Estimates of lake trout (Salvelinus namaycush) diet in Lake Ontario using two and three isotope mixing models

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A B S T R A C T

Recent development of multi-dimensional stable isotope models for estimating both foraging patterns and niches have presented the analytical tools to further assess the food webs of freshwater populations. One approach to refine predictions from these analyses is to include a third isotope to the more common two-isotope carbon and nitrogen mixing models to increase the power to resolve different prey sources. We compared predictions made with two-isotope carbon and nitrogen mixing models and three-isotope models that also included sulphur (34S) for the diets of Lake Ontario lake trout (Salvelinus namaycush). We determined the isotopic compositions of lake trout and potential prey fishes sampled from Lake Ontario and then used quantitative estimates of resource use generated by two- and three-isotope Bayesian mixing models (SIAR) to infer feeding patterns of lake trout. Both two- and three-isotope models indicated that alewife (Alosa pseudoharengus) and round goby (Neogobius melanostomus) were the primary prey items, but the three-isotope models were more consistent with recent measures of prey fish abundances and lake trout diets. The lake trout sample directly from the hatcheries had isotopic compositions derived from the hatchery food which were distinctively different from those derived from the natural prey sources. Those hatchery signals were retained for months after release, raising the possibility to distinguish hatchery-reared yearlings and similarly sized naturally reproduced lake trout based on isotopic compositions. Addition of a third-isotope resulted in mixing model results that confirmed round goby have become an important component of lake trout diet and may be overtaking alewife as a prey resource.

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Introduction

Stable isotope analysis has become a common method of inferring diet due to predictable relationships that link isotopic composition of consumer tissues to their resources (DeNiro and Epstein, 1981, 1978; Fry et al., 1978). Isotope analysis has moved away from qualitative comparisons of isotopic composition values to the quantification of dietary components through the development of isotope mixing models. Isotope models range from early linear mixing models (Post, 2002) to current Bayesian modelling techniques (Hopkins and Ferguson, 2012; Moore and Semmens, 2008; Parnell et al., 2010). These models rely on inherent isotopic variation between consumers and consumed resources making it possible to predict the relative contributions of multiple prey items to consumer diets (Parnell et al., 2010). As a result of the informative predictions generated by the Bayesian mixing model approach, this method is rapidly becoming a standard quantitative application for estimating diet resources. These models have been applied to foraging studies across a wide range of taxa, including mammals (Hopkins and Ferguson, 2012; Rodriguez and Gerardo Herrera, 2013), birds (Bond and Diamond, 2011; Moreno et al., 2010), amphibians (Gillespie, 2013), and fish (Locke et al., 2014), encompassing both terrestrial (Hopkins and Ferguson, 2012) and aquatic (Locke et al., 2014) habitats along with the interfaces between them (Moreno et al., 2010). As a result of continued development and application, isotope mixing models provide both accurate interpretation of diet and valuable information about the feeding patterns of organisms living in complex food webs.

Mixing models do have basic requirements that must be recognized and considered when designing experiments to assess foraging ecology. An essential forerunner to the successful application of all isotope studies is the presence of isotopic variation among members of the community examined. When possible prey sources do not differ in isotopic composition, it is not possible for mixing models to accurately distinguish among
these resources and subsequently to estimate consumer diets. In natural systems there are two methods to address low prey variability issues; (1) create general categories that combine prey types into broad, ecologically relevant units that differ in isotopic composition, e.g. between littoral and pelagic habitats within lakes (Harrod et. al., 2010; Hayden et. al., 2013), or (2) incorporate additional isotopes to increase the predictive capacity of mixing models when there are multiple potential prey species. For example, stable isotopes of sulphur ($\delta^{34}S$) have been incorporated into aquatic foraging studies of marine (Moreno et. al., 2010) and estuarine ecosystems (Deegan and Garritt, 1997) because $\delta^{34}S$ values typically differ between individuals that feed in the water column compared to those from the benthic substrate, and between anoxic and oxic sediments due to the actions of benthic sulphate consuming bacteria (Croisetiere et. al., 2009; Proulx and Hare, 2014). The use of $\delta^{34}S$ in freshwater systems has largely been focused on tracing the interactions between species or individuals moving between habitat types, i.e. between marine and freshwater, due to the disparity in sulphate concentrations between these systems (Fry, 2002) and the resulting effects on sulphur isotope values (e.g. MacAvoy et. al., 2000; Oifukeny et. al., 2012), but $\delta^{34}S$ has also been successfully used to distinguish among freshwater prey groups (Croisetiere et. al., 2009). As such, sulphur represents a candidate for the ‘third isotope’ role in freshwater studies aimed at increasing the discriminatory power of mixing models with multiple possible prey sources. Indeed, there is evidence from the marine yellow-legged gull (Larus michahellis) that incorporating sulphur into Bayesian mixing models already utilizing carbon and nitrogen can increase the power to distinguish between multiple food sources that were of both marine and terrestrial origin (Moreno et. al., 2010). Sulphur isotopes have been added to analyses of boreal lakes with carbon and nitrogen isotopes (Croisetiere et. al., 2009), but to our knowledge, the inclusion of sulphur in isotope mixing models has not yet been done in solely freshwater systems.

Large freshwater lakes are of special interest to researchers using stable isotope analysis because these bodies of water not only often support unique complex ecosystems with diverse species assemblages, but they are frequently impacted by a variety of human activities (e.g. Breffle et. al., 2013; Hartig et. al., 1996; Worthington and Lowe-McConnell, 1994). For example, there are ongoing efforts in the Laurentian Great Lakes of North America to prevent the loss and even restore native species (see Hartig et. al., 2014), and these efforts can be facilitated by detailed knowledge of the community interactions, e.g. food webs, that may determine the success or failure of conservation and management efforts. Stable isotope inferences of long-term diet are of particular use to community assessments, but can only be used to their full potential if there is enough power to distinguish between community members.

In this study, we focus on Lake Ontario lake trout (Salvelinus namaycush), a native species of fish that was extirpated from the lake during the early 20th century and has been the focus of international restoration efforts since the late 1960s (Dobiesz et. al., 2005; Mills et. al., 2003; Zimmerman et. al., 2009). Analyses of stomachs of lake trout collected during the 1970s, 1980s and 1990s identified alewife (Alosa pseudoharengus), rainbow smelt (Osmerus mordax), and slimy sculpin (Cottus cognatus) as the primary prey fish consumed, which represented a change from the historical diet linked to the collapse of native cisco (Coregonus spp.) and deepwater sculpin (Myoxocephalus quadricornis) populations during the 1940s (Christie et. al., 1987; Dietrich et. al., 2006). The 1990s and 2000s brought another period of shifting prey fish populations in Lake Ontario largely linked to the introduction and establishment of zebra and quagga mussels (Dreissena spp.) and round goby (Neogobius melanostomus; Lantry et. al., 2014a, 2014b; Mills et. al., 2005). In brief, slimy sculpin were found in high abundance in the benthic habitat of Lake Ontario until the early 1990s when their population began to decline and has continued to do so (Lantry et. al., 2014a). This decrease in slimy sculpin is likely related to the introduction of invasive dreissenids and subsequent reduction of Diporeia spp., the preferred prey of slimy sculpin (Lantry et. al., 2014a). Rainbow smelt were frequently the second largest component of salmonid and trout diets in Lake Ontario through the 1990s (Rand and Stewart, 1998; Lantry, 2001), but record low population sizes during the 2000s likely diminished their importance to lake trout diets thereafter (Lantry et. al., 2014a; Mills et. al., 2003, 2005). Finally, alewife populations have also declined in Lake Ontario, reaching abundances in 2007 that were estimated to be 1/3 the size of 1994 (Connerton et. al., 2014). At the same time that these prey fish numbers declined in Lake Ontario, round goby had become established throughout the Great Lakes region (e.g. Korns et. al., 2012). This new invasive benthic fish species has recently been found in the diets of predatory birds and all nearshore piscivorous fish within Lake Ontario, including lake trout (Dietrich et. al., 2006; Stewart et. al., 2014). There has been growing speculation that round goby could become an important component of lake trout diet (e.g. Mills et. al., 2003), and there are indicators that this may already be occurring (e.g. Dietrich et. al., 2006; Rush et. al., 2012). Given the number of potential prey species in the diets of Lake Ontario lake trout, the general interest in these fish, and the amount of information available, lake trout were chosen to examine diet predictions from both the two- and three-isotope mixing models.

We applied stable isotope analysis and SIAR (Stable Isotope Analysis in R; Parnell et. al., 2010) Bayesian mixing models to (1) estimate resource use of Lake Ontario lake trout approximately 10 years after the introduction of round goby and (2) compare the outcomes of two-isotope models that included only $\delta^{13}C$ and $\delta^{15}N$, the most common approach in isotope studies, to three-isotope models that also included $\delta^{34}S$ measures. We predicted that isotope mixing models would indicate that the diets across a wide size range of lake trout would contain primarily alewife, but also include substantial contributions from round goby. We also expected that measures of $\delta^{34}S$ concentration would enhance the ability to distinguish among the multiple candidate prey fishes by increasing the information available for isotope mixing models.

**Methods**

**Sample collection**

Samples of field-collected lake trout were selected from larger datasets of carbon and nitrogen isotope values ($n = 575$; Rush et. al., 2012; Yuille et. al., 2015) to provide a lake-wide representative sample of the isotopic variability. The lake trout selected were collected along the southern shore of Lake Ontario in September 2008 ($n = 14$) and July 2010 ($n = 23$; Fig. 1 and Electronic Supplementary Material (ESM) Table S1) using bottom-set graded mesh gill nets set in parallel to depth contours typically ranging from approximately 25–50 m depth (mesh sizes ranged from 38 to 151 mm; see Rush et. al., 2012 for details). Across the 37 samples of field-collected fish selected for this study, the mean $\delta^{13}C$ and $\delta^{15}N$ values were within 0.5% of these larger datasets providing a representative sample of the Lake Ontario population as a whole without considering spatial variation within Lake Ontario. Yearling lake trout (~150 mm total length) were also collected from the Ontario Ministry of Natural Resources and Forestry (OMNR) Harwood Fish Culture Station (Harwood, ON Canada) on the day fish were stocked into Lake Ontario in March 2013 ($n = 9$). Alewife, rainbow smelt, slimy sculpin and round goby, the primary forage fishes for lake trout, were collected with bottom trawls from US Geological Survey Lake Ontario prey fish assessments during April–May 2010 (ESM Table S2; Walsh and Weidel, 2013; Weidel et. al., 2013). Samples of hatchery feeds were obtained in 2010 from Harwood FCS and the USFWS Alleghany National Fish Hatchery (Warren, PA), the primary facilities supplying the lake trout for stocking into Lake Ontario. Because the marine fishes usually included in these feeds should differ from freshwater fish we expected there to be distinct isotopic variation between natural and hatchery food sources.

After collection, each lake trout was measured for total body length (mm) and wet mass (g). A sample of white muscle tissue from the...
anterior portion of the dorsal fin on the left side of each lake trout and whole body homogenates of prey fish were collected and kept on dry ice before being stored at $-80^\circ$C. Even though some studies have reported variation between specific tissue types, i.e. white muscle and whole body homogenates (e.g. Sotiropoulos et al., 2004), these are not consistently reported and could bias inferences of diet (e.g. Kurle et al., 2011; Logan and Lutcavage, 2008). In relation to our study, we were interested in the diets of lake trout that are digesting the entire prey fish, as such we were not concerned about isotopic biases related to using whole body homogenates of the prey fish.

Presence of lipids in fish tissues can deplete the observed $\delta^{13}$C values of tissues compared to that of pure protein (Boecklen et al., 2011; Fry et al., 2003). To correct for the presence of lipids, we first freeze-dried tissues for 48 h and homogenized them into a fine powder using liquid nitrogen and a mortar and pestle. Second, we performed lipid extractions on all samples (lake trout, prey fishes, and hatchery feed) using a 2:1 chloroform/methanol solution prior to measuring the isotopic composition (Bligh and Dyer, 1959). There is on-going debate regarding the use of chemical lipid extraction because it may alter $\delta^{15}$N values of tissues unrelated to the lipid content, but this effect is not consistently found across species or tissue types (e.g. Boecklen et al., 2011; Mateo et al., 2008; Sotiropoulos et al., 2004). Furthermore, a multi-trophic level comparison of nine temperate freshwater fish species from the Thousand Islands region (St. Lawrence–Lake Ontario interface) found that all species were similarly affected by lipid extraction and, therefore, the trophic relationships between species remained intact as long as the same lipid extraction methods were used on all samples (Murry et al., 2006). We used chemical lipid extraction to compare our results to previous analyses of Lake Ontario lake trout that used the same methods (e.g. Rush et al., 2012; Yuille et al., 2015). Caution should be taken when comparing the specific isotope values reported here to studies that did not lipid extract or used other extraction methods, and cross-study comparisons should focus on the overall trophic relationships between groups.

**Stable isotope analysis**

Stable isotope analysis of carbon ($^{13}$C/$^{12}$C) and nitrogen ($^{15}$N/$^{14}$N) was completed in the Trophic Ecology Laboratory at the University of Windsor’s Great Lakes Institute for Environmental Research (GLIER; Windsor, ON Canada) using a Delta Plus isotope-ratio mass spectrometer (ThermoFinnigan, San Jose, CA, U.S.A.) coupled to an elemental analyser (Costech, Valencia, CA, U.S.A.). Carbon and nitrogen isotope ratios were determined in relation to three internal laboratory standards and the #8414 bovine muscle NIST standard which were run every 12 samples. Stable isotope ratios of sulphur ($^{34}$S/$^{32}$S) were determined at the Environmental Isotope Laboratory at the University of Waterloo using an IsoChrom continuous flow isotope ratio mass spectrometer (Micromass, Wilmslow, U.K.) coupled to an elemental analyser (Costech, Valencia, CA, U.S.A.). The ratio of each isotope was determined by the difference between the measured sample and an international standard reference material:

$$iX = \frac{R_{\text{sample}}}{R_{\text{standard}} - 1}$$

where X is the isotope being measured ($^{13}$C, $^{15}$N, or $^{34}$S), R is the isotope ratio of interest, and $i$ is the measure of heavy to light isotope in a sample expressed as parts per mil ($\delta$). The international standard reference materials were Vienna Pee Dee Belemnite (VPDB), atmospheric nitrogen (AIR), and Vienna Cañon Diablo Triolite (VCDT) for $^{13}$C, $^{15}$N, and $^{34}$S, respectively.

**Statistical analysis and isotope mixing models**

Lake trout were classified into one of four groups based on collection year, source (i.e. field-collected or hatchery), and size characteristics. We categorized fish based on (1) the year of collection (2008, 2010, or 2013); (2) collection source (field-collected or hatchery-source); and (3) size of the fish (<250 mm or ≥250 mm total body length). We chose a size-range cut-off of 250 mm because it is possible for the smaller fish to still reflect the diet provided at the hatcheries and reflects previously identified size ranges for age-1 and -2 Lake Ontario lake trout around which shifts in stomach contents have been documented (e.g. Borgmann and Whittle, 1992; Pazzia et al., 2002). Based on these criteria we identified four lake trout groups for further analyses: (1) 2008 large field-collected (all >250 mm in length; n = 14); (2) 2010 large field-collected (>250 mm mm length; n = 11); (3) 2010 small field-collected (<250 mm in length; n = 12); and (4) 2013 small hatchery-source (<200 mm in length; n = 9).

Isotopic variation among the four lake trout groups was assessed separately for each isotope, i.e. $\delta^{13}$C, $\delta^{15}$N, and $\delta^{34}$S, using separate analysis of variance models (ANOVA; dependent factor: isotope values; independent factor: lake trout group) with Tukey’s post-hoc comparisons, when appropriate. We also compared the isotopic composition of the prey fishes (alewife, round goby, rainbow smelt, and slimy sculpin) collected in 2010 using ANOVA models (dependent factor: $\delta^{13}$C, $\delta^{15}$N, or $\delta^{34}$S values; independent factor: prey species) with Tukey’s post-hoc comparisons. All ANOVA models met the assumptions of normal distributions (Shapiro–Wilk, all P ≥ 0.07) and homogeneity of variances (Levene’s F-test, all F ≥ 0.27).

Contributions of each candidate diet source to total lake trout diet were modelled using SIAR (Stable Isotope Analysis in R; R version 2.14.2; R Development Core Team, 2012) mixing models for each of the four groups outlined above. The diets of larger fish, i.e. field-collected fish from 2008 and 2010 (>250 mm), were modelled using four-source mixing models that included alewife, round goby, slimy sculpin, and rainbow smelt as possible prey species (‘sources’ variable). Rush et al. (2012) reviewed stomach content analyses of lake trout and found that these four prey fishes represent at least 90% of their diet both
pre- and post-round goby invasion. Additionally, while other prey fishes, such as yellow perch (Perca flavescens) and shiners (Notropis spp.), have been found in lake trout stomachs the relative low frequency of their occurrence limits the potential contributions of these prey types to the longer-term diet estimates derived from stable isotopes (Brandt, 1986; Dietrich et al., 2006). Despite the inclusion of Mysis spp. in other models, we did not incorporate them here because it has been reported that lake trout become piscivorous immediately after release from hatcheries (body length approximately 150 mm) and modelled contributions of Mysis to lake trout diet have shown minimal contributions (Christie et al., 1987; Rush et al., 2012). The smaller lake trout, i.e. 2010 field-collected (<250 mm) and 2013 hatchery sampled fish, were modelled using five-source mixing models that included hatchery feed in addition to the four prey fishes above. Trophic fractionation was estimated using the mean diet-tissue discrimination factor (DTDF) reported for temperate freshwater carnivorous fishes (δ13C: 0.47 ± 1.23 ‰; δ15N: +3.23 ± 0.41 ‰) (Vander Zanden and Rasmussen, 2001). To our knowledge, specific δ34S DTDFs have not been measured for lake trout, but we applied a δ34S DTDF value of 0.5 ± 0.6 ‰ to our mixing models based on the mean values reported for rainbow trout (Onchorhyncus mykiss) and brook trout (Salvelinus fontinalis) (Jones et al., 2013; McCutchan et al., 2003).

Estimates of diet for individual fish were obtained for both two- and three-isotope models using the SIARSolo command in the SIAR analysis package. For this analysis we used the prey fishes as sources and the DTDF variables as above to generate 4-source diet estimates for each field-collected individual; only large field-collected fish (>250 mm) were included in this analysis. Models of each individual were run for 5 × 10⁵ iterations with a burn-in of 5 × 10⁴ iterations. Mean estimates of dietary contribution of round goby and alewife to individual lake trout were then used to test for a relationship between diet and body length. To test if there was a relationship between diet and body length of field-collected lake trout, analysis of covariance (ANCOVA) models were used to compare estimates of dietary contributions (single mean value for each individual lake trout from SIARSolo simulations above) from alewife and round goby to lake trout diet (dependent variable: proportion alewife or round goby; co-variates: total body length; independent variable: isotope model configuration, i.e. two- or three-isotope).

The δ13C and δ15N values of 2008 and 2010 field-collected lake trout were drawn from larger data sets of lake trout isotopic compositions previously published (Rush et al., 2012; Yuille et al., 2015), but all δ34S measures, statistical analyses, and isotope models using these data were completed for this work alone. All statistical analyses were completed in either JMP v. 11.2.0 (SAS Institute Inc., Cary NC USA, 2013) or R software (R version 3.1.1; R Development Core Team, 2014). Means are presented ± 1 standard deviation (SD), unless otherwise stated, and α = 0.05.

Results

Prey isotopic compositions

The prey fish species (alewife, round goby, rainbow smelt, slimy sculpin) differed significantly in δ13C (ANOVA; F3, 29 = 24.40, P < 0.01), δ15N (F3, 29 = 26.39, P < 0.01), and δ34S values (F3, 29 = 53.25, P < 0.01; Table 1, Fig. 2). Post-hoc comparisons indicated that δ13C values of alewife were greater than the three other prey fishes (Tukey’s, all P < 0.01), round goby and rainbow smelt had similar intermediate values (P = 0.99), and slimy sculpin had the lowest δ13C values (all P < 0.01). The δ15N values of alewife were the lowest of the prey fishes (all P ≤ 0.01), followed by round goby (all P ≤ 0.03), and the highest δ15N values were for rainbow smelt and slimy sculpin, which did not differ between each other (P = 0.93). Alewife and slimy sculpin had similar δ34S values (P = 0.91), as well as round goby and rainbow smelt (P = 0.99), but these two pairs of prey fishes differed between each other (all P < 0.01).

Lake trout isotopic compositions and mixing models

Comparing the isotopic compositions of the four lake trout groups (2008 field-collected, 2010 large field-collected, 2010 small field-collected, 2013 hatchery-source) indicated significant differences among groups for all three isotopes measured (δ13C: F3, 42 = 82.63, P < 0.01; δ15N: F3, 42 = 59.61, P < 0.01; δ34S: F3, 42 = 41.89, P < 0.01; Table 2, Fig. 2). Tukey’s post-hoc comparisons revealed that δ13C values differed among all lake trout groups (Table 2). The δ34S and δ34S values of the large field-collected lake trout did not differ between the 2008 and 2010 sampling years (Tukey’s, all P ≥ 0.06). The δ34S values of the 2010 small field-collected lake trout were significantly different from, but intermediate in value between the 2010 large field-collected lake trout and 2013 small hatchery-source lake trout (all P < 0.01). There were no significant differences in δ15N values between 2013 hatchery-source and the 2010 small field-collected lake trout (P = 0.35, Table 2).

Mixing models of the 2010 small field-collected and 2013 hatchery-source lake trout indicated that lake trout diet consisted of ≥32% hatchery food and was a significant contributor to diet for both two- and three-isotope mixing models of the two small lake trout groups (Fig. 3). The two- and three-isotope models of the hatchery-source fish provided similar estimates of hatchery food in their diets (two-isotope: 87%; three-isotope: 86%). The small 2010 field-collected lake trout also had diets estimated to be primarily from hatchery foods, but were lower compared to the hatchery-source fish for both two-isotope (field-collected: 65%; hatchery-source 87%) and three-isotope mixing models (field-collected 32%; hatchery-source 86%).

Two-isotope (δ13C & δ15N) mixing models of the large field-collected lake trout estimated relatively high dietary contributions of alewife in both sampling years (2008: 49%; 2010: 70%), followed by round goby (2008: 28%; 2010: 16%), rainbow smelt (both years: 8%), and slimy sculpin (2008: 15%; 2010: 6%) (Fig. 3). In comparison, the three-isotope (δ13C, δ15N & δ34S) models differed for both years, with lake trout diets consisting primarily of round goby (2008: 65%; 2010: 54%), followed by alewife (both years 22%), rainbow smelt (2008: 8%; 2010: 20%), and slimy sculpin (2008: 6%; 2010: 4%). Alewife consumption was lower by 27–48% between the two- and three-isotope models of the large trout and was redistributed largely to round goby (Fig. 3; ESM Table S3).

Estimates of alewife consumption by individual small (<250 mm) field-collected lake trout did not differ between two- and three-isotope model configuration (ANOVA; F1, 46 = 0.73, P = 0.40), but the model configurations (i.e. two- or three-isotope) did differ for round goby estimates (ANOVA; F1, 46 = 49.60, P < 0.01). There was no evidence of a relationship between lake trout size and consumption of either alewife (ANOVA; F1, 46 = 2.70, P = 0.11) or round goby (ANOVA; F1, 46 = 0.15, P = 0.71) and no interaction effects between any model variables (ANOVA; both F1, 46 ≤ 0.84, P ≥ 0.36; ESM Fig. S1).

Discussion

We examined the outcomes of two- and three-isotope mixing models to determine if stable isotope estimates of freshwater fish feeding patterns differed with the addition of sulphur isotopes (δ34S) to the carbon and nitrogen isotopes (δ13C & δ15N) typically used. We found that general patterns of estimated resource use remained relatively consistent between model configurations, but significant differences were found and these could have significant implications for our
understanding of lake trout foraging patterns. Here we discuss the implication of these mixing models both in the context of Lake Ontario lake trout feeding patterns and the use of sulphur isotopes in freshwater systems.

Consistent with the stocking of lake trout into Lake Ontario from fish hatcheries in both Canada and the USA, our isotope mixing models indicated that smaller field-collected lake trout (~250 mm in body length) had isotopic compositions consistent with hatchery feeds during rearing. The contribution of hatchery feed to isotopic composition was retained over a period of months based on the mixing models of smaller lake trout already released to Lake Ontario. The hatchery reared fish we sampled were an average length of 141 mm on the day of release, but we found significant hatchery contributions to diet in fish up to 250 mm in length. Based on USGS release and collection records, the 2010 small lake trout used in this study were released from hatcheries in mid-May and collected in July, a period of 1.5–2.5 months from release to sampling. The observed persistence, but significant decline (three-isotope mixing model), of the hatchery feed in lake trout tissues is consistent with both metabolic turnover of tissues over time and the formation of new tissues during growth after the fish were released into Lake Ontario (Hesslein et al., 1993; MacNeill et al., 2006). Residual influence of hatchery foods in yearling lake trout for at least their first summer of life in the lake indicates that it is possible to use stable isotopes of young fish to distinguish hatchery and natural reproduction of salmonids in the Great Lakes. Using the residual influence of hatchery foods in the tissues of stocked fish to distinguish between them and similarly sized naturally produced fishes will be of particular interest to individuals concerned with the establishment or level of natural reproduction of species that are frequently supplemented using hatchery-reared fish, but requires further refinement of the rates at which hatchery feeds dissipate from isotopic measurements.

Multiple disturbance events in the fish communities of the Laurentian Great Lakes, most recently the introduction and establishment of Dreissenid mussels in the 1990s and round goby in the early 2000s (e.g. Kornis et al., 2012; Strayer, 2008), have been linked to altered foraging patterns in lake trout (e.g. Rush et al., 2012). Based on dietary analyses of numerous Great Lakes species there has been an overall movement from a pelagic resource dominated food web to one that includes significant contributions from nearshore benthic energy sources (Dietrich et al., 2006; Turschak et al., 2014). In addition to the introduction of benthic round goby there may also be shifts in alewife to deeper waters (O’Gorman et al., 2000), a shift consistent with our alewife collection depth of 150 m (ESM Table S2). Our isotope mixing models of adult lake trout indicated that alewife and round goby were the two primary resource groups of all large field-collected lake trout regardless of the model configuration, supporting the general shift in lake trout diet away from one dominated by pelagic derived energy to one that is more linked to the benthic food web.

Diet estimates obtained from our three-isotope mixing models differed substantially from the two-isotope models for all the large field-collected lake trout leading to the question of which model configuration is the most accurate. To answer this question we consider four main observations about the Lake Ontario ecosystem. First, round goby abundance expanded rapidly during the 2000s and they have become the most common benthic prey fish in the lake at the same time that native slimy sculpin reached population lows (e.g. 2012 slimy sculpin density = 0.005 fish/m², round goby = 0.526 fish/m²; Dietrich et al., 2006; Weidel et al., 2013). Second, the prey fish community itself has not remained static in Lake Ontario and these changes are likely to affect other fishes throughout the food web. For example, the shift of pelagic alewife further offshore since the early 1990s (O’Gorman et al., 2000) may have decreased their exposure to demersal lake trout, forcing these piscivorous predators to rely more heavily on other species like round goby. Third, naturally produced young lake trout have begun to appear in substantial numbers (Lantry and Lantry, 2015) and this increase in natural reproduction may be due in part to a reduction in maternal lake trout consumption of alewife and rainbow smelt, prey that are both rich in thiaminase, an enzyme linked to reproductive failure for lake trout (Tillitt et al., 2005). Fourth, during 2003–2004, as round goby were becoming established in Lake Ontario, at population densities an order of magnitude lower than reached ten years later (2003–2004 ≤ 0.04 fish/m², Dietrich et al., 2006; 2012 = 0.5 fish/m²),
Weidel et al., 2013), they were found in the stomach contents of lake trout collected from the Kingston Basin (northeast Lake Ontario). At that time, Dietrich et al. (2006) not only found that round goby were already part of lake trout diet, but the goby were the primary diet item for lake trout 551–650 mm in length (mean round goby = 57% diet, mean alewife = 43%, n = 54 lake trout). Additionally, outside of Lake Ontario there are similar patterns of increased natural reproduction of lake trout in Lakes Huron (He et al., 2012; Riley et al., 2007) and Michigan (Hanson et al., 2013), in both cases linked to low alewife abundance. Diet shifts for lake trout in these other lakes away from a predominance of alewives was related to increases in both the levels of egg thiamine and the levels of successful natural reproduction (Riley et al., 2007; Hanson et al., 2013), and for Lake Huron round goby became a significant prey item in lake trout diet (Roseman et al., 2013), similar to what we surmise for Lake Ontario. Because our study was carried out in a natural system we do not have absolute certainty about which of our isotope mixing model configurations (two- or three-isotope) provide the most accurate information, but we argue that based on the emerging patterns both in Lake Ontario and the Great Lakes region in general it is likely that our three-isotope models provided the most accurate inferences of diet.

In this study, we found that inclusion of sulphur ($\delta^{34}S$) stable isotopes in mixing models of resource-use not only indicated higher levels of round goby consumption, but also provided evidence more consistent with emerging data about both prey fishes and lake trout in Lake Ontario. The results of this study indicate that the inclusion of a third isotope may be beneficial to studies of more complex food webs, e.g. in larger lakes with high species richness, but that their utility is dependent on there being variation among prey types for all three isotopes and a thorough consideration of the research questions being asked. Furthermore, our results provide additional support for the use of stable isotopes as a valuable tool for distinguishing between hatchery reared fish in their first few months at large and similarly sized naturally reproduced conspecifics due to the distinct isotopic compositions of hatchery feeds and the rates of isotopic change. Our data provide an indication of broad-scale diet patterns in Lake Ontario between two size

<table>
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<th>Trout group</th>
<th>n</th>
<th>Total body length (mm)</th>
<th>Mass (g)</th>
<th>$\delta^{13}C$</th>
<th>$\delta^{15}N$</th>
<th>$\delta^{34}S$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2008 field-collected</td>
<td>14</td>
<td>563.9 ± 219.2</td>
<td>2712.8 ± 2924.8</td>
<td>−24.0 ± 0.12A</td>
<td>+17.2 ± 1.4A</td>
<td>+4.7 ± 0.6A</td>
</tr>
<tr>
<td>2010 field-collected (&lt;250 mm)</td>
<td>12</td>
<td>334.6 ± 52.6</td>
<td>324.6 ± 144.2</td>
<td>−21.9 ± 1.1B</td>
<td>+16.3 ± 1.7B</td>
<td>+4.2 ± 0.6B</td>
</tr>
<tr>
<td>2010 field-collected (&gt;250 mm)</td>
<td>11</td>
<td>188.1 ± 34.6</td>
<td>24.7 ± 7.2</td>
<td>−18.7 ± 1.6C</td>
<td>+12.0 ± 1.9P</td>
<td>+5.7 ± 0.9P</td>
</tr>
<tr>
<td>2013 hatchery-source</td>
<td>9</td>
<td>140.9 ± 13.1</td>
<td>55.3 ± 23.1</td>
<td>−17.0 ± 0.1D</td>
<td>+11.0 ± 0.3B</td>
<td>+7.1 ± 0.2C</td>
</tr>
</tbody>
</table>

Table 2: Summary of the total body length, mass, and isotopic compositions of lake trout (Salvelinus namaycush) collected from Lake Ontario over multiple years. Mean ± 1 SD isotopic compositions for carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), and sulphur ($\delta^{34}S$) are presented for each group. Letters beside mean values represent the results of Tukey’s post-hoc comparisons with different letters indicating significant differences between the lake trout groups for a given isotope.

Fig. 3. SIAR stable isotope mixing model results estimating the proportion of different prey fish types to Lake Ontario lake trout (Salvelinus namaycush) diets. Lake trout were classified into one of four groups: (a) 2008 field-collected, (b) 2010 small field-collected, (c) 2010 large field-collected, or (d) 2013 hatchery-source (see Methods for details). SIAR models were run in both two-isotope ($\delta^{13}C$ and $\delta^{15}N$) and three-isotope ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$) configurations. Boxes represent the inner 50% of observations with a line indicating the mean value and the 95% Bayesian credibility intervals (BCI) are represented by the whiskers.
groups of lake trout, with the added knowledge that δ^{34}S is an informative dietary isotope in this and other systems where there are questions about diet variation across depth, size-classes, time and space. Such increased discriminatory power can inform continuing conservation and restoration efforts of lake trout in Lake Ontario. As researchers continue to pursue the use of stable isotopes to provide quantitative estimates of diet in freshwater food webs that contain multiple candidate prey species the inclusion of δ^{34}S may provide novel insights into feeding patterns not possible when using two-isotope carbon and nitrogen mixing models.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jglr.2016.03.010.

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Bond, A.L., Diamond, A.W., 2011. Recent Bayesian stable-isotope mixing models are highly increased discriminatory power can inform continuing conservation and management of fisheries and Aquatic Communities in Great Lakes Connecting Channels.


