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varied, on average, by ~40% across lake systems. The sources estimated to contribute most to fish biomass at the regional scale were sometimes unimportant in certain species and lake systems. Conversely, the least important sources at the regional scale were sometimes very important at the local scale. Spatial variation in the isotopic composition of production sources and fishes, and the proportional contributions of sources to fish biomass in the Amazon River floodplain, are probably influenced by multiple factors including variation in the quality and quantity of basal sources. Future stable isotope investigations of aquatic food webs of river–floodplain systems should consider not only suitable replication and appropriate temporal scale, but also spatial scale. [ABSTRACT FROM AUTHOR]

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Spatial variation in aquatic food webs in the Amazon River floodplain

Food webs are spatially variable and temporally dynamic in heterogeneous and species-rich river floodplains. However, empirical evidence that shows how food webs vary across landscapes and scales in river–floodplain ecosystems is limited, especially in the tropics. Here, we evaluate how the flow of energy and matter varies among food webs in aquatic habitats and across scales in the lower Amazon River floodplains. We surveyed 109 habitats across 19 floodplain units (lake systems) and analyzed the isotopic composition of primary production sources and fish tissues to estimate relative contributions of these sources to fish biomass at local and regional scales. Basal production sources and fish species each varied considerably in their carbon and

nitrogen isotopic ratios across the floodplain landscape. Aquatic macrophytes and suspended particulate organic material in the water column were inferred to be the principal basal sources contributing to the biomass of most fish species at the regional scale. However, the estimated contribution of different production sources to fish biomass varied, on average, by ~40% across lake systems. The sources estimated to contribute most to fish biomass at the regional scale were sometimes unimportant in certain species and lake systems. Conversely, the least important sources at the regional scale were sometimes very important at the local scale. Spatial variation in the isotopic composition of production sources and fishes, and the proportional contributions of sources to fish biomass in the Amazon River floodplain, are probably influenced by multiple factors including variation in the quality and quantity of basal sources. Future stable isotope investigations of aquatic food webs of river–floodplain systems should consider not only suitable replication and appropriate temporal scale, but also spatial scale.

Keywords: fish; freshwater; fluvial systems; stable isotope; carbon; nitrogen; landscape; spatial variation; production source; scale; tropics; Brazil

Food webs are spatially variable and temporally dynamic (Warren [78], Polis et al. [60]), particularly in the heterogeneous lowland rivers and floodplains in the tropics (Winemiller [81]). In these ecosystems, hydrology drives temporal variation in foodweb properties such as the number and intensity of predator-prey interactions, trophic link density, and food chain length (Winemiller [80]). Food webs also vary spatially in response to factors that change across landscape gradients, such as the sources of primary production, productivity, population abundance, or species interactions (Winemiller [80]). A growing body of work documents the temporal and spatial variation in foodweb structure in aquatic systems. However, few studies have used empirical data to test which mechanisms structure food webs across landscapes, and even fewer have evaluated how the spatial scale at which data are collected and analyzed influences foodweb patterns (Schoener [73], Thompson and Townsend [75]).

Most empirical studies of spatial variation in river food webs have compared sites with different watershed geochemistry, geomorphology, hydrology, land cover, human impacts, or some combination of these features (e.g., Benedito-Cecilio and Araujo-Lima [10], Jepsen and Winemiller [37], Hoeinghaus et al. [32], Ou and Winemiller [54], Alves et al. [1]). For example, patterns of material flow (e.g., availability of basal production sources and their contributions to fish biomass) diverge among rivers that have different levels of impact from hydroelectric dams (e.g., Hoeinghaus et al. [[32]], Upper Paraná Basin in Brazil; Ou and Winemiller [[54]], Lower Mekong Basin in Cambodia). In these cases, spatial variation in foodweb structure may be driven, either directly or indirectly, by differences in environmental conditions (e.g., biogeochemistry, topography, hydrology, land cover, etc.) as well as differences in productivity gradients and nutrient dynamics among reaches and watersheds associated to these conditions (Power [65], Polis and Hurd [61], Winemiller and Polis [85], Thompson and Townsend [74], DeAngelis [20]).

Currently, the extent to which foodweb structure and function varies along a given river–floodplain gradient is poorly understood and impossible to predict. In large river–floodplain systems, hydrological variation influences spatial habitat heterogeneity. This heterogeneity affects spatial variation among, and exchanges between, local food webs. Food web structure may be fairly constant across aquatic habitats present in a floodplain because flood pulses may facilitate dispersion of both aquatic organisms and their food sources across a large region (Junk et al. [39], Hurd et al. [34]). For example, a previous study described a lack of spatial variation in foodweb components in the Cinaruco River floodplain, which probably occurred because of the transport and exchange

of suspended particulate organic matter and lateral migrations of fishes during the flood pulse (Roach et al. [72]). Conversely, local food webs in a heterogeneous floodplain landscape may vary as functions of differences in the quality and quantity of food resources, assemblage composition, and species interactions (Hedges et al. [30], Winemiller [80], Polis et al. [60], Mortillaro et al. [51], Correa et al. [16], Arantes et al. [4]). Whether food webs vary across riverine landscapes has implications for foodweb structure and function at multiple scales—i.e., spatial variation in local food webs across the landscape may cause foodweb properties to differ at regional versus local scales (Martinez and Lawton [45], Polis et al. [60], Pillai et al. [59]).

Foodweb variation across environmental gradients in complex landscapes, such as tropical river–floodplain systems, can be explored with stable isotope analysis. Analysis of naturally occurring isotopic ratios of elements such as carbon (C) and nitrogen (N) can be used to infer trophic pathways (Peterson and Fry [56]), trophic niche breadth and overlap (Layman et al. [41]), and foodchain length (Post [63]). However, little is known about how isotopic composition of production sources and fishes vary spatially, although a few studies have used stable isotope analysis to evaluate temporal variation of primary C sources for some fish species in the floodplains of the Amazon River (i.e., Araujo-Lima et al. [5], Forsberg et al. [25], Benedito-Cecilio et al. [11], Benedito-Cecilio and Araujo-Lima [10], Oliveira et al. [53], Mortillaro et al. [50]). The limited information available shows evidence of upstream-downstream trends in the isotopic composition of production sources. This pattern has been found in $\delta^{13}C$ of C3 plants and C4 grasses (Martinelli et al. [43]), suspended particulate organic matter (Hedges et al. [30], Mortillaro et al. [51]), and the tissues of 2 fish species (*Prochilodus nigricans* and *Colossoma macropomum*; Benedito-Cecilio et al. [11]). However, spatial variation in the isotopic composition of production sources, fishes from different functional groups, and production source contributions to fish biomass have not yet been investigated in Amazon floodplains.

Here, we use stable isotope analysis to describe potential pathways of material transfer within food webs of aquatic habitats in floodplains of the lower Amazon River. We also investigate the extent to which variation in material transfer pathways is dependent on the spatial scale of analysis. We collected tissue samples of basal production sources and fishes for isotopic analysis from 109 floodplain habitats during the descending phase of the Amazon annual flood pulse in the Amazon floodplain. Our null hypothesis was that neither the isotopic composition of primary production sources, different fish trophic guilds, nor the relative contribution of these sources to fish biomass would vary across floodplain habitats. We expect our null hypothesis to be true when dispersal or transport among local habitats may cause high exchange of material and energy across the landscape. Several fish species are capable of dispersal over long distances and may therefore exploit resources from multiple locations (Arantes et al. [4]). Transport of nutrients and particulate organic matter also occurs, for example, via either downstream drift in the water or through movement of animals during migration (Winemiller and Jepsen [83], Winemiller et al. [84]). Therefore, variation among local habitats in isotopic composition of basal sources, fishes, and production sources assimilated by fishes could be minimal or nonexistent. In such a scenario, variation across scales (e.g., local vs. regional) would be minimal.

Our alternate hypothesis was that the isotopic composition of some production sources would vary among habitats, leading to spatial variation in isotopic composition of fishes and other consumers. In addition, the isotopic composition of fish tissue and the contribution of production sources to fish biomass could vary spatially if fish feeding behavior is sufficiently flexible to shift diets in response to variation in food quality or availability (Winemiller [80]). The magnitude of this spatial variation may depend on trophic guild. For example, tissues of herbivorous fishes (e.g., *Colossoma macropomum, Piaractus brachypomus*) feeding on basal production sources (e.g., fruits and seeds) may exhibit lower spatial variation in nitrogen isotopic ratios

compared with detritivores (e.g., *Curimata incompta*, *Potamorhina latior*) that consume organic matter of both autochthonous and allochthonous origin that can vary in both nutritional value (Goulding [28], [29], Bowen [12]) and isotopic composition (Benedito-Cecilio & Araujo-Lima [10]).

To test whether or not patterns are affected by spatial scale of analysis, we compare estimates for contributions of basal production to fish biomass at the scale of local habitats (lake systems) versus the broader regional landscape unit (lower Amazon). Thus, a basic objective of our study is to advance understanding of how spatial grain influences foodweb interpretations based on stable isotope analysis (Phillips et al. [58]). We also seek to improve knowledge of trophic ecology in the Amazon because this information is essential for managing human impacts that affect biodiversity and ecosystem services (Winemiller [82]).

Methods

Study area, data collection, and laboratory methods

This study was conducted within a 17,674-km^2 area in the floodplains of the lower Amazon River (referred to locally as *várzea*) in Brazil (Fig. 1). These floodplains are seasonally flooded by water that carries high loads of silt and nutrients that originate in the Andes Mountains. The study area contains a mosaic of forests, nonforested areas dominated by herbaceous vegetation, lakes, and secondary channels. The annual flood pulse produces gradual changes in water level from 4 to ~9 m annually as measured at the discharge gauges at Óbidos (mean = 5.7 m) and Parintins (mean = 5.4 m) (ANA [3]). The flood pulse reaches a maximum water level in June and a minimum in October [rising and high water levels: Jan–July; falling and low water level [Aug–Dec]) and causes shifts in habitat availability and environmental conditions. During the dry season, water is retained in channels and lakes, many of which are isolated, and during the wet, flood season, forests, pastures, channels, and lakes are well-connected.

Graph: Figure 1. Study area, local catchments (lake systems), and habitats sampled within lake systems in the lower Amazon River floodplain.

The sampling units were local catchments (or 'lake systems' sensu Castello et al. [13], Arantes et al. [4]) that contain lakes, interconnecting channels, forests, and areas with herbaceous vegetation and aquatic macrophytes that are hydrologically connected for 6 to 9 mo/y (Fig. 1). Lake systems are separated either by large flowing channels, natural levees, or a combination of these features.

We collected fish muscle tissue and common basal resources to capture the spatial variation in isotopic composition of both aquatic consumers and their potential food sources. We collected these samples from 109 locations that spanned a range of aquatic habitats (open water, flooded herbaceous vegetation, flooded forest) across 19 lake systems. These lake systems are distributed along ~250 km of the lower Amazon River (Fig. 1). We collected samples during the beginning of the falling-water stage of the annual hydrological cycle (August 2013, water level ~5.5 m) (Fig. 1), so the data should characterize the period of maximum lateral connectivity of aquatic floodplain habitats.

We collected fish with gillnets and took muscle tissue samples from 14 abundant species that represented various trophic guilds (Table 1). Fish muscle tissue samples were collected from the flank near the base of the dorsal fin, and we attempted to collect samples from 3–5 specimens per species in each lake system. The isotopic ratios in animal tissues reflect assimilation of elements from food over variable time intervals depending on tissue and isotopic/elemental turnover rates (Vander Zanden et al. [77]) rather than the isotopic

composition of the recent diet. The turnover time of fish muscle tissue typically ranges between 1–3 mo, depending on fish body size, age, or other factors (Jardine et al. [36], Weidel et al. [79]). Thus, we assumed that C and N isotopic ratios of fish muscle tissue reflect assimilation of sources during the ~3 mo preceding our surveys.

We classified fish species according to trophic guilds based on dietary information from published reports (e.g., Mérona and Mérona [47], Oliveira et al. [53], see Table 1). Four species were classified as herbivores-C3 (*n* = 165): *Colossoma macropomum*, *Mylossoma aureum*, *Piaractus brachypomus*, and *Rhytiodus microlepis* (Table 1). Herbivores-C3 feed predominantly on C3 plant material (seeds, fruits, or leaves) and on filamentous algae. Herbivores-C4 (1 species, *Schizodon fasciatus*, *n* = 75) feed predominantly on C4 plants (grasses and aquatic macrophytes). Two species classified as omnivores (*Hemiodus microlepis* and *Triportheus auritus*, *n* = 91) ingest combinations of plant material, detritus, and invertebrates. Detritivores (2 species, *Curimata incompta* and *Potamorhina latior*, *n* = 51) predominantly ingest fine particulate organic matter (POM) and filamentous algae. Planktivores (1 species, *Hypophthalmus marginatus*, *n* = 22) ingest variable combinations of phytoplankton, zooplankton, plant material, and detritus. Four species classified as piscivores (*Acestrorhynchus abbreviatus*, *Pellona castelnaeana*, *Plagioscion squamosissimus*, and *Pygocentrus nattereri*, *n* = 252) consume fish, scales and fins, or both, either whole or in pieces. Piscivores may also ingest small portions of terrestrial or aquatic macroinvertebrates (e.g., Ephemeroptera, Chironomidae, Coleoptera, Crustacea, etc.).

Graph

Table 1. Mean (±SD) values of carbon (C) and nitrogen (N) stable isotope ratios (δ 13 C and δ 15 N) for different fish species in the lower Amazon region. Results from analysis of variance (ANOVA) or analysis of covariance (ANCOVA) for groups of fish with similar trophic strategies are shown. ANCOVA was applied only for the detritivores, including Potamohina latior , and for the herbivore C4, Schizodon fasciatus. SL = standard length. Species trophic level estimates (mean ± CI) used in the trophic fractionation (TF) calculation (see Methods) and sample number (n) are also shown. * denotes statistically significant ($p \leq 0.01$) values.

Trophic strategy	$\delta^{13}C$ Pr(>F)	$\delta^{15}N$ Pr(>F)	Species/Trophic level	n	Mean $\delta^{13}C$	SD $\delta^{13}C$	Mean $\delta^{15}N$	SD $\delta^{15}N$	$\delta^{13}C$ Pr(>F)	$\delta^{15}N$ Pr(>F)
Herbivores C3	<0.01*	0.05	Colossoma macropomum	71	–27.1	2.2	9.1	1.1	<0.01*	<0.01*
3 (3.2–3.4)										
Piaractus brachypomus	25	–26.2	0.7	7.1	1.4	0.03	0.04			
2.6 (2.4–2.8)										
Mylossoma aureum	40	–27.1	1.3	8.6	0.9	0.08	0.06			
3.03 (2.8–3.2)										
Rhytiodus microlepis	29	–31.4	2.5	7.0	0.8	0.01*	<0.01*			
2.6 (2.4–2.7)										
Herbivore C4	0.03	<0.01*	Schizodon fasciatus	75	–20.9	4.4	8.2	1.4	–	–
0.6 (SL)	0.9 (SL)	3.8 (3.6–4.08)								
Omnivores	0.01*	0.09	Hemiodus microlepis	43	–33.1	2.0	8.5	0.8	0.01*	0.02
3.0 (2.8–3.2)										
Triportheus auritus	48	–27.1	1.9	10.5	0.8	0.03	<0.01*			
3.6 (3.4–3.8)										

Trophic strategy	$\delta^{13}C$ Pr(>F)	$\delta^{15}N$ Pr(>F)	Species/Trophic level	n	Mean $\delta^{13}C$	SD $\delta^{13}C$	Mean $\delta^{15}N$	SD $\delta^{15}N$	$\delta^{13}C$ Pr(>F)	$\delta^{15}N$ Pr(>F)
Detritivores	<0.01	<0.01	Curimata incompta	23	–31.7	2.2	8.1	0.7	0.02	0.60
0.1 (SL)	0.02 (SL)	2.9 (2.7–3.1)								
		Potamohina latior	28	–34.4	2.0	8.8	1.2	0.08	<0.01*	
3.1 (2.9–3.2)	0.21 (SL)	0.02 (SL)								
Planktivore	0.12	0.83	Hypophthalmus marginatus	22	–34.9	2.4	11.3	0.5	–	–
3.6 (3.8–4.1)										
Piscivores	<0.01*	<0.01*	Acestrorhynchus abbreviatus	54	–25.19	1.8	11.4	0.5	0.01*	<0.01*
3.9 (3.6–4.1)										
Pellona castelnaeana	50	–27.3	1.9	12.9	0.7	0.01*	0.05			
4.3 (4.1–4.54)										
Pygocentrus nattereri	100	–25.3	2.4	11.1	0.9	0.01*	0.37			
3.8 (3.6–4.0)										
Plagioscion squamosissimus	48	–25.6	1.9	11.8	0.6	<0.01*	<0.01*			
4.0 (3.8–4.2)										

We also collected terrestrial and aquatic plant material (macrophytes) to characterize the isotopic composition of basal food resources in Amazon floodplain food webs. These samples included leaves and fruits from the most common trees and shrubs (C3 terrestrial plants of the families Capparaceae and Lamiaceae), C3 aquatic macrophytes (e.g., *Eichhornia crassipes, Salvinia minima*), C4 grasses (*Paspalum repens*), suspended particulate organic material from the water column (POM), and benthic algae (hereafter, phytomicrobenthos). We collected samples of suspended POM by filtering water samples through pre-combusted Whatman GF/F filters (pore size 0.7 μm) with a vacuum system under low pressure. It is not always possible to trace the origin of suspended POM, but a previous analysis of POM in the lower Amazon suggested it was derived primarily from autochthonous material, including C3 aquatic plants and phytoplankton dominated by cyanobacteria (Mortillaro et al. [49]). Another study showed that vascular plant debris and soil humic material also contribute to the pool of suspended POM in the Amazon (Hedges et al. [30]). We collected benthic algae by gently scraping submerged tree branches. This sampling technique was unlikely to produce a pure sample of benthic algae, so we consider these phytomicrobenthos samples, which were probably composed of a combination of periphyton, fine particulate organic matter, and associated microorganisms. Phytomicrobenthos $\delta^{13}C$ values are highly variable in aquatic systems (Hladyz et al. [31]) and, therefore, the samples we collected from wood may not be representative of algal biofilms on other surfaces (e.g., soil or submerged macrophytes).

We also collected snails from floating aquatic vegetation. Snails often are considered algal grazers that can be used to estimate isotopic ratios of phytomicrobenthos, but our snail samples were insufficient (i.e., not found in every lake system) for use in verifying our phytomicrobenthos isotopic signatures. Our isotopic data for snails were, however, used as the primary consumer baseline for calculations of fish trophic position (see sections: Data analyses and Discussion: some limitations of stable isotope analysis for ecological inferences).

All samples were preserved in NaCl for later processing in the laboratory. We analyzed a total of 449 basal resource samples, 34 primary consumer samples (snails), and 656 fish muscle tissue samples for stable isotope ratios of C and N (Tables 1, 2). Nitrogen isotopic ratios ($^{15}N/^{14}N$ as expressed by $\delta^{15}N$) can be used to estimate the trophic position of an organism, because the proportion ^{15}N in tissues increases incrementally (usually by 2–4‰) as material is consumed and assimilated during successive steps in a food chain (Post [63], Caut et al. [14]). Carbon isotopic ratios ($^{13}C/^{12}C$, or $\delta^{13}C$) often are useful for identifying sources assimilated by consumers, because once C is fixed into the tissue of a plant, this ratio typically changes only 0.4–1‰ as material passes from 1 trophic level to the next (Fry [26]).

Graph

Table 2. Mean, minimum (Min), and maximum (Max) values of C and N stable isotope ratios (δ 13 C and δ 15 N) for basal resources in the lower Amazon region and results for multivariate analysis of variance (MANOVA) and analysis of variance (ANOVA) testing for differences in δ 13 C and δ 15 N of each production source across lake systems (n = number of samples). * = statistically significant result (p ≤ 0.01).

Sources of production	n	Mean $\delta^{13}C$	Mean $\delta^{15}N$	MANOVA	ANOVA				
(Min,Max)	(Min,Max)	df	F	Pr(>F)	$\delta^{13}C$ Pr(>F)	$\delta^{15}N$ Pr(>F)			
Phytomicrobenthos (–33.64, –21.12)	(1.1, 8.26)	24	–27.68	4.32	–	–	–	–	–
C3 aquatic macrophytes (–34.41, –27.10)	Eichhornia crassipes (0.53, 6.78)	79	–29.86	3.73	13	1.86	0.03	–	–
Salvinia minima			11	2.78	0.01*	0.02	0.05		
C4 grass (–16.04, –11.11)	(1.76, 8.44)	161	–12.88	4.72	18	4.45	0.01*	<0.01*	<0.01*
Terrestrial plants	Capparaceae	133	–29.8 (–34.09, –25.36)	5.02 (0.21, 11.17)	12	3.14	0.01*	0.06	0.01*
	Lamiaceae		10	2.23	0.01*	0.17	0.01*		
POM (–29.87, –22.27)	(–4.99, 4.88)	52	–25.13	0.40	18	3.15	0.01*	0.01*	0.01*

Prior to analysis, we soaked tissue samples in distilled water for 4 to 5 h, rinsed them, and dried them in an oven at 60° for ~48 h, following Arrington and Winemiller ([7]). After drying, we used a mortar and pestle to grind the samples into fine powder, divided the samples into subsamples and loaded into them into Ultra-Pure tin capsules (Costech Analytical, Valencia, California, USA). Each POM subsample was 15 to 20 mg, and fish muscle tissue and tissues from other sources were 1.5 to 3 mg. Samples were analyzed for C and N isotope ratios with mass spectrometry at the Analytical Chemistry Laboratory of the Institute of Ecology, University of Georgia, USA. We report isotope ratios in parts per thousand (‰; standardized in relation to reference material [Pee Dee Belemnite for C, atmospheric nitrogen for N]) as $\delta X = [(R_{sample}/R_{standard})\text{-}1)] \times 10^3$, where $R = {}^{13}C/{}^{12}C$ or $^{15}N/^{14}N$ (the ratio of heavy and light stable isotopes of C or N). No lipid corrections were necessary because the C:N ratios of these samples were relatively low (typically <3.5; Post et al. [64]).

Data analyses

Regional scale analyses

Prior to all analyses, we used analysis of variance (ANOVA) to test for differences in $\delta^{13}C$ and $\delta^{15}N$ of sources and consumers among habitats types within each lake system. Isotope values were similar among habitats ($p > 0.1$). We therefore used values of each source mean and standard deviation for a given lake system as the input for our analyses (i.e., Bayesian mixing model described below).

Then, we first evaluated general patterns of isotopic variation among fish species, trophic guilds, and primary producers with boxplots or biplots of $\delta^{13}C$ and $\delta^{15}N$. Second, we estimated the proportional contribution of each primary producer to fish biomass at a regional scale (i.e., combined data for all lake systems) with a Bayesian mixing model. This stable isotope mixing model uses Bayesian statistical techniques to incorporate uncertainty and variation in input parameters into the estimates of proportional contributions of sources to consumer biomass (Parnell et al. [55]). Prior to analysis, we evaluated the data to ensure it conformed to the mixing model assumptions. One assumption is that consumer isotopic values fall within the polygon (isospace) defined by C and N isotopic values of potential food sources when they are adjusted to account for trophic fractionation (TF) (Olive et al. [52], Phillips et al. [58]). We multiplied averaged values for TF of C and N reported in the literature (i.e., mean TF of C = 0.54 ± 0.53 and of N = 3.02 ± 0.47; e.g., Bastos et al. [9]) by the trophic level of the consumer (Reid et al. [68], Phillips et al. [58]) to adjust isotopic ratios of sources to create a polygon to test a given consumer. We used the R (version 3.4.0; R Foundation for Statistical Computing, Vienna, Austria) package *tRophicPosition* (Post [63], Quezada-Romegialli et al. [67]) to estimate consumer trophic position with an equation that uses an assumed value for $\delta^{15}N$ enrichment and the baseline trophic level first (see estimated trophic positions in Table 1). We used the mean $\delta^{15}N$ value of the primary consumers (snails) as our baseline estimates (see Post [63]). Biplots of $\delta^{13}C$ and $\delta^{15}N$ of basal production sources and TF-corrected fish isotopic values revealed that, for 5 of 14 fish species, the TF corrected values fell outside the polygon defined by potential basal sources. These species included 2 detritivores (*C. incompta*, *P. latior*), 1 planktivore (*H. marginatus*), 1 herbivore (*R. microlepis*), and 1 omnivore (*H. microlepis*). We therefore did not run mixing models for these 5 species, but only for the other 9 species that the TF corrected values fell inside the polygon defined by potential basal sources as follows. Biplots showed that 5 species (the piscivores *A. abbreviatus*, *P. nattereri*, and *P. squamosissimus*, the herbivore *S. fasciatus*, and the omnivore *T. auritus*) had isotopic values that fell mostly within the polygon defined by potential sources when we included ^{13}C-enriched C4 grass in the biplot. For these species, all 5 of our groups of basal sources (i.e., mean and standard deviation of isotope values of C3 terrestrial plants: Capparaceae and Lamiaceae, C3 aquatic macrophytes: *E. crassipes, S. minima*, C4 grasses, POM, and phytomicrobenthos) were included as inputs for the mixing model. Isotopic values for the remaining 4 species (*P. castelnaeana*, *C. macropomum*, *M. aureum*, *P. brachypomus*) plotted inside the polygon defined by isotopic values of 4 relatively ^{13}C-depleted production sources, but well beyond the range of ^{13}C-enriched C4 grasses. Previous studies analyzing gut contents and stable isotope data for Amazonian fishes have shown C4 plants to contribute little to Amazon fish diets and food chains supporting fish biomass (Araujo-Lima et al. [5], Forsberg et al. [25], Benedito-Cecilio et al. [11], Benedito-Cecilio and Araujo-Lima [10], Oliveira et al. [53]). Based on that information and our $\delta^{13}C$ and $\delta^{15}N$ biplots, we assumed these 4 species had not assimilated C from C4 grass, so we did not include C4 grass in the mixing model for these species. This assumption is reasonable since the discriminatory power of mixing models generally decreases with the number of sources (Phillips et al. [58]). Convergence diagnostics (Gelman diagnostics) were close to 1 (<1.1), indicating that the model performed well.

Local scale analyses and variations across the landscape

We 1st used ANOVAs to evaluate the among-lake-system variation in isotopic ratios ($\delta^{13}C$ and $\delta^{15}N$ as dependent variables) of each trophic guild. Whenever we observed significant differences, we used ANOVAs to test for among-lake differences in either the $\delta^{13}C$ or $\delta^{15}N$ of each fish species in that trophic guild. Between-site differences in fish tissue isotopic ratios could be influenced by differences in body size, because fish feeding and assimilation dynamics may shift ontogenetically. Therefore, prior to ANOVAs, we also tested for possible differences in the length–frequency distributions of each trophic guild and each species across lake systems with Fisher's exact test. Length–frequency distributions only differed significantly among lake systems for detritivores and the herbivore-C4 guild (represented only by *S. fasciatus*). Within trophic guilds, length–frequency distributions did not differ significantly ($p > 0.05$) among lake systems for any species pair except *P. latior* (detritivore) and *S. fasciatus* herbivore C4. Therefore, for detritivore and herbivore-C4 guilds, and for the species *P. latior*, analysis of isotopic differences among lake systems was done with standard length (SL) as a covariate in an analysis of covariance (ANCOVA).

We used multivariate analysis of variance (MANOVA) to test for differences in $\delta^{13}C$ and $\delta^{15}N$ of each production source across lake systems. Whenever MANOVA yielded significant differences in isotopic composition, we performed a univariate ANOVA to test for differences in either the $\delta^{13}C$ or $\delta^{15}N$ of each source. To ensure that possible interspecific variation in $\delta^{13}C$ and $\delta^{15}N$ of plants (Correa et al. [16]) would not influence results, we ran separate tests for each terrestrial (families Capparaceae and Lamiaceae) and aquatic (*E. crassipes, S. minima*) plant (Table 2). Phytomicrobenthos were not included in these analyses because of small sample sizes that resulted from the apparent scarcity of this material at most sites. Assumptions for these analyses (e.g., normally distributed residuals) were tested and met in all cases. Statistical significance of these tests were corrected for multiple comparisons (Bonferroni correction) at a significance level of $\alpha = 0.01$.

Finally, we assessed variation in the proportional contribution of each primary production source to the biomass of each fish species across lake systems. To do this we used Bayesian mixing models to estimate the proportional contributions of sources to the biomass of each species in each lake system and evaluated the coefficients of variation (CV) of these estimations across lake systems (involving 7–19 lake systems, depending on the species). Procedures for mixing model analyses were the same as described above for the regional scale analysis.

Results

Regional scale patterns

The basal production sources (C3 plants, C3 aquatic macrophytes, C4 grasses, phytomicrobenthos, POM), fish trophic guilds, and fish species all showed considerable variation in $\delta^{13}C$ and $\delta^{15}N$ values across the region (Tables 1, 2; Figs 2–4). Isotope values among all basal sources ranged from approximately –34 to –21‰ for C, and from –5 to 11‰ for N. The difference between minimum and maximum values of basal sources ranged from 12 (phytomicrobenthos) to 7.3‰ (aquatic macrophytes) for C and from 7 (grasses and phytomicrobenthos) to 11‰ (C3 terrestrial plants) for N.

Graph: Figure 2. Boxplots (median and quartiles) of δ13C and δ15N of fishes grouped by trophic guilds.

Carbon isotopic values among trophic guilds ranged from about –39‰ for the planktivore (*H. marginatus*) to –14‰ for the C4 herbivore (*S. fasciatus*). Carbon isotopic variability within trophic guilds ranged from 16‰ for herbivores-C4 to about 8‰ for planktivores (Fig. 2), and within-species values ranged from 16 (*R. microlepis*) to 3‰ (*P. brachypomus*) (Fig. 3, Table 1). The 5 fish species with isotopic values that fell outside the isospace

of our 5 basal sources (*C. incompta*, *P. latior*, *H. marginatus*, *H. microlepis*, *R. microlepis*) had relatively ^{13}C-depleted values (Fig. 3). 29% of fish had C isotopic signature values between those of C3 aquatic macrophytes and POM, and 38% had C isotopic signatures between those of the aquatic macrophytes and C4 grasses. Nitrogen isotopic signatures among trophic guilds and fish species ranged from ~4‰ for the C4 herbivore (*S. fasciatus*) to 15‰ for the piscivore *P. castelnaeana* (Figs 2, 4; Table 1). We found lower variability in the planktivore (~2‰) and detritivores (~4‰) than in herbivores (~8‰) and piscivores (~7‰) (Fig. 2). Intraspecific variability ranged from ~2 (*A. abbreviatus*) to ~6‰ (*S. fasciatus*, *C. macropomum*) (Fig. 4, Table 1).

Graph: Figure 3. Biplots of mean (±SE) δ13C and δ15N of primary producers, excluding C4 grasses, for different species of fish at the regional scale in the lower Amazon River floodplain. δ13C and δ15N of fishes are shown by black dots and are corrected for trophic fractionation (see Methods). Squares and lines represent mean and standard errors, respectively, of the isotopic signatures of the basal resources.

Graph: Figure 4. Biplots of mean (±SE) δ13C and δ15N of primary producers, excluding C4 grasses, for different species of fish at the regional scale in the lower Amazon River floodplain. δ13C and δ15N of fishes are shown by black dots and are corrected for trophic fractionation (see Methods). Squares and lines represent mean and standard errors, respectively, of the isotopic signatures of the basal resources.

The regional-scale mixing model indicated that C3 aquatic macrophytes and POM were the principal sources contributing to the biomass of most fish species, with C4 grasses having an important contribution only to *S. fasciatus* (Table 3, Fig. S1). Except for *S. fasciatus*, C4 grasses (when included in the model) and phytomicrobenthos appeared to be the least important basal production sources for fish biomass (Table 3, Fig. S1).

Graph

Table 3. Means and 1 st to 99 th percentile ranges of estimated contributions of basal production sources to fish biomass in the floodplains of the lower Amazon. CV = coefficient of variation of the proportional contribution of a given source across lake systems. PMB = Phytomicrobenthos, C3M = C3 aquatic macrophytes, TP = terrestrial plants, POM = particulate organic matter, C4G = C4 grasses.

Species	Source	Mean	1st	99th	CV (%)
Colossoma macropomum	PMB	0.09	0.02	0.21	39
	C3M	0.45	0.26	0.61	40
	TP	0.10	0.02	0.23	32
	POM	0.36	0.30	0.43	41
Piaractus brachypomus	PMB	0.13	0.03	0.26	52
	C3M	0.22	0.06	0.38	79
	TP	0.15	0.03	0.31	32
	POM	0.50	0.42	0.59	37
Mylossoma aureum	PMB	0.10	0.02	0.23	24
	C3M	0.45	0.27	0.61	26
	TP	0.10	0.02	0.25	23
	POM	0.35	0.28	0.41	21
Schizodon fasciatus	PMB	0.07	0.01	0.17	29
	C3M	0.07	0.01	0.16	23
	C4G	0.30	0.24	0.37	27

Species	Source	Mean	1st	99th	CV (%)
TP		0.06	0.01	0.1427	
POM		0.50	0.42	0.5726	
Pellona castelnaeana	PMB	0.10	0.02	0.2337	
C3M		0.55	0.37	0.7144	
TP		0.12	0.02	0.2531	
POM		0.23	0.16	0.3033	
Pygocentrus nattereri	PMB	0.14	0.03	0.2842	
C3M		0.37	0.21	0.5234	
C4G		0.07	0.04	0.1146	
TP		0.09	0.02	0.2047	
POM		0.34	0.28	0.3941	
Plagioscion squamosissimus	PMB	0.14	0.03	0.3026	
C3M		0.40	0.22	0.5627	
C4G		0.06	0.02	0.1056	
TP		0.10	0.02	0.2223	
POM		0.30	0.23	0.3730	
Acestrorhynchus abbreviatus	PMB	0.14	0.03	0.2840	
C3M		0.38	0.22	0.5233	
C4G		0.08	0.05	0.1248	
TP		0.09	0.02	0.2051	
POM		0.31	0.24	0.3742	
Triportheus auritus	PMB	0.07	0.01	0.1940	
C3M		0.55	0.35	0.6936	
C4G		0.02	0.00	0.0434	
TP		0.08	0.01	0.2238	
POM		0.28	0.21	0.3639	

Local scale patterns and variation across the landscape

Isotopic compositions varied significantly across lake systems for most basal production sources, trophic guilds, and fish species. The effect of lake system on $\delta^{13}C$ and $\delta^{15}N$ was significant for all basal sources (C4 grass, C3 terrestrial plants, and the aquatic macrophyte *S. minima*) except for the C3 aquatic macrophyte *E. crassipes* (MANOVA, $F_{11,28} = 1.86$, $p = 0.03$) (Table 2). ANOVA revealed significant differences across lake systems for the $\delta^{13}C$ and $\delta^{15}N$ values of both C4 grass and POM, whereas significant differences occurred in $\delta^{15}N$ only for terrestrial plants (Table 2).

The variation in $\delta^{13}C$ and $\delta^{15}N$ among lake systems differed by fish trophic guild. Both $\delta^{13}C$ and $\delta^{15}N$ of detritivores and piscivores differed significantly among lake systems (Table 1). Lake system was associated with variation in $\delta^{13}C$ only for herbivores and omnivores, and only $\delta^{15}N$ varied significantly among lake systems for the C4 herbivore (*S. fasciatus*). The planktivore (*H. marginatus*) had no significant among-lake system variation for either element (Table 1). Patterns of variation in $\delta^{13}C$ and $\delta^{15}N$ in fish species differed with lake system (Table 1). Most species, including *C. macropomum*, *R. microlepis*, *A. abbreviatus*, and *P. squamosissimus*, showed significant differences in both $\delta^{13}C$ and $\delta^{15}N$ across lake systems. *Hemiodus microlepis*, *P. castelnaeana* and *P. nattereri* showed differences only in $\delta^{13}C$, whereas *P. latior* and *T. auritus* had significant spatial variation only in $\delta^{15}N$. *P. brachypomus*, *M. aureum* and *C. incompta* did not show significant differences, either in $\delta^{13}C$ or $\delta^{15}N$ across lake systems. Standard length was not related to variation in $\delta^{13}C$ or $\delta^{15}N$ across lake systems for 2 trophic guilds (detritivores and herbivore C4), but it was related to the variation in $\delta^{15}N$ for 1 species (*P. latior*) (Table 1).

Mixing model results for the 9 species that data conformed to the mixing model assumptions (e.g., consumer isotopic values fell within isospace) for individual lake systems indicated that, although C3 aquatic macrophytes and POM were the principal sources contributing to biomass of most fish species and lake systems, there was considerable variation within fish species (Table 3). Coefficients of variation (CV) indicated that the mean proportional contributions of production sources to fish biomass varied on averaged 37% across all sources and species, with variation among some sources and species as high as 79% (C3 aquatic macrophytes for *P. brachypomus*) (see coefficients of variation, CV, Table 3). Species with the greatest coefficients of variation in source contributions among lake systems were *P. brachypomus* (range of 47%), *A. abbreviatus* (18%), and *P. nattereri* (13%). The smallest CV in source contributions across lake systems was observed for *S. fasciatus* (range of 6%) and *M. aureum* (5%). Relatively high CV values reflect high variation in the contributions of production sources to fish biomass within a given lake system. For example, phytomicrobenthos and C3 terrestrial plants, the least important sources at the regional scale, were the most important sources (contributing >30%) for 5 species in 5 lake systems, and for 4 other species in 7 lake systems. Conversely, C3 aquatic macrophytes and POM, the most important sources assimilated by fishes at the regional scale, were relatively unimportant (proportional contribution <7%) for 5 species in 4 lake systems, and 4 other species in 7 lake systems.

Discussion

Our results indicate that isotopic ratios of production sources, fish trophic guilds, and fish species vary across the Amazon floodplain landscape, even when connectivity has been high for ~7 mo. Our results also showed that contributions of alternative basal production sources to fish biomass, and therefore foodweb proprieties, vary with the spatial scale of observation. Taken together, these results are consistent with the view that networks of local food webs interact within broader landscapes to produce a regional trophic network (Holt [33], Winemiller and Jepsen [83], Pillai et al. [59]) and that ecological processes and interactions depend on mechanisms operating at multiple spatial scales (Leibold et al. [42], Presley et al. [66]). In addition, because outcomes from stable isotope analyses varied spatially and were sensitive to spatial scale, our study also supports the idea that sampling protocols for stable isotope analyses need to address spatial variability in isotopic composition (Correa et al. [16]). Finally, as described below, our findings on the spatial variation in the isotopic composition of production sources, fishes from different functional groups, and production source contributions to fish biomass advance understanding of the flow of matter and energy in aquatic habitats of the Amazon floodplain.

Regional scale patterns

Our results indicate that aquatic macrophytes and POM are important basal sources that contribute to fish biomass in the Amazonian floodplains, at least under the high-water conditions of the annual flood pulse. This result is consistent with findings for other rivers that carry high loads of suspended fine sediments (Roach [71], Ou and Winemiller [54]). These results are also consistent with studies that evaluated $\delta^{13}C$ values of tree parts, C3 aquatic macrophytes, and periphyton and phytoplankton, and found that C3 plants were the primary source of C for several Amazonian fish species (e.g., Araujo-Lima et al. [5], Forsberg et al. [25], Benedito-Cecilio et al. [11], Oliveira et al. [53]). C3 aquatic macrophyte leaves tend to have relatively high mineral and protein content, and along with fruits and seeds, are probably among the most nutritious plants in the Amazon floodplain (Forsberg et al. [25]). Herbivorous fishes associated with aquatic macrophyte beds that contain both C4 grasses (e.g., *Echinochloa polystachya*, *Paspalum repens*) and C3 macrophytes (e.g., *E. crassipes*, *Pistia stratiotes*, *Ceratopteris pteroides*, *S. minima*) were reported to avoid eating C4 grasses (Junk [38]). Instead, these fish consumed and, in many cases controlled, C3 plant biomass (Junk [38]). Our mixing model estimates

indicate that most fishes assimilated relatively little material from C3 terrestrial plants compared with aquatic C3 macrophytes. This result, however, does not discount the importance of fruits and seeds that are consumed directly by several herbivorous fishes, especially those from the family Serrasalmidae (Goulding [28], Correa et al. [15]). Most of our tissue samples for frugivorous species, such as *Colossoma macropomum*, were from subadults, which tend to be less-specialized frugivores and granivores than larger conspecifics (Forsberg et al. [25], Araujo-Lima and Goulding [6]). Given overlapping ranges of $\delta^{13}C$ values for C3 aquatic and terrestrial plants, it also is possible that our mixing model overestimated contributions from aquatic macrophytes versus C3 terrestrial plants. However, our analysis of the variation in proportional contributions of basal production sources to fish biomass across the landscape indicated that in some catchments C3 terrestrial plants were an important or a moderately important basal food source in food webs. C3 terrestrial plants were an important food source for *P. nattereri*, *T. auritus*, *A. abbreviatus*, and *P. castelnaeana*. C3 terrestrial plants were a moderately important food source for *C. macropomum*, *P. squamosissimus, M. aureum*, and *P. brachypomus*.

POM has not been previously reported to be an important production source supporting fish biomass in the Amazonian floodplains. Previous studies (Forsberg et al. [25]) have used similar methods for sampling suspended POM, but they found ^{13}C-depleted values that suggested a stronger contribution from phytoplankton. Conversely, our $\delta^{13}C$ values for POM are less ^{13}C-depleted and show intermediate values between those of C3 plants and C4 macrophytes. Since it is unlikely that this suspended material was derived from ^{13}C-depleted production sources, such as phytoplankton, the POM in our study could have been a mixture of detritus derived from aquatic and riparian plants with humic material from soil (Hedges et al. [30]).

Isotope studies have consistently shown that C4 grasses contribute little to the biomass of most fishes in the Amazon and other floodplain rivers (Araujo-Lima et al. [5], Forsberg et al. [25], Thorp et al. [76], Benedito-Cecilio et al. [11], Benedito-Cecilio and Araujo-Lima [10], Oliveira et al. [53], Zeug and Winemiller [86], Roach et al. [72], Mortillaro et al. [50], Ou and Winemiller [54]). Despite the fact that C4 grasses are responsible for much of the primary production in floodplain systems (Melack and Forsberg [46]), they seem to have low importance as a basal source in food chains supporting aquatic consumers. Their low importance may be caused by the relatively low digestibility of C4 grasses for many consumer taxa (Minson [48], Mortillaro et al. [50]). At the regional level, even *S. fasciatus*, a species known to feed extensively on grasses, appears to have assimilated large fractions of material that originated from POM. However, our analysis of the variation in proportional contributions of grasses to fish biomass across the landscape indicated that in many lake systems there were some fish species that had assimilated substantial amounts of material that originated from C4 grasses (e.g., coefficients of variation of assimilation of C4 grasses among lake systems for *P. nattereri* = 46%, *A. abbreviatus* = 48%, and *P. squamosissimus* = 56%)

The biplots and ranges of $\delta^{13}C$ and $\delta^{15}N$ values of fishes, particularly detritivores, indicate that biomass of 5 fish species we analyzed was probably derived, in part, by 1 or more relatively ^{13}C-depleted production source we did not sample. One candidate is phytoplankton. The range of $\delta^{13}C$ values for these fishes (–38.7 to –24.6‰) falls within the range of values reported for phytoplankton in the Amazon (Araujo-Lima et al. [5], Forsberg et al. [25], Martinelli et al. [44]). Phytoplankton is probably one of the main sources of C supporting biomass of several fish species in the Amazon floodplain, such as detritivores (Araujo-Lima et al. [5], Benedito-Cecilio et al. [11]), including *C. incompta* and *P. latior*, and the planktivorous catfish *H. marginatus*. Alternatively, the missing source in our analysis could be chemolithotrophic bacteria, which have $\delta^{13}C$ values significantly lower than phytoplankton (Peterson et al. [57], Fry and Sherr [27]) and could support higher consumers via detritus–microbial pathways. However, the consumer $\delta^{15}N$ values were not unusually low, as might be

expected if these fish consume chemolithotrophic bacteria. Improved knowledge of aquatic food webs in the Amazonian River and floodplains could be achieved by focusing research on the potential roles of phytoplankton and the microbial loop in supporting fish biomass.

Isotopic variation across the landscape

Our results demonstrated that $\delta^{13}C$ and $\delta^{15}N$ values of sources, fish trophic guilds, fish species, and the proportional contributions of production sources to fish biomass each varied across the landscape. These results are consistent with earlier claims about the variable nature of foodweb structure (Warren [78], Winemiller [80], [81]). Nitrogen and C isotopic values of all production sources, except for $\delta^{13}C$ of C3 terrestrial plants, varied spatially. This pattern is consistent with previous studies that found variation in the isotopic values of plants and POM along the Amazon River (Hedges et al. [30], Martinelli et al. [43], Mortillaro et al. [51]). Similar to these studies, our investigation produced evidence of a longitudinal gradient in which downriver $\delta^{13}C$ in aquatic macrophytes and phytomicrobenthos was lower than $\delta^{13}C$ for these groups in upstream reaches (Fig. S2). These patterns could be a result of the relatively limited number of samples we had at the ends of the longitudinal gradient for these sources, which may have obscured the potential variability in $\delta^{13}C$. However, the longitudinal trend of $\delta^{13}C$ in aquatic macrophytes could also be a result of greater fluxes of more ^{13}C-depleted dissolved CO_2 going from the river into the atmosphere in downstream reaches. Higher CO_2 output from downstream reaches would result in lower $\delta^{13}C$ in aquatic macrophytes that assimilate this dissolved CO_2 (Martinelli et al. [43], Benedito-Cecilio et al. [11]). Low values of $\delta^{13}C$ in phytomicrobenthos samples might be associated with CO_2 respired by ^{13}C-depleted aquatic macrophytes. The spatial variation in $\delta^{13}C$, as well as $\delta^{15}N$, for some sources of POM (Fig. S2) may be explained by variation in geomorphology and the extent of forest cover, which could result in differences in the quality of decomposing organic materials and soils (Renó et al. [69], Mortillaro et al. [51]).

Based on our results, we can only speculate on possible causes of $\delta^{13}C$ and $\delta^{15}N$ spatial variation. However, our findings provide a foundation for future research to tests these and other hypotheses. Multiple factors have either been shown or suggested to influence isotopic composition of basal production sources. These include variation in biogenic CO_2, differential diffusion rates of ^{13}C and ^{12}C during photosynthesis associated with variation in environmental conditions (e.g., river discharge, water velocity and canopy cover), pH and other abiotic factors, Chl *a* density, availability of dissolved inorganic C from various pools, physiological processes, genotype, etc. (Depetris and Kempre [21], Forsberg et al. [25], Finlay et al. [24], Evans [22], Finlay [23], Dawson et al. [19], Amundson et al. [2], Ishikawa et al. [35]).

Spatial variation in $\delta^{13}C$ for certain trophic guilds and species (herbivores-C3: *C. macropomum*, *R. microlepis*, and *H. microlepis*; piscivores: *A. abbreviatus*, *P. squamosissimus*, *P. castelnaeana* and *P. nattereri*), as well as variation in estimates of relative contribution of production sources to fish biomass in general, may reflect differences in trophic pathways at various locations within the landscape. Local food webs probably vary in the abundance and nutritional quality of alternative primary producers, detritus, and invertebrate prey as well as local environmental conditions. This hypothesis is supported by previous studies showing that tropical fishes have flexible feeding strategies that allow specialization when preferred resources become more available or when alternative resources become less available (Winemiller [80], Dabrowski and Portella [18], Correa and Winemiller [17]).

The spatial variation in $\delta^{15}N$ observed for certain trophic guilds and species (e.g., *C. macropomum*, *R. microlepis*, *S. fasciatus*, *P. latior*, *T. auritus*, *A. abbreviatus*, *P. squamosissimus*) could be influenced by

variations in $\delta^{15}N$ among sources or may reflect intraspecific differences in trophic position across the landscape. Our results that length–frequency distributions of most fish species were similar among lake systems suggest that there were no ontogenetic shifts in trophic positions in relation to location. However, the trophic level of an organism or average trophic level of a population should be dynamic rather than constant (Polis and Strong [62]). Many factors could affect spatial variation in trophic ecology, such as life history traits or the availability of alternative basal production sources and prey. Conversely, spatial variation in $\delta^{15}N$ of production sources was sometimes accompanied by similar variation in fish $\delta^{15}N$ values, indicating that these variations may covary in space (e.g., $\delta^{15}N$ of grass and *S. fasciatus* were positively related; Fig. S2).

Some limitations of stable isotope analysis for ecological inferences

The normal range of fish movement in the lower Amazonian floodplains is within lake systems, but some fishes migrate across lake systems during high-water conditions. Some of these species undergo seasonal migrations up to several hundred km (Ribeiro de Brito and Petrere [70], Barthem et al. [8]). Therefore, it is possible that $\delta^{13}C$ and $\delta^{15}N$ values in tissues of some fishes may actually reflect isotopic composition of production sources assimilated in inundated habitats or a different lake system. If this were the case, it could bias mixing model estimates of proportional contributions of local basal sources to fish biomass. Replication of this study across hydrological stages of the Amazon, particularly during the end of the dry season when lake systems have been isolated from each other for about 2 months, might reveal even stronger patterns of spatial variation in foodweb structure.

Stable isotope analysis has some practical limitations in general, including when it is used to estimate production sources and consumer diets in food webs. For example, there are uncertainties in the estimates of isotopic turnover time of tissues and trophic fractionation. There can also be missing basal resources, a lack of isotopic distinction among sources (end-members), and isotopic variability in sources and consumers (and see Layman et al. [40], Phillips et al. [58]). We used the same mixing model assumptions uniformly in our analyses, and results were based on a large number of samples obtained over a large region. However, we recognize that the aforementioned limitations could have influenced our results and interpretations.

In our study, potential sources of bias for mixing model estimates of proportional contributions of basal sources to fish biomass also include missing sources, exclusion of C4 grass as an end member, and low discrimination of certain sources based on $\delta^{13}C$ (e.g., aquatic vs terrestrial C3 plants). Relevant production sources could have been missing even for species that plotted inside an isospace polygon defined by source end members. As previously mentioned, missing sources might include phytoplankton and bacteria, as well as phytomicrobenthos samples from other microhabitats (see Methods). The exclusion of C4 grass from mixing models for certain species was based on our exploratory analysis of isotopic data and knowledge of the diets of these fishes, and it seems unlikely that its inclusion in models would significantly change estimated contributions of other basal sources to consumer biomass. The optimal number of sources to include in models always involves a tradeoff between sensitivity to missing sources versus lack of resolution associated with too many sources (Phillips et al. [58]). Ultimately, discriminatory power depends on the isotopic separation of sources. We note, however, that even if these factors influenced our estimates of proportional contributions of sources to fish biomass, it is unlikely that this bias would have systematically changed the major patterns of variation we observed within and among lake systems.

Conclusions

Aquatic food webs in the Amazon River floodplains vary spatially according to scales of resolution. The isotopic composition of basal production sources and fishes belonging to several trophic guilds differ among lake systems. Estimates of proportional contributions of basal production sources revealed the importance of C3 aquatic macrophytes and POM to biomass of most fish species. Grass was only important for 1 species, *S. fasciatus*, both at the regional scale and within most lake systems. Estimates for relative contributions of basal sources to biomass of most species varied among lake systems, with important sources assimilated at the regional scale sometimes being relatively unimportant in certain lake systems. Conversely, sources of low importance for most species at the regional scale (e.g., phytomicrobenthos, C3 terrestrial plants) sometimes were very important at the local scale. Several factors probably influence spatial variation in aquatic food webs of the Amazon floodplain, including variation in the quality and quantity of basal sources associated with gradients of watershed vegetation, heterogeneity of abiotic environmental conditions, and differences in structures of local populations and species assemblages. Although it would be difficult to do for a large system like the Amazon River and floodplains, it would be useful to investigate, at finer and broader spatial and temporal scales, the ways that these factors mediate variation in isotopic compositions of foodweb components and estimates of material transfer in food webs. Given that estimated contributions of production sources to fish biomass varied according to the spatial scale of analysis, researchers should be cautious when analyzing and interpreting isotopic data based on few samples. Depending on the hypothesis being tested, study designs should consider not only temporal replication, but also replication at appropriate spatial scales.

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Footnotes

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