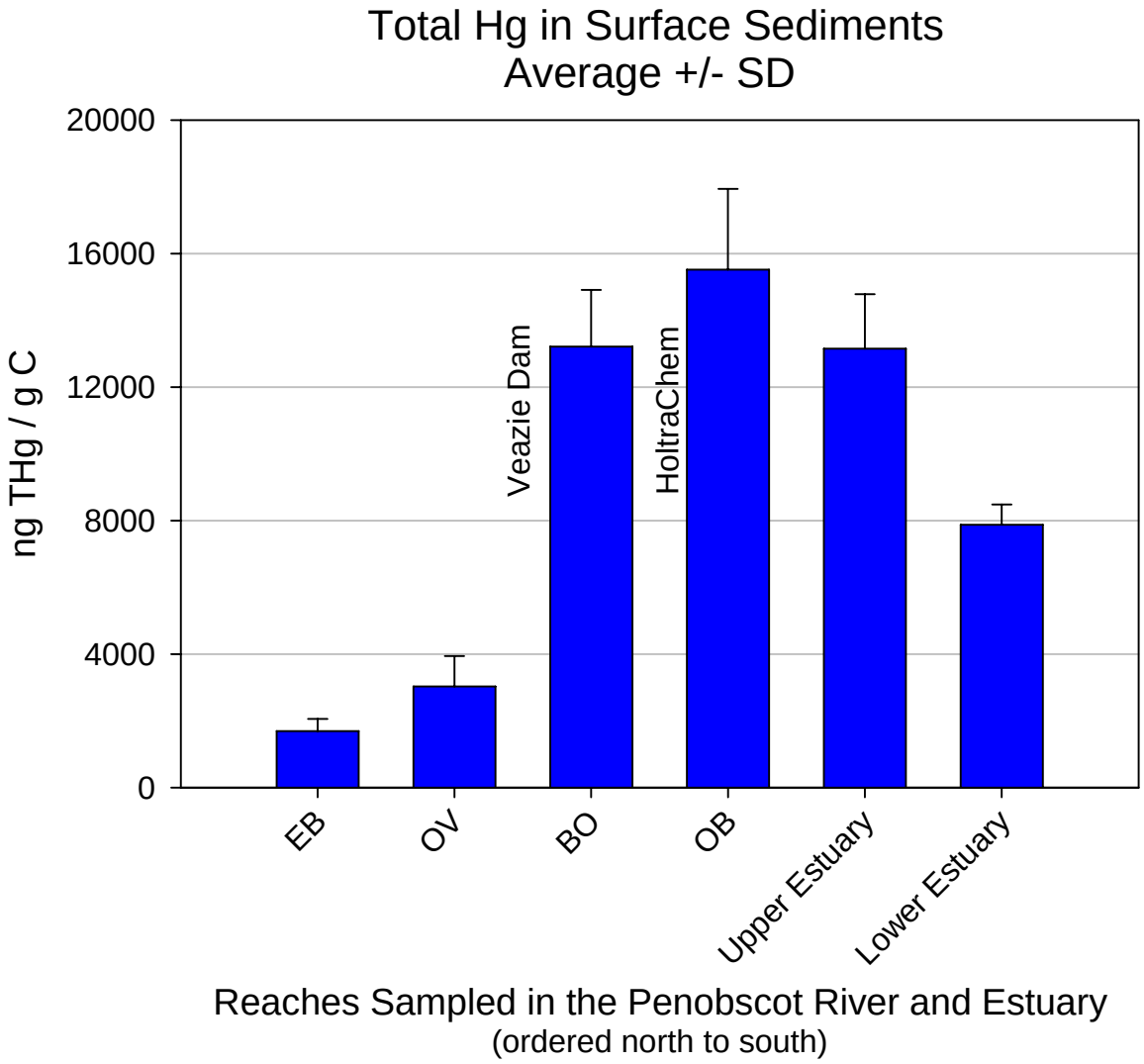


Figure ii. Concentrations of total Hg in surface sediments of the Penobscot River and Estuary. The East Branch of the Penobscot River (EB) is upstream of all known industrial point sources of Hg. The Old Town to Veazie reach (OV) is downstream of paper mills, which have used Hg in the past. High mercury 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[1] 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 a 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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[1] 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, perwinkles, 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 µg/g w.w. in blood, compared to levels of concern of 1.2 – 3 µg/g. This average concentration was similar to that found in 2006 (5.7 µg/g). Hg in Savannah sparrows and red-winged blackbirds sampled at Mendall Marsh was also high (3.2 and 3.8 µg/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 µg/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 µg/g in blood at Mendall Marsh, as compared to 0.14 µg/g at a reference area. Spotted sandpiper at Mendall were also high (3.3 µg/g in blood; however, only one bird was sampled) whereas Wilson's snipe and killdeer were lower (0.88 and 0.56 µg/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 µg/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 under-estimated. 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.

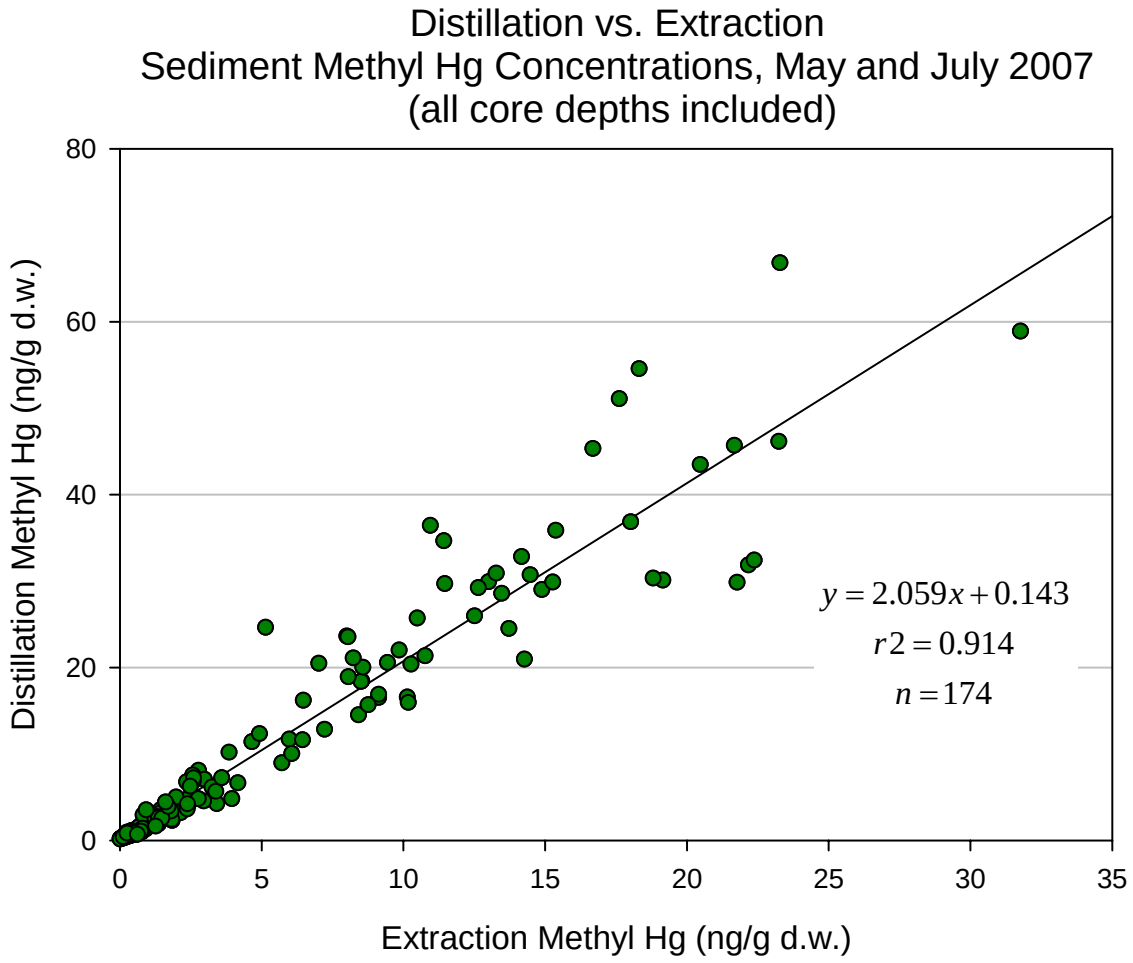

Figure 1. This graph illustrates the two-fold greater methyl Hg concentrations found in sediment using the distillation method as compared to the extraction method.

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.

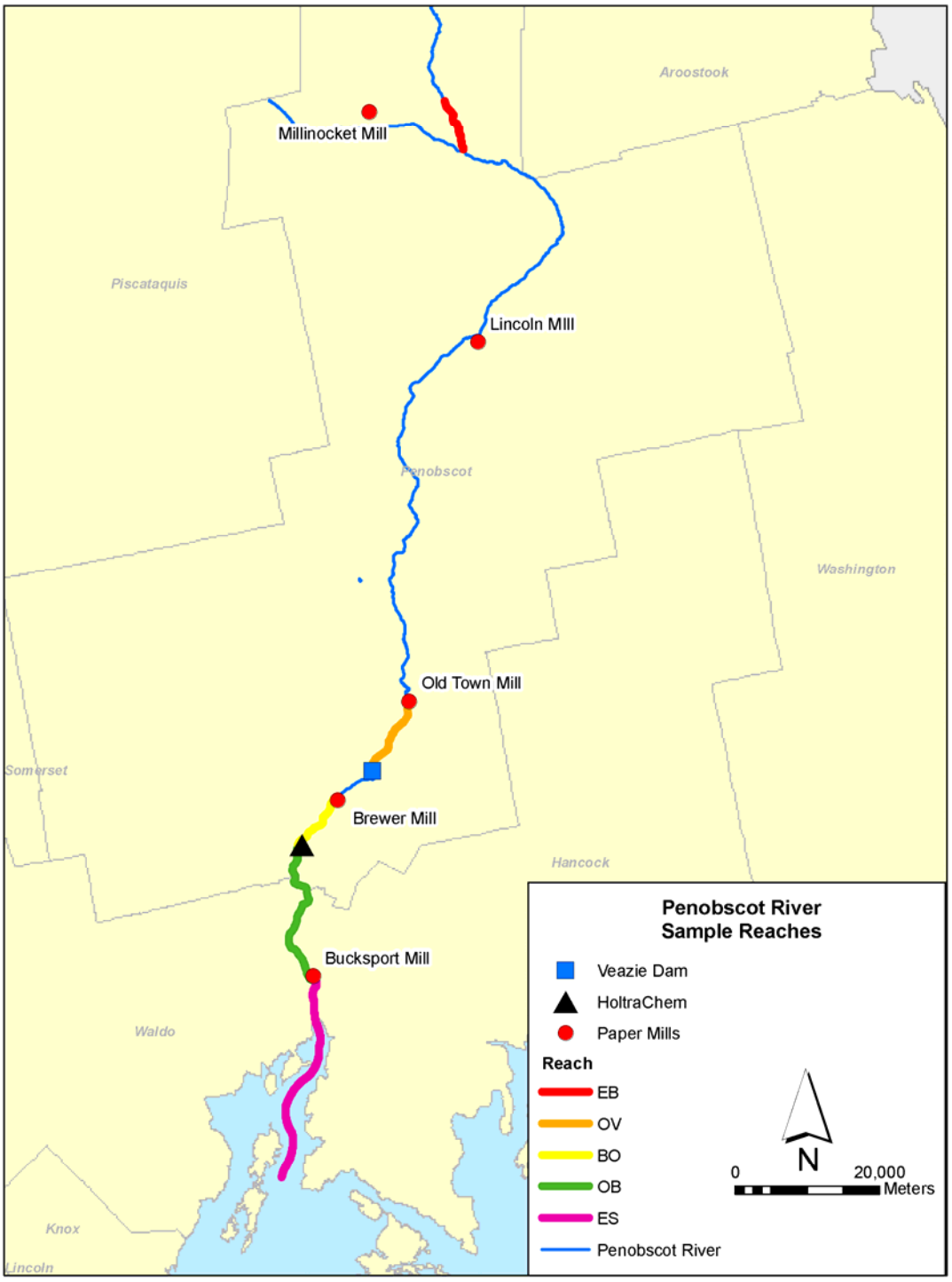

Figure 2. Map of the Penobscot River and estuary showing the locations of sampling reaches, active and inactive paper mills, the Veazie Dam, and the HoltraChem site at Orrington. The locations of sampling sites within each reach are shown in Figures 3 - 7. The river is tidal to a point located between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

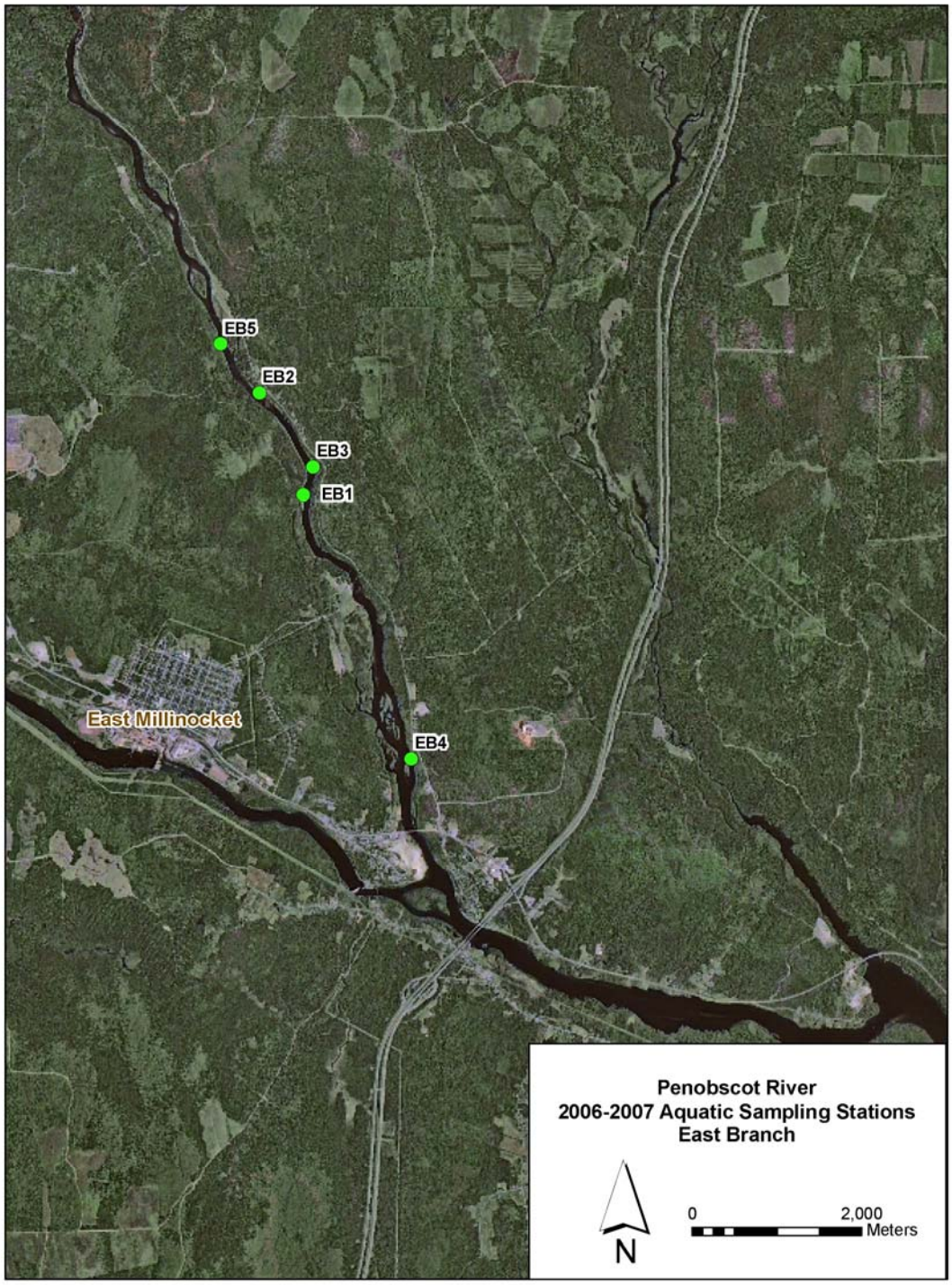

Figure 3. Location of East Branch sampling sites, 2006-2007.

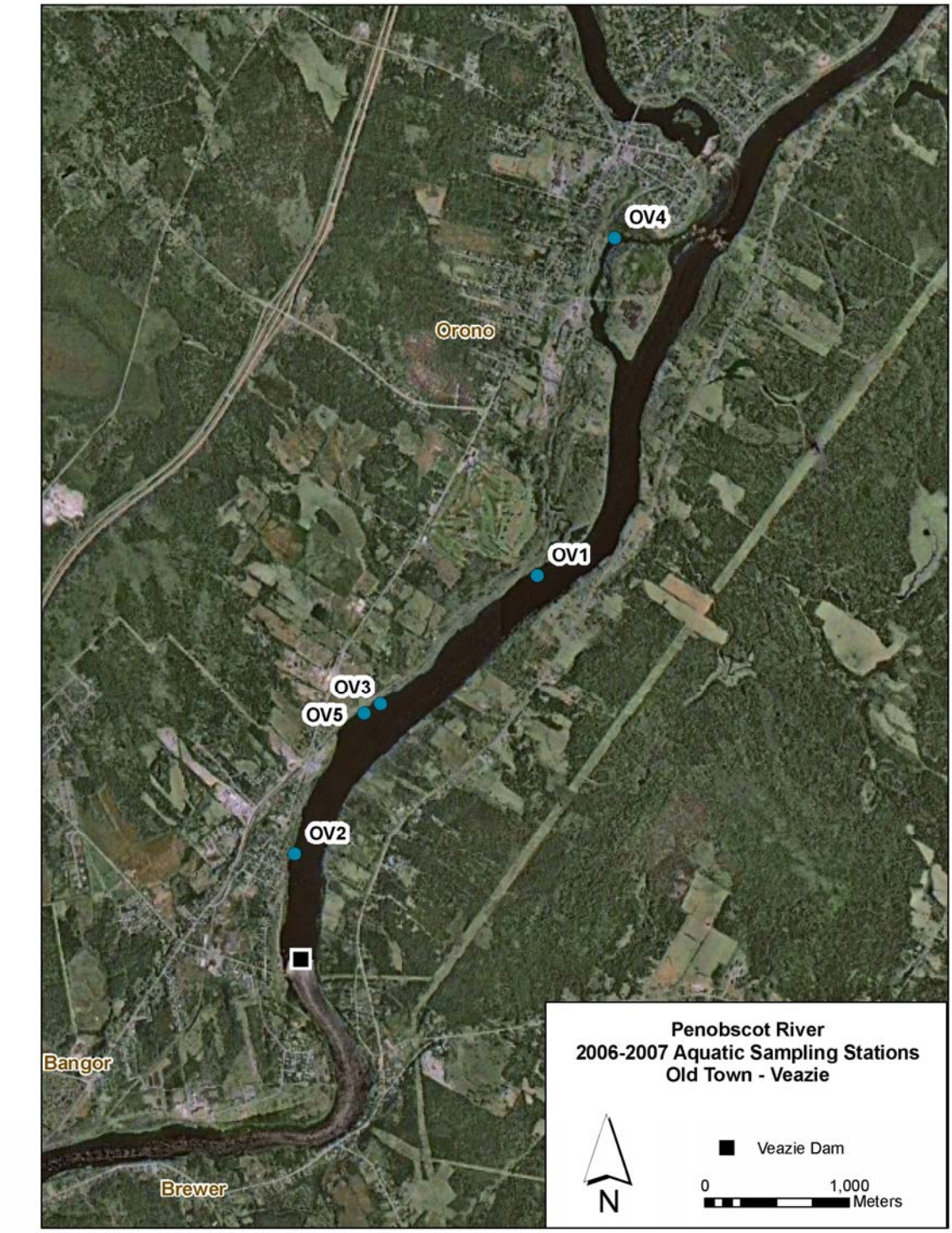

Figure 4. Location of Old Town – Veazie sampling sites, 2006-2007.

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

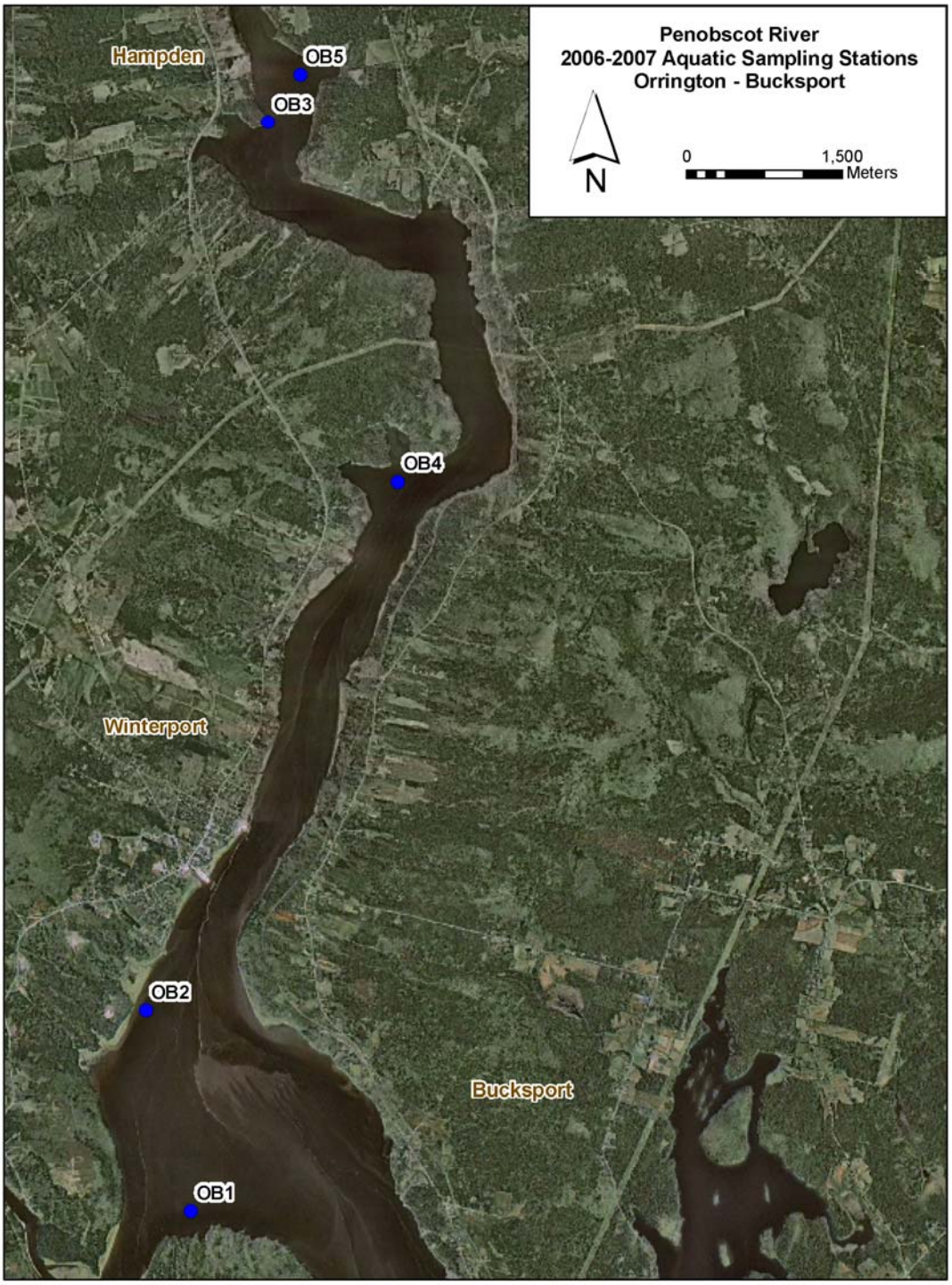

Figure 6. Location of Orrington – Bucksport sampling sites, 2006-2007.

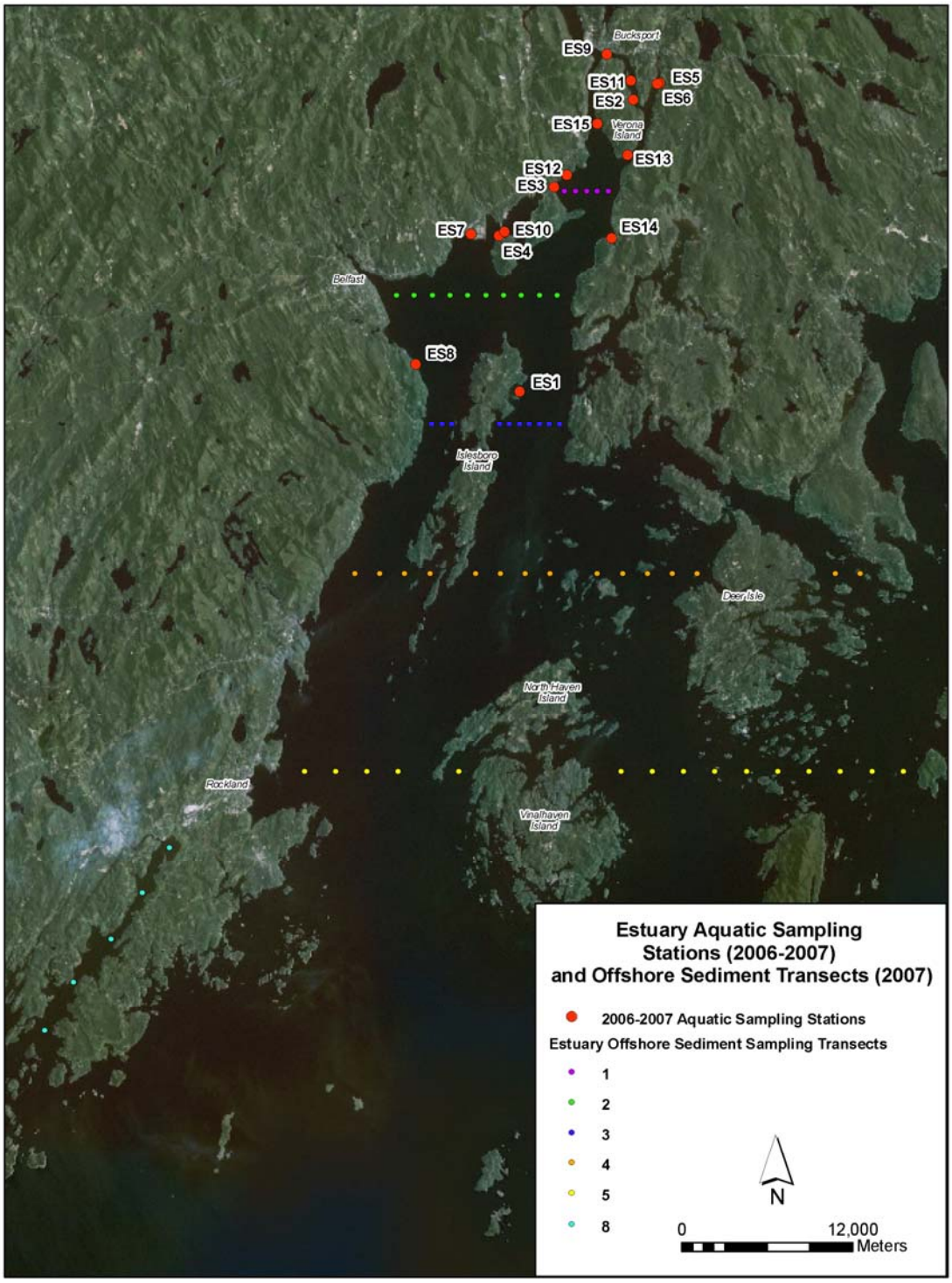

Figure 7. Location of Estuary (ES) aquatic sampling sites, 2006-2007. Also shown are the locations of sampling transects and sites in the estuary sampled for offshore surficial sediments in August 2007. Transects 1-5 are in the Penobscot estuary; Transect 8 is in the St. George estuary.

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 ± SD) 0.05 ± 0.01 to 0.08 ± 0.05 g d.w., while weights from Penobscot Bay sample sites were greater and more variable, ranging from 0.10 ± 0.09 g d.w. at ES09 at the north tip of Verona Island, to 0.61 ± 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 arenia*)

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.

Table 1. Summary of the invertebrate and fish samples collected and an outline of the statistical analyses performed. NS = not significant, Sig Var = significant variation, LOG = log transformation, SQRT = square root transformation.

SPECIES	SAMPLES COLLECTED by REACH and PERIOD												Total Hg					Methyl Hg					% Methyl Hg				Size			
	EB	OV		BO		OB				ES			THg transformed?	THg v. size	THg v. Period	THg v. Site	THg v. Reach	MeHg transformed?	MeHg v. size	MeHg v. Period	MeHg v. Site	MeHg v. Reach	% MeHg v. Size	% MeHg v. Period	% MeHg v. Site	% MeHg v. Reach	Size transformed?	Size vs. Period	Size vs. Site	Size vs. Reach
	III	III	VI	III	VI	I	II	III	VI	I	II	III																		
INVERTEBRATES																														
Nereis worms						10	12	12		70	116	114	LOG	Sig Var	Sig Var	Sig Var	Sig Var	LOG	NS	Sig Var	Sig Var	Sig Var	NS	NS	Sig Var	NS	LOG	Sig Var	Sig Var	Sig Var
Macoma clams							30	22			15	20	LOG	Sig Var	NS	NS	NS	SQRT	NS	NS	Sig Var	Sig Var	NS	NS	Sig Var	Sig Var	RAW	NS	Sig Var	NS
Soft-shelled clams *Mya arenia*							1			12	61	77	LOG	NS	Sig Var	Sig Var	—	LOG	NS	Sig Var	Sig Var	—	NS	NS	Sig Var	—	LOG	NS	Sig Var	—
Green Crab *Carcinus maenas*										9	76	68	LOG	Sig Var	Sig Var	Sig Var	—	LOG	Sig Var	Sig Var	Sig Var	—	NS	NS	NS	—	LOG	Sig Var	Sig Var	—
Rock Crab *Cancer irroratus*											57	32	LOG	NS	NS	Sig Var	—	LOG	NS	NS	Sig Var	—	NS	NS	Sig Var	—	RAW	Sig Var	Sig Var	—
FISH																														
Tomcod *Microgadus tomcod*				12				48			30	70	LOG	Sig Var	NS	Sig Var	Sig Var	RAW	Sig Var	NS	—	Sig Var	NS	NS	—	NS	LOG	NS	Sig Var	Sig Var
Banded killifish *Fundulus diaphanus*				26				9				1	RAW	Sig Var	—	Sig Var	Sig Var	RAW	Sig Var	—	—	NS	NS	—	—	NS	RAW	—	Sig Var	NS
Rainbow smelt *Osmerus mordax*								7				100	LOG	NS	—	Sig Var	Sig Var	RAW	NS	—	Sig Var	Sig Var	NS	—	NS	NS	RAW	—	Sig Var	Sig Var
Winter flounder *Pleuronectes americanus*												83	LOG	Sig Var	—	Sig Var	—	LOG	Sig Var	—	Sig Var	—	NS	—	NS	—	LOG	—	Sig Var	—
Golden shiner *Notemigonous crysoleucas*				11				9					RAW	NS	—	NS	NS	RAW	NS	—	—	NS	NS	—	—	NS	RAW	—	NS	NS
American eel *Anguilla rostrata*			24		78				46				LOG	Sig Var*	—	—	Sig Var	RAW	NS	—	—	NS	NS	—	—	NS	LOG	—	Sig Var	Sig Var

*age only

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 ES01on 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-variates.

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^{o}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^{o}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 co-variate 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^{o}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 ±

14.5 mm, mean weight 5.0 ± 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 ± 68 mm, mean weight 107 ± 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^{o}C at the lab. Muscle was sub-sampled from thawed fish for Hg analyses. Fish length (mean ± SD, 112.8 ± 21.1) and weight (15.5 ± 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 1st and July 1st, whereas the 2006 samples had been collected between July 13th and August 16th. 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.

		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										
		2007		2007			2007		07	2006		2007				2006		2007					2007										
BIRD SPECIES	tissue sample	June	July	June	July	August	June	July	June	July	August	May	June	July	August	July	August	June	July	August	September	May	June	July	August	THg distribution	THg v. size	THg vs. Sample Date	THg v. Age Class	THg v. Site	Size distribution	Size vs. Site
Double-crested cormorant *Phalacrocorax auritus*	egg															39	1	86	19							LOG	NS	NS	—	Sig Var	LOG	NS
Black guillemot *Cepphus grille*	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 *Ammodramus caudacutus*	blood									10	8		3	78									5	5		RAW	NS	NS	Sig Var	Sig Var	RAW	NS
	feathers									9	3		4	74												non-para metric	NS	NS	Sig Var	—	RAW	NS
Song sparrow *Melospiza meloodia*	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 *Melospizq georgiana*	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 *Agelaius phoeniceus*	blood												3	32												LOG	NS	NS	NS	Sig Var	RAW	NS
	feathers												3	32												LOG	NS	NS	Sig Var	NS	RAW	NS
Virginia Rail *Rallus limicola*	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)

		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									
		2007		2007			2007		07	2006		2007				2006		2007				2007									
BIRD SPECIES	tissue sample	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 *Ceryle haliaetus*	AD blood	2	2										3					2								LOG	NS	NS	Sig Var	—	—	—
	AD feathers	2	2										3					1								LOG	NS	NS	—	—	—	—
	JUV blood		26					2						13												LOG	NS	NS	Sig Var	Sig Var	RAW	Sig Var
Osprey *Pandion haliaetus*	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 *Haliaeetus leucocephalus*	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 3rd and September 2nd, 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[2]. In the top 3 cm of the sediment cores, while some sites were higher than others, there was little overall geographic trend trough 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-

[2] 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 realtionship 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.

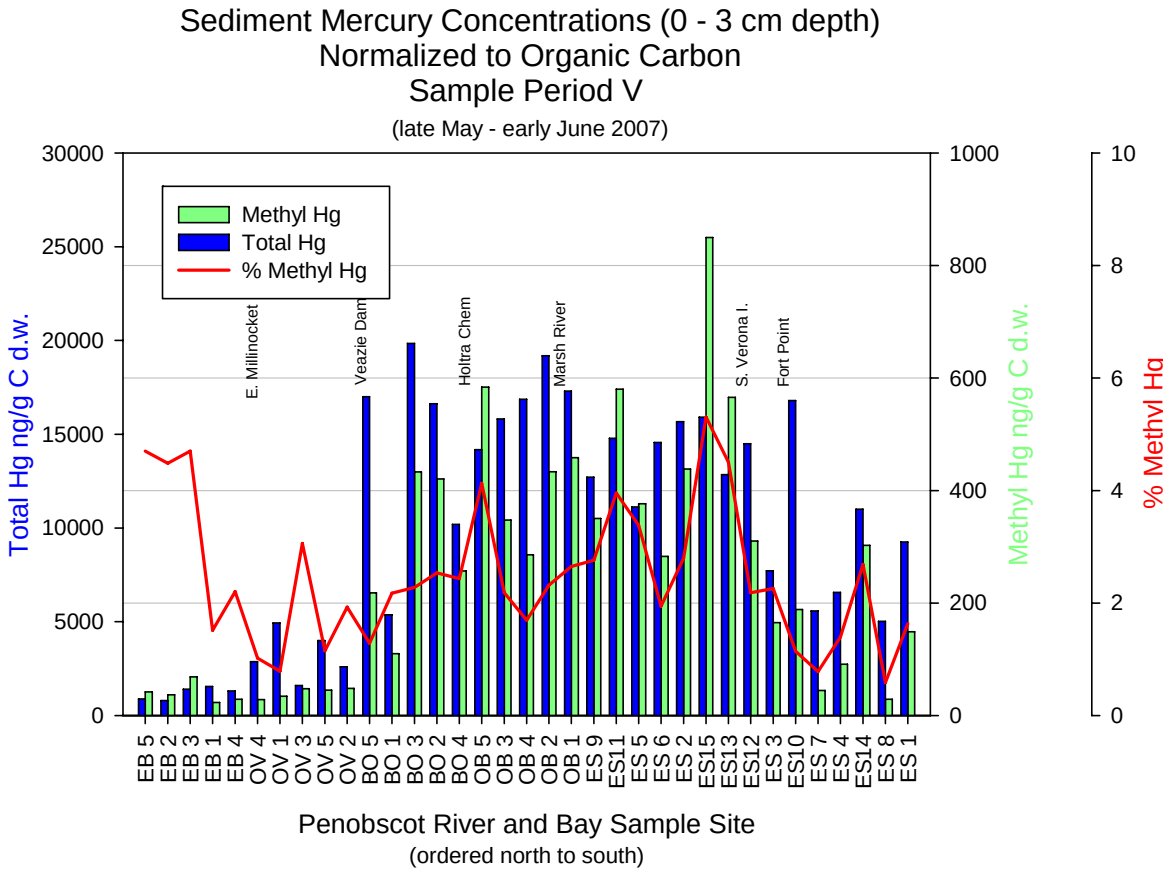

Figure 8. Total Hg concentrations, methyl Hg concentrations and % methyl Hg in the surface 3 cm of sediments in the Penobscot River and Estuary, Sampling Period V, May 2007. Total Hg concentrations were normalized to organic carbon. Sampling sites are ordered from north to south. The locations of sampling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

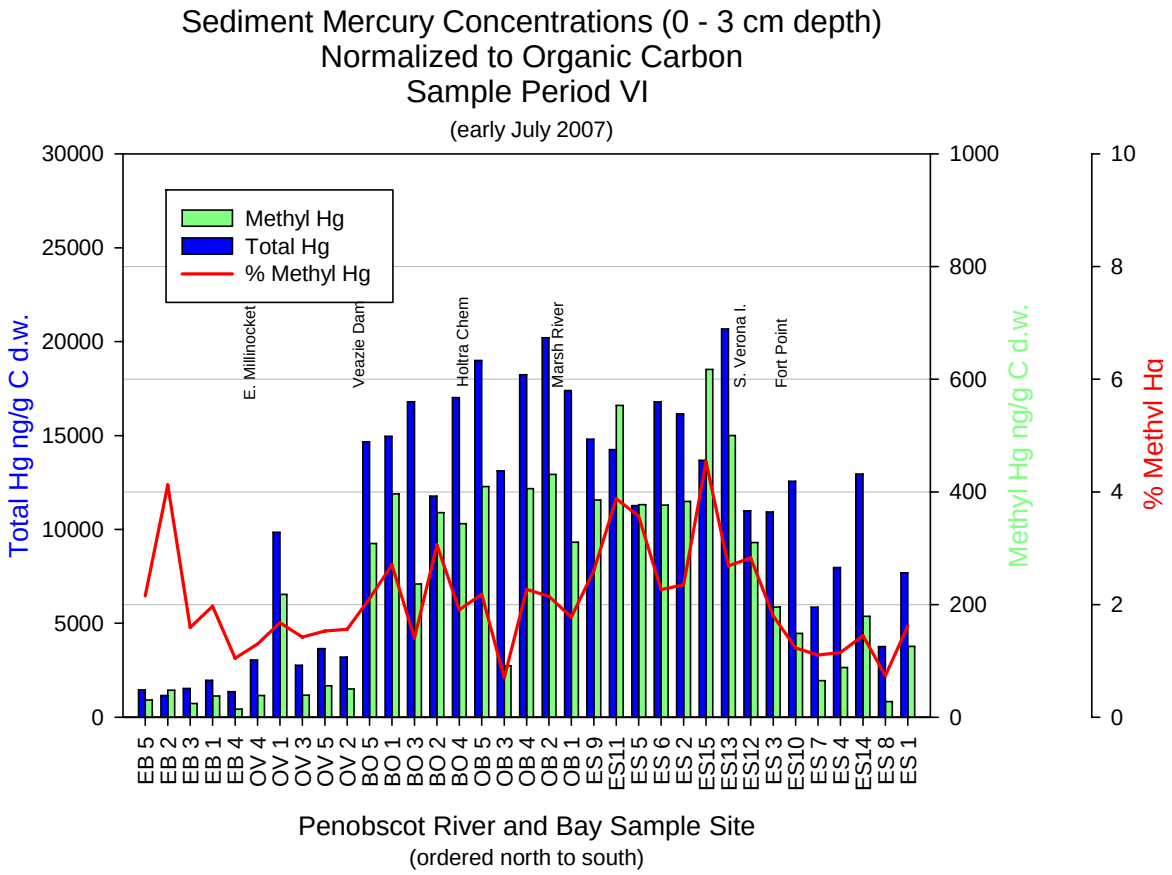

Figure 9. Total Hg concentrations, methyl Hg concentrations and % methyl Hg in the surface 3 cm of sediments in the Penobscot River and estuary, Sampling Period VI, July 2007. Total Hg concentrations were normalized to organic carbon. The location of sampling sites is shown in Figures 2-7.

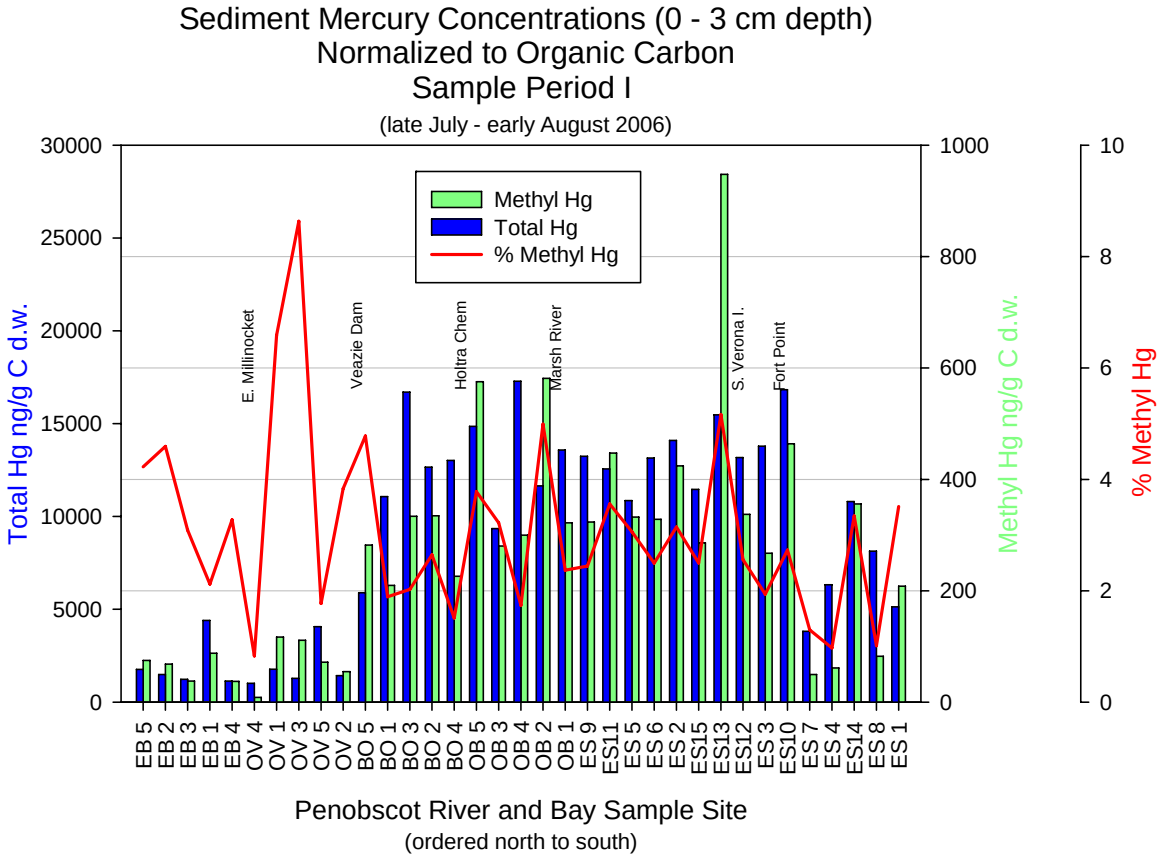

Figure 10. Total Hg concentrations, methyl Hg concentrations and % methyl Hg in the surface 3 cm of sediments in the Penobscot River and Estuary, Sampling Period I, August 2006. Total Hg concentrations were normalized to organic carbon. Sampling sites are ordered from north to south. The location of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

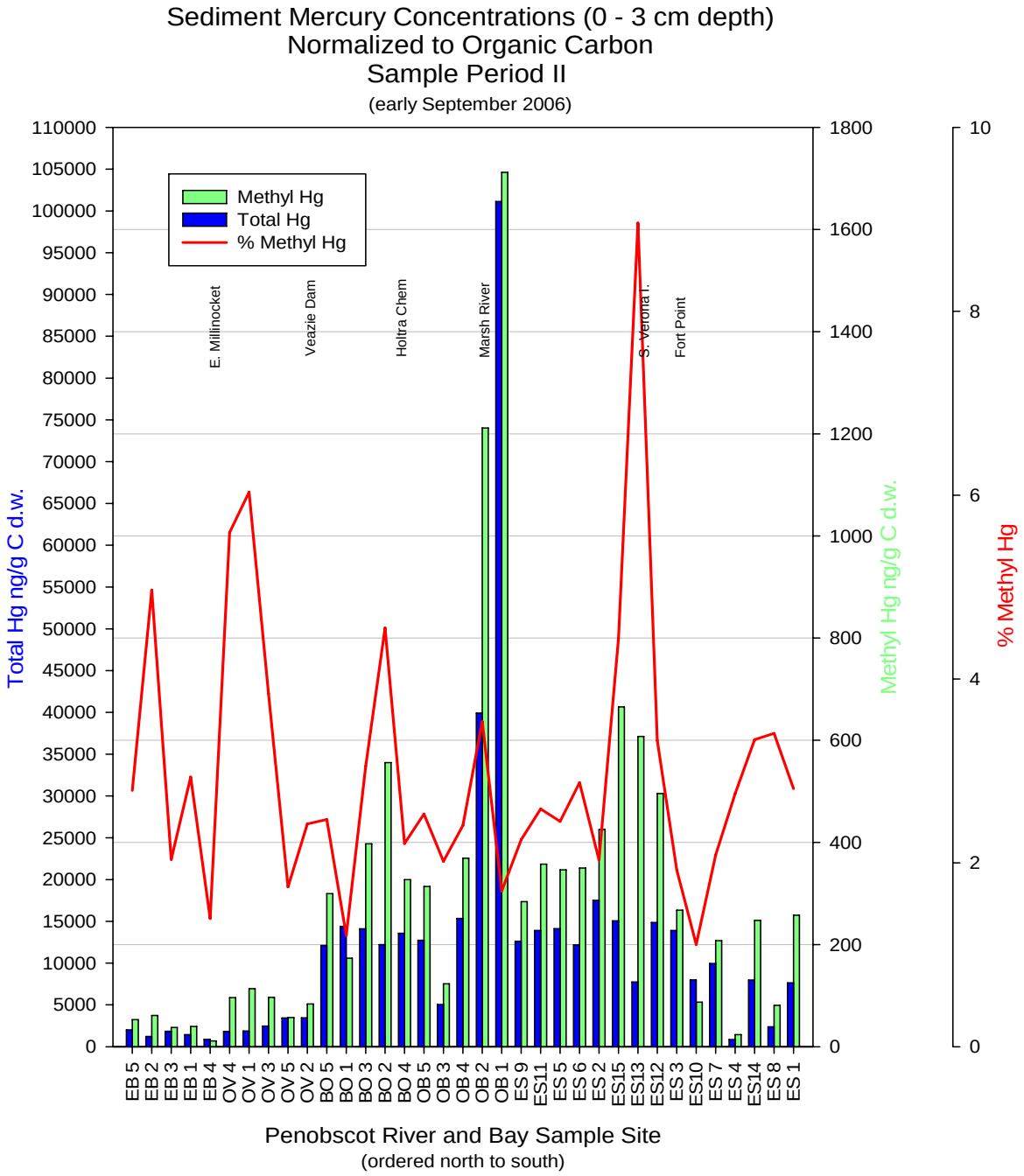

Figure 11. Total Hg concentrations, methyl Hg concentrations and % methyl Hg in the surface 3 cm of sediments in the Penobscot River and Estuary, Sampling Period II, early September 2006. Note difference in scale from other figures in this series. Total Hg concentrations were normalized to organic carbon.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

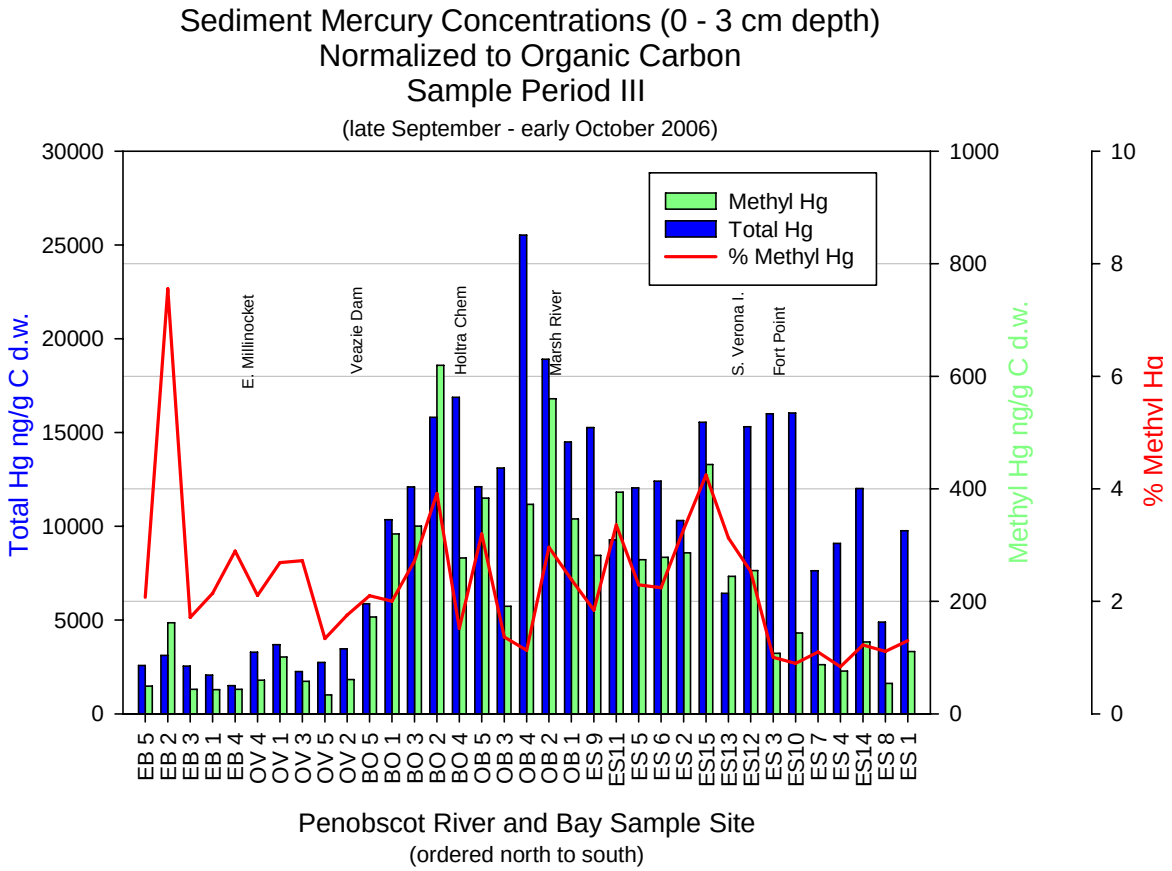

Figure 12. Total Hg concentrations, methyl Hg concentrations and % methyl Hg in the surface 3 cm of sediments in the Penobscot River and estuary, Sampling Period III, late September – early October 2006. Total Hg concentrations were normalized to organic carbon. Sampling sites are ordered from north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

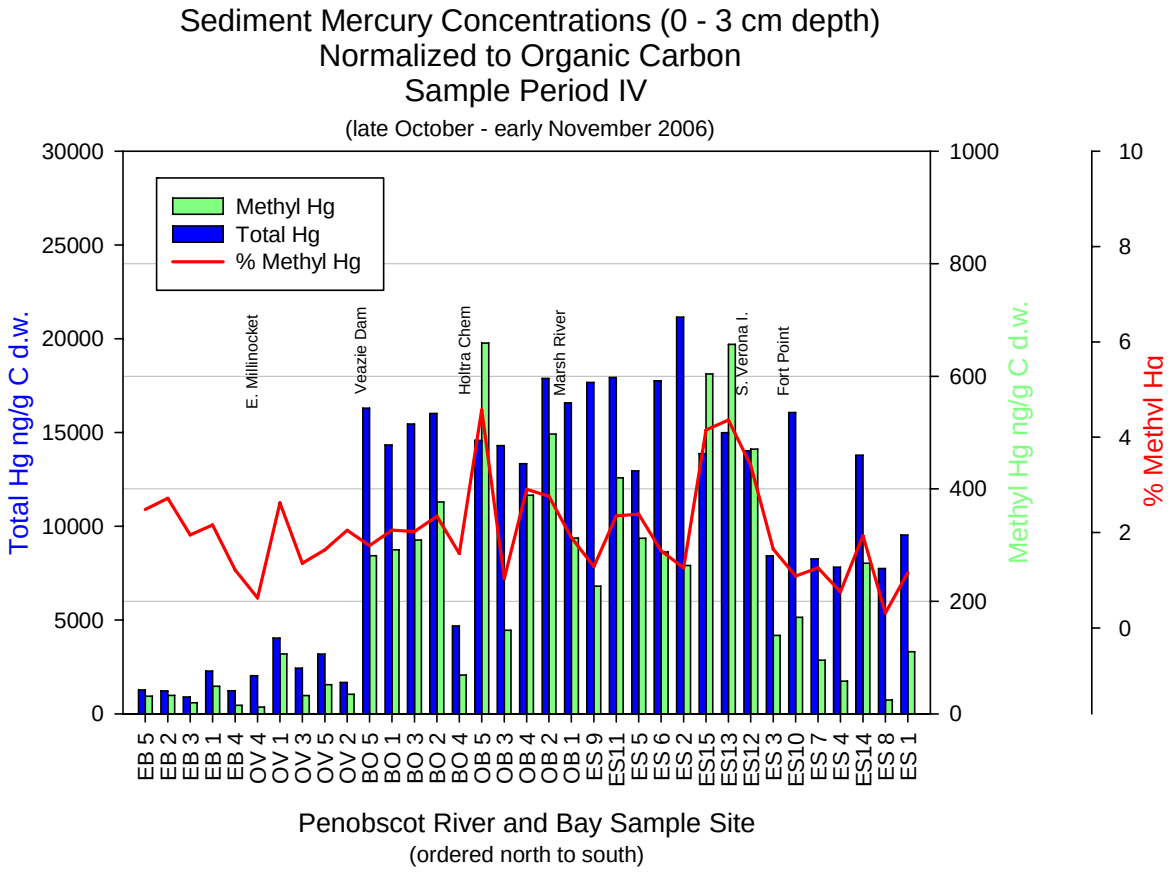

Figure 13. Total Hg concentrations, methyl Hg concentrations and % methyl Hg in the surface 3 cm of sediments in the Penobscot River and estuary, Sampling Period IV, late October 2006. Total Hg concentrations were normalized to organic carbon. Sampling sites are ordered from north to south. The location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

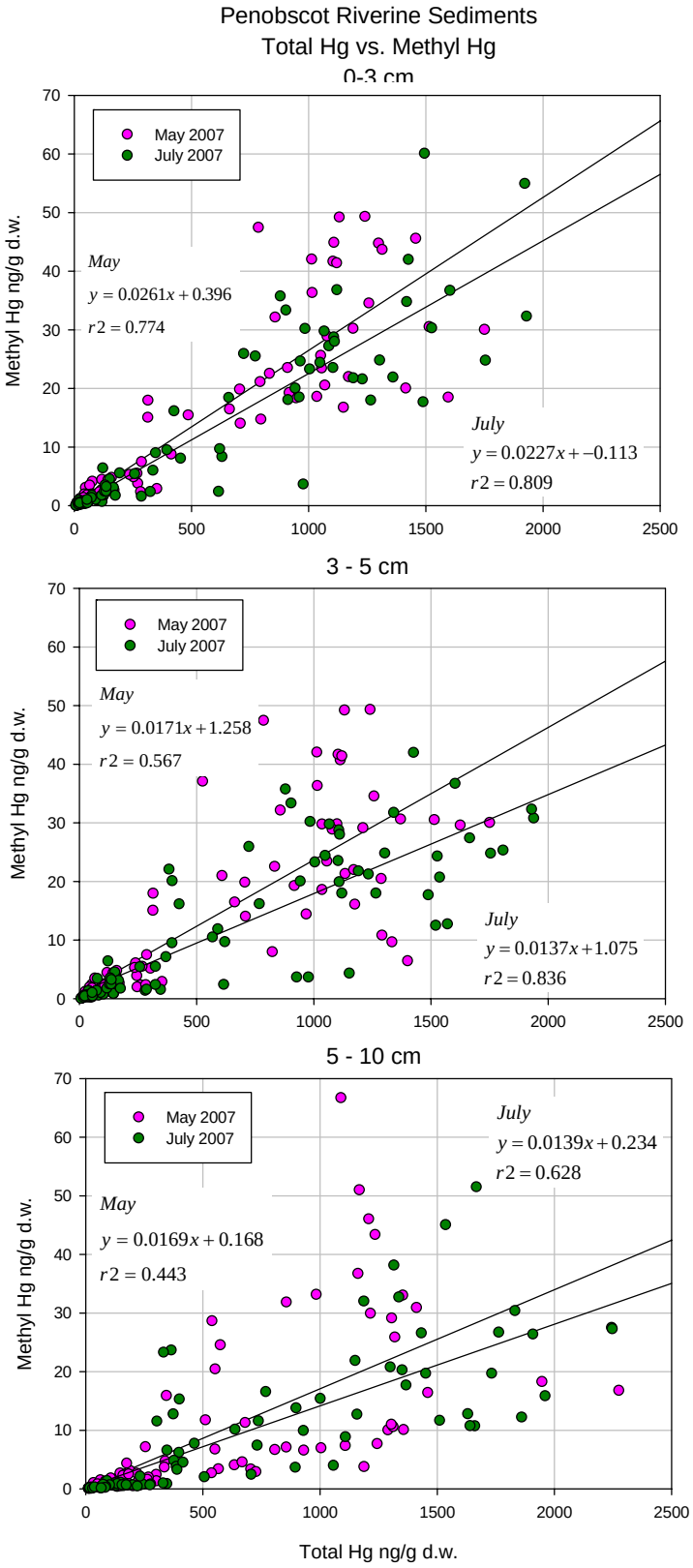

Figure 14. Relationships of methyl Hg to total Hg at three depths showing that methylation rates are greater in the sediments sampled at 0-3 cm

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.

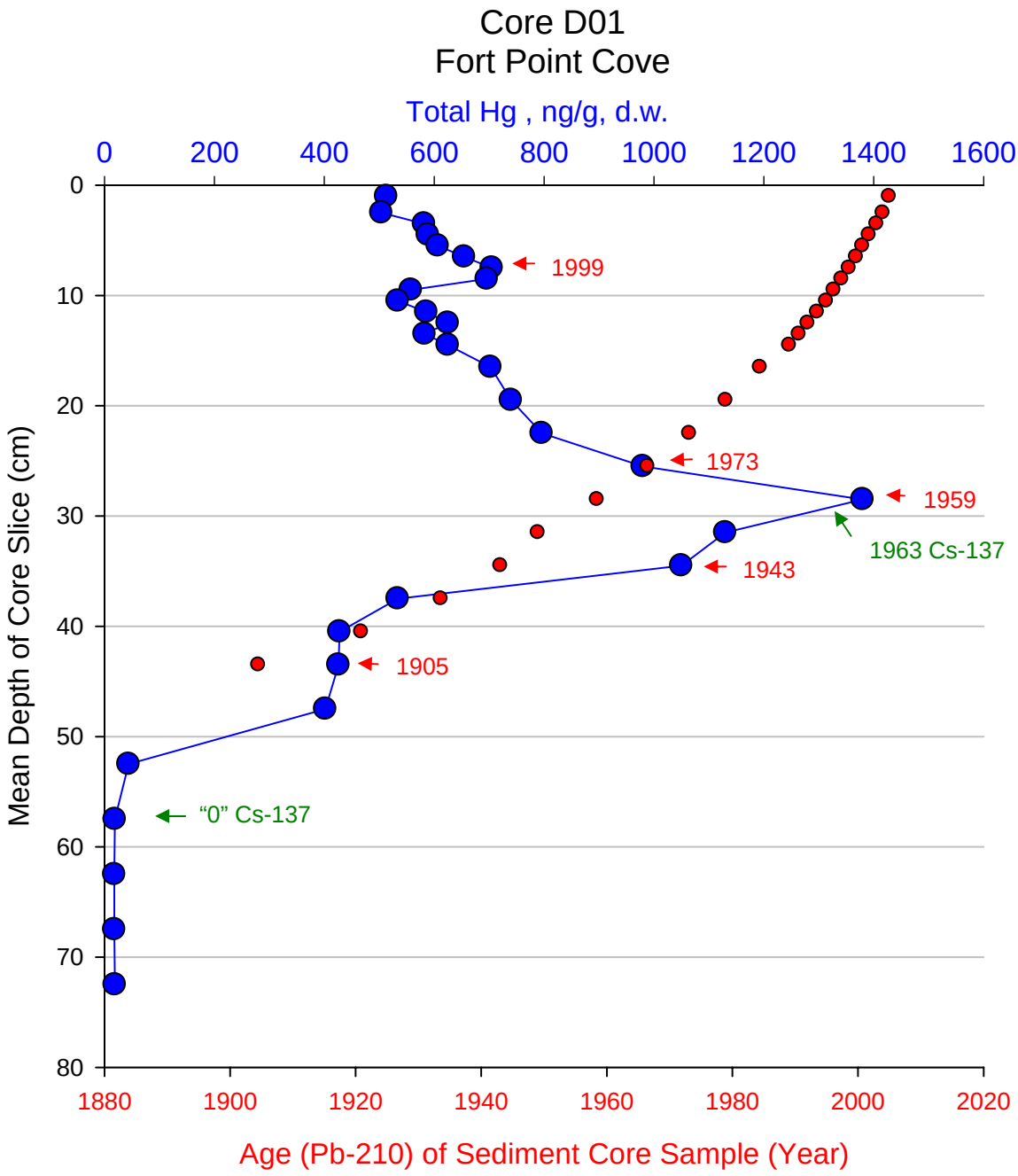

Figure 15. Total mercury concentrations, Pb-210, and Cs-137 dating of sediment core (D-01) taken at Fort Point Cove, N44.48233 W68.8087. August 20, 2007. Raw data given in Appendix 1.

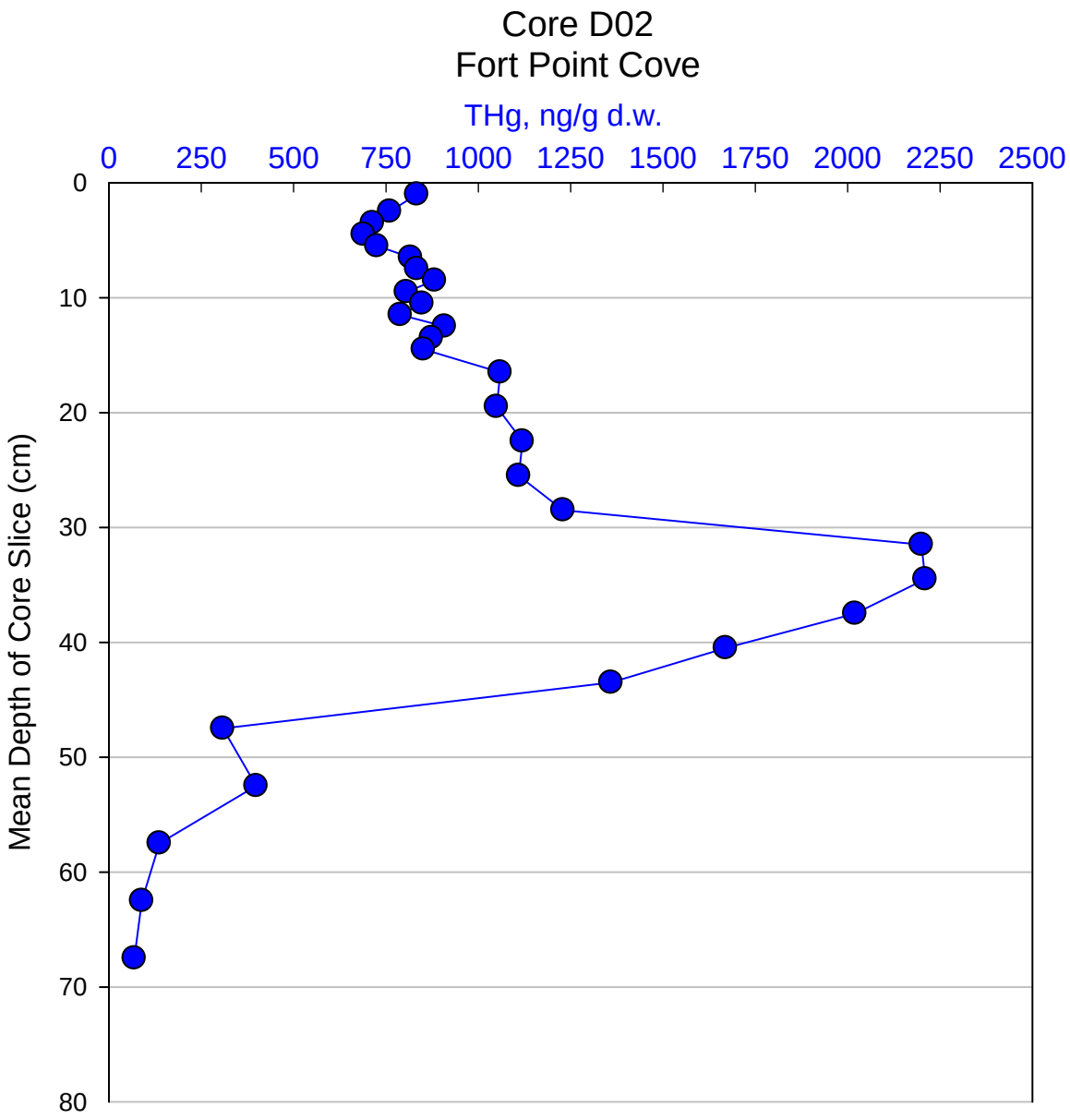

Figure 16. Total mercury concentrations in sediment core (D-02) taken at Fort Point Cove, (N44.48418, W68.81883), August 22, 2007. Raw data are given in Appendix 2.

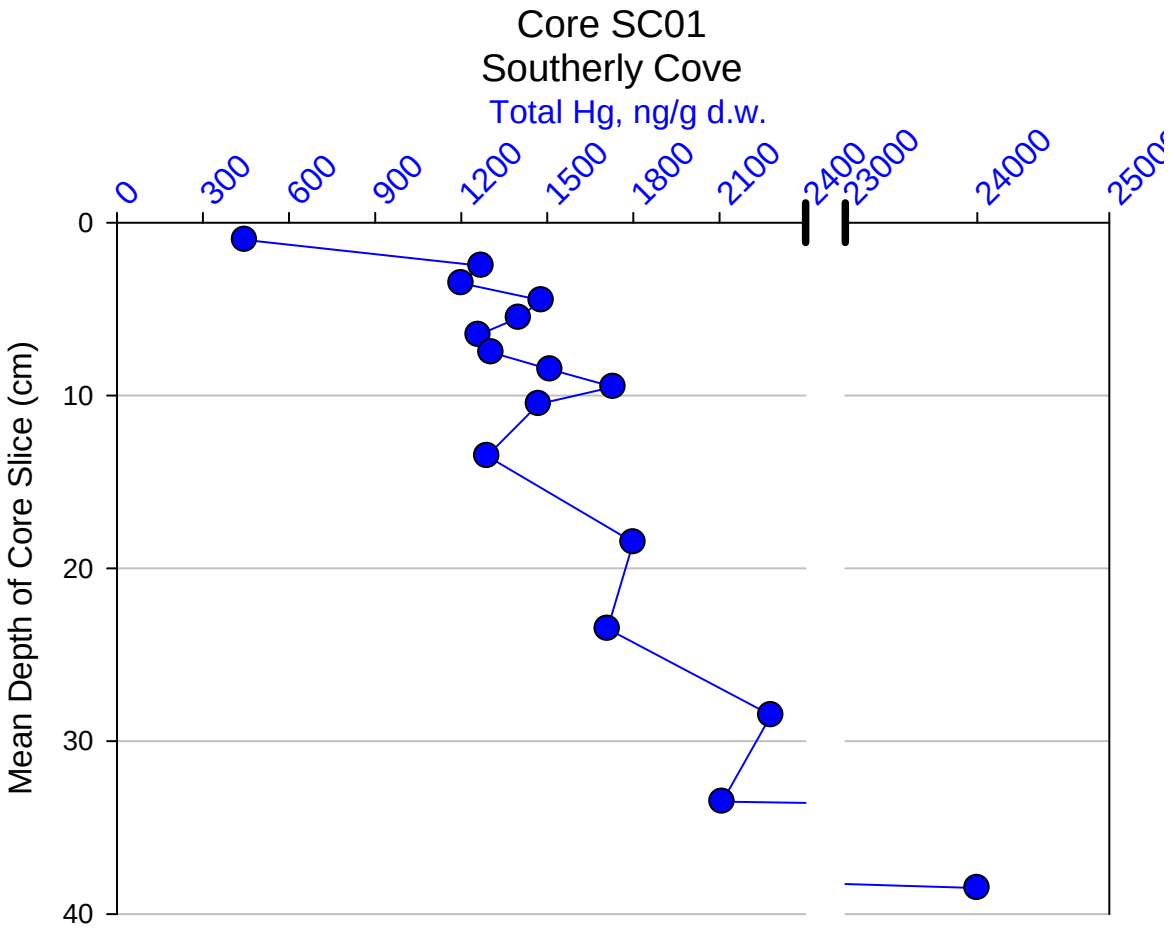

Figure 17. Total mercury concentrations in sediment core SC 01 taken at Southerly Cove (N44.73737, W68.82926), August 16, 2007. Raw data for this core are provided in Appendix 3.

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 know 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.

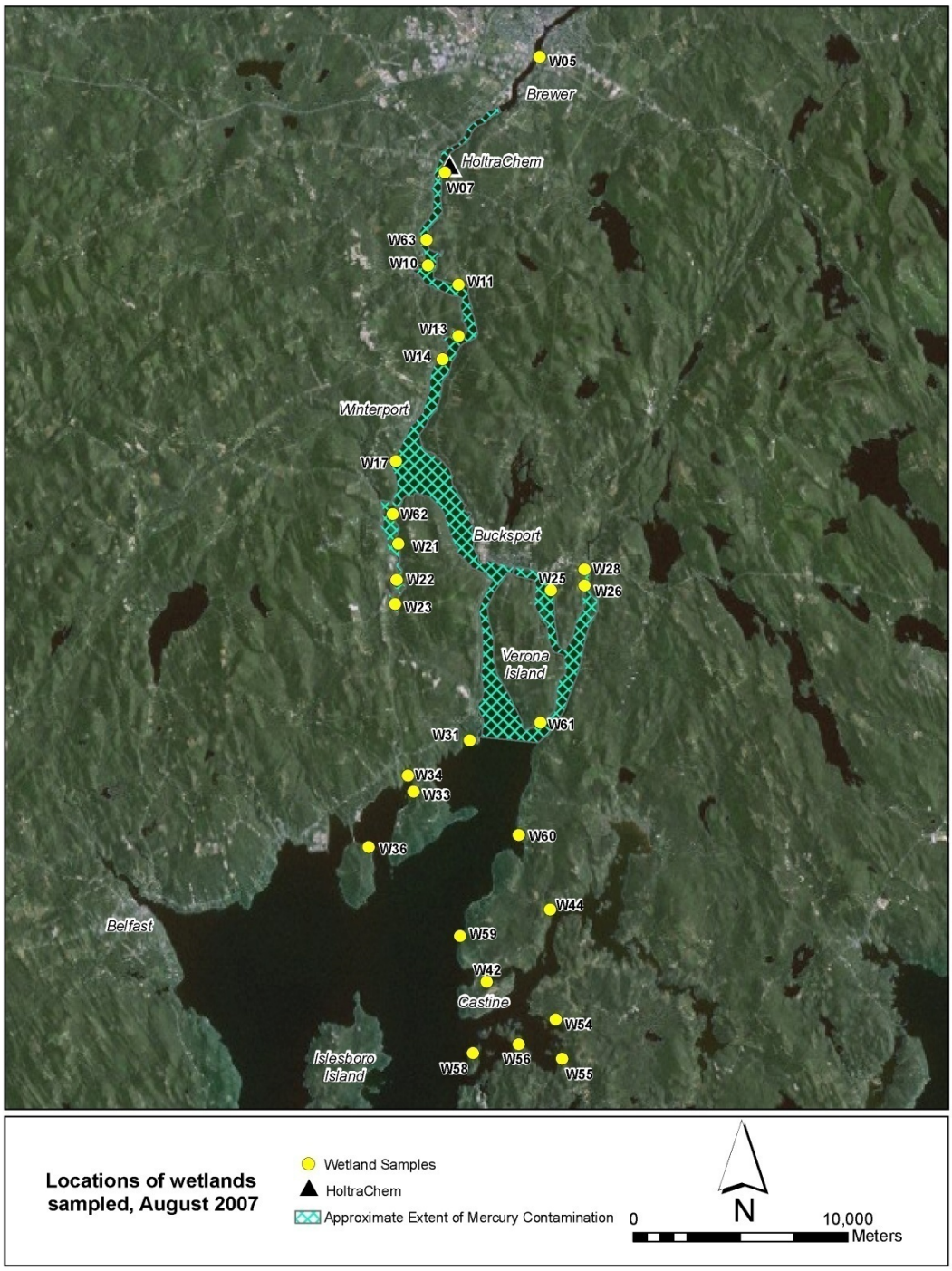

Figure 18. Map of wetland locations sampled for mercury in August 2007. Hatched area corresponds to the approximate extent of the most contaminated wetlands.

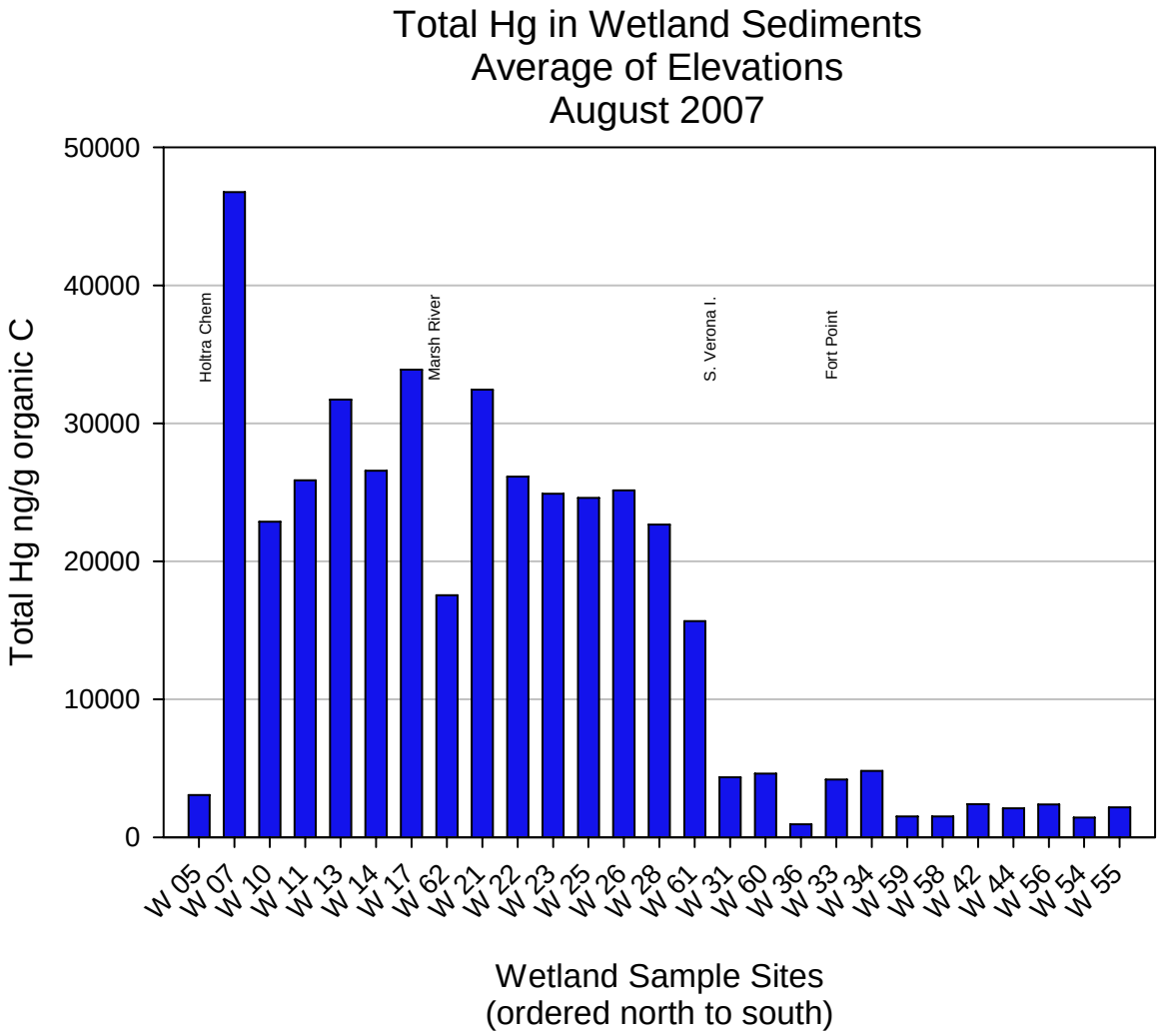

Figure 19. Concentrations of total Hg in soils of riparian wetlands in the lower Penobscot River and upper estuary. Sites are ordered from north to south. HoltraChem is adjacent to W 07 and Ft. Point lies south of W 31. Each wetland was sampled at four elevations, this graph presents the average total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

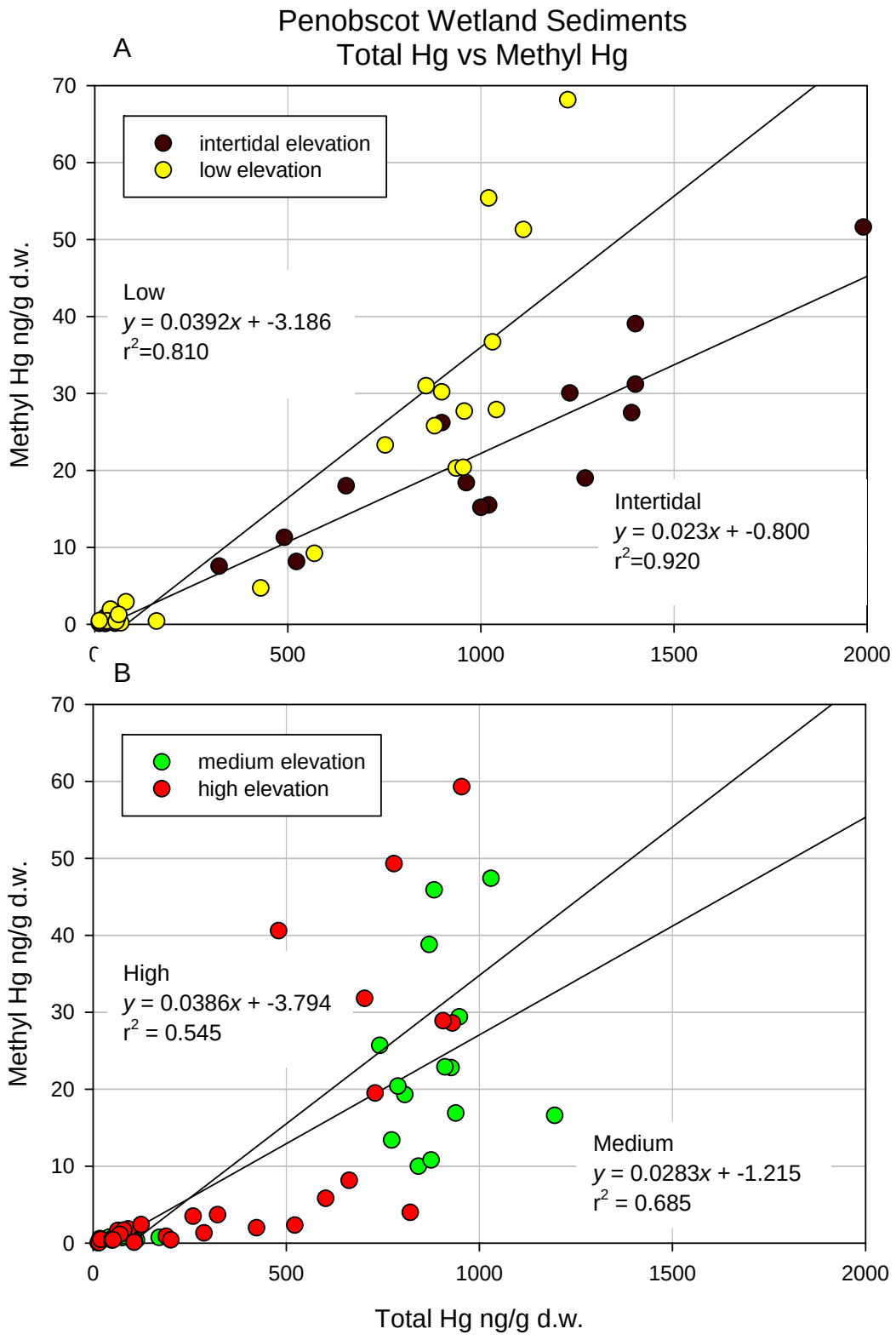

Figure 20. Relationships between methyl Hg and total Hg concentrations in wetland sediments at four different wetland elevations.

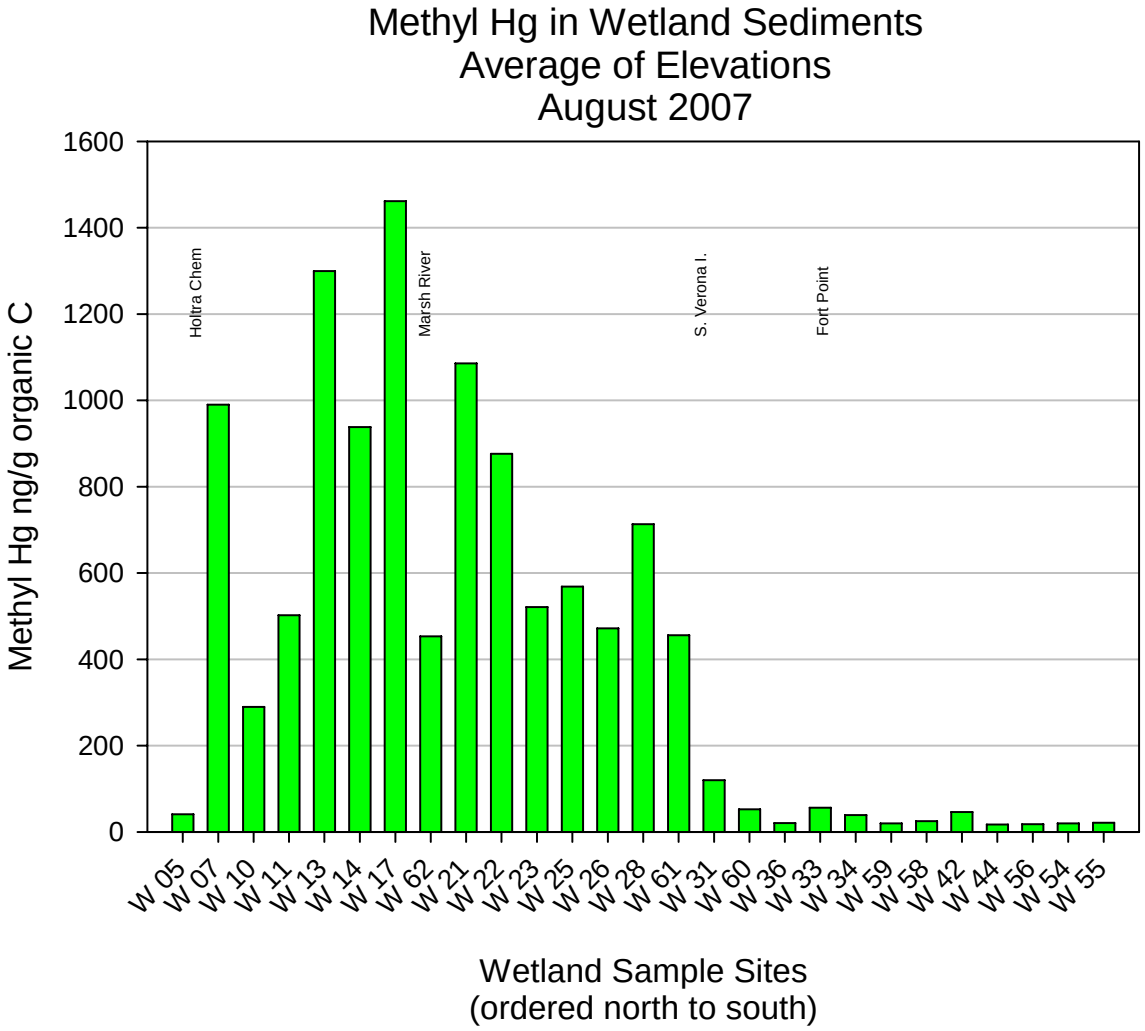

Figure 21. Concentrations of methyl Hg in soils of riparian wetlands in the lower Penobscot River and upper estuary. Sites are ordered from north to south. HoltraChem is adjacent to W 07 and Ft. Point lies south of W 31. Each wetland was sampled at four elevations, this graph presents the average methyl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

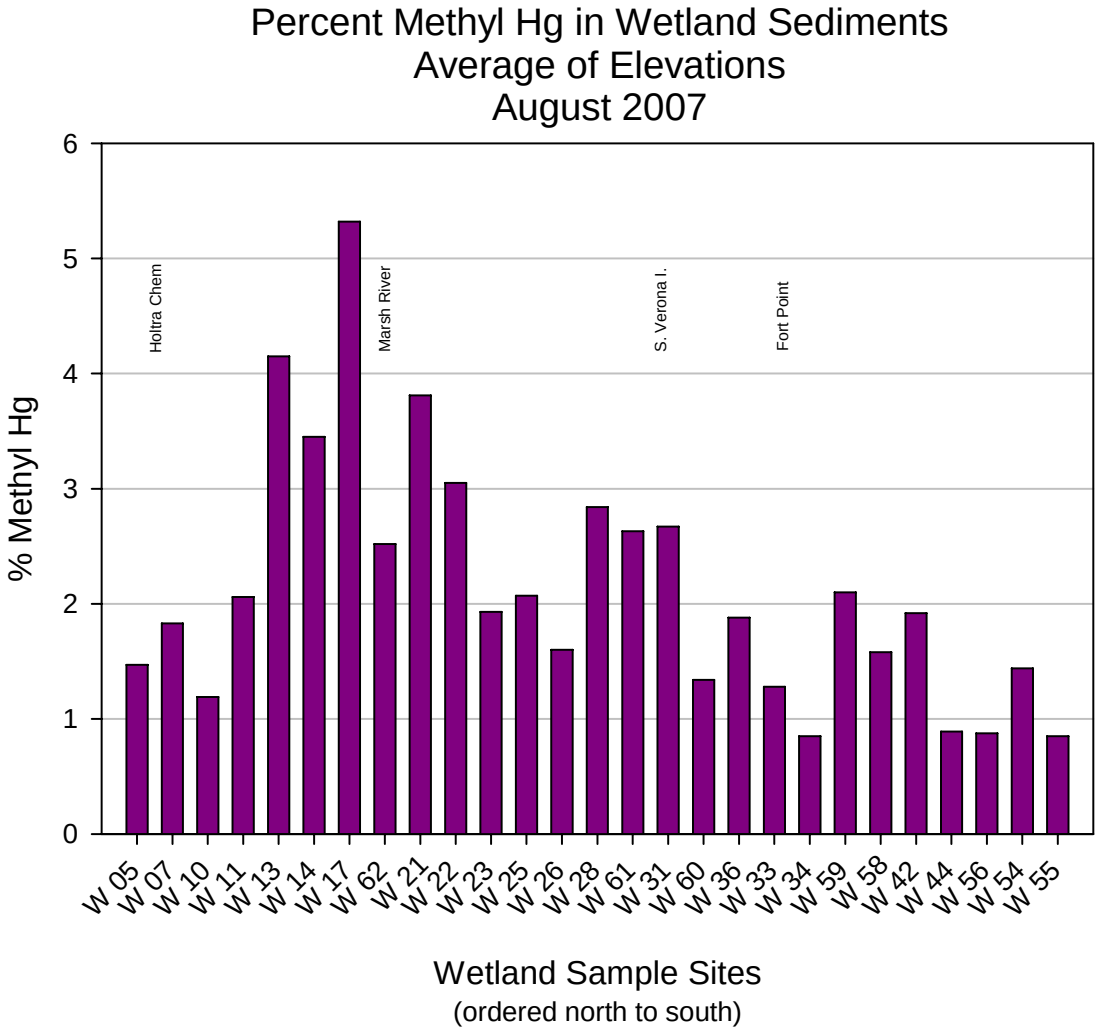

Figure 22. Percent methyl Hg in soils of riparian wetlands in the lower Penobscot River and upper estuary. Sites are ordered from north to south. HoltraChem is adjacent to W 07 and Ft. Point lies south of W 31. Each wetland was sampled at four elevations, this graph presents the average percent methyl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