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Effects of non-native fish on mercury biomagnification

Effects of non-native fish on lacustrine food web structure and mercury biomagnification along a dissolved organic carbon gradient

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Abstract: Though the introduction of non-native fish species has been shown to alter trophic ecology in aquatic ecosystems, there has been limited research on how invasive species alter methylmercury (MeHg) biomagnification in lacustrine food webs. We sampled surface water and biota from eight lakes in Quebec, Canada, spanning a range of dissolved organic carbon (DOC) concentrations (2.9 to 8.4 mg/L); four lakes were inhabited by native brook trout (*Salvelinus fontinalis*) and the remaining lakes contained

brook trout and a non-native fish, Allegheny pearl dace (*Margariscus margarita*). Periphyton, zooplankton, macroinvertebrates, and fish were analyzed for: 1) stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope ratios to delineate food webs, and 2) total Hg (THg) or MeHg. Compared to the brook trout from reference lakes, fish from invaded lakes had higher length-standardized THg concentrations as well as a narrower dietary range and elevated trophic level, inferred from unadjusted $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, respectively. The rate of Hg biomagnification was similar across invaded and reference lakes, implying little effect of the invasive fish on the trophic transfer of MeHg. Despite differences in food web structure due to pearl dace invasion, DOC was the strongest predictor of brook trout THg levels for all lakes, suggesting that underlying environmental factors exerted a stronger influence on brook trout THg concentrations than the presence of a non-native forage fish.

Keywords: Invasive species; stable isotopes; trophic ecology; brook trout; methylmercury

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INTRODUCTION

The introduction of non-native freshwater fish has increased 2-fold over the last 30 years, posing a major threat to aquatic ecosystems worldwide (Gozlan et al. 2010; Havel et al. 2015). The potential effects of these invasive species are wide-ranging and include the potential to alter aquatic community structure and degradation of ecosystem function (Havel et al. 2015). For example, non-native fish species can alter trophic linkages (Vander Zanden et al. 1999) and increase food chain length, which has implications for the biomagnification of contaminants in aquatic food webs (Rasmussen et al. 1990; Thomas et al. 2016; Eagles-Smith et al. 2018).

Mercury (Hg) is a pervasive and ubiquitous contaminant, which is released primarily through anthropogenic activities also threatening aquatic ecosystems globally (Driscoll et al. 2013; UNEP 2019). In its elemental form, Hg travels long distances in the atmosphere before it is deposited onto landscapes and waterbodies. In aquatic environments, inorganic Hg is methylated by microorganisms, producing methylmercury (MeHg), an organometal which bioaccumulates and biomagnifies (Driscoll et al. 2013). As a result, fish can accumulate high concentrations of MeHg ([MeHg]) in their tissues.

Due to its tendency to biomagnify, MeHg levels are generally higher in older and larger fish, and those that feed at upper trophic levels, (Sandheinrich and Wiener 2011) often inferred through stable nitrogen isotope ratios ($\delta^{15}\text{N}$) (Post 2002). Furthermore, because diet is the main source of MeHg for fish (Hall et al. 1997), feeding ecology is an important driver of fish MeHg levels. In general, fish feeding mainly on pelagic carbon sources in open-water environments tend to have higher Hg levels than those relying

more on littoral carbon sources (Eagles-Smith et al. 2008a; Kidd et al. 2012). Because non-native aquatic species alter food web dynamics (i.e., dietary carbon sources, food chain lengths, and trophic positions (Britton et al. 2010; Walsworth et al. 2013)) their presence can significantly affect MeHg levels in native fishes (Lepak et al. 2019).

However, even when size, age, or trophic level are accounted for, substantial variability in fish [MeHg] exists among lakes, even within the same geographic region (Rypel 2010; Lescord et al. 2015). This is due, in part, to several lake characteristics which influence Hg transportation, methylation, and bioavailability, processes which directly affect MeHg accumulation in fish (Fitzgerald and Lamborg 2007). For example, lakes with a greater abundance of wetlands in their catchments, large catchment to lake area ratios, and low pH or alkalinity tend to have fish with higher Hg levels (Paranjape and Hall 2017). In particular, aqueous dissolved organic carbon (DOC) increases the export of both inorganic Hg and MeHg from watersheds to waterbodies, and can stimulate microbial activity, leading to increased rates of Hg methylation (Fitzgerald and Lamborg 2007) and accumulation of MeHg in food webs (Lescord et al. 2018). Biotic Hg concentrations are often positively correlated with DOC concentrations ([DOC]), though recent work has shown the relationship is not always linear (French et al. 2014; Braaten et al. 2018). Therefore, to properly assess the impact of non-native species on aquatic food webs and the biomagnification of MeHg, some consideration must be given to the biogeochemical conditions of the systems, such as [DOC].

Though MeHg biomagnification in freshwater food webs is a well-studied phenomenon, limited research has explored how this process is influenced by ecosystem changes, such as species introductions (Eagles-Smith et al. 2018). In the present study we

assessed the presence of effect of non-native fish on aquatic food web and Hg dynamics in eight lakes in La Mauricie National Park (LMNP; Quebec, Canada; Figure 1). In four of the lakes, brook trout (*Salvelinus fontinalis*) were the only fish species present, whereas the remaining four lakes contained populations of both brook trout and non-native fish. We collected water samples (for DOC, particulate organic carbon (POC), THg, and MeHg) and biota across multiple trophic levels (periphyton, zooplankton, benthic macroinvertebrates, and fish) from each lake. All food web samples were analyzed for Hg concentrations (THg in fish; MeHg in invertebrates) as well as stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope ratios to discriminate between benthic and pelagic sources of carbon and to infer trophic positions, respectively. Using these data, we constructed models to understand if and why brook trout had higher levels of Hg in their tissue and different trophic ecology in the presence of non-native fish.

METHODS

Study site

LMNP is a Canadian federal park covering an area of about 540 km², located approximately 140 km from the city of Montreal. Following the last glaciation, the 139 lakes in the LMNP area were colonized mainly by brook trout, with fewer colonized by Arctic char (*Salvelinus alpinus*), spoonhead sculpin (*Cottus ricei*), and nine-spine stickleback (*Pungitius pungitius*). Near the end of the 19th century, almost all of the lakes supported populations of brook trout. Previous to the establishment of LMNP in 1970, the area was used by fishing clubs, which introduced as many as 19 different non-native fish species to certain lakes (Bertolo et al. 2008). The eight lakes (Caribou, Maréchal,

Baie Cobb, Onze Îles, Alphonse, Écarté, Besace, du Fou; Figure 1) included in the present study were selected based on their fish communities and their DOC levels, which have been monitored intermittently by park staff. Four of the lakes (Caribou, Maréchal, Baie Cobb, Onze Îles) support populations of brook trout, while four lakes (Alphonse, Écarté, Besace, du Fou) support populations of brook trout as well as non-native Allegheny pearl dace (*Margariscus margarita*; herein referred to as pearl dace; Table 1). One of these invaded lakes (Écarté) also contains non-native creek chub (*Semotilus atromaculatus*) but because only 1 creek chub was collected during this study, it was not included in any analysis.

Water sampling

Grab samples of surface water (n=4 per lake) were collected in 2012 for THg and MeHg analyses from a central location in each lake. Samples were collected in pre-cleaned (10% HCl) 250 ml amber glass bottles using clean methods (Kirk and St. Louis 2009). Water samples were acidified (0.2% v/v HCl) and stored at ~4 °C until analysis. Additional surface water samples were collected in pre-rinsed 500 ml HDPE bottles for analysis of DOC and POC from each lake. One field duplicate (du Fou) and two field blanks were included. Surface water aliquots for DOC analyses were passed through nitrocellulose membranes (0.45 µm) into certified clean amber glass bottles using a handheld vacuum pump in the field. Bottles were maintained at ~4 °C until analysis. For POC, a grab sample was filtered (pre-washed glass fiber filter) in the laboratory at INRS-ETE. The filter was retained, dried, and packed for stable isotope carbon and nitrogen analyses.

Food web sample collection

Sample sizes for the various food web samples are provided in Table 2. Brook trout were sampled from Caribou, Maréchal, Baie Cobb, and Onze Îles in early October of 2010 and 2012. Brook trout and non-native fish were collected from Alphonse, Ecarté, Besace, and du Fou in early October of 2011 and 2012. Park staff sampled fish from the littoral areas of the eight lakes using fyke nets set overnight. After capture, fish were transferred to square submersible fish cages (~1.2 m X 1.2 m), where they were held, until processing. Brook trout were killed by blunt force trauma to the head followed by cervical dislocation, and then immediately measured for length (cm) and weight (g). We classified brook trout as large and small fish based on fork lengths; mean fork lengths ranged for all lakes from 27.1 ± 3.0 to 31.7 ± 5.6 cm and 16.1 ± 5.0 to 21.6 ± 3.1 cm for large and small brook trout, respectively. Axial muscle was collected and stored at -20 °C for THg and bulk stable isotope (carbon and nitrogen) analyses. Pearl dace were killed by blunt force trauma to the head and frozen whole at -20 °C for THg and stable isotope analyses.

Periphyton was collected from the margins of lakes by scraping the undersides of rocks. Benthic macroinvertebrates and pelagic zooplankton were collected from the eight lakes in 2012 and 2013. Benthic macroinvertebrates were sampled from the littoral regions of the eight lakes using kick nets. Bulk invertebrate samples were sorted by taxa with identifications made to the level of order. Pelagic zooplankton were sampled from the water column of each lake by towing a hoop net (Wisconsin; 200 μ m) behind a small boat. Sorted benthic macroinvertebrate and zooplankton samples were identified using the taxonomic keys in Thorp and Covich (2009) and stored at -20 °C in 50 ml polypropylene tubes. Frozen brook trout axial muscle, whole non-native fish, benthic

macroinvertebrates, and zooplankton samples were freeze-dried and homogenized in preparation for stable isotope and Hg analyses.

Analysis of THg and MeHg in water

All sample processing was performed in a clean vertical laminar flow hood. THg in unfiltered and filtered water samples (n=8 each) were quantified at the University of Montreal according to United States Environmental Protection Agency (USEPA) Method 245.7 (USEPA 2005). Briefly, all Hg species were oxidized to Hg(II) with bromine monochloride (BrCl), then reduced to Hg(0) with stannous chloride (SnCl). Samples were then run on a Tekran® Series 2600 Automated Sample Analysis System, where the produced Hg(0) was purged with nitrogen, pre-concentrated onto a gold trap, thermally desorbed, and finally detected with a cold-vapor atomic fluorescence spectrometer (CVAFS). One blank and one sample duplicate were prepared and analyzed. During the analysis, instrument drift was checked with a blank and a calibration standard. The MDL was 0.2 ng/L for unfiltered water samples and 0.5 ng/L for filtered water samples. The relative percent difference between duplicates was 0.4%. The recovery of THg from a certified reference material was 94%.

Methylmercury concentrations in the unfiltered and filtered water samples (n=8 each) were determined according to USEPA Method 1630 (USEPA 1998). Briefly, water samples were purified by distillation at 125°C under nitrogen. Ascorbic acid was then added to the distillate and the pH was adjusted to 4.9 prior to ethylation of Hg species with sodium tetraethyl borate (NaBET₄). Ethylated samples were analyzed with a Brooks Rand MERX®-M Automated Methylmercury Analytical System, where the MeHg was

purged with nitrogen, pre-concentrated onto a graphitic carbon trap, thermally desorbed, converted into elemental Hg on a pyrolytic column, and finally detected with a CVAFS. A calibration curve was prepared with an undistilled MeHg standard. One blank, one matrix spike, and one sample duplicate were prepared, distilled and analyzed every 7 samples. The calibration curve was constructed with 7 standards and was checked with a certified reference material (TORT-2) digested based on the protocol described by Hammerschmidt and Fitzgerald (2005). Briefly, a precise weight of TORT-2 was digested for 12 hours at 60°C with 30% (v/v) HNO₃ (trace metal grade). The digestate was then diluted with ultrapure water, adjusted to pH 4.9 with KOH and acetate buffer, and ethylated with NaBET₄ prior to analysis. Percentages of recovery (mean ± SE) for the matrix spike (n=8) and certified reference material (n=4) were 80 ± 4% and 92 ± 1%, respectively. Duplicate relative percent differences (mean ± SE; n=7) were 11 ± 4%. During the analysis, instrument drift was checked with the measurement of an ethylation blank and a calibration standard every 10 samples. The MDL was 0.01 ng/L for unfiltered and filtered water samples.

Analysis of DOC

For DOC, water samples were analyzed within 48 h of collection at INRS-ETE with a Shimadzu TOC-V analyzer, which uses oxidative combustion-infrared analysis (Standard Method 5310). The MDL for DOC was 0.05 mg/L. The relative percent difference of DOC in duplicates (8.4 mg/L and 8.05 mg/L) collected from Lac du Fou was 4.3%. DOC values for the field blanks averaged 0.6 mg/L, indicating there was minor contamination of the filtered sample likely from the nitrocellulose filters. However, we chose not to

blank correct DOC values as the blank values were relatively low and our data were similar to data obtained by park staff.

Analysis of total mercury and methylmercury in biota

THg was determined in fish using direct mercury analyzers (DMA-80 and Nippon MA-3000) according to USEPA Method 7473 (Barst et al. 2013), which uses thermal decomposition, gold amalgamation, atomic absorption spectrophotometry. Quality assurance included the analysis of various standard reference materials (MESS-3, marine sediment, n=12; SRM 2976, mussel tissue, n=6; TORT-2 and TORT-3, lobster hepatopancreas, n=33 and n=5; DORM-4, fish muscle, n=6; DOLT-4, dogfish liver, n=13) from the National Research Council of Canada and National Institutes of Technology, duplicate samples, and blanks. The theoretical method detection limit (TMDL), calculated as 3X the standard deviation of replicate analyses of blank analytical vessels, was 0.66 ng Hg for the DMA-80 and 0.04 ng for the Nippon MA-3000. The mean percent recoveries of THg from certified reference materials were 98.8 ± 4.0 % for MESS-3, 103.0 ± 3.9 for SRM 2976, 100.8 ± 7.4 for TORT-2, 99.7 ± 7.8 for TORT-3, 100.5 ± 1.0 for DORM-4, and 99.9 ± 7.0 for DOLT-4. The mean relative percent difference of duplicate sample analyses (n=48) averaged 3.1 ± 2.8 %.

Methylmercury was measured in benthic macroinvertebrates and pelagic zooplankton according to USEPA 1630 (USEPA 1998). Briefly, freeze dried samples were accurately weighed into acid-washed polypropylene vials. Samples were digested at room temperature with a solution of 0.02 M L-cysteine in 4M HNO₃ for 24 h. Samples were then digested for another 48 h in an oven at 60 °C. Aliquots of the acid digests were

pipetted into 30 ml acid-washed vials along with ultrapure water and acetate buffer. Samples were ethylated with NaTEB₄ and analyzed with a Tekran 2700 GC-CVAFS. Standard reference materials (SRM 2976, n=3; DORM-4, n=4) were digested and analyzed with the samples. Additional quality control included duplicate samples, reagent blanks, and initial and ongoing precision recovery (OPR) samples (0.5 ng/L standard addition of MeHg). Reagent blanks were made of 4M HNO₃ / cysteine, but did not include any of the sample digest. The TMDL, calculated as 3X the standard deviation of replicate analyses of reagent blanks was 31 ng/L MeHg. The mean percent recoveries of MeHg from certified reference materials were 96.0 ± 24.1% for SRM 2976 and 107.6 ± 3.2% for DORM-4. The mean percent recovery of MeHg for OPR samples (n=13) was 96.0 ± 8.6%, and the mean relative percent difference of duplicate sample analyses (n=5) averaged 15.5 ± 11.4 %.

Stable isotope analysis of POC and food web samples

From each of the eight lakes, food web tissue samples were lyophilized and homogenized prior to analysis while POC samples were lyophilized and the entire sample was measured. Smaller invertebrate taxa were pooled to generate a sufficient sample size. Each sample was weighed into 5mm x 9 mm tin cups and packed. Stable isotope analyses were carried out in the Chemical Tracers Laboratory at the Great Lakes Institute for Environmental Research (GLIER; University of Windsor, ON Canada) and the Stable Isotope Facility (University of California, Davis). Samples were not lipid extracted or lipid corrected because the C:N ratio exceeded 3.5 in 93% of the samples. In both laboratories, an isotope-ratio mass spectrometer was used to measure the ratio of heavy-to-light carbon (¹³C/¹²C) and nitrogen (¹⁵N/¹⁴N) isotopes with the standard materials

Vienna Pee Dee Belemnite carbonate for CO₂ and atmospheric nitrogen for N₂ (Fry 2006). For the 104 samples run at the UC Davis facility (those collected in 2012) analysis was carried out using an Elementar Analysensysteme (GmbH, Hanau, Germany) interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) with the reference materials NIST bovine liver 1577c, USGS-41 L-glutamic acid, nylon 5, and NIST 1547 peach leaves. Precision of the internal standards measured $\leq 0.12\text{‰}$ for $\delta^{15}\text{N}$ and $\leq 0.11\text{‰}$ for $\delta^{13}\text{C}$ for all the standards ($n = 20$). Each 10th sample was run in duplicate (duplicates = 10, percent difference between duplicates was 4.0% for $\delta^{13}\text{C}$ and 4.0% for $\delta^{15}\text{N}$). Accuracy was based on deviation from the certified value of USGS 40 ($n=7$, difference from certified value 2.36 and 0.31 for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively).

For the 60 samples run at the Chemical Tracers Laboratory, a Delta Plus (ThermoFinnigan, San Jose, CA, U.S.A.) coupled with the elemental analyser (Costech, Valencia, CA, U.S.A.) was used in the Chemical Tracers Laboratory. Precision was assessed by the standard deviation of replicates (run every 15th sample) of the internal standards ($n=27$) NIST bovine liver 1577c, an internal lab standard (*Oreochromis niloticus*, tilapia muscle), and IVA33802174 urea. Precision of the internal standards measured $\leq 0.23\text{‰}$ for $\delta^{15}\text{N}$ and $\leq 0.17\text{‰}$ for $\delta^{13}\text{C}$ for all the standards. Each 13th sample was run in triplicate (standard deviation of the percent difference between triplicates was 4.3% for $\delta^{13}\text{C}$ and 5.24% for $\delta^{15}\text{N}$), and accuracy was based on deviation from certified value of USGS 40 ($n=27$, difference from certified value 0.01 and 0.02 for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively).

Ratios of isotopes were expressed in standard delta (δ) notation as parts per thousand (‰), as per equation (1):

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

where X is the isotope being measured (^{13}C , ^{15}N), R is the isotope ratio of interest (i.e., $^{13}\text{C} / ^{12}\text{C}$ or $^{15}\text{N} / ^{14}\text{N}$) (Peterson and Fry 1987; Fry 2006).

Data analyses

All statistical analyses and graphing were carried out using R package version 3.3.1 or JMP version 13, with alpha set at 0.05 for all tests. Mercury (unfiltered and filtered) and DOC concentrations in water are presented in ng/L and mg/L, respectively. Mercury concentrations in fish are reported as THg, while [MeHg] are reported for invertebrates; in both cases, concentrations are presented as $\mu\text{g/g}$ dry weight. For [Hg], [MeHg], and [DOC] we report means \pm standard deviations. Relationships between [Hg], [MeHg], and [DOC] were examined in bivariate scatterplots before testing linear regression models. Isotope values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of fish, invertebrates, periphyton, and POC within each lake were used to produce bivariate scatterplots to assess food web structure. Invertebrate catches differed substantially among lakes sampled, and only zooplankton (bulk), dragonfly larvae (infraorder Anisoptera), and mayfly larvae (order Ephemeroptera) were collected from most sites; samples from the orders Amphipoda, Hemiptera, Trichoptera, and Plecoptera were too sparse for use in subsequent tests or models, though MeHg and isotope data for these taxa are presented in the Supporting Information (SI) file. As such, only data from zooplankton, dragonfly larvae, and mayfly larvae were used for analyses and are presented in the main text; [MeHg] in dragonfly larvae, where sample sizes were

all > 2 for each lake (total n across lakes = 26), were pooled and compared between reference and invaded lakes using Linear Mixed Effects models (LMEs) including lake as a random effect (final model: dragonfly larvae [MeHg] ~ status + lake) using the R package *lme4* (Bates et al. 2014).

Because baseline values were similar among systems (e.g., ~25 ‰ in dragonfly larvae across lakes; see Figure 2) and the lakes are located in a relatively small geographic area, we used raw $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (referred to as $\delta^{13}\text{C}_{\text{raw}}$ and $\delta^{15}\text{N}_{\text{raw}}$) in our analyses. However, we also baseline corrected $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of fish by subtracting the signatures of dragonfly larvae within each lake; the results of analyses using these corrected values (referred to as $\delta^{13}\text{C}_{\text{cor}}$ and $\delta^{15}\text{N}_{\text{cor}}$) are also discussed and shown in the main text or SI file. Although baseline correction of $\delta^{13}\text{C}$ values is less common in the literature than that of $\delta^{15}\text{N}$, it is sometimes necessary when examining data across multiple lakes because, similar to nitrogen, basal carbon inputs can differ among systems (Glibert et al. 2019).

We estimated trophic niche by plotting $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values and fitting 95% confidence ellipses. We size-standardized [THg], $\delta^{13}\text{C}_{\text{cor}}$, and $\delta^{15}\text{N}_{\text{cor}}$ data to 30, 20, and 8.5 cm for each population of large brook trout, small brook trout, and pearl dace, respectively, using Analysis of Covariance (ANCOVA) models ([THg]/ $\delta^{13}\text{C}_{\text{cor}}$ / $\delta^{15}\text{N}_{\text{cor}}$ ~ fork length + lake + fork length*lake). Residuals from linear models between individual fish measurements of these dependant variables (i.e., [THg], $\delta^{13}\text{C}_{\text{cor}}$, and $\delta^{15}\text{N}_{\text{cor}}$) and fork length were also saved and used in subsequent LMEs to assess differences in these end-points, independent of fish size, between reference and invaded

lakes. In this analysis, the random effect of site was accounted for using LMEs, through the R package *lme4* (Bates et al. 2014), similar to the dragonfly model described above.

All food web metrics were calculated using baseline data from zooplankton samples as these were available from all lakes. Trophic positions (TPs) were calculated as per equations 1 and 2 from Garvey and Whiles (2016):

$$\alpha = (\delta^{13}C_{consumer} - \delta^{13}C_{zoopl}) / (\delta^{13}C_{dragonfly} - \delta^{13}C_{zoopl}) \quad (\text{equation 2})$$

$$TP = 2 + \frac{\{[\delta^{15}N_{consumer} - (\delta^{15}N_{dragonfly} * \alpha)] + [\delta^{15}N_{zoopl} * (1 - \alpha)]\}}{3.4} \quad (\text{equation 3})$$

Food chain length (FCL) was calculated as per equation 3 from Cabana and Rasmussen (1996):

$$FCL = \left[\frac{\delta^{15}N_{top\ adult\ brook} - \delta^{15}N_{zoopl}}{3.4} \right] + 2 \quad (\text{equation 4})$$

Food web magnification factors (FWMF) were calculated using equation 4 and 5 (modified from Fisk et al. (2001)):

$$\text{Where } \log MeHg = m * \delta^{15}N + b \quad (\text{equation 5})$$

$$\text{Then } FWMF = 3.4 (e^b) \quad (\text{equation 6})$$

Trophic magnification slopes (TMS) were calculated as per Lavoie et al. (2013): in each of the 8 lakes, biotic [Hg] (THg in fish and MeHg in invertebrates) were regressed against their raw $\delta^{15}N$ values. The resulting slope (i.e., the TMS) represents the rate at which MeHg is biomagnifying through the food web (Lavoie et al. 2013). Each lake model included data from zooplankton, dragonfly larvae, and mayfly larvae as representatives of the lower-trophic-level biota, as well as all large and small brook trout;

invaded lakes also included data from invasive pearl dace; creek chub were excluded from the analysis because they were only present in 1 lake. TMSs were compared among lakes using an ANCOVA model: $\log[\text{Hg}]$ (THg in fish, MeHg in invertebrates) $\sim \delta^{15}\text{N}_{\text{cor}} + \text{Lake} + \delta^{15}\text{N}_{\text{cor}} * \text{Lake}$, similar to Clayden et al. (2013).

To assess which parameters affect brook trout [THg] (standardized to 30 cm fork length) among invaded and reference lakes, we ran a series of regression models and performed AIC model averaging using the R-package *AICmodavg* (Mazerolle 2017) as per Grueber et al. (2011) and Lescord et al. (2019). A total of 7 models were run; one with an intercept and either lake status (i.e., invaded or reference), [DOC], FCL, TP, $\delta^{13}\text{C}$, or TMS as predictor variables (all normalized), as well as a null model with only an intercept term. These models were ranked using AICc, a form of AIC used when sample sizes are <100 (Burnham and Anderson 2002) and the coefficients of included predictor variables were averaged across all models with a delta AICc value less than 4.

RESULTS

DOC, THg, and MeHg in lake water

Overall, concentrations of DOC and Hg in water were different among invaded and reference lakes (Table 1). DOC concentrations ranged from 2.9 to 8.4 mg/L across all study lakes and mean [DOC] was higher from invaded (6.5 ± 2.2) compared to reference systems (3.9 ± 0.9 ; Table 1). Unfiltered [THg] and [MeHg] ranged from 0.62 to 3.36 ng/L and 0.02 to 0.21 ng/L, respectively. Filtered [THg] and [MeHg] lake water varied from 0.61 to 2.91 ng/L and 0.02 to 0.17 ng/L, respectively. MeHg comprised only a minor proportion of the THg present in filtered water, with percent MeHg values ranging from 2.5 to 4.7% and 3.9 to 6.4% for reference and invaded lakes, respectively. Similarly,

[MeHg] comprised 2.7 to 3.3% and 4.4 to 8.0% of the [THg] in unfiltered lake water from reference and invaded lakes, respectively. For all lakes, [DOC] was positively correlated with both unfiltered and filtered [THg] ($r^2=0.80$, $p=0.0029$ and $r^2=0.91$, $p=0.0002$) and [MeHg] ($r^2=0.73$, $p=0.0072$ and $r^2=0.93$, $p=0.0001$) (Figure S1).

THg and MeHg in biota

MeHg concentrations at the base of food webs (i.e., dragonfly larvae) ranged from 0.026 to 0.170 ($\mu\text{g/g}$ dry wt.) across all lakes (Table 2). Dragonfly larvae from invaded lakes had significantly higher (LME $p=0.002$) [MeHg] ($0.087 \pm 0.029 \mu\text{g/g}$ dry wt) when compared to reference lakes ($0.061 \pm 0.007 \mu\text{g/g}$ dry wt.; Table 2), though there was a notable amount of variation among sites. Similarly, length-standardized muscle [THg] was significantly higher in both small (LME $p=0.045$) and large (LME $p=0.002$) brook trout from invaded lakes (small and large brook trout: 1.03 ± 0.43 and $1.50 \pm 0.94 \mu\text{g/g}$ dry wt.) compared to those from reference lakes (small and large brook trout: 0.47 ± 0.08 and $0.81 \pm 0.12 \mu\text{g/g}$ dry wt.; Table 2). Across all lakes, there was no significant relationship between [DOC] and [MeHg] in dragonfly larvae. However, [DOC] in lake water was positively correlated with length-standardized muscle [THg] of both small ($r^2=0.72$, $p=0.008$) and large brook trout ($r^2=0.55$, $p=0.034$) (Figure S2). Similarly, length-standardized muscle [THg] of both small and large brook trout were positively correlated with [THg] and [MeHg] of filtered and unfiltered water samples ($r^2=0.59$ to 0.92 , $p=0.0001$ to 0.026) (Figures S3 and S4).

Food web metrics

For all lake food webs, mean $\delta^{15}\text{N}_{\text{raw}}$ values were lowest in periphyton samples (-0.913 to 1.28‰), higher in benthic invertebrates (0.497 to 4.30‰) and zooplankton (0.298 to

4.64‰), and highest in fish (6.06 to 8.50‰; Tables S1 and S2). Within lakes, mean $\delta^{15}\text{N}_{\text{raw}}$ values were similar for both small and large brook trout (generally less than 1‰ difference across lakes). Across lakes, mean $\delta^{15}\text{N}_{\text{raw}}$ values ranged from 6.32 ± 0.78 to 7.79 ± 0.34 ‰ for small brook trout and from 6.06 ± 0.78 to 8.50 ± 0.79 ‰ for large brook trout. Within invaded lakes, non-native pearl dace had slightly lower mean $\delta^{15}\text{N}_{\text{raw}}$ values than brook trout (generally less than 1‰ difference across lakes), except for Alphonse Lake, where $\delta^{15}\text{N}_{\text{raw}}$ values were similar (Figure 2). As expected, mean $\delta^{13}\text{C}_{\text{raw}}$ values were generally more negative in pelagic zooplankton (-36.73 to -31.08‰) compared to benthic invertebrates (-31.97 to -23.31‰) across lakes. In general, the ranges of $\delta^{13}\text{C}_{\text{raw}}$ values within each lake were similar among all fish (Figure 2).

The $\delta^{13}\text{C}_{\text{raw}}$ and $\delta^{15}\text{N}_{\text{raw}}$ values of large and small brook trout were significantly different between invaded and reference lakes; brook trout from invaded lakes having more enriched $\delta^{15}\text{N}_{\text{raw}}$ and $\delta^{13}\text{C}_{\text{raw}}$ values, suggesting these fish feed at higher trophic levels and further from shore (LME $p < 0.001$; Figure 3A). However, this was not the case for corrected stable isotope values (Figure 3B); neither $\delta^{15}\text{N}_{\text{cor}}$, $\delta^{13}\text{C}_{\text{cor}}$, nor trophic position of small and large brook trout were significantly different between invaded and reference lakes (LME $p = 0.332$ to 0.983). Overall, basal $\delta^{15}\text{N}_{\text{raw}}$ values in dragonfly larvae were similar between reference (mean $\delta^{15}\text{N}_{\text{raw}} = 2.8 \pm 1.7$) and invaded (mean $\delta^{15}\text{N}_{\text{raw}} = 3.3 \pm 1.5$) systems, suggesting that differences seen in uncorrected $\delta^{15}\text{N}$ values of brook trout from invaded and reference lakes (Figure 3A) were not simply due to a difference in baseline values. When comparing $\delta^{13}\text{C}$ signatures in brook trout from reference and invaded systems, fish from reference lakes had a greater span of both

corrected and raw values when compared to fish from invaded lakes, implying a narrower range of dietary selection for the latter fish (Figure 3A and 3B).

Predictors of brook trout THg and trophic magnification slopes

The slopes of the eight food web biomagnification models were significantly different from one another (ANCOVA interaction term $\delta^{15}\text{N}_{\text{cor}}*\text{Lake}$ $p < 0.001$; Supporting Information Table S3). The TMSs of invaded lakes were slightly higher than those from reference systems, with the exception of Maréchal and Alphonse lakes (Figure 4). Of the lake-level parameters assessed (i.e., FCL, FWMF, TMS, and [DOC]; Tables 1 and 3), only [DOC] varied notably among systems. Indeed, invaded lakes had higher [DOC] (mean: 6.5 ± 2.2 mg/L) than reference lakes (mean: 3.9 ± 0.9 mg/L; Table 1). The other parameters assessed, including TMS, were similar among invaded and reference lakes (e.g., $\text{FCL} = 3.77\pm 0.38$ and 3.70 ± 0.36 , $\text{TMS} = 0.196\pm 0.045$ and 0.198 ± 0.046 in reference and invaded sites, respectively; Table 3), suggesting no substantial difference in these endpoints relative to lake status.

Overall the multivariate averaging exercise showed that DOC and TMS were the only significant predictors of brook trout [THg] among the reference and invaded lakes. DOC concentrations were the stronger of these two predictors ($p = 0.029$, $r^2 = 0.55$), but the Hg biomagnification rate also influenced [THg] of large brook trout to some extent ($p = 0.099$, $r^2 = 0.41$) (Supporting Information Table S4). These modeling results suggest that brook trout from lakes with higher [DOC] and rates of Hg biomagnification (i.e., TMS) had higher [THg]. Notably, lake status, TP, $\delta^{13}\text{C}_{\text{cor}}$, and FCL were not shown to have a significant effect on [THg] in brook trout across our study lakes.

Discussion

In the present study we sampled the food webs of eight lakes (four reference and four invaded) in LMNP to determine if the presence of non-native fish influenced the [THg] of native brook trout. In addition to food web metrics, we also incorporated [DOC] in our sampling strategy and statistical models to account for potential site-specific environmental influences on Hg bioaccumulation and biomagnification.

Effect of non-native fish on aquatic food web structure

Overall, we found that the presence of invasive pearl dace altered the aquatic food webs of LMNP lakes. The $\delta^{13}\text{C}_{\text{raw}}$ values indicate that pearl dace, small brook trout, and large brook trout from all lakes, with the exception of Alphonse, rely mainly on benthic carbon sources; most of the pearl dace and brook trout from Alphonse had $\delta^{13}\text{C}_{\text{raw}}$ values more similar to zooplankton, suggesting more pelagic feeding by these fish. The similar within-lake $\delta^{15}\text{N}_{\text{raw}}$ values of small and large brook trout indicate that both size classes of fish fed at similar trophic positions. For Alphonse Lake, the substantial trophic niche overlap of non-native pearl dace and brook trout may be a result of similar resource use by the two species. This is consistent with previous studies that suggest trophic niche overlap between species of fish may be interpreted as an indication of competition for a shared food source (Vander Zanden et al. 1999; Pilger et al. 2010). Carbon and nitrogen isotopes have been used previously to assess trophic niche overlap and potential interactions between native and non-native fishes (Pilger et al. 2010; Córdova-Tapia et al. 2015). For example, Córdova-Tapia et al. (2015) documented trophic niche overlap between native and non-native species of fish in a Mexican lake. In that study, the authors argue that a substantial niche overlap implied shared resource use and potential competition among

native and non-native species. In the present study, however, the wider trophic niches of the non-native pearl dace suggests that the pearl dace are able to make use of various food sources, while the native brook trout feed on a smaller range of food sources and are therefore more strongly affected by competition.

In the three invaded lakes other than Alphonse, the typically higher $\delta^{15}\text{N}_{\text{raw}}$ values for brook trout relative to those of pearl dace suggest that brook trout feed at a higher trophic position and may utilize pearl dace as a food source, a frequently overlooked effect of lower-trophic-level invasive species (Puntilla-Dodd et al. 2019). Furthermore, brook trout from these invaded lakes tended to feed at higher trophic levels and fed on more off-shore sources than those from reference lakes. It is noteworthy that baseline-corrected stable isotope values indicated greater similarities between the feeding ecologies of brook trout from reference and invaded lakes. Both raw and corrected $\delta^{13}\text{C}$ values suggest that brook trout from invaded systems had narrower niches than brook trout from reference systems, potentially as a result of increased competition between brook trout and pearl dace in the invaded lakes. A narrowing of native fish $\delta^{13}\text{C}$ values has been suggested to occur as a result of non-native fish introductions in other systems (Córdova-Tapia et al. 2015).

Effect of non-native fish on Hg accumulation

Overall, [THg] of brook trout and pearl dace in LMNP were comparable to fish from other parts of North America (Depew et al. 2013). However, brook trout from invaded lakes had slightly higher [THg] when compared to fish from reference lakes (with the exception of Alphonse Lake, an invaded system with the lowest [THg] in brook trout). Despite substantial increases in non-native fish introductions and their impacts, few

studies have addressed how non-native fishes influence Hg dynamics in aquatic ecosystems (Eagles-Smith et al. 2018). Threadfin shad (*Dorosoma petenense*) introduction to a lake in California, USA caused native fish to shift from pelagic to benthic prey and increased their Hg concentrations by 50% (Eagles-Smith et al. 2008b). Several studies have been carried out to determine the influence of rainbow smelt (*Osmerus mordax*) introductions in the Laurentian Great Lakes and Hudson Bay drainages on contaminant levels in predatory fishes. While some research suggests that rainbow smelt caused food chains to lengthen and Hg levels to increase in predatory fishes, other research has produced conflicting results (Vander Zanden and Rasmussen 1996). For example, studies carried out in the Hudson Bay drainage demonstrated that smelt had elevated trophic positions and lower Hg relative to other forage fishes (Swanson et al. 2003). This in turn produced higher trophic positions in predatory fish, but no significant increases in their Hg levels (Johnston et al. 2003). Subsequent research concluded that rainbow smelt invasions would not likely result in Hg increases in predatory fishes, potentially due to a lack of Hg biomagnification at fine trophic scales (Swanson et al. 2006). Recent research from Lake Michigan demonstrates that food web shifts due to invasive species (dreissenid mussels and round goby (*Neogobius melanostomus*)) led to higher than expected Hg concentrations in predatory lake trout (*Salvelinus namaycush*) given declining atmospheric Hg emissions (Lepak et al. 2019).

Trophic magnification slopes, which ranged from 0.135-0.249, were comparable to other freshwater systems (global average = 0.16 - 0.24; (Lavoie et al. 2013)), suggesting that Hg undergoes a similar rate of trophic-transfer in LMNP lakes as in other aquatic food webs. To the best of our knowledge, this study is the first assessment of the

effect of non-native species on Hg TMSs. Our results show similar ranges of TMSs across both invaded and reference lakes, implying little effect of the invasive pearl dace on the rates of Hg biomagnification. In general, forage fish have complex trophic interactions and it is possible that the inclusion of other species in the TMS models would reveal more substantial effects on lower-food-web Hg biomagnification (Swanson et al. 2003).

Given the higher $\delta^{15}\text{N}_{\text{raw}}$ values of brook trout from the invaded lakes, we were surprised to find that $\delta^{15}\text{N}_{\text{raw}}$, $\delta^{15}\text{N}_{\text{cor}}$, and TP were not significant predictors of brook trout [THg] in our models. Rather, results of the statistical analyses demonstrate that DOC was the most consistent and influential predictor of brook trout [THg] across systems. A previous study on non-native rainbow smelt (*Osmerus mordax*) in Ontario lake similarly found an impact on food web structure, but no significant change in [THg] in the forage fish communities of invaded lakes (Swanson et al. 2003).

Overall, these model results imply that environmental influences over the Hg cycle are more important determinants of terminal piscine [THg] than alterations to the food web structure in LMNP lakes. Indeed, DOC plays a complex role in the bioavailability of Hg in freshwater ecosystems (Ravichandran 2004; Lavoie et al. 2019). Several studies have reported positive relationships between aqueous [THg] and/or [MeHg] with [DOC] in freshwaters (Driscoll et al. 1995; Scudder 2010; Braaten et al. 2014; Lescord et al. 2018; Lavoie et al. 2019; Wu et al. 2019). Dissolved organic matter (DOM) and/or DOC can influence Hg levels of freshwaters by facilitating the export of inorganic Hg and MeHg from watersheds to waterbodies (Driscoll et al. 1995; Hurley et al. 1995). Additionally, DOC plays a complex role in the bioavailability of Hg in

freshwater ecosystems (Ravichandran 2004). Hg methylation is mediated by microbial activity, thus factors that decrease or increase microbial uptake of inorganic Hg will affect the rate of MeHg production in freshwater environments. DOC complexation of inorganic Hg has been shown to decrease inorganic Hg uptake by bacteria (Gilmour and Henry 1991; Barkay et al. 1997). However, in certain freshwater environments DOC may stimulate microbial growth and microbial methylation (Benoit et al. 2003; Ravichandran 2004). Moreover, MeHg solubility has been shown to increase in the presence of DOC, leading to increased aqueous [MeHg] (Miskimmin 1991; Ravichandran 2004). The positive relationships between DOC and Hg in lake water and fish from LMNP lakes are consistent with a study of Adirondack lakes, where aqueous [THg], [MeHg], and fish [THg] increased over a similar range of [DOC] (Driscoll et al. 1995). It is noteworthy that the lakes in our dataset had low [DOC] (2.9-8.4 mg/L) and, given that the relationship between DOC and Hg in biota has been shown to be concentration-dependant, it is possible that the inclusion of lakes with higher [DOC] (i.e., <8-11 mg/L) may alter this finding (French et al. 2014; Braaten et al. 2018; Lescord et al. 2018).

Overall Conclusions

In the present study, we found that while Hg biomagnification in invaded and uninvaded systems was similar, there was some evidence of competition between invasive pearl dace and native brook trout. The presence of an additional forage fish species could theoretically increase predator fish contaminant concentrations by introducing an additional trophic level into the food web (Johnston et al. 2003; Wallace and Blersch 2015), but our work demonstrates the primary roles of the native and invasive species in LMNP lakes was not simply predator and prey. Competition between brook trout and

pearl dace would result in similar THg concentrations for the two species, as we observed for most individuals.

With respect to management decisions regarding invasive forage fish, neither this study nor the study by Johnston et al. (2003) indicate strong detrimental effects to native fish Hg levels due to invasive forage fish. Competition for prey resources appears to be of greater concern, with young-of-the-year and immature predator fish species being more susceptible to these effects (Eagles-Smith et al. 2008b). However, the interpretation of these effects is hindered by the scarcity of monitoring studies on this topic, and additional studies of the effects of invasive species on contaminant levels in piscivores are warranted. We highlight the need to include measures of DOC (and perhaps other biogeochemical measures) in future studies aimed at assessing Hg biomagnification in invaded lakes. Finally, we suggest that future studies on this topic may also benefit from a larger lake sample size; many of our statistical analyses were limited by a relatively small sample size (i.e., $n = 8$ for all population-level analyses) and a larger study may be able to detect more significant effects.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.xxxx.

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Figure captions

Figure 1. Location of La Mauricie National Park (Quebec, Canada; white star) and the eight lakes sampled for the present study. Lakes indicated with black arrows have populations of brook trout (*Salvelinus fontinalis*) as the only fish species present. Lakes indicated by white arrows have populations of brook trout and non-native fish.

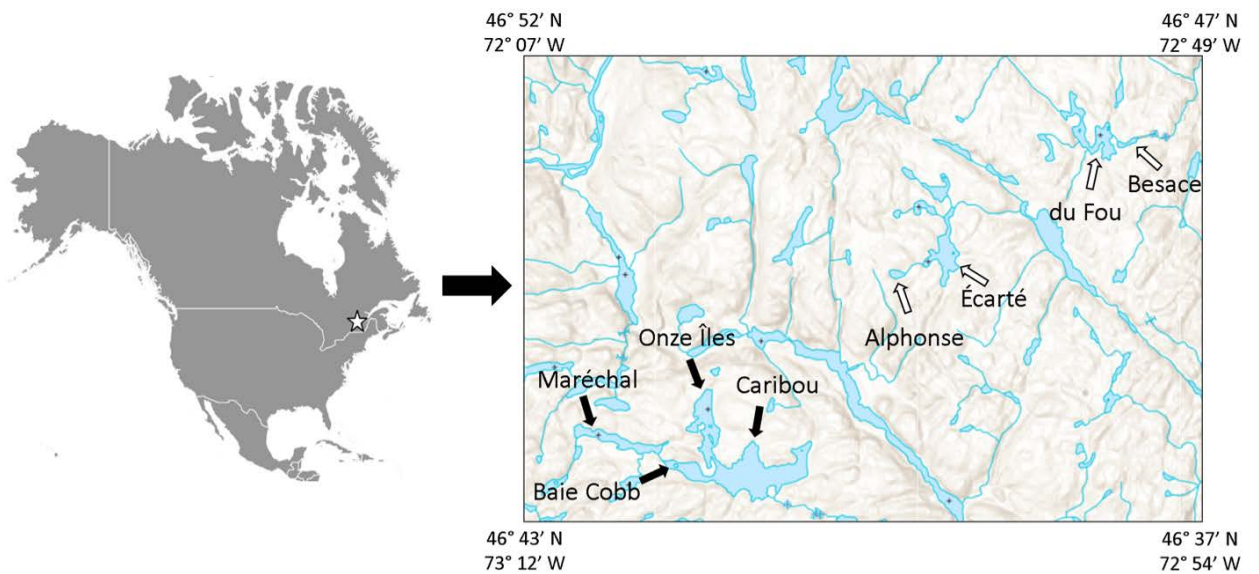


Figure 2: Stable carbon and nitrogen isotope ratio bi-plots for the eight food webs in La Mauricie National Park (Quebec, Canada).

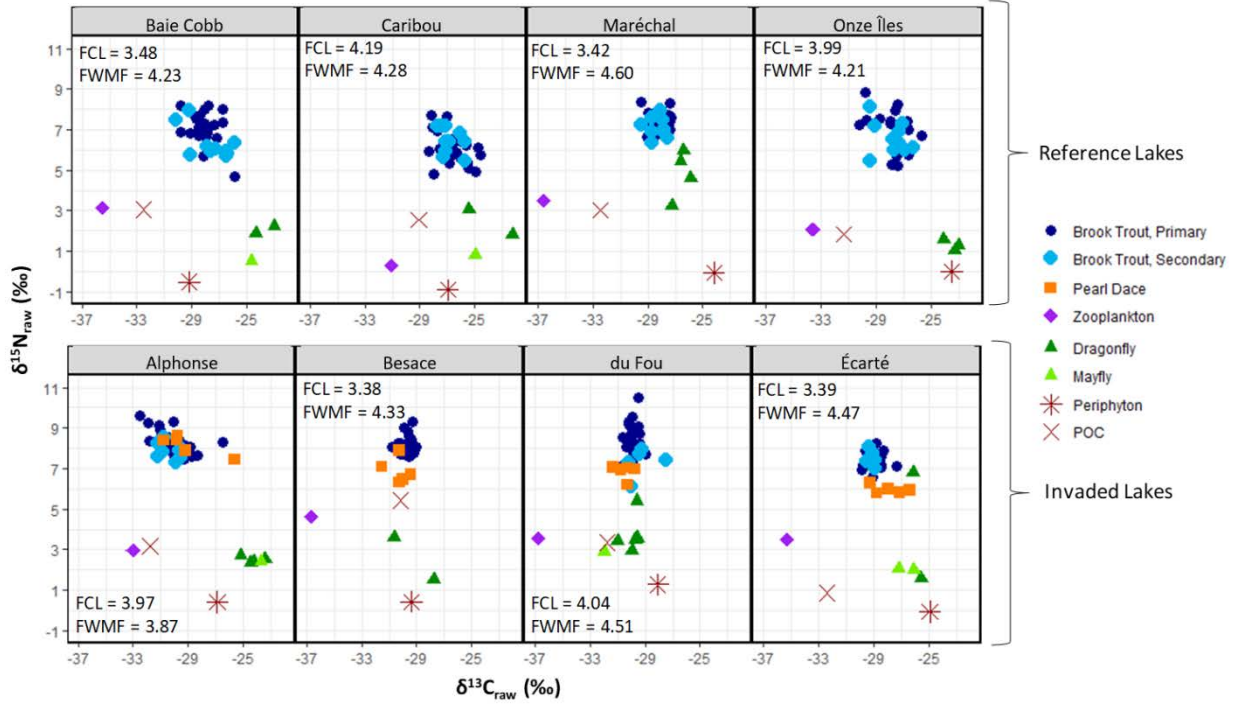


Figure 3: Trophic niche metrics of small and large brook trout (*Salvelinus fontinalis*) sampled from the eight study lakes in La Mauricie National Park (Quebec, Canada). Reference lakes are in blue and invaded lakes are in red. Panels A and B present raw and corrected stable isotope values, respectively.

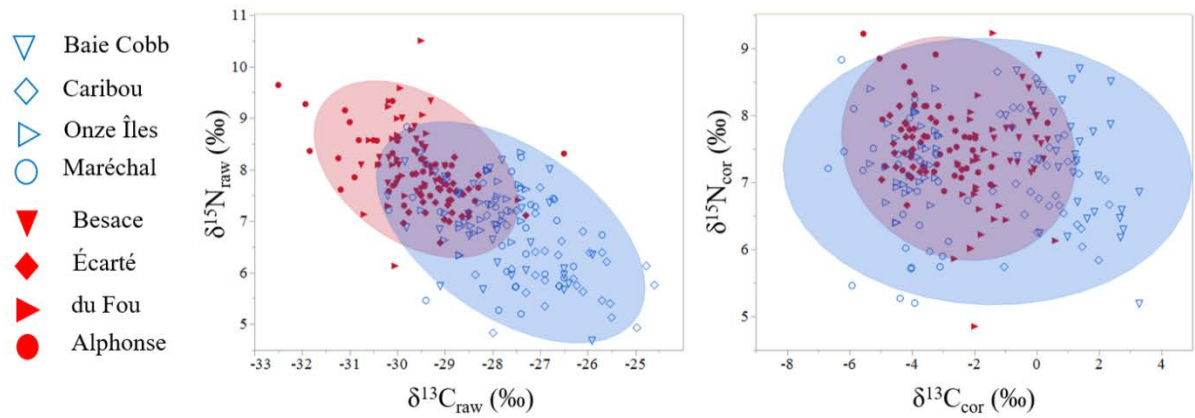


Figure 4: Biomagnification models for the eight food webs sampled from La Mauricie National Park (Quebec, Canada).

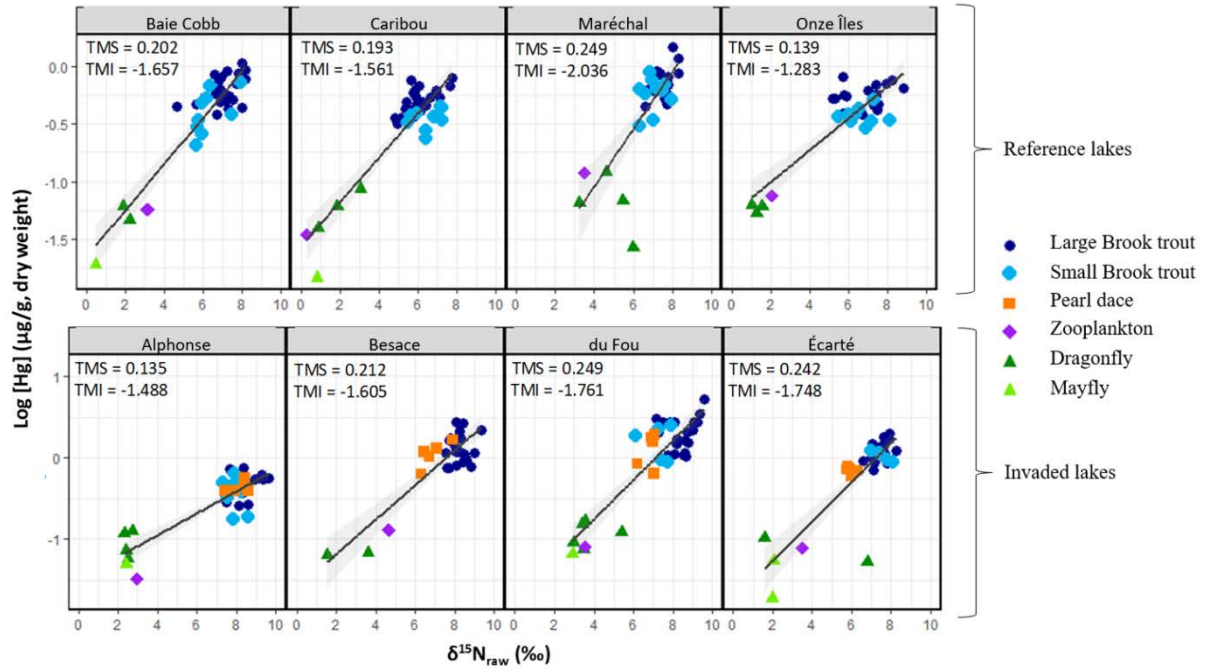


Table 1: Site characteristics and water data for eight lakes, four reference and four invaded, located in La Mauricie National Park (Quebec, Canada). All mercury measurements in water are presented as ng/L or ppt concentrations. Note: Ref. = reference lake; Inv. = invaded lake; BT = brook trout (*Salvelinus fontinalis*); APD = Allegheny pearl dace (*Margariscus margarita*); UF = unfiltered; F = filtered

Lake	Site Characteristics				Water Data					
	Stat	Surf Area (ha)	Max Depth (m)	Species Present	[DO C] (mg/ L)	[MeH g], UF	[MeH g], F	[THg] , UF	[THg] , F	%Me Hg ²
Baie Cobb	Ref .	0.63	15	BT	4.3	0.05	0.04	1.80	1.31	3.1
Caribo u	Ref .	3.96	35	BT	2.9	0.02	0.02	0.62	0.61	2.5
Maréc hal	Ref .	1.05	20	BT	5.0	0.06	0.07	1.73	1.50	4.7
Onze Îles	Ref .	1.41	30	BT	3.4	0.03	0.03	1.18	0.87	3.2
Mean	Ref	1.8±	25±		3.9±	0.04±	0.04±	1.33±	1.07±	3.4±0
±SD	.	1.5	9	----	0.9	0.02	0.02	0.55	0.40	.9

Alphonse	Inv.	0.13	15	BT, APD	4.9	0.04	0.03	0.81	0.76	3.9
Besace	Inv.	0.16	29	BT, APD	8.4	0.21	0.14	3.36	2.91	4.7
Écarté	Inv.	1.36	30	BT, APD	8.4	0.17	0.17	2.79	2.63	6.4
Fou	Inv.	1.34	3	BT, APD	4.4	0.15	0.05	1.87	0.99	5.0
Mean		0.7±	19±		6.5±	0.14±	0.10±	2.21±	1.82±	5.0±1
±SD	Inv.	0.7	13	----	2.2	0.08	0.07	1.11	1.10	.0

¹Maximum depths were provided by LMNP park staff.

$$^2([\text{MeHg}]_{\text{F}}/[\text{THg}]_{\text{F}})*100$$

Table 2: Average mercury concentrations in biota from the eight study lakes located in La Mauricie National Park (Quebec, Canada). Sample sizes of collected food web items are indicated by numbers in parentheses. Means and standard deviations are reported for samples with more than one replicate.

Lake	Stat	Invertebrates [MeHg] (µg/g dry wt)			Fish [THg] (µg/g dry wt)		
		Zooplankton	Mayfly larvae	Dragonfly larvae	Large brook trout ¹	Small brook trout ²	Pearl Dace ³
Baie	Ref	0.057	0.019	0.054±0	0.98±0.18	0.48±0.09	
Cobb	.	(1)	(1)	.010 (2)	(23)	(10)	---

Caribo	Ref	0.035	0.014	0.062±0	0.73±0.18	0.41±0.09	
u	.	(1)	(1)	.024 (3)	(22)	(11)	---
Maréc	Ref	0.120		0.070±0	0.71±0.18	0.58±0.09	
hal	.	(1)	---	.038 (4)	(22)	(10)	---
Onze	Ref	0.075		0.059±0	0.83±0.20	0.40±0.09	
Îles	.	(1)	---	.005 (3)	(19)	(10)	---
Mean±	Ref	0.072±0	0.017±0	0.061±0			
SD	.	.036	.003	.007	0.81±0.12	0.47±0.08	---
							0.54±
Alpho	Inv.	0.033	0.049	0.093±0			
nse		(1)	(1)	.032 (4)	0.27±0.18	0.43±0.10	0.15
					(22)	(13)	(5)
							1.22±
Besace	Inv.	0.129	---	0.052±0			
		(1)		.025 (3)	1.66±0.18	1.21±0.12	0.14
					(22)	(5)	(5)
							1.27±
du Fou	Inv.	0.080	0.066	0.122±0			
		(1)	(1)	.040 (5)	2.55±0.18	1.43±0.09	0.14
					(22)	(10)	(5)
							0.73±
Écarté	Inv.	0.079	0.037±0	0.079±0			
		(1)	.025 (2)	.037 (2)	1.53±0.18	1.07±0.12	0.14
					(24)	(5)	(5)
Mean±	Inv.	0.080±0	0.051±0	0.087±0			0.94±
SD		.039	.015	.029	1.50±0.94	1.03±0.43	0.36

¹Standardized to 30cm fork length; ²Standardized to 20cm fork length; ³Standardized to 8.5cm fork length

Table 3: Average stable isotope values (carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$)) for large brook trout and associated food web metrics. Note Ref. = reference lake; Inv. = invaded lake; TP = trophic position; FCL = food chain length; TMS = trophic magnification slope; TMI = trophic magnification intercept; FWMF = food web magnification factor.

Lake	Stat us	Food web metrics						
		$\delta^{15}\text{N}_{\text{raw}}$ (‰) ¹	$\delta^{13}\text{C}_{\text{raw}}$ (‰) ¹	TP ¹	FCL	TMS	TMI	FWMF
Baie Cobb	Ref.	7.32±0.	28.14±0	4.5±	3.48	0.202	-1.657	4.23
		13	.19	0.1				
Caribo u	Ref.	6.22±0.	26.61±0	3.8±	4.19	0.193	-1.561	4.28
		13	.19	0.1				
Maréch al	Ref.	7.40±0.	28.11±0	3.4±	3.42	0.249	-2.036	4.6
		13	.19	0.1				
Onze Îles	Ref.	7.12±0.	27.75±0	3.1±	3.99	0.139	-1.283	4.21
		14	.21	0.1				

Mean±			-		3.77±0.	0.196±0.	-	4.33
SD	Ref.	7.01±0.	27.65±0	3.7±	38	045	1.64±0.	±0.18
		54	.71	0.6			311	
Alphonse			-					
	Inv.	8.21±0.	29.86±0	4.7±	3.97	0.135	-1.488	3.87
		13	.19	0.1				
Besace			-					
	Inv.	8.37±0.	29.75±0	4.1±	3.38	0.212	-1.605	4.33
		14	.20	0.1				
du Fou			-					
	Inv.	8.41±0.	29.90±0	3.6±	4.04	0.249	-1.761	4.51
		13	.19	0.1				
Écarté			-					
	Inv.	7.61±0.	29.01±0	3.6±	3.39	0.242	-1.748	4.47
		13	.19	0.1				
Mean±			-		3.70	0.198±0.	-	4.30
SD	Inv.	8.15±0.	29.63±0	4.0±	±0.36	046	1.62±0.	±0.30
		37	.42	0.5			126	

¹Standardized to 30 cm fork length using an ANCOVA model: isotope = fork-length + lake + fork-length *lake