ABSTRACT

Title of Thesis:	THE IMPACT OF SEDIMENT RESUSPENSION ON MERCURY CYCLING AND THE BIOACCUMULATION OF METHYLMERCURY INTO BENTHIC AND PELAGIC ORGANISMS
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Sediment resuspension provides a potential mechanism for transferring Hg and MeHg from the sediment to filter feeding organisms and the pelagic food chain, and has been found to enhance Hg methylation. The study objective was to determine the effect of resuspension and clam density on Hg cycling and MeHg bioaccumulation into clams and zooplankton. Two, month-long mesocosm experiments were conducted. The Clam/No Clam Experiment compared resupension with clams (HDC1) versus without clams (NC). The Clam Density Experiment compared resuspension with a high-density population of clams (HDC2) compared with a low-density population of clams (LDC). In addition, a Hg stable isotope was added as a tracer to examine the complex trophic dynamics in the mesocosms. Results from the experiments suggest that clam density did not affect MeHg bioaccumulation into biota over the duration of the experiments. However, high clam density increased net production of MeHg in the water column.

THE IMPACT OF SEDIMENT RESUSPENSION ON MERCURY CYCLING AND

THE BIOACCUMULATION OF METHYLMERCURY INTO BENTHIC AND

PELAGIC ORGANISMS

By

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1.1 Introduction

Mercury (Hg) is a cause for concern for fish and wildlife health in both marine

and freshwater ecosystems, and ultimately humans who may consume them (USEPA,

1997). The coastal zone and estuaries are particularly impacted, especially those close to

major population centers that are strongly influenced by human activities, such as

Baltimore Harbor (Benoit et al., 1998; Mason et al., 2004). Contamination of these

ecosystems comes from both nearby inputs and long-range transport of Hg through the

atmosphere. Elevated concentrations of Hg in estuarine environments are predominantly

the result of anthropogenic activities in the form of urban runoff, industrial waste

discharge, and atmospheric deposition (Mason and Lawrence, 1999; USEPA, 1997).

Understanding the fate of Hg in estuaries is important in understanding the global Hg

biogeochemical cycle since estuaries link the terrestrial and marine environments, and

estuaries could be important sources of Hg to the coastal zone and the ocean. Studies in

the Chesapeake Bay, Hudson River and other estuaries have demonstrated that sediments

in these environments are the major repository for Hg and are the dominant site for Hg

methylation (Heyes et al., 2004; Mason and Lawrence, 1999). The large repository of Hg

in sediments suggest that these can act as a long-term source of Hg to the estuarine

environment (Benoit et al., 1998).

Many metals are toxic to organisms, but bioavailability and toxicity depends upon

the specific chemical form. The majority of Hg released into the environment is

inorganic, yet the most toxic and bioaccumulative form is methylmercury (MeHg). Thus,

for Hg, knowledge of the total concentration in the environment is inadequate to

accurately evaluate its toxicity. Production of MeHg is primarily a biologically-mediated reaction by anaerobic sulfate –reducing bacteria in the sediment, just below the oxic/anoxic interface (Benoit et al., 2003; Benoit et al., 1999; Mason, 2002). However, MeHg production also occurs at the sediment-water interface in well-mixed sediments, potentially providing a vector for MeHg entry to the water column and resulting in the exposure of organisms feeding at the sediment surface (Sunderland et al., 2004). The relationship between Hg inputs and concentrations in biota is influenced by factors that change the rate that inorganic Hg is converted to MeHg (Benoit et al., 2003; Sunderland et al., 2004), and those that influence the degree to which the MeHg produced can be bioaccumulated. As a result, benthic organisms in contact with contaminated sediment can accumulate high concentrations of Hg, especially MeHg, from porewater, overlying water, and food (Lawrence and Mason, 2001; Mason and Lawrence, 1999; USEPA, 1997). Furthermore, transfer of MeHg from the sediment to the water column also provides a source of MeHg to the pelagic food web.

Mercury, mainly as MeHg, bioaccumulates through all trophic levels of the aquatic food chain (Lindqvist et al., 1991; Watras and Bloom, 1992). In the U.S., 75% of all fish consumption advisories are due to Hg (USEPA, 2002). Lower trophic level organisms, which include many benthic organisms, are important in transferring Hg throughout the food web, especially since the greatest bioconcentration of MeHg occurs between water and phytoplankton (Lindqvist et al., 1991; Mason et al., 1996). Organisms that feed on benthic and pelagic microorganisms provide an important link between the base of the food web and higher trophic level organisms such as fish, birds and mammals,

and Hg uptake at the base of the food chain has primary control on the amount of Hg reaching higher trophic levels.

Benthic organisms' life histories range from deposit feeding directly on the sediments to filter feeding in the overlying water. Filter feeding bivalves are an important class of such organisms in estuarine environments that have the opportunity to accumulate pollutants from the particulate matter in the surrounding water they filter. The particulate material can contain MeHg that has been transferred directly or indirectly between the sediment and the water column. However, there is little information relating the physical, chemical and biological factors controlling bioaccumulation of Hg and MeHg in benthic organisms, and subsequent transfer to higher trophic levels, with the sediment and the water chemistry of their surrounding environment (Gagnon and Fisher, 1997; Wang et al., 1998; Watras and Bloom, 1992).

For benthic organisms living in shallow, tidal environments it is difficult to detect the route of accumulation due to sediment resuspension and mixing that result in a strong correlation between dissolved, suspended and surface sediment concentrations (Mason, 2002). The sediment characteristics controlling bioaccumulation (sediment, pore water, overlying water, or suspended matter) depend on both the specific metal of concern and the composition of the sediment since the binding phases, such as particulate organic carbon (POC), acid volatile sulfides (AVS), and metal-oxide phases, often determine bioavailability. However, benthic organisms accumulate most of their metal burden from food (Lee et al., 2000; Wang et al., 1998), and this is especially true for MeHg, as shown by Lawrence and Mason (2001).

Physiologically, assimilation is defined as the fraction of ingested contaminant that is incorporated into biological tissue, thus equaling adsorption minus excretion (Wang and Fisher, 1999). Biological factors that can influence contaminant assimilation include food quality (carbon content) and quantity, partitioning of contaminants in the food particles, and digestive physiology of the organisms. Other factors influencing assimilation include behavior of the chemical within the organism's gut and its associations with different geochemical fractions in the food particles. Organic carbon is important in controlling the bioaccumulation of Hg and MeHg in organisms. Hg and MeHg bind strongly to dissolved organic carbon (DOC) which can increase the dissolved concentrations, however, Hg and MeHg –DOC complexes are less efficiently taken up by methylating bacteria and phytoplankton (Mason, 2002), thus less likely to be trophically transferred. Particulate organic carbon (POC) has also been found to reduce bioavailability of Hg and MeHg from the sediment (Lawerence and Mason, 2001).

Solubilization studies with intestinal fluids of benthic invertebrates support the role of organic carbon in controlling Hg and MeHg bioaccumulation. These studies show a strong inverse correlation between the amount of MeHg released from the sediment and the organic content of the sediment. A greater percentage of MeHg is solubilized from the sediment compared to inorganic Hg, indicating that sediment associated MeHg is more readily available for uptake. In general, these results suggest that organic matter binds Hg and MeHg in the sediment, reducing solubilization within the intestinal tract, and bioaccumulation (Lawrence et al., 1999; McAlloon and Mason, 2003).

The relative importance of metal uptake from the dissolved and particulate (food) phases in organisms is also dependent on the metal's assimilation efficiency (AE).

Differences in AE's may also affect trophic transfer and biomagnification. Contaminants with low AE's are unlikely to be trophically transferred (Wang and Fisher, 1999). Hg has a relatively low AE (<30%) during trophic transfer from phytoplankton and zooplankton, but transfer efficiency is slightly higher for bivalves feeding on algae (Fisher and Reinfelder, 1995; Mason, 2002). However, MeHg has a much higher AE, and is efficiently transferred from phytoplankton to zooplankton (Mason et al., 1996). Gagnon and Fisher (1997) found AE's for the mussel, *Mytilus edulis*, to be 1-9% for Hg but >30% and up to 87% for MeHg, while Wang et al. (1998) found AE's for the deposit-feeding polychaete, *Nereis succinea*, to range from 7-30% for Hg and 43-83% for MeHg.

There are several processes by which contaminants can be transferred to the water column from the sediments, including diffusion and advection, sediment resuspension, and biotransfer from organisms that feed at the sediment-water interface into pelagic consumers. The cycling routes and primary pathways for Hg and MeHg among compartments of shallow estuarine environments are illustrated in Fig 1.1. Sediment is an important sink for Hg and MeHg, especially since Hg is a particle-reactive metal that, once released into the water, is likely scavenged by particles and removed to the sediments. However, as sediment is the main site for Hg methylation, it can also act as a net source of MeHg. Metals accumulated in the sediments may later become a source to the ecosystem (Shine et al., 1998) via diffusion and/or advection of porewaters, biotransfer, and resuspension of sediment . Since the chemistry of the surrounding environment affects the speciation of metals, bioavailability can often be determined by the composition of the sediments and the likelihood of resuspension (Cantwell et al., 2002). However, little is known about the extent to which Hg and MeHg can be
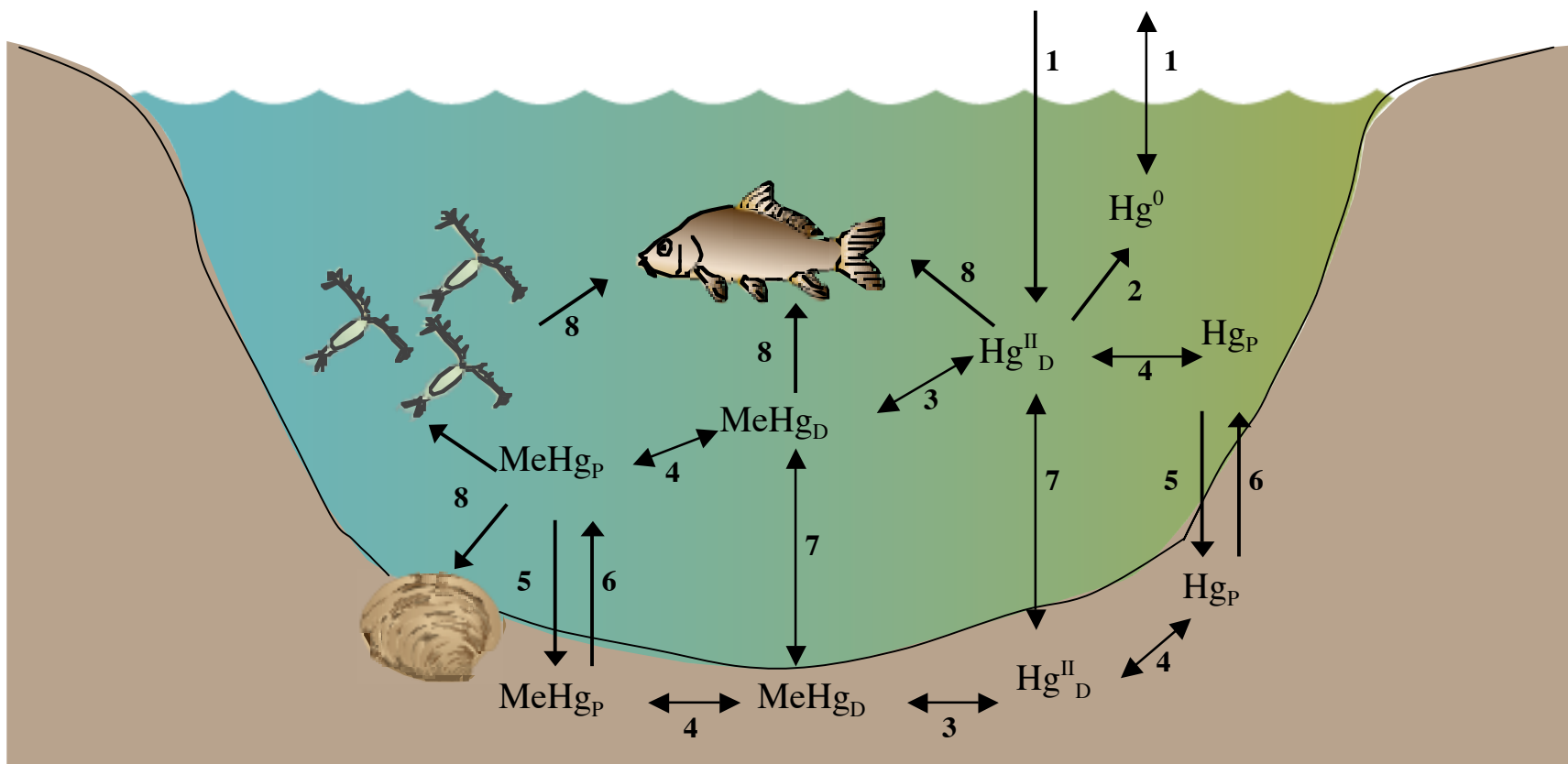
Figure 1.1: Mercury biogeochemical cycling (1) air-water exchange; (2) reduction; (3) methylation/demethylation; (4) adsorption/desorption; (5) particle settling; (6) resuspension; (7) diffusion; (8) bioaccumulation. Hg^0 - elemental Hg; Hg^{II}_D - dissolved inorganic Hg; Hg_P - particulate inorganic Hg; $MeHg_D$ - dissolved MeHg; $MeHg_P$ - particulate MeHg.

reintroduced into the water column by the processes discussed above (Mason and Lawrence, 1999). The flux of dissolved MeHg from sediments does not appear to be significant, except under conditions of low oxygen/hypoxic waters and/or reduced surface sediments (Gill et al., 1999; Mason et al., in press). Therefore, resuspension of the particles could be an important process influencing the impact of sedimentary Hg and MeHg on benthic and pelagic organisms. Previous studies by Kim et al. (2004; in revision) have highlighted some of the important pathways between Hg in sediment and MeHg in primary consumers. The current study was designed to further investigate these factors.

Sediment resuspension affects ecosystem processes in shallow estuarine environments by enhancing the link (benthic-pelagic coupling) between the sediment and the water column and is thought to influence the sediment characteristics and bioavailability of contaminants to organisms. Sediment resuspension can expose surface sediments to oxic bottom water, as well as exchange near-surface porewaters that can supply oxygen to previously anoxic, sub-surface sediments. This, in turn, can enhance aerobic mineralization rates (Cantwell et al., 2002). If biogeochemical conditions change as a result of bioturbation or resuspension, the redox conditions might change or oscillate from anoxic to oxic. For many metals, a change in redox conditions may lead to a change in solid-phase speciation, and thus a remoblization of metals. This might result in increased bioavailability and toxicity to benthic organisms (Cantwell et al., 2002; Sundelin and Eriksson, 2001). The fate of dissolved metals released from resuspended particles may follow one of several pathways. Metals from one phase may readily reabsorb to those of another phase, they may be exported from the estuarine or near-shore

environment to the coastal ocean, or they may be accumulated by biota (Benoit et al., 1998; Cantwell et al., 2002).

Macrofauna activities in the benthic environment greatly effect redox conditions in the surface sediments by altering the diagentic transport and chemistry during particle reworking, burrow formation, and irrigation. These processes play an important role in controlling net degradation of organic matter and nutrient cycling that can both effect metal cycling (Aller et al., 2001).

In estuarine sediments, bioturbation and irrigation, as well as resuspension due to tidal currents, increase the depth of oxygen penetration and create patches of nonequilibrium miocroenvironments where both oxidized and reduced forms of Fe, Mn, and S coexist under suboxic conditions (Simpson and Batley 2003). These physical disturbances change the time sequence of redox reactions by shifting sediments between the reducing and oxidizing conditions. Particle and fluid transport in these bioturbated areas influence overall rates of reactions, reaction distribution, degradation pathways, redox reaction balances, and the extent of organic matter degradation (Aller et al., 2001).

In undisturbed sediment, Hg methylation occurs in a subsurface layer between the redox transition to sulfate-reducing conditions and the depth at which sulfide levels become inhibitory to methylating bacteria. A peak in MeHg production and accumulation has been observed close to the sediment-water interface in freshwater and marine sediments. However, this same zone would not be found in sediments disturbed by benthic infauna since burrows would increase the extent and complexity of the zone of Hg methylation. In studies concerning infaunal burrow densities in Boston Harbor, Massachusetts, Benoit et al. (in press) found the depth of MeHg peak increased with

increasing burrow density since diffusion of oxidizing agents from the burrow walls into the surrounding sediment deepens the zones suitable for Hg methylation. Also, MeHg inventories were highest at intermediate burrow densities. The reduced inventory at high burrow densities was explained by an enhanced efflux of MeHg from near-surface sediments due to bioturbation and that the zone of sulfate reduction was found below the burrows as opposed to between them in the lower densities. The results from Benoit et al. (in press) provide strong evidence that bioturbation effects solid-phase MeHg profiles in marine sediments and suggests that infaunal burrows may influence MeHg production and accumulation through their influence on sediment redox chemistry.

Bioaccumulation of Hg is influenced by the propensity for transfer of MeHg from the sediments to the water column. In the absence of physical resuspension, Hg and MeHg are primarily introduced to the water column by diffusion from sediments. Relatively few studies have been published that incorporate the geochemical factors controlling the behavior of Hg and MeHg with physical disturbance such as sediment resuspension and bioturbation that changes the sediment redox state. Sediment resuspension is an important mechanism for contaminant transfer, especially for pollutants, such as Hg, that are strongly associated with the fine-grained, organic rich fraction of the sediment (Benoit et al., 1998), and provides a potential mechanism for transferring Hg and MeHg from the sediment to filter feeding organisms and the pelagic food chain. Filter feeders are exposed to contaminants in the water column primarily through ingesting phytoplankton that have accumulated the contaminants and/or by ingesting suspended material having had adsorbed contaminants. Previous studies have suggested that resuspension may both enhance the transfer of Hg and MeHg to the water

column in the dissolved phase, and increase the amount of particles available for consumption by filter feeders in the water column (Kim et al., 2004). The work described here expands upon previous studies by examining the role of the abundance of benthic filter feeders and their interactions with sediments on the fate, transport and bioaccumulation of MeHg in estuarine food chains.

1.2 Prior Research and Experimental Approach

This study was conducted as part of a larger project funded by the Hudson River Foundation (HRF) entitled "The Role of Resuspension in Enhancing the Remoblization and Bioaccumulation of Mercury and Methylmercury into Bivalves and Other Benthic Organisms". Mesocosms developed by Elka T. Porter were used to simulate sediment resuspension with realistic water column mixing and benthic boundary layer flow in order to examine physical, biological, and chemical processes simultaneously and their interactions, which are expected to be linear and non-linear (Porter et al., in prep). The study was intended to investigate the impact of sediment resuspension on nutrient cycling and productivity in shallow estuarine environments and how these affect the fate, transport, and bioaccumulation of Hg and MeHg into the food chain, as well as the role of benthic organisms in cycling Hg at the sediment-water interface. In addition, the role of resuspension in influencing Hg methylation was examined. Since experimental variability is high in the field, and small-scale, isolated laboratory experiments do not include indirect effects, the comparative mesocosm experiment approach used here allowed a more controlled study of these interactions.

1.2.1 Mesocosm Studies

To date, four outdoor, long-term experiments (3-4 weeks) with Baltimore Harbor

sediments have been conducted with and without benthic organisms to assess how the

coupling between sediment resuspension and benthic organisms affects nutrient dynamics

and Hg cycling and bioaccumulation (Table 1.1). The Hg and MeHg results of the first

two experiments focusing on sediment resuspension compared to no resuspension are

described in Kim et al. (2004; in revision), while the ecosystem functioning and nutrient

cycling is described in Porter et al. (in revision).

Table 1. Experimental Design for comparative ecosystem experiments in the resuspension mesocosms.
Mercenaria mercenaria was the clam species used.

Experiment	Treatment 1	Treatment 2	Date
1: Comparing Resuspension Regimes	Resuspension	No Resuspension	July 2001
2: Comparing Resuspension Regimes with Clams	Resuspension, Clams	No Resuspension, Clams	October 2001
3: Clam vs. No Clam	Resuspension, Clams	Resuspension	July 2002
4: Clam Density	Resuspension, High Density Clams	Resuspension, Low Density Clams	August 2003

The Resuspension Experiments (1 and 2) focused on the differences between

environments with tidal resuspension and environments where no resuspension occurs.

The hard clam, *Mercenaria mercenaria*, was introduced into the sediment for the second

Resuspension Experiment (2) to investigate the effects of resuspension on

bioaccumulation.

M. mercenaria is common to relatively turbid environments in the eastern coastal

and estuarine regions of USA (Stanley, 1985). They are suspension feeders that are able

to gather some nutrition from dissolved organic matter, but their primary food source is suspended particles, including plankton and detritus. Due to the clam's infaunal life habitat and short siphons, feeding typically occurs close to the bottom (Grizzle et al., 2001 and references therein).

Results from the Resuspension Experiments (1 and 2) show that particulate nitrogen, phosphorus, and carbon concentrations were significantly enhanced in the resuspension tanks verses the no resuspension tanks during mixing phase and increased linearly with increasing total suspended solids (TSS). Dissolved inorganic nitrogen, nitrate+nitrite, and soluble reactive phosphorous were also enhanced in the resuspension tanks. Phytoplankton biomass was enhanced by sediment resuspension due to the increase in nutrients to the water column even though light was limited in the resuspension tanks. However, resuspension did not allow algal growth at the sediment water interface and thus there was more microphytobenthos in the non-resuspension system than in the resuspension system. As a result, resuspension caused a transfer of primary productivity from the sediment surface to the water column. The addition of clams also destabilized the sediment and lead to enhanced sediment resuspension and higher water column TSS compared to systems without clams. In conclusion, Porter et al. (in revision) found that ecosystem processes are both directly and indirectly affected by tidal resuspension. Figure 1.2 illustrates some of the findings comparing resuspension and no resuspension, with and without clams, for the first three experiments.

In the Resuspension Experiments (1 and 2), Kim examined the differences in Hg cycling and methylation between resuspension and non-resuspension tanks. Particulate total Hg (HgT) was introduced to the water column by resuspension, however, particulate

MeHg was significantly lower in the resuspension tanks compared to the no resuspension tanks. Dissolved HgT and MeHg were similar between the two treatments and did not seem to be affected by sediment resuspension. Kim suggested that the dissolved and particulate phases for HgT and MeHg in the treatments cannot be explained by equilibrium partitioning only (Kim et al., 2004). Kim's results indicate that sediment resuspension has complex effects on Hg sediment chemistry and resuspension appears to enhance net Hg methylation within the system. Kim et al. (in revision) and Heyes et al. (in press) have used Hg isotopes to study the methylation and demethylation rates in sediments. They found that methylation of the isotope correlated well with *in situ* MeHg concentrations (Fig 1.3) and that MeHg concentration is a good indicator of recent net methylation activity in estuaries.

In terms of bioaccumulation, MeHg concentrations in zooplankton (>210 μm) increased over time in both systems (Fig 1.4). A significant accumulation of Hg or MeHg was also observed in the clams over the course of the experiments (see Table 2.5) (Kim et al., in revision). However, there was no difference in clam MeHg accumulation in the resuspension versus non-resuspension systems. Initial analysis of the results from the Resuspension Experiment (2) and the Clam/No Clam Experiment (Experiment 3) suggested that the clams were likely food limited. As a result, the Clam Density Experiment (Experiment 4) was designed to investigate the effect of clam density on ecosystem processes and MeHg bioaccumulation. This approach was confirmed by modeling studies of the Resuspension Experiment with clams (2) which supported the contention that the system was food-limited and that phytoplankton growth rate had a dramatic impact on both the growth rate of the clams and on their bioaccumulation of

MeHg (Kim, 2004). The modeling results suggest that "biodilution" is an important consideration in MeHg bioaccumulation in shallow ecosystems and thus the experimental design for the Clam Density Experiment (4) was chosen to examine this hypothesis further.

The current study focuses on the Clam/No Clam Experiment (Experiment 3; hereafter denoted as HDC1/NC) and the Clam Density Experiment (Experiment 4; hereafter denoted as HDC2/LDC). In this notation, HDC refers to a high density of clams (50 per tank); LDC refers to a low density of clams (10 per tank) and NC refers to tanks without clams. As the same density of clams was used in both experiments discussed in this thesis, to avoid confusion the different experiments will be denoted as HDC1 and HDC2. All experiments were with resuspension, in contrast to the experiments described above. In these experiments, tidally resuspended environments were mimicked in a 4 hour "on-phase", 2 hour "off-phase" cycle to investigate the impact of different densities of clam populations on Hg methylation in the system and bioaccumulation through the food chain. Specifically, the impact of resuspension on the accumulation of MeHg into filter feeding bivalves and zooplankton in the system was the focus of the study.
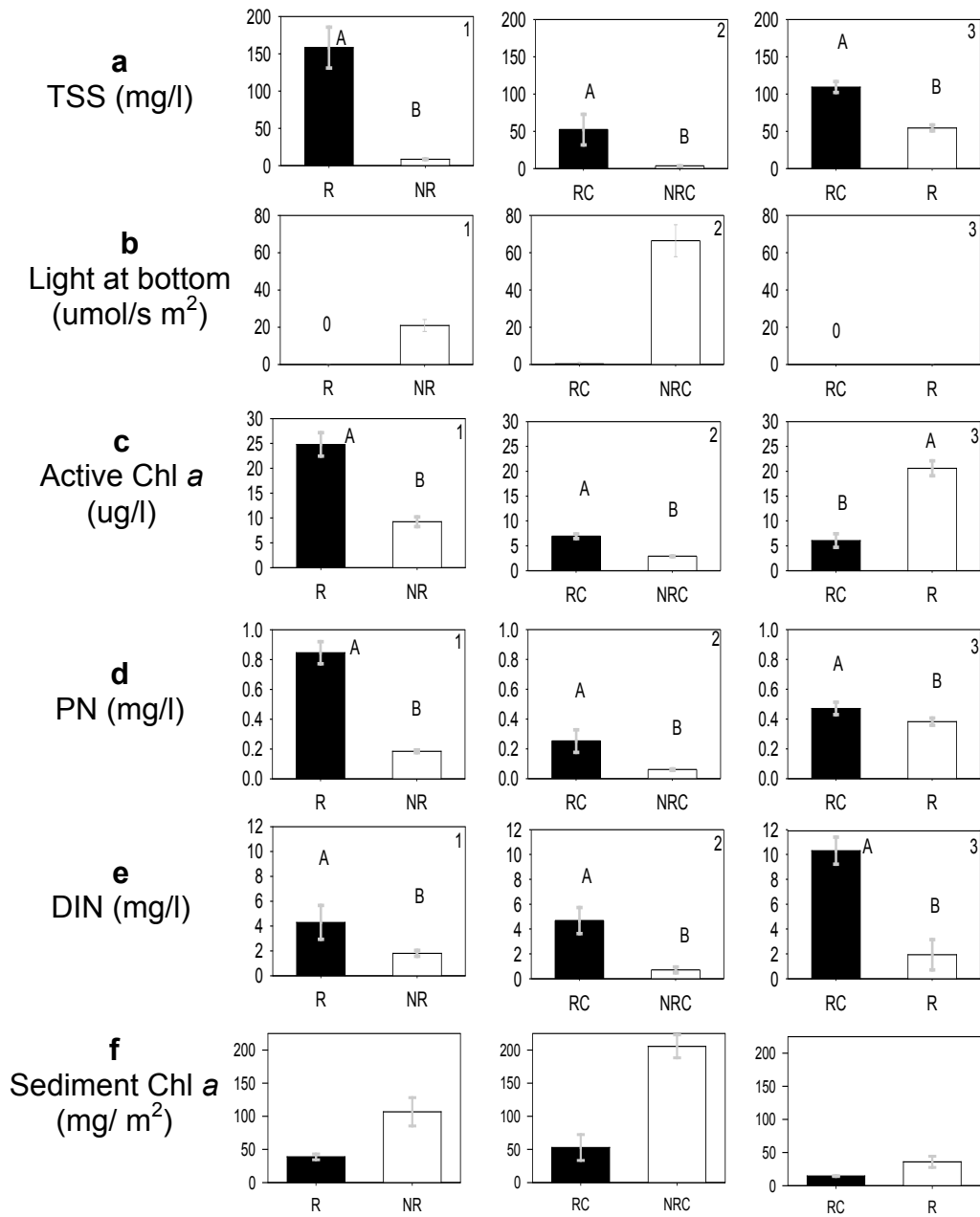
Figure 1.2: Results from three ecosystem experiments, averages (±SD) of day 7 to the end of each experiment (ca 3 weeks). a = Total Suspended Solids (TSS); b = light at the bottom; c = active chlorophyll *a*; d = particulate nitrogen (PN); e = dissolved inorganic nitrogen (DIN); f = sediment chlorophyll *a*. R=Tidal Resuspension, NR=No Resuspension, RC=Tidal Resuspension+clams, NRC=No Resuspension+clams. Data from Elka T. Porter.
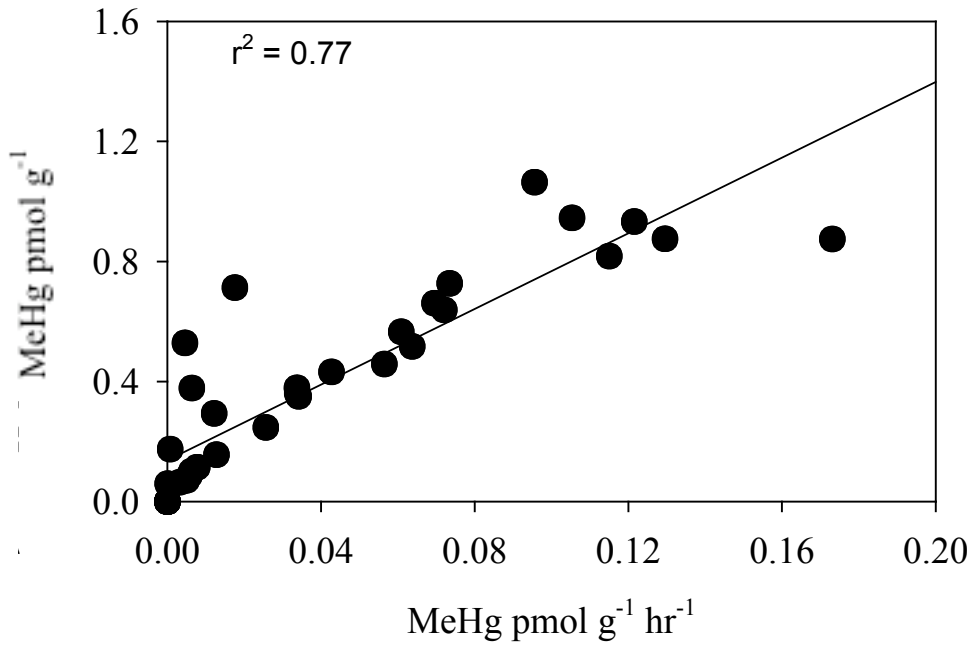
Figure 1.3: Methylation of Hg isotope and *in situ* MeHg concentration from the Patuxent River, MD. Data taken from Heyes et al. (in press).
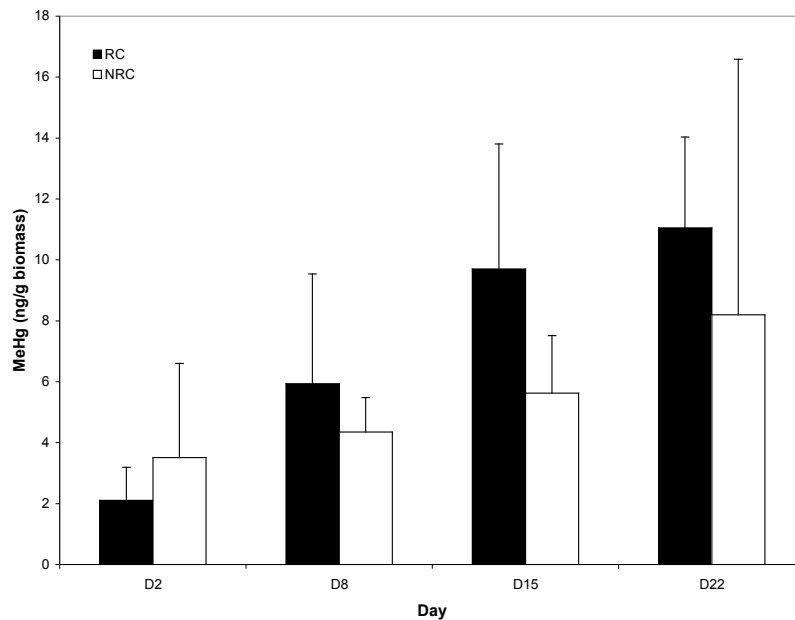
Figure 1.4: Experiment 2, MeHg in zooplankton (>210um).
RC = Resuspension with clams; NRC = No resuspension with clams.
Error bars represent standard deviations of 3 replicate samples in
each system. Data from Kim et al. (in revision).

1.2.2 Isotope Studies

In complex systems, where strong interactions exist between processes and multiple factors can influence the fate, transport and bioaccumulation of a chemical of interest, the use of tracers can provide information that cannot be obtained from measuring the total concentration of the chemical of interest. Previous studies have indicated that while measuring MeHg concentrations in the sediment may provide some insight into the potential for bioaccumulation, there is not a simple relationship between total MeHg concentration in the sediment and MeHg in benthic organisms (Lawrence and Mason, 2001; Mason and Lawrence, 1999). From previous studies, cycling of Hg through mesocosm systems is unclear due to the lack of strong changes in chemical concentration over time. As well, the sources and sinks of MeHg within the system were not well characterized. Thus, a stable isotope of Hg was added as a tracer to follow the Hg cycling through the system (e.g. Hintelmann and Evans, 1997). The goal of using the Hg stable isotope was to help clarify how Hg cycles through an experimentally resuspended environment and its impact on benthic organisms.

Stable isotope techniques using Hg have been successfully applied in both small-scale core incubations (Benoit et al., 2003) and larger scale mesocosm studies. Initial results from the Mercury Experiment to Assess Atmospheric Loading in Canada and the United States (METAALICUS) study in Canada have shown the power of stable isotope approaches in following the both the cycling of Hg, and its conversion to MeHg, as well as the rate at which the overall processes occur (Hintelmann et al., 2002). In the current study, a Hg stable isotope was added to the water column at the beginning of the Clam Density Experiment, in a concentration that did not significantly perturb the system, to

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trace the added Hg as it associated with water column and sedimentary particles, and was then transferred from the water column to the sediment. It was also expected that the rate of methylation in the sediment, the mechanisms by which MeHg becomes reintroduced into the water column, and its bioaccumulation into clams and other biota could be discerned from the tracer addition.

1.3 Rationale

Previous findings in mesocosm and field studies suggested that resuspension and bioturbation enhances methylation in the sediment (Benoit et al., in press; Kim et al., 2004), but that higher total suspended solids (TSS) associated with resuspension leads to an overall lower concentration of MeHg in the water column (on a ng g^{-1} basis) because the lower MeHg concentration sediment particles dilute the higher MeHg concentration biotic particles. Typically, MeHg accounts for 1% or less of the total Hg in estuarine sediments. However, phytoplankton can have a higher relative burden of MeHg, up to 10% of the total Hg due to bioconcentration of the metal (Benoit et al., 2003). Thus, in regions of low resuspension, or inorganic particle load, the overall average particulate MeHg concentration will likely be higher than the regions with high non-living (inorganic) particle loads, as was found by Kim et al. (2004). Water column MeHg concentrations in the dissolved phase, however, appeared to be relatively invariant over a range of TSS concentrations in this study, which implies there is little desorption, or that the rate of desorption is slow, from the inorganic sediment particles during resuspension (Kim et al., 2004).

In shallow systems, the concentrations of nutrients in the water column are enhanced, and phytoplankton-standing stocks are higher where sediment resuspension occurs. Overall, resuspension appears to result in a transfer of the majority of the new primary production from the benthos to the water column and this may have a strong impact on bioaccumulation. In the presence of filter feeders, their density will determine the steady state standing stock of primary producers. Thus, it is likely that at high filter feeder density, phytoplankton-standing stocks would be reduced. However, due to the enhanced supply of nutrients as a result of resuspension, phytoplankton growth rates would be higher. This would lead to the so-called "growth dilution" effect, in which faster growing individuals accumulate less contaminant than slower growing ones (Chen and Folt, 2005). Thus, if this growth enhancement effect occurred, then at high densities of filter feeders, bioaccumulation of Hg should be less than at lower densities. An alternative result would occur if there was a limitation to the amount of MeHg available in the water column to bioaccumulate into the phytoplankton, and this was the sole determinant on phytoplankton concentration. Under such a scenario, there would be more MeHg in the systems with lower standing stocks of phytoplankton (i.e. the opposite impact to that of growth dilution). However, based on previous results, this appears unlikely. Therefore, it was hypothesized that while the biological and chemical interactions are complex, in general, bioaccumulation of MeHg into filter feeders will decrease as filter feeder density increases under tidal resuspension conditions

1.4 Hypotheses

From our current understanding of Hg cycling and bioaccumulation, the following hypotheses were proposed:

1) Differences in clam densities will change the amount of MeHg bioaccumulated up the food chain. Specifically, MeHg concentrations in clams and zooplankton will decrease with increasing clam density.

2) Since clams destablize the sediments, an increase in clam density should result in increased methylation in the sediment.

3) Hg will be rapidly (within weeks) transferred from the water column to the methylation zones in the sediment due to the particle reactivity of Hg and sediment resuspension.

4) Food chain interactions are as important in determining MeHg concentrations in herbivores as biogeochemical processes.

1.5 Objectives

The objectives of this research were to test the above hypotheses by examining the effect of tidal resuspension on Hg cycling and the bioaccumulation of sedimentary Hg and MeHg to benthic organisms. These objectives were reached through first examining the impact of filter feeder density on the bioaccumulation of Hg, as well as using the Hg

stable isotope to trace the cycling of Hg through the system. The first and second hypotheses were investigated by analyzing water, sediment, and biota samples collected at regular intervals throughout the experiment. As stated above, it has been demonstrated that there is a strong relationship between *in situ* MeHg concentration and Hg methylation rate (Heyes et al., in press; Kim et al., in revision) and thus for these experiments the MeHg concentration in the upper sediment layer was used as a surrogate for net methylation rate. The third hypothesis was examined by using the Hg stable isotope to further clarify the Hg cycle within the mesocosms. The intent of this addition was to follow the transfer of the Hg through the system and address the issue of the rate at which 'new' Hg added to the water column is transferred to the zones of methylation. The redistribution of the Hg isotope between the dissolved and particulate phases in the water column, and in the sediments and biota could be used to provide estimates of the rates of various processes and this information can be used to update and further evaluate the model developed by Kim (2004) that was also used to investigate the fourth hypothesis.

1.6 Expected Results

In previous mesocosm studies, phytoplankton biomass was enhanced by sediment resuspension compared to no resuspension due to the increase in nutrients in the water column. The addition of clams destablized the sediment and lead to enhanced sediment resuspension and thus higher water column TSS concentrations and higher nutrients (Porter et al., in revision). This should enhance primary production, however, light limitation at high TSS has the potential to decrease primary production. From our data in

the Clam/No Clam Experiment, we predicted that higher numbers of clams would lead to greater removal of phytoplankton from the water column due to feeding. As a result, the rate of primary production will be further enhanced, especially if nutrient concentrations are increased by resuspension, as observed in previous mesocosm experiments. Generally, higher growth rates lead to lower concentrations of Hg in phytoplankton as accumulation is a function of both uptake rate and growth rate. If uptake rate is relatively constant, being related to water column speciation rather than to plankton growth rate (Kim, 2004; Mason et al., 1996), then the faster growing cells will have a lower Hg concentration (Chen and Folt, 2005; Pickhardt, 2002). In previous mesocosm studies, a significant accumulation of Hg or MeHg in the clams was not observed. It was therefore predicted that the high clam density led to lower Hg concentrations in phytoplankton, resulting in little growth and little change in Hg concentrations in the clams. In addition, due to the relatively high concentrations of Hg and MeHg in clams at the start of the experiments, changes in concentration were difficult to detect. The use of Hg stable isotopes should allow a better determination of the extent of uptake of MeHg even in the presence of a high background MeHg concentration.

If food is not limiting, clams feeding on phytoplankton with lower MeHg concentrations should accumulate less MeHg. As a result of high clam densities, it was believed that food was limiting in previous mesocosm experiments, since the clams did not grow or accumulate MeHg. However, at very low clam densities, phytoplankton may deplete the nutrients and grow slowly. MeHg concentrations in clams could be enhanced at these low growth rates and less competition for phytoplankton could result in a higher clam growth rate. Overall, it is expected that the interaction between biomass and

bioaccumulation of MeHg will be complex, and depend on both the system productivity and the impact of sediment disturbance on Hg methylation, as well as the transfer of MeHg from the sediments to the water column. The experiments outlined above were designed to investigate these interactions with a focus on their impact on bioaccumulation.

1.7 Materials, Methods and Experimental Approach

1.7.1 Mesocosm Set-up

The STORM (high bottom Shear realistic water column Turbulence Resuspension Mesocosm) facility used in this study consists of six 1000 L tanks with one m^2 sediment surface area. The mixing in the system is designed to generate uniform and realistic resuspension without producing excessive water column turbulence in a 4 hour "on-phase", 2 hour "off-phase" cycle (Porter et al., in prep). Muddy surface sediment was collected from Baltimore Harbor, MD, USA in the spring of 2002 and again in 2003 for the Clam/No Clam Experiment (HDC1/NC) and the Clam Density Experiment (HDC2/LDC), respectively. Hg concentrations in Baltimore Harbor sediment are, on average, of the same order of magnitude as other large, urbanized east coast estuarine systems (average HgT concentration is 450 ng g^{-1}) (Mason and Lawrence, 1999). The sediment was defaunated for two weeks prior to each experiment in an outdoor fiberglass holding tank covered with black plastic. The top 10 cm of the sediment in the holding tank was discarded before adding the sediment to the six mesocosms to form a sediment layer of 10 cm. The sediment was mixed and smoothed. Filtered (0.5 μm absolute) ambient seawater from the Patuxent River, a subestuary of the Chesapeake Bay, MD,

USA, was added to the tanks to a depth of 20 cm above the sediment surface without disturbing the sediment layer. A two-week equilibration period began with water column oxygenation and 50% daily water exchanges in order to flush out solutes, and allow for the sediment to regain steady state concentrations and distributions for the important parameters.

After the equilibration period, environmentally relevant densities of the benthic filter feeding bivalve, *Mercenaria mercenaria,* were added to the sediment by hand. The 40 mm long hard clams were allowed to borrow into the sediment overnight. The clams that had not burrowed by the following day were replaced with new clams, however the new clams that did not burrow by the second night were removed from the mesocosms and not replaced. Since negative effects on growth have been observed in clams at salinities below 15 ppt (Grizzle et al., 2001), the salinity was adjusted to approximately 18 ppt throughout the experiments.

Following clam additions, unfiltered ambient water from the Patuxent River was carefully added to the tanks without sediment disturbance to a total volume of 1000 L. Unfiltered water was used to initially fill the mesocosms so that representative phytoplankton and zooplankton communities would develop in the mesocosm tanks over time from these "seed" populations. For the remainder of the experimental period, 10% of the total volume of water was exchanged daily with filtered Patuxent River water to simulate tidal exchange within the system. The water exchange was performed at the end of the off-phase, when the mixing system was off, to minimize the loss of resuspended particles in the water column. Tank walls were cleaned every other day over the course of the experiment.

1.7.2 Experimental Design

Six mesocosm tanks were used in each experiment and two different experimental treatments were compared in triplicate. Tidal resuspension (4 hours on, 2 hours off) was simulated using the STORM tank mixing design in all the tanks (Porter et al., in prep). The HDC1/NC Experiment compared resuspension with clams (Tanks T1, T2, and T3) with resuspension without clams (Tanks T4, T5, and T6). In this experiment and in the previous mesocosm experiments involving the clams (Kim, in revision; Porter et al., submitted), a positive growth trend was not observed over the experiment. We believe that the clam populations were too large for the mesocosms and were therefore food limited for the duration of the experiments. Thus, in the Clam Density Experiment (HDC2/LDC), we examined differing population densities of clams to further assess this by comparing resuspension with a high density clam population (Tanks T1, T2, and T3) of approximately 50 clams (similar to the HDC1 treatment) and resuspension with a low density clam population (Tanks T4, T5, and T6) of 10 clams per tank (LDC). Both experiments were conducted for 4 weeks (28 days), however, the three tanks of the NC treatment of the HDC1/NC Experiment failed on D26.

1.7.3 Stable Isotope Addition

On day 2 of the HDC2/LDC Experiment, one tank from each treatment (Tanks T2 and T5) was spiked with 10 µg of the Hg stable isotope, giving an initial concentration of 10 ng/L. Hg stable isotope (^{199}Hg) was obtained from Oak Ridge National Laboratory (purity of 92%). The ^{199}Hg spike was prepared using the filtered ambient Patuxent River water. The isotope was added below the water surface during the mixing on-phase to ensure efficient adsorption to the particle phase, and in the evening to reduce the amount

of Hg loss to the atmosphere during the addition due to Hg reduction and subsequent evasion. Given a typical particle load of 100 mg l^{-1}, the added isotope represents, if all adsorbed, a concentration of 0.1 μg g^{-1}, or between 5-10% of the ambient concentration of Hg on the particles. The samples from the spiked tanks were analyzed using inductively coupled plasma mass spectrometry (ICP-MS), which can separate the different isotopes of Hg for detection. The ability of the ICP-MS to separate isotopes restricts the practical detection limit for one isotope of Hg to approximately 0.5% of the total ambient Hg concentration.

1.7.4 Sample Collection

Water

Between one and two liters of water was collected from each tank every 2-3 days during the resuspension on-phase by siphoning water 50 cm below the surface. Twice in the HDC1/NC Experiment and once in the HDC2/LDC Experiment, water samples were also collected repeatedly at the end of the resuspension on-phase as the particles settled out of the water column. Water samples were collected for typical water column parameters (temperature, dissolved oxygen, turbidity, fluorescence, seston) and nutrients, as well as for dissolved and particulate Hg and MeHg in the water column. All the Hg sample bottles were Teflon and acid cleaned according to established protocols before use (e.g. Mason et al., 1999). Samples were taken separately for Hg and other variables such as TSS, dissolved organic carbon (DOC), and chlorophyll *a* (Chl *a*). For particulate total Hg (HgT) and MeHg concentrations, water samples were filtered through 0.4 μm polycarbonate filters. The filters were stored in Petri dishes, double-bagged, and frozen until analysis. The filtrate was collected for dissolved HgT and MeHg in acid cleaned

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Teflon bottles and also frozen until analysis. Samples were filtered through pre-weighed

0.7 μm Whatman GF/F glass fiber filters for TSS, particulate organic matter (POM), Chl

a, and DOC. POM was calculated from loss on ignition at 450 °C for 4 hours after the

samples had been dried. Samples were sent to Analytical Services at the Chesapeake

Biological Laboratory (CBL) for analysis (www.cbl.umces.edu/nasl/index.htm) where

fluorescence and high temperature combustion methods were used to determine the Chl *a*

and DOC values, respectively.

Sediment

Sediment cores were taken for HgT and MeHg analysis during the resuspension

off-phase. The sediment cores were generally greater than 9 cm deep, taken in 25 cm

long acrylic tubes with a 3.2 cm diameter. The cores were immediately sliced at the

following intervals: 0-0.5, 0.5-1, 1-2, 2-3, 3-5, 5-7, and 7-9 cm. The sediment was

quickly frozen and stored until analysis. Percent organic matter in each interval of

sediment was determined by loss on ignition to 550 °C overnight.

In the HDC1/NC Experiment, initial sediment cores were taken from benthic

chambers so that the sediment surface in the tanks was not disturbed before the

experiment began. The separate cores underwent a two-week indoor equilibration period

similar to the STORM tanks representing similar initial conditions (Kim et al., in

revision). Sediment cores were also taken at the end of the experiment from each tank

(D26: T4, T5, and T6; D28: T1, T2, and T3) to determine the final Hg and MeHg

concentrations.

In the HDC2/LDC Experiment, initial sediment cores were taken from each tank

prior to filling the tanks with unfiltered Patuxent River water. Cores were taken in all

tanks in the middle of the experiment (D15) and at the end of the experiment (D28). Additionally, so we could monitor the isotope weekly, sediment cores were taken in the isotope-spiked tanks (T2 and T5) as well as one control tank (T4) on D8 and D22 up to 5cm.

Biota

Clams were shipped on ice from Cherrystone Aqua Farms, Cheriton, VA. They were kept in a holding tank with constant water circulation until the experiments began. They clams were cultured at a salinity of 21 ppt and thus were acclimated to the experimental conditions of 18-19 ppt by decreasing the salinity 1ppt per day. The levels of ammonium, nitrate, nitrite, salinity, and pH were measured daily for water quality assurance. The clams were fed algae paste (Aquaculture Supply USA) once a day until the beginning of the experiment. To obtain initial Hg and MeHg measurements, 10-15 clams from the holding tanks were sacrificed prior to the beginning of the experiment. The clams were recovered from all tanks at the end of the experiment. For Hg and MeHg analysis, tissue samples from 10-15 clams in each tank were homogenized and frozen in acid-cleaned containers.

Zooplankton samples were collected for Hg and MeHg analysis generally once a week using acid-cleaned polypropylene nets with a 210 μm mesh size. Zooplankton was collected with 63 μm mesh size to determine abundance of dominant taxa and age groups by direct counts. An electric pump was used for sampling that was specifically designed to sample 'gently' without destroying the zooplankton and fast enough so they did not escape. A sampling hose attached to a PVC rod was moved continuously throughout the water column to sample zooplankton as homogenously as possible. The zooplankton

were transferred from the nets to Teflon vials and filtered onto acid-clean polycarbonate filters. The filters were stored in Petri dishes, double bagged, and frozen until analysis.

1.7.5 Sample Analyses

Total mercury

Water samples were thawed and oxidized with bromine monochloride (BrCl) overnight to release Hg bound to particles. Zooplankton filter, particulate filter, sediment, and clam samples were thawed and digested in a solution of 7:3 sulfuric/nitric acid in Teflon vials at 60 °C over overnight before BrCl oxidation (for at least 1 hour) to ensure complete digestion of organic matter. For all samples, excess oxidant was neutralized with 10% hydroxylamine hyrochloride prior to analysis (Bloom and Crecelius, 1983). The samples were then reduced to elemental Hg by tin chloride. The elemental Hg was concentrated by gas sparging with argon and trapped on gold column. Quantification was completed by dual-stage gold-amalgamation/cold vapor atomic fluorescence (CVAFS) (Bloom and Fitzgerald, 1988) in accordance with protocols outlined in EPA method 1631 (USEPA, 1995). Samples from the isotope addition tanks were prepared in the same manner as described above, however, inductively coupled plasma-mass spectrometry (ICP-MS) was used for analysis rather than CVAFS (Heyes et al., in press; Hintelmann and Evans, 1997). A calibration curve with an r^2 of at least 0.99 was achieved daily. Detection limits for HgT were based on three standard deviations of blank measurements (digestion blanks for filters and sediment and $SnCl_2$ bubbler blanks for filtered water.) The detection limits for HgT were 0.023 ng l^{-1} for water samples, 2.10 ng g^{-1} for particulate filter samples, and 14.8 ng g^{-1} for sediment samples. Analysis of duplicate samples yielded an average relative percent difference (RPD) of less than 20%.

A recovery of estuarine sediment standard reference material (IAEA-405) was greater than 80 %. Spike recoveries also yielded greater than 80 % and the data were not corrected for less than 100% spike recovery.

Methylmercury

Water, zooplankton filters, particulate filters, sediment, and clam samples were thawed and distilled with a 50% sulfuric acid/20% potassium chloride solution (Horvat et al., 1993) in order to extract the MeHg from the particles associated with it. After distillation, the MeHg is ethylated with a sodium tetraethylborate solution to convert nonvolatile MeHg to gaseous methylethylmercury (Bloom, 1989). The gaseous Hg was purged from the solution and recollected on a Tenex column at room temperature. The methylethylmercury was thermally desorbed from the column and analyzed by isothermal gas chromatography before quantification by CVAFS (Bloom, 1989). Samples from the isotope addition tanks were prepared in the same manner as described above, however, quantification was performed by ICP-MS rather than CVAFS. A calibration curve with an r^2 of at least 0.99 was achieved daily. Detection limits for MeHg were based on three standard deviations of distillation blank measurements for waters, filters and sediment. The detection limits for MeHg were 0.018 ng l^{-1} for water samples, 0.018 ng g^{-1} for particulate filter samples, and 0.029 ng g^{-1} for sediment samples. Analysis of duplicate samples typically yeilded an average RPD of less than 20%. A recovery of estuarine sediment standard reference material (IAEA-405) was greater than 80 %. Spike recoveries yielded greater than 80 % and the data were not corrected for less than 100% spike recovery.

1.7.6 Statistical Analyses

ANOVA for repeated measures statistics was used to find significant differences between treatments and over time in the water column.   When factors were significant in the ANOVA model, Tukey's multiple pairwise comparisons were used to separate the levels of difference ($p \leq 0.05$). For the sediment, repeated measures statistics was used to find significant differences between treatments, over time, and at depth. Data were checked for normality and equal variances and log-transformed when necessary. Wilcoxon test, a non-parametric test, was used when the assumption of equal variances was not met. The Pearson product-moment correlation was used to obtain the correlation coefficient to see if there were linear relationships between variables. All the statistical results were reported as significant at the level of $p < 0.05$. Minitab (1999), version 13, by Minitab Inc., State College, PA, USA and JMP, version 4, by SAS Institute, Cary, NC, USA were used for the statistical analyses.

Chapter 2: The Effect of Clam Density on Mercury and Methylmercury Cycling in the Water Column and Sediment

2.1 Clam Density Experiment (HDC2/LDC)
Mercury and Methylmercury Concentrations

2.1.1 Water column characteristics

All samples were collected during the on-phase when the mixing system was actively resuspending the surface sediment. As shown in Fig 2.1a, total suspended solids (TSS) for HDC2 tanks averaged 90.4 ± 38.0 mg l^{-1}, which was significantly higher and than the LDC tanks, averaging 51.6 ± 14.5 mg l^{-1} (p =0.015). The two treatments differed significantly over time (p<0.001), and there was a significant interaction between treatment and day (p<0.001). Generally, over the course of the experiment, the differences in TSS decreased, and by the end of the experiment the TSS values were similar. Such changes have been attributed to the initial destabilization of the sediment by the clams. Over time, however, it appears that clam biodeposits and other factors lead to a restabilization of the sediment (Porter et al., submitted).

As with TSS, particulate organic matter (POM) concentrations were significantly higher and more variable for the HDC2 tanks, averageing16.0 ± 7.4 mg l^{-1}, compared to the LDC tanks, averaging 12.1 ± 3.5 mg l^{-1} (p =0.023). The two treatments differed significantly over time (p<0.001) and there was a significant interaction between treatment and day (p <0.001). In general, POM in HDC2 was higher than LDC until D14 where HDC2 continued to decrease while POM in LDC increased. This is consistent with the increase in TSS in the LDC treatment. Percent POM (percentage of TSS composed of POM) was higher, but not significantly different for the LDC tanks than the HDC2 tanks, averaging $24.3\pm4.5\%$ and $19.9\pm5.6\%$, respectively (Fig 2.1b). However,

the treatments differed significantly over time (p<0.001) and there was a significant interaction between treatment and day (p<0.001). The lower %POM in the HDC2 tanks is most likely due to the higher density of clams removing more phytoplankton from the water column. However, % POM also becomes similar between the treatments at the end of the experiment, so the initial difference could be attributed to the differences in the relative amount of resuspended material with an average % OM of 11.1±1.8% for the HDC2 tanks and 11.7±1.5% for the LDC tanks in surface sediment (Table 1.4) and plankton, which have a high OM content. Thus, differences and changes in time reflect mainly the changes in the relative amounts of the two particulate fractions (living and dead). POM was positively correlated with TSS in both HDC2 tanks (r^2=0.90) and LDC tanks (r^2= 0.74).

Water column total chlorophyll a (Chl $a)$ concentration (Fig 2.1c) was significantly higher in the LDC treatment, averaging 32.3±11.8 μg l^{-1}, than in the HDC2 treatment, averaging 22.0±6.9 μg l^{-1} (p =0.002), most likely due to the increased removal of phytoplankton by the higher density of clams. There was a significant interaction between treatment and day (p =0.001) and the two treatments differed significantly over time (p<0.001). Small phytoplankton blooms were observed in the LDC tanks on D13 and the HDC2 tanks on D23. The Chl a concentration in the HDC2 tanks did not change much over the course of the experiment (until D23), but the Chl a concentration increased in the LDC tanks. There were no significant correlations between Chl a and TSS or POM. However, the data for TSS and the lower % POM from the HDC2 tanks, along with the higher Chl a concentrations in the LDC tanks, suggest that clam feeding was removing the phytoplankton from the water column.
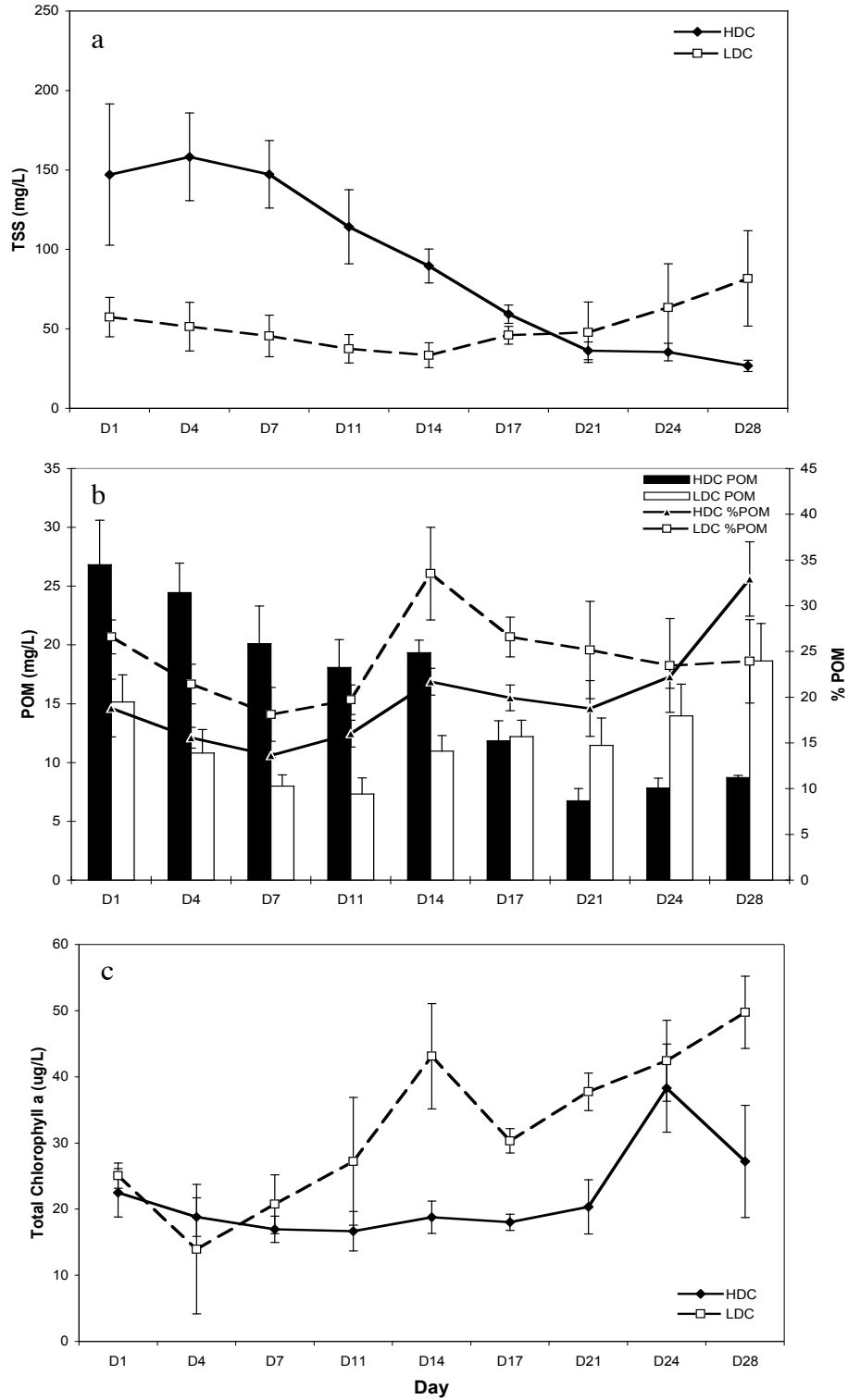
Figure 2.1: Average concentrations for water column variables in the HDC2 and LDC treatments of the Clam Density Experiment. (a) TSS concentration (b) POM and %POM concentration (c) Chl *a* concentration. Error bars show standard deviation of three replicate tanks in each system.

Dissolved organic carbon (DOC), on average, was not significantly different

between treatments, averaging 3.59±0.29 mg l^{-1} for the HDC2 tanks and 3.69±0.46 mg l^{-1}

for the LDC tanks. However the treatments differed significantly over time (p<0.001).

This is in contrast to the previous mesocosm experiments that found a significantly

higher DOC in the resuspension versus the non-resuspension treatments (Kim et al.,

2004). Resuspension appears to be a more important factor determining DOC

concentrations than clam density. DOC was positively correlated with TSS (r^2=0.54) and

POM (r^2=0.63) in the HDC2 tanks, but there was not a significant correlation in the LDC

tanks. However, DOC was negatively correlated with Chl *a* (r^2=0.79) in the LDC

treatment but there was no correlation in the HDC2 treatment.

Table 2.1 presents water chemical characteristics for the HDC2/LDC Experiment.

The measurements were made daily during the on-cycle. There was little difference

between treatments in terms of average salinity and temperature over the course of the

experiment. The pH and DO were slightly higher in the LDC treatment compared to the

HDC2 treatment.

Table 2.1: Average and standard deviation for ancillary parameters in the water column
during the course of the HDC2/LDC Experiment.

	Parameter	HDC	LDC
HDC2/LDC Experiment	DO mg l^{-1}	5.0 ± 1.5	7.2 ± 1.5
	Salinity (ppt)	18 ± 0.4	18 ± 0.6
	Temperature (°C)	24 ± 0.1	24 ± 0.08
	pH	7.9 ± 0.1	8.3 ± 0.2

2.1.2 Mercury distribution in the water column

Particulate HgT in the water column was not significantly different overall

between treatments in the HDC2/LDC Experiment, averaging 450±52 ng g^{-1} (HDC2) and

460±124 ng g^{-1} (LDC) (Fig 2.2a). Treatments differed significantly over time, however

(p<0.001) and there was a significant interaction between treatment and day (p= 0.005), but no clear pattern emerged. Despite the significant difference over time, Hg concentrations on particles were fairly consistent throughout the experiment, indicating that changes in TSS does not have a strong influence on HgT concentration. Particulate HgT concentrations were not correlated with TSS or POM, although particulate HgT concentration was negatively correlated with Chl a in the HDC2 tanks (r^2=0.44). The particles in the water column mostly come from the resuspended surface sediment (0-0.5 cm) of the tanks during the on-phase. Water column particulate HgT and surface sediment concentrations (Table 2.2) were similar where the final average concentrations for HDC2 and LDC treatments were 302±50 and 478±75 ng g^{-1}, respectively.

Dissolved HgT was not significantly different between the HDC2 tanks, averaging 1.45±0.33 ng l^{-1}, and the LDC tanks, averaging 0.92±0.29 ng l^{-1}, as seen in Fig 2.2b. Over the course of the experiment, the changes in dissolved HgT did not follow a noticeable trend and did not relate to changes in particulate HgT. Dissolved HgT was positively correlated with both TSS (r^2=0.49) and POM (r^2=0.49) in the HDC treatment. However, dissolved HgT was positively correlated with DOC (r^2=0.52) in the LDC treatment.

As mentioned earlier, 10% water exchanges were performed daily with filtered ambient water. This input water was collected at the same time as sample water throughout the experiment and the average dissolved HgT concentration was 0.77±0.37 ng l^{-1} (n=8). This concentration was similar and only slightly lower than the average concentrations in the tanks over time, and suggests that the input of Hg, as a result of the water changes, does not have a dramatic impact on the overall system. Since water

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Figure 2.2: Average concentrations of HgT in the particulate and dissolved phases in the HDC2 and LDC treatments of the Clam Density Experiment. (a) Particulate HgT concentration (b) Dissolved HgT concentration. Error bars show standard deviation of three replicate tanks in each system.

exchanges were performed after sampling, dissolved HgT in the mesocosms did not directly represent the concentration of HgT in the input water on the sampling day. However, as Kim et al. (2004) suggest in previous studies, dissolved HgT in the tanks may have been driven by both the change in concentration of the input water and the release of HgT from the particles during resuspension. The Hg added with the water addition is similar to that removed each day since the water removal occurred at the latter part of the off-cycle, where TSS levels were relatively low. However, the water removed was unfiltered and thus there was an overall net removal of Hg from the system with each water change. The HgT removed during water exchanges was estimated from off-cycle TSS and HgT concentrations on D14 and was 586 ng in the HDC2 tanks and 366 ng in the LDC tanks. Thus, there must have been some net input into the water column over time from the sediment to account for this small but consistent removal of Hg from the tanks.

The average concentration of particulate MeHg (on a mass basis) in the water column (Fig 2.3a) was higher and more variable in the LDC tanks, averaging 2.95 ± 2.77ng g^{-1}, but not significantly different from the HDC2 tanks, averaging 2.69 ± 1.96 ng g^{-1}. Particulate MeHg concentrations in the HDC2/LDC Experiment were higher than the surface sediment (0-0.5 cm) concentrations at the beginning and end of the experiment (Table 2.2) where the final averages for the HDC2 and LDC treatments were 0.50 ± 0.37 ng g^{-1} and 0.65 ± 0.41 ng g^{-1}, respectively. The HgT concentration on the particles reflect the surface sediment because inorganic Hg does not absorb into living organisms as well as MeHg so it is not as influenced by the presence and variation in the biotic fraction of the particles. MeHg, on the other hand, is more strongly absorbed by
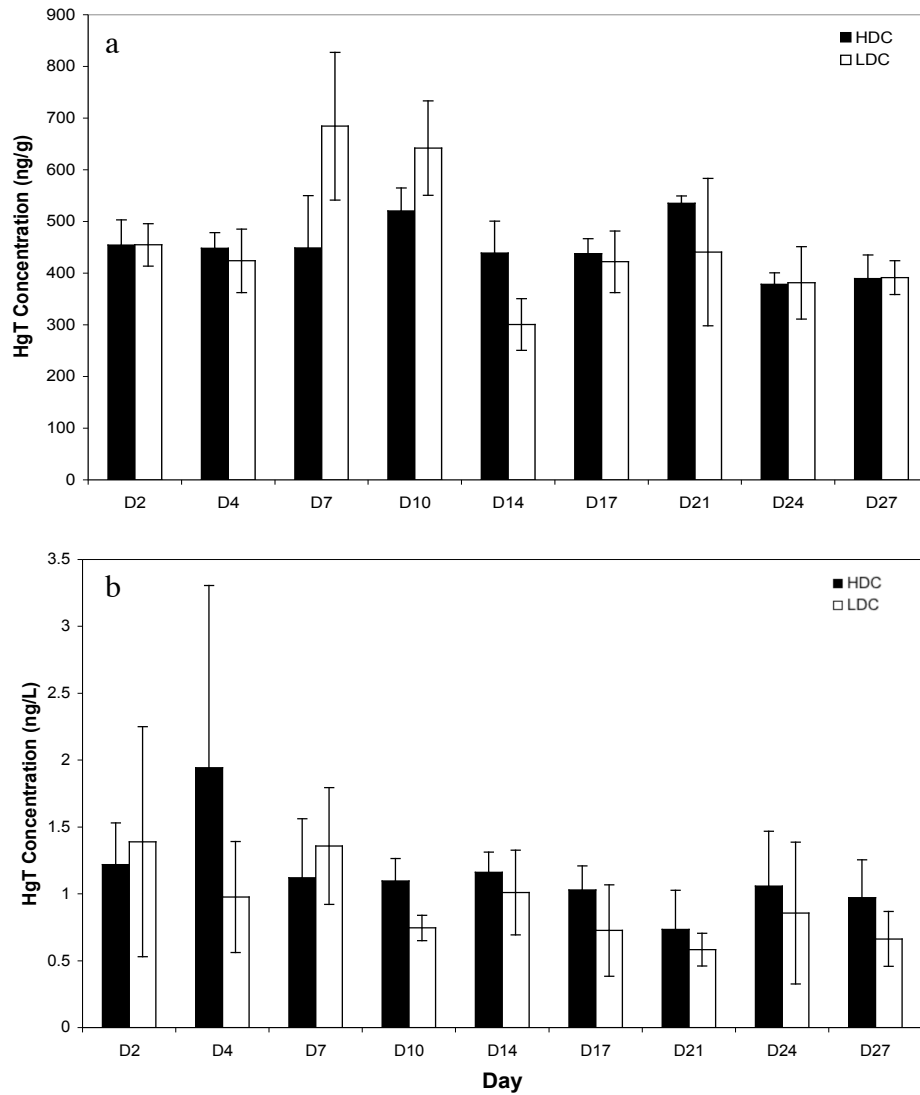
38
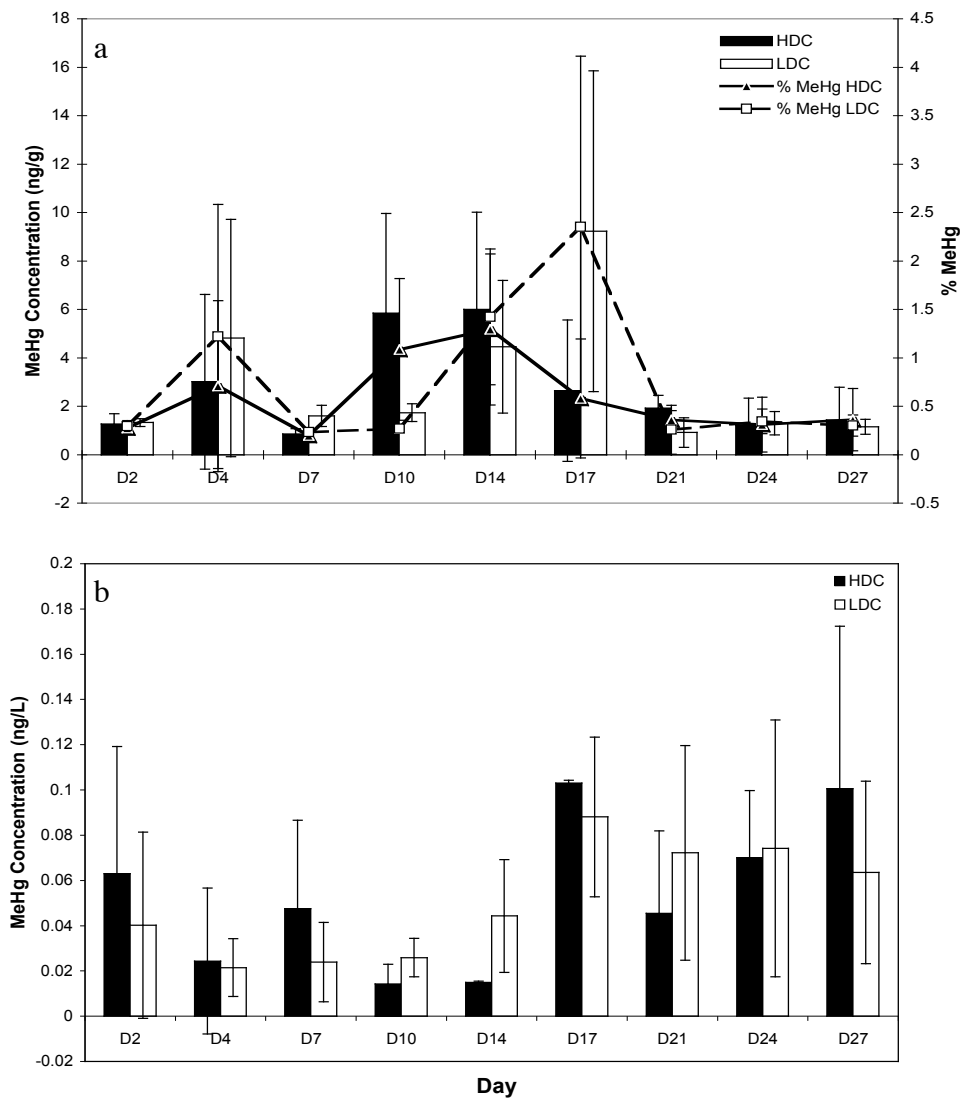
Figure 2.3: Average concentrations of MeHg in the particulate and dissolved phases in the HDC2 and LDC treatments of the Clam Density Experiment. (a) Particulate MeHg concentration and % MeHg (b) Dissolved MeHg concentration. Error bars show standard deviation of three replicate tanks in each system.

living matter. The MeHg concentrates in the biotic particles and increases the overall particulate MeHg concentration compared to the surface sediment even though the phytoplankton do not dominate the mass of particles. It has been found that 10% of the HgT in phytoplankton is MeHg, on average, compared to only 1% in sediment (Mason et al., 1996). Modeling studies of resuspension mesocosms provide supporting evidence for the larger MeHg concentration in phytoplankton compared to suspended sediments (Kim, 2004). Model estimates of the phytoplankton concentration of MeHg were 10-50 ng g^{-1} dry weight, much higher than that of the surface sediment. Similarly, zooplankton MeHg concentrations in the model were a factor of two higher. Further evidence can be found in the higher % MeHg values in the water column particles for the HDC2 (0.575±0.574%) and LDC (0.744±0.976%) than in the % MeHg values for the surface sediment at the end of the experiment (HDC2: 0.161±0.099% and LDC: 0.136±0.077%).

Percent MeHg on the particles is low in the mesocosms compared to other systems. Lawson et al. (2001) found % MeHg in riverine water columns to range between 1-5% of the HgT concentrations with a decrease in % MeHg as the flow rate and TSS increased. The % MeHg values in the HDC2/LDC Experiment are similar to the resuspension treatments in the Resuspension Experiments (1 and 2) (Kim et al., 2004), averaging 0.289±0.060% and 0.272±0.138%, respectively. The non-resuspension systems, with significantly less TSS but mostly composed of plankton, had higher average % MeHg values for the first (2.84±1.32%) and second (2.02±1.45%) Resuspension Experiments (Kim et al., 2004).

Table 2.2: Average concentrations of HgT, MeHg, % MeHg, and % organic matter with standard deviations from all the tanks in the HDC2/LDC Experiment.

Exp 4	Depth (cm)	HgT (ng g⁻¹)	MeHg (ng g⁻¹)	% MeHg	% Organic Matter	HgT (ng g⁻¹)	MeHg (ng g⁻¹)	% MeHg	% Organic Matter
		HDC2				**LDC**			
Initial	0-0.5	510±44	0.59±0.68	0.12±0.15	14.1±2.5	483±30	0.76±0.70	0.15±0.13	10.4±3.4
	0.5-1	426±47	0.62±0.37	0.15±0.09	9.6±1.8	531±109	0.79±0.29	0.15±0.10	10.3±3.0
	1-2	476±34	0.75±0.23	0,16±0.06	9.7±1.0	455±45	0.57±0.16	0.13±0.05	9.9±1.9
	2-3	435±36	0.72±0.14	0.17±0.02	10.3±1.7	435±71	0.75±0.44	0.19±0.15	8.9±2.4
	3-5	445±148	0.62±0.17	0.17±0.09	11.6±0.3	482±79	0.67±0.41	0.15±0.11	10.1±1.6
	5-7	551±105	0.79±0.23	0.15±0.07	11.2±0.3	487±97	0.66±0.39	0.15±0.11	9.8±2.0
	7-9	500±117	0.92±0.26	0.20±0.10	10.5	682±152	0.56±0.15	0.08±0.01	9.5±2.2
Mid	0-0.5	517±265	0.81±0.43	0.19±0.13	10.7±2.7	423±27	0.66±0.42	0.15±0.09	11.6±0.9
	0.5-1	403±71	0.62±0.25	0.16±0.07	11.7±0.7	436±4	0.57±0.18	0.13±0.04	10.5±2.4
	1-2	523±91	0.83±0.25	0.17±0.07	10.4±1.3	466±30	0.67±0.20	0.14±0.04	11.0±1.2
	2-3	452±27	0.84±0.23	0.18±0.05	10.4±1.2	536±120	0.78±0.49	0.14±0.07	10.6±0.3
	3-5	523±137	0.79±0.24	0.16±0.08	11.6±0.3	523±53	0.76±0.30	0.14±0.06	10.2±0.2
	5-7	527±141	0.95±0.43	0.20±0.13	11.3±0.3	434±114	0.66±0.37	0.16±0.11	11.3±0.2
	7-9	500±78	1.11±0.60	0.22±0.12	11.5±0.4	497±150	0.87±0.41	0.20±0.15	11.4±0.5
Final	0-0.5	302±50	0.50±0.37	0.16±0.10	11.1±1.8	478±75	0.65±0.41	0.14±0.08	11.7±1.5
	0.5-1	429±34	0.59±0.16	0.14±0.04	11.2±1.2	492±27	0.57±0.21	0.12±0.05	11.3±1.4
	1-2	507±99	0.74±0.15	0.15±0.06	10.8±0.6	458±64	0.69±0.35	0.15±0.06	10.1±1.2
	2-3	450±55	0.51±0.14	0.12±0.04	10.0±1.6	441±46	0.69±0.38	0.16±0.09	10.3±0.8
	3-5	474±62	0.79±0.32	0.17±0.07	11.2±0.8	473±37	0.51±0.07	0.11±0.02	10.5±1.2
	5-7	506±18	0.55±0.14	0.11±0.03	10.3±1.4	483±38	0.55±0.20	0.11±0.04	9.5±2.1
	7-9	590±206	0.87±0.24	0.16±0.10	10.2±1.6	479±13	0.54±0.30	0.11±0.07	10.2±1.6

The average concentration of dissolved MeHg in the water column was similar for the HDC2 treatment, averaging 0.054±0.034 ng l^{-1}, and not significantly different from the LDC treatment, averaging 0.050±0.025 ng l^{-1} (Fig 2.3b). Dissolved MeHg concentrations were variable in all systems throughout the experiment and was significantly different over time (p=0.001). Dissolved MeHg concentrations did not change in conjunction with particulate MeHg concentrations, however particulate and dissolved MeHg concentrations were negatively correlated (r^2=0.43) for the HDC2 treatment. The little change suggests dissolved MeHg concentrations are not controlled by equilibrium partitioning (Kim et al., 2004), and that desorption from sediments is not a substantial source of water column MeHg (Heyes et al., 2004). The average concentration of the input water, corresponding to the 10% daily water exchange, was 0.060±0.027 ng l^{-1} (n=9). Given the inputs and outputs from the tanks on a daily basis, and the similarity between the input water MeHg and the MeHg within the tank, the overall daily removal was 3.2 ng for the HDC2 tanks and 5.9 ng for the LDC tanks (estimated from D14), due primarily to the removal of particles from the tank each day during water exchanges.

2.1.3 Off Cycle Concentrations

Samples for water column characteristics were collected three times during the off-phase on D 6, 13, and 20. TSS concentrations were significantly lower in the off-phase compared to the on-phase in both the HDC2 tanks (11.0±2.3 mg l^{-1}) and the LDC tanks (11.0±2.8 mg l^{-1}). POM concentrations were also significantly lower in the off-phase, averaging 3.8±1.1 mg l^{-1} for the HDC and 5.1±1.8 mg l^{-1} for the LDC treatment. However, % POM was higher in the off-phase for both the HDC2 tanks (34.5±2.4%) and

LDC tanks (46.8±10.4%) due to the large amount of low organic matter sediment particles that rapidly settle during the off-phase.

Off-phase sampling for Hg analysis was only conducted on D14 in the HDC2/LDC Experiment. For HgT concentrations on the particles, the off-phase was similar to the on-phase for the HDC2 treatment (434±59 ng g^{-1}), but significantly lower in the LDC treatment (265±0.45 ng g^{-1}). The dissolved HgT concentrations for the off-phase were similar in both the HDC2 (0.93±0.24 ng l^{-1}) and LDC (1.22±0.38 ng l^{-1}) treatments to the on-phase average concentration.

The off-phase particulate MeHg concentrations were higher in the LDC tanks (4.25±0.74 ng g^{-1}) compared to the on-phase. The average off-phase concentration was similar to the on-phase for the HDC2 tanks (2.38±1.49 ng g^{-1}). Since most of the TSS settles quickly out of the water column once resuspension has been turned off, the increase in particulate MeHg concentrations during the off-phase suggests a greater proportion of living matter in the water column on a ng g^{-1} basis compared to the on-phase. Again, the average concentrations for dissolved MeHg for the off-phase are similar to the on-phase for both the HDC2 (0.064±0.040 ng l^{-1}) and LDC (0.083±0.004 ng l^{-1}) treatments.

2.1.4 Water Column Distribution Coefficients

Distribution coefficients (K_d) measure the relative distribution of Hg between the dissolved and particulate phases where a higher K_d value indicates a higher affinity for the particulate phase. With the approximation that the mass of one liter of water is one kg, this is a dimensionless unit.

$$\log K_d = \log \text{[particulate (ng/kg)]} / \text{[dissolved (ng/l)]}$$

The value of the K_d gives an indication of the extent to which the total metal concentration will be a function of TSS values. The particulate phase becomes the dominant phase above a TSS concentration of about 10 mg l^{-1} for a K_d of 10^5.

There was no significant difference between the HDC2 and LDC treatments in terms of distribution coefficients for both HgT and MeHg (Table 2.3), although the K_d's for the HDC2/LDC Experiment were within the same range as previous resuspension mesocosm experiments (Kim et al, 2004). There was also not a significant difference over time or with the interaction of treatment and time. The similarity between treatments is in contrast with previous mesocosm experiments that showed a difference in water column K_d for MeHg between resuspended and non-resuspended treatments (Kim et al., 2004). However, this is likely due to the fact that resuspension occurred in all systems, and the difference in the previous experiments is likely related to the different relative amounts of biotic particles in the non-resuspended versus the resuspended mesocosms. Clam density should not impact K_d's unless the clams change the sediment characteristics and their binding capacity. For example, K_d's were lower in the resuspension treatments with lower %POM compared to the non-resuspension treatments in the Resuspension Experiments (1 and 2) (Kim et al., 2004) since Hg has a high affinity to POM. Also, Mason and Sullivan (1998) demonstrated on the Anacostia River that the K_d for HgT and MeHg increased with increasing POM.

Lawson et al. (2001) found that Hg has the highest K_d value and was the only metal where K_d was a function of the organic content of the particulate matter. Thus, Hg concentrations should be strongly influenced by particulate load, even at low TSS since the particulate phase is the dominant fraction for Hg. This is true to a lesser extent for

MeHg as the log K_d values are lower. The difference is likely due to the relative higher

affinity of HgT to the particulate phase compared to MeHg. Lower K_d values were found

for MeHg than for HgT, similar to what was observed in the previous mesocosm

experiments (Kim et al., 2004), in the Patuxent River (Benoit et al., 1998), and other

locations (Lawson et al., 2001; Mason and Sullivan, 1998).

The log K_d values for the off-phase in the HDC2/LDC Experiment (Table 2.3)

were similar to the on-phase in both treatments and for HgT as well as MeHg, with the

exception of the HgT log K_d value for LDC which was lower than the on-phase value.

Table 2.3: Average water column distribution coefficient (log K_d) and standard deviation for HgT and MeHg in the HDC2/LDC Experiments.

	HDC2/LDC Experiment	
	HDC2	LDC
HgT –on [a]	5.62±0.12	5.73±0.15
HgT –off [b]	5.67±0.15	5.35±0.07
MeHg –on [a]	4.63±0.63	4.72±0.42
MeHg –off [b]	4.72±0.80	4.71±0.08

a: On-cycle when mixing system was on.
b: Off-cycle when mixing system was off. Average and standard deviation of replicate tanks for D14 only.

2.1.5 Mercury in the sediment

Table 2.2 shows the treatment average and standard deviation of HgT, MeHg, %

MeHg, and % organic matter for the HDC2/LDC Experiment. Sediment cores were

collected in all tanks before the experiment began (initial), on D15 (mid), and D28

(final). Percent organic matter remained relatively constant down core for the HDC2

(10.7±0.5%) and LDC (10.5±0.8%) tanks (final concentration). There were no

significant differences in the two treatments, over time, or at depth nor was there any

significant interactions. HgT in the sediment was also relatively constant down core with

no significant differences with depth, most likely due to the through homogenization of

the sediment prior to the beginning of the experiment. As would be expected, there were also no significant differences in HgT concentration over the course of the experiment since losses to the water column and through water exchange were trivial compared to the amount of Hg in the sediment. Concentrations of HgT are similar to those found in Baltimore Harbor by Mason and Lawrence (1999) with an average of 450 ng g^{-1}.

There were no significant differences in MeHg concentrations in the treatments, over time, or at depth. Generally, MeHg concentrations appear to decrease slightly over the course of the experiment suggesting net demethylation, however the decrease is not linear since the mid concentrations are often the highest. Kim et al. (in revision) found that the demethylation rate was higher than the methylation rate in the Resuspension Experiments (1 and 2), however, both demethylation and methylation rates were lower near the sediment surface than deeper sediments.

Percent MeHg remained relatively constant down core and throughout the course of the experiment with no significant differences in treatment, time, or depth. Percent MeHg in sediments has been shown to be strongly correlated with potential methylation rates measured in cores spiked with Hg isotopes, supporting the premise that % MeHg is a reasonable approximation of the relative rates of Hg methylation in the sediments (Fig 1.3) (Benoit et al., 2003; Heyes et al., in press; Sunderland et al., 2004). The % MeHg is low in the HDC2/LDC Experiment compared to 1% in the Chesapeake Bay (Mason and Lawrence, 1999) and 0.6% in Lavaca Bay, Texas (Bloom et al., 1999), indicating low methylation rates.

2.1.6 Summary

Clam density appeared to have the greatest impact on water column characteristics such as TSS, POM, and Chl *a*. However, clam density does not appear to affect HgT and MeHg in the dissolved or particulate phase of the water column. Sediment HgT and MeHg concentrations, as well as % MeHg and % organic matter, was similar between treatments reflecting the origin of the sediment and suggesting clam density had little impact on Hg cycling in the sediments over the course of the experiments.

Simple mass balance calculations of inputs and outputs of MeHg suggest there were a net production of MeHg in the HDC tanks, but a net loss in the LDC tanks. This suggests the higher density of clams may stimulate methylation by increased sediment destabilization, or increased oxygenation of the sediment through bioturbation. This agrees with findings from Hammerschmidt et al. (2004) that benthic infauna can affect the biogeochemical cycling of MeHg in coastal marine systems since mixing or irrigating the sediments appears to enhance MeHg production and extend zones of active Hg methylation.

2.2 The Investigation of Mercury and Methylmercury Cycling and Bioaccumulation Through the Use of a Mercury Stable Isotope

2.2.1 Background

Atmospheric deposition of natural and anthropogenically derived Hg is the main source to most aquatic systems either as direct deposition to the water surface or as runoff from the watershed (USEPA, 1997). Most of the Hg in atmospheric deposition is ionic (Hg^{II}) and MeHg is typically less than 1% of the total Hg concentration. However the

concentration of MeHg in rivers, lakes, and coastal waters is a larger fraction of the total

Hg concentration, so simple mass balance calculations suggest that most of the MeHg in

aquatic systems must be produced *in situ* with atmospheric deposition being an important

source of Hg for methylation (Mason et al., 1994). It is believed that Hg entering the

aqueous environment through direct deposition is more biologically available than *in situ*

Hg or Hg entering through runoff or groundwater since this Hg is more likely to be bound

to POM or DOM (Mason et al., 2000b).

Stable isotopes of Hg have been used to investigate the magnitude and time frame

of the response of MeHg in biota of aqueous systems to change in Hg loading, namely in

the whole-watershed Hg loading experiment (METAALICUS) in the Experimental Lakes

Area, Ontario and the mesocosm experiments in the Florida Evergades. Mercury was

added as an enriched stable isotope (Hg^{199}) to one tank from each treatment (T2: HDC2

and T5: LDC) in the HDC2/LDC Experiment. This allowed us to follow the newly added

Hg over time and distinguish it from the background Hg already in the mesocosm system.

The added isotope is referred to as 'Hg spike' or Hg^{199}, while the Hg in the system is

called 'background' Hg.

The addition of 10 µg of Hg^{199} to the water column, normalized to the surface area

of the mesocosms, is less than the average wet deposition of 25 µg m^{-2} y^{-1} measured at

Hart-Miller Island (HMI) that is located downwind of Baltimore's industrialized region

and near our sediment collection site. In comparison, the same study measured an

average wet deposition 13 µg Hg m^{-2} y^{-1} at the Chesapeake Biological Laboratory in

Solomons, MD, indicating there is a significant input of Hg to the atmosphere from the

Baltimore region (Mason et al., 1997). The Hg spike was added to simulate Hg entering

the aquatic environment through atmospheric deposition.   Since the spike was pre-equilibrated with Patuxent River water, the speciation of the spiked Hg added to the tanks should be similar to the background Hg in the tanks.

2.2.2 Hg Isotope Distribution

The addition of 10 μg of Hg^{199} to the water column was made in the evening of D2 during the on-phase. The first on-phase water samples were collected in the morning of D4. In that period, greater than 90% of the spiked Hg isotope became particle associated in the water column (Fig 2.4a, b). The amount of isotope measured in the particulate and dissolved phase for D4 was 3.84 μg and 4.68 μg for HDC2 and LDC, respectively. The measured values were similar, but less than, the "expected" added amount. In order to investigate further whether the correct amount of Hg isotope had been added to the tanks, first order kinetics were employed to back-calculate the initial amount of Hg spike added to the tanks (Fig 2.5) using these measured values through the following equation for a first order decay in concentration (Stumm and Morgan, 1996):

$$[A] = [A_0]\, e^{-kt}$$

where A is the sum of the isotope in the particulate and dissolved phase, A_0 is the initial amount of isotope added to the tanks, t is time (days), and k is the rate of isotope loss from the water column (day^{-1}). By using this equation, the initial spike amounts were estimated to be 11.1 μg and 6.8 μg for HDC2 and LDC, respectively. The rate of isotope loss from the water column (k) was remarkably similar in both systems. The half-life $(t_{1/2})$ of the Hg spike in the water column was calculated to be 1.3 days for both tanks using the following equation (Stumm and Morgan, 1996):

$$t_{1/2} = \ln 2\, /\, k$$

Since the first water column sampling coincided with the half-life of the Hg spike, the

mass balance estimations for D4 suggest little loss of the isotope from the system after

addition.
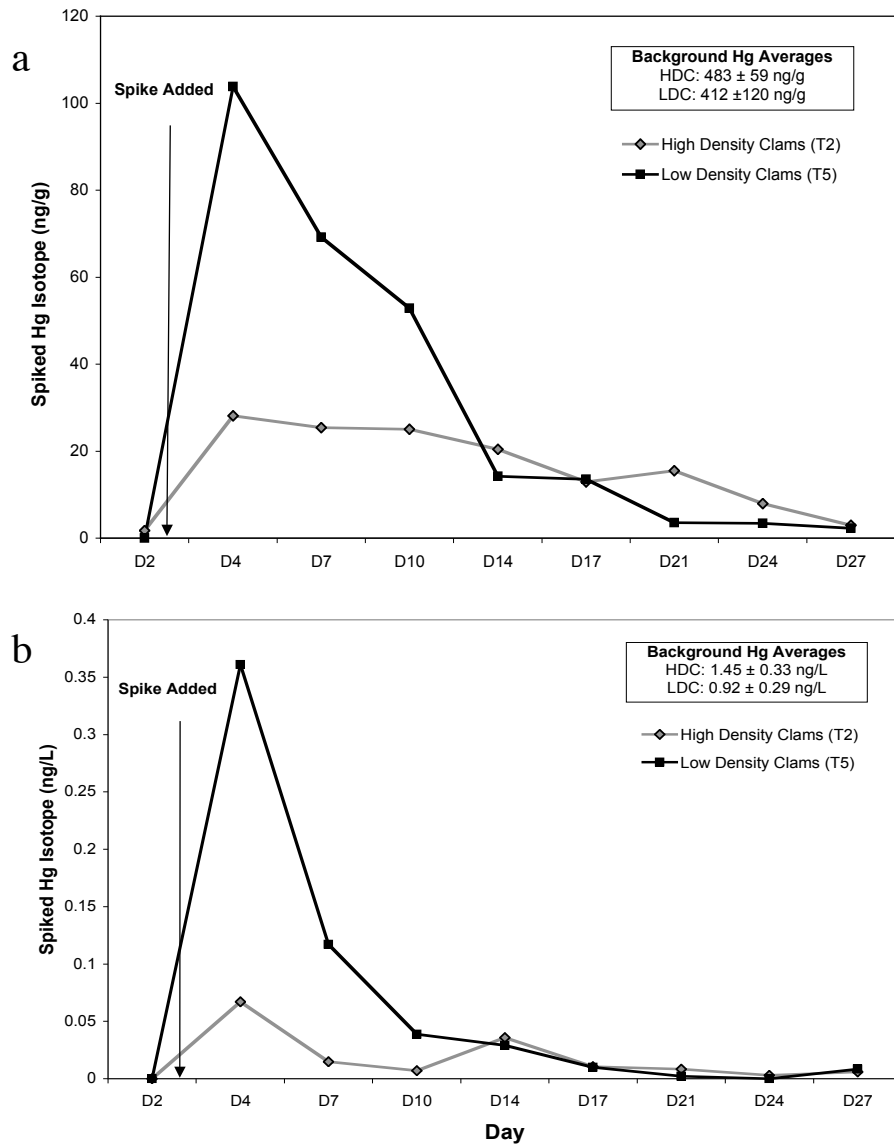
Figure 2.4: The fate of the Hg isotope in the water column of the Clam
Density Experiment. (a) Particulate phase (b) Dissolved phase

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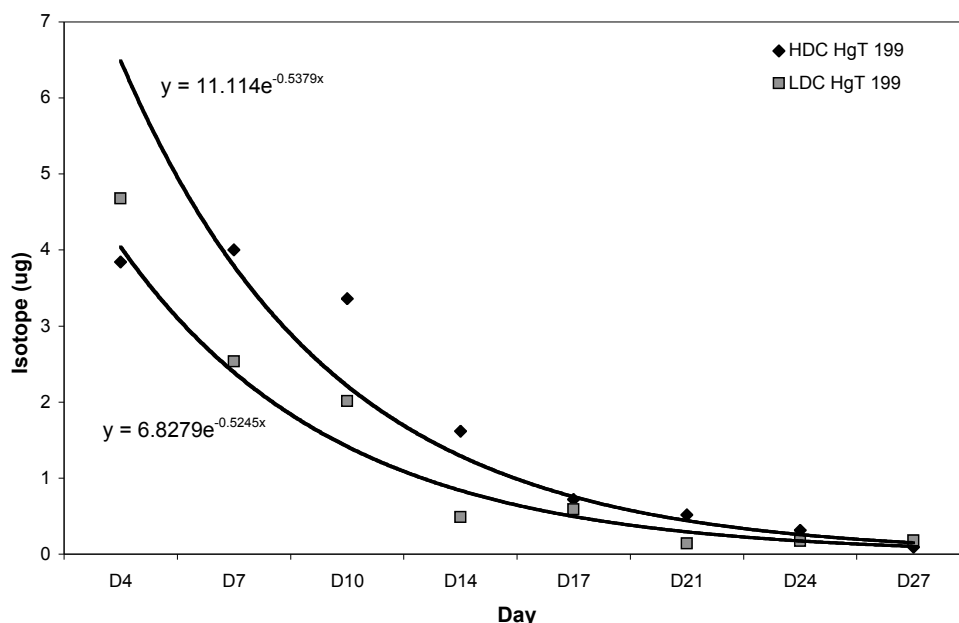
Figure 2.5: Loss of the Hg isotope from the water column (particulate and dissolved phase) over time.

The Hg spike concentration in the particulate and dissolved phase in the water column decreased over time (Fig 2.4a, b). There are several possibilities regarding the fate of the isotope, such as loss to the atmosphere, removal to the walls, removal to the sediment, or into organisms. As mentioned above, greater than 90% of the measured Hg spike was on the particles after the first day, providing evidence that the isotope most likely did not evade to the atmosphere. Evasion occurs when Hg is in the elemental form (Hg^0). Since Hg in this study was mainly particle bound and present was Hg^{II}, it would have to have been released from the particles, as only dissolved Hg^{II} is readily reduced, and reduced to Hg^0 to be lost to the atmosphere. This process is not thermodynamically probable due to the stability of the Hg^{II} complexes. The Hg spike may have adsorbed to the walls of the mesocoms, however, the tank walls were cleaned by scraping the accumulated particles off the sides of the tank every second day to minimize the loss of

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Hg. Another mechanism of removal could be accumulation of the isotope into organisms. The Hs spike was not detected in zooplankton. There was evidence of Hg spike in the clam samples, however only the clams from the LDC tank had an isotope concentration above the detection limit (> 0.5% of the background Hg concentration). However, the most likely removal mechanism was incorporation of the Hg spike into the sediment due to the mixing processes of resuspension and bioturbation by the clams. However, the Hg spike was not detected in the sediments. Again, this is most likely due to the relatively high concentration of background Hg in the sediment compared to the Hg spike. Since approximately only the top 2 mm of the sediment was being resuspended (Sanford, pers. comm.) and the interval of surface sediment sampled was 5 mm, the Hg spike was potentially 'diluted' by the background Hg in the sample. Also, our first sediment sampling did not occur until D8 that could have increased the opportunity for the Hg spike to be mixed deeper into the sediment and be undetected due to the high background concentration.

The percentage of isotope compared to the background Hg in the surface sediment can be estimated using the average Hg concentration on particles of 0.45 μg g^{-1}, an average sediment bulk density of 1.25 g cm^{-3} for the top 0.5 cm, a surface area in the tanks of 10^4 cm^{-2}, and a 0.5 cm depth of the surface sample. Assuming all of the 10 μg of isotope was particle associated and settled to the sediment surface (i.e. no mixing down core), it would account for less than 0.5% of the Hg in the sediment, which is below our detection limit for the isotope.

The Hg isotope concentration in the water column decreased over time (Fig 2.4a, b), presumably by being taken up onto particles and being buried in the sediment.

However, some of the isotope could have been lost through the daily water exchanges. To find the percent of the isotope removed during water exchanges, a constant, average TSS concentration was assumed from the off-phase of 11 mg l^{-1} for both the HDC2 and LDC tanks. It was also assumed that the particle Hg concentration in the off-phase reflected the particle concentration during the on-phase. Discrete sampling points support this assumption. There was an estimated Hg isotope loss of 6% for the HDC2 tank and 13% for the LDC tank. The amount of the isotope removed from the water column cannot be accounted for by the water exchanges, therefore this provides further evidence that the Hg spike was removed to the sediment below the resuspension layer. This also indicates that the resuspended material is not the same particles continuously resuspending and settling each cycle, but that there is a substantial exchange of material between the resuspended layer and deeper sediments.

The Hg spike was detected in both the dissolved and particulate phase in the water column. The spike concentration was higher (on a ng g^{-1} basis) in the water column in the LDC tank with the lower concentration of TSS (Fig 2.4a, b), but over time the Hg spike concentrations decreased and became similar due to particle mixing from resuspension and loss of the Hg spike to the sediment. The presence of Hg spike at the end of the experiment in the dissolved phase indicates continued partitioning between the dissolved and particulate phase over time that also suggests continued bioavailability.

There is evidence that the Hg isotope was methylated in the system since it was observed as MeHg in the water column particles. However, the concentration of MeHg^{199} was below or at the quantifiable detection limit of the method. Similar to HgT, the

methylated isotope would be difficult to see in the sediment due to the background concentration of MeHg.

2.2.3 Hg Isotope Distribution Coefficients

Water column distribution coefficients (K_d) were calculated for the Hg spike and compared to the background K_d values for Hg and are shown in Fig 2.6. There was no significant difference in average K_d values between the two treatments with the background Hg and they track each other well throughout the experiment. However, the K_d values for the isotope were higher over the course of the experiment, suggesting the Hg spike was more strongly bound to particles than the background Hg. This observation is counter to expectation since it is believed that the 'new' Hg binds quickly, but more weakly, to the surface of particles, and works its way into the matrix of the particles over time. Thus, the 'new' Hg becomes 'old' and less bioavailable with time. Thermodynamic calculations suggest that Hg in the dissolved phase will be mainly bound to DOC. Since the Hg spike is thought to be more reactive, it may be bound to the particles or preferentially incorporated into organisms in a higher proportion than the background Hg that may have a higher proportion of Hg strongly associated with DOC. This would result in higher K_d values for the Hg spike.
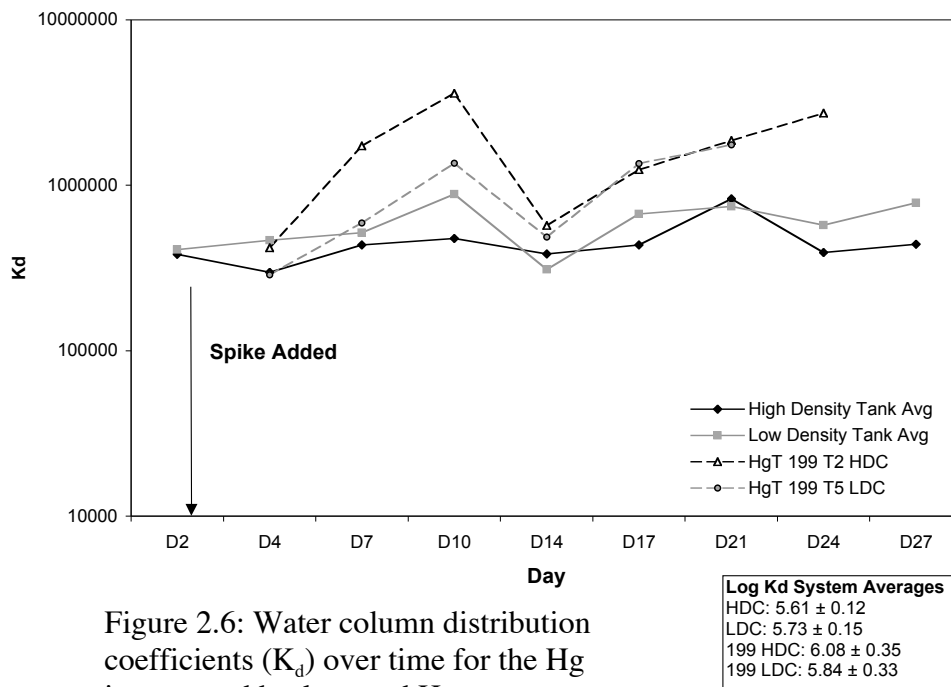

Figure 2.6: Water column distribution coefficients (K_d) over time for the Hg isotope and background Hg.

Log Kd System Averages
HDC: 5.61 ± 0.12
LDC: 5.73 ± 0.15
199 HDC: 6.08 ± 0.35
199 LDC: 5.84 ± 0.33

2.2.4 Hg Isotope Bioaccumulation

As mentioned above, the Hg spike was observed in the clams, but only at detectible concentrations in the LDC tank where there was a higher concentration of Hg spike in the water column dissolved and particulate phase due to the lower TSS concentrations. The higher concentration of Hg spike in the LDC tank is most likely due to the combination of more Hg spike on the particles in the water column and the lower biomass of clams feeding on the particles.

No Hg spike was detected in the zooplankton samples over the course of the experiment. This finding is not unexpected if we believe the Hg spike was more particle associated than the background Hg, since phytoplankton are thought to accumulate Hg from the dissolved phase (Mason et al., 1996).

MeHg^{199} was not detected in the biota. Since MeHg^{199} was barely seen on the particles, it is not surprising that it could not be detected in zooplankton or clams since it would be increasingly more difficult to see the isotope as you get up the food chain due to the increasingly higher concentrations of background MeHg.

The concentration of MeHg^{199} in clams was estimated using the MeHg^{199} concentration on the particles and the following equation:

MeHg^{199} in clams = clam biomass x clearance rate x phytoplankton POC concentration x

particulate MeHg^{199}/POC concencentration x POC AE x % feeding

The values for clam biomass, clearance rate, assimilation efficiency, and % feeding were taken from the model developed by Kim (2004). The estimated concentration in clams for the HDC tank was 1.37 ng MeHg^{199} g^{-1} and 2.36 ng MeHg^{199} g^{-1} for the LDC tank. For the HDC tank, this is close to 5% of the background Hg and 9% of the background Hg in

the LDC tank. These percentages are above the detection limit for the analytical method, indicating the methylated isotope should have been detected in the clams. However, this calculation assumed the maximum clearance rate for clams feeding 62% of the time, which could easily have overestimated the amount of MeHg[199] accumulated. Also, the assimilation efficiency used was for phytoplankton, which would be higher than the assimilation efficiency of resuspended material. Lastly, there is associated error with the concentration of MeHg[199] on particles since it was approaching our detection limit.

2.2.5 Summary

The amount of Hg stored in many ecosystems is far greater than the new mercury delivered by atmospheric deposition. The implication of greater mobility or bioavailablity to methylating bacteria of newly deposited Hg compared to 'old' Hg, stored in an ecosystem for many years, is that there could be a reduction in bioaccumulation of MeHg into biota if atmospheric deposition is reduced as a result of implementation of anthropogenic source reduction strategies. On the other hand, if all Hg is equally mobile and available for methylation, then changes in deposition rates will take a long time to affect levels of Hg in biota (Hintelmann et al., 2002).

Gilmour et al. (2004a; 2004b) have found several differences in the biogeochemical behavior of the newly added Hg from the behavior of background Hg such as differences in partitioning behavior in the sediments and water, as well as, bioavailability for methylation in the sediments and anoxic bottom waters. Our study supports these findings since the Hg spike was different from the background Hg in the water column leading to higher K_d values.

There was also evidence from our study that the Hg isotope was rapidly methylated within the system. This finding is supported by a study using in-lake mesocosms by Orihel et al. (2004) that found that the newly-deposited Hg spike was readily methylated and incorporated into the aquatic food web in less than 14 days. These results suggest that any changes in Hg entering the system will be relatively rapidly reflected in the food chain of the system. Thus, reductions in loadings of Hg to such a system should lead to a relatively rapid response in terms of the biota MeHg burden.

Chapter 3: The Effect of Clams on the Bioaccumulation of Mercury and Methylmercury

3.1 Clam/No Clam Experiment (HDC1/NC) Mercury and Methylmercury Concentrations

3.1.1 Water column characteristics

Total suspended solids (TSS) in the HDC1 tanks was significantly higher overall during resuspension (on-cycle), averaging 122 ± 27 mg l^{-1} than in the NC tanks, averaging 49 ± 15 mg l^{-1} (p<0.001). The two treatments differed significantly over time (p<0.001) and there was a significant interaction between treatment and day (p<0.001). As seen in Fig 2.1a, TSS was higher in the HDC1 tank in the beginning of the experiment. Similar to the HDC2/LDC Experiment, the HDC1 tanks appear to have had more sediment destabilization with the presence of clams. However, over time the differences in concentration decreased possibly due to clams removing particulate from the water column, or potentially due to the release of exudates by the clams that result in sediment stabilization.

As shown in Fig 3.1b, particulate organic matter (POM) was also significantly higher in the HDC1 tanks than the NC tanks (p<0.001), averaging 17 ± 5 mg l^{-1} and 10 ± 3 mg l^{-1}, respectively. The two treatments differed significantly over time (p<0.001) and there was a significant interaction between treatment and day (p<0.001). Like TSS, the values for HDC1 were higher in the beginning of the experiment, but came together over time. However, average % POM was significantly lower in the HDC1 tanks ($14.1\pm1.4\%$) than the NC tanks ($20.1\pm2.2\%$) (p<0.001). Again, the treatments were significantly different over time (p=0.026), and there was a significant interaction between treatment and day (p<0.001). Such differences could be attributed to the removal of phytoplankton

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by the clams or differences in the relative amount of resuspended material. The % POM is lower for the surface sediment (10.2±2.8% for the HDC1 tanks and 12.1±0.8% for the NC tanks) than that of the TSS (Table 3.1), and the presence of plankton accounts for the higher water column values. There was a significant positive correlation between TSS and POM in the HDC1 tanks ($r^2 = 91$), but the correlation was not significant for the NC tanks.

Total Chlorophyll a (Chl a), shown in Fig 3.1c, was significantly higher (p=0.002) in the NC tanks than the HDC1 tanks, averaging 27.2±8.6 µg l^{-1} and 16.6±3.4 µg l^{-1}, respectively. The treatments did not differ significantly over time. A small phytoplankton 'bloom' was observed in the NC treatment beginning on D14 and lasting until the end of the experiment, but Chl a concentration in the HDC1 treatment remained relatively constant. However, there was a significant interaction between treatment and day (p<0.001). The data for TSS and the lower %POM for the HDC tanks, along with the higher Chl a concentrations in the NC tanks, suggests that clam feeding was removing the phytoplankton from the water column. However, the Chl a concentration did not change over the experiment in the HDC tanks while it increased in the NC tanks, suggesting that the differences may not be entirely due to the presence of clams in the HDC tanks. Chl a was positively correlated with TSS for both the HDC1 (r^2=0.61) and NC (r^2=0.73) treatments as well as for POM for both the HDC1 (r^2=0.64) and NC (r^2=0.58) treatments.
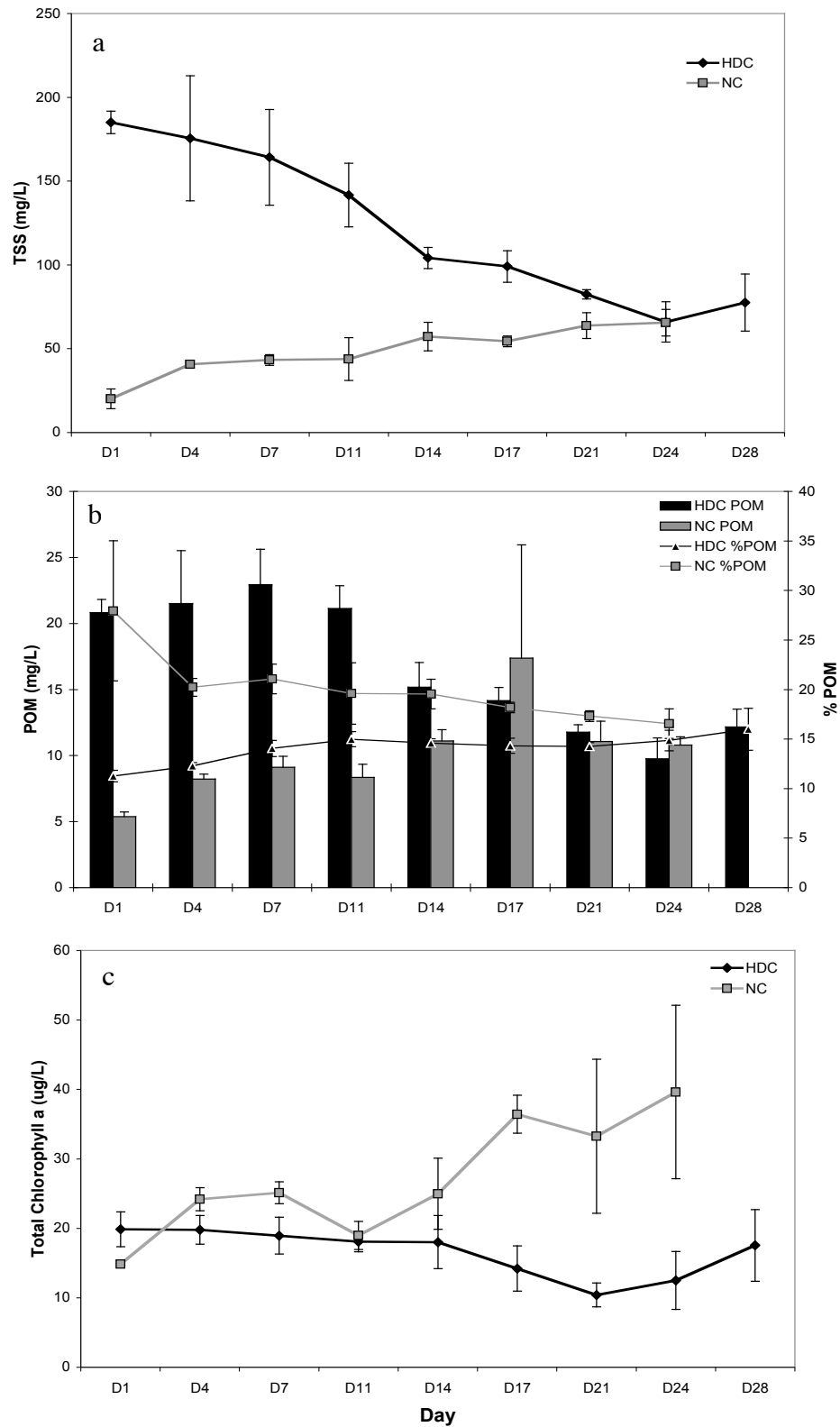
Figure 3.1: Average concentrations for water column variables in the HDC1 and NC treatments of the Clam/No Clam Experiment. (a) TSS concentration (b) POM and %POM concentration (c) Chl *a* concentration. Error bars show standard deviation of three replicate tanks in each system.

There was not a significant difference in dissolved organic carbon (DOC) found between the HDC1 (3.34 ± 0.46 mg l^{-1}) and NC (3.47 ± 0.38 mg l^{-1}) treatments, but the treatments differed significantly over time (p=0.003). There was a significant negative correlation between DOC and Chl a in the NC tanks (r^2=0.72), however there was a significant positive correlation between DOC and Chl a in the HDC1 tanks (r^2=0.68). Since DOC is not different between treatments, the Chl a concentrations drive the correlations.

Water chemical characteristics, measured daily during the on-cycle in the two treatments, are summarized in Table 3.2. There was little difference between systems in terms of average salinity and temperature over the course of the experiment. The pH was slightly higher and DO was considerable higher in the NC treatment.

Table 3.2: Average and standard deviation for ancillary parameters in the water column during the course of the HDC1/NC Experiment.

	Parameter	HDC1	NC
HDC1/NC Experiment	DO mg l^{-1}	4.8 ± 0.6	7.3 ± 0.6
	Salinity (ppt)	18 ± 0.2	18 ± 0.3
	Temperature (°C)	26 ± 0.02	26 ± 0.01
	pH	7.5 ± 0.2	8.0 ± 0.1

3.1.2 Mercury distribution in the water column

The average concentration of particulate HgT (on a mass basis) in the water column was not significantly different between treatments (Fig 3.2a), averaging 479±67 ng g^{-1} for the HDC1 tanks and 450±39 ng g^{-1} for the NC tanks. Particulate water column and surface sediment HgT concentrations (Table 3.1) were found to be similar, where the average final concentrations for HDC1 and NC were 469±56 ng g^{-1} and 390±49 ng g^{-1}, respectively.

Figure 3.2: Average concentrations of HgT in the particulate and dissolved phases in the HDC1 and NC treatments of the Clam/No Clam Experiment. (a) Particulate HgT concentration (b) Dissolved HgT concentration. Error bars show standard deviation of three replicate tanks in each system.

The average concentration of dissolved HgT in the water column was also not significantly different between the HDC1 tanks (1.94 ± 0.75 ng l^{-1}) and the NC tanks (1.84 ± 1.30 ng l^{-1}) (Fig 3.2b). The changes in dissolved HgT over time did not follow a discernable trend and did not correspond to changes in particulate HgT. Dissolved HgT in the input water was measured for the sampling days and averaged 1.74 ± 0.99 ng l^{-1} (n=6). As with the HDC2/LDC Experiment, this concentration was similar and only slightly lower than the average concentrations in the tanks over time. The HgT removed during water exchanges was estimated from off-cycle TSS and HgT concentrations on D17 and was 865 ng in the HDC1 tanks and 465 ng in the LDC tanks.

Particulate MeHg concentrations and % MeHg are shown in Fig 3.3a. The average concentration of particulate MeHg (on a mass basis) in the water column was higher and more variable in the NC tanks (1.87 ± 1.11 ng g^{-1}), but not significantly different from the HDC1 tanks (1.27 ± 0.16 ng g^{-1}). However, the two treatments differed significantly over time (p=0.004) and there was a significant interaction between treatment and day (p<0.001). Generally, the MeHg concentration appeared to be higher in the NC treatment in the beginning of the experiment, but decreased over time and both were similar at the end of the experiment. The lower TSS concentration, but higher Chl *a* concentration and %POM, indicates the TSS is most likely composed of more phytoplankton in the NC tanks. Kim (2004) showed that phytoplankton had a greater ability to accumulate MeHg and therefore had a higher MeHg concentration on an ng per g basis compared to sediment particles. This likely accounts for the lower %MeHg in the HDC1 tanks. Indeed, the average % MeHg was higher but not significantly different in

the NC treatment (0.422±0.294%) than the HDC1 treatment (0.271±0.097%), most likely

due to the significantly higher phytoplankton concentration in the NC treatment.
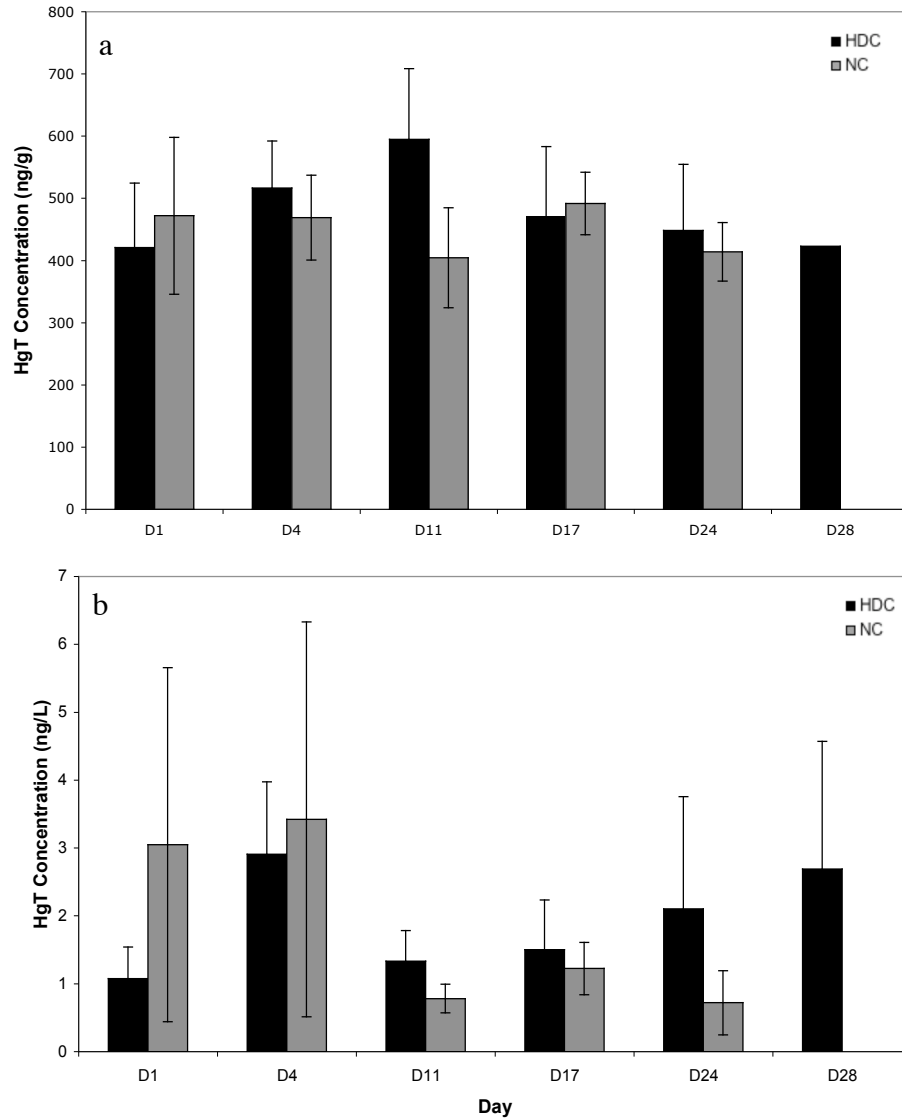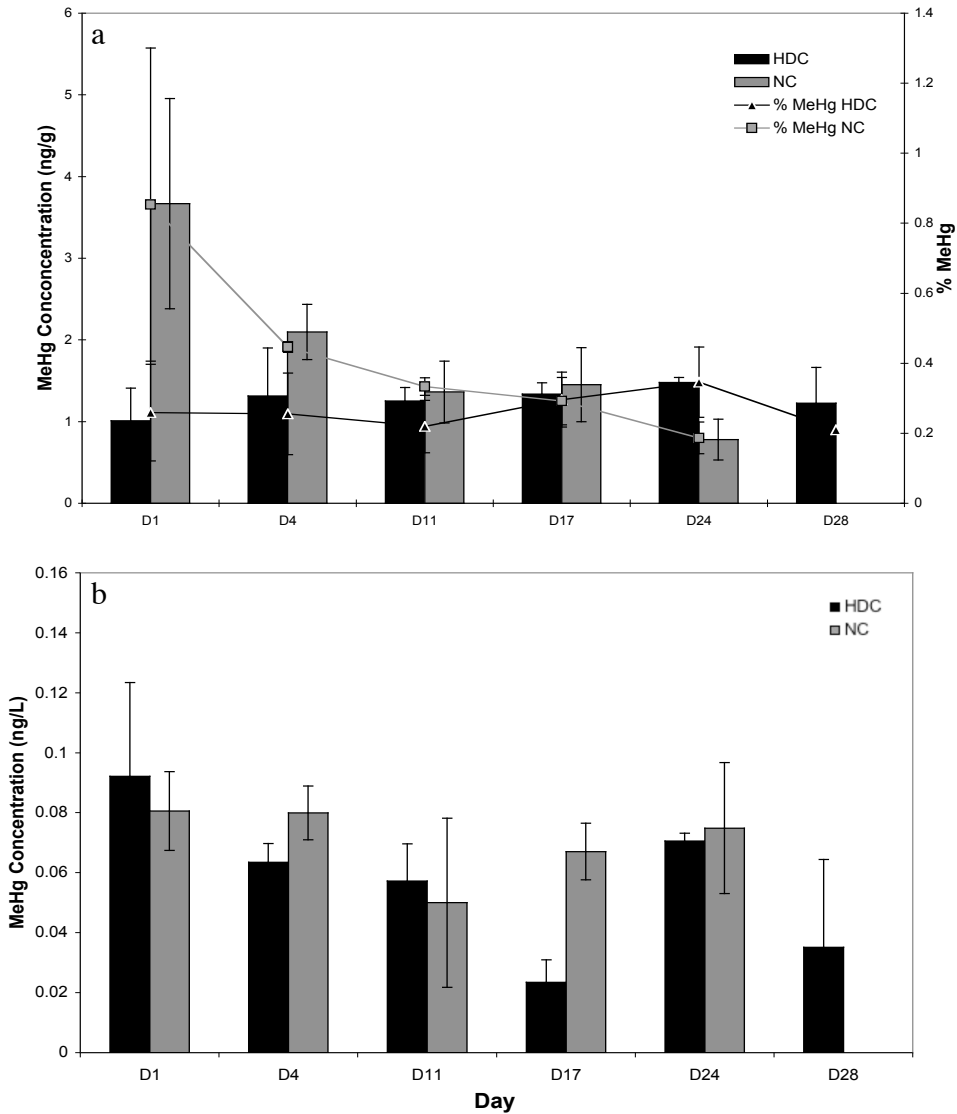

Figure 3.3: Average concentrations of MeHg in the particulate and dissolved phases in the HDC1 and NC treatments of the Clam/No Clam Experiment. (a) Particulate MeHg concentration and % MeHg (b) Dissolved MeHg concentration. Error bars show standard deviation of three replicate tanks in each system.

Surface sediment (0-0.5 cm) MeHg concentrations (Table 3.1) were lower in both the HDC1 tanks (0.53±0.17 ng g^{-1}) and NC tanks (0.51±0.16 ng g^{-1}) than the particulate concentrations, at both the beginning and the end of the experiment. % MeHg was also lower in the surface sediment in the HDC1 tanks (0.106±0.032%) and the NC tanks (0.135±0.057%) than in the resuspended particles, reflecting the higher proportion of living material (i.e. zooplankton and phytoplankton) which bioconcentrate MeHg in the water column to a higher concentration relative to that of the surface sediment.

The average concentration of dissolved MeHg in the water column was also slightly higher in the NC treatment (0.071±0.013 ng l^{-1}), but not significantly different than the HDC1 treatment (0.057±0.025 ng l^{-1}) (Fig 3.3b). However, the treatments did differ significantly over time (p=0.010). The average MeHg concentration for the input water was 0.054±0.037 ng l^{-1} (n=6). Similar to the previous experiments, dissolved MeHg concentrations were variable in all systems throughout the experiment and did not change in conjunction with particulate MeHg. The overall daily removal of MeHg due to water exchanges was 3.5 ng for the HDC1 tanks and 3.7 ng for the NC tanks (estimated from D17).

Table 3.2: Average concentrations of HgT, MeHg, % MeHg, and % organic matter with standard deviations from all the tanks in the HDC1/NC Experiment. The initial sediment cores are the same for both systems (see text, Chapter 1).

Exp 3	Depth (cm)	HDC1				NC			
		HgT (ng g^{-1})	MeHg (ng g^{-1})	% MeHg	% Organic Matter	HgT (ng g^{-1})	MeHg (ng g^{-1})	% MeHg	% Organic Matter
Initial	0-0.5	502	1.29	0.31	13.5	502	1.29	0.31	13.5
	0.5-1	466	0.93	0.22	12.6	466	0.93	0.22	12.6
	1-2	367	0.49	0.09	12.9	367	0.49	0.09	12.9
	2-3	432	0.99	0.19	12.9	432	0.99	0.19	12.9
	3-5	425	0.94	0.21	12.0	425	0.94	0.21	12.0
	5-7	448	1.37	0.28	12.7	448	1.37	0.28	12.7
	7-9								
Final	0-0.5	469±56	0.53±0.17	0.11±0.03	10.2±2.8	390±49	0.51±0.16	0.13±0.04	12.1±0.8
	0.5-1	475±103	0.44±0.17	0.09±0.02	11.8±1.3	471±116	0.38±0.16	0.08±0.01	12.6±0.2
	1-2	517±14	0.45±0.07	0.09±0.01	11.6±0.9	511±45	0.18±0.09	0.03±0.02	12.1±0.7
	2-3	555±126	0.93±0.20	0.17±0.02	12.0±0.1	542±10	0.96±0.11	0.18±0.02	12.3±0.2
	3-5	474±21	0.90±0.01	0.20±0.01	12.6±0.2	468±27	0.93±0.19	0.20±0.05	12.5±0.6
	5-7	543±54	0.74±0.12	0.14±0.03	12.7±0.5	509±33	1.09±0.27	0.22±0.06	12.7±0.3
	7-9	502±124	0.63±0.14	0.14±0.07	12.4±0.3	509±29	0.89±0.17	0.18±0.04	11.8±0.7

3.1.3 Off Cycle Concentrations

Sampling during the off-phase for water column characteristics occurred three times during the HDC1/NC Experiment (D7, 17, and 24). Average concentrations for TSS where significantly lower than the on-phase in both the HDC1 (11.99±1.79 mg l^{-1}) and NC (12.84±1.94 mg l^{-1}) treatments. POM was also significantly lower during the off-phase in both treatments, averaging 3.0±0.3 mg l^{-1} for the HDC tanks and 4.5±0.3 mg l^{-1} for the NC tanks. However, average %POM increased significantly in both the HDC1 (25.6±4.6%) and NC (34.9±2.8%) treatments compared to the on-cycle.

Off-phase samples were collected for Hg analysis on D17 and D24. On D17, unlike the on-phase average where the values were not significantly different, the HgT concentrations for the particles in HDC1 (685±164 ng g^{-1}) were significantly higher than the NC (309±24 ng g^{-1}) tanks and the on-phase average. On D24, the off-phase concentration for the HDC1 tanks (222±26 ng g^{-1}) had decreased and was less than the on-phase average, while the concentration for the NC tanks (352±49 ng g^{-1}) remained relatively constant.

The dissolved HgT concentrations for the off-phase were slightly lower in the HDC1 and NC treatments (0.84±0.35 ng l^{-1} and 0.78±0.21 ng l^{-1}, respectively) on D17 and comparable in the HDC1 and NC treatments to the on-phase average for D24 (1.91±0.75 ng l^{-1} and 2.68±1.87 ng l^{-1}, respectively).

Partculate MeHg concentrations during the off-phase were higher on both D17 and D24 and in all treatments. The concentrations on D17 were 2.73±0.53 ng g^{-1} for the HDC1 tanks and 2.48±0.68 ng g^{-1} for the NC tanks. On D24, the HDC tanks averaged 1.79 ±0.80 ng g^{-1} and the NC tanks averaged 2.70±0.21 ng g^{-1}. A higher % POM and a

higher particulate MeHg concentration is consistent with the notion of higher MeHg in living plankton, which are a relatively more dominant fraction of the TSS during the off-phase. Dissolved MeHg in the water column during the off-phase is similar to on-phase concentrations. The averages for HDC1 and NC treatments on D17 are 0.062 ± 0.003 ng l^{-1} and 0.045 ± 0.032 ng l^{-1}, respectively, and 0.073 ± 0.005 ng l^{-1} and 0.059 ± 0.039 ng l^{-1} for the HDC1 and NC tanks on D24.

3.1.4 Water Column Distribution Coefficients

Similar to the HDC2/LDC Experiment, the log K_d for HgT in the HDC1 and NC tanks were not significantly different from each other (Table 3.3). The log K_d's for MeHg were not significantly different for each treatment, in contrast with the previous experiments (Kim et al., in review) that showed a difference in water column K_d for MeHg between resuspended and non-resuspended treatments. The log K_d's for the HDC2/LDC Experiment were higher than the HDC1/NC Experiment, but all were within the same range as the previous mesocosm experiments (Kim et al., 2004). The differences between experiments were not significant for HgT, but they were significantly different for MeHg.

As with the HDC2/LDC Experiment, off-phase log K_d values were similar in both treatments for HgT and MeHg in the HDC1/NC Experiment (Table 3.3) indicating similar affinity to the particulate phase during the off-phase and on-phase.

Table 3.3: Average water column distribution coefficient (log K_d) and standard deviation for HgT and MeHg in the HDC1/NC Experiment.

	HDC1/NC Experiment	
	HDC	NC
HgT –on [a]	5.45±0.19	5.45±0.28
HgT –off [b]	5.61±0.49	5.41±0.37
MeHg –on [a]	4.41±0.25	4.38±0.23
MeHg –off [b]	4.50±0.21	4.81±0.47

a: On-cycle when mixing system was on.
b: Off-cycle when mixing system was off. Average and standard deviation of D17 and D24.

3.1.5 Mercury in the sediment

Table 3.1 shows the treatment average and standard deviation of HgT, MeHg, % MeHg and % organic matter for the HDC1/NC Experiment. There was only one core taken for the initial condition and no standard deviation is shown. Percent organic matter remained relatively constant down core, averaging 11.9±0.9% and 12.3±0.3% for the final sampling in the HDC1 and NC tanks, respectively and showed little change over time.

HgT in the sediment appears relatively constant down core and there was also little change in HgT concentration over the course of the experiment. There were no significant differences between treatments, over time, or down core for HgT concentration. MeHg concentrations appear to decrease slightly over the course of the experiment in the upper sediment sections, suggesting that there was overall net demethylation in these upper layers, however the differences were not significant. There were no significant differences between treatments or with depth. Percent MeHg also seems to decrease in the upper sediment sections in both the HDC1 and NC tanks, but again, the differences were not significant. However % MeHg is slightly lower than the

range of other systems, such as Lavaca Bay, Texas (0.65±0.34% for the upper 3 cm)
(Bloom et al., 1999).

3.2 Summary of Water Column and Sediment Concentrations in the Clam/No Clam and Clam Density Experiments

The two experiments, HDC1/NC and HDC2/LDC, suggest that clam density has the greatest impact on water column characteristics such as TSS, POM and Chl *a*. The HDC2/LDC Experiment most likely had a higher biomass of phytoplankton with the higher Chl *a* concentrations, higher % POM, and higher MeHg concentrations on the particulates. However, clam density does not appear to affect HgT in the dissolved or particulate phase of the water column. Dissolved MeHg concentrations were similar across both experiments and all treatments. Particulate MeHg concentrations and % MeHg, as mentioned above, were higher in the HDC2/LDC Experiment, most likely due to greater phytoplankton biomass in the water column. Distribution of Hg and MeHg within the water column was similar across experiments and treatments, indicating Hg's affinity for the particulate phase was not different. Sediment HgT and MeHg concentrations, as well as % MeHg and % organic matter, were similar between treatments and experiments suggesting that clam density had little impact on Hg cycling in the sediments over the course of the experiments.

As in the HDC2/LDC Experiment, simple mass balance calculations of inputs and outputs of MeHg suggest there was a net production of MeHg in the HDC1 treatments, but a net loss in the NC treatment. Again, this suggests that the higher density of clams may stimulate methylation.

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3.3 Mercury Bioaccumulation

3.3.1 Zooplankton

Total Hg concentration in zooplankton for the HDC1/NC Experiment is shown in Fig 3.4a. HgT concentration in zooplankton was not significantly different between treatments, or between time and treatments. Zooplankton in the Patuxent River water, added at the beginning of the experiment, was the source of zooplankton in the mesocosms for the duration of the experiment. Since the first sampling occurred on D10, the HgT concentration likely represents the *in situ* concentrations in the mesocosms and not the conditions in the Patuxent River. This is likely since a) the dominant zooplankton species, *Acartia tonsa* (Porter et al., submitted), reaches the adult stage in 14-16 days (Matias and Barata, 2004) and therefore the population has likely changed substantially during the first 10 days, and b) that studies have shown that small invertebrates reach a steady state concentration with their environment within a week (Lawrence and Mason, 2001). The little change in HgT concentrations may mask actual changes that are occurring in the different types of Hg, MeHg and inorganic Hg, as discussed below. The lower HgT concentrations in the middle of the experiment (D18, D25) correspond to higher zooplankton biomass (Fig 3.4c).
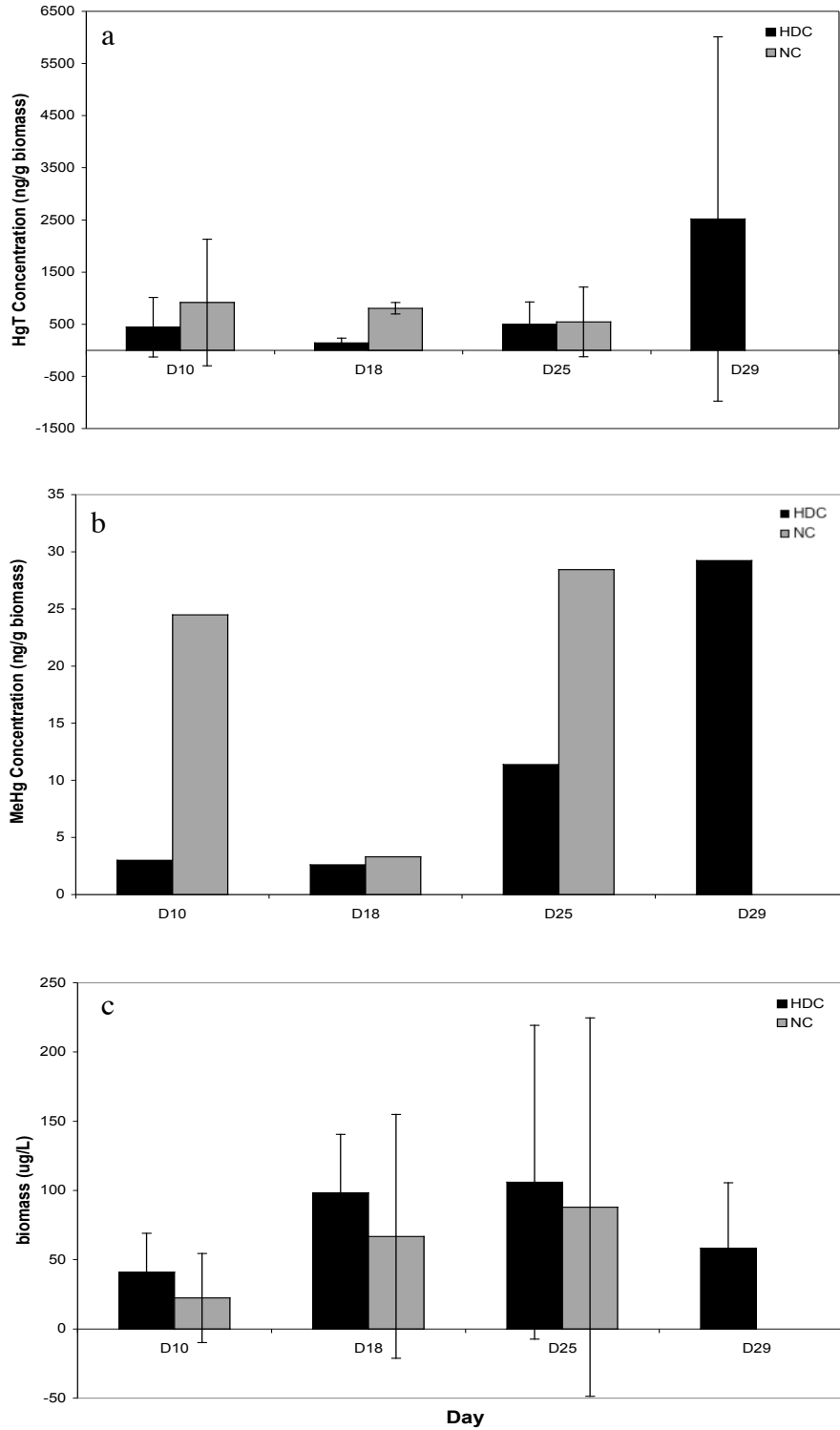
Figure 3.4: Zooplankton (>210 µm) data from the Clam/No Clam Experiment. (a) HgT concentration (b) MeHg concentration (c) Biomass. Error bars show standard deviation of three replicate tanks in each system.

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Similar to HgT, average zooplankton MeHg concentrations for HDC1/NC Experiment showed no significant difference between the two treatments (Fig 3.4b), but did show an overall increase in concentration with time, especially for the HDC1. Due to the low MeHg concentrations, the zooplankton samples from the three replicate tanks were combined for analysis, thus there are no error bars associated with the concentrations. Changes in zooplankton concentration did not mirror water column MeHg concentrations that were relatively constant over time and were not significantly different between treatments (Fig 3.3a, b).

The increase in MeHg in zooplankton and the relatively constant HgT concentrations suggest that over time, while the MeHg concentration is increasing, the inorganic Hg (HgT minus MeHg) is decreasing. This may be expected given that MeHg is more effectively stored within tissue, and has a much lower depuration rate than inorganic Hg (Mason, 2002; Mason et al., 2000a). Thus, if the inorganic Hg concentrations were higher initially, and were being diluted by growth effects during the experiments, then one may expect the trends in inorganic Hg and MeHg that are observed in the tanks, due to the specific conditions within the mesocosms.

Average % MeHg in the zooplankton of the NC tanks (2.76±2.40%) was higher, but more variable than the HDC1 tanks (1.49±0.71). The % MeHg values for zooplankton are 7 times higher than the % MeHg values for particles in the water column. In the NC treatment, the average concentration of MeHg was 1.6 times higher than the HDC1 treatment, but the zooplankton biomass is 1.3 times higher in the HDC1

tanks. This suggests that biomass dilution in the HDC1 tanks may have led to a slightly lower MeHg concentration.

In the HDC2/LDC Experiment, the zooplankton HgT concentration (Fig 3.5a) was not significantly different between the HDC2 and LDC treatments and there was no significant interaction with time. The higher HgT on D3 may represent the concentrations of the Patuxent River until growth, uptake, and depuration occur and concentrations reflect the conditions in the mesocosm. As with the HDC1/NC Experiment, the lower concentrations in the middle of the experiment (D9, 16, 23) correspond to higher zooplankton biomass for those days (Fig 3.5c).

As with the HDC1/NC Experiment, zooplankton samples from replicate tanks were combined for analysis and average MeHg concentrations were not significantly different between treatments. Again, zooplankton MeHg concentrations did not follow water column MeHg concentrations (Fig 2.3a, b). Average zooplankton % MeHg was higher than in the HDC1/NC Experiment, but more similar between treatments, although the LDC treatment (3.41±4.48%) was more variable than the HDC2 treatment (3.66±1.44%). The % MeHg values are 6.4 and 4.6 times higher in the HDC2 and LDC tanks, respectively, compared to the % MeHg in water column particles. The average concentration of MeHg in zooplankton in the HDC2 treatment was 1.5 times higher than the LDC treatment. However, the zooplankton biomass was only 1.1 times higher for the LDC treatment.
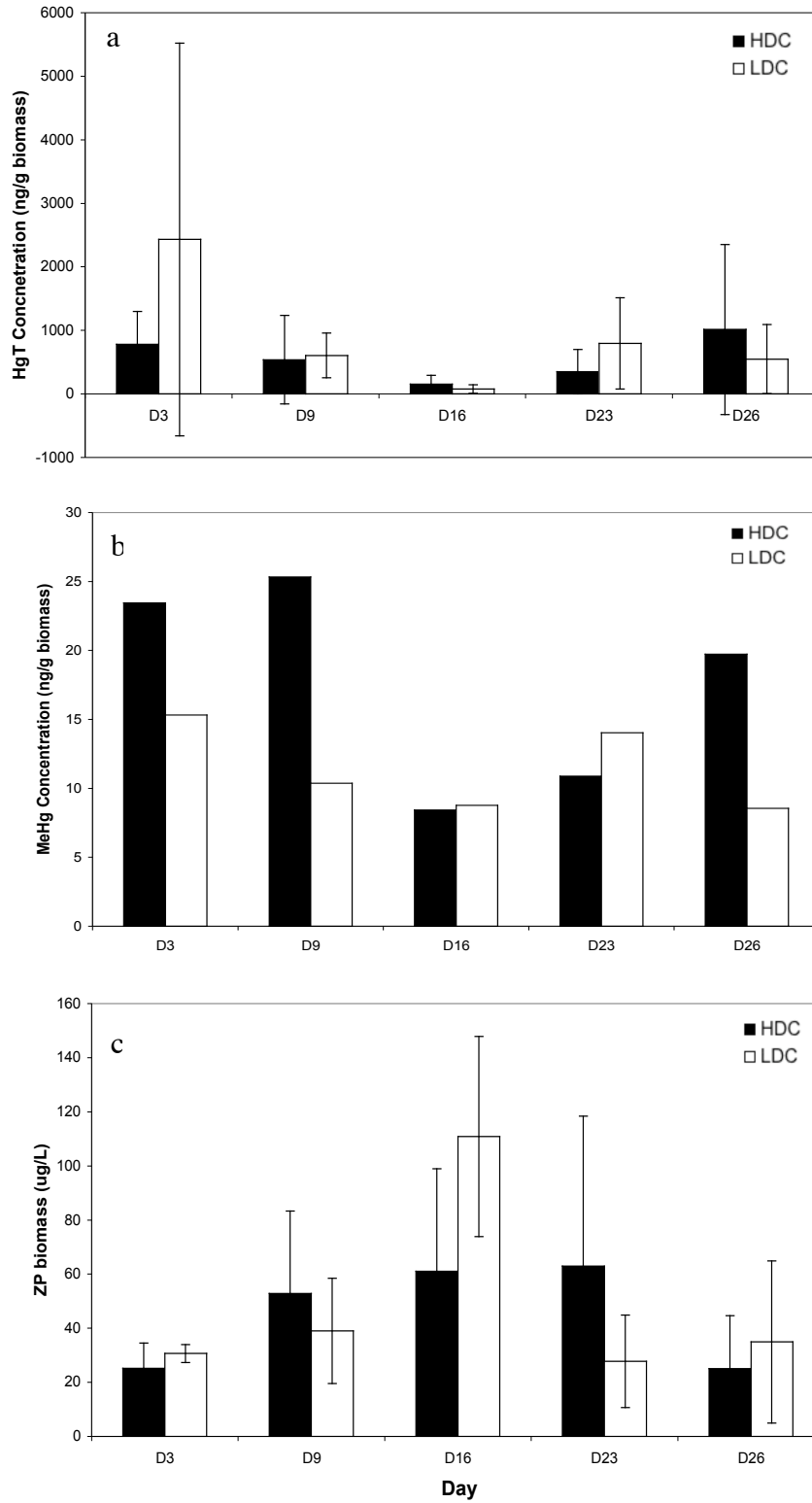
Figure 3.5: Zooplankton (>210 μm) data from the Clam Density Experiment. (a) HgT concentration (b) MeHg concentration (c) Biomass. Error bars show standard deviation of three replicate tanks in each system.

While Hg and MeHg concentrations were not measured in phytoplankton, biomass can be estimated from Chl *a* concentrations. These estimations show that phytoplankton make up 2-8% of the TSS in the water column. This is in agreement with the bioaccumulation model developed by Kim (2004) that found sediments accounted for a significant amount of POM in the water column, and 12% of POM, on average, was phytoplankton and zooplankton. As mentioned above, surface sediment and particulate HgT are similar in both experiments, however MeHg concentrations in the particulate are 2.5-6 times higher than surface sediment concentrations. This suggests that, while phytoplankton is a small percentage of the TSS in the systems, they have a higher MeHg concentration than the particles in the water column.

Percent MeHg in the zooplankton of both experiments is lower than that of other systems (Mason and Benoit, 2003 and references therein). This could indicate the zooplankton are not only feeding on phytoplankton, but also on other resuspended material. This was investigated by estimating the % MeHg in phytoplankton through the following equation:

$$\% \ MeHg_{phyto} = (\% \ MeHg_{part} - \% \ MeHg_{sed} \times fraction_{sed}) \ / \ fraction_{phyto}$$

Where the $fraction_{sed}$ is the amount of the particles in the water column that is resuspended from the sediment, $\% \ MeHg_{sed}$ is the % MeHg of the resuspended sediment particles, $fraction_{phyto}$ is the fraction of the total particle load that is phytoplankton, and $\% \ MeHg_{part}$ is the relative MeHg concentration of the total particle load in the water column. Since phytoplankton biomass estimations predict that 2-8 % of the TSS is phytoplankton, 5% was used as the fraction for the calculation. Thus the $fraction_{sed}$ is 95%. Percent

MeHg in the sediment was 0.15, on average, and MeHg concentration on the particles

was around 0.5 ng g^{-1}. Therefore, the % MeHg in phytoplankton would be approximately

7%. This is close to the % MeHg for the zooplankton, suggesting the zooplankton cannot

be solely feeding on phytoplankton otherwise their % MeHg would be much higher than

the value estimated for phytoplankton, as it has been shown in other studies that there is,

on average, about a factor of 3-5 increase in MeHg concentration per trophic level

(Mason, 2002).

The bioconcentration factor (BCF) is defined as the concentration of the

contaminant in the organism relative to the concentration of the medium in which it

resides. BCFs use tissue concentrations in the steady state to represent the maxiumum

level of accumulation that can be expected in an organism at a particular level of

exposure. BCFs were estimated for zooplankton for both HgT and MeHg (Fig 2.9 a, b)

using the following equation:

$$BCF = \log (C_b / C_w)$$

where C_b and C_w are the concentrations in biota and water, respectively, on a wet weight

basis.

The calculated BCFs were similar between treatments and experiments for both

Hg and MeHg (Fig 3.6 a, b). The BCFs for HgT are similar to other studies of

comparable organisms. Watras and Bloom (1992) found log BCF values ranging

between 4.8–5.2 for freshwater zooplankton for inorganic Hg. However, they found log

BCF values for MeHg close to 6.4, indicating that MeHg is bioconcentrated 10-100 times

higher than other Hg species, and MeHg becomes progressively more bioconcentrated

relative to the waterf or higher trophic level organisms. Results from Mason et al. (2000a)

also support this finding since log BCFs for stream invertebrates were 5-6 for MeHg.

MeHg's higher trophic transfer is most likely explained by the strong tendency to

accumulate in soft tissue, such as muscle, complexed to proteins (Mason et al., 2000a).
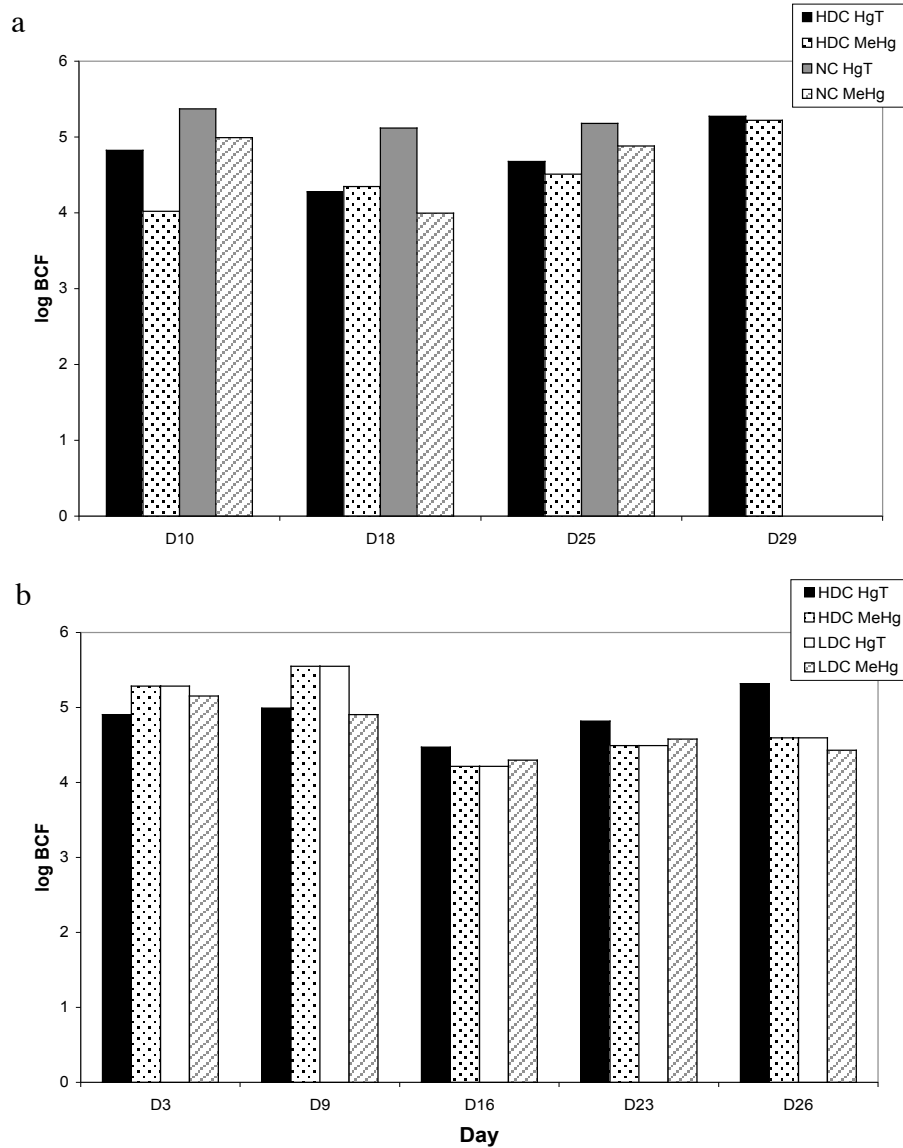

Figure 3.6: Log of the Bioconcentration Factors for HgT and MeHg in Zooplankton for (a) the Clam/No Clam Experiment and (b) the Clam Density Experiment.

MeHg in estuarine sediments is usually <1% of the total Hg and the BCFs for MeHg are about 10 times higher than inorganic Hg over environmentally relavent POC concentrations (Lawrence and Mason, 2001). It should be expected that MeHg would account for >10% of the total Hg in amphipods in the field, especially if the organisms were both filter and deposit feeding. However, field data from Baltimore Harbor and Lavaca Bay show % MeHg in amphipods much higher than predicted for benthic organisms. The MeHg BCFs for zooplankton in both experiments were on the same order of magnitude as the HgT BCFs. One possible explanation for the lack of difference in BCFs between total Hg and MeHg is the low % MeHg of the total Hg in our mesocosm systems, ranging from 1-4% for zooplankton, compared to zooplankton in other systems with a % MeHg range of 18-35% (Mason and Benoit, 2003 and references therein).

For Hg and MeHg, in both laboratory and field studies, the sediment BCF correlates best with POC (Lawrence and Mason, 2001; Mason and Lawrence, 1999). Sediment BCF (SBCF) is defined as the log (C_b / C_s) where C_s is the sediment concentration. Studies in Lavaca Bay, Texas (Bloom et al., 1999) and Chesapeake Bay (Benoit et al., 1998; Mason and Lawrence, 1999) have shown strong positive correlations between K_d and sediment POC for both Hg and MeHg, and a decrease in SBCF for benthic organisms with increasing POC. It has also been found that DOC plays an important role in bioaccumulation of Hg and MeHg. Dissolved concentrations in Hg and MeHg are often positively correlated with DOC, but negatively correlated with the BFC for phytoplankton, invertebrates, and fish (Lawrence and Mason, 2001; Mason, 2002). The Hg-DOC/POC relationships suggest that organic matter complexation makes Hg and

MeHg less bioavailable with increasing DOC/POC since the metal is more strongly bound to the organic matter (Mason, 2002).

Mason et al. (1996) suggests that Hg will be more strongly bound to POC than MeHg. Thus, at low POC, Hg and MeHg will mainly be bound to inorganic complexes and both are highly bioavailable. However, with high organic content, BCFs are small, indicating that both forms of Hg are tightly bound and relatively unavailable for assimilation (Mason, 2002). These findings support the low bioaccumulation observed in this study since the sediment used from Baltimore Harbor has a relatively high POC.

3.3.2 Clams

The concentration of HgT, MeHg and % MeHg in clams for the three mesocosm experiments (2, 3 and 4) are shown in Table 3.4. In the HDC1/NC Experiment, which compared systems with clams to those with no clams, the HgT and MeHg concentrations significantly increased in the HDC1 tanks compared to the initial concentrations. On average, the MeHg in the clams accounted for 41±8 % of the HgT concentration.

In the HDC2/LDC Experiment, the clams in the HDC2 treatment had a significantly higher concentration of HgT than the LDC treatment, possibly due to the greater TSS in the water column. However, neither treatment was significantly different from the initial clams. There were no differences in MeHg concentrations between treatments or with the initial clams. Both experiments had similar HgT and MeHg concentrations to the second Resuspension Experiment where Kim et al. (in revision) found clams had a significant increase in HgT in the resuspension tanks, but did not observe a significant increase in the non-resuspension tanks. However, MeHg concentrations increased in both the resuspension and non-resuspension treatments.

While the increase was significant it was relatively small (~20%). Given the larger variability in concentration between clams/tanks in the different experiments, such a small change may not have been discernible. Both experiments in this study yielded lower % MeHg values than the second Resuspension Experiment (Kim et al., in revision), however all experiments fall within the range for invertebrates where MeHg generally accounts for 20-80% of HgT (Claisse et al., 2001). The differences in %MeHg are driven mostly by the changes in the HgT between batches of clams, which were much more variable than that of MeHg, and likely reflects differences over time in the culture facility from which the clams were obtained.

The average water temperature and salinity for both experiments (Table 2.1 and 3.1) were within the range where there are generally no negative effects on pumping rate and growth of clams (Grizzle et al., 2001), however over the duration of the experiments, little clam growth was observed. In the HDC1/NC Experiment, clams grew 0.11±0.17 g in total live weight (0.5% of their total weight). None of the clams died and they were found to actively biodeposit (Porter et al., submitted). The energy budget equation for heterothrophic organisms has been described as (Grizzle et al., 2001),

$$\text{Ingestion} = \text{Growth Rate} + \text{Metabolic Rate} + \text{Egestion} + \text{Excretion}$$

Since we observed slight growth, no mortality, and some biodeposition, the first three variables are either positive or zero, indicating food must have been ingested by the clams in order to maintain or gain weight and overcome energy costs such as respiration. However, due to the minor change in weight, they may have been slightly food limited. If the clams were not feeding then they would have lost weight.

In the HDC1/NC Experiment, clam gape sensors were used to determine if the clams were feeding at the high TSS concentrations since previous laboratory research suggested that *M. mercenaria* does not feed at TSS levels above 44 mg l^{-1} (Grizzle et al., 2001). The results show that clams were open during the on-phase (62% of the time) (Porter et al., submitted). In addition, Chl *a* concentrations, an indicator of phytoplankton biomass, were reduced in the HDC1 system compared to the NC system, suggesting active phytoplankton removal by the clams (Fig 3.1c).

A bioenergetics-bioaccumulation model was developed for the mesocosm resuspension systems to examine carbon and contaminant flow through the food chain (Kim, 2004). The model was able to predict measured changes in phytoplankton, zooplankton, and clam biomass throughout the HDC1/NC Experiment. Although little clam growth was observed, clam biomass was predicted to change only slightly during the 4-week experiment, decreasing from 10.5 to 10.01 g carbon m[-3] (4.7%). Without active feeding, the model predicted that clam biomass would have decreased from 10.5 to 7.3 g carbon m[-3] wet weight (30%). The measured and modeled data suggest that the clams must have been feeding during the course of the mesocosm experiments, despite the high TSS levels (Porter et al., submitted).

In the HDC2/LDC Experiment, clams in both the HDC2 and LDC treatments grew more than the HDC1/NC Experiment, averaging 1.2±0.1 g (5.6% of their total weight) and 2.3±0.5 g (11.8% of their total weight), respectively. The little growth and lower phytoplankton biomass in the HDC1/NC Experiment indicates that food was probably limited compared to the HDC2LDC Experiment. Clams in the LDC tanks of the HDC2/LDC Experiment grew more, providing evidence that the HDC2 tanks were more

food limited in comparison to the LDC tanks.   This is further supported by comparing

the biomass of phytoplankton and zooplankton normalized to POM for the two

experiments (Fig 3.7a, b). In the HDC1/NC Experiment, phytoplankton biomass in the

NC treatment was much larger than the HDC1 treatment. The average zooplankton

biomass in the NC treatment was also higher than in the HDC1 treatment, presumably

since they did not have to compete with the clams for food. In the HDC2/LDC

Experiment, the phytoplankton biomass was higher, on average, in the LDC tanks than

the HDC2 tanks. The zooplankton biomass was similar between treatments, indicating

that the zooplankton were competing with the clams for food since the clams grew more

in the LDC tanks.

Table 3.4: Average concentrations of HgT, MeHg, and % MeHg in clams, *Mercenaria mercenaria*, with standard deviations of replicate tanks at the beginning and end of experiments.

	Treatment	HgT (ng g^{-1}, dry)	MeHg (ng g^{-1}, dry)	% MeHg
Resupension Experiment 2*	**Initial**	36.1	26.1	72
	RC	50.2 ± 8.0	32.1 ±1.2	65 ± 11
	NRC	46.1 ± 8.0	30.1 ± 2.0	64 ± 5.9
HDC1/NC Experiment 3	**Initial**	106	32.7	30.8
	HDC	103 ± 22.1	50.5 ± 12.3	44.0 ± 4.5
HDC2/LDC Experiment 4	**Initial**	56.6 ± 20.3	28.4 ± 9.3	56.8 ± 36.8
	HDC	66.4 ± 4.9	29.0 ± 5.0	44.9 ± 4.4
	LDC	51.7 ± 1.4	25.9 ± 18.0	50.7 ± 36.8

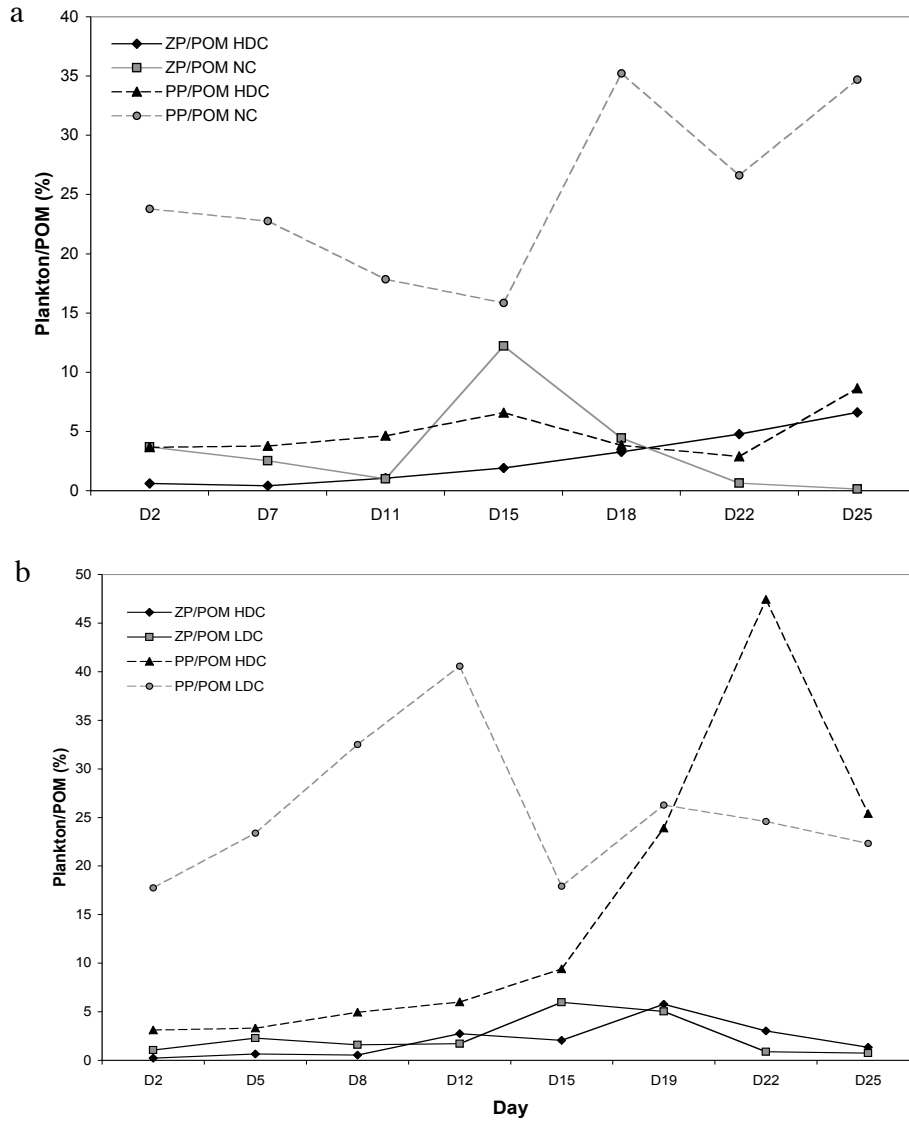* Data taken from Kim et al 2005 (RC= reuspension, clams; NRC= no-resuspension, clams)

Figure 3.7: Zooplankton and phytoplankton biomass normalized to POM in (a) the Clam/No Clam Experiment and (b) the Clam Density Experiment.

3.3.3 Bioaccumulation Model

Kim (2004) developed a carbon-based, multi-compartment bioaccumulation model for MeHg within a shallow estuarine system subject to resuspension to investigate the most important parameters controlling MeHg bioaccumulation into benthic and pelagic organisms. In previous mesocosm experiments, Kim (in revision) found MeHg concentrations in biota were not different between the resuspension and non-resuspension systems, but food availability and ingestion rates were important factors influencing the accumulation of MeHg into zooplankton and clams. The model was developed to investigate these interactions and processes in order to examine indirect effects of MeHg bioaccumulation. The model was calibrated and best fit to the observed data from the second Resuspension Experiment, and was then used to simulate other conditions.

From the results of the second Resuspension Experiment, it was concluded that resuspension did not increase dissolved MeHg, suggesting that MeHg desorption was not important (Kim et al., 2004). However, the model results showed that an increase in sediment MeHg, when resuspended, resulted in higher dissolved MeHg. Thus, MeHg in phytoplankton increased and led to higher MeHg in herbivores. Changes in the methylation rate had a greater effect on the MeHg burden in zooplankton than in clams. Since clams are the dominant biomass in the system, and given the amount of MeHg in the system, the clams would show little if any effect on their MeHg burden unless there was a substantial change in sediment methylation rate, or there was a longer time of exposure.

The model was run using inputs from the data in the HDC1/NC Experiment to establish how well the model could simulate these conditions. The HDC1/NC

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Experiment with the HDC1 and NC treatments was similar to the resuspension treatment of the second Resuspension Experiment. However, the HDC1/NC Experiment was conducted in the summer with a higher average temperature (26°C) than the Resuspension Experiment, conducted in October (20°C), which could affect the methylation rate, and other system parameters. The initial biomass of phytoplankton (PP), zooplankton (ZP) and microphytobenthos (MPB) were used from the data in the HDC1/NC Experiment. The clam (FF) biomass was the same as the second Resuspension Experiment. Initial conditions for dissolved MeHg, DOC, POC, and nutrient data were also used from the HDC1/NC Experiment.

The model results of phytoplankton biomass were in relatively good agreement with the data from the HDC1/NC Experiment in the beginning of the model run, however, the model failed to simulate the phytoplankton bloom later in the experiment (Fig 3.8a). Again, there was better agreement with the observed data in the early stage of the model for the zooplankton biomass (Fig 3.8a). The standing stock of phytoplankton was lower in the HDC1/NC Experiment compared to the HDC2/LDC Experiment and the first Resuspension Experiment, conducted in the summer, indicating that biomass was kept low by the competition between zooplankton and clams for limited food. This was supported by the model, since zooplankton biomass varied with phytoplankton biomass. Clam biomass, in the model, did not change substantially even with the depleted phytoplankton biomass at the end of the model run (Fig 3.8b). This agrees with the observed data that there was little clam growth over the course of the HDC1/NC Experiment.

MeHg in zooplankton showed a continuous increase over time in the modeled data. However, the observed data are at the low end of the model results (Fig 3.8c). The discrepancy between the modeled and observed results is likely the result of the fact that the model did not simulate the phytoplankton bloom later in the experiment and the corresponding increase in zooplankton (Kim 2004). The MeHg concentration in phytoplankton would have decreased as phytoplankton biomass increased, as supported by Pickhardt et al. (2002) that found algal blooms reduce MeHg uptake in zooplankton since increasing algae biomass decreases MeHg accumulation. Fig 3.8d shows the modeled results for MeHg in clams increased slightly (37%) during the course of the experiment and was in good agreement with the observed data.

Other model simulations were run to examine the effect of clams on phytoplankton and zooplankton biomass and MeHg accumulation. These conditions were similar to the HDC2/LDC Experiment. However, the modeled and observed data cannot be directly compared since initial inputs to the model were from the HDC1/NC Experiment and the low density clam biomass was modeled at 50% of the high density biomass while the HDC2LDC Experiment used 20% of the high density biomass. Regardless, changes in clam biomass had a significant impact on phytoplankton biomass, especially in the beginning of the model run (Fig 3.9a). This is in agreement with the Chl a concentrations (Fig 2.1c) and phytoplankton biomass estimates (2.75 mg l^{-1} for the HDC2 treatment and 4.04 mg l^{-1} for the LDC treatment) for the HDC2/LDC Experiment. Zooplankton biomass also increased with decreasing clam biomass (Fig 3.9b) most likely since phytoplankton became more available to zooplankton as clam biomass decreased. There was little difference in zooplankton biomass between the two treatments, as seen in

Fig 3.5c, however, the figure just describes the biomass for the >210μm size class while the model takes into account the smaller zooplankton (> 63-210 μm) as well. These results indicate that the effect of changes in clam biomass on zooplankton is indirect. Changes in MeHg burden in phytoplankton and zooplankton are not as greatly impacted by changes in clam biomass as that of plankton biomass (Fig 3.9c, d). This is in agreement with the observed data since there was not a significant difference in MeHg concentrations in zooplankton between the HDC and LDC treatments. Kim (2004) found that MeHg burden in phytoplankton was governed more directly by dissolved MeHg uptake rate and phytoplankton growth than to clam filtration rate. Similarly, MeHg in zooplankton was less affected by changes in clam biomass compared to phytoplankton biomass.

From sensitivity analyses, Kim (2004) found that phytoplankton population growth rate was a highly sensitive parameter influencing zooplankton biomass, but was not as sensitive a parameter in terms of clam biomass. Filtration rates of clams also had a great impact on plankton biomass and thus MeHg burden in biota. Since the zooplankton biomass was two orders of magnitude less than the clam biomass, it was more sensitive to changing parameters.

In conclusion, the model results suggest that sediment resuspension can play a role in transferring elevated MeHg on particles to the water column, thus increasing dissolved MeHg. Also, MeHg accumulation in plankton increases as uptake rate of dissolved MeHg by phytoplankton increase. The MeHg accumulation in clams is less affected by varying MeHg uptake rate by phytoplankton due to the larger biomass relative to phytoplankton and zooplankton.
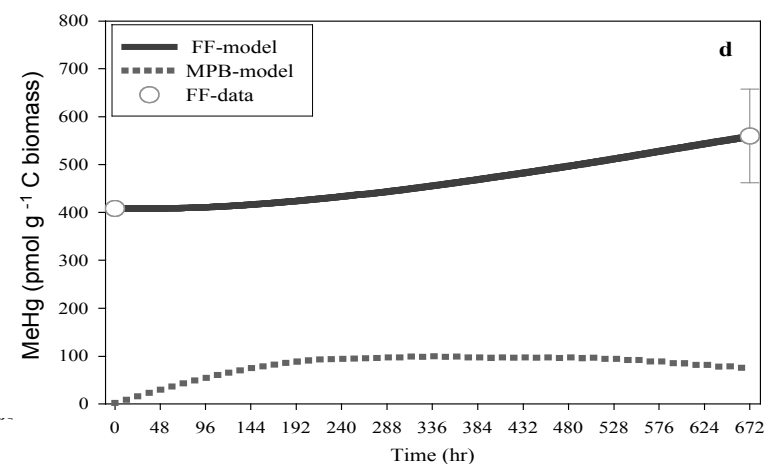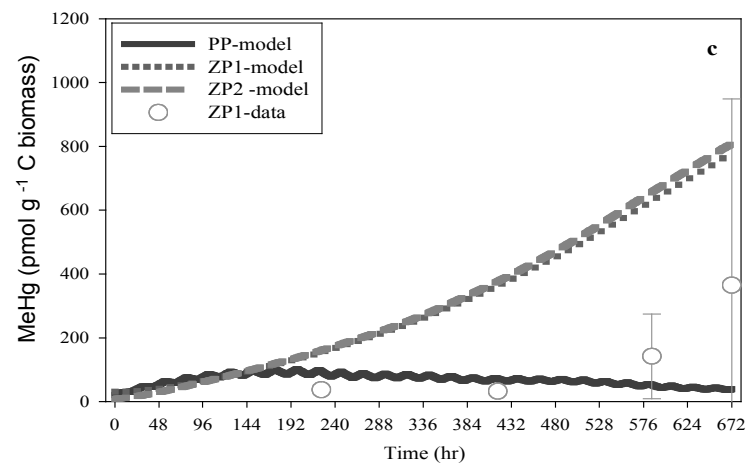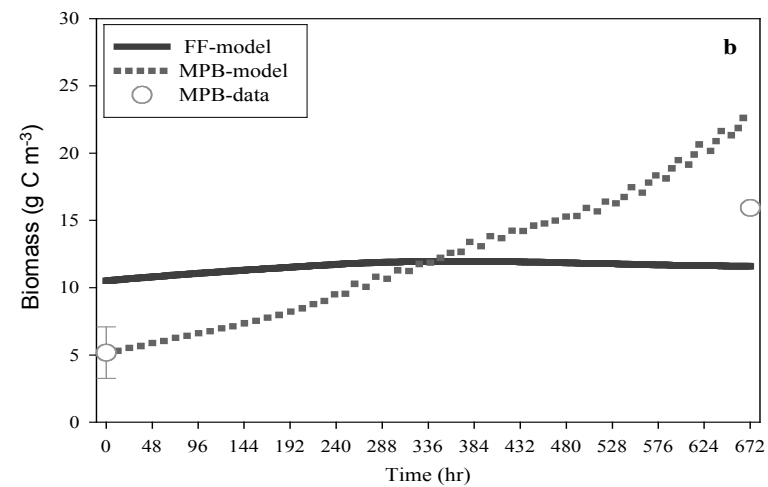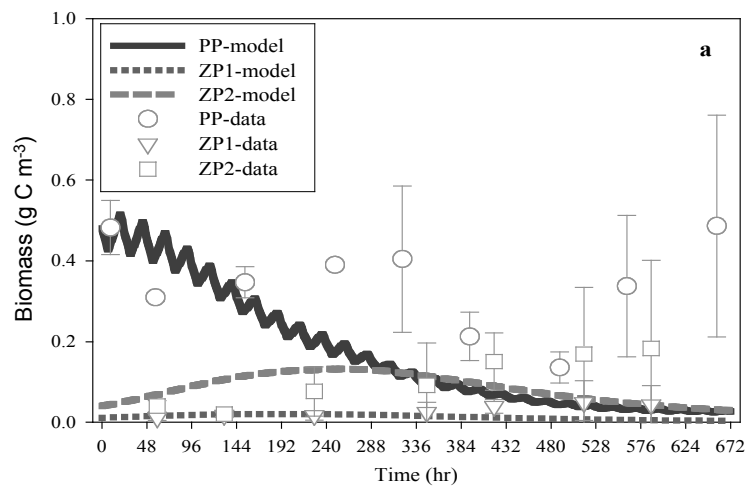
Figure 3.8: Model outputs for the the Clam/No Clam Experiment. (a) biomass in the water column (b) biomass in the sediment (c) MeHg in water column biota (d) MeHg in benthic biota. Error bars show the standard deviations of three replicate tanks. Data taken from Kim (2004).
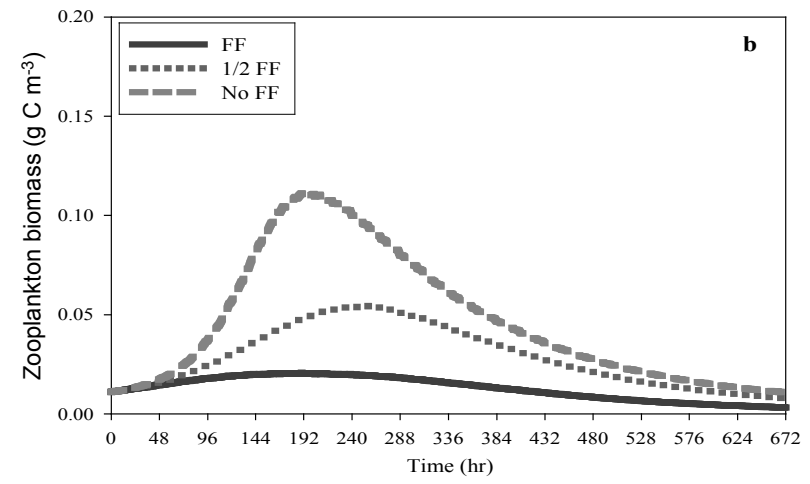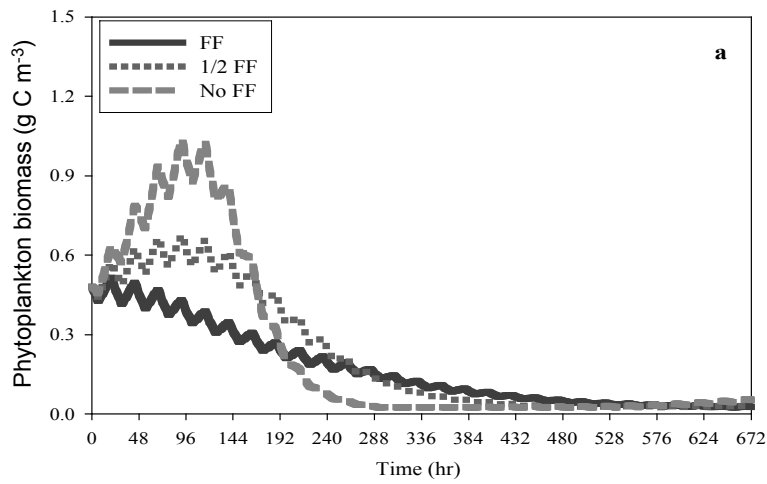
Figure 3.9: Effect of clam biomass model outputs. (a) Phytoplankton biomass (b) Zooplankton biomass (c) MeHg in phytoplankton (d) MeHg in zooplankton. The model run with clam (FF) biomass used in Experiments 2 and 3; 1/2 clam biomass; no clams. Data taken from Kim (2004).

3.3.4 Summary

Clam density did not appear to have an effect on Hg cycling or MeHg bioaccumulation into either zooplankton or clams for the duration of the experiment. Clam density did impact water column characteristics, 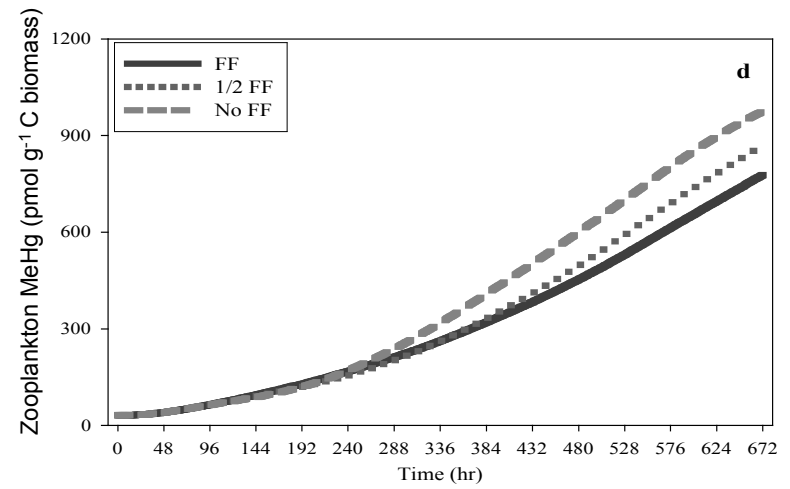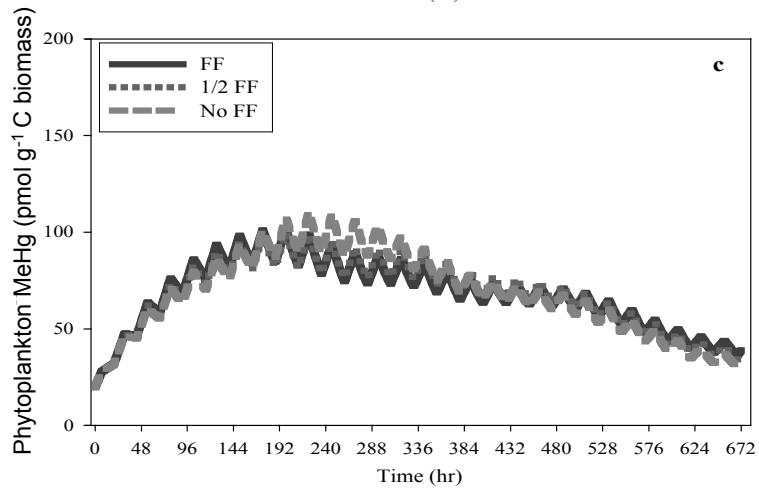such as phytoplankton biomass, and potentially phytoplankton growth rate. This could indirectly affect MeHg burden in phytoplankton and thus trophic bioaccumulation through a 'dilution effect' (Pickhardt, 2002). For example, the phytoplankton biomass was higher in the HDC2/LDC Experiment compared to the HDC1/NC experiment. In the HDC1/NC Experiment, there was little clam growth, but an increase in MeHg in the clams of HDC1. On the other hand, clam growth was significantly higher in the HDC2 and LDC treatments of the HDC2/LDC Experiment, but the were no significant differences in MeHg concentrations.

Chapter 4: Conclusions and Recommendations

4.1 Conclusions from the Clam/No Clam and Clam Density Experiments

The fate of Hg in estuaries is important in understanding the global Hg biogeochemical cycle, since estuaries link the terrestrial and marine environments. Only a small fraction of Hg transported in rivers is exported to the ocean due to the high retention of Hg in the estuarine environments with sedimentary removal as the primary sink (Mason et al., 1999). While under certain conditions the flux of dissolved MeHg from sediments may provide an important mechanism for transport of MeHg from sediment pore waters, the physical mechanism of mixing of the sediments may provide an additional vector for MeHg entry into the water column and therefore into the food web, both through organisms feeding at the sediment-water interface (Sunderland et al., 2004), and from uptake of the MeHg transferred to the water column by biota.

In previous experiments, it was found that sediment resuspension enhances Hg methylation in the sediment and plays an important role in transferring sediment MeHg to the water column, resulting in an increase of MeHg bioaccumulation (Kim, 2004). Sunderland et al. (2004) also found, in the well-mixed sediment of the Bay of Fundy, MeHg production occurs throughout the active sediment layer, not just at the oxic-anoxic boundary, suggesting that physical mixing in this location enhances the transfer of sulfate and carbon to depth and introduces more bioavailable inorganic Hg into the deeper sediments, potentially stimulating methylating bacteria.

The overall hypothesis for this research stated that, while the biological and chemical interactions are complex, the bioaccumulation of MeHg into filter feeders will decrease as filter feeder density increases under tidal resuspension conditions, due to the

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impact of the increase bivalve biomass on the phytoplankton standing stock and growth rate, and as a result, on the concentration of MeHg in the phytoplankton. The objectives of this thesis research were to investigate, and confirm or refute the hypothesis, and associated sub-hypotheses, by examining the effect of tidal resuspension on Hg cycling and the bioaccumulation of sedimentary Hg and MeHg to benthic organisms. These objectives were reached through examining the impact of filter feeder density on the bioaccumulation of Hg and MeHg, as well as using a Hg stable isotope addition to trace the cycling of Hg and MeHg through the system in more detail.

Two mesocosm experiments were conducted in July 2002 and August 2003 with two treatments each. The mesocosm system consists of six 1000L tanks with one m^2 sediment surface area, and a mixing system designed to generate uniform and realistic sediment resuspension without producing excessive water column turbulence (Porter et al., in prep). Sediment from Baltimore Harbor, MD was collected for the experiments and the hard clam, *Mercenaria mercenaria*, was used as a representative benthic filter feeder. The two treatments were compared in triplicate during the four-week experiments. The Clam/ No Clam Experiment compared resupension with clams (HDC1) with resuspension without clams (NC). The Clam Density Experiment compared resuspension with a high density population of clams (HDC2), similar to HDC1 of the Clam/No Clam Experiment, with resuspension with a low density population of clams (LDC).

The first hypothesis stated that differences in biota densities would change the amount of MeHg bioaccumulated up the food chain because of so-called 'bio-dilution effects'. Specifically, it was postulated that the MeHg concentration in clams and zooplankton would decrease with increasing clam density. The results of this study do

not however confirm the hypotheses, although it is similarly difficult to refute the hypothesis based on the study's results. Clam density did not appear to have an effect on MeHg bioaccumulation for the duration of the experiments into either the zooplankton or clams.

Clam density did impact phytoplankton biomass. Both HDC treatments had lower phytoplankton concentrations compared to the NC and LDC treatments, especially in the beginning of the experiment (Fig 2.10a, b). There was significantly lower dissolved inorganic nitrogen (DIN) (data not shown) in the NC treatment compared to the HDC1 treatment of the Clam/No Clam Experiment, which could suggest more removal by phytoplankton growth. DIN was also higher in the LDC treatment of the Clam Density Experiment than the HDC2 treatment, but the difference was not significant. The data show an increase in Chl a (Fig 2.1c, 2.4c) with decreasing clam density, suggesting a lower growth rate of phytoplankton if nutrients were limited, thus there is potentially higher phytoplankton growth rate at higher clam densities. This supports the proposed 'dilution effect' where biomass-specific concentrations of metals diminish as cells divide in rapidly growing phytoplankton resulting in a decrease in MeHg burden in biota (Pickhardt, 2002). Since the biomass of clams is so large, Kim (2004) suggests, based on her model results, that a substantial change in sediment methylation or a longer exposure time would have been required to have a significant impact on MeHg burden in the clams. Therefore, it is reasonable to conclude that while the differences in clam densities did not have a substantial impact on the concentration of MeHg in the secondary consumers, the experimental design was insufficient to allow a conclusive statement to be

made on the potential for phytoplankton growth rate to impact MeHg burdens in these organisms.

Secondly, it was hypothesized that there would be increased methylation in the sediment with an increase in clam density since clam density impacts sediment resuspension as clams destablize the sediment, and it was concluded by Kim et al. (in revisions) that sediment resuspension enhances Hg methylation. Sediment Hg methylation was observed through changes in sediment MeHg and % MeHg, as opposed to methylation rates, as it has been shown that these measures are correlated for estuarine sediments (Heyes et al., in press; Kim et al., in revisions). Significant differences in MeHg were not observed between the initial and final concentrations, or between treatments in the final sediment concentrations of the experiments. There were no significant differences in dissolved or particulate MeHg in the water column, as well. MeHg concentrations appear to have decreased in the surface sediment over the course of the Clam/ No Clam Experiment, indicating net demethylation. However, the initial core was not taken from the experimental mesocosms (see Chapter 1), so any variations between tanks are not represented in the initial concentration.

On the other hand, using the mass balance of inputs and outputs of MeHg for the mesocosm tanks, a net production of MeHg in the water column was calculated in the HDC treatments of both experiment 3, but not the NC or LDC treatments. Thus, these results support the hypothesis that increasing clam density leads to higher net methylation. It is likely that the higher density of clams increased the oxygenation of the sediment which reduces sediment acid volatile sulfides (AVS) and pore water sulfides. This environment can improve methylation by enhancing Hg bioavailability to bacteria

(Benoit et al., 1999; Hammerschmidt et al., 2004). However, the magnitude of increase in MeHg is not likely reflected in the sediment due to the high variability between replicate tanks, and the relatively small response.

Others have suggested that bioturbation in organic-rich sediments can enhance Hg methylation in the sediment (Benoit et al., in press). However, this might not be true for all sediments. For example, more oxygenation in sediments with low organic matter content might decrease methyaltion. Sulfate-reducing bacteria require low oxygen conditions and organic matter to covert inorganic Hg to MeHg. However, with higher bacterial activity there is greater reduction, and thus, a higher sulfide concentration. Hg is thought to be available to methylating bacteria through the neutral sulfur species (HgS^0), thus the sulfide concentration in the porewater control the Hg speciation of these complexes (Benoit et al., 1999). If sulfide levels become too high, the sulfide complexes are charged and become less available to the bacteria and methylation decreases. Oxygen reduces sulfide in the porewater, encouraging the neutral species, however, too much oxygen means there is no sulfide available to the bacteria.

The third hypothesis was that Hg would rapidly be transferred (within weeks) from the water column to the methylation zones in the sediment due to the particle reactivity of Hg and sediment resuspension events. The use of the Hg stable isotope in the Clam Density Experiment allowed us to observe that the Hg spike quickly associated with particles and was exponentially lost from the water column, most likely to the sediment. Although it was below quantifiable detection, we did observe the overall net methylation of the Hg spike, manifested as the presence of MeHg in the water column

particles, over the course of the experiment, suggesting the isotope was rapidly transferred to the sediment and methylated in the system.

Based on the methylation and demethylation rate constants of Kim et al. (in revision), $\sim 3 \times 10^{-2}$ d^{-1} and 16 d^{-1} respectively, for the mesocosm studies in the Resuspension Experiments (1 and 2), the Hg should have been methylated rapidly once it was transferred to the zone of methylation. The rapid removal of the Hg isotope from the water column suggests that it was transferred to depths below the resuspension layer within several days. Thus, the isotope was likely transported to the zone of methylation that is expected to occur below the actively resuspending surface layer. However, it is not the rate of methylation that is being measured with the experimental setup but the steady state MeHg concentration, which is equivalent to the ratio of the rate constants for a reversible first order reaction. This ratio is about 2×10^{-3} based on the rate constants above, or in terms of the Hg spike, about 30 ng of methylated spike. If the spike were spread over a depth of 1 cm of sediment, this would be equivalent to 1% or less of the *in situ* MeHg inventory. This is close to the detection capabilities of the instrumental method, and therefore undetectable. Thus, the lack of detection of the isotope in the sediments does not negate its rapid removal from the water column and its methylation. Overall, based on the results in terms of isotope in the various compartments of the mesocosm, it can be concluded that the transfer of newly added Hg to the zones of Hg methylation is rapid, on the order of days to weeks, for dynamics systems such as mimicked by the mesocosms. Therefore, any changes in Hg entering the system will be relatively rapidly reflected in the food chain of the system. Thus, reductions in loadings

of Hg to such a system should lead to a relatively rapid response in terms of the biota MeHg burden.

We had expected the results of the Hg isotope methylation and the redistribution of the Hg isotope between the dissolved and particulate phases in the water column, and in the sediments and biota, to provide estimates of the rates of various processes of Hg and MeHg cycling in the system. This information could be used to update and further evaluate the model developed by Kim (2004). However, since the methylated isotope was not observed in measurable quantities in most compartments, we could not use it to validate Kim's MeHg bioaccumulation model.

The last hypothesis states that food chain interactions are as important in determining MeHg concentrations in herbivores as biogeochemical processes. In the Clam/No Clam Experiment we observed an increase in MeHg in the clams of the HDC1 treatment, however clams growth was minute (0.5% of their total weight). In the Clam Density Experiment, there were no significant differences in MeHg concentrations between treatments or over time, however, the clam growth was significantly higher (5% and 11% of their total weight for the HDC2 and LDC tanks, respectively) than the Clam/No Clam Experiment. Since phytoplankton biomass was higher in the clam Density Experiment compared to the Clam/No Clam Experiment, the results suggests a 'dilution effect' with increasing phytoplankton biomass in the Clam Density Experiment.

Kim's (2004) modeling study confirmed the 'dilution effect' that increasing biomass resulted in a decrease in MeHg burden in biota. Overall, the model predicts that biomass and MeHg burden in biota are highly sensitive to varying phytoplankton production and the filtration rates of clams, which were the dominant biomass in the

system. However, the model predicts, despite the impact of clams on biomass of plankton, that the MeHg burden in plankton and zooplankton was governed more directly by the uptake rate of dissolved MeHg and plankton growth rate, than by other parameters. This conclusion is supported by the studies of Chen and Folt (2005) who found that differences in plankton density explain a significant amount of variation in Hg accumulation by fish across lakes, specifically that trophic transfer of Hg through the food web was reduced when phytoplankton and zooplankton density were high.

Overall, this study has provided further evidence that the biological and chemical interactions of Hg cycling and bioaccumulation is complex. Bioturbation by the clams may be as important as resuspension in increasing Hg methylation in the sediment. The clams in the Clam/No Clam Experiment increased the TSS concentrations by a factor of 9 compared to the treatments with no clams in the beginning of the experiment. Similarly, the higher density of clams in the Clam Density Experiment increased the TSS close to 3 times the low density of clams in the beginning of the experiment. However, differences in clam density had little impact on bioaccumulation of MeHg in benthic clams or pelagic zooplankton in these experiments. The duration of the study, as well the little relative change in MeHg concentration, may have limited our results from the experiment.

4.2 Recommendations for Future Research

The results from this study, along with small-scale incubations (Benoit et al., 2003; Heyes et al., in press), larger-scale mesocosm (Kim et al., in revision; Orihel et al., 2004) and ecosystem (Gilmour et al., 2004b; Hintelmann et al., 2002) studies, as well as food web studies (Pickhardt, 2002), show the power of using stable isotopes for Hg

research. One major advantage of using Hg isotopes is that the increase in Hg concentration does not have to be significant compared to the background Hg concentration in order to detect the addition. Also, multiple isotopes can be used within the same system to observe different processes. However, one disadvantage to using Hg isotopes is it may be unclear whether the newly added Hg is behaving in the same way as Hg in the environment would behave.

Our goal in adding the Hg isotope as a tracer was to clarify the sources and flows of MeHg in the system since cycling of Hg and MeHg was unclear in the previous experiments due to little changes in Hg concentrations over time. We were able to see the Hg spike in most compartments of the system, except the sediment and zooplankton. This showed, while the overall concentrations in the system did not change in a dramatic way, it did indicate that there were interactions. The system reached a steady state relatively rapidly, in terms of biogeochemical processes, compared to the timescale of the experiment. Conversely, the experimental duration did not appear long enough to observe the food chain bioaccumulation.

Little methylation of the isotope in the system was observed. However, the bioaccumulation model developed by Kim (2004) demonstrated that increasing Hg methylation resulted in higher MeHg burdern in the biota. One recommendation would be to use sediment with a greater methylation potential that would most likely increase the methylation rate and differences in MeHg concentration, and allow for a better comparison of effects between treatments.

Another recommendation for future studies would be to increase the isotope concentration added to the system in order to have a better chance at seeing the Hg spike

in all compartments and have a higher concentration of methylated spike in the system. Using the same calculation as described in Chapter 2 to determine the percent isotope compared to the background Hg concentration, doubling the added amount to 20 μg would raise the concentration to 0.7%, which is above the detection limit for analysis. This would not be an unreasonable increase since Mason et al. (1997) found wet deposition rate of 25 μg Hg m^{-2} y^{-1} at Hart-Miller Island near Baltimore. Also, the METALLICUS project, investigating whole ecosystem Hg processes, has added 3-4 times the annual deposition of Hg on the watershed in order to study the effect of Hg loading on the system (Gilmour et al., 2004a).

However, increasing the Hg concentration does not necessarily mean the methylation rate will increase since there are many variables, such as temperature and organic matter, which can affect the bioavailablity of Hg to methylating bacteria. Thus, experiments loading different amounts of Hg isotope to the mesocoms would be interesting in order to see how Hg concentration in the mesocosm system affects methylation rate and the resulting bioaccumulation into organisms. This information could be used to further refine the model developed by Kim (2004).

The mesocom experiments help to control the variability associated with field studies while still mimicking complex systems. Since there are many indirect effects on the cycling and biaccumulation of Hg that small-scale laboratory studies cannot include, the mesocosm approach can be informative. From the results of the study and the model developed by Kim (2004), longer-term experiments would be potentially helpful in understanding bioaccumulation in the system. However, since the system reached steady state relatively quickly, more sampling in the beginning of the experiment, especially

after the Hg spike addition, would provide a better understanding of the initial biogeochemical processes of 'new' Hg entering the system. More frequent and finer scale sampling of the sediment would be useful in detecting the Hg isotope, as well.

The impact of different levels of resuspension on Hg cycling and bioaccumulation would also be interesting to examine. The implications of storm or dredging events could be investigated with higher levels of resuspension. Lower levels of resuspension may favor bioaccumulation in the clams since there is a debate about their feeding rate at high levels of TSS (Grizzle et al., 2001; Porter et al., submitted). Also, the LDC tanks, with lower TSS, retained more of the isotope in the water column over the course of the experiment. Though the mesocosm system required muddy sediments, investigating different sediments, such as varying organic matter would be another interesting experiment.

Since food chain length is also important in the bioaccumulation of MeHg (Mason, 2002), using different organisms in the system could be valuable in examining food chain dynamics. Replacing the clams with small fish that would eat the zooplankton would give insight into bioaccumulation in the third trophic level.

The mesocosm experiments suggest that resuspension favors enhanced Hg methylation in the sediments, however, sediment resuspension does not appear to be a substantial menchanism for introducing dissolved MeHg into the water column due its high association with particles. Environments with increased resuspension and thus, increased methylation, may still have organisms with higher MeHg burdens if they are feeding on resuspended particles or sediment.

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