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

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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, Wright, and Devries, 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; Compeau and Bartha, 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 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, Fitzgerald, and Morel, 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, Wright, and Devries, 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).

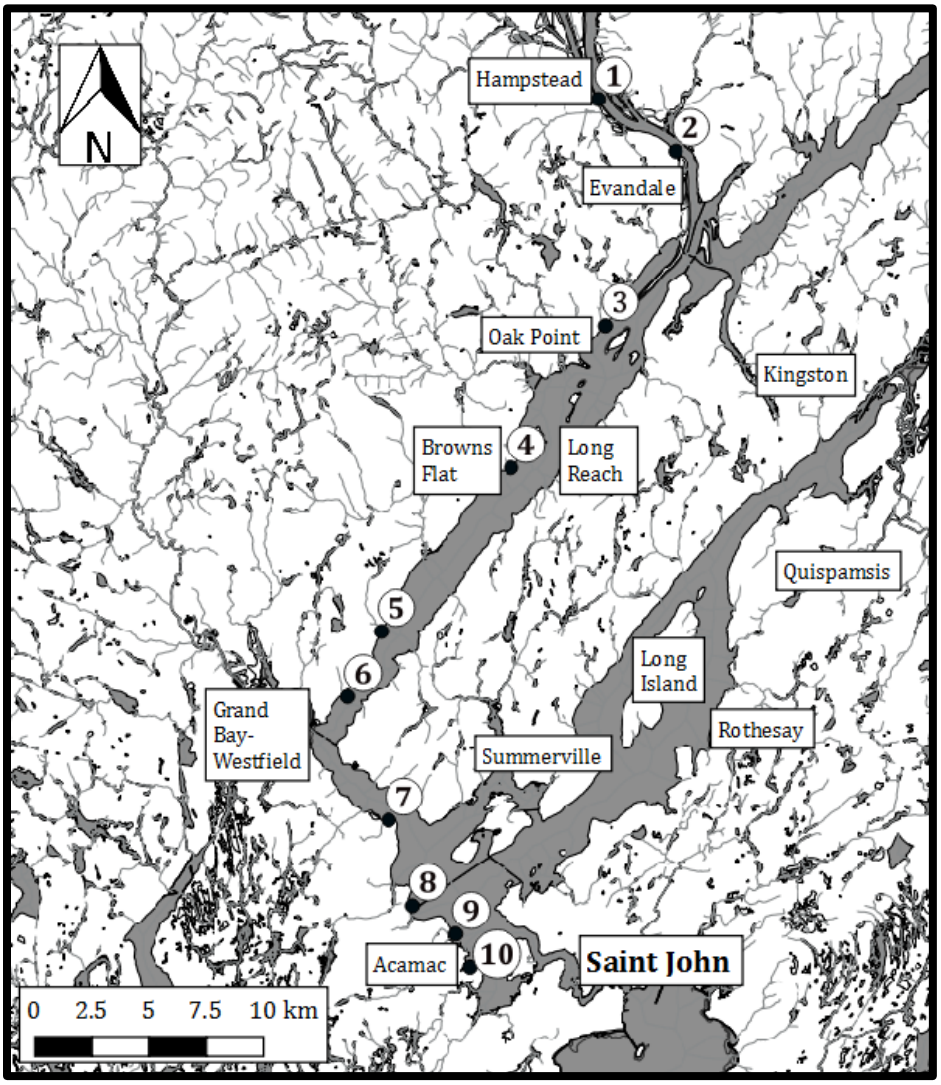
Stable isotopes can be used to identify diet and to trace MeHg biomagnification through food webs (Clayden et al., 2017; Kidd et al., 2012). Nitrogen isotope ratios ($^{15}N/^{14}N$; expressed as $\delta^{15}N$) assess relative trophic level of consumers because they retain more of the heavier isotope, 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 fuelling food webs (Fry, 2006; Svensson, Hyndes, and Lavery, 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; Fry, 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; Compeau and Bartha, 1987; Blum and Bartha, 1980; van der Velden et al., 2013; Evers et al., 2005; Fry and Chumchal 2012; Farmer, Wright, and Devries, 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

99 1.1 Study Area



100

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

101 The Saint John River has a drainage area >55,000 km^2 and flows into the Bay of
102 Fundy at the City of Saint John, New Brunswick, Canada (Kidd et al., 2011; Metcalfe et al.,
103 1976). The Bay of Fundy exhibits high tides up to 8 m in the Saint John Harbour and the
104 head of the tide occurs 135 km upstream (Kidd et al., 2011). The saline waters extend as far
105 as 60 km upstream of the river mouth, creating a continuous salinity gradient from
106 freshwater to the Harbour (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 five minutes for roughly 24 hours for two neap tide and three spring tide cycles (n = 5 data sets with n = 283, 5 minute samples). 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 10^{th} and 90^{th} 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

129 continuous HOBO measures in 2016 from the same site was 1.42 ± 1.56 (average ± SD will
130 be reported throughout text).

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

140 Invertebrate collections were done at each site using sweep nets, kick nets, and
141 rock-picking. Snails (genera *Physa, Fossaria,* and *Viviparus*) and amphipods (*Gammarus*)
142 were the common invertebrates found during collections in August 2015 (n = 1 sample per
143 site in 2015, except no snails were present at site 9 in 2015). We pooled all individuals to
144 make one pooled sample per site for both amphipods and snails to obtain sufficient mass
145 for all chemical analyses (>30 mg dry weight (dw)). In 2016, snails and amphipods were
146 collected on three dates from July to August (n = 3 samples per site per taxa). Chironomids
147 were also collected over this period in 2016 and pooled for adequate mass (n = 1 sample
148 per site). Invertebrates were placed on ice immediately after collection and were frozen
149 within five hours 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, 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 five 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 hours.

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 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).

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

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

205 Dried animal tissues (10 mg) were digested in a 25% KOH in methanol (MeOH)
206 solution, shaken for one hour, heated at 95 °C for one hour and cooled overnight at room
207 temperature (Edmonds et al., 2012). An aliquot of 20 – 40 µL of each digested sample was
208 analyzed for MeHg and Hg (II) (latter in 2016 only) as described above. Average percent
209 recovery of MeHg in CRM (DOLT-5 and DORM-4 from National Research Council of
210 Canada), 50 ng MeHg standards, and concentrations of calibration blanks were 105 ± 13%
211 (n = 11), 105 ± 7% (n = 20), and 7.98 x 10^{-6} µg/kg dw (n =43), respectively. Average
212 precision was within 13% (n = 9) for duplicate samples, with method detection limits
213 between 0.74 - 1.62 pg for the separate runs. The limits of detection were calculated as 3
214 times the SD of blanks for all MeHg analyses. For Hg (II), CRM DORM-4, standards, and
215 calibration blanks had average recoveries/concentrations of 105 ± 12% (n = 5), 86 ± 11%

(n = 13), and 8.22 x 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 methylmercury from sediments and soils, sediments (0.5 g) were extracted by shaking with 1.5 M KBr in 5% H_2SO_4 and 1 M $CuSO_4$ 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 hours 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 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.

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, based on three times the SD of blanks. 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 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. 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 Sulphur 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

283 statistical analyses of fish data. The biomagnification factor (BMF; Gobas et al., 2009),
284 which is the ratio of Hg in the consumer to that of the diet, was also calculated for
285 Fourspine Stickleback using their THg and amphipod MeHg values to represent consumed
286 food (amphipods were the main prey items - see Section 2.3). Invertebrate biota-sediment
287 accumulation factors (BSAF; ratio of MeHg in the consumer to that in sediment; Calle et al.,
288 2015) were also calculated for each site.

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

290 $$TP = \lambda + (\delta^{15}N_{consumer} - \delta^{15}N_{base})/\Delta_n \text{ (Post 2002)}$$

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

296 The proportion of diet from marine sources for the sticklebacks (% Marine; as for
297 $\delta^{13}C$ in Post, 2002) was calculated for each site using the fish $\delta^{34}S$ data and a simple, two-
298 end-member mixing model assuming no fractionation and based on average $\delta^{34}S$ values of
299 all invertebrates at the freshwater site (salinity = 0.06) and of those at the brackish site
300 (salinity = 6.96).

301 $$\%\ Marine = (\delta^{34}S_{consumer} - \delta^{34}S_{freshwater\ invertebrates})/(\delta^{34}S_{marine\ invertebrates} - \delta^{34}S_{freshwater\ invertebrates}) * 100$$

303 It was not calculated for invertebrates as there was no food source assessed. % Marine
304 values were held rounded between 0 and 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 ($\alpha = 0.05$) 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).

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	11346	7.75	< 0.50	0.015	1.7
9	45.284868	-66.156047	6.62	21.8	11852	8.08	< 0.50	0.015	1.8
10	45.271931	-66.148314	6.95	21.4	12523	6.40	< 0.05	0.023	1.5

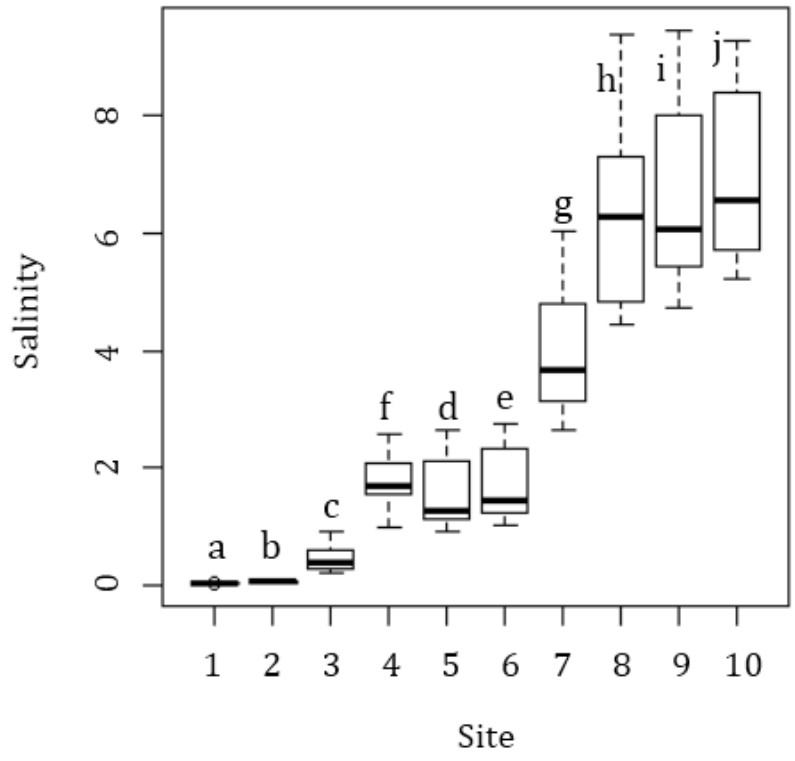


Fig. 2 – Continuous salinity measures taken at 10 sites in the Saint John River estuary, New Brunswick, Canada, in 2016 (see also Fig. 1). Statistically significant differences are represented by different letters (p<0.05).

328 2.2 Hg

329 THg in fish (THg-adj) differed between years with averages of 428 ± 24 µg/kg (in
330 dry weight unless otherwise stated) and 689 ± 18 µg/kg for 2015 and 2016, respectively
331 (year:site interaction $p < 0.03$, site effect $p < 0.01$, year effect $p < 0.01$). There were no
332 significant correlations between THg-adj and salinity within each year ($p > 0.05$; Fig 3;
333 Table 2). MeHg levels in amphipods, snails, chironomids, sediment, and water samples
334 were lower than those observed in fish, with averages of 85 ± 27 µg/kg, 71 ± 29 µg/kg, 52 ±
335 24 µg/kg, 0.34 ± 0.17 µg/kg, and 0.08 ± 0.03 ng/L, respectively, across all sites (Fig. 3;
336 Table 2). Only MeHg levels in chironomids were significantly correlated with salinity ($r =$
337 0.83, $p < 0.01$). Sediment MeHg increased and water MeHg decreased with salinity, but
338 these were not significant trends (sediment $p = 0.07$, water $p = 0.11$).

339 The average Hg (II) (2016 only) for stickleback across sites was 29 ± 21 µg/kg and
340 there was a negative trend with salinity ($r = -0.72$, $p < 0.02$; Fig. 4; Table 2). There were no
341 trends detected for invertebrates ($p > 0.12$). Average concentrations were 18 ± 7 µg/kg, 65
342 ± 18 µg/kg, and 46 ± 28 µg/kg in amphipods, snails, and chironomids, respectively.

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

347 The average BMF for stickleback was 6.6 and 7.5 for 2015 and 2016, respectively
348 (range = 3.4 to 13.9) and values were not significantly correlated with salinity across sites
349 ($p > 0.44$ for both years). The ranges in BSAFs were 171 to 1180, 137 to 246, and 68 to 431

350 for amphipods, snails and chironomids, respectively. These, too, did not change
351 significantly with salinity ($p > 0.17$ for all taxa).

352 The gut-cleared amphipods (average 16.2 ± 5 µg/kg) had similar Hg (II)
353 concentrations to those of the non-gut-cleared amphipods (average 15 ± 3 µg/kg) from the
354 same sites in 2016 ($p = 0.29$). MeHg was significantly different ($p = 0.05$) between gut-
355 cleared (average 109 ± 18 µg/kg) and non-gut cleared (average 100 ± 17 µg/kg) samples.
356 The average percent change in MeHg and Hg (II) from non-gut-cleared to gut-cleared was -
357 8 ± 12% and -4 ± 19%, respectively.

358

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	*-0.65		*-0.69	*-0.68	0.65	*-0.72		
	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	0.24		-0.35	-0.17	-0.46	-0.14	0.44	*0.88
	Chironomids	*0.77		*0.89	*0.73	-0.19	0.22	-0.47	0.35

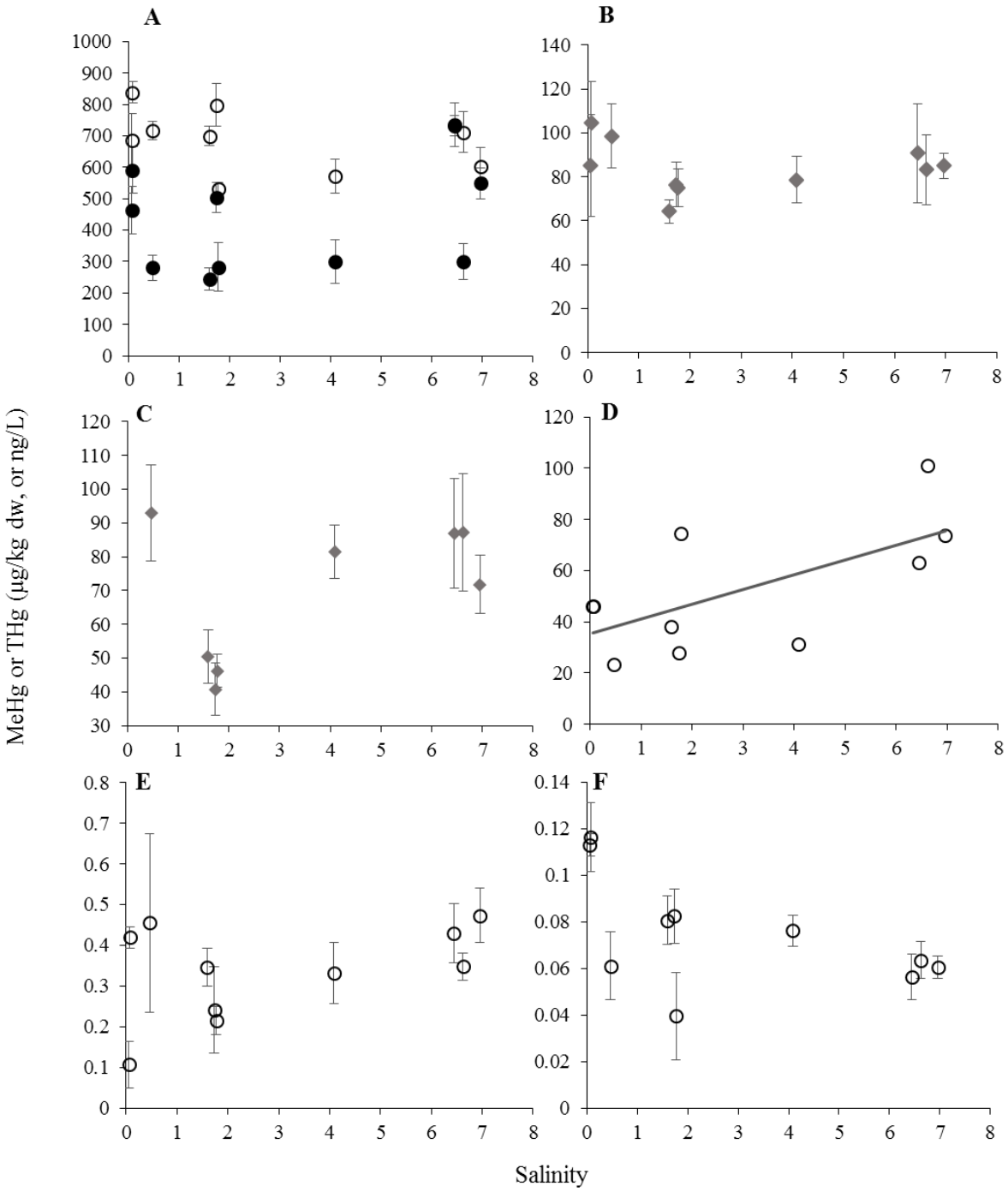


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Fig. 3 - THg (Fourspine Stickleback only) or MeHg in samples collected along a salinity gradient in the Saint John River estuary, New Brunswick, Canada. Levels are in µg/kg dry weight for Fourspine Stickleback (A; n = 7-10/site), amphipods (B; n = 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 (grey diamonds). Trend lines indicate a significant correlation ($p<0.05$).

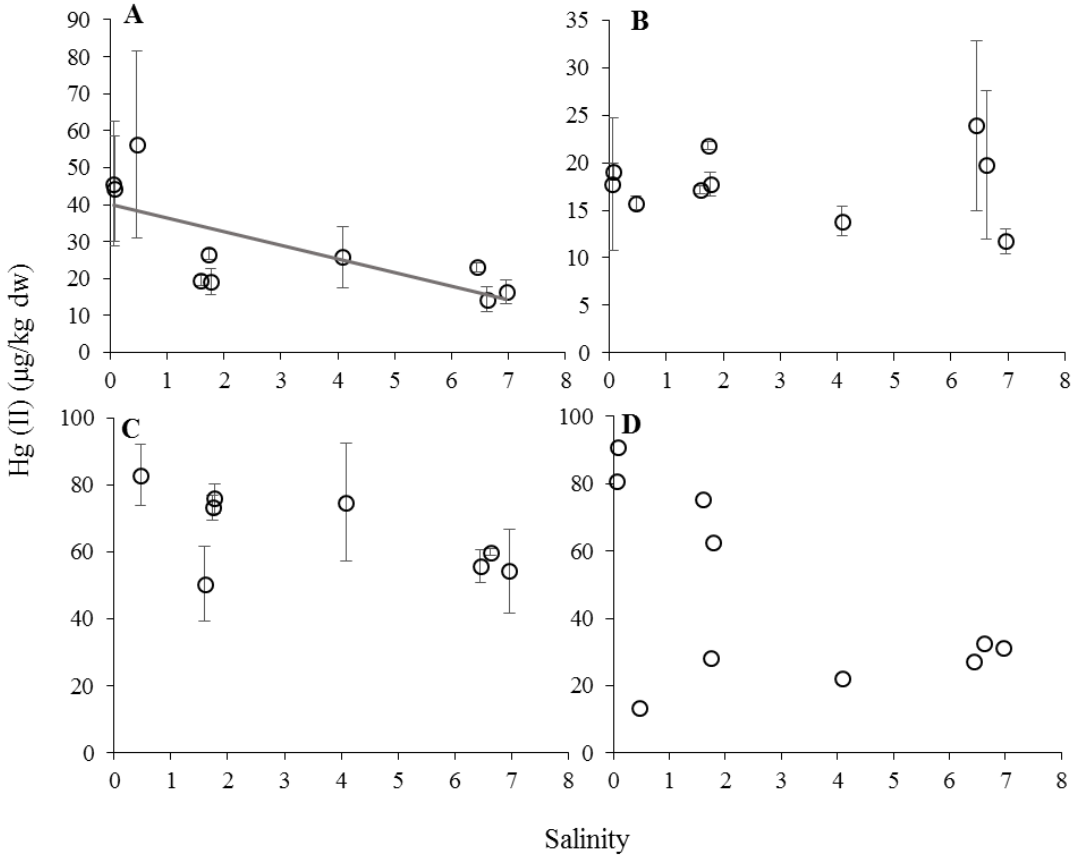

Fig. 4 - Hg (II) concentrations in biota sampled along a salinity gradient in the Saint John River estuary, New Brunswick, Canada, in 2016. Levels are µg/kg dry weight for Fourspine Stickleback (A; n = 10/site), amphipods (B; n = 2-3/site), snails (C; n = 3/site; excluding genus *Viviparus*), and chironomids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

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 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 taxa 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.

391 Average $\delta^{34}S$ values among sites ranged from 4 to 19‰, 3 to 18‰, 2 to 16‰, 3 to
392 16‰, and -11 to -2‰ for fish, amphipod, snails, chironomids, and sediments, respectively
393 (Fig. 7). There were similar spatial trends to those of $\delta^{13}C$, with an increase in $\delta^{34}S$ for all
394 biota from freshwater to higher salinity sites (Table 3). Biota sampled at sites with salinity
395 ≥ 1.59 (site 4 and those towards the estuary mouth) had consistently high $\delta^{34}S$ values,
396 reflective of marine sulfur sources, whereas biota sampled from sites 1 to 3 with salinities
397 <0.47 had $\delta^{34}S$ values that were distinctly lower. % Marine and $\delta^{34}S$ in 2015 stickleback
398 were positively correlated to salinity ($r = 0.70$, $p = 0.03$; $r = 0.70$, $p = 0.02$, respectively).
399 $\delta^{34}S$ was also correlated with salinity for amphipods ($r = 0.80$, $p < 0.01$), snails ($r = 0.74$, $p =$
400 0.01), and chironomids ($r = 0.76$, $p = 0.010$). Sediment values did not show the same trend
401 as biota and were not correlated with salinity (Fig. 7; $p = 0.09$).

402 Over both years, the diet (gut contents) of stickleback consisted of amphipods (in
403 79% of fish), chironomids (in 28% of fish), zooplankton (in 19% of fish), and other
404 dipterans (in 15% of fish) based on presence-absence information.

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

407 **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 a * ($p < 0.05$).**

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

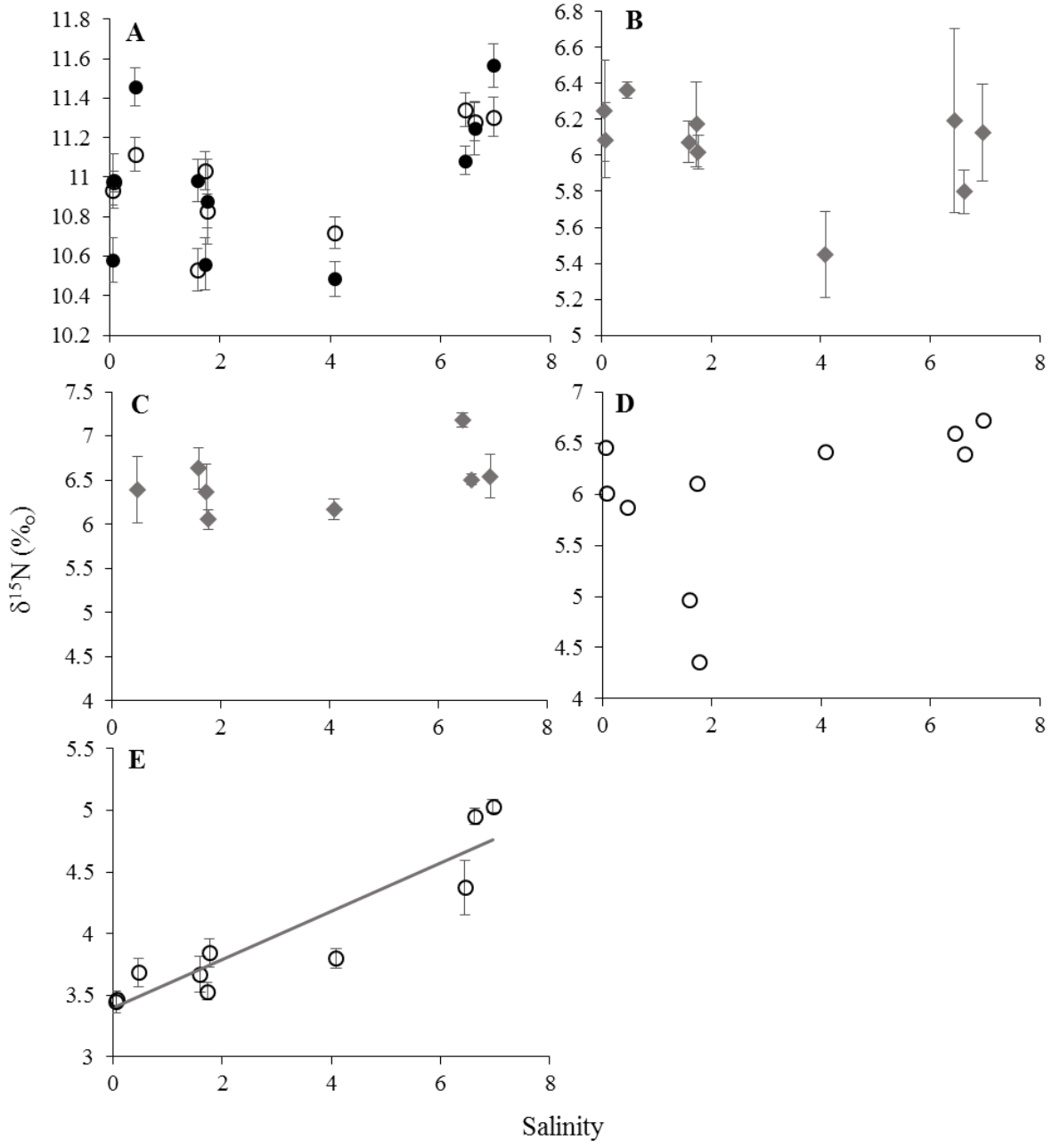

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Fig. 5 - Mean $\delta^{15}N$ values (‰) measured in Fourspine Stickleback (A), amphipods (B), snails (C, excluding genus *Viviparus*), chironomids (D), and sediments (E) along a salinity gradient within the Saint John River estuary, New Brunswick,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

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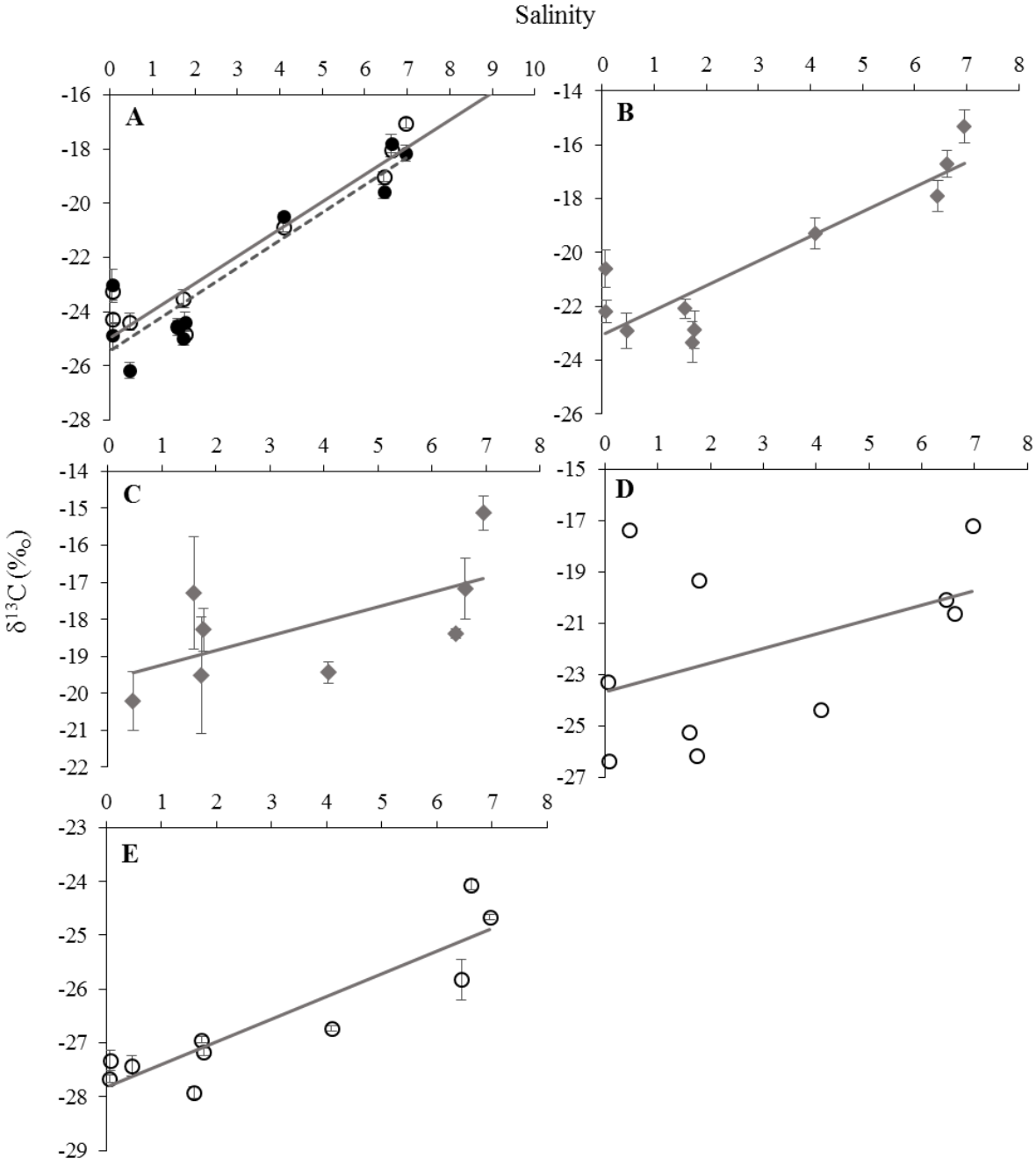

Fig. 6 - Mean $\delta^{13}C$ values (‰) measured in Fourspine Stickleback (A), amphipods (B), snails (C, excluding genus *Viviparus*), chironomids (D), and sediments (E) along a salinity gradient within the Saint John River estuary, New Brunswick, Canada. Figures show averages ± SE for 2015 (solid circles),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

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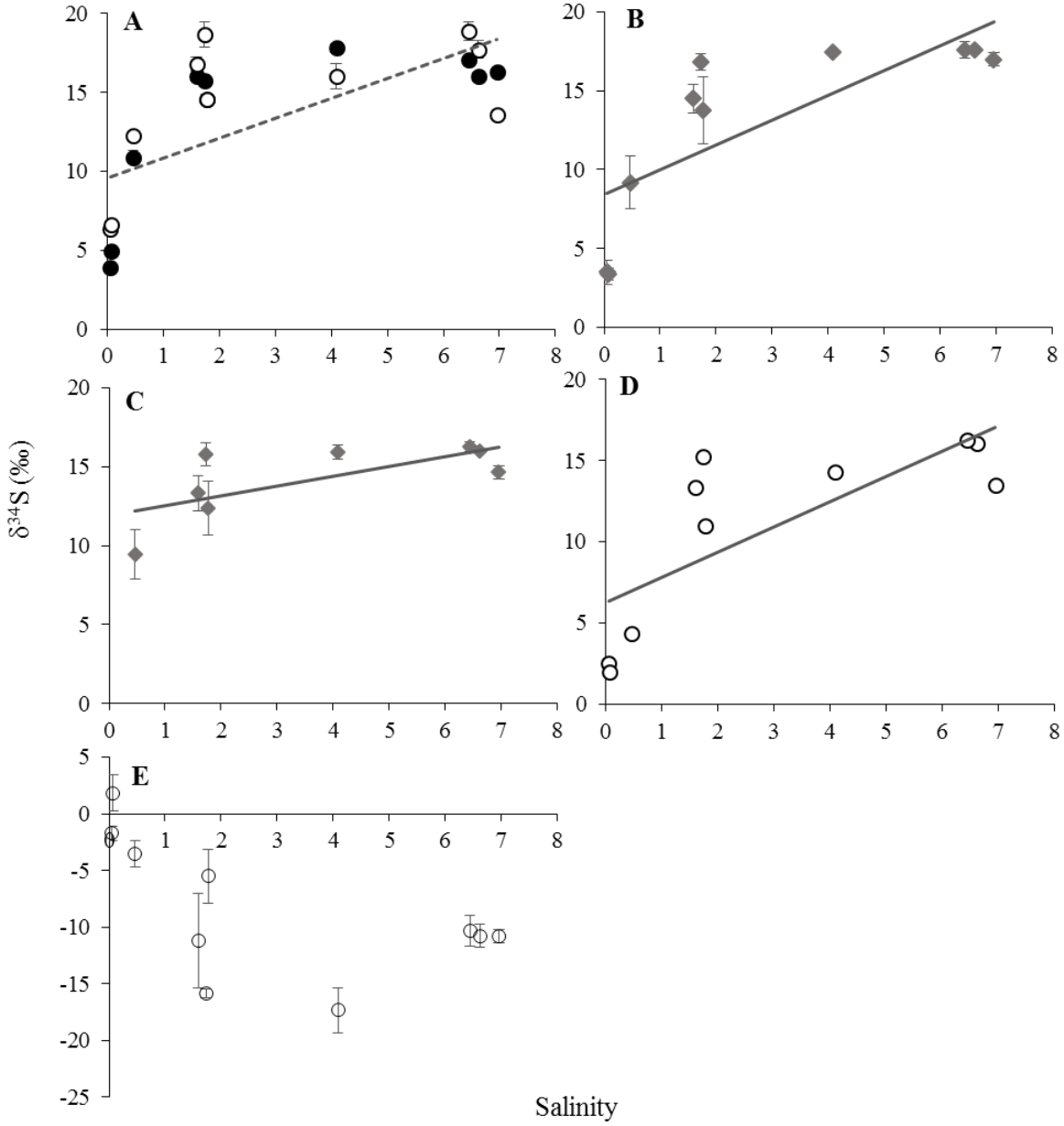

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Fig. 7 - Mean $\delta^{34}S$ values (‰) measured in Fourspine Stickleback (A), amphipods (B), snails (C, excluding genus *Viviparus*), chironomids (D), and sediments (E) along a salinity gradient within the Saint John River estuary, New Brunswick,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

413

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 in more saline waters, may also increase the production or bioavailability of MeHg (Gilmour, Henry, and Mitchell, 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.

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

436 and positive trends (described above) in biota MeHg with salinity is most probably
437 reflective of the complexity of Hg dynamics in an estuarine ecosystem. There may be
438 inconsistent effects of salinity on MeHg within the Saint John River ecosystem, or additional
439 environmental drivers influencing the production and uptake of MeHg within the estuary
440 that we did not capture in the endpoints assessed herein. Buckman et al. (2017) suggest
441 that environmental variables such as carbon sources, bioavailability, mixing, marsh
442 presence, level of human development, etc., can have a strong influence on MeHg
443 concentrations at different sites within a single estuary. These factors were not assessed in
444 the current study. In addition, the gradient in salinity in the Saint John River estuary may
445 have not been large enough to show an effect on MeHg bioavailability and bioaccumulation.
446 Other studies that show salinity influences on mercury endpoints in biotic and abiotic
447 samples had salinity ranges from 0.3 to 32.6 (Taylor et al., 2012), 0.1 to 20.8 (Buckman et
448 al., 2017), and 0 to 25 (Fry and Chumchal, 2012; Fry, personal communication, July 2017).
449 Therefore, other than for chironomids, we cannot provide any strong evidence to support
450 the hypothesis that salinity regulates MeHg concentrations in the Saint John River estuary,
451 thereby increasing or decreasing risk of its toxicity to most biota studied herein.

452 Though relationships were only significant for Fourspine Stickleback and snails,
453 there were decreasing concentrations of Hg (II) with salinity for each taxon in the Saint
454 John River estuary. No field studies were found that assessed Hg (II) concentrations along a
455 salinity gradient, but increased uptake of Hg (II) at lower salinities is commonly observed
456 in lab experiments on fish and invertebrates (Pan and Wang, 2004; Laporte et al., 1997;
457 Wang and Wang, 2010). This may be due to an increase in the unbound and bioavailable
458 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 wall 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 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 behaviour. 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.

481 In addition, these small-bodied stickleback are likely consumed by larger fishes such as
482 predatory Striped Bass (*Morone saxatilis*), Yellow Perch (*Perca flavescens*), and Chain
483 Pickerel (*Esox niger*) (Scott and Crossman, 1973) and some aquatic birds in the Saint John
484 River estuary. Although no measures of Hg have been made in the above mentioned
485 predatory fishes recently, they had elevated THg concentrations in the Saint John River
486 (0.70 to 2.13 mg/kg ww) in the 1970s (Dadswell, 1975) and may pose a current risk to
487 fish-consumers including humans.

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

496 MeHg concentrations found in invertebrates from the Saint John River estuary were
497 comparable to those observed in other estuarine studies. More specifically, THg in
498 *Gammarus* amphipods from the Narragansett Bay in Rhode Island, USA were 0.093 ± 0.022
499 mg/kg (Taylor et al., 2012) and 0.013 ± 0.002 mg/kg ww (0.065 mg/kg dw since they
500 reported 85% moisture content) (Payne and Taylor, 2010). In the Canadian Arctic and sub-
501 Arctic, Gammarid amphipods had 0.076 mg/kg THg in lake environments and between
502 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. Sediments 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 northestern 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. 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.

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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.

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.

Cabana, G., Rasmussen, J.B., 1996. Comparison of aquatic food chains using nitrogen isotopes. Proc. Natl. Acad. Sci. U. S. A. 93, 10844-10847.

Calle, P., Alvarado, O., Monserrate, L., Cevallos, J.M., Calle, N., Alava, J.J., 2015. Mercury accumulation in sediments and seabird feathers from the Antarctic Peninsula. Mar. Pollut. Bull. 91, 410-417.

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

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

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

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

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

606 Curry, R.A., Hughes, R.M., McMaster, M., Zafft, D., 2009. Chapter 9: Coldwater Fish in
607 Rivers. In Standard Sampling Methods for North American Freshwater Fishes. Edited
608 by Bonar, S., Hubert, W., Willis, D., AFS Publications, Bethesda, MD, USA. 139-158.

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

612 Department of Fisheries and Oceans Canada., 2009. Use of the lower Saint John River, New
613 Brunswick, as fish habitat during the spring freshet. DFO Can. Sci. Advis. Sec. Sci. Resp.
614 2009/014. 1-5.

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

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

620 Edmonds, S.T., O'Driscoll, N.J., Hillier, N.K., Atwood, J.L., Evers, D.C., 2012. Factors regulating
621 the bioavailability of methylmercury to breeding rusty blackbirds in northeastern
622 wetlands. Environ. Pollut. 171, 148-154.

623 Evans, D.W., Crumley, P.H., 2005. Mercury in Florida Bay fish: Spatial distribution of
624 elevated concentrations and possible linkages to Everglades restoration. Bull. Mar. Sci.
625 77, 321-345.

626 Evans, M.S., Lockhart, W.L., Doetzel, L., Low, G., Muir, D., Kidd, K., Stephens, G., Delaronde, J.,
627 2005. Elevated mercury concentrations in fish in lakes in the Mackenzie River Basin:
628 The role of physical, chemical, and biological factors. Sci. Total Environ. 351-352, 479-
629 500.

630 Evers, D.C., Burgess, N.M., Champoux, L., Hoskins, B., Major, A., Goodale, W.M., Taylor, R.J.,
631 Poppenga, R., Daigle, T., 2005. Patterns and interpretation of mercury exposure in
632 freshwater avian communities in northeastern North America. Ecotoxicology 14, 193-
633 221.

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

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

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

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

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

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

646 Fry, B., Chumchal, M.M., 2012. Mercury bioaccumulation in estuarine food webs. Ecol. Appl.
647 22, 606-623.

648 Gilmour, C.C., Henry, E.A., Mitchell, R., 1992. Sulfate stimulation of mercury methylation in
649 freshwater sediments. Environ. Sci. Technol. 26, 2281-2287.

650 Gobas, F.A.P.C., De Wolf, W., Burkhard, L.P., Verbruggen, E., Plotzke, K., 2009. Revisiting
651 bioaccumulation criteria for POPs and PBT assessments. Integr. Environ. Assess. Manag.
652 5, 624-637.

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

657 Kidd, S.D., Curry, R.A., Munkittrick, K.R., 2011. The Saint John River: A State of the
658 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.

Krabbenhoft, D.P., Olson, M.L., Dewild, J.F., Clow, D.W., Striegl, R.G., Dornblaser, M.M., 2002. Mercury loading and methylmercury production and cycling in high-altitude lakes from the Western United States. Water Air Soil Pollut. Focus 2, 233–249.

Laporte, J.M., Truchot, J.P., Ribeyre, F., Boudou, A., 1997. Combined effects of water pH and salinity on the bioaccumulation of inorganic mercury and methylmercury in the shore crab *Carcinus maenas*. Marine Pollution Bulletin 34, 880-893.

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.

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.

Mason, R.P., Lawrence, A.L., 1999. Concentration, distribution, and bioavailability of mercury and methylmercury in sediments of Baltimore Harbor and Chesapeake Bay, Maryland, USA. Environ. Toxicol. Chem. 18(11), 2438–2447.

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.

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.

Pan, J.F., Wang, W.X., 2004. Uptake of Hg(II) and methylmercury by the green mussel *Perna viridis* under different organic carbon conditions. Mar. Ecol. Prog. Ser. 276, 125-136.

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

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.

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

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

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

698 Smylie, M.S., McDonough, C.J., Reed, L.A., Shervette, V.R., 2016. Mercury bioaccumulation in
699 an estuarine predator: Biotic factors, abiotic factors, and assessments of fish health.
700 Environ. Pollut. 214, 169-176.

701 Stenzler, B., Hinz, A., Ruuskanen, M., Poulain, A.J., 2017. Ionic Strength Differentially Affects
702 the Bioavailability of Neutral and Negatively Charged Inorganic Hg Complexes. Environ.
703 Sci. Technol. 51, 9653-9662.

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

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

709 Taylor, D.L., Linehan, J.C., Murray, D.W., Prell, W.L., 2012. Indicators of sediment and biotic
710 mercury contamination in a southern New England estuary. Mar. Pollut. Bull. 64, 807-
711 819.

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

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

716 Turner, A., Millward, G.E., Le Roux, S.M., 2001. Sediment-water partitioning of inorganic
717 mercury in estuaries. Env. Sci. Technol. 35, 4648-4654.

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

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

723 Wang, R., Wang, W.X., 2010. Importance of speciation in understanding mercury
724 bioaccumulation in tilapia controlled by salinity and dissolved organic matter.
725 Environ. Sci. Technol. 44, 7964-7969.

726 Weinkauf, M., 2015. Conductivity2Salinity.xlsx.
727 https://www.marum.de/Software_and_Programs.html#Section28628. Accessed August
728 1, 2016.

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

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