RELATIONSHIPS BETWEEN POLLUTANT-INDUCED DIGESTIVE TOXICITY AND THE ASSIMILATION AND SUBCELLULAR PARTITIONING OF ELEMENTS BY GRASS SHRIMP *PALAEMONETES PUGIO*

by

DAVID R. SEEBAUGH

A dissertation submitted to the Graduate Faculty in Biology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

2010

This manuscript has been read and accepted for the
Graduate Faculty in Biology in satisfaction of the
dissertation requirement for the degree of Doctor of Philosophy

Dr. William G. Wallace, College of Staten Island

Date — Chair of Examining Committee

Dr. Laurel A. Eckhardt, Hunter College

Date — Executive Officer

Dr. William J. L'Amoreaux, College of Staten Island

Dr. Gillian M. Stewart, Queens College

Dr. Glenn R. Lopez, Stony Brook University

Dr. Judith S. Weis, Rutgers University
Supervisory Committee

THE CITY UNIVERSITY OF NEW YORK

Abstract

RELATIONSHIPS BETWEEN POLLUTANT-INDUCED DIGESTIVE TOXICITY AND THE ASSIMILATION AND SUBCELLULAR PARTITIONING OF ELEMENTS BY GRASS SHRIMP *PALAEMONETES PUGIO*

by

David R. Seebaugh

Advisor: Dr. William G. Wallace

Aquatic invertebrates inhabiting urbanized estuaries are typically exposed to pollutants through multiple pathways, including the diet. Biochemical and physical processes within invertebrate guts can be impacted by ingested pollutants, which may influence the assimilation of essential nutrients as well as pollutants. Pre-assimilatory digestive toxicity may result from pollutants circulating in gut fluid. Post-assimilatory toxicity could be due to incorporation of a pollutant into consumer tissues as a result of chronic exposure. This series of studies investigated the influence of chronic exposure to impacted field conditions or pre-exposure to dietary metal (Cd and Hg) in the laboratory on the assimilation of elements (organic carbon, Cd and Hg), subcellular partitioning of assimilated metal and digestive physiology (gut residence time [GRT], feces elimination rates [FER], gut pH and digestive protease activities) in the grass shrimp *Palaemonetes pugio*. Carbon and Cd assimilation and endpoints related to digestion were also assessed for naïve shrimp following ingestion of a pulse of Cd-contaminated food. Based on these studies, it appears that grass shrimp may be able to maintain carbon assimilation in the laboratory under different forms of pollutant-induced dietary stress. For field-collected shrimp, this phenomenon may be attributable to digestive plasticity (e.g., increased GRT to compensate for reduced digestive enzyme activities). Increased assimilation of Cd and

Hg was observed for shrimp collected along an impact gradient. Enhanced non-essential metal assimilation may have implications for accumulation and toxicity in impacted shrimp. In the case of Cd assimilation, a positive correlation with GRT and negative relationship with protease activities suggests that digestive plasticity may also influence assimilation of non-essential elements in the field. Variability in Cd assimilation by shrimp pre-exposed to dietary metal in the laboratory was not dose-dependent, which may be related to interactions between post-assimilatory impacts on gut physiology. Increased assimilation of Hg by Hg pre-exposed shrimp may have been related to a corresponding increase in gut pH (i.e., decreased concentrations of H^+ ions in circulating gut fluid).

ACKNOWLEDGEMENTS

The author thanks Dr. William G. Wallace for his generous patience and support during the design, implementation and interpretation of this research. All aspects of this work were substantially improved by advice and constructive criticism from dissertation supervisory committee members: Drs. William J. L'Amoreaux, Glenn R. Lopez, Gillian M. Stewart and Judith S. Weis.

This research was supported by Tibor T. Polgar Fellowships (2004 and 2007) from the Hudson River Foundation as well as a Science Fellowship and University Fellowships from the Graduate School and University Center of the City University of New York (CUNY). Funding was also provided through PSC-CUNY Research Awards 6427800, 6814500 and 6288800 to W.G. Wallace and CUNY Collaborative Incentive Research Grant 8020901 to G.M. Stewart and W.G. Wallace.

Access to radiation facilities was provided by the Department of Biology, College of Staten Island, CUNY and is greatly appreciated. Amphipods and grass shrimp were collected from Great Kills Harbor under permit from the Division of Natural Resources, National Park Service (Dr. George Frame). Shrimp were collected from Main Creek under permit from the New York City Department of Parks and Recreation (Dr. Ellen Pehek). Tuckerton, NJ seawater was provided by the Institute of Marine and Coastal Sciences, Rutgers University (Charlotte Fuller). Eileen Cullen, Nick Levintov, Allison Mass and Matt Perez assisted with collection and sorting of experimental animals. Amir Estephan participated in the subcellular fractionation of radiolabeled amphipods. Dr. Daisuke Goto provided access to sediment metal concentration and tissue burden data for polychaetes from the Staten Island study sites. D. Goto, Allison Mass and Caitlyn

Nichols assisted with stable metal analyses. Dr. Yalin Wang, Dr. Jimmie Fata, Jonathan Blaize and Charles Jenkins provided expert technical assistance with confocal microscopy. Access to NIR imaging equipment was generously provided by Dr. Probal Banerjee. The author also thanks Dr. Catherine McGlynn, Dr. John Waldman, William Nieder and several anonymous reviewers for valuable comments on manuscripts derived from research presented in this dissertation.

Completion of this research would not have been possible without the support of the students, faculty and administration of the Berkeley Carroll School, Brooklyn, NY.

This dissertation is dedicated to Eileen M. Cullen.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 2: Assimilation and subcellular partitioning of elements by grass shrimp collected along an impact gradient

CHAPTER 3: Assimilation, subcellular partitioning and accumulation of elements by grass shrimp pre-exposed to dietary metal

CHAPTER 1

General Introduction: Background and Study Objectives

General Background

Aquatic organisms inhabiting heavily industrialized estuaries such as the New York/New Jersey (NY/NJ) Harbor Estuary are typically exposed to suites of metal, organic and chemical pollutants through multiple pathways (i.e., dissolved, sediments and dietary sources) (Feng et al., 1998; Wang and Fisher, 1999; Steinberg et al., 2004). For metals (e.g., Cd, Cu, Hg, Pb and Zn), variability in total and bioavailable concentrations in sediments can vary considerably within an urban estuary, which may have implications for toxicity for resident populations as well as metal cycling (e.g., trophic transfer) in impacted communities (Berry et al., 1996; Wolfe et al., 1996; Ward, 2002; Nichols, 2004; Goto, 2009). Population differences in metal accumulation, detoxification (e.g., tissue metallothionein concentrations), sublethal toxicity (e.g., metabolism, prey capture and predator avoidance) and genetic resistance may be related to exposure to high metal loads in the field (Kraus and Kraus, 1986; Klerks and Levinton, 1989; Couillard et al., 1993; Khan and Weis, 1993; Paulson et al., 2003; Perez and Wallace, 2004; Manyin and Rowe, 2006). Chronic exposure can also impact bioavailability of metal in prey and assimilation by predators (Wallace and Lopez, 1997; Wallace et al., 1998; Rainbow et al., 2004a). In addition to understanding the effects of chronic pollutant exposure on organisms, it is equally important to consider impacts of exposure pathways independently, particularly since accumulation and sublethal toxicity (e.g., impacts on reproduction) can vary significantly depending upon the route of exposure (e.g., dissolved vs. diet) (Fisher et al., 1996; Hook and Fisher, 2001; Griscom et al., 2002; Rainbow et al., 2003; Shi et al., 2003; Campbell et al., 2005).

The assimilation of elements has been characterized for a variety of marine

invertebrates (e.g., bivalves, polychaetes and crustaceans) and fish (Reinfelder and Fisher 1991, 1994a, 1994b; Wang and Fisher, 1996; Wang et al., 1996; Baines et al., 2002; Nunez-Nogueira et al., 2006). Assimilation efficiencies (AE) may be influenced by many factors including diet composition, the subcellular partitioning of elements in food organisms, digestive physiology and exposure to contaminants (e.g., metals) (Reinfelder and Fisher, 1991; Wallace and Lopez, 1997; Rainbow et al., 2004b). Recent studies have demonstrated that metal AE can be impacted by previous or concurrent exposure to ingested metal (Shi et al., 2003; Rainbow et al., 2004b; Seebaugh et al., 2005). Few studies have attempted to relate variability in AE with metal-induced alterations in consumer digestive physiology (Selck et al., 1999). As suggested by Campbell et al. (2005), formulation of a unified theory of the processing, fate and toxicity of dietary metal for a taxonomic group requires comprehensive understanding of digestive processes in representative organisms. With baseline information, it may then be possible to assess impacts of metal exposure on digestive physiology as well as relationships between metal toxicity and the assimilation of essential as well as non-essential elements by consumers.

Ingested pollutants can elicit changes in digestive physiology that may be categorized according to whether or not a pollutant has been incorporated into tissues (i.e., assimilated) following absorption by the gut (Penry, 1998; Campbell et al., 2005; Wallace and Lopez, unpublished). Pre-assimilatory toxicity may be induced by pollutants circulating in gut fluid during a digestive cycle and include impacts on gut motility, enzyme activities or cellular mechanisms involved in absorption of the products of hydrolysis (De La Ruelle et al, 1992; Chen and Mayer, 1998; Chen et al., 2002; Campbell

et al., 2005; Wallace and Lopez, unpublished). These impacts could influence the assimilation of nutrients as well as ingested pollutants and would be related to availability of a pollutant in gut fluid, which is function of gut chemistry (e.g., pH and concentrations of pollutant-binding ligands) and not related to tissue burdens accumulated during previous exposures (Mayer et al., 1996, 1997, 2001; Wallace and Lopez, unpublished).

Post-assimilatory toxicity may result from incorporation of a pollutant into consumer tissues due to chronic exposure (e.g., impacted field conditions) (Penry, 1998; Wallace and Lopez, unpublished). Exposure may damage gut epithelial tissues and interfere with synthesis and discharge of digestive enzymes or absorption, transport and assimilation of nutrients and pollutants during subsequent digestive cycles (Wallace and Lopez, unpublished). Tissue damage may also impact energy reserves (e.g., glycogen and lipid stores) or nutrient circulation to other tissues via the blood or haemolymph (Icely and Nott, 1992). It may also be important to consider potential impacts of assimilated pollutants on mitotic activity and differentiation of embryonic cells from which enzyme-secreting and absorptive cells of the digestive epithelium are derived (Al-Mohanna et al., 1985). Impacts of pre- and post-assimilatory toxicity on predator digestive physiology may also be interactive (e.g., additive, synergistic or antagonistic), particularly under conditions where chronic exposure includes pollutant ingestion over successive digestive cycles (Wallace and Lopez, unpublished).

Cd and Hg are non-essential, class B metals (Cd is also considered a borderline metal) that can displace essential metal ions and impact functional characteristics of proteins (e.g., enzyme active sites) due to their high affinities for sulfydryl groups (Nieboer and Richardson, 1980; Mason and Jenkins, 1995). Exposure to either metal can

elicit toxicity at multiple levels of biological organization within estuarine communities. At the subcellular level, Cd exposure has been shown to impact gene expression for mitochondrial proteins and influence mitochondrial enzymes and bioenergetics in benthic fauna (Sokolova, et al., 2005; Lanning et al., 2006; Ivanina et al., 2008). Cd can also elicit oxidative stress, induce lysosomal membrane destabilization and may impact cellular integrity (Viarengo et al., 2000; Downs et al., 2001). Cd impacts at lower levels of organization (e.g., enzymes) may be manifested as impaired tissue function (e.g., egg production) (Hook and Fisher, 2002). At the organismal level, Cd exposure can influence oxygen consumption and whole-organism bioenergetics (Hutchenson et al., 1985; Lanning et al., 2006). Cd can also induce behavioral toxicity in benthic invertebrates that could impact individuals as well as populations (Wallace et al., 2000). Introduction of Cd at lower trophic levels may impact entire food webs, leading to community-wide consequences (Odum, 1985; Croteau et al., 2005).

Exposure to inorganic Hg can induce DNA damage, influence defenses against oxidative stress (e.g., glutathione) and impact organelle membrane stability in benthic invertebrate tissues (Regoli et al., 1997; Marchi et al., 2004; Tran et al., 2007). Hg exposure can elicit changes in organelle ultrastructure as well as tissue morphology that may be related to impacts on function or compensation for metal-induced cellular toxicity (Gregory et al., 1999; Yamuna et al., 2009). Reproductive success may be impacted by inorganic Hg through effects on tissues related to egg production, fertilization and embryonic development (Thain, 1984; Warnau et al., 1996; Hook and Fisher, 2002). Hg can also induce behavioral toxicity (e.g., impaired response to chemical stress or predator

avoidance) and may impact benthic community composition (Kraus and Kraus, 1986; Kádár et al., 2005; Goto and Wallace, 2010).

For invertebrates exposed to dietary metal, digestive toxicity at the cellular level may be related to the subcellular partitioning of absorbed metal within gut epithelial cells (Wallace et al., 2003; Cheung et al., 2006). For example, metal bound to metallothionein-like proteins (MTLP) or sequestered as insoluble metal-rich granules in gut tissues may be biologically-detoxified and would not be expected to interfere with cellular functions (e.g., absorption) (Hopkin and Nott, 1979; Wallace et al., 2003; Amiard et al., 2006). Conversely, ingested metal associated with metal-sensitive intracellular components (e.g., organelles and enzymes) may impact digestion (Wallace et al., 2003; Campbell et al., 2005).

Benthic invertebrates play a critical role in cycling of nutrients and organic matter and maintaining ecological efficiency within estuarine food webs through conversion of producer biomass, detritus and prey into dissolved organic matter, fecal material and invertebrate biomass consumed by organisms at multiple trophic levels (Nixon and Oviatt, 1973; Welsh, 1975; Haines and Montague, 1979; Sullivan and Moncreiff, 1990; Dittel et al., 2000; Quinones-Rivera and Fleeger, 2005). A potentially useful endpoint for evaluating sublethal toxicity in estuarine fauna may be impacts of exposure to chronic field conditions or dietary pollutants (e.g., non-essential metals) on the assimilation of organic carbon. Variability in carbon assimilation could potentially influence bioenergetics (energy allocation) within individuals and have implications for survival, growth and fitness that could impact population dynamics (Widdows et al., 1997; Vernberg and Piyatiratitivorakul, 1998; Reinsel et al., 2001; Forbes, 2005; Manyin and

Rowe, 2006). Changes in carbon assimilation by important nutrient-cycling species could, therefore, have community-wide consequences including impacts on community energetics (e.g., respiration and biomass), nutrient cycling as well as community structure/composition (Nixon and Oviatt, 1973; Odum, 1985; Sullivan and Moncreiff, 1990). Relatively few studies have investigated the influence of field exposure to contaminants on carbon assimilation by estuarine invertebrates (Widdows et al., 1997; Shuhong et al., 2005). The impacts of dietary exposure to specific pollutants (e.g., metals) on this important toxicological endpoint have received very little attention.

The studies described by this dissertation investigated the influence of chronic exposure to impacted field conditions or pre-exposure to dietary metal (Cd and Hg) on the assimilation of elements (organic carbon, Cd and Hg), subcellular partitioning of assimilated metal and digestive physiology (gut residence time [GRT], feces elimination rates [FER], gut pH and extracellular digestive protease activities) in grass shrimp *Palaemonetes pugio*. Additionally, carbon and Cd assimilation and endpoints related to digestive toxicity were assessed for naïve grass shrimp (i.e., shrimp from a reference site not subjected to previous laboratory exposure) following ingestion of a pulse of Cd-contaminated food. This experimental design facilitated comparison of impacts of dietary metal on digestive physiology prior to and following assimilation (i.e., pre- and post-assimilatory toxicity). Baseline data were obtained for shrimp collected from a reference site or appropriate laboratory controls.

Study organism

The daggerblade grass shrimp *Palaemonetes pugio* Holthius 1949 (Decapoda,

Caridea) is abundant in estuaries along the east coast of North America and persists under a wide range of conditions (e.g., dissolved oxygen, salinity, temperature and vegetation) (Wood, 1967; Nixon and Oviatt, 1973; Welsh, 1975; Alon and Stancyk, 1982; Kneib, 1987; Tashiro et al., 1994). This species has an omnivorous diet that varies according to seasonal abundances of food items and includes diatoms, epiphytic microalgae, plant detritus and an assortment of invertebrates (e.g., copepods, mysids, postlarval decapods, nematodes, oligochaetes and polychaetes) (Welsh, 1975; Bell and Coull, 1978; Morgan, 1980; Chambers, 1981; Kneib, 1985; Olmi and Lipcius, 1991; Tashiro et al., 1994; Gregg and Fleeger, 1998; Quinones-Rivera and Fleeger, 2005). Evidence suggests that grass shrimp may regulate benthic meiofauna as well as epiphytic microalgae and meiofauna associated with the cordgrass *Spartina alterniflora* (Bell and Coull, 1978; Morgan, 1980; Walters et al., 1996; Gregg and Fleeger, 1998; Fleeger et al., 1999; Quinones-Rivera and Fleeger, 2005). This species is also coprophagous and will readily consume its own fecal strands, which may contain high concentrations of assimilable organic material, as well as feces of other species (e.g., gastropods, crustaceans and polychaetes) (Johannes and Satomi, 1966; Frankenberg and Smith, 1967). Welsh (1975) concluded that grass shrimp play a vital role in nutrient cycling within estuarine food webs through conversion of detritus into fecal matter, dissolved organic matter and shrimp biomass and may increase ecological efficiency within these communities. This species is also important in the diets of key estuarine predators, including the mummichog *Fundulus heteroclitus* and striped bass *Morone saxatilis* and may serve as a vector of metals to higher trophic levels (Nixon and Oviatt, 1973; Kneib and Stiven, 1982; Posey and Hines, 1991; Davis et al., 2003; Carson and Merchant, 2005; Seebaugh et al., 2005).

In terms of tolerance (e.g., LC_{50} values), grass shrimp are sensitive to exposure to dissolved Cd, although uptake and mortality decrease with increasing Cd^{2+} complexation (e.g., by Cl^- in seawater) (Vernberg et al., 1977; Sunda et al., 1978; Engel and Fowler, 1979; Pesch and Stewart, 1980; Howard and Hacker, 1990). Exposure to Cd through solution induced increased levels of molecular biomarkers in grass shrimp (e.g., glutathione, lipid peroxide, manganese superoxide dismutases, αβ-crystallin and MTLP), indicative of metal-induced oxidative stress, impacts on cellular integrity or detoxification (Howard and Hacker, 1990; Downs et al., 2001; Amiard et al., 2006). Reduced oxygen consumption, metabolism and locomotor activity were reported for shrimp following exposure to dissolved Cd (Hutcheson et al., 1985; Manyin and Rowe, 2009). Cd accumulation by grass shrimp from the diet may be influenced by metal partitioning in prey (Nimmo et al., 1977; Wallace and Lopez, 1996, 1997; Wallace et al., 1998; Wallace et al., 2000; Seebaugh and Wallace, 2004; Seebaugh et al., 2005). For example, metal partitioned to a subcellular compartment containing trophically-available metal (TAM) (metal associated with heat-stable proteins [e.g., MTLP], heat-denatured proteins [e.g., enzymes] and organelles) may represent bioavailable Cd in invertebrate prey and serve as an estimate of Cd transfer to shrimp (Wallace and Luoma, 2003; Seebaugh and Wallace, 2004). Ingested Cd can also induce sublethal toxicity in this species. Wallace et al. (2000) reported that exposure to dietary Cd induced MTLP and that impaired prey capture was associated with increased partitioning of Cd to high molecular weight proteins (e.g., enzymes).

Impacts of Hg exposure on Hg uptake and toxicity in grass shrimp may be influenced by ecological factors, including previous field exposure and parasitism (Kraus

and Kraus, 1986, Kraus and Weis, 1987; Kraus et al., 1988; Bergey et al., 2002). For example, shrimp collected from a pristine reference site exhibited differences in predator avoidance behavior following exposure to $HgCl_2$ or methylmercury (Kraus and Kraus, 1986). Grass shrimp collected from a Hg-polluted site appeared to be more tolerant to Hg as avoidance was not impaired in this population following exposure to $HgCl_2$ (Kraus and Kraus, 1986). Similar field-dependent differences were observed with respect to larval metamorphosis and tolerance of adult shrimp (Kraus et al., 1988). MTLP can also be induced by exposure to Hg in the laboratory or the field (Kraus et al., 1988).

Metal exposure from contaminated field sediments would depend upon the extent to which metal ions are bound to ligands (e.g., acid volatile sulfides) *in situ* (Rubenstein et al., 1983; Rule, 1985; DiToro et al., 1992). Perez and Wallace (2004) observed that grass shrimp collected along an impact gradient exhibited impaired prey capture that may be related to high contaminant loads in sediments, including metals (e.g., Cd, Cu, Hg, Pb and Zn). Shrimp exposed to metal-rich coal combustion residues through contaminated sediment accumulated higher tissue concentrations of Cd, yet had lower Hg burdens relative to controls (Kuzmick et al., 2007). Chronic exposure to residues also resulted in higher frequencies of DNA damage in cells within the hepatopancreas and impacted larval survival (Kuzmick et al., 2007).

Field collection sites

Grass shrimp used for field-based components of this work were collected from salt marshes surrounding Staten Island, NY, located within the Harbor Core Area of the NY/NJ Harbor Estuary (Steinberg et al., 2004) (Chapter 2, Fig. 2-1). Three sites (Main

Creek, Mill Creek and Neck Creek) are located adjacent to the Arthur Kill (AK), which separates western Staten Island from New Jersey and links Newark Bay and Raritan Bay. Waters within the AK complex have long residence times and low rates of flushing and have been subjected to metal, organic pollutant and hazardous chemical discharges from industry, oil refineries, landfills and combined sewer overflows over many decades (Olsen et al.,1984; Oey et al., 1985; Gillis et al., 1993; Gunster et al., 1993; Crawford et al., 1995; Bergen et al., 2000). Lemon Creek is a tidal creek on the southern shore of Staten Island that empties directly into Raritan Bay. This site has had relatively low levels of pollution impacts compared to sites within the AK complex. The local reference site for these field studies was Great Kills Harbor, a small harbor located within the Gateway National Recreation area along the southeastern shore of Staten Island. This site is flushed with cleaner waters from Raritan Bay. While sediment metal concentrations (e.g., Cd and Hg at 0 to 5 cm) vary considerably among the Staten Island study sites, it is not yet known whether or not measurable gradients in organic pollutants (e.g., byproducts of petroleum processing or contaminants from landfill leachate) exist (Goto, 2009). Site-specific variability in digestive physiology or the assimilation and subcellular partitioning of elements could indeed be related to chronic exposure to metals, organic contaminants or interactive effects resulting from exposure to different pollutants.

Study objectives and hypotheses

Assimilation and subcellular partitioning of elements by grass shrimp collected along an impact gradient (Seebaugh and Wallace, 2009)

The objective of this study was to investigate intraspecific variation in the assimilation of elements (organic carbon, Cd and Hg) and subcellular partitioning of

assimilated metal in grass shrimp collected along an impact gradient within the NY/NJ Harbor Estuary. This work was conducted using radiotracer pulse-chase feeding experiments, where shrimp fed on ^{14}C-labeled meals or radiolabeled (^{109}Cd or ^{203}Hg) amphipod prey. The specific goals of this study were to 1) investigate impacts of field conditions on the assimilation of ingested elements by shrimp, 2) assess relationships between Cd and Hg assimilation by field-exposed shrimp and partitioning of metal radioisotopes to a subcellular compartment presumed to contain TAM in radiolabeled prey and 3) examine the influence of field exposure on subcellular distributions of assimilated metal by shrimp following 7 d depuration of ingested radioisotope (Wallace et al., 2003; Wallace and Luoma, 2003). It was hypothesized that shrimp exposed to impacted field conditions (i.e., within the AK complex) exhibit increased metal AE relative to shrimp from cleaner field sites. Reduced carbon assimilation was predicted for shrimp from impacted study sites. It was also predicted that increased partitioning of assimilated metal to the subcellular fraction containing heat-stable proteins (e.g., MTLP) would be observed for shrimp from within the AK complex. Additionally, Cd AE by field-exposed shrimp and Cd burdens in polychaetes from corresponding field sites were used to estimate site-specific doses of Cd to shrimp from ingestion of a single hypothetical meal *in situ*.

Assimilation, subcellular partitioning and accumulation of elements by grass shrimp pre-exposed to dietary metal

The primary goal of this work was to examine impacts of pre-exposure to dietary Cd or inorganic Hg on the assimilation of elements (organic carbon, Cd and Hg) and subcellular partitioning of assimilated metal by grass shrimp. This work was conducted

by feeding shrimp Cd- or Hg-contaminated oligochaetes for 15 d, followed by AE analyses using radiotracer (^{14}C, ^{109}Cd or ^{203}Hg) pulse-chase feeding techniques. The specific objectives of this study were to 1) investigate the influence of pre-exposure to dietary metal (followed by 2 d depuration) on the assimilation of elements by grass shrimp, 2) determine relationships between metal assimilated by shrimp and metal partitioning to TAM in radiolabeled prey and 3) examine the influence of pre-exposure to dietary Cd on the subcellular distribution of assimilated Cd and Hg in shrimp following 7 d depuration. It was hypothesized that previous exposure to dietary metal alters the subsequent assimilation and subcellular distribution of Cd and Hg by grass shrimp. Increased partitioning of assimilated metal to the heat stable fraction was predicted for shrimp pre-exposed to dietary metal. It was also predicted that carbon assimilation would decrease for shrimp across dietary Cd and Hg exposures. Relationships between stable metal accumulated by shrimp during pre-exposure and metal partitioning in oligochaete prey (i.e., to the TAM compartment) were assessed for evidence of bioenhancement of metal trophic transfer (Seebaugh et al., 2006).

Post-assimilatory digestive toxicity in grass shrimp *Palaemonetes pugio*

The objective of this investigation was to examine intraspecific variation in GRT, FER, gut pH and extracellular protease activities in grass shrimp collected along an impact gradient within the NY/NJ Harbor Estuary and to determine the influence of pre-exposure to dietary Cd or Hg on digestive toxicity. This work was conducted on grass shrimp *in vivo* using task-specific fluorescent or near-infrared (NIR) markers and non-invasive microfluorometric methods or a novel NIR imaging technique. Shrimp were also

assessed for molt stage following assays for digestive toxicity. It was hypothesized that field-collected and laboratory pre-exposed shrimp would exhibit treatment differences in GRT and FER, but that variability in gut pH or protease activities would not be observed. It was also predicted that endpoints related to digestive toxicity in grass shrimp would be influenced by molt stage.

Relationship between dietary cadmium absorption by grass shrimp (*Palaemonetes pugio*) and trophically available cadmium in amphipod (*Gammarus lawrencianus*) prey (Seebaugh et al., 2006)

The objective of this work was to investigate impacts of a pulse of Cd in food on Cd AE by naïve grass shrimp collected from a reference site. This work was conducted by exposing amphipods to a range of dissolved Cd concentrations with ^{109}Cd as a radiotracer of stable Cd, followed by radioanalysis and estimation of TAM-Cd in prey. Shrimp were then pulse-fed ^{109}Cd-labeled amphipods and assessed for Cd assimilation. A direct (~1:1) relationship between TAM Cd burdens in prey and Cd assimilation by grass shrimp was hypothesized (Wallace et al., 1998).

Carbon assimilation and digestive toxicity in naïve grass shrimp (*Palaemonetes pugio*) exposed to dietary Cd

The goal of this study was to investigate the influence of a pulse of dietary Cd on carbon AE, GRT, FER, extracellular protease activities and gut pH in naïve grass shrimp collected from a reference site. Shrimp were pulse fed Cd-contaminated meals containing ^{14}C-labeled diatoms, fluorescent or NIR markers and then assessed for AE or digestive toxicity through radioanalysis, microfluorometric methods or NIR imaging. Shrimp were also characterized for post-assay molt stage. It was hypothesized that carbon AE and

protease activities in grass shrimp would decrease with increasing dietary Cd exposure, but that GRT, FER, and gut pH would not exhibit variation among treatments. It was also predicted that molt stage would impact digestive physiology in grass shrimp.

CHAPTER 2

Assimilation and subcellular partitioning of elements by grass shrimp collected along an impact gradient

CHAPTER SUMMARY

Chronic exposure to polluted field conditions can impact metal bioavailability in prey and may influence metal transfer to predators. The present study investigated the assimilation of Cd, Hg and organic carbon by grass shrimp *Palaemonetes pugio*, collected along an impact gradient within the New York/New Jersey Harbor Estuary. Adult shrimp were collected from five Staten Island, New York study sites, fed ^{109}Cd- or ^{203}Hg-labeled amphipods or ^{14}C-labeled meals and analyzed for assimilation efficiencies (AE). Subsamples of amphipods and shrimp were subjected to subcellular fractionation to isolate metal associated with a compartment presumed to contain trophically available metal (TAM) (metal associated with heat-stable proteins [HSP – e.g., metallothionein-like proteins], heat-denatured proteins [HDP – e.g., enzymes] and organelles [ORG]). TAM-^{109}Cd% and TAM-^{203}Hg% in radiolabeled amphipods was ~64% and ~73%, respectively. Gradients in AE-^{109}Cd% (~54% to ~75%) and AE-^{203}Hg% (~61% to ~78%) were observed for grass shrimp, with the highest values exhibited by shrimp collected from sites within the heavily polluted Arthur Kill complex. Population differences in AE-^{14}C% were not observed. Assimilated ^{109}Cd% partitioned to the TAM compartment in grass shrimp varied between ~67% and ~75%. ^{109}Cd bound to HSP in shrimp varied between ~15% and ~47%, while ^{109}Cd associated with metal-sensitive HDP was ~17% to ~44%. Percentages of assimilated ^{109}Cd bound to ORG were constant at ~10%. Assimilated ^{203}Hg% associated with TAM in grass shrimp did not exhibit significant variation. Percentages of assimilated ^{203}Hg bound to HDP (~47%) and ORG (~11%) did not vary among populations and partitioning of ^{203}Hg to HSP was not observed. Using a simplified biokinetic model of metal accumulation from the diet, it is estimated that site-

specific variability in Cd AE by shrimp and tissue Cd burdens in field-collected prey (polychaetes *Nereis* spp.) could potentially result in up to ~3.2-fold differences in the dose of Cd assimilated by shrimp from a meal in the field. The results of this study also suggest that chronic field exposure can impact mechanisms of metal transport across the gut epithelium that do not influence carbon assimilation. Differences in the assimilation and subcellular partitioning of metal may have important implications for metal toxicity in impacted shrimp populations.

INTRODUCTION

Organisms within heavily industrialized estuaries such as the New York/New Jersey (NY/NJ) Harbor Estuary are typically exposed to suites of metal and organic pollutants through multiple pathways (i.e., dissolved, sediment and diet) (Feng et al., 1998; Steinberg et al., 2004). Metal concentrations in sediments can vary considerably within urban estuaries, which may have implications for metal cycling within impacted communities and toxicity to resident biota (Wolfe et al., 1996; Goto, 2009). Population differences in metal accumulation, toxicity and genetic resistance may be related to high contaminant loads in the field (Kraus and Kraus, 1986; Klerks and Levinton, 1989; Paulson et al., 2003). Field exposure can also impact bioavailability of metal in prey, which may influence transfer to predators (Wallace et al., 1998; Rainbow et al., 2004a).

Metal transfer along aquatic food chains may be more closely related to internal compartmentalization of metal than to whole tissue burdens in prey (Wallace and Luoma, 2003). For example, an ~1:1 relationship between metals (i.e., Cd and Zn) stored in specific subcellular fractions (i.e., heat-stable proteins [HSP - e.g., metallothionein-like proteins], heat-denatured proteins [HDP - e.g., enzymes] and organelles [ORG]) in invertebrate prey (oligochaetes, bivalves and brine shrimp) and assimilation by grass shrimp has been observed in several studies (Wallace et al., 1998; Wallace and Luoma, 2003; Seebaugh and Wallace, 2004). Due to this potential bioavailability to predators, metal associated with these fractions may be considered as a compartment containing trophically-available metal (TAM) (Wallace and Luoma, 2003).

The assimilation of ingested elements (e.g., metals and organic carbon) has been characterized for a variety of crustaceans, including decapods (Morgan, 1980; Reinfelder

and Fisher, 1991; Amouroux et al., 1997; Wallace and Luoma, 2003; Seebaugh and Wallace, 2004). Assimilation efficiencies (AE) may be influenced by biological and environmental factors including the partitioning of elements within food, previous exposure to contaminants and consumer digestive physiology (Reinfelder and Fisher, 1991; Rainbow et al., 2004b; Wallace et al., 2008). Toxicity of ingested metal may ultimately be related to subcellular partitioning following assimilation. For example, metal bound to metallothionein like proteins (MTLP) or sequestered as insoluble metal-rich granules is biologically-detoxified and would not be predicted to impact cellular functions, while metal associated with metal-sensitive intracellular components (e.g., enzymes and organelles) may elicit toxicity (Wallace et al., 2003; Amiard et al., 2006).

The daggerblade grass shrimp *Palaemonetes pugio* Holthius 1949 (Decapoda, Caridea) is abundant in estuaries along the eastern coast of North America and can persist under a wide range of ecological conditions (Wood, 1967; Welsh, 1975). This species has an omnivorous diet that can vary according to seasonal abundances of food items and includes diatoms, epiphytic microalgae, detritus and invertebrates (Welsh, 1975; Kneib, 1985; Quinones-Rivera and Fleeger, 2005). Grass shrimp are important in the diets of several finfish predators (e.g., *Fundulus heteroclitus* and *Morone saxatilis*) and may serve as a vector of metals to higher trophic levels (Davis et al., 2003). In previous work, Cd assimilation by grass shrimp was influenced by Cd burdens in laboratory-exposed invertebrate prey (Seebaugh et al., 2006). Pre-exposure to dietary metal also influences the subsequent assimilation of dietary Cd and Hg (Seebaugh and Wallace, unpublished).

The purpose of the present study was to investigate intraspecific variation in the assimilation of elements (Cd, Hg and organic carbon) and subcellular distribution of

assimilated metal by grass shrimp collected along an impact gradient within the NY/NJ Harbor Estuary. This work was conducted using radiotracer pulse feeding experiments and subcellular fractionation. The specific goals of this study were to 1) investigate the impact of field conditions on the assimilation of elements ingested by grass shrimp in the laboratory, 2) determine the relationship between metal assimilation by field-collected shrimp and the partitioning of metal to TAM in laboratory-exposed prey and 3) examine site-specific differences in the subcellular partitioning of assimilated metal following 7 d of depuration. Additionally, Cd AE and tissue Cd burdens in field-collected prey were used to estimate site-specific doses of Cd to shrimp from a hypothetical meal in the field.

MATERIALS AND METHODS

Field sampling

Grass shrimp for this study were collected from five salt marshes surrounding Staten Island, NY, located within the Harbor Core Area of the NY/NJ Harbor Estuary (Fig. 2-1) (Steinberg et al., 2004). Three sites are located within the Arthur Kill (AK) complex, which separates the western shore of Staten Island from New Jersey and connects Newark and Raritan Bays. Waters within the AK have long residence times, low rates of flushing and have been subjected to metal and organic pollutant discharges from industrial activities, oil refinery operations and combined sewer overflows over many decades (Gillis et al., 1993; Gunster et al., 1993; Crawford et al., 1995). Great Kills Harbor (GK) is located along the southeastern shore of Staten Island. This small harbor is not impacted by industrial activities directly, but may receive contaminants from runoff, marina operations and a sewage treatment plant. Lemon Creek (LC) is a small

tidal creek on the southern shore of Staten Island that empties into Raritan Bay, ~4 km east of the confluence of the AK and the Raritan River. Pollution impacts at LC are low compared to the sites within the AK complex, although sources of contamination may include runoff, marinas and remediation of a dental tool manufacturing site adjacent to the creek (Ward, 2002). Main Creek (MA) extends ~3 km from the AK and is susceptible to leachate contamination from landfills. Neck Creek (NC) extends ~0.6 km eastward from the AK and is likely impacted by general contamination from this waterway. Mill Creek (MI) is a small tidal creek extending ~0.5 km from the lower AK and ~1 km from Raritan Bay. This creek received discharges from a metal smelting facility from the 1930s until the 1970s (Ward, 2002). Data for metals (Ag, Cd, Cu, Hg, Ni, Pb and Zn) in sediments (0 to 5 cm) from the Staten Island study sites are presented in Table 2-1 (Goto, 2009).

Adult amphipods *Gammarus lawrencianus* (5 to 10 mm in length) were collected by dip net from GK and maintained in aerated, filtered seawater (1.0 μm filter, 10 ppt, 18-19 °C) from a reference site in Tuckerton, NJ (TK) (Fig. 2-1) (Weis et al., 2001). Amphipods were fed daily on commercial fish food, but were held within a 1-mm screen and allowed to clear their guts for 24 h prior to radiolabeling. Adult grass shrimp (~3 cm in length) were collected by dip net from the Staten Island study sites ~3 d prior to AE analysis (Fig. 2-1). Gravid females were identified in the field and excluded from analyses (Bauer and Abdalla, 2000). Shrimp were maintained in 38 l filtered, aerated seawater (63 μm, 10 ppt, 18-19 °C) from their respective collection sites. Site water was adjusted to 10 ppt with deionized water. To prevent ingestion of fecal strands, shrimp were housed above a 3-mm mesh partition positioned ~10 cm above the aquarium floor.

Shrimp were fed once on commercial fish food immediately following collection and allowed to clear their guts prior to feeding on radiolabeled amphipods or meals.

^{109}Cd- and ^{203}Hg labeling of amphipod prey

Amphipods were exposed in 4 l bottles containing 3 l aerated TK seawater, diluted with NANOpure® (Barnstead) deionized water (0.4 μm, 10 ppt, 18-19 °C) and spiked with $^{109}CdCl_2$ (in 0.5 N HCl) or $^{203}HgCl_2$ (in 1.0 N HCl) (Isotope Products) for 72 h or 48 h, respectively (~0.04 amphipods ml^{-1}). Reduced ^{203}Hg exposure times were necessary due to significant amphipod mortality observed during longer preliminary exposures. ^{109}Cd or ^{203}Hg activities of the radiolabeling solutions were 2.36 x 10^2 kBq l^{-1} (~0.025 μM Cd) or 9.25 kBq l^{-1} (~0.005 μM Hg) and were verified through analysis of 5 ml samples. Acidification of exposure solutions resulting from the addition of ^{109}Cd or ^{203}Hg stock solutions was offset by 0.5 or 1.0 N NaOH. Following exposure, amphipods were rinsed and stored frozen (-80 °C).

^{109}Cd and ^{203}Hg assimilation efficiency analyses

Following clearance of gut contents, grass shrimp were transferred to beakers containing TK seawater (1.0 μm, 10 ppt, 18-19 °C) and allowed to feed on ^{109}Cd- or ^{203}Hg-labeled amphipods for 45 min. Following feeding, shrimp were rinsed with clean seawater and analyzed for ^{109}Cd or ^{203}Hg activity (time [t] = 0) (Wallace et al., 1998). Shrimp that emitted sufficient ^{109}Cd or ^{203}Hg signals were housed in individual chambers within a 38 l aquarium containing filtered TK seawater, where they depurated ingested radioisotope for 7 d. Chambers were suspended above the aquarium floor to prevent consumption of fecal strands. Aquarium water was filtered through activated carbon to

reduce exposure to dissolved radioisotope. ^{109}Cd or ^{203}Hg activities in aquarium water were monitored through analysis of 5 ml samples and remained at background. Shrimp were fed daily on commercial fish food. Individual shrimp were analyzed for ^{109}Cd or ^{203}Hg activity at t = 2, 4, 8, 12, 24 and 48 h and approximately every 24 h thereafter. AE-^{109}Cd% and AE-^{203}Hg% were estimated as percentages of radioactivity retained in shrimp at 48 h, relative to t = 0 (Wallace and Luoma, 2003). A linear regression was fit to the physiological loss component of each retention curve (t > 24 h) and the corresponding slope was used to estimate the rate of physiological loss of ^{109}Cd or ^{203}Hg (Benayoun et al., 1974).

^{14}C-labeled meals

To ensure consistent ^{14}C signals in grass shrimp following pulse feeding, ^{14}C-labeled diatoms were concentrated and embedded in a modified gelatin-oligochaete homogenate mixture readily consumed by shrimp in previous work (Wallace and Lopez, 1996). In preliminary work for the present study, unlabeled diatoms remained intact (i.e., cell walls were not ruptured) following processing and freezing.

Axenic cultures of diatoms *Thalassiosira weissflogii* (CCMP 1336; Provasoli-Guillard Center for the Culture of Marine Phytoplankton) were grown in f/2 medium prepared with filtered TK seawater (0.22 µm, 32 ppt) (Guillard, 1983). Culture vessels were maintained in a walk-in incubator (18-19 °C) on a surface with a light irradiance of 100 µEinstein m^{-2} s^{-1} on a 14 h:10 h (light:dark) cycle (Miao and Wang, 2006). Diatoms were radiolabeled in f/2 medium containing $NaH^{14}CO_3$ (in deionized water) (American Radiolabeled Chemicals) for 7 d. The ^{14}C activity of the radiolabeling solution was 3.7 x

10^3 kBq l^{-1} and was verified through analysis of 1 ml samples (Roman, 1984). Gentle aeration was used to maintain diatoms in suspension (Dijkman and Kroon, 2002). Radiolabeled diatoms were harvested by centrifugation and stored frozen (-80 °C) (~3.41 x 10^7 cells ml^{-1}) (Fleeger et al., 1999). Oligochaetes *Tubifex tubifex* (Newman's Fish Food) were rinsed and homogenized in NANOpure® deionized water (0.66 g worm tissue ml^{-1}). A 1 ml portion of ^{14}C-labeled diatoms was combined with 1 ml worm homogenate, 0.33 ml cod liver oil (to enhance palatability) and 0.47 g gelatin crystals (Knox®). This mixture was sealed in a microcentrifuge tube, warmed with hot tap water, vortexed until uniform consistency was achieved and stored frozen (-80 °C). Individual meals consisted of 6 µl samples of the ^{14}C-labeled mixture, which were dispensed onto Nucleopore filters (Whatman®) and stored frozen (-80 °C) 24 h prior to feeding experiments (Wallace and Lopez, 1996).

^{14}C assimilation efficiency analysis

Following clearance of gut contents over ~3 d, grass shrimp were transferred to beakers containing clean TK seawater (1.0 µm, 10 ppt, 18-19 °C) and allowed to feed on ^{14}C-labeled meals for 45 min. Shrimp were then rinsed with clean seawater, transferred to individual defecation chambers housed within 38 l aquaria containing aerated seawater and allowed to feed *ad libitum* on commercial fish food. Fecal strands were collected as frequently as possible on GF/C filters (Whatman®) for 24 h. ^{14}C activities in aquarium water were monitored through analysis of 1 ml samples and remained at background. Shrimp were removed from defecation chambers at 24 h, rinsed with clean seawater and killed by freezing (-80 °C). Tissues were then minced with scissors, immersed in 1.5 ml

TS-2 tissue solubilizer (Research Products International) and digested for 4 d (Fleeger et al., 1999). ^{14}C- labeled feces from individual shrimp were digested in 1 ml TS-2 for 4 d. Samples were treated with 0.5 ml 30% H_2O_2 to reduce color quenching prior to the addition 10 ml BioSafe II liquid scintillation cocktail (RPI) and chemiluminescence was reduced by the addition of 70 µl glacial acetic acid (Dodson, 2002). ^{14}C AE was estimated using the mass balance method: $AE\text{-}^{14}C\% = (^{14}C\ retained/^{14}C\ ingested) \times 100$, where $^{14}C\ retained$ is ^{14}C activity in shrimp tissue at 24 h and $^{14}C\ ingested$ is the sum of $^{14}C\ retained$ and ^{14}C in feces (Wang and Fisher, 1996). Time courses in the retention of ^{14}C were not plotted for individual shrimp since liquid scintillation counting requires destruction of tissue samples (i.e., live shrimp cannot be counted repeatedly over time).

Subcellular fractionation

To characterize the fraction of ^{109}Cd or ^{203}Hg in amphipod prey potentially available to predators (i.e., TAM), subsamples of radiolabeled amphipods (n = 4 for ^{109}Cd, 5 for ^{203}Hg; 10 amphipods per replicate) were subjected to two-part subcellular fractionation. Amphipods were thawed on ice and homogenized in 3.3 ml cold Tris buffer (pH 7.6) using a Polytron® (Kinematica) tissue homogenizer. Homogenized tissue samples were centrifuged at 500 x g (15 min at 4°C) to separate the supernatant containing TAM from the pellet containing non-TAM fractions (Seebaugh and Wallace, 2004). TAM and non-TAM fractions were analyzed for ^{109}Cd or ^{203}Hg. After 7 d depuration of ingested ^{109}Cd or ^{203}Hg, grass shrimp (n = 3; 2 to 4 shrimp per replicate) were subjected to five-part subcellular fractionation to determine percentages of assimilated metal distributed among HSP, HDP, ORG, insoluble

components (INS – e.g., metal-rich granules) and cellular debris (CD – e.g., membranes) (Wallace et al., 2003; Seebaugh and Wallace, 2004). Following 500 x *g* centrifugation, the pellet was resuspended in 3 ml Tris buffer and heated at 100 °C for 2 min. An equal volume of 1 N NaOH was added and the suspension heated at 65 °C for 1 h. The suspension was centrifuged at 4500 x *g* (15 min at 4 °C) and the supernatant containing CD removed. Pellets containing INS were also recovered (Silverman et al., 1983). The 500 x *g* supernatants were centrifuged at 100,000 x *g* (1 h at 4 °C) to produce a pellet containing ORG. The resultant supernatants were heated at 80 °C for 10 min and cooled on ice for 1 h. The heat-treated cytosol was centrifuged at 38,000 x *g* (30 min at 4 °C) to pellatize HDP, while HSP remained in the supernatant (Bebianno et al., 1992). Isolated fractions were analyzed for ^{109}Cd or ^{203}Hg.

Radioanalyses

^{109}Cd- and ^{203}Hg-labeled samples were analyzed using a Wallac Wizard^{TM} 1480 automatic γ counter (Wallac Oy). ^{109}Cd or ^{203}Hg activities associated with HSP, HDP and ORG were used to reconstruct the TAM compartment (TAM-^{109}Cd% or TAM-^{203}Hg%) in grass shrimp (Wallace and Luoma, 2003). ^{14}C-labeled samples were analyzed using a Beckman LS 6500 liquid scintillation counter with an external quench monitor. Counting times for all samples were adjusted to maintain propagated counting errors of 5% or less.

Estimated dose of Cd to grass shrimp in the field

Site-specific doses of Cd to grass shrimp resulting from ingestion of prey in the field ($Cd_{dose,\ field}$) were estimated using a simplified biokinetic model of dietary metal accumulation: $Cd_{dose,\ field} = AE\text{-}^{109}Cd\% * Cd_{prey,\ field}$, where $AE\text{-}^{109}Cd\%$ is the site-specific

Cd AE obtained for shrimp in the present study and $Cd_{prey,\ field}$ is the whole tissue Cd burden in field-collected polychaetes *Nereis* spp. (Wang and Fisher, 1999a; Goto and Wallace, unpublished). Data for polychaetes were used in these calculations as amphipod tissue Cd data were not available for the Staten Island study sites. This model assumes equal rates of ingestion and physiological loss of assimilated metal for shrimp in the field (Wang and Fisher, 1999a). Additional limitations of this calculation are addressed in the Discussion. Proportional differences in $AE\text{-}^{109}Cd\%$, $Cd_{prey,\ field}$ and $Cd_{dose,\ field}$ were also calculated and normalized to values for GK (the reference site for this study).

Statistical analyses

The effects of study site on AE and the partitioning of assimilated metal to subcellular fractions in grass shrimp were analyzed using one-way analysis of variance (Sokal and Rohlf, 1995). Percentage data were arcsine transformed and normality tested using Shapiro-Wilk's *W* test. Homoscedasticity was tested using Levene's test. Unplanned comparisons in AE and the partitioning of metal were performed using Tukey-Kramer multiple comparisons (unequal *n*) and Tukey post-hoc tests (equal *n*), respectively (Sokal and Rohlf, 1995). Rates of physiological loss of metal were compared using an unplanned test of comparisons among regression coefficients (Tukey-Kramer method) (Sokal and Rohlf, 1995). AE-^{109}Cd% and AE-^{203}Hg% by shrimp and partitioning of metal to TAM in amphipods were compared using the *t*-test. Statistical analyses were performed using STATISTICA version 7.1 (StatSoft) and Instat version 3.0a (GraphPad).

RESULTS

TAM in amphipod prey

The percentages of ^{109}Cd or ^{203}Hg partitioned to the subcellular compartment containing TAM (i.e., metal associated with HSP, HDP and ORG) within amphipod prey were determined following tissue homogenization and centrifugation. TAM-^{109}Cd% and TAM-^{203}Hg% in amphipods were 63.8 ± 2.6% and 72.5 ± 2.1%, respectively.

^{109}Cd and ^{203}Hg assimilation by grass shrimp

AE-^{109}Cd% and AE-^{203}Hg% were determined for grass shrimp collected from five Staten Island study sites and pulse-fed radiolabeled amphipod prey. Depuration of ^{109}Cd and ^{203}Hg by shrimp was characterized by a three-compartment loss, with an initial rapid loss of metal from the release of radiolabeled fecal strands followed by a reduced rate of loss until ~48 h (Fig. 2-2). The slowest exchanging third compartment (t > 48 h) reflects physiological loss of assimilated metal. AE-^{109}Cd% or AE-^{203}Hg% were estimated as percentages of radioactivity retained in shrimp 48 h following ingestion of radiolabeled amphipods. Gradients in AE-^{109}Cd% (~54% to ~75%) and AE-^{203}Hg% (~61% to ~78%) were observed for grass shrimp, with the highest values for shrimp collected from sites within the AK complex (NC for ^{109}Cd; MA for ^{203}Hg) (Fig. 2-3). AE-^{109}Cd% by NC shrimp exceeded TAM-^{109}Cd% in amphipods, while AE-^{203}Hg% by GK and LC shrimp was less than TAM-^{203}Hg% in prey (Fig. 2-3). Physiological loss rates for ^{109}Cd (1.24 ± 1.22% to 2.45 ± 0.87% d^{-1}) and ^{203}Hg (2.59 ± 0.68% to 3.97 ± 0.89% d^{-1}) did not differ among populations (data not shown; unplanned test of comparisons among regression coefficients [Tukey-Kramer method]).

^{14}C assimilation by grass shrimp

In preliminary studies, grass shrimp fed ^{14}C-labeled meals released radiolabeled fecal strands for ~24 h (Fig. 2-4A). AE-^{14}C% was estimated at 24 h to minimize potential losses of ^{14}C through respiration. Population differences in AE-^{14}C% by shrimp collected from the Staten Island study sites were not observed (~82% for all sites) (Fig. 2-4B).

Subcellular partitioning of assimilated ^{109}Cd and ^{203}Hg

Following 7 d depuration of ingested radioisotope, grass shrimp were subjected to subcellular fractionation to estimate percentages of assimilated metal partitioned to the TAM compartment as well as individual fractions: HSP, HDP, ORG, INS and CD. TAM-^{109}Cd% in shrimp varied between ~67% and ~75% (Fig. 2-5A). Partitioning of assimilated ^{109}Cd bound to HSP in shrimp varied from ~15% to ~47%, with the highest values observed for shrimp collected from LC (~47%) and MI (~39%) (Fig. 2-5A). In contrast, percentages of ^{109}Cd associated with HDP were lowest for LC (~17%) and MI (~19%) shrimp. Partitioning of ^{109}Cd to ORG was constant among shrimp populations at ~10% (Fig. 2-5A). Percentages of ^{109}Cd associated with CD varied between ~25% and ~33% (ANOVA: $p < 0.05$; data not shown). ^{109}Cd signals associated with INS were below detection limits. TAM-^{203}Hg% in grass shrimp did not exhibit significant variation (Fig. 2-5B). Percentages of assimilated ^{203}Hg partitioned to individual fractions (HDP, ORG and CD) fractions did not differ among shrimp from the study sites (CD ANOVA: ns; data not shown) (Fig. 2-5B). ^{203}Hg associated with HSP and INS was below detection limits.

Estimated dose of Cd to grass shrimp in the field

The estimated dose of Cd to grass shrimp from a single meal in the field was calculated using a simplified biokinetic model of dietary metal accumulation (Table 2-2). Data and calculations are presented only for sites where TAM-Cd% in field-collected prey (polychaetes) was comparable to TAM-^{109}Cd% in laboratory-exposed amphipods (~64%). LC and MA polychaetes did not meet this criterion as TAM-^{109}Cd% was ~44% and ~26%, respectively. MI and NC polychaetes exhibited ~2-fold differences in whole tissue Cd burdens relative to worms collected from GK (Table 2-2; Goto and Wallace, unpublished). Site-specific differences in Cd AE (estimated from AE-^{109}Cd% in this study) and $Cd_{prey,\ field}$ could potentially result in increased doses of Cd assimilated by shrimp at sites within the AK complex, with the greatest increase (~3.2-fold) estimated for NC shrimp (Table 2-2).

DISCUSSION

The present study has demonstrated intraspecific variation in the assimilation of Cd and Hg by grass shrimp *Palaemonetes pugio* collected along an impact gradient within the NY/NJ Harbor Estuary. It must still be determined whether or not shrimp populations that inhabit waters within the AK complex and adjacent to Raritan Bay represent the same source population(s). If Staten Island shrimp have source populations in common, variability in metal assimilation may be attributable to differential exposure to pollutant (e.g., metal and organic) loads in the field. Differences in metal assimilation in impacted populations may also have an underlying genetic component, which may be manifested as intraspecific variation in digestion or internal metal processing following

absorption (Mayer et al., 2001; Levinton et al., 2003).

Patterns of ^{109}Cd and ^{203}Hg depuration by grass shrimp were similar to patterns observed for ^{109}Cd and ^{65}Zn in previous work and are characterized by a three-component loss, consistent with biphasic digestion (Decho and Luoma, 1996; Seebaugh and Wallace, 2004). The initial rapid loss compartment may represent the passage of food particles from the proventriculus, during which some radiotracer is purged due to particle sorting before reaching the hepatopancreas and expelled in feces (Icely and Nott, 1992). The second, slower loss compartment may represent expulsion of residual materials from the hepatopancreas resulting from intracellular digestion and loss of epithelial (possibly blister) cells prior to the next digestive cycle (Vogt, 1993; Wang and Fisher, 1999b). The slowest exchanging third compartment reflects physiological loss of assimilated metal (Wang and Fisher, 1999b). Differences in physiological ^{109}Cd or ^{203}Hg loss rates were not observed among shrimp from the study sites, suggesting that excretion of these accumulated non-essential metals (possibly stored in insoluble form by resorptive cells of the hepatopancreas) was not impacted by previous field exposure (Icely and Nott, 1992; Rainbow, 2002).

Carbon AE by grass shrimp following the consumption of ^{14}C-labeled meals (~82%) was similar to values estimated for *P. pugio* fed on diatoms (~79%) or seagrass (~83%) in previous studies, where losses of ingested ^{14}C or total carbon due to respiration were not considered in the mass balance calculation of AE (Johannes and Satomi, 1966; Morgan, 1980). Since ^{14}C-labeled fecal strands were not released by shrimp after 24 h in a preliminary study, AE-^{14}C% was estimated at this time point to minimize potential losses of ^{14}C through respiration. Amouroux et al. (1997) reported that ^{14}C released as

$^{14}CO_2$ by the prawn *Penaeus stylirostris* represented only a small fraction (~2.4%) of total radioactivity recovered 24 h after the consumption of ^{14}C-labeled mussel tissue. Carbon assimilation by grass shrimp does not appear to be influenced by temperature, salinity, meal composition or feeding time or related to variability in gut residence time (Johannes and Satomi, 1966; Morgan, 1980; Seebaugh and Wallace, unpublished).

Exposure to pollutants through the diet may elicit changes in predator digestive physiology that can be categorized according to whether or not a pollutant has been incorporated into tissues (i.e., assimilated) following absorption by the gut epithelium (Penry, 1998). Pre-assimilatory toxicity may be induced by ingestion of a pollutant and may include changes in gut retention time, extracellular digestive enzyme activities or absorption of the products of hydrolysis during the digestive cycle (Chen and Mayer, 1998; Campbell et al., 2005). These effects would be related to solubilization and availability of pollutants in gut fluid and not to pollutant burdens accumulated during previous exposures (Mayer et al., 2001). Post-assimilatory toxicity would result from incorporation of a pollutant as a consequence of chronic exposure (e.g., impacted field conditions in the present study). Previous exposure could impact synthesis and discharge of digestive enzymes as well as assimilation of nutrients and solubilized pollutants during subsequent digestive cycles (Icely and Nott, 1992; Vogt, 1993). The effects of pre- and post-assimilatory toxicity on digestive physiology may also be interactive, particularly under conditions where the gut is exposed during consecutive digestive cycles.

Grass shrimp used in the present study were fed immediately following collection, acclimated to laboratory conditions and allowed to clear their guts for 3 d prior to AE analysis. Since specimens were not fed during acclimation, additional nutrients and

pollutants were not available for absorption by the gut epithelium. Barring inherent population differences in digestion, increased assimilation of Cd and Hg by shrimp from sites within the AK complex may be related to post-assimilatory toxicity resulting from exposure to pollutants (e.g., metals, organic pollutants or leachate contamination from landfills) prior to collection. Despite population differences in the metal assimilation, intraspecific variability in carbon assimilation was not observed. This suggests that post-assimilatory toxicity in AK shrimp may impact mechanisms of divalent cation transport (possibly an amiloride-sensitive $2Na^{+}/1H^{+}$ antiporter) across the epithelium of the hepatopancreas that do not influence carbon assimilation (Ahearn et al., 1994).

Grass shrimp collected from Staten Island study sites with high rates of flushing with 'cleaner' waters from Raritan Bay (e.g., GK and LC) may experience the least impact from contaminants received from runoff, marina operations or nearby impacted waterways (e.g., the AK). Shrimp collected from tributaries within the AK complex (MA and NC) may be impacted by long-term exposure to metals, organic pollutants and leachate from landfills due to longer water residence times and reduced flushing due to concurrent tidal surges from Newark and Raritan Bays (Oey et al., 1985). The influence of this resonance effect on grass shrimp migration and population dynamics within the AK and its tributaries is unclear and must still be investigated. Shrimp collected from MI may represent transient populations as this site is located in close proximity to the confluence between the AK and Raritan Bay and is subjected to greater flushing than MA and NC. Increased flushing may also reduce accumulation of specific metals by resident biota despite exposure to localized, yet high sediment metal loads at MI (Goto, 2009). Interestingly, Paulson et al. (2003) reported tissue Cd and Hg burdens in ribbed mussels

collected from MI that were similar to specimens from a reference site near Raritan Bay (Sandy Hook, NJ).

Perez and Wallace (2004) observed a gradient in prey capture success by grass shrimp collected from GK, MI and a site located within a tributary of the AK, in close proximity to MA (Richmond Creek – RC) (Fig. 2-1). Prey capture rates for MI shrimp were intermediate between GK (highest success) and RC. Interestingly, MI has been subjected to recent (Spring 2009) site remediation efforts, which could potentially influence future metal concentrations in sediments and burdens in resident biota as well as toxicological impacts (e.g., differences in metal assimilation or prey capture) in grass shrimp (Levinton et al., 2003).

Previous studies suggest that TAM in prey may estimate the potential for Cd transfer to grass shrimp (Wallace et al., 1998; Wallace and Luoma, 2003; Seebaugh and Wallace, 2004). The relationship between AE-^{109}Cd% by Staten Island shrimp and TAM-^{109}Cd% in amphipods in the present study provides additional support for the hypothesis that TAM in prey may serve as a predictor of minimum Cd bioavailability to shrimp (Wallace and Luoma, 2003). The departure from the direct relationship between Cd AE and TAM in amphipods in the case of NC shrimp may be related to differences in predator digestion that influence solubilization and availability of metal associated with non-TAM fractions (INS and CD) in prey (Wallace et al., 2008). Metal solubilization and bioavailability in aquatic invertebrate guts may be related to specific biochemical characteristics of digestive fluids (e.g., surfactancy, amino acid concentrations and digestive enzyme activities), which can vary widely across taxa (Mayer et al., 2001). Exposure to contaminated field conditions (petroleum and metals) was shown to elicit

changes in gut fluid chemistry and morphological changes in the hepatopancreas of crayfish, which could potentially influence solubilization and assimilation of nutrients and metals (Anderson et al., 1997).

The partitioning of assimilated ^{109}Cd to biologically-detoxified HSP in grass shrimp following 7 d of depuration may reflect induction of MTLP or displacement of metal (e.g., essential Cu and Zn) from the existing MTLP pool (Wallace et al., 2003, Amiard et al., 2006). Enhanced partitioning of ^{109}Cd to HSP in LC and MI shrimp may provide additional protection for metal-sensitive HDP (e.g., enzymes) and ORG (Wallace et al., 2003). Although it is difficult to relate differences in the partitioning of ingested ^{109}Cd by grass shrimp to previous metal exposure without characterization of metal-specific MTLP, a dose-dependent increase in Cd-binding metallothioneins was reported for shrimp fed Cd-contaminated prey (Wallace et al., 2000). Intraspecific variability in total metallothionein concentrations was observed in bivalves collected along an environmental metal gradient and was correlated with tissue concentrations of Cd (but not Cu or Zn) as well as free Cd^{2+} concentrations at the water-sediment interface (Couillard et al., 1993). Population differences in partitioning of assimilated ^{203}Hg by shrimp following depuration were not observed. Additionally, ^{203}Hg associated with HSP was below detection limits. Kraus et al. (1988) reported induction of MTLP in grass shrimp exposed to high concentrations of dissolved $HgCl_2$. MTLP levels were also higher in shrimp collected from an Hg-contaminated site than for reference shrimp (Kraus et al., 1988). This suggests that tissue concentrations of ^{203}Hg assimilated by shrimp in the present study were not sufficient to induce MTLP or displace MTLP-bound metals (Amiard et al., 2006). Differences in the subcellular distributions of individual metals

(e.g., Cd vs. Hg) may have important toxicological implications for grass shrimp, particularly if a specific metal is not partitioned to biologically-detoxified fractions and is available to interact with metal-sensitive targets (Wallace et al., 2003).

Predicted doses of Cd to grass shrimp from a hypothetical meal in the field suggest that dietary Cd accumulation may be influenced by site-specific differences in AE as well as Cd burdens in prey. This calculation assumes, however, that AE-^{109}Cd% by shrimp pulse-fed ^{109}Cd-labeled amphipods reflects Cd AE following the ingestion of polychaetes *in situ* and that any effects of previous field exposure (e.g., post-assimilatory digestive toxicity) on shrimp subjected to AE analysis were not ameliorated by the loss of assimilated pollutants during a brief (3 d) period of acclimation to laboratory conditions. Metal accumulation by predators in the field may also be influenced by diet composition, ingestion, growth and efflux rates as well as exposure to pollutants through the dissolved phase (Wang and Fisher, 1999a).

The subsequent transfer of Cd and Hg from grass shrimp to predators (e.g., *Fundulus heteroclitus* and *Morone saxatilis*) may depend upon the extent to which assimilated metal is partitioned to TAM compartment in shrimp, which exhibited little variation in this study. Metal assimilation by predators may also be related to tissue metal burdens in shrimp as well as consumer digestive physiology (e.g., solubilization of metal in the gut fluid) in the field (Campbell et al., 2005; Wallace et al., 2008).

CONCLUSION

Previous studies have investigated interspecific differences in the assimilation of dietary metal among aquatic organisms (Chong and Wang, 2000). In the present study,

we have demonstrated intraspecific variability in Cd and Hg assimilation, but not carbon assimilation, by grass shrimp collected along an impact gradient within the NY/NJ Harbor Estuary. Differences in the assimilation of dietary metal may have important long-term toxicological consequences for aquatic invertebrate populations, including behavioral (e.g., impaired prey capture) and reproductive effects (e.g., decreased egg production) (Wallace et al., 2000; Hook and Fisher, 2001). Beyond direct effects of dietary metal on populations, metal exposure at lower trophic levels can impact food webs, resulting in community-wide consequences (Croteau et al., 2005). As a dominant epibenthic organism, the grass shrimp plays a key role in nutrient cycling within estuarine communities and may influence transfer of pollutants along food chains (Welsh, 1975). Since population differences in metal assimilation and partitioning cannot yet be attributed to specific changes in the digestive physiology of grass shrimp, future studies will assess the influence of impacted field conditions on gut residence time, digestive enzyme activities, gut pH and the functional morphology of the gut epithelium in this species.

Table 2-1. Metals in surface sediments (0 to 5 cm) at Staten Island, NY study sites (summer 2004).

	Concentration in sediment ($\mu g\ g^{-1}$)[a]				
Metal	GK	LC	MI	MA	NC
Ag	0.48	0.83	1.38	1.59	1.37
Cd	1.14	1.20	2.88	2.90	1.08
Cu	92.2	125	912	217	153
Hg	0.27	0.28	0.98	1.86	2.46
Ni	50.9	27.9	36.5	70.3	48.9
Pb	98.7	83.9	656	171	215
Zn	188	220	1193	411	339

[a]Mean dry wt. (n = 5) determined following HNO_3 digestion and analysis by graphite furnace atomic absorption spectrometry (Ag, Cd, Cu, Ni, Pb and Zn) or flow injection mercury system (Hg). Data from Goto, 2009.

Table 2-2. Estimated site-specific doses of Cd ($Cd_{dose,\ field}$) to grass shrimp *P. pugio* during a hypothetical meal in the field, calculated as:

$$Cd_{dose,\ field} = AE\text{-}^{109}Cd\% * Cd_{prey,\ field}$$

where $AE\text{-}^{109}Cd\%$ is the site-specific Cd AE observed for grass shrimp in the present study and $Cd_{prey,\ field}$ is the whole tissue Cd burden in field-collected prey (polychaetes *Nereis* spp.) from GK, MI and NC. Proportional differences in $AE\text{-}^{109}Cd\%$, $Cd_{prey,\ field}$ and $Cd_{dose,\ field}$ shown in parentheses are normalized to values for GK (the reference site). Percentages of Cd partitioned to the TAM compartment (TAM-Cd%) in polychaetes are also shown.

Study site	$AE\text{-}^{109}Cd\%$ *P. pugio*		$Cd_{prey,\ field}$[a] *Nereis* spp. (μg g^{-1}) (TAM-Cd%)		$Cd_{dose,\ field}$ (μg g^{-1} ingested tissue)
GK	53.76 (1.00x)	*	0.301 (1.00x) (74.78%)	=	0.162 (1.00x)
MI	74.12 (1.38x)		0.594† (1.97x) (60.26%)		0.440 (2.72x)
NC	75.26 (1.39x)		0.689† (2.29x) (63.67%)		0.519 (3.20x)

[a]Mean dry wt. ($n = 4$) determined following HNO_3 digestion and graphite furnace atomic absorption spectrometry (Goto and Wallace, unpublished). $Cd_{prey,\ field}$ ANOVA (log_{10} transformed data): $p < 0.05$. † = $Cd_{prey,\ field}$ differs significantly from GK (Tukey post-hoc test; $p < 0.05$).

Figure 2-1. Map indicating the location of collection sites for seawater, amphipods, *G. lawrencianus*, and grass shrimp *P. pugio*. Collection sites included Great Kills Harbor (GK), Lemon Creek (LC), Mill Creek (MI), Main Creek (MA), Neck Creek (NC), and Tuckerton, NJ (TK). Richmond Creek (RC) is also mentioned in the text. Labels for waterways in the region are also included: Newark Bay (NB), New York Harbor (NYH), Raritan Bay (RB), Raritan River (RR) and Arthur Kill (AK). Lines that transverse waterways represent major bridges. Adapted from Perez and Wallace, 2004.

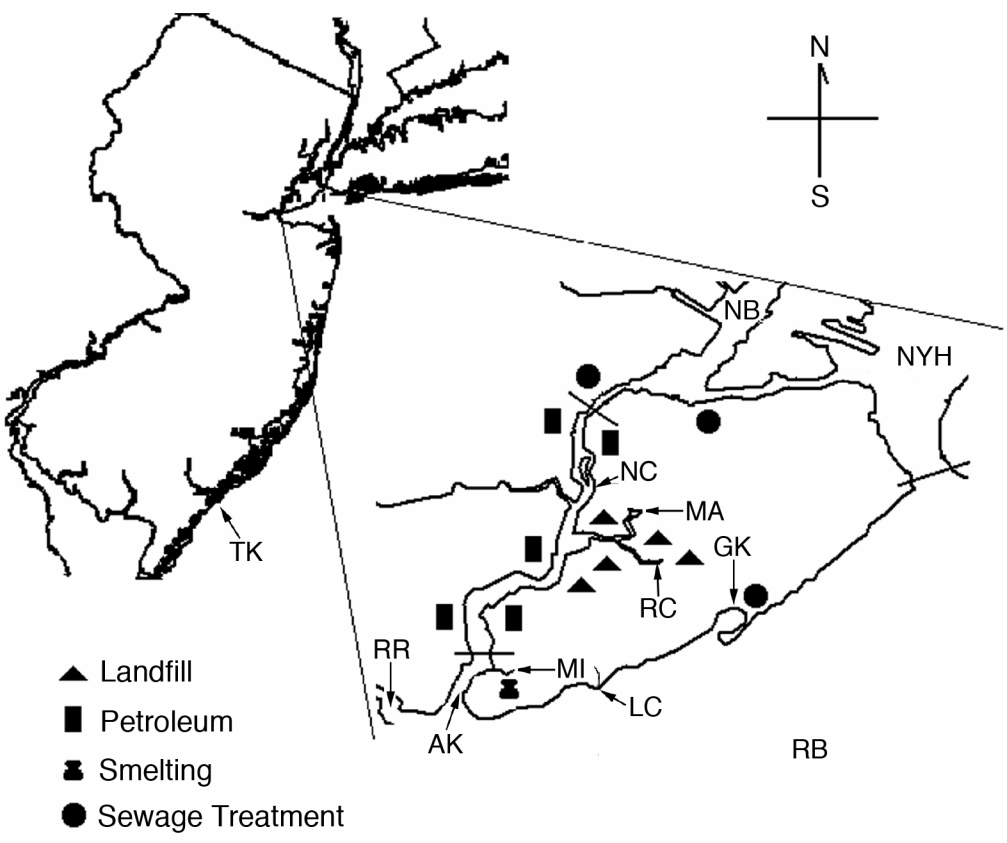
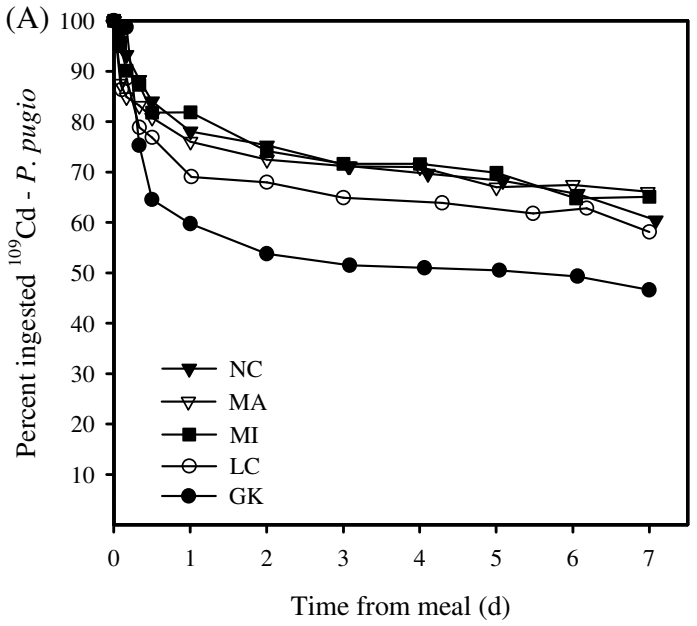


Figure 2-2. Time courses in the retention of (A) ^{109}Cd and (B) ^{203}Hg by grass shrimp *P. pugio* from GK, LC, MI, MA and NC (n = 6 to 11). Standard errors of calculated assimilation efficiencies (retention at t = 48 h) are shown in Fig. 3.



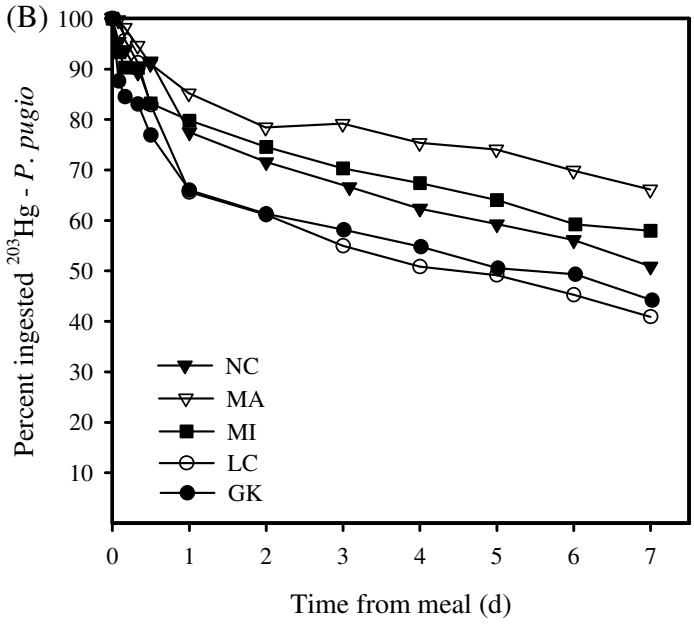


Figure 2-3. (A) AE-^{109}Cd% and (B) AE-^{203}Hg% by grass shrimp *P. pugio* collected from GK, LC, MI, MA and NC (n = 6 to 11; mean ± S.E.). Significant differences ($p < 0.05$) in AE-^{109}Cd% or AE-^{203}Hg% between sites (Tukey-Kramer multiple comparisons test) are indicated by different letters within each panel. † = AE-^{109}Cd% or AE-^{203}Hg% by shrimp differs ($p < 0.05$) from TAM-^{109}Cd% or TAM-^{203}Hg% (indicated by •; n = 4 or 5, respectively; mean ± S.E.) in amphipods *G. lawrencianus* (*t*-test, Welch corrected).

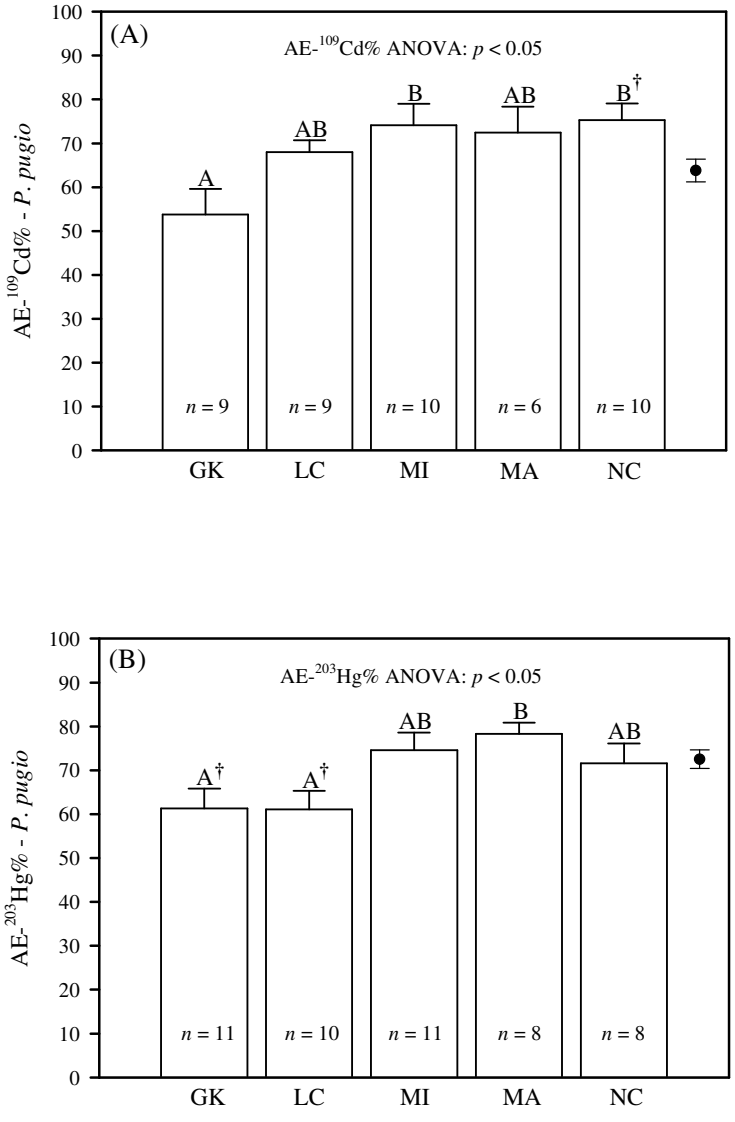
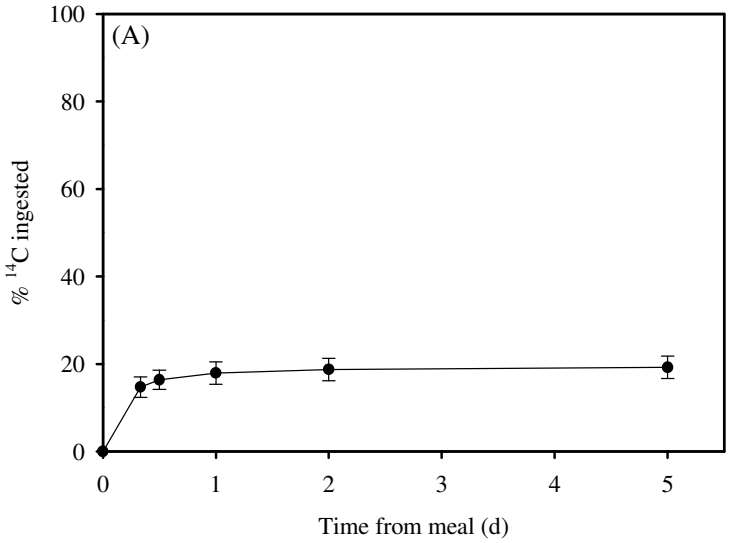


Figure 2-4. (A) Sample egestion curve showing cumulative ^{14}C activity in fecal strands released from grass shrimp *P. pugio* fed prepared ^{14}C-labeled meals during a preliminary study (n = 8). (B) AE-^{14}C% by grass shrimp collected from GK, LC, MI, MA and NC (n = 7 to 13; mean ± S.E.).



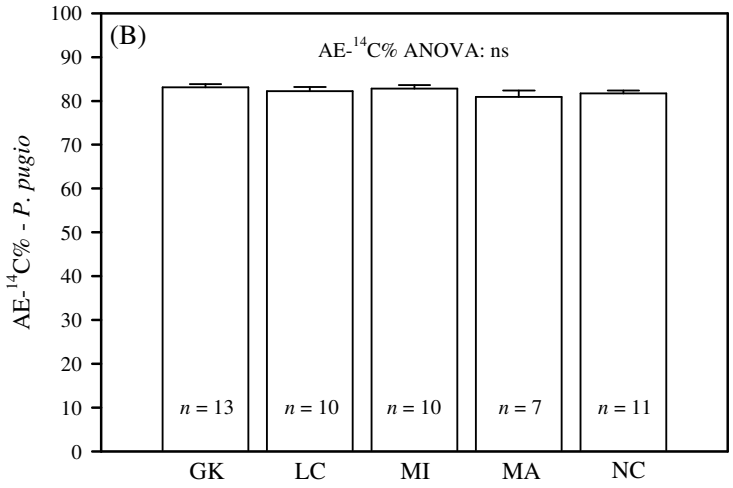
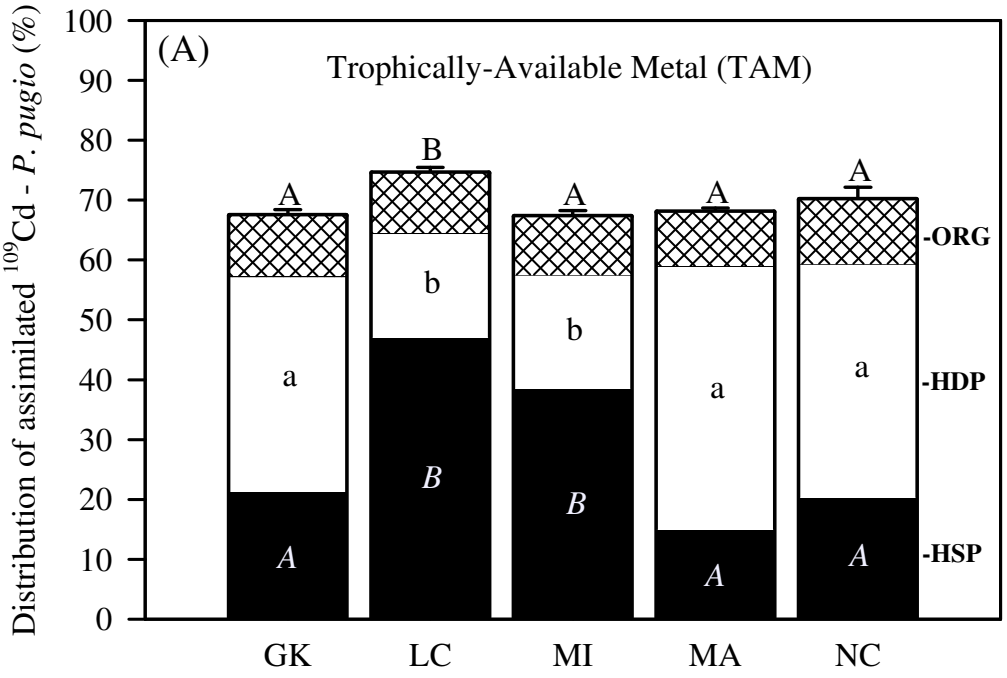
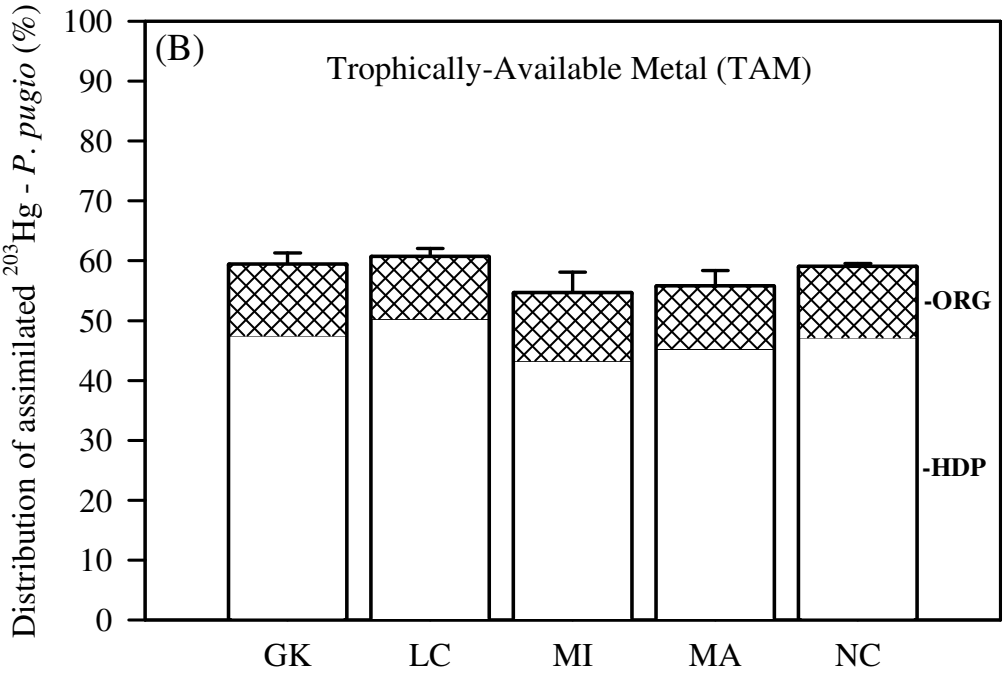


Figure 2-5. Distributions of assimilated (A) ^{109}Cd and (B) ^{203}Hg in the trophically-available metal (TAM) compartment (full bars) and individual subcellular fractions: heat-stable proteins (HSP), heat-denatured proteins (HDP) and organelles (ORG) (embedded within each bar) (n = 3; mean ± S.E.) in grass shrimp *P. pugio* collected from GK, LC, MI, MA and NC. In (A), TAM-^{109}Cd%, HSP and HDP ANOVA: $p <$ 0.05. ORG ANOVA: ns. Significant differences (p = 0.05) between sites (Tukey-Kramer post-hoc) are indicated by different letters. In (B), TAM-^{203}Hg%, HDP and ORG ANOVA: ns.




(B)
Trophically-Available Metal (TAM)
Distribution of assimilated ^{203}Hg - *P. pugio* (%)
100
90
80
70
60
50
40
30
20
10
0
GK
LC
MI
MA
NC
-ORG
-HDP

CHAPTER 3

Assimilation, subcellular partitioning and accumulation of elements by grass shrimp pre-exposed to dietary metal

CHAPTER SUMMARY

Metal assimilation by grass shrimp *Palaemonetes pugio* can be influenced by chronic exposure to impacted field conditions, which may be related to post-assimilatory digestive toxicity (e.g., changes in enzyme activities, gut motility or pH). The present study investigated impacts of pre-exposure to dietary Cd and Hg on the assimilation of Cd, Hg and organic carbon by this species. Shrimp were fed metal-contaminated oligochaetes *Tubifex tubifex* for 15 d, allowed to clear their guts and analyzed for assimilation efficiencies (AE) using radiotracer (^{109}Cd, ^{203}Hg or ^{14}C) pulse-feeding techniques. Subsamples of oligochaetes were subjected to subcellular fractionation to isolate metal associated with a compartment presumed to contain trophically-available metal (TAM). Following 7 d depuration of ingested metal radioisotopes, shrimp were subjected to fractionation to isolate assimilated ^{109}Cd or ^{203}Hg associated with individual subcellular fractions as well as TAM. Subsamples of prey and pre-exposed shrimp were also assessed for stable metal tissue burdens. Relationships between stable Cd partitioned to TAM in prey exposed to the highest Cd concentration and whole tissue Cd burdens in shrimp are consistent with bioenhancement of Cd trophic transfer (i.e., a greater than linear increase in Cd uptake by shrimp with respect to prey exposure) observed in previous work. Disproportionate increases in Hg accumulation by shrimp may be related to predator digestive physiology rather than TAM-Hg burdens in prey. AE-^{109}Cd% was impacted by pre-exposure to dietary Cd, but not in a dose-dependent manner. AE-^{203}Hg was not influenced by previous Cd ingestion. AE-^{109}Cd% exhibited variation that was not dose-dependent and AE-^{203}Hg% reached a plateau following pre-exposure to dietary Hg. AE-^{14}C% was not impacted by pre-exposure to either dietary metal. Moderate

repartitioning of assimilated ^{109}Cd to heat-stable proteins (e.g., metallothionein-like proteins) was observed for Cd pre-exposed shrimp. Variability in ^{203}Hg partitioning to organelles was evident for shrimp pre-exposed to Hg. Percentages of metal radioisotopes partitioned to the TAM compartment in shrimp were not impacted by pre-exposure to dietary metal. Differential impacts of previous dietary metal exposure on the assimilation of elements may be related to metal interactions with ion transport pathways. Grass shrimp may maintain nutrient assimilation by compensating for impacts of metal-induced post-assimilatory toxicity.

INTRODUCTION

Chronic exposure to contaminants in the field can influence the assimilation of elements by aquatic fauna, which may be related to partitioning of elements in food organisms as well as consumer-dependent processes (e.g., digestion) (Wallace et al., 1998; Rainbow et al., 2004a; Goto and Wallace, 2009). Organisms inhabiting impacted aquatic ecosystems may be exposed to contaminants through solution, sediments and the diet. For metals, patterns of accumulation and sublethal toxicity (e.g., effects on growth and reproduction) may vary depending upon the primary exposure pathway (Farag et al., 1999; Hook and Fisher, 2001; Griscom et al., 2002; De Schamphelaere and Janssen, 2004). It is, therefore, necessary to elucidate the independent effects of potential routes of exposure for specific pollutants (e.g., non-essential metals) before determining whether or not interactive (additive, synergistic or antagonistic) pathway effects are important in accumulation and eliciting toxicity in the field (Wang and Fisher, 1999a).

Ingested pollutants may exert digestive toxicity prior to incorporation into consumer tissues (pre-assimilatory toxicity) or as a consequence of tissue burdens resulting from chronic exposure (post-assimilatory toxicity) (Penry, 1998; Campbell et al., 2005; Seebaugh and Wallace, 2009). Pre-assimilatory toxicity may be related to solubilization and bioavailability of pollutants in gut fluid and could potentially impact hydrolysis and absorption of nutrients as well as pollutants during a digestive cycle (Chen and Mayer, 1998; Mayer et al., 2001). Post-assimilatory toxicity may impact enzymes and other components of the digestive milieu and influence nutrient and pollutant absorption by the gut epithelium during subsequent digestive cycles. The effects of pre- and post-assimilatory digestive toxicity on the assimilation of elements may also be

interactive in cases where organisms are exposed over successive digestive cycles (e.g., feeding in impacted field conditions).

The grass shrimp *Palaemonetes pugio* is common in estuaries along the Atlantic and Gulf coasts of North America and has an omnivorous diet that includes diatoms and invertebrates (Welsh, 1975; Kneib, 1985). This species is consumed by a number of finfish species and may play an important role in metal transfer along estuarine food chains (Posey and Hines, 1991; Davis et al., 2003; Seebaugh et al., 2005). In related work, Cd assimilation by naïve shrimp (i.e., shrimp collected from a reference site and not subjected to previous laboratory exposure) was influenced by Cd burdens in prey and may be related to impacts on digestion (Seebaugh et al., 2006). Intraspecific variability in Cd and Hg (but not carbon) assimilation was reported for shrimp collected from differentially-impacted sites within an urbanized estuary and may be related to post-assimilatory digestive toxicity in populations inhabiting industrialized waterways (Seebaugh and Wallace, 2009). Metal ingestion can also have behavioral impacts (e.g., impaired prey capture) on this species (Wallace et al., 2000).

The primary objective of this study was to investigate impacts of previous exposure to dietary metal on the assimilation of Cd, Hg and organic carbon by grass shrimp. This work was conducted by feeding shrimp on Cd- or Hg-contaminated oligochaete prey for 15 d, followed by assimilation efficiency (AE) analysis during radiotracer (^{109}Cd, ^{203}Hg or ^{14}C) pulse-feeding experiments. Relationships between metal radioisotope assimilation as well as stable metal accumulation by pre-exposed shrimp and potential metal availability in prey were examined through analysis of metal partitioning in oligochaetes to a subcellular compartment presumed to contain trophically-available metal (TAM).

TAM consists of metal associated with specific subcellular fractions: heat-stable proteins (HSP – e.g., metallothionein-like proteins [MTLP]), heat-denatured proteins (HDP – e.g., enzymes) and organelles (ORG) (Wallace and Luoma, 2003). Previous studies suggest that TAM may represent bioavailable metal in prey, although predator-dependent processes (e.g., digestion) can be important in determining the extent to which metal is transferred along aquatic food chains (Wallace and Luoma, 2003; Steen Redeker et al., 2007; Goto and Wallace, 2009). Subcellular fractionation techniques were also used to analyze partitioning of assimilated ^{109}Cd or ^{203}Hg to individual fractions as well as TAM in grass shrimp following ~7 d of depuration of ingested radioisotope.

MATERIALS AND METHODS

Field sampling

Adult grass shrimp (~3 cm from tip of rostrum to end of telson) were collected by dip net from Great Kills Harbor, Staten Island, New York, USA and maintained in 38 l glass aquaria containing aerated, filtered seawater (1.0 μm filter, 10 ppt, 18-19 °C) from a reference site in Tuckerton, New Jersey, USA (Weis et al., 2001). Gravid females were excluded from all analyses (Bauer and Abdalla, 2000). Shrimp were fed once on fish food (TetraMin®) following collection and allowed to clear their guts for ~3 d prior to feeding on metal-contaminated food. Additional seawater filtration was provided by an aquarium filter containing activated carbon.

Metal-contaminated prey

Oligochaetes *Tubifex tubifex* were obtained from a commercial supplier (Newman's Fish Food), rinsed with clean seawater (2.5 ppt) and acclimated in 19 l glass

aquaria containing filtered seawater (1.0 μm, 2.5 ppt, 18-19 °C) for ~3 d prior to stable metal exposures. Additional filtration was provided by an aquarium filter. Worms were not fed during acclimation. Exposure solutions were prepared by adding atomic absorption standards (1000 mg l^{-1} Cd or Hg in 3% HCl or 2% HNO_3, respectively) to 19 l aerated, filtered seawater (2.5 ppt, 18-19 °C) in polyethylene carboys. Worms were exposed at nominal metal concentrations: 0.22, 0.44 or 0.88 μM Cd; 0.007, 0.014 or 0.028 μM Hg for 96 h (~0.18 worm ml^{-1}). Exposure concentrations were selected based on worm mortality observed during preliminary exposures, published mortality data for *T. tubifex* and acute exposure concentration criteria for dissolved Cd and Hg (~0.36 μM and ~0.009 μM, respectively) in marine ecosystems (Bouché et al., 2000; US EPA, 1999, 2001). Seawater was spiked ~24 h prior to the addition of worms and collection of water samples for metal analysis. Exposure solutions were renewed at 48 h. Control worms were maintained in unspiked seawater for 96 h, with renewal at 48 h. Gentle aeration was provided by an air stone connected to an aquarium air pump. Following exposure, worms were rinsed, separated into ~2 g batches (~75 individuals), blotted with Kimwipes to remove excess seawater and stored frozen (-80 °C) in scintillation vials. Worms remained intact following freezing and subsequent thawing for feeding experiments. Subsamples of worms were analyzed for whole tissue stable Cd or Hg burdens as well as metal partitioned to TAM.

Pre-exposure to dietary metal

Following clearance of gut contents, shrimp (n = 16 per pre-exposure treatment) were housed in 38 l glass aquaria containing aerated, filtered seawater (1.0 μm, 10 ppt,

18-19 °C) and allowed to feed *ad libitum* on ~75 Cd- or Hg-exposed worms d^{-1} for 15 d (~5 worms $shrimp^{-1}$ d^{-1}). Additional water filtration was provided by aquarium filters containing activated carbon, but was suspended during feeding for ~1.5 h to prevent entrapment of worms within the filter mechanism. Aquarium water and filters were replaced after ~7 d to minimize exposure to metal through solution (Seebaugh et al., 2005). Seawater samples (including controls) were collected periodically, analyzed for Cd or Hg and concentrations of each metal remained at background. Shrimp mortality during pre-exposure was low (0% to ~6%). Pre-exposed shrimp were allowed to clear their gut contents for 2 d prior to feeding on radiolabeled (^{109}Cd or ^{203}Hg) worms or ^{14}C-labeled meals. Subsamples of pre-exposed shrimp were stored frozen (-80 °C) and analyzed for whole tissue Cd or Hg burdens.

Metal AE analyses

Subsamples of acclimated oligochaetes were radiolabeled in 4 l glass bottles containing 3 l aerated seawater, diluted with NANOpure® (Barnstead) deionized water (0.4 μm, 2.5 ppt, 18-19 °C) and spiked with $^{109}CdCl_2$ or $^{203}HgCl_2$ (Isotope Products) for 96 h (~0.18 worms ml^{-1}) without renewal of exposure solutions. ^{109}Cd or ^{203}Hg activities of exposure solutions were 2.22 x 10^2 kBq l^{-1} (~0.032 μM Cd) or 9.25 kBq l^{-1} (~0.005 μM Hg), which were verified through analysis of 5 ml seawater samples. Gentle aeration was provided by a glass pipette connected to an aquarium air pump by plastic tubing. Following exposure, worms were transferred to a 63-μm nylon mesh screen, rinsed with clean seawater and stored frozen (-80 °C). Subsamples of radiolabeled worms were analyzed for partitioning of ^{109}Cd or ^{203}Hg to TAM.

Following clearance of gut contents for 2 d , pre-exposed shrimp were subjected to ^{109}Cd or ^{203}Hg AE analysis as described by Seebaugh and Wallace (2009). Briefly, shrimp were fed on ^{109}Cd- or ^{203}Hg-labeled worms for 45 min, rinsed with seawater and analyzed for radioactivity for ~7 d. AE-^{109}Cd% and AE-^{203}Hg% were determined as percentages of radioactivity retained in shrimp at 48 h, relative to time (t) = 0. The slope of the linear regression fit to the physiological loss component of each retention curve (t > 24 h) was used to characterize loss of radioisotope by pre-exposed shrimp (Benayoun et al.,1974). Due to logistical constraints, retention of ^{109}Cd at t = 48 h was not determined for shrimp pre-exposed to dietary Hg and AE-^{109}Cd% was estimated using the y-intercept method (Wallace et al.,1998). Following ^{109}Cd or ^{203}Hg AE analysis, shrimp were frozen (-80 °C) and later subjected to subcellular fractionation.

Carbon AE analysis

Carbon AE by pre-exposed grass shrimp was estimated using the method of Seebaugh and Wallace (2009 and references therein). Briefly, diatoms *Thalassiosira weissflogii* were radiolabeled in f/2 medium containing $NaH^{14}CO_3$ (0.22 µm; 32 ppt; 18-19 °C; 3.7 x 10^3 kBq l^{-1}) for 7 d and harvested by centrifugation. Diatoms were then combined with homogenates of unexposed *T. tubifex*, cod liver oil and gelatin. Meals consisted of 6 µl portions of the ^{14}C-labeled mixture, which were dispensed onto polycarbonate filters and stored frozen (-80 °C) ~24 h prior to AE analysis. Gelatin-embedded diatoms remained intact following freezing and subsequent thawing for feeding experiments.

Following clearance of gut contents for 2 d, pre-exposed shrimp were fed on ^{14}C-labeled meals for 45 min and then transferred to individual defecation chambers. Fecal strands were collected periodically for 24 h. ^{14}C activities in solubilized shrimp tissues and cumulative feces at 24 h were determined by liquid scintillation counting. Counts were used to estimate ^{14}C AE: $AE\text{-}^{14}C\% = (^{14}C\ retained/^{14}C\ ingested) \times 100$, where $^{14}C\ retained$ is ^{14}C activity in shrimp tissues and $^{14}C\ ingested$ is the sum of $^{14}C\ retained$ and in cumulative feces (Wang and Fisher, 1996). Retention of ^{14}C over time could not be determined for individual shrimp as tissue samples are destroyed prior to scintillation counting (i.e., live organisms cannot be radioanalyzed repeatedly over time).

Subcellular fractionation

To characterize subcellular partitioning of stable metal or metal radioisotopes to the TAM compartment in oligochaetes, subsamples of worms were homogenized in 3.3 ml cold Tris buffer (pH 7.6) and centrifuged at 500 x *g* (15 min at 4 °C) to separate TAM from non-TAM fractions (Seebaugh and Wallace, 2004). Subsamples of homogenized tissues from stable metal exposures were removed prior to centrifugation and later analyzed for whole tissue Cd or Hg burdens. For quality assurance, procedural blanks were processed during fractionation of worms exposed to stable metal. Following ~7 d depuration of ingested ^{109}Cd or ^{203}Hg, shrimp were subjected to five-part fractionation to estimate percentages of assimilated radioisotopes distributed among individual subcellular fractions: HSP, HDP, ORG, insoluble components (INS – e.g., metal-rich granules [MRG]) and cellular debris (e.g., membranes) (Wallace et al., 2003).

Radioanalyses

^{109}Cd- or ^{203}Hg-labeled samples were analyzed by determining photon emissions (88 keV and 279 keV, respectively) using a Wallac Wizard™ 1480 automatic γ counter (Wallac Oy) equipped with a 7.6 cm NaI crystal. ^{109}Cd or ^{203}Hg activities associated with HSP, HDP and ORG were also used to reconstruct TAM-^{109}Cd% or TAM^{203}Hg% in pre-exposed grass shrimp (Wallace and Luoma, 2003). ^{14}C-labeled shrimp tissues and feces were analyzed with a Beckman LS 6500 liquid scintillation counter equipped with an external quench monitor. Counting times for all radiolabeled samples were adjusted to maintain propagated counting errors of 5% or less.

Stable metal analyses

Seawater samples collected during oligochaete exposures and shrimp pre-exposures were diluted with 2% HNO_3 (Cd) or 3% HCl (Hg) and analyzed for stable metal by atomic absorption spectrometry (AAS) (Perkin Elmer 3100 equipped with graphite furnace for Cd; Perkin Elmer FIMS-100 for Hg). Tissues and fractions from Cd-exposed worms and Cd pre-exposed shrimp were thawed, wet-weighed and dried in scintillation vials in a 60 °C oven. Following the addition of 7 mL trace metal-grade 70% HNO_3, samples stood overnight at room temperature and were then heated to complete the reflux reaction and dried. Dried samples were resuspended in 4 mL trace metal-grade 2% HNO_3, filtered (0.45 μM) and analyzed for Cd (Khoury et al., 2008). For quality assurance, method blanks were run during digestions and filtration along with Standard Reference Material 2976 mussel tissue (112% recovery).

Fractions from Hg-exposed worms and subsamples of Hg pre-exposed grass shrimp were thawed, wet-weighed and dried in scintillation vials in a 60 °C oven. Following the addition of trace metal-grade 95% H_2SO_4 and 70% HNO_3 (volume ratio = 4:1), vials were heated in a 60 °C water bath until samples were digested (Goto and Wallace, 2010). After cooling to 4 °C, 5% potassium permanganate was added to each sample until oxidizing conditions were maintained. Samples were allowed to stand overnight at 20 °C following the addition of 5 ml 5% potassium persulfate. Digested samples were decanted into volumetric flasks and 12% sodium chloride-hydroxylamine sulfate was added to reduce excess potassium permanganate (Hatch and Ott, 1968). Samples were brought to final volume with NANOpure® deionized water and analyzed for Hg using $SnCl_2$ as a reducing agent. Method blanks were run during digestions and filtration along with samples of Dogfish Muscle Certified Reference Material (DORM-2) (96% recovery).

Statistical analyses

Stable metal burdens in oligochaetes as well as the effects of pre-exposure to dietary metal on whole tissue metal burdens, AE and subcellular partitioning of metal radioisotopes by shrimp were analyzed using one-way analysis of variance (ANOVA) (Sokal and Rohlf, 1995; Zar, 1999). Metal concentration data were $\log_{10}$ transformed, percentage data were arcsine transformed and normality of transformed data was tested using Shapiro-Wilk's *W* test. Homoscedasticity was tested using Levene's test. Planned (*a priori*) comparisons of stable metal burdens worms and grass shrimp as well as AE and partitioning of metal radioisotopes by shrimp were performed using the *t*-test with

Bonferroni correction (Sokal and Rohlf, 1995). In the case where transformed AE-^{14}C% data (following pre-exposure to dietary Hg) did not meet ANOVA assumptions, treatment effects were analyzed using Kruskal-Wallis (K-W) ANOVA. The Mann-Whitney *U* test was used to compare grand means for AE-^{14}C% between the Cd and Hg pre-exposure treatment groups. Rates of physiological loss of metal radioisotopes were compared through planned testing of equality of slopes (*F*-test) (Sokal and Rohlf, 1995). AE-^{109}Cd% or AE-^{203}Hg% by shrimp and partitioning of radioisotope to TAM in worms were compared using the *t*-test. To evaluate homogeneity of radiolabeled food ingestion by grass shrimp, effects of pre-exposure treatment on ingested radioisotope (counts per minute [cpm] at $t = 0$ for ^{109}Cd and ^{203}Hg; sum of cpm retained by shrimp and in cumulative feces for ^{14}C) were analyzed using ANOVA. Correlations between AE and ingested cpm for shrimp were analyzed for treatments and treatment groups using Pearson product-moment correlation (r) or Spearman rank correlation coefficient (r_s) for non-normal data. Analyses were conducted using STATISTICA version 7.1 (StatSoft) and Instat version 3.10 (GraphPad).

RESULTS

Stable metal accumulation by oligochaetes and grass shrimp

Dissolved Cd concentrations in exposure solution samples collected ~24 h after seawater was spiked were: control (below AAS detection limits), 0.20, 0.38 and 0.81 µM Cd. Whole tissue burdens in oligochaetes increased over the range of Cd exposures from ~0.011 µg g^{-1} in controls to ~17.2 µg g^{-1} Cd (wet wt) in worms from the 0.81 µM Cd treatment (~1600-fold increase) (Fig. 3-1A). The tissue concentration of Cd in worms

exposed to 0.38 µM Cd was ~5.89x greater than for 0.20 µM Cd worms, suggesting greater than linear accumulation of Cd (~3.10x) with respect to exposure concentration. The whole tissue Cd burden in 0.81 µM Cd-exposed worms was ~2.23 x greater than for 0.38 µM Cd worms, indicating a proportional increase (~1.05x) in Cd accumulation with respect to exposure concentration. Tissue concentrations of Cd partitioned to the TAM compartment (TAM-[Cd] – i.e., the dose of Cd potentially available to predators) in oligochaete prey increased over the range of exposures from ~0.0032 to ~7.03 µg g^{-1} (~2200-fold increase) (Fig. 3-1A). Disproportionate increases in TAM-[Cd] with respect to dissolved Cd concentration were observed between the 0.20 and 0.38 µM Cd exposures (~2.22x) as well as between the 0.38 and 0.81 µM Cd exposures (~1.35x). Percentages of Cd partitioned to TAM did not vary significantly among treatments (TAM-Cd% ~28% to ~44%; ANOVA: ns) and were not correlated with exposure concentration or TAM-[Cd] (Pearson r: ns).

Whole tissue burdens in Cd grass shrimp that ingested Cd-contaminated worms for 15 d increased from ~0.0055 to ~0.90 µg g^{-1} (~164-fold increase) over the range of dietary pre-exposures (Fig. 3-1B). Accumulation of Cd by shrimp was less than linear with respect to TAM-[Cd] in prey between the control and 0.58 µg g^{-1} Cd treatments (~0.07x) and between the 0.58 and 2.45 µg g^{-1} (~0.42x) treatments. Shrimp did, however, exhibit a disproportionate (~2.41x) increase in tissue Cd burdens with respect to TAM-[Cd] in prey between the 2.45 and 7.03 µg g^{-1} Cd pre-exposures.

Dissolved Hg concentrations in exposure solution samples were: control (below AAS detection limits), 0.005, 0.02 and 0.03 µM Hg. Tissue Hg burdens in worms increased over the range of Hg exposures from ~0.098 µg g^{-1} in controls to ~0.63 µg g^{-1}

(wet wt) in worms from the 0.03 µM Hg treatment (~6.4-fold increase) (Fig. 3-2A). Hg uptake by worms exposed to 0.02 µM Hg was approximately twice that for 0.005 µM worms, indicating less than linear accumulation (~0.50x) with respect to Cd exposure concentration. Tissue burden in worms exposed to 0.03 µM Hg were ~1.73x than for 0.02 µM worms, suggesting a slightly greater than proportional increase in accumulation (1.15x) with respect to dissolved Hg exposure. Tissue concentrations of Hg associated with the TAM compartment in oligochaetes increases over the range of dissolved Hg exposures from ~0.05 to ~0.30 µg g^{-1} (Fig. 3-2A). Less than linear accumulation of Hg to TAM (~ 0.54x) was observed between 0.005 and 0.02 µM Hg exposures. TAM-[Hg] in 0.03 µM Hg- exposed worms was ~1.09x greater than that for 0.02 µM worms, with respect to dissolved Hg exposure. Percentages of Hg partitioned to TAM decreased from ~55% in controls to ~46 to 50% in worms from the remaining Hg treatments (ANOVA: $p < 0.05$, followed by Bonferonni *t*-test). TAM-Hg% in worms was not correlated with Hg exposure concentration or TAM-[Hg] (Pearson r: ns).

Tissue Hg concentrations in Hg pre-exposed shrimp increased from ~0.009 to ~0.09 µg g^{-1} (~11-fold increase) over the range of treatments (Fig. 3-2B). Hg accumulation was greater than linear with respect to TAM-[Hg] in oligochaetes between the control and 0.05 µg g^{-1} dietary pre- exposures (~1.34x) as well as between the 0.18 and 0.30 µg g^{-1} treatments (~1.35x).

Assimilation of elements by pre-exposed grass shrimp

TAM-^{109}Cd and TAM-^{203}Hg in radiolabeled oligochaete prey were ~83% and ~45%, respectively. AE-^{109}Cd% and AE-^{203}Hg% were determined for grass shrimp pre-

exposed to dietary Cd or Hg, allowed to clear their guts for 2 d and pulse-fed radiolabeled oligochaetes. Depuration was characterized by a three-component loss: a rapid loss of metal in radiolabeled feces, followed by a reduced rate of loss until ~48 h and then a slowly exchanging compartment beginning at $t > 48$ h (Fig. 3-3A to 3-3D). As reported in a related study, grass shrimp fed ^{14}C-labeled meals release radiolabeled feces for ~24 h (Seebaugh and Wallace, 2009). AE-^{14}C was estimated at this time point to minimize potential losses of ingested ^{14}C through respiration and excretion.

AE-^{109}Cd% by Cd pre-exposed grass shrimp decreased from ~45% in controls to ~30% in shrimp following consumption of oligochaetes with TAM-[Cd] of 0.58 µg g^{-1} (Fig. 3-4A). AE-^{109}Cd% increased over the remaining range of Cd pre-exposures with the highest value (~52%) for shrimp that consumed prey with TAM-[Cd] of 7.03 µg g^{-1}. AE-^{109}Cd% was less than TAM-^{109}Cd% in prey for all dietary Cd treatments (Fig. 3-4A). Although analysis of initial cpm in shrimp at $t = 0$ may suggest homogeneity of ^{109}Cd-labeled prey ingestion, AE-^{109}Cd% by Cd pre-exposed shrimp was negatively correlated with ingested cpm over the range of treatments (Table 3-1). AE-^{203}Hg did not vary for Cd pre-exposed shrimp and a direct (~1:1) relationship between AE ^{203}Hg% and TAM-^{203}Hg% in oligochaete prey was observed across Cd treatments (Fig. 3-4B). Homogeneity of ^{203}Hg-contaminated prey ingestion was observed for shrimp from this treatment group and a negative correlation between AE-^{203}Hg and initial cpm was detected over the range of Cd pre-exposures (Table 3-1). Physiological loss of ^{109}Cd (0.91 ± 0.49% to 1.92 ± 1.53% d^{-1}) or ^{203}Hg (2.03 ± 1.22% to 3.36 ± 0.99% d^{-1}) did not exhibit significant variation for Cd pre-exposed shrimp (data not shown; planned testing of equality of slopes [F-test: ns]). AE-^{14}C did not vary among dietary Cd treatments (~84% for all

treatments) (Fig. 3-4C). Homogeneity of ^{14}C-labeled meal ingestion was observed and AE-^{14}C was not correlated with ingested ^{14}C for the Cd pre-exposure treatment group (Table 3-1).

AE-^{109}Cd% by shrimp pre-exposed to dietary Hg was estimated using the *y*-intercept method and varied between ~40% and ~60%, with the greatest difference observed between controls and shrimp that consumed prey with TAM-[Hg] of 0.08 µg g^{-1} (Fig. 3-5A). AE-^{109}Cd% was equal (~48%) for shrimp that consumed prey with TAM-[Hg] of 0.18 and 0.30 µg g^{-1}. AE-^{109}Cd% by Hg pre-exposed shrimp was less than TAM-^{109}Cd% in prey (Fig. 3-5A). AE-^{203}Hg% (i.e., retention of ^{203}Hg at 48 h) by shrimp pre-exposed to dietary Hg increased from ~37% in controls to ~53% for the remaining treatments (Fig. 3-5B). AE-^{203}Hg% exceeded TAM-^{203}Hg% in oligochaetes for shrimp pre-exposed to the highest concentration of dietary Hg. Physiological loss rates for ^{109}Cd (1.18 ± 0.63% to 1.94 ± 0.79% d^{-1}) or ^{203}Hg (2.51 ± 0.60% to 3.96 ± 0.63% d^{-1}) did not differ significantly among dietary Hg treatments (data not shown; planned testing of equality of slopes [*F*-test: ns]). AE-^{14}C did not vary for Hg pre-exposed shrimp (~69% for all treatments) (Fig. 3-5C). Carbon assimilation by shrimp from the dietary Hg treatment group was, however, less than for Cd pre-exposed shrimp (Mann-Whitney *U* test on grand means: $p < 0.05$) (Fig. 3-4C and 3-5C). Homogeneity of radiolabeled prey or prepared meal ingestion was observed for all AE analyses following pre-exposure to Hg (Table 3-2). Assimilation of metal radioisotopes was not correlated with ingested cpm at $t = 0$. AE-^{14}C% was positively correlated with ingested ^{14}C across dietary Hg treatments.

Subcellular partitioning of assimilated ^{109}Cd and ^{203}Hg by grass shrimp

Following 7 d depuration of ingested metal radioisotope, shrimp were subjected to five-part subcellular fractionation to estimate percentages of assimilated metal associated with individual fractions (HSP, HDP, ORG, INS and cellular debris) as well as metal partitioned to TAM (TAM-^{109}Cd% or TAM-^{203}Hg% - i.e., percentages of assimilated metal potentially available to predators). Partitioning of ^{109}Cd to HSP (e.g., MTLP) in Cd pre-exposed shrimp varied between ~22% and ~33%, with the highest value observed following consumption of prey with TAM-[Cd] of 0.58 µg g^{-1} (Fig. 3-6A). Percentages of assimilated ^{109}Cd partitioned to HDP and ORG fractions did not vary significantly among dietary Cd treatments. TAM-^{109}Cd% in Cd pre-exposed shrimp was ~73%. Partitioning of assimilated ^{203}Hg to individual subcellular fractions or TAM-^{203}Hg% (~58%) did not vary for Cd pre-exposed shrimp (Fig. 3-6B). Partitioning of ^{109}Cd by Hg pre-exposed shrimp did not vary for individual fractions or TAM-^{109}Cd% (~76%) (Fig. 3-7A). Shrimp subjected to dietary Hg exposure exhibited differences in partitioning of ^{203}Hg to ORG, with the lowest value observed for shrimp pre-exposed to oligochaete prey with TAM-[Hg] of 0.08 µg g^{-1} (Fig. 3-7B). Partitioning of assimilated ^{203}Hg to HDP, ORG and TAM-^{203}Hg% (~59%) did not vary among Hg pre-exposure treatments. ^{203}Hg signals associated with HSP were below limits of detection for all Cd and Hg pre-exposed shrimp. ^{109}Cd and ^{203}Hg bound to INS (e.g., MRG) was below detection limits for all treatments. Partitioning of assimilated ^{109}Cd or ^{203}Hg to cellular debris (e.g., membranes) did not exhibit significant variability for any treatment group (ANOVA: ns; data not shown).

DISCUSSION

Whole tissue Cd burdens in oligochaetes *T. tubifex* increased disproportionately with respect to dissolved Cd between the 0.20 to 0.38 µM Cd exposures and did not reach a plateau over the entire range of Cd exposures. In previous studies of Cd accumulation by this species, neither concentration nor time-dependent plateaus in whole tissue burdens were observed following exposure to ~0.09 to 10 µM Cd through solution for up to 15 d (Bouché et al., 2000; Steen Redeker and Blust, 2004). Cd uptake by aquatic invertebrates can also be influenced by complexation with available ions (e.g., Cl^-) in exposure solutions (Sunda et al., 1978; Blust et al., 1992). Bouché et al. (2000) reported higher whole tissue Cd burdens in oligochaetes exposed to Cd dissolved in natural spring water despite using dissolved metal concentrations that were lower than used for this study (Cd dissolved in 2.5 ppt seawater). Tissue burdens estimated for Cd-exposed *T. tubifex* in the current study fell within the range of body burdens reported for oligochaetes *Limnodrilus hoffmeisteri* collected from reference and Cd-contaminated field sites (Wallace et al., 2000).

Although percentages of accumulated stable Cd in *T. tubifex* partitioned to the TAM did not exhibit significant variation, the observed increase in TAM-[Cd] tissue burdens was greater than linear with respect to dissolved Cd over the 0.20 to 0.81 µM Cd exposure range. TAM-[Cd] in Cd-exposed *T. tubifex* in this study was also comparable to estimates of bioavailable Cd reported for *L. hoffmeisteri* collected along a Cd gradient in the field (Wallace et al., 2000). Gillis et al. (2004) observed rapid induction of MTLP in *T. Tubifex* in response to Cd exposure through sediments. Increased MTLP concentrations were also reported in this species during exposure to dissolved Cd (0.1

μM) through solution, with levels reaching a plateau after 6 h (Steen Redeker et al., 2007). While oligochaete prey were not subjected to five-part subcellular fractionation to isolate metal associated with HSP (e.g., MTLP) in the present study, HSP-driven increases in Cd partitioning to TAM have been observed in invertebrate prey (brine shrimp) and can result in bioenhancement of Cd transfer along food chains that include grass shrimp (Seebaugh and Wallace, 2004; Seebaugh et al., 2005). It is also possible that any bioenhancement of Cd transfer from oligochaetes to shrimp in the present study may have been exacerbated by post-assimilatory digestive toxicity resulting from metal ingestion over 15 d. For the food chain based on the 0.81 μM Cd worm exposure, this combination of prey- and predator-dependent processes may have resulted in the observed ~3.25-fold increase in tissue Cd burdens in grass shrimp with respect to the 0.38 μM Cd food chain (Goto and Wallace, 2009).

Stable Hg burdens in oligochaetes did not reach saturation over the range of dissolved exposures. Genetic resistance to inorganic Hg has been reported for this species, however, it is not clear if this phenomenon is related to metal detoxification and storage (e.g., via MTLP or MRG) or Hg accumulated and lost via autotomy of caudal segments (Klerks and Bartholomew, 1991; Bouché et al., 2000; Vidal and Horne, 2003a, 2003b). Increases in TAM-[Hg] in worms were less than or roughly proportional with respect to dissolved Hg over 0.005 to 0.03 μM Hg exposure range. Since enhanced Hg partitioning to the TAM compartment in worms was not observed, the ~1.50-fold increase in shrimp Hg burdens between the 0.02 and 0.03 μM Hg food chains may be related to predator digestive physiology (e.g., gut pH) rather than to subcellular partitioning of Hg in prey (Goto and Wallace, 2009; Seebaugh and Wallace,

unpublished).

Patterns of ^{109}Cd assimilation by shrimp pre-exposed to dietary Cd or Hg suggest that effects of metal-induced post-assimilatory digestive toxicity on subsequent metal uptake may not be dose-dependent. Interactive effects on digestive parameters may be important, particularly if organisms are required to compensate for pollutant-impaired gut function to maintain adequate nutrient assimilation (Jumars, 2000). Compensatory mechanisms (e.g., increased gut transit time) may, in turn, influence pollutant assimilation (Seebaugh, et al., unpublished). AE-^{203}Hg% was not impacted by pre-exposure to dietary Cd and reached a plateau in shrimp pre-exposed to Hg. Hg assimilation by control shrimp from the Cd pre-exposure group was higher (~53%) than for controls from the dietary Hg treatments (~37%). These differences may be related to variability in digestion among population cohorts (shrimp pre-exposed to Cd were obtained ~1 month prior to specimens collected for dietary Hg experiments).

Rainbow et al. (2004b) reported that Cd assimilation by barnacles pulse-fed radiolabeled diatoms was not impacted by pre-exposure to dietary Ag, Cd or mixed-metal combinations for 19 d. Variability in Ag and Zn assimilation was observed following pre-exposure to dietary Ag. Interestingly, patterns of variability in metal assimilation by barnacles were similar to those for pre-exposed grass shrimp in the current study in that dose-dependent changes in AE were not observed (Rainbow et al., 2004b). Green mussels exhibited ~2-fold increases in Ag assimilation following pre-exposure to dietary Ag for 3 or 5 weeks (Shi et al., 2003). Cd AE increased while Zn assimilation decreased in whelks fed a Cd-enriched diet (Blackmore & Wang, 2004). Dose-dependent effects of pre-exposure to dietary metal on AE by mussels and whelks could not be established

since organisms in each study were subjected to two dietary treatments (Shi et al., 2003; Blackmore and Wang, 2004).

Depuration of ^{109}Cd and ^{203}Hg by pre-exposed grass shrimp was characterized by a three-compartment loss observed in previous work with this species and suggests a biphasic digestive cycle similar to that observed for other decapods (Icely and Nott, 1992; Decho and Luoma, 1991; Seebaugh and Wallace, 2009). The first compartment may reflect metal lost to particle sorting by cardiac setal screens and the gland filter before ingested materials reach the hepatopancreas (Icely and Nott, 1992; Wang and Fisher, 1999b). Rejected particles are circulated toward the midgut and packaged in feces (Dall and Moriarty, 1983; Felgenhauer, 1992). This compartment may also represent loss of residual materials from extracellular digestion (Decho and Luoma, 1991). The second compartment may reflect loss of metal absorbed by hepatopancreatic blister (B) cells, which are expelled and packaged in feces during the digestive cycle (Al-Mohanna and Nott, 1986; Vogt, 1993; Wang and Fisher, 1999b). Metal bound to ligands within the lumen of the hepatopancreas (unabsorbed materials or byproducts of intracellular digestion) may also be lost to feces during this phase. The slowest exchanging compartment represents physiological loss of assimilated metal (Wang and Fisher, 1999b). Rates of physiological ^{109}Cd or ^{203}Hg loss did not vary within pre-exposure treatment groups, indicating that elimination may not have been impacted by previous metal accumulation. This result also suggests that specific cell types involved in materials accumulation and storage (e.g., resorptive [R] cells of the hepatopancreatic epithelium) have a large capacity for metal sequestration (e.g., in insoluble MRG), a mechanism necessary for protection of sensitive intracellular components (e.g., enzymes and

organelles) as well as preservation of tissue functions (Brown, 1982; Al-Mohanna and Nott, 1987; Rainbow, 2002; Wallace et al., 2003).

Relationships between metal radioisotope ingestion and assimilation were not evident for grass shrimp exposed to a range of Cd burdens in prey or collected along a pollution gradient in previous work (unpublished analyses of data presented by Seebaugh et al., 2006 and Seebaugh and Wallace, 2009). If not a statistical artifact, the negative correlation between ingested radioactivity and ^{109}Cd assimilation by Cd pre-exposed shrimp in the current study suggests that inhibition of divalent cation transport across the hepatopancreatic epithelium may be important in determining the extent of Cd assimilation. Analyses of cation transport processes using brush border membrane vesicles isolated from lobster hepatopancreas suggest that Cd^{2+} ions may be transported across apical epithelial cell surfaces via an amiloride-sensitive $2Na^{+}/1H^{+}$ antiporter, one of several mechanisms involved in Ca^{2+} exchange (Ahearn et al., 1994; Zhuang and Ahearn, 1996). Verapamil-sensitive transmembrane Ca^{2+} channels have also been identified in the lobster as well as in B, but not R, cells in the hepatopancreas of a penaeid shrimp and may facilitate diffusion of elements with similar ionic radii (e.g., Cd^{2+}) across brush border surfaces (Simkiss, 1996; Zhuang and Ahearn, 1996; Zilli et al., 2000). If Ca^{2+} transport into the hepatopancreatic epithelium is impaired by previous Cd^{2+} exposure or inhibited by excess Cd^{2+} in gut fluid during a digestive cycle, available metal may not be absorbed and incorporated into grass shrimp tissues (Ahearn et al., 1994; Rainbow and Black, 2005).

In the case of grass shrimp that consumed prey with TAM-[Cd] of 0.58 $\mu g\ g^{-1}$, excess ^{109}Cd ingested during AE analysis and available in gut fluid may not have been

transported across epithelial membranes and made available for assimilation. Although this scenario may seem unlikely with trace Cd concentrations of used for AE analysis, impacts of post-assimilatory digestive toxicity on metal assimilation may be important, particularly if brush border surfaces of the hepatopancreas are damaged and mechanisms of Ca^{2+} exchange impaired by previous exposure to metal. Furthermore, although metal assimilation is assumed to be complete by 48 h after ingestion, it is not known whether or not all residual Cd from the previous digestive cycle (e.g., final pre-exposure feeding) would be cleared from hepatopancreatic tubules or potentially available to impact Ca^{2+} (and Cd^{2+}) transport across the gut epithelium during AE analysis (Icely and Nott, 1992).

In a companion study, median minimum gut residence time (GRT) estimated for shrimp following ingestion of prey with TAM-[Cd] of 7.03 μg g^{-1} (~350 min) was approximately half the values for shrimp from the 0.58 and 2.45 μg g^{-1} Cd treatments (~720 min) (Seebaugh et al., unpublished). AE-^{109}Cd% following pre-exposure to dietary Cd was highest for shrimp from the 7.03 μg g^{-1} treatment, which also ingested the least amount of radioisotope. Interestingly, ^{109}Cd assimilation by shrimp from this treatment may have been limited by a Cd-induced decrease in GRT, which could have reduced the time available for Cd^{2+} transport from gut fluid. Selck et al. (1999) reported that the direct relationship between Cd AE and GRT in polychaetes was reversed following pre-exposure exposure to Cd-contaminated sediments. These results suggest that previous exposure to dietary metal can induce changes in digestive physiology that, in turn, influence assimilation during subsequent feeding cycles. Fecal strand elimination rate (a possible surrogate for ingestion rate) as well as ingestion of meals used to analyze extracellular digestive protease activities did not vary for grass shrimp pre-exposed to

dietary Cd (Seebaugh et al., unpublished). An explanation for the variation in ^{109}Cd ingestion within this treatment group in the current study remains elusive. Previous exposure to pollutants (including metals) can also impact digestive enzyme activities as well as chemical characteristics of gut fluid (e.g., pH and surfactancy) that may influence hydrolysis and absorption of nutrients as well as solubilization and bioavailability of pollutants (Anderson, et al., 1997; Ahrens et al., 2001; Mayer et al., 2001; Seebaugh et al., unpublished).

Free intracellular Cd can bind to metallothioneins and be stored in detoxified form in insoluble, sulfur-containing MRG (Hopkin, 1989). This process appears to take place in R cells of the decapod hepatopancreas, which may be involved in accumulation, storage and elimination of metal over the course of the molt cycle (Hopkin and Nott, 1979; Al-Mohanna and Nott, 1987). Unsequestered intracellular Cd^{2+} or metal not bound to metal-sensitive targets (e.g., enzymes and organelles) could be available for transport across basolateral membranes of the hepatopancreatic epithelium to the haemolymph (via mechanisms of Ca^{2+} exchange), circulated and incorporated into other tissues (Zhuang and Ahearn, 1998; Ahearn et al., 2004). Metals can also be transported from the haemolymph to hepatopancreatic R cells for storage and eventual elimination (Al-Mohanna and Nott, 1987). Relationships between Ca^{2+} exchange pathways and Cd^{2+} transport across basolateral surfaces to and from the haemolymph are not well-understood and require additional study (Ahearn et al., 2004).

AE-^{203}Hg% did not vary for shrimp from the dietary Cd treatments, suggesting that Hg assimilation is not related to Ca^{2+} exchange. Inorganic Hg accumulation and flux across intestine isolated from blue crabs were influenced by temperature, inhibited by the

Na^+/K^+-ATPase inhibitor ouabain and impacted by organic ligands in a manner indicating that Hg transport across decapod gut epithelial surfaces may result from a combination of energy-dependent (e.g., a Na^+/K^+ pump) and passive ion exchange pathways (Andres et al., 2002; Laporte et al., 2002). Although Hg^{2+} and Ca^{2+} have similar ionic radii, studies of Hg accumulation in vertebrate cells have reported lack of competitive inhibition by other divalent cations (e.g., $Ca^{2+}$and Cd^{2+}) and no inhibitory effects of Ca^{2+} channel blockers that otherwise impact Cd accumulation (Blazka and Shaikh, 1991, 1992; Wicklund Glynn et al., 1994; Endo et al., 1997). These lines of evidence suggests that Hg transport may not involve transmembrane Ca^{2+} channels. It has been proposed that membrane fluidity, membrane potential and a HCO_3^-/Cl^- transporter (which may allow transport of negatively charged Hg-chloride complexes) are involved in Hg accumulation by epithelial tissues (Endo et al., 1997; Andres et al., 2002). Dissolved Hg exposure impacted Na^+ and Cl^- ion balance in Chinese mitten crab haemolymph and was likely related to impaired Na^+/K^+-ATPase activity and interference with Cl^- channels in gills (Péqueux et al., 1996). Haemolymph concentrations of Na^+ and Ca^{2+} in crayfish decreased following Hg ingestion and may have resulted from interactions between positive Hg complexes and mechanisms of ion transport (Wright and Welbourn, 1993; Andres et al., 2002). Although the pathway has not yet been identified, Hg can also be transported from haemolymph across basolateral membranes and accumulate in R cells of the hepatopancreas (Andersen and Baatrup, 1988).

Although Hg assimilation by grass shrimp was not influenced by pre-exposure to dietary Cd, AE-^{109}Cd% was impacted by prior Hg ingestion. Aduayom et al. (2003) investigated metal transport across human intestinal epithelial cells and reported that

initial Cd uptake was saturable and inhibited by Hg and *N*-ethylmaleimide (NEM; a sulfydryl group blocker). Hg accumulation did not reach initial saturation and was not inhibited by Cd or NEM, indicating that interactions between Cd and Hg are not reciprocal. This evidence suggests that uptake is through different pathways and that Hg can act as sulfydryl blocker, disrupting Cd absorption through interference with binding to sulfydryl groups within plasma membrane carrier proteins (Shaikh et al., 1995; Aduayom et al., 2003).

In a related study, pH within the proventriculus of shrimp increased over the range of dietary Hg pre-exposures and was accompanied by an inverse relationship between AE-^{203}Hg% and free H^+ ion concentrations in gut fluid (Seebaugh et al., unpublished). Competition between Hg^{2+} ions and protons in circulating gut fluid for ligands (e.g., sulfhydryls within the amino acid cysteine or ion transport channels) may have been reduced in Hg pre-exposed shrimp, resulting in increased Hg assimilation in the present study (Laporte et al., 2002). AE-^{203}Hg% reached a plateau across dietary Hg treatments, indicating that Hg transport into the hepatopancreatic epithelium can be saturated during a digestive cycle. Stable Hg accumulation by shrimp did not reach a plateau after 15 d ingestion of Hg-contaminated food, suggesting that Hg incorporation into tissues may not limited by saturable digestive processes over several digestive cycles. Additional research is required to determine whether Hg concentrations in specific or whole shrimp tissues reach equilibrium after long-term dietary exposure (i.e., feeding in Hg-impacted field conditions) (Galay Burgos and Rainbow, 1998; Rainbow, 2002)

Percentages of Cd partitioned to TAM in radiolabeled oligochaetes (TAM-^{109}Cd

~83%) were considerably higher than for worms exposed to stable Cd (TAM-Cd% ~44% or less). This variation may be related to differences in exposure protocol (radiolabeling solutions were not renewed), although this may not account for substantial differences between TAM-Cd% in ^{109}Cd-exposed worms and stable Cd controls (TAM-Cd% ~28%). Steen Redeker et al. (2007) observed corrected TAM-Cd% values (Cd associated with cellular debris was included in the original calculation) for *T. tubifex* exposed to 0.1 µM Cd (with renewal of exposure solutions) at ~67%. Good agreement between stable Cd partitioned to bioavailable fractions (i.e., TAM) and ^{109}Cd distributions following short-term exposure (i.e., ^{109}Cd followed the distribution of stable Cd) was found for field-collected *L. hoffmeisteri* (Wallace et al., 1998).

AE-^{109}Cd% by grass shrimp was significantly less than TAM-^{109}Cd% in oligochaete prey for all dietary treatments in the present study, including controls. This finding is inconsistent with previous work, where direct (~1:1) relationships between percentages of Cd assimilated by shrimp and TAM in prey were observed following consumption of field-collected oligochaetes or prey (brine shrimp or amphipods) from control treatments or field sites (Wallace et al., 1998; Seebaugh and Wallace, 2004, 2009). Relationships between AE by shrimp and TAM can also be impacted by tissue Cd burdens in prey, which may result from digestive toxicity (Seebaugh et al., 2005, 2006). AE-^{203}Hg% by grass shrimp exceeded TAM-^{203}Hg in prey for highest dietary Hg treatment, but was consistent with TAM for other pre-exposure treatments (including controls). In previous work, AE-^{203}Hg% by field-exposed shrimp did not exceed TAM-^{203}Hg% in prey (Seebaugh and Wallace, 2009). These lines of evidence suggest that while TAM may serve as an estimate of maximum metal (Cd, Hg and Zn) bioavailability

in invertebrate prey, correspondence between metal assimilation and TAM may also depend upon consumer digestion (Steen Redeker et al., 2007; Goto and Wallace, 2009; Seebaugh and Wallace, 2004, 2009).

Assimilation of organic carbon by grass shrimp was not impacted by previous exposure to dietary metal, although AE-^{14}C% by Hg pre-exposed shrimp (~69%) was lower than values estimated for Cd pre-exposed shrimp in previous studies (~79%-83%) using the mass balance calculation of AE as well as Cd pre-exposed shrimp in the present study (~84%) (Johannes and Satomi, 1966; Morgan, 1980; Seebaugh and Wallace, 2009). Carbon assimilation by this species does not appear to be correlated with variability in temperature, salinity, diet, ingestion rate or GRT (Johannes and Satomi, 1966; Morgan, 1980; Seebaugh et al., unpublished). ^{14}C-labeled diatoms from a single radiolabeling event were used to prepare all meals used for AE-^{14}C% experiments in this study. Since AE-^{14}C% analyses for Cd and Hg pre-exposure treatment groups were conducted one year apart, differences between groups may be related to yearly variation in assimilation of nutrients.

Nutrient absorption following extracellular digestion within the hepatopancreatic tubules has been attributed to R cells, which also store lipids and glycogen (Al-Mohanna and Nott, 1987; Sousa et al., 2005). Al-Mohanna and Nott (1987) did not observe evidence of endocytotic activity within apical regions of R cells in penaeid shrimp and proposed that soluble materials (e.g., amino acids, monosaccharides and lipid precursors) move across brush border surfaces via diffusion or active transport. Glucose transport across apical surfaces of R cells may be passive and driven by intracellular glucose concentrations that fluctuate during glycogen synthesis (Verri et al., 2001). Apical

endocytotic vesicles were observed in R cells of *Palaemonetes argentinus*, but any role in nutrient absorption remains to be determined (Sousa et al., 2005). An Na^+/D-glucose transporter has been implicated in glucose transport into B cells (Vilella et al., 2003). B cells also remove materials from the lumen of the hepatopancreas through endocytosis (Al-Mohanna and Nott, 1986; Sousa et al., 2005). This process may be related to nutrient absorption or clearance of residual waste materials in preparation for the next digestive cycle (Hopkin and Nott, 1980; Al-Mohanna and Nott, 1986; Vogt, 1993; Sousa et al., 2005). Intracellular digestion may also take place within B cells (Icely and Nott, 1992). Protein absorption by cells isolated from insect midgut epithelial tissues was shown to involve receptor-mediated endocytosis (Casartelli et al, 2008). Cd inhibition of this process in vertebrate kidney cell lines appears to be related to a defect in receptor recycling due to impaired endosomal acidification (Choi et al., 1999). Since AE-^{14}C% by grass shrimp was not impacted by pre-exposure to Cd or Hg, it appears that nutrient absorption and transport were not impaired by prior exposure or residual metal from earlier digestive cycles (Icely and Nott, 1992). This phenomenon was observed in shrimp subjected to chronic field exposure, although compensation for possible impacts of post-assimilatory toxicity (e.g., increased GRT to offset reduced digestive enzyme activities) may be important in maintaining adequate nutrient assimilation (Seebaugh and Wallace, 2009; Seebaugh et al., unpublished). In related work, carbon AE by naïve shrimp was not impacted by tissue Cd burdens in prey, indicating that nutrient absorption and transport may be unresponsive to metal exposure through gut fluid (Seebaugh, unpublished).

Partitioning of assimilated ^{109}Cd to the TAM compartment (the fraction of metal potentially available to finfish and other predators) in Cd pre-exposed shrimp remained

constant despite moderate repartitioning between the HSP and HDP fractions. Although repartitioning was not dose-dependent, increased ^{109}Cd associated with HSP for shrimp that consumed prey with TAM-[Cd] of 0.58 μg g^{-1} may indicate MTLP induction or displacement of metal (e.g., Cd or Hg from pre-exposure feeding) from the MTLP pool (Amiard et al., 2006). Increased partitioning of Cd to HSP may protect sensitive targets, including enzymes in the HDP fraction (Wallace et al., 2000). ^{203}Hg activities associated with the HSP fraction were below detection limits for all dietary metal treatments. This same phenomenon was observed for grass shrimp collected along a pollution gradient (Seebaugh and Wallace, 2009). Induction of MTLP in grass shrimp can be induced by Hg exposure, indicating that concentrations of ^{203}Hg assimilated by shrimp in the current study were not sufficient to induce MTLP or displace metal from existing MLTP (Kraus et al., 1988). Since ^{109}Cd and ^{203}Hg activities associated with the INS fraction were below detection limits, the importance of assimilated metal storage in insoluble MRG in pre-exposed shrimp cannot be determined from fractionation data and requires additional study (e.g., ultrastructural localization of metal in R cells) (Andersen and Baatrup, 1988). Substantial percentages of assimilated ^{109}Cd (24% to 27%) and ^{203}Hg (~42%) were associated with cellular debris. Although the toxicological significance of metal associated with this fraction is not known, metals with high affinities for functional groups (e.g., sulfydryls) within membranes could interfere with exchange of essential ions or impact organelle functions through membrane destabilization (Viarengo, et al., 2000; Aduayom et al., 2003; Wallace et al., 2003). A portion of metal associated with the cellular debris fraction may also be available to predators (Wallace and Lopez, 1997; Cheung and Wang, 2005).

CONCLUSION

Intraspecific variability in metal assimilation by grass shrimp may be related to digestive toxicity induced by previous exposure to pollutants in the field (Seebaugh and Wallace, 2009). Since populations in industrialized estuaries may be exposed to suites of contaminants through multiple routes of exposure, it is important to assess impacts of exposure to individual pollutants via specific pathways on subsequent accumulation and toxicity. The results of the present study suggest that previous exposure to dietary Cd and Hg can have differential impacts of the assimilation of elements that may be related to metal-specific mechanisms of entry into the gut epithelium as well as metal-metal interactions that influence uptake through ion transport pathways. Interactions that impact metal assimilation may have important implications for toxicity (e.g., effects on behavior or reproduction), particularly for aquatic organisms subjected to mixed metal exposure in the field (Wallace et al., 2000; Hook and Fisher, 2001; Goto, 2009). Carbon assimilation by grass shrimp was not impacted by pre-exposure to dietary metal, or chronic exposure to polluted field conditions in previous work, suggesting that this species may compensate for effects of post-assimilatory digestive toxicity by adjusting digestive parameters (e.g., GRT) to maintain adequate nutrient assimilation (Seebaugh and Wallace, 2009). Related studies will describe relationships between assimilation and digestive physiology (e.g., GRT, extracellular digestive enzyme activities, gut pH and functional morphology of gut tissue) in this species.

Table 3-1. Effects of pre-exposure to dietary Cd on radiolabeled food consumption by grass shrimp *P. pugio*, analyzed using one-way ANOVA. Ingested cpm for ^{109}Cd or ^{203}Hg is counts per minute at time (t) = 0 for individual shrimp during AE analysis. Ingested cpm for ^{14}C is the sum of ^{14}C retained and ^{14}C in cumulative feces for individual shrimp at t = 24 h. Correlations between AE and ingested cpm were analyzed for individual pre-exposure treatments as well as treatment groups using Pearson product-moment correlation (r, except where indicated). Data that did not fit a normal distribution were analyzed using Spearman rank correlation (r_s).

	Treatment (n)	Mean ingested cpm	AE vs. ingested cpm (r or r_s)
^{109}Cd:	Control[a] (9)	1498.5 ± 272.2	-0.5384 (ns)
	0.58 µg g^{-1} Cd (10)	2135.9 ± 280.1	-0.2816 (ns)
	2.45 µg g^{-1} Cd (11)	1832.9 ± 180.8	-0.3594 (ns)
	7.03 µg g^{-1} Cd (9)	1174.3 ± 241.9	-0.4924 (ns)
	All treatments	1681.4 ± 130.5	-0.5288 ($p < 0.05$)
	Ingested ^{109}Cd cpm ANOVA: ns (p = 0.0520)		
^{203}Hg:	Control (8)	427.2 ± 80.4	0.1656 (ns)
	0.58 µg g^{-1} Cd (9)	446.9 ± 43.5	-0.1317 (ns)
	2.45 µg g^{-1} Cd (10)	480.6 ± 49.1	-0.7089 ($p < 0.05$)
	7.03 µg g^{-1} Cd (10)	521.9 ± 53.9	-0.7630 ($p < 0.05$)
	All treatments	472.0 ± 27.7	-0.4507 ($p < 0.05$)
	Ingested ^{203}Hg cpm ANOVA: ns		
^{14}C:	Control (11)	7165.6 ± 1966.1	0.4308 (ns)
	0.58 µg g^{-1} Cd (8)	8896.6 ± 3204.3	-0.1422 (ns)
	2.45 µg g^{-1} Cd (9)	5333.7 ± 1247.9	-0.2053 (r_s; ns)
	7.03 µg g^{-1} Cd (10)	13417.6 ± 1765.1	-0.0376 (ns)
	All treatments	8741.5 ± 1113.5	0.1049 (r_s; ns)
	Ingested ^{14}C cpm ANOVA: ns		

[a] TAM-[Cd] in oligochaete prey (wet wt); control = 0.0032 µg g^{-1} Cd; [ns] ANOVA, r or r_s not significant

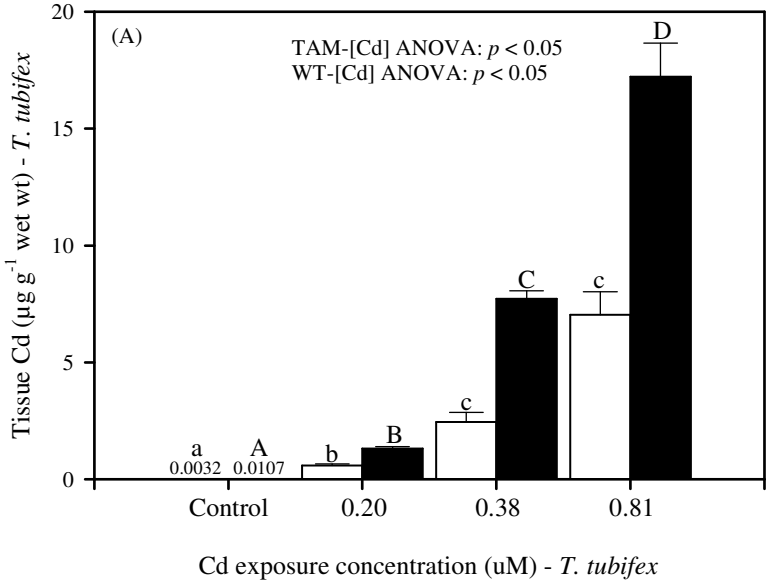
Table 3-2. Effects of pre-exposure to dietary Hg on radiolabeled food consumption by grass shrimp analyzed using one-way ANOVA. Details of analyses are as described for Table 3-1.

	Treatment (*n*)	Mean ingested cpm	AE vs. ingested cpm (r or r_s)
^{109}Cd:	Control[a] (9)	841.8 ± 143.3	-0.1041 (ns)
	0.08 µg g^{-1} Hg (10)	1287.5 ± 216.8	0.0219 (ns)
	0.18 µg g^{-1} Hg (8)	1124.6 ± 131.3	0.0199 (ns)
	0.30 µg g^{-1} Hg (9)	845.9 ± 95.4	-0.5718 (ns)
	All treatments	1029.5 ± 83.0	-0.1116 (ns)
	Ingested ^{109}Cd cpm ANOVA: ns		
^{203}Hg:	Control (7)	496.1 ± 76.7	0.1761 (ns)
	0.08 µg g^{-1} Hg (11)	556.1 ± 40.6	-0.2181 (ns)
	0.18 µg g^{-1} Hg (8)	574.3 ± 83.9	-0.5608 (ns)
	0.30 µg g^{-1} Hg (11)	442.2 ± 39.4	-0.0316 (ns)
	All treatments	514.8 ± 28.7	-0.1953 (ns)
	Ingested ^{203}Hg cpm ANOVA: ns		
^{14}C:	Control (11)	7609.6 ± 2390.1	0.8728 (r_s; $p < 0.05$)
	0.08 µg g^{-1} Hg (11)	10784.6 ± 2923.2	0.6707 ($p < 0.05$)
	0.18 µg g^{-1} Hg (9)	7499.9 ± 2220.3	0.5258 (ns)
	0.30 µg g^{-1} Hg (12)	6551.5 ± 1503.3	0.7210 ($p < 0.05$)
	All treatments	8103.5 ± 1137.2	0.7874 (r_s; $p < 0.05$)
	Ingested ^{14}C cpm ANOVA: ns		

[a] TAM-[Hg] in oligochaete prey (wet wt); control = 0.05 µg g^{-1} Hg

[ns] ANOVA, r or r_s not significant

Figure 3-1. Tissue Cd concentrations in (A) oligochaetes *T. tubifex* following exposure to dissolved Cd for 96 h and (B) grass shrimp *P. pugio* following consumption of dietary Cd (n = 4; mean ± S.E.). WT-[Cd] = whole tissue Cd burden in oligochaetes or shrimp (filled bars). TAM-[Cd] = Cd partitioned to the compartment containing trophically-available metal in oligochaetes (open bars). Significant differences ($p < 0.05$) in Cd burdens are indicated by different letters above each bar (t-test with Bonferroni correction).



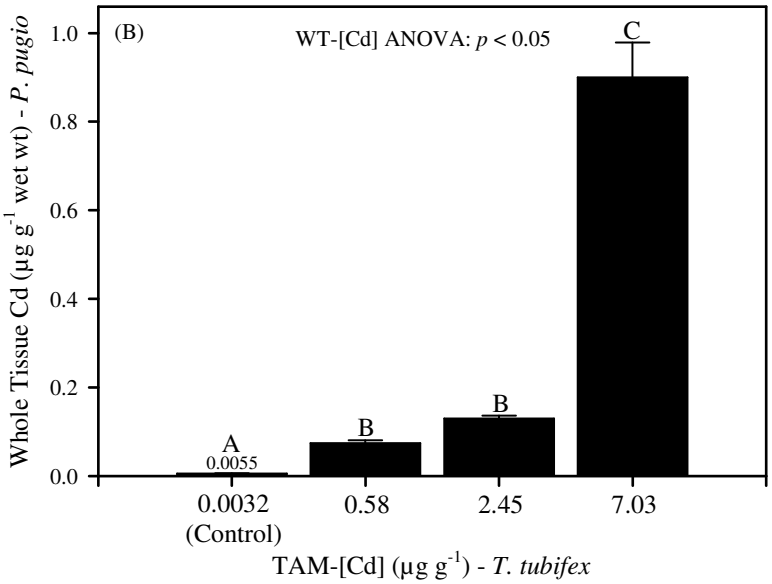
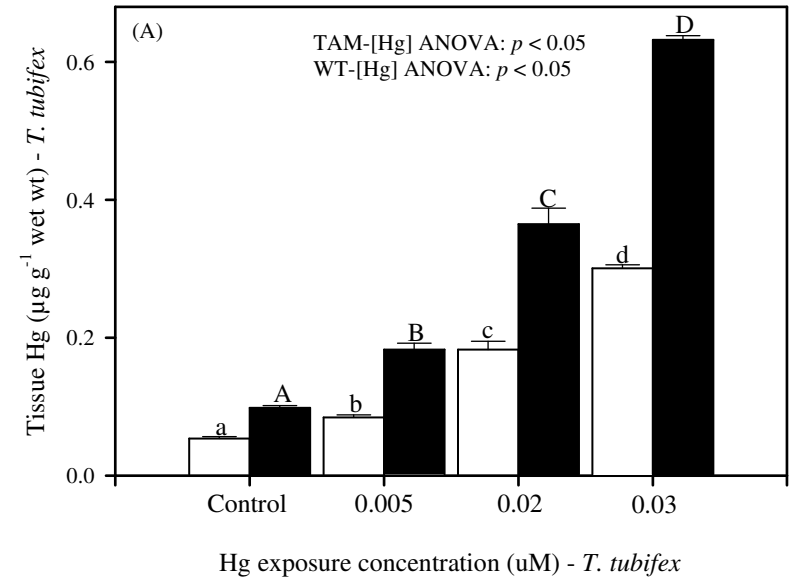


Figure 3-2. Tissue Hg concentrations in (A) oligochaetes *T. tubifex* following exposure to dissolved Hg for 96 h and (B) grass shrimp *P. pugio* following consumption of dietary Hg (n = 4; mean ± S.E.). WT-[Hg] = whole tissue Hg burden in oligochaetes or shrimp (filled bars). TAM-[Hg] = Hg partitioned to the compartment containing trophically-available metal in oligochaetes (open bars). Significant differences ($p < 0.05$) in Hg burdens are indicated by different letters above each bar (t-test with Bonferroni correction).



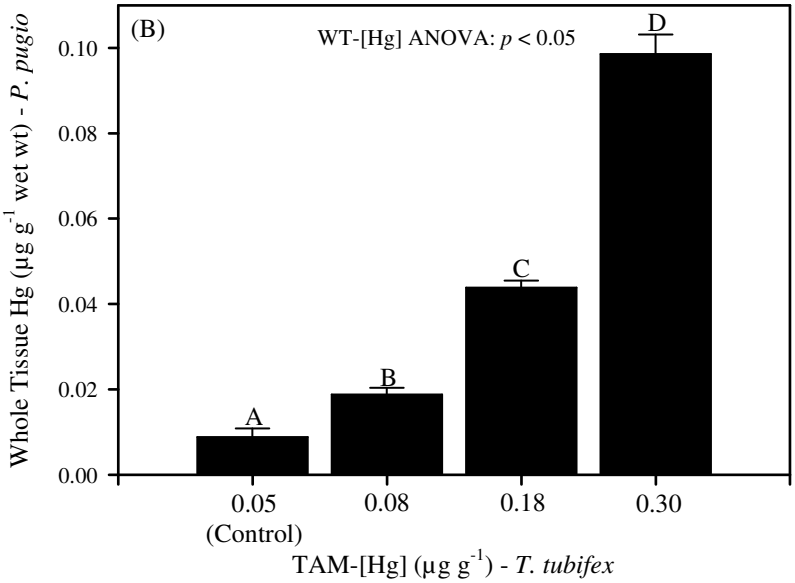
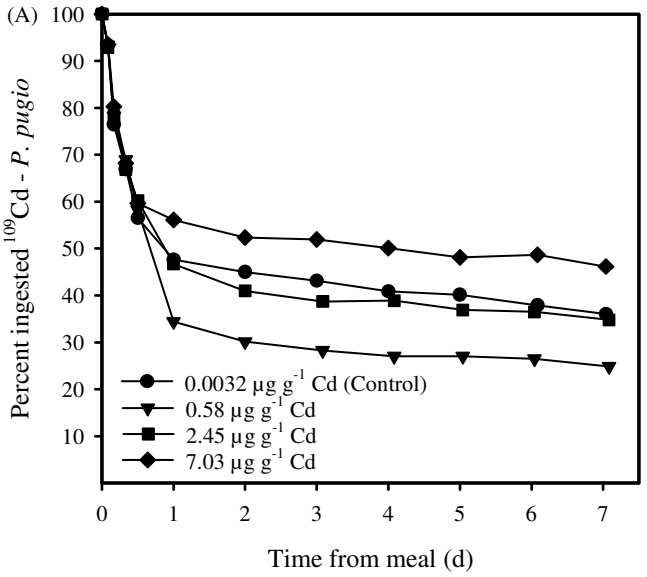


Figure 3-3A and 3-3B. Time courses in the retention of (A) ^{109}Cd or (B) ^{203}Hg by grass shrimp *P. pugio* pre-exposed to dietary Cd for 15 d (n = 8-11). Dietary treatments (TAM-[Cd] in oligochaete prey *T. tubifex*) are indicated by symbols shown in legend inset. Standard errors of calculated assimilation efficiencies (retention at t = 48 h) for A and B are shown in Fig. 4A and 4B, respectively.



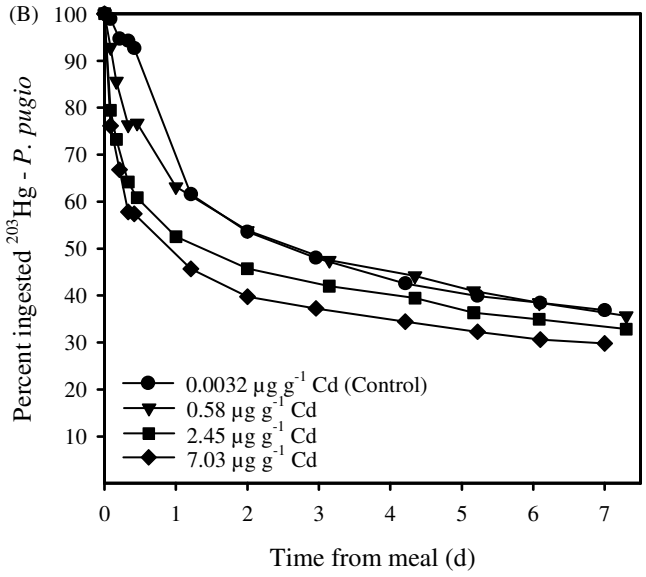
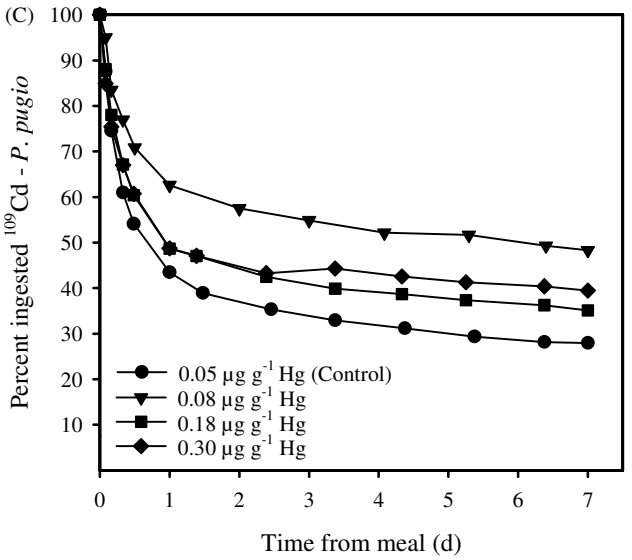


Figure 3-3C and 3-3D. Time courses in the retention of (C) ^{109}Cd or (D) ^{203}Hg by grass shrimp *P. pugio* pre-exposed to dietary Hg for 15 d (n = 8-11). Dietary treatments (TAM-[Cd] or TAM-[Hg] in oligochaete prey *T. tubifex*) are indicated by symbols shown in legend insets. Standard errors of calculated assimilation efficiencies (retention at t = 48 h) for (D) are shown in Fig. 5B. AE-^{109}Cd% by (C) shrimp pre-exposed to dietary Hg was estimated using the y-intercept method.



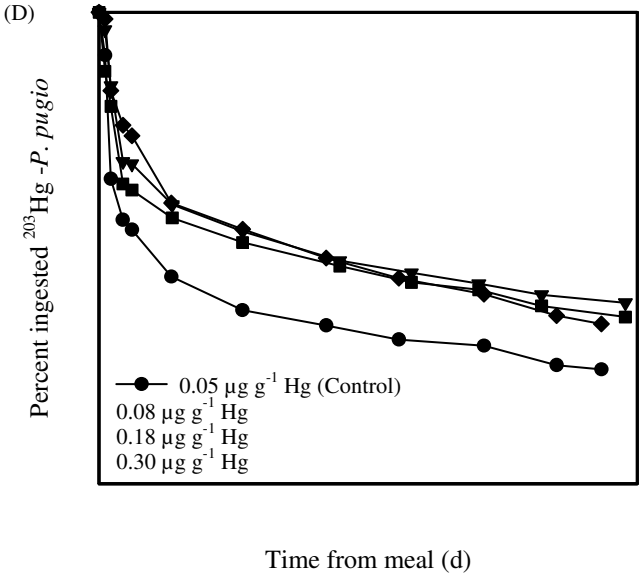
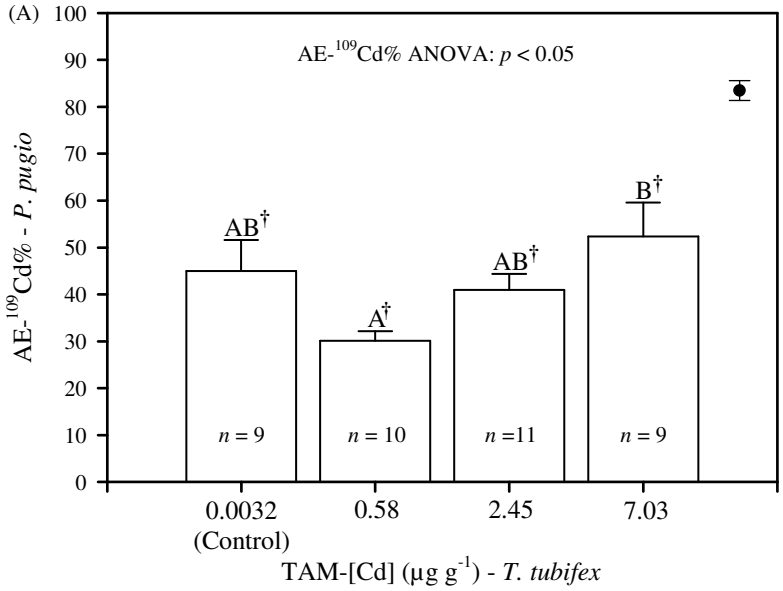
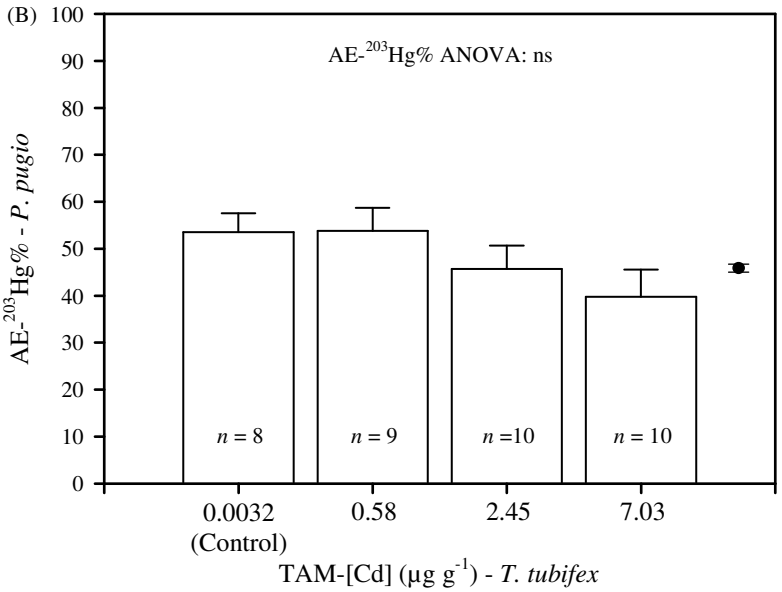


Figure 3-4. (A) AE-^{109}Cd%, (B) AE-^{203}Hg% and (C) AE-^{14}C% by grass shrimp *P. pugio* pre-exposed to dietary Cd for 15 d (n = 8-11; mean ± S.E.). Significant differences ($p < 0.05$) in AE between treatments (t-test with Bonferroni adjustment) are indicated by different letters within panels. † = AE-^{109}Cd% or AE-^{203}Hg% by *P. pugio* differs ($p < 0.05$) from TAM-^{109}Cd% or TAM-^{203}Hg% (indicated by • in panels A and B; n = 4; mean ± S.E.) in oligochaetes *T. tubifex* (t-test).





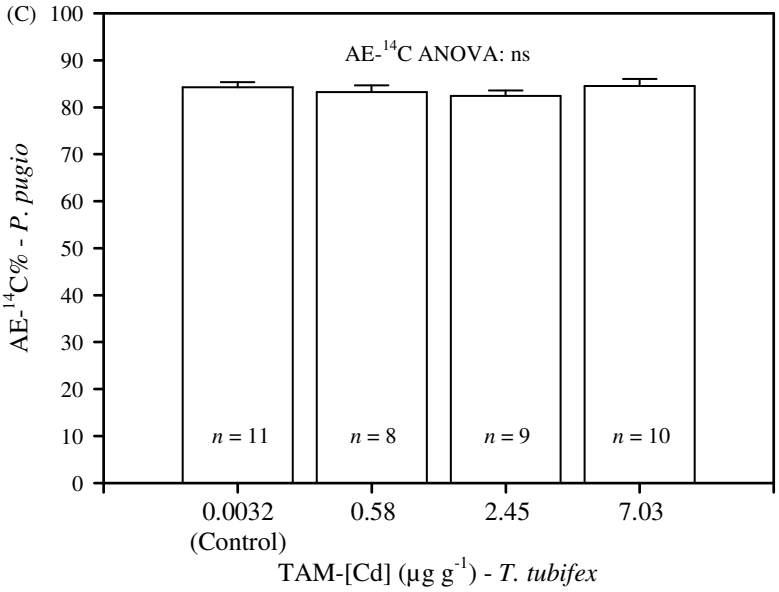
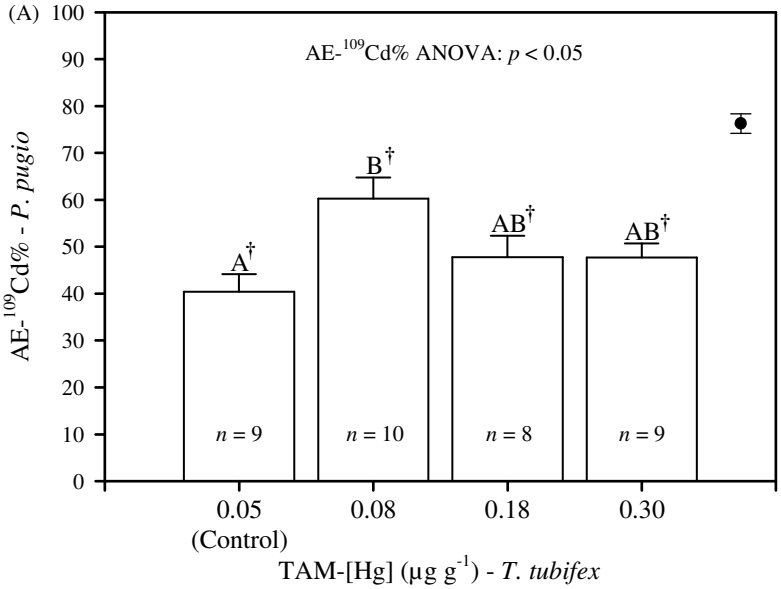
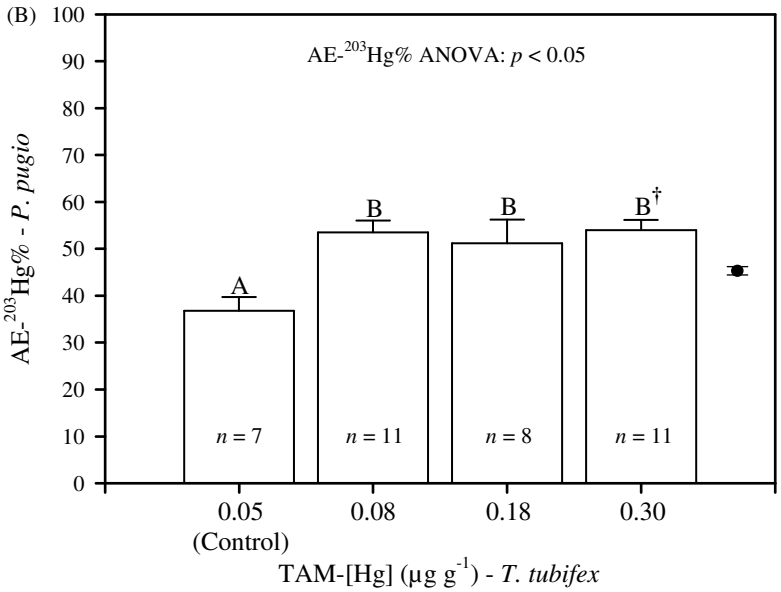

(C)
AE-14C ANOVA: ns
AE-14C% - P. pugio
100
90
80
70
60
50
40
30
20
10
0
n = 11
n = 8
n = 9
n = 10
0.0032
(Control)
0.58
2.45
7.03
TAM-[Cd] (µg g-1) - T. tubifex

Figure 3-5. (A) AE-^{109}Cd%, (B) AE-^{203}Hg% and (C) AE-^{14}C% by grass shrimp *P. pugio* pre-exposed to dietary Hg for 15 d (n = 7-12; mean ± S.E.). Significant differences ($p < 0.05$) in AE between treatments (t-test with Bonferroni adjustment) are indicated by different letters within panels. † = AE-^{109}Cd% or AE-^{203}Hg% by *P. pugio* differs ($p < 0.05$) from TAM-^{109}Cd% or TAM-^{203}Hg% (indicated by • in panels A and B; n = 4; mean ± S.E.) in oligochaetes *T. tubifex* (t-test).





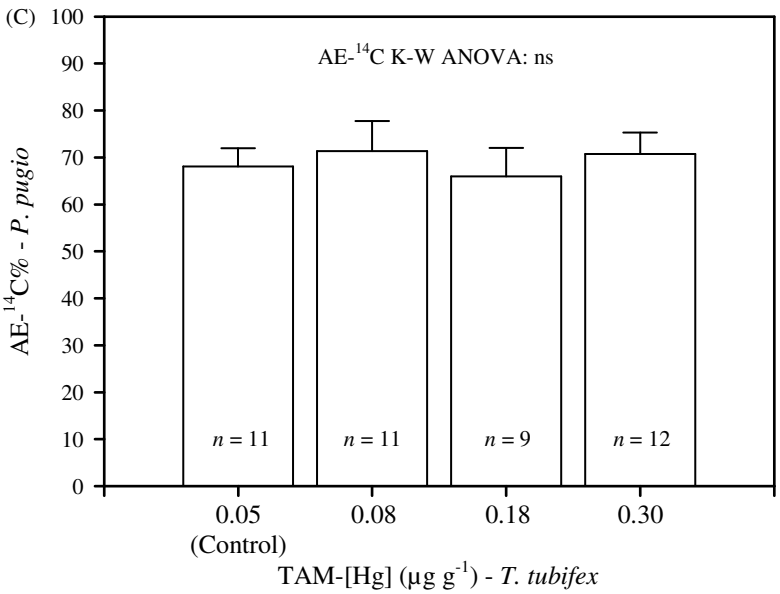
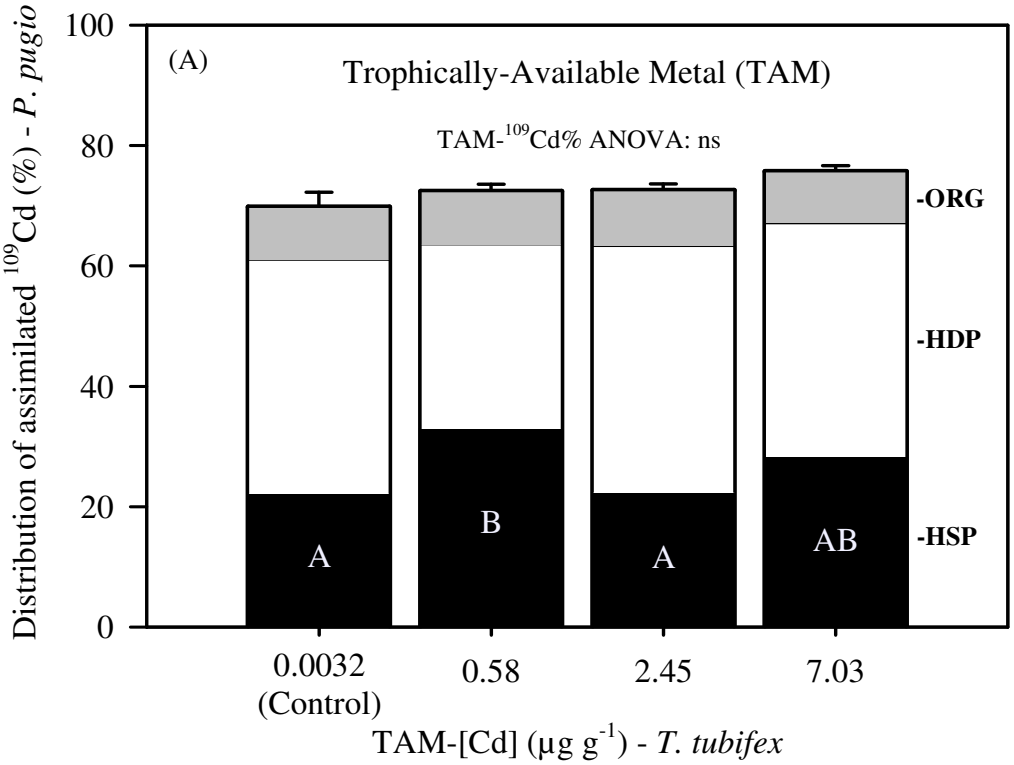

(C)
AE-14C K-W ANOVA: ns
AE-14C% - P. pugio
100
90
80
70
60
50
40
30
20
10
0
n = 11
n = 11
n = 9
n = 12
0.05
(Control)
0.08
0.18
0.30
TAM-[Hg] (µg g-1) - T. tubifex

Figure 3-6. Distributions of assimilated (A) ^{109}Cd and (B) ^{203}Hg in the trophically-available metal (TAM) compartment (full bars) and individual subcellular fractions: heat-stable proteins (HSP), heat-denatured proteins (HDP) and organelles (ORG) (embedded within each bar) (n = 4; mean ± S.E.) in grass shrimp *P. pugio* pre-exposed to dietary Cd for 15 d. In (A), HSP ANOVA: $p < 0.05$. TAM-^{109}Cd%, HDP and ORG ANOVA: ns. Significant differences ($p < 0.05$) between dietary treatments (t-test with Bonferroni correction) are indicated by different letters. In (B), TAM-^{203}Hg%, HDP and ORG ANOVA: ns.



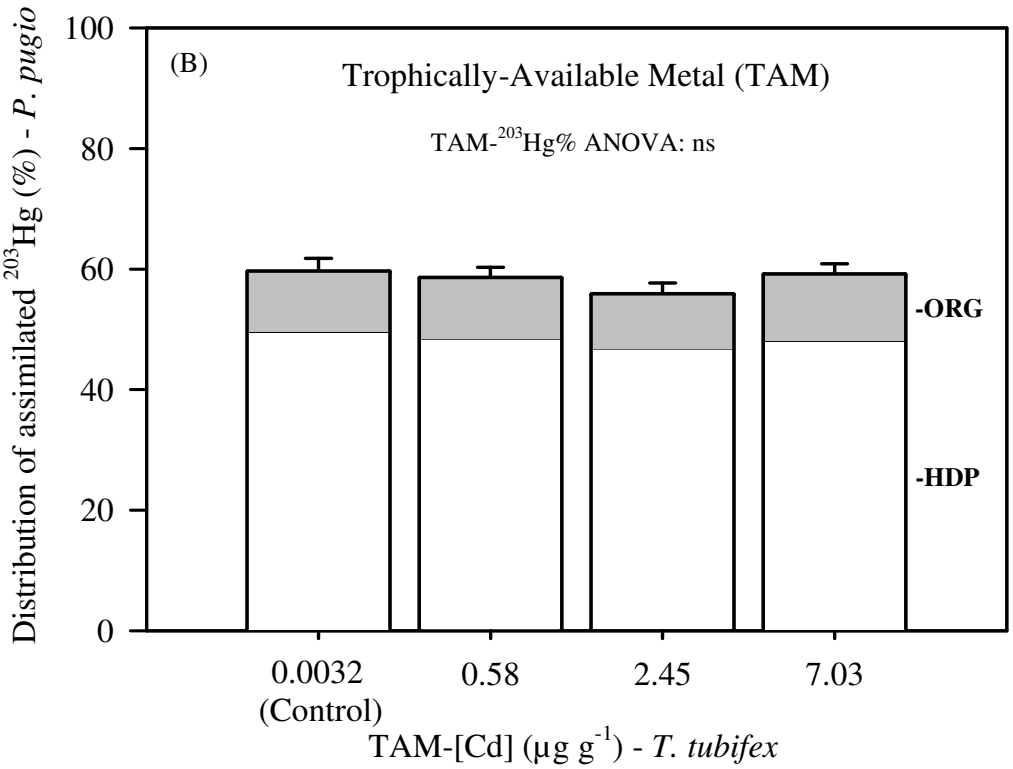
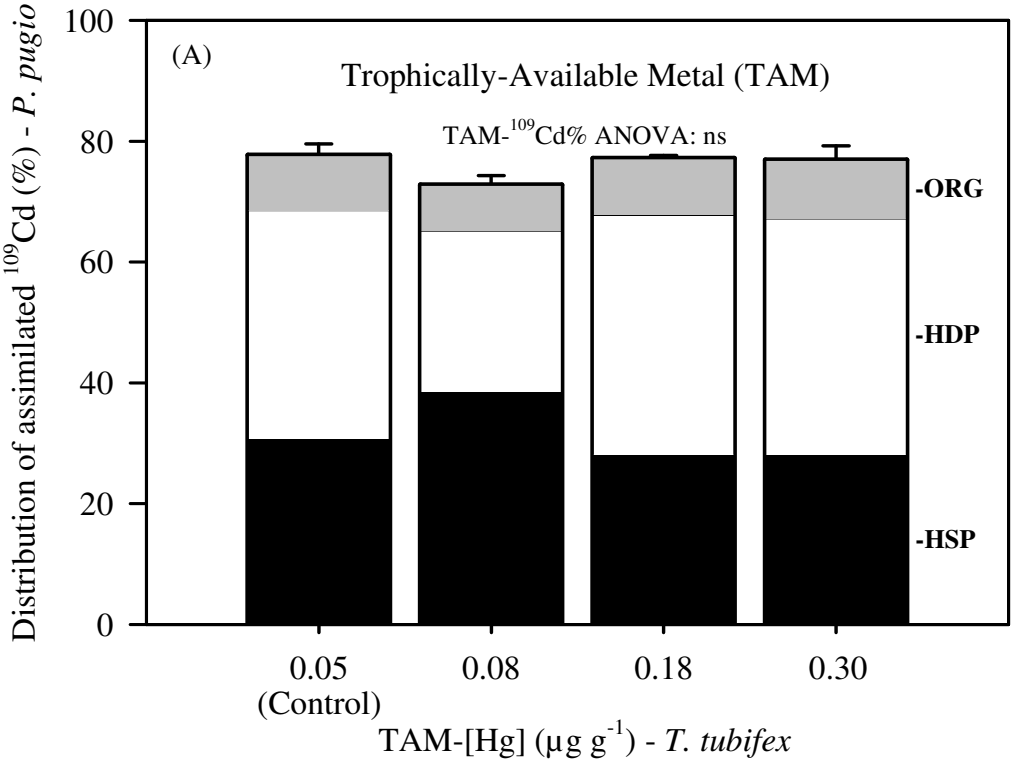
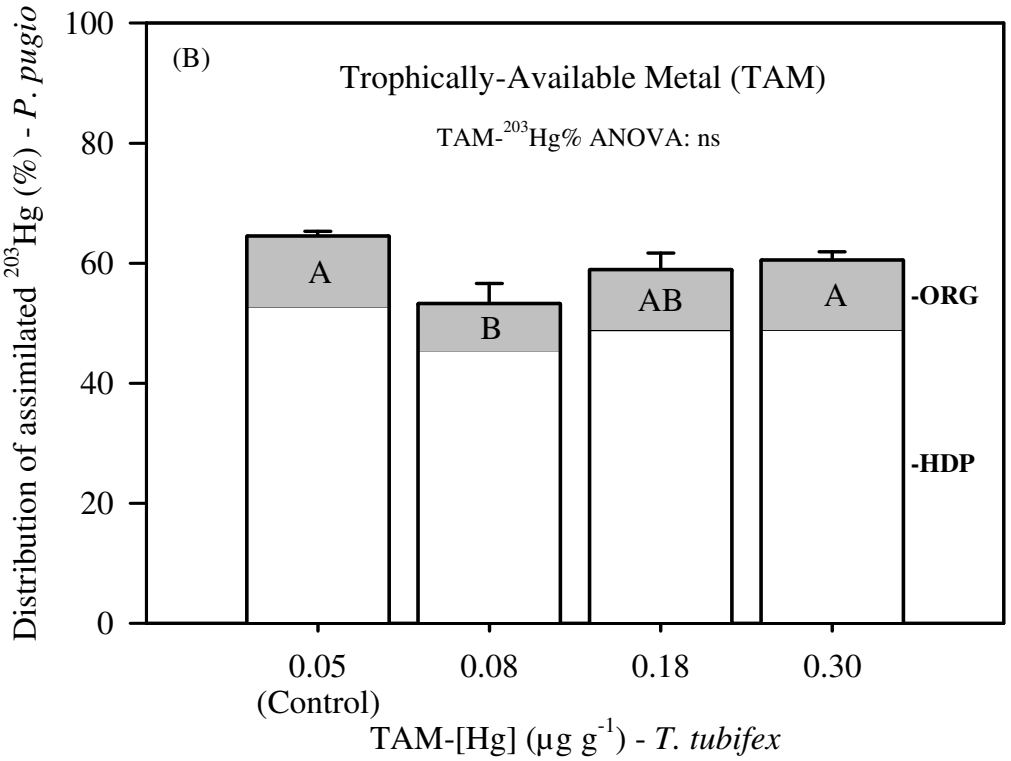

(B)
Trophically-Available Metal (TAM)
TAM-^{203}Hg% ANOVA: ns
Distribution of assimilated ^{203}Hg (%) - *P. pugio*
100
80
60
40
20
0
-ORG
-HDP
0.0032
(Control)
0.58
2.45
7.03
TAM-[Cd] (µg g^{-1}) - *T. tubifex*

Figure 3-7. Distributions of assimilated (A) ^{109}Cd and (B) ^{203}Hg in the trophically-available metal (TAM) compartment (full bars) and individual subcellular fractions: heat-stable proteins (HSP), heat-denatured proteins (HDP) and organelles (ORG) (embedded within each bar) (n = 4; mean ± S.E.) in grass shrimp *P. pugio* pre-exposed to dietary Hg for 15 d. In (A), TAM-^{109}Cd%, HSP, HDP and ORG ANOVA: ns. In (B), ORG ANOVA: $p < 0.05$. TAM-^{203}Hg% and HDP ANOVA: ns. Significant differences ($p < 0.05$) between dietary treatments (t-test with Bonferroni correction) are indicated by different letters.




(B)
Trophically-Available Metal (TAM)
TAM-^{203}Hg% ANOVA: ns
Distribution of assimilated ^{203}Hg (%) - *P. pugio*
100
80
60
40
20
0
A
B
AB
A
-ORG
-HDP
0.05
(Control)
0.08
0.18
0.30
TAM-[Hg] (µg g^{-1}) - *T. tubifex*

CHAPTER 4

Post-assimilatory digestive toxicity in grass shrimp *Palaemonetes pugio*

CHAPTER SUMMARY

Ingested pollutants (e.g., non-essential metals) may elicit digestive toxicity following incorporation into consumer tissues (i.e., assimilation). Post-assimilatory toxicity may include tissue damage influencing synthesis of digestive enzymes, gut transit times and absorption of nutrient and pollutants by the gut epithelium. The present study investigated impacts of chronic field exposure to impacted field conditions as well as laboratory pre-exposure to dietary metal (Cd and Hg) on gut residence time (GRT), feces elimination rate (FER), digestive protease activities and gut pH in the omnivorous grass shrimp *Palaemonetes pugio*. Adult shrimp were collected from differentially-impacted sites within the New York/New Jersey Harbor Estuary. Samples of naïve shrimp from a reference site were fed metal-contaminated oligochaetes for 15 d. Following clearance of gut contents, shrimp were fed prepared meals containing fluorescent or near-infrared markers and analyzed for digestive toxicity. Post-assay molt stage was also characterized for individual shrimp. Relationships between digestive parameters and assimilation efficiencies (AE) for Cd, Hg and organic carbon reported previously were also analyzed. Minimum GRT did not vary for field-collected shrimp, but was positively correlated with Cd (but not Hg or carbon) AE. Decreasing linear trends in GRT were observed over the range of Cd and Hg pre-exposures and a decrease in GRT was observed for the highest dietary Cd treatment, relative to the intermediate exposures. FER was not impacted by previous field exposure or exposure to dietary metal. Digestive protease activities exhibited a marked decrease in shrimp collected from impacted field sites relative to shrimp from a local reference site. Casein hydrolysis rates were negatively correlated with GRT and had an inverse relationship to Cd AE in field-

collected shrimp. Protease activities did not vary for grass shrimp pre-exposed to Cd. Enzyme activities were higher for shrimp from the highest Hg pre-exposure, relative to intermediate treatments. pH within the posterior region of the cardiac chamber of the proventriculus varied for field-collected and Cd pre-exposed shrimp. Both anterior and posterior region pH increased over the range of Hg pre-exposures and Hg AE exhibited a negative relationship to H^+ ion concentrations in the lumen of the proventriculus. Analysis of molt stage data did not reveal differences in digestion over the course of the molt cycle. Relationships between the assimilation of elements and digestive physiology in field-collected shrimp suggest the possibility that digestive plasticity (e.g., increasing GRT) may be important in compensating for post-assimilatory toxicity (i.e., reduced protease activities) and to maintain nutrient assimilation. Stress-induced population variability in digestive function may, in turn, enhance the assimilation of non-essential elements, including metals (e.g., Cd). Post-assimilatory impacts of dietary Hg exposure on gut pH may influence competition between Hg^{2+} and H^+ ions for binding sites, impacting availability and absorption of ingested Hg during subsequent digestive cycles.

INTRODUCTION

Digestive plasticity may allow organisms to maximize nutrient assimilation through changes in gut function (e.g., gut morphology, transit time, enzyme activities or chemistry of gut fluid) in response to external factors such as diet composition, intraspecific competition and predation (Bock and Mayer, 1999; Jumars, 2000; Relyea and Auld, 2004; Sabat et al., 2005). For organisms exposed to chemical stressors during feeding, plasticity may offset impacts on digestive physiology and allow individuals to maintain adequate assimilation of essential elements (e.g., organic carbon). This may be particularly important in settings (e.g., urbanized ecosystems) where the nutritional value of food organisms may also be compromised by pollutant exposure (Campbell et al., 2005). Any deleterious effects of ingested pollutants on digestive physiology and assimilation by species that play key roles in nutrient cycling and maintaining ecological efficiency may have important community-wide consequences (e.g., nutrient loss or changes in community structure) in impacted ecosystems (Nixon and Oviatt, 1973; Odum, 1985; Goto and Wallace, 2010).

Pollutants circulating in gastric fluid may interact with critical components (e.g., digestive enzymes, solubilizing agents and gut epithelial surfaces) involved in hydrolysis, solubilization and absorption of ingested matter (Chen and Mayer, 1998; Chen et al., 2002; Mayer et al., 1996, 2001; Seebaugh and Wallace, unpublished). These pre-assimilatory effects on digestion would result from exposure prior to incorporation of a pollutant into consumer tissues (Campbell et al., 2005). Digestive toxicity may also result from chronic exposure and accumulation of pollutants by tissues critical to gut functions. Post-assimilatory tissue damage may interfere with cellular machinery involved in

synthesis of enzymes and surfactants, impact regulation of peristalsis or influence transport of nutrients as well as pollutants across epithelial surfaces from the gut lumen and to the circulatory system. Toxicity associated with assimilated pollutants may also include impacts on other tissues, including those that influence behaviors associated with nutrient acquisition (Wallace et al., 2000; Perez and Wallace, 2004). For organisms that feed in polluted conditions, interactions between pre- and post-assimilatory toxicity may also be important in determining the extent to which ingested pollutants impact digestion and ultimately influence the assimilation of nutrients as well as pollutants.

In previous work with the daggerblade grass shrimp *Palaemonetes pugio* Holthius 1949 (Decapoda, Caridea), assimilation efficiencies (AE) for Cd and inorganic Hg were influenced by chronic exposure to impacted field conditions, indicating that pollutant-induced digestive toxicity (i.e., post-assimilatory toxicity) may influence the subsequent assimilation of dietary metal (Seebaugh and Wallace, 2009). Carbon assimilation was not impacted in field-exposed shrimp, suggesting that digestive plasticity may play a role in maintaining adequate nutrient assimilation. In related work, carbon assimilation by shrimp did not exhibit variation despite differences in Cd and Hg AE induced by previous exposure to dietary metal for 15 d (followed by 2 d depuration), suggesting that digestive plasticity may allow shrimp to compensate for metal-induced post-assimilatory toxicity (Seebaugh et al., unpublished). Grass shrimp play an important role in nutrient cycling within estuarine food webs through conversion of algae, detritus and invertebrate tissues into fecal matter utilized by bacteria and algae as well as shrimp biomass consumed by predators (e.g., the mummichog *Fundulus heteroclitus* and striped bass *Morone saxatilis*) (Welsh, 1975; Tashiro et al., 1994; Davis et al., 2003). Compensation for post-

assimilatory impacts of ingested pollutants on digestion and nutrient assimilation by shrimp may be important in distributing essential nutrients as well as maintaining adequate shrimp biomass within estuarine communities (Nixon and Oviatt, 1973; Welsh, 1975).

For the present study, impacts of chronic field exposure or pre-exposure to dietary Cd or inorganic Hg on endpoints related to digestive physiology (gut residence time [GRT], feces elimination rate [FER], digestive protease activities and gut pH) were investigated for grass shrimp. Shrimp were collected along an impact gradient within the New York/New Jersey Harbor Estuary or collected from a reference site and pre-exposed to dietary metal for 15 d, allowed to clear their guts and fed meals prepared with fluorescent or near-infrared (NIR) markers. Shrimp were assessed for digestive toxicity *in vivo* using microfluorometric methods (GRT/FER and pH) or a novel NIR imaging technique (protease activities). Baseline data were obtained for shrimp collected from a local reference site or appropriate laboratory controls. Post-assay molt stage was also characterized for individual shrimp to evaluate the potential effects of molt cycle on digestion.

MATERIALS AND METHODS

Field exposure to pollutants

Impacts of chronic field exposure on digestive physiology were assessed for adult grass shrimp (~3 cm in length), collected from three differentially-impacted sites surrounding Staten Island, New York, USA and maintained for ~3 d as described by Seebaugh and Wallace (2009). Great Kills Harbor (GK) is located along the southeastern shore of Staten Island, is flushed with cleaner waters from Raritan Bay and served as the reference site for this portion of the study (Fig. 4-1). Main Creek (MA) and Neck Creek (NC) are located within the heavily industrialized Arthur Kill (AK) complex, which separates Staten Island from New Jersey and links Raritan Bay and Newark Bay. Organisms within this network of waterways may be impacted by discharges from industry, byproducts of petroleum processing and combined sewer overflows as well as landfill leachate contamination (Gillis et al., 1993; Gunster et al., 1993; Crawford et al., 1995) (Fig. 4-1). Sediment metal (e.g., Cd and Hg) concentrations at these sites vary considerably (Goto, 2009). MA shrimp were not available for gut pH analysis.

Pre-exposure to dietary metal

Separate samples of GK shrimp were used to assess post-assimilatory digestive toxicity following pre-exposure to dietary metal as described by Seebaugh and Wallace (unpublished). Briefly, oligochaetes *Tubifex tubifex* were exposed to dissolved Cd (nominal concentrations: control, 0.22, 0.44 or 0.88 μM) or Hg (control, 0.007, 0.014 or 0.028 μM) for 96 h with renewal of exposure media at ~48 h. Following clearance of gut contents for ~3 d, shrimp (n = 16 per treatment) were fed ~75 metal-exposed worms d^{-1}

for 15 d. Pre-exposed shrimp were allowed to clear their guts for ~2 d prior to feeding on meals prepared to assess digestive toxicity.

Experimental meal preparation

Meals used for GRT/FER, protease activity and gut pH analyses were prepared by embedding task-specific fluorescent or near-infrared (NIR) markers in a gelatin-oligochaete tissue matrix readily consumed by shrimp in previous work (Seebaugh and Wallace, 2009). Unexposed oligochaetes were rinsed and homogenized in NANOpure® deionized water (0.66 g worm tissue ml^{-1}) using a Polytron® homogenizer (Kinematica). Meals used to estimate GRT consisted of 0.1 ml of 0.5 µm diameter Fluoresbrite® microspheres (Polysciences), combined with a 0.9 ml portion of concentrated diatoms *Thalassiosira weissflogii* (CCMP 1336; ~3.41 x 10^7 cells ml^{-1}), 1 ml oligochaete homogenate, 0.33 ml cod liver oil (to enhance palatability) and 0.47 g gelatin crystals (Knox®) (Nagano and Decamp, 2004; Seebaugh and Wallace, 2009). For protease activities, meals were prepared with oligochaete homogenate and gelatin in the same proportions as for GRT meals, with the remaining volume consisting of NANOpure® deionized water (pH 7.8) and IRDye® 800RS casein protease substrate in 50 mM TRIS-HCl (pH 7.8; preserved with 0.01% sodium azide) (Li-Cor). Hydrolysis of casein substrate releases IRDye-labeled peptides detectable by an NIR imaging system. Increases in NIR signals are proportional to the amount of casein hydrolyzed when substrate is supplied in excess. Individual meals contained ~0.003 nmol IRDye® and ~0.012 µg casein. Meals used to estimate gut pH were prepared by suspending 2.5 mg Zymosan A BioParticles® fluorescein conjugate (Molecular Probes) in 100 µl of a matrix

prepared by combining 125 µl worm homogenate, 166 µl NANOpure® deionized water and 0.059 g gelatin. Zymosan A consists of freeze-dried yeast *Saccharomyces cerevisiae* covalently labeled with fluorescein and has been used to estimate gut pH in deposit-feeding polychaetes based on the differential sensitivity of fluorescein emission intensities to pH at specific excitation wavelengths (isosbestic at 458 nm and pH-dependent at 496 nm) (Ahrens and Lopez, 2001; Molecular Probes, 2010). Each meal contained ~0.17 mg Zymosan A, which was usually sufficient to generate usable signals within the proventriculus. Diatoms and cod liver oil were not included in IRDye or fluorescein meals to reduce potential background fluorescence from these components. Fluorescent/NIR meal mixtures were sealed in a microcentrifuge tubes, warmed with tap water and vortexed to uniform consistency. Individual (6 µl) meals were dispensed onto Nucleopore filters (Whatman®) and stored frozen (-20 °C) ~2 h prior to feeding experiments (Wallace and Lopez, 1996).

GRT/FER analyses

Following clearance of gut contents, shrimp were transferred to aquaria containing clean seawater (1.0 µm, 10 ppt, 18-19 °C) and allowed to feed on Fluoresbrite microsphere-labeled meals for 30 min. Shrimp were then rinsed with seawater and the proventriculus of individuals inspected with a dissecting microscope to determine whether or not labeled food was consumed. Shrimp that consumed meals were transferred to individual defecation chambers housed within 38 l aquaria containing clean, aerated seawater and allowed to feed *ad libitum* on commercial fish food. Fecal strands were collected from individual shrimp on paper filters 1.5 h after feeding and

every 30 min thereafter for up to 13 h. Filters were air-dried and scanned for microspheres using an Axio Observer.Z1 inverted microscope (Zeiss) equipped with a fluorescent light source. Minimum GRT for individuals was estimated as time between introduction of fluorescent meals and first detection of microspheres in feces, to the nearest 30 min. Lengths of dried fecal strands were measured using a dissecting microscope (Olympus SZ40) equipped with a Moticam 1000 digital camera and Images Plus 2.0 (Motic). Software measurement tools were calibrated with a 1.5 mm diameter circle. As a quality control check, one fecal strand per filter was measured three times and individual measurements were within 2% of mean strand length. FER (mm h^{-1}) was calculated for individual shrimp for 2 h following minimum GRT.

Protease activities

Following clearance of gut contents, shrimp were transferred to clean seawater (1.0 µm, 10 ppt, 18-19 °C) and allowed to feed on IRDye-labeled casein meals for 14 min. Shrimp were then immobilized, dorsal-side-up, within 30 mm diameter glass tubes (0.6 mm wall-thickness; 5.3 mm inner diameter) cut from Pasteur pipettes and mounted to 100 mm diameter glass petri dishes with cyanoacrylate adhesive. Shrimp were scanned every 90 sec with an Odyssey® infrared imaging system (Li-Cor) (800 nm channel; 3.9 mm focus offset; 169 µm resolution; 10 ppt seawater in petri dishes; 18-19 °C within the scanning chamber) beginning at time (t) = 20 min from the introduction of IRDye-labeled meals. Whole shrimp were scanned during assays and NIR signals were only detected within the gut. Background signals from unlabeled shrimp tissues were characteristic of NIR imaging (i.e., negligible). Casein hydrolysis within the proventriculus and

hepatopancreas of individual shrimp was measured as the increase in integrated intensity over time, relative to t = 20 min. Shrimp that repositioned themselves during scanning could be identified through drastic changes in integrated intensity or visual inspection and were excluded from further analysis. A regression was fit to the linear portion of the curve (before substrate was exhausted, typically within 9 to 10.5 min of the initial scan) representing the mean increase in integrated intensity. Corresponding slopes were used to compare rates of casein hydrolysis for shrimp from each treatment.

Gut pH

Following clearance of gut contents, ~16 shrimp per treatment were transferred to a 9.5 l aquarium containing clean seawater (1.0 µm, 10 ppt, 18-19°C). One fluorescein meal was administered at a time. Shrimp that acquired a meal were allowed to feed for ~5 min, which was sufficient time for complete ingestion. Shrimp were then immobilized within 30 mm diameter glass tubes mounted to 20 mm x 40 mm chamber slides containing clean seawater (10 ppt). pH within the lumen of the anterior and posterior regions of the cardiac chamber of the proventriculus (~100 µm from the dorsal wall) was estimated using the 496 nm:458 nm intensity ratio (emissions at 530 ± 25 nm) method of Ahrens and Lopez (2001). Shrimp were scanned with a Leica DM IRE2 (inverted) or Leica DM RXA2 (upright) microscope (10x objective) attached to a Leica SP2 confocal microscope equipped with an argon/krypton laser. Fluorescent images for each excitation wavelength were captured and analyzed using Leica Confocal Software (LCS). Peristaltic contractions of the proventriculus typically subsided within 2 to 3 min after shrimp were removed from the feeding aquarium and did not interfere with image capture. The mean

baseline correction feature of LCS was used to correct for background fluorescence and was sufficient to correct for minimal autofluorescence generated within the proventriculus. Intensity ratios within the proventriculus were calibrated to standards prepared by adding 15 µl reconstituted Zymosan A (2.5 mg in 600 µl 50 mM TRIS-HCl; 0.01% sodium azide) to 200 µl of 100 mM MES (pK_a = 6.16), MOPS (pK_a = 7.28) or HEPES (pK_a = 7.55) buffers dissolved in NANOpure® deionized water. Buffers were adjusted over the range of pH 4.5 to pH 8.3 with 0.1 N HCl or 0.1 N NaOH. Calibration standards were drawn into glass capillary tubes and images acquired and analyzed as described for live shrimp. Borosilicate glassware was used for preparation of calibration standards to minimize effects of glass composition on pH. Intensity ratios obtained for the pH standards were regressed onto pH using a third-degree polynominal fit (Ahrens and Lopez, 2001). Standard calibration plots were produced for each set of analyses to control for fluctuations in instrumentation.

Statistical analyses

Effects of field site or pre-exposure to dietary metal on median minimum GRT in grass shrimp were tested using Kruskal-Wallis analysis of variance (K-W ANOVA) (Zar, 1999). Comparisons of GRT among Cd pre-exposure treatments were performed with Dunn's post test (Siegel and Castellan, 1988). Curves showing percentages of individuals with fluorescent microspheres in feces over time were compared with the Mantel-Cox test with comparisons among Cd treatments conducted using sequential Bonferonni correction (Dunn-Šidák method) (Altman, 1991; Sokal and Rohlf, 1995). Linear trends between treatments and median GRT were analyzed with the logrank test for trend.

Normality of $\log_{10}$ transformed FER data was tested with Shapiro-Wilk's *W* test and homoscedasticity tested using Levene's test. Treatment effects on FER were tested using one-way ANOVA or K-W ANOVA for data that did not meet assumptions (i.e., for shrimp pre-exposed to dietary Hg) (Sokal and Rohlf, 1995). Homogeneity of IRDye-labeled food ingestion (integrated intensity at t = 20 min) across dietary treatments was tested using ANOVA or K-W ANOVA. Differences in initial IRDye signals for field-collected shrimp were compared using the Tukey-Kramer multiple comparisons test. Casein hydrolysis rates were compared by unplanned testing of regression coefficients (slopes; Tukey-Kramer method) for field-collected shrimp or planned testing of equality of slopes (*F*-test) for shrimp pre-exposed to dietary metal (Sokal and Rohlf, 1995). Multiple comparisons of protease activities for Hg pre-exposed shrimp were conducted using the *t*-test with Bonferroni correction. Correlations between hydrolysis rates and initial NIR signals were analyzed using Pearson product-moment correlation or Spearman rank correlation for non-normal data. Gut pH data were converted to free H^+ concentrations, tested for normality and analyzed using the Mann-Whitney *U* test for field-collected shrimp or K-W ANOVA for metal pre-exposed shrimp (Murphy, 1981). Comparisons of proventriculus pH among dietary pre-exposure treatments were performed with Dunn's post test. Mann Whitney *U* was also used to compare anterior and posterior proventriculus pH for individual field or pre-exposure treatments. Gut pH data are reported as -log(mean $[H^+]$) with asymmetrical standard errors resulting from transformation following analyses for treatment effects (Hu et al., 2007). Post-assay molt stage (intermolt [C], premolt [D_0, D_1 or D_2] or postmolt) was determined via microscopic (100X magnification) examination of setal regions of uropod expodites for grass shrimp

assessed for digestive toxicity (Freeman and Bartell, 1975). Effects of molt stage on GRT, FER, protease activities and proventriculus pH within treatments and treatment groups were analyzed using K-W ANOVA (Mugnier and Justou, 2004). The influence of molt stage on anterior proventriculus pH could not be evaluated for NC shrimp due to insufficient sample sizes. After testing data for normality, Pearson product-moment correlation was used to evaluate relationships between Cd, Hg and carbon AE by field-collected and metal pre-exposed shrimp reported in related studies and gut physiology (Seebaugh and Wallace, 2009, unpublished). Gut pH in field-collected shrimp was not included in correlation analyses since data were available for only two study sites. Statistical analyses were performed using STATISTICA 7.1 (Statsoft), GraphPad Instat 3.10 and GraphPad Prism 5.03 (GraphPad).

Digestive toxicity data for grass shrimp pre-exposed to dietary Cd or Hg are plotted against concentrations of trophically-available metal (TAM-[Cd] or TAM-[Hg]) in subsamples of metal-exposed oligochaetes subjected to tissue digestion and stable metal analyses in related work (Seebaugh and Wallace, unpublished). TAM may represent bioavailable metal in prey and could serve as an estimate of the dose of Cd or Hg to shrimp during pre-exposure (Wallace and Luoma, 2003; Seebaugh and Wallace, 2004).

RESULTS

GRT

Minimum GRT for grass shrimp collected from differentially-impacted field sites or pre-exposed to dietary metal in the laboratory was estimated following ingestion of

meals containing 0.5 µm diameter fluorescent microspheres. Following ingestion of microspheres, shrimp typically consumed fish food during the course of the assay. Fluorescent markers were easily detectable in feces (Fig. 4-2A). Individual beads could not be identified within the proventriculus or hepatopancreatic tubules due to interference from surrounding tissues, although fluorescence within these organs could be distinguished from background (e.g., abdominal muscles) following ingestion (Fig. 4-2B to 4-2E).

Minimum GRT did not vary for grass shrimp from the Staten Island study sites (Fig. 4-3A). Analysis of percentages of shrimp with microspheres in fecal strands over time did not reveal variability among sites or a linear trend between impact gradient and GRT (Fig. 4-4A). GRT for shrimp that consumed worms with TAM-[Cd] of 7.03 µg g^{-1} during pre-exposure (350 min) was approximately half the value for shrimp from the 0.58 and 2.45 µg g^{-1} Cd treatments (720 min), but was not different from controls (600 min) (Fig. 4-3B). Curves showing percentages of shrimp with microspheres in fecal strands indicate a linear trend between pre-exposure to Cd and GRT, however, differences between individual curves could not be resolved following comparisons among dietary treatments (sequential Bonferonni correction following Mantel-Cox test: ns for all comparisons) (Fig. 4-4B). GRT did not vary for shrimp pre-exposed to dietary Hg, however, a linear trend between pre-exposure treatment and a reduction in median GRT was observed (Fig. 4-3C and 4-4C).

FER

FER was determined through microscopic measurement of fecal strand lengths

and was calculated for grass shrimp for 2 h following minimum GRT. FER did not vary significantly for shrimp collected along an impact gradient or pre-exposed to dietary Cd or Hg (Fig. 4-5).

Digestive protease activities

Field-exposed and metal pre-exposed shrimp were assessed for extracellular digestive protease activities following ingestion of meals containing casein substrate labeled with an NIR marker. Enzyme activities were estimated beginning at t = 20 min following the introduction IRDye-labeled meals to provide sufficient time for several shrimp to ingest food and to prepare animals for scanning. Casein hydrolysis rates were approximately linear from the time between initial scan and substrate exhaustion (Fig. 4-6 to 4-9). Exhaustion was not reached for some treatments (e.g., NC shrimp) and corresponding regression lines for treatment groups were plotted from t = 20 min to the earliest time point that shrimp from a treatment group reached a plateau in enzyme activity (e.g., 30.5 min for shrimp from the study sites). Homogeneity of IRDye-labeled meal ingestion was not evident for field-collected shrimp, but was apparent for shrimp pre-exposed to dietary metal (Table 4-1).

Shrimp collected from MA and NC exhibited a marked decrease in digestive protease activities relative to GK shrimp (Fig. 4-10A). Although rates of casein hydrolysis were positively correlated with initial IRDye signal in shrimp from the study sites, substrate hydrolysis in GK shrimp was ~3.14x the rate for MA shrimp, suggesting greater than linear enzyme activity with respect to ingestion (~2.09x) (Table 4-1). Protease activity in GK shrimp was ~1.46x greater than would be predicted had activity

been proportional to the difference in IRDye signal observed for NC shrimp. Protease activities did not vary for shrimp pre-exposed to dietary Cd (Fig. 4-10B). Casein hydrolysis in shrimp pre-exposed to oligochaetes with TAM-[Hg] of 0.30 µg g^{-1} took place at a faster rate than for shrimp from the 0.08 and 0.18 µg g^{-1} dietary treatments, but did not differ from controls (Fig. 4-10C).

Gut pH

Gut pH was estimated for grass shrimp following ingestion of meals prepared with fluorescein and was calibrated to 496 nm:458 nm intensity ratios obtained from pH standards. Calibration curves approximated the sigmoidal shape characteristic of pH-dependent variability in intensity ratios (emissions at 530 nm) (Fig. 4-11). Gut pH measurements were considered to be reliable if they fell within the range of pH ~5.15 to ~7.30 (see examples, Fig 4-12). pH within the proventriculus of grass shrimp was moderately acidic (pH 5.29 to 6.51) and differed from that of ambient seawater (~pH 8) (Table 4-2).

pH within the anterior region of the cardiac chamber of the proventriculus did not vary for shrimp from the Staten Island study sites, however, pH of the posterior region was higher in shrimp collected from NC (pH ~6.0) than for GK shrimp (pH ~5.76) (Table 2). pH within the anterior region of shrimp pre-exposed to dietary Cd did not exhibit variation, although pH within the posterior region varied between pH ~5.29 and ~6.02. Anterior and posterior region pH increased over the range of Hg pre-exposures (Table 4-2). pH within the anterior and posterior regions of the cardiac chamber did not vary for shrimp from the study sites or for individual dietary treatments (Mann-Whitney

U: ns for all comparisons).

Influence of molt stage on digestive physiology

Molt stages of grass shrimp from the Staten Island study sites were distributed: C: 15.5%, D_0: 48.3%, D_1: 15.5%, D_2: 18.9%, postmolt: 1.7%. For Cd pre-exposed shrimp, proportions of specimens in each stage of the molt cycle were: C: 18.1%, D_0: 39.1%, D_1: 20.9%, D_2: 16.4%, postmolt: 5.5%. Shrimp pre-exposed to dietary Hg were distributed: C: 16.1%, D_0: 28.6%, D_1: 25.9%, D_2: 22.3%, postmolt: 7.1%. For all shrimp evaluated for digestive toxicity (280 total specimens), distributions among molt cycle stages were: C: 16.8%, D_0: 36.8%, D_1: 21.8%, D_2: 19.3%, postmolt: 5.4%). GRT, FER or digestive protease activities were not influenced by molt stage for field-collected shrimp or shrimp pre-exposed to dietary Cd or Hg (Table 4-3 to 4-5). Gut pH was not influenced by molt stage for shrimp from the study sites or pre-exposed to dietary Cd. pH within the posterior region of the cardiac proventriculus in shrimp that consumed oligochaetes with TAM-[Hg] of 0.08 $\mu g\ g^{-1}$ was influenced by molt cycle, however, differences between individual stages could not be resolved by multiple comparisons among treatments (Table 4-5). Molt stage did not impact gut pH over the range of dietary Hg treatments.

Relationships between AE and digestive physiology

Relationships between metal and carbon AE by grass shrimp reported in related work and digestive physiology were evaluated through correlation analysis (Seebaugh and Wallace, 2009, unpublished). Significant correlations between Cd, Hg and carbon AE were not established for shrimp from three Staten Island study sites (Table 4-6). Cd assimilation was positively correlated with GRT. A negative relationship between Cd

assimilation and digestive protease activities is also suggested (r: $p = 0.059$). Correlations between Hg or carbon AE and gut physiology were not apparent. GRT and protease activities were negatively correlated for field-collected shrimp (Table 4-6). Correlation analysis did not reveal relationships between AE and digestion for shrimp pre-exposed to Cd (Table 4-7). pH within the anterior and posterior cardiac proventriculus was positively correlated for shrimp from the Cd treatments. A negative correlation between Hg AE and H^+ concentrations in the posterior region was established for shrimp pre-exposed to dietary Hg (Table 4-8). A negative relationship between Hg assimilation and free $[H^+]$ in the anterior region is also suggested (r: $p = 0.051$). pH within the anterior and posterior regions were positively correlated for shrimp from the dietary Hg treatments. Other relationships between AE and digestive physiology were not apparent for Hg pre-exposed shrimp (Table 4-8).

DISCUSSION

In the present study, grass shrimp collected across an impact gradient or pre-exposed to dietary metal were evaluated for digestive toxicity after clearing their gut contents for 2 or 3 d and then feeding on meals containing fluorescent or NIR markers. Previous work indicates that assimilation of metals and organic carbon by this species is complete by 48 h after ingestion (Seebaugh and Wallace, 2009, unpublished). Loss of elements beyond this point is related to physiological turnover and possibly loss of metal stored by resorptive [R] cells of the hepatopancreas (Al-Mohanna and Nott, 1987; Wang and Fisher, 1999). Although it is presumed that impacts of previous dietary exposure on digestion are related to post-assimilatory toxicity, it is possible that residual pollutants

from the previous digestive cycle (e.g., pollutants ingested in the field prior to collection or metal ingested during final pre-exposure feeding) may remain in the gut and are potentially available to interact with enzymes and other components within gut fluid (Icely and Nott, 1992). Estimates for other decapods (e.g., crabs and penaeid shrimp) indicate that ingested metals (e.g., Ag, Au and Th) clear the hepatopancreas 12 to 24 h after feeding (Icely and Nott, 1992). Blister (B) cell extrusion and increased mitotic activity of embryonic (E) cells take place during the excretory phase of digestion (24 to 48 h following ingestion) and the hepatopancreas is typically inactive by 48 h (Hopkin and Nott, 1980; Al-Mohanna and Nott, 1986). Considering patterns of radioisotope depuration for grass shrimp, it seems probable that a clearance time of 2 to 3 d was sufficient to remove ingested metals or other pollutants from the gut prior to assays for digestive toxicity (Seebaugh and Wallace, 2009, unpublished).

GRT in shrimp was determined following ingestion of meals containing 0.5 µm diameter microspheres, which are small enough to pass through setal screens within the cardiac chamber of the proventriculus as well as the gland filter along the floor of the pyloric chamber (Dall and Moriarty, 1983; Felgenhauer, 1992; Icely and Nott, 1992). Materials that pass through the gland filter then enter the hepatopancreas. Based on the size of fluorescent markers and fluorescence detected within the hepatopancreas following ingestion, it is presumed that GRT values reported in this study also represent the influence of hepatopancreatic processes that may ultimately determine timing of the decapod digestive cycle (Al-Mohanna and Nott, 1987). Hoyt et al. (2000) observed GRT from 0.5 to 2 h for *P. pugio* following ingestion of 2 to 4 µm diameter latex beads, which could not pass from the proventriculus to the hepatopancreas. GRT values for ingested

particles > 1 µm may only represent 'bulk' transit times and not have any direct bearing on the time required for digestion (Al-Mohanna and Nott, 1987; Icely and Nott, 1992; Hoyt et al., 2000; Beseres et al., 2006).

Minimum GRT did not exhibit significant variation for shrimp collected along an impact gradient, but was positively correlated with Cd assimilation observed previously (see additional discussion below) (Seebaugh and Wallace, 2009). GRT was reduced for shrimp that consumed meals containing oligochaetes with the highest concentration of dietary Cd, relative to the two intermediate treatments. Trends in decreasing GRT with increasing dietary Cd or Hg exposure were also observed. Although differences in GRT between controls and shrimp pre-exposed to intermediate concentrations of dietary Cd were not significant, the observed variability in GRT over the range of Cd exposures may represent a hormetic response to ingested metal. Similar, U-shaped dose responses have been observed for a wide variety of toxicological endpoints (Calabrese, 2005). For example, blowfly pupation rates were characterized by a hormetic dose-response to dietary Cd exposure (Nascarella et al., 2003). In related work, GRT in naïve shrimp was not influenced by ingestion of a pulse of Cd with food, suggesting that impacts of dietary metal on this toxicological endpoint may be related to post-assimilatory effects on the hepatopancreatic epithelium or musculature or perhaps tissues that regulate gut peristalsis (e.g., the stomatogastric nervous system innervating the proventriculus, abdominal ganglia as well as foregut and intestinal musculature) (Maynard and Dando, 1974; Meiss and Norman, 1977; Shuranova et al., 2006; Seebaugh et al., unpublished). Since FER was not impacted by pre-exposure to dietary metal, it seems probable that variation in GRT is related to alterations in hepatopancreas function (Al-Mohanna and

Nott, 1986, 1987). Variability in GRT can also be related to intrinsic features such as body size, ingestion rate, gut volume and morphology as well as extrinsic factors including temperature and diet (Lopez and Levinton, 1987; Chipps, 1988; Dam and Peterson, 1988; Penry and Jumars, 1990; Ahrens et al., 2001). In polychaetes *Capitella*, the direct relationship between Cd AE and GRT was reversed following exposure to Cd through spiked sediments (Selck et al., 1999).

FER by grass shrimp was not impacted by field exposure or pre-exposure to dietary metal in the laboratory. Variability in this endpoint may be related to ingestion rate or impacts on feces packaging and transport (Seebaugh et al., unpublished). In a related study, FER in naïve shrimp was impacted by a pulse of ingested Cd which may have been related to pre-assimilatory effects on midgut epithelial secretions (i.e., peritrophic membranes or mucopolysaccharides) (Forster, 1953; Seebaugh et al., unpublished). Any post-assimilatory effects of previous pollutant exposure on midgut secretions, gut musculature or abdominal ganglia in shrimp may not have reached the level of severity necessary to impact feces packaging, compaction and transport (Lovett and Felder, 1999; Shuranova et al., 2006). For grass shrimp subjected to chronic exposure (e.g., feeding in polluted field conditions), acclimation of midgut/hindgut functions may be necessary to sustain anal drinking and generate peristaltic/antiperistaltic contractions to maintain hydraulic pressure required for expansion of the hepatopancreatic tubules (Lovett and Felder, 1999). Chronic exposure may also require that shrimp compensate for pre-assimilatory impacts of circulating pollutants on midgut secretions in order to maintain intestinal function over successive digestive cycles. It is also possible that shrimp pre-exposed to dietary metal recovered from any pre-assimilatory impacts on midgut function

during the 2 d period between the final feeding on metal-contaminated oligochaetes and GRT/FER assays. In previous studies, feces production was not related to timing of the digestive cycle (i.e., marked by B cell extrusion and mitotic activity of E cells) for crabs or penaeid shrimp (Al-Mohanna and Nott, 1986).

Extracellular digestive enzymes, including carbohydrases (e.g., amylases, cellulase and chitinase), proteases (e.g., aminopeptidase and trypsin) and lipases have been characterized for a wide variety of decapod taxa (see reviews by van Weel,1970; Gibson and Barker, 1979; Dall and Moriarty, 1983). Specific enzymes are typically identified and activities characterized *in vitro* using gut (e.g., hepatopancreas) homogenates or gut fluid extracts (De La Ruelle et al., 1992; Glass and Stark, 1994; Ezquerra et al., 1997; Lemos et al., 2000, Muhlia-Almazán and García-Carreño, 2002). Relatively few studies have characterized enzymes using non-invasive methods (i.e., extracted from fecal strands) and little is known about enzyme activities within the confines of a functional proventriculus or hepatopancreas (Córdova-Murueta et al., 2003, 2004; Campbell et al. 2005). Extracellular digestive protease activities were characterized for field-collected and metal pre-exposed grass shrimp *in vitro*, in real time, using non-invasive methods. Since the time required to feed and prepare shrimp for NIR scanning was 20 min, initial casein hydrolysis rates (from $t = 0$) could not be monitored for linearity. Ahrens and Lopez (2001) reported that protease activities in the guts of deposit-feeding polychaetes monitored *in vivo* were approximately linear for 5 to 10 min following ingestion until available substrate (i.e., gelatin-embedded casein) was hydrolyzed. Protease activities in grass shrimp reached saturation for several sites and treatments within 29 to 30.5 min, indicating that casein substrate was exhausted. NIR imaging techniques in the present

study required longer scan times (90 sec) than methods involving fluorescent casein substrates used in previous studies (3.5 sec), resulting in reduced resolution over the time scale axis (Ahrens and Lopez, 2001).

Origins and functions of epithelial cell types that line decapod hepatopancreatic tubules have been the subject of significant controversy (particularly for B cells), however, it is widely-recognized that fibrillar (F) cells synthesize and secrete enzymes that catalyze extracellular hydrolysis of macromolecules in the proventriculus and lumen of the hepatopancreas (Gibson and Barker 1979; Al-Mohanna et al., 1985; Al-Mohanna and Nott, 1986; Vogt et al., 1989; Vogt, 1993). Vogt et al. (1989) determined that *Astacus* protease in crayfish is synthesized in F cells, transported to brush border surfaces and exocytosed into the hepatopancreatic tubules. Active enzyme is then accumulated and stored in the anterior region cardiac chamber of the proventriculus for the next feeding cycle (Vogt et al., 1989). Decapod digestive enzyme activities can be influenced by molt stage, feeding condition prior to analysis *in vitro* (Van Wormhoudt, 1974; Muhlia-Almazán and García-Carreño 2002). De La Ruelle et al. (1992) reported that metal (Co and Mn) exposure reduced activity of aminopeptidase isolated from crayfish.

Protease activities in grass shrimp collected from impacted collection sites (MA and NC) within the AK complex were reduced significantly relative to shrimp from a local reference site (GK). Casein hydrolysis rates were also disproportionately lower for AK shrimp with respect to initial IRDye signal, suggesting that any impacts of reduced food ingestion may be compounded by impaired digestive enzyme function. Since field-collected shrimp were allowed to clear their guts for 3 d prior to analysis, variability in protease activities may be related to post-assimilatory impacts on F cell machinery (e.g.,

ribosomes, endoplasmic reticulum, Golgi apparatus, vesicle transport or mechanisms of exocytosis) involved in synthesis and discharge of digestive enzymes (Vogt et al., 1989). Shrimp from the field sites investigated in the present study also exhibited differences in ^{14}C- labeled meal ingestion in related work (ingested cpm ANOVA: ns; followed by multiple comparisons using the Tukey-Kramer multiple comparisons test: GK > NC > MA) (analysis of unpublished data from Seebaugh and Wallace, 2009). Interestingly, homogeneity of radiolabeled amphipod ingestion was observed for shrimp from the study sites used in metal AE analysis, suggesting that dietary considerations (e.g., palatability of field-available prey vs. prepared meals) may be important in determining effects of dietary pollutant exposure on ingestion and digestive enzyme activities (analysis of unpublished data from Seebaugh and Wallace, 2009). Post-assimilatory impacts on other processes related to ingestion (e.g., mandibular mastication in decapods with reduced gastric armature) may also be important and require additional study (Felgenhauer and Abele, 1983; Sousa and Petriella, 2006). Perez and Wallace (2004) observed that impaired prey capture by AK shrimp may be related to behavioral efficiency, suggesting that effects of pollutant exposure on the assimilation of elements in the field may involve interactions between nutrient acquisition, ingestion and gut physiology.

Protease activities did not vary for shrimp pre-exposed to dietary Cd. In a related study, casein hydrolysis was impacted by ingestion of a pulse of Cd in food, which may have been related to pre-assimilatory impacts on F cell machinery involved in exocytosis or direct effects on enzyme conformation or active sites (Mitane et al., 1987; Stöcker et al., 1988; Vogt et al., 1989). Any post-assimilatory effects of dietary Cd exposure may not have been severe enough to impact F cell functions. Additionally, shrimp may have

recovered from pre-assimilatory effects on enzyme function during the 2 d period between the final feeding on Cd-contaminated worms and assays for protease activity. Casein hydrolysis rates were influenced by pre-exposure to Hg, suggesting intracellular impacts of metal not sequestered by F cell metallothioneins on enzyme production or transport (Chavez-Crooker et al., 2003; Biagioli et al., 2008).Variability in casein hydrolysis between the intermediate and highest dietary Hg treatments may have resulted from interactions between post-assimilatory impacts on enzyme availability and other gut parameters (e.g., pH) (Chen and Mayer, 1998).

Gut pH for grass shrimp was estimated *in vivo*, using a non-invasive technique based on differential sensitivities of fluorescein emissions within the lumen of the proventriculus (Ahrens and Lopez, 2001). Although direct measurements of gut juice with microelectrodes may provide greater accuracy in estimating pH (e.g., in polychaetes), microfluorometric methods allow for characterization of gut chemistry without risk of gut wall perforation (Ahrens and Lopez, 2001). Gastric juice pH (typically determined *in vitro*) is weakly acidic in many decapods, but may be neutral in some species (fiddler crabs *Uca*) (van Weel 1970, Gibson and Barker, 1979; Johnston and Yellowlees, 1998). Mechanisms for regulation of gut pH in decapods are poorly understood (Gibson and Barker, 1979). Since digestive enzymes are stored in active form within the cardiac proventriculus, the possibility that enzyme vacuoles released to the lumen of the hepatopancreas by F cells contain components that influence extracellular pH requires additional study (Zwilling and Neurath, 1981; Vogt et al., 1989). Dall and Moriarty (1983) suggested that factors regulating extracellular gut pH in decapods may

be produced by anterior midgut caeca. The structures have not yet been described for palaemonid shrimp (Sousa and Petriella, 2006).

Bioavailability and assimilation of ingested elements depends upon the extent to which they are desorbed from ligands in food, sediment particles or gut fluid, which may be related to internal pH (Griscom et al., 2002a). Changes in pH within the decapod hepatopancreas could potentially influence extracellular digestive processes, including activities of digestive enzymes. Divakaran and Ostrowski (1998) found that trypsin activity was not influenced by changes in pH (5.5 to 8.0) in hepatopancreatic extracts from peneaid shrimp. In many cases, digestive enzymes appear to have pH optima, which can vary considerably among species (van Weel, 1970 and references therein; Glass and Stark, 1994). Although it is not known if pH within B cell digestive vacuoles (which Vogt [1993] described as a huge lysosomes) is related to gut fluid pH, changes in free H^+ concentrations within this structure could potentially influence nutrient transfer to the haemolymph and other tissues (i.e., assuming that B cells are involved in nutrient assimilation) (Al-Mohanna and Nott, 1986).

pH (analyzed as H^+ concentrations) within the posterior region of the cardiac chamber of the proventriculus exhibited variation for grass shrimp from two Staten Island collection sites (GK and NC) and shrimp pre-exposed to dietary Cd (Murphy, 1981). pH within the anterior region did not vary for shrimp from either treatment group. Anterior and posterior region pH increased over the range of dietary Hg pre-exposures. In a related study, gut pH in naïve shrimp was not impacted by a pulse of ingested Cd, suggesting that post-assimilatory impacts of dietary pollutants (e.g., metals) may be important in

determining free proton concentrations in gut fluid. Intracellular exposure to metal (e.g., Cd^{2+} and Hg^{2+}) may induce lysosomal membrane destabilization and leakage of acid hydrolases into the cytoplasm causing cellular damage (Viarengo, et al., 2000). Furthermore, Hg^{2+} may inhibit a lysosomal proton pump mechanism due to interactions with membrane sulfhydryls, resulting in increased lysosomal pH (Viarengo et al., 2000). Post-assimilatory impacts of metal exposure on F cell machinery (e.g., lysosomes) may be important in determining gut pH and requires study. Damage to the gut epithelium may also impact mechanisms of ion exchange (e.g., an amiloride-sensitive electrogenic $2Na^{+}/1H^{+}$ antiporter) that may influence proton concentrations in gut fluid (Ahearn, 1987; Ahearn et al., 1994).

pH within the anterior and posterior regions of the cardiac chamber did not vary for individual collection sites or dietary metal treatments and peristalsis subsided shortly after ingestion, indicating that mixing of food, enzymes and other components of gut fluid was rapid and complete (Powell, 1974; Vogt et al., 1989; King and Alexander, 1994). Storage of enzymes in preparation for the next feeding cycle, thorough mixing of gut fluid and well-defined timing of events within the hepatopancreas (e.g., B cell extrusion and increased mitotic activity of E cells during the excretory phase) are consistent with a batch reactor model for extracellular digestion in decapods (Al-Mohanna and Nott, 1986; Vogt, 1993; Penry and Jumars, 1986, 1987). This model assumes feeding on discrete meals, pulsed input of reactants (e.g., extracellular digestive enzymes in the case of decapods) and that guts are cleared of products and reloaded with reactants prior to the next digestive cycle (Penry and Jumars, 1986, 1987). Digestion enzyme (e.g., protease) secretion by F cells can also be stimulated by feeding, which

may be necessary to supplement enzymes stored anteriorly in the proventriculus and to hydrolyze materials entering the hepatopancreas (Vogt et al., 1989). Terms related to GRT (influencing pulsed output of reactants, products of hydrolysis and expulsion of unprocessed materials) and enzyme activities (conversion of reactants to products over time) may be appropriate additions to a simple batch reactor model for grass shrimp, if this is indeed an appropriate model for this species (Penry and Jumars, 1987). It is not known whether plasticity of enzyme secretion by F cells or timing of the B cell cycle can accommodate feeding at irregular intervals, particularly during prolonged bouts of continuous ingestion (Vogt et al., 1989; Hopkin and Nott, 1980; Al-Mohanna and Nott, 1986). Patterns of irregular or continuous feeding may be modeled by other reactor designs (Penry and Jumars, 1986, 1987). Models of gut processes in decapods should also consider the influence of intracellular digestion on absorption and assimilation. Although intracellular digestion may take place in B cells, evidence for some species (e.g., *Palaemonetes argentinus*) suggests that endocytotic uptake of materials by this cell type may clear the hepatopancreatic tubules of waste materials between periods of nutrient absorption by R cells and enzyme secretion by F cells (Hopkin and Nott 1980; Al-Mohanna and Nott, 1986; Vogt, 1993; Sousa et al., 2005). B cells (and large digestive vacuoles) are later extruded from the epithelium of the hepatopancreas and voided in feces (Hopkin and Nott, 1980; Al-Mohanna and Nott, 1986). In addition to timing of the digestive cycle, hepatopancreatic processes may regulate input of reactants and output of reactions and products during extracellular digestion (Al-Mohanna and Nott, 1986; Penry and Jumars, 1986, 1987).

Distributions of post-assay grass shrimp among stages of the molt cycle were consistent with the relatively short intermolt and long premolt periods characteristic of diecdysic species (Freeman and Bartell, 1975). Palaemonids have a reduced foregut armature and rely on well-developed mandibles, rather than a gastric mill, for mastication (Felgenhauer and Abele, 1983; Sousa and Petriella, 2006). Shrimp from all stages consumed experimental meals indicating that cuticular rigidity of the feeding appendages, foregut (esophagus and proventriculus) and hindgut was sufficient for ingestion and to maintain digestive functions over the course of the molt cycle (i.e., except during molting). Further research is necessary to determine whether or not functional changes in gut cuticle occur from postmolt through premolt stage D_2. Although midgut organs are not lined with cuticle, hepatopancreatic glycogen is linked to the molt cycle, with peak levels reached during premolt to meet metabolic demands as well as generate sugar precursors for chitin synthesis (Heath and Barnes, 1970; Parvathy, 1970; Spindler-Barth, 1976). Interactions between variability in carbohydrate absorption during the molt cycle and pollutant-induced changes in hepatopancreatic functions (e.g., glucose transport into R and B cells) could influence metabolism, haemolymph glucose concentrations, glycogen storage by R cells and the molting process (Parvathy, 1971; Al-Mohanna and Nott, 1987; Lorenzon et al., 2000; Verri et al., 2001; Vilella et al., 2003). Metals stored in R cells and metallothioneins in the hepatopancreas also fluctuate with the molt cycle (Al-Mohanna and Nott, 1987, 1989; Engel, 1987). Preliminary evidence (due to small sample sizes) from the present work and a related study of digestion in naïve grass shrimp suggests that GRT, FER, extracellular protease activities and gut pH may not be influenced by the molt cycle (Seebaugh and Wallace, unpublished). In

contrast, activities of amylases and proteases isolated from the caridean shrimp *Palaemon serratus* fluctuated throughout the molt cycle, with maximum activities observed during intermolt and premolt (Van Wormhoudt, 1974). Feeding condition (i.e., starvation) may also impact digestive enzyme activities (Muhlia-Almazám and García-Carreño, 2002).

Although GRT did not vary significantly for grass shrimp collected along an impact gradient, this digestive parameter was positively correlated with Cd AE, but not carbon or Hg AE, reported previously (Seebaugh and Wallace, 2009). Extracellular digestive protease activities were negatively correlated with GRT and a negative relationship between casein hydrolysis rates and Cd AE was indicated for Staten Island shrimp populations. Digestive enzyme synthesis and activities in decapods can also be influenced by diet composition (e.g., protein content) (Rodriguez et al., 1994; Le Moullac et al., 1996). Interactions between post-assimilatory impacts of field exposure on F cell machinery and the nutritional content of food organisms may, therefore, be important in determining enzyme availability (Campbell et al., 2005). Assuming that shrimp inhabiting the AK complex do not possess the enzyme plasticity required to compensate for effects of ingested pollutants or diet, increased contact time between nutrients, gut fluid and absorptive surfaces of the gut epithelium may be necessary to maintain adequate nutrient assimilation (Bock and Mayer, 1999; Relyea and Auld, 2004; Lopez, 2005). AK shrimp assimilated nearly identical percentages of carbon from radiolabeled meals as shrimp from GK, suggesting that digestive plasticity (e.g., changes in hepatopancreatic processes that increase GRT) may be important in assimilation of essential elements (e.g., organic carbon) (Al-Mohanna and Nott, 1986, 1987; Seebaugh and Wallace, 2009). For impacted grass shrimp populations, a consequence of longer

GRT may be enhanced assimilation of non-essential elements, including pollutants (e.g., Cd). Lack of correspondence between GRT and Hg AE by field-collected grass shrimp could be related to metal-specific transport pathways across epithelial surfaces that may not be influenced by gut transit time (Zhang and Wang, 2006; Seebaugh and Wallace, unpublished).

It is not known whether or not grass shrimp inhabiting the AK complex (MM and NC) or sites flushed with cleaner waters from Raritan Bay (GK) represent the same source populations (Fig 4-1). Compensation for pollutant-impaired gut physiology (increased GRT to offset reduced enzyme function) among impacted shrimp populations may represent a phenotypic response to differential exposure to contaminant (e.g., metal and organic) loads in the field or may have an underlying genetic component (Klerks and Weis, 1987; Janssens et al., 2009). Rapid evolution and subsequent loss of genetic resistance was reported for benthic oligochaetes exposed to high concentrations of metals in sediments, which were removed during site remediation (Klerks and Levinton, 1989; Levinton et al., 2003). Adaptation of oligochaetes to metal-impacted conditions may have been attributable to genetic control of metal detoxification (Klerks and Bartholomew, 1990; Martínez and Levinton, 1996). The hepatopancreas plays an important role in regulation and storage of divalent cations (e.g., metals) as well as detoxification in decapods (Hopkin and Nott, 1979; Al-Mohanna and Nott, 1987; Chavez-Crooker et al., 2003). Adaptation of digestive physiology to dietary pollutant exposure may be manifested as intraspecific variation in grass shrimp gut function over successive generations. Transplant and selection studies may assist in determining whether population differences in digestive function are related to phenotypic or genotypic

plasticity. Gene flow may also be important in determining population responses to changes in environmental stress (e.g., metal pollutants) (Mackie et al., 2009). The possibility that migration of and gene flow between AK and Raritan Bay shrimp populations may be restricted by long water residence times resulting from concurrent tidal surges from Newark and Raritan Bays also requires investigation (Oey, et al, 1985; Mathews, 2007).

Relationships between gut physiology and Cd, Hg or carbon AE reported in related work were not apparent for shrimp pre-exposed to dietary Cd (Seebaugh and Wallace, unpublished). Proventriculus pH increased over the range of dietary Hg treatments and was accompanied by a negative relationship between Hg assimilation and H^+ concentrations in gut fluid (Seebaugh and Wallace, unpublished). Reduced competition between Hg^{2+} ions and protons for binding sites (perhaps sulfydryl groups associated with cysteine or ion transport pathways) may be important in determining Hg availability in gut fluid and transport across the hepatopancreatic epithelium in Hg pre-exposed shrimp (Mayer et al., 2001; Andres et al., 2002; Chen et al., 2002; Laporte et al., 2002).

CONCLUSION

In addition to routes of environmental pollutant exposure (e.g., dissolved vs. diet), it is important to consider the mode in which tissues are exposed once a pollutant is internalized (Hook and Fisher, 2001; Griscom et al., 2002b; Campbell et al., 2005). For dietary pollutants, toxicological impacts may vary depending upon whether exposure is through circulating gut fluid or follows assimilation by tissues. In related work, FER and protease activities in naïve grass shrimp were influenced by ingestion of a pulse of Cd

with food, indicating the potential importance of pre-assimilatory impacts of ingested metal on digestion. In the present study, we have demonstrated post-assimilatory impacts on digestion in grass shrimp collected along an environmental impact gradient or pre-exposed to dietary metal. These effects are presumed to be related to incorporation of pollutants into epithelial cells within the hepatopancreas (and possibly the midgut) that influence timing of digestion, synthesis and secretion of extracellular digestive enzymes and factors that may control gut pH. For shrimp inhabiting the AK complex, ingestion of organic pollutants (e.g., byproducts of petroleum processing and leachate contamination) may also be important in influencing gut functions. Post-assimilatory impacts of field exposure may also be related to interactions between different classes of ingested pollutants. Previous studies have investigated digestive plasticity (e.g., enzyme activities and surfactant secretion) in benthic invertebrates in response to variation in diet (Bock and Mayer, 1999). The results of the present study indicate that digestive plasticity may be important in maintaining adequate assimilation of essential nutrients (e.g., organic carbon) despite post-assimilatory impacts on digestion (e.g., reduced enzyme activities) (Seebaugh and Wallace, 2009). Since post-assimilatory toxicity is presumably related to functional changes in gut tissues, future work will investigate changes in the morphology and ultrastructural localization of ingested Cd and Hg in the grass shrimp hepatopancreas.

Table 4-1. Effects of chronic field exposure or pre-exposure to dietary Cd or Hg on initial IRDye signal in grass shrimp *P. pugio*. Collection sites included Great Kills Harbor (GK), Main Creek (MA) and Neck Creek (NC). Initial IRDye signal is integrated intensity at t = 20 min from the introduction of IRDye-labeled casein meals. Correlations between casein hydrolysis rate and initial IRDye signal were analyzed for individual treatments and treatment groups using Pearson product-moment (r, except where indicated). Data that did not fit a normal distribution were analyzed using Spearman rank correlation (r_s).

	Study site or Dietary treatment (n)	Initial IRDye signal	Casein hydrolysis rate vs. initial IRDye signal (r or r_s)
Study sites:	GK (9)	246.6 ± 31.5	-0.0824 (r_s; ns)
	MA (6)	164.2 ± 29.2	0.0003 (ns)
	NC (6)	43.2 ± 7.1	0.5244 (ns)
	All study sites	164.9 ± 24.3	0.6143 (r_s; $p < 0.05$)
	Ingested IRDye ANOVA: $p < 0.05$ (GK = MA > NC[b])		
Pre-exposure to dietary Cd:	Control[a] (9)	181.9 ± 38.4	0.0819 (ns)
	0.58 µg g^{-1} (10)	191.5 ± 24.3	0.2745 (ns)
	2.45 µg g^{-1} (6)	213.2 ± 42.7	0.6609 (ns)
	7.03 µg g^{-1} (10)	194.9 ± 39.4	-0.5349 (r_s; ns)
	All treatments	193.7 ± 17.3	0.0879 (r_s; ns)
	Ingested IRDye K-W ANOVA: ns		
Pre-exposure to dietary Hg:	Control (7)	170.4 ± 34.5	0.2562 (ns)
	0.08 µg g^{-1} (8)	260.9 ± 79.5	0.2251 (ns)
	0.18 µg g^{-1} (8)	277.5 ± 35.2	0.2509 (ns)
	0.30 µg g^{-1} (11)	177.3 ± 31.3	0.6781 ($p < 0.05$)
	All treatments	219.1 ± 24.2	0.0908 (r_s; ns)
	Ingested IRDye ANOVA: ns		

[a] Estimated dose of dietary metal as TAM-[Cd] or TAM-[Hg] in oligochaetes *T. tubifex* used to feed grass shrimp during 15 d pre-exposure (from Seebaugh and Wallace, unpublished). TAM- [Cd] in worms used to feed Cd controls = 0.0032 µg g^{-1}; TAM-[Hg] in worms used to feed Hg controls = 0.05 µg g^{-1}.

[b] Multiple comparisons conducted using Tukey-Kramer multiple comparisons test.

[ns] ANOVA, K-W ANOVA, r or r_s not significant.

Table 4-2. pH within the anterior and posterior regions of the cardiac chamber of the proventriculus in grass shrimp *P. pugio* collected from differentially-contaminated field sites or pre-exposed to dietary metal for 15 d.

Treatment (*n*)	Cardiac Chamber pH[†] Anterior	Posterior
Field sites:		
GK (8)	5.78 +0.07/-0.06[(ns)]	5.76 +0.04/-0.04[(a)]
NC (4)	5.59 +0.20/-0.14	6.00 +0.06/-0.06[(b)]
Pre-exposed to dietary Cd:		
Control[§] (6)	5.33 +0.24/-0.15[(ns)]	5.29 +0.21/-0.14[(a)]
0.58 µg g^{-1} Cd (10/9)	5.79 +0.13/-0.10	5.84 +0.11/-0.09[(ab)]
2.45 µg g^{-1} Cd (10/9)	5.90 +0.27/-0.17	6.02 +0.21/-0.14[(b)]
7.03 µg g^{-1} Cd (6)	5.87 +0.16/-0.12	5.64 +0.13/-0.10[(ab)]
Pre-exposed to dietary Hg:		
Control (6)	5.33 +0.24/-0.15[(a)]	5.29 +0.21/-0.14[(a)]
0.08 µg g^{-1} Hg (10)	5.74 +0.09/-0.07[(a)]	5.82 +0.10/-0.08[(ab)]
0.18 µg g^{-1} Hg (11/10)	5.76 +0.10/-0.08[(a)]	5.80 +0.07/-0.06[(ab)]
0.30 µg g^{-1} Hg (10)	6.51 +0.13/-0.10[(b)]	6.25 +0.14/-0.11[(b)]

[†] Reported as –log(mean [H^+])

[§] Estimated dose of dietary metal as TAM-[Cd] or TAM-[Hg] in oligochaetes *T. tubifex* used to feed grass shrimp during 15 d pre-exposure (from Seebaugh and Wallace, unpublished). TAM-[Cd] in worms used to feed Cd controls = 0.0032 µg g^{-1}; TAM-[Hg] in worms used to feed Hg controls = 0.05 µg g^{-1}.

[ns] Mann-Whitney *U* test or K-W ANOVA not significant (statistical analyses conducted on [H^+] data).

Significant differences in anterior or posterior cardiac chamber pH within each treatment group are indicated by different letters (Mann-Whitney *U* test for field sites; K-W ANOVA, followed by Dunn's post test for dietary metal treatments [$p < 0.05$]).

Table 4-3. Effects of molt stage on GRT, FER, protease activity and gut pH in grass shrimp *P. pugio* collected from differentially-impacted field sites.

	Study site			
Molt Stage[a]	GK (*n*)	MC (*n*)	NC (*n*)	All sites (*n*)
Median GRT (min)				
C	360 (2)$^{(ns)}$	n/a	n/a	360 (2)$^{(ns)}$
D_0	255 (2)	540 (4) $^{(ns)}$	585 (7) $^{(ns)}$	465 (13)
D_1	345 (1)	585 (2)	255 (3)	375 (6)
D_2	255 (1)	690 (2)	675 (1)	645 (4)
Post	345 (1)	n/a	n/a	345 (1)
Mean FER (mm h^{-1})				
C	2.28 ± 1.86 (2)$^{(ns)}$	n/a	n/a	2.28 ± 1.86 (2)$^{(ns)}$
D_0	0.43 ± 0.21 (2)	3.01 ± 1.05 (3) $^{(ns)}$	4.85 ± 1.39 (7) $^{(ns)}$	3.65 ± 0.95 (12)
D_1	9.34 ± 0.00 (1)	2.73 ± 0.00 (1)	5.37 ± 1.46 (3)	5.64 ± 1.33 (5)
D_2	8.35 ± 0.00 (1)	1.33 ± 0.00 (1)	0.98 ± 0.00 (1)	3.55 ± 2.40 (3)
Post	1.06 ± 0.00 (1)	n/a	n/a	1.06 ± 0.00 (1)
Casein hydrolysis rate (mean increase in integrated intensity)				
C	1.51 ± 0.54 (2)$^{(ns)}$	2.09 ± 0.13 (2)$^{(ns)}$	0.37 ± 0.11 (3)$^{(MWU)}$	1.18 ± 0.33 (7)$^{(ns)}$
D_0	5.49 ± 1.92 (6)	1.44 ± 0.00 (1)	0.60 ± 0.29 (3)	3.61 ± 1.35 (10)
D_1	n/a	0.48 ± 0.06 (2)	n/a	0.48 ± 0.06 (2)
D_2	0.58 ± 0.00 (1)	n/a	n/a	0.58 ± 0.00 (1)
Post	n/a	n/a	n/a	n/a
pH within anterior region of cardiac chamber [–log(mean $[H^+]$)]				
C	n/a		n/a	n/a
D_0	5.81 +0.09/-0.07 (3)$^{(ns)}$		5.63 +0.80/-0.27 (2)$^{(nd)}$	5.73 +0.20/-0.14 (5)$^{(ns)}$
D_1	5.61 +0.00/-0.00 (1)		n/a	5.61 +0.00/-0.00 (1)
D_2	5.81 +0.14/-0.10 (4)		5.55 +0.26/-0.16 (2)	5.70 +0.13/-0.10 (6)
post	n/a		n/a	n/a
pH within posterior region of cardiac chamber [–log(mean $[H^+]$)]				
C	n/a		n/a	n/a
D_0	5.82 +0.01/-0.01 (3)$^{(ns)}$		6.06 +0.17/-0.12 (2) $^{(ns)}$	5.90 +0.07/-0.06 (5) $^{(ns)}$
D_1	5.72 +0.00/-0.00 (1)		n/a	5.72 +0.00/-0.00 (1)
D_2	5.73 +0.17/-0.12 (4)		5.95 +0.03/-0.02 (2)	5.79 +0.07/-0.06 (6)
post	n/a		n/a	n/a

[a] Stages: intermolt (C), premolt (D_0, D_1 and D_2) and postmolt (post) determined by examination of setal region of uropod exopodites; $^{n/a}$ Shrimp from molt stage not identified following assay; ^{ns} K-W ANOVA not significant; ^{MWU} Comparison conducted with Mann-Whitney *U* test (ns); nd Analysis not performed due to insufficient sample size.

Table 4-4. Effects of molt stage on GRT, FER, protease activity and gut pH in grass shrimp *P. pugio* pre-exposed to dietary Cd.

	TAM-[Cd] in oligochaetes *T. tubifex* (µg g^{-1})				
Molt Stage[a]	Control (0.0032) (*n*)	0.58 (*n*)	2.45 (*n*)	7.03 (*n*)	All treatments (*n*)
Median GRT (min)					
C	405 (2)$^{(ns)}$	690 (3) $^{(ns)}$	675 (2)$^{(ns)}$	420 (2)$^{(ns)}$	630 (9)$^{(ns)}$
D_0	780 (2)	630 (2)	660 (5)	300 (6)	480 (15)
D_1	600 (6)	720 (5)	765 (2)	n/a	720 (13)
D_2	n/a	780 (1)	720 (1)	720 (1)	720 (3)
Pos	780 (1)	n/a	n/a	780 (2)	780 (3)
Mean FER (mm h^{-1})					
C	1.78 ± 0.59 (2)$^{(nd)}$	6.87 ± 1.95 (2) $^{(ns)}$	0.96 ± 0.12 (2)$^{(ns)}$	2.33 ± 1.82 (2)$^{(ns)}$	2.98 ± 1.00 (8)$^{(ns)}$
D_0	n/a	5.43 ± 0.00 (1)	5.39 ± 3.22 (4)	3.72 ± 1.32 (6)	4.47 ± 1.29 (11)
D_1	7.41 ± 0.79 (4)	3.02 ± 0.93 (3)	7.14 ± 0.00 (1)	n/a	5.73 ± 0.93 (8)
D_2	n/a	n/a	6.18 ± 0.00 (1)	9.92 ± 0.00 (1)	8.05 ± 1.87 (2)
post	n/a	n/a	n/a	n/a	n/a
Casein hydrolysis rate (mean increase in integrated intensity)					
C	2.71 ± 0.49 (3)$^{(ns)}$	2.09 ± 0.00 (1)$^{(ns)}$	n/a	1.47 ± 0.00 (1)$^{(ns)}$	2.33 ± 0.37 (5)$^{(ns)}$
D_0	5.23 ± 0.84 (2)	2.60 ± 0.89 (7)	4.44 ± 1.93 (2)$^{(ns)}$	2.17 ± 0.41 (4)	3.08 ± 0.54 (15)
D_1	2.74 ± 0.31 (2)	1.43 ± 0.00 (1)	4.77 ± 3.75 (2)	n/a	3.29 ± 1.36 (5)
D_2	3.57 ± 1.56 (2)	2.55 ± 0.00 (1)	1.95 ± 0.06 (2)	2.89 ± 0.75 (4)	2.79 ± 0.45 (9)
Post	n/a	n/a	n/a	4.02 ± 0.00 (1)	4.02 ± 0.00 (1)
pH within anterior region of cardiac chamber [–log(mean $[H^+]$)]					
C	n/a	5.58 +0.76/-0.26 (2) $^{(ns)}$	5.53 +1.14/-0.29 (2)$^{(ns)}$	5.91 +0.27/-0.17 (2) $^{(ns)}$	5.64 +0.24/-0.16 (6)$^{(ns)}$
D_0	5.39 +0.37/-0.20 (3)$^{(ns)}$	5.96 +0.14/-0.10 (5)	7.19 +0.17/-0.12 (2)	5.74 +0.18/-0.13 (3)	5.74 +0.19/-0.13 (13)
D_1	n/a	5.64 +0.12/-0.09 (2)	5.65 +0.27/-0.17 (2)	6.82 +0.00/-0.00 (1)	5.73 +0.16/-0.12 (5)
D_2	5.16 +0.78/-0.26 (2)	6.07 +0.00/-0.00 (1)	6.38 +0.28/-0.17 (3)	n/a	5.58 +0.61/-0.24 (6)
post	5.73 +0.00/-0.00 (1)	n/a	6.12 +0.00/-0.00 (1)	n/a	5.88 +0.24/-0.15 (2)
pH within posterior region of cardiac chamber [–log(mean $[H^+]$)]					
C	n/a	5.54 +0.00/-0.00 (1) $^{(ns)}$	5.65 +0.34/-0.19 (2)$^{(ns)}$	5.56 +0.11/-0.09 (2) $^{(ns)}$	5.57 +0.09/-0.07 (5)$^{(ns)}$
D_0	5.31 +0.36/-0.19 (3)$^{(ns)}$	5.98 +0.17/-0.12 (5)	6.61 +0.20/-0.13 (2)	5.56 +0.15/-0.11 (3)	5.66 +0.18/-0.13 (13)
D_1	n/a	5.87 +0.03/-0.03 (2)	5.78 +0.00/-0.00 (1)	6.83 +0.00/-0.00 (1)	5.95 +0.15/-0.11 (4)
D_2	5.15 +0.49/-0.23 (2)	5.86 +0.00/-0.00 (1)	6.59 +0.19/-0.13 (3)	n/a	5.56 +0.51/-0.23 (6)
post	5.76 +0.00/-0.00 (1)	n/a	5.93 +0.00/-0.00 (1)	n/a	5.84 +0.09/-0.08 (2)

[a] Stages: intermolt (C), premolt (D_0, D_1 and D_2) and postmolt (post) determined by examination of setal region of uropod exopodites; [n/a] Shrimp from molt stage not identified following assay; [nd] K-W ANOVA not performed due to insufficient sample size; [ns] K-W ANOVA not significant.

Table 4-5. Effects of molt stage on GRT, FER, protease activity and gut pH in grass shrimp *P. pugio* pre-exposed to dietary Hg.

	TAM-[Hg] in oligochaetes *T. tubifex* (μg g^{-1})				
Molt Stage[a]	Control (0.05) (*n*)	0.08 (*n*)	0.18 (*n*)	0.30 (*n*)	All treatments (*n*)
Median GRT (min)					
C	540 (1)[(ns)]	600 (2) [(ns)]	270 (1)[(ns)]	480 (2)[(ns)]	480 (6)[(ns)]
D_0	780 (5)	585 (2)	660 (1)	330 (2)	645 (10)
D_1	390 (2)	300 (2)	300 (4)	495 (2)	435 (10)
D_2	525 (2)	720 (4)	290 (3)	315 (4)	420 (13)
Post	690 (2)	410 (1)	n/a	n/a	600 (3)
Mean FER (mm h^{-1})					
C	1.83 ± 0.00 (1)[(ns)]	18.85 ± 0.00 (1)[(ns)]	7.67 ± 0.00 (1)[(ns)]	3.57 ± 2.73 (2)[(ns)]	7.09 ± 3.21 (5)[(ns)]
D_0	5.11 ± 1.19 (2)	13.41 ± 2.40 (2)	0.82 ± 0.00 (1)	14.62 ± 1.05 (2)	9.58 ± 2.26 (7)
D_1	5.68 ± 2.63 (2)	8.46 ± 7.98 (2)	11.32 ± 4.38 (4)	5.18 ± 0.05 (2)	8.39 ± 2.21 (10)
D_2	9.05 ± 8.27 (3)	5.32 ± 2.54 (3)	11.36 ± 6.93 (2)	5.59 ± 2.12 (4)	7.35 ± 2.24 (12)
post	0.49 ± 0.00 (1)	14.02 ± 0.00 (1)	n/a	n/a	7.27 ± 6.77 (2)
Casein hydrolysis rate (mean increase in integrated intensity)					
C	n/a	3.13 ± 0.00 (1)[(ns)]	2.25 ± 0.82 (3) [(ns)]	3.65 ± 0.83 (4)[(ns)]	3.06 ± 0.53 (8)[(ns)]
D_0	4.01 ± 1.15 (4) [(ns)]	2.09 ± 0.45 (3)	2.00 ± 0.93 (2)	6.49 ± 0.00 (1)	3.28 ± 0.66 (10)
D_1	n/a	0.97 ± 0.24 (3)	2.34 ± 0.35 (2)	3.24 ± 1.61 (2)	2.00 ± 0.54 (7)
D_2	2.08 ± 0.00 (1)	3.65 ± 0.00 (1)	n/a	3.53 ± 1.05 (3)	3.27 ± 0.64 (5)
Post	2.56 ± 0.57 (2)	n/a	1.67 ± 0.00 (1)	5.34 ± 0.00 (1)	3.03 ± 0.79 (4)
pH within anterior region of cardiac chamber [–log(mean $[H^+]$)]					
C	n/a	5.74 +0.00/-0.00 (1) [(ns)]	6.02 +0.48/-0.22 (2)[(ns)]	6.86 +0.00/-0.00 (1) [(ns)]	6.01 +0.25/-0.16 (4)[(ns)]
D_0	5.39 +0.37/-0.20 (3)[(ns)]	5.63 +0.10/-0.08 (4)	5.86 +0.17/-0.12 (4)	6.49 +0.00/-0.00 (1)	5.64 +0.14/-0.11 (12)
D_1	n/a	5.94 +0.53/-0.23 (3)	5.57 +0.11/-0.09 (4)	6.54 +0.31/-0.18 (5)	5.89 +0.16/-0.12 (12)
D_2	5.16 +0.78/-0.26	5.71 +0.22/-0.14 (2)	6.12 +0.00/-0.00 (1)	6.47 +0.40/-0.21 (2)	5.57 +0.41/-0.21 (7)
post	5.73 +0.00/-0.00	n/a	n/a	n/a	5.73 +0.00/-0.00 (1)
pH within posterior region of cardiac chamber [–log(mean $[H^+]$)]					
C	n/a	5.76 +0.00/-0.00 (1) [(†)]	5.84 +0.02/-0.02 (2)[(ns)]	7.28 +0.00/-0.00 (1) [(ns)]	5.93 +0.17/-0.12 (4)[(ns)]
D_0	5.31 +0.36/-0.19 (3)[(ns)]	5.62 +0.08/-0.07 (4)	5.86 +0.09/-0.08 (4)	6.01 +0.00/-0.00 (1)	5.59 +0.15/-0.11 (12)
D_1	n/a	6.38 +0.14/-0.11 (3)	5.69 +0.18/-0.13 (3)	6.24 +0.28/-0.17 (5)	6.03 +0.17/-0.12 (11)
D_2	5.15 +0.49/-0.23 (2)	5.88 +0.19/-0.14 (2)	5.93 +0.00/-0.00 (1)	6.35 +0.38/-0.20 (2)	5.57 +0.38/-0.20 (7)
post	5.76 +0.00/-0.00 (1)	n/a	n/a	n/a	5.76 +0.00/-0.00 (1)

[a] Stages: intermolt (C), premolt (D_0, D_1 and D_2) and postmolt (post) determined by examination of setal region of uropod exopodites; [n/a] Shrimp from molt stage not identified following assay; [ns] K-W ANOVA not significant; [†] K-W ANOVA: $p < 0.05$ (multiple comparisons with Dunn's post test: ns).

Table 4-6. Correlations between Cd, Hg and carbon assimilation efficiencies (AE)[a] and parameters related to digestive physiology (GRT, FER, digestive proteases [Prot]) in grass shrimp *P. pugio* collected from Staten Island study sites (GK, MA and NC). Statistical analyses conducted using Pearson product-moment correlation (r).

	Cd AE	Hg AE	C AE	GRT	FER
Cd AE	—	—	—	—	—
Hg AE	0.7458	—	—	—	—
C AE	-0.5893	-0.6200	—	—	—
GRT	0.9985[†]	0.8353	-0.8611	—	—
FER	0.2415	-0.2800	0.2328	0.2939	—
Prot	-0.9956[§]	-0.8131	0.8406	-0.9992[†]	-0.3311

[a] Cd, Hg and carbon AE data from Seebaugh and Wallace (2009)
[†] $p < 0.05$
[§] $p = 0.059$

Table 4-7. Correlations between Cd, Hg and carbon assimilation efficiencies (AE)[a] and parameters related to digestive physiology (GRT, FER, digestive proteases [Prot] and H^+ ion concentrations within the anterior and posterior regions of the cardiac chamber of the proventriculus [Ant H^+; Post H^+]) in grass shrimp *P. pugio* pre-exposed to dietary Cd. Analyses conducted using Pearson product-moment correlation (r).

	Cd AE	Hg AE	C AE	GRT	FER	Prot	Ant H^+
Cd AE	—	—	—	—	—	—	—
Hg AE	-0.7174	—	—	—	—	—	—
C AE	0.6319	-0.1657	—	—	—	—	—
GRT	-0.8514	0.6999	-0.8188	—	—	—	—
FER	-0.2308	0.8180	0.0677	0.4506	—	—	—
Prot	0.2719	0.0014	-0.3153	0.2727	0.4441	—	—
Ant H^+	0.1414	0.5869	0.4591	0.0358	0.9083	0.3708	—
Post H^+	0.3825	0.3643	0.6952	-0.2664	0.7402	0.2574	0.9535[†]

[a] Cd, Hg and carbon AE data from Seebaugh and Wallace (unpublished)
[†] $p < 0.05$

Table 4-8. Correlations between Cd, Hg and carbon assimilation efficiencies (AE)[a] and parameters related to digestive physiology (GRT, FER, digestive proteases [Prot] and H^+ ion concentrations within the anterior and posterior regions of the cardiac chamber of the proventriculus [Ant H^+; Post H^+]) in grass shrimp *P. pugio* pre-exposed to dietary Hg. Analyses conducted using Pearson product-moment correlation (r).

	Cd AE	Hg AE	C AE	GRT	FER	Prot	Ant H^+
Cd AE	—	—	—	—	—	—	—
Hg AE	0.6636	—	—	—	—	—	—
C AE	0.5132	0.3944	—	—	—	—	—
GRT	0.1213	-0.6302	0.2398	—	—	—	—
FER	0.8657	0.6874	0.0469	-0.1758	—	—	—
Prot	-0.6311	-0.1988	0.3113	-0.1494	-0.8445	—	—
Ant H^+	-0.4101	-0.9491[§]	-0.3729	0.7772	-0.4374	-0.0889	—
Post H^+	-0.5287	-0.9855[†]	-0.3550	0.7343	-0.5732	-0.0651	0.9876[†]

[a] Cd, Hg and carbon AE data from Seebaugh and Wallace (unpublished)
[†] $p < 0.05$
[§] $p = 0.051$

Figure 4-1. Map indicating the locations of Staten Island collection sites for grass shrimp *P. pugio*. Sites included Great Kills Harbor (GK), Main Creek (MA) and Neck Creek (NC). Labels for waterways in the region are also included: Newark Bay (NB), New York Harbor (NYH), Raritan Bay (RB), Raritan River (RR) and Arthur Kill (AK). Lines that transverse waterways represent major bridges. Potential sources of environmental contaminants are also indicated. Adapted from Perez and Wallace, 2004.

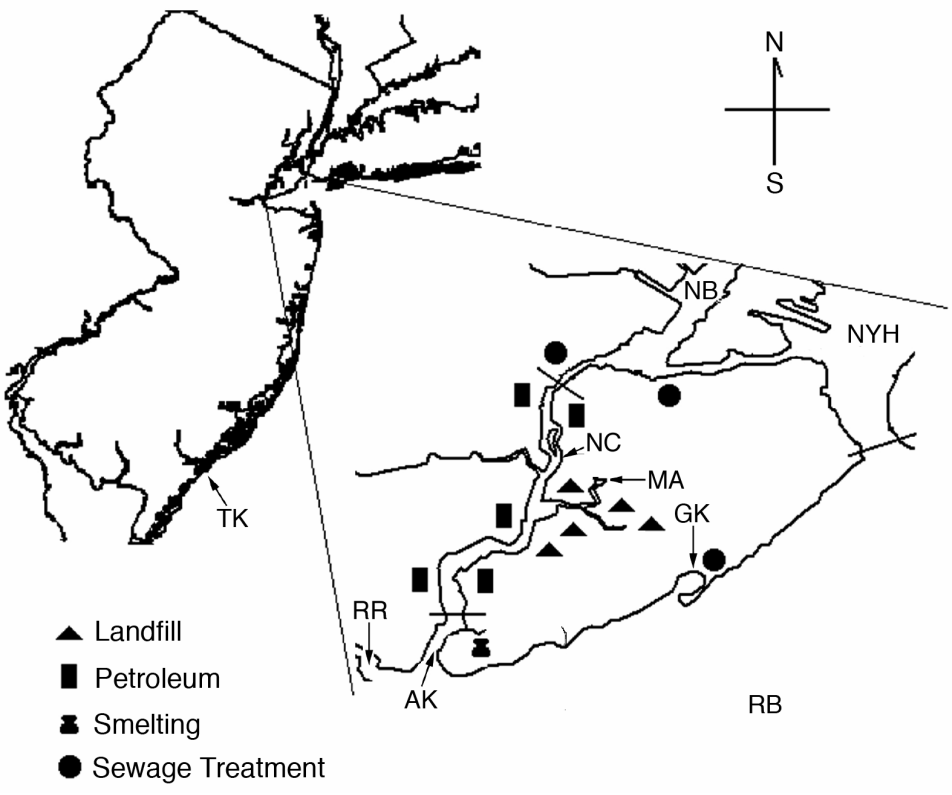
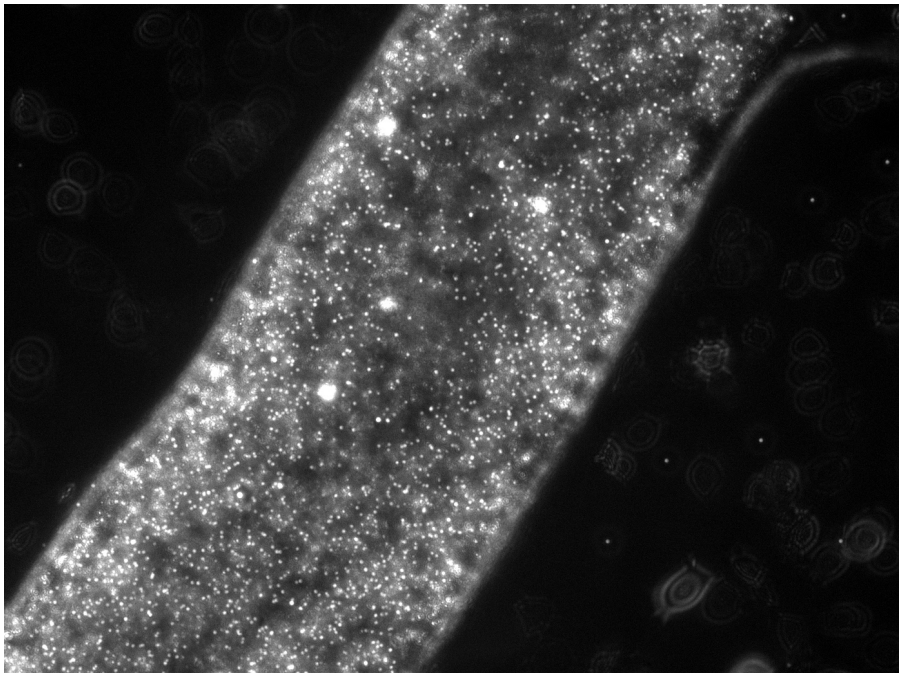
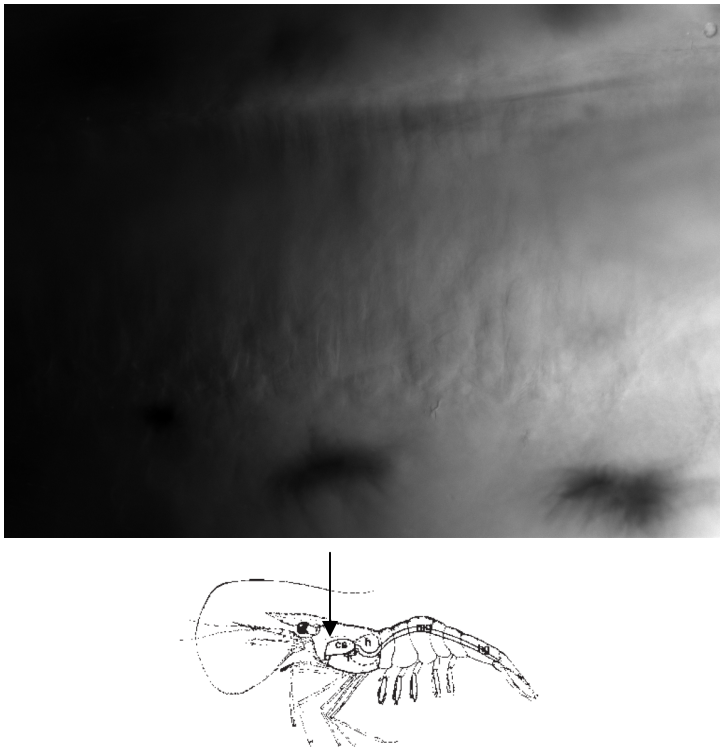


Figure 4-2. (A) Fecal strand released by grass shrimp *P. pugio* fed 0.5 µm diameter Fluoresbrite microspheres. Dorsal views showing fluorescence in (B) anterior region of the cardiac proventriculus, (C) posterior region, (D) above the hepatopancreas in an individual shrimp following ingestion of microspheres. (E) Dorsal view of posterior abdominal segment showing lack of fluorescence.

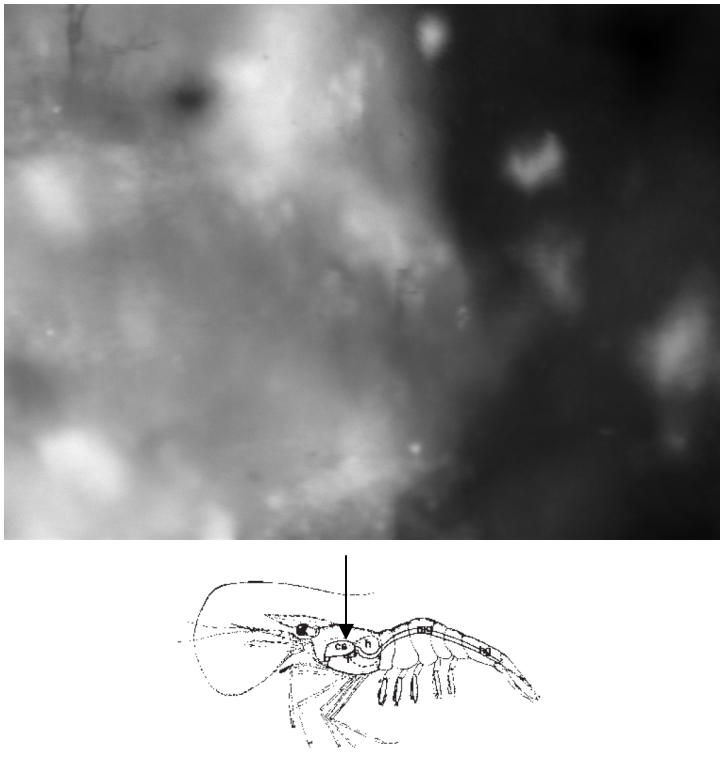
(A)



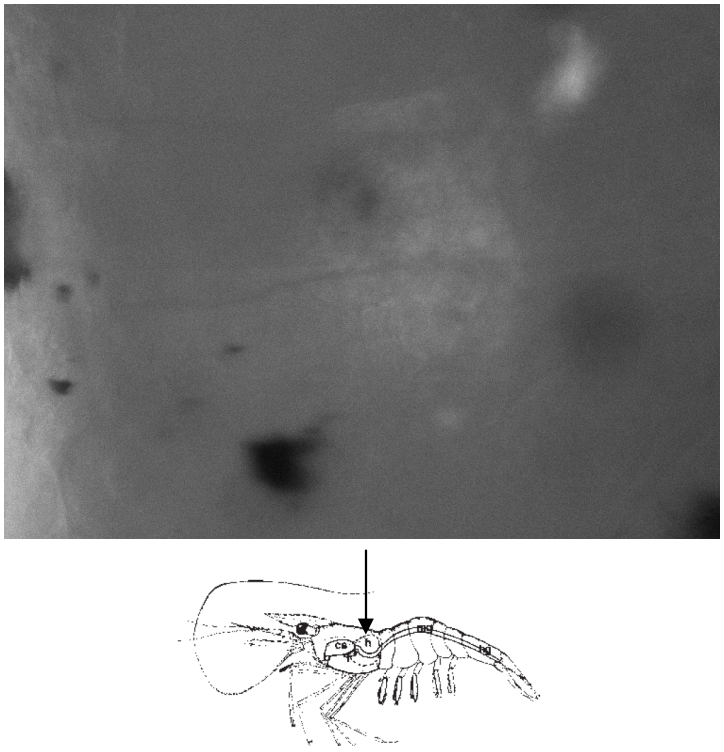
(B)



(C)



(D)



(E)

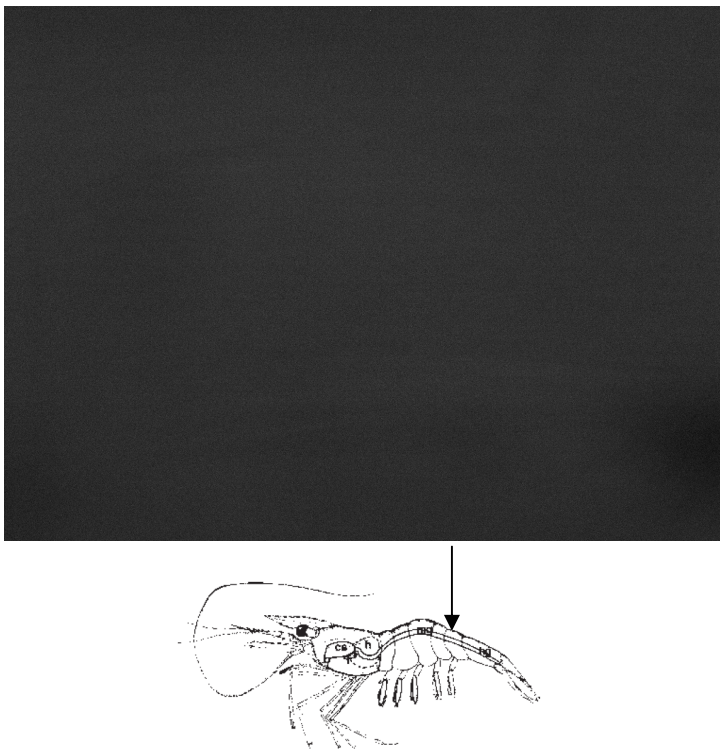
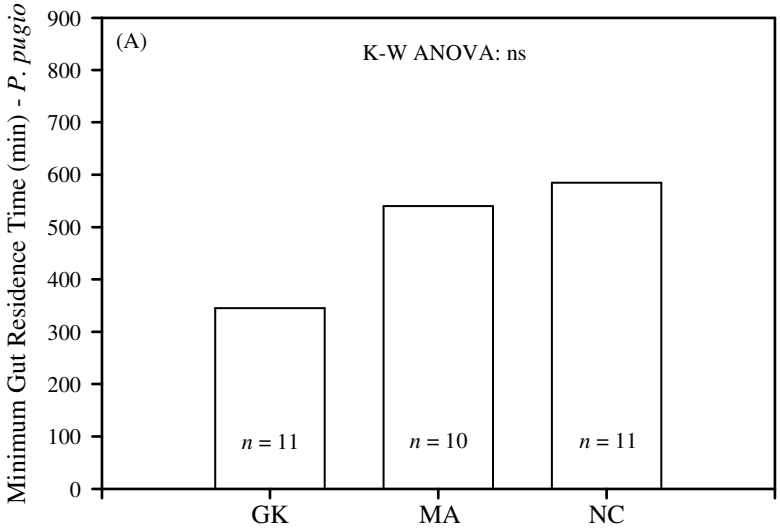
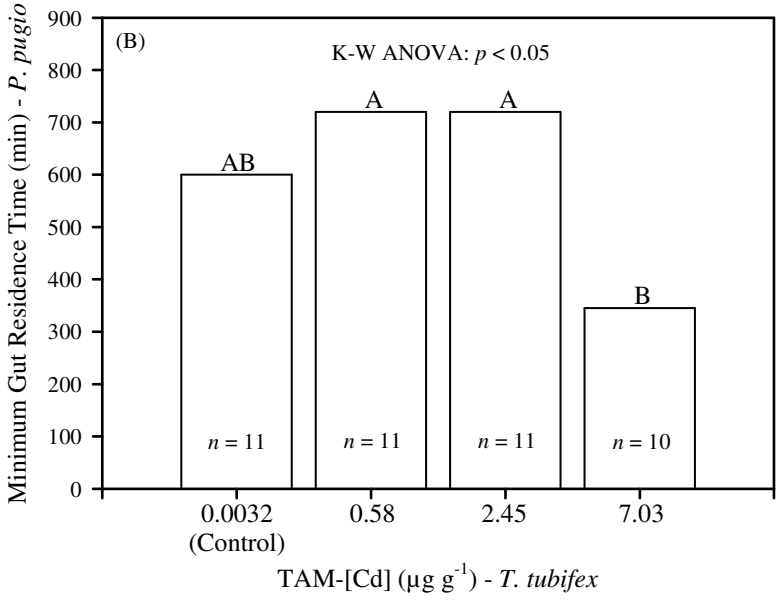


Figure 4-3. Minimum gut residence time in grass shrimp *P. pugio* (A) collected from Staten Island, NY study sites, (B) pre-exposed to dietary Cd or (C) pre-exposed to dietary Hg and fed meals containing 0.5 µm diameter Fluoresbrite microspheres (n = 9-12; median). In (B) significant differences ($p < 0.05$) between dietary treatments (Dunn's post test) are indicated by different letters.





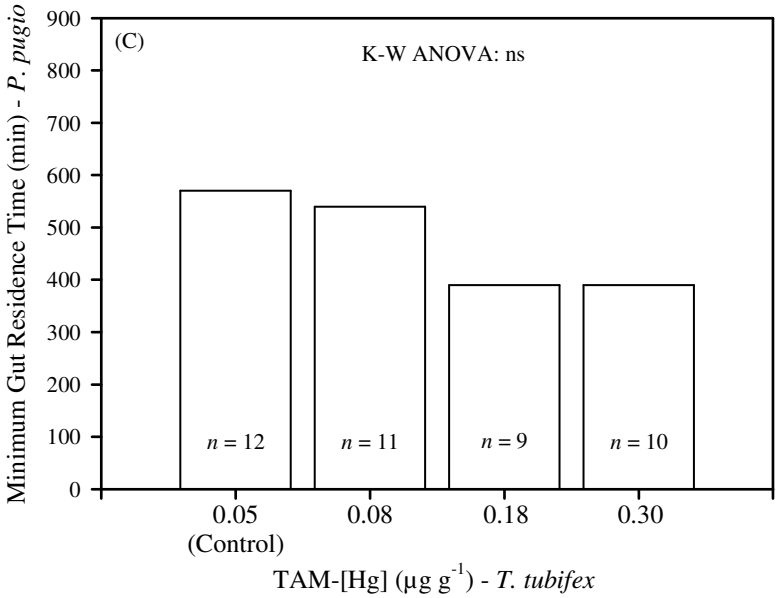
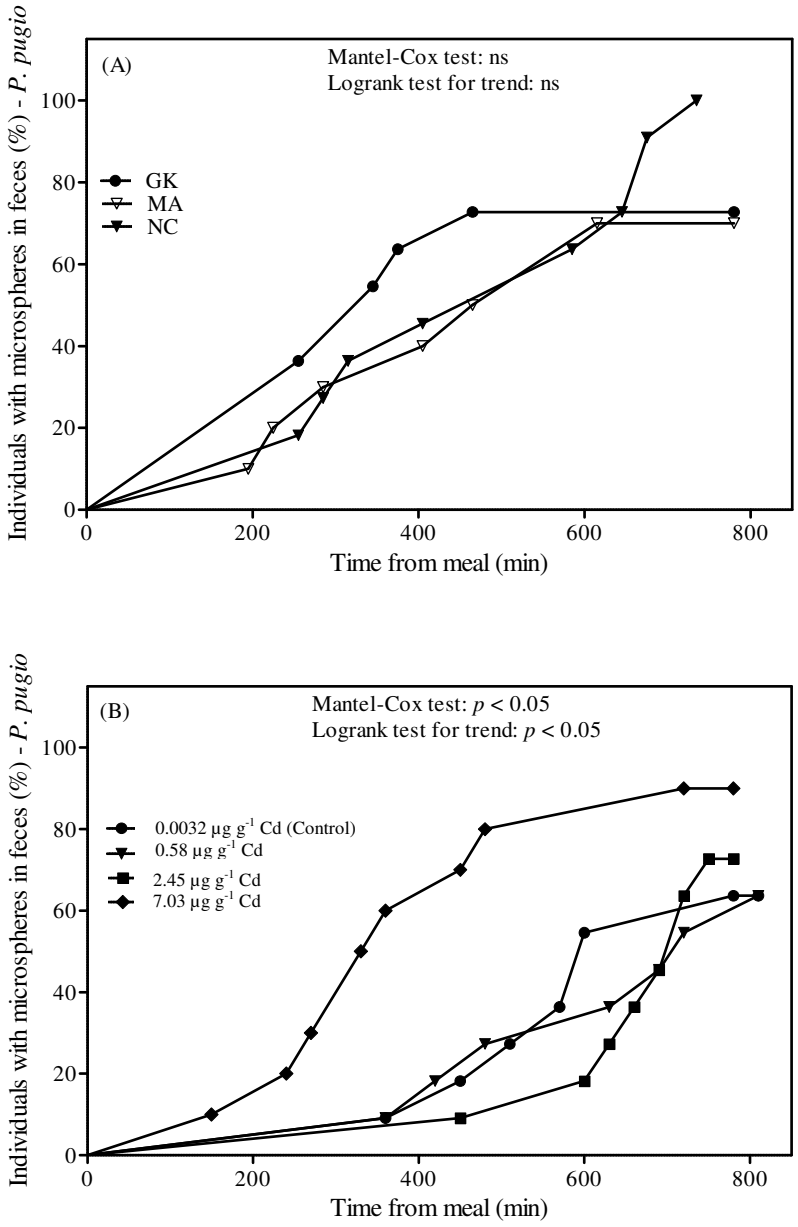

(C)
K-W ANOVA: ns
Minimum Gut Residence Time (min) - P. pugio
900
800
700
600
500
400
300
200
100
0
n = 12
n = 11
n = 9
n = 10
0.05
(Control)
0.08
0.18
0.30
TAM-[Hg] (µg g-1) - T. tubifex

Figure 4-4. Curves showing percentages of individuals with Fluoresbrite microspheres in feces over time for grass shrimp *P. pugio* (A) collected from Staten Island, NY study sites, (B) pre-exposed to dietary Cd or (C) pre-exposed to dietary Hg.



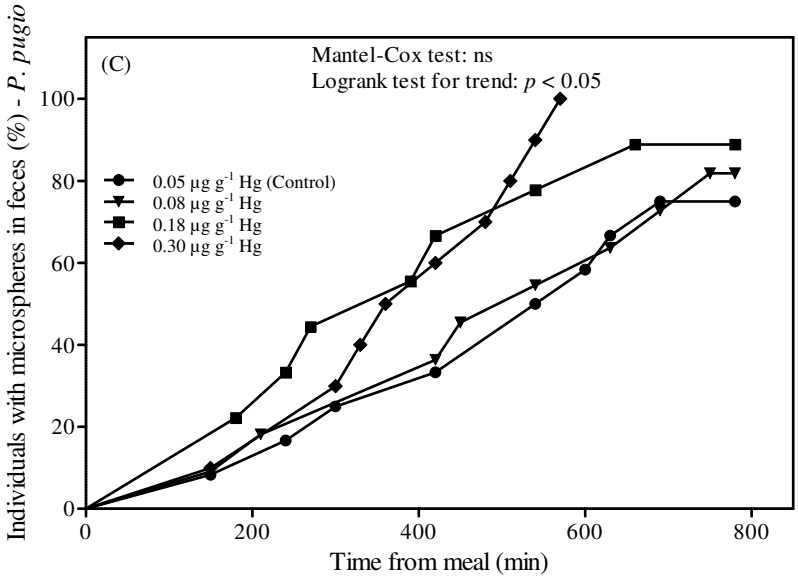
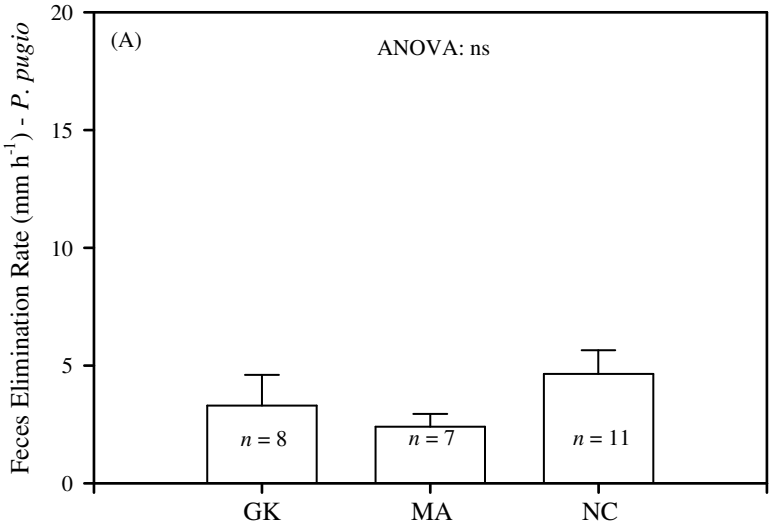
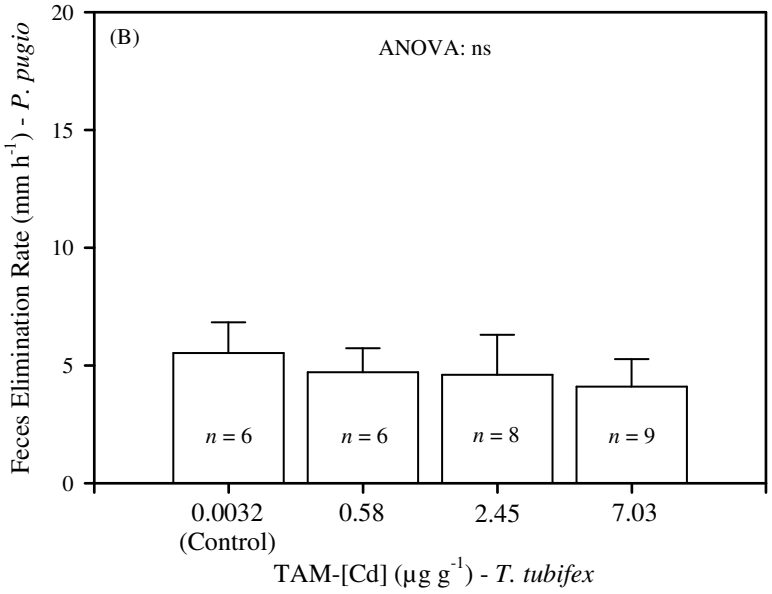

(C)
Mantel-Cox test: ns
Logrank test for trend: $p < 0.05$
0.05 µg g^{-1} Hg (Control)
0.08 µg g^{-1} Hg
0.18 µg g^{-1} Hg
0.30 µg g^{-1} Hg
Individuals with microspheres in feces (%) - *P. pugio*
Time from meal (min)
0
20
40
60
80
100
0
200
400
600
800

Figure 4-5. Feces elimination rate in grass shrimp *P. pugio* (A) collected from Staten Island, NY study sites, (B) pre-exposed to dietary Cd or (C) pre-exposed to dietary Hg (n = 7-11; mean ± S.E.).





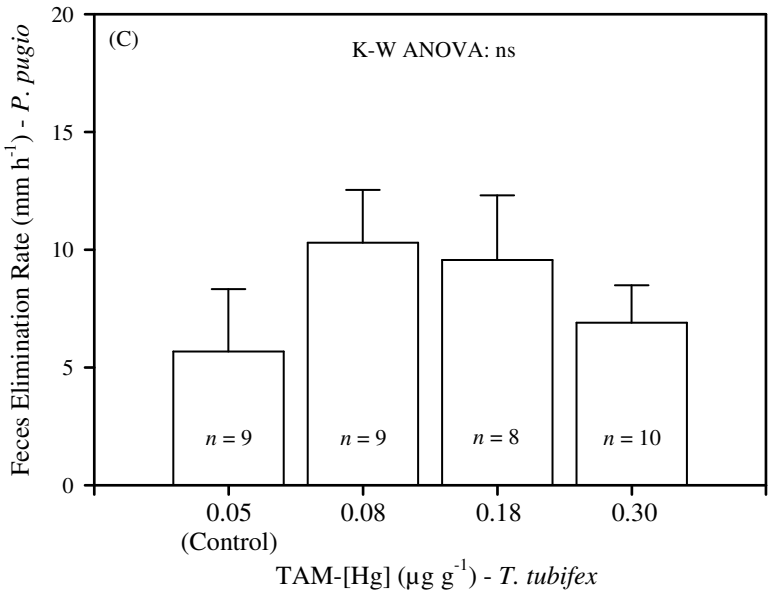
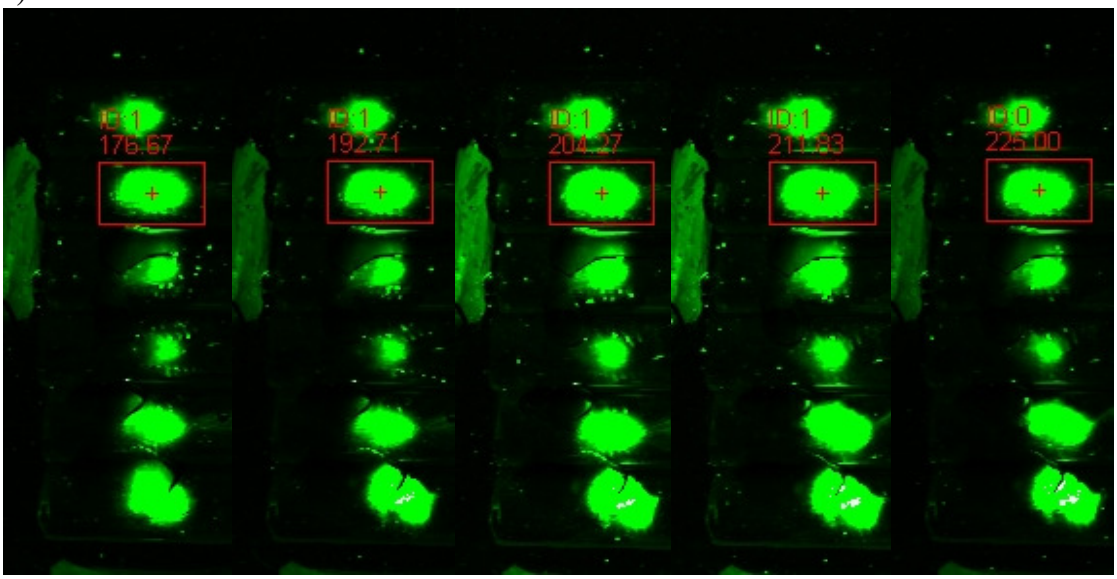
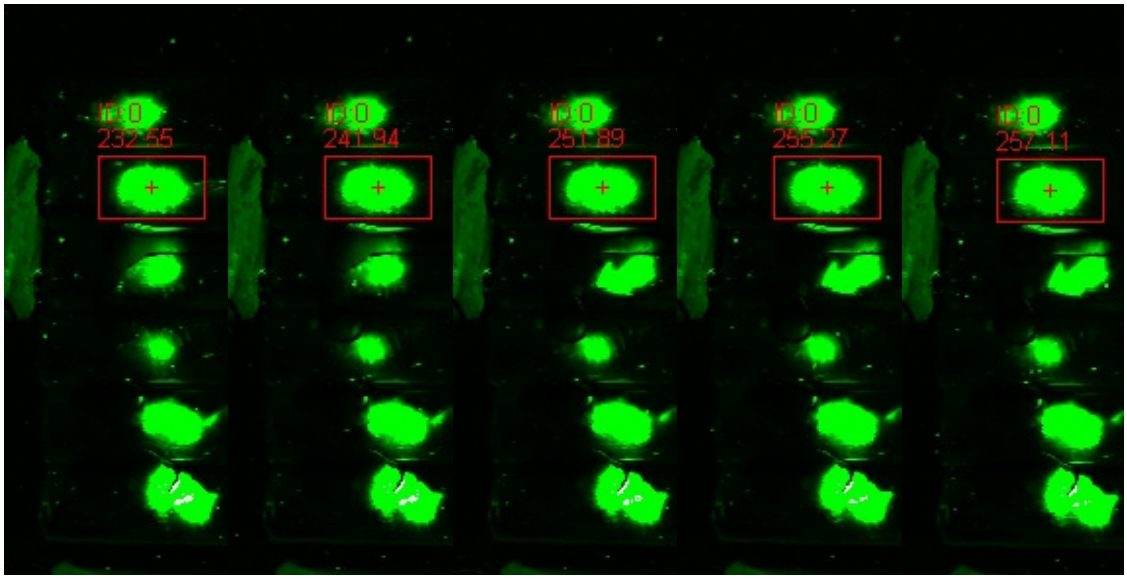

(C)
K-W ANOVA: ns
Feces Elimination Rate (mm h-1) - P. pugio
20
15
10
5
0
n = 9
n = 9
n = 8
n = 10
0.05
(Control)
0.08
0.18
0.30
TAM-[Hg] (µg g-1) - T. tubifex

Figure 4-6. (A) Pseudocolor images of IRDye-labeled casein substrate hydrolysis in grass shrimp *P. pugio* during NIR scanning (ventral view; anterior end of shrimp to left). Red boxes highlight the increase in integrated intensity for an individual shrimp (second image from top in each frame). (B) Plot of the increase in integrated intensity for shrimp highlighted in (A).

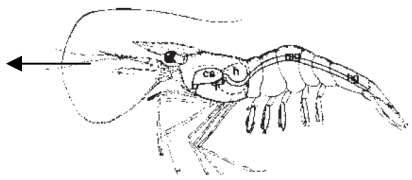
(A)



Time (min): 20.0	21.5	23.0	24.5	26.0
Intensity: 176.67	192.71	204.27	211.83	225.00



27.5	29.0	30.5	32.0	33.5
232.55	241.94	251.89	255.27	257.11



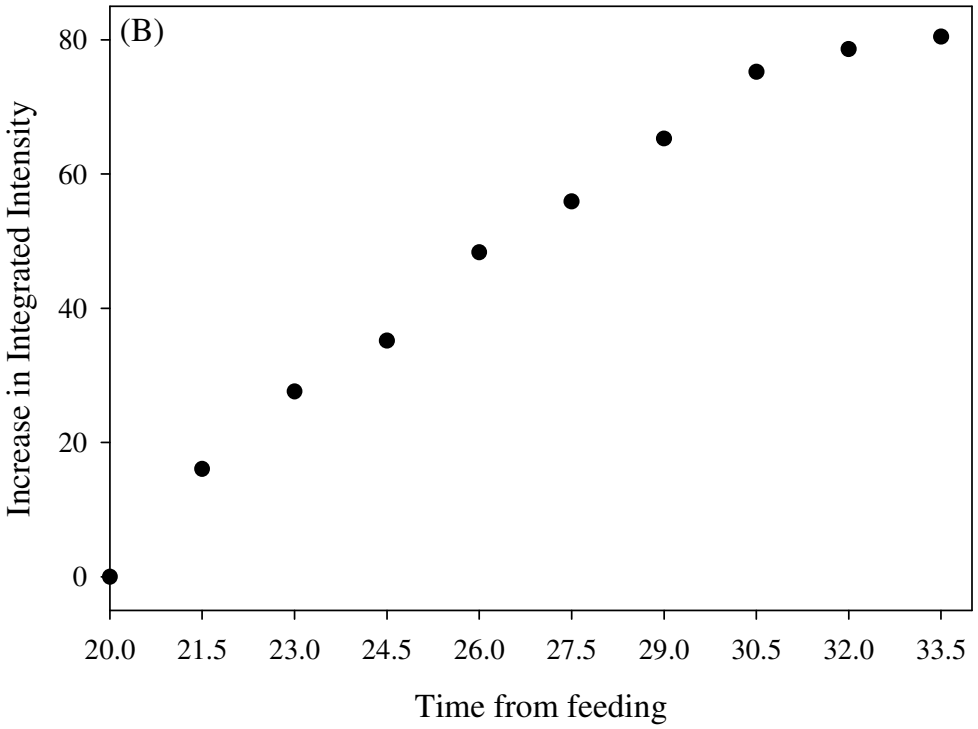
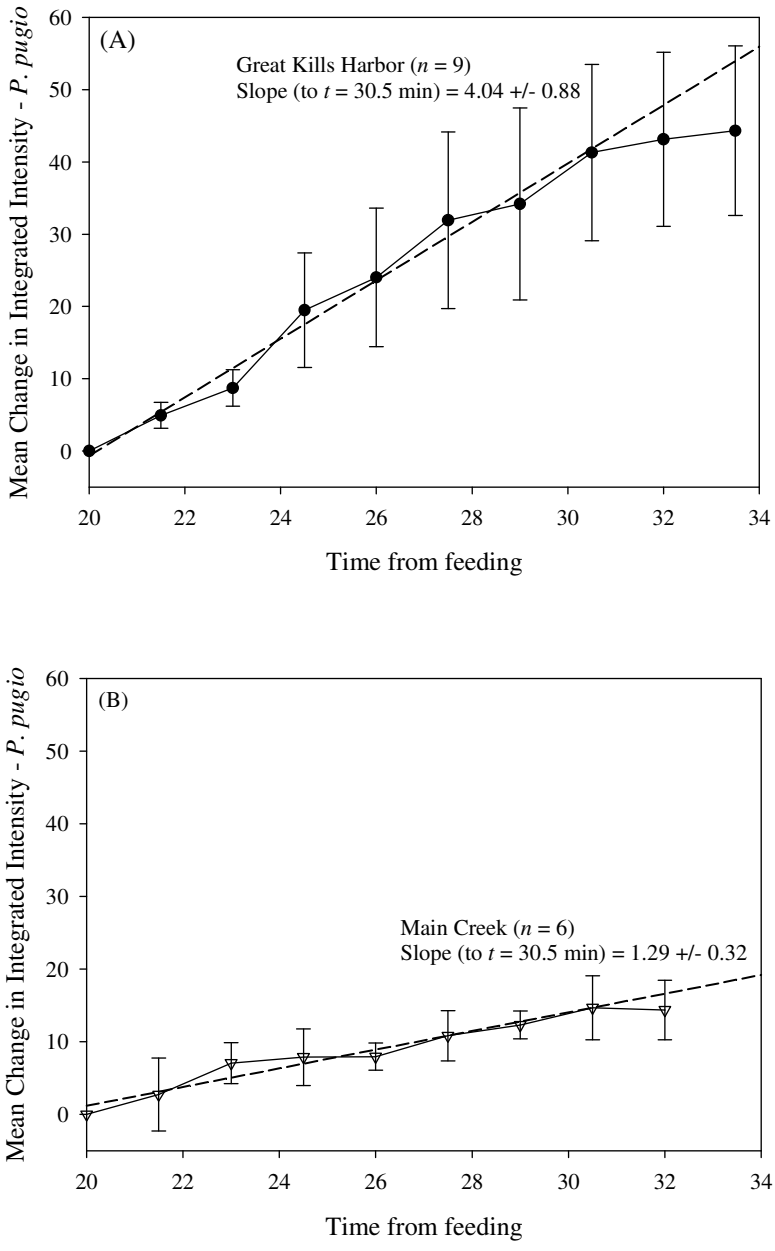

(B)
Increase in Integrated Intensity
0
20
40
60
80
20.0
21.5
23.0
24.5
26.0
27.5
29.0
30.5
32.0
33.5
Time from feeding

Figure 4-7. Mean change in integrated intensity in proventriculus and hepatopancreas of grass shrimp *P. pugio* collected from Staten Island study sites: (A) GK, (B) MC and (C) NC (n = 6-9).



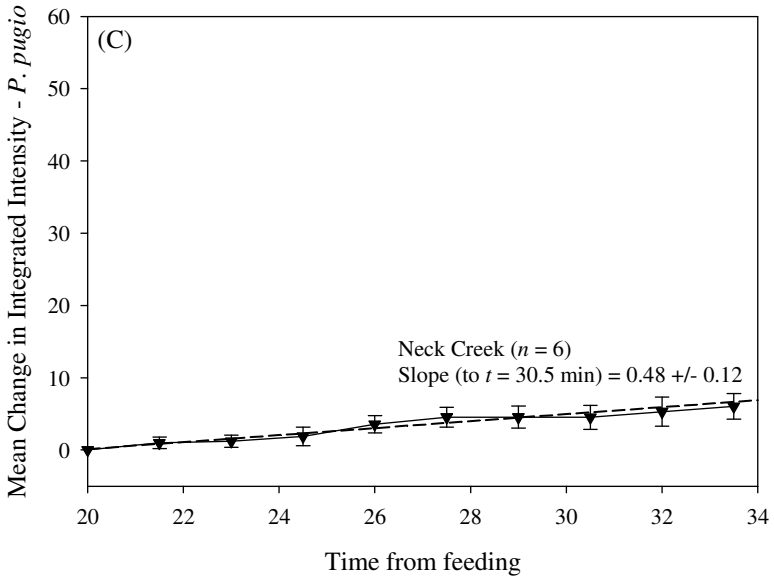
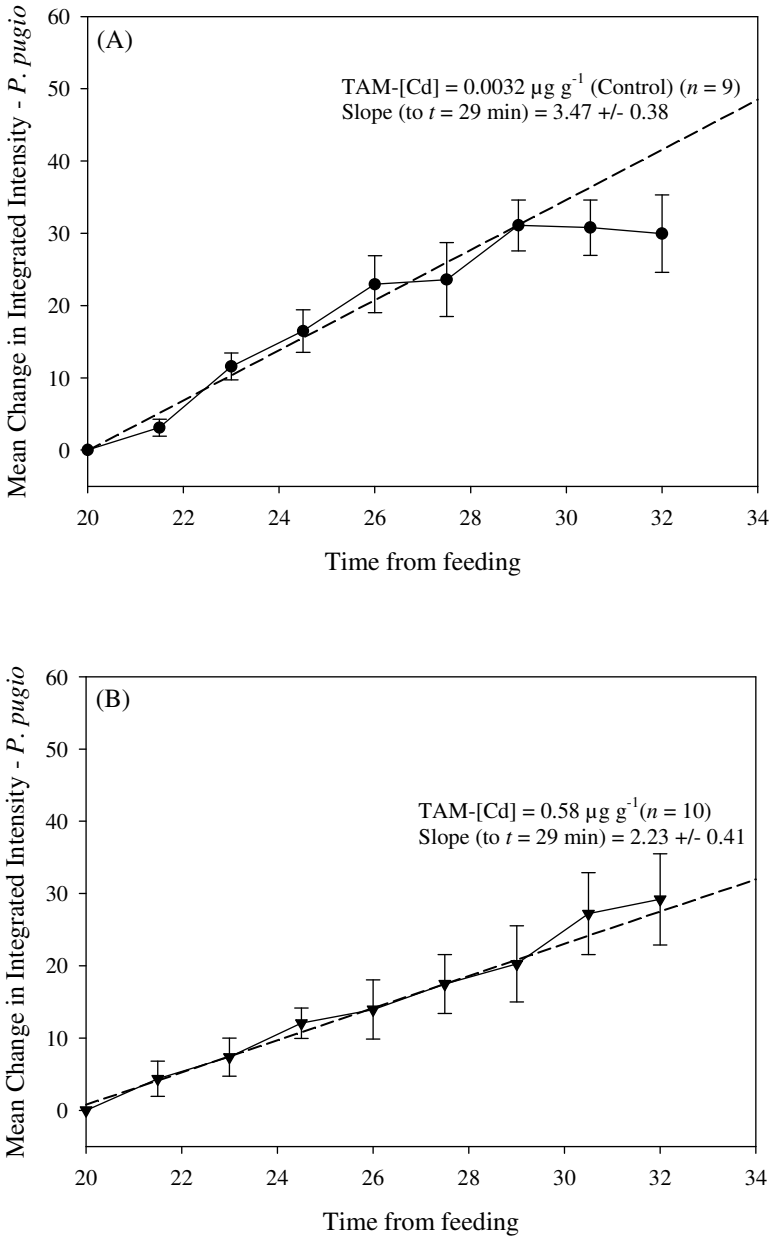
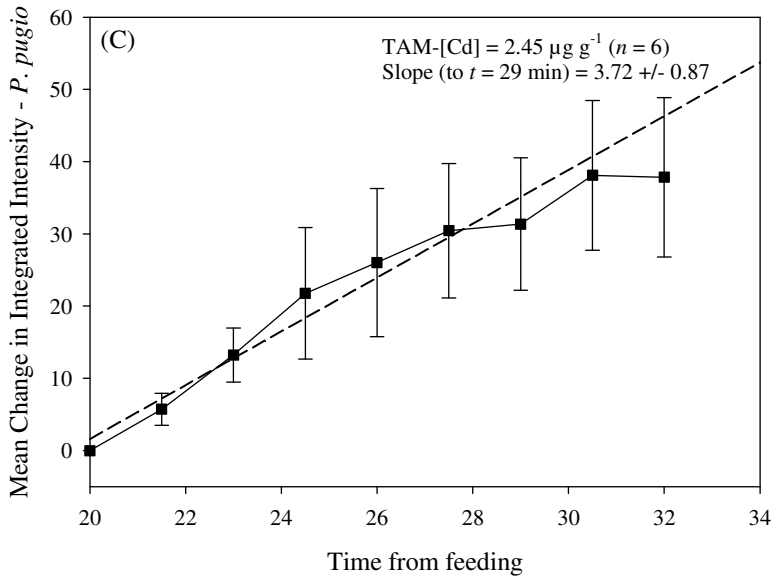

(C)
Mean Change in Integrated Intensity - P. pugio
60
50
40
30
20
10
0
Neck Creek (n = 6)
Slope (to t = 30.5 min) = 0.48 +/- 0.12
20
22
24
26
28
30
32
34
Time from feeding

Figure 4-8. Mean change in integrated intensity in proventriculus and hepatopancreas of grass shrimp *P. pugio* pre-exposed to dietary Cd and fed meals containing IRDye-labeled casein (n = 6-10).




(C)
TAM-[Cd] = 2.45 µg g-1 (n = 6)
Slope (to t = 29 min) = 3.72 +/- 0.87
Mean Change in Integrated Intensity - P. pugio
Time from feeding
0
10
20
30
40
50
60
20
22
24
26
28
30
32
34

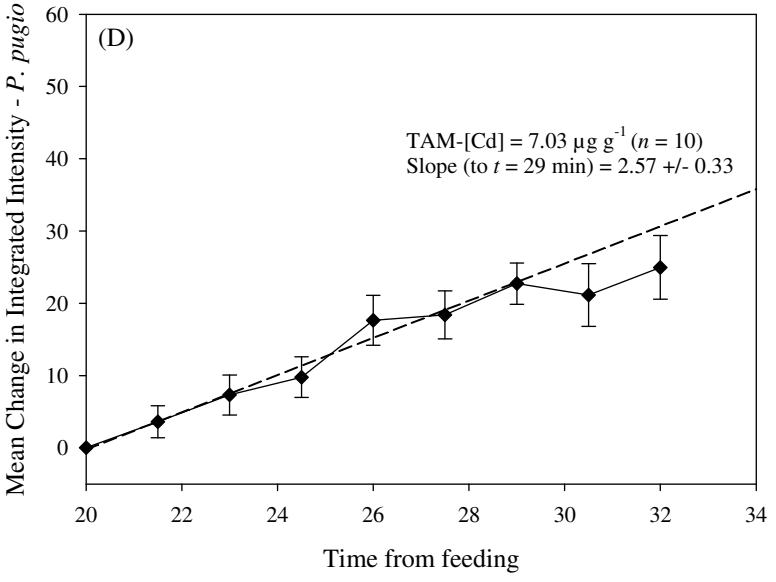
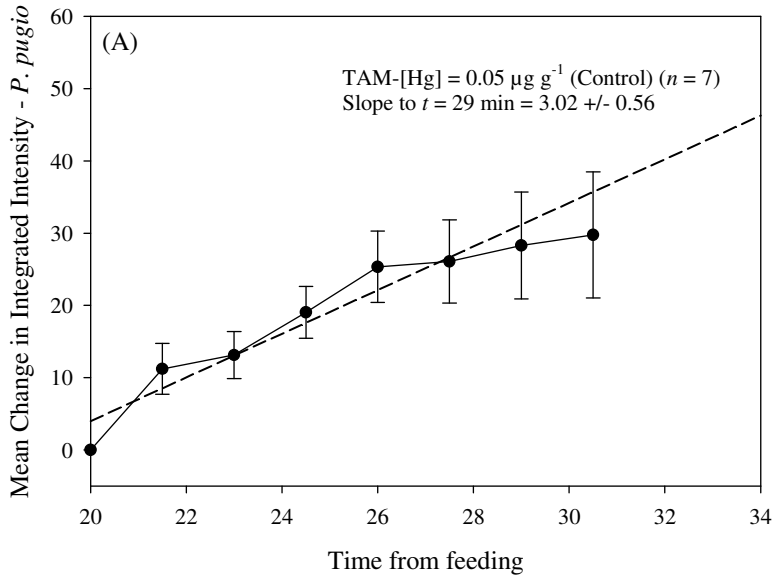
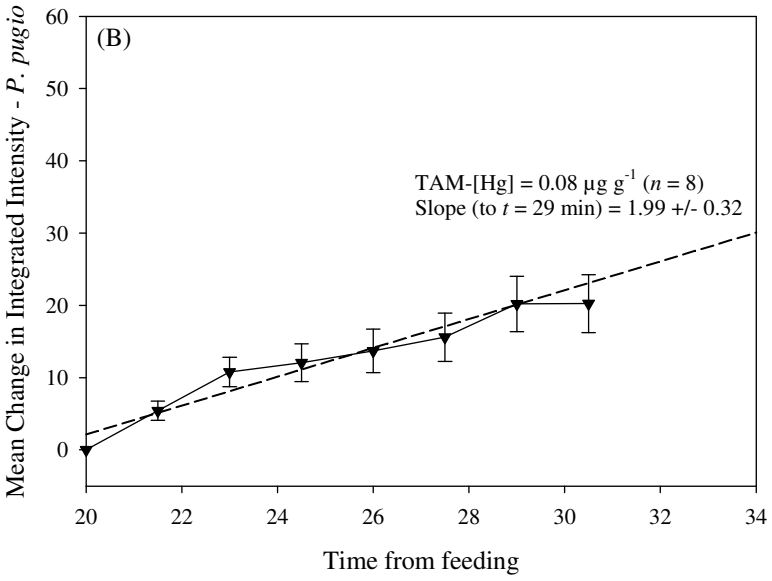
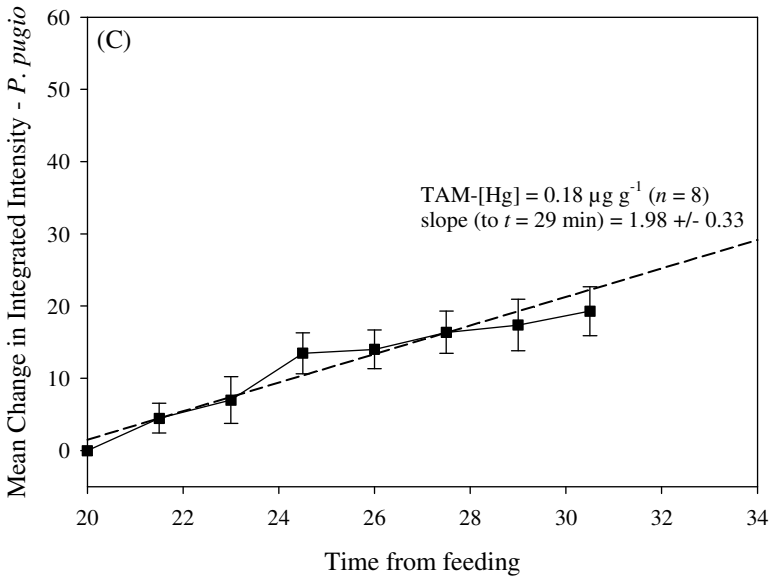

(D)
TAM-[Cd] = 7.03 µg g-1 (n = 10)
Slope (to t = 29 min) = 2.57 +/- 0.33
Mean Change in Integrated Intensity - P. pugio
Time from feeding
0
10
20
30
40
50
60
20
22
24
26
28
30
32
34

Figure 4-9. Mean change in integrated intensity in proventriculus and hepatopancreas of grass shrimp *P. pugio* pre-exposed to dietary Hg and fed meals containing IRDye-labeled casein (n = 7-11).






(C)
TAM-[Hg] = 0.18 µg g-1 (n = 8)
slope (to t = 29 min) = 1.98 +/- 0.33
Mean Change in Integrated Intensity - P. pugio
Time from feeding
0
10
20
30
40
50
60
20
22
24
26
28
30
32
34

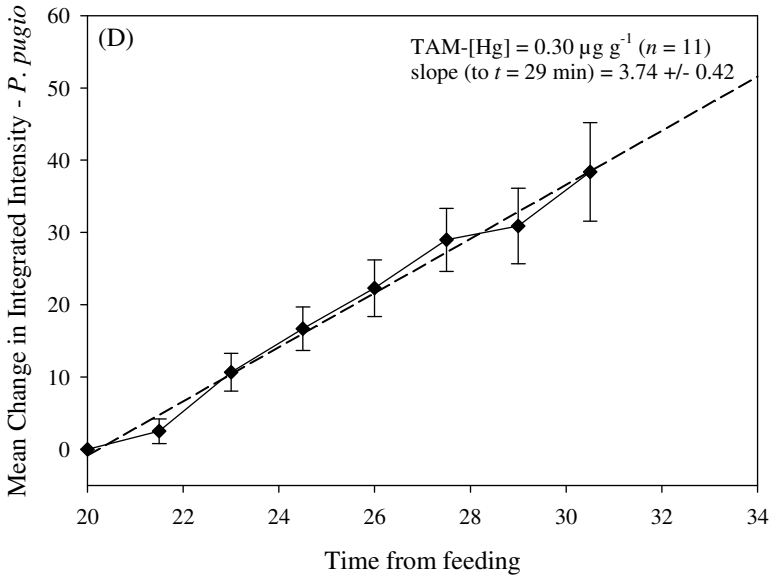
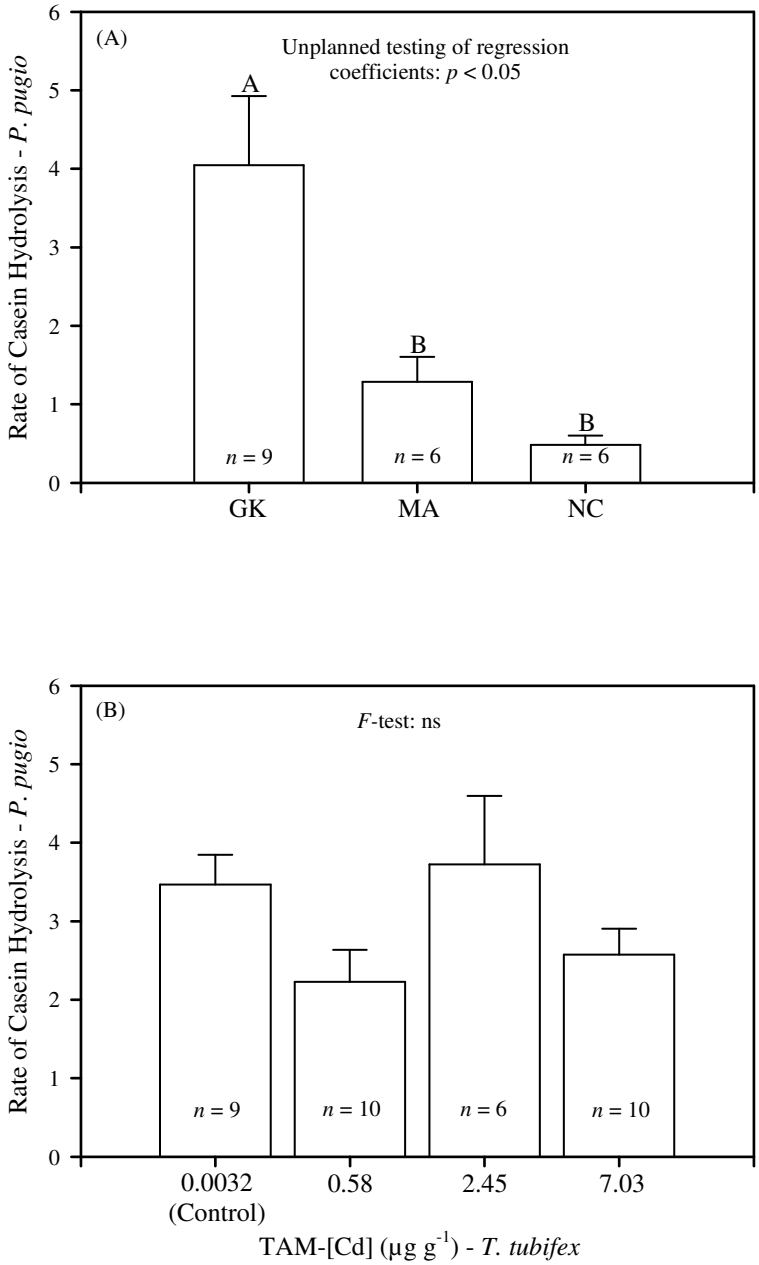

(D)
TAM-[Hg] = 0.30 µg g-1 (n = 11)
slope (to t = 29 min) = 3.74 +/- 0.42
Mean Change in Integrated Intensity - P. pugio
Time from feeding
0
10
20
30
40
50
60
20
22
24
26
28
30
32
34

Figure 4-10. Casein hydrolysis rates for grass shrimp *P. pugio* (A) collected from Staten Island study sites, (B) pre-exposed to dietary Cd or (C) pre-exposed to dietary Hg (n = 6-11). In (A) and (C), significant differences ($p < 0.05$) between sites (unplanned testing of regression coefficients: Tukey-Kramer method) or treatments (F-test, followed by t-test with Bonferroni correction) are indicated by different letters.



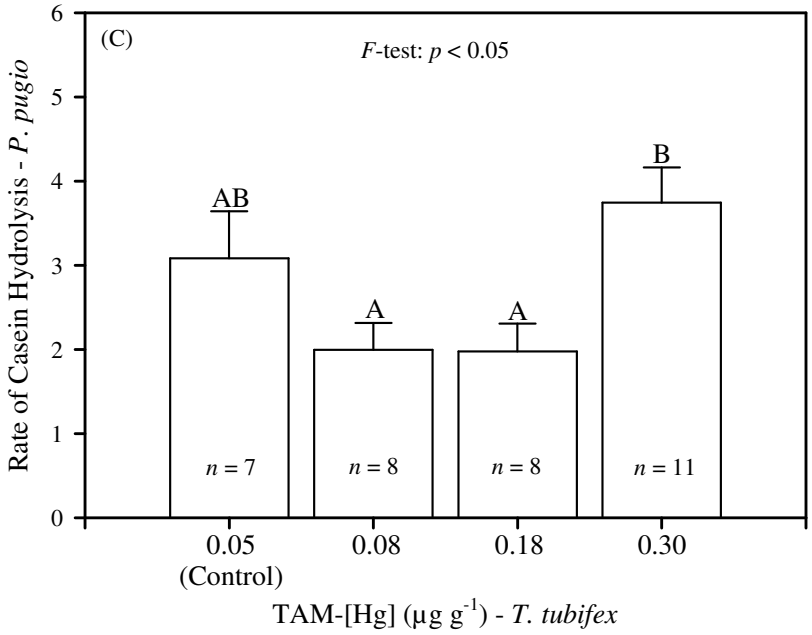

(C)
F-test: p < 0.05
Rate of Casein Hydrolysis - P. pugio
6
5
4
3
2
1
0
AB
A
A
B
n = 7
n = 8
n = 8
n = 11
0.05
(Control)
0.08
0.18
0.30
TAM-[Hg] (µg g-1) - T. tubifex

Figure 4-11. (A) Typical pH calibration curve for fluorescein based on 496 nm:458 nm intensity ratios (emissions at 530 nm) for buffered standards prepared with Zymosan A bioparticles.

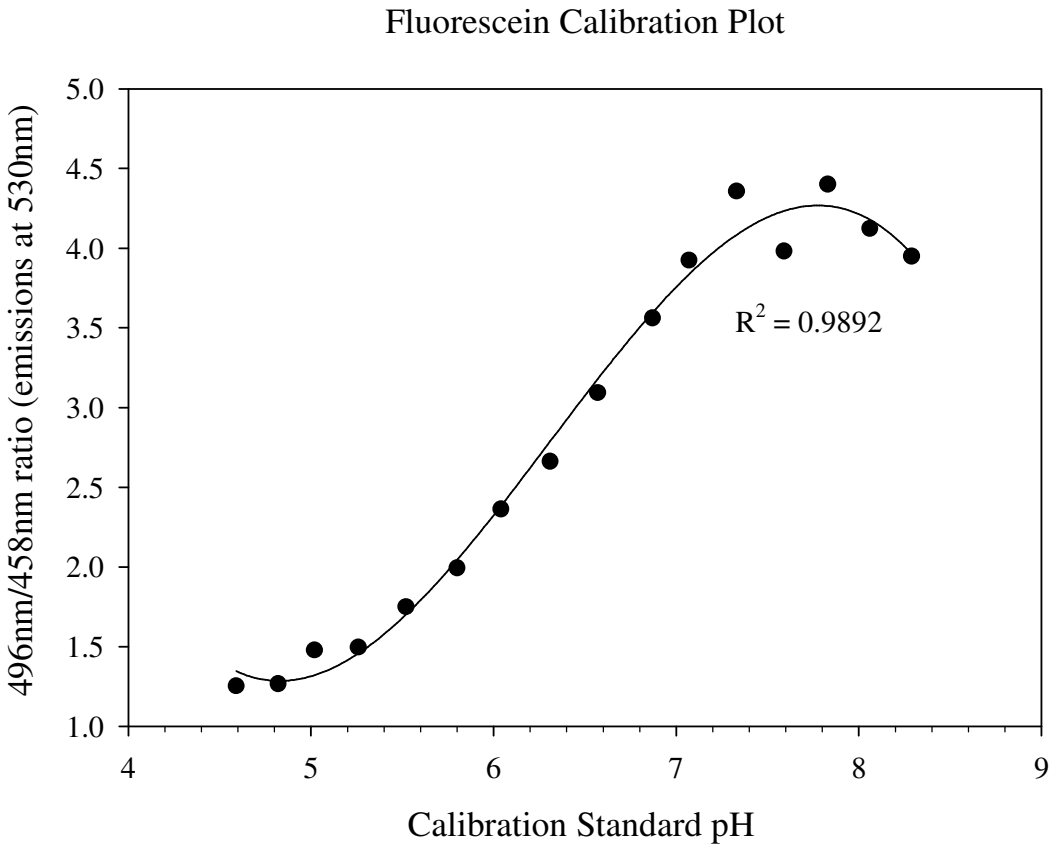
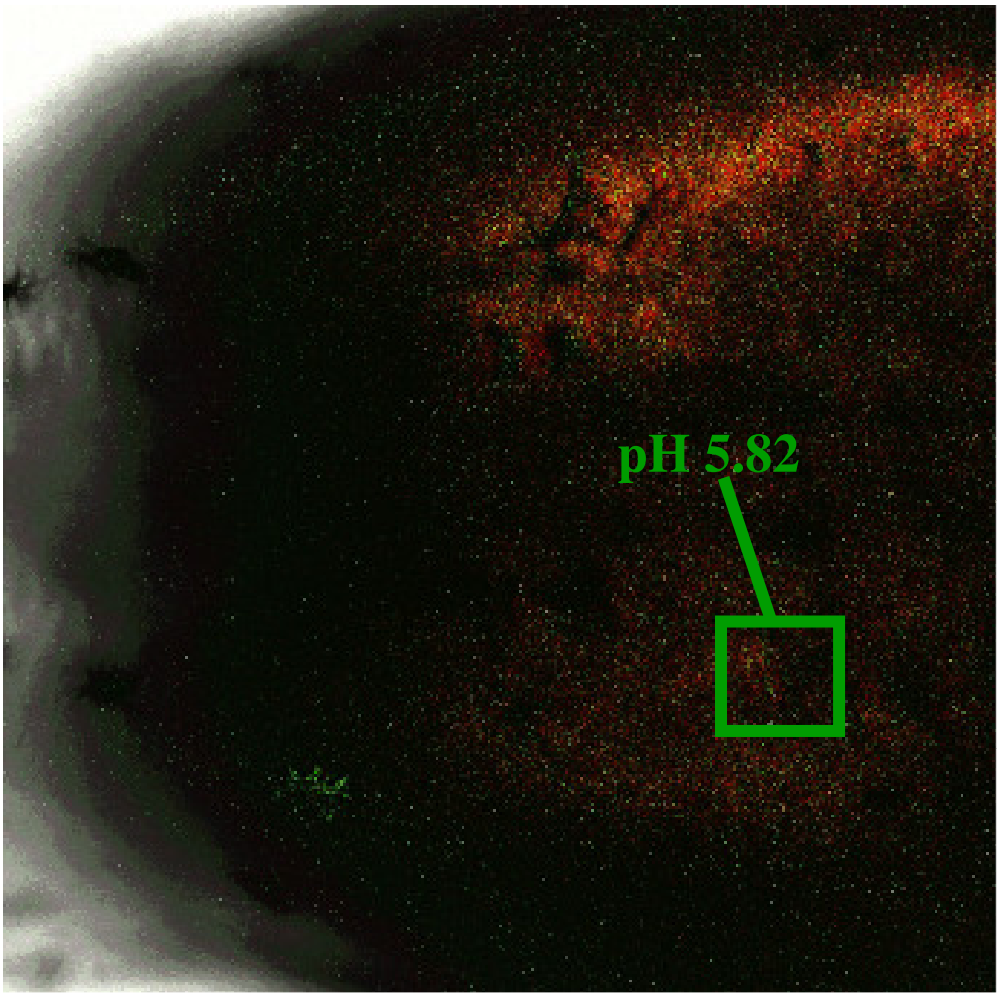
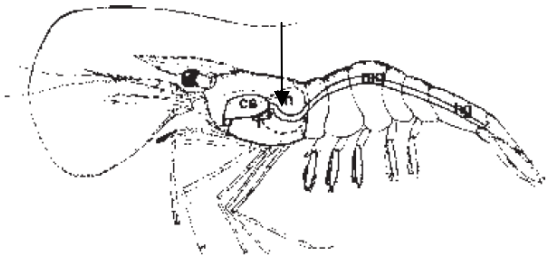


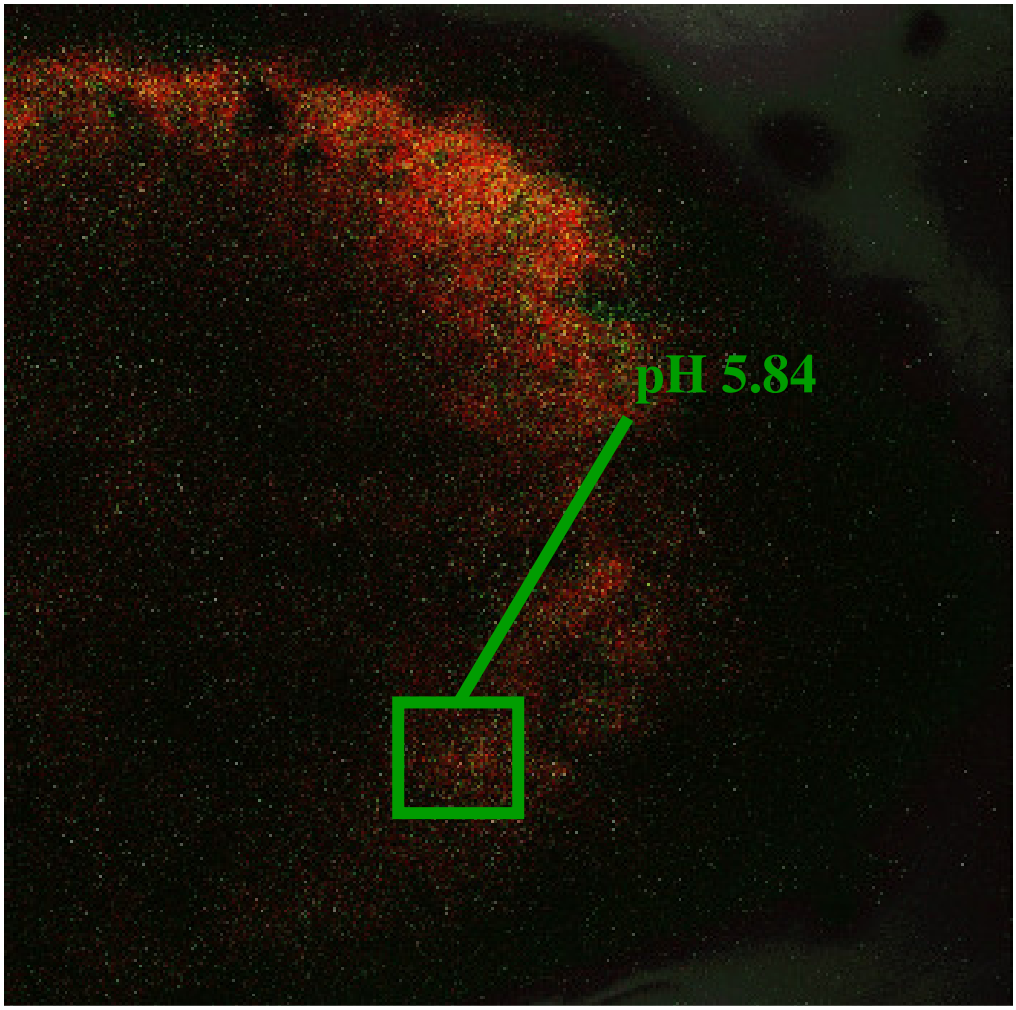
Figure 4-12. Pseudocolor images showing distribution of fluorescein within the cardiac proventriculus of grass shrimp *P. pugio* with estimates of pH within the lumen of the (A) anterior and (B) posterior region (~100 µm from dorsal wall).

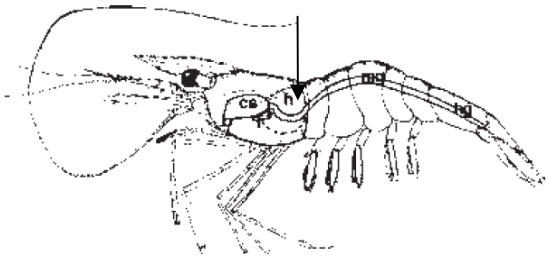
(A)





(B)





CHAPTER 5

Relationship between dietary cadmium absorption by grass shrimp (*Palaemonetes pugio*) and trophically available cadmium in amphipod (*Gammarus lawrencianus*) prey

With kind permission from Springer Science + Business Media: Bulletin of Environmental Contamination and Toxicology, Relationship between dietary cadmium absorption by grass shrimp (*Palaemonetes pugio*) and trophically available cadmium in amphipod (*Gammarus lawrencianus*) prey, Volume 76, 2006, Pages 16-23, D.R. Seebaugh, A. Estephan and W.G. Wallace.

CHAPTER SUMMARY

Previous studies of metal trophic transfer suggest that partitioning of metal to a compartment containing trophically-available metal (TAM) in invertebrate prey may be used to estimate dietary metal absorption by estuarine predators. The objective of this study was to investigate impacts of a pulse of Cd in food on Cd AE by naïve grass shrimp *Palaemonetes pugio* collected from a reference site. This work was conducted by exposing amphipods *Gammarus lawrencianus* to a range of dissolved Cd concentrations with ^{109}Cd as a radiotracer of stable Cd, followed by radioanalysis and estimation of TAM-Cd. Shrimp were then pulse-fed ^{109}Cd-labeled amphipods and assessed for Cd absorption. Although a direct (~1:1) correspondence between TAM Cd in prey and Cd absorption by shrimp was predicted for all dietary exposures, observed variability in this relationship suggests that digestive toxicity may be important in determining transfer of bioavailable metal in prey to predators. This study also provides additional support for the hypothesis that TAM may represent maximum bioavailable Cd in invertebrate prey.

INTRODUCTION

Recent studies have shown that the transfer of metals along estuarine food chains may be directly related to the subcellular distribution of metal within prey, indicating that the quantification of whole tissue metal burdens may not serve as a reliable predictor of metal trophic transfer (Wallace and Luoma, 2003; Seebaugh and Wallace, 2004). For example, the partitioning of metal (e.g., Cd and Zn) to a subcellular compartment containing trophically available metal (TAM) (i.e., metal bound to heat-stable proteins [HSP – e.g., metallothioneins], heat-denatured proteins [HDP – e.g., 'enzymes'] and organelles) has been quantified for several aquatic invertebrates, including brine shrimp, oligochaetes and bivalves (Wallace et al.,1998; Wallace and Luoma, 2003; Seebaugh and Wallace, 2004). A direct (~1:1) relationship between TAM in these organisms and metal absorption by grass shrimp predators (i.e., *Palaemonetes pugio* and *Palaemon macrodatylus*) suggests that TAM may be used to predict the transfer of metal to higher trophic levels (Wallace et al., 1998; Wallace and Luoma, 2003; Seebaugh and Wallace, 2004). Dietary metal absorption by decapod crustacean predators, however, may also be influenced by other factors, including multiple exposure pathways (e.g., dietary and dissolved metal), digestive physiology and pollutant-induced digestive toxicity (De La Ruelle et al., 1992; Rainbow, 1998; Reinfelder et al., 1998; Wang and Fisher, 1999a; Seebaugh and Wallace, 2005). In the present study, we investigate the influence of the partitioning of Cd to the TAM compartment within the gammaridean amphipod, *Gammarus lawrencianus*, on dietary Cd absorption by the daggerblade grass shrimp, *P. pugio*. These ecologically-important species are abundant in estuaries along the

northeastern coast of North America and may be at risk of exposure to metal contaminants, particularly in heavily-impacted, urban areas (Bousfield, 1973; Nixon and Oviatt, 1973; Perez and Wallace, 2004; Seebaugh and Wallace, 2005). Wallace and Estephan (2004) demonstrated that swimming activity in *G. lawrencianus* is sensitive to sublethal exposure to dissolved Cd. Reductions in prey capture success have been observed in *P. pugio* following the consumption of Cd-contaminated prey (Wallace et al., 2000). Each of these species also has the potential to serve as a vector of metal contaminants to higher trophic levels (Steele and Steele, 1970; Davis et al., 2003; Seebaugh et al., 2005).

MATERIALS AND METHODS

Amphipods, *G. lawrencianus*, were collected from Great Kills Harbor, Staten Island, New York, USA and maintained in culture over several generations. Each culture consisted of 1 cm of sieved sediment (< 300 μm) collected from Flax Pond, Old Field, New York, USA and ~7 l of filtered, aerated seawater (1.0 μm filter, 20 ppt, 21-22 °C) obtained from the Rutgers University Marine Field Station in Tuckerton, New Jersey, USA (Wallace and Estephan, 2004). Amphipod cultures were housed in a walk-in environmental chamber (12:12, light:dark cycle, 21-22 °C) and were fed weekly on a mixture of rice cereal (Gerber) and Tetramin® fish flakes (Tetra Sales) (Wallace and Estephan, 2004). Offspring produced by field-collected *G. lawrencianus* were removed periodically and used to establish laboratory cultures for use in feeding experiments.

Gammarus lawrencianus (3 to 5 mm in length) were removed from culture and held within a 1-mm screen for ~24 h to allow for the depuration of gut contents (20 ppt,

21-22 °C). Following depuration, *G. lawrencianus* (~40 amphipods per treatment) were exposed for 3 d in 4 l polycarbonate bottles containing 1 l of filtered, artificial seawater (Instant Ocean®, Aquarium Systems) (0.4 µm filter, 20 ppt, 21-22 °C), reagent-grade $CdCl_2$ and $^{109}CdCl_2$ (2.48 x 10^2 kBq l^{-1}) (Isotope Products) as a radiotracer of stable metal. Nominal Cd exposure concentrations (including the Cd contained in untreated artificial seawater and the radiotracer spike) were 0.01, 0.07, 0.13, 0.26 or 0.51 mg l^{-1}. The final specific activities for ^{109}Cd among the treatments ranged from ~0.07 to ~3.60 µg kBq^{-1}. Following exposure, surviving *G. lawrencianus* were rinsed 3 times with clean seawater (20 ppt) and stored frozen (-80 °C) in 20 ml scintillation vials.

In order to characterize the subcellular distribution of Cd within *G. lawrencianus*, amphipods from each treatment (n = 4, 4 animals per replicate) were subjected to homogenization, differential centrifugation and tissue digestion as described previously (Wallace and Luoma, 2003). This procedure resulted in the isolation of five operationally-defined subcellular fractions: HSP (e.g., metallothioneins), HDP (e.g., 'enzymes'), organelles, 'insoluble' components (e.g., exoskeleton and metal-rich granules) and cellular debris (Wallace and Luoma, 2003). Isolated fractions were transferred to 20 ml scintillation vials and analyzed for ^{109}Cd. A subcellular compartment containing TAM was reconstructed by combining the percentages of Cd associated with HSP, HDP and organelles fractions (i.e., TAM-Cd% = HSP% + HDP% + organelles%) (Wallace and Luoma, 2003; Seebaugh and Wallace, 2004).

Adult grass shrimp, *P. pugio*, (~3 cm in length), were collected from Great Kills Harbor, Staten Island, New York, USA and acclimated to laboratory conditions (20 ppt, 21-22 °C) for at least one week prior to absorption efficiency analysis. During

acclimation, *P. pugio* were fed daily on OSI® *Spirulina* fish flakes (OSI Marine Laboratory), but were not fed for 72 h prior to feeding on Cd-exposed *G. lawrencianus*. *P. pugio* (n = 5 to 9) were placed in individual 1000 ml polyethylene beakers containing 400 ml of clean seawater (20 ppt, 21-22 °C) and allowed to feed on 1 ^{109}Cd-labeled *G. lawrencianus* for 30 min (i.e., before the release of radiolabeled feces) (Wallace et al., 1998). Following the consumption of amphipod tissue, *P. pugio* were placed in 20 ml scintillation vials containing 10 ml of clean seawater (20 ppt, 21-22 °C) and radioanalyzed for ^{109}Cd (time = 0). *P. pugio* were housed in individual 3-mm mesh-lined chambers contained within a 76 l aquarium (20 ppt, 21-22 °C) and allowed to depurate ingested ^{109}Cd for 6 d (Wallace and Luoma, 2003; Seebaugh and Wallace, 2004). Grass shrimp were removed from the aquarium and analyzed for ^{109}Cd at time = 2, 4, 8, 12 and 24 h and approximately every 24 h thereafter. Filtration was provided by a Whisper® Junior filter (Tetra/Second Nature) in order to remove dissolved ^{109}Cd from the aquarium water resulting from depuration by *P. pugio*. ^{109}Cd activity in the aquarium water was monitored daily through radioanalysis of 5 ml samples and remained at background. A linear regression was fit to the physiological loss component of each retention curve (time > 24 h) and the corresponding y-intercept was used to estimate ^{109}Cd absorption efficiency (AE-Cd%) (at time = 0) for *P. pugio* from each dietary treatment (Wallace et al., 1998; Wang and Fisher, 1999b; Seebaugh and Wallace, 2004). The slope of each regression served as an estimate of the rate of physiological ^{109}Cd loss (Wallace et al., 1998; Seebaugh and Wallace, 2004).

All samples were analyzed for ^{109}Cd using a Wallace Wizard™ 7.6 cm 1480 automatic γ-counter (Wallac Oy). The counting efficiency for ^{109}Cd was ~55%. Counting

times for subcellular fractions within *G. lawrencianus* were 5 min and adjusted for live *P. pugio* (1 to 24 min) to maintain propagated counting errors of < 5% (Wallace and Luoma, 2003; Seebaugh and Wallace, 2004). Percentage subcellular distributions of ^{109}Cd in TAM fractions within *G. lawrencianus* were calculated based on the total radioactivity recovered subsequent to fractionation [i.e., (radioactivity in each subcellular fraction)/ (total radioactivity recovered)]. This method eliminates the impact of losses due to the fractionation process (i.e., homogenization and pipette transfers) and sets all replicates at 100% for the purpose of comparing proportional subcellular distributions (Wallace et al., 2003; Wallace and Luoma, 2003; Seebaugh and Wallace, 2004).

The normality of percentage data (i.e., total TAM-Cd% and Cd% within TAM fractions – HSP, HDP and organelles) and concentration data (TAM-[Cd]) for *G. lawrencianus* was verified using the Shapiro-Wilk's *W*-test. All treatment effects were analyzed using one-way analysis of variance (Sokal and Rohlf, 1981). Differences between means were compared using the Scheffé test and homogeneity of variances were analyzed using Levene's test. Differences between AE-Cd% and rates of physiological ^{109}Cd loss among *P. pugio*, and AE-Cd% in *P. pugio* and TAM-Cd% in *G. lawrencianus*, were compared using the unpaired *t*-test (Welch corrected) (Sokal and Rohlf, 1981). Linear regressions were generated using SigmaPlot, version 8.02 (SPSS, Inc.) and statistical analyses were performed using InStat, version 3.0 (GraphPad Software, Inc.) and STATISTICA, version 5.1 (Statsoft, Inc.).

RESULTS AND DISCUSSION

Trophically available Cd (TAM-Cd%) within *G. lawrencianus* was estimated

by combining the percentages of Cd associated with HSP (e.g., metallothioneins), HDP (e.g., 'enzymes') and organelles as shown in Fig. 5-1. TAM-Cd% was nearly constant at ~73% over the range of exposures from 0.01 to 0.26 mg l^{-1} Cd, but was reduced to ~61% at the 0.51 mg l^{-1} Cd exposure due to a 'shift' from HSP to both non-TAM fractions (i.e., 'insoluble' components and cellular debris - data not shown) for the storage of Cd. In terms of the concentrations of Cd available to predators of *G. lawrencianus*, TAM-[Cd] increased over the range of exposures from ~3 to ~6627 ng g wet wt^{-1} (Fig. 5-1, top of graph). Interestingly, TAM-[Cd] and whole body tissue Cd (data not shown) in *G. lawrencianus* did not fluctuate between the 0.13 and 0.26 mg l^{-1} Cd exposures, yet increased in a dose-dependent manner in amphipods exposed to 0.51 mg l^{-1} Cd.

Cd absorption efficiencies (AE-Cd%) for *P. pugio* were determined following the consumption of radiolabeled *G. lawrencianus* prey. Depuration of ^{109}Cd by *P. pugio* was characterized by a two-stage loss with an initial rapid loss of unassimilated metal due to the production of radiolabeled feces (see example, Fig. 5-2) (Wallace et al., 1998). This is consistent with earlier work, where grass shrimp consumed Cd-contaminated brine shrimp, oligochaete or bivalve prey (Wallace et al., 1998; Wallace and Luoma, 2003; Seebaugh and Wallace, 2004). AE-Cd% for *P. pugio* from each dietary exposure was determined using the *y*-intercept method and varied between ~33.1 and ~65.1% (Fig. 5-3). Dietary Cd absorption by *P. pugio* did not appear to be influenced by variability in metal excretion, as rates of physiological loss of ^{109}Cd by *P. pugio* did not differ among dietary treatments (Fig. 5-4).

The direct relationship between AE-Cd% by *P. pugio* and TAM-Cd% in *G. lawrencianus* exposed to 0.01 and 0.07 mg l^{-1} Cd suggests that TAM may be used to estimate Cd transfer from amphipod prey exposed to Cd concentrations that may be encountered in metal-impacted marine ecosystems (US EPA, 2001; Wallace and Luoma, 2003; Seebaugh and Wallace, 2004). AE-Cd% in *P. pugio* did not exceed TAM-Cd% in *G. lawrencianus* for any of the experimental food chains. This finding is consistent with previous studies and provides additional support for the hypothesis that TAM may represent maximum bioavailable Cd in invertebrate prey (Wallace et al., 1998; Wallace and Luoma, 2003; Seebaugh and Wallace, 2004; Seebaugh et al., 2005). Reduced dietary Cd absorption by *P. pugio* (i.e., relative to TAM-Cd% in prey) fed *G. lawrencianus* exposed to 0.13 and 0.26 mg l^{-1} Cd may be related to Cd-induced changes in digestive physiology (e.g., hepatopancreas function or gut passage time) and requires additional study. De La Ruelle et al. (1992) observed a reduction in the activity of aminopeptidase extracted from the hepatopancreas of the crayfish, *Procambarus clarkii*, and exposed to metals (e.g., Mn, Co and Hg). Cd-induced digestive toxicity in *P. pugio* would be expected to influence the assimilation of nutrients and TAM-Cd in prey during the initial rapid loss component of the depuration period (i.e., time < 24 h). If Cd absorption during this period is influenced by increasing exposure to dietary Cd, the observed relationship between AE-Cd% in grass shrimp and TAM-Cd% in amphipods exposed to 0.51 mg l^{-1} Cd may suggest the influence of other factors (e.g., gut pH) that could potentially influence the bioavailability of Cd in prey (Reinfelder et al., 1998; Wallace et al., 1998; Wallace and Luoma, 2003).

Figure 5-1. Subcellular partitioning of Cd as trophically available metal (TAM-Cd%) within *G. lawrencianus* following a 3 d aqueous exposure to 0.01, 0.07, 0.13, 0.26 or 0.51 mg l^{-1} Cd (n = 4; mean ± SE). TAM-Cd%, ORG-Cd% and HSP-Cd% ANOVA: $p < 0.05$; HDP-Cd% ANOVA: not significant. Significant differences ($p < 0.05$) in TAM-Cd% and Cd% among individual TAM fractions are indicated by different letters. Concentrations of Cd associated with the TAM compartment (i.e., TAM-[Cd]; mean ± SE) for each treatment are shown at the top of the graph. TAM-[Cd] ANOVA: $p < 0.05$. Asterisks (*) indicate that TAM-[Cd] in amphipods did not differ significantly among treatments ($p < 0.05$).

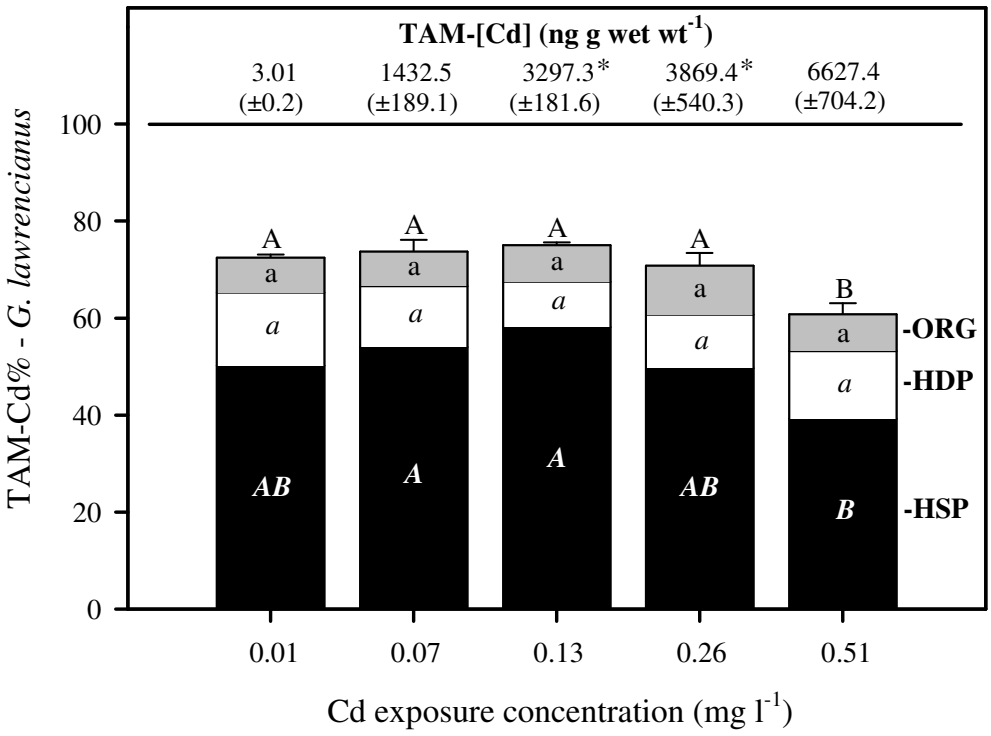


Figure 5-2. Time course in the retention of ^{109}Cd (n = 7; mean ± SE) by *P. pugio* following the consumption of *G. lawrencianus* prey exposed to 0.01 mg l^{-1} Cd through solution for 3 d.

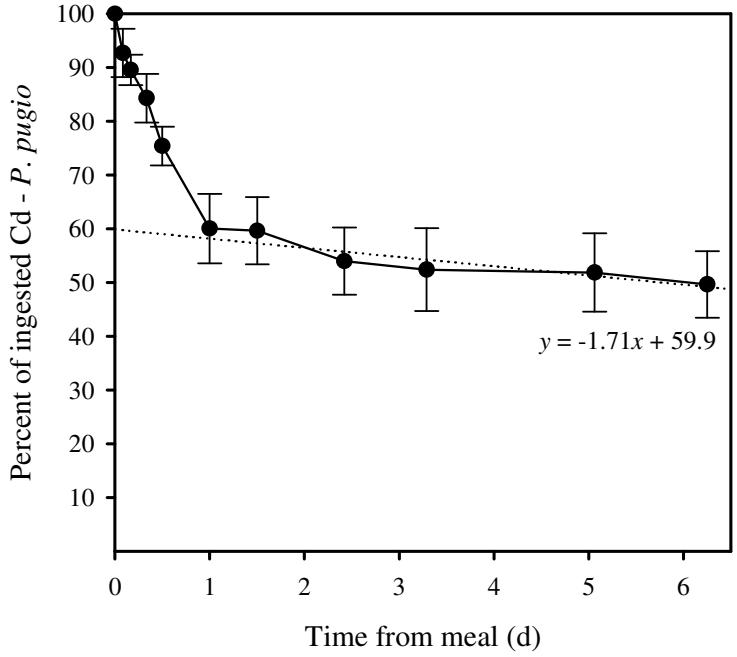


Figure 5-3. AE-Cd% (n = 6 - 9; mean ± SE; y-intercepts of linear regressions) by *P. pugio* following the consumption of *G. lawrencianus* prey exposed to Cd through solution. Significant differences ($p < 0.05$) in AE-Cd% are indicated by different letters. Daggers (†) indicate that AE-Cd% in *P. pugio* did not differ significantly from TAM-Cd% in *G. lawrencianus* (see Fig. 1).

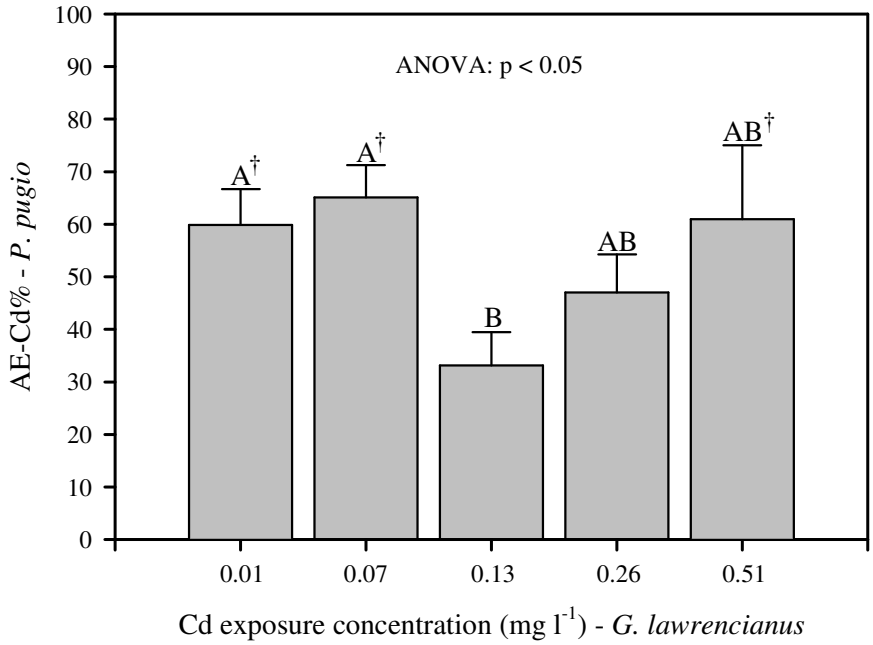
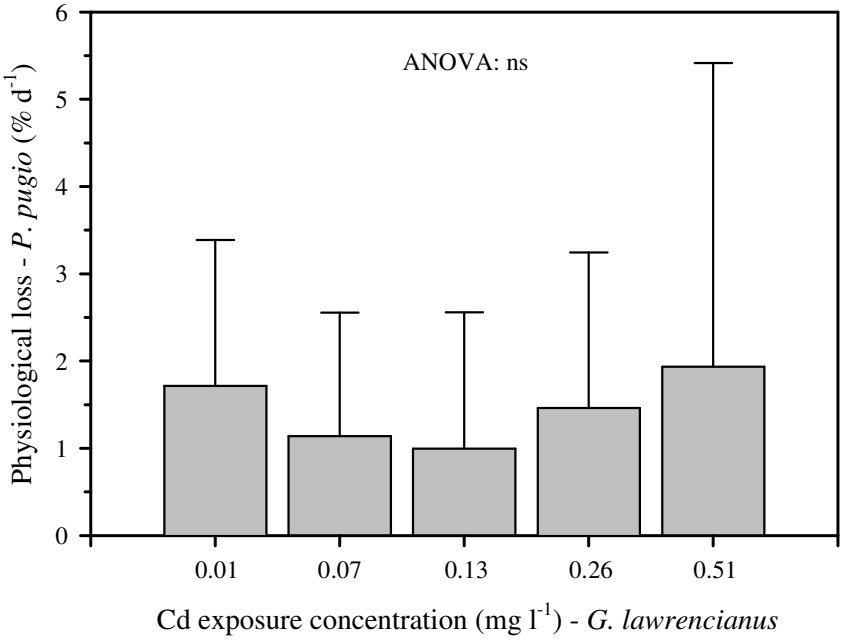


Figure 5-4. Rates of physiological ^{109}Cd loss (n = 6 - 9; mean ± SE; slopes of linear regressions) by *P. pugio* following the consumption of *G. lawrencianus* prey exposed to Cd through solution. ^{109}Cd loss rates among *P. pugio* did not differ significantly ($p > 0.05$). ns = not significant.



CHAPTER 6

Carbon assimilation and digestive toxicity in naïve grass shrimp (*Palaemonetes pugio*) exposed to dietary Cd

CHAPTER SUMMARY

Dietary metal exposure can induce digestive toxicity that may result from metal circulating in gut fluid (pre-assimilatory toxicity) or follow incorporation of metal into consumer tissues (post-assimilatory toxicity). The present study investigated impacts of a pulse of ingested Cd on carbon assimilation efficiency (AE), gut residence time (GRT), feces elimination rate (FER), extracellular digestive protease activities and gut pH in naïve grass shrimp *Palaemonetes pugio*. Adult shrimp were fed Cd-contaminated food containing ^{14}C-labeled diatoms or fluorescent/near infrared markers and analyzed for AE-^{14}C% or digestive toxicity. Additionally, molt stage was characterized for shrimp following assessment of digestive parameters. AE-^{14}C%, GRT and proventriculus pH were not impacted by Cd ingestion. A dose-dependent decrease in FER was observed for shrimp for 2 h following GRT. Protease activities increased over the range of dietary Cd exposures, however, this variation was not dose-dependent. Preliminary evidence suggests that indicators of digestive condition were not influenced by molt stage. Differential impacts of Cd exposure on carbon AE and Cd AE reported previously are consistent with studies involving shrimp subjected to chronic field exposure or pre-exposed to dietary metal. Impacts of ingested Cd on FER may be related to pre-assimilatory impacts on packaging, intestinal transport or release of feces. Digestive protease activities may have been influenced by pre-assimilatory interactions between available Cd^{2+} ions in gut fluid and enzyme-secreting fibrillar cells of the hepatopancreatic epithelium or direct impacts of metal on stored or circulating enzymes. The results of this study contrast observations from a related study, where Cd-induced variability in GRT and gut pH may be indicative of post-assimilatory toxicity.

INTRODUCTION

The assimilation of elements by aquatic invertebrates can be influenced by many factors including diet, ingestion rate, bioavailability of elements in gut fluid and digestive physiology (e.g., particle sorting, gut fluid chemistry, transit times and absorptive functions of the digestive epithelium) (Mayer et al. 1996, 1997; Ahrens et al., 2001; Salazar, 2003; Lopez, 2005). Exposure to pollutants (e.g., non-essential metals) through the diet may have significant implications for digestive toxicity that can impact assimilation of nutrients as well as pollutants (Campbell et al., 2005). It is, therefore, necessary to understand the influence of dietary pollutant exposure on key parameters related to digestive physiology, particularly for important nutrient-cycling organisms.

For metals, impacts on digestive function may depend upon the manner in which tissues or components of the digestive milieu are subject to exposure. Pre-assimilatory toxicity can be induced by metal circulating in gut fluid during a digestive cycle and may include impacts on digestive enzyme activities (Chen and Mayer, 1998; Campbell et al., 2005). Post-assimilatory toxicity from tissue metal burdens accumulated during chronic exposure can influence enzymes, gut motility or pH (Seebaugh et al., unpublished). Since impacts of pre- and post-assimilatory toxicity may be interactive (e.g., for organisms feeding in impacted field conditions), it is also important to assess independent effects of exposure to metal in gut fluid vs. assimilated metal on consumer digestion.

In previous work with the omnivorous grass shrimp *Palaemonetes pugio*, Cd assimilation efficiencies (AE) were influenced by tissue Cd burdens in laboratory-exposed prey, indicating that ingested metal can impact digestion in this species

(Seebaugh and Wallace, 2004; Seebaugh et al., 2005, 2006). Additionally, Cd AE was not dose-dependent, suggesting that metal impacts on gut functions may be variable or that interactivity between physiological processes may be important in determining the extent of assimilation. Carbon AE by shrimp was not influenced by chronic field exposure despite increased metal (i.e., Cd and Hg) assimilation by impacted populations, indicating that this species may compensate for pollutant-induced impacts on digestion in order to maintain adequate nutrient assimilation (Seebaugh and Wallace, 2009).

The present study investigated the influence of dietary Cd on carbon AE, gut residence time (GRT), feces elimination rate (FER), extracellular protease activities and gut pH in naïve grass shrimp, collected from a reference site used to assess impacts of pollutant exposure on populations within an urban estuary. This work was conducted through metal pulse feeding experiments, where shrimp consumed Cd-contaminated meals prepared with radiotracer (^{14}C) or task-specific fluorescent/near infrared (NIR) markers during a single feeding event. Shrimp were assessed for AE or digestive toxicity through radioanalysis, microfluorometric techniques or NIR imaging. Post-assay molt stage was determined for individuals to assess potential impacts of the molt cycle on grass shrimp digestion (Van Wormhoudt, 1974).

MATERIALS AND METHODS

Cd-contaminated meals

Meals used to assess carbon AE and parameters related to digestive physiology in shrimp were prepared with ^{14}C or fluorescent/NIR markers as described in related work (Seebaugh and Wallace, 2009; Seebaugh et al., unpublished). Briefly, oligochaetes

Tubifex tubifex were exposed to Cd (control, 1.76, 3.52 or 7.04 μM) (2.5 ppt, 18-19 °C) through solution for 96 h with renewal of exposure media at 48 h (~0.18 worms ml^{-1}). Exposed worms were stored frozen (-80 °C) in scintillation vials prior to homogenization and incorporation into experimental meals. Samples of worms from each treatment were digested in 70% HNO_3, dried, resuspended in 2% HNO_3, filtered and analyzed for tissue Cd burdens by graphite furnace atomic absorption spectrometry. Carbon AE was estimated following ingestion of meals containing ^{14}C-labeled diatoms *Thalassiosira weissflogii*, Cd-contaminated worm homogenate, cod liver oil and gelatin crystals. For GRT and FER analyses, the gelatin-worm matrix contained unlabeled diatoms and was amended with 0.5 μm diameter Fluoresbrite microspheres (Polysciences). Digestive protease activities were assessed using meals containing IRDye 800RS casein protease substrate (Li-Cor). Meals used for gut pH analysis included Zymosan A BioParticles fluorescein conjugate (Molecular Probes). Diatoms and cod liver oil were excluded from IRDye and fluorescein meals to reduce potential autofluorescence signal interference. Proportions of Cd-contaminated worm homogenate in prepared meals were equal for all experiments and treatments. Meal components were sealed in microcentrifuge tubes, heated to melt the gelatin and vortexed to uniform consistency. Individual (6 μl) portions were dispensed onto polycarbonate filters and stored frozen (-20 °C) 2 h prior to feeding experiments.

Assessment of carbon AE and digestive toxicity

Adult grass shrimp (~3 cm in length) were collected by dip net from Great Kills Harbor, Staten Island, New York and acclimated to laboratory conditions for 5 to 7 d in

clean, aerated seawater (10 ppt, 18-19 °C). This location served as a reference site in previous studies of assimilation, digestion and behavioral toxicity in shrimp exposed to impacted field conditions (Perez and Wallace, 2004; Seebaugh and Wallace, 2009; Seebaugh et al., unpublished). Shrimp were fed commercial fish food during acclimation and allowed to clear their gut contents for ~2 d prior to feeding on Cd-contaminated meals. A brief overview of the methods used to evaluate carbon AE and digestive toxicity is provided here as detailed descriptions are presented in related work (Seebaugh and Wallace, 2009; Seebaugh et al., unpublished). To estimate carbon AE, shrimp were fed ^{14}C-labeled meals for 45 min and transferred to defecation chambers. ^{14}C activities in solubilized shrimp tissues and cumulative feces at 24 h were determined by liquid scintillation counting and AE-^{14}C% was calculated using the mass balance method (Wang and Fisher, 1996). For GRT analysis, shrimp consumed meals containing fluorescent microspheres for 30 min. Feces were collected 1.5 h after feeding and every 30 min thereafter for up to 11.5 h. Dried feces were scanned for microspheres with a microscope equipped with a fluorescent light source (Zeiss Axio Observer.Z1). Minimum GRT for individuals was estimated as time between the introduction of meals and first detection of microspheres in feces, the nearest 30 min. Fecal strand lengths were determined using a dissecting microscope equipped with a digital imaging system and software measurement tools (Moticam 1000; Motic Images 2.0). FER for individual shrimp was calculated for 2 h following GRT. To determine extracellular protease activities, shrimp were fed IRDye-labeled casein meals for 14 min, immobilized and scanned with an NIR scanner (Li-Cor Odyssey) every 90 sec, from time (t) = 20 min following the introduction of food. Casein hydrolysis rates within the proventriculus and

hepatopancreas of shrimp were measured as increases in integrated intensity. A regression was fit to the linear portion of each plot (to t = 32 min) representing the mean increase in integrated intensity and the corresponding slope was used to compare rates among dietary treatments. To estimate gut pH, Zymosan A-labeled meals were introduced, one at a time, to an aquarium containing ~16 shrimp. Shrimp that acquired individual meals were isolated within the aquarium with a fine mesh net, allowed to feed for ~5 min, immobilized and then scanned with a confocal microscope (Leica SP2 with argon/krypton laser). pH within the anterior and posterior regions of the cardiac chamber of the proventriculus was estimated using the 496 nm:458 nm intensity ratio method (emissions at 530 nm) (Ahrens and Lopez, 2001). Ratios within the gut were calibrated to standards prepared by adding Zymosan A to buffers of known pH (range: pH 4.5 to pH 8.3). Shrimp were frozen (-20 °C) immediately following assays for digestive toxicity. Post-assay molt stage (intermolt [C], premolt [D_0, D_1 or D_2] or postmolt) was later characterized through microscopic examination (100x magnification) of setal regions of the uropods (Freeman and Bartell, 1975).

Statistical analyses

Where appropriate, normality of experimental data was analyzed using Shapiro-Wilk's W test and homoscedasticity tested with Levene's test. Tissue Cd burdens in oligochaetes were $\log_{10}$ transformed and analyzed using one way analysis of variance (ANOVA). Homogeneity of ^{14}C-labeled food ingestion (sum of ^{14}C retained and ^{14}C in cumulative feces) by grass shrimp was tested using Kruskal-Wallis ANOVA (K-W ANOVA) (Zar, 1999). Arcsine transformed AE-^{14}C% data were analyzed using K-W

ANOVA. Correlations between AE-^{14}C% and ingested ^{14}C were tested with Spearman rank correlation (r_s). Median GRT data were tested for treatment effects using K-W ANOVA. Curves showing percentages of shrimp with microspheres in feces over time were compared using the Mantel-Cox test (Altman, 1992). Linear trends between Cd exposure and median GRT were tested with the logrank test for trend. Log_{10}-transformed FER data were analyzed using ANOVA. Homogeneity of IRDye-labeled food ingestion (integrated intensity at t = 20 min) was tested using ANOVA. Effects of dietary Cd on protease activities were analyzed through planned testing of equality of slopes (F-test) (Sokal and Rohlf, 1995). Correlations between casein hydrolysis rates and initial IRDye signals were analyzed using r_s. Gut pH data were converted to hydrogen ion concentrations ($[H^+]$) and analyzed using K-W ANOVA (Murphy, 1981). Comparisons pH of the anterior and posterior regions of the cardiac proventriculus within dietary Cd treatments were performed using the Mann-Whitney U test. Gut pH data are reported as -log(mean $[H^+]$) with asymmetrical standard errors resulting from transformation (Hu et al., 2007). Effects of molt stage on digestive parameters within dietary Cd treatments as well as treatment groups were analyzed using K-W ANOVA (Mugnier and Justou, 2004). The influence of molt stage on gut pH could not be tested for individual treatments due to insufficient sample sizes. Multiple comparisons of metal burdens in worms as well as FER and protease activities in shrimp between treatments were performed using the t-test with Bonferroni correction (Sokal and Rohlf, 1995). Analyses were conducted using STATISTICA 7.1 (Statsoft), GraphPad Instat 3.10 and GraphPad Prism 5.03 (GraphPad).

RESULTS

Oligochaete tissue burdens

Whole tissue Cd burdens in oligochaetes used to prepare experimental meals increased over the range of dissolved Cd exposures from ~0.011 µg g^{-1} in controls to ~120.3 µg g^{-1} Cd (wet wt) in worms from the 7.04 µM Cd treatment (~11,000-fold increase) (Fig. 6-1). The tissue Cd burden in worms exposed to 3.52 µM Cd was ~1.53x that for 1.76 µM Cd worms, indicating less than linear accumulation (~0.77x) with respect to Cd exposure concentration. Increased Cd accumulation by worms exposed to 7.04 µM Cd was less than linear (~0.89x) relative to uptake by 3.52 µM Cd worms (i.e., with respect to dissolved Cd in exposure media).

^{14}C assimilation by grass shrimp

Carbon assimilation by grass shrimp was estimated following consumption of ^{14}C-labeled meals containing tissues from Cd-contaminated oligochaetes. Homogeneity of radiolabeled food ingestion among treatments was observed (Table 6-1). AE-^{14}C% did not exhibit variation and was ~83% across dietary treatments (Fig. 6-2). AE-^{14}C% was not correlated with ingested radioisotope for individual treatments or over the range of Cd exposures (Table 6-1).

Digestive physiology of grass shrimp

Minimum GRT by grass shrimp fed meals containing fluorescent microspheres was not influenced by Cd ingestion (Fig. 6-3A). Analyses of percentages of shrimp with microspheres in feces over time did not reveal differences among treatments or a linear trend between treatment and median GRT (Fig. 6-3B). A dose-dependent decrease in

FER was observed over the range of dietary Cd exposures (Fig. 6-4). FER for shrimp that ingested meals containing tissues from worms exposed to 7.04 µM Cd (~6.4 mm h^{-1}) was less than half the value for controls (~14.4 mm h^{-1}). Homogeneity of IRDye-labeled food ingestion was observed among dietary Cd treatments (Table 6-1). Mean changes in integrated intensity in the proventriculus and hepatopancreas of shrimp were approximately linear from t = 20 up to 33.5 min (Fig. 6-5). Substrate exhaustion was reached in control shrimp by ~32 min (Fig. 6-5A). Protease activities increased over the range of dietary Cd treatments, but did not vary in a dose-dependent manner (Fig. 6-6). Casein hydrolysis rates were correlated with initial IRDye signal in shrimp that consumed meals containing tissues from the 1.76 µM Cd worm exposure (Table 6-1). Hydrolysis rates were not correlated with initial signal for other dietary treatments or over the range of exposures. Gut pH varied between ~5.29 and ~6.01 and was not impacted by Cd ingestion (Table 6-2). pH within the anterior and posterior regions of the cardiac chamber of the proventriculus did not vary within individual treatments (Mann-Whitney U: ns for all comparisons). Molt stages of grass shrimp assessed for digestive toxicity were distributed as follows: C (~14%), D_0 (~40%), D_1 (~15%), D_2 (~21%), postmolt (~10%). GRT, FER or protease activities were not influenced by molt stage for any treatment or across dietary exposures (Table 6-3). Molt stage did not impact gut pH over the range of dietary Cd exposures.

DISCUSSION

In the present study, naïve grass shrimp from a reference site within an urban estuary were assessed for carbon AE or digestive toxicity following pulse-feeding on

meals containing homogenized tissues from Cd-contaminated oligochaetes. Elapsed time between Cd ingestion and data collection was subject to methodological constraints and varied from minutes (protease activities and gut pH) to 24 h (carbon AE). Previous work on metal assimilation by grass shrimp indicates that biphasic digestion and assimilation are completed by 48 h following pulse-feeding (Decho and Luoma, 1991; Icely and Nott, 1992; Seebaugh and Wallace, 2004, 2009). Short-term impacts of metal ingestion on digestive physiology may be related to metal in gut fluid prior to assimilation (pre-assimilatory toxicity). Impacts on digestion can also result from incorporation of metal into consumer tissues (post-assimilatory toxicity) (Seebaugh et al., unpublished). It may also be possible that toxicity can result from metal assimilated before completion of the digestive cycle.

Although whole tissue Cd burdens in oligochaetes *T. tubifex* did not reach saturation, accumulation was less than linear with respect to Cd exposure concentration. Interestingly, Cd uptake by worms increased disproportionately for exposures up to 0.38 µM Cd under the same experimental conditions (Seebaugh and Wallace, unpublished). Similar patterns in Cd uptake by this species have been observed over a comparable range of exposure concentrations in previous studies (Bouché et al., 2000; Steen Redeker and Blust, 2004). Concentration-dependent plateaus in Cd uptake have not been observed for *T. tubifex* at dissolved exposures up to 10 µM (Steen Redeker and Blust, 2004). The fraction of Cd associated with trophically-available metal (i.e., Cd associated with metallothionein-like proteins, enzymes and organelles) was not characterized for oligochaetes as in related work since metal in homogenized tissues may have also been bound to other components (e.g., diatoms, cod liver oil, casein or yeast) in meals ingested

by shrimp (Wallace and Luoma, 2003; Seebaugh and Wallace, unpublished). Based upon worm tissue Cd burdens presented in the current study, it is presumed that concentrations of available metal in shrimp gut fluid increased over the range of dietary treatments.

Carbon assimilation by shrimp at 24 h was not influenced by ingested Cd and AE-^{14}C% (~83%) was consistent with values reported elsewhere (Johannes and Satomi, 1966; Morgan, 1980; Seebaugh and Wallace, 2009). In previous work, Cd assimilation was impacted by tissue burdens in amphipod prey exposed to a comparable range of dissolved Cd concentrations (i.e., up to 4.54 μM) (Seebaugh et al., 2006). Following extracellular digestion within the hepatopancreas, nutrients may be transported from the lumen across brush border surfaces of resorptive (R) cells through diffusion or active transport or into blister (B) cells via active transport or endocytosis (Al-Mohanna and Nott, 1986; Verri et al., 2001; Vilella et al., 2003; Sousa et al, 2005). Cd^{2+} in gut fluid may be transported across apical surfaces via several pathways, including an $Na^{+}/1H^{+}$ antiporter and verapamil-sensitive transmembrane Ca^{2+} channels (Ahearn et al., 1994; Simkiss, 1996; Zilli et al., 2000). Differential impacts of exposure on carbon and Cd assimilation were also observed in related work where grass shrimp were collected along a pollution gradient or pre-exposed to dietary Cd or inorganic Hg (Seebaugh and Wallace, 2009, unpublished). Carbon AE was not impacted by previous or concurrent exposure, indicating that mechanisms of nutrient assimilation may be unresponsive to exposure to certain dietary pollutants (e.g., non-essential metals). Additionally, phenotypic digestive plasticity may allow shrimp to maintain assimilation of essential elements by altering gut function in response to dietary stressors (Bock and Mayer 1999, Relyea and Auld 2004; Sabat et al., 2005). For field-exposed shrimp, post-assimilatory impacts on protease

activities may have been offset by increased transit time, suggesting that plasticity could be important in maintaining nutrient assimilation by shrimp subjected to dietary stress (Seebaugh and Wallace, 2009; Seebaugh et al., unpublished).

Minimum GRT in shrimp was not impacted by ingestion of a pulse of Cd with food. In a related study, reduced GRT was influenced by pre-exposure to dietary Cd for 15 d followed by 2 d depuration (Seebaugh et al., unpublished). These results suggest that impacts of dietary Cd on transit time may necessitate metal incorporation into the hepatopancreatic epithelium or musculature or perhaps tissues that control peristalsis (e.g., the stomatogastric nervous system and musculature that control movements of the proventriculus) (Maynard and Dando, 1974; Al-Mohanna and Nott, 1986; Shuranova et al., 2006). Hoyt et al. (2000) reported minimum GRT for grass shrimp fed 2 to 4 µm diameter latex beads at 0.5 to 2 h. Beads were not detected in the hepatopancreas over the course of the digestive cycle (Hoyt et al., 2000). Particles within the proventriculus > 1 µm in diameter are sorted by cardiac setal screens (and possibly the gland filter), prevented from entering the hepatopancreas and circulated toward the midgut for elimination (Dall and Moriarty 1983, Felgenhauer, 1992; Icely and Nott, 1992). Fluorescence from 0.5 µm microspheres can be detected within the hepatopancreas, indicating that GRT values (median GRT for the range of dietary Cd treatments ~6.5 h) in the present study represent the influence of hepatopancreatic processes on transit time and not just 'bulk' passage times of larger particles rejected during sorting (Hoyt et al., 2000; Beseres et al., 2006; Seebaugh et al., unpublished).

It is generally assumed that increased assimilation is associated with prolonged exposure of ingested materials to digestive fluid as well as increased contact time

between nutrients and absorptive surfaces of the gut epithelium (Relyea and Auld, 2004; Lopez, 2005). Interestingly, relationships between assimilation and GRT may be species- or element-dependent. For marine fish, Se and Zn AE were correlated with increasing GRT, but this relationship did not hold for ingested Cd (Zhang and Wang, 2006). Cd assimilation was positively correlated with GRT in grass shrimp collected along an impact gradient, but similar relationships were not established for carbon or inorganic Hg (Seebaugh et al., unpublished). In polychaetes, a direct relationship between Cd assimilation and GRT was reversed by previous exposure to Cd in sediments (Selck et al., 1999). It does not appear that patterns of Cd assimilation across a range of Cd burdens in prey reported previously would be correlated with GRT based on the results of the current study (Seebaugh et al., 2006).

A dose-dependent decrease in FER was observed in shrimp for 2 h following minimum GRT. FER may be related to ingestion rate, although homogeneity of consumption of ^{14}C- and IRDye-labeled casein meals suggests that Cd-induced variability in this endpoint may result from interference with fecal strand packaging and transport. Materials rejected by particle sorting within the proventriculus as well as residual wastes and B cells extruded from the hepatopancreas later in the digestive cycle are packaged in a chitinous peritrophic membrane secreted by the midgut epithelium (Forster, 1953; Al-Mohanna and Nott, 1986). This membrane may protect the gut lining from abrasion and provide separation between water entering the gut from anal drinking and soluble materials and feces (Lovett and Felder 1990a). Bands of circular and longitudinal muscle that generate peristaltic and antiperistaltic waves that transport materials within the midgut appear to maintain positive hydraulic pressure required for expansion of the

hepatopancreatic tubules and compact feces (Lovett and Felder, 1990a; Sousa and Petriella, 2006). Mucopolysaccharides secreted into the midgut may facilitate movement of feces toward the hindgut, where muscles pump water into the digestive tract (Lovett and Felder, 1990b; Icely and Nott, 1992; Sousa and Petriella, 2006).

Pre-assimilatory impacts of ingested metal on secretions by the midgut epithelium could potentially interfere with packaging, intestinal transport and release of feces. Nunez-Nogueira et al.(2006) reported that assimilated Cd and Zn was incorporated into abdominal muscle tissues of penaeid shrimp ~2 to 5.6 d after ingestion. To influence FER, any impacts of Cd consumption on the intestinal musculature or associated abdominal ganglia would have required incorporation of metal into tissues before digestion of contaminated meals was complete (e.g., within 4.5 to 11.5 h for shrimp that ingested meals with tissues from worms exposed to 7.04 µM Cd) (Shuranova et al., 2006). It, therefore, seems plausible that fecal strand production was impacted by direct exposure to metal in gut fluid and not due to post-assimilatory toxicity. Al-Mohanna and Nott (1986) noted that rates of fecal strand production by decapods were not related to timing of the digestive cycle, which is dictated by processes within the hepatopancreas. Interestingly, FER for controls in the present study was ~2.5 to 4.4x higher than for control shrimp pre-exposed to dietary metal (i.e., Cd or Hg contaminated oligochaetes) for 15 d or shrimp collected from Great Kills Harbor, fed once on fish food and allowed to clear their guts in site water (Seebaugh and Wallace, unpublished). Controls in the current work were maintained in clean, filtered seawater and fed on fish food for 3 to 5 days prior to clearing their guts for 2 d. This disparity in FER among controls and reference shrimp may suggest that previous diet composition or acclimation

conditions can impact packaging and transport of feces.

Studies of decapod digestion have typically characterized extracellular enzyme activities *in vitro* using gut homogenates or fluid extracts (De La Ruelle et al., 1992; Glass and Stark 1994; Ezquerra et al., 1997; Lemos et al., 2000; Muhlia-Almazán and García-Carreño, 2002). These methods do not simulate actual gut conditions and may indeed render enzymes inactive (Dall and Moriarty, 1983). Few studies have characterized digestive enzyme activities in decapods using non-invasive techniques (e.g., enzymes recovered from feces) (Córdova-Murueta et al., 2003, 2004). Although functions of specific cell types within the decapod hepatopancreatic epithelium have been the subject of considerable debate (particularly for B cells), it is widely-accepted that fibrillar (F) cells synthesize and discharge enzymes that catalyze hydrolysis of ingested materials within the proventriculus and hepatopancreas (Al-Mohanna et al. 1985; Al-Mohanna and Nott, 1986; Vogt et al., 1989; Sousa et al., 2005). Vogt et al. (1989) used immunohistochemical, cytological and radiotracer methods to determine that a crayfish protease is synthesized F cells, transported to apical cell surfaces in vacuoles and exocytosed into the lumen of hepatopancreas. Active enzyme circulates throughout the hepatopancreas and foregut and may accumulate in the anterior region of the cardiac proventriculus in preparation for the next digestive cycle (Vogt et al., 1989).

Chen and Mayer (1998) reported Cu inhibition of protease activities in lugworm gut fluid incubated with contaminated sediments which may be due to interference with substrate access to enzyme active sites or changes in enzyme conformation. Protection from metal inhibition of digestive enzymes may be provided by ligands (e.g., amino acids) in gut fluid or competition from protons at low gut pH until threshold metal

concentrations are attained (Chen et al., 2002). Crayfish aminopeptidase activities were inhibited by metal exposure *in vitro* (De La Ruelle et al., 1992). For the current study, extracellular proteases activities in shrimp were characterized *in vivo* following ingestion of Cd-contaminated meals. Casein hydrolysis within the proventriculus and hepatopancreas was approximately linear and varied ~2.4-fold over the range of dietary treatments, but did not exhibit dose-dependency. Pre-assimilatory impacts on protease activities in live shrimp may be due to interactions between Cd^{2+} in gut fluid and apical F cell machinery involved in secretion or direct impacts on enzymes (e.g., topography or displacement of active site metal ions) stored or circulating in gut fluid (Mitane et al., 1987; Stöcker et al., 1988; Vogt et al., 1989; Casalino et al., 2002). Enzyme plasticity may be important in maintaining adequate nutrient assimilation, however, it is difficult to discern whether patterns of protease activities in Cd-exposed shrimp may be related to carbon AE, other measures of digestive toxicity or Cd AE reported in previous work (Bock and Mayer, 1999; Sabat et al., 2005; Seebaugh et al., 2006). The rate of casein hydrolysis in control shrimp was up to 4x slower than for controls pre-exposed to dietary metal (i.e., Cd or Hg) or collected from Great Kills Harbor in related work, suggesting that previous diet and acclimation conditions may influence digestive enzyme activities (Seebaugh and Wallace, unpublished).

Gut pH in shrimp was not impacted by Cd ingestion. Gastric juice pH is weakly acidic in many decapods and neutral in some taxa (e.g., fiddler crabs) (van Weel 1970, Gibson & Barker, 1979; Johnston and Yellowlees, 1998). pH values for shrimp estimated using microfluorometric methods *in vivo* were similar to values obtained for decapods via direct measurement of gastric juice with pH microelectrodes *in vitro* and were different

from seawater (i.e., pH 8) (Johnston and Yellowlees, 1998). In related work, pH of the posterior region of the cardiac chamber of the proventriculus was influenced by previous exposure to dietary Cd or chronic exposure to impacted field conditions (Seebaugh et al. unpublished). Both anterior and posterior region pH increased with pre-exposure to inorganic Hg (Seebaugh et al., unpublished). These results suggest that post-assimilatory impacts of pollutant (e.g., metal) exposure may be important in influencing extracellular gut pH. Pre-assimilatory exposure to Cd in the present study did not appear to impact concentrations of free protons in gut fluid. Mechanisms for regulation of gut pH in decapods are not well understood (Gibson and Barker, 1979). The possibility that enzyme vacuoles released to the lumen of the hepatopancreas by F cells contain factors that influence extracellular pH requires further study (Al-Mohanna et al., 1985; Vogt et al., 1989). It has been suggested that anterior midgut caeca may produce components in gut fluid that regulate pH in decapods that may be impacted by metal circulating in gut fluid (Dall and Moriarty, 1983). Midgut caeca have not been identified in palaemonids (Sousa and Petriella, 2006). Anterior and proventriculus pH in shrimp did not vary within individual dietary treatments, indicating that mixing of ingested materials, enzymes stored anteriorly and other components in gut fluid following meal consumption was rapid and thorough (Powell, 1974; Vogt et al., 1989; King and Alexander, 1994).

Evidence that GRT, FER, digestive protease activities and gut pH in grass shrimp may not be influenced by molt stage is considered preliminary due to small samples sizes or data missing for specific molt stages within individual dietary Cd treatments. It should be noted that premolt stage D_0 may include shrimp from stages eD_0 and lD_0 and D_1 may include specimens from D_1' and D_1'' (Freeman and Bartell, 1975; Mugnier and Justou,

2004). The majority of shrimp used to assess digestive toxicity (~76%) were identified as premolt, which is consistent with the short intermolt and relatively long premolt periods associated with diecdysic molt cycles (Freeman and Bartell, 1975). Interestingly, shrimp from all substages of the molt cycle were represented in the digestive toxicity portion of this study and consumed experimental meals. One shrimp molted during a GRT assay following meal ingestion and was excluded from further analysis. Molt condition may be expected to impact gut motility since the foregut and hindgut are lined with cuticle (Icely and Nott, 1992). While palaemonids have a reduced gastric armature and rely on well-developed mandibles for mastication, processes such as ingestion, peristalsis and adequate mixing of ingested materials, enzymes and gut fluid likely require rigidity of the proventricular cuticle (Felgenhauer and Abele, 1983; Lovett and Felder, 1990a; Sousa and Petriella, 2006). The hepatopancreas and midgut are not lined with cuticle and may not be impacted directly by molt stage, although accumulation and loss of stored materials (e.g., glycogen and metals) by R cells may take place in phase with the molt cycle (Al-Mohanna and Nott, 1987). Van Wormhoudt (1974) reported that amylase and protease activities isolated from *Palaemon serratus* oscillated throughout the molt cycle with maximum activities during intermolt and premolt. In penaeids, feeding condition may mask impacts of the molt cycle on digestive enzyme activities (Muhlia-Almazán and García-Carreño, 2002).

CONCLUSION

Metal accumulation and toxicity in aquatic invertebrates can vary significantly based on route of exposure (dissolved vs. dietary sources) (Hook and Fisher, 2001;

Griscom et al., 2002). For organisms exposed to dietary metal, digestive toxicity may result from metal circulating in gut fluid (pre-assimilatory toxicity) or metal incorporated into gut tissues (post-assimilatory toxicity). In the present study, we have demonstrated that ingestion of a pulse of Cd in food can impact FER and extracellular protease activities in naïve grass shrimp. These impacts may be related to interactions between ingested metal and gut epithelial tissues or direct impacts on components in gut fluid. Carbon AE, GRT and gut pH were not influenced by Cd ingestion. Pre-assimilatory toxicity may also be related to other digestive parameters not investigated in this study, including solubilization of pollutants (related to concentrations of dissolved organic matter) and gut fluid surfactancy (Mayer et al., 1997; Ahrens et al., 2001; Mayer et al., 2001; Chen et al., 2002). Since metal ingestion may induce damage to gut tissues, future work will examine impacts of pre-assimilatory exposure to Cd on the functional morphology of epithelial cells within the grass shrimp hepatopancreas (Khan et al., 2010).

Table 6-1. Effects of a pulse of Cd in meals on ingested ^{14}C or initial IRDye signal in grass shrimp *P. pugio*. Ingested ^{14}C is the sum of ^{14}C retained and ^{14}C in cumulative feces for individual shrimp at t = 24 h. Initial IRDye signal is integrated intensity at t = 20 min from the introduction of IRDye-labeled casein meals. Correlations between endpoints and ingested ^{14}C or initial IRDye signal were analyzed for individual dietary Cd treatments and treatment groups using Spearman rank correlation (r_s).

	Treatment (n)	Mean ingested ^{14}C or Initial IRDye signal	AE-^{14}C vs. ingested cpm or casein hydrolysis rate vs. initial IRDye signal (r_s)
^{14}C:	Control[a] (12)	8004.7 ± 1802.8	-0.0629 (ns)
	1.76 µM Cd (12)	7288.9 ± 1330.1	0.2727 (ns)
	3.52 µM Cd (3)	8633.8 ± 3460.2	0.5000 (ns)
	7.04 µM Cd (7)	8091.7 ± 1869.1	0.5000 (ns)
	All treatments	7825.5 ± 889.3	0.1392 (ns)
	Ingested ^{14}C cpm K-W ANOVA: ns		
IRDye:	Control (8)	131.1 ± 52.9	0.1429 (ns)
	1.76 µM Cd (7)	137.8 ± 47.6	0.7858 ($p < 0.05$)
	3.52 µM Cd (6)	112.3 ± 20.1	0.2571 (ns)
	7.04 µM Cd (6)	149.0 ± 18.3	0.4857 (ns)
	All treatments	132.6 ± 19.9	0.3736 (ns)
	Ingested IRDye ANOVA: ns		

[a] Dissolved Cd exposure concentration for oligochaetes *T. tubifex* used to prepare meals containing ^{14}C-labeled diatoms or IRDye-labeled casein.
[ns]KW-ANOVA, ANOVA or r_s not significant.

Table 6-2. pH within the anterior and posterior regions of the cardiac chamber of the proventriculus in grass shrimp *P. pugio* exposed to a pulse of dietary Cd during analysis.

Treatment (*n*)	Cardiac Chamber pH[a]	
	Anterior	Posterior
Control[b] (7)	5.59 +0.25/-0.16[(ns)]	5.36 +0.18/-0.13[(ns)]
1.76 µM Cd (3)	5.40 +0.33/-0.19	5.29 +0.14/-0.11
3.52 µM Cd (3)	5.85 +0.05/-0.04	6.01 +0.05/-0.05
7.04 µM Cd (4)	5.45 +0.30/-0.18	5.65 +0.22/-0.15

[a] Reported as –log(mean $[H^+]$).
[b] Dissolved Cd exposure concentration for oligochaetes *T. tubifex* used to prepare meals containing Zymosan A BioParticles fluorescein conjugate.
[ns]K-W ANOVA not significant (statistical analyses conducted on $[H^+]$ data).

Table 6-3. Effects of molt stage on GRT, FER, protease activity and gut pH in grass shrimp *P. pugio* exposed to a pulse of dietary Cd.

	Dietary Cd treatment (μM Cd) (*T. tubifex* exposure)				
Molt Stage[a]	Control (*n*)	1.76 (*n*)	3.52 (*n*)	7.04 (*n*)	All treatments (*n*)
Median GRT (min)					
C	465 (2)$^{(ns)}$	n/a	435 (2)$^{(ns)}$	510 (3)$^{(ns)}$	510 (7)$^{(ns)}$
D_0	585 (2)	360 (5)$^{(ns)}$	390 (5)	360 (3)	390 (15)
D_1	390 (3)	330 (1)	n/a	330 (3)	390 (7)
D_2	480 (2)	480 (2)	n/a	375 (2)	390 (6)
post	435 (2)	315 (2)	450 (2)	n/a	375 (6)
Mean FER (mm h^{-1})					
C	18.12 ± 3.36 (2)$^{(ns)}$	n/a	15.25 ± 7.81 (2)$^{(ns)}$	5.98 ± 3.56 (3)$^{(ns)}$	12.09 ± 3.18 (7)$^{(ns)}$
D_0	14.16 ± 2.27 (2)	11.15 ± 3.89 (5) $^{(ns)}$	7.94 ± 2.23 (5)	8.47 ± 2.84 (3)	9.79 ± 1.60 (15)
D_1	10.69 ± 4.84 (3)	8.94 ± 0.00 (1)	n/a	7.76 ± 1.06 (3)	9.18 ± 1.95 (7)
D_2	19.66 ± 2.31 (2)	5.23 ± 2.34 (2)	n/a	2.15 ± 0.14 (2)	9.02 ± 3.52 (6)
post	11.34 ± 5.32 (2)	13.08 ± 0.85 (2)	4.64 ± 0.94 (2)	n/a	9.68 ± 2.15 (6)
Casein hydrolysis rate (mean increase in integrated intensity)					
C	1.07 ± 0.42 (2)$^{(ns)}$	0.94 ± 0.00 (1)$^{(ns)}$	n/a	3.35 ± 0.00 (1)$^{(ns)}$	1.61 ± 0.61 (4)$^{(ns)}$
D_0	0.72 ± 0.35 (2)	1.46 ± 0.29 (2)	0.56 ± 0.26 (3)$^{(ns)}$	1.38 ± 0.12 (2)	0.97 ± 0.18 (9)
D_1	0.32 ± 0.00 (1)	0.98 ± 0.00 (1)	1.07 ± 0.00 (1)	1.60 ± 0.00 (1)	0.99 ± 0.26 (4)
D_2	0.93 ± 0.63 (2)	1.65 ± 0.99 (3)	0.47 ± 0.00 (1)	2.09 ± 1.41 (2)	1.43 ± 0.48 (8)
Post	1.11 ± 0.00 (1)	n/a	n/a	n/a	1.11 ± 0.00 (1)
pH within the anterior region of the cardiac chamber [–log(mean $[H^+]$)]					
C	n/a	n/a	5.93 +0.00/-0.00 (1)$^{(nd)}$	n/a	5.93 +0.00/-0.00 (1)$^{(ns)}$
D_0	5.58 +0.30/-0.17 (6)$^{(nd)}$	n/a	5.77 +0.00/-0.00 (1)	5.75 +0.07/-0.06 (2)$^{(nd)}$	5.63 +0.20/-0.14 (9)
D_1	5.69 +0.00/-0.00 (1)	5.45 +0.00/-0.00 (1)$^{(nd)}$	n/a	n/a	5.55 +0.14/-0.10 (2)
D_2	n/a	6.25 +0.00/-0.00 (1)	5.86 +0.00/-0.00 (1)	5.27 +0.40/-0.22 (2)	5.50 +0.41/-0.21 (4)
post	n/a	5.10 +0.00/-0.00 (1)	n/a	n/a	5.10 +0.00/-0.00 (1)
pH within the posterior region of the cardiac chamber [–log(mean $[H^+]$)]					
C	n/a	n/a	5.93 +0.00/-0.00 (1)$^{(nd)}$	n/a	5.93 +0.00/-0.00 (1)$^{(ns)}$
D_0	5.53 +0.18/-0.13 (6)$^{(nd)}$	n/a	6.09 +0.00/-0.00 (1)	5.91 +0.33/-0.19 (2)$^{(nd)}$	5.63 +0.16/-0.12 (9)
D_1	5.44 +0.00/-0.00 (1)	5.30 +0.00/-0.00 (1)$^{(nd)}$	n/a	n/a	5.36 +0.08/-0.06 (2)
D_2	n/a	6.90 +0.00/-0.00 (1)	6.04 +0.00/-0.00 (1)	5.49 +0.29/-0.17 (2)	5.73 +0.34/-0.19 (4)
post	n/a	5.11 +0.00/-0.00 (1)	n/a	n/a	5.11 +0.00/-0.00 (1)

[a] Stages: intermolt (C), premolt (D_0, D_1 and D_2) and postmolt (post) determined by examination of setal region of uropod exopodites; [n/a] Shrimp from molt stage not identified following assay; [nd] K-W ANOVA not performed due to insufficient sample size; [ns] K-W ANOVA not significant.

Figure 6-1. Whole tissue Cd concentrations in oligochaetes *T. tubifex* following exposure to Cd through solution for 96 h (n = 3-4; mean ± S.E.). Significant differences ($p < 0.05$) in Cd burdens are indicated by different letters above each bar (t-test with Bonferroni correction).

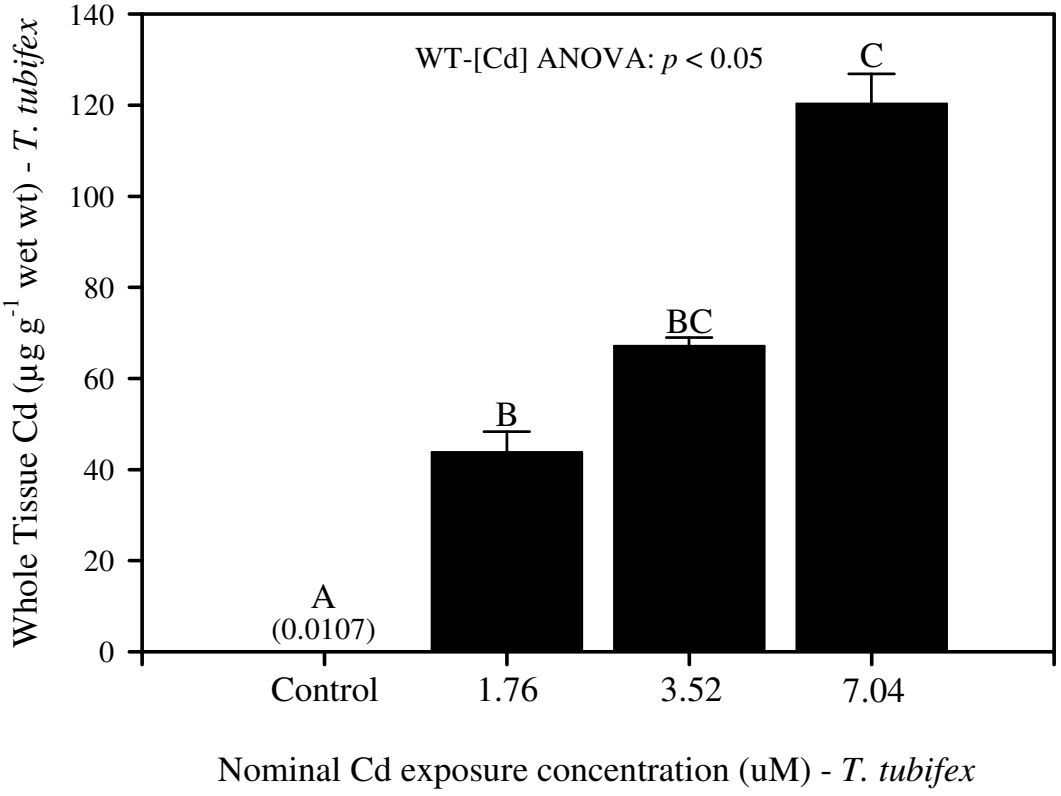


Figure 6-2. AE-^{14}C% by grass shrimp *P. pugio* following ingestion of a pulse of Cd in meals containing ^{14}C-labeled diatoms (n = 7-12; mean ± S.E.).

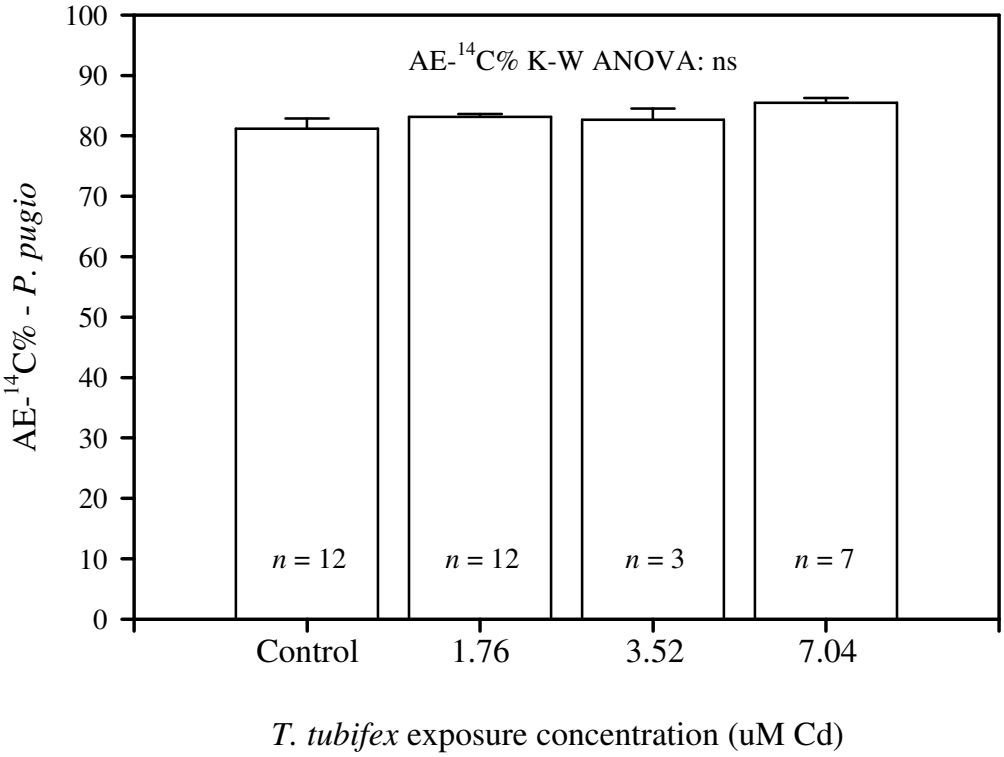
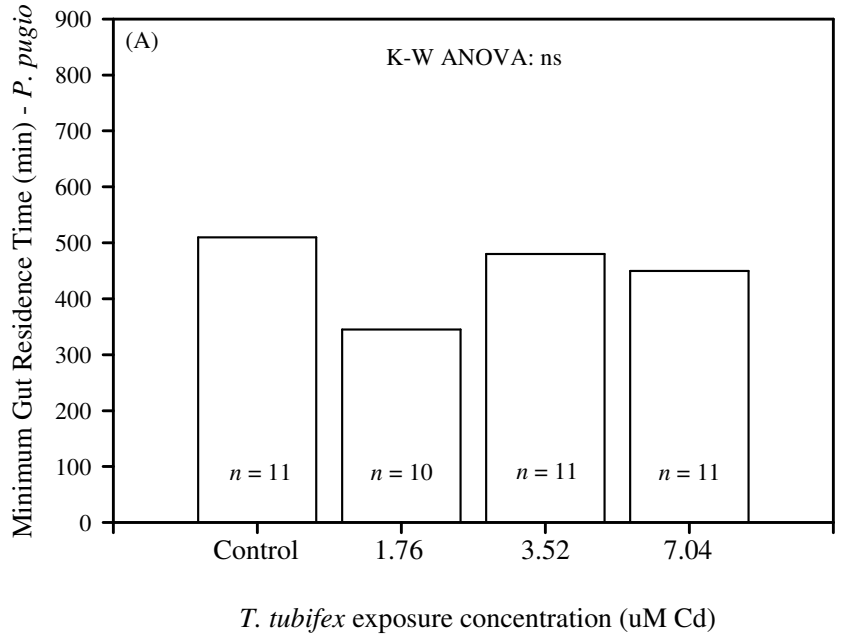


Figure 6-3. (A) Minimum gut residence time in grass shrimp *P. pugio* following ingestion of a pulse of Cd in meals containing 0.5 µm fluorescent microspheres (n = 10-11; median). (B) Curves showing percentages of grass shrimp with microspheres in feces over time.



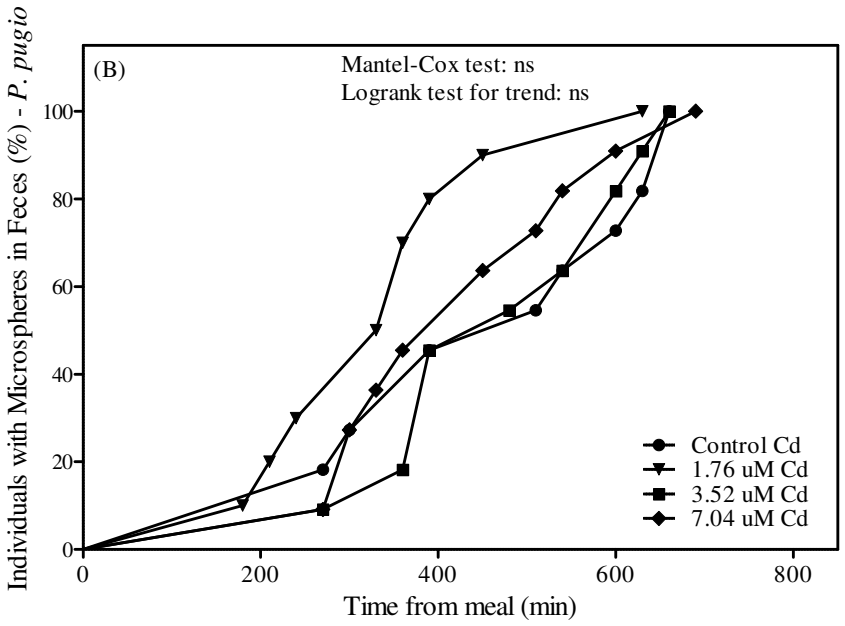


Figure 6-4. Feces elimination rate in grass shrimp *P. pugio* following ingestion of a pulse of Cd in meals containing 0.5 µm fluorescent microspheres (n = 10-11; mean ± S.E.). Significant differences ($p < 0.05$) between dietary treatments (t-test with Bonferroni correction) are indicated by different letters.

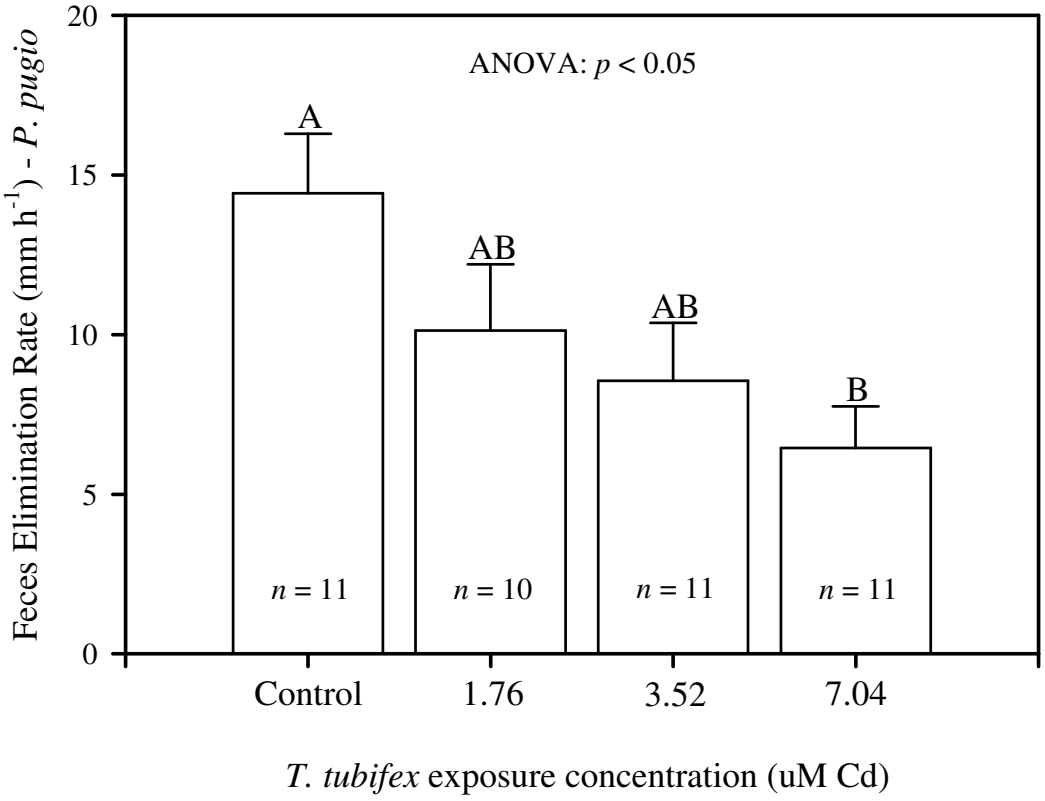
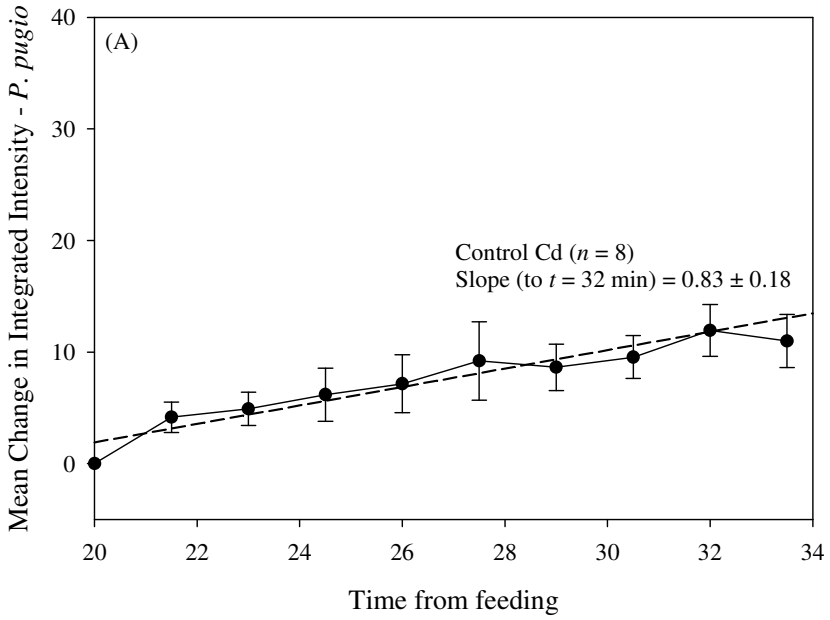
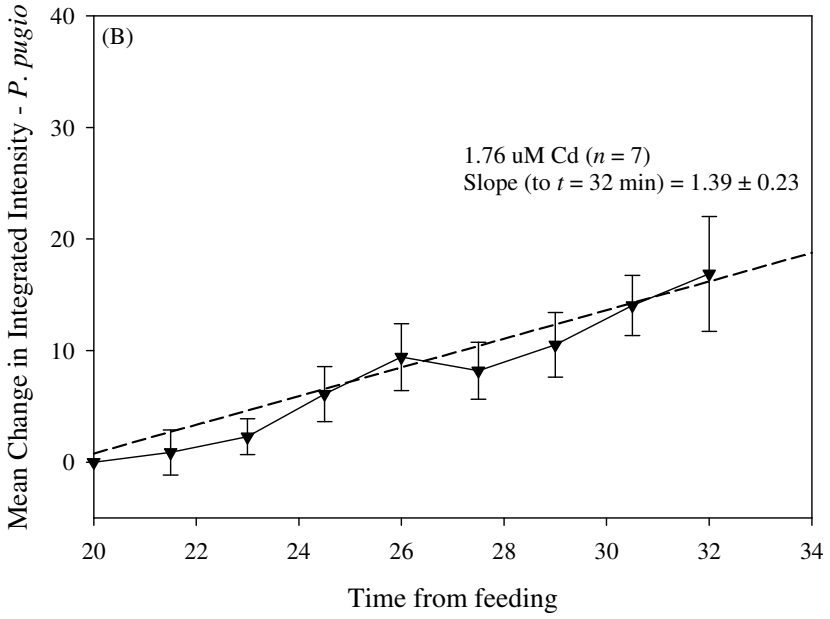
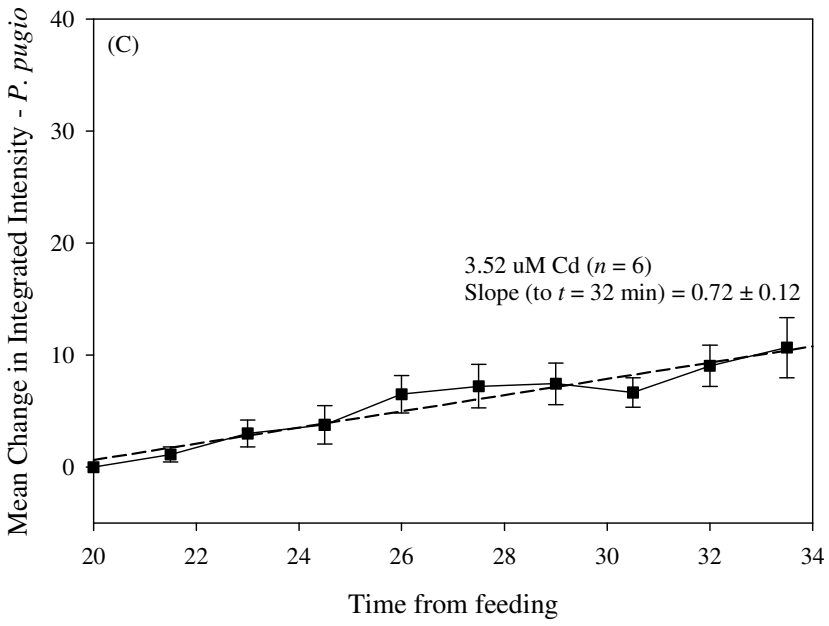


Figure 6-5. Mean change in integrated intensity in the proventriculus and hepatopancreas of grass shrimp *P. pugio* following consumption of a pulse of Cd in meals containing IRDye-labeled casein (n = 6-8).






(C)
3.52 uM Cd (n = 6)
Slope (to t = 32 min) = 0.72 ± 0.12
Mean Change in Integrated Intensity - P. pugio
Time from feeding
40
30
20
10
0
20
22
24
26
28
30
32
34

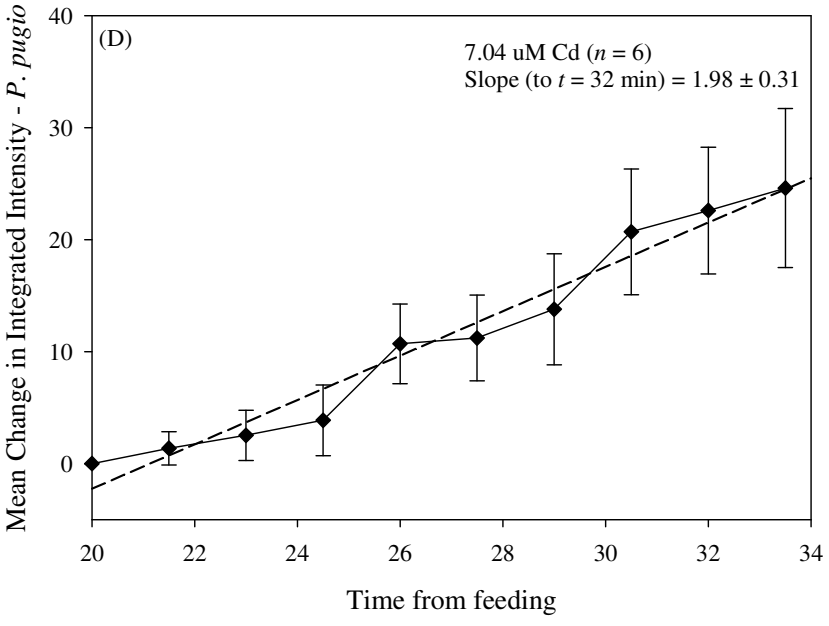

(D)
7.04 uM Cd (n = 6)
Slope (to t = 32 min) = 1.98 ± 0.31
Mean Change in Integrated Intensity - P. pugio
Time from feeding
40
30
20
10
0
20
22
24
26
28
30
32
34

Figure 6-6. Casein hydrolysis rates for grass shrimp *P. pugio* following consumption of a pulse of Cd in meals containing IRDye-labeled casein (n = 6-8). Significant differences ($p < 0.05$) between dietary treatments (t-test with Bonferroni correction) are indicated by different letters.

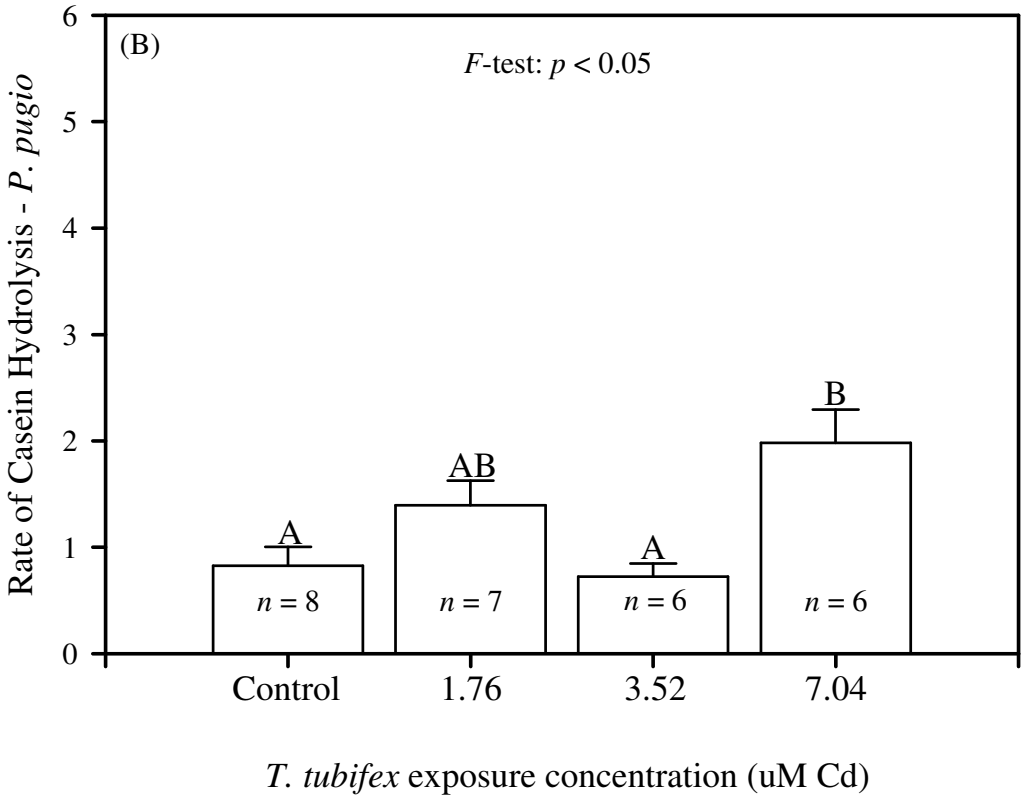


Fig. 6

CHAPTER 7

General conclusion

Biochemical and physical processes within invertebrate guts vary considerably across taxa and may be impacted by exposure to contaminants, including metals (Mayer et al., 1997, 2001; Chen and Mayer, 1998; Chen et al., 2002). Formulation of a unified theory of processing, fate and toxicological impacts of ingested pollutants within a taxon (e.g., palaemonid shrimp) requires comprehensive understanding of baseline gut physiology in representative organisms (e.g., grass shrimp *Palaemonetes*). This information can provide suitable basis for comparison while assessing relationships between gut function and assimilation of essential elements and pollutants by impacted organisms (Ahrens et al., 2001). Assimilation efficiencies and endpoints related to digestive toxicity in the present work (i.e., GRT/FER, digestive protease activities and gut pH) were assessed for shrimp following collection along an impact gradient, pre-exposure to metal (Cd or Hg) contaminated food or ingestion of a pulse of Cd in the case of naïve shrimp. Baseline data for each endpoint were obtained from shrimp collected from a local reference site or laboratory controls.

Increased assimilation of dietary metal (Cd and Hg) was predicted and observed for grass shrimp collected along an impact gradient within the NY/NJ Harbor Estuary. Enhancement of non-essential metal assimilation may have important implications for accumulation and toxicity (impacts on behavior and reproduction) in impacted shrimp (Kraus and Kraus, 1986; Wallace et al., 2000; Hook and Fisher, 2001; Griscom et al., 2002). In the case of Cd assimilation, a positive correlation with GRT and negative relationship with digestive protease activities suggests that digestive plasticity (perhaps important in maintaining carbon AE) may also influence assimilation of non-essential elements in the field. Variability in Cd AE by shrimp pre-exposed to dietary metal in the

laboratory was not dose-dependent, which may be related to interactions between post-assimilatory impacts on gut physiology. Hg AE was not influenced by pre-exposure to dietary Cd. Increased assimilation of Hg by Hg pre-exposed shrimp may have been related to a corresponding increase in gut pH (i.e., decreased concentrations of H^+ ions in circulating gut fluid).

Reduced carbon AE was predicted for grass shrimp collected from impacted sites within an urbanized estuary, pre-exposed to dietary metal or in naïve shrimp following ingestion of a pulse of Cd with food. Based on this series of studies, it appears that this species may indeed be able to maintain carbon assimilation in the laboratory under different forms of pollutant-induced dietary stress. For field-collected shrimp, this phenomenon may be attributable to digestive plasticity (i.e., possibly increased GRT to compensate for reduced digestive enzyme activities). Exposure to metal-contaminated sediments can elevate metabolism in decapods (e.g., crayfish and *Palaemonetes paludosus*), which may impact growth, energy storage and fitness due to increased energetic costs associated with physiological maintenance (Rowe, 1998; Rowe et al., 2001). Long-term, pollutant-induced changes in the assimilation of organic matter by shrimp in the field would have important implications for survival, growth and energy allocation and likely impact fitness as greater than 50% of the energy ingested by adults is allocated for reproduction under optimal salinity and temperature conditions (Alon and Stancyk, 1982; Vernberg and Piyatiratitivorakul, 1998; Reinsel et al., 2001). In a recent series of studies, Stout (2009) reported increased resource partitioning to reproduction by gravid grass shrimp collected from an oil- and metal-contaminated site relative to shrimp from a clean reference site. Interestingly, resource budget analyses also demonstrated that

despite decreased carbon consumption and increased respiration associated with chronic exposure, impacted shrimp in intermolt were able to maintain growth rates comparable to reference shrimp (Stout, 2009). Further research is required to investigate the impacts of quality of food consumed by grass shrimp in the field (e.g., high quality diatoms vs. low quality detritus from sources such as *Spartina*) on carbon assimilation or growth (Chervin, 1978; Wang and Fisher, 1996). Relative availability and nutritional quality of prey organisms may also vary among differentially impacted field sites and could potentially influence assimilation of essential nutrients (Campbell et al., 2005; Goto and Wallace, 2010).

Decapod digestion has been the subject of considerable research and debate, particularly with respect to hepatopancreas function (see reviews by Gibson and Barker, 1979; Dall and Moriarty, 1983; Icely and Nott, 1992 and references therein as well as recent research on *Palaemonetes argentinus* by Sousa et al., 2005). Studies using metal tracers (ferritin, colloidal gold and thorium dioxide) and X-ray microanalysis have been used to characterize uptake and ultrastructural localization of metals (including Cu, Pb and Zn) within B, R and F cells of the hepatopancreatic epithelium (Al-Mohanna and Nott, 1986, 1987, 1989; Al-Mohanna et al., 1985; Hopkin and Nott, 1979, 1980; Andersen and Baatrup, 1988). Although mechanisms of metal transport and storage (i.e., in metal-rich granules found in R cells) have been characterized for specific cell types, relationships between ultrastructural localization of metals, functional morphology of epithelial cells and gut physiology have not yet been established. In the present work, Cd-induced pre-assimilatory digestive toxicity (impacts on FER and protease activities) may have been related to metal interactions with apical epithelial cell surfaces (e.g., exocytotic

machinery). Post-assimilatory impacts of ingested metal on digestion (GRT, protease activities and gut pH) may have resulted from changes in organelle (e.g., mitochondrion or lysosome) or membrane structure or function following incorporation of metal by gut tissues (Viarengo et al., 2000; Cannino et al., 2009). Further research is required to determine if metal-induced variability in gut physiology may be related to interactions between metal circulating in gut fluid and apical cell surfaces in naïve shrimp or intracellular localization of assimilated metal and damage to gut tissues in metal pre-exposed shrimp (Andersen and Baatrup, 1988). For shrimp collected along an impact gradient, post-assimilatory tissue damage may have also been induced by exposure to organic contaminants (Gunster et al., 1993). Post-assimilatory toxicity may also include impacts of ingested pollutants on systems and processes beyond the digestive tract.

As a dominant epibenthic organism, the grass shrimp plays an important role in nutrient cycling within estuarine communities and may influence pollutant (e.g., metal) transfer along food chains (Nixon and Oviatt, 1973; Welsh, 1975; Davis et al., 2003; Seebaugh et al., 2005). For shrimp inhabiting the AK complex, it is not known whether impacts on food acquisition, gut physiology and assimilation are related specifically to ingested metals or organic pollutants (e.g., byproducts of petroleum processing or leachate from landfills) (Perez and Wallace, 2004). In palaemonids (e.g., *Palaemonetes pugio* and *P. argentinus*), environmental exposure to organic pollutants (e.g., pesticides) may impact gut function and influence the assimilation of elements (Doughtie and Rao, 1983; Sousa and Petriella, 2007). Dissolved pollutants (e.g., inorganic Hg) may also be incorporated by specific (e.g., nervous, muscle or gut) tissues involved in feeding or digestion (Anderson and Baatrup, 1988; Bianchini and Gilles, 2000). Additionally,

interactions between different classes of accumulated pollutants (e.g., metals and pesticides) as well as their respective exposure pathways could potentially influence digestion and assimilation (Wang and Fisher, 1999; Mortimer, 2000; Griscom et al., 2002).

In addition to pollutant concentrations in tissues, a wide variety of indicators have been suggested as biomarkers in environmental monitoring, including mechanisms of detoxification (e.g., metallothioneins), enzyme activities, functional morphology of specific tissues, energy reserves, reproductive output and behavior (Weis et al., 2001; Paulson et al., 2003; Amiard et al., 2006; Durou et al., 2007; Sousa and Petriella, 2007; Douhri and Sayah, 2009). Downs et al. (2001) reported impacts of heat stress, dissolved Cd, atrazine and fuel oil exposure on molecular biomarkers (including glutathione, lipid peroxide, heat-shock proteins, manganese superoxide dismustase and metallothionein-like proteins) indicative of oxidative stress, cellular integrity or detoxification in grass shrimp tissues. Since gut tissues are important targets in arthropods, assessment of gut functions (e.g., GRT, digestive enzyme activities and pH) in organisms from differentially-impacted field populations may have applications in environmental monitoring (Beaty et al., 2002; Campbell et al., 2005). With the exception of behavioral biomarkers (e.g., prey capture ability or predator avoidance), potential indicators of environmental contamination are not typically conducted using live organisms in real-time (Kraus and Kraus, 1986; Weis et al., 2001; Perez and Wallace, 2004). The non-invasive techniques developed in this and previous work on invertebrate gut physiology *in vivo* may complement existing biochemical, microscopic and behavioral methods for monitoring impacted aquatic ecosystems (Ahrens and Lopez, 2001).

Previous studies indicate that TAM in invertebrate prey may be used to estimate the potential for Cd, Zn and methylmercury transfer to predators, although predator-dependent processes may be important in determining assimilation (Wallace et al., 1998; Wallace and Luoma, 2003; Seebaugh and Wallace, 2004; Seebaugh et al., 2005; Goto and Wallace, 2009a). In grass shrimp, relationships between metal Cd AE and TAM-Cd were impacted by tissue metal concentrations in invertebrate prey (Seebaugh and Wallace, 2004; Seebaugh et al., 2005; the present work). Relationships between AE-^{203}Hg% by field-collected or metal pre-exposed shrimp and TAM-^{203}Hg% in amphipods/oligochaetes observed in the present work indicate that the TAM compartment may represent maximum bioavailable inorganic Hg in invertebrates. Hg circulating in gut fluid (i.e., prior to assimilation) could also influence correspondence between AE and TAM-Hg.

Methylation of inorganic Hg within the gut (e.g., by bacteria in fish intestine) could influence Hg speciation in gut fluid, physiological responses to ingested metal (e.g., mucous production in fish) and assimilation by tissues (Rudd et al., 1980; Farmanfarmaian, 1985; Handy et al., 2000; Goto and Wallace, 2009a). Although the decapod hepatopancreas is usually free of bacteria (pathogens can infect this organ), ingested microoganisms can be found in other digestive organs (Sugita et al., 1987; Vogt, 1997). While it does not appear that Hg methylation within benthic invertebrate tissues has been reported, the intriguing possibility that bacteria (e.g., *Pseudomonas*) observed in decapod guts may be capable of methylation requires study (Sugita et al., 1987; Farrell et al., 1991; Duran et al., 2008). Hg transformation in invertebrate guts could potentially influence Hg toxicity as well as Hg (or methylmercury) trophic transfer (Riisgard and

Hansen, 1990; Goto and Wallace, 2009a).

Doses of Cd to grass shrimp from a hypothetical meal in the field were estimated using a simplified biokinetic model of dietary metal accumulation and assumed equal rates of ingestion and physiological loss of metal for shrimp from the Staten Island study sites (Wang and Fisher, 1999). In addition to the limitations described in this study, this calculation can only provide a snapshot of metal accumulated during a single feeding. Estimates of ingestion rates, growth and efflux rates would be also necessary to determine if metal burdens in prey and AE can be used to predict steady-state burdens in shrimp (Thomann, 1981; Wang and Fisher, 1999). Diet composition (e.g., TAM in different prey items) may also be important in determining metal accumulation through feeding (Wallace and Luoma, 2003; Goto and Wallace, 2009b).

Metal concentrations in prey may also be applied to a dietary biotic ligand model (BLM) to estimate metal concentrations in food (i.e., with respect to binding strengths of competing cations for ligands) necessary to elicit toxicity at steady-state (Borgmann et al., 2005). Application of the BLM to pre-assimilatory toxicity would require estimates of metal concentrations in circulating gut fluid, concentrations of bound and free ligand in food particles or receptors along the gut epithelium as well as binding strengths for competing cations. A BLM model for post-assimilatory toxicity would also include rates of transport into epithelial tissues, concentrations of metal incorporated by gut tissues as well as excretion rates (Borgmann et al., 2005). Terms representing transport from the hepatopancreatic epithelium (e.g., from R or B cells) to the haemolymph and circulation to other tissues involved in digestion (e.g., enteric nervous system and gut musculature) may also be needed to model post-assimilatory impacts of ingested metal on gut

physiology (Al-Mohanna and Nott, 1987). Relationships between proventriculus pH and Hg AE by Hg pre-exposed shrimp observed in the present work indicate that interactions between pre- and post-assimilatory effects of ingested metal may be important in modeling dietary metal toxicity. The BLM approach may be supplemented by mechanistic and compartmental models of dietary metal toxicity which also consider metal transport into cells, metal partitioning among sensitive intracellular components and mechanisms of detoxification (Bell et al., 2002; Wallace et al., 2003; Borgmann et al., 2005).

Introduction of metals into food chains can influence population dynamics and may impact community composition (van Straalen et al., 1989; Goto and Wallace, 2010). Since toxicity (e.g., impacts on reproduction) can vary depending on the route of exposure, models of metal-induced effects on population growth should consider the potential impacts of dietary as well as dissolved sources (Hook and Fisher, 2001; Manyin and Rowe, 2008). Dietary metal accumulation may also vary with trophic level (i.e., due to biomagnification) and elicit differential effects on populations within impacted communities (Riisgard and Hansen, 1990; Crouteau et al., 2005).

REFERENCES

CHAPTER 1

Al-Mohanna, S.Y., Nott, J.A., Lane, D.J.W., 1985. Mitotic E- and secretory F-cells in the hepatopancreas of the shrimp *Penaeus semisulcatus* (Crustacea: Decapoda). J. Mar. Biol. Assoc. U.K. 65, 901-910.

Alon, N.C., Stancyk, S.E., 1982. Variation in life-history patterns of the grass shrimp *Palaemonetes pugio* in two South Carolina estuarine systems. Mar. Biol. 68, 265-276.

Amiard, J.-C., Amiard-Triquet, C., Barka, S., Pellerin, J., Rainbow, P.S., 2006. Metallothioneins in aquatic invertebrates: their role in metal detoxification and their use as biomarkers. Aquat. Toxicol. 76, 160-202.

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*). Limnol. Oceanogr. 47, 646-655.

Bergen, A., Alderson, C., Bergfors, R., Aquila, C., Matsil, M.A., 2000. Restoration of a *Spartina alterniflora* salt marsh following a fuel oil spill, New York City, NY. Wetland. Ecol. Manag. 8, 185-195.

Bell, S.S., Coull, B.C., 1978. Field evidence that shrimp predation regulates meiofauna. Oecologia 35, 141-148.

Bergey, L., Weis, J.S., Weis, P., 2002. Mercury uptake by the estuarine species *Palaemonetes pugio* and *Fundulus heteroclitus* compared with their parasites, *Probopyrus pandalicola* and *Eustrongylides* sp. Mar. Pollut. Bull. 44, 1046-1050.

Berry, W.J., Hansen, D.J., Mahony, J.D., Robson, D.L., DiToro, D.M., Shipley, B.P., Rogers, B., Corbin, J.M., Boothman, W.S., 1996. Predicting the toxicity of metal-spiked laboratory sediments using acid-volatile sulfide and interstitial water normalizations. Environ. Toxicol. Chem. 15, 2067-2079.

Campbell, P.G.C., Clearwater, S.J., Brown, P.B., Fisher, N.S., Hogstrand, C., Lopez, G.R., Mayer, L.M., Meyer, J.S., 2005. Digestive physiology, chemistry, and nutrition. In: Meyer, J.S., Adams, W.J., Brix, K.V., Luoma, S.N., Mount, D.R., Stubblefield, W.A., Wood, C.M. (Eds.), Toxicity of Dietborne Metals to Aquatic Organisms, Society of Environmental Toxicology and Chemistry, Pensacola, FL, pp. 13-57.

Carson, M.L., Merchant, H., 2005. A laboratory study of the behavior of two species of grass shrimp (*Palaemonetes pugio* Holthius and *Palaemonetes vulgaris* Holthius) and the killifish (*Fundulus heteroclitus* Linneaus). J. Exp. Mar. Biol. Ecol. 314, 187-201.

Chambers, R., 1981. Seasonal feeding and distribution of *Palaemonetes pugio* and *P. vulgaris* in Great Sippewissett salt marsh. Biol. Bull. 161, 324.

Chen, Z., Mayer, L.M., 1998. Digestive proteases of the lugworm (*Arenicola marina*) inhibited by Cu from contaminated sediments. Environ. Toxicol. Chem. 17, 433-438.

Chen, Z., Mayer, L.M., Weston, D.P., Bock, M.J., Jumars, P.A., 2002. Inhibition of digestive enzyme activities by copper in the guts of various marine benthic invertebrates. Environ. Toxicol. Chem. 21, 1243-1248.

Cheung, M.-S., Fok, E.M.W., Ng, T.Y., Yen, Y.-F., Wang, W.-X., 2006. Subcellular cadmium distribution, accumulation, and toxicity in a predatory gastropod, *Thais clavigera*, fed different prey. Environ. Toxicol. Chem. 25, 174-181.

Couillard, Y., Campbell, P.G.C., Tessier, A., 1993. Response of metallothionein concentrations in a freshwater bivalve (*Anodonta grandis*) along an environmental cadmium gradient. Limnol. Oceanogr. 38, 299-313.

Crawford, D.W., Bonnevie, N.L., Wenning, R.J., 1995. Sources of pollution and sediment contamination in Newark Bay, New Jersey. Ecotoxicol. Environ. Saf. 30, 85-100.

Croteau, M.N., Luoma, S.N., Stewart, A.R., 2005. Trophic transfer of metals along freshwater food webs: evidence of cadmium biomagnification in nature. Limnol. Oceanogr. 50, 1511-1519.

Davis, J.L.D., Metcalfe, W.J., Hines, A.H., 2003. Implications of fluctuating fish predator guilds on behavior, distribution, and abundance of a shared prey species: the grass shrimp *Palaemonetes pugio*. J. Exp. Mar. Biol. Ecol. 293, 23-40.

De La Ruelle, M., Hajjou, M., Van Herp, F., Le Gal, Y., 1992. Aminopeptidase activity from the hepatopancreas of *Procambarus clarkii*. Biochem. System. Ecol. 20, 331-337.

Dittel, A.I., Epifanio, C.E., Schwalm, S.M., Fantle, M.S., Fogel, M.L., 2000. Carbon and nitrogen sources for juvenile blue crabs *Callinectes sapidus* in coastal wetlands. Mar. Ecol. Prog. Ser. 194, 103-112.

DiToro, D.M., Mahony, J.D., Hansen, D.J., Scott, K.J., Carlson, A.R., Ankley, G.T., 1992. Acid volatile sulfide predicts the acute toxicity of cadmium and nickel in sediments. Environ. Sci. Technol. 26, 96-101.

Downs, C.A., Fauth, J.E., Woodley, C.M., 2001. Assessing the health of grass shrimp (*Palaemonetes pugio*) exposed to natural and anthropogenic stressors: a molecular biomarker system. Mar. Biotech. 3, 380-397.

Engel, D.W., Fowler, B.A., 1979. Factors influencing cadmium accumulation and its toxicity to marine organisms. Environ. Health. Perspect. 28, 81-88.

Feng, H., Cochran, J.K., Lwiza, H., Brownawell, B.J., Hirschberg, D.J., 1998. Distribution of heavy metals and PCB contamination in the sediments of an urban estuary: the Hudson River. Mar. Environ. Res. 45, 69-88.

Fisher, N.S., Teyssié, J.-L., Fowler, S.W., Wang, W.-X., 1996. Accumulation and retention of metals from food and water: a comparison under field and laboratory conditions. Environ. Sci. Tech. 30, 3232-3242.

Fleeger, J.W., Carman, K.R., Webb, S., Hilbun, N., Pace, M.C., 1999. Consumption of microalgae by the grass shrimp *Palaemonetes pugio*. J. Crustacean Biol. 19, 324-336.

Forbes, V.E., 2003. Contaminant effects on population demographics. In: Newman, M.C., Unger, M.A. Fundamentals of Ecotoxicology, Lewis Publishers, Boca Raton, FL, pp. 221-224.

Frankenberg, D., Smith, K.L., 1967. Coprophagy in marine animals. Limnol. Oceangr. 12, 443-450.

Gillis, C.A., Bonnevie, N.L., Wenning, R.J., 1993. Mercury contamination in the Newark Bay Estuary. Ecotoxicol. Environ. Saf. 25, 214-226.

Goto, D., 2009. Impacts of habitat degradation on *Fundulus heteroclitus* (Linneaus) in urban tidal salt marshes in New York. Ph.D. Dissertation. City University of New York, New York, NY.

Goto, D., Wallace, W.G., 2010. Relative importance of multiple environmental variables in structuring benthic macroinfaunal assemblages in chronically metal-polluted salt marshes. Mar. Environ. Pollut. 60, 363-375.

Gregg, C.S., Fleeger, J.W., 1998. Grass shrimp *Palaemonetes pugio* predation on sediment- and stem-dwelling meiofauna: field and laboratory experiments. Mar. Ecol. Prog. Ser. 175, 77-86.

Gregory, M.A., George, R.C., Marshall, D.J., Anandraj, A., McClurg, T.P., 1999. The effects of mercury exposure on the surface morphology of gill filaments in *Perna perna* (Mollusca: Bivalvia). Mar. Pollut. Bull. 39, 116-121.

Griscom, S.B., Fisher, N.S., Luoma, S.N., 2002. Kinetic modeling of Ag, Cd and Co bioaccumulation in the clam *Macoma balthica*: quantifying dietary and dissolved sources. Mar. Ecol. Prog. Ser. 240, 127-141.

Gunster, D.G., Bonnevie, N.L., Gillis, C.A., Wenning, R.J., 1993. Assessment of chemical loadings to Newark Bay, New Jersey from petroleum and hazardous chemical accidents occurring from 1986 to 1991. Ecotoxicol. Environ. Saf. 25, 202-213.

Haines, E.V., Montague, C.L., 1979. Food sources of estuarine invertebrates analyzed using $^{13}C/^{12}C$ ratios. Ecology 60, 48-56.

Hook, S., Fisher, N.S., 2001. Sublethal effects of silver on zooplankton: importance of exposure pathways and implications for toxicity testing. Environ. Toxicol. Chem. 20, 568-574.

Hook, S., Fisher, N.S., 2002. Relating the reproductive toxicity of five ingested metals in calanoid copepods with sulfur affinity. Mar. Environ. Res. 53, 161-174.

Hopkin, S.P., Nott, J.A., 1979. Some observations on concentrically structured intracellular granules in the hepatopancreas of the shore crab *Carcinus maenas* (L.). J. Mar. Biol. Assoc. U.K. 59, 867-77.

Howard, C.L., Hacker, C.S., 1990. Effects of salinity, temperature, and cadmium on cadmium-binding protein in the grass shrimp, *Palaemonetes pugio*. Arch. Environ. Contam. Toxicol. 19, 341-347.

Hutcheson, M., Miller, D.C., White, A.Q., 1985. Respiratory and behavior responses of the grass shrimp *Palaemonetes pugio* to cadmium and reduced dissolved oxygen. Mar. Biol. 88, 59-66.

Icely, J.D., Nott, J.A., 1992. Digestion and absorption: digestive system and associated organs. In: Harrison, F.W., Humes, A.G. (Eds.), Microscopic anatomy of invertebrates: Decapod, Crustacea. Vol. 10. Wiley-Liss, New York, pp. 147-201.

Ivanina, A.V., Habinck, E., Sokolova, I.M., 2008. Differential sensitivity to cadmium of key mitochondrial enzymes in the eastern oyster, *Crassostrea virginica* Gmelin (Bivalvia: Ostreidae). Comp. Biochem. Physiol. C. 148, 72-79.

Johannes, R.E., Satomi, M., 1966. Composition and nutritive value of fecal pellets of a marine crustacean. Limnol. Oceangr. 11, 191-197.

Kádár, E., Costa, V., Santos, R.S., Lopes, H., 2005. Behavioural response to the bioavailability of inorganic mercury in the hydrothermal mussel *Bathymodious azoricus*. J. Exp. Biol. 208, 505-513.

Khan, A.T., Weis, J.S., 1993. Bioaccumulation of heavy metals in two populations of mummichog (*Fundulus heteroclitus*). Bull. Environ. Contam. Toxicol. 51, 1-5.

Klerks, P.L., Levinton, J.S., 1989. Rapid evolution of metal resistance in a benthic oligochaete inhabiting a metal-polluted site. Biol. Bull. 176, 135-141.

Kneib, R.T., 1985. Predation and disturbance by grass shrimp, *Palaemonetes pugio* Holthius, in soft-substratum benthic invertebrate assemblages. J. Exp. Mar. Biol. Ecol. 93, 91-102.

Kneib, R.T., 1987. Seasonal abundance, distribution and growth of postlarval and juvenile grass shrimp (*Palaemonetes pugio*) in a Georgia, USA, salt marsh. Mar. Biol. 96, 215-223.

Kneib, R.T., Stiven, A.E., 1982. Benthic invertebrate responses to size and density manipulations of the common mummichog, *Fundulus heteroclitus*, in an intertidal salt marsh. Ecology 63, 1518-1532.

Kraus, M.L., Kraus, D.B., 1986. Differences in the effects of mercury on predator avoidance in two populations of the grass shrimp *Palaemonetes pugio*. Mar. Environ. Res. 18, 277-289.

Kraus, M.L., Weis, J.S., 1987. Differences in the effects of mercury on telson regeneration in two populations of the grass shrimp *Palaemonetes pugio*. Arch. Environ. Contam. Toxicol. 17, 115-120.

Kraus, M.L., Weis, J.S., Weis, P., 1988. Effects of mercury on larval and adult grass shrimp (*Palaemonetes pugio*). Arch. Environ. Contam. Toxicol. 17, 355-363.

Kuzmick, D.M., Mitchelmore, C.L., Hopkins, W.A., Rowe, C.L., 2007. Effects of coal combustion residues on survival, antioxidant potential, and genotoxicity resulting from full life-cycle exposure of grass shrimp (*Palaemonetes pugio* Holthius). Sci. Total Environ. 373, 420-430.

Lanning, G., Cherkasov, A.S., Sokolova, I.M., 2006. Temperature-dependent effects of cadmium on mitochondrial and whole-organism bioenergetics of oysters (*Crassostrea virginica*). Mar. Environ. Res. 62, S79-S82.

Manyin, T., Rowe, C.L., 2006. Chronic exposure of *Leptocheirus plumulosus* to Baltimore Harbor sediment: bioenergetic and population-level effects. Mar. Environ. Res. 62, 116-130.

Manyin, T., Rowe, C.L., 2009. Bioenergetic effects of aqueous copper and cadmium on the grass shrimp, *Palaemonetes pugio*. Comp. Biochem. Physiol. C. 150, 65-71.

Marchi, B., Burlando, B., Moore, M.N., Viarengo, A., 2004. Mercury- and copper-induced lysosomal membrane destabilisation depends on $[Ca2^{+}]_i$ dependent phospholipase A2 activation. Aquat. Toxicol. 66, 197-204.

Mason, A.Z., Jenkins, K.D., 1995. Metal detoxification in aquatic organisms. In: Tessier, A., Turner, D.R. (Eds.), Metal Speciation and Bioavailability in Aquatic Systems. John Wiley & Sons Ltd, West Sussex, England, pp. 479-608.

Mayer, L.M., Chen, Z., Findlay, R.H., Fang, J., Sampson, S., Self, R.F.L., Jumars, P.A., Quetél, C., Donard, O.F.X., 1996. Bioavailability of sedimentary contaminants subject to deposit-feeder digestion. Environ. Sci. Technol. 30, 2641-2645.

Mayer, L.M., Schick, L.L., Self, L.R.F., Jumars, P.A., Findlay, R.H., Chen, Z., Sampson, S., 1997. Digestive environments of benthic macroinvertebrate guts: enzymes, surfactants and dissolved organic matter. J. Mar. Res. 55, 785-812.

Mayer, L.M., Weston, D.P., Bock, M.J., 2001. Benzo[a]pyrene and zinc solubilization by digestive fluids of benthic invertebrates – a cross-phyletic study. Environ. Toxicol. Chem. 20, 1890-1900.

Morgan, M.D., 1980. Grazing and predation of the grass shrimp *Palaemonetes pugio*. Limnol. Oceanogr. 25, 896-902.

Nichols, C., 2004. Metal concentrations in salt marsh sediment from Staten Island, New York. Master's thesis. College of Staten Island, City University of New York, Staten Island, NY.

Nieboer, E., Richardson, D.H.S., 1980. The replacement of the nondescript term 'heavy metals' by a biologically and chemically significant classification of metal ions. Environ. Pollut. 1, 3-26.

Nimmo, D.R., Lightner, D.V., Bahner, L.H., 1977. Effects of cadmium on the shrimps, *Penaeus duorarum*, *Palaemonetes pugio* and *Palaemonetes vulgaris*. In: Vernberg, F.J., Calabrese, A., Thurberg, F.P., Vernberg, W.P. (Eds.), Physiological responses of marine biota to pollutants. Academic Press, New York, pp. 131-183.

Nixon, S,W., Oviatt, C.A., 1973. Ecology of a New England salt marsh. Ecol. Monogr. 43, 463-498.

Nunez-Nogueria, G., Rainbow, P.S., Smith, B.D., 2006. Assimilation efficiency of zinc and cadmium in the decapod crustacean *Penaeus indicus*. J. Exp. Mar. Biol. Ecol. 332, 75-83.

Odum, E.O., 1985. Trends expected in stressed ecosystems. Bioscience 35, 419-422.

Oey, L.Y., Mellor, G.L., Hires, R.L., 1985. Tidal modeling of the Hudson Raritan estuary. Estuar. Coast. Shelf. Sci. 20, 511-527.

Olmi, E.J., Lipcius, R.M., 1991. Predation on postlarvae of the blue crab *Callinectes sapidus* Rathbun by sand shrimp *Crangon septemspinosa* Say and grass *shrimp Palaemonetes pugio* Holthius. J. Exp. Mar. Biol. Ecol. 151, 169-183.

Olsen, D.R., Larson, I.L., Brewster, R.H., Cutshall, N.H., Bopp, R.P., Simpson, H.J., 1984. A geochemical assessment of sedimentation and contamination distributions in the Hudson River Estuary. National Oceanic and Atmospheric Administration, NOS, OMS2. Rockville, MD.

Paulson, A.J., Sharack, B., Zdanowicz, V., 2003. Trace metals in ribbed mussels from Arthur Kill New York/New Jersey, USA. Mar. Pollut. Bull. 46, 139-152.

Penry, D.L., 1998. Applications of efficiency measurements in bioaccumulation studies: definitions, clarifications, and a critique of methods. Environ. Toxicol. Chem, 17, 1633-1639.

Perez, M.H., Wallace, W.G., 2004. Differences in prey capture in grass shrimp, *Palaemonetes pugio*, collected along an environmental impact gradient. Arch. Environ. Contam. Toxicol. 46, 81-89.

Pesch, G.G., Stewart, N.E., 1980. Cadmium toxicity to three species of estuarine invertebrates. Mar. Environ. Res. 3, 145-156.

Posey, M.H., Hines, A.N., 1991. Complex predator-prey interactions within an estuarine benthic community. Ecology 72, 2155-2169.

Quinones-Rivera, Z.J., Fleeger, J.W., 2005. The grazing effects of grass shrimp, *Palaemonetes pugio*, on epiphytic microalgae associated with Spartina alterniflora. Estuaries 28, 274-285.

Rainbow, P.S., Blackmore, G., Wang, W.-X., 2003. Effects of previous field-exposure history on the uptake of trace metals from water and food by the barnacle *Balanus amphitrite*. Mar. Ecol. Prog. Ser. 259, 201-213.

Rainbow, P.S., Geffard, A., Jeantet, A.-Y., Smith, B.D., Amiard, J.C., Amiard-Triquet, C., 2004a. Enhanced food-chain transfer of copper-tolerant estuarine worms. Mar. Ecol. Prog. Ser. 271, 183-191.

Rainbow, P.S., Ng, T. Y.-T., Shi, D., Wang, W.-X., 2004b. Acute dietary pre-exposure and trace metal bioavailability to the barnacle *Balanus amphitrite*. J. Exp. Mar. Biol. Ecol. 311, 315-337.

Regoli, F., Nigro, M., Bertoli, E., Principato, G., Orlando, E., 1997. Defenses against oxidative stress in the Antarctic scallop *Adamussium colbecki* and effects of acute exposure to metals. Hydrobiologia 355, 139-144.

Reinfelder, J.R., Fisher, N.S., 1991. The assimilation of elements ingested by marine copepods. Science 251, 794-796.

Reinfelder, J.R., Fisher, N.S., 1994a. The assimilation of elements ingested by marine planktonic bivalve larvae. Limnol. Oceanogr. 39, 12-20.

Reinfelder, J.R., Fisher, N.S., 1994b. Retention of elements absorbed by juvenile fish (*Menidia menidia*, *Menidia beryllina*) from zooplankton prey. Limnol. Oceanogr. 39, 1783-1789.

Reinsel, K.A., Glas, P.S., Rayburn, J.R., Pritchard, M.K., Fisher, W.S., 2001. Effects of food availability on survival, growth and reproduction of the grass shrimp *Palaemonetes pugio*: a laboratory study. Mar. Ecol. Prog. Ser. 220, 231-239.

Rubinstein, N.I., Lores, E., Gregory, N.R., 1983. Accumulation of PCBs, mercury and cadmium by *Nereis virens*, *Mercenaria mercenaria* and *Palaemonetes pugio* from contaminated harbor sediments. Aquat. Toxicol. 3, 249-260.

Rule, J.H., 1985. Chemical extractions of heavy metals in sediments as related to metal uptake by grass shrimp (*Palaemonetes pugio*) and clam (*Mercenaria mercenaria*). Arch. Environ. Contam. Toxicol. 14, 749-757.

Seebaugh, D.R., Estephan, A., Wallace, W.G., 2006. Relationship between cadmium assimilation by grass shrimp (*Palaemonetes pugio*) and trophically-available cadmium in amphipod (*Gammarus lawrencianus*) prey. Bull. Environ. Contam. Toxicol 76, 16-23.

Seebaugh, D.R., Goto, D., Wallace, W.G., 2005. Bioenhancement of cadmium transfer along a multi-level food chain. Mar. Environ. Res. 59, 473-491.

Seebaugh, D.R., Wallace, W.G., 2004. Importance of metal-binding proteins in the partitioning of Cd and Zn as trophically-available metal (TAM) in the brine shrimp *Artemia franciscana*. Mar. Ecol. Prog. Ser. 272, 215-230.

Seebaugh, D.R. Wallace, W.G. 2009. Assimilation and subcellular partitioning of elements by grass shrimp collected along an impact gradient. Aquat. Toxicol. 93, 107-115.

Selck, H., Decho, A.W., Forbes, V.E., 1999. Effects of chronic metal exposure and sediment organic matter on digestive absorption efficiency of cadmium by the deposit-feeding polychaete *Capitella* species I. Environ. Toxicol. Chem. 18, 1289-1297.

Shi, D., Blackmore, G., Wang, W.-X., 2003. Effects of aqueous and dietary preexposure and resulting body burden on silver biokinetics in the green mussel *Perna viridis*. Environ. Sci. Technol. 37, 936-943.

Shuhong, W., Huasheng, H., Xinhong, W., 2005. Bioenergetic responses in green lipped mussels (*Perna viridis*) as indicators of pollution stress in Xiamen coastal waters, China. Mar. Environ. Res. 51, 738-743.

Sokolova, I.M., Sokolov, E.P., Ponnappa, K.M., 2005. Cadmium exposure affects mitochondrial bioenergetics and gene expression of key mitochondrial proteins in the eastern oyster *Crassostrea virginica* Gmelin (Bivalvia: Ostreidae). Aquat. Toxicol. 73, 242-255.

Steinberg, N., Suszkowski, D.J., Clark, L., Way, J., 2004. Health of the Harbor: The First Comprehensive Look at the State of the NY/NJ Harbor Estuary. A Report to the NY/NJ Harbor Estuary Program. Hudson River Foundation, New York, NY.

Sullivan, M.J., Moncreiff, C.A., 1990. Edaphic algae are an important component of salt marsh food-webs: evidence from multiple stable isotope analyses. Mar. Ecol. Prog. Ser. 62, 149-159.

Sunda, W.G., Engel, D.W., Thuotte, R.M., 1978. Effect of chemical speciation on toxicity of cadmium to grass shrimp, *Palaemonetes pugio*: importance of free cadmium ion. Environ. Sci. Tech. 12, 409-412.

Tashiro, J., Schmidt, R., Wallhäusser, I., 1994. *Palaemonetes pugio* in the Croton-Hudson confluence: grass shrimp as predators-detritivores and as prey of fish. Final Report. Hudsonia Limited, Bard College Field Station, Annandale-on-Hudson, NY.

Thain, J.E., 1984. Effects of mercury on the prosobranch mollusc *Crepidula fornicata*: acute lethal toxicity and effects on growth and reproduction of chronic exposure. Mar. Environ. Res. 12, 285-309.

Tran, D., Moody, A.J., Fisher, A.S., Foulkes, M.E., Jha, A.N., 2007. Protective effects of selenium on mercury-induced DNA damage in mussel haemocytes. Aquat. Toxicol. 84, 11-18.

Vernberg, W.B., DeCoursey, P.J., Kelly, M., Johns, D.M., 1977. Effects of sublethal concentrations of cadmium under static and flow-through conditions. Bull. Environ. Contam. Toxicol. 17, 16-23.

Vernberg, F.J., Piyatiratitivorakul, S., 1998. Effects of salinity and temperature on the bioenergetics of adult stages of the grass shrimp (*Palaemonetes pugio* Holthius) from the North Inlet Estuary, South Carolina. Estuaries 21, 176-193.

Viarengo, A., Marro, A., Marchi, B., Burlando, B., 2000. Single and combined effects of heavy metals and hormones on lysosomes of haemolymph cells from the mussel *Mytilus* galloprovincialis. 137, 907-912.

Wallace, W.G., Hoexum Brouwer, T.M., Brouwer, M., Lopez, G.R., 2000. Alterations in prey capture and induction of metallothioneins in grass shrimp fed cadmium-contaminated prey. Environ. Toxicol. Chem. 19, 962-971.

Wallace, W.G., Lee, B.G., Luoma, S.N., 2003. Subcellular compartmentalization of Cd and Zn in two bivalves. I. Significance of metal-sensitive fractions (MSF) and biologically-detoxified metal (BDM). Mar. Ecol. Prog. Ser. 249, 183-197.

Wallace, W.G., Lopez, G.R., 1996. Relationship between subcellular cadmium distribution in prey and cadmium trophic transfer to a predator. Estuaries 19, 923-930.

Wallace, W.G., Lopez, G.R., 1997. Bioavailability of biologically sequestered cadmium from an oligochaete to grass shrimp. Mar. Ecol. Prog. Ser. 147, 149-157.

Wallace, W.G., Lopez, G.R., Levinton, J.S., 1998. Cadmium resistance in an oligochaete and its effect on cadmium trophic transfer to an omnivorous shrimp. Mar. Ecol. Prog. Ser. 172, 225-237.

Wallace, W.G., Luoma, S.N., 2003. Subcellular compartmentalization of Cd and Zn in two bivalves. II. Significance of trophically-available metal (TAM). Mar. Ecol. Prog. Ser. 257, 125-137.

Walters, K., Jones, E., Etherington, L., 1996. Experimental studies of predation on metazoans inhabiting *Spartina alterniflora* stems. J. Exp. Mar. Biol. Ecol. 195, 251-265.

Wang, W.-X., Fisher, N.S., 1996. Assimilation of trace elements and carbon by the mussel *Mytilus edulis*: effects of food composition. Limnol. Oceanogr. 41, 197-207.

Wang, W.-X., Fisher, N.S., 1999. Delineating metal accumulation pathways for marine invertebrates. Sci. Total Environ. 237/238, 459-472.

Wang, W.-X., Reinfelder, J.R., Lee, B.-G., Fisher, N.S., 1996. Assimilation and regeneration of trace elements by marine copepods. Limnol. Oceanogr. 41, 70-81.

Ward, A., 2002. Metal contamination in sediments collected from marshes surrounding Staten Island, New York. Master's thesis. College of Staten Island, City University of New York, Staten Island, NY.

Warnau, M., Iaccarino, M., De Biase, A., Temara, A., Jangoux, M., Dubois, P., Pagano, G., 1996. Spermiotoxicity and embryotoxicity of heavy metals in the echinoid *Paracentrotus lividus*. Environ. Toxicol. Chem. 15, 1931-1936.

Welsh, B.L., 1975. The role of grass shrimp, *Palaemonetes pugio*, in a tidal marsh ecosystem. Ecology 56, 513-530.

Widdows, J., Nasci, C., Fossato, V.U., 1997. Effects of pollution on the scope for growth of mussels (*Mytilus galloprovincialis*) from the Venice Lagoon, Italy. Mar. Environ. Res. 43, 69-79.

Wolfe, D.A., Long, E.R., Thursby, G.B., 1996. Sediment toxicity in the Hudson-Raritan Estuary: distribution and correlations with chemical contamination. Estuaries 19, 901-912.

Wood, C.E., 1967. Physioecology of the grass shrimp, *Palaemonetes pugio*, in the Galveston Bay estuarine system. Contrib. Mar. Sci. Univ. TX 12, 54-77.

Yamuna, A., Saravana Bhavan, S., Geraldine, P., 2009. Ultrastructural observations in gills and hepatopancreas of prawn *Macrobrachium malcomsonii* exposed to mercury. J. Environ. Biol. 30, 693-699.

CHAPTER 2

Ahearn, G.A., Zhuang, A., Duerr, J., Pennington, V., 1994. Role of the invertebrate electrogenic $2Na^+/1H^+$ antiporter in monovalent and divalent cation transport. J. Exp. Biol. 196, 319-335.

Amiard, J.-C., Amiard-Triquet, C., Barka, S., Pellerin, J., Rainbow, P.S., 2006. Metallothioneins in aquatic invertebrates: their role in metal detoxification and their use as biomarkers. Aquat. Toxicol. 76, 160-202.

Amouroux, J.M., Cuzon, G., Grémare, A., 1997. Association of pulse chase design, compartmental analysis and analog modeling to assess absorption and assimilation efficiencies in *Penaeus stylirostris* fed an artificial diet. Aquaculture 149, 71-86.

Anderson, M.B., Reddy, P., Preslan, J.E., Fingerman, M., Bollinger, J., Jolibois, L., Maheshwarudu, G., George, W.J., 1997. Metal accumulation in crayfish, *Procambarus clarkii*, exposed to a petroleum-contaminated bayou in Louisiana. Ecotox. Environ. Saf. 37, 267-272.

Bauer, R.T., Abdalla, J.H., 2000. Patterns of brood production in the grass shrimp *Palaemonetes pugio* (Decapoda: Caridea). Invertebr. Reprod. Dev. 38, 107-113.

Bebianno, M.J., Langston, W.J., Simkiss, K., 1992. Metallothionein induction in *Littorina littorea* (Mollusca: Prosobranchia) on exposure to cadmium. J. Mar. Biol. Assoc. UK. 72, 329-342.

Benayoun, G., Fowler, S.W., Oregioni, B., 1974. Flux of cadmium through Euphausiids. Mar. Biol. 27, 205-212.

Campbell, P.G.C., Clearwater, S.J., Brown, P.B., Fisher, N.S., Hogstrand, C., Lopez, G.R., Mayer, L.M., Meyer, J.S., 2005. Digestive physiology, chemistry, and nutrition. In: Meyer, J.S., Adams, W.J., Brix, K.V., Luoma, S.N., Mount, D.R., Stubblefield, W.A., Wood, C.M. (Eds). Toxicity of Dietary Metals to Aquatic Organisms. Society of Environmental Toxicology and Chemistry, Pensacola, FL, pp. 13-57.

Chen, Z., Mayer, L.M., 1998. Digestive proteases of the lugworm, *Arenicola marina*, inhibited by Cu from contaminated sediments. Environ. Toxicol. Chem. 17, 433-438.

Chong, K., Wang, W.-X., 2000. Assimilation of cadmium, chromium, and zinc by the green mussel *Perna viridis* and the clam *Ruditapes philippinarum*. Environ. Toxicol. Chem. 19, 1660-1667.

Couillard, Y., Campbell, P.G.C., Tessier, A., 1993. Response of metallothionein concentrations in a freshwater bivalve (*Anodonta grandis*) along an environmental cadmium gradient. Limnol. Oceanogr. 38, 299-313.

Crawford. D.W., Bonnevie, N.L., Wenning, R.J., 1995. Sources of pollution and sediment contamination in Newark Bay, New Jersey. Ecotoxicol. Environ. Saf. 30, 85-100.

Croteau, M.N., Luoma, S.N., Stewart, A.R., 2005. Trophic transfer of metals along freshwater food webs: evidence of cadmium biomagnification in nature. Limnol. Oceanogr. 50, 1511-1519.

Davis, J.L.D., Metcalfe, W.J., Hines, A.H., 2003. Implications of fluctuating fish predator guilds on behavior, distribution, and abundance of a shared prey species: the grass shrimp *Palaemonetes pugio*. J. Exp. Mar. Biol. Ecol. 293, 23-40.

Decho, A.W., Luoma S.N., 1991. Time-courses in the retention of food material in the bivalves *Potamocorbula amurensis* and *Macoma balthica*: significance to the absorption of carbon and chromium. Mar. Ecol. Prog. Ser. 78, 303-314.

Dijkman, N.A., Kroon, B.M.A., 2002. Indications for chlororespiration in relation to light regime in the marine diatom *Thalassiosira weissflogii*. J. Photochem. Photobiol. B: Biol. 66, 179-187.

Dodson, C.L., 2002. The scintillation counter. In: Slater, R.J. (Ed). Radioisotopes in Biology, 2nd Edition. Oxford University Press, New York, pp. 85-129.

Feng, H., Cochran, J.K., Lwiza, H., Brownawell, B.J., Hirschberg, D.J., 1998. Distribution of heavy metal and PCB contaminants in the sediments of an urban estuary: the Hudson River. Mar. Environ. Res. 45, 69-88.

Fleeger, J.W., Carman, K.R., Webb, S., Hilbun, N., Pace, M.C., 1999. Consumption of microalgae by the grass shrimp *Palaemonetes pugio*. J. Crustacean. Biol. 19, 324-336.

Gillis, C.A., Bonnevie, N.L., Wenning, R.J., 1993. Mercury contamination in the Newark Bay Estuary. Ecotoxicol. Environ. Saf. 25, 214-226.

Goto, D., 2009. Impacts of habitat degradation on *Fundulus heteroclitus* (Linneaus) in urban tidal salt marshes in New York. Ph.D. Dissertation. City University of New York, New York, NY.

Guillard, R.R.L., 1983. Culture of phytoplankton for feeding marine invertebrates. In: Berg, C.J. (Ed). Culture of marine invertebrates. Hutchinson Ross, Stroudsburg, PA, pp. 108-132.

Gunster, D.G., Bonnevie, N.L., Gillis, C.A., Wenning, R.J., 1993. Assessment of chemical loadings to Newark Bay, New Jersey from petroleum and hazardous chemical accidents occurring from 1986 to 1991. Ecotoxicol. Environ. Saf. 25, 202-213.

Hook, S.E., Fisher, N.S., 2001. Reproductive toxicity of metals in calanoid copepods. Mar. Biol. 138, 1141-1152.

Icely, J.D., Nott, J.A., 1992. Digestion and absorption: digestive system and associated organs. In: Harrison, F.W., Humes, A.G. (Eds.). Microscopic anatomy of invertebrates: Decapod, Crustacea. Vol. 10. Wiley-Liss, New York, pp. 147-201.

Johannes, R.E., Satomi, M., 1966. Composition and nutritive value of fecal pellets of a marine crustacean. Limnol. Oceanogr. 11, 191-197.

Klerks, P.L., Levinton, J.S., 1989. Rapid evolution of metal resistance in a benthic oligochaete inhabiting a metal-polluted site. Biol. Bull. 176, 135-141.

Kneib, R.T., 1985. Predation and disturbance by grass shrimp, *Palaemonetes pugio* Holthius, in soft-substratum benthic invertebrate assemblages. J. Exp. Mar. Biol. Ecol. 93, 91-102.

Kraus, M.L., Kraus, D.B., 1986. Differences in the effects of mercury on predator avoidance in two populations of the grass shrimp *Palaemonetes pugio*. Mar. Environ. Res. 18, 277-289.

Kraus, M.L., Weis, J.S., Weis, P., 1988. Effects of mercury on larval and adult grass shrimp (*Palaemonetes pugio*). Arch. Environ. Contam. Toxicol. 17, 355-363.

Levinton, J.S., Suatoni, E., Wallace, W., Junkins, R., Kelaher, B., Allen, R.J., 2003. Rapid loss of genetically based resistance to metals after the cleanup of a Superfund site. Proc. Nat. Acad. Sci. USA 100, 9889-9891.

Mayer, L.M., Weston, D.P., Bock, M.J., 2001. Benzo[a]pyrene and zinc solubilization by digestive fluids of benthic invertebrates – a cross-phyletic study. Environ. Toxicol. Chem. 20, 1890-1900.

Miao, A.-J., Wang, W.-X., 2006. Cadmium toxicity to two marine phytoplankton under different nutrient conditions. Aquat. Toxicol. 78, 114-126.

Morgan, M.D., 1980. Grazing and predation of the grass shrimp *Palaemonetes pugio*. Limnol. Oceanogr. 25, 896-902.

Oey, L.Y., Mellor, G.L., Hires, R.I., 1985. Tidal modeling of the Hudson Raritan Estuary. Estuar. Coast. Shelf. Sci. 20, 511-528.

Paulson, A.J., Sharack, B., Zdanowicz, V., 2003. Trace metals in ribbed mussels from Arthur Kill New York/New Jersey, USA. Mar. Pollut. Bull. 46, 139-152.

Penry, D.L., 1998. Applications of efficiency measurements in bioaccumulation studies: definitions, clarifications, and a critique of methods. Environ. Toxicol. Chem. 17, 1633-1639.

Perez, M.H., Wallace, W.G., 2004. Differences in prey capture in grass shrimp, *Palaemonetes pugio*, collected along an environmental impact gradient. Arch. Environ. Contam. Toxicol. 46, 81-89.

Quinones-Rivera, Z.J., Fleeger, J.W., 2005. The grazing effects of grass shrimp, *Palaemonetes pugio*, on epiphytic microalgae associated with *Spartina alterniflora*. Estuaries 28, 274-285.

Rainbow, P.S., 2002. Trace metal concentrations in aquatic invertebrates: why and so what? Environ. Pollut. 120, 497-507.

Rainbow, P.S., Geffard, A., Jeantet, A.-Y., Smith, B.D., Amiard, J.C., Amiard-Triquet, C., 2004a. Enhanced food-chain transfer of copper-tolerant estuarine worms. Mar. Ecol. Prog. Ser. 271, 183-191.

Rainbow, P.S., Ng, T.Y.-T., Shi, D., Wang, W.-X., 2004b. Acute dietary pre-exposure and trace metal bioavailability to the barnacle *Balanus amphitrite*. J. Exp. Mar. Biol. Ecol. 311, 315-337.

Reinfelder, J.R., Fisher, N.S., 1991. The assimilation of elements ingested by marine copepods. Science 251, 794-796.

Roman, M., 1984. Utilization of detritus by the copepod, *Acartia tonsa*. Limnol. Oceanogr. 29, 949-959.

Seebaugh, D.R., Estephan, A., Wallace, W.G., 2006. Relationship between cadmium assimilation by grass shrimp (*Palaemonetes pugio*) and trophically-available cadmium in amphipod (*Gammarus lawrencianus*) prey. Bull. Environ. Contam. Toxicol 76, 16-23.

Seebaugh, D.R., Wallace, W.G., 2004. Importance of metal-binding proteins in the partitioning of Cd and Zn as trophically available metal (TAM) in the brine shrimp *Artemia franciscana*. Mar. Ecol. Prog. Ser. 272, 215-230.

Silverman, S., Steffans, W.L., Dietz, T.H., 1983. Calcium concretions in the gills of freshwater mussel serve as a calcium reservoir during periods of hypoxia. J. Exp. Zool. 227, 177-189.

Sokal, R.R., Rohlf, F.J., 1995. Biometry: The Principles and Practice of Statistics in Biological Research, 3rd edition. W.H. Freeman and Co., New York.

Steinberg, N., Suszkowski, D.J., Clark, L., Way, J., 2004. Health of the Harbor: The First Comprehensive Look at the State of the NY/NJ Harbor Estuary. A report to the NY/NJ Harbor Estuary Program. Hudson River Foundation, New York.

Vogt, G., 1993. Differentiation of B-cells in the hepatopancreas of the prawn *Penaeus monodon*. Acta Zool. 74, 51-60.

Wallace, W.G., Goto, D., Seebaugh, D.R., 2008. Metal assimilation results from the interaction of prey- and predator-dependent processes. Integrated Environ. Assess. Manag. 4, 375-377.

Wallace, W.G., Hoexum Brouwer, T.M., Brouwer, M., Lopez, G.R., 2000. Alterations in prey capture and induction of metallothioneins in grass shrimp fed cadmium-contaminated prey. Environ. Toxicol. Chem. 19, 962-971.

Wallace, W.G., Lee, B.G., Luoma, S.N., 2003. Subcellular compartmentalization of Cd and Zn in two bivalves. I. Significance of metal-sensitive fractions (MSF) and biologically detoxified metal (BDM). Mar. Ecol. Prog. Ser. 249, 183-197.

Wallace, W.G., Lopez, G.R., 1996. Relationship between subcellular cadmium distribution in prey and cadmium trophic transfer to a predator. Estuaries 19, 923-930.

Wallace, W.G., Lopez, G.R., Levinton, J.S., 1998. Cadmium resistance in an oligochaete and its effect on cadmium trophic transfer to an omnivorous shrimp. Mar. Ecol. Prog. Ser. 172, 225-237.

Wallace, W.G., Luoma, W.G., 2003. Subcellular compartmentalization of Cd and Zn in two bivalves. II. Significance of trophically-available metal (TAM). Mar. Ecol. Prog. Ser. 257, 125-137.

Wang, W.-X., Fisher, N.S., 1996. Assimilation of trace elements and carbon by the mussel *Mytilus edulis*: effects of food composition. Limnol. Oceanogr. 41, 197-207.

Wang, W.-X., Fisher, N.S., 1999a. Delineating metal accumulation pathways for marine invertebrates. Sci. Tot. Environ. 237/238, 459-472.

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

Ward, A., 2002. Metal contamination in sediments collected from marshes surrounding Staten Island, New York. Master's thesis. College of Staten Island, City University of New York, Staten Island, NY.

Weis, J.S., Smith, G., Santiago-Bass, C., Zhou, T., Weis, P., 2001. Effects of contaminants on behavior: biochemical mechanisms and ecological consequences. Bioscience 51, 209-217.

Welsh, B.L., 1975. The role of grass shrimp, *Palaemonetes pugio*, in a tidal marsh ecosystem. Ecology 56, 513-530.

Wolfe, D.A., Long, E.R., Thursby, G.B., 1996. Sediment toxicity in the Hudson-Raritan Estuary: distribution and correlations with chemical contamination. Estuaries 19, 901-912.

Wood, C.E., 1967. Physioecology of the grass shrimp, *Palaemonetes pugio*, in the Galveston Bay estuarine system. Contrib. Mar. Sci. Univ. Tex. 12, 54-77.

CHAPTER 3

Aduayom, I., Campbell, P.G.C., Denizeau, F., Jumarie, C., 2003. Different transport mechanisms for cadmium and mercury in Caco-2 cells: inhibition of Cd uptake by Hg without evidence for reciprocal effects. Toxicol. Appl. Pharmacol. 189, 56-67.

Ahearn, G.A., Zhuang, Z., Duerr, J., Pennington, V., 1994. Role of the invertebrate electrogenic $2Na^+/1H^+$ antiporter in monovalent and divalent cation transport. J. Exp. Biol. 196, 319-335.

Ahearn, G.A., Mandal, P.K., Mandal., A., 2004. Mechanisms of heavy-metal sequestration and detoxification in crustaceans: a review. J. Comp. Physiol. B. 174, 439-452.

Ahrens, M.J., Hertz, J., Lamoureux, E.M., Lopez, G.R., McElroy, A.E., Brownawell, B.J., 2001. The effect of body size on digestive chemistry and absorption efficiencies of food and sediment-bound organic contaminants in *Nereis succinea* (Polychaeta). J. Exp. Mar. Biol. Ecol. 263, 185-209.

Al-Mohanna, S.Y., Nott, J.A., 1986. B-cells and digestion in the hepatopancreas of *Penaeus semisulcatus* (Crustacea: Decapoda). J. Mar. Biol. Assoc. UK. 66, 403-414.

Al-Mohanna, S.Y., Nott, J.A., 1987. R-cells and the digestive cycle in *Penaeus semisulcatus* (Crustacea: Decapoda). Mar. Biol. 95, 129-137.

Amiard, J.-C., Amiard-Triquet, C., Barka, S., Pellerin, J., Rainbow, P.S., 2006. Metallothioneins in aquatic invertebrates: their role in metal detoxification and their use as biomarkers. Aquat. Toxicol. 76, 160-202.

Andersen, J.T., Baatrup, E., 1988. Ultrastructural localization of mercury accumulations in the gills, hepatopancreas, midgut, and antennal glands of the brown shrimp, *Crangon crangon*. Aquat. Toxicol. 13, 309-324.

Anderson, M.B., Reddy, P., Preslan, J.E., Fingerman, M., Bollinger, J., Jolibois, L., Maheshwardu, G., George, W.J., 1997. Metal accumulation in crayfish, *Procambarus clarkii*, exposed to a petroleum-contaminated bayou in Louisiana. Ecotox. Environ. Saf. 37, 267-272.

Andres, S., Laporte, J-M., Mason, R.P., 2002. Mercury accumulation and flux across the gills and the intestine of the blue crab (*Callinectes sapidus*). Aquat. Toxicol. 56, 303-330.

Bauer, R.T., Abdalla, J.H., 2000. Patterns of brood production in the grass shrimp *Palaemonetes pugio* (Decapoda: Caridea). Invertebr. Reprod. Dev. 38, 107-113.

Benayoun, G., Fowler, S.W., Oregioni, B., 1974. Flux of cadmium through Euphausiids. Mar. Biol. 27, 205-212.

Blackmore, G., Wang, W.-X., 2004. Relationships between metallothioneins and metal accumulation in the whelk *Thais clavigera*. Mar. Ecol. Prog. Ser. 277, 135-145.

Blazka, M.E., Shaikh, Z.A., 1991. Differences in cadmium and mercury uptakes by hepatocytes: role of calcium channels. Toxicol. Appl. Pharmacol. 110, 355-363.

Blazka, M.E., Shaikh, Z.A., 1992. Cadmium and mercury accumulation in rat hepatocytes: interactions with other metal ions. Toxicol. Appl. Pharmacol. 113, 118-125.

Blust, R., Kockelbergh, E., Baillieul, M., 1992. Effect of salinity on the uptake of cadmium by the brine shrimp *Artemia franciscana*. Mar. Ecol. Prog. Ser. 84, 245-254.

Bouché, M.-L., Habets, F., Biagianti-Risbourg, S., Vernet, G., 2000. Toxic effects and bioaccumulation of cadmium in the aquatic oligochaete *Tubifex tubifex*. Ecotoxicol. Environ. Saf. 46, 246-251.

Brown, B.E., 1982. The form and function of metal containing 'granules' in invertebrate tissues. Biol. Rev. 57, 621-667.

Campbell, P.G.C., Clearwater, S.J., Brown, P.B., Fisher, N.S., Hogstrand, C., Lopez, G.R., Mayer, L.M., Meyer, J.S., 2005. Digestive physiology, chemistry, and nutrition. In: Meyer, J.S., Adams, W.J., Brix, K.V., Luoma, S.N., Mount, D.R., Stubblefield, W.A., Wood, C.M. (Eds). Toxicity of Dietborne Metals to Aquatic Organisms. Society of Environmental Toxicology and Chemistry, Pensacola, FL, pp. 13-57.

Casartelli, M., Cermenati, G., Rodighiero, S., Pennacchio, F., Giordana, B., 2008. A megalin-like receptor is involved in protein endocytosis in the midgut of an insect (*Bombyx mori*, Lepidoptera). Am. J. Physiol. Regul. Integr. Comp. Physiol. 295, R1290-1300.

Chen, Z., Mayer, L.M., 1998. Digestive proteases of the lugworm, *Arenicola marina*, inhibited by Cu from contaminated sediments. Environ. Toxicol. Chem. 17, 433-438.

Cheung, M., Wang, W.-X., 2005. Influence of subcellular metal compartmentalization in different prey on the transfer of metals to a predatory gastropod. Mar. Ecol. Prog. Ser. 286, 155-166.

Choi, J.S., Kim, K.R., Ahn, D.W., Park, Y.S., 1999. Cadmium inhibits albumin endocytosis in opossum kidney epithelial cells. Toxicol. Appl. Pharmacol. 161, 146-152.

Dall, W., Moriarty, D.J.W., 1983. Functional aspects of nutrition and digestion. In: Bliss, D.E., Mantel, L.H. (Eds.), The Biology of Crustacea: Internal Anatomy and Physiological Regulation, Vol. 5. Academic Press, New York, pp. 215-261.

Davis, J.L.D., Metcalfe, W.J., Hines, A.H., 2003. Implications of fluctuating fish predator guilds on behavior, distribution, and abundance of a shared prey species: the grass shrimp *Palaemonetes pugio*. J. Exp. Mar. Biol. Ecol. 293, 23-40.

Decho, A.W., Luoma S.N., 1991. Time-courses in the retention of food material in the bivalves *Potamocorbula amurensis* and *Macoma balthica*: significance to the absorption of carbon and chromium. Mar. Ecol. Prog. Ser. 78, 303-314.

De Schamphelaere, K.A.C., Janssen, C.R., 2004. Effects of chronic dietary copper exposure on growth and reproduction of *Daphnia magna*. Environ. Toxicol. Chem. 23, 2038-2047.

Endo, T., Kimura, O., Sakata, M., Shaikh, Z.A., 1997. Mercury uptake by LLC-PK_1 cells: dependence on temperature and membrane potential. Toxicol. Appl. Pharmacol. 146, 294-298.

Farag, A.M., Woodward, D.F., Brumbaugh, W., Goldstein, J.N., MacConnell, E., Hogstrand, C., Barrows, F.T., 1999. Dietary effects of metals-contaminated invertebrates from the Coeur d'Alene River, Idaho, on cutthroat trout. Trans. Am. Fish. Soc. 128, 578-592.

Felgenhauer, B.E. 1992. Internal anatomy of the decapoda: an overview. In: Harrison, F.W., Humes, A.G. (Eds.), Microscopic anatomy of invertebrates: Decapod, Crustacea. Vol. 10. Wiley-Liss, New York, pp. 45-75.

Galay Burgos, M., Rainbow, P.S., 1998. Uptake, accumulation and excretion by *Corophium volutator* (Crustacea: Amphipoda) of zinc, cadmium and cobalt added to sewage sludge. Estuar. Coast. Shelf Sci. 47, 603-620.

Gillis, P.L., Dixon, G., Borgmann, U., Reynoldson, T.B., 2004. Uptake and depuration of cadmium, nickel, and lead in laboratory-exposed *Tubifex tubifex* and corresponding changes in the concentration of a metallothionein-like protein. Environ. Toxicol. Chem. 23, 76-85.

Goto, D., Wallace, W.G., 2009. Influences of prey- and predator-dependent processes on cadmium and methylmercury trophic transfer to mummichogs (*Fundulus heteroclitus*). Can. J. Aquat. Sci. 66, 836-846.

Goto, D., Wallace, W.G., 2010. Relative importance of multiple environmental variables in structuring benthic macroinfaunal assemblages in chronically metal-polluted salt marshes. Mar. Pollut. Bull. 60, 363-35.

Griscom, S.B., Fisher, N.S., Luoma, S.N., 2002. Kinetic modeling of Ag, Cd and Co bioaccumulation in the clam *Macoma balthica*: quantifying dietary and dissolved sources. Mar. Ecol. Prog. Ser. 240, 127-141.

Hatch, W.R., Ott, W.L., 1968. Determination of sub-microgram quantities of mercury by atomic absorption spectrophotometry. Anal. Chem. 40, 2085-2087.

Hook, S.E., Fisher, N.S., 2001. Sublethal effects of silver in zooplankton: importance of exposure pathways and implications for toxicity testing. Environ. Toxicol. Chem. 20, 568-574.

Hopkin, S.P., 1989. Ecophysiology of Metals in Terrestrial Invertebrates. Elsevier Applied Science, London.

Hopkin, S.P., Nott, J.A., 1979. Some observations on concentrically structured intracellular granules in the hepatopancreas of the shore crab *Carcinus maenas* (L.). J. Mar. Biol. Assoc. UK, 59, 867-877.

Icely, J.D., Nott, J.A., 1992. Digestion and absorption: digestive system and associated organs. In: Harrison, F.W., Humes, A.G. (Eds.), Microscopic anatomy of invertebrates: Decapod, Crustacea. Vol. 10. Wiley-Liss, New York, pp. 147-201.

Johannes, R.E., Satomi, M., 1966. Composition and nutritive value of fecal pellets of a marine crustacean. Limnol. Oceanogr. 11, 191-197.

Jumars, P.A., 2000. Animal guts as ideal chemical reactors: maximizing absorption rates. Am. Nat. 155, 527-543.

Khoury, J.N., Powers, E., Patniak, P., Wallace, W.G., 2009. Relating disparity in competitive foraging behavior between two populations of fiddler crabs to the subcellular partitioning of metals. Arch. Environ. Contam. Toxicol. 56, 489-499.

Klerks, P.L., Batholomew, P.R., 1990. Cadmium accumulation and detoxification in a Cd-resistant population of the oligochaete *Limnodrilus hoffmeisteri*. Aquat. Toxicol. 19, 97-112.

Kneib, R.T., 1985. Predation and disturbance by grass shrimp, *Palaemonetes pugio* Holthius, in soft-substratum benthic invertebrate assemblages. J. Exp. Mar. Biol. Ecol. 93, 91-102.

Kraus, M.L., Weis, J.S., Weis, P., 1988. Effects of mercury on larval and adult grass shrimp (*Palaemonetes pugio*). Arch. Environ. Contam. Toxicol. 17, 355-363.

Laporte, J.-M., Andres, S., Mason, R.P., 2002. Effects of ligands and other metals on the uptake of mercury and methylmercury across the gills and the intestine of the blue crab (*Callinectes sapidus*). Comp. Biochem. Physiol. C. 131, 185-196.

Mayer, L.M., Weston, D.P., Bock, M.J., 2001. Benzo[a]pyrene and zinc solubilization by digestive fluids of benthic invertebrates – a cross-phyletic study. Environ. Toxicol. Chem. 20, 1890-1900.

Morgan, M.D., 1980. Grazing and predation of the grass shrimp *Palaemonetes pugio*. Limnol. Oceanogr. 25, 896-902.

Penry, D.L., 1998. Applications of efficiency measurements in bioaccumulation studies: definitions, clarifications, and a critique of methods. Environ. Toxicol. Chem. 17, 1633-1639.

Péqueux, A., Bianchini, A., Gilles, R., 1996. Mercury and osmoregulation in the euryhaline crab, *Eriocheir sinensis*. Comp. Biochem. Physiol. C. 113, 149-155.

Posey, M.H., Hines, A.N., 1991. Complex predator-prey interactions within an estuarine benthic community. Ecology. 72, 2155-2169.

Rainbow, P.S., 2002. Trace metal concentrations in aquatic invertebrates: why and so what? Environ. Pollut. 120, 497-507.

Rainbow, P.S., Black, W.H. 2005. Cadmium, zinc and the uptake of calcium by two crabs, *Carcinus maenas* and *Eriocheir sinensis*. Aquat. Toxicol. 72, 45-65.

Rainbow, P.S., Geffard, A., Jeantet, A.-Y., Smith, B.D., Amiard, J.C., Amiard-Triquet, C., 2004a. Enhanced food-chain transfer of copper-tolerant estuarine worms. Mar. Ecol. Prog. Ser. 271, 183-191.

Rainbow, P.S., Ng, T.Y.-T., Shi, D., Wang, W.-X., 2004b. Acute dietary pre-exposure and trace metal bioavailability to the barnacle *Balanus amphitrite*. J. Exp. Mar. Biol. Ecol. 311, 315-337.

Seebaugh, D.R., Estephan, A., Wallace, W.G., 2006. Relationship between cadmium assimilation by grass shrimp (*Palaemonetes pugio*) and trophically-available cadmium in amphipod (*Gammarus lawrencianus*) prey. Bull. Environ. Contam. Toxicol 76, 16-23.

Seebaugh, D.R., Goto, D., Wallace, W.G., 2005. Bioenhancement of cadmium transfer along a multi-level food chain. Mar. Environ. Res. 59, 473-491.

Seebaugh, D.R., Wallace, W.G., 2004. Importance of metal-binding proteins in the partitioning of Cd and Zn as trophically available metal (TAM) in the brine shrimp *Artemia franciscana*. Mar. Ecol. Prog. Ser. 272, 215-230.

Seebaugh, D.R., Wallace, W.G. 2009. Assimilation and subcellular partitioning of elements by grass shrimp collected along an impact gradient. Aquat. Toxicol. 93, 107-115.

Selck, H., Decho, A.W., Forbes, V.E., 1999. Effects of chronic metal exposure and sediment organic matter on digestive absorption efficiency of cadmium by the deposit-feeding polychaete *Capitella* species I. Environ. Toxicol. Chem. 18, 1289-1297.

Shaikh, Z.A., Blazka, M.E., Endo, T., 1995. Metal transport in cells: cadmium uptake by rat hepatocytes and renal cortical epithelial cells. Environ. Health Perspect. 103, 73-75.

Shi, D., Blackmore, G., Wang, W.-X., 2003. Effects of aqueous and dietary preexposure and resulting body burden on silver biokinetics in the green mussel *Perna viridis*. Environ. Sci. Technol. 37, 936-943.

Simkiss, K., 1996. Ecotoxicants at the cell-membrane barrier. In: Newman, M.C., Jagoe, C.H. (Eds.), Ecotoxicology: A Hierarchical Treatment. Lewis Publishers, Boca Raton, FL, pp. 59-83.

Sokal, R.R., Rohlf, F.J., 1995. Biometry: The Principles and Practice of Statistics in Biological Research, 3rd edition. W.H. Freeman and Co., New York.

Sousa, L.G., Cuartas, E.I., Petriella, A.M.., 2005. Fine structural analysis of the epithelial cells in the hepatopancreas of *Palaemonetes argentinus* (Crustacea, Decapoda, Caridea) in intermoult. Biocell 29, 25-31.

Steen Redeker, E., Blust, R. 2004. Accumulation and toxicity of cadmium in the aquatic oligochaete *Tubifex tubifex*: a kinetic modeling approach. Environ. Sci. Technol. 38, 537-543.

Steen Redeker, E., van Campenhout, K., Bervoets, L., Reijnders, H., Blust, R., 2007. Subcellular distribution of Cd in the aquatic oligochaete *Tubifex tubifex*, implications for trophic availability and toxicity. Environ. Pollut. 148, 166-175.

Sunda, W.G., Engel, D.W., Thuotte, R.M., 1978. Effect of chemical speciation on toxicity of cadmium to grass shrimp, *Palaemonetes pugio*: importance of free cadmium ion. Environ. Sci. Tech. 12, 409-412.

United States Environmental Protection Agency, 1999. National recommended water quality criteria – correction. EPA-922-Z-99-001, April 1999.

United States Environmental Protection Agency, 2001. 2001 update of ambient water quality criteria for cadmium. EPA-822-R-01-001, April 2001.

Verri, T., Mandal, A., Zilli, L., Bossa, D., Mandal, P.K., Ingrosso, L., Zonno, V., Vilella, S., Ahearn, G.A., Storelli, C., 2001. D-glucose transport in decapod crustacean hepatopancreas. Comp. Biochem. Physiol. A. 130, 585-606.

Viarengo, A., Marro, A., Marchi, B., Burlando, B., 2000. Single and combined effects of heavy metals and hormones on lysosomes of haemolymph cells from the mussel *Mytilus galloprovincialis*. Mar. Biol. 137, 907-912.

Vidal, D.E., Horne, A.J., 2003a. Inheritance of mercury tolerance in the aquatic oligochaete *Tubifex tubifex*. Environ. Toxicol. Chem. 22, 2130-2135.

Vidal, D.E., Horne, A.J., 2003b. Mercury toxicity in the aquatic oligochaete *Sparganophilus pearsei* II: autotomy as a novel form of protection. Arch. Environ. Contam. Toxicol. 45, 462-467.

Vilella, S., Zilli, L., Ingrosso, L., Schiavone, R., Zonno, V., Verri, T., Storelli, C., 2003. Differential expression of Na^+/D-glucose cotransport in isolated cells of *Marsupenaeus japonicus* hepatopancreas. J. Comp. Physiol. B. 173, 679-686.

Vogt, G., 1993. Differentiation of B-cells in the hepatopancreas of the prawn *Penaeus monodon*. Acta Zool. 74, 51-60.

Wallace, W.G., Hoexum Brouwer, T.M., Brouwer, M., Lopez, G.R., 2000. Alterations in prey capture and induction of metallothioneins in grass shrimp fed cadmium-contaminated prey. Environ. Toxicol. Chem. 19, 962-971.

Wallace, W.G., Lee, B.G., Luoma, S.N., 2003. Subcellular compartmentalization of Cd and Zn in two bivalves. I. Significance of metal-sensitive fractions (MSF) and biologically detoxified metal (BDM). Mar. Ecol. Prog. Ser. 249, 183-197.

Wallace, W.G., Lopez, G.R., 1997. Bioavailability of biologically sequestered cadmium and the implications of metal detoxification. Mar. Ecol. Prog. Ser. 147, 149-157.

Wallace, W.G., Lopez, G.R., Levinton, J.S., 1998. Cadmium resistance in an oligochaete and its effect on cadmium trophic transfer to an omnivorous shrimp. Mar. Ecol. Prog. Ser. 172, 225-237.

Wallace, W.G., Luoma, W.G., 2003. Subcellular compartmentalization of Cd and Zn in two bivalves. II. Significance of trophically-available metal (TAM). Mar. Ecol. Prog. Ser. 257, 125-137.

Wang, W.-X., Fisher, N.S., 1996. Assimilation of trace elements and carbon by the mussel *Mytilus edulis*: effects of food composition. Limnol. Oceanogr. 41, 197-207.

Wang, W.-X., Fisher, N.S., 1999a. Delineating metal accumulation pathways for marine invertebrates. Sci. Tot. Environ. 237-238, 459-472.

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

Weis, J.S., Smith, G., Santiago-Bass, C., Zhou, T., Weis, P., 2001. Effects of contaminants on behavior: biochemical mechanisms and ecological consequences. Bioscience 51, 209-217.

Welsh, B.L., 1975. The role of grass shrimp, *Palaemonetes pugio*, in a tidal marsh ecosystem. Ecology 56, 513-530.

Wicklund Glynn, A., Norrgren, L., Müssener, A., 1994. Differences in uptake of inorganic mercury and cadmium in the gills of the zebrafish, *Brachydanio rerio*. Aquat. Toxicol. 30, 13-26.

Wright, D.A., Welbourn, P.M., 1993. Effects of mercury exposure on ionic regulation in the crayfish *Orconectes propinquus*. Environ. Pollut. 82, 139-142.

Zar, J.H., 1998. Biostatistical Analysis, 4th edition. Prentice Hall, Upper Saddle River,

Zhuang, Z., Ahearn, G.A., 1996. Ca^{2+} transport processes of lobster hepatopancreatic brush-border membrane vesicles. J. Exp. Biol. 199, 1195-1208.

Zhuang, Z., Ahearn, G.A., 1998. Energized Ca^{2+} transport by hepatopancreatic basolateral plasma membranes of *Homarus americanus*. J. Exp. Biol. 201, 211-220.

Zilli, L., Marsigliante, S., Zonno, V., Verri, T., Ahearn, G.A., Storelli, C., Vilella, S., 2000. Identification of calcium channels in B cells of *Penaeus japonicus* hepatopancreas. Comp. Biochem. Physiol. A. 126, S154.

CHAPTER 4

Ahearn, G.A., 1987. Nutrient transport by the crustacean gastrointestinal tract: recent advances with vesicle techniques. Biol. Rev. 62, 45-63.

Ahearn, G.A., Zhuang, Z., Duerr, J., Pennington, V., 1994. Role of the invertebrate electrogenic $2Na^{+}/1H^{+}$ antiporter in monovalent and divalent cation transport. J. Exp. Biol. 196, 319-335.

Ahrens, M.J., Hertz, J., Lamoureux, E.M., Lopez, G.R., McElroy, A.E., Brownawell, B.J., 2001. The effect of body size on digestive chemistry and absorption efficiencies of food and sediment-bound organic contaminants in *Nereis succinea* (Polychaeta). J. Exp. Mar. Biol. Ecol. 263, 185-209.

Ahrens, M.J., Lopez, G.R., 2001. *In vivo* characterization of the gut chemistry of small deposit-feeding polychaetes. In: Aller, J,Y., Woodin, S.A., Aller, R.C. (Eds.), Organism-sediment interactions. University of South Carolina Press, Columbia, SC, pp. 349-368.

Al-Mohanna, S.Y., Nott, J.A., 1986. B-cells and digestion in the hepatopancreas of *Penaeus semisulcatus* (Crustacea: Decapoda). J. Mar. Biol. Assoc. UK. 66, 403-414.

Al-Mohanna, S.Y., Nott, J.A., 1987. R-cells and the digestive cycle in *Penaeus semisulcatus* (Crustacea: Decapoda). Mar. Biol. 95, 129-137.

Al-Mohanna, S.Y., Nott, J.A., 1989. Functional cytology of the hepatopancreas of *Penaeus semisulcatus* (Crustacea: Decapoda) during the moult cycle. Mar. Biol. 101, 535-544.

Al-Mohanna, S.Y., Nott, J.A., Lane, D.J.W., 1985. Mitotic E- and secretory F-cells in the hepatopancreas of the shrimp *Penaeus semisulcatus* (Crustacea: Decapoda). J. Mar. Biol. Assoc. U.K. 65, 901-910.

Altman, D.G., 1991. Practical Statistics for Medical Research. Chapman and Hall/CRC Press, Boca Raton, FL.

Andres, S., Laporte, J-M., Mason, R.P., 2002. Mercury accumulation and flux across the gills and the intestine of the blue crab (*Callinectes sapidus*). Aquat. Toxicol. 56, 303-330.

Beseres, J.J., Lawrence, A.L., Feller, R.J., 2006. Practical equivalence of laboratory and field measurements of gut passage time in two penaeid shrimp species. Mar. Ecol. Prog. Ser. 309, 221-231.

Biagioli, M., Pifferi, S., Ragghianti, M., Bucci, S., Rizzuto, R., Pinton, P., 2008. Endoplasmic reticulum stress and alteration in calcium homeostasis are involved in cadmium-induced apoptosis. Cell Calcium 43, 184-195.

Bock, M.J., Mayer, L.M., 1999. Digestive plasticity of the marine benthic omnivore *Nereis virens*. J. Exp. Mar. Biol. Ecol. 240, 77-92.

Calabrese, E.J., 2005. Paradigm lost, paradigm found: the re-emergence of hormesis as a fundamental dose response model in the toxicological sciences. Environ. Pollut. 138, 378-411.

Campbell, P.G.C., Clearwater, S.J., Brown, P.B., Fisher, N.S., Hogstrand, C., Lopez, G.R., Mayer, L.M., Meyer, J.S., 2005. Digestive physiology, chemistry, and nutrition. In: Meyer, J.S., Adams, W.J., Brix, K.V., Luoma, S.N., Mount, D.R., Stubblefield, W.A., Wood, C.M. (Eds). Toxicity of Dietborne Metals to Aquatic Organisms. Society of Environmental Toxicology and Chemistry, Pensacola, FL, pp. 13-57.

Chavez-Crooker, P., Pozo, P., Castro, H., Dice, M.S., Boutet, I., Tanguy, A., Moraga, D., Ahearn, G.A., 2003. Cellular localization of calcium, heavy metals, and metallothionein in lobster (*Homarus americanus*) hepatopancreas. Comp. Biochem. Physiol. C. 136, 213-224.

Chen, Z., Mayer, L.M., 1998. Digestive proteases of the lugworm, *Arenicola marina*, inhibited by Cu from contaminated sediments. Environ. Toxicol. Chem. 17, 433-438.

Chen, Z., Mayer, L.M., Weston, D.P., Bock, M.J., Jumars, P.A., 2002. Inhibition of digestive enzyme activities by copper in the guts of various marine benthic invertebrates. Environ. Toxicol. Chem. 21, 1243-1248.

Chipps, S.R., 1998. Temperature-dependent consumption and gut-residence time in the opossum shrimp *Mysis relicta*. J. Plankton. Res. 20, 2401-2411.

Córdova-Murueta, J.H., García-Carreño, F.L., Navarette-del-Toro, M.A., 2003. Digestive enzymes present in crustacean feces as a tool for biochemical, physiological, and ecological studies. J. Exp. Mar. Biol. Ecol. 297, 43-56.

Córdova-Murueta, J.H., García-Carreño, F.L., Navarette-del-Toro, M.A., 2004. Effect of stressors on shrimp digestive enzymes from assays of feces: an alternate method of evaluation. Aquaculture 233, 439-449.

Crawford, D.W., Bonnevie, N.L., Wenning, R.J., 1995. Sources of pollution and sediment contamination in Newark Bay, New Jersey. Ecotoxicol. Environ. Saf. 30, 85-100.

Dall, W., Moriarty, D.J.W., 1983. Functional aspects of nutrition and digestion. In: Bliss, D.E., Mantel, L.H. (Eds.), The Biology of Crustacea: Internal Anatomy and Physiological Regulation, Vol. 5. Academic Press, New York, pp. 215-261.

Dam, H.G., Peterson, W.T., 1988. The effect of temperature on the gut clearance rate constant of planktonic copepods. J. Exp. Mar. Biol. Ecol. 123, 1-14.

Davis, J.L.D., Metcalfe, W.J., Hines, A.H., 2003. Implications of fluctuating fish predator guilds on behavior, distribution, and abundance of a shared prey species: the grass shrimp *Palaemonetes pugio*. J. Exp. Mar. Biol. Ecol. 293, 23-40.

De La Ruelle, M., Hajjou, M., Van Herp, F., Le Gal, Y., 1992. Aminopeptidase activity from the hepatopancreas of *Procambarus clarkii*. Biochem. System. Ecol. 20, 331-337.

Divakaran, S., Ostrowski, A.C., 1998. *In vitro* studies of the interaction of phytase with trypsin and amylase extracted from shrimp (*Penaeus vannamei*) hepatopancreas. J. Agric. Food. Chem. 46, 4973-4976.

Engel, D.W., 1987. Metal regulation and molting in the blue crab, *Callinectes sapidus*: metallothionein function in metal metabolism. Biol. Bull. 173, 239-251.

Ezquerra, J.M., García-Carreño, F.L., Civera, R., Haard, N.F., 1997. pH-stat method to predict protein digestibility in white shrimp (*Penaeus vannamei*). Aquaculture 157, 251-262.

Felgenhauer, B.E. 1992. Internal anatomy of the decapoda: an overview. In: Harrison, F.W., Humes, A.G. (Eds.), Microscopic anatomy of invertebrates: Decapod, Crustacea. Vol. 10. Wiley-Liss, New York, pp. 45-75.

Felgenhauer, B.E., Abele, L.G., 1983. Phylogenetic relationships among the shrimp-like decapods (Penaeoidea, Caridea, Stenopodidea). In: Schram, F.R. (Ed.), Crustacean Issues I: Crustacean Phylogeny. Balkema, Rotterdam, pp. 291-311.

Forster, G.R., 1953. Peritrophic membranes in the caridea (Crustacea Decapoda). J. Mar. Biol. Assoc. U.K. 32, 315-318.

Freeman, J.A., Bartell, C.K., 1975. Characterization of the molt cycle and its hormonal control in *Palaemonetes pugio* (Decapod, Caridea). Gen. Comp. Endocrinol. 25, 517-528.

Gibson, R., Barker, P.L., 1979. The decapod hepatopancreas. Oceanogr. Mar. Biol. Ann. Rev. 17, 285-364.

Gillis, C,A,, Bonnevie, N.L., Wenning, R.J., 1993. Mercury contamination in the Newark Bay Estuary. Ecotoxicol. Environ. Saf. 25, 214-226.

Glass, H.J., Stark, J.R., 1994. Protein digestion in the European lobster, *Homarus gammarus* (L.). Comp. Biochem. Physiol. B. 108, 225-235.

Goto, D., 2009. Impacts of habitat degradation on *Fundulus heteroclitus* (Linneaus) in urban tidal salt marshes in New York. Ph.D. Dissertation. City University of New York, New York, NY.

Goto, D., Wallace, W.G., 2010. Relative importance of multiple environmental variables in structuring benthic macroinfaunal assemblages in chronically metal-polluted salt marshes. Mar. Environ. Pollut. 60, 363-375.

Griscom, S.B., Fisher, N.S., Aller, R.C., Lee, B.-G., 2002a. Effects of gut chemistry in marine bivalves on the assimilation of metals from ingested sediment particles. J. Mar. Res. 60, 101-120.

Griscom, S.B., Fisher, N.S., Luoma, S.N., 2002b. Kinetic modeling of Ag, Cd and Co bioaccumulation in the clam *Macoma balthica*: quantifying dietary and dissolved sources. Mar. Ecol. Prog. Ser. 240, 127-141.

Gunster, D.G., Bonnevie, N.L., Gillis, C.A., Wenning, R.J., 1993. Assessment of chemical loadings to Newark Bay, New Jersey from petroleum and hazardous chemical accidents occurring from 1986 to 1991. Ecotoxicol. Environ. Saf. 25, 202-213.

Heath, J.R., Barnes, H., 1970. Some changes in biochemical composition with season and during the moulting cycle of the common shore crab, *Carcinus maenas*. J. Exp. Mar. Biol. Ecol. 5, 199-233.

Hook, S.E., Fisher, N.S., 2001. Sublethal effects of silver on zooplankton: importance of exposure pathways and implications for toxicity testing. Environ. Toxicol. Chem. 20, 568-574.

Hopkin, S.P., Nott, J.A., 1980. Studies on the digestive cycle of the shore crab *Carcinus maenus* (L.) with special reference to the B cells in the hepatopancreas. J. Mar. Biol. Assoc. UK. 60, 891-907.

Hoyt, M., Fleeger, J.W., Siebeling, R., Feller, R.J., 2000. Serological estimation of prey-protein gut-residence time and quantification of meal size for grass shrimp consuming meiofaunal copepods. J. Exp. Mar. Biol. Ecol. 248, 105-119.

Hu, W., Murphy, M.R., Constable, P.D., Block, E., 2007. Dietary cation-anion difference effects on performance and acid-base status in dairy cows postpartum. J. Dairy Sci. 90, 3367-3375.

Icely, J.D., Nott, J.A., 1992. Digestion and absorption: digestive system and associated organs. In: Harrison, F.W., Humes, A.G. (Eds.), Microscopic anatomy of invertebrates: Decapod, Crustacea. Vol. 10. Wiley-Liss, New York, pp. 147-201.

Janssens, T.K.S., Roelofs, D., van Straalen, N.M., 2009. Molecular mechanisms of heavy metal tolerance and evolution in invertebrates. Insect Sci. 16, 3-18.

Johnston, D.J., Yellowlees, D., 1998. Relationship between dietary preferences and digestive enzyme complement of the slipper lobster *Thenus orientalis* (Decapoda: Scyllaridae). J. Crustacean Biol. 18, 656-665.

Jumars, P.A., 2000. Animal guts as ideal chemical reactors: maximizing absorption rates. Am. Nat. 155, 527-543.

King, R.A., Alexander, C.G., 1994. Fluid extraction and circulation in the proventriculus of the banana prawn *Penaeus merguiensis* de man. J. Crustacean Biol. 14, 497-507.

Klerks, P.L., Batholomew, P.R., 1991. Cadmium accumulation and detoxification in a Cd-resistant population of the oligochaete *Limnodrilus hoffmeisteri*. Aquat. Toxicol. 19, 97-112.

Klerks, P.L., Levinton, J.S., 1989. Rapid evolution of metal resistance in a benthic oligochaete inhabiting a metal-polluted site. Biol. Bull. 176, 135-141.

Klerks, P.L., Weis, J.S., 1987. Genetic adaptation to heavy metals in aquatic organisms: a review. Environ. Pollut. 45, 173-205.

Laporte, J.-M., Andres, S., Mason, R.P., 2002. Effects of ligands and other metals on the uptake of mercury and methylmercury across the gills and the intestine of the blue crab (*Callinectes sapidus*). Comp. Biochem. Physiol. C. 131, 185-196.

Le Moullac, G., Klein, B., Sellos, D., Van Wormhoudt, A.N., 1996. Adaptation of trypsin, chymotrypsin, and α-amylase to casein level and protein source in *Penaeus vannamei* (Crustacea Decapoda). J. Exp. Mar. Biol. Ecol. 208, 107-125.

Lemos, D., Ezquerra, J.M., García-Carreño, F.L., 2000. Protein digestion in penaeid shrimp: digestive proteinases, proteinase inhibitors and feed digestibility. Aquaculture 186, 89-105.

Levinton, J.S., Suatoni, E., Wallace, W.G., Junkinsk, R., Kelaher, B., Allen, B.J., 2003. Rapid loss of genetically based resistance to metals after the cleanup of a Superfund site. Proc. Nat. Acad. Sci. 17, 9889-9891.

Lopez G.R., 2005. Diversity of structure and function of the digestive tract in aquatic invertebrates. In: Meyer, J.S., Adams, W.J., Brix, K.V., Luoma, S.N., Mount, D.R., Stubblefield, W.A., Wood, C.M. (Eds.), Toxicity of Dietborne Metals to Aquatic Organisms. Society of Environmental Toxicology and Chemistry, Pensacola, FL, pp. 227-232.

Lopez, G.R., Levinton, J.S., 1987. Ecology of deposit-feeding animals in marine sediments. Q. Rev. Biol. 62, 235-260.

Lorenzon, S., Francese, M., Ferrero, E.A., 2000. Heavy metal toxicity and differential effects on the hyperglycemic stress response in the shrimp *Palaemon elegans*. Arch. Environ. Contam. Toxicol. 39, 167-176.

Lovett, D.L., Felder, D.L., 1990. Ontogeny of kinematics in the gut of the white shrimp *Penaeus setiferus* (Decapoda: Penaeidae). J. Crustacean Biol. 10, 53-68.

Mackie, J.A., Levinton, J.S., Przeslawski, R., DeLambert, D., Wallace, W.G., 2009. Loss of evolutionary resistance by the oligochaete *Limnodrilus hoffmeisteri* to a toxic substance – cost or gene flow? Evolution 64, 152-165.

Martínez, D.E., Levinton, J.S., 1996. Adaptation to heavy metals in the aquatic oligochaete *Limnodrilus hoffmeisteri*: evidence for control by one gene. Evolution 50, 1339-1343.

Mathews, L.M., 2007. Evidence for restricted gene flow over small spatial scales in a marine snapping shrimp *Alpheus angulosus*. Mar. Biol. 152, 645-655.

Mayer, L.M., Chen, Z., Findlay, R.H., Fang, J., Sampson, S., Self, R.F.L., Jumars, P.A., Quetél, C., Donard, O.F.X., 1996. Bioavailability of sedimentary contaminants subject to deposit-feeder digestion. Environ. Sci. Technol. 30, 2641-2645.

Mayer, L.M., Weston, D.P., Bock, M.J., 2001. Benzo[a]pyrene and zinc solubilization by digestive fluids of benthic invertebrates – a cross-phyletic study. Environ. Toxicol. Chem. 20, 1890-1900.

Maynard, D.M., Dando, M.R., 1974. The structure of the stomatogastric neuromuscular system in *Callinectes sapidus*, *Homarus americanus* and *Panulirus argus* (Decapod Crustacea). Phil. Trans. Royal Soc. London. B. Biol. Sci. 268, 161-220.

Meiss, D.E., Norman, R.S., 1977. Comparative study of the stomatogastric system of the decapod Crustacea II: musculature. J. Morphol. 152, 55–76.

Mitane, Y., Aoki, Y., Suzuki, K.T., 1987. Cadmium inhibits protein secretion from cultured rat liver parenchymal cells. Biochem. Pharmacol. 36, 2647-2652.

Molecular Probes, Inc., 2010. Probes useful at near-neutral pH. Molecular Probes Handbook (www.invitrogen.com), section 20.2.

Mugnier, C., Justou, C., 2004. Combined effects of external ammonia and molt stage on the blue shrimp *Litopenaeus stylirostris* physiological response. J. Exp. Mar. Biol. Ecol. 309, 35-46.

Muhlia-Almazán, A., García-Carreño, F.L., 2002. Influence of molting and starvation on the synthesis of proteolytic enzymes in the midgut gland of the white shrimp *Penaeus vannamei*. Comp. Biochem. Physiol. B. 133, 383-394.

Murphy, M.R., 1981. Analyzing and presenting pH data. J. Dairy Sci. 65, 161-163.

Nagano, N., Decamp, O., 2004. Ingestion of a ciliated protozoa by first-feeding larval stage of Pacific white shrimp, *Litopenaeus vannamei* (Boone). Aquaculture Res. 35, 516-518.

Nascarella, M.A., Stoffolano Jr., J.G., Stanek III, E.J., Kostecki, P.T., Calabrese, E.J., 2003. Hormesis and stage specific toxicity induced by cadmium in an insect model, the queen blowfly, *Phormia regina* Meig. Environ. Pollut. 124, 357-262.

Nixon, S,W., Oviatt, C.A., 1973. Ecology of a New England salt marsh. Ecol. Monogr. 43, 463-498.

Odum, E.O., 1985. Trends expected in stressed ecosystems. Bioscience 35, 419-422.

Oey, L.Y., Mellor, G.L., Hires, R.I., 1985. Tidal modeling of the Hudson Raritan Estuary. Estuar. Coast. Shelf. Sci. 20, 511-528.

Parvathy, K., 1970. Blood sugars in relation to chitin synthesis during cutical formation in *Emerita asiatica*. Mar. Biol. 5, 108-112.

Parvathy, K., 1971. Glycogen storage in relation to the moult cycle in the two crustaceans *Emerita asiatica* and *Ligia exotica*. Mar. Biol. 10, 82-86.

Penry, D.L., Jumars, P.A., 1986. Chemical reactor analysis and optimal design. Bioscience 36, 310-315.

Penry, D.L., Jumars, P.A., 1987. Modeling animal guts as chemical reactors. Am. Nat. 129, 69-96.

Penry, D.L., Jumars, P.A., 1990. Gut architecture, digestive constraints and feeding ecology of deposit-feeding and carnivorous polychaetes. Oecologia 82, 1-11.

Perez, M.H., Wallace, W.G., 2004. Differences in prey capture in grass shrimp, *Palaemonetes pugio*, collected along an environmental impact gradient. Arch. Environ. Contam. Toxicol. 46, 81-89.

Powell, R.R. 1974. The functional morphology of the foreguts of the thalassinid crustaceans, *Callinassa californiensis* and *Upogebia pugettensis*. Univ. Calif. Pub. Zool. 102, 1-41.

Relyea, R.A., Auld, J.S., 2004. Having the guts to compete: how intestinal plasticity explains costs of inducible defenses. Ecol. Lett. 7, 869-875.

Rodriguez, A., LeVay, L., Mourente, G., Jones, D.A., 1994. Biochemical composition and digestive enzyme activity in larvae and postlarvae of *Penaeus japonicus* during herbivorous and carnivorous feeding. Mar. Biol. 118, 45-51.

Sabat, P., Riveros, J.M., López-Pinto, C., 2005. Phenotypic flexibility in the intestinal enzymes of the African clawed frog *Xenopus laevis*. Comp. Biochem. Physiol. A. 140, 135-139.

Seebaugh, D.R., Wallace, W.G., 2004. Importance of metal-binding proteins in the partitioning of Cd and Zn as trophically available metal (TAM) in the brine shrimp *Artemia franciscana*. Mar. Ecol. Prog. Ser. 272, 215-230.

Seebaugh, D.R. Wallace, W.G. 2009. Assimilation and subcellular partitioning of elements by grass shrimp collected along an impact gradient. Aquat. Toxicol. 93, 107-115.

Selck, H., Decho, A.W., Forbes, V.E., 1999. Effects of chronic metal exposure and sediment organic matter on digestive absorption efficiency of cadmium by the deposit-feeding polychaete *Capitella* species I. Environ. Toxicol. Chem. 18, 1289-1297.

Shuranova, Z.P., Burmistrov, Y.M., Strawn, J.R., Cooper, R.L., 2006. Evidence for an autonomic nervous system in decapod crustaceans. Int. J. Zool. Res. 2, 242-283.

Siegel, S., Castellan, N.J., 1988. Nonparametric Statistics for Behavioral Sciences, 2nd edition. McGraw-Hill, New York.

Sokal, R.R., Rohlf, F.J., 1995. Biometry: The Principles and Practice of Statistics in Biological Research, 3rd edition. W.H. Freeman and Co., New York.

Sousa, L.G., Cuartas, E.I., Petriella, A.M., 2005. Fine structural analysis of the epithelial cells in the hepatopancreas of *Palaemonetes argentinus* (Crustacea, Decapoda, Caridea) in intermoult. Biocell 29, 25-31.

Sousa, L., Petriella, A.M., 2006. Morphology and histology of *P. argentinus* (Crustacea, Decapoda, Caridea) digestive tract. Biocell 30, 287-294.

Spindler-Barth, M., 1976. Changes in the chemical composition of the common shore crab, *Carcinus maenas*, during the molting cycle. J. Comp. Physiol. 105, 197-205.

Stöcker, W., Wolz, R., Zwilling, R., Strydom, D.J., Auld, D.S., 1988. Astacus protease – a zinc metalloenzyme. Biochem. 27, 5026-5032.

Tashiro, J., Schmidt, R., Wallhäusser, I., 1994. *Palaemonetes pugio* in the Croton-Hudson confluence: grass shrimp as predators-detritivores and as prey of fish. Final Report. Hudsonia Limited, Bard College Field Station, NY.

van Weel, P.B., 1970. Digestion in crustacea. In: Florkin, M., Sheer, B.T. (Eds.), Chemical Zoology. Academic Press, New York, pp. 97-115.

Van Wormhoudt, A., 1974. Variations of the level of the digestive enzymes during the intermolt cycle of *Palaemon serratus*: influence of the season and effect of the eyestalk ablation. Comp. Biochem. Physiol. A. 49, 707-715.

Verri, T., Mandal, A., Zilli, L., Bossa, D., Mandal, P.K., Ingrosso, L., Zonno, V., Vilella, S., Ahearn, G.A., Storelli, C., 2001. D-glucose transport in decapod crustacean hepatopancreas. Comp. Biochem. Physiol. A. 130, 585-606.

Viarengo, A., Marro, A., Marchi, B., Burlando, B., 2000. Single and combined effects of heavy metals and hormones on lysosomes of haemolymph cells from the mussel *Mytilus galloprovincialis*. Mar. Biol. 137, 907-912.

Vilella, S., Zilli, L., Ingrosso, L., Schiavone, R., Zonno, V., Verri, T., Storelli, C., 2003. Differential expression of Na^+/D-glucose cotransport in isolated cells of *Marsupenaeus japonicus* hepatopancreas. J. Comp. Physiol. B. 173, 679-686.

Vogt, G., 1993. Differentiation of B-cells in the hepatopancreas of the prawn *Penaeus monodon*. Acta. Zool. 74, 51-60.

Vogt, G., Stöcker, W., Storch, V., Zwilling, R., 1989. Biosynthesis of *Astacus* protease, a digestive enzyme from crayfish. Histochemistry 91, 373-381.

Wallace, W.G., Hoexum Brouwer, T.M., Brouwer, M., Lopez, G.R., 2000. Alterations in prey capture and induction of metallothioneins in grass shrimp fed cadmium-contaminated prey. Environ. Toxicol. Chem. 19, 962-971.

Wallace, W.G., Lopez, G.R., 1996. Relationship between subcellular cadmium distribution in prey and cadmium trophic transfer to a predator. Estuaries 19, 923-930.

Wallace, W.G., Luoma, W.G., 2003. Subcellular compartmentalization of Cd and Zn in two bivalves. II. Significance of trophically-available metal (TAM). Mar. Ecol. Prog. Ser. 257, 125-137.

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

Welsh, B.L., 1975. The role of grass shrimp, *Palaemonetes pugio*, in a tidal marsh ecosystem. Ecology 56, 513-530.

Zar, J.H., 1998. Biostatistical Analysis, 4th edition. Prentice Hall, Upper Saddle River, NJ.

Zhang, L., Wang, W.-X., 2006. Significance of subcellular metal distribution in prey in influencing the trophic transfer of metals in a marine fish. Limnol. Oceangr. 51, 2008-2017.

Zwilling, R., Neurath, H., 1981. Invertebrate proteases. Methods Enzymol. 80, 633-664.

CHAPTER 5

Bousfield, E.L., 1973. Shallow-water Gammaridean Amphipoda of New England. Comstock, Cornell University Press, Ithaca, NY.

Davis, J.L.D., Metcalfe, W.J., Hines, A.H., 2003. Implications of a fluctuating fish predator guild on behavior, distribution, and abundance of a shared prey species: the grass shrimp *Palaemonetes pugio*. J. Exp. Mar. Biol. Ecol. 293, 22-40.

De La Ruelle, M., Hajjou, M., Van Herp, F., Le Gal, Y., 1992. Aminopeptidase activity from the hepatopancreas of *Procambarus clarkii*. Biochem. Sys. Ecol. 20, 331-337.

Nixon, S.W., Oviatt, C.A., 1973. Ecology of a New England salt marsh. Ecol. Monogr. 43, 463-498.

Perez, M.H., Wallace, W.G., 2004. Differences in prey capture in grass shrimp, *Palaemonetes pugio*, collected along an environmental impact gradient. Arch. Environ. Contam. Toxicol. 46, 81-89.

Rainbow, P.S., 1998. Phylogeny of trace metal accumulation in crustaceans. In: Langston WJ, Bebianno M (Eds.) Metal metabolism in aquatic environments. Chapman and Hall, London, pp. 285-320.

Reinfelder, J.R., Fisher, N.S., Luoma, S.N., Nichols, J.W., Wang, W.-X., 1998. Trace element trophic transfer in aquatic organisms: a critique of the kinetic model approach. Sci. Total Environ. 219, 117-135.

Seebaugh, D.R., Goto, D., Wallace, W.G., 2005. Bioenhancement of cadmium transfer along a multi-level food chain. Mar. Environ. Res. 59, 473-491.

Seebaugh, D.R., Wallace, W.G., 2004. Importance of metal-binding proteins in the partitioning of Cd and Zn as trophically available metal (TAM) in the brine shrimp *Artemia franciscana*. Mar. Ecol. Prog. Ser. 272, 215-230.

Seebaugh, D.R., Wallace, W.G., 2005. Absorption of dietary Cd by grass shrimp, *Palaemonetes* spp., collected along an environmental impact gradient. In: Waldman JR, Nieder WC (Eds.) Final Reports of the Tibor T. Polgar Fellowship Program, 2004, section VIII. Hudson River Foundation, New York, pp. 1-34.

Sokal, R.R., Rohlf, F.J., 1981. Biometry 2nd ed. WH Freeman & Co, New York.

Steele, D.H., Steele, V.J., 1970. The biology of *Gammarus* (Crustacea, Amphipoda) in the northwestern Atlantic. IV. *Gammarus lawrencianus* Bousfield. Can. J. Zoo. 48:1261-1267.

United States Environmental Protection Agency, 2001. 2001 update of ambient water quality criteria for cadmium. EPA-822-R-01-001, April 2001.

Wallace, W.G., Estephan, A., 2004. Differential susceptibility of horizontal and vertical swimming activity to cadmium exposure in a gammaridean amphipod (*Gammarus lawrencianus*). Aquat. Toxicol. 69, 289-297.

Wallace, W.G., Hoexum Brouwer, T.M., Brouwer, M., Lopez, G.R., 2000. Alterations in prey capture and induction of metallothioneins in grass shrimp fed cadmium-contaminated prey. Environ. Toxicol. Chem. 19, 962-971.

Wallace, W.G., Lee, B.G., Luoma, S.N., 2003. Subcellular compartmentalization of Cd and Zn in two bivalves. I. Significance of metal-sensitive fractions (MSF) and biologically detoxified metal (BDM). Mar. Ecol. Prog. Ser. 249, 183-197.

Wallace, W.G., Lopez, G.R., Levinton, J.S., 1998. Cadmium resistance in an oligochaete and its effect on cadmium trophic transfer to an omnivorous shrimp. Mar. Ecol. Prog. Ser. 172, 225-237.

Wallace, W.G., Luoma, S.N., 2003. Subcellular compartmentalization of Cd and Zn in two bivalves. II. Significance of trophically available metal (TAM). Mar. Ecol. Prog. Ser. 257, 125-137.

Wang, W.-X., Fisher, N.S., 1999a. Delineating metal accumulation pathways for marine invertebrates. Sci. Total Environ. 237/238, 459-472.

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

CHAPTER 6

Ahearn, G.A., Zhuang, Z., Duerr, J., Pennington, V., 1994. Role of the invertebrate electrogenic $2Na^{+}/1H^{+}$ antiporter in monovalent and divalent cation transport. J. Exp. Biol. 196, 319-335.

Ahrens, M.J., Hertz, J., Lamoureux, E.M., Lopez, G.R., McElroy, A.E., Brownawell, B.J., 2001. The effect of body size on digestive chemistry and absorption efficiencies of food and sediment-bound organic contaminants in *Nereis succinea* (Polychaeta). J. Exp. Mar. Biol. Ecol. 263, 185-209.

Ahrens, M.J., Lopez, G.R., 2001. In vivo characterization of the gut chemistry of small deposit-feeding polychaetes. In: Aller, J,Y., Woodin, S.A., Aller, R.C. (Eds.), Organism-sediment interactions. University of South Carolina Press, Columbia, SC, pp. 349-368.

Al-Mohanna, S.Y., Nott, J.A., 1986. B-cells and digestion in the hepatopancreas of *Penaeus semisulcatus* (Crustacea: Decapoda). J. Mar. Biol. Assoc. UK. 66, 403-414.

Al-Mohanna, S.Y., Nott, J.A., 1987. R-cells and the digestive cycle in *Penaeus semisulcatus* (Crustacea: Decapoda). Mar. Biol. 95, 129-137.

Al-Mohanna, S.Y., Nott, J.A., Lane, D.J.W., 1985. Mitotic E- and secretory F-cells in the hepatopancreas of the shrimp *Penaeus semisulcatus* (Crustacea: Decapoda). J. Mar. Biol. Assoc. U.K. 65, 901-910.

Altman, D.G., 1991. Practical Statistics for Medical Research. Chapman and Hall/CRC Press, Boca Raton, FL.

Beseres, J.J., Lawrence, A.L., Feller, R.J., 2006. Practical equivalence of laboratory and field measurements of gut passage time in two penaeid shrimp species. Mar. Ecol. Prog. Ser. 309, 221-231.

Bock, M.J., Mayer, L.M., 1999. Digestive plasticity of the marine benthic omnivore *Nereis virens*. J. Exp. Mar. Biol. Ecol. 240, 77-92.

Bouché, M.-L., Habets, F., Biagianti-Risbourg, S., Vernet, G., 2000. Toxic effects and bioaccumulation of cadmium in the aquatic oligochaete *Tubifex tubifex*. Ecotoxicol. Environ. Saf. 46, 246-251.

Campbell, P.G.C., Clearwater, S.J., Brown, P.B., Fisher, N.S., Hogstrand, C., Lopez, G.R., Mayer, L.M., Meyer, J.S., 2005. Digestive physiology, chemistry, and nutrition. In: Meyer, J.S., Adams, W.J., Brix, K.V., Luoma, S.N., Mount, D.R., Stubblefield, W.A., Wood, C.M. (Eds). Toxicity of Dietborne Metals to Aquatic Organisms. Society of Environmental Toxicology and Chemistry, Pensacola, FL, pp. 13-57.

Casalino, E., Calzaretti, G., Sblano, C., Landriscina, C., 2002. Molecular inhibitory mechanisms of antioxidant enzymes in rat liver and kidney by cadmium. Toxicology 179, 37-50.

Chen, Z., Mayer, L.M., 1998. Digestive proteases of the lugworm, *Arenicola marina*, inhibited by Cu from contaminated sediments. Environ. Toxicol. Chem. 17, 433-438.

Chen, Z., Mayer, L.M., Weston, D.P., Bock, M.J., Jumars, P.A., 2002. Inhibition of digestive enzyme activities by copper in the guts of various marine benthic invertebrates. Environ. Toxicol. Chem. 21, 1243-1248.

Córdova-Murueta, J.H., García-Carreño, F.L., Navarette-del-Toro, M.A., 2003. Digestive enzymes present in crustacean feces as a tool for biochemical, physiological, and ecological studies. J. Exp. Mar. Biol. Ecol. 297, 43-56.

Dall, W., Moriarty, D.J.W., 1983. Functional aspects of nutrition and digestion. In: Bliss, D.E., Mantel, L.H. (Eds.), The Biology of Crustacea: Internal Anatomy and Physiological Regulation, Vol. 5. Academic Press, New York, pp. 215-261.

Decho, A.W., Luoma S.N., 1991. Time-courses in the retention of food material in the bivalves *Potamocorbula amurensis* and *Macoma balthica*: significance to the absorption of carbon and chromium. Mar. Ecol. Prog. Ser. 78, 303-314.

De La Ruelle, M., Hajjou, M., Van Herp, F., Le Gal, Y., 1992. Aminopeptidase activity from the hepatopancreas of *Procambarus clarkii*. Biochem. System. Ecol. 20, 331-337.

Ezquerra, J.M., García-Carreño, F.L., Civera, R., Haard, N.F., 1997. pH-stat method to predict protein digestibility in white shrimp (*Penaeus vannamei*). Aquaculture 157, 251 262.

Felgenhauer, B.E. 1992. Internal anatomy of the decapoda: an overview. In: Harrison, F.W., Humes, A.G. (Eds.), Microscopic anatomy of invertebrates: Decapod, Crustacea. Vol. 10. Wiley-Liss, New York, pp. 45-75.

Felgenhauer, B.E., Abele, L.G., 1983. Phylogenetic relationships among the shrimp-like decapods (Penaeoidea, Caridea, Stenopodidea). In: Schram, F.R. (Ed.), Crustacean Issues I: Crustacean Phylogeny. Balkema, Rotterdam, pp. 291-311.

Forster, G.R., 1953. Peritrophic membranes in the caridea (Crustacea Decapoda). J. Mar. Biol. Assoc. U.K. 32, 315-318.

Freeman, J.A., Bartell, C.K., 1975. Characterization of the molt cycle and its hormonal control in *Palaemonetes pugio* (Decapod, Caridea). Gen. Comp. Endocrinol. 25, 517-528.

Gibson, R., Barker, P.L., 1979. The decapod hepatopancreas. Oceanogr. Mar. Biol. Ann. Rev. 17, 285-364.

Glass, H.J., Stark, J.R., 1994. Protein digestion in the European lobster, *Homarus gammarus* (L.). Comp. Biochem. Physiol. B. 108, 225-235.

Griscom, S.B., Fisher, N.S., Luoma, S.N., 2002. Kinetic modeling of Ag, Cd and Co bioaccumulation in the clam *Macoma balthica*: quantifying dietary and dissolved sources. Mar. Ecol. Prog. Ser. 240, 127-141.

Hook, S.E., Fisher, N.S., 2001. Sublethal effects of silver on zooplankton: importance of exposure pathways and implications for toxicity testing. Environ. Toxicol. Chem. 20, 568-574.

Hoyt, M., Fleeger, J.W., Siebeling, R., Feller, R.J., 2000. Serological estimation of prey-protein gut-residence time and quantification of meal size for grass shrimp consuming meiofaunal copepods. J. Exp. Mar. Biol. Ecol. 248, 105-119.

Hu, W., Murphy, M.R., Constable, P.D., Block, E., 2007. Dietary cation-anion difference effects on performance and acid-base status in dairy cows postpartum. J. Dairy Sci. 90, 3367-3375.

Icely, J.D., Nott, J.A., 1992. Digestion and absorption: digestive system and associated organs. In: Harrison, F.W., Humes, A.G. (Eds.), Microscopic anatomy of invertebrates: Decapod, Crustacea. Vol. 10. Wiley-Liss, New York, pp. 147-201.

Johannes, R.E., Satomi, M., 1966. Composition and nutritive value of fecal pellets of a marine crustacean. Limnol. Oceanogr. 11, 191-197.

Johnston, D.J., Yellowlees, D., 1998. Relationship between dietary preferences and digestive enzyme complement of the slipper lobster *Thenus orientalis* (Decapoda: Scyllaridae). J. Crustacean Biol. 18, 656-665.

Khan, F.R., Bury, N.R., Hogstrand, C., 2010. Cadmium bound to metal rich granules and exoskeleton from *Gammarus pulex* causes increased gut lipid peroxidation in zebrafish following single dietary exposure. Aquat. Toxicol. 96, 124-129.

King, R.A., Alexander, C.G., 1994. Fluid extraction and circulation in the proventriculus of the banana prawn *Penaeus merguiensis* de man. J. Crustacean Biol. 14, 497-507.

Lemos, D., Ezquerra, J.M., García-Carreño, F.L., 2000. Protein digestion in penaeid shrimp: digestive proteinases, proteinase inhibitors and feed digestibility. Aquaculture 186, 89-105.

Lopez G.R., 2005. Diversity of structure and function of the digestive tract in aquatic invertebrates. In: Meyer, J.S., Adams, W.J., Brix, K.V., Luoma, S.N., Mount, D.R., Stubblefield, W.A., Wood, C.M. (Eds.), Toxicity of Dietborne Metals to Aquatic Organisms. Society of Environmental Toxicology and Chemistry, Pensacola, FL, pp. 227-232.

Lovett, D.L., Felder, D.L., 1990a. Ontogeny of kinematics in the gut of the white shrimp *Penaeus setiferus* (Decapoda: Penaeidae). J. Crustacean Biol. 10, 53-68.

Lovett, D.L., Felder, D.L., 1990b. Ontogenetic changes in enzyme distribution and midgut function in developmental stages of *Penaeus setiferus* (Crustacea, Decapoda, Penaeidae). Biol. Bull. 178, 160-174.

Mayer, L.M., Chen, Z., Findlay, R.H., Fang, J., Sampson, S., Self., R.F.L., Jumars, P.A., Quetel, C., Donard, O.F.X., 1996. Bioavailability of sedimentary contaminants subject to deposit-feeder digestion. Environ. Sci. Technol. 30, 2641-2645.

Mayer, L.M., Schick, L.L., Self., R.F.L., Jumars, P.A., Findlay, R.H., Chen, Z., Sampson, S., 1997. Digestive environments of benthic macroinvertebrate guts: enzymes, surfactants and dissolved organic matter. J. Mar. Res. 55, 785-812.

Mayer, L.M., Weston, D.P., Bock, M.J., 2001. Benzo[a]pyrene and zinc solubilization by digestive fluids of benthic invertebrates – a cross-phyletic study. Environ. Toxicol. Chem. 20, 1890-1900.

Maynard, D.M., Dando, M.R., 1974. The structure of the stomatogastric neuromuscular system in *Callinectes sapidus*, *Homarus americanus* and *Panulirus argus* (Decapod Crustacea). Phil. Trans. Royal Soc. London. B. Biol. Sci. 268, 161-220.

Mitane, Y., Aoki, Y., Suzuki, K.T., 1987. Cadmium inhibits protein secretion from cultured rat liver parenchymal cells. Biochem. Pharmacol. 36, 2647-2652.

Morgan, M.D., 1980. Grazing and predation of the grass shrimp *Palaemonetes pugio*. Limnol. Oceanogr. 25, 896-902.

Mugnier, C., Justou, C., 2004. Combined effects of external ammonia and molt stage on the blue shrimp *Litopenaeus stylirostris* physiological response. J. Exp. Mar. Biol. Ecol. 309, 35-46.

Muhlia-Almazán, A., García-Carreño, F.L., 2002. Influence of molting and starvation on the synthesis of proteolytic enzymes in the midgut gland of the white shrimp *Penaeus vannamei*. Comp. Biochem. Physiol. B. 133, 383-394.

Murphy, M.R., 1981. Analyzing and presenting pH data. J. Dairy Sci. 65, 161-163.

Nunez-Nogueira, G., Rainbow, P.S., Smith, B.D., 2006. Assimilation efficiency of zinc and cadmium in the decapod crustacean *Penaeus indicus*. J. Exp. Mar. Biol. Ecol. 332, 75-83.

Perez, M.H., Wallace, W.G., 2004. Differences in prey capture in grass shrimp, *Palaemonetes pugio*, collected along an environmental impact gradient. Arch. Environ. Contam. Toxicol. 46, 81-89.

Powell, R.R. 1974. The functional morphology of the foreguts of the thalassinid crustaceans, *Callinassa californiensis* and *Upogebia pugettensis*. Univ. Calif. Pub. Zool. 102, 1-41.

Relyea, R.A., Auld, J.S., 2004. Having the guts to compete: how intestinal plasticity explains costs of inducible defenses. Ecol. Lett. 7, 869-875.

Sabat, P., Riveros, J.M., López-Pinto, C., 2005. Phenotypic flexibility in the intestinal enzymes of the African clawed frog *Xenopus laevis*. Comp. Biochem. Physiol. A. 140, 135-139.

Salazar, M.H., 2003. Biological factors influencing bioaccumulation in bivalves. In: Newman, M.C., Unger, M.A., Fundamentals of Ecotoxicology. Lewis Publishers, Boca Raton, FL, pp. 90-93.

Seebaugh, D.R., Estephan, A., Wallace, W.G., 2006. Relationship between cadmium assimilation by grass shrimp (*Palaemonetes pugio*) and trophically-available cadmium in amphipod (*Gammarus lawrencianus*) prey. Bull. Environ. Contam. Toxicol 76, 16-23.

Seebaugh, D.R., Goto, D., Wallace, W.G., 2005. Bioenhancement of cadmium transfer along a multi-level food chain. Mar. Environ. Res. 59, 473-491.

Seebaugh, D.R., Wallace, W.G., 2004. Importance of metal-binding proteins in the partitioning of Cd and Zn as trophically available metal (TAM) in the brine shrimp *Artemia franciscana*. Mar. Ecol. Prog. Ser. 272, 215-230.

Seebaugh, D.R. Wallace, W.G. 2009. Assimilation and subcellular partitioning of elements by grass shrimp collected along an impact gradient. Aquat. Toxicol. 93, 107-115.

Selck, H., Decho, A.W., Forbes, V.E., 1999. Effects of chronic metal exposure and sediment organic matter on digestive absorption efficiency of cadmium by the deposit-feeding polychaete *Capitella* species I. Environ. Toxicol. Chem. 18, 1289-1297.

Shuranova, Z.P., Burmistrov, Y.M., Strawn, J.R., Cooper, R.L., 2006. Evidence for an autonomic nervous system in decapod crustaceans. Int. J. Zool. Res. 2, 242-283.

Simkiss, K., 1996. Ecotoxicants at the cell-membrane barrier. In: Newman, M.C., Jagoe, C.H. (Eds.), Ecotoxicology: A Hierarchical Treatment. Lewis Publishers, Boca Raton, FL, pp. 59-83.

Sokal, R.R., Rohlf, F.J., 1995. Biometry: The Principles and Practice of Statistics in Biological Research, 3rd edition. W.H. Freeman and Co., New York.

Sousa, L.G., Cuartas, E.I., Petriella, A.M.., 2005. Fine structural analysis of the epithelial cells in the hepatopancreas of *Palaemonetes argentinus* (Crustacea, Decapoda, Caridea) in intermoult. Biocell 29, 25-31.

Sousa, L., Petriella, A.M., 2006. Morphology and histology of *P. argentinus* (Crustacea, Decapoda, Caridea) digestive tract. Biocell 30, 287-294.

Steen Redeker, E., Blust, R. 2004. Accumulation and toxicity of cadmium in the aquatic oligochaete *Tubifex tubifex*: a kinetic modeling approach. Environ. Sci. Technol. 38, 537-543.

Stöcker, W., Wolz, R., Zwilling, R., Strydom, D.J., Auld, D.S., 1988. Astacus protease – a zinc metalloenzyme. Biochem. 27, 5026-5032.

van Weel, P.B., 1970. Digestion in crustacea. In: Florkin, M., Sheer, B.T. (Eds.), Chemical Zoology. Academic Press, New York, pp. 97-115.

Van Wormhoudt, A., 1974. Variations of the level of the digestive enzymes during the intermolt cycle of *Palaemon serratus*: influence of the season and effect of the eyestalk ablation. Comp. Biochem. Physiol. A. 49, 707-715.

Verri, T., Mandal, A., Zilli, L., Bossa, D., Mandal, P.K., Ingrosso, L., Zonno, V., Vilella, S., Ahearn, G.A., Storelli, C., 2001. D-glucose transport in decapod crustacean hepatopancreas. Comp. Biochem. Physiol. A. 130, 585-606.

Vilella, S., Zilli, L., Ingrosso, L., Schiavone, R., Zonno, V., Verri, T., Storelli, C., 2003. Differential expression of Na^+/D-glucose cotransport in isolated cells of *Marsupenaeus japonicus* hepatopancreas. J. Comp. Physiol. B. 173, 679-686.

Vogt, G., Stöcker, W., Storch, V., Zwilling, R., 1989. Biosynthesis of *Astacus* protease, a digestive enzyme from crayfish. Histochemistry 91, 373-381.

Wallace, W.G., Luoma, W.G., 2003. Subcellular compartmentalization of Cd and Zn in two bivalves. II. Significance of trophically-available metal (TAM). Mar. Ecol. Prog. Ser. 257, 125-137.

Wang, W.-X., Fisher, N.S., 1996. Assimilation of trace elements and carbon by the mussel *Mytilus edulis*: effects of food composition. Limnol. Oceanogr. 41, 197-207.

Zar, J.H., 1998. Biostatistical Analysis, 4th edition. Prentice Hall, Upper Saddle River, NJ.

Zhang, L., Wang, W.-X., 2006. Significance of subcellular metal distribution in prey in influencing the trophic transfer of metals in a marine fish. Limnol. Oceangr. 51, 2008-2017.

Zilli, L., Marsigliante, S., Zonno, V., Verri, T., Ahearn, G.A., Storelli, C., Vilella, S., 2000. Identification of calcium channels in B cells of *Penaeus japonicus* hepatopancreas. Comp. Biochem. Physiol. A. 126, S154.

CHAPTER 7

Ahrens, M.J., Hertz, J., Lamoureux, E.M., Lopez, G.R., McElroy, A.E., Brownawell, B.J., 2001. The effect of body size on digestive chemistry and absorption efficiencies of food and sediment-bound organic contaminants in *Nereis succinea* (Polychaeta). J. Exp. Mar. Biol. Ecol. 263, 185-209.

Ahrens, M.J., Lopez, G.R., 2001. *In vivo* characterization of the gut chemistry of small deposit-feeding polychaetes. In: Aller, J,Y., Woodin, S.A., Aller, R.C. (Eds.), Organism-sediment interactions. University of South Carolina Press, Columbia, SC, pp. 349-368.

Al-Mohanna, S.Y., Nott, J.A., 1986. B-cells and digestion in the hepatopancreas of *Penaeus semisulcatus* (Crustacea: Decapoda). J. Mar. Biol. Assoc. UK. 66, 403-414.

Al-Mohanna, S.Y., Nott, J.A., 1987. R-cells and the digestive cycle in *Penaeus semisulcatus* (Crustacea: Decapoda). Mar. Biol. 95, 129-137.

Al-Mohanna, S.Y., Nott, J.A., 1989. Functional cytology of the hepatopancreas of *Penaeus semisulcatus* (Crustacea: Decapoda) during the moult cycle. Mar. Biol. 101, 535-544.

Al-Mohanna, S.Y., Nott, J.A., Lane, D.J.W., 1985. Mitotic E- and secretory F-cells in the hepatopancreas of the shrimp *Penaeus semisulcatus* (Crustacea: Decapoda). J. Mar. Biol. Assoc. U.K. 65, 901-910.

Alon, N.C., Stancyk, S.E., 1982. Variation in life-history patterns of the grass shrimp *Palaemonetes pugio* in two South Carolina estuarine systems. Mar. Biol. 68, 265-276.

Amiard, J.-C., Amiard-Triquet, C., Barka, S., Pellerin, J., Rainbow, P.S., 2006. Metallothioneins in aquatic invertebrates: their role in metal detoxification and their use as biomarkers. Aquat. Toxicol. 76, 160-202.

Andersen, J.T., Baatrup, E., 1988. Ultrastructural localization of mercury accumulations in the gills, hepatopancreas, midgut, and antennal glands of the brown shrimp, *Crangon crangon*. Aquat. Toxicol. 13, 309-324.

Beaty, B.J., Mackie, R.S., Mattingly, K.S., Carlson, J.O., Rayms-Keller, A., 2002. The midgut epithelium of aquatic arthropods: a critical target organ in environmental toxicology. Environ. Health. Perspect. 110, 911-914.

Bell, R.A., Ogden, N., Kramer, J.R., 2002. The biotic ligand model and a cellular approach to class B metal aquatic toxicity. Comp. Biochem. Physiol. C. 133, 175-188.

Bianchini, A., Gilles, R., 2000. Is the digestive tract an important access route for mercury in the Chinese crab *Eriocheir sinensis* (Crustacea, Decapoda)? Bull. Environ. Contam. Toxicol. 64, 412-417.

Borgmann, U., Janssen, C.R., Blust, R.J.P., Brix, K.V., Dwyer, R.L., Erickson, R.J., Hare, L., Luoma, S.N., Paquin, P.R., Roberts, C.A., Wang, W.-X., 2005. Incorporation of dietary metals exposure into regulatory frameworks. In: Meyer, J.S., Adams, W.J., Brix, K.V., Luoma, S.N., Mount, D.R., Stubblefield, W.A., Wood, C.M. (Eds.), Toxicity of Dietborne Metals to Aquatic Organisms, Society of Environmental Toxicology and Chemistry, Pensacola, FL, pp. 153-189.

Campbell, P.G.C., Clearwater, S.J., Brown, P.B., Fisher, N.S., Hogstrand, C., Lopez, G.R., Mayer, L.M., Meyer, J.S., 2005. Digestive physiology, chemistry, and nutrition. In: Meyer, J.S., Adams, W.J., Brix, K.V., Luoma, S.N., Mount, D.R., Stubblefield, W.A., Wood, C.M. (Eds.), Toxicity of Dietborne Metals to Aquatic Organisms, Society of Environmental Toxicology and Chemistry, Pensacola, FL, pp. 13-57.

Cannino, G., Ferruggia, E., Luparello, C., Rinaldi, A.M., 2009. Cadmium and mitochondria. Mitochondrion 9, 377-384.

Chen, Z., Mayer, L.M., 1998. Digestive proteases of the lugworm (*Arenicola marina*) inhibited by Cu from contaminated sediments. Environ. Toxicol. Chem. 17, 433-438.

Chen, Z., Mayer, L.M., Weston, D.P., Bock, M.J., Jumars, P.A., 2002. Inhibition of digestive enzyme activities by copper in the guts of various marine benthic invertebrates. Environ. Toxicol. Chem. 21, 1243-1248.

Chervin, M.B., 1978. Assimilation of particulate organic carbon by estuarine and coastal copepods. Mar. Biol. 49, 265-275.

Croteau, M.N., Luoma, S.N., Stewart, A.R., 2005. Trophic transfer of metals along freshwater food webs: evidence of cadmium biomagnification in nature. Limnol. Oceanogr. 50, 1511-1519.

Dall, W., Moriarty, D.J.W., 1983. Functional aspects of nutrition and digestion. In: Bliss, D.E., Mantel, L.H. (Eds.), The Biology of Crustacea: Internal Anatomy and Physiological Regulation, Vol. 5. Academic Press, New York, pp. 215-261.

Davis, J.L.D., Metcalfe, W.J., Hines, A.H., 2003. Implications of fluctuating fish predator guilds on behavior, distribution, and abundance of a shared prey species: the grass shrimp *Palaemonetes pugio*. J. Exp. Mar. Biol. Ecol. 293, 23-40.

Doughtie, D.G., Rao, K.R., 1983. Ultrastructural and histological study of degenerative changes in the antennal glands, hepatopancreas, and midgut of grass shrimp exposed to two dithiocarbamate biocides. J. Invert. Path. 41, 281-300.

Douhri, H., Sayah, F., 2009. The use of enzymatic biomarkers in two marine invertebrates *Nereis diversicolor* and *Patella vulgata* for the biomonitoring of Tangier's bay (Morocco). Ecotox. Environ. Saf. 72, 394-399.

Downs, C.A., Fauth, J.E., Woodley, C.M., 2001. Assessing the health of grass shrimp (*Palaemonetes pugio*) exposed to natural and anthropogenic stressors: a molecular biomarker system. Mar. Biotech. 3, 380-397.

Duran, R., Ranchou-Peyruse, M., Menuet, V., Monperrus, M., Bareille, G., Goñi, M.S., Salvado, J.C., Amouroux, D., Guyoneaud, R., Donar, O.F.X., Caumette, P., 2008. Mercury methylation by a microbial community from sediments of the Adour Estuary (Bay of Biscay, France). Environ. Pollut. 156, 951-958.

Durou, C., Smith, B.D., Roméo, M., Rainbow, P.S., Mouneyrac, C., Mouloud, M., Gnassia-Barelli, M., Gillet, P., Deutch, B., Amiard-Triquet, C., 2007. From biomarkers to population responses in *Nereis diversicolor*: assessment of stress in estuarine ecosystems. Ecotox. Environ. Saf. 66, 402-411.

Farrell, R.E., Huang, P.M., Germida, J.J., Hammer, U.T., Liaw, W.K., 1991. Influence of mercury (II) speciation on the methylation of mercury by *Pseudomonas fluorescens*. Internationale Vereinigung fuer Theoretische und Angewandte Limnologie. 24, 2190-2198.

Farmanfarmaian, A., 1985. Fractional distribution of $^{203}HgCl_2$ and $CH_3{}^{203}HgCl$ in the intestine of a marine fish. Mar. Environ. Res. 17, 176-180.

Gibson, R., Barker, P.L., 1979. The decapod hepatopancreas. Oceanogr. Mar. Biol. Ann. Rev. 17, 285-364.

Goto, D., Wallace, W.G., 2009a. Influences of pre- and predator-dependent processes on cadmium and methylmercury trophic transfer to mummichogs (*Fundulus heteroclitus*). Can. J. Aquat. Sci. 66, 836-846.

Goto, D., Wallace, W.G., 2009b. Relevance of intracellular partitioning of metals in prey to differential metal bioaccumulation among populations of mummichogs (*Fundulus heteroclitus*). Mar. Environ. Res. 68, 257-267.

Goto, D., Wallace, W.G., 2010. Relative importance of multiple environmental variables in structuring benthic macroinfaunal assemblages in chronically metal-polluted salt marshes. Mar. Environ. Pollut. 60, 363-375.

Griscom, S.B., Fisher, N.S., Luoma, S.N., 2002. Kinetic modeling of Ag, Cd and Co bioaccumulation in the clam *Macoma balthica*: quantifying dietary and dissolved sources. Mar. Ecol. Prog. Ser. 240, 127-141.

Gunster, D.G., Bonnevie, N.L., Gillis, C.A., Wenning, R.J., 1993. Assessment of chemical loadings to Newark Bay, New Jersey from petroleum and hazardous chemical accidents occurring from 1986 to 1991. Ecotoxicol. Environ. Saf. 25, 202-213.

Handy, R.D., Musonda, M.M., Phillips, C., Falla, S.J., 2000. Mechanisms of gastrointestinal copper absorption in the African walking catfish: copper dose-effects and a novel anion-dependent pathway in the intestine. J. Exp. Biol. 203, 2365-2377.

Hook, S., Fisher, N.S., 2001. Sublethal effects of silver on zooplankton: importance of exposure pathways and implications for toxicity testing. Environ. Toxicol. Chem. 20, 568-574.

Hopkin, S.P., Nott, J.A., 1979. Some observations on concentrically structured, intracellular granules in the hepatopancreas of the shore crab *Carcinus Maenas* (L.). J. Mar. Biol. Assoc. U.K. 59, 867-877.

Hopkin, S.P., Nott, J.A., 1980. Studies on the digestive cycle of the shore crab *Carcinus maenus* (L.) with special reference to the B cells in the hepatopancreas. J. Mar. Biol. Assoc. UK. 60, 891-907.

Icely, J.D., Nott, J.A., 1992. Digestion and absorption: digestive system and associated organs. In: Harrison, F.W., Humes, A.G. (Eds.), Microscopic anatomy of invertebrates: Decapod, Crustacea. Vol. 10. Wiley-Liss, New York, pp. 147-201.

Kraus, M.L., Kraus, D.B., 1986. Differences in the effects of mercury on predator avoidance in two populations of the grass shrimp *Palaemonetes pugio*. Mar. Environ. Res. 18, 277-289.

Manyin, T., Rowe, C.L., 2008. Modeling effects of cadmium on population growth of *Palaemonetes pugio*: results of full life cycle exposure. Aquat. Toxicol. 88, 111-120.

Mayer, L.M., Schick, L.L., Self, L.R.F., Jumars, P.A., Findlay, R.H., Chen, Z., Sampson, S, 1997. Digestive environments of benthic macroinvertebrate guts: enzymes, surfactants and dissolved organic matter. J. Mar. Res. 55, 785-812.

Mayer, L.M., Weston, D.P., Bock, M.J., 2001. Benzo[a]pyrene and zinc solubilization by digestive fluids of benthic invertebrates – a cross-phyletic study. Environ. Toxicol. Chem. 20, 1890-1900.

Mortimer, M.R., 2000. Pesticide and trace metal concentrations in Queensland estuarine crabs. Mar. Pollut. Bull. 41, 7-12.

Nixon, S,W., Oviatt, C.A., 1973. Ecology of a New England salt marsh. Ecol. Monogr. 43, 463-498.

Paulson, A.J., Sharack, B., Zdanowicz, V., 2003. Trace metals in ribbed mussels from Arthur Kill, New York/New Jersey, USA. Mar. Pollut. Bull. 46, 139-152.

Perez, M.H., Wallace, W.G., 2004. Differences in prey capture in grass shrimp, *Palaemonetes pugio*, collected along an environmental impact gradient. Arch. Environ. Contam. Toxicol. 46, 81-89.

Reinsel, K.A., Glas, P.S., Rayburn, J.R., Pritchard, M.K., Fisher, W.S., 2001. Effects of food availability on survival, growth and reproduction of the grass shrimp *Palaemonetes pugio*: a laboratory study. Mar. Ecol. Prog. Ser. 220, 231-239.

Riisgard, H.U., Hansen, S., 1990. Biomagnification of mercury in a marine grazing food-chain: algal cells *Phaeodactylum tricornutum,* mussels *Mytilis edulis* and flounders *Platichthys flesus* studied by means of a stepwise-reduction-CVAA method. Mar. Ecol. Prog. Ser. 62, 259-270.

Rowe, C.L., 1998. Elevated standard metabolic rate in a freshwater shrimp (*Palaemonetes paludosus*) exposed to trace element-rich coal combustion waste. Comp. Biochem. Physiol. A. 121, 299-304.

Rowe, C.L., Hopkins, W.A., Zehnder, C., Congdon, J.D., 2001. Metabolic costs incurred by crayfish (*Procambarus acutus*) in a trace element-polluted habitat: further evidence of similar responses among diverse taxonomic groups. Comp. Biochem. Physiol. C. 129, 275-283.

Rudd, J.W.M., Furutani, A., Turner, M.A., 1980. Mercury methylation by fish intestinal contents. App. Environ. Microbiol. 40, 777-782.

Seebaugh, D.R., Goto, D., Wallace, W.G., 2005. Bioenhancement of cadmium transfer along a multi-level food chain. Mar. Environ. Res. 59, 473-491.

Seebaugh, D.R., Wallace, W.G., 2004. Importance of metal-binding proteins in the partitioning of Cd and Zn as trophically-available metal (TAM) in the brine shrimp *Artemia franciscana*. Mar. Ecol. Prog. Ser. 272, 215-230.

Sousa, L.G., Cuartas, E.I., Petriella, A.M.., 2005. Fine structural analysis of the epithelial cells in the hepatopancreas of *Palaemonetes argentinus* (Crustacea, Decapoda, Caridea) in intermoult. Biocell 29, 25-31.

Sousa, L.G., Petriella, A.M., 2007. Functional morphology of the hepatopancreas of *Palaemonetes argentinus* (Crustacea: Decapoda): influence of environmental pollution. Rev. Biol. Trop. 55, 79-86.

Stout, J., 2009. Compensatory partitioning of physiological resource budgets by the grass shrimp (*Palaemonetes pugio*) in association with contaminants encountered in a marsh creek system. Ph.D. Dissertation. Rutgers The State University of New Jersey, New Brunswick, NJ.

Sugita, H., Ueda, R., Berger, L.R., Deguchi, Y., 1987. Microflora in the gut of Japanese coastal crustacea. Nippon Suisan Gakkaishi 53, 1647-1655.

Thomann, R.V., 1981. Equilibrium model of fate of microcontaminants in diverse aquatic food chains. Can. J. Fish. Aquat. Sci. 38, 280-296.

Van Straalen, N.M., Schobben, J.H.M., de Goede, R.G.M., 1989. Population consequences of cadmium toxicity in soil microarthropods. Ecotox. Environ. Saf. 17, 190-204.

Vernberg, F.J., Piyatiratitivorakul, S., 1998. Effects of salinity and temperature on the bioenergetics of adult stages of the grass shrimp (*Palaemonetes pugio* Holthius) from the North Inlet Estuary, South Carolina. Estuaries 21, 176-193.

Viarengo, A., Marro, A., Marchi, B., Burlando, B., 2000. Single and combined effects of heavy metals and hormones on lysosomes of haemolymph cells from the mussel *Mytilus galloprovincialis*. Mar. Biol. 137, 907-912.

Vogt, G., 1997. Hepatopancreatic brush border lysis (HBL) – a new bacterial disease of the shrimp *Palaemon elegans*. Dis. Aquatic. Org. 29, 151-155.

Wallace, W.G., Hoexum Brouwer, T.M., Brouwer, M., Lopez, G.R., 2000. Alterations in prey capture and induction of metallothioneins in grass shrimp fed cadmium-contaminated prey. Environ. Toxicol. Chem. 19, 962-971.

Wallace, W.G., Lee, B.G., Luoma, S.N., 2003. Subcellular compartmentalization of Cd and Zn in two bivalves. I. Significance of metal-sensitive fractions (MSF) and biologically-detoxified metal (BDM). Mar. Ecol. Prog. Ser. 249, 183-197.

Wallace, W.G., Lopez, G.R., Levinton, J.S., 1998. Cadmium resistance in an oligochaete and its effect on cadmium trophic transfer to an omnivorous shrimp. Mar. Ecol. Prog. Ser. 172, 225-237.

Wallace, W.G., Luoma, S.N., 2003. Subcellular compartmentalization of Cd and Zn in two bivalves. II. Significance of trophically-available metal (TAM). Mar. Ecol. Prog. Ser. 257,125-137.

Wang, W.-X., Fisher, N.S., 1996. Assimilation of trace elements and carbon by the mussel *Mytilus edulis*: effects of food composition. Limnol. Oceangr. 41, 197-207.

Wang, W.-X., Fisher, N.S., 1999. Delineating metal accumulation pathways for marine invertebrates. Sci. Total Environ. 237/238, 459-472.

Weis, J.S., Samson, J., Zhou, T., Skurnick, J., Weis, P., 2001. Prey capture ability of mummichogs (*Fundulus heteroclitus*) as a behavioral biomarker for contaminants in estuarine systems. Can. J. Fish. Aquat. Sci. 58, 1442-1452.

Welsh, B.L., 1975. The role of grass shrimp, *Palaemonetes pugio*, in a tidal marsh ecosystem. Ecology 56, 513-530.