Dietary vs non-dietary fatty acid profiles of lake trout morphs 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 organismal niche for a diversity of taxa. Most often, fatty acids examined include only those previously identified as “dietary” or “extended dietary”. Consequently, non-dietary fatty acids are commonly discarded, representing approximately half of the fatty acids that can be extracted from fish muscle. In this study, we explored whether non-dietary fatty acids can provide worthwhile information by assessing their ability to discriminate intraspecific diversity within and between lakes. Non-dietary fatty acids delineated variation geographically (between lakes), between locations within a lake, and among morphs within a species. Non-dietary fatty acids were useful in delineating morphs of lake trout in both lakes and, for Lake Superior, performed better than dietary fatty acids. Physiological differences that arise from dynamic differences in energy processing can be adaptive and linked to habitat use (i.e., shallow vs. deep depths) by species morphs, and likely explains why non-dietary fatty acids can be a relevant tool to delineate intraspecific diversity in lake trout, which are known to inhabit a large range of habitats. The level of differences in non-dietary fatty acids were as pronounced among morphs within Great Bear Lake as observed in Lake Superior. Little is known what causes non-dietary-mediated differences in fatty acid composition, but our results showed that assessing non-dietary fatty acids can be useful in delineating intraspecific diversity and spatial variation.

Introduction:

Constraints on traditional methods to investigate diets of organisms within aquatic systems have led to the use of biochemical tracers in contemporary investigations (Vinson & Budy, 2010). Among these, fatty acids have gained popularity as both qualitative and quantitative trophic markers that reflect foraging patterns and food-web dynamics (Iverson, 2009; Galloway *et al.*, 2014). As such, fatty acids have been used to characterize variation within populations and species (e.g., evolutionary units linked to trophic polymorphism; Logan *et al.*, 2008; Scharnweber *et al.*, 2016), and to explore geographical (Pomerleau *et al.*, 2014; Hiltunen *et al.*, 2016; Quérouil *et al.*, 2013) and temporal variation in trophic ecology (e.g., seasonal and annual variations; Eloranta *et al.*, 2013; Hartwich, Martin-Creuzburg & Wacker, 2012).

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 (Iverson, 2009; Budge, Iverson & Koopman, 2006). 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 *et al.*, 2004; Iverson, 2009). The storage patterns of fatty acids depend on the biochemical limitations of organisms to biosynthesize, modify chain-length, and introduce double bonds in fatty acids (e.g., with complexity culminating in vertebrates) (Iverson 2009). A critical analytical assumption is that storage characteristics of fatty acids within lipids are not being degraded during digestion, i.e., that they are stored in tissues in their basic form (Iverson, 2009). Fatty acids labelled as "dietary" or "extended dietary" tracers (Iverson, Frost & Lowry, 1997, 2004; Budge, Iverson & Koopman, 2006) have been the targets

for analyses in ecological studies. Consequently, approximately half of the fatty acids extracted from tissue samples are commonly omitted, with only those recognized to be typical markers of diet being analyzed.

The exclusion of these "non-dietary" fatty acids from analyses is not misguided, as the purpose of most studies is to accurately describe diet patterns, and this practice has resulted in reliable information being produced across taxa (Iverson *et al.*, 2004; Galloway *et al.*, 2014; Iverson, 2009). However, it is unknown whether valid information for other research questions may be lost when investigators discard a portion of the fatty acids in their samples and the potential discriminatory data that could have resulted if analysed. Biological (e.g., phenotypic and genetic) and environmental variables have been shown to affect lipid and fatty acid composition in fishes (Olsen & Skjervold, 1995). For example, temperature (Olsen, 1999; Farkas *et al.*, 1980), salinity (Borlongan & Benitez, 1992), and light (Ota & Yamada, 1971) have induced variation in lipid composition of fish tissue. 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 these questions, we compared "non-dietary" fatty acids among morphs of lake trout (*Salvelinus namaycush*) in Lake Superior to those among lake trout morphs in Great Bear Lake, as these morphs represent important intraspecific diversity in these lakes.

Salmonids, such as lake trout, inhabit evolutionarily young ecosystems believed to be 10,000 to 15,000 years old, e.g., post-glacial lakes colonized from non-glaciated refugia. The depauperate communities of post-glacial lakes are commonly characterized by reduced competition and predation, which allows colonizers access to a relatively wide array of resources, conditions that favour development of intraspecific diversity (Smith & Skulason, 1996; McPhail, 1993; Robinson & Wilson, 1994). This high level of ecological opportunity, together with an

increase in intraspecific competition after colonization, can promote individual specialization within a population, and the development of groups of individuals with similar patterns of resource use (Svanbäck *et al.*, 2007). Observable characteristics of individuals in such groups can evolve as niches expand to incorporate novel foraging resources, referred to as resource polymorphism (Skulason & Smith, 1995; Robinson & Parsons, 2002). Morphs (or ecotypes) can differ from each other in morphology, genetics, physiology, life-history, and/or behaviour, and these differences are typically associated with niche use (Schluter & McPhail, 1992; Smith & Skulason, 1996; Bolnick *et al.*, 2007).

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

A number of studies have assessed lake trout diets with fatty acids, either qualitatively (Chavarie *et al.*, 2016b; Hoffmann, 2017; Happel *et al.*, 2017b; Happel *et al.*, 2017a) or quantitatively (Happel *et al.*, 2016b; Happel *et al.*, 2016a), generating reliable information about dietary patterns. However, pronounced and systematic non-dietary-mediated differences in fatty acids composition have been reported in lake trout, supporting the rationale that non-dietary fatty acids could be important in delineating intraspecific diversity within this species. Goetz *et al.*

(2013) found physiological differences between lean and siscowet morphs of lake trout that reflected genetically based differences in lipid synthesis, metabolism, and transport (Goetz *et al.*, 2010), which suggests information from non-dietary fatty acids might be important, especially if they have genetic-based mechanisms. Consequently, where intraspecific diversity is manifested as physiological differences among morphs, non-dietary fatty acids could assist in identifying variation. Yet, little is known about the non-dietary-mediated differences in fatty acid composition of fish (or other taxa) in an ecological context.

To investigate the use of non-dietary fatty acids as an ecological tool, we analyzed a comprehensive set of non-dietary and dietary fatty acids from lake trout morphs collected from two lakes that sustain intraspecific diversity along different axes of intraspecific diversification (e.g., depth-dependent and depth-independent). Specifically, we compared: 1) non-dietary and dietary fatty acids among four morphs of lake trout from each of Great Bear Lake Lake Superior, and 2) how well non-dietary and dietary delineated intraspecific diversity within and between lakes. Answers to these questions will help determine if non-dietary fatty acids can discriminate fish geographically and among groups within a species, and if they offer different perspectives than dietary fatty acids.

Material 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), has a maximum depth of 446 m, 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. Samples of lake trout were collected from multiple locations, Great Bear Lake has five semi-isolated arms, but due to sample size, data were pooled across multiple sites (see Chavarie *et al.*, 2016b 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.*, 2016a). Currently, four lake trout morphs are described in Great Bear Lake; three are common (Morphs 1-3), and one is rare (Morph 4). Morph 1 has the smallest head and jaws, intermediate fin lengths and body shape intermediate between Morphs 2 and 3. Morph 2 has the largest head and jaws but the smallest fins (lean-like), and a streamlined body shape. Morph 3 has the longest fins, a robust body shape, and a sub-terminal mouth. Morph 4 has a thick and curved lower jaw, a streamlined body shape, and the smallest caudal peduncle among the morphs. Although Morph 4 is a specialist in the pelagic habitat, whereas Morphs 1-3 have more general feeding habits, with varying degrees of omnivory along a weak benthic-pelagic gradient in shallow-water (Chavarie *et al.*, 2016a; Chavarie *et al.*, 2016b).

Lake Superior is a post-glacial, oligotrophic lake shared 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 Horns *et al.*, 2003; Gorman, Yule & Stockwell, 2012; Hoffmann, 2017). 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), in Lake Superior (Fig. 1). Superior Shoal and Stannard Rock were known to support humper, lean, and siscowet Lake Trout morphs, whereas redfins, described by Muir *et al.* (2014), were not yet known to be present at these two sites.

Lake Superior supports one of the highest levels of sympatric diversity expressed within lake trout (Fig. 2). Four morphs are currently recognized; the siscowet, humper, and redfin morphs inhabit deep-water (> 70 m), whereas the lean morph occupies shallow-water habitats (< 70 m) (Moore & Bronte, 2001; Bronte & 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; Bronte & Moore, 2007; Moore & Bronte, 2001). The humper lake trout has a small head, short snout, short maxillae, large eyes, and short and narrow caudal peduncle. (Muir *et al.*, 2015; Moore & Bronte, 2001). 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 morphs (Muir *et al.*, 2014). Finally, the lean has 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; Muir *et al.*, 2015; Khan & Qadri, 1970).

Fatty acids

Dorsal muscle samples (Budge, Penney & Lall, 2011) from lake trout in both lakes were stored at -20 °C (Budge, Iverson & Koopman, 2006; Loseto *et al.*, 2009; Kavanagh *et al.*, 2010; Chavarie *et al.*, 2016b). Lipids were extracted from 1 g of the homogenate material; after passive overnight extraction (at -20) in 2:1 chloroform:methanol containing 0.01% BHT (v/v/w) (Folch, Lees & Sloane-Stanley, 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 0.88 NaCl solution were added because NaCl encourages 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 N H_2SO_4 in methanol) (Morrison & 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 I.D; 0.15 μm film thickness). The GC was coupled to a Flame Ionization Detector operating at 350 ^{o}C. Hydrogen was used as carrier gas flowing at 1.25 mL/min for 14 minutes, increasing to 2.5 mL/min for 5 minutes. The split/splitless injector was heated to 260 ^{o}C and run in splitless mode. The oven program was as follows: 60 ^{o}C for 0.66 min, increasing by 22.82 ^{o}C/min to 165 ^{o}C with a 1.97 min hold; increasing by 4.56 ^{o}C/min to 174 ^{o}C and by 7.61 ^{o}C/min to 200 ^{o}C with a 6 min hold. Peaks were quantified using Agilent Technologies ChemStation software. FA standards were obtained from Supelco (37 component FAME mix) and Nuchek (54 component mix GLC-463). FAMEs were identified via retention time and known standard mixtures and are reported as percentages of total fatty acids. All FA values were converted to a mass percentage of the total array, and were named according the IUPAC nomenclature as X:Y n-z, where X is the number of carbons in the FA, 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 MB.

For both lakes, tissue samples were taken from indivual lake trout previously identified to corresponding morphotypes: 126 samples were collected from Great Bear Lake (Morph 1 = 32, Morph 2 = 35, Morph 3 = 38, and Morph 4 = 21, see Chavarie *et al.*, 2016b 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, FA analysis procedures were divided in two steps, using non-dietary and dietary fatty acids (see Appendix 1), following the methods of Iverson, Frost and Lowry (1997, 2004) and Budge, Iverson and Koopman (2006) to identify dietary fatty acids. Thirty-eight dietary and 24 non-dietary fatty acids were found to be shared between the two lakes, and these were selected for further analyses (Table 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 ($z=x\mu/\sigma$). (Witten & Tibshirani, 2011; Clemmensen *et al.*, 2011). Principal Canonical Analysis (PCA) was performed on all dietary and non-dietary fatty acids to provide inference about patterns of variation among locations and morphs (Chavarie *et al.*, 2016b). For PCAs, 12 outlier individuals from Superior Shoal were excluded because PCAs are sensitive to outlier variation (but see Fig. A1 for PCAs with their inclusion) (Filzmoser, Hron & Reimann, 2009; Kriegel *et al.*, 2008).

To test for differences in fatty acid composition among morphs within each lake (dietary, non-dietary, and overall), Permutational Multivariate Analysis of Variance (PERMANOVA) was used (a non-parametric analog of Multivariate Analysis of Variance), followed by post-hoc comparisons with Bonferroni corrections. PERMANOVAs were performed in PAST 3 (Hammer, Harper & Ryan, 2001) using 9999 permutations. A similarity percentage routine (SIMPER) using

Bray-Curtis was used to determine which fatty acids (dietary, non-dietary) were primarily responsible for observed differences among morphs for each lake (King & Jackson, 1999). We also performed linear discriminant analysis with a jacknife validation procedure using 20% of our data as unknown on fatty acids (dietary, and non-dietary) from each location to evaluate their contribution to delineating intraspecific diversity in lake trout. Finally, PERMANOVA (using 9999 permutations), and linear discriminant analyses and jacknife validation procedures were performed on the ten most discriminating dietary and non-dietary fatty acids (20 fatty acids in total to reduce the number of vectors versus number of individuals tested for linear discriminant analysis); these fatty acids were identified from previous SIMPER analyses for each location.

Results

Combined lakes analyses

The first two axes of non-dietary fatty acids PCA explained 42.4 % of the variation among lake trout from Great Bear Lake and Lake Superior (Fig. 3). Lake trout from Great Bear Lake clustered together with 37 lake trout overlapped with those from Lake Superior, whereas those from Stannard Rock and Superior shoal overlapped. The separation between the two lakes appeared to be driven by the following fatty acids: 13:1, 14:1n-7, 15:0, 15:1n8, 15:1n6, 15:0 iso, 16:1n11, 17:0 iso, 20:0, 22:0, 20:2n9, and 24:1n9. Similar to the non-dietary fatty acids, the first two axes of the dietary fatty acids PCA explained 41.5% of the variation among lake trout from Great Bear Lake and Lake Superior. Lake trout from Great Bear Lake grouped together, with 32 individuals overlapped with Lake Superior lake trout, whereas lake trout from Stannard Rock totally overlapped with Superior Shoal individuals (Fig. 3). Separation between the two lakes 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 fatty acid associated with cannibalism or/and

carnivory (20:5n3; toward Great Bear Lake) (Appendix, Table 1). Finally, the first two axes of the PCA based on all fatty acids combined explained 39.0% of the variation among lake trout morphs from Great Bear Lake and Lake Superior. Lake trout from Great Bear Lake clustered together but with 15 lake trout overlapped with those from Lake Superior. Lake trout from Stannard Rock and Superior Shoal in Lake Superior overlapped completely.

Lake Trout intraspecific diversity of Great Bear Lake

Composition of both non-dietary and dietary fatty acids were able to discriminate among the four lake trout morphs from Bear Lake. Non-dietary fatty acids differed among the four Great Bear Lake morphs (one-way PERMANOVA, $F_{3,122} = 3.6$, $P < 0.01$). Similarly, comparison of non-dietary fatty acids showed that all pairs of morphs differed from one another ($P < 0.01$) except for morphs 1 and 3. The ten most discriminating non-dietary fatty acids 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 morph based on non-dietary fatty acids (Fig. 4). Dietary fatty acids also differed among the four lake trout morphs from Great Bear Lake (one-way PERMANOVA, $F_{3,122} = 2.95$, $P < 0.01$), and most pairs of morphs differed significantly from one another (all $P < 0.01$ except for morphs 1 vs. 3). The ten most discriminating dietary fatty acids 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 morph based on dietary fatty acids (Fig. 4). Finally, when the 10 dietary and 10 non-dietary most discriminating fatty acids were considered together, morphs differed from one another (PERMANOVA, $F_{3,122} = 2.8$, $P < 0.01$). All pairs of morphs differed ($P < 0.01$) except for morphs

1 and 3. The first two axes of the linear discriminant analysis explained 63.6% and 24.9% of the variation, and 54.8% of all individuals were correctly classified (Fig. 4).

Lake Trout intraspecific diversity of Lake Superior, Stannard Rock

Non-dietary fatty acids differed among the three morphs 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 non-dietary fatty acids from SIMPER explained 70.9% of the dissimilarity among morphs (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 morph based on non-dietary fatty acids (Fig. 4). In contrast to results from non-dietary fatty acid, no differences in dietary fatty acid composition occurred among the three lake trout morphs 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 morph based on dietary fatty acids (Fig. 4). Finally, the three morphs also differed when the 10 most discriminating non-dietary and dietary fatty acids were considered together (one-way PERMANOVA, $F_{2,87} = 2.1$, $P < 0.02$), and leans differed significantly from siscowets ($P < 0.01$). The first two axes of the linear discriminant analysis explained 73.3% and 26.8% of the variation, respectively, and 48.9% of all individuals were correctly classified to morph based on their dietary fatty acid profile (Fig. 4).

Lake Trout intraspecific diversity of Lake Superior, Superior Shoal

Like Stannard Rock, composition of non-dietary fatty acid differed significantly among the four morphs (one-way PERMANOVA, $F_{3,116} = 2.5$, $P < 0.01$), and leans differed significantly from redfins ($P < 0.01$) and were marginally significantly different between redfin and siscowet and between redfin and humper ($P < 0.06$). The ten most discriminating non-dietary fatty acids 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 morph using non-dietary fatty acids (Fig. 4). Based on dietary fatty acid composition, we found no differences among the four lake trout morphs from Superior Shoal (one-way PERMANOVA, $F_{3,116} = 0.8$, $P = 0.3$), similarly to what was found with fish from Stannard Rock. 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% and 35.6% of the variation, respectively, and 31.7% individuals were correctly classified to morph based on their dietary fatty acid profile (Fig. 4). In contrast to the results for Great Bear Lake and Stannard Rock, when the 10 non-dietary and 10 dietary most discriminating fatty acids were combined for lake trout from Superior Shoal, morphs did not differ significantly from one another (one-way PERMANOVA, $F_{3,116} = 1.3$, $P = 0.16$). The first two axes of the linear discriminant analysis explained 72.9% and 19.7% of the variation, respectively, and 42.5% of all individuals were correctly classified (Fig. 4).

Discussion

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” (Budge, Iverson & Koopman, 2006; Iverson *et al.*, 2004). However, including fatty acids associated with supplementary (non-

dietary) information might be worth considering for some studies. In this study, non-dietary fatty acids showed variation geographically (between lakes), between locations within a lake (i.e., Stannard Rock versus Superior Shoal), and among morphs within a species. Although some overlap was found, our results showed that when investigating intraspecific diversity, non-dietary fatty acids can be a useful tool to delineate groups, and that sometimes, such as at sites in Lake Superior, were more discriminatory than dietary fatty acids. Accordingly, our results suggested that discarding non-dietary 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.*, 2004; Iverson *et al.*, 1993). Metabolism can differ within species, as physiological differences often exists among sets of individuals, i.e., "morphs" (Pryke *et al.*, 2007; Miles *et al.*, 2007). Such physiological differences within a species may result in non-dietary fatty acids 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 morphs along a depth axis (Eschmeyer & Phillips, 1965; Eshenroder, 2008), and these lipid differences have been linked to genetic differences among morphs (Eschmeyer & Phillips, 1965; Goetz *et al.*, 2013; Goetz *et al.*, 2010). Thus, fatty acid deposition and metabolism appears to have undergone selection along a depth gradient likely in part to assist with buoyancy compensation, with differences reported between siscowet (deep-water morph) and lean (shallow-water morph) lake trout (Goetz *et al.*, 2010; Eschmeyer & Phillips, 1965). Until now, no information has been available for redfin and humper morphs.

In addition to the variation in lipid accumulation, Goetz *et al.* (2013) also found differences in lipid composition between siscowet and lean morphs. Because a common garden design was

used in the Goetz *et al.* (2013) 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 non-dietary fatty acids we observed among the four morphs of lake trout from Lake Superior was consistent with the concept of metabolotypes (Goetz *et al.*, 2013). Altogether, differences in energy processing and storage between lean and siscowet lake trout in Lake Superior are adaptive to their respective habitats, deep- vs. shallow-water, and to their life-histories (Goetz *et al.*, 2013). Lipid content, intertwined with buoyancy variations, have been linked to differences in depth distributions and swimming tactics among lake trout morphs (Zimmerman, Krueger & Eshenroder, 2006; Zimmerman, Krueger & Eshenroder, 2007; Zimmerman *et al.*, 2009). While deep-water morphs use hydrostatic lift related to lipid content to enhance vertical migration while foraging for *Mysis* and cisco (Henderson & Anderson, 2002), the shallow-water lean morph likely relies more on hydrodynamic lift, linked to the cruising movements of pelagic predators (Webb, 1984). Consistent with the findings of Goetz et al. (2013), we found that non-dietary fatty acids differed between shallow- and deep-water morphs at Stannard Rock. However, at Superior Shoal, non-dietary fatty acids differed only between the shallow-water lean morph and one deep-water morph – the redfin. This observation, along with some more subtle differences in non-dietary fatty acids among deep-water morphs, requires further study. At both Stannard Rock and Superior Shoal, diet overlap was found among morphs, and present and future diet overlap among morphs may be linked to ongoing changes in the prey base (Bronte *et al.*, 2003; Gorman *et al.*, 2013). Changes in the prey base occurred in Lake Superior prior to sampling, including loss of diversity of some coregonine forage fishes (Bronte *et al.*, 2003; Gorman *et al.*, 2013). Because conserving diversity of lake trout morphs is a priority for managers

(Zimmerman & Krueger, 2009), a deeper understanding how diet overlap among morphs responds to changes in the prey base also deserves more consideration.

The concept that dynamics of energy processing and storage are adaptive along a gradient that is at least partially driven by 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.*, 2016a; Chavarie *et al.*, 2018). The similarity of results between Great Bear Lake and Lake Superior is thus perplexing, as we were expected greater differences in non-dietary fatty acids among morphs in Lake Superior than in Great Bear Lake, due to known buoyancy variation associated with a depth gradient in Lake Superior. If lake trout morphs from Great Bear Lake are also under selection (Harris *et al.*, 2014), 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 morphs are independent of major habitat or resource partitioning (e.g., same question is pertinent for Lake Superior), especially because dietary fatty acids in Great Bear Lake were slightly better at delineating intraspecific groups than non-dietary fatty acids.

Ecological differences in allopatry are often found to be more pronounced than those in sympatry, due to disparate environments and isolation (Rundle & Nosil, 2005; Yoder *et al.*, 2010; Heggenes, 2002; Fraser *et al.*, 2011). In this study, differences between lakes in both dietary and non-dietary fatty acids appear to be greater than variation among morphs within a lake. 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, Vrede & Goedkoop, 2009; Budge, Iverson & Koopman, 2006; Dalsgaard *et al.*, 2003). Although benthic productivity generally dominates in

Arctic lakes (Chavarie *et al.*, 2018; Johnson, 1975), some lake trout from Great Bear Lake specialize on pelagic resources, including some individuals of Morph 2 and Morph 4, which have fatty acids signatures that overlap with lake trout from Lake Superior (Chavarie et al., submitted; Chavarie *et al.*, 2016a; Chavarie *et al.*, 2016b). With lake productivity in Arctic regions known to be benthic-oriented, combined with the known depth distributions in the deep-water habitat of lake trout morphs from Lake Superior (50-150 m; Muir *et al.*, 2014), our results reflected the expected ecology of this species from these two systems. Despite differences between lake trout inhabiting Great Bear Lake and Lake Superior, considerable overlap occurred among dietary (e.g., five out of 10) and non-dietary (e.g., 7 out of 10) fatty acids that were important in identifying intraspecific diversity in both lakes. The greater number of shared non-dietary fatty acids discriminating lake trout intraspecific diversity in the two lakes supports the idea of similar physiological differences (e.g., energy processing and storage) among morphs from both lakes.

A few caveats should be noted that could alter our interpretations. First, no spatial component was defined for the Great Bear Lake dataset, due to small sample sizes from each location, which probably introduced variation into the results. Spatial variation can become important when a large, complex system is studied (Chavarie *et al.*, 2015; Hoffmann, 2017) and environmental variables (e.g., temperature, light) are known to alter lipid composition in fish tissue (Olsen, 1999). Second, in Lake Superior, multiple life-stages, e.g., juvenile, mature, and resting individuals were included together in the analysis, and different life-stages can vary in lipid metabolism (Sheridan, 1989). In addition, lake trout length can also influence fatty acid composition. For example, in lakes Huron and Michigan, large lake trout relied more on nearshore-benthic food web resources than small lake trout (Happel *et al.*, 2017a). Finally, some fatty acids exist at very low amounts (≤ 2%), which can introduce error in interpreting differences among

fatty acids that are found in only trace amounts (e.g., peak shouldering). Despite these limitations, we found some consistent patterns with regards to intraspecific diversity between lakes and among morphs within lakes.

Conclusion

This study demonstrated the benefits of using both dietary and non-dietary fatty acids for delineating variation and diversity within a species. In some instances, non-dietary fatty acids were better for discriminating morphs than dietary fatty acids, in contrast to the popular maxim associated with trophic markers, "you are what you eat". Dietary fatty acids can determine the occurrence (or absence) of discrete niche use among sets of individuals (i.e., morphs) within a species (Chavarie *et al.*, 2016b). The fatty-acid composition of individuals reflecting their diet has been validated for lake trout (Happel *et al.*, 2016a; Happel *et al.*, 2016b). However, physiological differences in the dynamics of energy processing (e.g., metabolism) and storage can be adaptive among morphs (Goetz *et al.*, 2013; Goetz *et al.*, 2010; Eschmeyer & Phillips, 1965), and thus non-dietary fatty acids may be useful molecular biomarkers. Ultimately, the relative importance of dietary vs. non-dietary fatty acids depends on the question being asked. In our study, non-dietary fatty acids were valuable in delineating intraspecific variation within a lake, but also in examining differences between lakes. Non-dietary fatty acids can provide reliable information; therefore, one should carefully consider if such information is superfluous or not before data from these fatty acids are discarded.

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Data Availability Statement:

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

Conflict Of Interest

The authors declare that they have no conflict of interest.

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Table 1. The ten most discriminating non-dietary fatty acids from SIMPER analyses. Results are presented for each region, including percentage contribution to overall fatty acid dissimilarity among lake trout morphs, and their order of importance (1-10, in parentheses). 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 regions.

	Fatty acids	**Great Bear Lake** (63.8%)	**Stannard Rock** (70.9%)	**Superior Shoal** (69.9%)
Non-dietary Fatty Acids	12:0		3.6% (9)	6.1% (3)
	12:1	8.3% (3)	8.9% (2)	14.7% (2)
	13:1			
	14:1n-9	5.4% (6)	3.0% (10)	4.5% (6)
	14:1n-7	3.8% (9)		3.3% (10)
	14:1n-5			4.2% (8)
	14:0 iso		6.4% (4)	
	14:0 ante			
	15:0			
	15:1n-8	8.5 % (2)	25.9% (1)	18.8% (1)
	15:1n-6	8.2 % (4)	3.7% (7)	4.2% (7)
	15:0 iso	3.8% (10)	3.6% (8)	3.6% (9)
	16:1n-11			
	16:1n-9			
	16:1n-5			
	16:0 iso	7.1% (5)		
	7Methyl16:0	4.4% (7)		
	17:1			
	17:0 iso			
	18:1n-5			
	20:0		4.1% (6)	
	20:2n-9	10.2% (1)	4.5% (5)	5.2% (5)
	22:0	4.1% (8)	7.2% (3)	5.3% (4)
	24:1n-9			

Table 2. The ten most discriminating dietary fatty acids from SIMPER analyses. Results are presented for each region, including percentage contribution to overall fatty acid dissimilarity among lake trout morphs, and their order of importance (1-10, in parentheses). 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 regions.

	Fatty acids	**Great Bear Lake** (48.8%)	**Stannard Rock** (55.2%)	**Superior Shoal** (55.8%)
Dietary and Extended-Dietary Fatty Acids	14:0			
	16:0			
	16:1n-7			
	16:2n-6			
	16:2n-4			
	17:0			
	16:3n-4	4.0% (6)	2.5 % (9)	2.9% (10)
	16:4n-3	3.0% (10)	2.6% (8)	
	16:4n-1		7.3% (3)	6.3% (3)
	18:0			
	18:1n-9		2.4% (10)	
	18:1n-7			
	18:2n-6			
	18:2n-4			
	18:3n-6			
	18:3n-4			
	18:3n-3			
	18:3n-1	3.3% (9)		3.5% (7)
	18:4n-3			
	18:4n-1	3.5% (8)	10.5% (2)	11.1% (2)
	20:1n-11	4.0% (5)		
	20:1n-9			
	20:1n-7	3.6% (7)		
	20:2n-6			
	20:3n-6			
	20:4n-6			
	20:3n-3			
	20:4n-3			
	20:5n-3			
	22:1n-11	8.6% (1)	11.9% (1)	12.1 % (1)
	22:1n-9			
	22:1n-7		2.7% (6)	4.6% (5)
	22:2n-6	6.3% (3)		
	21:5n-3	7.5% (2)		4.7 % (4)
	22:5n-6		7.2% (4)	3.0% (9)
	22:4n-3	5.0% (4)	2.6% (7)	4.2% (6)
	22:5n-3			
	22:6n-3		5.5% (5)	3.4% (8)

List of Figures:

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.

Fig 2. A) The four morphotypes of Lake Trout in Lake Superior, the lean, humper, siscowet, and redfin (from Muir et al. 2014). B) The four shallow-water morphotypes of Lake Trout from Great Bear Lake, Morphs1-4 (from Chavarie et al. 2013, 2015, 2016a, 2016b).

Fig. 3. PCA of all fatty acids (top panels), dietary fatty acids (middle panels), and non-dietary 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 dietary fatty acids (top panel), non-dietary fatty acids (middle panel), and 10 most discriminating dietary and non-dietary fatty acids (bottom panel) 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.

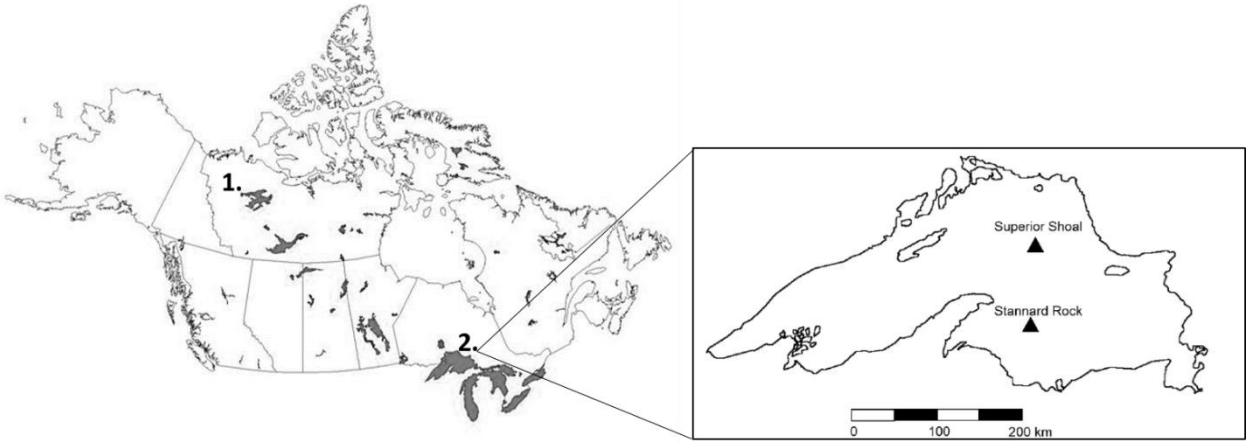
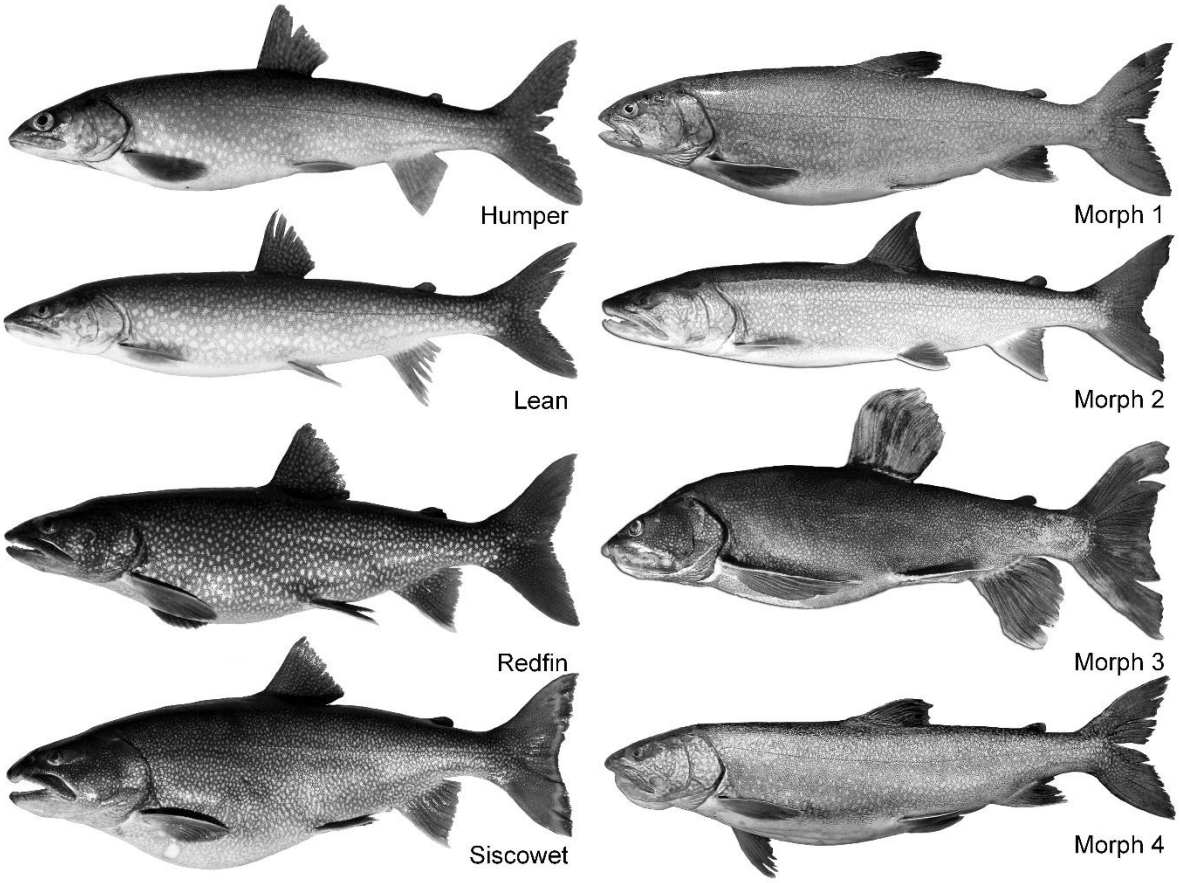


Fig. 1.



A) B)

Fig 2.

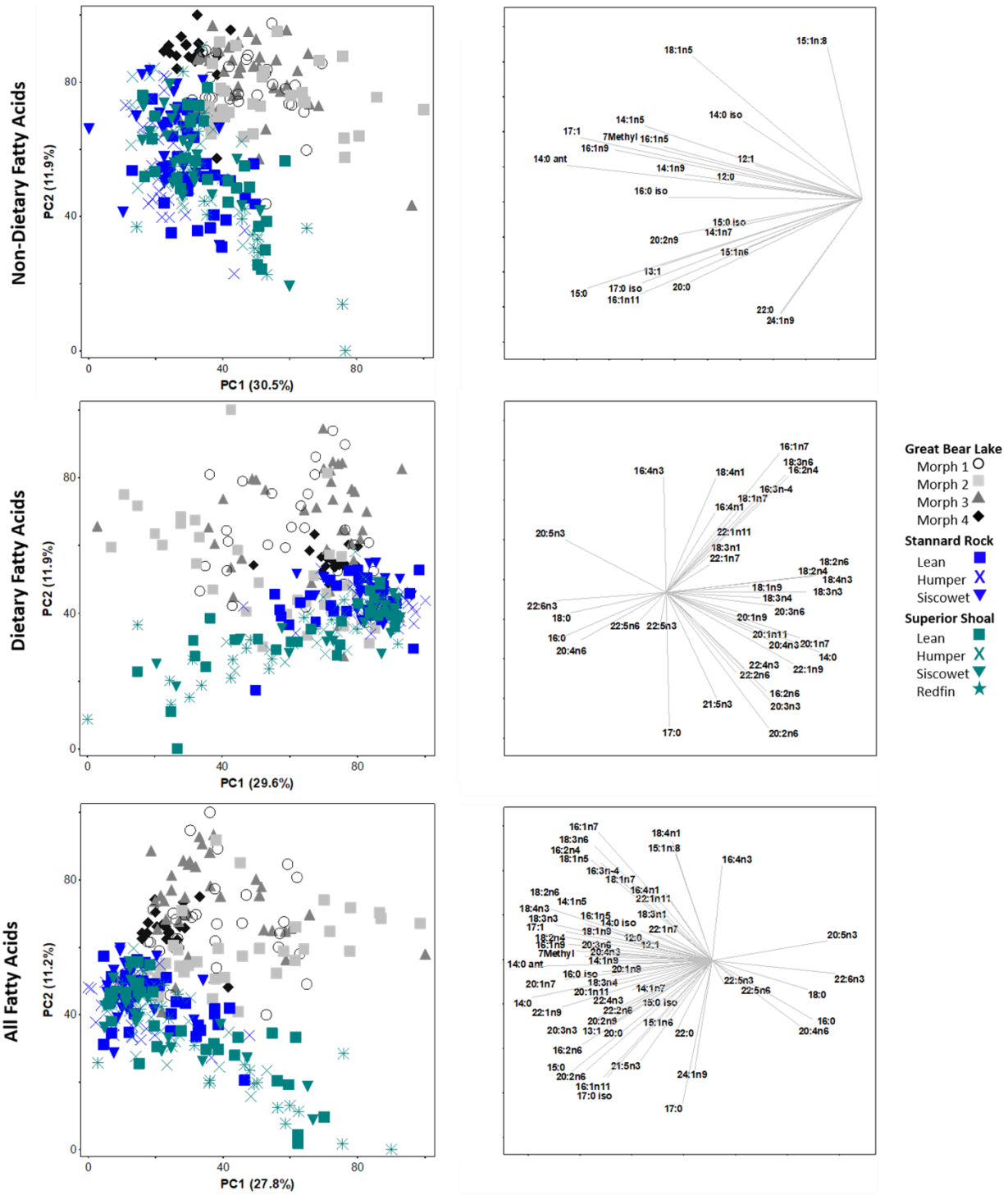

Non-Dietary Fatty Acids
Dietary Fatty Acids
All Fatty Acids
PC1 (30.5%)
PC2 (11.9%)
PC1 (29.6%)
PC2 (11.9%)
PC1 (27.8%)
PC2 (11.2%)
Great Bear Lake
Morph 1
Morph 2
Morph 3
Morph 4
Stannard Rock
Lean
Humper
Siscowet
Superior Shoal
Lean
Humper
Siscowet
Redfin

Fig. 3.

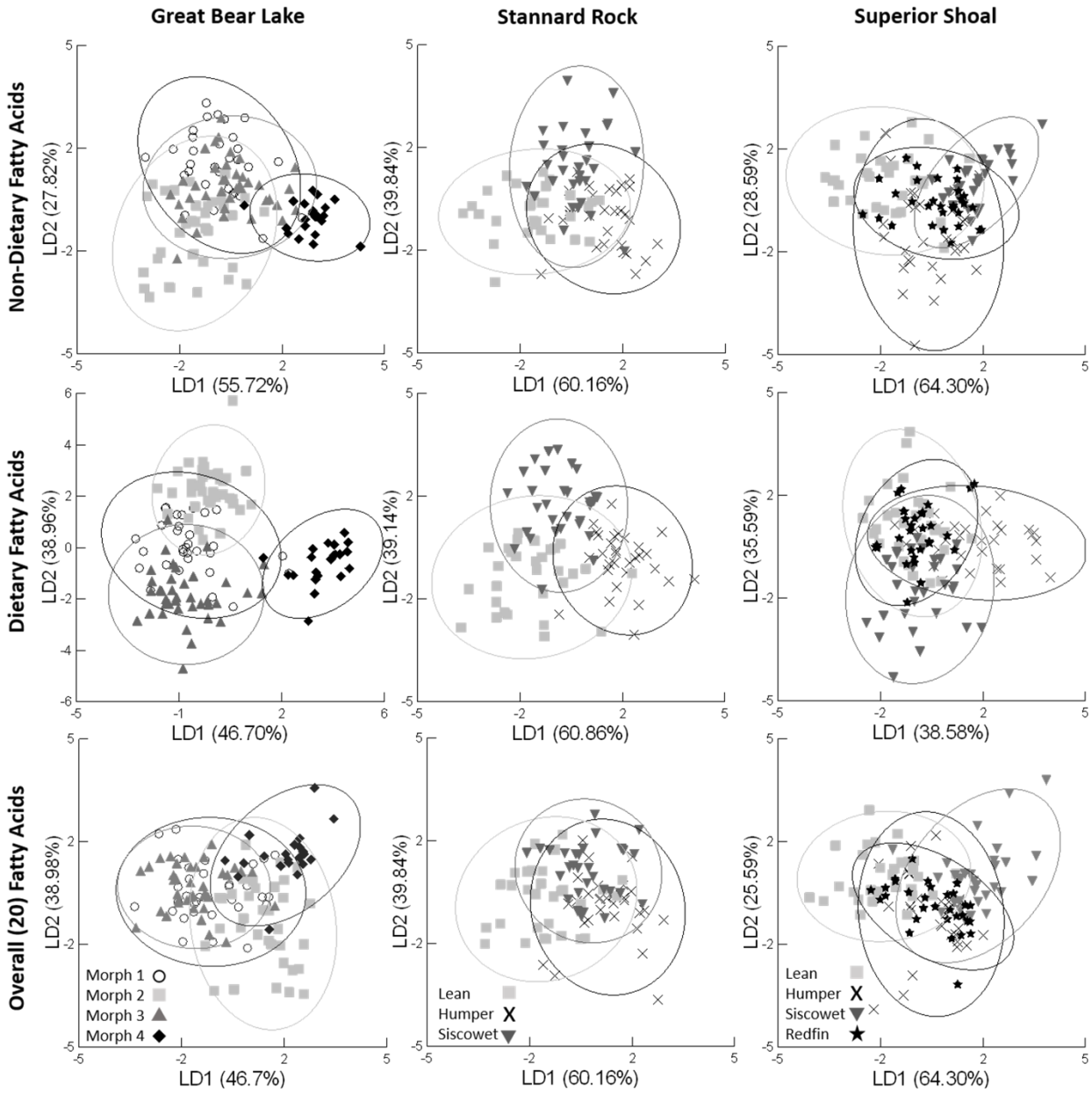

Great Bear Lake
Stannard Rock
Superior Shoal
Non-Dietary Fatty Acids
Dietary Fatty Acids
Overall (20) Fatty Acids
LD1 (55.72%)
LD2 (27.82%)
LD1 (60.16%)
LD2 (39.84%)
LD1 (64.30%)
LD2 (28.59%)
LD1 (46.70%)
LD2 (38.96%)
LD1 (60.86%)
LD2 (39.14%)
LD1 (38.58%)
LD2 (35.59%)
LD1 (46.7%)
LD2 (38.98%)
LD1 (60.16%)
LD2 (39.84%)
LD1 (64.30%)
LD2 (25.59%)
Morph 1
Morph 2
Morph 3
Morph 4
Lean
Humper
Siscowet
Redfin

Fig. 4.

Appendix:

Table A1. List of 38 of 41 fatty acids shared by the two datasets, considered as either "dietary" fatty acids or "extended-dietary" fatty acids and used in this study (see Iverson *et al*., 2004), and the dietary component they are associated with, based on literature: Sargent *et al*., 1995; Brett & 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*., 2009; ; Piché *et al*., 2010; Mariash *et al*., 2011. The fatty acids highlighted are the one discarded because they were not quantified for both lakes.

Dietary Fatty acids	**Dietary component**
14:0	Pelagic (Zooplankton) + diatom
16:0	Cannibalism or/and carnivorous
16:1n7	Benthic (bacterial synthesis + diatoms) Cannibalism or/and carnivorous
16:2n6	
16:2n4	Diatom
17:0	
16:3n-4	
16:4n3	
16:4n1	Diatom
18:0	Cannibalism or/and carnivorous
18:1n9	Pelagic (Zooplankton)
18:1n7	Benthic (bacterial synthesis + algal)
18:2n6	Terrestrial
18:2n4	
18:3n6	
18:3n4	
18:3n3	Terrestrial
18:3n1	
18:4n3	Pelagic (Zooplankton)
18:4n1	
20:0	*not used
20:1n11	Copepod (Iverson, 2009)
20:1n9	Pelagic (Calanoid copepods diet based)
20:1n7	Pelagic (Zooplankton)
20:2n9	*not used
20:2n6	
20:3n6	
20:4n6	Benthic (diatom)
20:3n3	
20:4n3	

20:5n3	Cannibalism or/and carnivorous
22:1n11	Copepod
22:1n9	Pelagic (Zooplankton)
22:1n7	
22:2n6	
21:5n3	
22:4n6	*not used
22:5n6	Pelagic (Calanoid copepods diet based)
22:4n3	
22:5n3	
22:6n3	Benthic (Pennate diatoms + dinoflagellates + bivalves) Cannibalism

Table A2. List of 24 fatty acids considered as "non-dietary" in this study.

Non-Dietary Fatty acids
12:0
12:1
13:1
14:1n9
14:1n7
14:1n5
14:0 iso
14:0 ante
15:0
15:1n8
15:1n6
15:0 iso
16:1n11
16:1n9
16:1n5
16:0 iso
7Methyl 16:0
17:1
17:0 iso
18:1n5
20:0
20:2n9
22:0
24:1n9

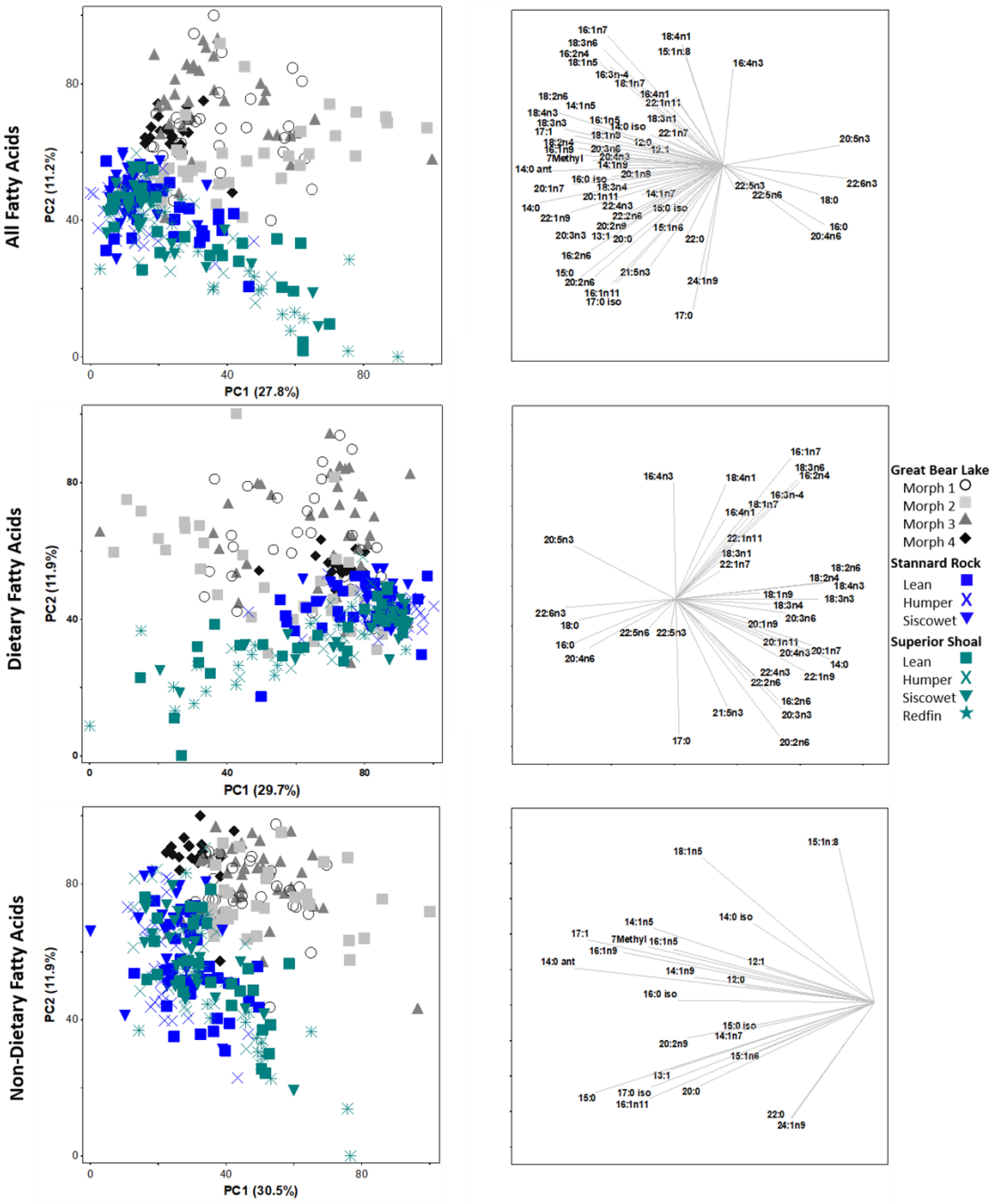


Fig. A1. PCA of all fatty acids (top panels), dietary fatty acids (middle panels), and non-dietary fatty acids (bottom panels) of each morph of Lake Trout from Great Bear Lake, Stannard Rock (Lake Superior), and Superior Shoal (Lake Superior), including the 12 outlier individuals. Vectors of individual 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.