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Ontogenetic variation in isotopic niche positions of aquatic consumers in boreal lakes

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Abstract

We used an extensive multispecies data set to examine how C and N stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) of aquatic consumers varied with body size in populations of northern lakes. Ontogenetic variation in tissue $\delta^{13}C$ and $\delta^{15}N$ was evident in a diversity of aquatic consumers. Relationships with body size tended to be stronger and more consistently positive for $\delta^{15}N$, and more variable in nature for $\delta^{13}C$. Among-population variation in ontogenetic slopes was greater for $\delta^{13}C$ than for $\delta^{15}N$ for most biota examined. Relationships between $\delta^{13}C$ and $\delta^{15}N$ and body size were still significant even after accounting for variation due to tissue C:N and body condition. Ontogenetic variation was more strongly linked to age than body size in some fishes, particularly for $\delta^{15}N$, but age effects, inferred from growth rate, also accounted for variation in $\delta^{13}C$ and $\delta^{15}N$ beyond body size effects; $\delta^{13}C$ tended to be positively related and $\delta^{15}N$ tended to be negatively related to growth rate. There was only limited evidence of concordance in ontogenetic slopes between co-habiting pairs of species, suggesting that the factors driving ontogenetic variation in stable isotope ratios may be largely species- or population-specific. However, ontogenetic slopes of individual taxa were related to various lake habitat features representing climate, ecosystem size, and water clarity. The proportion of isotopic niche space attributable to ontogenetic variation can be substantial and this has implications for applying and interpreting isotopic niche metrics. Isotopic analyses of freshwater food webs need to account for ontogenetic variation in component taxa.

Keywords: ontogenetic niche, trophic ecology, aquatic ecology, food webs

Introduction

The ecological niche is a unifying concept in ecology and is most-commonly described as a 'hyper-volume' in habitat and resource dimensions that a species occupies in the environment (Hutchinson 1957; Vandermeer 1972; Leibold 1995). Phenotypic variation within a species influences its distribution and function within the ecosystem, and thus demarcates the niche that it could potentially occupy (termed *fundamental niche*). However, various environmental constraints acting on a particular population, such as physical barriers, predators and competitors, ultimately reduce niche size to a smaller hyper-volume (termed *realized niche*). The realized niche can be conceptualized as a flexible volume that can shift in position, size and shape within the more rigid fundamental niche (Hutchinson 1957; Soberón and Arroyo-Peña 2017). Characteristics of the realized niche can be examined at the level of the species, population or individual, and determining what factors influence the realized niche furthers our understanding of community and food web structure and eco-evolutionary dynamics.

The realized niche of an individual is dependent not only on the abiotic and biotic conditions of its environment but also on its developmental stage. In many organisms, the habitats and resources that are utilized change through life as the organism ages, grows, and matures (Polis 1984). The trajectory of the resultant shift in position, size and shape of the realized niche as an individual passes through its life has been coined the ontogenetic niche (Werner and Gilliam 1984). It is well-recognized that many ecological and physiological processes vary with body size, particularly in aquatic ecosystems (Peters 1983; Werner and Gilliam 1984; Miller et al. 1988; Clarke and Johnston 1999). The size and type of prey that aquatic consumers could potentially ingest broadens as their body size increases and gape limitations are relaxed. Thus, the trophic component of their realized niche may also be expected to shift with increasing body size. Trophic ecologies and food web positions of aquatic consumers are increasingly being inferred from the chemical composition of their bodies, including the stable isotope ratios of tissues. Stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) provide time-integrated indices of assimilated matter with respect to primary production source and trophic elevation, respectively (Peterson and Fry 1987; Fry 2006; Boecklen et al. 2011). Accordingly, ontogenetic niche shifts of aquatic consumers are now often inferred from changes in their isotopic composition with increasing body size (Hammerschlag-Peyer et al. 2011; Krumsick and Fisher 2019).

Research to date has indicated that ontogenetic variation in isotopic composition of freshwater fishes is relatively strong in some species and/or populations but not others (Vander Zanden et al. 2000; Overman and Parrish 2001; Genner et al. 2003; Swanson et al. 2003; Zimmerman et al. 2009; Cott et al. 2011; Persaud et al. 2012). Furthermore, the variation may be more closely aligned with age in some species (Overman and Parrish 2001) but body size in others (Jardine and Curry 2006). Ontogenetic shifts in stable isotope compositions of consumers could be driven by changes in diet and/or food processing; as they develop, organisms may consume different things in different places, but they may also assimilate, metabolize, and allocate the ingested energy and nutrients differently (Shipley and Matich 2020). Regardless of the relative importance of these two factors, a broader and more comprehensive analysis of ontogenetic variation in stable isotope ratios of freshwater consumers would be beneficial to understanding the generality of these patterns, and potentially, some of the factors influencing them. Failure to properly account for ontogenetic variation could confound interpretation of isotopic compositions at other spatial or temporal scales, with far-reaching implications for research and management applications.

We leveraged a large database of stable isotope ratios, body size, and growth indices for 15 fish species and three macroinvertebrate taxa, representing more than 1000 populations from waterbodies in Canada's boreal shield region, to investigate broad patterns in ontogenetic niche shifts for key freshwater consumers. We had four major objectives in conducting this research. First, we wanted to quantify the nature, strength, and prevalence of size-based variation in C and N isotopic composition for a range of aquatic biota of varying trophic ecologies. This addressed the question of which species or feeding guilds exhibit stronger or more consistent ontogenetic variation in $\delta^{13}C$ and $\delta^{15}N$ than others. Second, we wanted to determine if age or growth may be stronger ontogenetic covariates than body size, or account for significant variation in addition to body size. This addressed the question of how the pace of development might contribute to observed isotopic variation, possibly through changes in isotopic discrimination or tissue turnover. Third, we wanted to determine if the strength or nature of ontogenetic variation could be related to physical characteristics of the ecosystems inhabited by these biota such as ecosystem size or visual environment. This addressed the question of whether ontogenetic patterns in isotopic shifts may be influenced by features of the habitat supporting the food web or influencing foraging conditions. Finally, we wanted to quantify the potential contribution of ontogenetic variation to estimates of trophic niche size based on dispersion of individuals in isotope ratio dimensions. This will inform proper consideration of ontogenetic variation in future research that applies isotopic niche metrics in trophic ecology and food web research.

Methods

Sample and data collection

Data were generated from biota sampled by multiple research projects and monitoring programs carried out from 1999 to 2020. We inferred food web positions (hereafter synonymous with trophic niche positions) from C and N stable isotope compositions of tissue samples from fishes and macroinvertebrates sampled from lakes in Ontario, Canada (Table S1). For very large lakes (e.g., Laurentian Great Lakes, Lake Nipigon), sampling was restricted to smaller regions within each lake. Lakes and lake regions were distributed over 13° latitude and varied widely in surface area (range 0.28 – 932 km^2), maximum depth (range 1.5 – 213 m), and water clarity (Secchi depth range 0.3 – 12.0 m) (Table S1).

Fish were sampled primarily in the open-water season with benthic multimesh monofilament gill nets of the NA1 (large mesh) and ON2 (small mesh) configurations (Sandstrom et al. 2018). Nets were set overnight at various depths in each lake. Supplemental fish sampling was also conducted with other types of gill nets, otter trawls, baited Gee traps, longlines, electrofishing gear and angling gear. Three macroinvertebrate taxa were also targeted. Unionid clams (Bivalvia, Unionidae) were collected from depositional zones by dip net or clam rake, crayfish (Malacostraca, Cambaridae) were captured in the nearshore zone with baited Gee traps, and snails (Gastropoda, mostly Lymnaeidae and Physidae) were picked from nearshore rocks and submerged woody debris. For each taxon, wide size ranges of individuals were selected from the catches for processing. For selected fish, total length (TL, mm) and whole body (round) weight (RWT, g wet) were measured, and axial skinless muscle samples were removed and placed in glass vials for isotope analysis. Selected clams were each measured for TL and RWT, and selected crayfish and snails were each measured for RWT only. Foot muscle was removed from clams, tail muscle was removed from crayfish, and whole viscera (body minus shell) was removed from snails and placed in glass vials for isotope analysis. All tissues were initially frozen at -20 °C, then freeze-dried (Labconco Freezone 12 L) for seven days and ground to a fine powder using a ball mill (Retsch MM 400). Ageing structures were also collected from a subset of the large-bodied fish species during processing. These were usually sagittal otoliths, but cleithra and pectoral fin rays were collected for some species. Ages were determined by counting annular growth rings on these structures following standard procedures (Pritchard et al. 2019). Further details on sample collection and processing are provided in the SI file.

Carbon and nitrogen isotopic compositions of all fish and macroinvertebrate tissues were analysed on a continuous-flow isotope ratio mass spectrometer (Thermo-Finnigan Delta Plus or Delta XP; ThermoFisher Scientific, Waltham, MA, USA) coupled to an elemental analyser (Carlo Erba NC2500; CEI, Wigan, UK, or Costech 4010; Costech Analytical Technologies, Valencia, CA, USA) at the Stable Isotopes in Nature Laboratory (University of New Brunswick, Fredericton, NB, Canada) following standard procedures (Jardine et al. 2003). Ratios of heavy to light isotopes were expressed in standard delta (δ) notation as parts per thousand (‰) relative to International Reference Standards of Vienna PeeDee Belemnite carbonate (V-PDB) and atmospheric nitrogen (AIR) for C and N, respectively. Mean coefficients of variation for duplicate analyses of sample material were 0.10% for $\delta^{13}C$ and 0.32% for $\delta^{15}N$. This analysis also provided estimates of tissue C and N contents (%) and we used the C:N ratio as an index of lipid content in subsequent analyses (Post et al. 2007; Logan et al. 2008).

Ontogenetic trends in isotopic variables were examined in relation to environmental variables representing ecosystem size, ecosystem productivity and climate - factors which may influence resource availability and use. Ecosystem size was characterized both horizontally using lake surface area (km^2) and vertically using lake maximum depth (m). Lake surface areas were determined from digitization of topographic maps, and maximum depths were determined from bathymetric surveys using sonar. Ecosystem productivity was inferred from Secchi depth (m), an indicator of nutrient concentrations and algal biomass (Carlson 1977; Nürnberg 1996; Brezonik et al. 2019). Secchi depths were determined over deep water near mid-lake during the summer stratification period. Secchi depth is also a direct measure of water clarity (Preisendorfer 1986) that can influence how habitat is perceived by visual consumers, and we interpreted our results with respect to both of these properties of Secchi depth. Climate was represented by mean growing degree-days greater than 5 ˚C (GDD) at the geographic coordinates of each lake for the period 1970-2000 calculated using a model developed by McKenney et al. (2006). Physical limnological data for sampled lakes were obtained primarily from the Aquatic Habitat Inventory (AHI) database, maintained by the Ontario Ministry of Natural Resources and Forestry (Peterborough, ON, Canada). These data originated from lake surveys conducted from the mid-1960s to the mid-1980s, updated with more recent data from MNRF sampling programs, where available. Environmental data could not be obtained for all lakes, in particular area and maximum depth data for regions of larger lakes.

Statistical analyses

Data were processed in several steps prior to analysis. Biota were first grouped into four guilds based on ecologies and life histories: piscivorous fishes, benthivorous fishes, forage fishes, and macroinvertebrates. Taxonomic categories within each of the three fish guilds were represented by single species, except for sculpins (*Cottus spp*) which arose from pooling slimy sculpin (*Cottus cognatus*) and mottled sculpin (*C. bairdii*). Though slimy sculpin appeared to dominate in the catches, both species were captured and the two could not be reliably separated for some lakes. Macroinvertebrates were left in the three broader taxonomic categories of clams, crayfish and snails. Scatter plots of $\delta^{13}C$ and $\delta^{15}N$ vs individual attributes at the taxon and population levels were used as a conservative approach to identify outliers, and these were only removed from analyses if deemed likely to have been a result of errors in sample handling or labelling, or data recording. Subsequently, we retained only those taxonomic categories for which we had data for 10 or more lakes, each with data for 10 or more individuals. The final data set was composed of 18 taxa including five for piscivorous fishes (lake trout, *Salvelinus namaycush*; burbot, *Lota lota*; walleye, *Sander vitreus*; northern pike, *Esox lucius*; smallmouth bass, *Micropterus dolomieu*), three for benthivorous fishes (lake whitefish, *Coregonus clupeaformis*; white sucker, *Catostomus commersoni*; longnose sucker, *Catostomus catostomus*), seven for forage fishes (cisco, *Coregonus artedi*; rainbow smelt, *Osmerus mordax*; sculpins; trout-perch, *Percopsis omiscomaycus*; yellow perch, *Perca flavescens*; spottail shiner, *Notropis hudsonius*; rock bass, *Ambloplites rupestris*), and three for macroinvertebrates (clams, crayfish, snails) (Table S2). The combined data represented 249 lakes and lake sectors (Table S1), 1116 populations and 14561 individuals (Table S2).

Statistical analyses used both frequentist and information-theoretic approaches, and analyses were performed using both SAS/STAT® software procedures (SAS Institute Inc 2013) and R packages and functions (R Core Team 2022). The primary response variables of interest were tissue $\delta^{13}C$ and $\delta^{15}N$ and the primary predictor variables representing body size were $\log_{10}$TL and $\log_{10}$RWT. Other predictor variables representing fish attributes included body condition (CONDIT), tissue C:N ratio, age and relative growth rate (RGR). These were selected as indices of tissue lipid content (C:N), short-term energy storage (CONDIT) and long-term structural growth (RGR). Body condition was calculated as the residual from $\log_{10}$RWT vs $\log_{10}$TL regressions (Kaufman et al. 2007) fitted by ordinary least-squares at the taxon level. Where age data were available, relative growth rate was calculated as the ratio of residual TL-at-age to predicted TL-at-age with both residual and predicted values derived from von Bertalanffy growth models (Chen et al. 1992; Lester et al. 2004) fitted by non-linear least-squares at the population level (taxon x lake).

To quantify the nature, strength and prevalence of size-based variation in stable isotope ratios (objective 1), we examined the relationships between $\delta^{13}C$ and $\delta^{15}N$ and each of four biotic predictors – $\log_{10}$TL, $\log_{10}$RWT, CONDIT and C:N. For each taxon, slopes of $\delta^{13}C$ and $\delta^{15}N$ vs each of these four predictors were calculated at the population level using OLS regression. We then determined mean slopes across populations and tested if the species-specific means differed from zero using single-sample t-tests if the slope distribution was normal (based on Shapiro-Wilks test) or Wilcoxon signed-rank tests if the distribution was not normal. Next, we compared models based on these four predictors using an information theoretic model selection approach (Johnson and Omland 2004; Anderson 2008). Predictors were normalized (converted to

Z-scores) prior to model fitting to reduce scaling effects. Linear mixed-effects models were fitted with lake as a random effect and all combinations of the predictors as fixed effects (maximum three per model), except for models that contained pairs of strongly correlated predictors. Fitted models were ranked by Akaike's Information Criterion adjusted for small sample size (AICc) and relative model strengths were assessed by Akaike weights (w_i) (Anderson 2008).

To determine if age or growth may be stronger ontogenetic covariates than body size, or account for significant variation in addition to body size (objective 2), we repeated the above analysis but included two additional predictors – fish age and RGR – and used the smaller subset of large-bodied fish species with age and growth data.

We looked for evidence of environmental influences on ontogenetic slopes across ecosystems (objective 3) in two steps. First, using population level slopes from each of δ^{13}C and δ^{15}N vs body size as generated from OLS regressions, we tested the significance of Pearson correlations between these slopes across lakes for each taxon pairing that was found in 20 or more lakes. Significant positive correlations between species slopes were interpreted as evidence of interspecific concordance in ontogenetic responses to environmental factors. Second, for selected species we compared simple and multiple regression models of isotope ratio vs body size slopes (our indicators of the strength and direction of ontogenetic diet shifts) as functions of the four environmental characteristics – lake area, lake maximum depth, Secchi depth and GDD – using a model selection approach. Predictors were normalized prior to model fitting and regression models used all combinations of the predictors. Fitted models were ranked by AICc and relative model strengths were assessed by Akaike weights (w_i).

Finally, we examined the contribution of ontogenetic variation to estimates of isotopic niche size (objective 4) by comparing estimates based on size-adjusted and unadjusted data. Isotopic niche size was estimated for selected taxa at the population level as the dispersion of individuals in $\delta^{15}N$ vs $\delta^{13}C$ space. Our dispersion index was the standard ellipse area adjusted for small sample size (SEAc) estimated using the SIBER package (Jackson et al. 2011). For each population, we estimated SEAc both before and after adjusting $\delta^{13}C$ and $\delta^{15}N$ to the mean body size of the sample, then calculated the relative difference between these two estimates as a percentage. These differences were then examined in relation to the body size variation in our samples, measured as the standard deviation of $\log_{10}$RWT, using OLS regressions.

Results

$\delta^{13}C$ and $\delta^{15}N$ vs body size and condition (objective #1)

Correlations among the four biotic predictors in this analysis are summarized in Table S3. Correlations between the two body size predictors - $\log_{10}$TL and $\log_{10}$RWT - were positive and strong ($r > 0.90$) for all taxa, as expected, whereas most correlations between tissue C:N and CONDIT and body size predictors were much weaker ($|r| < 0.40$)(Table S3). The C:N vs body size relationship tended to be positive for most piscivores and benthivores but negative for burbot, forage fishes and clams (Table S3). Correlations between tissue C:N and CONDIT were positive for most taxa, but strongest for the piscivores and benthivores (Table S3). In subsequent model comparisons, we did not include models that contained both $\log_{10}$TL and $\log_{10}$RWT.

Comparisons of δ^{13}C vs $\log_{10}$RWT slopes and δ^{15}N vs $\log_{10}$RWT slopes among taxa are illustrated for piscivores (Fig. 1), benthivores (Fig. 2), forage fishes (Fig. 3) and macroinvertebrates (Fig. 4). For all four guilds, mean (among-population) slopes were generally positive for δ^{15}N vs $\log_{10}$RWT but both positive and negative for δ^{13}C vs $\log_{10}$RWT (Figs. 1-4). Variation in slopes among populations, inferred from SD estimates, was also considerably higher for δ^{13}C vs $\log_{10}$RWT than for δ^{15}N vs $\log_{10}$RWT for most taxa (Figs. 1-4). Across taxa, δ^{15}N vs $\log_{10}$RWT relationships had both higher slope means (paired-comparisons t-test, $t = 3.218$, $n = 18$, $P = 0.0051$) and lower slope SD ($t = -5.348$, $n = 18$, $P < 0.001$) than δ^{13}C vs $\log_{10}$RWT relationships. Comparing among fish trophic guilds, among-population variation in both δ^{13}C vs $\log_{10}$RWT and δ^{15}N vs $\log_{10}$RWT slopes was lowest in the piscivores (Fig. 1), highest in the benthivores (Fig. 2) and intermediate in the forage fishes (Fig. 3). For δ^{13}C vs $\log_{10}$RWT, mean slopes differed significantly from 0 for six of 18 taxa examined; this included positive trends in one of five piscivores, one of three benthivores, and two of seven forage fishes, and negative trends in one of seven forage fishes and one of three macroinvertebrates (Figs. 1-4). For δ^{15}N vs $\log_{10}$RWT, mean slopes were significantly greater than 0 for 13 of 18 taxa examined, including all five piscivores, one of three benthivores, five of seven forage fishes, and two of three macroinvertebrates (Figs. 1-4). Using $\log_{10}$TL instead of $\log_{10}$RWT as the body size variable in this analysis changed the outcomes only slightly. For δ^{13}C, mean slope changed from not significant to significantly greater than 0 for lake trout, and from significantly less than 0 to not significant for cisco. For δ^{15}N, mean slope changed from not significant to significantly greater than 0 for trout-perch.

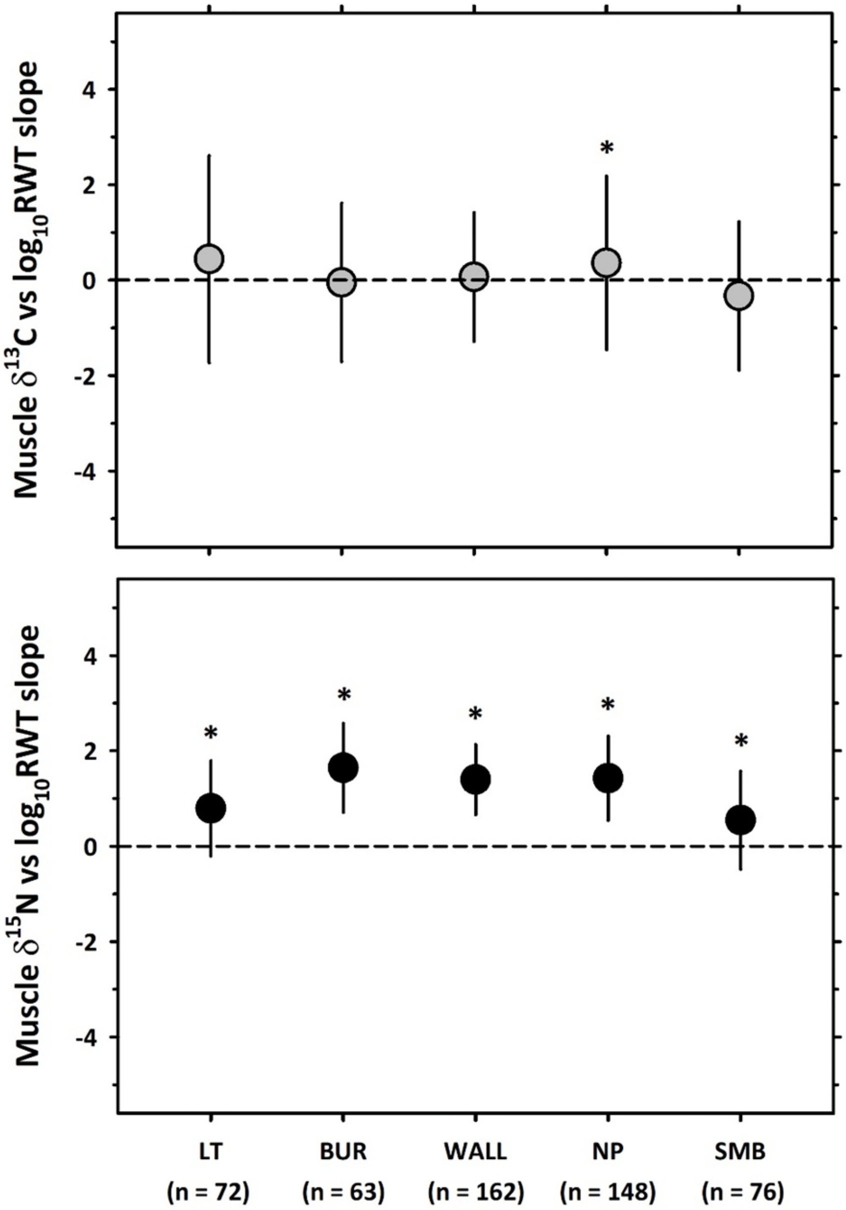

Fig. 1 Comparison of slopes of muscle stable isotope ratios vs body size ($\log_{10}$round weight) among five piscivorous fishes of northern lakes. Plots are for stable isotope ratios of carbon ($\delta^{13}C$, top) and nitrogen ($\delta^{15}N$, bottom). Slopes were calculated within each population and symbols represent among-population means (n = number of populations) ± 1 SD. Means differing significantly from zero are indicated * ($P < 0.05$; one-sample t-test or Wilcoxon signed-rank test). LT = lake trout, BUR = burbot, WALL = walleye, NP = northern pike, SMB = smallmouth bass

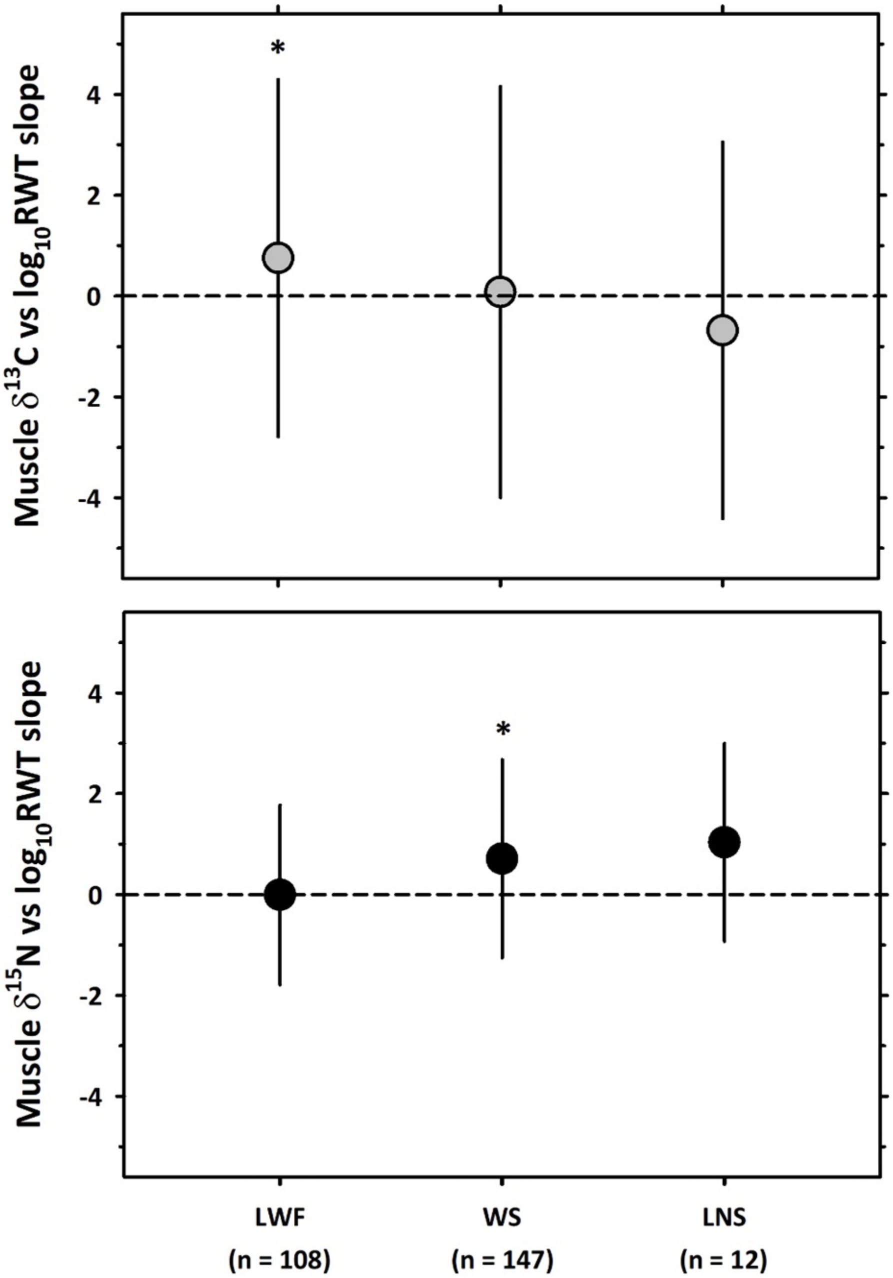

Fig. 2 Comparison of slopes of muscle stable isotope ratios vs body size ($\log_{10}$round weight) among three benthivorous fishes of northern lakes. Plots are for stable isotope ratios of carbon (δ^{13}C, top) and nitrogen (δ^{15}N, bottom). Slopes were calculated within each population and symbols represent among-population means (n = number of populations) ± 1 SD. Means differing significantly from zero are indicated * ($P < 0.05$; one-sample t-test or Wilcoxon signed-rank test). LWF = lake whitefish, WS = white sucker, LNS = longnose sucker

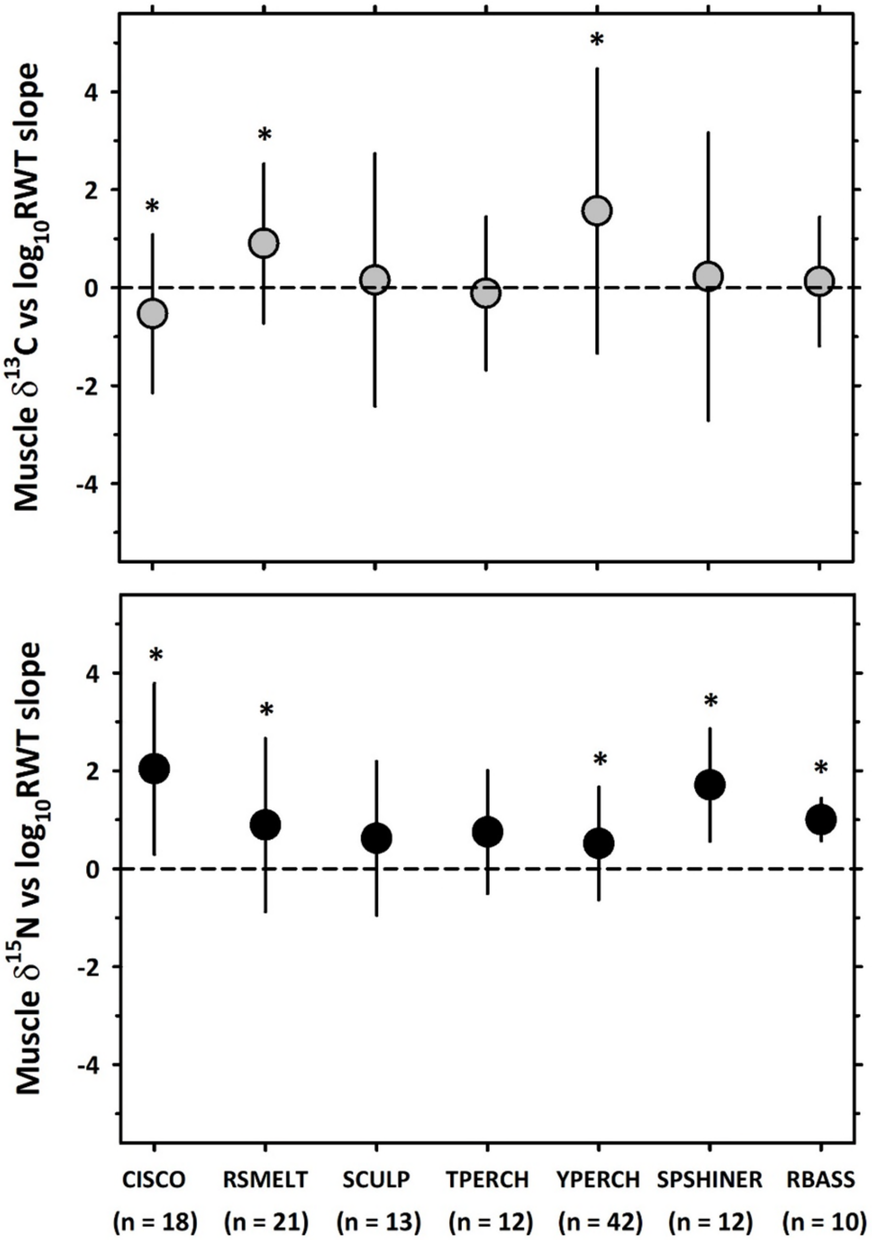

Fig. 3 Comparison of slopes of muscle stable isotope ratios vs body size ($\log_{10}$round weight) among seven small-bodied fishes of northern lakes. Plots are for stable isotope ratios of carbon (δ^{13}C, top) and nitrogen (δ^{15}N, bottom). Slopes were calculated within each population and symbols represent among-population means (n = number of populations) ± 1 SD. Means differing significantly from zero are indicated * ($P < 0.05$; one-sample t-test or Wilcoxon signed-rank test). CISCO = cisco, RSMELT = rainbow smelt, SCULP = sculpins, TPERCH = trout-perch, YPERCH = yellow perch, SPSHINER = spottail shiner, RBASS = rock bass

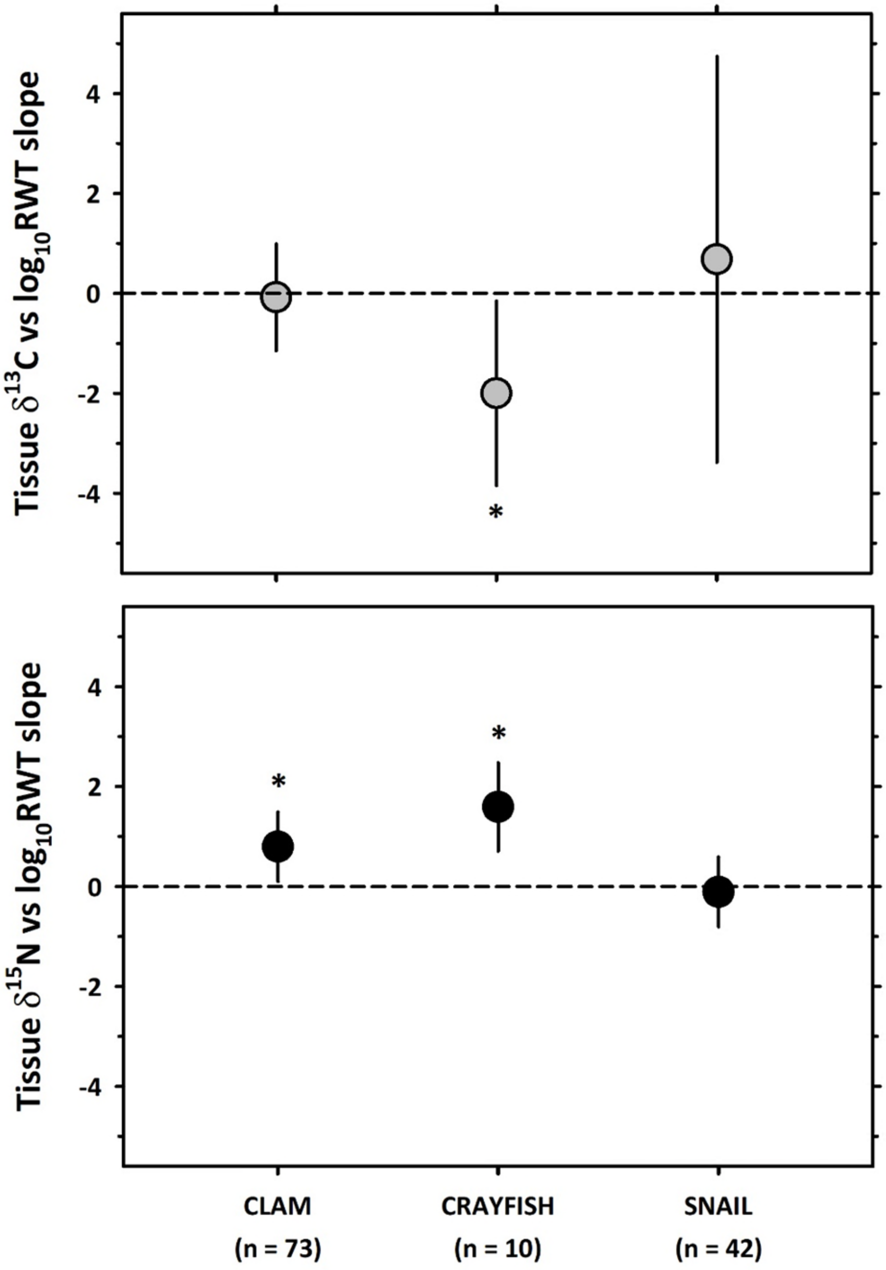

Fig. 4 Comparison of slopes of tissue stable isotope ratios vs body size (log$_{10}$round weight) among three macroinvertebrates of northern lakes. Plots are for stable isotope ratios of carbon (δ^{13}C, top) and nitrogen (δ^{15}N, bottom). Slopes were calculated within each population and symbols represent among-population means (n = number of populations) ± 1 SD. Means differing significantly from zero are indicated * ($P < 0.05$; one-sample t-test or Wilcoxon signed-rank test). CLAM = unionid clam (foot muscle), CRAYFISH = crayfish (tail muscle), SNAIL = snail (whole viscera)

Among-population mean slopes for relationships between each of δ^{13}C and δ^{15}N and each of the four biotic predictors are summarized in Table S4. There were significant negative relationships between δ^{13}C and tissue C:N for 11 of the 18 taxa examined; this was the most consistently strong relationship with δ^{13}C. In contrast, significant relationships between δ^{13}C and body size and between δ^{13}C and CONDIT were evident for six and two taxa, respectively. For δ^{15}N, significant relationships with tissue C:N and CONDIT were observed for six and two taxa, respectively, whereas significant positive relationships with body size were observed for 13 of 18 taxa. In light of the relatively strong C:N effect, we re-examined the isotope ratio vs log$_{10}$RWT slopes while accounting for C:N variation. Adding C:N as a second predictor to isotope ratio vs log$_{10}$RWT models tended to alter the log$_{10}$RWT slope for δ^{13}C models more than for δ^{15}N models. Accounting for C:N covariation shifted mean δ^{13}C vs log$_{10}$RWT slopes from not significant to significantly greater than 0 for lake trout and rock bass, and significantly less than 0 for clams; it also shifted mean δ^{13}C vs log$_{10}$RWT slopes from significantly less than 0 to not significant for cisco and crayfish. Accounting for C:N covariation only altered tests of mean δ^{15}N vs log$_{10}$RWT slopes for one taxon; the mean slope for rock bass shifted from significantly greater than 0 to not significantly different than 0.

Comparison of linear mixed-effects models indicated that variation in δ^{13}C and δ^{15}N was better explained by multiple predictors than single predictors for most taxa, particularly for δ^{13}C, and the best combination of predictors varied among taxa. Top-ranking models for 16 taxa (crayfish and snails are not included because TL and CONDIT data were lacking for both taxa) are summarized in Table S5 for δ^{13}C and in Table S6 for δ^{15}N. For δ^{13}C, the top-ranked models had multiple predictors for 14 of 16 taxa (10 models with two predictors, four models with three predictors). Body size, either $\log_{10}$TL or $\log_{10}$RWT, was included in the top-ranked δ^{13}C models for 15 of 16 taxa, but tissue C:N had the largest standardized coefficient for 10 of 16 models (Table S5). For δ^{15}N, the top-ranked models had multiple predictors for 10 of 16 taxa (seven models with two predictors, three models with three predictors). Body size, either $\log_{10}$TL or $\log_{10}$RWT, had the largest standardized coefficient in all top-ranked δ^{15}N models (Table S6).

Age and growth effects (objective #2)

Correlations among the six biotic predictors in this analysis are summarized in Table S7. Fish $\log_{10}$Age was most strongly correlated with the body size variables ($r > 0.70$), though correlations between $\log_{10}$TL and $\log_{10}$RWT were consistently stronger than either of their correlations with age (Table S7). Correlations were positive and moderately strong between RGR and the body size variables ($r = 0.31 – 0.56$) but not between RGR and $\log_{10}$Age ($|r| < 0.1$; Table S7). All other correlations between predictors were generally weak to moderate in strength ($|r| < 0.4$; Table S7). In subsequent model comparisons, we did not include models that contained any two of $\log_{10}$Age, $\log_{10}$TL and $\log_{10}$RWT.

When growth and age predictors were included in linear mixed-effects models in addition to body size predictors, model comparisons again indicated that variation in δ^{13}C was better explained by multiple predictors than single predictors for all seven fish species (Table 1). Akaike weights declined rapidly with decreasing model rank for most species, suggesting strong support for relatively few models; the exceptions were walleye and northern pike which had a broader range of plausible models (Table 1). The highest-ranked model had two predictors for each of walleye and northern pike, four predictors for lake whitefish and three predictors for each of the remaining species. For all species, the highest ranked three-predictor model included C:N and RGR with either age or size (Table 1). Age was the stronger ontogenetic variable for lake trout and white sucker, whereas TL or RWT was the stronger ontogenetic variable for the other five species. The RGR effect on δ^{13}C was positive for all species except smallmouth bass (Table 1). Within-population variation in δ^{13}C explained by the top-ranked three predictor models ranged from 34% for smallmouth bass to 61% for lake trout (Fig. 5). The increase in explained variation from the highest ranked single-predictor model to the highest ranked three-predictor model ranged from 19% for smallmouth bass to 28% for lake whitefish (Fig. 5).

Variation in δ^{15}N was also better explained by multiple predictors than single predictors for all seven fish species (Table 2). Akaike weights declined rapidly with decreasing model rank for burbot, walleye, northern pike and white sucker but not for the remaining three species (Table 1). Except for lake trout, the highest ranked model for each species included RGR with either age or size (Table 2). Age was the stronger ontogenetic variable for lake trout, walleye, lake whitefish and white sucker, whereas TL or RWT was the stronger ontogenetic variable for the other three species (Table 2). The RGR effect on δ^{15}N was negative for all species except walleye and smallmouth bass (Table 2). Within-population variation in δ^{15}N explained by the top-ranked three predictor models ranged from 36% for smallmouth bass to 64% for walleye (Fig. 6). The increase in explained variation from the highest ranked single-predictor model to the highest ranked three-predictor model ranged from 11% for walleye to 25% for lake whitefish (Fig. 6).

Table 1 Ranking of linear mixed effects models for muscle δ^{13}C as a function of various combinations of ontogenetic, growth and condition predictors for each of seven fishes. All models contained population (lake) as a random effect. For each species, all one, two, three and four predictor models were fitted and ranked except those containing any two or more of the size and age predictors. Subsets of the top-ranking models are shown. TL = total length, RWT = round weight, CONDIT = body condition, AICc = Akaike's Information Criterion corrected for small sample size, Δ_i = difference between AICc value of a given model and the top-ranked model, w_i = Akaike weight

	Standardized coefficients of predictors in model								
Model rank	**$\log_{10}$TL**	**$\log_{10}$RWT**	**$\log_{10}$AGE**	**RGR**	**CONDIT**	**C:N**	**AICc**	**Δ_i**	**w_i**
				Lake trout					
1	.	.	0.321	0.092	.	-0.791	1398.9	0.0	0.65
2	.	.	0.324	0.092	0.023	-0.799	1400.7	1.8	0.26
3	.	.	0.316	.	.	-0.761	1403.6	4.6	0.06
4	.	.	0.317	.	0.019	-0.768	1405.4	6.5	0.02
15	.	.	.	.	.	-0.710	1448.5	49.6	0.00
				Burbot					
1	.	-0.112	.	0.121	.	-0.087	1520.6	0.0	0.53
2	.	-0.125	.	0.126	0.035	-0.093	1521.7	1.1	0.32
3	.	-0.097	.	0.118	.	.	1524.0	3.4	0.10
4	.	-0.105	.	0.121	0.024	.	1525.6	5.0	0.04
8	.	-0.010	.	.	.	.	1533.9	13.3	0.00
				Walleye					
1	0.079	.	.	.	.	-0.233	5354.2	0.0	0.17
2	0.068	.	.	0.028	.	-0.231	5354.3	0.2	0.16
3	.	0.080	.	.	.	-0.234	5354.7	0.5	0.13
8	0.069	.	.	0.029	0.007	-0.232	5356.3	2.1	0.06
18	0.064	.	.	.	.	.	5429.8	75.6	0.00

Table 1 Ranked LME models for δ^{13}C, continued

	Standardized coefficients of predictors in model								
Rank	**$\log_{10}$TL**	**$\log_{10}$RWT**	**$\log_{10}$AGE**	**RGR**	**CONDIT**	**C:N**	**AICc**	**Δ_i**	**w_i**
				Northern pike					
1	0.143	.	.	.	.	-0.089	4214.9	0.0	0.27
2	0.127	.	.	0.033	.	-0.088	4215.6	0.6	0.20
3	0.145	.	.	.	0.017	-0.093	4216.7	1.7	0.11
4	0.128	.	.	0.033	0.017	-0.093	4217.3	2.4	0.08
5	0.137	.	.	.	.	.	4217.4	2.4	0.08
				Smallmouth bass					
1	-0.037	.	.	-0.088	.	-0.382	2555.4	0.0	0.45
2	.	-0.037	.	-0.086	.	-0.380	2557.2	1.8	0.18
3	-0.037	.	.	-0.088	0.001	-0.382	2557.4	2.0	0.16
4	-0.066	.	.	.	.	-0.389	2559.1	3.7	0.07
15	.	.	.	.	.	-0.407	2588.1	32.7	0.00
				Lake whitefish					
1	.	0.335	.	0.198	-0.181	-0.553	2947.9	0.0	0.53
2	0.325	.	.	0.201	.	-0.552	2950.3	2.4	0.16
3	0.336	.	.	0.196	-0.112	-0.544	2950.3	2.4	0.16
9	0.439	.	.	.	.	-0.532	2960.5	12.5	0.00
15	.	.	.	.	.	-0.376	3010.4	62.4	0.00
				White sucker					
1	.	.	0.036	0.193	.	-0.336	4458.7	0.0	0.70
2	.	.	0.038	0.195	0.018	-0.338	4460.7	2.0	0.26
3	0.005	.	.	0.184	.	-0.314	4465.7	7.0	0.02
5	.	.	0.032	.	.	-0.321	4468.2	9.5	0.01
18	.	.	-0.031	.	.	.	4486.7	28.0	0.00

Table 2 Ranking of linear mixed effects models for muscle δ^{15}N as a function of various combinations of ontogenetic, growth and condition predictors for each of seven fishes. All models contained population (lake) as a random effect. For each species, all one, two, three and four predictor models were fitted and ranked except those containing any two or more of the size and age predictors. Subsets of the top-ranking models are shown. TL = total length, RWT = round weight, CONDIT = body condition, AICc = Akaike's Information Criterion corrected for small sample size, Δ_i = difference between AICc value of a given model and the top-ranked model, w_i = Akaike weight.

	Standardized coefficients of predictors in model								
Model rank	**log$_{10}$TL**	**log$_{10}$Mass**	**log$_{10}$AGE**	**RGR**	**CONDIT**	**C:N**	**AICc**	**Δ_i**	**w_i**
				Lake trout					
1	.	.	0.220	.	.	0.052	980.0	0.0	0.21
2	.	.	0.229	.	.	.	980.6	0.7	0.15
3	.	0.267	.	-0.081	.	.	981.6	1.6	0.09
4	.	.	0.221	.	0.010	0.049	981.9	2.0	0.08
11	.	.	0.221	0.000	0.010	0.049	984.0	4.0	0.03
				Burbot					
1	.	0.713	.	-0.179	-0.105	.	969.9	0.0	0.37
2	0.696	.	.	-0.177	0.050	.	970.5	0.5	0.28
3	.	0.716	.	-0.179	-0.107	0.014	971.6	1.7	0.16
5	0.694	.	.	-0.176	.	.	973.8	3.9	0.05
16	0.560	.	.	.	.	.	1034.3	64.4	0.00
				Walleye					
1	.	.	0.611	0.045	0.035	.	3428.5	0.0	0.61
2	.	.	0.610	0.045	0.035	0.007	3430.3	1.8	0.25
3	.	.	0.604	0.043	.	.	3432.2	3.7	0.10
4	.	.	0.603	0.043	.	0.011	3433.7	5.2	0.05
7	.	.	0.602	.	.	.	3444.3	15.8	0.00

Table 2 Ranked LME models for δ^{15}N, continued

	Standardized coefficients of predictors in model								
Rank	**$\log_{10}$TL**	**$\log_{10}$RWT**	**$\log_{10}$AGE**	**RGR**	**CONDIT**	**C:N**	**AICc**	**Δ_i**	**w_i**
				Northern pike					
1	0.536	.	.	-0.125	.	0.120	2697.7	0.0	0.64
2	0.534	.	.	-0.125	-0.016	0.124	2698.9	1.3	0.34
3	.	0.541	.	-0.123	-0.111	0.126	2705.2	7.5	0.01
6	0.545	.	.	-0.125	.	.	2720.2	22.6	0.00
18	0.483	.	.	.	.	.	2775.8	78.2	0.00
				Smallmouth bass					
1	.	0.149	.	0.033	0.036	0.044	1462.9	0.0	0.19
2	.	0.149	.	0.033	.	0.047	1463.3	0.4	0.16
3	0.148	.	.	0.032	0.055	0.046	1464.0	1.1	0.11
5	.	0.160	.	.	.	0.049	1464.5	1.6	0.09
11	.	0.165	.	.	.	.	1467.2	4.3	0.02
				Lake whitefish					
1	.	.	0.090	-0.130	.	.	1931.1	0.0	0.25
2	.	.	0.083	-0.138	.	0.046	1931.2	0.0	0.24
3	.	.	0.085	-0.138	-0.046	0.050	1931.9	0.7	0.17
4	.	.	0.093	-0.130	-0.040	.	1932.2	1.0	0.15
13	.	.	0.092	.	.	.	1956.3	25.2	0.00
				White sucker					
1	.	.	0.268	-0.085	.	0.073	2604.5	0.0	0.53
2	.	.	0.268	-0.084	0.010	0.072	2606.4	1.9	0.21
3	0.315	.	.	-0.193	.	0.067	2607.8	3.3	0.10
4	.	.	0.282	-0.081	.	.	2609.0	4.5	0.06
15	.	.	0.283	.	.	.	2619.5	15.0	0.00

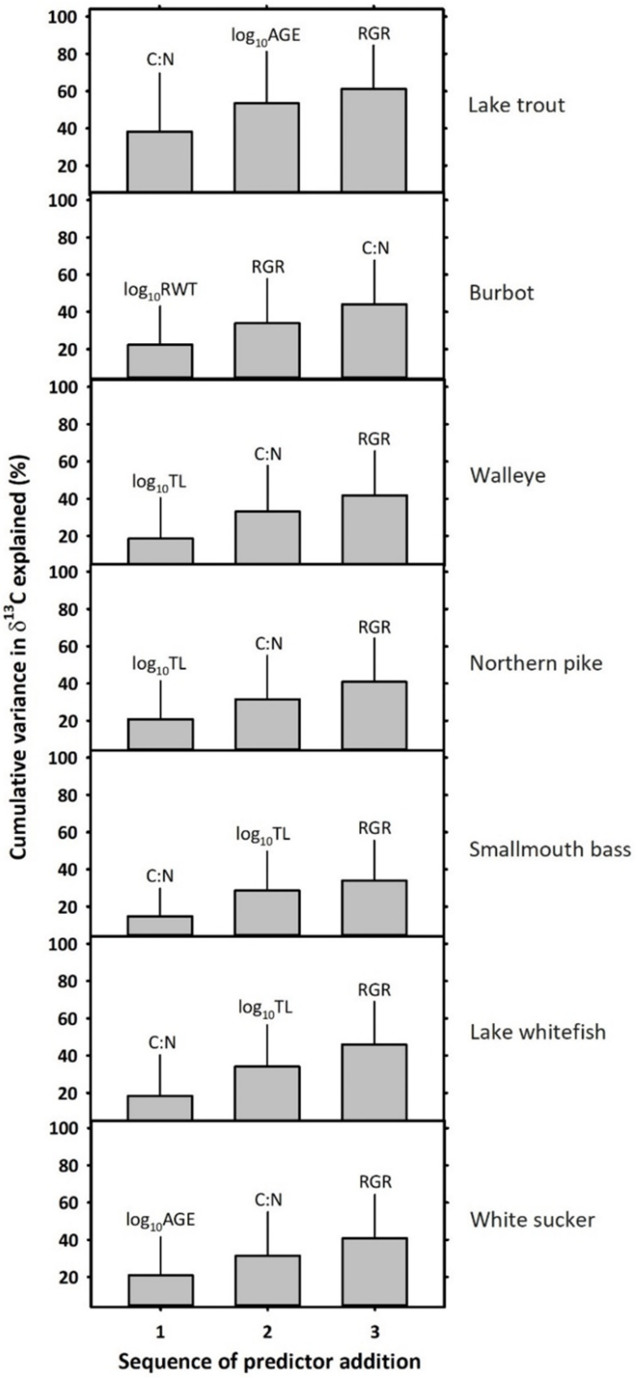

Fig. 5 Patterns of increasing explained variance (mean R^2) in fish muscle δ^{13}C for linear models with sequential addition of predictor variables (TL = fish total length, RWT = fish round weight, AGE = fish age, RGR = fish relative growth rate, CONDIT = fish body condition, C:N = fish muscle C:N; defined in text). Order of predictor addition is indicated for each species and was based on standardized model coefficients in the strongest three predictor model based on AICc ranking (Table 1)

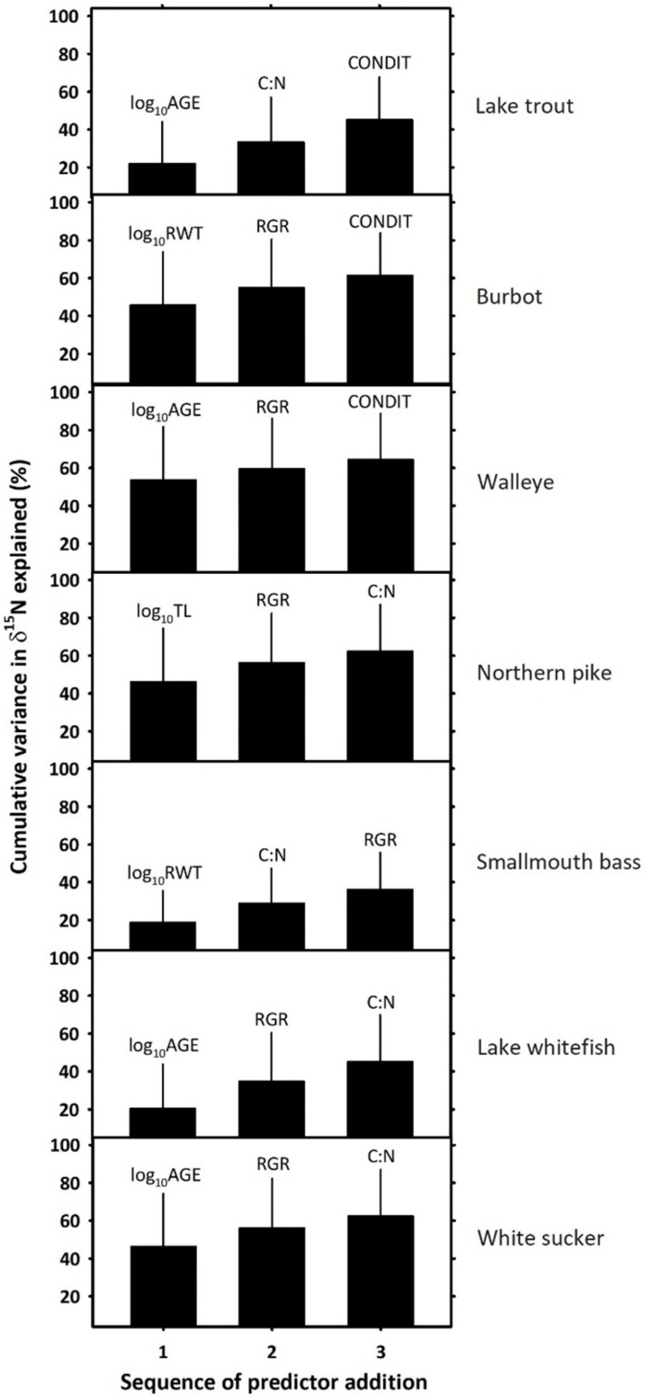

Fig. 6 Patterns of increasing explained variance (mean R^2) in fish muscle δ^{15}N for linear models with sequential addition of predictor variables (TL = fish total length, RWT = fish round weight, AGE = fish age, RGR = fish relative growth rate, CONDIT = fish body condition, C:N = fish muscle C:N; defined in text). Order of predictor addition is indicated for each species and was based on standardized model coefficients in the strongest three predictor model based on AICc ranking (Table 2)

Ontogenetic slopes vs environmental conditions (objective #3)

Sample sizes (i.e., number of lakes) for examining interspecific concordance in ontogenetic slopes were highly variable among species pairings. Across all 18 taxa there were 153 potential species pairings but only 37 had sample sizes ≥ 20 in our data set. Of these 37 correlations between $\delta^{13}C$ vs $\log_{10}RWT$ slopes, only the northern pike vs yellow perch pairing was statistically significant ($r = 0.62$, $n = 24$, $P = 0.0012$). The piscivore guild had relatively large sample sizes ($n > 30$) for all species pairings but these correlations were generally weak and non-significant ($|r| \leq 0.23$; Table 3). Trends ranged from weakly positive (burbot vs lake trout, $r = 0.20$) to weakly negative (burbot vs northern pike, $r = -0.23$)(Table 3). Similarly, correlations between $\delta^{15}N$ vs $\log_{10}RWT$ slopes were also generally weak with significant relationships seen in only 3 of 37 species pairings; lake trout vs lake whitefish ($r = -0.33$, $n = 53$, $P = 0.0015$), lake trout vs clams ($r = -0.44$, $n = 25$, $P = 0.027$), and burbot vs clams ($r = 0.45$, $n = 24$, $P = 0.027$). Within the piscivore guild, trends ranged from weakly positive (walleye vs smallmouth bass, $r = 0.18$) to weakly negative (lake trout vs burbot, $r = -0.26$) but none were statistically significant (Table 4).

Table 3 Pearson correlations (number of population pairings; p-value) between population-level δ^{13}C vs log$_{10}$-mass slopes for five piscivores (BUR = burbot, LT = lake trout, NP = northern pike, SMB = smallmouth bass, WALL = walleye).

	BUR	**WALL**	**NP**	**SMB**
LT	0.20 (46; P = 0.18)	-0.09 (44; P = 0.55)	-0.12 (51; P = 0.42)	0.18 (33; P = 0.33)
BUR		0.16 (57; P = 0.23)	-0.23 (54; P = 0.10)	0.14 (31; P = 0.47)
WALL			0.16 (124; P = 0.085)	0.08 (62; P = 0.52)
NP				0.04 (45; P = 0.80)

Table 4 Pearson correlations (number of population pairings; p-value) between population-level δ^{15}N vs $\log_{10}$-mass slopes for five piscivores (BUR = burbot, LT = lake trout, NP = northern pike, SMB = smallmouth bass, WALL = walleye)

	BUR	**WALL**	**NP**	**SMB**
LT	-0.26 (46; P = 0.084)	-0.21 (44; P = 0.18)	0.07 (51; P = 0.61)	0.10 (33; P = 0.60)
BUR		0.08 (57; P = 0.56)	-0.15 (54; P = 0.27)	-0.04 (31; P = 0.84)
WALL			0.14 (124; P = 0.12)	0.18 (62; P = 0.16)
NP				-0.03 (45; P = 0.86)

Relationships between ontogenetic slopes and physical characteristics of lakes were examined for individual taxa with data from > 30 lakes (10 of 18 taxa). Model selection results are summarized for analyses of δ^{13}C slopes and δ^{15}N slopes in Tables S8 and S9, respectively. For δ^{13}C ontogenetic slope models, the top-ranked model had no predictors (i.e., intercept only) for two taxa (northern pike, lake whitefish), two predictors for two taxa (white sucker, yellow perch), and one predictor for each of the six remaining taxa (Table S8). Akaike weights were generally low ($w_i < 0.30$) and declined slowly with decreasing rank, suggesting a wide range of plausible models for each taxon. Predictors that dominated top-ranked δ^{13}C models included Secchi depth for walleye (negative effect), maximum depth for smallmouth bass (negative effect) and yellow perch (positive effect), and lake area for white sucker (positive effect) (Table S8). For δ^{15}N ontogenetic slope models, Akaike weights were somewhat stronger, and multi-predictor models tended to rank higher (Table S9). Predictors that dominated top-ranked δ^{15}N models included GDD for burbot, lake whitefish and yellow perch (all positive effects), Secchi depth for walleye and northern pike (both positive effects), and maximum depth for smallmouth bass (positive effect), white sucker (negative effect), and clams (positive effect) (Table S9). Among the stronger relationships observed between ontogenetic slopes and environmental factors were walleye slopes vs Secchi depth (Fig. 7) and smallmouth bass slopes vs maximum depth (Fig. 8). For walleye, ontogenetic δ^{13}C slopes declined and ontogenetic δ^{15}N slopes increased significantly with increasing Secchi depth (Fig. 7). For smallmouth bass, a similar pattern was evident with increasing maximum depth (Fig. 8). Note that declining or increasing trends in these analyses are not relative to zero as population-level slopes range from strongly positive to strongly negative.

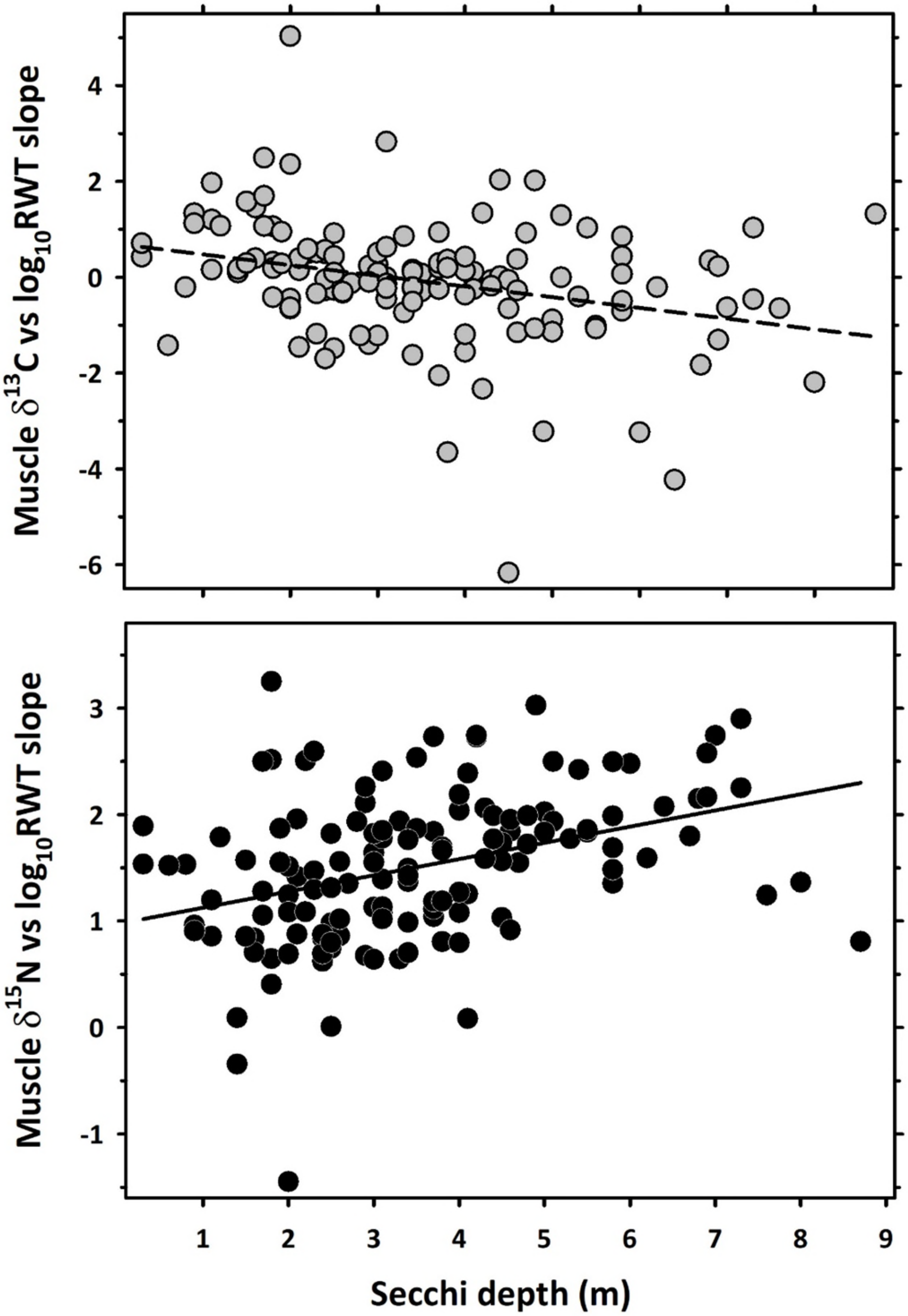

Fig. 7 Scatter plots of ontogenetic slopes for muscle δ^{13}C (top) and δ^{15}N (bottom) vs Secchi depth for walleye populations from 139 Ontario lakes. Lines are OLS fitted regressions (δ^{13}C slope = 0.702 – 0.223*Secchi, $F_{1,137}$ = 13.2, $P < 0.001$, adj-R^2 = 0.08; δ^{15}N slope = 0.973 + 0.152*Secchi, $F_{1,137}$ = 21.7, $P < 0.001$, adj-R^2 = 0.13)

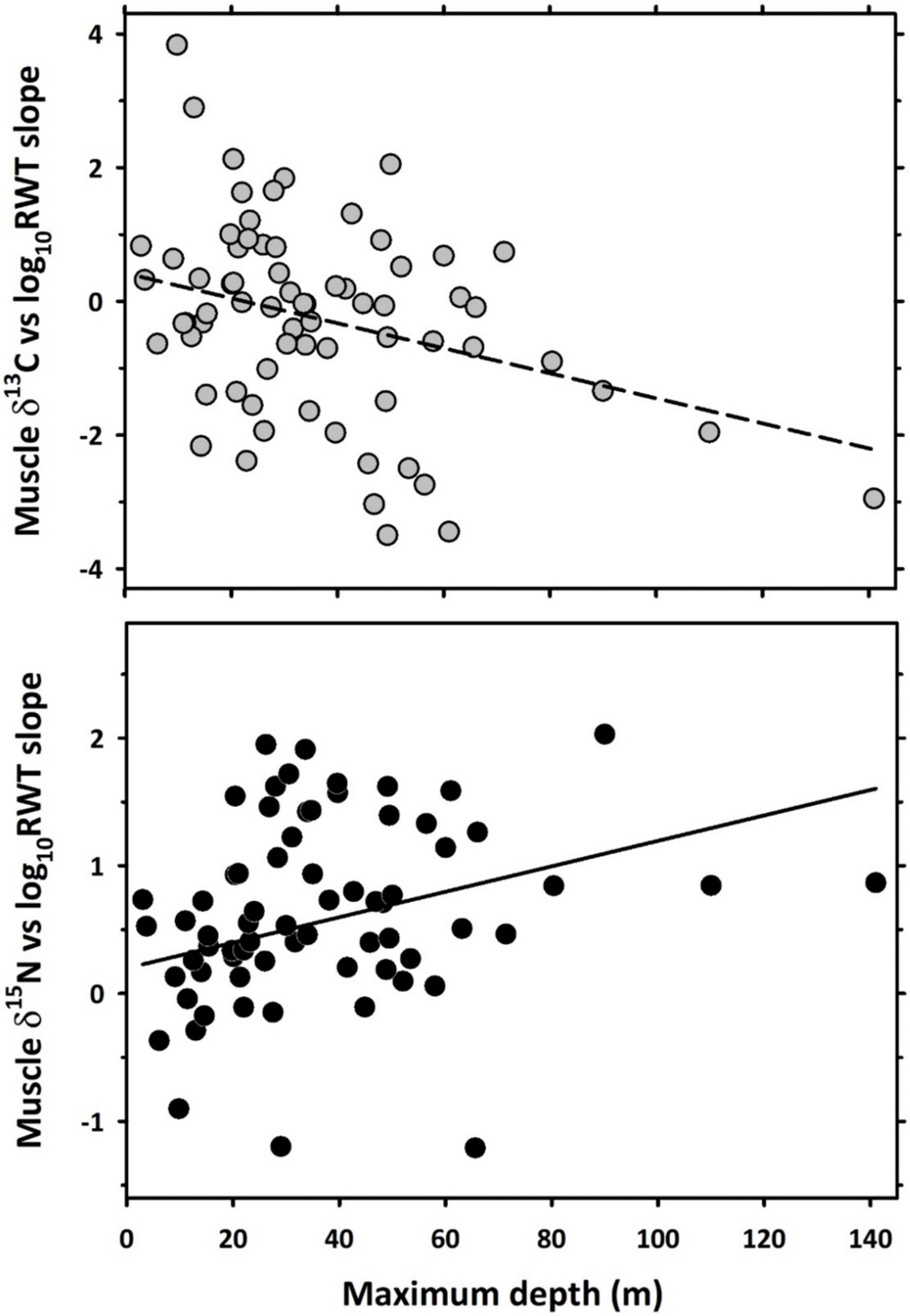

Fig. 8 Scatter plots of ontogenetic slopes for muscle δ^{13}C (top) and δ^{15}N (bottom) vs maximum depth for smallmouth bass populations from 71 Ontario lakes. Lines are OLS fitted regressions (δ^{13}C slope = 0.423 – 0.019*Z_{max}, $F_{1,69}$ = 13.7, $P < 0.001$, adj-R^2 = 0.15; δ^{15}N slope = 0.199 + 0.010* Z_{max}, $F_{1,69}$ = 7.64, P = 0.0073, adj-R^2 = 0.09). Data for two lakes are out of range and not displayed

Contribution of ontogenetic variation to isotopic niche size (objective #4)

The ontogenetic component of isotopic niche size was analyzed for individual taxa with data from > 30 lakes (10 of 18 taxa). The percentage of SEAc attributable to ontogenetic variation ranged from 0 to 85 % across 973 populations, and was more strongly related to ontogenetic slopes for $\delta^{15}N$ than for $\delta^{13}C$ for most taxa, except benthivorous fishes and snails. Body size variation within samples had significant effects in all models (Table S10). As expected, the influence of body size variation on the proportion of SEAc attributable to ontogenetic effects tended to be greater for taxa with stronger isotope vs body size relationships. At $\log_{10}$RWT SD = 0.3, the predicted percent of variation in SEAc attributable to ontogenetic variation ranged from 19% in lake whitefish, a species with weak ontogenetic variation, to 42% in burbot, a species with strong ontogenetic variation (Fig. 9).

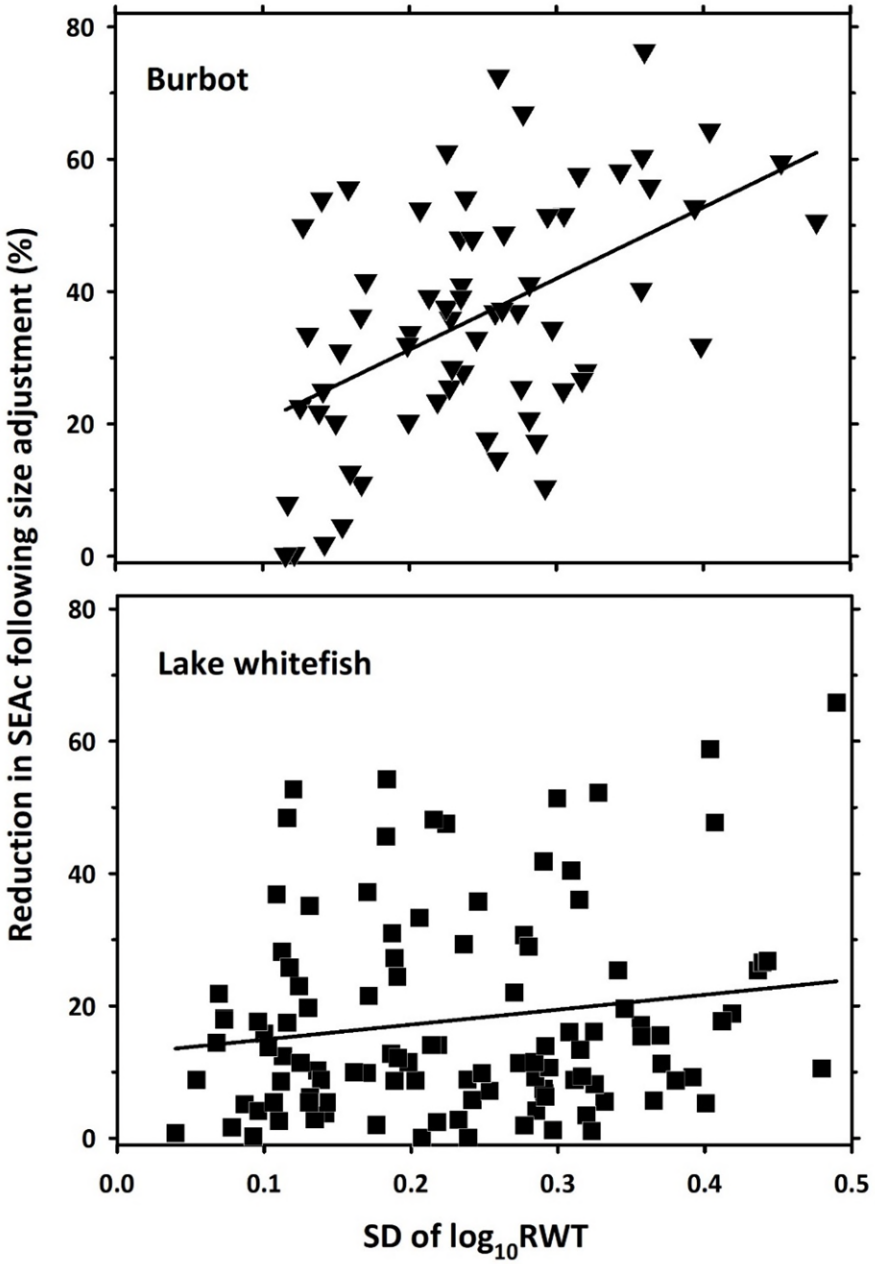

Fig. 9 Scatter plots of estimated ontogenetic contribution to isotopic niche size vs standard deviation (SD) in body size for samples from 66 burbot populations (top) and 111 lake whitefish populations (bottom) from Ontario lakes. Dependent variable was estimated as the difference between SEAc estimated without and with adjustment of δ^{13}C and δ^{15}N for ontogenetic ($\log_{10}$RWT) variation, as a percentage of SEAc without adjustment (see Methods). Trend lines are OLS fitted regressions and relationship was significant for burbot but not for lake whitefish (see Table S9)

Discussion

Body size has long been recognized as a key attribute influencing trophic interactions in aquatic food webs, and stable isotope analysis has become one of the primary approaches for elucidating these interactions and overall food web structure. We have provided the most comprehensive analysis to date of the relationships between C and N isotopic compositions and body size in freshwater consumers. Our analysis covered multiple trophic guilds and species, including some of the most common and economically valuable freshwater fish species in lakes of northeastern North America. Ontogenetic variation in tissue δ^{13}C and δ^{15}N appears to be widespread in a diversity of aquatic consumers, and we have provided the first field-based evidence that δ^{13}C and δ^{15}N may be under the combined influence of size and age in some fishes. The nature and strength of the isotopic ratio vs body size relationships were also found to be related to physical habitat characteristics of the lakes, though not necessarily the same lake characteristics for all species. Finally, our study provides the first quantification of how ontogenetic variation contributes to isotopic niche size.

Our results should first be considered in the context of the scope of the data used and the methods of analysis. The shape and strength of observed ontogenetic relationships will depend to some extent on the range of developmental stages examined; the wider the range, the more likely it will span shifts in habitat use, trophic ecology, or metabolism. But ontogenetic shifts do not necessarily follow the same body size trajectory for all species. For example, all fishes examined in this study begin feeding at very small body size (< 0.5 g) and all may be expected to consume progressively larger invertebrate prey during their early development, but piscivores would be expected to progress to the largest prey as adults. Our fish sampling only covered small body sizes for the forage fish guild. Most of the piscivores and benthivores analyzed for the current study were in the 200 – 2000 g size range which missed ontogenetic variation in early life; this may have had a bigger effect on detecting ontogenetic variation in benthivores than piscivores. We also assessed all ontogenetic patterns using a relatively simple isotope ratio vs log body mass linear model. Though this appeared to offer the best fit across the full data set, it may not have captured more subtle variability in ontogenetic patterns among species and populations.

The observed stable isotope composition of an individual consumer is a function of the isotopic composition of its diet, the isotopic discrimination as the diet is assimilated and incorporated into the consumer's body, and the time to reach equilibrium between the diet and body. Numerous factors may influence each of these components, and ultimately, the interpretation of stable isotope data (Shipley and Matich 2020). Ontogenetic patterns in stable isotope compositions of consumers are usually assumed to be diet related, though isotopic discrimination and tissue turnover may also vary with body size and could influence these relationships.

The positive $\delta^{15}N$ vs body size relationships we observed for most taxa were as expected. For aquatic consumers, and piscivores in particular, prey size tends to increase with predator size (Cohen et al. 1993; Brose et al. 2006; Gaeta et al. 2018). The ontogenetic increase in maximum prey size consumed may also be accompanied by an increase in the range of prey sizes consumed (Scharf et al. 2000). At the population level, ontogenetic shifts in both size and species compositions of diets have been identified through stomach content analysis for various freshwater fishes, including some of the species examined in the current study (Brandt 1986; Amundsen et al. 2003; Kahilainen and Lehtonen 2003). Increasingly, ontogenetic shifts in diet have been inferred from changes in tissue stable isotope ratios in a variety of fishes (Grey 2001; Overman and Parrish 2001; Murchie and Power 2004; Nakazawa et al. 2010; Hertz et al. 2016; Thelen et al. 2024). At the community level, larger organisms tend to have higher δ^{15}N in food webs of both boreal lakes (France et al. 1998; Persaud et al. 2012) and marine systems (Jennings et al. 2001). We found that within-population δ^{15}N vs body size slopes were stronger and more consistently positive than δ^{13}C slopes across the range of biota examined, and this is consistent with many earlier studies. Because δ^{15}N undergoes stepwise enrichment with each trophic transfer (Minagawa and Wada 1984), and trophic level is tightly linked to the size structure of aquatic food webs (Scarf et al. 2000), an individual consumer's trajectory along the δ^{15}N gradient is typically unidirectional and positive. We found significant positive relationships in all piscivores, most forage fishes and some benthivores and macroinvertebrates. Surprisingly, we even saw this trend in filter-feeding clams.

We expected that δ^{13}C vs body size relationships would be weaker and more variable than δ^{15}N relationships, both within and among taxa. The δ^{13}C of aquatic consumer tissues is tied to primary production source (France 1995; Hecky and Hesslein 1995), and consumer foraging behaviour can move bidirectionally along this gradient depending on prey availability or environmental conditions. The relative sizes and availabilities of prey items originating from allochthonous or pelagic primary production (^{13}C-depleted) and benthic primary production (^{13}C-enriched) are probably much more variable from lake to lake than the size-based food chain structure that influences $\delta^{15}N$. Accordingly, ontogenetic feeding shifts between offshore pelagic and nearshore benthic production are less predictable for most taxa, and the contributions from each source can become increasingly blended moving up the food chain (Schindler and Scheuerell 2002). Nevertheless, we did observe significant ontogenetic trends in $\delta^{13}C$ for some taxa and these trends were usually positive (northern pike, lake whitefish, rainbow smelt, yellow perch) suggesting an increasing reliance on benthic primary production with increasing body size. In contrast to our results, predatory fishes of the offshore shelf of northern Newfoundland show more consistent increases in both $\delta^{13}C$ and $\delta^{15}N$ with increasing body size (Krumsick and Fisher 2019) possibly reflecting differences in habitat gradients of $\delta^{13}C$ between marine and freshwater systems.

Variability in isotopic composition between consumer and prey, reflected in tissue-diet discrimination factors (TDDFs), may also play a role in our observed ontogenetic trends. Though many studies commonly assume fixed values of TDDFs for trophic transfers in aquatic food webs (e.g., + 0.8 ‰ for $\delta^{13}C$, + 3.4 ‰ for $\delta^{15}N$) laboratory experiments have shown them to be quite variable among different combinations of consumer and prey (McCutchan et al. 2003; Caut et al. 2009). In addition, fish that consume less food and grow slowly or have low body condition often have higher $\delta^{15}N$ (Trueman et al. 2005; Bowes et al. 2014; Doi et al. 2017), suggesting that TDDFs may vary inversely with growth. Laboratory experiments to determine TDDFs of fish usually focus on smaller and younger fish as they have high growth potential and can equilibrate with their diet relatively quickly. Such experiments rarely extend to larger and older fish and, as a result, it is unknown if TDDFs exhibit any systematic change with increasing body size in fishes that is independent of a change in diet.

The estimated isotopic discrimination between diet and consumer also depends in part on the tissue composition of the consumer considered (McCutchan et al. 2003). In particular, lipids tend to be ^{13}C-depleted relative to other tissue constituents (Focken and Becker 1998) and various methods have been proposed to account for this effect (Post et al. 2007; Logan et al. 2008). We used tissue C:N ratio as an index of lipid content in our analyses and most of the tissues analyzed were considered to have low lipid content (C:N < 4). Nevertheless, we detected significant negative relationships between δ^{13}C and C:N for many taxa, and including C:N as a second covariate in our ontogenetic models did alter δ^{13}C results somewhat. However, we still found significant relationships between δ^{13}C and δ^{15}N and body size for many taxa even after accounting for variation due to tissue C:N. This is probably because relationships between tissue C:N and body size were relatively weak and variable across our sampled taxa (Table S3) and thus C:N was less likely to confound stable isotope ratio vs body size relationships.

Isotopic change in consumers' bodies happens both through addition of new tissue (growth) and replacement of existing tissue (catabolic turnover), and these processes take time (Fry and Arnold 1982). Thus, the isotopic composition of a consumer's body does not necessarily reflect what was being consumed at the time of sampling but instead an averaging of what was consumed over some period prior to sampling. The length of this time period is related in part to the type of tissue analyzed because different tissues exhibit different isotopic turnover rates (Boecklen et al. 2011). We primarily used muscle, considered a good standard for isotope analysis because of its relatively slow turnover and consistent determinations (Hesslein et al. 1993; Pinnegar and Polunin 1999; Boecklen et al. 2011). Analysis of tissue with more rapid turnover (e.g., liver) may have provided data that better reflected recent diet but may also have provided noisier data due to seasonal variability (Perga and Gerdeaux 2005). Isotopic turnover rate slows with increasing body size for a wide range of taxa and tissue types (Thomas and Crowther 2015; Vander Zanden et al. 2015). Thus, isotopic compositions reflect a relatively longer pre-sampling period for large individuals than for small individuals in a population. This would tend to make isotope ratio vs body size slopes somewhat lower than would be expected if isotope turnover times were size invariant. Analyses of ontogenetic patterns in isotope ratios need to take account of ontogenetic changes in isotopic turnover that can introduce time lags and affect interpretation (Hertz et al. 2016).

Body size and age are closely related traits and their relationship over an organism's lifespan is typically curvilinear. Body size tends to increase linearly with age in the pre-maturation period and then increase at a decelerating rate following maturation as resource allocation shifts from somatic growth to reproduction (Lester et al. 2004). We may expect that tissue turnover slows and the ratio of growth to catabolic turnover also declines over this trajectory (Martinez del Rio et al. 2009). As expected, our analysis revealed that muscle δ^{13}C and δ^{15}N were related to age in large-bodied fishes, similar to their relationships with body size. Age was a stronger predictor than body size in some species, particularly for δ^{15}N. This was the case for lake trout, the piscivore with the widest age range in our data set, as well as for the piscivore walleye and the benthivores lake whitefish and white sucker. For most species we found evidence of a combined size and age effect, suggesting that observed ontogenetic variation in δ^{13}C and δ^{15}N may arise from growth-related changes in isotopic discrimination and/or tissue turnover, in addition to changes in diet. Few previous studies have reported relationships between C and N stable isotope ratios and growth rate in wild fishes (Johnston et al. 2021).

After accounting for age or body size effects, we found δ^{13}C tends to be positively related and δ^{15}N tends to be negatively related to growth rate. Similar trends have been reported between isotopic ratios and growth for laboratory-based experiments with invertebrates where growth was manipulated by either temperature (Power et al. 2003) or ration (Gorokhova 2018). The negative δ^{15}N vs growth rate trend is also consistent with results from laboratory growth studies on juvenile fish (Gaye-Siessegger et al. 2004; Trueman et al. 2005) and reports of elevated δ^{15}N in fasting animals (Doi et al. 2017).

We expected that ontogenetic trends in δ^{13}C and δ^{15}N for many aquatic consumers could be related to physical features of their habitats through their influence on foraging conditions and the biotic community composition of their food webs. Furthermore, we predicted that environmental conditions could have similar effects on the trophic ecologies of different species, and this may be manifested in interspecific concordance of ontogenetic slopes. With a few exceptions, we did not find strong concordance in ontogenetic slopes between co-habiting pairs of species within trophic guilds, nor between species deemed to be aligned on trophic pathways (e.g., piscivore and forage fish pairings). Similarly, a study of piscivores of northern Minnesota lakes found that interspecific concordance in slopes of isotopic-based trophic metrics was weak, despite relatively strong concordance between other isotopic-based food web metrics (Thelen et al. 2024). This suggests that the suite of factors driving ontogenetic trends in stable isotope ratios vary among species and populations. Though numerous studies have demonstrated significant relationships between isotopic niche metrics of northern fishes and habitat characteristics of the lakes they occupy (Dolson et al. 2009; Bartels et al. 2012; Hayden et al. 2014; Tunney et al. 2014; Stasko et al. 2015; Tunney et al. 2018; Johnston et al. 2019), relatively few have examined how ontogenetic patterns in isotopic compositions vary with respect to lake characteristics (Thelen et al. 2024). We found that ontogenetic slopes, particularly δ^{15}N vs body size, of various species were related to habitat characteristics representing ecosystem size and structure, water clarity and climate, though there was no clear combination of consistently strong predictors among the species examined.

Measuring dispersion of individual consumers in δ^{13}C - δ^{15}N space as an index of trophic niche size (Newsome et al. 2007) is now common in food web research and methods for quantifying this dispersion continue to evolve (Layman et al. 2007; Jackson et al. 2011; Cucherousset and Villéger 2015; Swanson et al. 2015; Eckrich et al. 2020). Such indices, often referred to as isotopic niche size, reflect net isotopic variation among individuals and is assumed to be primarily driven by diet. The relatively small isotopic niche sizes of captively-reared lake trout (fixed diet composition) compared to those of wild lake trout lends support to this assumption (Johnston et al. 2021). Our results demonstrate that isotopic niche size estimates for wild fish populations can be strongly influenced by ontogenetic variation in δ^{13}C and δ^{15}N. Because of the wide variability in the nature and strength of ontogenetic relationships, both among species and populations, the contribution of ontogenetic variation to isotopic niche space can also be highly variable. This introduces considerable scope for confounding effects of body size in studies comparing isotopic niches across spatio-temporal scales and steps should be taken to account for these effects.

Conclusion

Ontogenetic variation in isotopic compositions of aquatic consumers is both widespread and highly variable among species and populations in north temperate lakes. This variation needs to be recognized and accounted for when calculating and comparing isotopic-based food web metrics that characterize the position, size and shape of trophic niches. Our findings contribute to the application and interpretation of food web metrics, as well as to our understanding of trophic interactions and community dynamics in aquatic systems.

Supplementary Information The online version contains supplementary material available at https:// doi. org/ …….

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Declarations

Conflict of interest The authors declare that there are no conflicts of interest

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