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Spatial and temporal trends of mercury in the aquatic food web of the lower Penobscot River, Maine, USA, affected by a chlor-alkali plant

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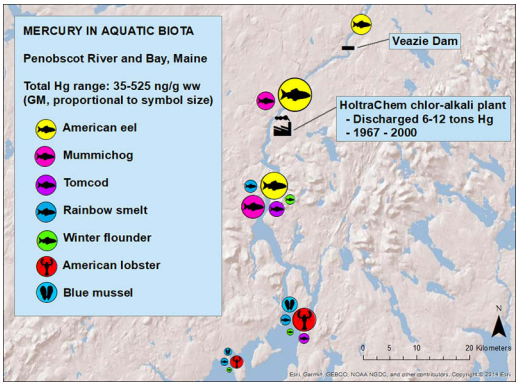
HIGHLIGHTS

- Elevated mercury residues persist in riverine biota 20 years after chlor-alkali plant closure
- Mercury in fish, lobster and blue mussels decrease with distance from plant site
- Evidence of declining trends in mercury concentrations in pelagic food web
- No change in mercury concentrations in benthic food web over time
- Elevated lobster mercury concentrations forced fishery closure in upper Penobscot Bay

GRAPHICAL ABSTRACT



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ABSTRACT

Mercury (Hg) concentrations in aquatic biota, including fish and shellfish, were measured over the period 2006–2012 in the lower Penobscot River and upper estuary (Maine, USA). The Penobscot is a system contaminated with Hg by a chlor-alkali plant that operated from 1967 to 2000, discharging 6–12 tons of mercury into the river. Mercury levels in aquatic biota were highest at sites downstream of the chlor-alkali plant and spatial trends were similar to those of sediments. Mean total Hg concentrations in fish muscle (adjusted for size or age) in the most affected areas were 521 (480, 566; 95% CI) ng/g ww in American eels, 321 (261,395) in mummichog, 121 (104, 140) in rainbow smelt, 155 (142,169) in tomcod, 55.2 (42.7,71.4) in winter flounder, and 328 (259,413) in American lobster tail and 522 (488,557) ng/g dw in blue mussel. Levels exceeded the 50 ng/g ww considered protective for piscivorous predators and were of concern for human health, with American eels and American lobster exceeding Maine's mercury action level of 200 ng/g ww. Calculations of trophic position (using nitrogen isotopes) suggested that the spatial patterns observed in total Hg concentrations were not due to changes in feeding habits of the species. Fish feeding in benthic food webs, as defined by stomach content and stable carbon isotope analyses, showed no change in Hg concentrations over time. In contrast, declining trends in Hg were found in two species dependent on pelagic food webs. The absence of declines in Hg

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American lobster (*Homarus americanus*)
Blue mussel (*Mytilus edulis*)
Stable isotopes

concentrations in the benthically-based food webs, despite the fact that most Hg was discharged into the system >40 years ago, is consistent with the long recovery predicted from dated sediment cores and from similar studies elsewhere.

1. Introduction

Mercury (Hg) remains a global concern for aquatic ecosystems (Krabbenhoft and Sunderland, 2013) due to anthropogenic loadings via atmospheric emissions (Fitzgerald et al., 1998) and Hg point sources discharging directly into freshwater and marine environments. Localized discharges from mercury-cell chlor-alkali plants are among the greatest point sources of Hg to aquatic systems (Bloom et al., 1999; Fimreite et al., 1971). While this production method has been largely discontinued, past discharges continue to pose a threat to aquatic systems long after plant operations have ceased (Munthe et al., 2007; Neff et al., 2012).

Inorganic Hg is the primary form of Hg discharged into aquatic systems yet, in the upper layers of aquatic sediment and in water, bacteria transform inorganic Hg into methyl Hg (Mitchell and Gilmour, 2008). Methyl Hg is highly bioaccumulative in organisms, and biomagnifies in aquatic food webs (Chen et al., 2008). This form of Hg is a potent neurotoxin that harms fish (Depew et al., 2012) and fish consumption remains the primary exposure route for humans (Zahir et al., 2005) and wildlife (Scheuhammer and Sandheinrich, 2008; Wolfe et al., 1998).

Mercury's biomagnification through an aquatic food web can be traced through the prey consumed at each trophic level. Methyl Hg, like carbon and nitrogen, enters the base of the aquatic food web through either benthic or pelagic primary producers (Chen et al., 2014). The source of methyl Hg and its transfer through the food web can be identified using stable isotope ratios of carbon and nitrogen which define the primary energy sources to and relative trophic levels of fish and other upper trophic level organisms (Lavoie et al., 2010; Peterson, 1999; Peterson et al., 2017). Identifying the source of methyl Hg to the food web through benthic or pelagic carbon may allow for focused and more effective remediation of a contaminated system.

The Penobscot River drains the largest watershed in New England, 22,600 km^2, and flows into Penobscot Bay along the central Maine coast. Between 1967 and 2000 the HoltraChem mercury-cell chlor-alkali plant operated along the east bank of the lower tidal portion of the river. This plant (HoltraChem) discharged an estimated 6–12 tons of Hg to the river via direct discharge of contaminated brine and via surface and subsurface runoff of Hg from the site (Turner et al., 2018). Assessments of the Hg contamination of the Penobscot system proceeded as unique court-ordered studies, and Hg levels in sediments, water and biota were determined with the ultimate goal of remediating the problem (Rudd et al., 2018).

Our study goals were to define the geographic extent of Hg contamination in aquatic biota in the lower Penobscot, identify temporal trends in Hg concentrations, and to define the accumulation and transfer of Hg (as total and methyl Hg) in the food web of the Penobscot River and estuary. We hypothesized that Hg concentrations in biota would diminish with distance from the chlor-alkali plant, that concentrations would decline following closure of the plant, and that Hg concentrations in target species feeding in the benthic food web would reflect sediment Hg concentrations.

2. Methods

2.1. Study area and sample collection

The study area extended for 43 km along the lower Penobscot River, from the town of Orono south to the river's mouth at Bucksport (Fig. 1) and south for an additional 27 km into Penobscot Bay. HoltraChem was located along the river in the town of Orrington, 23 km north of the river mouth. Samples were collected over several years (2006–2012; see Table 1) from five reaches within the study area, two upstream and three downstream of the plant site. The upstream OV reference reach was outside of the aquatic influence of the HoltraChem discharges in an impounded reservoir behind the former Veazie Dam, the first head-tide dam upstream of the river's mouth. All other reaches were under tidal influence. The BO reach extended upstream of the HoltraChem discharge site, the OB reach extended downstream, south, from the HoltraChem site to the river's mouth, upper ES ran south from the river's mouth at Bucksport to Fort Point in Penobscot Bay, and lower ES extended south into Penobscot Bay to the island of Islesboro (Fig.1).

The salinity gradient within the study area, from freshwater in the OV reach above the dam to full seawater in Penobscot Bay, imposed habitat boundaries on the species sampled and prevented sampling the same species throughout the entire study area (Fig. 1, Table 1). American eels (*Anguilla rostrata*) were sampled in the OV reference reach and the river upstream and downstream of the plant site (BO and OB reaches). Mummichog (*Fundulus heteroclitus*) and Atlantic tomcod (*Microgadus tomcod*) were collected both upstream and downstream of the plant site (BO and OB reaches). Rainbow smelt (*Osmerus mordax*) and winter flounder (*Pleuronectes americanus*) were sampled in the river downstream of the plant, and in both reaches of the Bay (OB and ES reaches). Blue mussels (*Mytilus edulis*) and American lobster (*Homarus americanus*) were sampled only in Penobscot Bay (ES reaches), except for one subtidal mussel collection made opportunistically in the OB reach.

Muscle or whole-body (see below) samples from all fish were analyzed for total Hg and methyl Hg was analyzed in 10% or more of the fish sampled. Fresh weight and total length were recorded from each fish prior to necropsy and sampling.

American eels were collected in the BO and OB reach using eelpots baited with salted herring during July in most years, with collections in August and September in 2008 and 2009. In the freshwater OV reference reach eels were electrofished between June and August, except for collections in September in 2008. Yellow, immature eels were exclusively sampled to determine Hg exposure at the sampling site. Sexually mature silver eels migrate through the study area in the fall from upstream lakes and rivers on their way to Atlantic spawning grounds. In 2008 several of the eels electrofished from the OV reach showed characteristics indicative of migratory silver eels. We collected additional external measurements and gonad weights from all OV eels and a subset of the BO eels, to confirm whether these eels were non-migratory yellow eels or silver eels migrating down the Penobscot River from upstream sites. One female eel, OV5-1, was found to be 16 years old, had a total length of 750 mm, weighed 890 g, had a gonadosomatic index of 4.96 (GSI; gonad weight/total weight $*$ 100), and a Pankhurst eye index of 11.7 (PEI; [((horizontal + vertical eye diameters/4)2 $*$ 3.14159)/body length] $*$ 100; Cottrill et al., 2002; Pankhurst, 1982)). Given the evidence this eel was determined to be a silver eel migrating downstream in the Penobscot River and omitted from the dataset. All eels were aged by counting the annular rings on otoliths necropsied from each eel; eel length is highly variable with age and could not be used as a surrogate for age.

Mummichog were collected in the Penobscot River and bordering wetlands by beach seine, bottom trawl and in unbaited minnow traps

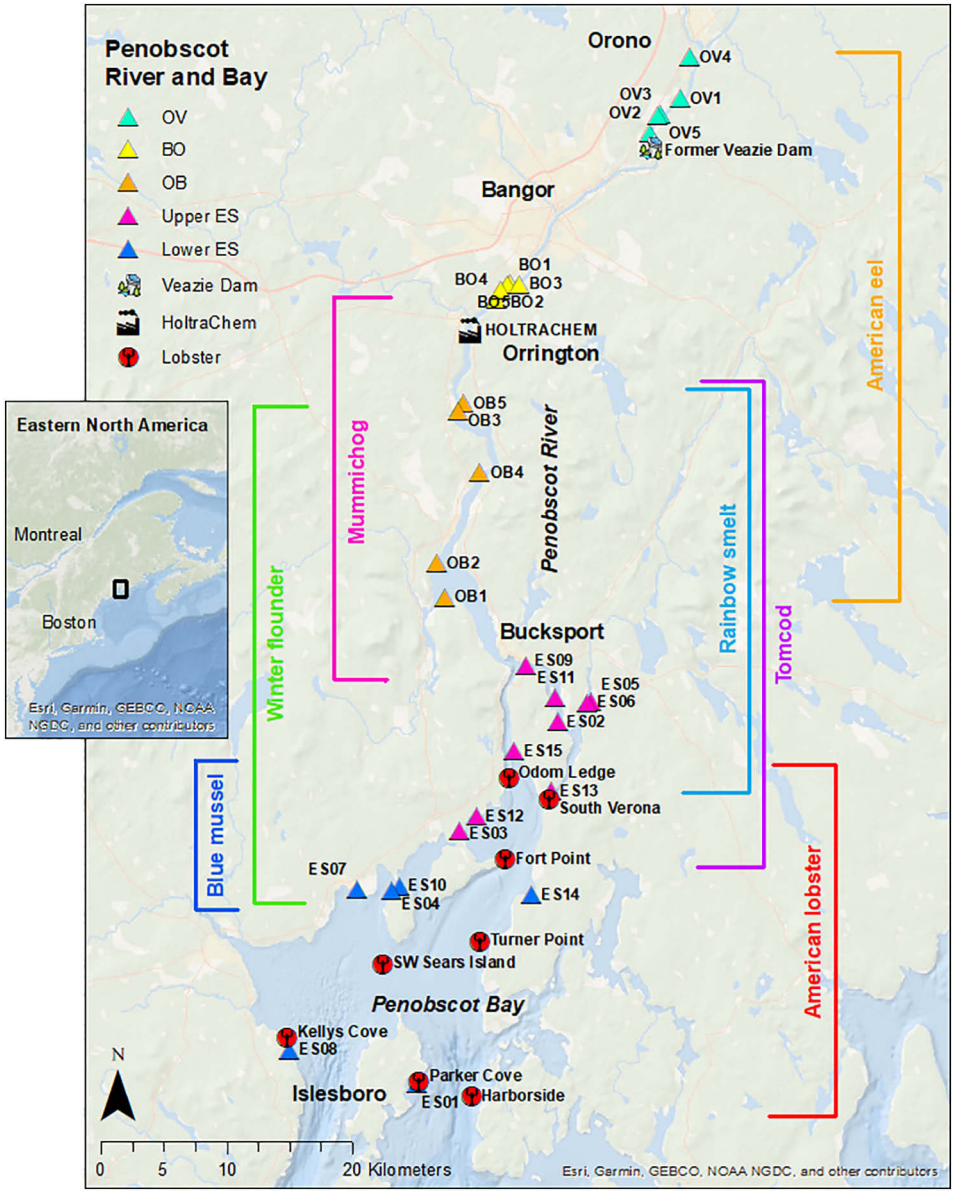

Fig. 1. Map of the lower Penobscot River and upper Penobscot Bay illustrating sample sites and the sample area of all species examined. The source of Hg contamination is the HoltraChem chlor-alkali plant. The former Veazie Dam defines the upstream limit of aquatic contamination.

placed in wetland slough channels. Most collections were made in late summer or early fall, with 2012 collections in July. Small fish, weighing ≤4 g, were gutted, heads removed and analyzed as such with skin intact.

Rainbow smelt, winter flounder, and Atlantic tomcod were collected in the Penobscot River and Penobscot Bay using trawl nets during September of each year. A subset of the winter flounder weighing <5 g were gutted, heads removed, and analyzed as such with attached skin.

Lobsters were collected in Penobscot Bay using commercial lobster traps baited with salted herring (marine derived) in both the upper and lower ES reaches. In most years lobster were sampled in September, with some collections in October of 2008 and 2009. Collections were made within plots ranging from 1 km^2 to 8 km^2. Water depth ranged from 3 to 11 m at the northern edge of the sample area, to 40 m in central portions of Penobscot Bay. We report findings for claw and tail muscle; tomalley was also sampled and those results are available in Kopec and Bodaly (2013a). Lobster fresh weight, carapace length, and sex were recorded. No gravid or notched females were sampled after 2006. In 2008 samples of tail and claw muscle from ten lobsters were analyzed for percent protein and percent total lipid at IEH-Warren Laboratory, Greeley, Colorado using standard methods.

Blue mussels were collected from the intertidal areas in Penobscot Bay and one opportunistic collection in 2010 from the subtidal channel in the OB reach of the Penobscot River. Mussels were collected in September of each year. Additional collections were made at selected sites in early spring (March 27–April 13) of 2012 and 2010 to compare seasonal variation in Hg concentrations. All mussels collected in 2010 were necropsied and categorized based on gonadal development as pre-spawning, spawning or post-spawning following Gosling (2003) to assess whether spawning stage influences Hg concentrations in mussel soft tissue.

Stomach content diet analyses were used to determine diet in four fish species, American eel, Atlantic tomcod, rainbow smelt, and mummichog. Sampled fish were chilled on wet ice in the field and stomach contents were removed during necropsy within three hours of collection and placed in glass vials filled with 6% formalin. When appropriate, formalin was injected into the stomach to arrest further deterioration of

Table 1
Sample schedule and locations for fish and shellfish from the lower Penobscot River and Penobscot Bay.

Penobscost reach	OV reference					BO					OB					Upper ES					Lower ES				
Species/year	2006	2007	2008	2009	2010	2012	2006	2007	2008	2009	2010	2012	2006	2007	2008	2009	2010	2012	2006	2007	2008	2009	2010	2012
American eel		X	X	X	X	X		X	X	X				X	X	X	X	X			X	X	X	X
Mummichug							X				X	X	X		X	X	X	X	X					
Rainbow smelt													X								X	X	X	X
Tomcod							X				X	X	X		X	X	X	X	X					
Winter flounder													X		X	X	X	X	X		X	X	X	X
Blue mussels															X	X	X	X			X	X	X	X
Amercian lobster															X	X	X	X			X	X	X	X

the contents. Following preservation with formalin, samples were transferred to ethanol for storage pending identification. Prey species were identified to the lowest possible taxon, to species where possible, and to higher taxa when species identification was limited by advanced digestion of the prey organism. Prey items were weighed to the nearest 0.01 g to determine relative weight contribution. Analysis of stomach content data used the percent weight contribution of each taxon identified.

Aquatic invertebrates collected in 2009 were used to establish isotopic values at the base of the benthic and pelagic foodwebs in the different reaches. For the base of the benthic food web Oligochaete worms were collected in July in the OV reference, BO and OB reaches and saltwater periwinkle snails (*Littorina*), and benthic worms, *Neanthes* and *Glycera* were collected in September in the ES reaches. The base of the pelagic food web was defined using filter-feeding *Elliptio* mussels from the OV reference reach, *Neomysis* shrimp from the OB reach and blue mussels (*Mytilus*), clams (*Mya*), and zooplankton collected in September from the ES reaches. *Gammarus* amphipods and *Crangon* shrimp were collected in the BO and OB reaches in September and used to define the isotopic values of secondary consumers with both benthic and pelagic carbon sources.

Stable isotopes were determined in fish, lobster and possible invertebrate prey collected in September 2009, and in archived fish and lobster samples collected in 2008. Muscle samples were analyzed in fish and lobster, the soft tissue was analyzed in bivalves and snails, and other invertebrates were analyzed whole. Composite samples were created when individual sample weights were insufficient for the analytical procedures to be performed. Following standard preservation methods for stable isotope analyses (Akin et al., 2011; Arrington and Winemiller, 2002), samples were frozen in cryogenic vials (Nalgene) at −20 °C prior to analysis. Appendix 1 lists the models used to calculate trophic position for primary consumers and secondary consumers feeding in one or two food webs, following Post (2002). The actual values used to estimate trophic position for the fish and shellfish target species are detailed in Appendix 2. In evaluating trophic level within site and species, years were combined if no significant difference was found between the two sample years (two-sample *t*-test, $p > 0.05$).

2.2. Laboratory analyses

All stable isotope analyses were performed at the Colorado Plateau Stable Isotope Laboratory at Northern Arizona University. Analyses for $\delta^{15}N$, $\delta^{13}C$, $\delta^{34}S$, %N and %C were run using a Thermo-Finnigan Delta^{plus} Advantage gas isotope-ratio mass spectrometer interfaced with a Costech Analytical ECS4010 elemental analyzer. The isotopic values for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ were expressed relative to the standard ratios for Vienna Pee Dee Belemnite for carbon, air for nitrogen, and Canyon Diablo Troilite for sulfur. A standard complement of quality control procedures were performed including duplicate analyses on 10% of the samples (0.7 ± 0.2% relative percent difference (RPD) mean ± SE), isotope (IAEA CH6, IAEA CH7, IAEA N1, IAEA N2, and IAEA S1–6) and elemental calibration standards, with recoveries of 98.6 ± 1.1% and 99.3 ± 0.3%, respectively and drift and linearity standards (NIST peach leaves; $\delta^{13}C$, −26.12 ± 0.08; $\delta^{15}N$, 1.93 ± 0.13, ±SD).

Both total and methyl Hg analyses were performed at one of two accredited analytical laboratories. Lobster and some fish samples were analyzed at the Marine Sciences Laboratory at Battelle Laboratories in Sequim, Washington. Mussels and most fish samples were analyzed at Flett Research, Ltd. in Winnipeg, Canada. Total Hg analyses followed a modified version of EPA method 1631e (US-EPA, 2002), using a Tekran Model 2500 Cold Vapor Atomic Fluorescence Spectrometer (CVAFS), and direct Hg combustion, using EPA method 7473 for a DMA80 Total Mercury Analyzer. Methyl Hg analyses were performed using EPA 1630 M, modified for tissues by digestion in 26% KOH in methanol. QA/QC analyses in each analytical batch included: three method blanks, two ongoing precision and recovery samples (OPR, blank spikes), one

sample of standard reference material (SRM; DORM-2,3, DOLT-2,3,or TORT 2,3), duplicates run on at least one sample or SRM (if sample mass insufficient) and four matrix spike samples. Data quality objectives included the method blank at ≤10× below the MDL (method detection limit; 0. 369 ng/g for total Hg, 0.276 for methyl Hg), the range of recovery at 70–130%, SRM/OPR accuracy at ≤30% and relative precision at ≤30. Recoveries for total Hg averaged (±SE) 101 ± 0.9% for SRMs, 101 ± 0.6% for blank spikes, and 101 ± 0.7% for matrix spikes, and there was a 3.4 ± 0.4% (RPD) for all duplicates and matrix spike duplicates. Recoveries for methyl Hg averaged 88 ± 1.6% for SRMs, 99 ± 1.4% for blank spikes and 98 ± 1.2% for matrix spikes, and there was a 5.8 ± 1.2% RPD for all duplicates and matrix spike duplicates. Total protein and total lipid (crude fat) content, percent total by mass, was determined for a subset ($n = 10$) of lobster tail and claw samples by IEH-Warren Laboratory in Greeley, Colorado using standard methods.

In 2009 methyl Hg concentrations were determined for all eel, blue mussel, and lobster tail samples, and 20 to 25% of the samples of mummichog, rainbow smelt, tomcod, and winter flounder. A smaller subset of samples were analyzed for methyl Hg in other years; those results are available in Kopec and Bodaly (2013a). Mercury concentrations are reported as ng/g wet (ww) weight for all tissue samples except blue mussels, which are reported as ng/g dry weight (dw).

2.3. Statistical analyses

Statistical and graphical analyses were conducted using R (R Core Team, 2014) or OriginPro (OriginLab, 2017). All Hg analytical results were log-transformed to meet statistical assumptions of normality. Trend analyses used a linear regression of Hg on year, adjusted for length, or eel age, within each sample site, with all regressions combined in one linear model with pooled variance. Geographic comparisons within species were initially made within individual years using an analysis of covariance, adjusted for size/age. Total Hg concentrations were summarized for each species by reach using an analysis of covariance adjusted across all sites for size, or age in the case of eels, and adjusted for sample year within each individual site. Unless noted, Hg concentrations are reported as the antilog of the least square mean adjusted for size or age, depending on the species.

3. Results

As described in more detail below, Hg concentrations in muscle from both fish and shellfish showed a geographic trend, with higher concentrations at sites near and downstream of the chlor-alkali plant and lower concentrations at more distant sites (ANOVA, adjusted for length or age, $P < 0.05$, Tukey pairwise test, $\alpha = 0.05$). This pattern was consistent across all years of the study. In the majority of species there was no trend in Hg concentrations over time, although significant declines were found at certain sites in species feeding in the pelagic food web (linear regression with pooled variance, adjusted for size or age, $P < 0.05$).

3.1. Mercury in fish and shellfish

Over 500 American eels were sampled from twelve sites in the three reaches along the lower Penobscot River between 2007 and 2012. Eels from the OV reference reach were significantly older (7.9 ± 2.6 years,) than eels from the BO (7.1 ± 2.2 years) and OB (6.8 ± 1.5 years) reaches ($F_{2562} = 9.9$, $P < 0.001$). Eel length differed significantly among all three reaches, being greatest in the OV reference reach (372 ± 119 mm), smaller in the BO reach (286 ± 68 mm) and intermediate in the OB reach (315 ± 60 mm)($F_{2562} = 47.7$, $P < 0.001$).

In general, total Hg concentrations in eel muscle did not vary temporally over the six-year period within each of the three reaches (Fig. 2). No significant trend in Hg concentrations in eel muscle was found in the upstream BO or downstream OB reaches from 2007 to 2012 (linear regression adjusted for age, $F_{25,540} = 9967$, $P > 0.05$). Total Hg did decline at one site in the OV reference reach (OV4), $P = 0.02$, but the site was outside of the aquatic influence of the Hg point source. Total Hg concentrations in eel muscle were significantly lower in the OV reference reach (312 ng/g ww CI [262,372]), than in the BO (521 ng/g ww CI [480, 566]) and OB (495 ng/g ww CI [449, 546]) reaches upstream and downstream of the plant site (ANCOVA adjusted for eel age and year, $F_{5,559} = 38{,}891$, $P < 0.001$). In 2009 methyl Hg analyses were run on all eel samples ($n = 135$); on average, methyl Hg comprised 96.1% of the total Hg in eel muscle.

Mummichog were collected in the BO and OB reaches ($n = 101$) along the Penobscot shoreline and in the marsh slough channels during mid-summer spawning runs. The mummichog were significantly longer in the downstream OB reach (69 ± 12 mm) than in the upstream BO reach (62 ± 9 mm; ANOVA, $F_{1100} = 9.1$, $p = 0.003$), while samples from both reaches were in the 1- to 2-year age classes (Collette and Klein-MacPhee, 2002). As shown in Fig. 3, significant declines in total Hg concentrations were found at the two sites sampled over three years (linear regression adjusted for length, $F_{7,94} = 14{,}376$, $P < 0.001$). Total Hg concentrations were significantly greater in the downstream OB reach (321 ng/g ww CI [261,395]) relative to the upstream BO reach (251 ng/g ww CI [204,308])(ANCOVA, adjusted for length and year, $F_{5,96} = 15{,}409$, $p < 0.001$). Methyl Hg in mummichog comprised 87.7% of the total Hg found in a subset of the mummichog samples analyzed in 2009.

Rainbow smelt were sampled between 2006 and 2012 throughout the downstream OB, and upper and lower ES reaches ($n = 520$). Mean lengths of smelt did not differ between the OB reach (118 ± 65 mm, $n = 105$) and the upper ES reach (114 ± 42, $n = 311$); smelt in the lower ES reach were significantly shorter (99 ± 26, n = 10) (ANOVA $F_{2,518} = 5.5$, $p = 0.004$) and were estimated to be in the 1- to 2-year age class (Collette and Klein-MacPhee, 2002). Total Hg declined significantly in smelt from the majority of sites sampled in three or more years (linear regression adjusted for length, $F_{33,486} = 483$, $P < 0.01$; Fig. 4). Declines were observed in all three reaches regardless of whether initial Hg concentrations were high or low. Total Hg concentrations in smelt muscle were significantly greater (ANCOVA adjusted for length and year, $F_{7512} = 20{,}064$, $p < 0.001$) in the downstream OB reach, closest to the Hg source, (121 ng/g ww, CI [104, 140]), followed by the upper ES reach (90 ng/g ww, CI [82,98]), with the lowest concentrations in the lower ES reach (73 ng/g ww, CI [64,83]. Methyl Hg comprised 75.5% of the total Hg in a subset ($n = 49$) of the 2009 rainbow smelt muscle samples analyzed.

Tomcod were sampled in the downstream OB ($n = 249$) and upper ES ($n = 270$) reaches. Tomcod were significantly longer in the downstream OB reach (150 ± 38 mm) compared to those sampled in the upper ES reach (136 ± 30 mm; ANOVA, $F_{1,518} = 23.0$, $p < 0.001$), and were primarily in the 1-year age class in both reaches (Collette and Klein-MacPhee, 2002). Significant declines in total Hg concentrations were found at two of the seven sites sampled in three or more years (Fig. 5; linear regression adjusted for length, $F_{25,494} = 4590$, $p = 0.01$). Tomcod from the downstream OB reach had significantly greater total Hg concentrations in muscle (155 ng/g ww CI [142,169]) than that found in fish from the upper ES reach (122 ng/g ww CI [113, 131]) (ANCOVA adjusted for length and year, $F_{5514} = 51{,}904$, $p < 0.001$). In 2009 methyl Hg accounted for 76.5% of the total Hg in tomcod muscle ($n = 49$).

Winter flounder collected in the downstream OB reach ($n = 143$, length = 125 ± 35 mm) were significantly larger than those sampled in both the upper ES ($n = 347$, length = 105 ± 36 mm) or lower ES reaches ($n = 122$, length = 98 ± 41 mm; ANOVA, $F_{2,609} = 19.7$, $p < 0.001$, Tukey HSD $\alpha = 0.05$). Most fish sampled were age 0 to age 1 fish (Pentilla et al., 1989). Total Hg concentrations in winter flounder muscle did not differ significantly over time at all nine sites sampled in three or more years (Fig. 6; linear regression adjusted for length, $F_{35,577} = 3809$, $P < 0.001$). Winter flounder sampled in the downstream OB reach had significantly greater Hg concentrations (55.2 ng/g ww CI

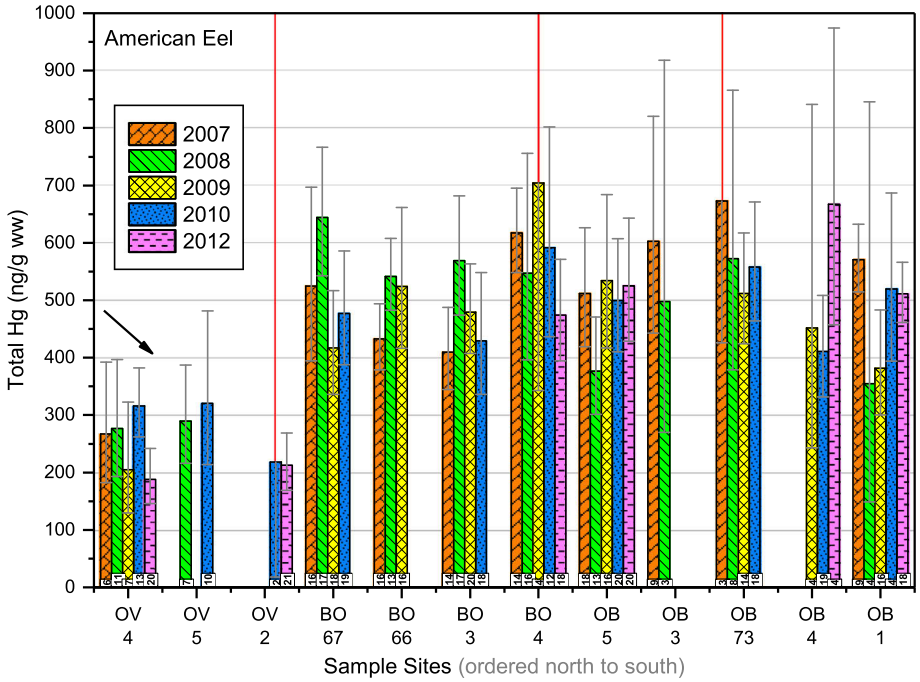

Fig. 2. Mean total Hg (ng/g ww, adjusted for age; 95% CI) in eel muscle collected from sites (see Fig. 1) upstream and downstream of a chlor-alkali plant on the Penobscot River, ME, between 2007 and 2012. OV sites were above a dam upstream of the discharges. An arrow indicates a significant decline at one OV site. Upper confidence intervals in red were truncated to fit the graph. Sample size is given at the base of each bar.

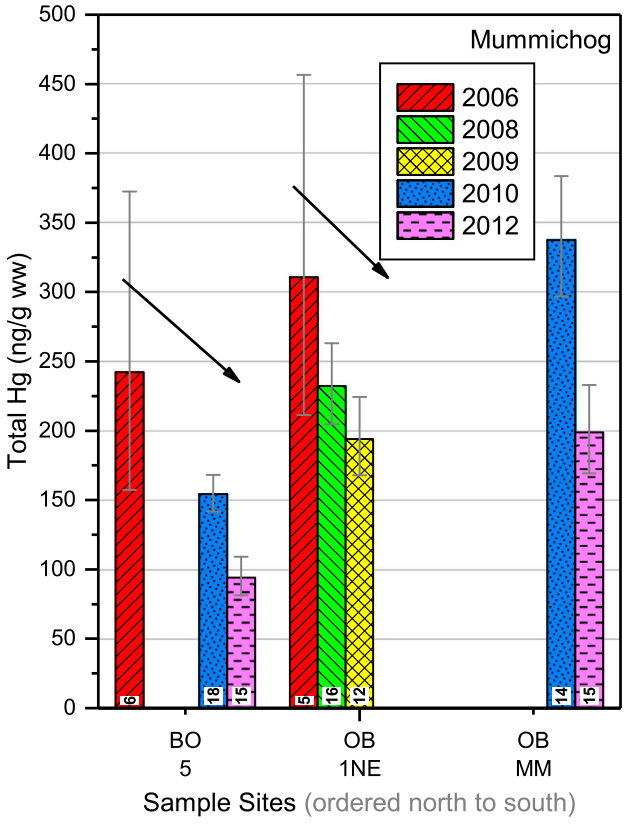

Fig. 3. Mean total Hg (ng/g ww, adjusted for length; 95% CI) in mummichog muscle collected from sites (see Fig. 1) upstream and downstream of a chlor-alkali plant on the Penobscot River, ME, between 2006 and 2012. Arrows indicate significant declines in Hg concentrations. Sample size is given at the base of each bar.

[42.7,71.4]), than found in flounder from the upper ES (42.9 ng/g ww CI [38.5, 47.8]) or lower ES reaches (35.1 ng/g ww CI [30.4,40.5]). In winter flounder muscle 70.4% of the total Hg was methyl Hg in a subset of the 2009 samples.

Here we report Hg concentrations in American lobster tail muscle; further details on Hg in claw and tomalley tissues are given in Kopec and Bodaly (2013a). Lobster carapace length differed significantly among the two ES reaches: 88.0 ± 9.1 mm in the upper ES reach and 85.4 ± 9.2 in the lower ES reach (ANOVA, $F_{1,602} = 10.0$, $p = 0.002$). Total Hg in lobster tail muscle declined significantly in three of the eight individual sites sampled between 2006 and 2012 (Fig. 7; ANCOVA adjusted for carapace length, $F_{17,586} = 7616$, $p < 0.001$). Tail muscle total Hg concentrations were significantly greater in the upper ES reach (328 ng/g ww CI [259,413]) relative to the lower ES (157 ng/g ww CI [137,180])(ANCOVA adjusted for carapace length and year, $F_{5,598} = 24{,}664$, $p < 0.001$). Total Hg was 2.3 times greater in tail muscle (GM = 157 ng/g ww) than in claw muscle (GM = 68 ng/g ww)(paired $t_{427} = 13.1$, $p < 0.001$). In a subset of 10 samples we examined whether the percent composition of protein or lipid in claw or tail muscle influenced the total Hg concentration in those tissues, but the results were inconclusive (Kopec and Bodaly, 2013a). In the 2009 lobster tail samples, 96.1% of the total Hg was methyl Hg ($n = 137$).

Blue mussels sampled in the fall from 2008 to 2012 were significantly larger in the lower ES reach (59.1 ± 9.8 mm) relative to those from the upper ES reach (55.1 ± 8.6 mm), while spring samples from the lower and upper ES were significantly larger (63.9 ± 4.8 mm) or of equivalent size (58.0 ± 4.7 mm), respectively, to the fall collections (ANOVA, $F_{3,618} = 17.0$, $p < 0.001$). Total Hg concentrations in mussels declined significantly over time in the three northern most sites, all in the upper ES reach, and there was a significant increase in total Hg concentrations at one lower ES site (Fig.8; linear regression adjusted for length, $F_{21,598} = 21{,}352$, $p < 0.01$). Overall, total Hg concentrations were significantly greater in samples from the upper ES reach (522 ng/g dw CI [488,557]) than in the lower ES reach (154 ng/g dw CI [142, 168])(ANCOVA, adjusted for length and year, $F_{5,544} = 51{,}751$,

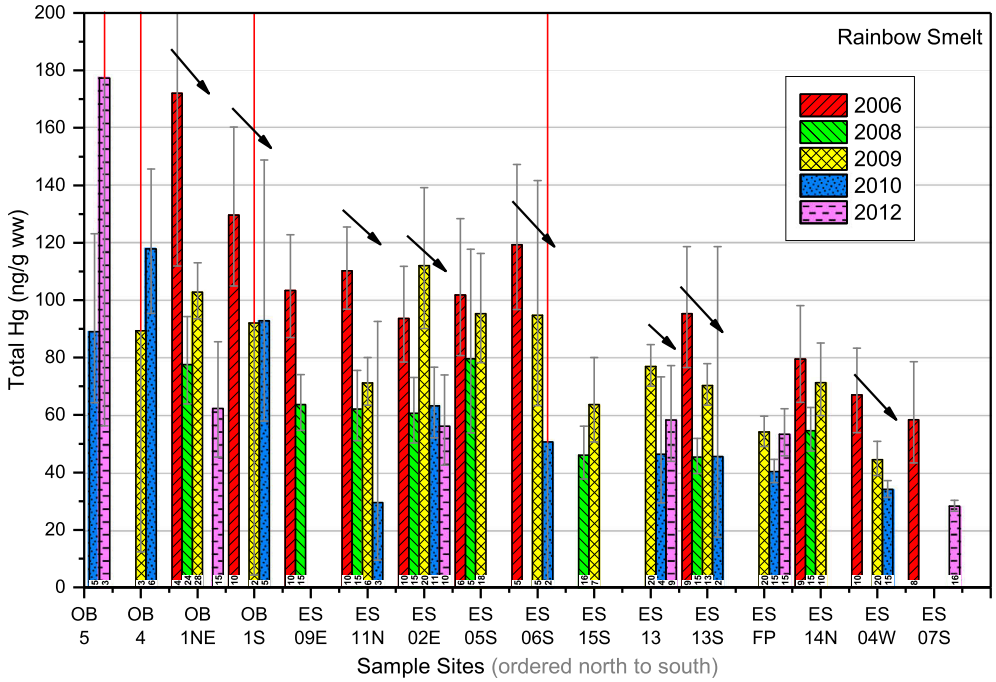

Fig. 4. Mean total Hg (ng/g ww, adjusted for length; 95% CI) in rainbow smelt muscle collected from sites (see Fig. 1) downstream of a chlor-alkali plant on the Penobscot River, ME, between 2006 and 2012. An arrow indicates a significant decline at individual sites. Upper confidence intervals in red were truncated to fit the graph. Sample size is given at the base of each bar.

$p < 0.001$). Seasonal changes in mussel total Hg concentrations were found at two sites in the ES reach. At ES13 in the upper ES reach, total Hg in mussels sampled in spring (812 ± 1.31 ng/g dw) were significantly greater than those sampled in the fall (454 ± 1.23 ng/g dw)($t_{63} = 9.5$, $p < 0.001$. At ES04, a site in the lower ES, total Hg in the spring and fall samples were not significantly different ($p = 0.20$) (Fig. 8). In 2009 methyl Hg comprised 51% of the total Hg present in blue mussel soft tissue ($n = 179$).

3.2. Fish stomach content analyses

In September 2009 a subset of the fish collected in each of the five reaches was necropsied ($n = 195$) and their stomach contents examined for prey analyses. Appendix 3 provides sampling details and the percent contribution by weight of each prey taxa, identified by species and reach. Benthic invertebrates dominated the diet of individual eels sampled in the three northern most reaches. In the OV reference reach, 61%

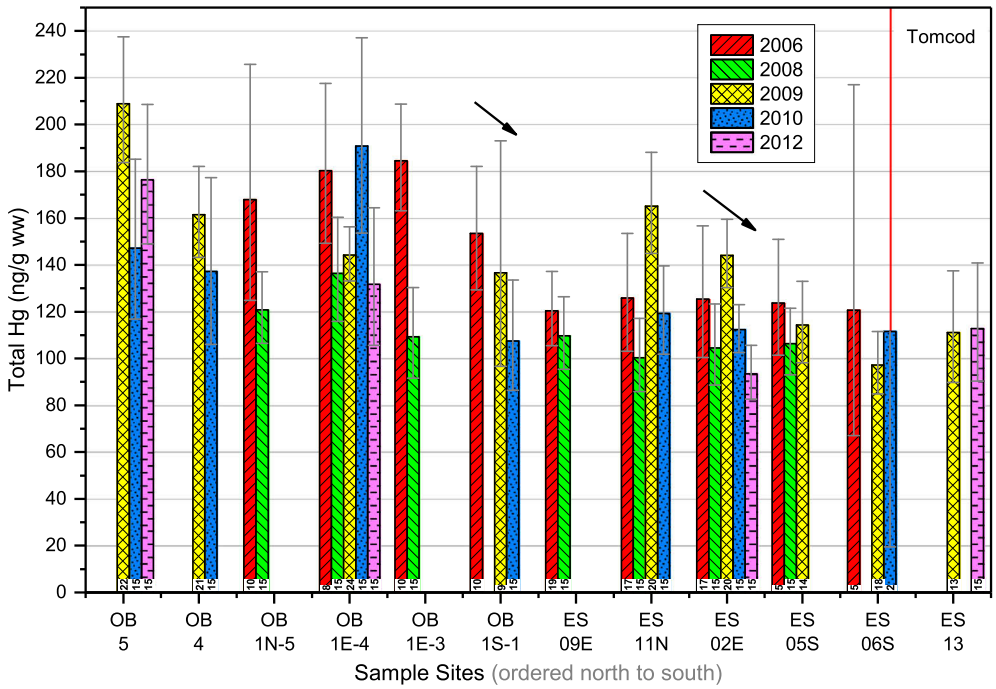

Fig. 5. Mean total Hg (ng/g ww, adjusted for length; 95% CI) in tomcod muscle collected from sites (see Fig. 1) downstream of a chlor-alkali plant on the Penobscot River, ME, between 2006 and 2012. An arrow indicates a significant decline at a site. The upper confidence interval shown in red was truncated to fit the graph. Sample size is given at the base of each bar.

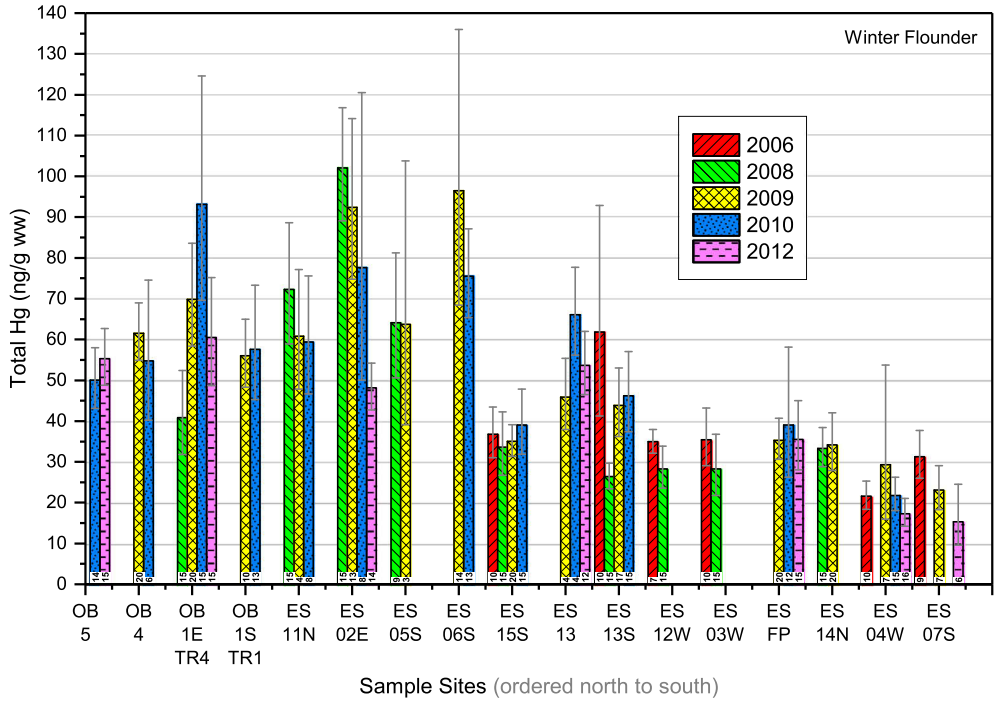

Fig. 6. Mean total Hg (ng/g ww, adjusted for length; 95% CI) in winter flounder muscle collected from sites (see Fig. 1) downstream of a chlor-alkali plant on the Penobscot River, ME, between 2006 and 2012. Sample size is given at the base of each bar.

of the diet was Oligochaete worms and 17% was decapod crustaceans, primarily *Orconectes* sp. (crayfish). In the upstream BO reach eel stomachs contained Nematode worms (46%) and stonefly (Perlidae) and other Insecta remains (43%). Decapod crustaceans, primarily *Crangon septemspinosa* (94%) dominated the eel diet in the downstream OB reach.

The tomcod diet in the downstream OB reach included epibenthic invertebrates (74%), primarily *Crangon septemspinosa*. and 19% was partially digested fish remains In the ES reach the tomcod diet shifted to zooplankton (47%), primarily *Neomysis americana* and epibenthic invertebrates (41%), primarily decapod shrimp.

Rainbow smelt in the downstream OB reach fed on Atlantic herring (*Clupea harengus*, 75%) semi-pelagic shrimp (*Neomysis americana*, 15%) and decapod crustaceans (*Crangon septemspinosa*, 9%). In the ES reaches the smelt diet shifted to the semi-pelagic invertebrates (90%), Calanoida copepods and shrimp, *Neomysis americana*.

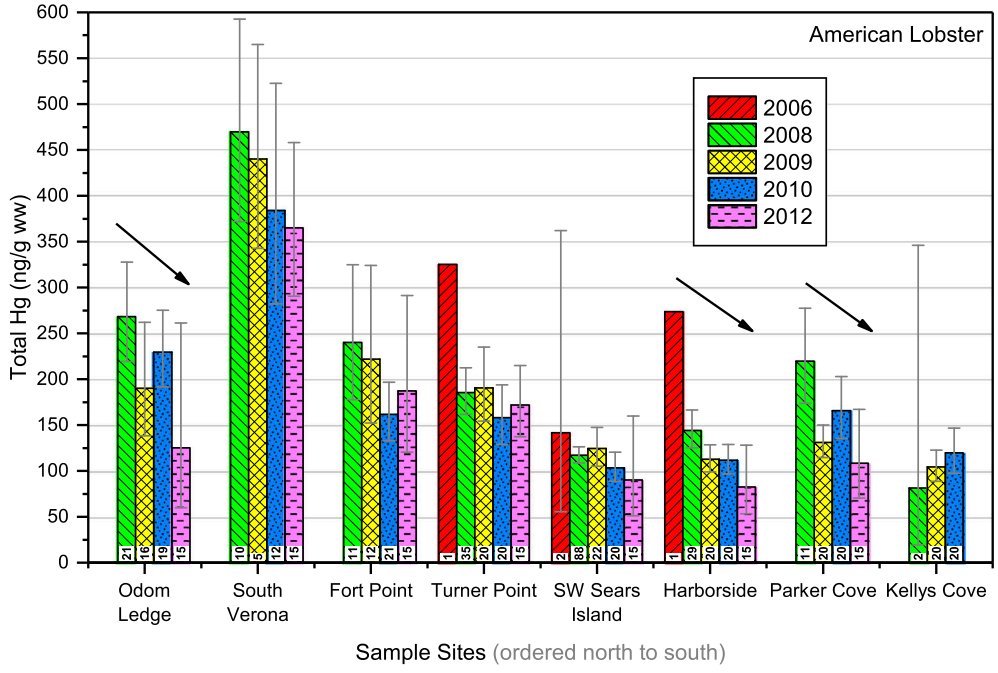

Fig. 7. Mean total Hg (ng/g ww, adjusted for length; 95% CI) in lobster tail muscle collected from sites (see Fig. 1) downstream of a chlor-alkali plant on the Penobscot River, ME, between 2006 and 2012. An arrow indicates a significant decline at a site. Sample size is given at the base of each bar.

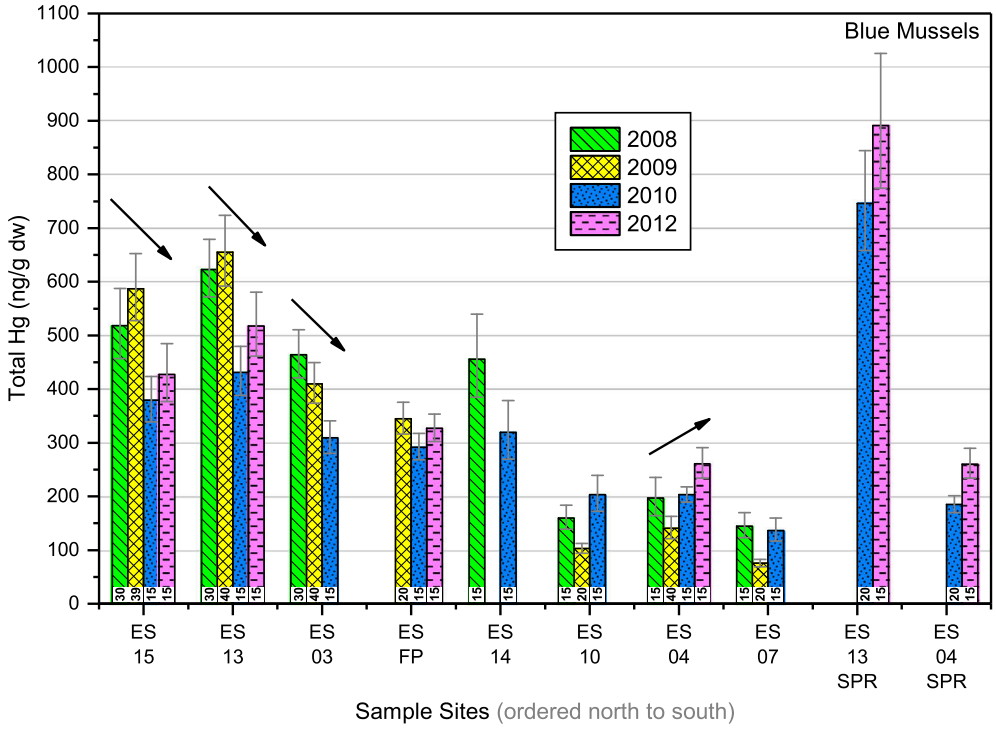

Fig. 8. Mean total (ng/g ww, adjusted for length; 95% CI) in blue mussel soft tissue collected from sites (see Fig. 1) downstream of a chlor-alkali plant on the Penobscot River, ME, between 2008 and 2012. Most collections occurred in the fall; two sites were also sampled in the spring (SPR). An arrow indicates a significant decline at a site. Sample size is given at the base of each bar.

The small mass of stomach contents (0.31 g) recovered from 12 mummichog stomachs sampled from the downstream OB reach was composed of partially digested insects (90%).

3.3. Stable isotope analyses

Stable isotope ratios of $\delta^{13}C$, $\delta^{34}S$ and $\delta^{15}N$ were determined for a subset of American eel, tomcod, rainbow smelt, mummichog and American lobster sampled in 2008 ($n = 249$) and in 2009 ($n = 168$). The stable isotope ratios of potential prey taxa were also determined in 2009 ($n = 179$). The stable isotope signatures of $\delta^{13}C$ and $\delta^{15}N$ were used to help identify whether the Penobscot target species fed in the benthic or pelagic food webs and their relative trophic position.

3.3.1. Carbon Base of target aquatic species

In the OV reference reach the $\delta^{13}C$ ratio for American eels (-26.7 ± 2.7‰) was more closely aligned with the Oligochaete worms sampled from the same reach (-28.1 ± 0.01‰) than the pelagic-feeding freshwater mussel (*Elliptio*), suggesting that these eels relied on the benthic food web (Fig. 9). The mean $\delta^{13}C$ value in eels was greater (-23.6 ± 1.0‰) further south, in the upstream BO reach, and equidistant between the values for Oligochaete worms (−24.7‰) and amphipods (−22.0‰), indicating continued foraging in the benthic food web. In the downstream OB reach, the $\delta^{13}C$ values for eels increased from -22.3 ± 1.6‰ to -18.8 ± 0.9‰ as the sampling sites became more brackish near the river's mouth. Amphipods sampled from the OB sites also had increasingly higher $\delta^{13}C$ ratios, from −18.8 to −17.1‰ (Fig. 9), similar to other benthic invertebrates (Appendix 5). The $\delta^{13}C$ values for *Crangon* shrimp also increased from -20.4 ± 0.9‰ to -18.6 ± 0.9‰ from upstream to downstream, and suggested that they at least partially contributed to the eel diet at the southern end of the reach.

The $\delta^{13}C$ values of tomcod also increased slightly from (-20.6 ± 0.6‰ to -19.4 ± 1.1‰) at sites progressively downstream in the OB reach as the marine influence increased closer to the river's mouth (Fig.9). These values were spatially aligned, though lower, than those found in *Crangon* shrimp, and indicated a reliance of tomcod on other prey with lower $\delta^{13}C$ values. In the upper ES reach the $\delta^{13}C$ values of tomcod (-15.8 ± 2.5‰) fell between that of zooplankton (−19.7‰) at the base of the pelagic food web and *Littorina* snails (-12.2 ± 0.4‰) at the base of the benthic food web.

Rainbow smelt sampled in the downstream OB reach had $\delta^{13}C$ values of -17.5 ± 0.4‰, that closely aligned with those found in benthic amphipods (-17.2 ± 0.5‰). In the lower ES reach in Penobscot Bay smelt had slightly lower $\delta^{13}C$ values of -18.0 ± 1.1‰, midway between the values of zooplankton (−19.7‰) and the filter-feeding softshell clam, *Mya* (-16.9 ± 0.4‰).

As seen in Fig. 9, the $\delta^{13}C$ values of American eels, tomcod and mummichog correlated spatially with the $\delta^{13}C$ values found in invertebrates sampled at the base of the benthic food web. No correlation was found between rainbow smelt and the base of the benthic food web. This correlation suggested a reliance of the former species on the benthic food web of this river.

Bi-plots of $\delta^{13}C$ v $\delta^{34}S$ for fish and lobster sampled in 2008 and 2009 in the lower Penobscot River and upper Penobscot Bay (Appendix 4) illustrate strong site fidelities in tomcod and mummichog and overlapping foraging ranges in rainbow smelt, American lobster and American eel, with the exception of eels sampled in OV4, upstream of the Veazie Dam. American lobster ratios would reflect both their natural benthic prey and the contribution from trap bait (pelagic herring), the latter of which is estimated to comprise up to 45% of the lobster diet (Grabowski et al., 2010). As seen in Appendix 5, the $\delta^{13}C$ and $\delta^{34}S$ values of Oligochaete worms, *Gammarus* amphipods, and *Crangon* shrimp increased from upstream to downstream as the sites approached the estuary. Less distinct trends were evident in the *Neanthes* worms, *Littorina* snails and *Mytilus* mussels sampled in the two ES reaches.

Detailed comparisons of $\delta^{15}N$ and $\delta^{13}C$ biplots for Penobscot target species and possible prey in both the benthic and pelagic food webs at individual sites in each reach of the Penobscot study area are presented in (Kopec and Bodaly, 2013b).

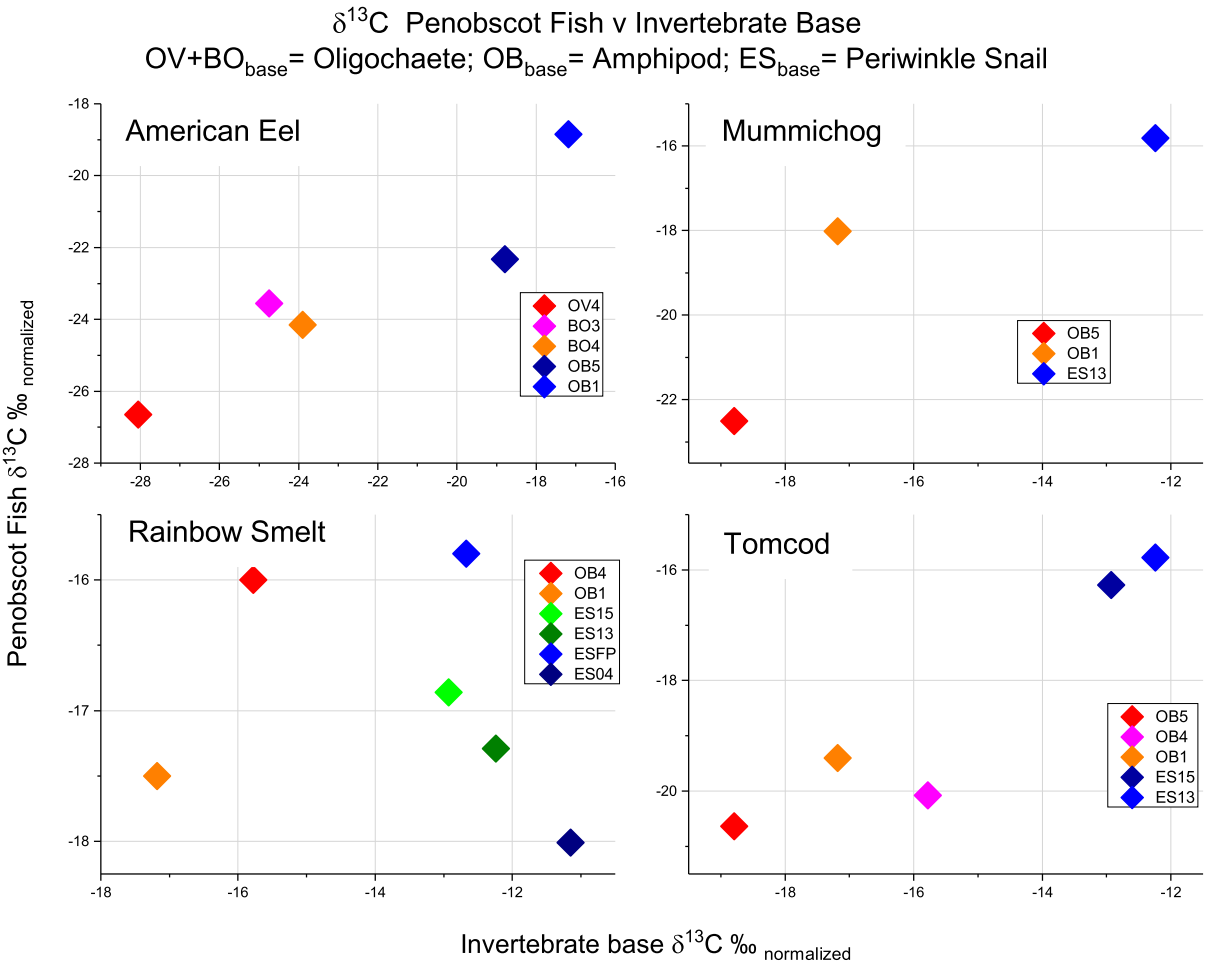

Fig. 9. Mean $\delta^{13}C$ (‰) of fish and benthic invertebrates sampled in the Penobscot River study area. A positive correlation was evident in the $\delta^{13}C$ values between the benthic invertebrate base and eel, mummichog and tomcod, all of which were found to feed in the benthic food web. No correlation was found between the $\delta^{13}C$ ratios of the benthic invertebrate base and rainbow smelt, which fed primarily in the pelagic food web.

3.3.2. Trophic levels and Hg concentrations

The trophic positions of American eel, tomcod, rainbow smelt, and lobster were estimated using established models (Post, 2002). Total Hg concentrations were not related to trophic level for any of the species examined, supporting the premise that spatial differences in Hg concentrations relate to differences in local Hg exposures and not to differences in food web structure among the different sample reaches. And, there was good agreement among the species trophic levels for tomcod (3.4–3.8 trophic units), rainbow smelt (3.6–3.9), and lobster (3.7–3.9) (Fig.10), despite the wide range in total Hg concentrations found in those three species. American eels generally fed at lower trophic levels than the other three species (2.8–3.7), yet they accumulated the greatest THg concentrations of any species sampled.

4. Discussion

4.1. Temporal trends in total Hg concentrations

In general, total Hg concentrations in benthic-foraging riverine and estuarine species in this study were stable from 2006 to 2012, suggesting little or no recovery from the contamination from the chlor-alkali plant that discharged to the Penobscot River until 2000. However, fish were sampled over a maximum of three or four years and mussels and lobster were sampled up to five years at individual sites, and this may not be long enough to detect temporal trends due to the interannual variation in Hg concentrations resulting from biological variability. A significant trend requires a minimum of three years of data and the decrease or increase must be found consistently in three subsequent years (Bignert et al., 2004; Bignert et al., 1993; Hebert and Weseloh, 2003; Krabbenhoft et al., 2007). A significant decline one year, followed by no change or an increase the next year, indicates year-to-year variation rather than a trend. Given the limited number of years sampled herein and the small sample sizes at each site, post hoc analyses of each species at individual sample sites produced a statistical power (1-β) generally below 0.1 (see Appendix 6 for more details). The highest power found was 0.34 for tomcod sampled at one site for five years. Continued monitoring of these sites is recommended to assess changes in Hg levels over time.

Statistically significant temporal trends in Hg varied among species and sites in the Penobscot River. American eels and winter flounder showed no change in Hg concentrations in areas under the aquatic influence of the HoltraChem discharges. Both species exhibit strong site fidelity (Fairchild et al., 2009; Parker, 1995) and rely on benthic food sources with limited home ranges, which may reduce variability in their Hg exposures. Eels are long-lived with an average age in this dataset of 6 to 10 years. Given that the Hg half-time in fish muscle is reported to exceed three years (van Walleghem et al., 2013) it is unlikely that eels would be sensitive indicators of changes in Hg exposure. Yet, the winter flounder sampled were either young-of-the-year or age-1 fish, and should be more sensitive to environmental change (Eagles-

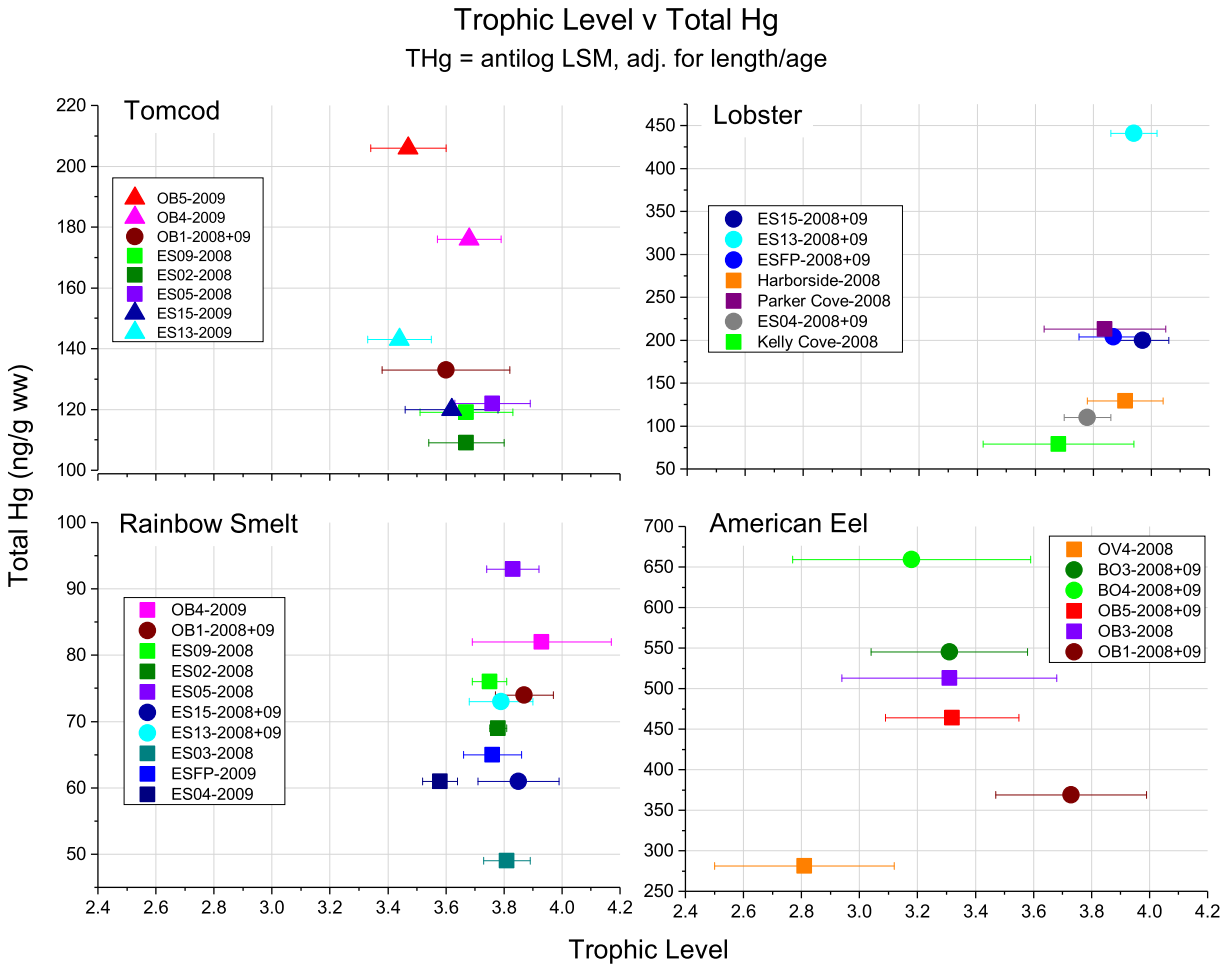

Fig. 10. Total Hg concentrations (ng/g ww) versus calculated trophic level (using $\delta^{15}N$) in fish and lobster sampled in the Penobscot River in 2008 and 2009. Note that the range of total Hg on the y-axis varies by species. Total Hg concentrations were not significantly correlated to trophic level within any of the species examined, further supporting the geographic trend of declining Hg concentrations with distance from the HoltraChem plant site.

Smith and Ackerman, 2009), and Hg concentrations in flounder did not change over time. Tomcod would also be sensitive to changes in Hg exposures as they were primarily age-1 fish in the current study; though significant declines in their Hg concentrations were found at two sites, adjacent sites did not and suggests limited larger-scale recovery. Lobster, also long-lived with an estimated age in the Penobscot dataset of four to six years (Hughes and Matthiessen, 1962; Wahle et al., 1996), showed a significant decline in total Hg concentrations in tail tissues at one site in the upper ES and at two sites in the lower ES reach. Lobsters are benthic feeders, yet in the heavily fished Gulf of Maine up to 45% of their diet comes from trap bait, primarily Atlantic herring (Grabowski et al., 2010; Jury et al., 2001) which complicate interpretation of this finding. Given the low power of the temporal trend analyses (Appendices 6 and 7), the absence of a trend may be mainly due to the shorter time period of this study. Alternatively, the lack of declines in benthic-feeding species at all sites downstream of the plant may be due to sustained elevated exposures to Hg-contaminated sediments.

Declines in Hg concentrations were evident in species foraging in the pelagic food web of the Penobscot River system. Rainbow smelt, which feed primarily in the pelagic food web (Buckley, 1989), showed consistent declines in total Hg concentrations over time and across sites (Fig. 4). These smelts were in the 1- to 2-year age classes (Chase et al., 2009) and are expected to respond quickly to changes in Hg exposures. Similarly, blue mussels filter-feed at the base of the pelagic food web. Mercury declined significantly in mussels at the three northern-most sites with the greatest Hg concentrations (Fig. 8). However, no changes in Hg concentrations were evident in blue mussels at less contaminated sites further to the south.

The declines in Hg concentrations in species foraging in the pelagic food web and persistent Hg concentrations, with some contradictory trends at individual sites, in biota feeding in the benthic food web are consistent with results in Hg-contaminated Lavaca Bay, Texas (Sager, 2002). Declines in Hg concentrations in the pelagic-feeding oysters (*Crassostrea virginica*) began soon after closure of the chlor-alkali plant Hg source, but were not consistent in crabs and finfish feeding in the benthic food web. Persistent Hg concentrations in Penobscot biota feeding in the benthic food web is consistent with the predicted decadal scale half times of Hg recovery in the Penobscot River sediment (Santschi et al., 2017).

In both 2010 and 2012 blue mussels were sampled from the Penobscot Estuary in early spring and fall to examine the influence of the summer spawning season as previous studies indicated a post-spawning decline in their Hg concentrations (Cossa and Rondeau, 1985; Lauenstein et al., 1993). Given that the timing of mussel spawning varies in estuarine habitats with annual changes in freshwater outflow and temperature (Newell, 1989), in 2010 we conducted a gross examination on all mussels to determine spawning stage. In the three upper ES sites that had elevated Hg concentrations in other biota (ES15, ES13, and ES03) there were higher Hg concentrations in mussels in the spring relative to the fall; no seasonal differences in mussel Hg

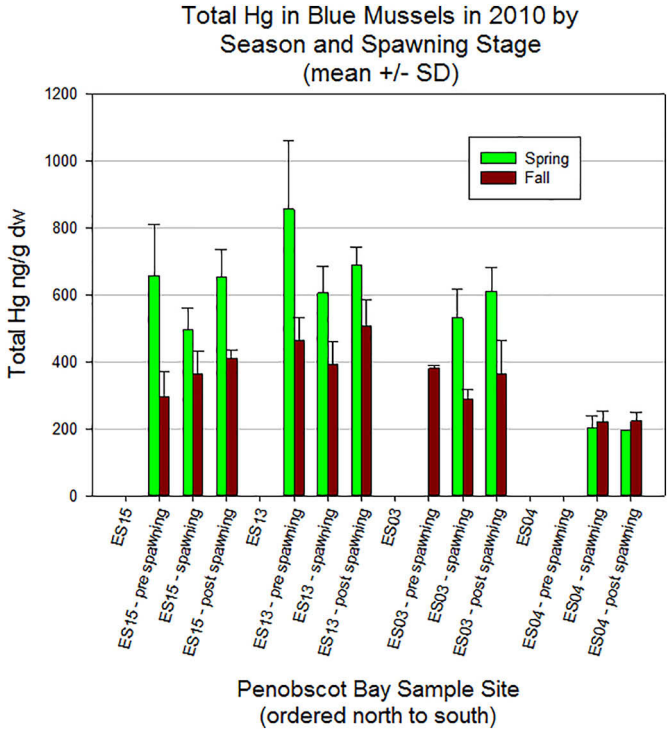

Fig. 11. Total Hg concentrations in the soft tissue of blue mussels grouped by spawning stage and sampled in both the spring and the fall of 2010. Total Hg concentrations in mussels were greater in the spring relative to the fall at three sites in the upper ES (ES15, ES13, ES03) while no seasonal difference was found at the reference site ES04 in the lower ES. Spawning stage had no influence on Hg concentrations in mussels sampled in Penobscot Bay in either the spring or the fall.

was found at the reference area (ES04). And as seen in Fig. 11, spawning stage did not influence Hg concentrations in mussels. Further work is needed to explain why Hg concentrations in mussels were only greater in the spring relative to the fall in the more contaminated sample areas.

4.2. Spatial trends in total Hg concentrations

The spatial variation in total Hg concentrations in most fish and shellfish from the Penobscot study area was consistent with the Hg source at the site of the former HoltraChem chlor-alkali facility on the eastern shore of the Penobscot River. Total Hg concentrations in fish and shellfish declined with distance from the plant site, with some exceptions for small scale habitat variation. This pattern reflected surface sediment (0–3 cm) total Hg concentrations in the Penobscot River which increased from roughly 100 ng/g dw in the OV reference reach to 900 to 1100 ng/g dw in the river sediment exposed to the HoltraChem discharges (Rudd et al., 2018). Total Hg concentrations in American eels were significantly greater near the plant site than in the OV reference reach upstream of the head-tide Veazie Dam (Table 2; Figs. 1 and 5). Given that immature yellow eels show strong site fidelity lasting 8–20 years, these eel Hg concentrations reflect long-term Hg exposure at the site sampled. Mummichog total Hg concentrations were significantly greater in the OB reach, downstream of the plant site, as were total Hg concentrations in tomcod, rainbow smelt, and winter flounder relative to sites further downstream (Table 2). Several factors contributed to the peak Hg concentrations in biota in the downstream OB reach, including the prevailing current from the upstream plant, increased Hg methylation in adjacent marshes and mudflats (Gilmour et al., 2018), and tidal resuspension of contaminated surface sediments in the lower river (Geyer and Ralston, 2018). Total Hg concentrations in

Table 2
Total Hg concentrations by reach in fish and shellfish in the Penobscot study area; geometric mean, adjusted for length or age (eels) and year, within each species; ng/g ww for all species except mussels, which are reported as * ng/g dw. The five reaches are ordered north to south, with the locations of the head-tide Veazie Dam and the HoltraChem plant noted. Bold values indicate the greatest mean total Hg concentration for each species.

Species \ Reach	OV Reference Reach	Veazie Dam	BO Upstream Reach	HoltraChem	OB Downstream Reach	Upper ES Reach	Lower ES Reach
American eel	312		**521**		495		
Mummichog			251		**321**		
Rainbow smelt					**121**	90	73
Tomcod					**155**	122	
Winter flounder					**55**	43	35
Blue mussels* (Fall)						**522***	154*
American lobster						**328**	157

lobster and blue mussels also showed a geographic decline with distance from the Hg source (Figs. 7 and 8).

In addition to these overall geographic trends, we observed an interesting spatial pattern in Hg exposures at individual lobster sample sites in the upper ES reach. The notably greater total Hg concentrations in lobster tail found consistently at the South Verona site (Fig. 7), with two times greater Hg than found at the Odom Ledge site 2.5 km to the northwest, is likely associated with enhanced Hg methylation (Gilmour et al., 2018) in the extensive complex of tidal marshes and mudflats directly upstream of the South Verona site. In addition, Yeager et al. (2018) reported that area to have the among the greatest surface sediment total Hg concentrations in the entire system. In contrast, the Odom Ledge lobster sample site lies near the southern end of the West Channel of the Penobscot River, which flows over a deep rocky cobble bottom (Fig. 1; Geyer and Ralston, 2018) and is unlikely to have enhanced methylation rates. The strong seasonal site fidelity in American lobster (Dunnington et al., 2005; Mercaldo-Allen et al., 2011) may also contribute to localized variation in Hg exposures and accumulations.

4.3. Regional comparisons of mercury concentrations in fish and shellfish

Mercury concentrations in eels from the lower Penobscot River below Veazie Dam were greater than those found in eels in North America and Europe, after adjusting for size, age and life-stage. Mercury concentrations in eels increase with age (Arleny et al., 2007), and eel life-stage determines mercury exposure. The strong site fidelity of yellow eels (Parker, 1995) limits their mercury exposure to the vicinity of capture, while Hg concentrations in migrating silver eels represent accumulations from lakes and tributaries upstream from where they were captured. Silver eels captured near the mouths of three small Maine rivers Leaman (1999) had average ages of 12–16 years, up to twice that of the non-migratory yellow eels from the lower Penobscot (6–8 years in OB and BO; 8–11 years of age in OV); however, Hg concentrations in eels from BO and OB reaches were equivalent to the greatest Hg concentrations for the silver eels (Leaman, 1999), indicating greater Hg exposure for eels from the lower Penobscot River.

Mercury in Penobscot eels exceeded levels reported in European eels (*Anguilla anguilla*), except for notably larger eels sampled near Liverpool, England at a site historically contaminated by the chlor-alkali industry. Total Hg levels in the European eel ranged from 60 ng/g ww in the Tiber River in Italy and 160 ng/g in Bosnia and Herzegovina (Has-Schon et al., 2008; Mancini et al., 2005) to 310 ng/g in estuarine waters with industrial exposure along the coast of France (Arleny et al., 2007). The greatest mean levels were reported in eels sampled in the early 1990s in the Mersey Estuary near Liverpool, reaching 1350 ng/g ww in individuals exceeding 500 mm in length. The larger size of eels from the Mersey Estuary

precludes a direct comparison with mercury levels in the smaller Penobscot River eels.

Other fish species from the Penobscot River and estuary were also elevated in Hg when compared to sites elsewhere in North America. Mercury in tomcod averaged 170 ng/g ww downstream of a Hg-elevated hydroelectric reservoir in Labrador, Canada (Anderson, 2011) and 140 ng/g ww in the Hg-contaminated Meadowlands, New Jersey, USA (Santoro and Koepp, 1986). Similarly, Hg concentrations in rainbow smelt from the downstream OB reach were greater than those reported in landlocked smelt in central Canada (mean total Hg 60 ng/g ww; Swanson et al., 2003, 2006). Mercury concentrations in mummichogs from the Penobscot River were four to ten times greater than reported for whole-body composites of mummichogs (20 ± 10 ng/g ww) collected in the lower Passaic River (New York, New Jersey), an area known for urban and industrial contamination (Ianuzzi et al., 2004). Hammerschmidt and Fitzgerald (2006) reported mean methyl Hg concentrations of 21 ± 18 ng/g ww in winter flounder muscle from Long Island Sound, about three times lower than was found in the more contaminated areas of the Penobscot study area, despite the larger size (mean length, 236 mm) of the Long Island Sound samples. In Boston Harbor a long-term monitoring study reported annual mean concentrations of total Hg in fillets from winter flounder (size not reported) that ranged from 40 to 90 ng/g, ww (Kane-Driscoll et al., 2008), equivalent to or lower than found in the OB and upper ES reaches of the Penobscot study area. Similarly, Gerhardt (1977) reported total Hg concentrations in winter flounder muscle from Delaware Bay of 57 ± 29 ng/g ww, up to 2 times lower than found in the more contaminated region of the Penobscot sample area. Finally, total Hg concentrations in winter flounder sampled in Frenchman Bay and Schoodic Point, Maine, directly east of Penobscot Bay, but outside of the aquatic influence of the Penobscot River, were an order of magnitude lower than found in the more contaminated areas of Penobscot Bay. In 2001 samples of winter flounder from Frenchman Bay averaged 11.1 ± 2.0 ng/g ww in muscle (assuming a 25% increase in Hg from whole fish to muscle samples), and 13.2 ± 4.9 ng/g ww from samples collected at Schoodic Point, for fish averaging 160 to 200 mm in length (Kopec, 2009).

The elevated Hg concentrations found in lobsters from the upper ES reach in Penobscot Bay prompted the Maine Department of Marine Resources to close the entire area to lobster and crab harvest (DMR, 2018). Further, Hg concentrations in raw lobster tissue may underestimate concentrations in boiled white lobster muscle (Perugini et al., 2013). Total Hg concentrations in lobster sampled in Maine estuaries outside of the Penobscot watershed averaged 82 to 208 ng/g ww (EPA, 2014). In the Long Island Sound area of New York Harbor, mean Hg concentrations in lobster muscle was 158–308 ng/g ww (Hammerschmidt and Fitzgerald, 2006; Roberts et al., 1982), lower than in the most contaminated areas of Penobscot Bay.

One of the Penobscot blue mussel sample sites in the lower ES reach, ES04, was adjacent to the NOAA Mussel Watch monitoring site of PBSI (Penobscot Bay Sears Island), permitting an assessment of their long-term trends in Hg concentrations. Mercury concentrations at ES04 between 2006 and 2010 were in the same range as reported for the Mussel Watch site PBSI in the mid-1980s (200 to 250 ng/g dw). However, the

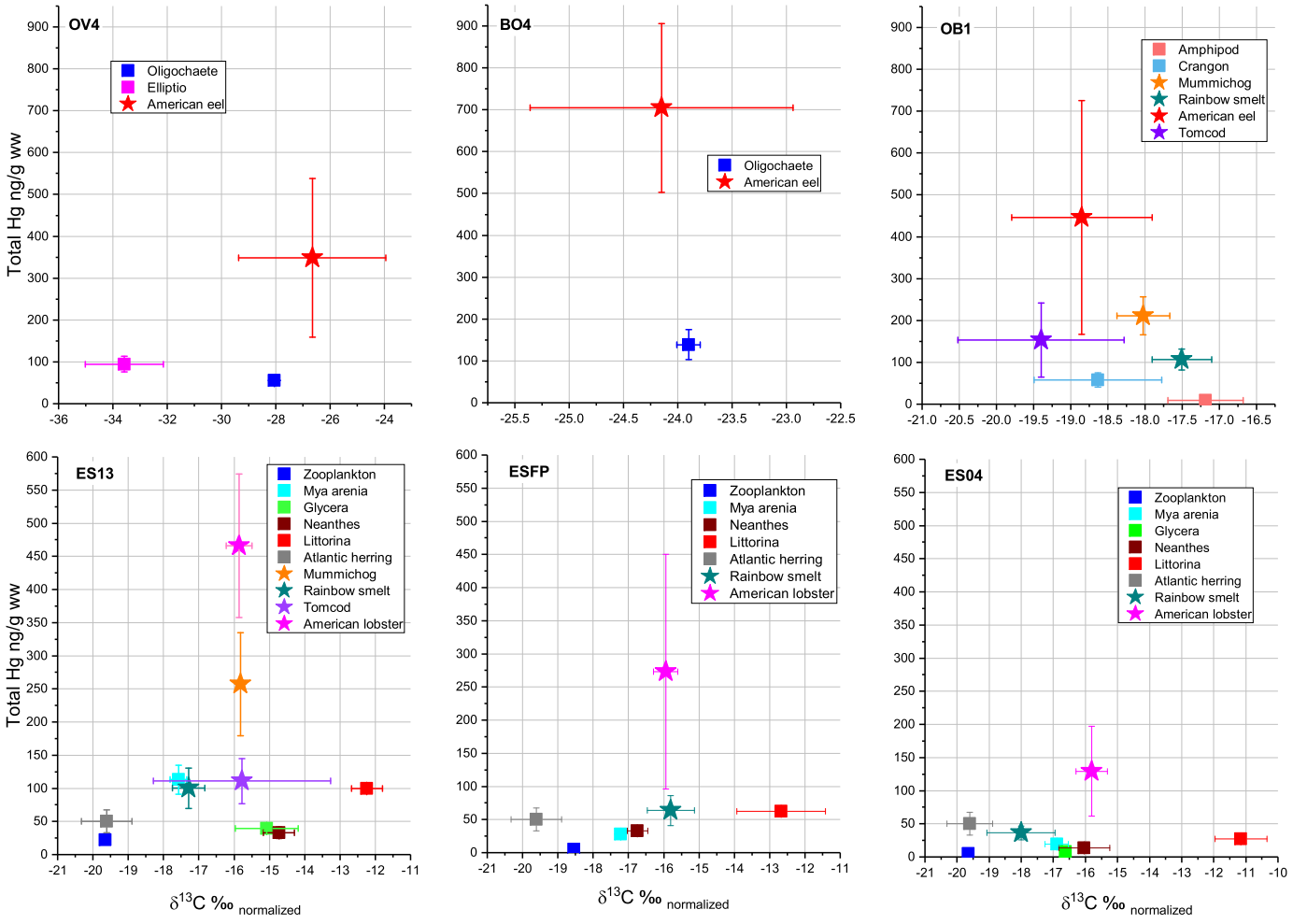

Fig. 12. Bi-plots comparing $\delta^{13}C$ values with mean total Hg concentrations in aquatic food webs at individual sites along the lower Penobscot River and into the ES reach of Penobscot Bay. Sites are ordered from north to south (Fig.1). Note the smaller Y-axis scale on the bottom row and the variable X-axes.

Mussel Watch data showed a large increase in total Hg concentrations in this area in the early 1990s, up to 560 ng/g dw, followed by a gradual decline to 40 ng/g dw by the early 2000s. These broad trends illustrate the need for long-term monitoring to accurately assess mercury concentrations over time.

4.4. Mercury in Penobscot fish and aquatic food webs

In the Penobscot study area, the combined results of the stable isotope and stomach content diet analyses found that most fish fed in the benthic food web at sites on the main stem of the river whereas in Penobscot Bay their diet was mainly pelagic. Mercury biomagnification in the food web is evident throughout the study area, as seen in the Fig. 12 plots comparing $\delta^{13}C$ with total Hg concentrations.

Eels fed primarily in the benthic food web along the entire lower Penobscot. There was good agreement between the $\delta^{13}C$ ratios in eels and the benthic invertebrates and the benthic prey items found in eel stomachs in the OV reference reach and the upstream BO reach (Fig. 12, Appendix 3). In the downstream OB reach the eel stomach contents were almost exclusively *Crangon* shrimp, while the stable isotope analyses indicated a broader summer diet. On average, total Hg concentrations in eels were seven times greater than found in their benthic prey (Fig. 12). However, the biomagnification factor was effectively greater given the low % methyl Hg found in benthic invertebrates (% methyl Hg in Oligochaete worms, 4.5% in the OV reach and 22% in the BO reach.).

Tomcod also fed on benthic prey in the lower river, with a gradual shift to a partial pelagic diet in Penobscot Bay. In the downstream OB reach tomcod stomach contents shifted from *Crangon* shrimp in the north to a diet of epi-benthic shrimp and pelagic fish in the areas closer to the river's mouth (Appendix 3). The stable isotope analyses in the OB reach were aligned with a benthic diet of *Crangon* shrimp and marine worms. In the upper ES reach, the tomcod stomach contents were a mix of pelagic *Neomysis* shrimp and epibenthic *Crangon* shrimp, matching the stable isotope values. The $\delta^{13}C$ values in tomcod muscle were equidistant between blue mussels, at the base of the pelagic food web, and the periwinkle snail, *Littorina*, at the base of the benthic food web. Total Hg concentrations in the tomcod sampled, from 120 to 160 ng/g ww, were two to three times greater than in their prey (Fig. 12).

In rainbow smelt we found strong evidence for a pelagic diet in the lower river and into Penobscot Bay. Smelt stomach contents from the southern end of the downstream OB reach were dominated by pelagic prey with *Crangon* shrimp contributing <10% to the diet (Appendix 3). However, the stable isotope signatures in smelt, integrating the diet over a longer time period, suggested *Crangon* shrimp as a central part of the smelt diet in that region. In the ES reaches, smelt stomach contents contained pelagic and epi-benthic shrimp, and planktonic copepods, in agreement with stable isotope values at the base of the pelagic food web. Total Hg concentrations in rainbow smelt, approximately 100 ng/g ww, were two to four times greater than potential prey.

In the ES reach there was a steady decline in total Hg concentrations in the entire food web, from lobster to zooplankton to *Littorina* snails (Fig. 12). In lobster tail total Hg concentrations decline from 440 to 125 ng/g ww, zooplankton total Hg declines from 22 to 5 ng/g ww and Hg in *Littorina* snails declines from 100 to 25 ng/g ww.

The importance of the benthic food web to fish and lobster in the lower Penobscot River and Bay reinforces the need to reduce the sediment concentrations of Hg in the contaminated area. If Hg is present in surface sediment a portion will be methylated and will enter the base of the food web. Once Hg enters either the pelagic or benthic food web it continues to biomagnify through each trophic level. Reducing sediment Hg concentrations is the only option for reducing contamination of upper trophic level biota feeding in the benthic food web.

5. Summary

Mercury contamination in the aquatic food web of the lower Penobscot River and upper Penobscot Bay remains as a legacy of approximately nine tons of Hg discharged into the lower river from the HoltraChem chlor-alkali plant operating from 1967 to 2000. Mercury concentrations in all target species were consistently greater at sites closer to the discharge than at more distant sites. Mercury concentrations in American eels, mummichogs, and American lobster exceeded the Maine mercury action level of 200 ng/g ww and the lobster fishery was closed by the state in upper Penobscot Bay due to Hg contamination. Food web analyses using both stomach contents and stable isotopes found most species foraged primarily in the benthic food web, with the exception of rainbow smelt and blue mussels. Significant declines in total Hg concentrations were found in species foraging in the pelagic food web at individual sample sites monitored between 2006 and 2012. Overall, these results suggest that there may be slow recovery of the system from Hg contamination and that ongoing monitoring is needed to assess these changes over time.

Acknowledgements

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Appendix 1. Models used to estimate trophic position of target fish and lobster using primary or secondary consumers as the $\delta^{15}N$ base

Models used to estimate trophic position of biota (Post 2002)
For primary consumers
$$\textit{Trophic Position} = \frac{\delta^{15}N}{3,4}$$
For secondary consumers feeding in one food web with one carbon source
$$\textit{Trophic Position} = \lambda + \frac{\delta^{15}N_{\,secondary\ consumer} - \delta^{15}N_{\,base}}{\Delta^{15}N}$$
For secondary consumers feeding in both benthic and pelagic food webs with two carbon sources
$$\textit{Trophic Position} = \lambda + \frac{(\delta^{15}N_{\,secondary\ consumer} - [\delta^{15}N_{base1} * \alpha + \delta^{15}N_{base2} * (1-\alpha)])}{\Delta^{15}N}$$
NOTE:
λ = trophic position of $\delta^{15}N_{base}$ (δ15N of primary consumer/3.4(aquatic) or /2.4 (terrestrial)
$\Delta^{15}N$ = enrichment of $\delta^{15}N$ per trophic level
α = percent contribution of the benthic or pelagic carbon source to the diet of the secondary consumer

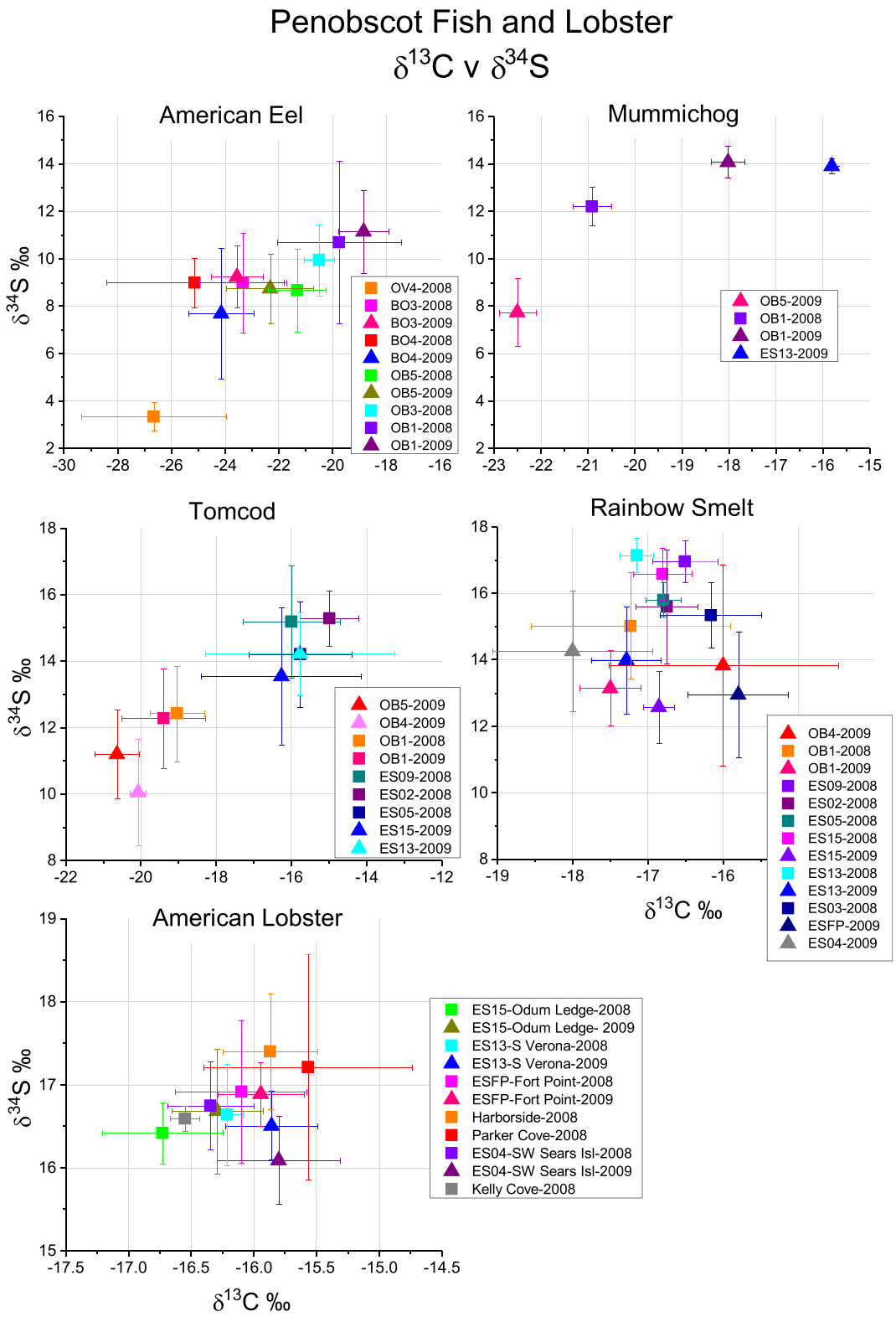
Appendix 2. Values used to estimate trophic position in fish and lobster using the models given in Appendix 1. Carbon sources are designated as benthic or pelagic, or a mixed contribution from both carbon sources (food webs). The taxa representing the $\delta^{15}N_{base}$ are given for each target species and sample site, along with the percent contribution of that carbon source (food web) to the diet (α). The value for the trophic position of the $\delta^{15}N_{base}$ (λ), from base 1, if only one carbon source is consumed, or from bases 1 and 2 if two carbon sources are consumed, is also given

FISH + SHELLFISH TARGET SPECIES		PREY BASE			$\delta^{15}N_{base1}$				$\delta^{15}N_{base2}$				
FOOD WEB SITE	TARGET SPECIES	1° Consumers	2° Consumers with 1 Carbon Source	2° Consumers with 2 Carbon Sources	TAXA	ESTIMATED TROPHIC LEVEL	$\delta^{15}N$ ‰	α	TAXA	ESTIMATED TROPHIC LEVEL	$\delta^{15}N$ ‰	1 - α	λ
		Carbon source	Carbon source	Carbon source									
BO3	American eel		benthic		Oligochaete	1.64	5.58	100				0	1.64
BO4	American eel		benthic		Oligochaete	1.81	6.14	100				0	1.81
OB1	American eel			benthic+pelagic	Amphipod	2.45	8.34	80	*Neomysis*	1.59	5.41	20	2.30
OB3	American eel			benthic+pelagic	(use OB5)								
OB5	American eel			benthic+pelagic	Amphipod	2.00	6.80	80	*Neomysis*	1.59	5.41	20	1.90
OV4	American eel		benthic		Oligochaete	1.04	3.50	100				0	1.04
ES13	Mummichog		benthic		*Littorina*	2.63	8.93	100				0	2.63
OB1	Mummichog		benthic		Amphipod	2.45	8.34	100				0	2.45
OB5	Mummichog		benthic		Amphipod	2.00	6.80	100				0	2.00
ES04	Lobster			benthic+pelagic	*Littorina*	2.26	7.67	53	*Mytilus*	1.89	6.42	47	2.09
ES13	Lobster			benthic+pelagic	*Littorina*	2.63	8.93	53	*Mytilus*	2.24	7.62	47	2.45
ES15	Lobster			benthic+pelagic	*Littorina*	2.70	9.19	53	*Mytilus*	2.31	7.87	47	2.52
ESFP	Lobster			benthic+pelagic	*Littorina*	2.51	8.55	53	*Mytilus*	2.23	7.59	47	2.38
H	Lobster			benthic+pelagic	(use ES04)								
KC	Lobster			benthic+pelagic	(use ES04)								
PC	Lobster			benthic+pelagic	(use ES04)								
ES02	Rainbow smelt		pelagic		*Mytilus*	(ES13)		100				0	
ES03	Rainbow smelt		pelagic		*Mytilus*	(ESFP)		100				0	
ES04	Rainbow smelt		pelagic		*Mytilus*	1.89	6.42	100				0	1.89
ES05	Rainbow smelt		pelagic		*Mytilus*	(ES13)		100				0	
ES09	Rainbow smelt		pelagic		*Mytilus*	(ES15)		100				0	
ES13	Rainbow smelt		pelagic		*Mytilus*	2.24	7.62	100				0	2.24
ES15	Rainbow smelt		pelagic		*Mytilus*	2.31	7.87	100				0	2.31
ESFP	Rainbow smelt		pelagic		*Mytilus*	2.23	7.59	100				0	2.23
OB1	Rainbow smelt		pelagic		*Neomysis*	1.59	5.41	100				0	1.59
OB4	Rainbow smelt		pelagic		*Neomysis*	1.59	5.41	100				0	1.59
ES02	Tomcod			benthic+pelagic	(use ES13)								
ES05	Tomcod			benthic+pelagic	(use ES13)								
ES09	Tomcod			benthic+pelagic	(use ES15)								
ES13	Tomcod			benthic+pelagic	*Littorina*	2.63	8.97	35	*Mytilus*	2.24	7.62	65	2.38
ES15	Tomcod			benthic+pelagic	*Littorina*	2.70	9.19	35	*Mytilus*	2.31	7.87	65	2.45
OB1	Tomcod			benthic+pelagic	Amphipod	2.45	8.34	50	*Neomysis*	1.59	5.41	50	2.02
OB4	Tomcod			benthic+pelagic	(OB1)								
OB5	Tomcod			benthic+pelagic	Amphipod	2.00	6.80	80	*Neomysis*	1.59	5.41	20	1.92
INVERTEBRATE + PREY TAXA													
ALL	Amphipod	benthic											
OB4	Worm (Annelid)	benthic											
ES	Atlantic Herring (lobster bait)		pelagic		*Mytilus*	2.23	7.38	100				0	2.23
ALL	Blue mussels (Mytilus)	pelagic											
OB1	Sand shrimp (*Crangon*)			benthic+pelagic	Amphipod	2.45	8.34	75	*Neomysis*	1.59	5.41	25	2.20
OB3	Sand shrimp (*Crangon*)			benthic+pelagic	(use OB5)								
OB4	Sand shrimp (*Crangon*)			benthic+pelagic	Amphipod	2.24	7.60	75	*Neomysis*	1.59	5.41	25	2.10
OB5	Sand shrimp (*Crangon*)			benthic+pelagic	Amphipod	2.00	6.80	75	*Neomysis*	1.59	5.41	25	1.90
OV4	Freshw ater mussel (Elliptio)	pelagic											
ES04	Blood w orm (*Glycera*)		benthic		*Littorina*	2.26	7.67	100				0	2.26
ES13	Blood w orm (*Glycera*)		benthic		*Littorina*	2.63	8.93	100				0	2.63
ES15	Blood w orm (*Glycera*)		benthic		*Littorina*	2.70	9.19	100				0	2.70
ALL	Periw inkle snail (*Littorina*)	benthic											
ALL	Soft-shell clam (*Mya*)	pelagic											
ES04	Sand w orm (*Neanthes*)		benthic		*Littorina*	2.26	7.67	100				0	2.26
ES13	Sand w orm (*Neanthes*)		benthic		*Littorina*	2.63	8.93	100				0	2.63
ESFP	Sand w orm (*Neanthes*)		benthic		*Littorina*	2.51	8.55	100				0	2.51
ALL	Insect larvae	benthic											
ALL	Opossum shrimp (*Neomysis*)	pelagic											
ALL	Worm (Oligochaete)	benthic											
ALL	Worm (Polychaete)	benthic											
ALL	Zooplankton	pelagic											

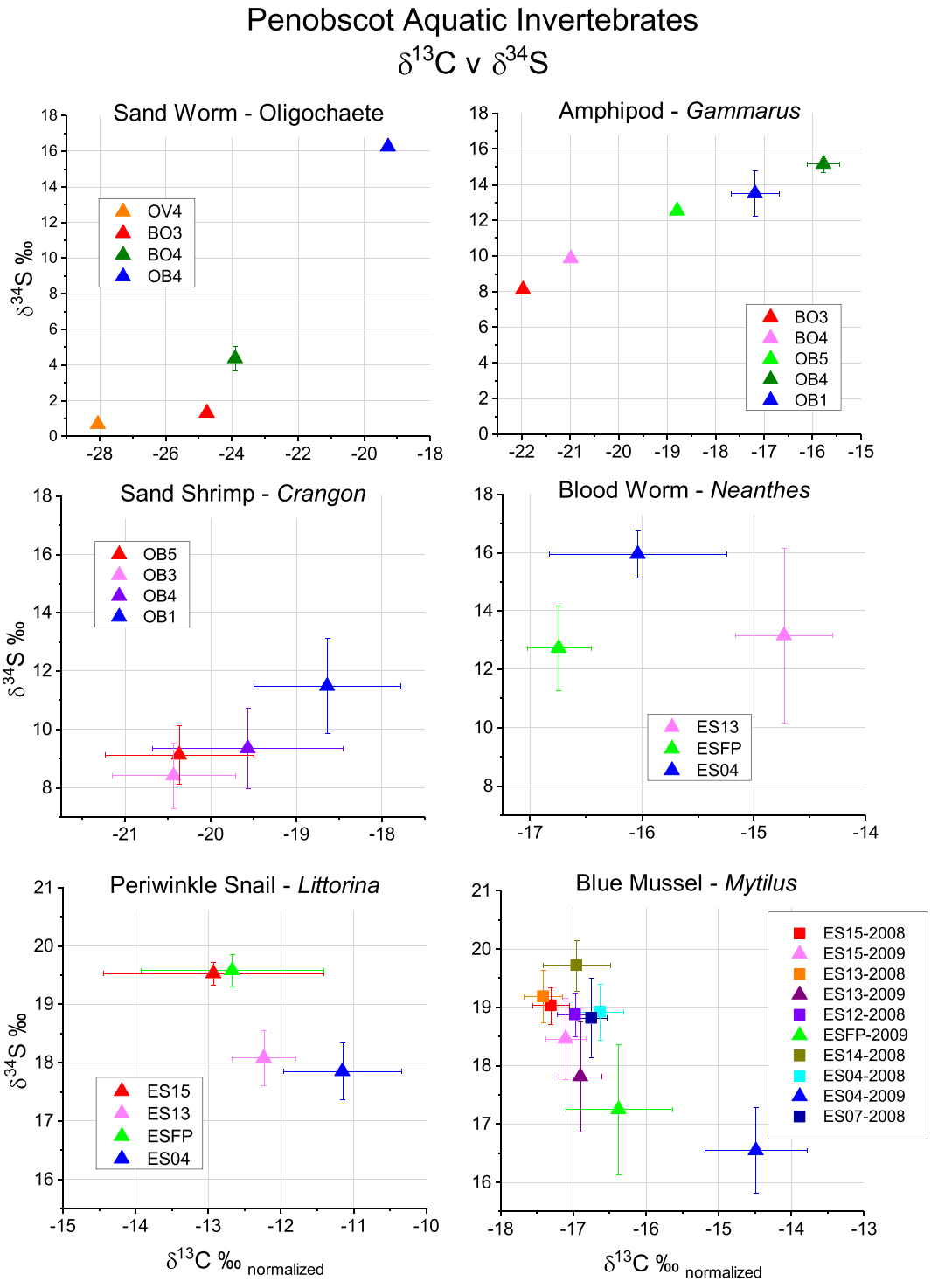
Appendix 3. Results of stomach content analyses (% mass of individual prey taxa) from four species of fish collected in September 2009 from the Penobscot River and Bay

Fish Prey Analysis, September 2009								
Reach	OV	BO	OB				ES	
Fish species	Eel	Eel	Eel	Tomcod	Rainbow smelt	Mummichog	Tomcod	Rainbow smelt
number of useable stomachs	7	7	26	55	8	12	19	34
total number of stomachs	7	12	44	59			19	
total stomach contents weight (g)	17.62	1.80	49.08	13.22	5.52	0.31	5.19	3.23
Fish prey items	% by weight	% by weight	% by weight	% by weight	% by weight	% by weight	% by weight	% by weight
SEMI-PELAGIC INVERTEBRATES			**0.12**	**4.16**	**14.95**	**0.00**	**47.40**	**90.07**
Zooplankton								
Calanoida								56.38
Chaetognatha (worms, could be in plankton or benthos)								0.15
Mysidacea				0.30				7.42
Mysis mixta			0.08	0.53	6.16		2.89	
Neomysis americana			0.04	3.33	8.79		44.51	26.12
EPI-BENTHIC INVERTEBRATES	**17.48**	**10.00**	**96.94**	**73.95**	**9.33**	**9.84**	**40.75**	**9.20**
Amphipods*								
Ampithoidae (amphipod)				0.08				
Ampelisca verilli (amphipod)				0.19				
Corophium sp. (Gammarid amphipod)				0.23			0.10	
Gammaridae				1.02		6.56		
Gammaridae remains				0.08				
Gammarus lawrencianus				1.13				
Gammarus sp.				1.17	0.09			
Isopods								
Anthuridea (isopod)			0.53					
Cirolana polita (isopod, scavenger on dead)				0.08				
Cyathura polita (isopod)			0.94	0.15				
Edotea phosphorea (isopod)				0.04			0.19	
Edotea sp. (isopod)							0.19	
Jaera marina (isopod)				0.04				
Snails								
Gastropoda	0.06							
Decapods								
Crangon septemspinosa			93.54	63.88	9.24		5.49	9.20
Crangon sp.				0.61			0.10	
Crustacea remains	0.17		0.01	4.92			3.08	
Decapoda remains			1.92					
Orconectes sp. (crayfish)	17.25							
Shrimp Decapoda		10.00		0.35		3.28	31.60	
ENDO-BENTHIC INVERTEBRATES	**61.58**	**46.11**		**0.04**			**3.09**	
Nematoda		46.11						
Oligochaeta	61.01							
Pectinaria sp. Tube (annelid worm)							0.10	
Polychaeta remains	0.57			0.04			2.99	
FISH			**1.53**	**19.40**	**74.82**			
Atlantic herring					74.82			
Mummichog			1.45					
Pisces remains			0.08	19.40				
SHELLFISH	**8.51**							
Mollusca	8.51							
INSECTS	**2.61**	**43.34**				**90.16**		
Digested Arthropoda		0.28						
Digested Insecta remains		20.28						
Insecta (ants?)						65.57		
Insecta pupa	0.62							
Perlidae (stonefly)		22.78						
Suborder Oniscidea (terrestrial sow bug)	1.76							
Terrestrial Insecta legs	0.23							
Winged Insecta						24.59		
ANIMAL	**9.19**		**0.11**	**1.36**			**0.10**	
Animal remains	9.19		0.11	1.36			0.10	
PLANT	**0.57**			**0.98**	**0.18**		**8.57**	**0.15**
Detritus				0.30				
Plant material				0.15				
Terrestrial detritus							8.48	
Wood fragments	0.57			0.53	0.18		0.10	0.15
Unidentified digested remains			0.49		0.91		0.10	0.31
*Amphipods possibly semi-pelagic								

Appendix 4. Bi-plots of $\delta^{13}C$ v $\delta^{34}S$ for fish and lobster sampled in 2008 and 2009 in the lower Penobscot River and upper Penobscot Bay, illustrating the distinct site fidelity evident in tomcod and mummichog ratios and the overlapping foraging ranges in rainbow smelt, American lobster and American eel, with the exception of eels sampled in OV4, upstream of the Veazie Dam. Sample sites for each species are ordered north to south



Appendix 5. Bi-plots of $\delta^{13}C$ and $\delta^{34}S$ in aquatic invertebrates sampled in 2009 along the lower Penobscot River and upper Penobscot Bay



Appendix 6. Temporal trend analyses with post hoc power analyses for Penobscot fish. Power was unacceptably low for all analyses, below 0.3

SPECIES	SITE		Total Hg, adjusted for size/age				Total Hg, adjusted for size/age		
			2006-2010				2006-2012		
		#	significant?	slope		#	significant?	slope	
		YRs	P =	incr(+)|decr(-)	1-β	YRs	P <0.05	incr(+)|decr(-)	1-β
Eel	OV4	4	no Δ		0.059	5	0.022	-0.1024	
	OV5	3	no Δ		0.045				
	OV2	1	NA			2	no Δ		0.063
	BO67	4	no Δ		0.086				
	BO66	3	no Δ		0.093				
	BO3	4	no Δ		0.105				
	BO4	4	no Δ		0.076	5	no Δ		0.134
	OB5	4	no Δ		0.085	5	no Δ		0.159
	OB3	2	no Δ		0.048				
	OB73	4	no Δ		0.081				
	OB4	2	no Δ		0.042	3	no Δ		0.059
	OB1	4	no Δ		0.066	5	no Δ		0.287
Tomcod	OB5	2	0.001	-0.515		3	no Δ		0.129
	OB4	2	no Δ		0.056				
	OB1N-5	2	0.012	-0.238					
	OB1E-4	4	no Δ		0.184	5	no Δ		0.337
	OB1E-3	2	<0.001	-0.379					
	OB1S-1	3	0.01	-0.121					
	ES09E	2	no Δ		0.075				
	ES11N	4	no Δ		0.086				
	ES02E	4	no Δ		0.085	5	0.032	-0.0572	
	ES05S	3	no Δ		0.061				
	ES06S	3	no Δ		0.059				
	ES13	1	—			2	no Δ		0.052
Rainbow Smelt	OB5	1	NA			2	0.0009	0.4781	
	OB4	2	no Δ		0.038				
	OB1NE	3	no Δ		0.095	4	<0.001	-0.1778	
	OB1S-1	3	0.010	-0.18					
	ES09E	2	<0.001	-0.382					
	ES11N	4	<0.001	-0.388					
	ES02E	4	no Δ		0.045	5	0.001	-0.1171	
	ES05S	3	no Δ		0.063				
	ES06S	3	0.005	-0.243					
	ES15S	2	0.040	0.457					
	ES13	2	0.013	-0.671		3	0.022	-0.1548	
	ES13S	4	0.001	-0.212					
	ES14N	3	no Δ		0.075				
	ESFP	2	0.020	-0.393		3	no Δ		0.115
	ES04W	3	<0.001	-0.235					
	ES07S	1	NA			2	<0.001	-0.1824	
Winter Flounder	OB5	1	NA			2	no Δ		0.160
	OB4	2	no Δ		0.049				
	OB1E4	3	<0.001	0.531		4	no Δ		0.107
	OB1S-1	2	no Δ		0.063				
	ES11N	3	no Δ		0.059				
	ES02E	3	no Δ		0.058	4	no Δ		0.080
	ES05S	2	no Δ		0.046				
	ES06S	2	0.004	-0.621					
	ES15S	4	no Δ		0.070				
	ES13	2	no Δ		0.066	3	no Δ		0.102
	ES13S	4	no Δ		0.069				
	ES14N	3	no Δ		0.072				
	ES12W	2	no Δ		0.048				
	ES03W	2	no Δ		0.046				
	ESFP	2	no Δ		0.055	3	no Δ		0.062
	ES04W	3	no Δ		0.055	4	no Δ		0.060
	ES07S	2	no Δ		0.062	3	no Δ		0.073
Mummichog	BO5	2	0.005	-0.124		3	<0.001	-0.2162	
	OBINE	3	0.001	-0.206					
	W21	1	NA			2	<0.001	-0.411	

Appendix 7. Temporal trend analyses with post hoc power analyses for Penobscot shellfish. Power was unacceptably low for all analyses, <0.3

SHELLFISH SPECIES	SITE		Total Hg, adjusted for size				Total Hg, adjusted for size		
			2006-2010				2006-2012		
		#	significant?	trend		#	significant?	trend	
		YR	P =	incr(+)|decr(-)	1-β	yr	P <0.05	incr(+)|decr(-)	1-β
Blue Mussel	ES15	4	<0.001	-0.133		5	<0.001	-0.1195	
	ES13	4	<0.001	-0.337		5	<0.001	-0.2309	
	ES12	2	<0.001	-0.479					
	ES03	4	<0.001	-0.249					
	ESFP	2	no Δ			3	no Δ		0.278
	ES14	3	<0.001	-0.274					
	ES10	4	no Δ		0.064				
	ES04	4	no Δ		0.056	5	0.012	0.066	
	ES07	4	no Δ		0.057				
	ES13-SPRING	1	NA			2	no Δ		0.052
	ES04-SPRING	1	NA			2	0.005	0.2342	
Lobster tail	Odom Ledge	3	no Δ		0.046	4	<0.001	-0.248	
	S. Verona	3	no Δ		0.049	4	no Δ		0.071
	Ft. Point	3	0.006	-0.293		4	no Δ		0.066
	Turner Point	4	no Δ		0.058	5	no Δ		0.067
	SW Sears Island	4	no Δ		0.056	5	no Δ		0.061
	Harborside	4	0.004	-0.229		5	<0.001	-0.2034	
	Parker Cove	3	no Δ		0.054	4	0.009	-0.1729	
	Kellys Cove	2	no Δ		0.059				

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