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Mercury bioaccumulation in aquatic biota along a salinity gradient in the Saint John River estuary

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ARTICLE INFO

Article history:
Received 12 October 2017
Revised 15 January 2018
Accepted 7 February 2018
Available online 6 March 2018

Keywords:
Stickleback
Amphipod
Methylmercury
Biomagnification
Stable isotopes
Food web

ABSTRACT

Although estuaries are critical habitats for many aquatic species, the spatial trends of toxic methylmercury (MeHg) in biota from fresh to marine waters are poorly understood. Our objective was to determine if MeHg concentrations in biota changed along a salinity gradient in an estuary. 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, with salinities ranging from 0.06 to 6.96. Total mercury (proxy for MeHg) was measured in whole fish and MeHg was measured in a subset of fish, pooled invertebrates, sediments, and water. Stable sulfur ($\delta^{34}S$), carbon ($\delta^{13}C$), and nitrogen ($\delta^{15}N$) isotope values were measured to assess energy sources (S, C) and relative trophic level (N). There were increases in biotic $\delta^{13}C$ and $\delta^{34}S$ from fresh to more saline sites and these measures were correlated with salinity. Though aqueous MeHg was higher at the freshwater than more saline sites, only chironomid MeHg increased significantly with salinity. In the Saint John River estuary, there was little evidence that MeHg and its associated risks increased along a salinity gradient.

Introduction

While methylmercury (MeHg) is often found at high concentrations in fish, concentrations are typically lower in marine than freshwater species of similar trophic levels (Zitko et al., 1971; Luten et al., 1980). However, MeHg in estuarine environments has been less studied. Previous work on MeHg in estuarine food webs include broader spatial studies on fish (van der Velden et al., 2013; Fry and Chumchal, 2012; Evans and Crumley, 2005), and intensive sampling in Arctic through sub-tropical estuaries (Buckman et al., 2017; Taylor et al., 2012; Farmer et al., 2010). There have been equivocal patterns in MeHg in biota along estuarine salinity gradients, but total mercury (THg) concentrations in fish (a proxy for MeHg) can be lower in individuals from higher salinity habitats (Fry and Chumchal, 2012; van der Velden et al., 2013; Smylie et al., 2016). In addition, sediments from lower salinity sites have higher mercury (Hg) methylation rates (Compeau and Bartha, 1984, 1987; Blum and Bartha, 1980). Much of the existing literature supports this negative trend between MeHg and salinity, which could be due to increased sulfide in saline waters binding inorganic mercury (Hg (II)), making it less

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https://doi.org/10.1016/j.jes.2018.02.024
1001-0742/

available for methylation within this environment (Compeau and Bartha, 1984). Also, the decreased deposition/increased dilution of Hg in marine waters compared to terrestrial environments (Mason et al., 1994) would likely contribute to lower MeHg concentrations in marine habitats and organisms. Studies have also found no relationship between THg in wild fish and salinity (Evans and Crumley, 2005) or that their THg increases with salinity (Dutton and Fisher, 2011; Farmer et al., 2010). The mixed results most probably reflect the complex relationships between estuarine environmental factors (*e.g.*, salinity, percent forest cover, level of human development, total suspended solids, dissolved organic carbon) and MeHg production or availability to biota (Buckman et al., 2017).

Stable isotopes can be used to identify diet and to trace MeHg biomagnification through food webs (Clayden et al., 2017; Kidd et al., 2012). Nitrogen isotope ratios ($^{15}N/^{14}N$; expressed as $\delta^{15}N$) assess relative trophic level of consumers because they retain more of the heavier isotope, and it is generally accepted that this ratio increases by 3.4‰ ± 0.98‰ (average ± SD) with each trophic level (Post, 2002). Because MeHg also increases with trophic level, $\delta^{15}N$ can be used to quantify and contrast its biomagnification in aquatic food webs (Kidd et al., 2012; Clayden et al., 2017). Beyond identifying primary production fueling food webs (Fry, 2006; Svensson et al., 2007), carbon isotopes ($\delta^{13}C$; $^{13}C/^{12}C$) can measure how much feeding takes place in freshwater or marine environments for biota as $\delta^{13}C$ values increase with salinity, reflecting the enriched $\delta^{13}C$ of marine CO_2 (Fry, 2002, 2006). In addition, $\delta^{13}C$ increases by 0.39‰ ± 1.3‰ in consumers compared to their food (Post, 2002). Marine sulfur has higher isotope values ($\delta^{34}S$; $^{34}S/^{32}S$) compared to freshwater sources and, thus, can distinguish whether animals have been feeding on marine or freshwater food sources (Fry, 2002) because their values will be similar to those of their diet, *i.e.*, little fractionation occurs (McCutchan et al., 2003; Fry, 2013; Fry and Chumchal, 2011).

This study investigated concentrations of Hg, measured as THg, MeHg and Hg(II), in fish and invertebrates along a salinity gradient in an estuary. We hypothesized that Hg bioaccumulation in estuarine food webs was regulated by the degree of marine influence. We predicted a negative correlation between salinity and MeHg in biota based on marine dilution of Hg inputs to aquatic systems and evidence of decreasing concentrations of MeHg from freshwater to marine environments commonly seen in the literature (Compeau and Bartha, 1984, 1987; Blum and Bartha, 1980; van der Velden et al., 2013; Evers et al., 2005; Fry and Chumchal, 2012; Farmer et al., 2010; Smylie et al., 2016). If this prediction is true, then marine influence could mitigate the risk of MeHg toxicity that exists for fishes found in estuarine environments.

1. Methods

1.1. Study area

The Saint John River has a drainage area > 55,000 km^2 and flows into the Bay of Fundy at the City of Saint John, New Brunswick, Canada (Kidd et al., 2011; Metcalfe et al., 1976). The Bay of Fundy exhibits high tides up to 8 m in the Saint John Harbor, and the head of the tide occurs 135 km upstream (Kidd et al., 2011). The saline waters extend as far as 60 km upstream of the river mouth, creating a continuous salinity gradient from freshwater to the harbor (Metcalfe et al., 1976). The Saint John River estuary is a nursery ground for fish and hosts 35 species (Department of Fisheries and Oceans Canada, 2009). THg concentrations in sediments and large-bodied fishes were measured in the 1970s, but no studies have assessed MeHg in the estuary (Dadswell, 1975; Travers, 1976).

1.2. Field collections

Ten sites within the Saint John River estuary were selected along the salinity gradient ranging from freshwater to brackish water near the mouth of the river (Fig. 1; see also Table 1). The sites had shallow beaches conducive to seining (Curry et al., 2009). Site physico-chemical characteristics were sampled within a three-hour window after the high tide measured at Saint John. Dissolved oxygen (DO) was measured at 0.75 m below the surface using a calibrated YSI Multi-Meter Model 85 (2015 and 2016). From July 26 to September 4, 2016, HOBO Conductivity Data Loggers were deployed at each site recording conductivity (0.1 μS/cm) and temperature (0.01°C) every 5 min for roughly 24 hr for two neap tide and three spring tide cycles (n = 5 data sets with n = 283-). The loggers were deployed 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. It was calculated from each conductivity and temperature reading (Weinkauf, 2015) based on algorithms outlined by UNESCO/ICES/SCOR/IAPSO (1981) and assuming a pressure of 1 standard atmosphere. For each site, the average of the salinity data between the 10th and 90th percentiles was used for all subsequent analysis to remove data extremes. These averages were also used to represent salinity at the sites for both years because only point measures of salinity were collected in 2015. The average absolute difference between one-time YSI probe salinity measures in 2015 and the average of continuous HOBO measures in 2016 from the same site was 1.42 ± 1.56 (average ± SD will be reported throughout text).

Seine nets were used to collect fish from each site (Curry et al., 2009). Fourspine Stickleback (*Apeltes quadracus*) occurred at all sites and thus was selected for Hg analyses. At each site, 7–10 fish were weighed (0.01 g), measured for total length (0.1 cm), euthanized using MS-222 and sacrificed using spinal severance according the University of New Brunswick's Animal Care protocol (2016-1S-01), and frozen within a few hours of collection. We analyzed MS-222, and no THg was found at a detection limit of 3.75 μg/kg. Fish collections were completed within a three-hour window after high tide (Saint John) between August and October 2015 and in August 2016. Clips were taken from the caudal fin of each fish and stored in 95% ethanol in a freezer for subsequent genetic analysis.

Invertebrate collections were done at each site using sweep nets, kick nets, and rock-picking. Snails (genera *Physa, Fossaria,* and *Viviparus*) and amphipods (*Gammarus*) were the common invertebrates found during collections in August 2015 (n = 1 sample per site in 2015, except no snails were present at site 9 in 2015). We pooled all individuals to make one pooled sample per site for both amphipods and snails to obtain sufficient mass for all chemical analyses (>30 mg dry weight (dw)). In 2016, snails and amphipods were collected on

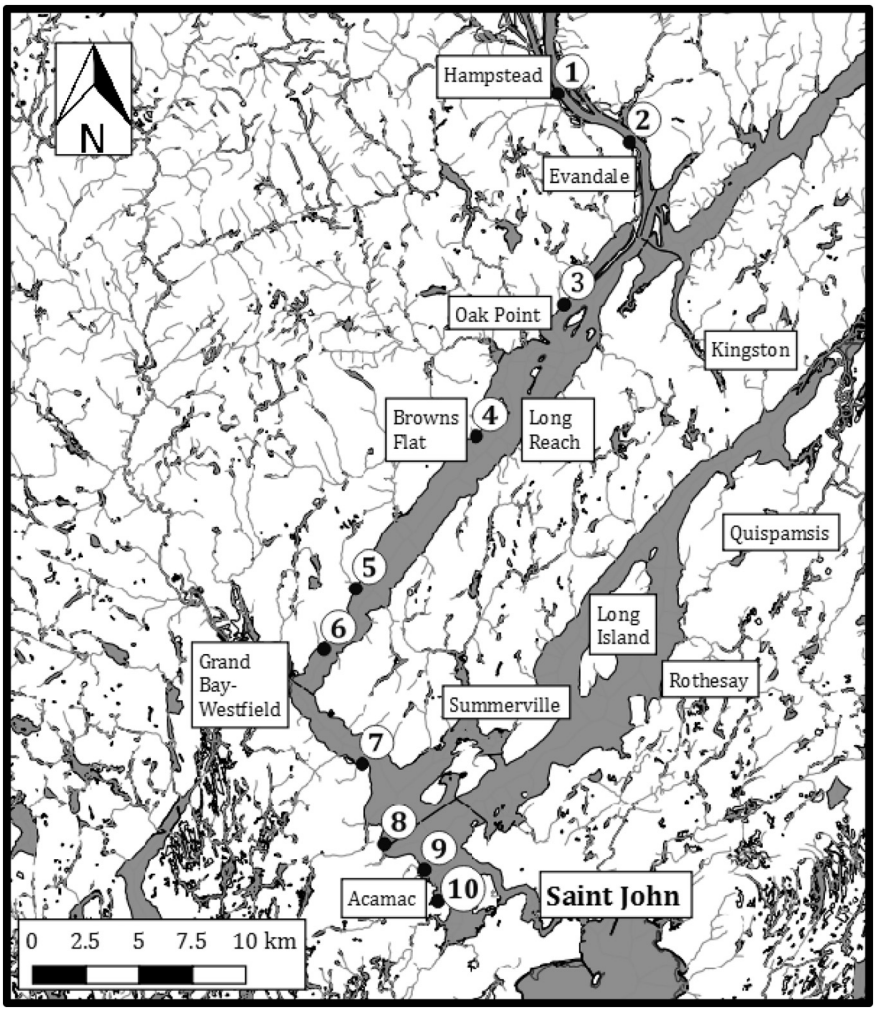

Fig. 1 – Ten sites sampled along the Saint John River estuary, New Brunswick, Canada, in 2015 and 2016 with local communities shown in boxes.

three dates from July to August (n = 3 samples per site per taxa). Chironomids were also collected over this period in 2016 and pooled for adequate mass (n = 1 sample per site). Invertebrates were placed on ice immediately after collection and were frozen within 5 hr until laboratory analyses were done.

On one occasion, amphipods were collected from each of the ten sites and either processed as described above or kept alive to clear their guts for comparisons of isotope and Hg measures between processed and gut-cleared samples. Amphipods were kept alive in aerated containers of site water overnight before sacrificing and processing the samples as described. A paired,

Table 1 – Location and water quality measures (2016 only) along the Saint John River estuary, New Brunswick, Canada. Salinity, temperature (Temp), and conductivity (Cond) were averages (5 min intervals, see Section 1.2), dissolved oxygen (DO) was a single measure, and nutrients were analyzed in the lab (n = 1/site). Data reading "<" are below detection limits.

Site	Latitude	Longitude	Salinity	Temp	Cond	DO	Nitrate + Nitrite	Total Phosphorus	Total Organic Carbon
	N	W		(°C)	(μS/cm)	(mg/L)	(mg/L)	(mg/L)	(mg/L)
1	45.612981	−66.074303	0.06	23.3	110	5.94	0.13	0.011	8.3
2	45.592456	−66.031182	0.07	23.2	152	6.73	0.14	0.013	6.8
3	45.523946	−66.071176	0.47	23.2	1008	6.34	0.10	0.017	5.6
4	45.468118	−66.124197	1.77	22.5	3431	7.94	0.10	0.014	3.2
5	45.404007	−66.197201	1.59	22.7	3118	6.78	0.11	0.026	4.4
6	45.378374	−66.215902	1.73	22.9	3372	8.15	0.14	0.013	3.6
7	45.329723	−66.193396	4.09	21.9	7404	8.93	< 0.05	0.012	2.3
8	45.296097	−66.180258	6.45	21.6	11,346	7.75	< 0.50	0.015	1.7
9	45.284868	−66.156047	6.62	21.8	11,852	8.08	< 0.50	0.015	1.8
10	45.271931	−66.148314	6.95	21.4	12,523	6.40	< 0.05	0.023	1.5

Welch's two sample *t*-test was used to assess differences in Hg or isotopes between gut-cleared *versus* non-gut-cleared amphipods.

Sediment was sampled at each site on August 30, 2016. The top 5 cm of sediment from a location adjacent to the seine sites was sampled in triplicate using an Ekman dredge and core tubes that were precleaned with 5% nitric acid and site water before each sample; the sediments were put into Ziploc bags and frozen. Water samples were also collected at each site with powder-free gloves at a depth of 10 cm in 3 pre-cleaned, 1- L amber bottles from August 6 to September 2, 2016. These were kept in coolers in the field and transported back to the laboratory, where they were filtered with 0.45- μm polyestersulfonate filters upon arrival, preserved with 1% concentrated trace-metal grade sulfuric acid, and stored at 4°C until analyzed. Field blanks (3) of ultra-pure water were also taken into the field, opened with gloves, closed, and returned to the lab for processing. A separate water sample (unfiltered) was also collected from each site between August 29 and September 2, 2016 and was submitted to an analytical lab for full inorganic chemistry analysis (Research and Productivity Council of New Brunswick in Fredericton, New Brunswick).

1.3. *Laboratory processing*

All glassware and utensils were washed with 5% nitric acid bath and rinsed with ultrapure water and dried before use in processing samples. In the lab, fish were dissected and their intestinal tracts were removed before THg and isotope analyses. 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 hr.

All fin clips taken from fish from each site in 2015 were genetically verified to be *Apeltes quadracus* (3% produced no results). 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.

For each invertebrate sample, snails and amphipods were identified to genus. For chironomids, predatory taxa were removed from the sample (Family Tanypodinae). Within pooled samples, n = 33–580, 4–15, and 69–565, for the number of individual amphipods, snails, and chironomids, respectively. All pooled samples were placed into glass vials and freeze dried for 48 hr, then homogenized using glass rods (invertebrates) or a ceramic knife on a glass cutting board (whole body fish).

1.4. *Hg analyses*

MeHg and inorganic Hg(II) analyses (latter analyzed in 2016 only) were 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 site) and for all invertebrate samples at the Center for Analytical Research on Environment laboratory at Acadia University (Wolfville, NS) with a Brooks Rand Inc. automated MERX system using aqueous-phase ethylation, purge and trap, a gas chromatograph (GC), and cold vapor atomic fluorescence spectrometry with a Model III fluorescence detector. MeHg analysis was also done for sediment and water samples. The methods used were adapted from the US EPA Method 1630 and Brooks Rand Analytical Notes. Hg species (elemental Hg(0), MeHg(I), and Hg(II)) are separated in the capillary GC before entering the pyrolysis column where they are combusted into elemental Hg(0) before entering the detector. The species reach the spectrometer at different times based on their original charge (MeHg(I) and Hg(II)), which determines the speed and attraction as it moves through the system. THg was calculated by adding MeHg and Hg(II) values together for 2016 samples. The quality assurance/quality control (QAQC) included method blanks, internal standards, certified reference materials (CRM), duplicates, and spiked samples (for water only).

Dried animal tissues (10 mg) were digested in a 25% KOH in methanol (MeOH) solution, shaken for 1 hr, heated at 95°C for 1 hr and cooled overnight at room temperature (Edmonds et al., 2012). An aliquot of 20–40 μL of each digested sample was analyzed for MeHg and Hg (II) (latter in 2016 only) as described above. Average percent recovery of MeHg in CRM (DOLT-5 and DORM-4 from National Research Council of Canada), 50- ng MeHg standards, and concentrations of calibration blanks were 105% ± 13% (n = 11), 105% ± 7% (n = 20), and 7.98×10^{-6} μg/kg dw (n = 43), respectively. Average 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 calculated as 3 times the SD of blanks for all MeHg analyses. For Hg(II), CRM DORM-4, standards, and calibration blanks had average recoveries/concentrations of 105% ± 12% (n = 5), 86% ± 11% (n = 13), and 8.22×10^{-6} μg/kg dw (n = 18), respectively. Average 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. %MeHg was calculated by adding MeHg and Hg (II) for invertebrates and fish.

Water samples (20 mL) were analyzed for MeHg using methods as in Klapstein et al. (2016), with a pH adjustment to 4.5 with 25% KOH. Matrix spike recoveries, replicates, calibration curves, and check standards were used to validate these analyses. There is currently no reliable CRM for MeHg in water available, therefore, spike recoveries in individual samples allow for direct ethylation recovery or efficiency corrections based on each individual water sample matrix. Calibration blanks, field blanks, and standards had average 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 samples (average recovery 100% ± 11%, n = 1 spike/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 statistical analysis. This was done for two samples from site 4 and for one sample from site 3.

As in the Brooks Rand Application Note: Extraction of MeHg from sediments and soils, sediments (0.5 g) were extracted by shaking with 1.5 mol/L KBr in 5% H_2SO_4 and

1 mol/L $CuSO_4$ into 10 mL of dichloromethane (DCM). A subsample of 2 mL of DCM extract was added to deionized water and mercury transferred to aqueous solution by heating to 70°C for 3 hr with Teflon boiling chips. Aqueous samples were then analyzed for MeHg using the same methods previously described. Calibration blanks, CRM (ERMCC580, European Reference Materials), and standards had average concentrations/recoveries of 8.7×10^{-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.

THg was analyzed in 10 mg of homogenized dry individual fish tissue at the University of New Brunswick Saint John using a Milestone Direct Mercury Analyzer. Wet weight THg concentrations were calculated using moisture content of individual fish. The average percent recovery was 90% ± 3% (n = 28) and 98% ± 7% (n = 28) for the certified reference material (CRM) DORM-4 (fish protein CRM, National Research Council of Canada) and the 10- ng liquid Hg standard, respectively. The average concentration of THg in the 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. All concentrations of THg and MeHg are reported in dry weight (dw = μg/kg) unless otherwise stated.

1.5. Stable isotope analyses

$\delta^{13}C$ and $\delta^{15}N$ levels 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 Stable Isotopes in Nature Laboratory (SINLAB), University of New Brunswick in Fredericton, NB. In 2015, sulfur isotope levels were analyzed in the SINLAB and in 2016 they were analyzed by the GG Hatch – Stable Isotope Laboratory using an Elementar Micro Cube Elemental Analyzer coupled with a DeltaPlus XP isotope ratio mass spectrometer. A subset of 2015 samples was re-run in 2016 for $\delta^{34}S$ to make an inter-lab comparison. The average difference from the SINLAB and Hatch measurements was −0.62% ± 0.46‰ (n = 12; paired t-test $p < 0.01$). Dried, homogenized samples were weighed into tin capsules and analyzed. Approximately 1 mg and 4–5 mg of animal tissues were used for $\delta^{13}C$, $\delta^{15}N$ 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 C and N analysis were ammonium sulfate with a known value of 20.3‰ and average of 20.45‰ ± 0.09‰ (n = 7), and polyethylene foil with a known value of −32.15‰ and an average of −32.19‰ ± 0.05‰ (n = 5, both certified by International Atomic Energy Agency). For S analysis, reference materials for the SINLAB were an in-house Bass Check Standard with a known value of 3.8‰ and average of 3.85‰ ± 0.58‰ (n = 12) and Pollock Sulfur Standard with a known value of 17.9‰ and average 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 averages between −0.42‰ ± 0.31‰ and −0.78‰ ± 0.18‰ (n = 24). The average absolute difference between duplicates was 0.21‰ ± 0.26‰ (n = 30) for N, 0.27‰ ± 0.60‰ for C (n = 30), 0.47‰ ± 0.36‰ for S (n = 11) at the SINLAB and 0.68‰ ± 0.59‰ for S (n = 21) at the Hatch Lab.

1.6. Data analyses

THg data in fish were length adjusted because THg increases with size (Evans et al., 2005), and fish were different in sizes among sites and years. This was done by adding the residuals from the linear relationship between THg and fish length to the THg values (as in Swanson and Kidd, 2010; THg-adj). These adjusted values were used in subsequent statistical analyses of fish data. The biomagnification factor (BMF; Gobas et al., 2009), which is the ratio of Hg in the consumer to that of the diet, was also calculated for Fourspine Stickleback using their THg and amphipod MeHg values to represent consumed food (amphipods were the main prey items — see Section 2.3). Invertebrate biota-sediment accumulation factors (BSAF; ratio of MeHg in the consumer to that in sediment; Calle et al., 2015) were also calculated for each site.

Trophic position (TP) of stickleback within each site was calculated.

$$TP = \lambda + \left(\delta^{15}N_{consumer} - \delta^{15}N_{base}\right)/\Delta_n \text{ (Post, 2002)}$$

where $\delta^{15}N_{consumer}$ was the stickleback value; $\delta^{15}N_{base}$ was the average value among invertebrates within each site; Δ_n was the enrichment factor for $\delta^{15}N$ which we assumed was 3.4‰ (Post, 2002), and λ = 2 was the trophic position of the primary consumers (Cabana and Rasmussen, 1996). For invertebrate TP calculations, sediment values were used for $\delta^{15}N_{base}$ and λ = 1.

The proportion of diet from marine sources for the sticklebacks (%Marine; as for $\delta^{13}C$ in Post, 2002) was calculated for each site using the fish $\delta^{34}S$ data and a two-end-member mixing model assuming no fractionation and based on average $\delta^{34}S$ values of all invertebrates at the freshwater site (salinity = 0.06) and of those at the brackish site (salinity = 6.96). It was not calculated for invertebrates as there was no food source assessed. %Marine values were held rounded between 0 and 100%.

$$\%\text{Marine} = \left(\delta^{34}S_{consumer} - \delta^{34}S_{freshwater\ invertebrates}\right) / \left(\delta^{34}S_{marine\ invertebrates} - \delta^{34}S_{freshwater\ invertebrates}\right) * 100\%$$

Each bivariate plot for mercury levels and the biotic/abiotic variables collected was examined to assess the potential linearity. The data sets were small (n = 10 to 96), and parametric tests were not appropriate. We chose to use a Pearson correlation coefficient (α = 0.05) to describe the bivariate associations. Our objective was not to describe relationships, rather it was to identify potential links between mercury and other variables that could be explored in future studies. Note that Spearman correlations resulted in very similar trends for those data that were not normal, but only Pearson's coefficients are reported herein. Comparisons among measures were based on individual samples, *e.g.*, Hg and isotope values measured in the same fish, or where there was a single value per site, such as for salinity and DO, average Hg values in biota or abiotic samples

were used. Any samples of *Viviparus* snails were excluded from these analyses because their MeHg concentrations were five times higher than other snail taxa, which was most probably related to different life histories (see Section 3.1). Analyses for stickleback were done separately for each year because there was a significant year-site ANOVA interaction for MeHg ($p = 0.03$, see Section 2.2). Years were combined for amphipods and snails because sample sizes were too small for separate analysis. Analysis for chironomids, water and sediments were from 2016 only.

2. Results

2.1. Water quality

Various water quality parameters are reported in Table 1. Average salinity of the sites ranged from 0.06 to 6.96 and sites were significantly different ($p < 0.001$; Fig. 2).

2.2. Hg

THg in fish (THg-adj) differed between years with averages of 428 ± 24 μg/kg (in dry weight unless otherwise stated) and 689 ± 18 μg/kg for 2015 and 2016, respectively (year:site interaction $p < 0.03$, site effect $p < 0.01$, year effect $p < 0.01$). There were no significant correlations between THg-adj and salinity within each year ($p > 0.05$; Fig. 3; Table 2). MeHg levels in amphipods, snails, chironomids, sediment, and water samples were lower than those observed in fish, with averages of 85 ± 27 μg/kg, 71 ± 29 μg/kg, 52 ± 24 μg/kg, 0.34 ± 0.17 μg/kg, and 0.08 ± 0.03 ng/L, respectively, across all sites (Fig. 3; Table 2). Only MeHg levels in chironomids were significantly correlated with salinity ($r = 0.83$, $p < 0.01$). Sediment MeHg increased and water MeHg decreased with salinity, but these were not significant trends (sediment $p = 0.07$, water $p = 0.11$).

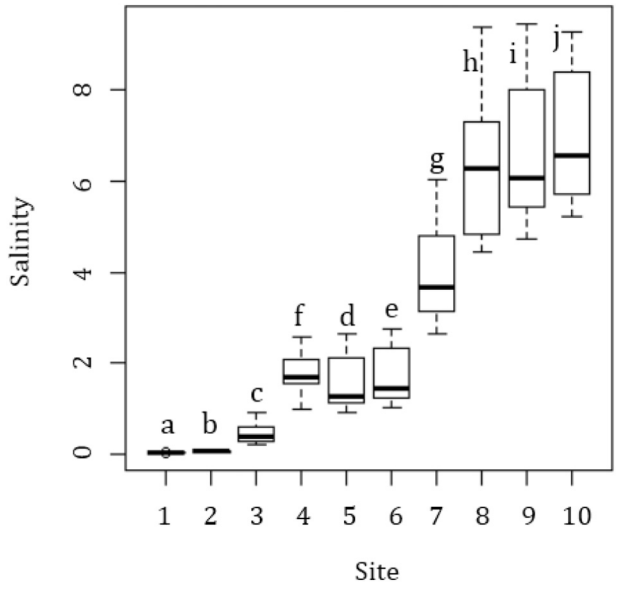

Fig. 2 – Continuous salinity measures taken at 10 sites in the Saint John River estuary, New Brunswick, Canada, in 2016 (see also Fig. 1). Statistically significant differences are represented by different letters ($p < 0.05$).

The average Hg (II) (2016 only) for stickleback across sites was 29 ± 21 μg/kg, and there was a negative trend with salinity ($r = -0.72$, $p < 0.02$; Fig. 4; Table 2). There were no trends detected for invertebrates ($p > 0.12$). Average concentrations were 18 ± 7 μg/kg, 65 ± 18 μg/kg, and 46 ± 28 μg/kg in amphipods, snails, and chironomids, respectively.

The % MeHg in biota ranged 88%–98%, 38%–91%, 24%–75%, and 33%–76% in stickleback, amphipods, snails, and chironomids, respectively. It increased significantly with increasing salinity in stickleback ($r = 0.67$, $p = 0.04$), snails ($r = 0.81$, $p = 0.02$), and chironomids ($r = 0.77$, $p = 0.01$), but there was no trend for amphipods ($p = 0.69$; Table 2).

The average BMF for stickleback was 6.6 and 7.5 for 2015 and 2016, respectively (range = 3.4 to 13.9), and values were not significantly correlated with salinity across sites ($p > 0.44$ for both years). The ranges in BSAFs were 171 to 1180, 137 to 246, and 68 to 431 for amphipods, snails, and chironomids, respectively. These, too, did not change significantly with salinity ($p > 0.17$ for all taxa).

The gut-cleared amphipods (average 16.2 ± 5 μg/kg) had similar Hg (II) concentrations to those of the non-gut-cleared amphipods (average 15 ± 3 μg/kg) from the same sites in 2016 ($p = 0.29$). MeHg was significantly different ($p = 0.05$) between gut-cleared (average 109 ± 18 μg/kg) and non-gut cleared (average 100 ± 17 μg/kg) samples. The average percent change in MeHg and Hg (II) from non-gut-cleared to gut-cleared was −8% ± 12% and −4% ± 19%, respectively.

Total Hg (THg-adj) in fish increased significantly with their trophic position (TP; $r = 0.37$, $p < 0.01$; 2015) and $\delta^{15}N$ (measured in 2015 $r = 0.27$, $p = 0.01$, and 2016 $r = 0.28$, $p = 0.01$). MeHg significantly increased with $\delta^{15}N$ values in sediments ($r = 0.48$, $p = 0.01$) and in chironomids ($r = 0.72$, $p = 0.02$). In stickleback from 2016, Hg(II) was negatively correlated with % Marine ($r = -0.72$, $p = 0.02$) and $\delta^{34}S$ values ($r = -0.70$, $p = 0.02$), while % MeHg increased with $\delta^{34}S$ ($r = 0.66$, $p = 0.04$). Similarly, Hg(II) decreased with $\delta^{34}S$ values in chironomids ($r = -0.78$, $p = 0.01$), while their % MeHg increased with $\delta^{34}S$ ($r = 0.89$, $p < 0.01$). Snail Hg(II) also showed a significant decrease with $\delta^{13}C$ values ($r = -0.52$, $p = 0.01$), while % MeHg increased significantly with $\delta^{13}C$ values in chironomids ($r = 0.73$, $p = 0.02$).

Hg measures in biota were also correlated with concentrations of this element in abiotic samples. THg-adj in fish ($r = 0.63$, $p = 0.05$) and MeHg in amphipods ($r = 0.78$, $p = 0.01$) significantly increased with water MeHg. Sediment MeHg was also positively related to MeHg ($r = 0.82$, $p = 0.01$) and % MeHg in snails ($r = 0.88$, $p < 0.01$). However, there were no significant correlations observed between average fish Hg measures and average invertebrate MeHg from each site.

2.3. Diet and trophic position of biota

Generally, the variation in $\delta^{15}N$ values was low within sites for each sample type (Fig. 5). Fourspine Stickleback had the highest $\delta^{15}N$ values of all samples, and their average TP was 3.48 ± 0.15 and 3.44 ± 0.09 in 2015 and 2016, respectively. TP was positively correlated with salinity for 2015 stickleback only ($r = 0.68$, $p = 0.01$; Table 3), and negatively correlated

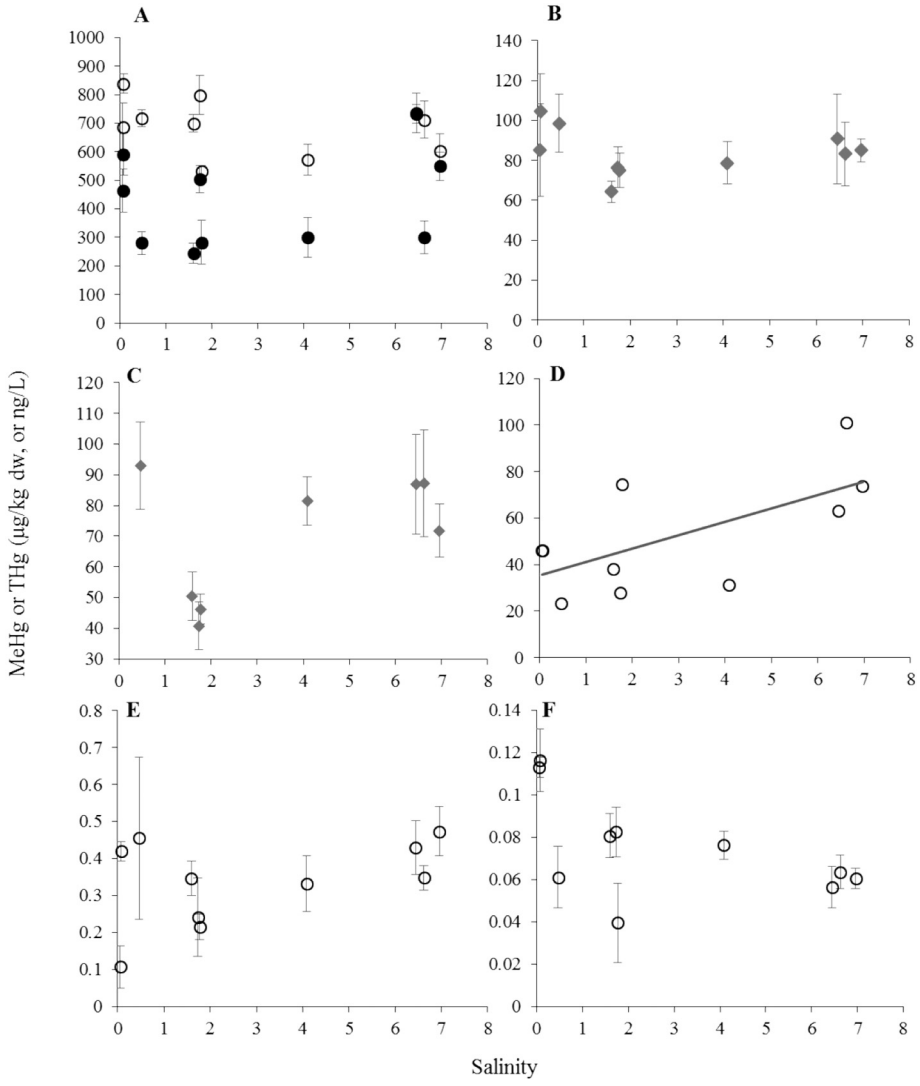

Fig. 3 – THg (Fourspine Stickleback only) or MeHg in samples collected along a salinity gradient in the Saint John River estuary, New Brunswick, Canada. Levels are in µg/kg dry weight for Fourspine Stickleback (A; *n* = 7–10/site), amphipods (B; *n* = 3–4/site), snails (C; *n* = 3–4/site; excluding genus *Viviparus*), chironomids (D; *n* = 1/site), and sediments (E; *n* = 3/site); water concentrations are in ng/L (F; *n* = 3/site). Values are averages ±1 SE for 2015 (solid circles), 2016 (open circles), or both years combined (gray diamonds). Trend lines indicate a significant correlation ($p < 0.05$).

Table 2 – Pearson correlation coefficients between mercury levels in different samples and measured biotic and abiotic variables from the Saint John River estuary, New Brunswick, Canada: MeHg (A); Hg(II) (B); and %MeHg (C). Significant correlations are identified with * $p < 0.05$.

	Sample type	Salinity	%Marine	$\delta^{34}S$	$\delta^{13}C$	TP	$\delta^{15}N$	Water MeHg	Sediment MeHg
A	Stickleback 2015	0.29	−0.19	−0.18	−0.02	*0.37	*0.27		
	Stickleback 2016	−0.28	−0.19	−0.14	−0.12	-0.18	*0.28	*0.63	0.04
	Amphipods	−0.26		−0.41	0.16	0.03	0.02	*0.78	−0.27
	Snails	0.65		−0.17	−0.26	−0.20	−0.06	−0.21	*0.82
	Chironomids	*0.83		0.41	0.44	0.16	*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	*−0.70	−0.54	0.33	−0.04		
	Amphipods	−0.20		0.01	−0.21	−0.04	0.31		
	Snails	*−0.60		*−0.04	*−0.52	0.65	*−0.38		
	Chironomids	−0.38		*−0.78	−0.49	0.39	0.26		
C	Stickleback 2016	*0.67	0.58	*0.66	0.52	−0.40	0.07	−0.29	0.03
	Amphipods	−0.15		−0.21	0.16	0.33	−0.23	0.42	0.01
	Snails	0.81		−0.07	−0.06	−0.46	−0.29	− 0.09	*0.88
	Chironomids	*0.77		*0.89	*0.73	−0.19	0.22	−0.47	0.35

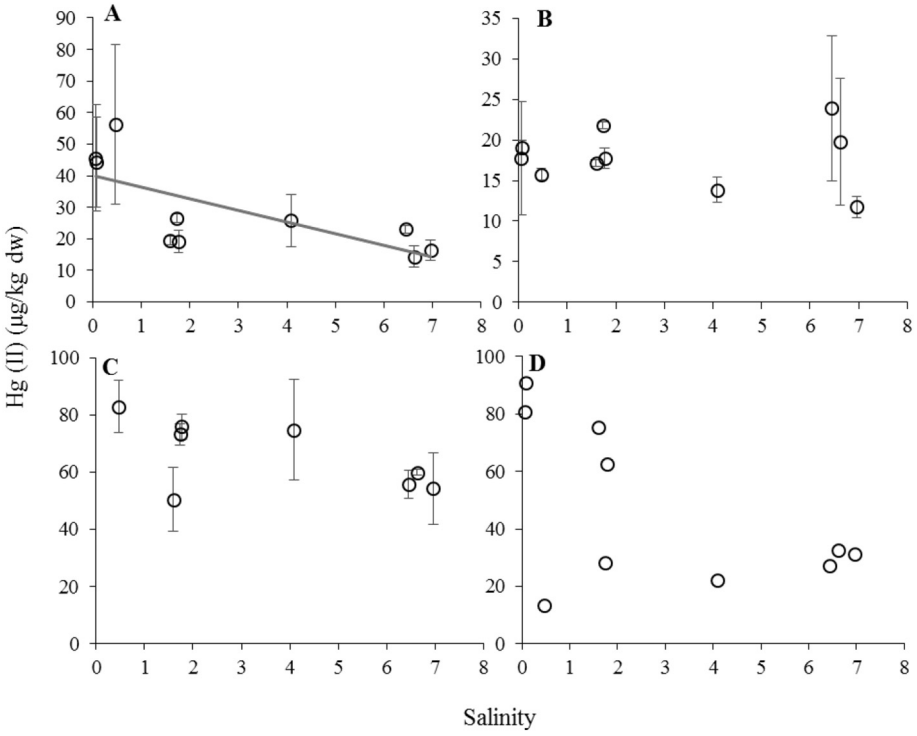

Fig. 4 – Hg (II) concentrations in biota sampled along a salinity gradient in the Saint John River estuary, New Brunswick, Canada, in 2016. Levels are µg/kg dry weight for Fourspine Stickleback (A; *n* = 10/site), amphipods (B; *n* = 2–3/site), snails (C; *n* = 3/site; excluding genus *Viviparus*), and chironomids (D; *n* = 1/site). Averages ±1 SE are reported for each value. Trend lines indicate a significant correlation (*p* < 0.05).

for amphipods ($r = -0.77$, $p = 0.01$). $\delta^{15}N$ values were only significantly correlated with salinity in sediments ($r = 0.92$, $p < 0.01$). Stickleback had average $\delta^{15}N$ values of 10.98‰ ± 0.50‰, and 11.01‰ ± 0.37‰ in 2015 and 2016, respectively. Amphipods, snails, and chironomids had averages of 6.05‰ ± 0.49‰, 6.21‰ ± 0.77‰, and 5.99‰ ± 0.76‰, respectively, and their overall TP values were 1.1 ± 0.20, 1.70 ± 0.17, and 1.59 ± 0.22, respectively. $\delta^{15}N$ values were lowest in sediments, with an average of 3.98‰ ± 0.60‰ across all sites.

Among site variability in $\delta^{13}C$ for each taxon was high (Fig. 6), and there were positive trends with salinity for all samples ($p < 0.04$; Table 3). The ranges in site averages 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.

Average $\delta^{34}S$ values among sites ranged 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 (Fig. 7). There were similar spatial trends to those of $\delta^{13}C$, with an increase in $\delta^{34}S$ for all biota from freshwater to higher salinity sites (Table 3). Biota sampled at sites with salinity ≥1.59 (site 4 and those towards the estuary mouth) had consistently high $\delta^{34}S$ values, reflective of marine sulfur sources, whereas biota sampled from sites 1 to 3 with salinities <0.47 had $\delta^{34}S$ values that were distinctly lower. % Marine and $\delta^{34}S$ in 2015 stickleback were positively correlated to salinity ($r = 0.70$, $p = 0.03$; $r = 0.70$, $p = 0.02$, respectively). $\delta^{34}S$ was also correlated with salinity for amphipods ($r = 0.80$, $p < 0.01$), snails ($r = 0.74$, $p = 0.01$), and chironomids ($r = 0.76$, $p = 0.01$). Sediment values did not show the same trend as biota and were not correlated with salinity (Fig. 7; $p = 0.09$).

Over both years, the diet (gut contents) of stickleback consisted of amphipods (in 79% of fish), chironomids (in 28% of fish), zooplankton (in 19% of fish), and other dipterans (in 15% of fish) based on presence-absence information.

There were no differences in $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ of amphipods that were gut-cleared and non-gut-cleared ($p > 0.20$).

3. Discussion

3.1. Hg

Although there was some evidence that MeHg concentrations in biota changed spatially along the Saint John River estuary – they were highest in chironomids from saline sites – fish, other invertebrates, sediments, and water did not show any spatial trends. Buckman et al. (2017) also found no relationship between salinity and MeHg in fish, sediments, or water. However, they did report that MeHg in suspended particulates and Grass Shrimp (*Palaemonetes* sp.) increased at more saline sites, mirroring the trends we observed for chironomids. Dutton and Fisher (2011) also report increased uptake of MeHg in the small-bodied fish *Fundulus heteroclitus* with increasing salinity. Buckman et al. (2017) mentioned that MeHg bound to marine dissolved organic carbon (DOC) may be more bioavailable than that bound to terrestrial DOC, thus explaining their spatial trends. In addition, sulfate and chloride ions, which are higher

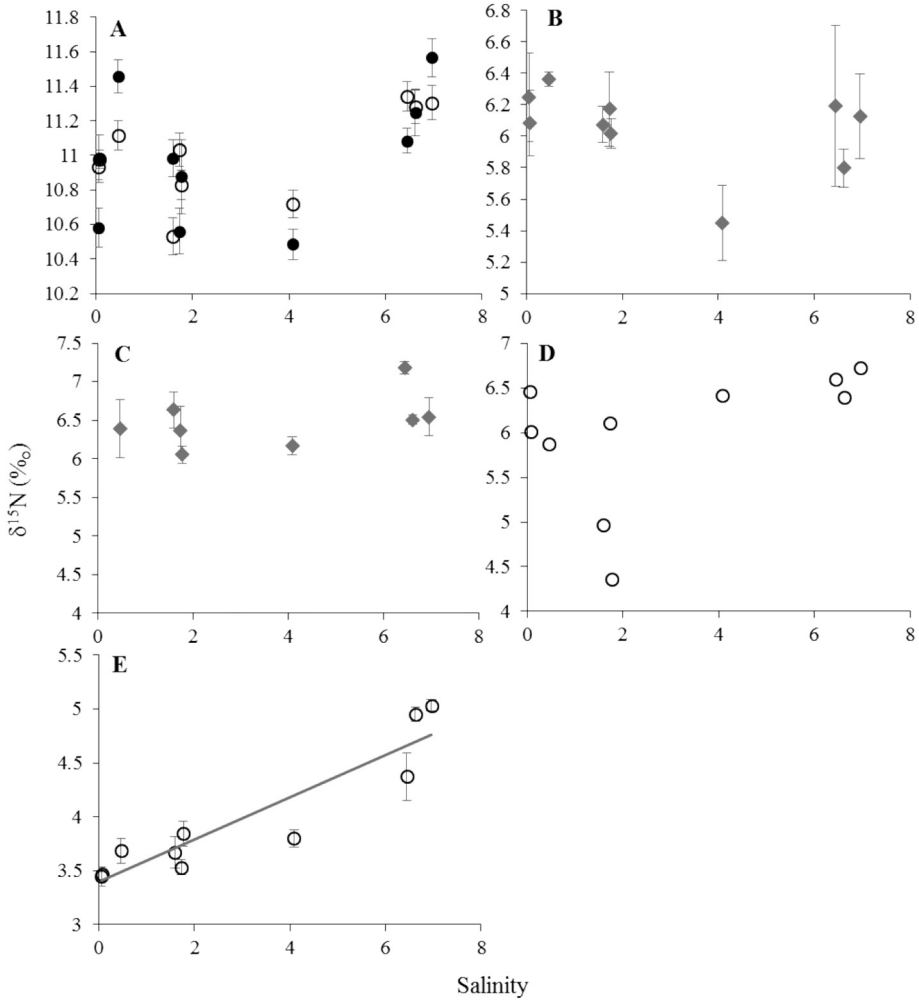

Fig. 5 – Mean $\delta^{15}N$ values (‰) measured in Fourspine Stickleback (A), amphipods (B), snails (C, excluding genus *Viviparus*), chironomids (D), and sediments (E) along a salinity gradient within the Saint John River estuary, New Brunswick, Canada. Figures show averages ± SE for 2015 (solid circles), 2016 (open circles), or both years combined (gray diamonds). Trend lines indicate a significant correlation ($p < 0.05$).

in more saline waters, may also increase the production or bioavailability of MeHg (Gilmour et al., 1992; Ndu et al., 2012). It is possible that these processes also affected MeHg bioaccumulation in chironomids at the brackish water sites in the Saint John River estuary, but it does not explain why other invertebrate taxa, water, and sediments from the higher salinity sites were not also higher in MeHg.

Table 3 – Pearson correlation coefficients of trends between salinity and isotope values ($\delta^{13}C$, $\delta^{15}N$, or $\delta^{34}S$) or derived values (% Marine and trophic position - TP) for samples from the Saint John River estuary, New Brunswick, Canada. Significant correlations are identified with * ($p < 0.05$).

Sample type	%Marine	$\delta^{34}S$	$\delta^{13}C$	TP	$\delta^{15}N$
Stickleback 2015	*0.70	*0.70	*0.91	*0.68	0.33
Stickleback 2016	0.59	0.60	*0.94	−0.48	0.58
Amphipods		*0.80	*0.91	*−0.77	−0.09
Snails		*0.74	*0.36	−0.45	0.62
Chironomids		*0.76	*0.69	−0.25	0.48
Sediments		−0.56	*0.90		*0.92

Although weak negative trends between salinity and MeHg concentrations were suggested for 2016 fish, amphipods, and water from this study, they were non-significant, and therefore, provided no evidence to support our original prediction that MeHg in the food web would decrease along the salinity gradient. This combination of non-significant and positive trends (described above) in biota MeHg with salinity is most probably reflective of the complexity of Hg dynamics in an estuarine ecosystem. There may be inconsistent effects of salinity on MeHg within the Saint John River ecosystem, or additional environmental drivers influencing the production and uptake of MeHg within the estuary that we did not capture in the endpoints assessed herein. Buckman et al. (2017) suggest that environmental variables such as carbon sources, bioavailability, mixing, marsh presence, level of human development, *etc.*, can have a strong influence on MeHg concentrations at different sites within a single estuary. These factors were not assessed in the current study. In addition, the gradient in salinity in the Saint John River estuary may have not been large enough to show an effect on

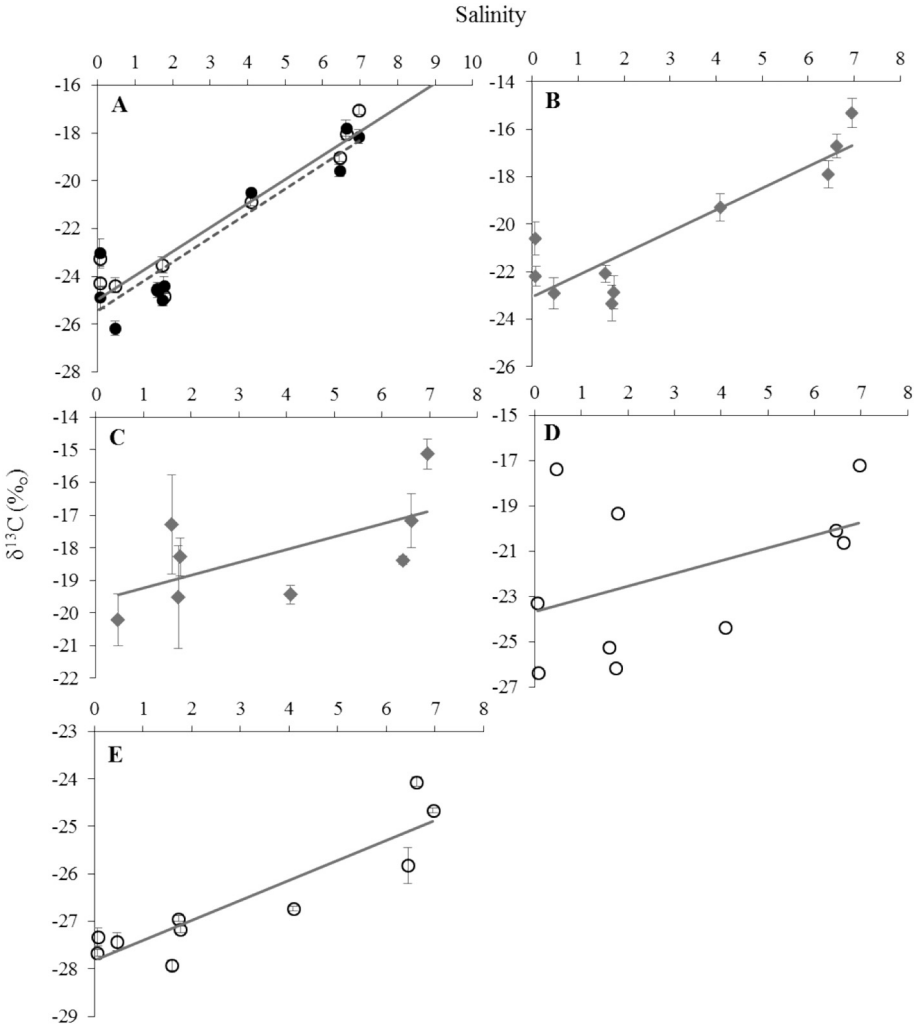

Fig. 6 – Mean $\delta^{13}C$ values (‰) measured in Fourspine Stickleback (A), amphipods (B), snails (C, excluding genus *Viviparus*), chironomids (D), and sediments (E) along a salinity gradient within the Saint John River estuary, New Brunswick, Canada. Figures show averages ± SE for 2015 (solid circles), 2016 (open circles), or both years combined (gray diamonds). Trend lines indicate a significant correlation ($p < 0.05$) and the dashed line represents 2015 stickleback only.

MeHg bioavailability and bioaccumulation. Other studies that show salinity influences on mercury endpoints in biotic and abiotic samples had salinity ranges from 0.3 to 32.6 (Taylor et al., 2012), 0.1 to 20.8 (Buckman et al., 2017), and 0 to 25 (Fry and Chumchal, 2012; Fry, personal communication, July 2017). Therefore, other than for chironomids, we cannot provide any strong evidence to support the hypothesis that salinity regulates MeHg concentrations in the Saint John River estuary, thereby increasing or decreasing risk of its toxicity to most biota studied herein.

Though relationships were only significant for Fourspine Stickleback and snails, there were decreasing concentrations of Hg(II) with salinity for each taxon in the Saint John River estuary. No field studies were found that assessed Hg(II) concentrations along a salinity gradient, but increased uptake of Hg(II) at lower salinities is commonly observed in lab experiments on fish and invertebrates (Pan and Wang, 2004; Laporte et al., 1997; Wang and Wang, 2010). This may be due to an increase in the unbound and bioavailable metal ions (as suggested by Pan and Wang, 2004) and reduced sediment partitioning of Hg(II) (Turner et al., 2001) at lower salinities; both likely contribute to the spatial patterns observed herein. In contrast, in lab studies Stenzler et al. (2017) showed that Hg (II) bioavailability to *Escherichia coli* increases with ionic strength. They suggest that ionic strength may alter properties of the bacterial cell membrane for uptake of the metal. Similarly, Dutton and Fisher (2011) found that Hg(II) uptake increased in fish *Fundulus heteroclitus* with increasing salinity in the lab. The decreasing concentrations of Hg(II) in biota from fresh to saline sites in the current study likely contributed to the increased % MeHg in chironomids and fish over this gradient, as well. Particulates, grass shrimp (Buckman et al., 2017), and sediments (Taylor et al., 2012) have also shown this same increase in % MeHg with increasing salinity.

To our knowledge, this is the first study on Hg in Fourspine Stickleback, but levels are similar to what has been found in closely-related species. Threespine Stickleback (*Gasterosteus*

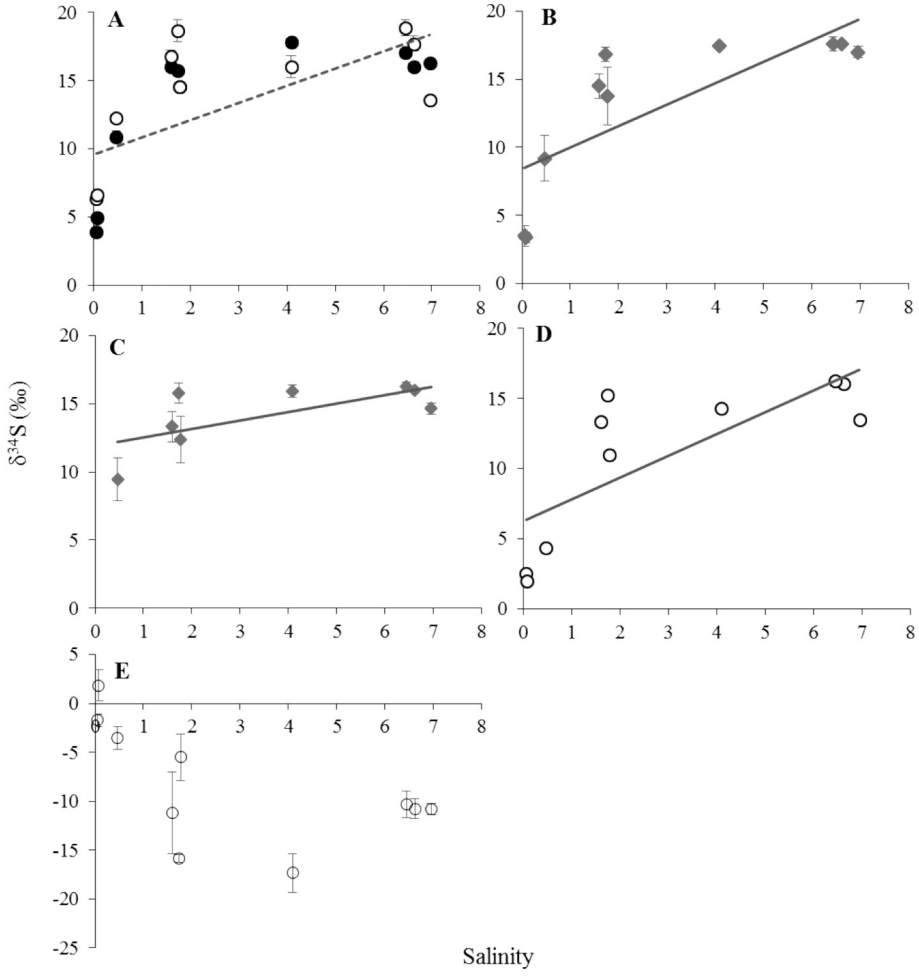

Fig. 7 – Mean $\delta^{34}S$ values (‰) measured in Fourspine Stickleback (A), amphipods (B), snails (C, excluding genus *Viviparus*), chironomids (D), and sediments (E) along a salinity gradient within the Saint John River estuary, New Brunswick, Canada. Figures show averages ± SE for 2015 (solid circles), 2016 (open circles), or both years combined (gray diamonds). Trend lines indicate a significant correlation ($p < 0.05$) and the dashed line represents 2015 stickleback only.

aculeatus) from the San Francisco Bay estuary and Gulf of Gdansk in Poland had average concentrations of 0.68 ± 0.03 mg/kg dw (Eagles-Smith and Ackerman, 2009) and 0.05 to 0.55 mg/kg dw (assuming 80% moisture content; Falandysz and Kowalewska, 1993).

Twenty of the 96 fish in 2015 and 29 of the 100 fish in 2016 had MeHg concentrations above 0.2 mg/kg ww. This is the whole-body 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 behavior. Because between 25% of the stickleback exceeded the toxicity threshold, it is possible that there may be some effects of MeHg on individuals in the Saint John River estuary, and this warrants further investigation. In addition, these small-bodied stickleback are likely consumed by larger fishes, such as predatory Striped Bass (*Morone saxatilis*), Yellow Perch (*Perca flavescens*), and Chain Pickerel (*Esox niger*) (Scott and Crossman, 1973) and some aquatic birds in the Saint John River estuary. Although no measures of Hg have been made in the above mentioned predatory fishes recently, they had elevated THg concentrations in the Saint John River (0.70 to 2.13 mg/kg ww) in the 1970s (Dadswell, 1975) and may pose a current risk to fish-consumers including humans.

In 2016, a different genus of snail was collected at the two most freshwater sites because the other two genera were not present. *Viviparus* snails had much higher concentrations of MeHg (~5-fold) when compared to the other genera and were therefore excluded from the statistical analyses. Most pulmonate taxa such as *Physa* and *Fossaria* reproduce once and only live for one year, while prosobranchs such as *Viviparus* reproduce multiple times over several years (Thorp and Covich, 2001). The *Viviparus* snails were most probably longer-lived or slower-growing in comparison to the other genera, allowing for more bioaccumulation of MeHg in the former taxon.

MeHg concentrations found in invertebrates from the Saint John River estuary were comparable to those observed in other estuarine studies. More specifically, THg in *Gammarus* amphipods from the Narragansett Bay in Rhode Island, USA were 0.093 ± 0.022 mg/kg (Taylor et al., 2012) and 0.013 ±

0.002 mg/kg ww (0.065 mg/kg dw since they reported 85% moisture content) (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 (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 (average 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 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).

Sediments and water from the present study had much lower concentrations of MeHg than biota, as expected, and were low or similar to concentrations found in other urban estuaries. Sediment MeHg levels were 1.85 μg/kg dw (average) in Narragansett Bay (Taylor et al., 2012) and between 0.08 to 4.90 μg/kg dw in the Delaware River estuary, with most of the sites falling under 1.60 μg/kg dw (Buckman et al., 2017). Ten estuaries in the northeastern U.S. had sediment MeHg concentrations ranging from 0.13 to 34.8 μg/kg dw (Chen et al., 2014). Dissolved MeHg in water ranged from 0.01 to 0.11 ng/L in an urbanized Delaware River estuary (Buckman et al., 2017) and from 0.001 to 0.025 ng/L in the ten estuaries sampled by Chen et al. (2014).

In the current study, MeHg in some taxa were related to its concentrations in sediments (for snails) or water (for fish and amphipods), as has been observed elsewhere. Chen et al. (2009) found that MeHg in sediments positively predicted some of the variation in biota MeHg, but that there were other factors, such as organic carbon in the sediments, that also explained some of the variation. Buckman et al. (2017) found that water particulate MeHg positively predicted MeHg in Blue Crab (*Callinectes sapidus*), juvenile White Perch (*Morone americana*), and Mummichog (*Fundulus heteroclitus*). Aside from particulate MeHg, other factors that predicted concentrations in Mummichog were the percent of land development and percent forest cover (Buckman et al., 2017). No models were built predicting MeHg concentrations using the environmental variables in the current study because of the low sample size (n = 10 sites).

Dissolved organic matter (DOM) and DOC were not measured herein, but can be correlated to MeHg and Hg (II) concentrations in systems (Krabbenhoft et al., 2002; Mason and Lawrence, 1999). Depending on the chemical composition of the organic material, it can affect binding or availability differently. For example, DOM with higher sulfur content is likely to bind more Hg, as it is typically found to be associated with sulfhydryl groups (Ravichandran, 2004). When larger DOM binds Hg(II) though, it can decrease the amount available for methylation by bacteria, but when MeHg binds to larger particles, it increases bioavailability to organisms (Paranjape and Hall, 2017). Measuring and characterizing DOM would be an important consideration for future studies.

3.2. Describing stable isotopes and diets

There were relationships between isotope values in biota and site salinity in the Saint John River estuary. Average $\delta^{13}C$ and $\delta^{34}S$ values were positively correlated with salinity for biota, as expected (Fry, 2002; Fry and Chumchal, 2011). Fish from all sites above 1.59 salinity had averages of over 90% marine sulfur in their diets according to the % Marine calculations, while those from below this salinity had values of 0–79%. The trends in these two isotopes and derived values supported the assumption that the biota sampled were living along a salinity gradient within the estuary.

The low among site variation in TP and $\delta^{15}N$ values and weak $\delta^{15}N$-salinity correlations for biota suggests they feed at a consistent trophic level along the river, which should indicate little influence of TP on MeHg concentrations along the gradient. The 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). Schein et al. (2013) found similar $\delta^{15}N$ values for Fourspine Stickleback and that they were more than one trophic level higher than amphipods too, despite amphipods making up the bulk of stickleback stomach contents.

4. Conclusion

Overall, we found little evidence to support our hypothesis that salinity regulates MeHg bioaccumulation in biota in the Saint John River estuary, Canada. While we cannot conclude that fish from freshwater or brackish water sites were at higher risk of MeHg toxicity, some individuals across all sites exceeded concentrations believed to be protective, suggesting that there may be effects of MeHg on fish within this system. In addition, concentrations of MeHg in higher-trophic-level fishes in this system are likely to exceed toxicity thresholds because of food web biomagnification, and this warrants further examination. MeHg remains a prominent issue within fish and fish-eating wildlife in North America and globally, and it is therefore important to seek a better understanding of its accumulation in complex ecosystems such as estuaries, as they typically host diverse wildlife, many of which are at the more vulnerable, juvenile life stages.

Acknowledgments

The Natural Sciences and Engineering Research Council of Canada (NSERC) Collaborative Research and Development program (#CRDPJ 462708-13), Discovery Program (#312237-2012), Canada Research Chairs (#950-230607) and Canada Foundation for Innovation (#201843) programs, and NB Power (#CRDPJ 462708-13) provided funding for this research. We would like to thank Michael van den Heuvel for lending equipment for this work as well as Sara Klapstein, Angella Mercer, Ryan Power, Stephanie Graves, Lindsay May, Bryce

Pippy, Nick Fraser, Laure Pezzatini, Nefertiti Roldán Wong, and Kelli Charbonneau for help in the field and lab with sampling and analysis.

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