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Energy channelling, food chain length and body condition in a northern lake predator

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Abstract

1. Identifying potential links between food web structure and animal body condition is fundamental for predicting the long-term persistence of populations under rapidly changing environments. Northern lakes, and the cold-water adapted species that inhabit them, are particularly vulnerable to a warming climate.

2. We explored relationships among body condition and energy channel use in lake trout (*Salvelinus namaycush*) and seasonal variation in food-chain length in the Lake of Two Rivers, Ontario, Canada. Bayesian mixing models using carbon isotope values ($\delta^{13}C$) of essential amino acids showed that individuals utilising multiple energy channels had higher gonad mass, suggesting that dietary diversity has reproductive fitness benefits.

3. Amino acid nitrogen isotope ($\delta^{15}N$) analysis revealed seasonal variation in food chain length, with extension of the food web during winter and spring and truncation of the food web during summer. Although these findings illustrate increased omnivory of lake trout during summer, there was no clear evidence that seasonal changes in food chain length had an impact on trout condition.

4. These findings provide new insight into potential relationships between food web structure, energy flow and consumer condition in freshwater ecosystems, thereby revealing potential responses of species to shifting ecosystem states under intensifying climate change at high latitudes.

KEYWORDS
climate change, compound-specific isotope analysis, ecosystem dynamics, food web, lake trout

1 | INTRODUCTION

Understanding fitness variation within animal populations is fundamental for predicting their ability to respond to environmental change, often termed adaptive capacity (Fragoso et al., 2020). Body condition, defined as the internal pool of acquired resources available for growth and reproduction, has been positively correlated with fitness in birds (Milenkaya et al., 2015) and is often calculated by normalising body mass to body length in fish (Thompson et al., 1991) or by measuring recirculating lipid metabolites (Shipley, Manlick, et al., 2022). The quantity and quality of resources available to consumers at different stages of the annual life cycle is known to affect consumer body condition and fitness (Fragoso et al., 2020; French et al., 2014; McLellan, 2011). Existing evidence suggests that longer food chains and feeding on a diversity of prey across multiple energy channels may result in higher body condition in certain contexts (Navarro-López et al., 2014; McLellan, 2011; Moorhouse-Gann et al., 2020). However, these examples are limited and thorough investigations into linkages between food web architecture and consumer body

condition are needed, especially for ecosystems undergoing rapid environmental change.

High latitude (>45°N) aquatic ecosystems are some of the most rapidly changing environments on Earth and are currently experiencing a suite of detrimental stressors such as rapid warming, hydrological regime shifts and intensification of seasonal flooding (Creed et al., 2017; Hansen et al., 2006; Kirtman et al., 2013). Given the well-documented sensitivity of aquatic ecosystems to variation in climate, they currently serve as strong sentinels of future change (Adrian et al., 2009; Schindler, 2009). These ecosystems offer a unique opportunity to explore relationships between food web structure, energy flow and consumer condition, as species must survive extreme seasonal shifts in water temperatures, light intensities and oxygen availability (Cavaliere et al., 2021; McMeans et al., 2015). Additionally, changing climate will be likely to alter the quantity and quality of resources available to lake consumers at different stages of their annual life cycle (Jeppesen et al., 2010), which has been found to alter life-history characteristics such as growth rate (Guzzo et al., 2017). While adaptative responses of lake consumers to seasonal changes in environmental conditions and resource availability are diverse and can include shifts in distribution, activity and/or diet (McMeans et al., 2015, 2020), the impacts of such variability on consumer fitness indicators, such as nutritional condition and reproductive success, are seldom studied. Given that increasing air temperatures will result in ice-free winters for 100,000 lakes worldwide (Woolway et al., 2020), understanding relationships between food web structure and body condition of consumers in these ecosystems may shed light on fitness variation, resilience under shifting ecosystem states, and potential mitigation strategies. Despite preliminary evidence and the urgency of further work, quantifying linkages between seasonal changes in food web structure, energy flow and consumer condition is inherently challenging in wild settings, particularly as these processes are temporally dynamic, highlighting a critical need for the application of novel monitoring techniques.

Recent advances in the isotopic analysis of individual compounds, such as amino acids (AAs), offer a novel means for discerning food-web structure (Pollierer et al., 2019; Ruiz-Cooley et al., 2017). For example, the carbon isotope (δ^{13}C) values of AAs can be used to trace energy flow within and among food webs, whereas the nitrogen isotope (δ^{15}N) values of AAs can be used to estimate consumer trophic position (TP) and total food chain length (FCL). Animals cannot synthesise the carbon skeletons of essential AAs and must therefore route them directly from diet or the gut microbiome with minimal isotopic fractionation (McMahon et al., 2010; Newsome et al., 2020). Pathways of *de novo* essential AA synthesis differ among plants, algae, bacteria and fungi, leading to unique essential AA δ^{13}C "fingerprints" among these potential energy channels (Besser et al., 2022; Elliott Smith et al., 2022; Larsen et al., 2009, 2012). Recent work using δ^{13}C analysis of essential AAs in consumer tissues has proven robust for tracing the contribution of different energy channels to individuals and communities (Elliott Smith et al., 2021; Manlick et al., 2023; Skinner et al., 2021). Because essential AA δ^{13}C fingerprinting relies upon the relative "spacing"

between the δ^{13}C values different essential AAs, and not measured δ^{13}C values, it is hypothesised that primary producers sampled from different geographical locations may indeed exhibit similar "fingerprints". This has been observed for autotrophs across extremely large spatial scales (Arsenault et al., 2022; Elliott Smith et al., 2022; Liew et al., 2019). Thus, provided that literature-derived essential AA δ^{13}C data are available and selected appropriately, minimal baseline sampling may be required for investigating energy flow to consumers-however, this requires additional rigorous validation.

With respect to δ^{15}N, AAs can be grouped into two broad categories that are loosely tied to patterns of kinetic isotope effects associated with nitrogen cycling during consumer metabolism. "Trophic" AAs undergo considerable transamination and deamination reactions and readily exchange nitrogen with the central glutamate pool during consumer metabolism, yielding large isotopic fractionation during trophic transfer (O'Connell, 2017). Conversely, "source" AAs undergo minimal trans- and deamination reactions, and thus reflect the δ^{15}N baseline values of a food chain (Nielsen et al., 2015; Ohkouchi et al., 2017). Offsets between trophic and source AA δ^{15}N values can serve as proxies for relative TP, and in apex consumers can be used to estimate FCL (e.g., Chua et al., 2021; Ruiz-Cooley et al., 2017). While compound-specific isotope approaches hold great potential for discerning many aspects of food web structure, a coupling of these techniques with indices of body and/or energetic condition remains scarce.

In a seasonally dynamic northern aquatic ecosystem, Lake of Two Rivers (Ontario, Canada), we used δ^{13}C and δ^{15}N values of individual AAs to explore how variability in food web structure, including energy channelling and FCL, influence the body condition of an apex generalist predator, lake trout (*Salvelinus namaycush*). We predicted that (1) individuals utilising a mix of energy channels would be of better condition as a result of the increased nutritional diversity of potential diet items (Cross et al., 2005), and (2) food-web truncation would lead individuals to forage on smaller prey, which also increases activity costs relative to foraging on larger prey (Cruz-Font et al., 2019) and could result in lower body condition based on studies in mammals (McLellan, 2011). This study sheds new light on the ecological responses of consumers to changes in food web structure and energy flow, with potential implications for species interactions, fitness and resilience of populations under intensifying environmental change.

2 | METHODS

2.1 | Field sampling and stomach content analysis

Research was conducted in the Lake of Two Rivers, Ontario, Canada (45°34.574′N, 78°28.842′W) from summer 2017 to winter 2019 (Appendix S1: Table S1). Lake trout were captured using angling and short sets of gill nets and euthanised using freezing, during four seasonal sampling periods (spring [$n=9$], summer [$n=9$], autumn [$n=10$] and winter [$n=12$]). Autumn sampling occurred

during post-spawning, and male and female lake trout were sampled to account for potential behavioural and physiological variation between sexes. For each individual, total length (TL, cm), fork length (FL, cm), round weight (RWT, g) and gonad mass (GON, g) were measured and recorded. Round weights and GON were normalised to individual fork lengths (e.g., $RWT_{normalised} = RWT/FL$), given allometric relationships between fish body size/mass, weight and GON. Fish were frozen immediately at −20°C before being transported on ice to a −80°C freezer at the University of Toronto Mississauga, where a sub-sample of liver tissue was taken (~1 g) for bulk and compound specific stable isotope analyses. Based on existing isotopic incorporation rates for in situ rainbow trout (*Oncorhynchus mykiss*), lake trout liver tissue was expected to integrate dietary inputs over ~3–5 months before sampling (Skinner et al., 2017). Fish were then dissected to obtain stomach contents for stomach-content analysis and liver tissue for stable-isotope analysis. Prey types present in each stomach were visually identified and recorded in the field, and empty stomachs were noted. Dietary fish were identified to the species level and invertebrates to order or genus.

2.2 | Stable isotope analysis of amino acids

All laboratory analyses were conducted at the University of New Mexico Center for Stable Isotopes (Albuquerque, New Mexico, USA). Liver tissues were freeze-dried and immersed in a 2:1 chloroform:methanol solvent solution (replaced every 24 hr) for >72 hr to remove lipids (Folch et al., 1957). After at least 72 hr, tissues were rinsed five times with DI water to remove excess solvent and dried at 50°C.

Approximately 0.3–0.5 mg of dried liver tissue was weighed into tin capsules for bulk tissue $\delta^{13}C$ and $\delta^{15}N$ analysis using a Costech 4010 elemental analyser coupled to a ThermoFisher Scientific Delta V Advantage isotope ratio mass spectrometer via a Conflo IV interface. Analyses were normalised to three in-house laboratory reference materials which were calibrated against IAEA N1, IAEA N2 and USGS 43 for $\delta^{15}N$ and NBS 21, NBS 22 and USGS 24 for $\delta^{13}C$. Analytical precision calculated from the analysis of standards was ±0.1‰ (1σ SD) for both $\delta^{13}C$ and $\delta^{15}N$.

Liver tissue samples from a total of 40 individuals were prepared for AA $\delta^{13}C$ analysis; a subset of these samples also was analysed for $\delta^{15}N$ (n = 20). The size range between fishes was low (FL = 345–445 mm) to reduce any potential impact of size-based feeding strategies on stable isotope values. For AA isotope analyses, ~4–20 mg of dried tissue was hydrolysed by immersing each sample in 1 ml of 6 N HCl at 110°C for 20 hr. This process converts glutamine to glutamic acid and asparagine to aspartic acid (Whiteman et al., 2019). Samples were then cooled and dried down to a fine film under a gentle stream of N_2 and then derivatised to N-trifluoroacetic acid isopropyl esters in a two-step procedure following Silfer et al. (1991). Firstly, the dried film was resuspended in a 4:1 solution of 2-propanol:acetyl chloride and heated for 1 hr at

110°C for esterification of the carboxyl group(s). The solution was then dried down under N_2 and rinsed twice with dichloromethane (DCM). Finally, each sample was reacted in a 1 ml solution of 1:1 trifluoracetic anhydride:DCM for 10 min at 110°C for acetylation of the amine group(s). Immediately before analysis, samples were dried under a gentle stream of N_2 and rinsed twice with DCM. After the second rinse, each sample was suspended in 50–150 μl of DCM and 1 μl of sample was injected onto a 60 m × 0.32 mm ID BPX5 × 1.0 μm column in a Thermo Scientific Trace 1310 gas chromatograph for separation of AAs. AAs were then combusted at 1000°C and oxidised to CO_2 or reduced to N_2 in a Thermo Scientific IsoLink II that was coupled to a Thermo Scientific Delta V Plus isotope ratio mass spectrometer. All samples were run as duplicate injections bracketed by one (for $\delta^{13}C$) or two (for $\delta^{15}N$) injection(s) of an in-house reference standard consisting of a mixture of powdered AAs purchased from Sigma Aldrich that were derivatised alongside each batch of unknown samples. $\delta^{13}C$ and $\delta^{15}N$ values of standard AAs were measured with a Costech 4010 elemental analyser coupled to a Thermo Scientific Delta V Plus isotope ratio mass spectrometer via a Conflo IV interface (Appendix S1, Table S2). Correction for the carbon added to AA side chains during derivatisation and associated fractionation was performed for each AA using the following equation (O'Brien et al., 2002):

$$\delta^{13}C_{USA} = \left(\delta^{13}C_{DSA} - \delta^{13}C_{DST}\left[\delta^{13}C_{UST} \times P\right]\right) \times P - 1. \quad (1)$$

where $\delta^{13}C_{USA}$ is the calculated $\delta^{13}C$ value of the underivatised AA in the sample, $\delta^{13}C_{DSA}$ is the $\delta^{13}C$ value measured for the derivatised AA in the sample, $\delta^{13}C_{DST}$ is the $\delta^{13}C$ value measured for the derivatised AA in the standard, $\delta^{13}C_{UST}$ is the $\delta^{13}C$ value of the underivatised AA in the standard (as measured via EA-IRMS), and P is the proportion of carbon measured native to the AA.

Measured AA $\delta^{15}N$ values of unknown samples were corrected with the equation:

$$\delta^{15}N_{USA} = \left(\delta^{15}N_{DSA} + \left[\delta^{15}N_{DST} - \delta^{15}N_{UST}\right]\right). \quad (2)$$

where DSA is the derivatised sample, DST is the derivatised standard, USA is the underivatised sample and UST is the underivatised standard (Whiteman et al., 2019, 2021). We determined $\delta^{13}C$ and $\delta^{15}N$ values for 13 individual AAs: Alanine (Ala), Glycine (Gly), Threonine (Thr), Serine (Ser), Valine (Val), Leucine (Leu), Isoleucine (Ile), Proline (Pro), Aspartic Acid/Asparagine (Asx), Glutamic Acid/Glutamine (Glx), Phenylalanine (Phe), Tyrosine (Tyr) and Lysine (Lys). The mean within-run precision of the in-house AA reference standard ranged from 0.3‰ to 0.5‰ for $\delta^{13}C$ and $\delta^{15}N$ (Appendix S1, Table S2).

2.3 | Statistical analyses

All statistical analyses were performed in the statistical programming software R (version 4.1.0).

2.3.1 | Energy channelling

We used essential AA $\delta^{13}C$ fingerprinting to define the predominant energy channels utilised by lake trout. Three energy channels most likely to support food-web biomass within the lake were identified: aquatic algal production (i.e., autochthonous phytoplankton and benthic algae), bacterially mediated production (e.g., cyanobacteria and recycled energy via the microbial loop) and terrestrial production (i.e., C_3 plants, herein "terrestrial C_3"). $\delta^{13}C$ values of six AA_{Ess} (Ile, Leu, Lys, Thr, Phe, Val) from sources that reflect these distinct energy channels were mined from two primary published datasets (Besser et al., 2022; Larsen et al., 2013, see Data S1). This method is appropriate given recent work suggesting that spatial variation in primary producer sampling does not significantly impact the uniqueness of isotopic fingerprints across broad primary producer groups (Arsenault et al., 2022; Elliott Smith et al., 2022; Liew et al., 2019). This implies that underpinning phylogenetic variation on which isotopic fingerprints are driven far exceeds that of spatial heterogeneity. Separations between sources were evaluated by reclassification using linear discriminant analysis (LDA) adopting a jack-knifed leave-one-out cross-validation (LOOic). Several recent studies have used a greater volume of data to generate LDA spaces for aquatic consumers than were included in this study. However, these studies are limited in that only $\delta^{13}C$ values for five essential AAs are used in the LDA. Therefore, we test the potential variation and effectiveness of LDA spaces by comparing reclassification rates of each primary producer from our initial dataset (126 primary producers with $\delta^{13}C$ values for six essential AAs) to an expanded global dataset (185 primary producers with $\delta^{13}C$ values for five essential AAs).

Because LDA cannot provide relative estimates of mixtures from multiple sources (i.e., an individual is probabilistically assigned to a single energy channel), we used Bayesian stable isotope mixing models implemented in the R package *MixSIAR* (Stock et al., 2018) to provide proportional estimates of energy channel reliance by individual lake trout. Linear discriminant coordinates (LD1 and LD2) for each energy channel were used to define the mixing space in which lake trout LD coordinates were examined. We generated mixing polygons based on $n = 10,000$ resampling iterations and removed individuals that displayed >95% probability of falling beyond the mixing space (Phillips et al., 2014; Smith et al., 2013). This resulted in removal of $n = 4$ individuals (10.0% of the total data; Figure 2b), which were sampled across spring, winter and autumn seasons. A further two individuals were removed from the final dataset as a consequence of missing GON data.

We evaluated potential body condition implications of variable energy channel use in lake trout using Bayesian isotope mixing models implemented in the R package *MixSIAR* (Stock et al., 2018). This approach uses LOOic and Akaike weights (*w*) to examine the relative degree to which different factors (both continuous and categorical) are influenced by the use of different resource pathways (Stock et al., 2018). We generated six candidate models that included categorical predictors of season and continuous effects of

FL, RWT, GON and $C:N_{Bulk}$ (as a proxy for liver lipid content). For models including RWT, GON and $C:N_{Bulk}$ we also included the effect of sex, given that the energetic allocation to body mass and reproduction may vary between male and female lake trout. Finally, we ran a model including the effects of season and GON, given that reproductive activity (and thus enlargement of reproductive organs) of lake trout typically occurs in the late spring and early summer before reproduction in the autumn (Blanchfield et al., 2009). Candidate models ($n = 34$ individuals) were compared to a null model including no covariates. Because mixing models were run using LDA coordinates based on essential AA $\delta^{13}C$ values (therefore assuming direct isotopic routing) trophic discrimination was assumed to be negligible and was therefore assigned values of 0 ± 0.

The most likely model was inferred through LOOic, *w* (Stock et al., 2018) and multiplicative error terms (ξ). Mixing models were run using uninformative priors across three separate Markov chains for 50,000 iterations, with a burn-in of 25,000 and thinning interval of 100. We assigned uninformative priors (0, 1) and incorporated residual and process error structures. Model convergence was assessed using Gelman-Rubin diagnostics, which should generally yield scores of <1.05 for model parameters (Gelman & Rubin, 1992; Stock et al., 2018).

2.3.2 | Food-chain length

Because lake trout are considered an apex predator in the lake ecosystem, we calculated their relative TP as a proxy for FCL using offsets in $\delta^{15}N$ values between several trophic–source pairs (i.e., $\Delta^{15}N_{trophic-source} = \delta^{15}N_{trophic} - \delta^{15}N_{source}$). We used the $\delta^{15}N$ values of Phe to discern the $\delta^{15}N$ baseline of the lake system for three reasons: (1) Phe is the most commonly employed source AA in the AA-SIA literature, (2) Phe $\delta^{15}N$ values of lake trout liver are more consistent across seasons than Lys $\delta^{15}N$ values, and (3) the algal, bacterial and terrestrial energy channels supporting this lake food web are unlikely to have upregulated Phe catabolic pathways that will increase variability in baseline Phe $\delta^{15}N$ values (Besser et al., 2022; Kendall et al., 2019, Ramirez et al., 2021). Offsets were calculated using several non-essential trophic AAs (Glx and Pro) in addition to several essential trophic AAs, given that the former (e.g., Pro) may be sensitive to physiological condition (Shipley, Olin, et al., 2022; Whiteman et al., 2021) as well as diet (McMahon & McCarthy, 2016). We did not calculate absolute TP (e.g., Chikaraishi et al., 2010; Nielsen et al., 2015), given that we did not calculate β values ($\Delta^{15}N_{trophic-source}$ for primary producers) for this specific system and recent studies have found considerable variability of *β* values (Besser et al., 2022; Ramirez et al., 2021).

We adopted Bayesian generalised linear models (BGLMs) to quantify relationships between FCL and several predictor variables in the R package *rstan* (Stan Development Team, 2021). Predictor variables were aligned with Bayesian mixing models and included season, FL, RWT, GON and $C:N_{Bulk}$. Model parameters were fitted using a Gaussian error distribution and uninformative priors (0, 1).

We derived posterior density distributions for model coefficients using a Markov chain Monte Carlo (MCMC) approach where parameter distributions were estimated from 100,000 model iterations across three markov chains. We used a model burn-in of 1000 iterations and thinning interval of 10.

2.3.3 | Analysis of stomach contents

We quantified stomach contents using percentage frequency of occurrence (Buckland et al., 2017), which is the percentage of non-empty stomachs that contain a certain prey type. Prey types were glassworms (*Chaoborus*), yellow perch, unidentified fish, macroinvertebrates and other. Unidentified fish could not be identified to a higher taxonomic resolution due to digestion. Items were placed in the "other" category when there was only one occurrence of that prey type.

3 | RESULTS

3.1 | Energy channelling

For the initial dataset, reclassification rates were 95%, 100% and 100% for algal production, bacterial production and terrestrial C_3 plants, respectively, indicating effective separation among energy channels in LDA space (Figure 1). Variation in LD1 (proportion of trace = 87.5%), as inferred through coefficients of linear discrimination (i.e., loadings), was primarily driven by the $\delta^{13}C$ values of Leu (−0.56) and Phe (0.43) (Table 1). Variation in LD2 (proportion of trace = 12.5%) was driven primarily by the $\delta^{13}C$ values of Lys (0.54) and Phe (−0.47) (Table 1). For the expanded dataset, despite an increased number of primary producers, the absence of Lysine $\delta^{13}C$ values resulted in poorer reclassification for each primary producer group, especially bacteria (algal production = 85%, bacterial production = 58%, terrestrial C_3 plants = 94%; Figure S1). Thus, we retained our initial dataset for all subsequent analyses. Essential AA $\delta^{13}C$ fingerprinting of liver tissue revealed consistent use of aquatic algal energy channels by lake trout across seasons (Figure 1). Across all individuals (*n* = 40), only a single female was probabilistically assigned to terrestrial C_3 plants in spring (Figure 1) with the remainder of individuals supported predominantly by algal production across all seasons.

Bayesian isotope mixing models revealed that aquatic algal production was the dominant energy channel supporting lake trout; however, individuals exhibited some reliance upon energy derived from terrestrial C_3 plants and bacteria (Figure 2; Appendix S1, Table S4). The most likely models included the effect of GON and sex (LOOic = 67.1, *w* = 0.61) (Table 2), and revealed that regardless of sex, individuals utilising a greater mix of energy channels had higher GON (Figure 2). The second and third most likely models were the null model and the model containing the effect of season and GON, which carried 20% and 16% of the model weights, respectively (Table 2). The latter suggested that a greater mix of resource pathways promoted higher GON, especially in the summer, but these models were associated with very high error at larger GON (Appendix S1, Figure S2). Multiplicative error terms for the top three candidate models were low ($\xi_{LD1} = 0.6$–0.8, $\xi_{LD2} = 1.2$–1.4), suggesting most of the variations in LD1 and LD2 were adequately explained by the selected predictor variables (Table 2; Stock et al., 2018).

3.2 | Analysis of stomach contents

Stomach contents revealed that lake trout switch their prey seasonally (Figure 3). Yellow perch were identified in over 75% of stomachs in the spring, autumn and winter but less than 20% of stomachs in the summer. By contrast, *Chaoborus* larvae were found in 73% of stomachs in the summer but in no other season. Macroinvertebrates were another seasonal prey type, found in approximately 70% of stomachs in the spring but less than 10% of stomachs in the summer and no stomachs in the autumn or winter.

3.3 | Food-chain length

Bayesian generalised linear models revealed several environmental and biological drivers of FCL inferred through $\Delta^{15}N_{trophic–source}$. Across all estimates of FCL we observed significant, positive effects of winter and near-significant positive effects of spring and RWT (Table 3, Figure 4). Models indicated a significant negative effect of summer, whereas the effects of GON and C:N on FCL were negligible (Table 3; Figure 4).

4 | DISCUSSION

We found strong relationships between food-web structure and individual body condition in lake trout from the Lake of Two Rivers. Bayesian mixing models revealed that individuals utilising a mix of available energy channels had higher GON, suggesting a positive relationship between multi-channelling and reproductive fitness. We also observed seasonal variability in FCL, with probable expansions during the winter and spring, and a truncation during the summer. We could not definitely conclude whether changes in FCL directly impacts overall lake trout condition, at least using estimates of fish mass relative to fish length. These findings provide timely insight into the seasonal variability of food web structure in northern lake ecosystems, and the extent to which alterations to FCL and energy flow impact fitness variation of cold-adapted northern lake consumers such as lake trout.

At the ecosystem level, the use of diverse energy channels by consumers has been shown to increase the stability and resilience of food webs to large-scale perturbations (Rooney et al., 2006; Rooney & McCann, 2012). However, few studies have assessed the direct

impacts of multi-channelling on consumer body condition and fitness. Applying a primary producer dataset that included $\delta^{13}C$ values from six essential AAs, we found links between multi-channelling and reproductive fitness in lake trout, whereby individuals exploiting a greater mix of energy channels had larger GON (Figure 3). This was largely driven by a marked increase in the contribution of
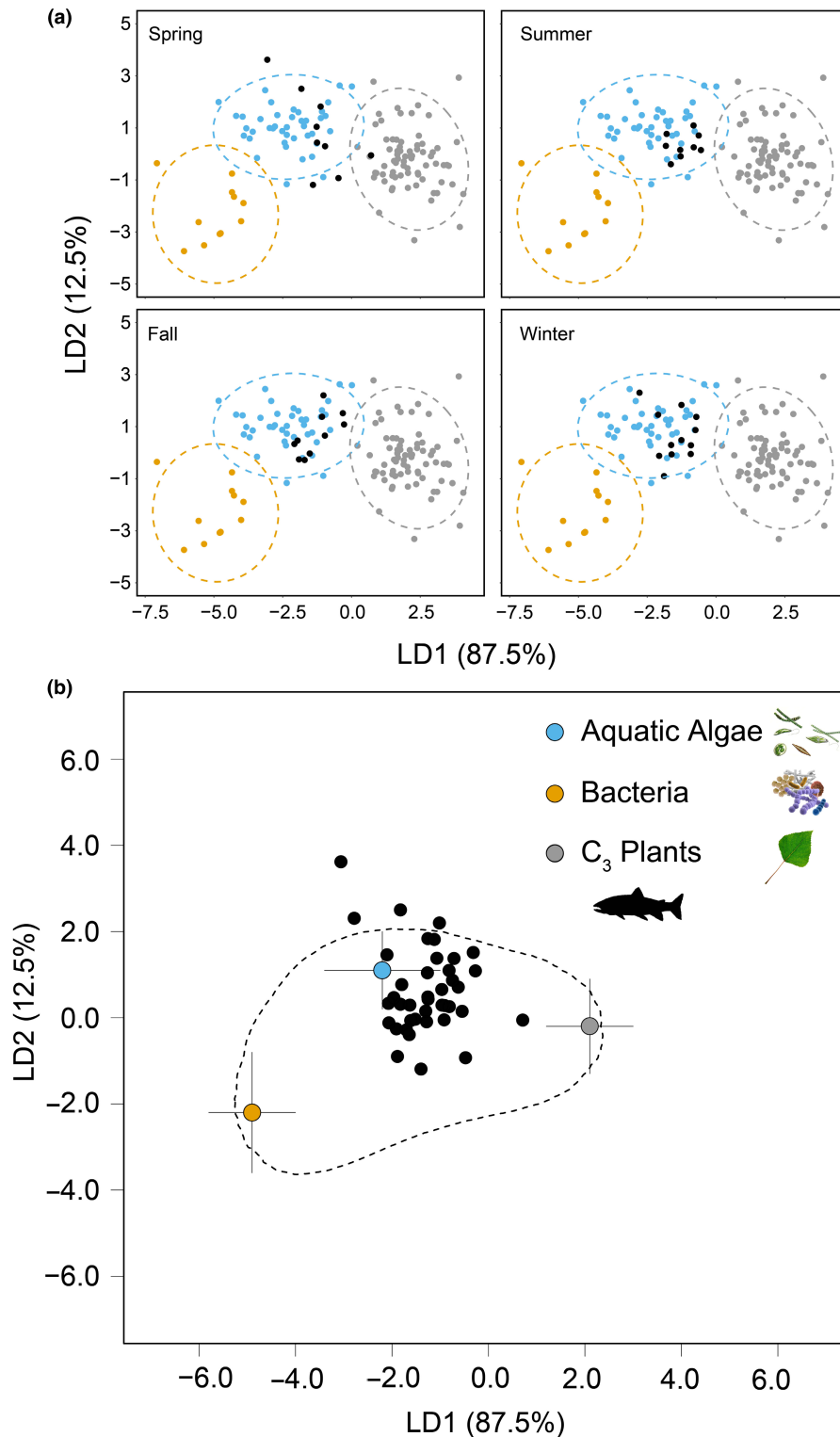
FIGURE 1 (a) First and second linear discriminants highlighting the use of three energy channels by male and female lake trout across four seasons; blue filled circles = aquatic algae, orange filled circles = bacterially mediated production, and grey filled circles = terrestrial C_3 production. Linear discriminant analyses were performed on the $\delta^{13}C$ values of six essential amino acids (Thr, Leu, Ile, Val, Phe and Lys). (b) Dietary mixing space derived from linear discriminant coordinates for the three energy channels and lake trout. Circles and whiskers represent the mean and standard deviation and dashed line highlights the 95% mixing region.
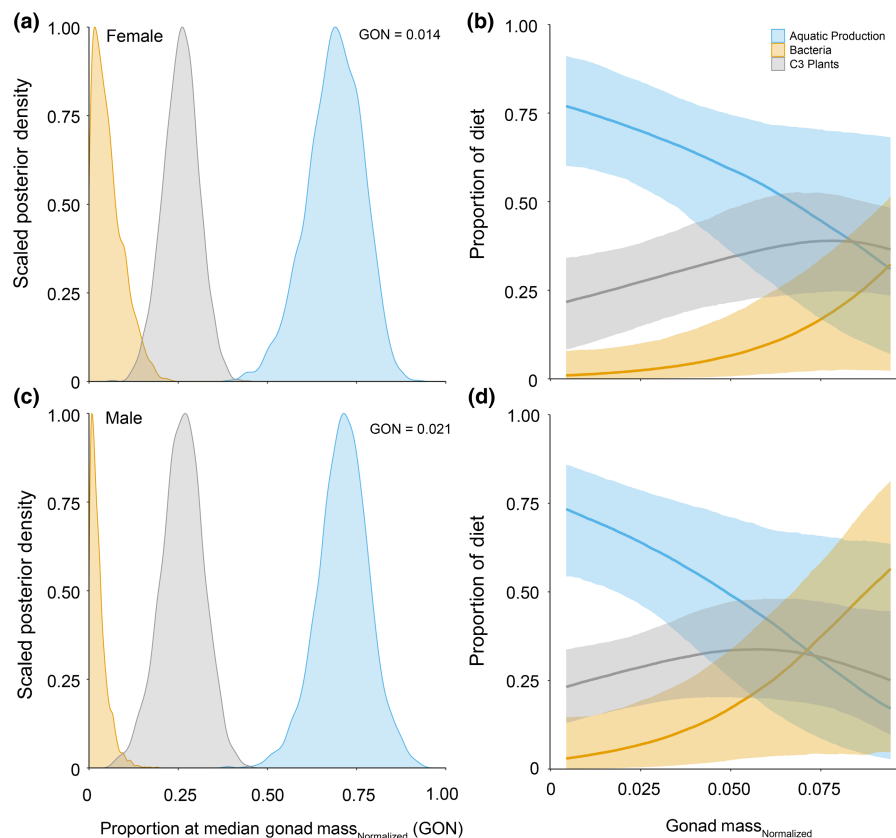
bacterially derived energy in individuals with larger GON. Although we observed higher credible intervals for individuals at larger GON, which may be a result of modest sample sizes, trends between multi-channelling and reproductive fitness appeared consistent across both male and female lake trout. This implies that the reproductive benefits of multi-channel foraging are unaffected by differences in sex-specific reproductive physiology.

Previous work has outlined how the use of both fast (e.g., algal production) and slow (e.g., microbially mediated detrital production) energy channels by consumers has the capacity to increase food-web stability, which may have knock-on nutritional

consequences (Rooney & McCann, 2012; Rooney et al., 2006). Owing to the lower nutritional quality of allochthonous terrestrial plants relative to aquatic algae (Brett et al., 2017), we find it unlikely that nutritional benefits result from the small increase of energy derived from terrestrial C_3 plants in individuals of larger GON (Figure 2). Instead, we propose that greater use of bacterially derived energy, channelled through the microbial loop (i.e., slow energy) may improve reproductive fitness in lake trout–this is especially evident for males (Figure 2). Although they may not provide omega-3 polyunsaturated fatty acids like algae, bacteria are still likely to provide key macronutrients such as protein and fat, and are becoming an increasingly recognised source of energy supporting lake biomass (Saboret et al., 2023). In this case, differences in energy channelling among individuals could be mediated by diet diversity (Weisse et al., 1990). It is well-established that bacteria play a critical role in the liberation and recycling of "slow" energy within aquatic food webs (Sherr & Sherr, 1988). Given the diverse forage base available to lake trout, it is certainly plausible that certain prey groups may be more reliant upon bacterially derived energy at the base of the food web. Here, diet diversity could play an important role in long-term fitness of lake trout, as seen as in other taxa such as birds (Navarro-López et al., 2014) and mammals (Manlick et al., 2021). We stress the need for further work exploring links between multi-channelling and body condition, given the paucity of empirical evidence and divided opinion

TABLE 1 Coefficients of linear discriminants for $\delta^{13}C$ values of essential amino acids (AA) used for isotopic fingerprinting. Values were derived from linear discriminant analyses of $n=88$ primary producers comprising terrestrial C_3, bacteria and aquatic algae.

AA	LD1	LD2
Ile	−0.01	0.16
Leu	−0.56	0.22
Lys	−0.07	0.54
Thr	0.08	0.00
Phe	0.43	−0.47
Val	0.00	0.02
Proportion of trace	0.87	0.13

FIGURE 2 (a,c) Scaled posterior density estimates of energy channel use for lake trout at median gonad mass (GON); (b,d) relationship between the use of energy channels and GON.

TABLE 2 Comparisons table of Bayesian isotope mixing models exploring the drivers of energy channel use in lake trout.

Model	LOOic	SE LOOic	ΔLOOic	SE ΔLOOic	w	ξ_{LD1}	ξ_{LD2}
~Gonad mass + Sex	67.1	16.0	0.0	NA	0.609	0.6	1.2
Null	69.5	13.7	2.4	4.8	0.183	0.8	1.5
~Gonad mass + Season	70.4	14.3	3.3	4.0	0.117	0.5	1.3
~Sex	73.1	11.8	6.0	6.8	0.030	0.7	1.4
~Round weight + Sex	73.4	16.3	6.3	4.4	0.026	0.8	1.2
~Season	74.3	17.5	7.2	4.5	0.017	0.7	1.4
~CNBulk + Sex	75.2	14.6	8.1	4.4	0.011	0.7	1.4
~Fork length + Sex	76.0	16.2	8.9	4.8	0.007	0.7	1.3

Note: For each model, the dependent variable is the proportion of energy derived from three energy channels. The most likely model is inferred from leave-one-out cross-validation scores (LOOic) and Akaike weights (*w*). Included are the standard errors of LOOic scores and Akaike weights and their differences (Δ) from the top model. ξ represent the multiplicative error terms for the first and second linear discriminants used to generate the mixing space.
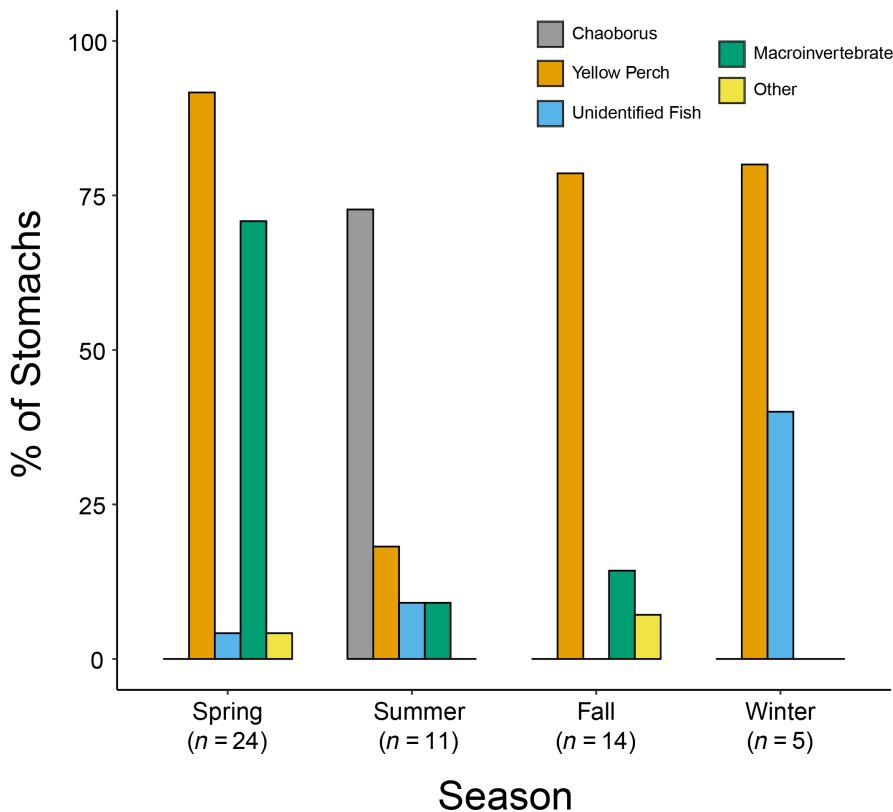

FIGURE 3 Seasonal diet of lake trout based on the stomach contents of individuals collected from Lake of Two Rivers in 2018 and 2019 (*n* = 54). Stomach contents are expressed as the percentage of non-empty stomachs that contained prey in the categories *Chaoborus* (grey), yellow perch (orange), unidentified fish (blue), macroinvertebrate (green) and other (yellow). The sample size is provided for each season. In the spring 100% of sampled fish had non-empty stomachs versus 84.6%, 70% and 41.7% in the summer, autumn and winter, respectively.

on the longer-term fitness consequences of generalist versus specialist foraging strategies (Griffith & Sultan, 2012; Manlick & Newsome, 2021; Navarro-López et al., 2014).

Considering the rate at which northern ecosystems are warming, it is possible that many lakes will shift toward extended summer conditions with little or no seasonal ice cover (Woolway et al., 2019). This is expected to alter key species interactions, mechanisms of co-existence (McMeans et al., 2020), and potentially impact rates of growth (Guzzo et al., 2017). For cold-adapted species like lake trout, this may extend periods in which individuals are restricted to deeper, offshore waters and forced to assume more omnivorous diets comprised of lower trophic level prey (e.g., zooplankton, Guzzo et al., 2017; Jeppesen et al., 2010). Our findings support this idea, illustrating a high degree of omnivory by lake trout during summer when individuals associate with deeper, offshore regions of the lake (McMeans et al., 2020), and switch from a diet comprised mostly of yellow perch to one that includes both fishes and macroinvertebrates (i.e., glassworms). Lake trout populations that omnivorously forage on smaller, invertebrate prey have been found to have higher energetic costs associated with longer periods of continuous prey searching (i.e., activity) compared to lake trout populations foraging on larger fish prey (Cruz-Font et al., 2019). Because lake trout are

TABLE 3 Median coefficient estimates (±95% credible intervals) of potential predictors of FCL derived from Bayesian generalised linear models.

| | FCL proxy | | | | |
	$\Delta^{15}N_{Glu-Phe}$	$\Delta^{15}N_{Pro-Phe}$	$\Delta^{15}N_{Ile-Phe}$	$\Delta^{15}N_{Leu-Phe}$	$\Delta^{15}N_{Val-Phe}$
Intercept	19.7 (17.0 to 22.6)	12.9 (9.6 to 16.4)	22.9 (20.0 to 26.0)	18.8 (15.8 to 22.3)	19.6 (15.7 to 23.7
RWT	0.7 (−0.6 to 2.0)	0.6 (−1.0 to 2.1)	0.2 (−1.2 to 1.5)	0.7 (−0.8 to 2.1)	0.0 (−1.7 to 1.6)
GON	0.0 (−1.9 to 2.0)	0.0 (−1.9 to 2.0)	0.0 (−1.9 to 2.0)	0.0 (−2.0 to 1.9)	0.0 (−2.0 to 1.9)
C:N	0.0 (−0.3 to 0.3)	0.2 (−0.2 to 0.5)	−0.4 (−0.7 to 0.0)	0.0 (−0.4 to 0.3)	0.1 (−0.4 to 0.6)
Spring	0.8 (−0.3 to 1.9)	0.9 (−0.5 to 2.2)	0.4 (−0.8 to 1.5)	0.5 (−0.8 to 1.7)	0.6 (−0.9 to 2.1)
Summer	−1.2 (−2.3 to −0.1)	−1.0 (−2.3 to 0.3)	−0.5 (−1.7 to 0.6)	−0.9 (−2.1 to 0.4)	−1.0 (−2.4 to 0.5)
Winter	0.7 (−0.4 to 1.8)	1.3 (−0.1 to 2.6)	0.7 (−0.5 to 1.8)	1.3 (0.0 to 2.5)	1.7 (0.1 to 3.2)

Note: Food-chain length is derived from offsets in nitrogen isotope ($\Delta^{15}N$) values between five trophic–source pairs of amino acids: Glu–Phe, Pro–Phe, Ile–Phe, Leu–Phe and Val–Phe.
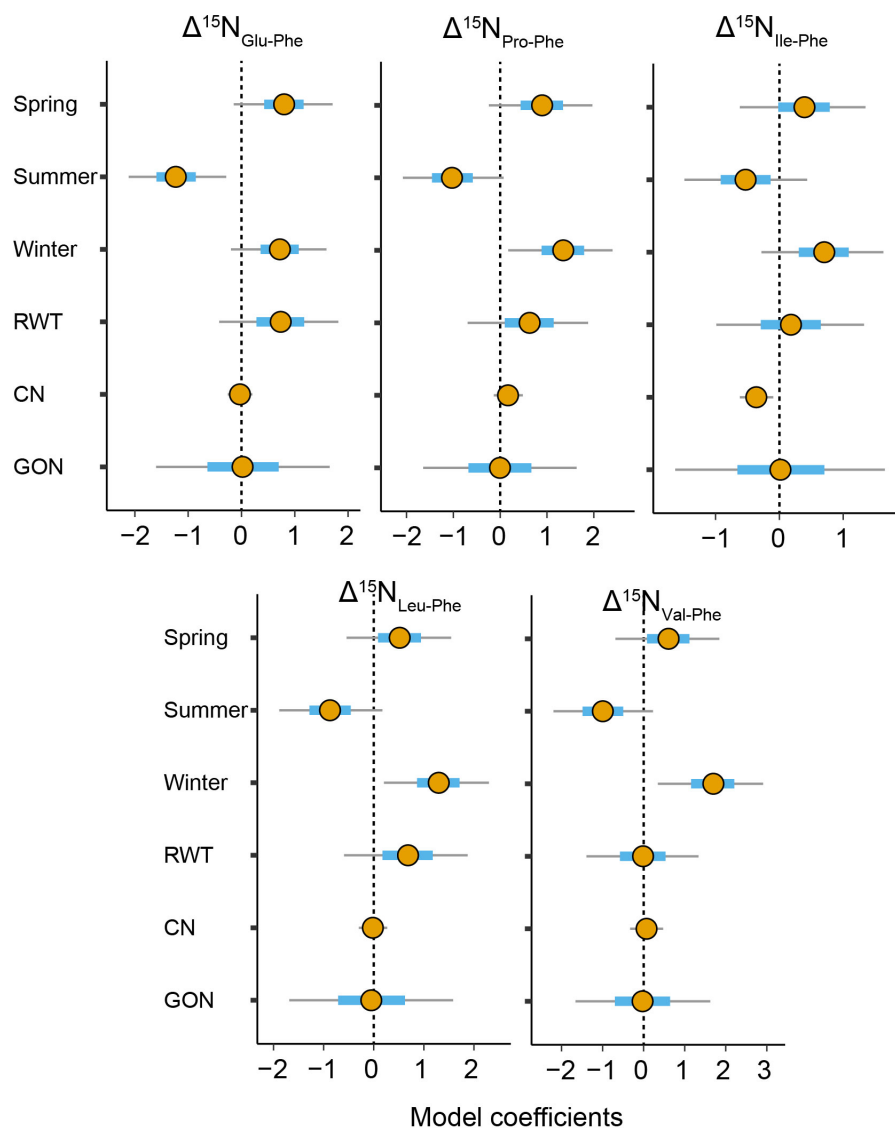
FIGURE 4 Model coefficients from Bayesian generalised linear models exploring the drivers of FCL using five proxies derived from offsets in nitrogen isotope values between trophic and source amino acids: $\Delta^{15}N_{GluPhe}$, $\Delta^{15}N_{ProPhe}$, $\Delta^{15}N_{IlePhe}$, $\Delta^{15}N_{LeuPhe}$ and $\Delta^{15}N_{ValPhe}$. Orange circles are median estimates of model coefficients, blue squares and grey whiskers are 75% and 95% credible intervals, respectively. CN, carbon to nitrogen ratio; GON, size-normalised gonad mass; RWT, size-normalised round weight.

cold-water adapted, significant energetic costs also may be associated with the requirement to forage in warmer waters during the summer relative to colder months. Combined, these factors would be expected to decrease the energetic state of lake trout during summer. Lake trout in our study lake are a smaller bodied population compared to other populations inhabiting lakes with offshore forage fish. However, summertime increases in foraging on smaller, lower trophic level prey did not have observable impacts on fish body condition in our study across seasons. From a fitness perspective, seasonal periods of omnivory are common in many lake predators (Jeppesen et al., 2010), but whether this has direct impacts on aspects of condition and longevity still remains inconclusive. Future studies could consider whether access to higher trophic position prey in colder seasons (spring, autumn, winter) is able to compensate for summertime restrictions toward smaller lower trophic prey in years with historically average summer durations, and whether lengthening the duration of summer (and omnivorous foraging) has negative effects on fish body condition. Furthermore, the application of additional metabolites that serve as proxies for condition, such as recirculating lipid metabolites (e.g., triglycerides and sterols) also should be explored. We note that seasonal changes in FCL may be exclusive to lakes specifically lacking offshore forage fishes (e.g., cisco, smelt and sculpins), such as Lake of Two Rivers, and appreciate that seasonal truncation of the lake food web may not be as pronounced in other settings where offshore forage fishes are more abundant. Additionally, greater information on overall prey availability, trout activity patterns and associated impacts on body condition are required to fully contextualise the energetic costs of seasonally variable food web characteristics.

Our findings suggested that $\Delta^{15}N_{trophic-source}$ can be used as a robust proxy for TP and FCL in lake ecosystems given the relatively uniform performance of both essential and non-essential trophic AAs in Bayesian GLMs (Figure 4). This is a notable finding given that the use of $\Delta^{15}N_{trophic-source}$ requires fewer assumptions than equations that convert such data into estimates of TP (Bowes & Thorp, 2015; Ohkouchi et al., 2017; Whiteman et al., 2019) and has yet to be applied to freshwater ecosystems fuelled by a combination of aquatic and terrestrial primary producers. We stress caution over the calculation of TP using standardised equations (e.g. Chikaraishi et al., 2010; Ohkouchi et al., 2017) because they require estimates of primary producer β values ($\Delta^{15}N_{trophic-source}$) and trophic–source AA discrimination factors that are not well-constrained in freshwater ecosystems. A recent meta-analysis of primary producer β values found significant variation between vascular terrestrial and non-vascular aquatic producers (Ramirez et al., 2021), which is challenging to integrate into TP equations for consumers supported by multiple energy channels (Chikaraishi et al., 2010). Further, estimates of trophic-source AA discrimination remains limited for many consumers, with high variation reported across taxa (McMahon & McCarthy, 2016; Nielsen et al., 2015). Finally, we acknowledge that physiological condition also can cause variation in $\Delta^{15}N_{trophic-source}$ and must be considered when using offsets to infer ecological information. For example, data from emperor penguins (*Aptenodytes forsteri*; Whiteman et al., 2021), southern elephant seals (*Mirounga leonine*, Lübcker et al., 2020) and bonnethead sharks (*Sphyrna tiburo*; Shipley, Olin, et al., 2022) have illustrated the sensitivity of AA $\delta^{15}N$ values to both catabolism and nitrogen conservation. Given the diversity of factors that could potentially influence $\Delta^{15}N_{trophic-source}$ that go beyond ecological processes, we recommend that studies aiming to quantify TP and FCL evaluate the performance of several trophic–source pairs, leveraging $\delta^{15}N$ data for both essential (e.g., Ile, Leu, Val) and non-essential (e.g., Glu and Pro) trophic AAs.

Finally, through comparison of multiple, literature-derived primary producer datasets, we emphasise the importance of scrutinising the relative loadings of AAs when applied to consumer fingerprinting. Our initial dataset ($n = 126$, six AAs) revealed the importance of Lysine $\delta^{13}C$ values in driving separation between energy channels in aquatic food webs. While the comparative global dataset ($n = 185$, five AAs) may have increased total sample size, the absence of Lysine $\delta^{13}C$ values resulted in poorer reclassification rates for primary producer groups—these observations bring to light important trade-offs between sample size and the number of essential AAs used for fingerprinting purposes. We therefore recommend greater generation of primary-producer essential AA $\delta^{13}C$ datasets across a diversity of habitats to rigorously explore the relative importance of individual AAs for deriving effective mixing spaces for consumer fingerprinting.

5 | CONCLUSIONS

Northern lake ecosystems provide a unique opportunity to explore relationships between food web structure and consumer fitness owing to extreme seasonal swings in environmental conditions that alter resource availability and species behaviour (McMeans et al., 2020). A greater incidence of multi-channel feeding was found to promote larger GON across individuals, underscoring the reproductive benefits of feeding across, rather than exclusively within, distinct energy channels. More broadly, this suggests that generalist foraging strategies have the capacity to increase reproductive success in lake consumers. While we did not detect seasonal signals in condition that were directly related to changes in food chain length, ongoing warming is beginning to force northern ecosystems away from periods of prolonged ice cover, which favour shorter food chains and greater omnivory in cold-tolerant species such as lake trout. Future work should focus on discerning linkages between food web properties and body condition if the adaptive capacity and longevity of keystone species are to be better understood.

AUTHOR CONTRIBUTIONS

Oliver N. Shipley, Bailey C. McMeans, Seth D. Newsome conceived project. Bailey C. McMeans and Emma J. Bloomfield conducted fieldwork and collected samples. Oliver N. Shipley and Alexi C. Besser performed laboratory analyses. Oliver N. Shipley analysed the data and wrote the paper with significant input from all authors. All authors approved the final version of the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

If accepted all data supporting this manuscript will be uploaded to the open-source, online repository IsoBank (www.isobank.org).

ANIMAL ETHICS STATEMENT

All sampling was conducted under University of Toronto Animal Use Protocol no. 20011951. Research did not involve manipulation of animals.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.