PHILOSOPHICAL TRANSACTIONS B

royalsocietypublishing.org/journal/rstb

Research

Cite this article: 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.
http://dx.doi.org/10.1098/rstb.2019.0639

Accepted: 7 February 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, biochemistry

Keywords:
controlled feeding studies, trophic modification, mixing models, polyunsaturated fatty acid, calibration coefficients

Author for correspondence:
Timothy D. Jardine
e-mail: tim.jardine@usask.ca

Electronic supplementary material is available online at https://doi.org/10.6084/m9.figshare.c.4978388.

THE ROYAL SOCIETY PUBLISHING

Unlocking the power of fatty acids as dietary tracers and metabolic signals in fishes and aquatic invertebrates

Timothy D. Jardine[1,2], Aaron W. E. Galloway[3] and Martin J. Kainz[4]

[1]School of Environment and Sustainability, University of Saskatchewan, Saskatoon, SK, Canada, S7N 5C8
[2]Canadian Rivers Institute, Fredericton, NB, Canada, E3B 5A3
[3]Oregon Institute of Marine Biology, University of Oregon, Charleston, OR 97420, USA
[4]Inter-university Center for Aquatic Ecosystems Research WasserCluster – Biologische Station Lunz, Lunz am See, Austria

TDJ, 0000-0002-5917-9792; AWEG, 0000-0001-7826-299X; MJK, 0000-0002-2388-1504

Determining the transfer and transformation of organic matter in food webs is a fundamental challenge that has implications for sustainable management of ecosystems. Fatty acids (FA) offer a potential approach for resolving complex diet mixtures of organisms because they provide a suite of molecular tracers. Yet, uncertainties in the degree of their biochemical modification by consumers, due to selective retention or metabolism, have limited their application. Here, we consolidated 316 controlled feeding studies of aquatic ectotherms (fishes and invertebrates) involving 1404 species–diet combinations to assess the degree of trophic modification of FA in muscle tissue. We found a high degree of variability within and among taxa in the %FA in consumer muscle tissue versus %FA in diet regression equations. Most saturated FA had weak relationships with the diet ($r^2 < 0.30$) and shallow slopes ($m < 0.30$), suggesting a lack of retention in muscle when fed in increasing amounts. Contrarily, several essential FA, including linoleic (18:2n-6) and α-linolenic acid (18:3n-3), exhibited significant relationships with the diet ($m > 0.35$, $r^2 > 0.50$), suggesting supply limitations and selective retention in muscle by consumers. For all FA, relationships strengthened with increasing taxonomic specificity. We also demonstrated the utility of new correction equations by calculating the potential contributions of approximately 20 prey items to the diet of selected species of generalist fishes using a FA mixing model. Our analyses further reveal how a broad range of fishes and invertebrates convert or store these compounds in muscle tissue to meet physiological needs and point to their power in resolving complex diets in aquatic food webs.

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

Fatty acids (FA) play important roles as key physiologically active compounds in heterotrophs and as tracers of organic matter pathways in food webs. In this regard, they are increasingly used as 'dietary biomarkers' for consumers [1]. FA can selectively be retained by organisms as structural components of cellular membranes (as phospholipids) and as storage lipids (e.g. triacylglycerols) and/or act as important precursors for regulatory compounds such as eicosanoid hormones [2]. The availability of certain FA, especially polyunsaturated FA (PUFA), differs among food sources [3,4], and animals vary in their ability to synthesize these compounds *de novo* [5]. As such, understanding origins and transformation pathways of FA in both natural ecosystems and animal production systems is important for identifying limits on the health and quality of wild and captive-reared animals [6–8].

Tracing the diets of wild animals has long been a subject of study because food webs underlie ecosystem structure, and the provision of food can limit animal populations. Several options exist for tracing animal diets [9] that range from traditional methods, such as direct observation or gut content analysis, to more recent techniques involving isotopic or molecular markers. For example, the analysis of stable isotope ratios of carbon and nitrogen in bulk tissues offers a useful means of identifying foraging behaviours, although it does not typically allow the identification of individual prey items because of limits on its resolving power. Most studies involve only two tracers ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) [10] and resources often have similar isotopic values. Compound-specific isotope analysis of FA and/or amino acids have high resolving power and are slowly becoming mainstream [11,12], but technical challenges remain and limit their widespread adoption (see [13]). Since the potential number of food items available for consumption can be vast, our ability to differentiate among single items will always be limited by the number of tracers that can be applied [9].

FA may offer a compromise between the ease of low-resolution bulk stable isotope analysis and the technical demands of high-resolution compound-specific isotope analysis. There are known differences in FA profiles among dietary sources within and among food webs [14–16]), with, for example, aquatic primary producers such as algae having greater long-chain ω-3 PUFA content than land plants [3]. Controlled feeding studies of fishes and invertebrates demonstrate strong correlations between dietary PUFA supply and PUFA in muscle tissues (e.g. [17–19]). Applying these principles to marine and aquatic mammals, Iverson *et al*. [20] first demonstrated how FA profiles in fat storage tissues (adipose tissue and blubber) could be used to estimate proportions of dietary resources retained in consumers. They developed quantitative FA signature analysis (QFASA) by feeding pinnipeds long-term single-source diets and subsequently measuring specific calibration coefficients, i.e. the ratio of FA proportions in the stored fat tissue of the consumer to FA proportions in the diet. They then applied these calibration coefficients to wild-caught individuals (marine mammals and seabirds) to estimate their diet (summarized in [21]). This approach, while successful, has been criticized because of potential species-to-species variation in the trophic modification of FA from the diet [22–24], and the choice of values for trophic modification has clear implications for mixing model outputs [25].

Different species have different FA requirements [5], especially among tissue types, and as such, it is likely that different species will alter the relative proportions of a given FA in a given tissue to varying degrees. Rearing the species of interest on relevant diets and building a prey FA 'reference library' is one way to directly account for trophic modification [26], but this is largely impractical for most species, especially in complex, species-rich food webs. In situations where the number of sources is large, new tools are available for applying FA data to estimate diet, all of which involve Bayesian frameworks that allow the creation of probabilistic distributions [27–29]. Regardless of the modelling approach used, an estimation of organism FA trophic modification is needed.

The search for replacements for fish oils in fish feeds has led to a proliferation of controlled feeding studies in fishes and invertebrates where FA supply is manipulated to determine minimal and optimal FA levels for growth, reproduction and survival [5,30]. Collectively, such studies offer a rich dataset with which to establish the degree of trophic modification of FA by ectothermic aquatic animals. We compiled a large database (23 391 observations) of controlled feeding studies to answer questions about the trophic modification of FA within and among fish and invertebrate taxa. Our overall goal was to compile a dataset of experimentally generated FA profiles that could be used to quantify calibration coefficients for a broad range of taxa and parametrize quantitative FA mixing model analyses. We hypothesized that ectothermic animals would selectively retain dietary PUFA in their muscle tissue, including the two essential PUFA linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3), as well as arachidonic acid (20:4n-6), eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3), because they cannot be synthesized *de novo* or are inefficiently converted. We used the slope of the regression of %FA in the consumer versus %FA in its diet as an indicator of FA retention, and predicted that the above-mentioned PUFA would have the highest slopes. We then demonstrated the application of the regression equations in estimating dietary source proportions for an Australian freshwater food web. We conducted these analyses to chart a path forward for applying FA contents in muscle tissue to determine animal diet, but also to yield insights on how animals integrate FA profiles from their diets to meet their physiological needs.

2. Materials and methods

We used Web of Science to find articles containing FA data in controlled feeding studies of fishes and invertebrates where animals were fed a single food source. We began by compiling all papers that cited Iverson *et al*. [20]. After exhausting that list, we used search terms that included 'PUFA' + 'fish' + 'feeding', 'PUFA' + 'shellfish' + 'feeding', 'PUFA' + 'shrimp' + 'feeding', etc. to cover most common aquatic taxa, from freshwater, lacustrine and marine systems. Altering terms slightly (e.g. 'PUFA' to 'fatty acid') returned similar results. We only used studies where tabular data were available for both diet and consumer. We focused on muscle tissue because this is the most commonly measured tissue in feeding studies of fish and invertebrates. We assumed that all consumers were 'in equilibrium' with their diet, meaning that any dietary FA signal was fully incorporated into the consumer tissues. This assumption likely did not hold in all cases, especially for short-term studies with adult organisms, and contributes to some of the scatter we observed in the regression analysis. In very few cases do we know the true tissue-turnover time for FA, which is an important area for future research [31].

Upon compiling the database, we narrowed it further by excluding rarely measured FA: any compound with less than 100 observations in the database was excluded. This left us with 35 individual FA ranging in sample size from $n = 144$ (24:0) to $n = 1440$ (18:2n-6). Of the 121 species represented in the database, the most common were Atlantic salmon (*Salmo salar*; $n = 149$ animal-diet data points), rainbow trout (*Oncorhynchus mykiss*; $n = 113$), European sea bass (*Dicentrarchus labrax*; $n = 83$), Nile tilapia (*Oreochromis niloticus*; $n = 65$), barramundi (*Lates calcarifer*; $n = 57$), gilthead sea bream (*Sparus aurata*; $n = 56$), Senegalese sole (*Solea senegalensis*; $n = 38$) and murray cod (*Maccullochella peelii*; $n = 31$), all finfish species. The most common non-finfish animals were giant tiger prawn (*Penaeus monodon*; $n = 26$), green abalone (*Haliotis fulgens*; $n = 18$), green sea urchin (*Strongylocentrotus droebachiensis*; $n = 14$),

Japanese disc abalone (*Haliotis discus hannai*; $n = 13$) and Chinese prawn (*Penaeus chinensis*; $n = 12$).

We calculated regression parameters (consumer %FA versus diet %FA) using the nlme package in R [32]. We began by calculating these statistics for all taxa combined (electronic supplementary material, table S1). We then recalculated the same regression parameters by class, order and family. In all cases, residuals were not distributed normally according to Shapiro–Wilk tests ($p < 0.05$), but regressions are robust to normality violations when sample sizes are large. A larger concern with the regressions was the increasing variance observed with increasing values of the independent variable for some FA (electronic supplementary material, figure S1). That is, error was greater when the proportion of the given FA was higher in the diet, indicating heteroscedasticity. This will be an ongoing challenge with the application of the method, as most data are clustered near the origin simply due to the diets fed to consumers in experimental trials, and we know comparatively less about consumer responses to being fed a diet with a high proportion of a single FA. We attempted logit transformations [33] for a subset of the regressions and were unsatisfied with the result (residuals remained non-normal), so we opted to use the untransformed proportions and accepted that this could introduce further error into the models.

To determine how the level of taxonomic specificity affected the resolution of the regression equations, we ran general linear models with %FA in the consumer as the response variable, % FA in the diet as a covariate and taxonomic classification as a fixed factor. For this fixed factor, we ran the model four separate times with different levels of specificity (no classification, class, order and family) and observed the change in model fit (r^2) with progressively more specific levels of taxonomy. Because we did not perform tests on these data or report *p*-values (focusing only on goodness of fit through r^2), we did not transform the data to improve normality and homoscedasticity.

To test for effects of habitat and diet, we focused on the fish portion of the dataset (approx. 80% of all observations), and using Fishbase [34], we classified each species as marine, freshwater or diadromous, and its diet as herbivorous, omnivorous or carnivorous. We then compiled slope estimates for species belonging to these categories and tested for differences among them using one-way ANOVAs in R, separately for each FA in the dataset. Since these data were also not normally distributed, we re-ran them using a non-parametric test (Kruskal–Wallis test with Dunn's test for *post hoc* comparisons).

To demonstrate the application of the new FA trophic modification correction equations in a mixing model, we analysed a dataset for a food web from Australian waterholes [35]. This dataset contained three dominant fish species—two native species (bony bream *Nematalosa erebi* and yellowbelly *Macquaria ambigua*) and invasive common carp (*Cyprinus carpio*). Previous work on this food web based on stable isotope ratios of C and N had calculated proportional estimates of diet from four organic matter pathways—C4 grasses, C3 leaf litter, plankton and periphyton. Model outputs had suggested that bony bream were most strongly connected to the pelagic zone (plankton), while common carp had a significant contribution of terrestrial organic matter (C3 and C4 plants) to the diet [35]. The use of a four-source, two-tracer ($\delta^{13}C$ and $\delta^{15}N$) mixing model in that study greatly simplified the complexity of this system because there were greater than 20 different diet items for which data were available. Thus, it offered an opportunity to apply a FA mixing model that could resolve more potential sources than were possible with isotopes alone.

With the model, we attempted to resolve the diets of the three fish species. We applied the Actinopterygii (ray-finned fishes) correction equations (table 1) to source data to create expected FA distributions in muscle for each species if they were feeding solely on each source. This is theoretically equivalent to creating a FA reference library (i.e. [26]), but by rearing each consumer on several diets and using the average trophic modifications across various diets as a universal correction for that class of consumer. Bony bream and common carp both feed on a mix of plant matter and invertebrates [36], so, from our dataset, we had 20 possible food sources available to their diet (seston, periphyton, grasses, leaf litter and herbaceous plants, plus 11 families of insects, three families of crustaceans and mussels). Yellowbelly do not feed on plants, instead preying on invertebrates and fishes [36], so there were 21 food sources available to their diet (11 families of insects, three families of crustaceans, and mussels, plus large and small bony bream, common carp, goldfish, Hyrtl's tandan and spangled perch). The equations were linear, so we substituted each food source's %FA value in the equation $\%FA_{consumer} = m * \%FA_{diet} + b$ to generate a predicted mixture value for that source. We used the 20 FA for which we had data that also had correction equations available, excluding those FA with weak explanatory power ($r^2 < 0.20$) (table 1). As an example to show how the correction equations influence FA distributions, we visualized the yellowbelly data with a non-metric multi-dimensional scaling (NMDS) plot in PRIMER-e that displays all FA in the consumer and its sources, both before and after applying the correction equations. We also showed data distributions for a subset of individual FA for the consumer and its sources, before and after correction. We built these plots to observe whether the mixture remained inside the 'mixing space' after correction for trophic modification, a key criterion for the successful application of mixing models [25,27].

After correcting the sources with our equations as described above, we ran a 20 FA mixing model (20 food sources for bony bream and carp and 21 food sources for yellowbelly). We used MixSIAR [29] with trophic enrichment factors of zero because the sources were already corrected using the equations. Correction occurs in a step prior to populating a file for use in MixSIAR, and the model, designed for stable isotope data, adds trophic enrichment factors to sources to create mixtures. To include error associated with the correction equations, we used the standard deviation of the slopes. This may underestimate error, so further work on optimizing this term is required. The large number of food sources and FA tracers meant that the MixSIAR model approached its practical computational capacity. The smallest consumer dataset (small bony bream, $n = 9$), run in a 'very short' mode, took approximately 6 h to complete. As such, model runs were limited to 'very short' mode conditions (chain length of 10 000, burn-in of 5000, thin of 5) and did not converge according to the Geweke and Gelman–Rubin test statistics. Therefore, results are preliminary and presented as a demonstration of the potential of the method.

3. Results

From our database summary, in captive rearing conditions, consumers retained some FA in muscle tissue but not others. When all taxa were combined, every FA, except for 17:0, 22:0 and 22:4n-6, had a significant regression between $\%FA_{consumer}$ and $\%FA_{diet}$ ($p < 0.05$, electronic supplementary material, figure S2 and table S1, and when corrected for multiple comparisons $p < 0.001$ (0.05/35)), though several of the significant regressions were weak, having $r^2 < 0.1$. The FA with the steepest slope ($m = 0.85$) was 22:6n-3. Other PUFA had modest slopes that ranged from 0.4 to 0.6, including 18:2n-6, 18:3n-3, 20:4n-6 and 20:5n-3, as did many of the monounsaturated FA (MUFA) such as 16:1n-9, 18:1n-7, 18:1n-9, 20:1n-7 and 20:1n-9 (electronic supplementary material, figure S2 and table S1). The shallowest slopes (less

Table 1. Parameters for ordinary least-squares regressions comparing FA composition of animals with the FA composition of their diets in controlled laboratory studies, separated by the class. Asterisks indicate those FA used in the model to estimate the diet of Australian fishes.

	Actinopterygii				Bivalvia				Echinoidea				Gastropoda				Malacostraca			
fatty acid	*b*	*m*	r^2	*n*	*b*	*m*	r^2	*n*	*b*	*m*	r^2	*n*	*b*	*m*	r^2	*n*	*b*	*m*	r^2	*n*
12:0	1.15	0.12	0.28	149	—	—	—	—	—	—	—	—	—	—	—		0.23	0.27	0.83	24
14:0*	1.01	0.48	0.53	950	3.07	0.15	0.08	38	3.93	0.91	0.39	35	2.39	0.12	0.04	56	0.66	0.70	0.13	101
15:0*	−0.04	0.89	0.62	218	0.51	0.18	0.15	25	0.45	−0.18	0.03	23	0.75	−0.39	0.22	26	0.37	0.14	0.10	46
16:0*	12.94	0.32	0.24	1043	7.97	0.51	0.47	38	7.15	0.33	0.21	35	17.87	0.08	0.11	56	14.20	0.20	0.06	120
17:0*	0.17	0.54	0.39	161	0.99	0.09	0.01	18	0.14	0.05	0.03	19	0.94	−0.11	0.12	39	0.60	−0.01	0.00	48
18:0	4.89	0.15	0.05	1009	6.02	0.21	0.08	35	3.40	−0.01	0.00	31	7.05	0.01	0.00	56	5.54	0.51	0.17	120
20:0*	0.06	0.87	0.62	317	0.30	0.02	0.18	11	3.08	−2.84	0.17	17	1.69	−3.12	0.11	44	0.43	−0.06	0.01	52
22:0	0.21	0.09	0.02	174	—	—	—	—	2.00	0.03	0.21	7	2.63	−7.33	0.20	23	0.31	−0.04	0.01	22
24:0*	0.03	0.67	0.40	103	—	—	—	—	—	—	—	—	−0.02	0.85	0.71	15	0.13	2.90	0.76	14
16:1n9*	0.50	0.77	0.64	93	−0.10	2.40	0.37	16	2.18	0.71	0.52	6	1.06	0.02	0.00	9	0.10	0.58	0.39	19
16:1n7*	2.73	0.44	0.33	715	2.10	0.26	0.47	32	1.82	0.28	0.31	28	2.86	−0.03	0.01	42	3.49	0.07	0.02	90
18:1n9*	9.61	0.63	0.59	948	3.08	0.41	0.38	38	0.74	0.25	0.70	31	5.23	0.09	0.09	56	9.21	0.46	0.56	112
18:1n7*	1.53	0.62	0.34	597	5.30	−0.15	0.01	38	2.51	−0.07	0.01	31	5.01	0.38	0.25	56	3.15	0.50	0.27	51
20:1n11	0.31	0.38	0.48	102	0.47	1.40	0.08	14	5.17	−1.08	0.01	17	3.19	1.46	0.03	23	0.57	2.48	0.51	17
20:1n9*	0.82	0.61	0.55	604	1.47	1.47	0.15	25	3.66	1.20	0.19	26	1.27	0.36	0.37	31	0.93	0.33	0.30	70
20:1n7	0.16	0.70	0.48	96	0.78	−0.03	0.00	24	2.48	0.73	0.34	19	0.60	−1.12	0.05	25	−0.09	0.90	0.96	9
22:1n11	0.32	0.49	0.55	284	—	—	—	—	0.10	−0.04	0.00	17	0.08	0.04	0.03	21	0.09	0.58	0.91	17
22:1n9	0.32	0.10	0.12	259	—	—	—	—	1.66	4.19	0.45	24	0.49	0.66	0.43	32	0.28	0.08	0.02	20
24:1n9*	0.10	0.99	0.31	173	—	—	—	—	—	—	—	—	—	—	—	—	0.77	0.18	0.79	8
16:2n4	0.18	0.02	0.00	111	0.45	0.06	0.02	13	—	—	—	—	0.10	0.22	0.40	18	0.41	0.00	0.00	15
16:3n4	0.13	0.08	0.02	89	1.82	0.25	0.09	20	—	—	—	—	—	—	—	—	0.49	0.03	0.06	19
18:2n6*	3.47	0.52	0.63	1135	1.24	0.38	0.86	43	−0.64	0.41	0.69	31	3.00	0.14	0.58	56	5.58	0.24	0.30	121
18:3n6	0.25	0.63	0.78	438	0.39	0.19	0.12	17	0.25	−0.01	0.00	21	0.11	0.00	0.01	29	0.53	−0.39	0.05	29
20:2n6	0.68	0.54	0.10	420	1.10	−0.61	0.04	24	2.21	0.54	0.00	26	1.45	−1.45	0.10	51	0.84	0.58	0.17	29
20:3n6	0.51	0.37	0.07	457	0.32	0.30	0.57	18	0.70	−0.13	0.03	10	0.71	−0.55	0.04	43	0.34	−0.03	0.00	13
20:4n6*	0.96	0.52	0.41	1061	2.65	0.28	0.25	42	7.09	0.28	0.15	31	5.40	0.33	0.27	56	3.63	0.63	0.32	111
22:4n6	0.34	0.00	0.00	234	0.49	0.46	0.12	18	—	—	—	—	0.92	−0.65	0.10	40	0.00	2.33	0.52	6

(*Continued.*)

Table 1. (*Continued.*)

	Actinopterygii				Bivalvia				Echinoidea				Gastropoda				Malacostraca			
fatty acid	*b*	*m*	r^2	*n*	*b*	*m*	r^2	*n*	*b*	*m*	r^2	*n*	*b*	*m*	r^2	*n*	*b*	*m*	r^2	*n*
22:5n6	0.68	0.56	0.51	244	−0.15	2.39	0.54	27	0.28	0.25	0.02	6	0.37	0.71	0.12	32	0.06	0.98	0.93	10
18:3n3*	0.86	0.41	0.72	1089	1.64	0.32	0.74	43	−0.45	0.31	0.88	31	1.57	0.11	0.27	56	0.30	0.45	0.70	120
18:4n3*	0.40	0.42	0.51	661	1.73	0.47	0.16	41	1.14	0.14	0.45	31	0.42	0.00	0.00	35	−0.07	0.19	0.75	39
20:3n3*	0.38	0.81	0.25	339	—	—	—	—	1.66	−11.62	0.05	25	0.37	0.17	0.05	18	0.53	0.62	0.07	23
20:4n3	0.36	0.57	0.42	532	0.23	0.62	0.26	18	1.55	−1.19	0.17	19	0.08	0.12	0.20	23	0.12	0.24	0.05	32
20:5n3*	1.40	0.51	0.55	1125	4.39	0.32	0.31	46	7.61	0.35	0.77	31	7.21	0.10	0.08	56	11.20	0.24	0.06	112
22:5n3*	0.95	0.88	0.35	869	1.43	−2.35	0.10	27	0.50	−0.10	0.03	25	6.60	2.10	0.24	53	0.51	0.15	0.08	43
22:6n3*	5.15	0.88	0.39	1135	8.21	0.41	0.08	46	1.01	0.32	0.47	28	1.51	0.26	0.10	56	5.40	0.59	0.40	112

than 0.2) were observed for some of the saturated FA (SAFA) including 12:0, 17:0, 18:0 and 22:0, and other PUFA such as 22:4n-6.

The use of class-specific regression equations led to improved model performance (figures 1 and 2). Sample sizes were relatively small for all classes except for the Actinopterygii, but each class had a unique slope for a given FA and the goodness-of-fit values for common PUFA were strong (figure 1 and table 1). For example, gastropods had shallow slopes (but relatively high r^2) for the two essential PUFA (18:2n-6 and 18:3n-3) when compared with the other FA groups (table 1). Even SAFA that had poor fits when all taxa were lumped together showed better fits when separated by class, with gastropods showing shallowest slopes (figure 2). The r^2 values for individual regressions were mostly higher when organisms were grouped according to taxonomy, and this was further observed by higher r^2 of overall models when the taxonomic level was specified as a factor, increasing consistently from no specification to the class, order, family and species level (figure 3). For all FA, the combination of %FA in the diet and species identity accounted for greater than 38% of the variation in %FA in the consumer's muscle tissue, with $r^2 > 75\%$ for some FA such as 18:2n-6, 18:3n-3, 20:4n-6 and 20:5n-3 (electronic supplementary material, table S2). Generally, MUFA and PUFA had tighter relationships between consumers and their diets (higher r^2) than did SAFA, irrespective of the level of taxonomic classification (figure 3).

There was little evidence for differences in slopes among fish diets or habitats; the results were largely driven by variability within categories. Marine fish had higher slopes than freshwater fish for only three (16:0, 18:4n-3 and 20:4n-3) of the 32 FA tested (electronic supplementary material, figure S3 and table S3), and only 18:4n-3 was significant after correcting for multiple comparisons ($p < 0.002$; 0.05/32). Applying non-parametric tests yielded similar results, with five of 32 FA (16:0, 18:2n-6, 18:4n-3, 20:4n-3 and 20:5n-3) showing significant differences among habitats (marine higher than freshwater), but none after correcting for multiple comparisons. In the diet comparisons, there were only higher slopes in four of the 32 FA in carnivores (18:2n-6, 18:4n-3, 22:4n-6 and 24:1n-9) compared with omnivores or herbivores, while omnivores were significantly higher for only one FA, 17:0 (electronic supplementary material, figure S4 and table S4). None of these tests were significant after accounting for multiple comparisons ($p < 0.002$; 0.05/32). The non-parametric tests yielded more significant results (eight of 32), and one of these (20:4n-6) remained significant after accounting for multiple comparisons; in that case, carnivores had a steeper slope than both omnivores and herbivores.

The correction of sources in the Australian waterhole food web, according to class-specific correction equations, reduced the overall dispersion of data, both for all FA (figure 4*a*) and for individual FA (figure 4*b*). FA profiles of the consumer (yellowbelly) were in similar multivariate space as the sources after correction (figure 4*a*), and for individual FA, the mean value for yellowbelly typically was near the minimum or maximum of the distribution of sources after correction.

MixSIAR model outputs differentiated the diets of the three fish species (electronic supplementary material, table S5). Dytiscid beetles were the top-ranked diet item for both

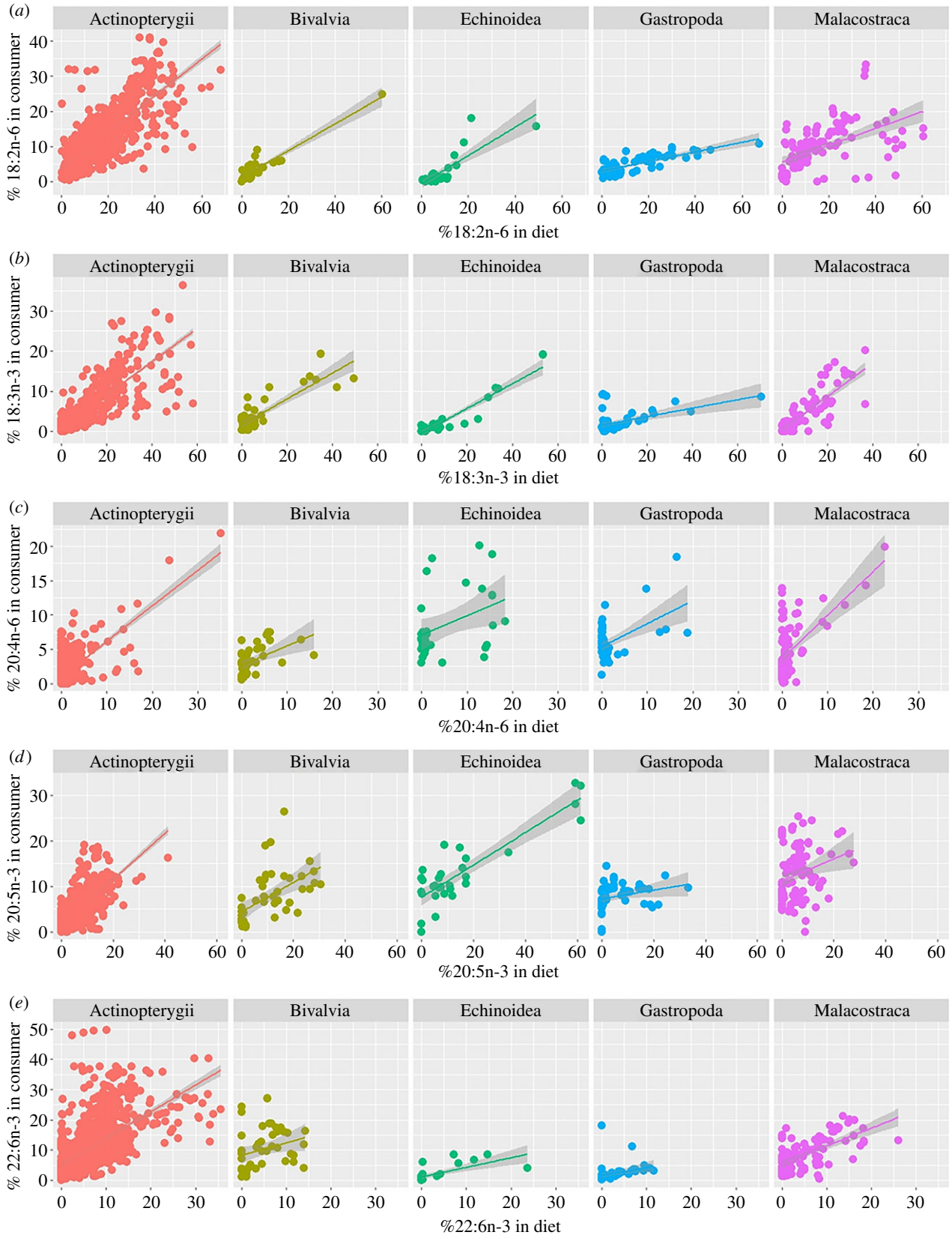

Figure 1. Composition (% of total FA) of animals, separated by class, for commonly measured PUFA as a function of the FA composition (%) of their diets in controlled laboratory studies. (*a*) 18:2n-6; (*b*) 18:3n-3; (*c*) 20:4n-6; (*d*) 20:5n-3; (*e*) 22:6n-3. Each point represents a unique animal-diet experiment. (Online version in colour.)

carp (proportion = 0.60) and large bony bream (0.51), and these two fish species also had mussels as the next-highest contributor. Small bony bream had caddisflies (Trichoptera) as the greatest proportion (0.26) followed by Dytiscidae (0.22). Yellowbelly had a mixed diet of caddisflies (0.25), mussels (0.19), dytiscids (0.19) and goldfish (0.14).

4. Discussion

There was a broad range of relationships between FA in consumer muscle tissue and their diets, with higher taxonomic specificity leading to tighter relationships. This exemplifies how organisms do not simply accumulate, but rather

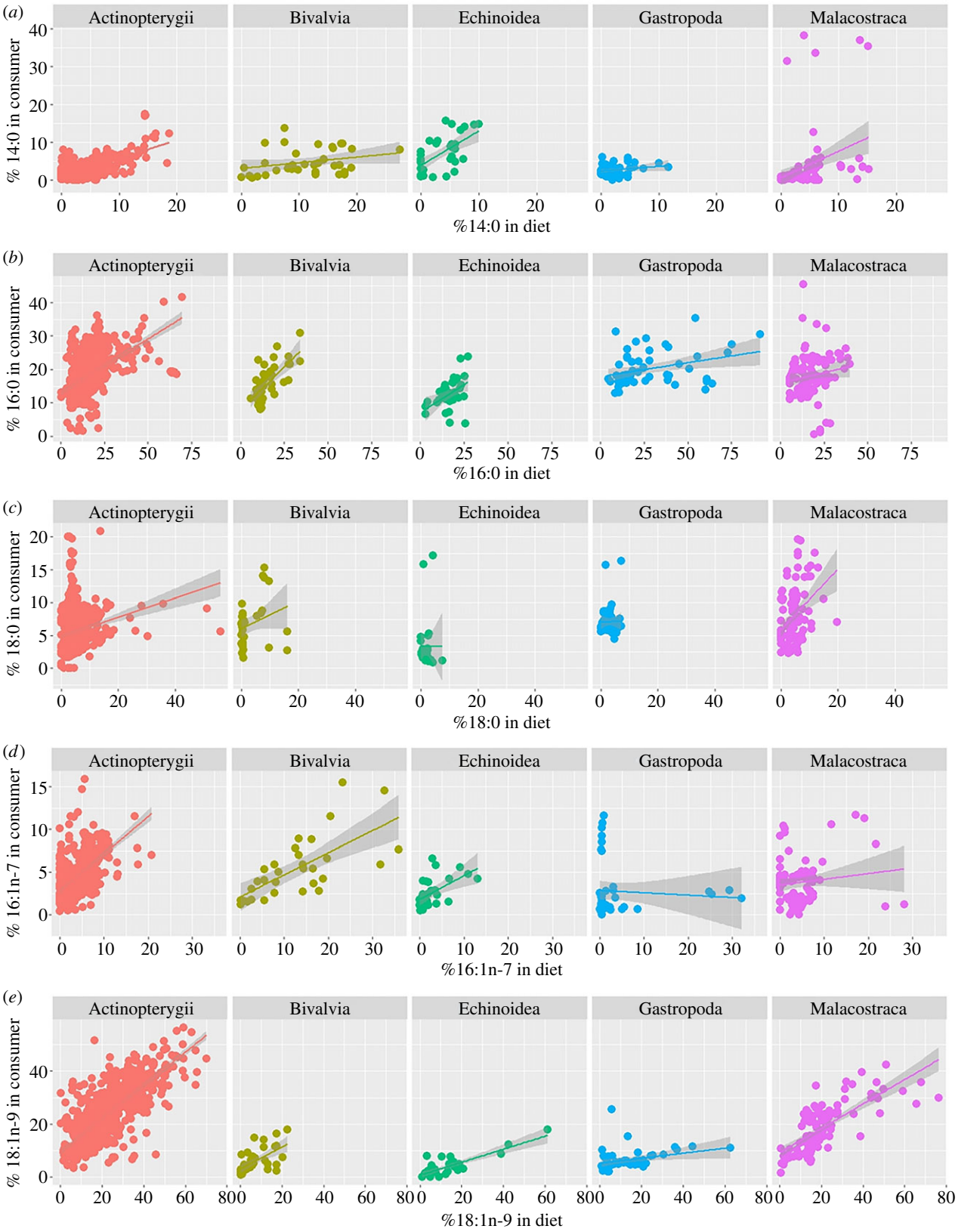

Figure 2. Composition (% of total FA) of animals, separated by class, for commonly measured SAFA and MUFA as a function of the FA composition of their diets in controlled laboratory studies. (*a*) 14:0; (*b*) 16:0; (*c*) 18:0; (*d*) 16:1n-7; (*e*) 18:1n-9. Each point represents a unique animal-diet experiment. (Online version in colour.)

transform and integrate dietary FA in muscle tissue to meet their physiological and metabolic needs. Our work highlights the need for additional controlled feeding studies with poorly studied organisms (i.e. those not commonly raised for aquaculture). Nevertheless, the present data allow the application of taxon-specific correction equations to several groups of organisms that lack data from controlled feeding studies, thus advancing the early work of Iverson *et al.* [20] to the use of muscle tissue in fishes and invertebrates and increasing the scope for dietary source tracing with FA.

The large number of FA examined here makes it difficult to focus on particular compounds, but several are worth mentioning. Out of all FA, the highest regression slope was found for 22:6n-3, which suggests that this long-chain ω-3

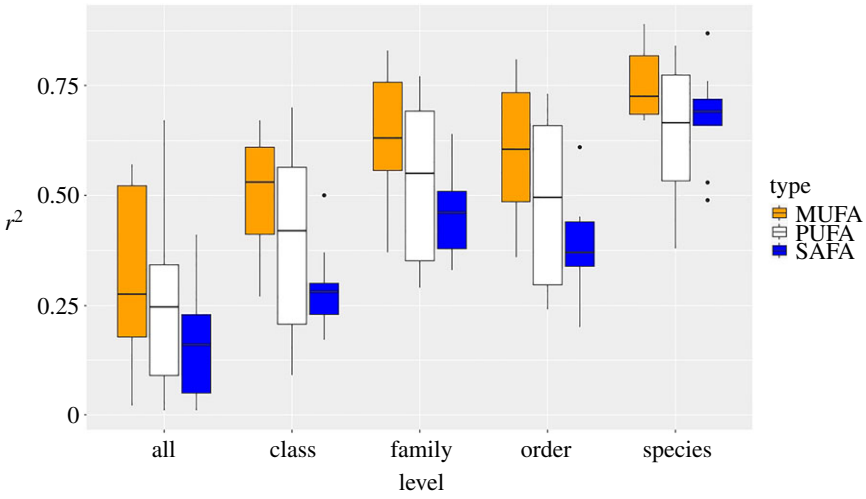

Figure 3. Coefficient of determination (r^2) for general linear models of %FA of animals as a function of the %FA of their diets, with increasing levels of taxonomic specificity included as terms in the models. (Online version in colour.)

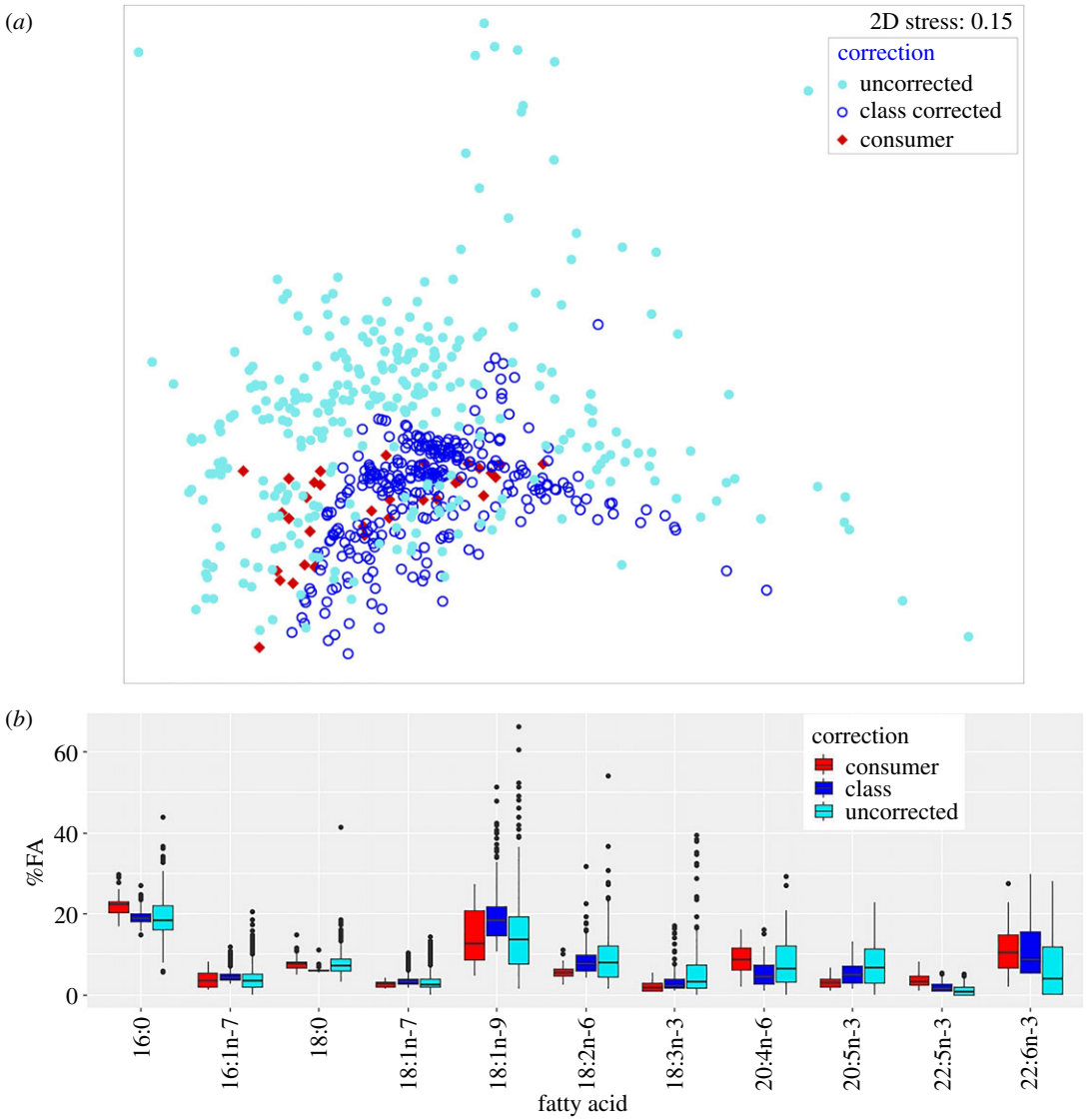

Figure 4. Application of FA regression equations to waterhole food web data from Jardine *et al.* [35]. (*a*) NMDS plot (Euclidean distance) showing the distribution of data for a predatory fish (yellowbelly) and potential sources with and without correction for trophic modification. (*b*) Box plots representing %FA for the 11 most common FA in yellowbelly and potential sources with and without correction for trophic modification. (Online version in colour.)

PUFA is more efficiently retained in muscle compared to other FA that are subject to higher FA catabolism. Because 22:6n-3 is a poor substrate for β-oxidation, this compound tends to be conserved in animals [5] and serves as a crucial molecule for fish reproduction [37]. It is a major FA in fish neural tissues and organs, including the retina and the

brain [38], and deficiencies can lead to symptoms such as reduced vision [39]. With a regression slope approaching one across all organisms considered, 22:6n-3 appears to be retained even when supplied at high proportions. One limitation of our data is that we used proportions as opposed to mass fractions (i.e. FA weight per unit biomass), as the former is the most common format in which FA data are reported. Had we used mass fractions, we could have tested whether true oversupply leads to limited retention. At the opposite end of the spectrum was 22:1n-11, which tends to be readily oxidized and, as such, should be in low proportions in consumers [5]. Yet, we calculated a relatively steep regression slope for this compound (0.48), suggesting that half of the supply is retained in the examined consumers. These observations point to the need for further research in determining the metabolic fate of different FA in muscle tissues.

Other FA that are involved in metabolic pathways leading to long-chain PUFA showed varying slopes across taxa in muscle. Most freshwater fish require approximately 1% of one or both of the two essential PUFA (18:2n-6 and 18:3n-3) in their diet [5]. The importance of these PUFA to fishes was corroborated by relatively high slopes (0.52 and 0.41, respectively) in this class of organisms. However, the other classes of organisms (Bivalvia, Malacostraca, Gastropoda and Echinoidea) had much lower regression slopes for 18:2n-6 and 18:3n-3, and gastropods in particular had shallow slopes. This could signify a less critical role for these compounds as, typically, they are either oxidized for fuel or rapidly converted to other PUFA. Two other key PUFA, 20:4n-6 and 20:5n3, are oxidized to eicosanoid hormones, and the latter is a key intermediate in the synthesis of 22:6n-3. Unsurprisingly, both of these long-chain PUFA had steep regression slopes in fishes (0.52 and 0.51, respectively). Arachidonic acid enhances the production of reproductive hormones of some fishes [40], but overall requirements for this ω-6 PUFA in fish are still poorly understood [41]. We note that the Malacostraca showed a steep slope (0.63) for this PUFA, much higher than that for 18:2n-6 (0.24), perhaps indicating a preference to convert the essential ω-6 PUFA 18:2n-6 to 20:4n-6 [42].

We predicted systematic differences in slopes for some FA in consumer muscle according to diet category and culturing habitat (with respect to salinity), but this hypothesis was not strongly supported in our results. Freshwater fish have greatest requirements for 18:2n-6 and 18:3n-3 [5]; thus, we expected steeper slopes for these two compounds. Marine species, meanwhile, are more likely to need 20:5n-3 and 22:6n-3 to be supplied by the diet because they evolved in environments with plankton that are high in these compounds and thus no longer faced selection pressure for the ability to elongate 18:3n-3. Long-chain PUFA are most limiting for marine species [5]. However, we found no strong difference among habitats for any of those four compounds. Likewise, there were limited differences between herbivores, omnivores and carnivores despite potential decreases in PUFA supply with increasing trophic level for lean top predators [43]. In freshwater, cold-adapted species (e.g. salmonids) require mainly 18:3n-3, while warm-water species (e.g. tilapia) require mainly 18:2n-6 [5]. Yet, the family Salmonidae had a greater slope for both 18:2n-6 and 18:3n-3 relative to Cichlidae. Recent work has shown that marine fishes in polar regions have greater PUFA contents than marine fishes from the tropics [8]. Additional work with our database could test for differences in slopes in warm- and cold-water species.

Some compounds had shallow regression slopes, but non-zero intercepts. This shows that the absence of the compound in the diet does not mean it will be absent in the consumer's muscle. Compounds such as 16:0, 18:0, 18:1n-9 and 22:6n-3 had intercepts of approximately 5 (%FA) or more when all taxa were combined, meaning that organisms that were deprived of these FA in controlled rearing conditions still maintained these compounds, likely as components of cell membranes or for key physiological processes. This could also be an artefact of experimental design, with few diets available that are low in these compounds to enable more data on responses at low levels. It may also indicate that consumers will maintain minimum levels of certain compounds through FA synthesis, desaturation or elongation from related precursor molecules (see [31]). Overall, the presence of non-zero intercepts further evidences the advantage of using correction equations as opposed to uniform calibration coefficients, because the latter would assume zero %FA in the consumer if there was zero %FA in the diet.

The application of the FA correction equations in a MixSIAR model for the Australian waterhole food webs yielded dietary source proportions that largely aligned with earlier stable isotope-based models [35], with greater specificity than was previously possible, but raised some caveats. The stable isotope model had estimated that carp had almost half their diet coming from C4 grasses and a top candidate as channelling this source to carp was grasshoppers; yet, while this source ranked fourth highest in the FA model, it had a low mean proportion (only 0.03). The large contribution of dytiscid beetles to the diet of carp could be responsible for the transfer of C4 carbon into the food web because dytiscids are semi-aquatic and stable isotopes had suggested that C4 plants were their greatest source. Stable isotope ratios estimated that yellowbelly consumed prey from a mixture of the planktonic (42%) and C4 grass (43%) pathways [35], in agreement with their consumption of filter feeders (i.e. mussels and caddisflies) and dytiscids shown here. However, the similarly high contribution of dytiscids to the diet of bony bream is difficult to explain because they are gape-limited and unlikely to feed on large-bodied dytiscids. Instead, they feed mostly in the water column when they are small (73% planktonic from the stable isotope model), which matches the relatively large contribution from Trichoptera we identified with FA. Stable isotope ratios implied that larger bony bream still fed in the water column (44%), but also fed more on periphyton (25%) than they did when they were small [35], but there was little evidence for a shift to benthic foraging in the larger bony bream in our FA model. Setting aside time for long-duration model runs would be necessary to finalize these source proportion outputs [29].

Our results chart a path forward for the application of FA mixing models using 'off-the-shelf' correction equations for taxa lacking experimental feeding trial data. By carefully incorporating the error associated with both food sources and trophic modification, we can produce more reliable estimates with appropriate uncertainty that resolve fish and invertebrate diets in complex food webs. Our data show that species-specific models are likely to be most appropriate for this purpose (electronic supplementary material, table S2,

[20,22,23]); however, we caution against using a species-specific equation unless it derives from robust data using the tissue of interest with a large sample size drawn from multiple studies. While higher-order taxonomic equations introduce greater scatter in regressions, they are less likely to be subject to artefacts of sample design (e.g. diet quality) that could skew results through the lack of consumption or incomplete assimilation of the diet by the consumer. For example, we were able to generate species-specific equations for carp based on three controlled studies [44–46], but opted not to use them because of the relatively small sample size ($n = 11$–15) and the presence of some negative slopes. Since we see no physiological basis for a negative slope, we assumed these were artefacts and chose the higher-order correction equation instead.

Our data also allow for the assessment of inclusion criteria for mixing models based on the goodness of fit of %FA in consumers as a function of their dietary %FA. Others have used a variety of approaches for screening that involve examining the magnitude of means and the degree of variance among diet sources [22,47,48]. While we cannot define a strict threshold of model fit that should determine whether a FA is included in a mixing model or not, generally any compound that has a $\%FA_{consumer}$ versus $\%FA_{diet}$ regression with $r^2 < 0.2$ should be treated with caution. For this reason, we excluded three FA (18:0, 22:0 and 20:3n-6) for which we had data, but the regression fits for the Actinopterygii equations were poor. Other reasons for excluding certain FA include proportions in consumers that are vastly higher than any measured source [49] that may owe to *de novo* synthesis from minor pathways (e.g. 18:1n-7 and 20:1n-7 by elongation from 16:1n-7 in bivales, [50,51]) as opposed to direct dietary supply. Finally, there is uncertainty due to the lack of complete FA turnover after short-term dietary switches in controlled studies [52], and in many instances, turnover is likely incomplete unless the consumer was raised on the diet from the post-larval stage. These limitations, which also affect other ecological tracers, will constrain future application of this model and depend largely on an individual researcher's willingness to relax or tighten criteria for inclusion.

We encourage others to add data to the database (available as the electronic supplementary material) to refine regression equations for existing taxa and to produce new equations for others not currently present, specifically for other tissues (e.g. adipose tissue) and for endotherms. Some of the regressions we presented here, including 18:2n-6 for bivalves and echinoderms, are driven by a small number of data points with high %FA in the diet, thus exerting strong leverage. More data will help strengthen these particular equations. We estimate that there are at least 50% more data available in the published literature than what we presented here. Since we limited our approach to tabulated data, all those studies with data in figures could have the data added by the primary authors of those studies. This includes data on adipose tissue in birds and mammals, which were reported in many of the foundational studies using a quantitative FA approach [21]. We also strongly encourage new controlled feeding studies for taxa that are poorly represented in the database, especially for non-salmonid fishes and invertebrates. Together, this will continue to advance our ability to resolve the diets of organisms in complex food webs and offer a window into the physiology and nutrition of aquatic consumers.

Data accessibility. The database used for the analysis is included in the electronic supplementary material.

Authors' contributions. T.D.J., A.W.E.G. and M.J.K. conceived the study. T.D.J. assembled the database. T.D.J. and A.W.E.G. performed analyses and prepared figures. T.D.J. wrote the first draft. A.W.E.G. and M.J.K. edited the manuscript.

Competing interests. We declare we have no competing interests.

Funding. We received no funding for this study.

Acknowledgements. We thank all the authors of the manuscripts who painstakingly produced tables of data that ultimately formed the FA database reported here, and Suzanne Budge and two anonymous reviewers for constructive comments that improved the manuscript.

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. Bell MV, Tocher DR. 2008 Biosynthesis of polyunsaturated fatty acids in aquatic ecosystems: general pathways and new directions. In *Lipids in aquatic ecosystems* (eds MT Arts *et al.*), pp. 211–236. New York, NY: Springer.
3. Hixson SM, Sharma B, Kainz MJ, Wacker A, Arts MA. 2015 Production, distribution, and abundance of long-chain omega-3 polyunsaturated fatty acids: a fundamental dichotomy between freshwater and terrestrial systems. *Environ. Rev.* **23**, 414–424. (doi:10.1139/er-2015-0029)
4. Twining CW, Brenna JT, Hairston Jr 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)
5. Tocher D. 2010 Fatty acid requirements in ontogeny of marine and freshwater fish. *Aquaculture Res.* **41**, 717–732. (doi:10.1111/j.1365-2109.2008.02150.x)
6. Tocher D. 2015 Omega-3 long-chain polyunsaturated fatty acids and aquaculture in perspective. *Aquaculture* **449**, 94–107. (doi:10.1016/j.aquaculture.2015.01.010)
7. Twining CW, Shipley JR, Winkler DW. 2018 Aquatic insects rich in omega-3 fatty acids drive breeding success in a widespread bird. *Ecol. Lett.* **21**, 1812–1820. (doi:10.1111/ele.13156)
8. Hicks CC *et al.* 2019 Harnessing global fisheries to tackle micronutrient deficiencies. *Nature* **574**, 95–98. (doi:10.1038/s41586-019-1592-6)
9. Nielsen JM, Clare EL, Hayden B, Brett MT, Kratina P. 2018 Diet tracing in ecology: method comparison and selection. *Methods Ecol. Evol.* **9**, 278–291. (doi:10.1111/2041-210X.12869)
10. Fry B. 2006 *Stable isotope ecology*. New York, NY: Springer.
11. McMahon KW, Thorrold SR, Houghton LA, Berumen ML. 2016 Tracing carbon flow through coral reef food webs using a compound-specific stable isotope approach. *Oecologia* **180**, 809–821. (doi:10.1007/s00442-015-3475-3)
12. Thorp JH, Bowes RE. 2017 Carbon sources in riverine food webs: new evidence from amino acid isotope techniques. *Ecosystems* **20**, 1029–1041. (doi:10.1007/s10021-016-0091-y)
13. 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)
14. Kelly JR, Scheibling RE. 2012 Fatty acids as dietary tracers in benthic food webs. *Marine Ecol. Progr. Ser.* **446**, 1–22. (doi:10.3354/meps09559)

15. 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)
16. Galloway AWE, Winder M. 2015 Partitioning the relative importance of phylogeny and environmental conditions on phytoplankton fatty acids. *PLoS ONE* **10**, e0130053. (doi:10.1371/journal.pone.0130053)
17. Brett MT, Muller-Navarra DC, Ballantyne AP, Ravet JL, Goldman CR. 2006 Daphnia fatty acid composition reflects that of their diet. *Limnol. Oceanogr.* **51**, 2428–2437. (doi:10.4319/lo.2006.51.5.2428)
18. Brett MT, Müller-Navarra DC, Persson J. 2009 Crustacean zooplankton fatty acid composition. In *Lipids in aquatic ecosystems* (eds MT Arts, MT Brett, MJ Kainz), pp. 115–146. New York, NY: Springer.
19. Benedito-Palos L *et al.* 2011 Modelling the predictable effects of dietary lipid sources on the fillet fatty acid composition of one-year-old gilthead sea bream (*Sparus aurata* L.). *Food Chem.* **124**, 538–544. (doi:10.1016/j.foodchem.2010.06.066)
20. 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)
21. .Iverson SJ. 2009 Tracing aquatic food webs using fatty acids: from qualitative indicators to quantitative determination. In *Lipids in aquatic ecosystems* (eds MT Arts *et al.*), pp. 281–307. New York, NY: Springer.
22. 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)
23. 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. Progr. Ser.* **467**, 263–276. (doi:10.3354/meps09934)
24. Bromaghin JF, Budge SM, Thiemann GW, Rode KD. 2017 Simultaneous estimation of diet composition and calibration coefficients with fatty acid signature data. *Ecol. Evol.* **7**, 6103–6113. (doi:10.1002/ece3.3179)
25. 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)
26. 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. Progr. Ser.* **507**, 219–232. (doi:10.3354/meps10860)
27. 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)
28. Bromaghin JF. 2017 QFASAR: quantitative fatty acid signature analysis with R. *Methods Ecol. Evol.* **8**, 1158–1162. (doi:10.1111/2041-210X.12740)
29. Stock BC, Jackson AL, Ward EJ, Parnell AC, Phillips DL, Semmens BX. 2018 Analysing mixing systems using a new generation of Bayesian tracer mixing models. *PeerJ* **6**, e5096. (doi:10.7717/peerj.5096)
30. Murray DS, Hager HH, Tocher DR, Kainz MJ. 2014 Effect of partial replacement of dietary fish meal and oil by pumpkin kernel cake and rapeseed oil on fatty acid composition and metabolism in Arctic charr (*Salvelinus alpinus*). *Aquaculture* **431**, 85–91. (doi:10.1016/j.aquaculture.2014.03.039)
31. Galloway AWE, Budge SM. 2020 The critical importance of experimentation in biomarker-based trophic ecology. *Phil. Trans. R. Soc. B* **375**, 20190638. (doi:10.1098/rstb.2019.0638)
32. Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team. 2019 nlme: linear and nonlinear mixed effects models. R package version 3.1–141. https://CRAN.R-project.org/package=nlme.
33. Wharton DI, Hui FKC. 2011 The arcsine is asinine: the analysis of proportions in ecology. *Ecology* **92**, 3–10. (doi:10.1890/10-0340.1)
34. Froese R, Pauly D (eds) 2019 *FishBase*. www.fishbase.org.
35. Jardine TD, Woods R, Marshall J, Fawcett J, Lobegeiger J, Valdez D, Kainz MJ. 2015 Reconciling the role of organic matter pathways in aquatic food webs by measuring multiple tracers in individuals. *Ecology* **96**, 3257–3269. (doi:10.1890/14-2153.1)
36. Pusey BJ, Kennard MJ, Arthington AH. 2004 *Freshwater fishes of north-eastern Australia*. Melbourne, Australia: CSIRO Publishing.
37. Izquierdo MS, Fernandez-Palacios H, Tacon AGJ. 2001 Effect of broodstock nutrition on reproductive performance of fish. *Aquaculture* **197**, 25–42. (doi:10.1016/S0044-8486(01)00581-6)
38. Stoknes IS, Okland HMW, Falch E, Synnes M. 2004 Fatty acid and lipid class composition in eyes and brain from teleosts and elasmobranchs. *Comp. Biochem. Physiol. B* **138**, 183–191. (doi:10.1016/j.cbpc.2004.03.009)
39. Bell MV, Batty RS, Dick JR, Fretwell K, Navarro JC, Sargent JR. 1995 Deitary deficiency of docosahexaenoic acid impairs vision at low light intensities in juvenile herring (*Clupea harengus* L.). *Lipids* **30**, 443–449. (doi:10.1007/BF02536303)
40. Norambuena F, Estevez A, Mananos E, Bell JG, Carazo I, Duncan N. 2013 Effects of graded levels of arachidonic acid on the reproductive physiology of Senegalese sole (*Solea senagalensis*): fatty acid composition, prostaglandins and steroid levels in the blood of broodstock bred in captivity. *Gen. Comp. Endocrinol.* **191**, 92–101. (doi:10.1016/j.ygcen.2013.06.006)
41. Bell MV, Sargent JR. 2003 Arachidonic acid in aquaculture feeds: current status and future opportunities. *Aquaculture* **218**, 491–499. (doi:10.1016/S0044-8486(02)00370-8)
42. Schlechtriem C, Arts MT, Zellmer ID. 2006 Effect of temperature on the fatty acid composition and temporal trajectories of fatty acids in fasting *Daphnia pulex* (Crustacea, Cladocera). *Lipids* **41**, 397–400. (doi:10.1007/s11745-006-5111-9)
43. Kainz MJ, Hager HH, Rasconi S, Kahilainen KK, Amundsen P-A, Hayden B. 2017 Polyunsaturated fatty acids in fishes increase with total lipids irrespective of feeding sources and trophic position. *Ecosphere* **8**, e01753. (doi:10.1002/ecs2.1753)
44. Guerden I, Bergot P, Van Ryckeghem K, Sorgeloos P. 1999 Phospholipid composition of common carp (*Cyprinus carpio*) larvae starved or fed different phospholipid classes. *Aquaculture* **171**, 93–107. (doi:10.1016/S0044-8486(98)00423-2)
45. Tian J-J, Lei C-X, Ji H. 2016 Influence of dietary linoleic acid (18:2n-6) and (α-linolenic acid (18:3n-3) ratio on fatty acid composition of different tissues in freshwater fish Songpu mirror carp, *Cyprinus carpio*. *Aquacult. Res.* **47**, 3811–3825. (doi:10.1111/are.12832)
46. Chen H, Tian J, Wang Y, Yang K, Ji H, Li J. 2017 Effects of dietary soybean oil replacement by silkworm, *Bombyx mori* L., chrysalis oil on growth performance, tissue fatty acid composition, and health status of juvenile Jian carp, *Cyprinus carpio* var. Jian. *J. World Aquacult. Soc.* **48**, 453–466. (doi:10.1111/jwas.12373)
47. Wang SW, Hollmen TE, Iverson SJ. 2010 Validating quantitative fatty acid signature analysis to estimate diet of spectacled and Steller's eiders (*Somateria fischeri* and *Polysticta stelleri*). *J. Comp. Physiol.* **180**, 125–139. (doi:10.1007/s00360-009-0393-x)
48. 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.
49. 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)
50. Ackman RG, Hooper SN. 1973 Non-methylene-interrupted fatty acids in lipids of shallow-water marine invertebrates: a comparison of two molluscs (*Littorina littorea* and *Lunatia triseriata*) with the sand shrimp (*Crangon septemspinosus*). *Comp. Biochem. Physiol. B* **46**, 153–165. (doi:10.1016/0305-0491(73)90057-6)
51. Zhukova NV. 1991 The pathway of biosynthesis of non-methylene-interrupted dienoic fatty acids in molluscs. *Comp. Biochem. Physiol. B* **100**, 801–804. (doi:10.1016/0305-0491(91)90293-M)
52. Robin JH, Regost C, Arzel J, Kaushik SJ. 2003 Fatty acid profile of fish following a change in dietary fatty acid composition with a dilution hypothesis. *Aquaculture* **225**, 283–293. (doi:10.1016/S0044-8486(03)00296-5)