

Penobscot River Mercury Study

Results of 2012 monitoring of mercury in Penobscot River and Bay With comparisons to previous years

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EXECUTIVE SUMMARY

The purpose of this report is to present data from the monitoring of mercury (Hg) in water, sediments and biota from 2012, and compare those data to those from previous years in the Penobscot system. Sampling in 2012 represented the second year (after 2010) of the proposed long term monitoring program of Hg in the Penobscot. Results from a number of sampling efforts prior to 2010 were also included where appropriate. We are especially trying to determine whether significant declines in Hg in water, surface sediments, aquatic biota, and marsh songbirds are evident over time since study of the system began in 2006.

In view of the long estimated recovery halftimes of the upper Penobscot estuary ($T_{1/2}$, 22 - 78 years - Phase II Report), we were not expecting to observe any statistically significant ecosystem level change in mercury concentrations in the upper estuary during the timeframe of 2006 and 2012, and as expected we saw no change. So far, the Long-Term Monitoring Program is accomplishing its goals, which are: 1) To confirm that the that recovery of the upper estuary from mercury contamination will continue to be a long-term multi-decadal process, as it has been in the past, and 2) To produce a data set, which can be used in the future, as a baseline of mercury concentrations in the ecosystem. This baseline will be used to confirm the success of any active remediation option, which may be applied to the upper estuary of the Penobscot in the future.

Mercury concentrations in water showed no trend over time in the period from 2006 through 2012. Dissolved total mercury was usually between 1 and 3 ng/L and dissolved methyl Hg was usually between 0.05 and 0.2 ng/L. Concentrations of Hg in particles coming into the estuary from the river flow at Veazie Dam were consistent over time, between 0.2 and 0.4 ug/gdw, while particulate Hg concentrations in the river water below Veazie Dam were generally greater and much more variable, with total Hg values ranging between 0.2 and 1.3 $\mu\text{g/gdw}$. Unfiltered total Hg and methyl Hg in water (ng/L) varied in response to TSS and particulate Hg concentrations..

Hg in surface sediments in the Penobscot did not show consistent trends over the period 2006 to 2012. In subtidal sediments near Fort Point, there was almost no change in total Hg or methyl Hg. In intertidal sediments sampled throughout the main stem of the river and into Penobscot Bay, there were no overall significant trends, although one site in the contaminated zone showed a significant decline in total Hg in the period ending in 2012. Methyl Hg was more variable than total Hg in intertidal sediments, showing declines at two sites in the contaminated zone, including the one site that showed a decline in total Hg. Similarly, in wetland sediments, there were no consistent patterns of change in total or methyl Hg at any of the four elevations sampled, while one site, W21 in Mendall Marsh, showed significant declines at the mudflat elevation in both 2010 and 2012. Similar geographic patterns were seen in 2012 as in previous years, with sites nearer to HoltraChem being higher in Hg.

Mercury concentrations in fish during the 2006-2012 monitoring period showed no significant changes in most species, with the exception of rainbow smelt. Rainbow smelt had significant declines in total Hg concentrations at most of the sites sampled in 2012. Total Hg concentrations were unchanged in eels and winter flounder. Tomcod and mummichogs did show significant declines at one site each.

In shellfish, significant declines in Hg in mussels were observed at sites in the northern part of the range of the species, closer to the HoltraChem site, but not at sites further to the south. This observation is consistent with the observed declines in Hg in rainbow smelt, which are pelagic feeders like mussels, perhaps indicating the beginning of decreases of Hg and methyl Hg in the water column. Mercury in lobster tail muscle declined significantly at three of seven sites sampled in 2012. Similar geographic patterns of Hg in shellfish were observed in 2012 as previously.

In birds, total Hg in blood sampled from Nelson's sparrows, swamp sparrows, and red-winged blackbirds sampled in marsh habitats in 2012 either increased or remained unchanged. In the contaminated sites along the lower Penobscot, Nelson's sparrows and red-winged blackbirds had, in 2012, the highest concentrations of Hg found during the 2007 through 2012 study period, while the blood from swamp sparrows was in the same range or lower than found in previous years.

Analysis of the ratios of stable nitrogen isotopes in shellfish, fish and birds found no evidence that differences in trophic levels within a species were the cause of geographic differences in Hg concentrations.

Power analyses were conducted to define the minimum monitoring period needed to provide enough power to confirm the absence of a significant trend in Hg in various media. Half times for recovery were taken from the latest estimates for different parts of the Penobscot system based on observations from dated sediment cores. For eels and other fish species, monitoring a minimum of 9 to 16 years would be required at reasonable sample sizes at most sites. Under similar assumptions about 12 years of monitoring would be required for most species of wetland birds at most sites, about 16 years for mussels and sediments, and about 20 years for black ducks, cormorants and lobsters.

INTRODUCTION

In 2006, the Penobscot River Mercury Study (PRMS) began a broad assessment of mercury (Hg) concentrations in biota, sediment and water in order to document Hg accumulations in the lower Penobscot River. The study was initiated in response to a 2003 decision in U.S. District Court on suspected Hg contamination of the lower Penobscot from Hg discharges from the HoltraChem chlor-alkali facility, located on the shore of the Penobscot River in the town of Orrington. Results presented in the PRMS Phase I report established geographic trends consistent with the HoltraChem site as the source of Hg contamination of the sediment in the lower river and upper portions of Penobscot Bay. The PRMS Phase I Update reported similar geographic trends in the majority of fish, shellfish and birds sampled in 2006 and 2007. Geographic trends in both sediment and biota were also found in subsequent years, 2008-2010, as described in the PRMS Phase II report, released in the spring of 2013.

In addition, the Phase II report described apparent trends in Hg concentrations in sediment and biota that were observed within the monitoring period 2006 through 2010. No significant trends in Hg concentrations over time were found at the majority of sites where fish, lobster and bird species were monitored between 2006 and 2010. There were some exceptions to this overall pattern. Between 2006 and 2010 significant declines in Hg concentrations were found in blue mussels sampled north of Fort Point, in the more contaminated portion of the study area. A similar decline in Hg concentrations was found in rainbow smelt sampled at sites east of Verona Island, but not at sites further to the north. Marsh birds showed extensive inter-annual variation, but no consistent trends were found in Hg at sites along the lower Penobscot. It is noteworthy, however, that any apparent temporal trends observed for Hg concentrations in biota over a short period (approximately five years) can be considered somewhat suspect because of interannual variations that arise as a result of numerous physiological and environmental conditions. While they may reflect a true change in time, longer trajectories are strongly advised in order to confirm these changes as temporal trends.

Mercury trends in both the sediment and the biota are important indicators of the recovery of the system. Several lines of evidence described in the Phase II report linked Hg concentrations in the sediment to Hg concentrations in most biota studied. Both stomach content and stable isotope analyses indicated that fish in the main stem of the Penobscot River fed in the benthic food web, with a gradual shift further south in the estuary to a pelagic food web for rainbow smelt and tomcod. Lobsters are naturally tied to the benthic food web, but access to bait in traps introduces pelagic fish into their diet. In contrast, blue mussels feed exclusively in the pelagic food web on phytoplankton. Marsh birds breeding in wetlands along the lower Penobscot, or ducks overwintering in those same marshes, feed on benthic invertebrates associated with either the aquatic sediment or marsh soils.

Mercury monitoring was continued in 2012 to document ongoing trends in the Penobscot study area. Decisions pending in Federal Court will define the response to the current levels of Hg

contamination in the lower Penobscot, either to enact active remediation to reduce Hg in sediment and biota, or to allow the natural attenuation of Hg concentrations in the contaminated area. Trends in Hg concentrations over time and space will indicate whether the chosen option reduces Hg accumulations in the system as planned.

The monitoring program was streamlined in 2012 in order to improve the strength of the data collected and to reduce the cost of the program. Fewer aquatic species and sites were retained in the 2012 monitoring design, which focused on maintaining a broad geographic coverage, sampling at sites with the longest sampling record, and standardizing the number of samples collected at each site. The marsh bird monitoring program was fundamentally changed, reducing the number of species and sites sampled, establishing specific sampling sites and dates, and locating a Downeast reference area outside of the aquatic influence of the HoltraChem plant.

METHODS

The 2012 monitoring design retained strategic elements of previous monitoring plans for the Penobscot study area, while reducing the number of species sampled and the number of sites monitored. Key sites were retained in each of the four study reaches (OV, Orono-Veazie; BO, Brewer-Orrington; OB, Orrington-Bucksport; ES, estuary). The sites where biota, sediment and water were sampled were co-located whenever possible in order to examine the movement of Hg from sediment and water to multiple levels of the aquatic and wetland food webs. Table 1 lists the specific sample types collected, sample sites, sample month and the number of samples collected from each site. Co-located and adjacent sites are grouped in the same line, illustrating the range of samples collected from each site. Figure 1 maps the locations of the monitoring sites within the lower Penobscot, but does not show distant reference sites. Figure 2 provides an overview of all of the 2012 sampling areas.

Biota Sample Collections

Fish - Five fish species were collected using the same range of methods used previously (Kopec and Bodaly 2013a). Eels (*Anguilla rostrata*) were electrofished in June in the freshwater OV reach, upstream of the Veazie Dam. Electrofishing was scheduled for June to avoid conflict with the federally-listed Atlantic salmon which migrate up the Penobscot later in the summer. In July, eels were collected from the brackish water downstream of the Veazie Dam, in the BO and OB reaches, using eel pots baited with salted bait herring. Mummichogs (*Fundulus heteroclitus*) were also collected in July, using non-baited minnow traps set in marsh slough channels. Traps were deployed at low tide and retrieved at high tide. Tomcod (*Microgadus tomcod*), winter flounder (*Pleuronectes americanus*) and rainbow smelt (*Osmerus mordax*) were collected using seine nets trawled along the bottom.

Biological data recorded from each fish included length and weight, with additional information collected from some species. Eels were directly aged using otoliths as their variable growth rates makes length or weight an unreliable indicator of age. Sex was determined for eels based on the presence of oocytes or spermatocytes (or undifferentiated cells) in gonadal tissue. Mummichogs were sexed using external morphology.

Samples of the axial muscle from each fish species were analyzed for total Hg. In very small specimens of mummichog and winter flounder the fish were gutted, and the head and tail removed prior to analysis.

Up to nine samples of each species from each monitoring site were also analyzed for the stable isotopes $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$, the results from $\delta^{15}\text{N}$ are reported here. An equipment malfunction caused a delay in completing the $\delta^{34}\text{S}$ analyses and the sulfur isotope signatures are not reported in this document.

Shellfish – Blue mussels (*Mytilus edulis*) and lobster (*Homarus americanus*) were collected from the ES reach, from Verona Island at the mouth of the Penobscot River south and west to Searsport. Mussels were sampled by hand from four established intertidal monitoring sites in September and from two sites in March. The spring collections further examined the increased Hg concentrations found in the spring, in 2010, at sites in the contaminated zone of the study area. Lobsters were collected in early September from lobster traps set by a commercial lobster fisherman in designated sample areas used since 2008. Field personnel noted the exact coordinates of each trap site.

Shellfish size, length of the mussel shell and carapace length in lobster were recorded and used to estimate age. Lobster weight and sex were recorded, though no gravid females or V-notched females were sampled, in accordance with the lobstermen's protocols to protect the population (despite permits that allowed such collections).

Shellfish were analyzed for Hg and stable isotopes. The soft tissue of the mussel was homogenized and analyzed for total Hg with a subset of roughly 30% analyzed for methyl Hg. Tail muscle was analyzed for total Hg from all lobster, and claw muscle and tomalley were sampled and archived from a representative subset of the lobster collected. Stable isotopes of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$ were analyzed from up to nine lobster and mussel samples from each monitoring site; the results from $\delta^{15}\text{N}$ are reported here.

Birds – The design of the bird sampling program was revised to focus on particular species and sites that best documented Hg concentrations in the Penobscot study area and at a nearby Downeast reference site outside of the aquatic influence of the river. Marsh birds, Nelson's sparrows (*Ammodramus nelsoni subvirgatus*), swamp sparrows (*Melospiza georgiana*), and red-winged blackbirds (*Agelaius phoeniceus*), were captured in mist nets at three marshes in a 14-day period in mid-July. Two marshes were sampled in the contaminated zone. The W17 marsh at

Hennessey Cove is directly south of Winterport on the west side of the Penobscot River. Mendall Marsh is a mile downstream from W17, also on the river's west side. At Mendall Marsh, collections were split between two large marsh areas, the Southwest marsh and the Southeast marsh, which are on opposite sides of the Marsh River, which bisects Mendall Marsh. We established a new reference area for marsh birds in the estuary of the Pleasant River, near the Downeast town of Addison. All three species were present at all sites monitored.

Eggs were collected from nesting double-crested cormorants (*Phalacrocorax auritus*) in late June, toward the end of the nesting season, but allowing sufficient time since cormorant arrival to the breeding sites for Hg concentrations to reflect current exposure levels. Collections were done in one day at two breeding colonies, one each in the contaminated zone (Sandy Point) and at a reference site on Thrumcap Island in lower Penobscot Bay. In January, American black ducks were captured in baited traps on Mendall Marsh, the South Verona marsh at ES13 and at a marsh near the mouth of the Jordan River in Frenchman Bay.

Standard measurements were recorded on each bird, including bill length, wing chord and weight, age class (HY, hatch year and AHY, after hatch year), and sex of the birds. Birds were banded with a USFWS aluminum band. A molt card was prepared on each bird to document whether the feathers that were sampled had been recently molted or were older feathers grown during the previous molt period.

Samples were collected for Hg and stable isotope analyses. Blood samples were collected into two heparinized microhematocrit capillary tubes, 10-50 μ l in each tube, following puncture of the cutaneous ulnar vein. Four feather samples were collected from each bird, the left and right first primary (P1) wing feather and the left and right R6 (sixth rectrices) outer tail feathers. Blood samples were analyzed for total Hg and the stable isotopes $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$; the results from $\delta^{15}\text{N}$ are reported here. One of each of the paired feather samples was analyzed for total Hg and the remaining feather was archived. Eggs sampled from cormorants were analyzed for total Hg and stable isotopes. Blood samples collected from American black ducks were analyzed for total Hg and stable isotopes. A P1 feather sample from each duck sampled was archived. The findings from the stable isotope analyses in ducks are reported below, Hg results were previously reported in Kopec and Bodaly (2013a).

Invertebrates – Both aquatic and wetland invertebrates were collected for stable isotope analyses in order to document variations in the isotopic signatures at the base of the food webs at different sites within the study area. At the aquatic monitoring sites, freshwater and brackish water snails were collected in July at the base of the benthic food web in OV, BO and OB and the saltwater periwinkle snails *Littorina* were collected in September in the ES reach. *Crangon* shrimp, also primarily at the base of the benthic food web were collected in September in the OB and ES reaches. The base of the pelagic food web was defined using filter-feeding mussels collected in July from the freshwater and brackish reaches of OV, BO and OB reaches, and in blue mussels collected in March and September in the ES reach.

Wetland invertebrates defined the isotopic signatures at the base of the wetland food web. Terrestrial snails and amphipods (*Gammarus* sp.) were sampled in June and July at the marsh bird sampling sites. At one site, Mendall Marsh SW, tadpoles were sampled from marsh pannes in June. Tadpoles were not present at the other wetland sites.

The results of stable isotope analyses for $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$ are reported here. For most taxa it was necessary to pool individuals into a composite sample of sufficient weight for the analyses. The pooled samples contained individuals of the same size range and genus whenever possible; one aquatic snail sample contained individual snails of the same size range but from different genera. In snails and mussels, the soft tissue was removed from each individual prior to analysis. Amphipods and Crangon shrimp were analyzed whole.

Sediment Sample Collections

All sediment samples were analyzed for total Hg and more than 50% of the samples were also analyzed for methyl Hg. Each sample was further characterized with analyses for total organic carbon (TOC) and grain size, emphasizing the % fines able to pass a 63 μm mesh sieve. Bulk samples were shipped to Flett Research Ltd. (Winnipeg, Manitoba, Canada) for homogenization and subsampling for Hg, TOC and grain size analyses. If homogenization was not possible on wet samples, the samples were freeze-dried prior to homogenization and sub-sampling.

Intertidal – Intertidal sediment samples were collected in August at low tide from sites in all four reaches of the Penobscot study area. Samples were collected by hand using stainless steel scoops from the top three centimeters of the sediment.

Subtidal – Subtidal samples were collected from three sites along the E01 transect (latitude 44.48200) which crosses Fort Point Cove from west to east. Samples were brought to the surface using a Van Veen dredge and a portion of the top three centimeters was subsampled from the contents of the dredge.

Wetland – Wetland sediments were collected from four marshes in the OB reach and from one marsh in the upper ES reach. The wetland sites were sampled at four elevations: high, the upper edge of the marsh platform just below the border with the adjacent upland; medium, the outer edge of the marsh platform immediately above the slope down to the mudflat; low, on the marsh slope at the outer edge of the marsh vegetation; mudflat, the outer edge of the mudflat at low tide. On the broad marsh platform at Mendall Marsh additional wetland sediment samples were collected at four sites designated as W21UM. Note that the high marsh elevation at W21 and W65 were approximately 15 m inland from the outer edge of the marsh platform, rather than at the uplands border sampled at other high elevation marsh sites.

Water Sample Collections

Surface water samples were collected from the main stem of the Penobscot River at four sites (Figure 1): 1) north of Veazie Dam, above the tidal influence of the Penobscot estuary (Veazie Dam or VZD); 2) in Orrington, downstream of the HoltraChem site (WQ1b-C); 3) near Winterport (WQ2 or WNTP); and 4) two miles upstream of the river's mouth at Bucksport (WQ3-C). All water samples collected below Veazie Dam were taken on a falling (ebb) tide.

Surface water samples were collected from the Penobscot River each month between June and September of 2012. Samples were collected using a high speed marine diaphragm pump through C-flex tubing weighted at the intake end. Unfiltered water samples were collected after the intake line was flushed for a minimum of one minute. Filtered (dissolved) samples were collected from water passed through an in-line filter (0.45 μ m pore filter). Both dissolved and unfiltered water samples were analyzed for total Hg and methyl Hg. An additional filtered water sample was analyzed for dissolved organic carbon (DOC), and an additional unfiltered water sample was collected for analysis of total suspended solids (TSS), used to calculate particulate Hg concentrations. At the beginning and end of each sampling event, equipment blanks of both unfiltered and filtered water were collected from 2.5L carboys of Poland Springs™ spring water, using the same pump, tubing and filters used to collect river water samples. Blanks were analyzed for both total and methyl Hg.

Stable Isotopes and Trophic Levels

Stable isotopes of nitrogen were used to estimate trophic levels of the aquatic and wetland target species examined in 2012. Raw $\delta^{15}\text{N}$ values were standardized using the $\delta^{15}\text{N}_{\text{base}}$ signature for each sample site or sample area, and for the appropriate food web (i.e., benthic or pelagic), using established models outlined in Chapter 16 of the Phase II Report. Tables 2 and 3 list the values used to estimate trophic position in fish, lobsters and birds using the formulas and methods detailed in Chapter 16 of the Phase II Report (Kopec and Bodaly, 2013b).

Analytical Methods

Mercury – Both total and methyl Hg analyses were performed at one of two accredited analytical laboratories used in previous years. Sediment, mussels and most fish samples were analyzed at Flett Research, Ltd. in Winnipeg, Manitoba using both cold vapor atomic fluorescence (CVAFS) and direct Hg combustion, as described in the PRMS Phase II report. Water, bird, lobster and some fish samples were analyzed at Battelle Laboratories in Sequim, Washington, also using CVAFS and direct Hg combustion as described previously.

Stable Isotopes - All stable isotope analyses were performed at the Colorado Plateau Stable Isotope Laboratory at Northern Arizona University. Analyses for $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, %N and %C were run using a Thermo-Finnigan Deltaplus Advantage gas isotope-ratio mass spectrometer interfaced with a Costech Analytical ECS4010 elemental analyzer. The isotopic signatures for $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$ were expressed relative to the standard ratios for Vienna Pee Dee Belemnite for carbon, and air for nitrogen. An equipment malfunction caused a delay in completing the $\delta^{34}\text{S}$ analyses and the carbon and sulfur isotope signatures are not reported in this document.

OC – Sediment organic carbon was determined at Columbia ALS in Kelso, Washington using EPA Method 9060, high temperature oxidation and coulometric detection. In 2010 this laboratory was determined to have excellent QA/QC procedures and to generate consistent and accurate data on sediment total organic carbon.

DOC – Dissolved organic carbon was determined on field-filtered water samples (0.45 μm in-line filters) at the University of Maine's Sawyer Environmental Research Laboratory in Orono, Maine. Following inorganic carbon removal by acidification with phosphoric acid, organic carbon was oxidized using sodium persulfate and heat, purged from solution and measured by a nondispersive infrared detector.

Grain size – Sediment grain size was also determined at the University of Maine's Sawyer Environmental Research Laboratory in Orono, Maine using simple fractionation of wet-sieved material to determine proportions of gravel (>2mm), sand (63 μm – 2mm), and mud (fines, <63 μm).

RESULTS AND DISCUSSION

Biota

Temporal Trends – The long-term monitoring program sought to identify trends in Hg concentrations over time, and distinguish between such trends and short-term, interannual variation. Countless natural factors influence Hg concentrations in the environment, especially in biota. While some of this natural variation can be mitigated with thoughtful study design (i.e., seasonal changes addressed by consistently monitoring in the same season each year), or statistical analyses (i.e., Hg in fish tissues adjusted for fish age), much of the short-term variability in natural systems cannot be defined or controlled. Because of this, even statistically significant trends identified from short-term monitoring may not describe an actual change in Hg concentrations over time, but instead interannual variation in Hg concentrations resulting from unexplained biological variation. A significant trend first requires a minimum of three years of data to calculate whether a trend exists. Further, for the trend to have biological meaning, the significant decrease or increase in concentration must be found consistently in three subsequent

years. A significant decline one year, followed by no change, or an increase the next year, indicates year-to-year variation, rather than a trend.

Since the monitoring of Hg concentrations in the lower Penobscot began in 2006 (fish and shellfish) and 2007 (birds), analysis of the 2010 data was generally the first opportunity to evaluate the presence of trends in biota Hg concentrations, and the 2012 data set created the next opportunity to examine the data for trends. Tables 4 through 7 compare whether significant trends identified in 2010 in Hg concentrations for specific species and sites were also found in the 2012 data set. As will be described in detail below, except for blue mussels, consistent trends in Hg concentrations were found at few sites. However, the short length of the monitoring program limits the conclusions about long-term trends in Hg in the lower Penobscot.

Temporal Trends in Fish – Mercury concentrations in fish during the 2006-2012 monitoring period showed no significant trends in most species, with the exception of Hg declines at certain sites for rainbow smelt and mummichog. This is essentially the same pattern that was found for the 2006 – 2010 monitoring period. Table 4 summarizes the results of the temporal trend analyses for both time periods (linear regression with pooled variance, adjusted for fish size or age, $P < 0.05$; this same table is presented with technical details in Appendix 1).

Total Hg concentrations were most consistent over time in eels (Figures 3 and 4) and winter flounder (Figures 5 and 6). Each species was found to have one site with a significant change in total Hg concentrations in one of the two time periods examined. Since the significant trend was not found in the other time period, for that site, the finding is apparently due to natural variation rather than a trend. The one site where eel Hg declined was at OV4, in the upstream reference area where Hg is not expected to change. Both species have strong site fidelity and fairly small home ranges, which may contribute to more consistent Hg exposures from their benthic prey. However, eels are long-lived, ranging in age in the 2012 data set from 4 to 12 years, and are not expected to quickly respond to changes in Hg in their diet. Conversely, the winter flounder sampled in 2012 and earlier were young fish, age 0 to age 1, with a mean length of 112 mm. These young flounder should respond quickly to changes in Hg exposure.

Tomcod sampled in 2006-2012 had a significant decline in total Hg at ES02, one of three sites where trend analysis was possible (Figures 7 and 8). At ES02E there had been no change in Hg concentrations in the earlier 2006-2010 time period. The decline in tomcod total Hg found at OB1S1 in 2006-2010 could not be further examined as it was not sampled in 2012. However, Hg concentrations did not change at the neighboring site, OB1NE, in either monitoring period. Tomcod's winter spawning runs to freshwater may increase the variability in their Hg exposure. The tomcod, like the winter flounder, were also age 0 and age 1 fish, with an average length of 140 mm, and were expected to quickly respond to changes in Hg in their diet.

Rainbow smelt had significant declines in total Hg concentrations at most of the sites sampled in 2012 (Table 4). While two consecutive years of significant declines were not found at any

individual sites, none of the sites showing a decline in 2006-2010 were sampled in 2012 (Figures 9 and 10). The sites showing a decline in 2012 either had no trend in 2010 (OB1NE, ES02E) or were sampled in only 2 years by 2010. However, several sites adjacent to those found to have a decline in 2012, also had significant declines in total Hg in 2010. These adjacent sites with declines in both years included OB1NE and OB1S-1, ES02E and ES11N, and ES13 and ES13S. These findings may indicate the beginnings of a decline in Hg concentrations in rainbow smelt; further monitoring is needed to confirm whether this is the case. Of note, given the decline found at most sites, Hg concentrations in smelt doubled at OB5, the site closest to the HoltraChem facility, but smelt have only been caught at that site in 2010 and 2012, so a trend analysis is not meaningful. As reported in Chapter 16 of the Phase II Report, rainbow smelt were the only fish sampled that fed almost exclusively in the pelagic food web. If the declines in Hg in smelt found to date continue into the future, it may indicate a decline in Hg in the pelagic food web, at least in the area near the mouth of the Penobscot River.

Mercury concentrations in mummichog declined at BO5, the only site sampled in three or more years in 2012 (Table 4). The site, BO5, is located in a pocket marsh just over 3 miles upstream of the HoltraChem site (Figure 11). Few sites were sampled in multiple years as consistent sampling started recently with a switch in collection methods to minnow traps in marsh sloughs (Figure 12). Mummichogs are benthic feeders known to forage on the marsh platform when flooded during spring tides.

Temporal Trends in Birds – Total Hg in blood sampled from three species of marsh birds in 2012 either increased or remained unchanged. Table 5 summarizes the results of trend analyses for the monitoring periods ending in 2010 and 2012. In 2012 both Nelson's sparrows (Figures 13 and 14) and red-winged blackbirds (Figures 15 and 16) had increased total Hg concentrations in their blood at the Mendall Marsh Southeast site. For the Nelson's this was the second year of significantly increased Hg concentrations at this site. The significant decrease in Nelson's found in 2010 at Mendall Marsh Southwest was not present in the 2012 dataset. There were no significant changes in total Hg concentrations in the blood from swamp sparrows sampled along the lower Penobscot in 2012, similar to the 2010 findings (Figures 17 and 18). The Downeast reference site on the Pleasant River was just established in 2012, so trend analysis was not possible at that site.

In the contaminated sites along the lower Penobscot, Nelson's sparrows and red-winged blackbirds had, in 2012, the highest concentrations of Hg found during the 2007 through 2012 study period, while the blood from swamp sparrows was in the same range or lower than found in previous years. While the increased concentrations in Nelson's and red-winged blackbirds likely indicate increased exposure from their invertebrate prey, other contributing factors should also be considered. In 2012 the marsh birds were sampled in mid-July, allowing for a longer residence time on the Penobscot marshes than occurred in some previous years. Given the birds' arrival dates to the Penobscot breeding marshes, both the Nelson's and the swamp sparrows were resident for a minimum of 6 weeks prior to sampling and the red-winged blackbirds were

resident a minimum of 8 weeks before sampled in mid-July. Longer residence times would have allowed higher Hg concentrations to accumulate in the birds' blood. However, swamp sparrows also had the same residence time, and no increase in Hg concentrations in 2012. Further, for the trend analyses, the blood Hg concentrations were adjusted for sample date (ANCOVA, adjusted for sample date, $P < 0.05$, Tukey pairwise test, $\alpha = 0.05$), to allow comparison with other years when the birds had a shorter residence time prior to sampling.

Yet, arrival times may still be a contributing factor. The winter of 2011-2012 was very mild, and spring temperatures came unusually early in 2012. It is possible that the birds arrived earlier in 2012 than in previous years; arrival dates were not monitored before 2012. The extreme concentrations found in Mendall Marsh in red-winged blackbirds, which had the longest residence time, support this hypothesis, and suggest the close relationship between extreme accumulations of Hg in marsh birds and their residence time along the lower Penobscot.

Total Hg concentrations in cormorant eggs held constant in 2012, as they did in 2010 (Figure 19). Black ducks have only been sampled in two years, precluding a statistical trend analysis.

Temporal Trends in Shellfish – During the 2006 through 2012 monitoring period, significant declines in Hg concentrations in blue mussels were again found at the more contaminated sites around Verona Island, but not at the mussel reference site near Searsport (Figure 20). As seen in Table 6 and in Figures 21 and 22, both of the northern-most mussel sample sites, ES15 and ES13, had significant declines in total Hg in both 2010 and in 2012. Methyl Hg also declined in both 2010 and 2012 at ES13, but not at the neighboring ES15 site. At ESFP (Fort Point), total Hg concentrations remained unchanged but methyl Hg declined significantly. The reference site ES04 showed a significant increase in total Hg concentrations but no change in methyl Hg concentrations. The trends described above used Hg data not adjusted for mussel size, in order to use the full dataset from 2006 through 2012. Shell length was not recorded in 2006.

However, for total Hg the same patterns of change were found using the 2008 through 2012 dataset, where the Hg data were adjusted for length. The trend results were slightly different for methyl Hg, with no change at ES13 instead of the decrease found in the 2006-2012 data set, and a significant increase in methyl Hg concentrations at the ES04 reference site, instead of the no change found in the larger data set.

Consistent declines in total Hg and methyl Hg concentrations in blue mussels at ES13, and in total Hg at ES15, both near Verona Island, may indicate the beginnings of a decline in Hg concentrations in the pelagic food web in that area. Mercury concentrations in rainbow smelt, which also feed primarily in the pelagic food web, also declined at ES13 in the 2012 dataset.

Mercury in lobster tail muscle declined significantly at three of seven sites sampled in 2012 (Table 6). At one site, Harborside, near the southern end of the lobster study area, declines were seen in both 2010 and in 2012. No decline had been observed in tail muscle in 2010 at the other

two sites, Odom Ledge, near Verona Island, and Parker Cove, on the east side of Islesboro (Figures 23 and 24).

Lobster feed in both the benthic and pelagic food webs. Unlike mussels and rainbow smelt, which filter pelagic invertebrates from the water where they reside, the pelagic portion of the lobster diet comes from trap bait, generally herring caught offshore. Herring bait would not reflect pelagic Hg exposure at specific, near shore sample sites. Further monitoring will show whether these declines persist.

Geographic and Seasonal Trends – Geographic trends in Hg concentrations point toward the influence of Hg released from the HoltraChem site on Hg accumulations in biota in the lower Penobscot relative to distant reference sites.

Geographic Trends in Fish – Mercury concentrations in most fish species were significantly greater at sites closest to the former HoltraChem facility (ANOVA, adjusted for fish length or age, $P < 0.05$, Tukey pairwise test, $\alpha = 0.05$). In rainbow smelt and tomcod (Figures 25 and 26), Hg concentrations at OB5, immediately south of HoltraChem, were significantly greater than at sites further downstream. However, the elevated Hg concentrations in rainbow smelt at OB5 may be related to ontogenetic changes (i.e., changes related to age) in the smelt diet. The smelt sampled at OB5 were relatively younger (mean length, 65 ± 8 mm) than the rainbow smelt sampled at sites further south (range of mean lengths 120 – 175 mm). Even though the Hg concentrations were adjusted for smelt length, ontogenetic changes in diet have been reported; younger smelt feed more in the benthic food web on worms and amphipods than older smelt that primarily filter-feed in the pelagic food web (Collette and Klein-McPhee 2002). A more benthic diet may alter the smelt's exposure to Hg relative to a pelagic diet. Winter flounder had significantly elevated Hg concentrations at sites in the OB reach and near Verona Island (Figure 27), relative to sites at Fort Point and further west in Penobscot Bay. Eels had significantly higher Hg in the BO and OB reach, which bracket the HoltraChem site, relative to sites upstream of the Veazie Dam. Mummichog, sampled at two sites (Figure 28), was the exception with Hg significantly greater in Mendall Marsh than a site upstream of HoltraChem.

Geographic Trends in Birds – Marsh birds breeding along the lower Penobscot accumulated 15 to 30 times more Hg in their blood than found in the same three species at the Pleasant River reference site. Figure 29 clearly illustrates the significantly greater Hg concentrations found at the two Mendall Marsh sites and the adjacent W17 marsh, relative to birds sampled at the Pleasant River marsh. All three marsh bird species sampled along the Pleasant River had significantly lower Hg concentrations in their blood than those sampled at marshes along the lower Penobscot (ANOVA, $P < 0.001$; Tukey pairwise, $\alpha = 0.05$). Among the Penobscot marshes, there were significant differences in Hg accumulations in some species. In swamp sparrows, Hg concentrations were significantly greater at the two Mendall Marsh sites relative to

the W17N marsh. In Nelson's sparrows concentrations were greatest at Mendall Southeast, followed by Mendall Southwest and then W17N. Red-winged blackbirds had statistically equivalent concentrations at all three Penobscot marshes.

In 2012 there was limited to no influence of bird sex on total Hg concentrations in blood, as had been found in previous years. Sex had no significant influence on blood Hg concentrations in Swamp sparrows at any of the three sites sampled (two-sample t-test with pooled variance, $P > 0.05$). In red-winged blackbirds, only males were sampled at W17N and at the Pleasant River reference site and no significant difference was found between males and females at Mendall Marsh ($P = 0.06$), though Hg concentrations in females were slightly greater. In Nelson's sparrows at W17N, sex was not a significant influence on blood Hg concentrations ($P = 0.43$), and only males were sampled at the Pleasant River site. At Mendall Marsh, males had significantly greater Hg concentrations than the two females sampled ($P = 0.004$).

Beyond looking at the variation in Hg concentrations among different sample sites, it is interesting to examine the variation among the species within each site. Figures 30 through 33 map the exact locations where marsh birds were sampled, and show the locations where capture nets were set but no birds were caught. For each adult bird sampled the blood total Hg concentration, rounded to the nearest whole number, is listed within the colored circles. Colored circles without a number shown the capture locations of hatch year birds (juvenile birds hatched in 2012). Note the overlap of the three species found at most sites, illustrating the blend of plant communities at most marshes. This was not found at Mendall Marsh Southeast (Figure 31) where two adjacent sampling sites were established to accommodate the birds' habitat preferences in less diverse plant communities. At all sites, red-winged blackbirds were captured in areas of cattail (*Typha angustifolia*) growing out of standing water, and the sparrows caught in drier areas vegetated with marsh grasses. The similarity in Hg concentrations within species and the variation in Hg among species sampled at the same site are notable, especially at Mendall Marsh Southwest (Figure 30). Whether this is due to differences in species' residence time, diet preferences, site fidelity, home range size or variable methylation rates in different microhabitats is worth exploring. This is in sharp contrast to the absence of variation in Hg accumulations in all three species at the Pleasant River reference site (Figure 33).

Geographic and Seasonal Trends in Shellfish – Mercury concentrations in blue mussels collected in September varied significantly among sites (Figure 34), with the greatest concentrations (490 ng/g dw) again at ES13, the site on the southeastern tip of Verona Island near the mouth of the Orland River. ES15, slightly to the north, on the western side of Verona Island, had somewhat lower Hg, but still significantly greater than sites further to the south. Spring mussel collections in March of 2012 found increased mussel Hg accumulations (mean total Hg, 900 ng/g dw), at ES13 in the contaminated area near Verona Island, relative to concentrations found in the fall (mean total Hg 490 ng/g dw), but no seasonal difference at the reference site ES04, near Searsport. In 2010 we found the same seasonal increase at the more contaminated sites, but no relationship between spawning stage and Hg concentrations in the mussels (Chapter 14, Figure

14-25). The source of this spring increase in Hg concentrations in mussels may relate to increased Hg in the phytoplankton consumed by filter-feeding mussels. While increased plankton densities in the spring may dilute the concentration of Hg in the phytoplankton (Chen and Folt 2005), methyl Hg release from thawing sediment may be great enough to negate the dilution of Hg by the increased plankton density.

Mercury concentrations in lobster tail muscle had a geographic pattern similar to that of mussels, with the greatest Hg concentrations at the South Verona site adjacent to ES13 (375 ng/g ww; ANCOVA, adjusted for carapace length, $P < 0.05$; Tukey pair-wise test, $\alpha = 0.05$; Figure 35). Of note, the two samples with the greatest Hg concentration, both exceeding 1,000 ng/g ww, were collected at the eastern edge of the South Verona sampling area, closest to ES13.

Concentrations at Odom Ledge (125 ng/g ww), adjacent to ES15, were markedly lower than in past years, equivalent to Hg in lobster at the far southern and western edge of the sample area.

Trophic Levels – Stable isotopes of nitrogen were used to estimate trophic levels of the aquatic and wetland target species examined in 2012. These analyses were undertaken to improve our interpretation of the results, especially to guard against concluding that changes in Hg in biota are due to changes in methyl Hg exposure, rather than due to changes in food chain length as defined by species' trophic level. We did not find evidence that differences in trophic levels within a species were the cause of geographic differences in Hg concentrations.

Note that only a subset of the fish and shellfish samples, up to 9 of the 15 samples collected at each site, were analyzed for stable isotopes. While the samples chosen for these analyses covered a range of fish lengths collected at each site, total Hg concentrations found in the subset often did not match the range of Hg concentrations found in the full sample set for each site.

Trophic Levels and Hg Concentrations in Fish – Mercury concentrations in American eels, winter flounder, and tomcod did not vary in relation to trophic level in 2012 (Figures 36 through 38). Mean trophic levels in eels ranged from 2.8, at OV2 to 3.3, at OV4, and both ends of the range of trophic levels were in the reference area above the Veazie Dam. Eels with the greatest length adjusted Hg concentrations were found at OB1, with a mean trophic level of 3.2. Most winter flounder had mean trophic levels between 2.6 and 2.9 while at one site, ES07, trophic level increased to roughly 3.4, with no relationship to Hg concentrations. Mean trophic levels in tomcod generally ranged between 3.0 and 3.4, except for OB5 which had a slightly elevated mean trophic level of 3.5.

In rainbow smelt, at one site, OB5, total Hg and trophic level were elevated relative to the other sites sampled (Figure 39). Mean trophic levels in most rainbow smelt ranged between 3.4 and 3.7, except at OB5 where the mean trophic level increased to 3.9 units. While these numbers may indicate that rainbow smelt fed at a somewhat higher trophic level at OB5, this may not be the case. As noted above in the discussion of geographic trends, the smelt sampled at OB5 were

relatively younger and expected to feed more in the benthic food web than older smelt, which may change their exposure to Hg and undermine our assumptions used to calculate trophic level (Table 2). Mummichog trophic levels were not significantly different between W21 (TL = 2.89 ± 0.26) and BO5 (TL = 2.67 ± 0.23 ; two-sample t-test, pooled variance, $P = 0.07$), while Hg concentrations were significantly greater at W21.

Trophic Levels and Hg Concentrations in Birds – In marsh birds we found no relationship between trophic level and Hg concentrations in bird blood. In fact, birds sampled at the reference site on the Pleasant River were feeding at trophic levels equal to or greater than the highest level found for the same species in the Penobscot marshes, where Hg concentrations were up to 30 times greater. Trophic levels in Nelson's sparrow ranged between 3.2 and 3.8 (Figure 40), between 2.9 and 3.4 in red-winged blackbirds (Figure 41), and between 2.5 and 3.2 in most swamp sparrows (Figure 42). The one swamp sparrow sampled at Mendall Marsh Southeast-1 had an estimated trophic level of 3.6 and the greatest Hg concentration in blood of any swamp sparrow sampled ($6 \mu\text{g/g ww}$). That individual swamp sparrow may have foraged exclusively on a predatory invertebrate or it may have consumed a normal diet, but in a particular foraging site with elevated Hg concentrations. The slight differences in trophic levels found in the three species likely reflect foraging preferences and contribute to their exposure to Hg.

In American black ducks and double-crested cormorants the estimated trophic levels had no relationship to Hg concentrations (Figure 43). Black ducks sampled in Penobscot marshes had mean trophic levels of 2.7 to 2.8 trophic units, rising to 3.1 in the ducks sampled in Frenchman Bay. The estimated trophic levels in cormorant eggs were consistent between the two breeding colonies sampled, 4.1 ± 0.1 at Sandy Point and 4.2 ± 0.2 at Thrumcap Island.

Trophic Levels and Hg Concentrations in Shellfish – As a primary consumer, trophic levels for blue mussels would directly reflect the geographic differences in the $\delta^{15}\text{N}$ signature found at each sample site, precluding a meaningful comparison of trophic levels and Hg. Figure 44 instead compares $\delta^{15}\text{N}$ ratios with mean Hg concentrations in blue mussels. The seasonal difference in $\delta^{15}\text{N}$ ratios is notable, though the cause is unknown. One possibility is an increased ingestion of protozoa (tiny zooplankton) filtered from the water in the late winter and early spring (Davenport et al. 2000). At ES13 the $\delta^{15}\text{N}$ ratio was significantly greater in the spring (mean $8.1 \pm 0.4 \text{ ‰}$) than six months later in the fall ($7.4 \pm 0.2 \text{ ‰}$, ANOVA, $P < 0.001$; Tukey pairwise test, $P < 0.05$). Hg concentrations were also significantly greater in the spring at that site. In contrast, the $\delta^{15}\text{N}$ ratios at ES04, the reference site near Searsport, were significantly lower in the spring ($6.2 \pm 0.3 \text{ ‰}$) relative to the fall ($6.9 \pm 0.3 \text{ ‰}$), while Hg concentrations did not vary with season.

Trophic levels in lobster varied little among our sample sites with mean levels near 3.4 trophic units at all sites except Fort Point, where it increased slightly to 3.6 (Figure 45). There was no relationship between trophic level and Hg concentration. Lobster bait, which comprises close to

45% of the lobster diet (Jury et al. 2001; Grabowski et al. 2010) and is shared across all sample sites, may contribute to the particularly uniform estimates of trophic level.

Sediment

Sediment Temporal Trends – Within our study period, 2006-2012, we have not detected a trend in Hg concentrations in the sediment of the lower Penobscot. In evaluating temporal trends in Hg concentrations in sediment, as in biota, it is important to recognize that interannual variation may briefly, but falsely, indicate the presence of a long-term trend. A minimum of three years of monitoring data is needed to establish the presence of trend, and the trend must be consistent in subsequent years to be considered real.

Calculations for the trend analyses given below used an independent linear model with pooled variance within each sediment type (subtidal, intertidal, and each of four wetland elevations). Mercury concentrations were log-normalized prior to analysis.

Temporal Trends in Subtidal Sediment – Subtidal sediments, collected along an east-west transect across Fort Point Cove, had the most consistent Hg concentrations over time of any sediment type examined. No significant trends in total Hg in subtidal sediments have been found to date (Figure 46 and Table 7). Further, in the period between 2007 and 2010, methyl Hg showed a significant decline at two sites, E01-1 and E01-4, but this decline did not continue as no decline in methyl Hg was evident in the period ending in 2012 (Figure 47).

Temporal Trends in Intertidal Sediment – No consistent trend in total Hg in intertidal sediments was found in the two monitoring periods ending in 2010 and 2012 (Table 7, Figures 48a and 48b). The significant increase in total Hg at ES13 between 2006 and 2010, driven largely by high total Hg concentrations found in 2010, did not continue in 2012. Significant declines in total Hg were found at two intertidal sites in 2012, OV2, in the reference reach above the Veazie Dam, and at BO5, immediately upstream of the HoltraChem site. Neither site had been sampled in sufficient years to define a trend in 2010.

Methyl Hg concentrations in intertidal sediments were more variable than total Hg concentrations. Significant declines in methyl Hg found at two sites in 2010, OV1, in the reference reach above Veazie Dam, and ES04, the Penobscot Bay reference site near Searsport, showed no decline in 2012 (Table 7, Figure 49). Declines in methyl Hg in 2012 were found at OV2 and BO5, sites where total Hg also declined, and at OB5, downstream of the HoltraChem site. The decline found at OB5 had not been present in 2010 and trends could not be assessed before 2012 at OV2 and BO5.

Reliable data on the total organic carbon (TOC) content of sediment samples is available for the years 2006, 2007, 2010 and 2012, coinciding with the years when intertidal sediments were

collected. Figures 50a and 50b, and Figure 51 illustrate the relationship in total Hg and methyl Hg among the intertidal sites after statistically adjusting for TOC. Adjusting the Hg concentration for TOC reduced the interannual variation within sites, and negated significant declines in total Hg at OV2, and in methyl Hg at BO5 and OB5 (Table 7). Significant declines at two sites, BO5 in total Hg, and OV2 for methyl Hg, were found whether Hg was adjusted for TOC or not.

Currently it appears that the significant changes in Hg concentrations in intertidal sediment found in the two study periods, 2006-2010 and 2006-2012, are the result of interannual variation rather than reflective of a long-term trend. No consistent Hg trend was found at any site in both 2010 and 2012.

Temporal Trends in Wetland Sediment – No consistent pattern of change across sites was found at any of the four elevations of wetland sediment sampled to date. One wetland site, W21, did have a consistent decline in Hg concentration at one elevation, and fluctuating declines at another elevation. Mercury concentrations also declined at W63 at two elevations in the period ending in 2012. Temporal Trends in wetland sediments could not be analyzed in relation to TOC or grain size due to gaps in the dataset. Wetland elevations were defined in the Methods section.

High Elevation - In 2012 both total Hg and methyl Hg declined at one high wetland site, W63, immediately south of the HoltraChem facility (Figures 52a and b, and Figure 53). Neither decline was present at W63 in the period ending in 2010. Three sites with significant increasing trends in MeHg concentrations in 2010 were not sampled in 2012.

Medium Elevation – Two significant declines in Hg concentrations were found at the medium marsh elevation in 2012, none of which were observed in 2010. There was a decline in total Hg at W21, along the west side of the Marsh River in Mendall Marsh. At W17, the marsh immediately south of Winterport on the west side of the river, methyl Hg declined significantly at the medium elevation (Figures 54a and b, and Figure 55).

Low Elevation - Total Hg declined at one wetland low elevation site in 2012, W63, immediately south of the HoltraChem facility (Figures 56a and b, and 57). No decline was found in 2010 at that site.

Mudflat Elevation – At the mudflat elevation of the wetland site W21, a consistent decline in both total and methyl Hg was found in both 2010 and in 2012. Methyl Hg declined significantly in 2012 at W17, but not in 2010 (Figures 58a and b, and Figure 59).

Sediment Geographic Trends – Significant geographic trends were found among the sediment sites within each of the three sediment categories. Sediment Hg concentration were analyzed as ng/g dw and additionally analyzed after statistical adjustment for TOC or TOC and grain size if either potential co-variate was found to have a significant influence on sediment Hg concentrations. The ANCOVA (analysis of co-variance) calculations generate individual

estimates of the Hg concentration as if each sample had the average concentration of TOC (or TOC and grain size) which was found for the entire dataset. This improves our ability to accurately compare concentrations among sites, by eliminating the influence of different sediment characteristics among the sites. However, the adjusted Hg concentrations do not represent the actual Hg concentration in the field which may be methylated and subsequently enter the food web at each site.

Geographic Trends in Subtidal Sediment – Total Hg concentrations in subtidal sediment, reported on a dry weight basis, declined significantly from the western mudflats (675 ng/g dw) to the eastern channel in Fort Point Cove (255 ng/g, dw; ANOVA, $P = 0.001$, $r^2 = 0.92$; Tukey pairwise test, $P < 0.05$; Figure 60). This geographic trend was almost reversed when subtidal sediment samples were adjusted for total organic carbon (TOC; Figure 61); TOC was significantly greater at E01-1 (6.0 %) relative to E01-4 (2.4%) (ANOVA, $P < 0.001$, Tukey pairwise test, $P < 0.05$). Total Hg adjusted for TOC was significantly lower at E01-1, at the western edge of the extensive mudflat in Fort Point Cove, relative to E01-3 and E01-4 (ANCOVA, adjusted for TOC, $r^2 = 0.97$, $P < 0.05$, Tukey pairwise test, $P < 0.05$). Grain size did not significantly influence total Hg concentrations in this sample set.

Geographic Trends in Intertidal Sediment – The greatest total Hg concentrations in intertidal sediment (980 to 1,050 ng/g dw; Figure 62) were found at OB5, directly downstream of the HoltraChem site, and at ES02, on the east side of Verona Island. Total Hg concentrations were below 375 (ng/g dw) at the other sites sampled. Adjusting the total Hg concentrations for TOC reduced the variability among sites and defined an area of elevated Hg concentrations from OB5, immediately downstream of HoltraChem, to ES13, on the southeast tip of Verona Island (ANCOVA, adjusted for TOC, $P < 0.001$, $r^2 = 0.986$; Tukey pairwise test, $P < 0.05$; Figure 63).

Geographic Trends in Wetland Sediment – Among the five wetland sediment sites sampled in 2012, the greatest variations in total Hg concentrations were found at the high, medium, and mudflat elevations while more uniform Hg concentrations were present in the sediment at the low elevation, at the end of the vegetated marsh slope at the upper edge of the mudflat. Adjusting the total Hg concentrations for the sediment characteristics of TOC and / or grain size did not dampen the variability found in wetland samples, but instead often switched the sites that had the highest or the lowest Hg concentration.

High Elevation – The greatest total Hg concentrations at the high marsh elevations were found at W17, directly south of Winterport (760 ng/g dw, geometric mean) and W21, in the southwest section of Mendall Marsh (580 ng/g dw; Figure 64). Concentrations dropped below 250 ng/g dw at the three remaining sites, most notably at W65, which is in Mendall Marsh, directly across the Marsh River from W21. After adjusting for TOC and grain size, total Hg concentrations at the high elevations were significantly greater at W63, the pocket marsh directly downstream of the HoltraChem site, compared to all other wetland sites (ANCOVA, adjusted for TOC and % fines, $P < 0.01$, $r^2 = 0.89$; Tukey pairwise test, $\alpha = 0.05$; Figure 65). The lowest total

Hg concentrations, both dry weight and adjusted for TOC and grain size were at W65, on the east side of Mendall Marsh.

Medium Elevation – At the medium elevation, located at each site where the marsh platform meets the vegetated marsh slope, total Hg concentrations were greatest at W63, downstream of HoltraChem (840 ng/g dw), and at W17, the marsh downstream of Winterport (810 ng/g dw; Figure 66). In Mendall Marsh Hg concentrations at this elevation were again high at W21, on the west side of the Marsh River (690 ng/g dw), and low at W65, on the east side of the Marsh River (325 ng/g dw). Adjusting the total Hg concentrations for TOC and grain size increased the relative Hg concentration at W61 on the southern tip of Verona Island (960 ng/g dw), but resulted in little change at the other wetland sites (Figure 67).

Low Elevation – Total Hg concentrations at the low elevation of the wetland sediments were fairly uniform, ranging from 750 to 930 (ng/g dw), in the area from W63, near HoltraChem, down to Mendall Marsh (Figure 68). Concentrations dropped to 500 ng/g dw at the southern tip of Verona Island, W61. Adjusting the Hg concentrations to TOC (ANCOVA, adjusted for TOC, $P < 0.001$; Tukey pairwise test, $\alpha = 0.05$) had little effect on the pattern of Hg concentrations (Figure 69).

Mudflat Elevation – Total Hg concentrations at W63 near HoltraChem (1,160 ng/g dw), were over 40% greater at this elevation than found at any other wetland sampled (Figure 70). After adjusting for TOC, the total Hg concentrations at the wetland mudflats were more uniform, ranging from 500 to 720 ng/g dw, yet significantly lower at W63 and W65 than the other three wetland sites (ANCOVA, adjusted for TOC, $P < 0.05$; Tukey pairwise test, $\alpha = 0.05$; Figure 71).

W21 Marsh Platform – Significant differences in total Hg concentrations were again found among sediment samples collected across the broad marsh platform at Mendall Marsh. Total Hg concentrations in the W21UM samples in the Mendall Marsh Southwest area ranged from 150 to 560 ng/g dw (Figure 72). Neither TOC nor grain size were significant co-variates with the total Hg concentrations.

Water – Surface water samples were collected from the Penobscot River at four sites extending from Veazie Dam, above the aquatic influence of the HoltraChem facility, to the southern-most site two miles upstream of the river's mouth at Bucksport (Figure 1). All water samples collected below Veazie Dam were taken on a falling (ebb) tide. Most water collections were made at low flow periods (Eddington gage height 2.75 to 4.75; USGS 2013), with the exception of the April and July 2009 samples collected during higher flows (Eddington gage height 7.25 to 10.0). There was unusually high precipitation during the summer of 2009.

Data from water samples collected in 2006 and 2007 at selected intertidal sites were added to the results of river water collected at the four water quality sites sampled in 2008 through 2012.

This extended the time period for evaluating changes in Hg concentrations in water. The intertidal sites matched to water quality sites were OV2 (Veazie Dam), OB5 (WQ1b-C), OB2 (WQ2/Winterport) and ES09 (WQ3-C).

Temporal Trends in Water - Dissolved total Hg and methyl Hg concentrations in Penobscot River surface water varied within a small range of values and often in synchrony among the sites, while particulate Hg concentrations were more variable over time. Small sample sizes limit statistical analyses of the temporal trends, yet several patterns emerge from the data.

Dissolved total Hg concentrations were generally synchronous with relatively greater concentrations in the waters above Veazie Dam and a sequential decline in concentrations at the three downstream sites (Figure 73), indicating limited input of dissolved total Hg below Veazie Dam. However, this general pattern shifted in samples collected after two weeks of unusually heavy rainfall in late June and July of 2009, when dissolved total Hg concentrations were slightly greater in surface water sampled at both WQ1b-C, downstream of HoltraChem, and at Winterport, relative to the water coming over Veazie Dam. The overall range in concentrations did not change over time.

Dissolved methyl Hg concentrations also varied in synchrony among the four sites sampled along the lower Penobscot River, with concentrations in a narrow range between 0.05 and 0.3 ng/L (Figure 74). Again, the greatest concentrations were found in the waters flowing over Veazie Dam, with sequentially lower concentrations at the downstream sites. The June -July 2009 storm event did not alter the relationships of dissolved methyl Hg concentrations among sites. The observation of higher dissolved THg at higher flows was also observed in a much larger data set on water coming over Veazie Dam in past (Phase II Report, Ch. 3, Figure 6, P. 3-116). No change in concentrations over time was evident.

Particulate total Hg concentrations (i.e. the concentration of Hg in particles, Figure 75) showed no trend over time as found for dissolved Hg, but in contrast with the dissolved Hg concentrations, the water coming over Veazie Dam was consistently lower in particulate Hg relative to the sampling sites downstream of the dam. Particulate Hg rarely exhibited synchrony in Hg concentrations among the sites. The one exception occurred again in the summer of 2009, when the particulate Hg concentration declined at all three downstream sites following the heavy rainfall in late June and early July of that year. At the two sites farthest downstream, Winterport and WQ3-C, the concentration continued to decline through early September. During the period of heavy rainfall, storm flows in tributary streams may have carried relatively clean sediments from the Penobscot watershed into the Penobscot River. As reported earlier (Turner et al. 2013), the particulate Hg concentrations declined during high-flow storm events in both the main stem of the Penobscot River (at Veazie Dam) and in streams tributary to the Penobscot. Those cleaner particles carried to the river during the storms may have mixed with the river's suspended sediment load and reduced the overall Hg concentration on the suspended particulates.

Geographic Trends in Water - In 2012 Penobscot River water samples were collected one day a month from June through September at Veazie Dam and at Winterport. The July sample event was expanded to the two additional sites, WQ1b-C and WQ3-C, sampled in past years. Figures 76 through 79 document the geographic distribution of Hg concentrations in river water found during each sample event in 2012. Several patterns are evident.

Water samples collected immediately above Veazie Dam (VZD) received no aquatic discharges from the HoltraChem facility but do contain the sediment and contaminant load from the extensive Penobscot watershed upstream of the dam, which is then carried by the river into the lower Penobscot study area. At this site, most mercury carried by the river is in the dissolved form (80 to 90%), as illustrated by the close agreement between the dissolved (filter passing) total Hg (THg Diss) and the combined concentration of the particulate and dissolved total Hg (THg Tot) in the Veazie Dam samples (Figures 76-79).

The predominance of dissolved Hg in the water coming over Veazie Dam is related to the relatively low particle load in the water upstream of the dam compared to downstream sites. Mean TSS (total suspended solids) was less than 2 mg/L on all Veazie Dam samples in 2012 (Figure 80). Further, dissolved organic carbon, which has a strong affinity for Hg, was relatively high in the Veazie water samples, ranging from 7.1 and 7.6 mg/L, and declined further downstream (Figure 81).

Dissolved Hg varied little at downstream sites relative to the concentration coming over Veazie Dam. Note that dilution from tributaries entering the Penobscot downstream of the Veazie Dam, and from tidal mixing, would be expected to dilute Hg concentrations.

Total Hg concentrations in water increased from Veazie Dam downstream to the mouth of the river at Bucksport; concentrations were two to seven times greater in the lower river than in the waters coming over Veazie Dam. This increase is driven largely by increased concentrations of particulate total Hg and increased TSS in the water sampled at the downstream sites. This occurrence is clearly illustrated in the June 2012 Winterport samples (Figure 76) where a high particulate Hg concentration (0.94 $\mu\text{g/g}$) coincided with an elevated TSS concentration (16.0 mg/L) to produce the greatest total Hg concentration found in 2012 (17.2 ng/L). This increased sediment load in June 2012 in the lower Penobscot was not related to a high flow storm event. The river gage height at Eddington was steady, and ranged from 2.75 to 4.5 feet at all four sample events in 2012. Instead, the increased sediment load appears to be connected to unconsolidated sediment picked up from the river bottom by the ebbing tidal current.

Methyl Hg concentrations were fairly consistent from Veazie Dam to the sites further downstream, with one exception. Methyl Hg, both dissolved and particulate, in the June sample at Winterport (0.47 ng/L) was over two times greater than the concentration coming over Veazie Dam. This increase was driven by an increased particulate methyl Hg concentration and a high TSS in the June sample. In summary, the increased Hg concentrations in the water of the lower

Penobscot River appear to be directly linked to increased loads of unconsolidated sediment picked up by tidal currents in the lower river.

Future Monitoring Study Design

Continued monitoring of Hg concentrations in sediment, water, and biota will document long-term changes in Hg contamination levels in the lower Penobscot. The design of the monitoring program will determine how quickly trends in Hg concentrations, or the absence of a trend, can be statistically verified. More frequent sampling and larger sample sizes reduce the time span needed to confirm whether change in Hg concentrations is occurring. Most authors recommend annual sampling as the most efficient sampling frequency to confirm trends in contaminant concentrations (Bignert et al. 1993; Hebert and Weseloh 2003; Bignert et al. 2004; Wiener et al. 2007). Less frequent sampling is compromised by short-term fluctuations that mask long-term trends, as illustrated in Bignert et al. (1993). The greater the time period needed to confirm the absence of a significant change in Hg concentrations, the less valuable the findings for evaluating the effectiveness of remediation or natural attenuation. Trend analyses are commonly designed to detect significant change within a five to ten year monitoring period to maximize the regulatory value of the findings.

Power Analyses - Power analyses were used to predict the minimum sample sizes needed to statistically confirm a change in Hg concentrations in biota and sediment. These *a priori* power tests were run using the coefficient of variation in the 2012 Hg concentrations for each species and site combination. Mercury concentrations in the target fish and shellfish species were log transformed and adjusted for size or age (eels) before variation within sites was determined. No adjustments for sample date were needed using the 2012 bird data as all collections of ducks and marsh birds were made within a single 7 to 14-day sampling period. Similarly, in 2012 cormorant eggs were collected on a single day from all sites. Sediment data were log transformed and total Hg concentrations were adjusted for TOC (subtidal and intertidal sediment), or particle size (wetland sediment) in order to determine the coefficient of variation within each site.

Estimates of the rate of change in Hg concentrations were based on the sediment Hg half-times developed in Chapter 6 of the Phase II report (Santschi 2013) - 22 years (Mendall Marsh), 31 years (Penobscot River), and 78 years (Orland River and Penobscot Estuary). Tables 9 through 15 present the sample sizes needed to confirm the absence of a trend, assuming a minimum power of 0.80, for a range of *minimum* monitoring periods. These minimum monitoring periods do not define the length of the monitoring period. Instead they are the minimum number of years in which samples must be collected before the results can statistically confirm the absence of a significant trend. The tests were also run using three different sampling frequencies, from annual sampling to sampling every two or three years.

The power analyses were run for a wide range of possible sample sizes. An upper limit was set for each sample type, estimated as the maximum number of samples that could conceivably be collected. This upper limit was set by a combination of sample availability and the logistics of sample collection. In many cases it was not considered possible to collect a sufficient number of samples to confirm change in the six or nine year minimum monitoring periods. This situation occurred more often when sampling frequency was extended to every two or three years.

The large sample sizes and extended minimum monitoring periods are largely driven by the long Hg half-times estimated for the lower Penobscot study area. If monitored natural attenuation is chosen to address the current levels of Hg contamination, the monitoring program will need careful design in order to generate meaningful results in a reasonable time period. For certain sample types it will take a minimum of 16 to 20 years to statistically confirm that Hg concentrations are not changing, given the estimated half-times in the Penobscot study area.

Most fish required annual sampling for a minimum sampling period of 16 years for the total Hg data generated to have sufficient power to statistically confirm that no trend is present (Tables 9 and 10). Mummichog and eels were the exceptions, as annual sampling of sample sizes ranging from 40 to 60 mummichogs and 10-50 eels per site was predicted to confirm that a trend was not present after six to nine years of sampling. Winter flounder, Atlantic tomcod and rainbow smelt required annual sampling of 10 to 50 fish per site for 16 years to confirm that no trend in total Hg was present.

In birds the minimum monitoring period varied among species (Tables 11 and 12). Nelson's sparrows monitored annually for six years would have sufficient power to confirm the absence of a trend with sample sizes of 5 to 40 birds per site. Swamp sparrows would need a minimum of nine years of annual sampling of 5 to 30 birds per site. Red-winged blackbirds would need at least 12 years of annual sampling of 5 to 40 birds per site. Black ducks were predicted to need nine years of annual monitoring of 7 muscle samples (collected in December) and a minimum of 12 years of annual sampling of 30 to 40 blood samples (collected in January) in order to confirm that total Hg concentrations were not changing (Table 13). Similarly, double-crested cormorants required sampling 7 to 40 eggs per site, each year for 12 years to confirm no change in total Hg concentrations.

Confirming the lack of a trend in Hg concentrations in shellfish required 12 to 20 years of annual monitoring. The absence of a trend in Hg concentrations in lobsters was predicted to require sampling 30 to 50 lobsters per site each year for 20 years. Mussel trends could be confirmed after 12 years of annual sampling of 15 to 60 mussels per site.

In sediment, minimum monitoring periods and sample sizes needed to confirm the lack of a trend in total Hg concentrations varied with sediment type. In general, annual sampling reduced the number of samples per site and / or the minimum sampling period needed (Tables 14 to 17). Annual collections of 10 to 30 subtidal sediment samples per site for nine years would confirm

the absence of a Hg trend. Three to 40 intertidal sediment samples per site, collected each year for 12 years, would confirm that Hg concentrations were not changing. Except for one site, wetland sediment samples of 3 to 30 samples per site would be needed to confirm the lack of a trend in total Hg concentrations within a 9 to 12 year monitoring period. Mercury concentrations at W61, where the estimated half-time of Hg in the sediment was 78 years, required sample sizes of 10 to more than 50 samples per site to confirm the absence of a trend in Hg concentrations.

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