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

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Abstract

Determining 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 1,404 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 vs. %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 alpha-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 ~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.

Key words: Controlled feeding studies, trophic modification, mixing models, PUFA, calibration coefficients

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 (Dalsgaard et al. 2003). FA can be selectively 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 (Bell and Tocher 2008). The availability of certain FA, especially polyunsaturated FA (PUFA), differs among food sources (Hixson et al. 2015, Twining et al. 2016), and animals vary in their ability to synthesize these compounds *de novo* (Tocher 2010). 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 (Tocher 2015, Twining et al. 2018, Hicks et al. 2019).

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 (Nielsen et al. 2018) 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 identification of individual prey items because of limits on its resolving power. Most studies involve only two tracers ($^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$) (Fry 2006), and resources often have similar isotopic values. Compound specific isotope analysis of fatty acids and/or amino acids have high resolving power and are slowly becoming mainstream (McMahon et al. 2016, Thorp

and Bowes 2017), but technical challenges remain and limit their widespread adoption (see Twining et al., this issue). 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 (Nielsen et al. 2018).

Fatty acids 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 (Kelly and Scheibling 2012, Galloway et al. 2012, Galloway and Winder 2015), with, for example, aquatic primary producers such as algae having greater long-chain omega-3 PUFA content than land plants (Hixson et al. 2015). Controlled feeding studies of fishes and invertebrates demonstrate strong correlations between dietary PUFA supply and PUFA in muscle tissues (e.g. Brett et al. 2006, 2009, Benedito-Palos et al. 2011). Applying these principles to marine and aquatic mammals, Iverson et al. (2004) first demonstrated how FA profiles in fat storage tissues (adipose tissue, blubber) could be used to estimate proportions of dietary resources retained in consumers. They developed quantitative fatty acid 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 Iverson 2009). This approach, while successful, has been criticised because of potential species-to-species variation in trophic modification of FA from the diet (Budge et al. 2012, Rosen and Tollit 2012, Bromaghin et al. 2017), and choice of values for trophic modification has clear implications for mixing model outputs (Phillips et al. 2014).

Different species have different FA requirements (Tocher 2010), 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 (Galloway et al. 2014), 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 (Galloway et al. 2015, Bromaghin 2017, Stock et al. 2018). Regardless of the modeling 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 (Tocher 2010; Murray et al. 2014). 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 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 parameterize 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 alpha-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.

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. (2004). 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 acids”) returned similar results. We only used studies where tabular data were available for both diet and consumer. We restricted our results to muscle tissue only because this is the most commonly measured tissue in feeding studies of fish and invertebrates. We assumed 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 (Galloway and Budge, this issue).

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-fish 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 vs diet %FA) using the nlme package in R (Pinheiro et al. 2019). We began by calculating these statistics for all taxa combined (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 (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 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 (Wharton and Hui 2011) 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 muscle 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 (approximately 80% of all observations) and, using Fishbase (Froese and Pauly 2019), 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 analyzed a dataset for a food web from Australian waterholes (Jardine et al. 2015). 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 (Jardine et al. 2015). The use of a four-source, two tracer ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) mixing model in that study greatly simplified the complexity of this system because there were >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., Galloway et al. 2014), 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 (Pusey et al. 2004), 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 (Pusey et al. 2004), 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 $\%FA_{\text{consumer}} = m * \%FA_{\text{diet}} + b$ 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 successful application of mixing models (Phillips et al. 2014, Galloway et al. 2015).

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, 21 food sources for yellowbelly). We used MixSIAR (Stock et al. 2018) 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 “very short” mode, took ~6 hours to complete. As such, model runs were limited to “very short” mode conditions (chain length of 10,000, burn-in of 5,000, 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.

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} ($p < 0.05$, Figure S2, 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 values < 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 (Figure S2, Table S1). The shallowest slopes (< 0.2) were observed for some of the saturated fatty acids (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 (Figure 1, 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, 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 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 $> 38\%$ 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 (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 (Figure S3, 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, 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 (Figure S4, 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.

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 4a) and for individual FA (Figure 4b). Fatty acid profiles of the consumer (yellowbelly) were in similar multivariate space as the sources after correction (Figure 4a), 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 (Table S5). Dytiscid beetles were the top-ranked diet item for both 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).

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 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. (2004) 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 make 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 omega-3 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 beta-oxidation, this compound tends to be conserved in animals (Tocher 2010) and serves as a crucial molecule for fish reproduction (Izquierdo et al. 2001). It is

a major FA in fish neural tissues and organs, including the retina and the brain (Stoknes et al. 2004) and deficiencies can lead to symptoms such as reduced vision (Bell et al. 1995). 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 (Tocher 2010). Yet, we calculated a relatively steep regression slope for this compound (0.48), suggesting 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 ~1% of one or both of the two essential PUFA (18:2n-6 and 18:3n-3) in their diet (Tocher 2010). 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 oxidised 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 (Norambuena et al. 2013), but overall

requirements for this omega-6 PUFA in fish are still poorly understood (Bell and Sargent 2003). 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 omega-6 PUFA 18:2n-6 to 20:4n-6 (Schlechtriem et al. 2006).

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 (Tocher 2010); 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 (Tocher 2010). 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 trophic level for lean top predators (Kainz et al. 2017). 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 (Tocher 2010). 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 (Hicks et al. 2019). 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 ~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 pre-cursor molecules (see Galloway and Budge, this issue). 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.

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 (Jardine et al. 2015), 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 channeling this source to carp was grasshoppers; yet, while this source ranked fourth highest, it had a low mean proportion (only 0.03). The large contribution of dytiscid beetles to the diet of carp could be responsible for 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 mix of the planktonic (42%) and C4 grass (43%) pathways (Jardine et al. 2015), 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 (Jardine et al. 2015), 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 (Stock et al. 2018).

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 (Table S2, Iverson et al. 2004, Budge et al. 2012, Rosen and Tollit 2012); 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 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 (Guerden et al. 1999, Tian et al. 2016, Chen et al. 2017), but opted not to use them because of the relatively small sample size ($n = 11$ to 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 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 degree of variance among diet sources (Wang et al. 2010, Budge et al. 2012, O'Donovan et al. 2018). 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} vs %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 (Bromaghin et al. 2015) 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 bivalves, Ackman and Hooper 1973, Zhukova 1991) as opposed to direct dietary supply. Finally, there is uncertainty due to lack of complete FA turnover after short-term dietary switches in controlled studies (Robin et al. 2003) 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 supplemental 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 (Iverson 2009). 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.

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Figures

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.

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.

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.

Figure 4. Application of FA regression equations to waterhole food web data from Jardine et al. (2015). (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.

611 **Table 1.** Parameters for ordinary least squares regressions comparing fatty acid composition of animals to the fatty acid composition
612 of their diets in controlled laboratory studies, separated by class. Asterisks indicate those FAs used in the model to estimate diet of
613 Australian fishes.

Fatty acid	Actinopterygii				Bivalvia				Echinoidea				Gastropoda				Malacostraca			
	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
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

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621 **Figure 1**

624 **Figure 2**

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Figure 3

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Supplementary Material

Table S1. Parameter estimates for ordinary least squares regressions of % fatty acids in consumers versus % fatty acids in their diets. All taxa combined.

Fatty acid	b	m	SE	r ²	n	p
12:0	1.04	0.12	0.01	0.30	178	<0.001
14:0	1.37	0.41	0.02	0.23	1222	<0.001
15:0	0.19	0.60	0.04	0.41	368	<0.001
16:0	13.97	0.24	0.01	0.16	1349	<0.001
17:0	0.54	0.04	0.02	0.01	313	0.094
18:0	5.15	0.18	0.02	0.05	1305	<0.001
20:0	0.35	0.53	0.05	0.20	453	<0.001
22:0	0.47	0.03	0.08	<0.01	236	0.674
24:0	0.19	0.29	0.08	0.08	144	<0.001
16:1n9	0.57	0.79	0.06	0.55	162	<0.001
16:1n7	3.02	0.33	0.02	0.27	950	<0.001
18:1n9	7.85	0.64	0.02	0.55	1221	<0.001
18:1n7	2.28	0.52	0.03	0.23	810	<0.001
20:1n11	1.34	0.26	0.13	0.02	181	0.041
20:1n9	1.08	0.53	0.02	0.44	781	<0.001
20:1n7	0.54	0.67	0.08	0.28	189	<0.001
22:1n11	0.26	0.48	0.02	0.57	351	<0.001
22:1n9	0.50	0.12	0.02	0.06	352	<0.001
24:1n9	0.42	0.36	0.06	0.16	204	<0.001
16:2n4	0.17	0.09	0.02	0.09	170	<0.001
16:3n4	0.25	0.28	0.04	0.22	156	<0.001
18:2n6	3.82	0.45	0.01	0.55	1440	<0.001
18:3n6	0.46	0.16	0.01	0.19	561	<0.001
20:2n6	0.84	0.44	0.08	0.06	563	<0.001
20:3n6	0.51	0.33	0.05	0.07	555	<0.001
20:4n6	1.50	0.59	0.02	0.33	1355	<0.001
22:4n6	0.42	0.02	0.06	<0.01	314	0.792
22:5n6	0.60	0.73	0.06	0.34	340	<0.001
18:3n3	0.90	0.39	0.01	0.67	1389	<0.001
18:4n3	0.58	0.31	0.02	0.33	834	<0.001
20:3n3	0.45	0.70	0.09	0.14	417	<0.001
20:4n3	0.39	0.52	0.03	0.27	635	<0.001
20:5n3	2.73	0.44	0.02	0.35	1421	<0.001
22:5n3	1.36	0.71	0.07	0.09	1044	<0.001
22:6n3	4.71	0.85	0.03	0.38	1428	<0.001

Figure S1. Residuals from equations relating % fatty acid in fish muscle tissues to % fatty acid in the diet, as a function of the dependent variable (% in diet). These 20 fatty acids were used to estimate the diets of fishes from Australian waterholes.

Figure S2. Fatty acid composition (%) of animals, all taxa combined, as a function of the fatty acid composition (%) of their diets in controlled laboratory studies. Each point represents a unique animal-diet experiment.

Table S2. 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.

Fatty acid	No factors	Class	Order	Family	Species
12:0	0.30	0.30	0.34	0.34	0.68
14:0	0.23	0.30	0.36	0.38	0.49
15:0	0.41	0.50	0.61	0.64	0.76
16:0	0.16	0.25	0.44	0.51	0.66
17:0	0.01	0.23	0.37	0.44	0.71
18:0	0.05	0.19	0.45	0.54	0.69
20:0	0.20	0.37	0.43	0.46	0.72
22:0	<0.01	0.28	0.30	0.33	0.87
24:0	0.08	0.17	0.20	0.49	0.53
16:1n9	0.55	0.63	0.75	0.77	0.89
16:1n7	0.27	0.45	0.48	0.54	0.72
18:1n9	0.55	0.67	0.72	0.75	0.81
18:1n7	0.23	0.40	0.50	0.62	0.68
20:1n11	0.02	0.54	0.74	0.76	0.82
20:1n9	0.44	0.52	0.59	0.61	0.68
20:1n7	0.28	0.62	0.81	0.83	0.88
22:1n11	0.57	0.58	0.62	0.64	0.67
22:1n9	0.06	0.32	0.40	0.44	0.73
24:1n9	0.16	0.27	0.36	0.37	0.70
16:2n4	0.09	0.17	0.30	0.47	0.74
16:3n4	0.22	0.44	0.54	0.55	0.64
18:2n6	0.55	0.61	0.66	0.70	0.77
18:3n6	0.19	0.22	0.24	0.36	0.38
20:2n6	0.06	0.15	0.32	0.33	0.56
20:3n6	0.07	0.09	0.27	0.29	0.42
20:4n6	0.33	0.55	0.67	0.72	0.81
22:4n6	<0.01	0.14	0.27	0.29	0.45
22:5n6	0.34	0.39	0.46	0.47	0.65
18:3n3	0.67	0.70	0.73	0.76	0.79
18:4n3	0.33	0.45	0.60	0.62	0.68
20:3n3	0.14	0.23	0.29	0.33	0.42
20:4n3	0.27	0.41	0.46	0.55	0.58
20:5n3	0.35	0.61	0.72	0.77	0.84
22:5n3	0.09	0.63	0.66	0.69	0.80
22:6n3	0.38	0.43	0.53	0.61	0.70

Figure S3. Boxplots of slopes of %FA consumer vs %FA diet for fishes residing in different habitats (diadromous, freshwater, marine). A – SAFA and MUFA; B – PUFA

B

Figure S4. Comparison of slopes of %FA consumer vs %FA diet for fishes occupying different trophic positions (carnivore, herbivore, omnivore). A – SAFA and MUFA; B – PUFA

B

Table S3. Results of one-way ANOVAs and Kruskal-Wallis tests evaluating differences in slopes (%FA in consumer vs. %FA in diet) among fishes from three different habitats (diadromous - D, freshwater - F, marine - M).

Fatty acid	n Diad	n Fresh	n Marine	ANOVA		Post-hoc	Kruskal-Wallis		Post-hoc
				F	p		X2	p	
12:0	1	6	3	0.483	0.636		0.327	0.849	
14:0	3	18	22	0.416	0.663		2.066	0.356	
15:0	0	7	8	0.436	0.521		0.054	0.817	
16:0	3	20	24	4.153	0.022	M>F	7.839	0.020	M>F
16:1n9	1	5	2	0.218	0.811		1.200	0.549	
16:1n7	3	12	20	0.539	0.589		0.587	0.746	
16:2n4	0	2	3	1.468	0.312		1.800	0.180	
17:0	2	9	6	0.717	0.505		2.662	0.264	
18:0	3	20	23	0.126	0.882		0.965	0.617	
18:1n9	3	17	24	0.465	0.631		1.428	0.490	
18:1n7	2	11	17	2.115	0.140		3.403	0.182	
18:2n6	3	20	25	1.791	0.178		6.461	0.040	M>F
18:3n6	3	11	8	0.137	0.873		0.071	0.965	
18:3n3	3	20	24	0.114	0.893		5.850	0.054	
18:4n3	3	9	16	8.571	0.001	D,M>F	11.383	0.003	M>F
20:0	1	10	8	0.324	0.728		1.221	0.543	
20:1n11	2	3	2	2.018	0.248		2.464	0.292	
20:1n9	2	12	11	0.177	0.839		1.676	0.433	
20:2n6	2	12	9	1.017	0.380		1.615	0.446	
20:3n6	1	10	8	0.569	0.577		0.771	0.680	
20:4n6	3	17	21	0.733	0.313		1.622	0.445	
20:3n3	1	10	8	0.795	0.468		0.455	0.797	
20:4n3	2	8	11	5.772	0.012	M>F	7.407	0.025	M>F
20:5n3	3	20	25	2.523	0.092		7.948	0.019	M>F
22:1n11	2	5	7	0.037	0.964		0.005	0.998	
22:1n9	2	7	7	0.298	0.748		1.628	0.443	
22:4n6	1	8	3	2.882	0.108		3.420	0.181	
22:5n6	1	6	6	0.306	0.743		1.780	0.411	
22:5n3	3	14	22	0.450	0.641		0.923	0.630	
22:6n3	3	20	25	0.608	0.549		2.409	0.300	
24:0	0	5	2	0.012	0.918		0.150	0.699	
24:1n9	2	6	2	0.034	0.967		0.073	0.964	

Table S4. Results of one-way ANOVAs testing and Kruskal-Wallis tests evaluating differences in slopes (%FA in consumer vs. %FA in diet) among fishes with three different diets (herbivorous - H, omnivorous - O, carnivorous - C).

Fatty acid	n herb	n omni	n carn	ANOVA		post hoc	Kruskal-Wallis		post hoc
				F	p		X2	p	
12:0	0	5	5	1.180	0.309		0.327	0.849	
14:0	3	12	28	1.629	0.209		4.315	0.116	
15:0	1	5	9	1.314	0.305		2.160	0.340	
16:0	3	13	31	2.619	0.084		9.632	0.008	C>H,O
16:1n9	2	1	5	1.225	0.369		4.050	0.132	
16:1n7	0	8	27	2.019	0.165		2.472	0.116	
17:0	2	4	11	6.038	0.013	O>C,H	6.563	0.038	O>H
18:0	3	13	30	1.073	0.351		6.867	0.032	O>H
18:1n9	3	11	30	1.245	0.299		2.918	0.233	
18:1n7	1	5	24	0.884	0.425		2.077	0.354	
18:2n6	3	13	32	3.922	0.027	C>O	10.565	0.005	C>H,O
18:3n6	2	7	13	1.327	0.289		4.646	0.098	
18:3n3	3	13	31	1.129	0.333		3.227	0.199	
18:4n3	2	5	21	3.633	0.041	C>H	4.396	0.111	
20:0	1	7	11	0.542	0.592		0.621	0.733	
20:1n9	2	6	17	0.413	0.667		0.288	0.866	
20:1n7	0	2	5	0.328	0.592		0.038	0.845	
20:2n6	1	9	13	1.098	0.353		1.871	0.392	
20:3n6	1	7	11	0.135	0.874		0.134	0.935	
20:4n6	2	11	28	0.883	0.422		15.299	0.000	C>H,O
20:3n3	2	5	12	0.913	0.421		2.838	0.242	
20:4n3	0	3	18	0.020	0.889		0.000	1.000	
20:5n3	3	13	32	2.580	0.087		8.182	0.017	C>O
22:0	0	4	3	0.884	0.390		1.125	0.289	
22:1n11	0	5	9	0.001	0.977		0.040	0.841	
22:1n9	1	4	11	0.423	0.664		1.533	0.465	
22:4n6	0	4	8	13.523	0.004	C>O	7.385	0.007	C>O
22:5n6	0	4	9	0.117	0.739		0.095	0.758	
22:5n3	3	8	28	2.003	0.150		4.120	0.128	
22:6n3	3	13	32	2.384	0.104		5.073	0.079	
24:0	0	3	4	3.996	0.102		3.125	0.077	
24:1n9	0	5	5	12.047	0.008	C>O	5.771	0.016	C>O

Table S5. MixSIAR model outputs from a 20 food source, 23 tracer model estimating most likely food items contributing to FA of bony bream, carp and yellowbelly. Values >0.10 are bolded for emphasis.

Source	Small bony bream Median (95% CI)	Large bony bream Median (95% CI)	Common carp Median (95% CI)	Yellowbelly Median (95% CI)
Grasses	0.02 (0.00,0.09)	0.02 (0.01,0.08)	0.00 (0.00,0.02)	-
Herbaceous plants	0.04 (0.00,0.10)	0.03 (0.00,0.07)	0.01 (0.00,0.03)	-
Leaf litter	0.01 (0.00,0.04)	0.02 (0.00,0.05)	0.00 (0.00,0.01)	-
Periphyton	0.03 (0.00,0.10)	0.04 (0.00,0.13)	0.00 (0.00,0.02)	-
Seston	0.03 (0.00,0.08)	0.01 (0.00,0.06)	0.04 (0.00,0.16)	-
Chironomidae	0.02 (0.00,0.08)	0.01 (0.00,0.04)	0.01 (0.00,0.02)	0.01 (0.00,0.03)
Coleoptera	0.02 (0.00,0.08)	0.02 (0.00,0.06)	0.01 (0.00,0.02)	0.02 (0.00,0.05)
Corixidae	0.04 (0.00,0.18)	0.04 (0.00,0.13)	0.01 (0.00,0.03)	0.01 (0.00,0.05)
Crayfish	0.03 (0.00,0.10)	0.02 (0.00,0.08)	0.01 (0.00,0.05)	0.02 (0.00,0.22)
Dytiscidae	0.22 (0.00,0.54)	0.51 (0.39,0.65)	0.60 (0.40,0.73)	0.19 (0.00,0.41)
Ephemeroptera	0.02 (0.00,0.10)	0.01 (0.00,0.06)	0.01 (0.00,0.03)	0.02 (0.00,0.07)
Formicidae	0.02 (0.00,0.06)	0.01 (0.00,0.04)	0.01 (0.00,0.03)	0.01 (0.00,0.04)
Gerridae	0.02 (0.00,0.07)	0.02 (0.00,0.06)	0.01 (0.00,0.04)	0.01 (0.00,0.05)
Macrobrachium	0.03 (0.00,0.09)	0.02 (0.00,0.07)	0.01 (0.00,0.04)	0.02 (0.00,0.06)
Mussels	0.06 (0.01,0.16)	0.06 (0.00,0.13)	0.19 (0.00,0.33)	0.19 (0.00,0.37)
Notonectidae	0.06 (0.00,0.19)	0.05 (0.00,0.17)	0.02 (0.00,0.05)	0.02 (0.00,0.09)
Odonata	0.02 (0.00,0.06)	0.01 (0.00,0.04)	0.01 (0.00,0.02)	0.01 (0.00,0.02)
Orthoptera	0.03 (0.00,0.19)	0.02 (0.00,0.08)	0.03 (0.00,0.17)	0.01 (0.00,0.05)
Trichoptera	0.26 (0.00,0.56)	0.04 (0.01,0.11)	0.02 (0.00,0.10)	0.25 (0.00,0.69)
Zooplankton	0.04 (0.00,0.13)	0.05 (0.01,0.04)	0.01 (0.00,0.04)	0.01 (0.00,0.05)
Bony bream (S)	-	-	-	0.00 (0.00,0.01)
Bony bream (L)	-	-	-	0.00 (0.00,0.02)
Carp	-	-	-	0.02 (0.00,0.06)
Goldfish	-	-	-	0.14 (0.00,0.28)
Hyrtl's tandan	-	-	-	0.04 (0.00,0.15)
Spangled perch	-	-	-	0.01 (0.00,0.03)