PHILOSOPHICAL TRANSACTIONS B

royalsocietypublishing.org/journal/rstb

Introduction

Cite this article: Galloway AWE, Budge SM. 2020 The critical importance of experimentation in biomarker-based trophic ecology. *Phil. Trans. R. Soc. B* **375**: 20190638. http://dx.doi.org/10.1098/rstb.2019.0638

Accepted: 19 May 2020

One contribution of 16 to a theme issue 'The next horizons for lipids as 'trophic biomarkers': evidence and significance of consumer modification of dietary fatty acids'.

Subject Areas:
ecology, developmental biology, environmental science, evolution, genetics, physiology

Keywords:
fatty acids, biomarkers, trophic tracers, trophic ecology, feeding experiments

Authors for correspondence:
Aaron W. E. Galloway
e-mail: agallow3@uoregon.edu
Suzanne M. Budge
e-mail: suzanne.budge@dal.ca

THE ROYAL SOCIETY PUBLISHING

The critical importance of experimentation in biomarker-based trophic ecology

Aaron W. E. Galloway[1] and Suzanne M. Budge[2]

[1]Department of Biology, Oregon Institute of Marine Biology, University of Oregon, Charleston, OR, USA
[2]Department of Process Engineering and Applied Science, Dalhousie University, Halifax, NS, Canada

AWEG, 0000-0001-7826-299X; SMB, 0000-0003-4984-7344

Fatty acids are commonly used as biomarkers for making inferences about trophic relationships in aquatic and soil food webs. However, researchers are often unaware of the physiological constraints within organisms on the trophic transfer and modification of dietary biomarkers in consumers. Fatty acids are bioactive molecules, which have diverse structures and functions that both complicate and enhance their value as trophic tracers. For instance, consumers may synthesize confounding non-dietary sourced markers from precursor molecules, and environmental conditions also affect fatty acid composition. There is a vital need for more research on the uptake and transfer of trophic biomarkers in individual organisms in order to advance the field and make meaningful use of these tools at the scale of populations or ecosystems. This special issue is focused on controlled feeding experiments on a diverse taxonomic breadth of model consumers from freshwater, marine and soil ecosystems with a goal of creating a more integrated understanding of the connection between consumer physiology and trophic ecology.

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

All heterotrophs contain fatty acids with diverse structures; some can be synthesized de novo but most are acquired through diet. Fatty acids can therefore be applied as trophic tracers or biomarkers to make inferences about the relative importance of different resources in aquatic food webs [1–3] (figure 1*a*,*b*). This tracer technique has developed largely along two lines, in inferring resource use; in basal food webs, questions have focused on identification of sources of primary production that support primary and secondary consumers, while in apex food webs, the interest has been in resource assimilation of predators.

Regardless of the trophic positioning of the consumers, there are three general types of research (reviewed in [3]): (i) qualitative–comparative (comparing across taxa, habitats, conditions); (ii) qualitative biomarker (inference of resource using unique biomarkers of those resources); and (iii) quantitative inference (calculating consumer diets). The qualitative approaches are based on interpreting the presence of fatty acid biomarkers in consumers as evidence for trophic support to the consumer [1,4]. The quantitative approach seeks to estimate actual proportional contribution of different resources to consumers using maths and experimentation [2,5].

The basic premise of all fatty acid trophic tracer approaches is that unique fatty acid biomarkers (depicted as red, yellow and blue coloured tracers for each algal producer in figure 1*b*) can be traced into the consumers in a food web. The approach is strongest when one can assume that transfer of the tracer signal is conserved across trophic levels [1] and requires that prey are sufficiently distinct in their biomarker signature to allow subsequent interpretation [6]. However, relatively little is known about the trophic modification of fatty acids (figure 2) and

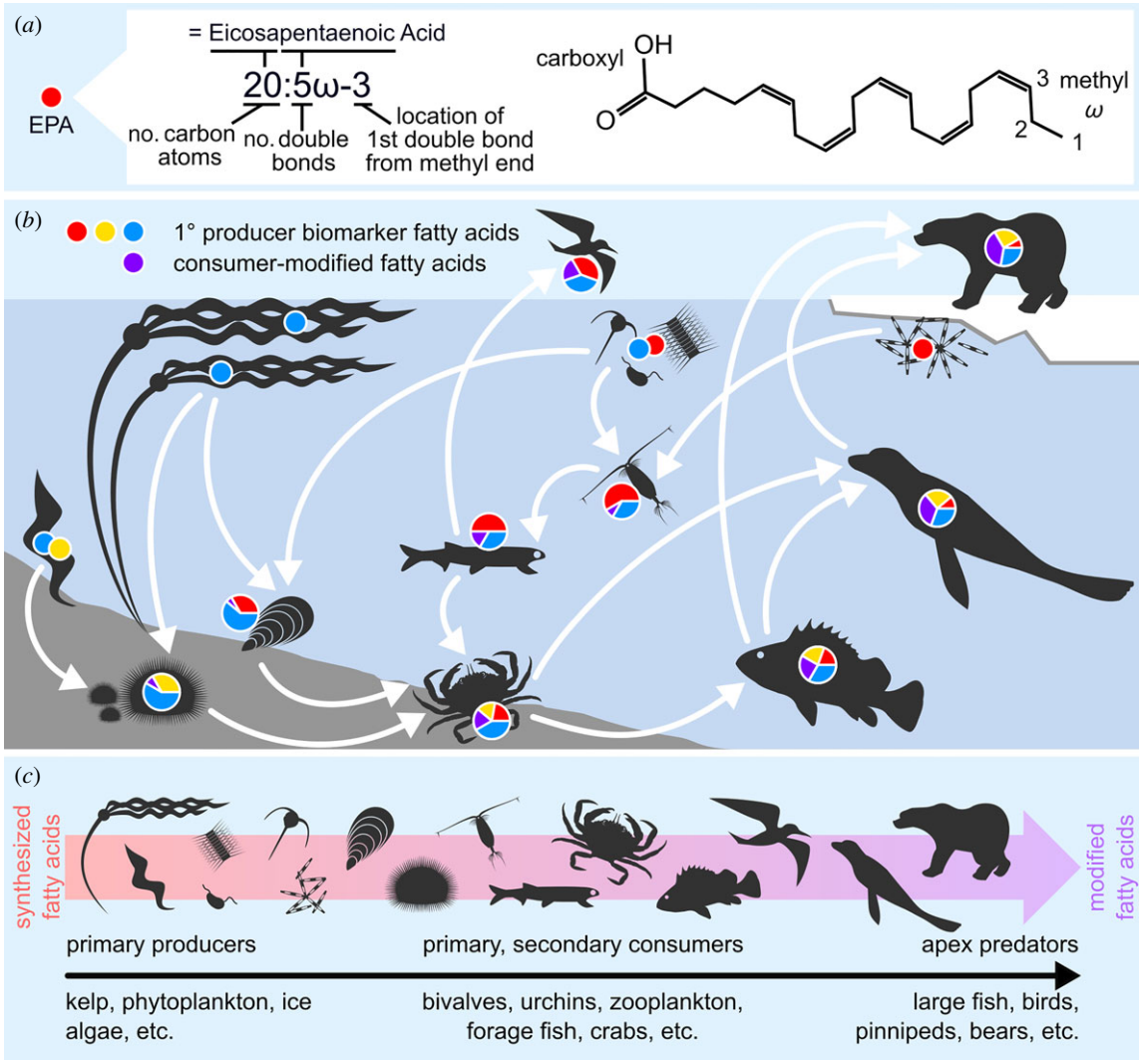

Figure 1. Fatty acids as trophic tracers or 'biomarkers'. (*a*) Structure of a polyunsaturated fatty acid (eicosapentaenoic acid; EPA). Researchers commonly use a version of either '*ω-x*' or '*n-x*', where x is the location of the first double bond from the methyl end of the molecule. (*b*) A cartoon of the path that producer-synthesized fatty acids may follow through a food web, where consumers are mixtures of proportions of biomarkers (pie charts). The cartoon only shows four tracers but actual producers and consumers may have up to 70 different fatty acids. (*c*) Continuum of algae-synthesized to highly modified fatty acids. Animals have differing, usually unknown, abilities to synthesize certain fatty acids de novo, resulting in higher predators having more derived, highly modified fatty acid profiles relative to basal consumers. Artwork by R. M. Yoshioka, commissioned by the authors. (Online version in colour.)

underlying consumer lipid metabolism or physiology of most organisms. Accounting for lipid trophic modification in consumers through project-specific feeding trials [5] or by measurement and estimation of general dietary 'calibration coefficients' [7, this issue; 8] is critical for quantitative biomarker applications [2,9]. However, understanding consumer trophic modification of their dietary fatty acids is relevant for all fatty acid biomarker applications [3].

Organisms may incorporate dietary sourced fatty acids into their tissues relatively unchanged, but they may also synthesize non-dietary sourced markers (e.g. purple tracer in figures 1 and 2) from precursor molecules. Fatty acids are extremely diverse in their structure and function (reviewed in [1,3,9]); some are important for structural and physiological needs, and others may be destined to be catabolized to meet energy demands [10,11]. While there are similarities in these needs among different heterotrophs, certain fatty acids are accumulated to a greater extent in some consumers than others. For example, docosahexaenoic acid (DHA; 22:6*ω*-3) appears to be selectively retained and critical for the survival of many copepods but is not retained or limiting for *Daphnia*, a cladoceran (reviewed in [12]).

2. A need for more experimentation on organism fatty acid metabolism

The theme of this special issue is experimental research and synthesis aimed at understanding the transfer and integration of dietary fatty acids into the tissues of consumers (figure 2). There is growing interest in the use of the trophic biomarker approach cartooned in figure 1 to make inferences about resources that are supporting specific consumers or populations. Fatty acids are very well poised to complement other long-used techniques for understanding the trophic ecology of wild consumers, which include direct observation of foraging, and analyses of stomach contents and stable isotopes [13]. However, feeding experiments are necessary to advance the field and make meaningful use of these tools at the scale of populations or ecosystems [14].

Fatty acids are a particularly appealing form of trophic tracer because of their diverse composition; organism fatty acid profiles often consist of 25–70 unique molecules [9]. Moreover, unlike stable isotope values, the multivariate fatty acid profiles of plants and animals are generally quite distinct, owing to differences in phylogeny and diet

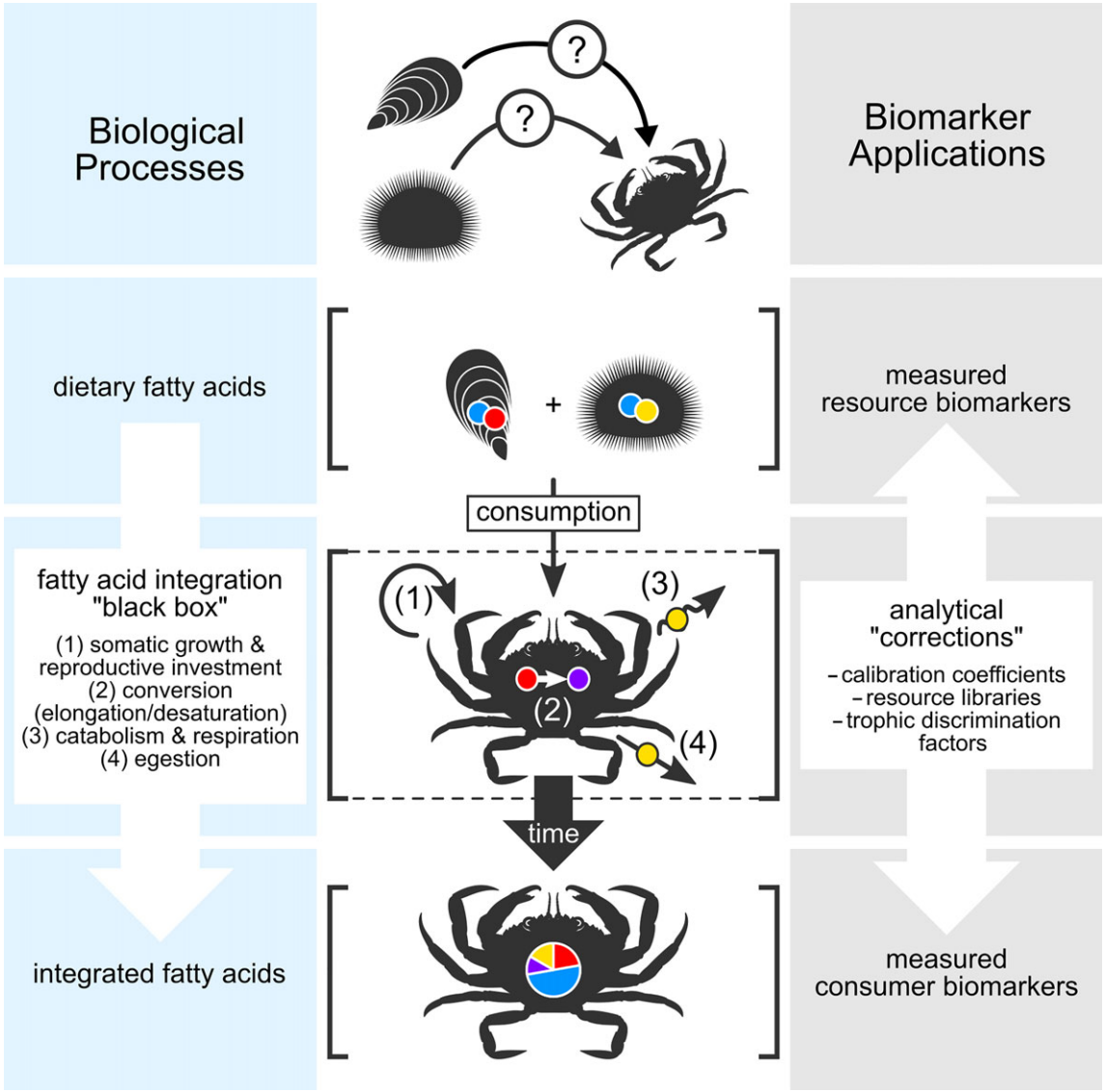

Figure 2. Biological processes (left column) and biomarker applications (right column) for an example consumer crab (centre column). In the cartoon, certain dietary fatty acids are preferentially retained (e.g. blue tracers) while others may be more likely to be converted (red to purple) or lost through crab respiration or catabolism. Feeding trials are necessary in order to interpret the integrated and measured consumer tracer fatty acids (bottom row). Artwork by R. M. Yoshioka, commissioned by the authors. (Online version in colour.)

[15–17]. However, this strength is also a key limitation; because fatty acids have diverse structures and functions, they are bioactive molecules. Much of the research using the fatty acid biomarker approach has only superficial acknowledgement of the uncertainty associated with the trophic transfer and storage of these tracers in consumers. Even when researchers have attempted to account for trophic modification in organism fatty acid profiles based on feeding trials designed to measure those modifications, as a field we have yet to understand the influence of ontogeny, physiological stress, competition, tissue turnover time and environmental factors such as temperature on biomarker trophic transfer and storage in consumers. Thus, we feel that advances of the approach have been limited by two major issues: (i) ecologists wishing to use this tool have not often appreciated the physiological limitations and issues underlying the utility of these biomarkers for tracing food webs and (ii) physiologists working on lipid metabolism within specific organisms have not generally made their work relevant for ecologists. The field seems primed for a more integrated understanding of the connection between consumer physiology and trophic ecology.

Recent genetic analyses have suggested that the extent that consumers are able to modify dietary fatty acids or synthesize new fatty acids is far greater than previously thought [18]. For instance, Kabeya *et al*. [19, this issue] demonstrate in a model polychaete consumer the approach of screening for genes that code for specific desaturases and elongases that enable polyunsaturated fatty acid (PUFA) synthesis. One might expect that the empirical studies we call for here may not be necessary with these recent advances in our understanding of the biochemical processes that organisms are capable of, given their genetics. However, such genetic screening is raising even more questions about the extent that organisms actually do biosynthesize fatty acids and does not supplant the need for lab experiments. Phenotypic expression is dependent on both genotype and environment, so it is not surprising to find that PUFA synthesis varies with dietary history [20, this issue] but not necessarily in ways that are anticipated based on genetic composition [21, this issue]. Importantly, when genetic analyses are paired with empirical feeding studies, it is possible to link demonstrated synthesis of particular fatty acids with upregulation of specific genes

[22, this issue]. Thus, the critical question is: does possession of the appropriate genes result in synthesis of biologically relevant amounts of long chain PUFA?

3. Key areas for future research

In this special issue, our focus is on experiments to study fatty acid trophic transfer and modification in organisms. Our primary aim is to convey the need to better understand physiological changes that consumers make to dietary lipids before application of biomarker approaches. We hope this special issue will increase efforts to conduct feeding trials that are designed to measure fatty acid trophic transfer and integration in diverse heterotrophs. While our focus is solely on fatty acids, many of these issues apply to other biomarkers that are in use, including stable isotope values of amino acids and other compounds. Robust application of those biomarkers faces the same difficulties as fatty acids, with a lack of information concerning trophic modification, and most studies limited to qualitative applications; we anticipate this special issue will highlight relevant issues for all biomarker fields.

Ultimately, experimental work is required for the development and refinement of biomarker models that can accurately estimate animal diets. Moreover, while fatty acids have been in use for many decades to infer trophic ecology [23–26], they are rarely used to determine predator–prey interactions which form the basis of ecosystem models. Indeed, a recent review describing potential improvements to ecosystem models cites the lack of information concerning consumer fatty acid metabolism as a major limitation in their inclusion [14]. Below we describe a number of key considerations when designing and interpreting empirical feeding studies with a goal of applying fatty acids in a quantitative manner to investigate consumer ecology. This does not represent an exhaustive list but, rather, provides a starting point for experimental design, drawing on examples of studies from this special issue and other areas.

(a) Matching composition of experimental and natural diets

It has been shown for both pinnipeds [27] and fish [28] that consumer assimilation and modification of dietary fatty acids varies according to the food consumed. Several articles in the present issue have further demonstrated diet specificity of fatty acid trophic assimilation in new model consumers [29–31]. In addition, Budge *et al*. [32, this issue] also demonstrate that the fat content of the diet affects consumer assimilation of dietary fatty acids. Thus, it is critical for both the proximate composition and fatty acid profile of experimental foods to be similar to that of natural diets to make realistic inferences about wild consumers that are informed from experimental feeding trials. With primary consumers, it is relatively easy to provide fresh, single species of phytoplankton or macroalgae as foods that are likely representative of nature since many short-lived primary consumers feed on temporally limited algal blooms. In this special issue, numerous authors employed this approach and focused on feeding pure algal diets [20,21,29,30,33,34]; with their springtail experiments Kühn *et al*. [35, this issue] were also able to offer mixed dried diets of bacteria, microalgae, fungi and plants.

At intermediate trophic levels, it can be difficult to meet the nutritional requirements of consumers by feeding single species, particularly for longer-duration experiments, since such predators are rarely so specialized [28,31]. With finfish and similar species that are raised for aquaculture, formulated feeds, tailored to satisfy nutritional requirements, can be employed [36]. With such an approach, the fatty acid composition of the diet can be carefully controlled to represent both single prey items (to establish extent of predator modification) and diet mixtures (to test mixing model performance), similar to Kühn's approach [35] with dried diets for springtails. However, feeds for aquaculture are usually designed to maximize growth and minimize cost of ingredients so they are typically higher in fat and lower in protein than encountered naturally [37].

Application of mixed and artificial types of diets in captive feeding experiments can lead to false conclusions concerning predator metabolism of dietary fatty acids [32, this issue] and care must be taken to ensure a realistic diet composition (e.g. [38]). In the case of Jardine *et al*. [7, this issue] the majority of the studies that were included in the synthesis took place in an aquaculture setting, with formulated diets that are likely to have been optimized for the particular consumer. A strength of that study is the large sample size of studies that contributed to the conclusions, but the generality of the patterns shown in Jardine *et al*. to consumers in the wild, which may have less optimal resources available, is still unknown. With top predators, such as pinnipeds, which are routinely held in captivity, feeding of single prey items is normal practice and does not seem to introduce nutritional deficiencies, leading to a wealth of data concerning the assimilation of dietary fatty acids by pinnipeds [2,27,39].

(b) Experimental duration

Intervention experiments must be conducted for an appropriately long period so that a consumer's tissues are sufficiently acclimated to the new food. However, because these types of experiments are often expensive and require close attention, competing pressures may cause researchers to terminate feeding studies too soon. Incorporation of dietary fatty acids follows a dilution model in finfish [40] so that fish can be fed until their mass increases two- or threefold to ensure the new food is fully incorporated (e.g. [32, this issue]). In small, short-lived species, particularly arthropods, a similar approach could be used, allowing the animals to progress through several growth stages or moults to ensure that a substantial mass gain has occurred. As with finfish, it can also be feasible to monitor a change in total mass [33]. A doubling or tripling in size is a rough indicator used for initial experiments with previously unstudied macroinvertebrates [6]; however, we note that in the six–eight week experiments with juvenile crabs by Thomas *et al*. [31, this issue], crabs that did not moult or grow appreciably still incorporated and reflected the fatty acid profiles of their different diets.

With large adult predators, such as pinnipeds and birds, where a dilution model cannot be applied, an alternative strategy is to compare fatty acid intake during the study with consumer fat stores. In the unlikely scenario where a predator's stored fatty acids would be exchanged or turned-over in a 1 : 1 relationship with consumed fatty acids, an experiment would have to proceed, at least, until the predator had consumed the same mass of fatty acids in its food as in its fat stores at the initiation of the experiment. Since selective mobilization and retention are known to occur in birds and mammals [41,42], it

would be prudent to allow experiments to proceed for a somewhat longer duration; however, there exists little literature to help determine the minimum duration required. Assessment of fatty acid intake relative to existing consumer fat stores is particularly important when a consumer fat depot is being sampled after consumption of a relatively low-fat diet.

A definitive answer to the question of feeding study duration is only possible through periodic sampling and determination of fatty acid profile of whole consumer or consumer tissue (e.g. [11]); unfortunately, such collection is often impractical because of fatal sampling or limitations on repeated sampling imposed by animal care requirements. The reliability of inferences about an organism's lipid metabolism derived from a feeding study depends on the sufficient assimilation of fatty acids in the field; certainly, greater attention should be given to this aspect in planning interventions. In the absence of empirical evidence of tissue turnover time with time-series sampling, we (Galloway and Budge) generally start with what we expect are 'long' experiments on individuals, before 'backing down' into shorter-duration trials. It is not possible to offer a prescriptive minimum feeding trial duration owing to the wide differences in growth rates among organisms, but we caution strongly against experiments that are too short for the consumer to integrate dietary biomarker signals.

(c) Tissue selection

While it is beyond the scope of this article to thoroughly review the analyst's choice of tissues to sample, we do highlight that the meaningful duration of a feeding trial is also dependent on the consumer tissue analysed. For example, while urchins are very long lived (e.g. more than 100 years) and slow growing, they can build gonads in a matter of weeks when conditions are favourable, and the fatty acids of these gonads are strongly influenced by their different macroalgal diets [43]. For very small consumers such as zooplankton it is often necessary to pool the whole bodies of individuals for each 'replicate' sample in order to meet the minimum biomass requirements for the fatty acid extraction technique being used by the analyst. For larger invertebrates, fishes, birds and mammals, it is common to target specific tissue types for biomarker analysis. Tissues to examine depend on the question, and usually the appropriate choice is determined by whether the analyst is examining the sample for its role in the food web (figure 1) as a predator or a prey [9]. If the sample is to characterize trophic ecology of a predator, it has been argued that a metabolically active energy storage tissue in the consumer should be sampled [9]; if the sample is for characterizing fatty acid signatures in prey it makes sense to use the homogenized, whole body of the organism as it would be consumed by the predator [15]. In practice, the most common tissue that analysts evaluate in individual organisms is muscle, fat, gonad, or liver (extensively reviewed in [3,9]). Because the different tissue types of a given organism are known to differ in their fatty acid compositions, for comparative studies across species, it is ideal to standardize tissue type in different consumers [44].

(d) Legacy fatty acids

'Legacy' fatty acid profiles are laid down in the consumer or its parents in the wild or while fed initial diets before an experiment starts. In a feeding study, legacy fatty acids can confuse interpretation, since certain fatty acids, particularly physiologically important essential fatty acids that are costly for the consumer to obtain via diet or biosynthesis, may be more likely to remain in the tissues of a consumer being fed new foods that are deficient in these nutrients. This can be a particular problem when the food consumed prior to the start of the experiment has a higher fat content than the new foods, requiring longer-duration experiments to replace the initial fatty acid signal. This effect is exacerbated if the fatty acids of interest are also in lower proportions in the experimental diet. Absence of a fatty acid in the initial diet may also influence our interpretation. For instance, Bell *et al*. [45] found no evidence of PUFA synthesis in wild *Calanus finmarchicus*; however, in the same species reared on phytoplankton lacking specific PUFA, Helenius *et al*. [20, this issue] demonstrated obvious synthesis of essential fatty acids. Legacy fatty acids can also be a result of maternal investment of lipids in eggs that feed lecithotrophic larvae and other consumers that prey upon the eggs [46,47]. In this issue, Hou *et al*. [48] capitalized on the deposition of legacy fatty acid in eggs to follow changes in maternal diets. The ideal path is to ensure that the diet fed to the consumer or mother before the experiment begins has a similar composition to both natural diets (if the outcomes are to be applied to natural populations) and the experimental diets in terms of fat content, and is nutritionally balanced and not deficient in essential fatty acids.

(e) Fatty acid selection

While up to 70 fatty acids are commonly identified and reported in trophic applications, inevitably some much smaller number is typically used in quantitative applications, often owing to statistical considerations, but also because of instrumental or biochemical uncertainties (e.g. see [2]). To determine diet, researchers may attempt to restrict applications to fatty acids that have a strictly dietary origin ('dietary' fatty acids) or can be both biosynthesized and acquired from diet ('extended dietary fatty acids'; [2]). From within those subsets, a number of approaches have been applied to further reduce the sets to yield the most reliable fatty acids for trophic inference. For instance, fatty acids have been excluded based on variation in their observed calibration coefficients (e.g. [39]) or in their proportions among prey species (e.g. [49,50]). Others have used simulation studies to test the ability of a model to predict a known diet, given a particular fatty acid dataset (e.g. [51]). A fourth approach compares the proportion of each fatty acid in all prey species with the corresponding fatty acid in the predator's tissue after adjustment for metabolism to identify predator fatty acid proportions that are greater than that of any prey item. Assuming that all important prey species have been sampled, such a situation implies that the adjustment of the predator fatty acid proportion to account for metabolism is unreliable and that certain fatty acids should be eliminated from modelling [52,53].

In this issue, Jardine *et al*. [7] offer a new means to identify fatty acids that are most likely to yield useful information about diet, through a large synthesis from 316 controlled feeding studies for a wide range of organisms. In their evaluation of the regressions of the fatty acid proportions in consumer tissue and diet, for some fatty acids, they found very weak relationships in all predator classes (i.e. 18:0), suggesting that these fatty acids convey little information about diet consumed, despite their prominence in predator and prey tissues [7]. Evaluation of individual correlations offers an objective solution to this issue of

fatty acid selection; without a strong relationship between fatty acid proportion in predator and prey, there is no reason to include that variable in modelling. Unfortunately, as Jardine *et al*. [7] point out, the minimum correlation for inclusion does remain a subjective decision, with their suggestion of 0.2 as a cut-off. A prudent approach might involve first selecting fatty acids based on correlation of predator and prey fatty acids, and then refinement following Bromaghin *et al*.'s [52] method of comparison of prey with adjusted predator profiles.

(f) Diet models

While the earliest quantitative applications of fatty acids to estimate diet used numerical optimization models (quantitative fatty acid signature analysis, QFASA; [2]), more recent applications have begun to draw on the wealth of Bayesian models employed in stable isotope ecology [5,50], leading to uncertainty about the most appropriate models to apply. Litmanen *et al*. [54, this issue] provides some guidance on this, with an initial critical comparison of the two classes of methods. These authors found that while all methods mostly performed well, QFASA was favoured in the scenarios they evaluated, in part because it had significantly shorter computation times and less ambiguity in the results [54]. The value in combining data from bulk stable isotope values and fatty acids in mixing model analyses has long been recognized, and modelling approaches that can incorporate both types of data are now available [49,55].

An obvious next extension is to combine fatty acid proportional data with stable isotope data of individual fatty acids, i.e. compound-specific isotope analysis (CSIA), offering greater power to resolve complex diets and address questions in trophic ecology (e.g. reviewed in [56, this issue]). However, as with applications of fatty acid proportional data, successful implementation of CSIA requires an understanding of the effects of predator metabolism on isotopic composition of fatty acids. As Burian *et al*. [33, this issue] and others [57,58] have demonstrated, such trophic fractionation is complex and rarely meets expectations based on fatty acid structure. We agree with Twining *et al*.'s [56, this issue] conclusion that much more effort must be placed on establishing reliable trophic fractionation factors, and we urge researchers to consider the criteria described above in their design; the same considerations will apply. Moreover, it is critical that the performance of these models is tested with increasingly complex empirically collected data from actual experiments, not just simulations, including mixtures of prey, and under a range of environmental conditions. New experiments are needed to determine if the trophic modification factors for a given consumer can reliably be approximated with the synthesis approach used in Jardine *et al*. [7], or if organism trophic modification is too specific to individual diet composition to warrant general calibration coefficients. These issues are important, because if estimates of consumer trophic modification for fatty acids are inaccurate and the results of analyses are sensitive to these limitations, it will not matter which mixing model approach is being used.

4. Conclusion

The use of fatty acids as trophic tracers requires bridging three distinct fields: ecology, physiology and chemistry. A fundamental limitation in the field at this point is that the experts in these very different disciplines may not understand the nuances of the other disciplines. For example, ecologists who wish to use fatty acids as trophic tracers may not fully appreciate the roles of different fatty acids in organism physiology and lipid metabolism. Likewise, a chemist who is well versed in the lipid analysis and physiological function of these fatty acids may still not understand the ecology or physiology of an organism. The path forward involves interdisciplinary collaboration and well-designed experimental studies. The contributions to this special issue provide many fine examples of these interdisciplinary approaches from leaders in the field with diverse areas of expertise.

Data accessibility. This article has no additional data.

Authors' contributions. A.W.E.G. and S.M.B. contributed equally to all aspects of the manuscript.

Competing interests. We declare we have no competing interests.

Funding. We received no funding for this study.

Acknowledgements. The authors thank Louise A. Copeman for reviewing the manuscript, and Reyn M. Yoshioka for working with us to develop the figure artwork, as a paid commission.

References

1. Dalsgaard J, St John M, Kattner G, Müller-Navarra DC, Hagen W. 2003 Fatty acid trophic markers in the pelagic marine environment. *Adv. Mar. Biol.* **46**, 225–340. (doi:10.1016/S0065-2881(03)46005-7)
2. Iverson SJ, Field C, Bowen WD, Blanchard W. 2004 Quantitative fatty acid signature analysis: a new method of estimating predator diets. *Ecol. Monogr.* **74**, 211–235. (doi:10.1890/02-4105)
3. Budge SM, Iverson SJ, Koopman HN. 2006 Studying trophic ecology in marine ecosystems using fatty acids: a primer on analysis and interpretation. *Mar. Mamm. Sci.* **22**, 759–801. (doi:10.1111/j.1748-7692.2006.00079.x)
4. Budge SM, Springer AM, Iverson SJ, Sheffield G. 2007 Fatty acid biomarkers reveal niche separation in an Arctic benthic food web. *Mar. Ecol. Prog. Ser.* **336**, 305–309. (doi:10.3354/meps336305)
5. Galloway AWE *et al*. 2015 A fatty acid based Bayesian approach for inferring diet in aquatic consumers. *PLoS ONE* **10**, e0129723. (doi:10.1371/journal.pone.0129723)
6. Galloway AWE, Eisenlord ME, Dethier MN, Holtgrieve GW, Brett MT. 2014 Quantitative estimates of isopod resource utilization using a Bayesian fatty acid mixing model. *Mar. Ecol. Prog. Ser.* **507**, 219–232. (doi:10.3354/meps10860)
7. Jardine TD, Galloway AWE, Kainz MJ. 2020 Unlocking the power of fatty acids as dietary tracers and metabolic signals in fishes and aquatic invertebrates. *Phil. Trans. R. Soc. B* **375**, 20190639. (doi:10.1098/rstb.2019.0639)
8. Budge SM, Penney SN, Lall SP. 2012 Estimating diets of Atlantic salmon (*Salmo salar*) using fatty acid signature analyses; validation with controlled feeding studies. *Can. J. Fish. Aquat. Sci.* **69**, 1033–1046. (doi:10.1139/f2012-039)
9. Iverson SJ. 2009 Tracing aquatic food webs using fatty acids: from qualitative indicators to quantitative determination. In *Lipids in aquatic ecosystems* (eds MT Arts, MT Brett, M Kainz). New York, NY: Springer.
10. Bell MV, Tocher DR. 2009 Biosynthesis of polyunsaturated fatty acids in aquatic ecosystems: general pathways and new directions. In *Lipids in aquatic ecosystems* (eds MT Arts, MT Brett, M Kainz), pp. 211–236. New York, NY: Springer.

11. 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)
12. Brett MT, Müller-Navarra DC, Persson J. 2009 Crustacean zooplankton fatty acid composition. In *Lipids in aquatic ecosystems* (eds MT Arts, MT Brett, M Kainz), pp. 115–146. New York, NY: Springer.
13. Boecklen WJ, Yarnes CT, Cook BA, James AC. 2011 On the use of stable isotopes in trophic ecology. *Annu. Rev. Ecol. Evol. Sys.* **42**, 411–440. (doi:10.1146/annurev-ecolsys-102209-144726)
14. Pethybridge HR, Choy CA, Polovina JJ, Fulton EA. 2018 Improving marine ecosystem models with biochemical tracers. *Annu. Rev. Mar. Sci.* **10**, 199–228. (doi:10.1146/annurev-marine-121916-063256)
15. 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)
16. Thiemann GW, Iverson SJ, Stirling I. 2008 Variation in blubber fatty acid composition among marine mammals in the Canadian Arctic. *Mar. Mamm. Sci.* **24**, 91–111. (doi:10.1111/j.1748-7692.2007.00165.x)
17. Galloway AWE, Britton-Simmons KH, Duggins DO, Gabrielson PW, Brett MT. 2012 Fatty acid signatures differentiate marine macrophytes at ordinal and family ranks. *J. Phycol.* **48**, 956–965. (doi:10.1111/j.1529-8817.2012.01173.x)
18. 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)
19. Kabeya N, Gür İ, Oboh A, Evjemo JO, Malzahn AM, Hontoria F, Navarro JC, Monroig Ó. 2020 Unique fatty acid desaturase capacities uncovered in *Hediste diversicolor* illustrate the roles of aquatic invertebrates in trophic upgrading. *Phil. Trans. R. Soc. B* **375**, 20190654. (doi:10.1098/rstb.2019.0654)
20. Helenius L, Budge SM, Nadeau H, Johnson CL. 2020 Ambient temperature and algal prey type affect essential fatty acid incorporation and trophic upgrading in a herbivorous marine copepod. *Phil. Trans. R. Soc. B* **375**, 20200039. (doi:10.1098/rstb.2020.0039)
21. Boyen J, Fink P, Mensens C, Hablützel PI, De Troch M. 2020 Fatty acid bioconversion in harpacticoid copepods in a changing environment: a transcriptomic approach. *Phil. Trans. R. Soc. B* **375**, 20190645. (doi:10.1098/rstb.2019.0645)
22. Emam M, Katan T, Caballero-Solares A, Taylor RG, Parrish KS, Rise ML, Parrish CC. 2020 Interaction between ω6 and ω3 fatty acids of different chain lengths regulates Atlantic salmon hepatic gene expression and muscle fatty acid profiles. *Phil. Trans. R. Soc. B* **375**, 20190648. (doi:10.1098/rstb.2019.0648)
23. Lovern JA. 1935 Fat metabolism in fishes. VI. The fats of some plankton Crustacea. *Biochem. J.* **29**, 847–849. (doi:10.1042/bj0290847)
24. Farkas T, Herodek S. 1959 Paper chromatographic studies on the fatty acid composition of some freshwater crustaceans. *Acta Biol. Acad. Sci. Hung* **10**, 85–90.
25. Ackman RG, Eaton CA. 1966 Lipids of the fin whale (*Balaenoptera physalus*) from North Atlantic waters: III. Occurrence of eicosenoic and docosenoic fatty acids in the zooplankter *Meganyctiphanes norvegica* (M. Sars) and their effect on whale oil composition. *Can. J. Biochem.* **44**, 1561–1566. (doi:10.1139/o66-176)
26. Lee RF, Nevenzel JC, Pfaffenhöfer GA. 1971 Importance of wax esters and other lipids in marine food chain: phytoplankton and copepods. *Mar. Biol.* **9**, 99–108. (doi:10.1007/bf00348249)
27. Rosen DAS, Tollit DJ. 2012 Effects of phylogeny and prey type on fatty acid calibration coefficients in three pinniped species: implications for the QFASA dietary quantification technique. *Mar. Ecol. Prog. Ser.* **467**, 263–276. (doi:10.3354/meps09934)
28. Happel A, Stratton L, Kolb C, Hays C, Rinchard J, Czesny S. 2016 Evaluating quantitative fatty acid signature analysis (QFASA) in fish using controlled feeding experiments. *Can. J. Fish. Aquat. Sci.* **73**, 1222–1229. (doi:10.1139/cjfas-2015-0328)
29. Schälicke S, Heim S, Martin-Creuzburg D, Wacker A. 2020 Inter- and intraspecific differences in rotifer fatty acid composition during acclimation to low-quality food. *Phil. Trans. R. Soc. B* **375**, 20190644. (doi:10.1098/rstb.2019.0644)
30. Strandberg U, Vesterinen J, Ilo T, Akkanen J, Melanen M, Kankaala P. 2020 Fatty acid metabolism and modifications in *Chironomus riparius*. *Phil. Trans. R. Soc. B* **375**, 20190643. (doi:10.1098/rstb.2019.0643)
31. Thomas MD, Schram JB, Clark-Henry ZF, Yednock BK, Shanks AL, Galloway AWE. 2020 Juvenile Dungeness crabs (*Metacarcinus magister*) selectively integrate and modify the fatty acids of their experimental diets. *Phil. Trans. R. Soc. B* **375**, 20200038. (doi:10.1098/rstb.2020.0038)
32. Budge SM, Townsend K, Lall SP, Bromaghin JF. 2020 Dietary fat concentrations influence fatty acid assimilation patterns in Atlantic pollock (*Pollachius virens*). *Phil. Trans. R. Soc. B* **375**, 20190649. (doi:10.1098/rstb.2019.0649)
33. Burian A, Nielsen JM, Hansen T, Bermudez R, Winder M. 2020 The potential of fatty acid isotopes to trace trophic transfer in aquatic food-webs. *Phil. Trans. R. Soc. B* **375**, 20190652. (doi:10.1098/rstb.2019.0652)
34. Graeve M, Boissonnot L, Niehoff B, Hagen W, Kattner G. 2020 Assimilation and turnover rates of lipid compounds in dominant Antarctic copepods fed with ^{13}C-enriched diatoms. *Phil. Trans. R. Soc. B* **375**, 20190647. (doi:10.1098/rstb.2019.0647)
35. Kühn J, Tobias K, Jähngen A, Ruess L. 2020 Shifting systems: prerequisites for the application of quantitative fatty acid signature analysis in soil food webs. *Phil. Trans. R. Soc. B* **375**, 20190650. (doi:10.1098/rstb.2019.0650)
36. Colombo SM, Foroutani MB, Parrish CC. In press. Fats and oils in aquafeed formulations. In *Bailey's industrial oil and fat products*, pp. 1–28. Chichester, UK: John Wiley & Sons. (doi:10.1002/047167849X.bio095)
37. Bell GB, Koppe W. 2010 Lipids in aquafeeds. In *Fish oil replacement and alternative lipid sources in aquaculture feeds* (eds GM Turchini, W-K Ng, DR Tocher), pp. 21–59. Boca Raton, FL: CRC Press.
38. Copeman LA, Laurel BJ, Parrish CC. 2013 Effect of temperature and tissue type on fatty acid signatures of two species of North Pacific juvenile gadids: a laboratory feeding study. *J. Exp. Mar. Biol. Ecol.* **448**, 188–196. (doi:10.1016/j.jembe.2013.07.008)
39. Nordstrom CA, Wilson LJ, Iverson SJ, Tollit DJ. 2008 Evaluating quantitative fatty acid signature analysis (QFASA) using harbour seals *Phoca vitulina richardsi* in captive feeding studies. *Mar. Ecol. Prog. Ser.* **360**, 245–263. (doi:10.3354/meps07378)
40. Jobling M. 2003 Do changes in Atlantic salmon, *Salmo salar* L., fillet fatty acids following a dietary switch represent wash-out or dilution? Test of a dilution model and its application. *Aquacult. Res.* **34**, 1215–1221. (doi:10.1046/j.1365-2109.2003.00965.x)
41. Raclot T. 2003 Selective mobilization of fatty acids from adipose tissue triacylglycerols. *Prog. Lipid Res.* **42**, 257–288. (doi:10.1016/s0163-7827(02)00066-8)
42. McWilliams SR, Guglielmo C, Pierce B, Klaassen M. 2004 Flying, fasting, and feeding in birds during migration: a nutritional and physiological ecology perspective. *J. Avian Biol.* **35**, 377–393. (doi:10.1111/j.0908-8857.2004.03378.x)
43. Schram JB, Kobelt JN, Dethier MN, Galloway AWE. 2018 Trophic transfer of macroalgal fatty acids in two urchin species: digestion, egestion, and tissue building. *Front. Ecol. Evol.* **6**, 83. (doi:10.3389/fevo.2018.00083)
44. Galloway AWE, Lowe AT, Sosik EA, Yeung JS, Duggins DO. 2013 Fatty acid and stable isotope biomarkers suggest microbe-induced differences in benthic food webs between depths. *Limnol. Oceanogr.* **58**, 1452–1462. (doi:10.4319/lo.2013.58.4.1451)
45. 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)
46. Fuiman LA, Faulk CK. 2013 Batch spawning facilitates transfer of an essential nutrient from diet to eggs in a marine fish. *Biol. Lett.* **9**, 20130593. (doi:10.1098/rsbl.2013.0593)
47. Fuiman LA, Connelly TL, Lowerre-Barbieri SK, McClelland JW. 2015 Egg boons: central components of marine fatty acid food webs. *Ecology* **96**, 362–372. (doi:10.1890/14-0571.1)
48. Hou Z, Faulk CK, Fuiman LA. 2020 Dynamics of diet–egg transfer of fatty acids in the teleost fish,

red drum (*Sciaenops ocellatus*). *Phil. Trans. R. Soc. B* **375**, 20190646. (doi:10.1098/rstb.2019.0646)
49. Neubauer P, Jensen OP. 2015 Bayesian estimation of predator diet composition from fatty acids and stable isotopes. *PeerJ* **3**, e920. (doi:10.7717/peerj.920)
50. O'Donovan SA, Budge SM, Hobson KA, Kelly AP, Derocher AE. 2018 Intrapopulation variability in wolf diet revealed using a combined stable isotope and fatty acid approach. *Ecosphere* **9**, e02420. (doi:10.1002/ecs2.2420)
51. Wang SW, Hollmen TE, Iverson SJ. 2010 Validating quantitative fatty acid signature analysis to estimate diets of spectacled and Steller's eiders (*Somateria fischeri* and *Polysticta stelleri*). *J. Comp. Physiol. B* **180**, 125–139. (doi:10.1007/s00360-009-0393-x)
52. Bromaghin JF, Rode KD, Budge SM, Thiemann GW. 2015 Distance measures and optimization spaces in quantitative fatty acid signature analysis. *Ecol. Evol.* **5**, 1249–1262. (doi:10.1002/ece3.1429)
53. Goetsch C, Conners MG, Budge SM, Mitani Y, Walker WA, Bromaghin JF, Simmons SE, Reichmuth C, Costa DP. 2018 Energy-rich mesopelagic fishes revealed as a critical prey resource for a deep-diving predator using quantitative fatty acid signature analysis. *Front. Mar. Sci.* **5**, 430. (doi:10.3389/fmars.2018.00430)
54. Litmanen JJ, Perälä TA, Taipale SJ. 2020 Comparison of Bayesian and numerical optimization-based diet estimation on herbivorous zooplankton. *Phil. Trans. R. Soc. B* **375**, 20190651. (doi:10.1098/rstb.2019.0651)
55. Stock BC, Semmens BX. 2016 *MixSIAR GUI user manual. Version 3.1*. See https://github.com/brianstock/MixSIAR.
56. Twining CW, Taipale SJ, Ruess L, Bec A, Martin-Creuzburg D, Kainz MJ. 2020 Stable isotopes of fatty acids: current and future perspectives for advancing trophic ecology. *Phil. Trans. R. Soc. B* **375**, 20190641. (doi:10.1098/rstb.2019.0641)
57. Bec A, Perga M-E, 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)
58. Budge SM, AuCoin LR, Ziegler SE, Lall SP. 2016 Fractionation of stable carbon isotopes of tissue fatty acids in Atlantic pollock (*Pollachius virens*). *Ecosphere* **7**, e01437. (doi:10.1002/ecs2.1437)