# Final Report

## Biological Processes Affecting Bioaccumulation, Transfer, And Toxicity of Metal Contaminants in Estuarine Sediments

SERDP Project ER-1503

December 2011

Celia Y. Chen
**Dartmouth College**

Nicholas S. Fisher
**Stonybrook University**

Joseph R. Shaw
**Indiana University**



SERDP
DOD ▪ EPA ▪ DOE

# REPORT DOCUMENTATION PAGE

*Form Approved*
*OMB No. 0704-0188*



**PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ORGANIZATION.**

| 1. REPORT DATE *(DD-MM-YYYY)* | 2. REPORT TYPE | 3. DATES COVERED *(From - To)* |
|---|---|---|
| 31-12-2011 | Final Report | 01-05-2006-31-12-2011 |

**4. TITLE AND SUBTITLE**

Biological processes affecting bioaccumulation, transfer, and toxicity of metal contaminants in estuarine sediments

**5a. CONTRACT NUMBER**

**5b. GRANT NUMBER**

**5c. PROGRAM ELEMENT NUMBER**

**6. AUTHOR(S)**

Celia Y. Chen
Nicholas S. Fisher
Joseph R. Shaw

**5d. PROJECT NUMBER**

ER-1503

**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**

Dartmouth College
Department of Biological Sciences
HB 6044, 406 Gilman Hall, Hanover, NH 03755

**8. PERFORMING ORGANIZATION REPORT NUMBER**

**9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)**

SERDP/ESTCP
901 North Stuart Street, Suite 303
Arlington, VA 22203

**10. SPONSOR/MONITOR'S ACRONYM(S)**

SERDP/ESTCP

**11. SPONSOR/MONITOR'S REPORT NUMBER(S)**

ER-1503

**12. DISTRIBUTION/AVAILABILITY STATEMENT**

Unlimited

**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**

The objectives of this research project were to 1) to characterize metal trophic transfer from sediments to intertidal food webs and determine food web structural variables that are sensitive to biological exposure and bioaccumulation in studies of reference vs. contaminated sites, 2) to understand processes regulating metal bioaccumulation in animals from contaminated sediments, to measure rates and routes of metal bioaccumulation in benthic animals, and to develop a predictive biokinetic model of metal bioaccumulation in benthic fauna representing different functional groups (deposit feeder, filter feeder, and omnivore/predator), and 3) to produce a set of robust genomic biomarkers that are predictive of the site-specific hazards posed by contaminated sediments.

**15. SUBJECT TERMS**

| 16. SECURITY CLASSIFICATION OF: | | | 17. LIMITATION OF ABSTRACT | 18. NUMBER OF PAGES | 19a. NAME OF RESPONSIBLE PERSON |
|---|---|---|---|---|---|
| a. REPORT | b. ABSTRACT | c. THIS PAGE | | 92 | Celia Y. Chen |
| | | | | | 19b. TELEPHONE NUMBER *(Include area code)* 603-646-2376 |

Reset

## TABLE OF CONTENTS



**Figures:**

**Tables:**

**Acknowledgments:** We thank Jason Williams, Jessica Dutton, Darren Ward, Vivien Taylor, and Deenie Bugge for their involvement in experimental and field work as well as data compilation and analyses. We also acknowledge contributions from the undergraduate students, Kate Labrum, Ed Moreno, Laura Coyle, Kaitlin Barber, for her help in conducting experiments.

**ABSTRACT**

The objectives of this research project were to 1) to characterize metal trophic transfer from sediments to intertidal food webs and determine food web structural variables that are sensitive to biological exposure and bioaccumulation in studies of reference vs. contaminated sites, 2) to understand processes regulating metal bioaccumulation in animals from contaminated sediments, to measure rates and routes of metal bioaccumulation in benthic animals, and to develop a predictive biokinetic model of metal bioaccumulation in benthic fauna representing different functional groups (deposit feeder, filter feeder, and omnivore/predator), and 3) to produce a set of robust genomic biomarkers that are predictive of the site-specific hazards posed by contaminated sediments. We used novel comparative approaches to link metal bioaccumulation in natural food webs to bioaccumulation and assimilation of metals by sentinel species in laboratory experiments and to genomic and physiological measures of metal toxicity in the species, *Fundulus heteroclitus*. These tools were applied in field sites ranging from relatively clean reference sites to industrialized contaminated sites. The ecosystem and community level fate of contamination was examined by relating metal burdens to food web structure and functional feeding groups. The transfer of metal contaminants in the food chain was investigated directly in bioaccumulation studies and development of a biokinetic model that is linked to metal trophic transfer measured in field studies. Conclusions: 1) In terms of factors determining bioavailability of metals in contaminated field sites, organic content of sediments plays an important role in decreasing bioavailability to pelagic and benthic feeding organisms. Moreover, metal concentrations of Cd, Zn, As, Pb, and Se in sediments do not predict concentrations in biota whereas sediment concentrations of Hg and MeHg do determine biotic exposures. Finally, pelagic fauna bioaccumulate more MeHg than benthic feeding fauna suggesting that chemical flux from contaminated sediments is important in determining bioaccumulation in pelagic food webs. Biokinetic models of metal bioaccumulation by the amphipod, *Leptocheirus plumulosus*, and the forage fish, *Fundulus heteroclitus*, show that food is an important route of uptake for particularly for MeHg. 2) Assimilation efficiencies of killifish are dependent on metal and on food species (amphipod vs. worm) and are highest for MeHg and lowest for Cd and Cr. For amphipods (*L. plumulosus*), feeding mode (particle feeding or deposit feeding) greatly determines the amount of metal bioaccumulation. At rates previously reported rates of deposit feeding, dietary metal sources account for greater than 90% of metal accumulated. Moreover, pelagic sources of particles account for much greater Cd and MeHg bioaccumulation than sediment associated particulates. 3)

## OBJECTIVE

This research broughts together a multi-disciplinary team (community ecology, metal biogeochemistry, ecotoxicology, applied genomics) to investigate "fundamental pathways and processes controlling the movement of contaminants from sediments to receptors". Four metal contaminants (Hg, As, Cd, and Pb) were investigated in intertidal sediments and associated benthic food webs. This study directly addressed several research priority needs identified by SERDP: 1) Tools to assess site-specific bioavailability; 2) Determination of ecosystem shift as a result of sediment contamination; 3) Effects of multiple metal contaminant interactions on fate and toxicity; 4) Improved understanding of contaminant transfer through the food chain. We investigated metal exposure, bioaccumulation, trophic transfer, and toxicity in the following

three Tasks: Task 1) to characterize metal trophic transfer from sediments to intertidal food webs and determine food web structural variables that are sensitive to biological exposure and bioaccumulation in studies of reference vs. contaminated sites; Task 2) to understand processes regulating metal bioaccumulation in animals from contaminated sediments, to measure rates and routes of metal bioaccumulation in benthic animals, and to develop a predictive biokinetic model of metal bioaccumulation in benthic fauna representing different functional groups (deposit feeder, filter feeder, and omnivore/predator); and Task 3) to produce a set of robust genomic biomarkers that are predictive of the site specific hazards posed by contaminated sediments.

## BACKGROUND

***Environmental Problem.*** Sediments in estuaries are the repositories of often high concentrations of metal contaminants from upland watersheds (MacKenzie, 2001; O'Connor, 1996). The fate of metals in these sediments is dependent on the physical regime of the estuary, the environmental chemistry of the sediments, and the movement of metals from sediments to biotic receptors through biological and ecological processes. Past studies of contaminants in estuarine food webs indicate that fish in the vicinity of contaminated sediments are subject to contaminant trophic transfer and bioaccumulation. (Francis et al., 1998; Peters et al., 1999). The biological and ecological processes that result in exposure of benthic and pelagic communities to metal uptake and toxicity are poorly understood and in need of study. This research project focuses on intertidal regions of estuaries that are often highly enriched in contaminants due to the complex geochemical processes from mixing of fresh and salt water and result in deposition of contaminants to sediments. Chemical transformations within intertidal sediments can influence the ultimate bioavailability of these contaminants (Rolfhus et al., 2003). For example, studies of Hg in aquatic systems have shown that Hg methylation rates are highest in intertidal sediments relative to sediments in deeper waters resulting in the formation of methylmercury (MeHg), the biologically available and toxic species of Hg (Marvin-DiPasquale et al., 2003). This zone of mixing can also cause the binding of metals such as Cd to particles reducing bioavailability (Cantwell et al., 2002; Garnier and Guieu, 2003).

Understanding the movement of metals from intertidal sediments to biotic receptors is important to the ecology of these systems as well as to understanding the potential for human exposure to metal contaminants through consumption of seafood. Estuarine systems provide important nursery grounds for many species of marine fish that develop and grow as they continue to move to deeper portions of the estuary. Marine fish obtain metal contaminants from water and from their food prey. These prey species reside for all or a portion of their life cycle in productive coastal and estuarine waters (Boesch and Turner, 1984; Deegan, 2000; Dionne et al., 1999). Beginning at the intertidal margin, different groups of resident and transient nekton species feed in habitats within the vegetated marsh (i.e., high marsh, low marsh, pools, creeks) and actively transport intertidal production from the marsh to the estuary. These resident species are those that spend their entire life cycle in areas of intertidal wetlands and are good site-specific bioindicators of contamination. Ontogenetic and trophic shifts of feeding across the estuary from intertidal to subtidal result in a horizontal transport of productivity and contaminants across the marsh surface known as the "nekton trophic relay" (Kneib, 1997; Kneib, 2000). Metal contaminants may be taken up by resident or transient nekton and trophically transferred to terrestrial (i.e., birds), estuarine and eventually coastal species that become prey for oceanic predators and humans. Thus, exposure of communities of marine organisms to metals

in the intertidal/subtidal sediments of the estuary can result in physical and trophic transfer of metals to coastal waters.

Metals in sediments are a major concern in DoD sites and in the environment globally. The DoD has over 17,000 sites across the US contaminated with a variety of compounds including heavy metals (Fredrickson et al. 2001). Metals comprise eight of the top fifty substances of concern on the Agency for Toxic Substances and Disease Registry (ATSDR) priority list, including the top three, As, Hg and Pb (ATSDR, 2011). Both Navy and Army sites contain repositories of contaminated sediments and remediation of these sites has been ongoing. Metal contaminants deposited on the landscape are ultimately transported to coastal rivers and estuaries where they are deposited and frequently resuspended in the water column when sediments are dredged to maintain navigable waterways. The vast majority of contaminated sediments contain low to moderate levels that are difficult to assess in terms of ecological risks (USEPA, 1996). Estuarine sediments are repositories for metal contaminants transported from watersheds and important habitat for many invertebrate and fish species. Benthic and epibentic organisms are exposed to metal concentrations producing molecular, population, and community level effects. These biota are also vectors to human exposure through the consumption of seafood.

We investigated As, Hg, Pb, and Cd because of their importance in DoD sites and other industrial point sources. Hg comes from the combustion of fossil fuels, cadmium from batteries, pigments, metal coatings, and plastics, arsenic as a wood preservative, and lead is used in the production of batteries and ammunition. They are all non-essential metals known to be toxic to aquatic organisms and they can mimic essential elements producing potentially complex and damaging effects (Cd mimics Zn; As mimics phosphate and sulfate; Hg binds to sulfhydryl groups).

***Past Research Projects.*** Past research projects have been conducted by the investigators in this team in the area of metal bioaccumulation and trophic transfer in freshwater lakes. Results of these studies show that metals differ in the degree to which they are bioaccumulated and biomagnified in the food web (Chen et al., 2000). Metals such as As and Pb have been shown to biodiminish in the food web whereas Hg biomagnifies from plankton up to fish and Cd and Zn biomagnify from small to large zooplankton (Chen and Folt, 2000; Chen et al., 2000). Earlier studies also show that pelagic food webs (plankton based) bioaccumulate higher concentrations of Hg than do benthic food webs in relatively uncontaminated systems (Karimi et al., 2007). However, whether this is true of contaminated marine sites, particularly those with contaminated sediments, is not known. Finally, metal concentrations in plankton have been shown to decrease with increased densities of phytoplankton and zooplankton resulting in a biodilution of metal (Chen and Folt, 2005; Pickhardt et al., 2002). This may be the reason that Hg concentrations in fish have been found to be negatively related to nutrient concentrations and human disturbance in lake watersheds. The increased disturbance may result in higher nutrient inputs, increased phytoplankton productivity, and decreased Hg concentrations in phytoplankton. At the community level, trophic transfer of metals is greatly affected by the structure of the food web and functional groups represented by the species (benthic vs. pelagic). For example, our research and work of others have shown that food webs with longer chain lengths biomagnify metals such as Hg and organic contaminants such as PCB's to a greater extent than shorter food chains (Cabana et al., 1994; Stemberger and Chen, 1998). Moreover, increased linkages appear to diminish the trophic transfer of Hg and As to fish (Stemberger and Chen, 1998).

Recent field studies on metal bioaccumulation in Gulf of Maine have shown significant differences in metal bioaccumulation across sites. Levels of some metals were substantially higher in industrialized Great Bay NH than in Wells or Mount Desert Island ME. Sediment concentrations measured by the USEPA-EMAP National Coastal Assessment monitoring program are also higher in Great Bay. However, both pelagic and benthic species had higher metal tissue concentrations in Great Bay even though aqueous concentrations were not different across the sites. This suggests that the source of metal to the food webs is the sediments even though pelagic feeders (fish and filter feeders) are exposed only indirectly by ingesting detritus and benthic infauna. In addition, Hg in contaminated sites does not increase with trophic level (as measured by $^{15}N$ signature) but does in a more pristine site. In uncontaminated sites, Hg may be predominantly taken up from water and biomagnified to higher trophic levels. Thus, contaminated sediments make benthic species more important receptors and transfer vectors of metals.

Past research by this research team includes experiments that used gamma-emitting radioisotopes to assess the bioavailability of metals from contaminated sediments to diverse suspension-feeding and deposit-feeding benthic bivalve molluscs. These studies have demonstrated that marine invertebrates and fish can accumulate appreciable quantities of contaminants, including metals, from their food (Fisher and Reinfelder, 1995; Fisher et al., 1996; Wang and Fisher, 1996; 1997). Other past studies conducted by members of this research team have also demonstrated that benthic invertebrates, both deposit-feeders and suspension-feeders, can assimilate metals directly out of sediments (Griscom and Fisher, 2004). To better evaluate the relative importance of the dietary and solute uptake pathways, Fisher and colleagues have developed and field-tested kinetic models (Baines et al., 2002; Fisher et al., 2000; Roditi et al., 2000; Wang and Fisher, 1996). Generally, site-specific model predictions of metal concentrations in animal tissues have been shown to be remarkably similar to independent field measurements. Thus. with this approach it has been possible to account for the major processes governing tissue concentrations of metals and to show that the lab-based kinetic parameters are applicable to field situations.

The use of microarrays as biomarkers of metal exposure is a relatively new approach that needs to be linked to higher level biological responses. SERDP investigators have been among the first to utilize these genetic tools to characterize genetic responses in aquatic organisms to metal exposures. Specifically, we have used this approach with the aquatic crustacean, *Daphnia pulex*, to discover metal regulated genes and to define biomarkers of complex metal exposures that link to physiological outcome and account for individual/clonal variability in pollutant response (Shaw et al., 2004). We had previously used high throughput functional genomics tools (e.g., microarrays) to identify and define the metal binding protein metallothionein in *Daphnia pulex* (Shaw et al., 2008). With the release of the genome assembly for *D. pulex* we have discovered three metallothionein genes and a potential splice variant. Our research has also linked quantitative changes in gene-response due to prolonged metal exposure with mechanisms of toxicity and costs at the population-level (Shaw et al., 2007a). We have also started to define metal response in killifish in terms of mechanisms of toxicity (Shaw et al., 2007b) and exposure related changes in accumulation (Shaw et al., 2007b).

***Scientific Questions.*** The scientific questions addressed in this study involve the bioavailability, bioaccumulation, trophic transfer, and ecotoxicology of metals in estuarine sediments and water. The three tasks in this project utilized techniques at three different levels of biological organization, community, individual, and genomic while addressing questions related to both

metal fate in food webs as well as metal effects on biota. The first task involved field studies of reference vs. contaminated estuarine sites in order to characterize metal trophic transfer from sediments to intertidal food webs and determine food web structural variables that are sensitive to biological exposure and bioaccumulation. The second task involved measuring rates and routes of metal bioaccumulation in benthic fauna in order to develop a predictive biokinetic model of bioaccumulation in fauna of different functional feeding groups. Finally, the third task investigated the genomic response of the model species, *Fundulus heteroclitus*, to metal exposure and linked genomic response to physiological endpoints in order to produce a set of robust genomic biomarkers that are predictive of the site-specific hazards posed by contaminated sediments.

## MATERIALS AND METHODS

***Task 1) to characterize metal trophic transfer from sediments to intertidal food webs and determine food web structural variables that are sensitive to biological exposure and bioaccumulation in studies of reference vs. contaminated sites.***

***Field sites.*** In order to characterize metal trophic transfer from sediment to intertidal food webs, metal bioaccumulation in estuarine food webs were investigated. Major benthic/salt marsh primary producers (benthic microalgae, salt marsh grasses), primary consumers (softshell clams, annelid worms, some polychaetes and oligochaetes, burrowing amphipods), and secondary consumers (sand shrimp, some polychaetes, resident benthic minnows, killifish). Field sites included 7 sampling sites located in 4 estuarine systems. Sites were Harbor Road and Drakes Island from the Webhannet River in Wells, ME; Portsmouth Naval Shipyard in Kittery, ME and Adams Point in Portsmouth, NH both in Great Bay; Somes Sound in Somesville, ME on Mount Desert Island; Greenwich Cove in Greenwich, RI and Bold Point in Providence, RI both in the Narragansett Bay Estuary. These sites were sampled at one time point in each of the years of 2006, 2007 and 2008. In 2007, the Great Bay NH and Wells Estuary ME sites were visited twice, both in summer and fall. Field work across a wider range of ten sites to capture greater contamination was conducted during summer 2008 as part of another related project. Additional sampling sites included Harbor Road and Drake's Island in Wells, ME, Bold Point in Providence, RI, Barn Island, CT, The Waquoit NERR, MA, Indian Cove Boatyard in Buzzard's Bay, MA, Portsmouth Naval Shipyard in Kittery, ME, Squamscott River, NH, Old Lyme, CT, Milford, CT, Jamaica Bay, NY, and Mill Creek in the NJ Meadowlands.

Drakes Island and Harbor Road were collected as two separate sites even though they are both part of the Webhannet River. The Webhannet River Estuary is a marsh-dominated system located 32 km north of the Maine/New Hampshire border. This estuary is relatively unimpacted and serves as a reference site for this study. Mount Desert Island is the most remote of the Gulf of Maine sites and most of the watersheds of the island's coves and bays are in the largely forested Acadia National Park. The only sources of contaminants are atmospheric. The Great Bay Estuary is a complex tidally dominated embayment located on the southern New Hampshire and Maine borders. The estuary is comprised of a variety of habitats including saltmarsh and subtidal eelgrass and extensive clam flats and oyster beds. The Portsmouth Naval Shipyard is toward the mouth of the bay in a largely industrialized area. Adams Point is located in a more forested region of the Bay. The dynamic mixing regime and tidal flux of Great Bay has resulted in the movement of contaminants back into the more remote reaches of the estuary. The two

sites located in the Narragansett Bay differ mainly in the local landuse. Bold Point is largely industrial and Greenwich Cove is a partially forested. Greenwich Cove is bordered by Goddard State Park on the east side and a public boat launch on the west. Bold Point is right in the city of Providence and is bordered by an oil refinery.

***Invertebrate and fish sampling.*** Invertebrates were sampled using a plastic coated Petite Ponar dredge sampler and a plastic shovel. Samples were sieved through a 0.5 mm, nylon coated mesh, returned to the lab on ice and separated from sediments in trace metal clean plastic trays. Invertebrates and benthic fish were then be sorted, counted, and identified to the lowest practical taxon. The most abundant invertebrates were analyzed for metal (inorganic Hg and MeHg, Cd, As, Pb) and C and N stable isotopes. All samples were handled with trace metal clean technique and samples were sorted and stored in acid cleaned teflon vials and frozen for later analysis. We used a variety of sampling gear (fish traps, cast nets, seine nets and minnow traps) to obtain an adequate sample of fish at all lower consumer trophic levels. Individuals over a range of sizes in each taxa were collected in order to determine if there were age or size relationships with metal concentration.

***Stable Isotopes.*** Isotopic signatures ($^{13}C/^{12}C$, $^{15}N/^{14}N$,) and total metal concentrations are being measured for primary producers (saltmarsh grass, benthic algae, phytoplankton, benthic invertebrates and fish). Samples were collected and stored in precombusted glass containers, freeze-dried and subsampled. Samples were analyzed at Colorado Plateau Stable Isotope Laboratory, Northern Arizona University.

***Sediment samples.*** Sediment samples were collected at each site using EPA-EMAP protocols in which the top 10 cm were collected using a polycarbonate tube. Multiple samples were composited into a single sample and subsamples of the composite removed for total metals (Hg, As, Cd, Pb, Ni, Zn, Cu, Cr, Ag), TOC, and grain size. Total metal samples were analyzed in the Trace Element Analysis Core Facility at Dartmouth College and TOC and grain size samples sent out to commercial laboratories. Samples for AVS-SEM were collected directly under water at low tide by inverting and removing an intact plug of sediment with trace metal clean glass sample jars. Samples were chilled and sent within 24 hours of collection to Battelle Laboratory in Seattle, WA.

***Metal analysis of biota***. All metal samples were analyzed by the Dartmouth Trace Element Analysis Core Facility using a magnetic sector inductively coupled plasma-mass spectrometer (ICP-MS ELEMENT2, Thermo-Finnigan, Waltham, MA). Total Hg samples were microwave digested for analysis with an aqua regia acid digestion (Optima, Fisher Scientific) . Total Hg in biota and sediments was analyzed using cold vapor-ICP-MS (instrument detection limits of ca. 0.1 ng $L^{-1}$). External quality control was achieved by digesting and analyzing similar amounts of standard reference materials (SRMs: NIST SRM 2976 mussel tissue and DORM-2, NRC-CNRC Canada). Average Hg recovery rates were 103.5$\pm$5% for the mussel SRM and 93% for DORM-2. For total Hg the method detection limit is approximately 2 ng $g^{-1}$ based on a sample weight of 30 mg and digestion volume of 10 ml. Field blanks for zooplankton were 16 ng $L^{-1}$, 100 times lower than the average sample concentrations. Biotic samples were analyzed for Hg speciation using isotope dilution gas chromatography-ICPMS. Samples were freeze-dried, spiked with an appropriate amount of enriched inorganic $^{199}Hg$ (HgI) and enriched methyl$^{201}Hg$ (MeHg) and

then extracted in 2-3 mls of KOH/methanol (25% w/v). One of two methods for Hg speciation was employed depending on the expected level of Hg in the original sample, a function of the initial available sample mass. For <20 mg the methodology involved purging with inert gas and trapping on a Tenax trap which was thermally desorbed and Hg species were quantified by isotope dilution GC-ICP-MS using a high sensitivity Element2 ICP-MS in low resolution mode. For >20 mg, samples were analyzed according to (Chen et al., 2009). The latter methodology is less time-consuming than the purge and trap method but has higher detection limits and is only suitable for larger initial sample masses. Quality control was conducted through the analysis of two SRM's: NIST 2976, mussel tissue with MeHg certified at 0.0278 ± 1.1 $\mu$g $g^{-1}$ and CRC (Ottawa, Canada) DORM-2, dogfish muscle, MeHg concentration of 4.47 ± 0.32 $\mu$g $g^{-1}$. Average recovery for MeHg in DORM2 was 108% (n=13, r.s.d. = 3.4%) and for NIST 2976 average recovery was 114% (n=12, r.s.d. = 10%). Method detection limits for MeHg analysis by isooctane extraction and capillary GC-ICP-MS (Agilent 7500c, Palo Alto, CA) are 5 ng $g^{-1}$ assuming an initial sample mass of 200 mg. For the purge and trap GC-ICP-MS (Element 2, Thermo-Fisher, Bremen, Germany) method detection limits are 0.2 ng $g^{-1}$ based on an initial sample weight of 25 mg.

Tissue and sediment samples for total Hg, As, Se, Cd, and Pb were acid digested with $HNO_3$ using a MARSxpress microwave digestion unit (CEM, Mathews, NC). Approximately 100 mg of sample was weighed into a Teflon digestion vessel and 2 ml of Optima $HNO_3$ was added. The vessel was heated to 180 °C with a 10 minute ramp and 10 minute hold. After digestion the sample was brought up to 25 ml volume with deionized water. Metals were analyzed by inductively coupled plasma mass spectrometry (ICP-MS, 7500cx, Agilent, Santa Clara, CA) using both collision cell and normal mode following the EPA 6020 protocol. . The digestion quality control included blank, duplicates and certified reference materials (SRM's: NIST SRM 2976 mussel tissue n=3, and TORT, NRC-CNRC Canada). Average metal recovery rates for mussel and TORT respectively were: THg 114.7+12.7, 99.6%; Cd 105+2.5, 110%; Pb 110.8+6.0, 129%; As 118.5+9.0, 106%; Se 114.6+13.6, 108.6%). Detection limits based on a 40 mg sample were: THg 0.015 mg/kg: Cd 0.158 mg/kg, Pb 0.016 mg/kg; As 0.128 mg/kg; Se 0.345 mg/kg.

***TOC analysis***. % TOC was determined at an external lab using thermal partitioning at 550°C (EPA Method 440.0). Total (organic + inorganic) C was determined at 1350°C combustion temp. A second sample was burned in a muffle furnace at 550°C to burn off organic C but leaving inorganic C. The residue from that procedure was put through the combustion analyzer at 1350°C to measure inorganic C. Organic C was calculated as the difference between the 2 determinations.

***Data analyses*.** We used $\log_{10}$-transformed metal concentrations in all analyses as this equalized variance and normalized residuals. All analyses were conducted using the statistical software program, JMP 5.01a. In addition, Biota-Sediment Concentration Factors (BSCF) were calculated for the five focal taxa collected in 2006 (BSCF = metal concentration in biota/metal concentration in sediment). The relationship between BSCF values and % TOC or SEM-AVS across sites were determined using analysis of covariance (ANCOVA) with species as an additive classification term. We used ANCOVA to test for a relationship between sediment metal vs. metal concentrations in the focal species across sites. The ANCOVA model included species

as a classification term and sediment metal as a covariate, with mean metal for each species at each site as the response. In ANCOVA analyses, we initially tested for interaction terms, and if none were significant, they were dropped from the models. We analyzed stable isotopes of N and C to identify organisms that feed more on pelagic resources (more depleted $^{13}C$) and feed at higher trophic levels (more enriched $^{15}N$). These patterns in stable isotopes reflect position within a food web, but there was confounding spatial variation in isotopic signatures across sites. Therefore, we included site as an additive blocking factor in an ANOVA to test for differences among the focal species. Thus, stable isotope values were compared between taxa within sites not across sites.

***Task 2) to understand processes regulating metal bioaccumulation in animals from contaminated sediments, to measure rates and routes of metal bioaccumulation in benthic animals, and to develop a predictive biokinetic model of metal bioaccumulation in benthic fauna representing different functional groups (deposit feeder, filter feeder, and omnivore/predator).***

***Effects of water exposure on metal uptake in killifish.*** Two liters of 0.2-μm filtered Norfolk, VA seawater (19.5 psu, 18°C) were labeled with radioisotopes. 73As and 109Cd were double labeled, while $^{51}Cr$, inorganic 203Hg and $^{210}Pb$ were single labeled. Metal concentrations were 1.06 nM for $^{73}As$, 0.68 nM for $^{109}Cd$, 0.18 nM for $^{51}Cr$, 0.33 nM for $^{203}Hg$ and 0.24 nM for $^{210}Pb$. After isotope addition the water equilibrated for at least 10 hours. Eight killifish were added to containers with 250 ml of the radiolabeled water (1 fish per container). The exposure time varied by metal with $^{203}Hg$ requiring a 4 hour exposure, $^{210}Pb$ a 25 hour exposure and $^{73}As$, $^{109}Cd$ and $^{51}Cr$ a 36 hour exposure. At regular intervals throughout the exposure fish were removed from the radiolabeled water, rinsed to remove excess radioisotope adsorbed to the body surface, and gamma-counted for 5 minutes in a large well NaI (T1) detector to calculate an accurate uptake rate. $^{73}As$ was detected at 53.4 keV, $^{109}Cd$ at 88 keV, $^{51}Cr$ at 320 keV, $^{203}Hg$ at 279 keV and $^{210}Pb$ at 46.5 keV.

At the end of the uptake exposure fish were added to 750 ml non-radiolabeled seawater and allowed to depurate for 10 days to calculate a loss rate. Timepoints were taken regularly for the first 48 hours and then every 24 hours thereafter. At each timepoint a 1 ml water sample and feces were collected and counted in an lkb gamma dectector. Water was changed after 24 hours and then every 3 days. The fish were fed a daily diet of frozen brine shrimp and bloodworms. After 10 days the fish were euthanized using MS-222, dissected, gamma-counted and dried at 60°C for 4 days to acquire dry weights. The fish were dissected into head (excl. gills), gills, internal organs (excl. liver), liver, skeleton, fillet and skin to assess tissue distribution.

***Aqueous Concentration Experiments.*** The purpose of these experiments was to investigate if metal uptake rate constants via an aqueous exposure ($k_u$), the subsequent loss of this metal ($k_{ew}$), and body distribution are influenced by metal concentration in the water. The uptake and efflux rate constants were used in a biokinetic model of metal bioaccumulation in *F. heteroclitus*. Five metal concentrations of Cd and Cr were chosen ranging from 0.2-3.5 nM, and five killifish used per concentration. Cd and Cr fish were labeled separately. Each fish was exposed to 250 ml of radiolabeled water at a given concentration and regular uptake timepoints were taken over a 3.5 day period. At the end of the Cd uptake the fish were killed and dissected, whereas the Cr fish underwent a six day depuration (with regular timepoints), and then killed and dissected. Both experiments were carried out in Norfolk, VA seawater (19.5 psu, 18°C).

***Aqueous exposures using water from three field sites.*** The purpose of these experiments was to investigate the influence of salinity and DOC on metal uptake, loss and body distribution using water from three locations with varying chemical characteristics. Water was collected from Baltimore Harbor, MD (BH); Norfolk, VA (NV); and Mare Island, CA (MI). Site water was radiolabeled with $^{73}$As, $^{109}$Cd, $^{51}$Cr and $^{203}$Hg. Cd and Hg were single labeled, As and Cr were double labeled. Salinity, DOC concentration and metal concentration added to each location is shown in Table 1. All experiments were carried out at 18°C. Each fish was exposed individually in 250 ml of radiolabeled site water.

| Location | Salinity (ppt) | DOC (µM) | Metal concentration (nM) | | | |
|---|---|---|---|---|---|---|
| | | | As | Cd | Cr | Hg |
| **Baltimore Harbor** | 7.6 | 103 | 0.27 | 0.45 | 0.43 | 0.01 |
| **Norfolk** | 19.5 | 117 | 1.06 | 0.45 | 0.43 | 0.01 |
| **Mare Island** | 23.0 | 42 | 0.34 | 0.45 | 0.85 | 0.03 |

**Table 1. Salinity, DOC and metal concentration additions**

Regular time points were taken over a 37 hour uptake period to calculate an accurate uptake rate. At the end of uptake the killifish were rinsed in non-radiolabeled water and allowed to depurate over 9 days with regular time points. At the end of depuration the fish were killed and dissected. 7-8 fish were used per treatment using Norfolk and Mare Island water. 25 fish were used for the Baltimore Harbor treatments and 5 fish were sacrificed every few days throughout the depuration period to monitor metal movement around the body.

***Trophic transfer from worms to killifish.*** Live California blackworms (*Lumbriculus variegatus*) were radiolabeled with As, Cd, Cr and Hg for 2.5 – 3 day and then fed to ten killifish (*Fundulus heteroclitus*) as described in Dutton et al. (2011) (Dutton and Fisher). At the end of feeding the initial radioactivity was recorded and regular timepoints were taken over 9 days while the fish were depurating. At the end of depuration fish were dissected into head, gills, internal organs (excl. liver), liver, skeleton (backbone and fins), fillet and skin to assess body distribution.

***Trophic transfer from amphipods to killifish.*** Experiments were conducted to investigate the trophic transfer of cadmium, inorganic mercury and methylmercury from phytoplankton to a benthic amphipod to fish, simulating a benthic food chain (see Dutton et al. 2011 (Dutton and Fisher). Depuration of ingested radioactivity occurred over 9 days, monitored regularly over time. 25 fish were used in this study; 5 fish were dissected every few days throughout the depuration period to assess the movement of each metal in the body over time.

***Killfish MeHg exposure via diet varying salinity and DOC.*** For killifish experiments, individuals were exposed to methylmercury (MeHg) via aqueous exposure in water of three

different salinities and DOC: Baltimore Harbor, MD (7.6 ppt, 103 μM DOC concentration); Norfolk, VA (19.5 ppt, 117 μM DOC concentration), and Mare Island, CA (22-23 ppt, 42 μM DOC concentration). MeHg addition was 0.13 nM at all field sites. After the water was radiolabeled and allowed to equilibrate (~10 hours), killifish were added to individual containers with 250 ml of water and allowed to take up MeHg for 37 hours, with bioaccumulation being checked regularly over time. At the end of uptake the fish were rinsed, counted for radioactivity, and allowed to depurate over a 9 day period, after which they were dissected. For the Norfolk and Mare Island treatments 8 fish were used. For the Baltimore Harbor treatment 25 fish were used, and 5 fish were dissected at regular intervals throughout the depuration period to investigate metal movement around the body over time.

***Killifish DOM experiments.*** For killifish aqueous exposures, DOM was purchased as humic acid isolated from the Suwannee River, Georgia. 1.25 L of DI water was adjusted to pH 10 using 1M NaOH for each DOM concentration. Humic acid was added at the following concentrations: 0 mg/L, 2.5 mg/L, 5 mg/L, 10 mg/L, and 20 mg/L, and sonicated for 15 minutes until dissolved. After sonication the pH was adjusted back to 7.8 – 8 using 2N HNO3. Water was then adjusted to 1 ppt salinity using Instant Ocean. Samples of each DOM concentration was taken for dissolved organic carbon (DOC) analysis (results show DOC is approximately 45-50% of the humic acid concentration). 250 ml of water were then poured into individual beakers (n = 5 per concentration) and left for 12 hours. Metals were then added and left to equilibrate for at least 8 hours. As and Cr were double labeled, and Cd, Hg and MeHg were single labeled. Each beaker was individually radiolabeled. Metal additions were 4.69 nM As, 0.43 nM Cr, 0.73 nM Cd, 0.72 nM for Hg, and 0.7 nM for MeHg. One fish was added per beaker and regular time points monitored metal uptake for 72 hours. At each time point the fish were rinsed in non-radiolabeled water to remove excess radioisotope before gamma-counting. At the end of uptake fish were dissected into head, gills, internal organs and body.

***Killifish salinity experiments.*** For these experiments, killfish were exposed to As, Cd, Cr, inorganic Hg [Hg(II)], and methylmercury (MeHg) dissolved in the aqueous phase at varying salinities (0 ppt, 2 ppt, 6ppt, 12 ppt and 25 ppt) according to Dutton et al. (2011) (Dutton and Fisher).

***Metal bioaccumulation from food and water by amphipods.*** *Leptocheirus metal exposures*. Filtered (0.2-$\mu$m) 20 ppt seawater collected from 1-2 m depths in the Elizabeth River in (Norfolk Harbor, Norfolk, VA USA) was used as experimental water during radioisotope spiking, uptake and depuration. $^{109}$Cd ($t_{1/2}$ = 462.3 d) and $^{73}$As (AsV, $t_{1/2}$ = 45.6 d) radioisotope stocks were obtained from the U.S. Department of Energy (Los Alamos, NM). $^{203}$Hg ($t_{1/2}$ = 45.6 d) used in inorganic Hg bioaccumulation experiments was obtained from Georgia State University. $^{203}$Hg ($t_{1/2}$ = 46.6 d) was obtained from Isotope Products (Valencia, CA) and then methylated ((Pickhardt et al., 2006) and references therein) for use in $CH_3Hg$ bioaccumulation experiments. When spiking water with radioisotope, a small volume of 1M NaOH was added to prevent changes in experimental water pH. In aqueous toxicity experiments (Cd, As), animals were exposed to stable metal only. A stock of stable 0.6 g $l^{-1}$ Cd was created by dissolving 0.12 g $CdCl_2$ (Sigma Aldrich) in 100 ml of deionized water. For As, an 1 g $l^{-1}$ stock of arsenate was obtained from Inorganic Ventures. Experimental water in each toxicity replicate was spiked with a metal stock to produce the desired concentration. Nominal water concentrations for stable

metal were validated by analyzing subsamples of water from each experimental replicate with ICPMS.

We used *L. plumulosus* adults in an all experiments, except for the Cd aqueous exposures where juveniles were used due to logistical constraints. Animals were obtained from a supplier (Cd aqueous uptake-Aquatic Biosystems, all other experiments-Aquatic Research Organisms). Amphipods used in the Cd aqueous exposure were 4-6 weeks old and were 3-5 mm in length. Adult amphipods used were over six weeks old and had a mean ± SE length of 5.54 ± 0.20 mm. To test for size differences in adult amphipods used in different exposures, we measured 30 randomly-chosen amphipods from each batch received from the supplier. A Tukey's HSD test showed that adult amphipods used in the experiment measuring inorganic Hg bioaccumulation from water (5.27 ± 0.19 mm) were significantly smaller than both amphipods used to measure inorganic Hg, Cd, and As bioaccumulation from food (6.50 ± 0.19 mm) and those used for measuring aqueous As and Cd aqueous uptake (6.14 ± 0.19 mm). No other significant differences in size among adults were detected.

We used radiotracer experiments to describe *L. plumulosus* metal bioaccumulation from the dissolved phase and from food (suspension-feeding alga *Isochrysis galbana*) for four metals: Cd, As, Hg, $CH_3Hg$. Separate experiments were conducted for each metal and uptake pathway according to Williams et al. (2010) (Williams et al., 2010). In order to evaluate the relative importance of water and food for metal bioaccumulation, parameters measured during radiotracer experiments were incorporated into a biokinetic model (Luoma and Rainbow, 2005; Wang and Fisher, 1999) as described in Williams et al. (2010) (Williams et al., 2010).

***Biokinetics and acute aqueous toxicity.*** Aqueous exposure experiments with Cd and As were designed to measure aqueous metal uptake and efflux at a range of concentrations, from low to acutely toxic. As a result, biokinetic parameters were measured five additional aqueous exposure concentrations. In these treatments, water radiolabeled with $^{109}Cd$ or $^{73}As$ was also spiked with stable metal to produce total water concentrations of 0.005, 0.015, 0.05, 0.15 and 0.45 mg/L for Cd and 0.45, 0.90, 1.80, 3.57 and 7.19 mg/L for As. In addition, for each metal, one treatment concentration (Cd: 0.005 mg/L, As: 1.80 mg/L) was repeated using Instant Ocean rather than Norfolk seawater to test for differences between natural and artificial media. We ran a parallel acute aqueous toxicity test using the same metal concentrations as in the aqueous radioisotope uptake/depuration experiments to quantify % survival and amphipod metal burden at a range of metal concentrations. In toxicity experiments, ten *L. plumulosus* were added to 200 ml of 0.2-$\mu$m filtered seawater spiked with stable metal only in each treatment replicate (N=5). Spiked water was subsampled, acidified with Optima $HN0_3$ to pH 2 and analyzed by ICP-MS to confirm experimental metal concentrations. After 96 h of exposure, the number of dead *L. plumulosus* in each replicate was tallied. Remaining live amphipods were rinsed three times with distilled water, frozen, and then later homogenized, acid-digested and analyzed by ICP-MS to quantify metal contents in amphipod tissues. All remaining live amphipods in a treatment were pooled in order to obtain enough biomass for metal analysis.

Radioactivity measurements and calculations were done according to Williams et al. (2010) (Williams et al., 2010).

***Hg bioaccumulation in amphipods via benthic vs. pelagic feeding.*** We conducted a MeHg and Hg stable isotope experiment to determine benthic vs. pelagic uptake by *Leptocheirus*

*plumulosus* using three different isotopes of Hg ($^{199}$Hg, $^{200}$Hg and $^{201}$Hg) For the MeHg experiment, the Hg isotopes were methylated. Different isotopes were used to label the two different routes of exposure: sediment and water column. Twenty liters of *Isochrysis galbana* was grown to high density in modified F/2 medium and spun down to 500 ml and split into two batches. Each batch was labeled with one of the stable isotopes and allowed to bioconcentrate the label for 24 hours. Each batch was then centrifuged in a high-speed centrifuge to form pellets and each algal pellet re-suspended in approx 50 ml of media. A subsample of each of these labeled solutions was taken to measure filtrate and filtered algae for MeHg isotope labeling. Twenty replicate jars contained labeled algae mixed into sediment ($^{200}$MeHg) and 50 mls of 20 ppt instant ocean spiked with $^{201}$MeHg labeled algae. Five adult amphipods (> 1mm) were added to each jar. At each of three time points, (16h, 24h, 40h), the incubation of 5 replicate jars was terminated and water samples, pore water samples, bulk sediment, and amphipod samples were taken for analysis of Hg isotope concentrations. Additional samples were also taken for unlabeled bulk sediments.

***Effect of salinity on metal toxicity and bioaccumulation in amphipods.*** 96-hr aqueous exposures were conducted for Cd and Zn at different salinities (10 ppt and 20 ptt). A range of metal concentrations was determined based on preliminary LC50 tests. Exposure chambers were filled with 200 ml artificial seawater (Instant Ocean) at a given salinity, populated with 20 individuals *L. plumulosus* (> 1mm) and each chamber was spiked to the given metal concentration. At the end of 96 hrs, mortality was observed and surviving individuals were rinsed, freeze-dried and sent for metal analysis at Dartmouth College Trace Element Analysis Core as above.

***Task 3) to produce a set of robust genomic biomarkers that are predictive of the site specific hazards posed by contaminated sediments.***

***Animals***. For all laboratory studies, *Fundulus heteroclitus*, were obtained from clean laboratory reared stocks from Diane Nacci of the USEPA laboratory at Narragansett or individuals caught in Northeast Creek near Mount Desert Biological Laboratory.

***Microarray FH.G.1 construction.*** *F. heteroclitus* microarrays were designed and produced at the Marine DNA Sequencing and Analysis Facility, a service core of the Mount Dessert Island Biological Laboratory (MDIBL), Salisbury Cove, ME. MDIBL has several ongoing sequencing (EST) projects (i.e., crab, killifish, lobster, shark, skate) and has produced several normalized (low redundancy) cDNA libraries for various tissues of the killifish. Based on sequencing of 40,363 expressed sequence tags (ESTs) obtained from heart and liver (Paschall et al., 2004) and 5,664 ESTs generated at MDIBL from a normalized multiple tissue cDNA library, 617 target genes were selected for an initial pass at producing a microarray. The ESTs representing these genes were used to design optimal 50-mer oligonucleotides via PICKY software. Oligonucleotides were synthesized by Integrated DNA Technologies, suspended in universal spotting solution (Corning), and printed on UltraGAPS slides (Corning) using a Gene Machines Accent arrayer, to produce eight replicate printings of the 617 oligonucleotides on each slide. The spot configuration was designed to provide four independent experimental units (hybridization zones) each containing the full compliment of oligonucleotides arrayed as

geographically independent duplicates. Quality control was assessed with the SpotQC system (Integrated DNA Technologies). Spot uniformity was high with minimal donut formation. A searchable deconvolution file relating EST accession number and functional identification to oligonucleotide spot location is available at http://www.mdibl.org/~dtowle/Fundulus/Fundulus4zoneDeconvol.gal.

***FH.G.1 Experimental design and data analyses***. Initial validation studies were conducted to determine the robustness of microarray response. The goal of these small-scale experiments is to test the technology in terms of performance (i.e., ability to detect differential gene expression in metal vs. control treated tissues from background noise). These will include anthropogenic (As, Cd, Pb) and natural metal (Cu and Zn). For these studies each metal was tested at low and no-effects concentrations defined for this duration of exposure. Exposures included exposures to single/individual metals; binary combinations of As, Cd, and Pb in combination with Cu at low and high concentrations; and complex mixtures of As, Cd, Pb, Cu and Zn.

**Transcriptome sequencing and assembly.** Killifish transrciptomes, representing 72 different conditions (i.e., life-stages, sexes, salinities, tissues, and metals were sequenced from two 454 (flex) sequencing runs. A normalized 454 cDNA library was prepared from total RNA pooled from equal molar concentrations of the 72 different conditions by the IU CGB, following custom methods similar to Meyer et al. 2009. One full PicoTitre(TM) plate of Roche/454 pyrosequencing was performed according to the manufacturer. Reads were assembled using Newbler 2.3 (Roche/454).

**Microarray FH.G.2 design, development, production.** The Center for Genomics and Bioinformatics - Genomics Core Facility collaborated with Roche NimbleGen Systems to design and manufacture a custom multiplex long-oligonucleotide Fundulus microarray. The 12 x 135 probe high-density microarray platform was prepared using Maskless Array Synthesizer (MAS), proprietary Roche NimbleGen Systems' technology. Temperature-balanced probe sets for annotated, known *Fundulus heteroclitus* genes of significance and unannotated transcriptionally active regions (TARs) derived from computational and empirical data (next-generation sequencing) were selected. In addition, Roche NimbleGen control probes and random probes designed by Roche NimbleGen to reflect the genome nucleotide frequencies and sequence by Markov modeling were arrayed arraying on HX12 x 135K format array by NimbleGen Systems of Iceland, LLC.

**FH.G.2 methods.** Total RNA primed with Oligo-dT-T7 Primer and converted to amplified RNA using MessageAmpII aRNA Amplification Kit (Ambion) per manufacturer's recommendations. Amplified RNA primed with Random hexamer Primer (Promega) and converted to ds cDNA using Double-stranded cDNA Synthesis Kit (Invitrogen). RNA degraded with RNaseA. ds cDNA purified with Phenol:Cholorform:IAA (Ambion) and recovered by glycogen-mediated ethanol precipiatation. Nanodrop ND-1000 (ThermoScientific) used to determine concentration. Bioanalyzer 2100 (Agilent Technologies) and DNA7500 Kit (Agilent Technologies) used to determine ds cDNA integrity. ds cDNA (1000ng) labeled using NimbleGen Dual Color Labeling Kit (Roche NimbleGen) per manufacture's recommendations to produce enough labeled product for 12 x 135K microarray chip. Hybridization and post-hybridization washing were performed using Hybridization Kit (Roche NimbleGen) and Wash Buffer Kit (Roche NimbleGen) per manufacturer's recommendations. Image acquisition with Axon GenePix 4200A Professional scanner, 5 micron resolution. Raw signal

intensities extracted with NimbleScan 2.4 Software (Roche NimbleGen) as PAIR files. Quantile normalization to convert raw to processed data.

***RNA Isolation***. RNA to be hybridized to the microarrays was column extracted (Qiagen) and DNAsed (DNA Free, Ambion). Following extraction it was quantified by spectrophotometry (Nanodrop Technologies) and its quality determined with a Bioanalyzer (Agilent).

***Killifish metal exposures.*** Killifish were exposed to metals in attempt to identify genetic biomarkers which may be used to identify differences in killifish response aqueous and dietary metal exposures. Experiments had 3 treatments 1) killifish fed metal-loaded amphipods (*Leptocheirus plumulosus*) while exposed to metal-free water, 2) killifish fed unloaded amphipods while exposed to metal in 32 ppt seawater and 3) killifish fed unloaded amphipods while exposed to metal-free water (control). After a 4 d exposure, individual fish from each treatment were euthanized by pithing of the brain followed by cervical dislocation and their gills and liver were dissected. The metal content in the carcass of each fish from each treatment and the pooled gills and livers from 5 fish (to provide sufficient biomass) was analyzed. The gills and liver of 5 additional fish from each treatment were preserved for later RNA analysis.

# RESULTS AND DISCUSSION

***Task 1: Field sampling***

***AVS-SEM Results***. AVS-SEM analyses showed that the sites varied greatly by metal. In addition, samples within site varied a great deal. Although the Providence River Estuary and Great Bay sites are by far the most industrialized, the SEM concentrations for all metals were not always the highest in those sites. In addition, the Greenwich Cove and Bold Point sites in the Providence River Estuary were not as contaminated as earlier RI state data suggested. However, in general the Great Bay sites (Portsmouth Naval Shipyard and Adams Point) had the highest concentration of metals (SEM) relative to the Mount Desert Island and RI sites. These higher metal concentrations were also balanced against the highest AVS values resulting in the greatest amount of binding of metals in the most contaminated sites. In fact, the SEM-AVS values show that the least industrialized site (Wells Estuary at Drakes Island) actually had the highest metal concentrations relative to AVS binding. The negative values of SEM-AVS indicate that all the metal was bound by AVS and theoretically not bioavailable. However, bioaccumulation in biota suggest otherwise.

| SITE | AVS | SEM Cd | SEM Cu | SEM Hg | SEM Ni | SEM Pb | SEM Zn | SUM SEM | SEM-AVS |
|---|---|---|---|---|---|---|---|---|---|
| Some Sound | 14.3067 | 0.0019 | 0.0203 | 0.000000 | 0.0151 | 0.0334 | 0.1564 | 0.2270674 | -14.079598 |
| Wells Harbor | 0.8453 | 0.0005 | 0.0027 | 0.000000 | 0.0037 | 0.0059 | 0.0623 | 1.00733545 | -11.657515 |
| Wells Drake | 15.5675 | 0.0018 | 0.0211 | 0.000005 | 0.0325 | 0.0408 | 0.3181 | 0.07694389 | -0.9521549 |
| Greenwich Cove | 1.1846 | 0.0004 | 0.0150 | 0.000002 | 0.0040 | 0.0485 | 0.1257 | 0.34664836 | -13.507186 |
| Bold Point | 29.7723 | 0.0033 | 0.1137 | 0.000013 | 0.0398 | 0.0995 | 0.9459 | 0.2015011 | -2.4178056 |
| Portsmouth | 29.6904 | 0.0060 | 0.1315 | 0.000065 | 0.0685 | 0.1998 | 1.2901 | 1.47460363 | -32.268002 |
| Adams Point | 12.6649 | 0.0037 | 0.0536 | 0.000019 | 0.0358 | 0.1210 | 0.7931 | 1.27133758 | -17.750417 |

**Table 2. AVS-SEM analysis of sediments.** All units in $\mu$moles/g DW.

*2006 field sampling*. The Great Bay sites and Bold Point RI had the greatest amount of organic carbon, but also the greatest concentrations of metal, particularly Pb, As, and Zn.

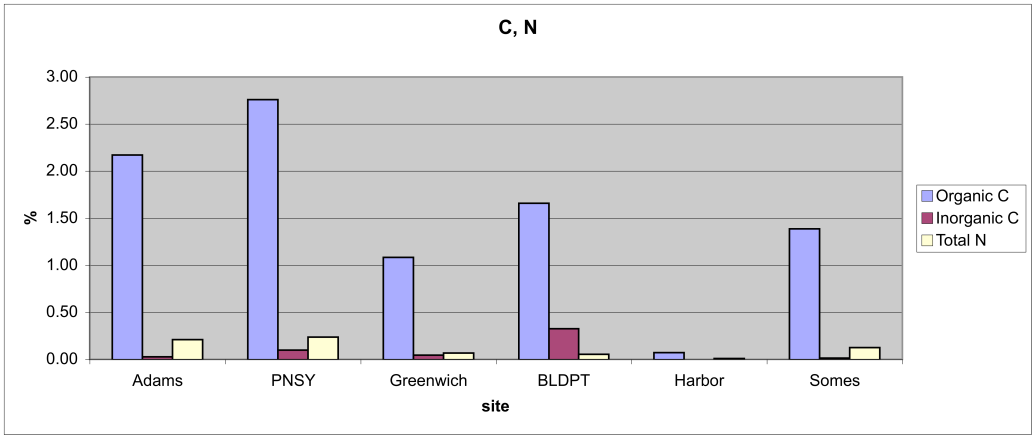



**Figure 1. Percent carbon and nitrogen in sediments across field sites.**

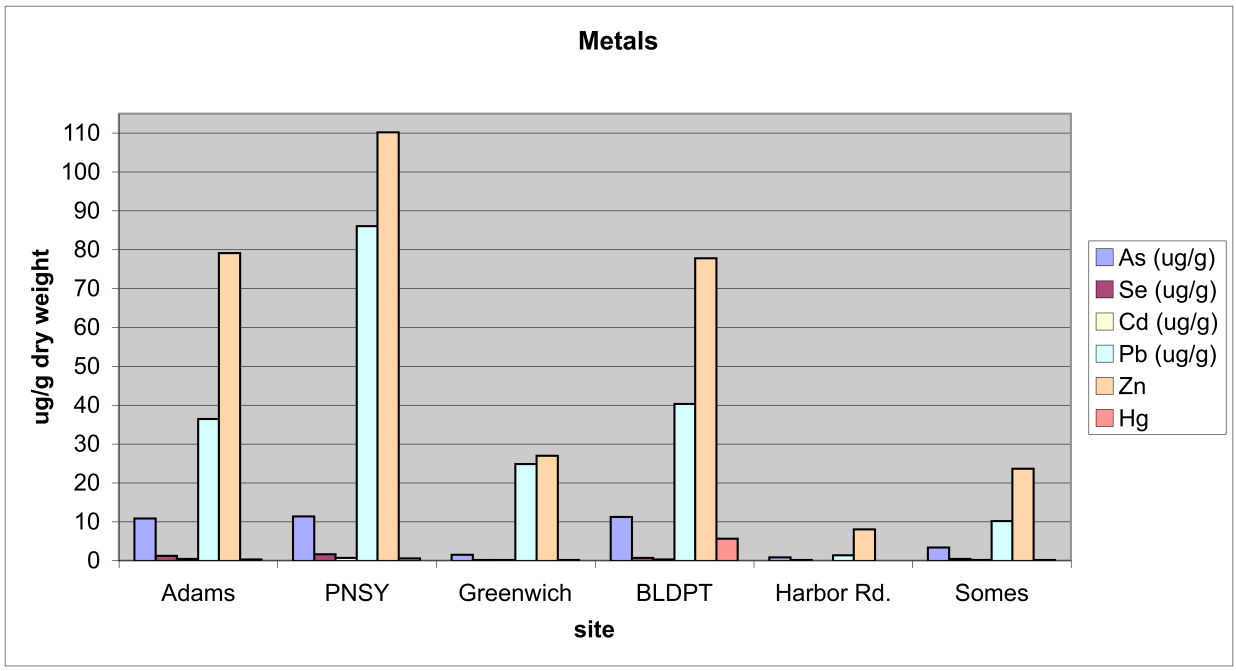



**Figure 2. Total metal concentrations in sediments across sites (ug metal/g dry weight of sediment).**

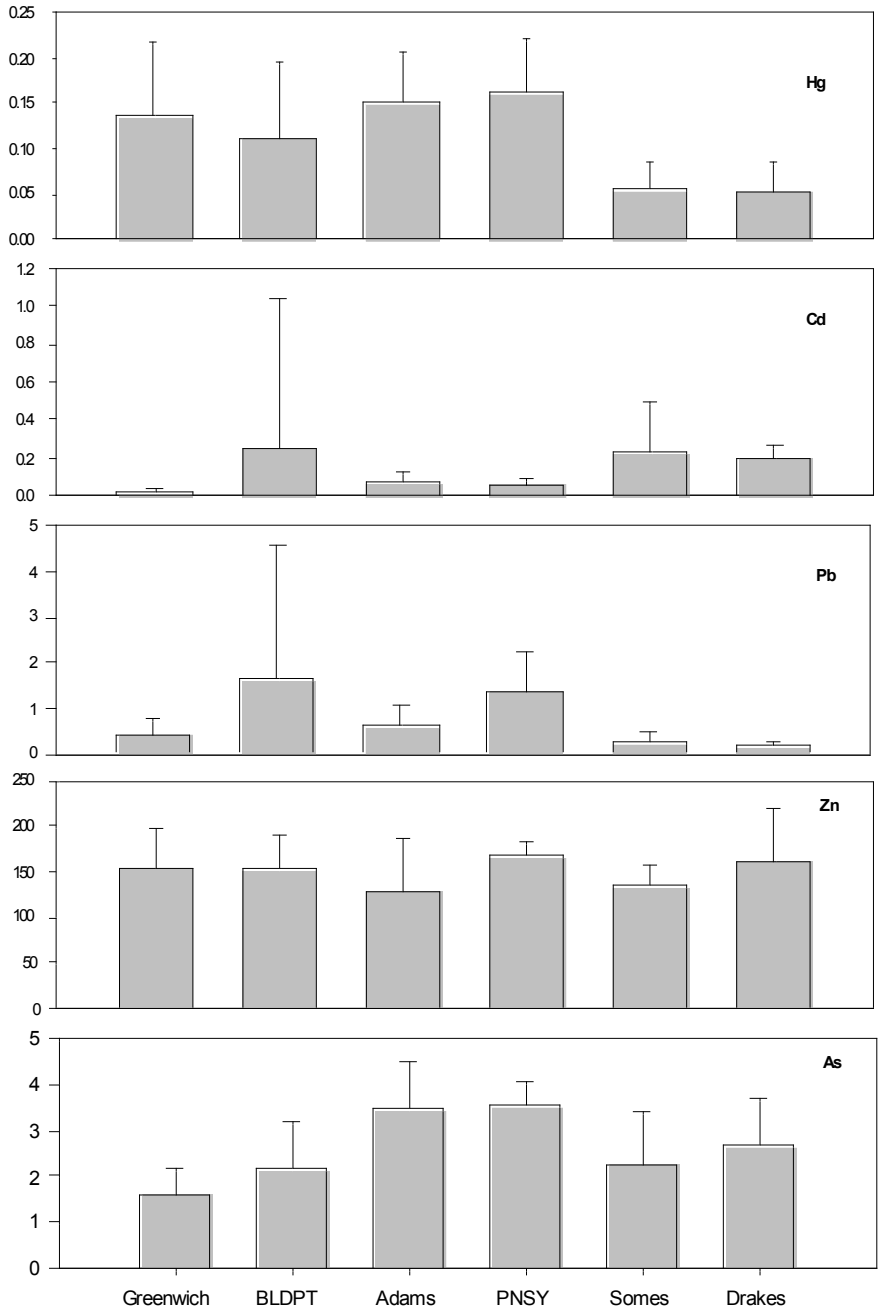
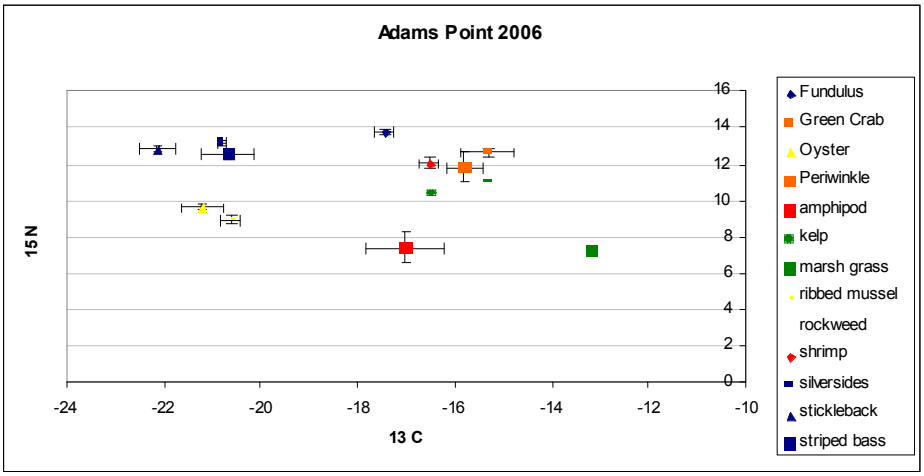


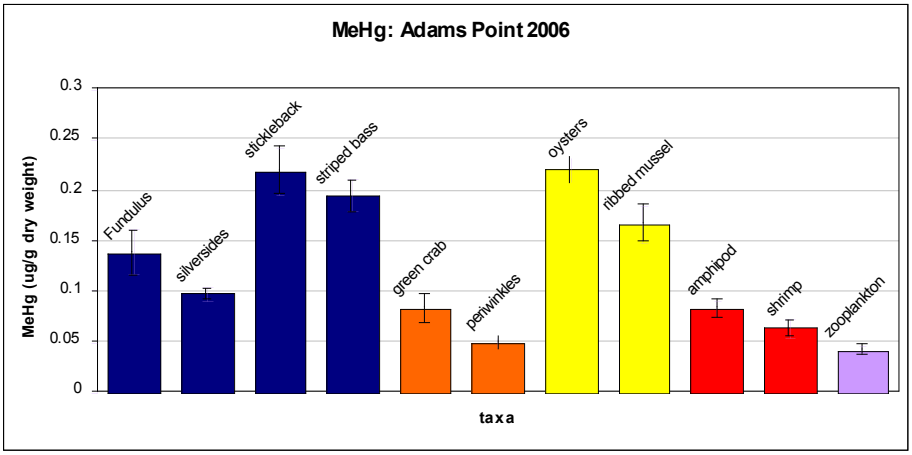

**Figure 3. Total metal concentrations in fish tissues (ug/g dry weight) across field sites**. N = 18 (Greenwich), N = 16 (Bold Point), N = 15 (Adams Point), N = 6 (Kittery), N = 6 (MIDBL-Somes), N = 12 (Wells-Drakes).

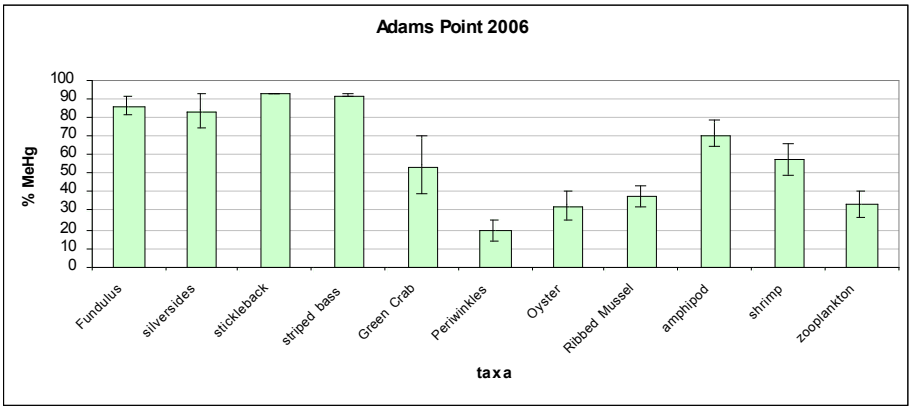
As seen in Figure 3, the Hg and Pb concentrations in 2006 were lower in Somes Sound at Mount Desert Island, ME and Drakes Island at Wells Estuary, ME. This is not surprising given that these two areas are the least industrialized. As concentrations in fish appear highest in Great Bay

NH at the Adams Point and Portsmouth Naval Shipyard sites. Pb and Cd appear higher at the Bold Point site in Providence River Estuary in RI.

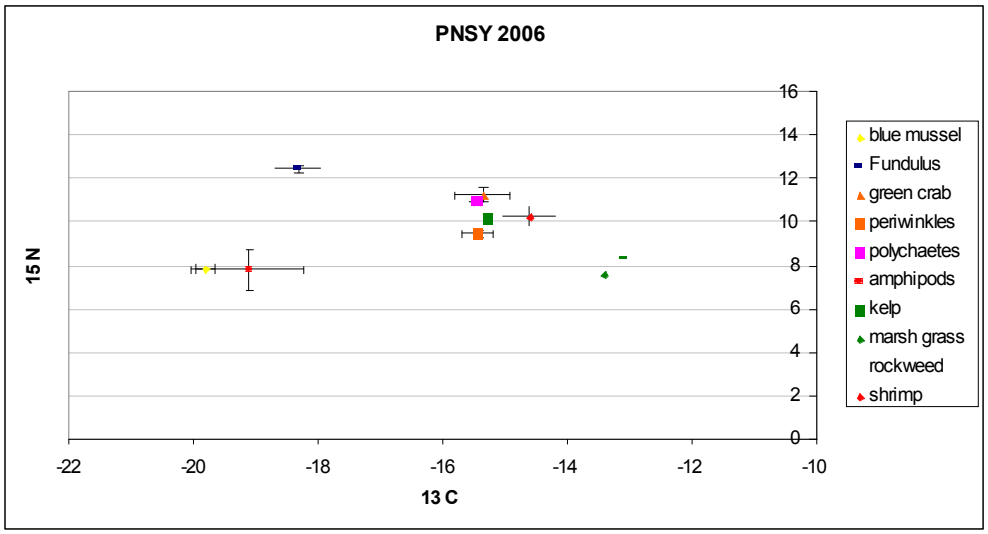
**Figure 4. Stable isotopes (delta $^{15}$N and $^{13}$C), MeHg, and % of total Hg as MeHg across taxa and sites (a-g).**
**a. Adams Point, NH**

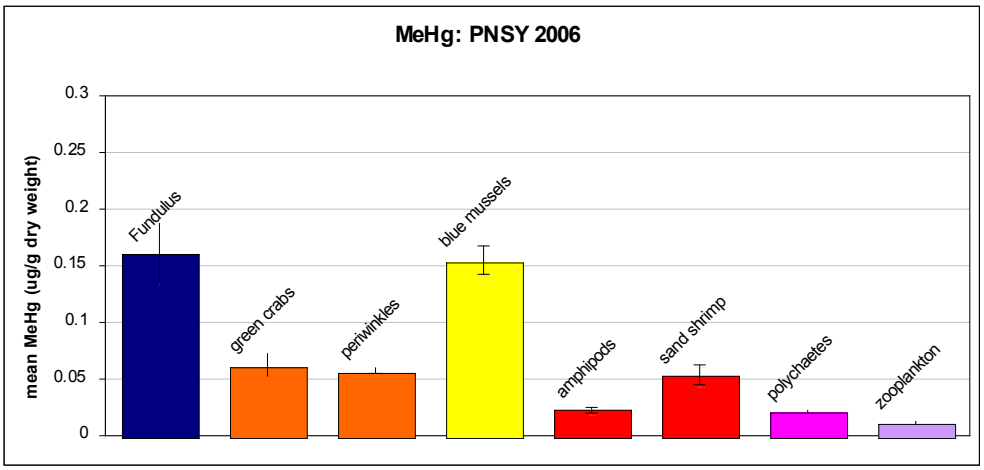


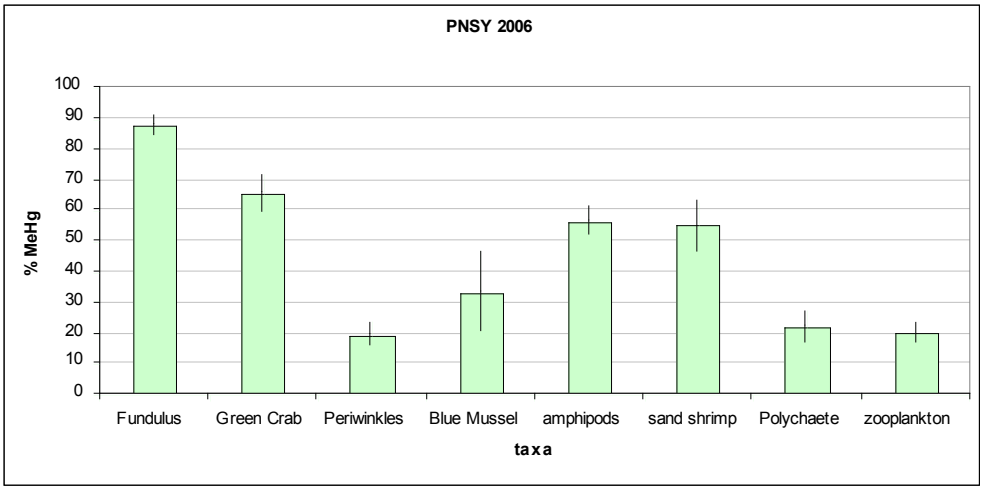



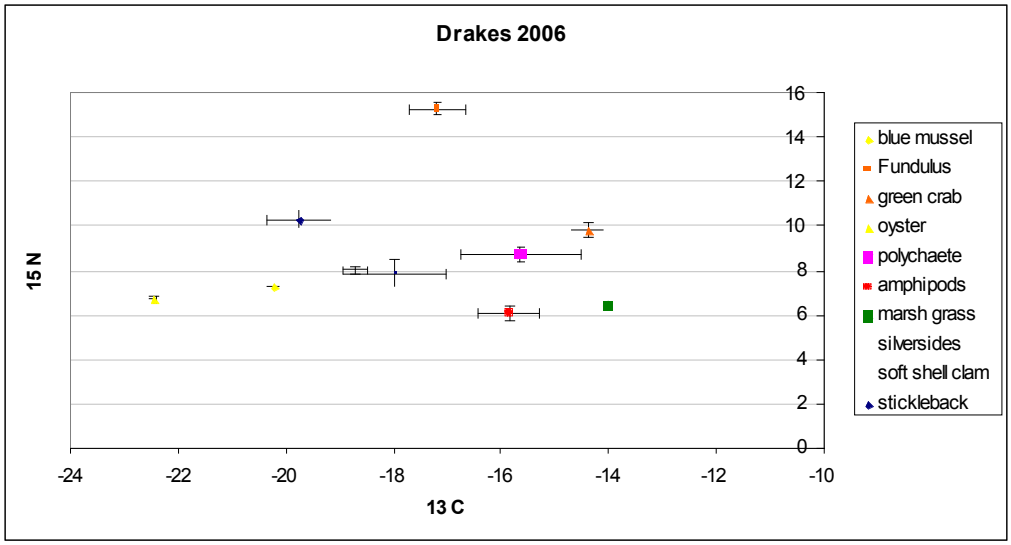
## b. Portsmouth Naval Shipyard (Great Bay NH)

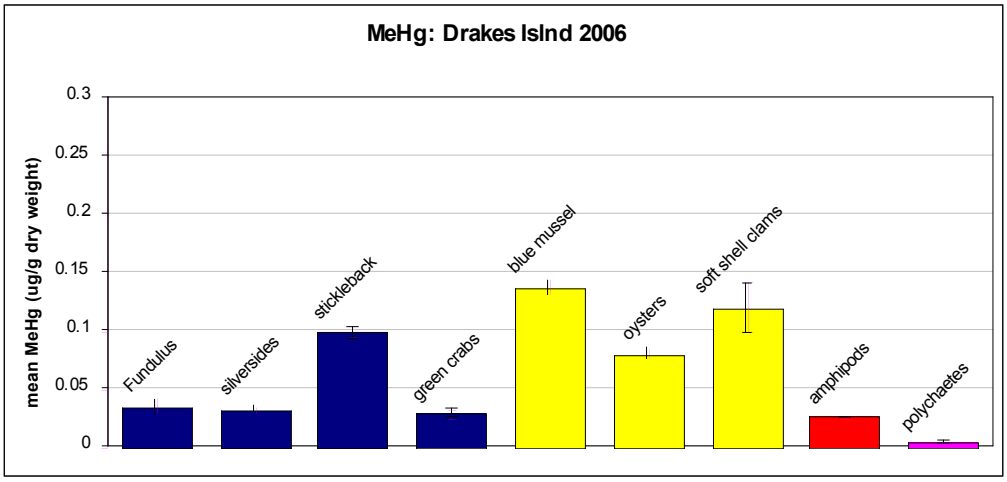


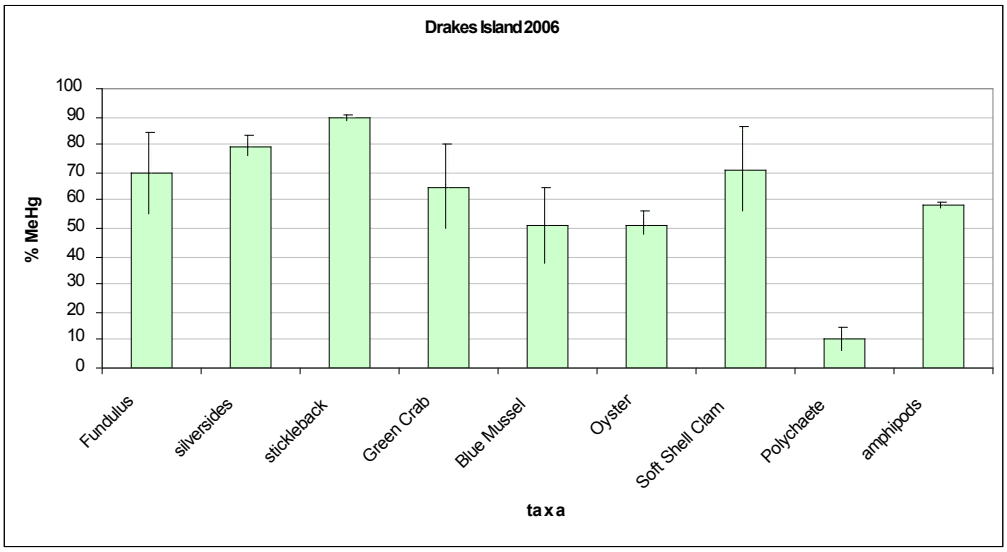



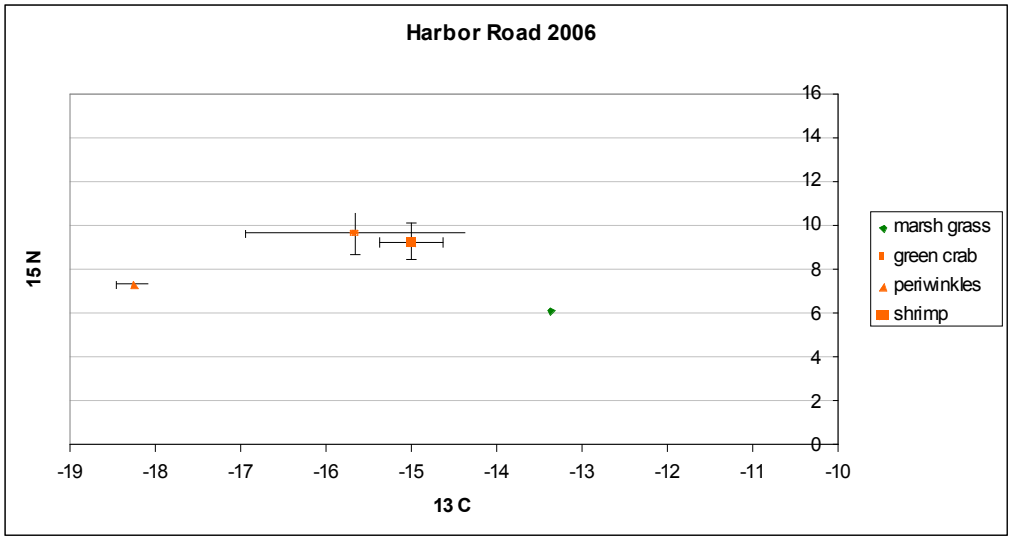
## c. Drakes Island (Wells Estuary ME)

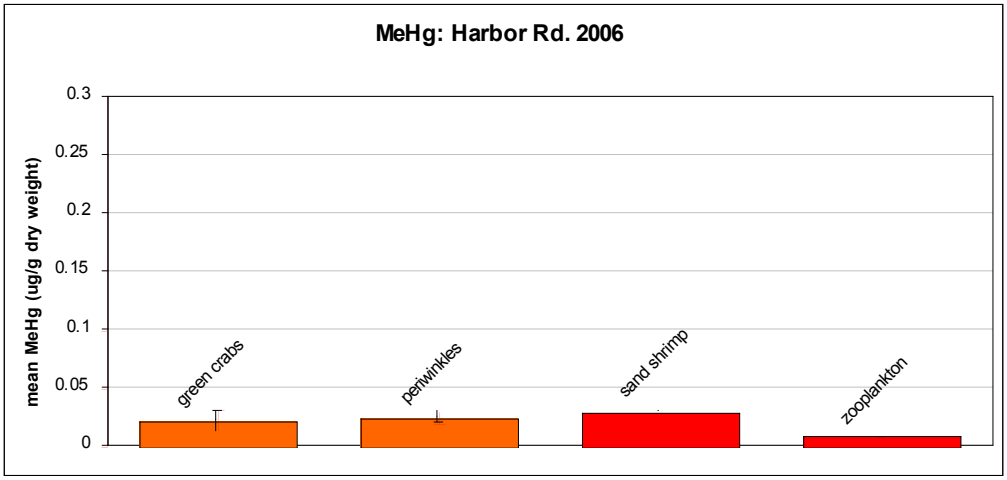


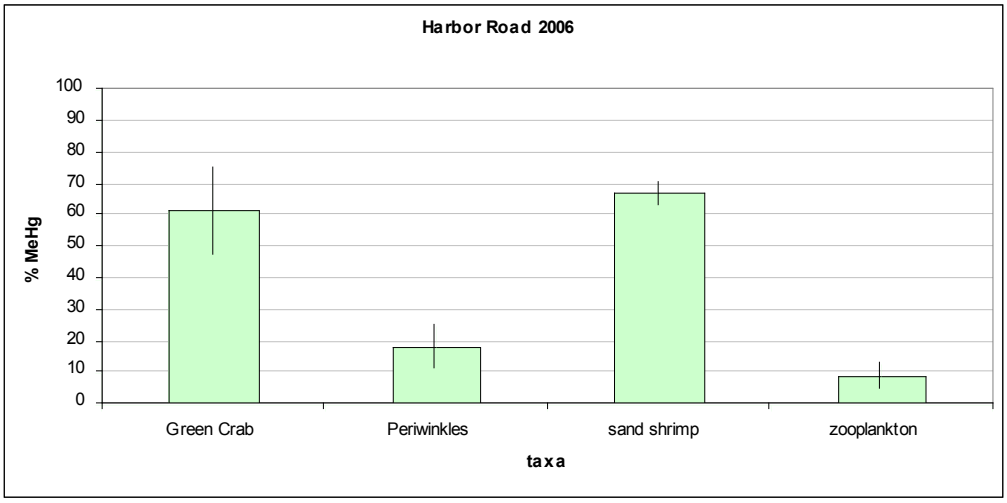



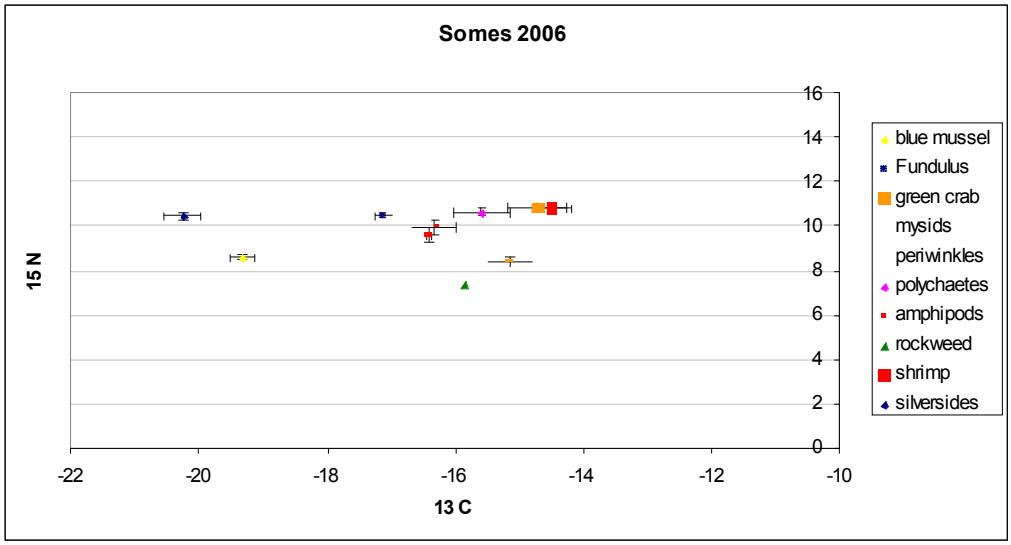
## d. Harbor Road (Wells Estuary ME)

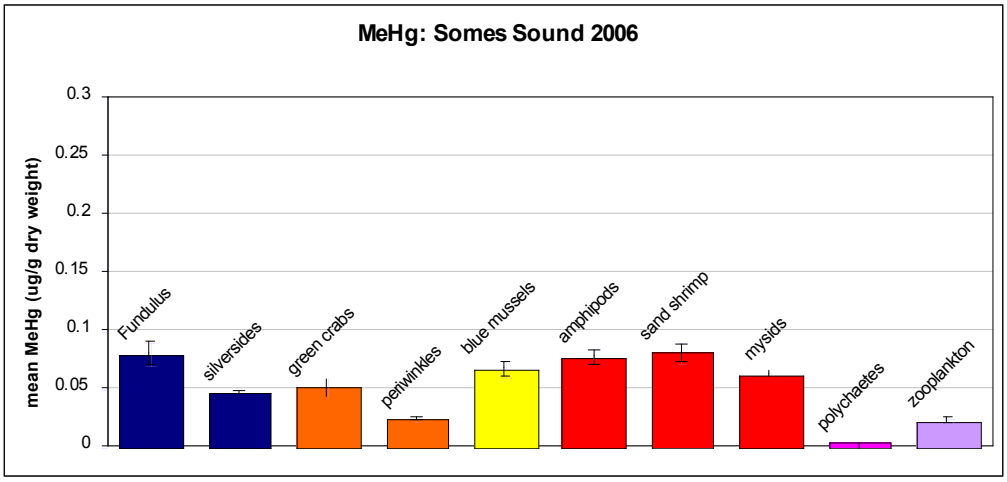


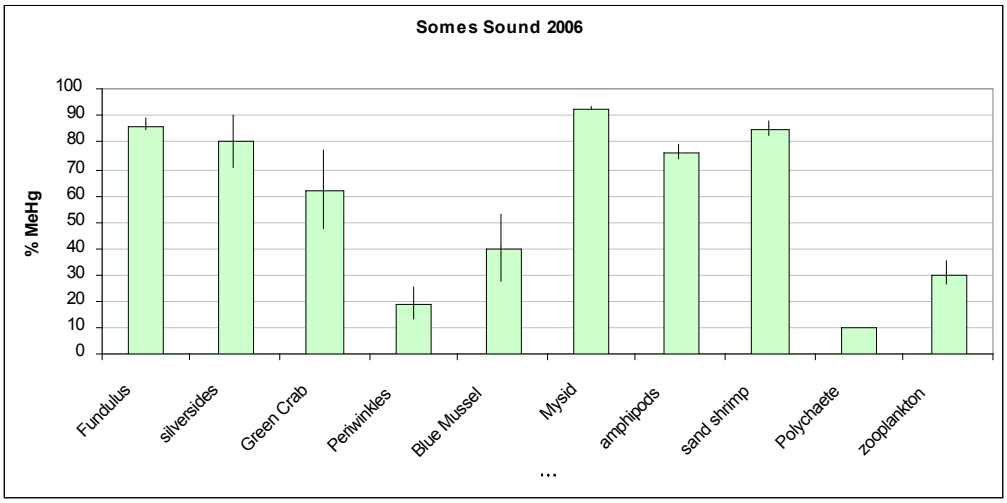



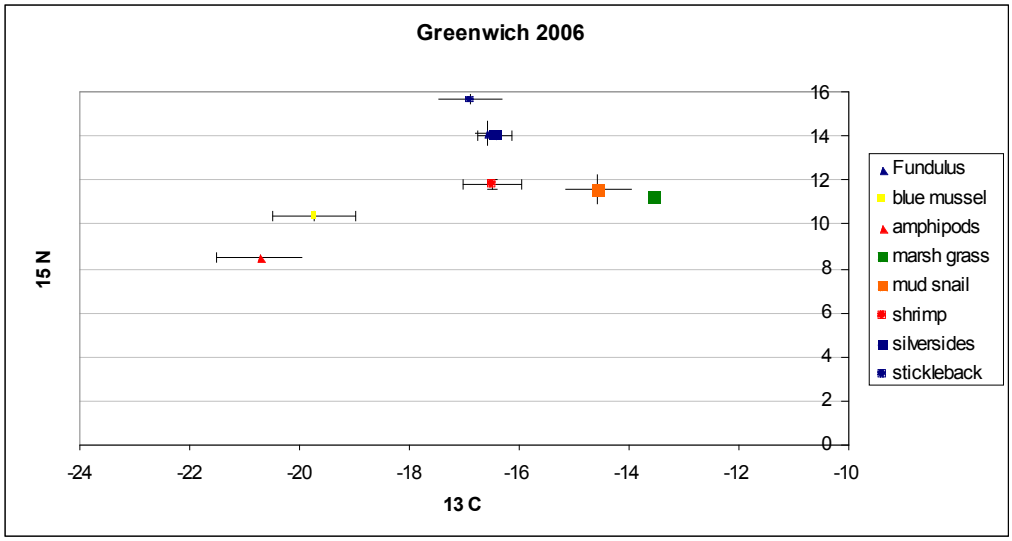
## e. Somes Sound (Mount Desert Island ME)

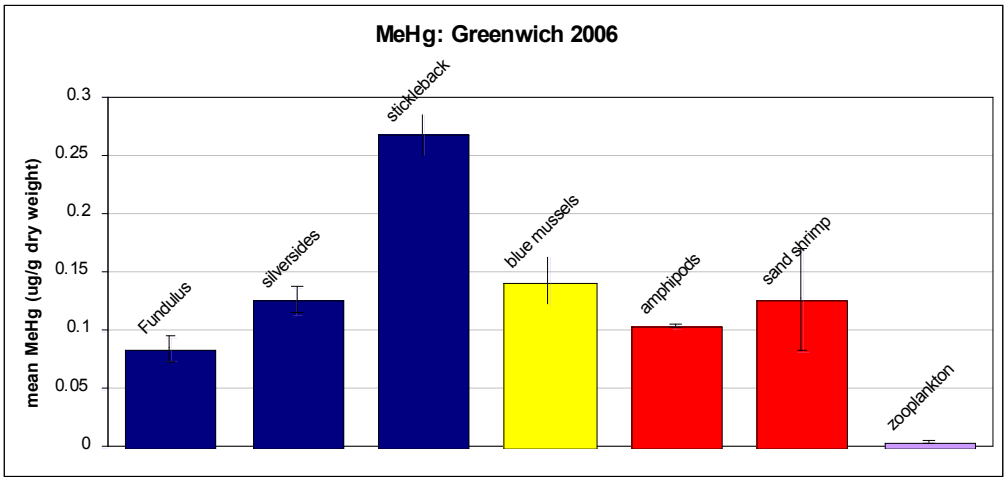


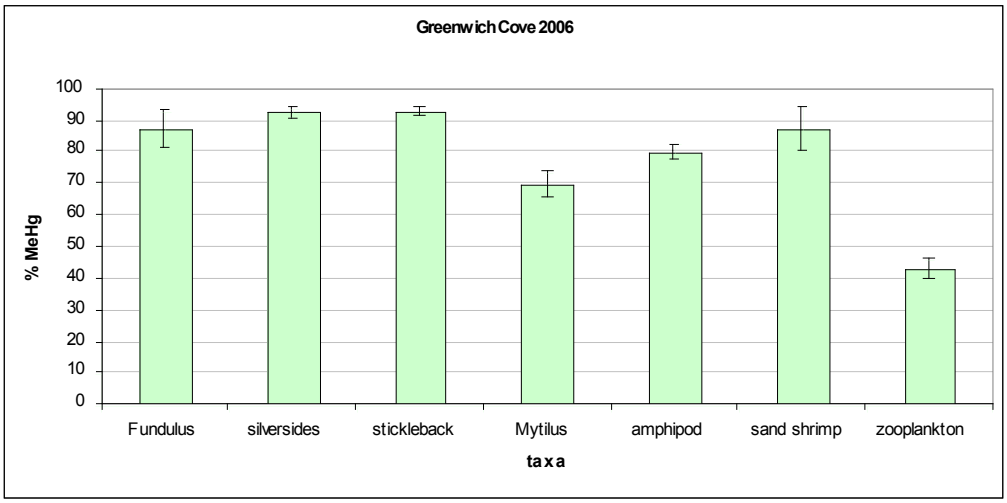



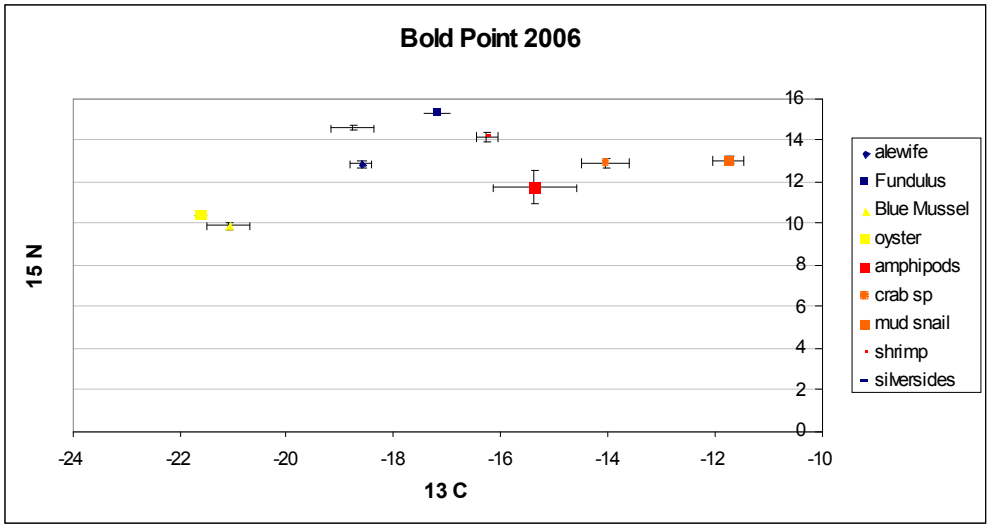
## f. Greenwich Cove (Providence River Estuary RI)

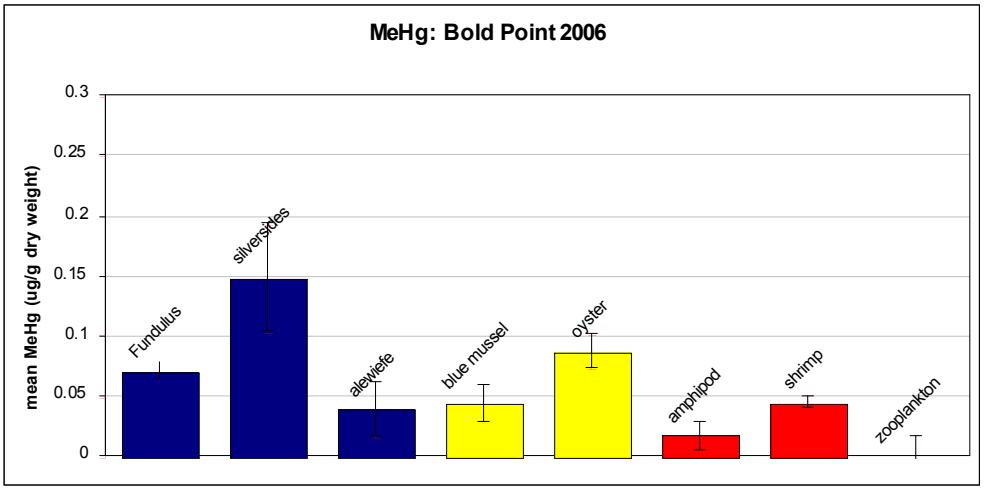


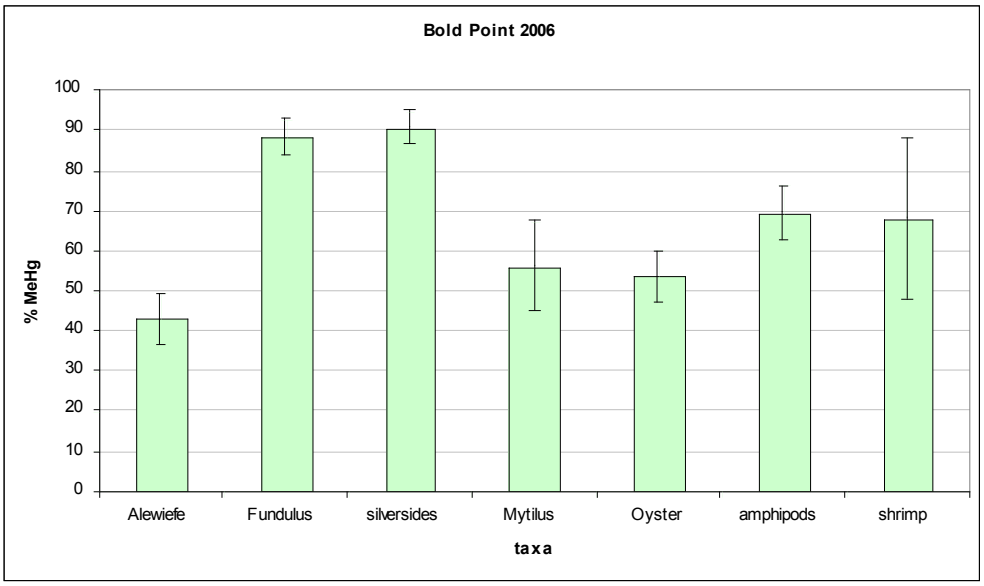



## g. Bold Point (Providence River Estuary RI)



As seen in Figures 4a-g, the pattern of stable isotopes values exhibited between sites in 2006 differs in terms of the magnitude of $^{15}N$ and $^{13}C$ values but some consistent relationships emerge. The pelagic and benthic fish species and filter feeders (Atlantic silversides, *Fundulus*, sticklebacks, blue mussels, oysters) tend to be more depleted (more negative values) in $^{13}C$ as expected suggesting a more autochthonous (phytoplankton based) food source whereas the benthic grazers and omnivores (green crabs, periwinkles, mudsnails, amphipods, shrimp) are less depleted suggesting a more allochthonous (benthic, terrestrial primary producers). The differences in trophic level as indicated by $^{15}N$ measurements do not differ greatly between taxa within a site but primary producers such as marsh grass and macroalgae range from 6.0-7.5 and *Fundulus* values range from 10.5-15.2. Generally, the difference between the $^{15}N$ signature for marsh grass samples and fish is no more than two trophic levels (~3.5 per trophic level). The percent of total Hg as MeHg appears to be higher for higher trophic level organisms such as the omnivorous fish species and green crabs and lower for filter feeders and benthic grazers.

Finally, the only metal other than MeHg that exhibited a relationship with stable isotope signatures was Pb concentrations in biota. Pb was negatively related to $^{15}N$ suggesting that Pb biodiminishes with increasing trophic levels (Figure 5, Pb shown in contrast to As). We have seen this trend in other freshwater food webs but to our knowledge, this is the first demonstration of this in marine food webs.

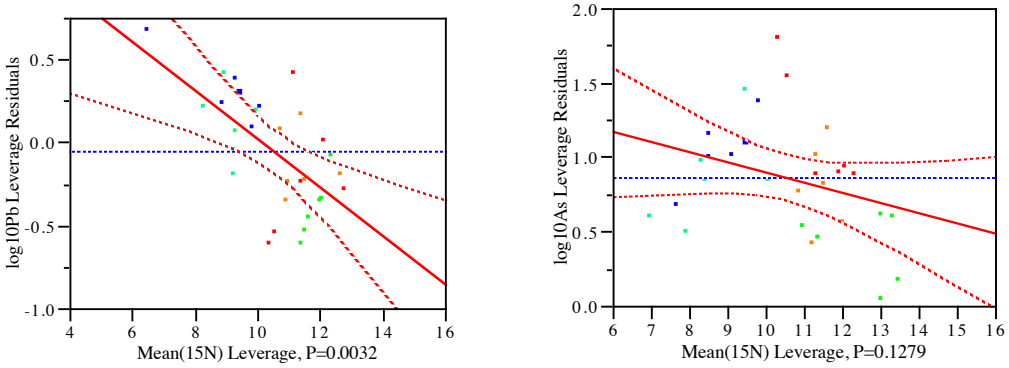



**Figure 5. Relationship of metal concentrations in biota to delta $^{15}N$.** Analyzed using ANCOVAs with site as additive term. a) Pb and b) As

For all metals (Hg, As, Cd, Zn, Pb, Se), concentration in sediments is highly correlated with total organic carbon (TOC) as is true for other datasets (USEPA EMAP-NCA). Moreover, the biota-sediment concentration factor (BSCF = concentration in biota/concentration in sediment) for these metals is negatively related to the TOC concentrations in sediments suggesting the important role of carbon in reducing the bioavailability of metals (Table 3 and Figure 6a and b, in all figures only MeHg or Hg and As are shown). However, there is no significant relationship between BSCF and SEM-AVS for any metals even though SEM-AVS is considered to be a good measure of bioavailability. For Hg, sediment concentrations vary by up to 100X between our pristine and our contaminated sites but concentrations in resident fauna at these sites differ in metal concentration by only 2-4 times (Chen et al., 2009). Nonetheless, Hg concentrations in sediments were significantly related to concentrations in biota (Table 3 and Figure 7a). For Cd, Pb, Zn, and As, there was no significant relationship between concentrations in biota and sediment concentrations (Table 3 and Figure 7b). All of these trends suggest that sediment

concentrations alone do not drive bioavailability of these metals and organic carbon has an important role in controlling bioavailability.

Our 2006 data also show that trophic position and feeding strategies determine uptake of MeHg and Hg; bioaccumulation is higher in pelagic feeding than benthic organisms (measured as delta$^{13}$C; Table 1 and Figure 8a). This finding suggests that the flux of contaminants into the water column is an important transfer route. In contrast to Hg, food source does not predict metal concentration in biota for any or the other metals (As, Cd, Pb, Zn, Se; Table 3 and Figure 8b). In addition, in contrast to Pb, %MeHg increases with increasing trophic level suggesting biomagnification.

| Y variable | X variable | Relationship of X to Y p-value | Model p-value | Interaction term |
|---|---|---|---|---|
| ANCOVA (by species2) | | | | |
| Log10 PbBSCF | TOC | .0038 | .0418 | Not sign./drop |
| | SEM-AVS | .5246 | .7086 | |
| Log10 CdBSCF | TOC | .0002 | .0004 | Not sign./drop |
| | SEM-AVS | .3840 | .1149 | |
| Log10 ZnBSCF | TOC | .0001 | .0004 | Not sign./drop |
| | SEM-AVS | .0341 | .1705 | |
| Log10 AsBSCF | TOC | .0001 | .0001 | Not sign./drop |
| | SEM-AVS | .0034 | .0668 | |
| Log10 SeBSCF | TOC | .0001 | .0001 | Not sign./drop |
| | SEM-AVS | .0281 | .1117 | |
| Log10HgBSCF | TOC | .0001 | .0001 | Not sign./drop |
| | SEM-AVS | .1307 | .3416 | |
| ANOVA | | | | |
| SedZn | TOC | | .0001 | |
| SedAs | TOC | | .0001 | |
| SedSe | TOC | | .0001 | |
| SedCd | TOC | | .0001 | |
| SedHg | TOC | | .0001 | |
| SedPb | TOC | | .0001 | |
| ANCOVA (by site) | | | | |
| Log10Zn conc | 13C | .4834 | .4955 | |
| Log10Se Conc | 13C | .0624 | .6604 | |
| Log10Cd Conc | 13C | .0293 | .1469 | |
| Log10Pb Conc | 13C | .0455 | .0750 | |
| Log10As Conc | 13C | .5760 | .7013 | |
| Log10Hg | 13C | .0019 | .0026 | Not sign./drop |
| Log10MeHg | 13C | .0109 | .0024 | Not sign./drop |
| %MeHg | 15N | .0043 | .0388 | Not sign./drop |
| Log10Pb Conc | 15N | .0199 | .0426 | Not sign./drop |
| ANCOVA (by species) | | | | |
| Log10Hg conc | MeansedHg | .0127 | .0003 | Not sign./drop |
| Log10As conc | MeansedAs | .8446 | .1420 | |
| Log10Zn conc | MeansedZn | .3849 | .1173 | |
| Log10Cd conc | MeansedCd | .6479 | .0048 | |

**Table 3. Statistical results of ANCOVAs and ANOVAs for 2006 field data across five sites (Mount Desert Island ME, Wells ME, Portsmouth Harbor NH, Adams Point NH, Providence RI, Greenwich RI).** Comparisons of: a) biota-sediment concentration factor (BSCF) to TOC and SEM-AVS; b) sediment concentration vs. TOC; c) concentrations in fauna vs. delta $^{13}C$ or $^{15}N$; d) concentrations in fauna vs. sediment concentrations. Yellow highlight denotes significant p-value

a) b)

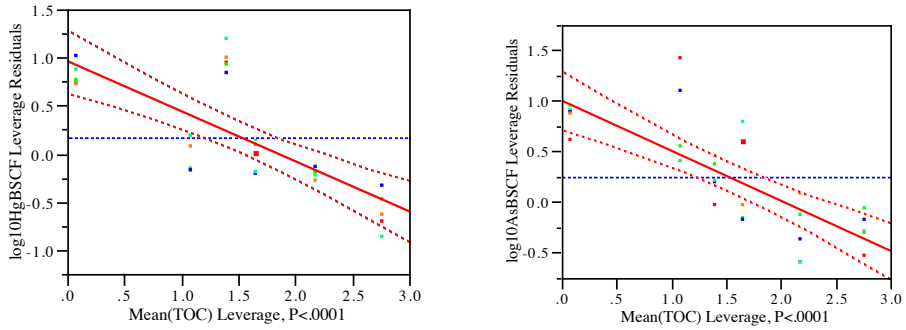
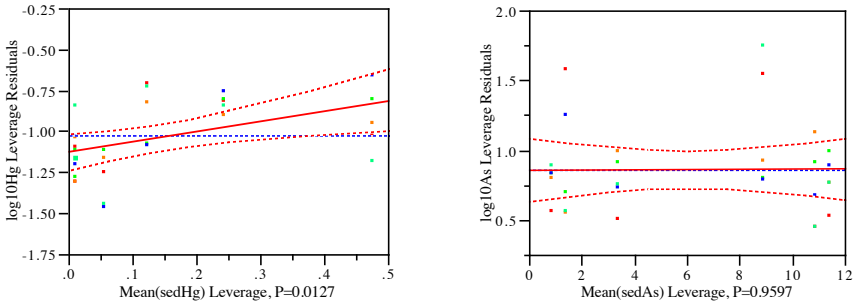


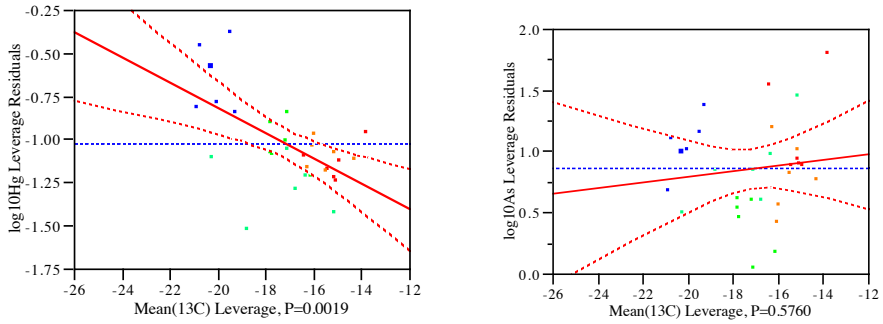

**Figure 6a and b**: **Relationship of biota-sediment concentration factor (BSCF) to total organic carbon (TOC) in 5 benthic species (green crab, killifish, mussel, shrimp, amphipod) across six field sites (listed above).** Analyzed using ANCOVAs with species as an additive term. a) Total Hg and b) As.

a) b)




**Figures 7a and b: Relationship of metals concentrations in biota to sediment concentrations.** ANCOVAs with species as an additive term. a) Total Hg and b) As.

a) b)




**Figures 8a and b: Relationship of metal concentrations in biota to delta 13C (more negative values indicate more pelagic than benthic food sources.** Analyzed using ANCOVAs with species as additive term. a) Total Hg and b) As.

***Task 2: Laboratory studies of metal bioaccumulation in Fundulus heteroclitus***

***Killifish aqueous metal exposures.*** In measurements of the uptake and loss in killifish of arsenic, cadmium, chromium, mercury and lead via the dissolved phase in contaminated seawater, mercury had the highest uptake rate (0.022 ± 0.003 L g d), followed by lead (0.0019 ± 0.0002 L g d), chromium (0.0007 ± 0.0002 L g d), cadmium (0.0006 ± 0.000 L g d) and arsenic had the lowest (0.0005 ± 0.000 L g d) (Table 4). Cadmium had the greatest percentage retained after 10 days (78 ± 1.0 %), followed by lead (66 ± 1.0 %), chromium (40 ± 1.1 %), mercury (39 ± 1.1 %) and arsenic had the lowest retention (26 ± 1.1). The efflux rate or loss rate was calculated after 48 hours of depuration. Arsenic had the highest loss rate followed by mercury, chromium, lead and cadmium (7.6 ± 0.8, 5.0 ± 0.6, 4.6 ± 1.1, 2.4 ± 0.3 and 1.8 ± 0.3 % d-1 respectively) (Table 4). After tissue dissection the lowest partitioning was found in the liver for arsenic, chromium, mercury and lead, and in the skeleton, fillet and skin for cadmium. For all metals except arsenic the greatest body burden was in the head. For arsenic it was in the fillet (Figure 9).

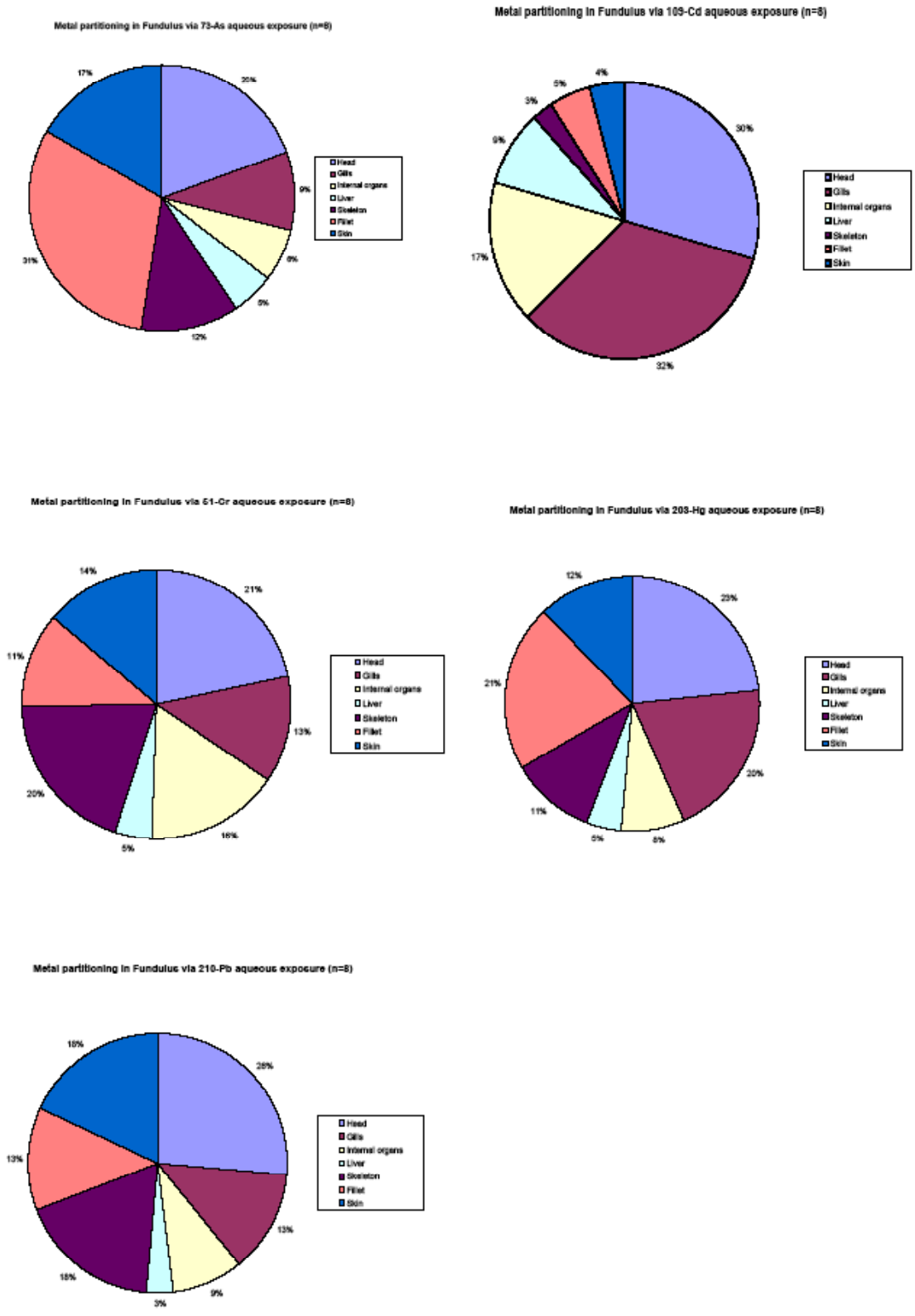



**Figure 9. Pie charts for metal partitioning in fish body tissues for As, Cd, Cr, Hg, and Pb.** Tissues: Head (light blue); gill (maroon); internal organs (ivory); liver (aqua); skeleton (purple); filet (pink); skin (blue).

**Model parameters (uptake rates and loss rates) for Norfolk, VA aqueous exposures:**

Mean ± 1 SE, n=8

| Metal | Ku (l g-1 d-1) | Ke (d-1) | Ke (% d-1) |
|---|---|---|---|
| As | 0.0005 ± 0.0000 | 0.076 ± 0.008 | 7.6 ± 0.8 |
| Cd | 0.0006 ± 0.0000 | 0.018 ± 0.003 | 1.8 ± 0.3 |
| Cr | 0.0007 ± 0.0002 | 0.046 ± 0.011 | 4.6 ± 1.1 |
| Hg | 0.022 ± 0.003 | 0.050 ± 0.006 | 5.0 ± 0.6 |
| Pb | 0.0019 ± 0.0002 | 0.024 ± 0.003 | 2.4 ± 0.3 |

**Table 4. Calculated rate constants for metal uptake and loss based on aqueous exposures.**

***Aqueous concentration dependence experiments.*** Killifish Cd uptake was linear throughout the exposure period, with metal concentrations in the fish at the end of uptake ranging from 42 pg/g dry wt when exposed to a 0.23 nM metal concentration to 518 pg/g dry wt when exposed to a 3.08 nM metal concentration (Figure 10). For Cr, the uptake curve leveled off after approximately 13 hours at all metal concentrations. Metal concentration in the fish at the end of uptake ranged from 12 pg/g dry wt when exposed to a 0.22 nM metal concentration to 190 pg/g dry wt when exposed to a 3.5 nM metal concentration (Figure 10).

For Cd, metal concentration in the water had limited influence on the uptake rate with $k_u$ ranging from 0.00040-0.00048 L $g^{-1}$ $d^{-1}$. For Cr, the metal concentration in the water had an influence on uptake rates at higher metal concentrations. At concentrations ranging from 0.22-1.75 nM the $k_u$ ranged from 0.00082-0.0010 L $g^{-1}$ $d^{-1}$. However at the 3.5 nM concentration the $k_u$ increased to 0.0017 L $g^{-1}$ $d^{-1}$ (Table 5).

For Cr, loss rate constants were similar for all metal concentrations with $k_{ew}$ ranging from 4.8-7.2% $d^{-1}$. The percentage retained at the end of depuration was greatest for the 3.5 nM concentration (55%, which corresponds with the slowest loss rate), compared to an average of 42% for the four lower concentrations (Table 6, Figure 11).

At the end of uptake (Cd) and at the end of depuration (Cr) the highest percentage of Cd was found in the gills, and the lowest percentage was in the liver and skeleton. For Cr, the greatest percentage body burden is found in the skeleton and the head.

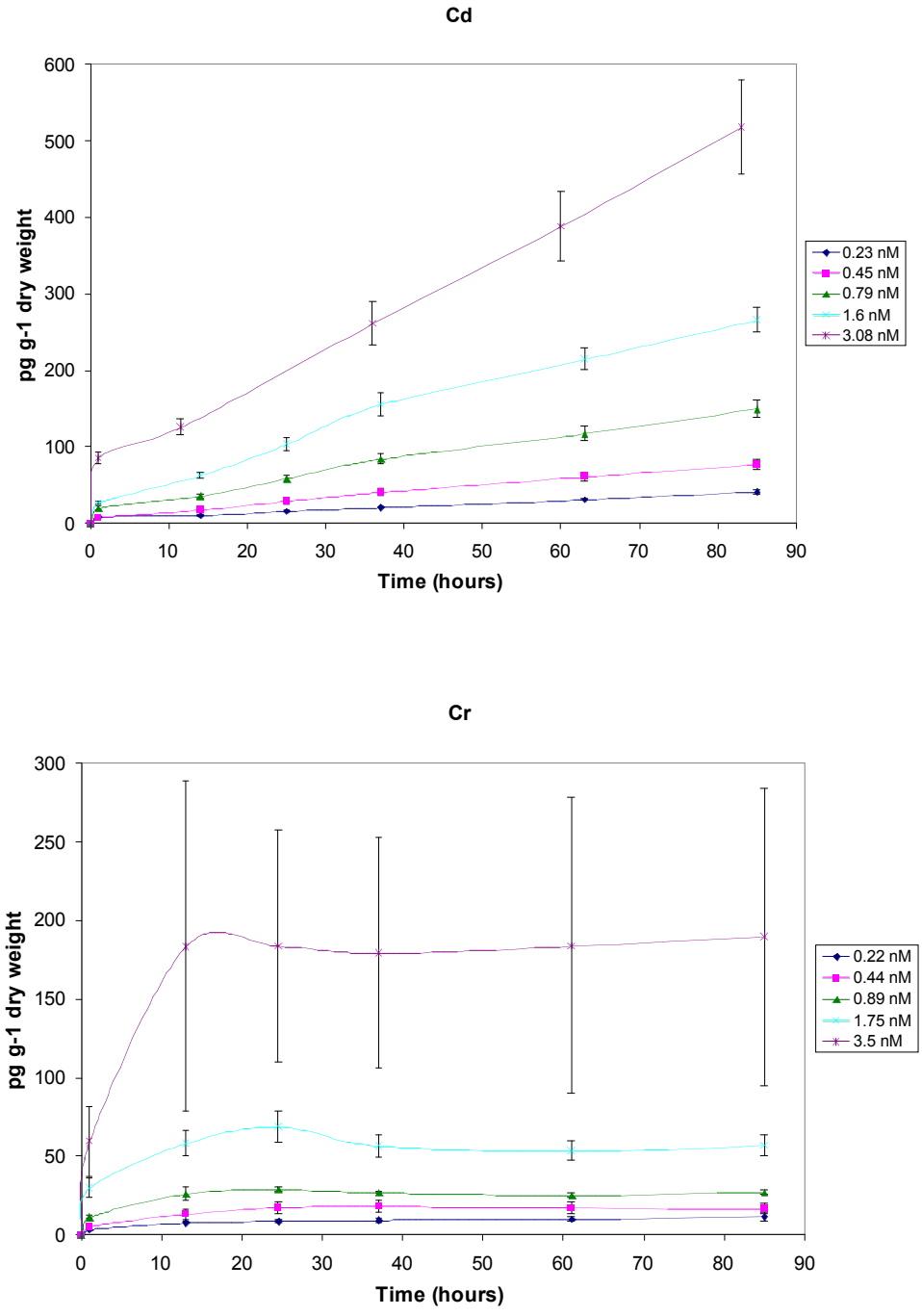



**Figure 10. Cd and Cr concentrations in killifish during uptake at different metal concentrations (n=5 per metal concentration), values are mean ± 1 SE.**

| Metal | Concentration (nM) | $k_u$ (L $g^{-1}$ $d^{-1}$) |
|---|---|---|
| Cd | 0.23 | 0.00041 ± 0.00004 |
| | 0.45 | 0.00046 ± 0.00006 |
| | 0.79 | 0.00048 ± 0.00004 |
| | 1.60 | 0.00047 ± 0.00005 |
| | 3.08 | 0.00040 ± 0.00005 |
| Cr | 0.22 | 0.0010 ± 0.0001 |
| | 0.44 | 0.0009 ± 0.0002 |
| | 0.89 | 0.0008 ± 0.0002 |
| | 1.75 | 0.0008 ± 0.0002 |
| | 3.50 | 0.0017 ± 0.0012 |

**Table 5. Cd and Cr $k_u$ values at different metal concentrations** (n=5 per concentration), values are mean ± 1 SE.

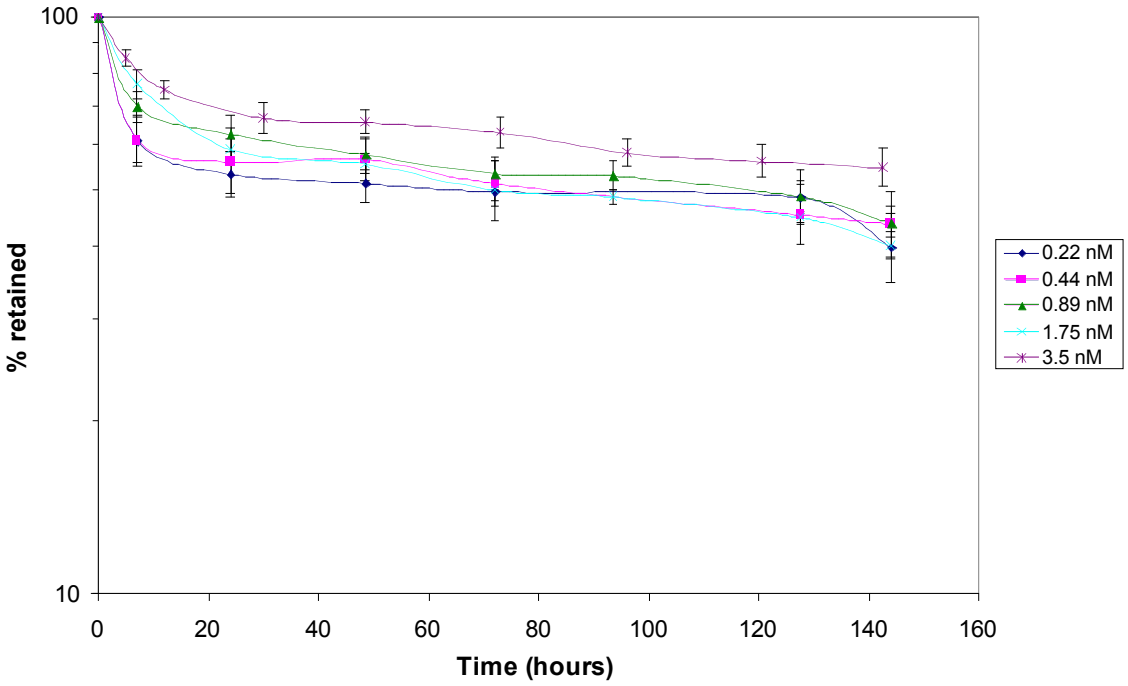


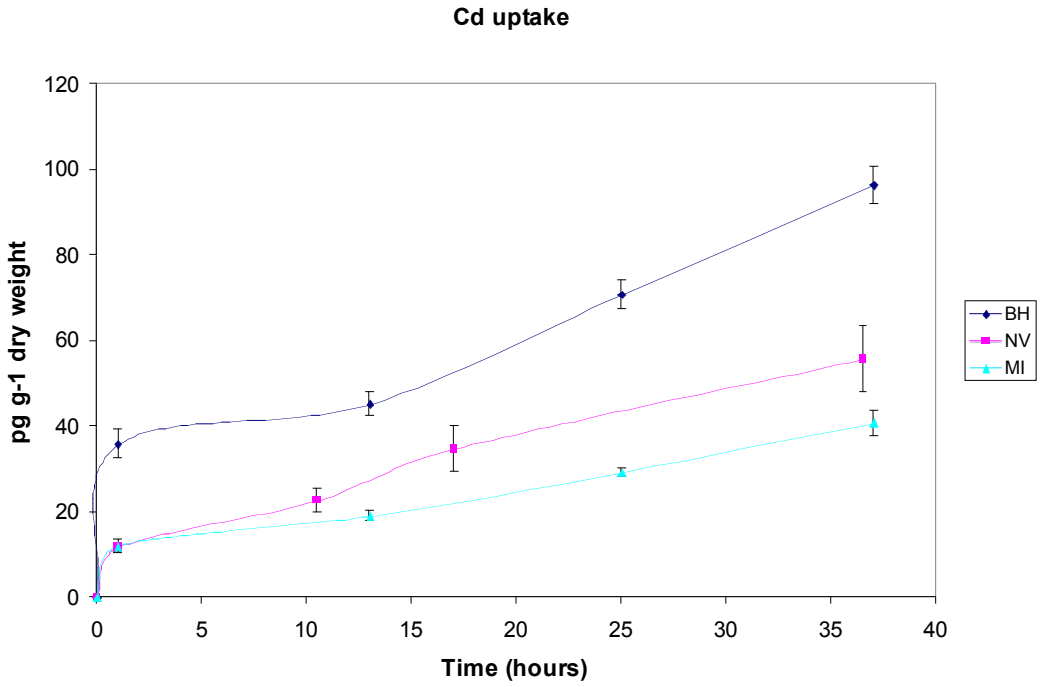

**Figure 11. Depuration of Cr at various metal concentrations (n=5 per concentration), values are mean ± 1 SE.**

| Concentration (nM) | $k_{ew}$ (% d-1) | % retained at end |
|---|---|---|
| 0.22 | 5.0 ± 1.1 | 40 ± 1.1 |
| 0.44 | 6.2 ± 2.9 | 44 ± 1.1 |
| 0.89 | 6.1 ± 1.3 | 44 ± 1.1 |
| 1.75 | 7.2 ± 0.5 | 40 ± 1.1 |
| 3.5 | 4.8 ± 0.8 | 55 ± 1.1 |

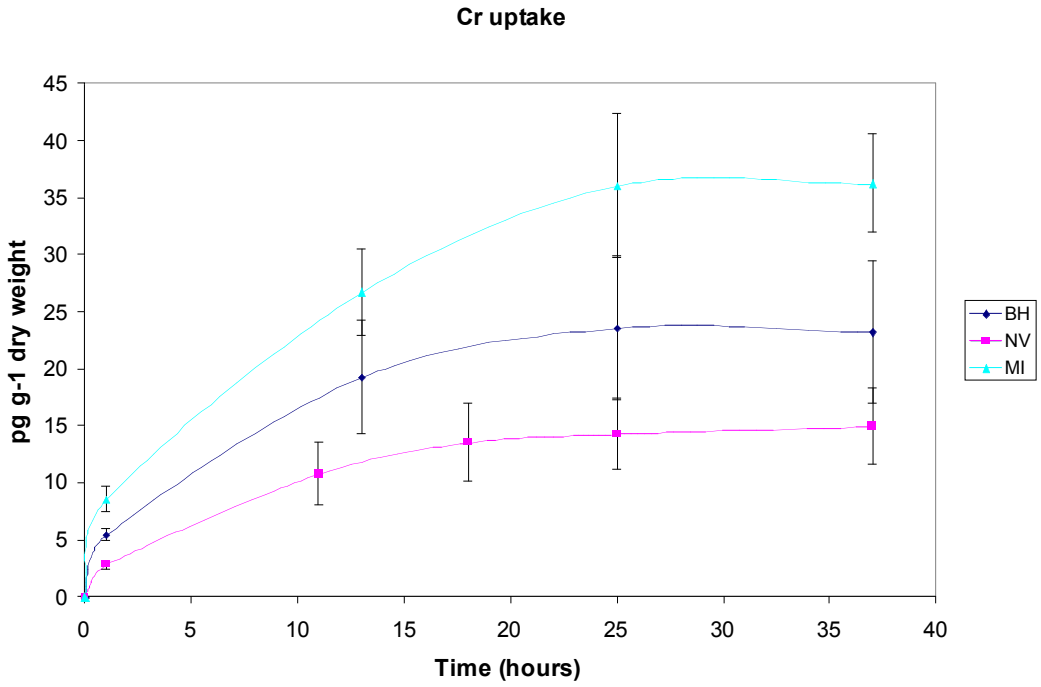
**Table 6. Cr $k_{ew}$ and % retained at the end of depuration at different metal concentrations (n=5 per concentration), values are mean ± 1 SE.**

***Aqueous exposures using water from three field sites.*** For Cd, as salinity increases Cd concentration in the fish decreases likely due to the complexation of cadmium with chloride. For Cr and Hg uptake is higher in water with a lower DOC concentration (Mare Island) (Figure 12). The source of exposure water did not affect the depuration curves for Cd, Cr or Hg. Cd uptake rates increased as salinity increased whereas efflux rates did not vary significantly per water source for each metal (Table 7). Dissection found that each metal is associated with the site of uptake i.e. head, gills and skin. A significant amount of metal was also associated with the internal organs. (For the following figures BH is Baltimore Harbor, NV is Norfolk and MI is Mare Island.)

a) Cadmium



b) Chromium




c) Mercury

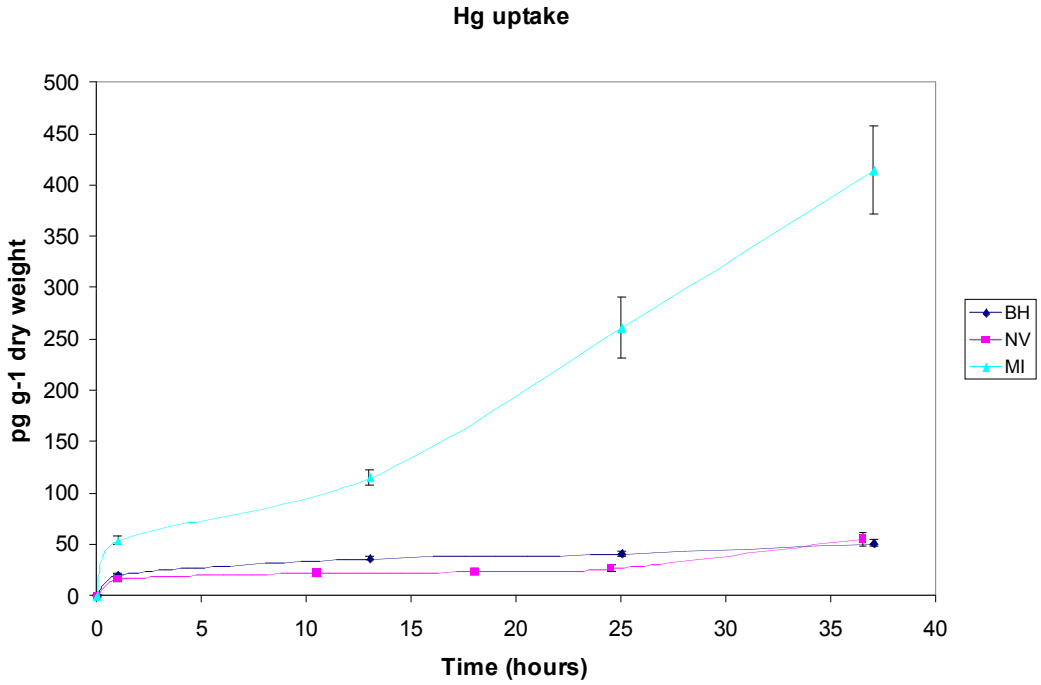
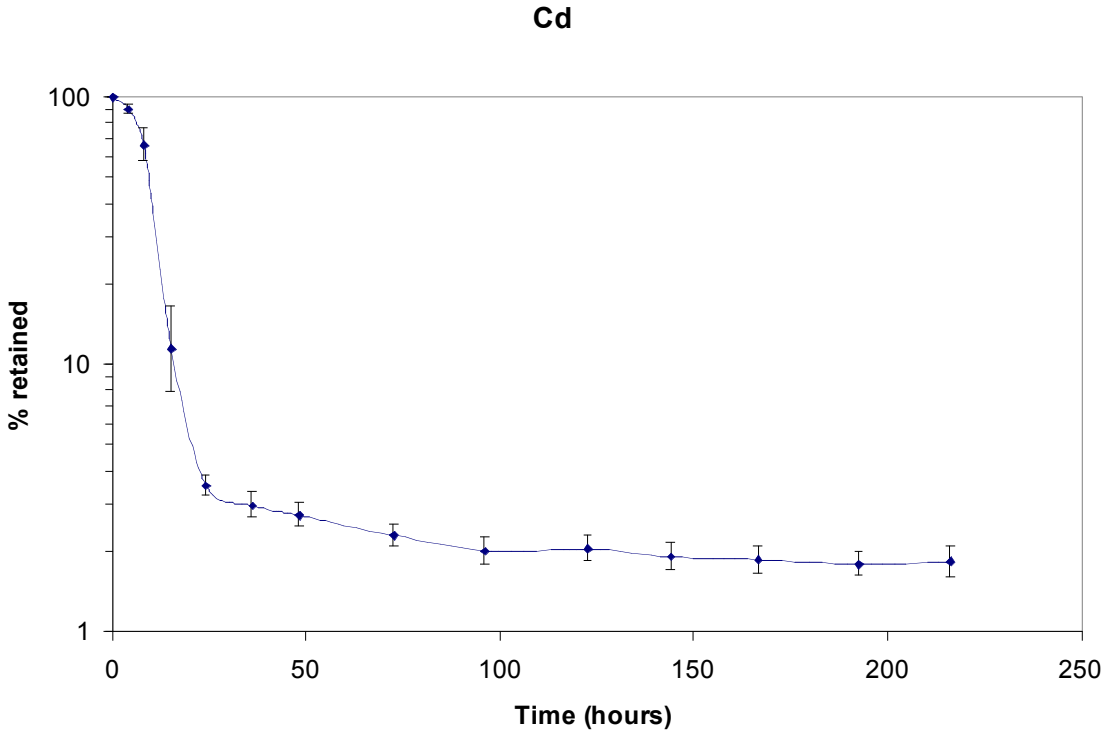


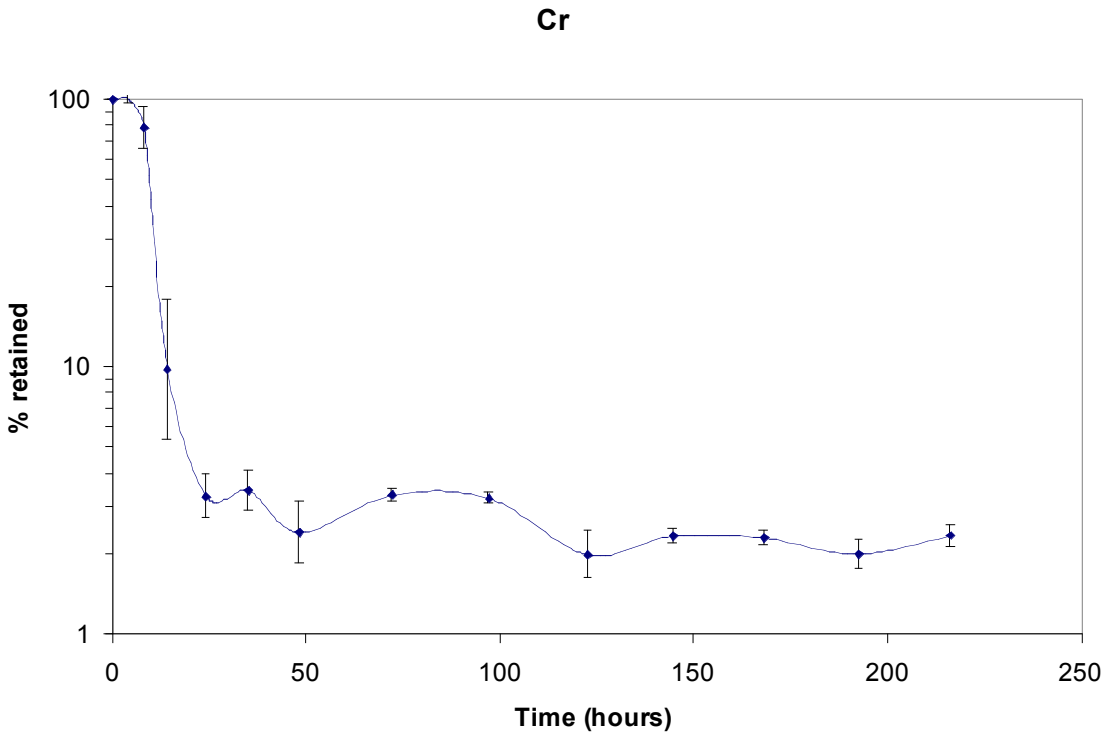

**Figure 12. Cd, Cr and Hg concentrations (pg $g^{-1}$ dry weight) in killifish during uptake**. Values are mean ± 1 SE.

| Metal | Location | $k_u$ (L $g^{-1}$ $d^{-1}$) | $k_{ew}$ (% $d^{-1}$) | % retained at the end |
|---|---|---|---|---|
| **Cd** | BH | 0.0011± 0.0001 | 1.7 ± 0.6 | 63 |
| | NV | 0.0007 ± 0.0001 | 1.5 ± 0.2 | 70 |
| | MI | 0.0004 ± 0.0001 | 1.8 ± 0.1 | 56 |
| **Cr** | BH | 0.0012 ± 0.0004 | 9.5 ± 0.7 | 18 |
| | NV | 0.0007 ± 0.0003 | 4.6 ± 1.1 | 39 |
| | MI | 0.0009 ± 0.0002 | 4.5 ± 0.9 | 20 |
| **Hg** | BH | 0.024 ± 0.002 | 4.6 ± 0.3 | 56 |
| | NV | 0.050 ± 0.005 | 2.3 ± 0.1 | 73 |
| | MI | 0.075 ± 0.010 | 3.7 ± 0.2 | 64 |

**Table 7. Kinetic parameters.** Values are mean ± 1 SE.

***Trophic transfer from worms to killifish.*** Killifish that were fed worms radiolabeled with Cd or Hg had strong Cd and Hg signals. Killifish fed worms radiolabeled with Cr or As had a low Cr signal and no As signal because worms took up very little Cr and no As. Not all fish ate resulting in killifish n values of 10 for Cd, 5 for Cr, 7 for Hg(II), and 8 for MeHg.After feeding on radiolabeled worms all metals were eliminated from killifish following a two-compartment loss pattern. During the first 24 h of depuration rapid loss corresponded to gut clearance of unassimilated metal, and the slower loss for the remainder of depuration corresponded to the physiological turnover of assimilated metal (Figure 13). Within the first 24 h 96% of Cd, 95% of Cr, 73% of Hg(II) was eliminated. At the end of the 9 day depuration period the fraction of the original metal retained in killifish 2% Cd, 2.5% Cr, 14% Hg, and 86% MeHg after feeding on worms (Figure 13). After 72 hours of depuration, AE (assimilation efficiency) is highest for Hg (23%), followed by Cr (3.7%) and lowest for Cd (2.6%). $k_{ef}$ (efflux rate after dietary exposure) was highest for Cr (6.8 % $d^{-1}$), followed by Hg (5.6 % $d^{-1}$) and Cd (3.6 % $d^{-1}$). At the end of depuration Hg had the highest % of initial radioactivity remaining (14%), followed by Cr (2.3%) and Cd (1.8%) (Table 8).


Cd
100
10
1
% retained
0
50
100
150
200
250
Time (hours)


Cr
100
10
1
% retained
0
50
100
150
200
250
Time (hours)

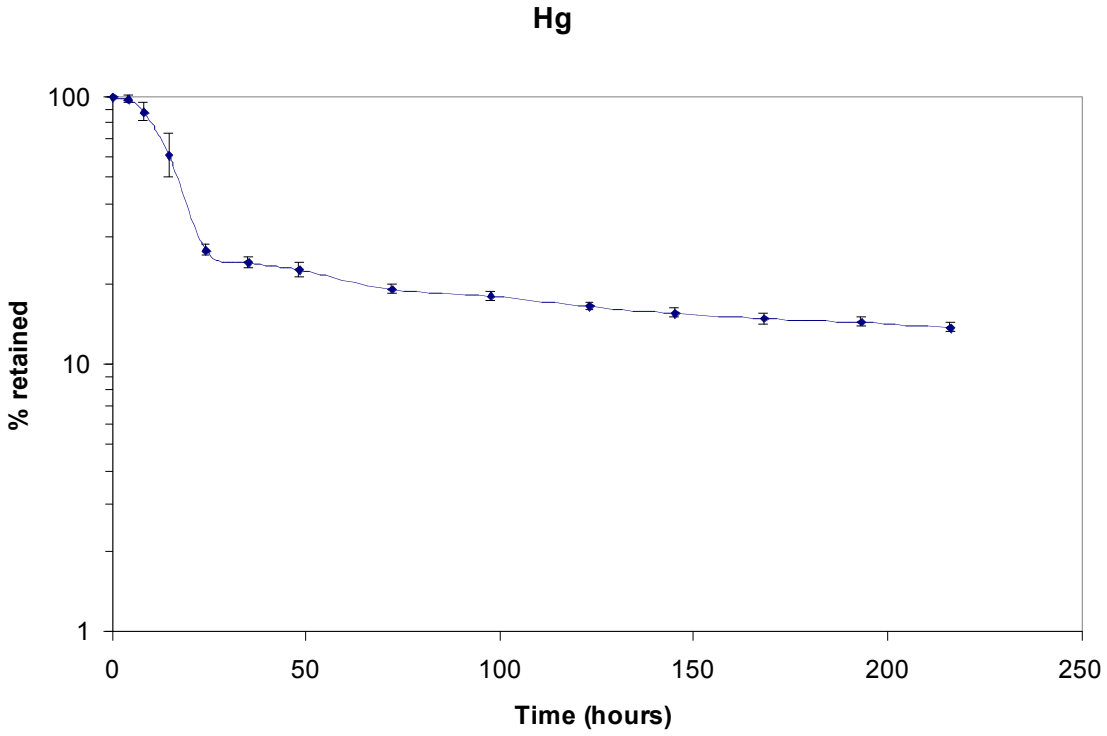
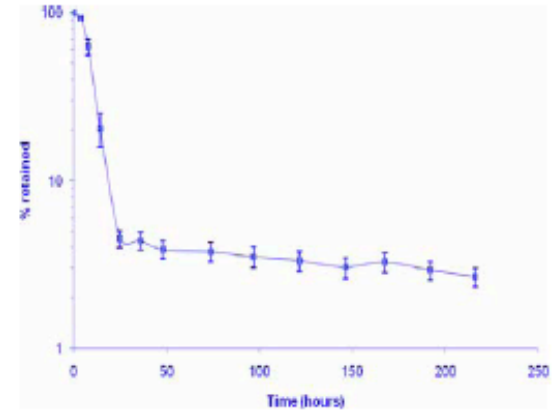



**Figure 13. Depuration curves for Cd (n=10), Cr (n=5) and Hg (n=7) after feeding on black worms for 9 days.** Values are mean ± 1 SE.

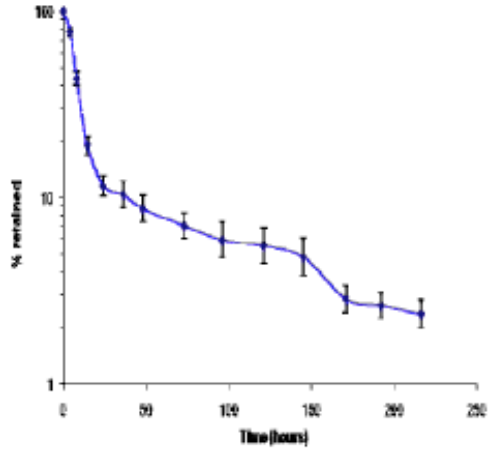
| Metal | AE (%) | $k_{ef}$ (% $d^{-1}$) | % retained at the end |
|---|---|---|---|
| **Cd** | 2.6 ± 0.3 | 3.6 ± 0.9 | 1.8 ± 1.1 |
| **Cr** | 3.7 ± 0.2 | 6.8 ± 1.1 | 2.3 ± 1.1 |
| **Hg** | 23 ± 1.2 | 5.6 ± 0.5 | 14 ± 1.0 |

**Table 8. Assimilation efficiencies (AE), loss rates after dietary exposure ($k_{ef}$) and % of initial radioactivity retained at the end of depuration for Cd (n=10), Cr (n=5) and Hg (n=7).** Values are mean ± 1 SE.

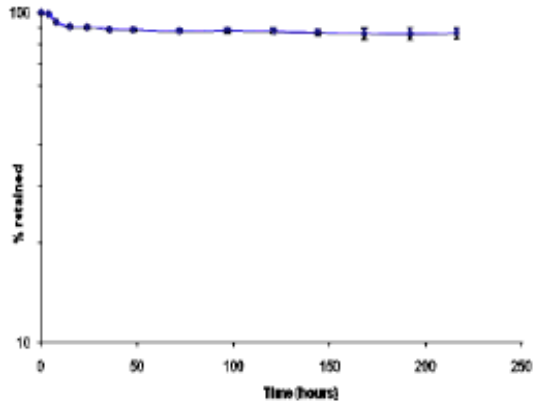
***Trophic transfer from amphipods to killifish.*** After 48 hours MeHg had the highest assimilation efficiency (92 ± 1.6 %), followed by Hg (14 ± 4.0 %), and lowest for Cd (4.5 ± 0.5 %). The efflux rate was highest for mercury (13 ± 1.1 % $d^{-1}$), followed by Cd (6.4 ± 1.0 % $d^{-1}$), and lowest by far for MeHg (0.8 ± 0.08 % $d^{-1}$). MeHg had the highest retention at the end of depuration (86%), and Cd and Hg had the lowest (both 2.5%) (Figure 14). Dissection data indicated that Cd and Hg remained associated with the intestine throughout depuration and very little was transported across the intestinal wall. MeHg was transported around the body within 4 hours of feeding and was the only metal to have a strong signal in the blood. At the end of depuration 36% of MeHg was associated with the fillet, 14% in both the head and intestine, and 9% in the liver.




Cd




Hg




MeHg

**Figure 14. Depuration curves for Cd, Hg, and MeHg after feeding on amphipods**. Values are means ± 1 SE.

In MeHg experiments conducted with *Isochrysis galbana*, the average assimilation efficiency was calculated to be 95% and the average efflux rate constant was 0.64 ± 0.15 % d-1. The trophic transfer factor for adult female killifish was determined to range from 1.49 to 14.9 when the ingestion rates varied from 0.01 - 0.1 g/g/d, indicating that this metal biomagnifies in food chains, consistent with other observations. The dissection found that 40% of the MeHg was transported to the fillet and only 1% to the ovary. However, when expressed on a concentration basis (normalized per gram) the heart contained the highest concentration of MeHg (153 ng/g) and the ovary (13.8 ng/g) and the concentration in the fillet (15.1 ng/g) were lower.

***Killfish MeHg exposure via diet varying salinity and DOC***. The uptake rate ($k_u$) of MeHg was highest in water from Baltimore Harbor (0.70 ± 0.09 L $g^{-1}$ $d^{-1}$), followed by Norfolk (0.38 ± 0.002 L $g^{-1}$ $d^{-1}$), and lowest in Mare Island (0.35 ± 0.05 L $g^{-1}$ $d^{-1}$) (Table 9). This suggests the MeHg binds to chloride ions in the water reducing bioavailability, because as salinity increased the uptake rate decreased. The efflux rate ($k_e$) did not vary with field location (0.7-0.9 % $d^{-1}$) and at the end of depuration, all 3 locations had retained 89% of the original radiolabel. This shows that the uptake of MeHg is controlled by water chemistry, but loss is a purely physiological process. Dissection data (not weight normalized) from all 3 locations showed that at the end of depuration, the greatest proportion of the metal was found in the fillet (31-34 %), followed by the head (21-23%), and the lowest proportion was found in the gills (4%). Dissection data from Baltimore Harbor showed that the proportion of MeHg in the gills decreased from 23% at the end of uptake to 4% at the end of depuration, and the proportion in the fillet increased from 9% at the end of uptake to 32% at the end of depuration. Of all the metals analyzed, MeHg was the only one to show a very clear signal in the blood.

| Location | *k*u (L g-1 d-1) | *k*ew (% d-1) | % retained at end |
|---|---|---|---|
| Baltimore Harbor | 0.7 ± 0.09 | 0.7 ± 0.1 | 89 ± 1 |
| Norfolk | 0.38 ± 0.02 | 0.9 ± 0.08 | 89 ± 1 |
| Mare Island | 0.35 ± 0.05 | 0.9 ± 0.08 | 89 ± 1 |

**Table 9. Kinetic parameters for MeHg.** Values are means ± 1 SE.

***Killifish DOM experiments.*** Data trends suggest there was no relationship between As and DOM concentration. Cr, Hg and MeHg uptake decreased as DOM concentration increased, suggesting DOM has protective properties. Cd had no relationship with DOM concentration except at 5 mg/L where uptake was significantly lower and there was a 24 hour delay in uptake. This suggests there is a switch over between the free ionic form at lower DOM concentrations, and the complex formed at higher DOM concentrations.

***Standardized salinity experiments.*** Data trends suggest that there was no relationship between

Cr and salinity, but As, Hg and MeHg uptake increased as salinity increased. Cd was the only metal where uptake decreased with increasing salinity.

***Metal bioaccumulation from food and water by amphipods.***

*Metal influx and efflux from the dissolved phase*. Metal influx ($K_u$, L $g^{-1}$ $d^{-1}$) and efflux ($K_{ew,}$ $d^{-1}$) rates are presented in Table 9 and depuration curves are presented in Figure 15. Influx rates (mean ± SE) for Cd (0.012 ± 0.002) and As (0.028 ± 0.003) were low. Correspondingly, mass balance calculations showed a decline in water concentration during uptake of less than 3% for Cd and less than 2% for As. In contrast, influx rates and changes in water concentration for inorganic Hg (1.14 ± 0.099, 42%) and $CH_3Hg$ (1.59 ± 0.13, 40%) were higher. For inorganic Hg, 10% of this decline was attributable to amphipod uptake, whereas for $CH_3Hg$, 88% was attributable to uptake. Efflux rates were much higher for Cd and As than for inorganic Hg and $CH_3Hg$ (Table 5). For all metals, amphipods showed an initial rapid loss within the first 4 h, followed by a slower loss rate through the remaining 68 h (Figure 16).

*Metal AE and efflux from I. galbana*. Metal assimilation efficiencies (AE) and efflux rates ($K_{ef}$) are presented in Table 9 and depuration curves are presented in Figure 15. During the 45 min uptake period, we observed a 40% change in metal concentration within uptake containers for inorganic Hg, 100% of which was attributable to amphipod uptake. Measured AE values were fourteen times higher for $CH_3Hg$ than for inorganic Hg and were higher for Cd than for As. For As, Cd and inorganic Hg, depuration followed a two-compartment pattern (Wang and Fisher 1999): an initial rapid loss (0-24 h), presumably due to gut passage of unavailable metals, followed by a slower loss rate (24-96 h), which activity of egested feces suggests resulted from digestive processes (Figure 15). $CH_3Hg$ showed a more accelerated two-compartment depuration pattern. Amphipods lost 17% of metal gained during uptake during the first 45 min of depuration and lost an additional 18% during the remaining 4 d. After 24 h of depuration, the activity of collected feces could not be distinguished from background levels. Efflux rates were 22 times lower for $CH_3Hg$ than for inorganic Hg and higher for As than for Cd (Figure 15).

*Biokinetic Modeling*. Measured values used to parameterize the biokinetic model are given in Table 9 and calculated $C_{ss}$, $R_f$ and $R_w$ values are shown in Table 10. The model indicated that, for the conditions tested, *I. galbana* would be the source of > 99% of bioaccumulated Cd and As, 70% of inorganic Hg and 91 % of bioaccumulated $CH_3Hg$. Compared to inorganic Hg, the $K_{ew}$ for $CH_3Hg$ (Table 9) was 175 times lower, which explains the relatively greater contribution of aqueous metal.

*Biokinetics & Acute Aqueous Toxicity*. For As and Cd, $K_u$ differed between metal concentrations (Table 11). For both As and Cd, a Tukey's HSD test showed that $K_{ew}$ was independent of aqueous metal concentration. Survival was greater than 94% for all treatment concentrations in the Cd acute aqueous toxicity test (Table 11). This is likely due to the relatively high (20 ppt) salinity used for the experiment. There was no difference in mortality between Instant Ocean and natural seawater, suggesting salinity, not DOC regulated toxicity (instant ocean has no DOC). For As, survival was >92% until concentrations reached > 3.5 mg/L (Table 12). Differences in As uptake rates did not show a clear relationship with toxicity patterns (Table 12). However, uptake rates at the lowest concentrations were significantly higher than uptake rates at the

highest concentration tested, where survival was 22%. The lowest and highest concentrations tested for Cd were also significantly different, but survival was not affected.

For As, radioactive counts dramatically increased at the 96 h time point for the two highest treatment concentrations. This may be the result of several factors: 1) As cpm values were, in general, very low at the last time point (75-100 cpm raw values, with background values of 62-64 cpm), indicating we were reaching the detection limit of the machine; 2) mortality was high in the two highest treatment concentrations (Table 11); and 3) in treatment replicates where mortality was very high, the remaining live amphipods had cpm values similar to those measured in the previous time point with less mortality. Because of this problem, $K_{ew}$ values were calculated using data collected between 12 h and 48 h of depuration. A 48 h uptake period was used for As, but data from the 24 h uptake time point was used to calculate $K_{ew}$ due to incomplete amphipod rinsing at the 48 h time point. Average count values obtained at 24 h were not significantly different from count values from replicate one at 48h (t-test, $\alpha$=0.05, df=12, p>.1602), suggesting uptake leveled off after 24 h.

| | aqueous | | | dietary | | | |
|---|---|---|---|---|---|---|---|
| **Metal** | **$C_w$** | **$K_u$** | **$K_{ew}$** | **$C_f$** | **AE** | **$K_{ef}$** | **IR** |
| | ($\mu$g $L^{-1}$) | (L $g^{-1}$ $d^{-1}$) | ($d^{-1}$) | ($\mu$g $mg^{-1}$) | (%) | ($d^{-1}$) | (mg g-1 $d^{-1}$) |
| **Cd** | 0.11 | 0.012 ± 0.002 | 2.1 ± 0.9 | $1.01x10^{-5}$ | 23.8 ± 3.74 | 0.31 ± 0.03 | 150.4 ± 5.7 |
| **As** | 0.0375 | 0.028 ± 0.003 | 9.1 ± 0.61 | $2.35x10^{-5}$ | 10.53 ± 1.66 | 5.9 ± 0.46 | 919.4 ± 82 |
| **Hg** | 0.340 | 1.14 ± 0.10 | 0.53 ± 0.06 | $8.35x10^{-5}$ | 6.28 ± 0.65 | 1.33 ± 1.22 | 4139 ± 626 |
| **$CH_3Hg$** | 0.1074 | 1.59 ± 0.13 | 0.003 ± 0.0008 | $5.36x10^{-5}$ | 80.48 ± 1.57 | 0.06 ± 0.01 | 469.8 ± 27 |

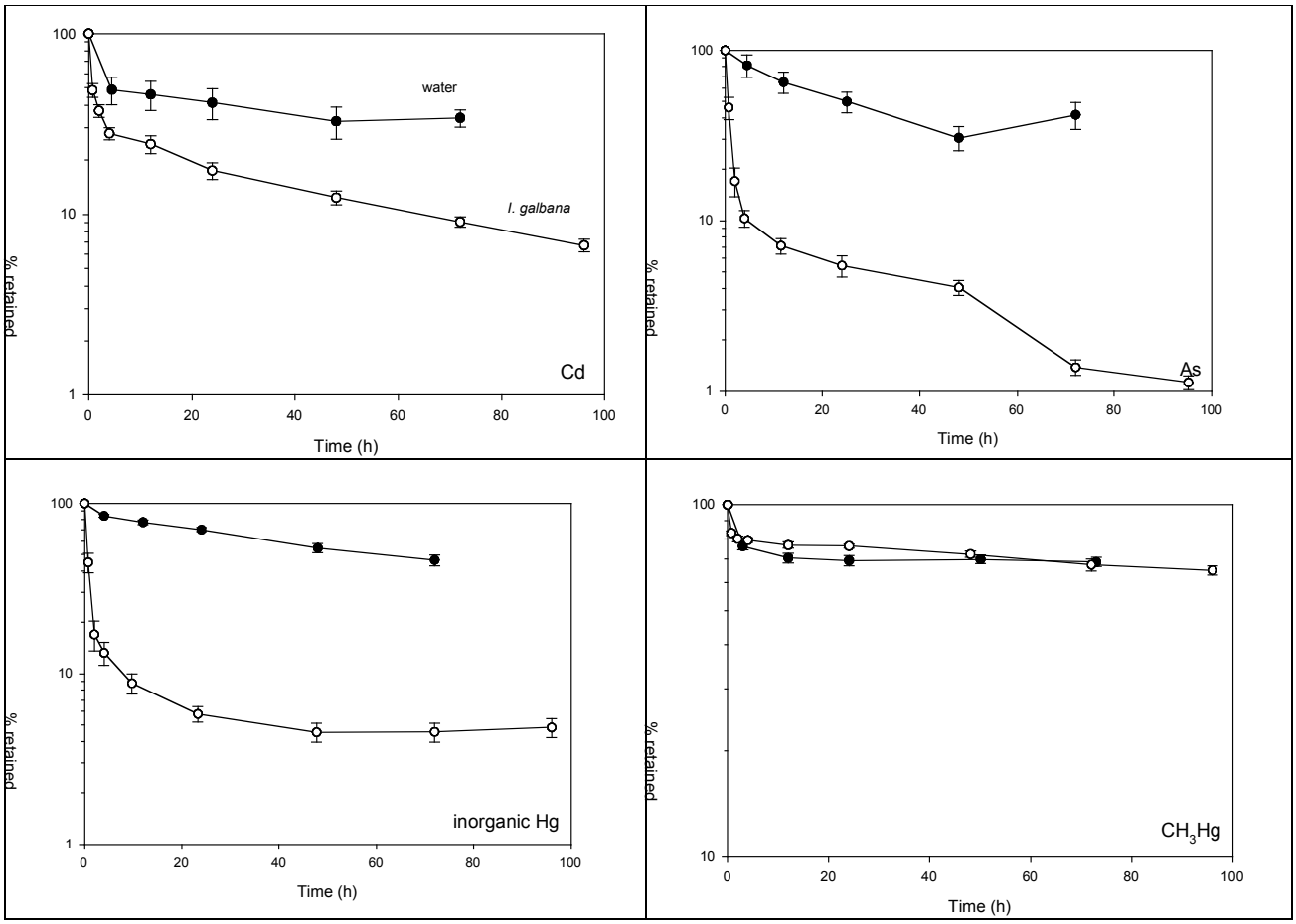
**Table 10. Biokinetic parameters measured for *L. plumulosus:* metal uptake rates ($K_u$), efflux rates ($K_{ew}$) from water, metal assimilation efficiencies (AE) and efflux rates ($K_{ef}$) after feeding on *I. galbana*, ingestion rate (IR) and metal concentration in *I. galbana* ($C_f$) and in water ($C_w$).**

| Metal | $C_{ss}$ (ug $g^{-1}$) | $R_f$ | $R_w$ |
|---|---|---|---|
| **Cd** | 0.102 | 0.99 | 0.01 |
| **As** | 0.038 | 0.997 | 0.003 |
| **Hg** | 2.18 | 0.70 | 0.30 |
| **$CH_3Hg$** | 27.22 | 0.91 | 0.09 |

**Table 11. Calculated mean values for the steady state metal concentrations in amphipods ($C_{ss}$) and the relative contribution of metal in *I. galbana* ($R_f$) and in water ($R_w$).**

| Metal | Nominal conc. (mg/L) | Measured conc. (mg/L) | $K_u$ (L $g^{-1}$ $d^{-1}$) | $K_{ew}$ ($d^{-1}$) | % Survival (Tox test) |
|---|---|---|---|---|---|
| **Cd** | control | b.d.l | | | 98 ± 2 |
| | 0.005 | 0.005 | 0.032 ± 0.005 $^{bc}$ | 3.6 ± 0.5 | 100 |
| | 0.015 | 0.015 | 0.024 ± 0.003 $^{abc}$ | 3.0 ± 0.5 | 96 ± 4 |
| | 0.05 | 0.04 | 0.040 ± 0.005 $^{ab}$ | 2.9 ± 1.4 | 98 ± 2 |
| | 0.15 | 0.18 | 0.053 ± 0.010 $^{ab}$ | 2.1 ± 1.1 | 94 ± 4 |
| | 0.45 | 0.52 | 0.049 ± 0.009 $^{ab}$ | 1.2 ± 1.3 | 98 ± 2 |
| | 0.05 (Instant Ocean) | 0.38 | 0.041 ± 0.004 $^{a}$ | 4.8 ± 0.9 | 94 ± 4 |
| **As** | | | | | |
| | control | b.d.l | | | 92 ± 6 |
| | 0.45 | 0.45 | 0.047 ± 0.014 $^{a}$ | 11.5 ± 2.4 | 96 ± 4 |
| | 0.90 | 0.96 | 0.013 ± 0.001 $^{b}$ | 12.0 ± 3.3 | 92 ± 6 |
| | 1.80 | 1.81 | 0.024 ± 0.004 $^{ab}$ | 15.1 ± 3.0 | 92 ± 5 |
| | 3.57 | 3.34 | 0.012 ± 0.004 $^{b}$ | 11.1 ± 4.9 | 68 ± 6 |
| | 7.19 | 6.70 | 0.021 ± 0.004 $^{ab}$ | 18.9 ± 4.9 | 22 ± 6 |
| | 1.80 (instant ocean) | 1.76 | 0.0017 ±0.003 $^{b}$ | 12.3 ± 1.0 | 92 ± 2 |

**Table 12. *L. plumulosus* uptake ($k_u$) and efflux ($k_{ew}$) rates from the dissolved phase for different As and Cd concentrations.** For each metal, $K_u$ values with different letters are significantly different ($\alpha$=0.05, ANOVA, Tukey's HSD). There are no significant differences between $K_{ew}$ values.




1

**Figure 15.** ***Leptocheirus plumulosus*** **depuration after uptake from** ***I. galbana*** **(open circles) and water (black squares) for Cd, As, inorganic Hg and $CH_3Hg$.** Metal concentrations (mg $L^{-1}$) in water were 1.12 x$10^{-4}$ (Cd), 3.75 x $10^{-5}$ (As), 3.39 x $10^{-4}$ (inorganic Hg) and 1.07 x $10^{-4}$ ($CH_3Hg$).

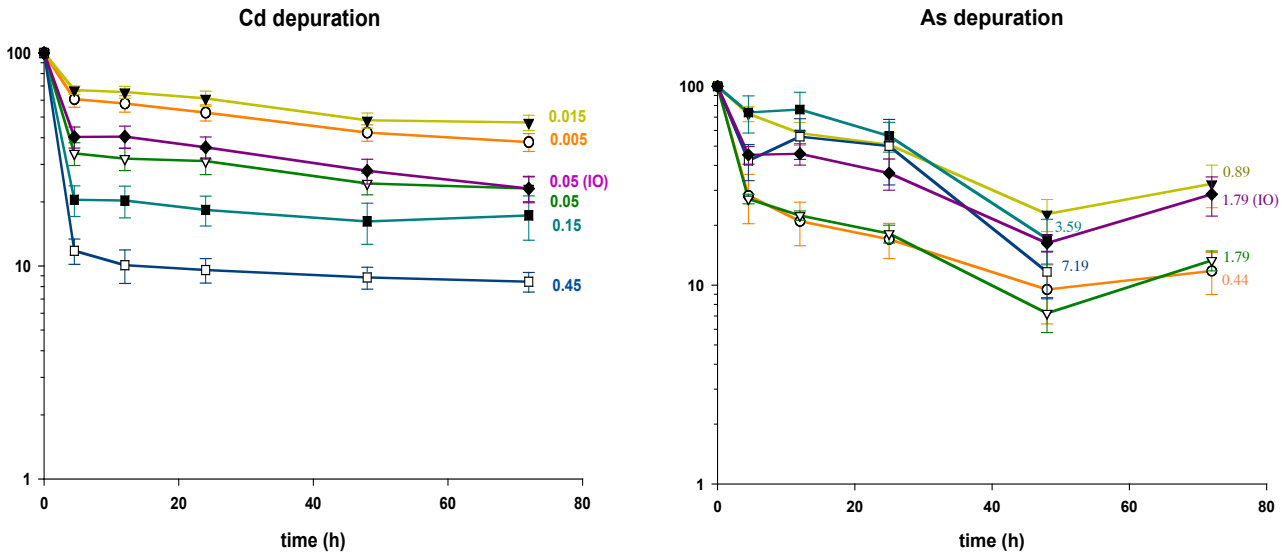
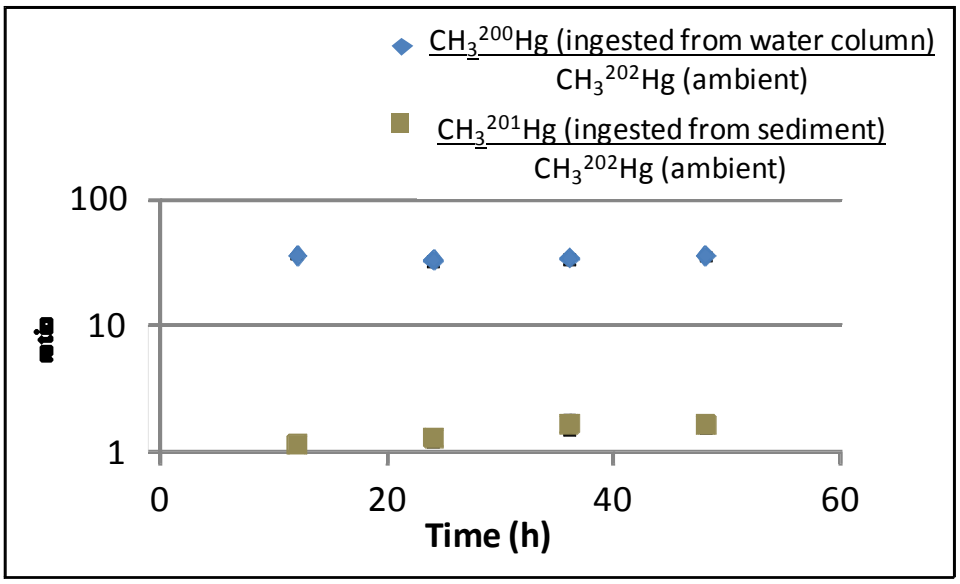



**Figure 16.** ***L. plumulosus*** **depuration after exposure to Cd and As in water at varying concentrations.**

***Hg bioaccumulation via benthic vs. pelagic feeding.*** The isotope ratio of $^{200}CH_3Hg$ to $^{202}CH_3Hg$ was greater than the ratio of $^{201}CH_3Hg$ to $^{202}CH_3Hg$ at all time points. The isotope ratio of $^{200}Hg$ to $^{202}Hg$ was greater than the ratio of $^{199}Hg$ to $^{202}Hg$ at all time points (Figure 9). Uptake factor, calculated as the ratio of isotopically enriched $CH_3Hg$ and Hg ingested by amphipods to $CH_3Hg$ and Hg added to algae mixed with the water column and sediment, is greater for algae that is added to water column versus algae that is added to sediments (Figure 10). These data indicate that the uptake of algae added to the water column was greater than uptake of algae in the sediment, suggesting that the pelagic route of MeHg uptake is more important than uptake directly from sediments.



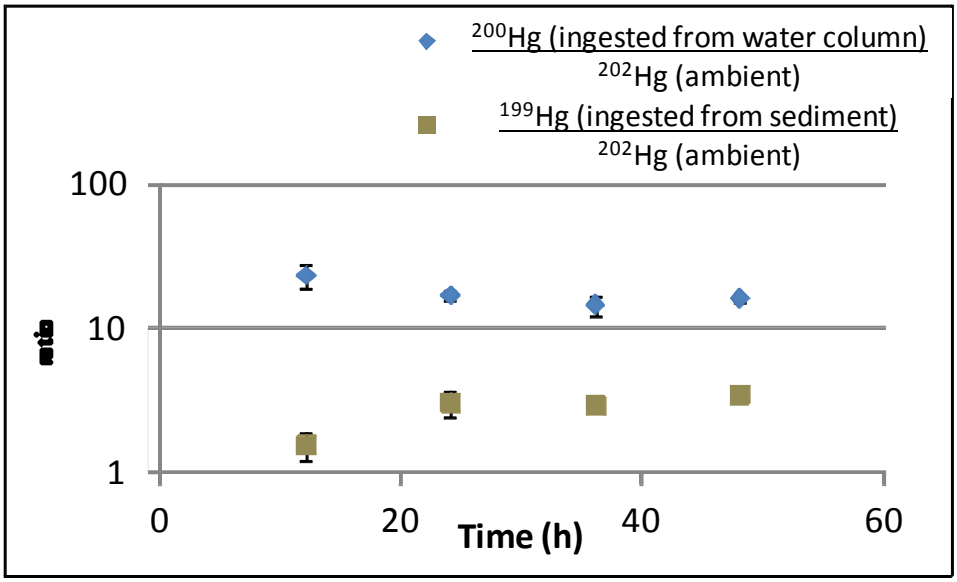



**Figure 17. Isotope ratio of enriched a) $CH_3{}^{200}Hg$ and $CH_3{}^{201}Hg$ to $CH_3{}^{202}Hg$ and b) $^{200}Hg$ and $^{199}Hg$ to $^{202}Hg$ in amphipods incubated in microcosms for 12, 24, 36 and 48 hrs**. $CH_3{}^{200}Hg$ and $CH_3{}^{201}Hg$ were ingested from algae added to the water column and sediment, respectively, and $CH_3{}^{202}Hg$ was present from natural Hg in the system. $^{200}Hg$ and $^{199}Hg$ were ingested from algae added to the water column and sediment, respectively, and $^{202}Hg$ was present from natural Hg in the system (n = 4).

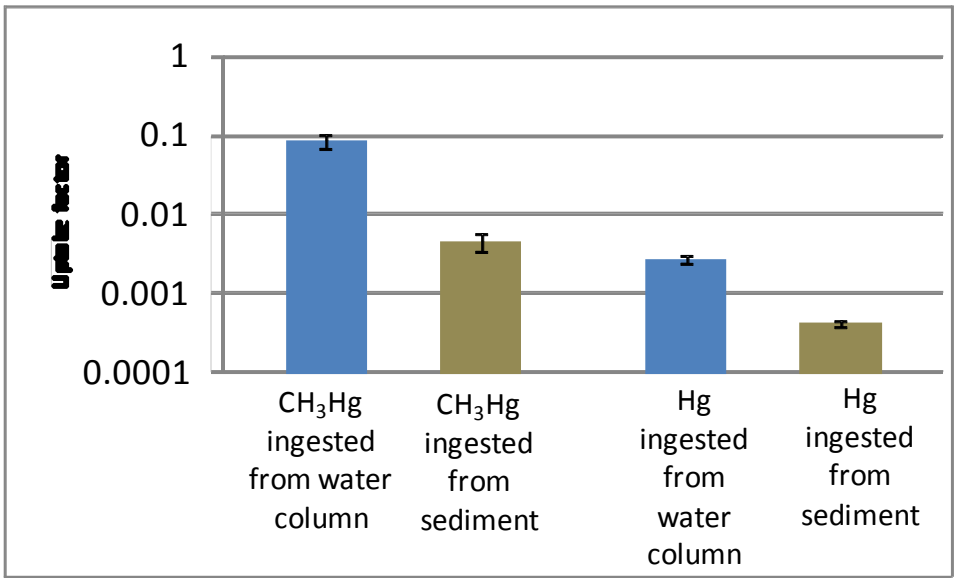



**Figure 18. Uptake factor, calculated as the ratio of isotopically enriched $CH_3Hg$ and Hg ingested by amphipods to $CH_3Hg$ and Hg added to algae mixed with the water column and sediment, after 48 hr incubation in microcosms** (n = 4).

***Effect of salinity on metal toxicity and bioaccumulation in amphipods.*** In 96-hr aqueous Cd exposures, at the lower salinity (10 ppt), found that toxicity increased as bioaccumulation

increased, except at the highest concentrations of 4.8 $\mu$g/kg where there were no live animals left for measuring Cd concentrations (Figure 19a). In 20 ppt exposures, where toxicity at the two highest concentrations (2.4 and 4.8 $\mu$g/kg) were lower than in 10 ppt, the toxicity also increased as bioaccumulation of Cd increased although bioaccumulation at the highest concentration was not proportionally as high as the mortality (Figure 19b). This may be due to the lack of replication in this treatment of animals for metal analysis since very few survived. However, these data suggest that as animals are exposed to higher concentrations they not only take up more metal but also die from the increased uptake.

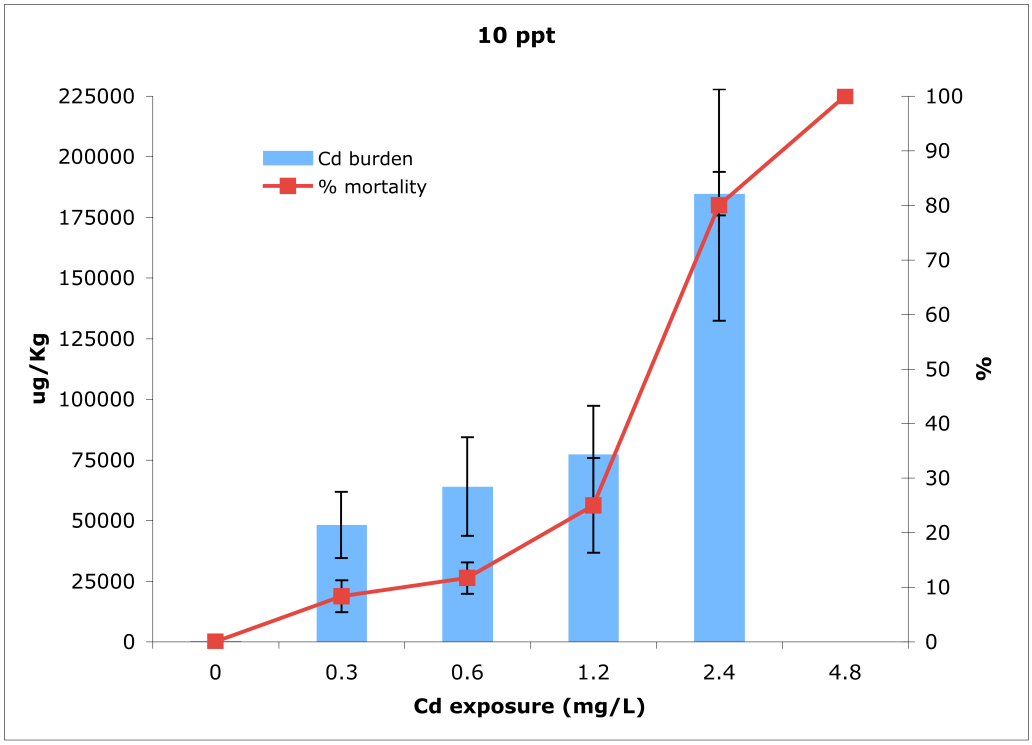



**Figure 19a. Bioaccumulation of Cd and survival of *L. plumulosus* in response to Cd exposures in 10 ppt saltwater (Instant Ocean) in a 96-hr acute toxicity test** (n = 3).

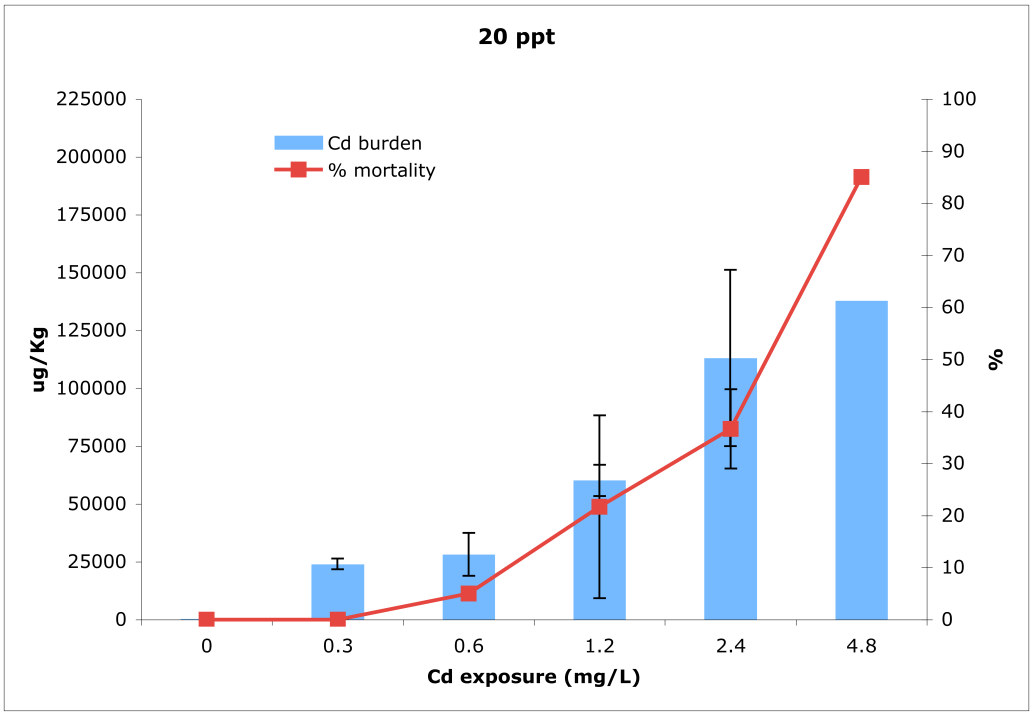
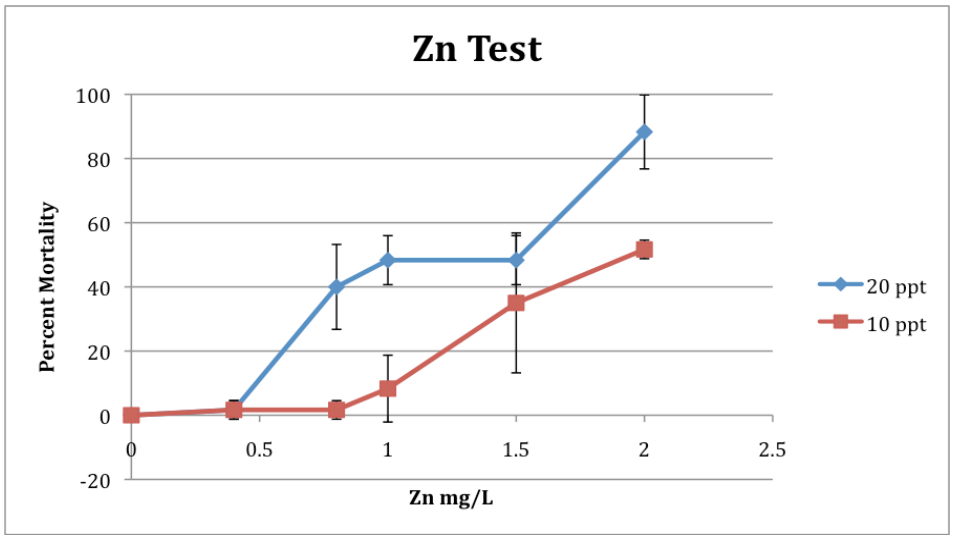



**Figure 19b. Bioaccumulation of Cd and survival of *L. plumulosus* in response to Cd exposures in 20 ppt saltwater (Instant Ocean) in a 96-hr acute toxicity test** (n = 3).

In 96-hr aqueous Zn exposures, at concentrations greater than 0.5 mg/L, toxicity was greater at the higher salinity (20 ppt). Data also suggest a higher bioaccumulation of Zn in higher salinities (n = 3).



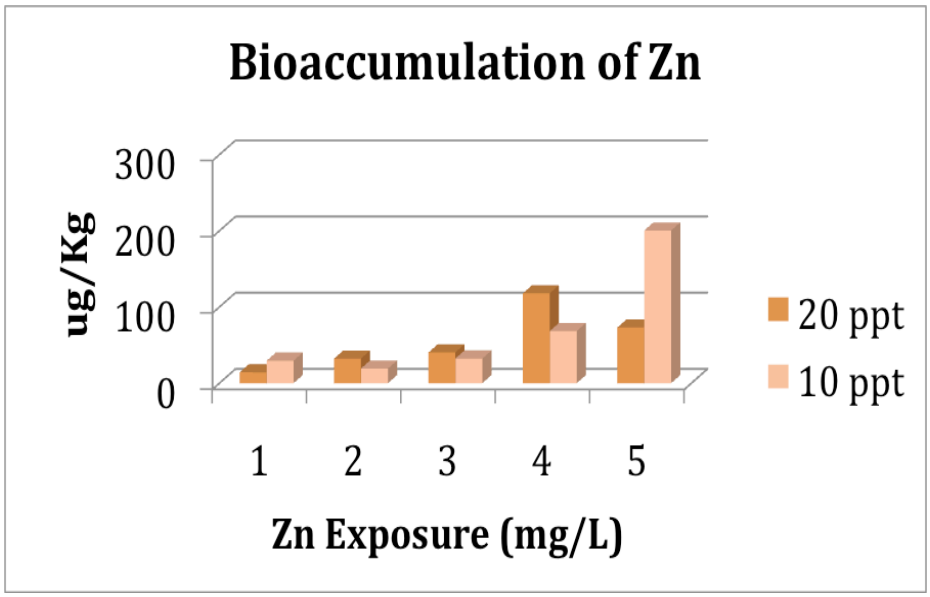



**Figure 20. Percent mortality and bioaccumulation of Zn in *L. plumulosus* exposed to a range of Zn concentrations in a 96-hr toxicity test** (n = 3).

***Task 3: Fundulus metal exposure and microarray experiments***

**Generation 1 microarray experiments.** The first generation killifish microarray included 617 oligonucleotides designed from predicted and unique gene products obtained from EST sequencing. These were spotted on Corning GAPSII slides using a GeneMachines OmniGrid Accent arrayer. The spot configuration was designed to provide four independent experimental units (hybridization zones) each containing the full compliment of oligonucleotides arrayed as geographically independent duplicates. Quality control was assessed with the SpotQC system (Integrated DNA Technologies). Spot uniformity was high with minimal donut formation (Figure 21).

Results from initial validation experiments conducted with the first generation microarrays demonstrated uniform spot morphologies, equal fluorescent intensities for the two pools of RNA labeled with different fluorescent markers, and these results were consistent across arrays and slides.

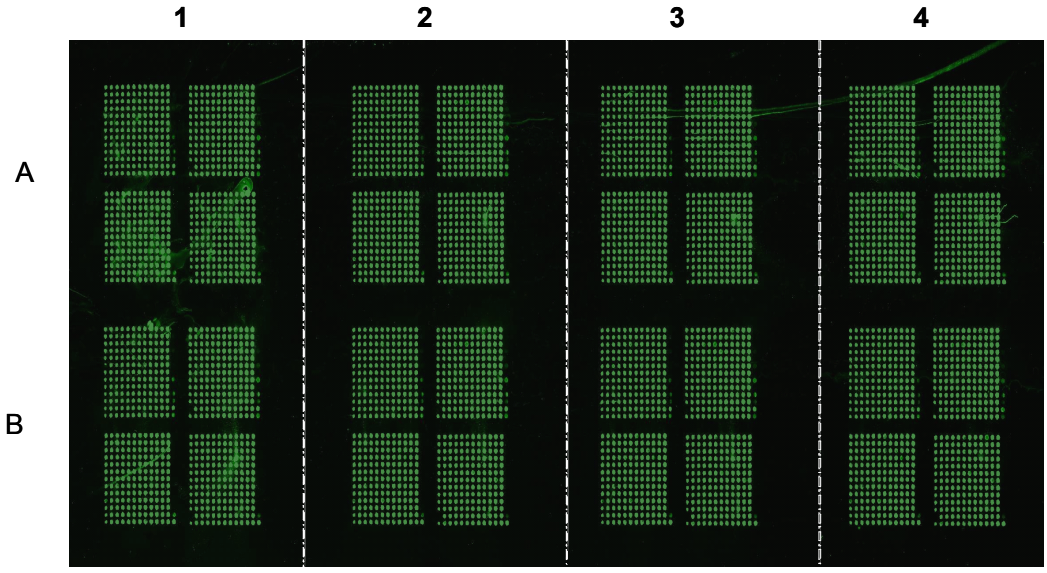



**Figure 21. Quality control image of a 617-feature oligonucleotide microarray of sequences selected from *Fundulus heteroclitus* EST libraries.** The slide, containing 8 replicates of the 617 features, was incubated with a Cy3-labeled oligonucleotide in the SpotQC system (Integrated DNA Technologies) and scanned at 532 nm with a GenePix 4000B scanner (Axon Instruments). The design provides 4 experimental units (1-4) each consisting of two geographically independent replicate arrays (A, B) containing the full compliment of 617 unique oligonucleotide probes.

Exposures were completed for initial validation experiment. The goals of these experiments were to **(*i*)** define As, Cd, Cu, Pb and Zn gene response profiles in gill and liver tissue following 96-h exposure; **(*ii*)** isolate As, Cd, and Pb specific gene responses in the presence of Cu; **(*iii*)** isolate As, Cd, and Pb specific gene responses in complex metal mixtures. For these studies each metal was tested at low and no-effects concentrations defined for this duration of exposure. Exposures included exposures to single/individual metals; binary combinations of As, Cd, and Pb in combination with Cu at low and high concentrations; and complex mixtures of As, Cd, Pb, Cu and Zn. Exposures were completed and tissues were isolated and stored in RNA later.

Results from microarray experiments conducted to examine differences in gene expression between killifish exposed to arsenic, copper, and combinations of each metal demonstrated genes that were uniquely regulated by copper (i.e., 21) and arsenic (i.e., 28) and those that were common between the two (i.e., 17) following exposures to the individual metals (Figure22). Furthermore, when killifish were exposed to arsenic and copper in combination, subsets of these patterns were still identifiable. For example, nine of the seventeen genes that were observed to be regulated by copper were identified when copper was co-administered with arsenic. Similarly, 16 of the 28 genes identified as arsenic signatures were still present in the combined exposure. These results look promising for biomarker applications in that we were able to identify an arsenic signature in the presence of the essential metal copper.

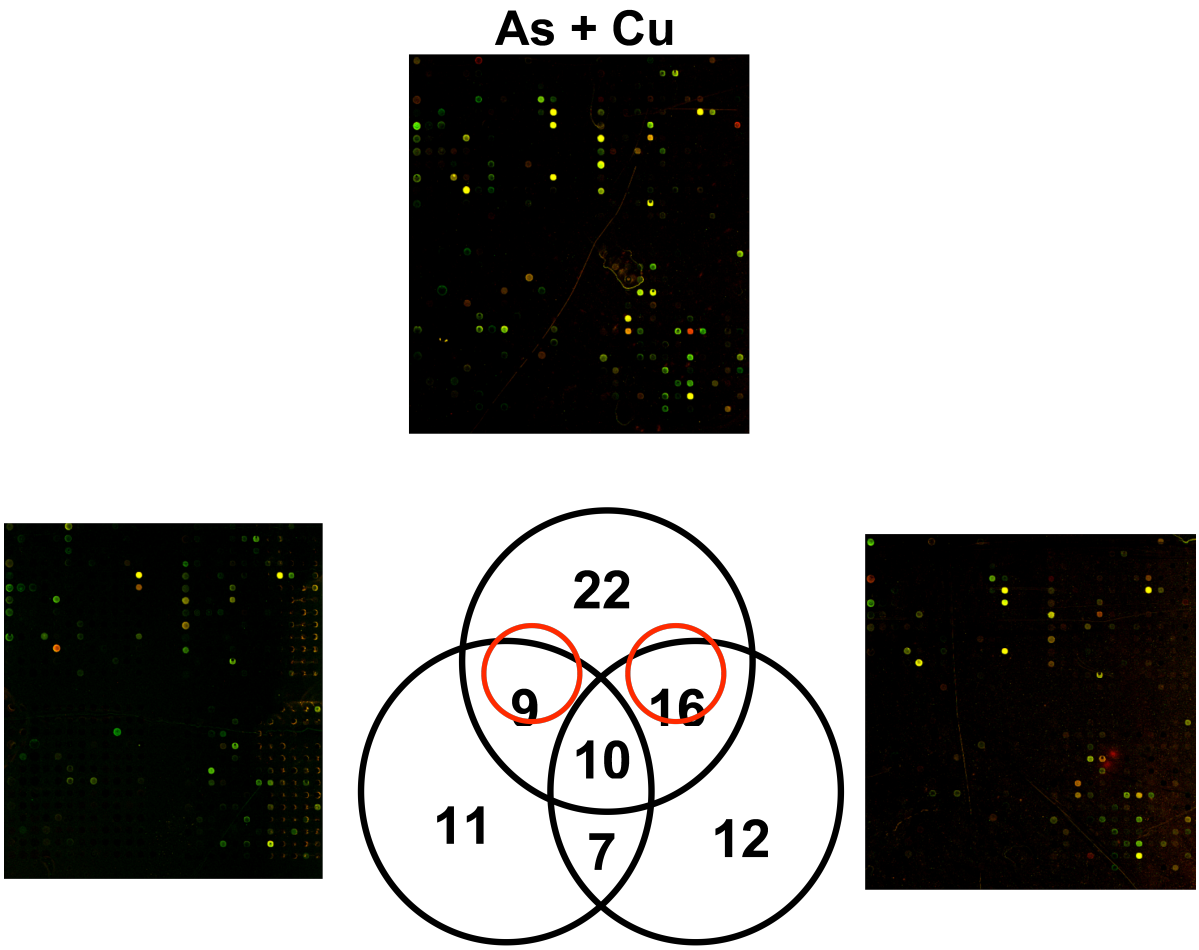
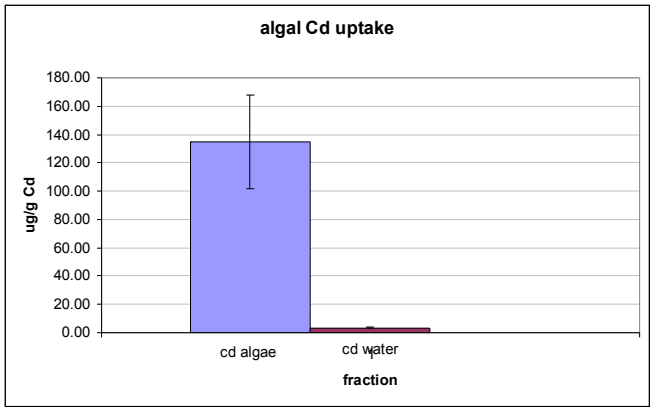
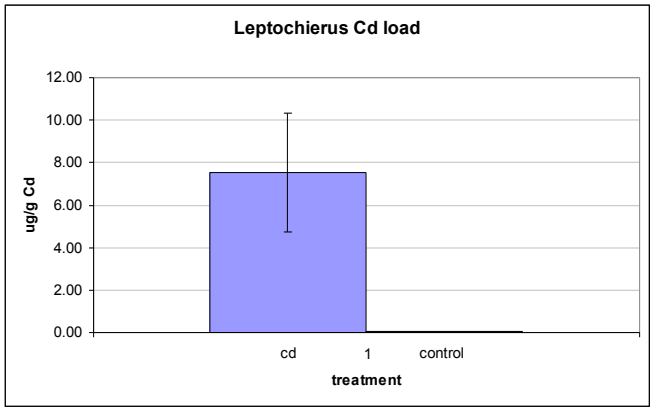



**Figure 22. *Fundulus* genes uniquely regulated by As and Cu and combinations of each metal (As + Cu).**

***Metal uptake in amphipods.*** In a pilot experiment, dietary and water-based uptake of Cd was examined. Dietary Cd-exposed fish were fed benthic amphipods (*Leptocheirus plumulosus*) that were previously fed a Cd-spiked culture of the diatom *Isochrysis galbana*. Cd spiked into the *Isochrysis* culture at 2 ppm resulted in 343 ug/g Cd in algae, 9.6 ug/g in amphipods, and 0.2 ug/g in dietary exposed Cd fish. Fish exposed to water with 514 ug/L Cd, in contrast had an average Cd burden of 1.8 ug/g. The dietary and water-exposed fish treatments were significantly different from a control and significantly different from one another (Figure 23).



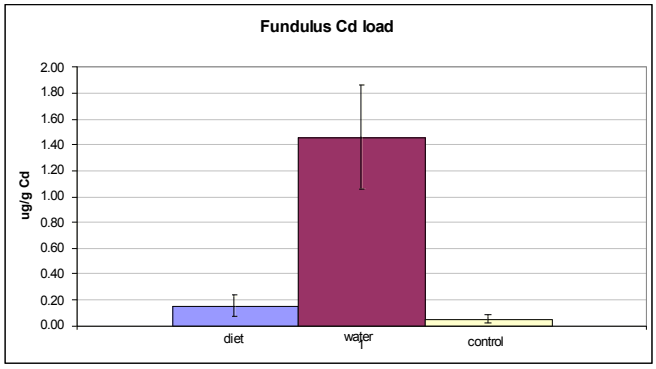




**Figure 23. *Fundulus* Cd uptake experiments via water and *Leptocheirus*.** a) algal uptake of Cd (N=2, bars indicate range); b) *Leptocheirus* uptake from Cd spiked algae vs. control algae (N=2, bars indicate range); c) *Fundulus* uptake of Cd from Cd loaded *Leptocheirus* vs. water exposure (N=4, bars indicate SD).

***Killifish metal uptake.*** A dietary exposure treatment (gills or livers of five fish pooled to obtain sufficient tissue for metal analysis) showed that gills from fish fed Cd-loaded amphipods had approximately 10 times higher Cd concentrations than fish fed control amphipods (Figure 24). Livers from dietary-exposed fish showed elevated Cd concentrations also, but control livers showed evidence of sample contamination (Figure 23). Results indicate that pooling organs and increasing dietary loading produced elevated gill and liver tissue concentrations necessary for

comparing gene expression patterns. Similar killifish metal exposures were conducted for As and Pb.

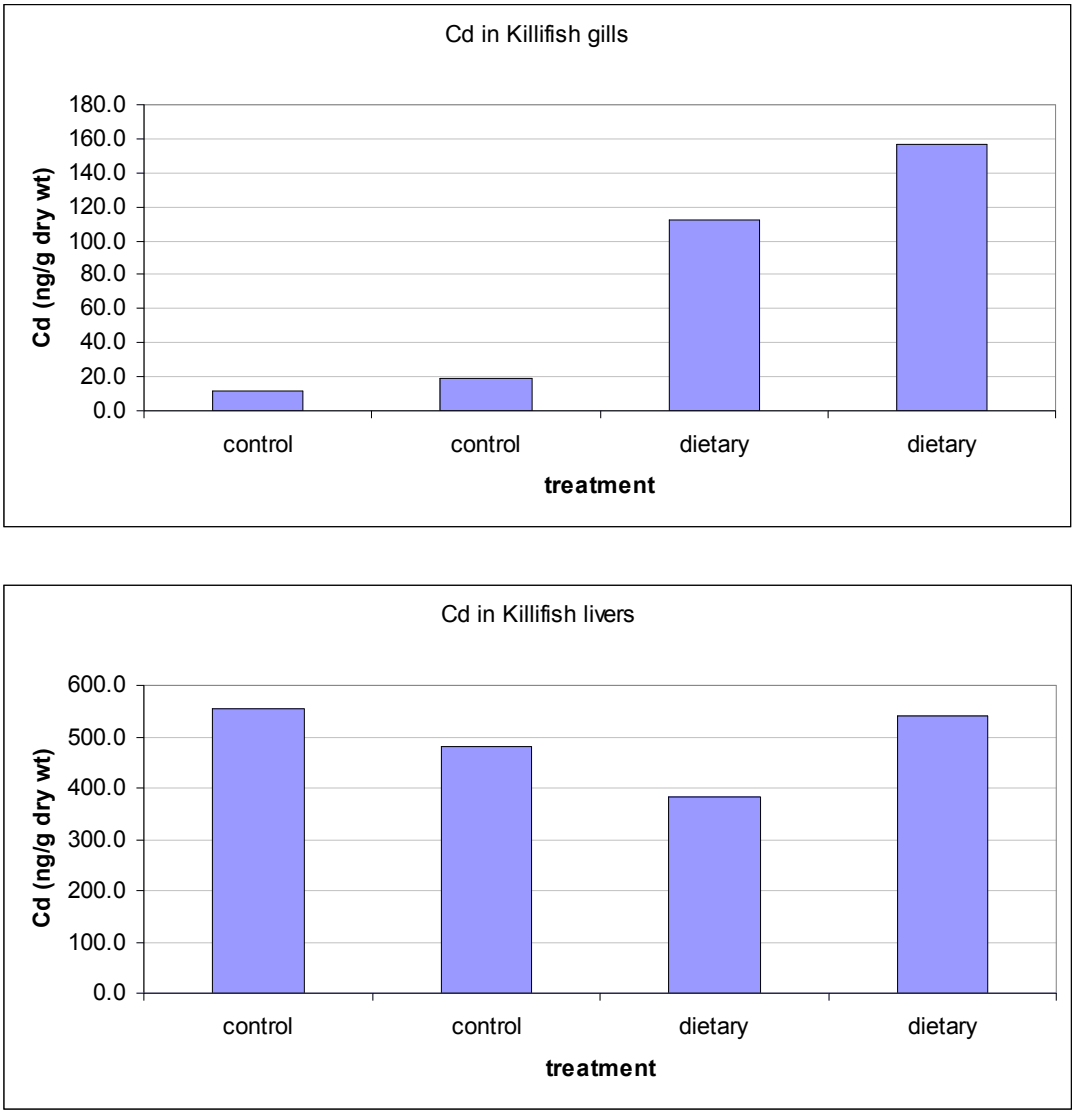



**Figure 24. Cd concentrations (ng/g dry wt.) in killifish gill and liver tissue.** Each bar represents one replicate (gills or livers pooled from 5).

**Generation 2 microarray experiments.**

**Transcriptome sequencing and assembly.** Killifish transcirptomes, representing 72 different conditions (i.e., life-stages, sexes, salinities, tissues, and metals) were sequenced from two 454 (flex) sequencing runs. The two runs produced a total of 1,340,048 expressed sequence tags (EST) containing 273,469,835bp. Of these ESTs, 1,302,633 EST exceeded our minimal quality standards, which represents 229,144,736bp that were used for the assembly. Eighty-seven (1,132,718 EST) percent of the high quality ESTs were compiled into 38,673 contigs (18,682,616bp) with an average length of 483bp and N50 of 646bp. The average depth of coverage of the contigs was 9.8X per nucleotide positions. Of these contigs, about one-third

(11,873) were considered large (>500bp) and within this group the average contig size was 950bp and the N50 was 1028bp. The majority of contigs (35,261) contained predicted open reading frames (ORFs) that averaged 196bp and there were more than 12,000 ORFs greater than 200bp in length. The remaining 14% (169,915 ESTs) of sequence reads were not homologous with other sequences and were retained as singletons.

The assembled transcritprome is robust. It contains >850 of the 924 single copy genes conserved in all vertebrates. We directly compared the similarity of gene sequences between killifish and zebrafish and extended orthologous comparisions with all model organisms using OrthoDB (http://cegg.unige.ch/orthodb4). The majority of killifish genes in our database shared homology with those from zebrafish, including 14,868 of the 16,410 zebrafish genes whose functions are known. We have partitioned these into paralogous genes residing in gene families and can now easily make comparisons between all model species. For example, 8,258 genes that share known functions with genes in mice.

Arsenic signature: We determined that arsenic induces salinity dependent transcriptomic responses that are associated with its physiological effects during seawater acclimation. For these studies killifish were acclimated to freshwater and the transcriptomic response was measured as a function of salinity, time and arsenic exposure (100 ppb) following immediate transfer to seawater. We employed a replicated, factorial design and differentially regulated genes were determined by fit to a linear model. We have previously reported that arsenic constrains physiological plasticity by inhibiting seawater acclimation and this was captured in the transcriptomic response, where gene-response profiles obtained following 1-h and 24-h in seawater in the presence of arsenic more closely resembled those of freshwater fish (Fig. 25). Not surprisingly these included genes known to be involved in mitochondiral function, cellular organization and remodeling, cell signaling, immune function, and arsenic toxicity, as well as, many novel genes. Perhaps the most striking finding was the emergence of a large interactive transcriptome –gene responses not predicted by simple addition of their activity in arsenic or seawater alone (Fig. 26). These responses represent environment dependent gene by arsenic interactions, and highlight the critical need for environmentally responsive model systems, such as killifish, in defining critical toxicity pathways that influence natural populations, which live in variable environments. In other words, we have identified signatures that are predictive of arsenic exposure in freshwater and seawater-exposed fish (note the 83 genes significantly regulated by arsenic irrespective of salinity in Fig. 26). However, the functional significance of these genes is not fully realized until they are viewed in context of the large number of genes that show significant interactions with environmental variables, in this case salinity.

Of major interest to the current project which is focused on the identification of molecular signatures that are predictive of exposure to toxic compounds and their effects within the organism, we were able to harness the power of our gene library with the improved functional listings in order to construct networks of co-regulated genes that are biologically related. This network analysis makes use of the largest curated knowledge base of previously published finding on mammalian biology from the public literature (Ingenuity Systems). Networks were constructed by combing the knowledge of functional interactions between mammalian genes with our understanding of gene functions from our database of killifish genes in order to infer biochemical interactions mediated by the effects of experimental treatments on

gene expression. For example, the genes that show salinity dependent expression following arsenic exposure (i.e., significant interaction term), form a functional network of coupled genes (Fig. 27a) that mutes the arsenic response (Fig. 27b) and the salinity response (Fig. 27b).

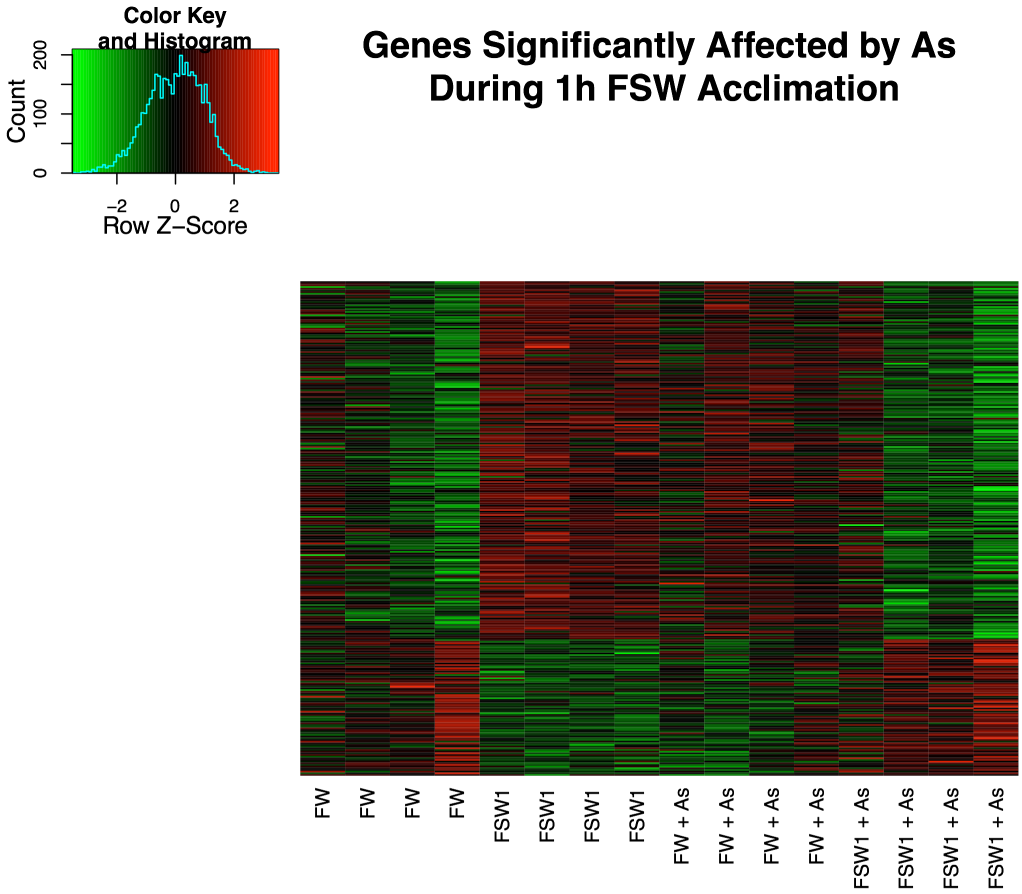



**Figure 25. Arsenic inhibits transcriptomic response seen during seawater acclimation: Heat map illustrating the response patterns of genes significantly affected by arsenic following the immediate transition from freshwater (FW) to seawater for 1 hour (FSW1) SW when combined with arsenic exposure (FSW1+As).**

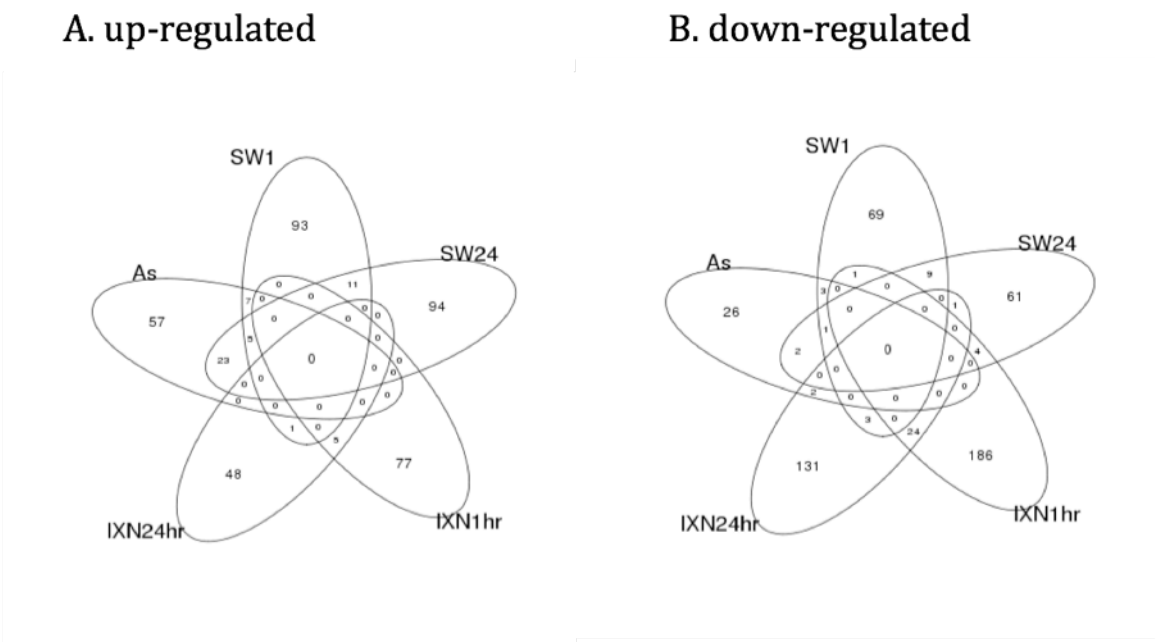
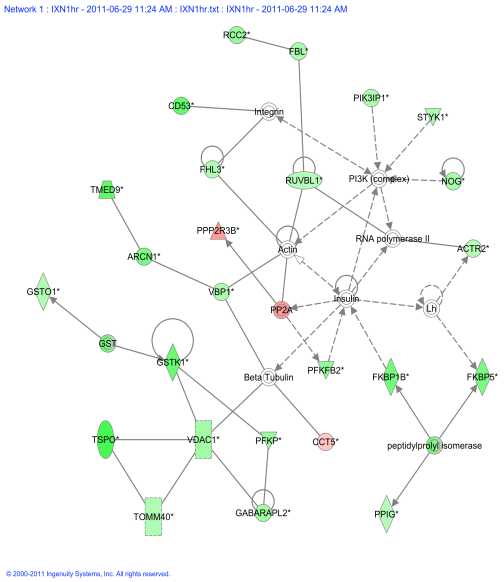



**Figure 26. Interactive transcriptome revealed. Transcriptomic response to arsenic during seawater acclimation reveals salinity dependent, arsenic mediated gene expression. Numbers represent differently regulated genes (p<0.05 and estimated fold change >2) as determined by fit to a linear model, for the following treatments: arsenic (As), 1hr in seawater (SW1), 24 hr in seawater (SW24), interactions between arsenic and SW1 (1XN24hr). Interaction effects are effects not predicted by simple addition of effects attributable to arsenic or seawater alone.**




**C.**

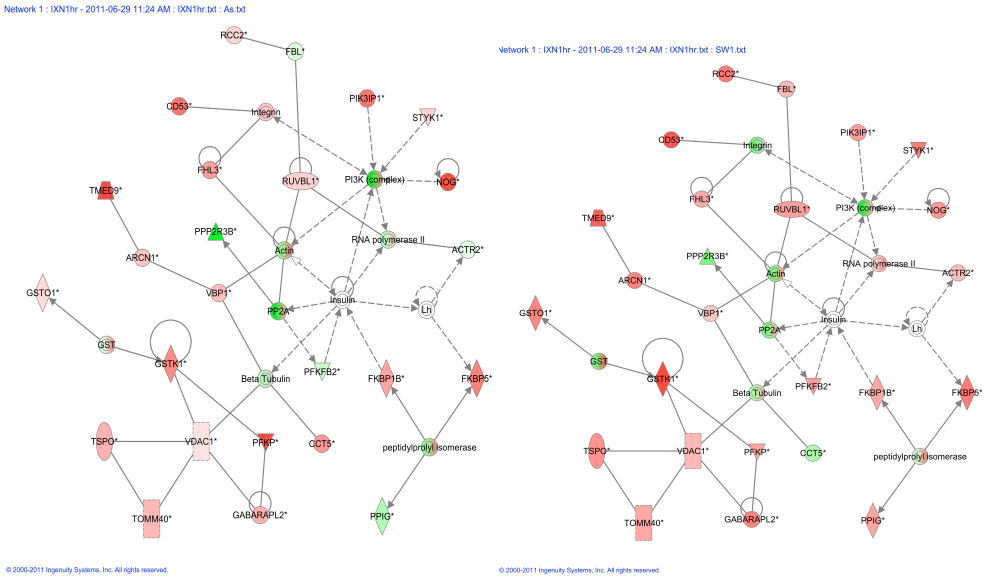



**Figure 27. Functional network of interacting genes. The network was constructed based on co-regulated genes determined from the microarray experiments using Ingenuity software. Color indicates direction of response with the red highlight up-regulated genes and green those that are down-regulated. Intensity is proportional to magnitude of response. Panel A is the interaction network, Panel B and C visualize the arsenic and salinity responses within the interaction network.**

## CONCLUSIONS AND IMPLICATIONS

*Task 1:* The past field data and the sediment data analyzed for the 2006 field season indicate that the concentrations in the seven sites across four estuarine systems do differ and that the Great Bay sites have the highest metal bioavailability in sediments. However, the differences in metal concentrations in the food web do not vary strongly with sediment concentration. In fact, benthic-sediment concentration factors for sediments vary significantly with TOC and not with SEM-AVS. When comparing the various invertebrate and vertebrate taxa, concentrations in the filter feeders, ribbed and blue mussels, are highest for all metals. Moreover, the biota that are more depleted in $^{13}C$ appear to have higher concentrations of MeHg suggesting that pelagic feeding organisms bioaccumulate more MeHg than benthic feeding organisms. Finally, relationships of metal concentrations in biota with trophic level (measured as $^{15}N$) are only apparent with %MeHg which indicates biomagnification and with Pb which decreases in concentration with increasing trophic level indicating biodiminution as has been seen in freshwater systems.

*Task 2:* The bioaccumulation experiments indicate that there are differences in the retention of metals and their distribution in tissues dependent on the metal. In general as metal concentrations in water increase, metal concentrations in fish also increase but $K_u$ does not. Salinity and DOC also affect the amount of uptake and rate of uptake for Cd and Cr. Ingestion of worms by killifish results in bioaccumulation of Hg but less so for Cd and Cr. Hg had the fastest uptake rate followed by Pb, Cr, Cd and As. Arsenic had the fastest loss rate followed by Hg, Cr, Pb and Cd. After ten days Cd had the highest retention followed by Pb, Cr, Hg and As. Following tissue dissection the lowest partitioning was found in the liver for As, Cr, Hg and Pb, and in the skeleton, fillet and skin for Cd. For all metals except As the greatest body burden was in the head. For As it was in the fillet. The assimilation of MeHg was higher in killifish exposed via food than assimilation of Hg (inorganic) or Cd. The high levels of MeHg retention were largely due to low levels of efflux during depuration. MeHg was also the only metal shown to be concentrating in the blood. In comparisons of bioaccumulation of MeHg when exposed in different waters (varying in salinity and DOC), experiments showed that there was a decrease in uptake with increasing salinity suggesting that the Cl- reduces the bioavailability of MeHg but that efflux was not influenced by water chemistry. However, standardized salinity and DOC experiments suggest that salinity may enhance the uptake of metals. In all experiments, retention of MeHg was extremely high (~90%) and was largely found in the head and filet of the killifish. Biokinetic parameters have been calculated for metal bioaccumulation by *L. plumulosus*. For the bioaccumulation of Cd, As, Hg, and MeHg, the assimilation efficiencies varied greatly with lowest AE of Hg (6%) and highest of MeHg at 80%. Cd AE was 24% and As AE was 11%. The resulting steady state metal concentrations also varied enormously with MeHg being from one to three orders of magnitude higher than the other metals. This is largely due to the high retention of MeHg as compared to the other metals.

*Task 3*. The first generation microarray included 617 oligonucleotides designed from predicted and unique gene products obtained from EST sequencing. In addition, exposures of *Fundulus* were completed for initial validation experiments including exposures to single/individual metals; binary combinations of As, Cd, and Pb in combination with Cu at low and high

concentrations; and complex mixtures of As, Cd, Pb, Cu and Zn. Exposures were completed, tissue isolated, and stored in RNA later.

Initial validation experiments were conducted as a QA step to ensure the appropriate function of microarray technology. These experiments successfully addressed a checkpoint (go/no go) to determine that the microarray response was appropriate before proceeding with detailed laboratory and field experiments. We determined that microarrays generated differential responses in metal treated and control groups that could distinguished the response to Cd from the response to the essential metal Cu.

Given the success of the early studies, we sequenced and assembled a transcriptome database for killifish. We used this information to upgrade our microarray platform from individual 50 base-pair oligonucleotides representing only 500 genes, to over 35,000 genes and gene fragments represented by multiple long oligonucleotides (i.e., ~95000 elements). This array platform was employed to successfully obtain functionally significant biomarkers of exposure that were able to differentiate individual (i.e., genetic) variation and physical modifiers of effects (e.g., salinity). The tools constructed from this grant complement other resources (including a complete genome sequence for this species) that make the killifish a model system for environmental genomics and enhance the ability to detect and determine the effects of exposures to environmental toxicants.

## LITERATURE CITED


ATSDR, 2011. Priority List of Hazardous Substances Agency for Toxic Substances and Disease Registry.

Baines, S.B., Fisher, N.S., Stewart, R., 2002. Assimilation and retention of selenium and other trace elements from crustacean food by juvenile striped bass (Morone saxatilis). Limnology and Oceanography 47, 646-655.

Boesch, D.F., Turner, R.E., 1984. Dependence of Fishery Species on Salt Marshes - the Role of Food and Refuge. Estuaries 7, 460-468.

Cabana, G., Tremblay, A., Kalff, J., Rasmussen, J.B., 1994. Pelagic Food-Chain Structure in Ontario Lakes - a Determinant of Mercury Levels in Lake Trout (Salvelinus-Namaycush). Canadian Journal of Fisheries and Aquatic Sciences 51, 381-389.

Cantwell, M.G., Burgess, R.M., Kester, D.R., 2002. Release and phase partitioning of metals from anoxic estuarine sediments during periods of simulated resuspension. Environmental Science & Technology 36, 5328-5334.

Chen, C.Y., Folt, C.L., 2000. Bioaccumulation and diminution of arsenic and lead in a freshwater food web. Environ. Sci. Technol 34, 3878-3884.

Chen, C.Y., Folt, C.L., 2005. High plankton densities reduce mercury biomagnification. Environ. Sci. Technol. 39, 115-121.

Chen, C.Y., Folt, C.L., Stemberger, R.S., Blum, J.D., Klaue, B., Pickhardt, P.C., 2000. Accumulation of heavy metals in food web components across a gradient of lakes. Limnol. Oceanogr 45, 1525-1536.

Chen, C.Y., Dionne, M., Mayes, B.M., Ward, D.M., Sturup, S., Jackson, B.P., 2009. Mercury Bioavailability and Bioaccumulation in Estuarine Food Webs in the Gulf of Maine. Environ. Sci. Technol. 43, 1804-1810.

Deegan, L.A., Hughes, J. E., Rountree, R. A., 2000. Salt marsh ecosystem support of marine transient species. In: Weinstein, M.P., Kreeger, D. A. (Ed.), Concepts and Controversies in Tidal Marsh Ecology. Kluwer Academic Publishers, Boston, pp. 333-365.

Dionne, M., Short, F.T., Burdick, D.M., 1999. Fish utilization of restored, created and reference salt-marsh habitat in the Gulf of Maine., American Fisheries Society Symposium, pp. 384-404.

Dutton, J., Fisher, N.S., Bioaccumulation of As, Cd, Cr, Hg(II), and MeHg in killifish (Fundulus heteroclitus) from amphipod and worm prey. Sci. Total Environ. 409, 3438-3447.

Dutton, J., Fisher, N.S., Salinity Effects on the Bioavailability of Aqueous Metals for the Estuarine Killifish Fundulus Heteroclitus. Environ. Toxicol. Chem. 30, 2107-2114.

Fisher, N.S., Reinfelder, J.R., 1995. The Trophic Transfer of Metals in Marine Systems. In: Tessier, A., Turner, D.R. (Eds.), Metal Speciation and Bioavailability in Aquatic Systems. John Wiley & Sons Ltd, pp. 363-406.

Fisher, N.S., Teyssie, J.L., Fowler, S.W., Wang, W.X., 1996. Accumulation and retention of metals in mussels from food and water: A comparison under field and laboratory conditions. Environmental Science & Technology 30, 3232-3242.

Fisher, N.S., Stupakoff, I., Sanudo-Wilhelmy, S., Wang, W.X., Teyssie, J.L., Fowler, S.W., Crusius, J., 2000. Trace metals in marine copepods: a field test of a bioaccumulation model coupled to laboratory uptake kinetics data. Marine Ecology-Progress Series 194, 211-218.

Francis, D.R., Jude, D.J., Barres, J.A., 1998. Mercury distribution in the biota of a Great Lakes estuary: Old Woman Creek, Ohio. Journal of Great Lakes Research 24, 595-607.

Garnier, J.M., Guieu, C., 2003. Release of cadmium in the Danube estuary: contribution of physical and chemical processes as determined by an experimental approach. Marine Environmental Research 55, 5-25.

Griscom, S.B., Fisher, N.S., 2004. Bioavailability of sediment-bound metals to marine bivalve molluscs: An overview. Estuaries 27, 826-838.

Karimi, R., Chen, C.Y., Pickhardt, P.C., Fisher, N.S., Folt, C.L., 2007. Stoichiometric controls of mercury dilution by growth. P Natl Acad Sci USA 104, 7477-7482.

Kneib, R.T., 1997. The role of tidal marshes in the ecology of estuarine nekton. Oceanography and Marine Biology: an Annual Review 35, 163-330.

Kneib, R.T., 2000. Salt Marsh Ecoscapes and production transfers by estuarine nekton in the southeastern United States. In: Kreeger, M.P.W.a.D.A. (Ed.), Concepts and Contoversies in Tidal Marsh Ecology. Kluwer Academic Publishers, Boston, pp. 267-291.

Luoma, S.N., Rainbow, P.S., 2005. Why is metal bioaccumulation so variable? Biodynamics as a unifying concept. Environ. Sci. Technol. 39, 1921-1931.

MacKenzie, R.A., 2001. Great Lakes coastal wetlands-estuarine systems: invertebrate communities, particle dynamics, and biogeochemical cycles. Univeristy of Wisconsin at Milwaukee, Milwaukee, Wisconsin.

Marvin-DiPasquale, M.C., Agee, J.L., Bouse, R.M., Jaffe, B.E., 2003. Microbial cycling of mercury in contaminated pelagic and wetland sediments of San Pablo Bay, California. Environmental Geology 43, 260-267.

O'Connor, T.P., 1996. Chapter 13: Coastal Sediment Contamination in the Northeast Shelf Large Marine Ecosystem. In: K. Sherman, N.A.J., and T. J. Smayda (Ed.), The Northeast Shelf Ecosystem: Assessment, Sustainability, and Management. Blackwell Science, Cambridge, MA, pp. 239-257.

Peters, G.M., Maher, W.A., Krikowa, F., Roach, A.C., Jeswani, H.K., Barford, J.P., Gomes, V.G., Reible, D.D., 1999. Selenium in sediments, pore waters and benthic infauna of Lake Macquarie, New South Wales, Australia. Marine Environmental Research 47, 491-508.

Pickhardt, P.C., Stepanova, M., Fisher, N.S., 2006. Contrasting uptake routes and tissue distributions of inorganic and methylmercury in mosquitofish (Gambusia affinis) and redear sunfish (Lepomis microlophus). Environ. Toxicol. Chem. 25, 2132-2142.

Pickhardt, P.C., Folt, C.L., Chen, C.Y., Klaue, B., Blum, J.D., 2002. Algal blooms reduce the uptake of toxic methylmercury in freshwater food webs. P Natl Acad Sci USA 99, 4419-4423.

Roditi, H.A., Fisher, N.S., Sanudo-Wilhelmy, S.A., 2000. Field testing a metal bioaccumulation model for zebra mussels. Environmental Science & Technology 34, 2817-2825.

Rolfhus, K.R., Lamborg, C.H., Fitzgerald, W.F., Balcom, P.H., 2003. Evidence for enhanced mercury reactivity in response to estuarine mixing. Journal of Geophysical Research-Oceans 108.

Shaw, J.R., Glaholt, S.P., Greenberg, N.S., Sierra-Alvarez, R., Folt, C.L., 2007a. Acute toxicity of arsenic to Daphnia pulex: Influence of organic functional groups and oxidation state. Environ. Toxicol. Chem. 26, 1532-1537.

Shaw, J.R., Chen, C.Y., Davey, J., C., F., Hamilton, J.A., Colbourne, J.K., 2004. Using microarrays to determine metal induced gene-response in Daphnia pulex, Fouth SETAC World Congress: 25th Annual Meeting in North America, Portland, OR.

Shaw, J.R., Jackson, B., Gabor, K., Stanton, S., Hamilton, J.W., Stanton, B.A., 2007b. The influence of exposure history on arsenic accumulation and toxicity in the killifish, Fundulus heteroclitus. Environ. Toxicol. Chem. 26, 2704-2709.

Shaw, J.R., Sato, J.D., VanderHeide, J., LaCasse, T., Stanton, C.R., Lankowski, A., Stanton, S.E., Chapline, C., Coutermarsh, B., Barnaby, R., Karlson, K., Stanton, B.A., 2008. The role of SGK and CFTR in acute adaptation to seawater in Fundulus heteroclitus. Cell. Physiol. Biochem. 22, 69-78.

Stemberger, R.S., Chen, C.Y., 1998. Fish tissue metals and zooplankton assemblages of northeastern US lakes. Canadian Journal of Fisheries and Aquatic Sciences 55, 339-352.

USEPA, 1996. National sediment quality servey - a report to congress on the extent and severity of sediment contamination in surface waters of the United States.

Wang, W.X., Fisher, N.S., 1996. Assimilation of trace elements and carbon by the mussel Mytilus edulis: Effects of food composition. Limnology and Oceanography 41, 197-207.

Wang, W.X., Fisher, N.S., 1997. Modeling the influence of body size on trace element accumulation in the mussel Mytilus edulis. Marine Ecology-Progress Series 161, 103-115.

Wang, W.X., Fisher, N.S., 1999. Assimilation efficiencies of chemical contaminants in aquatic invertebrates: A synthesis. Environ. Toxicol. Chem. 18, 2034-2045.

Williams, J.J., Dutton, J., Chen, C.Y., Fisher, N.S., 2010. Metal (As, Cd, Hg, and CH(3)Hg) bioaccumulation from water and food by the benthic amphipod *Leptocheirus plumulosus* Environ. Toxicol. Chem. 29, 1755-1761.

**APPENDIX B**

## LIST OF SCIENTIFIC/TECHNICAL PUBLICATIONS


Chen, C., Dionne, M., Mayes, B., Ward, D., Sturup, S., Jackson, B. 2009. Mercury bioavailability and bioaccumulation in estuarine food webs in the Gulf of Maine. Environ. Sci. Technol 43: 1804-1810.

Chen, C., Amirbahman, A., Fisher, N., Harding, G., Lamborg, C., Nacci, D., Taylor, D. 2008. Methylmercury in marine ecosystems: spatial patterns and processes of production, bioaccumulation, and biomagnification. Ecohealth 5(4): 399-408.

Chen, C.Y., Hathaway, K.M., Thompson, D.G., Folt, C.L. 2008. Multiple stressor effects of Release, pH, and food on the zooplankton, *Simocephalus vetulus* and larval amphibian, *Rana pipiens*. Ecotoxicol and Environ Safety 71: 209-218.

Chen, C.Y., Serrell, N., Evers, D.C., Fleishman, B.J., Lambert, K.F., Weiss, J., Mason, R.P., Bank, M.S. 2008. Meeting report: Methylmercury in marine ecosystems - From sources to seafood consumers. Environ Health Perspectives 116(12): 1706-1712.

Dutton, J. and Fisher, N.S. 2011. The bioaccumulation of As, Cd, Cr, Hg, and MeHg in the killifish (*Fundulus heteroclitus*) from worm and amphipod prey. Science of the Total Environment 409 (18): 3438-3447.

Dutton, J. and Fisher, N.S. 2011. Salinity effects on the bioavailability of aqueous metals for the estuarine killifish *Fundulus heteroclitus*. Environmental Toxicology and Chemistry 30(9): 2107-2014.

Dutton, J. and Fisher, N.S. 2010. Intraspecific comparisons of metal bioaccumulation in the juvenile Atlantic silverside *Menidia menidia*. Aquatic Biology 10: 211-226.

Evers, D.C., Mason, R.P., Kamman, N.C., Chen, C.Y., Bogomolni, A.L., Taylor, D.L., Hammerschmidt, C.R., Jones, S.H., Burgess, N.M., Munney, K. Parson, K.C. 2008. An integrated mercury monitoring program for temperate estuarine and marine ecosystems on the North American Atlantic Coast. Ecohealth 5(4): 426-441.

Shaw, J.R., Bomberger, J., VanderHeide, J., LaCasse, T., Stanton, S., Coutermarsh, B., Barnaby, R., Stanton, B.A. 2010. Arsenic Inhibits SGK-1 Activation of CFTR $Cl^-$ Channels in killifish, *Fundulus heterclitus*. Aquatic Toxicology 98(2): 157-164.

Shaw, J.R., Sato, D., VanderHeide, J., LaCasse, T., Stanton, C.R., Lankowski, A., Stanton, S.E., Chapline, C., Coutermarsh, B., Barnaby, R., Karlson, K., Stanton, B.A. 2008. The role of SGK and CFTR in acute adaptation to seawater in *Fundulus heteroclitus*. Cell Physiol Biochem 22: 69-78

Shaw, J.R., Colbourne, J.K., Davey, J.C., Glaholt, S.P., Hampton, T.H., Chen, C.Y., Folt, C.L. Hamilton, J.W. 2007. Gene response profiles for *Daphnia pulex* exposed to the environmental stressor cadmium reveals novel crustacean metallothioneins. BMC Genomics 8:477.

Towle, D.W., Shaw, J.R. 2007. An oligonucleotide microarray for gene expression studies in the killifish *Fundulus heteroclitus*. The Bulletin, Mount Desert Island Biological Laboratory 46: 70.

Williams, J.J., Dutton, J., Chen, C.Y., Fisher, N.S. 2010. Metal (As, Cd, Hg, and $CH_3Hg$) bioaccumulation from water and food by the benthic amphipod *Leptocheirus plumulosus*. Environmental Toxicology and Chemistry 29(8): 1755-1761.

**Appendix C: Manuscript in Review**

**Factors controlling metal bioaccumulation in estuarine food webs across a contamination gradient**

**Celia Y. Chen†, Jason J. Williams‡, Nicholas S. Fisher§, Darren M. Ward‖**

† Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire 03755, USA, celia.chen@dartmouth.edu (Corresponding Author)

‡ TerraGraphics Environmental Engineering, 121 S Jackson St, Moscow, ID 83843, USA, jjwill04@gmail.com

§ School of Marine and Atmospheric Sciences, Stony Brook University, Stony Brook, New York 11794, USA, nfisher@notes.cc.sunysb.edu

‖ Department of Fisheries Biology, Humboldt State University, Arcata, CA 95521, USA darren.ward@humboldt.edu

**Abstract**

Estuarine sediments are important sources of metal bioaccumulation to pelagic and benthic organisms that are consumed by humans. We investigated chemical and ecological factors influencing the bioaccumulation of Hg, methyl-Hg (MeHg), Cd, Se, Pb, and As by primary and secondary consumers across a gradient of estuarine sites in New England that varied in sediment metal concentrations. In sediments, we measured metal concentrations and TOC. In biota, we measured metal concentrations and delta $^{15}N$ and delta $^{13}C$ signatures. We also calculated Benthic-Sediment Concentration Factors (BSCFs) for each metal. Bioavailability of all metals was lower in more contaminated sites, likely due to factors related to higher levels of organic carbon in the sediments. Carbon normalized sediment concentrations were related to biotic metal concentration only for Hg, indicating that, unlike for other metals, sediment concentrations of Hg are controlling factors of Hg bioavailability. Pelagic feeding resulted in higher Hg and MeHg concentrations than benthic feeding, and MeHg concentrations were higher in higher trophic level organisms. Our research highlights the importance of biogeochemistry and food web factors for understanding metal bioavailability, particularly for Hg and MeHg.

Key words: metals bioaccumulation, metal bioavailability, estuarine ecology, methylmercury, food web

## Introduction

Metal contamination is a major concern in the environment globally. Metals comprise four of the top ten substances of concern on the 2007 CERCLA Priority List of Hazardous Substances, with As, Pb, and Hg comprising the top three (ATSDR 2007). Metals enter the environment from both natural and anthropogenic sources. Mobilized metals that are deposited on the landscape, lakes, or rivers are ultimately transported hydrologically to coastal rivers and estuaries, where they often accumulate in the sediment. As a result, sediments in estuaries are repositories for metal contaminants from upland watersheds and represent a potentially enriched source of bioavailable metal for pelagic and benthic organisms. Benthic fauna living in contaminated sediments accumulate metals and potentially transfer them to their predators. Metal contaminants in sediments can also flux into the water column, where they are available to the pelagic food web.

Sediment and water concentrations alone do not determine availability or uptake of metals by organisms. Metal bioavailability to ecological receptors is controlled by complex physical, chemical, and biological factors that affect exposure and uptake patterns (NRC 2003). These factors include metal speciation (controlled by redox, organic matter, sulfides), metal concentration in aqueous and particulate (food) phases, and ecological processes such as feeding strategies and trophic position of exposed organisms (Luoma and Rainbow 2008). Metal bioaccumulation can be used as an endpoint for evaluating bioavailability and in some cases can be related to metal-induced effects at the organism and population levels (Ankley 1996; Borgmann and Norwood 2002; Borgmann et al. 2004; Marsden and Rainbow 2004; Lee and Lee 2005).

An understanding of processes and mechanisms controlling bioavailability is critical to predicting metal bioaccumulation in aquatic food webs and successfully linking bioavailability of metals to effects relevant to human exposure through consumption of seafood. In order to fully evaluate bioavailability and bioaccumulation of metals, net accumulation in tissue from exposure to all environmental sources must be considered (Newman 1998). Here we use tissue concentrations of metals in estuarine food webs, as well as biotic concentrations normalized to sediment concentrations (benthic-sediment concentration factors) as indicators of bioavailability and we relate metal concentrations to benthic and pelagic pathways of exposure and trophic position.

In this study we examined the bioavailability of metal contaminants (Hg, MeHg, Se, As, Cd, and Pb) to benthic and pelagic biotic receptors across a gradient of intertidal sites in New England estuaries encompassing a broad range of watershed urbanization and sediment metal concentrations. All of the metals are prevalent at anthropogenically contaminated estuarine sites, but the metals differ in their routes of uptake and modes of toxicity (Chapman et al. 2003). We measured metal concentrations in lower trophic level organisms, as these taxa link metals in sediments and the water column to pelagic fisheries that are important vectors of human exposure to metal contaminants. Our goal was to compare the factors controlling bioaccumulation of different metals in estuarine organisms. Specifically, we address three research questions: 1) Does the relationship of sediment concentration to biotic concentration differ between metals? 2) Does total organic carbon in sediments influence bioavailability of different metals similarly?; and 3) Do pathways of uptake in estuarine fauna differ across metals?

## Methods

We studied six sites that encompassed a broad range of watershed land use and urbanization. Four sites were located in the Gulf of Maine, and two sites in Narragansett Bay, RI. The sites included: 1) Adams Point in southeast Great Bay NH, where direct industrial inputs are low (Jones 2000) and land use is relatively forested; 2) the Portsmouth Harbor Region of Great Bay, which is highly industrialized and adjacent to numerous contaminated sites; 3) the Webhannet Estuary in Wells ME, which is undeveloped except for some residential areas (mostly seasonal); 4) Somes Sound on Mount Desert Island Maine adjacent to Acadia National Park, which is pristine but receives relatively high atmospheric inputs of Hg (Kahl et al. 2007)*;* 5) Greenwich Cove, a residentially developed site with a large boat marina on the eastern side of Narragansett Bay and 6) Providence River Estuary a highly industrialized site and shipping channel at the head of Narragansett Bay. At all sites, we sampled intertidal areas with similar patterns of tidal inundation.

*Sediment samples:* Sediment samples were collected in summer 2006 at each site using a 6 cm diameter coring tube. The top 2 cm of nine sediment cores were composited into a single sample. Aliquots of the composite were freeze-dried, homogenized, and analyzed for total Hg, MeHg, Se, Cd, As, and Pb and total organic carbon as described below.

*TOC analysis of sediments*. TOC was analyzed using thermal partitioning at 550°C (EPA 440.0). Total (organic + inorganic) C was determined at 1350°C combustion temperature. A second sample was combusted at 550°C to burn off organic C but leaving inorganic C. The residue from that procedure was put through the combustion analyzer at 1350°C to measure inorganic C. Organic C was calculated as the difference between the two determinations.

*Biotic samples*. We measured inorganic Hg, MeHg, Se, As, Cd, and Pb in benthic and pelagic fish and invertebrates and compared animal metal concentrations to *in situ* sediment concentrations and to animal stable isotope signatures ($^{13}C$ and $^{15}N$). At each site, we collected animals from a range of feeding groups and trophic levels, including filter feeders (blue mussels and ribbed mussels; *Mytilus edulis and Geukensia demissa*), detritivores (amphipods), and omnivorous fish and invertebrates (killifish, *Fundulus heteroclitus*;, green crabs, *Carcinus maenas*; and shrimp, *Paleomonetes pugio, Crangon septemspinosus*).

Invertebrates were sampled using plastic trowels, minnow traps, D nets, pitfall traps, collected by hand, or collected by sieving sediments through a 0.5 mm nylon coated mesh. All collected invertebrates were returned to the lab where they were sorted the same day and identified to the lowest practical taxon. All samples were handled with trace metal clean technique and stored in either acid cleaned plastic bags or acid cleaned teflon vials (for smaller organisms) and frozen. Later, frozen samples were thawed, rinsed with ultra-clean water, weighed, and freeze-dried and homogenized prior to metal analysis. Mussels were removed from their shells prior to freeze-drying. Amphipods collected at each site were pooled in order to obtain at least 3 samples with enough dry weight for metal analysis.

Fish sampling was conducted at mid to high tide levels using fish seines, fyke nets, and minnow traps. Fish were handled and euthanized with protocols approved by the Dartmouth College IACUC (Institutional Animal Care and Use Committee). Fish total lengths and wet weights were measured. Sizes of individuals for each fish and invertebrate species were standardized for metal

tissue samples to reduce the influence of size on feeding habits and trophic position. Fish were frozen in acid rinsed plastic bags for storage and processed in the lab according to Chen et al. (Chen et al. 2009). Individuals processed for metals were selected to keep the mean size as similar as possible across sites to reduce the potential for confounding effects due to size-related changes in feeding habits or trophic position.

*Stable Isotope Analysis*. Whole fish tissue, whole invertebrates, and mussels without shells all sampled in 2006 were analyzed for stable isotopes at the Colorado Plateau Stable Isotope Laboratory. Animal samples were freeze-dried, ground and homogenized. Isotopic signatures ($^{13}C/^{12}C$, $^{15}N/^{14}N$) were measured for each species. $^{13}C$ was used to identify food sources (Peterson and Fry 1987) such as benthic vs. pelagic production (Stribling and Cornwell 1997) or marsh plants versus phytoplankton (Sullivan and Moncreiff 1990). $^{15}N$ was used to identify the relative trophic levels of the organisms within each site (Peterson and Fry 1987).

*Metal analysis of biotic and sediment samples*. All sample metal analyses (Hg, MeHg, Se, As, Cd, Pb,) were conducted by the Dartmouth Trace Element Core Facility using a magnetic sector inductively coupled plasma-mass spectrometer (ICP-MS ELEMENT2, Thermo-Finnigan, Waltham, MA). Biotic samples were analyzed for Hg speciation using isotope dilution gas chromatography-ICPMS. Samples were freeze-dried and homogenized, spiked with an appropriate amount of enriched inorganic $^{199}Hg$ (HgI) and enriched methyl$^{201}Hg$ (MeHg) and then extracted in 2-3 ml of KOH/methanol (25% w/v). One of two methods for Hg speciation was employed depending on the expected level of Hg in the original sample, a function of the initial available sample mass. For samples <20 mg, the methodology involved purging with inert

gas and trapping on a Tenax trap which was thermally desorbed and Hg species were quantified by isotope dilution GC-ICP-MS using a high sensitivity Element2 ICP-MS in low resolution mode. For samples >20 mg, samples were analyzed according to Lorenzo et al. (Perna et al. 2005). The latter methodology is less time-consuming than the purge and trap method but has higher detection limits and is only suitable for larger initial sample masses. Quality control was conducted through the analysis of two SRM's: NIST 2976, mussel tissue with MeHg certified at 0.0278 ± 1.1 $\mu$g $g^{-1}$ and CRC (Ottawa, Canada) DORM-2, dogfish muscle, MeHg concentration of 4.47 ± 0.32 $\mu$g $g^{-1}$. Average recovery for MeHg in DORM-2 was 108% (n=13, r.s.d. = 3.4%) and for NIST 2976 average recovery was 114% (n=12, r.s.d. = 10%). Method detection limits for MeHg analysis by isooctane extraction and capillary GC-ICP-MS (Agilent 7500c, Palo Alto, CA) are 5 ng $g^{-1}$ assuming an initial sample mass of 200 mg. For the purge and trap GC-ICP-MS (Element 2, Thermo-Fisher, Bremen, Germany) method detection limits are 0.2 ng $g^{-1}$ based on an initial sample weight of 25 mg.

Tissue and sediment samples for total Hg, As, Se, Cd, and Pb were acid digested with $HNO_3$ using a MARSxpress microwave digestion unit (CEM, Mathews, NC). Approximately 100 mg of sample was weighed into a Teflon digestion vessel and 2 ml of Optima $HNO_3$ was added. The vessel was heated to 180 °C with a 10 minute ramp and 10 minute hold. After digestion the sample was brought up to 25 ml volume with deionized water. Metals were analyzed by inductively coupled plasma mass spectrometry (ICP-MS, 7500cx, Agilent, Santa Clara, CA) using both collision cell and normal mode following the EPA 6020 protocol. . The digestion quality control included blank, duplicates and certified reference materials (SRM's: NIST SRM 2976? mussel tissue n=3, and TORT, NRC-CNRC Canada). Average metal recovery

rates for mussel and TORT respectively were: THg 114.7+12.7, 99.6%; Cd 105+2.5, 110%; Pb 110.8+6.0, 129%; As 118.5+9.0, 106%; Se 114.6+13.6, 108.6%). Detection limits based on a 40 mg sample were: THg 0.015 mg/kg: Cd 0.158 mg/kg, Pb 0.016 mg/kg; As 0.128 mg/kg; Se 0.345 mg/kg.

*Data Analysis*. Because the same mussel, shrimp, and amphipod species were not collected at all sites, biotic data was pooled into five general taxonomic groups for data analysis: mussels, amphipods, *Fundulus,* crabs, and shrimp. The ratio of the maximum to minimum metal concentrations in sediments to each taxonomic group was calculated for each metal to compare the variation within abiotic and biotic compartments across sites. Biota-Sediment Concentration Factors (BSCFs) were calculated for each species group or metal per site as (BSCF = metal concentration in organism ($\mu$g $g^{-1}$ dry wt) / metal concentration in sediment ($\mu$g $g^{-1}$ dry wt)).

We used general linear models (analysis of covariance) to evaluate the relationship between sediment characteristics at each site and element concentrations in organisms. The response variable was the mean $\log_{10}$-transformed element concentration for each species at each site, with separate analyses for each element. We accounted for variation in metal concentrations among taxonomic groups by including taxa as a nominal term in all models. We considered sediment element concentration, TOC-normalized sediment element concentration, and sediment TOC as predictors variables, with a single continuous predictor in each model. Sediments were characterized at the site level, not independently for each taxonomic group at each site, so we conservatively took the number of sites as the degrees of freedom for the continuous predictors.

We used general linear models (GLM) to evaluate the relationship between element concentrations in organisms and two food web variables, trophic level (as indexed by $^{15}N$) and pelagic feeding (as indexed by $^{13}C$). The response variable was the mean $\log_{10}$-transformed element concentration for each species at each site, with a separate analysis for each element. We accounted for site-to-site variation in metal concentrations and isotopic baselines by including site as a nominal term in the models. This approach assumes that, within each site, $^{15}N$ is linearly related to trophic position (Peterson and Fry 1987) and $^{13}C$ is linearly related to the relative proportion of pelagic resources in the diet (Stribling and Cornwell 1997).

**Results**

Sediment metal concentrations ranged widely across sites (Table 1). Across all metals, concentrations in sediments increased with the %TOC (Fig. 1a). The maximum:minimum ratio for sediments across sites was much greater than variation in biotic concentrations for Hg, MeHg, and Se, but variations in sediment and biotic concentrations were similar for Cd, As, and Pb (Table 2). Table 3 shows the results of the ANCOVA testing the relationship between response variables (metal concentrations in biota, BSCF) and predictor variables (sediment metal concentrations, TOC, TOC-normalized sediment metal concentrations). Sediment concentrations were marginally predictive of biotic concentrations for only Hg, both as total bulk sediment concentrations (Table 3, $P = 0.079$) or TOC-normalized concentrations (Table 3, $P = 0.018$). Other metals in biota were not predicted by sediment metal concentrations or TOC.

Trace metal concentrations in organisms were significantly different across sites and species, but there was an interaction between site and species such that no site had elevated trace

metal concentrations for all species (Table 4). Unlike the other metals, sites did not differ significantly in terms of their Se concentrations in biota.

Ecological measures ($^{13}C$ and $^{15}N$, Table S2, Supplementary Data) were predictive of biotic metal concentrations for Hg and MeHg but not for the other metals (Table 5). MeHg and Hg concentrations were highest in organisms that were more depleted in $^{13}C$ indicating that pelagic food sources resulted in higher metal bioaccumulation than benthic food sources. Mussels had the most pelagic signature, possibly accounting for their consistently high MeHg concentrations. Higher trophic level organisms, as revealed by $^{15}N$ enrichment, also had higher MeHg concentrations and higher percent of total Hg as MeHg. *Fundulus* had the highest trophic level of the five taxonomic groups.

$Log_{10}$ BSCFs, reflecting metal bioaccumulation for each animal relative to sediment concentrations at each site, ranged from -0.60 to 1.82 (As), -1.19 to 1.09 (Cd), -0.91 to 0.86 (Hg), 0.87 to 3.05 (MeHg), -2.464 to -0.33 (Pb) , and -0.18 to 1.26 (Se) (Fig. 1b, mean values presented). $Log_{10}$ BSCF values greater than zero indicated that organisms concentrated metals to levels greater than the sediment from which they came. All the $log_{10}$ BSCFs for MeHg were >0 and all were <0 for Pb, but both negative and positive for the other metals. In the case of Se, only one species type at one site had a negative $Log_{10}$ BSCF (amphipods in Portsmouth, Great Bay NH). Across sites, BSCFs were generally more positive for the three less urbanized and contaminated sites (Wells ME, Somes Sound ME, and Greenwich RI) than more urbanized and contaminated sites (Adams Point and Portsmouth in Great Bay NH, Bold Point in Providence RI). For all metals, BSCFs were inversely related to sediment TOC (Figure 1b, Table 3).

**Discussion**

Bioavailability of contaminants is a key concept in the evaluation of ecological risk in contaminated sites and the environmental fate of metals in marine ecosystems. Sediment and porewater metal concentrations are considered to be important routes of exposure in coastal food webs (Luoma & Rainbow 2008). In this study, variation in sediment vs. biotic concentrations differed greatly across metals. However, organic carbon was positively related to concentrations of all metals across sites while bioavailability measured as BSCF was greatest in the least contaminated sites, suggesting that metal binding with organic carbon plays a role in reducing metal transfer from contaminated sediments to the food web. While metal inputs to coastal sediments are an important source to coastal food webs (Luoma and Rainbow 2008), the loading of carbon in coastal systems greatly mediates bioavailability of metals and decreases bioaccumulation of metals particularly in human impacted sites (Amiard et al. 2007; Chen et al. 2009; Baumann and Fisher 2011).

In this study, Hg, MeHg, and Se concentrations in sediments vary across sites to a much greater degree than concentrations in biota. These metals are taken up predominantly through trophic transfer from food and this may reduce the variability in ratios of metal concentrations in tissues to sediments (Fisher and Reinfelder 1995; Harris and Bodaly 1998; Mason et al. 2000; Chen et al. 2008). For Cd, As, and Pb, sediment variation and biotic variation are similar. In fish, Cd is known to be taken up from water via the gills (Lee and Lee 2005) and especially from food (Mathews and Fisher 2008) whereas Pb uptake in bivalves is via ingestion of particulates (Metian et al. 2009). MeHg and Se BSCFs for all taxonomic groups are greater than 1.0

indicating that biotic concentrations are all higher than sediment concentrations. This may be due to higher assimilation efficiencies and/or greater retention of MeHg and Se for a variety of taxonomic groups. This contrasts with low assimilation efficiencies of Pb which bioaccumulates in organisms to much lower levels than concentrations in sediments (Sotos-Jiménez et al. 2011).

For all metals (Hg, MeHg, As, Cd, Se, Pb), concentration in sediments is highly correlated with organic carbon as is true for other datasets (USEPA EMAP-NCA). Moreover, the BSCFs for these metals are negatively related to the organic carbon concentrations in sediments suggesting a role of organic carbon related factors in reducing the bioavailability of metals. This relationship is consistent with some but not all earlier studies (Cantwell and Burgess 2001; Desrosiers et al. 2008). Although the relationship of BSCF is due in part to the strong positive relationship of TOC to metal concentration in sediments, carbon normalized sediment concentrations are related to biotic metal concentrations for only Hg indicating that sediment Hg concentration is a significant predictor for Hg bioaccumulation. Sediment metal concentrations for the other metals do not predict bioaccumulation.

In our study, measurements of stable isotope signatures of food web organisms showed relationships between food sources and metal bioaccumulation for only Hg and MeHg, as seen previously (Chen et al. 2009). In all cases, organisms deriving their food from pelagic sources (more depleted in $^{13}C$) had higher Hg and MeHg concentrations than those feeding on less depleted sources. This is similar to results of earlier studies in estuaries and in freshwater systems (Power et al. 2002; Chen et al. 2009). Moreover, organisms with more enriched delta $^{15}N$ also had higher MeHg concentrations and percent of total Hg as MeHg. This has been shown for other food webs in marine and freshwater ecosystems (Campbell et al. 2005; Hammerschmidt

and Fitzgerald 2006; Driscoll et al. 2007; Chen et al. 2009). Although not significant, there was also a trend of decreasing Pb concentration with increasing trophic level that has been shown in other freshwater studies as well (Chen and Folt 2000; Chen et al. 2000). This is likely a function of the fact that Pb shows low assimilation in animals (Sotos-Jiménez et al. 2011).

In conclusion, higher organic carbon loading in more human impacted estuarine sites reduces the bioavailability of Hg to a greater extent than in more pristine systems with lower metal and carbon loading. Concentrations of other metals in biota are not correlated with metal concentrations in sediment and as a result, higher metal concentrations in sediments do not result in higher concentrations of metals in benthic and pelagic organisms across these systems. Across all sites, organisms consistently bioaccumulate MeHg, and Se to higher concentrations than in sediment, whereas for Pb, concentrations in biota are always lower than sediment concentrations. Finally, Hg and MeHg are unique among the metals studied here in that food web variables predict bioaccumulation: stable isotope signatures of carbon indicate that bioaccumulation is greater in organisms deriving their food from pelagic as opposed to benthic food. This result suggests that Hg and MeHg flux from sediments to the water column in addition to bulk concentrations in sediments may be important in determining their bioavailability to estuarine food webs.

**Acknowledgments**

We are grateful to Brandon Mayes for conducting fieldwork and to Amy Higgins for gathering research materials and creating field site maps. We also thank Brian Jackson, Vivien Taylor, and Arthur Baker of the Dartmouth Trace Element Analysis Core for metal analysis of all our samples. This research was funded by the U.S. Department of Defense Strategic Environmental Research and Development Program (SERDP) Project ER-1503 and by National Institutes of Health Grant P42 ESO7373 from the National Institute of Environmental Health Sciences.

Table 1. Sediment attributes across six sites in the Gulf of Maine and Narragansett Bay.

| Site | As ($\mu$g g$^{-1}$) | Cd ($\mu$g g$^{-1}$) | Hg ($\mu$g g$^{-1}$) | MeHg ($\mu$g g$^{-1}$) | Pb ($\mu$g g$^{-1}$) | Se ($\mu$g g$^{-1}$) | TOC (%) |
|---|---|---|---|---|---|---|---|
| **Adams Pt. NH** | 11.2 | 0.4 | 0.2 | 0.002 | 37.3 | 1.1 | 2.1 |
| **Bold Point RI** | 8.8 | 0.5 | 0.08 | 0.0006 | 75.9 | 0.6 | 3.2 |
| **Greenwich RI** | 1.2 | 0.08 | 0.05 | 0.0001 | 17.4 | 0.3 | 0.8 |
| **MDI ME** | 3.6 | 0.2 | 0.04 | 0.0004 | 11.1 | 0.5 | 1.6 |
| **Portsmouth Harbor NH** | 12.1 | 0.6 | 0.3 | 0.003 | 79.6 | 1.3 | 2.8 |
| **Wells ME** | 2.2 | 0.1 | 0.03 | 0.0003 | 4.7 | 0.3 | 0.5 |

Table 2. Ratio of maximum to minimum values across all sites for sediment metal concentrations and biotic concentrations.

| | **As** | **Cd** | **Hg** | **MeHg** | **Pb** | **Se** |
|---|---|---|---|---|---|---|
| Sediment | 10.5 | 6.9 | 10 | 24.5 | 17 | 5 |
| Amphipod | 19.9 | 6.2 | 5.3 | 5.9 | 2.7 | 2.8 |
| Crab | 13.4 | 9.5 | 4 | 3.2 | 36.2 | 3.4 |
| *Fundulus* | 2 | 30.7 | 3 | 4.5 | 7.4 | 3 |
| Mussel | 3.8 | 14.7 | 6.4 | 3.7 | 5.1 | 1.8 |
| Shrimp | 3.7 | 5.7 | 3 | 4.2 | 11.4 | 4 |

Table 3. Summary of ANCOVA analyses for relationships between metals in biota, BSCF's, sediment concentrations, TOC-normalized sediment concentrations, and TOC. Cases where the predictor effect was statistically significant are shaded grey.

| Response (metal in biota) | Predictor Variable | R2 | Full model P-value | Taxa P-value | Predictor slope | Slope SE | Predictor P-value |
|---|---|---|---|---|---|---|---|
| As | Sed. Conc | 72% | <0.001 | <0.001 | 0.07 | 0.06 | 0.25 |
| Cd | Sed. Conc | 60% | <0.001 | <0.001 | 0.16 | 0.22 | 0.49 |
| Hg | Sed. Conc | 56% | 0.001 | 0.001 | 0.24 | 0.12 | 0.08 |
| MeHg | Sed. Conc | 29% | 0.11 | 0.07 | 0.04 | 0.1 | 0.72 |
| Pb | Sed. Conc | 42% | 0.02 | 0.02 | 0.22 | 0.14 | 0.17 |
| Se | Sed. Conc | 55% | 0.001 | 0.001 | -0.07 | 0.13 | 0.62 |
| As | TOC-normalized Sed. Conc. | 72% | <0.001 | <0.001 | 0.11 | 0.12 | 0.36 |
| Cd | TOC- normalized Sed. Conc. | 61% | <0.001 | <0.001 | 0.69 | 0.59 | 0.28 |
| Hg | TOC- normalized Sed. Conc. | 64% | <0.001 | <0.001 | 0.52 | 0.16 | 0.02 |
| MeHg | TOC- normalized Sed. Conc. | 30% | 0.11 | 0.07 | 0.07 | 0.14 | 0.64 |
| Pb | TOC- normalized Sed. Conc. | 37% | 0.04 | 0.02 | 0.2 | 0.3 | 0.54 |
| Se | TOC- normalized Sed. Conc. | 55% | 0.001 | 0.001 | -0.04 | 0.18 | 0.84 |
| AsBSCF | TOC | 71% | <0.001 | <0.001 | -0.3 | 0.05 | <0.001 |
| CdBSCF | TOC | 66% | <0.001 | <0.001 | -0.27 | 0.07 | 0.01 |
| HgBSCF | TOC | 77% | <0.001 | 0.001 | -0.28 | 0.04 | <0.001 |
| MeHgBSCF | TOC | 54% | 0.001 | 0.42 | -0.36 | 0.07 | 0.003 |
| PbBSCF | TOC | 52% | 0.002 | 0.06 | -0.3 | 0.08 | 0.01 |
| SeBSCF | TOC | 54% | 0.002 | 0.04 | -0.2 | 0.05 | 0.01 |

Table 4. Element concentrations (ng $g^{-1}$) for all sites and taxa, reported as mean (standard deviation). N=3 for each species type from each site. Standard deviations reported as 0 are less than 0.01.

| Site | Taxa | As | Cd | Hg | MeHg | Pb | Se |
|---|---|---|---|---|---|---|---|
| Adams Pt. NH | Amphipod | 2.91 (0.69) | 0.04 (0.01) | 0.08 (0.02) | 0.08 (0.01) | 0.81 (0.25) | 1.39 (0.16) |
| Adams Pt. NH | Crab | 5.61 (1.45) | 0.28 (0.13) | 0.12 (0.05) | 0.08 (0.04) | 4.24 (3.01) | 1.79 (0.5) |
| Adams Pt. NH | Fundulus | 2.92 (0.18) | 0.03 (0.01) | 0.16 (0.04) | 0.14 (0.04) | 0.55 (0.34) | 1.93 (0.04) |
| Adams Pt. NH | Mussel | 7.46 (0.72) | 0.85 (0.46) | 0.43 (0.15) | 0.17 (0.04) | 1.07 (0.28) | 2.41 (0.43) |
| Adams Pt. NH | Shrimp | 5.65 (1.02) | 0.2 (0.06) | 0.11 (0.02) | 0.06 (0.02) | 0.65 (0.41) | 1.88 (0.22) |
| Bold Point, RI | Amphipod | 10.56 (0.05) | 0.1 (0.01) | 0.02 (0) | 0.02 (0) | 1.62 (0.39) | 1.09 (0.19) |
| Bold Point, RI | Crab | 7.04 (2.54) | 0.05 (0.01) | 0.04 (0.02) | 0.03 (0.01) | 0.26 (0.16) | 3.63 (1.05) |
| Bold Point, RI | Fundulus | 2.23 (1.05) | 0.43 (1.12) | 0.08 (0.02) | 0.07 (0.02) | 0.31 (0.13) | 1.5 (0.64) |
| Bold Point, RI | Mussel | 9.68 (5.51) | 3.67 (1.31) | 0.08 (0.01) | 0.05 (0.01) | 2.18 (0.65) | 3.58 (0.98) |
| Bold Point, RI | Shrimp | 7.29 (0.81) | 0.37 (0.36) | 0.06 (0.01) | 0.05 (0.02) | 1.15 (0.79) | 1.25 (0.04) |
| Greenwich, RI | Amphipod | 3.76 (0.39) | 0.2 (0.01) | 0.11 (0) | 0.1 (0) | 1.76 (0.32) | 1.96 (0.24) |
| Greenwich, RI | Crab | 7.54 (3.8) | 0.14 (0.09) | 0.16 (0.07) | 0.07 (0.03) | 0.12 (0.06) | 4.64 (1.94) |
| Greenwich, RI | Fundulus | 1.79 (0.6) | 0.01 (0.02) | 0.09 (0.04) | 0.08 (0.03) | 0.26 (0.12) | 1.03 (0.31) |
| Greenwich, RI | Mussel | 7.94 (3.78) | 0.25 (0.06) | 0.2 (0.02) | 0.14 (0.02) | 0.84 (0.34) | 4.37 (2.44) |
| Greenwich, RI | Shrimp | 3.16 (0.94) | 0.16 (0) | 0.13 (0.07) | 0.13 (0.08) | 0.24 (0.1) | 1.07 (1.25) |

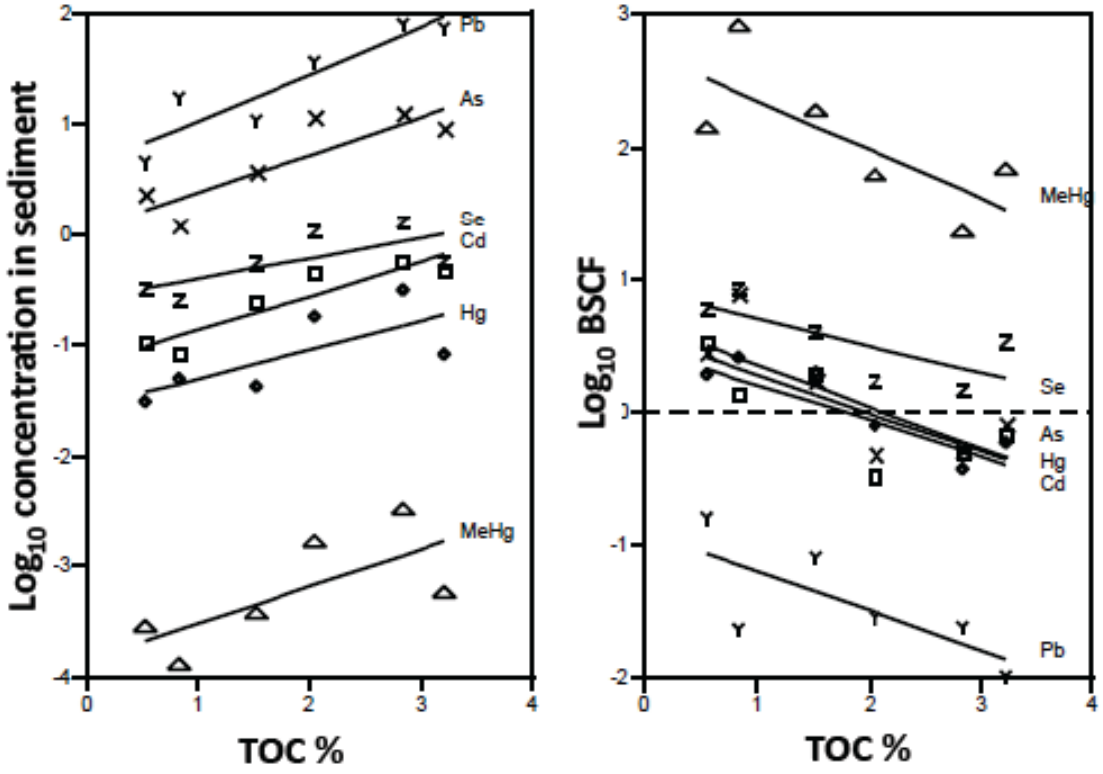
Table 5. Summary of General Linear Model (GLM) analyses for relationships between metals in biota and stable isotope signatures ($^{13}C$ and $^{15}N$) across sites.

| Response (metal in biota) | $R^2$ | Model P-value | Site P-value | $^{13}C$ slope | $^{13}C$ SE | $^{13}C$ P-value | $^{15}N$ slope | $^{15}N$ SE | $^{15}N$ P-value |
|---|---|---|---|---|---|---|---|---|---|
| As | 31% | 0.24 | 0.14 | 0.08 | 0.04 | 0.09 | -0.11 | 0.05 | 0.03 |
| Cd | 35% | 0.16 | 0.37 | -0.08 | 0.06 | 0.18 | -0.07 | 0.06 | 0.29 |
| Hg | 58% | <0.001 | 0.03 | -0.09 | 0.02 | <0.001 | 0.03 | 0.03 | 0.24 |
| MeHg | 66% | <0.001 | <0.001 | -0.08 | 0.02 | <0.001 | 0.06 | 0.02 | 0.02 |
| Pb | 43% | 0.05 | 0.15 | -0.04 | 0.04 | 0.32 | -0.07 | 0.04 | 0.13 |
| Se | 15% | 0.77 | 0.99 | -0.04 | 0.03 | 0.14 | 0 | 0.03 | 0.88 |
| %MeHg | 41% | 0.07 | 0.41 | -0.08 | 1.46 | 0.95 | 4.39 | 1.66 | 0.01 |

**Figure Legend**

Figure 1. Relationship of TOC to: a) metal concentrations in sediments ($log_{10}$ concentration) and b) Mean Benthic Sediment Concentration Factor ($log_{10}$ BSCF), averaged across all species at each site. Each metal is represented by a different symbol: triangle=MeHg, Z=Se, X=As, cross=Hg, square=Cd, and Y=Pb.

Figure 1


Log10 concentration in sediment
TOC %
Pb
As
Se
Cd
Hg
MeHg
Log10 BSCF
TOC %
MeHg
Se
As
Hg
Cd
Pb

**References**


Amiard, J. C., A. Geffard, C. Amiard-Triquet and C. Crouzet. 2007. Relationship between the lability of sediment-bound metals (Cd, Cu, Zn) and their bioaccumulation in benthic invertebrates. *Estuarine Coastal and Shelf Science* 72(3): 511-521.

Ankley, G. T. 1996. Evaluation of metal/acid-volatile sulfide relationships in the prediction of metal bioaccumulation by benthic macroinvertebrates. *Environmental Toxicology and Chemistry* 15(12): 2138-2146.

ATSDR. (2007). "CERCLA Priority List of Hazardous Substances." from http://www.atsdr.cdc.gov/cercla/07list.html.

Baumann, Z. and N. S. Fisher. 2011. Relating the sediment phase speciation of arsenic, cadmium, and chromium with their bioavailability for the deposit-feeding polychaete *Nereis succinea*. *Environmental Toxicology and Chemistry* 30: 747-756.

Borgmann, U. and W. P. Norwood. 2002. Metal bioavailability and toxicity through a sediment core. *Environmental Pollution* 116(1): 159-168.

Borgmann, U., W. P. Norwood and D. G. Dixon. 2004. Re-evaluation of metal bioaccumulation and chronic toxicity in Hyalella azteca using saturation curves and the biotic ligand model. *Environmental Pollution* 131(3): 469-484.

Campbell, L. M., R. J. Norstrom, K. A. Hobson, D. C. G. Muir, S. Backus and A. T. Fisk. 2005. Mercury and other trace elements in a pelagic Arctic marine food web (Northwater Polynya, Baffin Bay). *Science of the Total Environment* 351: 247-263.

Cantwell, M. G. and R. M. Burgess. 2001. Metal-colloid partitioning in artificial interstitial waters of marine sediments: Influences of salinity, pH, and colloidal organic carbon concentration. *Environmental Toxicology and Chemistry* 20(11): 2420-2427.

Chapman, P. M., F. Y. Wang, C. R. Janssen, R. R. Goulet and C. N. Kamunde. 2003. Conducting ecological risk assessments of inorganic metals and metalloids: Current status. *Human and Ecological Risk Assessment* 9(4): 641-697.

Chen, C., A. Amirbahman, N. Fisher, G. Harding, C. Lamborg, D. Nacci and D. Taylor. 2008. Methylmercury in Marine Ecosystems: Spatial Patterns and Processes of Production, Bioaccumulation, and Biomagnification. *EcoHealth* 5: 399-408.

Chen, C. Y., M. Dionne, B. M. Mayes, D. M. Ward, S. Sturup and B. P. Jackson. 2009. Mercury Bioavailability and Bioaccumulation in Estuarine Food Webs in the Gulf of Maine. *Environmental Science & Technology* 43(6): 1804-1810.

Chen, C. Y. and C. L. Folt. 2000. Bioaccumulation and diminution of arsenic and lead in a freshwater food web. *Environmental Science & Technology* 34(18): 3878-3884.

Chen, C. Y., C. L. Folt, R. S. Stemberger, J. D. Blum, B. Klaue and P. C. Pickhardt. 2000. Accumulation of heavy metals in food web components across a gradient of lakes. *Limnol. Oceanogr* 45: 1525-1536.

Desrosiers, M., C. Gagnon, S. Masson, L. Martel and M. P. Babut. 2008. Relationships among total recoverable and reactive metals and metalloid in St. Lawrence River sediment: Bioaccumulation by chironomids and implications for ecological risk assessment. *Science of the Total Environment* 389(1): 101-114.

Driscoll, C. T., Y. J. Han, C. Y. Chen, D. C. Evers, K. F. Lambert, T. M. Holsen, N. C. Kamman and R. K. Munson. 2007. Mercury contamination in forest and freshwater ecosystems in the Northeastern United States. *Bioscience* 57(1): 17-28.

Fisher, N. S., V. T. Breslin and M. Levandowsky. 1995. Accumulation of Silver and Lead in Estuarine Microzooplankton. *Marine Ecology-Progress Series* 116(1-3): 207-215.

Fisher, N. S. and J. R. Reinfelder. 1995. The Trophic Transfer of Metals in Marine Systems. *Metal Speciation and Bioavailability in Aquatic Systems*. ed. A. Tessier and D. R. Turner, John Wiley & Sons Ltd**:** 363-406.

Hammerschmidt, C. R. and W. F. Fitzgerald. 2006. Bioaccumulation and trophic transfer of methylmercury in Long Island Sound. *Archives of Environmental Contamination and Toxicology* 51(3): 416-424.

Hammerschmidt, C. R., W. F. Fitzgerald, C. H. Lamborg, P. H. Balcom and P. T. Visscher. 2004. Biogeochemistry of methylmercury in sediments of Long Island Sound. *Marine Chemistry* 90(1-4): 31-52.

Harris, R. C. and R. A. Bodaly. 1998. Temperature, growth and dietary effects on fish mercury dynamics in two Ontario lakes. *Biogeochemistry* 40(2-3): 175-187.

Jones, S., H. (2000). A technical characterization of estuarine and coastal New Hampshire., New Hampshire Estuaries Project**:** 274.

Kahl, J. S., S. J. Nelson, I. Fernandez, T. Haines, S. Norton, G. B. Wiersma, G. Jacobson, A. Amirbahman, K. Johnson, M. Schauffler, L. Rustad, K. Tonnessen, R. Lent, M. Bank, J. Elvir, J. Eckhoff, H. Caron, P. Ruck, J. Parker, J. Campbell, D. Manski, R. Breen, K. Sheehan and A. Grygo. 2007. Watershed nitrogen and mercury geochemical fluxes integrate landscape factors in long-term research watersheds at Acadia National Park, Maine, USA. *Environmental Monitoring and Assessment* 126(1-3): 9-25.

Lee, J. S. and J. H. Lee. 2005. Influence of acid volatile sulfides and simultaneously extracted metals on the bioavailability and toxicity of a mixture of sediment-associated Cd, Ni, and Zn to polychaetes Neanthes arenaceodentata. *Science of the Total Environment* 338(3): 229-241.

Luoma, S. N. and P. S. Rainbow. 2008. *Metal contamination in aquatic environments: science and lateral management*. Cambridge: Cambridge University Press.

Marsden, I. D. and P. S. Rainbow. 2004. Does the accumulation of trace metals in crustaceans affect their ecology - the amphipod example? *Journal of Experimental Marine Biology and Ecology* 300(1-2): 373-408.

Mason, R. 2002. The bioaccumulation of mercury, methylmercury and other toxic elements into pelagic and benthic organisms. *Coastal and estuarine risk assessment*. ed. N. MC, R. MH and H. RC, CRC/Lewis Publishers**:** 127–149.

Mason, R. P., J. M. Laporte and S. Andres. 2000. Factors controlling the bioaccumulation of mercury, methylmercury, arsenic, selenium, and cadmium by freshwater invertebrates and fish. *Archives of Environmental Contamination and Toxicology* 38(3): 283-297.

Mathews, T. and N. S. Fisher. 2008. Evaluating the trophic transfer of cadmium, polonium, and methylmercury in an estuarine food chain. *Environmental Toxicology and Chemistry* 27(5): 1093-1101.

Mathews, T. and N. S. Fisher. 2008. Trophic transfer of seven trace metals in a four-step marine food chain. *Marine Ecology-Progress Series* 367: 23-33.

Metian, M., M. Warnau, F. Oberhansli and P. Bustamante. 2009. Delineation of Pb contamination pathways in two Pectinidae: The variegated scallop Chlamys varia and the king scallop Pecten maximus. *Science of the Total Environment* 407(11): 3503-3509.

Newman, M. C. 1998. *Fundamentals of Ecotoxicology*. Chelsea, MI: Ann Arbor Press.

NRC (2003). Bioavailability of Contaminants in Soils and Sediments: Processes, Tools, and Applications, National Academies Press, Washington, D.C.

O'Connor, T. P. 1996. Coastal sediment contamination in the Northeast shelf large marine ecosystem. *The Northeast shelf ecosystem: assessment, sustainability, and management*. ed. K. Sherman, N. A. Jaworski and T. J. Smayda. Cambridge, MA, Blackwell Science**:** 239-257.

Perna, L., A. LaCroix-Fralish and S. Sturup. 2005. Determination of inorganic mercury and methylmercury in zooplankton and fish samples by speciated isotopic dilution GC-ICP-MS after alkaline digestion. *Journal of Analytical Atomic Spectrometry* 20(3): 236-238.

Peterson, B. J. and B. Fry. 1987. Stable Isotopes in Ecosystem Studies. *Annual Review of Ecology and Systematics* 18: 293-320.

Power, M., G. M. Klein, K. Guiguer and M. K. H. Kwan. 2002. Mercury accumulation in the fish community of a sub-Arctic lake in relation to trophic position and carbon sources. *Journal of Applied Ecology* 39(5): 819-830.

Sotos-Jiménez, M. F., C. Arellano-Fiore, R. Rocha-Velarde, M. E. Jara-Marini, J. Ruelas-Inzunza and F. Páez-Osuna. 2011. Trophic transfer of lead through a model marine four-level food chain: Tetraselmis suecica, Artemia franscscana, Litopenaeus vannamei, and Haemulon scudderi. *Archives of Environnmental Contamination and Toxicology* 61: 280-291.

Stribling, J. M. and J. C. Cornwell. 1997. Identification of important primary producers in a Chesapeake Bay tidal creek system using stable isotopes of carbon and sulfur. *Estuaries* 20(1): 77-85.

Sullivan, M. J. and C. A. Moncreiff. 1990. Edaphic Algae Are an Important Component of Salt-Marsh Food-Webs - Evidence from Multiple Stable Isotope Analyses. *Marine Ecology-Progress Series* 62(1-2): 149-159.