

# Reconciling the role of organic matter pathways in aquatic food webs by measuring multiple tracers in individuals

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**Abstract.** Few studies measure multiple ecological tracers in individual organisms, thus limiting our ability to differentiate among organic matter source pathways and understand consequences of dietary variation and the use of external subsidies in complex food webs. We combined two tracers, stable isotope (SI) ratios and fatty acids (FA), to investigate linkages among ecological compartments (water column, benthos, riparian zone) in food webs in waterholes of a dryland river network, the Border Rivers in southwestern Queensland, Australia. Comprehensive analyses of sources (plankton, periphyton, leaf litter, riparian grasses) and animals (benthic insects, mollusks, large crustaceans, fishes) for SI and FA showed that all three zones contribute to animal biomass, depending on species and life stage. Large fishes derived a subsidy from the riparian/floodplain zone, likely through the consumption of terrestrial and semi-aquatic insects and prawns that fed on detritivorous insects. Importantly, post-larval bony bream (*Nematalosa erebi*) and golden perch (*Macquaria ambigua*) were tightly connected to the water column, as evidenced by <sup>13</sup>C-depleted, <sup>15</sup>N-enriched isotope ratios and a high content of plankton-derived polyunsaturated fatty acids (eicosapentaenoic acid [EPA; 20:5ω3] and docosahexaenoic acid [DHA; 22:6ω3]). These observations were consistent with expectations from nutritional requirements of fish early life stages and habitat changes associated with maturity. These results highlight the importance of high-quality foods during early development of fishes, and show that attempting to attribute food-web production to a single source pathway overlooks important but often subtle subsidies that maintain viable populations. A complete understanding of food-web dynamics must consider both quantity and quality of different available organic matter sources. This understanding can be achieved with a combined SI and FA approach, but more controlled dietary studies are needed to estimate how FA profiles are modified by animals when consuming a diverse range of diets of variable quality.

**Key words:** arachidonic acid; benthic habitat; Border Rivers, Queensland, Australia; diet quality; docosahexaenoic acid; eicosapentaenoic acid; essential fatty acids; food-web subsidies; polyunsaturated fatty acids; stable isotopes; terrestrial organic matter; water column.

## INTRODUCTION

The provision of organic matter to food webs exerts strong controls on secondary and tertiary production and predator–prey interactions, ultimately dictating food-web structure ( Hairston and Hairston 1993). Identifying underlying sources of production and constraints on its transfer through food webs (Müller-Navarra et al. 2004, Karlsson et al. 2009) can aid in modeling sustainable yields of exploited animal populations (Pauly and Christensen 1995) as well as predicting how management activities or landscape change might influence biodiversity and overall ecosystem function (Bunn et al. 1999, Greig et al. 2011). One component of

organic matter flow, subsidies from one habitat to another, has the potential to sustain animal populations above levels that would be possible in the absence of such subsidies (Polis et al. 1997, Nakano and Murakami 2001). There is thus great interest in identifying the magnitude of subsidies from donor systems because they may benefit or otherwise influence recipient systems (Moore et al. 2004, Marczak et al. 2007, Wipfli and Baxter 2010).

Contrasting observations and perspectives have led to divergent views about the relative importance of different organic matter pathways in fueling aquatic food webs. Proponents of terrestrial–aquatic linkages (allochthony) point to detectable contributions of terrestrially derived particulate organic matter to the tissues of zooplankton, zoobenthos, and fishes in temperate lakes as evidence for a terrestrial subsidy (Cole et al. 2011, Solomon et al. 2011). Others, however,

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argue that aquatic sources of organic matter production (autochthony), via algae, provide dietary resources that enter the food web well out of proportion to their biomass and production because of their superior nutritional quality, and that terrestrial sources are unlikely to sustain animals (Thorp and Delong 2002, Bunn et al. 2003, Brett et al. 2009). Similarly, within aquatic systems there is heterogeneity in resource use among subhabitats (Vander Zanden et al. 2011), with some demonstrating that carbon produced by periphyton is more readily transferred up food webs compared to phytoplankton-derived carbon (Bunn et al. 2003, Vander Zanden et al. 2011, Jardine et al. 2013). Diet quality is often poorly considered in food-web studies, and usually only includes aspects such as food lability and palatability (Marecchelli et al. 2011, Mineau et al. 2012) or search and handling times and energy gains from consuming certain prey (e.g., Wipfli and Baxter 2010, Giacomini et al. 2013). This is despite the fact that there are nutritional aspects of diet governed by its biochemical composition; including N and P content, protein, vitamins and minerals, and polyunsaturated fatty acids (PUFA), that, if not provided as required, constrain animal growth and reproduction (Holt 2011).

Polyunsaturated fatty acids play important physiological roles in cell membranes. Metazoans, especially vertebrates, lack the necessary enzymes ( $\Delta 12$  and  $\Delta 15$  desaturases) to produce the essential PUFA, i.e., linoleic acid (LIN; 18:2 $\omega$ 6) and alpha-linolenic acid (ALA; 18:3 $\omega$ 3) that are only synthesized in plants; thus, these compounds must be supplied in the diet (Bell and Tocher 2009). Conversion of these 18-carbon PUFA to longer-carbon-chain PUFA, including eicosapentaenoic acid (EPA; 20:5 $\omega$ 3), docosahexaenoic acid (DHA; 22:6 $\omega$ 3), and arachidonic acid (ARA; 20:4 $\omega$ 6) can be achieved with varying efficiencies among species (Bell and Tocher 2009). Though some freshwater fishes manage to convert precursor fatty acids (FA) to EPA, DHA, and ARA, albeit mostly inefficiently, dietary supply of these long-chain PUFA is the most reliable means of obtaining these critical compounds. Thus, PUFA are preferentially retained in many animals, resulting in higher concentrations relative to diet (Kainz et al. 2004). Importantly, PUFA are thought to play an ecological role in aquatic food webs because high concentrations can optimize certain physiological functions such as somatic growth (Müller-Navarra et al. 2004, Brett et al. 2009), cold and heat tolerance, immune response (Arts and Kohler 2009), and neurological development. Thus, identifying the key pathways for uptake and trophic transfer of PUFA becomes critical for understanding how aquatic animals optimize survival, growth, and reproduction (Brett et al. 2009).

Despite the great potential for combining measures of diet quality with ecological tracers, simultaneous measurements of PUFA and tracers such as stable isotopes (SI) of C and N are limited (Perga et al. 2006, Carreon-Palau et al. 2013, Lau et al. 2014). Of these few

examples, only Lau et al. (2014) explicitly linked SI and PUFA measurements, revealing a positive relationship between mean autochthonous contribution to the diet and PUFA concentrations in insects and fish from small boreal lakes. At the heart of the application of any chemical tracer to determine dietary source pathways are two main concerns: (1) large differences in chemical profiles must exist among organic matter sources; and (2) there must be limited or predictable bioconversion or routing (referred to as fractionation in the SI literature) from the sources to animals. In this sense, combining SI and FA analyses as multiple lines of evidence may provide the needed resolution to track organic matter through food webs. This is because FA profiles differ strongly among sources (e.g., algae will always have higher EPA than higher plants; Brett et al. 2009), but we know less about their bioconversion because of the many complex biochemical pathways involved (Bell and Tocher 2009). Conversely, SI profiles can often overlap among sources, but controlled laboratory studies tell us that isotopic differences between an animal and its diet are reasonably consistent (e.g., 0.4‰  $\pm$  1.3‰ for  $\delta^{13}\text{C}$  and 3.4‰  $\pm$  1.0‰ for  $\delta^{15}\text{N}$ ; Post 2002).

Food-web reconstructions with tracers also tend to examine species means, thereby failing to consider individual variation in diet. Yet we know that many animals, especially gape-limited predators such as fishes, change their diet and habitat as they grow (Post 2003, Nunn et al. 2012). Individual variation in diet has important ecological and evolutionary consequences because selection acts on individuals (Araujo et al. 2011) and body size can drive this dietary variability (Woodward et al. 2005), yet it is an underreported measure in food-web studies. Thus, we must look beyond simple measures of overall community organic matter use and consider how, for example, the dietary patterns and organic matter sources of small, post-larval individuals allow them to pass through the bottlenecks present at critical life stages where growth rates can be slowed and mortality can be high (Post 2003).

Our objective was to study organic matter pathways leading to individuals in freshwater food webs, with a focus on the use of external subsidies and its nutritional consequences, using a model river system in southwestern Queensland, Australia. We examined how PUFA are supplied and transferred in an aquatic food web that included higher plants, algae, invertebrates, reptiles, and fishes. We evaluated the relative importance of different organic matter pathways (water column, benthos, riparian zone) in contributing to biomass and PUFA content by quantifying individual diet within and among the dominant fish and invertebrate species. Specifically, we asked whether shifts in animal diet with body size were accompanied by corresponding changes in the delivery of PUFA to body tissues. By doing so, we aimed to reconcile divergent views of the sources of organic matter fueling food webs of small lentic systems

(Brett et al. 2009, Cole et al. 2011, Vander Zanden et al. 2011).

## METHODS

### *Study location*

Our sampling took place several months after a prolonged flood event in the Weir, Narran, and Balonne rivers, in southwestern Queensland, Australia (Appendix A), in the headwaters of the Murray-Darling Basin. We consider this a model ecosystem to integrate SI and FA tracers because the food web is relatively simple. While 46 fish species are present in the basin, fewer than 20 are known to occur in the upper reaches where our sampling was conducted, and the number of potential food-source pathways is limited because aquatic macrophytes are extremely rare due to steep-sided banks and high turbidity. Due to large stream distances and the presence of many artificial barriers, there is no migratory connectivity from the upstream location of the sites in the basin to estuaries and marine habitats for mobile animals that could confound interpretation of the tracer data. Five sites were selected based on a combination of nearby gauging stations, permission to sample by station owners, and reasonable road access.

### *Environmental characteristics*

We sampled during low flow conditions when all five sites were generally narrow, shallow, turbid, and productive (Appendix A: Table A1). During the sample period, sites ranged from 17 to 81 m wide and 2 to 6 m deep. Turbidity ranged from 80 to 1250 Nephelometric turbidity units (NTU), resulting in high rates of light extinction (measured  $K_d$  values of 17–28  $m^{-1}$ ) and euphotic depths of 16–25 cm. High nutrient and chlorophyll concentrations categorized these study systems as meso- to hypereutrophic (Smith et al. 1999). Total phosphorus (range 15–58  $mg/m^3$ ), total nitrogen (500–4700  $mg/m^3$ ), and algal biomass in the water column (chlorophyll *a* range 5–170  $mg/m^3$ ) were all at the upper end of the observed range for flowing waters. Benthic chlorophyll *a* sampled from sediments (epipelton) in the narrow littoral fringe of the photic zone (cf Bunn et al. 2003) classified these sites as oligotrophic (Smith et al. 1999), with concentrations ranging from 1 to 18  $mg/m^2$ , but algal biomass was higher on sparse woody debris (epixylon), with most concentrations between 20 and 60  $mg/m^2$  (minimum of 1, maximum of 115  $mg/m^2$ ).

### *Sampling for stable isotopes and fatty acids*

We collected plant and animal samples at the five sites on three occasions (Appendix A). All food-web samples were split equally for analysis of SI (Griffith University, Nathan, Queensland) and FA (WasserCluster Lunz, Lunz am See, Austria). Aliquots for SI were frozen immediately in portable freezers at  $-20^{\circ}C$  and aliquots for FA were flash-frozen in liquid  $N_2$  on site. While we analyzed >1200 samples for SI, we selected a subset of

the samples ( $n = 571$ ) to be analyzed for FA that represented different taxa across sites and times.

### *Laboratory processing*

*Stable isotope analysis.*—Oven-dried samples were ground to fine powder and weighed into tin capsules for analysis of stable C and N isotopes. Animal material was weighed at 1 mg and plant material at 4 mg. Samples were combusted in an EA 3000 elemental analyzer (Eurovector, Milan, Italy). The resultant  $CO_2$  and  $N_2$  gases were delivered to an Isoprime mass spectrometer (GV Instruments, Manchester, UK). Samples were corrected with liquid standards previously calibrated against IAEA standards (CH6, CH7, N1, and N2). Duplicates within runs yielded mean differences of 0.4‰ for  $\delta^{13}C$  and 0.3‰ for  $\delta^{15}N$  ( $n = 27$ ).

*Lipid and fatty acid analysis.*—Freeze-dried samples (1–20 mg) were homogenized and stored in chloroform (2 mL) under an  $N_2$  atmosphere overnight at  $-80^{\circ}C$  to further break up tissues and enhance lipid extraction efficiency. Lipids were extracted using standard techniques (Appendix A) and fatty acid methyl esters (FAME) were analyzed using a gas chromatograph (Trace GC Thermo, Thermo Fisher, Waltham, Massachusetts, USA) with FID 260°C detector, 1 mL/min He as carrier gas, 40 mL/min  $H_2$  and 45 mL/min  $N_2$  as detector gases, air at 450 mL/min, and a temperature ramp of 140°C (for 5 min), increasing by 4°C/min (24 min) to 240°C (20 min) for a total 50 min, and equipped with a temperature-programmable injector and an autosampler. A Supelco (Sigma Aldrich, St. Louis, Missouri, USA) SP-2560 column (100 m, 25 mm inside diameter, 0.2- $\mu m$  film thickness) was used for FAME separation. Excalibur 1.4 (Excalibur Systems, Elmont, New York, USA) was used for calculation and, if necessary, manual resetting of the chromatograms. Fatty acid concentrations were calculated using calibration curves based on known standard concentrations (for details, see Heissenberger et al. [2010]).

### *Isotope mixing models, fatty acid profiling, and statistical analyses*

Using the C and N isotope data, we examined the contribution of four basal organic matter sources (plankton, periphyton,  $C_3$  detritus, and  $C_4$  detritus) to food webs using SIAR (Parnell et al. 2010, Appendix A) and applied biomass estimates from quantitative catch data to calculate the total contribution of each source to fish community biomass (Jardine et al. 2013, Jardine 2014, Appendix A). Our second tier of analysis was to examine individual variation in diet by using the SIARsolo function (Parnell et al. 2010). This allowed most-probable dietary source proportion estimates for individuals and enabled an examination of variation in diet across the lifespan of the most commonly analyzed organisms. We compared source proportion estimates with body size for prawns and three common and abundant fish species (carp (*Cyprinus carpio*), golden

TABLE 1. Percentage of total fish biomass and SIAR (Parnell et al. 2010, Appendix A) posterior distributions of dietary source contributions for the most dominant fish species in waterholes of the Border Rivers system, southwestern Queensland, Australia.

Species	Biomass (%)	<i>n</i>	Dietary source contribution (%)				Terrestrial contribution to biomass (%)
			Plankton	Periphyton	C <sub>3</sub> detritus	C <sub>4</sub> detritus	
Common carp ( <i>Cyprinus carpio</i> )	58	104	15 (8–21) (95% CI)	24 (13–34) (95% CI)	14 (4–25) (95% CI)	47 (44–50) (95% CI)	35 (28–44) (95% CI)
Golden perch ( <i>Macquaria ambigua</i> )	15	88	42 (36–48) (95% CI)	7 (1–17) (95% CI)	7 (1–17) (95% CI)	43 (40–46) (95% CI)	8 (6–9) (95% CI)
Bony bream ( <i>Nematalosa erebi</i> )							
<100 mm	1	64	73 (66–80) (95% CI)	5 (0–15) (95% CI)	8 (0–19) (95% CI)	13 (9–17) (95% CI)	0 (95% CI)
>100 mm	12	110	44 (38–50) (95% CI)	25 (8–40) (95% CI)	13 (2–31) (95% CI)	17 (14–20) (95% CI)	4 (2–6) (95% CI)
Hyrtil's tandan ( <i>Neosilurus hyrtilii</i> )	5	6	27 (5–45) (95% CI)	15 (1–40) (95% CI)	16 (1–44) (95% CI)	39 (30–49) (95% CI)	3 (2–5) (95% CI)
Spangled perch ( <i>Leiopotherapon unicolor</i> )	4	12	35 (20–48) (95% CI)	10 (1–28) (95% CI)	12 (1–33) (95% CI)	41 (35–48) (95% CI)	2 (1–3) (95% CI)
Goldfish ( <i>Carassius auratus</i> )	4	12	29 (12–44) (95% CI)	19 (1–40) (95% CI)	16 (1–39) (95% CI)	35 (28–42) (95% CI)	2 (1–3) (95% CI)
Total		99					53 (40–70) (95% CI)

Notes: Biomass percentage is shown as a mean. Other values are shown as medians, with 95% credible intervals in parentheses; *n* is the number of individuals analyzed for stable isotopes.

perch (*Macquaria ambigua*), and bony bream (*Nematalosa erebi*), using log-transformed body mass (g) as our measure of size. In each case, we used ordinary least squares (OLS) regressions and tested the fit of three models (linear, exponential, and quadratic).

Fatty acids identified for sources and animals were classified as saturated fatty acids (SAFA), monounsaturated fatty acids (MUFA), and PUFA. We also examined specific groups of FA markers including bacterial FA (i.e., 15:0 and 17:0 with their iso- and anteiso-branches, and 16:1 $\omega$ 9, 18:1 $\omega$ 12, 18:1 $\omega$ 7, 18:1 $\omega$ 6, and 16 $\Delta$ 9,10) and terrestrial FA (typically long-chain SAFA, including C22:0, C23:0, C24:0, C26:0, and longer). To determine how certain FA are metabolized or accumulated, we used a discriminant function analysis to discriminate among different taxa according to their FA profiles during each sampling period. This analysis creates functions that are combinations of predictors (in this case, FA) that maximize the difference among groups. We used this approach because we expected differences among groups (taxa) to be greater than those within groups (taxa) across sites and times (Lau et al. 2012).

To link the SI data with FA data, we first grouped plankton and periphyton source proportions together and collectively referred to them as aquatic sources. We then performed OLS linear regressions of percentage contribution of individual PUFA to total FA profiles (percentage FA) against proportion aquatic diet, separately for herbivorous and predatory insects (Lau et al. 2012), prawns and crayfish, and fishes. We regressed known PUFA (LIN, ALA, ARA, EPA, and DHA) as well as other dietary biomarkers including  $\omega$ 3/ $\omega$ 6 ratios. These ratios can differ between sources of organic

matter, with algae having higher  $\omega$ 3/ $\omega$ 6 than terrestrial detritus (Torres-Ruiz et al. 2007). Results were considered significant at  $P < 0.05$ .

## RESULTS

### Dietary source proportions

Insects, crustaceans, mollusks, and reptiles used all four organic matter pathways (plankton, periphyton, C<sub>3</sub> detritus, and C<sub>4</sub> detritus) in varying proportions. Though there was considerable variation within and among taxa (Appendix A: Fig. A4), known grazers and filter-feeders such as mayflies (Ephemeroptera), cased caddisflies (Trichoptera; Appendix A: Fig. A4), and mussels (Hyriidae; Appendix A: Fig. A4) had strong contributions from the plankton organic matter pathway (>60%), and predatory dragonflies (Odonata) also derived the majority of their diet from this pathway (Appendix B: Table B1). As expected, terrestrial insects had diets dominated by terrestrial sources (Appendix A: Fig. A4), with C<sub>4</sub> grasses contributing the highest amounts for both ants and grasshoppers (Appendix B: Table B1). Other taxa, including chironomids, herbivorous and predatory beetles, crayfish, prawns, and turtles, had mixed diets (Appendix B: Table B1).

Driven by the numerical dominance of carp, terrestrial inputs largely supported fish biomass. Because carp had a diet that was almost one-half made up of prey from the C<sub>4</sub> pathway (47%), this source was responsible for the greatest fraction of overall standing fish biomass (Table 1). Golden perch, the second most dominant species, also had a large contribution from this terrestrial pathway (43%). Even though the C<sub>3</sub> detrital pathway contributed very little to fish diet ( $\leq$ 16% for all species), the combination of C<sub>3</sub> and C<sub>4</sub> detrital pathways

accounted for 53% of the mean fish biomass (Table 1), a result that was consistent across sites and times (Appendix B: Table B2). Periphyton contributed surprisingly little to fish biomass, accounting for  $\leq 25\%$  of the diet in all species.

*Individual measurements: body size and organic matter pathways*

Dietary organic matter pathways varied according to body size for three of the four most common and abundant taxa (Fig. 1) and were generally well described by log-linear functions, though good fits by quadratic functions suggested that large initial changes in diet at small body sizes were followed by smaller changes at larger body sizes (Appendix B: Table B3). For *Macrobrachium* spp., the contribution of the planktonic pathway declined with body mass from 40% at a body size of 30 mg to 25% at a body size of 5 g ( $r^2 = 0.31$ ,  $F_{1,134} = 59.82$ ,  $P < 0.001$ ) while the use of the  $C_4$  detrital pathway increased from 20% to 35% over this size range ( $r^2 = 0.16$ ,  $F_{1,134} = 25.79$ ,  $P < 0.001$ ; Fig. 1a). Golden perch exhibited a similar pattern, with the contribution from plankton declining with mass from 35% at 100 mg to 20% at 1 kg ( $r^2 = 0.34$ ,  $F_{1,86} = 43.48$ ,  $P < 0.001$ ), and the  $C_4$  detritus contribution increasing with mass from 10% to 40% ( $r^2 = 0.38$ ,  $F_{1,86} = 53.84$ ,  $P < 0.001$ ; Fig. 1b). Carp did not display variation in diet associated with size ( $P > 0.05$  for all regressions; Fig. 1c; Appendix B: Table B3), but data are lacking for very small individuals because they were not captured in our fyke nets. Bony bream exhibited a strong decline in the planktonic contribution with increasing size from almost 50% at 1 g to 25% at 500 g ( $r^2 = 0.35$ ,  $F_{1,171} = 93.92$ ,  $P < 0.001$ ; Fig. 1d). The contribution from  $C_4$  detritus marginally increased with body size (from 10% to 20%;  $r^2 = 0.12$ ,  $F_{1,171} = 23.41$ ,  $P < 0.001$ ) and was paralleled by a consistent increase in the contribution from periphyton (from 15% to 30%;  $r^2 = 0.44$ ,  $F_{1,171} = 132.30$ ,  $P < 0.001$ , data not shown) and to a lesser extent from  $C_3$  detritus with increasing body size (Appendix B: Table B3).

*Fatty acids in sources and animals*

Fatty acid profiles differed strongly among sources. SAFA were highest in leaf litter and aquatic sources, suggesting the latter contained a mix of algae, terrestrial detritus, fungi, and bacteria (Appendix B: Table B4). Long-chain SAFA (22:0, 23:0, 24:0, 26:0, 28:0) were, however, restricted to leaf litter and to a lesser extent, grasses (Fig. 2). Aquatic sources were highest in MUFA, again suggesting they represented mixed sources. Fresh terrestrial plants (herbaceous vegetation and pasture grasses) had high short-chain PUFA because of a large contribution from ALA and LIN (Appendix B: Table B4). While all sources contained appreciable quantities of these two precursors ( $>3\%$  of total), because terrestrial sources were particularly rich in ALA and LIN ( $>7\%$  of total), there was limited differentiation in

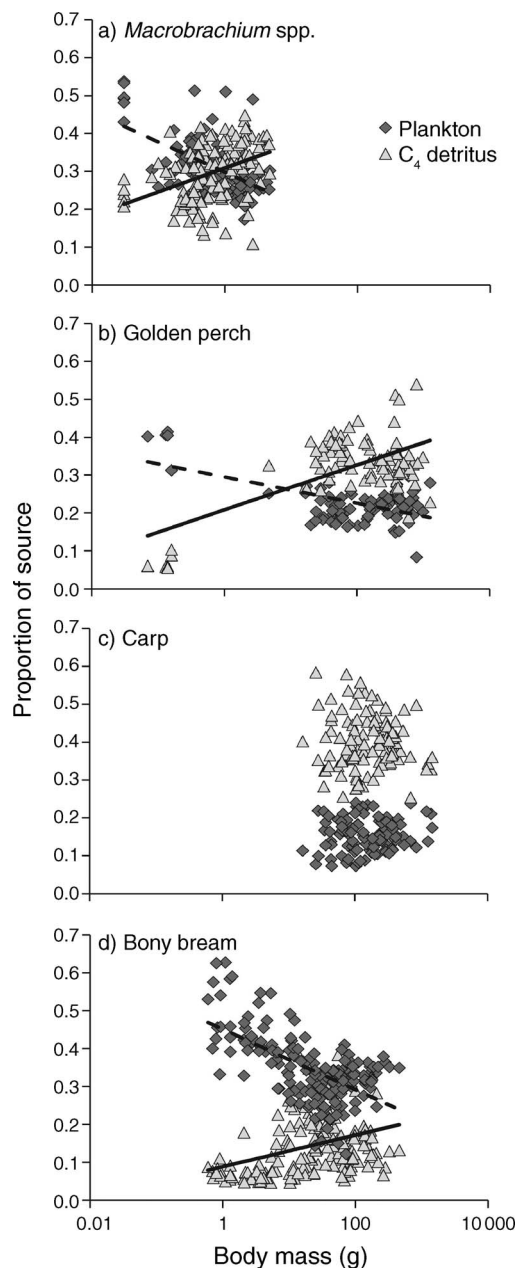


FIG. 1. Change in dietary source proportions with body size (presented on a log scale) for (a) *Macrobrachium australiense*, (b) golden perch (*Macquaria ambigua*), (c) common carp (*Cyprinus carpio*), and (d) bony bream (*Nematalosa erebi*) of the Border Rivers, Queensland, Australia. Lines indicate significant ( $P < 0.05$ ) best-fit log-linear regressions for plankton vs. body size (dashed line) and  $C_4$  detritus vs. body size (solid line), with regression parameters and fit described in Appendix B: Table B3.

$\omega 3/\omega 6$  ratios between aquatic and terrestrial sources (Appendix B: Table B4). While all sources had low percentages of the  $\omega 6$  PUFA ARA ( $<1.5\%$ ), only aquatic sources contained the  $\omega 3$  PUFA EPA and DHA (2.7% and 5.2% EPA in seston and periphyton, respectively).

Transfer of dietary FAs to animals varied among taxa. Zooplankton accumulated PUFA that accounted for 55% of total FAs, and their DHA content vastly exceeded that of all other taxa, leading to the highest  $\omega 3/\omega 6$  ratios of any taxon (Appendix B: Table B5). EPA was also highest in zooplankton, though other invertebrates such as mayflies, corixids, notonectids, and dragonflies also had a high relative contribution from this PUFA. Grasshoppers and crickets obtained high proportions of the precursors ALA and LIN from their diet of grasses, but all other PUFA were absent in these taxa. Ants had high LIN, but very low ALA, and also an extremely high contribution from a single FA, 18:1 $\omega 9$  (45%). All fish species maintained high proportions of DHA in their tissues ( $>10\%$ ) and PUFA exceeded SAFA and MUFA for all species except Hyrtl's tandan (*Neosilurus hyrtl*) (Appendix B: Table B6). Bony bream had the highest ALA and EPA. Terrestrial FA were virtually absent ( $\leq 1\%$ ) in all taxa, and overall  $\omega 3/\omega 6$  ratios were relatively low ( $<2.5$  in all species).

When considering full FA profiles, groups were clearly separated based on Function 2 of the discriminant function analysis, which included  $\omega 3$  and  $\omega 6$  precursors and PUFA (Fig. 2). Fishes were differentiated from other taxa based on their high proportions of long-chain C20 and C22 PUFA at all sampling times. Zooplankton had profiles that were most similar to fishes, and zoobenthos formed an intermediate group between aquatic and terrestrial sources and periphyton and seston (Fig. 2). Terrestrial invertebrates were grouped with terrestrial plants and characterized by high ALA and LIN. These patterns did not vary considerably across the three sample periods (Fig. 2).

#### *Individual measurements: combining isotopes and fatty acids*

By integrating SI with FA, the nutritional implications of differential resource use within and among species became apparent. PUFA profiles were strongly linked to the contribution of aquatic sources to the diet, with high ALA, DHA, EPA, and  $\omega 3/\omega 6$  ratios and low LIN and ARA in animals that fed on the plankton and periphyton source pathways (Fig. 3; Appendix B: Table B7). While the strength and significance of the relationships between individual PUFA and dietary source contributions varied among taxa, coefficients were all positive for  $\omega 3$  PUFA and all negative for  $\omega 6$  PUFA (Fig. 3; Appendix B: Table B7). As a result, all taxa had significantly higher  $\omega 3/\omega 6$  ratios when they fed more on the aquatic source pathway. Relationships between  $\omega 3/\omega 6$  ratios and proportion aquatic diet were significant ( $P < 0.05$ ) for herbivorous insects ( $r^2 = 0.11$ ,  $F_{1,44} = 5.62$ ,  $P = 0.022$ ), predatory insects ( $r^2 = 0.26$ ,  $F_{1,32} = 11.57$ ,  $P = 0.002$ ), prawns ( $r^2 = 0.40$ ,  $F_{1,28} = 18.53$ ,  $P < 0.001$ ), and fishes ( $r^2 = 0.42$ ,  $F_{1,128} = 92.71$ ,  $P < 0.001$ ; Fig. 3) and for all taxa corresponded to increases from  $\omega 3/\omega 6 < 1$  when proportion aquatic was equal to 0.20 to  $\omega 3/\omega 6 > 2$  when proportion aquatic was equal to 0.70.

## DISCUSSION

By measuring dietary source pathways leading to individuals, our results show that the full spectrum of organic matter sources available to these river food webs is used to some degree, consistent with findings from small temperate lakes (Solomon et al. 2011, Lau et al. 2014). Animals exploited these different pathways during different life stages, with smaller individuals coupled to the water column where they fed on plankton rich in PUFA that supported somatic growth and development (Arts and Kohler 2009). Large fishes, however, retained a large portion of terrestrial diet sources, suggesting a considerable terrestrial subsidy for these food webs. While simple measures of biomass (Jardine et al. 2013, Jardine 2014) result in an equal contribution of terrestrial and aquatic sources to the food web, they ignore important resources (i.e., plankton) that are critical for post-larval life stages. We suggest that prior admonitions for the importance of particular organic matter sources in sustaining food webs (Brett et al. 2009, Cole et al. 2011) are misdirected. Instead, because most food webs consist of animals that exhibit mixed source use, a more fruitful path forward will be to identify environmental conditions that can promote or hinder dependence on particular pathways or subsidies (Müller-Navarra et al. 2004, Karlsson et al. 2009, Marcarelli et al. 2011, Solomon et al. 2011, Roach 2013) and how that dependency varies among species and life stages (Marczak et al. 2007).

Terrestrial inputs to freshwater food webs, as suggested by stable isotopes, are viewed as subsidies that contribute a significant fraction of the trophic base for animals (Solomon et al. 2011, Bartels et al. 2012, Scharnweber et al. 2014). We found considerable use of a terrestrial subsidy by insects, crustaceans, reptiles, and fishes, largely originating with pasture grasses that use the C<sub>4</sub> photosynthetic pathway. Floods that preceded the study likely delivered large volumes of terrestrial material into the river channel, including both detrital matter and trapped insects (Junk et al. 1989, Roach 2013). Conditioning of this detritus to dissolved organic matter could allow its indirect entry to the food web (Cole et al. 2011). Further inputs of insects likely occurred post-flood, aided by the long and narrow shape of these waterholes, resulting in high perimeter to area ratios and thus large interfaces for interaction between aquatic and terrestrial environments (Marczak et al. 2007). This strong contribution to the long-term requirements of adult organisms reinforces the role of detritus as a stable, persistent reservoir of organic matter in aquatic food webs (Moore et al. 2004).

Periphyton played a surprisingly limited role in the food web. Rivers such as ours that transport large volumes of suspended sediment are heterotrophic during and immediately after periods of high flow (Bunn et al. 2006, Roach et al. 2014). Benthic algal growth in the narrow littoral fringe was limited, as evidenced by moderate epilimnetic chlorophyll *a* concentrations that are

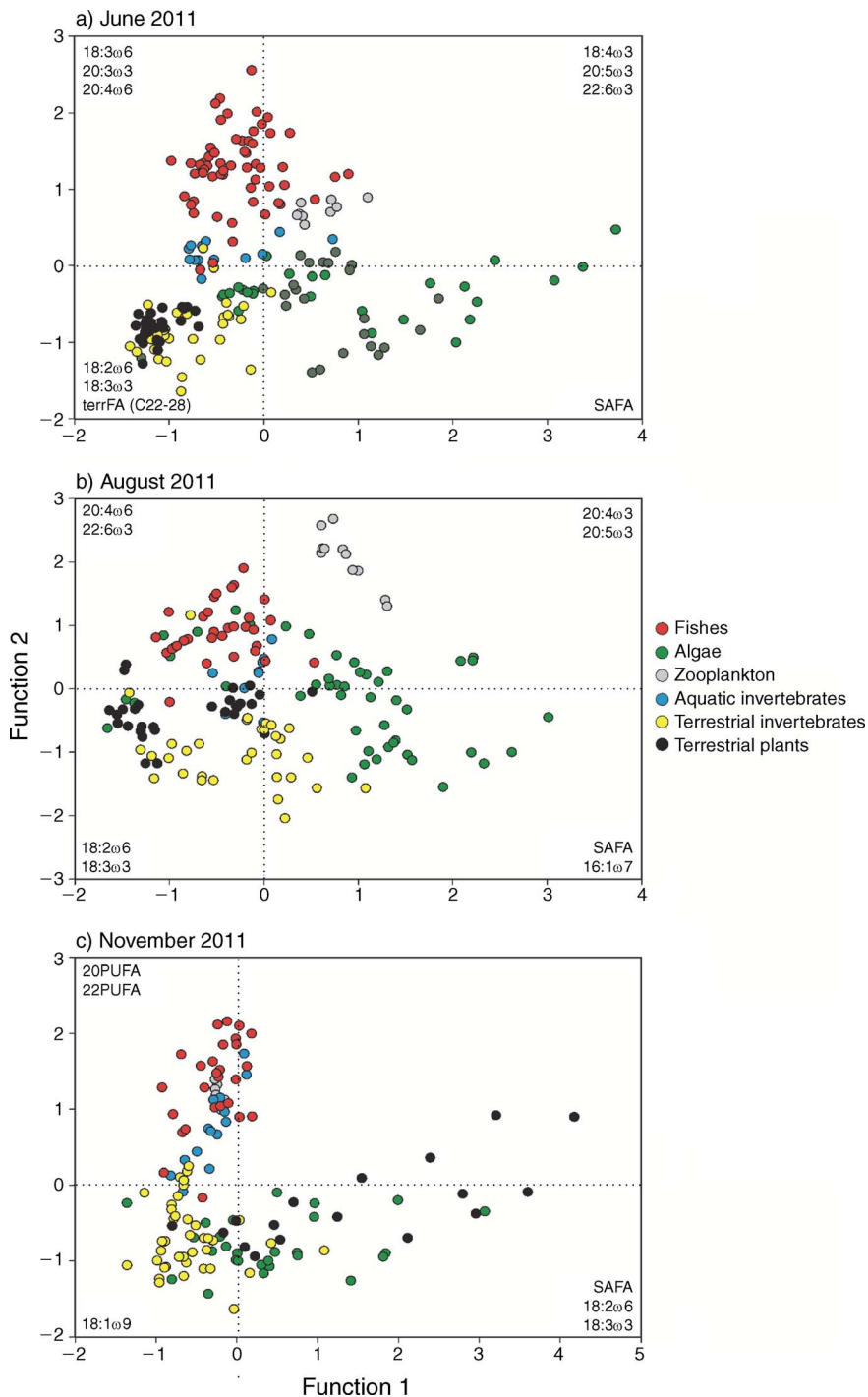


FIG. 2. Discriminant function analysis of fatty acids (FA) for sources and animals in waterholes sampled (a) four months post flood, (b) six months post flood, and (c) nine months post flood. At all sampling periods, fishes and zooplankton are clearly discriminated from other groups based on Function 2 (*y*-axis), which indicates high proportions of physiologically active, long-chain polyunsaturated fatty acids (PUFA) such as ARA (arachidonic acid; 20:4 $\omega$ 6), EPA (eicosapentaenoic acid; 20:5 $\omega$ 3), and DHA (docosahexaenoic acid; 22:6 $\omega$ 3), whereas algae, terrestrial plants, and terrestrial invertebrates have higher proportions of short-chain PUFA such as LIN (linoleic acid; 18:2 $\omega$ 6) and ALA (alpha-linolenic acid; 18:3 $\omega$ 3), as well as saturated fatty acids (SAFA). Zoobenthos (aquatic invertebrates) display an intermediate mixture of these FA.

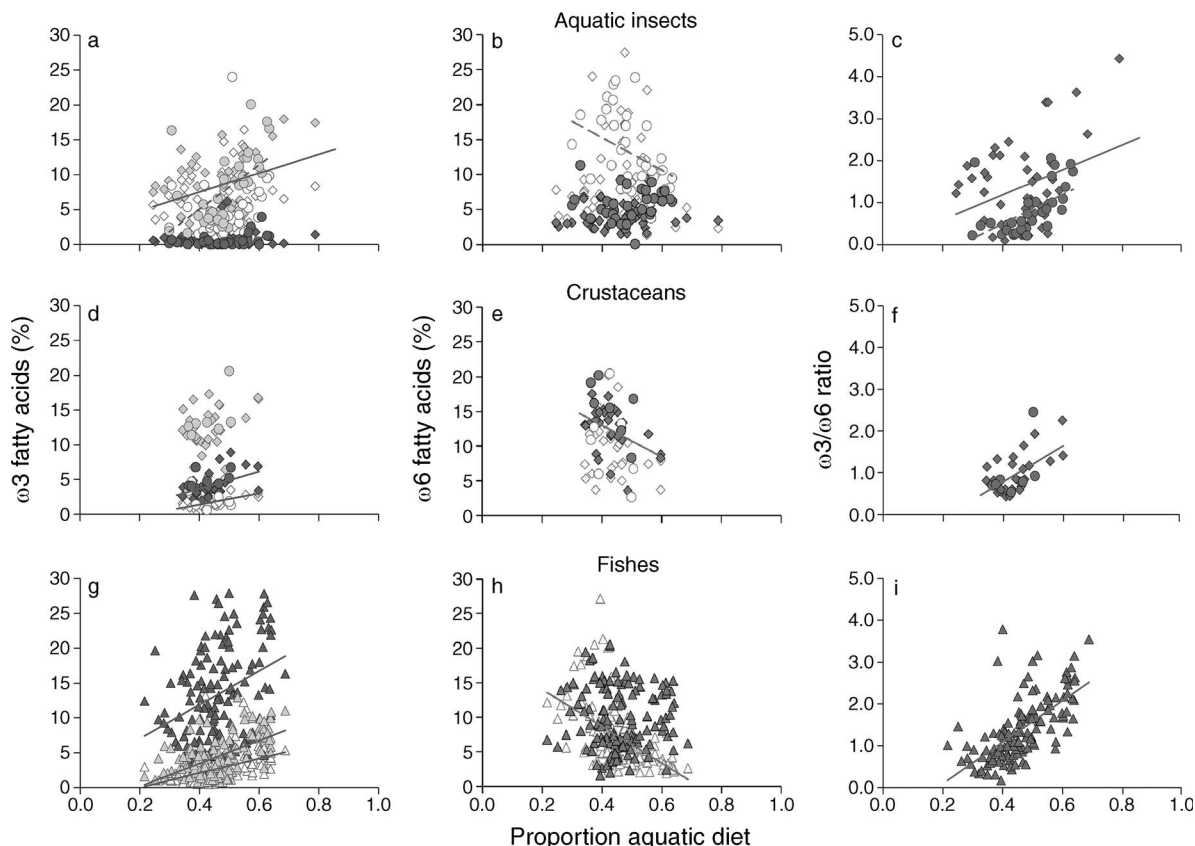


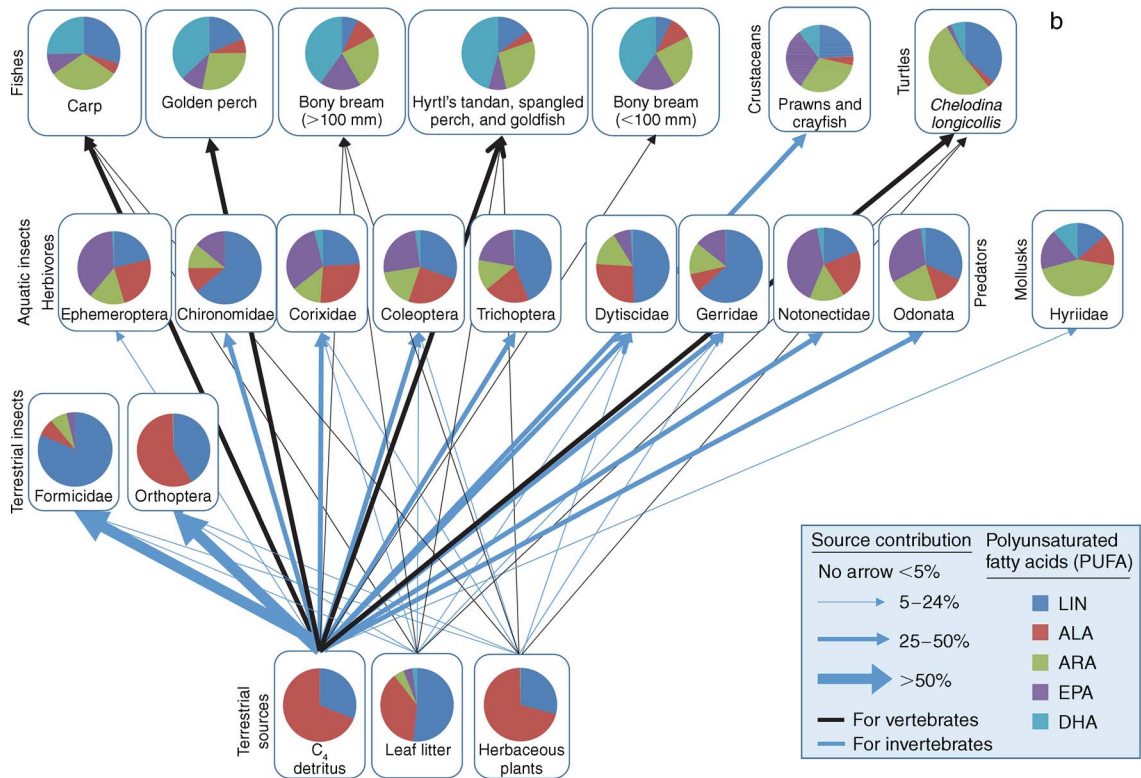
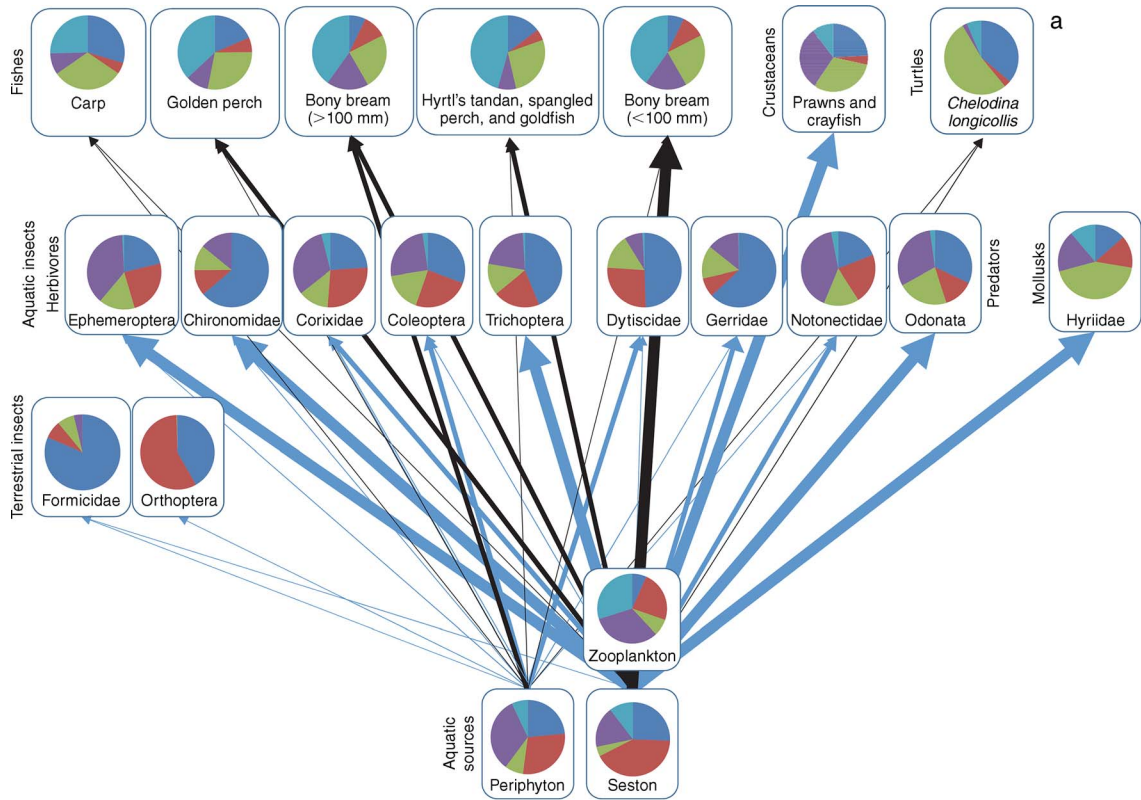
FIG. 3. Relationship between PUFA and the proportional contribution to the diet from the aquatic source pathways (phytoplankton + periphyton) for aquatic insects (a–c, diamonds show herbivores, circles show predators), crustaceans (d–f, diamonds show *M. australiense*, circles show *Cherax* spp.) and fishes (g–i). Symbols in (a, d, g) are  $\omega 3$  PUFA (open are ALA, shaded are EPA, solid are DHA), symbols in (b, e, h) are  $\omega 6$  PUFA (open are LIN, solid are ARA), and symbols in (c, f, i) are  $\omega 3/\omega 6$  ratios (secondary y-axis). Significant ( $P < 0.05$ ) regressions are indicated with solid and dashed lines and are described in Appendix B: Table B6.

unlikely to contribute substantially to food-web maintenance, unlike other dryland rivers (Bunn et al. 2003). Owing to high turbidity, our measures of light extinction ( $17\text{--}28\text{ m}^{-1}$ ) were vastly higher than values attributed to dissolved organic matter that were previously hypothesized to limit benthic production ( $0.3\text{--}4.1\text{ m}^{-1}$  [Ask et al. 2009],  $0.6\text{--}1.4\text{ m}^{-1}$  [Solomon et al. 2011]). Our epipelonal and epixylon assemblages were also dominated by chlorophytes (filamentous green algae) that are considered to be of limited nutritional quality compared to Bacillariophytes (diatoms) because the former lack C20 and C22 PUFA (Brett and Müller-Navarra 1997, Napolitano 1999).

Though terrestrial sources did not contain C20 and C22 PUFA, they had high contents of C18 PUFA (ALA and LIN), the precursors for C20 and C22 PUFA synthesis. Terrestrial vegetation, particularly grasses and herbaceous plants, had high ALA, in line with results for trees from diverse biomes (mangroves [Carreon-Palau et al. 2013], alder and hemlock [Volk and Kiffney 2012]). Highly processed terrestrial material tends to be lower in ALA (Torres-Ruiz et al. 2007), much like we observed for leaf litter. Terrestrial insects (orthopterans and lepidopterans) also had high ALA, probably due to feeding directly on ALA-rich live plant material, which is consistent with recent meta-analyses that included

FIG. 4. A PUFA source food web illustrating dietary source percentages (arrows) for animals including terrestrial insects, aquatic insects, mollusks, crustaceans, turtles, and fishes, calculated using stable isotope (SI) ratios. Fishes include Hyrtl's tandan (*Neosilurus hyrtlii*), spangled perch (*Leiopotherapon unicolor*), and goldfish (*Carassius auratus*). Pie charts show the relative proportion of three physiologically active PUFA (ARA, EPA, DHA) and their two precursors (LIN and ALA) in the sources and animals. Plankton source proportions were estimated using zooplankton as a proxy for pure phytoplankton (arrows originate with seston, an impure representation). C<sub>3</sub> detritus source proportions were split equally between herbaceous plants and leaf litter because their SI ratios were similar but they had different fatty acid profiles.





these taxa (Fontaneto et al. 2011, Rumpold and Schluter 2013). Ants, however, had high LIN, but <2% ALA, EPA, and DHA, and such low content is likely to be typical of this insect family (Sihamala et al. 2010). These differences among taxa within broader categories of organic matter pathways thus reinforce our notion that the quality of a given subsidy must be taken into account when considering its role in recipient food webs (Marcarelli et al. 2011).

Dietary PUFA requirements vary according to life stage, a feature that has long been recognized in aquaculture (Tocher 2010), yet is not often considered in models of growth and development of wild fishes, even though shifts from planktivory to benthivory and piscivory are common in fishes (Nunn et al. 2012). These shifts are ascribed to expansion of visual fields, improved prey handling ability, and the development of the fins and the alimentary tract (Nunn et al. 2012) that lead to peaks in foraging ability on small zooplankton at small body sizes in generalist predators. All of these developmental changes could be enhanced by the delivery of PUFA (Arts and Kohler 2009), and our results suggest that wild fishes are adapted to exploit PUFA-rich dietary sources in their early development. The changes we observed in golden perch diets with size are consistent with hatchery studies on this species. Post-larval perch with a diet supplemented with EPA (8.4%) had higher survival and were longer and heavier compared to the control diet (0.9% EPA; Sheikh-Eldin et al. 1997). This difference in PUFA content is similar to the difference between zooplankton (13.7% EPA) and terrestrial insects (<1% EPA) in our food webs. We suggest that the importance of the water column for feeding by post-larval fishes (Nunn et al. 2012) is a general phenomenon that occurs globally in small freshwater lakes and lowland rivers. Large fishes are rarely supported solely by the water column (Bunn et al. 2003, Solomon et al. 2011, Vander Zanden et al. 2011, Jardine et al. 2013, this study), yet many species are known to shift their diet from planktivory to benthivory during their development (Appendix C: Table C1). Organisms may also make a return switch to  $\omega$ 3-rich sources prior to reproduction, and partition this high-quality diet to gonadal development, as typically observed in controlled fish-feeding studies (e.g., Tocher 2010). In our data set, bony bream shifted from 50% reliance on planktonic sources in early life to a minimum of 20% reliance by the time they reached a size of ~125 mm, coincident with the onset of maturation in this species (Puckridge and Walker 1990). After this size, planktonic C again increased in importance to ~35%, suggesting that EPA- and DHA-rich plankton once again featured in the diet, perhaps through the consumption of detrital material in the deep-water zone of the waterholes. This highlights an important area for further research, an examination of the dietary sources fueling reproduction using measurements of FA (Gomes et al. 2010) and SI.

The  $\omega$ 3/ $\omega$ 6 ratio was a strong indicator of aquatic vs. terrestrial source reliance. There were significant relationships between proportion aquatic diet and  $\omega$ 3/ $\omega$ 6 for all taxa examined, thus providing the most comprehensive evidence of this ratio as a food-web marker (Napolitano 1999, Koussoropolis et al. 2008). Despite these trends, insects and fishes in our study generally had low  $\omega$ 3/ $\omega$ 6 compared to taxa examined in colder aquatic ecosystems (Torres-Ruiz et al. 2007, Ahlgren et al. 2009, Volk and Kiffney 2012), which could be due to remoteness of the study sites from  $\omega$ 3-rich marine sources (e.g., anadromous fishes; Rinella et al. 2013), the relatively high contribution of  $\omega$ 6-rich terrestrial sources to the diets of invertebrates and fishes (Solomon et al. 2011), and/or a lower physiological requirement of insects and fishes in subtropical ecosystems for high amounts of  $\omega$ 3, as apparently is the case in organisms of colder ecosystems.

Optimal  $\omega$ 3/ $\omega$ 6 ratios are likely species specific (Ahlgren et al. 2009), and our data show that internal regulation of this ratio and of physiologically active PUFA (EPA, DHA, ALA) can be limited due to dietary supply constraints (Fig. 3). Though animals can bioconvert and preferentially retain particular FAs when they are in low dietary supply, these adaptations are not enough to overcome dietary limitation, as is observed for laboratory-reared isopods fed diets of varying quality (EPA-rich and EPA-poor; Galloway et al. 2014). Much of the scatter in our PUFA vs. proportion aquatic diet relationships (Fig. 3) is therefore likely due to preferential retention of dietary PUFA even when supply is low. This internal conversion (akin to fractionation of stable isotopes) has limited the application of FAs as source tracers in food webs because these metabolic modifications make it difficult to confidently trace back to the original source of production. The application of calibration coefficients, or the proportional change in a particular FA from diet to animal, can allow back-calculation of dietary source proportions in predators with high-quality diets (Iverson et al. 2004). With a few exceptions, most FAs exhibit limited proportional change from diet to predator (between 0.5 and 2.0) that is consistent across several species of birds and mammals (Iverson 2009). Conversely, grazers and omnivores that consume diets of variable quality are more likely to exhibit variable calibration coefficients (Galloway et al. 2014). As such, recent quantitative FA models to identify dietary sources use resource libraries developed from controlled feeding studies of the species of interest on all possible diets (Galloway et al. 2014), thus negating the need for calibration coefficients. Use of this approach in a context with multiple animal species such as ours, however, is clearly premature without extensive laboratory validation. Because many controlled PUFA dietary studies have already been conducted with a multitude of taxa (e.g., Atlantic salmon *Salmo salar*; Budge et al. 2012), we need a meta-analytic approach such as those conducted with

stable isotopes (e.g., Post 2002) to establish mean calibration coefficients and the error among species and diets to unlock the massive potential of FAs as quantitative tracers.

Until we are able to generalize across multiple species, a synthesis of SI and FA data into a single mixing model will remain elusive. Instead, we propose a focus on the physiologically active PUFA and their application in PUFA source food webs (Fig. 4; cf flow food web Cross et al. 2013). This shifts the use of PUFA as tracers to their use as consequences of dietary variation, allowing a better appreciation for the role of quality in determining food-web patterns and strength of subsidies (Wipfli and Baxter 2010). These food-web representations could be constructed using either stomach content analyses or stable isotopes, and they show how PUFA are synthesized and transferred, in concert with bulk C and N, and how differential routing of dietary components can paint different food-web pictures (Brett et al. 2009, Cole et al. 2011). From this representation, we can see that zooplankton clearly play a central role in providing EPA and DHA to higher trophic levels (Fig. 4), whereas terrestrial sources (both plant detritus and insects), may offer a bulk subsidy based on SI, but their ability to supply physiologically active PUFA is limited. In addition to quantity, timing, and spatial components of subsidies (Baxter et al. 2005, Yang et al. 2010, Marcarelli et al. 2011) predictive food-web models therefore need to explicitly consider food quality and examine kinetics of both bulk C and N transfer and PUFA and resultant growth of animals (e.g., zooplankton; Perhar et al. 2012). While these models are an important step toward recognizing the role of PUFA (Perhar et al. 2012), they need to be extended beyond the water column, especially given the clear evidence for the use of benthic and terrestrial sources in aquatic food webs that often provide additional resources in sustaining animals (Bunn et al. 2003, Solomon et al. 2011, Vander Zanden et al. 2011, Jardine et al. 2013, this study).

Consideration of PUFA in food-web models becomes particularly important when environmental change alters the flow of organic matter and essential compounds to higher trophic levels (Bunn et al. 1999, Greig et al. 2011). Beyond the replacement of native with invasive species (Mineau et al. 2012), human-induced changes to aquatic ecosystems have the potential to limit PUFA supply to the top of the food web (Müller-Navarra et al. 2004). In-stream barriers to anadromous fish migration and large-scale clearing of riparian zones can interrupt PUFA delivery from subsidies. Further effects from wholesale landscape alterations include proliferation of light-tolerant and bloom-forming taxa (Bunn et al. 1999, Müller-Navarra et al. 2004) and erosion, both of which may shade PUFA-rich diatoms in the benthos. Light extinction in our study waterholes was high due to high turbidity, vastly exceeding the light limitations induced by organic matter in many temper-

ate oligotrophic lakes (Karlsson et al. 2009, Solomon et al. 2011). This shading limited benthic production (Roach et al. 2014) and the transfer of periphyton to higher trophic levels. Because animals in unperturbed aquatic systems are likely adapted to use a mix of available resources, including subsidies, it is imperative that systems are managed to retain a balance of available food sources to native species. More comprehensively considering the role of PUFA in food-web structure and function, and how subsidies may deliver PUFA, will assist in achieving these management objectives (Marczak et al. 2007, Wipfli and Baxter 2010).

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## SUPPLEMENTAL MATERIAL

## Ecological Archives

Appendices A–C are available online: <http://dx.doi.org/10.1890/14-2153.1.sm>