Mercury in fish and invertebrates in the Saint John River estuary

by

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ABSTRACT

Methylmercury (MeHg) is a common toxin that affects freshwater fish and fish consumers in North America, but its accumulation has not been well studied in estuarine systems. Therefore, the spatial trends of MeHg in biota from fresh to marine waters are poorly understood. This study investigated patterns of MeHg along a salinity gradient ranging from freshwater to brackish water within the Saint John River estuary, New Brunswick, Canada. The objective was to identify how MeHg concentrations in organisms relate to this salinity gradient and other biological or environmental variables. Samples collected in 2015 and 2016 included fourspine stickleback (*Apeltes quadracus*), *Gammarus* amphipods, freshwater snails, chironomids, sediments, and water. With increasing salinity, MeHg increased in chironomids but showed no strong correlations for other samples. This research showed that salinity was not strongly associated with MeHg in abiotic and biotic compartments of this estuary and that the risk of MeHg toxicity cannot be identified based on habitat salinity. However, this work is important for building future studies predicting MeHg in estuarine food webs.

DEDICATION

Dedicated to my Poppy, Buddy Reinhart,

who was a devoted fisherman of the Saint John River watershed

and would have loved to see me out fishing and playing with bugs.

1934 – 2017



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1. Mercury in the environment

1.1 Mercury sources and deposition

Mercury (Hg) is a naturally occurring metal found in geologic deposits, including cinnabar ore and fossil fuels (UNEP, 2013). Weathering of these geologic sources, volcanic activity and volatilization from soils and the ocean can release Hg into the atmosphere as gaseous elemental Hg (0) (Selin et al., 2007; UNEP, 2013). However, since the beginning of industrialization and use of fossil fuels, the rate of Hg released to the atmosphere, as Hg (0) and ionic divalent Hg (II) (the oxidized and more reactive form of gaseous Hg (0); Selin et al., 2007), has increased drastically (Fitzgerald et al., 1998; Slemr and Langer, 1992) and is predicted to keep rising (Pacyna et al., 2010). Human activities are responsible for an estimated 70 - 80 % of Hg currently in the atmosphere (Mason et al., 1994), and those that are linked to the release of Hg, either as a by-product or through intentional use, include the extraction and burning of coal and other fossil fuels, as well as other industrial activities (Pacyna et al., 2006). These latter activities include, but are not limited to, the mining or production of certain metals (gold, zinc, copper, and lead), caustic soda, chlorine (chlor-alkali processes), cement, fungicides, pesticides, and waste incineration (Pacyna et al., 2006; UNEP, 2013).

Hg (II) in the atmosphere is mainly deposited locally, whereas Hg (0) has a longer atmospheric lifetime and can either travel tens of thousands of kilometers from its source and/or be oxidized to Hg (II) and then deposited (Mason et al., 1994; Schroeder and Munthe, 1998). Deposition of Hg (II) is primarily into terrestrial soils where it can be slowly released into aquatic environments, part of the first steps

leading to its transformation and uptake into the aquatic food web (section 1.2). However, it can also be deposited directly to aquatic environments from the atmosphere (Mason et al., 1994). Once in aquatic environments, Hg (II) can be reduced to Hg (0) and re-volatilized back to the atmosphere or it can remain in the water, bind with ligands, and settle into the sediment (Mason et al., 1994). Hg (II) can form ligands with anions (OH^-, SO_4^{2-}, Cl^-, S^{2-}, etc.) and dissolved organic matter (DOM) (HsuKim et al., 2013; Ravichandran, 2004). Some of these neutrally charged ligands can be more easily taken up by bacteria that convert the metal to its most toxic form for biota, the organic monomethylmercury (MeHg). This process, methylation, is done primarily by anaerobic sulfate reducing bacteria in the top sediment layer, although it can also be carried out by iron reducing bacteria and periphyton (Compeau and Bartha, 1985; Fleming et al., 2006; Hamelin et al., 2011).

There are different environmental factors that can affect the amount of MeHg available for uptake into organisms at the base of the food web. Methylation is mainly a bacterial process and environmental factors can either alter the Hg (II) available for methylation and/or bacterial productivity (Paranjape and Hall, 2017). DOM present in the water can increase or decrease the bioavailability of Hg (II) depending on its chemical characteristics. Material with higher sulfur content is likely to bind more Hg (II), as Hg is typically found to be associated with sulfhydryl groups (Ravichandran, 2004). When large DOM binds Hg (II), it can decrease the amount available for methylation (bacteria are more likely to take in smaller sized complexes), but when bound to MeHg, it can increase its bioavailability (Paranjape and Hall, 2017). Dissolved oxygen (DO) in water is inversely correlated with

methylation of Hg (II), presumably because anaerobic methylators are more active at lower DO concentrations (Taylor et al., 2012). Similarly, low pH water seems to favour methylation (Ullrich et al., 2001), and increasing chloride ions in water increases the bioavailability of MeHg to *E. coli* (Ndu et al., 2012). Alternatively, there is a negative relationship between sulfide content in sediment pore waters and the availability of Hg (II) for methylation (Benoit et al., 1999).

1.2 Bioaccumulation and biomagnification of MeHg

The bioaccumulation of MeHg happens because it concentrates in animal tissues faster than it can be eliminated because of its tendency to cross cell membranes and bind with thiol groups within amino acids of animal tissues (Wiener et al., 2003; Clarkson et al., 2007). In consumers such as fish, MeHg accumulates at high concentrations compared to Hg (II) and is eliminated from the body more slowly, so the former poses more of a threat (Bloom, 1992). Older, slower growing fish also show higher mercury accumulation than faster growing, younger fish because of their longer exposure period (Sandheinrich and Drevnick, 2016). Consumers are exposed to MeHg primarily through their diet and, because it bioaccumulates, prey to predator transfer of the toxin happens efficiently (Hall et al., 1997). Fish can assimilate a large portion of the MeHg contained in their food (up to 80%) and can even accumulate high concentrations from waterborne exposures in the lab (Wiener et al., 2003).

MeHg biomagnifies, meaning it is found in much higher concentrations in upper-trophic-level animals compared to those lower in the food web (Chumchal et al., 2011). MeHg concentrations increase by an average factor of eight across trophic

levels (Lavoie et al., 2013). The overall percentage of total Hg (THg; both Hg (II) plus MeHg) that is MeHg also increases with trophic level. While sediment THg is found to be 2 – 2.8 % MeHg (Marvin-Dipasquale et al., 2009), primary producers (i.e. algae and seagrasses) typically have 5 – 13 % MeHg, primary consumers (i.e. gastropods) have 33 - 72 % MeHg, and fish typically have > 90 % MeHg (Bloom, 1992; Francesconi and Lenanton, 1992). Since THg is almost all MeHg in fish, THg is often used as a proxy for MeHg.

1.3 Toxicity of MeHg

Fish are often studied when looking at the toxicity of MeHg because of the high mobility of the toxin in aquatic food webs, and because consumption of fish is the primary source of MeHg exposure for humans. MeHg is a neurotoxin that leads to oxidative stress, neuron degradation, and brain lesions in Atlantic salmon parr (*Salmo salar*) exposed to MeHg in their diets (Berntssen et al., 2003). MeHg can also alter the behaviours of some species and cause reduced levels of feeding, swimming activity, and predator avoidance (Berntssen et al., 2003; Wiener et al., 2003; Webber and Haines, 2003). High exposures of MeHg in water (0.93 µg/L) can lead to deformities and mortality in brook trout (*Salvelinus fontinalis*) (McKim et al., 1976). Reproductive effects in fish from MeHg exposure include impaired gonadal development, reduced spawning, and deformities in offspring (McKim et al., 1976; Friedmann et al., 1996). An analysis of toxic effects observed in multiple lab studies of fish led to the development of a toxicity threshold for whole body THg of 0.2 µg/g wet weight (Beckvar et al., 2005). Fish with tissue levels below this threshold are considered to be protected from negative effects of MeHg on growth, reproduction,

development, and behaviour. Toxic effects of MeHg can also be seen in fish-eating wildlife at environmental concentrations. For example, common loons (*Gavia immer*) exposed to high MeHg in Nova Scotia, Canada display reduced reproductive success and adult survival (Burgess and Meyer, 2008).

Beyond toxic effects in wildlife, there is concern for humans who consume fish often. Many communities around the globe, for example Indigenous Peoples in Canada, depend on fish as a protein source for cultural or economic reasons (Berti et al., 1998; Valera et al., 2011). While there are health benefits from eating fish protein, the concerns over its toxicity has led to different regional and national fish consumption advisories to protect human health (Health Canada, 2007; Food and Agriculture Organization and World Health Organization, 2010). In adult humans, where the nervous system is the primary target for Hg toxicity, sensory disturbances, loss of motor skills, slurred speech, and developmental retardation result from non-lethal Hg poisoning (Mergler et al., 2007). Human fetuses and young children are at high risk for damage to the central nervous system, kidneys, cardiovascular system, etc. from Hg toxicity, and there are links to cerebral-palsy or developmental delays (Ronchetti et al., 2006; Brown and Austin, 2012). Since the toxin can be transferred maternally to fetuses there are specific fish consumption guidelines for pregnant women or women of child-bearing age in Canada (Health Canada, 2007).

1.4 Salinity and MeHg

While some environmental variables, such as pH and sulfur species, are known to influence concentrations of MeHg in organisms of aquatic food webs as

previously mentioned, the effect of salinity on MeHg concentrations in organisms is less understood. Lab studies suggest freshwater sediments have greater potential for methylation of Hg (II) into the more toxic form MeHg, compared to more saline sediments from estuaries (Compeau and Bartha, 1984; Compeau and Bartha, 1987; Blum and Bartha, 1980). This suggests that these freshwater sites could be at higher risk of MeHg uptake into food webs. Alternatively, chloride ions which are abundant in seawater compared to freshwater, can also increase MeHg bioavailability. MeHg-chloride ligands (CH_3HgCl) that are neutrally charged can be more bioavailable for uptake into bacteria compared to MeHg on its own (CH_3Hg^{2+}; Ndu et al., 2012).

There is some evidence to support that biota from freshwater sites may be exposed to or accumulate higher concentrations of MeHg than biota from marine environments. van der Velden et al. (2013) found that Canadian Arctic and sub-Arctic lake-dwelling Arctic char (*Salvelinus alpinus*), threespine stickleback (*Gasterosteus aculeatus*), ninespine stickleback (*Pungitius pungitius*), Gammarid amphipods, snails, etc. had significantly higher THg concentrations in their tissues than the same groups sampled from marine environments. Evers et al. (2005) also analyzed a large dataset of THg in birds and found that concentrations were highest in the belted kingfisher and bald eagles from freshwater environments (marine < estuarine < riverine < lakes). On the same note, Fry and Chumchal (2012) measured THg in over 2100 fish of various species from two Louisiana estuaries over five years. They reported that average THg concentrations in fish were 2.4 times higher in the low salinity upper reaches (typically under 3 on the Practical Salinity Scale)

when compared to fish from the higher salinity areas (typically above 15 on the Practical Salinity Scale; Fry, personal communication, July 2017).

MeHg does not always decrease in biota with increasing salinity though. In a lab experiment by Dutton and Fisher (2011), there was a positive correlation between salinity (ranging from 0 to 25 ppt) and MeHg uptake into Killifish (*Fundulus heteroclitus*). Also, in an Alabama estuary Largemouth Bass (*Micropterus salmoides*) had the highest THg concentrations in freshwater sites when compared to bass from higher salinity sites, but the opposite trend was found for southern flounder (Farmer et al., 2010). Evans and Crumley (2005) found no significant relationship between THg concentrations and water salinity for various fish species. However, these authors did not look at freshwater sites for comparison. Taylor et al. (2012) also found that % MeHg increased (the % of THg represented by MeHg) in sediments with increasing salinity. Since the trend of MeHg or THg in sediments or biota along increasing salinity gradients is not consistent and estuaries are important areas for juvenile fish as well as fish consumers, more research in this area would be beneficial.

1.5 Stable isotopes as indicators of diet and residency

Stable isotopes have become a tool used to help identify diet of organisms and linking that diet with MeHg accumulation (Kidd et al., 2012; Clayden et al., 2017). Among other uses, stable isotopes help identify dietary sources and fate of nutrients within a system. Isotopes are forms of the same element that differ in the number of neutrons in their nuclei, making them slightly different in their physical nature. Those elements that vary in neutrons by only a small amount have stable

nuclei and persist in nature, remaining measurable and acting as natural forms of tracers (Fry, 2006). A mixture of these heavy (more neutrons) and light (fewer neutrons) isotopes circulate between inorganic and organic materials in predictable ways, making them useful in tracing the sources of certain materials (Fry, 2006; Hoefs, 1980). The most common elements that are used for isotope studies in ecology are hydrogen, carbon, nitrogen, oxygen, and sulfur. When reporting stable isotopes, the value represents a difference in the isotopic ratio of a sample compared to that of a standard.

$$\delta = [(R_{Sample} / R_{Standard}) - 1] * 1000$$

The R_{Sample} represents the ratio of heavy to light isotope in the sample and $R_{Standard}$ represents the same ratio in the international standard for that element (Hoefs, 1980; Fry, 2006). Examples of some stable isotopes are ^{13}C, ^{12}C, ^{15}N, ^{14}N, ^{34}S and ^{32}S. These transfer in a predictable way from food source to consumer. One use of these ratios of heavy to light isotopes ($^{13}C/^{12}C$, $^{15}N/^{14}N$, and $^{34}S/^{32}S$) is to measure them in various trophic levels of a food web to identify sources of nutrients and diet to biota (Fry, 2006). The ratios are then expressed in δ notation as $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$, respectively, as shown above.

One use of nitrogen isotope values ($\delta^{15}N$) is to assess relative trophic position of animals within a food web. Consumers tend to retain more of the heavier nitrogen isotope compared to the lighter isotope when compared to their prey, so $\delta^{15}N$ values increase (by roughly 3.4‰) with each trophic level (Vander Zanden and Rasmussen, 2001). Since MeHg accumulates in fish primarily through their diets and biomagnifies between trophic levels, and stable nitrogen isotopes are an indicator of

trophic level, they are also used to assess MeHg contamination in aquatic food webs (Lavoie et al., 2013; Kidd et al., 1995). They can quantify biomagnification of MeHg, as $\delta^{15}N$ is positively related to log-MeHg or THg concentrations in lake food webs (Kidd et al., 2012; Clayden et al., 2017). $\delta^{15}N$ values may either increase or decrease with salinity depending on nutrient loading into the system (Fry 2002).

$\delta^{13}C$ values tend to remain similar or become slightly enriched (heavier) from prey to consumer, so when measured in biota, they can help determine the primary carbon sources in their diet (Post, 2002). Primary producers may be enriched or depleted in the heavier ^{13}C isotope, resulting in higher or lower $\delta^{13}C$ values, respectively, depending on the source of their CO_2 for photosynthesis (Fry, 2006). Svensson et al. (2007) used $\delta^{13}C$ values to identify which primary production sources (microphytobenthos, saltmarsh seagrasses, and seston) were fueling temperate estuarine food webs. Carbon isotopes can also be used to trace freshwater or marine influences in animal diets, as there tends to be an increase of $\delta^{13}C$ with increasing salinity. This is reflective of the enriched $\delta^{13}C$ values of marine dissolved CO_2 (Fry, 2002; Fry, 2006).

Like $\delta^{13}C$, $\delta^{34}S$ values are typically only slightly enriched between trophic levels so they are good indicators of dietary sulfur sources (McCutchan et al., 2003). $\delta^{34}S$ values are strong indicators of site-fidelity and residency within estuaries (Fry, 2013; Fry and Chumchal, 2011), as values increase in a predictable way with increasing salinity (Fry, 2002). This is because high sulfate levels in the marine waters have a distinct $\delta^{34}S$ signal from that found in freshwaters (Fry and Chumchal,

2011). Biota that rely on primary production in areas with higher marine sulfate can easily be distinguished from those that rely on freshwater primary production (Fry and Chumchal, 2011). This is useful because when measuring contaminants in biota in the wild, it is important to know if animals are residents of their site of capture and, therefore, whether the exposure is from a local source or not. $\delta^{34}S$ values can also help distinguish which source of primary production fuels aquatic food webs. The distinction can be made between benthic primary production, pelagic primary production or sedimentary detritus, as these sources can have distinct isotope values (Lamontagne et al., 2016; Croisetière et al., 2009).

1.6 Saint John River estuary

The Saint John River estuary is a dynamic tidal system at the mouth of a large river draining over 55,000 km^2 (Kidd et al., 2011; Metcalfe et al., 1976). The estuarine portion of the river primarily runs through the Valley Lowlands ecoregion within the Atlantic Maritime Ecozone (as classified by New Brunswick Ecological Land Classification - NBELC). This region is comprised primarily of mixed wood Acadian forests and shallow glacial sedimentary overburden (Kidd et al., 2011). The estuary is also characterized by various sills, deeper basins, and small islands. The mouth of the river contains a shallow sill and narrow opening that restricts some movement of water into or out of the estuary at Reversing Falls (Metcalfe et al., 1976). The salt wedge that moves upstream with the tides can reach as far as 60 km upriver near Gagetown, NB, in dry summer months, but this varies throughout the

year. In months with higher flows (typically spring) the salt wedge may not progress much further than Reversing Falls (Metcalfe et al., 1976).

The current sources of Hg to the Saint John River include diffuse atmospheric sources, run-off from the land, and industrial or municipal discharges. There is also a natural geologic deposit of Hg on Taylor's peninsula within the Saint John Harbour (Travers, 1976). Most of the direct use of Hg in agricultural applications, chlor-alkali production, gold-mining, pharmaceuticals, and paints in the Canadian Maritimes were phased out in the late 1900's by regulation changes, and by 1995 the main source of Hg to the atmosphere in the region was municipal waste incineration (Sunderland and Chmura, 2000). Fossil fuel combustion is also an important regional source of the metal to the atmosphere (Sunderland and Chmura, 2000).

The only study on THg concentrations in fish in the Saint John River estuary was done by Dadswell (1975). Without reporting the length of the fish, they found that average concentrations of THg within seven of the 15 species were above the established upper limit for human consumption in Canada (0.5 µg/g wet weight (ww); Health Canada, 2007). The average concentrations of the 15 species ranged from 0.07 µg/g ww in alewife (*Alosa pseudoharengus*) to 2.13 µg/g ww in striped bass (*Morone saxatilis*). When these were converted to whole body concentrations ($THg_{whole\ body} = 0.6THg_{fillet}$; Wyn et al. 2009), all but five of the 15 species were above the 0.2 µg/g whole body wet weight toxicity threshold (Beckvar et al., 2005). Individual striped bass (large fish samples ranging from 1 – 12 kg) were as high as 3.82 µg/g THg within the estuary. Given that mercury in fish from the Saint John

River estuary has not been investigated since 1975, additional measurements would be beneficial.

1.7 Objectives

One objective of this research was to examine whether MeHg concentrations change in food webs along a salinity gradient in the Saint John River estuary. I hypothesized that the bioaccumulation of Hg in the food web is regulated by marine influence, as measured by salinity, and predicted that MeHg concentrations (THg in fish) in biota would decrease with increasing salinity. If methylation of Hg is lower in the more saline sites, then it is also expected that the proportion of THg found as MeHg in biota will decrease with increasing salinity. Another objective was to see how various environmental or biological variables relate to MeHg or THg concentrations within fish and invertebrates along the gradient to identify those that may be useful in predicting MeHg. These variables include water salinity, dissolved oxygen, $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values (or derived values), and MeHg from sediment, water, and invertebrates (fish only). While some studies have assessed MeHg in fish from across a range of salinities (Farmer et al., 2010; van der Velden, 2013; Fry and Chumchal, 2012; Taylor et al., 2012; Evans and Crumley, 2005), a consistent trend has not yet been established for MeHg accumulation in food webs along a salinity or marine gradient. This study is an important contribution to investigating the relationship between salinity and MeHg concentrations in estuarine biotic and abiotic compartments and for future studies trying to identify sites in estuaries that may be at higher risk of MeHg toxicity.

1.8 References

Beckvar, N., Dillon, T.M., Read, L.B., 2005. Approaches for linking whole-body fish tissue residues of mercury or DDT to biological effects thresholds. Environ. Toxicol. Chem. 24, 2094-2105.

Benoit, J.M., Mason, R.P., Gilmour, C.C., 1999. Estimation of mercury-sulfide speciation in sediment pore waters using octanol-water partitioning and implications for availability to methylating bacteria. Environ. Toxicol. Chem. 18, 2138-2141.

Berntssen, M., Aatland, A., Handy, R., 2003. Chronic dietary mercury exposure causes oxidative stress, brain lesions, and altered behaviour in Atlantic salmon (*Salmo salar*) parr. Aquatic Toxicology 65, 55-72.

Berti, P.R., Receveur, O., Chan, H.M., Kuhnlein, H.V., 1998. Dietary exposure to chemical contaminants from traditional food among adult dene/metis in the western Northwest Territories, Canada. Environ. Res. 76, 131-142.

Bloom, N.S., 1992. On the chemical form of mercury in edible fish and marine invertebrate tissue. Can. J. Fish. Aquatic Sci. 49(5), 1010–1017.

Blum, J.E., Bartha, R., 1980. Effect of salinity on methylation of mercury. Bull. Environ. Contam. Toxicol. 25, 404-408.

Brown, I.A., Austin, D.W., 2012. Maternal transfer of mercury to the developing embryo/fetus: Is there a safe level?. Toxicol. Environ. Chem. 94, 1610-1627.

Burgess, N.M., Meyer, M.W., 2008. Methylmercury exposure associated with reduced productivity in common loons. Ecotoxicology 17, 83-91.

Chumchal, M.M., Rainwater, T.R., Osborn, S.C., Roberts, A.P., Abel, M.T., Cobb, G.P., Smith, P.N., Bailey, F.C., 2011. Mercury speciation and biomagnification in the food web of Caddo Lake, Texas and Louisiana, USA, a subtropical freshwater ecosystem. Environ. Toxicol. Chem. 30, 1153-1162.

Clarkson, T.W., Vyas, J.B., Ballatori, N., 2007. Mechanisms of mercury disposition in the body. Am. J. Ind. Med. 50, 757-764.

Clayden, M.G., Lescord, G.L., Kidd, K.A., Wang, X., Muir, D.C., O'Driscoll, N.J., 2017. Using sulfur stable isotopes to assess mercury bioaccumulation and biomagnification in temperate lake food webs. Environ. Toxicol. Chem. 661-670.

Compeau, G., Bartha, R., 1984. Methylation and demethylation of mercury under controlled redox, pH, and salinity conditions. Appl. Environ. Microbiol. 48, 1203-1207.

Compeau, G.C., Bartha, R., 1985. Sulfate-reducing bacteria: Principal methylators of mercury in anoxic estuarine sediment. Appl. Environ. Microbiol. 50, 498-502.

Compeau, G.C., Bartha, R., 1987. Effect of salinity on mercury-methylating activity of sulfate-reducing bacteria in estuarine sediments. Appl. Environ. Microbiol. 53, 261-265.

Croisetière, L., Hare, L., Tessier, A., Cabana, G., 2009. Sulphur stable isotopes can distinguish trophic dependence on sediments and plankton in boreal lakes. Freshw. Biol. 54, 1006-1015.

Dadswell, M., 1975. Mercury, DDT and PCB content of certain fishes from the Saint John River estuary, New Brunswick Paper presented to meeting of Canadian Society of Environmental Biologists, Fredericton, New Brunswick.

Dutton, J., Fisher, N.S., 2011. Salinity effects on the bioavailability of aqueous metals for the estuarine killifish *Fundulus heteroclitus*. Environ. Toxicol. Chem. 30, 2107-2114.

Evans, D.W., Crumley, P.H., 2005. Mercury in Florida Bay fish: Spatial distribution of elevated concentrations and possible linkages to Everglades restoration. Bull. Mar. Sci. 77, 321-345.

Evers, D.C., Burgess, N.M., Champoux, L., Hoskins, B., Major, A., Goodale, W.M., Taylor, R.J., Poppenga, R., Daigle, T., 2005. Patterns and interpretation of mercury exposure in freshwater avian communities in northeastern North America. Ecotoxicology 14, 193-221.

Farmer, T.M., Wright, R.A., Devries, D.R., 2010. Mercury concentration in two estuarine fish populations across a seasonal salinity gradient. Trans. Am. Fish. Soc. 139, 1896-1912.

Fitzgerald, W.F., Engstrom, D.R., Mason, R.P., Nater, E.A., 1998. The case for atmospheric mercury contamination in remote areas. Environ. Sci. Technol. 32, 1-7.

Fleming, E.J, Mack, E.E, Green, P.G, Nelson, D.C., 2006. Mercury methylation from unexpected sources: Molybdate-inhibited freshwater sediments and an iron-reducing bacterium. Appl. Environ. Microbiol. 72(1), 457–464.

Food and Agriculture Organization, World Health Organization, 2010. Joint FAO/WHO expert consultation on the risks and benefits of fish consumption.

Report 978, 1-49. http://www.fao.org/docrep/014/ba0136e/ba0136e00.pdf. Accessed February 2, 2017.

Francesconi, K.A., Lenanton, R.C.J., 1992. Mercury contamination in a semi-enclosed marine embayment: Organic and inorganic mercury content of biota, and factors influencing mercury levels in fish. Mar. Environ. Res. 33, 189-212.

Friedmann, A.S., Watzin, M.C., Brinck-Johnsen, T., Leiter, J.C., 1996. Low levels of dietary methylmercury inhibit growth and gonadal development in juvenile walleye (*Stizostedion vitreum*). Aquatic Toxicology 35, 265-278.

Fry, B., 2002. Conservative mixing of stable isotopes across estuarine salinity gradients: A conceptual framework for monitoring watershed influences on downstream fisheries production. Estuaries 25, 264-271.

Fry, B., 2006. Stable Isotope Ecology. Springer, New York, NY.

Fry, B., Chumchal, M.M., 2011. Sulfur stable isotope indicators of residency in estuarine fish. Limnol. Oceanogr. 56, 1563-1576.

Fry, B., Chumchal, M.M., 2012. Mercury bioaccumulation in estuarine food webs. Ecol. Appl. 22, 606-623.

Fry, B., 2013. Using stable CNS isotopes to evaluate estuarine fisheries condition and health. Isot. Environ. Health Stud. 49, 295-304.

Hall, B., Bodaly, R., Fudge, R., Rudd, J., Rosenberg, D., 1997. Food as the dominant pathway of methylmercury uptake by fish. Water Air Soil Pollut. 100(1-2), 13-24.

Hamelin, S., Amyot, M., Barkay, T., Wang, Y.P., Planas, D., 2011. Methanogens: Principal methylators of mercury in lake periphyton. Environ. Sci. Technol. 45(18), 7693–7700.

Health Canada, 2007. Human Health Risk Assessment of Mercury in Fish and Health Benefits of Fish Consumption. 1–70. http://www.hc-sc.gc.ca/fn-an/alt_formats/hpfb-dgpsa/pdf/nutrition/merc_fish_poisson-eng.pdf. Accessed February 2, 2017.

Hoefs, J., 1980. Stable Isotope Geochemistry, 2nd edition. Springer-Verlag, New York, NY.

Hsu-Kim, H., Kucharzyk, K.H., Zhang, T., Deshusses, M.A., 2013. Mechanisms regulating mercury bioavailability for methylating microorganisms in the aquatic environment: A critical review. Environ. Sci. Technol. 47, 2441-2456.

Kidd, K.A., Hesslein, R.H., Fudge, R.J.P., Hallard, K.A., 1995. The influence of trophic level as measured by delta-N-15 on mercury concentrations in fresh-water organisms. Water Air Soil Pollut. 80(1-4), 1011-1015.

Kidd, K.A., Muir, D.C.G., Evans, M.S., Wang, X., Whittle, M., Swanson, H.K., Johnston, T., Guildford, S., 2012. Biomagnification of mercury through lake trout (*Salvelinus namaycush*) food webs of lakes with different physical, chemical and biological characteristics. Sci. Total Environ. 438, 135-143.

Kidd, S.D., Curry, R.A., Munkittrick, K.R., 2011. The Saint John River: A State of the Environment Report, 1–166.

Lamontagne, S., Deegan, B.M., Aldridge, K.T., Brookes, J.D., Geddes, M.C., 2016. Fish diets in a freshwater-deprived semiarid estuary (The Coorong, Australia) as inferred by stable isotope analysis. Estuar. Coast. Shelf Sci. 178, 1-11.

Lavoie, R.A., Jardine, T.D., Chumchal, M.M., Kidd, K.A., Campbell, L.M., 2013. Biomagnification of mercury in aquatic food webs: A worldwide meta-analysis. Environ. Sci. Technol. 47, 13385-13394.

Marvin-Dipasquale, M., Lutz, M.A., Brigham, M.E., Krabbenhoft, D.P., Aiken, G.R., Orem, W.H., Hall, B.D., 2009. Mercury cycling in stream ecosystems. 2. Benthic methylmercury production and bed sediment - Pore water partitioning. Environ. Sci. Technol. 43, 2726-2732.

Mason, R.P., Fitzgerald, W.F., Morel, F.M.M., 1994. The biogeochemical cycling of elemental mercury: Anthropogenic influences. Geochim. Cosmochim. Acta 58, 3191-3198.

McCutchan Jr., J.H., Lewis Jr., W.M., Kendall, C., McGrath, C.C., 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos 102, 378-390.

McKim, J.M., Olson, G.F., Holcombe, G.W., Hunt, E.P., 1976. Long term effects of methylmercuric chloride on three generations of brook trout (*Salvelinus fontinalis*): Toxicity, accumulation, distribution, and elimination. J. Fish. Res. Board Canada 33, 2726-2739.

Mergler, D., Anderson, H.A., Chan, L.H.M., Mahaffey, K.R., Murray, M., Sakamoto, M., Stern, A.H., 2007. Methylmercury exposure and health effects in humans: A worldwide concern. Ambio 36, 3-11.

Metcalfe, C.D., Dadswell, M.J., Gillis, G.F., Thomas, M.L.H., 1976. Physical, chemical, and biological parameters of the Saint John River Estuary, New Brunswick, Canada. Environment Canada Technical Report number 686, 1-41.

Ndu, U., Mason, R.P., Zhang, H., Lin, S., Visscher, P.T., 2012. Effect of inorganic and organic ligands on the bioavailability of methylmercury as determined by using a mer-lux bioreporter. Appl. Environ. Microbiol. 78, 7276-7282.

Pacyna, E.G., Pacyna, J.M., Steenhuisen, F., Wilson, S., 2006. Global anthropogenic mercury emission inventory for 2000. Atmos. Environ. 40, 4048-4063.

Pacyna, E.G., Pacyna, J.M., Sundseth, K., Munthe, J., Kindbom, K., Wilson, S., Steenhuisen, F., Maxson, P., 2010. Global emission of mercury to the atmosphere from anthropogenic sources in 2005 and projections to 2020. Atmos. Environ. 44, 2487-2499.

Paranjape, A.R., Hall, B.D., 2017. Recent advances in the study of mercury methylation in aquatic systems. Facets 2, 85-119.

Post, D.M., 2002. Using stable isotopes to estimate trophic position: Models, methods, and assumptions. Ecology 83, 703-718.

Ravichandran, M., 2004. Interactions between mercury and dissolved organic matter - A review. Chemosphere 55, 319-331.

Ronchetti, R., Zuurbier, M., Jesenak, M., Koppe, J.G., Ahmed, U.F., Ceccatelli, S., Villa, M.P., 2006. Children's health and mercury exposure. Acta Paediatr. Int. J. Paediatr. 95, 36-44.

Sandheinrich, M.B., Drevnick, P.E., 2016. Relationship among mercury concentration, growth rate, and condition of northern pike: A tautology resolved?. Environ. Toxicol. Chem. 35, 2910-2915.

Schroeder, W.H., Munthe, J., 1998. Atmospheric mercury - An overview. Atmos. Environ. 32, 809-822.

Selin, N.E., Jacob, D.J., Park, R.J., Yantosca, R.M., Strode, S., Jaeglé, L., Jaffe, D.A., 2007. Chemical cycling and deposition of atmospheric mercury: Global constraints from observations. J. Geophys. Res. 112, D02308.

Slemr, F., Langer, E., 1992. Increase in global atmospheric concentrations of mercury inferred from measurements over the Atlantic Ocean. Nature 355(6359), 434-437.

Sunderland, E., Chmura, G., 2000. An inventory of historical mercury emissions in Maritime Canada: implications for present and future contamination. Sci. Total Environ. 256, 39-57.

Svensson, C.J., Hyndes, G.A., Lavery, P.S., 2007. Food web analysis in two permanently open temperate estuaries: Consequences of saltmarsh loss? Mar. Environ. Res. 64, 286-304.

Taylor, D.L., Linehan, J.C., Murray, D.W., Prell, W.L., 2012. Indicators of sediment and biotic mercury contamination in a southern New England estuary. Mar. Pollut. Bull. 64, 807-819.

Travers, I., 1976. Mercury in the sediments of the Saint John River basin. Environmental Protection Service Report, 1-53.

Ullrich, S.M., Tanton, T.W., Abdrashitova, S.A., 2001. Mercury in the aquatic environment: A review of factors affecting methylation. Crit. Rev. Environ. Sci. Technol. 31, 241-293.

UNEP. 2013. Global mercury assessment 2013: Sources, emissions, releases and environmental transport. UNEP Chemicals Branch, Geneva, Switzerland.

Valera, B., Dewailly, E., Poirier, P., 2011. Impact of mercury exposure on blood pressure and cardiac autonomic activity among Cree adults (James Bay, Quebec, Canada). Environ. Res. 111, 1265-1270.

van der Velden, S., Dempson, J.B., Evans, M.S., Muir, D.C.G., Power, M., 2013. Basal mercury concentrations and biomagnification rates in freshwater and marine food webs: Effects on Arctic charr (*Salvelinus alpinus*) from eastern Canada. Sci. Total Environ. 444, 531-542.

Vander Zanden, M.J., Rasmussen, J.B., 2001. Variation in d15N and d13C trophic fractionation: Implications for aquatic food web studies. Limnol. Oceanogr. 46, 2061-2066.

Webber, H.M., Haines, T.A., 2003. Mercury effects on predator avoidance behaviour of a forage fish, golden shiner (*Notemigonus crysoleucas*). Environ. Toxicol. Chem. 22, 1556-1561.

Wiener, J.G., Krabbenhoft, D.P., Heinz, G.H., Scheuhammer, A.M., 2003. Ecotoxicology of mercury. In Hoffman DJ, Rattner BA, Burton GA, Cairns J, eds, Handbook of Ecotoxicology, 2nd ed. CRC Press, Boca Raton, FL, USA, 409–463.

Wyn, B., Kidd, K.A., Burgess, N.M., Curry, R.A., 2009. Mercury biomagnification in the food webs of acidic lakes in Kejimkujik National Park and National Historic Site, Nova Scotia. Can. J. Fish. Aquatic Sci. 66, 1532-1545.

2 Mercury in fish and invertebrates in the Saint John River Estuary

2.1 ABSTRACT

The spatial trends of MeHg in biota from fresh to marine waters are poorly understood. The objective of the current research was to determine if MeHg concentrations in biota decrease along salinity gradients in estuaries. Fourspine stickleback (*Apeltes quadracus*), invertebrates (snails, amphipods, and chironomids), sediments and water were collected from ten sites along the Saint John River estuary, New Brunswick, Canada, in 2015 and 2016. Total mercury was measured in whole fourspine stickleback and MeHg was measured in pooled invertebrates, sediments, and water. Stable sulfur ($\delta^{34}S$), carbon ($\delta^{13}C$), and nitrogen isotope values ($\delta^{15}N$) were also measured, to help establish the relationship of diet to salinity and Hg measures. Salinity of sites ranged from 0.06 to 6.96. There was an increasing trend in MeHg with salinity in chironomids, but no correlations were seen for other sample types. Salinity therefore did not exhibit a consistent relationship with MeHg in biotic and abiotic compartments of this estuary.

2.2 Introduction

Mercury (Hg) is one of the most common toxins that can affect freshwater fish and fish consumers in North America. Methylmercury (MeHg), the organic form of the metal, is a neurological and reproductive toxin that bioaccumulates to high concentrations in animal tissues (Wiener et al., 2003; Berntssen et al., 2003; Friedmann et al., 1996). This means that it concentrates in tissues, such as muscle in fish, faster than it is excreted. MeHg enters animals primarily through their diet

(Hall et al., 1997), and it also biomagnifies with each increasing trophic level in aquatic systems (Chumchal et al., 2011). This puts top consumers, including humans, at the highest risk for MeHg toxicity.

While MeHg is often found at very high concentrations in fish, it can be much lower in marine than freshwater species (Zitko et al., 1971; Luten et al., 1980). There have been broader spatial studies assessing Hg in biota in different habitats within estuaries (van der Velden et al., 2013; Fry and Chumchal, 2012; Evans and Crumley, 2005), or focussed on intensive sampling within an estuary along a salinity gradient (Buckman et al., 2017; Taylor et al., 2012; Farmer et al., 2010). There has not been a consistent trend observed with MeHg increasing or decreasing with estuarine salinity gradients in biota. Some studies have found that THg concentrations (a proxy for MeHg in fish) in wild fish are negatively correlated to increasing salinity (Fry and Chumchal, 2012; van der Velden et al., 2013). Similarly, sediments collected from estuarine sites with lower salinities have been shown to have higher Hg methylation rates, producing more MeHg when compared to freshwater sites (Compeau and Bartha, 1984; Compeau and Bartha, 1987; Blum and Bartha, 1980). Other studies have found that there is no relationship between THg in wild fish and salinity in estuaries (Evans and Crumley, 2005), or that they are positively correlated (Dutton and Fisher, 2011; Farmer et al., 2010). The variable results of these studies likely reflect a complex relationship between MeHg availability to biota and various biogeochemical factors within estuaries (Buckman et al., 2017).

Stable isotopes are used to identify diet and trace MeHg biomagnification through food webs (Clayden et al. 2017; Kidd et al. 2012). Nitrogen isotope values (measured as $\delta^{15}N$) are used in aquatic systems to assess relative trophic level of consumers, because they retain more of the heavier isotope, increasing by 3.4 ± 0.98‰ with each level (Post, 2002). Because MeHg also increases with trophic level, $\delta^{15}N$ values can be used to predict MeHg concentrations in aquatic food webs and quantify its biomagnification (Kidd et al., 2012; Clayden et al., 2017). Carbon isotopes values ($\delta^{13}C$) identify which primary producers (grass, algae, etc.) are fuelling aquatic food webs (Fry, 2006; Svensson et al., 2007). $\delta^{13}C$ values increase by 0.39 ± 1.3‰ in consumers compared to their food (Post, 2002). Carbon isotopes can also indicate whether animals have been feeding in freshwater or marine environments as $\delta^{13}C$ values increase with salinity in estuaries, reflecting the enriched $\delta^{13}C$ of marine CO_2 (Fry, 2002; Fry, 2006). Sulfur isotope values ($\delta^{34}S$) in fish are also positively correlated with salinity in estuaries, as marine sulfur has a distinctly enriched $\delta^{34}S$ signal compared to freshwater sources. Measuring these isotopes can therefore also help distinguish whether animals have been feeding in marine or freshwater food sources in estuaries (Fry, 2002), as consumer values will be similar to their diet (very little fractionation; McCutchan et al., 2003). For this reason, $\delta^{34}S$ values can identify whether fish caught at sites of specific salinities are residents or if they feed elsewhere (Fry, 2013; Fry and Chumchal, 2011).

This study in the Saint John River estuary investigated concentrations of Hg in fish along a salinity gradient. I hypothesized that marine influence, represented by salinity, is related to Hg bioaccumulation in aquatic food webs and predicted a

negative correlation between salinity and MeHg (THg in fish). If this prediction is true, then freshwater fish are at higher risk of mercury toxicity than fish with more marine influence in their diet. It is important to identify which fish may be exposed to fewer toxins within their environment and, therefore, which would be safest for fish-eating wildlife and humans to consume. Sites ranging from freshwater to brackish were sampled for fish, various invertebrate taxa, sediments, and water in the Saint John River estuary in 2015 and 2016 to assess the MeHg levels within the food web.

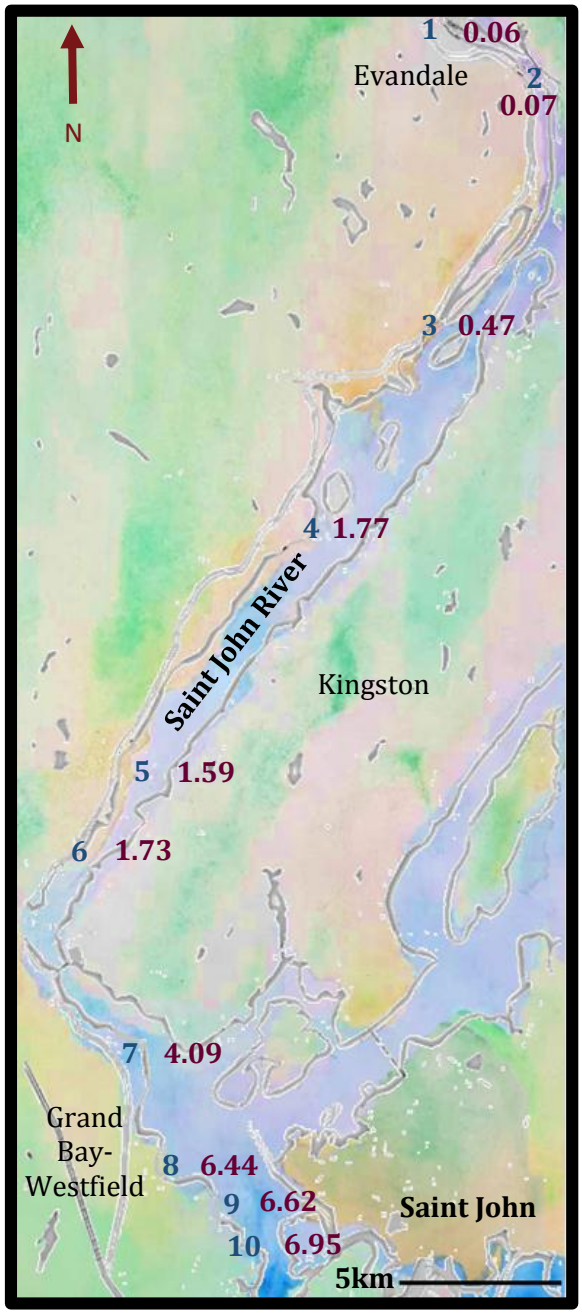

Fig. 1 – Original watercolor painting of ten sites sampled and their average salinities along the Saint John River estuary.

2.3 Methods

2.3.1 Study Area

The Saint John River is a very large system with a drainage area greater than 55,000 km^2 that opens up to the Saint John Harbour, at Reversing Falls in Saint John, New Brunswick, Canada (Kidd et al., 2011; Metcalfe et al., 1976). The Bay of Fundy, where the Saint John Harbour is located, exhibits some of the highest tides in the world (up to

8 m in the Saint John Harbour) and the river is tidal up to Fredericton, New Brunswick (135 km upstream; Kidd et al., 2011). The salt wedge extends as far as 60 km upstream of Reversing Falls in dry summer months and there is a continuous salinity gradient from freshwater toward the mouth of the river where the water is brackish (Metcalfe et al., 1976). The Saint John River estuary hosts around 35 species of fish (DFO, 2009). Travers (1976) documented THg concentrations in sediments throughout the watershed and Dadswell (1975) found elevated concentrations of THg in large predatory fish such as Striped Bass (*Morone saxatilis*), Yellow Perch (*Perca flavescens*), and Chain Pickerel (*Esox niger*).

2.3.2 Field Collections

Ten sites within the Saint John River estuary were selected along the salinity gradient ranging from freshwater to more marine influenced, or brackish water near the mouth of river (Fig. 1). The sites were located on beaches conducive to seining and were between two and eight km apart. Within a three-hour window after the Saint John high tide, the water quality (dissolved oxygen, conductivity, and temperature) was measured during sampling in both 2015 and 2016 at 0.75 m below the surface using a calibrated YSI Multi-Meter Model 85. From July 26 to September 4, 2016, HOBO Conductivity Data Loggers were deployed at each site for 24 hours at a time, taking measurements every five minutes, for two neap tide and three spring tide cycles. The loggers were deployed within a three-day window surrounding the peak tide time and were located 50 cm above the substrate at a total depth of 0.5 – 1.5 m (depending on the tide's height). Salinity (Practical Salinity Scale) was used as a proxy of marine influence within the sampled habitats and was calculated from each

conductivity reading using a calculator created by Weinkauf (2015), based on algorithms outlined by Unesco/ICES/SCOR/IAPSO (1981), and assuming a pressure of 101,325 Pa. Salinity data between the 10th and 90th percentiles for each site were used for analysis to remove data extremes, and these were confirmed to be statistically different along the gradient using an Analysis of Variance (ANOVA) and Tukey's Post Hoc Tests. The means of these 10 – 90th percentile data within each site was used for subsequent analysis. The 2016 values were used in the analysis of 2015 data, too, as there were not sufficient salinity data collected in 2015.

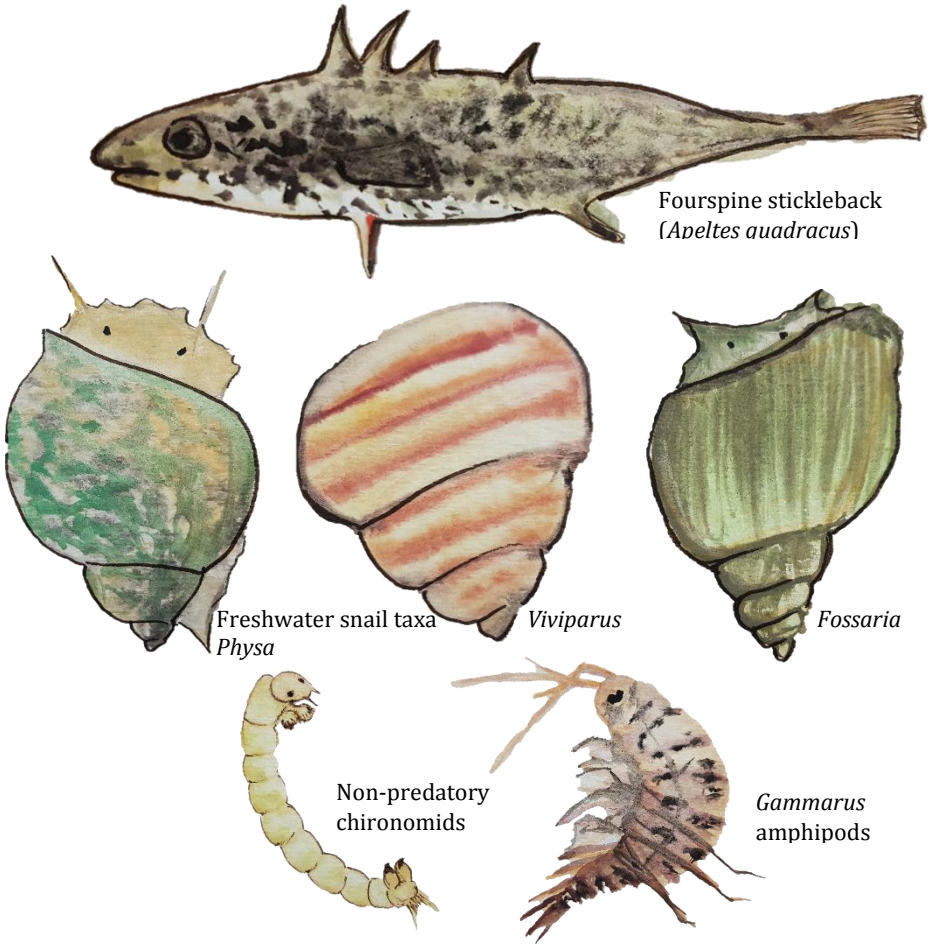

Fig. 2 – Original watercolor paintings of the taxa collected from the Saint John River estuary.

Seine nets were used to collect fish from each site (Fig. 2). Fourspine stickleback (*Apeltes quadracus*; 7 - 10 fish per site) were weighed, measured, euthanized using MS-222 and spinal severance according the University of New Brunswick's Animal Care protocol (MS-222 had no detectable THg; data not shown), and frozen within a few hours of collection. This species was found at each site. Fish collections were all done within a three-hour window after high tide at the mouth of the river, and occurred between August and October 2015, and in August of 2016. Fin clips were taken from the caudal fin of each fish and stored in 95% ethanol in a freezer for subsequent genetic analysis.

Invertebrate collections at each site were done using sweep nets, kick nets, and rock-picking. Snails (genus *Physa, Fossaria, and Viviparus*) and amphipods (*Gammarus*), which were common invertebrates found at each site, were collected in August 2015 and were limited to one pooled sample per site for both amphipods and snails (Fig. 2). Snails were collected at 9 of the 10 sites in 2015 (absent from site 9). On three separate dates in July – August 2016, snails and amphipods were collected (three pooled samples per site; 1 per site per date). Chironomids were collected over several dates through this period in 2016 to gain enough mass for analysis and pooled into one sample per site (Chironomidae excluding predatory family Tanypodinae). The invertebrates were placed on ice immediately after collection and were frozen within 5 hours until used for further analysis.

During one of the amphipod collections, enough mass was collected to create two samples. One sample of amphipods was placed in a Whirl-pak® bag and frozen

within hours of collection while the others were kept alive in aerated water (from the site of collection) overnight to presumably clear their guts. This was done to compare the isotope and mercury endpoints measured in gut-cleared amphipod samples versus non-cleared samples, as the latter were mainly used in this study.

At each site on August 30, 2016, the top five cm of sediment was sampled in triplicate using an Ekman dredge and core tubes that were precleaned with 5% nitric acid and site water before each sample, put into Ziploc bags, and frozen. Surface water samples were also collected in pre-cleaned one litre amber bottles in triplicate from August 6 to September 2, 2016 at each site. These were collected roughly ten cm below the surface with powder-free gloves, filtered with 0.45 micron polyestersulfonate filters within a few hours of collection, preserved with 1% concentrated trace-metal grade sulfuric acid and stored at 4°C until analyzed. Field blanks (three) of ultra-pure water were also taken into the field, opened with gloves, closed, and returned to the lab. A separate water sample was also collected from each site between the dates of August 29 to September 2, 2016 with the same methods, and was submitted to an analytical lab (RPC in Fredericton, New Brunswick) for additional water chemistry analysis.

2.3.3 Laboratory Processing

All glass vials used for sample processing and analysis were soaked in a 5% nitric acid bath and rinsed with ultrapure water. Petri dishes, glass rods, cutting boards, ceramic knives, and other utensils used for sample processing were rinsed with 5% nitric acid, ultrapure water, and dried with Kimwipes® between samples.

In the lab fish were dissected and their intestinal tracts were removed before THg and isotope analysis. Gut contents were examined qualitatively using a dissecting microscope to identify prey items. The whole body of each fish, minus the intestinal tract, was placed into a pre-weighed vial and freeze-dried using a Labconco Freezone 12 for 48 hours.

Fin clips collected in 2015 across all sites (93 of 96 fin clips were successful) were genetically verified to be from *Apeltes quadracus*. Polymerase chain reactions (PCR) were done using primers VF2 and FishR2 (Integrated DNA Technologies) to sequence the CO1 barcode region of the DNA. PCRs were Sanger sequenced by Genome Québec at McGill University in Montréal, Québec, Canada. This analysis was not repeated in 2016 because field identifications from 2015 were confirmed to be 100% accurate.

Snails and amphipods were identified to genus. Chironomids were partially thawed and predatory taxa were removed from the sample (family Tanypodinae). For each collection date and site, individuals of a taxon were pooled to obtain enough mass for all chemical analyses (minimum 30 mg dry weight). Within a pooled sample, n = 33 – 580, 4 – 15, and 69 – 565, for amphipods, snails, and chironomids, respectively. All pooled samples were placed into glass vials and freeze dried for 48 hours. Samples were weighed before and after freeze drying to calculate moisture content and then homogenized using glass rods (invertebrates) or a ceramic knife on a glass cutting board (whole body fish).

2.3.4 Hg Analysis

THg was analyzed at the University of New Brunswick Saint John using a Milestone Direct Mercury Analyzer. This analysis was done using 10 mg of homogenized, individual dried fish samples. Wet weight THg concentrations were calculated using moisture content of individual fish. Quality assurance and quality control (QAQC) included blanks, duplicates, and various standards/certified reference materials (CRM) (intra-lab standard of dried fish tissue, 10 ng liquid Hg standard and DORM-4 fish protein CRM from National Research Council of Canada). The mean percent recovery of the CRM was 90 ± 3% (n = 28) and for the 10 ng liquid Hg standard was 98 ± 7% (n = 28). The mean concentration of THg in method blanks, assuming a 10 mg sample, was 1.15 ± 1.25 µg/kg dw (n = 28). Average precision was within 14% (n = 20) for duplicate samples, with an overall method detection limit of 3.75 µg/kg. The limit of detection was three times the standard deviation of blanks. All concentrations of THg and MeHg are reported as dry weight unless otherwise stated.

MeHg and Hg (II) analysis (Hg (II) for 2016 samples only) was done for a subset of individual fish (n = 9 in 2015, 3 fish each from sites 1, 6, and 8; n = 30 in 2016, 3 from each of the 10 sites) and for all invertebrate, sediment, and water samples at the Environmental Biogeochemistry Lab at Acadia University (Wolfville, NS) using a Brooks Rand automated MERX system with a Model III fluorescence detector. The methods used were adapted from the US EPA Method 1630 and Brooks Rand Analytical Notes. Digested samples (see below for details) were analyzed through aqueous-phase ethylation, purge-and-trap gas chromatography prior to atomic fluorescence spectrometry. THg was calculated by adding MeHg and

Hg (II) values together for 2016 samples. The relative percent difference of these THg values and the values obtained from the Direct Mercury Analyzer for the 2016 fourspine stickleback was 25 ± 14%. The QAQC included method blanks, internal standards, CRM, duplicates, and spiked samples (for water only).

Dried animal tissues (10 mg) were digested in a 25% KOH in methanol (MeOH) solution. All samples were shaken for one hour, heated at 95 °C for one hour and cooled overnight at room temperature. An aliquot of 20 – 40 µL of each digested sample was analyzed for MeHg and Hg (II) (in 2016 only). Mean percent recovery of MeHg in CRM (DOLT-5 and DORM-4 from National Research Council of Canada), 50 ng standards, and concentrations of calibration blanks were 105 ± 13% (n = 11), 105 ± 7% (n = 20), and 7.98 x 10^{-6} µg/kg dw (n =43), respectively. Mean precision was within 13% (n = 9) for duplicate samples, with method detection limits between 0.74 - 1.62 pg for the separate runs. The limits of detection were 3 times the standard deviation of blanks for all analyses. For Hg (II), CRM DORM-4, standards, and calibration blanks had mean recoveries/concentrations of 105 ± 12% (n = 5), 86 ± 11% (n = 13), and 8.22 x 10^{-6} µg/kg dw (n = 18), respectively. Mean precision for Hg (II) duplicate measurements was within 22% (n = 7), and the limits of detection were 0.92 and 1.29 pg for the separate runs.

Water samples (20 mL) were analyzed for MeHg using the same methods with a pH adjustment to 4.5 with 25% KOH to ensure efficiency of the 2 M acetate buffer and ethylating agent (Klapstein et al., 2016). Calibration blanks, field blanks, and standards had mean concentrations/recoveries of 0.02 ± 0.02 ng/L (n = 17),

0.03 ± 0.02 ng/L (n = 3), and 106 ± 7% (n = 10), respectively. MeHg concentrations were corrected for spike recoveries if they fell outside of the 80 – 120% range, and this was done for two of the sample measurements (average recovery 100 ± 11%, n = 1/sample). Average precision was within 3% (n = 3) for duplicate samples, with an overall method detection limit of 0.72 pg. Samples that fell below this value were assigned random numbers between zero and the detection limit for the analysis. This was the case for two samples from site 4 and one of the samples from site 3.

Sediments were digested in 1.5 M KBr in 5% H_2SO_4 and 1 M $CuSO_4$, then analyzed for MeHg using the same methods previously described. Calibration blanks, certified reference material (ERMCC580, European Reference Materials), and standards had mean concentrations/recoveries of 8.7 x 10^{-6} µg/kg (n = 13), 71 ± 4% (n = 3), and 100 ± 2% (n = 5), respectively. All sediment MeHg concentrations were corrected for CRM recoveries. Average precision was within 20% (n = 3) for duplicate samples, with an overall method detection limit of 0.98 pg.

2.3.5 Stable Isotope Analysis

Carbon and nitrogen stable isotope ratios were measured in all fish, invertebrate, and sediment samples on a Costech 4010 elemental analyzer coupled with a Thermo-Finnigan DeltaPlus isotope ratio mass spectrometer at the at the Stable Isotopes in Nature Laboratory (SINLAB), University of New Brunswick in Fredericton, NB. In 2015, $\delta^{34}S$ analyses for the samples were analyzed in the SINLAB as well, and in 2016 they were done by the GG Hatch – Stable Isotope Laboratory using an Elementar Micro Cube Elemental Analyser coupled with a DeltaPlus XP

isotope ratio mass spectrometer. A subset of 2015 samples were re-run in 2016 for $\delta^{34}S$ to make an inter-lab comparison (mean absolute difference in measurements among labs was 0.58 ± 0.37‰ (n = 12)). Isotopic ratios reported are in the 'delta δ notation' using the equation:

$$\delta X = [(R_{sample} / R_{standard}) - 1] \times 1000$$

where R is the ratio of heavy to light isotope of each element ($^{13}C/^{12}C$, $^{15}N/^{14}N$, and $^{34}S/^{32}S$) either within the sample or an international standard for each separate element. For C, N, and S the international standards are PeeDee Belemnite, air, and Canyon Diablo Troilite, respectively. Dried, homogenized samples were weighed into tin capsules and analyzed. Approximately 1 mg and 4 - 5 mg of animal tissues were used for $\delta^{13}C/\delta^{15}N$ analysis and $\delta^{34}S$ analysis, respectively. Approximately 20 mg of sediment was used for all isotope analyses. The laboratory standards reported from the SINLAB for $\delta^{13}C/\delta^{15}N$ analysis were ammonium sulfate with a known value of 20.3‰ and mean measurement of 20.45 ± 0.09‰ (n = 7), and polyethylene foil with a known value of -32.15‰ and a mean measurement of -32.19 ± 0.05‰ (n = 5, both certified by International Atomic Energy Agency). For $\delta^{34}S$ analysis they were an in-house Bass Check Standard with a known value of 3.8‰ and mean measurement of 3.85 ± 0.58‰ (n = 12)), and Pollock Sulphur Standard with a known value of 17.9‰ and mean measurement of 17.42 ± 0.57‰ (n = 16)). The Hatch Lab used an in-house Silver Sulfide standard with an expected value of -0.65‰ and mean measurements between -0.42 ± 0.31‰ and -0.78 ± 0.18‰ (n = 24). The mean absolute difference between duplicates was 0.21 ± 0.26‰ (n = 30)

for $\delta^{15}N$, 0.27 ± 0.60‰ for $\delta^{13}C$ (n = 30), 0.47 ± 0.36‰ for $\delta^{34}S$ (n = 11) at the SINLAB and 0.68 ± 0.59‰ for $\delta^{34}S$ (n = 21) at the Hatch Lab.

2.3.6 Data Analyses

Some data were adjusted or derived for subsequent analysis. THg data were length adjusted within sites because THg accumulation in fish increases with size (Evans et al., 2005), and there were significantly different lengths of fish among sites ($p < 0.001$). The residuals from the THg-length regression relationship for each year were added onto the mean THg per site, giving the new variable THg-adj (as in Swanson and Kidd, 2010). These values were used in the subsequent analyses for fish. The biomagnification factor (BMF), which is the ratio of Hg found in a consumer to that in its diet (Gobas et al. 2009), was also calculated for fourspine stickleback THg, using amphipod MeHg values to represent consumed food. This is assuming that they are consuming 100% amphipods, for simplicity's sake.

Trophic position (TP) was also calculated using the $\delta^{15}N$ values of animals and sediments. The equation to calculate TP was $\lambda + (\delta^{15}N_{consumer} - \delta^{15}N_{base})/\Delta_n$ (Post 2002). The consumer is the organism you are determining the trophic position of, base is the food source, Δ_n is the enrichment of $\delta^{15}N$ from food source to consumer and λ is the trophic position of the base used. The enrichment factor used was 3.4‰ (Post 2002), average $\delta^{15}N$ values across all invertebrates within each site ($\lambda = 2$) were used as the diet base for fourspine stickleback, and average sediment values ($\lambda = 1$) were used as a base for invertebrate TP.

The final derived value was the percentage of the fourspine stickleback diet that was marine based on its sulfur content. A simple two-end-member mixing model, assuming no fractionation, was used with $\delta^{34}S$, and the end members were freshwater and brackish invertebrates as food sources for fish, since these have distinct $\delta^{34}S$ values. The average $\delta^{34}S$ values of all invertebrates from the most freshwater site (0.06 salinity) and from the most saline site (6.96 salinity) were used. The equation that was used for % Marine was:

$$(\delta^{34}S_{consumer} - \delta^{34}S_{freshwater\ invertebrates})/(\delta^{34}S_{marine\ invertebrates} - \delta^{34}S_{freshwater\ invertebrates})*100$$

(example with $\delta^{13}C$ in the introduction of Post, 2002). This new variable called % Marine was used in place of raw $\delta^{34}S$ data for fourspine stickleback statistics only. It was not calculated for invertebrates as there was no food source $\delta^{34}S$ data and sediment data did not follow a similar trend as for the animals. Any values of % Marine that were below zero were rounded up to zero and any values above 100% were rounded down to 100% for statistical analysis.

ANOVA and Tukey's Post-Hoc tests were used to look for among-site differences in THg-adj in fish (n = 7-10/site) and MeHg in snails (n = 3-4/site excluding *Viviparus* snails from sites 1 and 2; see explanation in paragraph below), amphipods, sediments, and water (n = 3-4/site). This and subsequent analysis for fish were done separately for each year because there was a significant interaction between year and site ($p = 0.03$). T-tests were used within sites for fish to assess among-year differences. One amphipod sample from site 1 in 2016 had an abnormally high concentration of Hg (II) (roughly 4 times higher than other

samples) and an abnormally low concentration of MeHg (roughly 10 times lower than other samples). Contamination was suspected and the sample was excluded from subsequent analysis. Assumptions of normality and homoscedasticity of all ANOVA models were examined and data were determined to be approximately normal. Alpha was set to 0.05. Paired Welch two sample t-tests were also used to identify differences between Hg or isotope measures in gut cleared versus non-gut cleared amphipods.

Finally, Pearson's correlation matrices were used to examine relationships between various independent variables and THg-adj in fish or MeHg in other biota among sites. The independent variables in the matrices included salinity, DO, % Marine (fish only) or $\delta^{34}S$, TP (fish only) or $\delta^{15}N$, $\delta^{13}C$, average invertebrate MeHg (fish only), and for 2016 data average sediment MeHg, average water MeHg, Hg (II), and % MeHg were included. All comparisons among measures that were paired for an individual sample (i.e. various Hg measures and isotope values measured in the same fish) were done using raw data, whereas correlations including measures with just one value per site (salinity, DO, average invert MeHg, etc.) were done using within site mean Hg data. Any pooled samples of the genus *Viviparus* for snails were excluded from these correlations because they had mean MeHg concentrations roughly five times higher than the mean of other snail taxa, likely due to differing life histories. *Viviparus* were only collected at sites 1 and 2 in 2016, so only these data were excluded from subsequent analyses.

2.4 Results

2.4.1 Hg concentrations

From highest to lowest average concentrations of THg (fish) or MeHg (other samples), the order was fourspine stickleback > amphipods > snails (excluding *Viviparus*) > chironomids > sediments > water. Even after length adjusting the fish data, THg concentrations in 2016 (mean 689 ± 18 µg/kg; mean length 4.51 ± 0.04 cm) were consistently higher than they were in 2015 (mean 428 ± 25 µg/kg; mean length 4.24 ± 0.17 cm; concentrations reported in dw unless otherwise stated). Within site t-tests showed that five of the sites (sites 3, 5, 6, 7, and 9) were significantly higher in THg-adj in 2016 compared to 2015 ($p \leq 0.036$), while the other five showed no differences. There were no significant correlations between THg-adj and salinity for fourspine stickleback in 2015 ($r = 0.29$, $p = 0.75$) or 2016 ($r = -0.28$, $p = 0.43$; Fig. 3; Table 1). The mean BMFs for fourspine stickleback in 2015 and 2016 were 6.6 and 7.5, respectively, ranged from 3.4 to 13.9, and showed no significant correlations with salinity ($r = -0.22$ and $p = 0.44$ in 2015; $r = -0.03$ and $p = 0.92$ in 2016).

MeHg levels in invertebrate, sediment, and water samples were much lower than those of fish and only occasionally related to the salinity gradient (Fig. 3; Table 1). Invertebrate concentrations of MeHg were between three and eight times lower than those of fourspine stickleback (THg). Amphipods had an overall mean MeHg concentration of 85 ± 27 µg/kg and site means were not correlated with salinity ($r = -0.26$, $p = 0.35$). Except for the low salinity sites, MeHg concentrations in snails were similar in range to that found for amphipods. Snails (excluding *Viviparus*) had an overall mean MeHg concentration of 71 ± 29 µg/kg and site means showed no strong correlation with salinity ($r = 0.65$, $p = 0.48$). Meanwhile, *Viviparus* samples

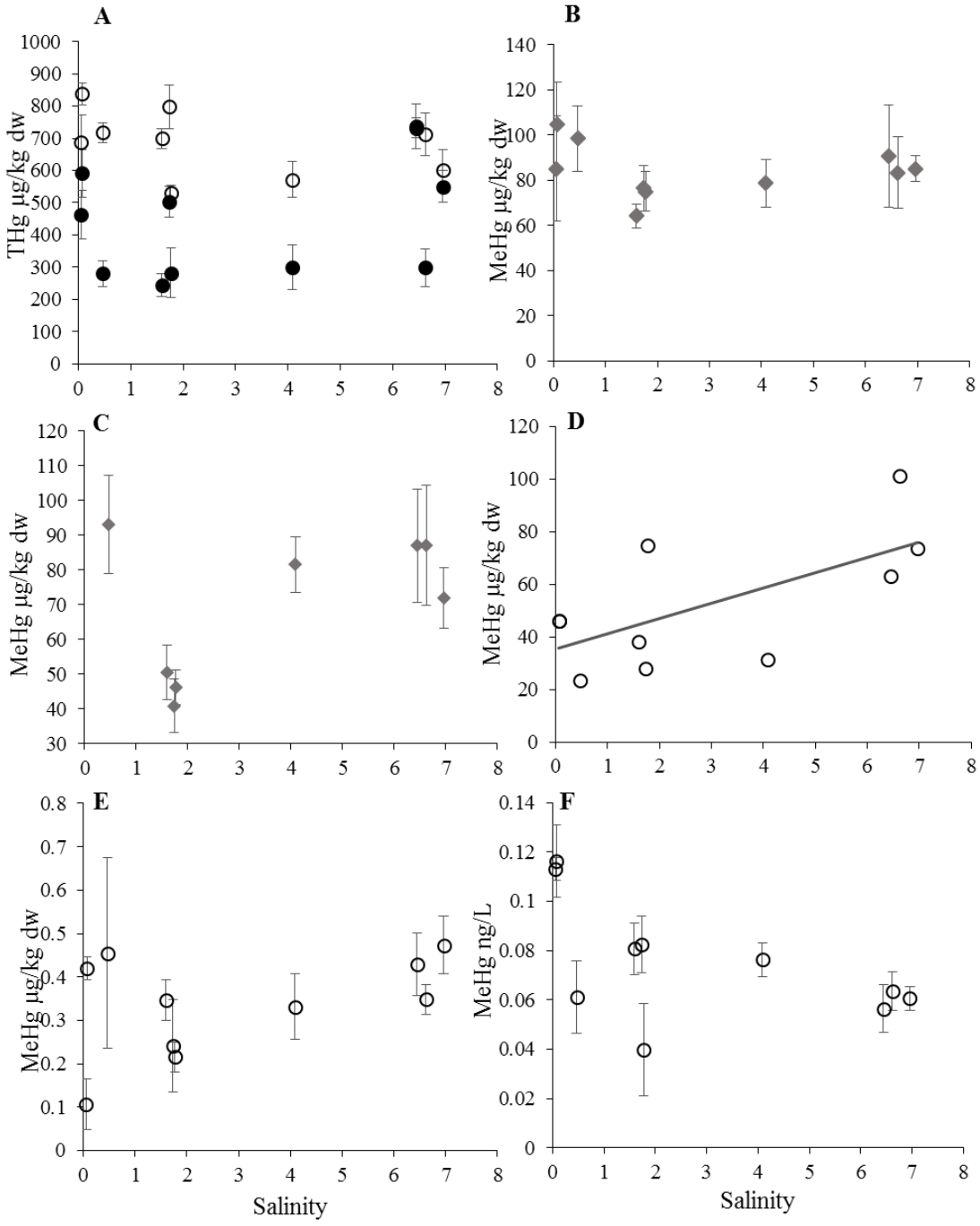

Fig. 3-Total mercury (THg – fourspine stickleback only) or methylmercury (MeHg) in samples collected along a salinity gradient in the Saint John River estuary. Concentrations reported in µg/kg dry weight (dw) or ng/L (for water samples only) for fourspine stickleback (A; n = 7-10/site), amphipods (B; n = 3-4/site), snails (C; n = 3-4/site; excluding genus Viviparus), chironomids (D; n = 1/site), sediments (E; n = 3/site), and water (F; n = 3/site). Data are means ± standard error in 2015 (solid circles), 2016 (open circles) or both years combined (grey diamonds). Trend lines indicate a significant correlation (p<0.05).

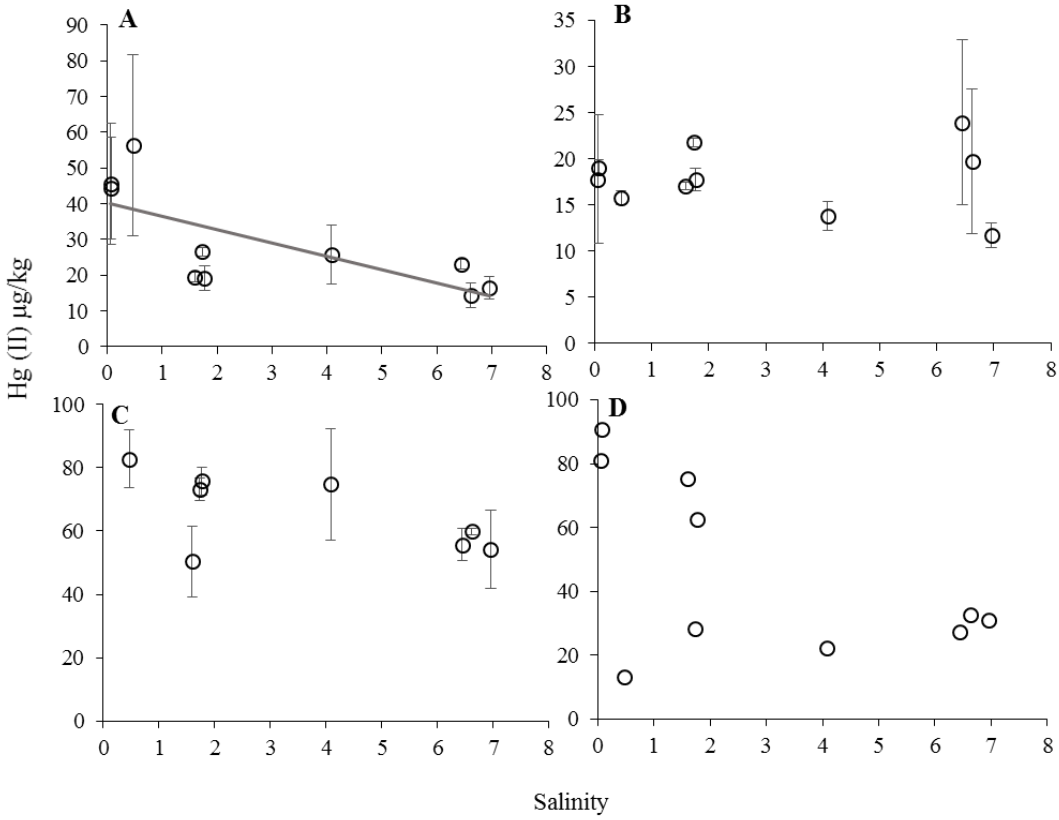

Fig. 4- Hg (II) concentrations in biota sampled along a salinity gradient in the Saint John River estuary in 2016. Concentrations reported in µg/kg dry weight (dw), measured in fourspine stickleback (A; n = 10/site), amphipods (B; n = 2-3/site), snails (C; n = 3/site; excluding genus Viviparus), and chironomids (D; n = 1/site). Error bars show standard error of the mean for each salinity value. Trend lines indicate a significant correlation (p<0.05).

had a much higher mean of 363 ± 186 µg/kg at the two freshwater sites in 2016. MeHg in chironomids increased significantly with salinity (r = 0.83, p = 0.003), and the mean was 52 ± 24 µg/kg. Sediments had a comparatively low overall mean MeHg concentration of 0.34 ± 0.17 µg/kg and were also not strongly correlated with salinity (r = 0.59, p = 0.07). Finally, MeHg in water had a low overall mean concentration of 0.08 ± 0.03 ng/L and there was no strong correlation with salinity (r = -0.54, p = 0.11).

The order of taxa from highest to lowest mean Hg (II) concentrations was snails > chironomids > fourspine stickleback > amphipods (Fig. 4). Mean Hg (II) in

Table 1 - Correlation coefficients between independent variables and MeHg (A), Hg (II) (B), and %MeHg (C) in biotic and abiotic samples from the Saint John River estuary. Significant correlations are identified with *, p < 0.05.

	Sample Type	Salinity	%Marine (fish only) or $\delta^{34}S$	$\delta^{13}C$	TP (fish only) or $\delta^{15}N$	Water MeHg	Sediment MeHg
A	Stickleback 2015	0.29	-0.19	-0.02	*0.37	-	-
	Stickleback 2016	-0.28	-0.19	-0.12	-0.18	*0.63	0.04
	Amphipods	-0.26	-0.41	0.16	0.02	*0.78	-0.27
	Snails	0.65	-0.17	-0.26	0.06	-0.21	*0.82
	Chironomids	*0.83	0.41	0.44	*0.72	-0.30	0.55
	Sediments	0.59	-0.12	0.32	*0.48	-0.42	-
	Water	-0.54	-	-	-	-	-0.42
B	Stickleback 2016	*-0.72	*-0.72	-0.54	0.33	0.51	-0.17
	Amphipods	-0.20	0.01	-0.21	0.31	0.10	-0.37
	Snails	-0.60	-0.04	*-0.52	-0.38	-0.15	-0.38
	Chironomids	-0.38	*-0.78	-0.49	0.26	0.40	-0.01
C	Stickleback 2016	*0.67	0.58	0.52	-0.40	-0.29	0.03
	Amphipods	-0.15	-0.21	0.16	-0.23	0.42	0.01
	Snails	*0.81	-0.17	-0.26	0.06	-0.08	*0.88
	Chironomids	*0.77	*0.89	*0.73	0.22	-0.47	0.35

fourspine stickleback across all sites was 29 ± 21 µg/kg and there was a negative trend with salinity (r = -0.72, p = 0.019; Table 1). Amphipods had a lower mean concentration of Hg (II) at 18 ± 7 µg/kg, and there was no strong trend with salinity (r = -0.20, p = 0.58). Similarly, there was no strong trend in snail Hg (II) with salinity (r = -0.60, p = 0.12), with a mean (excluding *Viviparus*) of 65 ± 18 µg/kg. As for MeHg, Hg (II) in *Viviparus* had a much higher mean of 193 ± 64 µg/kg. The overall mean Hg (II) in chironomids was 46 ± 28 µg/kg, again, showing no strong correlation with salinity (r = -0.38, p = 0.99). The gut-cleared amphipods had similar Hg (II) concentrations to those of the non-gut-cleared amphipods from the same sites in 2016, suggesting comparable results (paired t-test p = 0.29). In contrast, there was a difference in mean MeHg between gut-cleared and non-gut cleared (p =

0.05). The mean overall percent change in MeHg and Hg (II) from non-gut-cleared to gut-cleared was -8 ± 12% and -4 ± 19%, respectively.

The % MeHg in some biota increased with salinity (Table 1). This measure significantly increased with salinity in fourspine stickleback ($r = 0.67$, $p = 0.036$) and the range in means between sites was 94 – 98% (mean 97 ± 2%). This relationship was stronger for snails ($r = 0.81$, $p = 0.016$) and chironomids ($r = 0.77$, $p = 0.009$), where mean % MeHg ranged from 38 to 63%, and 33 to 75%, respectively. Alternatively, there were no strong correlations between % MeHg and salinity for amphipods, with means ranging from 70 – 90% ($r = -0.15$, $p = 0.69$).

There were among site differences observed within some, but not all, of the sample types (Fig. 5). ANOVAs of THg-adj in fish showed significant differences in concentrations across sites with years analyzed separately ($p < 0.001$ and $p = 0.006$ for 2015 and 2016, respectively). Years were kept separate because of the significant interaction between year and site ($p = 0.05$). There were also significant site differences for snails ($p = 0.016$; *Viviparus* excluded) and water ($p = 0.002$), but not sediments ($p = 0.164$), or amphipods ($p = 0.66$). However, these among-site differences were not related salinity, as outlined above in this section and the differences among sites were not similar among sample types.

2.4.2 Water Quality

Average salinity of the sites ranged from 0.06 to 6.96, and each site was significantly different from all other sites according to the Tukey's Post Hoc Test (p

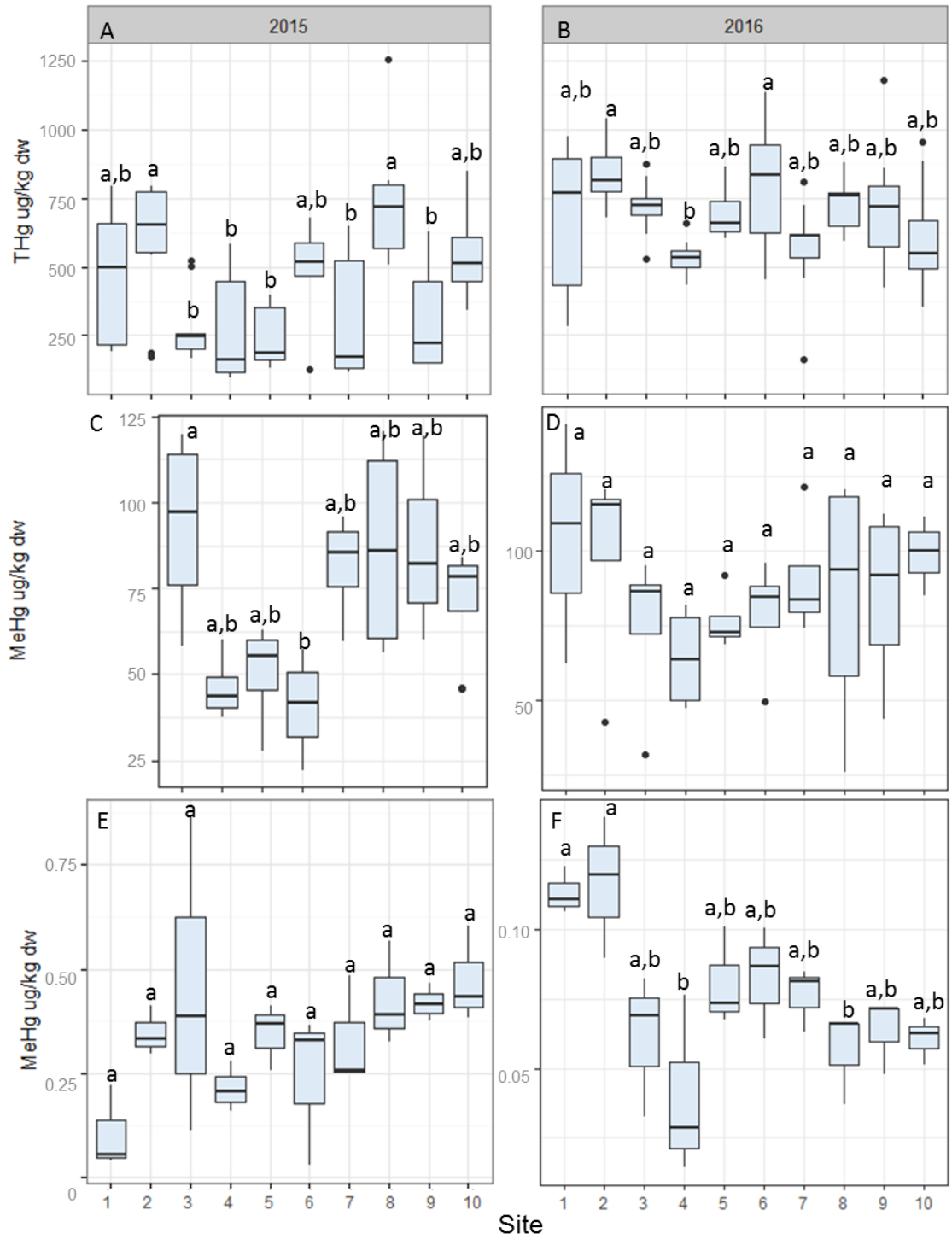

Fig. 5- Differences in total mercury (THg) or methylmercury (MeHg) in samples from ten sites along a salinity gradient in the Saint John River estuary. Concentrations reported in µg/kg dry weight (dw) or ng/L (for water samples only) measured in fourspine stickleback (A and B; n = 7-10/site), snails (C; n = 3-4/site; data excluding sites 1 and 2 with Viviparus), amphipods (D; n = 3-4/site), sediments (E; n = 3/site), and water (F; n = 3/site). Sites are ordered from lowest salinity (site 1) to highest salinity (site 10). Data that do not share a common letter label are significantly different, α = 0.05.

< 0.001; Fig. 6). Average temperature decreased from 23.3°C to 21.4°C along the salinity gradient and total organic carbon decreased from 8.3 to 1.5 mg/L. In contrast, water increased in calcium, chloride, and sulfate concentrations from freshwater to brackish water. Other metals such as boron (B), iron (Fe), lithium (Li), magnesium (Mg), manganese (Mn), molybdenum (Mo), potassium (K), rubidium (Rb), sodium (Na), strontium (Sr), and vanadium (V) also increased (Table 2).

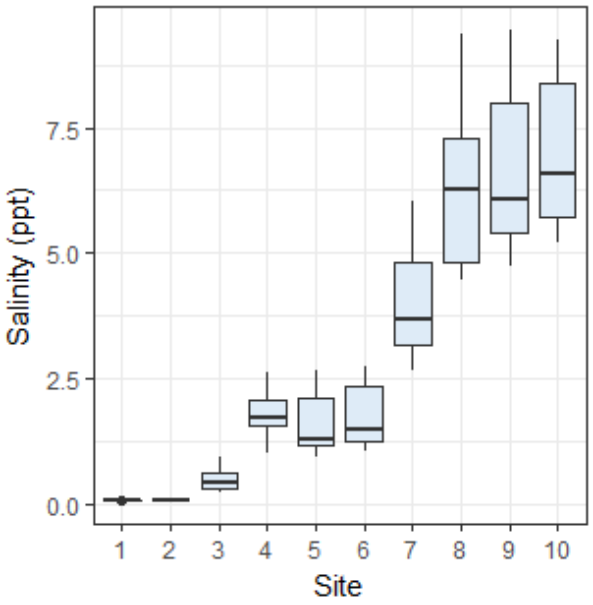

Fig. 6- Continuous salinity measures taken at sites 1 to 10 in the Saint John River estuary in 2016. All site salinities were statistically different from one another (p<0.001).

2.4.3 Diet and trophic position of biota

Generally, the standard deviations in $\delta^{15}N$ values were low within sites for each sample type (Fig. 7; Table 3). Stickleback had the highest $\delta^{15}N$ values of all samples. Mean TP occupied by fourspine stickleback was 3.48 ± 0.15 in 2015 and 3.44 ± 0.09 in 2016 across all sites. TP was significantly and positively correlated with salinity in 2015 stickleback (r = 0.68, p = 0.012), but there was no significant trend for 2016 (r = -0.46, p = 0.160; Table 4). Amphipods had an overall mean $\delta^{15}N$ of 6.05 ± 0.49‰ and there was no correlation with salinity (r = -0.09, p = 0.160). Snails had similar values with a mean $\delta^{15}N$ of 6.21 ± 0.77‰ but no significant correlation with salinity (r = 0.62, p = 0.35). There was also no strong correlation between $\delta^{15}N$ values and salinity for chironomids (r = 0.48, p = 0.160), with a mean

Table 2: Location and various water quality measures (2016 only) of sites (see also Fig. 1) along the Saint John River estuary. Salinity, temperature (Temp), and conductivity (cond) were from HOBO continuous measures, dissolved oxygen (DO) was from the YSI measures (n=1/site) and the other endpoints were measured in water samples sent to RPC in Fredericton, NB, Canada (n=1/site). If <, then measure was below detection limit.

Site	Latitude	Longitude	Mean Salinity	Mean Temp	Cond	DO	Calcium	pH	Alkalinity (as $CaCO_3$)	Chloride	Sulfate	Nitrate + Nitrite
	N	W		°C	µS/cm	mg/L	mg/L	units	mg/L	mg/L	mg/L	mg/L
1	45.612981	-66.074303	0.06	23.3	110	5.94	14.7	7.8	43	5	2	0.13
2	45.592456	-66.031182	0.07	23.2	152	6.73	14.8	7.8	38	5	6	0.14
3	45.523946	-66.071176	0.47	23.2	1008	6.34	18.7	8.0	36	181	26	0.10
4	45.468118	-66.124197	1.77	22.5	3431	7.94	35.8	7.8	39	870	120	0.10
5	45.404007	-66.197201	1.59	22.7	3118	6.78	28.1	8.1	38	486	74	0.11
6	45.378374	-66.215902	1.73	22.9	3372	8.15	32.9	7.9	40	780	109	0.14
7	45.329723	-66.193396	4.09	21.9	7404	8.93	57.6	8.0	45	1800	230	< 0.05
8	45.296097	-66.180258	6.45	21.6	11346	7.75	85.7	7.7	50	3090	460	< 0.50
9	45.284868	-66.156047	6.62	21.8	11852	8.08	81.9	7.7	53	2940	440	< 0.50
10	45.271931	-66.148314	6.95	21.4	12523	6.40	91.1	7.9	48	3090	440	< 0.05

Site	Total Organic Carbon	B	Fe	Li	Mg	Mn	Mo	K	Rb	Na	Sr	V
	mg/L	µg/L	µg/L	µg/L	mg/L	µg/L	µg/L	mg/L	µg/L	mg/L	µg/L	µg/L
1	8.3	5	80	0.5	2	12	0.10	0.6	0.7	4	106	< 1
2	6.8	5	80	0.5	2	12	0.1	0.6	0.7	3	106	< 1
3	5.6	50	130	2.2	15	23	0.3	4.5	1.8	103	190	2
4	3.2	216	160	8.7	64	28	0.8	20.2	6.0	502	506	5
5	4.4	127	240	5.3	39	34	0.5	12.5	3.9	284	352	4
6	3.6	194	140	7.8	57	20	0.7	18.0	5.3	454	452	5
7	2.3	472	300	18.4	138	25	1.5	44.0	12.1	942	956	12
8	1.7	800	< 200	31.0	231	30	2.0	70.3	19.0	1650	1510	10
9	1.8	780	< 200	29.0	217	20	2.0	66.6	18.0	1540	1400	10
10	1.5	850	400	33.0	247	20	3.0	78.0	21.0	1760	1640	20

of 5.99 ± 0.76‰. Finally, $\delta^{15}N$ values in sediments were lowest and were significantly and positively correlated with salinity ($r = 0.92$, $p < 0.001$), with an overall mean of 3.98 ± 0.60‰.

Among site variability for each taxa was higher for $\delta^{13}C$ than for $\delta^{15}N$ (Table 3), but there were generally positive trends with salinity, with animals from the high salinity sites having the heaviest $\delta^{13}C$ signals (Fig. 8; Table 4). There were significant positive correlations between $\delta^{13}C$ in stickleback and salinity for both 2015 ($r = 0.91$, $p < 0.001$) and 2016 ($r = 0.94$, $p < 0.001$). The same correlations were seen for amphipods ($r = 0.91$, $p < 0.001$), snails ($r = 0.36$, $p = 0.039$), chironomids ($r = 0.69$, $p = 0.026$), and sediments ($r = 0.90$, $p < 0.001$). The ranges in site means were -26 to -17‰, -23 to -15‰, -21 to -15‰, -26 to -17‰, and -28 to -24‰, respectively, for fish, amphipods, snails, chironomids, and sediments.

Among site variability was high for mean $\delta^{34}S$ for all sample types (Fig. 9; Table 3), but there were generally similar spatial trends to those of $\delta^{13}C$. There was a positive trend in $\delta^{34}S$ from freshwater to higher salinity sites for all biota (Table 4; Fig. 9). Biota sampled at sites with salinity of 1.59 or higher (site 4 and up) had consistently high $\delta^{34}S$ values reflective of marine sulfur sources. Biota sampled from sites 1 to 3 with salinities from 0.06 - 0.47 had $\delta^{34}S$ values distinctly lower than the higher salinity sites (Fig. 9). For stickleback, % Marine (derived from $\delta^{34}S$ values) was positively correlated to salinity in 2015 ($r = 0.70$, $p = 0.025$; Table 4), and but there was no strong correlation in 2016 ($r = 0.59$; $p = 0.073$). There were also significant positive correlations between $\delta^{34}S$ values and salinity for amphipods (r =

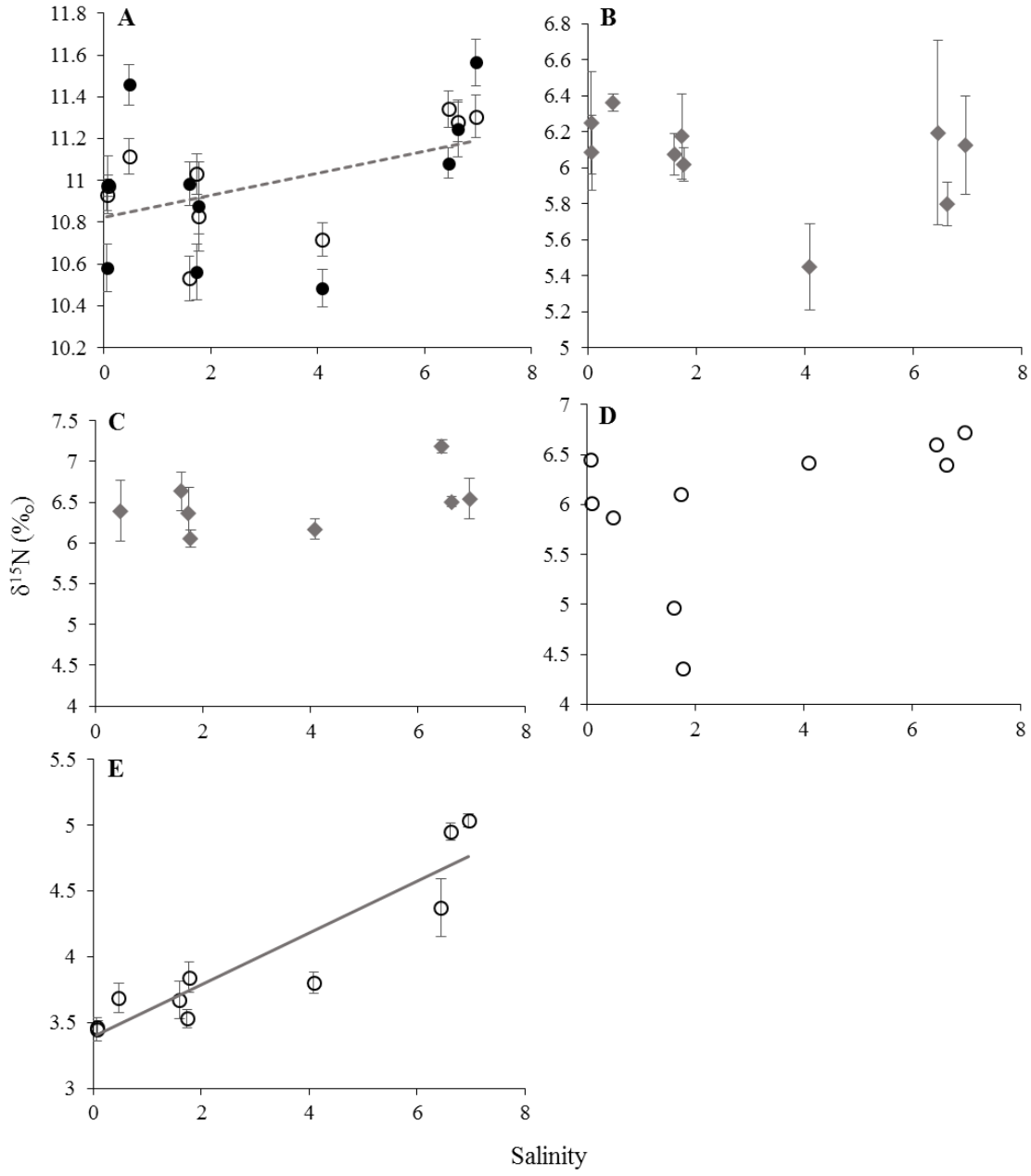

Fig.7- Mean $\delta^{15}N$ values (‰) measured in fourspine stickleback (A), amphipods (B), snails (C, excluding genus Viviparus), chironomids (D), and sediments (E) along a salinity gradient within the Saint John River estuary, New Brunswick, Canada. Figure shows averages ± standard error for 2015 (solid circles), 2016 (open circles), or both years combined (grey diamonds). Trend lines indicate a significant correlation ($p<0.05$) and the dashed line represents 2015 stickleback only.

Table 3- Mean and standard deviation (SD) of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values (‰) measured in fourspine stickleback, amphipods, snails, chironomids, and sediments along a salinity gradient within the Saint John River estuary, New Brunswick, Canada in 2015 or 2016.

Fourspine Stickleback			$\delta^{13}C$ ‰		$\delta^{15}N$ ‰		$\delta^{34}S$ ‰	
	Site	n	Mean	SD	Mean	SD	Mean	SD
2015	1	10	-23.00	1.82	10.58	0.36	3.92	0.64
	2	10	-24.87	1.47	10.98	0.44	4.97	0.81
	3	10	-26.18	0.91	11.46	0.31	10.90	1.32
	4	7	-24.39	0.95	10.88	0.57	14.59	0.84
	5	10	-24.54	0.67	10.98	0.33	16.06	1.02
	6	10	-24.98	0.86	10.56	0.42	15.72	0.47
	7	10	-20.50	0.51	10.48	0.28	17.81	0.80
	8	10	-19.59	0.83	11.08	0.23	17.04	0.42
	9	10	-17.79	1.05	11.25	0.43	16.00	0.92
	10	9	-18.16	0.87	11.57	0.33	16.28	0.66
2016	1	10	-23.25	1.26	10.93	0.23	6.37	1.08
	2	10	-24.27	0.62	10.98	0.17	6.65	0.36
	3	10	-24.37	0.97	11.12	0.27	12.23	0.74
	4	10	-24.82	0.99	10.83	0.27	14.56	0.56
	5	10	-24.56	1.02	10.53	0.34	16.78	1.50
	6	10	-23.53	1.08	11.03	0.31	18.68	2.53
	7	10	-20.87	0.62	10.72	0.26	16.00	2.53
	8	10	-19.02	0.53	11.34	0.27	18.88	1.78
	9	10	-18.02	0.81	11.28	0.30	17.72	1.76
	10	10	-17.05	0.57	11.31	0.32	13.59	0.88

Amphipods			$\delta^{13}C$ ‰		$\delta^{15}N$ ‰		$\delta^{34}S$ ‰	
	Site	n	Mean	SD	Mean	SD	Mean	SD
2015	1	1	-19.88		5.71		4.85	
	2	1	-21.06		5.62		4.46	
	3	1	-23.89		6.45		12.14	
	4	1	-21.71		5.76		16.52	
	5	1	-21.91		6.37		15.70	
	6	1	-24.55		5.49		17.93	
	7	1	-17.84		4.84		17.66	
	8	1	-16.79		4.67		16.08	
	9	1	-15.26		5.50		17.44	
	10	1	-13.65		5.45		17.56	
2016	1	2	-20.97	1.49	6.52	0.23	2.82	0.86
	2	3	-22.58	0.45	6.24	0.34	3.00	0.45
	3	3	-22.57	1.36	6.33	0.09	8.20	3.33
	4	3	-23.25	1.45	6.10	0.08	12.87	4.70
	5	3	-22.15	0.88	5.98	0.16	14.10	2.01
	6	3	-22.92	1.57	6.40	0.16	16.46	0.82
	7	3	-19.78	0.73	5.65	0.32	17.38	0.41
	8	3	-18.27	1.14	6.70	0.15	18.09	0.26
	9	3	-17.19	0.27	5.90	0.17	17.66	0.51
	10	3	-15.88	0.63	6.35	0.37	16.77	0.88

Table 3: Continued…

	Site	n	$\delta^{13}C$ ‰ Mean	$\delta^{13}C$ ‰ SD	$\delta^{15}N$ ‰ Mean	$\delta^{15}N$ ‰ SD	$\delta^{34}S$ ‰ Mean	$\delta^{34}S$ ‰ SD
Snails								
2015	1	1	-18.63		5.90		3.75	
	2	1	-17.79		6.25		5.31	
	3	1	-21.36		7.42		13.07	
	4	1	-19.48		5.76		16.40	
	5	1	-21.81		7.33		15.56	
	6	1	-23.35		5.56		17.65	
	7	1	-19.43		6.45		15.75	
	8	1	-18.49		6.95		15.30	
	10	1	-13.89		6.11		15.59	
2016	1	3	-20.76	1.02	5.31	0.36	2.07	0.61
	2	3	-22.07	0.54	4.43	0.21	1.07	0.29
	3	3	-19.82	1.67	6.05	0.37	8.28	2.45
	4	3	-17.89	1.00	6.15	0.12	11.04	2.54
	5	3	-15.79	0.34	6.40	0.10	12.59	2.00
	6	3	-18.25	2.27	6.64	0.37	15.14	0.88
	7	3	-19.44	0.70	6.08	0.18	15.96	1.14
	8	3	-18.36	0.31	7.26	0.03	16.57	0.12
	9	3	-17.17	1.41	6.50	0.11	16.02	0.14
	10	3	-15.54	0.50	6.69	0.48	14.35	0.70

	Site	n	$\delta^{13}C$ ‰ Mean	$\delta^{13}C$ ‰ SD	$\delta^{15}N$ ‰ Mean	$\delta^{15}N$ ‰ SD	$\delta^{34}S$ ‰ Mean	$\delta^{34}S$ ‰ SD
Chironomids								
2016	1	1	-23.26		6.45		2.51	
	2	1	-25.25		6.01		1.97	
	3	1	-26.34		5.87		4.35	
	4	1	-26.14		4.36		10.97	
	5	1	-17.34		4.97		13.34	
	6	1	-24.37		6.11		15.27	
	7	1	-20.07		6.42		14.27	
	8	1	-20.62		6.60		16.27	
	9	1	-19.30		6.40		16.05	
	10	1	-17.17		6.73		13.46	
Sediments								
2016	1	3	-27.66	0.12	3.45	0.15	-1.74	0.87
	2	3	-27.33	0.33	3.46	0.09	1.83	2.77
	3	3	-27.43	0.32	3.69	0.20	-3.53	2.04
	4	3	-27.17	0.13	3.84	0.20	-5.50	4.10
	5	3	-27.92	0.18	3.67	0.25	-11.19	7.16
	6	3	-26.94	0.10	3.53	0.12	-15.87	0.71
	7	3	-26.74	0.09	3.80	0.14	-17.35	3.43
	8	3	-25.82	0.65	4.37	0.38	-10.31	2.31
	9	3	-24.06	0.18	4.95	0.12	-10.77	1.73
	10	3	-24.66	0.08	5.03	0.09	-10.84	1.00

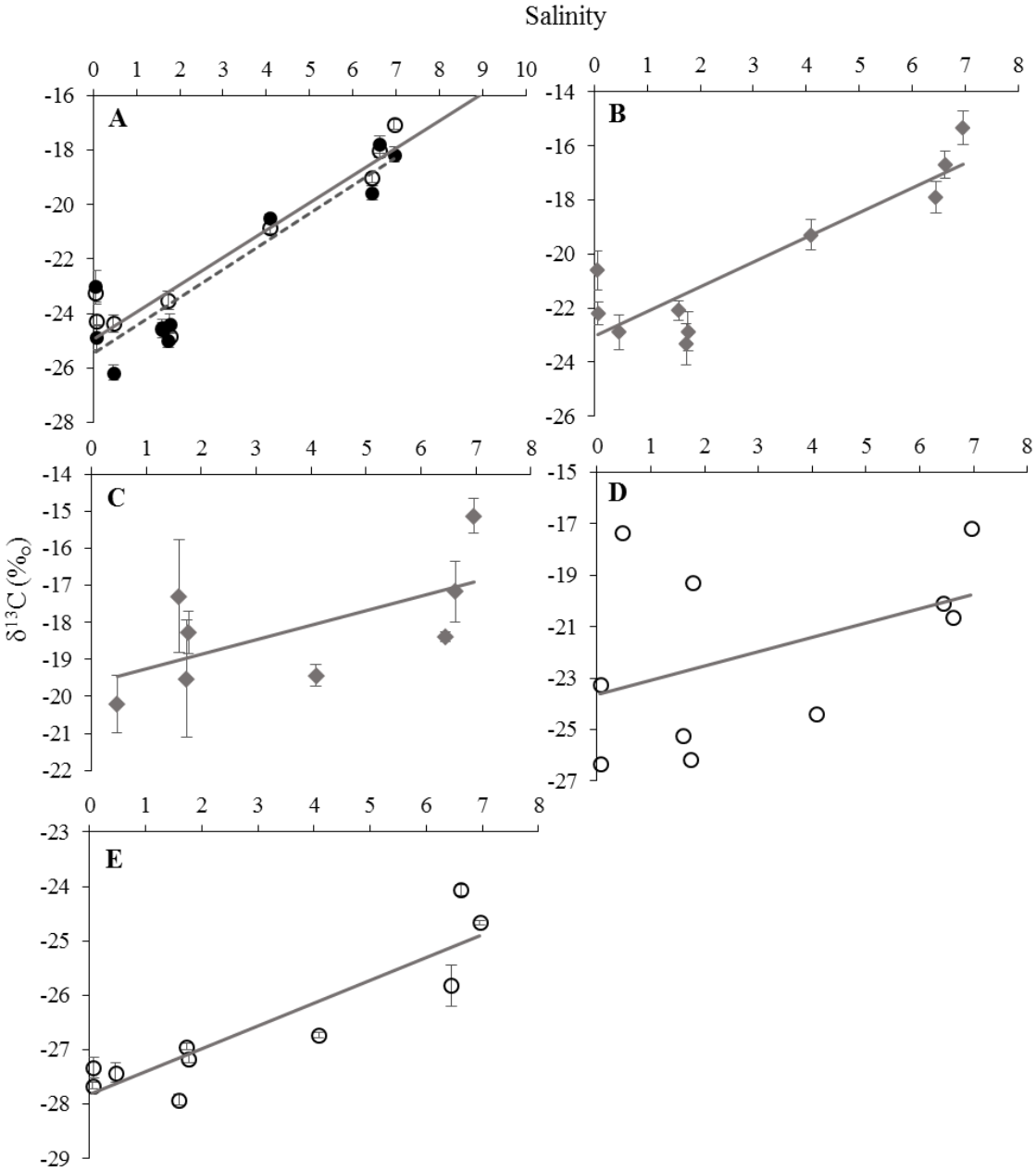

Fig. 8- Mean $\delta^{13}C$ values (‰) measured in fourspine stickleback (A), amphipods (B), snails (C, excluding genus *Viviparus*), chironomids (D), and sediments (E) along a salinity gradient within the Saint John River estuary, New Brunswick, Canada. Showing averages ± standard error for 2015 (solid circles), 2016 (open circles), or both years combined (grey diamonds). Trend lines indicate a significant correlation ($p<0.05$) and the dashed line represents 2015 stickleback only.

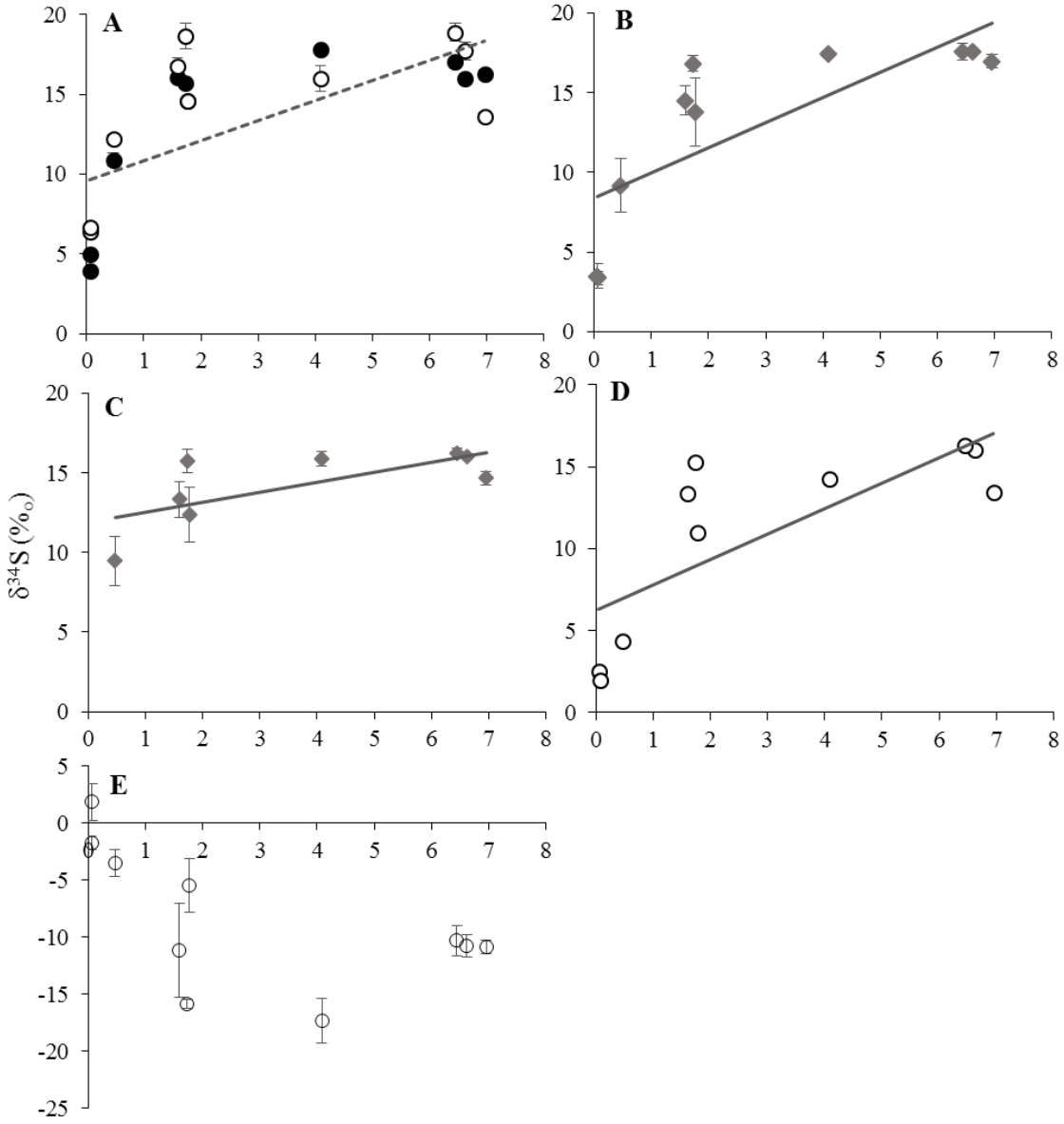

Fig. 9- Mean $\delta^{34}S$ values (‰) measured in fourspine stickleback (A), amphipods (B), snails (C, excluding genus *Viviparus*), chironomids (D), and sediments (E) along a salinity gradient within the Saint John River estuary, New Brunswick, Canada. Showing averages ± standard error for 2015 (solid circles), 2016 (open circles), or both years combined (grey diamonds). Trend lines indicate a significant correlation ($p<0.05$) and the dashed line represents 2015 stickleback only.

Table 4 – Correlation coefficients between salinity and $\delta^{13}C$, $\delta^{15}N$, or $\delta^{34}S$ values or derived values (%Marine and trophic position - TP) for samples from the Saint John River estuary. Significant correlations are identified with a *, p < 0.05.

Sample Type	%Marine (fish only) or $\delta^{34}S$	$\delta^{13}C$	TP (fish only) or $\delta^{15}N$
Stickleback 2015	*0.70	*0.91	*0.68
Stickleback 2016	0.59	*0.94	-0.48
Amphipods	*0.80	*0.91	-0.09
Snails	*0.74	*0.36	0.62
Chironomids	*0.76	*0.69	0.48
Sediments	-0.56	*0.90	*0.92

0.80, $p < 0.001$), snails ($r = 0.74$, $p = 0.013$), and chironomids ($r = 0.76$, $p = 0.010$). Finally, sediments had a different trend to that of biota (Fig. 9) with heavier mean $\delta^{34}S$ values that were not related to salinity ($r = -0.56$, $p = 0.093$). Ranges in mean $\delta^{34}S$ values among sites were from 4 to 19‰, 3 to 18‰, 2 to 16‰, 3 to 16‰, and -11 to -2‰ for fish, amphipod, snails, chironomids, and sediments, respectively.

Qualitative analysis of fourspine stickleback gut contents showed that they were mainly feeding on amphipods, chironomids, and zooplankton. Although not all the gut contents were identifiable under the microscope, the harder parts of these invertebrates were easily recognized. In 2015 the fourspine stickleback guts contained primarily amphipods (found in 67 of the 96 fish), chironomids (in 10 fish), and zooplankton (in 13 fish). In 2016 the identifiable material in the guts also consisted mainly of amphipods (in 88 of the 100 fish), chironomids (in 45 fish), and zooplankton (in 24 fish), but also of other dipterans (in 30 fish).

Paired t-test between gut-cleared and non-gut cleared amphipods show no significant differences between the two sample types for each isotope measure ($p = 0.19$, 0.33, and 0.78, respectively, for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ comparisons). Differences

between paired samples (gut cleared to non-gut cleared within each site) ranged from -0.26 to 0.46‰, -0.30 to 0.57‰, and -0.98 to 1.11‰ for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$, respectively.

2.4.4 Additional correlations with Hg

Aside from the Hg measures being variably correlated to salinity measures (section 2.4.1), there were also relationships observed with isotope measures (Table 1). THg-adj in fish significantly increased with TP, derived from $\delta^{15}N$ values ($r = 0.37$, $p < 0.001$) in 2015. Similarly, MeHg significantly increased with $\delta^{15}N$ values in sediments ($r = 0.48$, $p = 0.006$) and chironomids ($r = 0.72$, $p = 0.018$). In fourspine stickleback in 2016, Hg (II) was significantly negatively correlated with % Marine, derived from $\delta^{34}S$ values ($r = -0.72$, $p = 0.019$). Again, Hg (II) decreased significantly with $\delta^{34}S$ values in chironomids ($r = -0.78$, $p = 0.007$), while their % MeHg significantly increased with this measure ($r = 0.89$, $p = 0.001$). Snail Hg (II) also showed a significant decrease with $\delta^{13}C$ values ($r = -0.52$, $p = 0.008$), while % MeHg increased significantly with $\delta^{13}C$ values in chironomids ($r = 0.73$, $p = 0.016$).

Hg measures in biota were also correlated with concentrations of this element in abiotic samples. THg-adj in fish ($r = 0.63$, $p = 0.050$) and MeHg in amphipods ($r = 0.78$, $p = 0.007$) significantly increased with water MeHg. Sediment MeHg was also positively related to MeHg ($r = 0.82$, $p = 0.012$) and %MeHg in snails ($r = 0.88$, $p = 0.004$). However, there were no significant correlations observed between average fish Hg measures and average invertebrate MeHg from each site.

2.5 Discussion

2.5.1 Hg-salinity relationships

Firstly, I confirmed that there is a true salinity gradient in the Saint John River estuary and, as such, the biotic and abiotic compartments at the sites I selected were exposed to varying degrees of marine influence. As salinity was a key variable in this study, it was critical to confirm the statistical differences and a true gradient in this measure among sites. The $\delta^{13}C$ and $\delta^{34}S$ data in biota were also evidence for a true gradient, as they increased as expected with salinity (Fry, 2002; Fry and Chumchal, 2011). It was, however, a low gradient. Including additional sites along this gradient as well as higher salinity sites may increase the likelihood of seeing biological differences and an effect from salinity.

I predicted a negative correlation between MeHg bioaccumulation in the food web and salinity based on previous literature (Fry and Chumchal, 2012; van der Velden et al., 2013; Compeau and Bartha, 1984; Compeau and Bartha, 1987; Blum and Bartha, 1980), but did not observe this trend. Although there was some evidence that Hg concentrations in biota changed spatially along the Saint John River estuary, the relationships with salinity were not consistent across taxa or years and this concurs with the literature. Firstly, there were no significant correlations between salinity and THg in stickleback or between salinity and MeHg in amphipods, snails, sediments, or water. This is similar to what was found by Buckman et al. (2017), where salinity was not a main predictor of MeHg in fish, sediments or water. Meanwhile, the same study found that MeHg in suspended particulates and grass shrimp increased at the most saline sites, mirroring the

significant positive trends with salinity observed for MeHg in chironomids in the present study. It could be that the mercury at these higher salinity sites was more bioavailable to these invertebrates. Buckman et al. (2017) also mentioned that increased bioavailability of MeHg bound to marine dissolved organic carbon (DOC) versus terrestrial DOC can be a driver of this relationship. Negative trends between MeHg and salinity were expected since Hg methylation tends to decrease with increasing salinity within estuaries (Blum and Bartha, 1980; Compeau and Bartha, 1984), and this negative relationship has been documented in various fish, invertebrate, and bird species ranging from freshwater to marine environments (Evers et al., 2005; van der Velden et al., 2013). Although weak negative trends were exhibited by amphipods, 2016 fish, and water in my study, they were non-significant relationships. Overall though, this inconsistency in directionality of MeHg-salinity trends has been previously documented in estuarine biota. This may be reflective of differing dietary carbon sources among sites or years for taxa, or there may be additional biogeochemical drivers influencing the production and uptake of MeHg within the estuary that I did not capture. As mentioned above, the gradient in salinity may also not be large enough to show effects on biological uptake of Hg. I therefore, cannot provide any evidence to support that salinity, or marine influence, regulates MeHg concentrations, thereby increasing or decreasing risk of toxicity, to any biota sampled herein.

My hypothesis was based on previous work that observed that Hg methylation rates were higher in freshwater sediments in an estuary compared to sediments from more saline sites (Compeau and Bartha, 1984; Blum and Bartha,

1980). I found no evidence that methylation was higher at the freshwater sites in the Saint John River estuary, although there were trends opposing this prediction for Hg (II) and % MeHg for biota. It is possible that there are lower Hg (II) concentrations in stickleback samples from the higher salinity sites because of a change in food source along the river or additional inorganic Hg inputs into the upstream portions of the river. It is also possible that there is increased tidal flushing of Hg (II) downstream, reduced uptake or higher methylation rates at these higher salinity sites. Similar to what was found for fourspine stickleback, snails, and chironomids in the present study, an increase in % MeHg in particulates, grass shrimp (*Palaemonetes sp.*; Buckman et al., 2017), and sediments (Taylor et al., 2012) with increasing salinity has also been documented in other estuarine studies. This trend is contrary to what was predicted, because if methylation decreased with salinity, % MeHg should also decrease.

Beyond just salinity, there were other measures of marine influence that correlated with Hg (II) in organisms in the Saint John River estuary that may be useful for future studies assessing Hg in estuaries. The high correlations between salinity and either $\delta^{34}S$, % Marine or $\delta^{13}C$ values indicate these are potential proxies for habitat salinity in biotic samples. Though salinity did not show a significant correlation with Hg measures for some samples, there were significant relationships between isotope values and Hg measures. In addition to including $\delta^{15}N$ because of its relationship to Hg biomagnification in food webs, it is recommended for future studies to measure $\delta^{34}S$ and $\delta^{13}C$ values for their potential to predict Hg in estuarine biota.

This complex relationship between MeHg in biota and environmental factors (e.g. salinity) is not unique to the Saint John River estuary. Buckman et al. (2017) similarly sampled a complex estuarine system and found that the best environmental predictors of Hg were not consistent across all taxa within the estuary. So, while the Saint John River estuary is a highly tidal, complex system, it is also likely representative of a typical dynamic and large estuary. Buckman et al. suggest that environmental variables such as carbon sources, bioavailability, mixing, marsh presence, etc., can have a strong influence on MeHg concentrations at different sites within a single estuary. In the present study variables such as salinity, water MeHg, sediment MeHg, and isotope values showed correlations with Hg measures in one or more taxa, but again, relationships were not always consistent between taxa or years. Estuaries are complex systems and there are many biogeochemical factors that change with salinity (chlorine, sulfate, metals, etc.). These factors may also interact with the different forms of Hg in different ways, making it difficult to identify the marine influence on MeHg concentrations and which variables drive changes in the food web. It is therefore recommended to have additional variables as potential predictors beyond those used herein, or to conduct lab studies to try and isolate the primary factors influencing Hg availability. Some used by Buckman et al. (2017) included land cover data such as % marsh, % forest, and level of development. It would also be good to identify chemical characteristics of measures that are known to affect MeHg bioavailability such as dissolved organic material or organic carbon within the water or sediments (Wiener et al., 2003). With enough replication and characterization of sites within an estuary, a predictive

model could be built for identifying MeHg accumulation at sites based on these environmental variables.

2.5.2 Comparing Hg in the Saint John River estuary to other studies

No published studies could be found on fourspine stickleback Hg concentrations, but coastal or estuarine threespine stickleback (*Gasterosteus aculeatus*) show similar concentrations to those that I sampled from the Saint John River estuary. Eagles-Smith and Ackerman (2009) found average concentrations of 0.68 ± 0.03 mg/kg dw in fish from the San Francisco Bay estuary that were similar to those from the present study of 0.43 ± 0.24 mg/kg dw in 2015 and 0.69 ± 0.18 mg/kg dw in 2016. Wet weight concentrations of THg in threespine stickleback from the Gulf of Gdansk in Poland were similar or slightly lower, with concentrations between 0.01 and 0.11 mg/kg ww (approximately 0.05 to 0.55 mg/kg dw if 80% moisture content is assumed; Falandysz and Kowalewska, 1993). The BMFs of MeHg from predatory invertebrates to blacknose dace (*Rhinichthys atratulus*) sampled in ranged from 1.8 to 9.4 (mean 4.5 ± 2.3) in a study on food webs in streams in New Brunswick, Canada (Jardine et al. 2013). The range (3.4 to 13.9) for fourspine stickleback from the present study overlaps with these values (mean 6.6 ± 3.1), suggesting this species assimilates MeHg similarly to the blacknose dace.

On average, stickleback from this study did not exceed Hg guidelines. THg concentrations in fourspine stickleback ranged from 0.03 to 0.41 mg/kg (moisture content ranged from 52 – 92% in fourspine stickleback; mean 71%) and 20 of the 96

fish in 2015 and 29 of the 100 fish in 2016 had concentrations above 0.2 mg/kg ww. This is the toxicity threshold determined by Beckvar et al. (2005), below which fish are likely protected from the negative impacts of MeHg on growth, reproduction, development, and behaviour. The highest concentration of THg in stickleback from the present study was 0.46 mg/kg ww, so all individuals had levels that fell below the 0.5 mg/kg ww health advisory for humans consuming fish (Health Canada, 2007). These small-bodied stickleback would not typically be eaten by humans. But, when fish this size have high THg concentrations, it is likely that their predators will have much higher concentrations due to biomagnification. An example would be the predatory striped bass or pickerel sampled in 1975 from the Saint John River (Dadswell, 1975). Therefore, it is important to recognize that while fourspine stickleback are under Health Canada's safe consumption threshold, their predators may not be. Though there is little information on the predators of fourspine stickleback, other species of stickleback (threespine stickleback and brook stickleback) are consumed by aquatic birds and many larger fish species, such as perch and bass (Scott and Crossman, 1973), which are recreationally fished within the Saint John River. It would be interesting to have more current THg concentrations from these fish species in the Saint John River estuary.

Contrary to my findings of low concentrations in fish, Dadswell (1975) reported THg concentrations in 15 fish species from the Saint John River estuary and, though he did not sample fourspine stickleback, all fish species were over the whole body toxicity threshold (after conversion using the equation $THg_{whole\ body} = 0.6THg_{fillet}$; Wyn et al. 2009) of 0.2 mg/kg ww except anadromous Atlantic Salmon

(*Salmo salar*), American Shad (*Alosa sapidissima*), and Alewife (*Alosa pseudoharengus*). Predatory species such as Striped Bass (*Morone saxatilis*), Chain Pickerel (*Esox niger*), American Eel (*Anguilla rostrata*), and Yellow Perch (*Perca flavescens*) were higher than this threshold, with averages of 2.13, 1.00, 0.65, and 0.70 mg/kg ww THg in their tissues, respectively. While these concentrations are much higher than those measured in fourspine stickleback in the present study (range of 0.04 to 0.41 mg/kg ww), they could be reflective of the biomagnification of THg concentrations from stickleback, or similar small-bodied fish, as a prey item to these larger, predatory species. The biomagnification of Hg to piscivorous fish from prey fish is typically between 4 and 9 fold (Wiener et al., 2003). Using the mean THg concentrations (0.15 mg/kg ww) of fourspine stickleback from the Saint John River estuary and the average increase in Hg for predatory fish outlined by Weiner et al. (4 to 9-fold), it can be roughly estimated that fish preying on fourspine stickleback within the Saint John River currently have between 0.60 and 1.35 mg/kg ww, values that are also above both guidelines. As such, further monitoring of Hg in predatory fishes in the estuary is warranted.

In 2016, a different genus of snail was collected at the two most freshwater sites (*Viviparus* from sites 1 and 2) because the other two genera were not present during sampling. These were the only sites where *Viviparus* was collected, but these snails had very high concentrations of MeHg in their tissues when compared to the other genera. Average MeHg concentrations from the furthest upstream sites in this year were roughly five times higher than the average concentrations of the other snail taxa. I excluded these data as their concentrations were not directly

comparable. Most pulmonate taxa such as *Physa* and *Fossaria* reproduce once and only live for one year, while prosobranchs such as *Viviparus* reproduce many times over several years (Thorp and Covich, 2001). Therefore, it is possible that these *Viviparus* snails were longer-lived or slower-growing in comparison to the other genera, allowing for more bioaccumulation of MeHg.

MeHg concentrations found in Saint John River invertebrates were comparable to levels observed in other estuarine studies, indicating no extreme contamination within the estuary. THg concentrations in *Gammarus* amphipods from the Narragansett Bay in Rhode Island, USA were 0.093 ± 0.022 mg/kg in one study (Taylor et al., 2012) and 0.013 ± 0.002 mg/kg ww (0.065 mg/kg dw since they reported 85% moisture content) in another (Payne and Taylor, 2010). In the Canadian Arctic and sub-Arctic Gammarid amphipods had 0.076 mg/kg THg in lake environments and between 0.016 and 0.049 mg/kg in marine habitats (van der Velden et al., 2013), which are slightly lower values than those measured in Saint John River amphipods, at 0.109 ± 0.023 mg/kg (for invertebrates THg = MeHg + Hg (II) concentrations from 2016). The snails *Littorina littorea* and *Massarius obsoletus* from the Narragansett Bay had 0.090 and 0.177 mg/kg THg, respectively (Taylor et al. 2012). van der Velden et al. (2013) also reported that freshwater gastropods had 0.085 mg/kg THg, whereas marine gastropods had 0.045 mg/kg. While these do overlap with Saint John River snail concentrations of THg (mean 0.138 ± 0.035 mg/kg), the *Viviparus* snails had much higher concentrations of THg (0.555 ± 0.233 mg/kg). In freshwater environments, chironomids sampled by van der Velden et al. (2013) had 0.162 to 0.232 mg/kg in average THg, which is higher than the Saint

John River chironomids that ranged from 0.036 to 0.137 mg/kg THg (average 0.099 ± 0.038 mg/kg). So again, there was likely low contamination of invertebrates from this system in comparison to other urbanized estuaries.

Sediments and water from the present study had much lower concentrations of MeHg than biota, as expected, and were low in comparison to concentrations in other urban estuaries. Sediments had mean MeHg concentrations of 1.85 μg/kg in the Narragansett Bay (Taylor et al., 2012), and in the urbanized Delaware River estuary mean concentrations ranged from 0.08 to 4.90 μg/kg, with most of the sites falling under 1.60 μg/kg (Buckman et al., 2017). A study of ten different estuaries reported sediments with MeHg concentrations ranging from 0.13 to 34.8 μg/kg (Chen et al. 2014). The sediments from the Saint John River estuary fall on the lower end of this range with a mean of 0.34 ± 0.17 μg/kg. In the case of the present study, sediment MeHg was strongly correlated with MeHg in snails, indicating that, for the most part, bioaccumulation in other taxa sampled may be independent of sediment concentrations. Chen et al. (2009) found that while sediments positively predict some of the variation in biota MeHg, there are other factors, such as organic carbon in the sediments, that also explain a lot of the variation (stated that higher organic carbon decreases MeHg bioaccumulation). Dissolved MeHg in water from the lowest, mid, and highest salinity sites (salinities of < 1, 1 to 4 and > 4, respectively) in the urbanized Delaware River estuary were from 0.01 to 0.11 ng/L, 0.01 to 0.09 ng/L and 0.01 to 0.07 ng/L, respectively (Buckman et al., 2017). In the ten estuaries sampled by Chen et al. (2014) these concentrations were 0.001 to 0.025 ng/L, and overlap with those from the Saint John River estuary (0.04 to 0.12 ng/L). So, water

and sediments of the Saint John River estuary are similar in MeHg concentrations to other urbanized estuaries. In the present study, average water MeHg across sites was significantly positively correlated with MeHg in stickleback and amphipods, suggesting that food web bioaccumulation was linked to water MeHg concentrations. Buckman et al. (2017) found a similar trend, where water particulate MeHg positively predicted MeHg in Blue Crab (*Callinectes sapidus*) and juvenile White Perch (*Morone americana*).

2.5.3 Describing stable isotopes and diets

The $\delta^{15}N$ values of fourspine stickleback from the present study, which increase with trophic position (TP), are in line with those found elsewhere in the literature. In a study by Schein et al. (2013), fourspine stickleback from the Stanley River estuary, PEI, Canada had $\delta^{15}N$ values around 12‰, which is similar but slightly higher than results for the Saint John River estuary. Fourspine stickleback in the Schein et al. study were found to be closely linked to the benthic habitat and Gammarid amphipods made up the bulk of their gut contents, as for the same species from the current study. The low among-site variation in TP values for fourspine stickleback suggests they feed at a consistent trophic level along the river, which should indicate little influence of TP on MeHg concentrations along the gradient. This was the case for fish where there was only a weak correlation between TP and MeHg for 2015. Fourspine stickleback were roughly 1.8 trophic levels higher than invertebrates sampled in the present study (assuming a trophic enrichment of 3.4‰; Post, 2002). This value was similar to what Schein et al. (2013) found, where fourspine stickleback were also more than one trophic level

higher than amphipods, despite amphipods being abundant in their guts. They also found that fish eggs were prevalent in the stomach contents of fourspine stickleback, which could be increasing the $\delta^{15}N$ values of the fish. It is likely then that even though there were mainly amphipods and chironomids found in the guts of the stickleback from the Saint John River estuary, there is another food source with a higher $\delta^{15}N$ value that was not measured. Alternatively, the enrichment factor for this food web may be higher than the commonly used 3.4‰ estimation (Post, 2002).

The generally low among site standard deviations for $\delta^{15}N$ in amphipods, snails, and chironomids also suggests they are feeding at consistent trophic levels along the salinity gradient. The values measured in the Saint John River estuary are also comparable to other studies. Amphipods from the Stanley River estuary in PEI, Canada had $\delta^{15}N$ values of 7‰, which is again, slightly higher than those from the Saint John River estuary. In this study, snails and chironomids showed similar values to those of the amphipods, but seem to have higher values than the same taxa from other studies. Kidd et al. (1995) found that snails from family Lymnaeidae and chironomids from subfamily Chironominae collected from a freshwater lake had $\delta^{15}N$ values around 2‰ and 4‰, respectively. Doi et al. (2006) also found that snails (Japanese freshwater species *Semisulcospira libertina*) and chironomids had $\delta^{15}N$ values all between 2 and 3‰. This could be reflective of primary production sources from these other studies having lower baseline $\delta^{15}N$ values, but primary production sources were not measured in the current study so this comparison cannot be made.

Overall there was a broad range of $\delta^{13}C$ and $\delta^{34}S$ values for biota observed within sites, not just across the salinity gradient. For fourspine stickleback, the $\delta^{13}C$ values differed by over 5‰ within some sites. This variability was also observed for amphipods and snails, which had up to 3‰ and 5‰ differences within a single site, respectively. It is possible that this variability is reflective of the high mixing and tidal effects within the estuary and that these are causing primary production sources to be variable within a site. For $\delta^{34}S$ values, differences up to 7, 9, and 5‰ in fourspine stickleback, amphipods, and snails, respectively, were also found within sites. For sediments this was even higher with 13‰ differences among samples from the same site. However, since there is a distinct difference between freshwater and marine $\delta^{34}S$ values in biota (Fry and Chumchal, 2011), the trend with salinity could still be clearly shown with the mean site values. This high within site variability, again, could be due to the contrasting high and low tidal effects and mixing of marine waters and freshwater along the estuary.

There were also relationships between isotope values in biota and site salinity. Average $\delta^{13}C$ values were correlated with salinity for all samples. This positive correlation was expected, as it was also found by Fry (2002) for clams and dissolved inorganic carbon from a salinity gradient in the San Francisco Bay, where samples from higher salinities had marine carbon enriched in $\delta^{13}C$ (range of zero to 30 practical salinity units). This is in support of the assumption that the biota sampled were, in fact, living along a salinity gradient within the Saint John River estuary. This is also to say that average $\delta^{13}C$ values could serve as a proxy for habitat salinity. The same clams from the San Francisco Bay showed no consistent increase

or decrease in $\delta^{15}N$ along the salinity gradient though (Fry, 2002). This is true too for the biota in the present study, where there was no consistent trend between their $\delta^{15}N$ and salinity.

Like average $\delta^{13}C$ values, average $\delta^{34}S$ values were positively correlated with salinity for all biota sampled. The biota inhabiting brackish water typically have near 100% marine-sourced sulfur in their diets when considering these isotope values. This is supported by the calculations of % marine in the diets of fourspine stickleback within each site. This data showed that fish from all sites above 1.59 had means of over 90% marine sulfur in their diets. The fish from sites below this salinity had mean % Marine values of 0 – 54% in 2015 and higher values of 31 – 79% in 2016. Fry and Chumchal (2011) documented the same trend for other estuarine fish living along a salinity gradient, where the fish ranged between -3 and 3‰ $\delta^{34}S$ at the most freshwater sites (salinity near 0 on Practical Salinity Scale) and gradually increased up to 16‰ for the marine sites. These are similar values to data from the Saint John River estuary, where all sites above 1.63 salinity had mean $\delta^{34}S$ values above 14‰, and sites below this salinity had values below 12‰. There was no specific shifting point in $\delta^{34}S$ values within the fish data of Fry and Chumchal (2011) but more of a gradual increase from freshwater to marine. Again, this positive trend observed in the biota sampled from the Saint John River estuary supports the prediction that they lived along a salinity gradient that ranges from freshwater to brackish water.

Though there are plenty of variables that change from upstream to downstream in an estuary, I am confident that the most prominent differences

among my sites were that of a chemical freshwater to marine gradient rather than physical changes in water flow across sites (riverine flows at the freshwater sites and more lentic conditions at the higher salinity sites). This chemical relationship is exhibited by the distinct freshwater to marine trend in $\delta^{34}S$ values seen for all biota, which is the relationship expected based on the literature (Fry and Chumchal, 2011). $\delta^{34}S$ values in fish from strictly lake and river habitats are found in the range between -8 and 6 ‰, while those from the present study ranged from 3 to 21‰ in individual fish (Carr et al., 2017). This, in combination with the statistically distinct gradient in salinity is evidence of a real marine chemical gradient within these Saint John River estuary sites.

2.5.4 Conclusion

Overall, I did not find evidence to support my hypothesis that salinity regulates Hg bioaccumulation in aquatic food webs. The relationships between MeHg and salinity or related proxies were not always similar among taxa within the present study, depicting an inconsistent relationship between environmental variables and MeHg accumulation in biota within the Saint John River estuary. Therefore, I cannot conclude that fish from a certain salinity were at higher risk of mercury toxicity. This work will be helpful though for future studies considering the variables influencing accumulation of MeHg in biota within other estuarine systems. MeHg remains a prominent issue within fish and fish-eating wildlife in North America and it is therefore important to still try to understand and study its accumulation in nature, identifying animals with the lowest potential toxicity to their consumers. The accumulation of MeHg within urban estuaries is especially

important as these typically host diverse wildlife at juvenile stages, are fished by humans, and are understudied in terms of MeHg accumulation into these food webs. Mean MeHg or THg concentrations in biota, water, and sediments from the Saint John River estuary overlapped with what has been found in other urban estuaries, indicating mercury levels in this river are not exceptionally high in comparison. However, the biota measured herein were only primary or secondary consumers; sampling higher trophic levels in the Saint John River estuary would be beneficial as it would provide knowledge of Hg exposures for fish-eating wildlife or humans.

2.6 References

Beckvar, N., Dillon, T.M., Read, L.B., 2005. Approaches for linking whole-body fish tissue residues of mercury or DDT to biological effects thresholds. Environ. Toxicol. Chem. 24, 2094-2105.

Berntssen, M., Aatland, A., Handy, R., 2003. Chronic dietary mercury exposure causes oxidative stress, brain lesions, and altered behaviour in Atlantic salmon (*Salmo salar*) parr. Aquatic Toxicology 65, 55-72.

Blum, J.E., Bartha, R., 1980. Effect of salinity on methylation of mercury. Bull. Environ. Contam. Toxicol. 25, 404-408.

Buckman, K., Taylor, V., Broadley, H., Hocking, D., Balcom, P., Mason, R., Nislow, K., Chen, C., 2017. Methylmercury Bioaccumulation in an Urban Estuary: Delaware River, USA. Estuar. Coasts, 1-13.

Carr, M.K., Jardine, T.D., Doig, L.E., Jones, P.D., Bharadwaj, L., Tendler, B., Chételat, J., Cott, P., Lindenschmidt, K-E., 2017. Stable sulfur isotopes identify habitat-specific foraging and mercury exposure in a highly mobile fish community. Sci. Total Environ. 586, 338-346.

Chen, C.Y., Dionne, M., Mayes, B.M., Ward, D.M., Sturup, S., Jackson, B.P., 2009. Mercury bioavailability and bioaccumulation in estuarine food webs in the Gulf of Maine. Environ. Sci. Technol. 43, 1804-1810.

Chen, C.Y., Borsuk, M.E., Bugge, D.M., Hollweg, T., Balcom, P.H., Ward, D.M., Williams, J., Mason, R.P., 2014. Benthic and pelagic pathways of methylmercury bioaccumulation in estuarine food webs of the Northeast United States. PLoS ONE 9.

Chumchal, M.M., Rainwater, T.R., Osborn, S.C., Roberts, A.P., Abel, M.T., Cobb, G.P., Smith, P.N., Bailey, F.C., 2011. Mercury speciation and biomagnification in the food web of Caddo Lake, Texas and Louisiana, USA, a subtropical freshwater ecosystem. Environ. Toxicol. Chem. 30, 1153-1162.

Clayden, M.G., Lescord, G.L., Kidd, K.A., Wang, X., Muir, D.C., O'Driscoll, N.J., 2017. Using sulfur stable isotopes to assess mercury bioaccumulation and biomagnification in temperate lake food webs. Environ. Toxicol. Chem. 36(3), 661-670.

Compeau, G., Bartha, R., 1984. Methylation and demethylation of mercury under controlled redox, pH, and salinity conditions. Appl. Environ. Microbiol. 48, 1203-1207.

Compeau, G.C., Bartha, R., 1987. Effect of salinity on mercury-methylating activity of sulfate-reducing bacteria in estuarine sediments. Appl. Environ. Microbiol. 53, 261-265.

Dadswell, M., 1975. Mercury, DDT and PCB content of certain fishes from the Saint John River estuary, New Brunswick Paper presented to meeting of Canadian Society of Environmental Biologists, Fredericton, New Brunswick.

DFO., 2009. Use of the lower Saint John River, New Brunswick, as fish habitat during the spring freshet. DFO Can. Sci. Advis. Sec. Sci. Resp. 2009/014.

Doi, H., Takagi, A., Mizota, C., Okano, J., Nakano, S., Kikuchi, E., 2006. Contribution of chemoautotrophic production to freshwater macroinvertebrates in a headwater stream using multiple stable isotopes. Int. Rev. Hydrobiol. 91, 501-508.

Dutton, J., Fisher, N.S., 2011. Salinity effects on the bioavailability of aqueous metals for the estuarine killifish *Fundulus heteroclitus*. Environ. Toxicol. Chem. 30, 2107-2114.

Eagles-Smith, C.A., Ackerman, J.T., 2009. Rapid changes in small fish mercury concentrations in estuarine wetlands: Implications for wildlife risk and monitoring programs. Environ. Sci. Technol. 43, 8658-8664.

Evans, D.W., Crumley, P.H., 2005. Mercury in Florida Bay fish: Spatial distribution of elevated concentrations and possible linkages to Everglades restoration. Bull. Mar. Sci. 77, 321-345.

Evans, M.S., Lockhart, W.L., Doetzel, L., Low, G., Muir, D., Kidd, K., Stephens, G., Delaronde, J., 2005. Elevated mercury concentrations in fish in lakes in the Mackenzie River Basin: The role of physical, chemical, and biological factors. Sci. Total Environ. 351-352, 479-500.

Evers, D.C., Burgess, N.M., Champoux, L., Hoskins, B., Major, A., Goodale, W.M., Taylor, R.J., Poppenga, R., Daigle, T., 2005. Patterns and interpretation of mercury exposure in freshwater avian communities in northeastern North America. Ecotoxicology 14, 193-221.

Falandysz, J., Kowalewska, M., 1993. Mercury concentration of stickleback *Gasterosteus aculeatus* from the Gulf of Gdansk. Bull. Environ. Contam. Toxicol. 51, 710-715.

Farmer, T.M., Wright, R.A., Devries, D.R., 2010. Mercury concentration in two estuarine fish populations across a seasonal salinity gradient. Trans. Am. Fish. Soc. 139, 1896-1912.

Friedmann, A.S., Watzin, M.C., Brinck-Johnsen, T., Leiter, J.C., 1996. Low levels of dietary methylmercury inhibit growth and gonadal development in juvenile walleye (*Stizostedion vitreum*). Aquatic Toxicology 35, 265-278.

Fry, B., 2002. Conservative mixing of stable isotopes across estuarine salinity gradients: A conceptual framework for monitoring watershed influences on downstream fisheries production. Estuaries 25, 264-271.

Fry, B., 2006. Stable Isotope Ecology. Springer, New York, NY, USA.

Fry, B., 2013. Using stable CNS isotopes to evaluate estuarine fisheries condition and health. Isot. Environ. Health Stud. 49, 295-304.

Fry, B., Chumchal, M.M., 2011. Sulfur stable isotope indicators of residency in estuarine fish. Limnol. Oceanogr. 56, 1563-1576.

Fry, B., Chumchal, M.M., 2012. Mercury bioaccumulation in estuarine food webs. Ecol. Appl. 22, 606-623.

Gobas, F.A.P.C., De Wolf, W., Burkhard, L.P., Verbruggen, E., Plotzke, K., 2009. Revisiting bioaccumulation criteria for POPs and PBT assessments. Integr. Environ. assess. manage. 5, 624-637.

Hall, B., Bodaly, R., Fudge, R., Rudd, J., Rosenberg, D., 1997. Food as the dominant pathway of methylmercury uptake by fish. Water Air Soil Poll. 100(1-2), 13-24.

Health Canada, 2007. Human Health Risk Assessment of Mercury in Fish and Health Benefits of Fish Consumption. 1–70. http://www.hc-sc.gc.ca/fn-an/alt_formats/hpfb-dgpsa/pdf/nutrition/merc_fish_poisson-eng.pdf. Accessed February 2, 2017.

Jardine, T.D., Kidd, K.A., O'Driscoll, N., 2013. Food web analysis reveals effects of pH on mercury bioaccumulation at multiple trophic levels in streams. Aquat. Toxicol. 132-133, 46-52.

Kidd, K.A., Schindler, D.W., Hesslein, R.H., Muir, D.C.G., 1995. Correlation between stable nitrogen isotope ratios and concentrations of organochlorines in biota from a freshwater food web. Sci. Total Environ. 160-161, 381-390.

Kidd, K.A., Muir, D.C.G., Evans, M.S., Wang, X., Whittle, M., Swanson, H.K., Johnston, T., Guildford, S., 2012. Biomagnification of mercury through lake trout (*Salvelinus namaycush*) food webs of lakes with different physical, chemical and biological characteristics. Sci. Total Environ. 438, 135-143.

Kidd, S.D., Curry, R.A., Munkittrick, K.R., 2011. The Saint John River: A State of the Environment Report, 1–166.

Klapstein, S.J., Ziegler, S.E., Risk, D.A., O'Driscoll, N.J., 2016. Quantifying the effects of photoreactive dissolved organic matter on methylmercury photodemethylation rates in freshwaters. Environ. Toxicol. Chem. 1493-1502.

Luten, J.B., Ruiter, A., Ritskes, T.M., Rauchbaar, A.B., Riekwel-Booy, G., 1980. Mercury and selenium in marine and freshwater fish. J. Food Sci. 45, 416-419.

McCutchan Jr., J.H., Lewis Jr., W.M., Kendall, C., McGrath, C.C., 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos 102, 378-390.

Metcalfe, C.D., Dadswell, M.J., Gillis, G.F., Thomas, M.L.H., 1976. Physical, chemical, and biological parameters of the Saint John River Estuary, New Brunswick, Canada. Environment Canada Technical Report number. 686, 1-41.

Payne, E.J., Taylor, D.L., 2010. Effects of diet composition and trophic structure on mercury bioaccumulation in temperate flatfishes. Arch. Environ. Contam. Toxicol. 58, 431-443.

Post, D.M., 2002. Using stable isotopes to estimate trophic position: Models, methods, and assumptions. Ecology 83, 703-718.

Schein, A., Courtenay, S.C., Kidd, K.A., Campbell, K.A., van den Heuvel, M.R., 2013. Food web structure within an estuary of the southern Gulf of St. Lawrence undergoing eutrophication. Can. J. Fish. Aquatic Sci. 70, 1805-1812.

Scott, W.B., Crossman, E.J., 1973. Freshwater Fishes of Canada. Fisheries Research Board of Canada, Ottowa, 658-668.

Svensson, C.J., Hyndes, G.A., Lavery, P.S., 2007. Food web analysis in two permanently open temperate estuaries: Consequences of saltmarsh loss?. Mar. Environ. Res. 64, 286-304.

Swanson, H.K., Kidd, K.A., 2010. Mercury concentrations in arctic food fishes reflect the presence of anadromous arctic charr (*Salvelinus alpinus*), species, and life history. Environ. Sci. Technol. 44, 3286-3292.

Taylor, D.L., Linehan, J.C., Murray, D.W., Prell, W.L., 2012. Indicators of sediment and biotic mercury contamination in a southern New England estuary. Mar. Pollut. Bull. 64, 807-819.

Thorp, J.H., Covich, A.P., 2001. Ecology and classification of North American freshwater invertebrates. Academic Press, San Diego, California, USA. 297 – 325.

Travers, I., 1976. Mercury in the sediments of the Saint John River basin. Environmental Protection Service Report, 1-53.

Unesco, ICES, SCOR, IAPSO. 1981. Tenth report of the joint panel on oceanographic tables and standards. Unesco technical papers in marine science 36, 15-19.

van der Velden, S., Dempson, J.B., Evans, M.S., Muir, D.C.G., Power, M., 2013. Basal mercury concentrations and biomagnification rates in freshwater and marine food webs: Effects on Arctic charr (*Salvelinus alpinus*) from eastern Canada. Sci. Total Environ. 444, 531-542.

Weinkauf, M. 2015. Conductivity2Salinity.xlsx. https://www.marum.de/Software_and_Programs.html#Section28628. Accessed August 1, 2016.

Wiener, J.G., Krabbenhoft, D.P., Heinz, G.H., Scheuhammer, A.M., 2003. Ecotoxicology of mercury. In Hoffman DJ, Rattner BA, Burton GA, Cairns J, eds, Handbook of Ecotoxicology, 2nd ed. CRC Press, Boca Raton, FL, USA, 409–463.

Wyn, B., Kidd, K.A., Burgess, N.M., Allen Curry, R., 2009. Mercury biomagnification in the food webs of acidic lakes in Kejimkujik National Park and National Historic Site, Nova Scotia. Can. J. Fish. Aquatic Sci. 66, 1532-1545.

Zitko, V., Finlayson, B.J., Wildish, D.J., Anderson, J.M., Kohler, A.C., 1971. Methylmercury in Freshwater and Marine Fishes in New Brunswick, in the Bay of Fundy, and on the Nova Scotia Banks. Journal of the Fisheries Research Board of Canada. 28, 1285-1291.

3 General conclusions and future research

3.1 Summary of findings, implications, and future work

Overall, there was not a consistent trend in MeHg among taxa with increasing salinity in the Saint John River estuary. An increase in MeHg with salinity was observed for chironomids but no apparent relationship was observed for fish, amphipods, snails, sediments, and water. This inconsistency in trends was not as expected, as the hypothesis was that MeHg is regulated by salinity or marine influence and a decrease in MeHg with increasing salinity was predicted. Overall, my results do not support this hypothesis and I cannot identify sites where fish may pose a higher risk to their consumers based on salinity. Mine is one of only a few studies that has examined the site to site variability in MeHg within a single estuarine food web and assessed the small-scale changes along a single marine gradient. It is likely that there are many complex relationships with MeHg accumulation in aquatic biota and their environment. It is not just salinity that changed chemically along this gradient, but many factors, including some that were likely not measured in the present study. This work, however, will be very useful for future studies trying to isolate the environmental variables that predict MeHg in biota in other estuaries and the species that may be more conducive to answering that question. In future studies, it is recommended to also include additional predictor variables such as land use, land cover, and the chemical characteristics of dissolved organic matter or carbon (Buckman et al. 2017).

The MeHg or THg measured in biota, sediments, and water in the present study was similar to what has been found for similar species in other estuaries

(Eagles-Smith and Ackerman, 2009; Falandysz and Kowalewska, 1993; Payne and Taylor, 2010; van der Velden et al., 2013; Taylor et al., 2012; Buckman et al., 2017). This indicates that, in comparison, abiotic and biotic compartments of the Saint John River estuary do not exhibit MeHg contamination more than what is typically seen in an urbanized estuary. The majority of the fourspine stickleback were below toxicity thresholds for negative impacts on fish health (Beckvar et al., 2005) or below consumption guidelines (Health Canada, 2007). Again, this indicates that this species may not be adversely affected by MeHg. However, it is important to keep in mind that these stickleback are a small prey fish species, and it is likely that their consumers, such as predatory Striped Bass, Yellow Perch, American Eel, etc., would have much higher concentrations as a result of biomagnification of MeHg. In fact, these species have been shown to have very high THg concentrations within the Saint John River in a study published in 1975 (Dadswell, 1975), but current data are lacking and needed.

The biota used in this study exhibited characteristics that were appropriate for monitoring contaminants within an estuary. $\delta^{34}S$ and $\delta^{13}C$ isotope values of each taxa increased as expected along the marine gradient in biota, indicating they were typically residents at their site of capture (Fry and Chumchal, 2011; Fry, 2013). This makes them very useful for looking at specific site effects and for monitoring of contaminants as their concentrations may be linked to their habitat characteristics. The ease of capture of fourspine stickleback and their tolerance for freshwater, brackish, and marine environments makes them great for estuarine studies. In addition, Gammarid amphipods and chironomids, the common prey items for

fourspine stickleback, are easy to collect, making it easier to link contaminants from the diet to consumer.

A recommendation for future work on MeHg along a salinity gradient would be to expand the study to encompass additional freshwater sites and higher salinities, extending the gradient. The trends of MeHg accumulation with taxa observed in the present study were inconsistent with increasing salinity. However, in future studies these trends may be more clear or consistent if additional freshwater or marine sites beyond the brackish zone were added, making it a larger gradient in salinity and increasing the power for statistical analysis and the likelihood of seeing a biological effect. However, collecting the same species from freshwater to full marine environments may be difficult. Again, since fourspine stickleback and amphipods were linked in the food web, and can inhabit a broad range of salinities, these could be ideal test species for future work comparing accumulation across salinities. Beyond expanding to other freshwater sites and to marine environments, it would be interesting to study additional estuaries. In New Brunswick there are not many other rivers with a natural and gradual salinity gradient without barriers such as waterfalls or dams. However, additional estuaries with a gradient such as this, similar habitat characteristics and species could also be used to investigate the influence of salinity or estuarine gradients on MeHg.

The present study is the first study looking at MeHg in the Saint John River estuary since the mid-1970s (Travers, 1976; Dadswell, 1975). It is also the only study that examined MeHg concentrations at the base of the food web within the

estuary. The fish analyzed, fourspine stickleback, are a prey fish, rather than a fish that would be eaten by humans. The MeHg within these fish would therefore, be directly affecting larger predatory fish but not humans. A future investigation on the MeHg levels in larger predatory fish or fish-eating wildlife, such as loons, herons, or osprey, within the estuary would be an excellent addition to this research. Measuring the contaminants in Striped Bass, Atlantic Salmon, Yellow Perch, etc. would be more relevant for the indigenous communities or recreational fishers of New Brunswick who rely on freshwater fish of the Saint John River as a food source. While these larger fish and wildlife consumers are more relevant for investigating higher trophic level and human exposure to MeHg, they are also more mobile and would not be as easy to link to strictly freshwater or marine habitats because of this. Finally, these data can serve as a baseline for future MeHg contamination studies within the Saint John River estuary, which will become increasingly important as future use of the river and its resources continues to grow.

3.2 References

Beckvar, N., Dillon, T.M., Read, L.B., 2005. Approaches for linking whole-body fish tissue residues of mercury or DDT to biological effects thresholds. Environ. Toxicol. Chem. 24, 2094-2105.

Buckman, K., Taylor, V., Broadley, H., Hocking, D., Balcom, P., Mason, R., Nislow, K., Chen, C., 2017. Methylmercury Bioaccumulation in an Urban Estuary: Delaware River, USA. Estuar. Coasts, 1-13.

Dadswell, M., 1975. Mercury, DDT and PCB content of certain fishes from the Saint John River estuary, New Brunswick Paper presented to meeting of Canadian Society of Environmental Biologists, Fredericton, New Brunswick.

Eagles-Smith, C.A., Ackerman, J.T., 2009. Rapid changes in small fish mercury concentrations in estuarine wetlands: Implications for wildlife risk and monitoring programs. Environ. Sci. Technol. 43, 8658-8664.

Falandysz, J., Kowalewska, M., 1993. Mercury concentration of stickleback *Gasterosteus aculeatus* from the Gulf of Gdansk. Bull. Environ. Contam. Toxicol. 51, 710-715.

Fry, B., Chumchal, M.M., 2011. Sulfur stable isotope indicators of residency in estuarine fish. Limnol. Oceanogr. 56, 1563-1576.

Fry, B., 2013. Using stable CNS isotopes to evaluate estuarine fisheries condition and health. Isot. Environ. Health Stud. 49, 295-304.

Health Canada, 2007. Human Health Risk Assessment of Mercury in Fish and Health Benefits of Fish Consumption. 1–70. http://www.hc-sc.gc.ca/fn-an/alt_formats/hpfb-dgpsa/pdf/nutrition/merc_fish_poisson-eng.pdf. Accessed February 2, 2017.

Payne, E.J., Taylor, D.L., 2010. Effects of diet composition and trophic structure on mercury bioaccumulation in temperate flatfishes. Arch. Environ. Contam. Toxicol. 58, 431-443.

Taylor, D.L., Linehan, J.C., Murray, D.W., Prell, W.L., 2012. Indicators of sediment and biotic mercury contamination in a southern New England estuary. Mar. Pollut. Bull. 64, 807-819.

Travers, I., 1976. Mercury in the sediments of the Saint John River basin. Environmental Protection Service Report, 1-53.

van der Velden, S., Dempson, J.B., Evans, M.S., Muir, D.C.G., Power, M., 2013. Basal mercury concentrations and biomagnification rates in freshwater and marine

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Mercury in fish and invertebrates in the Saint John River estuary. Reinhart, B., Kidd, K., O'Driscoll, N., Curry, R.A.C., Pavey, S., 15-Jul-2017. International Conference on Mercury as a Global Pollutant, Providence, RI, USA.