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Comparisons among three diet analyses demonstrate multiple patterns in the estimated adult diet of a freshwater piscivore, *Salvelinus namaycush*

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ABSTRACT

Understanding trophic interactions is critical for successful resource management. However, studying diet patterns (e.g., spatial and seasonal changes) can require extensive effort. Using individual analyses to interpret patterns may be further complicated by assumptions and limitations of the analytical approach. We investigated and compared predicted adult lake trout (*Salvelinus namaycush*) diet composition and patterns using stomach content analysis (SCA), fatty acid analysis (FAS), and stable isotope analysis (SIA) individually and simultaneously. The three analyses were conducted for fall-captured fish in Lake Ontario and provided different diet composition estimates; SCA suggested alewife (*Alosa pseudoharengus*) was dominant by frequency and mass, while FAA and SIA suggested rainbow smelt (*Osmerus mordax*) contributed the most based on similarity among fatty acid signatures and two-stable isotope (carbon and nitrogen) mixing models, respectively. We hypothesize the disagreement among diet estimates is a result of a seasonal shift in diet variably expressed due to differing extent of time reflected by the diet metric: hours to days for SCA, weeks to months for FAA and several months for SIA. Despite variability in diet composition estimates among methods, similar patterns in lake trout diet were observed among the three diet analyses; the contribution of alewife in lake trout diet was greater for larger individuals and for males compared to females, particularly in the east and northeast regions of the lake where alewife density was relatively low. Thus, the complementary results from the three analyses suggest that length, location, sex, and season all influence lake trout diet. Individually, analyses often failed to identify these patterns in lake trout diet with significance, and some of the patterns have not been observed in previous studies of lake trout diet in Lake Ontario. The thorough description of lake trout diet obtained from a single sampling season demonstrates how simultaneous use of multiple diet analyses may allow investigation of spatial and seasonal diet composition and with reduced sampling effort.

1. Introduction

Quantifying fish diets can be challenging due to limited direct observation of feeding. Knowledge of predator–prey relationships, however, is a critical component for understanding species interactions and maintaining balance in managed ecosystems (Christensen, 1996; Kitchell et al., 2000). For many decades, researchers have investigated diet composition of fishes using stomach content analyses (SCA) (e.g., Hynes 1950), while various biochemical tracers have continued to develop and expand in their application (Nielsen et al., 2018). Studies have also included multiple diet analyses to develop a stronger, more complex understanding of predator–prey interactions (e.g., Happel

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et al., 2015; Young et al., 2018).

Examination of stomach contents has been used frequently and provides information such as frequency and mass of diet items (e.g., Hyslop, 1980; Ahlbeck et al., 2012). Analysis of stomach contents arguably provides the greatest taxonomic resolution due to the direct observation of prey. However, SCA can be limited in its application due to a short-term representation of diet (hours to days; He and Wurtsbaugh 1993) as well as potential error from partially digested, and often unidentifiable, remains (Tirasin and Jørgensen, 1999; Baker et al., 2014). Digestion rates can also vary among consumed prey creating challenges for quantifying diet (Ahlbeck et al., 2012). In addition, stomachs may be empty at the time of capture or influenced by regurgitation, depending on the method of capture and species of interest (Bowman, 1986; Sutton et al., 2004). Therefore, to obtain a full understanding of a species' diet composition based on stomach contents alone, sampling events that target many individuals are likely needed multiple times throughout the year.

Biochemical methods including fatty acid (FAA) and stable isotope (SIA) analyses are alternative techniques that provide estimates of diet composition based on information obtained from every individual sampled (Budge et al., 2006; Boecklen et al., 2011). These methods represent an assimilation of diet items consumed over an extended period, with the specific timeframe dependent on the predator species and tissues sampled (Vander Zanden et al., 2015; Happel et al., 2016). When biochemical data are available for predators and their expected prey, these methods can be used to investigate predator–prey interactions and estimate the relative contributions of prey species with mixing models (e.g., MixSIAR, Stock et al., 2018; QFASAR, Bromaghin 2017). However, mixing models require information regarding differential assimilation and turn-over rates of isotopes and fatty acids following consumption (Nielsen et al., 2018). Additional challenges for biochemical methods include overlap of biochemical signatures among prey species and costly instrumentation for laboratory analyses.

Fatty acids are transferred relatively conservatively from prey to predators (Lovern 1935), allowing diet estimates to be generated based on predator fatty acid composition (e.g., Iverson, 1993; Budge et al., 2006; Happel et al., 2016). During digestion, fatty acids remain largely intact and are retained within the predator for days to months depending on metabolic demands (Iverson 2009). For salmonines specifically, studies have shown that the majority of an individual's fatty acid composition likely represents prey consumed within the previous 8–12 weeks but may include prey consumed up to 22 weeks prior (Budge et al., 2011; Happel et al., 2016). To study trophic interactions with fatty acid data, fatty acids signatures commonly composed of 20 + individual fatty acids can be used to determine similarities among individuals or species (Budge et al., 2006) or quantitative estimates of specific prey items (Iverson et al., 2004). Alternatively, individual fatty acids have been used to demonstrate prey origins (benthic vs pelagic) (Kelly and Scheibling, 2012; Paterson et al., 2014) or, in relatively simple systems with few prey options, associations with specific prey items (Happel et al., 2017).

Stable isotope analyses estimate trophic interactions based on distinct isotopic ratios. Carbon (δ^{13}C) and nitrogen (δ^{15}N) isotope ratios have been used frequently to determine food web interactions and provide insight into an individual's original energy sources (e.g., benthic or pelagic) and trophic positioning, respectively (Boecklen et al., 2011). Turnover rates for stable isotopes are dependent on tissue accumulation (i.e., growth rate) and metabolic replacement (Hesslein et al., 1993). Therefore, the isotopic signature of individuals with a fast growth rate are generally plastic and could reflect a change in diet while the signature of slow-growing individuals would likely represent a long-term summary of diet with changes dependent on metabolic turnover rates (Hesslein et al., 1993; Rush et al., 2012). Isotopic turnover rates also vary based on many factors that influence tissue accumulation and metabolic replacement including taxa, tissue, life stage, and body size (Boecklen et al., 2011; Vander Zanden et al., 2015). For fishes, white muscle tissue is commonly used in isotope analyses and has an isotopic half-life that ranges from days to potentially years, depending on the life stage, growth rate, body size, and catabolic tissue replacement rate of the individual (Weidel et al., 2011; Vander Zander et al., 2015).

Throughout the Great Lakes, food web structure has been altered frequently by the introduction of non-native species, particularly in the prey fish community (e.g., Bronte et al., 2003; Mills et al., 2003). In addition, restoration of native lake trout (*Salvelinus namaycush*) has been a major objective with varying degrees of success among the lakes (Krueger et al., 1995). In Lake Ontario, regional natural recruitment has occurred, but the overall low abundance of naturally-produced adult lake trout suggests that impediments to lake trout restoration are still present, many of which may be diet related (e.g., prey quantity and quality) (Lantry et al., 2014, 2019). Therefore, obtaining a thorough understanding of lake trout diet may help understand the significance of proposed impediments to restoration efforts. Numerous studies have investigated adult lake trout diet in Lake Ontario using SCA (e.g., Lantry, 2001; Dietrich et al., 2006; Mumby et al., 2018), FAA (Happel et al., 2017; Futia et al., 2019), and SIA (Rush et al., 2012; Colborne et al., 2016; Mumby et al., 2018) and have generally identified three main prey species: alewife, *Alosa pseudoharengus*; rainbow smelt, *Osmerus mordax*; and round goby, *Neogobius melanostomus*. Across these studies, results demonstrate that lake trout diets are primarily composed of prey species invasive to Lake Ontario; however, no studies to our knowledge have investigated lake trout diet in Lake Ontario using the three analyses simultaneously.

The goal of this study was to determine if simultaneous use of stomach content, fatty acid, and stable isotope analyses can provide new insight into predator diet, and to provide a more robust interpretation of lake trout diet in Lake Ontario to potentially facilitate future restoration efforts. To address this goal, prey contributions to lake trout diet were estimated using SCA, FAA, and SIA; patterns in diet composition based on three common environmental factors (location of capture, predator total length, and predator sex) were investigated; and results from the three diet analyses were compared among each other and to previous studies investigating lake trout diet in Lake Ontario.

2. Materials and methods

2.1. Fish collection and sampling

Lake trout were collected using bottom-set, monofilament gill nets set overnight at 12 sites in Lake Ontario between August and October 2013 by the U.S. Geological Survey (USGS), the New York State Department of Environmental Conservation (NYSDEC), and the Ontario Ministry of Natural Resources and Forestry (OMNRF) (Fig. 1; Table 1). Survey gill nets consisted of nine 15.2- x 2.4-m panels of 51- to 151-mm

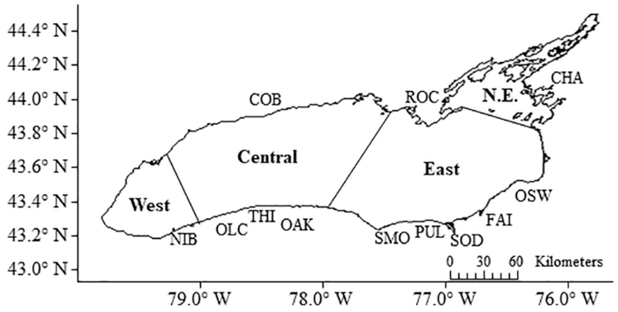

Fig. 1. Approximate locations of 12 sampling sites within four regions (bold font) of Lake Ontario. The Northeast region is abbreviated as N.E. and sampling locations are abbreviated as follows: Cobourg, COB; Rocky Point, ROC; Charity, CHA; Oswego, OSW; Fair Haven, FAI; Sodus, SOD; Pultneyville, PUL; Smoky Point, SMO; Oak Orchard, OAK; Thirty Mile Point, THI; Olcott, OLC; and Niagara, NIA.

Table 1
Sample summary including sampling locations, regions, date of collection, sample size (N), range of total length, and mass (average ± standard deviation) of lake trout collected throughout Lake Ontario. The agency responsible for each sampling event is included as well (Ontario Ministry of Natural Resources and Forestry: OMNRF; U.S. Geological Survey: USGS; New York State Department of Environmental Conservation: NYSDEC). Samples are listed based on their geographic positions starting at the western-most Canadian location and moving clockwise around the lake and ending at the western-most location within the United States.

Sampling location	Region	Date	N	Total length (mm)	Mass (g)	Agency
Cobourg	Central	09/03/2013	10	665–780	4,630 ± 972	OMNRF
Rocky Point	East	09/24/2013	3	635–692	3,459 ± 388	OMNRF
Rocky Point	East	10/17/2013	4	710–740	4,369 ± 569	OMNRF
Charity	Northeast	09/06/2013	15	626–801	4,652 ± 1,123	NYSDEC
Oswego	East	09/15/2013	10	576–766	3,828 ± 995	USGS
Fairhaven	East	09/16/2013	13	501–788	3,385 ± 1,043	NYSDEC
Sodus	East	09/14/2013	10	609–776	3,822 ± 1,111	USGS
Pultneyville	East	09/15/2013	14	594–747	3,724 ± 805	NYSDEC
Smokey point	East	09/10/2013	15	603–816	3,541 ± 973	NYSDEC
Oak Orchard	Central	09/10/2013	10	634–826	3,804 ± 1,185	USGS
Thirty Mile Point	Central	09/09/2013	11	629–761	3,801 ± 856	USGS
Olcott	Central	09/06/2013	10	576–817	3,829 ± 1,115	USGS
Niagara Bar	West	09/08/2013	10	648–760	4,247 ± 749	USGS

mesh (stretched measure) in 12.5-mm increments. At 11 of the 12 sites, four survey nets were fished along randomly chosen transects, parallel to contours beginning at the 10 °C isotherm and proceeding deeper in 10-m increments. At the one site located in the eastern outlet basin (CHA in Fig. 1) three nets were fished due to the depth of the thermocline. Sites were grouped into four regions (Northeast, East, Central, West; Fig. 1) based on thermal regions defined by Stewart and Robertson (1991). Individual lake trout may have moved between these regions based on typical dispersal ranges – within 100 km of spawning locations – observed in Lake Ontario and other systems in the Great Lakes (reviewed in Binder et al., 2021; Ivanova et al., 2021). However, such movements would have been limited to individuals captured near regional boundaries given the range of longitudinal movements is lowest in the months when our sampling occurred (S. Ivanova *pers. comm.*). Upon capture, total length (mm), mass (g), and sex of lake trout were recorded. Few immature fish were captured so only adult fish (≥500 mm) were included in this study ($N = 135$; Table 1). Samples of belly flap and muscle were collected, frozen on dry ice, and transported to SUNY Brockport where they were stored at −80 °C until processing. All sampling and handling of fish was carried out in accordance with guidelines for the care and use of fishes by the American Fisheries Society (Use of Fishes in Research Committee, 2014).

2.2. *Stomach content analysis*

Stomach contents were extracted from lake trout directly after capture and intact prey fish were identified to species, counted, and measured for total length when possible. Remaining identifiable prey (i.e., invertebrates) were also counted. Estimates of lake trout diet composition were quantified using mass-based methods, which provide an accurate representation of diet when prey are large-sized (Ahlbeck et al., 2012). For samples collected by NYSDEC and USGS, approximate mass of intact prey fish was determined with length-weight models based on prey data obtained from assessment surveys conducted in 2013 (Walsh and Connerton, 2014; Weidel and Connerton, 2014; Weidel et al., 2014). For prey fish whose length could not be measured, approximate mass was estimated using a species-specific mean mass calculated from the estimated mass of all intact prey fish collected from lake trout stomachs in 2013 (Lantry 2001). For samples collected by the OMNRF, mass of each diet item was quantified by volume displacement, assuming a density of 1 g/mL.

2.3. *Fatty acid analysis*

In comparisons between belly flap and muscle tissues, belly flap provided more accurate estimates of diet based on fatty acid composition (Budge et al., 2011; Happel et al., 2020) and, therefore, was used for FAA. Skin was removed from each frozen belly flap tissue sample and the remaining tissue was homogenized, from which a 0.5 g sample was taken. Total lipids were extracted with chloroform/methanol solution (2:1, v/v) containing 0.01% butylated hydroxytoluene as an antioxidant (Folch et al., 1957). After extraction, the solvent was evaporated under a stream of nitrogen gas and total lipid content was determined. A known amount of internal standard (nonadecanoic acid) was then added to each sample based on the lipid content of the sample (1 mL of standard for 8 mg of total lipids) (Czesny and Dabrowski 1998). Fatty acid methyl esters (FAMEs) were prepared using 1.5 mL of 0.5 M NaOH in methanol and 2 mL of borontrifluoride methanol with each sample (Metcalfe and Schmitz 1961). FAMEs were separated using a gas chromatograph/mass spectrometer (Agilent 7890A Gas Chromatograph, Agilent Technologies, Inc., Wilmington, DE, USA) with a mass selective detector (Agilent Technologies, Inc., 5975C), a capillary column (Omegawax^{TM} 320, 30 m × 0.32 mm × 0.25 μm film thickness, Supelco, Bellefonte, PA, USA), and an autoinjector (Agilent Technologies, Inc., 7693). Instrument blank samples were run after every 10 samples for quality assurance. Twenty-six individual FAMEs were identified by fragment ions and comparing their retention times to the ones of authentic standard mixtures (FAME mix 37 components, Supelco). The proportion of each FAME was determined as the concentration of an individual FAME, quantified by comparing peak areas with that of the internal standard, relative to the total concentration of all FAMEs detected. Fatty acid data were compared by proportions rather than concentrations to limit the effect of factors that influence total lipid content and are not related to diet composition (e.g., starvation). Simultaneous consideration of the proportions for each fatty acid represents the fatty acid signature (FAS) of an individual fish. Data for the fatty acid composition of common prey fishes (alewife, rainbow smelt, and round goby) captured during spring, summer, and fall of 2013 in the central region of Lake Ontario were obtained from Happel et al. (2017).

2.4. *Stable isotope analysis*

A skinless and boneless section of white dorsal muscle was sampled from each fish for SIA. Muscle tissue was processed and analyzed at the Trophic Ecology Laboratory at the University of Windsor’s Great Lakes

Institute for Environmental Research (Windsor, ON, CA; Colborne et al., 2016). Briefly, samples were freeze-dried then homogenized into a fine powder. Chemical extraction of lipids was performed with a 2:1 chloroform methanol solution (Bligh and Dyer 1959). Analysis of carbon (^{13}C:^{12}C) and nitrogen (^{15}N:^{14}N) isotopes were performed using a Delta Plus isotope-ratio mass spectrometer (ThermoFinnigan, San Jose, CA, USA) coupled to an elemental analyzer (Costech, Valencia, CA, USA). Carbon and nitrogen isotope standards were run every 12 samples for quality assurance. The ratio of each isotope (i.e., heavy to light isotope; δ) was determined as the difference between the muscle sample and an international standard material: $\delta X = (R_{sample}/R_{standard}\text{-}1)$ where X is the isotope (^{13}C or ^{15}N) and R is the ratio of interest ($\delta^{13}C$ or $\delta^{15}N$). Isotopic ratios are expressed as parts per mil (‰). The standard materials for carbon and nitrogen isotopes were Vienna Pee Dee Belemnite and atmospheric nitrogen, respectively. Lipid-corrected stable isotope data for common prey fishes captured from all regions of Lake Ontario between April and November 2013 were obtained from Mumby et al. (2018).

2.5. *Statistical analysis*

For stomach contents, the percent contribution of each prey fish species within individual stomachs was calculated based on frequency and mass. Invertebrate percent contributions were excluded from statistical comparisons due to low frequency ($n = 1$) and average percent contribution (<0.1%). Therefore, the percent by mass reported for each fish species was calculated as the summed mass of all identifiable individuals of a species relative to the total mass of all identifiable fish within that stomach. Empty stomachs and stomachs only containing unidentifiable prey were excluded from analyses.

Fatty acid data could not be analyzed using mixing models because appropriate calibration coefficients are currently unavailable. Therefore, quantitative diet estimates could not be determined from these data. Comparisons among FASs were investigated with multivariate analyses using PRIMER v.6 (Primer-E, Plymouth, U.K.). The importance of each prey fish species towards lake trout diet composition was estimated by an analysis of similarity (ANOSIM; test statistic = *R*), which determined the pairwise dissimilarity between the FASs of lake trout and each prey fish species based on a Bray-Curtis similarity matrix. Values of *R* between 0.25 and 0.5 were considered different with some overlap, 0.5 to 0.75 as different, and 0.75 to 1.0 as highly different. To investigate patterns in lake trout diet composition, relative contributions for each prey fish species were estimated from ratios of prey-specific fatty acids (i.e., fatty acids with the greatest interspecies variation) (Happel et al., 2017). The equation used to estimate relative contributions of each prey species was $rc_z = \frac{FattyAcid_z}{FattyAcid_y + FattyAcid_x}$, where rc_z is the relative contribution of species z, and the three fatty acid values (z, y, and x) represent the percent contribution of the three prey-specific fatty acids. Prey-specific fatty acids were identified as the individual fatty acids that contributed most to differences in FASs between prey fish species based on similarity percentages (SIMPER) and were the most influential for distinguishing prey species based on loading values from principal component analysis (PCA). The PCA generated five principal components (PC), of which the two PCs that explained the greatest amount of variation among prey species were retained; a biplot of these two PCs provided a visual of the dissimilarity among samples. To ensure a reduced model including only the three prey-specific fatty acids could discriminate the three prey fish species, global ANOSIM results were compared between the reduced model and a full model that included data for all 26 fatty acids.

Stable isotope results for lake trout were estimated using Bayesian two-source isotope mixing models in the MixSIAR package (Stock et al., 2018). Isotope mixing models were used to determine lake trout diet based on lipid-corrected $\delta^{13}C$ and $\delta^{15}N$ values of alewife ($N = 802$), rainbow smelt ($N = 355$), and round goby ($N = 448$) as possible prey species ("sources" variable). Trophic fractionation estimates for $\delta^{13}C$ and $\delta^{15}N$ were 0.39‰ (SD = 1.30) and 3.40‰ (SD = 0.98), respectively (Post 2002). To estimate prey contributions for each lake trout separately, a fixed effect variable with a unique value for each lake trout (i.e., fish identification number) was included. The model was run for 1×10^5 simulations with a 5×10^4 burn-in and thinning of 50. Diet proportion estimates for each lake trout were obtained from the model output by extracting the *p.fac1* parameter and these estimates were used in subsequent analyses.

The relationships between various independent variables (lake trout region, length, and sex) and estimated prey contribution were determined for all three diet analyses. Diet estimates from SCA were compared using univariate statistics because low sample sizes for combinations of all independent variables prevented multivariate comparisons. Contributions of each prey fish species by mass and frequency failed normality assumptions, so comparisons among regions were performed using Kruskal-Wallis tests (test statistic = χ). Significant results were followed by Dunn's test with Bonferroni corrections for pairwise comparisons (Dinno 2017). Correlations between length and contributions of each prey fish species were analyzed with Spearman's rank correlation (test statistic = r). Lastly, differences in the contributions of each prey fish species by lake trout sex were analyzed using Fisher's exact test for frequency data and Mann-Whitney *U* test (test statistic = *U*) for mass data.

For FAA and SIA diet estimates, the relationships among estimated contributions of each prey fish species and predator length, sex, and region of capture (i.e., main and interaction effects) were determined by stepwise multiple linear regression using R (R Core Team 2018). Multiple linear regression was performed instead of analysis of covariance due to failed assumptions for homogeneity of regression slopes. Significance of the individual predictor variables was determined by analysis of variance (ANOVA, test statistic = partial *F*). To determine the magnitude of significant relationships between predictor variables and diet contributions, regression coefficients (β) and corresponding p values were determined. For interpretation of categorical variables (sex and region), one category was set as the baseline that other categories were compared to (e.g., regression coefficients for the west, east, and northeast regions are represented as the change relative to the central region). To interpret interaction effects between variables, simple linear regression and ANOVA were conducted. The SIA data, measured as proportions, were logit transformed for the regression analyses to improve normality and homogeneity of the residuals (Warton and Hui 2011).

Diet estimates for individuals that were common to all three analyses ($n = 60$) did not differ significantly (Mann-Whitney U, $U \geq 1{,}810$, $p \geq 0.2267$) from the diet estimates for the remaining fish included for each analysis (Supplemental Table 1). Therefore, all diet estimates from each analysis were included in comparisons. For all analyses, significance was set at $p < 0.05$ although marginal significance is noted for p values between 0.05 and 0.10. Common abbreviations used throughout the paper are defined in Table 2 and samples sizes by region and sex are listed in Table 3.

3. Results

3.1. *Stomach content analysis*

Stomach contents were collected and analyzed for 131 lake trout. Sixty-five (50%) of the lake trout (total length range: 501–826 mm) contained prey items in their stomachs, of which 51 contained only identifiable fish, four contained only unidentifiable remains, and the remaining 10 contained some combination of identifiable fish, unidentifiable remains, or invertebrates (Table 4). Among all stomachs, there were three fish species (alewife, rainbow smelt, and round goby) and one invertebrate species (undetermined species of tapeworm) observed. Only a single identifiable prey species was present in 47 of the 65 stomachs containing prey (alewife: 41; rainbow smelt: 1; round goby: 5). In the 61 stomachs containing identifiable prey, alewife was the most

Table 2
Definitions for common abbreviations for diet analyses, statistical test, and biochemical variables.

Diet Analyses	
FAA	Fatty acid analysis
SCA	Stomach contents analysis
SIA	Stable isotope analysis
Statistical Tests	
ANOSIM	Analysis of similarity
ANOVA	Analysis of variance
PC	Principal component
PCA	Principal component analysis
SIMPER	Similarity percentages
Biochemical Variables	
FAS	Fatty acid signature
DHA	Docosahexaenoic acid
OA	Oleic acid
POA	Palmitoleic acid

Table 3
Sample sizes by region and sex for lake trout included in statistical comparison of diet composition. Values are included for three separate diet analyses: stomach contents analysis (SCA), fatty acid analysis (FAA), and stable isotope analysis (SIA).

	SCA		FAA		SIA	
	Female	Male	Female	Male	Female	Male
West	3	0	10	0	9	0
Central	14	3	34	7	33	6
East	15	15	41	27	38	24
Northeast	6	5	9	6	9	6

Table 4
Lake trout diet estimates based on stomach content analysis (SCA; N = 61), fatty acid analysis (FAA; N = 135), and stable isotope analysis (SIA; N = 119). SCA only included stomachs that contained diet items and are presented as the number of lake trout containing each diet item (frequency) and the percent contribution by mass of each diet item. Invertebrates and unidentified remains (UIR) were not included in calculations for mass. Results for the FAA are presented as ratios of fatty acid proportions that correspond to prey fish species (alewife: oleic acid ratio, rainbow smelt: docosahexaenoic acid ratio; round goby: palmitoleic acid ratio). Results from the SIA are presented as the percent contribution of each prey fish species towards overall lake trout diet based on mixing model estimates. Results for SCA by mass, FAA ratios, and SIA percentages are reported as average ± standard deviation.

		Alewife	Rainbow smelt	Round goby	Invertebrate	UIR
SCA	Frequency	55	4	6	1	14
	Mass (%)	88.9 ± 30.0	2.5 ± 13.3	8.6 ± 27.7	<0.1	–
FAA	Ratio	1.43 ± 0.13	0.25 ± 0.04	0.27 ± 0.05	–	–
SIA	Percentage	20.1 ± 1.5	61.3 ± 2.4	18.6 ± 1.3	–	–

frequent prey fish, occurring in 55 (90.2%) stomachs, followed by round goby (6, 9.8%) then rainbow smelt (4, 6.6%). Alewife was also the most abundant prey by mass, followed by round goby then rainbow smelt (Table 4).

Stomach content composition differed significantly based on region and, with marginal significance, based on sex. Rainbow smelt contributions to lake trout stomach contents differed significantly among regions by frequency (Kruskal-Wallis, $\chi = 9.07$, $p = 0.024$) and mass (Kruskal-Wallis, $\chi = 9.07$, $p = 0.028$), with significantly larger contributions in the northeast region compared to the central (Dunn's test, frequency: $p = 0.014$, mass: $p = 0.016$) and east (Dunn's test, frequency: $p = 0.014$, mass: $p = 0.016$) regions. No significant spatial differences occurred for contributions of alewife or round goby (Kruskal-Wallis, $\chi < 3.50$, $p > 0.340$). Based on sex, the frequency of each prey fish species did not differ significantly (Fisher's exact test, $p \geq 0.250$); however, contributions of rainbow smelt and round goby combined were greater for females (24%) than males (4%) with marginal significance (Fisher's exact test, $p = 0.074$). By mass, alewife contributions to stomach contents were higher for males than for females with marginal significance (Mann-Whitney U, $U = 519.5$, $p = 0.058$) while no notable differences were observed for contributions of rainbow smelt or round goby. Lastly, there were no significant relationships between lake trout length and any prey fish contributions, based on frequency and mass (Spearman's rank correlation, $-0.09 \leq r < 0.06$, $p \geq 0.457$).

3.2. *Fatty acid analysis*

The FASs of prey fish species were different (ANOSIM, Global $R = 0.683$) and mainly separated by three fatty acids: palmitoleic acid (POA), oleic acid (OA), and docosahexaenoic acid (DHA) (SIMPER and PCA; Fig. 2A). These fatty acids were prey-specific in that each was substantially greater in one species than the other two (alewife: OA, round goby: POA, rainbow smelt: DHA). Using the two first PCs, nearly 75% of the original variability among prey FASs was explained, with PC_1 most strongly correlated with DHA (rainbow smelt; PCA, loading = 0.74) and OA (alewife; PCA, loading = -0.51) and PC_2 most strongly correlated with POA (round goby; PCA, loading = -0.79) and DHA

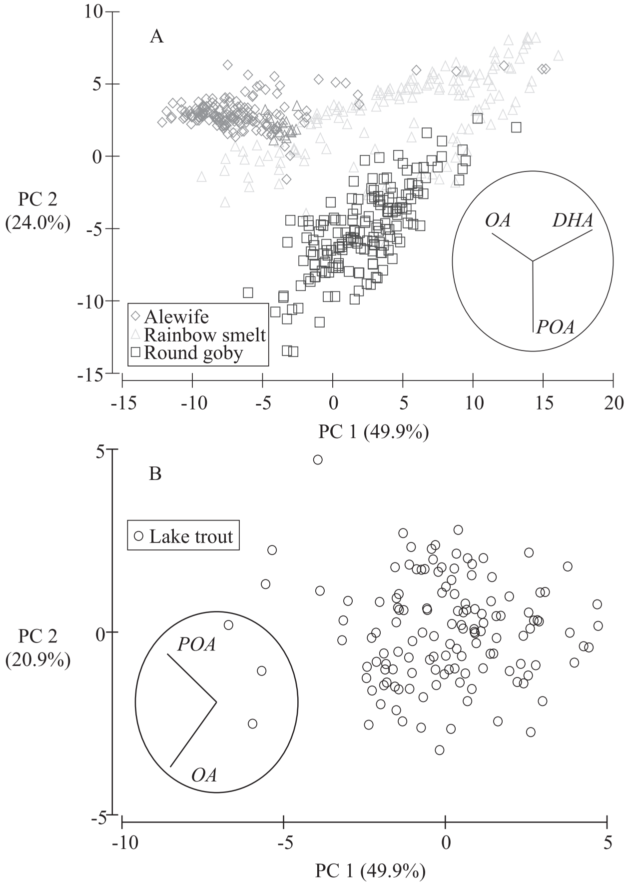

Fig. 2. Principal component analyses for fatty acid signatures of three common prey fish species (A) and lake trout (B) from Lake Ontario. Vectors for oleic acid (OA), docosahexaenoic acid (DHA), and palmitoleic acid (POA) are included based on their correlations with each principal component (PC). The percent of the original variation among fatty acid signatures that is accounted for by each PC is included in parentheses on the axis title.

(rainbow smelt; PCA, loading = 0.35). In addition, dissimilarity among prey species was maintained when only the three prey-specific fatty acids were compared (ANOSIM, Global *R* = 0.529).

The FASs were determined for all 135 lake trout sampled and were highly different from those of each prey species. Rainbow smelt FASs had the lowest dissimilarity compared to lake trout FAS (ANOSIM, *R* = 0.852), suggesting rainbow smelt had the greatest contribution towards lake trout diet, followed by alewife (ANOSIM, *R* = 0.916), and lastly round goby (ANOSIM, *R* = 0.927). Variability among lake trout FASs was mainly driven by POA and OA based on PCA (Fig. 2B; Supplemental Table 2). Together, PC_1 and PC_2 accounted for 70% of the original variability among lake trout FASs with both PC_1 and PC_2 being correlated with POA (PC_1: loading = −0.61, PC_2: loading = 0.54) and OA (PC_1: loading = −0.57, PC_2: loading = −0.72).

Based on the ratios of prey-specific fatty acids, predator length was the only significant predictor variable of diet contribution and was only significant for relative contributions of alewife and rainbow smelt (Fig. 3, Table 5). Regression coefficients indicate that, overall, alewife contributions to lake trout diets increased significantly with predator length, while rainbow smelt contributions decreased significantly with length (Table 6). Significant and marginally significant interactions were also present based on ratios of prey-specific fatty acids, including interactions between predator length and sex as well as length and region for rainbow smelt and round goby relative contributions (Table 5). When separated by sex, female total length had no significant correlations with rainbow smelt or round goby contributions while male total length had a significant negative correlation with rainbow smelt contributions (simple linear regression, $\beta = -3.01\ E^{-4}$; ANOVA, *F* = 5.77, p = 0.021) and a marginally significant positive correlation with round goby contributions (simple linear regression, $\beta = 2.70\ E^{-4}$; ANOVA, *F* = 3.81, p = 0.058). When separated by region, predator length had significant, negative correlations with rainbow smelt contributions in the northeast (simple linear regression, $\beta = -5.99\ E^{-4}$; ANOVA, *F* = 11.05, p = 0.005) and east regions (simple linear regression, $\beta = -1.80\ E^{-4}$; ANOVA, *F* = 4.05, p = 0.048); a significant positive correlation was also present between length and round goby contributions in the northeast region (simple linear regression, $\beta = 7.97\ E^{-4}$; ANOVA, *F* = 5.95, p = 0.030).

3.3. *Stable isotope analysis*

The carbon and nitrogen isotopic ratios were determined for 125 of the lake trout collected ($\delta^{13}C$ = –22.09 ± 0.37, $\delta^{15}N$ = 18.15 ± 0.36; Fig. 4). Based on our stable isotope mixing models, rainbow smelt contributions averaged over half of the estimated lake trout diet composition (61.3%, SD = 2.4), while alewife (20.1%, SD = 1.5) and round goby (18.6%, SD = 1.3) contributions were lower and similar to each other (Table 4). Using the estimated diet proportions from the mixing models, multiple linear regression models demonstrated significant or marginally significant patterns in diet estimates based on region and length (Fig. 5, Table 5). For the multiple regression model estimating the proportion of alewife, length was the most significant predictor variable followed by region (Table 5). The regression coefficient for length indicated a significant increase in estimated alewife proportions as lake trout length increased. Based on region, alewife proportions were significantly greater in the northeast relative to the central region but did not differ between other region comparisons (Fig. 5A, Table 6). Length was also the most significant predictor variable for rainbow smelt proportions, followed by region (Table 5). The estimated proportion of rainbow smelt in lake trout diet was significantly lower for larger individuals, and larger for lake trout captured in the east region relative to those from the central region with marginal significance (Fig. 5B, Table 6). Lastly, region was a significant predictor variable of round goby proportions in lake trout diet, with significantly lower proportions for lake trout from the east region relative to those captured from the central region (Fig. 5C, Tables 5 & 6).

4. Discussion

4.1. *Comparison of diet estimates*

Estimated lake trout diet composition varied among SCA, FAA, and SIA, including conflicting conclusions for the importance of different prey fish species. Results from the SCA strongly support alewife as the dominant prey of lake trout between August and October 2013, which is consistent with the substantially high abundance of alewife relative to other offshore prey fishes in Lake Ontario during 2013 (Walsh and Connerton, 2014; Weidel and Connerton, 2014; Weidel et al., 2014). Previous studies have also suggested alewife are the dominant prey of lake trout in Lake Ontario based on SCA (e.g., Dietrich et al., 2006; Mumby et al., 2018), FAA (Happel et al., 2017) and SIA (Rush et al., 2012; Mumby et al., 2018). In contrast, FAA and SIA results from our study suggested rainbow smelt had the largest contribution to lake trout diet (based on similarity of FAS), and contributions of alewife and round

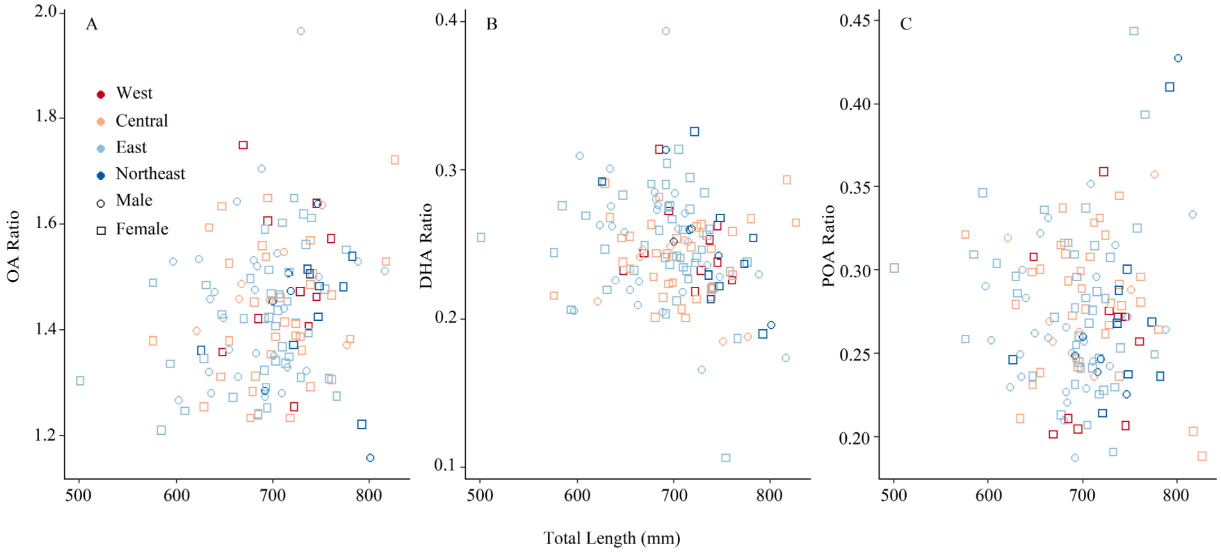

Fig. 3. Ratios of prey-specific fatty acids for alewife (oleic acid, OA; frame A), rainbow smelt (docosahexaenoic acid, DHA; frame B), and round goby (palmitoleic acid, POA; frame C) in lake trout stomach contents as a function of total length. Data are grouped by region (color) and sex (shape).

Table 5

Partial F statistics and corresponding p-values for main (predator length, sex, and region) and interaction effects from six multiple linear regression models. The dependent variable for each regression model was the estimated contribution for one of three common prey species (alewife, rainbow smelt, or round goby) in lake trout diet based on fatty acid analysis (FAA) or stable isotope analysis (SIA). The coefficient of determination (R^2) for each model is included as well. Significant results are in bold font and results with marginal significance (p value between 0.05 and 0.10) are denoted by a cross.

		Alewife		Rainbow smelt		Round goby	
		F	p	F	p	F	p
FAA	Length	5.27	**0.02**	6.20	**0.01**	0.22	0.64
	Sex	1.34	0.25	0.08	0.78	0.56	0.45
	Region	1.02	0.39	0.92	0.43	0.88	0.45
	Length*Sex	0.19	0.66	2.75	0.10†	3.50	0.06†
	Length*Region	0.52	0.67	2.49	0.06†	3.00	**0.03**
	Sex*Region	0.30	0.74	0.68	0.51	0.33	0.72
	R^2	0.10		0.15		0.13	
SIA	Length	12.88	**<0.01**	9.87	**<0.01**	0.38	0.54
	Sex	1.89	0.17	1.30	0.26	0.37	0.54
	Region	2.53	0.06†	3.20	**0.03**	6.87	**< 0.01**
	Length*Sex	0.56	0.46	< 0.01	0.99	2.01	0.16
	Length*Region	1.28	0.28	1.41	0.24	1.18	0.32
	Sex*Region	0.10	0.91	0.93	0.40	1.88	0.16
	R^2	0.20		0.20		0.22	

Table 6

Regression coefficients (β) for predictor variables from reduced linear regression models that only included significant and marginally significant variables (see Table 5). Regression models predicted the estimated contributions of a common prey species in lake trout diet based on fatty acid analysis (FAA) data or logit transformed stable isotope analysis (SIA) data generated from mixing models. The central region was set as the baseline that other regions were compared to (e.g., estimated proportions of round goby based on SIA increase by 0.07 for lake trout in the northeast compared to lake trout in the central region). Significant ($p < 0.05$) regression coefficients are in bold font and coefficients with marginal significance ($0.05 < p < 0.10$) are noted with a cross. The coefficient of determination (R^2) is also included for each reduced regression model.

		R^2	West β	Central β	East β	Northeast β	Length (mm) β
FAA	Alewife	0.04	–	–	–	–	**4.97 E^{-4}**
	Rainbow smelt	0.04	–	–	–	–	**−1.43 E^{-4}**
SIA	Alewife	0.15	0.05	**−1.71**	0.01	**0.07**	**4.51 E^{-4}**
	Rainbow smelt	0.14	−0.02	**0.70**	0.04†	−0.04	**−3.61 E^{-4}**
	Round goby	0.14	−0.02	**−1.44**	**−0.07**	−0.00	–

goby were lower. The high estimates for rainbow smelt contributions were unexpected as studies in recent years generally produce estimates <20%, regardless of the analysis used (Rush et al., 2012; Colborne et al., 2016; Mumby et al., 2018). However, SIA analyses have shown that lake trout have a diverse diet relative to other salmonines in Lake Ontario (Rush et al., 2012; Yuille et al., 2015; Mumby et al., 2018) and, while infrequent, rainbow smelt can dominate lake trout stomach contents (Nawrocki et al., in press).

The different diet estimates among analytical methods used in this study may be influenced by a difference in the timeframe represented by each analysis and a seasonal shift in lake trout diet. Lake trout stomach contents likely represented diet items consumed within the previous 2–3 days based on evacuation rates for piscivorous brown trout (*Salmo trutta*) (He and Wurtsbaugh 1993). Fatty acid turnover rates based on Atlantic salmon (Budge et al., 2011) suggest FASs of lake trout in the present study likely incorporate diet over the previous 12–22 weeks (i.e., late spring/summer through fall). For the stable isotope data, lake trout results likely had a strong influence of prey consumed since spring and potentially winter, based on the average estimated isotopic half-life of the individuals in this study (162 days based on equation 2 from Vander Zanden et al., 2015). As adult lake trout have a slow growth rate, dietary assimilation likely has a reduced impact on their isotope turnover rate relative to smaller-sized individuals developing new tissues during growth (Rush et al., 2012).

Due to the different timeframes represented by each diet analysis, a seasonal shift in lake trout diet could explain the different estimates observed among analyses. Considering results from each diet analysis conducted, consumption of round goby and rainbow smelt prior to summer stratification (i.e., during winter and spring) may be relatively high, then decrease during the summer and fall as dominant alewife contributions further increase. Various studies provide support of a seasonal diet shift in lake trout. For example, seasonal trends in lake tout diet have been reported in Lake Ontario with significantly lower contributions of alewife stomach contents collected during April and May compared to those collected during June through September (Lantry 2001). Similarly, higher proportions of round goby have been observed in stomach contents of lake trout from Lake Ontario collected during the spring as compared to those collected in the summer (Dietrich et al., 2006). In Lake Michigan, shifts in lake trout diet have been observed during the winter, changing from primarily alewife at the beginning of winter to benthic prey (slimy sculpin, *Cottus cognatus*) by early spring (Eck and Wells 1986). Round goby also dominated the stomach contents of lake trout from Lake Michigan in May and June, while alewife were the main diet item from July through October (Luo et al., 2019). Increased consumption of species other than alewife during the winter and spring may be influenced by increased spatial overlap as prey often overwinter offshore (Lynch and Mensinger, 2012; Blair et al., 2018) and lake trout can occupy a larger depth range prior to stratification (Eck and Wells 1983). Lastly, lake trout continue to feed and grow during winter (Eck and Wells, 1986; McMeans et al., 2020), suggesting their biochemical signatures are likely influenced by winter feeding, unlike other species with reduced foraging during winter that may have little to no representation of this season in diet analyses (Perga and Gerdeaux 2005).

Dissimilarity in estimated diet composition among methods may, in part, be related to the prey species samples used in each biochemical analysis. The high estimated contribution of rainbow smelt in both FAA and SIA analyses is questionable because the proportion of rainbow smelt in the pelagic fish caught during 2013 was low compared to previous years (3.1%) and compared to the proportion of alewife (>90%) in

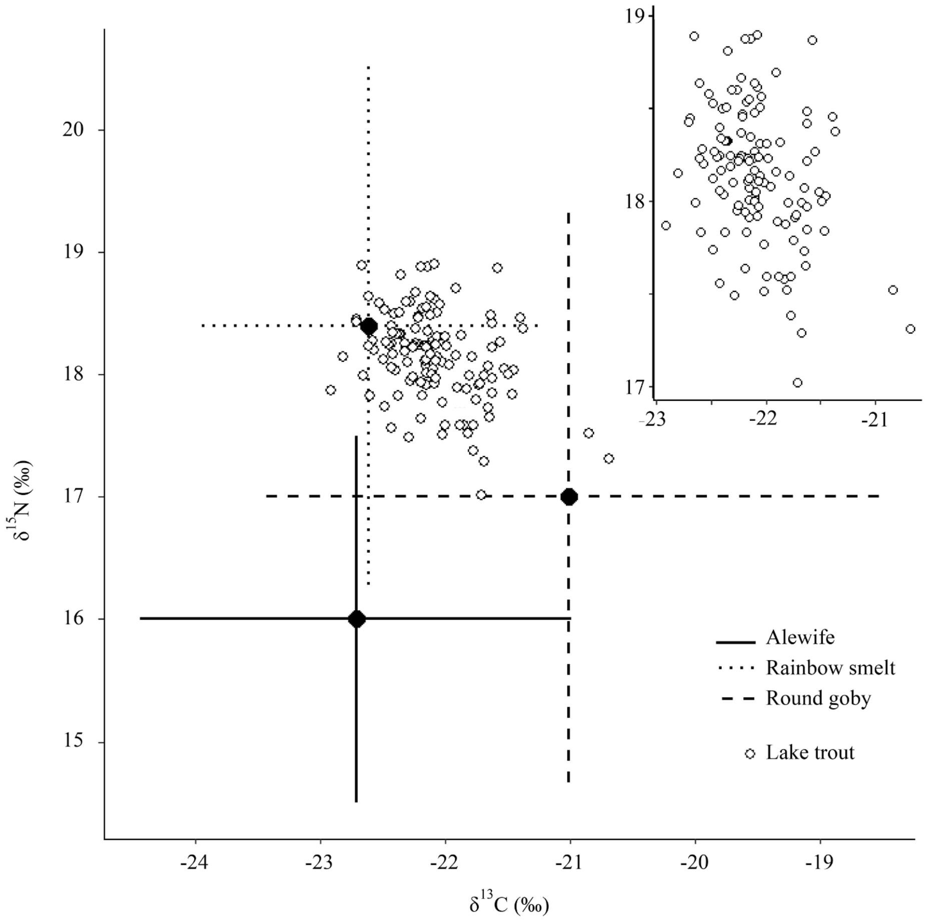

Fig. 4. Stable isotope composition of Lake Ontario lake trout in relation to average (±1 SD) alewife, rainbow smelt, and round goby isotope composition after adjustments for trophic discrimination. The inset figure focuses on lake trout stable isotope composition.

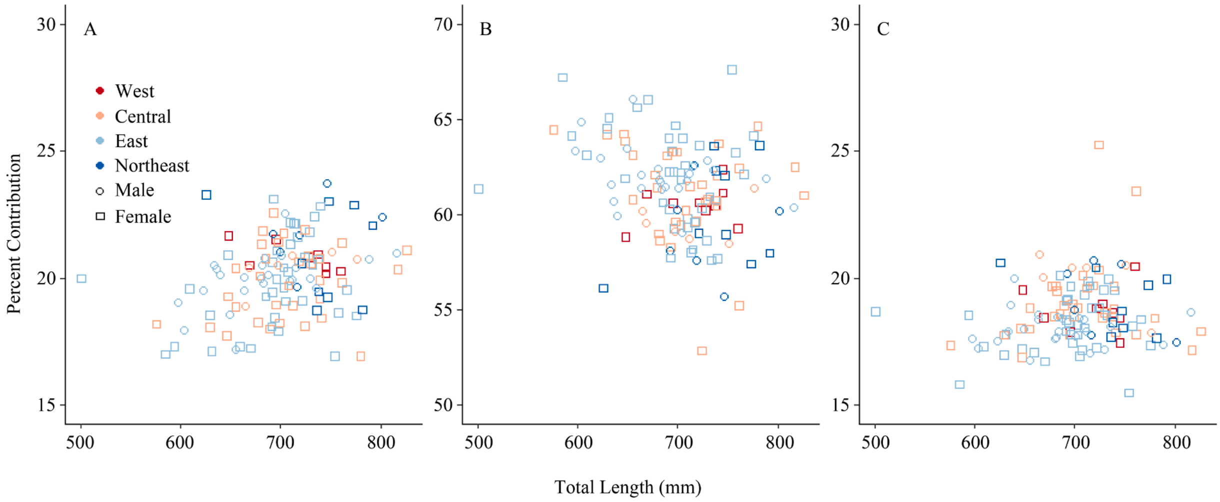

Fig. 5. Estimated percent contribution of alewife (A), rainbow smelt (B), and round goby (C) in lake trout diet, based on stable isotope mixing models, as a function of total length. Data are grouped by region (color) and sex (shape).

the pelagic catch (Weidel and Connerton 2014). For both the prey fatty acid and stable isotope data, rainbow smelt had an intermediate signature between alewife and round goby, potentially resulting in an inflated rainbow smelt representation. For FAA specifically, DHA can be generated from linolenic acid in freshwater fishes (Watanabe 1982), which may have inflated the proportion of this fatty acid in lake trout signatures. As a result, the relatively high similarity between lake trout and rainbow smelt FASs would include factors unrelated to predator–prey interactions. For SIA, using non-species-specific trophic discrimination factors with mixing models can introduce significant variation in SIA diet estimates (Bond and Diamond 2011), but to our knowledge lake trout DTDFs have not been established. However, using alternative discrimination values (Vander Zanden and Rasmussen, 2001; Mumby et al., 2018) produced similarly high estimates of rainbow smelt. Lastly, prey contributions could have been inflated inaccurately by misclassified prey not included in our FAA or SIA. However, additional prey species not included in our models had minimal contributions to lake trout stomach contents in recent studies (Dietrich et al., 2006; Mumby et al., 2018). Therefore, we do not expect misrepresentation of additional species to have a substantial influence on our results.

4.2. Patterns in estimated lake trout diet

While estimated diet composition differed among the three diet analyses, similar patterns were generally observed among methods, particularly based on lake trout length and region. Results from the FAA and SIA suggest that the proportion of alewife in lake trout diet increased with length. Previous studies have investigated changes in adult lake trout diet in Lake Ontario corresponding to lake trout length with little to no evidence for differences in species consumed (Lantry, 2001; Mumby et al., 2018). Dietrich f2006) examined lake trout stomach contents in April through September and observed individuals with a fork length between 551 and 650 mm mainly consumed round goby (57%, N = 54 lake trout), although all other size classes between 450 and 849 mm (i.e., 450–550 mm and 651–849 mm) had alewife as their main diet item. Differences in diet composition have been observed, however, based on the size of alewife with a significant, positive correlation between lake trout length and alewife length (Nawrocki et al., in press). While feeding on different age classes of alewife may cause variation in predator biochemical concentrations, these differences would likely be minimal to differences caused by consuming alternative species (Happel et al., 2017; Mumby et al., 2018). In other Great Lakes, evidence for diet shifts in lake trout based on length has been demonstrated through FAA, with increased reliance on nearshore-benthic food web components (i.e., round goby) with greater total length in lakes Huron and Michigan (Happel et al., 2018).

The significance of spatial differences in lake trout diet was most supported by SCA and SIA. The SCA data indicated that rainbow smelt importance increased in the northeast region relative to the east and central regions. Similarly, the SIA results demonstrated greater consumption of rainbow smelt in the east region relative to the central region. These spatial differences in rainbow smelt consumption correspond with hydroacoustic assessments of pelagic prey species conducted during 2013; rainbow smelt densities were highest in the northeast and eastern regions, while alewife densities were lowest in the northeast and southern east regions (Ontario Ministry of Natural Resources 2014). However, the biological significance of these spatial difference in rainbow smelt consumption are likely minimal as sample size for the stomach contents was limited and the difference based on SIA was only approximately 4%. Interestingly, SIA results also indicated lake trout in the northeast region consumed more alewife compared to lake trout captured from the central region. The lake trout collected in the northeast region were large compared to those captured from the other regions, suggesting alewife likely comprised a relatively large component of their overall diet. In addition, while alewife density was low in the northeast region, lake trout captured from this region may have spent a considerable amount of time in adjacent areas where alewife were more abundant. Studies have demonstrated that adult lake trout home range in the Great Lakes may cover 100 km or more (e.g., Schmalz et al., 2002; Elrod, 1987; Binder et al., 2017). Therefore, while the lake trout were captured in the northeast region, the northern portion of the central and east regions where alewife are more abundant (Ontario Ministry of Natural Resources 2014) would likely be within their home range. As a result, the long-term interpretation of diet represented by SIA would have a strong signal for alewife. In contrast, the short-term interpretation of stomach contents would only represent prey consumed while lake trout were present in the northeast region. The SIA results also suggested a high contribution of round goby for lake trout from the central basin relative to lake trout captured in the east region. Similarly, benthic trawl sampling conducted along the southern shore of Lake Ontario during 2013 demonstrated round goby densities were greatest east of the Niagara River mouth, near the Olcott sampling site in the central region (Weidel et al., 2014). Previous diet studies have found minimal differences in lake trout stomach content composition based on location within Lake Ontario (Rand and Stewart, 1998; Lantry, 2001), although round goby likely had little to no presence in Lake Ontario when these studies were conducted. More recently, spatial variation was observed in lake trout stomach contents, but these differences may have been confounded by lake trout size and differed from SIA results that demonstrated comparable isotopic niche size and orientation throughout the lake (Nawrocki et al., in press).

Results from the SCA indicated slight sex-based differences in lake trout diet, with female stomach contents containing higher contributions of prey species other than alewife compared to male stomach contents. While alewife were the most abundant prey consumed by both females and males based on SCA, nine of the ten lake trout that consumed rainbow smelt or round goby were females. Similarly, the average percent contribution of alewife in lake trout stomach contents by mass was 85% and 96% for female and male lake trout, respectively. However, this difference appears to be dependent on region, with most rainbow smelt and round goby consumed in the east and northeast regions. As previously mentioned, these regions have the lowest densities of alewife and highest densities of rainbow smelt (Ontario Ministry of Natural Resources 2014). Therefore, sex-based differences in diet may be greatest when alewife density is reduced, or diversity of the prey base increases, indicating female lake trout may have a more generalist foraging behavior than males. As sex-based differences in diet were not observed for either of the long-term biochemical analyses, it is possible that these differences also vary seasonally, and are limited to the fall. To our knowledge, sex-based differences have not been previously reported for lake trout diet in Lake Ontario.

By considering results from all three diet analyses, four factors (location, lake trout length, lake trout sex, and season) that likely influence lake trout diet were identified, while individual analyses identified no more than two significant factors. Overall, our results indicated adult lake trout primarily consumed alewife during fall; however, rainbow smelt and round goby increased in relative importance, especially for females, for smaller lake trout and in areas with lower alewife abundance. Our results also provide evidence that diet may vary by season, with increased diet diversity during winter and spring; however, direct observation of lake trout stomach contents collected seasonally is needed to confirm seasonal diet shift. The SIA provided the most statistical evidence for patterns in estimated lake trout diet, identifying significant results for predator length and location. While FAA only identified one significant predictor variable (length), the significant interactions with region and sex demonstrate complex spatial and sex-based differences in diet estimates obtained from this method. The SCA was able to provide evidence for spatial and sex-based differences; however, the necessity to pool our stomach contents data due to low sample size prevented consideration of interactions among predictor variables. No single method could provide insight into seasonal changes in diet because all fish were collected during the fall.

The detailed patterns derived from the simultaneous use of the three diet analyses provide novel information about lake trout diet in Lake Ontario that reflects the complexity of resource use throughout the lake and may assist restoration efforts. For example, these results provide evidence that female lake trout may have a greater response to changes in the Lake Ontario prey base than males. The tendency for lake trout to consume alternative prey (i.e., prey other than alewife) likely impacts the resilience of the lake trout population considering the alewife population in Lake Ontario is relatively unstable due to large winter die-offs of yearling alewife following severe winters (Walsh et al., 2016) and high selectivity for alewife by other piscivorous salmonids in Lake Ontario (e.g., Chinook and coho salmon; Mumby et al., 2018).

Furthermore, consuming prey other than alewife may support lake trout restoration efforts as alewife consumption has been associated with a nutritional deficiency (Thiamine Deficiency Complex) that impairs offspring survival (Brown et al., 2005; Fitzsimons et al., 2009; Futia et al., 2019). Thus, if females remain in locations with lower alewife abundance where they are more likely to consume alternative prey, the probability for localized natural recruitment would increase.

4.3. Limitations of diet analyses

While each analysis provided a unique perspective of diet composition, they each had limitations. For SCA, the high percentage of empty stomachs (~50%) substantially reduced the sample size for analyzing diet composition. As a result, only univariate statistical comparisons could be conducted and significance of interactions among predictor variables could not be quantified. In addition, unidentifiable stomach contents were excluded from statistical comparisons, potentially influencing diet estimates. Our sampling method likely increased the frequency of empty stomachs and unidentified remains due to gillnet set duration (approximately 24 h) during which fish could digest and regurgitate their stomach contents (Bowman, 1986; Ahlbeck et al., 2012).

For both FAA and SIA, diet composition was based on statistical estimates with wide confidence limits rather than direct observation. Due to the lack of direct observation, estimates from these analyses required prior knowledge of lake trout diet composition and available prey species to determine which species were included in the models. By only including the most abundant prey species in these models, alternative diet items were excluded. In addition, the diet estimates produced by these models can be impaired by misrepresentation of prey species. Misrepresentation can occur if tissue discrimination factors are inaccurate or prey species have indistinct biochemical signatures (Nielsen et al., 2018). To analyze biochemical tracers, expensive instrumentation is required and processing time for individual samples is long compared to the SCA, resulting in higher costs. Quantitative diet estimates using biochemical analyses are also dependent on available and accurate tissue discrimination factors. Limited knowledge on the metabolism of individual fatty acids in freshwater fishes prevented quantitative fatty acid signature analysis with mixing models. Such models incorporate fatty acid metabolism with correlation coefficients for each fatty acid included in the model. While correlation coefficients of individual fatty acids have been determined for juvenile lake trout fed various invertebrate-based diets, multiple coefficients had significant differences among diets leading to the conclusion that additional research on fatty acid metabolism of freshwater fishes is needed (Happel et al., 2016). Once appropriate correlation coefficients become available, fatty acid mixing models, like stable isotope mixing models, can be used to estimate diet composition of every individual sampled (Choy et al., 2019).

5. Conclusions

We estimated diet composition and observed various patterns in adult lake trout diet composition using three different diet analyzes in unison. Our results indicate that simultaneous use of SCA, FAA, and SIA may provide insight into the year-round diet of slow-growing fish with less field work and smaller sampling requirements than individual analyses would require. For Lake Ontario, our results suggest that alewife were the most frequently consumed prey species in the fall. However, alternative species such as rainbow smelt and round goby were likely consumed in higher proportions during other seasons (i.e., winter and spring). Furthermore, diet patterns based on size and sex were observed, in addition to regional patterns likely associated with unequal distribution of prey species among regions. Overall, this three-pronged approach incorporating SCA, FAA, and SIA for diet estimation demonstrated multiple patterns that were often underestimated by individual analyses and previous studies. These results demonstrate the value of using multiple analyses to investigate complex and potentially subtle patterns in predator diet. The patterns we have described for adult lake trout diet in Lake Ontario can inform future predator–prey interactions and guide restoration efforts as stressors such as invasive species and climate change continue to alter the system.

CRediT authorship contribution statement

Matthew H. Futia: Conceptualization, Formal analysis, Methodology, Writing - original draft, Writing - review & editing. **Scott F. Colborne:** Formal analysis, Methodology, Writing - review & editing. **Aaron T. Fisk:** Data curation, Formal analysis, Funding acquisition, Methodology, Resources, Writing - review & editing. **Dimitry Gorsky:** Funding acquisition, Writing - review & editing. **Timothy B. Johnson:** Data curation, Funding acquisition, Methodology, Writing - review & editing. **Brian F. Lantry:** Data curation, Formal analysis, Methodology, Writing - review & editing. **Jana R. Lantry:** Data curation, Formal analysis, Writing - review & editing. **Jacques Rinchard:** Conceptualization, Data curation, Methodology, Resources, Software, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2021.107728.

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