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ARTICLE

Dietary versus nondietary fatty acid profiles of lake trout ecotypes from Lake Superior and Great Bear Lake: Are fish really what they eat?

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Abstract: Fatty acids are well-established biomarkers used to characterize trophic ecology, food-web linkages, and the ecological niche of many different taxa. Most often, fatty acids that are examined include only those previously identified as "dietary" or "extended dietary" biomarkers. Fatty acids considered as nondietary biomarkers, however, represent numerous fatty acids that can be extracted. Some studies may include nondietary fatty acids (i.e., combined with dietary fatty acids), but do not specifically assess them, whereas in other studies, these data are discarded. In this study, we explored whether nondietary biomarker fatty acids can provide worthwhile information by assessing their ability to discriminate intraspecific diversity within and between lakes. Nondietary fatty acids used as biomarkers delineated variation among regions, among locations within a lake, and among ecotypes within a species. Physiological differences that arise from differences in energy processing can be adaptive and linked to habitat use by a species' ecotype and likely explains why nondietary fatty acid biomarkers can be a relevant tool to delineate intraspecific diversity. Little is known about the nondietary-mediated differences in fatty acid composition, but our results showed that nondietary fatty acid biomarkers can be useful tool in identifying variation.

Résumé : Les acides gras sont des biomarqueurs bien établis qui sont utilisés pour caractériser l'écologie trophique, les liens au sein de réseaux trophiques et les niches écologiques de nombreux taxons. Dans la plupart des cas, les acides gras examinés comprennent seulement ceux déjà identifiés comme étant des biomarqueurs « alimentaires » ou « alimentaires élargis ». Les acides gras considérés comme étant des biomarqueurs non alimentaires comprennent toutefois de nombreux acides gras pouvant être extraits. Certaines études peuvent inclure des acides gras non alimentaires (c.-à-d. combinés à des acides gras alimentaires), mais ne les évaluent pas spécifiquement, alors que, dans d'autres études, ces données sont écartées. Nous avons examiné si des acides gras biomarqueurs non alimentaires peuvent fournir de l'information utile en évaluant leur capacité de discrimination de la diversité intraspécifique à l'intérieur d'un lac et entre lacs. Des acides gras non alimentaires utilisés comme biomarqueurs ont permis de cerner des variations entre régions, entre différents sites dans un même lac et entre écotypes d'une même espèce. Des différences physiologiques découlant de différences dans les processus de transformation énergétique peuvent être adaptatives et reliées à l'utilisation de l'habitat par des écotypes d'une espèce, et expliqueraient vraisemblablement pourquoi les acides gras biomarqueurs non alimentaires peuvent être des outils pertinents pour circonscrire la diversité intraspécifique. Les connaissances sur les variations de la composition des acides gras non médiés par le régime alimentaire sont très limitées, mais nos résultats montrent que les acides gras biomarqueurs non alimentaires peuvent constituer un outil valable pour cerner les variations. [Traduit par la Rédaction]

Introduction

Constraints on traditional methods to investigate diets of organisms within aquatic systems have led to the development and use of biochemical tracers (Vinson and Budy 2010). Among these, fatty acids have gained popularity as both qualitative and quantitative trophic markers that reflect foraging patterns and food-web dynamics (Galloway et al. 2014; Iverson 2009). As such, fatty acids have been used to characterize variation within populations and

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species (e.g., evolutionary units linked to trophic polymorphism; Logan et al. 2000; Scharnweber et al. 2016) and to explore geographical (Hiltunen et al. 2016; Pomerleau et al. 2014; Quérouil et al. 2013) and temporal variation in trophic ecology (e.g., seasonal and annual variations; Eloranta et al. 2013; Hartwich et al. 2013).

Fatty acids are the "building blocks" of lipids and represent the largest constituent of neutral lipids (e.g., triacylglycerols and wax esters) and polar phospholipids (Iverson 2009). The array of fatty acids present in nature is exceptionally complex; routinely ~70 fatty acids can be identified within an organism (Budge et al. 2006; Iverson 2009). The utility of fatty acid analyses to reflect foraging patterns and food-web dynamics relies on the assumption that lipids are broken down into their constituent fatty acids and incorporated relatively unchanged into consumer tissues (Howell et al. 2003; Iverson 2009; Iverson et al. 2004). The storage patterns of fatty acids depend on the biochemical limitations of organisms to biosynthesize, modify chain length, and introduce double bonds into fatty acids, which culminate in vertebrates (Iverson 2009). Fatty acids that are stored in predator tissues, with no or little modification from their prey, have been labelled as "dietary" or "extended dietary" tracers (Budge et al. 2006; Iverson et al. 1997, 2004) and have been the target of analyses in ecological studies. Accordingly, for purposes of this study, we use the terminology of "dietary" versus "nondietary" derived fatty acids based on the classification of Iverson et al. (2004), who identified typical dietary fatty acid markers that are now used across a variety taxa. Often, nondietary fatty acids extracted from tissue samples are not examined, and only those recognized as dietary are analyzed. In other studies, dietary and nondietary fatty acids may be combined to characterize food-web relationships, but information gained from inclusion of nondietary fatty acids is not specifically addressed (Hiltunen et al. 2019; Mariash et al. 2017; McMeans et al. 2015; Taipale et al. 2019).

The exclusion or ignoring nondietary fatty acids from analyses is not misguided, as the purpose of most studies is to describe diet patterns, and this practice has resulted in reliable information being produced across taxa (Galloway et al. 2014; Grosbois et al. 2017; Iverson 2009; Iverson et al. 2004). However, it is unknown whether valid information for other research questions is lost when investigators discard nondietary fatty acids. Biological (e.g., phenotypic and genetic) and environmental variables can affect lipid and fatty acid composition in fishes (Olsen and Skjervold 1995), including temperature (Farkas et al. 1980; Olsen 1999), salinity (Borlongan and Benitez 1992), and light (Ota and Yamada 1971). Thus, fatty acids not labelled as "dietary" markers could be useful when the aim of a study is to delineate or better understand intraspecific diversity. To investigate this, we compared nondietary with dietary fatty acid biomarkers among ecotypes of lake trout (*Salvelinus namaycush*) in Lake Superior and Great Bear Lake, as these ecotypes represent important intraspecific diversity in these lakes.

Salmonids, such as lake trout, inhabit young ecosystems believed to be 10 000 to 15 000 years old (e.g., postglacial lakes colonized from nonglaciated refugia). The depauperate communities of postglacial lakes are commonly characterized by reduced interspecific competition and predation, which allows colonizers access to a relatively wide array of resources, conditions that favour development of intraspecific diversity (McPhail 1993; Robinson and Wilson 1994; Smith and Skulason 1996). This high level of ecological opportunity, together with an increase in intraspecific competition after colonization, can promote specialization and divergence within a population (e.g., the development of groups of individuals with similar patterns of resource use; Svanbäck et al. 2008). Phenotypic characteristics of individuals in such groups may evolve as niches incorporate novel foraging resources, referred to as resource divergence (Robinson and Parsons 2002; Skulason and Smith 1995). As niches diverge, ecotypes can develop differences in morphology, genetics, physiology, life history, and (or) behaviour (Bolnick et al. 2007; Schluter and McPhail 1992; Smith and Skulason 1996).

Intraspecific diversity in lake trout has been mostly linked to differences in depth distribution and, not surprisingly, is best known from large (>500 km^2), deep lakes, such as Lake Superior (Moore and Bronte 2001; Muir et al. 2014), Lake Mistassini (Zimmerman et al. 2007), Great Slave Lake (Zimmerman et al. 2006), and Great Bear Lake (Chavarie et al. 2013). Although lake trout diversification has often focused on isolation-by-depth (without excluding isolation-by-adaptation), diversification also occurs in small lakes or within shallow-water habitats (Bernatchez et al. 2016; Chavarie et al. 2013, 2016*c*; Morissette et al. 2018).

A number of studies have assessed lake trout diets with fatty acids, either qualitatively (Chavarie et al. 2016*b*; Happel et al. 2017, 2018; Hoffmann 2017) or quantitatively (Happel et al. 2016*a*, 2016*b*), and facilitated informed inferences about lake trout dietary patterns. However, pronounced and systematic nondietary-mediated differences in composition of fatty acids have been reported in lake trout, supporting the rationale that nondietary fatty acid biomarkers could be important in delineating intraspecific diversity within this species. Goetz et al. (2014) found physiological differences between lean and siscowet ecotypes of lake trout that reflected genetically based differences in lipid synthesis, metabolism, and transport (Goetz et al. 2010), which suggests information from nondietary fatty acid biomarkers might be important, especially if they have genetic-based mechanisms. Consequently, where intraspecific diversity is manifested as physiological differences among ecotypes, nondietary fatty acid biomarkers could assist in identifying variation. Yet, little is known about the nondietary-mediated differences in fatty acid composition of fish (or other taxa) in an ecological context.

To investigate the use of nondietary fatty acid biomarkers as an ecological tool, we analyzed a set of nondietary and dietary fatty acid biomarkers (as classified by the literature) from lake trout ecotypes collected from two lakes that sustain intraspecific diversity along different axes of intraspecific diversification (e.g., depth-dependent and depth-independent). Specifically, we compared (*i*) nondietary and dietary fatty acid biomarkers among four ecotypes of lake trout from each of Great Bear Lake and Lake Superior and (*ii*) how well nondietary and dietary biomarkers delineated intraspecific diversity within and between lakes. Results of these comparisons will help determine whether nondietary fatty acid biomarkers can discriminate fish geographically and among groups within a species and whether they offer different perspectives than dietary fatty acid biomarkers.

Materials and methods

Study systems and intraspecific diversity of lake trout

Located in the northeast corner of Northwest Territories (Canada; 65°92′N, 120°82′W), Great Bear Lake is the most northerly lake of its size (~31 000 km^2) and is the fifteenth deepest freshwater lake in the world (Fig. 1; Johnson 1975). A UNESCO biosphere reserve, Great Bear Lake is 250 km south of the Arctic Ocean and has characteristics typical of an Arctic lake. The lake is ultra-oligotrophic and, despite its size, has a simple food web, supporting only 15 fish species (Johnson 1975; MacDonald et al. 2004). The lake and its biota have remained relatively isolated and unexploited and is one of the most pristine large lakes in North America. Great Bear Lake has five semi-isolated arms, but due to sample sizes, data were pooled across multiple sites (see Chavarie et al. 2016*b* for details).

Great Bear Lake sustains a noteworthy example of lake trout divergence (Fig. 2). With its intraspecific diversity independent of depth-based segregation, the lake also presents an unusual ecological framework for lake trout differentiation (Chavarie et al. 2016*a*). Currently, four shallow-water ecotypes for lake trout are

Fig. 1. Location of (1) Great Bear Lake (Canada) and (2) Lake Superior (Canada–USA). Two sampling sites were defined in Lake Superior to account for spatial variation: Superior Shoal and Stannard Rock (QGIS 3.0, Canada Basemap; Hoffmann 2017).

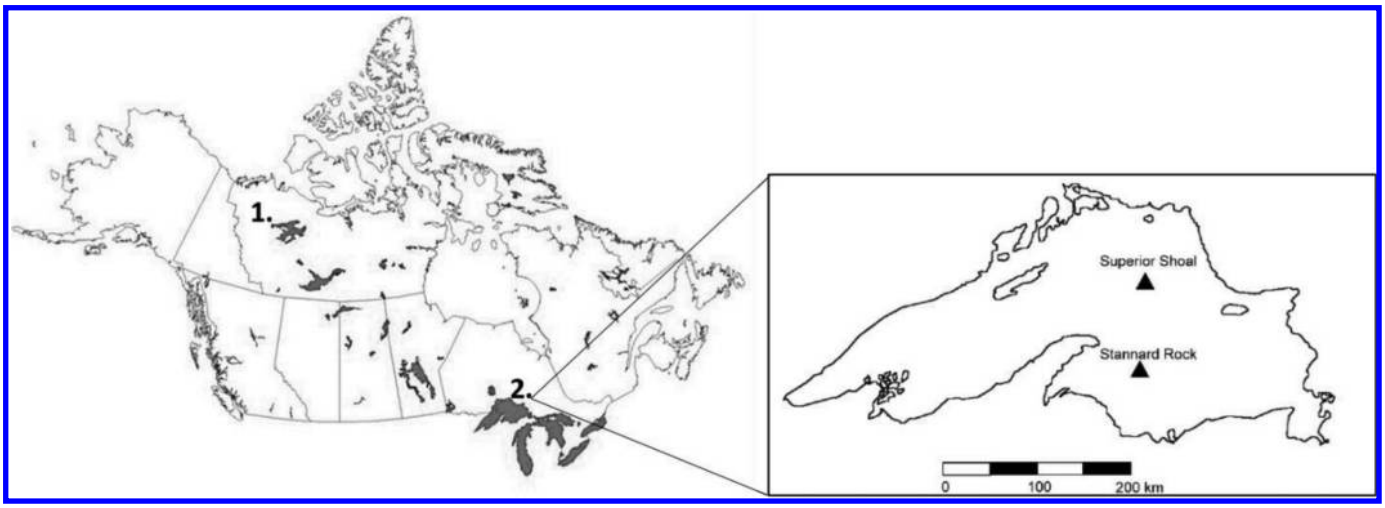
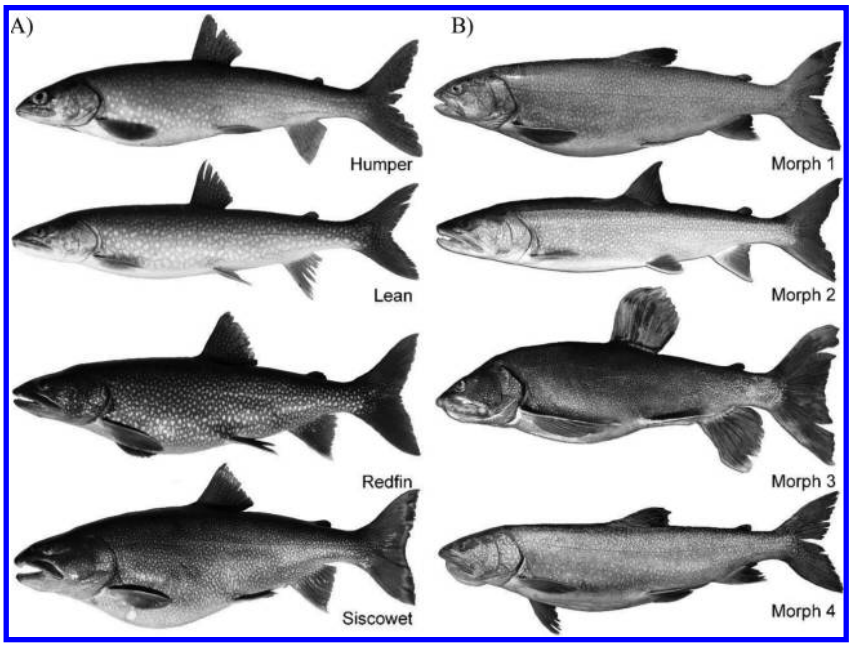


Fig. 2. (A) The four morphotypes of lake trout in Lake Superior: the lean, humper, siscowet, and redfin (defined in Muir et al. 2014). (B) The four shallow-water morphotypes of lake trout from Great Bear Lake: Morphs1–4 (defined in Chavarie et al. 2013).



described in Great Bear Lake; three are common (Ecotypes 1–3), and one is rare (Ecotype 4). Ecotype 1 has the smallest head and jaws, intermediate fin lengths, and body shape intermediate between Ecotypes 2 and 3. The lean-like Ecotype 2 has the largest head and jaws but the smallest fins and a streamlined body shape. Ecotype 3 has the longest fins, a robust body shape, and a subterminal mouth. Ecotype 4 has a thick and curved lower jaw, a streamlined body shape, and the smallest caudal peduncle among the ecotypes. Although Ecotype 4 is a pelagic specialist, Ecotypes 1–3 have more general feeding habits, with varying degrees of omnivory along a weak benthic–pelagic gradient (Chavarie et al. 2016*a*, 2016*b*).

Lake Superior is a postglacial, oligotrophic lake between Canada and the USA (47°43′N, 86°56′W; Fig. 1). Lake Superior is the largest lake in the world by surface area (82 100 km^2). Most of the waters of Lake Superior can be classified as offshore; 77% of the total area is greater than 80 m deep (maximum depth = 406 m; see Gorman et al. 2012; Hoffmann 2017; Horns et al. 2003). Lake Superior supports 87 fish species, with lake trout as a main target of the commercial fishery since the 1800s. Lake trout were sampled from two sites: Superior Shoal (48°3′43.54″N, 87°8′52.57″W) and Stannard Rock (47°12′26.26″N, 87°12′3.82″W) (Fig. 1).

Lake Superior supports one of the highest levels of sympatric diversity expressed within lake trout (Fig. 2). Four ecotypes are currently recognized; the siscowet, humper, and redfin ecotypes inhabit deep water (>70 m), whereas the lean ecotype occupies shallow-water habitats (<70 m) (Bronte et al. 2003; Bronte and Moore 2007; Muir et al. 2014). The siscowet is characterized by a large head, short snout, long maxilla, large eye, short and deep caudle peduncle, and moderately long paired fins (Muir et al. 2015). Humpers have a small head, short snout, short maxillae, large eyes, and short and narrow caudal peduncle (Moore and Bronte 2001; Muir et al. 2015). The redfin has the largest head, snout, and eyes, the longest and deepest caudal peduncle, and much longer pelvic and pectoral fins than the other ecotypes (Muir et al. 2014). Finally, lean lake trout have a large, narrow, and

pointed head, long snout, small eyes, long and narrow caudal peduncle, short paired fins, and low body lipid content (Endler 1978; Khan and Qadri 1970; Muir et al. 2015). As vertical-migrating visual predators, the three deepwater ecotypes are likely feeding mostly on *Mysis* and deepwater ciscoes (*Coregonus artedi* complex; Hoffmann 2017; Hrabik et al. 2006; Muir et al. 2014), whereas the lean ecotype is adapted for daytime predation on pelagic fishes in shallow-water habitats (piscivorous feeding strategy; Harvey and Kitchell 2000; Harvey et al. 2003; Janhunen et al. 2009).

Fatty acids

Dorsal muscle samples (Budge et al. 2011) from lake trout in both lakes were stored at –20 °C (Budge et al. 2006; Chavarie et al. 2016*b*; Kavanagh et al. 2010; Loseto et al. 2009). Lipids were extracted from 1 g of the homogenate material; after passive overnight extraction (at –20 °C) in 2:1 chloroform–methanol containing 0.01% butylated hydroxytoluene (BHT) (*v*/*v*/*w*) (Folch et al. 1957), samples were filtered through Whatman Grade 1 Qualitative filter paper, and the filter paper sample was rinsed twice with 2 mL of 2:1 chloroform–methanol. Sample extract was collected in a test tube, and 7 mL of 0.88 NaCl solution was added to encourage fatty acids to move into the organic (chloroform) layer. The aqueous layer was discarded, after which the chloroform was dried with sodium sulfate prior to total lipid determination. The extracted lipid was used to prepare fatty acid methyl esters (FAME) by transesterification with Hilditch reagent (0.5 mol·L^{-1} H_2SO_4 in methanol; Morrison and Smith 1964). Samples were heated for 1 h at 100 °C. Gas chromatographic (GC) analysis was performed on an Agilent Technologies 7890N GC equipped with a 30 m J&W DB-23 column (0.25 mm inside diameter; 0.15 μm film thickness). The GC was coupled to a Flame Ionization Detector operating at 350 °C. Hydrogen was used as carrier gas flowing at 1.25 mL·min^{-1} for 14 min, increasing to 2.5 mL·min^{-1} for 5 min. The split–splitless injector was heated to 260 °C and run in splitless mode. The oven program was as follows: 60 °C for 0.66 min, increasing by 22.82 °C·min^{-1} to 165 °C with a 1.97 min hold; increasing by 4.56 °C·min^{-1} to 174 °C and by 7.61 °C·min^{-1} to 200 °C with a 6 min hold. Peaks were quantified using Agilent Technologies ChemStation software. Fatty acid standards were obtained from Supelco (Oakville Ontario, Canada) (37 component FAME mix) and Nuchek (Elysian Minnesota, USA) (54 component mix GLC-463). FAMEs were identified via retention time and known standard mixtures and are reported as percentages of total fatty acids. Fatty acid standards were obtained from Supelco (37 component FAME mix) and Nuchek (54 component mix GLC-463). Standards were run to allow creation of a four-level calibration curve for each set of samples at the start of each sample set. A standard was repeated every 10 samples thereafter. Every 10th sample was injected in duplicate. All fatty acid values were converted to a mass percentage of the total array and were named according the IUPAC nomenclature as X:Yn-z, where X is the number of carbons in the fatty acid, Y is the number of methylene-interrupted double bonds present in the chain, and n-z denotes the position of the last double bond relative to the methyl terminus (Ronconi et al. 2010). All laboratory analyses were conducted at the Freshwater Institute, Fisheries and Oceans Canada, Winnipeg, Manitoba.

For both lakes, tissue samples were taken from individual lake trout previously identified to corresponding ecotypes: 126 samples were collected from Great Bear Lake (Ecotype 1 = 32, Ecotype 2 = 35, Ecotype 3 = 38, and Ecotype 4 = 21; see Chavarie et al. 2016*b* for more details), and 210 samples were collected from Lake Superior (60 siscowet, 60 humper, 30 redfin, and 60 lean; see Hoffmann 2017 for more details). Overall, fatty acid analysis procedures were divided into two steps, using nondietary and dietary fatty acid biomarkers (see Appendix A), following the methods of Iverson et al. (1997, 2004) and Budge et al. (2006) to identify dietary fatty acid biomarkers. Dietary biomarkers were defined as fatty acids with ≥14 carbons that are generally incorporated into animal tissue from the diet with no or little modification (e.g., rather than from biosynthesis; Iverson 2009). Thirty-eight dietary and 24 nondietary fatty acid biomarkers were found to be shared between the two lakes, and these were selected for further analyses (Tables A1 and A2).

Statistical analyses

Unless noted otherwise, statistical analyses were conducted using R software version 3.5.3 (R Core Team 2017). Prior to analysis, fatty acid concentrations were logit-transformed ($\log[p/(1-p)]$) to normalize the data and then scaled and centered using a *z*-score transformation ($z = x\mu/\sigma$) (Clemmensen et al. 2011; Witten and Tibshirani 2011). Principal component analysis (PCA) was performed on all dietary and nondietary fatty acid biomarkers to provide inference about patterns of variation among locations and ecotypes (Chavarie et al. 2016*b*). PCA summarizes similarities and differences among individuals, based on their fatty acid profiles, independent of ecotype and location (Chavarie et al. 2016*b*). For PCAs, 12 outlier individuals from Superior Shoal were excluded because PCAs are sensitive to outlier variation (but see Fig. A1 for PCAs with outliers included; Filzmoser et al. 2009; Kriegel et al. 2008).

To test for differences in fatty acid composition among ecotypes within each lake (dietary and nondietary), we used permutational multivariate analysis of variance (PERMANOVA; a nonparametric analog of multivariate analysis of variance), followed by post hoc comparisons with Bonferroni corrections. PERMANOVAs were performed in PAST 3 (Hammer et al. 2001) using 9999 permutations. A similarity percentage routine (SIMPER) using Bray–Curtis was used to determine which fatty acids (dietary and nondietary) were primarily responsible for observed differences among ecotypes for each lake (King and Jackson 1999). We also performed linear discriminant analysis on fatty acids (dietary and nondietary) to delineate differences among ecotypes at each location. A jacknife validation procedure, using 20% of our data as unknown, provided a classification success metric to assess how distinct ecotypes appeared in each fatty acid dataset (dietary versus nondietary).

Results

Combined lakes analyses

The first two axes of the PCAs explained 42.4% and 41.5%, respectively, of the variation among individuals in their nondietary and dietary fatty acid biomarkers (Fig. 3). In both PCAs, lake trout from Great Bear Lake were largely distinct from Lake Superior trout (only ~30 individuals from Great Bear Lake overlapped with individuals from Lake Superior), but trout from the two Lake Superior sites, Stannard Rock and Superior Shoal, overlapped. The nondietary fatty acids 13:1, 14:1n-7, 15:0, 15:1n-8, 15:1n-6, 15:0 iso, 16:1n-11, 17:0 iso, 20:0, 22:0, 20:2n-9, and 24:1n-9 contributed to the separation between the two lakes. Separation between the two lakes in the dietary PCA appeared to be driven by fatty acids associated with pelagic habitat (14:0, 20:1n-9, 20:1n-7, 20:1n-11, and 22:1n-9; toward Lake Superior) versus one dietary fatty acid associated with cannibalism and (or) carnivory (20:5n-3; toward Great Bear Lake) (Appendix A, Table A1). Finally, the first two axes of the PCA based on all fatty acids combined explained 39.0% of the variation among lake trout ecotypes from Great Bear Lake and Lake Superior. As before, lake trout from Great Bear Lake were largely separated from the Lake Superior trout, whereas lake trout from Stannard Rock and Superior Shoal in Lake Superior overlapped completely.

Lake trout intraspecific diversity of Great Bear Lake

Composition of both nondietary and dietary fatty acid biomarkers were able to discriminate among the four lake trout ecotypes from Great Bear Lake. Nondietary fatty acid biomarkers differed among the four Great Bear Lake ecotypes (one-way PERMANOVA, $F_{[3,122]} = 3.6$, $P < 0.01$). Similarly, comparison of nondietary fatty

Fig. 3. Principal component analysis (PCA) of nondietary fatty acid biomarkers (top panels), dietary fatty acid biomarkers (middle panels), and all fatty acids (bottom panels) for individual lake trout collected from Great Bear Lake, Stannard Rock (Lake Superior), and Superior Shoal (Lake Superior). Vectors of individual fatty acids important to the positioning of lake trout are represented to the right of each PCA. Angles and lengths of vectors represent the direction and strength of relationships, respectively, between variables and the principal components.

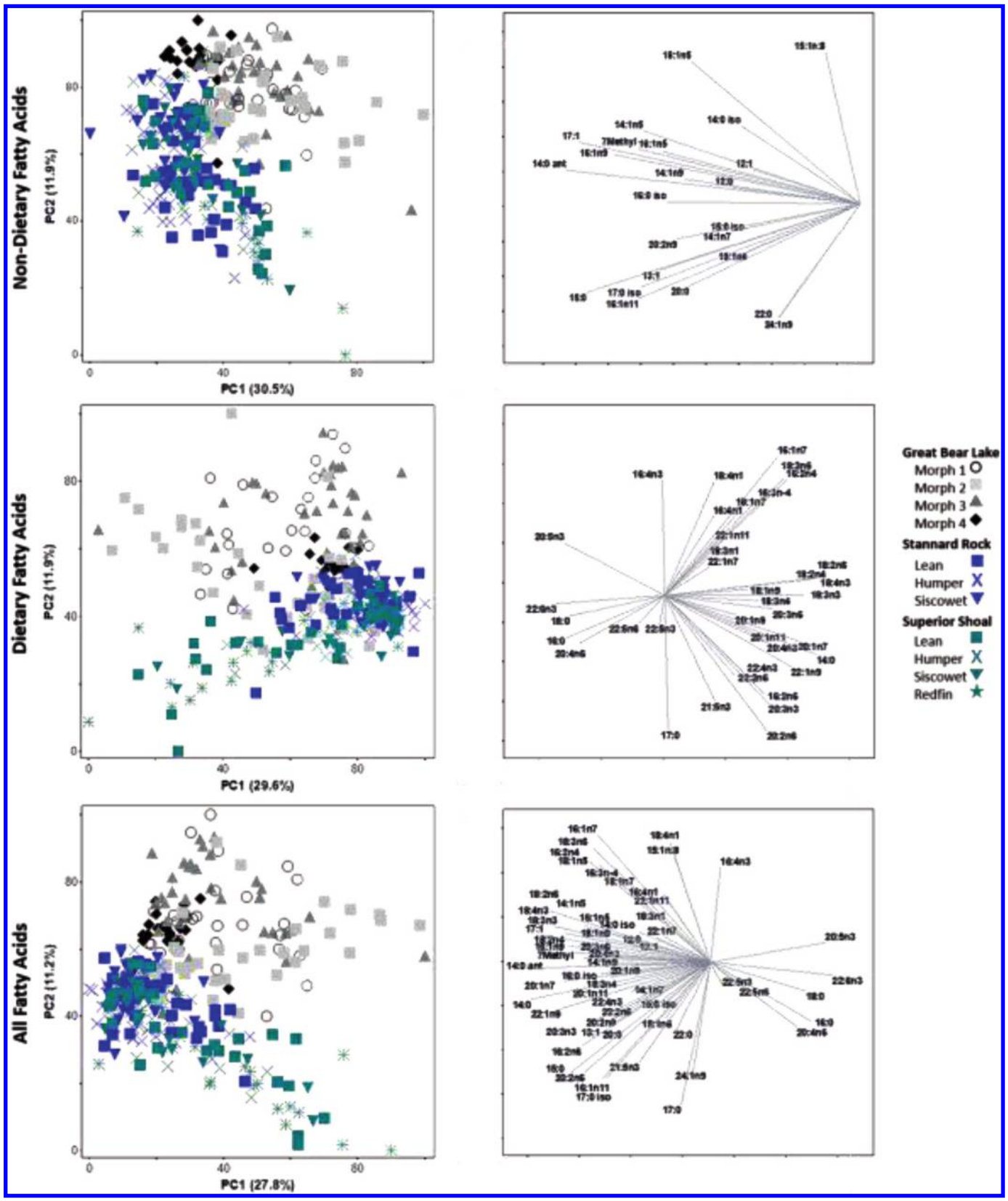
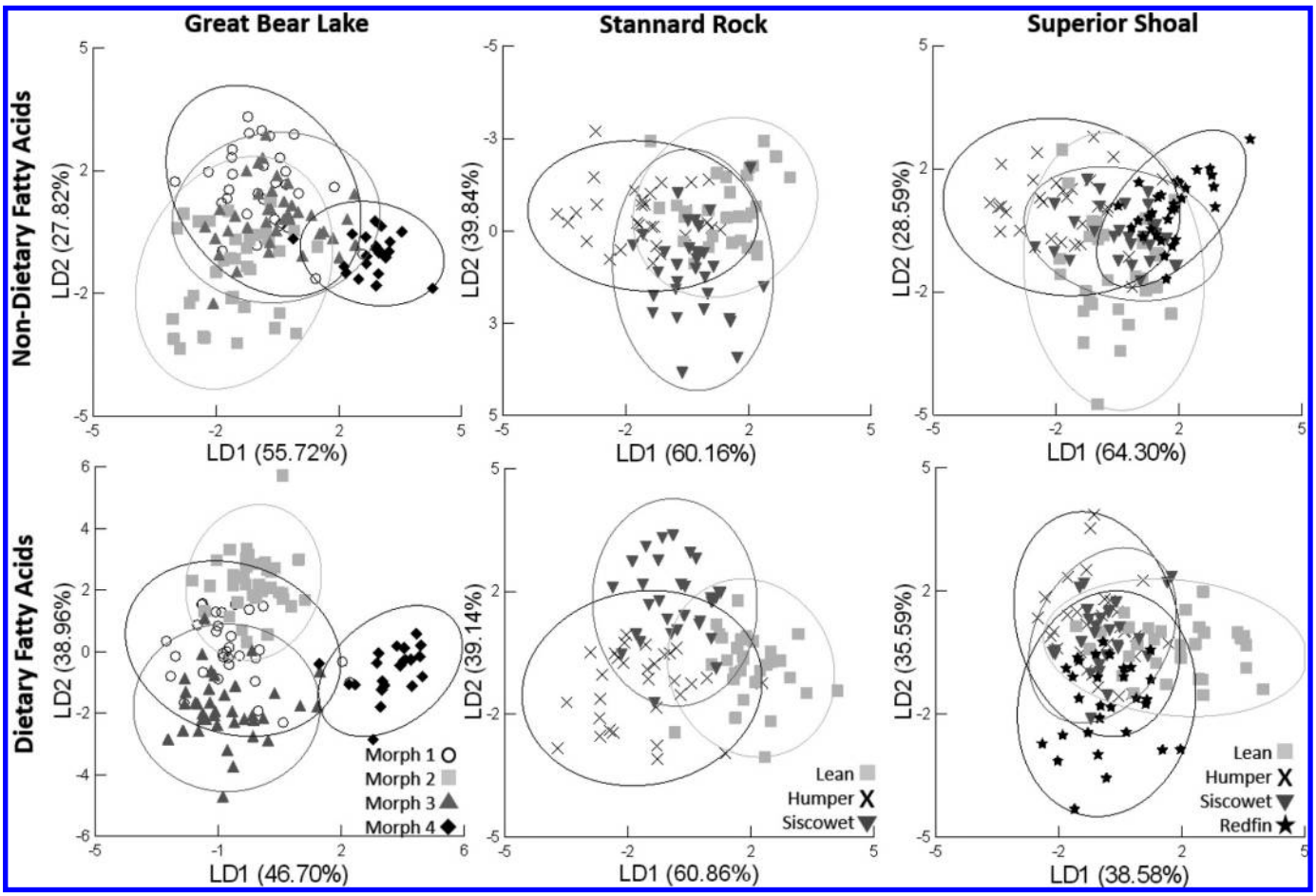


Fig. 4. Results of linear discriminant function analyses of nondietary fatty acid biomarkers (top panels) and dietary fatty acid biomarkers (bottom panels) for lake trout collected from Great Bear Lake, Stannard Rock (Lake Superior), and Superior Shoal (Lake Superior). The 95% ellipse of each morph is also provided.



acid biomarkers showed that all pairs of ecotypes differed from one another ($P < 0.01$) except for Ecotypes 1 and 3. The ten most discriminating nondietary fatty acid biomarkers from SIMPER explained 63.8% of the dissimilarity among groups (Table 1). The first two axes of the linear discriminant analysis explained 55.7% and 27.8% of the variation, and 57.9% of all individuals were correctly classified to ecotype based on nondietary fatty acid biomarkers (Fig. 4). Dietary fatty acid biomarkers also differed among the four ecotypes from Great Bear Lake (one-way PERMANOVA, $F_{[3,122]} = 2.95$, $P < 0.01$), and most ecotypes differed from each other (all pairwise $P < 0.01$ except for Ecotypes 1 versus 3). The ten most discriminating dietary fatty acid biomarkers from SIMPER explained 48.8% of the dissimilarity among groups (Table 2). The first two axes of the linear discriminant analysis explained 46.7% and 39.0% of the variation, and 68.3% of all individuals were correctly classified to ecotype based on dietary fatty acid biomarkers (Fig. 4).

Lake trout intraspecific diversity of Lake Superior, Stannard Rock

Nondietary fatty acid biomarkers differed among the three ecotypes of lake trout from Stannard Rock (one-way PERMANOVA, $F_{[2,87]} = 3.9$, $P < 0.01$), and leans differed significantly from both siscowets and humpers ($P < 0.01$). The ten most discriminating nondietary fatty acid biomarkers from SIMPER explained 70.9% of the dissimilarity among ecotypes (Table 1). The first two axes of the linear discriminant analysis explained 60.2% and 39.8% of the variation, respectively, and 52.2% of all individuals were correctly classified to ecotype based on nondietary fatty acid biomarkers (Fig. 4). In contrast with results from nondietary fatty acid biomarkers, no differences in dietary fatty acid composition occurred among the three lake trout ecotypes from Stannard Rock, Lake Superior (one-way PERMANOVA, $F_{[2,87]} = 1.12$, $P = 0.3$). The ten most discriminating fatty acids from SIMPER explained 55.2% of the dissimilarity among groups (Table 2), and the first two axes of the linear discriminant analysis explained 60.9% and 39.1% of the variation. Fifty percent of individuals were correctly classified to ecotype based on dietary fatty acid biomarkers (Fig. 4).

Lake trout intraspecific diversity of Lake Superior, Superior Shoal

Like Stannard Rock, composition of nondietary fatty acid biomarkers differed significantly among the four ecotypes (one-way PERMANOVA, $F_{[3,116]} = 2.5$, $P < 0.01$); leans differed from redfins ($P < 0.01$), and differences were marginal between redfin and siscowet and between redfin and humper ($P < 0.06$). The ten most discriminating nondietary fatty acid biomarkers from SIMPER explained ~69.9% of the dissimilarity among groups (Table 1). The first two axes of the linear discriminant analysis explained 64.3% and 25.6% of the variation, and 45.0% individuals were correctly classified to ecotype using nondietary fatty acid biomarkers (Fig. 4). Similar to what was found at Stannard Rock, we found no differences among the four ecotypes from Superior Shoal based on dietary fatty acid composition (one-way PERMANOVA, $F_{[3,116]} = 0.8$, $P = 0.3$). The ten most discriminating fatty acids from SIMPER explained 55.8% of the dissimilarity among groups (Table 2). The first two axes of the linear discriminant analysis explained 38.6%

Table 1. The ten most discriminating nondietary fatty acid biomarkers from SIMPER analyses to determine which fatty acids were primarily responsible for observed differences.

Fatty acid	Great Bear Lake (63.8%)	Stannard Rock (70.9%)	Superior Shoal (69.9%)
12:0		3.6%	6.1%
12:1	8.3%	8.9%	14.7%
13:1			
14:1n-9	5.4%	3.0%	4.5%
14:1n-7	3.8%		3.3%
14:1n-5			4.2%
14:0 iso		6.4%	
14:0 ante			
15:0			
15:1n-8	8.5%	25.9%	18.8%
15:1n-6	8.2%	3.7%	4.2%
15:0 iso	3.8%	3.6%	3.6%
16:1n-11			
16:1n-9			
16:1n-5			
16:0 iso	7.1%		
7Methyl16:0	4.4%		
17:1			
17:0 iso			
18:1n-5			
20:0		4.1%	
20:2n-9	10.2%	4.5%	5.2%
22:0	4.1%	7.2%	5.3%
24:1n-9			

Note: Results are presented for each region, including percent contribution to overall fatty acid dissimilarity among lake trout morphs. Fatty acids are listed in order of elution; those highlighted in grey are shared among the study three regions. The total percentage of the ten most discriminating fatty acids are given for each region.

Table 2. The ten most discriminating dietary and extended dietary fatty acid biomarkers from SIMPER analyses to determine which fatty acids were primarily responsible for observed differences.

Fatty acid	Great Bear Lake (48.8%)	Stannard Rock (55.2%)	Superior Shoal (55.8%)
14:0			
16:0			
16:1n-7			
16:2n-6			
16:2n-4			
17:0			
16:3n-4	4.0%	2.5%	2.9%
16:4n-3	3.0%	2.6%	
16:4n-1		7.3%	6.3%
18:0			
18:1n-9		2.4%	
18:1n-7			
18:2n-6			
18:2n-4			
18:3n-6			
18:3n-4			
18:3n-3			
18:3n-1	3.3%		3.5%
18:4n-3			
18:4n-1	3.5%	10.5%	11.1%
20:1n-11	4.0%		
20:1n-9			
20:1n-7	3.6%		
20:2n-6			
20:3n-6			
20:4n-6			
20:3n-3			
20:4n-3			
20:5n-3			
22:1n-11	8.6%	11.9%	12.1%
22:1n-9			
22:1n-7		2.7%	4.6%
22:2n-6	6.3%		
21:5n-3	7.5%		4.7%
22:5n-6		7.2%	3.0%
22:4n-3	5.0%	2.6%	4.2%
22:5n-3			
22:6n-3		5.5%	3.4%

Note: Results are presented for each region, including percent contribution to overall fatty acid dissimilarity among lake trout morphs. Fatty acids are listed in order of elution; those highlighted in grey are shared among the study three regions. The total percentage of the ten most discriminating fatty acids are given for each region.

and 35.6% of the variation, respectively, and 31.7% of fish were correctly classified to ecotype based on their dietary fatty acids (Fig. 4).

Discussion

In this study, nondietary fatty acids showed variation geographically (between lakes), between locations within a lake (i.e., Stannard Rock versus Superior Shoal), and among ecotypes within a lake or location. Although some overlap existed (which reduced the power to discriminate), our results showed that when investigating intraspecific diversity, nondietary fatty acid biomarkers can be a useful tool to delineate groups, and that sometimes, such as at sites in Lake Superior, these biomarkers were more discriminatory than dietary fatty acids. While characterizing trophic divergence, food-web linkages, and time-integrated niche use across a large array of taxa, most investigators use a common set of fatty acids that are selected from lists that classify fatty acids as "dietary" or "extended dietary" biomarkers (Budge et al. 2006; Iverson et al. 2004). However, our results suggest that discarding nondietary fatty acid data as a matter of course may result in inadvertent loss of information.

Not all fatty acids provide equivalent information about diet due to metabolism and de novo synthesis (Iverson et al. 1993, 2004). Metabolism, however, can differ within species, as physiological differences often exist among sets of individuals (i.e., ecotypes; Miles et al. 2007; Pryke et al. 2007). Such physiological differences within a species may result in nondietary fatty acid biomarkers being a relevant tool to delineate intraspecific diversity, as we show here for lake trout. In Lake Superior, fat content has long been recognized as an important characteristic that distinguishes lake trout ecotypes along a depth axis (Eschmeyer and Phillips 1965; Eshenroder 2008), and these lipid differences have been linked to genetic differences among ecotypes (Eschmeyer and Phillips 1965; Goetz et al. 2010, 2014). Thus, fatty acid deposition and metabolism appear to have undergone selection along a depth gradient likely in part to contribute to buoyancy compensation, with differences reported between siscowet (deepwater ecotype) and lean (shallow-water ecotype) lake trout (Eschmeyer and Phillips 1965; Goetz et al. 2010). Until now, no information has been available for redfin and humper ecotypes.

In addition to the variation in lipid accumulation, Goetz et al. (2014) also found differences in lipid composition between siscowet and lean ecotypes. Because a common garden design was used in their experiment, the higher proportion of polyunsaturated fatty acids (PUFAs) found in the muscle lipid profile of siscowet than in lean lake trout could not be attributed to differences in diet. The differences in nondietary fatty acid biomarkers we observed among the four ecotypes of lake trout from Lake Superior are consistent with the concept of metabolotypes (Goetz et al. 2014). Altogether, differences in energy processing and storage between lean and siscowet lake trout in Lake Superior are adaptive to their respective habitats, deep versus shallow water, and to their life histories (Goetz et al. 2014). Lipid content,

intertwined with buoyancy variations, has been linked to differences in depth distributions and swimming tactics among lake trout ecotypes (Zimmerman et al. 2006, 2007, 2009). While deepwater ecotypes use hydrostatic lift related to lipid content to enhance vertical migration while foraging for *Mysis* and cisco (Henderson and Anderson 2002), the shallow-water lean ecotype likely relies more on hydrodynamic lift, linked to the cruising movements of pelagic predators (Webb 1984). Consistent with the findings of Goetz et al. (2014), we found that nondietary fatty acid biomarkers differed between shallow- and deepwater ecotypes at Stannard Rock. However, at Superior Shoal, nondietary fatty acid biomarkers differed only between the shallow-water lean ecotype and one deepwater ecotype: the redfin. This observation, along with some more subtle differences in nondietary fatty acid biomarkers among deepwater ecotypes, requires further study, as our current knowledge is limited. It is presently unclear whether these lake trout ecotypes have adapted physiologically to their habitat leading to metabolotypes and how diets differ among all ecotypes temporally and spatially within Lake Superior. As such, feeding on the same item by different ecotypes might produce different fatty acid accumulations resulting not only in physiologically interesting questions but also questioning the accuracy of predictions about diet composition.

The concept that dynamics of energy processing and storage are adaptive along a gradient associated with depth does not apply to the intraspecific diversity of lake trout in Great Bear Lake. This diversity is limited to shallow-water habitat and appears to be independent of major habitat and resource partitioning (Chavarie et al. 2016*a*, 2018). The similarity of results between Great Bear Lake and Lake Superior is thus perplexing, as we were expecting greater differences in nondietary fatty acid biomarkers among ecotypes in Lake Superior than in Great Bear Lake, due to known buoyancy variation associated with a depth gradient in Lake Superior. If lake trout ecotypes from Great Bear Lake are also under selection (Harris et al. 2015), differences in energy processing and storage may be as pronounced as those that have been observed in Lake Superior. Another question raised by our results is the extent to which ecotypes are independent of major habitat or resource axes (the same question is pertinent for Lake Superior), especially because dietary fatty acid biomarkers were slightly better than nondietary biomarkers at delineating intraspecific groups in Great Bear Lake, but not in Lake Superior.

Differences between morphs and sites may be influenced by the total content of fatty acids in muscle, rather than by their composition (%). Tissue-specific storage of fatty acids can vary across space or time, and some fish store lipids as modified adipose or lipid pockets in their muscle (e.g., salmonids; Iverson 2009, Sasaki et al. 1989). In a comparison of fatty acid content between dorsal muscle versus belly tissue, Happel et al. (2020) found a threshold response; the tissues became increasingly dissimilar when lipid content of muscle was ≳10%. Nevertheless, Happel et al. (2020) found that fatty acid profiles were specific to each of the five lakes they examined (i.e., lake trout displayed broad variation among locations).

Ecological differences in allopatry are often more pronounced than those in sympatry, due to disparate environments and isolation (Fraser et al. 2011; Heggenes 2002; Rundle and Nosil 2005; Yoder et al. 2010). Our results were consistent with this trend (i.e., differences between lakes were greater than differences among ecotypes within a lake for both dietary and nondietary fatty acid biomarkers). For dietary fatty acids, differences between the two lakes appeared to be due to fatty acids associated with a pelagic environment, such as C20 and C22 monounsaturates, that can be used as biomarkers of food webs based on pelagic copepods (Ahlgren et al. 2009; Budge et al. 2006; Dalsgaard et al. 2003). Although benthic productivity generally dominates in Arctic lakes (Chavarie et al. 2018; Johnson 1975), few lake trout in Great Bear Lake specialized on pelagic resources (i.e., few Ecotypes 2 and 4 had fatty acid signatures that overlapped with lake trout from Lake Superior; Chavarie et al. 2016*a*, 2016*b*).

With the general benthic orientation of lake productivity in Arctic regions, combined with the known distributions in deepwater habitats of lake trout ecotypes from Lake Superior (50–150 m; Muir et al. 2014), our overall results reflected the expected ecological differences of this species from these two systems. Despite these interlake differences, similar dietary (e.g., 5 out of 10) and nondietary (e.g., 7 out of 10) fatty acid biomarkers were important in identifying intraspecific diversity within each lake. The greater number of shared nondietary fatty acid biomarkers discriminating lake trout intraspecific diversity in the two lakes supports the idea of similar physiological differences (e.g., energy processing and storage) among ecotypes from both lakes.

A few caveats should be noted that could alter interpretations of this study. First, we cannot ensure the fatty acid biomarkers defined based on Iverson et al. (2004) truly reflect dietary versus nondietary origins, due to the lack of taxa-specific studies on the integration of prey fatty acids. Despite this uncertainty, the aim of this paper was to examine loss of information from discarding fatty acids generally considered as nondietary biomarkers by the literature. Second, no spatial component was defined for the Great Bear Lake dataset, due to small sample sizes from each location, which may have introduced variation into the results. The importance of spatial variation in large, complex systems was shown here for Lake Superior (Chavarie et al. 2015; Hoffmann 2017), and environmental variables (e.g., temperature, light), which may vary spatially, are known to alter lipid composition in fish tissue (Olsen 1999). Third, multiple sizes and life stages (e.g., juvenile, mature, and resting individuals) were included in the Lake Superior analysis; different life stages can vary in lipid metabolism (Sheridan 1989), and large lake trout can rely more on nearshore–benthic food-web resources than small lake trout (Happel et al. 2018). Finally, some fatty acids exist at very low amounts (≤2%), which can introduce error when interpreting differences among fatty acids that are found in only trace amounts (e.g., peak shouldering; Christie 1998). Despite these limitations, we found some consistent patterns with regards to intraspecific diversity between lakes and among ecotypes within lakes from two distinct datasets.

Conclusion

Our study demonstrated the potential benefits of using both dietary and nondietary fatty acid biomarkers for delineating variation within a species. In some instances, nondietary fatty acids were better for discriminating ecotypes than dietary ones, in contrast with the popular maxim associated with trophic markers: "you are what you eat". Dietary fatty acid biomarkers can document the occurrence of discrete niche use among sets of individuals (i.e., ecotypes) within a species (Chavarie et al. 2016*b*). The fatty acid composition of individuals that reflects their diet has been validated for lake trout (Happel et al. 2016*a*, 2016*b*). However, physiological differences in the dynamics of energy processing (e.g., metabolism) and storage can also be adaptive among ecotypes (Eschmeyer and Phillips 1965; Goetz et al. 2010, 2014), which may result in differences in nondietary fatty acids (e.g., greater than dietary fatty acids) and thus be useful molecular tools. The lack of information on nondietary fatty acids in lake trout (and other taxa), however, raises questions about their physiological role and whether consumers acquire them or not from their diet (e.g., synthesis de novo, elongation). This would be a fruitful area for future research.

Ultimately, the relative importance of dietary versus nondietary fatty acid biomarkers depends on the question being asked. In our study, nondietary fatty acids were valuable in delineating intraspecific variation within a lake, but also in examining differences between lakes. Nondietary fatty acid biomarkers can pro-

vide useful information; therefore, one should carefully consider whether such information is superfluous or not before data from these fatty acids are discarded.

Data availability statement

All data presented are available by request via e-mail to the first author.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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References

Ahlgren, G., Vrede, T., and Goedkoop, W. 2009. Fatty acid ratios in freshwater fish, zooplankton and zoobenthos — are there specific optima? *In* Lipids in aquatic ecosystems. *Edited by* M. Kainz, M.T. Brett, and M.T. Arts. Springer, New York. pp. 147–178.

Alfaro, A.C., Thomas, F., Sergent, L., and Duxbury, M. 2006. Identification of trophic interactions within an estuarine food web (northern New Zealand) using fatty acid biomarkers and stable isotopes. Estuar. Coast. Shelf Sci. **70**(1–2): 271–286. doi:10.1016/j.ecss.2006.06.017.

Bernatchez, S., Laporte, M., Perrier, C., Sirois, P., and Bernatchez, L. 2016. Investigating genomic and phenotypic parallelism between piscivorous and planktivorous lake trout (*Salvelinus namaycush*) ecotypes by means of RADseq and morphometrics analyses. Mol. Ecol. **25**(19): 4773–4792. doi:10.1111/mec.13795. PMID:27497114.

Bolnick, D.I., Svanbäck, R., Araújo, M.S., and Persson, L. 2007. Comparative support for the niche variation hypothesis that more generalized populations also are more heterogeneous. Proc. Natl. Acad. Sci. **104**(24): 10075–10079. doi:10.1073/pnas.0703743104. PMID:17537912.

Borlongan, I.G., and Benitez, L.V. 1992. Lipid and fatty acid composition of milkfish (*Chanos chanos* Forsskal) grown in freshwater and seawater. Aquaculture, **104**(1–2): 79–89. doi:10.1016/0044-8486(92)90139-C.

Brett, M.T., and Müller-Navarra, D.C. 1997. The role of highly unsaturated fatty acids in aquatic foodweb processes. Freshw. Biol. **38**: 483–499.

Bronte, C.R., and Moore, S.A. 2007. Morphological variation of siscowet lake trout in Lake Superior. Trans. Am. Fish. Soc. **136**(2): 509–517. doi:10.1577/T06-098.1.

Bronte, C.R., Ebener, M.P., Schreiner, D.R., DeVault, D.S., Petzold, M.M., Jensen, D.A., et al. 2003. Fish community change in Lake Superior, 1970–2000. Can. J. Fish. Aquat. Sci. **60**(12): 1552–1574. doi:10.1139/f03-136.

Budge, S.M., Iverson, S.J., Bowen, W.D., and Ackman, R.G. 2002. Among- and within-species variability in fatty acid signatures of marine fish and invertebrates on the Scotian Shelf, Georges Bank, and southern Gulf of St. Lawrence. Can. J. Fish. Aquat. Sci. **59**(5): 886–898. doi:10.1139/f02-062.

Budge, S.M., Iverson, S.J., and Koopman, H.N. 2006. Studying trophic ecology in marine ecosystems using fatty acids: a primer on analysis and interpretation. Mar. Mammal Sci. **22**(4): 759–801. doi:10.1111/j.1748-7692.2006.00079.x.

Budge, S.M., Penney, S.N., and Lall, S.P. 2011. Response of tissue lipids to diet variation in Atlantic salmon (*Salmo salar*): implications for estimating diets with fatty acid analysis. J. Exp. Mar. Biol. Ecol. **409**(1–2): 267–274. doi:10.1016/j.jembe.2011.09.002.

Chavarie, L., Howland, K.L., and Tonn, W.M. 2013. Sympatric polymorphism in lake trout: the coexistence of multiple shallow-water morphotypes in Great Bear Lake. Trans. Am. Fish. Soc. **142**(3): 814–823. doi:10.1080/00028487.2013.763855.

Chavarie, L., Howland, K., Harris, L., and Tonn, W. 2015. Polymorphism in lake trout in Great Bear Lake: intra-lake morphological diversification at two spatial scales. Biol. J. Linn. Soc. **114**(1): 109–125. doi:10.1111/bij.12398.

Chavarie, L., Harford, W.J., Howland, K.L., Fitzsimons, J., Muir, A.M., Krueger, C.C., and Tonn, W.M. 2016*a*. Multiple generalist morphs of Lake Trout: Avoiding constraints on the evolution of intraspecific divergence? Ecol. Evol. **6**: 7727–7741. doi:10.1002/ece3.2506. PMID:30128124.

Chavarie, L., Howland, K., Gallagher, C., and Tonn, W. 2016*b*. Fatty acid signatures and stomach contents of four sympatric Lake Trout: assessment of trophic patterns among morphotypes in Great Bear Lake. Ecol. Freshw. Fish, **25**: 109–124. doi:10.1111/eff.12195.

Chavarie, L., Muir, A.M., Zimmerman, M.S., Baillie, S.M., Hansen, M.J., Nate, N.A., et al. 2016*c*. Challenge to the model of lake charr evolution: shallow- and deep-water morphs exist within a small postglacial lake. Biol. J. Linn. Soc. [In press.] doi:10.1111/bij.12913.

Chavarie, L., Howland, K.L., Harris, L.N., Hansen, M.J., Harford, W.J., Gallagher, C.P., et al. 2018. From top to bottom: do Lake Trout diversify along a depth gradient in Great Bear Lake, NT, Canada? PLoS ONE, **13**(3): e0193925. doi:10.1371/journal.pone.0193925. PMID:29566015.

Christie, W.W. 1998. Gas chromatography-mass spectrometry methods for structural analysis of fatty acids. Lipids, **33**(4): 343–353. doi:10.1007/s11745-998-0214-x.

Clemmensen, L., Hastie, T., Witten, D., and Ersbøll, B. 2011. Sparse discriminant analysis. Technometrics, **53**(4): 406–413. doi:10.1198/TECH.2011.08118.

Dalsgaard, J., St. John, M., Kattner, G., Müller-Navarra, D., and Hagen, W. 2003. Fatty acid trophic markers in the pelagic marine environment. Adv. Mar. Biol. **46**: 225–340. doi:10.1016/S0065-2881(03)46005-7.

Eloranta, A.P., Mariash, H.L., Rautio, M., and Power, M. 2013. Lipid-rich zooplankton subsidise the winter diet of benthivorous Arctic charr (*Salvelinus alpinus*) in a subarctic lake. Freshw. Biol. **58**(12): 2541–2554. doi:10.1111/fwb.12231.

Endler, J.A. 1978. A predator's view of animal color patterns. *In* Evolutionary biology. *Edited by* M.K. Hecht, W.C. Steere, and B. Wallace. Plenum Press, New York and London.

Eschmeyer, P.H., and Phillips, A.M., Jr. 1965. Fat content of the flesh of siscowets and lake trout from Lake Superior. Trans. Am. Fish. Soc. **94**: 62–74. doi:10.1577/1548-8659(1965)94[62:FCOTFO]2.0.CO;2.

Eshenroder, R.L. 2008. Differentiation of deep-water lake charr *Salvelinus namaycush* in North American lakes. Environ. Biol. Fishes, **83**: 77–90. doi:10.1007/s10641-007-9265-y.

Farkas, T., Csengeri, I., Majoros, F., and Oláh, J. 1980. Metabolism of fatty acids in fish: III. Combined effect of environmental temperature and diet on formation and deposition of fatty acids in the carp, *Cyprinus carpio* Linnaeus 1758. Aquaculture, **20**(1): 29–40. doi:10.1016/0044-8486(80)90059-9.

Filzmoser, P., Hron, K., and Reimann, C. 2009. Principal component analysis for compositional data with outliers. Environmetrics, **20**(6): 621–632. doi:10.1002/env.966.

Folch, J., Lees, M., and Sloane-Stanley, G. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. **226**(1): 497–509. PMID:13428781.

Fraser, D.J., Weir, L.K., Bernatchez, L., Hansen, M.M., and Taylor, E.B. 2011. Extent and scale of local adaptation in salmonid fishes: review and meta-analysis. Heredity, **106**: 404–420. doi:10.1038/hdy.2010.167. PMID:21224881.

Galloway, A.W.E., Eisenlord, M.E., Dethier, M.N., Holtgrieve, G.W., and Brett, M.T. 2014. Quantitative estimates of isopod resource utilization using a Bayesian fatty acid mixing model. Mar. Ecol. Prog. Ser. **507**: 219–232. doi:10.3354/meps10860.

Gladyshev, M.I., Sushchik, N.N., Anishchenko, O.V., Makhutova, O.N., Kalachova, G.S., and Gribovskaya, I.V. 2009. Benefit–risk ratio of food fish intake as the source of essential fatty acids vs. heavy metals: a case study of Siberian grayling from the Yenisei River. Food Chem. **115**(2): 545–550. doi:10.1016/j.foodchem.2008.12.062.

Goetz, F., Rosauer, D., Sitar, S.P., Goetz, G., Simchick, C., Roberts, S., et al. 2010. A genetic basis for the phenotypic differentiation between siscowet and lean lake trout (*Salvelinus namaycush*). Mol. Ecol. **19**(Suppl. 1): 176–196. doi:10.1111/j.1365-294X.2009.04481.x. PMID:20331779.

Goetz, F., Jasonowicz, A., Johnson, R., Biga, P., Fischer, G., and Sitar, S. 2014. Physiological differences between lean and siscowet lake trout morphotypes: Are these metabolotypes? Can. J. Fish. Aquat. Sci. **71**(3): 427–435. doi:10.1139/cjfas-2013-0463.

Gorman, O.T., Yule, D.L., and Stockwell, J.D. 2012. Habitat use by fishes of Lake Superior. II. Consequences of diel habitat use for habitat linkages and habitat coupling in nearshore and offshore waters. Aquat. Ecosyst. Health Manage. **15**(3): 355–368. doi:10.1080/14634988.2012.711664.

Grosbois, G., Mariash, H., Schneider, T., and Rautio, M. 2017. Under-ice availability of phytoplankton lipids is key to freshwater zooplankton winter survival. Sci. Rep. **7**(1): 11543. doi:10.1038/s41598-017-10956-0. PMID:28912552.

Hammer, Ø., Harper, D., and Ryan, P. 2001. Past: paleontological statistics software package for education and data analysis. Palaeontol. Electron. **4**: 1–9.

Happel, A., Stratton, L., Kolb, C., Hays, C., Rinchard, J., and Czesny, S. 2016*a*. Evaluating quantitative fatty acid signature analysis (QFASA) in fish using controlled feeding experiments. Can. J. Fish. Aquat. Sci. **73**(8): 1222–1229. doi:10.1139/cjfas-2015-0328.

Happel, A., Stratton, L., Pattridge, R., Rinchard, J., and Czesny, S. 2016*b*. Fatty-acid profiles of juvenile lake trout reflect experimental diets consisting of natural prey. Freshw. Biol. **61**(9): 1466–1476. doi:10.1111/fwb.12786.

Happel, A., Pattridge, R., Walsh, M., and Rinchard, J. 2017. Assessing diet com-

positions of Lake Ontario predators using fatty acid profiles of prey fishes. J. Gt. Lakes Res. **43**(5): 838–845. doi:10.1016/j.jglr.2016.12.008.

Happel, A., Jonas, J.L., McKenna, P., Rinchard, J., He, J.X., and Czesny, S.J. 2018. Spatial variability of lake trout diets in Lakes Huron and Michigan revealed by stomach content and fatty acid profiles. Can. J. Fish. Aquat. Sci. **75**(1): 95–105. doi:10.1139/cjfas-2016-0202.

Happel, A., Stafford, C.P., Rinchard, J., and Czesny, S. 2020. Fatty acid profiles of lake trout reveal the importance of lipid content for interpreting trophic relationships within and across lakes. J. Gt. Lakes Res. **46**(1): 188–197. doi:10.1016/j.jglr.2019.10.015.

Harris, L.N., Chavarie, L., Bajno, R., Howland, K.L., Wiley, S.H., Tonn, W.M., and Taylor, E.B. 2015. Evolution and origin of sympatric shallow-water morphotypes of Lake Trout, *Salvelinus namaycush*, in Canada's Great Bear Lake. Heredity, **114**: 94–106. doi:10.1038/hdy.2014.74. PMID:25204304.

Hartwich, M., Martin-Creuzburg, D., and Wacker, A. 2013. Seasonal changes in the accumulation of polyunsaturated fatty acids in zooplankton. J. Plankton Res. **35**(1): 121–134. doi:10.1093/plankt/fbs078.

Harvey, C.J., and Kitchell, J.F. 2000. A stable isotope evaluation of the structure and spatial heterogeneity of a Lake Superior food web. Can. J. Fish. Aquat. Sci. **57**(7): 1395–1403. doi:10.1139/f00-072.

Harvey, C.J., Schram, S.T., and Kitchell, J.F. 2003. Trophic relationships among lean and siscowet lake trout in Lake Superior. Trans. Am. Fish. Soc. **132**(2): 219–228. doi:10.1577/1548-8659(2003)132<0219:TRALAS>2.0.CO;2.

Heggenes, J. 2002. Flexible summer habitat selection by wild, allopatric brown trout in lotic environments. Trans. Am. Fish. Soc. **131**(2): 287–298. doi:10.1577/1548-8659(2002)131<0287:FSHSBW>2.0.CO;2.

Henderson, B.A., and Anderson, D.M. 2002. Phenotypic differences in buoyancy and energetics of lean and siscowet lake charr in Lake Superior. Environ. Biol. Fishes, **64**: 203–209. doi:10.1023/A:1016023027437.

Hiltunen, M., Taipale, S.J., Strandberg, U., Kahilainen, K.K., and Kankaala, P. 2016. High intraspecific variation in fatty acids of *Eudiaptomus* in boreal and subarctic lakes. J. Plankton Res. **38**(3): 468–477. doi:10.1093/plankt/fbw008.

Hiltunen, M., Peltomaa, E., Brett, M.T., Aalto, S.L., Strandberg, U., Oudenampsen, J., et al. 2019. Terrestrial organic matter quantity or decomposition state does not compensate for its poor nutritional quality for *Daphnia*. Freshw. Biol. **64**(10): 1769–1786. doi:10.1111/fwb.13368.

Hoffmann, J.M. 2017. Investigating trophic ecology and dietary niche overlap among morphs of Lake Trout in Lake Superior. University of Waterloo, Waterloo, Ontario.

Horns, W., Bronte, C.R., Busiahn, T.R., Ebener, M.P., Eshenroder, R.L., Gorenflo, T., et al. 2003. Fish-community objectives for Lake Superior. Great Lakes Fishery Commission Special Publication 03-01, Ann Arbor, Michigan.

Howell, K.L., Pond, D.W., Billett, D.S., and Tyler, P.A. 2003. Feeding ecology of deep-sea seastars (Echinodermata: Asteroidea): a fatty-acid biomarker approach. Mar. Ecol. Prog. Ser. **255**: 193–206. doi:10.3354/meps255193.

Hrabik, T.R., Jensen, O.P., Martell, S.J.D., Walters, C.J., and Kitchell, J.F. 2006. Diel vertical migration in the Lake Superior pelagic community. I. Changes in vertical migration of coregonids in response to varying predation risk. Can. J. Fish. Aquat. Sci. **63**(10): 2286–2295. doi:10.1139/f06-124.

Iverson, S.J. 2009. Tracing aquatic food webs using fatty acids: from qualitative indicators to quantitative determination. *In* Lipids in aquatic ecosystems. *Edited by* M.T. Arts, M.T. Brett, and M. Kainz. Springer. pp. 281–308.

Iverson, S.J., Bowen, W.D., Boness, D.J., and Oftedal, O.T. 1993. The effect of maternal size and milk energy output on pup growth in grey seals (*Halichoerus grypus*). Physiol. Zool. **66**(1): 61–88. doi:10.1086/physzool.66.1.30158287.

Iverson, S.J., Frost, K.J., and Lowry, L.F. 1997. Fatty acid signatures reveal fine scale structure of foraging distribution of harbor seals and their prey in Prince William Sound, Alaska. Mar. Ecol. Prog. Ser. **151**: 255–271. doi:10.3354/meps151255.

Iverson, S.J., Field, C., Bowen, W.D., and Blanchard, W. 2004. Quantitative fatty acid signature analysis: a new method of estimating predator diets. Ecol. Monogr. **74**: 211–235. doi:10.1890/02-4105.

Janhunen, M., Peuhkuri, N., and Piironen, J. 2009. Morphological variability among three geographically distinct Arctic charr (*Salvelinus alpinus* L.) populations reared in a common hatchery environment. Ecol. Freshw. Fish, **18**(1): 106–116. doi:10.1111/j.1600-0633.2008.00329.x.

Johnson, L. 1975. Distribution of fish species in Great Bear Lake, Northwest Territories, with reference to zooplankton, benthic invertebrates, and environmental conditions. J. Fish. Res. Board Can. **32**(11): 1989–2004. doi:10.1139/f75-235.

Käkelä, R., Käkelä, A., Kahle, S., Becker, P.H., Kelly, A., and Furness, R.W. 2005. Fatty acid signatures in plasma of captive herring gulls as indicators of demersal or pelagic fish diet. Mar. Ecol. Prog. Ser. **293**: 191–200. doi:10.3354/meps293191.

Kattner, G., Hagen, W., Graeve, M., and Albers, C. 1998. Exceptional lipids and fatty acids in the pteropod *Clione limacina* (Gastropoda) from both polar oceans. Mar. Chem. **61**(3–4): 219–228. doi:10.1016/S0304-4203(98)00013-9.

Kavanagh, A.S., Cronin, M.A., Walton, M., and Rogan, E. 2010. Diet of the harbour seal (*Phoca vitulina vitulina*) in the west and south-west of Ireland. J. Mar. Biol. Assoc. U.K. **90**(8): 1517–1527. doi:10.1017/S0025315410000974.

Khan, N.Y., and Qadri, S.U. 1970. Morphological differences in Lake Superior lake char. J. Fish. Res. Board Can. **27**(1): 161–167. doi:10.1139/f70-017.

King, J.R., and Jackson, D.A. 1999. Variable selection in large environmental data sets using principal components analysis. Environmetrics, **10**(1): 67–77. doi:10.1002/(SICI)1099-095X(199901/02)10:1<67::AID-ENV336>3.0.CO;2-0.

Kriegel, H.-P., Kröger, P., Schubert, E., and Zimek, A. 2008. A General Framework for increasing the robustness of PCA-based correlation clustering algorithms. Springer, Berlin Heidelberg. pp. 418–435.

Logan, M.S., Iverson, S.J., Ruzzante, D.E., Walde, S.J., Macchi, P.J., Alonso, M.F., and Cussac, V.E. 2000. Long term diet differences between morphs in trophically polymorphic *Percichthys trucha* (Pisces: Percichthyidae) populations from the southern Andes. Biol. J. Linn. Soc. **69**(4): 599–616. doi:10.1006/bijl.1999.0383.

Loseto, L., Stern, G., Connelly, T., Deibel, D., Gemmill, B., Prokopowicz, A., et al. 2009. Summer diet of beluga whales inferred by fatty acid analysis of the eastern Beaufort Sea food web. J. Exp. Mar. Biol. Ecol. **374**(1): 12–18. doi:10.1016/j.jembe.2009.03.015.

MacDonald, D., Levy, D., Czarnecki, A., Low, G., and Richea, N. 2004. State of the aquatic knowledge of Great Bear Lake watershed. Report to Indian and Northern Affairs Canada. Water Resources Division, MacDonald Environmental Sciences, Nanaimo, British Columbia.

Mariash, H.L., Cazzanelli, M., Kainz, M.J., and Rautio, M. 2011. Food sources and lipid retention of zooplankton in subarctic ponds. Freshw. Biol. **56**(9): 1850–1862. doi:10.1111/j.1365-2427.2011.02625.x.

Mariash, H.L., Cusson, M., and Rautio, M. 2017. Fall composition of storage lipids is associated with the overwintering strategy of *Daphnia*. Lipids, **52**(1): 83–91. doi:10.1007/s11745-016-4219-9. PMID:27981494.

McMeans, B.C., McCann, K.S., Humphries, M., Rooney, N., and Fisk, A.T. 2015. Food web structure in temporally-forced ecosystems. Trends Ecol. Evol. **30**(11): 662–672. doi:10.1016/j.tree.2015.09.001. PMID:26452520.

McPhail, J.D. 1993. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): origin of the species pairs. Can. J. Zool. **71**(3): 515–523. doi:10.1139/z93-072.

Miles, D.B., Sinervo, B., Hazard, L.C., Svensson, E.I., and Costa, D. 2007. Relating endocrinology, physiology and behaviour using species with alternative mating strategies. Funct. Ecol. **21**(4): 653–665. doi:10.1111/j.1365-2435.2007.01304.x.

Moore, S.A., and Bronte, C.R. 2001. Delineation of sympatric morphotypes of lake trout in Lake Superior. Trans. Am. Fish. Soc. **130**(6): 1233–1240. doi:10.1577/1548-8659(2001)130<1233:DOSMOL>2.0.CO;2.

Morissette, O., Sirois, P., Lester, N.P., Wilson, C.C., and Bernatchez, L. 2018. Supplementation stocking of Lake Trout (*Salvelinus namaycush*) in small boreal lakes: Ecotypes influence on growth and condition. PLoS ONE, **13**(7): e0200599. doi:10.1371/journal.pone.0200599. PMID:30001412.

Morrison, W.R., and Smith, L.M. 1964. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride–methanol. J. Lipid Res. **5**(4): 600–608. PMID:14221106.

Muir, A.M., Bronte, C.R., Zimmerman, M.S., Quinlan, H.R., Glase, J.D., and Krueger, C.C. 2014. Ecomorphological diversity of Lake Trout at Isle Royale, Lake Superior. Trans. Am. Fish. Soc. **143**(4): 972–987. doi:10.1080/00028487.2014.900823.

Muir, A.M., Hansen, M.J., Bronte, C.R., and Krueger, C.C. 2015. If Arctic charr *Salvelinus alpinus* is 'the most diverse vertebrate', what is the lake charr *Salvelinus namaycush*? Fish Fish. **17**(4): 1194–1207. doi:10.1111/faf.12114.

Olsen, Y. 1999. Lipids and essential fatty acids in aquatic food webs: what can freshwater ecologists learn from mariculture? *In* Lipids in freshwater ecosystems. *Edited by* M.T. Arts and B.C. Wainman. Springer-Verlag, New York. pp. 161–202.

Olsen, Y., and Skjervold, H. 1995. Variation in content of Ω3 fatty acids in farmed Atlantic salmon, with special emphasis on effects of non-dietary factors. Aquacult. Int. **3**(1): 22–35. doi:10.1007/BF00240918.

Ota, T., and Yamada, M. 1971. Lipids of Masu salmon *Oncorhynchus masou*. I. Variations of the lipid content and the fatty acid composition of juvenile Masu salmon during the period of smolt-transformation, and on the influence of light upon those variations. Hokkaido Univ. Fac. Fish. Bull. pp. 151–158.

Piché, J., Iverson, S.J., Parrish, F.A., and Dollar, R. 2010. Characterization of forage fish and invertebrates in the northwestern Hawaiian Islands using fatty acid signatures: species and ecological groups. Mar. Ecol. Prog. Ser. **418**: 1–15. doi:10.3354/meps08814.

Pomerleau, C., Lesage, V., Winkler, G., Rosenberg, B., and Ferguson, S.H. 2014. Contemporary diet of bowhead whales (*Balaena mysticetus*) from the Eastern Canadian Arctic inferred from fatty acid biomarkers. Arctic, **67**(1): 84–92. doi:10.14430/arctic4366.

Pryke, S.R., Astheimer, L.B., Buttemer, W.A., and Griffith, S.C. 2007. Frequency-dependent physiological trade-offs between competing colour morphs. Biol. Lett. **3**(5): 494–497. doi:10.1098/rsbl.2007.0213. PMID:17609174.

Quérouil, S., Kiszka, J., Cordeiro, A.R., Cascão, I., Freitas, L., Dinis, A., et al. 2013. Investigating stock structure and trophic relationships among island-associated dolphins in the oceanic waters of the North Atlantic using fatty acid and stable isotope analyses. Mar. Biol. **160**(6): 1325–1337. doi:10.1007/s00227-013-2184-x.

R Core Team. 2017. R: a language and environment for statistical computing [online]. Available from http://www.R-project.org/.

Robinson, B.W., and Parsons, K.J. 2002. Changing times, spaces, and faces: tests and implications of adaptive morphological plasticity in the fishes of northern postglacial lakes. Can. J. Fish. Aquat. Sci. **59**(11): 1819–1833. doi:10.1139/f02-144.

Robinson, B.W., and Wilson, D.S. 1994. Character release and displacement in fishes: a neglected literature. Am. Nat. **144**: 596–627. doi:10.1086/285696.

Ronconi, R., Koopman, H.N., McKinstry, C., Wong, S., and Westgate, A.J. 2010. Inter-annual variability in diet of non-breeding pelagic seabirds *Puffinus* spp. at migratory staging areas: evidence from stable isotopes and fatty acids. Mar. Ecol. Prog. Ser. **419**: 267–282. doi:10.3354/meps08860.

Rundle, H.D., and Nosil, P. 2005. Ecological speciation. Ecol. Lett. **8**(3): 336–352. doi:10.1111/j.1461-0248.2004.00715.x.

Sargent, J.R., Bell, J.G., Bell, M.V., Henderson, R.J., and Tocher, D.R. 1995. Requirement criteria for essential fatty acids. Symposium of European Inland Fisheries Advisory Commission. Journal of Applied Ichthyology, **11**: 183–198.

Sasaki, S., Ota, T., and Takagi, T. 1989. Compositions of Fatty Acids in the Lipids of Chum Salmon during Spawning Migration. Nippon Suisan Gakkaishi, **55**(12): 2191–2197. doi:10.2331/suisan.55.2191.

Scharnweber, K., Strandberg, U., Marklund, M.H.K., and Eklöv, P. 2016. Combining resource use assessment techniques reveals trade-offs in trophic specialization of polymorphic perch. Ecosphere, **7**(8): e01387. doi:10.1002/ecs2.1387.

Schluter, D., and McPhail, J.D. 1992. Ecological character displacement and speciation in sticklebacks. Am. Nat. **140**: 85–108. doi:10.1086/285404. PMID: 19426066.

Sheridan, M.A. 1989. Alterations in lipid metabolism accompanying smoltification and seawater adaptation of salmonid fish. Aquaculture, **82**(1–4): 191–203. doi:10.1016/0044-8486(89)90408-0.

Skulason, S., and Smith, T.B. 1995. Resource polymorphisms in vertebrates. Trends Ecol. Evol. **10**: 366–370. doi:10.1016/S0169-5347(00)89135-1. PMID: 21237070.

Smith, T.B., and Skulason, S. 1996. Evolutionary significance of resource polymorphisms in fishes, amphibians, and birds. Annu. Rev. Ecol. Syst. **27**: 111–133. doi:10.1146/annurev.ecolsys.27.1.111.

Stowasser, G., Graham, J.P., Moffat, C.F., Collins, M.A., and Forsythe, J.W. 2006. Experimental study on the effect of diet on fatty acid and stable isotope profiles of the squid *Lolliguncula brevis*. J. Exp. Mar. Biol. Ecol. **333**: 97–114.

Svanbäck, R., Eklöv, P., Fransson, R., and Holmgren, K. 2008. Intraspecific competition drives multiple species resource polymorphism in fish communities. Oikos, **117**(1): 114–124. doi:10.1111/j.2007.0030-1299.16267.x.

Taipale, S.J., Vuorio, K., Aalto, S.L., Peltomaa, E., and Tiirola, M. 2019. Eutrophication reduces the nutritional value of phytoplankton in boreal lakes. Environ. Res. **179**: 108836. doi:10.1016/j.envres.2019.108836. PMID:31708172.

Tucker, S., Bowen, W.D., and Iverson, S.J. 2008. Convergence of diet estimates derived from fatty acids and stable isotopes within individual grey seals. Mar. Ecol. Prog. Ser. **354**: 267–276. doi:10.3354/meps07263.

Vinson, M.R., and Budy, P. 2010. Sources of variability and comparability between salmonid stomach contents and isotopic analyses: study design lessons and recommendations. Can. J. Fish. Aquat. Sci. **68**(1): 137–151. doi:10.1139/F10-117.

Virtue, P., Mayzaud, P., Albessard, E., and Nichols, P. 2000. Use of fatty acids as dietary indicators in northern krill, *Meganyctiphanes norvegica*, from northeastern Atlantic, Kattegat and Mediterranean waters. Canadian J. Fish. Aquat. Sci. **57**(Suppl. 3): 104–114. doi:10.1139/f00-182.

Webb, P.W. 1984. Body form, locomotion and foraging in aquatic vertebrates. Am. Zool. **24**: 107–120. doi:10.1093/icb/24.1.107.

Witten, D.M., and Tibshirani, R. 2011. Penalized classification using Fisher's linear discriminant. J. R. Stat. Soc. Ser. B Stat. Methodol. **73**(5): 753–772. doi:10.1111/j.1467-9868.2011.00783.x. PMID:22323898.

Yoder, J.B., Clancey, E., Des Roches, S., Eastman, J.M., Gentry, L., Godsoe, W., et al. 2010. Ecological opportunity and the origin of adaptive radiations. J. Evol. Biol. **23**(8): 1581–1596. doi:10.1111/j.1420-9101.2010.02029.x. PMID: 20561138.

Zimmerman, M.S., Krueger, C.C., and Eshenroder, R.L. 2006. Phenotypic diversity of lake trout in Great Slave Lake: differences in morphology, buoyancy, and habitat depth. Trans. Am. Fish. Soc. **135**: 1056–1067. doi:10.1577/T05-237.1.

Zimmerman, M.S., Krueger, C.C., and Eshenroder, R.L. 2007. Morphological and ecological differences between shallow- and deep-water lake trout in Lake Mistassini, Quebec. J. Gt. Lakes Res. **33**: 156–169. doi:10.3394/0380-1330(2007)33[156:MAEDBS]2.0.CO;2.

Zimmerman, M.S., Schmidt, S.N., Krueger, C.C., Vander Zanden, M.J., and Eshenroder, R.L. 2009. Ontogenetic niche shifts and resource partitioning of lake trout morphotypes. Can. J. Fish. Aquat. Sci. **66**(6): 1007–1018. doi:10.1139/F09-060.

Appendix A

Appendix Tables A1 and A2 and Fig. A1 appear on the following pages.

Table A1. List of 38 of 41 fatty acids shared by the two datasets, considered as either "dietary" or "extended dietary" fatty acid biomarkers and used in this study (see Iverson et al. 2004) and the dietary component they are associated with, based on literature listed below.

Dietary fatty acid	Dietary component
14:0	Pelagic (zooplankton) + diatom
16:0	Cannibalism and (or) carnivorous
16:1n-7	Benthic (bacterial synthesis + diatoms); cannibalism and (or) carnivorous
16:2n-6	
16:2n-4	Diatom
17:0	
16:3n-4	
16:4n-3	
16:4n-1	Diatom
18:0	Cannibalism and (or) carnivorous
18:1n-9	Pelagic (zooplankton)
18:1n-7	Benthic (bacterial synthesis + algal)
18:2n-6	Terrestrial
18:2n-4	
18:3n-6	
18:3n-4	
18:3n-3	Terrestrial
18:3n-1	
18:4n-3	Pelagic (zooplankton)
18:4n-1	
20:0	Not used*
20:1n-11	Copepod (Iverson 2009)
20:1n-9	Pelagic (calanoid copepods diet-based)
20:1n-7	Pelagic (zooplankton)
20:2n-9	Not used*
20:2n-6	
20:3n-6	
20:4n-6	Benthic (diatom)
20:3n-3	
20:4n-3	
20:5n-3	Cannibalism and (or) carnivorous
22:1n-11	Copepod
22:1n-9	Pelagic (zooplankton)
22:1n-7	
22:2n-6	
21:5n-3	
22:4n-6	Not used*
22:5n-6	Pelagic (calanoid copepods diet-based)
22:4n-3	
22:5n-3	
22:6n-3	Benthic (pennate diatoms + dinoflagellates + bivalves); cannibalism

Note: Sargent et al. 1995; Brett and Müller-Navarra 1997; Kattner et al. 1998; Virtue et al. 2000; Budge et al. 2002; Dalsgaard et al. 2003; Iverson et al. 2004; Käkelä et al. 2005; Alfaro et al. 2006; Tucker et al. 2008; Ahlgren et al. 2009; Gladyshev et al. 2009; Loseto et al. 2009; Stowasser et al. 2006; Piché et al. 2010; Mariash et al. 2011.

*These fatty acids were discarded because they were not quantified for both lakes.

Table A2. List of 24 fatty acids considered as "nondietary" biomarkers in this study.

12:0	15:0	7Methyl 16:0
12:1	15:1n-8	17:1
13:1	15:1n-6	17:0 iso
14:1n-9	15:0 iso	18:1n-5
14:1n-7	16:1n-11	20:0
14:1n-5	16:1n-9	20:2n-9
14:0 iso	16:1n-5	22:0
14:0 ante	16:0 iso	24:1n-9

Fig. A1. PCA of all fatty acids (top panels), dietary fatty acid biomarkers (middle panels), and nondietary fatty acid biomarkers (bottom panels) of each morph of lake trout from Great Bear Lake, Stannard Rock (Lake Superior), and Superior Shoal (Lake Superior), including the 12 outlier individuals. Vectors of individual fatty acids important to the positioning of lake trout are represented to the right of each PCA. Angles and lengths of vectors represent the direction and the strength, respectively, of relationships between variables and the principal components. [Colour online.]