BIOACCUMULATION OF TRACE ELEMENTS BY BIVALVES IN THE ALTAMAHA RIVER SYSTEM

by

W. AARON SHOULTS-WILSON

(Under the Direction of Marsha C. Black)

ABSTRACT

Trace elements occur naturally within the Earth's crust but human activity can introduce them into aquatic environments, resulting in elevated concentrations, potentially leading to toxicity. The purpose of this dissertation was to evaluate a biomonitor approach for assessing trace elements in the Altamaha River system. First, bioaccumulation of trace elements in the Asian clam, *Corbicula fluminea,* was compared to that of the co-occurring native mussel, *Elliptio hopetonensis. Corbicula* was shown to accumulate higher concentrations of several potentially toxic trace elements (As, Cd, Cu and Hg) than *E. hopetonensis*. When compared across sites, concentrations of As, Cd, Cu, Hg and Pb were correlated between the two species, supporting *Corbicula's* suitability as an indicator of concentrations in *E. hopetonensis*. A wider survey of bioaccumulation using only *Corbicula* found significant sources of Cd, Cr, Cu, Hg and Zn within the Altamaha system. Controlling for natural variation in environmental and individual parameters eliminated some statistical significance in this survey. Site location in the watershed, dissolved oxygen, sediment composition, concentrations of elements in the sediment and organism length and condition were factors that influenced trace element bioaccumulation. Finally, *E. hopetonensis* shells were sectioned and the trace element profiles of annuli were analyzed using laser ablation inductively coupled plasma-mass spectrometry (LA ICP-MS). Mn was found to accumulate in the shell in a seasonal manner. Using Mn profiles to compare shells from the same site aligned peaks in Pb concentration were between shells. This indicates that freshwater bivalve shells could serve as archives of the local environment. Overall, these findings elucidate some of the aspects needed for a successful and informative biomonitoring program for trace metals using freshwater bivalves, as well as suggesting some future methods of biomonitoring.

INDEX WORDS: Altamaha River, biomonitor, bioaccumulation, *Corbicula fluminea*, arsenic, cadmium, copper, mercury, lead, zinc, laser ablation, freshwater mussel, sediment, water chemistry.

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DEDICATION

In loving acknowledgement of some of my greatest teachers, my grandparents: June Meece (botany); Russell Meece (land management); Bill Wilson (zoology); Mary Wilson (music).

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CHAPTER ONE

INTRODUCTION

The course of evolutionary and human history has rendered potentially toxic trace elements such as arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), lead (Pb) and zinc (Zn) among the hardest contaminants to monitor, mitigate and regulate. Unlike organic xenobiotic contaminants (e.g. pesticides, pharmaceuticals, etc.) potentially toxic trace elements occur naturally within the earth's crust. With xenobiotic pollutants, the ultimate source of contaminants is rarely in question: human beings manufactured these molecules and human activity released them into the environment. On the other hand, certain mineral and soil formations may carry naturally high levels of trace elements. These may leach into local streams and wetlands and be taken up by plants and animals, masking the additions caused by human activities.

Over time xenobiotic molecules decay, being metabolized by higher organisms or degraded by microorganisms into smaller components. Trace elements do not degrade in the same manner. Rather, they are passed from one environmental compartment to another, used as catalytic centers by biomolecules, sequestered in soils and bioinorganic structures, transformed into oxides, sulfides and nitrates and carried on air and waterborne particles: they remain indestructible. Therefore, the pollution of the past continues to haunt us in the present. The large amounts of trace elements belched up by the smokestacks of the industrial revolution leave records in tree rings, peat bogs and stratified layers of sediment (Shotyk et al. 2003, Heim et al. 2004, Abreu et al. 2008). Attributing trace elements to current human activity must contend with the distribution of elements of the geologic past, as well as all the redistributions during human history.

Furthermore, xenobiotics are new chemicals. While their properties may be similar to those of natural molecules, chemical alterations make them more likely to act as toxicants by interacting with biochemical pathways in expected or unexpected manners. With trace elements, the route between exposure and toxicity is less direct. Exposure throughout evolutionary history has resulted in many trace elements (e.g. Ca, Cu, Fe, Mg, Mn, Se, Zn, etc) becoming a vital part of the cellular machinery of living organisms (Silva and Williams 2001). These essential elements are carefully regulated by biochemical mechanisms of uptake, sequestration, accumulation, utilization and elimination in order to maintain ideal concentrations within the tissues of organisms (Newman 1998). In fact, in some instance, trace element deficiency in an environment can be as much or more detrimental to organisms as increased exposure.

A separate class of trace elements has no known biological function (e.g. Cd, Hg, Pb). These non-essential elements can cause toxicity at low levels by disrupting biochemical pathways and tend to accumulate to higher levels than essential elements because of relatively inefficient biochemical detoxification and elimination pathways (Silva and Williams 2001). With the notable exceptions of the non-essential elements, most trace elements are not inherently toxic. However, for many of them (e.g. Ag, Al, As, Cr, Cu, Ni, Se, Sn, Zn) the potential for toxicity is there, if organisms are exposed to higher levels than they are able to cope with.

Therefore, human activities that increase the levels of these trace elements in the environment have the potential to adversely affect all exposed organisms. Currently, human sources constitute large percentages of the trace elements that enter environmental compartments (Callender 2005). Human activities have therefore caused a redistribution of trace elements on a global scale, introducing them into systems where they would naturally exist in small amounts and making them more readily available to organisms by extracting them from normally inaccessible minerals.

In the southeastern United States, industry and urbanization, intensive cotton cultivation and clear cutting have all led to vast shifts in environmental structure in the last two centuries (Merchant 2007). One particular adverse effect of these environmental changes is the endangerment of the world's most diverse assemblage of freshwater mussel species (Williams et al. 1993, Neves 1999, Strayer et al. 2004). As a hotspot for biodiversity, the southeast also holds a large proportion of the nation's endangered or threatened mussel species (Williams et al. 1993). Even as conservation organizations attempt to preserve these species, the extreme drought of 2007 has demonstrated the difficulty in balancing human demands with those of endangered species (AP 2007).

At the crossroads of biodiversity preservation and monitoring of contaminants lies biomonitoring, an attempt to use living organisms as sensors of contamination. Using biomonitors allows investigators to identify increases of contaminants in a biologically relevant way by monitoring an organism's interaction with this contaminant. This is especially important for trace elements, when interactions between contaminant and organism can be subtle and complex. Freshwater bivalves, including both native and invasive species, have long been considered compelling candidates to be used as biomonitor organisms (Doherty 1990, Elder 1991). They could prove especially compelling monitors of pollution in the same areas that hold threatened mussel species, because the physiology is bound to be more similar between two bivalve species than between two less similar organisms.

In this dissertation, I apply the principles of biomonitoring using freshwater bivalves in the Altamaha River, a Georgia watershed serving as habitat for threatened endemic freshwater mussel species (Meador 2008). Chapter Two reviews some of the recent literature dealing with the bioaccumulation of trace elements in freshwater mussels in general and in particular the invasive Asian clam *Corbicula fluminea.* Special attention is given to the use of bivalve shells as archival records of the environment and trace element exposure. In Chapter Three, the use of *Corbicula* bioaccumulation as an indication of trace element contamination is compared to bioaccumulation in a co-occurring native freshwater mussel (*Elliptio hopetonensis*). Species-specific differences in tissue concentrations of trace elements, inter-site correlations between the two species, spatial distribution of elements and potential confounding factors are analyzed and discussed. Sampling over a wider area in Chapter Four, a method using bioaccumulation of trace elements in *Corbicula* to analyze sources of contamination in the Altamaha River watershed is investigated. Linear hierarchical models are used to remove naturally occurring variation in tissue concentrations of trace elements. Finally, in Chapter Five, trace metals in the shells of the freshwater mussel *E. hopetonensis* are analyzed as archival records of environmental conditions and exposure to potentially toxic trace elements.

References

Abreu, S. N., A. M. V. M. Soares, A. J. A. Nogueira, and F. Morgado. 2008. Tree rings, *Populus nigra L.*, as mercury data logger in aquatic environments: Case study of an historically contaminated environment. Bulletin of Environmental Contamination and Toxicology **80**:294-299.

AP. 2007. Georgia's governor declares drought emergency. http://www.msnbc.msn.com/id/21393296/. Associated Press.

Callender, E. 2005. Heavy metals in the environment--historical trends in environmental chemistry, Lollar B.S. ed. 1 edition. Elsevier, Amsterdam, The Netherlands.

Doherty, F. G. 1990. The Asiatic clam, *Corbicula Spp*, as a biological monitor in freshwater environments. Environmental Monitoring and Assessment **15**:143-181.

Elder, J., Collins, JJ. 1991. Freshwater molluscs as indicators of bioavailability and toxicity of metals in surface-water systems. . Reviews Environ Contam Tox **122**:37-79.

Heim, S., J. Schwaubauer, A. Kronimus, R. Littke, C. Woda, and A. Mangini. 2004. Geochronology of anthropogenic pollutants in riparian wetland sediments of the Lippe River (Germany). Organic Geochemistry **35**:1409-1425.

Meador, J. R. 2008. The development and evaluation of a freshwater mussel sampling protocol for a large lowland river. University of Georgia, Athens, GA.

Merchant, C. 2007. American environmental history : an introduction. Columbia University Press, New York.

Neves, R. J. 1999. Conservation and commerce: management of freshwater mussel (Bivalvia : Unionoidea) resources in the United States. Malacologia **41**:461-474.

Newman, M. C. 1998. Fundamentals of ecotoxicology. Ann Arbor Press, Chelsea, MI.

Shotyk, W., M. E. Goodsite, F. Roos-Barraclough, R. Frei, J. Heinemeier, G. Asmund, C. Lohse, and T. S. Hansen. 2003. Anthropogenic contributions to atmospheric Hg, Pb and As accumulation recorded by peat cores from southern Greenland and Denmark dated using the 14C "bomb pulse curve". Geochimica Et Cosmochimica Acta **67**:3991-4011.

Silva, J. J. R. F. d. and R. J. P. Williams. 2001. The biological chemistry of the elements : The inorganic chemistry of life. 2nd edition. Oxford University Press, Oxford ; New York.

Strayer, D. L., J. A. Downing, W. R. Haag, T. L. King, J. B. Layzer, T. J. Newton, and S. J. Nichols. 2004. Changing perspectives on pearly mussels, North America's most imperiled animals. Bioscience **54**:429-439.

Williams, J. D., M. L. Warren, K. S. Cummings, J. L. Harris, and R. J. Neves. 1993. Conservation status of freshwater mussels of the United States and Canada. Fisheries **18**:6-22.

CHAPTER TWO

FRESHWATER MUSSELS (FAMILY UNIONIDAE), ASIAN CLAMS (*CORBICULA FLUMINEA*), AND BIVALVE (CLASS BIVALVIA) SHELLS AS BIOMONITORS OF TRACE ELEMENT CONTAMINATION[1]

Abstract

Freshwater bivalves have received widespread interest as biomonitor organisms of contaminants. This paper reviews recent studies using freshwater bivalves in general and the Asian clam *Corbicula fluminea* in particular as biomonitors of potentially toxic trace element contamination. Individual growth and size, the effects of seasonal fluctuations in temperature and reproductive state, water and sediment chemistry and the differing biological and chemical characteristics of trace elements have been reported to affect how bivalves accumulate trace elements. Fewer attempts have been made to definitively link trace element accumulation to adverse effects at higher levels of biological organization. A new approach to biomonitoring analyzes growth annuli in bivalve shells for archival records of trace element exposure. Further research in the field should focus on 1) explaining the effects of confounding factors such as size and season of collection and how much variation they explain compared to anthropogenic inputs; 2) linking bioaccumulation to adverse effects; and 3) exploring the use of bivalve shells as archives of trace element exposure.

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[1] Shoults-Wilson WA. Portions to be submitted to *Hydrobiologia.*

Introduction

A biomonitor organism is a species that has been chosen to provide indications of the potential impacts of anthropogenic activities on wild populations. Anthropogenic impacts on natural communities are numerous and include alteration of habitat, direct harvest of certain species, disruption of naturally occurring nutrient cycles and the introduction of increased amounts of toxic elements or compounds. Typically, biomonitor organisms are employed to determine the impacts of contaminants such as pesticides, other organic xenobiotic compounds and potentially toxic trace elements that are released into a system due to human activities.

Several criteria must be considered in order to determine the suitability of candidate biomonitor species based on Elder (1991): 1) tolerance to the contaminants in question; 2) mobility, with less mobile organisms preferable; 3) a life span long enough to allow sampling of multiple age-classes; 4) ubiquity and abundance at sites of interest; 5) ability to obtain enough tissue for appropriate analyses; 6) ability to survive sampling and laboratory testing disturbances; 7) ease of identification and sampling; 8) display of a metric of exposure to the contaminant(s) of interest; 9) a link between this metric and metrics of environmental health or special concern (Table 2.1).

One group of organisms that have become common biomonitors in both marine (Farrington 1983) and freshwater (Elder 1991) systems are bivalve species [class Bivalvia (McMahon 2001)]. Bivalves are mollusks, which secrete a calcium carbonate shells in two halves (or valves) with an external hinge. They are primarily filter feeders, drawing water in through a siphon and removing suspended food particles, although they can use their muscular “foot” to sweep food particles into their shell cavity (Reid et al. 1992, McMahon 2001).

The suitability of freshwater bivalves as biomonitors according to the criteria listed previously is summarized in Table 2.1. Briefly, freshwater mussels are large, mostly sedentary organisms, which burrow in the sediment of aquatic systems and move very little even during storm surges (Imlay 1982, Schwalb and Pusch 2007). Mussels in the family Unionidae are typically long lived (up to 20 years maximum age on average), while other freshwater bivalves (such as clams of the family Corbiculidae) have shorter life spans (up to 3-4 years maximum age) (Vaughn and Hakenkamp 2001). They are usually resistant to a wide array of contaminants and can survive short-term disturbances, although these characteristics are species specific (Elder 1991). Freshwater mussels typically colonize aquatic habitat in areas of dense aggregation known as beds, making abundance within a system extremely patchy (Strayer 1999, Morales et al. 2006, Strayer 2008). While they can easily be sampled from wadeable portions of streams, it can be difficult to distinguish certain species (Campbell et al. 2005). The final two criteria (metric of exposure and correlation to environmental health) are the subject of most research on the suitability of bivalve species as biomonitor organisms. Findings vary between studies, species and contaminants of concern.

The purpose of this review is first to summarize research that has investigated the use of freshwater bivalves as biomonitor of a diverse group of potentially toxic contaminants: trace elements (e.g. As, Cd, Cr, Cu Hg, Pb, Zn, etc). Several reviews have previously been published on trace metal accumulation in bivalves, including one focusing on all freshwater mollusks (Elder 1991); another specific to the use of the invasive Asian clam *Corbicula fluminea* (Doherty 1990); another specific to employing bivalve shells to monitor metals (Imlay 1982); and more recently a mini-review devoted to the ecotoxicology of metals on freshwater mussels (Naimo 1995). This review will include elements of all of these reviews and synthesize the issues involving investigating the bioaccumulation of trace elements by freshwater mussels and *Corbicula fluminea* (hereafter *Corbicula*) and the use of mussel shells as biomonitor surrogates. This final section will be given special attention, with a focus on the analysis of growth annuli in bivalve shells for trace element composition.

Overview of Exposure Routes and Bioaccumulation of Trace Elements in Bivalves

A bewildering array of natural processes define how trace elements move in the environment and are eventually accumulated by aquatic organisms. As described in Foster and Charlesworth (1996), trace elements entering fluvial systems are primarily sorbed to particulate matter and sediment, especially very fine sediment. Trace metals in sediments can exist in a variety of chemical states and complexes, which may be more or less available for uptake by benthic organisms (Salomons et al. 1987, Tessier and Campbell 1987, Foster and Charlesworth 1996). Freshwater bivalves, as organisms that actively burrow in sediment and feed on detrital deposits, are more likely than many other organisms to be exposed to trace elements bound to sediments. For instance, Tessier et al. (1984) found that Cu, Pb and Zn bound to iron (Fe) oxides, organic matter and sulfides were best predictive of accumulation in *Elliptio complanata.* Applying a more rigorous approach, Tessier et al. (1993) developed a model for Cd accumulation in *Anodonta grandis* that included Fe oxide, organic matter, pH and Cd concentration in lake sediments as predictors. These biogeochemical models indicate that the major factors affecting bioaccumulation are related to the chemistry of the surficial sediment that provides bivalves with habitat.

Alternatively, the biodynamics concept has been proposed as a means of understanding the effects of four primary factors on bioaccumulation: “metal specificity, environmental influences, exposure route, and species-specific characteristics” (Luoma and Rainbow 2005).

This approach typically uses controlled, laboratory experiments to determine the kinetics of uptake and loss of trace elements via various pathways. These results are used in the dynamic multi-pathway bioaccumulation model (DYM-BAM) to predict the concentration of contaminants in an organism at steady state (C_{SS}):

$$C_{SS} = [(k_u * C_W) + (AE * IR * C_F)] / (k_e + g)$$

Where k_u is a constant of uptake, C_W is the water concentration of the element in questions, AE is a measure of assimilation efficiency from food for that element, IR is a rate of food intake, C_F is the concentration in food of the element of question, k_e is a constant of elimination, and g is the rate of growth of the organism (Luoma and Rainbow 2005).

The DYN-BAM model of accumulation has been applied to a wide variety of aquatic invertebrates, as reviewed and synthesized by Rainbow (2002) and Wang & Fisher (1999). A principle finding from these studies has been that metal accumulation is proportional to the assimilation efficiency (AE) of metals from food (Wang and Fisher 1999). The implication of this finding is that most bioaccumulation of metal occurs from exposure. This is not surprising, considering that the concentrations of metals found in food resources, sediment and particulates are usually orders of magnitude higher than aqueous concentrations.

Most of the biodynamic modeling to date has focused on marine species of bivalves such as the blue mussel *Mytilus edulis* (Wang and Fisher 1999), the clam *Macoma balthica* (Griscom et al. 2002) and the green mussel *Perna viridis* (Shi and Wang 2005). However, the same conclusions appear to hold true for freshwater bivalves. For instance, Roditi & Fisher (1999) reported that the freshwater zebra mussel, *Dreissena polymorpha* accumulate up to 100 times the amount of the elements Ag, C, Cd, Hg and Se from food than water. A further experiment in the field found that *D. polymorpha* accumulates most of its Ag, Cd and Hg from ingested particulate matter and dissolved organic carbon (Roditi et al. 2000b, a).

Tissue Bioaccumulation of Trace Elements by Freshwater Bivalves:

The bioaccumulation of trace elements by freshwater mussels has been more exhaustively reviewed in Elder and Collins (1991), while the use of *Corbicula* as a biomonitor for trace elements was first reviewed by Doherty (1990). Therefore, this paper will review more recent studies using freshwater bivalves (especially *Corbicula*) as biomonitors of trace elements, with a focus on the issues involved in interpreting those results.

Tissue Specific Bioaccumulation

Bioaccumulation of trace metals has been shown to occur at different concentrations in different tissues of freshwater mussels, although the distinct tissues vary from study to study. For instance, Adams et al. (1981) found Cd and Zn concentrated in the gills of transplanted *Amblema perplicata* when those tissues, along with foot, mantle viscera, were analyzed separately. The zebra mussel *D. polymorpha* and a co-occurring mussel *Mytilopsis leucophaeta* have been found to accumulate more Cd, Cu and Zn in the byssus (proteinaceous threads used to attach to hard substrate) than the soft body tissue or shell (Van der Velde 1992). Gundacker (2000) divided *Unio pictorum* and *Anodonta* sp. into viscera, gill, mantle, adductor muscle and shell. The gills of both species had some of the highest concentrations of Cd, Cu, Pb and Zn, while the shell tended to have the lowest concentrations of these elements.

Investigations into the tissue-specific accumulation of trace elements in *Corbicula* have been limited and primarily investigated in a laboratory environment, probably due to the small size of the organisms. In a study that combined water and sediment exposures, inorganic and methylated Hg and Cd predominantly accumulated in the viscera and gills, rather than the mantle, foot or kidney tissues (Inza et al. 1997). Similar results were obtained in another laboratory study (Baudrimont et al. 1997) but with the gills showing higher accumulation of Cd and Hg, followed by viscera and the other tissues. These studies both indicate that *Corbicula* probably accumulates trace elements in a manner similar to freshwater mussels, with gills accumulating higher concentrations, although mussel viscera have not been shown to have significantly higher concentrations of Cd, Pb, or Zn (Adams et al. 1981, Gundacker 2000). Conners et al. (1999) reported Pb concentrations in *Corbicula* shells up to 89% greater than that of the adductor muscle and foot tissues. Such high concentrations in shells contradicts to the findings of Gundacker (2000) who discerned no significant difference in Pb concentrations between shells and adductor muscles in two freshwater mussels. This discrepancy may be explained by the difference between controlled laboratory experiments (Sturresson 1976, Almeida et al. 1998, Conners 1999) that have regularly reported Pb concentrations in bivalve shells 1 to 2 orders of magnitudes higher than those found by field studies (Bourgoin and Risk 1987, Bourgoin 1990, Gundacker 2000). In other words, a controlled laboratory environment introducing environmental media enriched in trace elements may influence the distribution of those elements in the tissues of biomonitor organisms. One possible explanation is that the coping mechanisms used by bivalves to survive acute doses of trace elements may differ from those used in dealing with the chronic exposure that is more common in the natural environment. Furthermore, it may be related to the preference in laboratory studies for aqueous exposure, while most environmental accumulation is related to dietary exposure.

A little understood aspect of trace element bioaccumulation is the effect that the presence of other trace elements has on mechanisms of accumulation. Studies into tissue-specific distributions of trace elements have touched on this issue, which is undoubtedly a factor in the environment where all trace elements naturally occur, although usually absent in most laboratory studies which are often concerned with only a single element. A 1986 laboratory study (Cassini et al.) found Cd exposure to increase Zn concentration in the gills of *Anodonta cygnea* but not *Unio elongatulus.* Other studies suggest that rather than Cd affecting Zn accumulation, Zn exerts an antagonistic effect on Cd accumulation, not surprising given that both elements favor a 2+ oxidation state and have a similar ionic radius. The conclusions of Hemelraad et al. (1987) indicate that *A. cygnea* exposed to Zn as well as Cd accumulated lower concentrations of Cd overall, specifically in the gills, with slightly higher levels of Cd accumulated in the midgut. A more recent study reached similar conclusions for *D. polymorpha* and *Corbicula*, with Zn/Cd mixtures causing the bivalves to accumulate lower concentrations of Cd than exposure to Cd-only solution (Marie et al. 2006). These studies demonstrate just one of the many interactions that may occur between trace elements in the environment.

Seasonal Differences in Accumulation

It is necessary to consider the season of collection when using freshwater bivalves as monitors of trace elements. It is commonly assumed that seasonal changes in organism tissue mass (caused by the reproductive cycle) and environmental concentrations of trace metals (caused by high flow events) will result in different tissue concentrations of trace elements (Abaychi and Mustafa 1988, Luoma et al. 1990). However Couillard et al. (1995b) found during the course of a year-long transplant study using *Pyganodon grandis* that while the mussels' condition (tissue dry wt / shell wt) changed in a seasonal manner, tissue concentrations of Cd and Zn were more closely related to environmental concentrations of those metals. A study using the Brazilian mussel *Anodontites trapesialis* found seasonal differences in the concentration of Cd and Pb only at two sites (Tomazelli et al. 2003). Again, point source proximity was more important in explaining Pb accumulation. Finally, while Angelo et al. (2007) found evidence of seasonally variable water and fine sediment concentrations of Cd, Pb and Zn caused by high-flow events, they concluded that these affected metal accumulation less than anthropogenic inputs when compared across a wide area.

Seasonality has also been implicated as a factor influencing the accumulation of trace elements in *Corbicula*. This is a reasonable assumption, since *Corbicula* only feed and grow for a certain portion of the year when the water temperature is above 14° C (Joy 1985). Studies in artificial streams have shown increased accumulation of Cd with increased temperature (Graney et al. 1984). Biodynamic models for *Corbicula* indicate that it accumulates Cu primarily from dietary sources (Croteau et al. 2004, Croteau and Luoma 2005). Therefore the biodynamic theory predicts seasonal differences in accumulation based on metabolic activity and sources of food, such as seasonal algal blooms.

Actual field verification of seasonality in trace metal bioaccumulation has been more conclusive for *Corbicula* than for other freshwater mussel species. Abaychi and Mustafa (1988) found temporal variability in the concentrations of Cd, Cu, Fe, Mn, Ni, Pb, V and Zn in water, suspended particles and *Corbicula* tissue. However, they did not determine the statistical significance of this fluctuation or the causal agent, limiting the strength of their conclusions. In a 3-year study in Suisun Bay, Luoma et al. (1990) showed seasonal fluctuations in *Corbicula* condition (calculated dry weight of a 3.5 cm clam) and Cd, Cr, Cu and Zn in both *Corbicula* and fine sediment. They found a correlation between Cd and Cu with the flow regime of the Sacramento River but determined that temporal change in Cr accumulation was driven by emissions from a local steel plant. They recommended temporally intensive monitoring, with yearly averages being used to determine spatial trends in trace element contamination.

Relations Between Trace Element Accumulation and Bivalve Age and/or Size

Another oft-sited consideration in freshwater bivalve biomonitor studies is the size or age of the organism (Millington 1983, Elder 1991). Studies using freshwater mussels that address this factor are aptly summarized in Metcalfe-Smith et al. (1996). Few general conclusions can be drawn about the relation between mussel size and metal accumulation. Metcalfe-Smith et al. concluded that size and age should be included when designing a monitoring program but that distinctions should be made between "contaminated" and "clean" sites, which will show different ranges of accumulation. They also suggested that condition (tissue dry weight / shell volume) serves as a better indicator of size than shell length for many elements (e.g. Cd, Cu, Mn and Pb). However, Perceval et al. (2006) found that age was not a good indicator of metal accumulation in *P. grandis.*

With regards to shell length as a variable affecting accumulation, broad conclusions are also difficult to draw. Naimo et al. (1992) found Zn to be significantly concentrated and Cu to be significantly less concentrated in larger individuals of *Amblema plicata plicata.* However, Cd and Hg accumulation showed different relationships to growth depending on the sampling site. A potential confounding factor in the results presented by this study was their use of size classes, which essentially transformed length from a continuous variable into a discrete variable. They also did not allow mussels to depurate before dissection, which meant that they measured metals sorbed to gut contents as well as those assimilated into tissue. A similarly limited study found a larger size class of *Mytilaster lineatus* to accumulate significantly higher concentrations of Cu and lower concentrations of Pb than a smaller size class (Pourang 1996).

Comparing size classes to determine correlations between accumulation and length has also been used in studies utilizing *Corbicula* as a biomonitor, probably to facilitate pooling of tissues in for analysis. The size class approach was used by Abaychi and Mustafa (1988) to conclude that smaller *Corbicula* accumulated significantly higher concentrations of Cu, Ni, Pb and Zn as well as lower concentrations of Cd. In clams sampled from the Rio de lat Plata (Argentina), Bilos et al. (1998) found larger size classes to accumulate higher concentrations of Cu and lower concentrations of Zn. Sebesvari et al. (2005) showed a positive correlation between size class and Sn accumulation but not As accumulation. In their study of a mining-impacted watershed, Angelo et al. (2007) found lower concentrations of Cd in smaller size classes but larger concentrations of Pb. Comparing size classes from a relatively uncontaminated site to data from more contaminated sites, they concluded that environmental factors, rather than individual clam size, accounted for more spatial variation.

The problem with assessing the effects of organism size using size classes is that *Corbicula* have been shown to spawn in distinct, homogenously sized cohorts in certain systems (Stites et al. 1995). In other words, effects on accumulation implied to be a function of growth may be a function of cohort age. Because *Corbicula* grow at different rates under different conditions, size classes cannot easily be applied across multiple sites where cohorts may grow at different rates (i.e. this would involve comparing older, slower growing cohorts to younger, faster growing cohorts). Using lengths and tissue concentrations of individuals, Peltier et al. (2008) found a significant negative relationship between length and Cd accumulation but no correlation with As, Cu, Hg, Se or Zn. Because length only explained 3-9% of variation in trace element concentrations, they concluded that land cover was a better predictor. Clearly, more work needs to be done in examining the relationship between size and the accumulation of trace elements. Advanced analytical techniques can analyze individual organisms, allowing size to be used as a continuous, rather than a discrete variable. Removing the variability related to size from bioaccumulation data will ensure that erroneous conclusions are not drawn about the relative contamination of different sites.

Relating Bioaccumulation to Water and Sediment Sources of Trace Elements

A central idea behind many biomonitoring efforts is that biomonitor organisms take up trace elements from the environment and thus their levels of bioaccumulation can be correlated to concentrations of that element in the local environment. This approach has been employed to some extent with various freshwater mussels. Adams et al. (1981) found *A. perplicata* to accumulate higher concentrations of Zn and Cd at sites with higher concentrations of those elements in the sediments. *D. polymorpha* from 20 sites on the Mosel River showed no correlation between tissue and water concentrations of Cd, Cr, Cu and Zn but did show a correlation for Pb (Mersch 1992). Five sites in the Mirgenbach reservoir showed a tissue/water correlation with Cu but with none of the other metals. Looking at several tissues and several environmental compartments, Gundacker (2000) correlated tissue concentrations of Pb and Zn in *Anodonta* to fine (< 23 μm) sediments, and Cd in *U. pictorum* tissues to concentrations in the sediments. The general trend seen in data gathered from freshwater mussels is for stronger correlations between bioaccumulated trace elements and the concentrations in local sediments, rather than those in ambient waters at the time of sampling.

Many more biomonitoring studies of this type have been conducted using *Corbicula*, with many of them reviewed by Doherty (1990). For example, laboratory studies have demonstrated that *Corbicula* can accumulate Cd, Cu and Zn from water exposure in a dose-dependent manner (Graney et al. 1983); accumulate Cu from water in a dose-dependent manner (Harrison et al. 1984); accumulate Pb but not Cd from sediments (Tatem 1986); and accumulate Cd and Hg from the water column in a dose-dependent manner (Inza et al. 1998). Most studies carried out in aquaria and artificial streams tend to focus on the overlying water column as the primary source of metals. Taking this approach is complicated by the natural response of *Corbicula* to contaminants, which is to close valves and lower respiration in order to limit exposure (Doherty et al. 1987). In other words, *Corbicula* will bioaccumulate trace elements in a manner that is more likely to reflect chronic exposure to trace elements rather than brief exposure. As a longer term depository of trace elements, sediments are more commonly correlated to *Corbicula* tissues in field studies (Foster and Charlesworth 1996).

In a field study on the Apalachicola River, Elder and Mattraw (1984) found a significant correlation between fine sediment and *Corbicula* tissue concentrations for As but not for Cd, Cr, Cu, Pb or Zn. This finding contradicts several studies and is most likely due to Elder and Mattraw's difficulty in detecting elements in *Corbicula* tissues. They also collected *Corbicula* and sediments from separate, nearby sites but treated them as though they came from the same site. More recently, Villar et al. (1999) concluded that Cd accumulation in *Corbicula* and *Limnoperna fortunei* was linked with sediment loads, while Cu and Zn accumulation was not related to sediment concentrations. Andrès et al. (1999) found that Cd accumulated in relation to the amount of Cd in the water column but that Zn showed no similar correlation. A number of other studies previously discussed in this review have shown poor agreement between *Corbicula* and water Cd, Cu, Fe, Mn, Ni, Pb, V and Zn (Abaychi and Mustafa 1988); apparent, but non-significant, correlations between sediment and *Corbicula* Cd, Cr, Cu, Pb and Zn (Luoma et al. 1990); and stronger relationships between bioaccumulation and free ion concentrations (calculated from sediment) for Cd than for Cu and Zn (Perceval et al. 2006). A repeated finding in field surveys of *Corbicula* is that trace element concentrations in clam tissues are more likely to be correlated to sediment concentrations than water. However, essential metals such as Cu and Zn seem to be less likely to show a correlation between tissue and environmental compartments than nonessential elements such as Cd. This is not surprising, considering the higher biochemical controls that are exerted on Cu and Zn concentrations. Conclusions on the environmental compartments that can be linked to bioaccumulation must therefore be made on an element-by-element basis.

Relating Bioaccumulation to Adverse Effects

The ultimate goal for biomonitoring studies it to relate metrics that are analyzed (e.g. tissue concentrations of a metal) with both organism impairment and human activity. In this way, the amount of impairment caused by human activities can be quantified and monitored. Attempts to connect mussel bioaccumulation of metals to deleterious effects in the environment have met with mixed results. For instance, while Vinot & Pihan (2005) found that *D. polymorpha* accumulated Cu from algae and transferred it to molluscivorous fish, they could only speculate on potential ecotoxicological effects. Using *P. grandis*, Couillard et al. (1995a) attempted to link bioaccumulation to all levels of organization. Their study examined biomarkers (cellular level), growth (individual level) and reproductive (population level) endpoints in *P. grandis* with increased Cd and Zn accumulation. They found increased oxidative stress and decreased growth with metal accumulation but could not unambiguously make conclusions at the population level because of the difference in response between transplanted and local mussels (Couillard et al. 1995a). A later study using the same protocol found that the calculated free ion of Cd^{2+} could be correlated to decreased population and fecundity endpoints (Perceval et al. 2004). However, much of the variation in Cd^{2+} could be explained by environmental factors such as dissolved organic matter and degree days (a function of time that varies by temperature), thus indicating that lake morphometry could be structuring populations more than Cd contamination. A 13-year study in the same smelter-impacted region of Canada found that metal accumulation in *P. grandis* decreased as smelter emissions decreased (Perceval et al. 2006) proving its effectiveness as a biomonitor of smelter pollution. Finally, in one of the more convincing studies, Angelo et al. (2007) found that local extirpation or decreased richness of native mussel assemblages was correlated to higher levels of Cd, Pb and Zn in mussel tissues and fine sediments.

These studies demonstrate the difficulties in initiating and interpreting the results of a biomonitoring program based on the bioaccumulation of trace elements by mussels. In addition to the considerations already discussed, numerous papers have noted differences in accumulation between different mussel species sampled at the same site (Van der Velde 1992, Metcalfe-Smith et al. 1996, Gundacker 2000, Angelo et al. 2007). Because freshwater species can have fairly restricted ranges, it can be difficult to compare the results of one biomonitor to another in locations where different species overlap.

As with freshwater mussels, few studies have attempted to link trace metal bioaccumulation to endpoints that demonstrate a detrimental effect to *Corbicula*. Returning to Luoma et al. (1990), this study correlated decreased condition (calculated dry weight of a 3.5 cm individual at a certain sampling site/date) with Cd and Cu in sediment and *Corbicula* tissue. Paggi et al. (2006) examined the entire benthic community in a contaminant plume and found *Corbicula* to be a sensitive species to Cr pollution but did not measure bioaccumulation. Other studies have found *Corbicula* survival and growth to react to inputs of acid mine drainage and nutrients (Soucek et al. 2001), as well as impacts from other coal-related activities such as mining, burning, and waste disposal (Hull et al. 2006). In these studies, bioaccumulation of trace metals was not measured, although contaminant sources were likely to contain increased loads of trace elements. There is a large gap in our understanding between the bioaccumulation of trace elements by biomonitor bivalves and the effects on the health of the aquatic system the biomonitor organism represents.

Bioaccumulation of Trace Elements in the Shell Annuli of Bivalves

The calcium carbonate shells of freshwater bivalves have also been described as potentially useful in the biomonitoring of trace element contamination (Imlay 1982). As they grow, bivalves secrete sequential layer of shell to contain their increasing tissue mass. In many climates, shell growth occurs in an annual fashion, with most growth occurring during periods of the warmest water temperatures (Dunca et al. 2005). The patterns laid down by these layers are typically used to age the bivalves, with sectioning the shell to reveal internal growth rings considered the most accurate method (Neves and Moyer 1988). During shell growth, many trace elements are incidentally incorporated into the shells of bivalves (Imlay 1982), making them suitable as components of a biomonitoring study. More so than tissues, shells could theoretically serve as long-term records of trace element exposure.

Early investigations into the trace metal accumulation in freshwater mussels are reviewed thoroughly by Imlay (1982). These studies mainly used whole-shell analysis for metal concentration, an approach that has not been wholly abandoned. Studies previously mentioned in this study (Van der Velde 1992, Gundacker 2000) compared the trace element concentrations in tissues of mussels collected in the field to concentrations in shells. In all instances, the shell was found to accumulate (in some cases significantly) lower concentrations of trace elements. Markich et al. (2002) found significant correlations between *Velesunio angasi* shell concentrations of Co, Cu, Mn, Ni, U and Zn and the concentration found in the surface water (all concentrations standardized by Ca). They further found significant correlation between Cu, Ni and Zn concentrations in the shell to those in the sediment (sediment concentrations standardized by Fe). Carroll and Romanek (2008) found that Mn and Sr in the shells of *E. complanata* from various sites correlated to concentrations collected in the overlying water over a 2-year period.

Non-Toxic Trace Elements in Bivalve Shells: Ba, Mg, Mn and Sr

More detailed investigations of bivalve shells have approached them as recorders of environmental condition and sampled within individual growth rings, attempting to determine seasonal or annual differences in trace element concentration (see Table 2.2 for a summary of studies). Earlier attempts at analyzing separate annuli relied on ashing shells and physically breaking annuli apart (Imlay 1982, Neves and Moyer 1988). These approaches have been superseded by newer instrumental techniques, which can analyze elemental profiles over a very small area of shell. The principle technique used in this manner is laser ablation inductively coupled plasma-mass spectroscopy (LA ICP-MS). The many analytical uses of LA ICP-MS are reviewed in Russo et al. (2002), while the issues surrounding calibrating LA ICP-MS for studies of biogenic calcium carbonate are explored in several papers (Vander Putten et al. 1999, Bellotto and Miekeley 2000, Craig et al. 2000, Barats et al. 2007). These advances in analytical capability have made it possible to analyze small fluctuations in the elemental composition of shell annuli, potentially identifying seasonal trends or anomalous events resulting from increased exposure to trace elements.

Strong evidence has emerged from studies on estuarine and marine bivalves that non-toxic trace elements such as Ba, Mg, Mn and Sr are deposited into the shell in a manner driven by environmental parameters. The estuarine clam *Mya arenaria* showed clear correlation between peaks in Sr/Ca ratios and winter (low temperature) annuli (Palacios et al. 1994). The same relationship was found in *Spisula solidissima*, while *Mercenaria mercenaria* shells showed the opposite relationship to season (Stecher et al. 1996). Regular Sr peaks corresponding to seasonal cycles of growth, have also been found in *Arctica islandica* (Toland et al. 2000), *M. edulis* (Vander Putten et al. 2000), mangrove *Isognomon ephippium* (Lazareth et al. 2003)*,* *Protothaca staminea* (Takesue and Van Geen 2004) and in the scallop *Pectens maximus* (Freitas et al. 2006). However, the cause of this annual fluctuation is a matter of debate. While Stecher et al. (1996) demonstrated a correlation between Sr and δ^{18}O [a standard measure of water temperature at the time of shell formation; (Andrus and Crowe 2000)], Takesue and van Geen (2004) argued that Sr peaks are more closely related to shell growth rate because their data could not be well correlated to recorded water temperatures. Likewise, Vander Putten et al. (2000) found that Sr did not co-vary with temperature. Modeling a larger number of environmental parameters, Freitas et al. (2006) found temperature to best explain Sr/Ca shell formed in the spring, while Mg/Ca best explained Sr/Ca incorporated in the summer, possibly due to the lattice structure of calcium carbonate. These distinctions, while they do not change the seasonal patterns of peaks, do indicate that Sr/Ca concentrations are the result of complex biogeochemical processes.

Another environmental indicator analyzed in estuarine shells is Ba concentration. Ba peaks in shell annuli are believed to represent times of greatly increased productivity, such as periods of algal blooms (Stecher et al. 1996). In the same study discussed above, Stecher et al. (1996) noted “sharp, episodic maxima” in Ba/Ca profiles that they associated with “episodic phytoplankton blooms associated with increased nutrients and light fluxes in the spring.” Toland et al. (2000) and Vander Putten et al. (2000) reported findings that agreed with this hypothesis, while Lazareth et al. (2003) similarly found Ba/Ca peaks in a tropical coastal system were likely driven by freshwater/nutrient inputs associated with the monsoon regime. A more thorough approach by Gillikin et al. (2006) involving both laboratory studies and field transplants of *M. edulis* firmly linked $[Ba/Ca]_{shell}$ to $[Ba/Ca]_{Water}$. Although they could not link episodic Ba maxima to chlorophyll *a* maxima at all sites, they concluded that barite formation during algal blooms is one of the most likely answers for this phenomenon. Overall, the literature appears to agree that Ba profiles in bivalve shells are governed by primary productivity and nutrient availability.

A less well-researched element in bivalve annuli is Mg. Toland et al. (2000) assumed a relationship between temperature and Mg defined the seasonal trends they found in the shells of *A. islandica.* Year round Mg/Ca relationships to temperature were demonstrated by Lazareth et al. (2003) and Takesue and van Geen (2004). However, Vander Putten (2000) found a strong relationship between Mg/Ca in the shells of transplanted *M. edulis* and water temperature in the spring that “broke down” during the summer months. Likewise, while Freitas et al. (2006) observed a weak but significant correlation between temperature and Mg/Ca, they found that the relationship differed during winter and summer months. The available findings therefore seem to indicate that different controls affect Mg/Ca concentrations in marine or estuarine bivalves, with temperature being a major factor for at least part of the year.

A final element thought to indicate environmental conditions is Mn. The first paper to correlate Mn to estuarine environmental parameters is Vander Putten et al. (2000), who found Mn to co-vary with Ba and chlorophyll *a*, thus linking Mn/Ca to primary productivity. The findings of this study were seconded by that of Lazareth et al. (2003). However, Freitas et al. (2006) determined that the seasonal Mn peaks seen in their study did not match local chlorophyll *a* peaks or periods of Mn oxidation, but were more likely related to dissolved and particulate concentrations of Mn. Unfortunately, this assessment was not based on directly measured Mn concentrations during the study period but rather on records of seasonal Mn concentrations in the study area from 20 years previous. Therefore, it may be more fitting to look at their findings in a similar light to Ba analyses, in which Ba/Ca peaks could not be well correlated to the exact dates of chlorophyll *a* peaks (Vander Putten et al. 2000, Gillikin et al. 2006).

Similar studies in freshwater bivalves have only been pursued in a few limited instances. A study using *Anodonta* demonstrated possible seasonal fluctuations in Mn concentrations (Lindh et al. 1988). The authors speculated that spring snow melt mobilized Mn, which was then incorporated into the shell. A study employing freshwater pearl mussels, *Margaritifera margaritifera*, found a poor relation of Sr/Ca to temperature but still found higher Sr concentrations in winter growth bands and disturbance lines (Nystrom et al. 1996). Further, they observed Mn peaks during times of high temperature and low dissolved oxygen, although they did not attempt to correlate the two directly. Profiles of Mn obtained from *Hyridella depressa* also showed seasonal peaks in Mn associated with organic-rich bands of winter growth (Siegele et al. 2001). Langlet et al. (2007) found seasonal peaks of Mn in the shells of *Pleiodon spekii* from Lake Tanganyika that they related to seasonal Mn upwellings and increased primary productivity linked to the monsoon cycle. In their study of *E. complanata*, Carroll and Romanek (2008) found that shells downstream of a lake showed regular peaks in Ba and Mn that they speculated could correspond to phytoplankton availability. Furthermore, they found that a site near a coal-fired power plant provided very different Ba, Mn and Sr profiles than those found at other sites, which may indicate that pollution can alter the manner in which these trace elements are incorporated into shells.

Explanations of trace element profiles in bivalve shells have in some cases agreed with the conclusions of marine and estuarine researchers, for instance the finding that Mn is primarily influenced by phytoplankton availability (Langlet et al. 2007, Carroll and Romanek 2008). However, Nystrom et al. (1996) speculated that Sr was incorporated during growth cessation to create higher density crystal lattices for protective purposes. They also saw a link in seasonal lows of DO with peaks of Mn, caused by redox chemistry that made Mn more bioavailable. With all of the many biogeochemical processes that go into bioavailability of trace elements and their incorporation into bivalve shells, it is possible that freshwater bivalves act in a different manner than their marine relations.

Potentially Toxic Trace Elements in Bivalve Shells

Trace elements linked to industrial contamination have not been studied as extensively as Ba, Mg, Mn and Sr. Using only the most recent annuli of *M. arenaria*, Pitts and Walker (1994) found direct correlation between $[Pb]_{shell}$ and $[Pb]_{Water}$ collected over a 2-year period. An early use of LA ICP-MS by Raith et al. (1996) used a single *A. islandica* shell to identify Pb peaks that may have correlated to increased loads entering Cardigan Bay (UK). Four individuals of *Cerastoderma edule* were collected from 4 sites around the UK in Price and Pearce (1997). The authors characterized As, Cu, Pb, U and Zn and found that metal profiles rarely matched one another in the same shell and that metal concentrations often increased after winter growth bands. Their complete lack of statistical analysis renders any relationship merely speculative. Analysis of *Crassotrea virginica* shells by Huanxin et al. (2000) revealed variations in Cr, Fe, Mn, Pb and Zn but the authors did not attempt to described the reasons behind the variation. In a more concerted attempt to characterize trace element pollution using bivalve shells, Richardson et al. (2001) compared three *Modiolus modiolus* from a North Sea (UK) site of known industrial dumping to three shells from a non-dumping sites. Cu, Pb and Zn concentrations in the shells were significantly higher at the dumping site. The metal profiles showed good agreement between the shells at the same site and may have reflected a temporal decrease in trace metal exposure following a ban on dumping industrial waste at sea. A similar approach was taken by Liehr et al. (2005), using *A. islandica* from the Mecklenburg Bight (Baltic Sea; Germany/Denmark). They also found higher Cu, Pb and Zn concentrations at the dumping site but saw no pattern.

In a study limited by its minimal sampling, Chiffoleau et al. (2004) found V to have been significantly enriched in a single specimen of *P. maximus* in the annulus laid down directly following an oil spill. The laser ablation study was strengthened by biomonitor data from mussels and oysters that showed increased tissue concentrations of Ni and V following the spill but weakened by the use of only a single sample shell. Nevertheless, this is one of the few studies that links trace element enrichment in shell annuli directly to a pollution event. In an attempt to determine impacts of accelerated glacier loss and increased human activity in the Antarctic, Dick et al. (2007) examined shells of *Laternula elliptica*. No increases in Al, Cu, Fe, Mn or Pb were found in more recent annuli, although resolution was limited by the narrowness of the growth rings being analyzed.

Lead has been more intensely analyzed than other potentially toxic trace elements. In an attempt to validate incorporation of Pb into growing shell, MacFarlane et al. (2006) determined that the pearl mussel *Pinctada imbricata* incorporated increased Pb concentrations into shell during both sustained and chronic aqueous exposures. Vander Putten et al. (2000) found underlying seasonal trends (broad maxima in spring and early summer) in Pb/Ca concentrations in *M. edulis* but were unable to correlate them to seasonal fluctuations in Pb concentration in water. *Mercenaria mercenaria* shells, both modern and archival, collected from Pamlico Sound (NC, USA) were used to develop a long-term chronology by Gillikin et al. (2005). They found significantly higher Pb/Ca in annuli coinciding with dates directly before the ban on tetra-ethyl lead in gasoline but found no other intra- or inter-annual trends. Unfortunately, their "annual" data was a mean of averaged intra-annual data that was then averaged again between shells from sites that had different environments and significantly different Pb/Ca concentrations. A visual inspection of their intra-annual data for four shells indicates apparent seasonal trends, similar to those previously described in Vander Putten et al. (2000). The overall implication is that their conclusion that bivalve shells do not constitute suitable archives of environmental Pb may be mistaken.

Again, the literature is depaupurate when it comes to freshwater studies of the sort discussed above, with a few studies using a wide variety of instrumental approaches. An early study by Carrell et al. (1987) using a proton microprobe (μ-PIXE) found newer *M. margaratifera* shells showed significantly lower levels of Ag, Au, Co and Fe than 19th century samples taken from annuli laid down when mining in the region was active. Subsequently, a similar study showed possible seasonal fluctuations in Al, Cu, Fe and Zn in *Anodonta* (Lindh et al. 1988). Fossil *D. polymorpha* shells sampled in lake sediment cores were analyzed with LA ICP-MS for Cd, Cu, Pb and Zn by Schettler and Pearce (1996). This study showed that older shells located in less contaminated sediments accumulated lower levels of Cd, Cu, Pb and Zn, but annuli were not sampled individually. Al-Aasm et al. (1998) digested whole annuli of *D. polymorpha* from Lake Erie and determined that Cd, Cu, Fe, Mg, Mn and V increased during periods of warmer temperatures and were higher at sites closer to sources of industrial contaminants, although they supplied no statistical analysis to support this. A study utilizing micro-XRF spectrometry on a modern (1992) and archival (1917) specimen of *Unio crassus retzius* was undertaken by Kurunczi et al. (2001). This approach allowed only a qualitative comparison, with the newer shell surface showing As, Cu and Pb not present in the archived sample. Metal profiles were attributed to the dubious provenance of sediments preserved on the shell surface, limiting their usefulness. Markich et al. (2002) found significantly higher concentrations of Co, Cu, Mn, U and Zn in *V. angasi* from a site impacted by mine waste when compared to reference sites, with shell concentrations linearly correlated to water concentrations. They also found that Cu and Zn in annual layers declined after the mine-waste site was remediated. Carroll (2008) used LA ICP-MS to analyze Cu in *E. complanata* and found increased concentrations at organic-rich winter growth bands, implying that Cu was associated with the organic fraction of the shell, which tends to increase during winter growth.

Summary

That environmental factors exert strong controls on incorporation of Ba, Mg, Mn and Sr into bivalve shells is supported by numerous studies. The challenges for the future in this field will be two-fold. First, the individual agents of environmental control need to be investigated further, so that trace element profiles can be used as reliable chronographs of environmental conditions at a given time in an organism's life. Secondly, the same approaches that have successfully identified potential environmental controls in estuarine and marine systems should be applied more extensively to freshwater systems, especially lotic systems. Applying this approach to lotic systems may prove more challenging because the more variable environment of freshwater, especially lotic, systems may lead to more variable data.

Summarizing the current findings for metals such as Cu, Pb and Zn is more difficult than those for Ba, Mg, Mn and Sr. That Cu, Pb and Zn can become enriched in the shells of bivalves with increased exposure is supported by several studies but the utility of retroactively monitoring shell annuli is not always clear. Much work needs to be done in order to use bivalve shell annuli as archives of trace element pollution, especially in freshwater systems. Firstly, seasonal environmental parameters controlling contaminant uptake need to be taken into account to eliminate naturally occurring peaks in concentrations. Secondly, the regularity with which bivalves record the impacts of short-term pollution events needs to be validated. Validation could be performed through laboratory and mesocosm studies or by surveying populations that have recently seen increased exposure. By validating these approaches, bivalve shells could be transformed into long-term, retroactive records of trace element pollution that would require much less time and effort to sample than traditional biomonitoring approaches.

Conclusions

A large body of work has grown up around the idea of using freshwater bivalves in general and *Corbicula fluminea,* in particular, as biomonitors of potentially toxic trace elements. Although it has been well established that bivalves exposed to higher levels of trace elements will accumulate higher concentrations in their tissues, the many factors that affect bioaccumulation are not fully understood. Two leading models of trace element bioaccumulation (biodynamic and biogeochemical) indicate that element sources in particulates and sediments contribute the most to accumulation. Together, these studies call into question the importance of bioaccumulation and toxicity studies using water as a primary source. Likewise, individual size and season of collection have been implicated as affecting bioaccumulation but the specific effects of these factors remain limited to the species, element, site and methodology of a particular study. Meanwhile, numerous field studies have indicated that anthropogenic inputs (as measured in particulates and sediments especially) of trace elements better determine bioaccumulation than other confounding affects. Continued investigation into the individual and environmental factors that influence trace element accumulation in bivalves, as well as studies that link bioaccumulation directly to adverse effects in wild populations, will greatly increase their usefulness as biomonitors. Meanwhile, a promising aspect of biomonitoring is analyzing the accumulation of trace elements in bivalve shells. Understanding the environmental controls of this process and validating the ability of bivalve shells to record fluctuations in metal concentrations may eventually enable retroactive monitoring of trace element pollution.

Table 2.1. A table summarizing the criteria of good biomonitors, how closely they are met by freshwater bivalves, with references relating to the appropriate point.

Criterion	Suitability of Bivalves	References
1. Tolerance of contaminants	Typically resilient to contaminants; can be dependent on species and contaminant.	(Elder 1991, Naimo 1995)
2. Limited mobility	Mostly sedentary; move very little even during storm surges	(Imlay 1982, Schwalb and Pusch 2007)
3. Long life span	Unionidae: up to 100 years; Corbiculidae: 1-4 years	(McMahon 2001, Vaughn and Hakenkamp 2001)
4. Ubiquity and abundance	Typically colonize in large, dense “beds”; distribution can be patchy	(Strayer 1999, Morales et al. 2006, Strayer 2008)
5. Tissue amount	Wide range of sizes; smaller species may have to be analyzed in composite samples	(Elder 1991, McMahon 2001, Vaughn and Hakenkamp 2001)
6. Ability to survive disturbance	Can be dependent on species; by sealing its valves, a bivalve can survive most short-term disturbances	(Elder 1991, Vaughn and Hakenkamp 2001)
7. Easily identified and sampled	Sampling can occur in wadeable portions of streams; identification of certain species may be problematic	(Campbell et al. 2005, Meador 2008)
8. Displays a metric of exposure	Metrics can include: Biomarkers, bioaccumulation, growth, fecundity, acute toxicity, etc.	Numerous: See this chapter
9. The metric correlates to environmental health	May be problematic; see this chapter for more detailed analysis.	Numerous: See this chapter

Table 2.2. Field studies that have analyzed intra- and/or inter-annual variability in trace element concentrations of bivalve shells.

Environment	**Species**	**Instrumentation**	**Elements**	**Citation**
Marine	*Arctica islandica*	LA ICP-MS	Sr, Pb	Raith et al. 1996
	Spisula solidissima	LA ICP-MS	Ba, Sr	Stecher et al. 1996
	Cerastoderma edule	LA ICP-MS	As, Cu, Pb, U, Zn	Price and Pearce 1997
	Crassotrea virgina	GF-AAS	Cd, Cu, Cr, Fe, Mn, Pb, Zn	Huanxi et al. 2000
	Arctica islandica	LA ICP-MS	Ba, Mg, Sr	Toland et al. 2000
	Modiolus modiolus	LA ICP-MS	Cu, Pb, Zn	Richardson et al. 2001
	Pecten maximus	LA ICP-MS	Ni, V	Chiffoleau et al. 2004
	Protothaca staminea	LA ICP-MS	Mg, Sr	Takesue and van Geen 2004
	Mercenaria mercenaria	LA ICP-MS	Pb	Gillikin et al. 2005
	Arctica islandica	LA ICP-MS	Cu, Pb, Zn	Liehr et al. 2005
	Pecten maximus	LA ICP-MS	Mg, Mn, Sr	Freitas et al. 2006
	Laternula elliptica	LA ICP-MS	Al, Cu, Fe, Mn, Pb and U	Dick et al. 2007
Estuarine	*Mya arenaria*	LA ICP-MS	Sr	Palacios et al. 1994
	Mercenaria mercenaria	LA ICP-MS	Ba, Sr	Stecher et al. 1996
	Mytilus edulis	LA ICP-MS	Ba, Mg, Mn, Pb, Sr	Vander Putten et al. 2000
	Isognomon ephippium	LA ICP-MS	Ba, Mg, Mn, Sr	Lazareth et al. 2003
	Mytilus edulis	LA ICP-MS	Ba	Gillikin et al. 2006
Lentic Freshwater	*Anodonta*	μ-PIXE	Al, Cu, Fe, Mg, Mn, Ni, Sr, Zn	Lindh et al. 1988
	Dreissena polymorpha	LA ICP-MS	Cd, Cu, Pb, Zn	Schettler and Pearce 1996
	Dreissena polymorpha	ICP-MS	Cd, Cu, Fe, Mg, Mn, Pb, V	Al-Aasm et al. 1998
	Pleiodon spekii	LA ICP-MS	Mn	Langlet et al. 2007
Lotic Freshwater	*Margaratifera margaratifera*	μ-PIXE	Al, Cu, Fe, Mg, Mn, Ni, Zn	Carrell et al. 1987
	Margaratifera margaratifera	SLIM-UP	Mn, Sr	Nystrom et al. 1996
	Unio crassus retzius	μ-XRF	Cu, Fe, Mn, Pb, Sr, Zn	Kurunczi et al. 2001
	Hyridella depressa	μ-PIXE	Mn	Siegele et al. 2001
	Velesunio angasi	SIMS	Cu, Co, Fe, Mn, Ni, Pb, U, Zn	Markich et al. 2002

References

Abaychi, J. K. and Y. Z. Mustafa. 1988. The Asiatic clam, *Corbicula fluminea* - an indicator of trace metal pollution in the Shatt Al-Arab River, Iraq. Environmental Pollution **54**:109-122.

Adams, T. G., G. J. Atchison, and R. J. Vetter. 1981. The use of the three-ridge clam (*Amblema perplicata*) to monitor trace metal contamination. Hydrobiologia **83**:67-72.

Al-Aasm, I. S., J. D. Clarke, and B. J. Fryer. 1998. Stable isotopes and heavy metal distribution in *Dreissena polymorpha* (Zebra mussels) from western basin of Lake Erie, Canada. Environmental Geology **33**:122-129.

Almeida, M. J., G. Moura, T. Pinheiro, J. Machado, and J. Coimbra. 1998. Modifications in *Crassostrea gigas* shell composition exposed to high concentrations of lead. Aquatic Toxicology **40**:323-334.

Andres, S., M. Baudrimont, Y. Lapaquellerie, F. Ribeyre, N. Maillet, C. Latouche, and A. Boudou. 1999. Field transplantation of the freshwater bivalve *Corbicula fluminea* along a polymetallic contamination gradient (river Lot, France): I. Geochemical characteristics of the sampling sites and cadmium and zinc bioaccumulation kinetics. Environmental Toxicology and Chemistry **18**:2462-2471.

Andrus, C. F. T. and D. E. Crowe. 2000. Geochemical analysis of *Crassostrea virginica* as a method to determine season of capture. Journal of Archaeological Science **27**:33-42.

Angelo, R. T., M. S. Cringan, D. L. Chamberlain, A. J. Stahl, S. G. Haslouer, and C. A. Goodrich. 2007. Residual effects of lead and zinc mining on freshwater mussels in the Spring River Basin (Kansas, Missouri, and Oklahoma, USA). Science of the Total Environment **384**:467-496.

Barats, A., C. Pecheyran, D. Amouroux, S. Dubascoux, L. Chauvaud, and O. F. X. Donard. 2007. Matrix-matched quantitative analysis of trace-elements in calcium carbonate shells by laser-ablation ICP-MS: application to the determination of daily scale profiles in scallop shell (*Pecten maximus*). Analytical and Bioanalytical Chemistry **387**:1131-1140.

Baudrimont, M., J. Metivaud, R. MauryBrachet, F. Ribeyre, and A. Boudou. 1997. Bioaccumulation and metallothionein response in the Asiatic clam (*Corbicula fluminea*) after experimental exposure to cadmium and inorganic mercury. Environmental Toxicology and Chemistry **16**:2096-2105.

Bellotto, V. R. and N. Miekeley. 2000. Improvements in calibration procedures for the quantitative determination of trace elements in carbonate material (mussel shells) by laser ablation ICP-MS. Fresenius Journal of Analytical Chemistry **367**:635-640.

Bilos, C., J. C. Colombo, and M. J. R. Presa. 1998. Trace metals in suspended particles, sediments and Asiatic clams (*Corbicula fluminea*) of the Rio de la Plata Estuary, Argentina. Environmental Pollution **99**:1-11.

Bourgoin, B. P. 1990. *Mytilus edulis* shell as a bioindicator of lead pollution - Considerations on bioavailability and variability. Marine Ecology-Progress Series **61**:253-262.

Bourgoin, B. P. and M. J. Risk. 1987. Historical changes in lead in the Eastern Canadian Arctic, determined from fossil and modern *Mya truncata* shells. Science of the Total Environment **67**:287-291.

Campbell, D. C., J. M. Serb, J. E. Buhay, K. J. Roe, R. L. Minton, and C. Lydeard. 2005. Phylogeny of North American amblemines (Bivalvia, Unionoida): Prodigious polyphyly proves pervasive across genera. Invertebrate Biology **124**:131-164.

Carell, B., S. Forberg, E. Grundelius, L. Henrikson, A. Johnels, U. Lindh, H. Mutvei, M. Olsson, K. Svardstrom, and T. Westermark. 1987. Can mussel shells reveal environmental history? Ambio **16**:2-10.

Carroll, M. and C. S. Romanek. 2008. Shell layer variation in trace element concentration for the freshwater bivalve *Elliptio complanata*. Geo-Marine Letters **In Press**.

Cassini, A., L. Tallandini, N. Favero, and V. Albergoni. 1986. Cadmium bioaccumulation studies in the freshwater mollusks *Anodonta cygnea* and *Unio elongatulus*. Comparative Biochemistry and Physiology C-Pharmacology Toxicology & Endocrinology **84**:35-41.

Chiffoleau, J. F., L. Chauvaud, D. Amouroux, A. Barats, A. Dufour, C. Pecheyran, and N. Roux. 2004. Nickel and vanadium contamination of benthic invertebrates following the "Erika" wreck. Aquatic Living Resources **17**:273-280.

Conners, D., Westerfield, SM, Feyko, A, Black, MC 1999. Lead accumulation in soft tissues and shells of Asiatic clams (*Corbicula fluminea*). Proceedings of the 1999 Georgia Water Resources Conference.

Couillard, Y., P. G. C. Campbell, J. Pellerinmassicotte, and J. C. Auclair. 1995a. Field transplantation of a freshwater bivalve, *Pyganodon grandis*, across a metal contamination gradient .2. Metallothionein response to Cd and Zn exposure, evidence for cytotoxicity, and links to effects at higher levels of biological organization. Canadian Journal of Fisheries and Aquatic Sciences **52**:703-715.

Couillard, Y., P. G. C. Campbell, A. Tessier, J. Pellerinmassicotte, and J. C. Auclair. 1995b. Field transplantation of a freshwater bivalve, *Pyganodon grandis*, across a metal contamination gradient .1. Temporal changes in metallothionein and metal (Cd, Cu, and Zn) concentrations in soft tissues. Canadian Journal of Fisheries and Aquatic Sciences **52**:690-702.

Craig, C. A., K. E. Jarvis, and L. J. Clarke. 2000. An assessment of calibration strategies for the quantitative and semi-quantitative analysis of calcium carbonate matrices by laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS). Journal of Analytical Atomic Spectrometry **15**:1001-1008.

Croteau, M. N. and S. N. Luoma. 2005. Delineating copper accumulation pathways for the freshwater bivalve *Corbicula* using stable copper isotopes. Environmental Toxicology and Chemistry **24**:2871-2878.

Croteau, M. N., S. N. Luoma, B. R. Topping, and C. B. Lopez. 2004. Stable metal isotopes reveal copper accumulation and less dynamics in the freshwater bivalve *Corbicula*. Environmental Science & Technology **38**:5002-5009.

Dick, D., E. Philipp, M. Kriews, and D. Abele. 2007. Is the umbo matrix of bivalve shells (*Laternula elliptica*) a climate archive? Aquatic Toxicology **84**:450-456.

Doherty, F. G. 1990. The Asiatic clam, *Corbicula Spp*, as a biological monitor in freshwater environments. Environmental Monitoring and Assessment **15**:143-181.

Doherty, F. G., D. S. Cherry, and J. Cairns. 1987. Valve closure responses of the Asiatic clam *Corbicula fluminea* exposed to cadmium and zinc. Hydrobiologia **153**:159-167.

Dunca, E., B. R. Schone, and H. Mutvei. 2005. Freshwater bivalves tell of past climates: But how clearly do shells from polluted rivers speak? Palaeogeography Palaeoclimatology Palaeoecology **228**:43-57.

Elder, J., Collins, JJ. 1991. Freshwater molluscs as indicators of bioavailability and toxicity of metals in surface-water systems. . Reviews Environ Contam Tox **122**:37-79.

Elder, J. F. and H. C. Mattraw. 1984. Accumulation of trace elements, pesticides, and polychlorinated biphenyls in sediments and the clam *Corbicula manilensis* of the Apalachicola River, Florida. Archives of Environmental Contamination and Toxicology **13**:453-469.

Farrington, J. W. 1983. Bivalves as sentinels of coastal chemical pollution - the mussel (and oyster) watch. Oceanus **26**:18-29.

Foster, I. D. L. and S. M. Charlesworth. 1996. Heavy metals in the hydrological cycle: Trends and explanation. Hydrological Processes **10**:227-261.

Freitas, P. S., L. J. Clarke, H. Kennedy, C. A. Richardson, and F. Abrantes. 2006. Environmental and biological controls on elemental (Mg/Ca, Sr/Ca and Mn/Ca) ratios in shells of the king scallop *Pecten maximus*. Geochimica Et Cosmochimica Acta **70**:5119-5133.

Gillikin, D. P., F. Dehairs, W. Baeyens, J. Navez, A. Lorrain, and L. Andre. 2005. Inter- and intra-annual variations of Pb/Ca ratios in clam shells (*Mercenaria mercenaria*): A record of anthropogenic lead pollution? Marine Pollution Bulletin **50**:1530-1540.

Gillikin, D. P., F. Dehairs, A. Lorrain, D. Steenmans, W. Baeyens, and L. Andre. 2006. Barium uptake into the shells of the common mussel (*Mytilus edulis*) and the potential for estuarine paleo-chemistry reconstruction. Geochimica Et Cosmochimica Acta **70**:395-407.

Graney, R. L., D. S. Cherry, and J. Cairns. 1983. Heavy metal indicator potential of the Asiatic clam (*Corbicula fluminea*) in artificial stream systems. Hydrobiologia **102**:81-88.

Graney, R. L., D. S. Cherry, and J. Cairns. 1984. The influence of substrate, pH, diet and temperature upon cadmium accumulation in the Asiatic clam (*Corbicula fluminea*) in laboratory artificial streams. Water Research **18**:833-842.

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

Gundacker, C. 2000. Comparison of heavy metal bioaccumulation in freshwater molluscs of urban river habitats in Vienna. Environmental Pollution **110**:61-71.

Harrison, F. L., J. P. Knezovich, and D. W. Rice. 1984. The toxicity of copper to the adult and early life stages of the freshwater clam, *Corbicula manilensis*. Archives of Environmental Contamination and Toxicology **13**:85-92.

Hemelraad, J., Kleinveld, HA, de Roos, AM, Holwerda, DA, Zandee, DI 1987. Cadmium kinetics in freshwater clams. III. Effects of zinc on uptake and distribution of cadmium in *Anodonta cygnea*. Arch. Environ. Contam. Toxicol. **16**:95-101.

Huanxin, W., Z. Lejun, and B. J. Presley. 2000. Bioaccumulation of heavy metals in oyster (*Crassostrea virginica*) tissue and shell. Environmental Geology **39**:1216-1226.

Hull, M. S., D. S. Cherry, and R. J. Neves. 2006. Use of bivalve metrics to quantify influences of coal-related activities in the Clinch River watershed, virginia. Hydrobiologia **556**:341-355.

Imlay, M. 1982. Use of shells of freshwater mussels in monitoring heavy metals and environmental stresses: a review. . Ecological Review **15**:1-14.

Inza, B., F. Ribeyre, and A. Boudou. 1998. Dynamics of cadmium and mercury compounds (inorganic mercury or methylmercury): uptake and depuration in *Corbicula fluminea*. Effects of temperature and pH. Aquatic Toxicology **43**:273-285.

Inza, B., F. Ribeyre, R. MauryBrachet, and A. Boudou. 1997. Tissue distribution of inorganic mercury, methylmercury and cadmium in the Asiatic clam (*Corbicula fluminea*) in relation to the contamination levels of the water column and sediment. Chemosphere **35**:2817-2836.

Joy, J. E. 1985. A 40-week study on growth of the Asian clam, *Corbicula fluminea* (Muller), in the Kanawha River, West-Virginia. Nautilus **99**:110-116.

Kurunczi, S., S. Torok, and P. Chevallier. 2001. A micro-XRF study of the element distribution on the growth front of mussel shell (Species of *Unio crassus Retzius*). Mikrochimica Acta **137**:41-48.

Langlet, D., L. Y. Alleman, P. D. Plisnier, H. Hughes, and L. Andre. 2007. Manganese content records seasonal upwelling in Lake Tanganyika mussels. Biogeosciences **4**:195-203.

Lazareth, C. E., E. Vander Putten, L. Andre, and F. Dehairs. 2003. High-resolution trace element profiles in shells of the mangrove bivalve *Isognomon ephippium*: a record of environmental spatio-temporal variations? Estuarine Coastal and Shelf Science **57**:1103-1114.

Liehr, G. A., M. L. Zettler, T. Leipe, and G. Witt. 2005. The ocean quahog *Arctica islandica L.*: a bioindicator for contaminated sediments. Marine Biology **147**:671-679.

Lindh, U., H. Mutvei, T. Sunde, and T. Westermark. 1988. Environmental history told by mussel shells. Nuclear Instruments & Methods in Physics Research Section B-Beam Interactions with Materials and Atoms **30**:388-392.

Luoma, S. N., R. Dagovitz, and E. Axtmann. 1990. Temporally intensive study of trace metals in sediments and bivalves from a large river, estuarine system - Suisun Bay Delta in San-Francisco Bay. Science of the Total Environment **97-8**:685-712.

Luoma, S. N. and P. S. Rainbow. 2005. Why is metal bioaccumulation so variable? Biodynamics as a unifying concept. Environmental Science & Technology **39**:1921-1931.

MacFarlane, G. R., S. J. Markich, K. Linz, S. Gifford, R. H. Dunstan, W. O'Connor, and R. A. Russell. 2006. The Akoya pearl oyster shell as an archival monitor of lead exposure. Environmental Pollution **143**:166-173.

Marie, V., P. Gonzalez, M. Baudrimont, J. P. Bourdineaud, and A. Boudou. 2006. Metallothionein response to cadmium and zinc exposures compared in two freshwater bivalves, *Dreissena polymorpha* and *Corbicula fluminea*. Biometals **19**:399-407.

Markich, S. J., R. A. Jeffree, and P. T. Burke. 2002. Freshwater bivalve shells as archival indicators of metal pollution from a copper-uranium mine in tropical northern Australia. Environmental Science & Technology **36**:821-832.

McMahon, R. F. 2001. "Mollusca: Bivalvia", in Ecology and classification of North American freshwater invertebrates, Thorp JH and Covich AP ed. Pages 315-399. Academic Press, San Diego.

Meador, J. R. 2008. The development and evaluation of a freshwater mussel sampling protocol for a large lowland river. University of Georgia, Athens, GA.

Mersch, J., Jeanjean, A, Spor, H, Pihan, J-C. 1992. The freshwater mussel *Dreissena polymorpha* as a bioindicator for trace metals, organochlorines and radionuclides. Limnologie aktuell **4**:227-244.

Metcalfe-Smith, J. L., R. H. Green, and L. C. Grapentine. 1996. Influence of biological factors on concentrations of metals in the tissues of freshwater mussels (*Elliptio complanata* and *Lampsilis radiata radiata*) from the St Lawrence River. Canadian Journal of Fisheries and Aquatic Sciences **53**:205-219.

Millington, P., Walker, KF 1983. Australian freshwater mussel *Velsunio ambiguous* (Philippi) as a biological monitor for zinc, iron, and manganese. . Aust. J. mar. freshwat. Res. **34**:873-892.

Morales, Y., L. J. Weber, A. E. Mynett, and T. J. Newton. 2006. Effects of substrate and hydrodynamic conditions on the formation of mussel beds in a large river. Journal of the North American Benthological Society **25**:664-676.

Naimo, T. J. 1995. A review of the effects of heavy metals on freshwater mussels. Ecotoxicology **4**:341-362.

Naimo, T. J., D. L. Waller, and L. E. Hollandbartels. 1992. Heavy metals in the threeridge mussel *Amblema plicata plicata* (Say, 1817) in the Upper Mississippi River. Journal of Freshwater Ecology **7**:209-217.

Neves, R. J. and S. N. Moyer. 1988. Evaluation of techniques for age determination of freshwater Mussels (Unionidae). American Malacological Bulletin **6**:179-188.

Nystrom, J., E. Dunca, H. Mutvei, and U. Lindh. 1996. Environmental history as reflected by freshwater pearl mussels in the River Vramsan, southern Sweden. Ambio **25**:350-355.

Paggi, A. C., C. Ocon, M. Tangorra, and A. R. Capitulo. 2006. Response of the zoobenthos community along the dispersion plume of a highly polluted stream in the receiving waters of a large river (Rio de la Plata, Argentina). Hydrobiologia **568**:1-14.

Palacios, R., J. M. Orensanz, and D. A. Armstrong. 1994. Seasonal and lifelong variation of Sr/Ca ratio in shells of *Mya arenaria* from Grays Harbor (Washington) - An ancillary criterion in demographic studies. Estuarine Coastal and Shelf Science **39**:313-327.

Peltier, G. L., J. L. Meyer, C. H. Jagoe, and W. A. Hopkins. 2008. Using trace element concentrations in *Corbicula fluminea* to identify potential sources of contamination in an urban river. Environmental Pollution **154**:283-290.

Perceval, O., Y. Couillard, B. Pinel-Alloul, E. Bonneris, and P. G. C. Campbell. 2006. Long-term trends in accumulated metals (Cd, Cu and Zn) and metallothionein in bivalves from lakes within a smelter-impacted region. Science of the Total Environment **369**:403-418.

Perceval, O., Y. Couillard, B. Pinel-Alloul, A. Giguere, and P. G. C. Campbell. 2004. Metal-induced stress in bivalves living along a gradient of Cd contamination: relating sub-cellular metal distribution to population-level responses. Aquatic Toxicology **69**:327-345.

Pitts, L. C. and G. T. Wallace. 1994. Lead deposition in the shell of the bivalve, *Mya arenaria* - An indicator of dissolved lead in seawater. Estuarine Coastal and Shelf Science **39**:93-104.

Pourang, N. 1996. Heavy metal concentrations in surficial sediments and benthic macroinvertebrates from Anzali wetland, Iran. Hydrobiologia **331**:53-61.

Price, G., Pearce, NJG 1997. Biomonitoring of pollution by *Cerastoderma edule* from the British Isles: A laser ablation ICP-MS study. . Marine Pollution Bulletin **34**:1025-1031.

Rainbow, P. S. 2002. Trace metal concentrations in aquatic invertebrates: Why and so what? Environmental Pollution **120**:497-507.

Raith, A., W. T. Perkins, N. J. G. Pearce, and T. E. Jeffries. 1996. Environmental monitoring on shellfish using UV laser ablation ICP-MS. Fresenius Journal of Analytical Chemistry **355**:789-792.

Reid, R. G. B., R. F. McMahon, D. O. Foighil, and R. Finnigan. 1992. Anterior inhalant currents and pedal feeding in bivalves. Veliger **35**:93-104.

Richardson, C. A., S. R. N. Chenery, and J. M. Cook. 2001. Assessing the history of trace metal (Cu, Zn,Pb) contamination in the North Sea through laser ablation ICP-MS of horse mussel *Modiolus modiolus* shells. Marine Ecology-Progress Series **211**:157-167.

Roditi, H. A. and N. S. Fisher. 1999. Rates and routes of trace element uptake in zebra mussels. Limnology and Oceanography **44**:1730-1749.

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

Roditi, H. A., N. S. Fisher, and S. A. Sanudo-Wilhelmy. 2000b. Uptake of dissolved organic carbon and trace elements by zebra mussels. Nature **407**:78-80.

Russo, R. E., X. L. Mao, H. C. Liu, J. Gonzalez, and S. S. Mao. 2002. Laser ablation in analytical chemistry - A review. Talanta **57**:425-451.

Salomons, W., N. M. Derooij, H. Kerdijk, and J. Bril. 1987. Sediments as a source for contaminants. Hydrobiologia **149**:13-30.

Schettler, G. and N. J. G. Pearce. 1996. Metal pollution recorded in extinct *Dreissena polymorpha* communities, Lake Breitling, Havel Lakes system, Germany: A laser ablation inductively coupled plasma mass spectrometry study. Hydrobiologia **317**:1-11.

Schwalb, A. N. and M. T. Pusch. 2007. Horizontal and vertical movements of unionid mussels in a lowland river. Journal of the North American Benthological Society **26**:261-272.

Sebesvari, Z., K. F. Ettwig, and H. Emons. 2005. Biomonitoring of tin and arsenic in different compartments of a limnic ecosystem with emphasis on *Corbicula fluminea* and *Dikerogammarus villosus*. Journal of Environmental Monitoring **7**:388-388.

Shi, D. L. and W. X. Wang. 2005. Uptake of aqueous and dietary metals by mussel *Perna viridis* with different Cd exposure histories. Environmental Science & Technology **39**:9363-9369.

Siegele, R., I. Orlic, D. D. Cohen, S. J. Markich, and R. A. Jeffree. 2001. Manganese profiles in freshwater mussel shells. Nuclear Instruments & Methods in Physics Research Section B-Beam Interactions with Materials and Atoms **181**:593-597.

Soucek, D. J., T. S. Schmidt, and D. S. Cherry. 2001. In situ studies with Asian clams (*Corbicula fluminea*) detect acid mine drainage and nutrient inputs in low-order streams. Canadian Journal of Fisheries and Aquatic Sciences **58**:602-608.

Stecher, H. A., D. E. Krantz, C. J. Lord, G. W. Luther, and K. W. Bock. 1996. Profiles of strontium and barium in *Mercenaria mercenaria* and *Spisula solidissima* shells. Geochimica Et Cosmochimica Acta **60**:3445-3456.

Stites, D. L., A. C. Benke, and D. M. Gillespie. 1995. Population dynamics, growth, and production of the Asiatic clam, *Corbicula fluminea*, in a blackwater river. Canadian Journal of Fisheries and Aquatic Sciences **52**:425-437.

Strayer, D. L. 1999. Use of flow refuges by Unionid mussels in rivers. Journal of the North American Benthological Society **18**:468-476.

Strayer, D. L. 2008. Freshwater mussel ecology : A multifactor approach to distribution and abundance. University of California Press, Berkeley.

Sturresson, U. 1976. Lead enrichment in shells of *Mytulis edulis*. Ambio **5**:253-256.

Takesue, R. K. and A. Van Geen. 2004. Mg/Ca, Sr/Ca, and stable isotopes in modern and holocene *Protothaca staminea* shells from a northern California coastal upwelling region. Geochimica Et Cosmochimica Acta **68**:3845-3861.

Tatem, H. E. 1986. Bioaccumulation of polychlorinated biphenyls and metals from contaminated sediment by freshwater prawns, *Macrobrachium rosenbergii* and Clams, *Corbicula fluminea*. Archives of Environmental Contamination and Toxicology **15**:171-183.

Tessier, A. and P. G. C. Campbell. 1987. Partitioning of trace metals in sediments - Relationships with bioavailability. Hydrobiologia **149**:43-52.

Tessier, A., P. G. C. Campbell, J. C. Auclair, and M. Bisson. 1984. Relationships between the partitioning of trace metals in sediments and their accumulation in the tissues of the freshwater mollusk *Elliptio complanata* in a mining area. Canadian Journal of Fisheries and Aquatic Sciences **41**:1463-1472.

Tessier, A., Y. Couillard, P. G. C. Campbell, and J. C. Auclair. 1993. Modeling Cd partitioning in oxic lake sediments and Cd concentrations in the freshwater bivalve *Anodonta grandis*. Limnology and Oceanography **38**:1-17.

Toland, H., B. Perkins, N. Pearce, F. Keenan, and M. J. Leng. 2000. A study of sclerochronology by laser ablation ICP-MS. Journal of Analytical Atomic Spectrometry **15**:1143-1148.

Tomazelli, A. C., L. A. Martinelli, W. E. P. Avelar, P. B. de Camargo, A. H. Fostier, E. S. B. Ferraz, F. J. Krug, and D. S. Junior. 2003. Biomonitoring of Pb and Cd in two impacted watersheds in southeast Brazil, using the freshwater mussel *Anodontites trapesialis* (Lamarck, 1819) (Bivalvia : Mycetopodidae) as a biological monitor. Brazilian Archives of Biology and Technology **46**:673-684.

Van der Velde, G., Hermus, K, Van der Gaag, M, Jenner, HA 1992. Cadmium, zinc, and copper in the body, byssus and shell of the mussels, *Mytilopsis leucophaeta* and *Dreissena* *polymorpha* in the brackish Noordzeekanaal of the Netherlands. Limnologie aktuell **4**:213-226.

Vander Putten, E., F. Dehairs, L. Andre, and W. Baeyens. 1999. Quantitative in situ microanalysis of minor and trace elements in biogenic calcite using infrared laser ablation - inductively coupled plasma mass spectrometry: A critical evaluation. Analytica Chimica Acta **378**:261-272.

Vander Putten, E., F. Dehairs, E. Keppens, and W. Baeyens. 2000. High resolution distribution of trace elements in the calcite shell layer of modern *Mytilus edulis*: Environmental and biological controls. Geochimica Et Cosmochimica Acta **64**:997-1011.

Vaughn, C. C. and C. C. Hakenkamp. 2001. The functional role of burrowing bivalves in freshwater ecosystems. Freshwater Biology **46**:1431-1446.

Villar, C., J. Stripeikis, L. D'Huicque, M. Tudino, O. Troccoli, and C. Bonetto. 1999. Cd, Cu and Zn concentrations in sediments and the invasive bivalves *Limnoperna fortunei* and *Corbicula fluminea* at the Rio de la Plata basin, Argentina. Hydrobiologia **416**:41-49.

Vinot, I. and J. C. Pihan. 2005. Circulation of copper in the biotic compartments of a freshwater dammed reservoir. Environmental Pollution **133**:169-182.

Wang, W. X. and N. S. Fisher. 1999. Assimilation efficiencies of chemical contaminants in aquatic invertebrates: A synthesis. Environmental Toxicology and Chemistry **18**:2034-2045.

CHAPTER THREE

BIOACCUMULATION OF TRACE ELEMENTS BY *CORBICULA FLUMINEA* AND *ELLIPTIO HOPETONENSIS* IN THE ALTAMAHA RIVER SYSTEM[1]

Abstract

The Asian clam, *Corbicula fluminea,* has frequently been used as a biomonitor of a wide array of pollutants, including potentially toxic trace elements. This study analyzed the tissue concentrations of As, Cd, Cr, Cu, Hg, Mn, Ni, Pb and Zn in *Corbicula* and the native mussel *Elliptio hopetonensis* from 14 sites in the Altamaha River system, in Georgia, USA. Concentrations (µg/g) of As, Cd, Cu, Hg and Pb were found to be significantly positively correlated ($\alpha = 0.05$) between the two species, indicating that *Corbicula* bioaccumulation can be used as a proxy for co-occurring *E. hopetonensis.* When calculated as body burdens (g), As, Cd, Cu, Hg and Zn were significantly positively correlated ($\alpha = 0.05$) between the two species. The trace element tissue concentrations and body burdens in both *Corbicula* and *E. hopetonensis* were correlated to a number of environmental and growth parameters. Lower water temperature, dissolved oxygen, alkalinity, and hardness tended to lead to higher concentrations of trace elements, while larger organisms tended to accumulate higher concentrations. Only Cd, Cu, Ni and Pb showed any correlation between tissue concentrations of either species and concentrations in water and/or sediment. No obvious longitudinal trends could be seen from the data. This study supports the idea that bioaccumulation of trace elements by *Corbicula* can be

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used to approximate levels accumulated by co-occurring native mussel species as part of a biomonitoring program.

Introduction

Native mussel species have been declining across the southeast (Williams et al. 1993). The Altamaha River in southeastern Georgia contains a native assemblage of 19 mussels. The federally endangered species *Elliptio spinosa* is of special concern (Meador 2008). Threats to native mussels include sedimentation and habitat alteration, loss of host fish species, exposure to anthropogenic contaminants and competition from invasive species (Williams et al. 1993, Naimo 1995). As an invasive species, *Corbicula* has been implicated as a factor in native mussel decline through competition for food resources (Sickel 1973, Kraemer 1979, Elder 1991).

A widespread invasive freshwater bivalve, the Asian clam (*Corbicula fluminea*; hereafter *Corbicula*) was first found along the Pacific coast of the United States in 1937 (Kraemer 1979). Since then it has spread to 33 of the 49 continental states, its range limited by colder winter temperatures (Stites et al. 1995). Spreading to the southeastern states, *Corbicula* became established in all Georgia watersheds, including the largest Atlantic Slope drainage, the Altamaha River (Sickel 1973, Stites et al. 1995). It has since established itself ubiquitously and abundantly throughout the Altamaha and its tributaries.

Corbicula has been studied extensively as a biomonitor, or an organism whose accumulation of or reaction to contaminants is used as a measure of the impacts of those contaminants on the environment. It has become popular as a biomonitor for both potentially toxic trace elements (Caldwell and Buhler 1983, Graney et al. 1983, Elder and Mattraw 1984, Graney et al. 1984, Tatem 1986, Abaychi and Mustafa 1988, Doherty et al. 1990, Luoma et al. 1990, Pourang 1996, Baudrimont et al. 1997, Andres et al. 1999, Villar et al. 1999, Angelo et al.

2007) and organic compounds (Elder and Mattraw 1984, Tatem 1986, Doherty 1990). The general consensus found in the literature is that *Corbicula* can serve as a good biomonitor organism for a variety of contaminants due to its ubiquity, high abundance, low mobility, rapid accumulation of contaminants and tolerance to high amounts of contamination (Doherty 1990). Of primary interest in past studies has been the bioaccumulation of contaminants by *Corbicula* following exposure (Graney et al. 1983, Doherty 1990). Elevated bioaccumulation of trace elements by freshwater bivalves has been linked to adverse effects such as oxidative stress, reduced fecundity and local extinctions of native mussels (Couillard et al. 1995, Perceval et al. 2004, Angelo et al. 2007). Few studies have made direct comparison between the accumulation of contaminants by *Corbicula* and that of co-occurring native mussel species (Caldwell and Buhler 1983, Angelo et al. 2007).

In order to better evaluate the utility of *Corbicula* as a biomonitor organism, this study examined 1) the difference (if any) in the amount of trace metals accumulated by co-occurring *Corbicula* and the native Unionid mussel species *Elliptio hopetonensis* at multiple sites within the Altamaha River system; 2) the ability to draw significant correlations between *Corbicula* accumulation and that of a native species; 3) spatial and environmental factors that may influence bioaccumulation. By elucidating the link between *Corbicula* and *E. hopetonensis* in their bioaccumulation of trace metals, the groundwork could be laid for trace metal biomonitoring programs using *Corbicula* exclusively. Such a program would allow the assessment of the threat of trace metals to native mussels without disrupting their communities with destructive sampling.

Materials and Methods

Study Area

The Altamaha River drainage is completely contained within the state of Georgia. The Altamaha River proper is formed by the confluence of the Ocmulgee and Oconee Rivers, whose headwaters are in the piedmont phyto-geographic province and cross the fall line at Macon and Milledgeville GA, respectively (Reese and Batzer 2007). The Altamaha proper has a largely unaltered channel, bordered by wide areas of bottomland forest, while the Oconee and Ocmulgee both have reservoirs in their middle or upper reaches. Other major tributaries are the Little Ocmulgee and Ohoopee Rivers. While many of its smaller tributaries below the fall line could be considered to be blackwater systems (Patrick 1994), the Altamaha mainstem is not a classic example of a blackwater river (Stites et al. 1995).

Fourteen sites were chosen for this study, two on the lower Oconee (OCN), four on the middle and lower Ocmulgee (OMG), one on the lower Ohoopee (OHP), and seven on the main channel of the Altamaha (ALT; Figure 3.1). These sites were chosen based on their location within the Altamaha system (for broad representation), their downstream proximity to potential sources of trace metals (population centers and industry) and the co-occurrence of *Corbicula* and *E. hopetonensis.* Habitats sampled ranged from depositional sloughs separated from the river by a small sandbar, to the leading edges of sandbars, to rocky shelves of the unique “Altamaha grit” microhabitat. GIS coordinates were taken and recorded for each site at the time of sampling (Magellan explorist 210; Thales Navigation, San Diego CA), and a sketch of the site was made indicating major features of substrate and local morphology.

Water Quality and Stream Parameters

At each site, the following water quality parameters were measured immediately upstream of the sampling site: temperature and dissolved oxygen (YSI Model 55; Yellow Springs OH, USA), conductivity (YSI Model 85), and pH (Orion Model 720A; Beverly MA, USA). Nalgene bottles previously washed in 10% trace metal grade HNO_3 were used to collect 500 mL of water at each site. The water was kept cool and refrigerated upon return to the lab. Subsamples (50 mL) were used to determine alkalinity and hardness titrametrically (Hach®, Loveland CO, USA).

Sediment and Bivalve Collection

Sediment was collected at each site using a petite Ponar grab sampler until 3 grabs had been made or >500 mL of sediment had been acquired. Composite sediments were homogenized in an acid-rinsed plastic container and 500 mL was subsampled into a 10% trace metal grade HNO_3 washed nalgene bottle. The sediment was stored on ice for transport back to the lab and stored at 4°C. Further processing took place within 2 weeks of collection.

At each site, *Corbicula* (25-35) and *E. hopetonensis* (5) were sampled by hand and deposited in labeled mesh bags. They were stored in a bucket of aerated river water, which was placed in a cooler with small amounts of ice to limit stress to the organisms. Upon transportation to the lab, they were transferred to a climate controlled room and allowed to depurate gut contents for ~12-36 h.

Sample Processing

Water samples were filtered through a 0.5 µm paper filter that had previously been rinsed with 10% trace metal grade HNO_3 and dried. Approximately 50 mL of each filtered sample were poured into an acid-washed nalgene bottle and acidified with 0.5 mL of trace metal grade

HNO_3 in order to maintain the integrity of trace metal concentrations. Samples were stored at room temperature until analysis.

All sediment samples were processed using a protocol adapted from Luoma et al. (1990). Sediment samples were re-homogenized and poured through a 1 mm acid-washed plastic sieve. The resulting < 1 mm fraction was placed in acid-washed nalgene containers and stored at –20°C until they could be processed further. Frozen samples were thawed under refrigeration at a later date and wet-sieved again through a 105 µm Nytex mesh (Spectrum Mesh; Rancho Dominguez CA, USA) into an acid-washed 600 mL beaker. Fine fractions were dried at temperatures ranging from room temperature to 95°C, homogenized using a dry acid-washed mortar and pestle and stored in acid washed plastic centrifuge tubes under anhydrous conditions until digestion.

Bivalves were measured for maximum length, height, and depth of shells while still alive. Whole tissues (8-10 *Corbicula* and 5 *E. hopetonensis*) were dissected from their shells and placed on pre-weighed aluminum pans. Wet weights of tissues were measured and the tissues were then dried at 100 °C for 48 h or until completely dry, at which point they were reweighed. Because of its much larger tissue mass, *E. hopetonensis* tissues were homogenized using an acid-washed mortar and pestle. *Corbicula* samples were stored as whole tissues. All dried tissues were stored in acid-washed plastic containers until digestion. Shells were allowed to dry, then weighed, labeled in pencil and stored in plastic bags. Left valves of shells were filled with sand of a known density and weighed in order to estimate shell volume (Kesler 2004).

Acid-Digestion

Between 0.25 and 0.5 g of dried fine fraction sediment were completely digested in 8 mL trace metal grade HNO_3 and 3 mL HF, using a MARS 5 HP 500 Plus system (CEM Corp; Matthews NC, USA), under the conditions of EPA protocol 3052 (USEPA 1996). Three replicate samples were digested for each sediment composite. All digestions were diluted in 24 mL of Milli-Q water to ~33% acid. Blanks and standard sediments (MESS-3, National Research Council of Canada, Ottawa ON, Canada; NIST 1645, National Bureau of Standards, Gaithersburg MD, USA) were also digested with each run. All samples, blanks and standards were stored at room temperature in acid-washed centrifuge tubes until analysis.

Whole tissues of *Corbicula* and 0.10-0.25 g homogenized tissues of *E. hopetonensis* were completely digested in 5 mL of trace metal grade HNO_3, using a MARS 5 HP 500 Plus system (CEM Corp; Matthews NC, USA), under the conditions of EPA protocol 3015 (USEPA 1994). Three replicate digestions were performed for each individual *E. hopetonensis.* Digestions were diluted with 10 mL Milli-Q water to ~33% acid. Exact concentrations were calculated using dry tissue mass and the mass of the final solution. One blank (acid only) and one standard reference material (SRM) of ~0.075 g TORT-2 lobster hepatopancreas (National Research Council of Canada, Ottawa ON, Canada) were digested in each run. Dilute samples, blanks and standard were stored at room temperature until analysis.

Sample Analysis

Analyses of digested samples were carried out as described in Unrine et al. (2007). Trace element concentrations in digests (As, Cd, Cr, Cu, Hg, Mn, Ni, Pb and Zn) were quantified using a Perkin-Elmer Sciex Elan DRC Plus inductively coupled plasma-mass spectrometer (ICP-MS; Norwalk CT, USA) operating in standard mode. Method detection limits (MDLs) were calculated using blank digests. Mean MDLs ranged from 0.0250 µg/g for Cd to 3.360 µg/g for Cu. Recoveries of SRMs were in good agreement with certified values and averages ranged between 85.27% for As and 130.04% for Pb. However Cr recovery was highly variable because of carbon interference and Cr concentrations from those samples were not used in data analysis.

Relative percent difference (RPD) of replicate dilutions of sub-samples was used to approximate analytical error. On average (n=34) RPD ranged from 2.01% for As and Zn to 12.80% for Pb. Spikes of ~2-5 times the un-spiked sample concentration had mean recoveries ranging from 91.61% for Cr and 101.87% for Ni (n=33).

Statistical Analysis

Concentrations of metals were calculated on a µg/g dry tissue weight basis for both *Corbicula* and *E. hopetonensis* and were compared at each site. Analysis of variance (ANOVA) was used to determine significant differences in metal concentrations between species followed by Duncan's post hoc test ($\alpha = 0.05$). Calculations were performed using Statistical Analysis Software (SAS v9.1, SAS Institute, Cary NC, USA). Similarly, ANOVA was used to determine a significant difference in accumulation of an element between a site and the closest upstream site. Tissue concentrations of each element were averaged for a given species at each sites. The mean concentrations of an element for each species were correlated across sites using linear regression and an F-test performed to determine the significance ($\alpha = 0.05$) of the correlation. Finally, tissue element concentrations at each site were compared to environmental concentrations of the element (water and sediment) at each site, as well as the water quality parameters (temperature, pH, etc) at each site using linear regression. F-Tests were used to determine any significant ($\alpha = 0.05$) correlations between these outside factors and accumulation of trace metals in the two species.

Results

The results of the comparisons of element accumulation between *Corbicula* and *E. hopetonensis* are summarized in Table 3.1 (mean concentrations of elements, organized by site and species, are included in Appendix 2.1). Overall, *Corbicula* tended to accumulate significantly higher concentrations of As, Cd, Cu, and Hg. *E. hopetonensis* tended to accumulate significantly higher concentrations of the elements Mn. Samples that were below detection limits reduced the number of sites at which a comparison could be made for Ni and Pb (to 11 and 5 respectively). Highly variable Cr recovery from SRMs decreased the number of sites with usable data (to 7). These elements, as well as Zn, did not reveal trends of accumulation that were clear, although *Corbicula* tended to accumulate higher concentrations of Cr and Ni, while *E. hopetonensis* tended to accumulate higher levels of Zn. It should be noted that at sites where Cr, Ni, and Pb were below detection in *Corbicula*, they were readily detectable in *E. hopetonensis.*

The log transformed concentrations of the elements As, Cd, Cu, Hg and Pb found in *Corbicula* were significantly ($\alpha = 0.05$) correlated to those found in *E. hopetonensis* (Figure 3.2). The log transformed body burdens of the elements As, Cd, Cu, Hg and Zn found in *Corbicula* were significantly ($p < 0.05$) correlated to those found in *E. hopetonensis* (Figure 3.3). All correlations were positive, with higher concentrations and body burdens of an element in *Corbicula* corresponding to higher concentrations and body burdens in *E. hopetonensis.*

Upstream/downstream comparisons for selected elements (As, Cd, Cu, Hg, Pb, and Zn) are represented in Figure 3.4. No clear longitudinal trends can be drawn from these data. Notably, the site on the Ohoopee River (OHP1) exhibited significantly elevated levels of Cd, Cu, Hg, Pb, and Zn. The two species also tended to show increased or decreased element concentrations in tandem with one another. This further supports the use of *Corbicula* as a surrogate of *E. hopetonensis*.

There were numerous correlations between environmental parameters and the mean concentration of accumulated elements. The significant ($\alpha = 0.05$) correlations are listed in Table 3.2. Water quality parameters (pH, temperature, dissolved oxygen, alkalinity and hardness) were negatively correlated, while size metrics [shell length, height, depth, thickness index and condition index: dry tissue mass / shell volume (Kesler 2004)] and concentrations of the elements in water and sediment typically formed positively correlated. Body burdens of both species had a greater tendency to correlate with size metrics and were also more likely to be correlated with certain water quality parameters, such as temperature and dissolved oxygen. On the other hand, tissue concentration was more likely to be correlated with water alkalinity and hardness. With respect to the concentrations or burdens of an element reflecting the amounts found in the environment, only Cd, Cu, Ni, and Pb showed any correlation between amount accumulated and the amount found in water and/or sediment samples. Overall, *Corbicula* tended to correlate with fewer parameters, whether growth or environmental, than *E. hopetonensis*.

Discussion

Comparison of trace element bioaccumulation between Asian clams and native mussels

It has been suggested that the Asian clam *Corbicula* is a suitable biological monitor of potentially toxic trace metals (Doherty 1990). This assertion comes primarily from comparisons between *Corbicula* and environmental concentrations of metals in compartments such as water and sediment. The typical approach has been to validate the ability of tissue concentrations in *Corbicula* to indicate increased environmental concentrations. This study has attempted to validate *Corbicula's* suitability as a biomonitor by comparing it to another species, in particular that of a native Unionid mussel collected in the field.

Angelo et al. (2007) investigated the accumulation of Cd, Pb, and Zn by *Corbicula* as well as various co-occurring native mussel species (14 species not differentiated in their data analysis). They found that *Corbicula* tissue concentrations significantly correlated to mussel concentrations. Similarly, in this study, accumulation of higher concentrations of As, Cd, Cu, Hg, and Pb by *Corbicula* were correlated with higher concentrations in *E. hopetonensis* (Figure 3.2). This supports the idea that metal concentrations accumulated by *Corbicula* can be used to estimate those accumulated by co-occurring populations of native mussels. It is likely that biomonitoring programs that solely sample *Corbicula* can provide an accurate representation of the exposure of native Unionids to trace element pollution.

While mean concentrations are well correlated when compared between sites, our study also showed that *Corbicula* typically accumulated higher concentrations of As, Cd, Cu, and Hg, while *E. hopetonensis* typically accumulated higher concentrations of Mn and Zn (Table 3.1). This differs from past studies, which found *Corbicula* to accumulate significantly higher concentrations of Pb, while accumulating similar amounts of Cd and Zn to various native species in Oklahoma (Angelo et al. 2007) or accumulating similar concentrations of Cd, Hg, and Zn as other bivalves in the Columbia River estuary, Oregon (Caldwell and Buhler 1983). In both of these studies, multiple species were averaged together, with few of the samples being from *Elliptio* species, a fact that may account for this discrepancy. Our comparison of Pb accumulation was also hampered by difficulty in quantifying concentrations of Pb in *Corbicula.* This may be due to the small tissue mass of the organisms or low environmental availability in the Altamaha system.

Spatial distribution of trace elements

Comparing sites to those immediately upstream showed no underlying longitudinal trend in trace element bioaccumulation within the system (Figure 3.4). A few trends can be seen. The site OMG3 tended to show significantly lower levels of bioaccumulation than OMG2, the site directly upstream (Figure 3.4). Likewise, the site OCN2 showed significantly lower concentrations of several trace elements than its nearest upstream neighbor, OCN1. Both of these sites (OMG3 and OMG2) were situated in marginal habitat, with specimens that were on average smaller than those of the nearest neighboring sites.

Abaychi and Mustafa (1988) reported smaller clams collected in the field had accumulated significantly higher concentrations of Cu, Ni, Pb, and Zn, while larger clams accumulated significantly higher concentrations of Cd. Luoma et al. 1990 reported Cd, Cr, and Cu concentrations were positively correlated with *Corbicula* length. No mean size metrics were correlated with tissue metal concentrations of Cd, Cu, Hg, and Pb in either species in our study, although *E. hopetonensis* displayed a positive correlation between shell length and Cr concentration (Table 3.2). Because each of these previous studies assigned individuals to size classes that were then analyzed in composite, the observed correlation between accumulation and size (in both cases shell length) could be an artifact of aggregation. Conversely, by selecting individuals of representative sizes for analysis and then averaging by site, we may be unable to observe the effects of size. Significantly lower accumulation could also be the result of more frequent valve closures to survive within marginal habitats. Valve closure has been shown to be a common response in *Corbicula* to toxic trace elements that limits exposure and deleterious effects (Doherty et al. 1987).

Also of interest is the site on the Ohoopee, OHP1, which typically showed significantly higher concentrations of elements than the nearest downstream site, ALT4 (Figure 3.4). Further, ALT4 frequently had significantly higher concentrations of these elements than its downstream neighbor, ALT5 and showed significantly higher bioaccumulation than the nearest site upstream ALT3. This may provide evidence of a source of trace metals (particularly Cd and Hg) within the Ohoopee drainage that is then transferred to the Altamaha. It may also indicate that the water chemistry parameters (low alkalinity and hardness) of the Ohoopee may increase bioavailability and therefore accumulation of these trace elements.

One difficulty in using biomonitor organisms to assess the distribution of contaminants is that in natural environments, a large amount of habitat heterogeneity can exist in a small area, leading to fine spatial differences in the conditions under which bioaccumulation takes place. This leads to the comparison of tissue trace element concentrations from ALT1 and ALT2 (Figure 3.4). These sites are ~500 m apart and were collected on the same day. *Corbicula* at ALT2 had significantly higher concentrations of As, Cd, Cu, Hg, and Zn than those at ALT1. ALT1 was a swifter flowing site of packed sand, while ALT2 was a shallower site with little current, whose sediment was silty sand and dead bivalve shells. The differences in environmental conditions between sites could have led to the observed differences in trace element accumulation. This illustrates the kind of fine scale spatial variability that makes it difficult to draw conclusions on anthropogenic sources of trace elements based on upstream/downstream comparisons.

Correlation between bioaccumulation and environmental parameters

Bioaccumulation of trace elements by both species was significantly correlated to a variety of environmental factors (Table 3.2). Two factors frequently negatively correlated with bioaccumulation were water hardness and alkalinity (e.g. Cd, Cu, Hg, Pb, Zn). With lower alkalinity and hardness, trace metal concentrations in tissues were found to be higher. This agrees with predictions of the biotic ligand model (BLM), in which Ca and Mg ions compete with metals for uptake (Paquin et al. 2002). As discussed earlier, this is important when considering the reasons for the high metal concentrations found at the site of OHP1, which also had lower water alkalinity and hardness than all other sites (data not shown).

Environmental concentrations of trace metals were correlated with tissue concentrations only in some instances (Table 3.2). It has been reported that *Corbicula* accumulate very low amounts of trace metals from sediment (Doherty 1990). However, the trace elements Cu and Pb were significantly correlated between concentrations in *Corbicula* tissues and sediment, while Cd and Pb were correlated between concentrations in *E. hopetonensis* tissues and in sediment. Elder and Mattraw (1984) found no correlation between *Corbicula* accumulation of Cd, Cr, Cu, Pb, or Zn and sediment from sites in the Apalachicola River in Florida, although they did find a correlation with As. However, they analyzed very fine clay particles (<23-µm) rather than the more inclusive fine fraction we analyzed (<105-µm). Also, their methods resulted in limited detection of As and Cd from *Corbicula* tissue, which may have skewed their results. Tatem (1986) found that *Corbicula* did not accumulate Cd from contaminated sediment, although they did find an accumulation of Pb, as did this study. Luoma et al. (1990) using methods similar to those used in this study found strong correlations between concentrations of Cu, Cd, Pb, and Zn in *Corbicula* and fine (<100-µm) sediments sampled from Suisun Bay, California. However, the correlation was only significant when monthly data were aggregated on a yearly or tri-yearly basis. In any case, the findings of this study indicate that *Corbicula* may take up some trace elements from the fine fraction of sediment it inhabits. Similarly, the native mussel *E. hopetonensis* may be accumulating certain toxic trace elements (Cd, Pb) from exposure via fine sediment sources. Future biomonitoring programs would be advised to consider incorporating sediment into any sampling protocol.

Some past studies (Abaychi and Mustafa 1988, Luoma et al. 1990, Angelo et al. 2007) have indicated that trace element tissue concentrations in *Corbicula*, as well as other bivalves (Cain and Luoma 1990) fluctuate on a seasonal basis. These changes have been attributed to increased water flow and trace element exposure during seasonal high flows (Abaychi and Mustafa 1988) and seasonal changes in body mass resulting from growth and reproductive strategies (Luoma et al. 1990). Our data shows significant negative correlation between mean *E. hopetonensis* tissue concentrations of the essential elements Ni and Zn with water temperature, an indicator of season, while only Zn shows such a correlation in *Corbicula* (Table 3.2). In this instance, it seems that essential elements may be more greatly affected by seasonal changes in the local environment. One proposed approach to eliminating the seasonal changes of tissue mass that can influence concentration is to calculate body burden as opposed to tissue concentration (Luoma et al. 1990). However, body burdens of Cd, Cr, Cu, Mn, Ni and Zn are all significantly correlated to temperature in *E. hopetonensis*, while no element body burdens are correlated in *Corbicula*. Body burden is also more often significantly correlated to organism size metrics, causing small differences in body size to bias data. Therefore, body burden is not necessarily a robust metric to use when comparing sites sampled on different dates. The work of Angelo et al. (2007) concluded that seasonal effects on *Corbicula* accumulation were secondary to the effects of anthropogenic inputs into the system. Our study supports this conclusion, which suggests that sampling season should be a secondary concern when instituting a spatially intensive biomonitoring program.

Conclusions

This study has provided evidence that the Asian clam *Corbicula* can be used as a biomonitoring proxy of trace element accumulation for the native freshwater mussel *E. hopetonensis* and adds to previous evidence that *Corbicula* is a suitable biomonitor of trace elements. This study also provides the first survey of trace metal accumulation in the bivalves of the Altamaha River system and indicates some areas that may exhibit increased exposure to trace elements, either through anthropogenic activity or through increased bioavailability caused by differences in water chemistry. Clearly, more intensive sampling needs to be conducted in this system to explore the distribution and potential impacts of trace elements on native mussel assemblages. This study shows that both of the bivalves studied, *Corbicula* and *E. hopetonensis*, may accumulate trace elements from the fine fraction of sediment, suggesting that analysis of sediment for trace elements should be included in any biomonitoring program. Finally, correlations between tissue concentrations of trace elements in both species and environmental factors indicate that local heterogeneity in water chemistry, habitat and organism size play important rolls and bioaccumulation and should be taken into account when analyzing bioaccumulation data.

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Table 3.1. Mean concentration of each element as it was accumulated by each species over all sites. The number of sites in which a comparison between species was possible is given and the number of sites at which each species accumulated significantly higher concentrations of each element.

Element	Species	Concentration (µg/g)	Number of sites	Significantly higher sites
As	*Corbicula*	4.55±0.20	14	14
	E. hopetonensis	2.34±0.06		0
Cd	*Corbicula*	2.34±0.27	14	9
	E. hopetonensis	1.27±0.08		0
Cr	*Corbicula*	4.18±0.40	7	4
	E. hopetonensis	2.61±0.18		0
Cu	*Corbicula*	67.8±3.8	14	14
	E. hopetonensis	5.62±0.12		0
Hg	*Corbicula*	1.296±0.195	14	10
	E. hopetonensis	0.357±0.028		1
Mn	*Corbicula*	50.9±8.3	14	0
	E. hopetonensis	4098±191		14
Ni	*Corbicula*	3.84±0.67	11	5
	E. hopetonensis	1.91±0.14		0
Pb	*Corbicula*	0.553±0.082	5	1
	E. hopetonensis	0.852±0.129		2
Zn	*Corbicula*	122±3	14	1
	E. hopetonensis	167±15		6

Table 3.2. A list of physical and environmental parameters significantly ($p < 0.05$) correlated to the mean amounts of each element at each site as calculated by tissue concentration (µg/g) or body burden (g). The individual factors use the following abbreviations: **Wat** = concentration of the element in the water; **Sed** = concentration of the element in the sediment; pH; Temp = water temperature; DO = water dissolved oxygen; Alk = water alkalinity; Hard = water hardness; L = mean shell length; H = mean shell height; D = mean shell depth; TM = thickness metric (shell mass / shell length); CI = condition index (tissue dry mass / shell volume).

Element	Species	(+) Correlation with Concentration	(-) Correlation with Concentration	(+) Correlation with Body Burden	(-) Correlation with Body Burden
As	*C. fluminea*	None	None	L, H, D, TM, CI	None
	E. hopetonensis	TM, CI	None	L, H, D, TM, CI	None
Cd	*C. fluminea*	None	Hard	**Sed**	None
	E. hopetonensis	**Sed**	Alk, Hard	L, H, D, TM	Temp
Cr	*C. fluminea*	None	None	L, H, D, TM	None
	E. hopetonensis	L, H, TM	None	L, H, D, TM, CI	Temp, DO
Cu	*C. fluminea*	**Sed**	Alk, Hard	**Wat**, **Sed**, L, H, D, TM	None
	E. hopetonensis	None	Hard	L, H, D, TM	Temp, DO, CI
Hg	*C. fluminea*	None	Alk, Hard	None	Hard
	E. hopetonensis	None	Alk, Hard	H, D, TM	None
Mn	*C. fluminea*	None	None	L, H, D	None
	E. hopetonensis	TM	None	H, D, TM, CI	Temp
Ni	*C. fluminea*	None	None	None	None
	E. hopetonensis	**Wat**	Temp, Alk, Hard	L, H, D, TM, CI	Temp, DO
Pb	*C. fluminea*	**Sed**	pH, Alk, Hard	None	None
	E. hopetonensis	**Wat**, **Sed**	Alk, Hard	**Wat**, **Sed**	Alk, Hard
Zn	*C. fluminea*	None	L, H, D, Temp, Alk, Hard	L, H, D, TM, CI	None
	E. hopetonensis	H, D, TM, CI	Temp	H, D, TM, CI	Temp

Figure 3.1. A map of sites sampled within the Altamaha River system. The following abbreviations map sites to the river they were sampled from: OMG = Ocmulgee; OCN = Oconee; OHP = Ohoopee; ALT = Altamaha Mainstem.

Figure 3.2. The natural log of mean metal(loid) concentration in the tissue of *E. hopetonensis* at each site, plotted as a function of the natural log mean concentration in the tissue of *Corbicula.* All linear regressions are significant ($p < 0.05$) with the following r^2 values: As = 0.5441; Cd = 0.5807; Cu = 0.3444; Hg = 0.7524; Pb = 0.6686.

Figure 3.3. The natural log of mean metal(loid) body burden of *E. hopetonensis* at each site, plotted as a function of the natural log mean body burden of *Corbicula.* All linear regressions are significant ($p < 0.05$) with the following r^2 values: As = 0.7226; Cd = 0.6303; Cu = 0.5694; Hg = 0.5172; Zn = 0.7962.

Figure 3.4. Mean tissue concentrations found in *Corbicula* and *E. hopetonensis* of A) As and Cd; B) Cu and Zn; C) Hg and Pb at each site, arranged up to downstream. Values that are significantly ($p < 0.05$) higher than those of the site directly upstream are indicated with ↑, while those that are significantly lower are indicated by ↓. River convergences are indicated using lines and large arrows. In the cases of significant differences between two upstream sites, two arrows are used.

Figure 3.1.

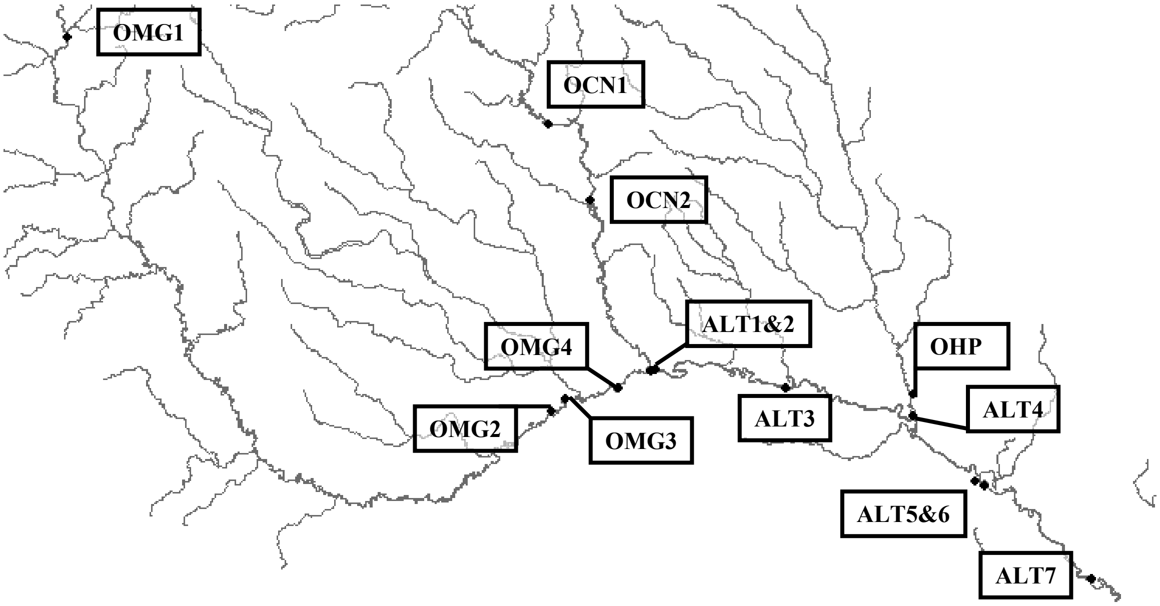
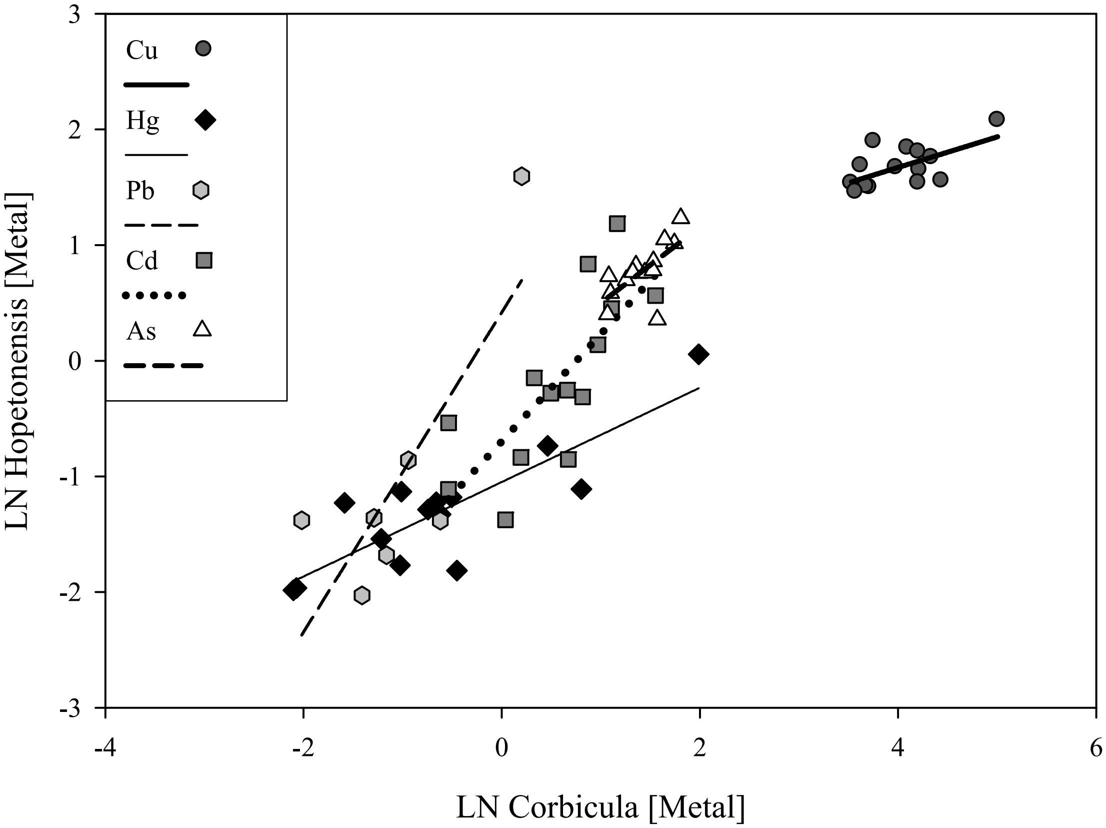


Figure 3.2.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Hg
Pb
Cd
As
LN Hopetonensis [Metal]
LN Corbicula [Metal]
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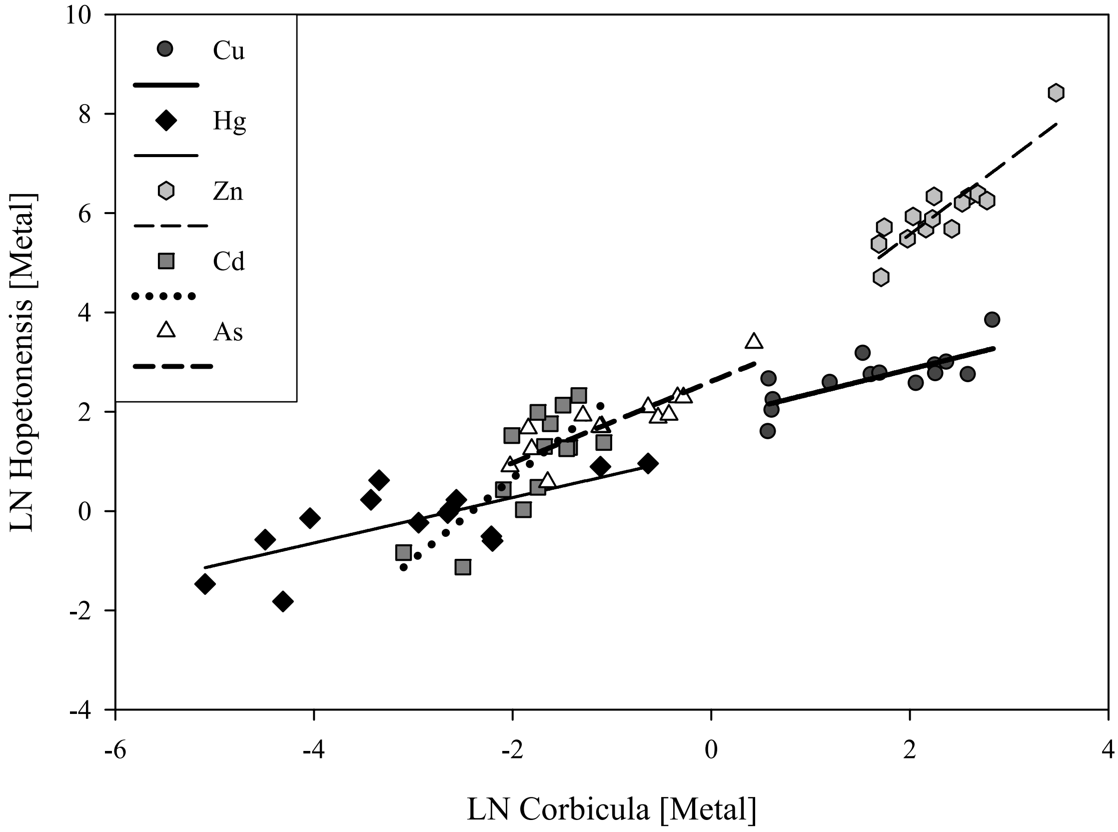
0
-1
-2
-3
-4
-2
0
2
4
6

Figure 3.3.


Cu
Hg
Zn
Cd
As
LN Hopetonensis [Metal]
LN Corbicula [Metal]
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6
4
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0
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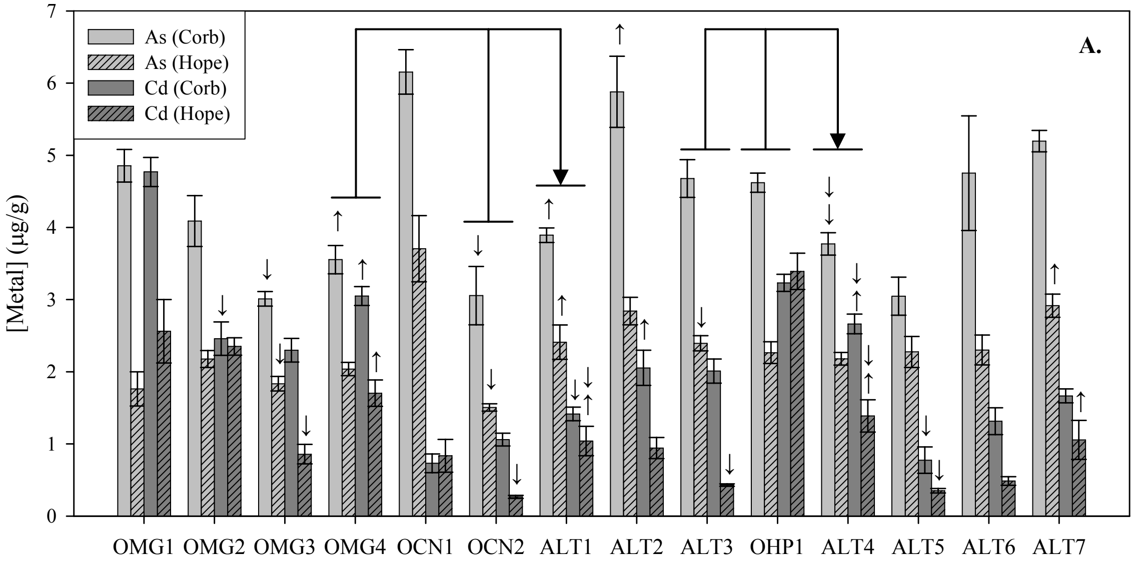
-4
-6
-4
-2
0
2
4

Figure 3.4.


A.
As (Corb)
As (Hope)
Cd (Corb)
Cd (Hope)
[Metal] (µg/g)
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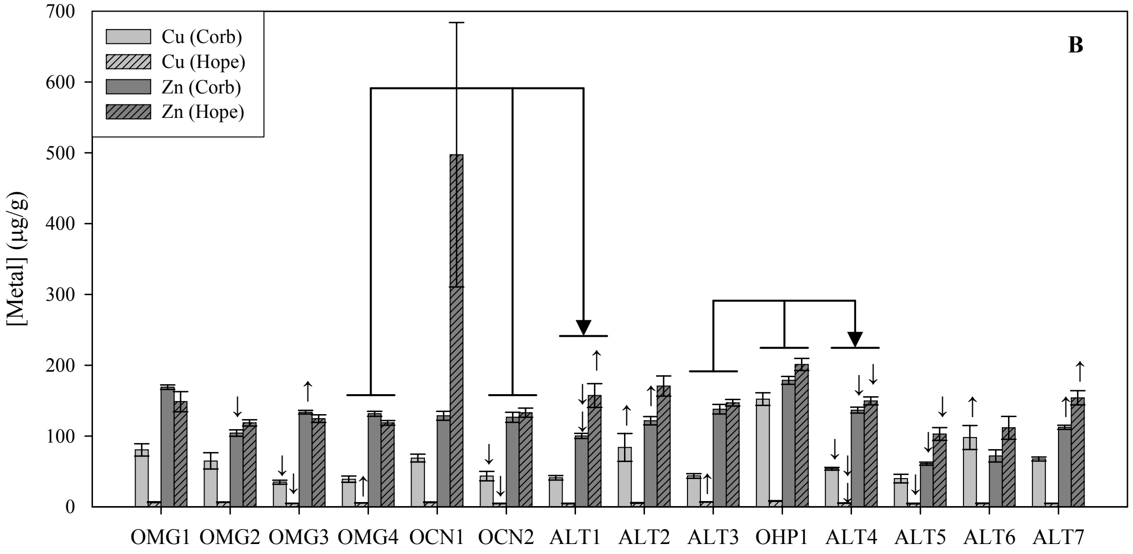
1
2
3
4
5
6
7
OMG1
OMG2
OMG3
OMG4
OCN1
OCN2
ALT1
ALT2
ALT3
OHP1
ALT4
ALT5
ALT6
ALT7


B
Cu (Corb)
Cu (Hope)
Zn (Corb)
Zn (Hope)
[Metal] (µg/g)
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300
400
500
600
700
OMG1
OMG2
OMG3
OMG4
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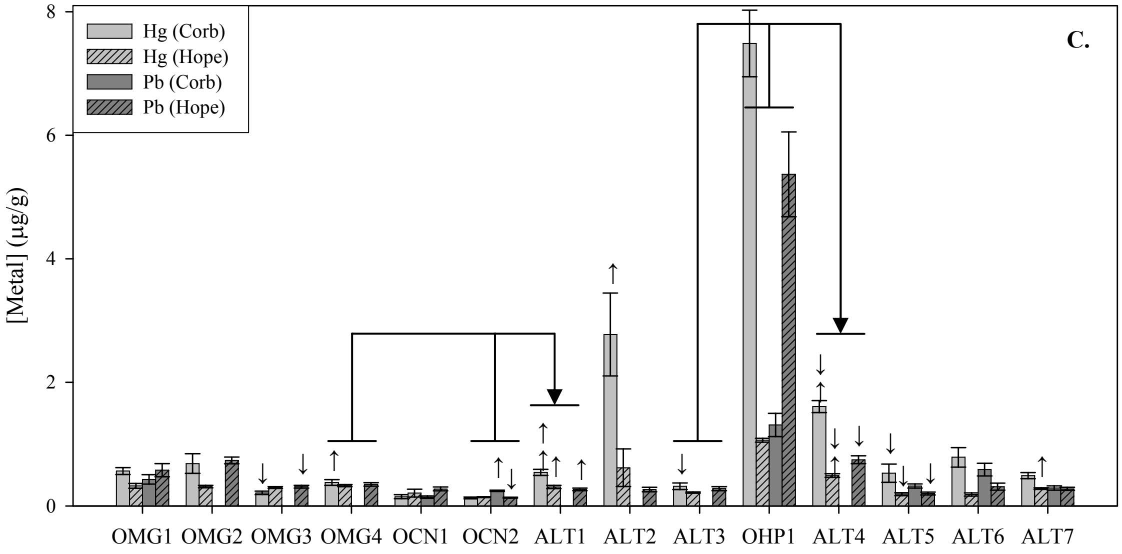
OCN2
ALT1
ALT2
ALT3
OHP1
ALT4
ALT5
ALT6
ALT7

Figure 3.4 (cont.)



References

Abaychi, J. K. and Y. Z. Mustafa. 1988. The Asiatic clam, *Corbicula fluminea* - an indicator of trace metal pollution in the Shatt Al-Arab River, Iraq. Environmental Pollution **54**:109-122.

Andres, S., M. Baudrimont, Y. Lapaquellerie, F. Ribeyre, N. Maillet, C. Latouche, and A. Boudou. 1999. Field transplantation of the freshwater bivalve *Corbicula fluminea* along a polymetallic contamination gradient (river Lot, France): I. Geochemical characteristics of the sampling sites and cadmium and zinc bioaccumulation kinetics. Environmental Toxicology and Chemistry **18**:2462-2471.

Angelo, R. T., M. S. Cringan, D. L. Chamberlain, A. J. Stahl, S. G. Haslouer, and C. A. Goodrich. 2007. Residual effects of lead and zinc mining on freshwater mussels in the Spring River Basin (Kansas, Missouri, and Oklahoma, USA). Science of the Total Environment **384**:467-496.

Baudrimont, M., J. Metivaud, R. MauryBrachet, F. Ribeyre, and A. Boudou. 1997. Bioaccumulation and metallothionein response in the Asiatic clam (*Corbicula fluminea*) after experimental exposure to cadmium and inorganic mercury. Environmental Toxicology and Chemistry **16**:2096-2105.

Cain, D. J. and S. N. Luoma. 1990. Influence of seasonal growth, age, and environmental exposure on Cu and Ag in a bivalve indicator, *Macoma balthica*, in San-Francisco Bay. Marine Ecology-Progress Series **60**:45-55.

Caldwell, R. S. and D. R. Buhler. 1983. Heavy metals in estuarine shellfish from Oregon. Archives of Environmental Contamination and Toxicology **12**:15-23.

Couillard, Y., P. G. C. Campbell, J. Pellerinmassicotte, and J. C. Auclair. 1995. Field transplantation of a freshwater bivalve, *Pyganodon grandis*, across a metal contamination gradient .2. Metallothionein response to Cd and Zn exposure, evidence for cytotoxicity, and links to effects at higher levels of biological organization. Canadian Journal of Fisheries and Aquatic Sciences **52**:703-715.

Doherty, F. G. 1990. The Asiatic clam, *Corbicula Spp*, as a biological monitor in freshwater environments. Environmental Monitoring and Assessment **15**:143-181.

Doherty, F. G., D. S. Cherry, and J. Cairns. 1987. Valve closure responses of the Asiatic clam *Corbicula fluminea* exposed to cadmium and zinc. Hydrobiologia **153**:159-167.

Doherty, F. G., D. S. Cherry, and J. Cairns. 1990. Multiseasonal tissue-growth trends in *Corbicula fluminea* (Bivalvia, Corbiculidae) from the New River, Virginia. Nautilus **104**:10-15.

Elder, J., Collins, JJ. 1991. Freshwater molluscs as indicators of bioavailability and toxicity of metals in surface-water systems. . Reviews Environ Contam Tox **122**:37-79.

Elder, J. F. and H. C. Mattraw. 1984. Accumulation of trace elements, pesticides, and polychlorinated biphenyls in sediments and the clam *Corbicula manilensis* of the Apalachicola River, Florida. Archives of Environmental Contamination and Toxicology **13**:453-469.

Graney, R. L., D. S. Cherry, and J. Cairns. 1983. Heavy metal indicator potential of the Asiatic clam (*Corbicula fluminea*) in artificial stream systems. Hydrobiologia **102**:81-88.

Graney, R. L., D. S. Cherry, and J. Cairns. 1984. The influence of substrate, pH, diet and temperature upon cadmium accumulation in the Asiatic clam (*Corbicula fluminea*) in laboratory artificial streams. Water Research **18**:833-842.

Kesler, D. H. 2004. Influence of a lentic area on condition indices of *Corbicula fluminea* in the Wolf River, Tennessee. Journal of Freshwater Ecology **19**:445-453.

Kraemer, L. R. 1979. *Corbicula* (Bivalvia, Sphaeriacea) vs indigenous mussels (Bivalvia, Unionacea) in United-States Rivers - A hard case for interspecific competition? American Zoologist **19**:1085-1096.

Luoma, S. N., R. Dagovitz, and E. Axtmann. 1990. Temporally intensive study of trace metals in sediments and bivalves from a large river, estuarine system - Suisun Bay Delta in San-Francisco Bay. Science of the Total Environment **97-8**:685-712.

Meador, J. R. 2008. The development and evaluation of a freshwater mussel sampling protocol for a large lowland river. University of Georgia, Athens, GA.

Naimo, T. J. 1995. A review of the effects of heavy metals on freshwater mussels. Ecotoxicology **4**:341-362.

Paquin, P. R., J. W. Gorsuch, S. Apte, G. E. Batley, K. C. Bowles, P. G. C. Campbell, C. G. Delos, D. M. Di Toro, R. L. Dwyer, F. Galvez, R. W. Gensemer, G. G. Goss, C. Hogstrand, C. R. Janssen, J. C. McGeer, R. B. Naddy, R. C. Playle, R. C. Santore, U.

Schneider, W. A. Stubblefield, C. M. Wood, and K. B. Wu. 2002. The biotic ligand model: a historical overview. Comparative Biochemistry and Physiology C-Toxicology & Pharmacology **133**:3-35.

Patrick, R. 1994. Rivers of the United States. J. Wiley, New York.

Perceval, O., Y. Couillard, B. Pinel-Alloul, A. Giguere, and P. G. C. Campbell. 2004. Metal-induced stress in bivalves living along a gradient of Cd contamination: relating sub-cellular metal distribution to population-level responses. Aquatic Toxicology **69**:327-345.

Pourang, N. 1996. Heavy metal concentrations in surficial sediments and benthic macroinvertebrates from Anzali wetland, Iran. Hydrobiologia **331**:53-61.

Reese, E. G. and D. P. Batzer. 2007. Do invertebrate communities in floodplains change predictably along a river's length? Freshwater Biology **52**:226-239.

Sickel, J. 1973. A new record of *Corbicula manilensis* (philippi) in the southern Atlantic slope region of Georgia. . Nautilus **87**:11-12.

Stites, D. L., A. C. Benke, and D. M. Gillespie. 1995. Population dynamics, growth, and production of the Asiatic clam, *Corbicula fluminea*, in a blackwater river. Canadian Journal of Fisheries and Aquatic Sciences **52**:425-437.

Tatem, H. E. 1986. Bioaccumulation of polychlorinated biphenyls and metals from contaminated sediment by freshwater prawns, *Macrobrachium rosenbergii* and Clams, *Corbicula fluminea*. Archives of Environmental Contamination and Toxicology **15**:171-183.

Unrine, J. M., W. A. Hopkins, C. S. Romanek, and B. P. Jackson. 2007. Bioaccumulation of trace elements in omnivorous amphibian larvae: Implications for amphibian health and contaminant transport. Environmental Pollution **149**:182-192.

USEPA. 1994. EPA Method 3015. Microwave assisted acid digestion of aqueous samples and extracts. U.S. Environmental Protection Agency, Washington, D.C.

USEPA. 1996. EPA Method 3052. Microwave assisted digestion of siliceous and organically based matrices. Revision 0 (December 1996). U.S. Environmental Protection Agency, Washington, D.C.

Villar, C., J. Stripeikis, L. D'Huicque, M. Tudino, O. Troccoli, and C. Bonetto. 1999. Cd, Cu and Zn concentrations in sediments and the invasive bivalves *Limnoperna fortunei* and *Corbicula fluminea* at the Rio de la Plata basin, Argentina. Hydrobiologia **416**:41-49.

Williams, J. D., M. L. Warren, K. S. Cummings, J. L. Harris, and R. J. Neves. 1993. Conservation status of freshwater mussels of the United States and Canada. Fisheries **18**:6-22.

Appendix 3.1. Element concentrations (in μg/g) in the tissues of each bivalve species, given as the mean of each site, plus or minus standard error. Underlined values indicate a mean that is significantly higher than the mean for the other species co-occurring at that site. These sites are those on the major tributaries of the Altamaha.

Site	Species	As	Cd	Cr	Cu	Hg	Mn	Ni	Pb	Zn
OCN1	*C. flum.*	6.35±0.31	0.83±0.13	UR	71.7±5.48	0.163±0.031	20.0±4.6	1.50±0.30	0.154±0.019	137±6
	E. hope.	3.70±0.46	0.84±0.23	UR	6.33±0.51	0.209±0.064	7171±942	1.31±0.19	0.275±0.031	497±187
OCN2	*C. flum.*	3.05±0.40	1.06±0.09	1.50±0.18	43.2±6.7	0.129±0.016	18.0±1.8	2.02±0.37	0.245±0.015	126±7
	E. hope.	1.51±0.05	0.27±0.02	0.55±0.07	4.62±0.33	0.142±0.007	2259±190	1.09±0.08	0.134±0.012	132±7
OMG1	*C. flum.*	4.85±0.23	4.77±0.20	UR	80.4±8.7	0.566±0.056	81.0±9.5	1.65±0.16	0.429±0.076	169±3
	E. hope.	1.76±0.24	2.56±0.44	UR	6.47±0.60	0.326±0.040	3901±700	3.67±1.13	0.580±0.106	148±14
OMG2	*C. flum.*	3.01±0.35	2.30±0.23	3.11±0.63	34.7±11.6	0.212±0.160	18.2±1.6	9.23±1.86	BD	134±5
	E. hope.	1.83±0.12	0.86±0.12	3.29±0.58	4.71±0.28	0.298±0.018	2897±327	1.68±0.22	0.312±0.053	125±4
OMG3	*C. flum.*	4.09±0.10	2.46±0.16	1.94±0.16	64.8±2.8	0.687±0.026	16.8±2.6	4.25±5.66	BD	104±3
	E. hope.	2.18±0.11	2.35±0.14	2.45±0.30	6.38±0.22	0.314±0.148	4323±349	1.97±0.10	0.737±0.025	119±6
OMG4	*C. flum.*	3.55±0.20	3.05±0.13	2.39±0.15	38.9±4.3	0.381±0.046	64.6±11.3	2.78±0.32	BD	131±3
	E. hope.	2.04±0.09	1.70±0.18	2.52±0.25	5.50±0.28	0.328±0.017	3148±231	1.94±0.26	0.348±0.031	118±3
OHP1	*C. flum.*	4.62±0.13	3.23±0.12	UR	152.1±9.1	7.49±0.54	45.1±11.2	7.77±4.38	1.31±0.188	179±5
	E. hope.	2.26±0.15	3.39±0.25	UR	8.14±0.41	1.06±0.032	7715±755	3.08±0.21	5.37±0.687	201±9

* Standard error could not be calculated for this element at this site.

BD = All samples were below detection for this element at this site.

UR = All samples were unreliable for this element at this site.

Appendix 3.1 (cont). Element concentrations (in µg/g) in the tissues of each bivalve species, given as the mean of each site, plus or minus standard error. Underlined values indicate a mean that is significantly higher than the mean for the other species co-occurring at that site. These sites are those on the main channel of the Altamaha River.

Site	Species	As	Cd	Cr	Cu	Hg	Mn	Ni	Pb	Zn
ALT1	*C. flum.*	3.89±0.10	1.42±0.09	UR	41.0±3.3	0.543±0.049	41.3±3.9	BD	BD	100±4
	E. hope.	2.41±0.23	1.04±0.20	UR	4.72±0.37	0.307±0.026	3473±543	1.17±0.27	0.268±0.022	157±16
ALT2	*C. flum.*	5.88±0.49	2.05±0.24	UR	83.8±19.7	2.776±0.671	105±23.3	BD	BD	122±6
	E. hope.	2.84±0.19	0.94±0.15	UR	5.45±0.41	0.620±0.304	4753±761	0.94±0.19	0.266±0.041	171±14
ALT3	*C. flum.*	4.68±0.26	2.00±0.17	8.46±0.63	43.4±3.2	0.318±0.054	35.8±2.0	5.61±0.59	BD	138±7
	E. hope.	2.39±0.10	0.43±0.02	1.73±0.09	6.78±0.34	0.217±0.011	4061±212	2.30±0.42	0.281±0.049	147±5
ALT4	*C. flum.*	6.22±2.45	6.37±3.70	4.95±1.20	63.0±9.5	1.456±0.175	19.2±1.0	3.19±0.56	0.608*	144±9
	E. hope.	2.18±0.09	1.39±0.22	2.35±0.39	5.37±0.21	0.492±0.031	4318±497	1.53±0.13	0.750±0.063	150±6
ALT5	*C. flum.*	3.05±0.26	0.78±0.18	UR	39.8±6.1	0.529±0.147	30.7±10.5	BD	0.322*	60.9±2
	E. hope.	2.28±0.21	0.35±0.03	UR	4.50±0.34	0.191±0.023	1358±217	1.25±0.39	0.200±0.030	103±9
ALT6	*C. flum.*	4.75±0.82	1.32±0.19	UR	97.8±16.9	0.787±0.156	151±96.1	2.59*	0.589*	71.8±9
	E. hope.	2.30±0.21	0.49±0.06	UR	4.90±0.34	0.187±0.025	2141±723	0.86±0.20	0.313±0.063	112±16
ALT7	*C. flum.*	5.20±0.15	1.67±0.10	5.59±0.60	67.4±2.9	0.491±0.049	31.0±3.9	3.22±0.30	0.291±0.301	112±3
	E. hope.	2.92±0.16	1.06±0.27	2.96±0.31	4.73±0.23	0.282±0.014	5619±661	1.43±0.11	0.277±0.036	154±10

* Standard error could not be calculated for this element at this site.

BD = All samples were below detection for this element at this site.

UR = All samples were unreliable for this element at this site.

CHAPTER FOUR

THE ASIAN CLAM *CORBICULA FLUMINEA* AS A BIOMONITOR OF TRACE ELEMENT CONTAMINATION: ACCOUNTING FOR NATURAL VARIATION WHEN IDENTIFYING ANTHROPOGENIC SOURCES[1]

Abstract

Widespread decline of freshwater mussel populations has led to interest in monitoring anthropogenic contaminants that could potentially be harming native mussel populations. In this study, specimens of the invasive clam, *Corbicula fluminea,* were collected above and below possible sources of potentially toxic trace elements (As, Cd, Cr, Cu, Hg, Pb and Zn) in the Altamaha River System. Their bioaccumulation of these elements was quantified, along with environmental (water and sediment) concentrations. Hierarchical linear models were used to decrease variability in concentrations related to environmental (site water chemistry and sediment characteristics) and individual (growth metrics) variables. This study found significantly elevated bioaccumulation of Cd, Cu and Hg below the outfall of kaolin processing facilities, Zn below a tire cording facility, and Cr below a nuclear power plant and a paper pulp mill. Hierarchical linear models found correlations between trace element accumulation and variables such as upstream distance, dissolved oxygen, % silt and clay in the sediment, elemental sediment concentration, shell length and bivalve condition index. Taking into account this variability reduced the number of sites with significantly elevated trace elements. This finding

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shows that conclusions about trace element distribution cannot be drawn from bioaccumulation studies without taking into account environmental and individual sources of variation.

Introduction

An unfortunate recent development in aquatic ecology has been the widespread decline of native mussel populations throughout the United States (Williams et al. 1993). There are numerous possible explanations for these declines, including habitat alteration from sedimentation and impoundment, changes in fish-host populations and distributions, competition from invasive species and exposure to anthropogenic toxicants (Williams et al. 1993, Naimo 1995, Vaughn and Taylor 1999).

Toxic trace elements such as arsenic (As), cadmium (Cd), chromium (Cr) copper (Cu), mercury (Hg), lead (Pb) and zinc (Zn) have been shown to exert acute and chronic toxic effects on various freshwater bivalves (Elder 1991, Naimo 1995). Responses can include altered valve movement (Doherty et al. 1987), inhibition of growth (Belanger et al. 1986), reduced fecundity (Perceval et al. 2004) and mortality (Harrison et al. 1984, Elder 1991, Naimo 1995). Because of their ability to accumulate trace elements following exposure, freshwater bivalves, particularly the invasive Asian clam *Corbicula fluminea* (hereafter *Corbicula*), are frequently used as biological monitors of trace elements contamination (Millington 1983, Abaychi and Mustafa 1988, Doherty 1990, Luoma et al. 1990, Pourang 1996, Gundacker 2000, Tomazelli et al. 2003, Sebesvari et al. 2005, Angelo et al. 2007).

In biomonitoring studies, an increased concentration of trace elements in the tissues of the biomonitor species is typically considered an indicator of increased environmental availability of that element. However, a host of environmental factors that include size and age (MetcalfeSmith et al. 1996), sampling season (Luoma et al. 1990) and the chemistry of surrounding water and sediment (Graney et al. 1984, Angelo et al. 2007) have all been shown to be correlated to the bioaccumulation of various trace elements. This makes it difficult to draw conclusions about contaminant levels based solely on tissue concentrations of trace elements in the biomonitor organism.

The Altamaha River system is the largest drainage basin encompassed by the state of Georgia and is home to 3 imperiled native mussel species (Meador 2008). The Oconee River, a primary tributary of the Altamaha is also home to an extensive kaolin mining and processing industry (Pruett 2000). The kaolin industry in Georgia and abroad, has been implicated for increasing loads of Zn and Hg in local sediments (Jordao et al. 2002, Lasier et al. 2004), with potential detrimental implications to native mussels. Other potential point sources for trace elements in this region include a former tire cording plant, which has been fined for excessive discharge of Zn and Cu, a nuclear power plant, a paper mill and a state prison. The area is also subject to non-point sources, although its lower reaches have only small urban areas and little light industry.

This study examined 1) whether proximity to various industrial activities increases the concentrations of trace elements in resident bivalves; 2) the utility of hierarchical linear modeling in accounting for variability in trace elements accumulation; and 3) the amount of variability in elemental concentrations that could be accounted for by environmental factors or individual growth rather than human activity. This would not only indicate sources of stressors to bivalves in the Altamaha River but it would also provide a more accurate indication of human influence on trace element accumulation.

Materials and Methods

Study Area

The Altamaha River drainage is completely contained within the state of Georgia, USA. The mainstem of the Altamaha River is formed by the confluence of the Ocmulgee and Oconee Rivers, whose headwaters are in the piedmont phyto-geographic province and cross the fall line at Macon and Milledgeville GA, respectively (Reese and Batzer 2007). Study sites were chosen based on potential point sources of trace elements and to provide coverage of the majority of the watershed area.

Twenty-two sites were chosen for this study, with sites located upstream and downstream of 6 potential point sources of trace elements: Little Commissioner's Creek (LCC) which carries effluent from a kaolin processing facility, Kettle Creek (KC) which drains an area with active kaolin mines, the former Amercord tire facility (AMC) on the Ocmulgee River, Plant Hatch Nuclear Power Station (PH) and the Rayonier paper mill (RAY) on the Altamaha River and the Reidsville State Penitentiary (RSP) on the Ohoopee River (Figure 4.1). The remaining 10 sites were spread out among the rivers and their tributaries, with emphasis placed on the Oconee because of the large amount of kaolin mines within its drainage. Habitats sampled included depositional sloughs, edges of sandbars, rocky shelves of the unique "Altamaha grit" microhabitat and gravel seams in the main channel. GIS coordinates were taken and recorded for each site at the time of sampling (Magellan explorist 210; Thales Navigation, San Diego CA), and a sketch of the site was made indicating major features of substrate and local morphology. Sampling occurred during the spring and early summer of 2007.

Sample Collection

Sample collection is described in detail in Chapter 3. Briefly, water temperature, dissolved oxygen, pH and conductivity were determined on site, while water alkalinity and hardness were determined later in the laboratory. Grab samples of water (~500 mL), sediment (~500 mL) and *Corbicula* (5-35 individuals) were collected at each site. A petite ponar grab was used to collect sediment and was also used to collect *Corbicula* during high flows. All samples were kept in a cooler that contained small amounts of ice until they could be transported back to the laboratory.

Sample Processing

Water and *Corbicula* processing are described in more detail in Chapter 3. Water samples were filtered (0.5 µm filter) and acidified (10%; trace metal grade HNO_3). *Corbicula* were measured for length, height, depth and shell mass. Whole tissues were removed from shells, dried, massed and stored in acid-washed vessels. Shell valves were measured for volume as described in Kesler (2004).

Sediment samples were split into two separate sub-samples. One sub-sample was re-homogenized and poured through a 1 mm acid-washed plastic sieve. The resulting < 1 mm fractions were placed in acid-washed nalgene containers and stored at –20°C. Frozen samples were thawed under refrigeration at a later date and wet-sieved again through a 105 µm Nytex mesh (Spectrum Mesh; Rancho Dominguez CA, USA) into an acid-washed 600 mL beaker as in (Luoma et al. 1990). Fine fractions were dried at temperatures ranging from room temperature to 50°C, homogenized using a dry, acid-washed mortar and pestle and stored in acid washed centrifuge tubes under anhydrous conditions until digestion.

The other sub-sample was refrigerated until it could be homogenized and dried. It was then put through nested sieves (4 mm, 2 mm, 1 mm, 500 µm, 250 µm, 125 µm and 64 µm mesh) as recommended by Plumb (1981). These sediment fractions were carefully transferred to weigh boats and massed to determine the percentage of each size fraction in the overall sample. At least 98% of the original material was accounted for.

Acid-Digestion

Between 0.25 and 0.5 g of dried fine fraction sediment were completely digested in 8 mL trace metal grade HNO_3 and 3 mL HF, using a MARS 5 HP 500 Plus system (CEM Corp; Matthews NC, USA), under the conditions of EPA protocol 3052 (USEPA 1996). Three replicate samples were digested for each of the sediment composites. All digestions were diluted in 24 mL of Milli-Q water to ~33% acid. Blanks and standard sediments (MESS-3, National Research Council of Canada, Ottawa ON, Canada; NIST 1645, National Bureau of Standards, Gaithersburg MD, USA) were also digested with each run. All samples, blanks and standards were stored at room temperature in acid-washed centrifuge tubes until analysis.

Whole tissues of *Corbicula* were completely digested in 5 mL of trace metal grade HNO_3, using a MARS 5 HP 500 Plus system (CEM Corp; Matthews NC, USA), under the conditions of EPA protocol 3015 (USEPA 1994). Digestions were diluted with 10 mL Milli-Q water to ~33% acid. Exact concentrations were calculated using dry tissue mass and the mass of the final solution. One blank (acid only) and one standard reference material (SRM) of ~0.075 g TORT-2 lobster hepatopancreas (National Research Council of Canada, Ottawa ON, Canada) were digested in each run. Dilute samples, blanks and standard were stored at room temperature until analysis.

Sample Analysis

Analyses of digested samples were carried out as described in Unrine et al. (2007). Trace element concentrations in digests (As, Cd, Cr, Cu, Hg, Mn, Ni, Pb and Zn) were quantified using a Perkin-Elmer Sciex Elan DRC Plus inductively coupled plasma-mass spectrometer (ICP-MS; Norwalk CT, USA) operating in standard mode. Method detection limits (MDLs) were calculated using blank digests. Mean MDLs ranged from 0.0250 µg/g for Cd to 3.360 µg/g for Cu. Recoveries of SRMs were in good agreement with certified values, mean recoveries ranging from 85.27% for As and 130.04% for Pb. Relative percent difference (RPD) of replicate dilutions of sub-samples was used to approximate analytical error. On average (n=34) RPD ranged from 2.01% for As and Zn to 12.80% for Pb. Spikes of ~2-5 times the un-spiked sample concentration had mean recoveries ranging from 91.61% for Cr and 101.87% for Ni (n=33).

Statistical Analysis

Initially, analysis of variance (ANOVA) followed by Duncan's post hoc test were calculated using Statistical Analysis Software (SAS v9.1, SAS Institute, Cary NC, USA). These tests determine significant ($\alpha = 0.05$) differences between metal concentrations in clams collected both upstream and downstream of potential trace element sources (Fig. 4.1).

In order to reduce variation in the data caused by natural factors, we employed hierarchical linear models. Hierarchical linear models are able to reduce variation at multiple levels of biological organization, such as the individual level, the site (i.e. local environment) level, the regional (e.g. watershed) level, etc. By reducing naturally occurring variation, this approach can then test what differences in bioaccumulation are more likely due to human activities. This approach also allows multiple models to be applied to the data and ranked for their usefulness (Burnham and Anderson 2001).

In our approach to limiting natural variation in bioaccumulation, we used predictors at two levels of biological organization. Individual (Level-1) predictors were: shell length, height and depth; shell thickness index [shell mass / shell length (Griffiths and Cyr 2006)]; condition index [tissue dry weight / shell volume; (Kesler 2004)]; tissue dry weight. Site specific (Level-2) predictors were: latitude, longitude and upstream distance from the estuary; day of the year collected; water temperature, DO, pH, conductivity, alkalinity and hardness; percent sediment fraction (silts/clays, very fine sand, fine sand, medium sand, coarse sand, very coarse sand, fine gravel, gravel); sediment sorting index (standard deviation of sediment fractions); sediment coarseness index (weighted average of sediment fractions); and water and sediment concentrations of the element being modeled. Incorporating Level-1 predictors into a model decreases variability within a site, while incorporating Level-2 predictors into a model decreases variability between sites.

All level-2 predictors were considered singly and the decrease in inter-site variation calculated. All combinations of the Level-2 predictors that decreased inter-site variation were then considered in two-variable models unless they were correlated to each other (Pearson's coefficient squared < 0.30). Level-1 predictors were then considered singly and the decrease in intra-site variation calculated for each. Fully hierarchical models of 1 Level-1 predictor and 1 Level-2 predictor were also considered for all Level-1 predictors, combined with most Level-2 predictors (only the 3 sediment fractions which decreased intra-site variation the most and %silt/clay were considered in this manner).

The Aikake Information Criteria (AIC) were calculated for all models using the following formula:

$$\mathrm{AIC} = -2*\ln(\mathrm{likelihood}) + 2*K$$

Where K = the number of parameters in the model (Burnham and Anderson 2001).

Data sets where $n/K < 40$ had AICs bias-adjusted using the following formula:

$$\mathrm{AIC_C} = -2*\ln(\mathrm{likelihood}) + 2*K + [2*K*(K+1)]/(n-K-1)$$

AIC values were used to rank models, with the model with the lowest AIC being the best fitting model. Subtracting the lowest AIC from all AICs resulted in ranked Δi values, such that the best fitting model had $\Delta i = 0$. Finally Aikake weights were calculated to quantify the plausibility of the model, using the following equation:

$$w_i = \frac{exp(-0.5*\Delta i)}{\Sigma^{R}_{r=1}\, exp(-0.5*\Delta_r)}$$

Only models in which $w_{i(\Delta i>0)} > (w_{i(\Delta i=0)} / 2)$ were considered for the selected model. In other words, only models for which the best-fit model was less than twice as likely were considered. A final model for each element was selected based on low Aikake weights, the environmental importance of the predictors used in the model and the ability to make up/downstream comparisons using the model (see below).

Using the final models to take into account inter- and/or intra-site variability, the difference of the intercepts (i.e. mean tissue concentrations after the model is fit) for all the up/downstream pairs was calculated. The 95% confidence intervals of all difference were also calculated. Significantly non-zero ($p < 0.05$) differences were those whose 95% confidence intervals did not overlap zero. A non-zero difference indicated that the mean concentrations of the up/downstream pair were significantly different.

Results

Mean concentrations of elements in all three compartments (water, sediment, *Corbicula tissues*) are compiled in Appendix 4.1. The up/downstream comparisons of mean tissue concentrations made using ANOVA with Duncan's post hoc test are represented in Figure 4.2. Of the six up/downstream comparisons, the site downstream of LCC is the only one that consistently showed significantly ($p < 0.05$) higher tissue concentrations of trace elements (Cd, Cu, Hg, and Zn) than in the tissues of clams collected from the site upstream (Figure 4.2). All other sites demonstrated no definitive trends between elements, showing significantly higher concentrations in clams for one or more element both upstream and down.

Best fitting hierarchical linear models for each element are given in Table 4.1 with AIC, Δi and w_i values. The final hierarchical models selected for each element are given in Table 4.2 with estimates for each parameter and an estimation of the reduction in variability resulting from using the model. Models with $\Delta i > 0$ were chosen over models with $\Delta i = 0$ when $w_{i(\Delta i>0)} > (w_{i(\Delta i=0)} / 2)$ and when their model parameters were deemed more environmentally relevant. For instance, upstream distance was considered to be more environmentally relevant to stream spatial organization than longitude or latitude and was preferred to these two (As and Cr models; Table 4.1). In one instance (Cd model) a final model in which $\Delta i > 0$ was selected because the model in which $\Delta i = 0$ could not calculate all up/downstream comparisons (Table 4.1).

The differences in the final model intercepts (mean concentrations of elements) between clam samples at each up/downstream pair are given in Figure 4.3. Note that the 95% confidence interval (CI_{95}) displayed in this graph provides a visual indication of significant differences. If the CI_{95} overlaps with zero, that indicates there is no difference between the concentrations found at each site after the final model reduces variability.

Discussion

Applying the hierarchical linear models to the bioaccumulation data from the 22 sites reduced overall variability in the data to varying degrees (Table 4.2). When these models were used to assess six up/downstream comparisons, fewer significant differences between sites were found than when the tissue concentrations of these sites were compared directly. In this section, we will discuss the effects of the model on our conclusions and how they relate to previous studies.

Arsenic (As)

When ANOVA was used to compare the six up/downstream comparisons in our study, two were found to be significantly different, indicating higher exposure to As at one site rather than the other (Figure 4.2B). Following the selection of a final model (Table 4.1 & 4.2), variability related to DO and upstream distance was controlled and the resulting comparisons revealed no significant differences between any up/downstream pair (Figure 4.3B).

Few studies have used *Corbicula* as a biomonitor species for As, although past work has shown no relation between As accumulation and the length or shell weight of organisms (Sebesvari et al. 2005). This agrees with our finding that organism growth metrics did not explain As accumulation in *Corbicula*. On the other hand, our findings indicate that DO influences As accumulation, with clams collected from more oxygenated surface waters portending higher accumulation. This increased accumulation may be due to increased availability of the arsenate ion, the dominant form of As under oxic conditions. It could also be related to a seasonal fluctuation in As accumulation, since higher DO accompanies higher flows and colder temperatures. The selected model also incorporated stream distance to decrease inter-site variability, with upstream sites tending to show lower levels of bioaccumulation. The sites that we sampled on smaller, blackwater tributaries tended to be located further upstream, while sites sampled on river mainstems were on average located further downstream. Thus, these differences in accumulation could be attributed to differences in the chemistry between small, blackwater tributaries and main river channels. In either case, it appears that variations in As bioaccumulation at these site pairs can be primarily attributed to environmental differences at each site rather than human activity.

Cadmium (Cd)

Using ANOVA, *Corbicula* collected from a site directly downstream of LCC were found to have accumulated significantly higher concentrations, while the reverse was true for sites above and below RAY (Figure 4.2B). However, using a linear hierarchical model incorporating the length of individual clams and the % of silt and clay (<64 μm) particles at each site, the difference between the RAY sites was no longer significant at $\alpha = 0.05$ (Figure 4.3B).

These Cd data illustrate the importance of taking into account environmental conditions at each site before attributing significant differences to human activities. The site upstream of RAY was predominantly very coarse sand (47.85%) with little silt/clay (1.26%). Meanwhile, the downstream site was located in a slough with predominantly very fine sand (49.16%) and a large amount of silt/clay (16.28%). Past studies using artificial streams have found that *Corbicula* accumulate significantly less Cd in a predominantly silt/clay system than in systems that were predominantly or completely sandy in composition (Graney et al. 1984). Past studies also support our finding that longer *Corbicula* accumulate higher concentrations of Cd than smaller individuals (Abaychi and Mustafa 1988, Metcalfe-Smith et al. 1996, Angelo et al. 2007).

However, even with the model taking into account some of the variation in observed concentrations, clams collected from the site below LCC accumulated significantly higher Cd concentrations than the upstream site. This indicates that effluent from the kaolin-processing plant or another local industry may be introducing bioavailable Cd to Commissioner's Creek (an Oconee tributary) via LCC. This is somewhat surprising, given that sediments potentially impacted by kaolin processing activity typically show concentrations of Cd below detection (Jordao et al. 2002, Lasier et al. 2004). The concentrations (Appendix 4.1) of Cd found in sediments from our study sites on the Oconee were 1-2 orders of magnitude greater than those found by Lasier et al. in the same area in 1998, using similar methodos. This may indicate a new source of Cd in the area or a change in industrial practices.

Chromium (Cr)

Clams sampled at sites downstream of both the Plant Hatch (PH) nuclear power facility and Rayonier paper mill (RAY) accumulated significantly higher concentrations of Cr than their upstream counterparts when compared using ANOVA (Figure 4.2B). Subsequent hierarchical modeling resulted in a model using organism condition and stream distance as the primary controls for variability, such that *Corbicula* with a better condition that were located further downstream, accumulated greater concentrations of Cr. Even after the model took into account the variation caused by condition and stream distance, significant differences were still found between the paired sites at PH and RAY (Figure 4.3B).

Past studies have shown a correlation between Cr accumulation in *Corbicula* and organism size, the season of collection and sediment loads of Cr (Luoma et al. 1990). In our study, indicators of season (temperature, DO, day of year) and $[Cr]_{Sediment}$ did not produce one of the best-fit models. Condition of the organism [tissue dry weight / shell volume; (Kesler 2004)] was used in the selected model, with organisms in poorer condition showing higher accumulation of Cr. Because dry weights of *Corbicula* can fluctuate seasonally (Luoma et al. 1990), this may indicate some seasonal fluctuation in Cr accumulation. As with As, organisms in smaller tributaries tended to accumulate lower concentrations of Cr, probably leading to the influence of stream distance.

The significant difference between organisms above and below PH is most likely due to the fact that the nuclear power station is a permitted discharger of Cr and Zn (USEPA 2006) and elevated tissue concentrations of these elements may be expected. RAY, a paper fibers mill, has permits to discharge Hg, Mn, Ni, Pb, V and Zn but not Cr (USEPA 2006). However, Cr has been found bound to a potentially bioavailable fraction of pulp and paper waste from a mill in Finland (Poykio et al. 2007). It is also closely associated with the ferro-manganese oxide (FMO) fraction of freshwater sediment (Koretsky et al. 2006). By releasing large amounts of Mn compounds [110000 lbs/year; (USEPA 2006)], the mill may be increasing bioavailability of Cr.

Copper (Cu)

ANOVA comparisons indicated significant differences between two site pairs (AMC and LCC; Figure 4.2A). Model selection resulted in a best-fit model using shell length and upstream distance as the variables. After controlling for these variables, both differences were still found to be significant, while a third site pair was found to be significantly different (RSP; Figure 4.3A).

Like Cr, Cu has also been reported to vary with organism size, season, and sediment concentrations of the metal (Luoma et al. 1990). Also like Cr, our best-fit model used stream distance along with a growth metric (length) to reduce variation. With respect to the site pairs showing significant differences, the site downstream of LCC showed significantly higher concentrations of Cu. Sediment in the Oconee River has been shown to be enriched with Cu (Lasier et al. 2004) but the authors could not link these levels to kaolin activities. Our findings indicate a source of Cu emanating from LCC that causes increased concentrations of Cu in downstream organisms. Copper readily sorbs to kaolin particles, with increased adsorption in the presence of humic acids (Arias et al. 2002) and neutral pH (Katsumata et al. 2003) such as can be found in some blackwater systems (Patrick 1994). Because bivalves preferentially filter fine particles (Vaughn and Hakenkamp 2001), the presence of kaolin particles in solution could be mobilizing copper and other metals. This is in agreement with some of the conclusions of the biodynamic model of trace element bioaccumulation, which states that assimilation from food is the primary source of metals to aquatic organisms (Luoma and Rainbow 2005). This has been found to be true for Cu accumulation in *Corbicula* in particular (Croteau et al. 2004, Croteau and Luoma 2005).

The other two site pairs (AMC and RSP) had upstream sites with significantly higher concentrations of Cu. In the case of AMC, the former Amercord tire plant was fined for illegal release of Cu compounds (ARK 2003), which would imply that organisms below its effluent release may show increased accumulation. The Cu compounds released by the Amercord plant apparently did not have a significant impact on accumulation at this site at the time of sampling. Operations ceased at this plant in 2001, so discharges since that time have been limited to rain overflow of chemical storage tanks (Spinrad 2003). This may mean that Cu at this site has been diluted or sequestered with time, limiting the bioaccumulation that we observed, although there was no difference in the Cu concentrations found in the sediment (Appendix 4.1). Sediment sequestration of metals can occur fairly quickly and while it may decrease exposure in the short term, contaminated sediment may serve as a long-term source (Salomons et al. 1987, Foster and Charlesworth 1996).

The last site with significant differences after the model was taken into account (RSP) is unique in being the only site whose difference was *not* shown to be significant using ANOVA. The reason our linear hierarchical model indicated that the differences were significant was because the downstream organisms were on average larger, while mean concentrations of Cu were similar between the two sites. Controlling for shell length accentuated the slight difference between the two sites. In other words, the upstream organisms accumulated higher levels of Cu despite being smaller than the downstream organisms. Because Cu accumulation normally increases with length, this small difference in concentration becomes important.

Mercury (Hg)

Of our six site pairs compared using ANOVA, three upstream sites were found to have significantly higher levels of bioaccumulation of Hg than their downstream counterpart, while one downstream site had significantly higher Hg concentrations (Figure 4.2C). The best-fit linear hierarchical model for Hg used only the variable of shell length. Past studies have also found bivalves with longer shells to accumulate higher concentrations of Hg (MetcalfeSmith et al. 1996). Because larger individuals are older (Stites et al. 1995), this is probably due to fact that the methylated form of Hg is readily accumulated in *Corbicula* tissues over time, with much slower elimination that inorganic Hg (Inza et al. 1998). Older individuals will have more time to accumulate Hg but will be unable to eliminate it.

After taking into account organism length, only two up/downstream pairs showed significant differences in Hg bioaccumulation (Figure 4.3C). One pair consisted of the sites above and below KC. In this case, the upstream site showed higher concentrations. This probably reflects elevated methylation that takes place in wetlands (Rudd 1995), especially humic-rich, blackwater wetlands (Jagoe et al. 1998), such as those that make up the riparian zone of Buffalo Creek, where the sites were located. A recent survey of trace elements accumulated by *Corbicula* in the Chattahoochee and Broad Rivers (Peltier et al. 2008) found the highest Hg accumulation in catchments with higher percentages of forest cover. The authors attributed this to the greater organic materials associated with forested catchments.

The other pair were again the sites above and below LCC. The downstream clams accumulated significantly more Hg than the upstream site. This is not surprising, considering that Lasier et al. (2004) found that sediments in the Oconee River directly below the confluence with Commissioner's Creek had concentrations of Hg higher than 83% of the sediments in the National Status and Trends data set. Our sediment samples were mostly below detection for Hg, but one site (OCN2) within the same region sampled by Lasier et al. contained similar concentrations of Hg as those found by that study (Appendix 4.1). Commissioner's Creek seems to carry Hg to the Oconee River and that Hg enters the creek after the confluence with LCC. As with other metals, Hg readily sorbs to kaolin in freshwater (Bilinski et al. 1991). It is therefore possible that kaolin-processing activities may be responsible for increased Hg bioaccumulation and concentration in the fine fractions of sediment. Again, this is in accordance with the biodynamic model of bioaccumulation (Roditi et al. 2000).

Lead (Pb)

Lead accumulation showed no significant differences in any up/downstream pair analyzed, either using ANOVA or a linear hierarchical model. This could be due to low bioavailability of Pb in the environment, as evidenced by how frequently we encountered tissue samples below detection. The best fitting model for Pb used, like Hg, shell length alone. In this case, larger individuals were found to have accumulated lower concentrations of Pb, possibly due to growth dilution or older organisms sequestering Pb in their shells. This finding is similar to previous studies that have found higher concentrations of Pb in smaller *Corbicula* (Abaychi and Mustafa 1988, Angelo et al. 2007). Previous studies of kaolin-processing activity have also not found that it substantially increases the concentrations of Pb in nearby sediments (Jordao et al. 2002, Lasier et al. 2004).

Zinc (Zn)

ANOVA analysis showed mean tissue concentrations of Zn to be variable at five of the six paired sites, making it the most significant differences of any element I analyzed (Figure 4.2A). The best-fit model for Zn used the variables clam condition (tissue dry mass / shell volume) and sediment Zn concentrations (Table 4.2). Taking those variables into account, only one pair remained significantly different (Fig. 4.3A). This pair was made up of the sites on the Ocmulgee above and below the outfall of the former Amercord tire plant (AMC), with the downstream site having higher concentrations. This is to be expected, since Amercord was fined in 2003 for discharging excessive amounts of Cu and Zn compounds (ARK 2003). Increased Zn concentrations in *Corbicula* tissues probably reflect this increased exposure to metals.

Two of the other site pairs which showed higher concentrations downstream were those above and below LCC and KC. In both cases, the downstream sediment had much higher concentrations of Zn (Appendix 4.1). While taking into account Zn sediment concentrations rendered these differences non-significant, it should be noted that kaolin processing uses large amounts of Zn compounds and is known to increase concentrations of Zn found in sediment (Jordao et al. 2002, Lasier et al. 2004). Therefore, if Zn bioaccumulation in *Corbicula* is strongly tied to exposure via sediment, kaolin mining and processing activities could still account for the different Zn concentrations found at the downstream sites. This agrees with the findings of some biogeochemical models, which are based on the idea that easily extractable metals and sediment chemistry are some of the major factors in bioaccumulation by bivalves (Tessier et al. 1984).

Conclusions

Several conclusions can be drawn from the results of this study.

1) Size of individual clams can be used to predict the accumulation of many trace elements within a site. Our selected models for all elements but As included shell length or organism condition as a variable.

2) Location within a river system also greatly affects accumulation. Our selected models for As, Cr and Cu all used stream distance as a variable, with *Corbicula* collected from upstream sites in smaller tributaries accumulating lower concentrations. This result warns against using smaller upstream tributaries as “reference” sites and comparing them to contaminated sites in larger rivers. While this approach lends itself to the null hypothesis testing approach used in ANOVA and other common statistical tests, it could produce inappropriate comparisons. In other words, “reference” sites could show lower bioaccumulation not just because of less human activity but also because of different chemicophysical characteristics.

3) A significant source of the potentially toxic metals Cd, Cu, Hg and Zn appears to enter the Altamaha river system in the vicinity of the confluence of Little Commissioner’s (LCC) and Commissioner’s Creeks. However, significant amounts of metals do not appear to be entering the system at the confluence of Kettle (KC) and Buffalo Creeks. The evident conclusion is that effluent from kaolin processing at LCC is increasing bioaccumulation of trace elements in the system, while kaolin-mining activities in the watershed of KC does not seem to affect accumulation. More studies should be conducted to determine the range and extent of kaolin processing impacts on trace element contamination.

4) Our findings show agreement with some of the tenets of two of the principle models for metal bioaccumulation in aquatic invertebrates: biogeochemical and biodynamic models. Because bivalves both filter out particulate matter as their principle foot and interact closely with this sediment, it is probable that both contribute to bioaccumulation of trace elements. Kaolin, as easily suspended fine particles that readily sorb a variety of metals, may mobilize potentially toxic trace elements as well as increasing their bioavailability. More research in this system should be conducted to analyze the affect of particulate matter on bioaccumulation and the role of kaolin effluent on particulate matter.

Finally, this study demonstrates the utility and necessity of taking into account both individual and environmental differences when comparing biomonitor populations. Simply comparing tissue concentrations between two populations can lead to erroneous conclusions and misattribution of contamination to human activities.

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Table 4.1. Best fitting models for each metal, with the model's parameters, the number of parameters (K), the residual sum of squares (RSS), the Aikake Information Criteria (AIC_C), difference between the AIC of the model and that of the model with the lowest AIC (Δi), and the Aikake weight (w_i). Note that only models with w_i within 10% of the lowest w_i are included for each element (in the case of Zn there was no other model that qualified). Models selected as the final model for each element are in bold.

Element	Model	K	RSS	AIC_c	Δi	w_i
As	Longitude, DO	5	9166	723.6	0.0	0.1269
As	DO	4	9303	724.0	0.4	0.1060
As	Latitude, DO	5	9186	724.0	0.4	0.1060
As	**Stream Distance, DO**	**5**	**9196**	**724.2**	**0.6**	**0.0940**
Cd	Thickness Index, $[Cd]_{Sediment}$	7	34172	967.0	0.0	0.5433
Cd	**Length, % Silt/Clay**	**7**	**34342**	**968.0**	**0.9**	**0.3464**
Cd	Weight, $[Cd]_{Sediment}$	7	34836	971.0	3.5	0.0944
Cr	Condition, Longitude	7	4947	536.7	0.0	0.0839
Cr	Longitude, Alkalinity	5	5113	537.2	0.5	0.0645
Cr	Longitude	4	5189	537.3	0.6	0.0627
Cr	**Condition, Stream Distance**	**7**	**4967**	**537.3**	**0.6**	**0.0627**
Cu	**Length, Stream Distance**	**7**	**762735**	**1532.64**	**0.0**	**0.3679**
Cu	Length, Longitude	7	765254	1533.24	0.6	0.2369
Cu	Length, $[Cu]_{Sediment}$	7	772011	1534.84	2.2	0.0861
Hg	**Length**	**5**	**689**	**250.6**	**0.0**	**0.5814**
Hg	Length, % Medium Sand	7	682	253.0	2.3	0.1282
Hg	Length, Coarseness Index	7	689	254.9	4.2	0.0126
Pb	**Length**	**5**	**779**	**226.9**	**0.0**	**0.4124**
Pb	Dry Weight, % Silt/Clay	7	760	228.6	1.7	0.1787
Pb	Height, % Silt/Clay	7	770	230.1	3.2	0.0844
Zn	**Condition, $[Zn]_{Sediment}$**	**7**	**1452246**	**1649.8**	**0.0**	**0.9930**

Table 4.2. Selected linear hierarchical models for each trace element, with the predictors for each model and percent decrease of τ_{00} (intrer-site variability) and σ^2 (intra-site variability). The values in parentheses indicate the percentage of the total variability decreased.

Element	**Level-2 Predictor**	**Level-2 Predictor**	**Level-1 Predictor**	**% decrease in τ_{00}**	**% decrease in σ^2**
As	Stream Dist -0.4256	DO 0.6717	*None*	49.84% (19.15%)	-0.08% (-0.05%)
Cd	% Silt/Clay -2.437	*None*	Length 0.2299	-72.05% (-16.35%)	20.73% (16.01%)
Cr	Stream Dist -0.7738	*None*	Condition -0.5720	-0.30% (-0.20%)	17.79% (6.11%)
Cu	Stream Dist -5.8349	*None*	Length 11.399	27.19% (12.97%)	24.48% (12.81%)
Hg	*None*	*None*	Length 0.2251	4.03% (3.39%)	34.66% (5.49%)
Pb	*None*	*None*	Length -0.0017	-33.56% (-2.93%)	23.32% (18.55%)
Zn	$[\mathrm{Zn}]_{\mathrm{Sediment}}$ 25.095	*None*	Condition 3.2799	63.63% (45.41%)	11.21% (3.21%)

Figure 4.1. Map of the locations sampled in this study. The labels refer to the six site-pairs used to make up/downstream comparisons of trace element bioaccumulation at sites of interest: Amercord tires (AMC), Little Commissioner's Creek (LCC), Kettle Creek (KC), Plant Hatch (PH), Rayonier (RAY), and Reidsville State Penitentiary (RSP).

Figure 4.2. Average *Corbicula* tissue concentrations of (A) Cu and Zn; (B) As, Cd and Cr; and (C) Hg and Pb of clams found upstream and downstream of sites of interest: Amercord tires (AMC), Little Commissioner's Creek (LCC), Kettle Creek (KC), Plant Hatch (PH), Rayonier (RAY), and Reidsville State Penitentiary (RSP). Upstream locations are in black, while downstream locations are in gray. The error bars represent standard error of concentrations. Sites with an * have significantly higher concentrations than their paired site. Significance was determined using ANOVA with Duncan's post hoc test ($\alpha = 0.05$).

Figure 4.3. Differences in mean *Corbicula* tissue concentrations of (A) Cu and Zn; (B) As, Cd and Cr; and (C) Hg and Pb between clams found upstream and downstream of sites of interest: Amercord tires (AMC), Little Commissioner's Creek (LCC), Kettle Creek (KC), Plant Hatch (PH), Rayonier (RAY), and Reidsville State Penitentiary (RSP). Mean *Corbicula* tissues at all sites had variation in each element controlled by the final model. Error bars represent 95% confidence intervals (CI_{95}).
↑ = Upstream site has a significantly higher concentration.
↓ = Downstream site has a significantly higher concentration.

Figure 4.1

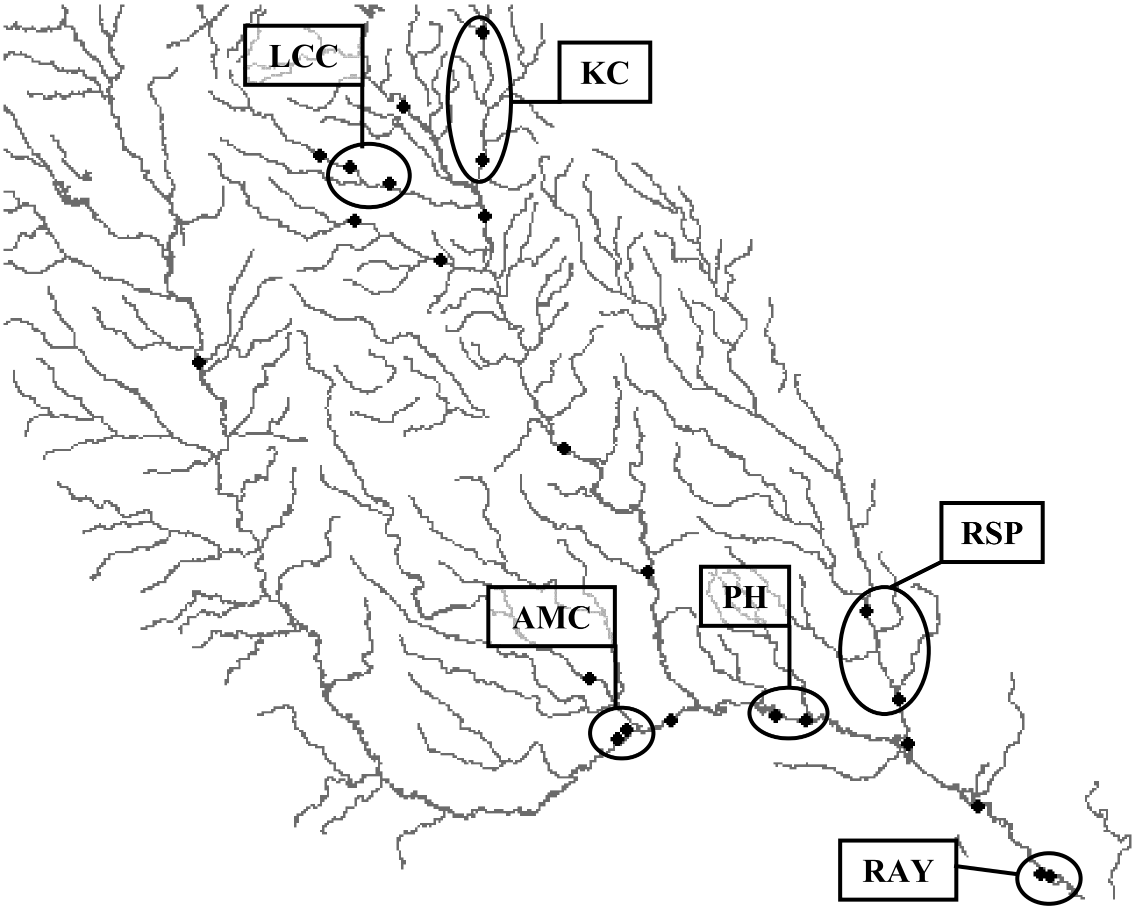
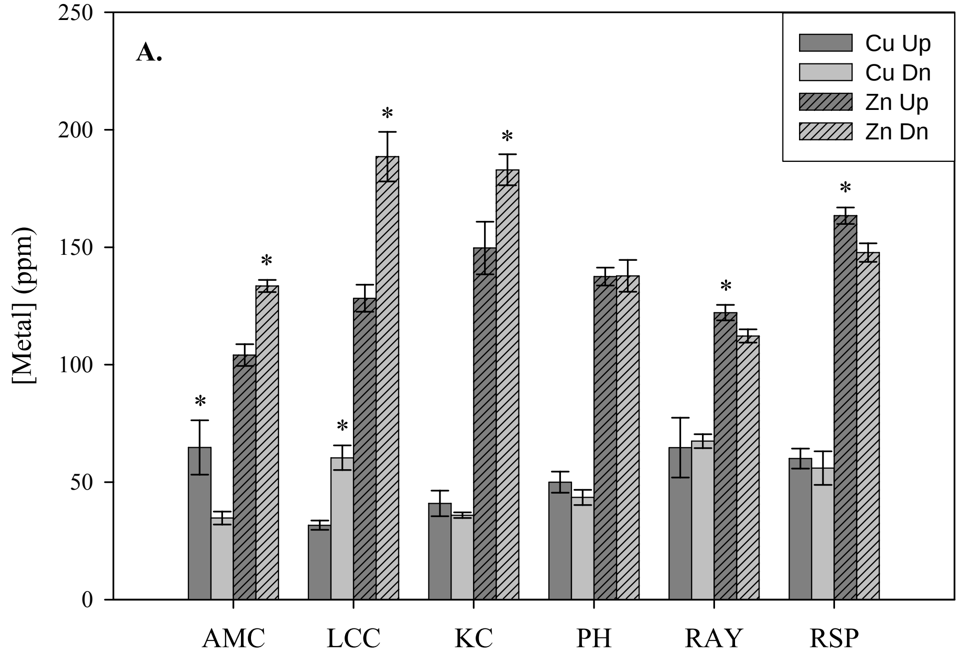


Figure 4.2


A.
[Metal] (ppm)
0
50
100
150
200
250
AMC
LCC
KC
PH
RAY
RSP
Cu Up
Cu Dn
Zn Up
Zn Dn
*

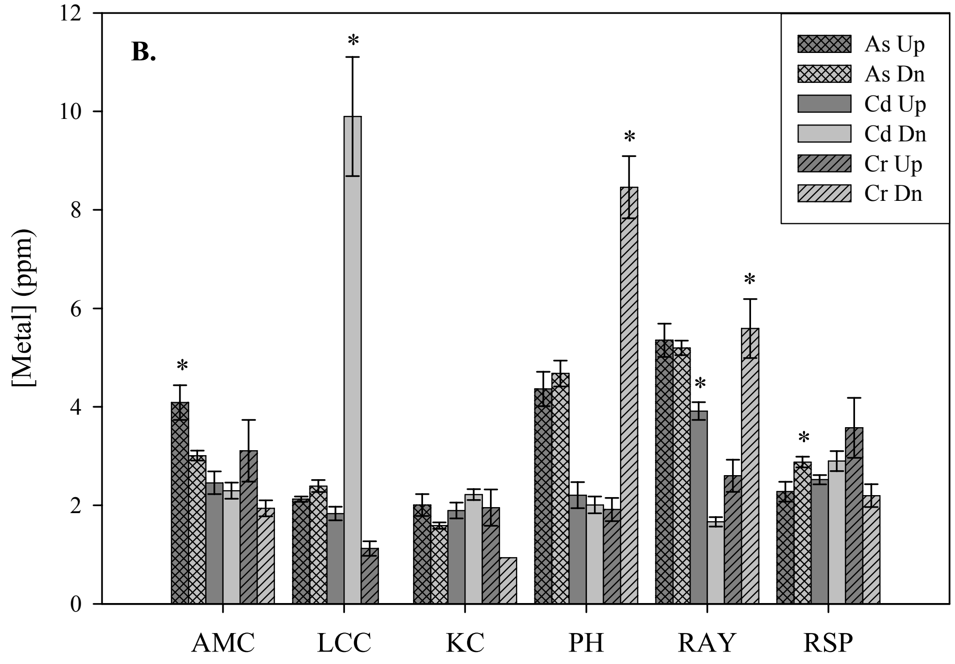

B.
[Metal] (ppm)
0
2
4
6
8
10
12
AMC
LCC
KC
PH
RAY
RSP
As Up
As Dn
Cd Up
Cd Dn
Cr Up
Cr Dn
*

Figure 4.2 (cont.)

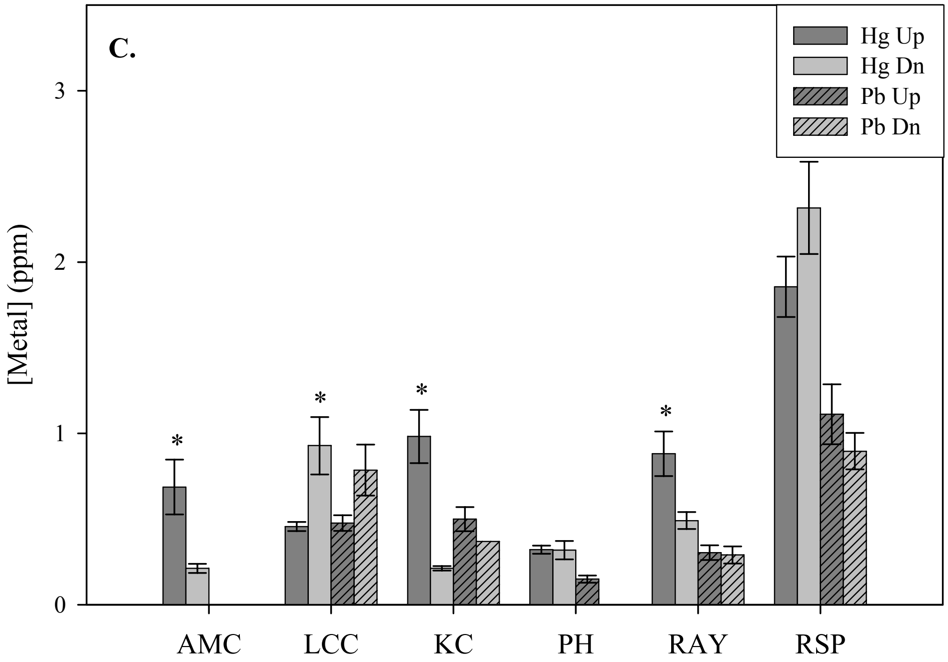
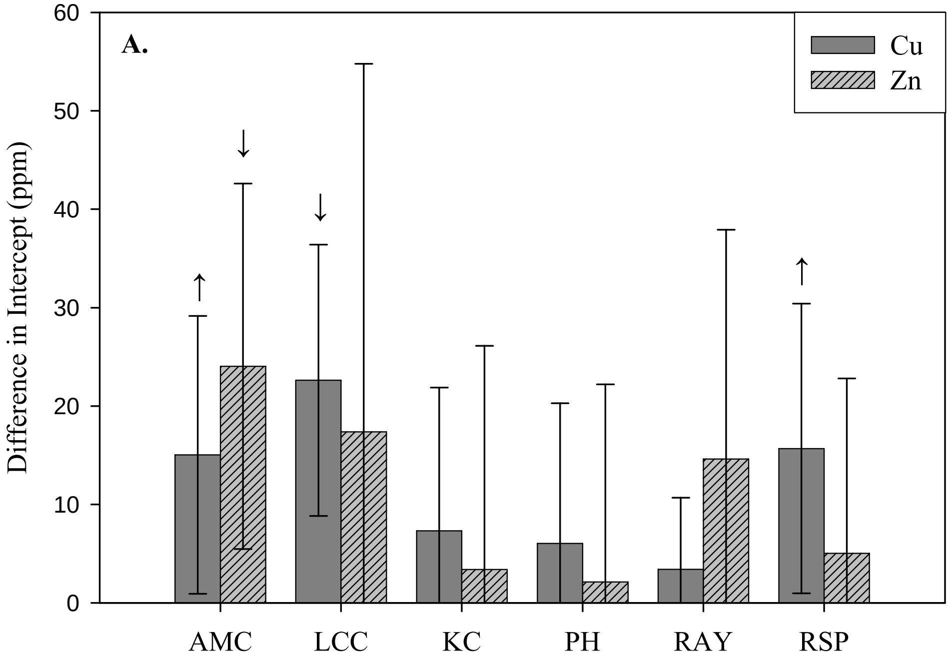


Figure 4.3


A.
Cu
Zn
Difference in Intercept (ppm)
0
10
20
30
40
50
60
AMC
LCC
KC
PH
RAY
RSP
↑
↓
↓
↑

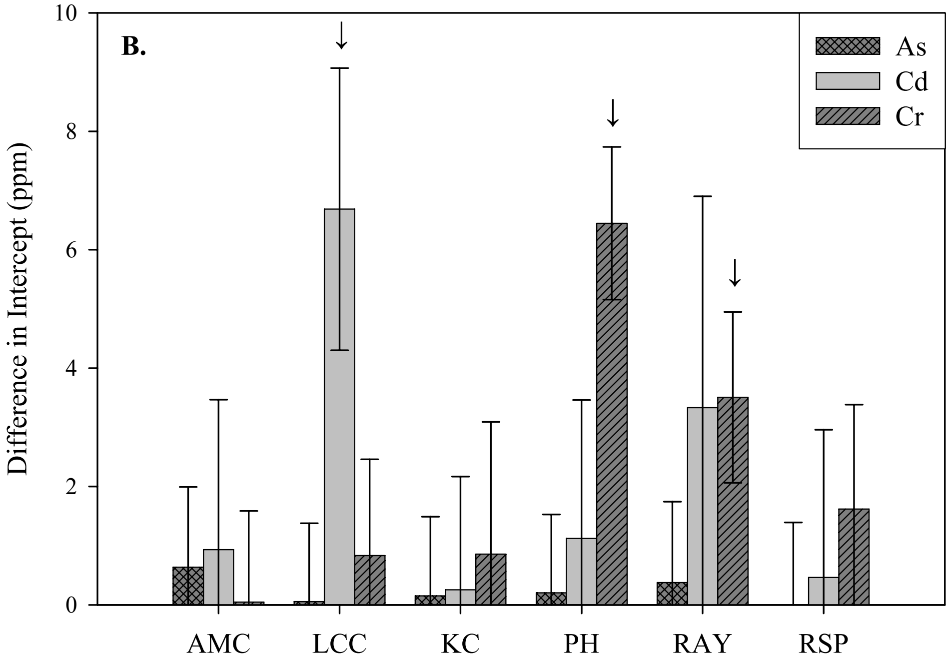
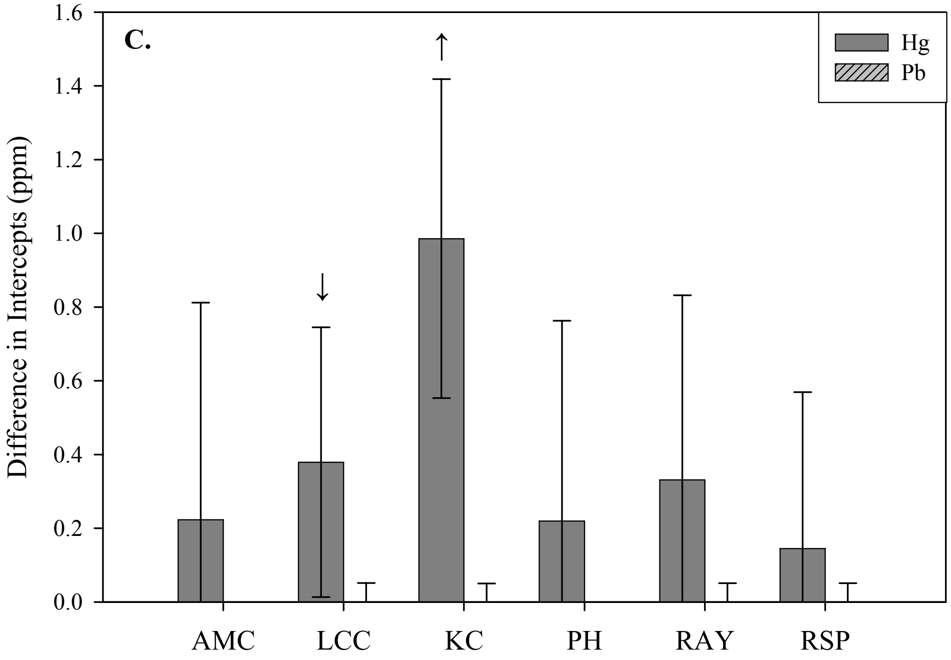

B.
As
Cd
Cr
Difference in Intercept (ppm)
0
2
4
6
8
10
AMC
LCC
KC
PH
RAY
RSP
↓
↓
↓

Figure 4.3 (cont)



References

Abaychi, J. K. and Y. Z. Mustafa. 1988. The Asiatic clam, *Corbicula fluminea* - an indicator of trace metal pollution in the Shatt Al-Arab River, Iraq. Environmental Pollution **54**:109-122.

Angelo, R. T., M. S. Cringan, D. L. Chamberlain, A. J. Stahl, S. G. Haslouer, and C. A. Goodrich. 2007. Residual effects of lead and zinc mining on freshwater mussels in the Spring River Basin (Kansas, Missouri, and Oklahoma, USA). Science of the Total Environment **384**:467-496.

Arias, M., M. T. Barral, and J. C. Mejuto. 2002. Enhancement of copper and cadmium adsorption on kaolin by the presence of humic acids. Chemosphere **48**:1081-1088.

ARK. 2003. Business must pay the price: Federal judge fines south Georgia company $1 million for polluting Ocmulgee River. Altamaha River Keeper.

Belanger, S. E., J. L. Farris, D. S. Cherry, and J. Cairns. 1986. Growth of Asiatic clams (*Corbicula Sp*) during and after long-term zinc exposure in field-located and laboratory artificial streams. Archives of Environmental Contamination and Toxicology **15**:427-434.

Bilinski, H., S. Kozar, M. Plavsic, Z. Kwokal, and M. Branica. 1991. Trace metal adsorption on inorganic solid-phases under estuarine conditions. Marine Chemistry **32**:225-233.

Burnham, K. P. and D. R. Anderson. 2001. Kullback-Leibler information as a basis for strong inference in ecological studies. Wildlife Research **28**:111-119.

Croteau, M. N. and S. N. Luoma. 2005. Delineating copper accumulation pathways for the freshwater bivalve *Corbicula* using stable copper isotopes. Environmental Toxicology and Chemistry **24**:2871-2878.

Croteau, M. N., S. N. Luoma, B. R. Topping, and C. B. Lopez. 2004. Stable metal isotopes reveal copper accumulation and less dynamics in the freshwater bivalve *Corbicula*. Environmental Science & Technology **38**:5002-5009.

Doherty, F. G. 1990. The Asiatic clam, *Corbicula Spp*, as a biological monitor in freshwater environments. Environmental Monitoring and Assessment **15**:143-181.

Doherty, F. G., D. S. Cherry, and J. Cairns. 1987. Valve closure responses of the Asiatic clam *Corbicula fluminea* exposed to cadmium and zinc. Hydrobiologia **153**:159-167.

Elder, J., Collins, JJ. 1991. Freshwater molluscs as indicators of bioavailability and toxicity of metals in surface-water systems. . Reviews Environ Contam Tox **122**:37-79.

Foster, I. D. L. and S. M. Charlesworth. 1996. Heavy metals in the hydrological cycle: Trends and explanation. Hydrological Processes **10**:227-261.

Graney, R. L., D. S. Cherry, and J. Cairns. 1984. The influence of substrate, pH, diet and temperature upon cadmium accumulation in the Asiatic clam (*Corbicula fluminea*) in laboratory artificial streams. Water Research **18**:833-842.

Griffiths, N. A. and H. Cyr. 2006. Are there hot spots for *Elliptio complanata* in the shallow littoral zone of a large Canadian Shield lake? Canadian Journal of Fisheries and Aquatic Sciences **63**:2137-2147.

Gundacker, C. 2000. Comparison of heavy metal bioaccumulation in freshwater molluscs of urban river habitats in Vienna. Environmental Pollution **110**:61-71.

Harrison, F. L., J. P. Knezovich, and D. W. Rice. 1984. The toxicity of copper to the adult and early life stages of the freshwater clam, *Corbicula manilensis*. Archives of Environmental Contamination and Toxicology **13**:85-92.

Inza, B., F. Ribeyre, and A. Boudou. 1998. Dynamics of cadmium and mercury compounds (inorganic mercury or methylmercury): uptake and depuration in *Corbicula fluminea*. Effects of temperature and pH. Aquatic Toxicology **43**:273-285.

Jagoe, C. H., B. Arnold-Hill, G. M. Yanochko, P. V. Winger, and I. L. Brisbin. 1998. Mercury in alligators (*Alligator mississippiensis*) in the southeastern United States. Science of the Total Environment **213**:255-262.

Jordao, C. P., M. G. Pereira, and J. L. Pereira. 2002. Metal contamination of river waters and sediments from effluents of kaolin processing in Brazil. Water Air and Soil Pollution **140**:119-138.

Katsumata, H., S. Kaneco, K. Inomata, K. Itoh, K. Funasaka, K. Masuyama, T. Suzuki, and K. Ohta. 2003. Removal of heavy metals in rinsing wastewater from plating factory by adsorption with economical viable materials. Journal of Environmental Management **69**:187-191.

Kesler, D. H. 2004. Influence of a lentic area on condition indices of *Corbicula fluminea* in the Wolf River, Tennessee. Journal of Freshwater Ecology **19**:445-453.

Koretsky, C. M., J. R. Haas, N. T. Ndenga, and D. Miller. 2006. Seasonal variations in vertical redox stratification and potential influence on trace metal speciation in minerotrophic peat sediments. Water Air and Soil Pollution **173**:373-403.

Lasier, P. J., P. V. Winger, J. L. Shelton, and K. J. Bogenrieder. 2004. Sediment-quality assessment of the lower Oconee River. Southeastern Naturalist **3**:139-154.

Luoma, S. N., R. Dagovitz, and E. Axtmann. 1990. Temporally intensive study of trace metals in sediments and bivalves from a large river, estuarine system - Suisun Bay Delta in San-Francisco Bay. Science of the Total Environment **97-8**:685-712.

Luoma, S. N. and P. S. Rainbow. 2005. Why is metal bioaccumulation so variable? Biodynamics as a unifying concept. Environmental Science & Technology **39**:1921-1931.

Meador, J. R. 2008. The development and evaluation of a freshwater mussel sampling protocol for a large lowland river. University of Georgia, Athens, GA.

Metcalfe-Smith, J. L., R. H. Green, and L. C. Grapentine. 1996. Influence of biological factors on concentrations of metals in the tissues of freshwater mussels (*Elliptio complanata* and *Lampsilis radiata radiata*) from the St Lawrence River. Canadian Journal of Fisheries and Aquatic Sciences **53**:205-219.

MetcalfeSmith, J. L., R. H. Green, and L. C. Grapentine. 1996. Influence of biological factors on concentrations of metals in the tissues of freshwater mussels (Elliptio complanata and Lampsilis radiata radiata) from the St Lawrence River. Canadian Journal of Fisheries and Aquatic Sciences **53**:205-219.

Millington, P., Walker, KF 1983. Australian freshwater mussel *Velsunio ambiguous* (Philippi) as a biological monitor for zinc, iron, and manganese. . Aust. J. mar. freshwat. Res. **34**:873-892.

Naimo, T. J. 1995. A review of the effects of heavy metals on freshwater mussels. Ecotoxicology **4**:341-362.

Patrick, R. 1994. Rivers of the United States. J. Wiley, New York.

Peltier, G. L., J. L. Meyer, C. H. Jagoe, and W. A. Hopkins. 2008. Using trace element concentrations in *Corbicula fluminea* to identify potential sources of contamination in an urban river. Environmental Pollution **154**:283-290.

Perceval, O., Y. Couillard, B. Pinel-Alloul, A. Giguere, and P. G. C. Campbell. 2004. Metal-induced stress in bivalves living along a gradient of Cd contamination: relating sub-cellular metal distribution to population-level responses. Aquatic Toxicology **69**:327-345.

Plumb, R. 1981. Procedures for handling and chemical analysis of sediment and water samples. U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

Pourang, N. 1996. Heavy metal concentrations in surficial sediments and benthic macroinvertebrates from Anzali wetland, Iran. Hydrobiologia **331**:53-61.

Poykio, R., H. Nurmesniemi, and R. L. Keiski. 2007. Environmental risk assessment of heavy metal extractability in a biosludge from the biological wastewater treatment plant of a pulp and paper mill. Environmental Monitoring and Assessment **128**:153-164.

Pruett, R. J. 2000. Georgia kaolin: development of a leading industrial mineral. Mining Engineering **52**:21-27.

Reese, E. G. and D. P. Batzer. 2007. Do invertebrate communities in floodplains change predictably along a river's length? Freshwater Biology **52**:226-239.

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

Rudd, J. W. M. 1995. Sources of methyl mercury to freshwater ecosystems - A review. Water Air and Soil Pollution **80**:697-713.

Salomons, W., N. M. Derooij, H. Kerdijk, and J. Bril. 1987. Sediments as a source for contaminants. Hydrobiologia **149**:13-30.

Sebesvari, Z., K. F. Ettwig, and H. Emons. 2005. Biomonitoring of tin and arsenic in different compartments of a limnic ecosystem with emphasis on *Corbicula fluminea* and *Dikerogammarus villosus*. Journal of Environmental Monitoring **7**:388-388.

Spinrad, J. C. 2003. Federal court fines Georgia manufacturer $1 million for Clean Water Act violations. Georgia Environmental Law Excerpts. Arnall Golden Gregory LLP.

Stites, D. L., A. C. Benke, and D. M. Gillespie. 1995. Population dynamics, growth, and production of the Asiatic clam, *Corbicula fluminea*, in a blackwater river. Canadian Journal of Fisheries and Aquatic Sciences **52**:425-437.

Tessier, A., P. G. C. Campbell, J. C. Auclair, and M. Bisson. 1984. Relationships between the partitioning of trace metals in sediments and their accumulation in the tissues of the freshwater mollusk *Elliptio complanata* in a mining area. Canadian Journal of Fisheries and Aquatic Sciences **41**:1463-1472.

Tomazelli, A. C., L. A. Martinelli, W. E. P. Avelar, P. B. de Camargo, A. H. Fostier, E. S. B. Ferraz, F. J. Krug, and D. S. Junior. 2003. Biomonitoring of Pb and Cd in two impacted watersheds in southeast Brazil, using the freshwater mussel *Anodontites trapesialis* (Lamarck, 1819) (Bivalvia : Mycetopodidae) as a biological monitor. Brazilian Archives of Biology and Technology **46**:673-684.

Unrine, J. M., W. A. Hopkins, C. S. Romanek, and B. P. Jackson. 2007. Bioaccumulation of trace elements in omnivorous amphibian larvae: Implications for amphibian health and contaminant transport. Environmental Pollution **149**:182-192.

USEPA. 1994. EPA Method 3015. Microwave assisted acid digestion of aqueous samples and extracts. U.S. Environmental Protection Agency, Washington, D.C.

USEPA. 1996. EPA Method 3052. Microwave assisted digestion of siliceous and organically based matrices. Revision 0 (December 1996). U.S. Environmental Protection Agency, Washington, D.C.

USEPA. 2006. Envirofacts Data Warehouse.

Vaughn, C. C. and C. C. Hakenkamp. 2001. The functional role of burrowing bivalves in freshwater ecosystems. Freshwater Biology **46**:1431-1446.

Vaughn, C. C. and C. M. Taylor. 1999. Impoundments and the decline of freshwater mussels: A case study of an extinction gradient. Conservation Biology **13**:912-920.

Williams, J. D., M. L. Warren, K. S. Cummings, J. L. Harris, and R. J. Neves. 1993. Conservation status of freshwater mussels of the United States and Canada. Fisheries **18**:6-22.

Appendix 4.1. Concentrations (ppm, µg/g) of elements at each site in three compartments: water, sediment and *Corbicula* tissues. Water concentrations come from a single sample, while sediments are means of 3 sub-samples from a single sample and tissue values come from whole tissue digestions of individual *Corbicula* (n = 1-8).

	OMG1	**OMG2**	**OMG3**	**OMG4**	**LOMG**	**OCN1**	**OCN2**	**OCN3**	**OCN4**	**COM1**	**COM2**	**COM3**
[As]$_{\mathrm{H2O}}$	0.6764	0.4881	0.5568	0.4424	0.8412	0.4227	0.2675	0.3285	0.3752	0.5762	0.4903	0.4531
[As]$_{\mathrm{Sed}}$	6.081	*BD*	*BD*	4.879	6.895	8.269	5.025	10.68	*BD*	13.21	7.348	7.728
[As]$_{\mathrm{Clam}}$	3.005	4.087	3.009	3.552	2.467	6.220	3.072	4.206	3.054	3.975	2.126	2.392
[Cd]$_{\mathrm{H2O}}$	0.06055	0.02420	0.06598	0.1761	0.03312	0.07191	0.02934	0.03521	0.05005	0.08729	0.1139	0.1006
[Cd]$_{\mathrm{Sed}}$	0.6118	0.4741	0.4495	0.1919	0.1397	0.2276	0.3190	2.391	0.1795	0.0281	0.1185	1.241
[Cd]$_{\mathrm{Clam}}$	5.145	2.458	2.297	3.049	1.521	1.457	5.730	6.967	1.059	0.4814	1.833	9.896
[Cr]$_{\mathrm{H2O}}$	1.465	0.7413	1.429	1.379	0.8700	2.182	0.9098	1.008	0.8412	2.586	2.704	1.016
[Cr]$_{\mathrm{Sed}}$	67.64	61.31	70.02	74.39	49.63	68.93	59.42	64.62	62.35	66.36	62.46	81.49
[Cr]$_{\mathrm{Clam}}$	2.311	3.107	1.940	2.387	1.569	3.098	1.830	3.115	1.495	1.442	1.124	*BD*
[Cu]$_{\mathrm{H2O}}$	2.275	1.398	2.127	2.035	1.241	2.672	1.984	1.943	1.406	2.164	1.782	2.319
[Cu]$_{\mathrm{Sed}}$	31.15	22.02	22.06	21.55	12.56	45.26	26.90	32.56	22.51	25.77	29.50	24.45
[Cu]$_{\mathrm{Clam}}$	54.034	64.77	34.66	38.91	108.36	60.67	58.78	47.49	43.24	24.24	31.64	60.35
[Hg]$_{\mathrm{H2O}}$	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
[Hg]$_{\mathrm{Sed}}$	*BD*	*BD*	*BD*	*BD*	*BD*	*BD*	*BD*	0.3088	*BD*	*BD*	*BD*	*BD*
[Hg]$_{\mathrm{Clam}}$	0.2548	0.6865	0.2122	0.3806	4.341	0.1738	0.1750	0.2393	0.1294	0.0838	0.4559	0.9281
[Pb]$_{\mathrm{H2O}}$	0.4461	0.5044	0.8655	0.2514	0.7260	0.7820	0.2550	0.3481	0.1705	0.3063	0.3644	0.1740
[Pb]$_{\mathrm{Sed}}$	39.89	27.96	34.77	29.96	42.62	33.82	27.24	29.31	28.53	24.72	37.13	35.35
[Pb]$_{\mathrm{Clam}}$	0.7593	*BD*	*BD*	*BD*	0.4763	0.2423	1.136	0.7144	0.2451	0.2330	0.4686	0.7541
[Zn]$_{\mathrm{H2O}}$	440.7	115.1	660.0	883.2	266.2	576.3	223.4	168.0	352.2	1526	2403	1156
[Zn]$_{\mathrm{Sed}}$	130.2	115.0	129.6	88.92	79.18	122.9	301.2	413.1	174.4	63.04	97.91	506.7
[Zn]$_{\mathrm{Clam}}$	133.2	104.1	133.5	131.4	145.7	115.9	149.2	269.6	126.4	101.9	128.3	188.5

ND = Not detected. This element was not analyzed because of predicted low detection.

BD = Below detection. All analytical values for this element from this site were below the detection limit.

Appendix 4.1 (cont). Concentrations (ppm, µg/g) of elements at each site in three compartments: water, sediment and *Corbicula* tissues. Water concentrations come from a single sample, while sediments are means of 3 sub-samples from a single sample and tissue values come from whole tissue digestions of individual *Corbicula* (n = 1-8).

	BUF1	**BUF2**	**BSC1**	**BSC2**	**ALT1**	**ALT2**	**ALT3**	**ALT4**	**ALT5**	**ALT6**	**OHP1**	**OHP2**
[As]$_{H2O}$	0.7050	2.721	0.8807	0.6713	0.3181	0.4736	0.4685	0.3269	0.4067	0.3081	0.5329	0.8291
[As]$_{Sed}$	13.11	8.963	7.984	7.842	6.481	*BD*	10.65	8.020	14.23	6.902	*BD*	8.005
[As]$_{Clam}$	2.006	1.588	1.711	3.351	4.363	4.677	3.770	5.465	5.352	5.195	2.335	2.879
[Cd]$_{H2O}$	0.04158	0.03338	0.02240	0.04398	0.1399	0.06531	0.03357	0.04529	0.04303	0.06603	0.03170	0.1243
[Cd]$_{Sed}$	0.0921	0.7487	0.5718	0.3358	0.3412	0.2705	0.5142	0.3161	0.6243	0.3441	0.2420	2.089
[Cd]$_{Clam}$	1.865	2.218	2.902	2.183	2.168	2.009	2.662	4.569	3.842	1.665	2.573	2.853
[Cr]$_{H2O}$	0.0978	0.3508	0.2443	0.7006	1.973	1.047	0.7152	0.4709	0.7559	0.9822	0.3900	1.744
[Cr]$_{Sed}$	68.43	63.35	77.24	73.59	70.92	56.19	60.58	95.23	114.9	69.95	53.87	37.84
[Cr]$_{Clam}$	1.954	0.934	1.013	1.145	1.916	8.460	4.949	3.685	2.601	5.589	3.574	2.198
[Cu]$_{H2O}$	1.164	2.387	0.4314	0.8068	2.521	1.505	1.021	33.70	2.083	1.448	0.7324	1.262
[Cu]$_{Sed}$	21.82	20.90	15.98	14.70	25.36	19.16	21.38	18.39	26.37	23.77	15.26	8.253
[Cu]$_{Clam}$	40.87	35.88	39.29	55.02	49.97	43.44	53.63	36.24	64.68	67.43	59.33	55.99
[Hg]$_{H2O}$	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
[Hg]$_{Sed}$	0.3998	*BD*	*BD*	*BD*	*BD*	*BD*	0.3342	0.3040	*BD*	*BD*	*BD*	*BD*
[Hg]$_{Clam}$	0.9822	0.2126	2.037	1.882	0.3210	0.3180	1.610	0.4097	0.8811	0.4909	1.837	2.316
[Pb]$_{H2O}$	0.2264	0.2848	0.08869	0.3426	0.6404	0.2534	0.1629	0.3646	0.2851	0.1631	0.2109	0.8185
[Pb]$_{Sed}$	23.25	20.99	28.32	32.82	29.04	25.39	31.66	29.56	34.66	29.47	39.46	27.98
[Pb]$_{Clam}$	0.4991	0.3693	0.6610	0.6059	0.1495	*BD*	*BD*	1.237	0.3037	0.2907	1.176	0.8956
[Zn]$_{H2O}$	219.2	41.08	126.2	317.8	1169	235.6	447.0	155.4	306.9	466.8	106.9	496.0
[Zn]$_{Sed}$	96.36	269.7	156.2	123.3	164.3	137.7	181.4	109.0	113.9	169.2	96.16	62.12
[Zn]$_{Clam}$	149.7	182.9	145.2	135.4	137.5	137.8	136.6	121.1	122.1	112.2	162.7	147.7

ND = Not detected. This element was not analyzed because of predicted low detection.

BD = Below detection. All analytical values for this element from this site were below the detection limit.

CHAPTER FIVE

USE OF AUTOREGRESSION TO ANALYZE LASER ABLATION INDUCTIVELY COUPLED PLASMA-MASS SPECTROSCOPY DATA FROM BIVALVE SHELLS[1]

Abstract

Bivalves secrete their shells in an annual fashion, forming discrete bands of growth. In doing so, they incorporate trace elements in concentrations based on environmental conditions and exposure, thus making it possible to use them as archives of environmental information. In this study, we used laser ablation inductively coupled plasma-mass spectrometry to analyze trace elements (Cd, Cu, Mn, Pb and Zn) on a transect perpendicular to the growth annuli of the freshwater bivalve *Elliptio hopetonensis.* Concentrations of Mn from multiple shells at each site were correlated and average Mn data series were formed. Periodicity of Mn data was determined and sampling errors removed using an autoregression model. The data series at each site were shown to have regular fluctuations of high and low concentrations. Fluctuations were similar between the shells from the same site but different between shells from different sites. Using Mn correlations to offset Pb series from the same site aligns Pb peaks from multiple shells. This study shows that Mn deposition in the shells of *E. hopetonensis* is a regular, seasonal process but that growth differs between sites with different environments. It also shows that Mn series can be used to align Pb peaks between multiple shells. By using autoregression models to remove

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sampling error, bivalve shells have the potential to be used as archives of both essential (i.e. Mn) and potentially toxic (i.e. Pb) trace element uptake by the organism.

Introduction

Bivalve biomonitoring approaches are often employed to determine the extent and magnitude of anthropogenic trace element release into the aquatic environment. In some places, these programs build long term data sets that record the changes in environmental availability of trace elements over time (Farrington 1983, Beliaeff et al. 1998, Perceval et al. 2006). In fact, past research has indicated that long-term baseline data may be necessary for understanding the environmental parameters affecting trace element bioaccumulation by biomonitor species (Luoma et al. 1990). While temporally intensive studies are certainly preferable to studies conducted on a limited time scale, the added expense from repeated sampling can make this infeasible.

It has been shown in numerous studies that bivalves incorporate higher levels of trace elements into their shells when exposed to higher concentrations in the laboratory (Sturresson 1976, Sturresson 1978, Imlay 1982, Bourgoin 1990, Almeida et al. 1998, Conners 1999) or in the environment (Imlay 1982, Koide 1982, Carell et al. 1987, Schettler and Pearce 1996, Gundacker 2000, Richardson et al. 2001, Markich et al. 2002, Chiffoleau et al. 2004, Liehr et al. 2005). Unlike tissues, trace elements deposited into the shell become locked into a matrix with much slower turnover (Yap et al. 2003).

Furthermore, the shells of bivalves are secreted in an annual fashion, usually with distinct bands marking periods of rapid growth (warm months) and periods of slower growth (winter months) (Dunca et al. 2005). This means that bivalve shells can potentially serve as an archive of trace element exposure that stretches backwards in time from the date of collection. This would allow temporally intensive biomonitoring of trace metals without necessitating time intensive sampling strategies.

Accessing these archives is dependent on understanding the complex biogeochemical controls that influence the movement of trace elements from the environment into the shell. These may include temperature (Stecher et al. 1996), growth (Takesue and Van Geen 2004), dissolved oxygen (Nystrom et al. 1996), precipitation (Lazareth et al. 2003), nutrients/primary productivity (Vander Putten et al. 2000, Langlet et al. 2007) and exposure to pollutants (Richardson et al. 2001, Gillikin et al. 2005, Liehr et al. 2005). The trace element most commonly investigated in freshwater bivalve shells is Mn. It has been shown to regularly peak in the portion of annuli corresponding to early or midsummer, either due to anoxic release of Mn from sediments (Nystrom et al. 1996) or turbation in anoxic sediments (Langlet et al. 2007) or seasonal phytoplankton blooms (Lazareth et al. 2003).

Using laser ablation inductively coupled plasma-mass spectroscopy (LA ICP-MS), small fluctuations in trace element concentrations in shells can be quantified (Vander Putten et al. 1999, Bellotto and Miekeley 2000, Barats et al. 2007). By using this technique to sample within the annuli of bivalves, intra-annual (seasonal) and inter-annual (yearly) differences in trace element accumulation can be studied. Theoretically, with enough resolution, anomalous events (extreme weather events, pollution events, etc) recorded in bivalve shells could also be analyzed.

To date, this approach has been primarily applied to marine and estuarine bivalves, while little work has focused on freshwater bivalves (Nystrom et al. 1996, Langlet et al. 2007). Even fewer studies have attempted to analyze potentially toxic trace metals (e.g. Cd, Cu, Pb and Zn) in freshwater bivalve shells (Markich et al. 2002). In this study, we analyze shells of the freshwater mussel *Elliptio hopetonensis* from the Altamaha River (Georgia, USA) to 1) demonstrate the utility of autoregression analysis when analyzing trace element data from bivalve shells, 2) determine whether regular patterns of the trace element Mn can be discerned using straight-line ablation and compared between different individuals and 3) determine whether the potentially toxic elements Cd, Cu, Pb and Zn can be detected in the shells and related to those patterns.

Materials and Methods

Sampling

Live specimens of *E. hopetonensis* were collected from three sites on the lower Oconee River (OCN) and upper Altamaha River (ALT) in southern Georgia, USA (Figure 5.1). Locations for sampling were chosen based on previous records of *E. hopetonensis* populations living along a gradient downstream of the principal source of trace elements in the watershed, kaolin mining on the middle Oconee River (Lasier et al. 2004). Five specimens were collected at each site and placed in a bucket of aerated site water for transport to the lab. All specimens were measured for maximum length, height and depth before having their soft tissues removed from the shells. The shells were then massed, labeled in pencil and dried at room temperature.

Sample Processing

The left valves of each shell were cut into several 1 mm sections along the axis of least growth, using an Isomet® Low Speed Saw (Buehler, Lake Bluff IL, USA). This axis was chosen to allow samples to fit on a standard petrographic slide without cutting them into segments. All sectioning was done perpendicular to growth lines. The sections were then polished with 600, 400, 320 and 240 grit SiC and cleaned in an ultrasonic bath using 90% EtOH. One section from each shell was mounted on a petrographic slide for LA ICP-MS analysis.

LA ICP-MS Analysis

The LA ICP-MS system used consists of a Merchantek^{TM} UP frequency quintupled Neodymium doped Yttrium Aluminum Garnet (Nd-YAG) operating at 213 nm, coupled to a Perkin-Elmer Sciex Elan DRC Plus ICP-MS (Norwalk CT, USA). Ablations were made in a straight line as close to orthogonal to growth lines as possible. A pre-ablation pass to remove surface contamination [as recommended by Vander Putten et al. (1999)] was performed at a spot size of 75 µm, a repetition rate of 20 Hz and a scanning speed of 25 µm/s. The second, analytical pass was made with similar operating parameters but at a slower speed of 5 µm/s. In some cases, a single line was ablated, while in larger shells, a second line was ablated at a different angle in order to account for curvature in the shell. Isotopes that were analyzed in this manner were ^{55}Mn, ^{65}Cu, ^{68}Zn, ^{114}Cd, and ^{208}Pb. Data were calibrated using a series of calcium carbonate standards prepared in house and having concentrations ranging from 0 to 100 ppm for all elements analyzed [as recommended by Bellotto and Miekeley (2000)]. Standards were analyzed along a 900 µm transect for a total of 80 values per calibration. To allow comparison between standards and samples, all values were calibrated using Ca^{43} as an internal standard (Vander Putten et al. 1999).

Statistical Analysis

Different trace metals within the same shell were correlated with each other using Excel (Microsoft, Redmond WA, USA), in order to determine if there was any direct relation between accumulation of different elements within the shell. The element Mn was chosen to construct models for all shells because past studies (Nystrom et al. 1996, Langlet et al. 2007) and our own preliminary study (data not shown) indicated the regular, seasonal nature of its incorporation into freshwater bivalve shells. The data series for Mn that we collected also showed some of the strongest correlations between shells at the same site, which made them amenable to combination (see below). Preliminary studies (data not shown) indicated that trace element data collected from bivalve shells is highly autocorrelated—data points are correlated to earlier data points in the same series. In order to account for autocorrelation, we treated the data as a time series, incorporating "seasonal" (cyclical) data into an autoregression model that removed errors caused by autocorrelated data (Chatfield 2004).

The first step in model building was to create mean Mn data series for each site. Observations of Mn concentration in shells from the same sites were correlated to one another at different lags (differences in observation number). Best-fit lags were determined based on: 1) minimizing the lags between shells; 2) obtaining correlations above 0.250 between as many shells as possible; 3) visual inspection of the mean time series to determine that the number of peaks have not greatly increased nor decreased when compared to the Mn data series of individual shells. For values that were at the upper limit of detection, Mn concentrations as a function of Ca were calculated for each shell and a standard value was substituted for above detection observations. This was only extensively used for shells from the site OCN1 (Fig 5.1).

Average Mn data series were then examined for cyclical patterns in concentration. In cases where more than one ablation line was used, these data series were separated and analyzed individually. After estimation of peak distances, the linear autocorrelation function of each data series was determined using an autoregressive integrated moving average (ARIMA) procedure in Statistical Analysis Software (SAS Institute, Cary NC, USA). This procedure measures correlation between points within the same data series at different distances apart within the series (e.g. different lags). Autocorrelation data was plotted at lags up to 360, with correlations >

|2*Standard Error| being considered significant. Lags that were indicated to have significant correlation were introduced into the data series in the form of a "cycle" value (i.e. for a lag of three, observations 1-3 would be assigned a cycle parameter of 1-3, but observation 4 would be assigned a 1, observation 5 a 2, observation 6 a 3, observation 7 at 1, etc). The mean values of all observations with the same cycle value then became separate parameters of the regression.

Bayes Information Criteria were calculated for all models to determine the appropriate lag for autoregression. Autoregression takes into account errors caused by data series whose values are autocorrelated. Following autoregression at the appropriate lag, the p-values of all cyclical parameters were assessed for significance. The residuals of the autoregression models were assessed for normality using the Kolmogorov-Smirnov test ($\alpha = 0.05$) with no more than 1.5% of residuals removed as outliers. Lingering correlation was assessed using the autocorrelation function as described above. Models were determined to be appropriate for use if the mean square error (MSE) was less than that of the least squares (linear, non-cyclical) autoregression model and the residuals were considered normal and uncorrelated. The model with the lowest MSE was then chosen as the final autoregression model.

Finally, the lags used to construct the mean Mn data series were applied to trace metals Cd, Cu, Pb and Zn and correlation between shells analyzed, in order to determine if the Mn data series could be used to match up peaks of potentially toxic element accumulation within the shell.

Results

General parameters for each shell are collected in Table 5.1. The site OCN1 consisted of older, larger individuals with thicker shells, while OCN2 was a population of smaller, younger individuals.

The correlation between different elements within each shell are listed in Appendix 5.1. Elements rarely showed correlation with another element. However, Mn concentrations were consistently correlated with Zn concentration, although this correlation was in some cases negative and in others positive (Appendix 5.1). This is probably because Zn behaved differently from Cd, Cu and Pb, declining in a linear or exponential manner with occasional broad peaks as the ablation moved away from the growth edge of the shell. The elements Cd, Cu and Pb were characterized by a flat baseline with occasional, sharp peaks. Also, concentrations of Cd showed occasional correlation to Pb concentrations.

Graphs of the individual Mn data series, grouped by site and offset by lags of greatest collective correlation, and the mean Mn data series are displayed in Figure 5.2. The correlations between Mn from different shells at the chosen lags are listed in Table 5.2. Only one correlation between shells at the same site was less than |0.25| (shells M1 and M2 from OCN2).

The fit of the least squares regression are given in Table 5.3. Notice that for the sites OCN1 and ALT1, two least squares regressions are given, which represent the two separate ablations used to follow the curvature of the shell. For most of the data series, the concentration of Mn generally decreased as the ablations moved towards the umbo (i.e. towards the older parts of the shell).

The final models determined for each data series are described in Table 5.4. All data series include a cyclical component which, when included in the autoregression, decreased the MSE as compared to the linear least squares autoregression (Table 5.4). In the case of the first ablation of OCN1, this cyclical component was relatively weak, with none of the cyclical parameters being significant ($\alpha = 0.10$). This is probably because most of the data in this range was above the upper limit of detection for all four shells and was substituted with standard values. The data series and their linear regressions with cyclical components are compared in Figure 5.3. Cyclical components do not match exactly with all peaks in a given data series, being selected based on reducing MSE.

There were no strong correlations between Cd, Cu, or Pb from different shells at the lags used to create the average Mn series (Appendix 5.2) or at any other lags (data not shown). This is probably because of the irregular, “spiky” nature of the data. Zn did show correlation between different shells but often showed higher correlation at lags other than those that were used for the average Mn series. However, visual inspection of Pb data from the shells at site OCN2 indicate that lags used for the average Mn data series aligned peaks from multiple shells, as shown in Figure 5.4. Shells from OCN1 also exhibited some alignment of Pb peaks (Figure 5.4). Only shells from ALT1 did not show this trend, probably due to the fact that only one shell from that site showed discernible Pb peaks. Visual inspection of Cd and Cu did not reveal any obvious peak alignment as was the case for Pb.

Discussion

Utility of Autoregression Models

This is the first study that the authors are aware of in which trace element data from bivalve shell annuli were analyzed using an autoregression function. This is surprising, considering that several studies have concluded that trace elements in bivalve shells are linked to regular, seasonal cycles of temperature, growth, precipitation, primary productivity or some combination (Nystrom et al. 1996, Vander Putten et al. 2000, Lazareth et al. 2003, Freitas et al. 2006, Langlet et al. 2007). Seasonal parameters such as these fluctuate in an autocorrelated manner: considering a measurement of such a parameter independent from previous measurements introduces errors. Because bivalves secrete shell in a continuous manner, dependent on environmental parameters such as temperature and pollution levels (Dunca et al. 2005), statistical treatment of data from shell annuli must use statistical methods that removes autocorrelation errors.

Strong autocorrelation was found in all data series of Mn in our study. As shown by Table 4, a model that took autocorrelation into account fit the date much better than a linear least squares model for each data series. This clearly demonstrates the utility of autoregression when analyzing trace metal concentrations collected from bivalve shells. Our study also found that, on the whole, Mn concentrations declined as the ablation continued into the older annuli of the shell. This has previously been reported in the literature and has been explained as an ontogenetic effect of the mussel's age affecting shell formation (Imlay 1982).

Seasonality of Mn Incorporation in Bivalve Shells

Our study also shows that the freshwater bivalve *E. hopetonensis* incorporates Mn into its shell in a cyclical fashion. The size of these cycles (number of data points contained in one cycle) differed between data series that came from different angles of ablation (if more than one was used) or different sites (Table 5.4). However, it seems that cycle size is conserved between shells originating from the same site, as demonstrated by the between-shell correlations in Table 5.2. This could indicate that local environmental factors affect the shell growth and Mn incorporation of individuals.

Our study was also the first to determine the strength of observed seasonal trends. As demonstrated by Table 5.4, some cyclical trends were stronger than others, with significant components ranging from 0-71.93%. It could be argued that the weakness of the cyclic parameters for the first ablation line of shells from OCN1 indicates that the cyclical model is inappropriate. However, this weakness is probably due to the fact that many points in these shells were at the upper limit of detection and were replaced by a standard value, an approach known to cause distortions (Park et al. 2007).

With regards to whether Mn concentration in bivalve shells fluctuates in a seasonal manner, our method of data collection does not allow us to directly determine where individual peaks occur within a growth annuli. However, our estimates for the number of annuli that are ablated (Table 5.1) agree to some degree with the number of peaks found in each mean Mn series. For instance, the series for OCN2 shows 4 peaks (Figure 5.4), while 2-3 annuli were ablated in each shell. The series for ALT1 shows 15 peaks from shells that had an average of 8 annuli ablated. However, peaks from the first ablation seem to represent a pattern of large and small peaks that may indicate some kind of biannual trend. In this case, the data represents 7-8 seasons, which agrees with annuli counts. Because of values that were uniformly above detection, it is difficult to count peaks in OCN1. Overall, comparing mean Mn peaks to annuli counts agrees with other studies that have found Mn accumulation to show a strictly seasonal trend (Nystrom et al. 1996, Vander Putten et al. 2000, Siegele et al. 2001, Freitas et al. 2006, Langlet et al. 2007).

Past researchers have suggested that increased Mn concentrations in bivalve shells could be the result of increased summer concentrations of Mn caused by anoxic waters (Nystrom et al. 1996), early summer increases in primary productivity (Vander Putten et al. 2000, Lazareth et al. 2003) or a combination of the two (Langlet et al. 2007). While we do not have environmental monitoring data that could allow us to test either of these hypotheses, we can make a few observations, the shells from OCN1 which routinely had Mn concentrations above the instrumental detection were collected from a small slack water on the bankward side of a sandbar. Periodic disconnection at this site could result in slower moving, more anoxic water in the summer than at OCN2 (undercut bank with strong current) or ALT (fast flowing and sandy). Data collected by USGS at sites (Doctortown and Everett GA) downstream of those sampled in this study indicate average water concentrations of Mn to be higher in summer months (July and August), while dissolved oxygen is lower (USGS 2008a, b). Because Mn is associated with oxides, it is readily mobilized under anoxic conditions (Koretsky et al. 2006, Poykio et al. 2007). Therefore, it is plausible that later summer anoxia leads to the seasonal increases in Mn concentrations that we found in the shells of *E. hopetonensis.*

Bivalve Shells as Long-Term Records of Trace Element Pollution

Past studies have shown that higher concentrations of Cd, Cu, Pb and Zn in the annuli of bivalve shells can be linked to higher concentrations of those trace elements in the environment (Schettler and Pearce 1996, Richardson et al. 2001, Markich et al. 2002, Liehr et al. 2005). However, past attempts to use annuli as archives of past contamination have given mixed results. Markich et al. (2002) analyzed the trace elements in the freshwater species, *Velesunio angasi,* at sites downstream of a mine waste site that had recently been remediated. They found annuli laid down after remediation had significantly lower concentrations of Cu and Zn than annuli laid down prior to remediation. Gillikin et al. (2005) constructed a 53-year series of Pb found in the shells of *Mercenaria mercenaria* from a coastal island. They found that Pb concentrations in annuli corresponding to the years 1977-1981 were significantly higher than years before and after that time period. They found no other trends, not even seasonal trends in Pb seen by other researchers (Vander Putten et al. 2000, Freitas et al. 2006).

Our specimens showed very low concentrations of the elements Cd, Cu and Pb, with only a few individuals showing discernable peaks in Pb concentration (Figure 5.4). This could be a function of the species, *E. hopetonensis*, being less likely to incorporate these elements into its shell than other species that have been studied. Or it could be the result of low environmental concentrations of the elements in the system, since the Lower Oconee/Upper Altamaha has little urbanization and only a few significant local sources of potentially toxic elements [see Chapter 4; (Lasier et al. 2004)]. Our findings did indicate that aligning Pb data series from different shells based on the lags of best agreement for Mn data series seems to match peaks between shells (Figure 5.4A,B). This is especially obvious when looking at the younger shells from OCN2 (Figure 5.4B), where an increase in observed Pb concentrations in 3-4 of the 4 shells accompanies the mean series' most prominent peak in Pb concentrations. This concurrence indicates that the seasonal trends seen in Mn accumulation could be used to match up many elemental data series between shells. By doing this, periods of increased Pb accumulation are also aligned. As seasonal Mn incorporation into bivalve shells becomes better understood, it should be used to determine dates of increased accumulation of elements such as Pb.

Future Directions

This study illustrates one of the weaknesses in using straight-line rather than spot ablation when analyzing bivalve shells. Unlike spot ablation, data points collected using straight-line ablation cannot be easily related to a specific portion of shell annuli. However, straight-line ablation provides a higher degree of resolution, and is more capable of detecting small fluctuations in trace element concentration that could indicate temporary exposure to increased concentrations of trace elements.

This is one of several issues to be addressed by future analysis of the data generated in this study and in future studies. Firstly, a means of directly linking trace element data that demonstrates a seasonal nature to dates as explained by annual growth rings would allow anomalies to be related to environmental disturbances or, potentially, pollution events.

Secondly, a more robust model must be developed that allows for differences in individual shells, rather than smoothing them out by averaging them together. Such a model would be able to take into account above detection values and changes in the angle of ablation. It could also elucidate the differences not just between individuals at a single site, but between sites. Thirdly, having developed a new, more robust approach, it should further be applied to other trace elements such as barium (Ba) and strontium (Sr), which were not considered in this study.

Conclusions

This study has shown that straight-line laser ablation can be used to reveal regular patterns in Mn concentration in the shells of the freshwater bivalve *E. hopetonensis.* The data series generated by this study can be well correlated between individuals from the same site and in doing so, the data series of the potentially toxic trace element Pb are aligned with one another. Furthermore, the necessity of taking into account autocorrelation when analyzing data generated from bivalve shells was demonstrated by the fit of autoregression when compared to that of least squares linear regression. The shells of freshwater bivalves continue to be a promising source of archival data of trace element pollution in a given region. As we better understand the links between the accumulation of elements such as Ba, Mn and Sr in bivalve shells and seasonal, environmental parameters, we can better understand the factors behind accumulation of potentially toxic trace elements such as Cd, Cu, Pb and Zn. Understanding these relationships will require a combination of controlled laboratory experiments, caging and field studies under a variety of conditions. It will also require the development of robust statistical models that can account for autocorrelation errors, the environmental differences between different sites and the growth history of individuals.

Acknowledgments

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Table 5.1. Mean concentrations of elements (μg/g of Ca^{43}), size parameters (shell length, height, depth and shell weight) and estimated number of annuli ablated (i.e. number of years the trace element data represents) of individual shells analyzed in this study. Average values for each site are given in bold.

Site	Shell	Length (mm)	Height (mm)	Depth (mm)	Shell Wt. (g)	Annuli Ablated	[Cd/Ca] (μg/g)	[Cu/Ca] (μg/g)	[Mn/Ca] (μg/g)	[Pb/Ca] (μg/g)	[Zn/Ca] (μg/g)
OCN1	M1	105.5	63.5	35.0	111.55	15	4.390	2.481	351.3	2.821	14.98
	M2	108.9	56.4	31.2	86.72	18	4.392	2.492	445.1	2.821	17.99
	M3	114.2	61.4	34.3	113.68	7	4.407	2.506	412.1	2.848	10.06
	M4	121.4	66.0	35.3	118.34	19	4.393	2.476	415.0	2.849	16.30
	AVG	**112.5**	**61.8**	**34.0**	**107.57**	**14.75**	**4.396**	**2.489**	**405.9**	**2.835**	**14.83**
OCN2	M1	92.0	43.2	24.9	29.01	3	4.396	2.492	137.1	2.833	12.25
	M2	90.1	49.0	23.5	27.60	2	4.387	2.472	126.2	2.846	8.70
	M3	90.5	41.1	19.4	19.91	2	4.394	2.463	167.0	2.835	13.47
	M4	88.6	41.6	19.9	25.54	3	4.383	2.425	134.9	2.816	1.23
	AVG	**90.3**	**43.7**	**21.9**	**25.51**	**2.5**	**4.390**	**2.463**	**141.3**	**2.833**	**8.91**
ALT1	M1	106.8	50.8	29.0	57.64	9	4.440	2.566	227.1	3.430	4.86
	M2	102.8	51.4	31.0	26.19	10	4.395	2.471	177.9	2.841	7.90
	M3	92.6	49.7	25.7	50.63	5	4.382	2.443	153.2	2.815	10.60
	AVG	**100.7**	**50.6**	**28.6**	**44.82**	**8**	**4.406**	**2.493**	**186.1**	**3.029**	**7.79**

Table 5.2. Correlations between Mn data series for shells within a given site, at the lags selected for greatest average correlation. Values in bold are greater than 0.25 or less than –0.25. Correlations between the same data sets = 1.000.

Site	**Shell**	M1	M2	M3	M4
OCN1	M1	1.000			
	M2	**0.431**	1.000		
	M3	**0.266**	**0.647**	1.000	
	M4	**0.363**	**0.379**	**0.351**	1.000
OCN2	M1	1.000			
	M2	-0.013	1.000		
	M3	**0.601**	**0.316**	1.000	
	M4	**0.332**	**0.401**	**0.251**	1.000
ALT1	M1	1.000			
	M2	**0.472**	1.000		
	M3	**0.505**	**0.527**	1.000	

Table 5.3. The fit of the least squares lines for the Mn data series, calculated for a linear regression (Lin) and a linear autoreggression (Auto).

Site	Data Points	Intercept (Lin)	Slope (Lin)	r^2 (Lin)	Intercept (Auto)	Slope (Auto)	r^2 (Auto)
OCN1	1-950	484.05	-0.046	0.086	474.16	-0.028	0.918
	951-2073	441.37	-0.184	0.634	452.06	-0.208	0.989
OCN2	1-1253	114.88	0.043	0.243	125.71	0.027	0.993
ALT1	1-538	284.44	-0.145	0.171	283.94	-0.149	0.994
	539-1099	124.42	0.026	0.067	126.16	0.012	0.984

Table 5.4. Autoregression models for Mn data series, including number of cyclical components, % of cyclical components that are significant ($\alpha = 0.10$) and MSE for both the least squares (LS) model and the least squares model with cyclical components (LSCC).

Site	**Data Points**	**Cyclical Components**	**% Significant Components**	**MSE (LS)**	**MSE (LSCC)**
OCN1	1-950	91	0.00%	154.05	149.79
	951-2073	292	37.33%	63.066	59.897
OCN2	1-1253	285	71.93%	7.0953	6.8595
ALT1	1-538	49	57.14%	18.628	17.906
	538-1098	126	30.95%	4.1078	3.8545

Figure 5.1. Sampling locations on the Oconee (OCN) and Altamaha (ALT) Rivers.

Figure 5.2. Mn data series, offset by lags of highest overall concentration. The gray lines are individual shells, while the black line is the mean series. Vertical lines indicate the end of the first ablation line. The individual plots represent the three sites OCN1, OCN2, and ALT1 in a downstream manner.

Figure 5.3. Regressions (black lines) of mean Mn series (gray lines). The regressions include cyclical components as described in Table 5. The individual plots represent the three sites OCN1, OCN2, and ALT1 in a downstream manner.

Figure 5.4. Mean Pb series (black line), offset by the same lags as the mean Mn series. The bars at the bottom indicate the number of shells with above average Pb values at each point on the series. Gray boxes highlight peaks in the mean Pb series that coincide with above average values in several shells. The individual plots represent the three sites OCN1, OCN2, and ALT1 in a downstream manner.

Figure 5.1

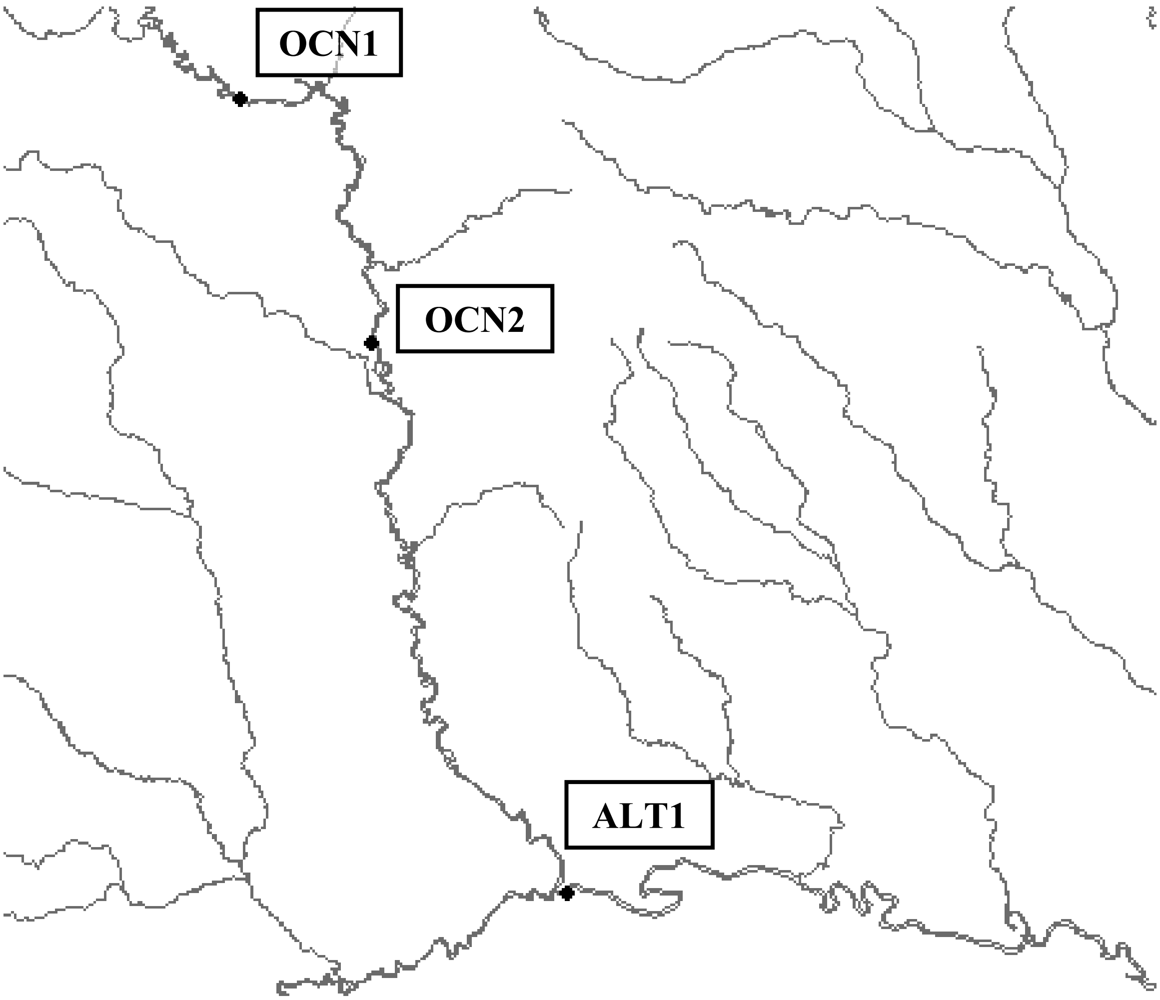
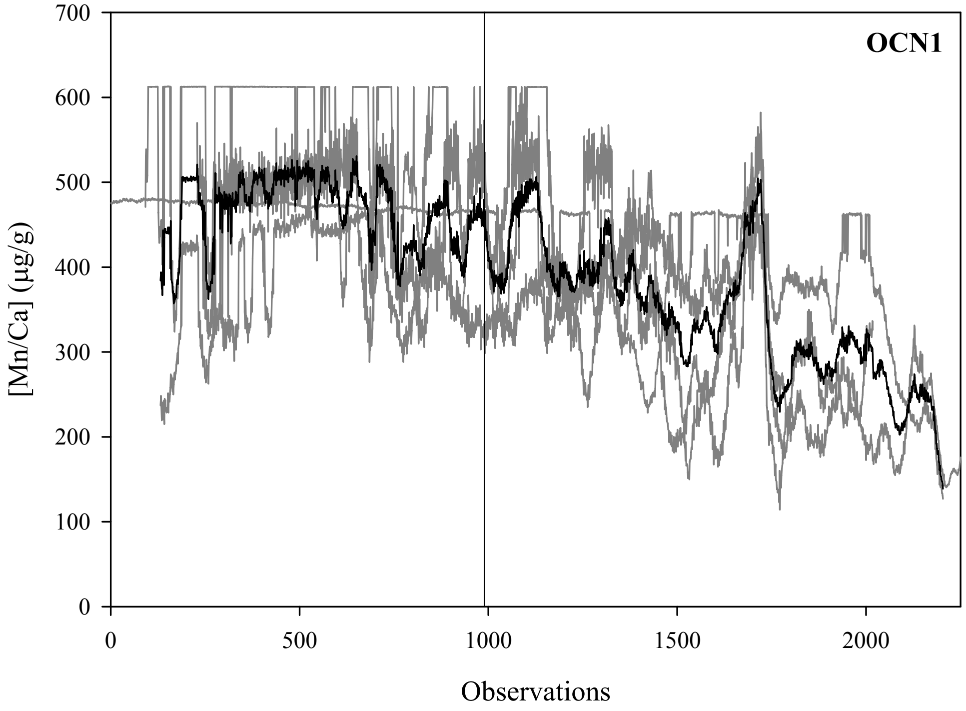


Figure 5.2.


OCN1
[Mn/Ca] (µg/g)
700
600
500
400
300
200
100
0
0
500
1000
1500
2000
Observations

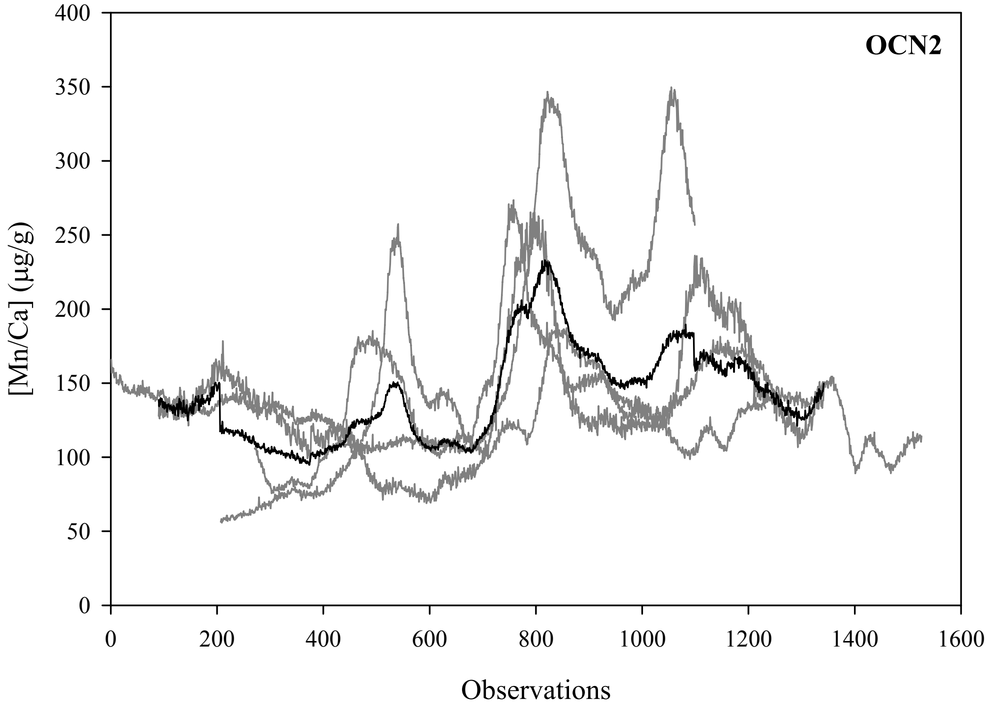

OCN2
[Mn/Ca] (µg/g)
400
350
300
250
200
150
100
50
0
0
200
400
600
800
1000
1200
1400
1600
Observations

Figure 5.2 (cont).

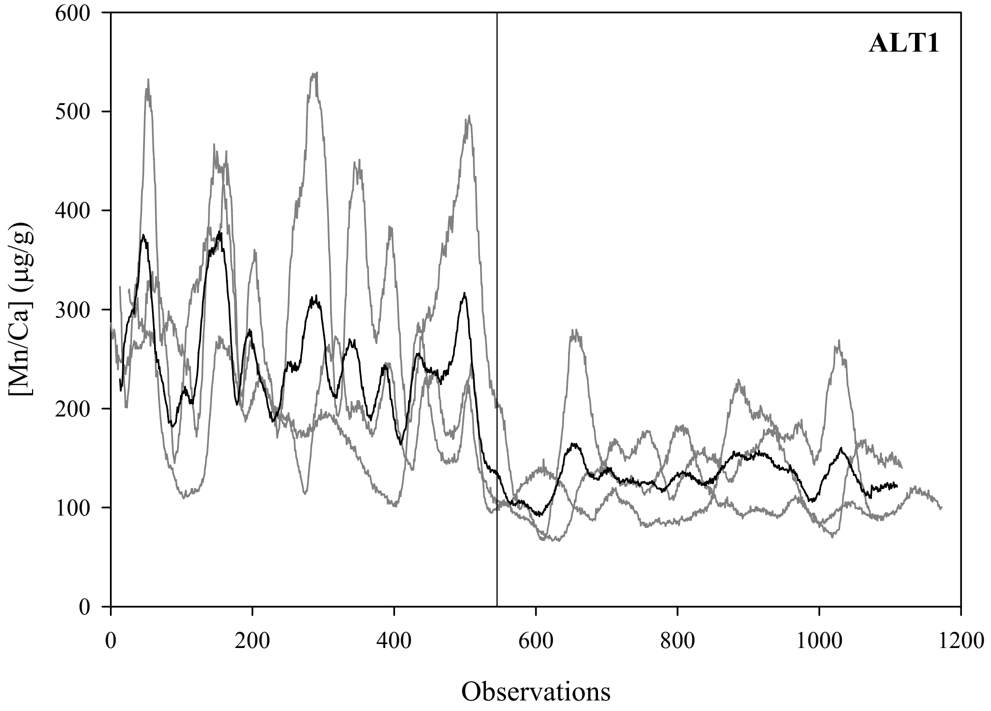
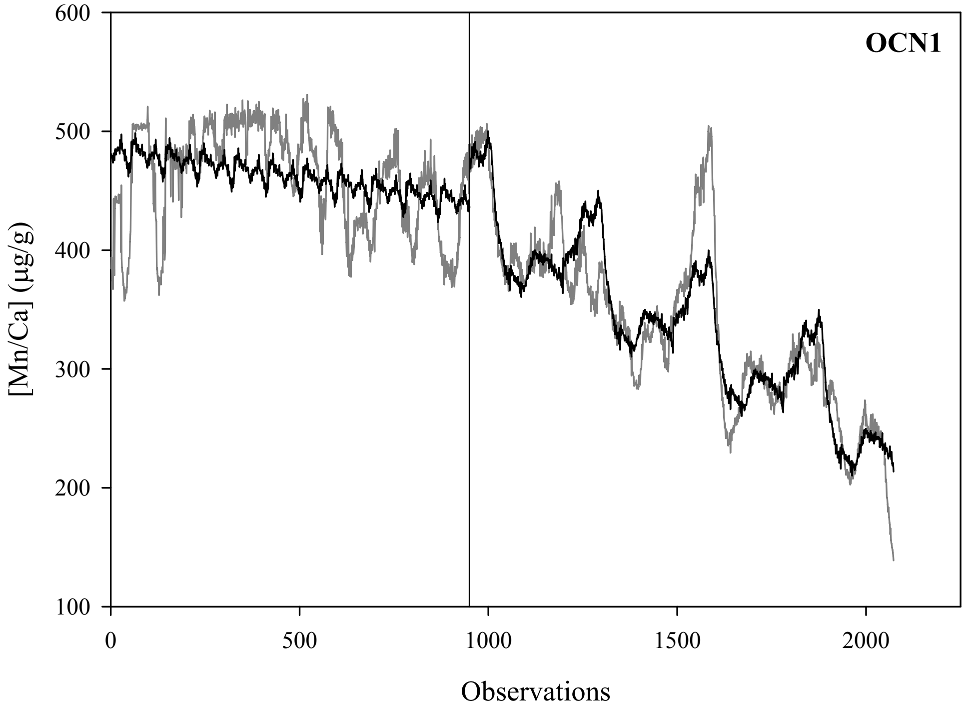


Figure 5.3.


OCN1
600
500
400
300
200
100
[Mn/Ca] (µg/g)
0
500
1000
1500
2000
Observations

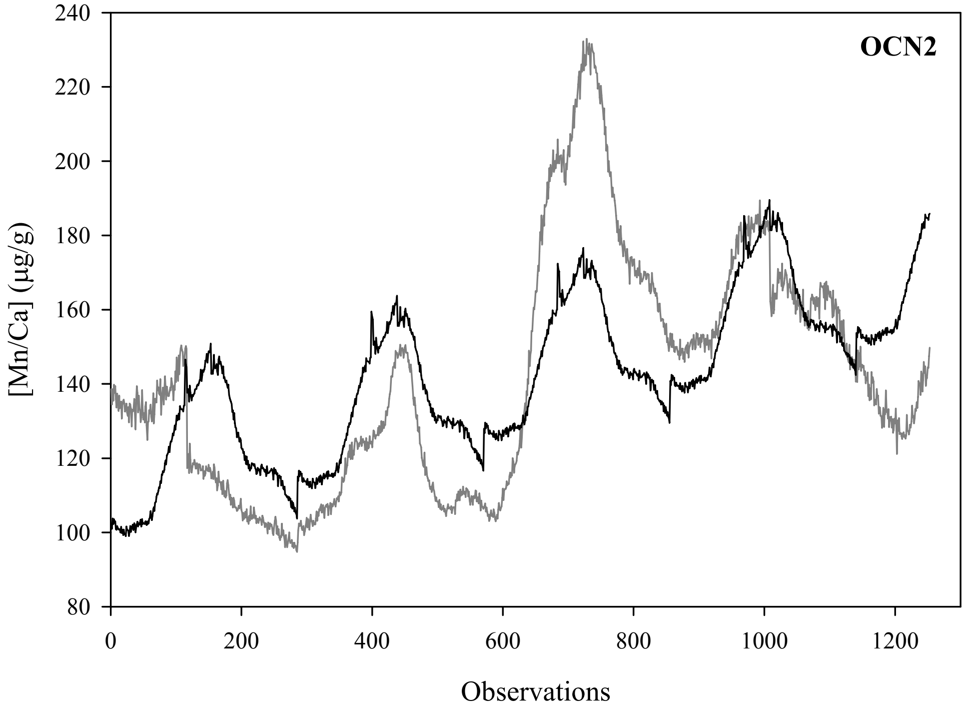

OCN2
240
220
200
180
160
140
120
100
80
[Mn/Ca] (µg/g)
0
200
400
600
800
1000
1200
Observations

Figure 5.3 (cont).

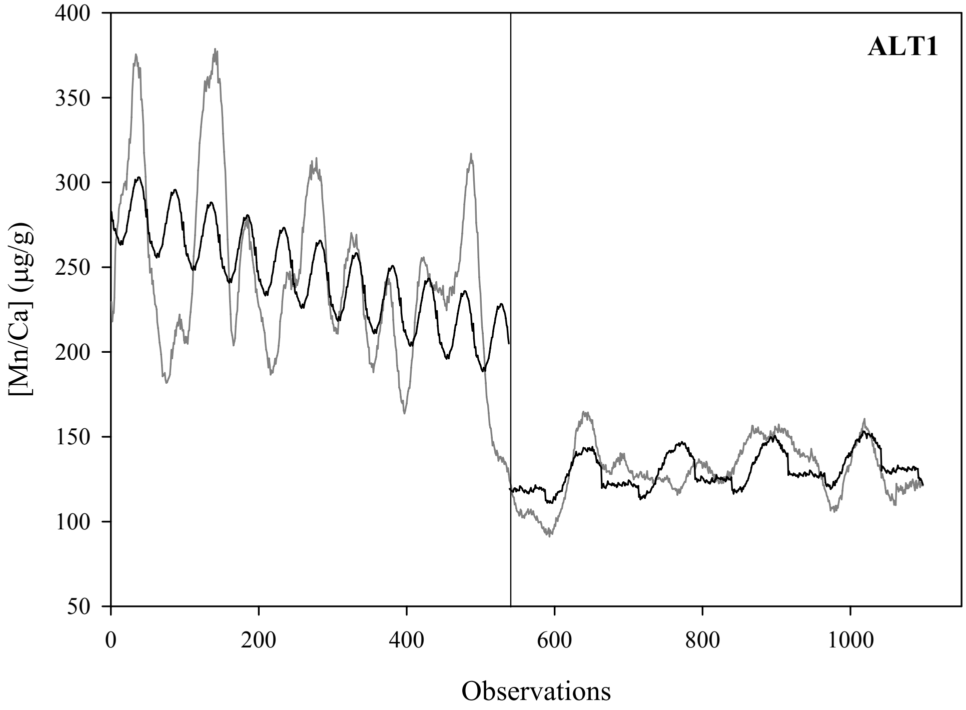
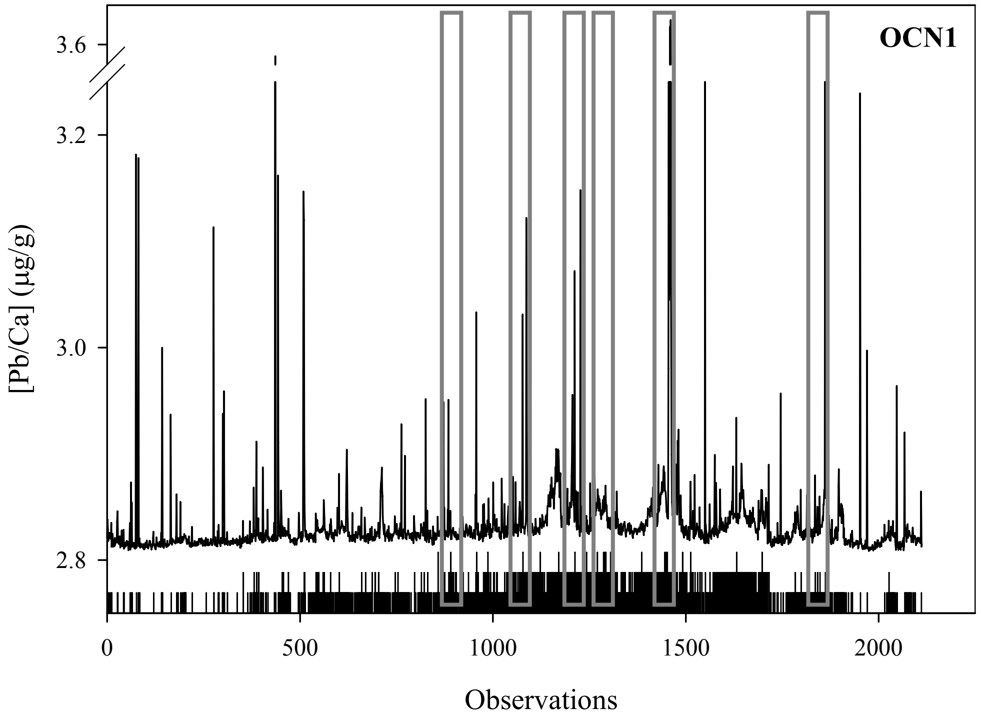


Figure 5.4.


OCN1
[Pb/Ca] (µg/g)
3.6
3.2
3.0
2.8
0
500
1000
1500
2000
Observations

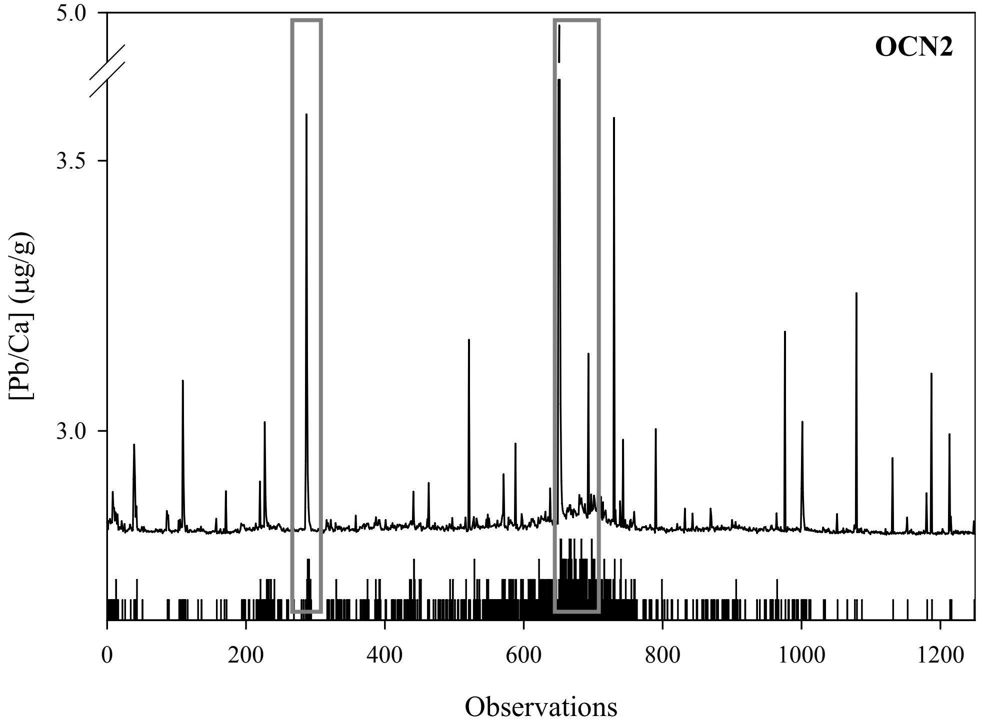
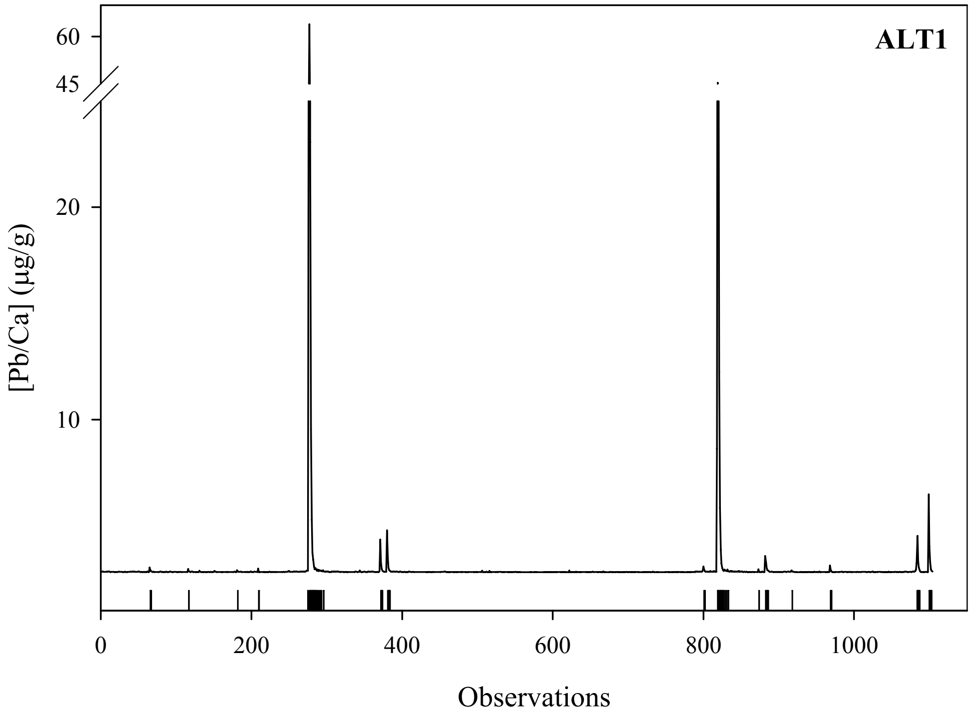

OCN2
[Pb/Ca] (µg/g)
5.0
3.5
3.0
0
200
400
600
800
1000
1200
Observations

Figure 5.4 (cont).



References

Almeida, M. J., G. Moura, T. Pinheiro, J. Machado, and J. Coimbra. 1998. Modifications in *Crassostrea gigas* shell composition exposed to high concentrations of lead. Aquatic Toxicology **40**:323-334.

Barats, A., C. Pecheyran, D. Amouroux, S. Dubascoux, L. Chauvaud, and O. F. X. Donard. 2007. Matrix-matched quantitative analysis of trace-elements in calcium carbonate shells by laser-ablation ICP-MS: application to the determination of daily scale profiles in scallop shell (*Pecten maximus*). Analytical and Bioanalytical Chemistry **387**:1131-1140.

Beliaeff, B., T. P. O'Connor, and D. Claisse. 1998. Comparison of chemical concentrations in mussels and oysters from the United States and France. Environmental Monitoring and Assessment **49**:87-95.

Bellotto, V. R. and N. Miekeley. 2000. Improvements in calibration procedures for the quantitative determination of trace elements in carbonate material (mussel shells) by laser ablation ICP-MS. Fresenius Journal of Analytical Chemistry **367**:635-640.

Bourgoin, B. P. 1990. Mytilus-Edulis Shell as a Bioindicator of Lead Pollution - Considerations on Bioavailability and Variability. Marine Ecology-Progress Series **61**:253-262.

Carell, B., S. Forberg, E. Grundelius, L. Henrikson, A. Johnels, U. Lindh, H. Mutvei, M. Olsson, K. Svardstrom, and T. Westermark. 1987. Can mussel shells reveal environmental history? Ambio **16**:2-10.

Chatfield, C. 2004. The analysis of time series : an introduction. 6th edition. Chapman & Hall/CRC, Boca Raton.

Chiffoleau, J. F., L. Chauvaud, D. Amouroux, A. Barats, A. Dufour, C. Pecheyran, and N. Roux. 2004. Nickel and vanadium contamination of benthic invertebrates following the "Erika" wreck. Aquatic Living Resources **17**:273-280.

Conners, D., Westerfield, SM, Feyko, A, Black, MC 1999. Lead accumulation in soft tissues and shells of Asiatic clams (*Corbicula fluminea*). Proceedings of the 1999 Georgia Water Resources Conference.

Dunca, E., B. R. Schone, and H. Mutvei. 2005. Freshwater bivalves tell of past climates: But how clearly do shells from polluted rivers speak? Palaeogeography Palaeoclimatology Palaeoecology **228**:43-57.

Farrington, J. W. 1983. Bivalves as sentinels of coastal chemical pollution - the mussel (and oyster) watch. Oceanus **26**:18-29.

Freitas, P. S., L. J. Clarke, H. Kennedy, C. A. Richardson, and F. Abrantes. 2006. Environmental and biological controls on elemental (Mg/Ca, Sr/Ca and Mn/Ca) ratios in shells of the king scallop *Pecten maximus*. Geochimica Et Cosmochimica Acta **70**:5119-5133.

Gillikin, D. P., F. Dehairs, W. Baeyens, J. Navez, A. Lorrain, and L. Andre. 2005. Inter- and intra-annual variations of Pb/Ca ratios in clam shells (*Mercenaria mercenaria*): A record of anthropogenic lead pollution? Marine Pollution Bulletin **50**:1530-1540.

Gundacker, C. 2000. Comparison of heavy metal bioaccumulation in freshwater molluscs of urban river habitats in Vienna. Environmental Pollution **110**:61-71.

Imlay, M. 1982. Use of shells of freshwater mussels in monitoring heavy metals and environmental stresses: a review. . Ecological Review **15**:1-14.

Koide, M., Lee, DS, Goldber, ED. 1982. Metal and transuranic records in mussel shells, byssal threads, and tissues. . Estuarine, Coastal, and Shelf Sci **15**:679-695.

Koretsky, C. M., J. R. Haas, N. T. Ndenga, and D. Miller. 2006. Seasonal variations in vertical redox stratification and potential influence on trace metal speciation in minerotrophic peat sediments. Water Air and Soil Pollution **173**:373-403.

Langlet, D., L. Y. Alleman, P. D. Plisnier, H. Hughes, and L. Andre. 2007. Manganese content records seasonal upwelling in Lake Tanganyika mussels. Biogeosciences **4**:195-203.

Lasier, P. J., P. V. Winger, J. L. Shelton, and K. J. Bogenrieder. 2004. Sediment-quality assessment of the lower Oconee River. Southeastern Naturalist **3**:139-154.

Lazareth, C. E., E. Vander Putten, L. Andre, and F. Dehairs. 2003. High-resolution trace element profiles in shells of the mangrove bivalve *Isognomon ephippium*: a record of environmental spatio-temporal variations? Estuarine Coastal and Shelf Science **57**:1103-1114.

Liehr, G. A., M. L. Zettler, T. Leipe, and G. Witt. 2005. The ocean quahog *Arctica islandica L.*: a bioindicator for contaminated sediments. Marine Biology **147**:671-679.

Luoma, S. N., R. Dagovitz, and E. Axtmann. 1990. Temporally intensive study of trace metals in sediments and bivalves from a large river, estuarine system - Suisun Bay Delta in San-Francisco Bay. Science of the Total Environment **97-8**:685-712.

Markich, S. J., R. A. Jeffree, and P. T. Burke. 2002. Freshwater bivalve shells as archival indicators of metal pollution from a copper-uranium mine in tropical northern Australia. Environmental Science & Technology **36**:821-832.

Nystrom, J., E. Dunca, H. Mutvei, and U. Lindh. 1996. Environmental history as reflected by freshwater pearl mussels in the River Vramsan, southern Sweden. Ambio **25**:350-355.

Park, J. W., M. G. Genton, and S. K. Ghosh. 2007. Censored time series analysis with autoregressive moving average models. Canadian Journal of Statistics-Revue Canadienne De Statistique **35**:151-168.

Perceval, O., Y. Couillard, B. Pinel-Alloul, E. Bonneris, and P. G. C. Campbell. 2006. Long-term trends in accumulated metals (Cd, Cu and Zn) and metallothionein in bivalves from lakes within a smelter-impacted region. Science of the Total Environment **369**:403-418.

Poykio, R., H. Nurmesniemi, and R. L. Keiski. 2007. Environmental risk assessment of heavy metal extractability in a biosludge from the biological wastewater treatment plant of a pulp and paper mill. Environmental Monitoring and Assessment **128**:153-164.

Richardson, C. A., S. R. N. Chenery, and J. M. Cook. 2001. Assessing the history of trace metal (Cu, Zn,Pb) contamination in the North Sea through laser ablation ICP-MS of horse mussel *Modiolus modiolus* shells. Marine Ecology-Progress Series **211**:157-167.

Schettler, G. and N. J. G. Pearce. 1996. Metal pollution recorded in extinct *Dreissena polymorpha* communities, Lake Breitling, Havel Lakes system, Germany: A laser ablation inductively coupled plasma mass spectrometry study. Hydrobiologia **317**:1-11.

Siegele, R., I. Orlic, D. D. Cohen, S. J. Markich, and R. A. Jeffree. 2001. Manganese profiles in freshwater mussel shells. Nuclear Instruments & Methods in Physics Research Section B-Beam Interactions with Materials and Atoms **181**:593-597.

Stecher, H. A., D. E. Krantz, C. J. Lord, G. W. Luther, and K. W. Bock. 1996. Profiles of strontium and barium in *Mercenaria mercenaria* and *Spisula solidissima* shells. Geochimica Et Cosmochimica Acta **60**:3445-3456.

Sturresson, U. 1976. Lead enrichment in shells of *Mytulis edulis*. Ambio **5**:253-256.

Sturresson, U. 1978. Cadmium enrichment in shells of *Mytulis edulis*. Ambio **7**:122-125.

Takesue, R. K. and A. Van Geen. 2004. Mg/Ca, Sr/Ca, and stable isotopes in modern and holocene *Protothaca staminea* shells from a northern California coastal upwelling region. Geochimica Et Cosmochimica Acta **68**:3845-3861.

USGS. 2008a. USGS 02226000 Altamaha River At Doctortown, GA.

USGS. 2008b. USGS 02226160 Altamaha River Near Everett City, GA.

Vander Putten, E., F. Dehairs, L. Andre, and W. Baeyens. 1999. Quantitative in situ microanalysis of minor and trace elements in biogenic calcite using infrared laser ablation - inductively coupled plasma mass spectrometry: A critical evaluation. Analytica Chimica Acta **378**:261-272.

Vander Putten, E., F. Dehairs, E. Keppens, and W. Baeyens. 2000. High resolution distribution of trace elements in the calcite shell layer of modern *Mytilus edulis*: Environmental and biological controls. Geochimica Et Cosmochimica Acta **64**:997-1011.

Yap, C. K., A. Ismail, S. G. Tan, and I. A. Rahim. 2003. Can the shell of the green-lipped mussel *Perna viridis* from the west coast of Peninsular Malaysia be a potential biomonitoring material for Cd, Pb and Zn? Estuarine Coastal and Shelf Science **57**:623-630.

Appendix 5.1 Correlations between different elements within the same shell. Values in bold are greater than 0.25 or less than –0.25.

Site	Shell	Mn/Cd	Mn/Cu	Mn/Pb	Mn/Zn	Cd/Cu	Cd/Pb	Cd/Zn	Cu/Pb	Cu/Zn	Pb/Zn
OCN1	M1	0.0457	0.0425	0.0592	**0.2942**	0.0076	0.0797	-0.0091	-0.0117	0.0547	0.0691
	M2	-0.0417	0.0107	0.0047	**0.7024**	-0.0177	0.0084	-0.0435	0.0037	0.0457	0.0219
	M3	-0.0074	-0.0782	-0.1175	**0.2646**	0.0182	0.1652	0.0319	0.1586	0.0843	0.0252
	M4	0.0068	-0.0966	0.0161	**0.5516**	-0.0083	0.2048	-0.0319	0.0006	-0.0473	-0.1409
OCN2	M1	0.0445	-0.0106	0.2142	-0.1778	-0.0119	0.1582	-0.0485	0.0192	0.0753	-0.1340
	M2	0.0030	0.0223	-0.0228	**-0.3901**	0.0360	**0.2544**	-0.0147	-0.0129	0.0368	-0.0206
	M3	0.0364	0.0972	0.0097	**-0.5749**	0.2450	0.0367	-0.0493	-0.0361	-0.1051	0.0496
	M4	0.0482	-0.0139	0.0657	**0.3735**	-0.0153	**0.4681**	0.1361	-0.0373	-0.0245	0.0963
ALT	M1	0.0867	0.0635	0.0771	**0.5557**	0.0004	**0.9010**	0.0136	0.0010	-0.0008	0.0073
	M2	0.0345	-0.0973	-0.0492	**0.6134**	0.1091	0.1851	0.0864	-0.0222	-0.0091	-0.0492
	M3	-0.0281	-0.0252	0.0401	**0.6292**	-0.0160	-0.0004	-0.0446	0.0054	-0.0090	0.0542

Appendix 5.2. The correlation between Cd, Cu, Pb and Zn from different shells at the lags determined by correlations between Mn data series. Values in bold are greater than 0.25 or less than –0.25. Correlations between the same data sets = 1.000.

		Cd				**Cu**				**Pb**				**Zn**			
Site	**Shell**	M1	M2	M3	M4	M1	M2	M3	M4	M1	M2	M3	M4	M1	M2	M3	M4
OCN1	M1	1.000				1.000				1.000				1.000			
	M2	0.016	1.000			-0.021	1.000			-0.007	1.000			**0.730**	1.000		
	M3	0.066	0.010	1.000		-0.007	-0.022	1.000		-0.021	-0.008	1.000		-0.001	0.139	1.000	
	M4	-0.006	-0.008	0.003	1.000	-0.017	-0.011	-0.008	1.000	-0.011	-0.008	0.002	1.000	**0.735**	**0.741**	0.025	1.000
OCN2	M1	1.000				1.000				1.000				1.000			
	M2	-0.008	1.000			0.016	1.000			0.025	1.000			**0.862**	1.000		
	M3	-0.002	-0.008	1.000		-0.052	0.023	1.000		0.034	-0.004	1.000		**-0.405**	**0.602**	1.000	
	M4	0.011	0.020	-0.018	1.000	-0.013	-0.005	0.010	1.000	0.016	0.004	-0.002	1.000	**-0.628**	**-0.530**	**0.604**	1.000
ALT1	M1	1.000				1.000				1.000				1.000			
	M2	0.008	1.000			-0.006	1.000			-0.005	1.000			**0.803**	1.000		
	M3	0.002	-0.012	1.000		-0.031	-0.013	1.000		-0.012	-0.028	1.000		**0.803**	**0.912**	1.000	

CHAPTER SIX

CONCLUDING REMARKS

This dissertation has attempted to address a number of the devilish details that can hinder efforts to monitor potentially toxic trace element contamination in lotic systems. The first goal was to determine whether bioaccumulation of trace elements in the tissues of the Asian clam *Corbicula fluminea* accurately reflected the accumulation in the tissues of a co-occurring native mussel species (*Elliptio hopetonensis*) at the same sites. While this seems like an obvious mode of inquiry, there is a sad lack of direct comparisons in the literature between freshwater mussels and the invasive species that are assumed to represent them as biomonitors. This dissertation determined that several trace elements (As, Cd, Cu, Hg, Pb and Zn) showed similar trends between each bivalve species. However, the study revealed that a number of other factors such as size, season of collection (represented by temperature and to some extent dissolved oxygen) and water chemistry may be in some way influencing accumulation. Without taking into account these factors, it would be difficult to draw firm conclusions about the *cause* of increased accumulation of trace elements found at any given site.

The next step, then, was to expand the spatial range of the study and the number of environmental parameters that were analyzed along with the trace elements. The key to implementing this step was also in employing hierarchical linear models as a means of accounting for individual variation in trace element accumulation related to growth as well as site-specific variation related to location within the watershed and local water and sediment characteristics. That hydrological, chemical, physical and biological parameters should affect bioaccumulation should come as no surprise. What is needed in biomonitoring efforts such as this one is not just the recognition that those parameters affect the outcome but an attempt to remove naturally occurring variation. In other words, more sophisticated and flexible statistical models than the typical null hypothesis testing approaches need to be employed. By removing uncertainty caused by naturally occurring variation, biomonitoring approaches can truly achieve their ostensible goal of identifying the presence and impacts of anthropogenic pollution.

In its final study, I attempted to use freshwater bivalve shells as archives of exposure to trace elements, as well as a record of seasonal environmental processes. The results lend credence to the idea that trace element accumulation in annuli of bivalve shells can eventually be tapped as a source of environmental data. Bivalve shells may be recording information on climate, hydrology, water chemistry and pollution events within their shells. It will require an array of validating studies as well as specialized statistical models to access this data and fully understand its implications. Because bivalve shells are secreted in a sequential manner, their trace element concentrations are autocorrelated and again, cannot be dealt with using null hypothesis testing. Despite these challenges, the benefits of using shells as environmental archives is clear, with implications including bivalve aging (the current techniques of which are in dispute), monitoring the effects of climate change as they directly impact an organism and possibly, retroactive monitoring of pollution events.

Overall, this dissertation makes the case for a carefully considered, appropriately analyzed biomonitoring protocol when investigating trace elements. Naturally occurring variation in bioaccumulation needs to be controlled for accurate conclusions to be drawn. Potential biomonitor organisms need to meet a variety of criteria, including validation of their suitability that takes place under the variable conditions that biomonitor programs would expect to encounter in the field. At the same time, biomonitoring programs need to keep in mind their ultimate goals, as in these studies, which attempted to identify sources of potentially toxic trace elements to threatened native bivalves.

The mussels of the Altamaha River system are a highly unique and in some instances imperiled group of organisms that inhabit a large, relatively unaltered drainage. Concurrent to the studies reported on here, other researchers were investigating the genetics of Altamaha mussels, their populations and our ability to accurately assess them and the movement of organic xenobiotics within the Altamaha and its tributaries. At the same time, the Altamaha spinymussel, *Elliptio spinosa*, was finally slated for federal listing as an endangered species, underscoring the necessity of conservation and monitoring of this system. And so the work continues, with a concerted effort to determine fish hosts for all the endemic species of the Altamaha. Between the efforts of researchers to understand the unique mussel populations and the stressors that they face and the actions of conservation-minded citizens groups such as the Altamaha Riverkeeper, it is entirely possible that mussels and their habitat, will be preserved.