AN EXPERIMENTAL APPROACH TO UNDERSTAND THE RESPONSES OF BENTHIC FORAMINIFERA TO Cd, Pb, Hg, AND Zn

by

ELLEN BROUILLETTE

(Under the Direction of Susan T. Goldstein)

ABSTRACT

Foraminifera respond to a large suite of environmental parameters in addition to anthropogenic influences. To successfully apply foraminifera as bio-indicators we must be able to establish how they respond to individual parameters, including pollutants, in a controlled environment. Only then will it be possible to apply foraminifers as bio-indicators in specific environments. Experiments were conducted to study the responses of common coastal benthic foraminiferans to Cd, Pb, Hg and Zn. Foraminiferal propagule banks used in these experiments were collected from relatively pristine mudflats, located near the lighthouse on Sapelo Island, Georgia. Results document change in assemblage abundance, diversity, evenness and the construction of aberrant test morphologies in response to different levels of exposure to selected heavy metals. *Haynesina germanica* and *Ammonia tepida* are more resistant to high concentrations of heavy metals. *Ovammina opaca* and *Psammophaga simplora* are able to withstand exposure to small concentrations of heavy metals. In general increased exposure to Pb, Zn, and Cd resulted in a decrease in abundances, and species richness. Variable results were observed in evenness with increasing concentrations. Zn was the only metal in the study that produced aberrant test morphologies. *A. tepida* deformed the most frequently and exhibited a distinctive enlarged aperture. Results suggest foraminifera are potential bio-indicators in stressed polluted environments.

INDEX WORDS: Benthic Foraminifera, Georgia Coast, Heavy Metal Pollution, Bio-Indicators, *Haynesina germanica, Ammonia tepida, Psammophaga simplora, Ovammina opaca*

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CHAPTER 1

INTRODUCTION

This thesis is written as a manuscript and is intended for submission to the *Journal of Foraminiferal Research.* As a result this thesis is best read as a single chapter. Chapter two will discuss the previous literature, field site, methods, results, discussion, and conclusion.

To monitor the responses of common coastal benthic foraminifera to Cd, Pb, Hg and Zn this study compares total abundances, species richness, evenness, individual species trends, and aberrant test morphologies. Numerous previous studies focused on *in-situ* field based approaches that attempt to correlate foraminiferal responses with the presence of heavy metals in the environment. This approach alone, however; does not distinguish between the influences of pollutants and environmental parameters (e.g., salinity, temperature, depth, pH, and grain size) that can effect foraminiferal distributions and morphology. By taking an experimental approach we controlled many of the environmental parameters and gained a better understanding of the responses of foraminifera to heavy metals.

These experiments provide valuable insight to how foraminifera respond to specific concentrations of individual heavy metals. This information, along with previous laboratory and *in-situ* studies, will aid in the development of foraminifera as bio-indicators for polluted coastal environments and provide a foundation for future experimental and culture-based studies.

CHAPTER 2

INTRODUCTION

Resig (1960) and Watkins (1961) first documented the response of benthic foraminifera to environmental pollution. Since, many field-based studies conducted globally have demonstrated the effects of heavy metal and organic contaminants on benthic foraminifera (reviewed by Alve, 1995; Nigam, 2006; Table 1). Recent studies have further contended that modern foraminifera, sensitive to a wide range of environmental parameters, are useful as cost-effective bio-indicators in monitoring polluted marine environments and environmental change (e.g., Kramer and others, 1991; Coccioni, 2000; Scott and others, 2001). Foraminiferal responses to heavy-metal exposure can be traced in foraminiferal assemblages through the appearance of aberrant test morphologies (Zampi and D’Onofrio, 1984; Almogi-Labin and others, 1992) and changes in assemblage diversity and abundance (Ellison and others, 1986; Yanko and others, 1998; Samir, 2000). However, impacted settings often contain multiple contaminants and are subject to natural environmental fluctuations. The relationship between foraminifera and change in a single environmental parameter or contaminant using field-based studies alone is difficult to document.

Environments, particularly those of the coastal zone, are ever-changing, and environmental parameters often shift in complex ways that may impact foraminiferal distributions, abundance, diversity, and the occurrence of aberrant test morphologies (Boltovskoy and others, 1991; Stouff and others, 1999; Geslin and others, 2000; Geslin and others, 2002). As a result, numerous field-based studies have reported various responses to the exposure of heavy metals. Coccioni (2000) and Ferraro and others (2005) documented a decline in abundance, which in some cases resulted in a dead zone, with exposure to multiple heavy-metal contaminants. Châtelet and others (2004) also observed a decline in abundance but contribute the overall distribution of foraminifera to salinity. Banerji (1990) and Ferraro and others (2005) both examined locales with multiple heavy metal contaminants and found that species richness and diversity decline at sites with increased exposure to heavy metals. However, Coccioni (2000) reported that sediment type corelated with a decrease in diversity rather than the presence of heavy metals.

Ferraro and others (2005) reported aberrant test morphologies in up to 10% of the assemblage with exposure to heavy metals. Coccioni (2000) reported that test deformations are more prevalent in areas of fluctuating salinity, regardless to the presence of heavy metals. In addition, Geslin and others (2000) suggested that foraminifera respond in the same manner, including the growth of aberrant tests, to any environmental stress. Here, we work with a suite of heavy metals individually to determine whether different species of foraminifera produce generic or specific responses to different pollutants. Exposure to individual heavy metals over a range of concentrations will allow the identification of harmful levels of contaminants to the benthic foraminiferal community and to identify those heavy metals that have the greatest affect on benthic foraminifera.

The goal of this study is to experimentally asses the responses of common coastal foraminifera grown with exposure to specific heavy metals (Pb, Cd, Zn, Hg) over a range of concentrations based on the EPA's National Recommended Water Quality Criteria for Saltwater. To do this, we utilized an experimental approach that allowed us to control several environmental factors (salinity, temperature, illumination, sediment source) while applying heavy metals individually at specific concentrations. Thus, we can assess the impacts of contaminants without the confounding effects of environmental fluctuations. Assemblages of foraminifera were grown from propagules, the numerous tiny juveniles that occur naturally in fine-grained sediments (Alve and Goldstein, 2003; Goldstein and Alve, 2006), with exposure to a specific metal. Using propagule banks provides a novel, simplistic experimental approach to this problem and permits the cost-effective assessment of the common taxa present in an assemblage of foraminifera. Those potential responses to heavy metal exposure examined during the course of this study include: a) the impact on both abundance and species richness within experimentally grown assemblages, b) species-specific effects and the identification of potential bio-indicator species, and c) the occurrence in aberrant test morphologies.

TRACE METALS

Heavy metal concentrations in marsh environments are controlled by reactions that occur on the surface of sediment particles that include sorption, reduction/oxidation, and solid solution chemistry (Luoma, 1990). Sediment size, texture and composition can affect the binding site density. Numerous studies demonstrated that study areas with finer sediment fractions and finer textures maintain higher organic content and greater concentrations of trace metals (Loring, 1982; Williams and others, 1978; Ackerman, 1980; Forstner and Salomons, 1980; Wheeler and others, 1980; Ackerman and others, 1983). These processes regulate the activity of free metal ions in estuarine waters and provide a pathway for uptake by estuarine inhabitants, including foraminifera. Trace metal sorption occurs on organic matter, hydrated Fe, and Mn (oxy) hydroxides in estuarine sediments. Adsorption occurs when a solute binds to a solid surface due to weak electrical forces (Sadiq, 1992).

Cadmium (Cd) most commonly occurs in the II oxidation state in nature and has an average concentration of 0.15 to 0.20 ppm in the Earth's crust. Anthropogenic uses of Cd include the smelting and refining of zinc, as an additive to pigments, polyvinyl plastics, and batteries and a corrosion inhibitor for metals (Bradl, 2005). Sources for Cd pollution include phosphate fertilizers, municipal sewage, and mining. Cd adsorption occurs quickly and is mainly affected by pH, ionic strength and cation exchange capacity. Over 95% of adsorption occurs in the first 10 minutes, and equilibrium is reached within an hour (Santillan-Medrano and Jurinak, 1975). As pH increases, Cd sorption triples (Christensen, 1984). Conflicting studies have been reported for Cd adsorption in marine environments. Studies show positive and negative correlations between sediment size and the presence of Fe and Mn (oxy)hydroxides in sediments (Sadiq, 1992). Rather, the independent chemistry of an environment controls Cd adsorption, sediment size, organic matter and carbonate surfaces, all which influence adsorption rates (Sadiq, 1992).

Lead concentrations in modern environments range between 0.01 and 1000 ppm. The variance results primarily from anthropogenic contributions which stem from gasoline, batteries, cable covering, and other chemicals. Lead forms an insoluble solid phase in highly concentrated areas by adsorbing onto particulates in seawater. Pb commonly exists in the II oxidation state allowing it to quickly adsorb onto environmental particles including clays, carbonates, oxides, organic matter and hydroxides of Fe and Mn.

Zinc (Zn) occurs in the crust at approximately 70 ppm. Fertilizers, insecticides, mining and sewage sludge are sources of anthropogenic Zn pollution. Zn adsorption is governed by pH, clay content, organic matter and cation exchange capacity (CEC). Accumulation of Zn is primarily controlled by pH, sediment size, and organic content (Bradl et al., 2005). As with all heavy metals, the type of clays present will control Zn adsorption based on CEC, surface area, and texture. Surface charges on hydrous oxides depends greatly on pH and increases with pH, therefore Zn adsorption is also affected by the presence of oxide surfaces whose clay fractions have silica components (McBride and Blasiak, 1979).

Mercury (Hg) is very toxic to marine organisms, and pristine habitats generally host less than 0.001 ppm Hg. Anthropogenic sources of Hg include electrical products, chloralkali plants, and other miscellaneous instruments. Hg can exist in several forms that can all speciate with one another readily in marine environments (Sadiq, 1992). Deposit feeders free Hg adsorbed onto sediments, thus reintroducing Hg into the water column making it bio-available to other marine organisms. In marine environments Hg occurs as a positively charged ion, and readily reacts with clays, organics, oxides, carbonates and hydroxides of Fe and Mn. The order of importance of these is not yet clearly understood however the adsorption is known to be a relatively fast reaction.

MATERIALS AND METHODS

Assemblages of adult foraminifera were grown from a “propagule” bank that contains abundant juveniles present in the fine sediment fraction. Sapelo Island, Georgia was chosen as a field site based on its accessibility and general lack of anthropogenic pollution and disturbances. Sediment samples were collected from the Lighthouse mudflats (Fig 1), located on the southern end of the island.

Sediment collected in October 2007, July 2008, and December 2008 is used in this study. Surface sediment was collected from the upper few mm, avoiding the sulfidic subsurface sediments, using a spatula. The sediment was sieved at the University of Georgia's Marine Institute. A larger 850-micron sieve was used to separate plant debris and gastropods from the fine-sediment fraction. The < 63 micron fraction was retained and transported to the University of Georgia. Twenty-mL aliquots of the < 63 micron fraction were placed in culture containers along with 40 mL Instant Ocean that was diluted with distilled water to adjust the salinity to that at the time of collection (~32 psu). Concentrations of heavy metal contaminants were added to the culture containers based on the United States Environmental Protection Agency's National Recommended Water Quality Criteria for Saltwater Criteria Maximum Concentration (Cd 0.040 ppm; Hg 0.0018 ppm; Pb 0.210 ppm; and Zn 0.090 ppm) and increased by an order of magnitude for four or more concentration levels of each individual heavy metal (Table 2). The Criteria Maximum Concentration (CMC) is the amount of a specific heavy metal that an "aquatic community can be exposed to briefly without resulting in an unacceptable effect" (United States EPA, 2006). Heavy metals were added as dissolved chlorides with the exception of Pb which was added as a carbonate, acidified prior to use, based on the environment, toxicity, and the ability of the compound to dissolve in solution. Replicates of each concentration level along with controls, in which no metals were added, were also run. The containers were kept at a constant temperature of 18ºC, and illuminated on a

12-hour cycle. After four weeks the experimental and control containers were harvested concurrently, preserved using 10% buffered formalin and stained with 1 g/L rose Bengal. A total of 52 samples were picked wet for foraminifera, identified and counted.

At the time of harvesting, water in each culture container was decanted and stored in centrifuge tubes for approximately a year prior to analysis using inductively coupled plasma optical emission spectrometry (ICP-OES). Samples were filtered through a 0.45 μM membrane filter (Whatman, USA) and acidified to 10% with analytical-reagent grade HNO_3. The analytical determination of metals (Cd, Pb and Zn) was carried out using inductively coupled plasma optical emission spectrometry (ICP-OES;4300DV; PerkinElmer-Sciex, Waltham, MA USA).

For ICP-OES analysis, samples were dissolved and diluted in 0.16 M ultra high purity HNO_3. High-purity water (electrical resistivity >18.2 MΩ cm) was produced with a Milli-Q system (Millipore, MA, USA). After ICP-OES analysis, all experiments treated with Hg yielded levels below the instrument detection limits; therefore, no graphical interpretation of Hg and foraminifers are included in this report. Total foraminiferal abundances ranged from 1470 to 771 individuals (Appendix 1) with no apparent relationship to the initial metal concentrations added.

Calibration was obtained with NIST traceable external standards. Standard solutions were prepared by diluting a 1000 mg. L^{-1} multielement solution (ICP Multielement standards, PlasmaCal, Canada and Inorganic Ventures, USA) with 0.16 M ultra high purity $HNO_{3.}$ Duplicates samples, blanks and internal calibration verification (ICVs) were analyzed at a rate of 15% of total samples and were accepted at a 90% accuracy rate.

The instrument detection limit (IDL) was calculated by obtaining 7 to 10 consecutive measurements of reagent blank solution. This parameter was calculated as the average of blank readings plus 3 times their standard deviation. All metal concentrations were determined in triplicates. Duplicates samples, internal calibration verification (ICVs), and blanks were analyzed at a rate of 15% of total samples.

Because nitric acid was not added to the October 2007 and July 2008 samples until October 2008, metals may have adsorbed to the containers, thus reducing their concentration prior to analysis. To address this problem, sediment samples were recollected in December 2008, to determine a correction factor to account for adsorption that may have occurred in the untreated October 2007 and July 2008 water samples. The December 2008 experiments were conducted using the same protocols described previously with identical concentrations of heavy metals. When the December 2008 water samples were analyzed following the above procedures, metal concentrations were significantly lower than the October 2007 and July 2008 samples, which was not expected. Nonetheless, the trends in the data set are the same, in that the higher concentrations of heavy metals added experimentally yielded higher measured ICP-OES results. We expected to see higher concentrations of heavy metals in the ICP-OES analysis for the calibration suite of samples because they were treated and analyzed immediately after harvesting thus reducing adsorption onto the container walls and suspended fine sediment. This was not the case, and concentrations were significantly lower. Although the October 2007 and July 2008 water samples were left untreated they must contain at least the measured amount if not slightly higher. Therefore, the overall trends are accurate and represent minimum values for the experimental heavy metal concentrations.

Still images of specimens were obtained using Scanning Electron Microscopy (SEM) at the University of Georgia's School of Veterinary Science and The Center for Ultrastructure Research at the Franklin college of Arts and Sciences. Images of specimens with aberrant test morphologies were examined to characterize how the test morphology may be altered.

Statistical analyses and graphical interpretation were performed using R version 2.5.1 (R Development Core Team, 2007). Individual scatter plots include abundance (total individuals in an assemblage), species richness (number of species in a sample), evenness measured by Simpson's Diversity index (Simpson, 1949), and total individuals of each of the four most abundant species vs. Cd, Zn and Pb ICP-OES heavy metal concentrations. For concentrations that fall bellow the detection limits of the instrument (Cd 0.0192 ppm, Pb 0.0275 ppm, Zn 0.027 ppm), half of the detection limit is used and plotted to the left of the detection limit boundary. Linear regressions and exponential regressions are fitted to each data set and the exponential regression is illustrated on the graphs. For this study, an R^2 value of 0.600, $p \leq 0.0005$ or greater is considered significant.

RESULTS

Foraminifers respond negatively to high concentrations of Cd, Zn and Pb. The negative response is observed in total abundances, species trends and diversity measurements. Pb has the most acute effect on total abundance, abundances of the four most common species (*Ovammina opaca, Psammophaga simplora, Ammonia tepida, Haynesina germanica)*, species richness, and Simpson's Index. The monothalamids are more resilient to low concentrations of Cd, Zn, and Pb than the rotaliids and suffer the greatest negative effects at the highest concentrations of all the metals in the study. The rotaliids are sensitive at lower concentrations of Cd, Zn and Pb than the monothalamids, but have much larger populations in the highest treatments of Cd and Zn.

The heavy metal concentrations added to the experiments at the start of the study are based on the EPA's guidelines for saltwater environments (Table 2). However, concentrations measured at the end of the 4-week experiment (Table 2) are in many cases significantly less. This may be the result of adsorption onto sediment and organic particles, adsorption onto the container walls, and interaction with living microbiota. Therefore, all analyses are based on the heavy metal concentrations measured by ICP-OES at the end of the 4-week experiment.

Three parameters are used to characterize and compare the effects of exposure to individual heavy metals: 1) the measured concentration that results in an acute effect on the assemblage, 2) the concentration at which no effects of the heavy metals are observed and results are similar to the controls, and 3) the acuteness of Cd, Zn, and Pb as indicated by the slope of the regression. In addition, the acute effect, if below the EPA's guideline, is noted (Table 4).

TRENDS IN ABUNDANCE

On average, 1319 individual foraminiferans grew from 20 mL of sediment in the controls. *Ovammina opaca*, *Psammophaga simplora, Haynesina germanica*, and *Ammonia tepida* dominate the assemblages in both the controls and experimental treatments. Along with *Buliminella elegantissima*, and *Miliammina fusca* these species comprise at least 80% of most samples. A total of 8 controls (no metals added) were conducted (4 from October 2008, 2 from July 2009, and 2 from December 2009). Total abundance in the controls ranged from 1099-1571 individuals. In all treatments, assemblages grown with exposure to high concentrations of metals yielded fewer foraminiferans than those of the controls.

In treatments with Cd concentrations up to 0.55 ppm, total abundances are similar to those of the controls. Cd concentrations of 3.6 ppm result in an acute effect on the total abundance (Fig 2.1; Appendix 1). The slope of the regression is -0.134 (Table 3). Total abundances grown with exposure to Zn are comparable to those of the controls at concentrations up to 0.027 ppm (Fig 2.2; Appendix 1). An acute response occurs at a Zn concentration of 0.12 ppm and generates a negative slope of -0.195 (Table 3). Of the metals examined, exposure to Pb results in the most acute effects on total abundance producing a regression slope of -0.772 (Table 3). Compared to the controls, exposure to small concentrations of Pb (0.07-0.13 ppm) do not correspond to a reduction in total foraminiferal abundance (Fig 2.3; Appendix 1). An acute effect, reflected in a sharp reduction in abundance, is observed at a Pb concentration of 1.94 ppm. At higher concentrations of Pb (2.1-14 ppm), no greater negative effect is observed.

SPECIES RICHNESS AND SIMPSON’S INDEX

To compare samples of uneven abundances, species richness and Simpson’s Diversity index are used to best describe the foraminiferal assemblages (Simpson, 1949). Species richness in the controls ranged from 12-16. Sixteen is the highest species richness observed in the study. The lowest values for species richness are recorded in treatments and not in controls.

In treatments containing Cd, richness values are slightly less (9-15 species) than those of the controls (Fig 3.1; Appendix 1). As a result, no acute effect on species richness is observed in the Cd treatments. The slope of the regression is only slightly negative (-0.029; Table 3). Exposure to Zn at concentrations up to 3449.18 ppm produced variable results (Fig 3.2; Appendix 1). At a concentration of 1.01 ppm species richness totaled 4, whereas 12 species are recorded at a concentration of 3429.4 ppm and 3 species at 3449.18 ppm. Because of the variation in the data no acute response in species richness is identified. The resulting slope of the regression is just slightly negative (-0.057; Table 3). In treatments containing Pb, species richness remained comparable to that of the controls up to concentrations of 0.33 ppm (Fig 3.3; Appendix 1). Pb concentrations result in the most acute affect on species richness at 1.94 ppm with a slope of -0.245 (Fig 3.3; Table 3). Assemblages grown with exposure to Pb concentrations of ≥ 1.94 ppm contain only 2-6 species.

Simpson's index is used to evaluate species evenness in the controls and experimental treatments. In the controls evenness varies from 0.738-0.80. In the Cd treatments, values ranged from a high of ~0.8 at exposures up to 0.36 ppm, to a low of ~0.52 at higher concentrations (Fig 4.1; Appendix 1). Simpson's index does decline in exposure to Cd, however; there is no abrupt change. Therefore, an acute effect on the Simpson's index is not observed with exposure to Cd. The resulting slope of the regression is -0.025 (Table 3). Exposure to Zn concentrations up to 3449.18 ppm had no significant effect on evenness (Fig 4.2; Appendix 1). At all concentrations of Zn (< 0.027-3449.18 ppm), Simpson's index values are only slightly less than those of the controls. As a result, the slope of the regression is -0.008 (Table 3). No acute effect is observed in evenness in Zn treatments. Exposure to Pb resulted in the most acute effect on Simpson's index at 1.94 ppm. The slope of the regression is only slightly negative, -0.063 (Table 3). In treatments with Pb concentrations of 0.33 ppm or less, Simpson's Index values are broadly comparable to those of the controls. At higher concentrations, (1.94 ppm or greater) evenness is 0.46-0.67; 0.46 is the lowest Simpson's index values recorded in the study (Fig 4.3; Appendix 1).

SPECIES TRENDS

Trends are summarized for the four most abundant species that grew in the controls and treatments: two monothalamids, *Ovammina opaca and Psammophaga* cf. *P. simplora*, and two rotaliids, *Ammonia tepida* and *Haynesina germanica.* These four species dominate the controls. Several additional species are generally common in the controls, though far less abundant: *Buliminella elegantissima, Miliammina fusca, Textularia candeiana, Textularia nitens,* and *Quinqueloculina jugosa* (Appendix 1).

In the controls, *O. opaca* abundances range from 109-209 individuals with an average of 167 specimens. *O. opaca* responds negatively to all of the metals examined. At low Cd concentrations (< 0.0192-3.60 ppm) the population sizes of *O. opaca* are greater or similar to those of the controls (Fig 5.1; Appendix 1) reaching a maximum of 328 individuals. Higher concentrations of Cd (10.36-1599.31ppm) have an acute effect resulting in 46 or fewer individuals. The slope of the regression is -0.34 (Table 3). Exposure to Zn concentrations up to 0.12 ppm result in abundances of *O. opaca* similar or higher (154-283 individuals) than those of the controls (Fig 5.2; Appendix 1). Zn concentrations ≥1.01 ppm have a strong acute effect on *O. opaca* resulting in no more than 2 individuals per treatment. The resulting slope of the regression is -0.46 (Table 3).

In Pb treatments, *O. opaca*'s population size is comparable to that found in the controls up to concentrations of 0.33 ppm (Fig 5.3; Appendix 1). Pb treatments of 1.94 ppm or greater result in a "dead zone" for *O. opaca* (where no individuals grew from the propagule bank). The slope of the regression (-1.00) represents the most acute effect recorded for any species in the study.

Populations of *P. simplora* range from 109-197 individuals in the controls but are significantly reduced in all heavy metal treatments. The 4 highest treatments of Cd, Zn, and Pb result in ≤ 13 individuals of *P. simplora*. Cd treatments up to 3.6 ppm result in population sizes (129-216 individuals) similar to those of the controls (Fig 6.1; Appendix 1). Cd concentrations of 10.36 ppm result in an acute response. The slope of the regression is -0.262 (Table 3). Higher concentrations (up to 1599.31 ppm) result in ≤ 97 individuals of *P. simplora*. Zn treatments of < 0.027-0.12 ppm, have populations of *P. simplora* (134-196 individuals) similar to those of the controls (Fig 6.2, Appendix 1). Zn concentrations of 1.01 ppm result in an acute response. Higher concentrations of Zn (158.6-3449.2 ppm) result in 10 or fewer individuals of *P. simplora*. The resulting slope of the regression is -0.406 (Table 3). Treatments of Pb ranging from 0.07-0.33 ppm contained populations of *P. simplora* equivalent (119-197 individuals) to those of the controls (Fig 6.3; Appendix 1). Treatments with greater concentrations of Pb (1.94-14 ppm) result in an acute affect with 5 or fewer individuals. Pb treatments have the greatest acute effect on *P. simplora* (regression slope-0.902; Table 3).

In the controls, *A. tepida* is among the most abundant calcareous species. Population sizes range from 187-323 individuals (average of 276). Cd concentrations from < 0.0192-0.55 ppm result in *A. tepida* abundances similar to those of the controls (Fig 7.1;

Appendix 1). However, exposure to Cd concentrations of 3.6-1599.3 ppm result in variable population sizes (12-187 individuals). As a result, the slope of the regression is -0.102 (Table 3) and no acute effect is observed. Zn concentrations < 0.027 result in abundances similar to those of the controls (Fig 7.2; Appendix 1). Exposure to Zn treatments of 0.12 ppm result in an acute response of *A. tepida.* At higher concentrations of Zn (1.01- 3449.2 ppm) only 25-97 individuals are present. The slope of the regression is -0.132 (Table 3). Pb treatments have the most acute effect on *A. tepida* producing a slope of -0.737 (Table 3). Concentrations of Pb up to 0.13 ppm result in abundances similar to those of the controls. Exposure to Pb concentrations of 0.21-14 ppm (the EPA's guideline for Pb is 0.21 ppm; Table 4) result in an acute effect on the abundance of *A. tepida* (6-48 individuals).

H. germanica is the most abundant calcareous species in the controls and most of the experimental treatments. In the controls, populations of *H. germanica* range from 452-622 individuals. Cd treatments produce variable results. Concentrations up to 0.55 ppm result in populations of 224-750 individuals (Fig 8.1; Appendix 1). Exposure to Cd concentrations of 10.36-11.2 ppm correspond to 14-15 individuals while concentrations of 25.61-1599.3 ppm result in 128-187 individuals. As a result of the variation, no acute affect can be determined. The resulting slope of the regression is only slightly negative, -0.134 (Table 3). Zn concentrations of < 0.027 result in abundances similar to or slightly less than those of the controls (Fig 8.2; Appendix 1). Exposure to Zn concentrations of 0.12 results in an acute affect on *H. germanica*. The slope of the regression is -0.241 (Table 3). Greater concentrations, up to 3449.18 ppm, result in 53 or fewer individuals. Exposure to Pb, again produces the greatest acute effect with a regression slope of -0.646.

Small concentrations of Pb (0.08-0.33 ppm) result in abundances of *H. germanica* that are less (94-295 individuals) than those of the controls. An acute affect occurs at 1.94 ppm resulting in populations of 10-20 individuals.

SHELL DEFORMITIES

Following the terminology of Alve (1991), the aberrant test morphology of *A. tepida* most commonly observed in this study (55% of all deformations) is classified as an enlarged aperture with extreme deformation forming pustules and aberrant calcium carbonate formation (Fig 10). In addition, some specimens exhibit sutures that are covered with an additional layer of calcite (Fig 10.9, 10.10) compared to a normal test (Fig 9).

Deformed tests occurred in the controls at a low frequency (0.91-0.27%) which is comparable to levels reported for naturally occurring assemblages (Alve, 1991; Boltovskoy and others, 1991). Of the heavy metals examined, high proportions of abnormally constructed tests occurred only in those assemblages grown with exposure to Zn. However, concentrations of Zn do not positively correlate to the percent of the deformed shells in the assemblage (Fig 12), contradicting what is expected and reported in numerous field studies. Zn concentrations of < 0.027 ppm result in 12.2% of the assemblages having aberrant test morphologies, the highest observed in this study. Higher concentrations of Zn (158.57- 3449.18) ppm result in lower percentages of deformed tests, 0.9-2.7% however, they are still comparable or higher than those of the controls. Some species had much higher frequencies of deformed tests than others. The monothalamids, *O. opaca* and *P. simplora,* produced no aberrant test morphologies in any of the experiments. Exposure to Zn < 0.027 ppm, however, produced deformations in several calcareous species: 54.4% of *A. tepida* specimens, 24% of *H. germanica*, and 25% of *Q. jugosa* (Fig 5). Very few, < 0.1%, of multilocular agglutinated foraminifers produced deformed tests.

DISCUSSION

TOTAL ABUNDANCE TRENDS

Many field studies report that while pollution may control species trends in foraminifera, other environmental factors effect the assemblages as well including substrate, salinity and hydrodynamics (Coccioni, 2000; Cearreta and others, 2002; Geslin and others, 2002; Hayward and others, 2004; Ruiz and others, 2004; Romano, and others, 2008). The goal of this study is to control environmental parameters, and isolate the responses to heavy metals. Our results are consistent to those of numerous field studies in that we found: 1) a decline in abundance at moderately polluted sites/treatments, 2) extreme degradation of foraminifer assemblages at the highest contaminated sites/treatments and, 3) the presence of aberrant test morphologies.

Field studies conducted in environments subjected to heavy metal pollution report a decline in total foraminiferal abundances (e.g. Schafer, 1973). Therefore it is not surprising that our results show a decline in total abundances with exposure to the highest concentrations of Cd, Zn and Pb. Of interest, however, are the unique responses that occurred to individual heavy metals. Cd treatments, even at the highest concentrations, have the least acute affect on total abundances. The response reflects an increase of opportunistic species (*H. germanica* and *A. tepida*) corresponding to a decline in the monothalamids. Foraminifera are very sensitive to Zn in small concentrations. Exposure to higher concentrations of Zn result in significantly reduced, yet substantial, assemblages dominated by the rotaliids. Though the opportunistic/tolerant species are still able to grow with exposure to high concentrations of Zn, the smaller abundances may reflect reduced tolerances, leaving them unable to grow as quickly and rapidly colonize the available habitat. Ultimately, high concentrations of Pb have the greatest acute effect on all species; no species, including the opportunistic taxa, are able to grow healthy populations under these conditions.

Cadre and Debenay (2006) reported the cessation of growth in response to cultures stressed by high concentrations of Cu. This may also occur in response to other heavy metals. As a result the propagules present in the source sediment may have tolerated the heavy metals but did not grow. This, in a field study or laboratory-based study, can produce a dead zone resulting from stunted growth and the lack of reproduction. In this study, at least a few foraminiferans grew when exposed to Cd, Zn ,and Pb at any concentration.

RICHNESS AND EVENNESS

Field-based studies report decreases in diversity measurements as the exposure to heavy metals increase (Coccioni, 2000; Armynot du Châtelet and others, 2004; Kfouri and others, 2005). In this study, species richness and the Simpson's index both are reduced in high concentrations of Pb. However, with exposure to Zn and Cd there was no acute effect on species richness and Simpson's index. The decline in species richness is a result of the absence of the monothalamids and rare taxa at higher concentrations. As this pattern continues we would expect to see an increase in Simpson's index as the individuals become evenly distributed among the opportunistic/tolerant species as reported by field studies (Murray, 1973). However Simpson's values decline in higher treatments of Cd and Pb. This is influenced by the persistence of some rare taxa even in treatments with the highest metal concentrations.

SPECIES TRENDS

Individual species are important when evaluating an assemblage for responses to pollution. Over the past 50 years, studies on foraminiferal distributions in stressed and polluted environments demonstrate the tolerance and sensitivity of many individual species. Responses observed in the four most abundant species found in this study vary depending on the individual heavy metal.

Few studies address the response of monothalamid foraminiferans to anthropogenic pollution. *O. opaca* and *P. simplora* are not mentioned in pollution studies, most likely a result of research location and sample preparation methods. However *O. opaca* and *P. simplora* are good bio-indicators given their absence or small population size at sites where coastal pollution is a problem. In the treatments of Cd, Zn, and Pb with the highest concentrations, very few if any monothalamids grew. However, in treatments with low metal concentrations, the monothalamids thrived and even exceeded the abundances found in the controls. This may be an opportunistic response resulting from slight declines in the dominant rotaliids or other microbial competitors. Because of their generalized or simplistic test structure, test deformations, if present, are difficult to recognize and were not observed in this study.

The reduced abundances of agglutinated taxa with exposure to heavy metals has been documented in many heavy metal field studies (Banerji, 1992 especially with Zn; Debenay and others, 2001; Bergin and others, 2006), and results of the present study are consistent. “Dead zones have even been rported with exposure to metals (Ellison, 1986; Kfouri and others, 2005; Ferraro and others, 2006; Ruiz and others, 2008).

A. tepida is an opportunistic species that is tolerant to a variety of environmental conditions (Almogi-Labin and others, 1995; Alve and Murray 1999). *H. germanica* is also resistant to pollutants, including metals, and is an opportunistic species (Debenay and others, 2001; Armynot du Châtelet and others, 2004). Our results concur: *H. germanica* and *A. tepida*, are the most resilient species when exposed to high concentrations of Cd, Zn and Pb. The presence of calcareous species and absence of agglutinated species suggest that a calcareous test is beneficial in polluted environments.

Previous studies report an increase of opportunistic/tolerant taxa at polluted sites (Watkins, 1961; Schafer and Cole, 1974; Samir, 2000; Luan and Debenay, 2005;Di Leonardo and others, 2007; Mojtahid and others, 2008; Romano and others, 2008; Valenti and others, 2008). These studies attribute these increases to the exploitation of sediment type, food abundance, and habitat space as less tolerant species decline. In this experiment, many environmental parameters were controlled, leading us to believe that the increased availability of habitat space and perhaps other resources, coupled with lack of competition allow opportunistic/tolerant species to grow.

Previous studies on foraminifera and pollution that focused on sewage outfalls report that the diet of foraminifers may contribute to changes in abundance and diversity (e.g. Watkins, 1961; Bandy, O.L. and others, 1964; Topping, J.N. and others, 2006). In the present study, *H. germanica* sequesters diatom chloroplasts (Lopez, 1979); *P. simplora* ingests sand grains (Arnold, 1982); and *A. tepida* ingests clay particles and microorganisms (Goldstein and Corliss, 1994). The most abundant foraminiferal species encountered in this study therefore have different diets. However, the rapid decline of all species, with the exception of *A. tepida* and *H. germanica* in Cd treatments, suggests that diet alone is not the most important limiting factor.

The EPA's National Recommended Water Quality Criteria for Saltwater, Criteria Maximum Concentration (CMC) (Cd 0.040 ppm, Hg 0.0018 ppm, Pb 0.210 ppm, and Zn 0.090 ppm) are the highest concentration of a heavy metal that an aquatic community can be exposed to "without resulting in an unacceptable affect." All of the acute responses observed in total abundance, species richness, Simpson's index and the four most abundant species are at concentrations equal to or higher than the EPA standards. Therefore because the acute effects are consistent with the EPA standards, foraminifers are useful bio-indicators.

SHELL DEFORMITIES

Many field based studies examining heavy metal contaminants and foraminifera report test deformations. The underlying causes of the deformations however, are often ambiguous because environmental conditions vary widely within the coastal zone, and typically multiple contaminants are present. Many different environmental parameters reportedly can cause test deformations, including fluctuations in salinity, temperature, pH, and grain size (Boltovskoy and others, 1991; Debenay and others, 2001). The experimental design of this study, allowed us to eliminate variations in several of these environmental parameters, therefore limiting the effects to individual heavy metals.

In the experimental treatments *A. tepida* produced the most prevalent test deformation but only in the presence of Zn. Exposure to other metals (Cd, Pb) did not result in abnormally formed tests. The type of test deformation observed, an enlarged aperture, has been documented previously but less dramatically and not in *A. tepida*. Polovodova and Schönfeld (2008) also noted an enlarged aperture in *Ammonia beccarii* specimens, but attributed the deformation to changes in salinity, a factor which was controlled in the present study. The extremely enlarged aperture therefore may be a specific response to Zn and not changes in salinity.

Aberrant test morphologies in our study, resulting from abnormal chamber formation and growth, occurred in adult foraminifera grown with exposure to exposure to Zn. Yanko (1998) stated that the presence of heavy metals strongly affects the cytoskeleton of foraminifera. The cytoskeleton defines the shape of the foraminiferal test during growth. Cytoplasmic filaments form the template for each additional chamber that is formed. Calcification of the foraminiferal test occurs after the development of a glycoprotein organic matrix that provides the surface on which calcium carbonate crystallizes (Hemleben and others, 1977). Results of this study suggest that Zn has a greater impact on the cytoskeleton or calcification resulting in test deformations.

Along with calcium, a variety of trace elements can be included in the foraminiferal test/calcite (Lea and Boyle, 1989; Fritz and others, 1992). Several studies found higher concentrations of heavy metals in foraminiferal tests (including Cd, Zn, and Pb) in environments with elevated heavy metals (Rathburn and others, 2008; Romano and others, 2008; Bloundi and others, 2009). In addition, deformed tests have higher heavy metal concentrations than normal tests (Sharifi and others, 1991; El Din, 2001) suggesting that the incorporation of metals into the lattice structure of the calcite results in deformation.

Calcification studies found the presence of Zn and can slow or cease calcification in high concentrations (Meyer, 1984; Ghizellaoui and others, 2007). A study by Ghizellaoui and others (2007) found that only 1.000 ppm of Zn inhibited 100% of abiotic calcification in experimental studies. Lower concentrations (< 0.2 ppm) result in a 20% reduction in calcification. In addition, these authors note that the presence of Zn also affects the crystal growth which results in significantly smaller crystals. This suggests exposure to Zn may inhibit calcite formation resulting in deformed tests.

While there is debate regarding the causes of aberrant test morphologies in foraminifera, it is clear that the presence of Cd and Pb alone do not cause abnormalities. Zn produced a specific aberrant test morphology in *A. tepida*. Because deformations only resulted from exposure to Zn, results of this study suggest aberrant test morphologies alone are not good indicators of stressed environments. Instead the patterns and strong correlations observed in total abundance and species trends along with test deformations are more indicative of anthropogenically polluted environments. As we continue to gain a better understating of how and why foraminifers respond to pollutants in controlled environments, it will be important to understand how all of the parameters shown to cause disturbances in assemblages interact so that a working model can be created for field studies.

CONCLUSIONS

Coastal environments are ever-changing complex systems. Studies have been conducted assessing the potential of foraminifers as bio-indicators of anthropogenically stressed environments. To develop foraminifera as bio-indicators, controlled experimental studies are necessary to better assess how foraminiferans respond to selected pollutants. In this study assemblages of coastal foraminifera grew under controlled environmental conditions in the lab with exposure to a series of heavy metal (Cd, Zn, Pb) concentrations. Results show that: 1) The exposure of foraminifers to Cd, Zn and Pb result in negative responses, although each response is dependent upon the species and heavy metal, 2) Zn causes distinct aberrant test morphologies in *A. tepida* only, 3) The deformation may be caused by interference with the cytoskeleton or the inhibition of calcification by Zn, 4) Pb has the most acute effect on total abundance, species richness, Simpson's index, and the four most abundant species, 5) *A. tepida* and *H. germanica* are valuable bio-indicators for Zn, Cd and Pb in modern and past environments, 6) *O. opaca* and *P. simplora* are also excellent bio-indicators in anthropogenically stressed environments, however, the lack of a hard test lowers the chances of fossilization and reduces their usefulness of studying the onset and recovery of anthropogenic pollution in the historical record.

CHAPTER 3

FUTURE WORK

With this study and the contributions of numerous previous studies, much is still unknown regarding the responses of foraminifera to anthropogenic stresses. Laboratory studies offer the ability to control environmental parameters that can cause changes in foraminiferal assemblages, thus isolating the response of foraminifera to added contaminants. Very little is known about the response of foraminifera to individual pollutants in a controlled environment. Future laboratory research to be conducted will include working with a larger suite of contaminants. Contaminants will include organic wastes such as organic compounds from agriculture and fertilizer runoff, cellulose, and lignin; additional heavy metals such as arsenic, copper, chromium and nickel; and additional chemicals associated with anthropogenic use including oil. In addition a combination of these pollutants will be added to individual experiments as we gain better understanding of their individual impacts.

Little attention has been given to the cytological responses of foraminifera to pollutants. With a better understanding of these aspects we can gain knowledge of how and why foraminiferan respond negatively to contaminants. Transmission electron microscopy will be conducted on different species exposed to a suite of contaminants to monitor individual species responses.

In addition, the shell chemistry of the foraminiferan will be examined to monitor if heavy metals are incorporated in the test and if the concentrations are reflective of the treatments in which they grew. Shell chemistry will be studied using bulk Iductively Coupled Plasma-Mass Spectrometry (ICP-MS) of the entire foraminiferal test. To analyze more precisely where in the foraminiferal test concentrations are the greatest (e.g. normal versus deformed chambers of a single specimen), Laser Ablation ICP-MS will be used on individual specimens.

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Yanko, V., Kronfield, J., Flexer, A., 1994, Response of benthic foraminiferal tests in response to pollution by heavy metals: implications for pollution monitoring. Journal of Foraminiferal Research, v. 24, p. 73-97.

Yanko, V., Ahmad, M., Kaminski, M., 1998, Morphological deformities of benthic foraminiferal tests in response to pollution by heavy metals: implications for pollution monitoring. Journal of Foraminiferal Research, v. 28, no. 3, p. 177-200.

Table 1---Summary of papers published on the response of Foraminifera to heavy metal pollution.

Year	Author	Pollutants	Comments
2009	Bloundi, M., Duplay., Quaranta, G.	Zn, Cu, Pb, V, Cr, Co, As, Ni	Test abnormalities present and uptake of heavy metals. Decrease of Foraminifera Abundance.
2008	Romano, E., Bergamin, L., Grazia Finoia, M., Gabriella Carboni, M., Ausili, A., Gabellini, M.	PAHs, Mn, Pb, Zn, Fe	Low faunal abundance, increase in opportunistic species (*H. germanica, Q. parvula, M, subrotunda*) near high polluted sites. Increase in test deformation and incorporation of Fe
2008	Valenti, D., Tranchina, L., Brai, M., Caruso, A., Cosentino, C., Spagnolo, B.	Zn, Cu, Cr, Fe, Pb, Hg	Negative correlation between heavy metal and abundance. Opportunistic behavior observed in *Elphidium* spp. Suggests it is controlled by environmental parameters
2008	Carnahan, E., Hoare, A., Hallock, p., Lidz, B., Reich, D.	Cu, Zn, Cr, Hg, Pb, Ni, As, Sb, Sn, Ag	Richness and diversity negatively correlated with metals. Species correlated differently with metals. Noted environmental variables play a role.
2008	Mojtahid, M., Jorissen, F., Pearson, T.H.	Sewage Outfall	Species tolerant to low oxygen strive near disposal site along with opportunistic species. Slowly equilibrium taxa replace the opportunistic taxa
2008	Ruiz, F., Borrego, J., Gonzalez-Regalado, M.L., Lopez Gonzalez, N., Carro, B., Abad, M.,	Mining (Cu, Zn, Pb)	Extremely low numbers of Foraminifera and no mention of aberrant test morphologies
2007	Di Leonardo, R., Bellanca, A., Capotondi, L., Cundy, A., Neri, R.	Hg and PAHs	Reduction in total Foraminifera. Increase in aberrant test morphologies. Dominance of opportunistic species.
2007	Mikulie, N., Orescanin, V., Elez, L., Pavicic, L., Pezelj, D., Lovrencic, I., Lulic., S.	Cr, Cu, Zn, Pb	Very low numbers of *Ammonia* spp (the only foraminifer the paper discusses) and no test deformations
2007	J. de Nooijer, L., Reichart, G., Duenas-Bohorquez, A., Wolthers, M., Ernst, S., Mason, S., van der Zwaan G.,	Cu incorporation culture experiment	Cu is incorporated into Calcite at 0.25+/-0.15 based on seawater chemistry. No species specific differentiation, temperature or salinity difference were noted
2007	Frontalini, F., Coccioni, R.	As, Cd, Cr, Cu, Hg, Mn, Ni, Pb, Zn, V	Defined more tolerant and less tolerant species found along a pollution gradient. Noted the appearance of test deformations and supports the use as foraminifera as bioindicators of heavy

			metals in coastal environments
2007	Luciani	Large suite of heavy metals and pesticides	Appearance of opportunistic species through time. Deformed test deformations were present at all of the sites studied and supports the use of foraminifera as bioindicators
2006	Bergin, F., Kucuksezgin, F., Uluturhan, E., Barut, I.F., Meric, E., Avsar, N., Nazik, A.	Hg, Cd, Pb, Cr, Zn, Cu, Ni, Mn Organics	Noted that in higher contaminated environments the number of species decreases and indicator taxa appear. Also made correlations between deformed tests and organic pollutants
2006	Brouillette, E., Rathburn, A., Perez, E., Kluesner, J., Gray, C., Basak, C., Gieskes, J.	Hg, Cd, Pb, Cr, Zn, Cu, Ni, Mn, Mg, As	Decrease in foraminiferal diversity and abundance along the pollution gradient. Also noted an increase in the test deformations with increases in heavy metal concentrations
2006	Kluesner, J., Rathburn, A., Perez, E., Brouillette, E., Gray, C., Gieskes, J.	Hg, Cd, Pb, Cr, Zn, Cu, Ni, Mn, Mg, As	Reported initial findings showing a relationship between heavy metal concentrations in the sediments and incorporation of heavy metals in the foraminiferal test
2006	Burone, L., Venturini, N., Sprechman, P., Valente, P., Muniz, P.	Sewage, hydrocarbons, Cr, Pb	Decrease in foraminifera density along a pollution gradient. Increase in deformed tests in contaminated areas, and notes that this may be linked to salinity and pollution variation
2006	Cadre, V., Debenay, J.P.	Cu (culture experiment)	Sensitivity to low and high levels of Cu. High levels resulted in delayed growth and reproduction, and the appearance of deformed tests with cytological modification
2006	Ferraro, L., Sprovieri, M., Alberico, I., Lirer, F., Prevedello, L., Marsella, E.	Ni, Pb, Zn, Hg, Cd, As, Cr, Cu, V	Suggests a possible control of heavy metals on foraminiferal assemblages. High levels of pollutants show an increase in tolerant species, and 10% deformed populations.
2005	Nigam, R	Summary	Summary of work done on all Pollutants
2005	Kfouri, P.B.P, Figueira, R.C.L., Figueiredo, A.M.G., Souza, S.H.M., Eichler, B.B.	As, Cr, Zn, Ba, Fe, Br, Co, Cs, Rb, Sb, Se	Increase in abundance of opportunistic species in areas with high levels of heavy metals. Decrease in foraminifera diversity and complete absence was observed in high polluted areas
2004	Armynot du Chatelet, E., Debenay, J.P., Soulard, R.	As, Cd, Cr, Cu, Hg, Ni, Pb, Zn and 13 PAH	Areas with increased levels of heavy metal pollutants showed lower density and species richness. No correlation between foraminiferal distribution and organic pollutants
2004	Hayward, B., Grenfall,	Zn, Cu, Pb, Ni,	Reports that foraminiferal test

	H., Nicholson, K., Parker, R., Wilmhurst, J., Horrocks, M., Swales, A., Sabaa, A.	N, P	deformations are not influenced by the presence of heavy metals. Foraminiferal assemblages and abundances are also not affected by heavy metals and the salinity may be the controlling factor
2004	Ruiz, F., Gonzalez-Regalado, M.L., Borrego, J., Abad, M., Pendon, J.G.	Cr, Pb, Zn, Cu, fertilizers, petroleum byproducts	High levels of pollution are unfavorable conditions for foraminiferal assemblages along with grain size, subaerial exposure, and frequency of dredging
2004	Saraswat, R., Kurtarkar, S.R., Mazumder, A., Nigam, R.	Hg culture experiment	Observed that with increased levels of Hg, the growth rate decreased, and abnormal chambers were produced. At the highest levels of Hg no growth was observed.
2002	Cearreta, A., Irabien, M.J., Ulibarri, I., Yusta, I., Croudance, I.W., Cundy, A.B.	Fe, Mn, Ti, P, Zn, Pb, Cu, Ni, Cr, As	No major correlation between the presence of heavy metals and foraminiferal assemblages. Suggests elevation above mean sea level, and salinity control foraminiferal distribution
2002	Geslin, E., Debenay, J.P., Duleba, W., Bonetti, C.	Cu, Ni, Zn, Pb, Hg, Cd, organics, oil	Greater percentage of deformed tests did not correlate with increased pollutants. The regeneration of deformed/damaged tests may be correlated to strong hydrodynamics
2001	Debenay, J.P., Tsakiridis, E., Soulard, R., Grossel, H.	Al, As, Cd, Cr, Cu, Hg, Ni, Pb, Zn, PAHs, PCBs	Areas with high heavy metal contamination showed the presence of tolerant pioneer species. Lower polluted areas proved difficult to determine what controlled foraminiferal distribution and assemblages
2000	Coccioni, R.	Sc, V, Cr, Co, Ni, Cu, Zn, Nb, Pb, Hg, Ga, Sr, Ba, Y, Zr, Rb, La, Ce, Th, S, As, Br	Foraminiferal assemblages respond quickly to environmental stresses. Environmental stress correlated with an increase in test abnormalities and a decrease in diversity. Heavy metals are not correlated with short term effects. Notes the difficulty of isolating individual factors and recommends culture experiments.
2000	Samir, A.M.	Pb, Zn, Cu, Cr, Cd, agricultural wastes	Direct correlation between foraminiferal assemblages and abundances to the presence of heavy metals and not agricultural wastes. Observed the presence and increase of tolerant species in more contaminated areas along with deformed tests
1999	Alve, E., Olsgard, F.	Cu	Colonization experiments using Cu. Levels of >900ppm showed a decrease in assemblage diversity and affects on

			reproduction
1999	Geen, A., Luoma, S.	Cd	Implements the use of Cd/Ca ratio of foraminiferal test through time to reconstruct polluted environments of the past
1998	Yanko, V., Ahmad, M., Kaminski, M.	Cd, Cr, Ti, Pb, As, Cu, Zn, Co, Ni, V	Known indicator species increase along with the appearance of deformed tests to increased heavy metal concentrations. Recommends culture experiments to confirm findings
1995	Alve, E.	Summary	Review of various types of pollution on foraminiferal assemblages and diversity
1995	Bresler, V., Yanko, V.,	Cd, Cu, Hg	Foraminifera attached to seaweed are less sensitive to heavy metal concentrations compared to foraminifera that are attached to seaweed
1991	Alve, E.	Cu, Pb, Zn, Cd, Hg, organic matter	The use of box cores to trace pollution through time. Shift in assemblage with increased pollution, migration of species due to pollution, and presence of deformed tests
1991	Boltovskoy, E., Scott, D.B., Mediolo, F.S.	Summary	Review of all ecological parameters and pollutants that may cause morphological variations in foraminifera
1987	Nagy, J., Alve, E.	Zn, paper mill	Reduction in calcareous taxa, increase in total population overtime
1986	Ellison, R., Broome, R., Ogilvie, R.	Zn, Cr, V, Mn	Increase of opportunistic species in more contaminated areas. Strong correlation with pollution and changes in density and diversity of foraminifera, and migration downstream and near absence all together in highly contaminated areas
1961	Watkins, J	Sewage Outfall	Foraminifers increase closer to the outfall. Arenaceous inhabit shallower sediment. Abnormality increases towards the outfall.

Figure 1: Map of Sapelo Island. Modified from the National Park Service. The Georgia coast is 5 miles to the east of Sapelo Island. The Lighthouse Mudflats are located on the southern end of the island.

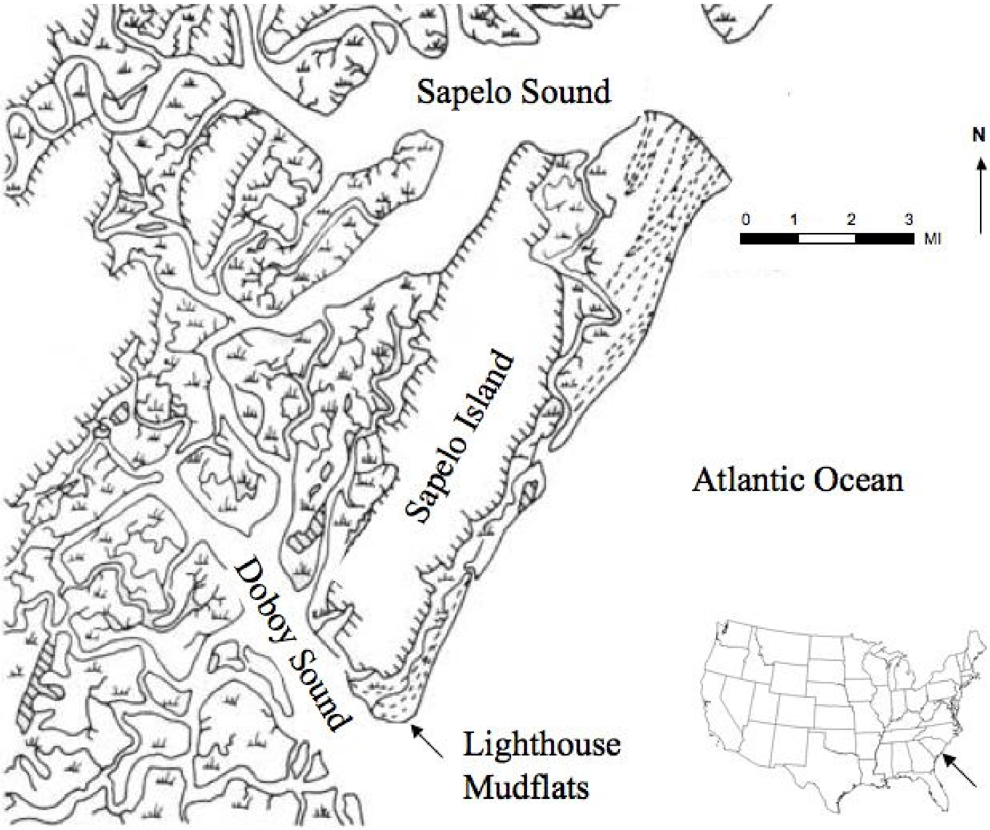

Sapelo Sound
N
0 1 2 3
MI
Sapelo Island
Atlantic Ocean
Doboy Sound
Lighthouse
Mudflats

Table 2--- Heavy metal concentrations

Cadmium Detection Limit 0.0192 ppm

Experimentally added metals Oct 2008	Heavy metal concentration after 4 weeks Oct 2008	Replicate	December 2008 Samples	December 2008 Replicate
0.024	0.04	< 0.0192	0.03	0.02
0.24	0.36	0.36	< 0.0192	< 0.0192
2.4	3.60	0.55	< 0.0192	0.02
24	11.20	10.36	< 0.0192	0.03
240	39.60*	25.61*	0.87	0.62
2400	1582.37*	1599.31*	32.07	26.97

Zinc Detection Limit 0.027 ppm

Experimentally added metals Oct 2008	Heavy metal concentration after 4 weeks Oct 2008	Replicate	December 2008 Samples	December 2008 Replicate
0.0432	< 0.027	< 0.027	< 0.027	< 0.027
0.432	< 0.027	< 0.027	< 0.027	< 0.027
4.32	< 0.027	< 0.027	< 0.027	< 0.027
43.2	1.01	0.12	< 0.027	< 0.027
432	170.14*	158.57*	< 0.027	< 0.027
4,320	3429.38*	3449.18*	10.11	5.202

Lead Detection Limit 0.0275 ppm

Experimentally added metals Oct 2008	Heavy metal concentration after 4 weeks Oct 2008	Replicate	December 2008 Samples	December 2008 Replicate
0.162	0.09	0.11	0.11	0.10
1.62	0.07	0.09	0.10	0.09
16.2	0.08	0.13	0.09	0.09
162	0.33	0.21	0.17	0.17
1620	2.10**	1.94**	2.10	1.94
16200	13.97**	12.80**	13.97	12.80

*Samples were collected in July 2008

**Samples were collected in December 2008

Table 3--- R^2 Values of linear and exponential regressions and exponential slope values

	R^2 Values		Exponential Slope
	Linear	Exponential	
Total Foraminifera			
Cadmium	0.6659***	0.4697**	-0.134
Zinc	0.7133***	0.8466***	-0.195
Lead	0.7944***	0.8232***	-0.772
Species Richness			
Cadmium	0.313**	0.333**	-0.029
Zinc	0.287*	0.2605*	-0.057
Lead	0.605***	0.6071***	-0.245
Simpson's			
Cd	0.5723***	0.5598***	-0.025
Zinc	0.280*	0.2912*	-0.008
Lead	0.6162***	0.5877***	-0.063
O. opaca			
Cadmium	0.3729**	0.5574***	-0.34
Zinc	0.6344***	0.8403***	-0.46
Lead	0.5291**	0.7936***	-1
P. simplora			
Cadmium	0.586**	0.6441***	-0.262
Zinc	0.8276***	0.8341***	-0.406
Lead	0.7349***	0.7403***	-0.902
A. tepida			
Cadmium	0.3831**	0.166	-0.102
Zinc	0.545***	0.5591**	-0.132
Lead	0.593**	0.8263***	-0.737
H. germanica			
Cadmium	0.3296*	0.2079*	-0.134
Zinc	0.4835*	0.5807**	-0.241
Lead	0.4922**	0.7399***	-0.646

* $P \leq 0.05$
** $P \leq 0.005$
*** $P \leq 0.0005$

Figure 2: Total Foraminifera Vs. Individual Heavy Metals. The dashed vertical line is the U.S. EPA CMC limit. ICPOES measurements that are below the instrument detection limits are plotted to the left of the vertical line.

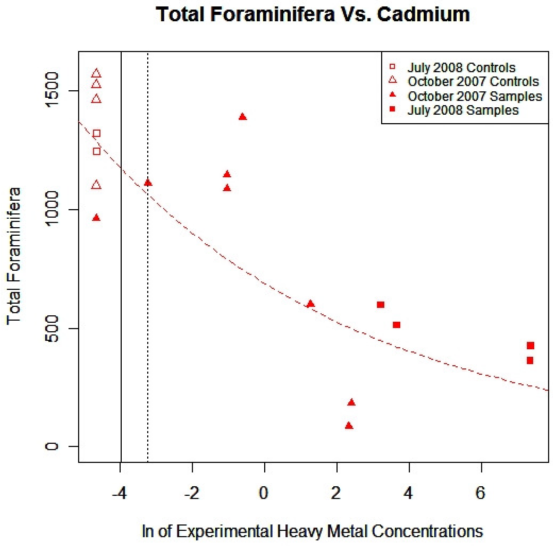


Fig 2.1

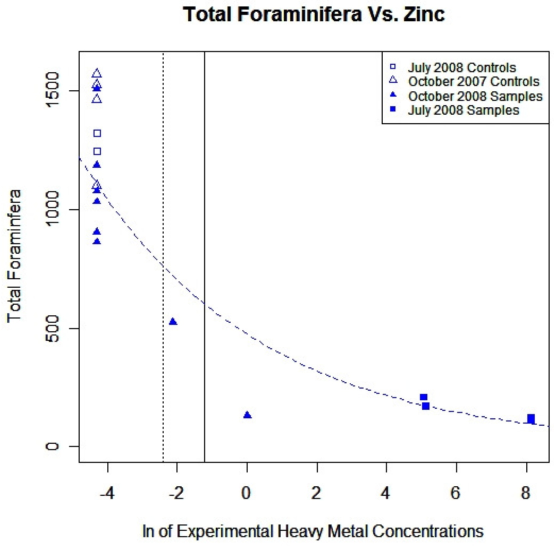


Fig 2.2

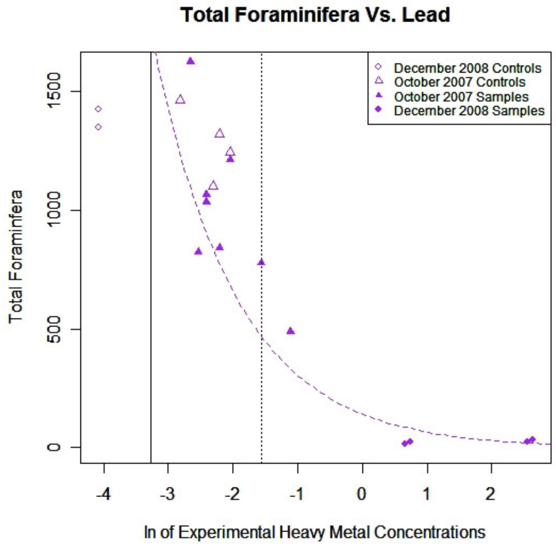


Fig 2.3

Figure 3: Species Richness Vs. Individual Heavy Metals. The dashed vertical line is the U.S. EPA CMC limit. ICPOES measurements that are below the instrument detection limits are plotted to the left of the vertical line.

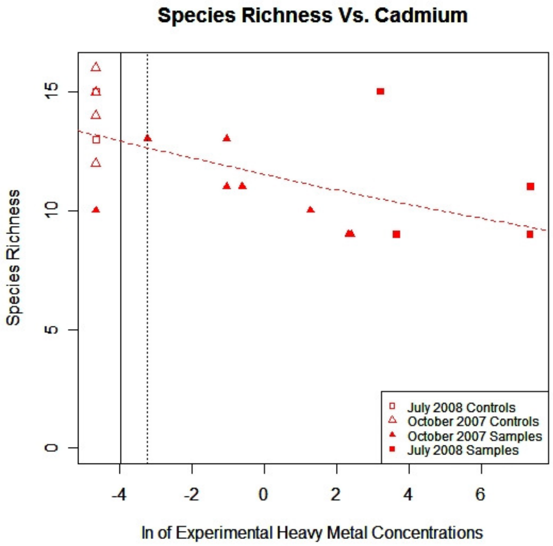


Fig 3.1

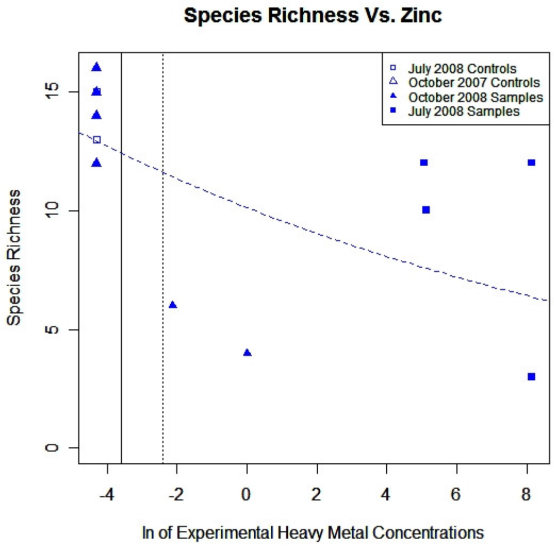


Fig 3.2

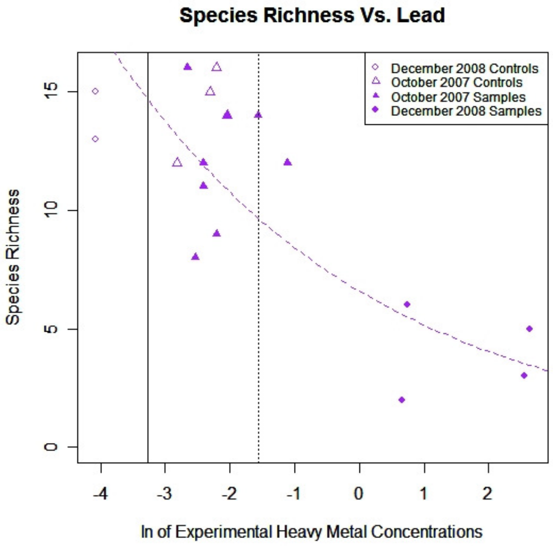


Fig 3.3

Figure 4: Simpson’s Diversity Indices Vs. Individual Heavy Metals. The dashed vertical line is the U.S. EPA CMC limit. ICPOES measurements that are below the instrument detection limits are plotted to the left of the vertical line.

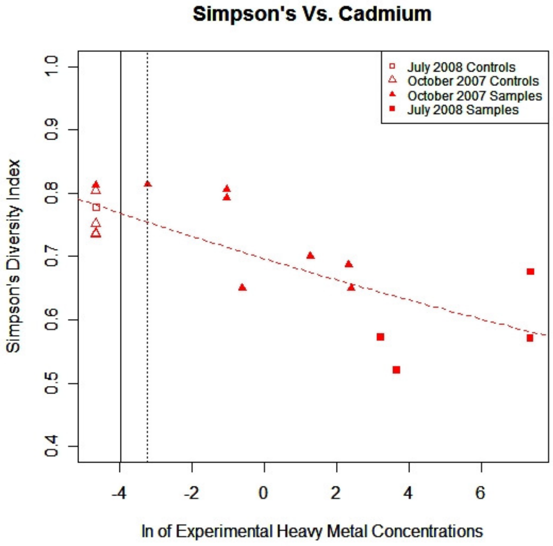


Fig 4.1

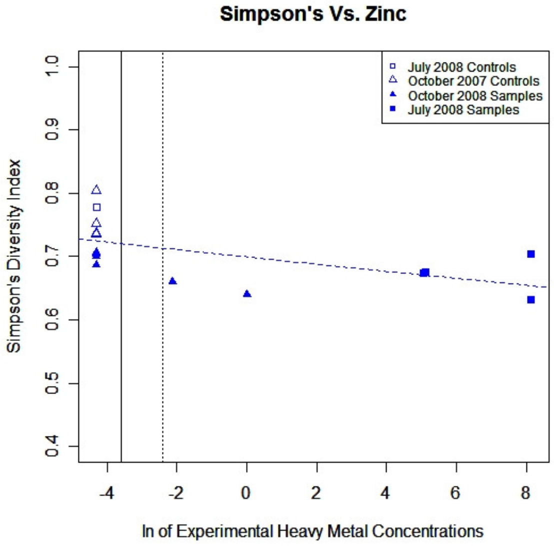


Fig 4.2

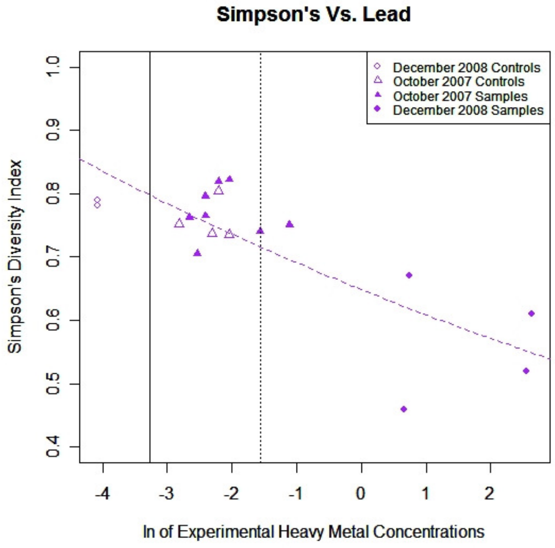


Fig 4.3

Figure 5*: Ovammina opaca* Vs. Individual Heavy Metals. The dashed vertical line is the U.S. EPA CMC limit. ICPOES measurements that are below the instrument detection limits are plotted to the left of the vertical line.

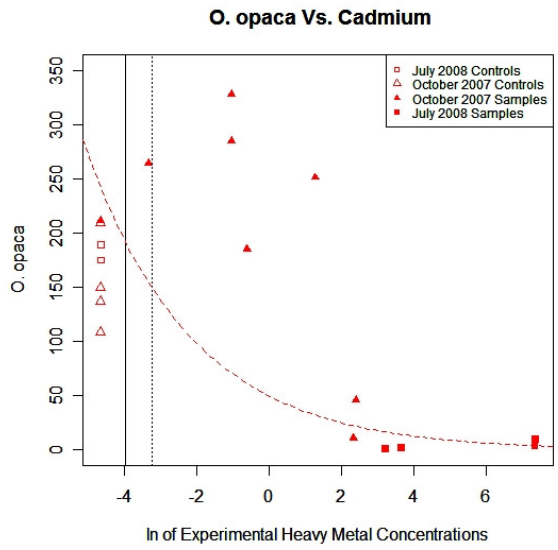


Fig 5.1

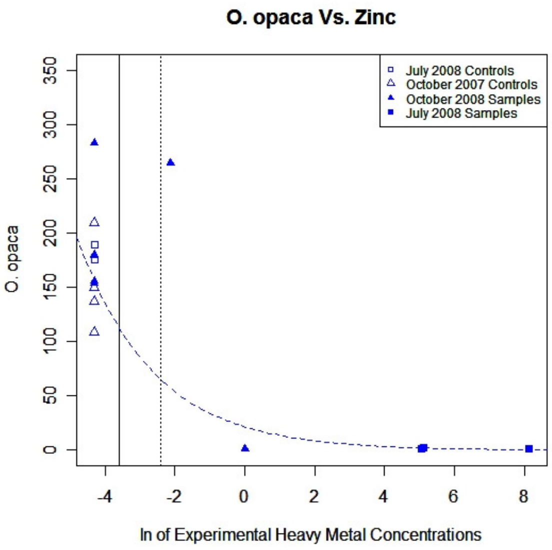


Fig 5.2

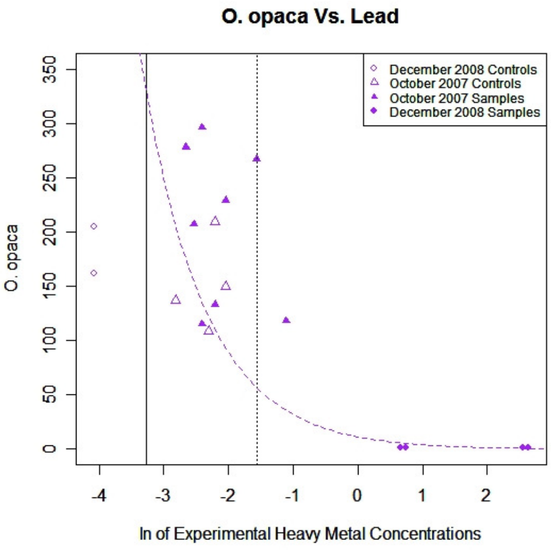


Fig 5.3

Figure 6: *Psammophaga simplora* Vs. Individual Heavy Metals. The dashed vertical line is the U.S. EPA CMC limit. ICPOES measurements that are below the instrument detection limits are plotted to the left of the vertical line.

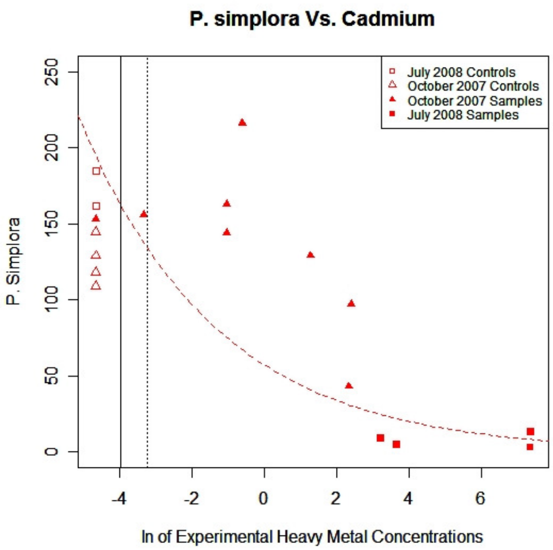


Fig 6.1

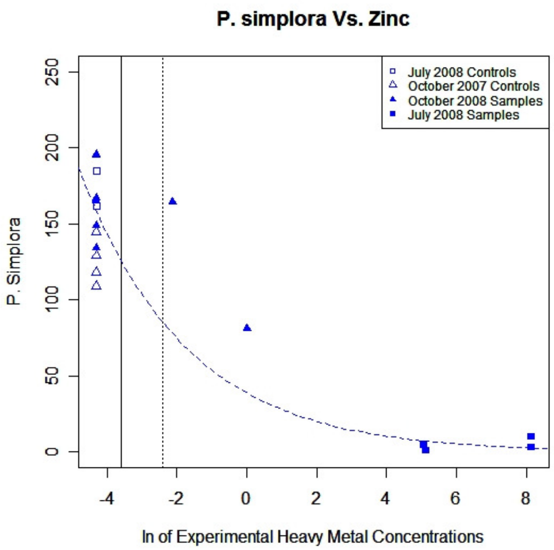


Fig 6.2

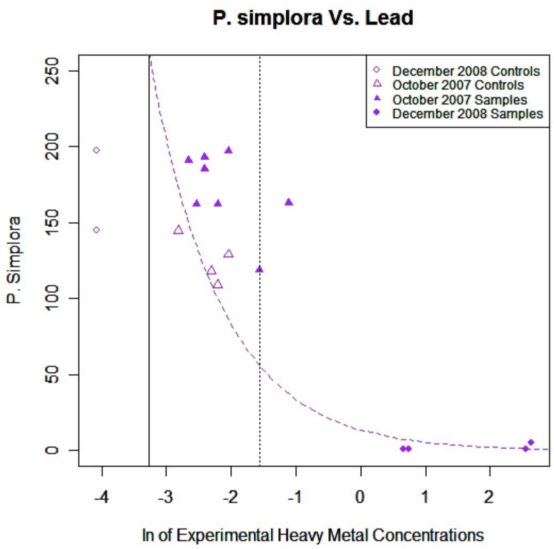


Fig 6.3

Figure 7: *Ammonia tepida* Vs. Individual Heavy Metals. The dashed vertical line is the U.S. EPA CMC limit. ICPOES measurements that are below the instrument detection limits are plotted to the left of the vertical line.

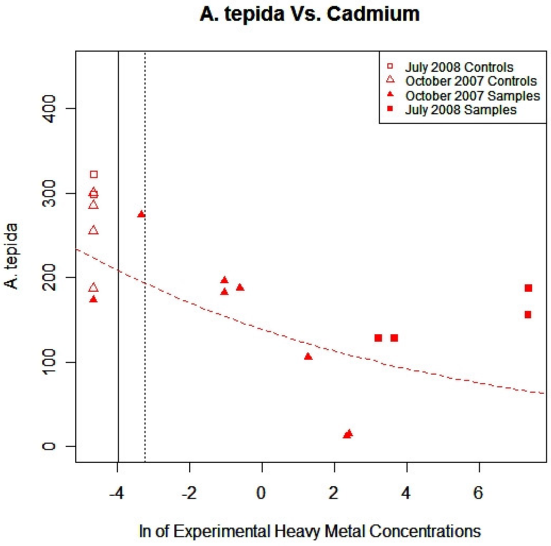


Fig 7.1

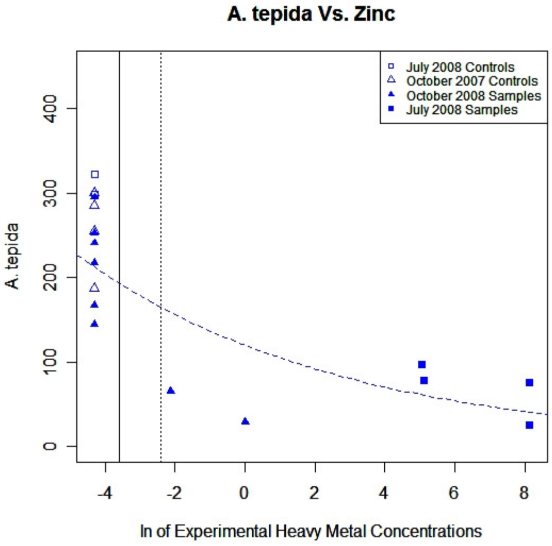


Fig 7.2

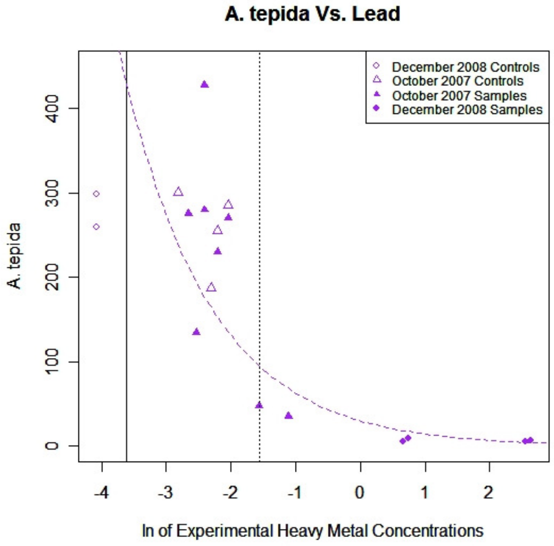


Fig 7.3

Figure 8: *Haynesina germanica* Vs. Individual Heavy Metals. The dashed vertical line is the U.S. EPA CMC limit. ICPOES measurements that are below the instrument detection limits are plotted to the left of the vertical line.

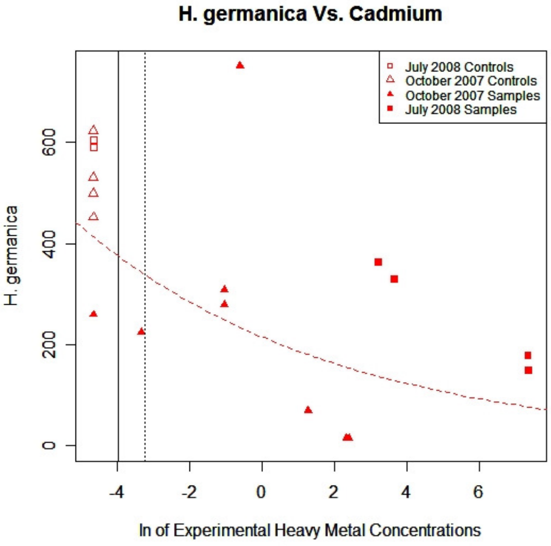


Fig 8.1

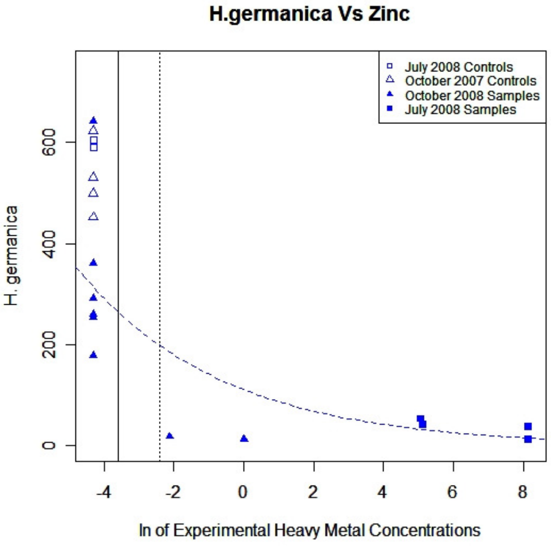


Fig 8.2

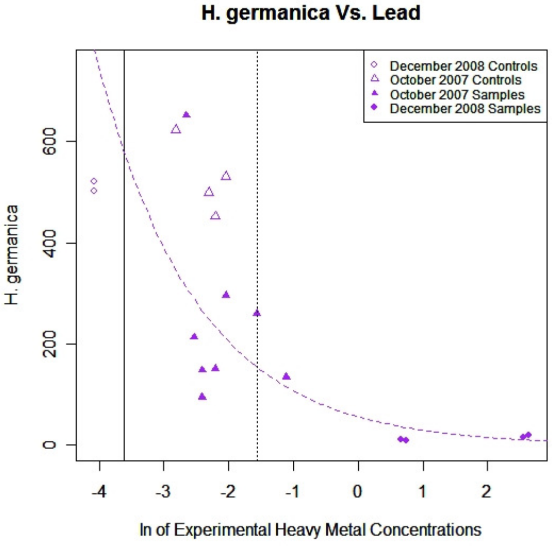


Fig 8.3

Figure 9: Normal morphologies of common taxa in the experiments. 9.1 *Ovammina opaca;* 9.2 *Psammophaga simplora;* 9.3 *Buliminella elegantissima*; 9.4 and 9.5 *Haynesina germanica*; 9.6 *Ammonia tepida*; 9.7 *Quinqueloculina jugosa*; 9.8 *Textularia nitens*; 9.9 *Miliammina fusca*.

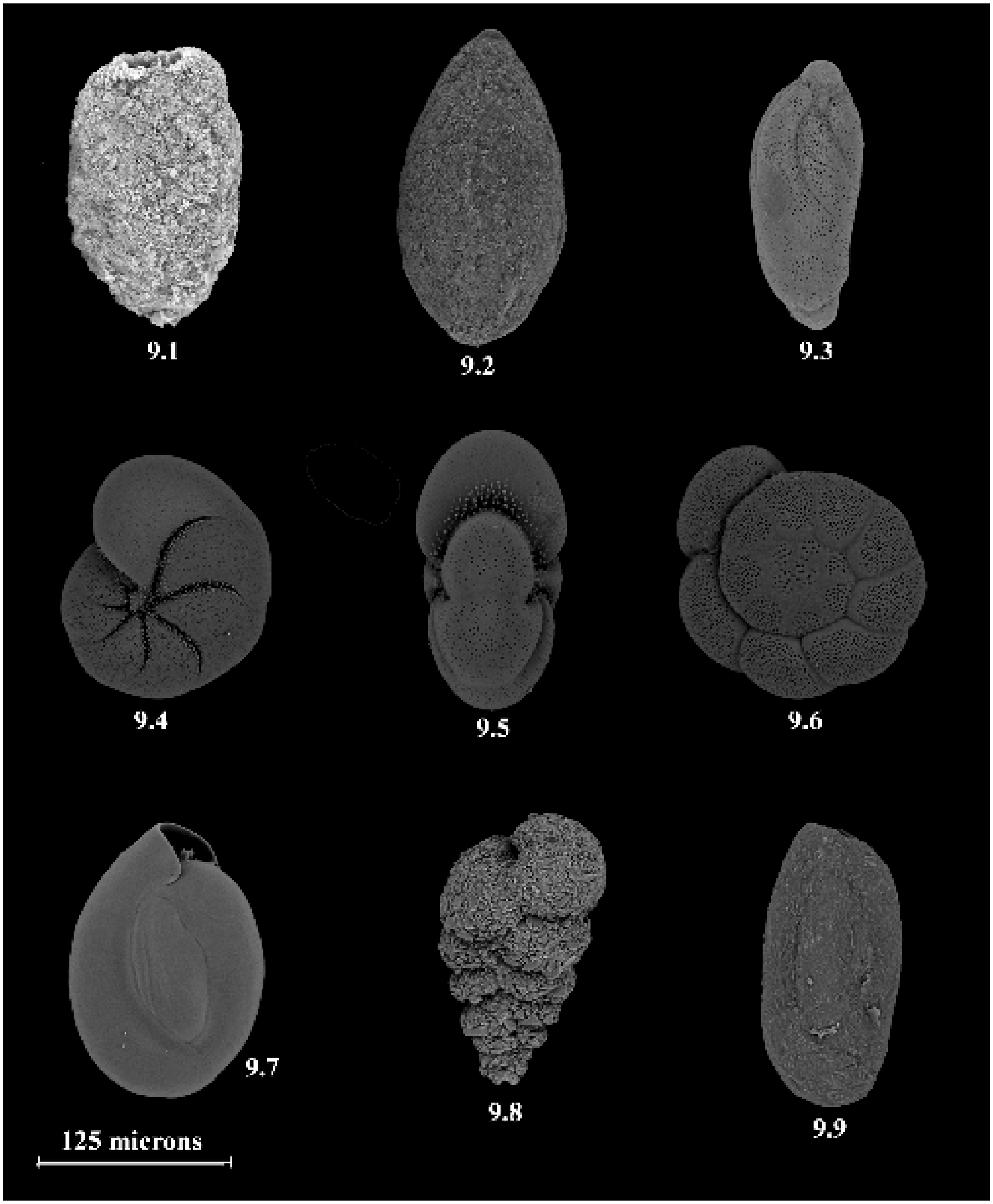
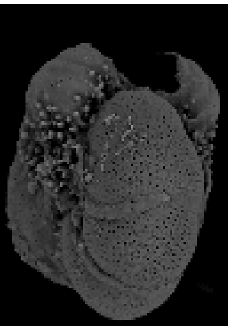
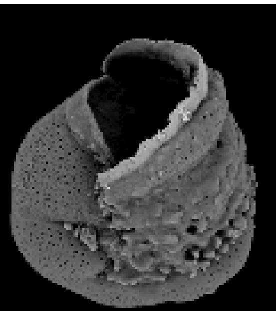

9.1
9.2
9.3
9.4
9.5
9.6
9.7
9.8
9.9
125 microns

Figure 10: Aberrant test morphologies of *Ammonia tepida*. Produced in concentrations of Zn displaying extremely enlarged apertures, irregular calcification, and increased pustules.


10.1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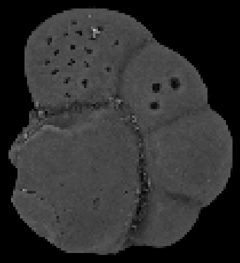
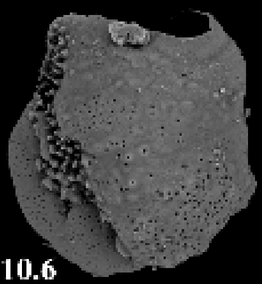
10.3


10.4


10.5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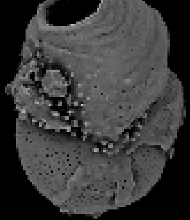
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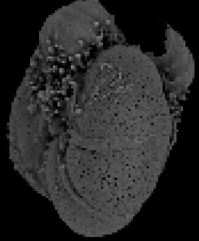
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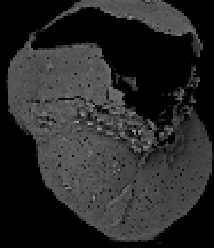
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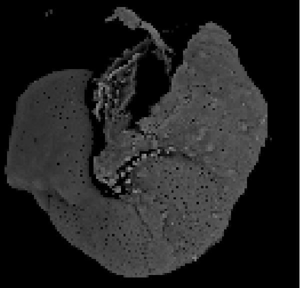
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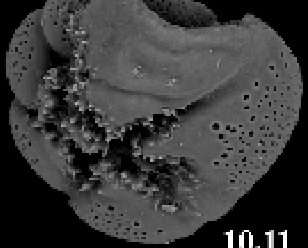
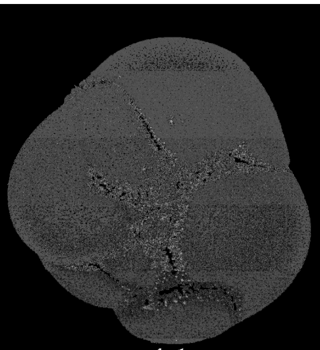

10.11


10.12


10.13

Figure 11: Aberrant test morphologies. 11.1 and 11.2 *Haynesina germanica,* twisted and distorted chamber arrangement; 11.3 *Ammonia tepida,* twisted test; 11.4 *Ammonia tepida,* loosely coiled and overdeveloped chambers; 11.5 and 11.6 *Ammonia tepida,* convexed umbilical; 11.7 *Ammonia tepida,* distorted chamber shape and arrangement; 11.8 and 11.9 *Ammonia tepida,* overdeveloped chambers; 11.10 *Ammonia tepida,* distorted chamber shape; 11.11 *Haynesina germanica,* overdeveloped chambers; 11.12 distorted chamber formation.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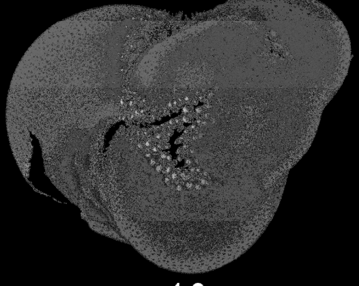
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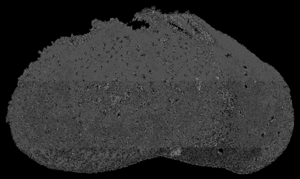
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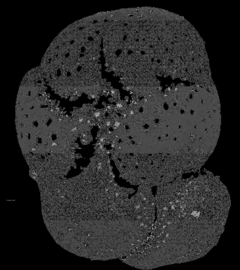
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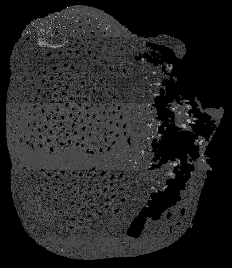
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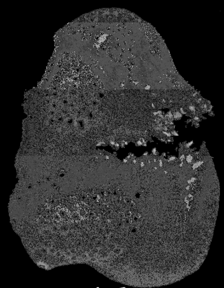
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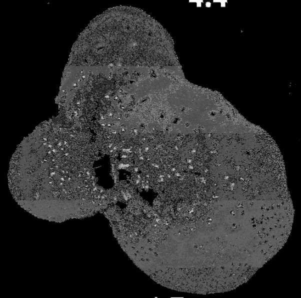
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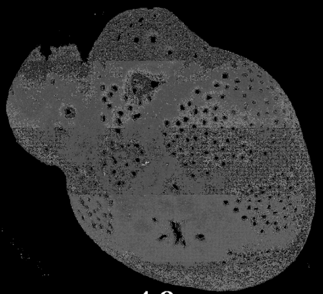
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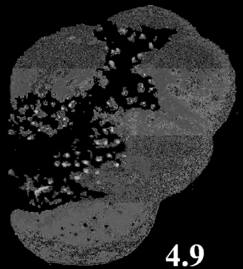
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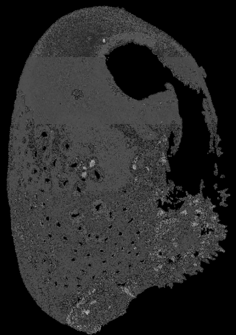
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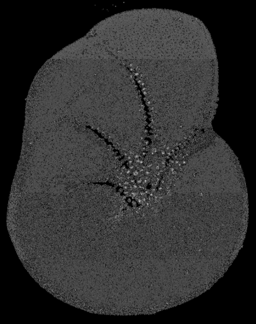
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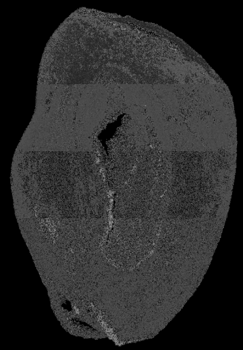
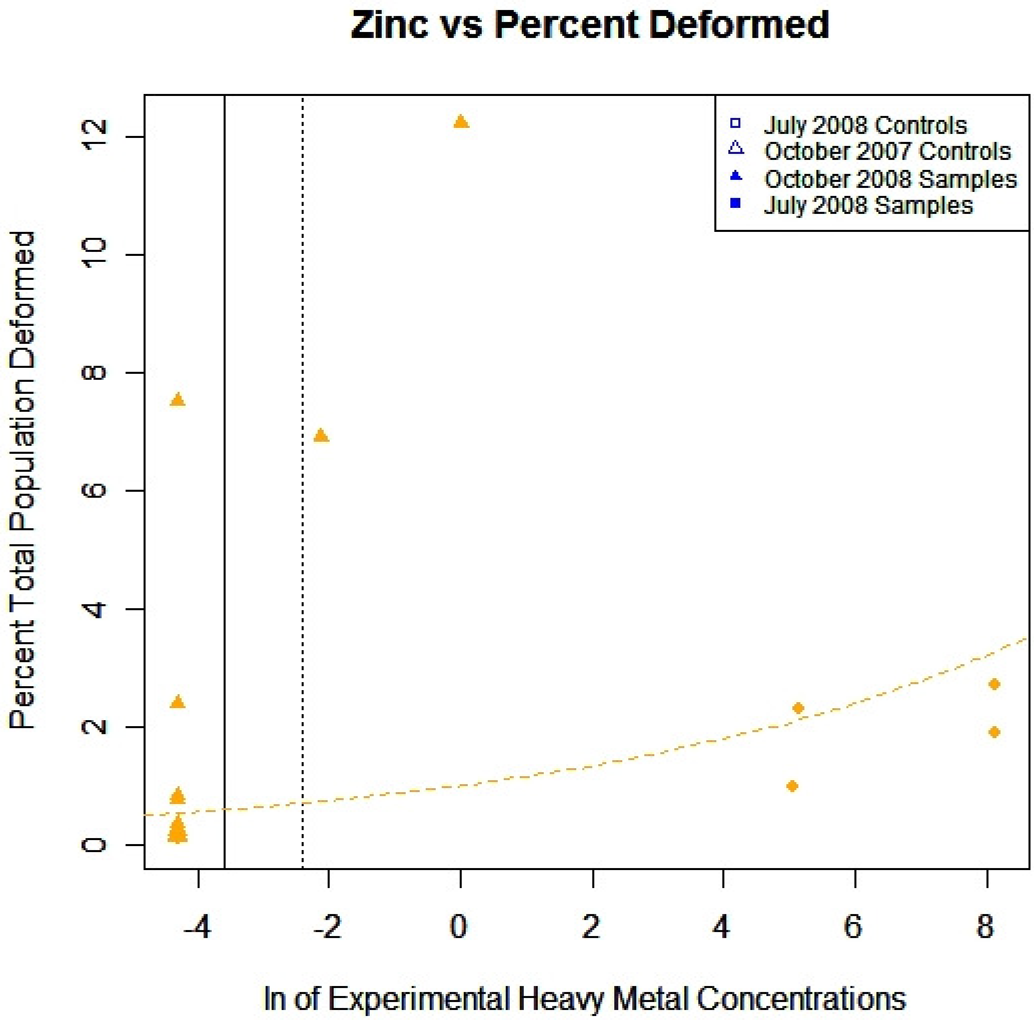

4.12

Figure 12: Correlation of aberrant test morphologies to Zn.



Zinc vs Percent Deformed
□ July 2008 Controls
△ October 2007 Controls
▲ October 2008 Samples
■ July 2008 Samples
Percent Total Population Deformed
0
2
4
6
8
10
12
-4
-2
0
2
4
6
8
In of Experimental Heavy Metal Concentrations

Figure 13: Aberrant test morphologies produced in Zn 2.82 ppm

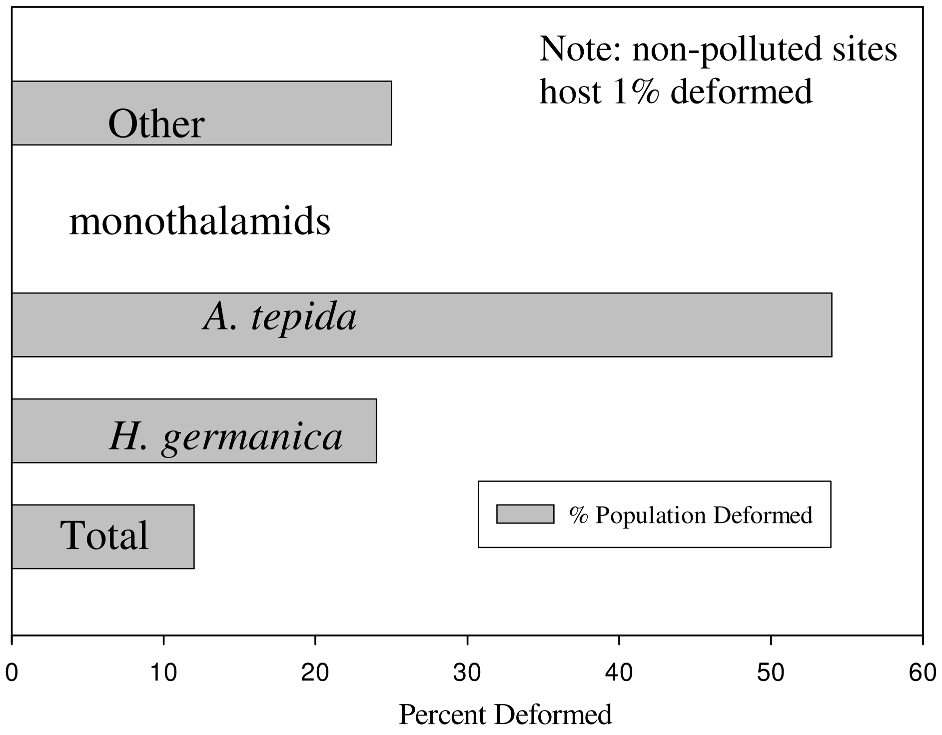

Note: non-polluted sites host 1% deformed
Other
monothalamids
A. tepida
H. germanica
Total
% Population Deformed
0
10
20
30
40
50
60
Percent Deformed

Table 4--- Heavy metal concentrations that result in an acute effect on foraminifers

Variable	Cd ppm	EPA CMC 0.04 ppm	Zn ppm	EPA CMC 0.09 ppm	Pb ppm	EPA CMC 0.21 ppm
Total	3.6		0.12		1.94	
Richness	N/A		N/A		1.94	
Simpson's Index	N/A		N/A		1.94	
O. opaca	10.36		1.01		1.94	
P. simplora	10.36		1.01		1.94	
A. tepida	N/A		0.12		0.21	*
H. germanica	N/A		0.12		1.94	

* Represents concentration exposures below or equal to the EPA's CMC

Appendix I: Foraminifera data

Controls								
Sample Date	Oct-08	Oct-08	Oct-08	Oct-08	Jul-09	Jul-09	Dec-09	Dec-09
Replicate	1/4	2/4	3/4	4/4	1/2	2/2	1/2	2/2
N=	1099	1462	1244	1319	1571	1524	1350	1425
Richness	15	12	14	16	15	13	13	15
Simpson's	0.738	0.7521	0.73491	0.80422	0.77807	0.778	0.780668	0.78955
Species								
Ovammina opaca	109	137	150	209	189	175	162	205
Psammophaga simplora	118	145	129	109	162	185	145	197
Ammonia tepida	187	301	286	255	323	299	299	260
Haynesina germanica	499	622	531	452	605	589	502	520
Buliminella elegantissima	58	65	71	75	81	69	65	52
Miliammina fusca	16	52	44	59	32	62	45	40
Textularia candeiana	39	62	17	61	68	39	48	46
Textularia nitens	11	22	6	41	25	32	28	27
Textularia palustris	5		1	4	21	12		17
Triloculina oblonga	2	5	1	3	1		1	11
Reophax cf. R. arcticus				1		3		
Ammottium salsum	2		1		1		1	1
Reophax cf. R. nana	1			1				
Quinqueloculina jugosa	37	29	5	32	42	56	39	27
Quinqueloculina poeyana	9	21		12	19		14	12
Quinqueloculina bosciana	6		1					9
Ammodiscus catinus			1	4		1		
Elphidium excavutum				1	1		1	1
Trochammina inflata		1			1	2		

Cadmium Experiments

Heavy metal (ppm)	bidl	0.04	0.36	0.36	0.55	3.6	10.36	11.2	25.61	39.6	1582	1599
N=	962	1108	1146	1089	1388	598	86	184	597	511	362	425
Richness	10	13	13	11	11	10	9	9	15	9	9	11
Simpson's	0.81	0.81	0.81	0.79	0.65	0.7	0.687	0.65	0.573	0.52	0.571	0.68
Species												
Ovammina opaca	211	264	285	328	185	251	11	46		2	4	10
Psammophaga simplora	153	156	163	144	216	129	43	97	9	5	3	13
Ammonia tepida	174	274	196	182	187	106	12	15	128	128	156	187
Haynesina germanica	259	224	308	279	750	70	14	15	363	329	178	149
Buliminella elegantissima	51	74	79	44	32	30			1			3
Miliammina fusca	20	22	6	6			1		15	8	5	10
Textularia candeiana	55	58	81	47	7	2	1	2	5	2	1	5
Textularia nitens	36	22	12	52	1	5		2	3	2		1
Textularia palustris	2	4	2	3	5	2			1			
Triloculina oblonga	1	3	1	3	1				1			
Reophax cf. R. arcticus		2							2			
Ammottium salsum		3										
Reophax cf. R. nana												
Quinqueloculina jugosa			3	1	3	1	2	2	60	33	10	29
Quinqueloculina poeyana		2	5		1	2	1	4	6	2	4	12
Quinqueloculina bosciana			5				1	1				
Ammodiscus catinus									1			
Elphidium excavutum												
Trochammina inflata									2		1	6

Zinc Experiments

Heavy metal (ppm)	bidl 1	bidl 2	bidl 3	bidl 4	bidl 5	bidl 6	0.12	1.01	159	170.1	3429	3449
N=	1507	905	1031	861	1076	1186	525	131	207	169	111	122
Richness	15	12	12	14	16	15	6	4	12	10	12	3
Simpson's	0.69	0.7	0.7	0.71	0.7	0.7	0.66	0.64	0.673	0.675	0.704	0.631
Species												
Ovammina opaca	156	154	283	154	180	179	264		1	2		
Psammophaga simplora	196	149	195	134	165	167	164	81	5		3	10
Ammonia tepida	295	167	145	218	253	241	65	29	97	78	25	75
Haynesina germanica	641	260	254	178	292	360	18	13	53	42	12	37
Buliminella elegantissima	71	61	75	43	51	58			1			
Miliammina fusca	28	26	20	27	19	8	1		28	24	34	
Textularia candeiana	45	23	20		29	80	13	8	4	4	1	
Textularia nitens	25	32	15	32	19	33					3	
Textularia palustris	2	1	3	40	16	3					7	
Triloculina oblonga	27			2	2	2			8		2	
Reophax cf. R. arcticus	5			3	2	4					3	
Ammottium salsum	6				1					1		
Reophax cf. R. nana					3					7		
Quinqueloculina jugosa	6	18	5	11	38	20			3	1	4	
Quinqueloculina poeyana	2	12	13	17	1	29			1	3	1	
Quinqueloculina bosciana	2	2	3	1	5	1						
Ammodiscus catinus									3	7		
Elphidium excavutum				1		1					16	
Trochammina inflata									3			

Lead Experiments

Heavy metal (ppm)	0.07	0.08	0.09	0.09	0.11	0.13	0.21	0.33	1.94	2.1	12.8	14
N=	1625	823	1065	1034	840	1211	776	487	17	24	25	35
Richness	16	8	11	12	9	14	14	12	2	6	3	5
Simpson's	0.762	0.705	0.796	0.765	0.82	0.82	0.74	0.75	0.46	0.67	0.52	0.61
Species												
Ovammina opaca	278	207	296	115	133	229	267	118				
Psammophaga simplora	191	162	193	185	162	197	119	163				5
Ammonia tepida	276	135	280	428	230	270	48	35	6	9	6	7
Haynesina germanica	651	213	148	94	151	295	260	135	11	10	16	20
Buliminella elegantissima	104	77	65	78	65	71	5	4		2		
Miliammina fusca	22	14	21	17	26	20	16	4				
Textularia candeiana	56	13	28	50	46	55	9	7			3	1
Textularia nitens	18	2	22	54	25	33	12	5		1		2
Textularia palustris	2		2	2	2	6	1	2				
Triloculina oblonga	3		1	7		4	1					
Reophax cf. R. arcticus	3											
Ammottium salsum	7											
Reophax cf. R. nana	4											
Quinqueloculina jugosa	4		9	2		15	20	8		1		
Quinqueloculina poeyana	2			2		12	16	4		1		
Quinqueloculina bosciana	4					1	1	2				
Ammodiscus catinus												
Elphidium excavutum						3	1					
Trochammina inflata												

Mercury Experiments								
Heavy metal (ppm)	1.8	1.8	18	18	180	180	1800	1800
N=	1141	863	1290	829	1470	1059	771	1111
Richness	15	9	18	9	15	11	16	11
Simpson's	0.815	0.806	0.743	0.82	0.73	0.811	0.83	0.814101
Species								
Ovammina opaca	186	235	191	158	235	194	189	165
Psammophaga simplora	164	153	135	165	190	175	100	145
Ammonia tepida	254	195	217	218	191	196	111	184
Haynesina germanica	314	150	565	138	655	316	184	355
Buliminella elegantissima	114	53	57	40	66	48	37	42
Miliammina fusca	21	26	15	23	27	22	17	26
Textularia candeiana	54	40	44	24	50	36	77	122
Textularia nitens	15	10	12	60	31	27	7	63
Textularia palustris	2	1	30	3	11	2	36	3
Triloculina oblonga	8		3		1		1	5
Reophax cf. R. arcticus	2		5		6		3	
Ammottium salsum			1				2	
Reophax cf. R. nana							2	
Quinqueloculina jugosa	4		5		3	20	1	1
Quinqueloculina poeyana	1		3		2	22	3	
Quinqueloculina bosciana	1		2		2	1	1	
Ammodiscus catinus			1					
Elphidium excavutum	1		2					
Trochammina inflata			2					