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Uptake and Trophic Transfer for Mercury and Methylmercury at the Base of Marine Food Webs

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Methylmercury (MeHg) is bioconcentrated in phytoplankton and transferred via consumption to zooplankton, planktivorous fish, and eventually larger predators. This dissertation research investigated the transfer of Hg and MeHg from phytoplankton to zooplankton through laboratory experiments and field measurements for several different realms of the marine environment, including coastal, oceanic, and polar regions. Phytoplankton samples were size fractioned into 0.2-5 μm, 5-20 μm, and seston of >20 μm samples, and demonstrate distinctive regional and global variations. The MeHg bioconcentration factors (logBCF) for phytoplankton in the Pacific Ocean had logBCF values that averaged 5.7 ± 1.0 and were higher than the range for Long Island Sound and coastal regions (2.6-5). Zooplankton samples were analyzed for Hg and MeHg at size fractionations of 0.2-0.5 mm, 0.5-1.0 mm, 1.0-2.0 mm and >2.0 mm. The %MeHg in the organisms was typically highest in the largest size class, displaying MeHg bioaccumulation for increasing zooplankton sizes. The carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$) and sulfur ($\delta^{34}S$) isotopes were also evaluated, and demonstrate characteristic feeding sources and patterns for zooplankton Hg and MeHg accumulation. Feeding experiments were performed using stable isotopes of inorganic Hg (^{200}Hg) and MeHg ($CH_3{}^{199}Hg$) in order to determine assimilation efficiencies (AE) and uptake and transfer of Hg and MeHg into marine diatoms and the copepod *Acartia tonsa*. The average AEs were much greater for $CH_3{}^{199}Hg$ (~85%) than for ^{200}Hg (~35%) demonstrating more efficient transfer of MeHg at the planktonic level. Furthermore, these experiments gave evidence for active uptake of MeHg into algae at low (~pM) concentrations. Coastal estuarine uptake experiments for Hg isotope species directly from sediment were also undertaken, and demonstrate that sedimentary sources have a limited and highly variable impact on coastal phytoplankton MeHg accumulation.

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Uptake and Trophic Transfer for Mercury and Methylmercury at the Base of Marine Food Webs

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Introduction

1.1 Background & Relevance

Mercury (Hg), a naturally occurring element has been tied to human health and history since the days of alchemy, when it was believed that blending Hg and sulfur would convert it into gold. However, being naturally present in the environment does not inevitably mean Hg is harmless. As a result of anthropogenic practices, such as combustible coal burning, cement manufacturing, landfill diffusion and gold mining (Branch, 2008; Driscoll et al., 2013), Hg levels have risen substantially in the modern era. Modern emissions are at least three times the natural emissions from geological sources which were once the primary source of Hg to the atmosphere. Though the ratio of natural to anthropogenic emissions is not exactly established, and has been reported to range from 2 to 6 (Nriagu and Becker, 2003; Gustin et al., 2000; Amos et al., 2013; 2015). Mercury is a dynamic and potent metal, quickly shifting forms and configurations in order to reside in nearly every reservoir and in biota throughout the world. The gaseous elemental form of Hg (Hg^0) and divalent mercury (Hg(II)) are especially ubiquitous, because these reactive gaseous mercury species are quickly dispersed through global and local atmospheric circulation, including relatively untouched pristine environments such as the polar ecosystems (Chaulk et al., 2011; Tedesco et al., 2012) and the open ocean environment (Lamborg et al, 2014). Mercury primarily exists as Hg^0 within the atmosphere. Divalent mercury tends to bind with other elements, most prominently halogens (i.e. $HgCl_2$, $HgBr_2$) and organic ligands (Ulrich et al., 2001), and is the dominant form in water and soils. Due to its strong combining ability Hg(II) tends to rain and deposit out quicker from the atmosphere than the longer expanse of Hg^0. Thus, the combination of these species results in the prominent global reach and regional impact of Hg emissions.

Mercury's ability to steadily move globally is a concern; however one of the most notorious Hg species is the organic form methylmercury (CH_3Hg^+; MeHg), the species which dominates in biota. Methylmercury is a highly bioavailable and a damaging neurotoxin (Clarkson and Magos, 2006), which makes it a substantiated global concern for ecosystems. The biological hazards of MeHg in fish and higher predators have been well documented (Berntssen et al, 2003; Watras et al., 1998; Lavoie et al., 2013), and MeHg remains a serious threat to the health of consumers throughout the world. However, the majority of bioaccumulation studies have been undertaken in freshwater systems (Pickhardt and Fisher, 2007; Berntssen et al, 2003; Watras et al, 1998). Though freshwater ecosystem health is a valid concern, oceanic fish represent a greater source of methylmercury into the human diet (Sunderland, 2007; Chen et al., 2008). Furthermore, little research has been undertaken at mercury's point of entry into marine food webs, primarily due to the fact that it is extremely difficult to measure MeHg uptake and tropic transfer under natural conditions. Determining total Hg and MeHg concentrations in water, phytoplankton and zooplankton is still a formidable analytical challenge (Fitzgerald and Watras, 1989; Gill and Fitzgerald, 1985) since open ocean concentrations of inorganic Hg and MeHg are extremely low (<1 pM for MeHg) (Munson et al., 2015; Mason et al., 2001; Gill and Fitzgerald, 1985), and close to historical analytical detection limits. As a result, uncertainty still exists concerning behavior of Hg and MeHg in the lower levels of the food chain in marine ecosystems, and the exact processes which drive and control bioaccumulation and trophic transfer are unresolved. Recent analytical advances have improved detection limits, making studies of the relatively low concentrations found in marine ecosystems possible (Munson et al., 2014). Although there are still some analytical limitations, there is now a greater opportunity to

better understandthe mechanisms which control Hg and MeHg uptake into the marine food chain.

Bioaccumulation into marine food webs is a prominent concern because dietary intake of fish and seafood is the dominant exposure to the toxicant MeHg for humans and seafood consumers. As the movement of MeHg bioaccumulation within the lower trophic levels of marine ecosystems is relatively unknown, this is the focus of this research. Several factors make this research relevant to human and marine food web health. The most prominent consequences of MeHg poisoning include serious adverse effects on the nervous system, including paresthesias, tremors, motor difficulties and sensory disturbances, in addition to heart, kidney and brain disease and impairment (Clarkson and Magos, 2006). Attention was first focused on MeHg poisoning when a large outbreak occurred in Minamata, Japan during the 1950s. The source of this outbreak was exposure from the consumption of local fish with MeHg levels orders of magnitude greater than natural levels (Kudo and Turner, 1999). A nearby chemical plant was manufacturing acetaldehyde using inorganic Hg as a catalyst. The catalytic reaction resulted in prominent industrial MeHg production, where discharge was being pumped into the coastal water system in immense amounts (~14.6 tons; Clarkson and Magos, 2006). Although it is possible that a minor amount of MeHg was also produced naturally in the environment, the sheer volume of MeHg drained into the coastal environment likely overwhelmed any natural methylation. As the consumed seafood was orders of magnitude higher in MeHg than natural concentrations, the acute toxicity resulted in the pertinent poisoning of higher consumers (humans and felines). This case was the first modern instance to bring focus to the ability of MeHg to bioaccumulate in aquatic food chains.

Although awareness and regulatory measures make it unlikely that environmental MeHg levels will reach those found in Minamata, it is still imperative that we understand the basics of bioaccumulation, especially at the lower trophic levels. Increasing climate emissions from fossil fuel consumption and modern practices, and associated model predictions (e.g. Amos et al., 2013; 2015; Driscoll et al., 2013) indicate that concentrations of Hg will only increase in the environment during the near future. Thus, it is also crucial that we establish baseline measurements of Hg and MeHg for natural plankton assemblages, and begin to understand how these levels impact aquatic systems.

1.2 Bioaccumulation Parameters

Methylmercury's permeation into the base of food chains from water is the largest concentration step, and it is only partially understood. Firstly, MeHg complexation and availability are influenced by organic matter (Driscoll et al., 1995; Benoit et al, 2003; Ulrich et al., 2001). Dissolved organic matter (DOC) has an antagonistic and complex relationship with Hg bioavailability. Sulfur-containing functional groups, such as thiols (i.e. cysteine), preferentially bind to mercury species. Mercury tends to bind moderately with nitrogen containing ligands and only weakly with oxygen-containing ligands. According to Ravichandran (2004) a system with elevated levels of sulfur-containing organic matter favors alliances with inorganic Hg, which then helps to appropriate Hg out of the system, since Hg is less bioavailable for organism uptake or methylation. There is clear evidence that MeHg is more soluble under acidic conditions, and decreasing pH yields a net production of MeHg (Ullrich et al., 2001), but in natural (uncontaminated) marine waters and sediment these conditions are less of a factor as

the pH is usually less extreme then what is enacted under laboratory experiments. This relationship between Hg and DOC is dependent on a complex interaction of redox conditions, biological activity, available organic matter and its composition, reduction and volatilization of elemental Hg, as well as pH (Ravichandran, 2004).

The conversion of Hg to MeHg is a critical component of Hg movement in aquatic systems, as MeHg is the form that biomagnifies and is toxic to organisms (Ullrich et al, 2001; Chen et al., 2008; Lavoie et al., 2013). Schaffer et al (2011) found higher rates of Hg uptake and methylation in the presence of strongly complexing low molecular weight thiols for both iron reducing bacteria (FeRB) and sulfate reducing bacteria (SRB) in culture, indicating a tight coupling between Hg methylation and MeHg export out of the bacteria cell in anaerobic environments. Their results indicated that Hg methylation could serve to help avoid cellular buildup of toxic Hg for some bacteria species. Najera et al (2005) demonstrated that facilitated uptake of complexed charged Hg species ($Hg(NH_3)^{2-}$), as well as passive diffusion of neutral Hg species ($HgCl_2$ and $Hg(OH)_2$) were both important for uptake into planktonic *Escherichia coli* cells.

Coastal ecosystems act as a major source of MeHg production via microbial sedimentary processes (Hollweg et al., 2009; Hammerschmidt et al., 2004). Inevitable, coastal areas thus provide a significant source of anthropogenic mercury enrichment to marine ecosystems (Hammerschmidt et al., 2008; Chen et al., 2014), though they can also act as a sink for Hg entering from outside sources. It is therefore essential to understand factors leading to both MeHg production and uptake at the base of the food web in order to remediate environments and to adopt policies to protect humans and wildlife.

There are variations in MeHg and inorganic mercury (Hg) in coastal ecosystem biota, particularly in primary and secondary trophic levels (Pickardt and Fisher, 2007). These variations could be associated with: 1) distinctive methylation potentials for diverse coastal environments which are related to microbial activity; and 2) differences in Hg speciation, and be dependent on organic matter speciation and sulfur content in the sediment, as well as redox potential of pore waters (Chen et al., 2008; Ravichandran, 2004; Ullrich et al., 2001) which influence sediment fluxes. Methylation and demethylation rates of Hg have been measured in sediment (Hollweg et al., 2009; Hammerschmidt et al., 2004). Flux estimates using various approaches (e.g. Hollweg et al., 2009; Hammerschmidt et al., 2008) suggest that there is not a strong correlation between the extent of Hg methylation in sediments and MeHg flux to the water column. Gosnell et al., (2015) noted a strong association between higher MeHg in the water column and enhanced seasonal Hg methylation in the sediments of the Delaware estuary. In contrast, Balcom et al. (2015) found no correlation between sediments and particulate Hg for several estuaries along the north east coast of the US. Furthermore, direct determination of the Hg and MeHg transfer from the sediment-water interface into plankton has not previously been measured. It is important to demonstrate and quantify Hg and MeHg movement from sedimentary sources into phytoplankton, as primary producers are where pelatic bioaccumulation initiates (Mason et al., 1996; Pickardt and Fisher, 2007; Le Faucheur et al., 2014). Consequentially, Hg uptake by the plankton determines transport to upper levels of the food web; thus determining potential exposure for humans (Chen et al., 2008; Lavoie et al., 2013; Chen et al., 2014).

Complexation of MeHg with inorganic ligands also has a strong influence on bioavailability. For example, MeHg complexed with chloride (CH_3HgCl) is much more readily

taken up by organisms than if it is complexed with hydroxide (CH_3HgOH) (Ravichandran, 2004; Mason et al., 1996). Thus, the formation of natural complexes influences uptake by organisms, as phytoplankton MeHg uptake is thought to occur via passive diffusion across a cell membrane (Pickhardt and Fisher, 2007; Gorski et al., 2006; Fisher and Reinfelder, 1995; Lawson and Mason, 1998). Subsequently, MeHg is bioconcentrated in phytoplankton and transferred via consumption through the food chain to herbivorous zooplankton, planktivorous fish, and eventually larger predators (Foster et al., 2012; Kim et al, 2004; Berntssen et al, 2003). Assimilation efficiencies (AE) of MeHg are much greater than those for inorganic Hg (Watras et al., 1998; Tsui et al., 2009; Wang, 2002). Pickhardt et al. (2006) observed that while Hg uptake into algae was not affected by DOC, MeHg concentrations in algae were much greater in DOC-enriched water. Moreover, Schartup et al. (2015) noted that the type of DOC could potentially be impacting uptake into algae in coastal marine systems.

There are few measurements of AE's for plankton at environmentally relevant concentrations. To increase understanding of the assimilation of Hg and MeHg into marine food webs it is important to examine factors influencing assimilation into various phytoplankton species. Previous trophic transfer experiments used mostly mercury radioisotopes as the chemical tracer (Rouleau and Block, 1997; Fisher, 1985; Fisher et al., 1995). Although radioisotope techniques are well established and radioisotopes make excellent tracers, it is not possible to perform experiments using both Hg and MeHg during the same exposure as the same isotope radiotracer is used for both. Furthermore, concentrations required to make those experiments feasible have often been much greater than natural Hg levels. Moye et al., (2002) found uptake of CH_3HgCl seemed to involve different transporters into freshwater algae cells depending on the exposure level, possibly suggestive of active uptake at lower concentrations

(<10 nM). Thus, a distinct benefit of using stable Hg isotopes is the ability to investigate uptake under more environmentally realistic conditions of Hg and MeHg in the same culture for marine systems, as well as utlize different species of Hg (i.e. MeHg and Hg) concurrently, potentially highlighting positive or negative speciation interactions. Though experiments preformed here are still above natural concentrations due to remaining detection limit limitations, they still represent a further step towards measuring assimilatin efficiencies more realistic environmental levels.

1.3 Environmental Parameters

A separate issue which needs to be addressed through this research is whether there are any seasonal feedbacks for Hg and MeHg uptake and bioaccumulation. Methylation potential of an environment is its capacity to convert inorganic Hg into MeHg. The methylation potential for a region is a function of the supply of inorganic Hg and the bioavailability of Hg to methylating organisms, which is affected by factors such as organic matter content and sulfide levels (Benoit et al, 2003; Chen et al., 2008b, Ullrich et al., 2001), and the microbial activity. Temperature can also be a factor, as SRB activity tends to increase in warmer seasons (King et al., 1999). Coastal systems tend to have higher concentrations of DOC and nutrients than open ocean waters. Methylation is believed to take place in anoxic sediments in coastal regions and estuaries (Hammerschmidt et al., 2004), whereas methylation is more important within the water column in the open ocean (Mason et al., 2012). In temperate climates the oxygen, Hg and organic matter levels can have large seasonal variations (Balcom et al., 2008). During spring there is enhanced primary productivity in the water column as bottom nutrients are stirred up into the euphotic

zone, and this input amplifies growth of plankton into a spring bloom. As nutrient supplies decrease through uptake, the spring bloom eventually decays. The phytoplankton degradation after the spring bloom can decrease oxygen concentrations in the water, and result in low-oxygen and anoxic regions during late summer. Though every region does not become completely anoxic, these seasonal blooms and crashes could still affect the methylation potential (Fisher and Wente, 1993). Furthermore, the degradation of algae blooms can deliver refractory or reactive carbon to the benthos, modifying the redox chemistry and methylation potential of sediments. A fall plankton bloom is also relatively common in temperate coastal regions, such as the northeast and northwest United States. Organic matter present during the fall bloom could therefore be from sources remineralized at lower oxygen levels, thus springtime DOC could be characteristically different than the fall. If methylation potential is affected by the types of organics and oxygen levels in the water column, then it is possible that more MeHg is available to be taken up by organisms in fall blooms than for the spring.

Laboratory experiments by Chen and Folt (2005) have noted that larger phytoplankton blooms can lead to reduced uptake of Hg and MeHg into individual species via a 'dilution effect'. It is important to investigate if this effect occurs in the natural environment as well. It is possible that there could be growth dilution during the seasonal plankton blooms, while mercury becomes concentrated in the intermittent summer season. Therefore, it is constructive to investigate if Hg concentrations and trophic transfer follow any consistent pattern through the seasons, as this could further influence the initiation of mercury into the marine food chain. By measuring plankton through the seasonal blooms it possible to investigate if there are measurable differences between %MeHg (proportion of Hg as MeHg) in the spring and fall, versus the summer, when productivity and bloom sizes of plankton are different.

It is important to quantify Hg and MeHg movement from sedimentary sources into phytoplankton, as this is where some of the initial biomagnification occurs in coastal ecosystems (Mason et al., 1996; Pickardt and Fisher, 2007), thereby affecting the marine food web and human and coastal ecological health (Chen et al., 2008). Understanding and quantifying this initial transmission will provide a basis for assessing MeHg and Hg uptake throughout the food chain of coastal ecosystems. Levels of MeHg transfer have not been established for primary and secondary producers (zooplankton) or for open ocean populations or over time and spatially, and relatively little data have previously been published on concentrations of Hg in coastal ocean plankton (Hammerschmidt et al., 2013; Burt et al., 2013; Foster et al., 2012).

Hypotheses:

Based on the above, the following hypotheses were defined to focus this research:

1) Mercury and methylmercury bioaccumulation into plankton is dependent on exposure concentration, and the bioconcentration factor will decrease with increasing exposure concentration. Therefore, given that most previous work has been done at higher exposure concentrations, bioaccumulation factors are likely higher than previously assessed.

2) The volume and size of primary producers will impact the concentration of Hg and MeHg assimilated into plankton. This hypothesis is predicated on the notion that uptake is related to surface area, and concentration to volume, so larger plankton should have lower concentrations of MeHg per unit volume.

3) Environmental parameters such as physical characteristics and nutrient concentration, can impact trophic transfer and bioaccumulation into plankton. Furthermore, attributes of the food web structure, such as the presence of intermediate consumers and multiple size fractions, as well as transitional interactions and planktonic food web length, are major determinants of bioaccumulation by zooplankton.

Different laboratory experiments and field experiments were completed in order to address these hypotheses. Experimental hypothesis (1) was tested by varying the exposure Hg and MeHg isotope concentrations by 10x in two separate algae cultures. The stable ^{200}Hg isotope was used for inorganic Hg, and $CH_3{}^{199}Hg$ was used for MeHg measurements. Algal uptake of isotopes was measured for water concentrations of 10 pM MeHg and 20 pM Hg, and a separate experiment assessed levels of 100 pM MeHg and 200 pM Hg. Algae with different concentrations were fed to copepods to see if Hg and MeHg bioaccumulation was continual in the next trophic level, and if exposure concentration has any impact on trophic transfer (Lawson and Mason, 1998; Pickhardt and Fisher, 2007).

Hypothesis (2) was tested by using different algae species, also of different sizes. The physiology and size relationship has been tested on freshwater algae (Pickhardt and Fisher, 2007), however it has not yet been demonstrated with marine species, nor has AE's of MeHg by zooplankton feeding on algae. Using smaller algae species will yield a higher [size/volume], and should therefore yield a higher uptake of Hg and MeHg in the smaller species. The two different algae were fed to copepods in order to see if trophic transfer of Hg and MeHg was also enhanced in the smaller algae species.

Hypothesis (3) was investigated by performing a seasonal study of Hg and MeHg dynamics in the plankton community of Long Island Sound. Stable isotopes of carbon (C^{12}, C^{13}), nitrogen (N^{14}, N^{15}) and sulfur (S^{32}, S^{34}) were additionally measured in order to determine separate food chain dynamics and consumption patterns for the various plankton of each season.

Additionally, the potential for phytoplankton uptake directly from the release of MeHg and Hg from sediment was examined in conjunction with assays of methylation and demethylation to test the sediment-water column link towards bioaccumulation initiation. This latter experiment was an environmental expression of hypothesis (1) and hypothesis (3).

The three hypotheses were verified with field data. By sampling phytoplankton and zooplankton from various size fractions, the potential impacts of MeHg and Hg in larger or more omnivorous zooplankton could be compared to the smaller species. Samples from various ocean regimes were examined by collecting size fractions of phytoplankton and zooplankton and measuring the MeHg and Hg concentrations within, and relating to the water column concentrations. In order to better understand Hg and MeHg bioaccumulation into the lower trophic levels of marine ecosystems, both coastal and open ocean systems were investigated. Size fractionations of phytoplankton and zooplankton were collected from a variety of ecosystems, and throughout several different regions of the world, including coastal regions and the open Pacific Ocean. Furthermore, in situ experiments for uptake into algae and primary feeding experiments were completed to understand the effects of concentration, from the base of the food chain to secondary predators. This research primarily examines Hg and MeHg uptake, bioaccumulation and trophic transfer in the coastal and open ocean environment, focusing on the primary and secondary levels of the pelagic food chain.

Chapter 2:

Mercury and Methylmercury Incidence and Bioaccumulation in Plankton from the Central Pacific Ocean

2.1 Introduction

Mercury (Hg) is a substantiated global concern due to the high bioavailability of its organic form methylmercury (MeHg), which is a damaging neurotoxin (Clarkson and Magos, 2006), and highly bioaccumulative in aquatic food chains (Chen et al., 2008; Sunda, 2012). Mercury emissions have increased above natural environmental levels due to anthropogenic practices, such as high temperature combustion, artisanal gold mining and cement manufacturing (National Research Council, 2000). Due to its long atmospheric residence time, Hg has the ability to travel throughout the world, impacting remote regions such as the open ocean (Lamborg et al., 2014; Mason et al., 2012; Mason et al., 1994). Oceanic fish represent a critical source of MeHg into diets, as most humans consume seafood in higher quantities than freshwater fish (Sunderland, 2007; Chen et al., 2008). Nevertheless, little research has been undertaken at mercury's point of entry into open ocean marine food webs, primarily due to the fact that it is difficult to measure MeHg uptake and tropic transfer under natural conditions, as open ocean concentrations of inorganic Hg and MeHg are extremely low (<1 pM for MeHg) (Mason et al., 2012; Gill and Fitzgerald, 1985; Lamborg et al., 2014). As a result, uncertainty still exists related to the behavior of Hg and MeHg in lower trophic levels of the food chain in marine ecosystems. Likewise, the exact processes which drive and control natural bioaccumulation and trophic transfer are still unresolved for the open ocean.

It is imperative to fully comprehend the transport of Hg and MeHg into the lower oceanic trophic levels, as these reflect the inception of exposure. Algae accrue nutrients, including essential metals (e.g. iron, zinc) and unessential elements (e.g. Hg, lead, arsenic) from the surrounding media, and also release compounds that influence metal speciation. Phytoplankton population and composition therefore has a significant impact on the levels and availability of

trace metals in marine waters. The remineralisation of algae during sinking and bloom degradation also impact metal speciation and availability. The resultant formation of natural complexes influences uptake of many metals by biota. Prior research suggests that phytoplankton MeHg uptake occurs primarily via passive diffusion of neutral complexes across a cell membrane (Pickhardt and Fisher, 2007; Gorski et al., 2006; Fisher and Reinfelder, 1995; Lawson and Mason, 1998; Mason et al., 1996). Passive uptake of both species could also result after sorption of Hg and MeHg to cell surfaces (Fisher, 1985).

However, the possibility also exists that phytoplankton assimilate Hg and MeHg via active or facilitated transport. Active uptake of MeHg remains unspecified. It is probable that MeHg is likewise accumulated when cells acquire organic compounds which are strongly bound to MeHg, such as cysteine or other thiols (Moye et al., 2002; Lawson and Mason, 1998), as has been found to occur in bacteria (Ndu et al., 2012: Schaefer et al., 2011). Pickhardt and Fisher (2007) demonstrated that live cells accumulated more MeHg than dead cells in freshwater phytoplankton, suggesting possible uptake via active processes. This was not the case for inorganic Hg. Because MeHg permeates into cellular cytoplasm rather than being bound with the cellular membrane (Mason et al., 1996; Pickhardt and Fisher, 2007), transfer and accumulation into the marine food web is greater for MeHg than for inorganic Hg (Reinfelder and Fisher, 1991; Mason et al., 1996). Subsequently, MeHg is bioconcentrated in phytoplankton and transferred via consumption through the food chain to herbivorous zooplankton, planktivorous fish, and eventually larger predators, with the fraction of MeHg amplifying at each trophic level (Foster et al., 2012; Kim et al, 2004; Berntssen et al, 2003). Therefore, it is crucial to quantify Hg and MeHg movement from ocean waters into primary and secondary consumers,

as this is where the principal biomagnification occurs (Pickardt and Fisher, 2007; Mason, 2002; Le Faucher et al., 2014).

Relatively little data have been published on natural concentrations of Hg in coastal ocean plankton (Foster et al., 2012; Hammerschmidt et al., 2013; Mason et al., 2012), and there is even less information concerning the open ocean. In addition, there has been little study of the concentrations of MeHg in different size fractions of plankton, except for those published recently by Hammerschmidt et al. (2013). Furthermore, quantified amounts of MeHg transfer have not been established for primary (phytoplankton) and secondary producers (zooplankton) for open ocean populations. Even though coastal ecosystems represent a large fraction of overall productivity for marine systems, the global expanse of the open ocean, and in particular the more productive regions such as the equatorial Pacific, warrant consideration in terms of importance towards Hg and MeHg bioaccumulation. This study presents measurements of size fractionated Hg and MeHg concentrations for open ocean phytoplankton and zooplankton. The results of this study indicate that concentrations in zooplankton increase with organism size and that the bioaccumulation factor for phytoplankton decrease as suspended particle concentration increase. Determining these values have helped to establish levels of Hg and MeHg at the base of the open ocean food chain, at the onset of marine bioaccumulation. Additionally, these data provide information to managers and modelers concerned with the bioaccumulation of MeHg into open ocean fish consumed by humans.

2.2 Methods

2.2.1 Sample Collection

Phytoplankton and zooplankton samples were collected along a transect from Oahu, Hawaii to Apia, Western Samoa, with a brief stopover at the Island republic of Kiribati (Figure 2.1). The cruise occurred on the R/V Kilo Moana during October 3-23, 2011. The cruise transect traveled through a High Nutrient Low Chlorophyll (HNLC) zone (~station 3) and the highly productive equatorial upwelling region (station 5). Eight (out of twelve) stations were sampled for plankton, and Table 1 details the coordinates for each station sampled. Two stations, station 3 and 5, were occupied for a 3-day period, which yielded the opportunity to collect multiple temporal zooplankton samples, and compare concentrations in night and day zooplankton collections. Separate day and night net tows were collected in order to investigate any differences due to the possible occurrence of diurnal migrating zooplankton. Water and plankton were sampled using trace-metal clean techniques.

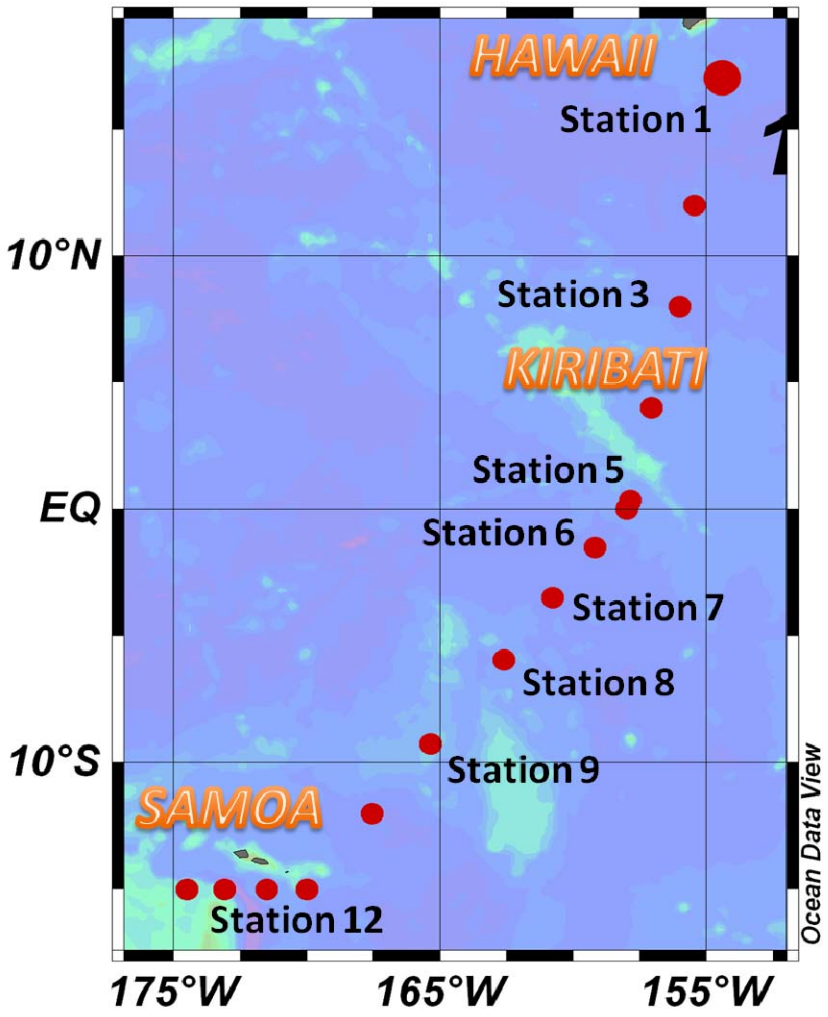

Figure 2.1. Cruise track starting from Honolulu, Hawaii to Apia, Samoa, with a brief stopover in the Island Republic of Kiribati. Stations which were sampled for plankton are noted on the map, and locations are detailed in Table 2.1.

Phytoplankton (seston) samples were amassed from known volumes of water collected out of Go-Flo bottles at the depth of the chlorophyll maximum of each station (Table 2.1). Phytoplankton was concentrated on acid-cleaned polycarbonate filters for various size fractionations by filtering seawater through 0.2 µm, 5 µm, and occasionally 20 µm filters. Potential occurrence of zooplankton in these fractions was impeded using a 200 µm mesh shield

prior to filtering. Volumes filtered ranged from 0.5-2 L for the smallest fraction to 2-5.5 L for the larger fractions. Given this fractionation, phytoplankton size classes will be referenced as <5 μm (for 0.2-5 μm fractions), 5-20 μm and >20 μm. The 20 μm fraction represents plankton of >20 μm but <200 μm. Samples were collected at each size fraction for fluorescence, which was measured on board using a handheld Turner aquafluor fluorometer. In order to assess phytoplankton concentrations on a mass basis, biomass was calculated using both the chlorophyll and phaeopigment measurements, assuming a constant carbon:chlorophyll ratio of 100 for the region (Taylor et al., 2011; Claustre et al., 1999; Wang et al., 2013). These values were further converted into biomass by assuming 40% carbon in biomass. The calculated phytoplankton biomass values were scaled by a factor of 3 to account for biomass from sources other than phytoplankton, such as bacteria, inorganic particles, fecal pellets and other detritus (Church, 2008; Libes, 1992). Thus, the reported concentrations reflect those of the different seston sizes. These concentration values are comparable to those collected by others using size fractionation approaches (e.g. Hammerschmidt et al., 2013).

Table 2.1. Coordinates for plankton stations, and chlorophyll maximum depth sampled for phytoplankton, during the central Pacific research cruise.

Station #	Longitude (^{o}W)	Latitude (^{o}S)	Chl Max. depth (m)
1	154.40	-17.00	115
3	156.00	-8.00	80
5	157.87	-0.36	40
6	160.77	3.50	75
7	162.61	5.96	75
8	165.36	9.25	79
9	167.56	12.00	60
12	173.10	15.00	125

Water profiles were collected at each station using trace metal clean techniques and a trace metal-free rosette. They were analyzed on board for mercury speciation concentrations, including total Hg and dissolved gaseous Hg (elemental and dimethylmercury). Samples were also collected and analyzed for nutrients, dissolved oxygen, temperature and salinity by the geochemistry group at the Woods Hole Oceanography Institute (Munson, K., 2014; Lamborg et al, 2014).

Zooplankton collections were completed using a vertically towed 200 μm mesh opening/closing net, hauled on a Kevlar line, from depths of 200 m up to ~10 m. The net was closed at ~10 m below the keel of the ship in order to minimize potential vessel contamination. Zooplankton size fractionations were separated by using multiple acid-cleaned nylon mesh screens on a bench covered with plastic sheeting. Zooplankton was divided into sizes of 0.2-0.5 mm (200-500 μm), 0.5-1.0 mm, 1.0-2.0 mm and >2.0 mm. Zooplankton size fractions were divided into multiple subsamples when large biota volumes (i.e. Station 5) permitted. Subsamples of the zooplankton fractions for carbon, nitrogen and sulfur determinations (C, N, S) were also collected on pre-combusted 25 mm GF/F filters to assess cellular content of these nutrients.

2.2.2 Analytical

Samples were extracted in acid cleaned 15 ml centrifuge tubes with dilute 4.51 M HNO_3 (7 ml total) in a 60 ^{o}C water bath for ~12 hours prior to analysis (Hammerschmidt and Fitzgerald, 2005). For MeHg measurements, sample aliquots were neutralized using 10 N potassium hydroxide, and buffered with 4 M ammonium acetate (800 μL) to maintain the optimum pH of 4.9 in a sparging flask containing ~100 mL ultra pure (18.2 MΩ) water. Sodium

tetraethylborate (200 µL; 1% w/v) was added to form derivative Hg species. Volatile ethylated derivatives were purged onto a Tenax trap using ultra high purity nitrogen gas (N_2). The sample was then thermally desorbed from the Tenax, releasing and separating the ethylated Hg derivatives via isothermal gas chromatography (GC). Methylmercury samples are ultimately quantified by Tekran 2500 cold vapor atomic fluorescence (CVAFS), based on the methylethylmercury peak area using an external calibration curve (r^2 >0.998). There was not sufficient volume to perform substantial replicate sample analysis; however, standard addition and other QA/QC practices were incorporated into the analytical protocols to ensure measurements accuracy and precision. The RSD based on repeat analysis of recovered matrix standards was 6.7%. The detection limit was 0.016 pmol MeHg.

In order to completely extract all Hg for total analysis, brominemonochloride (BrCl, 40µl; 0.2% v/v) was added to the remaining 4.51 M HNO_3 sample aliquot, which was further diluted with 18.2 MΩ water, and digested >24 hours. Hydroxylamine hydrochloride (20 µl; 0.1% v/v) was then added to reduce any remaining BrCl prior to Hg analysis. A sample aliquot was added to a sparging flask filled with ~ 100 mL 18.2 MΩ water to which tin chloride reductant (100 µl; $SnCl_2$) was added to reduce iconic Hg. The solution was then purged with N_2 to transfer elemental Hg onto a trap containing gold-coated beads (dual amalgamation). Once the trap was heated, it released gaseous Hg^0 which was measured using Tekran 2500 CVAFS. The concentration was determined using an external calibration curve (r^2 >0.999). Replicate analyses were completed when possible, and standard addition and other QA/QC practices were integrated into the analytical practices to ensure accuracy and precision. The RSD for total Hg based on repeat standard recovery was 2.9%, and the detection limit was 0.091 pmol.

Chlorophyll concentrations were determined on board as described above. Zooplankton samples collected on 25 mm GF/F filters were dried at ~60 ^{o}C for approximately 36 hours, and wrapped in tin foil capsules. These dried samples were then analyzed on a Fisions Instrument EA 1108 CHNS elemental analyzer for C, N and S measurements using an external standard calibration curve (r^2 >0.999). Sulfur measurements were only completed for samples from Station 5. The precision of the measurements were ± 2 μmol, and the detection limit was 0.4 μmol for the EA analysis.

2.3 Results

2.3.1 Phytoplankton

Chlorophyll concentrations were highest in the <5 μm fraction at all stations and were >90% of the estimated seston biomass, confirming the dominance of small plankton in this ocean region. Chlorophyll concentrations were somewhat higher but comparable to other recent measurements in the region (e.g. Taylor et al., 2011), as expected given that sampling was at the chlorophyll maximum at each station. The ratio of chl a/pheopigment (Chl/Phaeo) was between 1 and 2.5, with the higher ratios, signifying less degradation, being at the equatorial and near equatorial stations. The estimated suspended particulate concentrations for the <200 μm fraction ranged from 0.5 to 1.6 mg/L and was highest at Stations 5 and 12, and lowest at Stations 7-9. The higher concentration at Station 12 may appear surprising given that it was furthest from the equator in the Southern Hemisphere; however, this station was relatively close to the island of Samoa. Seston MeHg concentrations were highest in the 5-20 μm and >20 μm size fractions at stations 1, 3, and 6, and did not exceed 10 pmol g-biomass^{-1} (wet weight) for the remaining size

classes. The <5 µm size fraction exhibited the highest concentration at station 1 (8 pmol g-biomass^{-1} (wet weight)), and decreased throughout the cruise transect from north to south, with relatively minor variations in concentration. As the <5 µm fraction dominated the biomass, the overall MeHg concentrations for the <200 µm seston fraction was similar, ranging up to 8.2 pmol g^{-1} wet weight and was highest at stations 1 and 3 and lowest at station 12 (Figure 2.2a).

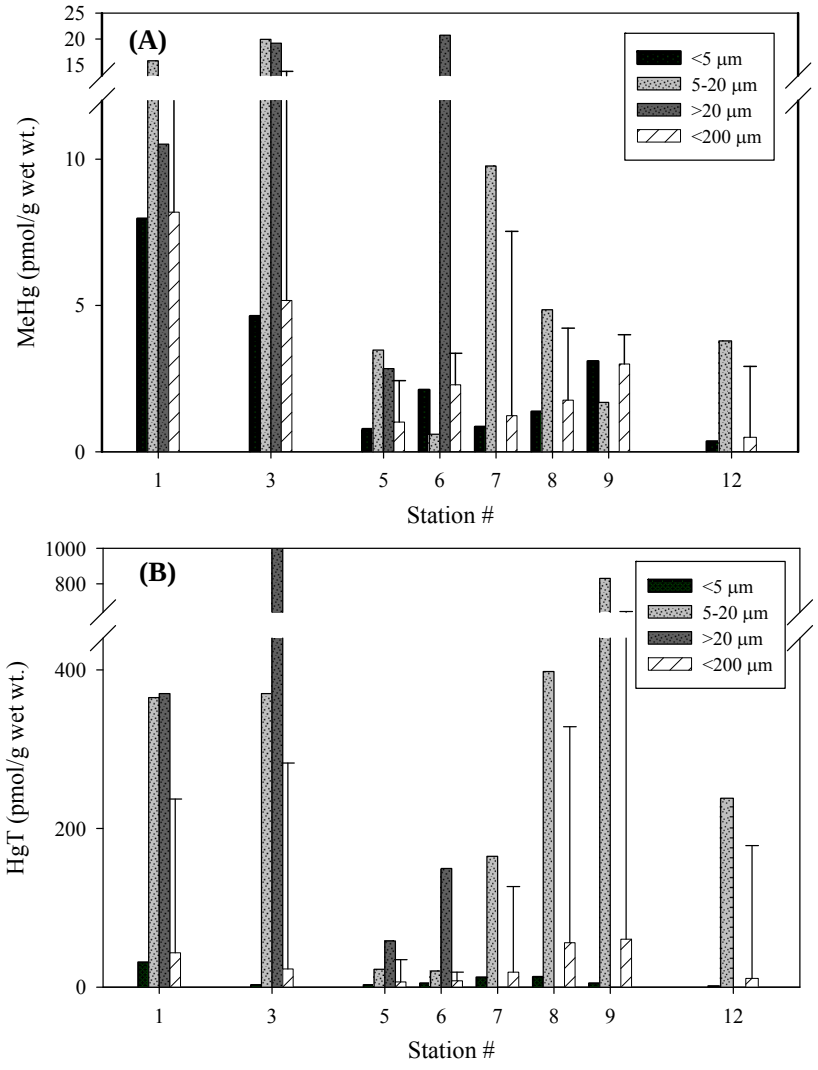

Figure 2.2. **(A)** Methylmercury (MeHg) and **(B)** total mercury (HgT) concentrations (pmol/g wet wt.) for phytoplankton collected in the eastern central Pacific. Size classes are noted as <5 µm for the measured 0.2-5 µm fractions, 5-20 µm, >20 µm, and <200 µm (including standard deviation error bars) for the entire phytoplankton average for each station.

Inorganic Hg values were more variable, with highest concentrations for each station always in the >20 μm (58-1437 pmol g-biomass^{-1} (wet weight)) or 5-20 μm (20.3-830 pmol g-biomass^{-1} (wet weight)) size classes, and lowest concentrations (1.31-31.4 pmol g-biomass^{-1} (wet weight)) were in the <5 μm fraction throughout the transect (Figure 2.2b). The extremely high value for >20 μm at station 3 (1437 pmol g-biomass^{-1} (wet weight)) could be a result of mass estimation error due to the particularly low biomass compromising the calculation for HgT. Thus, it was concluded that this value represents an outlier and was not included in the figure. For the combined fractions (<200 μm) the concentrations ranged from 6 to 60 pmol g-biomass^{-1} (wet weight).

As a potential explanation for some of the measured higher HgT values, there was an inverse relationship between HgT concentration in the various seston size classes and the Chl/Phaeo ratio in the fraction. For samples where the ratio was >1, reflecting actively growing biomass, the HgT concentration was lowest. The highest HgT concentrations were found for samples where the ratio was <1 (indicating relatively degraded biomass). In all cases this was for the largest size fractions. Furthermore, the Chl/Phaeo ratio was >1, and mostly >2, for every <5 μm fraction, suggesting that this fraction represented actively growing plankton biomass. There was some relationship between MeHg in seston and the Chl/Phaeo ratio, but it was less apparent than for HgT.

Notably, the percentage as MeHg (%MeHg; [MeHg]/[HgT]) in the seston was always highest in the <5 μm phytoplankton size class for each station (Figure 2.3a). Only stations 7 and 8 had %MeHg values that were under 20% for the <5 μm size class, while all partitions for the 5-20 μm and >20 μm size classes had values below 20% for %MeHg. Considering the overall

<200 μm fraction, the %MeHg ranged from 3 to 29%, comparable to values found in other studies (<2 to 15%; Mason et al., 2012; Hammerschmidt et al., 2013), and similar to that found for suspended particulate during the cruise (up to 25% for the upper water column; Munson, 2014). As shown in Figure 2.3a, the %MeHg concentrations for phytoplankton were higher than in the water (Figure 2.3c; Munson, 2014), which is expected given that MeHg is accumulated more readily into phytoplankton over inorganic Hg (e.g. Mason et al., 1996). Moreover, the %MeHg in the dissolved fraction is relatively high for the stations occupied during the cruise compared to other ocean regions (Mason et al., 2012; Munson et al., 2015). Thus, the higher %MeHg in the seston of this study appears credible.

Ratios of the concentration in an organism to what was in the water column (Bioconcentrations factors; BCFs (L/kg)) were also calculated for all fractions at each station of the cruise transect. The water to phytoplankton step is the greatest concentration step in the trophic transfer of MeHg. The logBCFs ranged from ~3.8-7 for HgT, and ~4.1-6.8 for MeHg for all <200 μm size classes (Table 2.2). There was a slight trend in increasing logBCF values with increasing seston size class for HgT, but no trend with size class was evident for MeHg. Specifically, the HgT logBCF values were almost always greater than the MeHg log BCF values for both the >20 μm and 5-20 μm size fractions, but the majority of <5 μm fractions had larger MeHg log BCF values. The measured filtered HgT concentrations for the depths of the chlorophyll maximum were <0.2 pM in many instances in this study (Munson et al., 2015). These relatively low values, compared to previous measurements (0.5-2 pM; Mason and Fitzgerald, 1993; 0.3-1.5 pM Laurier et al., 2004 for stations around 25 ^{o}N) may be a reason for the higher estimated values in this instance. The higher logBCF for MeHg in the <5 μm fraction compliments the result that the highest %MeHg value for phytoplankton was always higher for

the <5 µm size class. Average logBCF's for the entire seston (<200 µm fraction) ranged from 4.4 to 6.2.

Table 2.2. Bioconcentration factors (logBCFs) calculated for each phytoplankton size class collected throughout the cruise transect. LogBCFs were calculated for both HgT and MeHg levels for each size class filtered.

Station #	**LogBCF HgT** ***0.2-5µm***	**LogBCF MeHg** ***0.2-5µm***	**LogBCF HgT** ***5-20µm***	**LogBCF MeHg** ***5-20µm***	**LogBCF HgT** ***>20µm***	**LogBCF MeHg** ***>20µm***
1	4.46	5.60	5.64	5.90	5.65	5.72
3		6.12	6.44	6.75	7.05	6.74
5	4.48	4.53	5.45	5.18	5.91	5.10
6	5.30	5.11	6.16	4.56	6.98	6.10
7	5.50	4.72	6.63	5.77		
8	5.20	4.88	6.74	5.42		
9	4.04	5.40	6.67	5.14		
12	3.77	4.33	6.16	5.36		

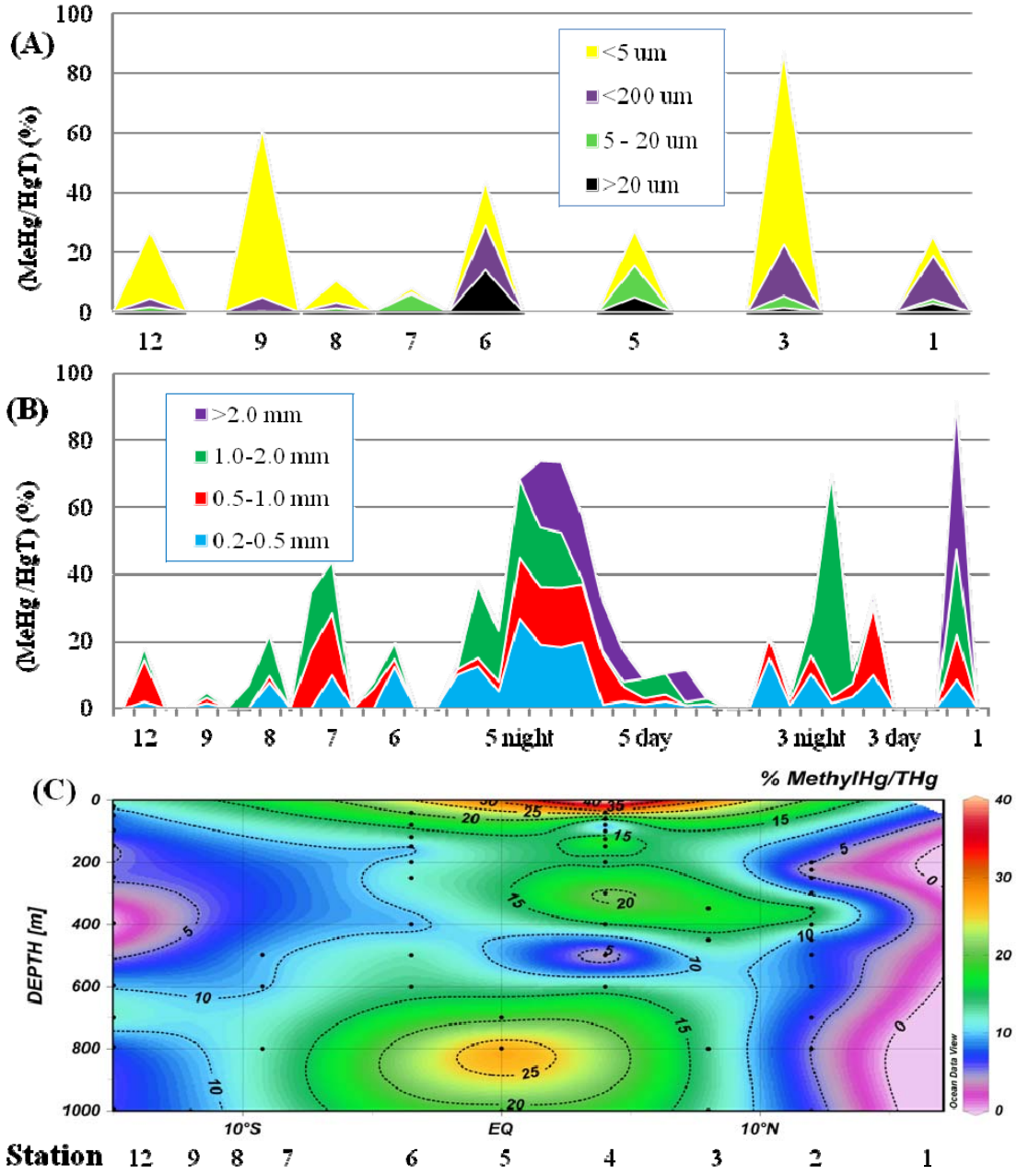

Figure 2.3. **(A)** Percent of phytoplankton biomass as MeHg (%MeHg; [MeHg]/[Hg]) displayed for each size class, for each of the stations. **(B)** The percent of zooplankton biomass as MeHg demonstrated for each size class, for each station sampled. This graph includes all multiple vertical net tows completed at each station, as coordinated along the cruise tract. **(C)** The concentration of %MeHg in the water column for the cruise transect, displayed in depth from surface to 1000 m. Stations are approximately aligned with latitued, and numbered along the horizontal axis for each section. Station 5 (at equator) represents an equatorial upwelling zone, and displays some of the highest %MeHg in the biomass, as well as deeper within in the water column. Water column data for (C) was taken from Munson (2014), and used with permission of the author.

The carbon:chlorophyll ratio (C:Chl) is typically not consistent throughout productive marine regions both spatially and temporally, therefore additional ratio values of 75 and 125 C:Chl, which cover typical concentration ratios for the open ocean, were compared to the chosen C:Chl of 100. These different ratios will impact the estimated concentrations for Hg and MeHg. Notably, the lower 75 C:Chl would increase both the Hg and MeHg concentrations by approximately 30%. Therefore, the 5-20 μm size fraction at station 5 would shift from 3.5 pmol/g (wet wt.) up to 4.6 pmol/g (wet wt.) MeHg, with corresponding Hg concentrations that would increase from 22.4 pmol/g (wet wt.) up to 29.9 pmol/g (wet wt.) at the 75 C:Chl. Conversly, using the greater ratio if 125 C:Chl would have the reverse effect, with concentrations decreasing by approximately 20% for all reported Hg and MeHg levels. Thus, at the greater C:Chl of 125, the 5-20 μm size fraction at station 5 would decrease down to 2.8 pmol/g (wet wt.) MeHg, and shift down to Hg levels of 17.9 pmol/g (wet wt.). On the other hand, the %MeHg is impervious to different C:Chl ratios, and the logBCF values were less affected by alteration of the C:Chl, and consistent with the directional shifts seen in the calculated concentrations. For example, the MeHg logBCF values for the station 5 5-20 μm size were 5.31 (75 C:Chl), 5.18 (100 C:Chl) and 5.09 (125 C:Chl), with comparable Hg log BCF shifts of 5.57 (75 C:Chl), 5.45 (100 C:Chl) and 5.35 (125 C:Chl). As the selected 100 C:Chl was aligned with previously reported measurements in the investigated region (Taylor et al., 2011; Claustre et al., 1999; Wang et al., 2013), that is what was reported for this study. Nevertheless, it is important to be aware of regional and measured C:Chl levels when calculating the Hg and MeHg concentrations in plankton via this method.

2.3.2 Zooplankton

The C:N values for all size classes of zooplankton ranged from 4.4 to 8.5, representing a large distribution in cell nutritional content. The Redfield ratio for C:N is 6.6, therefore higher values indicated consumption of more degraded material or N limitation, as N is typically recycled faster than C. Theoretically, smaller C:N values could indicate that zooplankton species were consuming more nitrogen and possibly eating higher on the food chain, as C:N values decrease in higher trophic level consumers. However, there was no consistent relationship between size class and C:N values for the transect (Figure 2.4a). In terms of other parameters measured, there was a correlation for sulfur content and MeHg for Station 5 (mmol:pmol; r^2 = 0.5102; p = .0006; n=12) where S was measured (Figure 2.5). However, no correlation was found with S and HgT (mmol:pmol; r^2 = 0.0359; n=12).

Total Hg concentrations for zooplankton size fractions ranged from 5 - 135 pmol g-biomass^{-1} (wet weight; Figure 2.4b), while MeHg concentrations ranged from ~1 - 17 pmol g-biomass^{-1} (wet weight; Figure 2.4c). Zooplankton abundance and MeHg concentrations both peaked at stations 3 and 5 (in the upwelling region), and lowest MeHg concentrations were found at the open ocean stations 7 through 12 (2 – 7 pmol g-biomass^{-1} (wet weight)). The overall %MeHg in zooplankton was greatest at stations 1, 3, 5 and 7, and consistently increased with increasing size class (Figure 2.3b). There was variability in zooplankton HgT and MeHg concentrations throughout the transect, which is consistent with previous investigations (Hammerschmidt et al, 2013; Foster et al, 2012; Chen et al, 2009). Multiple measurements at each station agreed within the variability for HgT replicate measurements (±0.3-11.3 pmol g-biomass^{-1}) and MeHg (± 0.06-2.2 pmol g-biomass^{-1}), although there were larger deviations at some stations (Figure 2.4).

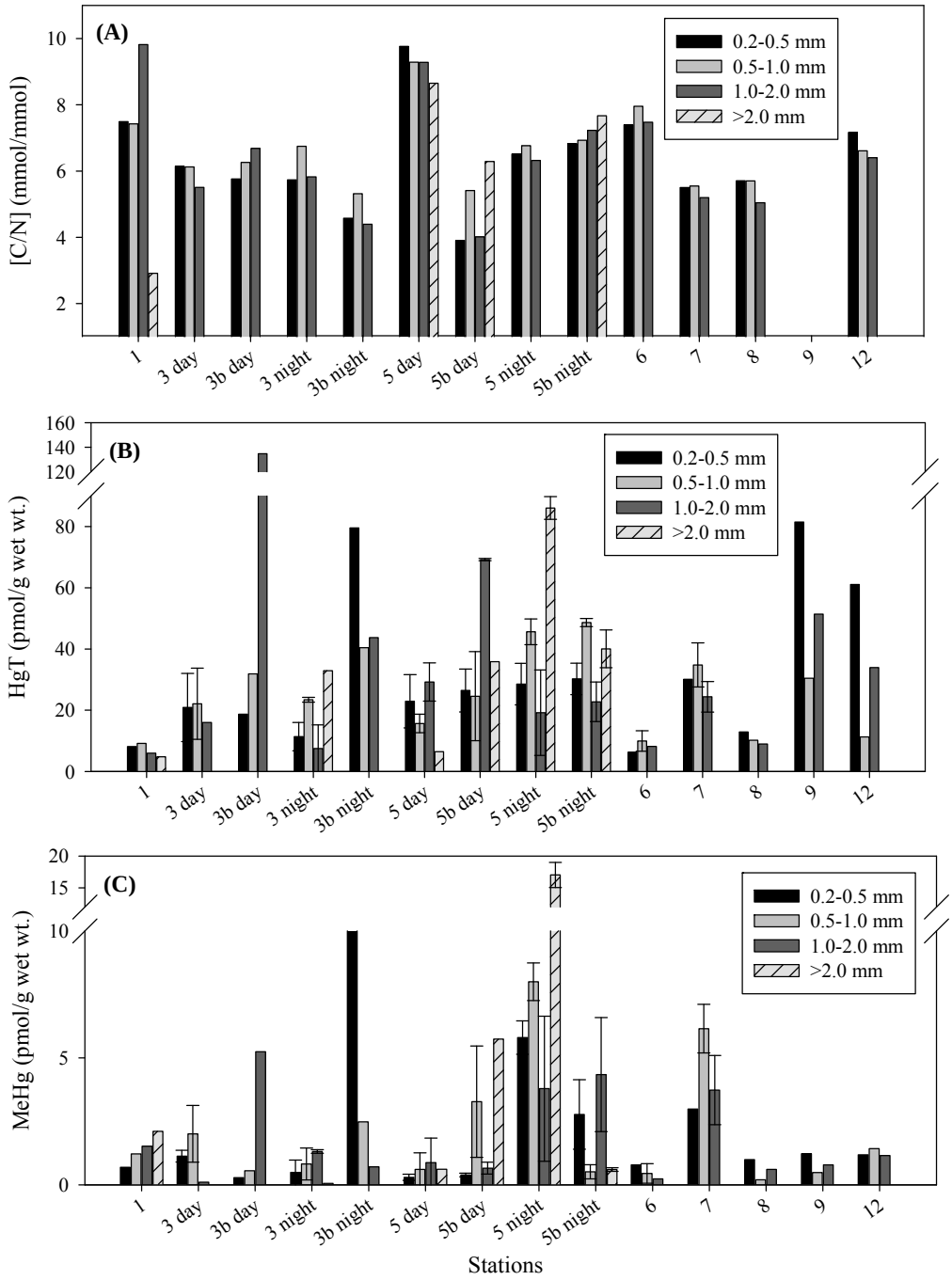

Figure 2.4. **(A)** The carbon: nitrogen (C:N) values for each zooplankton size class collected during the cruise. Size classes were divided as sizes of 0.2-0.5 mm (200-500 μm), 0.5-1.0 mm, 1.0-2.0 mm and > 2.0 mm. **(B)** Total mercury (HgT) and **(C)** methylmercury (MeHg) concentrations (pmol/g wet wt.) for multiple zooplankton size fractions at each station. Error bars indicate standard deviation values for duplicate subsamples for each size fraction, when measured. Stations 3 and 5 had multiple net tows for different days, including different tows at day and night time, and for separate days at the 3 day stations. Tows collected on a second day of the 3 day station are noted with 'b' after the station number.

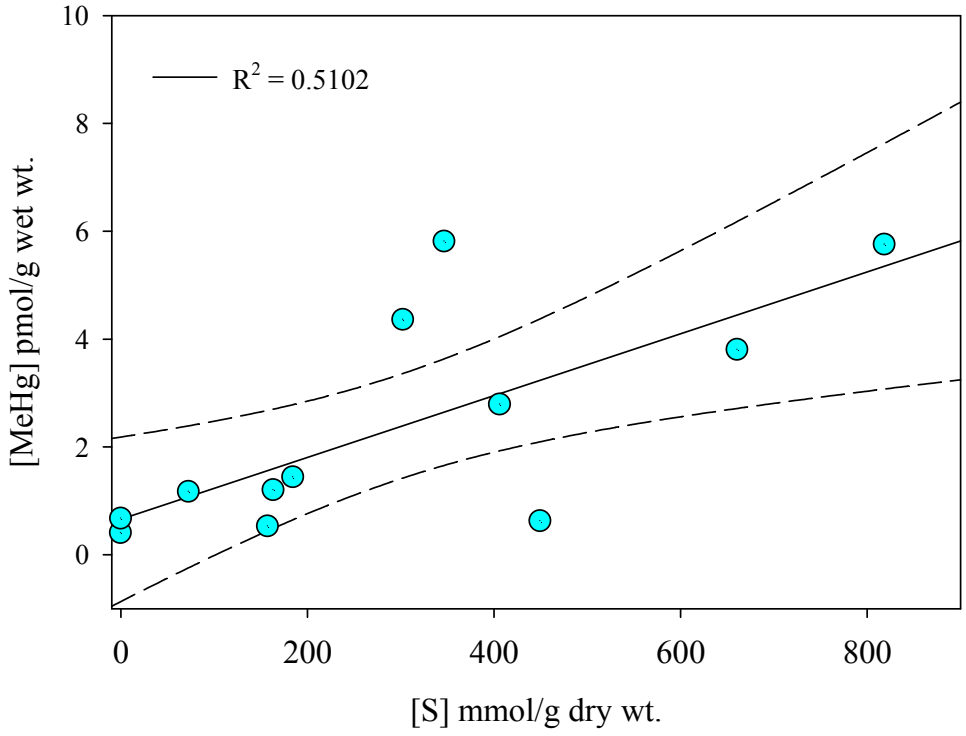

Figure 2.5. **(top)** Linear correlation (and 95% confidence intervals) of elemental sulfur (mmol/g dry wt.) to methylmercury (pmol/g wet wt.) in zooplankton of all size classes at station 5. Linear correlation is significant ($r^2 = 0.5102$; $p = 0.0006$; $n = 12$), indicates that there is a correlation with organic S, presumably within proteins, and MeHg concentration. There was no correlation for elemental sulfur (mmol/g dry wt.) with total mercury (pmol/g wet wt.) in zooplankton ($r^2 = 0.0359$; $n = 12$).

No distinctive difference emerged between day and night zooplankton collections for HgT for all size classes at stations 3 (T-test, p=0.608, n=14) and 5 (T-test, p=0.714, n=34). There was also no difference for MeHg concentrations for all sizes of station 3 (T-test, p=0.561, n=14). On the other hand, all size fractions at station 5 contained higher concentrations of MeHg for night collections of zooplankton (T-test, p=0.0001, n=34). These results were consistent when each size class was evaluated separately. Therefore, it appears that there is no major impact on HgT concentrations in diel migrating zooplankton for this study. However, migration

could influence the MeHg concentrations for zooplankton. The results suggest that zooplankton residing deeper in the water column around the productive upwelling zone of station 5 have higher MeHg concentrations.

Bioconcentration factors for zooplankton were also calculated based on water concentrations for each station, and are reported in Table 2.3. The logBCF values range from 3.7-6.1 for HgT and 4.1-6.5 for MeHg. The logBCFs exhibit a much smaller range than those calculated for phytoplankton, as the overall average was ~5.3. Values are consistent with, though slightly higher than, previously reported numbers, as coastal and estuarine MeHg logBCFs range from 3.8 to 5.3 (Mason et al., 2012). The slightly elevated values are consistent with the higher BCF values found for phytoplankton. Average logBCF values for all size classes are greater for MeHg than HgT at station 1, though conversely all other stations exhibit the opposite tendency. Overall no clear trends emerged based on station or size fractions measured for zooplankton logBCFs. As zooplanktons are believed to acquire Hg and MeHg primarily from their feeding activities, the lack of variation is not unexpected.

Table 2.3. Bioconcentrations factors (logBCFs) calculated for each zooplankton size class, including separate day and night stations, collected throughout the cruise transect. BCFs were calculated based on measured water concentrations for each station (logBCF = zooplankton concentration/water concentration). Both HgT and MeHg values were calculated for each size class measured.

Station	LogBCF HgT *0.2-0.5 mm*	LogBCF MeHg *0.2-0.5 mm*	LogBCF HgT *0.5-1.0 mm*	LogBCF MeHg *0.5-1.0 mm*	LogBCF HgT *1.0-2.0 mm*	LogBCF MeHg *1.0-2.0 mm*	LogBCF HgT *>2.0 mm*	LogBCF MeHg *>2.0 mm*
1	4.00	4.54	4.05	4.79	3.87	4.88	3.77	5.02
3 day	5.22	5.51	5.24	5.76	5.10	4.48		
3b day	5.17	4.91	5.40	5.20	6.03	6.18		
3 night	4.96	5.14	5.27	5.37	4.77	5.57	5.42	4.24
3b night	5.80	6.53	5.51	5.85	5.54	5.30		
5 day	5.53	4.11	5.37	4.43	5.64	4.59	4.98	4.43
5b day	5.60	4.23	5.56	5.16	6.01	4.46	5.73	5.40
5 night	5.63	5.41	5.83	5.55	5.46	5.22	6.11	5.87
5b night	5.65	5.09	5.86	4.35	5.53	5.28	5.78	4.42
6	5.67	4.68	5.86	4.44	5.78	4.15		
7	5.92	5.26	5.98	5.58	5.83	5.36		
8	5.26	4.74	5.15	4.04	5.10	4.52		
9	5.66	5.00	5.23	4.60	5.46	4.81		
12	5.57	4.85	4.84	4.93	5.32	4.84		

2.4 Discussion

2.4.1 Phytoplankton

Previous studies in estuarine and coastal waters have reported logBCF values for MeHg from 3.3 to 4.2, lower than found in this study. Hammerschmidt et al. (2013) found that their values for MeHg logBCF increased from low values around 3.6 to 4.0 to values up to 5.0 at low suspended solid (TSS) concentrations, with the increase becoming apparent at TSS concentrations of <1 mg/L. As estimated TSS concentrations for the equatorial Pacific during our cruise were mostly <1 mg/L, it is apparent that the logBCF's reported here are consistent

with those found in their study off the western North Atlantic Ocean shelf. In addition, concentrations found here (0.5-8.2 pmol/g wet wt) are overall higher than those reported by Hammerschmidt et al. (2013) for their seston at the lower TSS concentrations (0.3-2 pmol/g). Hammerschmidt and Bowman (2012) estimated a concentration for mixed layer seston at the Pacific Ocean intercalibration SAFe site of 4 pmol/g wet wt, which is equivalent to a logBCF of 5.3 based on their measured filtered MeHg concentrations, and analogous to values reported here. Overall, our results and the other limited data for the open ocean suggest that relative concentrations of MeHg in open ocean phytoplankton and the associated BCF's are higher than that of coastal waters, though differences are less than an order of magnitude between coastal and offshore regions.

Higher concentrations for the open ocean, where plankton biomass are lower, are consistent with the idea that concentrations decrease as biomass increases, and growth rates increase (i.e. growth dilution; Pickhard et al., 2004; Hammerschmidt et al., 2013; Driscoll et al., 2012). It is evident that phytoplankton concentrate more Hg and MeHg from the water in the oligotrophic open ocean, suggesting that the MeHg may be more bioavailable in offshore waters. The higher MeHg and Hg concentrations in the larger sized classes of phytoplankton around the HNLC region could be a consequence of those plankton accruing more Hg and MeHg as they attempt to accumulate the limited essential metals (i.e. iron, zinc) of that section. Additionally, there is the potential for enhanced bioaccumulation in offshore waters due to carbon recycling through the biomagnifications of the microbial loop.

Alaternatively, the data could be explained by a further difference between coastal and offshore waters dissolved organic carbon (DOC) concentrations and composition, which is lower, more biologically derived, and less degraded for subtropical and equatorial Pacific waters

(<80 μM; Taki and Suzuki, 2001) compared to coastal and shelf environments (Hansell and Carlson, 2002). These waters likely bind the Hg and MeHg less strongly than terrestrial-derived and humic-rich organic matter, increasing their bioavailability to plankton, as demonstrated by Schartup et al. (2015) when comparing coastal and offshore waters of the North Atlantic shelf.

Another explanation for the relatively high MeHg BCFs is that the area is dominated by small microbes and phytoplankton (< 5μm), which have previously been shown to accumulate higher MeHg than larger organisms. Kim et al. (2012) found MeHg logBCF values, estimated from their reported volume concentration factors and organism size data, to range from 5.1 for the cyanobacteria *Chroococcus minutus* to 3.9 for a large diatom *Stephanopyxis palmeriana*. Overall, the bioconcentration factor increased as cell size decreased. In freshwater, Pichhardt and Fisher (2007) found a similar range in values for the logBCF, from 5.0 for the cyanobacteria *Synechocystis* to 4.3 for larger algal species. Through bioaccumulation modeling Driscoll et al. (2012) showed that the concentration in small phytoplankton (<3 μm) could be up to 5 times that of larger species, a result consistent with culture studies. These differences are equivalent with values found in this study.

The %MeHg in phytoplankton size classes offer interesting insights into possible bioconcentration mechanisms. Methylmercury has been found to reside predominantly in the cytoplasm (>60%) and bind to the proteins within cells (Wu and Wang, 2011; Mason et al., 1996), while inorganic mercury binds preferentially to the outer membranes of cells (>90%), and is thus assimilated at a less efficient rate (Reinfield and Fisher, 1991). Hence, the surface to volume ratio of phytoplankton may be the factor influencing %MeHg for phytoplankton, as smaller phytoplankton have a greater surface to volume ratio, and therefore their MeHg is more bioavailable for accumulation into zooplankton relative to HgT. That appears to be the case with

our measurements, as the <5 µm size class has greatest %MeHg throughout the transect, even though that size class has the lowest concentrations of MeHg. Alternatively the higher %MeHg in that fraction could be an indication of Hg being methylated by bacteria which would be part of the <5 µm fraction. Lehnherr et al. (2011) demonstrated methylation within the high chlorophyll region in the Arctic Ocean, thus it is possible that it could also be occurring in the equatorial open ocean region. Unfortunately no microbial identification was undertaken during this cruise, so differentiation of potential methylating microbes between size fractions remains unknown for this region. However, based on incubation experiments using unfiltered waters, Munson (2014) found methylation at all stations in the chlorophyll maximum zone, suggesting that in situ methylation may account for the higher %MeHg in the <5 µm fraction.

Nutrients and dissolved oxygen water measurements did not bestow insights into factors controlling the variability of phytoplankton Hg and MeHg. However, there were correlations between MeHg concentrations in the phytoplankton and the corresponding water %MeHg, total Hg and MeHg concentrations. Peaks in the %MeHg in the plankton were at stations 3 and 9, which actually had relatively low levels of measured %MeHg in the water column (Figure 2.3c). This may suggest that MeHg uptake into phytoplankton and particulate export reduced the locally dissolved water MeHg concentrations for these sites. Alternatively, the low TSS and likely lower DOC at these sites resulted in higher bioaccumulation. Hammerschmidt et al (2013) reported no correlation between dissolved MeHg and MeHg in microseston (<200 µm), and currently few studies have been able to demonstrate strong relationships between marine water column MeHg and phytoplankton MeHg (Luengen and Flegal, 2009; Sunda, 2012). This markedly reflects the fact that one major driver of MeHg bioavailability in oxic surface waters is DOC concentration, which likely does not vary in a consistent manner with dissolved MeHg.

Freshwater studies have shown that the bioconcentration of MeHg decreases with the DOC concentration based on studies using DOC extracted from numerous locations (Luengen et al., 2012). Variations in DOC potentially accounts for the lack of correlation in the present study.

2.4.2 Zooplankton

Reduced sulfur resides in cellular protein, and is known to bind very strongly to Hg and MeHg through thiol ligands (Ravichandran, 2004; Yoon et al., 2005). The weak association with S indicates that diet and other factors are additionally important to zooplankton MeHg bioaccumulation, and predominantly more so for HgT bioaccumulation for these organisms. Chen et al (2008) noted that physical and ecological processes were more important than sources regarding MeHg transfer and bioaccumulation in marine food webs among estuarine and coastal regions. Additionally, laboratory experiments by Lawson and Mason (1998) showed that while dissolved speciation of MeHg impacted assimilation into phytoplankton, trophic transfer to zooplankton was not influenced by water column speciation. Our results from the analysis of open ocean plankton further strengthen this conclusion.

Only Station 1 appeared to display an increase in MeHg concentrations and corresponding decrease in HgT concentrations for increasing size classes. For the rest of the stations, this trend of increasing bioaccumulation with larger size fractions of zooplankton was inconsistent. Though the measured concentrations were variable from station to station, the %MeHg for zooplankton was relatively continual across the transect. The smallest size class (<0.5 mm) consistently had the lowest %MeHg. Furthermore, the %MeHg for the larger size classes amplified with increasing size fractions (Figure 2.3b). This trend was most prominent at station 1, which was the station closest to Hawaii, and station 5, the upwelling zone. The greater

%MeHg in the largest zooplankton is suggestive that these animals could be consuming smaller zooplankton rather than phytoplankton, or phytoplanktons which contain more %MeHg.

Due to the commonly higher MeHg concentrations in sub-thermocline waters compared to the mixed layer (Mason et al., 1990; Munson et al., 2015; Mason et al., 2012) it was hypothesized that zooplankton living at depth would have higher concentrations than those at the surface. The results obtained here suggest that their vertical migration and feeding at the surface mitigates any influence of residing in deeper waters during the day for station 3. This could also be a reflection of the relatively consistent MeHg concentrations through the water column at station 3. Nonetheless, the distinctly higher concentrations of MeHg in night collections of zooplankton at station 5 could be demonstrating this effect. The highest MeHg concentration for water was found below ~600 m at station 5, much deeper then zooplankton collection for this study. However, zooplankton can swim hundreds of meters within several hours (Brierley, 2014; De Robertis et al., 2003), which could be sufficient for animals residing in the deeper, high MeHg waters to have migrated into nighttime capture.

Water concentrations of HgT and MeHg were fairly low throughout the transect (Munson et al., 2015). As noted above, there was a slight increase in both the MeHg and %MeHg for station 5 (>0.05 pM and >10%, relatively), which corresponds to the increase in zooplankton %MeHg at that station (Figure 2.3b). While there was an additional %MeHg enhancement measured in all size classes of phytoplankton at station 5, there were additional peaks in stations 3 and 9 as well for the <5 μm phytoplankton size class, which did not further correspond with water or zooplankton trends. However, these results should not be over-interpreted, considering that phytoplankton were collected from only one depth, while zooplankton are likely feeding throughout the mixed layer.

Cumulative biomass collected for each tow at station 5 was substantially more plentiful than any other station, arriving aboard looking like a thick plankton stew brewed in the cod end of the deep, which was a striking visual indication of productivity for the upwelling region of the equatorial Pacific. Considerable labile dissolved organic material can be produced via 'sloppy feeding' by zooplankton (Steinberg et al, 2004), in addition to ample fecal material and discharge resulting from turnover of dissolved organic carbon through the microbial loop (Møller and Nielsen, 2003). The presence of zooplankton has been shown to increase microbial productivity, likely as a result of increased derivatives of labile dissolved material (Peduzzi and Herndl, 1992). Algal decay has also been shown to release MeHg in natural waters (Luengen and Flegal, 2009), thus it is possible that ruptured and processed algae particles can further contributed to enhanced water MeHg within the mixed layer. As a result, it is feasible that the abundant zooplankton in this region of Station 5 could have contributed to amplified MeHg within the deeper water column by providing a significant input of processed and labile organic matter raining down from the surface ocean. This could potentially fuel microbial methylation in the deeper waters, especially below the oxygen rich euphotic zone. Higher sub-thermocline MeHg concentrations are found in the upwelling region, and mass balance estimations indicated it is mostly derived from in situ production, rather than due to transfer with sinking particulate (Sunderland et al., 2009; Mason and Fitzgerald, 1993). These potential sources of MeHg are evident in the large peak of %MeHg in the water column at the surface and directly deep below the prolific upwelling zone in Figure 2.3c (Munson, 2014).

This research reports on the first attempt at quantifying Hg and MeHg in different phytoplankton and zooplankton size fractions in the open ocean, a region where plankton are relatively scarce compared to the more scrutinized coastal environment. Overall the results

reported provide important clues into the sources and uptake of Hg and MeHg into phytoplankton, and transfer to zooplankton, as well as how plankton size impacts these relationships in the open ocean. However, results presented here have also highlighted the many gaps and questions that plankton Hg and MeHg bioaccumulation research needs to further investigate. Future research and modeling studies should build on these initial results to further elucidate open ocean Hg and MeHg cycling at the base of the marine food chain.

Chapter 3:

Seasonal Trophic Transfer Dynamics of Mercury species in Zooplankton and Phytoplankton of Long Island Sound

3.1 Introduction

Methylmercury (MeHg) is a notorious bioaccumulating neurotoxin, which causes brain and neurological damage in humans and detrimental effects on wildlife (Clarkson and Magos, 2002; Wolfe and Daniel, 1998). For this reason MeHg is a major concern for ecological and human health, and a topic which should especially be an issue for prominent fish consumers. The foundation of MeHg bioaccumulation begins at the base of the food chain in marine systems, where phytoplankton and zooplankton initiate the accretion inventory. Uptake at the base of the food chain is influenced by mercury (Hg) concentrations and speciation in the water column, as well as MeHg complexation (Mason, 2002; Chen et al., 2008).

In estuaries methylation is believed to take place in anoxic sulfide rich sediments (Merritt and Amirbahman, 2009; Heyes et al., 2006; Hammerschmidt et al., 2008), though there is evidence that it is also occurring within the water column (Lehnherr et al., 2011; Pucko et al., 2014; Schartup et al., 2015b; Ortiz et al., 2015). Methylation potential for a region can be driven by the supply of inorganic Hg and the bioavailability of Hg to methylating organisms, which is affected by factors such as organic matter content and sulfide levels (Benoit et al, 2003; Lambertsson and Nilsson, 2006). Temperature can also be a driver of methylation potential, due to the effective positive response of Sulfide Reducing Bacteria (SRB) to warmer environments (King et al., 1999). The role of organic matter in the water has been highlighted as an unknown factor for MeHg dynamics in marine systems and food chains (Luengen et al., 2012; Ravichandran, 2004). As a result, there is evidence for higher MeHg concentrations in sediment during warmer seasons (Balcom et al., 2008; Gosnell et al., 2015), which can be further released into the coastal system. Hypothetically, higher concentrations of MeHg within the water column could result in more uptake of MeHg at the base of the food chain.

In temperate climates Hg, MeHg, oxygen and organic matter levels can all experience large variations with season (Anderson and Taylor, 2001; Balcom et al., 2008; Gosnell et al., 2015). During the spring there is enhanced primary productivity in the water column as bottom nutrients are stirred up into the euphotic zone, and this input increases growth of plankton into a spring bloom. Springtime euphotic waters can have amplified dissolved oxygen concentrations resulting from increased photosynthesis rates. As nutrient supplies decrease through uptake, the spring bloom eventually begins to wane and productivity dies off. Subsequently, the remineralization of these organisms can decrease oxygen concentrations in the water, and result in low-oxygen and anoxic regions in the late summer (Anderson and Taylor, 2001; Latimer et al., 2014). The seasonal flux of carbon into the sediments can also influence microbial activity, by providing nutritional substrate and influencing habitual redox conditions. Fall plankton blooms are also possible for temperate regions, when passing storms (and winds) stir up residing nutrients which have been amplified from decaying summer blooms. If methylation potential is affected by the types of organics and oxygen levels in the water column, then it is possible that the MeHg being taken up in fall plankton blooms has a different signature than MeHg entering the spring bloom. While organic matter in the LIS fall bloom is typicall remineralized at low oxygen levels, organics formed in the springtime are sourced differently. Hypothetically, if lower oxygen and warmer temperatures enhance methylation in the sediment and water column, then there could be more Hg methylation being undertaken in the summer. As there tends to be less biomass during the summer, there could also be enhanced uptake within the available plankton, resulting in increased %MeHg within biomass.

Chen and Folt (2005) experimentally demonstrated that larger plankton blooms can lead to decreased MeHg uptake via ‘bloom dilution’. As plankton tend to follow a seasonal pattern of

prospering and attenuating, it is important to investigate if seasonal blooms in the natural environment can help to reduce bioaccumulation of MeHg within the plankton population. Methylmercury bioconcentration in phytoplankton is the most concentrated step in the marine food chain, thus it is important to determine potential impacts on the water column where plankton accrue MeHg and Hg. Methylmercury is then transferred trophically to zooplankton, and on to smaller fish, and then MeHg is biomagnified at each trophic level on the way up to larger predators, including humans (Mason, 2002; Kim et al, 2004; Berntssen et al, 2003). Relatively little data has been published on in situ factors influencing MeHg transfer from primary producers to secondary consumers in coastal systems (Hammerschmidt et al., 2013; Foster et al., 2013). Furthermore, there have not been investigations into any potential seasonal effects in plankton bioaccumulation. Thus, little data has been collected to determine how nutritional and physical parameters in the water column facilitate MeHg uptake into the lower trophic levels of the food chain, and if there are any seasonal effects on uptake and biomagnification. Thus, considerable uncertainty exists for the behavior of Hg and MeHg at the lower levels in these marine ecosystems beyond a single investigation collection.

For this study we present different size fractions of phytoplankton and zooplankton for the spring, summer and fall seasons within Long Island Sound (LIS). We also collected stable isotope data for C, N and S to measure trophic level and consumption patterns within the zooplankton. Stable N isotopes ($[^{15}N/^{14}N]$; $\delta^{15}N$ ‰) have been used through numerous studies as a proxy for trophic positon (Foster et al, 2012; Post, 2002; Fry, 1988; Senn et al., 2010), with heavier isotopes indicating consumption higher on the food chain. Stable C isotopes ($[^{13}C/^{12}C]$; $\delta^{13}C$ ‰) can help elucidate the different sources of food for the consumers (Fry, 2006; Michener and Shell, 1994). Depleted $\delta^{13}C$ ‰ values in plankton could be related to terrigenously derived

carbon, while marine sources tend to be more enriched (Peterson et al., 1985). However, it is also expected that zooplankton $\delta^{13}C$ values reflect dietary sources from phytoplankton and particulate material (Michener and Shell, 1994; Fry, 1988). Stable S isotopes ($[^{34}S/^{32}S]$; $\delta\,^{34}S$ ‰) have previously been used to discern benthic sources into the food chain (Fry, 1988; Peterson et al., 1985).

In LIS, located off the north east coast of the United States, bottom waters of the western and eastern endpoints typically experience distinguishable variations in summer hypoxia. The western end of the sound (WLIS) is more enclosed, and situated proximate to dense urban populations and contaminants (including Hg), as well as ample nutrient runoff. The WLIS thus tends to experience hypoxic regions during the summer compared to the eastern end (Anderson and Taylor, 2001; Latimer et al., 2014), which is less populated and more pristine. The eastern end of LIS (ELIS) is near to the race, where Atlantic shelf water cycles through LIS via tidal and circulative forcing. An estuarine circulation pattern (bottom inflow and surface outflow) exists in ELIS throughout the year. During winter bottom waters can travel well into central LIS to upwell against the coast, but summertime westward progress of bottom waters typically does not extend past the central sound region (Latimer et al., 2014). Numerous rivers empty into LIS in addition to the marine water flushing in from the rise, thus there are several possible nutritional and material sources into LIS. It was hypothesized that these regional and seasonal variations could factor into the methylation potential and accumulation within the various size fractions of plankton.

3.2 Methods

3.2.1 Sample Collection

Samples were collected during 2014 to represent three separate seasons: May 20 - 22 (Spring), August 2 - 5 (Summer) and September 9 - 11 (Fall) in LIS. Plankton and water samples were collected from stations in the Western (WLIS) and Eastern (ELIS) Sound in the spring and summer, while the fall cruise was extended to include stations within the Central Long Island Sound (CLIS), in addition to a station along the shelf break (SB) and mid-shelf (MS) region along the Eastern coast outside of Long Island Sound. Locations of the stations are displayed in Figure 3.1. Triplicate substations were collected during the spring and summer seasons for WLIS and ELIS approximately one mile apart at each site, while duplicate substations were collected at WLIS, ELIS and MS during the fall.

Water samples were collected using a GoFlo bottle attached to a Kevlar line, and deployed by hand off the side of the vessel. A trace-metal clean rosette system was used for the fall cruise. Water was transferred into clean cuvette containers for the cruise duration, and kept cool and dark until being processed on board or back in the laboratory at UConn. Water samples were collected for one substation or station at each site. Water samples were collected at the depth where phytoplankton was sampled, the surface chlorophyll max, in addition to a deeper collection for each station (except CLIS). Water samples were filtered through 0.22 μm quartz fiber filters (QFF) using a 1 L trace-metal clean Nalgene filter apparatus. Water samples were collected for nutrients, chlorophyll a, total suspended solids (TSS), dissolved organic carbon (DOC), dissolved Hg and MeHg, as well as particulate Hg and MeHg. A CTD attached to the Kevlar line was also deployed at each station, and recorded the fluorescence, oxygen, temperature and salinity for each station in profile.

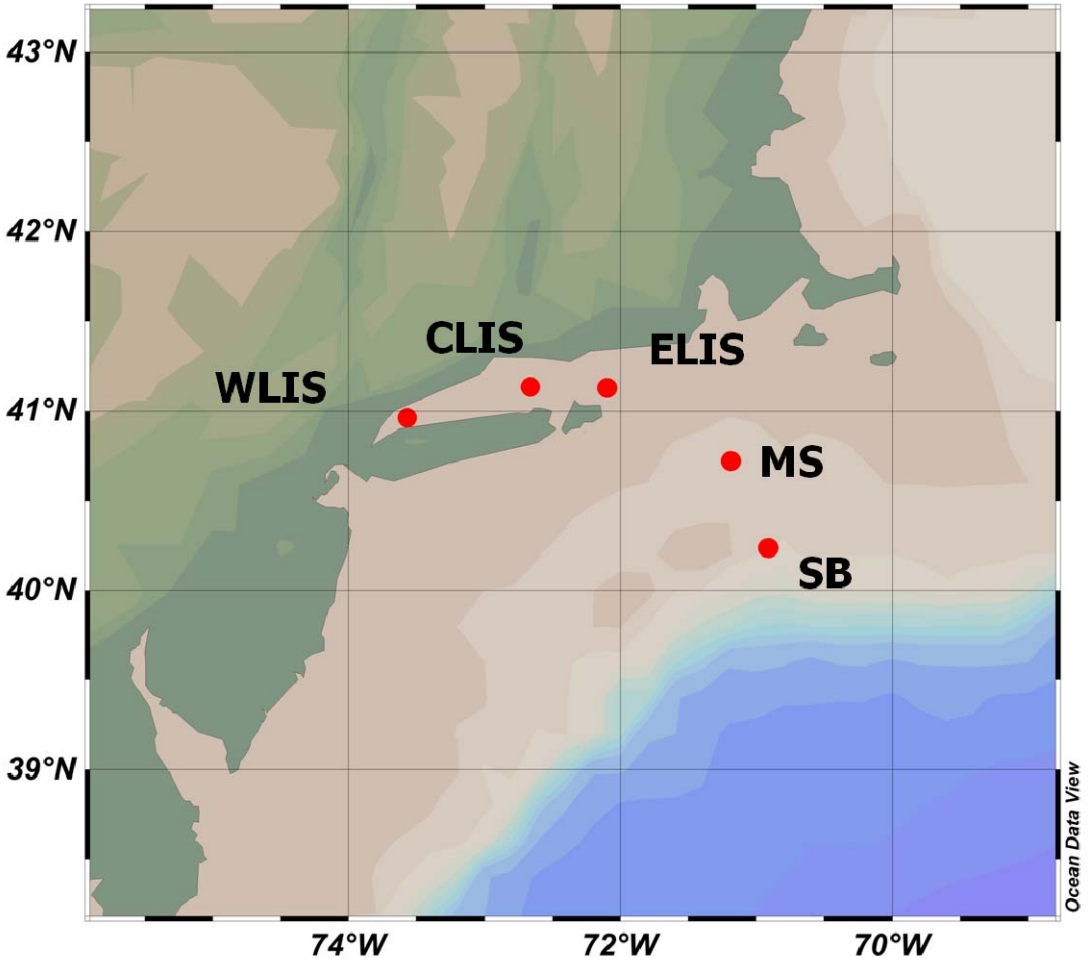

Figure 3.1. Map of stations sampled for each season in Long Island Sound (LIS) and during the fall cruise during 2014.

Water used for collecting phytoplankton was obtained out of trace-metal clean GoFlo bottles. Separate phytoplankton fractions were obtained by filtering the seawater through polycarbonate filters of sizes 0.2 µm, 5 µm and 20 µm. Larger potential contaminants or organisms were obstructed by initially filtering the samples with a 200 µm mesh. Chlorophyll a samples were collected for each size fraction of phytoplankton using the approximate same

volume of water. This resulted in size fractions of phytoplankton of 0.2 – 5 μm (which includes pelagic microbes), 5 – 20 μm, and larger seston of 20 – 200 μm (referenced as >20 μm).

Zooplankton were collected at each station by attaching an opening-closing 200 μm net (Seatec) to the Kevlar line and deploying it to ~2 m above the bottom, then up to ~1 m from the surface, where it was closed and hauled aboard. Depths deployed ranged from 11 m in LIS, to 100 m deep along the MS and SB stations. Zooplankton were separated into size fractions of 0.2 – 0.5 mm (200 – 500 μm), 0.5 – 1.0 mm, 1.0 – 2.0 mm and >2.0 mm via mesh screens of the different noted sizes. For sample fractions with substantial biota, the sample was divided into multiple subsamples for analysis. Multiple subsamples of zooplankton biota were also collected at every size fraction for stable isotope analysis of C, N, and S on pre-combusted 25 mm glass fiber filters (GFF; 0.45 μm).

3.2.2 Analytical

Phytoplankton and zooplankton were measured for Hg and MeHg for each seasonal size fraction. Each sample was weighed, then digested in 4.51 M HNO_3 acid solution in a 60 ^oC water bath for at least 12 hours prior to analysis. For MeHg analysis, a 1 mL aliquot of the digest was neutralized with ~550 μL of 10 N KOH to a reactive pH of ~4.9, and diluted to a final volume of 30 mL in a glass vial using ultra pure 18.2 MΩ water. Ammonium acetate buffer (4M; 225 μL) and sodium tetraethylborate (30 μL) were added to each sample to induce derivative Hg species formation. Samples were then analyzed on a Tekran auto-methylmercury analyzer using cold vapor atomic fluorescence (CVAFS), based on an external calibration curve (r^2>0.998). Replicate samples were analyzed during each analysis, with relatively good agreement. The RSD was 6.7%, and the detection limit was 0.016 pmol MeHg.

For total Hg analysis (HgT), the remaining digest was diluted using with 18 MΩ water, and brominemonochloride (50 µL) was added for at least 24 hours preceding analysis to further decompose organic matter and convert all Hg into the ionic form. Hydroxylamine hydrochloride (25 µL) was added to each sample at least 1 hour before analysis to degrade excess oxidant. A sample aliquot was added to a sparging flask filled with ~ 100 mL 18.2 MΩ water with 100 µL of a tin chloride reductant, then purged with N_2 onto a trap containing gold-coated beads. The mercury was quantified and measured using a Tekran 2500 by CVAFS, with an external calibration curve (r^2>0.999). The RSD was determined to be 2.9% for total Hg, with a detection limit of 0.091 pmol.

Chlorophyll analysis was completed for all of water and phytoplankton samples. Chlorophyll was extracted by digesting the filters in 5 mL of 90% acetone for 18 hours in the dark (5 ^{o}C). Filters were removed, and chlorophyll was measured on a Trilogy fluorometer. After all of the initial fluorescence was recorded, ~10% HCl was added to each sample, and phaeopigment values were measured after 90 seconds. Water samples were measured for nitrate and nitrite on a Unity Scientifics' SmartChem nutrient analyzer, and DOC was measured on a Shimandzu TOC/TN instrument.

Zooplankton subsamples for δ^{13}C and δ^{15}N isotopes were dried on the 25 mm GF/F filters at ~60 ^{o}C for at ~48 hours, then weighed and wrapped in tin foil capsules. Dried samples were analyzed on a Costech 4010 elemental analyzer coupled with a thermo Scientific Delta V Advantage IRMS operator in Continuous Flow mode (EA-IRMS), using both USGS 40 and USGS 41 (L-Glutamic Acid) stable isotope standards for quantification. Samples were analyzed in batches of 20, with blanks ran before and after each sample to minimize potential carryover.

Sulfur isotope analysis was separately undertaken at the University of Purdue using the IAEA S1, IAEA S2 and IAEA S3 standards.

3.3 Results

3.3.1 Phytoplankton & Water Column

Phytoplankton concentrations of HgT were consistently lowest in the 0.2-5 μm size fraction, and did not exceed 1.5 pmol/g (wet wt.) for any season at both WLIS and ELIS (Figure 3.2 and 3.3). Concentrations increased with larger fractions, and the largest size fraction of seston (> 20 μm) had the highest concentrations of HgT throughout all LIS seasons. Overall, HgT concentrations in all size fractions of ELIS phytoplankton were much higher than for WLIS, for both HgT and MeHg. When each specific size fraction was compared seasonally, there was a significant difference between WLIS and ELIS phytoplankton Hg and MeHg concentrations for spring and summer (t-test, $p<0.05$), but not for the fall season 0.2-5 μm fractions (t-test, $p>0.1$). Even though water concentrations were overall lower for ELIS (Table 3.1), the %MeHg (MeHg/Hg) in the phytoplankton (3-13%; Table 3.2) was higher than that of the water (mostly <1%; Table 3.1) indicating a preferential accumulation of MeHg over inorganic Hg for phytoplankton in LIS.

Concentrations of MeHg in phytoplankton of the 0.2-5 μm size fraction were also lowest for both WLIS and ELIS, and consistently low for all seasons. The >20 μm size fraction also had the highest concentrations of MeHg for all seasons, though it was less pronounced for ELIS during the spring, as the 5-20 μm size was of comparable magnitude (Figure 3.3). As a result, there was no indication of seasonal impact of water column methylation on the MeHg

concentration within the size fraction which would include microbes (0.2-5 μm) in WLIS (Figure 3.2), though there was evidence for a potential summer season increase in %MeHg in the more highly flushed waters waters of ELIS. This is also true for the larger size fractions, which would include any 'marine snow' or aggregates that were found to be important location for in situ methylation in laboratory experiments by Ortiz et al. (2015).

Clear trends are visible for concentrations of HgT and MeHg in the size fractions collected during the fall cruise (Figure 3.4). The largest size class collected is consistently highest for each station, with station ELIS containing the highest concentration measured for phytoplankton HgT (15.4 pmol/g (wet. wt.)). The intermediate size fraction is relatively consistent through the LIS stations, slightly increases at the MS, and markedly increases at the SB station of the less turbid marine waters. The 0.2-5 μm size class is highest at the SB station, exceeding 2.1 pmol/g (wet wt.), while the 5-20 μm size class is clearly highest at the SB station at 12 pmol/g (wet wt.) for HgT. For specific size class comparisons of the fall cruise, there was a significant difference between stations for the 0.2-5 μm MeHg and 5-20 μm HgT concentrations (ANOVA, $p<0.001$), but no statistical significant difference found for 0.2-5 μm HgT or 5-20 μm MeHg concentrations (ANOVA, $p>0.2$). Furthermore, there was a significant difference between the LIS stations and shelf stations (t-test, $p<0.05$).

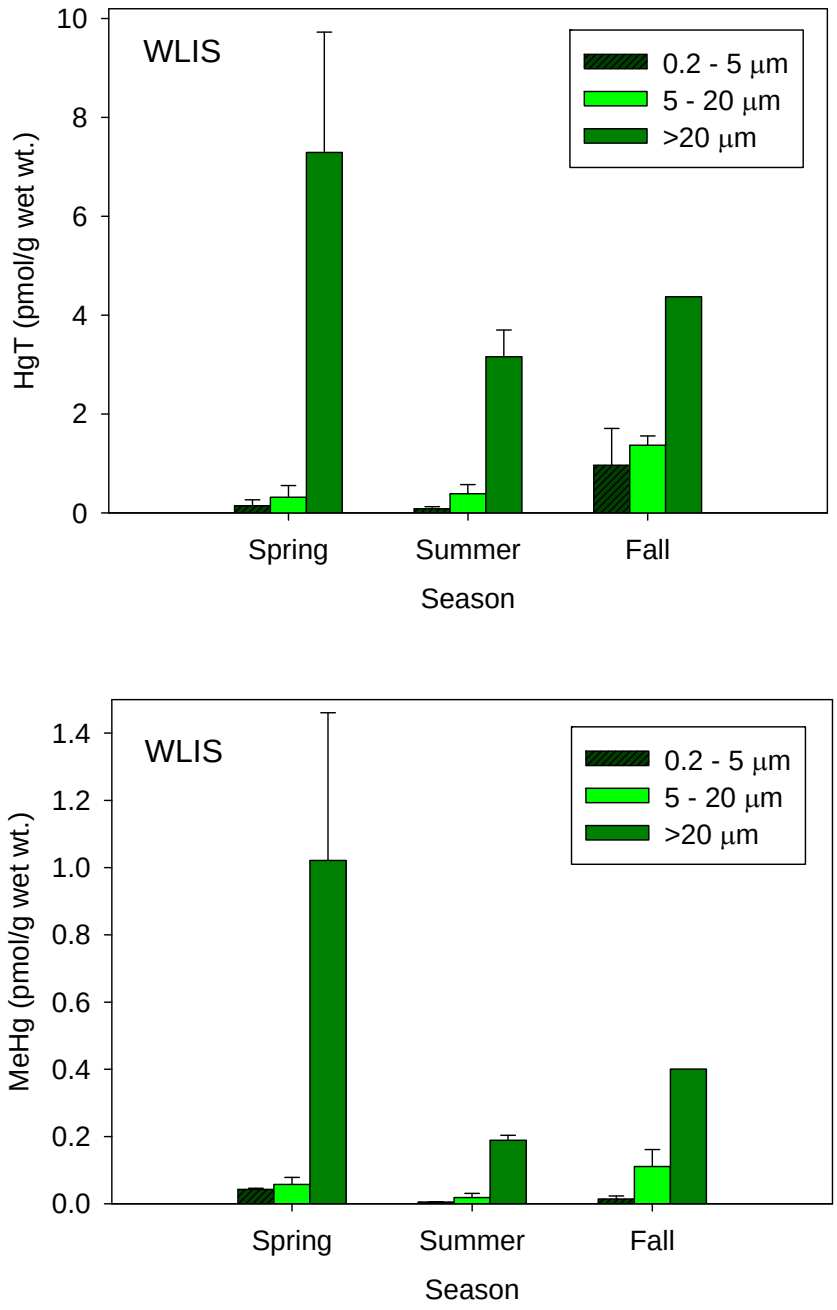

Figure 3.2. Inorganic mercury (HgT; top) and methylmercury (MeHg; bottom) concentrations for each size fraction of phytoplankton measured per season at WLIS. Error bars reflect standard deviation between 3 separate substations at each site.

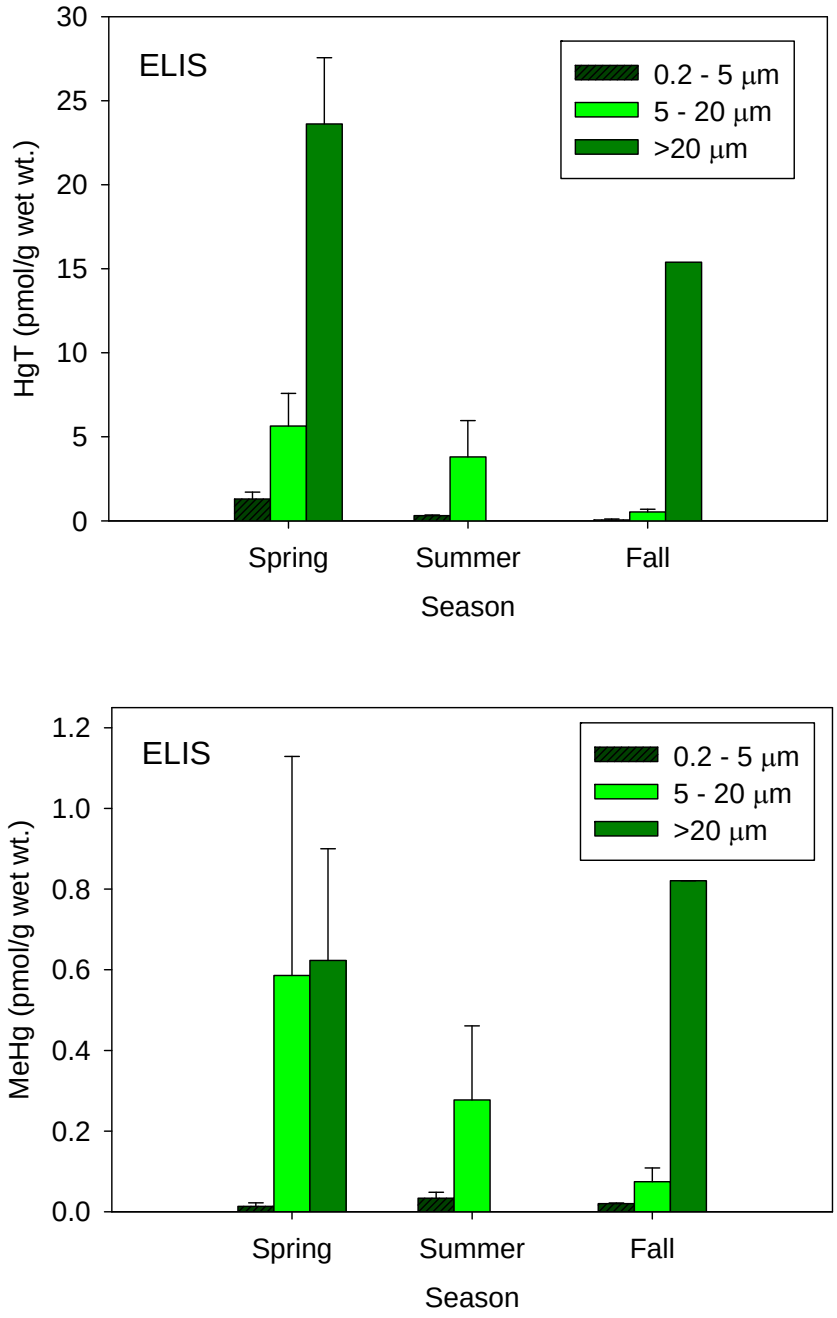

Figure 3.3. Inorganic mercury (HgT; top) and methylmercury (MeHg; bottom) concentrations for each size fraction of phytoplankton measured per season at ELIS. Error bars reflect standard deviation between 3 separate substations at each site.

Methylmercury concentrations during the fall cruise also indicate a difference between shelf and sound stations, as the MS and SB stations are respectively 0.5 and 0.6 pmol/g (wet wt.), much higher for the 5-20 μm sizes than the ~ 0.1 pmol/g (wet wt.) concentrations measured in LIS (Figure 3.4). As was seen for the HgT, the highest concentration measured for MeHg was in the >20 μm fraction at ELIS in fall (0.82 pmol/g (wet wt.)). The MS station had the highest concentration for the 0.2-5 μm size fraction. These higher concentrations in phytoplankton could be a direct indication of the higher HgT and MeHg concentrations in water being advected into the surface, as sub-thermocline water masses containing higher concentrations of Hg and MeHg could be transported and upwelled along the coastal shelf along the shelf break (Hammerschmidt et al., 2013). Nonetheless, the MeHg was relatively low in the water measured during collection (<0.009 pM; Table 3.1). However this lower concentration could be a result of MeHg being transferred into the plankton as biomass increases rapidly leaving less remaining in the water column, rather than consistently low values.

Water HgT concentrations were highest during the spring at WLIS (4.31 pM), though WLIS had lower HgT concentrations in the summer and fall (Table 3.1). The ELIS stations had more consistent HgT concentrations, though were also lower during fall (1.22 pM). The shelf stations had lower HgT values than LIS, but MeHg concentrations were comparable to those in LIS. Overall the highest MeHg water concentrations were measured in WLIS spring (0.02 pM) and fall (0.022 pM) seasons, and summertime MeHg water concentrations were actually lowest for both WLIS and ELIS. The %MeHg values were below 1% for nearly all the LIS water measurements, as well as the MS station. The only %MeHg which was significantly high was the deep (50 m) SB sample, at 2.9%.

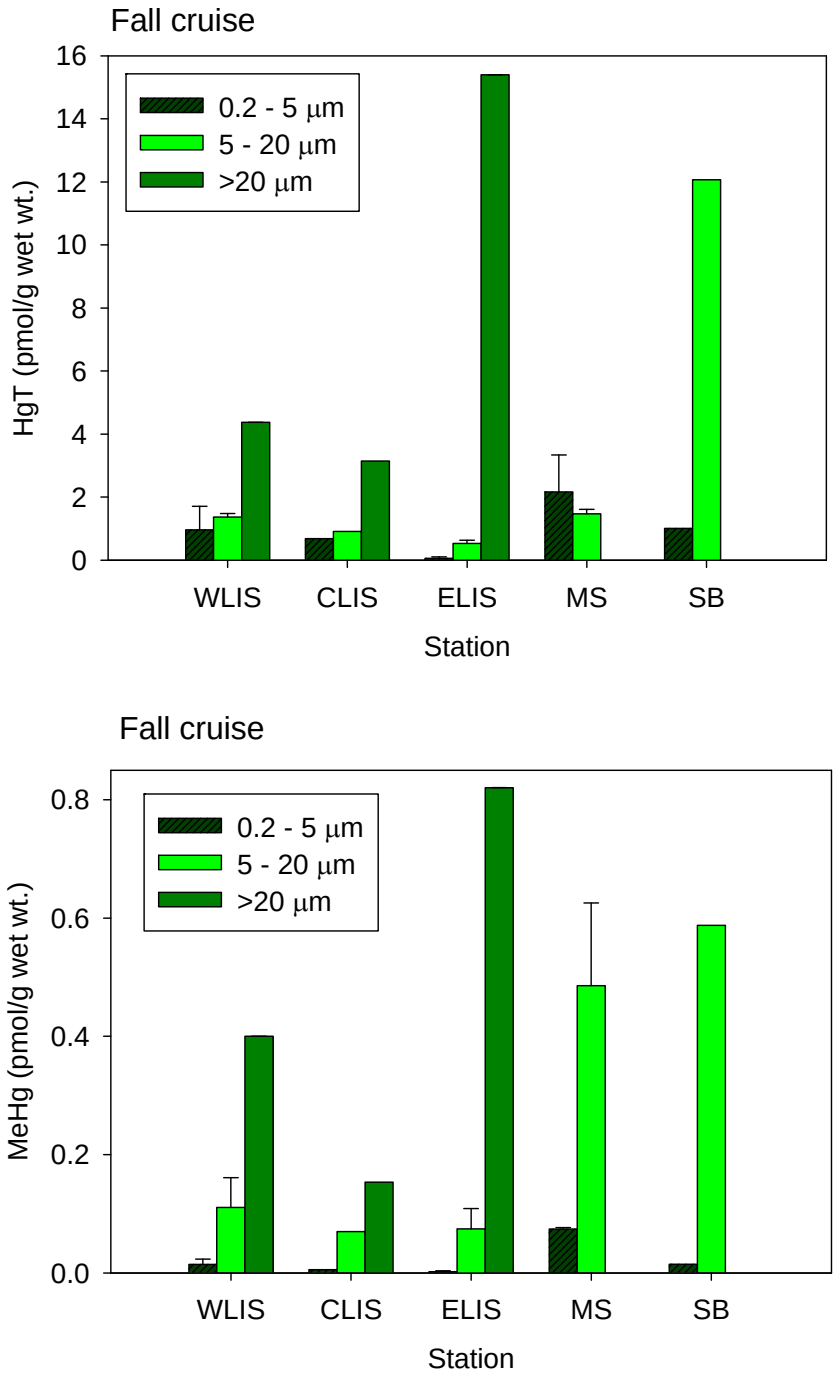

Figure 3.4. Inorganic mercury (HgT; top) and methylmercury (MeHg; bottom) concentrations for each size fraction of phytoplankton measured at each station during the fall cruise.

Table 3.1. Water column measurements for LIS and shelf break sampling through the seasons. Depth which phytoplankton and surface water were collected from is also presented. The other deeper depth water collected and measured at each station is also displayed in parenthesis.

Station - Season	Depth (m)	HgT (pM)	MeHg (pM)	%MeHg (%)	DOC (µM)	NO_3^- (µM)	Chl a (µg/L)
WLIS – Sp.	10 (15)	4.31 (2.66)	0.02 (0.023)	<1 (<1)	246 (225)	nd	1.19 (0.28)
WLIS – Sum.	10 (15)	1.56 (1.68)	0.016 (0.009)	1.03 (<1)	160.9 (199.3)	nd	0.94 (2.78)
ELIS – Sp.	4 (12)	2.45 (2.35)	0.014 (0.009)	<1 (<1)	128.8 (151)	nd	0.04 (0.05)
ELIS – Sum.	4 (12)	2.68 (1.75)	0.002 (0.02)	<1 (<1)	172.2 (173.6)	nd	0.98 (1.31)
WLIS – Fa.	10 (15)	1.89 (1.72)	0.022 (0.033)	1.16 (1.75)	209 (212)	3.65 (1.22)	0.73
CLIS – Fa.	7	1.77	0.015	<1	200	0.9	0.88
ELIS – Fa.	4 (12)	1.22 (1.49)	0.015 (0.009)	1.23 (<1)	222 (200)	nd	0.18
MS – Fa.	10 (40)	1.03 (0.67)	<0.009 (0.01)	<1 (<1)	80.3 (177.7)	nd	0.02
SB – Fa.	10 (50)	0.75 (1.07)	0.013 (0.031)	1.73 (2.9)	210.6 (210.2)	nd	0.002

Nitrate (NO_3^-) and nitrite (NO_2^-) concentrations were measured for the waters of each station and season, and the majority of measurements were below detection, primarily during spring and summer seasons (Table 3.1). During fall, concentrations were measured in surface samples of WLIS at 3.65 µM NO_3^- (and 1.8 µM NO_2^-), while the 12 m depth contained 1.22 µM NO_3^- (0.76 µM NO_2^-). The CLIS station also had measurable levels of ~0.9 µM NO_3^- (0.1 µM NO_2^-). Clearly only fall WLIS and CLIS had an excess of NO_3^- and NO_2^- where it could be detected in the water column, and was not all utilized by algae. Any available nutrients at the other stations and seasons could have had limited availability, or were apparently consumed by phytoplankton, leaving nothing measureable in the water column.

The DOC was highest in WLIS during the spring (264 µM), and decreased during the summer before increasing again in fall (Table 3.1). At ELIS the DOC was highest during fall (222 µM). Both WLIS and ELIS had relatively consistent DOC concentrations with depth. The MS station had much lower DOC than any other station (80.3 µM), though the SB station was comparable to LIS concentrations with levels reaching 210 µM DOC.

The ratio of organism to water concentrations (Bioconcentration factors; logBCFs) for phytoplankton HgT are presented in Table 3.2, while the values for MeHg are in Table 3.3. Overall the ranges are relatively consistent, and the values for some some of the larger algae fractions fall in line with previously reported coastal values (Hammerschmidt et al., 2013), though the 0.2-5 µm size fractions are all lower, besides the MS station. The logBCFs for HgT range from ~2.4 to 5 while the MeHg values are slightly higher at ~ 2.6 to 5.5. Furthermore, the logBCF values consistently increase with each larger size fraction for both Hg and MeHg, and this trend is present throughout the seasons and in both WLIS and ELIS.

Table 3.2. Average HgT bioconcentration values (logBCF) for phytoplankton during the seasonal LIS and fall shelf cruises.

Size	WLIS Spring	WLIS Sum.	WLIS Fall	ELIS Spring	ELIS Sum.	ELIS Fall	CLIS Fall	MS Fall	SB Fall
0.2-5 µm	2.38 ± 0.14	2.94 ± 0.32	2.62 ± 0.28	3.19 ± 0.20	2.65 ± 0.18	2.81 ± 0.21	2.56	3.61 ± 0.31	3.12
5-20 µm	2.61 ± 0.54	3.03 ± 0.61	3.26 ± 0.21	3.56 ± 0.31	3.24 ± 0.34	3.26 ± 0.27	3.66	3.53 ± 0.35	4.21
20-200 µm	4.79 ± 0.22	4.47 ± 0.31	4.31	4.98 ± 0.06		4.11	4.01		

Table 3.3. Average MeHg bioconcentration values (logBCF) for phytoplankton during the seasonal LIS and fall shelf cruises.

Size	WLIS Spring	WLIS Sum.	WLIS Fall	ELIS Spring	ELIS Sum.	ELIS Fall	CLIS Fall	MS Fall	SB Fall
0.2-5 µm	2.87 ± 0.78	2.55 ± 0.15	2.77 ± 0.28	2.92 ± 0.26	3.25 ± 0.16	2.87 ± 0.21	2.58	4.41 ± 0.65	2.67
5-20 µm	3.91 ± 0.89	3.49 ± 0.23	4.13 ± 0.21	4.81 ± 0.31	4.41 ± 0.21	3.67 ± 0.36	2.72	4.47 ± 0.63	4.28
20-200 µm	4.79 ± 0.35	4.76 ± 0.21	5.03	5.47 ± 0.23		5.11	3.24		

3.3.2 Zooplankton

The HgT in WLIS zooplankton was quite low, residing below 20 pmol/g (wet wt.) for all size classes of zooplankton for both spring and summer (Figure 3.5). Concentrations of HgT in WLIS increased prominently in the fall, with the 0.5-1.0 mm and 1.0-2.0 mm size classes showing the greatest increase. Fall WLIS HgT concentrations were equivalent to those found in ELIS (t-test, $p>0.1$) for all size classes.

Methylmercury WLIS concentrations on the other hand, tended to be highest (~5.5 pmol/g (wet wt.)) in the intermediate size fractions except in the fall season, where the >2.0 mm size fraction increased to ~5.9 pmol/g (wet wt.). Statistical differences for MeHg concentrations between WLIS and ELIS were less robust, with only the spring 0.2-0.5 mm and 1.0-2.0 mm, and summer 0.5-1.0 mm fractions demonstrating significant divergence (t-test, $p<0.05$). However, comparing seasons between each station for specific size classes did demonstrate that there were significant differences for MeHg concentrations for 1.0-2.0 mm zooplankton in WLIS (ANOVA, $p<0.001$), and the 0.2-0.5 mm and 0.5-1.0 mm fractions for ELIS (ANOVA, $p<0.03$). During the 2014 summer there was a prominent ctenophora bloom throughout LIS, and this resulted in the

very low ~0.16 pmol/g (wet wt.) MeHg value for the >2.0 mm size fraction during that season, as measurements are calculated on a wet mass basis (ctenophores are ~95% water).

In ELIS almost all the HgT zooplankton size fractions were much higher than in WLIS and clustered around 20 – 45 pmol/g (wet wt.) concentrations for both the spring and summer seasons (Figure 3.6). Examining comparisons for the specific WLIS versus ELIS size classes yielded that population concentrations were statistically different for the spring and the summer (t-test, $p<0.05$), excluding the 0.5-1.0 mm size which displayed no difference between the LIS endpoints (t-test, $p>0.1$). None of the zooplankton size fractions were statistically different for Hg and MeHg concentrations between WLIS and ELIS for fall sampling (t-test, $p>0.5$).

During the fall cruise, the 0.2-0.5 mm size fraction remained relatively consistent in HgT for all sites (ANOVA, $p>0.5$), while the three larger size fractions increased from the summer concentrations. The increase was most prominent in the 0.5-1.0 mm size fraction during fall (t-test, $p<0.05$), nearly doubling the HgT concentration up to ~80 pmol/g (wet wt.). The LIS net collections tended to not have ample amounts of large zooplankton present, and were primarily dominated by smaller and intermediate copepod species.

The ELIS MeHg concentrations were more variable for each size fraction throughout the seasons, although there is a clear increase with size in the spring. The 0.2-0.5 mm size fraction displayed the highest concentrations during the summer (~7 pmol/g (wet wt.)), while the larger size fractions were greatest in the spring (~5 to 8.7 pmol/g (wet wt.)). Though there were several pipefish in the >2.0 mm size fraction, it did not yield a higher MeHg concentration (~7 pmol/g (wet wt.)). The largest size fraction fell into the range for zooplankton values during summer in ELIS, and was actually lower than concentration measured in the spring >2.0 mm size fraction.

Though the smaller sized zooplankton in ELIS might suggest greater MeHg intake during the summer, the overall range in seasonal concentrations yields more variability than clear answers.

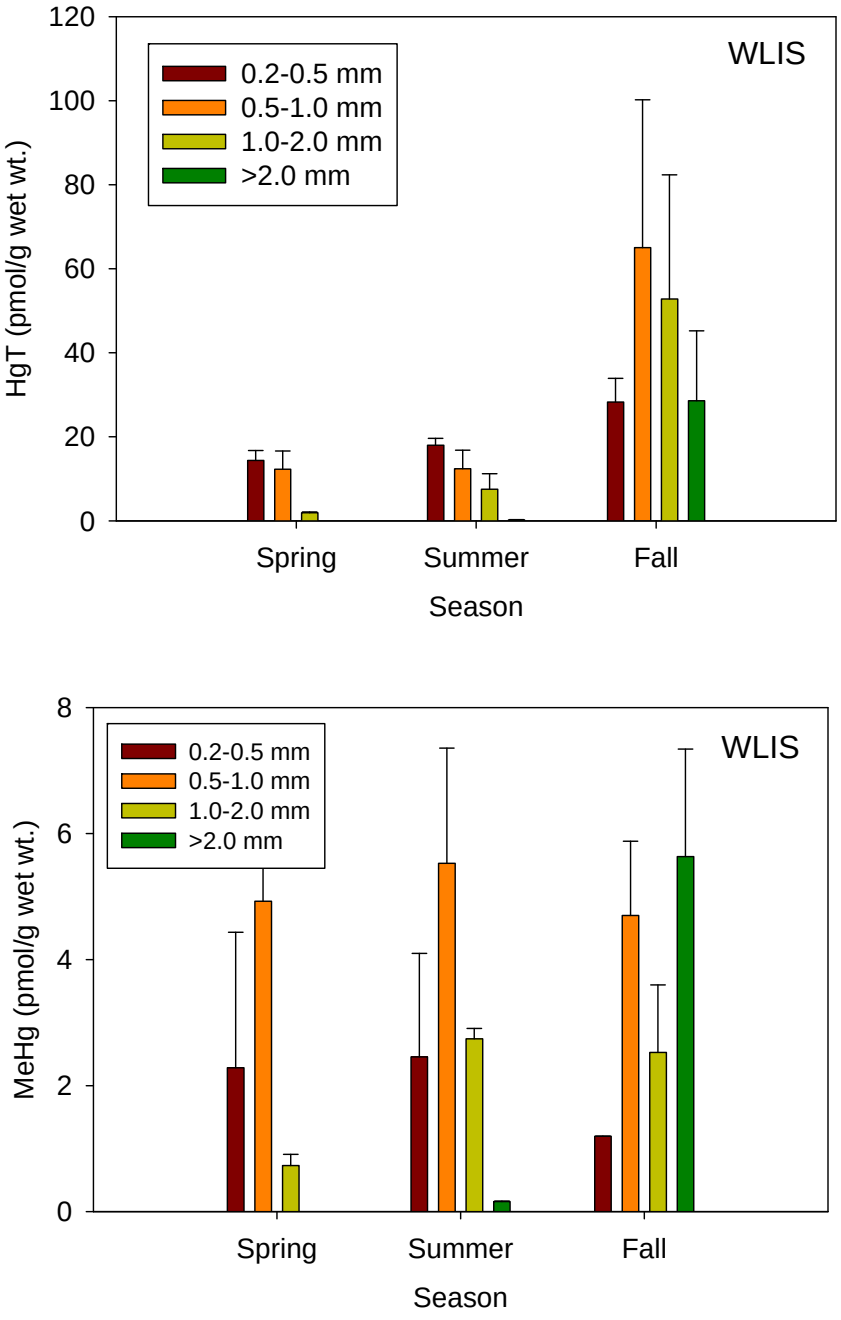

Figure 3.5. Inorganic mercury (HgT; top) and methylmercury (MeHg; bottom) concentrations for each size fraction of zooplankton measured per season at WLIS. Error bars reflect standard deviation between 3 separate substations at each site.

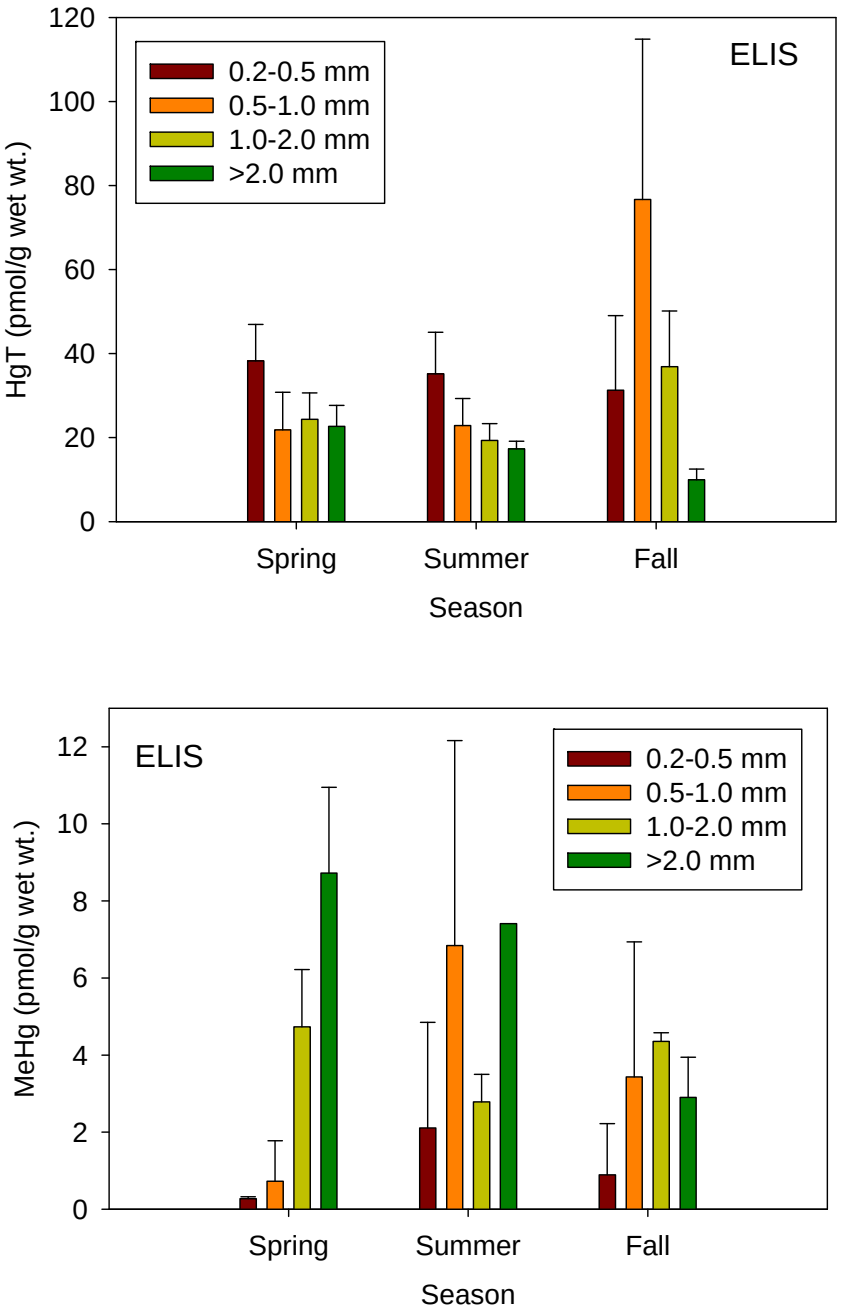

Figure 3.6. Inorganic mercury (HgT; top) and methylmercury (MeHg; bottom) concentrations for each size fraction of zooplankton measured per season at ELIS. Error bars reflect standard deviation between 3 seperate substations at each site.

For the fall cruise, the HgT concentrations tend to be consistent in the 0.2-0.5 mm size class (Figure 3.7), excluding the CLIS station which has the highest concentration measured (126 pmol/g (wet wt.)). The size 0.2-0.5 mm class is also highest than the other LIS measurements and size classes at the SB station (Figure 3.7). Most of the zooplankton size classes exhibit decreasing HgT concentrations for the three largest size fractions for all stations, besides the SB station. Notably, the largest size class always contains the lowest HgT concentration for each station. The LIS stations tend to have overall higher and more variable HgT concentrations than the two shelf stations.

Markedly, the 0.2-0.5 mm size class contained the lowest concentration of MeHg for each station during the fall cruise (Figure 3.7), even though the CLIS station measurement was again the highest at 9.6 pmol/g (wet wt.). There were no specific trends in size class concentrations for the remaining size classes, as the highest concentration was variable, and different for each station. There appeared to be some indications of increasing MeHg concentrations with increasing size classes, though this was not extended to the largest size class. The >2.0 mm size class only exhibited the highest average MeHg concentration for the WLIS station at 5.6 pmol/g (wet wt.).

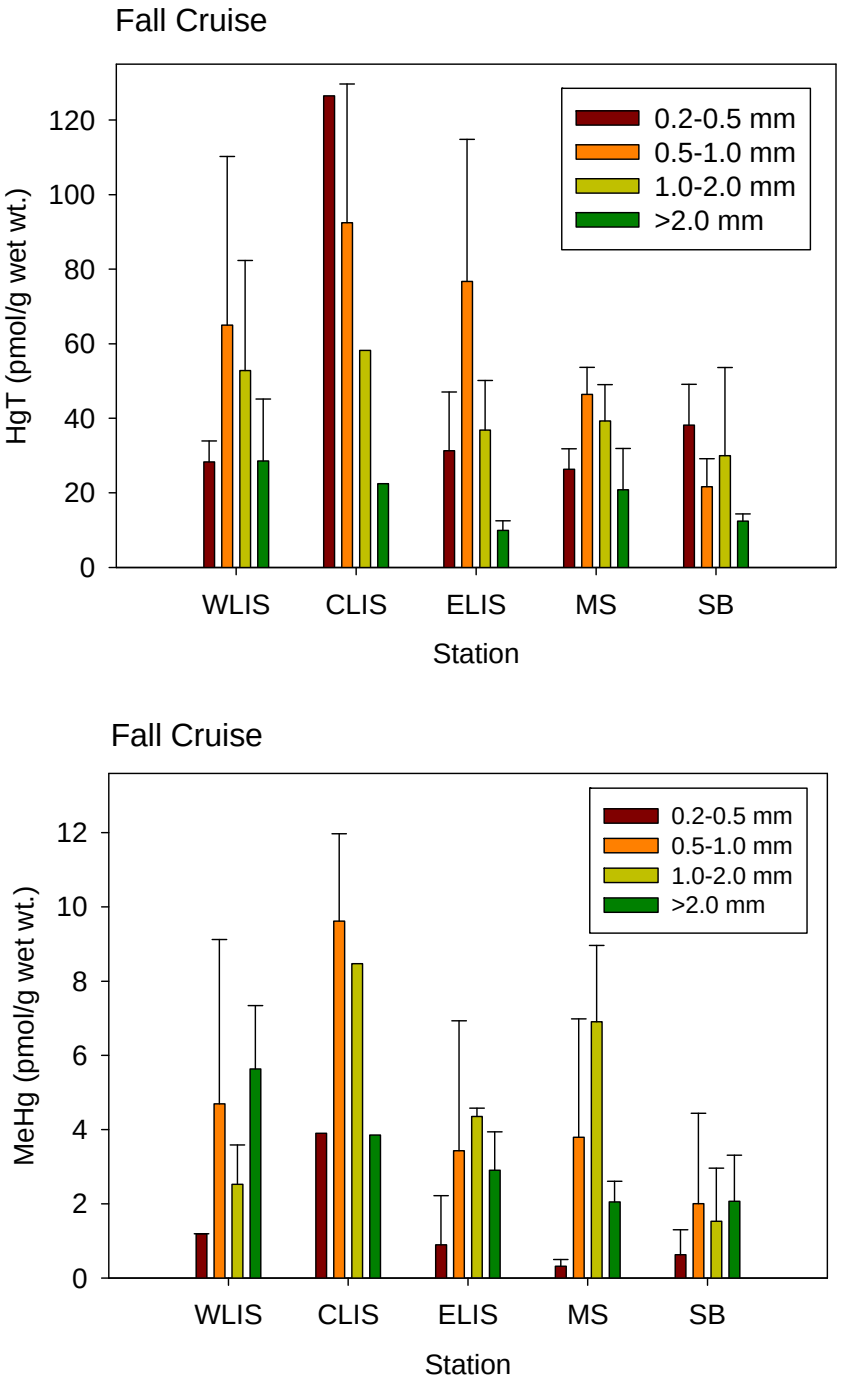

Figure 3.7. Inorganic mercury (HgT; top) and methylmercury (MeHg; bottom) concentrations for each size fraction of zooplankton measured during the fall cruise out to the shelf. Error bars reflect standard deviation between 2 different substations or multiple samples for the size class.

Zooplankotn bioconcentration factors (logBCFs) are presented for HgT in Table 3.4, and the MeHg values are in Table 3.5. Overall the range span is larger than what was found for phytoplankton, and there is no stark difference between LIS and shelf values. However, the values for HgT at WLIS during the spring and summer are lower for the largest size class of each season (2.65 and 2.11, respectively). The MeHg logBCF value was also lower at WLIS during the summer (2.9). Conversely, the MeHg values at the summer ELIS station were higher than all the other measurements, ~ 5.8 to 6.4 (excluding the >2.0 mm size class, which was 3.8). Besides those mentioned, the logBCFs for HgT range from ~3.8 to 4.8 while the MeHg values additionally skew higher at ~ 3.9 to 5.9.

Table 3.4. Average HgT bioconcentration values (logBCF) for zooplankton during the seasonal LIS and fall shelf cruises.

Size	WLIS Spring	WLIS Sum.	WLIS Fall	ELIS Spring	ELIS Sum.	ELIS Fall	CLIS Fall	MS Fall	SB Fall
0.2-0.5 mm	3.51 ± 0.13	4.05 ± 0.09	4.17 ± 0.09	4.19 ± 0.09	4.10± 0.13	4.35 ± 0.29	4.85	4.40 ± 0.08	4.69 ± 0.12
0.5-1.0 mm	3.43 ± 0.15	3.91± 0.17	4.51 ± 0.34	3.92 0.16	3.92 ± 0.14	4.77 ± 0.20	4.69 ± 0.16	4.65 ± 0.07	4.45 ± 0.15
1.0-2.0 mm	2.65 ±0.04	3.63 ± 0.26	4.29 ± 0.31	3.99 ± 0.11	3.85 ± 0.09	4.46 ± 0.18	4.51	4.57 ± 0.13	4.52 ± 0.38
>2.0 mm		2.11± 0.14	4.13± 0.27	3.95 ± 0.11	3.81	3.90 ± 0.11	4.10	4.26 ± 0.20	4.22 ± 0.07

Table 3.5. Average MeHg bioconcentration values (logBCF) for zooplankton during the seasonal LIS and fall shelf cruises.

Size	WLIS Spring	WLIS Sum.	WLIS Fall	ELIS Spring	ELIS Sum.	ELIS Fall	CLIS Fall	MS Fall	SB Fall
0.2-0.5 mm	4.91 ± 0.42	5.10 ± 0.26	3.95 ± <0.001	4.28 ± 0.08	5.75 ± 0.59	4.31 ± 0.82	4.85	4.49 ± 0.29	4.50 ± 0.60
0.5-1.0 mm	5.12 ± 0.73	5.41 ± 0.32	5.20 ± 0.21	4.32 ± 0.71	6.44 ± 0.34	5.21 ± 0.43	5.79 ± 0.11	5.40 ± 0.61	4.89 ± 0.79
1.0-2.0 mm	4.55 ± 0.11	5.23 ± 0.02	5.04 ± 0.18	5.52 ± 0.13	6.13 ± 0.11	5.46 ± 0.02	4.52	5.87 ± 0.12	4.94 ± 0.49
>2.0 mm		2.90 ± 0.39	5.40 ± 0.13	5.78 ± 0.10	3.81	5.26 ± 0.14	4.10	5.34 ± 0.14	4.98 ± 0.60

Examining the %MeHg (MeHg/HgT) eliminates some of the variation between the separate plankton size fractions. The %MeHg values for all of the seasons for LIS phytoplankton and zooplankton size fractions are displayed in Table 3.6 (WLIS) and Table 3.7 (ELIS). Through the %MeHg we can clearly see some differences between the western and eastern LIS regions, as well as some potential seasonal affects. In WLIS, it is clear that the spring displays the highest %MeHg for all the plankton, from the smallest phytoplankton class to the largest zooplankton. The summer exhibits some of the lowest %MeHg values for all sizes, except for the 0.2-5 μm and 1.0 – 2.0 mm sizes which actually increased during the summer. However, the summer season also displayed increasing %MeHg values with increasing size fractions, except for the highest and lowest size fractions.

Table 3.6. The %MeHg (MeHg/HgT) values throughout the seasons for all plankton size classes at WLIS

WLIS	Spring	Summer	Fall
0.2-5 µm	4.4	7.2	2.7
5-20 µm	22.9	4.9	8.4
20-200 µm	12.6	6.1	9.2
0.2-0.5 mm	17.2	16.2	2.1
0.5-1.0 mm	24.9	21.7	6.4
1.0-2.0 mm	38.1	45.3	9.8
>2.0 mm		8.6	27.6

Table 3.7. The %MeHg (MeHg/HgT) values throughout the seasons for all plankton size classes at ELIS

ELIS	Spring	Summer	Fall
0.2-5 µm	1.2	9.8	7.2
5-20 µm	8.1	18.1	13
20-200 µm	1.8		5.2
0.2-0.5 mm	1.1	5.5	2.2
0.5-1.0 mm	3.2	28	5.1
1.0-2.0 mm	21.1	26.5	13.4
>2.0 mm	39.2	44.7	30.7

In ELIS on the other hand, the spring exhibited some of the lowest %MeHg concentrations for the size classes. Every size class increased and contained the highest %MeHg during the summer collections, excluding the 1.0 – 2.0 mm. Furthermore, the ELIS stations further exhibited increasing %MeHg with increasing size classes for the zooplankton measurements. The highest %MeHg at 44.7% was found during the summer in the >2.0 mm fraction, which included small pipefish, which were not present in other collections. Phytoplankton in the larger 5-20 µm size class also consistently displayed greater %MeHg than the smaller 0.2-5 µm size class in ELIS.

The fall cruise %MeHg is presented in Table 3.8. The %MeHg is more variable for the comparable LIS and shelf stations. Except for the MS station, the %MeHg increases with increasing sizes for the zooplankton size fractions, and the highest %MeHg was always in the largest size fraction. At 38.8%, the highest %MeHg was found at the MS 5-20 µm phytoplankton fraction during the fall.

Table 3.8. The %MeHg (MeHg/HgT) values for every stations complete size fraction range for plankton collected during the fall cruise.

Fall cruise	WLIS	CLIS	ELIS	MS	SB
0.2-5 µm	2.7	1.2	7.2	6.2	2.2
5-20 µm	8.4	7.7	13	38.8	5.2
20-200 µm	9.2	4.8	5.2		
0.2-0.5 mm	2.1	3.8	2.2	1.4	1.5
0.5-1.0 mm	6.4	12	5.1	8.3	11.9
1.0-2.0 mm	9.8	14.5	13.4	19.4	10.2
>2.0 mm	27.6	17.2	30.7	14.1	16.9

3.3.3 Stable Isotopes

The fractionation of stable C isotopes (δ^{13}C ‰) was plotted against the methylmercury concentrations for each zooplankton size fraction seasonally for both WLIS and ELIS (Figure 3.8). Typically more depleted values of δ^{13}C indicate sources of more terriginous food material. Different trends were apparent for each different LIS location, indicating that there are some differences between C source material or the C assimilated into phytoplankton. Values in ELIS shift toward more depleted (-16 to -26 ‰ δ^{13}C) values than those in WLIS (-17 to -23 ‰ δ^{13}C), although there is substantial overlap between locations. Correlation trends in WLIS were also more clustered by season, with fall values being less depleted (~ -18 ‰ δ^{13}C), and indicating a

different marine source than summer, which contained more depleted material (~ -22 ‰ $\delta^{13}C$) than other sampling times. The $\delta^{13}C$ ratios of spring were a mix of those endpoints. The correlations for each season in ELIS tended to be more scattered, especially in the summer. However, there were also groupings of less depleted $\delta^{13}C$ indicative of marine material for the fall, and more depleted values clustered around $\delta^{13}C$ values ranging from -20 to -24 ‰ in the spring.

During the fall cruise trends of $\delta^{13}C$ indicated that the values for the two shelf stations were more depleted than LIS stations (Figure 3.9), and were somewhat consistent throughout the size fractions. This could be an indication that the marine stations' zooplankton contained some terreginous derived materials sourced from LIS waters. However, as ranges in $\delta^{13}C$ for marine phytoplankton have been found around -18 to -24 ‰ $\delta^{13}C$ (Fry and Sherr, 1984), this is likely more of a reflection of the zooplanktons diet. The MS station spanned the largest $\delta^{13}C$ range, with values reaching up to -18 ‰ $\delta^{13}C$. The LIS stations tended to be scattered around $\delta^{13}C$ values within the scale of -16 to -19.5 ‰.

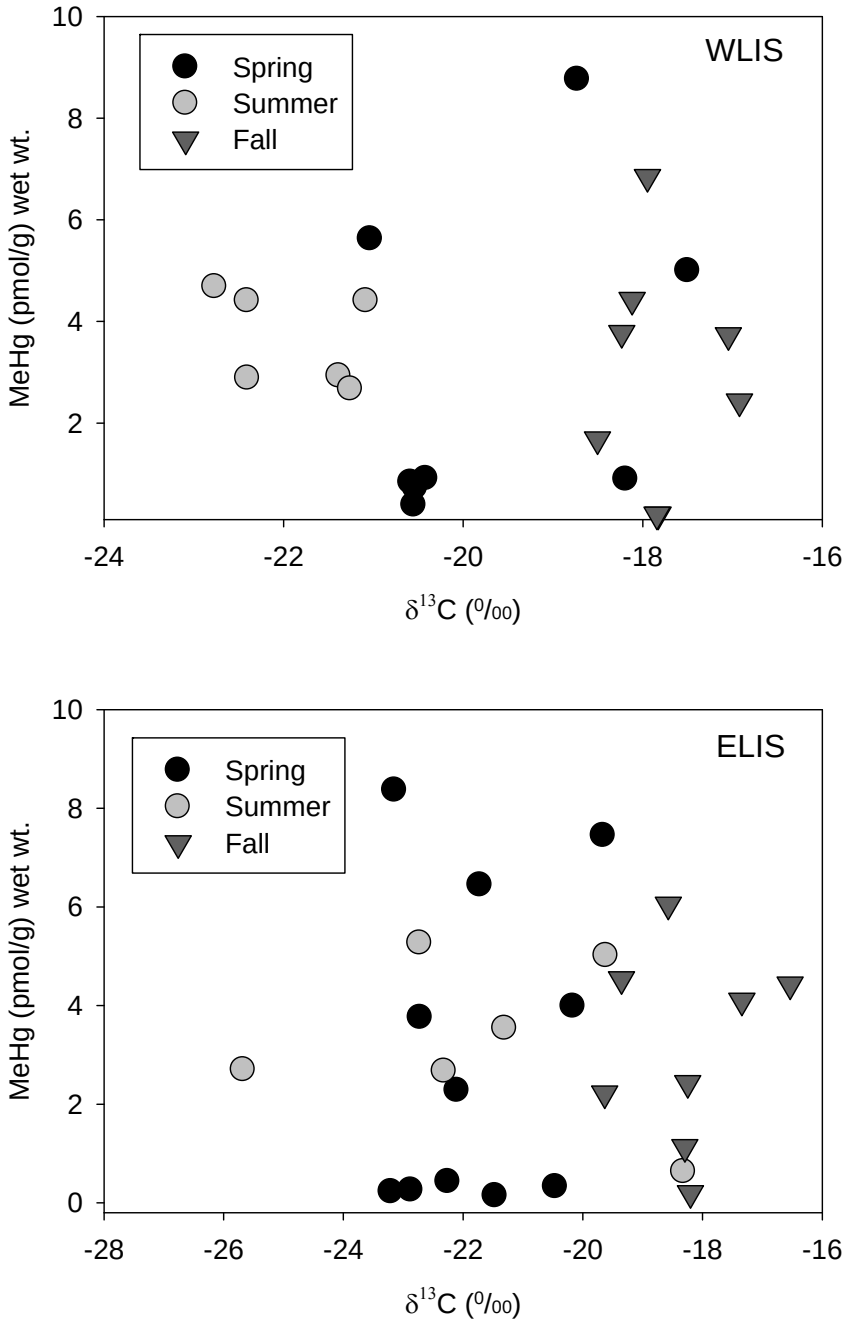

Figure 3.8. Methylmercury (MeHg) concentrations (pmol/g wet wt.) associated with $\delta^{13}C$ values for each season for WLIS (top) and ELIS (bottom).

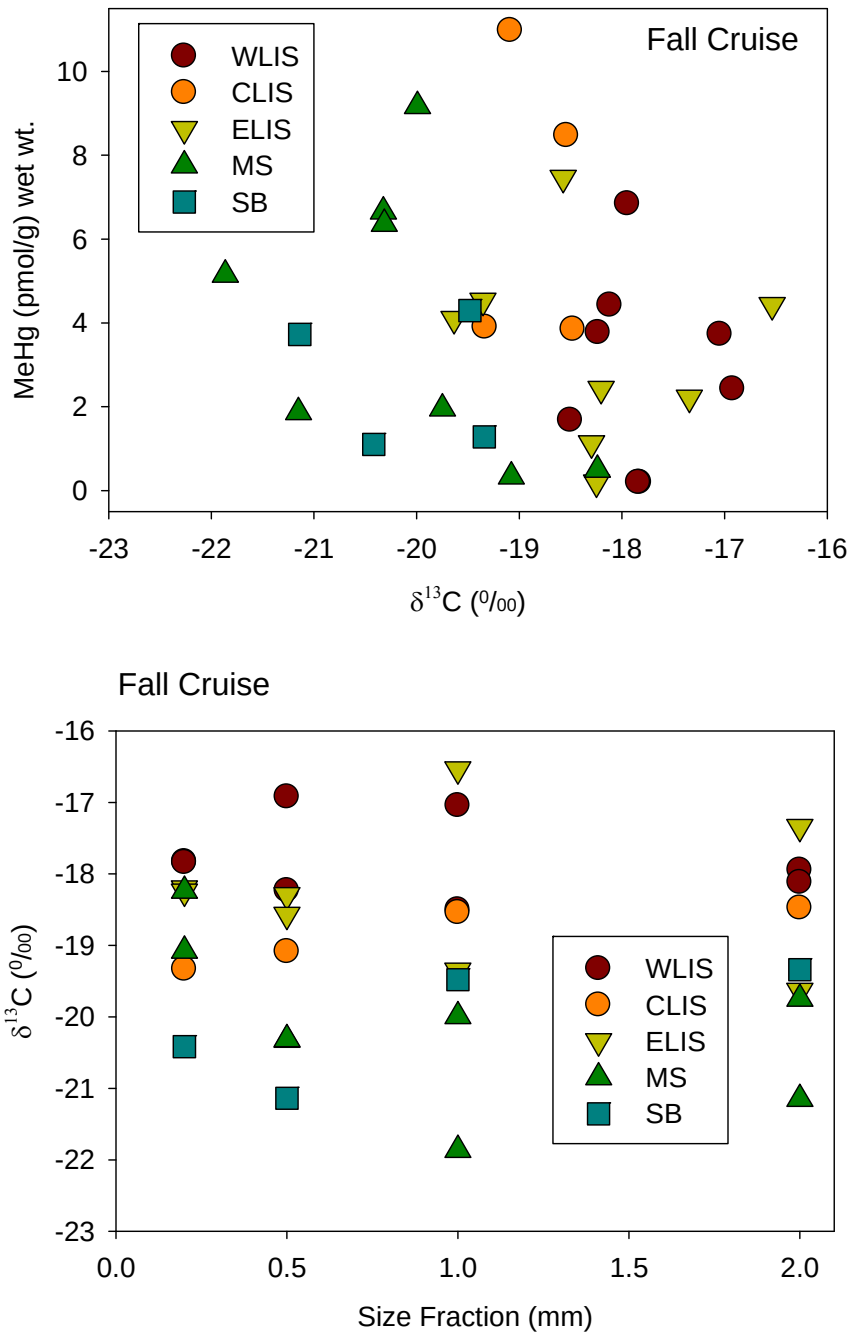

Figure 3.9. Methylmercury (MeHg) concentrations (pmol/g wet wt.) associated with $\delta^{13}C$ values for each site during the fall cruise (top). Stable $\delta^{13}C$ values are also displayed for each size class (bottom).

Zooplankton methylmercury concentrations were plotted against stable N isotopes ($\delta^{15}N$ ‰) and are displayed in Figure 3.10. The ranges are different for WLIS and ELIS, with $\delta^{15}N$ ratios in WLIS overall higher (8 to 16 ‰ $\delta^{15}N$) than ELIS (~7 to 12 ‰ $\delta^{15}N$), and overall the trends for seasons were somewhat consistent. Thus, it appears that zooplankton consumed differently in the terms of trophic levels of their food depending on the season, or that the source of N assimilation into phytoplankton changed with season. For both WLIS and ELIS it appeared that the spring species were feeding at approximately the same trophic level relative to bioaccumulation, as there is little change in MeHg concentratins for the size fractions across the $\delta^{15}N$ ratios. Summer $\delta^{15}N$ isotopes in WLIS and ELIS were lower than fall $\delta^{15}N$ values, indicating that the fall plankton were possibly consuming species higher in the trophic cascade, or that there is a relative difference in N isotope signature with season. In ELIS and WLIS $\delta^{15}N$ values in the spring were lower than $\delta^{15}N$ ratios measured for fall.

The fall cruise appeared to represent clear groups of different trophic consumption for each station (Figure 3.11). Both shelf stations had the lowest $\delta^{15}N$ ratios, with the primarily marine SB station plankton having the lowest $\delta^{15}N$ (~4 to 6 ‰ $\delta^{15}N$) out of all the zooplankton measured. Notably, comparisons of signatures for zooplankton collected from most marine sites to those from the relatively enclosed end of WLIS, shows that the $\delta^{15}N$ ratios became increasingly heavier (~15 ‰ $\delta^{15}N$ at WLIS).

Plotting the %MeHg against $\delta^{15}N$ (‰) appears to indicate that the shelf species were separated from the LIS zooplankton in terms of sources of their food and MeHg (Figure 3.12). The shelf stations have a slight positive correlation between %MeHg and higher $\delta^{15}N$ (r^2=0.273, p = 0.006, n = 22), while the fall LIS stations were overall higher in $\delta^{15}N$, but displayed little change across the %MeHg range and lacked any correlation (r^2=0.017, p = 0.88, n =38). The

%MeHg was not at all correlated with $\delta^{13}C$, and there was no clear distinction between the shelf and LIS stations (Figure 3.12).

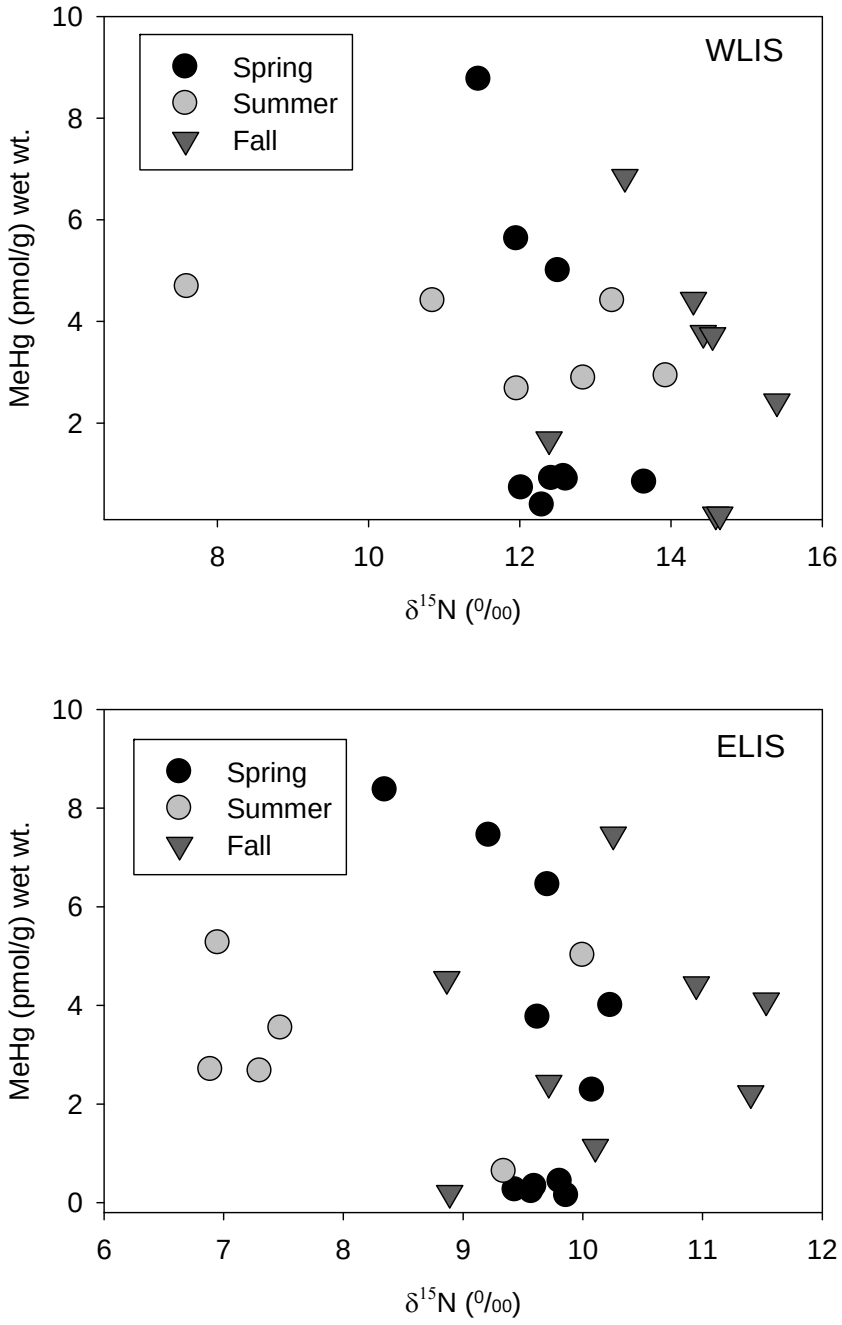

Figure 3.10. Methylmercury (MeHg) concentrations (pmol/g wet wt.) associated with $\delta^{15}N$ values for each season for WLIS (top) and ELIS (bottom).

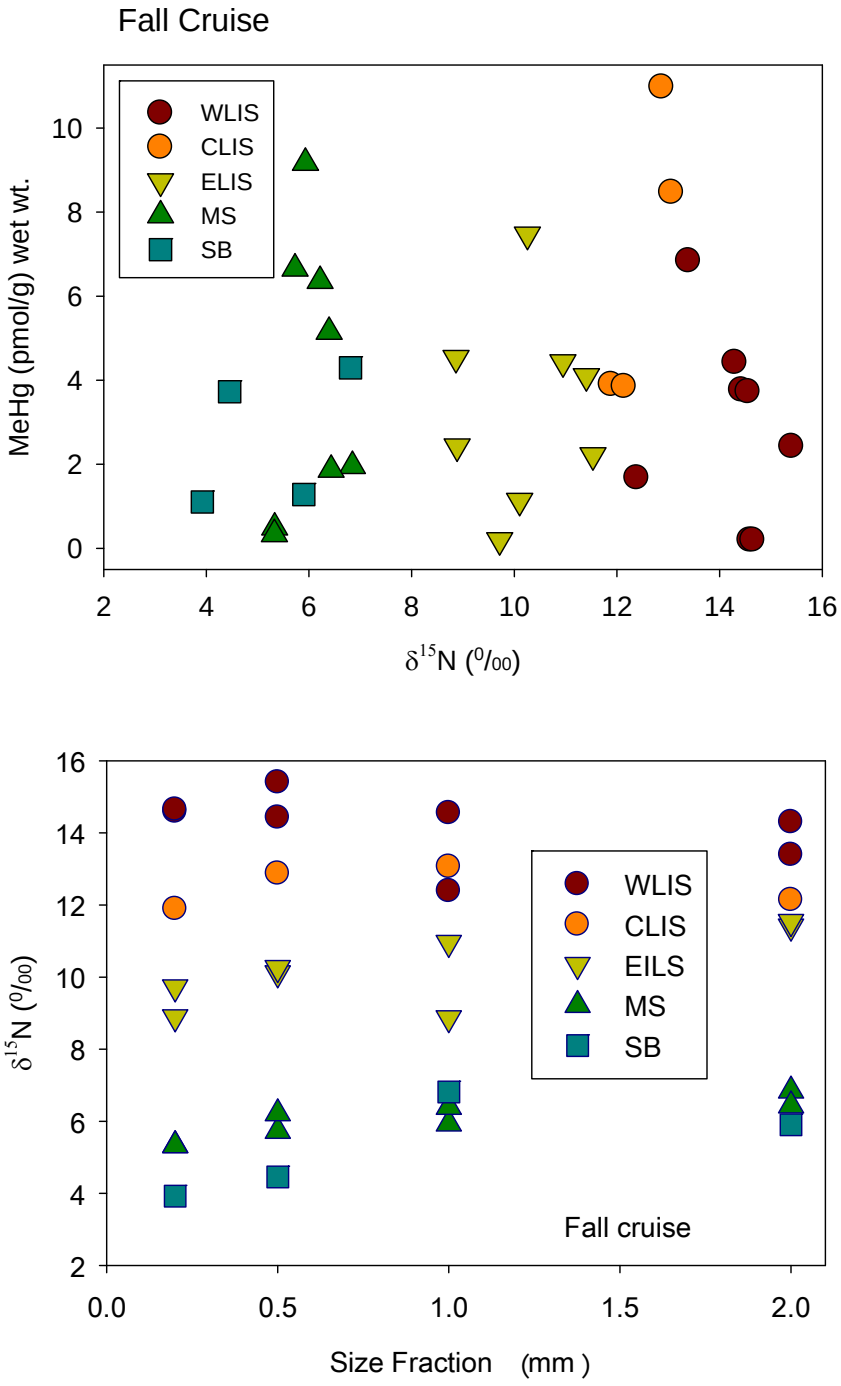

Figure 3.11. Methylmercury (MeHg) concentrations (pmol/g wet wt.) associated with $\delta^{15}N$ values for each site during the fall cruise (top). Stable $\delta^{15}N$ values are also displayed for each size class (bottom).

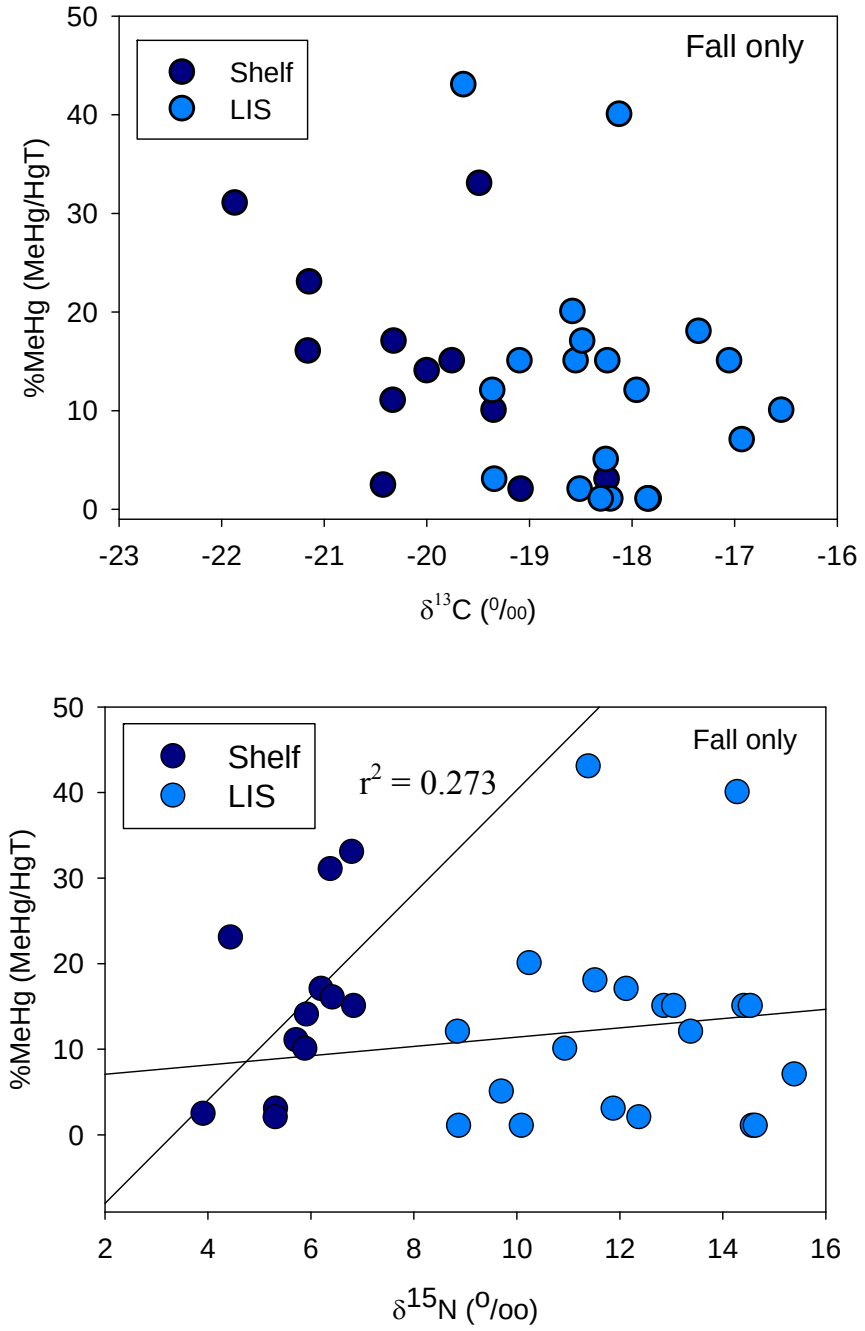

Figure 3.12. Correlation between %MeHg (MeHg/HgT) and $\delta^{13}C$ values (top) and values $\delta^{15}N$ (bottom). Shelf stations are significantly correlated between %MeHg and $\delta^{15}N$ (r^2=0.273, p = 0.006, n = 22), but no other groups yield a significant relationship.

Stable isotopes of S ($\delta^{34}S$) collected from spring and fall cruises from the WLIS and ELIS, in addition to the other fall cruise stations, were plotted against the associated $\delta^{13}C$ isotopes (Figure 3.13). There was not sufficient sample amount to obtain $\delta^{34}S$ values from the summer zooplatnkon samples. Peterson et al. (1985) noted that there tends to be a large difference between $\delta^{34}S$ values found in upland C-3 plants (4.7 ± 0.9‰) and pelagic phytoplankton (~18.6 ± 0.6 ‰). Sedimentary sulfides produced via microbial sulfate become isotopically lighter (-24‰), while porewater sulfates are more variable and heavier (~60‰) than water column sulfates (Connolly et al., 2004). Planktonic algae typically yield values of 20.3 ‰ from seawater S uptake, which correspond to the majority of the measurements in this study. As $\delta^{34}S$ fractionates very little between consumers and food sources (~1‰), it is evident that most of the zooplankton had $\delta^{34}S$ values reflecting a mix of marine diatoms and phytoplankton, with minor amounts of detritus complimenting their diets. The few noticible fractions with lower values (17.9 and 18.9‰) from WLIS fall (0.2-0.5 mm) and ELIS spring (0.5-1.0 mm & 1.0-2.0 mm) conceivably indicate that these animals were consuming primarily phytoplankton.

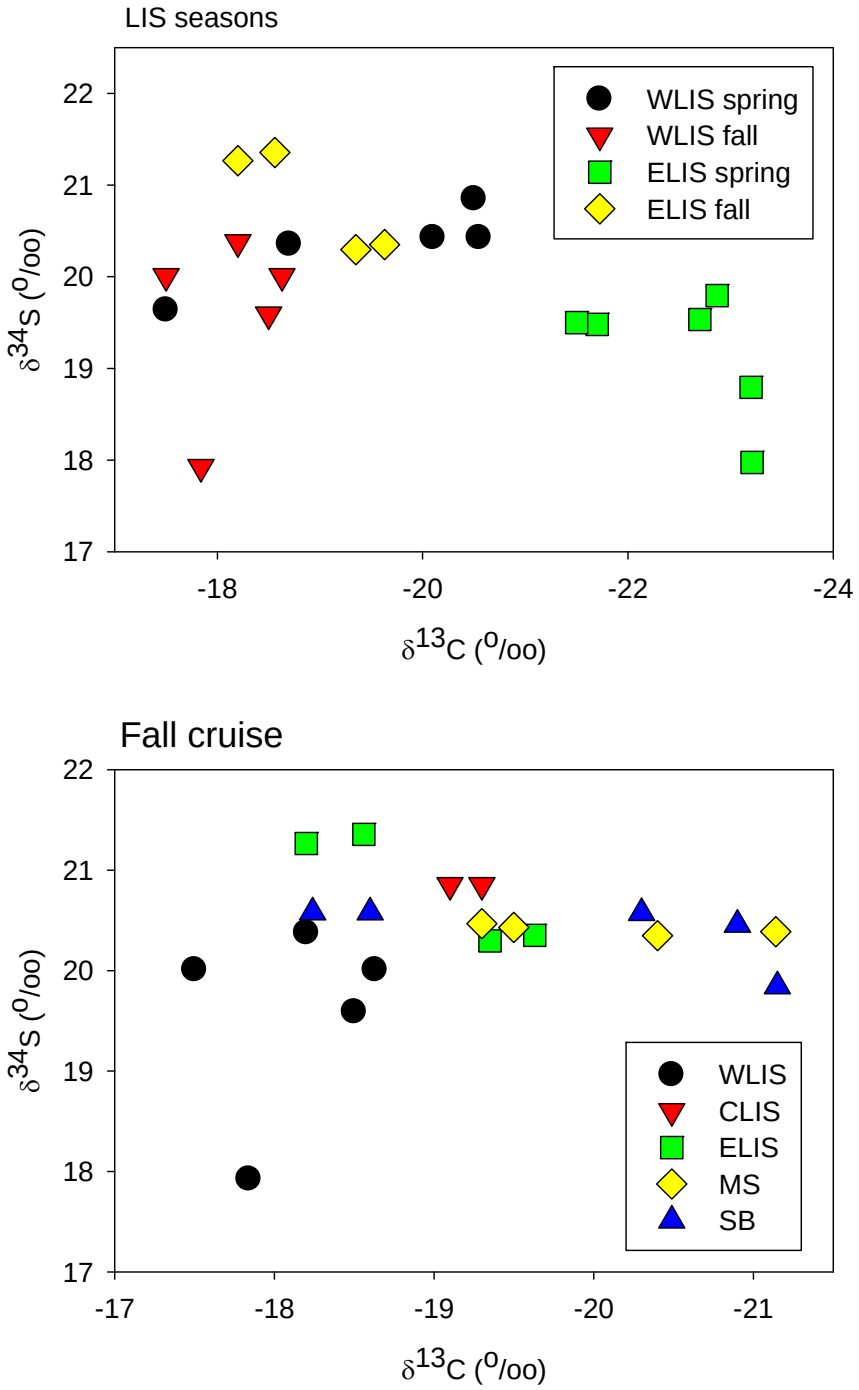

Figure 3.13. Association between $\delta^{34}S$ and $\delta^{13}C$ values for the spring and fall seasons in WLIS and ELIS (top) and during the fall cruise (bottom) for collected zooplankton size fractions.

3.4 Discussion

3.4.1 Phytoplankton

The spring season contained the overall highest concentrations of Hg and MeHg for all phytoplankton fractions. This could be a result of the relatively low biomass growth in the winter season around LIS, due to limiting sunlight, temperature, and nutrients. Fewer algae could be accumulating most of the burden of all of the residual Hg and MeHg within LIS, resulting in higher overall concentrations. It is notable that the concentrations in ELIS are much higher than found in WLIS. This was slightly surprising, as WLIS is closer to high urban populations and surrounded by a more industrialized region of LIS, and has slightly higher MeHg concentrations in the water (Table 3.1) and in sediments (Hammerschmidt et al., 2004; Balcom et al., 2008). In contrast, ELIS is open to more relatively pristine coastal marine waters exchange with LIS. Though the shelf data is limited, there are no distinct differences in water MeHg values between LIS sites and offshore sites.

The DOC values were higher by a factor of two in WLIS than ELIS during the spring, but not at other times of the year. It is possible that there was more of the Hg and MeHg bound up with DOC in WLIS, and less available for uptake into algae due to the higher organic content. Lamborg et al. (2004) noted that DOC within WLIS was primarily from terrestrial organic matter and phytoplankton exudates, with other Hg-binding ligand sources including rivers, sewage and sediments. As the DOC within LIS is somewhat homogeneous, this suggests that the major driver would be concentration differences between stations.

However, this HgT and MeHg variability could also be a result of the higher eutrophication which typically plagues WLIS. Though higher nutrient burdens can eventually yield low oxygen levels and inhospitable environments for some organisms, the higher nutrients

from urban and terresterial runoff typical of WLIS could also benefit algae growth, at least for some species. In this way, the prominent lower Hg and MeHg values measured in WLIS could be a result of growth dilution, where the ELIS phytoplankton take up more Hg and MeHg per organism. Chen et al. (2005) found the phenomenon of phytoplankton density reducing Hg and MeHg per phytoplankton cell was more important than other prominent water chemistry factors such as pH or DOM for 20 northeastern American lakes. Though especially high chl a was not measured for every station during sampling, WLIS annually has seasonal blooms yielding concentrations greater than 30 µg/L, compared to the peak bloom concentrations of 8 µg/L for ELIS (Latimer et al., 2014). The fluorescence was measured during each season, and exhibited ranges of 1-10 mg m^{-3} (µg/L) in ELIS, but had higher ranges of 8-26 mg m^{-3} for WLIS during sampling. Thus it is feasible that more nutrients had resulted in vaster residual blooms in WLIS, and could be responsible for growth dilution effects of Hg and MeHg in phytoplankton. Notably, though the WLIS has experienced summer hypoxia since the early 1970s (Anderson and Tyler, 2001), there was no prominent lengthy hypoxia during the summer of 2014. Thus, any potential increase in anoxia-driven sediment methylation in WLIS and its subsequent transport to the mixed layer would have been dampened during this study.

Plankton concentrations of HgT and MeHg were substantially lower in the summer compared to both the spring and fall collections. This could be a possible consequence of seasonal growth dilution, as a spring algae bloom could have diluted concentrations for the organisms to accumulate during summertime. Speciation of phytoplankton could also be a factor in differences seen during the seasons, but this is unlikely. Diatoms species dominate LIS year round, accounting for half of the total biomass (Latimer et al., 2014). However, during summer dinoflagellates and other smaller phytoplankton such as cyanobacteria (i.e. *Synechococcus*) tend

to become the majority in LIS (Latimer et al., 2014). Thus, it should not be ruled out that the differences in seasonal Hg and MeHg concentrations for phytoplankton could be attributed to changing patterns of dominant biota. Furthermore, smaller organisms would have a different size/volume physiology, resulting in different pathways for Hg and MeHg accumulation (Chapter 4). However, it is interesting to note that the %MeHg values increased during the summer season for the 0.2-5 μm size fraction for both the WLIS and ELIS stations, indicating potential methylation by the pelagic microbes additionally amassed in that fraction. Higher %MeHg has been measured in that size class in the central Pacific Ocean (Gosnell and Mason, 2015; Chapter 2) and water column methylation has been measured in different regions' water columns for both coastal waters and those of the open ocean (Lehnherr et al., 2011; Pucko et al., 2014; Schartup et al., 2015b; Ortiz et al., 2015). The WLIS station had the highest %MeHg in the larger size fractions of phytoplankton, which was a different trend than was seen in ELIS, and also different from what was found in the open Pacific Ocean, where the smallest fraction consistently had this highest %MeHg (Gosnell and Mason, 2015; Chapter 2). It was possible that pelagic methylation could have occurred in association with the >20 μm size fraction of phytoplankton, as Hg methylation was seen in Ortiz et al. (2015) in the same flocculate size fraction in laboratory experiments. However, the %MeHg was not higher in the summer >20 μm indicating that this was evident. While the reasons for the various trends are not entirely apparent, these measurements are adding to the increasing data that suggests the potential importance of water column methylation in the overall MeHg marine dynamics in marine waters.

During the fall the concentrations in algae increased again, though not quite as high as was seen during spring. The trends seen in stations during the fall cruise could add evidence to the hypothesis of bloom dilution effects, as the algae Hg and MeHg concentrations tended to

increase moving from more coastal and enclosed stations out to the more marine stations. The ELIS station had the highest concentrations for algae for the largest >20 μm size class, though relatively low %MeHg values. Waters along the continental shelf have previously been found to have higher concentrations of Hg and MeHg (Hammerschmidt et al., 2013), but substantially higher MeHg concentrations were not measured in the deep shelf waters during this study, and were highest at WLIS. The lowest HgT values for water were recorded at MS (1.03 pM) and SB (0.75 pM) stations in fall. The MS 5-20 μm size fraction had the highest %MeHg of the plankton, though the water concentrations were not comparatively higher in MeHg (<0.01 pM). The MS station also had the lowest DOC concentration, thus there could have been more MeHg available for uptake if less was bound to organic ligands, possibly illustrated in the higher phytoplankton concentrations for those stations. The higher MeHg concentrations in the algae along the shelf may be a reflection of higher water concentration, while the intermediate concentrations measured in algae residing in higher MeHg waters of WLIS could reflect growth dilution effects. The DOC fluorescence was analyzed for the WLIS and SB stations for the fall cruise, and revealed that characteristics between the two station endpoints were different (N. Mazrui, personal communication). Expectedly, the DOC in WLIS was more terrestrial derived, while the SB DOC specified a more marine origin. This could be a primary factor in differences between algae Hg and MeHg uptake for shelf and LIS species. However, besides the MS station, the DOC values were measured consistently around 200 μM for the other stations during the fall, suggesting that DOC concentration were less influential in controlling variations in algae uptake within LIS.

Methylmercury bioconcentrations factors (logBCFs) ranged from 2.6 to 5.5 for LIS phytoplankton, and 2.7 to 4.5 for the shelf stations. The HgT logBCFs fell slightly lower, and

had minimal variation between LIS and shelf stations. Values were 2.4 to 5 in LIS and 3.5 to 4.2 for the shelf stations for HgT logBCFs. These values span a lower range, but are consistent with previous measurements by Hammerschmidt et al (2013) off the Atlantic shelf (3.9 to 4.6 for MeHg logBCFs) and with the typical range of measurements of 4 – 5 logBCFs (Mason et al., 2012). There was a relative increase with logBCFs with increasing sizes, indicating that larger species were concentrating more Hg and MeHg out of the water in LIS. However, these values are lower than what has been found in the more sparsely populated open ocean waters (up to ~7 logBCF; Gosnell and Mason, 2015; Chapter 2). These measurements further hint that there is less concentration of Hg species in coastal phytoplankton compared to their open ocean counterparts.

3.4.2 Zooplankton

3.4.2.1 Long Island Sound

The highest seasonal concentrations for HgT in zooplankton were measured in the fall for both WLIS and ELIS, and are more pronounced for WLIS. It is notable that the zooplankton mimicked the phytoplankton HgT concentrations in that the WLIS concentrations were half those of ELIS for spring and summer. Thus, the higher nutrient concentrations typically found in the western end of the sound could also be the benefit of a greater biomass, and the resultant growth dilution of zooplankton, as zooplankton in WLIS would be feeding on the more depleted phytoplankton residing in surrounding waters. It is additionally possible that due to the prominent summertime ctenophore bloom, and general consumption of biomass by other zooplanton, that measured concentrations were higher in the fall due to the increased uptake burden into the remaining individual species. The MeHg concentrations were much more

variable, especially in ELIS. Overall, the 0.5 – 1.0 mm size class had relatively consistent MeHg concentrations throughout all seasons in WLIS. The highest concentrations tended to be found in the two intermediate size fractions at both sites. These size fractions were dominated by adult copepod and euphausii species, though predatory chaetognathas (arrow worms) were also found in the larger size fractions.

For WLIS, the %MeHg was highest in the spring for all but the largest size fraction measured for each season, thus there was no indication of a seasonal increase in MeHg body accumulation during the summer season. Generally the summer WLIS plankton %MeHg indicate little change between the spring and summer seasons, followed by a decrease in %MeHg in the fall. The fall season had relatively little variation between the %MeHg for the different size fractions in WLIS, indicating no clear bioaccumulation pattern throughout the plankton levels. However, the spring and summer seasons in WLIS did display increasing %MeHg with increasing trophic position throughout the zooplankton sizes, indicating that the bioaccumulation of MeHg is reflecting that larger organisms are feeding on not only phytoplankton, but also higher trophic level organisms.

The %MeHg in summer zooplankton of ELIS were clearly elevated compared to the other seasons for all size classes. The increase in MeHg logBCFs at ELIS during the summer further helps confirm that the zooplankton were accumulating more MeHg during that season (Table 3.5). The increasing %MeHg with larger size classes is prominent throughout all the plankton fractions, excluding the comparably large value for the 5-20 μm phytoplankton group. The ELIS station was located around the marine end of LIS, so there is possibly more water passage and flushing, and less urban nutrient input than the WLIS stations. These values provide some potential support for the theory that there could be MeHg uptake and enhancement in

plankton during the summer season. It also appears that the effect is more pronounced for the more marine ELIS station. Thus, it is possible that regions which are more pristine and contain less biomass could be more affected by potential enhanced summer methylation, or other inputs of MeHg to the water column, such as fluvial sources or from atmospheric deposition.

Alternatively, the differences in quality and quantity of DOC in the various regions could also impact the accumulation at the base of the food chain, and this could be more important given the relative binding strengths of inorganic Hg compared to MeHg. Lamborg et al (2004) found that the Hg binding ligand intensity was approximately an order of magnitude greater for waters from LIS compared to DOC from the mid-Atlantic shelf, inferring that coastal ocean organic matter has a lower Hg-binding capacity. Furthermore, abundant terrestrial-derived organic matter, which tends to be humic-rich, more securely binds to Hg and MeHg, and could possibly lead to a decrease in bioavailability to coastal species of phytoplankton (Schartup et al., 2015). Thus, the impact of DOC on uptake might not be consistent for the different forms of Hg, which could influence the %MeHg in the phytoplankton and zooplankton.

While there is some variation in the assimilation efficiency (AE) for different zooplankton, it is instructive to consider impacts that the %MeHg in food has on the %MeHg in zooplankton. Typical experimental values for AE are ~80% for MeHg and 15% for inorganic Hg. Therefore, an organism feeding on food with 2% MeHg, approximately the low end of measurements in LIS phytoplankton, would have a %MeHg around 10% based on estimated relative differences in AE. Similarly, feeding on food with 10 %MeHg would yield 37 %MeHg in zooplankton. These calculations do not take into account growth effects or differences in Hg and MeHg depuration, but do indicate that the %MeHg levels in larger zooplankton could be

derived from consumption of large phytoplankton, or a combination of larger phytoplankton and smaller zooplankton fractions.

Stable $\delta^{15}N$ ratios tend to be larger if animals are feeding higher on the food chain (Fry, 1988), and enrichment of 3-4 ‰ per trophic level is possible. Copepods and eyphausiids were the dominant species found in LIS, and have been found to have $\delta^{15}N$ values around 10 ‰ (Senn et al, 2010; Fry, 1988). The stations at ELIS had species with $\delta^{15}N$ values around 9 – 10 ‰, which fall in this range, but at WLIS the $\delta^{15}N$ values tended to skew much higher, primarily around 12 – 14 ‰. Senn et al (2010) measured values consistent with these for estuarine zooplanktivorous species in the Gulf of Mexico. Thus, the WLIS species appear to be consuming higher on the tropic feeding chains than species found in ELIS. Alternatively, and more feasible, it is possible that the nitrate assimilated at the base of the food chain in WLIS is isotopically heavier due to human inputs, specifically waste water discharge which is more explicit in WLIS. Modern riverine water $\delta^{15}N$ mean ratios average around ~5 ‰, while waste water values span heavier at 10 to 12 ‰ $\delta^{15}N$ (Latimer et al., 2014). Pertaining to the pelagic nutrient basis, waste water treatment plants output has NO_3^- $\delta^{15}N$ ranging from -9 to 16 ‰ (Latimer et al., 2014). Thus, it is highly probably that WLIS zooplanktons are consuming phytoplankton and particulate matter which has a higher initial base of $\delta^{15}N$ isotopes.

To support the bioaccumulation narrative it would be expected that higher values would correspond to organisms with the most MeHg or %MeHg. However, there were no clear increasing correlations when $\delta^{15}N$ was plotted against MeHg concentrations, and no correlation between %MeHg and $\delta^{15}N$ (‰) in LIS for this study. The lack of correlation helps support the idea of differences in sources of inorganic nitrogen to WLIS and ELIS, and also that zooplankton were consuming resources with variable amounts of Hg and MeHg. However, the $\delta^{15}N$ values

do appear to indicate that there were separate feeding linkages during each season. This is most prominent in WLIS where spacing is more apparent. Foster et al (2012) plotted MeHg concentrations against $\delta^{15}N$ ‰ values to establish separate trophic links within specific species of zooplankton in the Hudson Bay. Though specific species groups were not defined in this study, the separate linkages for each season indicated different predator-prey or omnivorous connections that were apparent for each season's size fractions. The changing trends in food chains were consistent for both WLIS and ELIS, though the extent of variability for the two food chains was different.

In ELIS there is much more overlap between the seasons and $\delta^{15}N$ values. These feeding structures aptly resemble more complicated food webs and omnivorous relationships present in more oligotrophic regions then strict linear trophic feeding relationships. Fittingly, the lack of strong correlation between MeHg and $\delta^{15}N$ species could be further indicative of the complexity of the food web structure. More opportunistic feeders would not consistently retain strong bioaccumulation of MeHg for bigger zooplankton sizes akin to species which are primarily predatory towards lower trophic levels. Thus, the food web structure could dilute expected effects of trophic transfer in terms of $\delta^{15}N$ and %MeHg increase, as lower MeHg from different microalgae and particulate matter would add variability into the diets of zooplankton. Zooplanktons feeding from multiple sources in a web structure are likely to reflect these effects in the measured MeHg and HgT concentrations for all the stations, but this trend is evidently most pronounced in the variability found at ELIS.

The stable $\delta^{13}C$ ratios are also supportive of narrative that WLIS and ELIS zooplankton were receiving differently sources of food depending on the season, as the groups were clustered around distinctive values. Copepod and euphausiids tend to have $\delta^{13}C$ values clustered around -

20 ‰, whereas amphipods residing in the benthos tend to have less depleted values around -17 ‰ $\delta^{13}C$ (Fry, 1988). The summer $\delta^{13}C$ in WLIS was much more depleted, which indicates possibly more terrigenous sources were being incorporated into biomass during that season. Terrigenous inputs can have values for $\delta^{13}C$ values of -23 to -30 ‰ for C_3 plant material (Michener and Shell, 1994), and this has been reflected by particulate matter values around -24 ‰ in Narragansett Bay (Gearing et al., 1984). For the western end of the sound, this is not entirely surprising, as WLIS is mostly surrounded by land sources and would have less seasonal storm-driven circulation to flush these local inputs out during the summer. Though summertime is not usually a high flow season for the rivers draining into LIS, the residual $\delta^{13}C$ remaining from spring runoff could reflect carbon that was earlier accumulated into the zooplankton. Previous studies have noted that higher productivity and diatom abundance can produce phytoplankton with higher C-13 content (Fry, 1988; Michener and Shell, 1994; Gearing et al., 1984). The spring and fall seasons tend to encompas less stagnant waters, and the enhanced turbidity and LIS water circulation could explain the slightly less depleted marine sources which are present in zooplankton $\delta^{13}C$. For ELIS, the more depleted signals which are present in the spring and summer could be a reflection of the water outflow patterns, which would contain some riverine terresterial sources from within LIS.

Variations in $\delta^{13}C$ can be a result of phytoplankton food resources with different isotopic compositions (Fry, 2006). Compositions of animal carbon isotopes tend to reflect the diet within 1‰ (Michener and Shell, 1994). Notably the $\delta^{13}C$ values do not make ideal trophic position indicators as they tend to be conservative between trophic levels and have large ranges overlapping source values, though 1.1‰ enrichment per trophic level has been reported (Michener and Shell, 1994). Furthermore, it should be noted that this interpretation can be

muddled in regions with multiple sources of overlapping isotope values, such as was seen in LIS for this study. However $\delta^{13}C$ values do help discern the diet sources of zooplankton. Typical values in estuarine systems experience large variations, especially for marine microalgae which can span ranges of $\delta^{13}C$ from -8 to -15 ‰ (Fry and Sherr, 1984). Marine phytoplankton can fall between -18 to -24 ‰ $\delta^{13}C$ (Fry and Sherr, 1984), which could represent intrinsic food for zooplankton species measured throughout the seasons at both ELIS and WLIS. The $\delta^{13}C$ values from each site also could indicate that the ELIS species were feeding opportunistically across many more different sources for each season than WLIS, though the signatures indicate consumption of more phytoplankton and nanoplankton then marine microalgae. Furthermore, more depleted values tend to indicate animals feeding on particulate organic carbon (Fry, 1988), so this illustrates that summer and spring species were potentially consuming more degraded matter then in fall. Gearing et al (1984) also demonstrated that diverse seasonal phytoplankton populations yielded different isotopic compositions in nearby Narragansett Bay (RI), with nanoplankton dominating the summer (~-23 ‰ $\delta^{13}C$), and heavier diatoms present during winter and spring. Phytoplankton that have larger cell sizes and faster growth rates can also ensue enriched C-13 (Michener and Shell, 1994). The variations in seasonal $\delta^{13}C$ values in LIS zooplankton most likely also reflect seasonal changes in phytoplankton compositions. However, it is likely that the most prominent indication from the different $\delta^{13}C$ ranges for LIS is that zooplankton were feeding from a variety of sources, from bacteria and particulate matter, up to predating on smaller zooplankton for some of the larger species.

Average isotopic signatures of C and N for the WLIS and ELIS seasons are presented in Figure 3.14. Fry (1988) initially found a general trend of increasing $\delta^{13}C$ and $\delta^{15}N$ with increasing trophic levels, but no correlation between $\delta^{13}C$ and $\delta^{15}N$ ratios. However, Foster et al.

(2012) was able to demonstrate some omnivorous links within the feeding system of Hudson Bay in the sub-arctic region.

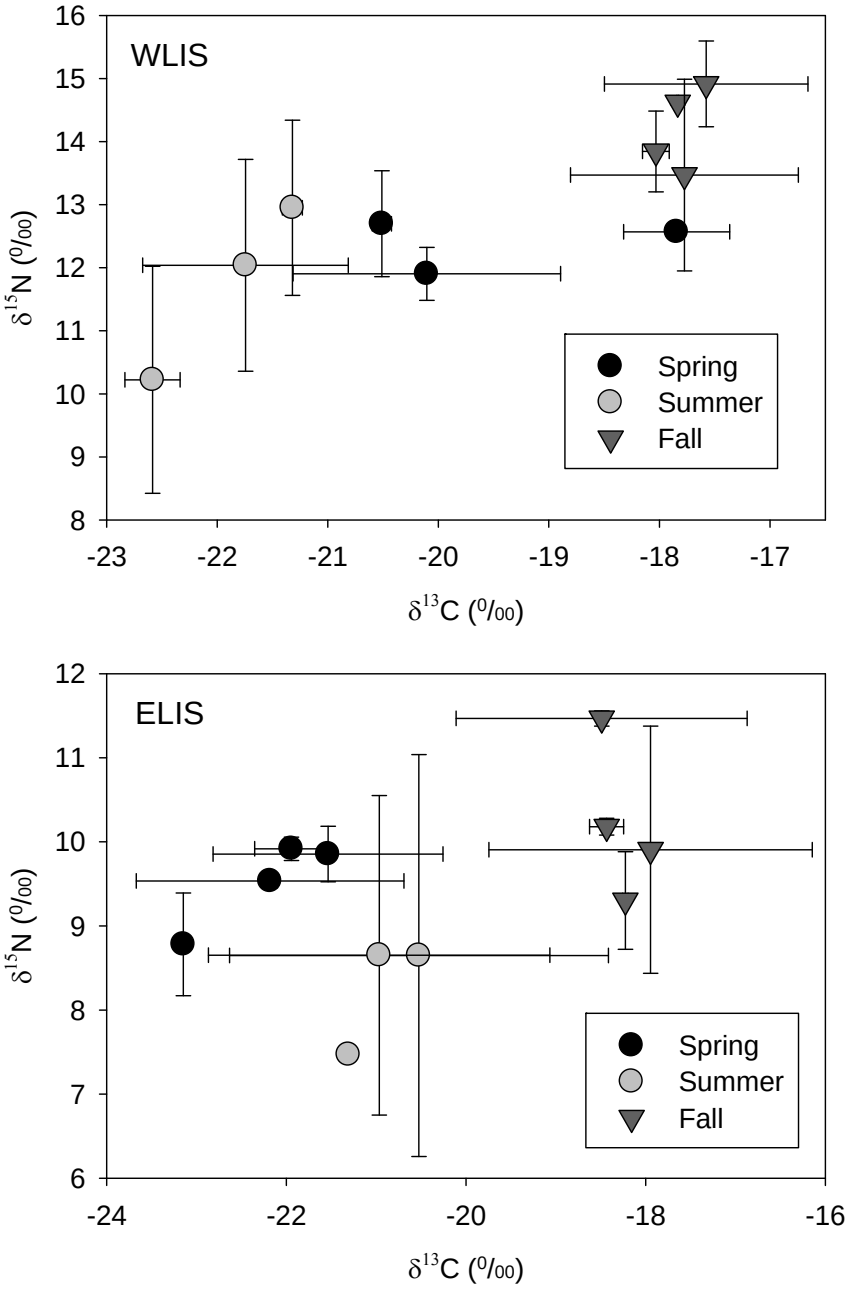

Figure 3.14. Stable $\delta^{15}N$ versus $\delta^{13}C$ values plotted for each season for WLIS (top) and ELIS (bottom). Error bars reflect variation between 3 substations for each site.

From these plots it appears that there were distinctive food chains for each season in WLIS. It seems that the spring zooplankton might have had more of an omnivorous feeding relationship, while the summer and fall might have followed some slightly more predatory linkages. The higher range in $\delta^{15}N$ values in WLIS measurements compared to ELIS indicates that these species were consuming higher in the food links than at ELIS, or food sourced with a higher base of $\delta^{15}N$ levels (i.e. sewage).

The signatures are different in ELIS, and contain much more overlap between isotopes, especially in the summer. This could signify that there was more of an herbivorous and omnivorous feeding web structure for most of the year, except during fall where it appears there might have been some predator interactions. The seasonal feeding patterns do appear somewhat consistent for WLIS and ELIS, though the spring in ELIS is shifted towards more depleted terrigenous sources than at WLIS. As the estuarine circulation pattern is consistently bringing different water masses passing through ELIS, it is not unexpected to see large variations in the zooplankton diets.

3.4.2.2 Shelf stations

Overall the HgT concentrations were higher in LIS than at marine stations during the fall cruise, peaking in the smallest zooplankton size classes at the intermediate CLIS station. The CLIS station also had the overall highest MeHg concentrations for both the 0.5-1.0 mm and 1.0-2.0 mm fractions. Though the 0.2-0.5 mm size class consistently was the lowest in MeHg for all stations (~1-4 pmol/g (wet wt.)), overall there was little indication of increasing MeHg concentrations with increasing sizes. The largest size class had the most MeHg at WLIS (~5.8 pmol/g (wet wt.)), though it was less than the 0.5-1.0 mm size at CLIS (~10 pmol/g (wet wt.)).

The phytoplankton values at the MS and SB stations both had larger values represented in at least one size fraction for phytoplankton MeHg and HgT, but these greater concentrations were not reflected in the zooplankton.

The %MeHg values on the other hand, indicate that there was increasing bioaccumulation of MeHg through the zooplankton at each station, excluding the value for the >2.0 mm size at MS. The largest zooplankton %MeHg was found at WLIS (27.6 %) and ELIS (30.7 %). The smallest 0.2-0.5 mm sizes were relatively consistently low across the stations from LIS to the shelf (~1.4 to 3.8 %), and contained primarily small copepods throughout. Therefore it is likely that these species were feeding on similar materials, and very low on the food chain, as even most of the measured phytoplankton had greater %MeHg values.

Measurements for $\delta^{15}N$ isotopes tend to further exemplify that consumption went higher into the food chain in LIS compared to the shelf station during the fall. The $\delta^{15}N$ measurements were highest at WLIS (~14 to 16 ‰ $\delta^{15}N$), decreasing as the stations moved eastward and out to the shelf stations. The MS station was much lower than at ELIS, collectively around 5-7 ‰ $\delta^{15}N$, but the SB stations had the lowest $\delta^{15}N$ measured during the fall (~4 to 5 ‰ $\delta^{15}N$). Furthermore, there is nearly no overlap between the LIS and shelf stations in $\delta^{15}N$ values. Thus, the marine species are apparently consuming much lower on the food chain, probably more particulate material and fewer larger phytoplankton or small zooplankton. It was also evident that the anthropogenic enhanced $\delta^{15}N$ influence perceptibly decreased from WLIS out to the SB station.

The $\delta^{13}C$ values were much more depleted for the marine stations than LIS during fall. There was slight overlap around -20 to -19 ‰ $\delta^{13}C$, which is not unexpected as some circulating marine algae sources likely drifted into LIS. There was no real indication of any correlation

between MeHg of HgT concentrations and $\delta^{13}C$ depletion. Trends across the different size fractions for $\delta^{13}C$ were relatively flat, indicating that all sizes were possibly feeding on particulate matter and algae for distinctive sources moving from WLIS out to the SB. As both marine phytoplankton (-18 to -24 ‰ $\delta^{13}C$) and microalgae (-8 to -27 ‰ $\delta^{13}C$) fall into the ranges measured (Fry and Sherr, 1984), it is likely that both were food sources throughout the transect.

Examining the average signatures of $\delta^{13}C$ and $\delta^{15}N$ isotope for the fall cruise indicates divergent groups of isotopic signatures (Figure 3.15). Two distinctive groups are apparent, and they clearly represent LIS stations and shelf stations. There is relatively large overlap in $\delta^{13}C$ values, especially at ELIS, but this is not unexpected as there are many different sources with overlapping $\delta^{13}C$ values. The ELIS station would likely have the most overlap of the stations as it is at the entrance to LIS, thus it experiences both fluctuating terresterial output and marine input into the surface waters. The isotopic separation between values at each station is not large enough to represent trophic transfer, but it does help confirm that zooplankton had distinctive body burdens comparing LIS and the marine shelf.

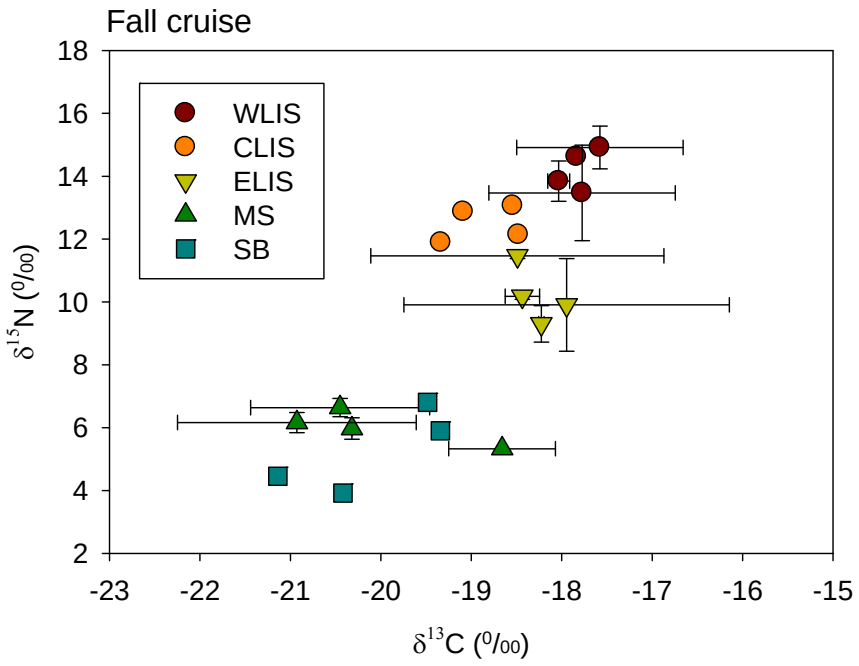

Figure 3.15. Stable $\delta^{15}N$ versus $\delta^{13}C$ values plotted for each station during the fall cruise. Error bars reflect variation between substations for each site.

3.5 Conclusions

Phytoplankton concentrations of Hg and MeHg were surprisingly lowest in summer compared to during spring and fall. Though there was higher %MeHg in the 0.2-5 µm size class of WLIS compared to the other fractions, there was no clear indications of a seasonal increase in MeHg measured in phytoplankton during the summer. There was also no increase in MeHg measured in LIS waters during the summer. Furthermore, methyltion rates were established as low in WLIS by Hammerschmidt et al (2008), possibly due to abundant organic carbon sequestering Hg to the substrate. Thus, though an increase in sediment SRB methylation during the summer season is possible, this does not appear to be reflected in pelagic algae or waters collected in LIS for this study. One notable result is the much higher concentrations of both HgT

and MeHg were measured in ELIS than WLIS phytoplankton. As WLIS tends to have higher continuous nutrient levels in the water, and therefore more potential algae growth, this suggests that phytoplankton abundance could be a factor, and demonstrative of growth dilution (Chen and Folt, 2005). If there are enough nutrients to sustain continuous algae growth, then the HgT and MeHg would be more diluted then in the regions where there is less nutrient supply and plankton growth. However, another likely scenarios is that the DOC in WLIS has a stronger capacity to form complexes with Hg and MeHg (Lamborg et al., 2004; Hammerschmidt et al., 2008), making it less available for uptake. It is unclear which factor could be the most important, however both theories likely contribute to the trends detected.

There are some apparent changes in the composition of phytoplankton and zooplankton HgT and MeHg concentrations for each season. The HgT concentrations were relatively consistent for spring and summer at both the ELIS and WLIS, but increased in the fall for both regions, especially at WLIS. The MeHg concentrations were more variable, but experienced some overall increase in the summer of ELIS. In WLIS it appears that there is no distinct seasonal increase in %MeHg during the summer, and a more distinctive increase in %MeHg with increasing trophic levels in the spring and summer than during the fall season. There does seem to be an increase in the summertime %MeHg for zooplankton in ELIS. This could be a reflection of increasing bioaccumulation during the summer for species residing around that station. However, it is notable that this increase is more likely due to less overall biomass then to any measured increase in MeHg into the water column, as there were no discernable changes in ELIS water or concentrations of MeHg. Changes in water concentrations coinciding with higher summertime sediment methylation rates have been seen in other regions (Gosnell et al., 2015), but were not apparent during 2014 in LIS.

It does appear that there are different groups of plankton dominating and sourcing the food for zooplankton for each season in LIS. This is confirmed by the $\delta^{13}C$ isotopic measurements, as there are different values for each seasonal alliance. This was more apparent at WLIS then ELIS, possibly indicating some linear feeding patterns at WLIS, and weaker marine water input through the seasons. Though there is apparently some omnivorous web feeding at both stations through the seasons, the web-like overlap is much more prominent at ELIS. Carbon isotope food webs can be altered by seasonal and environmental changes, and different phytoplankton dominating the base of the structure (Gearing et al., 1984; Paterson et al., 1985). Thus there is not one clear source which can be discerned to dominate the zooplankton. However it is evident that there are different sources into WLIS then ELIS, and that they vary by season. The $\delta^{15}N$ values also indicated that there was some trophic transfer feeding in the zooplankton of LIS, and that the species in WLIS were consuming much higher on the food chain, and more anthropogenically sourced $\delta^{15}N$, then species in ELIS. It is likely that zooplankton in ELIS are more subject to the sparser marine water cycling then WLIS, and therefore feed lower and more opportunistic depending on what drifts into their region. Though to further highlight the potential dilution effect, the zooplankton in WLIS also had some of the lowest %MeHg values during fall, even though the $\delta^{15}N$ indicated that they had been consuming higher in the food linkage. The $\delta^{15}N$ values also indicate that there were different patterns depending on the season for each station, and that there was much more overlap in values at ELIS. This likely effects the HgT and MeHg biota concentrations, though there was no correlation between %MeHg and $\delta^{15}N$ values. The lack of clear correlations between size fractions and HgT and MeHg concentrations is conceivably explained by the overlap in $\delta^{13}C$ and $\delta^{15}N$ values, as a more omnivorous diet could dilute any clear bioaccumulation signals at the

plankton level. However more evidence is needed to confirm this. There were very minor differences in the $\delta^{34}S$ isotopes within zooplankton measured, though most zooplankton had values around 20.4 ‰, corresponding to typical phytoplankton measurements (Peterson et al., 1985). Thus, even in the shallower system of LIS, the zooplankton were primarily feeding on pelagic food sources, and not dipping down to the benthos for their nutritional needs.

Overall this study highlights how the amount and prominence of organisms can affect HgT and MeHg bioaccumulation. At ELIS and on the shelf stations there appeared to be much more bioconcentration of Hg species into the plankton during the summer. This was further confirmed by the measurements of logBCFs throughout the different seasons. The fall cruise extending out into the marine waters off the shelf of LIS demonstrated the clear differences between coastal and marine plankton species. This was most prominent in the $\delta^{15}N$ and $\delta^{13}C$ measurements, as all plots for stations in the fall cruise displayed distinctions between stations moving from WLIS out to the SB.

Thus it is possible that seasonal methylation, or general MeHg uptake, could be more of a factor in more pristine systems and open ocean systems, and less of a factor in regions with relatively prominent amounts of planktonic biomass, such as eutrophic coastal systems. Long Island Sound might not be the easiest places to discern distinctive Hg differences in plankton, as it contain relatively low levels of Hg and MeHg compared to some other regions, and the water column is continuously circulating fresh inputs and outputs. Seasonal methylation could possibly be more of a factor for smaller enclosed estuaries which have large organic matter changes, or relatively pristine regions such as the arctic. As climate change is expected to affect both the arctic and open ocean prominently, it is possible that those organisms could be subjected to more of a burden of seasonally driven increases in MeHg uptake into fewer species.

Chapter 4:

Stable Isotope Quantification of Trophic Transfer for Inorganic and Methylmercury from Marine Diatoms to the copepod *Acartia tonsa* and Native LIS Zooplankton Species

4.1 Introduction

Mercury (Hg) levels in the environment have been enhanced from natural levels due to anthropogenic emissions and practices, such as combustible coal burning, cement manufacturing and gold mining (National Research Council, 2000). Methylmercury (CH_3Hg; MeHg) acts as a neurotoxin, and is an established human health concern as humans reside as apex consumers in the aquatic food web (National Research Council, 2000; Clarkson and Magos, 2006). The toxic effects of CH_3Hg on humans and marine organisms are enhanced through lipid membrane penetration (Clarkson and Magos, 2006; Bustamante et al., 2006), and tends to be enhanced higher on the food chain due to the greater affinity of CH_3Hg to cellular proteins. Marine food webs tend to bioconcentrate CH_3Hg, therefore toxicity becomes magnified with increased trophic position (Watras et al, 1998).

Relatively little data has been published on the effects of CH_3Hg transfer from algae to primary consumers (zooplankton), or on Hg speciation trophic transfer in coastal marine systems (Chen et al., 2008). The majority of research has been undertaken in freshwater systems (Surette et al., 2003; Pickhardt and Fisher, 2007; Mohr et al., 2002). Furthermore, many previous studies have used high and relatively unrealistic exposure levels (Mason et al., 1996; Pickhardt and Fisher, 2007; Mathews and Fisher, 2008; Zhang et al., 2013). Thus, there is the need to further investigate the uptake and trophic transfer of CH_3Hg in marine plankton at more realistic concentrations. Humans tend to consume much greater quantities of oceanic fish where ambient Hg levels are extremely low, thus it is imperative to understand Hg accumulation dynamics at lower exposures. Additionally, there is a great deal of unexplained variation in CH_3Hg levels in the biota of coastal ecosystems, especially in primary consumers, which must be understood in order to properly assess factors influencing the bioaccumulation processes.

It is important to understand the mechanisms of transfer of Hg and CH_3Hg through the lower trophic levels, as this is where the highest biomagnifications occurs (Mason et al., 1996; Mason, 2002; Moye et al., 2002; Fisher et al., 1984). Recent analytical advances have decreased detection limits for determining the Hg and CH_3Hg concentrations in aquatic systems. Using stable Hg isotopes, it is now possible to do experiments at substantially lower and environmentally realistic values. Understanding these mechanisms at more realistic Hg concentrations will provide a more solid basis for assessing varying impacts of Hg and CH_3Hg concentrations in the natural environment, and at the base of the food chain where bioaccumulation initiates. Additionally, through these laboratory-based trophic transfer experiments using stable Hg isotopes we were able to simultaneously measure the movement of Hg and CH_3Hg through marine primary producers into secondary consumers.

One principal aim of these experiments was to see if measured levels of Hg and CH_3Hg in plankton are a function of the exposure concentration. A separate aim of this research was to determine if algae size would impact the uptake, and subsequently trophic transfer of Hg and CH_3Hg from phytoplankton to zooplankton. Two different diatoms were used to be representative of different sizes of phytoplankton which are commonly found in marine environments. The diatoms *Thalassiosira weissflogii* (~10-17 μm) and *Thalassiosiria pseudonona* (~3-10 μm) were chosen, as both have previously been used in feeding experiments (Siuda and Dam, 2010) as well as in uptake experiments using higher Hg and CH_3Hg concentrations (Fisher et al., 1984; Mason et al., 1996). They were also chosen as it was likely that they would be consumed by the zooplankton used in these studies. The algae were cultured and allowed to accumulate under two different concentrations, near environmental levels (pM), of Hg (^{200}Hg) and enriched CH_3Hg ($CH_3{}^{199}Hg$). As copepods are the predominant zooplankton

in the coastal ocean, the species *Acartia tonsa* (~1 mm), a prevailing zooplankton in the nearby vicinity of Long Island Sound, was the grazer chosen for feeding experiments.

In order to compare laboratory bioaccumulation and transfer to what could occur in the coastal environment; additional transfer experiments were preformed using natural zooplankton of various sizes. Zooplankton were collected during two separate seasonal cruise excursions in Long Island Sound (LIS; Chapter 3) and used as various secondary consumers. These' *in situ*' feeding experiments provided an additional basis for what can be experienced in natural assemblages of the coastal marine environments.

4.2 Methods

4.2.1 Experimental

The copepod *Acartia tonsa* was cultured in filtered seawater in a walk in chambers at 18^{o}C at the University of Connecticut, and kept alive on a diet of feeding phytoplankton *Thalassiosiria weissflogii* (Tw) and *Tetraselmis impellucida* at a concentration of approximately 500 µg C/L. For each feeding experiment approximately 350 adult female copepods were selected and kept in fresh filtered seawater without food for one day prior to the experiment. For the beginning of each feeding experiment, 30 copepods were collected from and placed in a 1 L sized wide-mouthed feeding bottle containing freshly filtered seawater.

The two *Thalassiosiria* cultures of algae were grown under f/4 culture medium levels with trace metals reduced to f/8 levels, in a light-dark cycle at 18 ^{o}C. Algae cellular concentrations were monitored using a Beckman Coulter particle analyzer. The particle analyzer was also used to assess concentrations of algae which would be fed to copepods in order to keep

algae amounts equivalent to natural assemblages. Based on natural bloom magnitudes in Long Island Sound and feeding experiment concentrations used in Siuda and Dam (2010), phytoplankton cell concentrations fed to each grazers for 24 hours were estimated to be ~3000 cells mL^{-1} for Tw and and ~18000 cells mL^{-1} for *Thalassiosira pseudonona* (Tp). Thirty copepod species feeding for 24 hours thus required ~ 90000 cells (Tw) or ~270000 (Tp), and the necessary volume for each treatment was calculated from the algae being used during the experiment.

Algae cultures were spiked with specific amount of enriched $CH_3{}^{199}Hg$ and ^{200}Hg at the approximate exponential phases for each culture, and cellular incorporation was allowed to occur for 24 hours prior to feeding and sampling. Lower addition experiments were undertaken with concentration combos of 10 pM $CH_3{}^{199}Hg$ and 100 pM ^{200}Hg . Separate higher concentration experiments were completed using magnitudes of 100 pM $CH_3{}^{199}Hg$ and 200 pM of ^{200}Hg. Initial samples were collected for *Thalassiosira* and *A. tonsa* in order to assess background mercury levels of algae and grazers. Algae samples were also collected for both 'clean' and spiked feeding treatments at the initiation of each feeding experiment to measure actual and background algae concentrations.

Algae for each treatment was gently transferred on a 5 μm polypropylene filter (or 1 μm for Tp), and resuspended into 30 mL of clean filtered seawater in order to remove any potential dissolved isotope sources from the culture medium. The algae were then carefully added back to each specific treatment bottle. Isotopically treated algae were added to 5 bottles, while untreated ('clean') algae were added to 3 bottles for control treatments. Three bottles also contained only algae of the same cell concentration added to each treatment in order to account for any potential

cellular growth during the experiment. All of the treatment bottles were placed on a feeding wheel situated in an 18^{o}C walk-in chamber for 24 hours. Algae were fed to copepods for 24 h.

For the complementary in situ feeding experiments, zooplankton were collected during the summer and fall legs of the seasonal study in LIS (chapter 3). The zooplankton were kept alive and separated into different size fractions to measure the potential differences in the assimilation efficiency for different size classes. In order to minimize additional variables, only *T. weissflogii* was used as the food in these field experiments, and only the higher concentration combination was investigated (100 pM $CH_3{}^{199}Hg$ & 200 pM ^{200}Hg). Each zooplankton size class was transferred into individual 1L wide mouth bottles filled with clean filtered seawater and estimated amounts of re-suspended Tw algae, and allowed to feed for 24 hours. Clearly dead zooplankton were removed prior to initiating the experiment.

After the zooplankton were allowed to feed, the bottles were removed and processed. Bottles containing only algae were measured on the coulter counter. Species were separated via filtering each treatment through size fractioned cascading filters of 63 μm, to collect the adult copepods (or '*in situ*' LIS field zooplankton), and 20 μm to collect the feces. These collections were then reverse filtered using clean filtered seawater, onto 20 μm or 5 μm polypropylene filters, and frozen until ^{200}Hg and $CH_3{}^{199}Hg$ analysis. Copepod feces were collected on 5 μm polypropylene filters in order to measure the assimilation efficiency of ^{200}Hg and $CH_3{}^{199}Hg$. Any remaining algae were collected on 5 μm or 1 μm polycarbonate filters to measure any uneaten $CH_3{}^{199}Hg$ and ^{200}Hg levels, and kept frozen until analysis.

4.2.2 Analytical

Mercury isotope determinations for experimental biota were analyzed at the University of Connecticut using the high sensitivity Perkin-Elmer ELAN DRCII Inductively coupled plasma mass spectrometer (ICP-MS) in low resolution mode. Using gas chromatography coupled with cold-vapor atomic fluorescence spectrometry (CVAFS) for Hg and CH_3Hg quantification. Biota samples were weighed and digested in 4.51 M HNO_3 acid solution in a 60 ^oC water bath for ~12 hours prior to analysis. A 1 mL aliquot of the digest was neutralized with ~550 µL of 10 N KOH to a reactive pH of ~4.9 for $CH_3{}^{xxx}Hg$ analysis, and made up to 30 mL in a acid-cleaned glass vial with ultra pure 18.2 MΩ water. Ammonium acetate buffer (4M; 225 µL) and sodium tetraethylborate (30 µL) were added to each sample to induce Hg ethylation. Samples were then analyzed on a Tekran auto-methylmercury (CVAFS) coupled with the ICP-MS. Water samples were additionally distilled and quantified using a separate $CH_3{}^{201}Hg$ isotope dilution tracer. The CH_3Hg recovery was also assessed for recovery from standard additions into a matrix, and the 91± 7% was within the range accepted (71-125%) based on EPA method 1638. The $CH_3{}^{xxx}Hg$ peaks were integrated by Perkin-Elmer Cromera software. The method detection limit (MDL) was assessed to be 0.02 pmol for these measurements.

The ICP-MS was coupled with FIAS for ^{yyy}Hg analysis. The remaining digest was diluted up to 10-14 mL using with 18 MΩ water. Digests which contained potential blockages (i.e. zooplankton) were transferred to clean tubes. Brominemonochloride (BrCl; 40 µL) was added for at least 24 hours preceding analysis to oxidize remaining tests and algae. Inorganic mercury for water samples was digested in 10 mL aliquots with ~2.91 M HNO_3 and BrCl for at least 48 hrs prior to analysis. A stable isotope ^{201}Hg tracer was added to each sample (at 0.5 nM concentration) and hydroxylamine hydrochloride (20 µL) were added to each sample prior to

analysis. Calibration curves were achieved to $r^2 = 0.99$ for each analysis. Instrument precision and accuracy were also monitored by standard and analytical duplicates ~ every 10 samples, in addition to laboratory reagent blanks, and RSD was <10% while the MDL was 1.1 pmol.

4.3 Results & Discussion

4.3.1 Algal Uptake

Uptake into algae was clearly driven by both the initial concentration and algal species. Uptake concentrations displayed in Figure 4.1 and Figure 4.2 represent the average and standard deviation of all of the feeding experiments performed, including algae used in field feeding experiment. A total of 8 different experiments were completed over 3 years. As far as the ^{200}Hg spikes, the Tp algae contained concentrations ~0.001 amol/cell after exposure to the lower 100 pM spike, and increased to 0.15 amol/cell for the 200 pM spike. In contrast, the Tw algae contained higher concentrations than had been measured for either Tp measurement, at 0.25 amol/cell for the 100 pM addition and 14.24 amol/cell for the 200 pM addition (Figure 4.1). Algae uptake statistically differed by species and initial concentration, in addition to being distinct when compared to initial concentrations ($p<0.03$, $n=24$, Two-factor Anova). Surprisingly, though the ^{200}Hg concentrations were only doubled, the uptake concentrations increases were greater than this for the higher experiment additions, reflecting a significant additional uptake for both algae species.

The $CH_3{}^{199}Hg$ additions were an order of magnitude different for the separate feeding experiments (Figure 4.2). Uptake concentrations into the Tw algae clearly reflected this magnitude of difference, as the +10 pM addition contained 0.14 amol/cell and the +100 pM had

1.02 amol/cell. The Tp algae displayed a difference, and although both concentrations were also less than what was in the Tw cells, differences were not proportional as they were with Tw. The lower exposure concentration of $CH_3^{199}Hg$ resulted in uptake of 0.014 amol/cell for Tp, and the culture with the higher addition contained 0.08 amol/cell. Overall the $CH_3^{199}Hg$ concentrations per cell were strongly statistically different between uptake concentrations and algae species ($p<0.001$, $n=24$, Two-factor Anova).

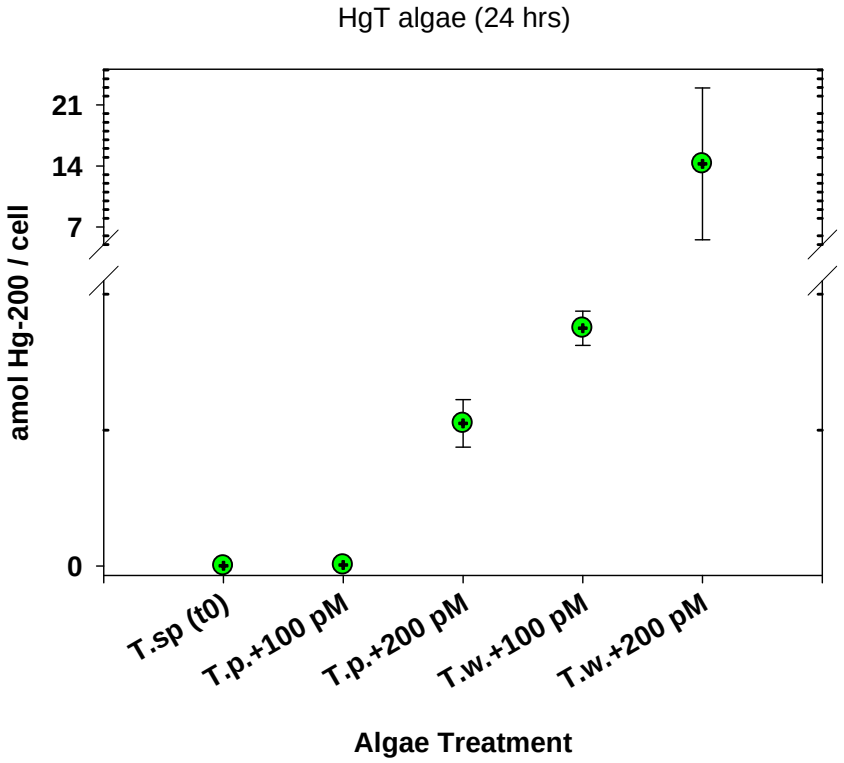

Figure 4.1. Concentration of ^{200}Hg (amol/cell) in algae for each feeding experiment preformed. Initial and control concentrations of Hg^{200} in algae (T.sp (t0)) is also plotted for reference.

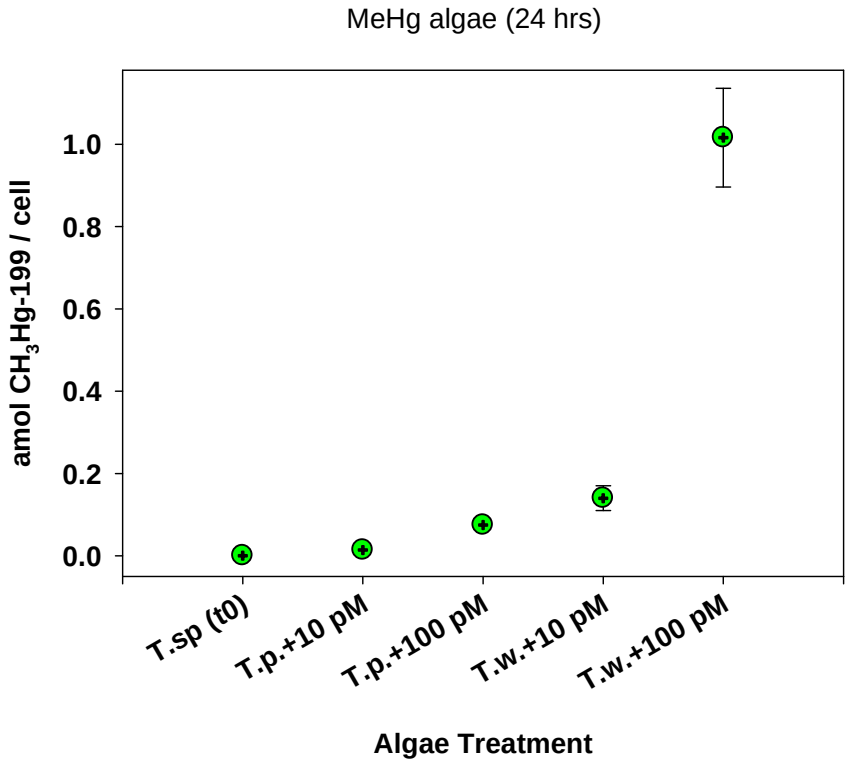

Figure 4.2. Concentration of $CH_3{}^{199}Hg$ (amol/cell) in algae for each feeding experiment performed. Initial and control concentrations of CH_3Hg in algae (T.sp (t0)) is also plotted for reference.

Volume concentration factors (VCFs; concentration in algal cell per cellular volume/concentration in solution) were calculated for each isotope concentration based on the volume of each species of algae (Table 4.1; dimensionless VCF $x10^3$), which was estimated by the size dimensions and confirmed by literature assessments using *T. psuedonona* and T.*weissflogii* VCFs (Fisher et al., 1984; Mason et al., 1996). The VCFs were significantly lower for the smaller isotope spike, which is unexpected as VCFs theoretically should be independent of concentration. The difference was much more pronounced for the *T. pseudonona* algae, though the *T. weissflogii* VCFs also somewhat reflect a magnitude of difference. One interesting trend was that the overall VCF values for the *T. pseudonona* algae had similar relative amplification between concentrations for both isotope species, suggesting much higher uptake

for both forms of Hg at the higher exposure concentration. This result could have important implications about the impact of size; i.e. smaller algae species behave differently towards specified Hg and CH_3Hg uptake than larger species.

Table 4.1. Volume concentration factors (VCF x 10^3; VCF = [algae] / ([water]*volume algae)) calculated for each isotope concentration used for the two separate algae species.

Treatment	+100 pM ^{200}Hg	+200 pM ^{200}Hg	+10 pM $CH_3{}^{199}Hg$	+100 pM $CH_3{}^{199}Hg$
T.pseudonona	**72.1 ± 0.8**	**1038.1 ± 101.5**	**35.8 ± 21.7**	**1332.0 ± 101.6**
T.weissflogii	**11.9 ± 0.8**	**212.8 ± 83.6**	**77.4 ± 10.2**	**142.1 ± 16.5**

Most researchers suggest that algae primarily take up Hg and CH_3Hg via passive absorption (Fisher et al., 1984; Mason et al., 1996; Luengen et al., 2012; Le Faucher et al., 2014), though active absorption is expected for essential metals, such as Fe and Zn, and it is possible that non-essential metals such as Hg are inadvertently accumulated via these mechanisms as well. Also, as neither Hg nor CH_3Hg are present in solution in seawater dominantly as a free ion, the speciation in solution will have an impact on the rate of uptake and whether uptake is passive or active. When accumulated into phytoplankton actively or via facilitated transport, metals must initially diffuse through the cellular boundary layer from solution onto the plasma membrane, and attach to specific transporters to actively cross the membrane into cells. Metals tend to bind to intercellular ligands which make up the usual algal metabolic processes such as photosynthesis, and then be incorporated into cellular functions.

Isotope concentrations remaining in solution indicate that nearly all of the isotopes were taken up into the algae after 24 hours (Figure 4.3). More isotopes were taken up into the Tp

cultures, as would be expected taking into account the denser cultures. Interestingly, there were no significant differences in remaining water levels between ^{200}Hg concentrations for either algae species, even though initial additions were twice as high for the higher exposure experiments. Higher experimental concentrations yielded more relative uptake for both isotope concentrations. The most noticeable difference was between the $CH_3{}^{199}Hg$ concentrations remaining for the Tw species (1.5 pM compared to 6.7 pM), though it was nowhere near initial addition concentration differences. It is likely that all of the isotopes would have continued being accumulated by the algae cultures until there is none remaining in solution with more time and continued cellular growth. The relative similar ^{200}Hg concentrations remaining in solution could be a potential indication of the comparative dependence of Hg uptake on algae species prevailing over initial addition.

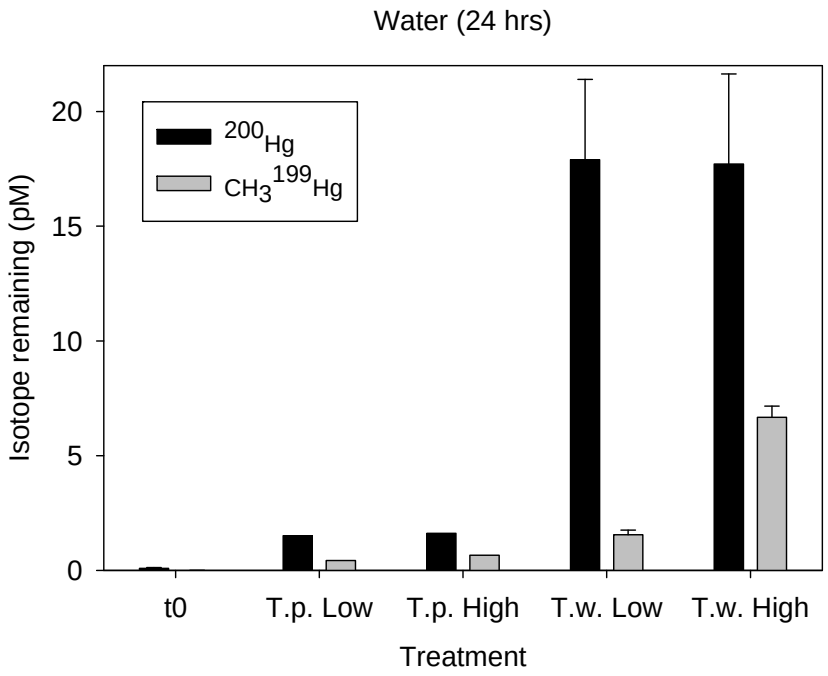

Figure 4.3. Remaining isotope water concentrations for ^{200}Hg and $CH_3{}^{199}Hg$ (pM) after 24 hours for each algae species.

The estimated recovery of isotopes for all experiments were calculated based on the measured amol/cell concentrations, cell count, and remaining concentration of isotopes in solution. All of the treatments for both diatoms had $CH_3{}^{199}Hg$ recoveries around 100%, indicating that all of the $CH_3{}^{199}Hg$ was accounted for in the algae and water. The ^{200}Hg had much more variable recoveries, as the Tw species had low recovery for the +100 pM ^{200}Hg addition (54%), but higher recovery for the +200 pM ^{200}Hg addition (134%). Both ^{200}Hg addition levels for the Tp species were ~100% recovery.

Average uptake rates for $CH_3{}^{199}Hg$ and ^{200}Hg into the different diatoms (amol $cell^{-1}$ hr^{-1}) were calculated, and are available in Table 4.2. The uptake rates were more than a factor greater between species than initial additions would suggest, further confirming algae size differences. The Tp species uptake rates related with the initial relative ratio of Hg species additions for the high exposures, and to a lesser extent the lower exposure ratios as well. But, for the Tw species uptake seemed more of a function of initial exposure concentrations difference. For comparison, the relative exposure concentrations (amol $cell^{-1}$ hr^{-1} pM^{-1}) were also calculated, based on the final water concentration, for all the experimental treatments and are presented in Table 4.2. When evaluated to other relative uptake rates for Tw reported in Le Faucheur et al (2014) it is evident that the numbers are much lower than found for experiments preformed at the higher exposure levels (i.e. nM), which were orders of magnitude greater (2-6 amol $cell^{-1}$ hr^{-1} pM^{-1}). However, values in these experiments are equivalent to rates calculated from some field measurements at lower concentrations in various freshwater species (Le Faucheur et al., 2014). This further emphasizes the possibility that experiments preformed at higher concentrations could have yielded overestimated or unrealistic uptake rates in some instances. The relative

exposure concentrations indicate that greater uptake occurred at higher exposures for $CH_3{}^{199}Hg$, and this was much more pronounced for Tw algae. However, relative exposure concentrations clearly show that ^{200}Hg uptake was more dependent on algae species, as the values are analogous, independent of exposure concentration in both Tp (0.06-0.07 amol $cell^{-1}$ hr^{-1} pM^{-1}) and Tw (~0.75 amol $cell^{-1}$ hr^{-1} pM^{-1}). This possibly demonstrates the inorganic Hg affinity is more dependent on algae size (i.e outer cell wall surface area), while the CH_3Hg is more driven by exposure concentration, and could possibly be more effectively taken up into algae.

Table 4.2. The average uptake rate (amol/cell/hr) and exposure concentrations (amol/cell/pM) for each treatment of $CH_3{}^{199}Hg$ and inorganic ^{200}Hg for the two different algae species.

Treatment *algae*	$CH_3{}^{199}Hg$ (amol $cell^{-1}$ hr^{-1})	^{200}Hg (amol $cell^{-1}$ hr^{-1})	$CH_3{}^{199}Hg$ (amol $cell^{-1}$ hr^{-1} pM^{-1})	^{200}Hg (amol $cell^{-1}$ hr^{-1} pM^{-1})
Low ***T.pseudonona***	0.0001	0.0004	0.018	0.064
High ***T.pseudonona***	0.0033	0.0063	0.027	0.067
Low ***T.weissflogii***	0.0058	0.0104	0.065	0.747
High ***T.weissflogii***	0.0459	0.2503	0.278	0.738

The involvement of effective transport by active uptake or facilitated mechanisms is known as a possibility for additional algae uptake of metals. For example, Pickhardt and Fisher (2007) demonstrated that heat-killed diatoms contained less phytoplasm CH_3Hg, suggesting a metabolically controlled uptake into diatoms. Fisher et al (1984) also reported that living cells took up more Hg then dead or dying cells. Experiments preformed on bacterial mer-lux bioreporters demonstrated facilitated transport at more realistic trace levels (<50 pM), indicating

that facilitated Hg uptake is possible in pelagic eukaryotic cells (Golding et al., 2002; Golding et al., 2007). Facilitated uptake has been shown to be more of a factor at lower solution Hg concentrations, as was shown by Moye et al. (2002) for a freshwater species. That research demonstrated that at lower concentrations of CH_3HgCl (<10 nM) the uptake curve was steeper than that at higher concentrations by a factor of ~10 for the species *Selenastrum capricornutum*. Moye et al. (2002) also provided hints that algal uptake at lower concentrations could be preformed via different mechanisms for the lower concentrations than their initial experiments which were completed under exceptionally high concentrations. The results presented from our uptake measurements did not address the difference between passive and active uptake, as living cells were consistently used for optimal feeding. However, the question of active uptake into marine algae is still being resolved, though it does appear to have a possible influence on enhanced CH_3Hg and Hg accumulation into algae.

Methylmercury and Hg species are known to bind especially strong to sulfur and selenium proteins (Clarkson and Magos, 2006), which assists in the high accumulation of Hg species even though there are no known metabolic benefits to containing more Hg. There has been some demonstration of detrimental effects on algae which accumulate excessive Hg and CH_3Hg, though these experiments were all preformed at unrealistic environmental concentrations (i.e. μM), thus it is unlikely that most marine populations are ever detrimentally impacted by ambient Hg concentrations. Also, there have also been no ill effects reported on species for experiments below excessive experimental concentrations (Golding et al., 2007; Le Faucher et al., 2014). In natural aquatic systems Hg and CH_3Hg species tend to be available complexed to various neutral ligands (i.e. $HgCl_2$; CH_3HgCl), and the presence of alternative binding ligands such as DOM and EDTA in solution has been shown to reduce metal

bioavailability to algae (Pickhard and Fisher, 2007; Luengen et al., 2012). However, once inside the cells it has been noted that the presence of intercellular ligands can induce dissociation of any accumulated Hg complexes (Clarkson and Magos, 2006; LeFaucher et al, 2014). This is viable as there is a relatively weak affinity between Hg^{+2} and Cl^{-} compard to the stronger binding capacity of intercellular thiols, resulting in the dissociation of the $HgCl_2$ complexes (LeFaucher et al., 2014).

Mason et al (1996) initially established that passive diffusion of $HgCl_2$and CH_3HgCl through Tw membrane was the primary mechanism of uptake into the diatom, by demonstrating the linear relationship between Hg and CH_3Hg and the overall octanol-water partitioning coefficients. These experiments were done at much higher Hg levels than is found in the natural environment, prompting some recent inquiries done at more realistic uptake levels. These results appear to demonstrate that lower trace level concentrations of CH_3Hg and Hg still tend to result in relatively equivalent accumulation for Tw species, confirming that uptake continues to be concentration dependent at low concentrations. This is what was found in the experiments reported here.

Algae physiology has also been strongly suggested to play a role in which uptake mechanism dominates for different species. For example, the size and volume dimensions of algae have been suggested to impact uptake of Hg and CH_3Hg species into various algae species. It is apparent that species of algae does impact uptake, as the same concentration in the water did not yield similar results in cellular concentrations for the two *Thalassiosira* species tested here. The smaller sized Tp species consistently contained less Hg and CH_3Hg then Tw. Even the lower addition of Hg isotopes yielded higher concentrations within the Tw than was found in the higher addition for the Tp algae. It has been demonstrated that inorganic Hg tends to bind

preferentially to the outer cellular walls and algal membranes, while the CH_3Hg resides better into cellular cytoplasm (Reinfelder and Fisher, 1991; Mason et al., 1996; Le Faucher et al., 2014). The two algae species of different dimensions do tend to have different VCFs for each Hg isotope form. The larger algae species Tw has a much lower VCF then the smaller Tp species as far as ^{200}Hg. This indicates that the smaller algae cell contained a magnitude more ^{200}Hg accumulated to the cell then the larger species. If the size to volume ratio for the smaller species is larger, which would be true given their dimentions, than it is possible that there is more relative area for the ^{200}Hg to attach per cell volume. However the cellular cytoplasm bound $CH_3{}^{199}Hg$ VCF is relatively closer, demonstrating that the two different sizes of algae contain more similar levels of $CH_3{}^{199}Hg$ depending on the initial concentration.

It is also viable that a 'bloom dilution' effect factored into the dissimilar uptake concentration differences between algae species. The smaller Tp species tended to grow in much greater cellular numbers prior to isotope addition than Tw (~1000000 for Tp versus ~70000 for Tw). It has been demonstrated that greater densities of algae tends to dilute the Hg and CH_3Hg species within a cell, as it is distributed more abundantly throughout the culture medium (Pickhardt et al., 2002; Chen and Folt, 2005). Thus, it is possible that the denser cultures of Tp likely also contribute to the much lower concentrations within those cells of ^{200}Hg and $CH_3{}^{199}Hg$ compared to Tw. However, even with higher overall culture densities, the differences between the uptake concentrations and when compared to background concentrations were still apparent.

4.3.2 Feeding Copepods

The grazers reflected the differences in the ^{200}Hg algae concentrations clearly for the Tp species, though they were less related for the Tw algae (Figure 4.4). For the lower Tw treatment

the grazer concentration was more aligned with the initial and control concentrations than the algae, though the higher treatment more clearly reflected the much higher algal concentration in the grazers at 52.3 ± 11.0 pmol/g ^{200}Hg. The *A.tonsa* feeding on Tp reflected clearer differences, with the lower treatment copepods containing 1.49 ± 1.0 pmol/g ^{200}Hg compared to the higher treatment with 9.64 ± 6.8 pmol/g ^{200}Hg. Overall the feeding treatments for measurements of ^{200}Hg were statistically different from controls and each experiment concentration ($p<0.001$, n=24, Two-factor Anova). Though differences between the lower concentration Tw feeding treatment and controls appeared overlapping in Figure 4.4, the variances were 0.48 and 0.14 respectively.

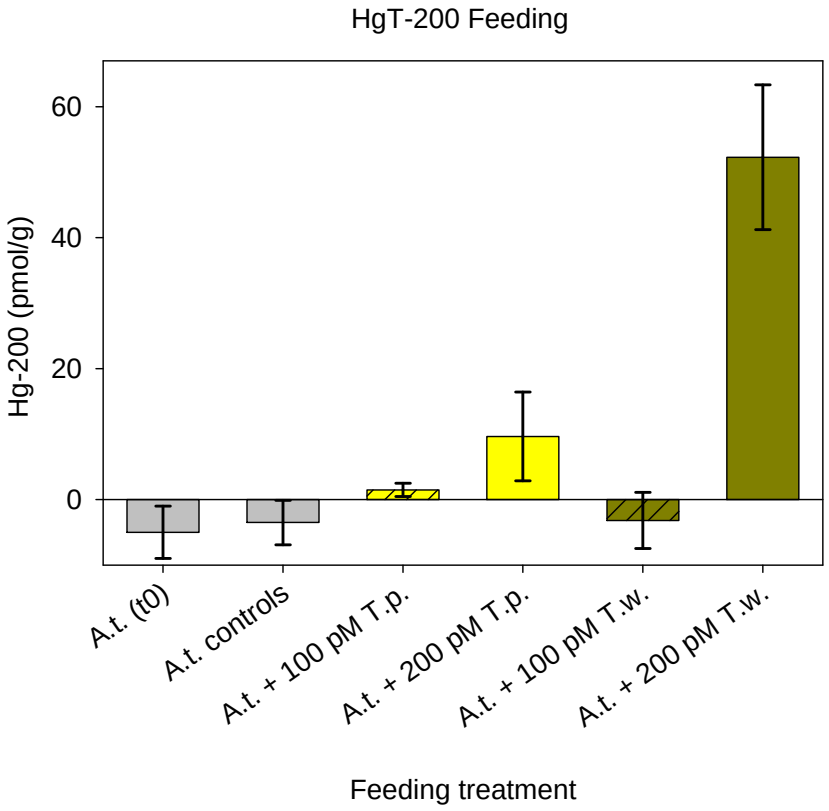

Figure 4.4. Concentration of ^{200}Hg (pmol/g) measured in copepods after feeding on spiked algae for 24 hours, for each feeding experiment preformed. Initial concentration of ^{200}Hg in copepods (A.t. (t0)), and control treatments using untreated algae (A.t. controls) are also plotted.

Grazer concentrations of $CH_3^{199}Hg$ are a relative reflection of algae isotope concentrations (Figure 4.5). Though there appears to be some minor overlap with control values and their standard deviations, the *A. tonsa* fed the lower treatment of Tp contained 1.1 ± 1.0 pmol/g $CH_3^{199}Hg$, while the higher treatment yielded 2.2 ± 1.3 pmol/g. Differences for $CH_3^{199}Hg$ consumption were statistically significant between controls and treatment levels ($p<0.001$, Two-factor Anova). Divergence between treatment concentrations was much clearer for copepods consuming Tw species. The lower treatment copepods contained 2.61 ± 0.9 pmol/g $CH_3^{199}Hg$ while the higher treatment yielded 24.8 ± 5.8 pmol/g $CH_3^{199}Hg$.

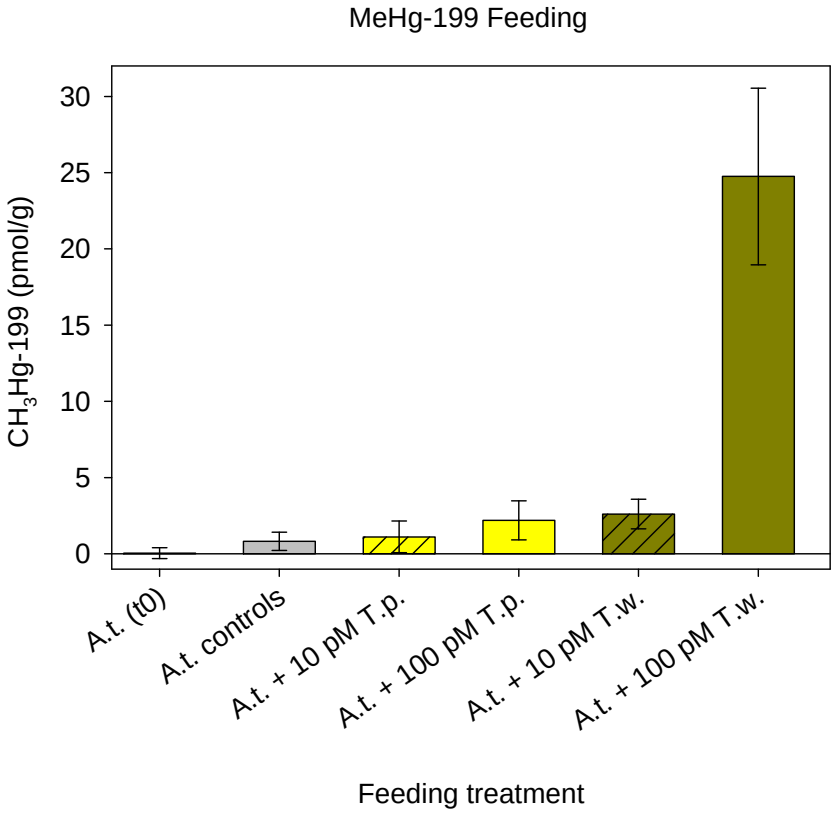

Figure 4.5. Concentration of $CH_3^{199}Hg$ (pmol/g) measured in copepods after feeding on spiked algae for 24 hours, for each feeding experiment preformed. Initial concentration of $CH_3^{199}Hg$ in copepods (A.t. (t0)), and control treatments (*A.tonsa* control) are also plotted for reference.

Assimilation efficiencies were calculated based on the concentrations in the *A.tonsa* treatments and their corresponding feces collection (Table 4.3). Assumptions in these measurements were that all of the feces were collected, thus these values yield a conservative estimate. As the remaining water of each experiment was filtered to collect any uneaten algae, it is possible that any escaped feces fragments could have ended up in that fraction. However, when the remaining algae concentrations were assessed, there were no significantly different values to indicate that feces were measured with the algae. Furthermore, each filter was visually inspected to ensure that there were feces on it, and that there were no prominent feces present on the algae or copepod fractions filters.

Table 4.3. Assimilation efficiencies (AE = [copepods/(copepods+feces)]) for each feeding experiment preformed. Treatments are separated by isotope species addition and concentration.

Treatment	+100 pM ^{200}Hg	+200 pM ^{200}Hg	+10 pM $CH_3{}^{199}Hg$	+100 pM $CH_3{}^{199}Hg$
A.tonsa* + *T.p.	**31.1 ± 10.8**	**41.9 ± 14.7**	**61.8 ± 21.6**	**71.2 ± 8.8**
A.tonsa* + *T.w.	**23.9 ± 11.4**	**32.3 ± 17.7**	**67.6 ± 9.2**	**88.6 ± 3.4**

For the Tp feeding experiments, the $CH_3{}^{199}Hg$ isotope AEs were relatively consistent ranging from ~60-70 %, when considering the large overlap in the +10 pM values. The Tw and Tp AEs exhibited clearer differences between the $CH_3{}^{199}Hg$ additions, as the lower accumulation ranged from ~61-68%, though the range spanned was larger for Tp. However, the higher addition yielded much larger values at ~71-88%, which are more akin to reported values in experiments using much higher concentrations (Mason et al., 1996; Fisher et al., 1984). The

^{200}Hg AEs were overall lower than those for $CH_3{}^{199}Hg$ across the species and ranges. There was some differences between the concentration differences for Tp but only about a ~10% increase was apparent for the higher ^{200}Hg concentration. The Tw experiments on the other hand, yielded relatively lower AEs for both concentrations (~24-33%), though the difference of ~10% between concentrations for ^{200}Hg was consistent. These values possibly indicate lower assimilation of ^{200}Hg in *A.tonsa* from both algae species was only slightly dependent on initial uptake levels. However, it is also notable that the AEs for inorganic Hg are higher than has been previously found (Mason et al., 1996; Fisher et al., 1984), indicating that there could be more active uptake and assimilation for lower, more environmental concentrations.

Primary consumers of algae containing accumulated Hg and CH_3Hg represent the ingress of trophic transfer into aquatic food chains. However, consumers do not necessarily consume everything available; therefore transfer can also depend on Hg speciation, algal quality as well as density (Reinfelder and Fisher, 1991; Pickhardt et al., 2002; Zhang et al., 2013; LeFaucher et al., 2014). It is also possible that for laboratory feeding experiments the transfer of Hg species can depend on preferential consumption of specific algae species by the grazer.

Metal form-specific behavior in trophic transfer has been demonstrated via various experiments (Mason et al., 1996; Fisher et al., 1984; Reinfelder and Fisher, 1991; Pickhardt et al., 2002). However, experiments below 100 pM for Hg or CH_3Hg have not previously been verified for marine primary producers and consumers. The form-specific behavior of Hg and CH_3Hg trophic transfer entitles that these species accumulate differently in the consumer. Metals which preferentially bind to cellular membranes such as Hg^{+2} tend to be assimilated less efficiently in consumers (Reinfelder and Fisher, 1991). But species such as CH_3Hg become incorporated into the cytoplasm, and as a result are conducted much more efficiently. For

example, Mason et al. (1996) found only about a 15% AE for copepods feeding on Hg^{+2}, compared to much higher levels of CH_3Hg assimilation (62%). The AEs reported in the lower concentrations of ^{200}Hg additions are higher than previous experimental assessments, however, they are clearly lower then AEs for $CH_3{}^{199}Hg$ across the spectrum of algal species and concentrations. Demonstrating a possible volume or species effect towards accumulation, the Tp AEs were higher for Hg^{200} than the Tw species (i.e. ~31% for Tp vs 24% for Tw), but lower for $CH_3{}^{199}Hg$. This effect corresponds to the algal VCF measurements. Thus it is possible that inorganic Hg is transferred more efficiently for smaller denser species, and less efficiently for larger diatoms such as Tw. Larger algal species likewise contain more cytoplasm where CH_3Hg species accrue, which is why it is more significantly assimilated. As the cellular cytoplasm additionally supplies a nutritional base, grazers metabolisms are accustomed to accumulating substance out of cellular cytoplasm. This is essentially the basis of bioaccumulation, as CH_3Hg is transferred and retained more efficiently up the food chain via its connection and stability to essential proteins and cellular content. The highest AE was markedly in the Tw species with the higher $CH_3{}^{199}Hg$ addition at ~88%. This demonstrates that lower CH_3Hg concentrations can induce lower assimilation, though trophic transfer is still relatively efficient and enhanced when compared to Hg.

If algae continued to grow during the experiment, then it would be possible that the initial concentrations present in the algae cells would have been diluted due to cell division. However, measurements of cell abundance on the coulter counter before and after the feeding period indicated that there was little if any growth during the experiments. Notably, the Tw species didn't grow during the feeding experiment time period as cell counts were measurably the same. It is possible that the algae refrained from growing due to the lack of culture medium within the

filtered seawater, as the algae culture remaining in the culture bottles persisted in relative healthy and abundant growth during the same corresponding time period. Dead cells can remineralize accumulated metals, however Fisher et al (1984) reported that this tended to happen only after >48 hours had passed. There was some minor growth for the Tp species during the separate feeding experiment time periods, but it was less than 0.1 day^{-1}, and not significant enough to account for large differences in measurements. Furthermore, any uneaten algae measured proceeding feeding contained approximately the same concentrations of ^{200}Hg and $CH_3{}^{199}Hg$ as at the beginning of the experiment.

4.3.3 Field Feeding Experiments

The field feeding zooplankton tended to yield assimilation concentrations much higher than the *A.tonsa* levels. There were also differences in the assemblages depending on the season and station collected (Figure 4.6), though the ^{200}Hg was consistently greater then $CH_3{}^{199}Hg$. For the different seasons collected in ELIS, it appears that the 0.2-0.5 mm size fraction accumulated more of both isotope species in the summer than during the fall. However, the 0.5-1.0 mm and 1.0-2.0 mm size fractions displayed the opposite trend, with accumulation levels being much higher than summer. The summertime ELIS collection displays decreasing concentrations of both species with increasing size fractions, while the fall collection peaks in concentration at the middle 0.5-1.0 mm fraction. The one surviving size fraction collected from the Fall mid-shelf station had much higher ^{200}Hg accumulation then either ELIS collection, and concurrently very low $CH_3{}^{199}Hg$ accumulation. The $\%CH_3Hg$ ($CH_3{}^{199}Hg$ /(^{200}Hg + $CH_3{}^{199}Hg$)) was calculated for each zooplankton size fraction and is displayed in Table 4.3. The $\%CH_3Hg$ for natural

assemblages collected during the field cruises are also available in parenthesis for comparison in each size fraction.

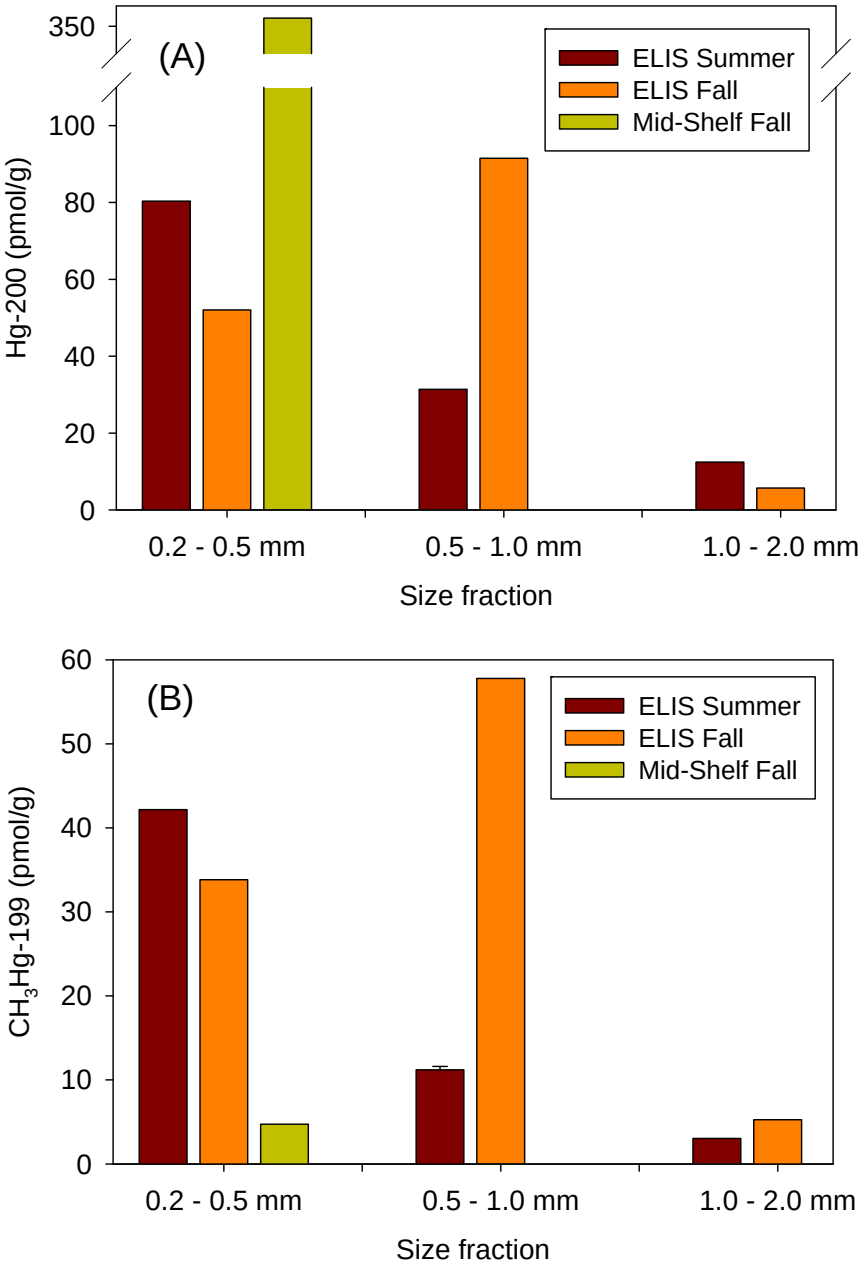

Figure 4.6. Measured concentration of (A) ^{200}Hg (pmol/g) and (B) $CH_3{}^{199}Hg$ (pmol/g) in the separate size fractions collected from Long Island Sound after feeding on spiked algae for 24 hours. Separate zooplankton station collections are represented by different colored bars as noted in the legend, while the different size fractions are grouped together.

Nearly all of the feeding experiment isotope $\%CH_3Hg$ values are much higher than those found naturally, which is not surprising considering the experimental algae contained much higher magnitudes than natural levels. Especially for the $CH_3{}^{199}Hg$ isotope, as CH_3Hg tends to be approximately 10% of natural water Hg levels (Mason, 2002; Chen et al., 2008), but was added at 50% for the experiments. The extent of increased consumption was most stark for the fall zooplankton, which display much greater experimental levels than those found in the environment. However, it is notable that the mid-shelf station contained the exact same $\%CH_3Hg$ for experimental isotope collections and field levels (1.4%). Also, the summer ELIS collections are closer in range for the 0.5-1.0 mm and 1.0-2.0 mm size fractions, yielding differences of less than 10%.

Zhang et al (2013) demonstrated zooplankton feeding selectivity on species of different microalgae resulted in various levels of Hg accumulation. Their experiment was extended to fish, which also contained a range of levels attributed to selective feeding on the experimental zooplankton. Although one type of algae was used during these feeding experiments, it is possible that selective feeding and specific zooplankton dependent assimilation contributes to the variable levels of accumulation for the different size fractions.

Based on the same algae experimental additions, the $\%CH_3Hg$ was variable for *A. tonsa* species. This demonstrates that lower levels of accumulation in the algae species does translate up the food chain to consumption as well. However, the $\%CH_3Hg$ is closer in range between algae species than the actual concentrations initially illustrate (Figure 4.6). The calculated values of experimental $\%CH_3Hg$ also further confirm the differences between algae Hg species transfer, and the strength of bioaccumulation for the various species. The *A. tonsa* fed Tp ended up with

experimental levels of 29.9 ± 9.8 % for the lower feeding addition, and 25.1 ± 8.5 % of $\%CH_3Hg$ for the higher concentration addition. While the copepods feeding on Tw were more variable, and contained higher additions of $\%CH_3Hg$ of 29.9 ± 8.9 % , compared to lower experimental additions of 18.6 ± 6.5 % of $\%CH_3Hg$. The impacts of initial concentrations appear to impact the larger Tw species more than the smaller Tp algae. This demonstrates that algae size and uptake does translate up the food chain to trophic consumption, and that speciation and accumulation can impact grazers $\%CH_3Hg$.

However, it is also likely that variability in differences between $\%CH_3Hg$ for the experimental *A. tonsa* levels is somewhat driven by the assimilation of ^{200}Hg. The AEs for ^{200}Hg were lower for the Tw species, which could be why there were more distinct differences between $\%CH_3Hg$ levels. Therefore, it is highly probably that the high $\%CH_3Hg$ values for the field zooplankton could be more influenced by lower ^{200}Hg assimilation in those zooplankton species.

As accumulation of different microalgae can impact differences in $\%CH_3Hg$ up the food chain, it is also clear that consumers impact bioaccumulation of Hg species. The copepods *A .tonsa* are one of the dominant species in LIS during the summer (Siuda and Dam, 2010), where field zooplankton were collected. Thus it is probably that there were abundant *A. tonsa* within the field tows, especially for the 0.2-0.5 mm size fraction. Though the field zooplankton did contained more $\%CH_3Hg$, the similar smaller size fraction values (34.4% and 39.4%) could also be a reflection of cellular and metabolic factors which influence bioaccumulation for copepod species, as experimental *A. tonsa* had $\%CH_3Hg$ values which overlapped with field values. If cellular material, which theoretically contains more $CH_3{}^{199}Hg$, is assimilated and utilized more in metabolic processes of grazers, then it is probably that they will bioaccumulate more $CH_3{}^{199}Hg$.

Unfortunately the concentrations in the fecal pellets for field zooplankton were not detectable for $CH_3{}^{199}Hg$, thus there was no assimilation efficiency estimates possible for comparison to laboratory experiments. Surprisingly, feces samples were measured for ^{200}Hg, and indicated that most of the size fractions had assimilations greater than 89% for nearly all fractions, which is incredibly efficient. However, this measurement should be taken cautiously, as it is possible that the filter size used for feces was not adequate for field species feces collection. Nonetheless, the summer 0.2-0.5 mm fraction, and the mid-shelf 0.2-0.5 mm sample had AEs of 2.6 and 8.4 respectively, which could be reflective of realistic collections, and also consistent with reduced assimilation in smaller species. On the other hand, the lack of measurable feces for relatively large comparable amounts of zooplankton could also confirm that the species were assimilating most of the algae, and especially the $CH_3{}^{199}Hg$ out of the cellular algae matter.

The algae which were fed to the size fractioned zooplankton contained measured levels of $\%CH_3Hg$ at ~ 5%. As a result, none of the zooplankton exhibit direct assimilation reflective of algae, as nearly all of the zooplankton had higher $\%CH_3Hg$, besides the mid-shelf species. It is apparent from all of the size fractions %MeHg values that the zooplankton are continuously assimilating more CH_3Hg then Hg. However the inclinations for size fractions were contrasting depending on the season they were collected. The 0.2-0.5 mm size fraction had the greatest $\%CH_3Hg$ for that season, and the levels decrease in tandem with larger fractions. This could be a result of more overall accumulation at the smallest size, as it is possible that these species were quite starved in the summer waters which contained less phytoplankton. It is also possible that this size assimilated much more $CH_3{}^{199}Hg$, as this fraction actually had a very low estimated AE for ^{200}Hg (2.6%). The larger size fractions during the summer could have been more sated,

possibly from previously feeding on smaller zooplankton, and thus not processing as much algae. However, it is noteworthy that the two larger size fractions in summer had relatively similar %CH_3Hg to what was measured in the natural field collections (Table 4.4). This similarity indicates that there could be internal cytoplasm attachment, or metabolic functions within the various zooplankton size classes that are accruing $CH_3{}^{199}Hg$ and ^{200}Hg with some consistency.

The zooplankton measured during the fall exhibited increasing measured %CH_3Hg with increasing size fractions, and this trend was also present in the ambient field zooplankton. The 1.0-2.0 mm sized zooplanktons collected during the fall have the highest %CH_3Hg (47.9%) of any fraction for the seasons. These values signify that larger zooplankton bioaccumulated more CH_3Hg than smaller zooplankton. It is likely that during this season the larger species present were more voracious and generally exhibited greater consumption then smaller species, or that cellular matter in larger species present in the fall was more accommodating to the CH_3Hg species out of algae cells. An alternative possibility is that diatoms used for feeding field zooplankton were larger then the algae which they were consuming in LIS. As was demonstrated in the laboratory experiments, the larger algae more efficiently transferred $CH_3{}^{199}Hg$ to *A.tonsa*. Thus it is possible that by feeding on larger algae, the field zooplankton accumulated higher levels of %CH_3Hg than was seen in the field populations with variable algae.

Table 4.4. The % CH_3Hg ($CH_3{}^{199}Hg/(^{200}Hg + CH_3{}^{199}Hg)$) for each size fraction measured for the zooplankton collected in the field for in situ feeding experiments. The %CH_3Hg for the populations of zooplankton measured for the populations from field measurements is displayed in parenthesis for each size fraction and sample.

Size fraction	ELIS Summer	ELIS Fall	MS Fall
0.2 – 0.5 mm	34.4 % (5.5%)	39.4 % (2.2 %)	1.3 % (1.4%)
0.5 – 1.0 mm	26.8 % (28.2%)	38.7 % (5.1%)	
1.0 – 2.0 mm	19.7 % (15.1 %)	47.9 % (13.4%)	

The mid-shelf species were entirely different from the ELIS species, as was evident from the measurements. It is possible that the mid-shelf zooplankton fraction was feeding more on lysed algae or fecal material which did not contain the full concentration strength which were found within the algae, or assimilating much less of the available Hg species as was seen for the ^{200}Hg AE. However, it is also likely that there was more mortality and agitations which affected or skewed the measurements, since the zooplankton from these samples were kept much longer at transport prior to feeding then the other zooplankton. Though attempts were made to remove deceased zooplankton preceding the feeding experiment, it is highly probably that some were missed. Furthermore, some zooplankton could have expired shortly after starting due to the stress and handling during the long journey back to the lab, and skewed the results more towards ambient levels.

A relative trophic transfer value was calculated for each lab experiments and field feeding treatment (RTT factor = [%CH_3Hg_{zoop}] / [%CH_3Hg_{algae}]), and results are displayed in Table 4.5. The *A. tonsa* used for the laboratory experiments were equivalent to the smallest size class used in field experiments, thus results are focused on those sizes. These values confirmed that the field zooplankton had a higher fraction of transfer than laboratory zooplankton, besides the field zooplankton from the open waters of the shelf (MS fall) which yielded a very low amount of transfer. The field zooplankton in the summer and the 1.0-2.0 mm fall species had the highest amount of transfer from algae into zooplankton. This could possibly indicate that there are physiological and metabolic constituents in larger zooplankton which facilitate enhanced trophic transfer compared to smaller species, and more efficient accretion of CH_3Hg. But further research is necessary to confirm any of these initial theories.

Table 4.5. Relative Trophic transfer factor ([%CH_3Hg_{zoop}] / [%CH_3Hg_{algae}]). Low treatments are the lower concentration combo (10 pM$CH_3{}^{199}Hg$ &100 pM ^{200}Hg) while high treatments are greater combo (100 pM $CH_3{}^{199}Hg$ & 200pM^{200}Hg). Field feeding experiments used *T.weissflogii* algae at the higher treatment.

Treatment - algae	RTT factor
Low *T.pseudonona*	1.9 ± 0.81
High *T.pseudonona*	0.78 ± 0.26
Low *T.weissflogii*	0.44 ± 0.24
High *T.weissflogii*	2.46 ± 0.74
MS fall 0.2 – 0.5 mm	0.26
ELIS summer 0.2 – 0.5 mm	8.63
ELIS fall 0.2 – 0.5 mm	6.60
ELIS summer 0.5 – 1.0 mm	4.95
ELIS fall 0.5 – 1.0 mm	7.67
ELIS summer 1.0 – 2.0 mm	7.53
ELIS fall 1.0 – 2.0 mm	9.33

There is a clear difference in the RTT values for the concentration differences in experiments for the algae, confirming that exposure concentrations in algae impacts the amount transferred. However, it is interesting that the lower concentration for the smaller algae species (*T. pseudonona*) is higher than the greater exposure concentrations, indicating that smaller sized algae could facilitate bioaccumulation differently than larger species. Although this could be a product of the more effectively assimilated ^{200}Hg driving down the algal %CH_3Hg for the lower exposure, and therefore yielding higher RTT values upon calculation.

This work represents some initial attempts to separate Hg speciation trophic transfer effects at more realistic concentrations in the plankton level. It is clear that ambient water concentrations of Hg and CH_3Hg influence the uptake into algae, which is then transferred up the food chain into the grazers. There is also evidence that algae species matters, though impacts of

size remain to be teased out of speciation and bloom density effects for further clarity. These measurements represent some of the first steps to determine effects of various zooplankton sizes and species at lower levels. One of the clear things to emerge is that zooplankton will accumulate more Hg species if there are higher levels in the algae. Thus, and it seems that there are a lot of metabolic and cellular variables remaining to decipher for trophic transfer at the primary accumulation levels of bioaccumulation. However, it is evident that higher ambient concentrations beget higher levels of accumulation at lower environmentally realistic levels, and this effect can be transferred up the food chain and influence marine species throughout the oceans. Thus, it is probably that bioaccumulation will continue to be enhanced if steps are not taken to mitigate excess anthropogenic Hg additions into marine waters.

Chapter 5:

Factors Controlling Uptake of Mercury and Methylmercury into Algae from Sediment

5.1 Introduction

Mercury (Hg) in the environment have been steadily increasing above natural levels as a result of rising anthropogenic emissions from multiple modern sources, most prominently combustible coal burning and gold mining. Although, there has been some indication of recent declines in emissions in North America (Driscoll et al., 2013), the legacy of Hg deposition throughout the world merits continuous investigation of Hg dynamics in the environment. Methylmercury (CH_3Hg; MeHg) is a neurotoxin and a significant health concern for humans, as it is present in high concentrations in fish (FDA, 2006; National Research Council, 2000), particularly apex predator species such as Tuna and Swordfish. Coastal estuarine and marine environments provide the largest supply of fish for human consumption throughout the world (FDA, 2006). Marine food webs have a tendency to bioconcentrate CH_3Hg, magnifying toxicity with increasing trophic position (Chen et al., 2008; Fitzgerald et al., 2007). Thus, understanding the factors that influence the release of CH_3Hg from sediments where it is formed, and uptake into the primary producers of coastal ecosystems is incredibly important to understanding the overall bioaccumulation narrative in coastal food webs.

Coastal ecosystems act as a major source of CH_3Hg production via microbial sedimentary production (King et al., 1999; Lambertsson and Nilsson, 2006; Hammerschmidt et al., 2004; Hollweg et al., 2009). Furthermore, abundant supply and runoff of ample bioavailable organic matter can positively influence methylation potential in coastal sediments (Benoit et al., 1998; Merritt and Amirbahman, 2009). Inevitably, coastal areas can provide a significant source of anthropogenic mercury enrichment to marine ecosystems, and potential sources of CH_3Hg (Merritt and Amirbahman, 2009; Chen et al., 2008; Fitzgerald et al., 2007; Hammerschmidt et al., 2004). It is therefore essential to understand factors leading to both CH_3Hg production and

uptake at the base of the food web, as this is where the coastal bioaccumulation narrative initiates.

There are variations in CH_3Hg and inorganic mercury (Hg) in coastal ecosystem biota, particularly in primary and secondary trophic levels (Pickardt and Fisher, 2007; Chen et al., 2008; Chen et al., 2014). Variations could be associated with distinctive methylation potentials for diverse coastal environments, and further dependent on organic matter speciation and sulfur content in the sediment, as well as redox potential of pore waters (Benoit et al., 1999; Hollweg et al., 2009) which influence bioavailability for methylation and sediment CH_3Hg and Hg fluxes. Methylation and demethylation rates of Hg have been measured in sediment (Hollweg et al., 2009; Hammerschmidt et al., 2004; Lambertsson and Nilsson, 2006), based on the formation rates of the pseudo first-order kinetic reaction as discussed in Hintelmann et al. (2000). Flux estimates for dissolved CH_3Hg efflux from sediments using various approaches suggest that there is not a strong correlation between the extent of Hg methylation in sediment and CH_3Hg flux to the water column (Chen et al., 2014; Balcom et al., 2015). However, it has also been demonstrated that strong methylation during warmer seasons can increase water CH_3Hg concentrations (Gosnell et al., 2015), suggesting that increased Sulfate Reducing Bacteria (SRB) activity driven enhanced methylation within the sediment could viably produce more CH_3Hg flux out of sediments and into the water column and biota in some regions.

Direct determination of the Hg and CH_3Hg transfer into biota from the sediment-water interface has not previously been determined or measured. Some previous research has utilized methylation rates (Hammerschmidt and Fitzgerald, 2006) or limited core incubation experiments (Hammerschmidt et al., 2004; Hollweg et al., 2009) to estimate flux from the sediment into biota. However, there have been no actual measurements to confirm Hg and CH_3Hg movement from

sediments into organisms. It is therefore important to quantify the hypothesized Hg and CH_3Hg movement from sedimentary sources into phytoplankton, as primary producers are where the initial biomagnification into the pelagic food web transpires (Mason et al., 1996; Pickhardt and Fisher, 2007). Consequentially, uptake into phytoplankton influences the greater marine food web, eventually controlling human and coastal ecological health (Mason, 2002; Chen et al., 2008; Driscoll et al., 2012). Understanding and quantifying this initial transmission from sediment sources will provide a basis for assessing the importance of sediments in CH_3Hg and Hg uptake throughout the food chain of coastal and estuarine ecosystems.

5.2 Methods

5.2.1 Experimental Setup

Previous research has indicated that coastal regions can experience a seasonal increase in methylation rates during the summer season (Balcom et al., 2008; Hollweg et al., 2009; Gosnell et al., 2015). Thus, several different sites were sampled along the coast in relative 'pristine' environments during the warmer summer season in order to assess potential differences between sediment Hg methylation in natural coastal environments. Individual sediment cores were extracted using acid-cleaned polycarbonate tubes from 3 separate coastal locations along the coast of the Northeastern United States (Figure 5.1). Cores were collected from Acadia National Park in the states of Maine (ME) and along the eastern shore of Connecticut (CT) during the summer of 2013. Cores were also collected from the coast of southern Delaware (DE) during the summer of 2015. These sites were selected in order to investigate effects of different near shore environments and temperatures. Sediment characteristics additionally measured for each site

included initial CH_3Hg and Hg and sulfate concentration of sediment and pore waters, organic matter content (%LOI), and methylation and demethylation rates.

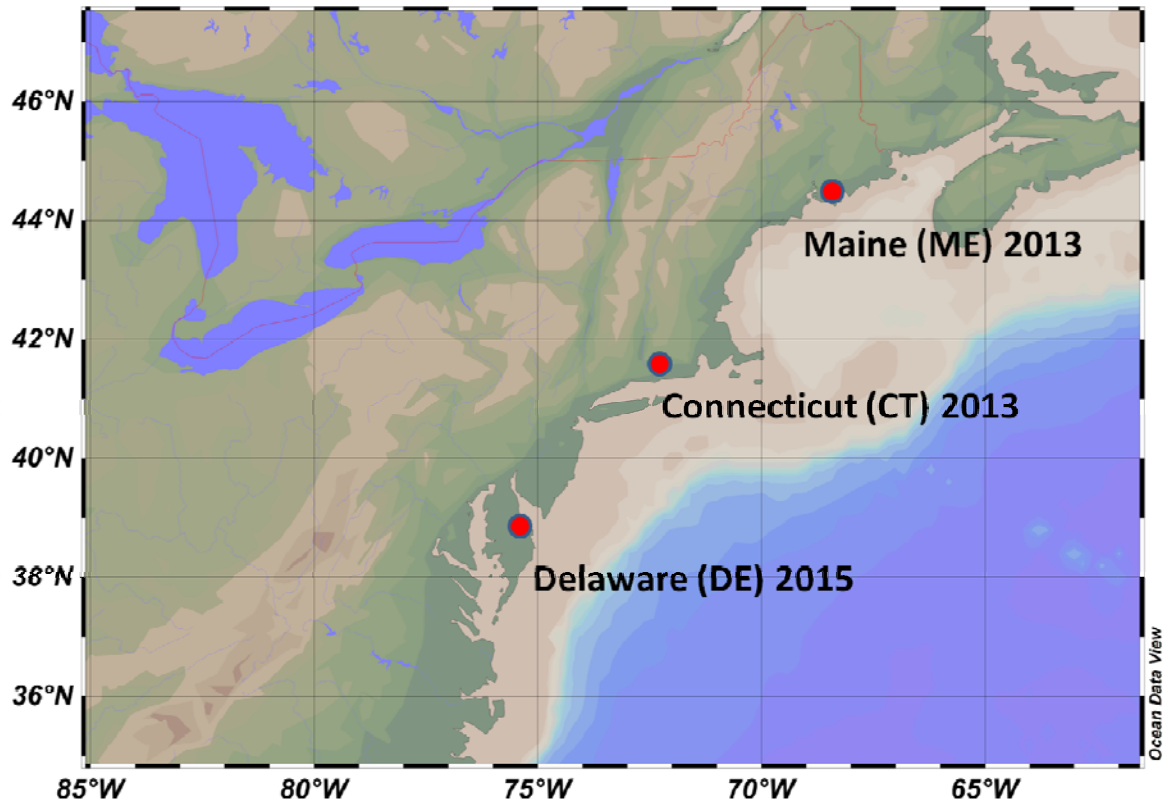

Figure 5.1. Map of the northern eastern seaboard (USA) where cores were collected from. Sites pictured are from the states of Maine (ME) and Connecticut (CT) in 2013, and Delaware (DE) in 2015.

Five treatments were investigated for each site using a separate isotopic scheme for each core: (1) no Hg isotope spikes (control), (2) Inorganic ^{200}Hg only, (3) Methyl $CH_3{}^{199}Hg$ isotope only, (4) Both the ^{200}Hg and $CH_3{}^{199}Hg$ isotopes, (5) Both ^{200}Hg and $CH_3{}^{199}Hg$ isotopes into the water column only (no sediment). Each treatment had duplicate cores. Each individual sediment core was spiked at 1 cm depth intervals with each separate isotope treatment prior to algae

addition, from the surface of the sediment down to ~ 6 cm below the surface. The calculated isotope addition per each cm were 0.061 nmol $CH_3{}^{199}Hg$ and 4.6 nmol ^{200}Hg. The addition into the water core (treatment 5) was additionally this amount for ME and CT, though concentrations in the water were reduced to 6.1 $CH_3{}^{199}Hg$ pmol and 0.23 nmol ^{200}Hg for the DE site in 2015.

The robust coastal diatom *Thalassiosiria weissflogii* was chosen as the representative algae for uptake measurements. The *T. weissflogii* was cultured under trace metal clean conditions until it reached the pre-exponential growth phase prior to each experiment. Diatoms were then gently transferred into individual core tubes, and separated from the sediment border with a 10 μm mesh membrane placed in the water, and above the water-sediment interface. Algae cell counts were measured on a Beckman Coulter particle analyzer prior to algae addition, and for each core treatment upon the experiments conclusion. Cores remained unsealed to allow sufficient air and gas exchange, though a bag was loosely attached over the top of each core to prevent potential contaminants from falling into the water and algae (Figure 5.2A). Cores were than secured upright on a gently moving table in order to keep algae from settling for the duration of incubation (Figure 5.2B).

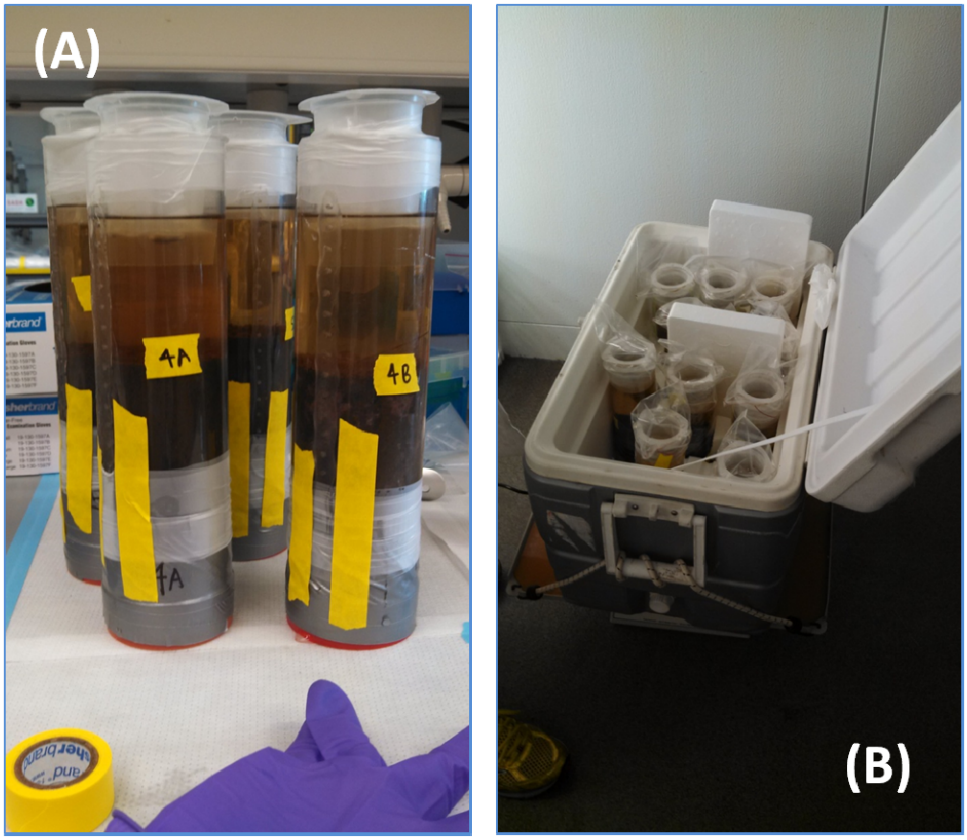

Figure 5.2. (A) Setup of filters containing algae secured to the top of cores, with sufficient air exchange between overlying water and atmosphere. (B) Cores secured upright and open to light (at 12 hour light-dark cycle) in a temperature controlled chamber. (Photo credit: V. Tanguay)

Sediment cores were incubated for 3 days in a walk-in chamber equilibrated to the approximate temperature range of cores from each site (~18-20 ^{o}C), in order to allow time for the estimated transfer of the Hg species into the overlying core water. After incubation the diatoms were gently pipetted out of each overlying section and collected onto 5 µm polycarbonate filters, than frozen until analysis. Approximately 15 mL of algae was collected on each filter, with a minimum of 2 separate filters samples collected per core. Filtered seawater from the algae was also collected and frozen until analysis. Once the alga was removed, the top 0-2 cm (0-4 cm for DE) of the sediment core was separately collected, freeze dried, and frozen until analysis.

5.2.2 Analytical

Mercury and methylmercury isotopes were analyzed via gas chromatography coupled with cold-vapor atomic fluorescence spectrometry (CVAFS), at the University of Connecticut using a Perkin-Elmer ELAN DRCII Inductively coupled plasma mass spectrometer (ICP-MS) in low resolution mode. Water and sediment samples were separately homogenized, weighed and steam distilled for $CH_3{}^{xxx}Hg$ analysis after the addition of 0.2 mL 20% KCl and 1 mL 9 M H_2SO_4 into 40 mL 18.2 MΩ water, using a stable $CH_3{}^{201}Hg$ isotope tracer to assess volume collected. Aliquots of distillate were made up to 30 mL in acid-cleaned glass vial with ultra pure 18.2 MΩ water. Algae samples were digested in 4.51 M HNO_3 acid solution in a 60 ^oC water bath for ~12 hours prior to analysis, and a 1 mL aliquot of the digest was neutralized with ~550 µL of 10 N KOH (~4.9), and made up to 30 mL using 18.2 MΩ water. Ammonium acetate buffer (4M; 225 µL) and sodium tetraethylborate (30 µL) were added to each sample to induce Hg ethylation for $CH_3{}^{xxx}Hg$ analysis. Samples were analyzed on a Tekran auto-methylmercury (CVAFS) coupled with the ICP-MS, and $CH_3{}^{xxx}Hg$ peaks were integrated via Perkin-Elmer Cromera software. The CH_3Hg recovery was quantified using a $CH_3{}^{201}Hg$ isotope tracer and by standard addition matrix recovery, and was within the accepted range (71-125%) based on EPA method 1638. At least one sediment and water replicate were completed for each analysis, while numerous algae replicates were completed. Method detection limit (MDL) was assessed to be 0.02 pmol for these measurements.

The ICP-MS was coupled with automatic flow-injection (FIAS) for ^{yyy}Hg analysis. Sediments were weighed and microwave digested in a 3:7 mixture of $HCl:HNO_3$ matrix, diluted with 18.2 MΩ water to 30 mL, and allowed to further degrade with 200 µL Brominemonochloride (BrCl) for 48 hours prior to analysis. A 1-2 mL aliquot of sediment

digest was used for analysis. Water samples were digested in 10 mL aliquots with ~2.91 M $HNO_3$and 100 µL BrCl for at least 48 hours. Remaining algae digest was diluted up to 10-14 mL using with 18 MΩ water prior to analysis, and 40 µL BrCl was added for at least 24 hours preceding analysis. A stable isotope ^{201}Hg tracer was added to each sample (at 0.5 nM concentration) and hydroxylamine hydrochloride (40 µL) was added to each sample prior to analysis. Calibration curves were achieved to $r^2 = 0.99$ preceding each daily analysis. Instrument precision and accuracy were further monitored by standard and analytical duplicates ~ every 10 samples, in addition to laboratory reagent blanks, and RSD was <10% while the MDL was 1.1 pmol.

It should be noted that though all of the analytical checks for FIAS were sufficient, the sample replicate measurements of inorganic Hg for ME algae treatment 5 (^{200}Hg and $CH_3{}^{199}Hg$ into overlying water) were poor; resulting in more variability in those samples than is preferred. Thus, some of the measurements for the ^{200}Hg and ^{199}Hg in ME algae for treatment 5 should be deemed conservative, and outlying replicate measurements were excluded from calculations. However, the CH_3Hg measurements for ME algae treatment 5, which tend to be more important for bioaccumulation assessment, had very good replication and recovery, and yielded sound results.

5.3 Results

There were leaking issues with some experiments, as the cores contained injection holes on the side where water could slowly drip out once the experiment commenced. As a result, when the core was found to have leaked at the end of the experiment, it was not sampled or

measured analytically, as it was assumed that any results would be flawed. Thus, when a core leaked there is no measurement reported, resulting in an empty space for the core labeled in the figures. Most of the cores for the CT site were leakers, and only 4 survived, and were able to have corresponding water and algae measurements. However, nearly all of the cores remained intact for the experiments for both the ME and DE sites, as only one core leaked at each of those sites.

5.3.1 Treatment 1 (background control)

The cores collected from the three sites had relatively low natural variability of CH_3Hg and HgT within the sediment. Cores from the three sites ranged from background excess isotope concentrations of <0.01- 0.3 pmol/g (dry wt.) for CH_3Hg, and ~0.02-0.2 pmol/g (dry wt.) of HgT. Water concentrations tended to fall within a small range, somewhat independent of the site, but were all consistently low. Inorganic Hg levels ranged from below detection limits to 0.3 pM, while the CH_3Hg concentrations were from below detection (<0.02 pM) to <0.2 pM. Background (initial) concentrations of CH_3Hg in the algae fell within the narrow range of <0.002 amol/cell to 0.008amol/cell. Control concentrations for most of the algae were very low, as CT and ME yielded concentrations below 0.005 amol/cell, and DE levels were even lower at <0.002 amol/cell. The levels of inorganic Hg were noticeably higher in the algae for one of the control cores for the ME site at 29 amol/cell. This amount was still relatively low compared to the exposure value, and hinted that there may be a greater potential for inorganic Hg release out of the ME sediments. However, for the most part there was no measureable difference between the low levels of control and initial algae concentrations of CH_3Hg and Hg for any of the experimental cores, besides the one higher measurement in one noted ME core.

5.3.2 Treatment 2 (^{200}Hg isotope into sediment)

Though there were significant (measureable) levels of ^{200}Hg methylation in the sediments for both CT and ME, there was apparently not sufficient production to drive substantial release from the sediment and accumulation into the algae over the incubation period (Figure 5.3), as there were no detectable $CH_3{}^{200}Hg$ concentrations in ME or CT algae. The DE site had much lower levels of excess isotopes in the sediment, and the negative excess $CH_3{}^{199}Hg$ and $CH_3{}^{200}Hg$ concentrations were below detection limits, indicating no measurable production. Thus, the concentrations were equivalent to background levels for that site (~0.002-0.008 amol/cell), and did not clearly indicate any excessive $CH_3{}^{200}Hg$ uptake sourced from the sediments.

The amounts of background Hg and un-methylated ^{200}Hg was significantly higher in the ME site compared to DE and CT (Figure 5.4). There was clearly a release of ^{200}Hg from the sediment into the water column for both CT and ME, and it was variable between the collected cores. The CT site incubation cores had algae with lower ^{200}Hg than both ME cores (~13 pmol/cell). However the ME cores were considerably different, as the ME-2 core contained significantly more ^{200}Hg in the water (0.58 pM versus 107 pM) and algae measurements (28 amol/cell versus 169 amol/cell). Sediment cores tend to be spatially variable (Hammerschmidt et al., 2004; Hollweg et al., 2009; Merritt and Amirbahman, 2009), thus it is not unexpected that there is variation in the release of Hg and CH_3Hg species depending on the cores, even if collected in close proximity, for some occurrences.

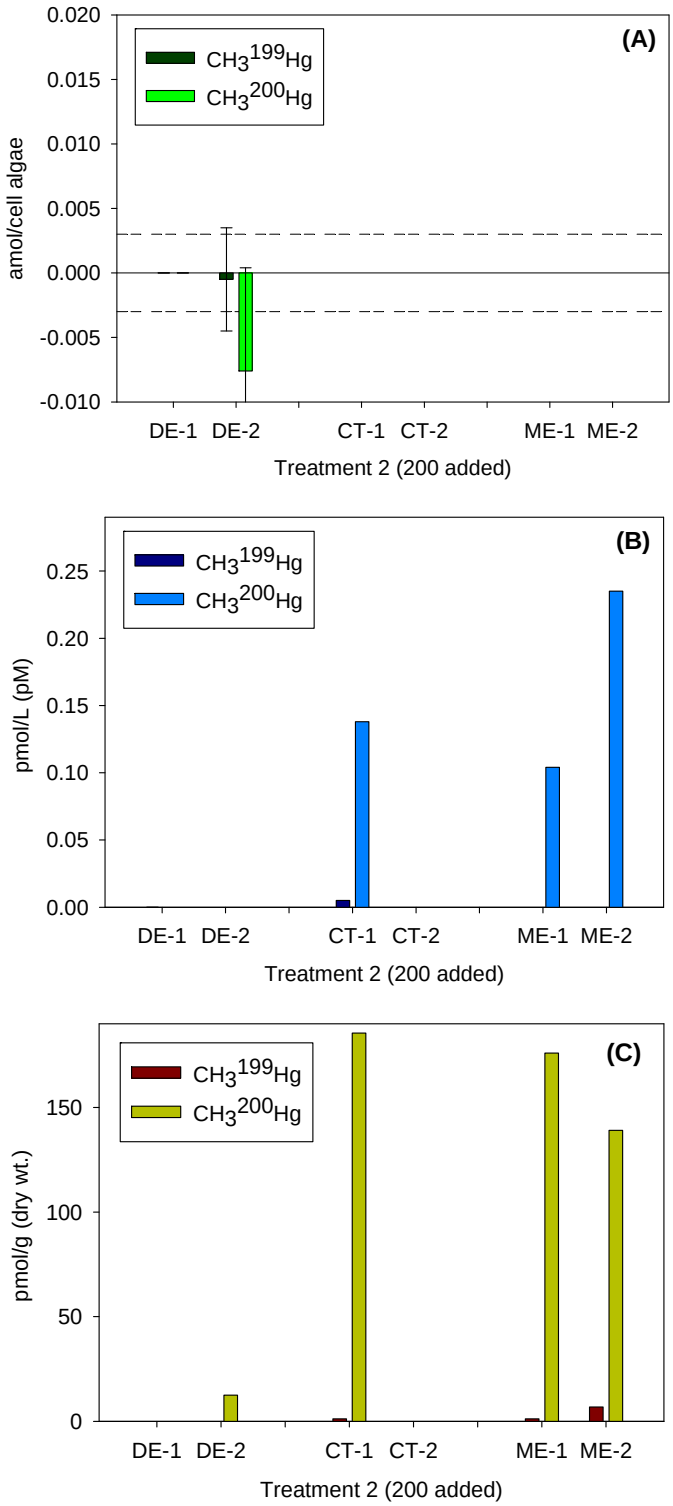

Figure 5.3. Core treatments 2 with ^{200}Hg isotopes added to duplicate cores collected from DE, CT and ME. Concentrations of $CH_3{}^{199}Hg$ and $CH_3{}^{200}Hg$ are shown in measured excess isotopic concentrations for (A) algae treatment (amol/cell), including detection limit line (0±SD) for comparison, (B) water above sediment core (pM), and (C) sediments (pmol/g dry wt.).

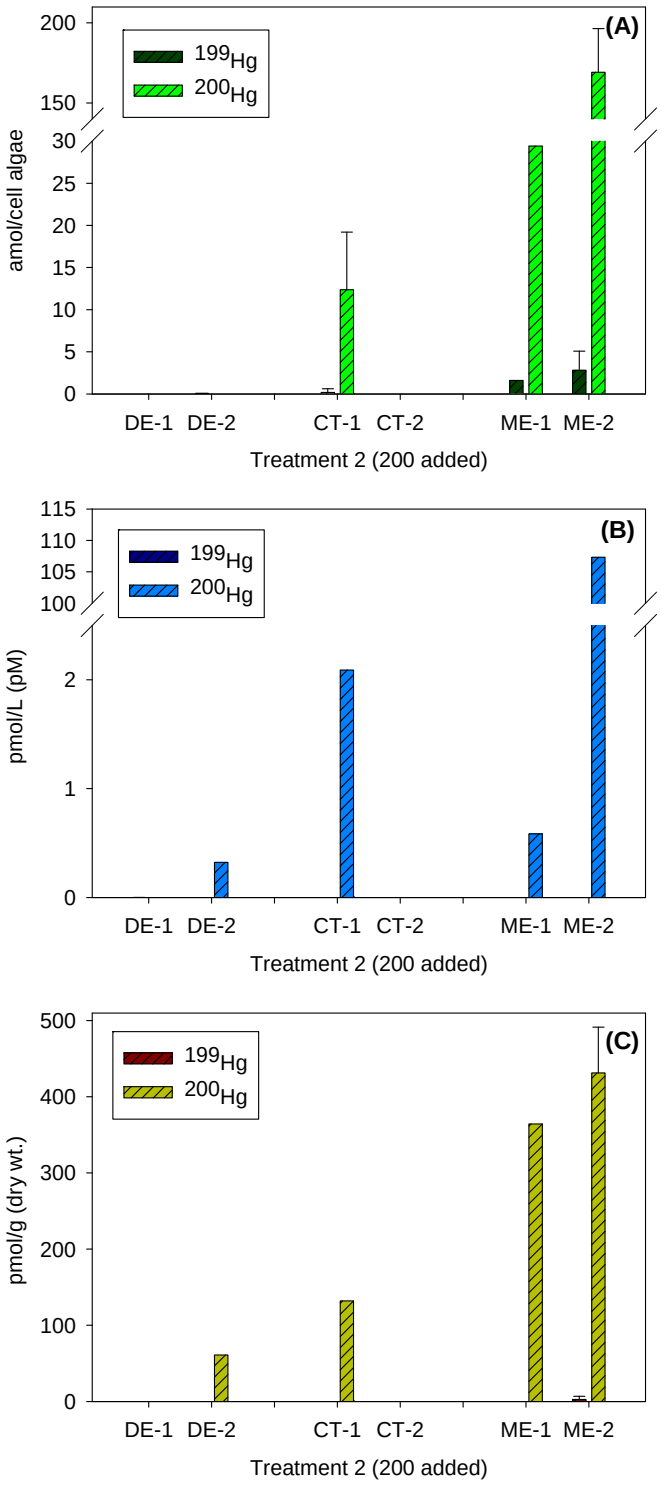

Figure 5.4. Core treatments 2 with ^{200}Hg isotopes added to duplicate cores collected from DE, CT and ME. Concentrations of ^{199}Hg and ^{200}Hg are shown in measured excess isotopic concentrations for (A) algae treatment (amol/cell), (B) water above sediment core (pM), and (C) sediments (pmol/g dry wt.).

5.3.3 Treatment 3 ($CH_3^{199}Hg$ isotope into sediment)

The ME cores indicated much higher levels of $CH_3^{199}Hg$ flux into the overlying water and algae (Figure 5.5). The measured isotopic excess values for DE algae and water were slightly detectable above the control treatment for one core, but below detection in the other core algae. Unfortunately both of the CT cores leaked away for this treatment, thus there was no possible intermediate measurement to further compare the sites. Variability is again demonstrated between cores, as the ME-1 core has significantly more $CH_3^{199}Hg$ measured in the water (17 pM) and algae (0.28 amol/cell) than ME-2 (1.8 pM and 0.01 amol/cell). Normalizing to the water concentrations, the uptake rates were, 0.016 and 0.006 amol/cell/pM, respectively, suggesting that the algae body burden in each case was relatively related uniformly to the exposure concentrations. Interestingly, it appears that ME-2 had higher levels of $CH_3^{199}Hg$ remaining in the sediment, possibly confirming that most of the $CH_3^{199}Hg$ was transported into the water column and algae for ME-1. Surprisingly, there was apparently considerable levels of excess $CH_3^{200}Hg$ measured in the ME and DE sediments, waters and algae, as this was not apparently added in the experiment. Even though isotope treatments are significantly pure (typically >90%), there is always a minor fraction of other isotopes which are present, though not enough to represent that a significant amount be detected in excess during analysis. Thus, it is likely that the presence of excess $CH_3^{200}Hg$ is a result of contamination from carryover between treatment spike additions. This is likely given that the higher levels measured in the algae tend indicate it is more likely carryover of isotope contamination, rather than measurements which reflect natural production in the sediment.

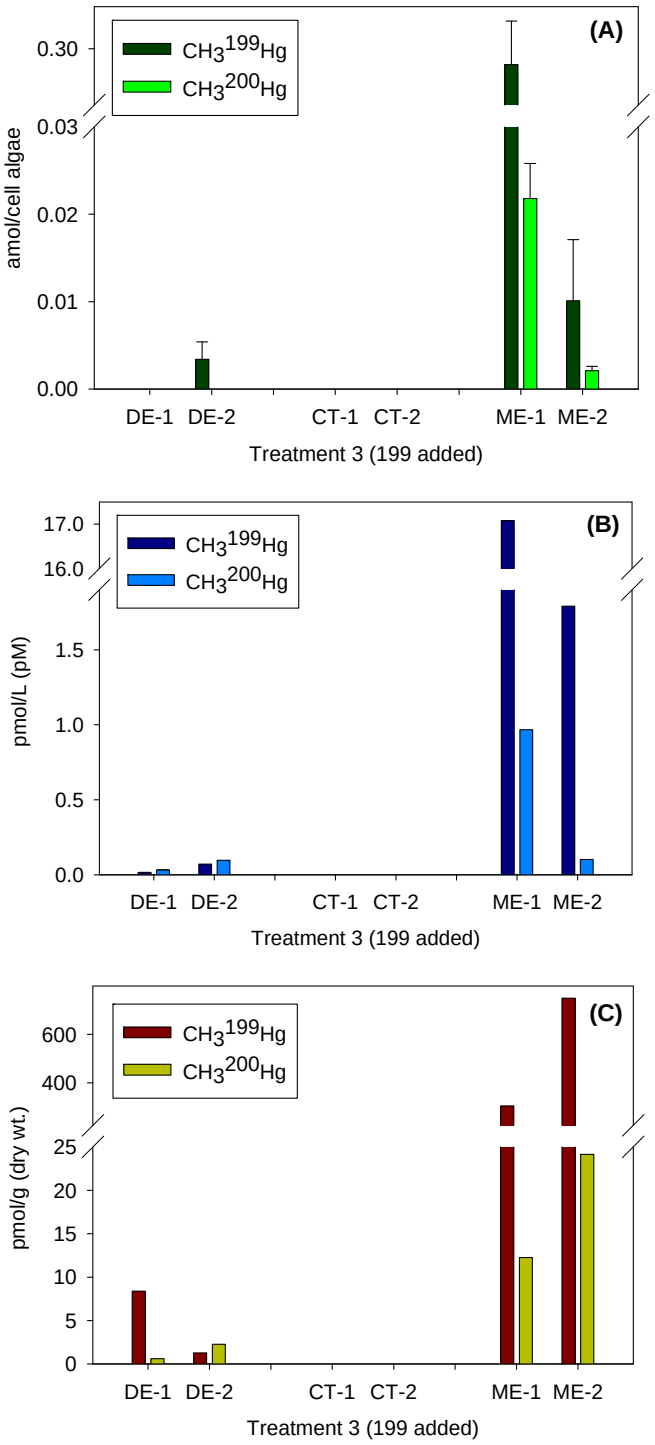

Figure 5.5. Core treatments 3 with $CH_3{}^{199}Hg$ isotopes added to duplicate cores collected from DE, CT and ME. Concentrations of $CH_3{}^{199}Hg$ and $CH_3{}^{200}Hg$ are shown in measured excess isotopic concentrations for (A) algae treatment (amol/cell), (B) water above sediment core (pM), and (C) sediments (pmol/g dry wt.)

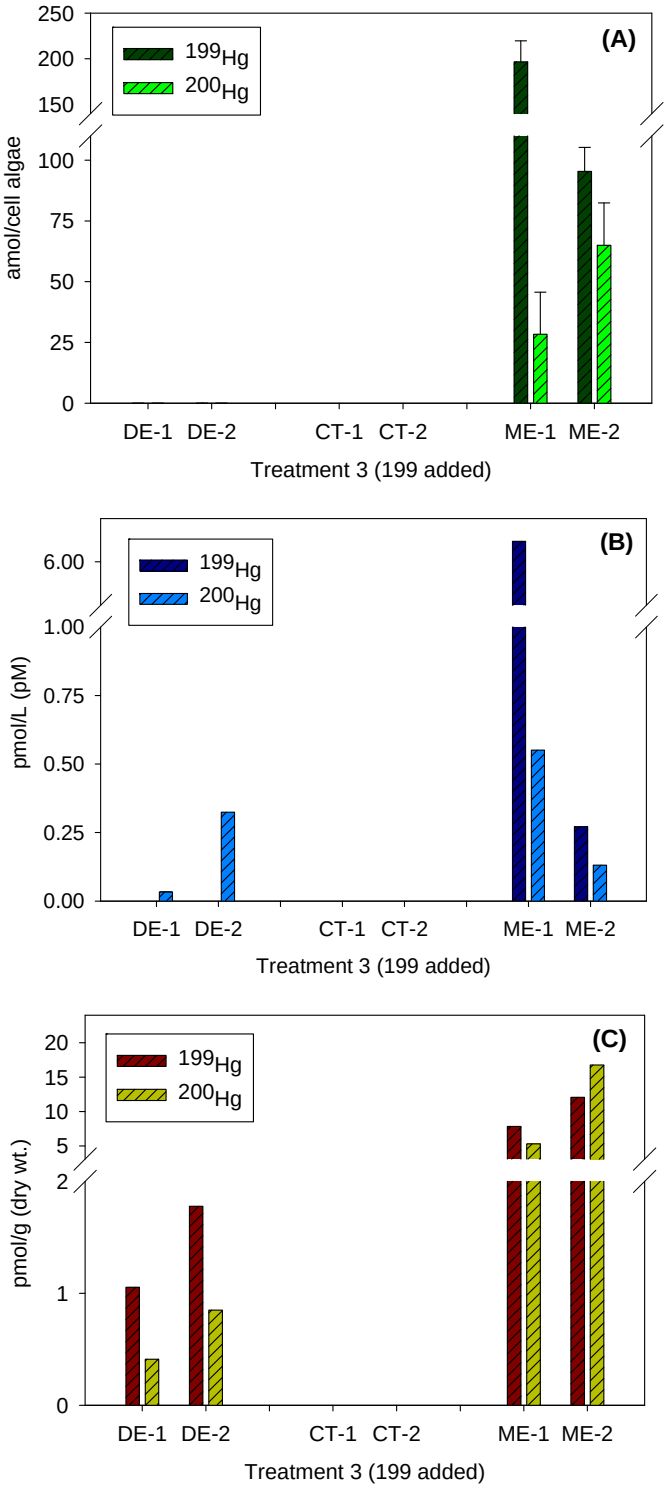

Figure 5.6. Core treatments 3 with $CH_3{}^{199}Hg$ isotopes added to duplicate cores collected from DE, CT and ME. Concentrations of ^{199}Hg and ^{200}Hg are shown in measured excess isotopic concentrations for (A) algae treatment (amol/cell), (B) water above sediment core (pM), and (C) sediments (pmol/g dry wt.).

There also appeared to be significantly more demethylation of $CH_3{}^{199}Hg$ at the ME compared to DE (Figure 5.6). The ME-1 core released significantly more demethylated ^{199}Hg into the water column (6.7 pM) and into residual algae (196 amol/cell), confirming that the conditions in this core resulted in more Hg species transport from sediment than ME-2. Levels of excess ^{200}Hg are similar to ^{199}Hg in the cores, possibly indicating that the $CH_3{}^{199}Hg$ experienced a measurable amount of demethylylation. There was no significant detectible release of demethylated ^{199}Hg for the DE cores, and levels were much lower in the sediment.

5.3.4 Treatment 4 (^{200}Hg & $CH_3{}^{199}Hg$ isotopes into sediment)

Propensity between sediment Hg species concentrations, water values, and algae uptake are more clearly displayed between the 3 coastal sites for these experiments (Figure 5.7 & Figure 5.8). The DE sites had the lowest levels of methylation, demethylation and speciation transport of the 3 sites, and the algae collected from DE cores tended to have significantly lower levels of CH_3Hg and Hg species, and appeared to be the sites with the least isotope releases from the sediments into the overlying water. The CT sites tended to generate intermediate levels, while the ME sites had the highest concentration sets of each site.

At both the CT and ME sites there were differences between the release of $CH_3{}^{199}Hg$ species from the sediment into the overlying water column between cores. The CT-1 core had much higher concentrations remaining in the sediment than CT-2, though the ME sites were higher than both CT cores. The water concentrations changes tended to more closely mimic the algae trends in ME more than in CT. Though the CT-2 water concentrations were lower in remaining isotope concentrations than for CT-1, the algae uptake tended to have similar uptake

levels of $CH_3{}^{199}Hg$, yielding concentrations of 0.03 and 0.044 amol/cell. The ME cores resulted in significantly higher uptake levels, as concentrations were 0.08 and 0.17 amol/cell of $CH_3{}^{199}Hg$ in the algae (Figure 5.7A).

Though it appeared there were similar levels of ^{200}Hg methylated in the CT-1 sediment, there was more remaining in the water than at CT-2, and also higher levels of $CH_3{}^{200}Hg$ in the algae at CT-1 (0.01 amol/cell) than CT-2 (0.005 amol/cell). There was significant methylation of the ^{200}Hg isotope in both the CT cores and ME cores, and especially prominent methylation in ME-2where concentrations reached 649 pmol/g $CH_3{}^{200}Hg$ in the sediment (Figure 5.7C). The significant levels of methylation in ME-2 sediment prominently transferred through the water and into the algae. Water concentrations were 7.4 pM, and the algae contained 0.18 amol/cell of $CH_3{}^{200}Hg$ at the end of the experiment.

The cores had similar ^{200}Hg levels (~57-112 pmol/g) remaining, aside from ME-2 which was substantally greater (~550 pmol/g). Alternatively, levels of ^{200}Hg were much higher in ME-1 water than ME-2, and the uptake into the algae was significantly higher as well (43 amol/cell versus 133 amol/cell). Thus, the differences between cores imply that ^{200}Hg concentrations out of ME-1 sediment were more efficiently transported into the water and than accumulated into algae than ME-2 (Figure 5.8).

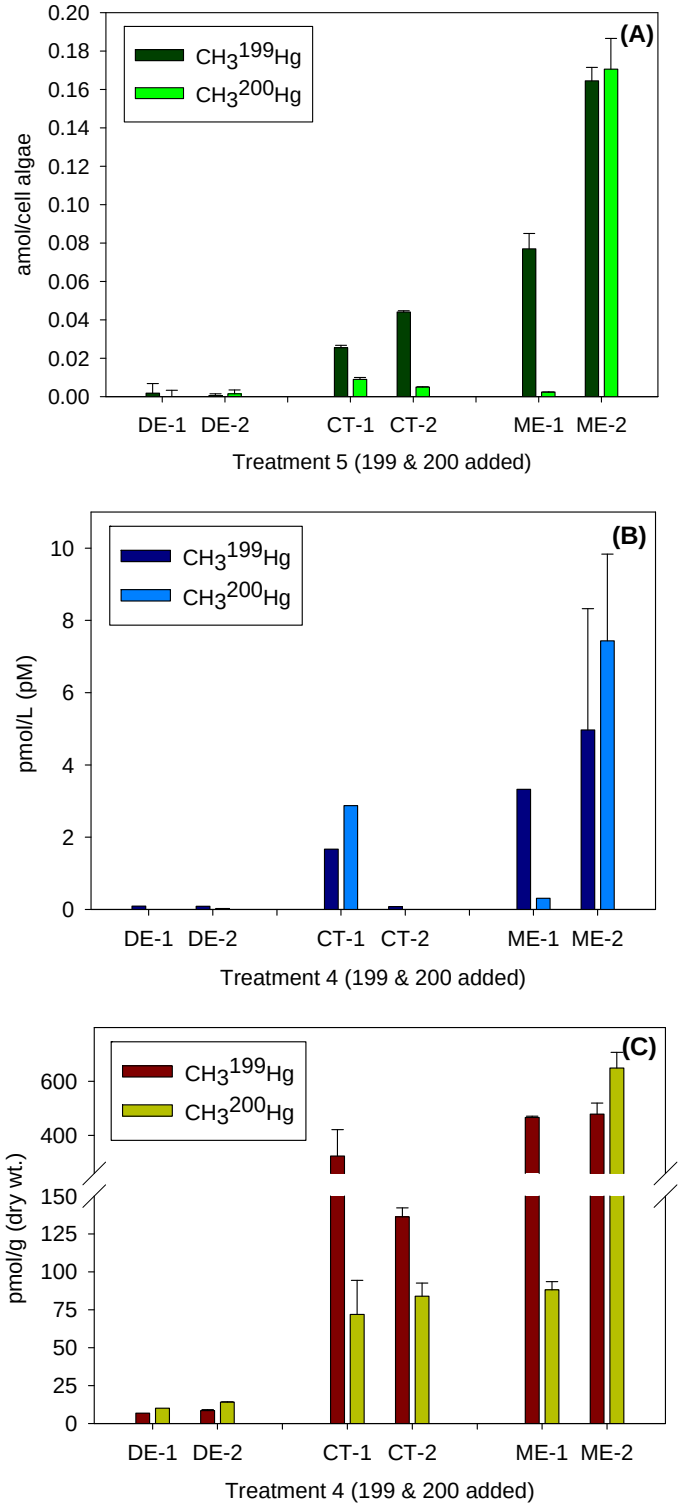

Figure 5.7. Core treatments 4 with both $CH_3{}^{199}Hg$ and ^{200}Hg isotopes added to duplicate cores collected from DE, CT and ME. Concentrations of $CH_3{}^{199}Hg$ and $CH_3{}^{200}Hg$ are shown in measured excess isotopic concentrations for (A) algae treatment (amol/cell), (B) water above sediment core (pM), and (C) sediments (pmol/g dry wt.).

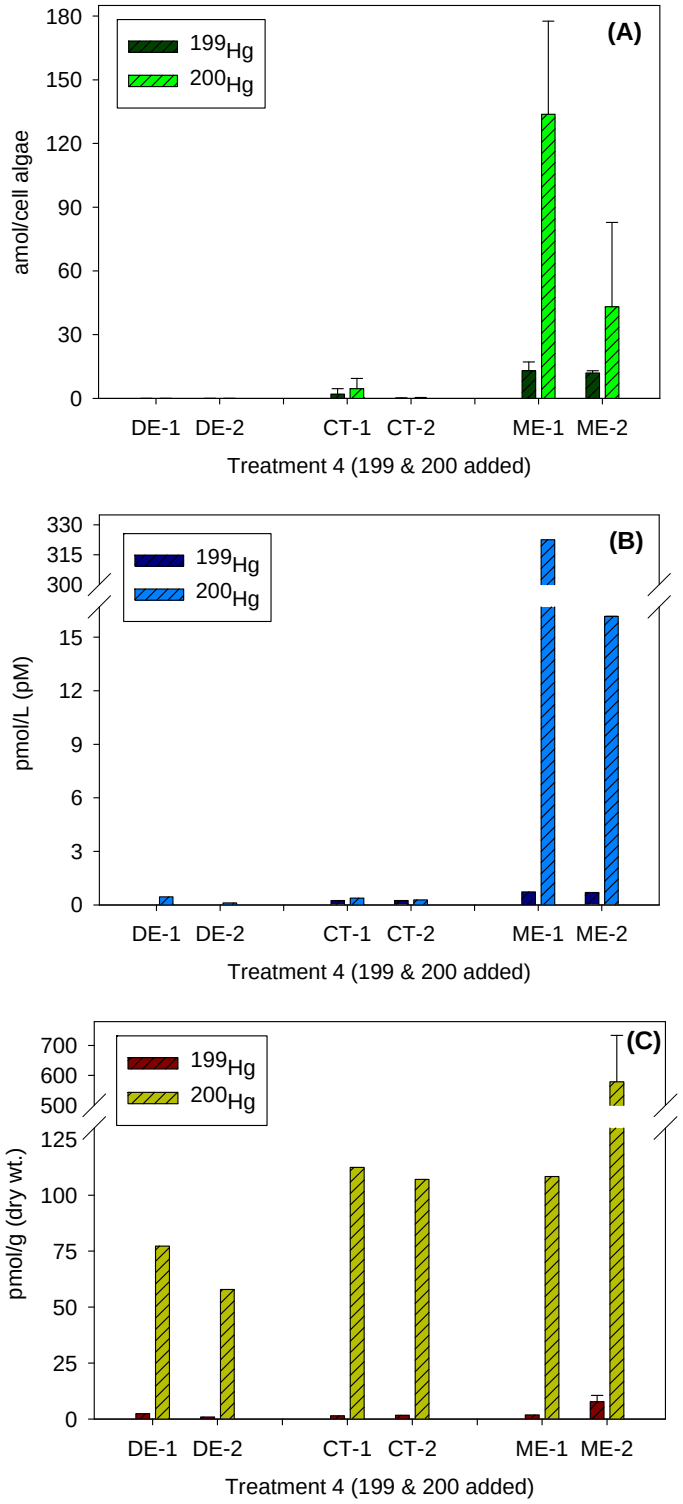

Figure 5.8. Core treatments 4 with both $CH_3{}^{199}Hg$ and ^{200}Hg isotopes added to duplicate cores collected from DE, CT and ME. Concentrations of ^{199}Hg and ^{200}Hg are shown in measured excess isotopic concentrations for (A) algae treatment (amol/cell), (B) water above sediment core (pM), and (C) sediments (pmol/g dry wt.).

Concentrations of demethylated ^{199}Hg in the sediments were consistent and relatively low across the sites (~1-5.7 pmol/g), though water concentrations at ME revealed higher levels than was found at DE or CT (0.2 pM versus 0.7 pM). Concurrently, it appears there was slightly more demethylated ^{199}Hg taken up into algae from ME cores as well, with measured concentrations of 11-13 amol/cell, compared to 2 amol/cell at CT-1(Figure 5.8A).

5.3.5 Treatment 5 (^{200}Hg & $CH_3{}^{199}Hg$ isotopes into overlying water)

Extremely high concentrations of isotopes were injected into the overlying water of the ME cores in treatment 5 (4.6 nmol ^{200}Hg and 0.61 nmol $CH_3{}^{199}Hg$), and this is clearly reflected in the water and algae (Figure 5.9 & Figure 5.10). High concentrations also remained in the water, indicating that the algae had become somewhat saturated with metal uptake, as there was relatively low cell concentration in each core compared to normal experimental culture volumes. The water injections into the DE cores were modified to lower, slightly more realistic water concentrations compated to the previous experiments in 2013, thus the lower values in the algae (~0.02 amol/cell $CH_3{}^{199}Hg$ and ~1.0 amol/cell ^{200}Hg) also reflect what would be expected.

Notably, a large amount of the isotopes which were not taken up by algae were deposited into the sediment. As the ME site has much higher concentrations, there are significantly higher levels for those cores, resulting in 22-37 pmol/g $CH_3{}^{199}Hg$ and 100-500 pmol/g ^{200}Hg concentrations measured in the sediment. Another striking thing is that there was significant methylation in the sediment of the excessive ^{200}Hg which flowed within the pore waters, resulting in concentrations of 70-110 pmol/g dry wt $CH_3{}^{200}Hg$. Even the lower injections of

isotopes for the DE treatment resulted in significant amounts being transferred into the sediment, and prominent methylation of ^{200}Hg resulting in levels of ~6 pmol/g $CH_3{}^{200}Hg$.

The CT cores again both leaked, thus there was no analogous algae and water measurements. However, the sediment values were assessed for this CT treatment in order to compare how much was residing as an estimate to compated with the other sites, and appear to fall within an expected range. There was ~6-9 pmol/g $CH_3{}^{199}Hg$ and 22-39 pmol/g ^{200}Hg in the leaked CT cores.

Though there was prominent transport of isotopes into the sediment and taken up in the algae, there was still significant amounts of isotopes residing in the overlying water. The ME cores contained very high levels of 30-35 pM $CH_3{}^{199}Hg$ and 300-600 pM ^{200}Hg. The DE cores also had significant water concentrations, at ~22-27 pM ^{200}Hg (Figure 5.9B), though only 0.4-1.6 pM of $CH_3{}^{199}Hg$ was measured (Figure 5.9B).

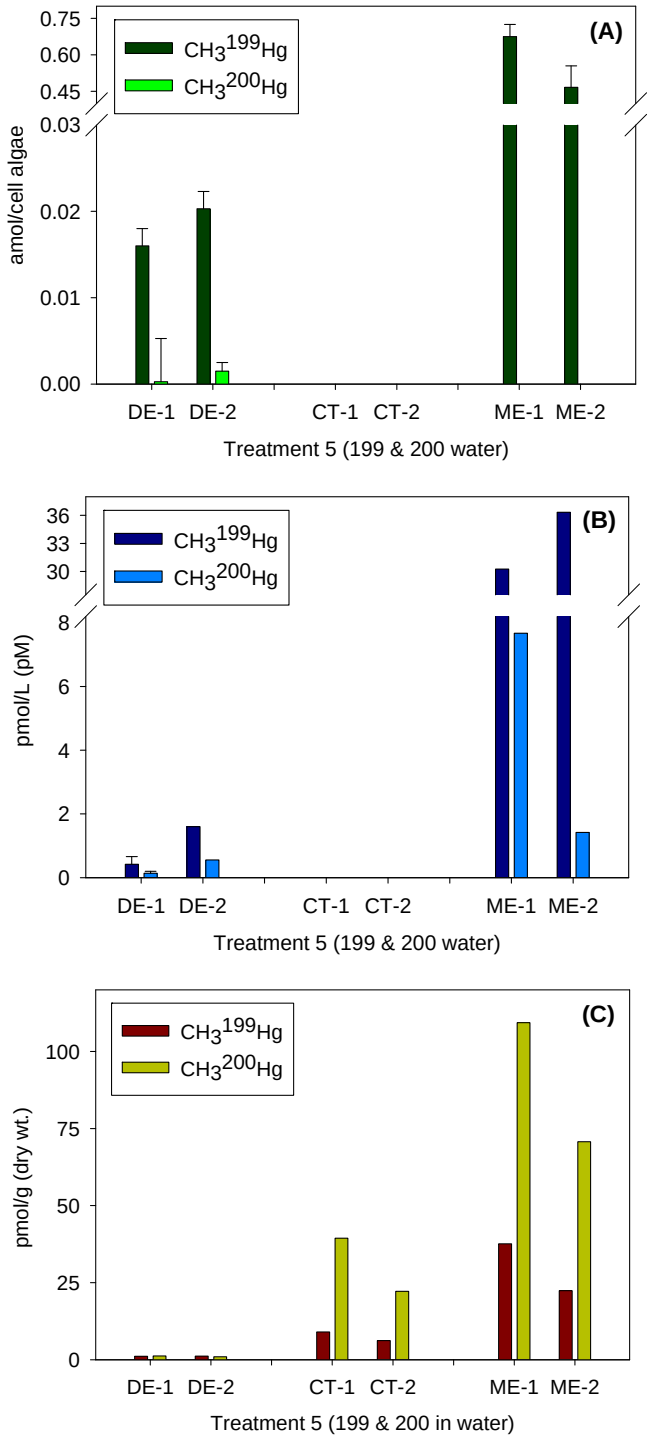

Figure 5.9. Core treatments 5 with both $CH_3^{199}Hg$ and ^{200}Hg isotopes added to the water above duplicate cores collected from DE, CT and ME. Concentrations of $CH_3^{199}Hg$ and $CH_3^{200}Hg$ are shown in measured excess isotopic concentrations for (A) algae treatment (amol/cell), (B) water above sediment core (pM), and (C) sediments (pmol/g dry wt.). Cores from the CT site leaked, thus the sediment concentrations represent a conservative estimate.

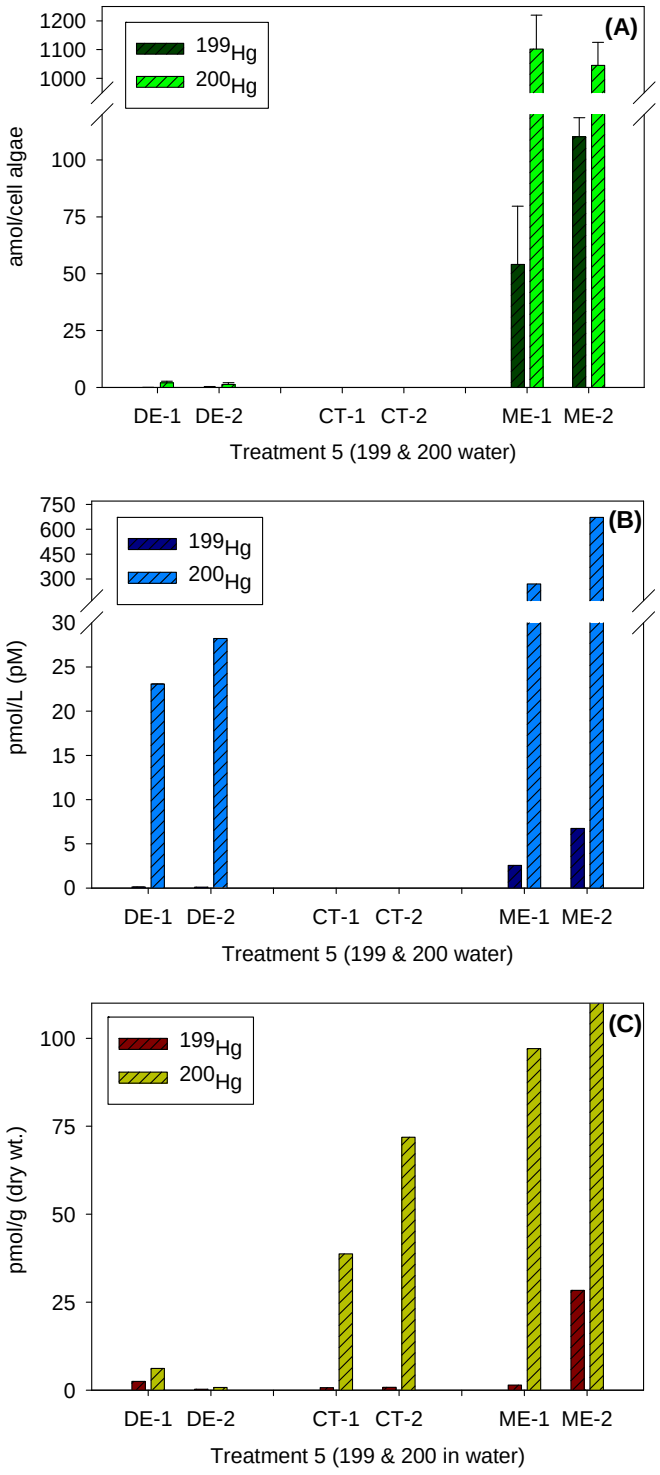

Figure 5.10. Core treatments 5 with both $CH_3{}^{199}Hg$ and ^{200}Hg isotopes added to the water above duplicate cores collected from DE, CT and ME. Concentrations of ^{199}Hg and ^{200}Hg are shown in measured excess isotopic concentrations for (A) algae treatment (amol/cell), (B) water above sediment core (pM), and (C) sediments (pmol/g dry wt.). Cores from the CT site leaked, thus the sediment concentrations represent a conservative estimate.

5.4 Discussion

There were clearly differences between the sites and the amount of dissolved species of CH_3Hg and Hg flux from the sediment and into algae. Nearly all of the sites locations were in relatively 'pristine' parks, and theoretically removed from excessive contamination, although there is some contamination in the large watersheds of the Delaware River and LIS. However, the background levels of Hg and CH_3Hg were variable at each site (Mazrui et al., in prep; Table 5.1), and seemed to yield a minor aspect for Hg species transfer into algae.

The upper DE estuary has a long history of industrial contamination and 'legacy' Hg embedded within the sediments. Therefore, around the DE beaches closer to the eastern seaboard where sediment cores were collected for this study, there were higher residual Hg and CH_3Hg concentrations. Concentrations are actually higher than were found at the upper DE estuary (Gosnell et al., 2015), where the upper DE estuarine sediment had demonstrated relative high seasonal sediment methylation , which influenced those regional water concentrations. However, this was not the case in the lower DE estuary region. Very low methylation rates were measured in the sediments for DE during this experiment (Table 5.2). The methylation rates calculated for net formation (2 hours; Table 5.1) overlap with the 3 day experiment rates for the CT and ME sites, which include longer cycling of methylation and demethylation of the isotope spike. The DE rates are only estimated from the longer 3 day experiment measurements. However, it is not always viable to compare net formation with the longer rates, as there can be sufficient demethylation of the newly formed CH_3Hg over the longer term, which would change the final isotope reservoirs used in the calculations. Therefore, the DE rates are more

conservative than those for CT and DE. Yet the lower rates for DE do seem to be representative, as modest levels of Hg and CH_3Hg were transported from the sediment into the overlying water.

Table 5.1. Average background parameters measured in top 0-2 cm sediments for each site (data from Mazrui et al., in prep). The methylation (k_{meth}) and demethylation rates (k_{demeth}) are calculated for a 2 hour net formation rate for CT and ME, and averaged for the 3-day experiment for the DE site.

Site	HgT (nmol/g)	CH_3Hg (pmol/g)	%MeHg (CH_3Hg/HgT)	%LOI	k_{meth} (% day^{-1})	k_{demeth} (% day^{-1})
DE	NA	13.1 ± 0.3	NA	9.5± 0.3	0.21 ± 0.06	0.043
CT	0.51 ± 0.2	0.39 ± 0.01	0.08	9.9 ± 3.7	1.18 ±0.39	7.72
ME	0.70 ± 0.2	1.51 ± 0.3	0.22	27.1 ± 4.1	1.25 ±0.67	5.26

Table 5.2. Calculated methylation (k_{meth}) and demethylation rates (k_{demeth}) for each site and core. Rates are calculated for the 3 day experiment duration.

Core treatment	DE k_{meth} (% day^{-1})	CT k_{meth} (% day^{-1})	ME k_{meth} (% day^{-1})	DE k_{demeth} (% day^{-1})	CT k_{demeth} (% day^{-1})	ME k_{demeth} (% day^{-1})
2 - 1	-	1.57	0.67	-	-	-
2 - 2	0.17	-	0. 41	-	-	-
3 – 1	-	-	-	0.01	-	-0.051
3 - 2	-	-	-	-0.029	-	-0.057
4 - 1	0.18	0.89	1.13	-0.026	-0.075	-0.077
4 - 2	0.27	1.09	1.97	-0.031	-0.061	-0.059

The background concentrations of HgT and CH_3Hg within the sediments were evidently a minor factor for how much Hg species were accumulated within the algae. At first this might seem unexpected, as higher concentrations would seemingly beget greater concentrations within the ecosystem and biota. However, Chen et al. (2014) demonstrated that there is actually not a strong relationship between sediment concentrations and estuarine biota across multiple

estuaries. The DE sediments contained much higher levels of CH_3Hg than CT or ME (Table 5.1), though ME was slightly higher than CT. Though DE concentrations were highest out of the three sites, it should also be noted that all three sites were considered relatively 'pristine', and equivalent to many other various coastal sites along the eastern seaboard and elsewhere (Fitzgerald et al., 2007; Chen et al., 2014; Gosnell et al., 2015). Furthermore, it is also evident that higher concentrations of CH_3Hg were not primarily the only factor in greater uptake in the algae, as control algae from the three sites contained relatively low and consistent concentrations of CH_3Hg from the three sites, even as the natural levels of CH_3Hg within the DE sediment were higher (Table 5.1). However it does seem that the geochemical and physical characteristics within ME sediment did yield the ability to influence greater background uptake into algae as was evident results from the ME-2 core control algae.

Though the injected concentrations were supposed to be consistent for each site, measured levels of excess isotopes indicate that the injections into the DE sediments seemed to be consistently lower than those for CT and ME in 2013. Thus, it is possible that the unexpected higher background concentrations for DE sediments dampened any affect which would have been seen from the isotope additions. Therefore the comparably lower injection concentrations for DE could likely have also been a factor towards the poorer release and unmeasured uptake in algae from that site. However, it could also be a factor of the geochemical dynamics of the organic matter within the sediments, as DE was characteristically very different from ME.

Organic matter content has also been suggested as a strong control for sediment MeHg and HgT (Lambertsson and Nilsson, 2006; Ullrich et al., 2001; Hammerschmidt et al., 2008; Merritt and Amirbahman, 2009; Schartup et al., 2013). Mercury species bind strongly to organic matter containing reduced S- groups (e.g. thiols and sulfides), and also adhere to O- and N-

functional groups within organic matter. If there is abundant organic matter and humic substances within an environment it can contribute to enrichment by maintaining strong absorption to Hg and CH_3Hg. Hammerschmidt et al. (2004) found a strong positive relationship between organic matter content (%LOI) and CH_3Hg and Hg species in Long Island Sound (CT), further hypothesizing that CH_3Hg partitioning was controlled by adsorption and desorption with organic ligands for that site. If dissolved organic matter is quickly fluctuating through the sediment and pore waters than it is possible that the Hg species will be traveling with that associated ligand movement. The ME sites had much higher %LOI than CT and DE, with measurements of 27.1% versus only 10% LOI found in CT. The DE site was not much lower than CT, with LOI measured to be ~9%. Physical characteristics of the sediment confirmed the measurements, as the ME site was visibly full of sticks and fluffy unrefined flocculate speckled through the sediment cores. While the CT cores had some visual indications of organic material, the DE sediment appeared to have nearly no organic matter, and essentially had the appearance of uniform fine-ground clay particles (Figure 5.11C&D). The organic matter content of the sediment was also somewhat evident during the digestion for HgT analysis, as the ME and CT sites produced abundant fuming upon the addition of HNO_3, though the DE sediment scarcely reacted to acid addition. Though the CT sediments only had a slightly lower k_{meth} than ME for treatment 5 (Figure 5.7), there was much less $CH_3{}^{200}Hg$ transferred into the water and algae. Thus this could be an indication of the importance in ample organic matter for Hg speciation transport. However, it is evident that organic material was not the primarily controlling factor for CH_3Hg and Hg flux through the sediments, as the DE site had much lower methyltion and Hg species movement into the water column, but similar levels of %LOI to CT. Thus the source and types of organic matter characteristics could also factor into methylation potentials of sediment.

Hollweg et al. (2009) found that organic content alone did not support differences in Hg and CH_3Hg partitioning within the sediments and pore waters in Chesapeake Bay. Also, there was no connection found between sediment Hg and CH_3Hg levels and %LOI in the upper DE estuary (Gosnell et al., 2015). In most sediment CH_3Hg production is greatest at depths just below the oxic/anoxic transition zone. This principle methylating region tends to have microbial sulfate reduction, but very little methylation-inhibiting sulfide available. Shallow sediments from the upper Bay of the Chesapeake demonstrated that decreases in both k_{meth} and %MeHg were found to coincide with increasing sulfide concentrations and decreases in sulfate reduction rates (Hollweg et al., 2009). Schartup et al. (2013) further advocated that total S was a better net Hg methylation predictor across eastern ecosystems then organic content. As a result, CH_3Hg production in sediments can be further controlled by the redoxcline depth as well as organic matter quality and degradation status, especially the redox form of S species. The Hg and CH_3Hg species can bind strongly to the smaller sulfide molecule (Ullrich et al., 2001; Lambertsson and Nilsson, 2006), making it easier for those species to flux out of the sediment when bound up with sulfide.

Methylation of Hg is primarily driven in the sediment by SRB (King et al, 1999; King et al., 2000; Barkay et al., 1997), though iron reducers and other anaerobic species can also contribute (Kerin et al., 2006). Though bacteria are too small to see, there was stark evidence of a microbial presence in ME in the form of purple SRB colonies through the sediments (Figure 5.11A&B). The existence of abundant sulfur species in the sediment was additionally confirmed by odor, as both the ME and CT cores had a distinctive smell of reduced sulfur during sampling and preparation. This is also a possible additional explanation for the higher background values of %MeHg found within the sediments in that relatively 'pristine' region, as these microbes are

continuously at work methylating Hg when conditions are favorable. Lambertsson and Nilsson (2006) noted that the availability of fresh organic substrate could purportedly be a dominant factor affecting Hg methylation by SRB. Thus it is possible that the abundant rich organic matter in ME, including humic substances evident by the tea-colored tannins percolated into the overflowing waters, could have provided fresher substrate for Hg species reallocation.

The net methyation rates (k_{meth}) within sediments was clearly instigative in how much Hg was methylated and released into the water column overlying the sediment cores (Table 5.2). The DE sediments had a k_{meth} which was a factor lower than found at CT and ME (Table 5.1), and there was nearly no CH_3Hg species found within the algae for any of those cores. The k_{meth} was evidently not the only factor, as CT and ME had relatively similar k_{meth} rates, but there was nearly always more CH_3Hg and Hg taken up into the algae at ME than CT. Furthermore, some k_{meth} calculations would indicate that particular cores had more prominent rates, but there were not strong corresponding amounts of CH_3Hg within the algae or waters, such as core CT-1 (Figure 5.3A). Concurrently the lower k_{meth} in ME also yielded no enhanced $CH_3{}^{200}Hg$ within the waters and algae of both cores in Figure 5.3A. Thus, it is clear that sediment characteristics influencing flux are also important for Hg species transport. Nonetheless there was also evidence that stronger methylations rates could factor into greater uptake for ME cores. The k_{meth} over the 3 day experiment was greater in ME-2 (1.97 % day^{-1}) than ME-1 (1.13 % day^{-1}), and the resultant rates produced relatively more ample $CH_3{}^{200}Hg$ within the algae for that core (Figure 5.7A).

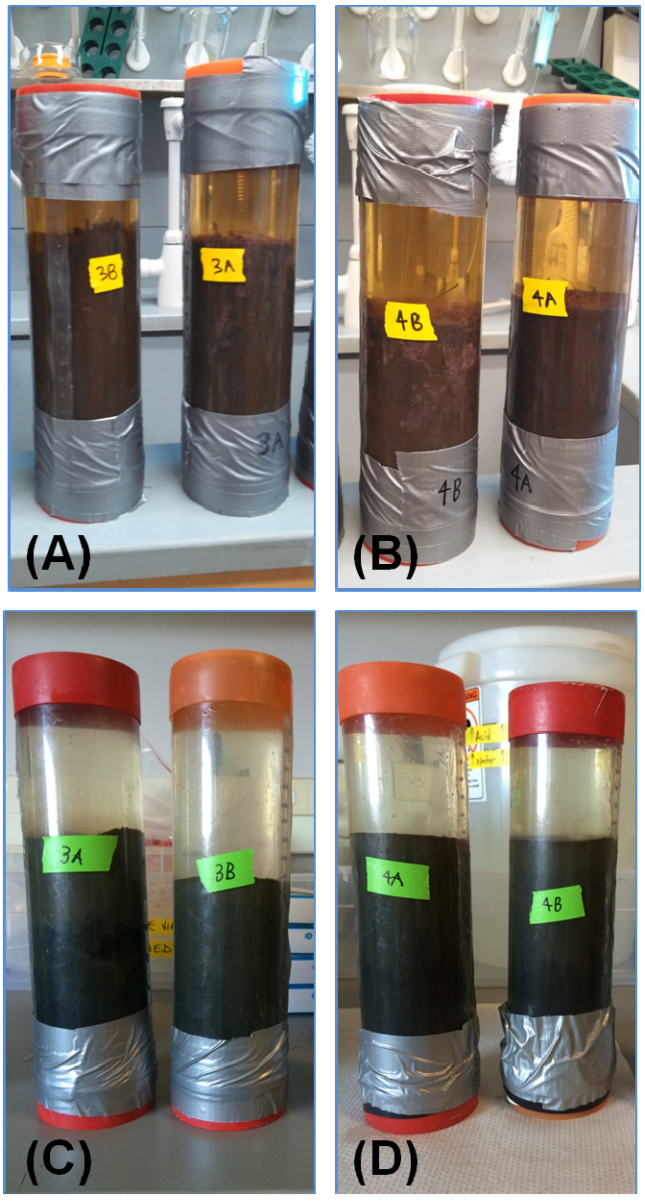

Figure 5.11. Differences between the ME sites (A) & (B) and DE sites (C) & (D) are clearly evident in the sediment cores. The ME sediment (top) contains much more organic material then the DE sediment. There is also characteristic streaks of purple sulfur reducing bacteria clearly visible in the ME sediment cores, which were not found at DE. While the DE cores are relatively uniform and made of fine grained clay-like sediments (bottom). (Photo credit: V. Tanguay & B. DiMento)

It is likely that the pore waters and pore spaces between sediments also contribute to the leakiness of Hg species from sediments into waters. Theoretically Hg and CH_3Hg species would need to distribute into pore waters prior to being dissolved into overlying waters. It is possible that the floculate organic-rich sediments of ME contained slightly more spacious pores for dissolved species to maneuver out into the covering water, which would contribute to the relative greater success of transport for those cores. Though the measured %LOI indicated that the DE and CT sites were relative consistent for organic matter concentrations, the appearance of the sediment was relatively different. The fine-grained clay-like particles of DE could have sealed closer together once settled, and this might have contributed to reduced movement for dissolved species out. Furthermore, it has also been demonstrated that the ligand and S- speciation within organic matter is also important to Hg transformation, not just the overall %LOI (Ullrich et al., 2001; Lambertsson and Nilsson, 2006; Fitzgerald et al., 2007; Merritt and Amirbahman, 2009; Schartup et al., 2013).

Organic matter content, porosity and tortuousity also likely influence the amounts of inorganic Hg which are transferred from sediments into algae uptake, as the ME sites were also remarkably dominant in that aspect. There was also liberal release of isotopic species which had not undergone transformation ($CH_3{}^{199}Hg$ and ^{200}Hg). This is clearly evident in Figure 5.4, where nearly no evident $CH_3{}^{200}Hg$ was released, but levels of ^{200}Hg were detected within the algae for both CT and ME.

The possibility exists that some of the isotope spike was closer to the sediment surface, and therefore more easily transported independent of sediment characteristics. This could be a factor in why ME-2 uptake is much greater than ME-1 in Figure 5.4A, and why there is significant more $CH3^{199}Hg$ in ME-1 algae for Figure 5.5A. However the excessive

concentrations measured in Figures 5.9 and 5.10 help to confirm that there was likely not a direct (accidental) addition into the water instead of the sediment. Though it is still probable that some variation is due to certain isotopes traveling a shorter distance through sediment than others, this effect is likely minor when compared to sediment geochemistry. Algae uptake tends to perceptibly reflect large differences in concentrations of Hg and CH_3Hg within the water they inhabit, as they primarily take up metal species via passive absorption (Le Faucheur et al., 2014; Mason et al., 1996; Pickardt and Fisher, 2007; Chapter 4), though some facilitated uptake is possibly likely at lower concentrations (Chapter 4; Mohr et al., 2002). The concentrations of $CH_3{}^{199}Hg$ when isotope was added directly to the water at levels injected into the sediments are factors higher than any other measurement (Figure 5.9A). And the difference is particularly striking for the ^{200}Hg algae concentrations in Figure 5.10. Furthermore, when lower concentrations were added to the water, as was done for the DE treatments, there was much less uptake in the algae, and much less isotope deposited into the sediments.

It is evident that there was a strong association between sedimentary isotopic release into the overlying water and uptake in algae, as is demonstrated in Figure 5.12. The correlation between algae concentrations (amol/cell) and water concentrations (pM) was significant for $CH_3{}^{199}Hg$ and $CH_3{}^{200}Hg$ ($r^2=0.9002$, $p<0.0001$, $n=14$). Furthermore, there was significance between inorganic Hg concentrations for algae (amol/cell) and water (pM), pertaining to ^{199}Hg via demethylation ($r^2=0.733$, $p=.001$, $n=10$), and a weaker significance for ^{200}Hg release ($r^2=0.537$, $p=0.03$, $n=8$), as long as treatment 5 (the direct injection into water) was not included. These correlations were also seen in the CT and DE algae and water measurements to a lesser extent. However, there were no other significant correlations between sediments, water and algae. Chen et al. (2014) found a correlation with algae and water CH_3Hg concentrations, but no

correlation with sediment, along several Northeast US estuaries. These results help to confirm what was seen in those field measurements.

The demethylation rates for isotopes concentrations at the end of the experiment seemed to be unreliable (Table 5.2). Though formation rates have successfully been calculated using other site core measurements for a 2 hour period (Table 5.1), the calculations for the 3 day period were not at all similar. As demethylation occurs relatively quickly within the sediments, and the Hg produced could be additionally remethylated over a longer time period, it is possible that the isotopes had reached equilibrium after the longer 3 day experiment period. Therefore it is likely the k_{demeth} rate calculation was no longer viable for this longer experiment duration.

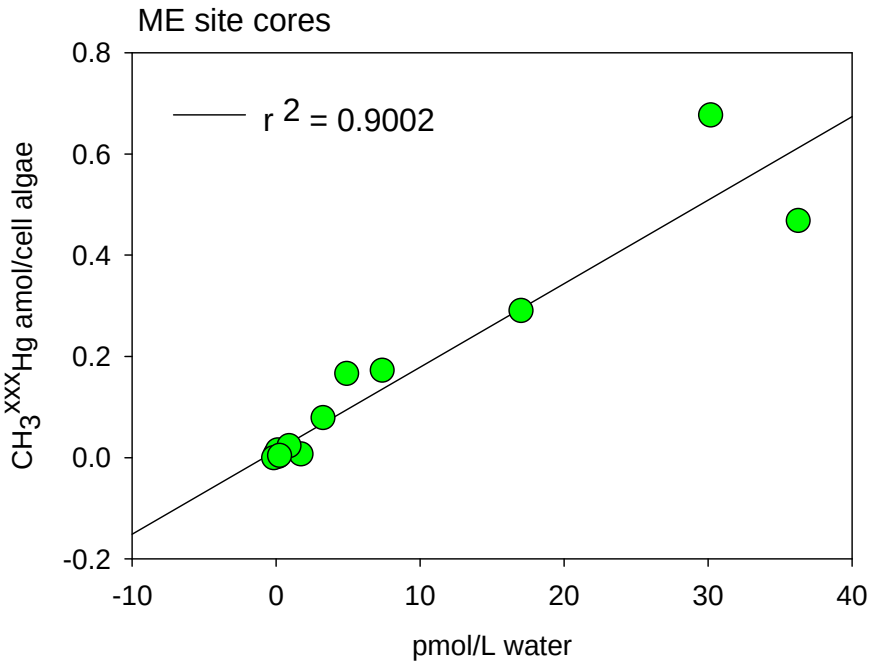

Figure 5.12. Correlation for concentrations of CH_3Hg in algae (amol/cell) against CH_3Hg water (pM) for all core treatments for the ME site.

In order to quantify differences between estimated upper sediment (1 mm) output from the 3 sites, the fraction of $CH_3{}^{199}Hg$ and $CH_3{}^{200}Hg$ accumulated in the waters (%) and algae (%) was calculated for treatment 4 (both isotopes into sediment), and results are displayed in Table 5.3. Results from analogous calculations for the inorganic isotopes (^{199}Hg and ^{200}Hg) from sediments into water and algae are in Table 5.4. It is evident that there is still significant amounts of CH_3Hg being released from sediments into overlying waters and algae, with the algae accumulating approximately 10% of water levels. However, primarily it is clear that majority of the isotopic Hg species were remaining in the sediments. The DE sites, which were significantly low, tended to have 0.003-0.02 % of isotope transport into the algae, while the intermediate CT sites had 0.01-0.06 %. Though the ME sites seemed significantly higher, the amount transported was similar to the other sites, ranging from 0.01-0.06 % in the algae. Though these amounts seem minor at first glance, these amounts are calculated for a small slice of sediment and the overlying water column. When average uptake concentrations were calculated for the experiment duration, the CT sites had 0.07 – 0.27 pM, and ME sites ranged from 0.06- 1.43 pM CH_3Hg accrued in all the algae. Thus, it is possible that in some ideal cases sediment output of methylated Hg could be influential towards adding to the CH_3Hg burden within resident phytoplankton, however more information about alternative input sources is needed prior to determining the significance of each competing factor on the influences for estuarine CH_3Hg bioaccumulation burdens.

Table 5.3. Calculated amount of estimated transfer for methyl-Hg isotopes ($CH_3^{199}Hg$ &$CH_3^{200}Hg$) from top 1 mm of sediment cores into water (%) and algae (%) from treatment 4 cores at the three sites.

Site	$CH_3^{199}Hg$ 1 mm (pmol)	$CH_3^{199}Hg$ Water %	$CH_3^{199}Hg$ % in algae	$CH_3^{200}Hg$ 1 mm (pmol)	$CH_3^{200}Hg$ Water %	$CH_3^{200}Hg$ % in algae
DE-1	8.85	0.011	0.005	13.15	0.018	0.003
DE-2	14.10	0.007	0.013	23.27	0.019	0.023
CT-1	329.18	0.101	0.017	73.02	0.079	0.032
CT-2	169.05	0.009	0.062	104.16	0.001	0.011
ME-1	376.69	0.176	0.017	71.22	0.086	0.003
ME-2	422.83	0.234	0.059	573.66	0.259	0.045

Table 5.4. Calculated amount of estimated transfer for inorganic Hg isotopes (^{199}Hg &^{200}Hg) from top 1 mm of sediment cores into water (%) and algae (%) from treatment 4 cores at the three sites.

Site	^{199}Hg 1 mm (pmol)	^{199}Hg Water %	^{199}Hg % in algae	^{200}Hg 1 mm (pmol)	^{200}Hg Water %	^{200}Hg % in algae
DE-1	3.15	0.019	0.352	100.36	0.090	0.074
DE-2	1.46	0.029	0.604	95.49	0.023	0.064
CT-1	1.45	0.853	73.894	114.18	0.062	7.741
CT-2	2.10	0.202	14.708	132.86	0.043	0.291
ME-1	1.50	1.171	86.915	87.52	24.430	42.425
ME-2	5.04	0.586	77.664	414.21	0.671	13.468

Rates of release were calculated for each site considering the measured levels of isotopic species into the overlying water and algae after 3 days. The DE site had the lowest rate for all species, as the rates were primarily negative, or consistent with values ~0.0002 pmol/h for all excess background flux. The CT site was intermediately higher, with rates of 0.004 ± 0.0008 pmol/h for $CH_3^{199}Hg$, and also yielded a measurable methylation output of 0.001 ± 0.0004 pmol/h for $CH_3^{200}Hg$. As the ME site had the greatest output and k_{meth}, the rates were also much

greater at 0.017 ± 0.01 pmol/h for $CH_3{}^{199}Hg$, and more importantly were 0.017 ± 0.009 pmol/h for the $CH_3{}^{200}Hg$, which represents methylation output from the sediment. The inorganic species had higher rates for the sites. Demethylation output (^{199}Hg) was 0.19 ± 0.15 pmol/h for CT and 0.67 ± 0.23 pmol/h for ME. The rate of flux for excess ^{200}Hg was 0.30 ± 0.23 pmol/h for CT and quite high rates of 3.44 ± 1.44 pmol/h for ME.

The amount of inorganic Hg transported into algae was greater and more variable than what was seen for CH_3Hg species. The DE sites seemed to have very little release, as the amounts in algae ranged from 0.06-0.6 % for both isotope species. From these numbers it was evident that there had been substantial demethylation of CH_3Hg within the sediments, as the CT and ME algae contained 14.7-86.9% ^{199}Hg. There was also adequate direct transport of ^{200}Hg, with levels ranging from 0.3-7.7 % in CT algae, and 13.5-42.4 % in ME algae. Thus, there were significantly higher amounts of inorganic Hg transported out of the sediment into overlying water and biota within each core. The amount of ^{200}Hg and ^{199}Hg from the water into algae was much higher than was measured for CH_3Hg. It was estimated that all the algae accumulated concentrations ranging from 0.3 pM up to 0.9 nM over the 3 day experiment. Levels were extremely variable, as only the ME-1 core exhibited high ^{200}Hg fractions in the overlying water (24 %) and algae (42 %), but the remaining cores contained much lower water levels (<1%). Thus, even though it is evident that inorganic Hg was transported from sediments into water and overlying biota at much greater levels then CH_3Hg, it was also more variable from site to site and within cores.

Mercury can occasionally be an adhesive metal, especially in the inorganic form. Thus it is also possible that some species of Hg and CH_3Hg were released from the sediment but did not make it into algae due to adhering to the barrier filter or sides of the sediment core. Though it is

unlikely that this affected one site (e.g. DE) more than the others, it is also probably that some variation between algae uptake is due to isotopes being unintentionally taken out of the mass balance. As the filter was necessary to keep algae from intermingling with suspended sediment and unidentified pelagic biota, this was unavoidable for this initial set of experiments. However, it could be worthy to attempt this investigation with no potentially complicating barriers in future investigations.

In conclusion, it does seem that sediments can be a contributing factor in algae uptake. Although sediments seem to only contribute a minor fraction of their CH_3Hg towards bioaccumulation, and exhibit highly variable output, it is still evident that over a course of a month or over a large sediment basin there could be substantial levels of Hg species produced from sediment sources, under the right conditions. However, the documented spatial variability in sedimentary Hg and CH_3Hg output indicates that patchiness would result in large spatial differences, even if a site seems ideally situated for methylation. More obvious factors such as higher concentrations of Hg and CH_3Hg in the sediment only slightly factor into more flux into the water column, and overall the concenttation does not seem to be the primary factor controlling Hg release, as would be expected. The k_{meth} seems to be a factor related to some CH_3Hg release from some sediments (i.e. ME), but increased output is also subjective to the many other variables which can influence metal species release form sediments, such as porosity and metal affinity. Many different variables and factors likely influence successful output of Hg species from the sediments, and there are still many unknowns that need to be discerned for sediments influence in pelagic bioaccumulation. It does seem that organic matter content is a factor in sediment methylation and productivity, as sediments which contain higher %LOI also had higher flux output. However higher levels of organic matter do not automatically yield

higher methylation, as the type of organic matter also factors into SRB activity and Hg species movement. It is evident that sources other than sediment Hg methylation also influence the CH_3Hg amounts in the water and biota. For example, although Gosnell et al. (2015) demonstrated an increase of CH_3Hg in DE water coinciding with enhanced seasonal SRB methylation, it was also concluded that inputs of CH_3Hg were primarily from fluvial and other outside sources during alternative times of year. Thus, it is important to consider beyond coastal sediment when deliberating the source of bioaccumulative Hg species into algae, but it should not be ruled out entirely.

Chapter 6:

Current Regional and Global Overview for Trophic Transfer of Mercury Species in Plankton: Proposed areas for Future Research

6.1 Introduction

It is probable that different regions throughout the oceans and coasts of the world will have distinctive bioaccumulation burdens as each marine expanse is characterized differently with large and small scale circulation influences, differences in food chain structure, and chemical and physical signatures varying from the Arctic regions to the southern hemisphere. However, only a few different regions have been explored in terms of bioaccumulation of Hg and MeHg at the bottom of the food webs. From these, some results and signatures are starting to emergeconcerning the factors which could be influencing bioaccumulation at the planktonic level. This chapter presents and compares some of the other field work which was undertaken to understand the differences in plankton bioaccumulation amongst ecosystems. Though more questions remain than have been answered to date, the overall view from the results presented through this research has made a contribution towards improved understanding of this area of study. Below the similarities and differences across ecosystems are explored using data from a variety of studies.

6.2 Coastal Systems

Size fractionated zooplankton samples were collected from Narragansett Bay out to the outer shelf, in concurrence with a microbial research cruise during July of 2012 (Figure 6.1). The cruise started off Block Island (RI) and traveled out onto the Atlantic shelf break, then returned along the same course over a period of 3 days. Zooplankton were measured for Hg and MeHg at size fractions of 200-500 μm (0.2-0.5 mm), 0.5-1.0 mm, 1.0-2.0 mm and >2.0 mm. Net

tows were deployed from approximately 50 m deep up to the surface. At Station 24 the tow extended to 120 m into the deeper shelf waters.

The first two stations were collected in the relatively sheltered waters closer to land, while station 33 and 15 were situated along the shelf, and station 24 was in the marine waters of the shelf break. Stable isotopes of carbon (C) and nitrogen (N) were also measured for select zooplankton fractions, and provide an additional dimension for understanding potential regional and dietary effects on zooplankton bioaccumulation of Hg and MeHg in this coastal system. Unfortunately there were equipment problems with water collection, therefore sufficient measurements were not taken for the smallest plankton sizes or waters concentrations during this cruise. Zooplanktons samples were rinsed thoroughly with clean filtered seawater prior to filtering, and the usual steps were taken to keep everything as clean as possible, though the net was not deployed completely 'trace metal clean' as no Kevlar line was used. As this research is specifically focused on zooplankton along the Atlantic shelf, it provides useful comparison to the seasonal sampling undertaken in Long Island Sound (Chapter 3).

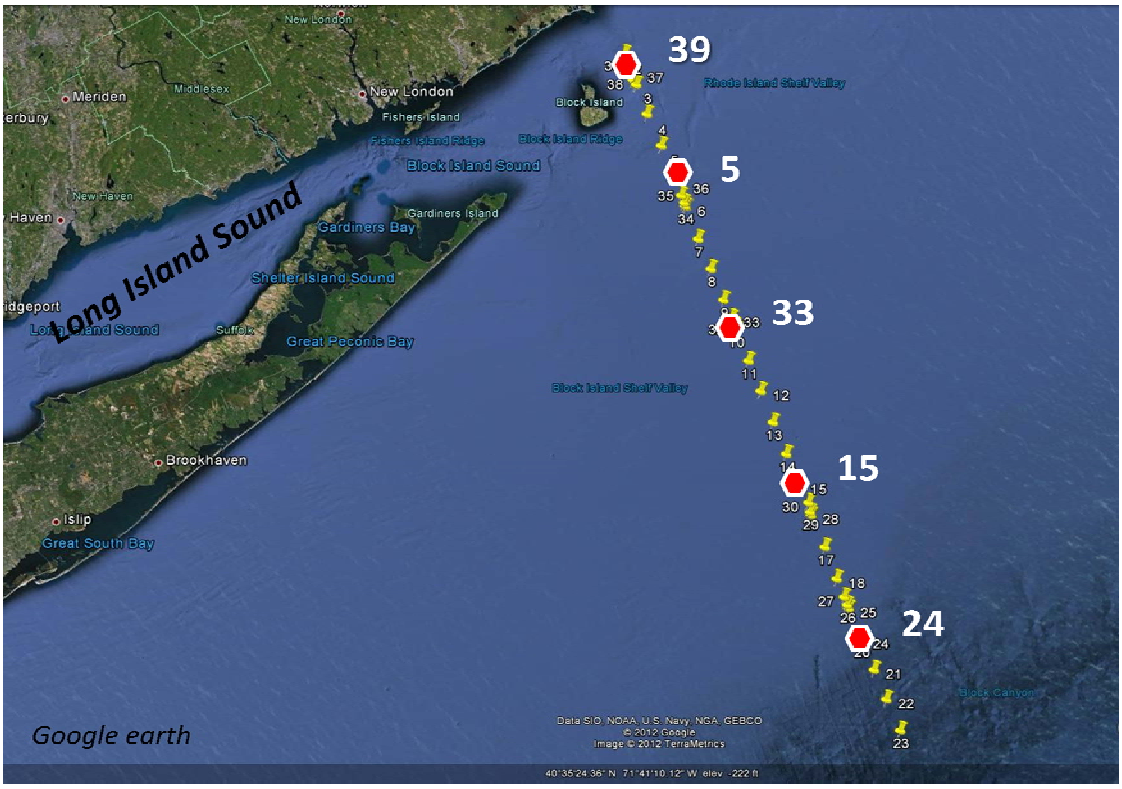

Figure 6.1. Map of cruise out to the Atlantic shelf transect during July of 2012. Stations where zooplankton was collected for Hg and MeHg measurements are noted and labeled along the cruise path. (Map provided with permission by G. McManus)

The MeHg concentrations ranged from 0.1 – 10 pmol/g (wet wt.) for all the stations and size fractions measured (Figure 6.2B). The 0.2-0.5 mm size fraction tended to be the lowest in MeHg for all the stations excluding Staion 5. Two of the stations collected closer to shore, stations 39 and 33, had relatively similar concentrations across all but one size fraction, and the highest concentration measured for that fraction was much greater than the rest of the fractions.

Concentrations of total Hg in the zooplankton ranged from ~10 to 85 pmol/g (wet wt) Hg in the zooplankton size fractions (Figure 6.2A). There appeared to be very little correlation between the size fractions and concentration, as high variability was evident within each of the fractions. The highest Hg concentrations were found in the >2.0 mm size fraction for stations 15

and 33, as well as the1.0-2.0 mm fraction for station 39. The highest values were in the >2.0 mm size at shelf station 15 for both Hg and MeHg.

Concentrations in zooplankton for the Atlantic shelf in 2012 are comparable to those collected during the summer in 2014 at ELIS and along the shelf in the fall (Chapter 3). Furthermore, the measurements in the summer zooplankton on the Atlantic shelf are much higher than zooplankton collected at WLIS during the summer, and tended to have analogous levels to those found in the ELIS fractions collected. Thus, these data provide further evidence that zooplankton away from coast, such as along the shelf (Chapter 3) and in open ocean waters (Gosnell and Mason, 2015; Chapter 2) accumulate more MeHg and Hg than animals residing within the more nutrient rich waters nearshore and in estuarine waters.

The %MeHg (MeHg/HgT) has been a useful measurement to discern if some size fractions are bioaccumulating more MeHg relative to inorganic Hg. The %MeHg for the zooplankton size fractions for the Atlantic cruise is displayed in Figure 6.3. Theoretically smaller species and animals feeding lower on the food chain should have a smaller %MeHg burden than animals consuming higher up the trophic level. The smallest size fraction of 0.2-0.5 mm did have the lowest %MeHg across the stations during this cruise. However, the largest fraction of zooplankton, >2.0 mm, only had the greatest %MeHg at station 33, while the 1.0-2.0 mm size fraction was greatest at all other stations. As a result, it seems that there is possibly overlap between feeding patterns in the two larger size classes sampled in this region. This could be due to differences in the types of organism in these fractions and their feeding preferences. In other words, it is possible that the Atlantic shelf species consume food containing varying levels of MeHg, and the more predatory species are found within the two highest size classes.

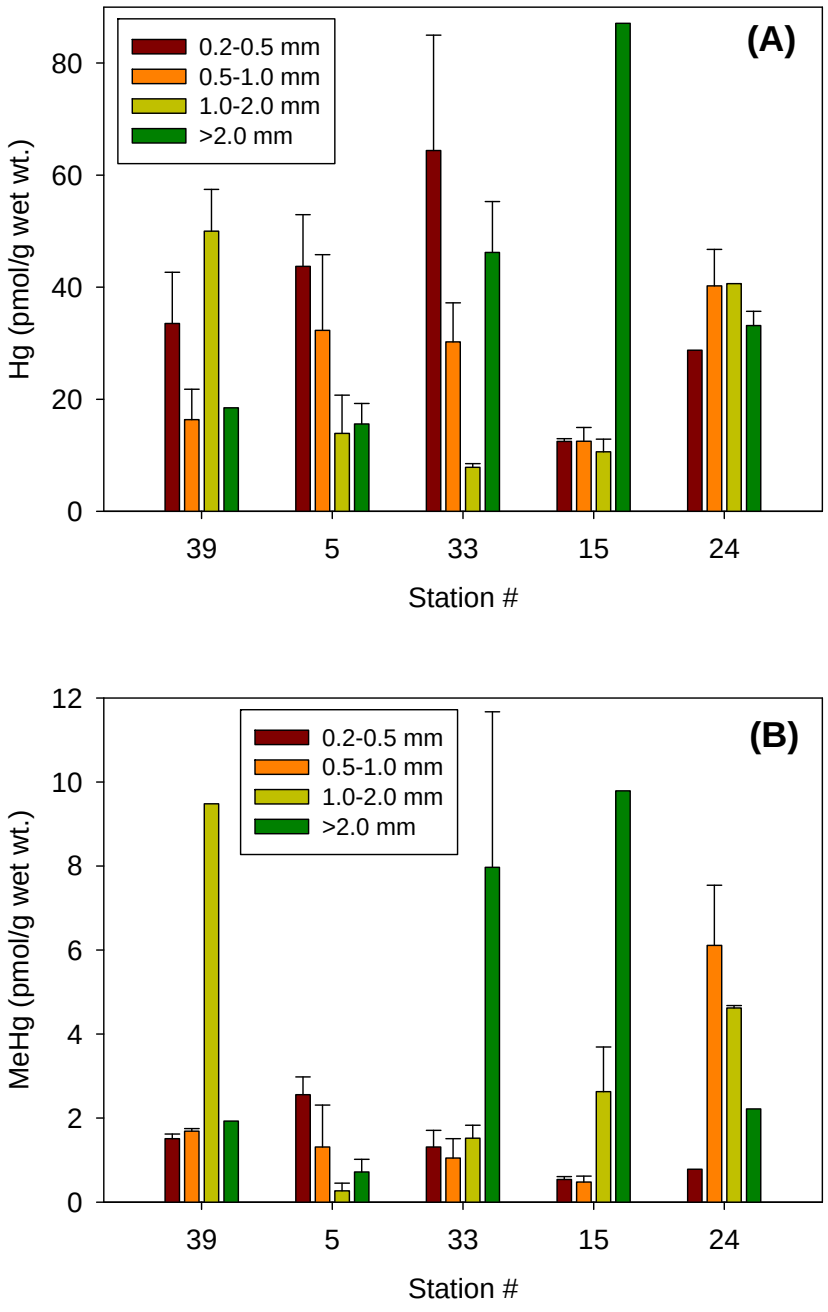

Figure 6.2. Concentrations (pmol/g wet wt.) of Hg (A) and MeHg (B) in zooplankton from Block Island (RI) to the shelf break during the summer of 2012.

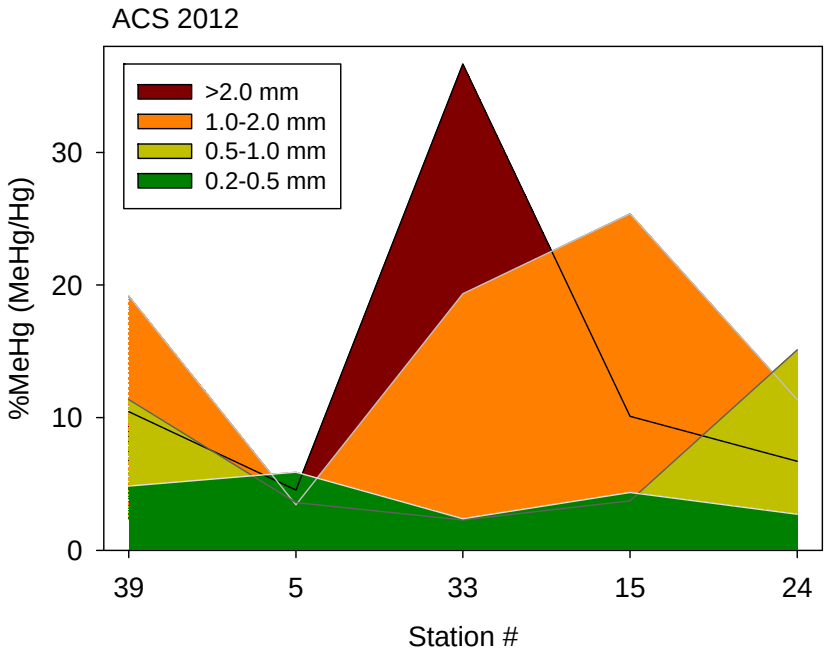

Figure 6.3. Values of %MeHg (MeHg/HgT) for zooplankton size fractions from off Block Island (RI) to the shelf break during the summer of 2012.

The %MeHg for the Atlantic shelf 0.2-0.5 mm zooplankton (~5-34%) was higher than shelf species collected during the fall 2014 cruise, which tended to be ~ 2%. However, this size fraction was comparable to the ELIS summer measurements, which were also ~5%. The rest of the size fractions actually had lower %MeHg values than was found at ELIS in 2014, even the > 2.0 mm size at station 33 was only within 10% of the ELIS measurements. Based on chlorophyll measurements collected throughout the cruise transit (Figure 6.4), it is clear that there was much more phytoplankton biomass closer to the coast than out in the shelf waters. As a result, there could have been more diluted distributions of Hg and MeHg within the nearshore stations, as was possibly seen in WLIS during that study (Chapter 3). Therefore it is possible that the zooplankton collected from the shelf waters had did not demonstrate the proposed summertime amplification in %MeHg than species closer to shore, suggesting less impact of sediment and

enclosed water column methylation than the more nearshore species of LIS. The water measurements from the Atlantic shelf collected in 2014 (Chapter 3) indicated there was no seasonal increase in MeHg water levels during that summer, as they were relatively low. However, as summertime amplification of MeHg accumulation is still not completely determined, this result is not conclusive.

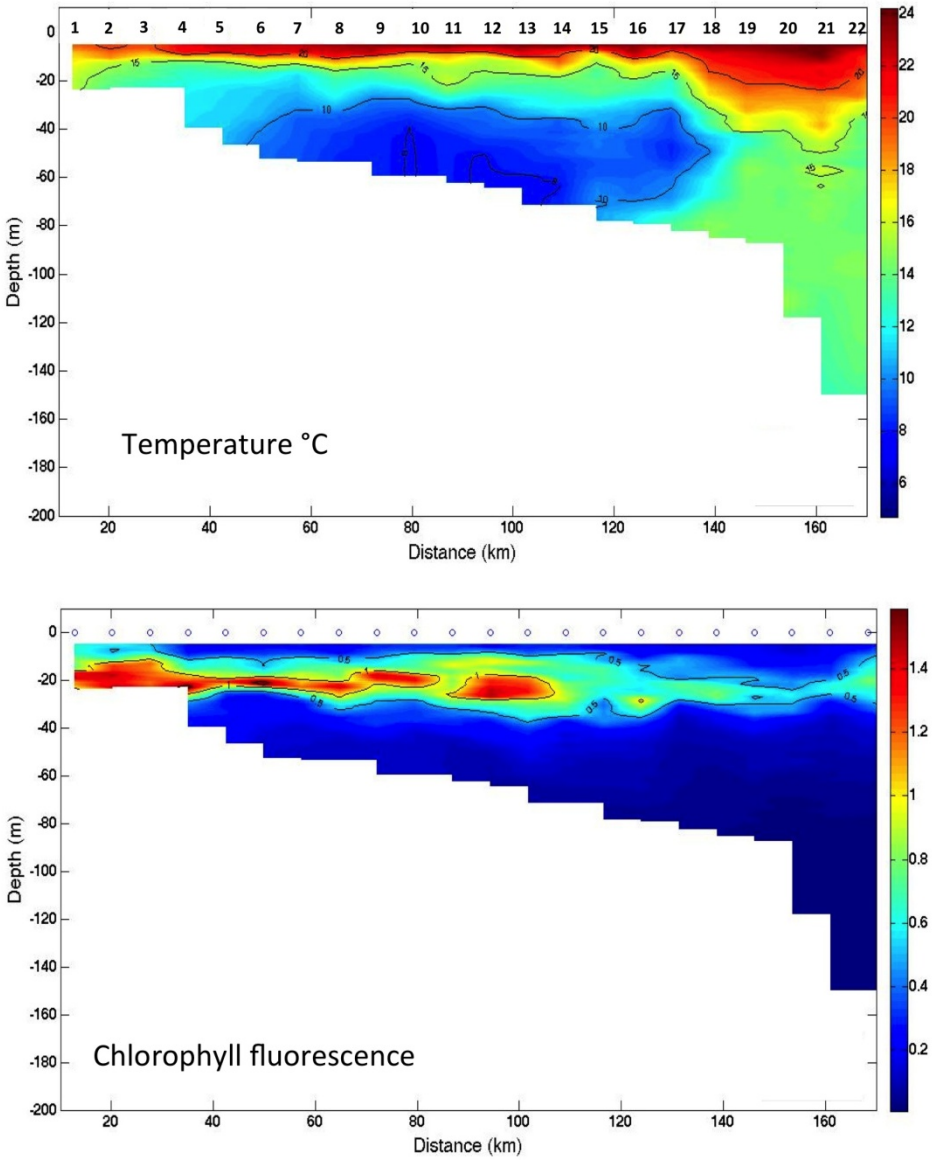

Figure 6.4. Temperature profiles (top) and chlorophyll a measurements for the stations in the outgoing cruise tract of July 2012. (Data and figure provided by G. McManus)

Another interesting feature in the %MeHg is the comparab low amounts in all size fractions at station 5. Station 5 was located farther off Block Island, though essentially within range of circulating outgoing Long Island Sound waters and incoming marine shelf waters. It is possible that the zooplankton at this location were consuming phytoplankton and particles which had a lower MeHg burden, either from enhanced nutrient inputs off the shelf attributing to growth dilution, or from the more pristine marine waters.

One of the more prominent features of the concentrations of the MeHg in the zooplankton is the relative consistency between station size fractions, except for one larger value in a size fraction at each station. This is evident at stations 39, 33 and 15 for the >2.0 mm or 1.0-2.0 mm size fractions (Figure 6.2A). This would possibly indicate that the large values have a specific driver accounting for the greater MeHg than the average assemblages of that station. Thus, it is prominent that some variables which were not quantified using the size fraction approach could be contributing to the variation.

Zooplankton were not separated into species for each size fraction, therefore this is logically a factor which could contribute to variability within measured concentrations. At station 33 there were significant euphausiids in the >2.0 size fraction. When this size fraction was filtered, these euphausiids were separated out and collected for separate analysis from the replicates of mixed assemblages, and the results of these different species are given in Figure 6.5. Some small fish fry were also collected in the net tow of station 33, and are displayed for additional comparison. It seems evident from Figure 6.5 that differences in biota species can yield a clear difference towards MeHg and Hg concentrations in some instances. However, the euphausiids in this example seem especially high as far as the rest of the >2.0 mm sizes, by

comparison. As these animals were significantly outliers with the rest of the assemblages concentrations, this measurement was not included with averages calculated in Figure 6.2. Thus, this example is not entirely reflective of all of the rest of the zooplankton measured, as they were even higher than the fish concentrations on a per mass basis (which were also >2.0 mm). However, this presents further information on the possiblity that other distinctive zooplankton with a higher bioconcentration capacity were sampled within the assemblages at some of the stations in this cruise, and possibly these differences are responsible for the higher measurements at some stations within single size classes. Though this is just one isolated instance, it does demonstrate that species type is likely a prominent factor in influencing bioaccumulation at the zooplankton level.

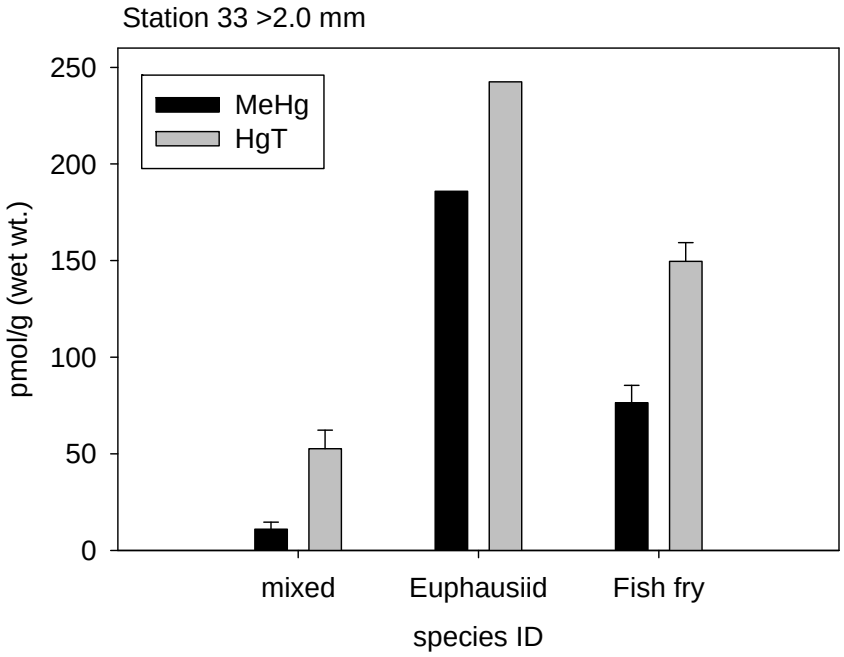

Figure 6.5. Differences in concentrations for MeHg and HgT (pmol/g wet wt.) for mixed zooplankton and individual euphausiids, versus a small fish fry captured at that station. Samples are all of the >2.0 mm size fraction and were collected at Station 33. The Euphausiid measurements were not included in Figure 6.2 & 6.3 averages.

The zooplankton collected during this cruise were dominated by copepods, especially in the 0.2-0.5 mm size fractions for each station. There were also abundant euphausiids, and some amphipods, in the larger fractions. A few chaetognaths were collected in the >2.0 mm size at stations 5 and 33, which could have specifically impacted the MeHg and Hg concentrations at station 33. However, as they were mixed with a large assemblage of euphausiids, amphipods and larger copepods for that fraction, there is no distinction in which animals are responsible for the strikingly higher measurements at station 33. However, as the >2.0 mm size fraction at station 15 also contained mostly larger euphausiids and amphipods (Figure 6.6), it is possible that those zooplankton present a larger bioaccumulation burden than the surrounding animals.

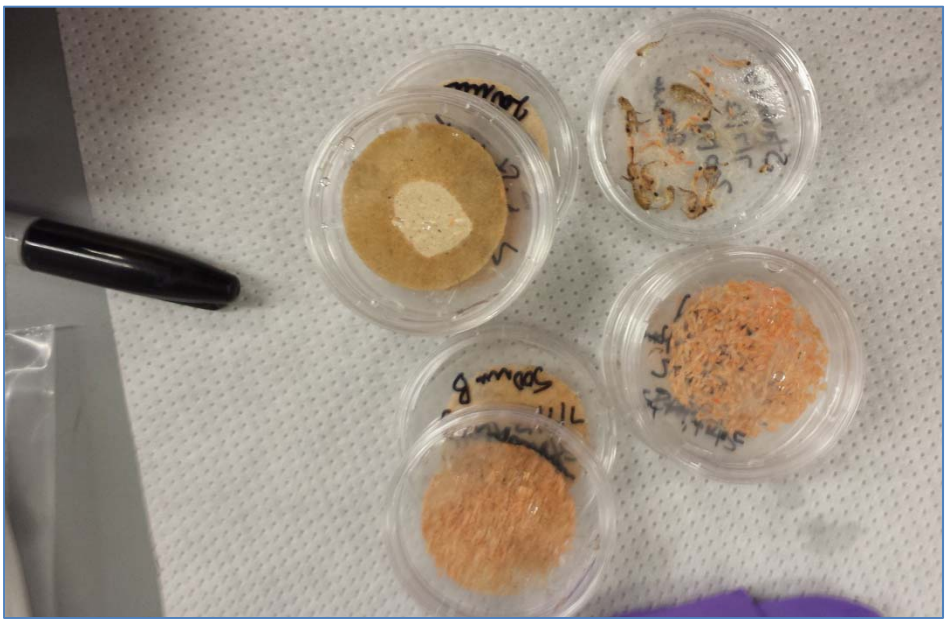

Figure 6.6. Zooplankton collected in the different size fractions for Hg and MeHg analysis, from station 15 of the Atlantic Shelf cruise in 2012. The >2.0 mm zooplankton are distinctively larger and different species than the other sizes, and contained higher concentrations of Hg and MeHg.

Stable isotopes of C (^{12}C, ^{13}C) and N (^{14}N, ^{15}N) were also collected for selected size fractions of zooplankton. These values are useful for discerning differences between diet and food sources in multiple investigations (Fry and Sherr, 1984; Gearing et al., 1984). Stable isotopes of C ($\delta^{13}C$ ‰) can indicate regional sources of food for plankton, as more depleted values indicate terriginous materials (Chapter 3; Fry, 1988). The $\delta^{13}C$ ‰ values of marine phytoplankton have been found around -18 to -24 ‰ $\delta^{13}C$ (Fry and Sherr, 1984), indicating that depletion in zooplankton can also be a result of food sources to the secondary consumers. The AC zooplankton $\delta^{13}C$ ‰ tended to range between -19 to -26 in this study, and there was no clear relation between station sampled and $\delta^{13}C$ ‰ value. The lack of correlation is indicative that the zooplanktons along this shelf were viably feeding on similar materials coming from the surrounding land and river runoffs, or from primary production associated with marine phytoplankton residing along the shelf. Gearing et al. (1984) reported values of -24‰ $\delta^{13}C$ as representative of particulate material in Narragansett Bay, which was regionally close to where zooplanktons were collected along the Atlantic shelf. The $\delta^{13}C$ ‰ values in this study were overall more depleted then those seen in LIS during 2014, although there was some overlap with values measured in ELIS during the summer (-16 to -26 ‰ $\delta^{13}C$). These measurements further indicate that the shelf waters of 2012 far outside of Narragansett Harbor had different food sources then those of WLIS, and also help confirm the intersection to animals collected with the cycling waters of ELIS during 2014.

Stable N isotope ratios ($\delta^{15}N$ ‰) were plotted against zooplankton MeHg and Hg concentrations and are displayed in Figure 6.7. Unlike what was found with $\delta^{13}C$ ‰ ratios, there does seem to be some coorespondence between station and trends. The zooplankton collected

closest to shore, at station 39, have the highest $\delta^{15}N$ ‰ values (~9 ‰), indicating that they were consuming higher on the food chain, or consuming food with a N source that is more impacted by human activity (i.e. sewage treatment plants). Concurrently, station 24, which was located at the shelf break had the lowest grouping, with values ranging from 5-6 ‰ $\delta^{15}N$.

Though there is overlap for the middle stations, the stations tend to decrease in $\delta^{15}N$ ‰ values moving offshore onto the shelf. As the three middle stations are dispersed throughout the marine shelf waters, it is not unexpected that the organisms sampled could be feeding on similar available food of that region. The zooplankton collected on the shelf during the fall cruise of 2014 had a larger range of values, changing from ~10 ‰ $\delta^{15}N$ around ELIS to ~4 ‰ $\delta^{15}N$ at the shelf break. Thus, the trend was somewhat consistent with the current data, but it seems that the summertime animals of 2012 were possibly consuming lower on the food chain for this region. Furthermore, there do not appear to be clear inclinations for MeHg and Hg concentration correlations against $\delta^{15}N$ ‰ for zooplankton, besides a slight positive increase for station 15. This seems to indicate that there was more omnivorous feeding on the stations of the shelf waters, yielding less conclusive trends then was seen in the various stations of LIS and the shelf in 2014.

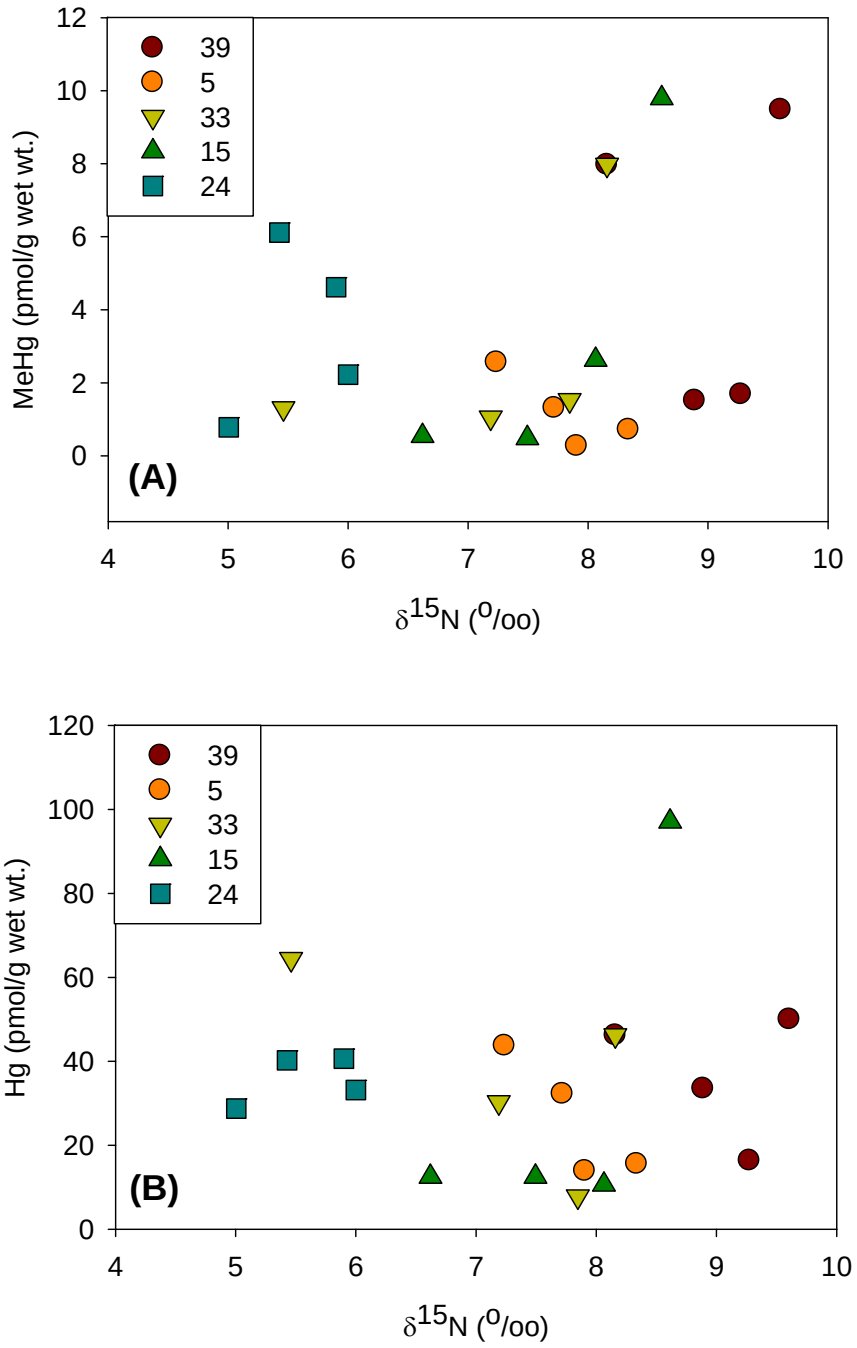

Figure 6.7. Values of $\delta^{15}N$ (‰) versus MeHg (A) and Hg (B) for zooplankton size fractions from off Block Island (RI) to the shelf break, collected during the summer of 2012.

6.3 Open Ocean Systems

Though open ocean waters contain very sparse biomass compared to coastal systems, it is clear that there can be significant bioaccumulation at the base of the food chain which expectedly impacts open ocean fish concentrations, and global Hg cycling in marine waters.

Phytoplankton had inorganic mercury concentrations ranging from ~1.3 up to >400 pmol/g (wet wt.), and MeHg concentrations found at 0.5 pmol/g (wet wt.) up to nearly 20 pmol/g (wet wt.) at some stations in the open equatorial Pacific Ocean, primarily in the biota poor HNLC region (Chapter 2; Gosnell and Mason, 2015). Though the open ocean is more pristine compared to coastal stations, the phytoplankton had much higher concentrations than what has been seen along the coast. Notably, the logBCF levels for phytoplankton in the Pacific were higher (5.69 ± 0.98) than coastal measurements (~4 log BCF; Hammerschmidt et al., 2013; Chapter 3), indicating that open ocean phytoplankton especially concentrate available Hg species within marine waters

On the other hand, phytoplankton sampled at several stations around BATS in Bermuda (western Atlantic) during August of 2010 were somewhat lower in concentration than what was seen in the Pacific Ocean. These algae had approximate values of ~1 to 44 pmol/g (wet wt.) concentrations of Hg, with extremely low levels MeHg ranging from 0.05 to 2 pmol/g (wet wt.). As a result, the %MeHg only ranged from <1% to 5% for the oceanic Atlantic phytoplankton. These values are conservative estimates, as abundance was so low that seston mass was difficult to measure accurately.

Zooplankton concentrations in the Pacific Ocean were highly variable through the cruise transit, falling between ~10- 120 pmol/g (wet wt.) for Hg, and ~1-18 pmol/g (wet wt.) for MeHg

overall. These concentrations were not excessively high when compared to some particular size fractions in the coastal concentrations; however they were overall higher than what has been seen for near shore values (Hammerschmidt et al., 2013; Chapter 3). They also ranged higher than what was seen in Bermuda zooplankton collected around BATS. The open ocean Atlantic animals had Hg concentration of 0.8 pmol/g (wet wt) to 37 pmol/g (wet wt.) and MeHg levels ranging from 0.7 to 12.8 pmol/g (wet wt.).

One evident feature about open ocean Pacific zooplankton was that there was consistent increase in %MeHg with increasing size for the zooplankton, unlike coastal measurements where the %MeHg values had slightly more variability. The Bermuda zooplankton only partially exhibited this trend, ranging in %MeHg of 5-33%, but with more overlap between sizes than what was seen in the Pacific. It is probable that open ocean Pacific zooplankton are feeding on species which have a higher %MeHg burden, as some of the phytoplankton collected in the Pacific had relatively high %MeHg levels. But other factors could also be affecting these results, such as more limited growth from limited food sources, or different omnivorous behavior for these organisms compared to coastal species. Stable isotopes could potentially be a useful tool in future investigations for open ocean plankton, as the [C/N] values did not offer clear correlations or understanding of differences, though they don't always offer exact trend evaluations, which was evident from the LIS measurements (Chapter 3).

It was also significant that zooplankton collected at night seemed to have different bioaccumulation burdens than day time collected organisms at the highly productive upwelling station along the equatorial Pacific. This demonstrated that deeper dwelling animals could be impacted by the increased burden of MeHg found in some deep waters, or by enhanced Hg methylation within low oxygen AOU regions as was suggested in Fitzgerald et al., 2007. Thus,

it is not only phytoplankton which can be affected by exposure concentrations of Hg species in the water column, but zooplankton as well. It is clear from these initial results that the open ocean represents a region where more investigation is necessary to understand differences in trophic transfer of Hg at the plankton level. It is important that we understand more of the open ocean Hg cycling and affects on biota, as marine systems represent most of the water systems, and are the source of mos fish consumed by humans.

6.4 Arctic Regions

Arctic systems are far from source points of anthropogenic emissions of Hg, however there is still ample concentrations of Hg found within the environment and biota of the polar hemispheres (Stern et al., 2012; Burt et al., 2013; Schroeder et al., 1998). Gaseous mercury has a relatively consistent residence time in the atmosphere, which gives it the ability to travel throughout the world. One of the primary mechanisms Hg transfers into Arctic ecosystems is via atmospheric mercury depletion events (AMDEs), which are unique to the polar marine environments. The AMDEs area result of facilitated sunlight-induced reactions of elemental Hg (Hg(0)) with atmospheric bromine and bromine oxides. These events oxidize Hg (0) to reactive gaseous Hg (RGM), which is a more form of Hg(II) that is more easily removed by wet and dry deposition, as it is highly soluble, and because it is highly particle reactive can assist in particulate Hg formation. The particulate Hg and RGM are easily scavenged from the atmosphere and deposited onto open marine, lake and river waters, as well as snow and ice surfaces. Through numerous phase transformation and concentrated transport, very high concentrations of Hg have been reported in Arctic snow and ices (Burt et al., 2013).

Plankton was collected for Hg and MeHg bioaccumulation measurements from Lake Melville, an estuarine fjord located along the northern Atlantic coast in the provence of Labrador, Canada (Figure 6.8; SI: Schartup et al., 2015b). Though the Arctic is a relatively pristine region with few anthropogenic Hg sources, Arctic plankton tends to have elevated concentrations of MeHg (Stern et al., 2012; Burt et al., 2013; Pućko et al., 2014). Zooplankton were collected using an opening-closing 200 μm mesh net (Seatec), from depths of approximately 30 m to just below the surface in and around Goose Bay, and sampling was extended down to 150 m for the deeper lake and Groswater Bay tows. Phytoplankton were amassed on polycarbonate filters (0.2 μm & 5 μm) using filtered seawater collected from an acid-cleaned GoFlo bottle attached to a Kevlar line. In addition to plankton samples, sediment and water column measurements were also collected in order to discern Hg cycling and dynamics throughout the lake region (Figure 6.8; from Schartup et al., 2015b).

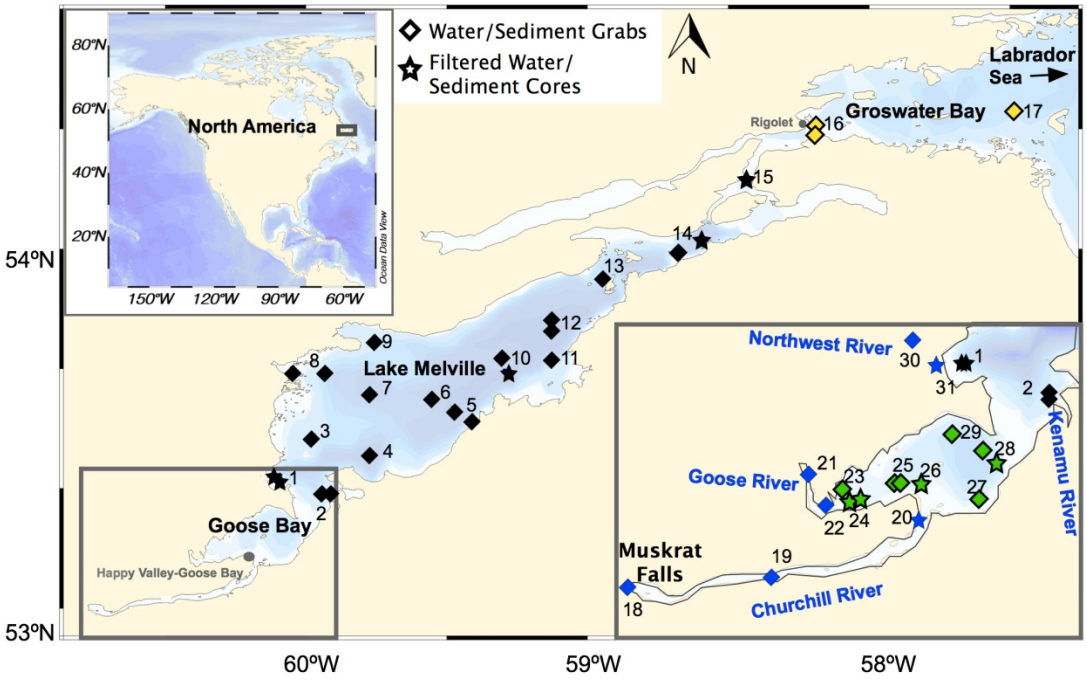

Figure 6.8. Map of Lake Melville sampling sites for water and sediments, as well as the location of the new hydroelectric facility (Muskrat Falls), which will flood the main estuary tributary, the Churchill River, in 2017. Different regions are defined by colors: black (Lake Melville), green (Goose Bay) and yellow (Groswater Bay). Tributary rivers are noted by the blue symbols. Plankton was collected from sites 1, 6, 10, 14, 16, 17 and 28. (Figure from SI in Schartup et al., 2015b)

Lake Melville is an expansive and deep semi-enclosed estuarine system (180 km length, mean depth 83.5 m). Throughout winter and fall season the lake is primarily ice covered. During spring runoff it tends to obtain substantial freshwater from several surrounding rivers, primarily the Churchill which yields greater than 60% of the input (Bobbit and Akenhead, 1982). A prominent physical year-round feature of Melville is a low-salinity layer approximately 2-15 m below the surface. The stratification between low-salinity and brackish waters extends throughout the lake, but is most pronounced at the imput regions of the primary tributary sources, and least pronounced where marine waters initially enter Melville. This distinctive salinity difference tends to support slightly different species of zooplankton, as well as cause

small scale mixing fronts where marine snow flocs can form, and where proposed enhanced methylation could occur (Ortiz et al., 2015; Schartup et al., 2015b).

Benthic processes are known to produce MeHg via microbial methylation by anaerobic bacteria (King et al., 1999). This production in sediments has been thought of as a primary source towards MeHg bioaccumulation in coastal and estuarine systems. Methylation in the open ocean is thought to occur in subsurface waters where particulate remineralization is occurring, and bacterial activity is high (Mason et al., 2012). Water column methylation was measured using isotopic incubations in Schartup et al., (2015b), and methylation in marine snow has also been shown to occur using coastal waters (Ortiz et al., 2015). Thus, there is evidence that pelagic methylation processes can also be an important source of MeHg into plankton in coastal waters, and further contributing to their bioaccumulation load in the Arctic.

There were clear differences in plankton MeHg concentrations collected from the different regions of Lake Melville (Figure 6.9A). Plankton collected from the sheltered region of Goose Bay, which receives considerable river discharge from the main Churchill River, had much higher concentrations throughout the size classes than those collected from the main lake or Groswater Bay stations. The %MeHg values approached 80% in the intermediate size fractions (500-1000 μm and 1000-2000 μm), and the other fractions were still relatively higher than values found for other coastal systems (i.e. LIS and the Atlantic shelf). Furthermore, the methylation rates measured in Goose Bay tended to be enhanced compared to other regional water column rates (Schartup et al., 2015b).

Goose Bay has stable year-round stratification proximate to the Churchill River discharge, which helps to form an organic matter layer as well as flocs of marine snow, where potential methylation in the water column has a stronger base for development. Furthermore,

river discharge into Lake Melville is the dominant source of total Hg into the system (Schartup et al., 2015b; Sunderland et al., 2010), thus there is also a broader foundation for Hg methylation closer to the greatest source. It was hypothesized that the buildup of detritus and remineralized nutrients from algal degradation likely fueled microbial activity in the water column, enhancing methylation and uptake into residing zooplankton. Furthermore, the bioaccumulation factors (BAF; plankton [MeHg]/water [MeHg]) were also slightly higher for the Goose Bay plankton compared to the other plankton in Lake Melville, indicating there was further enhancement from the water column into zooplankton of Goose Bay (Figure 6.9B).

The zooplankton collected from the open waters of the fjord had similar low levels to other enclosed oligiotrophic regions (Chapter 3), while the coastal plankton contained extremely low MeHg levels. The MeHg BAFs of Lake Melville and Grosswater Bay samples (~log 3-5) were consistent with coastal measurements along the Atlantic (Chapter 3; Hammerschmidt et al., 2013). The animals collected from the lake and coast are reflective of lower seawater Hg and MeHg, and relatively organic poor waters along the northern Atlantic shelf. However, there was an increase in measured water MeHg levels in June throughout the study region. This increase in MeHg in the waters could have been dampened in the biota accumulation throughout the Lake Melville region, as there is sufficiently more biomass in the Arctic summertime than any other time of the year. Thus it is possible that river discharge has the ability to enhance physical processes which drive methylation and uptake into plankton.

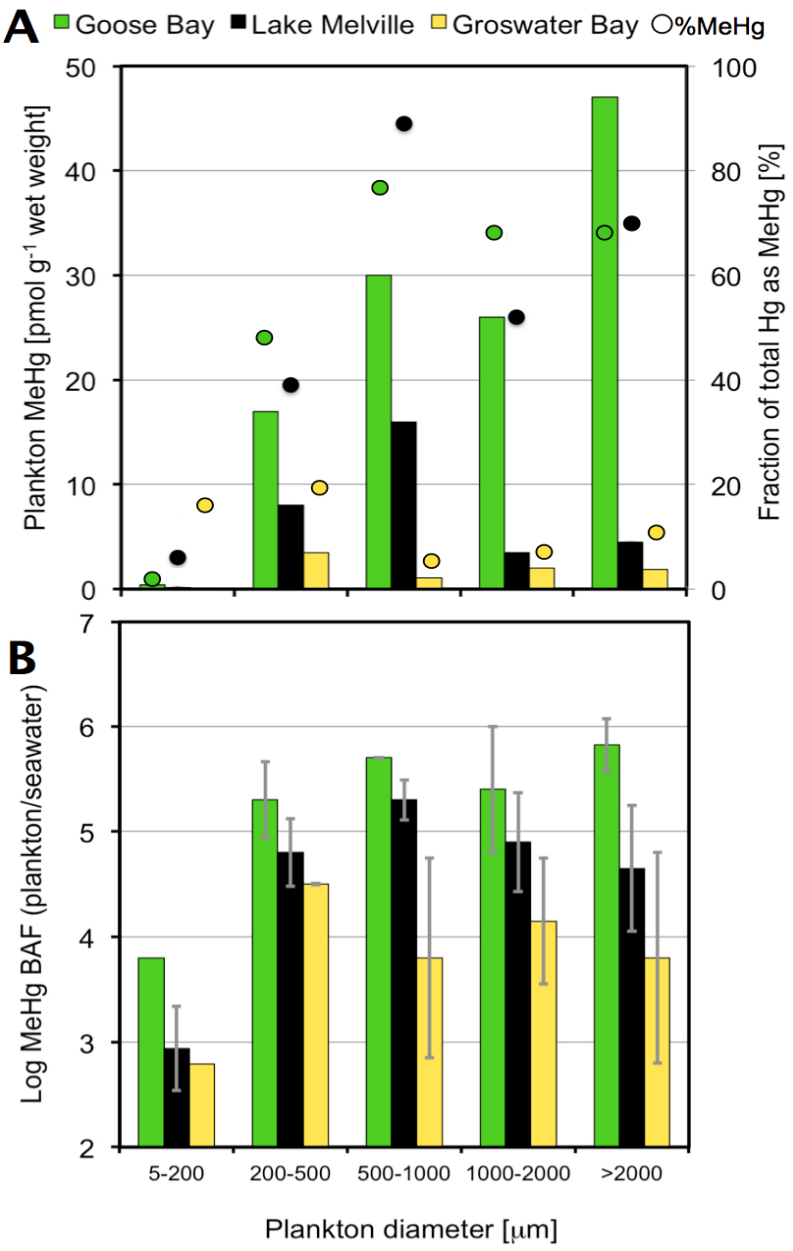

Figure 6.9. (A) The MeHg concentrations for the different size classes collected across the different sampling regions. Phytoplanton are represented as the 5-200 µm size class. Bar colors represent the different sites sampled: black (Lake Melville), green (Goose Bay) and yellow (Groswater Bay). (B) The bioaccumulation factors (plankton [MeHg]/water [MeHg]; BAF) calculated for each plankton size fraction across the sampling regions. (Figure from Schartup et al., 2015b).

Schartup et al. (2015b) additionally investigated the effects of soil flooding towards MeHg production around the lake, as a hydroelectric dam has been proposed in the region. It was demonstrated that methylmercury inputs would likely increase by 25-200% by flooding the soils through reservoir creation. Thus, the MeHg burden into the plankton, up to the fish and marine mammals, and therefore human consumers will likely rise with the proposed hydroelectric power completion, and provide additional sedimentary sources to the pelagic methylation.

The zooplankton collected throughout the Lake Melville sampling were somewhat variable in composition moving from the stratified bay waters out to the coast, though there were consistently abundant amounts of copepods, chaetognathas and euphausiids. At station 14, which was located in Lake Melville, there was prominent variety within the zooplankton of the >2000 μm size class, and some species were separated out into specific measurements for Hg and MeHg in Figure 6.10. There were distinctively two different copepods, which are noted as 'copepods' and 'big red copepods' in Figure 6.10. The larger 'big red copepods' were not confirmed as a species, but they were likely *Calanus hyperboreus.*

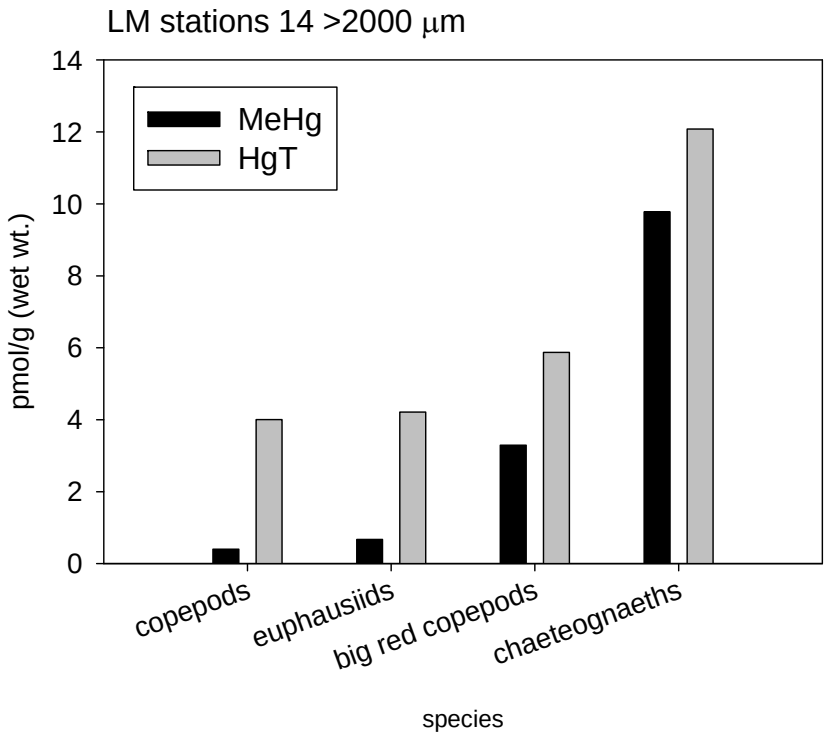

Figure 6.10. Differences in species Hg and MeHg composition in several different >2000 μm zooplankton collected from station 14 in Lake Melville.

There were very distinctive differences between Hg and MeHg concentrations for the species measured. The chaetognathas, which are predatory zooplanktivores, had significantly greater concentrations of both Hg species, and also the highest %MeHg. Within this same >2000 μm size class, the larger copepod species had greater concentrations than both 'smaller' copepods and the euphausiids measured at the station. Based on these measurements, it seems that larger predatory zooplankton could possibly have greater MeHg bioaccumulation compared to larger zooplanktons which are more omnivorous. However, as there have only been a few measurements thus far, this theory represents an adequate area for future research.

6.5 Current Overview & Future Research

It is evident that there is significant uptake and trophic transfer of Hg and MeHg species at the plankton levels in marine systems. Summary measurements of HgT and MeHg collected for various phytoplankton are presented in Table 6.1, while zooplankton concentrations are available in Table 6.2. Size fractions are not distinguished for these values. When comparing coastal collections to the open ocean, it is evident that open ocean levels are much greater then what is found in coastal communities, which is especially pronounced for the phytoplankton in the Pacific HNLC region and zooplankton in the Pacific upwelling region. This overview hints that organisms dwelling in more sparsely populated oligotrophic regions could potentially be bioaccumulating greater burderns of available Hg and MeHg, while more intense blooms of plankton could be weaking the burden of Hg and MeHg bioaccumulation. However, this is not necessarily a promotion of coastal nutrient enhancement, as it is evident that there are still many unknowns factoring into these measurements. Furthermore, the values in Tables 6.1 and 6.2 further demonstrate variability within regional measurements, as even different ends of LIS yield dissimilar Hg burdens, and the plankton in Lake Melville had different Hg bioaccumulation evidently dependent on where they were residing in the fjord.

Table 6.1. Phytoplantkon concentrations of HgT (pmol/g wet wt.) and MeHg (pmol/g wet wt.) for selected assemblages throughout the world. The various %MeHg burdens are also displayed for each region. Though plankton were size fractioned, those differences are not displayed.

Location	Site	HgT (pmol/g)	MeHg (pmol/g)	%MeHg
Atlantic Fjord	Goose Bay	2.28-4.34	0.04-0.1	2
	Lake Melville	0.48-1.45	0.01-0.1	2-6
	Labrador Shelf	0.25-0.8	0.05-0.09	12-16
CT coast	WLIS	0.08-7.3	0.02-1.02	2-23
	ELIS	0.06-23.6	0.02-0.84	1-18
	Shelf	1-12	0.02-0.59	2-39
Atlantic coast		1-14	0.1-1.3	2-6
Atlantic Ocean	Bermuda	9.2-44.6	0.05-1.8	1-5
Pacific Ocean	HNLC	5.7-370	4.7-20	2-83
	Upwelling	2.8-23	0.7-3.8	5-28
	Open	1.8-338	1.6-10	1-61

Table 6.2. Zooplantkon concentrations of HgT (pmol/g wet wt.) and MeHg (pmol/g wet wt.) for selected net collections throughout the world. The various %MeHg burdens are also displayed for each region. Though plankton were initially size fractioned, those differences are not displayed. Atlantic coast values are from Hammerschmidt et al. (2013).

Location	Site	HgT (pmol/g)	MeHg (pmol/g)	%MeHg
Atlantic Fjord	Goose Bay	7.5-45.5	6.4-45.5	29-95
	Lake Melville	4.1-27.1	0.6-18.4	15-84
	Labrador Shelf	7.3-54.4	0.1-10.9	5-75
CT coast	WLIS	0.6-65	0.2-5.6	2-45
	ELIS	19.3-76.7	0.3-6.9	1-45
	Shelf	12.4-46.4	0.3-6.9	2-19
Atlantic coast		10-85	0.1-10	5-37
Atlantic Ocean	Bermuda	0.7-37.9	0.7-5.1	5-33
Pacific Ocean	HNLC	81-437	5-118	5-61
	Upwelling	72-893	3-283	5-76
	Open	54-398	3-227	5-49

Differences in global and regional values are not limited to measured concentrations within plankton, as there are furthermore distinctions in the bioconcentration factors (logBCF s) for phytoplankton (Table 6.3) and zooplankton (Table 6.4). The logBCF values help to

normalize for differences in water concentration, as they are a measurement of the ratio of what is in the organism to what is measured in the water column. These measurements further confirm that open ocean plankton are concentrating more available Hg and MeHg, and it is especially prominent for the phytoplankton. The zooplankton open Pacific logBCFs are elevated compared to coastal stations, but higher values are also found for LIS shelf and ELIS species, indicating that diet and speciation are also controlling factors in bioconcentation form the water.

Table 6.3. Ratio values for organism to water column concentrations (Bioconcentrations factors; logBCFs (L/kg)) for HgT and MeHg for selected phytoplankton measurements throughout the world.

Location	Site	HgT (10^)	MeHg (10^)
Atlantic Fjord	Goose Bay	2.8	3.9
	Lake Melville	2.5	2.9
	Labrador Shelf	2.7	2.6
CT coast	WLIS	2.3 – 4.8	2.6 – 5
	ELIS	2.6– 5	2.7– 5
	Shelf	3.6 – 4.2	2.7 - 4.5
Pacific Ocean	HNLC	6– 7	6.4 – 6.8
	Upwelling	4.5 – 5.9	4.5 – 5.5
	Open	4 – 6.7	4.3 – 5.8

Size fractioning the zooplankton has yielded important insight into some trophic transport trends and influences. For example, it is evident that there is more omnivorous behavior in coastal systems compared to open oceans and out along the coastal shelf, and this behavior can possibly dampen amounts of Hg and MeHg accumulated by zooplankton. It seems that more predatory zooplankton bioaccumulate more MeHg, which has been seen for fish species and invertebrates. However, it is apparent that more research is needed to confirm and better understand these results. There is some initial evidence that speciation of the different

zooplankton is relevant to the Hg levels accumulated (Figures 6.5 & 6.10), thus separating out the zooplankton into their species groups represents a natural next step in this research.

Table 6.4. Ratio values for organism to water column concentrations (Bioconcentrations factors; logBCFs (L/kg)) for HgT and MeHg for selected zooplantkon measurements throughout the world.

Location	Site	HgT (10^)	MeHg (10^)
Atlantic Fjord	Goose Bay	3.3– 4	5.3– 6
	Lake Melville	3 – 4	4.7 – 5.4
	Labrador Shelf	3.6– 4.5	3.8 – 4.5
CT coast	WLIS	2 – 4.5	2.9– 5.4
	ELIS	3.8 – 4.8	3.8– 6.4
	Shelf	4.2 – 4.7	4.5 – 5.9
Pacific Ocean	HNLC	4.7– 6	4.9 – 6.2
	Upwelling	5.5 – 6.1	4 – 5.9
	Open	5.1 – 6	4 – 5.4

For example, the mixed LIS zooplankton fed identical levels of Hg and MeHg isotopes in Chapter 4 yielded variable %MeHg concentrations. There were some hints that metabolic processes or specific physiological proteins within the zooplankton fractions influenced uptake, as some %MeHg values were similar to field measurements. However it is clear that there is very little understanding of what occurs to make some zooplankton accumulate more MeHg than others, besides carnivorous behavior or higher consumption.

Furthermore, it is evident from both the laboratory experiments and field samples that different sized algae accumulate Hg and MeHg at variable levels of uptake. It is also clear that algae take in Hg species reflective of exposure concentration, and this effect is especially prominent for MeHg species. However, it is also apparent from field sampling that

phytoplankton collected do not necessarily reflect what the zooplankton are consuming. This is partially due to the mobility of zooplankton, but it is also a result of the specified sampling scheme. It would potentially be beneficial to sample phytoplankton from several different depths besides only the chlorophyll maximum to help discern correlations between phytoplankton and zooplankton in marine systems. Thus, future investigations should attempt to broaden the collection scheme, and it is feasible that could be efficient by utilizing particle pumps. Also, as detection limits continue to improve, it is also possible that this would be easier to undertake in the near future. It would further be beneficial if some phytoplankton species identification was undertaken concurrently with field samples, though it is clear that would be quite difficult to carry out within the realms of sparse biomass, such as open ocean measurements.

More research is needed on the effects organic matter and DOC on Hg and MeHg uptake into algae species. Organic matter has been shown to have a contrasting relationship in environmental systems. In some instances, it seems that high organic matter contents could sequester Hg from easy transport or mehtylation within sediments of marine systems (Hammerschmidt et al., 2008). Pickhardt et al., (2006) implied that enriched DOC could enhance MeHg algal uptake, however, Schartup et al. (2015) indicated that coastally derived DOC influenced uptake differently then marine sourced DOC along the Atlantic coast. Thus, it is clear that there is still relatively little confirmed about the influence of concentration and type of DOC on algae uptake, especially algae in marine systems or within the open ocean.

Another important future undertaking for phytoplankton Hg trophic transfer is the southern hemisphere, as no plankton Hg bioaccumulation research has been reported in that region of the world. Besides the few stations collected in the south central Pacific Ocean (Chapter 2), there are no known measurements of plankton Hg concentrations within the

southern hemisphere. Moreover, it is unknown what is happening concerning bioaccumulation in the Southern Ocean and within the pelagic biota of Antarctica. As Hg is a ubiquitous global traveler it is critical to discern bioaccumulation within that half of the globe as well. It would be interesting to investigate if there is potentially more water column Hg methylation impacts within the half of the world which contains greater water expanse. Alternatively, it would also be important to investigate if there is enhanced bioaccumulation affects off the coast and within the open waters of southern Asia, which are proximate to the massive forest fires burning down rainforests in Indonesia and substantial coal-fired plants of China. These anthropogenic practices release immense amounts of Hg, therefore it would be important to discern if there are proximate effects in the biota of the region.

Arctic systems are incredibly important, and also incredibly vulnerable to warming seas and changing circulation physical patterns. Burt et al. (2013) examined mercury uptake into algal sea ice during a spring bloom, and found that Hg uptake was enhanced with algae residing on ice surfaces. Sea ice is relatively heterogeneous, and tends to be made up of a patchwork of ice crystals, liquid brine and air pockets. This structure tends to provide a unique polar niche where enhanced Hg concentrations have been measured (Chaulk et al., 2011). Furthermore, enhanced Hg levels within sea ice tend to bioconcentrate into the algae, which could be responsible for greater Arctic bioaccumulation by providing an enhanced vector for accumulation of Hg into zooplankton of the Arctic. Sea ice tends to contribute greatly to the primary production by providing pulses of nutrients and biomass as it melts. Therefore, it also provides a strong basis for Hg accumulation and transformation to plankton.

The sea ice community productivity is projected to increase with climate change dampening temperatures in the Arctic (Tadesco et al., 2012). However, there is no strong

conclusion on how this will affect the greater plankton community towards Hg bioaccumulation. On one hand, it is possible that enhanced growth could yield more biodilution of Hg species as has been seen in Pickhardt et al. (2002). Alternatively, it is possible that this chemical transformation and release of nutrients could results in enhanced methylation to pelagic microbes, resulting in an increased burden towards arctic plankton. As arctic systems tend to have higher burdens of Hg then would be thought for such pristine regions, these regions offer an exceptionally important focus of future research.

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