

Intra- and interlake comparisons of select fishes in the Laurentian Great Lakes using morphometry, stable isotopes, and mercury

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Intra- and interlake comparisons of select fishes in the Laurentian Great Lakes using morphometry, stable isotopes, and mercury

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Abstract

Food web structure is known to vary among the Laurentian Great Lakes. Here, we present evidence that food web structure also varies within each Great Lake. We compiled fish morphometry and ecological (stable isotope niche areas) and bioaccumulative (trophic magnification slope) metrics on 12 fish taxa from 5 ecoregions across 4 Great Lakes. Data were summarized taxonomically, environmentally, and by trophic guild to deduce patterns in ecological and bioaccumulative metrics among ecoregions and lakes. The results reinforce the spatial variability associated with trophic relationships both within and among the Great Lakes and provide rationale for considering sub-lake spatial units to achieve ecosystem-based management objectives.

Résumé

Comparaisons intra-lacs et interlacs de poissons sélectionnés dans les Grands Lacs laurentiens à l'aide de la morphométrie, des isotopes stables et du mercure

Il est connu que la structure du réseau alimentaire varie entre les Grands Lacs laurentiens. Ici, nous présentons des preuves que la structure du réseau alimentaire varie également au sein de chaque Grand Lac. Nous avons dressé la morphométrie des poissons et les mesures écologiques (niches d'isotopes stables) et bioaccumulatives (pente d'amplification trophique) pour 12 taxons de poissons provenant de 5 écorégions dans 4 Grands Lacs. Les données ont été résumées sur les plans taxonomique et environnemental ainsi que par guilde trophique afin de déduire des modèles de mesures écologiques et bioaccumulatives entre les écorégions et les lacs. Les résultats renforcent la variabilité spatiale associée aux relations trophiques, à la fois dans les Grands Lacs et entre ces derniers, et justifient la prise en compte des unités spatiales sous-lacustres pour atteindre les objectifs de gestion écosystémique.

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Introduction

The Laurentian Great Lakes basin is a large and spatially complex freshwater system, providing ecological and economical services such as drinking water, recreational use, fishing, and commerce to more than 34 million people (Baustian et al. 2014). Among and within these lakes, differing physical and chemical (intrinsic) properties such as depth, temperature, and productivity contribute to differences in fish habitat, community assemblages, and food web structure (Ives et al. 2019, Wegher et al. 2019).

These lakes are also constantly changing due to stressors such as climate change, aquatic invasive species (AIS), and nutrient and contaminant loadings that confound our interpretation of their condition or state. Given the variation in intrinsic properties and stressors over time and space, we consider whether it is possible to compare fish health and fisheries both within and among lakes. For example, we might expect walleye (a coolwater species) production to be lower in Lake Superior than in Lake Erie due to Lake Superior's largely cold water habitats and lower primary productivity, but likewise if stressors (e.g., increasing water temperature induced by climate change) are impeding production, should we expect the capacity for increased production in Lake Superior to be as high as in Lake Erie? Likewise, are fishes from different guilds or trophic levels comparably impacted by these intrinsic and external forces? Understanding the degree to which intra- and interlake variation contributes to different ecological metrics might enable us to better understand both the magnitude of impact and the potential for recovery (e.g., historical impacts of AIS) at whole lake and basin wide scales.

Various research efforts have been devoted to developing indicators of lake health (e.g., Niemi and McDonald 2004, Brazner et al. 2007, Allan et al. 2013, ECCC/US EPA 2022). Current monitoring programs are continuously being challenged to collect, analyze, and interpret more data with fewer resources, thus we need to consider which indicators would be best to make broad interpretations about lake health. Here, we focus on broad integrative ecological tools — morphometrics, stable isotopes, and trophic magnification slopes (TMS) — that capture the state variable but also the mechanistic response of fishes, and that can be more easily interpreted by resource management partners (Table 1). We also propose the use of ecoregions — sub-lake spatial units with definable properties — as a method to reflect perceived differences in intrinsic lake properties and external stressors that may reveal underlying ecological differences both within and among Great Lakes.

To track and account for the differing biogeochemical processes of ecoregions in the Great Lakes, we utilize stable isotopes. Stable isotopes are an ecological tool that integrates a mixture of organic and inorganic sources for the isotope under consideration, typically carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotopes. Differences in surrounding geology, water chemistry, and environmental inputs (e.g., watershed, ground water, and anthropogenic sources) result in spatially distinct stable isotopes among and within Great Lakes. $\delta^{13}\text{C}$ stable isotopes represent the degree of nearshore or offshore feeding while $\delta^{15}\text{N}$ stable isotopes are used to estimate food chain length. Standardizing the differences among such heterogenous environments can be accomplished using baseline organisms — lower trophic level organisms before any or limited trophic enrichment (transfer of energy in food webs) between predator and prey occurs. Once the isotope enters the trophic pathway as predator consumes prey, the isotopic ratios change in predictable ways and this information is used by ecologists to interpret

relevant ecology processes (diet, trophic position, niche partitioning, etc.). However, the entire trophic pathway in the ecoregion is grounded in the stable isotopes of its baseline. In other words, baseline differences are incorporated in all organisms associated with that isotopic source. Hence the need to account for baselines before comparing species or food webs.

In addition to stable isotopes, TMS uses the relationship between nitrogen ($\delta^{15}\text{N}$) stable isotopes and methylmercury to measure energy transfer efficiency throughout food webs from prey to predators. When consumed, methylmercury bioaccumulates in fish tissue and is not expelled, making it an effective ecological tracer to scale against $\delta^{15}\text{N}$, which is indicative of trophic position (a measure of how high up a species is feeding in a food web). Global TMS values are typically ~0.2 (total mercury; Zhang et al. 2012, Lavoie et al. 2013), where higher values suggest metabolic inefficiency in a system (i.e., a need to consume more prey/energy to meet the energy demands of predators) whereas lower values suggest that energy for growth is obtained more efficiently. TMS values may differ among and within Great Lakes and can be used as a bioaccumulative metric to interpret different energy transfer efficiency strengths.

Our goal was to generate ecological (stable isotope) and bioaccumulative (TMS) metrics to compare health and functional attributes of Great Lakes fishes. Chosen fish species were those commonly encountered in sampling programs but that represent different habitats and thermal and trophic guilds. After standardizing data to account for intrinsic biases, we generated a data set that better facilitates comparison across the Great Lakes both to assess current fish health (i.e., an integrative measure of fish condition or performance) and to understand the relative impairment or recovery following ecological or anthropogenic perturbation.

Methods

Data collection and measurements

Samples were obtained from multiple agencies as part of the Cooperative Science and Monitoring Initiative (CSMI) conducted on each lake. In consultation with lake-specific agencies, standardized ecoregions were established to describe different fish assemblages; ecoregion designations for each lake were inlet, anthropogenic, open coastal, embayment, and outlet (Figure 1, Table 2). Sample collections were coordinated to achieve representation of different trophic guilds in these different fish community structures and were categorized into piscivore (fish-eating), omnivore (broad diet), insectivore (insect-eating), and planktivore (plankton-eating) trophic guilds (Hoyle et al. 2012). Fish taxa included walleye (*Sander vitreus*), lake trout (*Salvelinus namaycush*), smallmouth bass (*Micropterus dolomieu*), yellow perch (*Perca flavescens*), cisco (*Coregonus artedi*), lake whitefish (*Coregonus clupeaformis*), alewife (*Alosa pseudoharengus*), rainbow smelt (*Osmerus mordax*), emerald and spottail shiner (*Notropis* sp.), deepwater sculpin (*Myoxocephalus thompsonii*), slimy sculpin (*Cottus cognatus*), and round goby (*Neogobius melanostomus*). Collected fish were mostly from adult life stages. These species were selected because of their commonness among and within most of the Great Lakes. Dreissenids were also collected as a baseline to adjust for biogeochemical differences within and among lakes (Grigorovich et al. 2008, Burlakova et al. 2018).

Binational agencies collected fish using gill nets and trawls, while dreissenid mussels were collected from trawls or ponar devices from various sites in each lake and ecoregion (Table 3).

All samples were sent to the University of Windsor and Canadian Nuclear Laboratories for processing, where they were identified, measured, weighed, and a small piece of white muscle tissue was removed anterior to the dorsal fin. Muscle tissue samples were freeze dried, homogenized into fine powder, and analyzed for carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotopes, and for total mercury.

Morphometric analyses were determined and presented to provide basic description and context for comparability of fish species collected across ecoregions and among lakes (e.g., comparing Lake Ontario-open coastal alewife to Lake Huron-embayment alewife). Whole fish were weighed (g) and measured (mm), stomachs were removed, and fish were frozen for further processing. Fulton's condition factor (K) was calculated using the equation:

$$K = \frac{10^5 \text{RWT}}{\text{TLEN}^3}$$

where TLEN represents total length (mm) and RWT represents round weight (g). Fish species measuring <30 mm or having a Fulton's K condition factor <0.4 or >1.7 were not included in this analysis (<1% of samples collected). These samples were omitted because they represented unrealistic fish condition. Observed mean ($\pm\text{SE}$) TLEN, RWT, and K values were reported for each fish taxa for each ecoregion and lake examined in this study (TLEN, Table 4; RWT, Table 5; K, Table 6; Barnham and Baxter 1998, Chipps and Garvey 2007). These mean values are representative of the samples provided to us by agencies and do not necessarily reflect population-level patterns in size or condition.

Stable isotope analysis

White muscle samples (~1 cm³) were removed anterior to the dorsal fin of each fish and freeze dried for 48 hours at -48 °C and 133 x 10⁻³ mbar. Samples and standards were weighed into 5x9 mm tin cups to obtain a final sample mass (400–600 µg, fish; 600–800 µg, invertebrates). A 4010 Elemental Combustion Analyzer (Costech Instruments, Valencia, CA, USA) was used to combust samples into gaseous carbon and nitrogen products that are carried via helium gas into the Delta V Advantage Thermoscientific Continuous Flow Mass Spectrometer (Thermo Scientific, Bremen, Germany). Stable isotope values are reported as units per mil (‰) and were calculated using the equation:

$$\delta X = \left(\left[\frac{R_{\text{sample}}}{R_{\text{standard}}} \right] - 1 \right) \times 1000$$

where X represents either ^{13}C or ^{15}N , and R is represented by the ratio of heavy to light elements ($^{13}\text{C}:\text{C}$ or $^{15}\text{N}:\text{N}$). Vienna Pee Dee Belemnite (VPDB) and atmospheric nitrogen were used as standards (R standard) for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively. Instrument accuracy measured throughout the period by NIST standards for $\delta^{15}\text{N}$ were within 0.1‰ (NIST 8573), 0.4‰ (NIST 8548), and <0.1‰ (NIST 8549), and for $\delta^{13}\text{C}$ were within 0.2‰ (NIST 8542) and 0.1‰ (NIST 8573) of certified values. Precision for laboratory standards (NIST 1577c and tilapia muscle, n=221 for each), run after every 15 samples, were 0.1‰ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. The precision of replicate tissue samples (every 14th sample was analyzed in triplicate) was within the acceptable $\pm 0.2\text{‰}$ range (0.1‰ for $\delta^{13}\text{C}$ and 0.1‰ for $\delta^{15}\text{N}$; n=30).

Lipid extraction

Except for Lake Ontario, all fish and invertebrate samples were run in bulk and not chemically lipid extracted. Since lipid extraction can influence $\delta^{15}\text{N}$, Lake Ontario samples were $\delta^{15}\text{N}$ -adjusted from lipid extracted to bulk using a fish-specific regression line (Larocque et al. 2021), while $\delta^{13}\text{C}$ values remained unchanged. To remove the influence of ^{13}C -depleted lipids in muscle tissue in all other lakes, fish samples were mathematically adjusted for $\delta^{13}\text{C}$ ($\text{C:N}>3.4$; Hoffman et al. 2015) using the equation:

$$\delta^{13}\text{C}_{\text{Lipid Free}} = \delta^{13}\text{C}_{\text{Bulk}} + \frac{\Delta\delta^{13}\text{C}_{\text{Lipid-Bulk}} * (\text{C:N}_{\text{Lipid Free}} - \text{C:N}_{\text{Bulk}})}{\text{C:N}_{\text{Bulk}}}$$

where $\Delta\delta^{13}\text{C}_{\text{Lipid-Bulk}}=-6.5\text{\textperthousand}$ (Hoffman et al. 2015) and $\text{C:N}_{\text{Lipid Free}}=3.4\text{\textperthousand}$ (McConaughey and McRoy 1979). Invertebrate samples were also mathematically adjusted using the general aquatic organism equation:

$$\delta^{13}\text{C}_{\text{Lipid Free}} = \delta^{13}\text{C}_{\text{Bulk}} + \left(\frac{a * \text{C:N}_{\text{Bulk}} + b}{\text{C:N}_{\text{Bulk}} + c} \right)$$

where coefficients $a=7.415\text{\textperthousand}$, $b=-22.732\text{\textperthousand}$, and $c=0.736$, were determined from general freshwater aquatic invertebrates (Logan et al. 2008).

Baseline adjustment

To adjust for differences in fish isotope values among lakes, dreissenid $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were used because of long tissue-turnover times in this organism that are more consistent across season and comparable to fish metabolic tissue-turnover times (Cabana and Rasmussen 1996, Vander Zanden and Rasmussen 1999), as well as their ubiquity in all ecoregions across most of the Great Lakes (Bunnell et al. 2014, Burlakova et al. 2018). Due to baseline differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values with depth (Power et al. 2003, Uzarski 2021), only dreissenids collected between 10 and 80 m were used in each ecoregion. For either $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ at each lake and ecoregion, we removed extreme values that were not within the 10th and 90th percentile of data distribution (Jackson and Britton 2014, Pettitt-Wade et al. 2018). Ecoregion-specific mean dreissenid isotope values were then used to correct and adjust lake- and ecoregion-specific fish stable isotope values using the equation:

$$\delta X_{\text{fish}} = (\delta X_{\text{fish}} - \delta X_{\text{mean dreissenid}})$$

where X represents either ^{13}C or ^{15}N . Ecoregion-specific baseline corrections were applied to the same ecoregion in which fish were collected (tables 10 and 11).

Due to the lack of available dreissenid baselines in all ecoregions of Lake Superior and Lake Huron, the results of conservative ($p<\alpha/2$; $\alpha=0.05$) non-parametric statistical tests (Kruskal-Wallis + Dunn tests) were used to infer a mean ($\pm 1 \text{ SD}$) dreissenid stable isotope value for Lake Superior embayment and outlet ecoregion values, as well as Lake Huron anthropogenic ecoregion values. Since differences were not significant for any anthropogenic-open coastal pairwise comparisons in lakes Erie and Ontario ($Z<2.7$; $p>0.03$), we assumed an equal mean open coastal ecoregion $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ($\pm 1 \text{ SD}$) value for the anthropogenic ecoregion in Lake

Huron. Most pairwise comparisons revealed there to be no significant differences in embayment-open coastal ecoregion $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values ($\delta^{13}\text{C}$ Lakes Huron, Erie, Ontario: $Z<0.8$, $p=1.00$; $\delta^{15}\text{N}$ Lakes Huron, Erie: $Z<2.4$, $p\geq0.07$), therefore we assumed an equal mean open coastal ecoregion $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (± 1 SD) value for the Lake Superior embayment ecoregion. Additionally, we assumed Lake Superior outlet-anthropogenic $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values to be equal since most pairwise comparisons in other lakes did not reveal significant differences ($\delta^{13}\text{C}$ Lakes Erie or Ontario: $Z<2.7$, $p\geq0.03$; $\delta^{15}\text{N}$ Lake Erie: $Z=0.8$; $p=1.00$). Results of Wilcoxon pairwise comparison tests were not used to infer missing mean ecoregion $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values due to unequal number of samples being compared ($n=2$) relative to Kruskal-Wallis tests ($n=4$ or 5).

Total mercury analysis

Fish from lakes Huron, Ontario, and Superior were analyzed for total mercury using a Milestone DMA-80 direct mercury analyzer in accordance with US-EPA method 7473 (SW-846; US EPA 1998), while Lake Erie fish were analyzed using cold vapour-flameless atomic absorption spectroscopy (CV-FAAS; Bhavsar et al. 2010). Results are found to be comparable between these mercury-based analytical methods (Rennie et al. 2010). At least 5 certified reference materials were analyzed for each DMA-80 run to confirm accuracy and precision (NRC standard: TORT-3, internal standard: lobster hepatopancreas). Mean estimates of TORT-3 across all runs were $288 \pm 15 \mu\text{g/kg}$, which is within range of the certified reference material (CRM) for TORT-3 ($292 \pm 22 \mu\text{g/kg}$). CV-FAAS standards were also analyzed for accuracy and precision and fell within the acceptable range. All total mercury concentrations were reported as wet $\mu\text{g/kg}$.

The data in this report reflect what has been collected by multiple agencies as a part of the CSMI program and are not reported in other databases.

Isotopic niche area calculations

Lake food web structure (interspecific)

To interpret ecological conclusions from stable isotope data, we have provided a visual reference guide (Figure 2). Both (a) absolute (unadjusted; Figure 3) and (b) relative (baseline-adjusted; Figure 4) mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were determined for each fish taxa in each lake. Isotopic niche area was determined for each fish taxa in each lake using SIAR (Stable Isotope Analysis in R, v.4.2; Stock and Semmens 2013) and SIBER (Stable Isotope Bayesian Ellipses in R V2.1.6; Jackson et al. 2011) packages in R. Corrected standard ellipse area (SEA_C) was calculated using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ to generate ellipses that represent the core (40% spread of bivariate data) isotopic niche (Parnell et al. 2010).

Ecoregion food web structure (intraspecific)

Corrected standard ellipse areas (SEA_C) were calculated for each fish taxa in each ecoregion and lake using both (a) absolute and (b) relative $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. Multivariate normal distributions were fitted to each relative (baseline-adjusted) grouping, and a posterior distribution for each covariance matrix was produced. The posterior estimates for each ellipse (SEA_B ; % o^2) were estimated using Bayesian statistics over 10,000 iterations (Jackson et al. 2011).

Relative $\delta^{15}\text{N}$ and total mercury regressions

While methylmercury is known to bioaccumulate throughout food webs (see Table 1), we relied on total mercury as a proxy to determine trophic magnification from prey to predator fish (Bloom 1992, Lavoie et al. 2013). Total mercury (Hg) values were adjusted for moisture content, where dry weight was back-calculated to wet weight using a general value of 75% water content as reported in other studies (Lavoie et al. 2010, 2013) and were log transformed (\log_{10}). Trophic magnification slopes (TMS) were calculated separately for warm + cool- (emerald shiner, round goby, yellow perch, smallmouth bass, walleye) and coldwater (alewife, rainbow smelt, slimy sculpin, cisco, lake trout) assemblages. We only reported the transfer efficiency amongst fishes, thus baselines were not included in these calculations. Log-transformed Hg was regressed against relative $\delta^{15}\text{N}$ values using the equation:

$$\log_{10}[\text{Hg}] = \delta^{15}\text{N}_{\text{relative}} * m + b$$

where the slope (m) represents the TMS and (b) represents the y-intercept. Only significant slopes ($m > 0$; $p < 0.05$) were reported. Deepwater sculpin were also reported but not included in TMS calculations due to perceived strong depth effects on $\delta^{15}\text{N}$.

Deducing patterns

Global mean (± 1 SD) TLEN, RWT, Fulton's K, absolute $\delta^{13}\text{C}$, absolute $\delta^{15}\text{N}$, relative $\delta^{13}\text{C}$, and relative $\delta^{15}\text{N}$ values were calculated for each species across each lake and ecoregion. Mean ecoregion-specific values that were greater or less than ± 1 SD of the global mean are highlighted and bolded throughout the tables (tables 4–10) and summarized in Appendix 5.

Results

Measurements

Across ecoregions and lakes, fish TLEN, RWT, and K values ranged from 34–934 mm (Table 4), 0.3–8800.0 g (Table 5), and 0.4–1.7 (Table 6), respectively. In general, insectivores had the smallest mean TLEN (slimy sculpin, Lake Superior anthropogenic and open coastal: 56 ± 4 mm and 56 ± 2 mm; round goby, Lake Huron inlet: 52 ± 2 mm) and RWT (deepwater sculpin, Lake Superior anthropogenic: 1 ± 0 g; round goby, Lake Huron inlet: 1.0 ± 0 g) values. Piscivores had the largest mean TLEN (lake trout, Lake Ontario embayment, open coastal, outlet: 668 ± 19 mm, 705 ± 6 mm, 677 ± 15 mm; walleye, Lake Ontario embayment: 644 ± 12 mm) and RWT (lake trout, Lake Ontario embayment, open coastal, outlet: 3703 ± 304 g, 4309 ± 126 g, 3863 ± 275 g) values. Rainbow smelt generally had the lowest mean Fulton's K condition factors (e.g., Lake Superior outlet – 0.50 ± 0.02 ; Lake Ontario anthropogenic – 0.46 ± 0.01), while smallmouth bass had the highest mean Fulton's K condition factors (e.g., Lake Erie inlet – 1.68 ± 0.00 ; outlet – 1.53 ± 0.03).

Carbon and nitrogen stable isotopes

Absolute stable isotopes

In general, differences in absolute carbon and nitrogen stable isotopes existed across all lakes. Fish absolute $\delta^{13}\text{C}$ ranged from $-31.4\text{\textperthousand}$ to $-14.5\text{\textperthousand}$ (CR=16.9%; Table 8, Figure 5) and $\delta^{15}\text{N}$ ranged from $2.3\text{\textperthousand}$ to $18.8\text{\textperthousand}$ (NR=16.5%; Table 9, Figure 6) across the entire Great Lakes Basin. Cisco, lake whitefish, and slimy sculpin had the lowest absolute mean $\delta^{13}\text{C}$ (cisco, Lake Ontario inlet: $-28.7 \pm 0.5\text{\textperthousand}$; lake whitefish, Lake Erie embayment: $-26.0 \pm 0.7\text{\textperthousand}$; slimy sculpin, Lake Superior embayment: $-27.4 \pm 0.7\text{\textperthousand}$), while smallmouth bass and round goby had the highest absolute mean $\delta^{13}\text{C}$ (smallmouth bass, Lake Huron embayment: $-16.7 \pm 0.2\text{\textperthousand}$; round goby, Lake Huron embayment: $-18.8 \pm 0.5\text{\textperthousand}$). Fish taxa from Lake Superior had the lowest absolute $\delta^{15}\text{N}$ (cisco, Lake Superior open coastal: $6.2 \pm 0.2\text{\textperthousand}$; lake whitefish, Lake Superior open coastal: $6.3 \pm 0.1\text{\textperthousand}$), while taxa from Lakes Ontario (walleye, inlet: $17.9 \pm 0.2\text{\textperthousand}$; lake trout, anthropogenic: $17.0 \pm 0.1\text{\textperthousand}$) and Erie (lake trout, embayment: $17.0 \pm 0.6\text{\textperthousand}$; open coastal: $17.4 \pm 0.3\text{\textperthousand}$) had the greatest $\delta^{15}\text{N}$ values. While these differences were most pronounced across lakes, many differences existed in ecoregion-specific absolute carbon (Table 8, Figure 5) and nitrogen stable isotopes (Table 9, Figure 6).

Relative stable isotopes

After adjusting for baseline differences in ecoregions and lakes, some lake and ecoregion-specific differences remained in relative carbon and nitrogen stable isotope values (see Table 1 for approach to interpretation). However, relative $\delta^{13}\text{C}$ (16/129; Appendix A5.6) and $\delta^{15}\text{N}$ (41/129; Appendix A5.7) instances deviating from the global mean occurred less often compared to absolute $\delta^{13}\text{C}$ (24/129; Appendix A5.4) and $\delta^{15}\text{N}$ (52/129; Appendix A5.5) instances. Relative $\delta^{13}\text{C}$ ranged from $-5.3\text{\textperthousand}$ to $11.6\text{\textperthousand}$ (CR=16.8%; Figure 5) and $\delta^{15}\text{N}$ ranged from $-1.7\text{\textperthousand}$ to $14.4\text{\textperthousand}$ (NR=16.1%) (Figure 6, Table 10). In general, the trend in the magnitude of relative $\delta^{13}\text{C}$ values are near identical to absolute $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. Cisco, lake whitefish, and slimy sculpin had the lowest relative mean $\delta^{13}\text{C}$ (cisco, Lake Ontario inlet: $-3.3 \pm 0.5\text{\textperthousand}$; lake whitefish, Lake Erie embayment: $-0.8 \pm 0.7\text{\textperthousand}$; slimy sculpin, Lake Superior embayment: $-0.5 \pm 0.7\text{\textperthousand}$), while smallmouth bass and round goby had the highest relative $\delta^{13}\text{C}$ (smallmouth bass, Lake Huron embayment: $9.3 \pm 0.2\text{\textperthousand}$; round goby, Lake Huron embayment: $7.3 \pm 0.4\text{\textperthousand}$; smallmouth bass, Lake Ontario open coastal: $7.2 \pm 0.2\text{\textperthousand}$). Unlike the similarity between absolute and relative $\delta^{13}\text{C}$, relative $\delta^{15}\text{N}$ patterns were markedly different from absolute $\delta^{15}\text{N}$, with relative $\delta^{15}\text{N}$ being lowest in Lake Ontario (alewife, anthropogenic: $0.4 \pm 0.1\text{\textperthousand}$; open coastal: $1.8 \pm 0.1\text{\textperthousand}$; round goby, anthropogenic: $1.7 \pm 0.3\text{\textperthousand}$) and highest in Lake Huron (lake trout, inlet: $10.3 \pm 0.1\text{\textperthousand}$; open coastal: $10.9 \pm 0.1\text{\textperthousand}$; walleye, open coastal: $10.8 \pm 0.2\text{\textperthousand}$) (Table 11).

Spatial orientation of $\delta^{15}\text{N}$ values changed following baseline adjustments. For example, rainbow smelt from the anthropogenic ecoregion of Lake Ontario had the highest absolute $\delta^{15}\text{N}$ ($14.4 \pm 0.3\text{\textperthousand}$) but the lowest relative $\delta^{15}\text{N}$ ($2.6 \pm 0.3\text{\textperthousand}$) among all Lake Ontario ecoregions. Other examples of large shifts in ecoregion-specific orientation include Lake Ontario lake trout and slimy sculpin, Lake Erie embayment walleye and *Notropis* sp., and Lake Huron round goby, walleye, and lake whitefish. This re-orientation of ecoregion-specific $\delta^{15}\text{N}$ values was further manifested in isotopic niches and thus the overlap among ecoregions changed after adjusting for baseline values to estimate relative $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (figures 7.1–7.12).

Across all lakes and ecoregions, SEA_B values ranged from 0.1–16.6‰² (range=16.5‰²) (Table 12). No clear ecoregion-specific patterns in SEA_B sizes were evident for any fish taxa but Lake Huron inlet SEA_B were usually smaller than other ecoregion-specific SEA_B for each fish taxa. The biggest range in ecoregion SEA_B occurred in Lake Huron (yellow perch, walleye, round goby), while the smallest range in ecoregion SEA_B differences occurred in Lake Ontario (deepwater sculpin, slimy sculpin). SEA_B did not increase with trophic guild or vary by lake environment.

Total mercury

Across ecoregions and lakes, fish Hg ranged from 11–743 µg/kg (Table 13). In general, piscivores (lake trout, smallmouth bass, walleye) had the highest mean Hg values, while planktivores (alewife, emerald shiner) had the lowest mean Hg values among lakes. Insectivore Hg varied by species, where lake whitefish, slimy sculpin, and deepwater sculpin had relatively higher Hg compared to round goby and rainbow smelt. No consistent patterns in Hg values were evident with respect to ecoregion or fish taxa; however, Lake Superior fish taxa Hg was generally greater (range: 128–666 µg/kg) than that in lakes Huron (55–743 µg/kg), Erie (11–308 µg/kg), and Ontario (20–280 µg/kg).

Relative $\delta^{15}\text{N}$ and total mercury trophic magnification slope

Across all lakes and ecoregions, where significant TMS slopes existed, they were all positive and ranged from 0.05 to 0.39 ($p<0.05$). Except for Lake Superior (for which specimens representing the warmwater thermal guild were unavailable), all Great Lakes had measurable ecoregion-specific TMS in both warm + cool- (Figure 8.1) and coldwater (Figure 8.2) environments. No hierarchy in ecoregion-specific TMS magnitudes was discernable for either warm + cool- or coldwater environments. When present, anthropogenic and outlet TMS were always >0.15 ($p<0.05$).

Implications

We present information about traditional fisheries metrics and ecological and bioaccumulative metrics for 2 thermal and 4 trophic guilds, spanning 12 fish taxa across 5 ecoregions in lakes Superior, Huron, Erie, and Ontario. Similar to management units that are segregated into spatially discrete fisheries designations, we described ecologically defined units (ecoregions; Table 2) to illustrate the importance of considering spatial sub-units to characterize the food web ecology of the heterogenous Great Lakes (Riseng et al. 2017). The designation of ecoregions is standardized among lakes, and they are characterized by similar environmental and hydrodynamic variables, providing a consistent framework when undertaking inter- and intralake ecological metric comparisons.

After adjusting for differences in baseline, many of the fish taxa-specific ecological and bioaccumulative metrics in this study differed by lake and ecoregion. In some instances, this difference may be indicative of larger fish mobility, resulting in seasonal movements among ecoregions. Taxa-specific relative stable isotope values differed, reflecting differences in nearshore or offshore habitat use ($\delta^{13}\text{C}$) or fishes feeding on lower or higher trophic level prey ($\delta^{15}\text{N}$; Table 1). Baseline adjustment removed some of the variation in intrinsic properties (environmental and hydrodynamic variables; Table 2) and produced ecological metrics more reflective of ecological function than biogeochemical differences among regions. This

approach provides ecological metrics that are less likely to produce erroneous conclusions surrounding fish health.

Our study results serve to reinforce the consideration of incorporating broader integrative ecological metrics into lake and fisheries management decisions (Table 1). For example, using ecological metrics to monitor historical and contemporary trends in forage fish resource use in Lake Huron (changing ecosystem: declining nutrients, increased water clarity) could provide more insight into changing trophic energy transfer up food chains and across lake zones. Current sampling protocols already collect the same tissue samples for other ministries (i.e., contaminant monitoring for Ministry of the Environment, Conservation, and Parks; Beech and Brown 2022) and can be expanded to collect samples for stable isotope analysis from the same fish. Having paired contaminant and stable isotope samples allow for straightforward analyses and determination of ecological and bioaccumulative metrics, as demonstrated in this study. The findings presented here also demonstrate the importance of collecting lower trophic level/baseline organisms in conjunction with fish sampling efforts to facilitate more refined ecological and bioaccumulative metrics, better reflecting ecological processes related to energy transfer in regions of interest among and within the Great Lakes.

Few studies have quantified spatial differences in ecological metrics not only among (Dobiesz et al. 2010, Bunnell et al. 2014, Riseng et al. 2017) but within (Johnson et al. 2007, Ives et al. 2019, Wegher 2019) large, heterogenous aquatic ecosystems. In addition to traditional fisheries metrics (length, weight, condition factor), we advocate for the collection of paired stable isotope and contaminant data that can provide insight into how functional lake properties may drive differences in food web structure within or among lakes. Without understanding biogeochemical differences in ecosystems, it becomes more difficult to manage uncertainty in fisheries. Acquiring these data will allow scientists to explore future ecosystem-level changes (e.g., predator-prey relationships, niches, and food web structure). Refining these ecological and bioaccumulative metrics will inform management goals and objectives as they relate to understanding how structural lake properties influence fisheries at a more local scale.

Table 1. General interpretation of ecological and bioaccumulative metrics used to describe food web structure.

Ecological/ bioaccumulative metric	Purpose	Interpretation	Unit
Baseline	Establishment of the baseline and use as an adjustment factor is needed before fish stable isotopes can be correctly interpreted	Isotopic signatures of abundant low trophic level (primary producer) organisms ¹ . Reflects differences in intrinsic lake properties (productivity, depth, temperature) as well as the effects of external stressors (run off, climate change, AIS)	$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$; mean \pm SE ‰
Absolute $\delta^{13}\text{C}$	The habitat use (primary production pathway) by a species. Does not account for intrinsic differences in the system (i.e., not adjusted for baseline) ²	Determines whether fish are feeding more nearshore (less negative) or offshore (more negative); comparisons only valid among species collected from the same lake and ecoregion	$\delta^{13}\text{C}$; mean \pm SE ‰
Absolute $\delta^{15}\text{N}$	The relative trophic position of a species. Does not account for intrinsic differences in the system (i.e., not adjusted for baseline) ³	Determines whether fish are feeding on lower trophic (lower $\delta^{15}\text{N}$) or higher trophic (higher $\delta^{15}\text{N}$) level prey; comparisons only valid among species collected from the same lake and ecoregion	$\delta^{15}\text{N}$; mean \pm SE ‰
Relative $\delta^{13}\text{C}$	The habitat use (primary production pathway) by a species adjusted by baseline $\delta^{13}\text{C}$ values ¹	Determines whether fish are feeding more nearshore (less negative) or offshore (more negative) after adjusting for baseline. Comparisons across lake and ecoregion are valid	$\delta^{13}\text{C}$; mean \pm SE ‰
Relative $\delta^{15}\text{N}$	The relative trophic position of a species adjusted by baseline $\delta^{15}\text{N}$ values ¹	Determines whether fish are feeding on lower trophic (lower $\delta^{15}\text{N}$) or higher (higher $\delta^{15}\text{N}$) trophic level prey after adjusting for	$\delta^{15}\text{N}$; mean \pm SE ‰

Ecological/ bioaccumulative metric	Purpose	Interpretation	Unit
Carbon range (CR)	Absolute carbon range - Comparing carbon ranges can reveal whether species are utilizing resources from certain primary production pathways (nearshore, offshore, or both) (i.e., comparing different species collected from same lake and ecoregion)	baseline. Comparisons across lake and ecoregion are valid	
	Relative carbon range - Comparing carbon ranges among systems can reveal whether fish populations are occupying the same trophic role (i.e., same primary production pathway, comparing among species across lakes or ecoregions)	The greater the range in CR, the broader the habitat use (nearshore/offshore) of a species ²	$\delta^{13}\text{C}$; ‰ (presented as a range)
Nitrogen range (NR)	Absolute nitrogen range - Comparing nitrogen ranges can reveal whether species are utilizing resources from certain trophic levels (consuming primary producers, primary consumers, secondary consumers or omnivorous) (i.e., comparing different species collected from same lake and ecoregion)	The greater the range in NR, the broader the species' prey/resource use (feeding on prey from multiple trophic levels) ³	$\delta^{15}\text{N}$; ‰ (presented as a range)
	Relative nitrogen range - Comparing nitrogen ranges among systems can reveal whether fish populations are		

Ecological/ bioaccumulative metric	Purpose	Interpretation	Unit
Niche area	occupying the same trophic role (i.e., occupying the same trophic level; comparing among species across lakes or ecoregions)		
	Comparing niche areas within a system (i.e., comparing among species) can reveal adjustments to predator/prey stocking	Larger niche areas suggest a broader range	SEA_B ; % ² (quantified along both the $\delta^{13}C$ and $\delta^{15}N$ axes)
	Comparing niche areas among systems can reveal whether managing lake or ecoregion-specific fish stocks can be treated the same (i.e., comparing among species across lakes or ecoregions)	in habitat and resource use. Can be compared among different species or within populations ^{4,5}	
Mercury	For similar feeding pathways, can reflect the energetic demands of consumers (higher accumulation reflects higher feeding rate)	Represents the total mercury concentration of fish consumers ⁶	$\mu\text{g Hg/kg}$ wet body weight
Trophic magnification slope (TMS)	Used in concert with $\delta^{15}N$ to estimate the amount of bioaccumulation which is reflective of metabolic efficiency and growth of the fish (i.e., fish with higher mercury per $\delta^{15}N$ are using energy less efficiently (consuming more energy per unit growth)	Quantifies biomagnification (transfer of available mercury throughout a food web) ⁷	$(\log \text{Hg})/\delta^{15}\text{N}$

¹Cabana and Rasmussen 1996; ²Peterson and Fry 1987; ³DeNiro and Epstein 1981; ⁴Layman et al. 2007; ⁵Jackson et al. 2011; ⁶Campbell et al. 2005; ⁷Kidd et al. 2011

Table 2. Summary of ecoregion classifications guided by differing intrinsic properties (environmental hydrodynamic variables) and the anticipated fisheries response using morphometry and ecological metrics (stable isotopes (SIs) and trophic magnification slopes (TMS)). (NA=not applicable)

Ecoregion	Environmental variables	Hydrodynamic variables	Anticipated ecological metric response		
			Morphometry	SIs	TMS
Inlet	NA	Accumulation of hydrodynamic and chemical properties from upstream sources (i.e., Lake Huron inlet composition similar to Lake Superior outlet ecoregion) ¹	NA	Comparable to outlet ecoregion of previous lake	Comparable to previous lake's outlet ecoregion
Anthropogenic	Strongly influenced by urban and agricultural run-off ²	NA	Fulton's K condition factor would be lower than other ecoregions	SIs would be higher than other ecoregions	Hg and TMS would be higher than other ecoregions
Open coastal	Deepest water column ³ , highest temperature and light range ⁴ ; least influenced by anthropogenic stressors; lack of vegetation; moderate wave action ³	Influenced by stratification, circulation, and up-/downwelling events ²	Fulton's K condition factor would be higher than other ecoregions	SIs would be lower than other ecoregions	Hg and TMS would be lower than other ecoregions
Embayment	Shallower, warmer water; abundance of wetlands ⁴	Protected shorelines; least influenced by mechanical wave action ⁴	Fulton's K condition factor would be higher than other ecoregions	Carbon SIs would be comparatively higher reflecting a nearshore benthic signal	NA
Outlet	NA	Accumulation of hydrodynamic flow and mixture of properties from all other ecoregions (i.e.,	NA	SIs would be an average of all other ecoregions	Hg and TMS would be an average

Ecoregion	Environmental variables	Hydrodynamic variables	Anticipated ecological metric response		
			Morphometry	SI _s	TMS
		Lake Erie outlet is combination of Lake Erie inlet, anthropogenic, open coastal, and embayment ecoregions) ¹			of all other ecoregions

¹Ives et al. 2019, ²Kidd et al. 2011, ³Johnson et al. 2007, ⁴Riseng et al. 2017

Table 3. Monitoring sample collection gear types, date and depth (m) ranges, and agencies for fish and invertebrate samples collected across ecoregions (IN = inlet, AN = anthropogenic, EB = embayment, OC = open coastal, OU = outlet) in the Laurentian Great Lakes (Superior, Huron, Erie, Ontario) between 2016 and 2019. Filter-feeder dreissenids were collected to represent a standardized isotopic baseline.

Lake	Year	Date range	Gear type	Depth range (m)	Trophic guild	Agency source by ecoregion				
						IN	AN	EB	OC	OU
Superior	2016, 2017, 2019	June 01– September 30 2016, 2017, 2019	Ponar	16	Filter feeder (dreissenids)	NA	1	NA	2	NA
			Trawl Gillnet	9–152	Planktivore	NA	3,4	5	3	3,5
			Trawl	5–226	Invertivore	NA	3	5	3	3,5
			Gillnet	10–136	Piscivore	NA	3,4	5	3,4,6,7	5,8
Huron	2017	April 24– September 30 2017	Trawl	16–82	Filter feeder (dreissenids)	3	NA	3	3	3
			Trawl	2–35	Planktivore	5	NA	5	NA	NA
			Gillnet	2–82	Invertivore	3,5	3	3,5	3,5	5
				2–29	Omnivore	5	NA	5	3,5	5
				2–120	Piscivore	5	3,9	5	3,5,9	3,5,9
Erie	2019	May 2– November 14 2019	Ponar	9–39	Filter feeder (dreissenids)	10	5	5	5	10
			Trawl	1–2	Planktivore	5	11	5	NA	5,12
			Gillnet	10–205	Invertivore	3,5	5	3,5	3,5,12	3,5
				1–25	Omnivore	11	11	5	5,11	5,12
				2–57	Piscivore	11	11	5	5,11	5,12

Lake	Year	Date range	Gear type	Depth range (m)	Trophic guild	Agency source by ecoregion				
						IN	AN	EB	OC	OU
Ontario	2018	April 09–November 20 2018	Trawl	5–82	Filter feeder (dreissenids)	5	5	5	5	5
				7–150	Planktivore	3,5	5	5	3,5,12	5,12
			Gillnet	2.4–30	Invertivore	5,12	12	5	5,12	12
				17–50	Omnivore	5	5	5	5	5
				5–80	Piscivore	5	5	3,5	3,5,12	5,12

¹Northland College, ²U.S. National Park Service, ³U.S. Geological Survey, ⁴Wisconsin Department of Natural Resources, ⁵Ontario Ministry of Northern Development, Mines, Natural Resources and Forestry, ⁶U.S. Fish and Wildlife Service, ⁷Great Lakes Indian Fish and Wildlife Commission, ⁸Bay Mills Indian Community, ⁹Michigan Department of Natural Resources, ¹⁰University of Windsor, ¹¹Ohio Department of Natural Resources, ¹²New York State Department of Environmental Conservation

Table 4. Observed mean total length (TLEN \pm SE; mm) of 12 fish taxa collected across ecoregions (IN = inlet, AN = anthropogenic, EB = embayment, OC = open coastal, OU = outlet) in the Laurentian Great Lakes (Superior, Huron, Erie, Ontario) between 2016 and 2019. Highlighted values represent samples that are more than ± 1 SD from the species global mean (species average combining all lakes and ecoregions).

Lake	Environment	Trophic guild	Species	IN	AN	EB	OC	OU	
Superior	Pelagic	Planktivore	Alewife ¹	NA	NA	NA	NA	NA	
			Cisco ²	NA	226 \pm 11	266 \pm 7	163 \pm 17	277 \pm 14	
		Insectivore	Rainbow smelt ³	NA	119 \pm 4	114 \pm 5	113 \pm 26	110 \pm 5	
		Piscivore	Lake trout ⁴	NA	403 \pm 20	468 \pm 18	512 \pm 34	489 \pm 23	
		Planktivore	<i>Notropis</i> spp. ⁵	NA	NA	NA	NA	NA	
	Benthopelagic	Insectivore	Lake whitefish ⁶	NA	319 \pm 16	391 \pm 9	169 \pm 17	384 \pm 14	
		Omnivore	Yellow perch ⁷	NA	NA	NA	NA	NA	
		Piscivore	Smallmouth bass ⁸	NA	NA	NA	NA	NA	
			Walleye ⁹	NA	NA	NA	NA	NA	
		Benthic	Round goby ¹⁰	NA	NA	NA	NA	NA	
	Huron		Insectivore	Slimy sculpin ¹¹	NA	56 \pm 4	70 \pm 2	56 \pm 2	
			Deepwater sculpin ¹²	NA	NA	NA	83 \pm 4	NA	
			Planktivore	Alewife	140 \pm 14	NA	141 \pm 2	NA	
			Cisco	383 \pm 18	NA	163 \pm 9	427 \pm 0	289 \pm 32	
	Pelagic	Insectivore	Rainbow smelt	93 \pm 1	NA	130 \pm 2	137 \pm 4		
		Piscivore	Lake trout	411 \pm 11	NA	511 \pm 16	606 \pm 10		
	Benthopelagic	Planktivore	<i>Notropis</i> spp.	NA	NA	NA	NA		
		Insectivore	Lake whitefish	497 \pm 7	591 \pm 11	505 \pm 12	486 \pm 16		
		Omnivore	Yellow perch	220 \pm 7	NA	159 \pm 3	180 \pm 5		
		Piscivore	Smallmouth bass	NA	NA	168 \pm 13	NA		
			Walleye	447 \pm 23	470 \pm 11	417 \pm 11	467 \pm 12		
	Benthic	Insectivore	Round goby	52 \pm 2	NA	88 \pm 2	66 \pm 5	90 \pm 18	
			Slimy sculpin	58 \pm 3	NA	NA	NA	63 \pm 5	
		Deepwater sculpin	NA	NA	NA	NA	82 \pm 3	NA	

Lake	Environment	Trophic guild	Species	IN	AN	EB	OC	OU
Erie	Pelagic	Planktivore	Alewife	NA	NA	NA	NA	117 ± 2
			Cisco	NA	NA	NA	NA	NA
		Insectivore	Rainbow smelt	NA	41 ± 0	132 ± 10	107 ± 9	120 ± 12
		Piscivore	Lake trout	NA	NA	466 ± 45	541 ± 56	NA
		Planktivore	<i>Notropis</i> spp.	103 ± 5	76 ± 0	89 ± 2	NA	85 ± 4
	Benthopelagic	Insectivore	Lake whitefish	NA	NA	124 ± 2	87 ± 3	323 ± 41
		Omnivore	Yellow perch	141 ± 26	NA	173 ± 15	207 ± 12	225 ± 13
		Piscivore	Smallmouth bass	206 ± 0	NA	104 ± 0	373 ± 49	346 ± 23
			Walleye	283 ± 15	334 ± 28	319 ± 29	291 ± 15	307 ± 27
			Round goby	61 ± 14	169 ± 0	75 ± 11	75 ± 6	75 ± 16
Ontario	Benthic	Insectivore	Slimy sculpin	NA	NA	NA	NA	NA
			Deepwater sculpin	NA	NA	NA	NA	NA
		Pelagic	Planktivore	Alewife	153 ± 5	142 ± 8	NA	148 ± 4
			Cisco	199 ± 25	NA	366 ± 9	391 ± 29	355 ± 3
			Insectivore	Rainbow smelt	126 ± 7	133 ± 6	102 ± 3	111 ± 2
			Piscivore	Lake trout	636 ± 17	489 ± 41	668 ± 19	705 ± 6
			Planktivore	<i>Notropis</i> spp.	NA	NA	NA	NA
	Benthopelagic	Insectivore	Lake whitefish	NA	NA	515 ± 15	501 ± 6	513 ± 43
			Yellow perch	72 ± 0	NA	156 ± 0	181 ± 11	155 ± 11
		Piscivore	Smallmouth bass	NA	NA	328 ± 71	381 ± 23	385 ± 20
			Walleye	562 ± 55	NA	644 ± 12	631 ± 13	477 ± 53
			Round goby	88 ± 3	101 ± 7	72 ± 2	97 ± 3	99 ± 11
Benthic	Insectivore	Slimy sculpin	99 ± 4	92 ± 12	NA	98 ± 2	NA	NA
		Deepwater sculpin	NA	NA	NA	129 ± 4	NA	NA

¹*Alosa pseudoharengus*, ²*Coregonus artedi*, ³*Osmerus mordax*, ⁴*Salvelinus namaycush*, ⁵*Notropis* spp.*, ⁶*Coregonus clupeaformis*, ⁷*Perca flavescens*, ⁸*Micropterus dolomieu*, ⁹*Sander vitreus*, ¹⁰*Neogobius melanostomus*, ¹¹*Cottus cognatus*, ¹²*Myoxocephalus thompsonii*

**Notropis* spp. consisted of both emerald shiner (*Notropis atherinoides*) and spottail shiner (*Notropis hudsonius*).

Table 5. Observed mean round weight (RWT \pm SE; g) of 12 fish taxa collected across ecoregions (IN = inlet, AN = anthropogenic, EB = embayment, OC = open coastal, OU = outlet) in the Laurentian Great Lakes (Superior, Huron, Erie, Ontario) between 2016 and 2019. Highlighted values represent samples that are more than \pm 1SD from the species global mean (species average combining all lakes and ecoregions).

Lake	Environment	Trophic guild	Species	IN	AN	EB	OC	OU
Superior	Pelagic	Planktivore	Alewife ¹	NA	NA	NA	NA	NA
			Cisco ²	NA	127 \pm 19	153 \pm 16	62 \pm 31	460 \pm 140
		Insectivore	Rainbow smelt ³	NA	11 \pm 1	11 \pm 1	8 \pm 1	9 \pm 1
		Piscivore	Lake trout ⁴	NA	947 \pm 191	1260 \pm 140	1319 \pm 293	1063 \pm 145
		Planktivore	<i>Notropis</i> spp. ⁵	NA	NA	NA	NA	NA
	Benthopelagic	Insectivore	Lake whitefish ⁶	NA	388 \pm 63	583 \pm 43	72 \pm 31	605 \pm 76
		Omnivore	Yellow perch ⁷	NA	NA	NA	NA	NA
		Piscivore	Smallmouth bass ⁸	NA	NA	NA	NA	NA
			Walleye ⁹	NA	NA	NA	NA	NA
			Round goby ¹⁰	NA	NA	NA	NA	NA
Huron	Benthic	Insectivore	Slimy sculpin ¹¹	NA	2 \pm 0	3 \pm 0	2 \pm 0	2 \pm 0
			Deepwater sculpin ¹²	NA	NA	NA	5 \pm 0	NA
		Planktivore	Alewife	21 \pm 4	NA	20 \pm 1	NA	NA
			Cisco	592 \pm 84	NA	31 \pm 7	675 \pm 0	201 \pm 51
		Insectivore	Rainbow smelt	5 \pm 0	NA	13 \pm 1	19 \pm 1	23 \pm 5
	Benthopelagic	Piscivore	Lake trout	726 \pm 68	NA	505 \pm 135	2363 \pm 119	2333 \pm 196
		Planktivore	<i>Notropis</i> spp.	NA	NA	NA	NA	NA
		Insectivore	Lake whitefish	1223 \pm 64	1895 \pm 117	1299 \pm 113	1219 \pm 131	918 \pm 109
		Omnivore	Yellow perch	139 \pm 15	NA	58 \pm 4	104 \pm 9	108 \pm 21
		Piscivore	Smallmouth bass	NA	NA	67 \pm 15	NA	NA
			Walleye	977 \pm 145	964 \pm 68	930 \pm 73	1039 \pm 94	798 \pm 100
	Benthic	Insectivore	Round goby	1 \pm 0	NA	8 \pm 0	5 \pm 1	12 \pm 5

Lake	Environment	Trophic guild	Species	IN	AN	EB	OC	OU
Erie	Pelagic	Planktivore	Slimy sculpin	2 ± 0	NA	NA	NA	3 ± 1
			Deepwater sculpin	NA	NA	NA	5 ± 0	NA
		Alewife		NA	NA	NA	NA	13 ± 1
		Cisco		NA	NA	NA	NA	NA
		Insectivore	Rainbow smelt	NA	4 ± 0	16 ± 4	10 ± 2	15 ± 2
	Benthopelagic	Piscivore	Lake trout	NA	NA	1358 ± 261	1947 ± 571	NA
		Planktivore	<i>Notropis</i> spp.	11 ± 1	4 ± 0	4 ± 0	NA	5 ± 0
		Insectivore	Lake whitefish	NA	NA	18 ± 3	5 ± 0	678 ± 141
		Omnivore	Yellow perch	61 ± 20	NA	86 ± 15	134 ± 21	152 ± 30
		Piscivore	Smallmouth bass	147 ± 0	NA	16 ± 0	776 ± 357	849 ± 166
			Walleye	171 ± 24	284 ± 72	449 ± 98	387 ± 62	426 ± 107
Ontario	Benthic	Insectivore	Round goby	6 ± 5	70 ± 0	6 ± 4	8 ± 3	10 ± 5
			Slimy sculpin	NA	NA	NA	NA	NA
			Deepwater sculpin	NA	NA	NA	NA	NA
		Planktivore	Alewife	24 ± 2	22 ± 3	NA	26 ± 1	16 ± 1
			Cisco	67 ± 25	NA	504 ± 40	728 ± 146	397 ± 18
	Benthopelagic	Insectivore	Rainbow smelt	12 ± 2	14 ± 2	5 ± 0	7 ± 0	8 ± 0
		Piscivore	Lake trout	3276 ± 242	1888 ± 382	3703 ± 304	4309 ± 126	3863 ± 275
		Planktivore	<i>Notropis</i> spp.	NA	NA	NA	NA	10 ± 2
		Insectivore	Lake whitefish	NA	NA	1134 ± 86	1075 ± 36	1434 ± 323
		Omnivore	Yellow perch	2 ± 0	NA	44 ± 0	69 ± 9	52 ± 9
Benthic	Piscivore	Smallmouth bass	NA	NA	290 ± 180	746 ± 197	878 ± 184	
			Walleye	2172 ± 599	NA	3179 ± 180	3057 ± 191	1526 ± 367
	Insectivore	Round goby	9 ± 1	18 ± 3	4 ± 0	15 ± 1	13 ± 4	
		Slimy sculpin	12 ± 2	9 ± 2	NA	13 ± 0	NA	

Lake	Environment	Trophic guild	Species	IN	AN	EB	OC	OU
			Deepwater sculpin	NA	NA	NA	21 ± 2	NA

¹*Alosa pseudoharengus*, ²*Coregonus artedi*, ³*Osmerus mordax*, ⁴*Salvelinus namaycush*, ⁵*Notropis* spp.*, ⁶*Coregonus clupeaformis*, ⁷*Perca flavescens*, ⁸*Micropterus dolomieu*, ⁹*Sander vitreus*, ¹⁰*Neogobius melanostomus*, ¹¹*Cottus cognatus*, ¹²*Myoxocephalus thompsonii*

**Notropis* spp. consisted of both emerald shiner (*Notropis atherinoides*) and spottail shiner (*Notropis hudsonius*).

Table 6. Calculated Fulton's K condition factor (\pm SE) from observed total length (TLEN) and round weight (RWT) of 12 fish taxa collected across ecoregions (IN = inlet, AN = anthropogenic, EB = embayment, OC = open coastal, OU = outlet) in the Laurentian Great Lakes (Superior, Huron, Erie, Ontario) between 2016 and 2019. Highlighted values represent samples that are more than \pm 1SD from the species global mean (species average combining all lakes and ecoregions).

Lake	Environment	Trophic guild	Species	IN	AN	EB	OC	OU
Superior	Pelagic	Planktivore	Alewife ¹	NA	NA	NA	NA	NA
			Cisco ²	NA	0.69 \pm 0.01	0.69 \pm 0.01	0.64 \pm 0.02	0.86 \pm 0.06
		Insectivore	Rainbow smelt ³	NA	0.54 \pm 0.01	0.53 \pm 0.03	0.52 \pm 0.01	0.50 \pm 0.02
		Piscivore	Lake trout ⁴	NA	0.82 \pm 0.03	0.91 \pm 0.02	0.86 \pm 0.04	0.88 \pm 0.05
		Planktivore	<i>Notropis</i> spp. ⁵	NA	NA	NA	NA	NA
	Benthopelagic	Insectivore	Lake whitefish ⁶	NA	0.81 \pm 0.02	0.86 \pm 0.01	0.62 \pm 0.02	0.92 \pm 0.02
		Omnivore	Yellow perch ⁷	NA	NA	NA	NA	NA
		Piscivore	Smallmouth bass ⁸	NA	NA	NA	NA	NA
			Walleye ⁹	NA	NA	NA	NA	NA
		Benthic	Round goby ¹⁰	NA	NA	NA	NA	NA
			Slimy sculpin ¹¹	NA	1.06 \pm 0.07	0.94 \pm 0.06	0.94 \pm 0.06	1.01 \pm 0.07
			Deepwater sculpin ¹²	NA	NA	NA	0.79 \pm 0.05	NA
Huron	Pelagic	Planktivore	Alewife	0.77 \pm 0.05	NA	0.71 \pm 0.02	NA	NA
			Cisco	1.01 \pm 0.04	NA	0.64 \pm 0.05	0.87 \pm 0	0.79 \pm 0.04
		Insectivore	Rainbow smelt	0.51 \pm 0.00	NA	0.52 \pm 0.01	0.61 \pm 0.01	0.56 \pm 0.01
		Piscivore	Lake trout	0.96 \pm 0.01	NA	0.96 \pm 0.02	0.97 \pm 0.01	0.91 \pm 0.01
		Planktivore	<i>Notropis</i> spp.	NA	NA	NA	NA	NA
	Benthopelagic	Insectivore	Lake whitefish	0.97 \pm 0.02	0.91 \pm 0.03	0.90 \pm 0.02	0.90 \pm 0.02	0.85 \pm 0.02
		Omnivore	Yellow perch	1.14 \pm 0.02	NA	1.04 \pm 0.01	1.16 \pm 0.02	0.91 \pm 0.03
		Piscivore	Smallmouth bass	NA	NA	1.34 \pm 0.06	NA	NA
			Walleye	0.93 \pm 0.01	0.90 \pm 0.01	0.96 \pm 0.01	0.91 \pm 0.01	0.84 \pm 0.01

Lake	Environment	Trophic guild	Species	IN	AN	EB	OC	OU
Erie	Benthic	Insectivore	Round goby	0.72 ± 0.08	NA	1.15 ± 0.04	1.32 ± 0.07	1.29 ± 0.10
			Slimy sculpin	1.15 ± 0.09	NA	NA	NA	1.28 ± 0.09
			Deepwater sculpin	NA	NA	NA	0.88 ± 0.04	NA
	Pelagic	Planktivore	Alewife	NA	NA	NA	NA	0.84 ± 0.03
			Cisco	NA	NA	NA	NA	NA
		Insectivore	Rainbow smelt	NA	0.5 ± 0.0	0.54 ± 0.01	0.53 ± 0.02	0.57 ± 0.02
	Benthopelagic	Piscivore	Lake trout	NA	NA	1.15 ± 0.09	1.13 ± 0.04	NA
		Planktivore	<i>Notropis</i> spp.	0.83 ± 0.03	0.91 ± 0	0.68 ± 0.01	NA	0.84 ± 0.04
		Insectivore	Lake whitefish	NA	NA	0.96 ± 0.14	0.81 ± 0.03	0.98 ± 0.06
	Benthopelagic	Omnivore	Yellow perch	1.04 ± 0.03	NA	1.19 ± 0.06	1.23 ± 0.03	1.24 ± 0.03
		Piscivore	Smallmouth bass	1.68 ± 0.00	NA	1.43 ± 0.00	1.48 ± 0.09	1.53 ± 0.03
			Walleye	0.72 ± 0.04	0.79 ± 0.03	0.85 ± 0.05	0.77 ± 0.01	0.85 ± 0.01
Ontario	Benthic	Insectivore	Round goby	1.25 ± 0.06	1.45 ± 0	1.22 ± 0.06	1.26 ± 0.04	1.22 ± 0.16
			Slimy sculpin	NA	NA	NA	NA	NA
			Deepwater sculpin	NA	NA	NA	NA	NA
	Pelagic	Planktivore	Alewife	0.67 ± 0.03	0.72 ± 0.03	NA	0.69 ± 0.02	0.86 ± 0.03
			Cisco	0.79 ± 0.03	NA	0.97 ± 0.03	0.98 ± 0.04	0.85 ± 0.01
		Insectivore	Rainbow smelt	0.53 ± 0.01	0.46 ± 0.01	0.54 ± 0.02	0.53 ± 0.01	0.56 ± 0.02
	Benthopelagic	Piscivore	Lake trout	1.11 ± 0.02	0.99 ± 0.03	1.10 ± 0.02	1.16 ± 0.01	1.14 ± 0.02
		Planktivore	<i>Notropis</i> spp.	NA	NA	NA	NA	0.82 ± 0.04
		Insectivore	Lake whitefish	NA	NA	0.78 ± 0.01	0.85 ± 0.01	0.89 ± 0.04
	Benthopelagic	Omnivore	Yellow perch	0.57 ± 0.02	NA	1.17 ± 0	0.99 ± 0.03	1.18 ± 0.06
		Piscivore	Smallmouth bass	NA	NA	1.29 ± 0.02	1.46 ± 0.05	1.57 ± 0.02
			Walleye	1.17 ± 0.04	NA	1.15 ± 0.01	1.16 ± 0.03	0.99 ± 0.05
	Benthic	Insectivore	Round goby	1.19 ± 0.06	1.35 ± 0.05	1.20 ± 0.03	1.28 ± 0.03	1.15 ± 0.11

Lake	Environment	Trophic guild	Species	IN	AN	EB	OC	OU
			Slimy sculpin	1.15 ± 0.04	0.99 ± 0.06	NA	1.47 ± 0.03	NA
			Deepwater sculpin	NA	NA	NA	0.94 ± 0.04	NA

¹*Alosa pseudoharengus*, ²*Coregonus artedi*, ³*Osmerus mordax*, ⁴*Salvelinus namaycush*, ⁵*Notropis* spp.*, ⁶*Coregonus clupeaformis*, ⁷*Perca flavescens*, ⁸*Micropterus dolomieu*, ⁹*Sander vitreus*, ¹⁰*Neogobius melanostomus*, ¹¹*Cottus cognatus*, ¹²*Myoxocephalus thompsonii*

**Notropis* spp. consisted of both emerald shiner (*Notropis atherinoides*) and spottail shiner (*Notropis hudsonius*).

Table 7. Calculated mean (± 1 SD) dreissenid stable isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) baseline adjustment factors across ecoregions (IN = inlet, AN = anthropogenic, EB = embayment, OC = open coastal, OU = outlet) in the Laurentian Great Lakes (Superior, Huron, Erie, Ontario) between 2016 and 2019. Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were calculated based on data values within the 10th and 90th percentile. Different superscripts denote significant differences in mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope values across ecoregion specific dreissenid values ($p < \alpha/2$; $\alpha = 0.05$).

Lake	IN		AN		EB		OC		OU	
	n	$\delta^{13}\text{C}$	n	$\delta^{13}\text{C}$	n	$\delta^{13}\text{C}$	n	$\delta^{13}\text{C}$	n	$\delta^{13}\text{C}$
Superior	NA	NA	10	-25.84 \pm 1.32	NA	-26.89 \pm 1.05 ¹	10	-26.89 \pm 1.05	NA	-25.84 \pm 1.32 ²
Huron	17	-25.93 \pm 0.88	NA	-26.14 \pm 0.78 ³	5	-26.04 \pm 0.69	10	-26.14 \pm 0.78	22	-25.96 \pm 1.03
Erie	12	-24.93 \pm 0.97	40	-24.07 \pm 1.31	13	-25.23 \pm 0.85	23	-24.86 \pm 1.05	7	-25.10 \pm 1.92
Ontario	5	-25.42 \pm 0.36 ^{AB}	6	-24.83 \pm 0.92 ^{ABC}	9	-24.91 \pm 0.63 ^{ABC}	20	-25.08 \pm 0.84 ^{AB}	43	-24.44 \pm 0.64 ^{AC}
	n	$\delta^{15}\text{N}$	n	$\delta^{15}\text{N}$	n	$\delta^{15}\text{N}$	n	$\delta^{15}\text{N}$	n	$\delta^{15}\text{N}$
	NA	NA	10	0.28 \pm 1.56	NA	0.23 \pm 1.82 ¹	10	0.23 \pm 1.82	NA	0.28 \pm 1.56 ²
Superior	NA	NA	10	0.28 \pm 1.56	NA	0.23 \pm 1.82 ¹	10	0.23 \pm 1.82	NA	0.28 \pm 1.56 ²
Huron	17	2.87 \pm 1.42 ^A	NA	1.96 \pm 0.33 ³	8	4.11 \pm 3.87 ^A	7	1.96 \pm 0.33 ^{AB}	24	3.87 \pm 1.34 ^{AC}
Erie	12	7.30 \pm 0.91 ^A	40	7.79 \pm 1.56 ^{AC}	14	11.00 \pm 1.53 ^B	24	9.29 \pm 1.87 ^B	7	8.62 \pm 2.43 ^{ABC}
Ontario	5	10.34 \pm 0.72 ^A	6	11.82 \pm 0.66 ^{AB}	9	9.45 \pm 0.20 ^{AC}	16	10.56 \pm 1.21 ^{AB}	43	9.86 \pm 0.61 ^{AC}

¹Lake Superior $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ embayment values were informed by Lake Superior open coastal values.

²Lake Superior $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ outlet values were informed by Lake Superior anthropogenic values.

³Lake Huron $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ anthropogenic values were informed by Lake Huron open coastal values.

Table 8. Absolute $\delta^{13}\text{C}$ values of 12 fish taxa collected across ecoregions (IN = inlet, AN = anthropogenic, EB = embayment, OC = open coastal, OU = outlet) in the Laurentian Great Lakes (Superior, Huron, Erie, Ontario) between 2016 and 2019. To remove the influence of ^{13}C -depleted lipids in muscle tissue, samples were mathematically adjusted for $\delta^{13}\text{C}$ for Lakes Superior, Huron, and Erie, while Lake Ontario muscle samples were chemically lipid-adjusted. Highlighted values represent samples that are more than $\pm 1\text{SD}$ from the species global mean (species average combining all lakes and ecoregions).

Lake	Environment	Trophic Guild	Species	IN	AN	EB	OC	OU
Superior	Pelagic	Planktivore	Alewife ¹	NA	NA	NA	NA	NA
			Cisco ²	NA	-25.3 ± 0.1	-24.9 ± 0.1	-25.1 ± 0.3	-24.6 ± 0.5
		Insectivore	Rainbow smelt ³	NA	-25.4 ± 0.1	-25.3 ± 0.1	-25.2 ± 0.2	-24.6 ± 0.1
		Piscivore	Lake trout ⁴	NA	-24.2 ± 0.1	-24.0 ± 0.1	-23.9 ± 0.4	-23.5 ± 0.1
		Planktivore	<i>Notropis</i> spp. ⁵	NA	NA	NA	NA	NA
	Benthopelagic	Insectivore	Lake whitefish ⁶	NA	-24.5 ± 0.1	-23.3 ± 0.2	-24.1 ± 0.1	-22.9 ± 0.3
		Omnivore	Yellow perch ⁷	NA	NA	NA	NA	NA
		Piscivore	Smallmouth bass ⁸	NA	NA	NA	NA	NA
			Walleye ⁹	NA	NA	NA	NA	NA
		Benthic	Round goby ¹⁰	NA	NA	NA	NA	NA
			Slimy sculpin ¹¹	NA	-24.6 ± 0.3	-27.4 ± 0.7	-23.4 ± 0.3	-22.9 ± 0.6
Huron	Pelagic	Planktivore	Deepwater sculpin ¹²	NA	NA	NA	-24.9 ± 0.0	NA
			Alewife	-25.8 ± 1.9	NA	-24.7 ± 0.8	NA	NA
			Cisco	-21.8 ± 0.4	NA	-24.5 ± 0.8	-23.0 ± 0.0	-23.6 ± 0.1
		Insectivore	Rainbow smelt	-24.8 ± 0.1	NA	-24.6 ± 0.1	-23.7 ± 0.2	-23.9 ± 0.1
		Piscivore	Lake trout	-22.4 ± 0.2	NA	-22.9 ± 0.1	-22.7 ± 0.1	-22.3 ± 0.2
	Benthopelagic	Planktivore	<i>Notropis</i> spp.	NA	NA	NA	NA	NA
		Insectivore	Lake whitefish	-20.4 ± 0.3	NA	-21.5 ± 0.4	-20.7 ± 0.4	-22.0 ± 0.5
		Omnivore	Yellow perch	-20.5 ± 0.2	NA	-21.3 ± 0.2	-21.6 ± 0.3	-20.5 ± 0.2
		Piscivore	Smallmouth bass	NA	NA	-16.7 ± 0.2	NA	NA

Lake	Environment	Trophic Guild	Species	IN	AN	EB	OC	OU
Erie	Benthic	Insectivore	Walleye	-22.7 ± 0.2	-24.0 ± 0.1	-23.1 ± 0.3	-21.2 ± 0.2	-21.9 ± 0.1
			Round goby	-19.8 ± 0.2	NA	-18.8 ± 0.5	-21.8 ± 0.5	-20.7 ± 0.4
			Slimy sculpin	-24.5 ± 0.2	NA	NA	NA	-24.4 ± 0.4
			Deepwater sculpin	NA	NA	NA	-23.9 ± 0.1	NA
	Pelagic	Planktivore	Alewife	NA	NA	NA	NA	-22.9 ± 0.1
			Cisco	NA	NA	NA	NA	NA
		Insectivore	Rainbow smelt	NA	NA	-24.0 ± 0.1	-24.0 ± 0.1	-23.9 ± 0.1
		Piscivore	Lake trout	NA	NA	-22.8 ± 0.3	-22.6 ± 0.1	NA
	Benthopelagic	Planktivore	<i>Notropis</i> spp.	-22.9 ± 0.1	-25.2 ± 0.0	-23.6 ± 0.1	NA	-22.2 ± 0.4
			Lake whitefish	NA	NA	-26.0 ± 0.7	-23.9 ± 0.1	-24.2 ± 0.1
		Omnivore	Yellow perch	-22.6 ± 0.4	NA	-22.6 ± 0.3	-22.8 ± 0.2	-23.5 ± 0.3
		Piscivore	Smallmouth bass	-20.5 ± 0.0	NA	-22.6 ± 0.0	-21.3 ± 0.3	-21.4 ± 0.1
			Walleye	-22.3 ± 0.2	-21.9 ± 0.2	-22.3 ± 0.1	-22.7 ± 0.1	-22.3 ± 0.1
			Round goby	-23.7 ± 0.1	-24.1 ± 0.0	-22.9 ± 0.5	-23.4 ± 0.1	-24.2 ± 0.3
	Benthic	Insectivore	Slimy sculpin	NA	NA	NA	NA	NA
Ontario	Pelagic	Planktivore	Deepwater sculpin	NA	NA	NA	NA	NA
			Alewife	-22.9 ± 0.1	-22.7 ± 0.1	NA	-23.0 ± 0.1	-22.7 ± 0.1
			Cisco	-28.7 ± 0.5	NA	-22.3 ± 0.2	-21.6 ± 0.3	-22.3 ± 0.1
		Insectivore	Rainbow smelt	-23.2 ± 0.1	-23.0 ± 0.2	-23.4 ± 0.2	-23.3 ± 0.1	-22.9 ± 0.2
		Piscivore	Lake trout	-22.5 ± 0.2	-23.2 ± 0.3	-22.4 ± 0.2	-22.4 ± 0.1	-22.3 ± 0.1
	Benthopelagic	Planktivore	<i>Notropis</i> spp.	NA	NA	NA	NA	-22.5 ± 0.6
		Insectivore	Lake whitefish	NA	NA	-21.7 ± 0.3	-22.1 ± 0.3	-21.1 ± 0.4
		Omnivore	Yellow perch	-19.5 ± 0.2	NA	-21.8 ± 0.0	-19.9 ± 0.5	-21.9 ± 0.4
		Piscivore	Smallmouth bass	NA	NA	-19.9 ± 0.4	-19.1 ± 0.1	-20.0 ± 0.2
			Walleye	-22.9 ± 0.0	NA	-21.7 ± 0.1	-21.8 ± 0.1	-22.5 ± 0.6

Lake	Environment	Trophic Guild	Species	IN	AN	EB	OC	OU
Benthic	Insectivore		Round goby	-21.8 ± 0.2	-22.4 ± 0.3	-21.1 ± 0.5	-22.6 ± 0.3	-22.1 ± 0.4
			Slimy sculpin	-23.8 ± 0.0	-23.5 ± 0.3	NA	-23.4 ± 0.1	NA
			Deepwater sculpin	NA	NA	NA	-23.7 ± 0.1	NA

¹*Alosa pseudoharengus*, ²*Coregonus artedi*, ³*Osmerus mordax*, ⁴*Salvelinus namaycush*, ⁵*Notropis* spp.*, ⁶*Coregonus clupeaformis*, ⁷*Perca flavescens*, ⁸*Micropterus dolomieu*, ⁹*Sander vitreus*, ¹⁰*Neogobius melanostomus*, ¹¹*Cottus cognatus*, ¹²*Myoxocephalus thompsonii*

**Notropis* spp. consisted of both emerald shiner (*Notropis atherinoides*) and spottail shiner (*Notropis hudsonius*).

Table 9. Absolute $\delta^{15}\text{N}$ values of 12 fish taxa collected across ecoregions (IN = inlet, AN = anthropogenic, EB = embayment, OC = open coastal, OU = outlet) in the Laurentian Great Lakes (Superior, Huron, Erie, Ontario) between 2016 and 2019. Lake Ontario bulk $\delta^{15}\text{N}$ values were back-calculated from chemically extracted values using a fish-specific regression line (Larocque et al., 2021). Highlighted values represent samples that are more than $\pm 1\text{SD}$ from the species global mean (species average combining all lakes and ecoregions).

Lake	Environment	Trophic Guild	Species	IN	AN	EB	OC	OU
Superior	Pelagic	Planktivore	Alewife ¹	NA	NA	NA	NA	NA
			Cisco ²	NA	7.0 ± 0.2	6.6 ± 0.1	6.2 ± 0.2	6.7 ± 0.2
		Insectivore	Rainbow smelt ³	NA	6.9 ± 0.1	7.0 ± 0.1	6.8 ± 0.1	7.0 ± 0.2
			Piscivore	Lake trout ⁴	NA	10.5 ± 0.1	9.8 ± 0.1	9.1 ± 0.4
	Benthopelagic	Planktivore	<i>Notropis</i> spp. ⁵	NA	NA	NA	NA	NA
			Insectivore	Lake whitefish ⁶	NA	7.7 ± 0.1	7.7 ± 0.1	6.3 ± 0.1
		Omnivore	Yellow perch ⁷	NA	NA	NA	NA	NA
		Piscivore	Smallmouth bass ⁸	NA	NA	NA	NA	NA
			Walleye ⁹	NA	NA	NA	NA	NA
			Round goby ¹⁰	NA	NA	NA	NA	NA
Huron	Benthic	Insectivore	Slimy sculpin ¹¹	NA	9.3 ± 0.4	8.8 ± 0.2	7.7 ± 0.3	8.0 ± 0.2
			Deepwater sculpin ¹²	NA	NA	NA	10.3 ± 0.1	NA
		Planktivore	Alewife	8.8 ± 0.4	NA	10.0 ± 0.1	NA	NA
			Cisco	9.9 ± 0.1	NA	10.4 ± 0.7	8.9 ± 0.0	8.4 ± 0.3
			Insectivore	Rainbow smelt	10.2 ± 0.1	NA	10.3 ± 0.1	8.8 ± 0.1
	Benthopelagic	Piscivore	Lake trout	13.2 ± 0.1	NA	13.0 ± 0.2	12.8 ± 0.1	12.3 ± 0.1
		Planktivore	<i>Notropis</i> spp.	NA	NA	NA	NA	NA
		Insectivore	Lake whitefish	11.2 ± 0.1	NA	10.4 ± 0.2	9.8 ± 0.2	9.6 ± 0.3
		Omnivore	Yellow perch	10.9 ± 0.1	NA	10.3 ± 0.1	10.9 ± 0.3	9.0 ± 0.2
		Piscivore	Smallmouth bass	NA	NA	10.2 ± 0.2	NA	NA
			Walleye	12.0 ± 0.2	16.1 ± 0.2	11.6 ± 0.1	12.8 ± 0.2	14.4 ± 0.3

Lake	Environment	Trophic Guild	Species	IN	AN	EB	OC	OU
Erie	Benthic	Insectivore	Round goby	8.7 ± 0.1	NA	9.3 ± 0.2	8.4 ± 0.6	8.7 ± 0.8
			Slimy sculpin	11.9 ± 0.2	NA	NA	NA	11.9 ± 0.7
			Deepwater sculpin	NA	NA	NA	11.3 ± 0.1	NA
	Pelagic	Planktivore	Alewife	NA	NA	NA	NA	14.4 ± 0.1
			Cisco	NA	NA	NA	NA	NA
		Insectivore	Rainbow smelt	NA	NA	16.7 ± 0.3	15.7 ± 0.3	14.8 ± 0.2
		Piscivore	Lake trout	NA	NA	17.0 ± 0.6	17.4 ± 0.3	NA
			Planktivore	<i>Notropis</i> spp.	12.5 ± 0.1	14.9 ± 0.0	14.1 ± 0.2	NA
			Insectivore	Lake whitefish	NA	NA	13.6 ± 0.8	14.8 ± 0.2
Ontario	Benthopelagic	Omnivore	Yellow perch	13.0 ± 0.3	NA	13.6 ± 0.4	14.4 ± 0.3	14.0 ± 0.4
			Piscivore	Smallmouth bass	13.0 ± 0.0	NA	13.9 ± 0.0	14.7 ± 0.4
			Walleye	14.5 ± 0.2	15.2 ± 0.2	16.0 ± 0.2	15.5 ± 0.1	15.3 ± 0.2
		Benthic	Round goby	11.8 ± 0.2	13.7 ± 0.0	13.6 ± 0.6	14.0 ± 0.1	13.6 ± 0.3
			Insectivore	Slimy sculpin	NA	NA	NA	NA
			Deepwater sculpin	NA	NA	NA	NA	NA
	Pelagic	Planktivore	Alewife	12.6 ± 0.1	12.2 ± 0.1	NA	12.4 ± 0.1	12.3 ± 0.1
			Cisco	13.1 ± 0.3	NA	14.3 ± 0.1	14.3 ± 0.3	14.3 ± 0.1
		Insectivore	Rainbow smelt	13.9 ± 0.3	14.4 ± 0.3	13.9 ± 0.2	13.7 ± 0.2	13.5 ± 0.2
		Piscivore	Lake trout	16.8 ± 0.1	17.0 ± 0.1	16.8 ± 0.1	16.7 ± 0.1	16.7 ± 0.1
			Planktivore	<i>Notropis</i> spp.	NA	NA	NA	13.0 ± 0.1
			Insectivore	Lake whitefish	NA	NA	15.3 ± 0.2	15.1 ± 0.1
Benthopelagic	Omnivore	Yellow perch	12.7 ± 0.6	NA	14.2 ± 0.0	15.0 ± 0.2	14.3 ± 0.1	
		Piscivore	Smallmouth bass	NA	NA	15.1 ± 0.1	15.2 ± 0.1	14.9 ± 0.1
			Walleye	17.9 ± 0.2	NA	15.6 ± 0.1	15.7 ± 0.1	15.2 ± 0.2
	Benthic	Insectivore	Round goby	13.5 ± 0.2	13.5 ± 0.3	13.8 ± 0.3	15.0 ± 0.2	14.0 ± 0.4

Lake	Environment	Trophic Guild	Species	IN	AN	EB	OC	OU
			Slimy sculpin	16.3 ± 0.1	16.3 ± 0.2	NA	15.3 ± 0.2	NA
			Deepwater sculpin	NA	NA	NA	16.3 ± 0.2	NA

¹*Alosa pseudoharengus*, ²*Coregonus artedi*, ³*Osmerus mordax*, ⁴*Salvelinus namaycush*, ⁵*Notropis* spp.*, ⁶*Coregonus clupeaformis*, ⁷*Perca flavescens*, ⁸*Micropterus dolomieu*, ⁹*Sander vitreus*, ¹⁰*Neogobius melanostomus*, ¹¹*Cottus cognatus*, ¹²*Myoxocephalus thompsonii*

**Notropis* spp. consisted of both emerald shiner (*Notropis atherinoides*) and spottail shiner (*Notropis hudsonius*).

Table 10. Relative $\delta^{13}\text{C}$ values of 12 fish taxa collected across ecoregions (IN = inlet, AN = anthropogenic, EB = embayment, OC = open coastal, OU = outlet) in the Laurentian Great Lakes (Superior, Huron, Erie, Ontario) between 2016 and 2019. To remove the influence of $\delta^{13}\text{C}$ -depleted lipids in muscle tissue, samples were mathematically adjusted for $\delta^{13}\text{C}$ for Lakes Superior, Huron, and Erie, while Lake Ontario muscle samples were chemically lipid-adjusted. Mean $\delta^{13}\text{C}$ Dreissenid values from each ecoregion and lake in Table 5 were used to calculate relative $\delta^{13}\text{C}$ values. Highlighted values represent samples that are more than $\pm 1\text{SD}$ from the species global mean (species average combining all lakes and ecoregions).

Lake	Environment	Trophic Guild	Species	IN	AN	EB	OC	OU
Superior	Pelagic	Planktivore	Alewife ¹	N/A	N/A	N/A	N/A	N/A
			Cisco ²	N/A	0.5 \pm 0.1	1.9 \pm 0.1	1.7 \pm 0.3	1.2 \pm 0.5
		Insectivore	Rainbow smelt ³	N/A	0.4 \pm 0.1	1.6 \pm 0.1	1.7 \pm 0.2	1.3 \pm 0.1
		Piscivore	Lake trout ⁴	N/A	1.7 \pm 0.1	2.8 \pm 0.1	3.0 \pm 0.3	2.3 \pm 0.1
		Planktivore	<i>Notropis</i> spp. ⁵	N/A	N/A	N/A	N/A	N/A
	Benthopelagic	Insectivore	Lake whitefish ⁶	N/A	1.3 \pm 0.0	3.6 \pm 0.2	2.8 \pm 0.1	3.0 \pm 0.3
		Omnivore	Yellow perch ⁷	N/A	N/A	N/A	N/A	N/A
		Piscivore	Smallmouth bass ⁸	N/A	N/A	N/A	N/A	N/A
			Walleye ⁹	N/A	N/A	N/A	N/A	N/A
		Benthic	Round goby ¹⁰	N/A	N/A	N/A	N/A	N/A
			Slimy sculpin ¹¹	N/A	1.3 \pm 0.3	-0.5 \pm 0.7	3.5 \pm 0.3	2.9 \pm 0.6
Huron	Pelagic	Planktivore	Alewife	0.1 \pm 2.0	N/A	1.8 \pm 0.2	N/A	N/A
			Cisco	4.1 \pm 0.4	N/A	1.3 \pm 0.8	3.2 \pm 0.0	2.4 \pm 0.1
		Insectivore	Rainbow smelt	1.1 \pm 0.1	N/A	1.4 \pm 0.2	2.5 \pm 0.2	2.1 \pm 0.1
		Piscivore	Lake trout	3.6 \pm 0.2	N/A	3.1 \pm 0.1	3.4 \pm 0.1	3.6 \pm 0.2
	Benthopelagic	Planktivore	<i>Notropis</i> spp.	N/A	N/A	N/A	N/A	N/A
		Insectivore	Lake whitefish	5.6 \pm 0.3	N/A	4.6 \pm 0.4	5.5 \pm 0.4	4.0 \pm 0.5
		Omnivore	Yellow perch	5.5 \pm 0.2	N/A	4.8 \pm 0.2	4.5 \pm 0.3	5.4 \pm 0.2

Lake	Environment	Trophic Guild	Species	IN	AN	EB	OC	OU
Erie	Benthic	Piscivore	Smallmouth bass	N/A	N/A	9.3 ± 0.2	N/A	N/A
			Walleye	3.2 ± 0.2	2.1 ± 0.1	3.0 ± 0.3	4.9 ± 0.2	4.0 ± 0.1
		Insectivore	Round goby	6.1 ± 0.5	N/A	7.2 ± 0.5	4.3 ± 0.5	5.2 ± 0.4
			Slimy sculpin	1.5 ± 0.2	N/A	N/A	N/A	1.5 ± 0.4
			Deepwater sculpin	NA	N/A	NA	2.2 ± 0.1	NA
	Pelagic	Planktivore	Alewife	N/A	N/A	N/A	N/A	2.1 ± 0.1
			Cisco	N/A	N/A	N/A	N/A	N/A
		Insectivore	Rainbow smelt	N/A	N/A	1.2 ± 0.1	0.8 ± 0.1	1.1 ± 0.1
			Piscivore	Lake trout	N/A	2.4 ± 0.3	2.3 ± 0.1	N/A
		Planktivore	<i>Notropis</i> spp.	1.9 ± 0.1	-1.1 ± 0.0	1.6 ± 0.1	N/A	2.9 ± 0.4
Ontario	Benthopelagic	Insectivore	Lake whitefish	N/A	N/A	-0.8 ± 0.7	0.9 ± 0.1	0.9 ± 0.1
			Yellow perch	2.3 ± 0.4	N/A	2.7 ± 0.3	2.1 ± 0.2	1.6 ± 0.3
		Piscivore	Smallmouth bass	4.4 ± 0.0	N/A	2.6 ± 0.0	3.6 ± 0.3	3.7 ± 0.1
			Walleye	2.6 ± 0.2	2.2 ± 0.2	2.9 ± 0.1	2.2 ± 0.1	2.8 ± 0.1
			Round goby	1.3 ± 0.1	-0.0 ± 0.0	2.4 ± 0.5	1.4 ± 0.1	0.9 ± 0.3
	Benthic	Insectivore	Slimy sculpin	N/A	N/A	N/A	N/A	N/A
			Deepwater sculpin	N/A	N/A	N/A	N/A	N/A
		Planktivore	Alewife	2.5 ± 0.1	2.1 ± 0.1	N/A	2.0 ± 0.1	1.7 ± 0.1
			Cisco	-3.3 ± 0.5	N/A	2.6 ± 0.2	3.5 ± 0.3	2.2 ± 0.1
		Insectivore	Rainbow smelt	2.2 ± 0.1	1.8 ± 0.2	1.5 ± 0.2	1.7 ± 0.1	1.6 ± 0.2
	Pelagic	Piscivore	Lake trout	3.0 ± 0.2	1.6 ± 0.3	2.6 ± 0.2	2.7 ± 0.1	2.1 ± 0.2
		Planktivore	<i>Notropis</i> spp.	N/A	N/A	N/A	N/A	2.0 ± 0.6
		Insectivore	Lake whitefish	N/A	N/A	3.3 ± 0.3	3.0 ± 0.3	3.3 ± 0.4
		Omnivore	Yellow perch	5.9 ± 0.2	N/A	3.1 ± 0.0	5.2 ± 0.5	2.6 ± 0.4
		Piscivore	Smallmouth bass	N/A	N/A	5.0 ± 0.4	6.0 ± 0.1	4.4 ± 0.2

Lake	Environment	Trophic Guild	Species	IN	AN	EB	OC	OU
Benthic	Insectivore		Walleye	2.5 ± 0.0	N/A	3.3 ± 0.1	3.3 ± 0.1	1.9 ± 0.6
			Round goby	3.6 ± 0.2	2.4 ± 0.3	3.8 ± 0.5	2.5 ± 0.3	2.4 ± 0.4
			Slimy sculpin	1.7 ± 0.0	1.4 ± 0.3	N/A	1.7 ± 0.1	N/A
			Deepwater sculpin	NA	NA	N/A	1.4 ± 0.1	N/A

¹*Alosa pseudoharengus*, ²*Coregonus artedi*, ³*Osmerus mordax*, ⁴*Salvelinus namaycush*, ⁵*Notropis* spp.*, ⁶*Coregonus clupeaformis*, ⁷*Perca flavescens*, ⁸*Micropterus dolomieu*, ⁹*Sander vitreus*, ¹⁰*Neogobius melanostomus*, ¹¹*Cottus cognatus*, ¹²*Myoxocephalus thompsonii*

**Notropis* spp. consisted of both emerald shiner (*Notropis atherinoides*) and spottail shiner (*Notropis hudsonius*).

Table 11. Relative $\delta^{15}\text{N}$ values of 12 fish taxa collected across ecoregions (IN = inlet, AN = anthropogenic, EB = embayment, OC = open coastal, OU = outlet) in the Laurentian Great Lakes (Superior, Huron, Erie, Ontario) between 2016 and 2019. Lake Ontario bulk $\delta^{15}\text{N}$ values were back-calculated from chemically extracted values using a fish-specific regression line (Larocque et al., 2021). Mean $\delta^{15}\text{N}$ Dreissenid values from each ecoregion and lake in Table 5 were used to calculate relative $\delta^{15}\text{N}$ values. Highlighted values represent samples that are more than $\pm 1\text{SD}$ from the species global mean (species average combining all lakes and ecoregions).

Lake	Environment	Trophic guild	Species	IN	AN	EB	OC	OU
Superior	Pelagic	Planktivore	Alewife ¹	NA	NA	NA	NA	NA
			Cisco ²	NA	6.8 ± 0.2	6.4 ± 0.1	5.9 ± 0.2	6.5 ± 0.2
		Insectivore	Rainbow smelt ³	NA	6.6 ± 0.1	6.7 ± 0.1	6.5 ± 0.1	6.7 ± 0.2
		Piscivore	Lake trout ⁴	NA	10.2 ± 0.1	9.6 ± 0.1	8.9 ± 0.4	9.0 ± 0.2
		Planktivore	<i>Notropis</i> spp. ⁵	NA	NA	NA	NA	NA
	Benthopelagic	Insectivore	Lake whitefish ⁶	NA	7.4 ± 0.1	7.5 ± 0.1	6.1 ± 0.1	7.6 ± 0.2
		Omnivore	Yellow perch ⁷	NA	NA	NA	NA	NA
		Piscivore	Smallmouth bass ⁸	NA	NA	NA	NA	NA
			Walleye ⁹	NA	NA	NA	NA	NA
		Benthic	Round goby ¹⁰	NA	NA	NA	NA	NA
			Slimy sculpin ¹¹	NA	9.0 ± 0.4	8.6 ± 0.2	7.4 ± 0.3	7.8 ± 0.2
Huron	Pelagic	Planktivore	Alewife	5.9 ± 0.4	NA	5.9 ± 0.1	NA	NA
			Cisco	7.1 ± 0.1	NA	6.3 ± 0.7	7.0 ± 0.0	4.6 ± 0.3
		Insectivore	Rainbow smelt	7.3 ± 0.1	NA	6.2 ± 0.1	6.8 ± 0.1	4.6 ± 0.2
		Piscivore	Lake trout	10.3 ± 0.1	NA	8.9 ± 0.2	10.9 ± 0.1	8.4 ± 0.1
	Benthopelagic	Planktivore	<i>Notropis</i> spp.	NA	NA	NA	NA	NA
		Insectivore	Lake whitefish	8.3 ± 0.1	NA	6.3 ± 0.2	7.8 ± 0.2	5.7 ± 0.3
		Omnivore	Yellow perch	8.0 ± 0.1	NA	6.2 ± 0.1	8.9 ± 0.3	5.2 ± 0.2
		Piscivore	Smallmouth bass	NA	NA	6.1 ± 0.2	NA	NA

Lake	Environment	Trophic guild	Species	IN	AN	EB	OC	OU
Erie	Benthic	Insectivore	Walleye	9.2 ± 0.2	4.1 ± 0.2	7.4 ± 0.1	10.8 ± 0.2	10.6 ± 0.3
			Round goby	5.8 ± 0.0	NA	5.2 ± 0.2	6.5 ± 0.6	4.8 ± 0.8
			Slimy sculpin	9.0 ± 0.2	NA	NA	NA	8.0 ± 0.7
			Deepwater sculpin	NA	NA	NA	9.3 ± 0.1	NA
	Pelagic	Planktivore	Alewife	NA	NA	NA	NA	5.8 ± 0.1
			Cisco	NA	NA	NA	NA	NA
		Insectivore	Rainbow smelt	NA	NA	5.7 ± 0.3	6.4 ± 0.3	6.2 ± 0.2
		Piscivore	Lake trout	NA	NA	6.0 ± 0.6	8.1 ± 0.3	NA
	Benthopelagic	Planktivore	<i>Notropis</i> spp.	5.2 ± 0.1	7.1 ± 0.0	3.1 ± 0.2	NA	4.3 ± 0.2
		Insectivore	Lake whitefish	NA	NA	2.6 ± 0.8	5.5 ± 0.2	7.1 ± 0.1
		Omnivore	Yellow perch	5.7 ± 0.3	NA	2.6 ± 0.4	5.2 ± 0.3	5.4 ± 0.4
		Piscivore	Smallmouth bass	5.7 ± 0.0	NA	2.9 ± 0.0	5.4 ± 0.4	7.2 ± 0.2
Ontario	Benthic	Insectivore	Walleye	7.2 ± 0.1	7.4 ± 0.2	5.0 ± 0.2	6.3 ± 0.1	6.7 ± 0.2
			Round goby	4.5 ± 0.2	5.4 ± 0.0	2.6 ± 0.6	4.7 ± 0.1	4.9 ± 0.3
		Insectivore	Slimy sculpin	NA	NA	NA	NA	NA
			Deepwater sculpin	NA	NA	NA	NA	NA
	Pelagic	Planktivore	Alewife	2.2 ± 0.1	0.4 ± 0.1	NA	1.8 ± 0.1	2.4 ± 0.1
			Cisco	2.8 ± 0.3	NA	4.8 ± 0.1	3.7 ± 0.3	4.4 ± 0.1
		Insectivore	Rainbow smelt	3.6 ± 0.3	2.6 ± 0.3	4.5 ± 0.2	3.2 ± 0.2	3.6 ± 0.2
		Piscivore	Lake trout	6.5 ± 0.1	5.2 ± 0.1	7.3 ± 0.1	6.1 ± 0.1	6.8 ± 0.1
	Benthopelagic	Planktivore	<i>Notropis</i> spp.	NA	NA	NA	NA	3.1 ± 0.1
		Insectivore	Lake whitefish	NA	NA	5.9 ± 0.2	4.5 ± 0.1	5.6 ± 0.2
		Omnivore	Yellow perch	2.3 ± 0.6	NA	4.7 ± 0.0	4.4 ± 0.2	4.4 ± 0.1
		Piscivore	Smallmouth bass	NA	NA	5.7 ± 0.1	4.6 ± 0.1	5.1 ± 0.1
			Walleye	7.5 ± 0.2	NA	6.2 ± 0.1	5.2 ± 0.1	5.3 ± 0.2

Lake	Environment	Trophic guild	Species	IN	AN	EB	OC	OU
Benthic	Insectivore		Round goby	3.1 ± 0.2	1.7 ± 0.3	4.3 ± 0.3	4.5 ± 0.2	4.2 ± 0.4
			Slimy sculpin	6.0 ± 0.1	4.5 ± 0.2	NA	4.8 ± 0.2	NA
			Deepwater sculpin	NA	NA	NA	5.8 ± 0.2	NA

¹*Alosa pseudoharengus*, ²*Coregonus artedi*, ³*Osmerus mordax*, ⁴*Salvelinus namaycush*, ⁵*Notropis* spp.*, ⁶*Coregonus clupeaformis*, ⁷*Perca flavescens*, ⁸*Micropterus dolomieu*, ⁹*Sander vitreus*, ¹⁰*Neogobius melanostomus*, ¹¹*Cottus cognatus*, ¹²*Myoxocephalus thompsonii*

**Notropis* spp. consisted of both emerald shiner (*Notropis atherinoides*) and spottail shiner (*Notropis hudsonius*).

Table 12. Modal Bayesian standard ellipse area (SEA_B) values of posterior distribution-estimated relative $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of 12 fish taxa collected across ecoregions (IN = inlet, AN = anthropogenic, EB = embayment, OC = open coastal, OU = outlet) in the Laurentian Great Lakes (Superior, Huron, Erie, Ontario) between 2016 and 2019.

Lake	Environment	Trophic guild	Species	IN	AN	EB	OC	OU
Superior	Benthopelagic	Pelagic	Planktivore	Alewife ¹	NA	NA	NA	NA
				Cisco ²	NA	1.9	3.6	2.6
			Insectivore	Rainbow smelt ³	NA	0.9	0.7	1.5
			Piscivore	Lake trout ⁴	NA	1.1	1.7	2.3
			Planktivore	<i>Notropis</i> spp. ⁵	NA	NA	NA	NA
		Benthic	Insectivore	Lake whitefish ⁶	NA	0.3	3.9	0.8
			Omnivore	Yellow perch ⁷	NA	NA	NA	NA
			Piscivore	Smallmouth bass ⁸	NA	NA	NA	NA
				Walleye ⁹	NA	NA	NA	NA
			Insectivore	Round goby ¹⁰	NA	NA	NA	NA
				Slimy sculpin ¹¹	NA	2.6	3.2	3.2
Huron	Benthopelagic	Pelagic	Planktivore	Deepwater sculpin ¹²	NA	1.8	NA	0.1
				Alewife	4.0	NA	1.8	NA
				Cisco	0.4	NA	10.0	NA
			Insectivore	Rainbow smelt	2.2	NA	6.6	3.2
			Piscivore	Lake trout	1.2	NA	4.3	2.0
		Benthic	Planktivore	<i>Notropis</i> spp.	NA	NA	NA	NA
			Insectivore	Lake whitefish	0.6	NA	3.6	3.3
			Omnivore	Yellow perch	1.7	NA	11.2	16.6
			Piscivore	Smallmouth bass	NA	NA	0.4	NA
				Walleye	1.7	2.1	10.3	3.5
			Insectivore	Round goby	NA	NA	8.2	10.0
				Slimy sculpin	0.8	NA	NA	NA
				Deepwater sculpin	NA	NA	NA	0.2

Lake	Environment	Trophic guild	Species	IN	AN	EB	OC	OU
Erie	Pelagic	Planktivore	Alewife	NA	NA	NA	NA	0.2
			Cisco	NA	NA	NA	NA	N/A
		Insectivore	Rainbow smelt	NA	NA	2.0	0.8	0.4
		Piscivore	Lake trout	NA	NA	2.5	0.2	NA
		Planktivore	<i>Notropis</i> spp.	0.9	NA	0.6	NA	3.5
	Benthopelagic	Insectivore	Lake whitefish	NA	NA	5.3	0.5	0.7
		Omnivore	Yellow perch	2.4	NA	3.0	3.8	1.5
		Piscivore	Smallmouth bass	NA	NA	NA	NA	1.4
			Walleye	0.7	1.6	1.3	2.9	1.5
		Benthic	Round goby	NA	NA	5.3	0.5	0.7
			Slimy sculpin	NA	NA	NA	NA	NA
			Deepwater sculpin	NA	NA	NA	NA	NA
Ontario	Pelagic	Planktivore	Alewife	0.3	0.4	NA	1.1	0.1
			Cisco	0.8	NA	1.7	2.5	2.0
		Insectivore	Rainbow smelt	0.7	1.3	0.3	0.5	0.7
		Piscivore	Lake trout	2.1	1.9	1.7	2.0	1.3
		Planktivore	<i>Notropis</i> spp.	NA	NA	NA	NA	2.1
	Benthopelagic	Insectivore	Lake whitefish	NA	NA	3.9	2.3	2.3
		Omnivore	Yellow perch	0.8	NA	1.7	2.5	2.0
		Piscivore	Smallmouth bass	NA	NA	0.1	0.5	0.8
			Walleye	NA	NA	0.9	1.2	3.6
		Benthic	Round goby	2.9	3.3	1.9	3.1	2.8
			Slimy sculpin	0.2	0.6	NA	0.3	NA
			Deepwater sculpin	NA	NA	NA	0.8	NA

¹*Alosa pseudoharengus*, ²*Coregonus artedi*, ³*Osmerus mordax*, ⁴*Salvelinus namaycush*, ⁵*Notropis* spp.*, ⁶*Coregonus clupeaformis*, ⁷*Perca flavescens*, ⁸*Micropterus dolomieu*, ⁹*Sander vitreus*, ¹⁰*Neogobius melanostomus*, ¹¹*Cottus cognatus*, ¹²*Myoxocephalus thompsonii*

**Notropis* spp. consisted of both emerald shiner (*Notropis atherinoides*) and spottail shiner (*Notropis hudsonius*).

Table 13. Total mercury ($\mu\text{g}/\text{kg}$; wet weight) values of 12 fish taxa collected across ecoregions (IN = inlet, AN = anthropogenic, EB = embayment, OC = open coastal, OU = outlet) in the Laurentian Great Lakes (Superior, Huron, Erie, Ontario) between 2016 and 2019.

Lake	Environment	Trophic guild	Species	IN	AN	EB	OC	OU
Superior	Pelagic	Planktivore	Alewife ¹	NA	NA	NA	NA	NA
			Cisco ²	NA	215 \pm 24	313 \pm 28	128 \pm 33	246 \pm 36
		Insectivore	Rainbow smelt ³	NA	179 \pm 12	218 \pm 14	158 \pm 8	270 \pm 35
		Piscivore	Lake trout ⁴	NA	664 \pm 73	548 \pm 34	666 \pm 236	293 \pm 30
		Planktivore	<i>Notropis</i> spp. ⁵	NA	NA	NA	NA	NA
	Benthopelagic	Insectivore	Lake whitefish ⁶	NA	163 \pm 7	595 \pm 51	138 \pm 9	360 \pm 35
		Omnivore	Yellow perch ⁷	NA	NA	NA	NA	NA
		Piscivore	Smallmouth bass ⁸	NA	NA	NA	NA	NA
			Walleye ⁹	NA	NA	NA	NA	NA
		Benthic	Round goby ¹⁰	NA	NA	NA	NA	NA
Huron	Pelagic	Insectivore	Slimy sculpin ¹¹	NA	219 \pm 40	288 \pm 29	245 \pm 34	191 \pm 24
			Deepwater sculpin ¹²	NA	NA	NA	294 \pm 43	NA
		Planktivore	Alewife	67 \pm 15	NA	67 \pm 8	NA	NA
			Cisco	75 \pm 15	NA	108 \pm 37	212 \pm 0	58 \pm 6
		Insectivore	Rainbow smelt	55 \pm 15	NA	95 \pm 36	58 \pm 7	57 \pm 0
	Benthopelagic	Piscivore	Lake trout	92 \pm 6	NA	120 \pm 20	174 \pm 32	157 \pm 32
		Planktivore	<i>Notropis</i> spp.	NA	NA	NA	NA	116 \pm 17
		Insectivore	Lake whitefish	60 \pm 5	NA	48 \pm 10	60 \pm 7	60 \pm 18
		Omnivore	Yellow perch	105 \pm 13	NA	99 \pm 21	144 \pm 40	202 \pm 73
		Piscivore	Smallmouth bass	NA	NA	153 \pm 58	264 \pm 159	NA
			Walleye	234 \pm 35	743 \pm 213	187 \pm 43	290 \pm 77	131 \pm 10
Benthic	Insectivore	Round goby	18 \pm 0	NA	78 \pm 34	94 \pm 15	69 \pm 0	
			Slimy sculpin	54 \pm 9	NA	NA	NA	30 \pm 3
	Insectivore	Deepwater sculpin	NA	NA	NA	79 \pm 10	NA	

Lake	Environment	Trophic guild	Species	IN	AN	EB	OC	OU	
Erie	Pelagic	Planktivore	Alewife	NA	NA	NA	NA	NA	
			Cisco	NA	NA	NA	NA	NA	
		Insectivore	Rainbow smelt	NA	NA	63 ± 9	23 ± 3	20 ± 6	
		Piscivore	Lake trout	NA	NA	83 ± 15	240 ± 91	NA	
		Planktivore	<i>Notropis</i> spp.	102 ± 19	60 ± 0	110 ± 17	NA	NA	
	Benthopelagic	Insectivore	Lake whitefish	NA	NA	20 ± 0	12 ± 2	36 ± 5	
		Omnivore	Yellow perch	118 ± 6	NA	31 ± 5	78 ± 18	52 ± 7	
		Piscivore	Smallmouth bass	NA	NA	NA	308 ± 181	120 ± 47	
			Walleye	57 ± 6	116 ± 16	116 ± 19	149 ± 46	62 ± 18	
		Benthic	Round goby	20 ± 0	20 ± 0	19 ± 1	23 ± 3	11 ± 1	
	Ontario		Slimy sculpin	NA	NA	NA	NA	NA	
			Deepwater sculpin	NA	NA	NA	NA	NA	
	Pelagic	Alewife	54 ± 8	57 ± 18	NA	62 ± 9	28 ± 0		
		Cisco	NA	NA	61 ± 8	66 ± 15	42 ± 8		
		Rainbow smelt	48 ± 9	40 ± 6	31 ± 10	23 ± 2	23 ± 1		
		Lake trout	82 ± 9	80 ± 26	90 ± 10	99 ± 7	102 ± 10		
		<i>Notropis</i> spp.	NA	NA	NA	NA	NA		
	Benthopelagic	Insectivore	NA	NA	38 ± 0	23 ± 0	96 ± 42		
		Lake whitefish	NA	NA	57 ± 0	91 ± 16	102 ± 15		
		Omnivore	NA	NA	20 ± 13	102 ± 29	NA		
		Smallmouth bass	NA	NA	270 ± 22	280 ± 26	20 ± 1		
		Walleye	179 ± 0	NA	NA	NA	NA		
	Benthic	Insectivore	Round goby	35 ± 6	24 ± 6	NA	25 ± 2	59 ± 0	
			Slimy sculpin	126 ± 31	182 ± 34	NA	106 ± 17	NA	
			Deepwater sculpin	NA	NA	NA	51 ± 9	NA	

¹*Alosa pseudoharengus*, ²*Coregonus artedi*, ³*Osmerus mordax*, ⁴*Salvelinus namaycush*, ⁵*Notropis atherinoides*, ⁶*Coregonus clupeaformis*, ⁷*Perca flavescens*, ⁸*Micropterus dolomieu*, ⁹*Sander vitreus*, ¹⁰*Neogobius melanostomus*, ¹¹*Cottus cognatus*, ¹²*Myoxocephalus thompsonii*

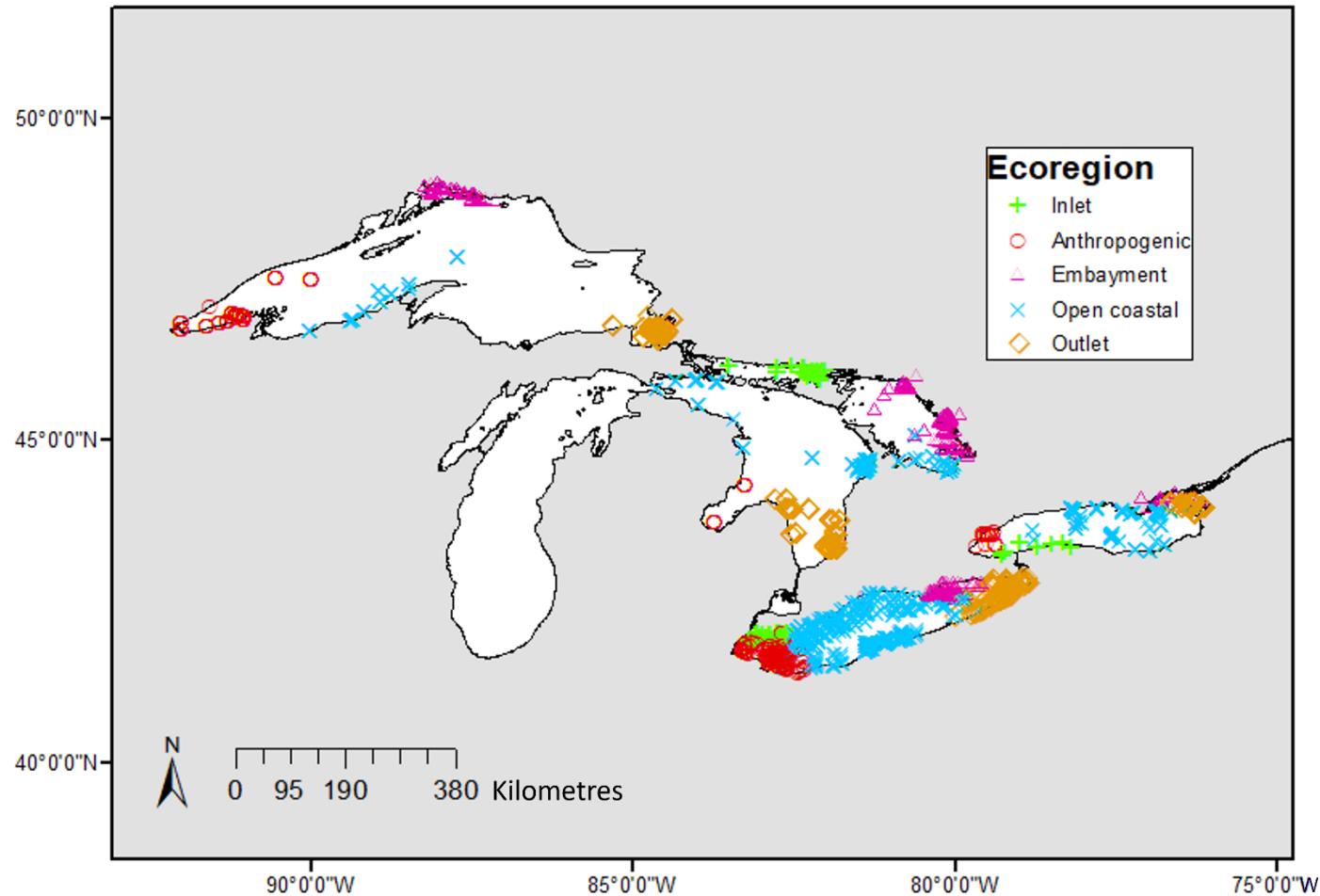


Figure 1. Sample collection sites for fish taxa and dreissenids across 5 ecoregions (inlet, anthropogenic, embayment, open coastal, and outlet) among lakes Superior, Huron, Erie, and Ontario between 2016 and 2019.

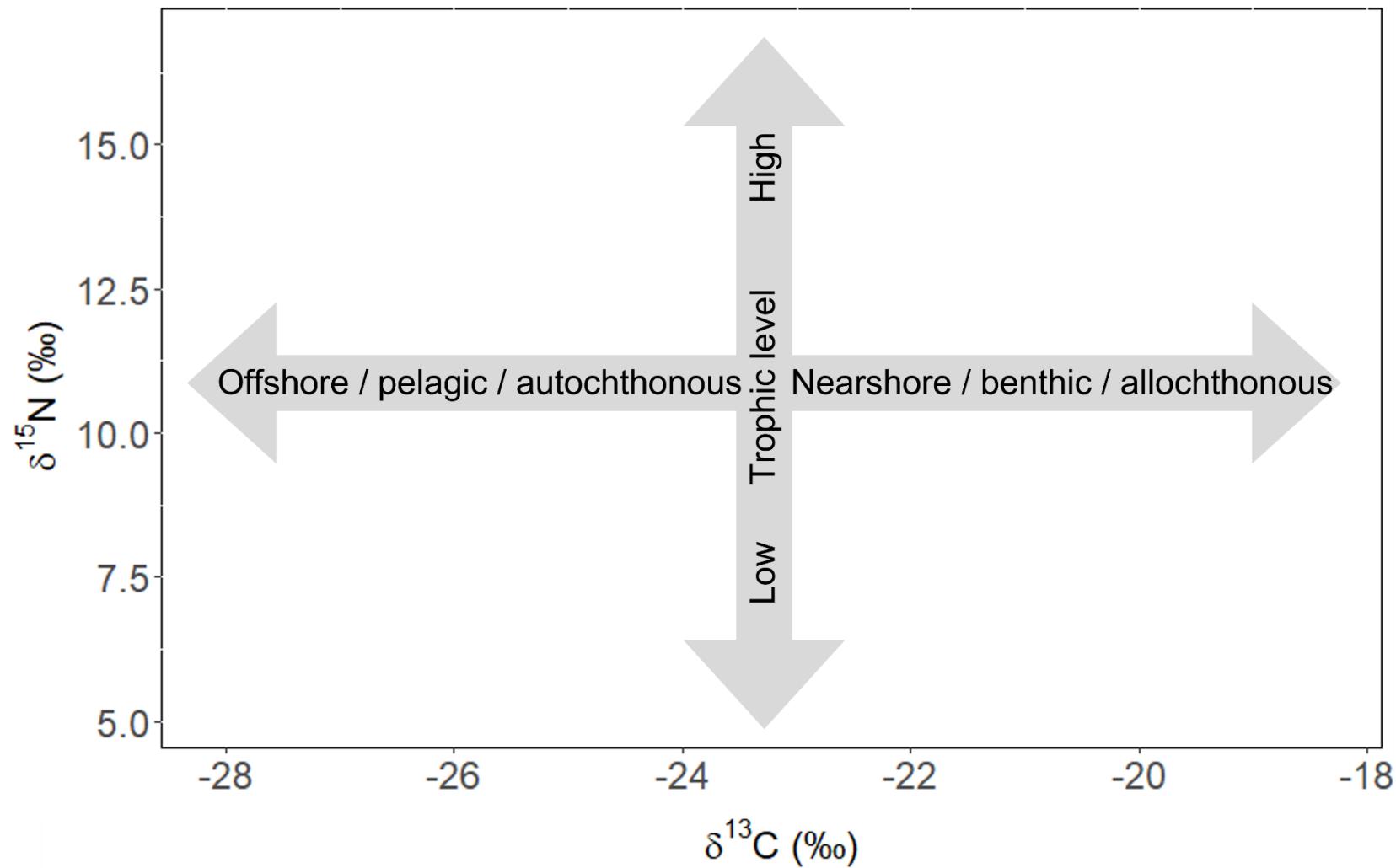


Figure 2. Interpretation guide of bivariate carbon ($\delta^{13}\text{C}$; x-axis) and nitrogen ($\delta^{15}\text{N}$; y-axis) isotope biplots for fish species collected in lakes Superior, Huron, Erie, and Ontario. This guide applies to figures 3, 4, and 7.1–7.12.

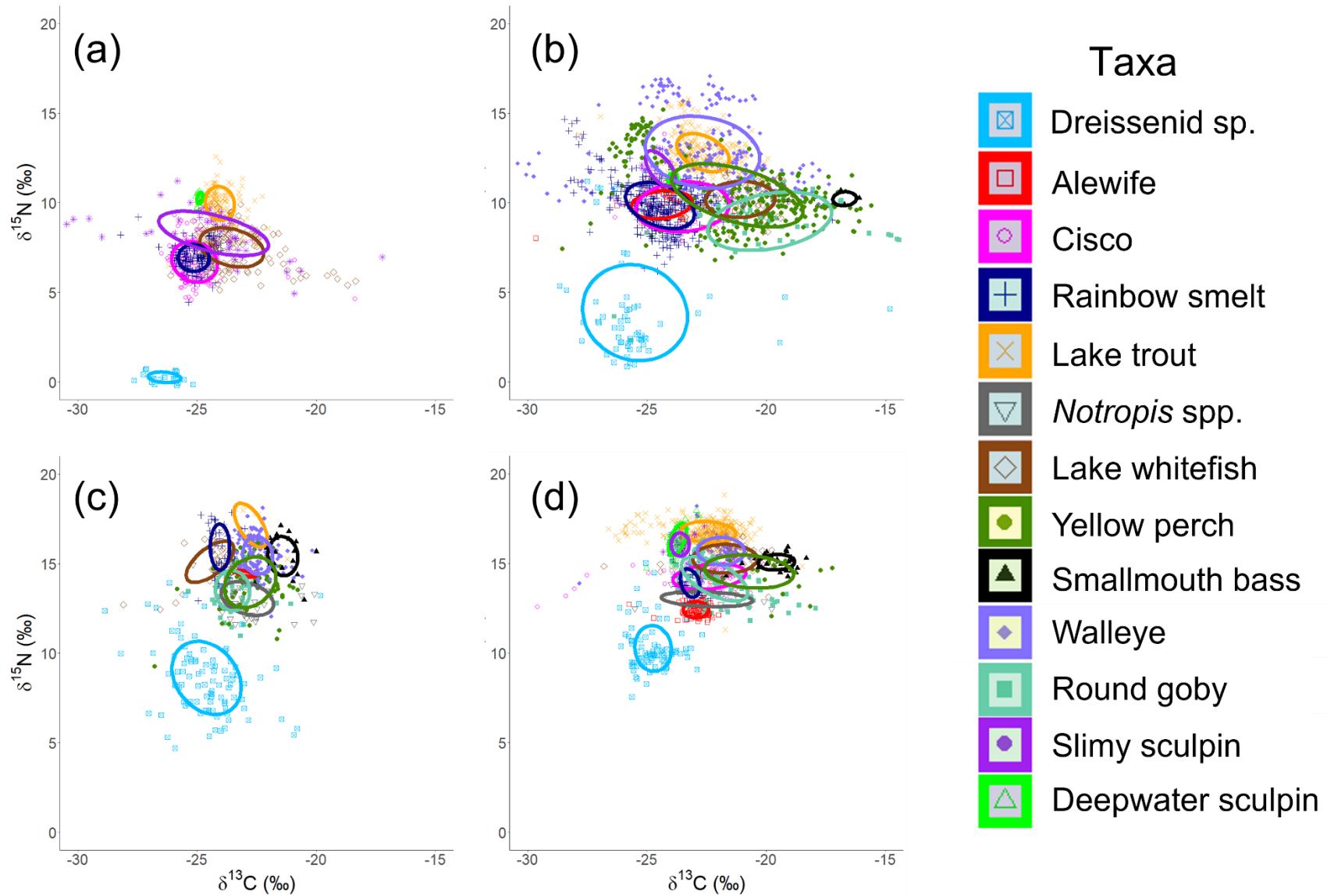


Figure 3. Bivariate absolute stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopes of 12 fish taxa and dreissenids across lakes (a) Superior, (b) Huron, (c) Erie, and (d) Ontario.

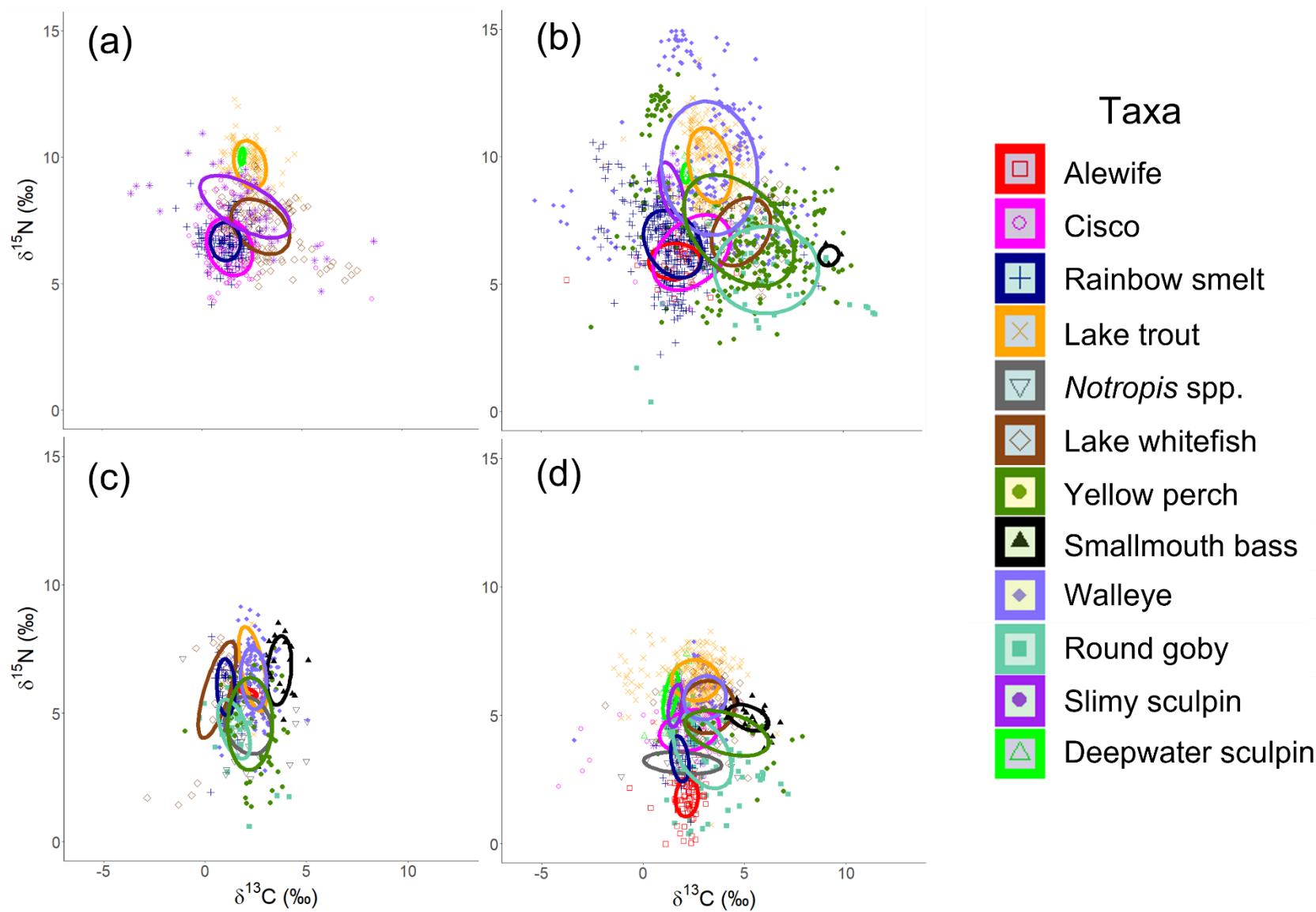


Figure 4. Bivariate relative stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopes of 12 fish taxa across lakes (a) Superior, (b) Huron, (c) Erie, and (d) Ontario.

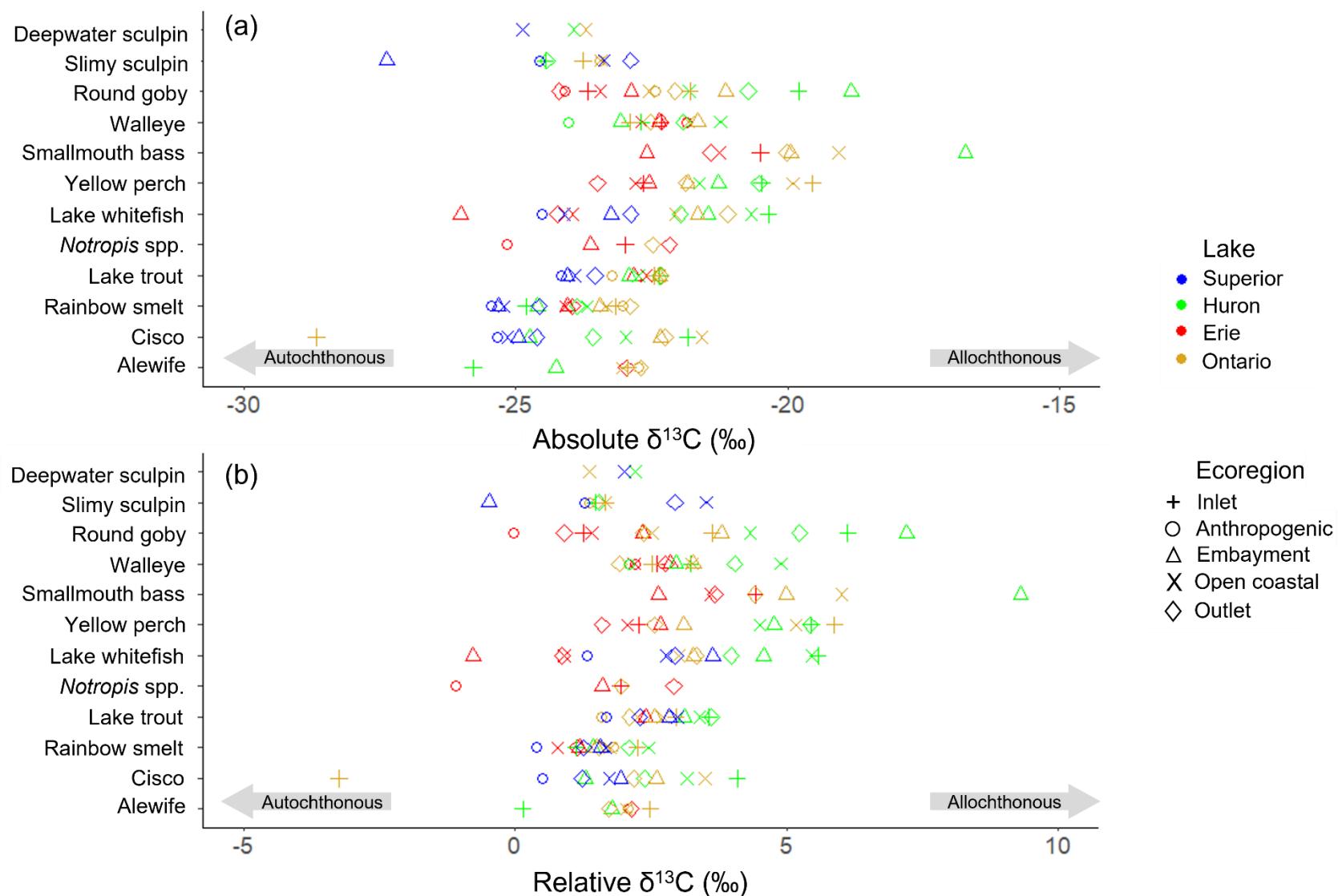


Figure 5. Range of (a) absolute and (b) relative stable carbon ($\delta^{13}\text{C}$) isotope values for 12 fish species collected across 5 ecoregions (inlet, anthropogenic, embayment, open coastal, outlet) in lakes Superior, Huron, Erie, and Ontario.

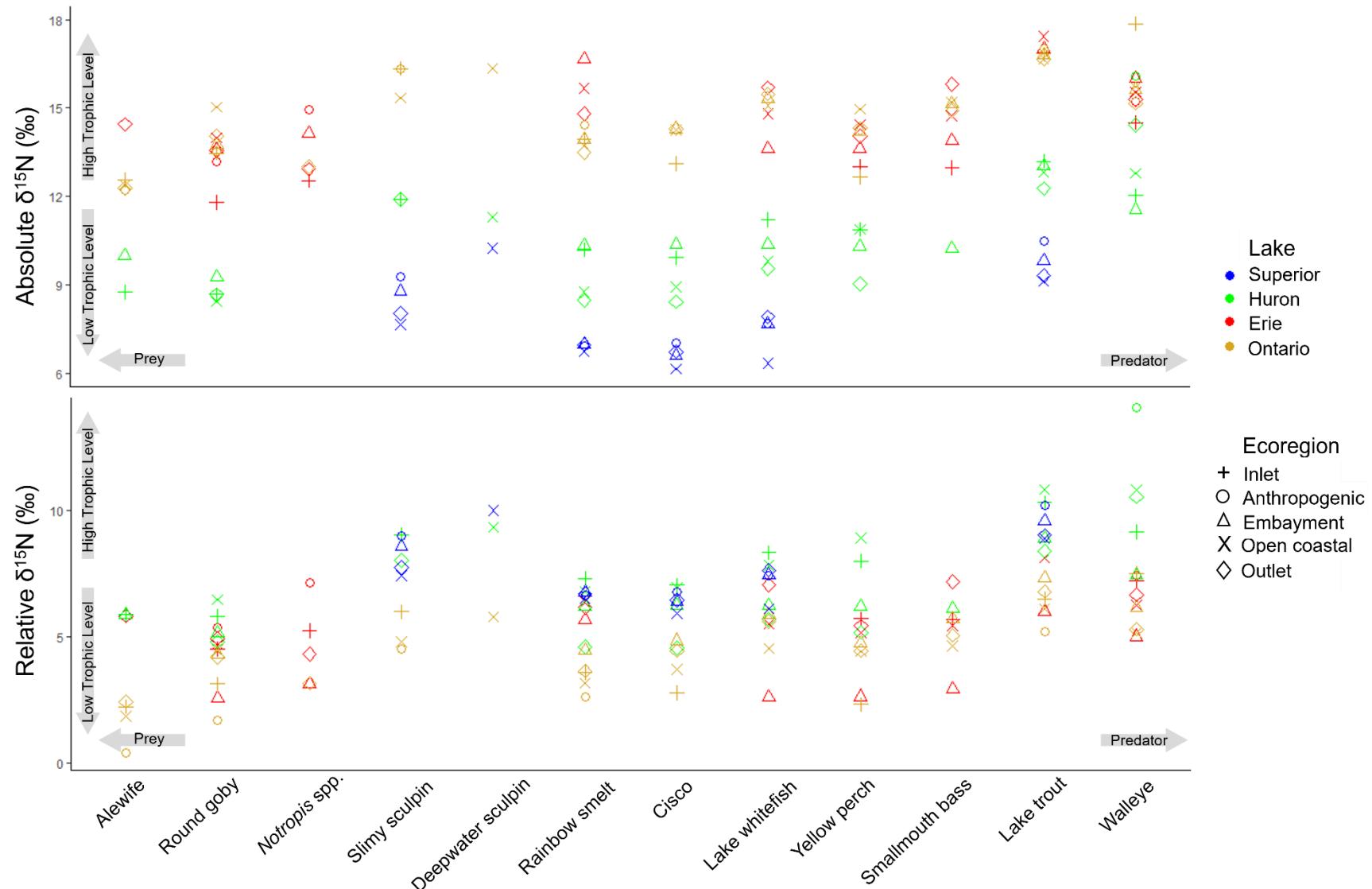


Figure 6. Range of (a) absolute and (b) relative stable nitrogen ($\delta^{15}\text{N}$) isotope values for 12 fish species collected across 5 ecoregions (inlet, anthropogenic, embayment, open coastal, outlet) in lakes Superior, Huron, Erie, and Ontario.

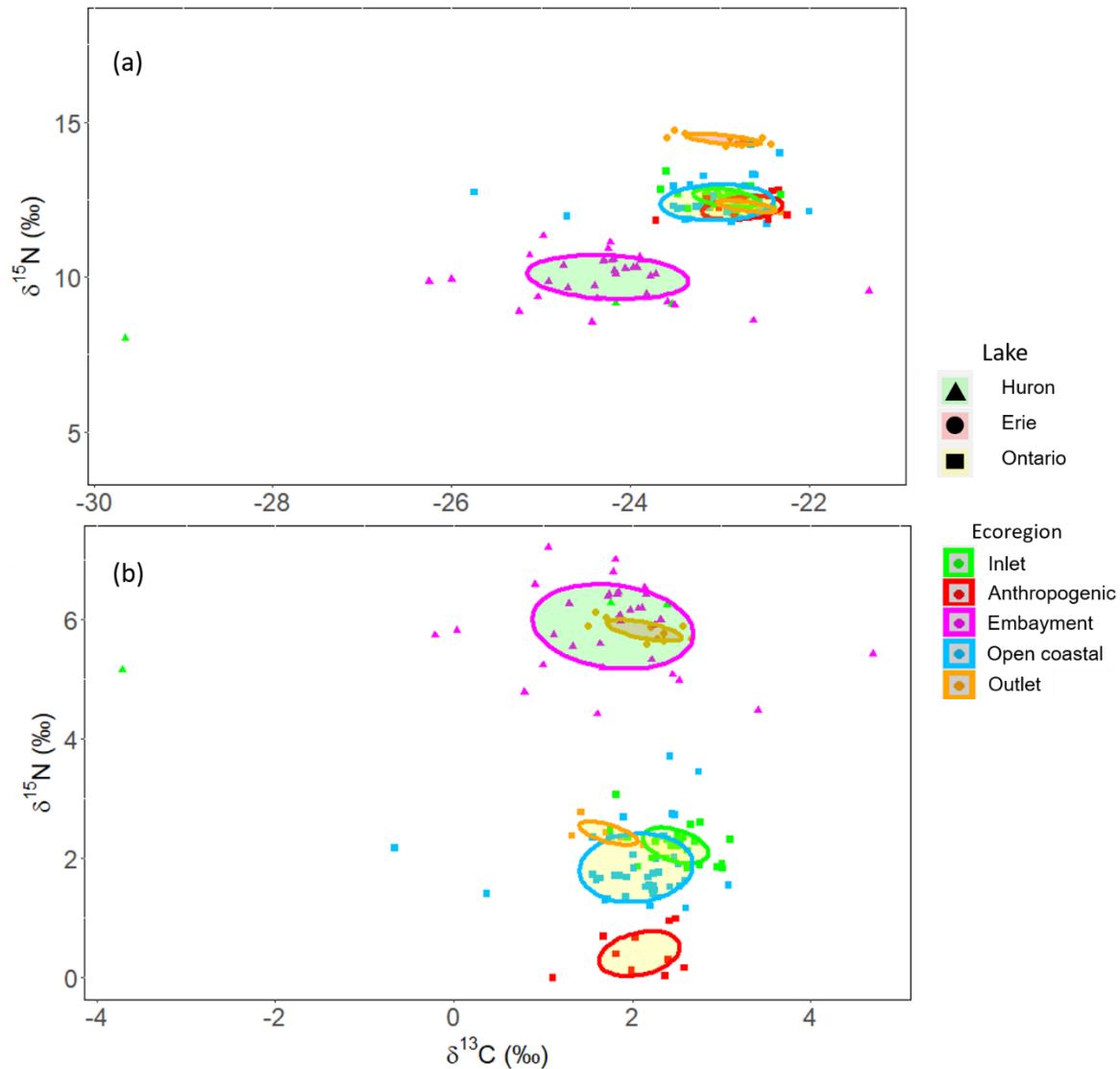


Figure 7.1. Bivariate (a) absolute and (b) relative stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopes of alewife (*Alosa pseudoharengus*) across 5 ecoregions (inlet, anthropogenic, embayment, open coastal, outlet) in lakes Superior, Huron, Erie, and Ontario.

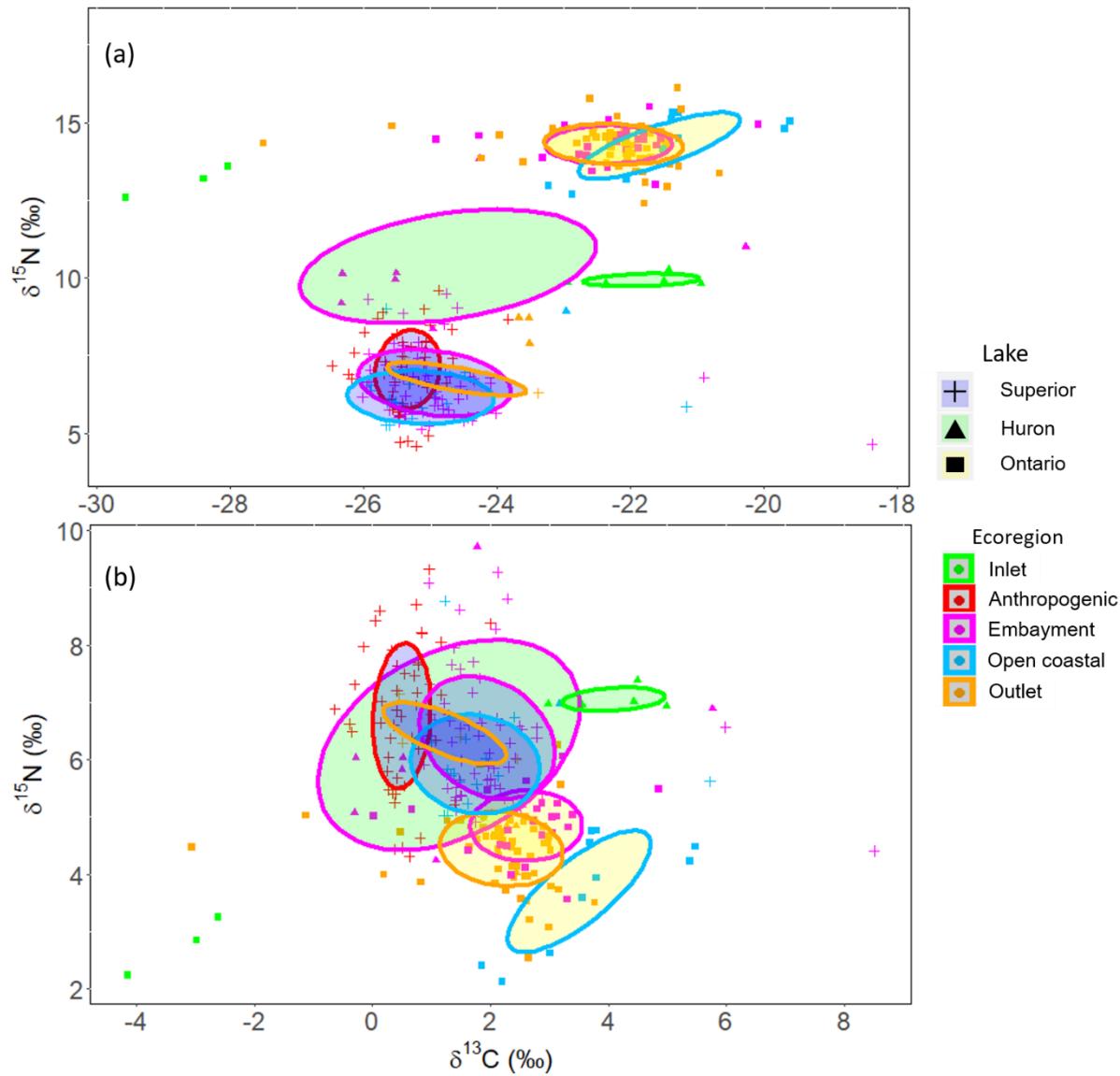


Figure 7.2. Bivariate (a) absolute and (b) relative stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopes of cisco (*Coregonus artedi*) across 5 ecoregions (inlet, anthropogenic, embayment, open coastal, outlet) in lakes Superior, Huron, Erie, and Ontario.

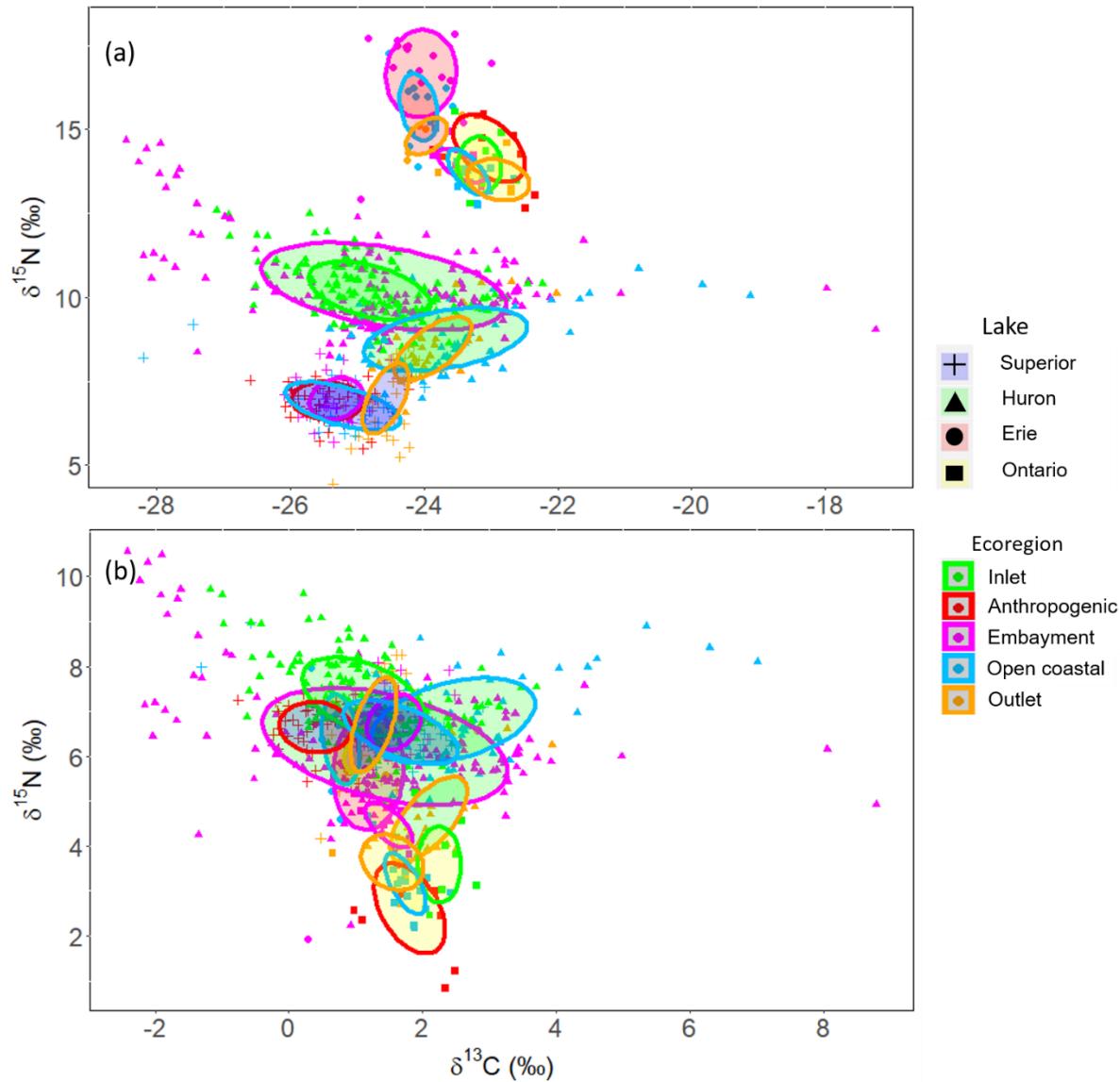


Figure 7.3. Bivariate (a) absolute and (b) relative stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopes of rainbow smelt (*Osmerus mordax*) across 5 ecoregions (inlet, anthropogenic, embayment, open coastal, outlet) in lakes Superior, Huron, Erie, and Ontario.

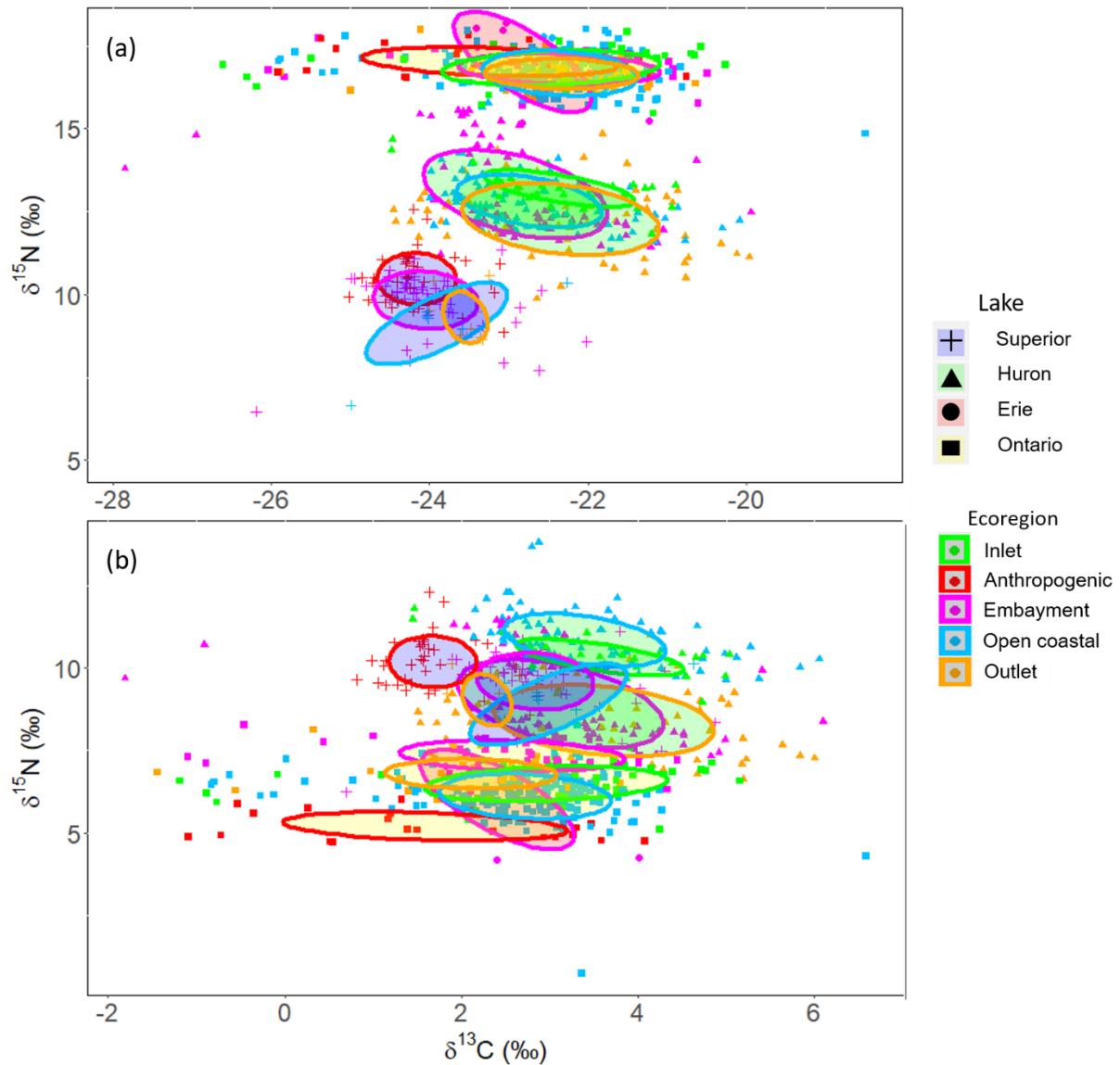


Figure 7.4. Bivariate (a) absolute and (b) relative stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopes of lake trout (*Salvelinus namaycush*) across 5 ecoregions (inlet, anthropogenic, embayment, open coastal, outlet) in lakes Superior, Huron, Erie, and Ontario.

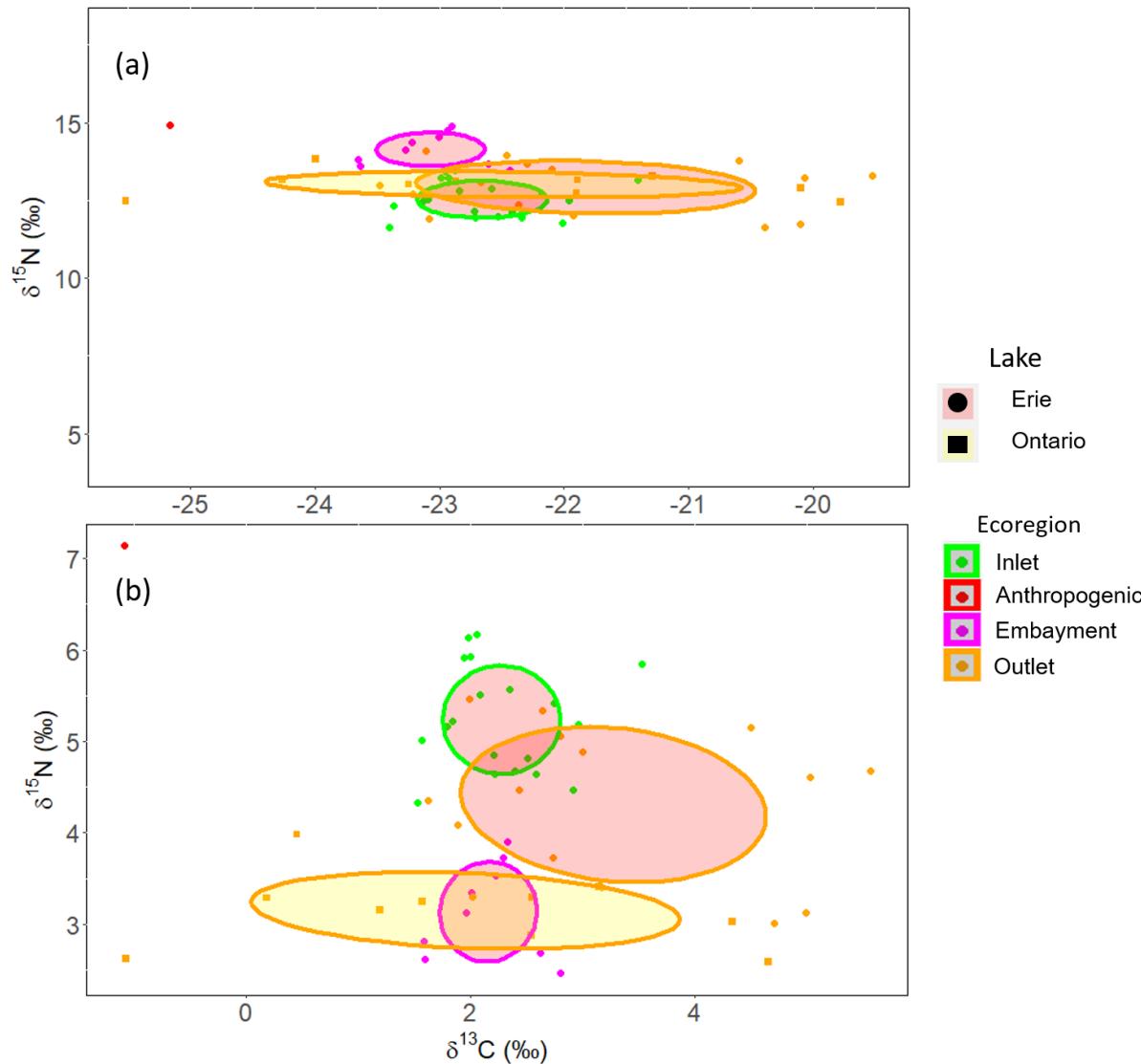


Figure 7.5. Bivariate (a) absolute and (b) relative stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopes of *Notropis* spp. (emerald shiner (*Notropis atherinoides*) and spottail shiner (*Notropis hudsonius*)) across 5 ecoregions (inlet, anthropogenic, embayment, open coastal, outlet) in lakes Superior, Huron, Erie, and Ontario.

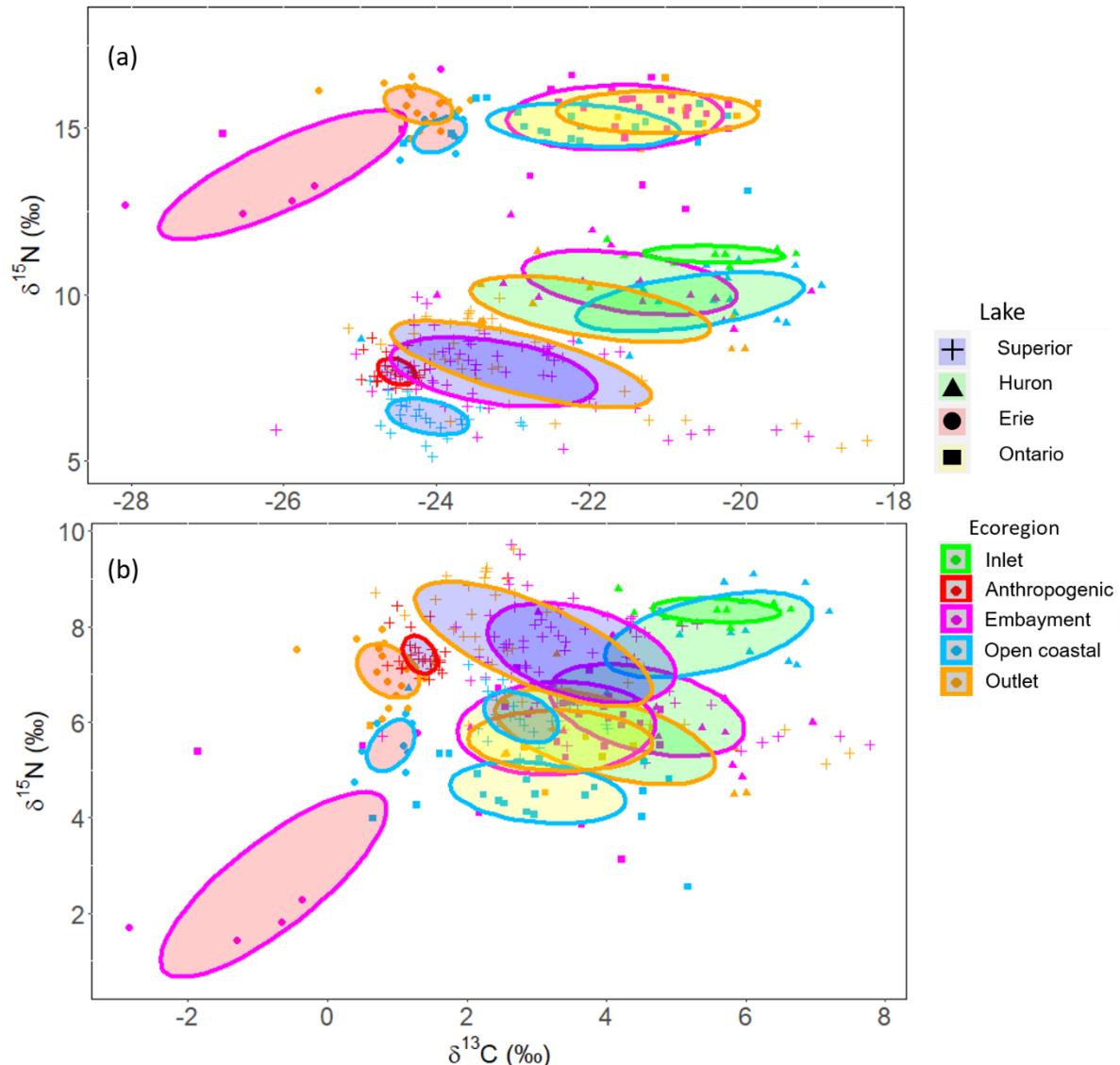


Figure 7.6. Bivariate (a) absolute and (b) relative stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopes of lake whitefish (*Coregonus clupeaformis*) across 5 ecoregions (inlet, anthropogenic, embayment, open coastal, outlet) in lakes Superior, Huron, Erie, and Ontario.

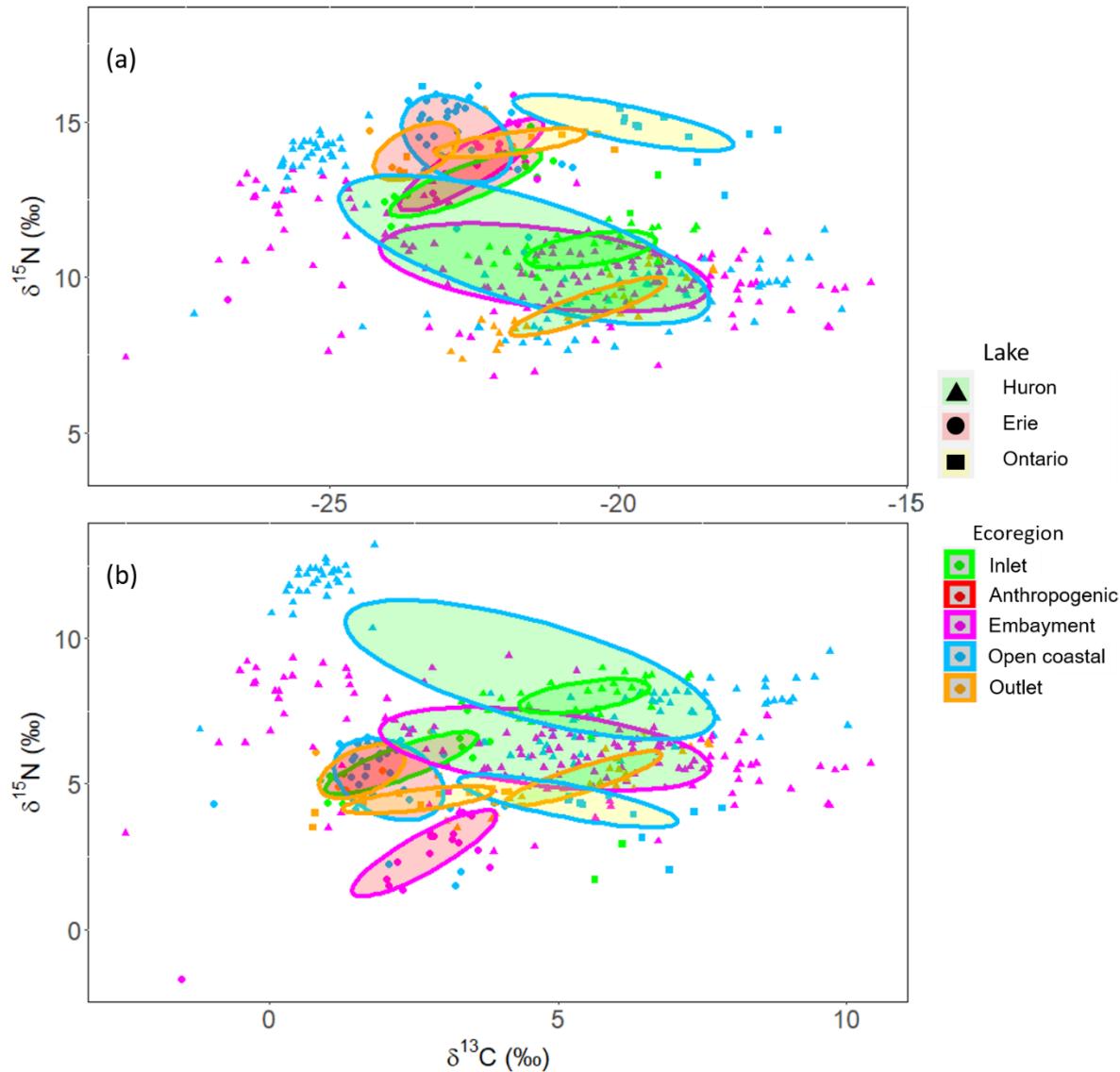


Figure 7.7. Bivariate (a) absolute and (b) relative stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopes of yellow perch (*Perca flavescens*) across 5 ecoregions (inlet, anthropogenic, embayment, open coastal, outlet) in lakes Superior, Huron, Erie, and Ontario.

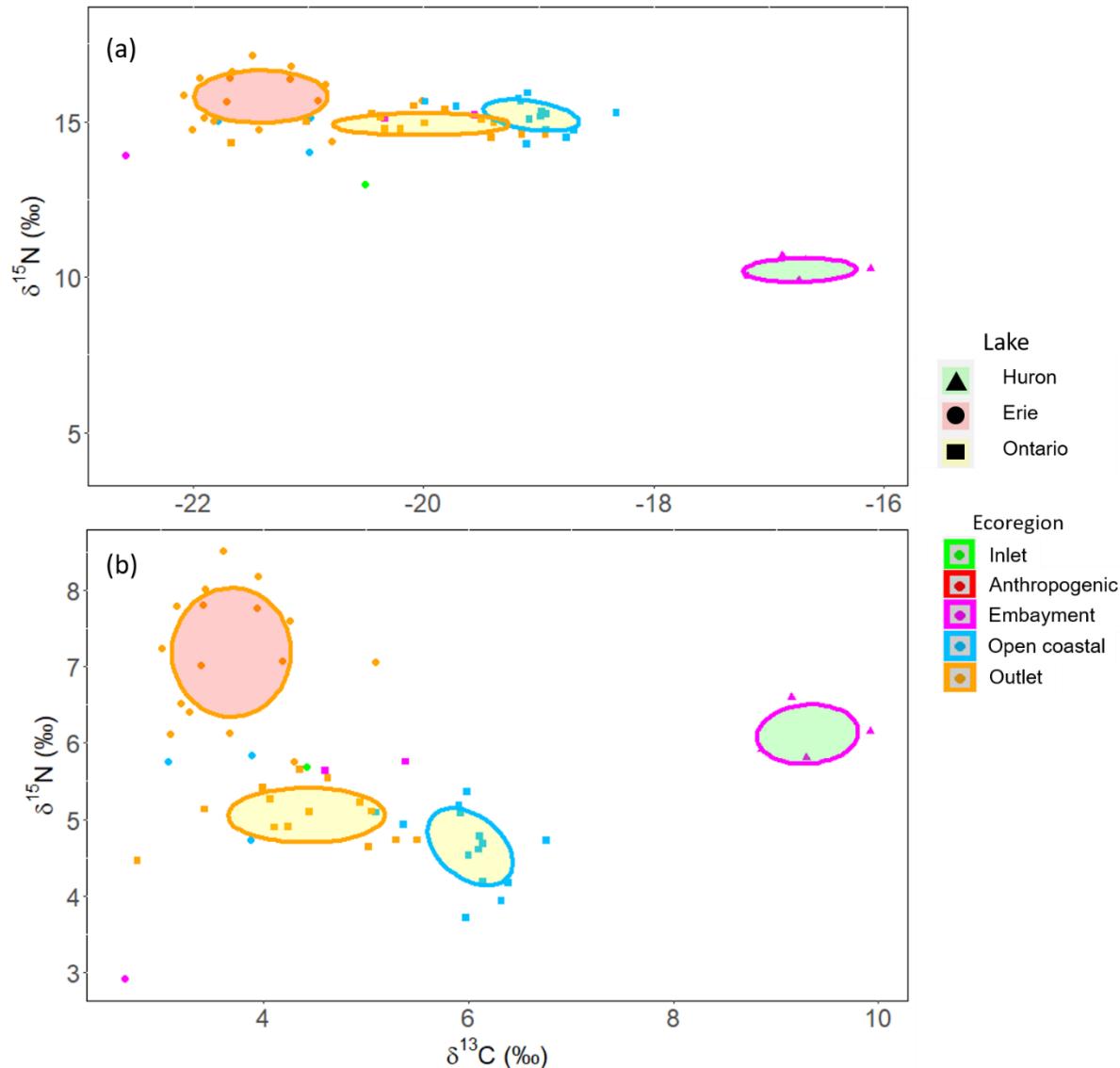


Figure 7.8. Bivariate (a) absolute and (b) relative stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopes of smallmouth bass (*Micropterus salmoides*) across 5 ecoregions (inlet, anthropogenic, embayment, open coastal, outlet) in lakes Superior, Huron, Erie, and Ontario.

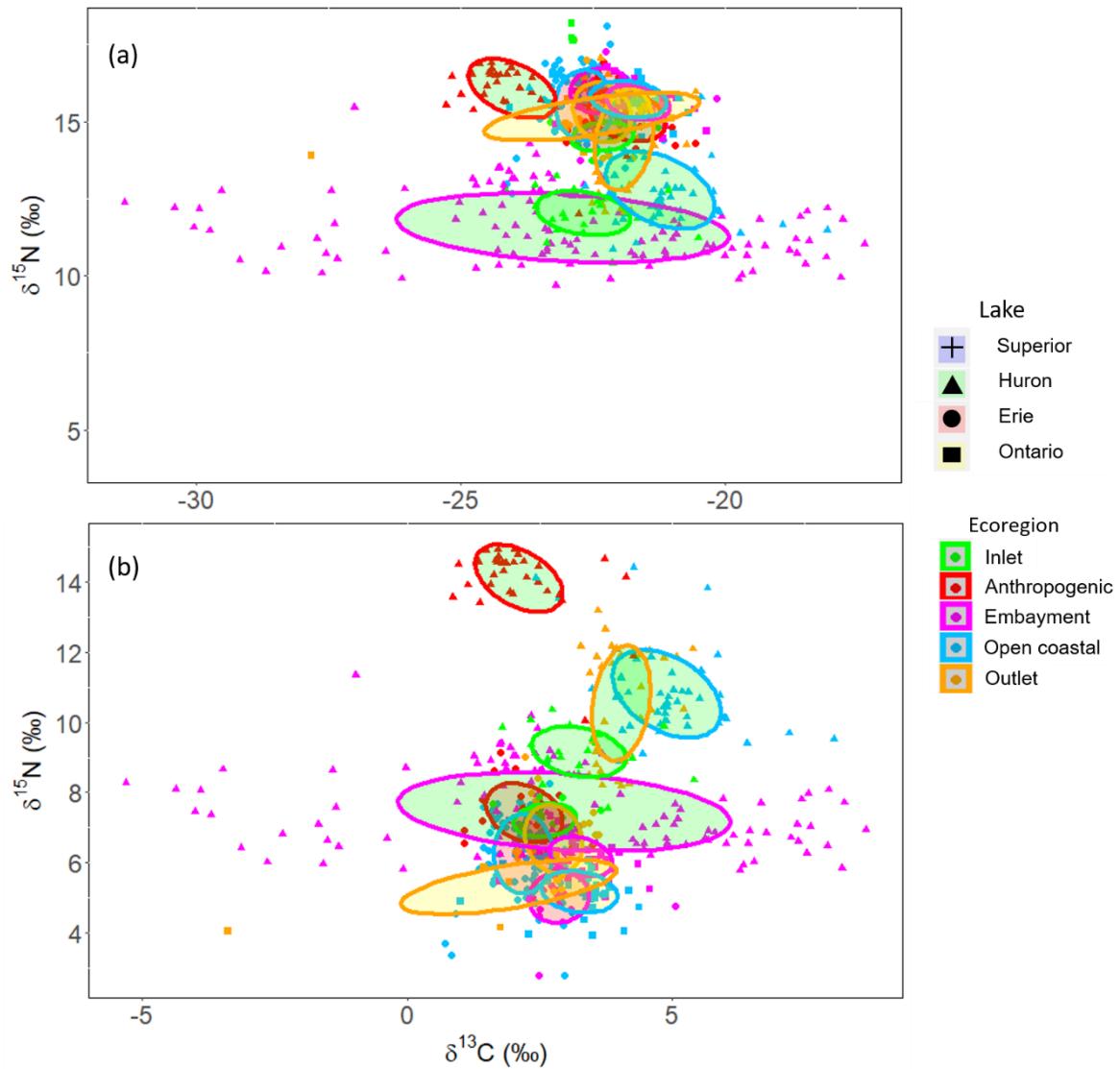


Figure 7.9. Bivariate (a) absolute and (b) relative stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopes of walleye (*Sander vitreus*) across 5 ecoregions (inlet, anthropogenic, embayment, open coastal, outlet) in lakes Superior, Huron, Erie, and Ontario.

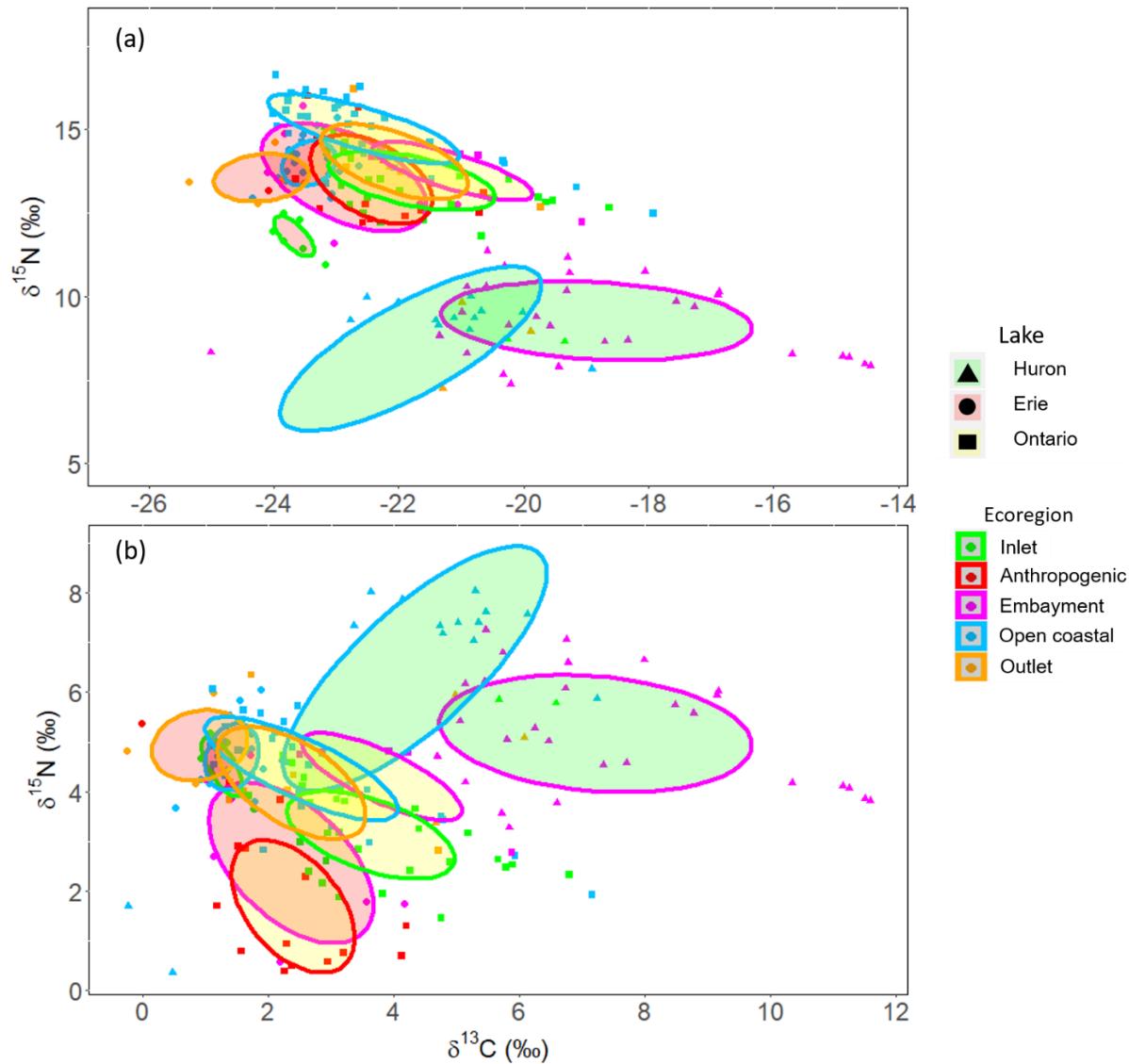


Figure 7.10. Bivariate (a) absolute and (b) relative stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopes of round goby (*Neogobius melanostomus*) across 5 ecoregions (inlet, anthropogenic, embayment, open coastal, outlet) in lakes Superior, Huron, Erie, and Ontario.

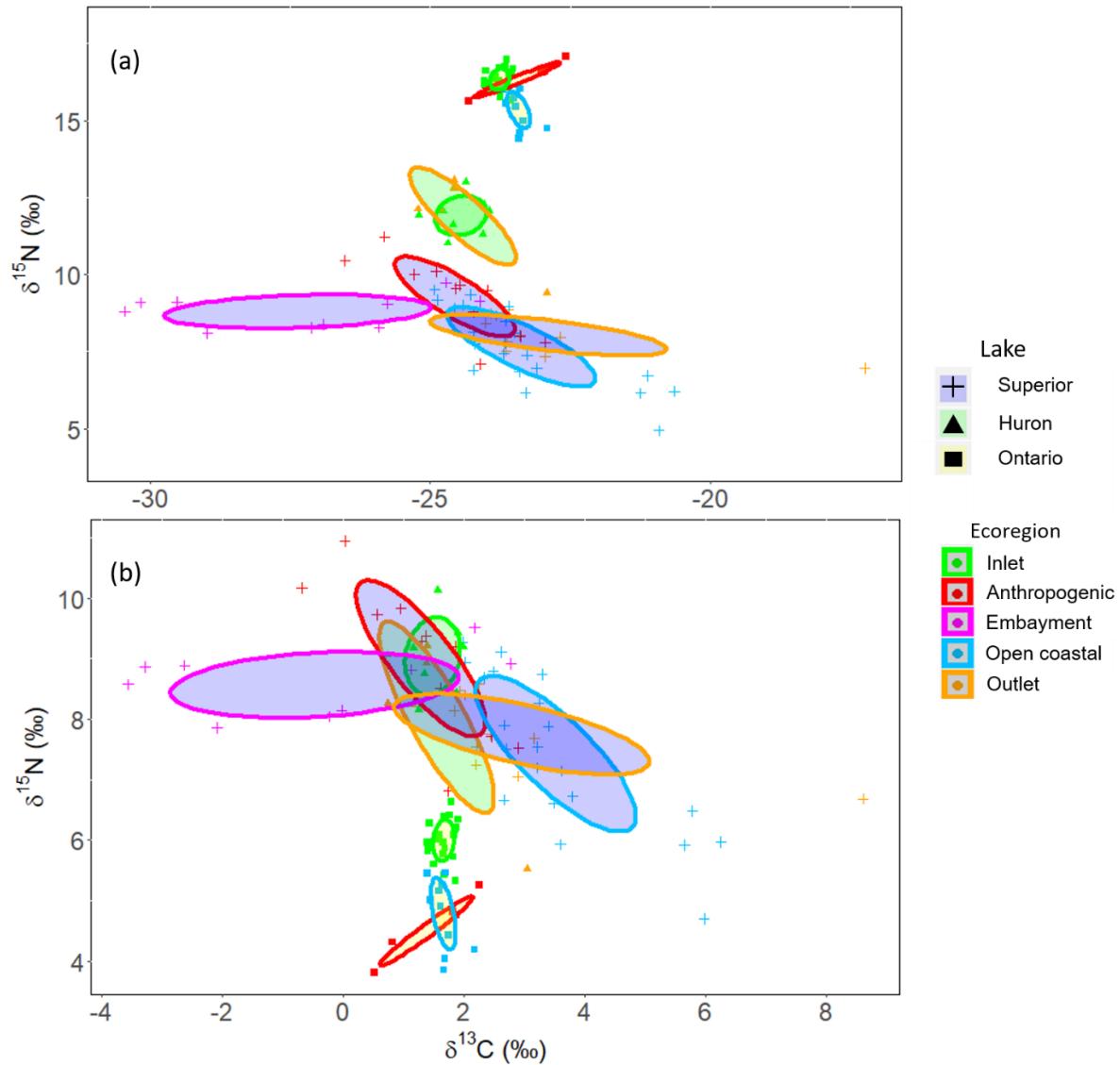


Figure 7.11. Bivariate (a) absolute and (b) relative stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopes of slimy sculpin (*Cottus cognatus*) across 5 ecoregions (inlet, anthropogenic, embayment, open coastal, outlet) in lakes Superior, Huron, Erie, and Ontario.

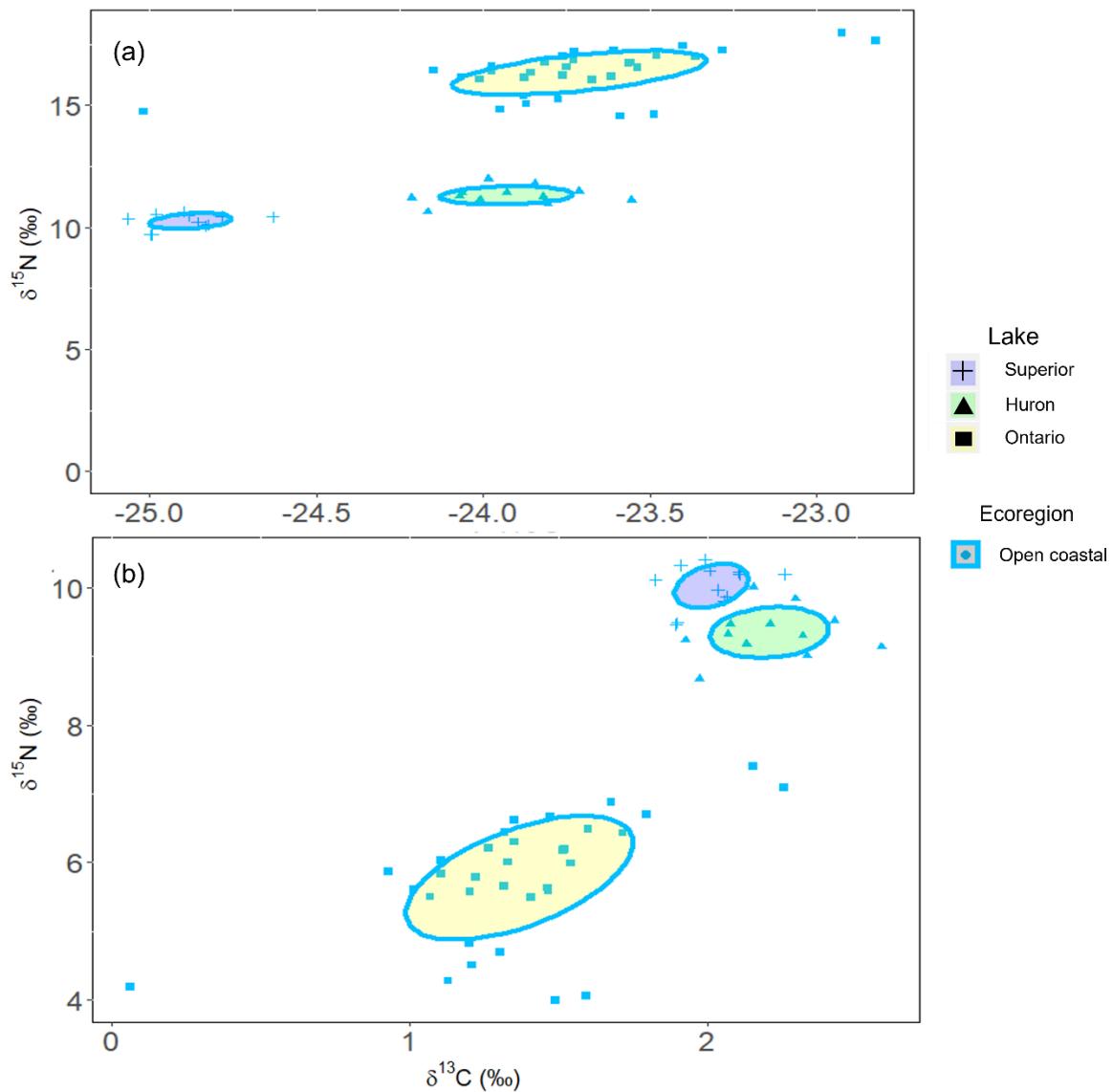


Figure 7.12. Bivariate (a) absolute and (b) relative stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopes of deepwater sculpin (*Myoxocephalus thompsonii*) across 5 ecoregions (inlet, anthropogenic, embayment, open coastal, outlet) in lakes Superior, Huron, Erie, and Ontario.

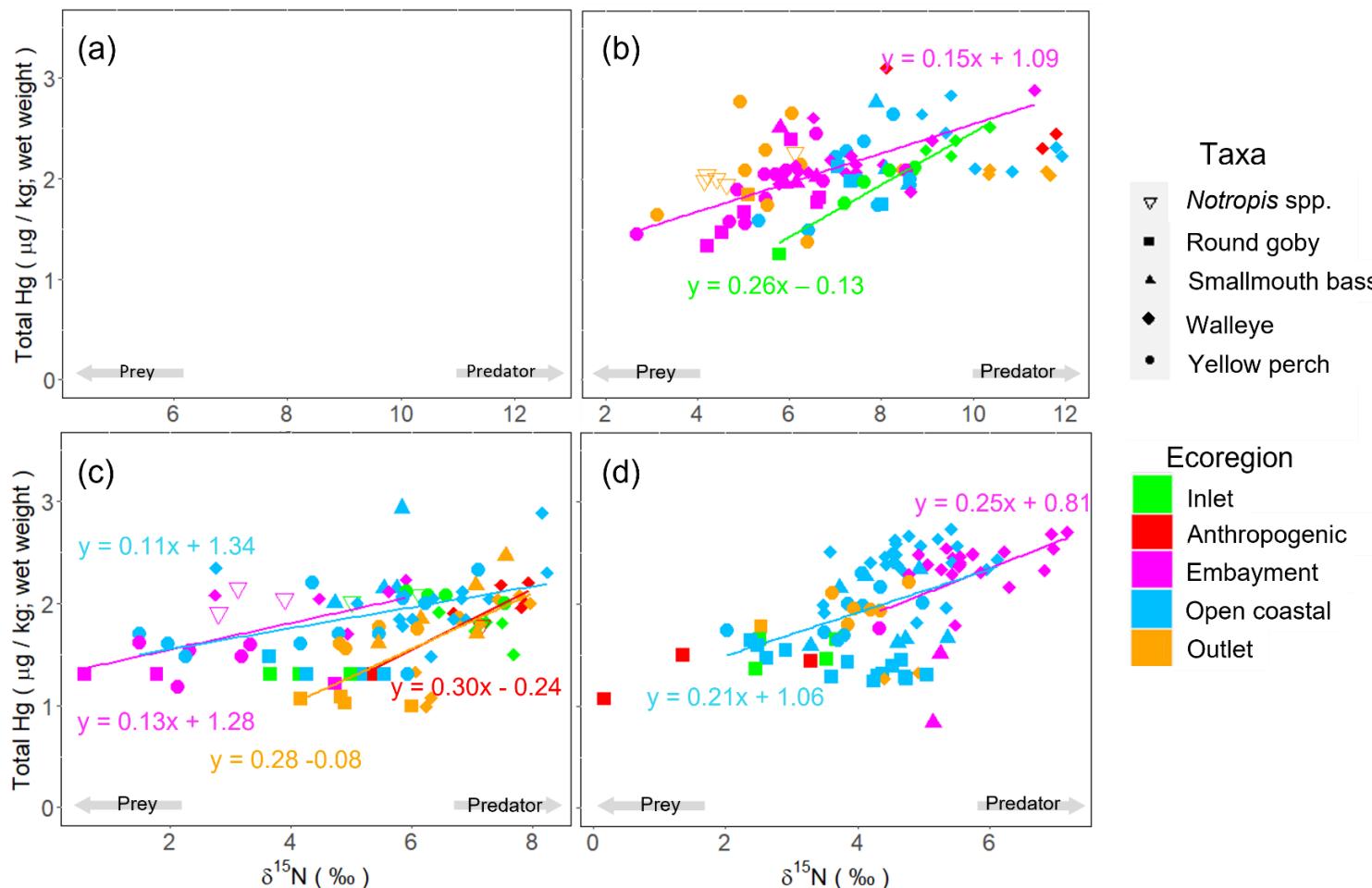


Figure 8.1. Warm + cool-water trophic magnification slopes (TMS) calculated using total $\log_{10}\text{Hg}$ (mercury) and relative $\delta^{15}\text{N}$ (nitrogen) stable isotope values for benthopelagic — emerald shiner (*Notropis atherinoides*), yellow perch (*Perca flavescens*), smallmouth bass (*Micropterus dolomieu*), and walleye (*Sander vitreus*) — and benthic — round goby (*Neogobius melanostomus*) — fish taxa across 5 ecoregions (inlet, anthropogenic, embayment, open coastal, outlet) in lakes (a) Superior (no fish collected), (b) Huron, (c) Erie, and (d) Ontario. For each ecoregion and lake, only significant regression slopes ($m > 0$; $p < 0.05$) were plotted. Regression equations also listed in Table A3.1.

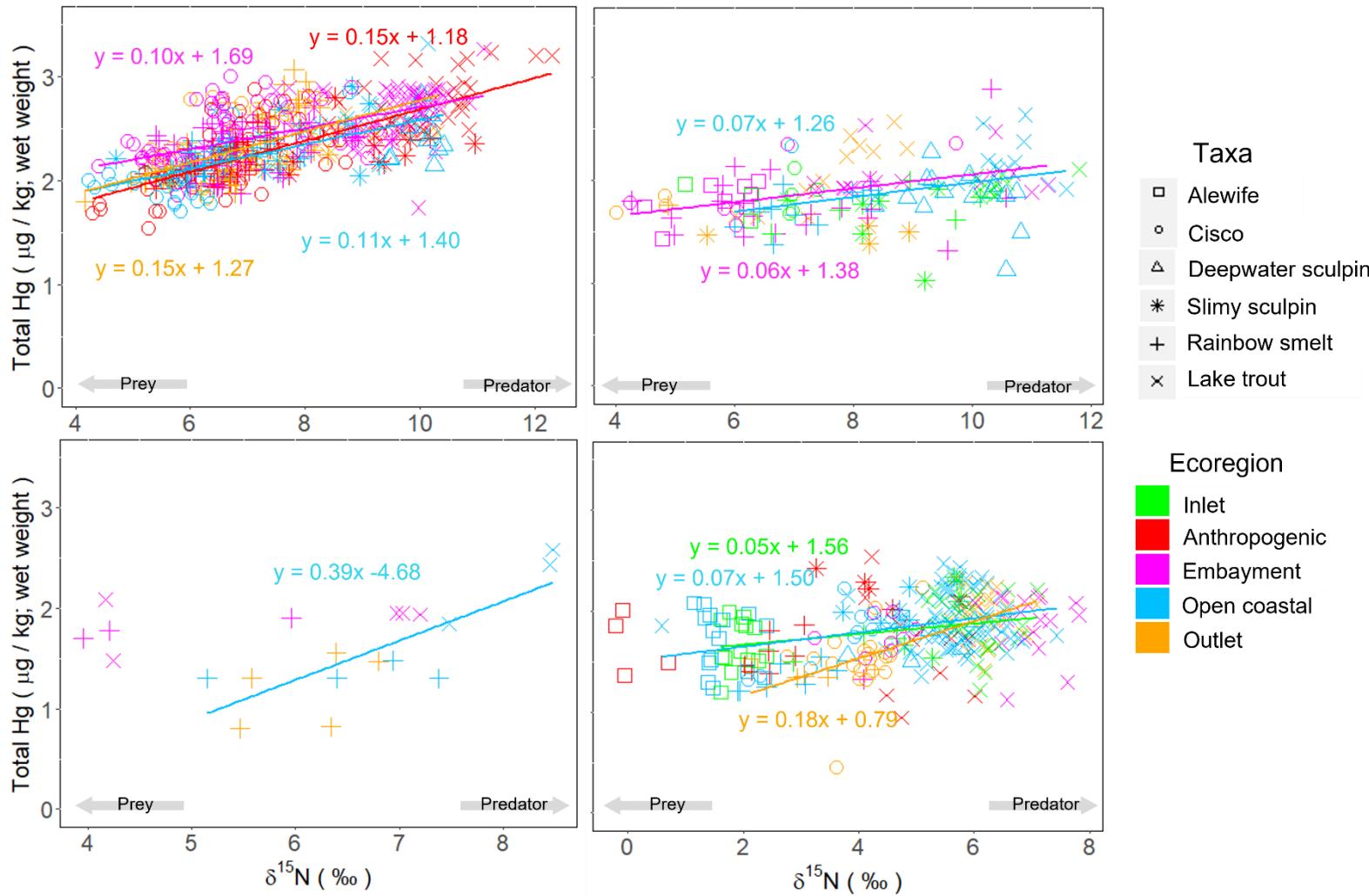


Figure 8.2. Coldwater trophic magnification slopes (TMS) calculated using total $\log_{10}\text{Hg}$ (mercury) and relative $\delta^{15}\text{N}$ (nitrogen) stable isotope values for pelagic — alewife (*Alosa pseudoharengus*), cisco (*Coregonus artedi*), deepwater sculpin (*Myoxocephalus thompsonii*), slimy sculpin (*Cottus cognatus*), rainbow smelt (*Osmerus mordax*), and lake trout (*Salvelinus namaycush*) — fish taxa across 5 ecoregions (inlet, anthropogenic, embayment, open coastal, outlet) in lakes (a) Superior, (b) Huron, (c) Erie, and (d) Ontario. For each ecoregion and lake, only significant regressions slopes ($m > 0$; $p < 0.05$) were plotted. Regression equations also listed in Table A3.2.

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Appendix 1. Glossary

Absolute $\delta^{13}\text{C}$ – Indicates whether fish are feeding more nearshore (less negative) or offshore (more negative); comparisons only valid among species collected from the same lake and ecoregion.

Absolute $\delta^{15}\text{N}$ – Indicates whether fish are feeding on lower trophic (lower $\delta^{15}\text{N}$) or higher (higher $\delta^{15}\text{N}$) trophic level prey; comparisons only valid among species collected from the same lake and ecoregion.

Baseline – A low trophic level, abundant species that is collected and used to adjust for isotopic differences in food webs to make appropriate comparisons within and among areas, in this case lakes. Also referred to as *isotopic baseline*.

Benthic – Nearshore lake environment that is typically influenced by allochthonous carbon from the watershed.

Benthopelagic – Transition zone between benthic and pelagic lake environments that contain a mixture of allochthonous (introduced) and autochthonous (natural) carbon sources from watershed and phytoplankton.

Carbon range (CR) – The greater the range in CR, the broader the habitat use (nearshore/offshore) of a species. Comparing carbon ranges can reveal whether species are using specific primary production pathways or vary in their resource use.

Ecological tracers – Stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopes, as well as mercury, that move in predictable ways through food webs to reflect functional properties and processes.

Ecoregion – Designated sub-lake spatial unit that is standardized across an area and defined by specific intrinsic properties (e.g., for lakes depth, temperature) or the presence of external stressors (e.g., urban and agricultural loadings).

External stressors – Stressors that are caused by human activities, including climate change, introduction of aquatic invasive species, and urban and agricultural runoff into the watershed.

Food web structure – Characterization of predator-prey relationships (and the flow of energy through predation) in an ecosystem (from primary producers to secondary consumers) and limnological environments (offshore, i.e., pelagic and nearshore, i.e., benthic).

Insectivore – Trophic guild classification referring to insect-eating species.

Intrinsic properties – Physical and chemical properties, in this case of lakes, such as depth, temperature, productivity, and circulation that may influence fish habitat, community assemblages, and food web structure.

Isotopic niche width – Determined by calculating the area represented by carbon and nitrogen stable isotopes on a biplot. Larger niche areas suggest a broader range in habitat and resource use. Can be compared among different species or within populations. Represented by SEA_B.

Mercury – A commonly assessed chemical originating from anthropogenic sources that accumulates in organisms with limited excretion (i.e., it bioaccumulates). Mercury concentration reflects feeding rate relative to prey mercury concentration.

Nitrogen range (NR) – The greater the NR, the broader the prey/resource use of a species (consuming prey from across the entire food chain from multiple trophic levels). Comparing

nitrogen ranges can reveal whether species are occupying a specific trophic role (e.g., only piscivore) or consume prey across all trophic levels (more omnivory).

Omnivore – Trophic guild classification referring to the consumption of prey across different trophic levels (consumption of planktivores, insectivores, and piscivores).

Pelagic – Offshore lake environment characterized by autochthonous carbon driven by phytoplankton.

Piscivore – Trophic guild classification referring to fish-eating species.

Planktivore – Trophic guild classification referring to plankton-eating species.

Relative $\delta^{13}\text{C}$ – Indicates whether fish are feeding more nearshore (less negative) or offshore (more negative) after adjusting for baseline. Comparisons across lake and ecoregion are valid.

Relative $\delta^{15}\text{N}$ – Indicates whether fish are feeding on lower trophic (lower $\delta^{15}\text{N}$) or higher (higher $\delta^{15}\text{N}$) trophic level prey after adjusting for baseline. Comparisons across lake and ecoregion are valid.

Stable carbon ($\delta^{13}\text{C}$) isotopes – Ecological tracer used to quantify habitat and resource use of individuals. More negative values indicate resources are being obtained from pelagic environments driven by offshore phytoplankton, while more positive values indicate resources are being obtained from benthic environments, driven by watershed contributions.

Stable nitrogen ($\delta^{15}\text{N}$) isotopes – Ecological tracer used to quantify trophic position of an individual. Lower values indicate a lower trophic position, found in planktivores, while greater values indicate a higher trophic position, typically found in piscivores.

Trophic enrichment – The transfer of energy between predator and prey throughout a food web that can be estimated using stable nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) isotopes.

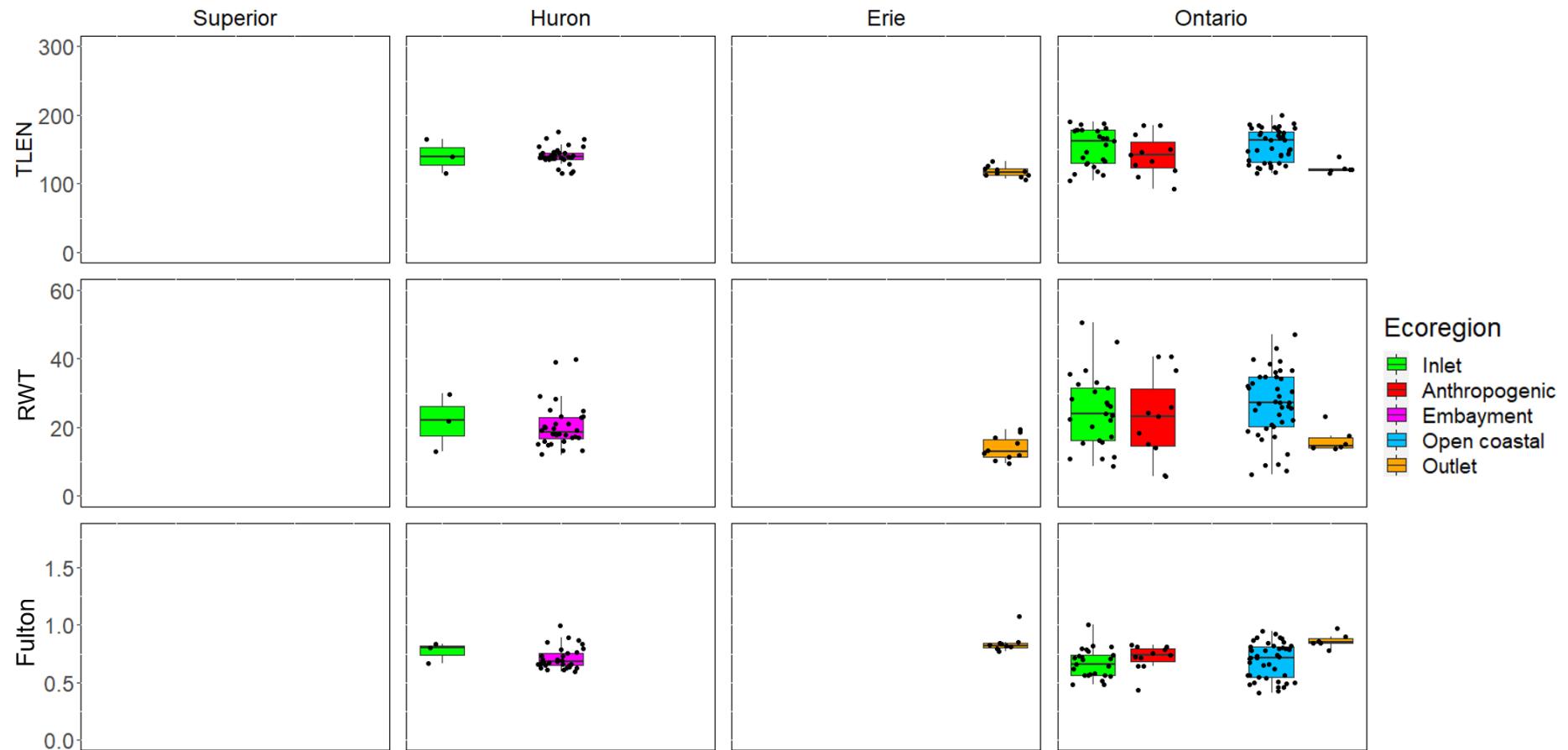
Trophic guild – Groupings of species with similar diet compositions (e.g., walleye, lake trout, and smallmouth bass all primarily consume fish; thus, we group them all under the trophic guild term “piscivores”).

Trophic level (TL) – Numerical representation of food chain length corresponding to the flow of energy from the base of the food web (primary producers; TL=1) to the top consumer (secondary consumers; TL=4).

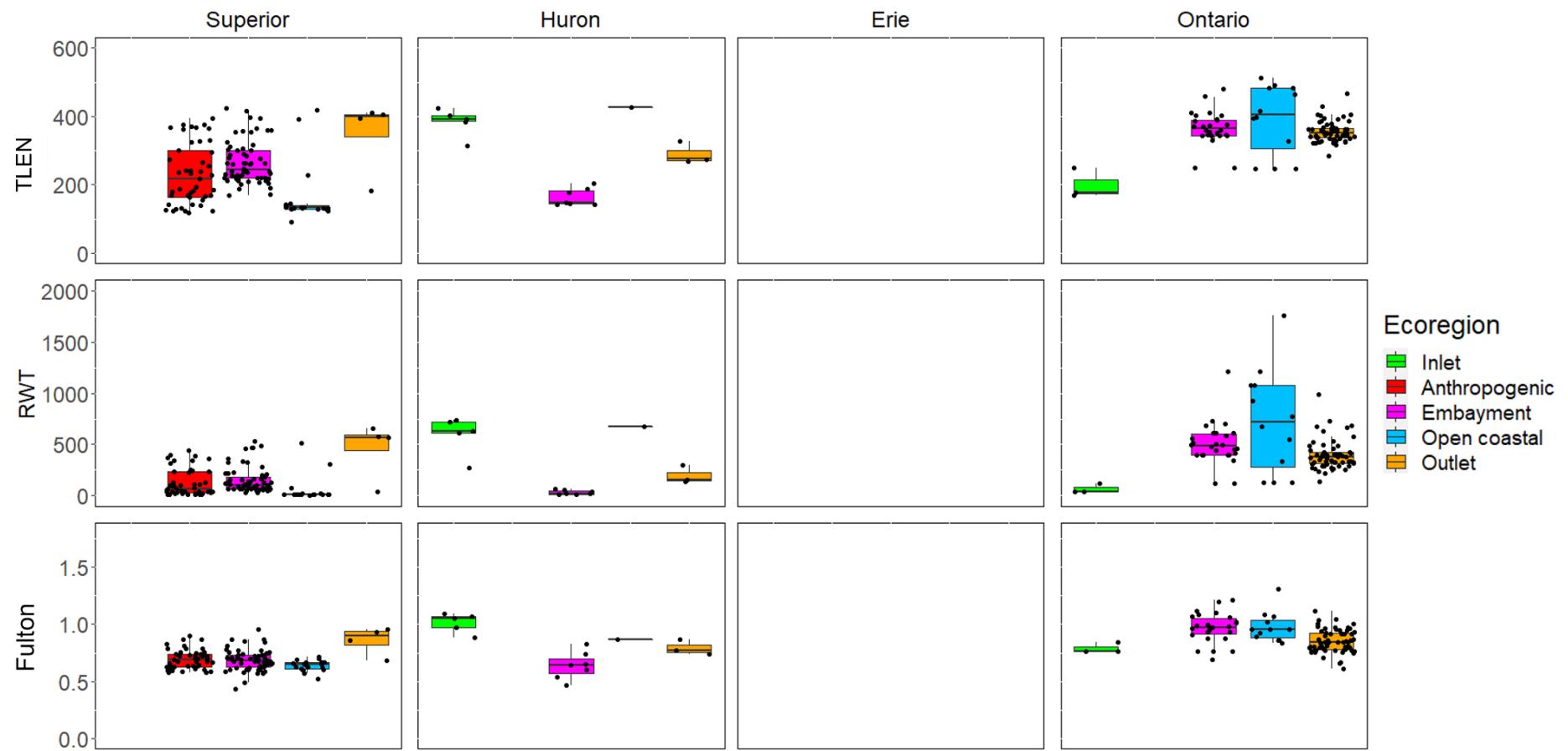
Trophic magnification slope (TMS) – The slope of the relationship between $\delta^{15}\text{N}$ (trophic position) and the natural logarithm of total mercury.

Trophic position (TP) – Continuous measurement of TL that accounts for omnivory. A top consumer may consume equal parts primary consumers (TL=3) and secondary consumers (TL=4), therefore their trophic position would be ~3.5, reflecting the mixed diet.

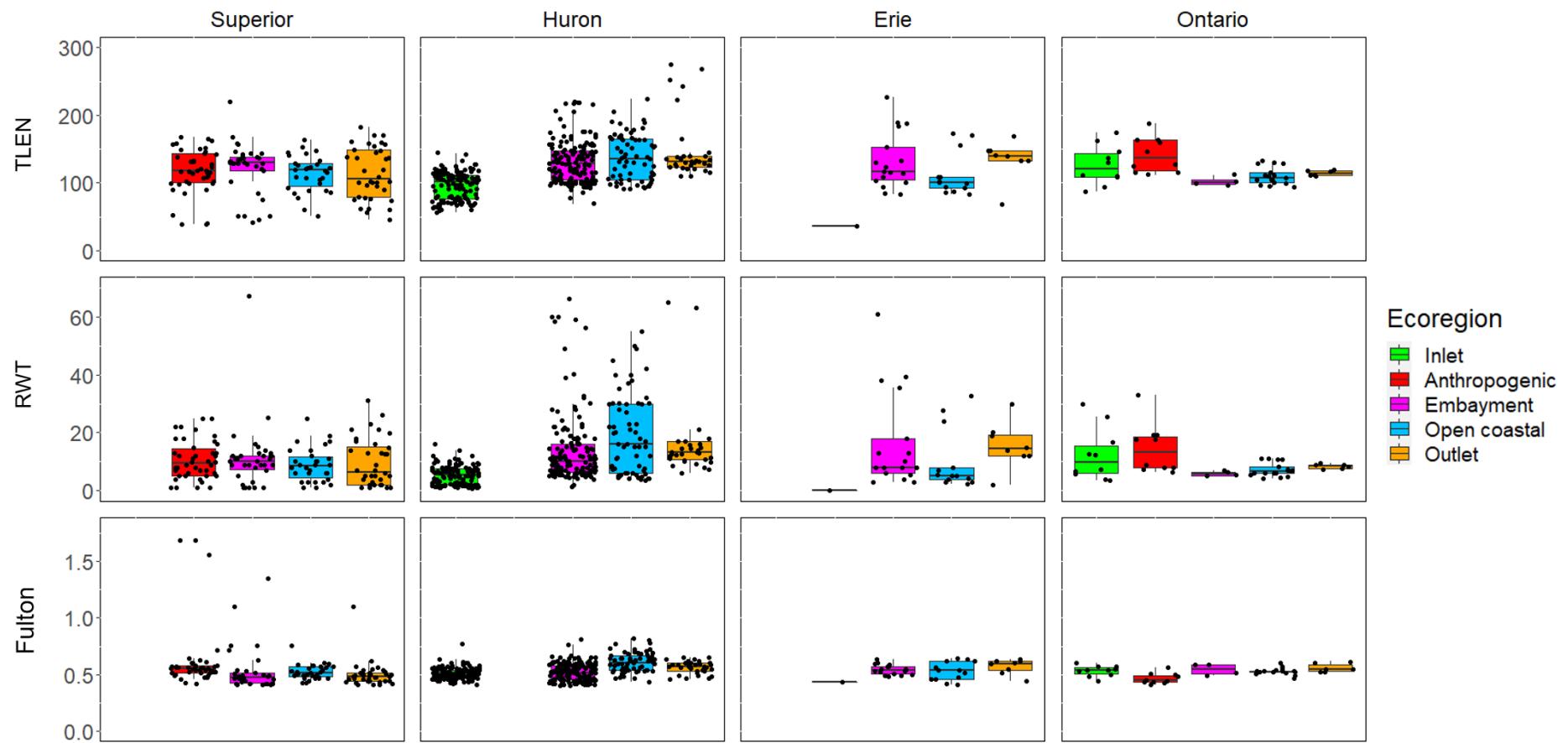
Appendix 2. Morphometrics



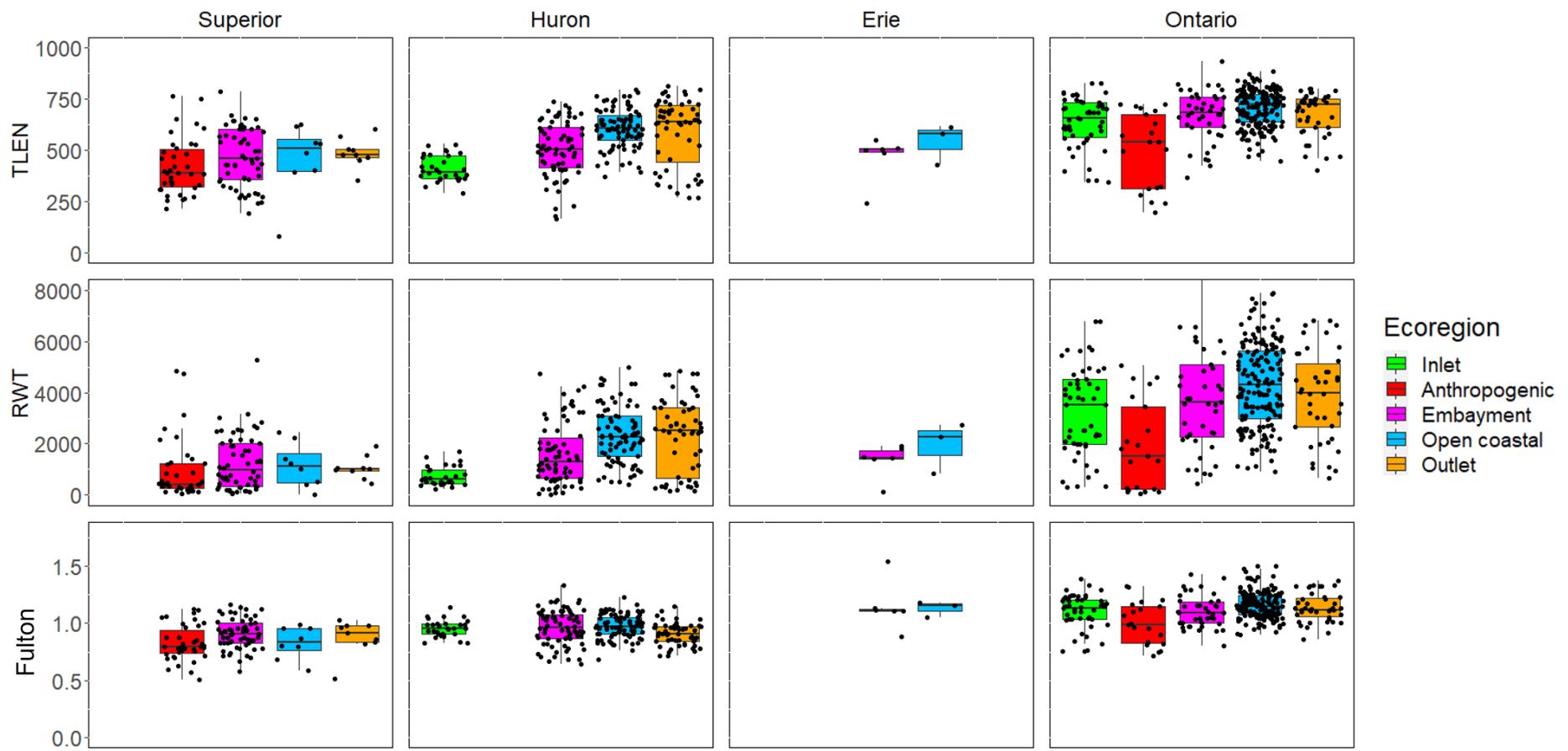
A2.1. Total length (TLEN; mm), round weight (RWT; g), and Fulton's K condition factor (Fulton) of alewife (*Alosa pseudoharengus*) across 5 ecoregions (inlet, anthropogenic, embayment, open coastal, outlet) in lakes Superior, Huron, Erie, and Ontario. Box plot horizontal ends represent 25th and 75th percentiles, while mean values are represented with a horizontal line.



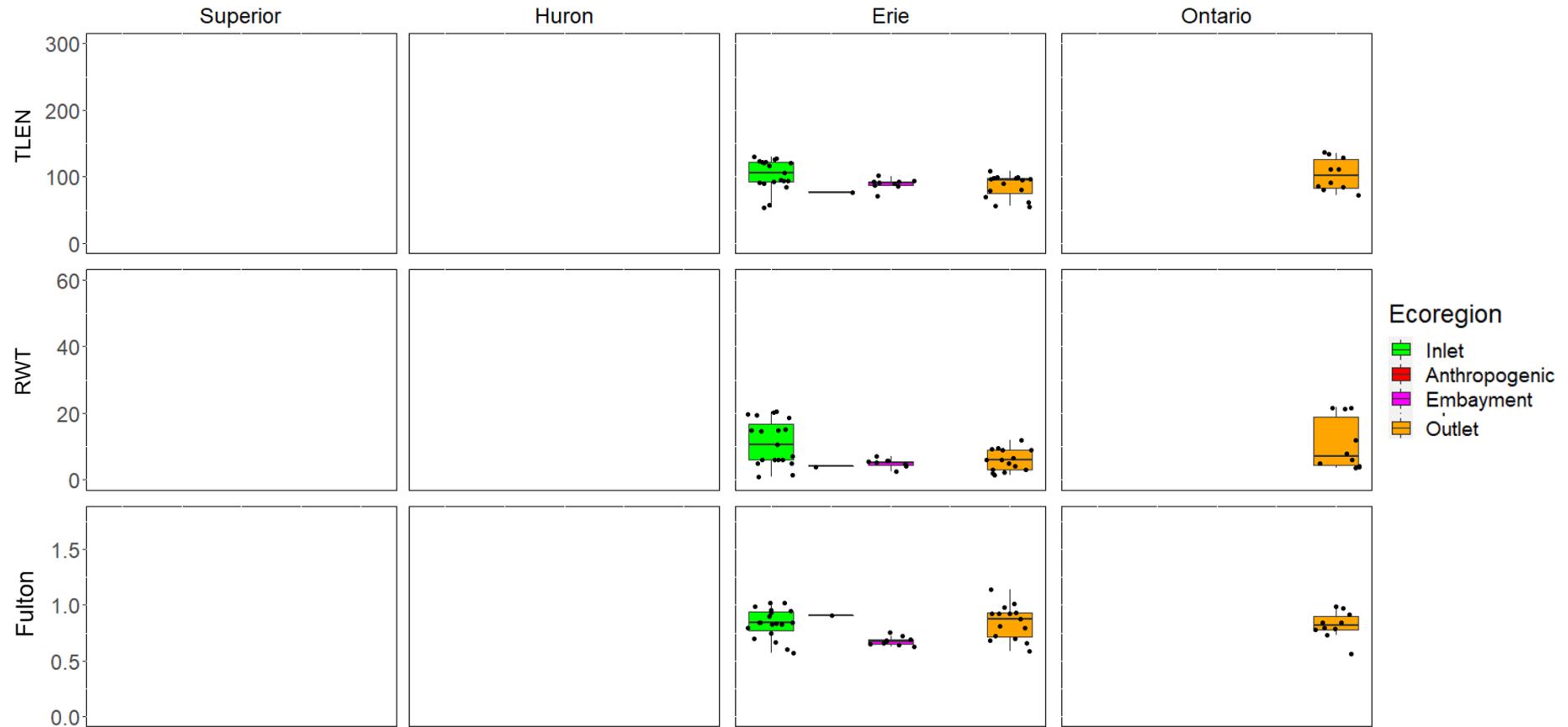
A2.2. Total length (TLEN; mm), round weight (RWT; g), and Fulton's K condition factor (Fulton) of cisco (*Coregonus artedi*) across 5 ecoregions (inlet, anthropogenic, embayment, open coastal, outlet) in lakes Superior, Huron, Erie, and Ontario. Box plot horizontal ends represent 25th and 75th percentiles, while mean values are represented with a horizontal line.



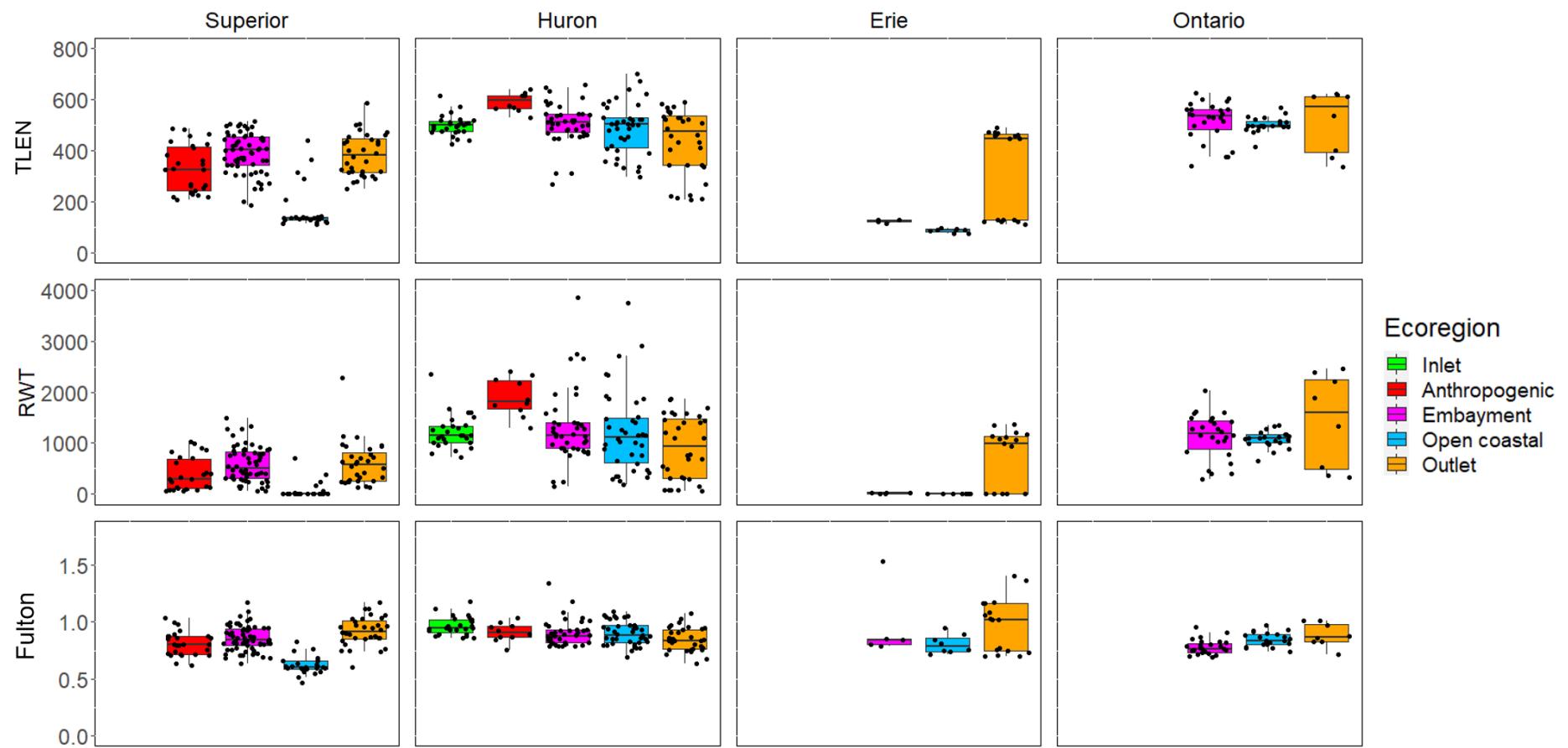
A2.3. Total length (TLEN; mm), round weight (RWT; g), and Fulton's K condition factor (Fulton) of rainbow smelt (*Osmerus mordax*) across 5 ecoregions (inlet, anthropogenic, embayment, open coastal, outlet) in lakes Superior, Huron, Erie, and Ontario. Box plot horizontal ends represent 25th and 75th percent quartiles, while mean values are represented with a horizontal line.



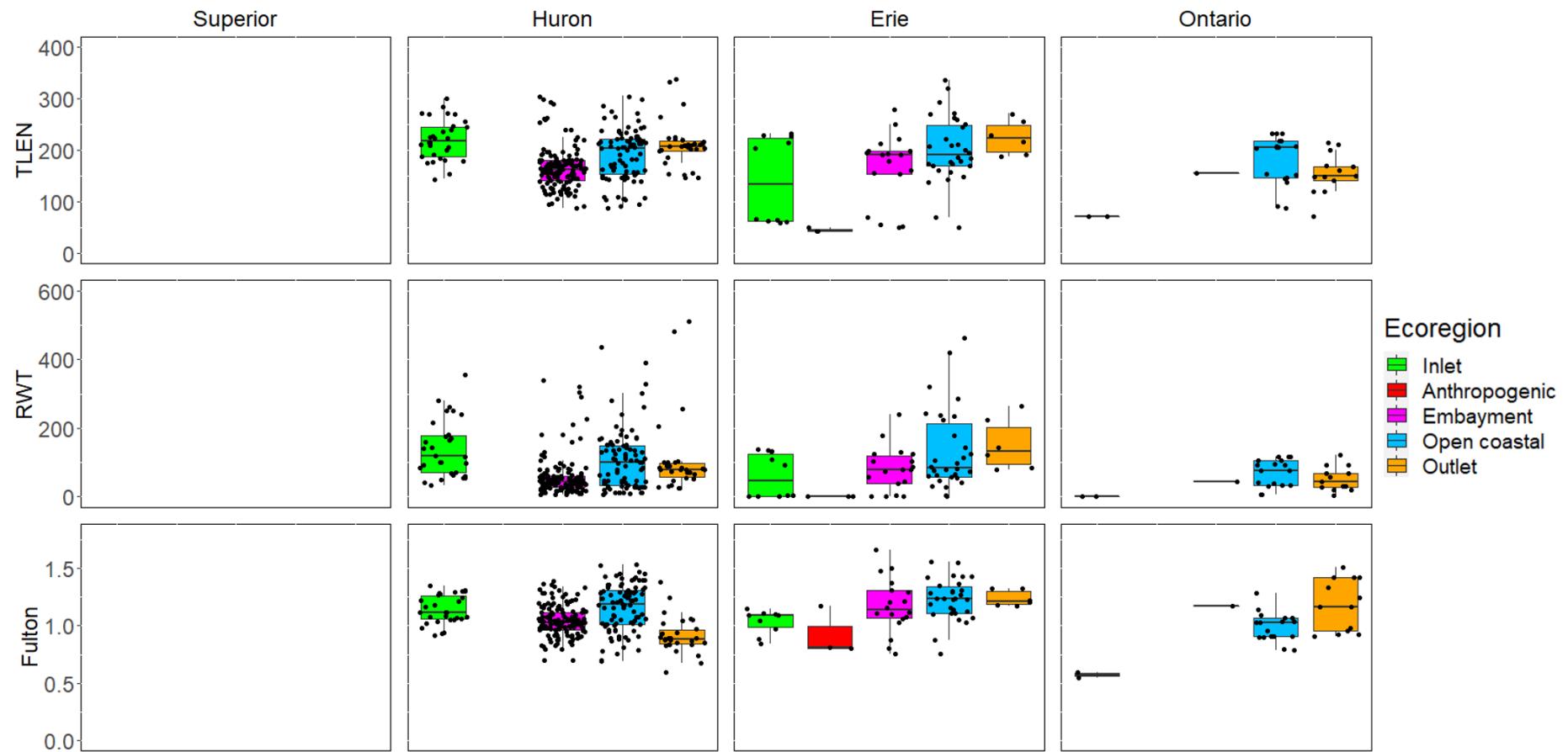
A2.4. Total length (TLEN; mm), round weight (RWT; g), and Fulton's K condition factor (Fulton) of lake trout (*Salvelinus namaycush*) across 5 ecoregions (inlet, anthropogenic, embayment, open coastal, outlet) in lakes Superior, Huron, Erie, and Ontario. Box plot horizontal ends represent 25th and 75th percent quartiles, while mean values are represented with a horizontal line.



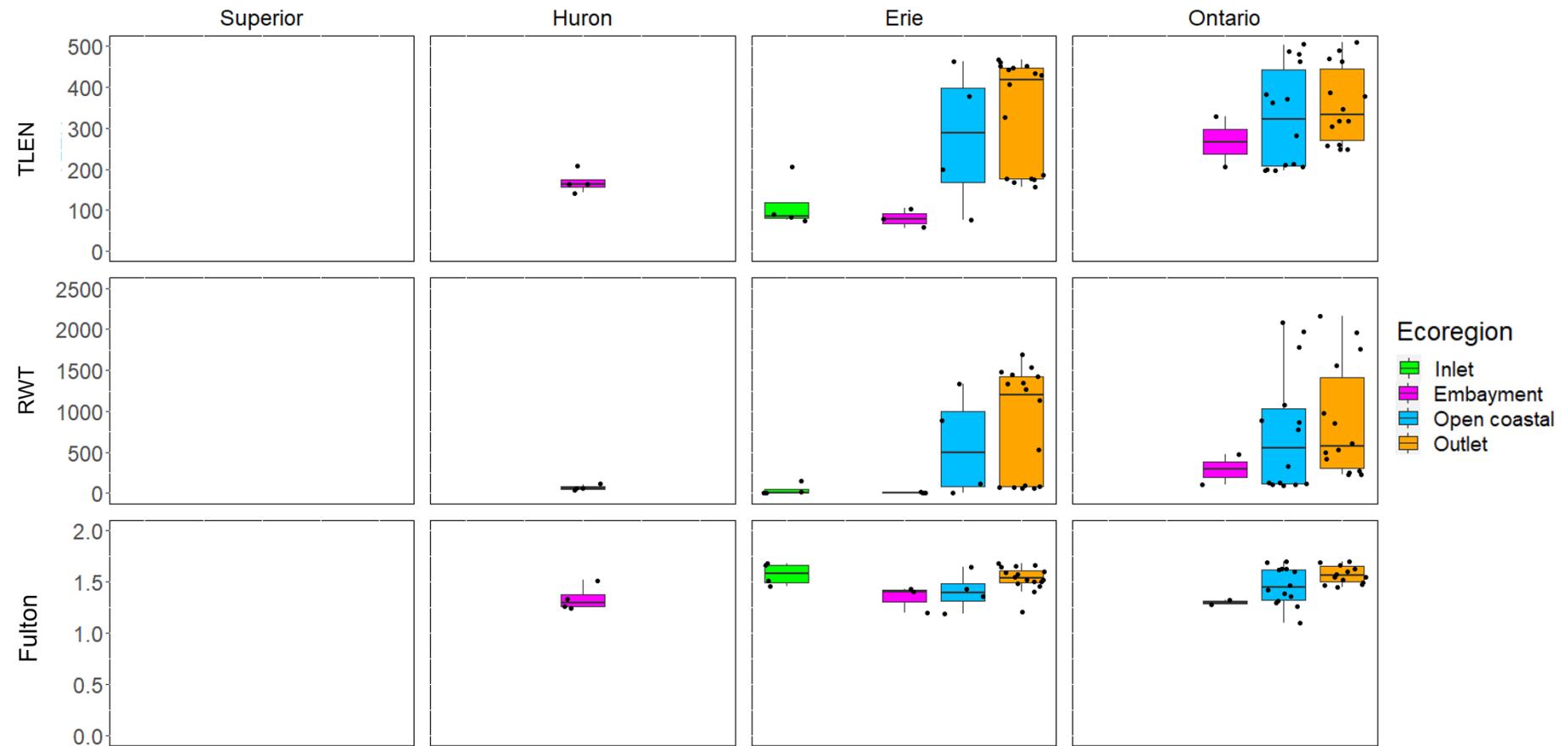
A2.5. Total length (TLEN; mm), round weight (RWT; g), and Fulton's K condition factor (Fulton) of *Notropis* spp. (emerald shiner (*Notropis atherinoides*) and spottail shiner (*Notropis hudsonius*)) across 5 ecoregions (inlet, anthropogenic, embayment, open coastal, outlet) in lakes Superior, Huron, Erie, and Ontario. Box plot horizontal ends represent 25th and 75th percent quartiles, while mean values are represented with a horizontal line.



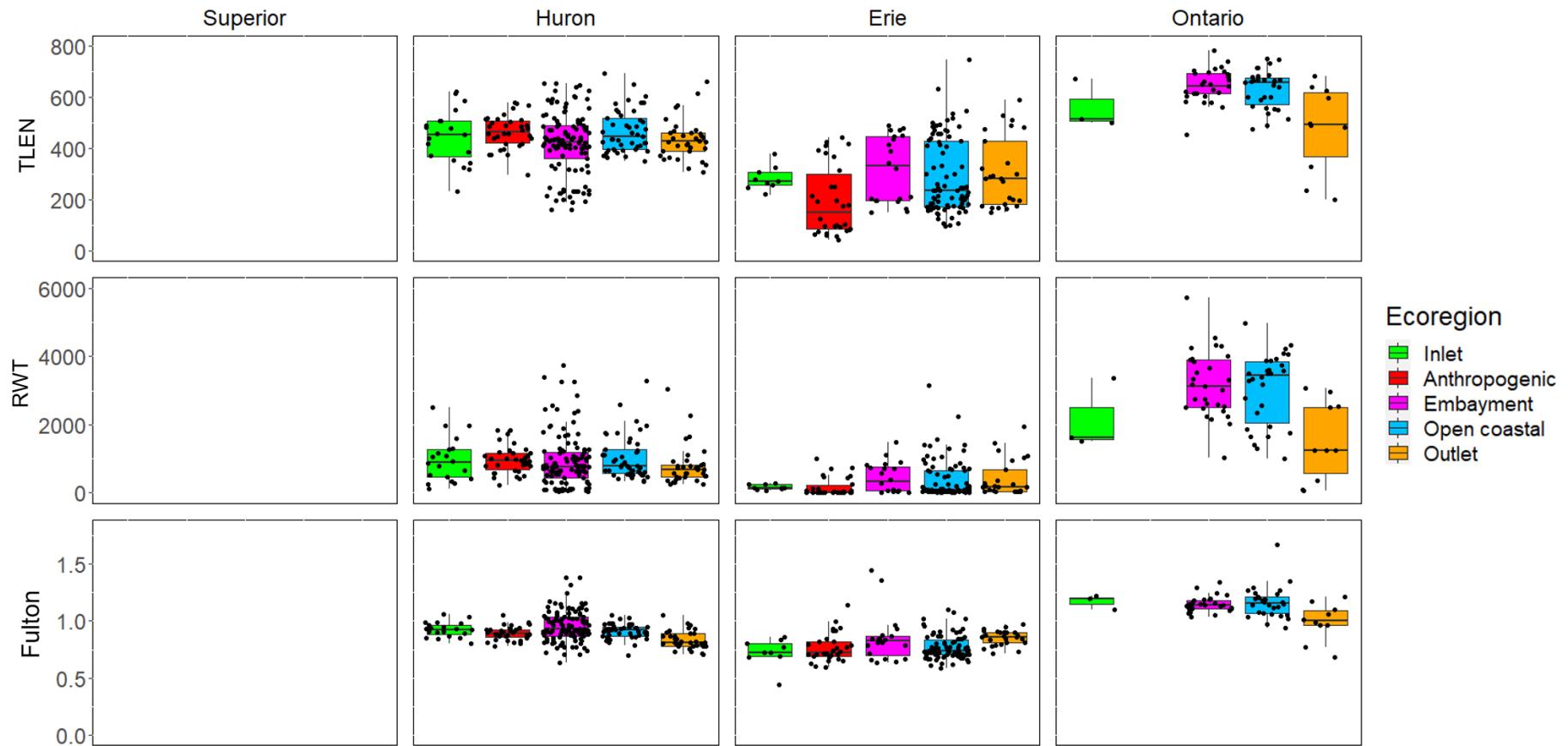
A2.6. Total length (TLEN; mm), round weight (RWT; g), and Fulton's K condition factor (Fulton) of lake whitefish (*Coregonus clupeaformis*) across 5 ecoregions (inlet, anthropogenic, embayment, open coastal, outlet) in lakes Superior, Huron, Erie, and Ontario. Box plot horizontal ends represent 25th and 75th percent quartiles, while mean values are represented with a horizontal line.



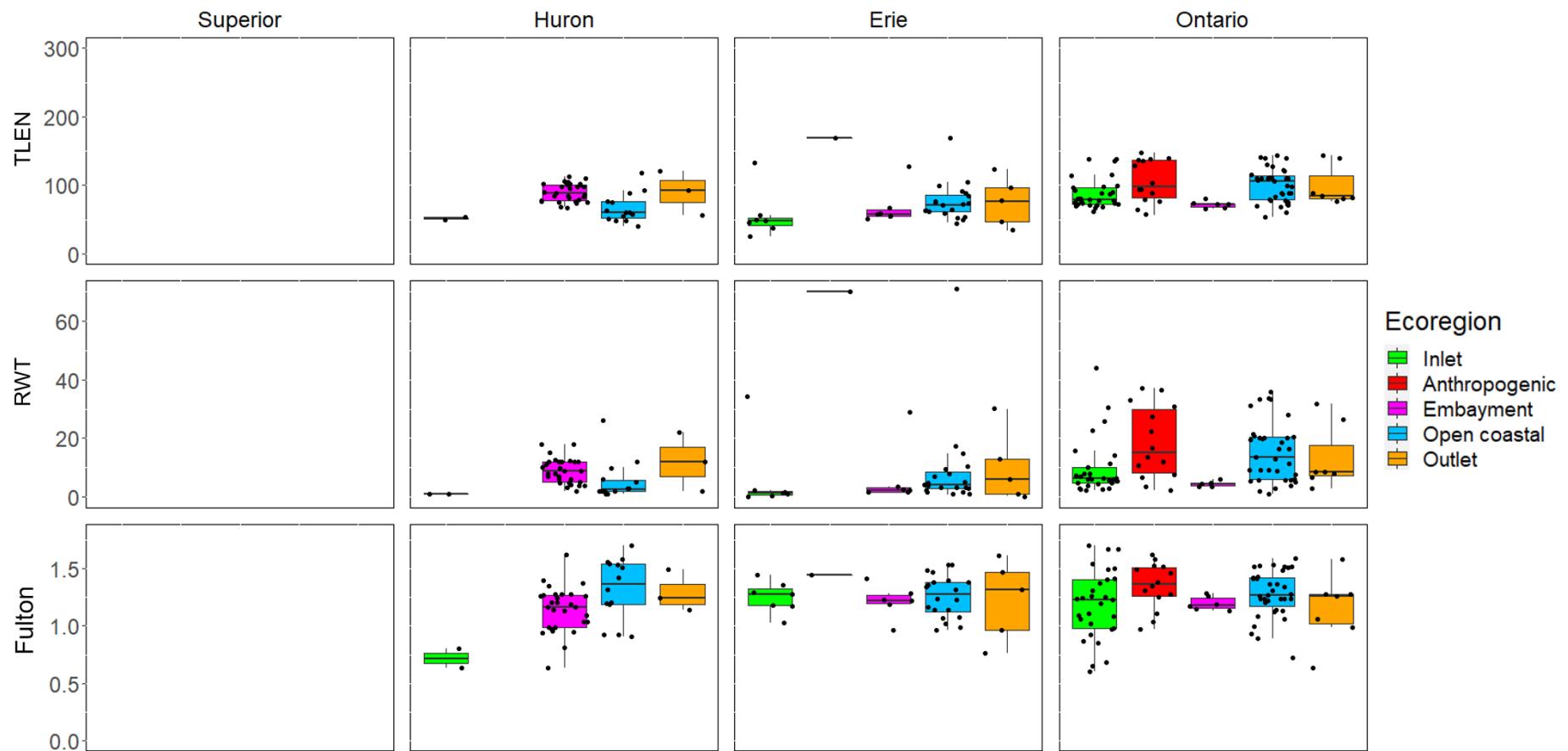
A2.7. Total length (TLEN; mm), round weight (RWT; g), and Fulton's K condition factor (Fulton) of yellow perch (*Perca flavescens*) across 5 ecoregions (inlet, anthropogenic, embayment, open coastal, outlet) in lakes Superior, Huron, Erie, and Ontario. Box plot horizontal ends represent 25th and 75th percentiles, while mean values are represented with a horizontal line.



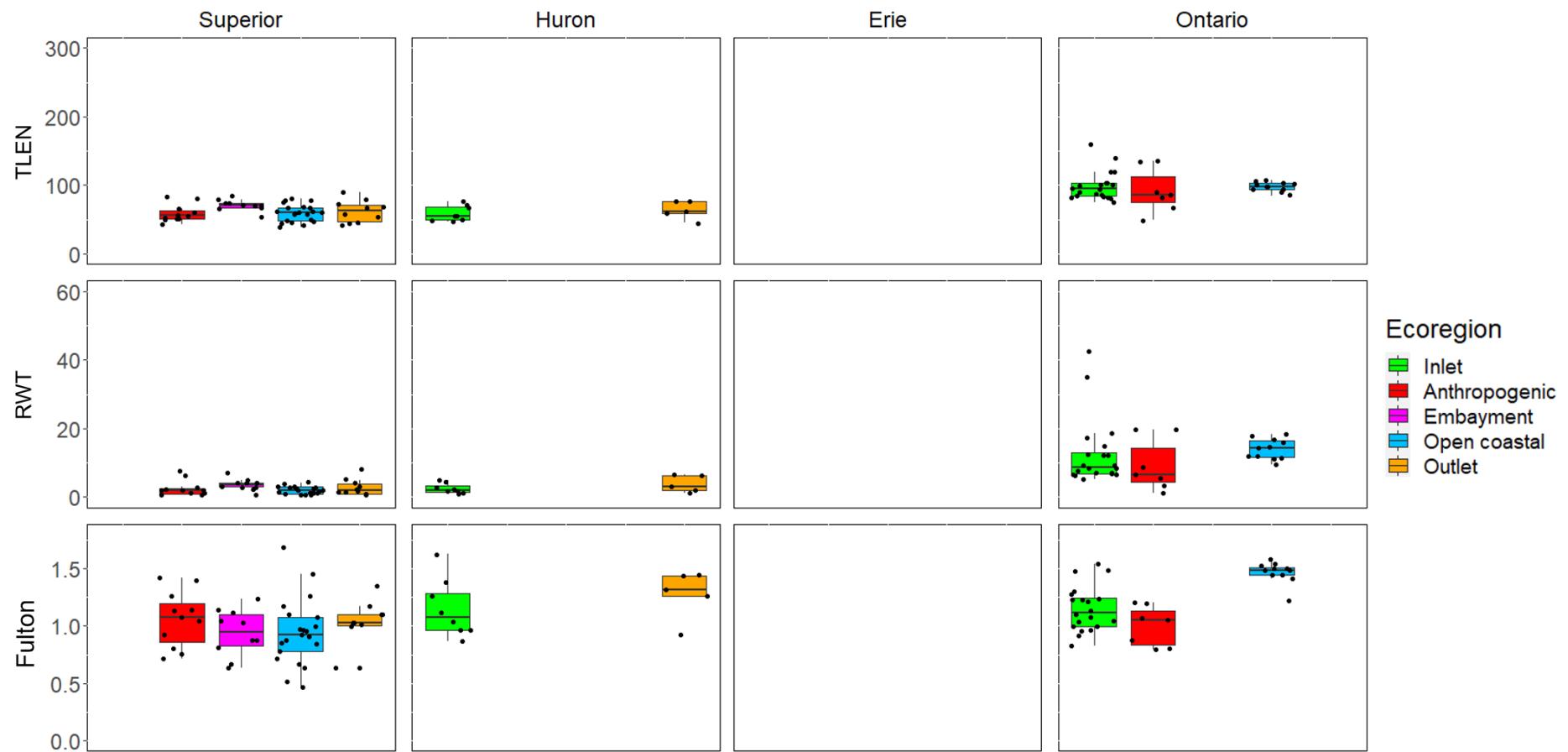
A2.8. Total length (TLEN; mm), round weight (RWT; g), and Fulton's K condition factor (Fulton) of smallmouth bass (*Micropterus dolomieu*) across 5 ecoregions (inlet, anthropogenic, embayment, open coastal, outlet) in lakes Superior, Huron, Erie, and Ontario. Box plot horizontal ends represent 25th and 75th percent quartiles, while mean values are represented with a horizontal line.



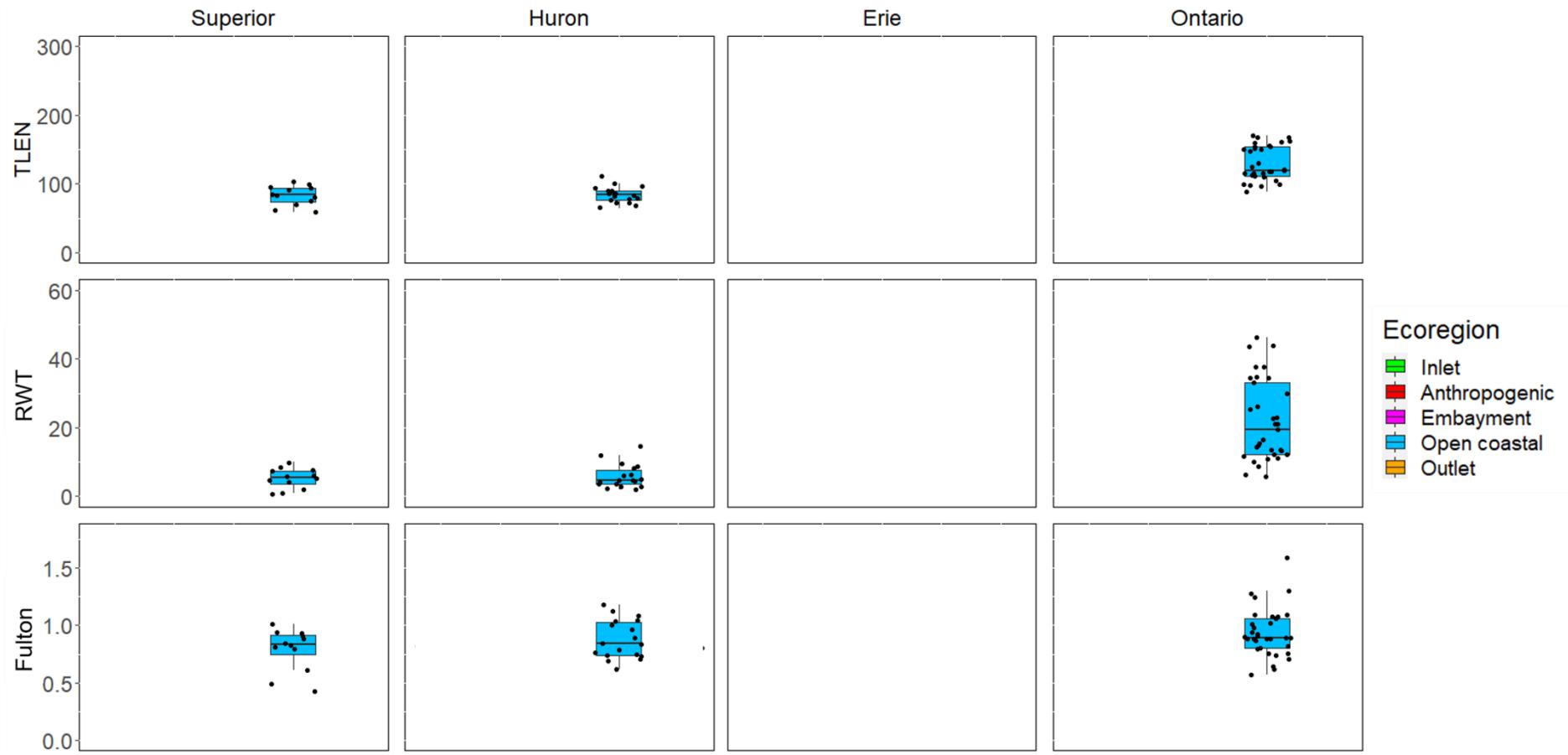
A2.9. Total length (TLEN; mm), round weight (RWT; g), and Fulton's K condition factor (Fulton) of walleye (*Sander vitreus*) across 5 ecoregions (inlet, anthropogenic, embayment, open coastal, outlet) in lakes Superior, Huron, Erie, and Ontario. Box plot horizontal ends represent 25th and 75th percent quartiles, while mean values are represented with a horizontal line.



A2.10. Total length (TLEN; mm), round weight (RWT; g), and Fulton's K condition factor (Fulton) of round goby (*Neogobius melanostomus*) across 5 ecoregions (inlet, anthropogenic, embayment, open coastal, outlet) in lakes Superior, Huron, Erie, and Ontario. Box plot horizontal ends represent 25th and 75th percent quartiles, while mean values are represented with a horizontal line.



A2.11. Total length (TLEN; mm), round weight (RWT; g), and Fulton's K condition factor (Fulton) of slimy sculpin (*Cottus cognatus*) across 5 ecoregions (inlet, anthropogenic, embayment, open coastal, outlet) in lakes Superior, Huron, Erie, and Ontario. Box plot horizontal ends represent 25th and 75th percent quartiles, while mean values are represented with a horizontal line.



A2.12. Total length (TLEN; mm), round weight (RWT; g), and Fulton's K condition factor (Fulton) of deepwater sculpin (*Myoxocephalus thompsonii*) across 5 ecoregions (inlet, anthropogenic, embayment, open coastal, outlet) in lakes Superior, Huron, Erie, and Ontario. Box plot horizontal ends represent 25th and 75th percent quartiles, while mean values are represented with a horizontal line.

Appendix 3. Bioaccumulative metrics

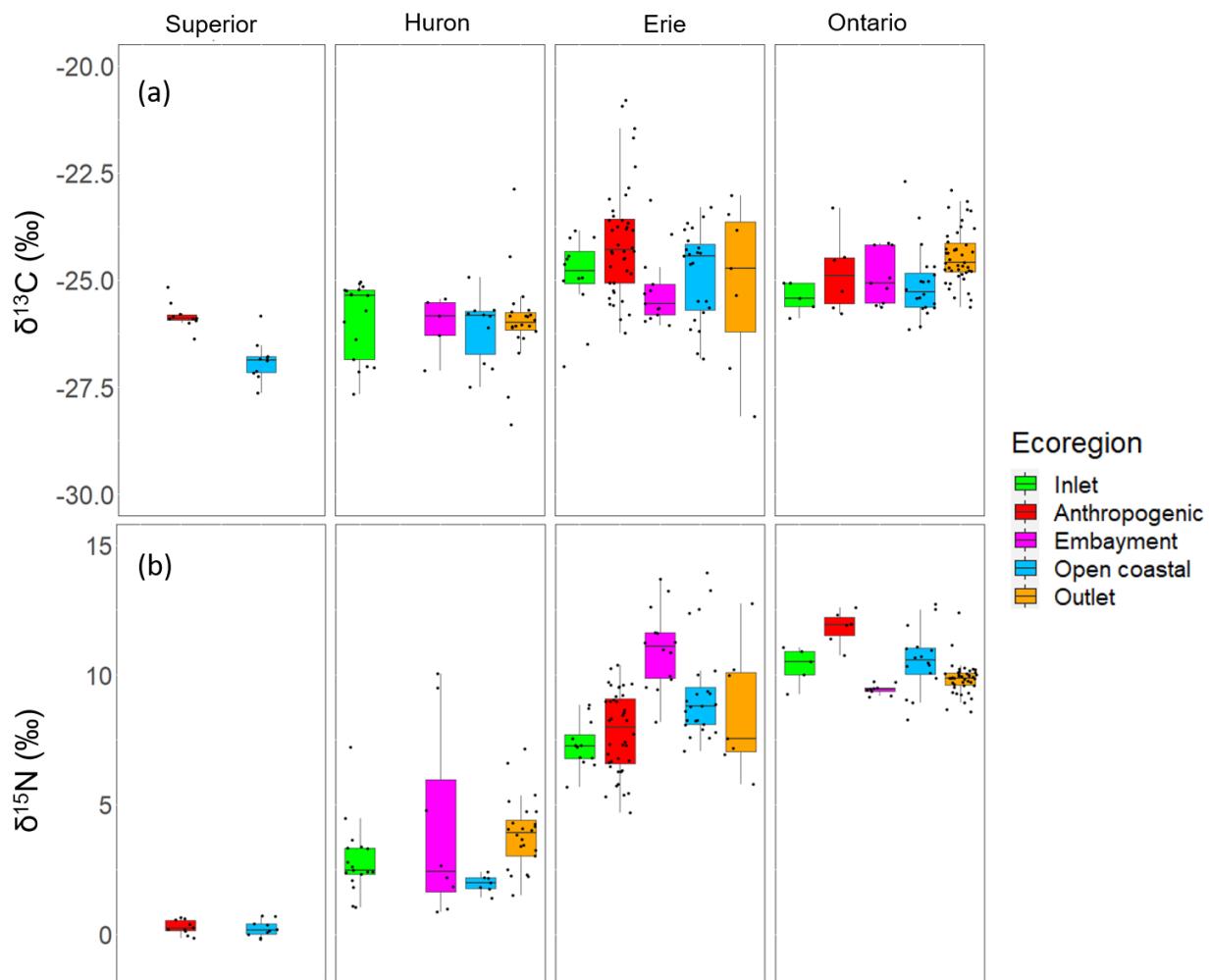
A3.1. Warmwater trophic magnification slopes (TMS) calculated using total log₁₀Hg (mercury) and δ₁₅N (nitrogen) stable isotope values for benthopelagic — emerald shiner (*Notropis atherinoides*), yellow perch (*Perca flavescens*), smallmouth bass (*Micropterus dolomieu*), and walleye (*Sander vitreus*) — and benthic — round goby (*Neogobius melanostomus*) — fish taxa across 5 ecoregions (inlet, anthropogenic, embayment, open coastal, outlet) in lakes (a) Superior (no warmwater fish collected), (b) Huron, (c) Erie, and (d) Ontario.

Lake/ecoregion	Regression equation	p (slope)	p (intercept)
Superior			
Inlet	NA	NA	NA
Anthropogenic	NA	NA	NA
Embayment	NA	NA	NA
Open coastal	NA	NA	NA
Outlet	NA	NA	NA
Huron			
Inlet	y = 0.26x + 0.02	0.00*	0.48
Anthropogenic	y = -0.04x + 3.25	0.71	0.08
Embayment	y = 0.15x + 1.09	0.00*	0.00*
Open coastal	y = 0.08x + 1.45	0.08	0.00*
Outlet	y = 0.01x + 1.97	0.53	0.00*
Erie			
Inlet	y = 0.11x + 1.09	0.08	0.01*
Anthropogenic	y = 0.30x - 0.24	0.01*	0.63
Embayment	y = 0.13x + 1.28	0.03*	0.00*
Open coastal	y = 0.10x + 1.34	0.01*	0.00*
Outlet	y = 0.27x - 0.08	0.00*	0.85
Ontario			
Inlet	y = 0.06x + 1.34	0.73	0.10
Anthropogenic	y = 0.10x + 1.16	0.49	0.11
Embayment	y = 0.25x + 0.81	0.03*	0.19
Open coastal	y = 0.21x + 1.06	0.00*	0.00*
Outlet	y = -0.09x + 2.17	0.61	0.02*

A3.2. Coldwater trophic magnification slopes (TMS) calculated using total log₁₀Hg (mercury) and δ¹⁵N (nitrogen) stable isotope values for pelagic — alewife (*Alosa pseudoharengus*), cisco (*Coregonus artedi*), deepwater sculpin (*Myoxocephalus thompsonii*), slimy sculpin (*Cottus cognatus*), rainbow smelt (*Osmerus mordax*), and lake trout (*Salvelinus namaycush*) — fish taxa across 5 ecoregions (inlet, anthropogenic, embayment, open coastal, outlet) in lakes (a) Superior (no warmwater fish collected), (b) Huron, (c) Erie, and (d) Ontario.

Lake/ecoregion	Regression equation	p (slope)	p (intercept)
Superior			
Inlet	NA	NA	NA
Anthropogenic	y = 0.15x + 1.18	0.00*	0.00*
Embayment	y = 0.10x + 1.69	0.00*	0.00*
Open coastal	y = 0.11x + 1.40	0.00*	0.00*
Outlet	y = 0.15x + 1.27	0.00*	0.00*
Huron			
Inlet	y = 0.03x + 1.50	0.14	0.00*
Anthropogenic	NA	NA	NA
Embayment	y = 0.06x + 1.38	0.00*	0.00*
Open coastal	y = 0.07x + 1.27	0.00*	0.02*
Outlet	y = 0.10x + 1.22	0.07	0.00*
Erie			
Inlet	NA	NA	NA
Anthropogenic	NA	NA	NA
Embayment	y = 0.06x + 1.49	0.22	0.00*
Open coastal	y = 0.39x - 1.50	0.02*	0.28
Outlet	y = 0.31x - 0.68	0.40	0.74
Ontario			
Inlet	y = 0.05x + 1.56	0.01*	0.00*
Anthropogenic	y = 0.01x + 1.70	0.80	0.00*
Embayment	y = 0.05x + 1.49	0.18	0.00*
Open coastal	y = 0.07x + 1.50	0.00*	0.00*
Outlet	y = 0.18x + 0.79	0.00*	0.00*

Appendix 4. Baseline stable isotopes



A4.1. Spread of Dreissenid (a) $\delta^{13}\text{C}$ and (b) $\delta^{15}\text{N}$ stable isotope values across 5 ecoregions (inlet, anthropogenic, embayment, open coastal, outlet) in lakes Superior, Huron, Erie, and Ontario. Values represented fell within the 10th and 90th percentile of data distribution.

A4.2. Results of Kruskal-Wallis tests on $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ ecoregion-specific Dreissenid values for lakes Superior, Huron, Erie, and Ontario.

Lake	$\delta^{13}\text{C}$				$\delta^{15}\text{N}$			
	n	χ^2	df	p	n	χ^2	df	p
Superior	20	NA	2 ^A	0*	20	NA	2 ^A	0.7
Huron	54	2.66	3	0.45	56	14.25	3	0*
Erie	95	10.05	4	0.04	97	32.18	4	0*
Ontario	83	18.97	4	0*	79	27.8	4	0*

^AFor Lake Superior, a pairwise Wilcox comparison test was used given only 2 ecoregions (Anthropogenic and Open coastal) to compare.

A4.3. Dunn test post-hoc comparisons ($p < \alpha/2$; $\alpha = 0.05$) of Kruskal-Wallis findings from A4.1 for lakes Huron, Erie, and Ontario $\delta^{13}\text{C}$ values. Significant post-hoc comparisons were denoted with a (*).

Post-hoc comparison pairings by lake	z	p
Lake Huron		
Embayment-Inlet	0.81	1.00
Embayment-Open coastal	1.41	0.47
Embayment-Outlet	1.41	0.47
Inlet-Open coastal	0.79	1.00
Inlet-Outlet	0.71	1.00
Open coastal-Outlet	0.24	1.00
Lake Erie		
Anthropogenic-Embayment	2.61	0.04
Anthropogenic-Inlet	1.85	0.32
Anthropogenic-Open coastal	2.25	0.12
Anthropogenic-Outlet	1.18	1.00
Embayment-Inlet	0.51	1.00
Embayment-Open coastal	0.68	1.00
Embayment-Outlet	0.71	1.00
Inlet-Open coastal	0.08	1.00
Inlet-Outlet	0.26	1.00
Open coastal-Outlet	0.23	1.00
Lake Ontario		
Anthropogenic-Embayment	0.02	1.00
Anthropogenic-Inlet	1.29	1.00
Anthropogenic-Open coastal	0.69	1.00
Anthropogenic-Outlet	1.44	0.73
Embayment-Inlet	1.39	0.82
Embayment-Open coastal	0.76	1.00
Embayment-Outlet	1.75	0.39
Inlet-Open coastal	0.92	1.00
Inlet-Outlet	3.00	0.01*
Open coastal-Outlet	3.52	0.00*

A4.4. Dunn test post-hoc comparisons ($p < \alpha/2$; $\alpha = 0.05$) of Kruskal-Wallis findings from A4.1 for Lake Huron, Erie, and Ontario $\delta^{15}\text{N}$ values. Significant post-hoc comparisons were denoted with a (*).

Post-hoc comparison pairings by lake	Z	p
Lake Huron		
Embayment-Inlet	0.25	1.00
Embayment-Open coastal	1.81	0.21
Embayment-Outlet	1.45	0.43
Inlet-Open coastal	1.84	1.19
Inlet-Outlet	2.22	0.08
Open coastal-Outlet	3.56	0.001*
Lake Erie		
Anthropogenic-Embayment	4.80	0*
Anthropogenic-Inlet	1.19	1.00
Anthropogenic-Open coastal	2.59	0.05
Anthropogenic-Outlet	0.80	1.00
Embayment-Inlet	4.79	0*
Embayment-Open coastal	2.44	0.07
Embayment-Outlet	2.51	0.06
Inlet-Open coastal	3.00	0.01*
Inlet-Outlet	1.52	0.64
Open coastal-Outlet	0.79	1.00
Lake Ontario		
Anthropogenic-Embayment	4.54	0*
Anthropogenic-Inlet	1.62	0.51
Anthropogenic-Open coastal	1.89	0.29
Anthropogenic-Outlet	3.75	0.00*
Embayment-Inlet	2.52	0.06
Embayment-Open coastal	3.57	0.00*
Embayment-Outlet	2.07	0.19
Inlet-Open coastal	0.16	1.00
Inlet-Outlet	1.37	0.84
Open coastal-Outlet	2.48	0.06

Appendix 5. Global mean morphometrics and ecological metrics

A5.1. Proportion of ecoregions where mean total length (TLEN) deviated more than ± 1 SD from the TLEN global mean (grouped by (a) Great Lake and (b) ecoregion) for each species. Proportions in italic font represent instances where mean TLEN never deviated from the global mean, proportions highlighted in yellow represent mean TLEN deviating from the global mean more than half the time, and proportions highlighted in green (bold font) represent mean TLEN always deviating from the global mean.

(a)	Superior	Huron	Erie	Ontario	sum	(b)	Superior	Huron	Erie	Ontario	Sum
Alewife	N/A	0/2	1/1	0/4	1/7	Inlet	N/A	2/9	0/5	2/8	3/22
Cisco	1/4	2/4	N/A	1/4	4/12	Anthropogenic	1/5	1/2	2/4	0/5	4/16
Rainbow smelt	0/4	0/4	1/4	0/5	1/17	Embayment	0/5	2/9	2/8	1/8	5/30
Lake trout	1/4	1/4	0/2	0/5	2/15	Open coastal	2/6	1/8	1/7	3/11	7/32
<i>Notropis</i> spp.	N/A	N/A	0/4	0/1	0/5	Outlet	0/5	0/8	1/8	0/10	1/31
Lake whitefish	1/4	1/5	2/3	0/3	4/15	sum	3/21	6/36	6/32	6/42	21/131
Yellow perch	N/A	0/4	0/4	1/4	1/12						
Smallmouth bass	N/A	1/1	1/4	0/3	2/8						
Walleye	N/A	0/5	0/5	2/4	2/14						
Round goby	N/A	1/4	1/5	0/5	2/14						
Slimy sculpin	0/4	0/2	N/A	2/3	2/9						
Deepwater sculpin	0/1	0/1	N/A	0/1	0/3						
sum	3/21	6/36	6/32	6/42	21/131						

A5.2. Proportion of ecoregions where mean round weight (RWT) deviated more than ± 1 SD from the RWT global mean (grouped by (a) Great Lake and (b) ecoregion) for each species. Proportions in italic font represent instances where mean RWT never deviated from the global mean, proportions highlighted in yellow represent mean RWT deviating from the global mean more than half the time, and proportions highlighted in green (bold font) represent mean RWT always deviating from the global mean.

(a)	Superior	Huron	Erie	Ontario	sum	(b)	Superior	Huron	Erie	Ontario	Sum
Alewife	N/A	0/2	1/1	0/4	1/7	Inlet	N/A	2/9	0/5	1/8	3/22
Cisco	0/4	2/4	N/A	1/4	3/12	Anthropogenic	0/5	1/2	1/4	0/5	2/16
Rainbow smelt	0/4	0/4	0/4	0/5	0/17	Embayment	0/5	0/9	2/8	1/8	3/30
Lake trout	0/4	1/4	0/2	0/5	1/15	Open coastal	1/6	1/8	1/7	2/11	5/32
<i>Notropis</i> spp.	N/A	N/A	0/4	0/1	0/5	Outlet	0/5	0/8	1/8	1/10	2/31
Lake whitefish	1/4	1/5	2/3	1/3	5/15	sum	1/21	4/36	5/32	5/42	15/131
Yellow perch	N/A	0/4	0/4	1/4	1/12						
Smallmouth bass	N/A	0/1	1/4	0/3	1/8						
Walleye	N/A	0/5	0/5	2/4	2/14						
Round goby	N/A	0/4	1/5	0/5	1/14						
Slimy sculpin	0/4	0/2	N/A	0/3	0/9						
Deepwater sculpin	0/1	0/1	N/A	0/1	0/3						
sum	1/21	4/36	5/32	5/42	15/131						

A5.3. Proportion of ecoregions where mean Fulton's K condition factor (K) deviated more than ± 1 SD from the K global mean (grouped by (a) Great Lake and (b) ecoregion) for each species. Proportions in italic font represent instances where mean K never deviated from the global mean, proportions highlighted in yellow represent mean K deviating from the global mean more than half the time, and proportions highlighted in green (bold font) represent mean K always deviating from the global mean.

(a)	Superior	Huron	Erie	Ontario	sum	(b)	Superior	Huron	Erie	Ontario	Sum
Alewife	N/A	0/2	1/1	1/4	2/7	Inlet	N/A	2/9	2/5	2/8	6/22
Cisco	0/4	1/4	N/A	2/4	3/12	Anthropogenic	1/5	0/2	0/4	0/5	1/15
Rainbow smelt	0/4	1/4	0/4	0/5	1/17	Embayment	0/5	1/9	0/8	3/8	4/30
Lake trout	1/4	0/4	0/2	0/5	1/15	Open coastal	1/6	1/8	0/7	3/11	5/32
<i>Notropis</i> spp.	N/A	N/A	0/4	0/1	0/5	Outlet	0/5	0/8	1/8	1/10	2/31
Lake whitefish	1/4	0/5	0/3	0/3	1/15	sum	2/21	4/36	3/31	9/42	18/131
Yellow perch	N/A	0/4	0/4	1/4	1/12						
Smallmouth bass	N/A	1/1	1/4	1/3	3/8						
Walleye	N/A	0/5	1/5	3/4	4/14						
Round goby	N/A	1/4	0/5	0/5	1/14						
Slimy sculpin	0/4	0/2	N/A	1/3	1/9						
Deepwater sculpin	0/1	0/1	N/A	0/1	0/3						
sum	2/21	4/36	3/32	9/42	18/131						

A5.4. Proportion of ecoregions where mean absolute $\delta^{13}\text{C}$ deviated more than ± 1 SD from the absolute $\delta^{13}\text{C}$ global mean (grouped by (a) Great Lake and (b) ecoregion) for each species. Proportions in italic font represent instances where mean absolute $\delta^{13}\text{C}$ never deviated from the global mean, proportions highlighted in yellow represent mean absolute $\delta^{13}\text{C}$ deviating from the global mean more than half the time, and proportions highlighted in green (bold font) represent mean absolute $\delta^{13}\text{C}$ always deviating from the global mean.

(a)	Superior	Huron	Erie	Ontario	sum	(b)	Superior	Huron	Erie	Ontario	Sum
Alewife	N/A	2/2	0/1	0/4	2/7	Inlet	N/A	3/9	0/5	2/8	5/22
Cisco	0/4	2/4	N/A	2/4	4/12	Anthropogenic	2/5	0/1	2/3	1/5	5/14
Rainbow smelt	0/4	0/4	0/3	3/5	3/16	Embayment	2/5	3/9	2/8	0/8	7/30
Lake trout	3/4	0/4	0/2	0/5	3/15	Open coastal	2/6	1/8	0/7	1/11	4/32
<i>Notropis</i> spp.	N/A	N/A	1/4	0/1	1/5	Outlet	0/5	0/8	1/8	2/10	3/31
Lake whitefish	1/4	2/4	1/3	1/3	5/14	sum	6/21	7/35	5/31	6/42	24/129
Yellow perch	N/A	0/4	0/4	0/4	0/12						
Smallmouth bass	N/A	1/1	1/4	0/3	2/8						
Walleye	N/A	0/5	0/5	0/4	0/14						
Round goby	N/A	1/4	2/5	0/5	3/14						
Slimy sculpin	1/4	0/2	N/A	0/3	1/9						
Deepwater sculpin	1/1	0/1	N/A	0/1	1/3						
sum	6/21	8/35	5/31	6/42	25/129						

A5.5. Proportion of ecoregions where mean absolute $\delta^{15}\text{N}$ deviated more than ± 1 SD from the absolute $\delta^{15}\text{N}$ global mean (grouped by (a) Great Lake and (b) ecoregion) for each species. Proportions in italic font represent instances where mean absolute $\delta^{15}\text{N}$ never deviated from the global mean, proportions highlighted in yellow represent mean absolute $\delta^{15}\text{N}$ deviating from the global mean more than half the time, and proportions highlighted in green (bold font) represent mean absolute $\delta^{15}\text{N}$ always deviating from the global mean.

(a)	Superior	Huron	Erie	Ontario	sum	(b)	Superior	Huron	Erie	Ontario	Sum
Alewife	N/A	2/2	1/1	0/4	3/7	Inlet	N/A	3/9	0/5	2/8	5/22
Cisco	1/4	0/4	N/A	3/4	4/12	Anthropogenic	2/5	0/1	1/3	2/5	5/14
Rainbow smelt	4/4	0/4	3/3	5/5	12/16	Embayment	2/5	4/9	3/8	4/8	13/30
Lake trout	4/4	0/4	2/2	1/5	7/15	Open coastal	6/6	1/8	4/7	6/11	16/32
<i>Notropis</i> spp.	N/A	N/A	2/4	0/1	2/5	Outlet	2/5	2/8	4/8	4/10	12/31
Lake whitefish	1/4	0/4	2/3	3/3	6/14	sum	12/21	10/35	12/31	18/42	52/129
Yellow perch	N/A	1/4	2/4	3/4	6/12						
Smallmouth bass	N/A	1/1	0/4	0/3	1/8						
Walleye	N/A	2/5	0/5	1/4	3/14						
Round goby	N/A	4/4	0/5	0/5	4/14						
Slimy sculpin	1/4	0/2	N/A	1/3	2/9						
Deepwater sculpin	1/1	0/1	N/A	1/1	2/3						
sum	12/21	10/35	12/31	18/42	52/129						

A5.6. Proportion of ecoregions where mean relative $\delta^{13}\text{C}$ deviated more than ± 1 SD from the relative $\delta^{13}\text{C}$ global mean (grouped by (a) Great Lake and (b) ecoregion) for each species. Proportions in italic font represent instances where mean relative $\delta^{13}\text{C}$ never deviated from the global mean, proportions highlighted in yellow represent mean relative $\delta^{13}\text{C}$ deviating from the global mean more than half the time, and proportions highlighted in green (bold font) represent mean relative $\delta^{13}\text{C}$ always deviating from the global mean.

(a)	Superior	Huron	Erie	Ontario	sum	(b)	Superior	Huron	Erie	Ontario	Sum
Alewife	N/A	1/2	0/1	0/4	1/7	Inlet	N/A	4/9	0/5	1/8	5/22
Cisco	0/4	1/4	N/A	2/4	3/12	Anthropogenic	0/5	0/1	2/3	0/5	2/14
Rainbow smelt	0/4	0/4	0/3	0/5	0/16	Embayment	1/5	2/9	1/8	0/8	4/30
Lake trout	0/4	0/4	0/2	0/5	0/15	Open coastal	0/6	2/8	0/7	1/11	3/32
<i>Notropis</i> spp.	N/A	N/A	1/4	0/1	1/5	Outlet	0/5	0/8	2/8	0/10	2/31
Lake whitefish	0/4	2/4	1/3	0/3	3/14	sum	1/21	8/35	5/31	2/42	16/129
Yellow perch	N/A	0/4	1/4	0/4	1/12						
Smallmouth bass	N/A	1/1	0/4	0/3	1/8						
Walleye	N/A	1/5	0/5	0/4	1/14						
Round goby	N/A	2/4	2/5	0/5	4/14						
Slimy sculpin	1/4	0/2	N/A	0/3	1/9						
Deepwater sculpin	0/1	0/1	N/A	0/1	0/3						
sum	1/21	8/35	5/31	2/42	16/129						

A5.7. Proportion of ecoregions where mean relative $\delta^{15}\text{N}$ deviated more than ± 1 SD from the relative $\delta^{15}\text{N}$ global mean (grouped by (a) Great Lake and (b) ecoregion) for each species. Proportions in italic font represent instances where mean relative $\delta^{15}\text{N}$ never deviated from the global mean, proportions highlighted in yellow represent mean relative $\delta^{15}\text{N}$ deviating from the global mean more than half the time, and proportions highlighted in green (bold font) represent mean relative $\delta^{15}\text{N}$ always deviating from the global mean.

(a)	Superior	Huron	Erie	Ontario	sum	(b)	Superior	Huron	Erie	Ontario	Sum
Alewife	N/A	2/2	1/1	1/4	4/7	Inlet	N/A	5/9	0/5	2/8	7/22
Cisco	0/4	1/4	N/A	0/4	1/12	Anthropogenic	2/5	1/1	1/3	4/5	8/14
Rainbow smelt	0/4	1/4	0/3	5/5	6/16	Embayment	0/5	1/9	2/8	1/8	9/30
Lake trout	1/4	2/4	1/2	2/5	6/15	Open coastal	1/6	5/8	0/7	5/11	11/32
<i>Notropis</i> spp.	N/A	N/A	2/4	1/1	3/5	Outlet	0/5	2/8	2/8	2/10	6/31
Lake whitefish	0/4	1/4	1/3	1/3	3/14	sum	3/21	14/35	10/31	14/42	41/129
Yellow perch	N/A	1/4	1/4	1/4	3/12						
Smallmouth bass	N/A	0/1	2/4	0/3	2/8						
Walleye	N/A	3/5	1/5	1/4	5/14						
Round goby	N/A	1/4	1/5	0/5	2/14						
Slimy sculpin	1/4	1/2	N/A	2/3	4/9						
Deepwater sculpin	1/1	1/1	N/A	0/1	2/3						
sum	3/21	14/35	10/31	14/42	41/129						

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