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Stable isotopes of fatty acids: current and future perspectives for advancing trophic ecology

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To understand consumer dietary requirements and resource use across ecosystems, researchers have employed a variety of methods, including bulk stable isotope and fatty acid composition analyses. Compound-specific stable isotope analysis (CSIA) of fatty acids combines both of these tools into an even more powerful method with the capacity to broaden our understanding of food web ecology and nutritional dynamics. Here, we provide an overview of the potential that CSIA studies hold and their constraints. We first review the use of fatty acid CSIA in ecology at the natural abundance level as well as enriched physiological tracers, and highlight the unique insights that CSIA of fatty acids can provide. Next, we evaluate methodological best practices when generating and interpreting CSIA data. We then introduce three cutting-edge methods: hydrogen CSIA of fatty acids, and fatty acid isotopomer and isotopologue analyses, which are not yet widely used in ecological studies, but hold the potential to address some of the limitations of current techniques. Finally, we address future priorities in the field of CSIA including: generating more data across a wider range of taxa; lowering costs and increasing laboratory availability; working across disciplinary and methodological boundaries; and combining approaches to answer macroevolutionary questions.

This article is part of the theme issue 'The next horizons for lipids as 'trophic biomarkers': evidence and significance of consumer modification of dietary fatty acids'.

1. Introduction

Understanding consumer dietary requirements and resources is critical for understanding the structure of food webs across ecosystems. Researchers have employed a variety of methods, ranging from gut content investigations to stable isotope analyses to DNA-metabarcoding, in order to understand consumer diets [1]. Analysing the stable isotope ratios (e.g. the ratio of ^{13}C to ^{12}C isotopes) of whole tissues from consumers and their potential resources is a particularly popular method for reconstructing diet because these analyses are relatively inexpensive and require minimal sample preparation. Bulk stable isotope analyses of carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$) and hydrogen ($\delta^{2}H$), in which all forms of carbon, nitrogen or hydrogen in a sample are analysed, are commonly used in nutritional studies and food web reconstructions (figure 1*a*; [2]). Carbon and hydrogen stable isotope ratios can assess an animal's diet sources [3,4], while nitrogen stable isotope values can provide information on trophic position as well as dietary resources, especially proteins, and nutritional

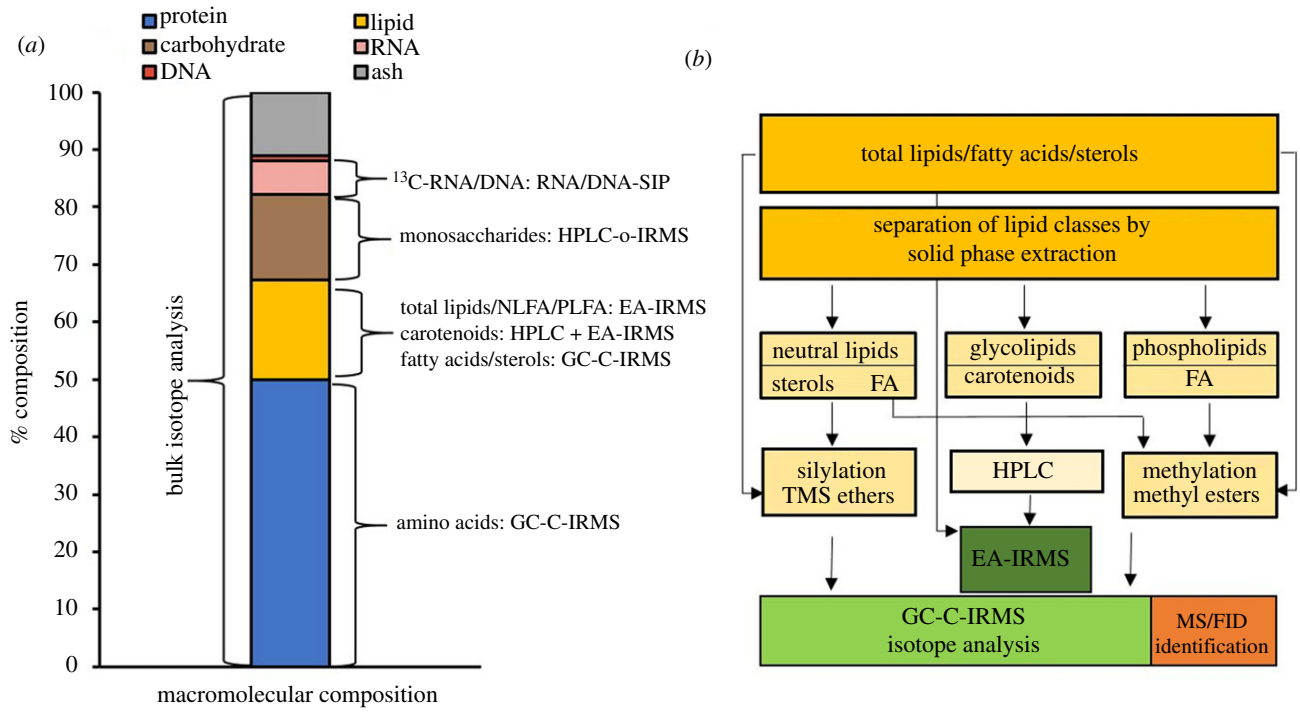

Figure 1. (*a*) Organic compounds included in bulk versus CSIA and analytical procedures used for different groups of organic compounds, and (*b*) analytical procedures used in CSIA of different lipid classes. Bulk lipids or fractionated lipids (neutral lipids, glycolipids, phospholipids) can be directly measured with EA-IRMS or fatty acid and sterols in different fractions can be derivatized and run with GC-C-IRMS. Carotenoids can be first separated with HPLC and then collected fractions can be run with EA-IRMS. SIP, stable isotope probing; EA, elemental analyzer; HPLC, high-performance liquid chromatography; IRMS, stable isotope ratio mass spectrometry; FA, fatty acids; GC-C, gas chromatography-combustion; NLFA, neutral lipid fatty acids; PLFA, phospholipid fatty acids; TMS, trimethylsilyl ethers; MS/FID, mass spectrometry/flame ionization detector. (Online version in colour.)

status [5,6]. Thus, by comparing consumer and resource stable isotope data, researchers can make inferences about what an animal has consumed.

When reconstructing animal diets in nature, researchers rely upon differences in the stable isotope values of potential resource pools to discriminate among resources [7]. For example, the fate of methane in food webs can be efficiently traced due to its naturally depleted ^{13}C value [8,9], while ^{2}H values have been used to trace migration across large geographical scales based upon precipitation gradients [10]. Researchers can use stable isotope mixing models based upon the isotopic values of consumer and resource plus empirically derived information on consumer stable isotope fractionation and trophic discrimination patterns to estimate the contributions of multiple resources to an animal's diet [11].

All bulk stable isotope-based diet estimates face two inherent methodological limits: (i) they require separation between the stable isotope values of potential resources and (ii) mixing model estimates have low precision for diets composed of many more potential food resources than the total number of bulk isotopes used (e.g. more than four isotopically distinct resources in a model based upon $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$). Unfortunately, these conditions are often not met [11]. Dietary resources frequently have overlapping stable isotope values, meaning that mixing models may not be able to discriminate between any potential resources or may not be able to discriminate between any resources. At the same time, animals often consume more than the two to four resources capable of being reliably resolved with high precision (i.e. without underdetermination), even with recent Bayesian mixing models (e.g. [12–14]). Consequently, in order to produce more precise estimates of diet, researchers are often forced to lump isotopically similar resources together into a single main resource or are limited to making inferences about diet at coarser-grain resolutions, which may obscure quantitatively minor, but nutritionally important dietary resources.

Fatty acid analyses have emerged as a complementary or alternative technique to bulk stable isotope analyses for examining trophic interactions and nutritional constraints. Numerous studies have used fatty acid composition analyses descriptively to distinguish between resources with overlapping bulk stable isotope values (e.g. as reviewed in [15]). Because most organisms contain a great diversity of fatty acids (up to 70; as reviewed in [15]), resources are less likely to overlap completely in their lipid composition, allowing researchers to identify potential dietary items at the species level. For example, Budge *et al*. [16] found high interspecific variation in the fatty acid composition of fish and identified 16 different fish at the species level with high accuracy using discriminant analysis. Fatty acid composition is also useful for understanding broader-scale patterns, such as the degree to which animals consume aquatic or terrestrial resources. Specifically, several major groups of algae contain omega-3 long-chain polyunsaturated fatty acids (*n*-3 LC-PUFAs), while most vascular plants and cyanobacteria only contain PUFAs with 18 carbon atoms, such as α-linolenic acid (ALA 18:3*n*-3) or linoleic acid (LIN; 18:2*n*-6), which are the precursors to LC-PUFAs [17,18]. Based upon this, Koussoroplis *et al*. [19] used the ratio of docosahexaenoic acid (DHA; 22:6*n*-3) to LIN to identify mammalian carnivores that varied in their aquatic resource use. Because a diverse array of animals require dietary sources of both DHA and eicosapentaenoic acid (EPA; 20:5*n*-3) (e.g. [20–24]), numerous studies have also used fatty acid composition analyses to evaluate food quality for consumers. Most notably, researchers have used fatty acid analyses

to argue for the nutritional importance of autochthonous algal resources over allochthonous terrestrial resources for consumers in aquatic food webs (e.g. [25]).

Compound-specific stable isotope analyses (CSIA; figure 1*a*,*b*), in which researchers determine the stable isotope values of individual organic compounds, like amino acids (e.g. [26,27]), sterols (e.g. [28,29]) or fatty acids [30], offer potential solutions to some of the limitations of bulk stable isotope analyses because even without distinct bulk signals, resources can contain isotopically distinct compounds. Isotopic separation at the compound-specific level may occur as a result of differing synthesis pathways or when prey derive or 'route' individual compounds from specific dietary sources that differ from those of the bulk diet [31,32]. Consequently, trophic interactions that cannot be resolved at the bulk level may be able to be resolved with CSIA. Using organic compounds also expands the potential number of resources under consideration if each compound has isotopically distinct values that allow it to be traced. Finally, CSIA can provide insights into sources and synthesis pathways for organic compounds.

In our synthesis and review of CSIA of fatty acids, we aim to provide readers with a broad overview of the promise that CSIA-based studies hold as well as the reality of their constraints. While CSIA can be used for a wide array of organic compounds, we focus on CSIA of fatty acids because this technique is particularly well suited as a complement to more widely used tools like bulk stable isotope and fatty acid composition analyses. We hope that this review will provide readers with a thorough understanding of what we have learned so far using CSIA of fatty acids and what readers should be aware of if they want to use this method in their own research. CSIA of fatty acids is still a relatively new and underused method in ecological research, and thus, we hope that this review piques others' interests in continuing to explore the boundaries of CSIA methods and applications.

2. Insights from previous studies: what we have learned

(a) Natural abundance studies

Researchers have used natural abundance CSIA in a growing number of observational and experimental studies. To date, these studies have largely focused on three main areas: (i) freshwater, (ii) marine and (iii) soil food webs, but are gradually expanding beyond these focal areas to provide insights into food webs from additional systems, such as riparian areas. Across systems, observational compound-specific stable isotope studies have helped enhance our understanding of consumer diet composition (e.g. [33–37]) and subsidies to consumers (e.g. [38,39]). For example, in their pioneering work, Murphy & Abrajano [40] demonstrated the potential of using CSIA to trace carbon flow through aquatic food webs. More recently, CSIA-based studies have revealed uncoupling between essential compounds and organic matter transfer, thus highlighting the nutritional importance of minor food sources [39,41]. Researchers have also employed natural abundance CSIA in experimental diet manipulation studies across a variety of systems to examine both trophic ecology (e.g. [30,42–46]) and nutritional physiology (e.g. [44,47–49]).

One of the advantages of using CSIA is its potential to reveal isotopic variation at the natural abundance level among individual compounds even when variation is not present at the bulk element scale (e.g. [50]). As in bulk stable isotope studies, all fatty acid CSIA-based studies require that the fatty acids of resources have distinctive stable isotope values (e.g. [51]). Researchers can then use these fine-scale differences at the natural abundance level to trace the movement of specific fatty acids or food resources with isotopically distinctive fatty acids. For example, Budge *et al.* [34] found that arctic marine consumers had isotopic values that were generally intermediate between the distinctive $\delta^{13}C$ values for 16:4*n*-1 and EPA (20:5*n*-3) of ice algae and pelagic phytoplankton. Although previous studies have generally used CSIA to estimate consumer reliance on two potential food sources (but see [52]), CSIA theoretically offers the advantage of being able to discriminate between a far greater array of potential resources ($n_{\text{fatty acids}}+1$) with high precision.

CSIA can be performed on total fatty acids or on fatty acids from specific lipid classes like phospholipids (polar) or neutral lipids (figure 1). Phospholipids are structural membrane-bound lipids within cells while neutral lipids, such as triacylglycerols, represent stored fats. CSIA of these individual lipid classes may particularly advance our current understanding of fatty acid metabolism in consumers. For example, changes in stable isotope values of FA in neutral or polar lipids in consumers relative to their dietary fatty acid stable isotope composition can indicate how storage or structural fatty acids, respectively, are modified within consumers (e.g. [30,53,54]). Neutral lipids show diet-based shifts in fatty acid sources faster than phospholipids (e.g. [41,53,55]). In addition, because phospholipids are used to maintain membrane fluidity, their composition can reflect regulation in response to environmental factors like temperature, while neutral lipids reflect stored dietary lipids and are thus preferred for examining trophic structure (e.g. [56]). The limited number of studies that have included isotopic composition data on multiple lipid fractions (i.e. neutral and polar lipids) found that for consumers of a given species fed the same diet, lipid fractions showed similar long-term overall patterns (e.g. [30,54]), suggesting that total fatty acids alone may potentially provide an accurate representation of overall diet and metabolic processes. However, additional research is needed before researchers can assume that these findings are representative for consumers generally.

Phospholipid fatty acids (PLFA) have proved particularly useful for identifying microbial (i.e. bacteria, fungi, algae) populations based upon patterns generated through the biogeochemical processes that different microbes are involved with (e.g. [57]). For example, compound-specific analysis of PLFA has been used to understand animal diets [51,58–60] and microbial interactions across both aquatic and terrestrial ecosystems (e.g. [61–63]). CSIA of PLFA can also serve as a valuable method for defining substrate use by microbes, providing important insights into biogeochemical processes and environmental change (e.g. [64,65]). However, interpreting PLFA results is usually only possible for broad taxonomic groups, such as gram-positive or gram-negative bacteria, fungi and phytoplankton at the class level [61,66–68].

While CSIA can be an especially powerful tool for studies of trophic ecology, it is critical that researchers understand lipid metabolism and isotopic turnover within their consumers of interest when employing CSIA in trophic ecology [49]. Fatty acids within consumer tissues are the result of: (i) assimilation from diet and incorporation directly into tissues as entire

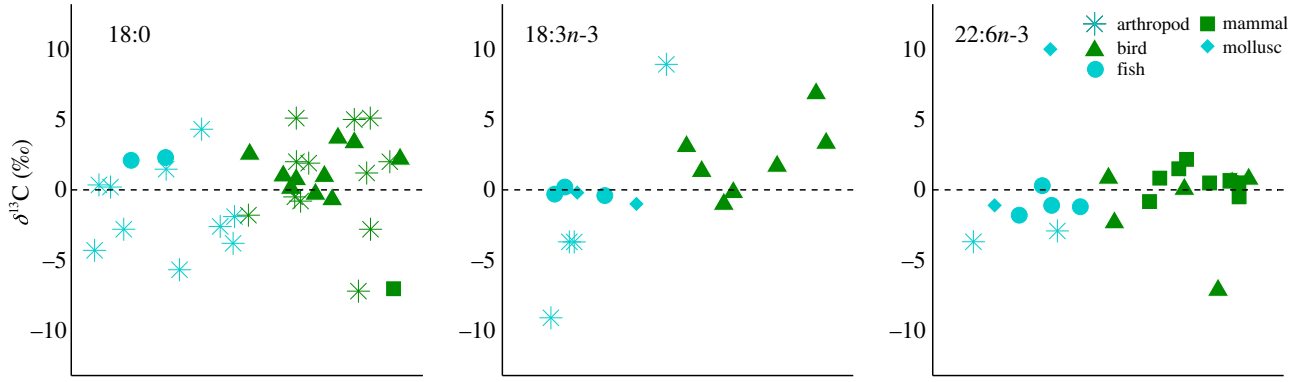

Figure 2. Mean differences between diet and consumer isotopic values for three fatty acids (18:0, a saturated fatty acid; ALA or 18:3*n*-3, a shorter-chain *n*-3 PUFA; and DHA or 22:6*n*-3, a long-chain *n*-3 PUFA) across a diversity of aquatic (blue) and terrestrial (green) taxa. Data sources used: [30,43–45,47,49,71,76–79]; CW Twining and JR Shipley 2020, unpublished data.

Table 1. Common sources and magnitude of isotopic shifts in the $^{13}C/^{12}C$ ratio of fatty acids between diet and consumers.

source	rationale	Δ ^{13}C (‰)	reference
de novo synthesis	fractionation by pyruvate dehydrogenase, carbon from various dietary pools	−6–8‰	DeNiro & Epstein [70]
relative markers	fatty acid pool consists of dietary and synthesized molecules	±2.3–3.0‰	Chamberlain *et al.* [28]
absolute markers	species-specific differences in closely related consumers	± 0.41‰ ($n = 30$)	Chamberlain *et al.* [71]
chain elongation	fractionation of the catalyst involved, $\delta^{13}C$ value of acetyl-CoA	$\delta^{13}C$ abundance in entire molecule: PLFA: 0.1–2.6% NLFA: 0–0.3%	Menzel *et al.* [72]

molecules through the process of 'dietary routing' [69]; (ii) synthesis from fatty acid precursors (e.g. ALA elongation and desaturation to EPA) followed by incorporation into tissue; or (iii) synthesis from carbohydrates in diet through carbohydrate to lipid routing (e.g. conversion of carbohydrates into 16:0) with or without further modification. Consequently, the stable isotope values of consumer fatty acids may represent the values of dietary items directly or those of dietary items mixed with molecules originating from consumer metabolism, which are subject to fractionation (table 1). The most common shifts in the $^{13}C/^{12}C$ ratio of fatty acids during lipid metabolism in consumers are presented in table 1. For example, there is often considerable fractionation and mixing for fatty acids in which the fatty acid pool consists of both dietary and synthesized molecules, but less for fatty acids taken up directly from diet [73]. When interpreting compound-specific stable isotope data, it thus is crucial to understand whether the fatty acids of interest can be synthesized de novo or from specific precursors or are strictly essential. For example, as a result of enzymatic fractionation, fatty acids synthesized de novo from carbohydrates (e.g. palmitic acid, 16:0) by the enzyme pyruvate dehydrogenase, which produces the fatty acid precursor acetyl-CoA, are depleted in ^{13}C and exhibit 6–8‰ lower $\delta^{13}C$ values compared to dietary carbon sources [70] (table 1). Moreover, the precision of the instrument (GC-C-IRMS) adds a variation of ±0.54‰ ($n = 147$; [74]) to the measured values in both diet and consumer. Qualitative assignment of trophic links (e.g. feeding strategies) with CSIA can work well for all fatty acids, whereas quantitative assessments (e.g. carbon flux rates or fatty acid metabolism) that include fatty acids originating from consumer metabolism require a strong ^{13}C label that clearly illuminates the dietary route of fatty acids (see the section below).

While admittedly limited in scope, the existing experimental natural abundance compound-specific stable isotope studies can provide insights into how lipid metabolism and incorporation into tissues vary among fatty acids, among taxa and among systems and trophic levels. Understanding all post-ingestive processes is crucial for drawing inferences about food web interactions in compound-specific stable isotope-based studies just as it is in bulk stable isotope studies [49,75]. Without an accurate understanding of trophic discrimination factors (TDF) and lipid metabolism, researchers may draw spurious conclusions about the origins of dietary resources, including the sources of individual compounds. For example, differences between diet and consumer tissue for the saturated fatty acids palmitic acid (16:0) and stearic acid (18:0) range from −10‰ to over 5‰, even within the same studies (figure 2; electronic supplementary material, figure S1). As discussed above, the stable isotope values of fatty acids in consumers not only reflect processes of ingestion, digestion and preferential routing, incorporation or excretion, but can also reflect additional post-digestive processes, including the break-down or synthesis of organic compounds [30]. Consequently, essential fatty acids that consumers must derive from diet make better trophic markers than those that can be derived either from diet, dietary precursors, or de novo synthesis. We, therefore, recommend that researchers focus upon fatty acids of known dietary origin, especially for quantitative dietary reconstruction studies.

Many food web studies have assumed that trophic fractionation should be close to negligible for fats for animals that cannot synthesize them de novo (e.g. [80–82]). However, experimental studies suggest that even fatty acids like PUFAs that are often essential for consumers can be subject to trophic fractionation when being incorporated into lipids in tissue (e.g.

[30,83]). For example, while the isotopic values of omega-3 and omega-6 PUFAs show much less variation between diet and consumer tissues compared to saturated fatty acids (figure 2), which consumers may either derive from diet or de novo biosynthesis, differences in the isotopic values of PUFAs between consumers and their diets can still vary from over −5 to +5‰ (figure 2), even in the same taxa fed different experimental diets (electronic supplementary material, figure S1). For example, Lau *et al*. [43] found that *Macrobrachium hainanense* shrimp fed higher ALA, lower EPA prey had tissue ALA values that were approximately −9‰ lower than dietary ALA, while those fed higher EPA, lower ALA prey had ALA values that were around +9‰ compared to diet. By contrast, CW Twining and JR Shipley (2020, unpublished data) and Fujibayashi *et al*. [45] found that tree swallow (*Tachycineta bicolor*) chicks and zebrafish (*Danio reno*) fed lower ALA, higher EPA diets had ALA values that were depleted relative to diet, while those on higher ALA, lower EPA diets had ALA values that were enriched relative to diet. Overall, current studies suggest that there is no clear one-size-fits-all TDF-type value for fatty acids akin to the TDF patterns seen across broad taxonomic groups for bulk stable isotopes.

Based on the current state of knowledge, we echo previous calls to develop empirically based species- and diet-specific TDF for use in bulk stable isotope studies and urge researchers to develop similar estimates of diet to tissue variation for CSIA. For example, given the limited number of current studies examining specific lipid classes, empirical studies that compare fatty acid turnover and incorporation from diet among lipid classes are particularly needed (e.g. [53]). Due to the large degree of variation in lipid metabolism even within species, we also recommend that researchers be conservative in their interpretation of compound-specific stable isotope data, especially in the absence of additional supporting evidence (e.g. substantial differences in the availability of a given fatty acid between potential resources or empirical evidence that consumers have limited abilities to synthesize a given fatty acid). Using generalized TDF-type values across taxa and making assumptions about synthesis abilities when the nutritional physiology of the consumer is unknown has a high potential for inaccurate interpretations. Unless researchers have a thorough empirical knowledge of dietary effects on compound-specific isotopic values for their individual study species (e.g. [30,42,44]), applying mixing models to compound-specific data from the field to determine the exact proportions of individual fatty acids from different resources is inappropriate. We also urge readers to couple observational CSIA-based studies with experimental studies for their consumers of interest.

(b) Experimental labelling studies

^{2}H-, ^{13}C- and ^{15}N-labelling can be used to trace biogeochemical processes including hydrogen, carbon and nitrogen cycles within the laboratory and at the whole ecosystem scale (e.g. [84,85]). Isotopic labelling to increase differences among carbon sources followed by CSIA allows researchers to detect even slight carbon isotopic changes (0.001%) in organic compounds at nanomolar concentrations and to comprehensively track the fate of fixed carbon from basal producers to upper trophic levels [61]. By contrast, ordinary quadrupole GC mass spectrometry (MS) requires a greater than 1% change in ^{13}C-content [61], while DNA and RNA stable isotope probing (SIP) require 15–20 atom % ^{13}C-labelling, making CSIA over 1000 times more sensitive than GC-MS and 20 000 times more sensitive than DNA or RNA SIP [86–89].

Combining CSIA with ^{13}C-isotope-labelling experiments (e.g. ^{13}C-bicarbonate in lakes and streams or free air $^{13}CO_2$ for terrestrial primary producers) has allowed researchers to more precisely trace nutrient cycles, assess food web structure and understand the transfer of bulk and individual molecules from diet to consumers [84,85,90–93]. CSIA of PLFA following labelling has been used to define group-specific primary production, individual microbial contributions to diet and microbial carbon cycling (e.g. [79,94–97]). For instance, PLFA-based studies on terrestrial microorganisms [98] have investigated biogeochemical pathways with ^{13}C-labelled substrates [99,100]. CSIA following ^{13}C-labelling in aquatic systems has enabled researchers to distinguish among terrestrial, phytoplankton, biofilm and bacterial carbon sources [58,59,94,95]. Similarly, studies using CSIA in terrestrial systems have demonstrated the potential of ^{13}C-labelling to disentangle complex food webs within the myco-rhizosphere, such as whether consumers derive carbon from specific soil bacteria or fungi [101–105]. Given its relative ease and low cost, $^{13}CO_2$ pulse labelling of primary producers followed by CSIA is a promising tool for introducing strong ^{13}C signals into food webs in trophic studies across ecosystems (e.g. [79,84,85]).

At the individual or species level, labelled individual fatty acids or dietary items can also be used to make inferences about lipid metabolism. While controlled dietary studies on various performance metrics can hint at whether or not specific fatty acids are growth limiting, enriched compound-specific stable isotope studies are necessary to confirm whether or not specific fatty acids are strictly essential (i.e. cannot be synthesized at all from molecular precursors) or physiologically essential (i.e. necessary quantities for survival and normal functioning cannot be synthesized from molecular precursors available in natural dietary items). By dosing an animal with an enriched fatty acid in the hundreds of per mil and then examining the stable isotope values of the given fatty acid (e.g. ALA) and its potential synthesis products (e.g. EPA and DHA) and correcting these values based on the stable isotope values of un-dosed control animals, researchers can trace carbon sources throughout fatty acid conversion processes like elongation and desaturation [106]. While biomedical researchers and human nutritionists first developed these methods, ecologists have since adapted them for use in wild animals in both laboratory (e.g. [72,107–109]) and natural settings (e.g. [110]). For example, using δ^{13}C- and δ^{2}H-enriched dietary items, researchers have found that harpacticoid copepods are capable of converting dietary EPA into DHA [111], while calanoid copepods are unable to synthesize DHA from ALA at rates necessary for normal growth and reproduction [108,112,113]. Similarly, researchers have used both ^{13}C- and ^{2}H-enriched ALA to study the ability of juvenile rainbow trout (*Oncorhynchus mykiss*) to synthesize DHA from ALA [46,107].

An advantage of using highly enriched fatty acids over those with more modest differences at the natural abundance level (e.g. from C_3 versus C_4 plants) is that researchers can account for very low rates of synthesis that may not be apparent relative to background levels of variance in natural abundance studies. However, precision is lower for highly enriched compounds, due to the high variation in ^{13}C-labelling, which can result from organisms with a mix of natural and ^{13}C-labelled compounds in tissue. In order to minimize

variation during labelling, ^{13}C-labelling of over 10% ^{13}C should typically be avoided and ^{13}C-label pulses should be repeated until a stable level of label incorporation is achieved. Alternatively, researchers can use high dose, 99% ^{13}C-label with GC-MS isotopologue profiling (see 'Emerging techniques') to generate more detailed information on consumer metabolism [72]. Unfortunately, highly enriched fatty acids are relatively expensive compared to natural abundance dietary items that vary more modestly in the δ^{13}C values of their fatty acids. In addition, highly ^{13}C-enriched commercial standards are not readily available. Another inherent methodological challenge is that in order to calculate exact rates of conversion of specific compounds to a final product, it is also necessary to either (i) label only one potential source compound per individual or (ii) label multiple potential source compounds with different isotopes. For example, to determine if a species is capable of synthesizing DHA from both ALA and EPA, separate individuals must either be dosed with ^{13}C-enriched ALA or EPA or the same individual must be dosed with ^{13}C-enriched ALA and ^{2}H-enriched EPA or vice versa, both of which add to costs.

In spite of these challenges, researchers have gained uniquely valuable insights into the physiological black boxes of their study organisms by coupling highly enriched fatty acid diets with CSIA. For example, although earlier diet manipulation-based studies demonstrated that certain consumers, such as *Daphnia* [114,115], have limited abilities to synthesize EPA and DHA from dietary ALA, combining fatty acid concentration data with compound-specific stable isotope data has enabled researchers to calculate conversion rates with more precise efficiency (e.g. 1% for *Daphnia*; [91]). Similarly, although an initial captive diet study suggested that tree swallow chicks were likely EPA- and DHA-limited [24], a follow-up ^{13}C-enriched ALA tracer study demonstrated that tree swallow chick EPA and DHA synthesis from ALA was indeed insufficient to meet chick demand [110]. Furthermore, both studies provide strong evidence that ALA, EPA and/or DHA exhibit limited modification from diet and thus appear to be reliable markers of diet for these species. We encourage fellow researchers to use insights about conversion, assimilation and turnover gleaned from experimental diet enrichment studies like these to inform future natural abundance studies.

3. Best practices: what you should know

Since the 1990s, CSIA has expanded the range of research questions that can be answered across disciplines through a combination of gas or liquid chromatography with isotope ratio MS (e.g. [106]). Because lipids predominantly consist of carbon, CSIA allows researchers to combine fatty acid and carbon stable isotope analytical techniques (figure 1). CSIA of fatty acids is conducted via GC-C-IRMS (figure 1). For high accuracy (close to absolute value), repeatability (within-run precision) and reproducibility (between-run precision), researchers should be aware of several important technical aspects of these analyses.

First, in order to have high reproducibility with a normal GC analysis, fatty acid methyl esters (FAME) should be analysed with GC-C-IRMS relatively soon after being extracted from samples and derivatized. As for any GC analysis, injection mode influences peak size and column phase influences the peak shape and resolution (e.g. [116,117]), and efficient oxidation is therefore crucial for obtaining accurate δ^{13}C values of fatty acids. To prevent drift during an analysis, the oxidation reactor needs to be initially oxidized and given an oxygen pulse before each sample (e.g. with Thermo equipment) or a continuous flow of oxygen (e.g. with Elementar equipment) needs to be used. If repeatability is low, the length of the oxidation pulse can be lengthened. This is crucial for DHA, which is close to the last compound in a typical FAME analysis (i.e. for fatty acids with less than or equal to 24 carbon chain lengths), especially when analysing FAME with long columns (60 m). If the temperature programme is very long (greater than 60 min), it can also be beneficial to have internal standards at the beginning and end of the analysis. Readers looking for additional technical recommendations for CSIA in general may wish to consult chapter 3 of Jochmann & Schmidt [118].

Previous studies [60,119] have shown higher variance in the δ^{13}C values of fatty acids generated by GC-C-IRMS compared to bulk δ^{13}C values generated by EA-IRMS. EA-IRMS accuracy is routinely 0.2‰, whereas accuracy in GC-C-IRMS is 0.5–1‰ [72,91]. However, precision for individual fatty acids may vary from 0.4 to 5.4‰ [51]. This high variance can be the result of technical issues (poor oxidation, carry-over problems or co-elution of peaks) or biological differences among replicates. Quantifying the ^{13}C to ^{12}C ratio in food web studies employing a strong isotopic label, such as pulse labelling a primary producer with $^{13}CO_2$ or offering a highly ^{13}C-labelled fatty acid diet to consumers, makes IRMS analyses particularly difficult. After the labelled substrate is incorporated into the trophic network, isotopic analyses are hindered by 'carry over effects' of the ^{13}C signal in key metabolites, which generally achieve heavy enrichment. In practice, this means that if chromatographic peaks are not baseline separated, the ^{13}C introduced especially by a highly labelled compound (e.g. 16:0) can feed considerably into the signal in its close neighbour (e.g. 16:1*n*-7). To check for this, researchers can swap the polarity of the column (i.e. from polar to non-polar or vice versa) or use longer columns to assess the ^{13}C signal of the key metabolites. If these refinements do not resolve issues, mass spectrometric analyses via isotopologue profiling may prove to be a more useful form of analysis (see the section below).

In order to obtain accurate natural abundance values, it is also crucial that raw δ^{13}C or δ^{2}H (see the next section) values of fatty acids be corrected based upon standards. However, this currently presents a major challenge for CSIA-based studies because the International Atomy Energy Association does not provide GC-C-IRMS standards. Thus, users must either find private supplies of standards (e.g. alkane and fatty acid standards for δ^{13}C correction from the University of Indiana) or use internal FAME standards with a known isotopic composition determined by EA-IRMS for correction. After data generation, the raw δ^{13}C values of natural abundances of fatty acids from GC-C-IRMS need to be corrected by those of the derivatization reagent (e.g. methanol) used during fatty acid extraction. For example, the δ^{13}C value of FAME need to be corrected for the methyl group generated during methylation (e.g. [30]).

4. Emerging techniques: what we are learning

(a) Compound-specific stable isotopes of hydrogen

From a chemical point of view, fatty acids are almost uniquely composed of carbon and hydrogen. So far, researchers using

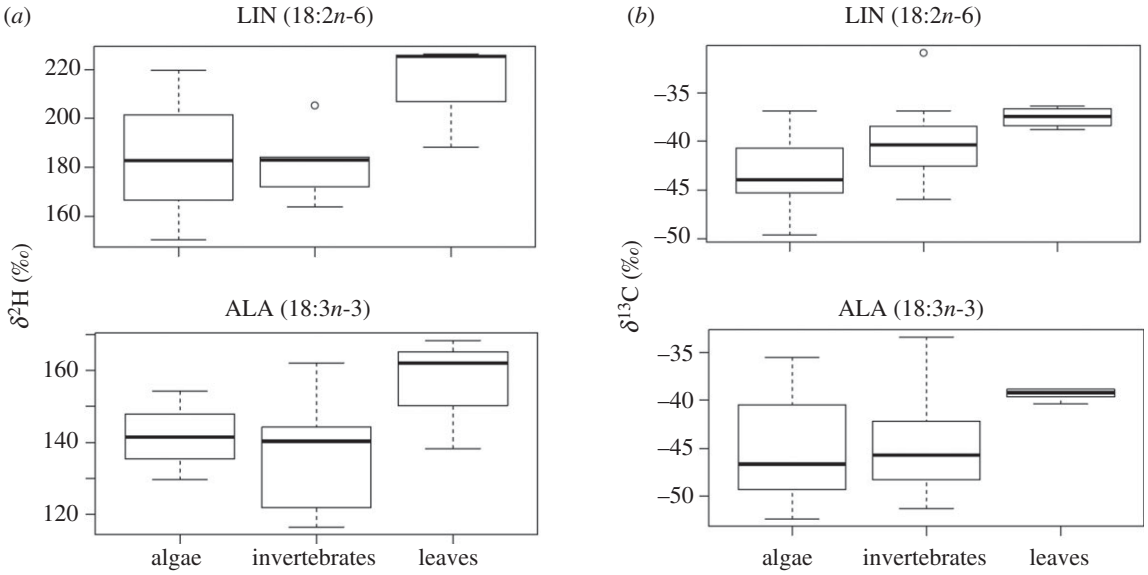

Figure 3. Stable isotope ratios of (*a*) hydrogen (δ^2H) and (*b*) carbon (δ^{13}C) of the essential omega-6 fatty acid linoleic acid (LIN) and the essential omega-3 fatty acid α-linolenic acid (ALA) (MJ Kainz, K Winter, M Pilecky 2020, unpublished data). The mean isotopic difference in δ^2H-LIN and δ^2H-ALA was approximately 40‰ and approximately 20‰ between consumers and submerged terrestrial leaves and algae, respectively; the mean isotopic difference in δ^{13}C-LIN and δ^{13}C-ALA was approximately 6‰ and approximately 5‰ between consumers and submerged terrestrial leaves and algae, respectively, in subalpine Austrian streams.

CSIA have mostly focused on identifying the δ^{13}C values of specific fatty acids (discussed above), whereas ecological research on stable hydrogen isotope ratios (δ^2H or δD) of fatty acids is still in its infancy. Previous studies using bulk hydrogen isotopes have demonstrated that ^{2}H values typically have larger isotopic separation between diet sources and consumers than the more traditional isotopes used in trophic ecology (i.e. δ^{13}C or δ^{15}N) as a result of the greater relative mass differences associated with such a light isotope (e.g. [4]). For example, in their classic study, Doucett *et al*. [120] found that autochthonous aquatic organic matter was approximately 100‰ more depleted in ^{2}H compared to allochthonous terrestrial organic matter. Doucett *et al*. [120] suggested that such large isotopic differences were likely a result of: (i) algal fractionation against ^{2}H during photosynthesis; (ii) isotopic enrichment in terrestrial leaves due to evaporation particularly in ecosystems with low relative humidity and stomatal conductance; and/or (iii) because lipids are depleted in ^{2}H, the higher lipid contents in algae compared to terrestrial leaves may cause more depleted δ^2H values in algae. More recently, Soto *et al*. [121] reported isotopic differences in bulk δ^2H of approximately 40‰ between aquatic and terrestrial plants, whereas differences in δ^{13}C values for the same samples were only approximately 2‰. Based on these enormous differences in δ^2H values between sources, it is tempting to prefer hydrogen over carbon isotopes to assess the retention of various diet sources in consumers. However, large differences in bulk hydrogen isotopes in consumers may also result from changes in hydrogen isotopes of environmental water that can consequently affect δ^2H values in consumers [4,122], including at the compound-specific level [123]. For example, Fogel *et al*. [123] found that δ^2H values of amino acids in *Escherichia coli* grown on different substrates reflected diet for essential amino acids and environmental water for non-essential or dispensable amino acids. Such risks must be taken into account when dealing with bulk hydrogen isotopes in trophic ecology and may be, to some degree, lower when using hydrogen isotopes of fatty acids.

Hydrogen stable isotopes of fatty acids are still rarely used as dietary source biomarkers, but hold clear advantages over more commonly used approaches like carbon stable isotope analyses of fatty acids. One of the advantages of compound-specific δ^2H analyses include the irreplaceable H–C bond of the fatty acyl chain. This means that once an H–C bond is formed, this link does not exchange and consequently δ^2H values should remain unchanged because fractionation occurs with the breaking or formation of bonds. Sessions *et al*. [124] reported that such H–C links can be changed over geologic time scales, but such time frames are less relevant for questions of current trophic ecology. Because both terrestrial plants and algae are able to synthesize ALA [125,126], terrestrial plant- or algal-derived ALA isotopic δ^2H values should remain unchanged during trophic transfer. Consumers grazing entirely on algae should have the same δ^2H values in their ALA as algal-ALA because very few consumers can synthesize ALA de novo. The same principle applies to LIN, the essential omega-6 PUFA, which can be synthesized in both terrestrial plants and algae. Results from recent field studies in subalpine stream catchments (MJ Kainz, K Winter, M Pilecky 2020, unpublished data) indicate an approximately 20‰ isotopic difference in the δ^2H values of ALA and an approximately 40‰ difference in those of LIN between terrestrial plants and algae collected in streams (figure 3), which is within the range of differences reported for bulk hydrogen isotopes from terrestrial and aquatic sources (see [121]). Assuming no trophic alternation of ALA or LIN in consumers, these data suggest that stream macroinvertebrates (here grazers and collectors) mostly rely on algae-derived ALA and LIN because the δ^2H values of ALA and LIN in the consumers are, on average, very closely related to δ^2H values of these PUFA in algae (figure 3*a*). When comparing these δ^2H values with δ^{13}C values of ALA and LIN in terrestrial plants and algae (figure 3*b*), a much smaller isotopic difference in δ^{13}C-ALA and δ^{13}C-LIN values between terrestrial plants and algae becomes evident (i.e. approx. 5 and 6‰ isotopic difference, respectively). In contrast with δ^2H-LIN, δ^{13}C-LIN values of

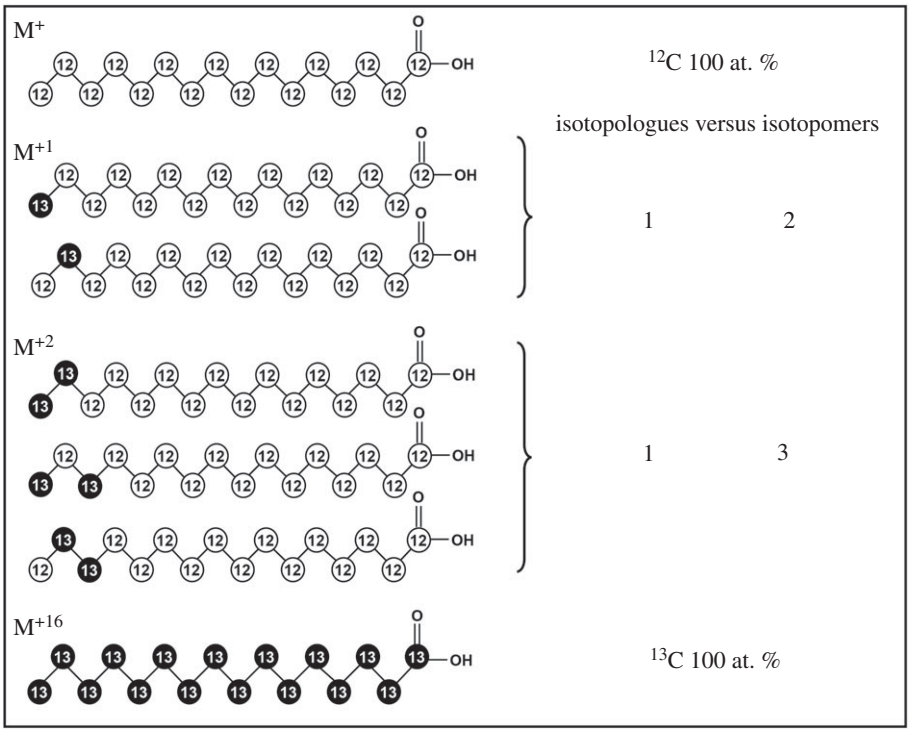

Figure 4. Combinations of possible isotopomers for ^{13}C mass isotopologues of palmitic acid (16:0). The molecule ion (M^+) and the mass isotopologues M^{+1}, M^{+2} and M^{+16} are presented as examples. The distribution pattern of labelled carbon (^{13}C) is shown using filled circles.

grazers and collectors are between the δ^{13}C-LIN values of algae and submerged terrestrial leaves, while the mean δ^{13}C values of ALA in these macroinvertebrates are more closely related to δ^{13}C-ALA values in algae, as in the case of δ^2H-ALA values. These initial results suggest that stable hydrogen isotopes for essential PUFA have the potential to provide a larger isotopic differentiation than carbon isotopes of fatty acids (see above), but more work is required to better understand changes in δ^2H values of fatty acids between potential diet sources and consumers.

Hydrogen stable isotopes of fatty acids are not without their limitations, chief among which are analytical novelty and a lack of understanding about variation in the δ^2H values of PUFAs and other fatty acids in organisms at various trophic levels. In addition, from an analytical point of view, the low atomic mass of hydrogen provides a challenge for analysis via GC-high temperature conversion-IRMS, in particular when the content of target fatty acids is low [127]. From a physiological point of view, it is fairly well understood that hydrogen fractionation occurs during lipid synthesis [128,129], resulting in isotopically lighter δ^2H values of lipids. However, isotopic variation in the δ^2H values of PUFAs is not well understood. This is because any conversion of fatty acids (e.g. from ALA to longer-chain and/or more unsaturated PUFA) may entail fractionation of hydrogen isotopes during the addition or removal of constituent hydrogen within the fatty acyl chain as is the case for carbon (e.g. [83,130]). For example, little is known about how δ^2H is processed by consumers, such as benthivorous stream fish [131], that derive DHA from precursor PUFAs rather than diet. The enzymatic processes required to convert precursors to DHA, often involving a series of elongation, desaturation and even retro-conversion (β-oxidation) steps, are likely to involve changes in δ^2H values between dietary PUFAs and DHA. Therefore, as compound-specific δ^2H analysis of fatty acids becomes established as a method, complementary research on consumer physiology for species of interest is also needed.

(b) Analyses of fatty acid isotopomers (position-specific) and isotopologues (mass-specific)

Intramolecular isotope pattern studies are emerging as another promising tool. Using enriched compounds as precursors or $^{13}CO_2$ pulse chase experiments, isotopic patterns within molecules can provide insights into resource fluxes in food webs (e.g. [132]) as well as into consumers' metabolic pathways [133,134]. Within a molecule's isotopic 'fine structure', isotopomers and isotopologues can be distinguished. Isotopomers are similar in isotopic composition, but differ in the position of isotopes within the molecule, while isotopologues are molecules that differ in their isotope composition (figure 4). Isotopomer measurements have been used to study pathways' kinetics and dynamics, such as the regulatory effects inherent in normal metabolism as well as disease [135,136] because position-specific stable isotope measurements enable researchers to precisely track molecular dynamics [137]. The ^{13}C profiles of metabolites are determined by nuclear magnetic resonance (NMR) spectroscopy or MS [135]. Researchers have typically used enriched substrates of glucose, pyruvate or acetate to investigate carbohydrate metabolism, and glycine to investigate amino acid metabolism. The pattern of labelling in isotopomers of these molecules can reveal enzyme activities and the nature of re-ordering reactions that direct metabolism [138]. For instance, ^{13}C-labelled pyruvate and glucose have been used to characterize the potential for bacteria to ferment sugars to ethanol [139]. A major challenge for expanding isotopomer analyses beyond medical research to ecological studies is that labelled standards with exact structures are not readily accessible at affordable prices. However, isotopomers have the potential to distinguish between dietary fatty acids that overlap in compound-specific isotopic values, but differ in position-specific values as a consequence of synthesis through different metabolic pathways. For instance, Scandellari *et al.* [140] supplied ectomycorrhizal fungi with six-position-specific

^{13}C-labelled isotopomers of glucose, resulting in a parametrized metabolic model of pathways in lipid biosynthesis. Using two labelled substrates, acetate and glycerol, Pollard *et al*. [141] performed an analyses of the biosynthetic fluxes and rates in lipid metabolism of *Camelina sativa* seeds. Overall, isotopomer labelling studies are a valuable tool to describe the distribution of isotopic tracers in metabolic networks [142], but have yet to be applied in studies of trophic ecology.

While isotopomer analysis is the preferred tool to investigate metabolic pathways, and thus trophic interactions, isotopologue profiling offers more information on carbon fluxes and dietary routing of metabolites [135]. Typical CSIA gives isotopic values for all of a certain molecule while isotopologue profiling additionally assigns where heavy atoms are located within the molecule (figure 4). Briefly, the carbon skeleton of a fatty acid is a mosaic of assembled building blocks derived from acetyl-CoA, and covalent linkages can be formed by ^{13}C-labelled or unlabelled ^{12}C fragments. By comparing the ^{13}C isotopologue profiles of metabolites from diet and consumers, relative resource use and metabolic pathways can be gleaned [143]. Thus, in contrast with isotopomer analysis, no position-specific labelled substrate is needed. Instead any perturbation of the natural isotope equilibrium (e.g. with a CO_2 pulse labelling) or a relatively inexpensive entire labelled resource (glucose, cellulose), which spreads in the experimental system, allows for analyses of a broad spectrum of metabolic dynamics [144]. This approach developed in microbiology, starting with studies on environmental degradation processes [145,146]. Ecological studies have used this approach to assess carbon flux pattern through soil microbial networks [147] or lipid transfer from plants to arbuscular mycorrhiza fungi [148]. For trophic interactions, isotopologue profiling has also been applied to quantify carbon flux via fatty acids in aquatic protists [149] and soil Collembola [72,150].

Isotopologue profiling is based on the labelling pattern or mass distribution vector, which describes the fractional abundances of all isotopologues with different masses in a molecule. For example, incorporation of ^{13}C from a labelled precursor into the molecular ion (M^+) leads to the formation of a series of isotopologues (i.e. M^{+1}, M^{+2}, etc.; [137,145]; figure 4). This provides information on the specific ^{13}C distribution (called isotopologe pattern or profile) in the labelled metabolite, as every different isotopic composition has a different mass ([144,150]; electronic supplementary material, table S2). Next, the total incorporation of ^{13}C can be calculated by the abundance ratio summation of all target isotopologues [132]. Consequently, isotopologue profiling delivers both ^{13}C position and flux, but does not distinguish between isotopomers. Quantitative NMR spectroscopy as well as GC-MS or liquid chromatography-MS are commonly used to determine the ^{13}C profiles of metabolites (e.g. to assign the use of host-derived glucose or amino acids in pathogenic bacteria), with MS favoured due to its higher sensitivity [143]. For metabolic networks, this method is particularly well established in amino acids [151], but can be applied to any labelled metabolite detectable by these analytical techniques, including fatty acids.

In their pioneering study, Menzel *et al*. [72] investigated the trophic transfer and fate of labelled palmitic acid (^{13}C16:0, 99 atomic %) in Collembola consumers. Based on these data, electronic supplementary material, table S2 presents the intramolecular ^{13}C distribution in dominant fatty acids. Comparable with the GC-C-IRMS analysis, isotopologue profiling assigned the dietary routing of the entire ^{13}C16:0 molecule into consumers. Additionally, isotopologue profiling revealed 16:0 desaturation to palmitic acid (16:1*n*-7) and chain elongation to stearic acid (18:0), both indicated by the high relative abundance of M^{+16} ([72]; see electronic supplementary material, table S2). Further, an increase in the abundance of M^{+2} demonstrated de novo synthesis of oleic acid (18:1*n*-9) and arachidonic acid (20:4*n*-6) from ^{13}C-acetyl-CoA. Menzel *et al*. [150] give a step-by-step protocol on how to perform isotopologue profiling with fatty acids using GC-MS. However, despite the high sensitivity of modern MS instruments, obtaining accurate ^{13}C patterns still remains a challenge, especially for long-chain fatty acids with low abundance of the molecular ion (M^+) and isotopologues (M^{+X}). Overall, isotopologue profiling allows researchers to determine: (i) the incorporation rate and percentage usage of any supplied ^{13}C-labelled carbon resource in the formation of consumer fatty acids (e.g. capability for de novo synthesis); (ii) the contribution of a fatty acid precursor to pathways leading to the formation of specific (e.g. essential) fatty acids; (iii) the metabolic transformations of a dietary routed fatty acid in consumer metabolism (e.g. chain elongation, desaturation); and (iv) the flux of C derived from a dietary routed fatty acid through intermediary reactions and pathways. Consequently, like GC-C-IRMS ^{13}C analysis of entire fatty acids, isotopologue profiling of fatty acids assigns the relative carbon flow from diet to consumer, but also provides additional detailed structural information on consumer lipid pathways.

5. Future research priorities: what we still want to know

There is much that we still want to know about compound-specific stable isotopes of fatty acids. In particular, there is enormous potential to contribute to the development of new techniques using CSIA to study trophic ecology. For example, future studies using stable hydrogen isotopes of fatty acids need to address basic questions including: (i) how variable are δ^2H values in fatty acids of difference sources over time and space; (ii) how 'stable' are δ^2H values in fatty acids from one trophic level to the next (i.e. trophic discrimination); and finally, (iii) how can analytical limitations in analyses of stable hydrogen isotopes in fatty acids be overcome? These same questions also remain open for isotopologues and present another exciting frontier for future studies. Position-specific stable isotope or isotopomer analyses have yet to be applied to studies of trophic ecology, yet they offer finer-scale resolutions than CSIA, such as when dietary fatty acids also overlap in compound-specific isotopic values for either δ^{13}C or δ^2H.

Nevertheless, many questions still remain for 'classic' CSIA of carbon from entire fatty acid molecules. In particular, the following are urgently needed: (i) more experimental studies across a diversity of taxa, life stages and habitats to quantify diet to tissue variation empirically; and (ii) more comprehensive studies evaluating how factors, such as diet, nutritional status, sex and age, influence the isotopic composition of individual fatty acids. The existing compound-specific stable isotope literature is heavily biased towards aquatic organisms (i.e. fish and zooplankton), soil microorganisms, and riparian or marine-associated birds (figure 2), while eco-physiological CSIA-based studies on wild terrestrial vertebrates, in particular mammals, amphibians and reptiles, as well as insects are scarce

or non-existent, except for humans. For example, CSIA-based studies on humans have revealed that females of reproductive age have higher ALA to EPA and DHA conversion capabilities compared to males [152,153] and that ALA to DHA conversion decreases with age in infants (e.g. as reviewed in [154]). However, it remains to be seen how representative these findings are across a diversity of taxa in the wild and how the effects of factors like age and sex compare to those like diet and nutritional status, which are heavily influenced by environmental conditions.

Moreover, the vast majority of ecological studies using CSIA of fatty acids are observational rather than empirical, making it difficult to isolate the impact of factors like dietary variation and providing little to no reliable information on processes like trophic discrimination. While bulk stable isotope research also suffers from many of these same deficits [75], the wide range of dietary fatty acids and synthesis capacities across consumers make it even more critical to understand the influence of these effects on compound-specific stable isotope patterns when using CSIA to study trophic ecology. We suggest that additional studies using labelled fatty acid diets coupled with CSIA will help researchers gain insights into the physiological black boxes of their study organisms. For example, while recent research shows that a diversity of invertebrates possess the genes necessary for de novo ALA synthesis [155], suggesting that ALA and LC-PUFA availability may not limit consumers to the degree once thought (e.g. [156]), these synthesis abilities have yet to be quantified empirically at the whole animal level. Together, experimental and observational approaches will allow researchers to test key assumptions like these about lipid metabolism that are critically important for informing future studies of complex food web dynamics.

Finally, two of the existing barriers to the increased use and development of CSIA are accessibility-based: costs and laboratory availability. As noted, CSIA is methodologically sensitive and time-consuming. CSIA requires specialized intricate instrumentation and highly trained personnel to run and troubleshoot said instrumentation and to process data. In addition, most labelling approaches are rather expensive and require a high minimum mass for analyses, making this type of work especially challenging for small-scale consumers. Together, these factors create a high barrier of entry for those seeking to enter the field or set up their own facilities. Furthermore, even for those who want to generate compound-specific isotopic data with collaborators, the small number of laboratories that run CSIA, especially those prepared to work with non-model organism samples from natural ecosystems, limits the potential for the CSIA field to expand further.

(a) Challenges and steps forward

In this review, we have shown that CSIA is a powerful tool for broadening our understanding of both food web ecology and nutritional requirements across a diversity of species. However, there is still considerable work to be done. First, we need to break traditional barriers across disciplines (e.g. human nutrition, biochemistry, microbiology and ecology) and subdisciplines (e.g. limnology, ornithology and soil ecology) in order to understand trophic interactions and metabolism at the scale of whole food webs. We also need to cross new methodological frontiers, such as routinely combining empirical eco-physiological studies with food web analyses, in order to achieve deeper insight in how dietary fatty acids move through consumers, within food webs and across ecosystems. Future studies spanning disciplines and combining methods can also help us to ask large-scale questions about the evolution of lipid metabolism, which remains largely unexplored in most wild species. For instance, combining CSIA-based eco-physiological studies on individual species with CSIA-based food web studies within a phylogenetic framework can help us to understand how trophic ecology and habitat have acted selectively upon traits related to fatty acid synthesis across consumers. Understanding how physiological requirements for fatty acids have evolved across consumers will help reveal which resources are most likely to be limiting to which consumers, ultimately informing future studies of trophic ecology.

Data accessibility. This manuscript includes preliminary data for a larger study that is currently in preparation for publication elsewhere.

Authors' contributions. C.W.T., S.J.T., L.R., A.B., D.M.-C. and M.J.K. conceived the ideas and wrote the manuscript; C.W.T. led the writing process and all authors contributed critically to the drafts and gave final approval for publication.

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References

1. Majdi N *et al.* 2018 There's no harm in having too much: a comprehensive toolbox of methods in trophic ecology. *Food Webs* **17**, e00100. (doi:10.1016/j.fooweb.2018.e00100)
2. Martínez del Rio C, Wolf N, Carleton SA, Gannes LZ. 2009 Isotopic ecology ten years after a call for more laboratory experiments. *Biol. Rev.* **84**, 91–111. (doi:10.1111/j.1469-185X.2008.00064.x)
3. DeNiro MJ, Epstein S. 1978 Influence of diet on the distribution of carbon isotopes in animals. *Geochim. Cosmochim. Acta* **42**, 495–506. (doi:10.1016/0016-7037(78)90199-0)
4. Macko SA, Estep ML, Lee WY. 1983 Stable hydrogen isotope analysis of foodwebs on laboratory and field populations of marine amphipods. *J. Exp. Mar. Biol. Ecol.* **72**, 243–249. (doi:10.1016/0022-0981(83)90109-0)
5. Deniro MJ, Epstein S. 1981 Influence of diet on the distribution of nitrogen isotopes in animals. *Geochim. Cosmochim. Acta* **45**, 341–351. (doi:10.1016/0016-7037(81)90244-1)
6. Hobson KA, Alisauskas RT, Clark RG. 1993 Stable-nitrogen isotope enrichment in avian tissues due to fasting and nutritional stress: implications for isotopic analyses of diet. *Condor* **95**, 388–394. (doi:10.2307/1369361)
7. Fry B. 2006 *Stable isotope ecology*. Berlin, Germany: Springer.
8. Kankaala P, Taipale S, Grey J, Sonninen E, Arvola L, Jones RI. 2006 Experimental d13C evidence for a contribution of methane to pelagic food webs in lakes. *Limnol. Oceanogr.* **51**, 2821–2827. (doi:10.4319/lo.2006.51.6.2821)
9. Jones RI, Grey J. 2011 Biogenic methane in freshwater food webs. *Freshwater Biol.*

56, 213–229. (doi:10.1111/j.1365-2427.2010.02494.x)

10. Hobson KA. 1999 Tracing origins and migration of wildlife using stable isotopes: a review. *Oecologia* **120**, 314–326. (doi:10.1007/s004420050865)
11. Phillips DL, Inger R, Bearhop S, Jackson AL, Moore JW, Parnell AC, Semmens BX, Ward EJ. 2014 Best practices for use of stable isotope mixing models in food-web studies. *Can. J. Zool.* **92**, 823–835. (doi:10.1139/cjz-2014-0127)
12. Brett MT. 2014 Resource polygon geometry predicts Bayesian stable isotope mixing model bias. *Mar. Ecol. Prog. Ser.* **514**, 1–12. (doi:10.3354/meps11017)
13. Brett MT, Eisenlord ME, Galloway AWE. 2016 Using multiple tracers and directly accounting for trophic modification improves dietary mixing-model performance. *Ecosphere* **7**, e01440. (doi:10.1002/ecs2.1440)
14. Stock BC, Jackson AL, Ward EJ, Parnell AC, Phillips DL, Semmens BX. 2018 Analyzing mixing systems using a new generation of Bayesian tracer mixing models. *Peer J.* **6**, e5096. (doi:10.7717/peerj.5096)
15. Iverson SJ. 2009 Tracing aquatic food webs using fatty acids: from qualitative indicators to quantitative determination. In *Lipids in aquatic ecosystems* (eds M Arts, M Brett, M Kainz), 281–308. London, UK: Springer.
16. Budge SM, Iverson SJ, Bowen WD, Ackman RG. 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**, 886–898. (doi:10.1139/f02-062)
17. Hixson SM, Sharma B, Kainz MJ, Wacker A, Arts MT. 2015 Production, distribution, and abundance of long-chain omega-3 polyunsaturated fatty acids: a fundamental dichotomy between freshwater and terrestrial ecosystems. *Environ. Rev.* **23**, 414–424. (doi:10.1139/er-2015-0029)
18. Twining CW, Brenna JT, Hairston NG, Flecker AS. 2016 Highly unsaturated fatty acids in nature: what we know and what we need to learn. *Oikos* **125**, 749–760. (doi:10.1111/oik.02910)
19. Koussoroplis A-M, Lemarchand C, Bec A, Desvilettes C, Amblard C, Fournier C, Berny P, Bourdier G. 2008 From aquatic to terrestrial food webs: decrease of the docosahexaenoic acid/linoleic acid ratio. *Lipids* **43**, 461–466. (doi:10.1007/s11745-008-3166-5)
20. Rivers JPW, Sinclair AJ, Crawford MA. 1975 Inability of the cat to desaturate essential fatty acids. *Nature* **258**, 171–173. (doi:10.1038/258171a0)
21. Goulden CE, Place AR. 1990 Fatty acid synthesis and accumulation rates in daphniids. *J. Exp. Zool.* **256**, 168-178. (doi:10.1002/jez.1402560207)
22. Sargent J, Bell G, McEvoy L, Tocher D, Estevez A. 1999 Recent developments in the essential fatty acid nutrition of fish. *Aquaculture* **177**, 191–199. (doi:10.1016/S0044-8486(99)00083-6)
23. Martin-Creuzburg D, Wacker A, Basen T. 2010 Interactions between limiting nutrients: consequences for somatic and population growth of *Daphnia magna*. *Limnol. Oceanogr.* **55**, 2597–2607. (doi:10.4319/lo.2010.55.6.2597)
24. Twining CW, Brenna JT, Lawrence P, Shipley JR, Tollefson TN, Winkler DW. 2016 Omega-3 long-chain polyunsaturated fatty acids support aerial insectivore performance more than food quantity. *Proc. Natl Acad. Sci. USA* **113**, 10 920–10 925. (doi:10.1073/pnas.1603998113)
25. Brett MT, Kainz MJ, Taipale SJ, Seshan H. 2009 Phytoplankton, not allochthonous carbon, sustains herbivorous zooplankton production. *Proc. Natl Acad. Sci. USA* **106**, 21 197–21 201. (doi:10.1073/pnas.0904129106)
26. Silfer JA, Engel MH, Macko SA, Jumeau EJ. 1991 Stable carbon isotope analysis of amino acid enantiomers by conventional isotope ratio mass spectrometry and combined gas chromatography/isotope ratio mass spectrometry. *Anal. Chem.* **63**, 370–374. (doi:10.1021/ac00004a014)
27. Whiteman JP, Smith EAE, Besser AC, Newsome SD. 2019 A guide to using compound-specific stable isotope analysis to study the fates of molecules in organisms and ecosystems. *Diversity* **11**, 18. (doi:10.3390/d11010008)
28. Chamberlain PM, Bull ID, Black HIJ, Ineson P, Evershed RP. 2004 Lipid content and carbon assimilation in Collembola: implications for the use of compound-specific carbon isotope analysis in animal dietary studies. *Oecologia* **139**, 325–335. (doi:10.1007/s00442-003-1422-1)
29. Gergs R, Steinberger N, Beck B, Basen T, Yohannes E, Schulz R, Martin-Creuzburg D. 2015 Compound-specific δ13C analyses reveal sterol metabolic constraints in an aquatic invertebrate. *Rapid Commun. Mass Spectrom.* **29**, 1789–1794. (doi:10.1002/rcm.7279)
30. Bec A, Perga ME, Koussoroplis A, Bardoux G, Desvilettes C, Bourdier G, Mariotti A. 2011 Assessing the reliability of fatty acid-specific stable isotope analysis for trophic studies. *Methods Ecol. Evol.* **2**, 651–659. (doi:10.1111/j.2041-210X.2011.00111.x)
31. Schwarcz HP. 1991 Some theoretical aspects of isotope paleodiet studies. *J. Archaeol. Sci.* **18**, 261–275. (doi:10.1016/0305-4403(91)90065-W)
32. Gannes LZ, O'Brien DM, del Rio CM. 1997 Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. *Ecology* **78**, 1271–1276. (doi:10.1890/0012-9658(1997)078[1271:SIIAEA]2.0.CO;2)
33. Rieley G, Van Dover C, Hedrick D, Eglinton G. 1999 Trophic ecology of *Rimicaris exoculata*: a combined lipid abundance/stable isotope approach. *Mar. Biol.* **133**, 495–499. (doi:10.1007/s002270050489)
34. Budge SM, Wooller MJ, Springer AM, Iverson SJ, McRoy CP, Divoky GJ. 2008 Tracing carbon flow in an arctic marine food web using fatty acid-stable isotope analysis. *Oecologia* **157**, 117–129. (doi:10.1007/s00442-008-1053-7)
35. Braeckman U, Provoost P, Sabbe K, Soetaert K, Middelburg JJ, Vincx M, Vanaverbeke J. 2012 Temporal dynamics in the diet of two marine polychaetes as inferred from fatty acid biomarkers. *J. Sea Res.* **68**, 6–19. (doi:10.1016/j.seares.2011.11.003)
36. Graham C, Oxtoby L, Wang SW, Budge SM, Wooller MJ. 2014 Sourcing fatty acids to juvenile polar cod (*Boreogadus saida*) in the Beaufort Sea using compound-specific stable carbon isotope analyses. *Polar Biol.* **37**, 697–705. (doi:10.1007/s00300-014-1470-5)
37. Taipale SJ, Vuorio K, Brett MT, Peltomaa E, Hiltunen M, Kankaala P. 2016 Lake zooplankton δ13C values are strongly correlated with the δ13C values of distinct phytoplankton taxa. *Ecosphere* **7**, e01392. (doi:10.1002/ecs2.1392)
38. Colombo S, Parrish C, Whiticar M. 2016 Fatty acid stable isotope signatures of molluscs exposed to finfish farming outputs. *Aquacult. Environ. Interact.* **8**, 611–617. (doi:10.3354/aei00202)
39. Twining CW, Brenna JT, Lawrence P, Winkler DW, Flecker AS, Hairston Jr NG. 2019 Aquatic and terrestrial resources are not nutritionally reciprocal for consumers. *Funct. Ecol.* **33**, 2042–2052. (doi:10.1111/1365-2435.13401)
40. Murphy DE, Abrajano Jr TA. 1994 Carbon isotope compositions of fatty acids in mussels from Newfoundland estuaries. *Estuarine Coastal Shelf Sci.* **39**, 261–272. (doi:10.1006/ecss.1994.1063)
41. Koussoroplis AM, Bec A, Perga ME, Koutrakis E, Desvilettes C, Bourdier G. 2010 Nutritional importance of minor dietary sources for leaping grey mullet *Liza saliens* (Mugilidae) during settlement: insights from fatty acid delta C-13 analysis. *Mar. Ecol. Prog. Ser.* **404**, 207–217. (doi:10.3354/meps08485)
42. Pond DW, Leakey RJG, Fallick AE. 2006 Monitoring microbial predator–prey interactions: an experimental study using fatty acid biomarker and compound-specific stable isotope techniques. *J. Plankton Res.* **28**, 419–427. (doi:10.1093/plankt/fbi130)
43. Lau DCP, Leung KMY, Dudgeon D. 2009 Are autochthonous foods more important than allochthonous resources to benthic consumers in tropical headwater streams? *J. North Am. Benthol. Soc.* **28**, 426–439. (doi:10.1899/07-079.1)
44. Budge SM, Wang SW, Hollmén TE, Wooller MJ. 2011 Carbon isotopic fractionation in eider adipose tissue varies with fatty acid structure: implications for trophic studies. *J. Exp. Biol.* **214**, 3790–3800. (doi:10.1242/jeb.057596)
45. Fujibayashi M, Sakamaki T, Shin W, Nishimura O. 2016 Food utilization of shell-attached algae contributes to the growth of host mud snail, *Bellamya chinensis*: evidence from fatty acid biomarkers and carbon stable isotope analysis. *Limnologica* **57**, 66–72. (doi:10.1016/j.limno.2015.11.006)
46. Taipale SJ, Kahilainen KK, Holtgrieve GW, Peltomaa ET. 2018 Simulated eutrophication and browning alters zooplankton nutritional quality and determines juvenile fish growth and survival. *Ecol. Evol.* **8**, 2671–2687. (doi:10.1002/ece3.3832)
47. Stott AW, Davies E, Evershed RP, Tuross N. 1997 Monitoring the routing of dietary and biosynthesised lipids through compound-specific stable isotope (δ13C) measurements at natural

abundance. *Naturwissenschaften* **84**, 82–86. (doi:10.1007/s001140050354)

48. Teece MA, Fogel ML, Dollhopf ME, Nealson KH. 1999 Isotopic fractionation associated with biosynthesis of fatty acids by a marine bacterium under oxic and anoxic conditions. *Org. Geochem.* **30**, 1571–1579. (doi:10.1016/S0146-6380(99)00108-4)
49. Carter WA, Whiteman JP, Cooper-Mullin C, Newsome SD, McWilliams SR. 2019 Dynamics of individual fatty acids in muscle fat stores and membranes of a songbird and its functional and ecological importance. *Physiol. Biochem. Zool.* **92**, 239–251. (doi:10.1086/702667)
50. Kohl L, Laganière J, Kate A, Sharon A, Penny L, Morrill P, Van Biesen G, Ziegler SE. 2015 Distinct fungal and bacterial d13C signatures as potential drivers of increasing d13C of soil organic matter with depth. *Biogeochemistry* **124**, 13–26. (doi:10.1007/s10533-015-0107-2)
51. Taipale SJ, Peltomaa E, Hiltunen M, Jones RI, Hahn MW, Biasi C, Brett MT. 2015 Inferring phytoplankton, terrestrial plant and bacteria bulk δ^{13}C values from compound specific analyses of lipids and fatty acids. *PLoS ONE* **10**, e0133974. (doi:10.1371/journal.pone.0133974)
52. Gaillard B, Meziane T, Tremblay R, Archambault P, Blicher ME, Chauvaud L, Rysgaard S, Olivier F. 2017 Food resources of the bivalve *Astarte elliptica* in a sub-Arctic fjord: a multi-biomarker approach. *Mar. Ecol. Prog. Ser.* **567**, 139–156. (doi:10.3354/meps12036)
53. Carter WA, Cooper-Mullin C, McWilliams SR. 2018 Turnover of muscle lipids and response to exercise differ between neutral and polar fractions in a model songbird, the zebra finch. *J. Exp. Biol.* **221**, jeb168823. (doi:10.1242/jeb.168823)
54. Carter W, Bauchinger U, McWilliams S. 2019 The importance of isotopic turnover for understanding key aspects of animal ecology and nutrition. *Diversity* **11**, 84. (doi:10.3390/d11050084)
55. Menzel R, Geweiler D, Sass A, Simsek D, Ruess L. 2018 Nematodes as important source for omega-3 long-chain fatty acids in the soil food web and the impact in nutrition for higher trophic levels. *Front. Ecol. Evol.* **6**, 96. (doi:10.3389/fevo.2018.00096)
56. Haubert D, Häggblom MM, Scheu S, Ruess L. 2008 Effects of temperature and life stage on the fatty acid composition of Collembola. *Eur. J. Soil Biol.* **44**, 213–219. (doi:10.1016/j.ejsobi.2007.09.003)
57. Werne JP, Baas M, Sinninghe Damsté JS. 2002 Molecular isotopic tracing of carbon flow and trophic relationships in a methane-supported benthic microbial community. *Limnol. Oceanogr.* **47**, 1694–1701. (doi:10.4319/lo.2002.47.6.1694)
58. Kankaala P, Taipale S, Li L, Jones RI. 2010 Diets of crustacean zooplankton, inferred from stable carbon and nitrogen isotope analyses, in lakes with varying allochthonous dissolved organic carbon content. *Aquat. Ecol.* **44**, 781–795. (doi:10.1007/s10452-010-9316-x)
59. Berggren M, Ziegler SE, St-Gelais NF, Beisner BE, del Giorgio PA. 2014 Contrasting patterns of allochthony among three major groups of crustacean zooplankton in boreal and temperate lakes. *Ecology* **95**, 1947–1959. (doi:10.1890/13-0615.1)
60. Taipale SJ, Hiltunen M, Vuorio K, Peltomaa E. 2016 Suitability of phytosterols alongside fatty acids as chemotaxonomic biomarkers for phytoplankton. *Front. Plant Sci.* **7**, 212. (doi:10.3389/fpls.2016.00212)
61. Boschker HTS, Middelburg JJ. 2002 Stable isotopes and biomarkers in microbial ecology. *FEMS Microbiol. Ecol.* **40**, 85–95. (doi:10.1111/j.1574-6941.2002.tb00940.x)
62. Pancost RD, Damsté JSS. 2003 Carbon isotopic compositions of prokaryotic lipids as tracers of carbon cycling in diverse settings. *Chem. Geol.* **195**, 29–58. (doi:10.1016/S0009-2541(02)00387-X)
63. Cowie BR, Slater GF, Bernier L, Warren LA. 2009 Carbon isotope fractionation in phospholipid fatty acid biomarkers of bacteria and fungi native to an acid mine drainage lake. *Org. Geochem.* **40**, 956–962. (doi:10.1016/j.orggeochem.2009.06.004)
64. Ben-David EA, Holden PJ, Stone DJM, Harch BD, Foster LJ. 2004 The use of phospholipid fatty acid analysis to measure impact of acid rock drainage on microbial communities in sediments. *Microb. Ecol.* **48**, 300–315. (doi:10.1007/s00248-003-1045-4)
65. Drerup SA, Vis ML. 2016 Responses of stream biofilm phospholipid fatty acid profiles to acid mine drainage impairment and remediation. *Water Air Soil Pollut.* **227**, 159. (doi:10.1007/s11270-016-2856-5)
66. Zelles L. 1999 Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. *Biol. Fertil. Soils* **29**, 111–129. (doi:10.1007/s003740050533)
67. Taipale S, Strandberg U, Peltomaa E, Galloway AW, Ojala A, Brett MT. 2013 Fatty acid composition as biomarkers of freshwater microalgae: analysis of 37 strains of microalgae in 22 genera and in seven classes. *Aquat. Microb. Ecol.* **71**, 165–178. (doi:10.3354/ame01671)
68. Willers C, Jansen van Rensburg P, Claassens S. 2015 Phospholipid fatty acid profiling of microbial communities—a review of interpretations and recent applications. *J. Appl. Microbiol.* **119**, 1207–1218. (doi:10.1111/jam.12902)
69. Pond CM. 1981 Storage. In *Physiological ecology: an evolutionary approach to resource use* (eds CR Townsend, P Calow). London, UK: Blackwell.
70. DeNiro MJ, Epstein S. 1977 Mechanism of carbon isotope fractionation associated with lipid synthesis. *Science* **197**, 261–263. (doi:10.1126/science.327543)
71. Chamberlain PM, Bull ID, Black HIJ, Ineson P, Evershed RP. 2006 The effect of diet on isotopic turnover in Collembola examined using the stable carbon isotopic compositions of lipids. *Soil Biol. Biochem.* **38**, 1146–1157. (doi:10.1016/j.soilbio.2005.09.015)
72. Menzel R, Ngosong C, Ruess L. 2017 Isotopologue profiling enables insights into dietary routing and metabolism of trophic biomarker fatty acids. *Chemoecology* **27**, 101–114. (doi:10.1007/s00049-017-0236-2)
73. Traugott M, Kamenova S, Ruess L, Seeber J, Plantegenest M. 2013 Empirically characterising trophic networks: what emerging DNA-based methods, stable isotope and fatty acid analyses can offer. *Adv. Ecol. Res.* **49**, 177–224. (doi:10.1016/B978-0-12-420002-9.00003-2)
74. Haubert D, Birkhofer K, Fließbach A, Gehre M, Scheu S, Ruess L. 2009 Trophic structure and major trophic links in conventional versus organic farming systems as indicated by carbon stable isotope ratios of fatty acids. *Oikos* **118**, 1579–1589. (doi:10.1111/j.1600-0706.2009.17587.x)
75. Wolf N, Carleton SA, del Rio CM. 2009 Ten years of experimental animal isotopic ecology. *Funct. Ecol.* **23**, 17–26. (doi:10.1111/j.1365-2435.2009.01529.x)
76. Howland MR, Corr LT, Young SM, Jones V, Jim S, Van Der Merwe NJ, Mitchell AD, Evershed RP. 2003 Expression of the dietary isotope signal in the compound-specific δ13C values of pig bone lipids and amino acids. *Int. J. Osteoarchaeol.* **13**, 54–65. (doi:10.1002/oa.658)
77. Katan T, Caballero-Solares A, Taylor RG, Rise ML, Parrish CC. 2019 Effect of plant-based diets with varying ratios of omega 6 to omega 3 fatty acids on growth performance, tissue composition, fatty acid biosynthesis and lipid-related gene expression in Atlantic salmon (*Salmo solar*). *Comp. Biochem. Physiol. D Genom. Proteom.* **30**, 290–304. (doi:10.1016/j.cbd.2019.03.004)
78. Lacombe RJS, Giuliano V, Colombo SM, Arts MT, Bazinet RP. 2017 Compound-specific isotope analysis resolves the dietary origin of docosahexaenoic acid in the mouse brain. *J. Lipid Res.* **58**, 2071–2081. (doi:10.1194/jlr.D077990)
79. Ruess L, Tiunov A, Haubert D, Richnow HH, Häggblom MM, Scheu S. 2005 Carbon stable isotope fractionation and trophic transfer of fatty acids in fungal based soil food chains. *Soil Biol. Biochem.* **37**, 945–953. (doi:10.1016/j.soilbio.2004.09.015)
80. Pond DW, Sargent JR, Fallick AE, Allen C, Bell MV, Dixon DR. 2000 δ13C values of lipids from phototrophic zone microplankton and bathypelagic shrimps at the Azores sector of the Mid-Atlantic Ridge. *Deep Sea Res. Part I* **47**, 121–136. (doi:10.1016/S0967-0637(99)00050-3)
81. Veefkind R. 2003 Carbon isotope ratios and composition of fatty acids: tags and trophic markers in pelagic organisms. PhD thesis, University of Victoria.
82. Bec A. 2003 Flux de matière dans le réseau trophique planctonique du lac d'Annecy: approche expérimentale et in situ par les acides gras marqueurs et leurs rapports isotopiques (δ13C). PhD thesis, University of Savoie Chambéry.
83. Gladyshev MI, Makhutova ON, Kravchuk ES, Anishchenko OV, Sushchik NN. 2016 Stable isotope fractionation of fatty acids of *Daphnia* fed laboratory cultures of microalgae. *Limnologica* **56**, 23–29. (doi:10.1016/j.limno.2015.12.001)
84. Pace ML *et al.* 2004 Whole-lake carbon-13 additions reveal terrestrial support of aquatic food webs. *Nature* **427**, 240–243. (doi:10.1038/nature02227)

85. Billings SA, Ziegler SE. 2005 Linking microbial activity and soil organic matter transformations in forest soils under elevated CO_2. *Glob. Change Biol.* **11**, 203–212. (doi:10.1111/j.1365-2486.2005.00909.x)
86. Radajewski S, Ineson P, Parekh NR, Murrell JC. 2000 Stable-isotope probing as a tool in microbial ecology. *Nature* **403**, 646. (doi:10.1038/35001054)
87. Radajewski S, McDonald IR, Murrell JC. 2003 Stable-isotope probing of nucleic acids: a window to the function of uncultured microorganisms. *Curr. Opin. Biotechnol.* **14**, 296–302. (doi:10.1016/S0958-1669(03)00064-8)
88. Manefield M, Whiteley AS, Griffiths RI, Bailey MJ. 2002 RNA stable isotope probing, a novel means of linking microbial community function to phylogeny. *Appl. Environ. Microbiol.* **68**, 5367–5373. (doi:10.1128/AEM.68.11.5367-5373.2002)
89. Uhlik O, Leewis M-C, Strejcek M, Musilova L, Mackova M, Leigh MB, Macek T. 2013 Stable isotope probing in the metagenomics era: a bridge towards improved bioremediation. *Biotechnol. Adv.* **31**, 154–165. (doi:10.1016/j.biotechadv.2012.09.003)
90. Carpenter SR. 2005 Eutrophication of aquatic ecosystems: bistability and soil phosphorus. *Proc. Natl Acad. Sci. USA* **102**, 10 002–10 005. (doi:10.1073/pnas.0503959102)
91. Taipale SJ, Kainz MJ, Brett MT. 2011 Diet-switching experiments show rapid accumulation and preferential retention of highly unsaturated fatty acids in *Daphnia*. *Oikos* **120**, 1674–1682. (doi:10.1111/j.1600-0706.2011.19415.x)
92. Streit K *et al.* 2014 Soil warming alters microbial substrate use in alpine soils. *Global Change Biol.* **20**, 1327–1338. (doi:10.1111/gcb.12396)
93. Scharnweber K, Syväranta J, Hilt S, Brauns M, Vanni MJ, Brothers S, Köhler J, Knežević-Jarić J, Mehner T. 2014 Whole-lake experiments reveal the fate of terrestrial particulate organic carbon in benthic food webs of shallow lakes. *Ecology* **95**, 1496–1505. (doi:10.1890/13-0390.1)
94. Pace ML *et al.* 2007 Does terrestrial organic carbon subsidize the planktonic food web in a clear-water lake? *Limnol. Oceanogr.* **52**, 2177–2189. (doi:10.4319/lo.2007.52.5.2177)
95. Lyon DR, Ziegler SE. 2009 Carbon cycling within epilithic biofilm communities across a nutrient gradient of headwater streams. *Limnol. Oceanogr.* **54**, 439–449. (doi:10.4319/lo.2009.54.2.0439)
96. Dijkman NA, Boschker HT, Middelburg JJ, Kromkamp JC. 2009 Group-specific primary production based on stable-isotope labeling of phospholipid-derived fatty acids. *Limnol. Oceanogr. Methods* **7**, 612–625. (doi:10.4319/lom.2009.7.612)
97. Wiegner TN, Kaplan LA, Ziegler SE, Findlay RH. 2015 Consumption of terrestrial dissolved organic carbon by stream microorganisms. *Aquat. Microb. Ecol.* **75**, 225–237. (doi:10.3354/ame01761)
98. Neufeld JD, Dumont MG, Vohra J, Murrell JC. 2007 Methodological considerations for the use of stable isotope probing in microbial ecology. *Microb. Ecol.* **53**, 435–442. (doi:10.1007/s00248-006-9125-x)
99. Ladygina N, Hedlund K. 2010 Plant species influence microbial diversity and carbon allocation in the rhizosphere. *Soil Biol. Biochem.* **42**, 162–168. (doi:10.1016/j.soilbio.2009.10.009)
100. Müller K, Kramer S, Haslwimmer H, Marhan S, Scheunemann N, Butenschön O, Scheu S, Kandeler E. 2016 Carbon transfer from maize roots and litter into bacteria and fungi depends on soil depth and time. *Soil Biol. Biochem.* **93**, 79–89. (doi:10.1016/j.soilbio.2015.10.015)
101. Poll J, Marhan S, Haase S, Hallmann J, Kandeler E, Ruess L. 2007 Low amounts of herbivory by root-knot nematodes affect microbial community dynamics and carbon allocation in the rhizosphere. *FEMS Microbiol. Ecol.* **62**, 268–279. (doi:10.1111/j.1574-6941.2007.00383.x)
102. Drigo B *et al.* 2010 Shifting carbon flow from roots into associated microbial communities in response to elevated atmospheric CO_2. *Proc. Natl Acad. Sci. USA* **107**, 10 938–10 942. (doi:10.1073/pnas.0912421107)
103. Tavi NM, Martikainen PJ, Lokko K, Kontro M, Wild B, Richter A, Biasi C. 2013 Linking microbial community structure and allocation of plant-derived carbon in an organic agricultural soil using $13CO_2$ pulse-chase labelling combined with 13C-PLFA profiling. *Soil Biol. Biochem.* **58**, 207–215. (doi:10.1016/j.soilbio.2012.11.013)
104. Maboreke HR, Graf M, Grams TEE, Herrmann S, Scheu S, Ruess L. 2017 Multitrophic interactions in the rhizosphere of a temperate forest tree affect plant carbon flow into the belowground food web. *Soil Biol. Biochem.* **115**, 526–536. (doi:10.1016/j.soilbio.2017.09.002)
105. Sommer J, Dippold MA, Zieger SL, Handke A, Scheu S, Kuzyakov Y. 2017 The tree species matters: belowground carbon input and utilization in the myco-rhizosphere. *Eur. J. Soil Biol.* **81**, 100–107. (doi:10.1016/j.ejsobi.2017.07.001)
106. Goodman KJ, Brenna JT. 1992 High sensitivity tracer detection using high-precision gas chromatography-combustion isotope ratio mass spectrometry and highly enriched uniformly carbon-13 labeled precursors. *Anal. Chem.* **64**, 1088–1095. (doi:10.1021/ac00034a004)
107. Bell LS, Cox G, Sealy J. 2001 Determining isotopic life history trajectories using bone density fractionation and stable isotope measurements: a new approach. *Am. J. Phys. Anthropol.* **116**, 66–79. (doi:10.1002/ajpa.1103)
108. Bell MV, Dick JR, Anderson TR, Pond DW. 2007 Application of liposome and stable isotope tracer techniques to study polyunsaturated fatty acid biosynthesis in marine zooplankton. *J. Plankton Res.* **29**, 417–422. (doi:10.1093/plankt/fbm025)
109. Malcicka M, Ruther J, Ellers J. 2017 De novo synthesis of linoleic acid in multiple Collembola species. *J. Chem. Ecol.* **43**, 911–919. (doi:10.1007/s10886-017-0878-0)
110. Twining CW, Lawrence P, Winkler DW, Flecker AS, Brenna JT. 2018 Conversion efficiency of α-linolenic acid to omega-3 highly unsaturated fatty acids in aerial insectivore chicks. *J. Exp. Biol.* **221**, jeb165373. (doi:10.1242/jeb.165373)
111. De Troch M, Boeckx P, Cnudde C, Van Gansbeke D, Vanreusel A, Vincx M, Caramujo MJ. 2012 Bioconversion of fatty acids at the basis of marine food webs: insights from a compound-specific stable isotope analysis. *Mar. Ecol. Prog. Ser.* **465**, 53–67. (doi:10.3354/meps09920)
112. Graeve M, Albers C, Kattner G. 2005 Assimilation and biosynthesis of lipids in Arctic *Calanus* species based on feeding experiments with a 13C labelled diatom. *J. Exp. Mar. Biol. Ecol.* **317**, 109–125. (doi:10.1016/j.jembe.2004.11.016)
113. Boissonnot L, Niehoff B, Hagen W, Søreide JE, Graeve M. 2016 Lipid turnover reflects life-cycle strategies of small-sized Arctic copepods. *J. Plankton Res.* **38**, 1420–1432. (doi:10.1093/plankt/fbw076)
114. Weers P, Siewertsen K, Gulati R. 1997 Is the fatty acid composition of *Daphnia galeata* determined by the fatty acid composition of the ingested diet? *Freshwater Biol.* **38**, 731–738. (doi:10.1046/j.1365-2427.1997.00238.x)
115. von Elert E. 2002 Determination of limiting polyunsaturated fatty acids in *Daphnia galeata* using a new method to enrich food algae with single fatty acids. *Limnol. Oceanogr.* **47**, 1764–1773. (doi:10.4319/lo.2002.47.6.1764)
116. Sessions AL. 2006 Isotope-ratio detection for gas chromatography. *J. Sep. Sci.* **29**, 1946–1961. (doi:10.1002/jssc.200600002)
117. Blessing M, Jochmann MA, Schmidt TC. 2008 Pitfalls in compound-specific isotope analysis of environmental samples. *Anal. Bioanal. Chem.* **390**, 591–603. (doi:10.1007/s00216-007-1588-1)
118. Jochmann MA, Schmidt TC. 2015 *Compound-specific stable isotope analysis*. Cambridge, UK: Royal Society of Chemistry.
119. Middelburg J, Barranguet C, Boschker H, Herman P, Moens T, Heip C. 2000 The fate of intertidal microphytobenthos carbon: an in situ 13C-labeling study. *Limnology and Oceanography.* **45**, 1224–1234. (doi:10.4319/lo.2000.45.6.1224)
120. Doucett RR, Marks JC, Blinn DW, Caron M, Hungate BA. 2007 Measuring terrestrial subsidies to aquatic food webs using stable isotopes of hydrogen. *Ecology* **88**, 1587–1592. (doi:10.1890/06-1184)
121. Soto DX, Decru E, Snoeks J, Verheyen E, Van de Walle L, Bamps J, Mambo T, Bouillon S. 2019 Terrestrial contributions to Afrotropical aquatic food webs: the Congo River case. *Ecol. Evol.* **9**, 10 746–10 757. (doi:10.1002/ece3.5594)
122. Solomon C, Cole J, Doucett R, Pace M, Preston N, Smith L, Weidel BC. 2009 The influence of environmental water on the hydrogen stable isotope ratio in aquatic consumers. *Oecologia* **161**, 313–324. (doi:10.1007/s00442-009-1370-5)
123. Fogel ML, Griffin PL, Newsome SD. 2016 Hydrogen isotopes in individual amino acids reflect differentiated pools of hydrogen from food and water in *Escherichia coli*. *Proc. Natl Acad. Sci. USA* **113**, E4648–E4653. (doi:10.1073/pnas.1525703113)

124. Sessions AL, Sylva SP, Summons RE, Hayes JM. 2004 Isotopic exchange of carbon-bound hydrogen over geologic timescales. *Geochim. Cosmochim. Acta* **68**, 1545–1559. (doi:10.1016/j.gca.2003.06.004)
125. Domergue F, Spiekermann P, Lerchl J, Beckmann C, Kilian O, Kroth PG, Boland W, Zähringer U, Heinz E. 2003 New insight into *Phaeodactylum tricornutum* fatty acid metabolism. Cloning and functional characterization of plastidial and microsomal delta12-fatty acid desaturases. *Plant Physiol.* **131**, 1648–1660. (doi:10.1104/pp.102.018317)
126. Guschina IA, Harwood JL. 2006 Lipids and lipid metabolism in eukaryotic algae. *Prog. Lipid Res.* **45**, 160–186. (doi:10.1016/j.plipres.2006.01.001)
127. Renpenning J, Kummel S, Hitzfeld KL, Schimmelmann A, Gehre M. 2015 Compound-specific hydrogen isotope analysis of heteroatom-bearing compounds via gas chromatography-chromium-based high-temperature conversion (Cr/HTC)-isotope ratio mass spectrometry. *Anal. Chem.* **87**, 9443–9450. (doi:10.1021/acs.analchem.5b02475)
128. Smith B, Ziegler H. 1990 Isotopic fractionation of hydrogen in plants. *Bot. Acta* **103**, 335–342. (doi:10.1111/j.1438-8677.1990.tb00171.x)
129. Liu J, Liu W, An Z, Yang H. 2016 Different hydrogen isotope fractionations during lipid formation in higher plants: implications for paleohydrology reconstruction at a global scale. *Sci. Rep.* **6**, 19711. (doi:10.1038/srep19711)
130. Gladyshev MI, Sushchik NN, Kalachova GS, Makhutova ON. 2012 Stable isotope composition of fatty acids in organisms of different trophic levels in the Yenisei River. *PLoS ONE* **7**, e34059. (doi:10.1371/journal.pone.0034059)
131. Guo F, Bunn SE, Brett MT, Fry B, Hager H, Ouyang X, Kainz MJ. 2018 Feeding strategies for the acquisition of high-quality food sources in stream macroinvertebrates: collecting, integrating, and mixed feeding. *Limnol. Oceanogr.* **63**, 1964–1978. (doi:10.1002/lno.10818)
132. Zhang X, He H, Amelung W. 2007 A GC/MS method for the assessment of 15N and 13C incorporation into soil amino acid enantiomers. *Soil Biol. Biochem.* **39**, 2785–2796. (doi:10.1016/j.soilbio.2007.06.001)
133. Tcherkez G, Mahé A, Hodges M. 2011 12C/13C fractionations in plant primary metabolism. *Trends Plant Sci.* **16**, 499–506. (doi:10.1016/j.tplants.2011.05.010)
134. Crown SB, Antoniewicz MR. 2013 Publishing 13C metabolic flux analysis studies: a review and future perspectives. *Metab. Eng.* **20**, 42–48. (doi:10.1016/j.ymben.2013.08.005)
135. Postle AD, Hunt AN. 2009 Dynamic lipidomics with stable isotope labelling. *J. Chromatogr. B* **877**, 2716–2721. (doi:10.1016/j.jchromb.2009.03.046)
136. Paglialunga S, Dehn CA. 2016 Clinical assessment of hepatic de novo lipogenesis in non-alcoholic fatty liver disease. *Lipids Health Dis.* **15**, 1–10. (doi:10.1186/s12944-016-0321-5)
137. Bacher A, Chen F, Eisenreich W. 2016 Decoding biosynthetic pathways in plants by pulse-chase strategies using $13CO_2$ as a universal tracer. *Metabolites* **6**, 21. (doi:10.3390/metabo6030021)
138. Kuchel PW, Philp DJ. 2008 Isotopomer subspaces as indicators of metabolic-pathway structure. *J. Theor. Biol.* **252**, 391–401. (doi:10.1016/j.jtbi.2007.05.039)
139. Feng X *et al.* 2009 Characterization of the central metabolic pathways in *Thermoanaerobacter* sp. strain X514 via isotopomer-assisted metabolite analysis. *Appl. Environ. Microbiol.* **75**, 5001–5008. (doi:10.1128/AEM.00715-09)
140. Scandellari F, Hobbie EA, Ouimette AP, Stucker VK. 2009 Tracing metabolic pathways of lipid biosynthesis in ectomycorrhizal fungi from position-specific 13C-labelling in glucose. *Environ. Microbiol.* **11**, 3087–3095. (doi:10.1111/j.1462-2920.2009.02013.x)
141. Pollard M, Delamarter D, Martin TM, Shachar-Hill Y. 2015 Lipid labeling from acetate or glycerol in cultured embryos of *Camelina sativa* seeds: a tale of two substrates. *Phytochemistry* **118**, 192–203. (doi:10.1016/j.phytochem.2015.07.021)
142. Wiechert W, Nöh K, Weitzel M. 2013 Metabolic isotopomer labeling systems. Part III: Path tracing. *Math. Biosci.* **244**, 1–12. (doi:10.1016/j.mbs.2013.02.012)
143. Eisenreich W, Dandekar T, Heesemann J, Goebel W. 2010 Carbon metabolism of intracellular bacterial pathogens and possible links to virulence. *Nat. Rev. Microbiol.* **8**, 401. (doi:10.1038/nrmicro2351)
144. Fuchs TM, Eisenreich W, Kern T, Dandekar T. 2012 Toward a systemic understanding of listeria monocytogenes metabolism during infection. *Front. Microbiol.* **3**, 23. (doi:10.3389/fmicb.2012.00023)
145. Fang J, Lovanh N, Alvarez PJJ. 2004 The use of isotopic and lipid analysis techniques linking toluene degradation to specific microorganisms: applications and limitations. *Water Res.* **38**, 2529–2536. (doi:10.1016/j.watres.2004.03.006)
146. Jehmlich N, Schmidt F, Von Bergen M, Richnow H-H, Vogt C. 2008 Protein-based stable isotope probing (Protein-SIP) reveals active species within anoxic mixed cultures. *ISME J.* **2**, 1122. (doi:10.1038/ismej.2008.64)
147. Dijkstra P, Dalder JJ, Selmants PC, Hart SC, Koch GW, Schwartz E, Hungate BA. 2011 Modeling soil metabolic processes using isotopologue pairs of position-specific 13C-labeled glucose and pyruvate. *Soil Biol. Biochem.* **43**, 1848–1857. (doi:10.1016/j.soilbio.2011.05.001)
148. Keymer A *et al.* 2017 Lipid transfer from plants to arbuscular mycorrhiza fungi. *eLife* **6**, e29107. (doi:10.7554/eLife.29107)
149. Kuppardt S, Chatzinotas A, Kästner M. 2010 Development of a fatty acid and RNA stable isotope probing-based method for tracking protist grazing on bacteria in wastewater. *Appl. Environ. Microbiol.* **76**, 8222–8230. (doi:10.1128/AEM.01632-10)
150. Menzel R, Nehring R, Simsek D, Ruess L. 2018 Fatty acid 13C isotopologue profiling provides insight into trophic carbon transfer and lipid metabolism of invertebrate consumers. *J. Vis. Exp.* **134**, e57110. (doi:10.3791/57110)
151. Sauer U. 2006 Metabolic networks in motion: 13C-based flux analysis. *Mol. Syst. Biol.* **2**, 62. (doi:10.1038/msb4100109)
152. Burdge GC, Jones AE, Wootton SA. 2002 Eicosapentaenoic and docosapentaenoic acids are the principal products of alpha-linolenic acid metabolism in young men. *Br. J. Nutr.* **88**, 355–363. (doi:10.1079/BJN2002662)
153. Burdge GC, Wootton SA. 2002 Conversion of alpha-linolenic acid to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women. *Br. J. Nutr.* **88**, 411–420. (doi:10.1079/BJN2002689)
154. Brenna JT, Salem Jr N, Sinclair AJ, Cunnane SC. 2009 α-Linolenic acid supplementation and conversion to n-3 long-chain polyunsaturated fatty acids in humans. *Prostaglandins Leukot. Essent. Fatty Acids* **80**, 85–91. (doi:10.1016/j.plefa.2009.01.004)
155. Kabeya N, Fonseca MM, Ferrier DEK, Navarro JC, Bay LK, Francis DS, Tocher DR, Castro LFC, Monroig Ó. 2018 Genes for de novo biosynthesis of omega-3 polyunsaturated fatty acids are widespread in animals. *Sci. Adv.* **4**, eaar6849. (doi:10.1126/sciadv.aar6849)
156. Ruess L, Mueller-Navarra D. 2019 Essential biomolecules in food webs. *Front. Ecol. Evol.* **2019**. (doi:10.3389/fevo.2019.00269)