

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 ¹³C-depleted, ¹⁵N-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,

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 ω 6) and alpha-linolenic acid (ALA; 18:3 ω 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 ω 3), docosahexaenoic acid (DHA; 22:6 ω 3), and arachidonic acid (ARA; 20:4 ω 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- μm 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 ₃ detritus	C ₄ 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 ω 9, 18:1 ω 12, 18:1 ω 7, 18:1 ω 6, and 16 Δ 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 ω 3/ ω 6 ratios. These ratios can differ between sources of organic

matter, with algae having higher ω 3/ ω 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, C₃ detritus, and C₄ 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 C₄ 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 C₄ 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 C₃ detrital pathway contributed very little to fish diet (\leq 16% for all species), the combination of C₃ and C₄ 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

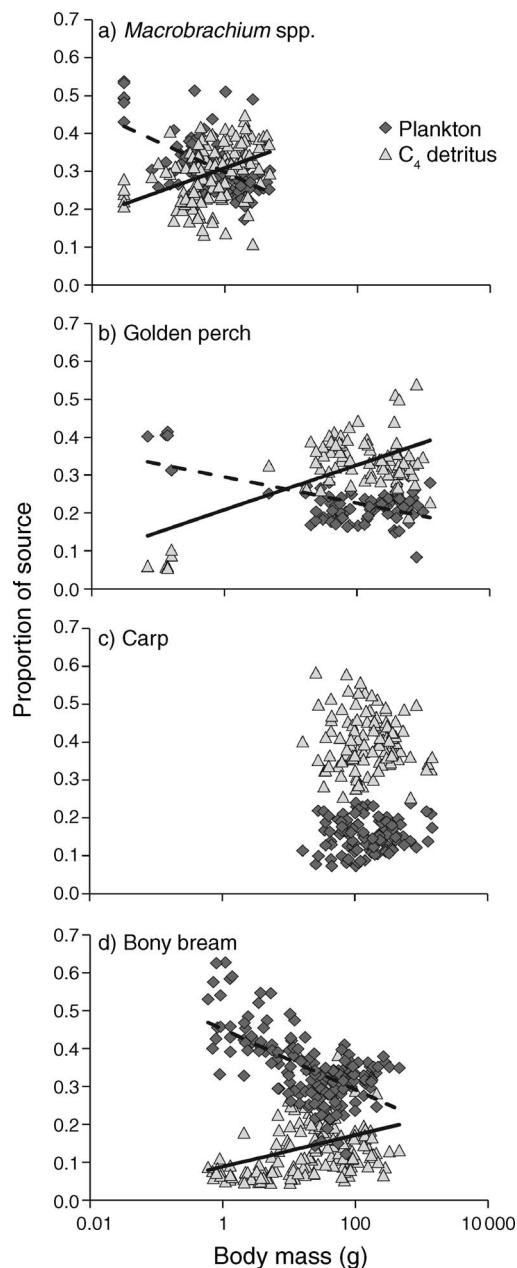


FIG. 1. Change in dietary source proportions with body size (presented on a log scale) for (a) *Macrobrachium australiense*, (b) golden perch (*Macquaria ambigua*), (c) common carp (*Cyprinus carpio*), and (d) bony bream (*Nematalosa erebi*) of the Border Rivers, Queensland, Australia. Lines indicate 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 C₄ 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

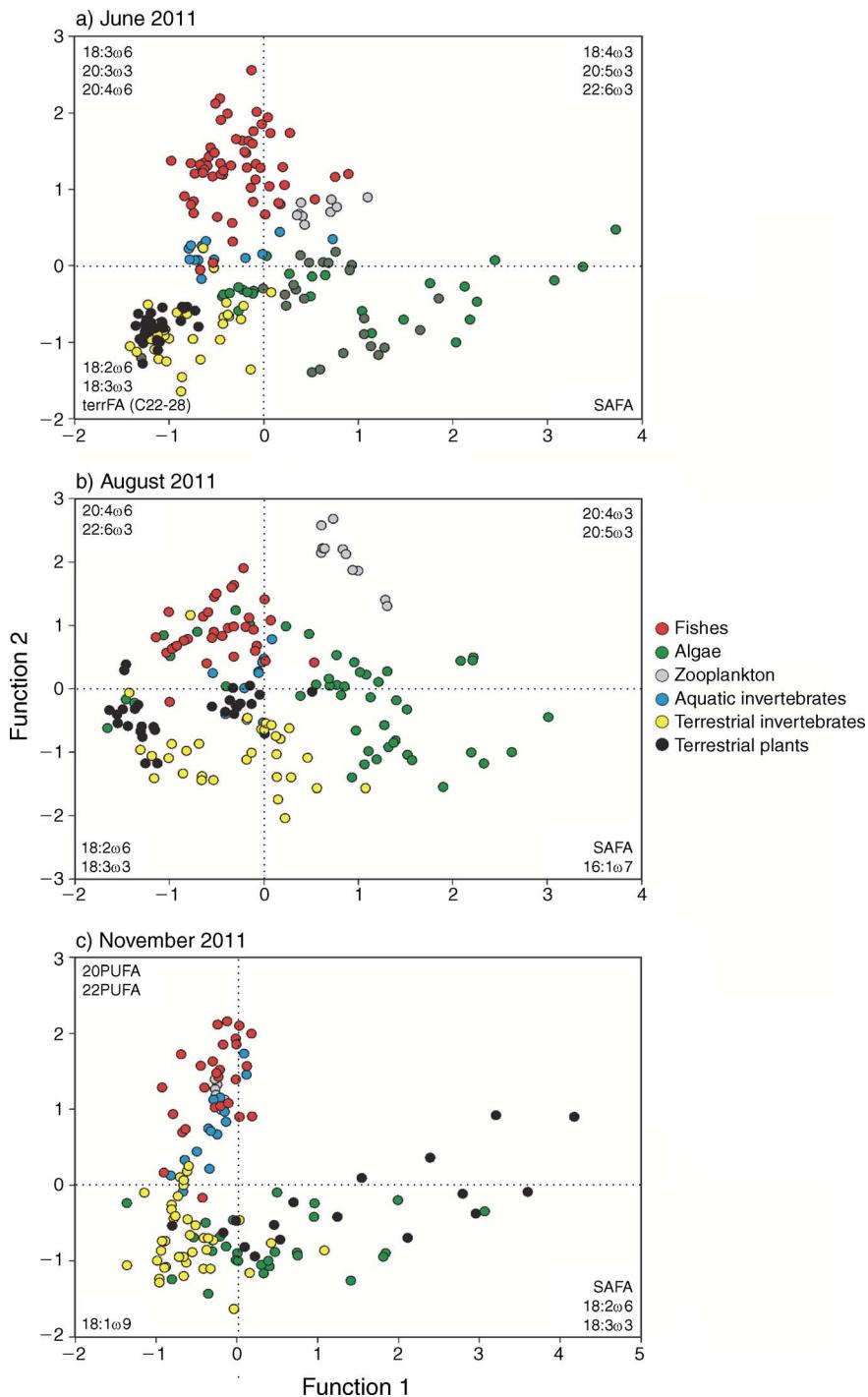


FIG. 2. Discriminant function analysis of fatty acids (FA) for sources and animals in waterholes sampled (a) four months post flood, (b) six months post flood, and (c) nine months post flood. At all sampling periods, fishes and zooplankton are clearly discriminated from other groups based on Function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

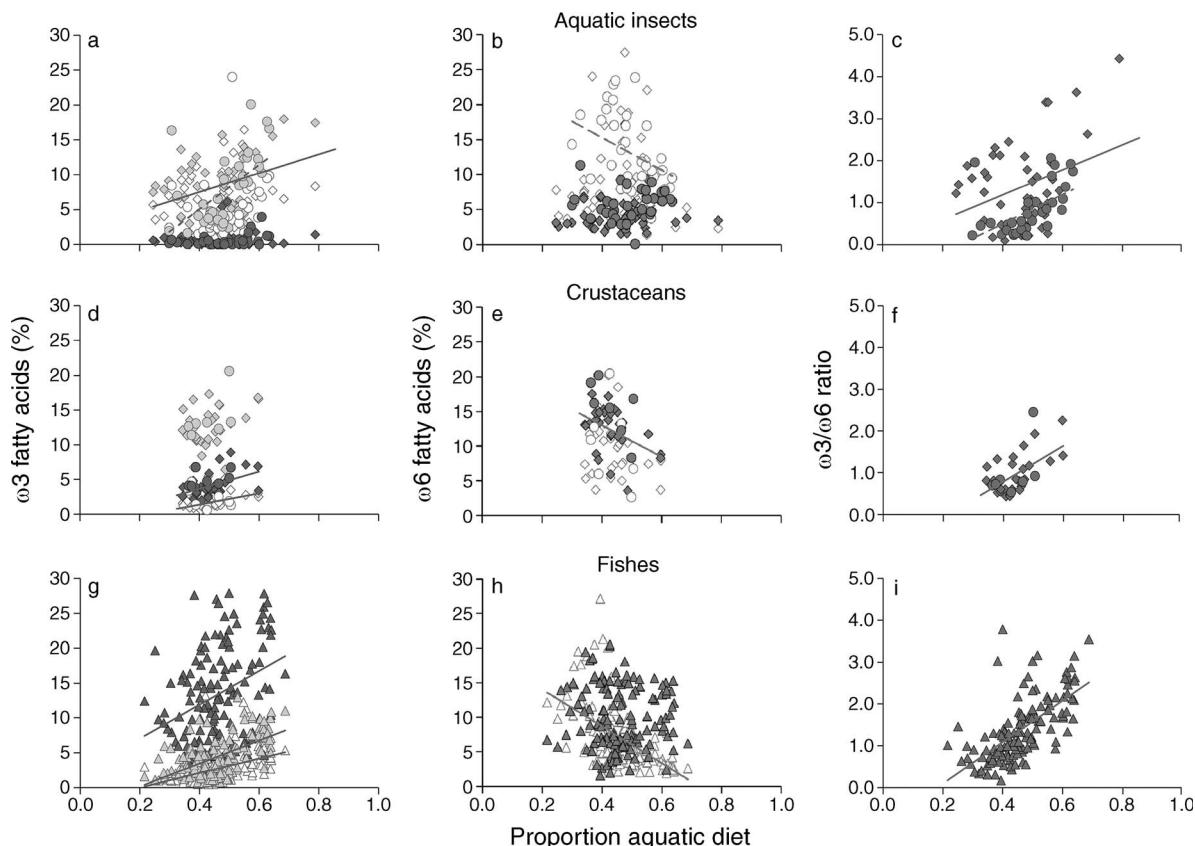
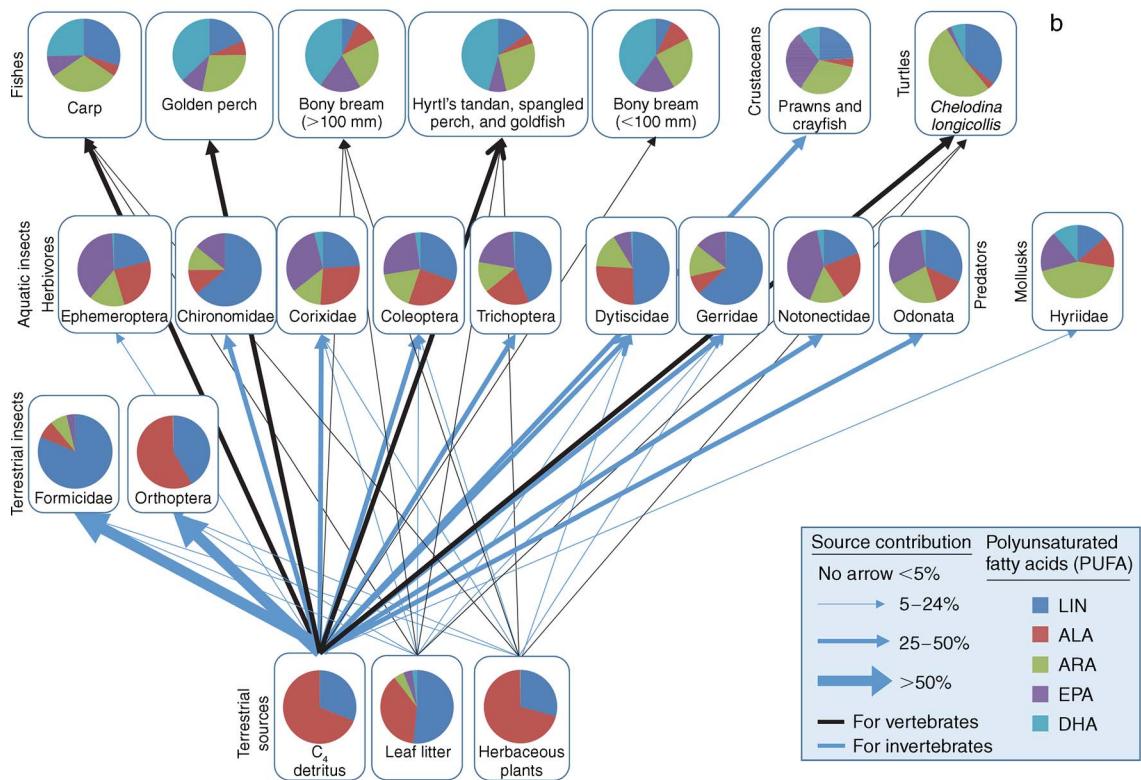
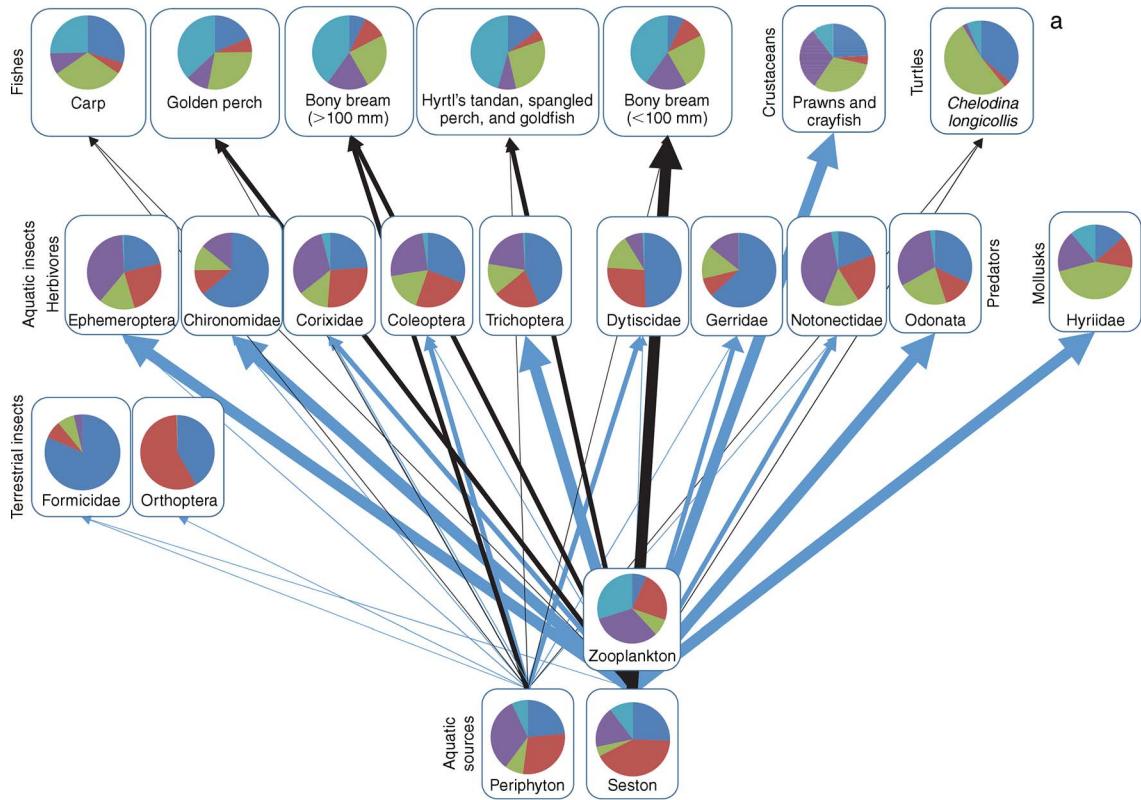


FIG. 3. Relationship between PUFA and the proportional contribution to the diet from the aquatic source pathways (phytoplankton + periphyton) for aquatic insects (a–c, diamonds show herbivores, circles show predators), crustaceans (d–f, diamonds show *M. australiense*, circles show *Cherax* spp.) and 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). C₃ 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.



Source contribution	Polyunsaturated fatty acids (PUFA)
No arrow	<5%
Light blue arrow	5–24%
Medium blue arrow	25–50%
Dark blue arrow	>50%
Thick black arrow	For vertebrates
Blue arrow	For invertebrates
Blue square	LIN
Red square	ALA
Green square	ARA
Purple square	EPA
Cyan square	DHA

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 ω 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 ω 3/ ω 6 ratio was a strong indicator of aquatic vs. terrestrial source reliance. There were significant relationships between proportion aquatic diet and ω 3/ ω 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 ω 3/ ω 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 ω 3-rich marine sources (e.g., anadromous fishes; Rinella et al. 2013), the relatively high contribution of ω 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 ω 3, as apparently is the case in organisms of colder ecosystems.

Optimal ω 3/ ω 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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LITERATURE CITED

- Ahlgren, G., T. Vrede, and W. Goedkoop. 2009. Fatty acid ratios in freshwater fish, zooplankton and zoobenthos—are there specific optima? Pages 147–178 in M. T. Arts, M. T. Brett, and M. J. Kainz, editors. *Lipids in aquatic ecosystems*. Springer-Verlag, New York, New York, USA.
- Araujo, M. S., D. I. Bolnick, and C. A. Layman. 2011. The ecological causes of individual specialisation. *Ecology Letters* 14:948–958.
- Arts, M. T., and C. C. Kohler. 2009. Health and condition in fish: the influence of lipids on membrane competency and immune response. Pages 237–256 in M. T. Arts, M. T. Brett, and M. J. Kainz, editors. *Lipids in aquatic ecosystems*. Springer-Verlag, New York, New York, USA.
- Ask, J., J. Karlsson, L. Persson, P. Ask, P. Bystrom, and M. Jansson. 2009. Terrestrial organic matter and light penetration: effects on bacterial and primary production in lakes. *Limnology and Oceanography* 54:2034–2040.
- Bartels, P., J. Cucherousset, K. Steger, P. Eklov, L. J. Tranvik, and H. Hillebrand. 2012. Reciprocal subsidies between freshwater and terrestrial ecosystems structure consumer resource dynamics. *Ecology* 93:1173–1182.
- Baxter, C. V., K. D. Fausch, and W. C. Saunders. 2005. Tangled webs: reciprocal flows of invertebrate prey link streams and riparian zones. *Freshwater Biology* 50:201–220.
- Bell, M. V., and D. R. Tocher. 2009. Biosynthesis of polyunsaturated fatty acids in aquatic ecosystems: general pathways and new directions. Pages 211–236 in M. T. Arts, M. T. Brett, and M. J. Kainz, editors. *Lipids in aquatic ecosystems*. Springer-Verlag, New York, New York, USA.
- Brett, M. T., M. J. Kainz, S. J. Taipale, and H. Seshan. 2009. Phytoplankton, not allochthonous carbon, sustains herbivorous zooplankton production. *Proceedings of the National Academy of Sciences USA* 106:21197–21201.
- Brett, M. T., and D. C. Müller-Navarra. 1997. The role of highly unsaturated fatty acids in aquatic foodweb processes. *Freshwater Biology* 38:483–499.
- Budge, S. M., S. N. Penney, and S. P. Lall. 2012. Estimating diets of Atlantic salmon (*Salmo salar*) using fatty acid signature analyses: validation with controlled feeding studies. *Canadian Journal of Fisheries and Aquatic Sciences* 69:1033–1046.
- Bunn, S. E., P. M. Davies, and T. D. Mosisch. 1999. Ecosystem measures of river health and their response to riparian and catchment degradation. *Freshwater Biology* 41:333–345.

- Bunn, S. E., P. M. Davies, and M. Winning. 2003. Sources of organic carbon supporting the food web of an arid zone floodplain river. *Freshwater Biology* 48:619–635.
- Bunn, S. E., M. C. Thoms, S. K. Hamilton, and S. J. Capon. 2006. Flow variability in dryland rivers: boom, bust and the bits in between. *River Research and Applications* 22:179–186.
- Carreon-Palau, L., C. C. Parrish, J. A. del Angel-Rodriguez, H. Perez-Espana, and S. Aguiniga-Garcia. 2013. Revealing organic carbon sources fueling a coral reef food web in the Gulf of Mexico using stable isotopes and fatty acids. *Limnology and Oceanography* 58:593–612.
- Cole, J. J., S. R. Carpenter, J. F. Kitchell, M. L. Pace, C. T. Solomon, and B. C. Weidel. 2011. Strong evidence for terrestrial support of zooplankton in small lakes based on stable isotopes of carbon, nitrogen, and hydrogen. *Proceedings of the National Academy of Sciences USA* 108:1975–1980.
- Cross, W. F., C. V. Baxter, E. J. Rosi-Marshall, R. O. Hall, T. A. Kennedy, K. C. Donner, H. A. W. Kelly, S. E. Z. Seegert, K. E. Behn, and M. D. Yard. 2013. Food-web dynamics in a large river discontinuum. *Ecological Monographs* 83:311–337.
- Fontaneto, D., M. Tommaseo-Ponzetta, C. Galli, P. Rise, R. H. Glew, and M. G. Paoletti. 2011. Differences in fatty acid composition between aquatic and terrestrial insects used as food in human nutrition. *Ecology of Food and Nutrition* 50:351–367.
- Galloway, A. W. E., M. E. Eisenlord, M. N. Dethier, G. W. Holtgrieve, and M. T. Brett. 2014. Quantitative estimates of isopod resource utilization using a Bayesian fatty acid mixing model. *Marine Ecology Progress Series* 507:219–232.
- Giacomini, H. C., B. J. Shuter, and N. P. Lester. 2013. Predator bioenergetics and the prey size spectrum: do foraging costs determine fish production? *Journal of Theoretical Biology* 332:249–260.
- Gomes, A. D., T. G. Correia, and R. G. Moreira. 2010. Fatty acids as trophic biomarkers in vitellogenic females in an impounded tropical river. *Fish Physiology and Biochemistry* 36:699–718.
- Greig, H. S., P. Kratina, P. L. Thompson, W. J. Palen, J. S. Richardson, and J. B. Shurin. 2011. Warming, eutrophication, and predator loss amplify subsidies between aquatic and terrestrial systems. *Global Change Biology* 18:504–514.
- Hairton, N. G., and N. G. Hairton. 1993. Cause-effect relationships in energy flow, trophic structure, and interspecific interactions. *American Naturalist* 142:379–411.
- Heissenberger, M., J. Watzke, and M. J. Kainz. 2010. Effect of nutrition on fatty acid profiles of riverine, lacustrine and aquaculture-raised salmonids of pre-alpine habitats. *Hydrobiologia* 650:243–254.
- Holt, G. J., editor. 2011. Larval fish nutrition. John Wiley & Sons, New York, New York, USA.
- Iverson, S. J. 2009. Tracing aquatic food webs using fatty acids: from qualitative indicators to quantitative determination. Pages 281–307 in M. T. Arts, M. T. Brett, and M. J. Kainz, editors. *Lipids in aquatic ecosystems*. Springer-Verlag, New York, New York, USA.
- Iverson, S. J., C. Field, W. D. Bowen, and W. Blanchard. 2004. Quantitative fatty acid signature analysis: a new method of estimating predator diets. *Ecological Monographs* 74:211–235.
- Jardine, T. D. 2014. Organic matter sources and size structuring in stream invertebrate food webs across a tropical to temperate gradient. *Freshwater Biology* 59:1509–1521.
- Jardine, T. D., R. J. Hunt, S. J. Faggotter, D. Valdez, M. A. Burford, and S. E. Bunn. 2013. Carbon from periphyton supports fish biomass in a wet-dry tropical river. *River Research and Applications* 29:560–573.
- Junk, W. J., P. B. Bayley, and R. E. Sparks. 1989. The flood pulse concept in river-floodplain systems. Pages 110–127 in *Proceedings of the International Large River Symposium*. Canadian Special Publication of Fisheries and Aquatic Sciences, Fisheries and Oceans Canada, Ottawa, Ontario, Canada.
- Kainz, M., M. T. Arts, and A. Mazumder. 2004. Essential fatty acids in the planktonic food web and their ecological role for higher trophic levels. *Limnology and Oceanography* 49:1784–1793.
- Karlsson, J., P. Bystrom, J. Ask, P. Ask, L. Persson, and M. Jansson. 2009. Light limitation of nutrient-poor lake ecosystems. *Nature* 460:506–510.
- Koussoropolis, A. M., C. Lemarchand, A. Bec, C. Desvillettes, C. Amblard, C. Fournier, P. Berny, and G. Bourdier. 2008. From aquatic to terrestrial food webs: decrease of the docosahexaenoic acid/linoleic acid ratio. *Lipids* 43:461–466.
- Lau, D. C. P., I. Sundh, T. Vrede, J. Pickova, and W. Goedkoop. 2014. Autochthonous resources are the main driver of consumer production in dystrophic boreal lakes. *Ecology* 95:1506–1519.
- Lau, D. C. P., T. Vrede, J. Pickova, and W. Goedkoop. 2012. Fatty acid composition of consumers in boreal lakes—variation across species, space and time. *Freshwater Biology* 57:24–38.
- Marcarelli, A. M., C. V. Baxter, M. M. Mineau, and R. O. Hall, Jr. 2011. Quantity and quality: unifying food web and ecosystem perspectives on the role of resource subsidies in freshwaters. *Ecology* 92:1215–1225.
- Marczak, L. B., R. M. Thompson, and J. S. Richardson. 2007. Meta-analysis: trophic level, habitat, and productivity shape the food web effects of resource subsidies. *Ecology* 88:140–148.
- Mineau, M. M., C. V. Baxter, A. M. Marcarelli, and G. W. Minshall. 2012. An invasive riparian tree reduces stream ecosystem efficiency via a recalcitrant organic matter subsidy. *Ecology* 93:1501–1508.
- Moore, J. C., et al. 2004. Detritus, trophic dynamics and biodiversity. *Ecology Letters* 7:584–600.
- Müller-Navarra, D. C., M. T. Brett, S. Park, S. Chandra, A. P. Ballantyne, E. Zorita, and C. R. Goldman. 2004. Unsaturated fatty acid content in seston and tropho-dynamic coupling in lakes. *Nature* 427:69–72.
- Nakano, S., and M. Murakami. 2001. Reciprocal subsidies: dynamic interdependence between terrestrial and aquatic food webs. *Proceedings of the National Academy of Sciences USA* 98:166–170.
- Napolitano, G. E. 1999. Fatty acids as trophic and chemical markers in freshwater ecosystems. Pages 21–37 in M. T. Arts and B. C. Wainman, editors. *Lipids in freshwater ecosystems*. Springer-Verlag, New York, New York, USA.
- Nunn, A. D., L. H. Tewson, and I. G. Cowx. 2012. The foraging ecology of larval and juvenile fishes. *Reviews in Fish Biology and Fisheries* 22:377–408.
- Parnell, A. C., R. Inger, S. Bearhop, and A. L. Jackson. 2010. Source partitioning using stable isotopes: coping with too much variation. *PLoS ONE* 5(3):e9672.
- Pauly, D., and V. Christensen. 1995. Primary production required to sustain global fisheries. *Nature* 374:255–257.
- Perga, M. E., M. Kainz, B. Matthews, and A. Mazumder. 2006. Carbon pathways to zooplankton: insights from the combined use of stable isotope and fatty acid biomarkers. *Freshwater Biology* 51:2041–2051.
- Perhar, G., G. B. Arhonditsis, and M. T. Brett. 2012. Modelling the role of highly unsaturated fatty acids in planktonic food web processes: a mechanistic approach. *Environmental Reviews* 20:155–172.
- Polis, G. A., W. B. Anderson, and R. D. Holt. 1997. Toward an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. *Annual Review of Ecology and Systematics* 28:289–316.

- Post, D. M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* 83: 703–718.
- Post, D. M. 2003. Individual variation in the timing of ontogenetic niche shifts in largemouth bass. *Ecology* 84: 1298–1310.
- Puckridge, J. T., and K. F. Walker. 1990. Reproductive biology and larval development of a gizzard shad, *Nematalosa erebi* (Gunther) (Dorosomatinae, Teleostei), in the River Murray, South Australia. *Australian Journal of Marine and Freshwater Research* 41:695–712.
- Rinella, D. J., M. S. Wipfli, C. M. Walker, C. A. Stricker, and R. A. Heintz. 2013. Seasonal persistence of marine-derived nutrients in south-central Alaskan salmon streams. *Ecosphere* 4:art122.
- Roach, K. A. 2013. Environmental factors affecting incorporation of terrestrial material into large river food webs. *Freshwater Science* 32:283–298.
- Roach, K. A., K. O. Winemiller, and S. E. Davis III. 2014. Autochthonous production in shallow littoral zones of five floodplain rivers: effects of flow, turbidity and nutrients. *Freshwater Biology* 59:1278–1293.
- Rumpold, B. A., and O. K. Schluter. 2013. Nutritional composition and safety aspects of edible insects. *Molecular Nutrition & Food Research* 57:802–823.
- Scharnweber, K., J. Syväranta, S. Hilt, M. Brauns, M. J. Vanni, S. Brothers, J. Köhler, J. Knežević-Jarić, and T. Mehner. 2014. Whole-lake experiments reveal the fate of terrestrial particulate organic carbon in benthic food webs of shallow lakes. *Ecology* 95:1496–1505.
- Sheikh-Eldin, M., S. S. De Silva, and B. A. Ingram. 1997. Effects of diets and feeding rate on the survival and growth of Macquarie perch (*Macquaria australasica*) larvae, a threatened Australian native fish. *Aquaculture* 157:35–50.
- Sihamala, O., S. Bhulaidok, L. R. Shen, and D. Li. 2010. Lipids and fatty acid composition of dried edible red and black ants. *Agricultural Sciences in China* 9:1072–1077.
- Smith, V. H., G. D. Tilman, and J. C. Nekola. 1999. Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. *Environmental Pollution* 100:179–196.
- Solomon, C. T., S. R. Carpenter, M. K. Clayton, J. J. Cole, J. J. Coloso, M. L. Pace, M. J. Vander Zanden, and B. C. Weidel. 2011. Terrestrial, benthic, and pelagic resource use in lakes: results from a three-isotope Bayesian mixing model. *Ecology* 92:1115–1125.
- Thorp, J. H., and A. D. Delong. 2002. Dominance of autochthonous autotrophic carbon in food webs of heterotrophic rivers. *Oikos* 96:543–550.
- Tocher, D. R. 2010. Fatty acid requirements in ontogeny of marine and freshwater fish. *Aquaculture Research* 41:717–732.
- Torres-Ruiz, M., J. D. Wehr, and A. A. Perrone. 2007. Trophic relations in a stream food web: importance of fatty acids for macroinvertebrate consumers. *Journal of the North American Benthological Society* 26:509–522.
- Vander Zanden, M. J., Y. Vadeboncoeur, and S. Chandra. 2011. Fish reliance on littoral–benthic resources and the distribution of primary production in lakes. *Ecosystems* 14: 894–903.
- Volk, C., and P. Kiffney. 2012. Comparison of fatty acids and elemental nutrients in periphyton, invertebrates, and cutthroat trout (*Oncorhynchus clarki*) in conifer and alder streams of western Washington State. *Aquatic Ecology* 46: 85–99.
- Wipfli, M. S., and C. V. Baxter. 2010. Linking ecosystems, food webs, and fish production: subsidies in salmonid watersheds. *Fisheries* 35:373–387.
- Woodward, G., B. Ebenman, M. Emmerson, J. M. Montoya, J. M. Olesen, A. Valido, and P. H. Warren. 2005. Body size in ecological networks. *Trends in Ecology & Evolution* 20:402–409.
- Yang, L. H., K. F. Edwards, J. E. Byrnes, J. L. Bastow, A. N. Wright, and K. O. Spence. 2010. A meta-analysis of resource pulse–consumer interactions. *Ecological Monographs* 80: 125–151.

SUPPLEMENTAL MATERIAL

Ecological Archives

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