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Carbon Sources in Riverine Food Webs: New Evidence from Amino Acid Isotope Techniques

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Abstract

A nearly 40-year debate on the origins of carbon supporting animal production in lotic systems has spawned numerous conceptual theories emphasizing the importance of autochthonous carbon, terrestrial carbon, or both (depending on river stage height). Testing theories has been hampered by lack of adequate analytical methods to distinguish in consumer tissue between ultimate autochthonous and allochthonous carbon. Investigators initially relied on assimilation efficiencies of gut contents and later on bulk tissue stable isotope analysis or fatty acid methods. The newest technique in amino acid, compound specific, stable isotope analysis (AA-CSIA), however, enables investigators to link consumers to food sources by tracing essential amino acids from producers to consumers. We used AA-CSIA to evaluate nutrient sources for 5 invertivorous and 6 piscivorous species in 2 hydrogeomorphically contrasting large rivers: the anastomosing Upper Mississippi River (UMR) and the mostly constricted lower Ohio River (LOR). Museum specimens we analyzed isotopically had been collected by other investigators over many decades (UMR: 1900–1969; LOR: 1931–1970). Our results demonstrate that on average algae contributed 58.5% (LOR) to 75.6% (UMR) of fish diets. The next highest estimated contributions of food sources were from C_3 terrestrial plants (21.1 and 11.5% for the LOR and UMR, respectively). Moreover, results from 11 individually examined species consistently demonstrated the importance of algae for most fish species in these trophic guilds. Differences among rivers in relative food source availability resulting from contrasting hydrogeomorphic complexity may account for relative proportions of amino acids derived from algae.

Key words: Flood Pulse Concept; Ohio River; River Continuum Concept; Riverine Ecosystem Synthesis; River Wave Concept; Upper Mississippi River.

Introduction

Aquatic ecologists have been debating the carbon basis of food webs in lotic systems for nearly half a century. Resulting perspectives and published concepts have been influenced by the investigator's: (a) choice of methods (short-term gut contents or longer-term measures using fatty acids and either bulk tissue or amino acid stable isotopes); (b) selection of dependent variables (secondary production, species diversity, or system metabolism);

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and (c) focus on system size (headwaters, mid-order streams, large rivers, or floodscapes), flood characteristics (timing and length), or the river's physical structure (hydrogeomorphic or network connections). The primary food source models developed over this period are the River Continuum Concept (RCC; Vannote and others 1980; Sedell and others 1989), the Flood Pulse Concept (FPC; Junk and others 1989; Junk and Wantzen 2004), the Riverine Productivity Model (RPM; Thorp and Delong 1994, 2002), the Riverine Ecosystem Synthesis (RES; Thorp and others 2006, 2008), and the River Wave Concept (RWC; Humphries and others 2014).

Most of these theories, other than the RPM and RES, emphasized the dominant importance to large river food webs of allochthonous carbon from the riparian zone and broader floodscape, with its lentic habitats and normally dry terrestrial floodplains. The RCC initially theorized (Vannote and others 1980) that these food webs were supported primarily by leakage of mostly allochthonous, dissolved and fine particulate carbon from upstream and the terrestrial environment—a source that would consist primarily of recalcitrant carbon. After the FPC (Junk and others 1989) proposed instead that food webs in large rivers were supported by carbon from flooded forests (based on studies in the Amazon, but with predictions for the Mississippi), the RCC was revised to indicate that floodplain carbon was the primary source fueling large river food webs (Sedell and others 1989). The RPM disputed these conclusions (Thorp and Delong 1994, 2002), proposing instead that large river food webs were primarily fueled by carbon from autochthonous primary production in the main channel and lateral slackwaters. Later, the RES emphasized the broad ecological influence of hydrogeomorphic structure from headwaters to the river terminus (Thorp and others 2006, 2008). The RWC (Humphries and others 2014) suggested that all four models were correct, depending on the river's hydrographic stage.

Our purpose here is to focus on a single question: ''Which source of carbon (autochthonous or allochthonous) is most important to eukaryotic food webs in the riverscape of two large rivers with contrasting hydrogeomorphic structures, one with a complex anastomosing channel (Upper Mississippi River, or UMR) and the other with a relatively simple, constricted channel (the lower Ohio River, or LOR)?'' To answer this question, we employed the newest analytical method (amino acid compound-specific isotope analysis—increasingly known as AA-CSIA), which was unavailable to authors of the RCC, FPC, RPM, and RES when their hypotheses were first published.

Our analysis is primarily limited here to riverscapes (main channel and lateral slackwaters below flood stage) of ''large'' rivers, which we operationally define here as follows: (a) being wide enough so that riparian shading covers less than 10% of the main channel width; (b) having a stream order of six or larger; and (c) characterized by temporal flood pulses ranging from asynchronous and short-lived to highly synchronous and lasting months. We emphasize sources of carbon influencing animal production rather than overall system metabolism.

In our study, we evaluated food sources supporting invertivorous and piscivorous fishes in the UMR and LOR using AA-CSIA on museum specimens. The lowhead dams in both rivers stabilize water levels during periods of low discharge, maintaining a minimum stage height (depth) needed for barge navigation. The magnitude and duration of high flows in these ''run-of-the-river'' systems are relatively unchanged, however, because the dams are overtopped or the gates are opened during periods of high discharge (Chen and Simons 1986; Alexander and others 2012). Thus, the ecological/hydrological conditions are much more similar to unregulated rivers than rivers impounded by high dams.

Methods

Using AA-CSIA Analysis to Determine Food Sources

We analyzed sources of carbon in food webs using AA-CSIA, a relatively new ecological technique employed mostly in marine studies (for example, Chikaraishi and others 2007, 2009; Popp and others 2007; Hannides and others 2009; McMahon and others 2015b). Although previous methods are still useful, the AA-CSIA offers greater analytical precision and flexibility while providing more tracers to delineate food sources. The specific advantages of this method over bulk tissue stable isotope analysis (or ''bulk tissue analysis'') and/or fatty acid analysis are as follows: (1) It can more precisely (compared to bulk tissue techniques) distinguish in consumer tissue the ultimate source of organic matter based on the presence of both essential and non-essential amino acids (Bowes and Thorp 2015); (2) trophic position can be determined by measurement of nitrogen isotopes (not possible with lipid analysis); (3) the source signature is not altered significantly by sample location or collec-

tion time (in contrast to bulk tissue techniques); (4) the signature of primary consumers (herbivores) need not be analyzed in order to identify organic sources for higher level consumers (common approach in most bulk tissue analyses); (5) the results are very precise (low standard error) compared to bulk tissue analysis (Bowes and Thorp 2015); (6) field samples can be preserved in salt, alcohol, or formalin (Hannides and others 2009; González-Bergonzoni and others 2014), thereby allowing use of both field and museum samples; (7) rapid field processing to preserve the signature is not required (in contrast to fatty acid analysis); and (8) the signature reflects the long term average or feeding patterns (comparable to bulk tissue and fatty acid techniques but unlike the rapid turnover in gut content analysis). The disadvantages of AA-CSIA are that samples require extensive laboratory preparation (true also for fatty acid techniques) prior to analysis on an IRMS (isotope-ratio mass spectrometer), and relatively few laboratories currently perform these services for outside investigators. Both these limitations contribute to the currently high analytical costs of AA-CSIA, which is presently 10–20× or greater than for most bulk tissue analysis.

Museum Fish Samples

Museum collections and species surveys by government agencies provide data potentially useful for analyzing long-term environmental impacts (Vander Zanden and others 2003; Gido and others 2010) as well as spatially dispersed ecological processes. Although present-day investigators are limited by historic variations in the species preserved, collection dates, and specific sites sampled, these restrictions can be ameliorated by careful development of the study scope and the spatial and temporal scales over which specimens are selected (for example, Vander Zanden and others 2003).

We analyzed food sources and trophic state of piscivorous and invertivorous fishes from 300^{+} km stretches of the UMR (Wabasha, Minnesota to Savanna, IL USA) and LOR (Evansville, Indiana to Cairo, IL USA) using preserved specimens from museums. Samples were donated by the Bell Museum, Field Museum, Illinois Natural History Survey, Illinois State Museum, Milwaukee Public Museum, Ohio State University Museum of Biological Diversity, Southern Illinois University, University of Michigan Museum of Zoology, and University of Wisconsin–Stevens Point. To establish initial diet tendencies of species, we consulted state taxonomic keys for Missouri, Tennessee, and Wisconsin (Etnier 1993; Pflieger 1997; Becker 1983). In total, we analyzed food sources for 4 species (29 individuals) of invertivores and 5 species (37 individuals) of piscivores originally collected (1900–1969) from the UMR, and 4 species (30 individuals) of invertivores and 4 species (24 individuals) of piscivores originally sampled (1931–1970) from the LOR (Table 1). The largest preserved specimens were chosen for tissue harvesting; however, museum fish specimens are often relatively small, reflecting the need to conserve limited shelf space. Because of body size restrictions, it is likely that some of these piscivores were more omnivorous (that is, feeding also on invertebrates) than their guild placement would suggest (see Table 1). In some analyses, therefore, we combined these groups.

Sample Processing and Isotope Analysis of Fish Tissue

For isotope analysis, we first extracted muscle tissues from an area between the lateral line and dorsal fin of adult fish preserved in various museums and then stored these samples on ice until returned to the lab. They were then rinsed with deionized water, placed in pre-combusted glass vials, dried at 60°C for 48 h, and ground into a fine, homogenized powder using a Wig-L-Bug® mixer/amalgamator.

Processed samples were later shipped to the UC-Davis Stable Isotope Facility for analysis of amino acid stable isotope signatures. General techniques for AA-CSIA are summarized below and extensively described by Walsh and others (2014). Sample preparation involves acid hydrolysis for the liberation of amino acids from proteins and derivatization by methyl chloroformate to produce compounds amenable to GC analysis. Amino acid derivatives are injected in split (^{13}C) or splitless (^{15}N) mode and separated on an Agilent J&W factor FOUR VF-23 ms column (30 m × 0.25 mm ID, 0.25 micron film thickness). Once separated, amino acid derivatives are quantitatively converted to CO_2 and NO_x in an oxidation reactor at 950°C, and NO_x are subsequently reduced to N_2 in a reduction reactor at 650°C. Following water removal through a nafion dryer, N_2 or CO_2 enters the isotope-ratio mass spectrometer. A pure reference gas (CO_2 or N_2) is used to calculate provisional δ-values of each sample peak. Next, isotopic values are adjusted to an internal standard (for example, norleucine) of known isotopic composition. Final δ-values are obtained after adjusting the provisional values for changes in linearity and instrumental drift such that correct δ-values for laboratory standards are

Table 1. Numbers of Species Analyzed from the Mississippi and Ohio Rivers

	Number sampled		Trophic position	
	UMR	LOR	UMR	LOR
Presumptive piscivores				
Esox americanus (Redfin Pickerel)	9	0	2.67 ± 0.06	–
Micropterus punctulatus (Spotted Bass)	0	6	–	3.25 ± 0.05
Morone chrysops (White Bass)	8	5	2.94 ± 0.13	3.14 ± 0.32
Morone mississippiensis (Yellow Bass)	4	1	3.08 ± 0.18	3.54
Sander canadensis (Sauger)	9	12	3.12 ± 0.09	3.14 ± 0.14
Sander vitreus (Walleye)	7	0	3.18 ± 0.13	–
Mean for Piscivores	37	24	3.00 ± 0.06	3.20 ± 0.08
Presumptive invertivores				
Aplodinotus grunniens (Freshwater Drum)	5	6	2.59 ± 0.12	2.79 ± 0.07
Ictalurus furcatus (Blue Catfish)	0	6	–	3.04 ± 0.11
Percina shumardi (River Darter)	5	0	2.59 ± 0.14	–
Pomoxis annularis (White Crappie)	8	8	3.32 ± 0.05	3.17 ± 0.09
Pomoxis nigromaculatus (Black Crappie)	11	10	3.14 ± 0.09	2.99 ± 0.08
Mean for invertivores	29	30	2.95 ± 0.07	3.01 ± 0.05

Numbers of different fish species analyzed from the Upper Mississippi (UMR) and lower Ohio Rivers (LOR) using AA-CSIA and their trophic position as calculated by their mean $\delta^{15}N$ value ±1 SE.

obtained. Signatures of both $\delta^{13}C$ and $\delta^{15}N$ were determined for the following amino acids and expressed as per mil (‰): alanine, aspartic acid, glutamic acid, glycine, isoleucine, lysine, methionine, phenylalanine, proline, tyrosine, and valine. Tyrosine signatures were excluded from analyses due to missing measurements caused by concentrations below detection limits.

To calculate trophic position of consumers from AA-CSIA data, we employed the following formula: $TP = [(($ ^{15}N of glutamic acid-^{15}N of phenylalanine$)-3.4) \div 7.6] + 1$ (for example, Chikaraishi and others 2007, 2009, 2014; Popp and others 2007; Hannides and others 2009; Steffan and others 2013; Bowes and Thorp 2015).

Food Source Calculations

To match the AA-CSIA signature in the consumers with potential food sources, we measured isotopic signatures in nine potential aquatic (autochthonous) and terrestrial (allochthonous) food sources obtained commercially or from our local area (Table 2). Such sources could easily vary in species from those currently present in our LOR and UMR sample areas as well as those present over the last 100 years. These differences would obviate proper use of ''bulk tissue isotope techniques'' because of confounding differences in bulk tissue signatures by species, location, and time. However, the same is not true for AA-CSIA where close proximity in space and time is not required because the chemical signature of the essential and non-essential amino acids are highly conserved among broad taxonomic groups and not influenced significantly by location (Larsen and others 2009, 2013). For example, Larsen and others (2014) found at most subtle differences in $\delta^{13}C$ AA values among axenic cultures of 4 distinct algal groups (for example, 2 chlorophytes, 2 chrysophytes, 5 diatoms, and 3 haptophytes) and among species from diverse freshwater, estuarine, and marine habitats. Moreover, they reported no systematic differences in $\delta^{13}C$ AA patterns between plants raised in greenhouses and similar species collected from boreal and mangrove ecosystems.

Amino acids can be considered essential (4 in our study), conditionally non-essential (2), and non-essential (3), with ''essential'' in biology referring to amino acids that animals cannot manufacture. The two non-essential amino acid categories can be useful in identifying organic sources (including from bacteria), as they have also been shown to reveal significant interactions between taxa and amino acid identities (Larsen and others 2009). However, investigators need to be cautious in their interpretations because some trophic modifications can occur in animals for amino acids not considered essential (McMahon and others 2015a). We recommend use of both essential and non-essential amino acids for studies where the number of independent variables (for example, food sources) would otherwise exceed the number of dependent variables (quantity of essential amino acids). If the number of independent variables is five or fewer,

Table 2. Isotope Values for Essential and Non-essential Amino Acids

Food source	Genus	Essential amino acids				Conditionally non-essential		Definitely non-essential		
		ile	leu	phe	val	gly	pro	ala	asp	Glu
CYA	*Spirulina*	−26.74 ± 0.46	−31.05 ± 1.11	−26.35 ± 0.75	−28.07 ± 0.44	−17.83 ± 0.71	−16.37 ± 0.40	−17.85 ± 0.36	−09.20 ± 0.26	−19.75 ± 1.04
GRE	*Chlorella*	−31.29 ± 0.40	−34.42 ± 0.54	−35.17 ± 0.90	−35.62 ± 0.29	−21.04 ± 1.16	−24.30 ± 0.46	−24.44 ± 0.63	−21.33 ± 0.76	−29.24 ± 0.64
FUN	*Saccharomyces*	−12.75 ± 0.75	−16.78 ± 0.37	−11.34 ± 0.66	−11.33 ± 0.59	−15.43 ± 0.45	−12.43 ± 0.87	−07.98 ± 0.22	−12.08 ± 2.36	−12.20 ± 0.24
C_3	*Elymu, Populus, Glycine, Vallisneria*	−27.06 ± 0.48	−34.45 ± 0.42	−30.07 ± 1.11	−32.36 ± 0.85	−04.47 ± 0.69	−19.05 ± 0.27	−22.85 ± 0.63	−15.77 ± 0.95	−25.67 ± 0.72
C_4	*Zea*	−12.69 ± 0.58	−19.66 ± 0.90	−16.47 ± 1.29	−15.51 ± 1.69	−03.13 ± 1.02	−01.84 ± 0.55	−09.15 ± 0.16	−14.52 ± 0.71	−14.48 ± 2.10

Isotope ($\delta^{13}C$) values shown for the 4 essential and 5 non-essential amino acids shown for potential food sources for fishes in the UMR and LOR. Data represent mean $\delta^{13}C$ values ±1 SE, based on 3 replicates of each genus sampled. Essential amino acids are ile isoleucine, leu leucine, phe phenylalanine, val valine. Conditionally non-essential amino acids are gly glycine, pro proline. Non-essential amino acids: ala alanine, asp aspartic acid, glu glutamic acid. Food sources are CYA cyanobacteria, GRE green algae, FUN fungi, C_3 C_3 terrestrial grass, tree leaves, soybean, and aquatic macrophyte, C_4 C_4 terrestrial corn.

then the investigator could use just essential amino acids.

To calculate the amino acid composition of food sources, we measured isotopic signatures using $\delta^{13}C$ AA-CSIA for three ''replicates'' of the following potential aquatic (autochthonous) and terrestrial (allochthonous) food sources, as represented biochemically by cyanobacteria (*Spirulina*), green algae (*Chlorella* sp.), fungi (baker's yeast or *Saccharomyces cerevisiae*), C_3 plants (C_3 grass (*Elymus* sp., probably *E. virginicus*), C_3 tree leaves (cottonwood, *Populus deltoides*), C_3 crop (soybean, *Glycine max),* and C_3 aquatic vascular macrophyte (wild celery, *Vallisneria americana*), and a C_4 terrestrial plant (corn, *Zea mays*). These specific food sources were chosen as they represent common food sources available in rivers across the USA. The terrestrial sources were collected in Lawrence, Kansas, and aquatic sources were ordered from laboratory cultures (PureBulk.com). These new signatures were used in conjunction with data from other aquatic studies (Larsen and others 2009, 2013) to determine classification and specific isotopic fingerprints of the different food sources (Figures 1 and 2).

The $\delta^{13}C$ values of each amino acid were normalized (Figure 1) to their respective sample means ($\delta^{13}C_{AA}$-mean $\delta^{13}C$) and tested for univariate normality. Normalizing the values to the means removes any effect of growth media among different food sources. To explore patterns and determine producer food groups, we performed principal component analysis on normalized $\delta^{13}C$ signatures of all available amino acids. The results showed that samples clustered according to major phylogenetic associations, with five major groups identified: cyanobacteria, algae, fungi, C_3 plants, and C_4 terrestrial plants (Figure 2). Differences in each amino acid $\delta^{13}C$ signature among producer groups were tested with ANOVA. We then ran linear discriminant function analysis on $\delta^{13}C$ AA-CSIA to determine the combination of $\delta^{13}C$ AA-CSIA values as independent variables (9 amino acids) that best explained differences between food sources (categorical variables determined by principal component analysis). The reference $\delta^{13}C$ isotopic composition of the 9 amino acids as well as their associated uncertainties (±1 SE) of the 8 food sources are provided in Table 2. We used a leave-one-out cross validation approach to calculate the probability of food source group membership of the classifier samples. To test that there were no difference in classification among groups, Pillai-Bartlett trace (MANOVA) was applied. All preliminary analyses on food sources were done in Minitab 14 (Minitab Inc., State College, PA, USA).

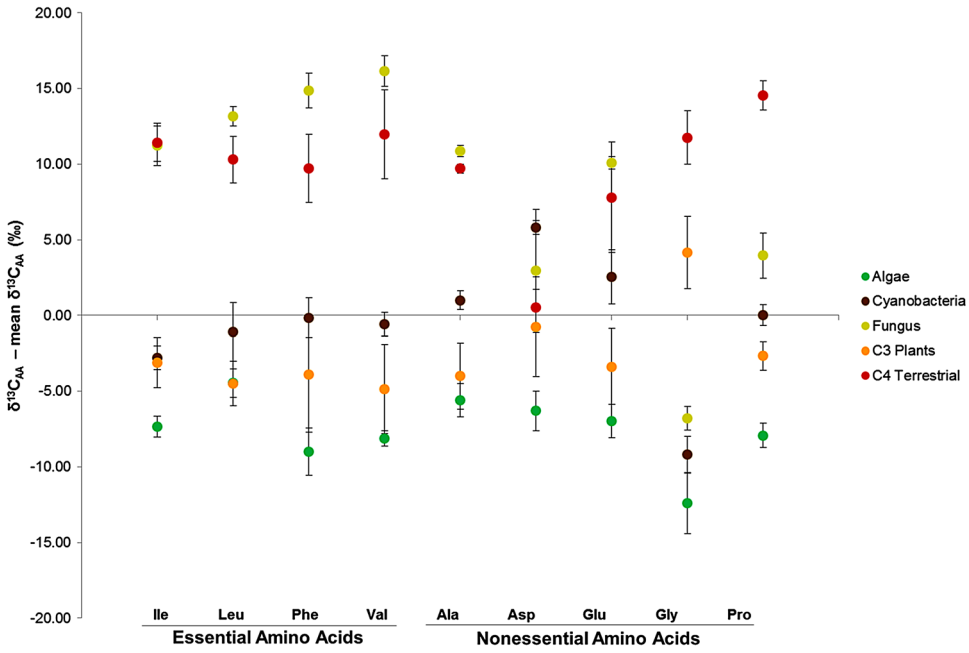

Figure 1. The $\delta^{13}C$ values of individual amino acids for each of 5 food source groups, relative to their respective mean $\delta^{13}C$. *Error bars* indicate ±SD of each taxon.

Relative contributions of dietary amino acids to consumers were estimated using the software ''Food Reconstruction Using Isotopic Transferred Signals'' (or FRUITS; Fernandes and others 2014, 2015). Normalized $\delta^{13}C$ values as well as their associated uncertainties (±1 SD) for each consumer species and potential food sources (Table 1) in the river were incorporated in the FRUITS model. FRUITS accounts for dietary routing, that is, the contribution of different original primary production sources towards the consumer's amino acids signals. It was assumed that all food sources were equally likely and had the potential to make up 100% of the diet of the consumer. No other priors were used in the model. FRUITS is executed with a software package for performing ''Bayesian inference Using Gibbs Sampling'' (or BUGS). It considers biochemical composition of sources and which sources dominate (see http://www.mrc-bsu.cam.ac.uk/software/bugs/). The FRUITS output is a summary of percent contributions of each potential food source to the consumer's diet along with standard deviation and confidence intervals. FRUITS version 2.0 (http://sourceforge.net/projects/fruits/) was used for estimating food source contributions. Sensitivity analyses were conducted to evaluate the reliability of the results by taking into account posterior uncertainties in the proportional contributions of different food sources and food source combinations (Fernandez and others 2014).

Results

Food Source Classifications

An analysis of the nine tested food sources revealed 5 distinct groups: cyanobacteria, algae, fungi, C_3 plants (including both terrestrial plants and aquatic macrophytes), and C_4 terrestrial plants, with each exhibiting very different patterns of $\delta^{13}C$ variation for both essential and nonessential amino acids (Figure 1). For both essential and nonessential amino acids, taxon identity, amino acids identity, and their interaction had highly significant effects on amino acid $\delta^{13}C$ (all $p < 0.001$). The presence of a highly significant interaction between taxon and amino acid demonstrates that isotopic variations among individual amino acids were taxon dependent. Linear discriminant analysis revealed highly significant differences between taxa, based on non-normalized $\delta^{13}C$ values from 4 essential (Pillai trace = 2.18455, $F_{4,23} = 5.716$, $p = 0.001$) and 5 non-essential (Pillai trace = 3.03819, $F_{4,23} = 11.372$, $p = 0.001$) amino acids. Five food sources had distinct isotopic clusters for each taxa when graphed with the first (accounting for 80.1% of the variation) and second (accounting for 14.3% of the variation) discriminant axes (Figure 2). All food groups classified with greater than 99.99% certainty and posterior probability with their own groups (Figure 2).

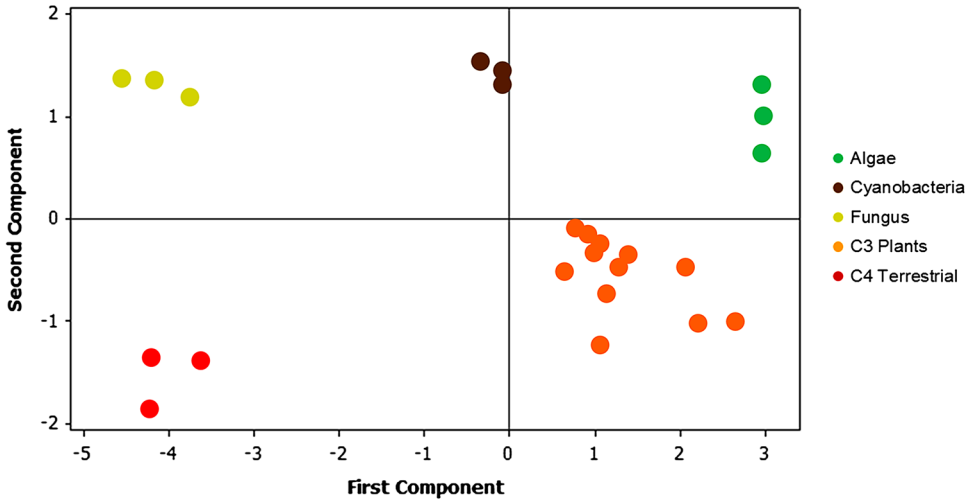

Figure 2. Principal component analysis of food sources using $\delta^{13}C$ variations among four essential amino acids (*ile* isoleucine, *leu* leucine, *phe* phenylalanine, *val* valine), two conditionally non-essential amino acids (*gly* glycine, *pro* proline) and three non-essential amino acids (*ala* alanine, *asp* aspartic acid, *glu* glutamic acid). Five food source groups are shown based on the first and second principal components. Terrestrial C3 plants are grouped with aquatic C3 macrophytes.

Trophic Position

Based solely on available museum specimens, the species composition of piscivores and invertivores in the UMR and LOR seemed to overlap considerably, with 4 of 6 species of piscivores and 3 of 5 invertivores occurring in both rivers (Table 1). This is not surprising, given that the mouth of the Ohio is only about 700 river miles and 6 degrees latitude from the lower sample site on the Upper Mississippi, and both are tributaries of the lower Mississippi River. Based on AA-CSIA data, the piscivores in general had only a slightly higher trophic position than the invertivores in both the UMR (TP: 3.00 vs. 2.95; $F_{1,76} = 0.24$, $p = 0.628$) and the LOR (TP: 3.20 vs. 3.01; $F_{1,58} = 3.67$, $p = 0.060$) (Table 1). The mean values were sufficiently close to suggest a more general diet for the piscivores in this data set than their name would suggest. The small size of the piscivores in the museum collections undoubtedly contributed to the minimal separation of the two functional groups, but our long-standing observations of gut contents of freshly caught fish in these rivers also suggest that many ''piscivores'' (for example, smallmouth bass), including large individuals, also consume some invertebrates, such as omnivorous crayfish.

Food Sources

The major basal food source identified for fish that had originally been collected over decades from both rivers was strongly associated with autochthonous primary production (UMR: $F_{5,48} = 3387.2$, $p = 0.001$; LOR: $F_{5,42} = 1363.4$, $p = 0.001$). Diets of combined piscivores and invertivores were ultimately linked by amino acids to eukaryotic algae (58 and 78%, for the LOR and UMR, respectively), whether we analyzed all 9 amino acids for which we have complete isotopic signature data (Figures 3B and 4B) or just the 4 essential amino acids (Figures 3A and 4A). Fishes in the UMR relied to a greater degree on algae as an ultimate food source than those in the LOR ($F_{1,15} = 29.52$, $p = 0.0001$). All remaining sources of nutrients in the UMR contributed no more than 12% to the diet of these fishes as a group (Figure 3), but 21% of food sources in the LOR came from C_3 plants (terrestrial and aquatic macrophytes combined) (Figure 4). As another autochthonous source, cyanobacteria appeared to contribute 6–9% of the amino acids to fishes in these large rivers.

Analysis of food sources by species also revealed a stronger reliance in most species on algae by both trophic levels but major differences among rivers. Eight of nine species in the UMR derived more than 60% of their amino acids from algae, with only river darters (*Percina shumardi*) having a mean value under 30% (Figure 5A). In contrast, although three of eight species collected from LOR relied on algae for 76–81% of their amino acids (Figure 5B), the remainder had mean values in the 41–62% range and generally greater variability among species in algal use. In the six cases where the same species were collected from both rivers, those in the UMR showed either a greater reliance on algae or were essentially equal, with only one exception (yellow bass: 69% in the UMR vs. 81% in LOR).

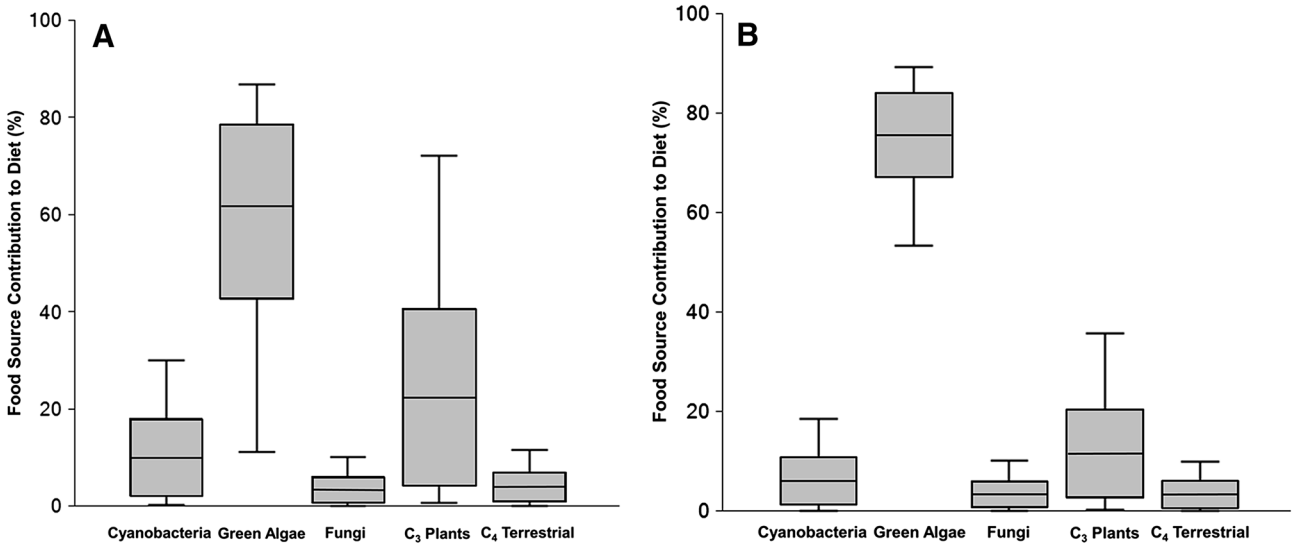

Figure 3. Box plots of the reliance of fish (combined presumptive invertivores and piscivores) from the Upper Mississippi River on five food sources, based on AA-CSIA of amino acids that are known to be (**A**) essential; or (**B**) a combination of essential and non-essential. Fishes are listed in Table 1, and potential food sources are given in Table 2. *Solid bar* in center of box is the mean; *upper* and *lower* bounds of box are ±1 SD; and whiskers are 95% confidence interval.

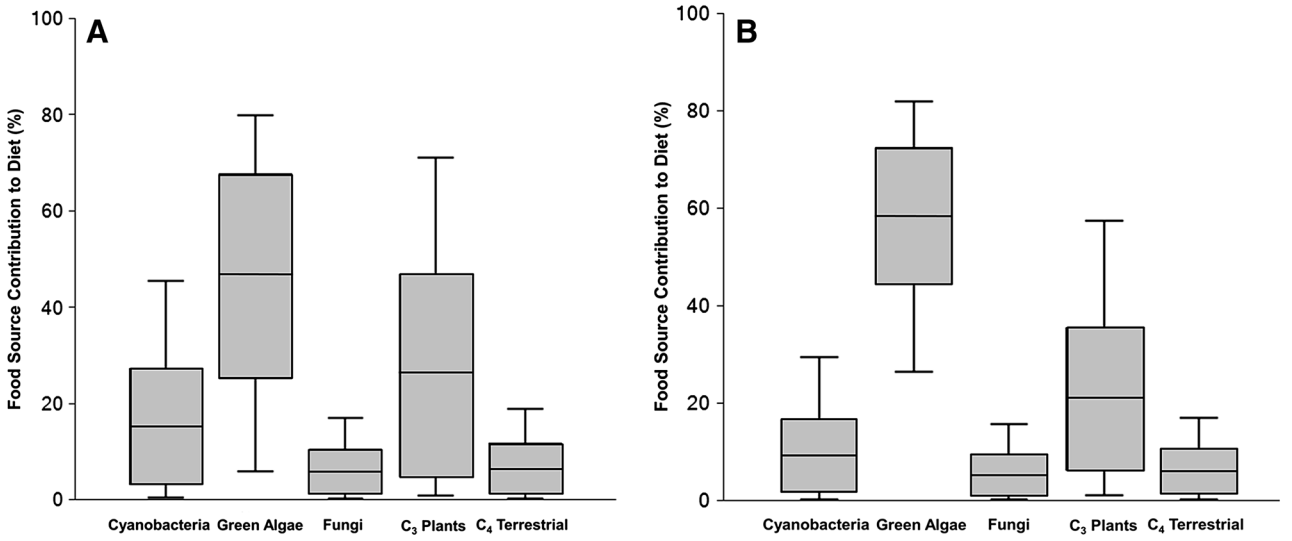

Figure 4. Box plots of the reliance of fish (combined presumptive invertivores and piscivores) from the Ohio River on five food sources, based on AA-CSIA of amino acids that are known to be (**A**) essential; or (**B**) a combination of essential and non-essential. Fishes are listed in Table 1, and potential food sources are given in Table 2. *Solid bar* in center of box is the mean; *upper* and *lower* bounds of box are ±1 SD and whiskers are 95% confidence interval.

Discussion

General Conclusions

Our results demonstrate that algae are the dominant original biotic source of carbon for piscivorous and invertivorous fish collected from these large river sections of the anastomosing UMR and the constricted LOR. Use of algae was especially dominant in the shallower, more structurally diverse UMR. This ecological influence of a river's hydrogeomorphic structure is consistent with predictions of Poole (2002, 2010) and Thorp and others (2006, 2008). The degree of importance of algae to metazoan production will likely vary somewhat among sections of rivers with different hydrogeomorphic structure, but we predict that autochthonous car-

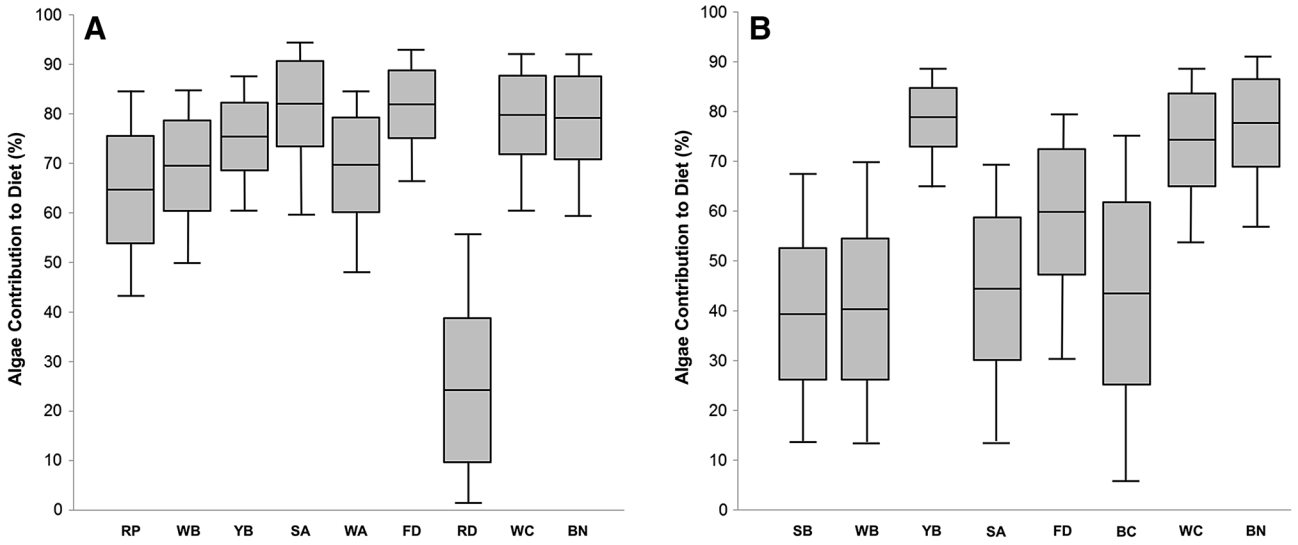

Figure 5. (**A**) Percent reliance on algae as a food source by fish in the Upper Mississippi River. Shown are five species of piscivores: *RP* (redfin pickerel, *Esox americanus*), *WB* (white bass, *Morone chrysops*), *YB* (yellow bass, *Morone mississippiensis*), *SA* (sauger, *Sander canadensis*), and *WA* (walleye, *Sander vitreus*). Also shown are four species of invertivores: *FD* (freshwater drum, *Aplodinotus grunniens*), *RD* (river darter, *Percina shumardi*), *WC* (white crappie, *Pomoxis annularis*), and *BN* (black crappie, *Pomoxis nigromaculatus*). (**B**) Percent reliance on algae as a food source by fish in the lower Ohio River. Shown are four species of piscivores: *SB* (spotted bass, *Micropterus punctulatus*), *WB* (white bass, *Morone chrysops*), *YB* (yellow bass), and *SA* (sauger). Also shown are four species of invertivores: *FD* (freshwater drum), *BC* (blue catfish, *Ictalurus furcatus*), *WC* (white crappie), and *BN* (black crappie, *Pomoxis nigromaculatus*). *Solid bar* in center of box is the mean; *upper* and *lower* bounds of box are ±1 SD; and whiskers are 95% confidence interval.

bon will provide the primary support of metazoan production in most large rivers, with the possible exception of rivers where instream autotrophy is substantially suppressed by light limitations resulting from high turbidity, great depth, or perhaps extensive riparian cover. Seasonal differences will alter algal contributions somewhat; but on an annual basis, autochthony should still dominate.

These conclusions do not necessarily pertain to the importance of terrestrial carbon to bacterial production, but see perspectives on the ''heterotrophy paradox'' in rivers (Thorp and Delong 2002) as well as arguments against a significant role of allochthony for bacterial metabolism in Brett and others (unpublished).

Why are there Differences Between the Upper Mississippi and Ohio Rivers?

Although algae represented the dominant, original carbon source in both rivers, autochthony contributed a greater percentage of the amino acids to fish in the UMR than in the LOR. The differences between these two large rivers may reflect effects of stage height (river depth) and hydrogeomorphic complexity on the absolute and relative amount of benthic and suspended algae. Compared to the LOR, the anastomosing UMR contains much more abundant shallow, slowly flowing slackwaters where downstream transport and helical flow are reduced and more light reaches the bottom, thereby increasing algal availability for consumers. Ecologists have demonstrated high levels of productivity in slackwaters of the main and side channels (for example, Reynolds and others 1991; Schiemer and others 2001; Ochs and others 2013). In contrast, the LOR is largely a single channel river with shallow areas primarily confined to main channel banks. Moreover, since construction of low-head dams on these two rivers, the UMR has gained in hydrogeomorphic complexity whereas the LOR (with taller, low-head dams) has mostly remained the same or perhaps lost a few islands (Bowes and others, unpublished manuscript). Following construction of low-head dams in the LOR, a regime shift occurred where the food web was shifted from one supported by benthic algae to one more heavily sustained by pelagic basal resources (Delong and others, unpublished). This change in algal type and river position could have altered the dependence on autochthony as well as the species and diet of the fish community. No comparable regime shift has thus far occurred in the UMR, but models indicate the system is at a regime tipping

point should the U.S. Army Corps of Engineers implement proposed increases in minimum river stage height (Delong and others, unpublished).

Evidence from Other Lotic Studies

The debate on the relative importance of autochthonous and allochthonous carbon in rivers and lakes has continued for nearly four decades, during which time most scientists have progressively shifted from gut content analysis (but see Benke and Wallace 2015) to fatty acid or bulk-tissue stable isotope analysis, while also changing their focus from daily consumption (gut contents) to longer term feeding trends (weeks to months) using lipids and stable isotopes. Only recently have a few aquatic studies (mostly marine) adopted the newest AA-CSIA techniques. Differences in the nature and reliability of these older techniques can account for some variation in conclusions, but there are also verifiable differences in use of aquatic and terrestrial carbon sources based on river hydrogeomorphic type (Thorp and others 2006, 2008), climatic zone (for example, tropical rivers, dryland rivers, and temperate zone rivers in different biomes; for example, Dudgeon and others 2010; Pettit and others 2011), season, flood stage, turbidity, location in either the riverscape (main channel or lateral slackwaters) or floodscape (for example, oxbow lakes and wetlands), and organism studied. Consequently, Roach (2013) recommended analyzing basal production sources in terms of hydrologic and physicochemical attributes.

Nearly, all previous studies of food sources in large rivers have suffered from less precise analytical techniques, with the result that the data are less definitive than ecologists would wish. Nonetheless, the importance of autochthony in rivers has been repeatedly demonstrated for many types of rivers around the globe. While a few studies have emphasized a dominant role for allochthonous carbon in food webs of turbid rivers (for example, Zeug and Winemiller 2008; Wellard Kelly and others 2013), most published studies in this century have concluded that the primary carbon source in large rivers is of autochthonous origin, especially true algae (for example, Winemiller 2005; Delong and Thorp 2006; Hadwen and others 2010; Pingram and others 2012; Roach 2013; Sullivan 2013) or, at a minimum, there is high selectivity for autochthonous carbon (for example, Cole and Solomon 2012). Moreover, a currently unpublished study in the unregulated Wabash River using AA-CSIA also demonstrated that all fish examined depended on algae for the majority of their essential amino acids (for example, >80% in gar and spotted bass) (Mark Pyron, personal communication; July 2016). Although it seems clear that algal production in the main channel is usually insufficient to support total secondary production and a P/R ratio above 1 (for example, Ochs and others 2013), lateral riverscape sites can be a major route for energy inputs to secondary consumers (for example, Cole and Solomon 2012).

Biochemical Reasons for the Relative Importance of Algae to Metazoa

Terrigenous carbon inputs from plants into rivers and lakes can substantially influence the physicochemical properties of the aquatic system in various important ways, such as attenuation of PAR and UVR (Schindler and others 1997; von Einem and Granéli 2010; Holgerson and others 2016), and they can inhibit primary production (Jones 1992; Karlsson and others 2009). A large proportion of this carbon (for example, 89–90% of total POC), however, is highly recalcitrant, lignocellulose. This material slows both microbial degradation (for example, Moran and others 1989) and later synthesis into metazoan biomass as a result of its kinetic properties rather than inherent energy content (Brett and others, unpublished). Because of its very high proportion of lignocellulose, this terrestrial carbon from higher plants is a poor food source for metazoa. Moreover, terrestrial plants and heterotrophic bacteria lack some conditionally indispensable polyunsaturated fatty acids (Cunnane 2000; Brett and Müller-Navarra 1997; Brett and others 2009) and other nutrients that are critical for rapid growth in aquatic invertebrates and fishes. However, this allochthonous material might serve as a metabolic ''lifeboat'' (Wetzel 1995) that allows metazoans to survive until more nutritious algal resources are more abundant. In addition, the more recalcitrant but energetically high glucose from terrestrial POC might be employed under nutritional stress for energy while simultaneously using fatty acids and amino acids for somatic growth and reproduction (Taipale, unpublished in Brett and others, unpublished).

What are the Implications for Various Models?

The overwhelming evidence from laboratory biochemical analyses, laboratory experiments, and field studies using AA-CSIA, bulk tissue stable isotopes, and fatty acids seems to confirm that the primary organic source supporting metazoan food

webs in small to large rivers is derived from true algae. However, there are several caveats to this conclusion. First, this conclusion does not necessarily extend at this time to forested headwaters because of an insufficient amount of studies employing the most modern chemical techniques (but see evidence of autochthony for small streams in France 1995; Brito and others 2006; McNeely and others 2007; Lau and others 2009). Nonetheless, the biochemical characteristics of allochthonous carbon inputs from land, as discussed by Brett and others (unpublished), merit a reevaluation of the relative importance of carbon sources to metazoans in headwaters. Second, most fish we analyzed were collected in warmer periods of the year (when field taxonomists and ecologists are typically more active), and thus the conclusions on the relative importance of algae versus terrestrial plants may need to be seasonally analyzed. However, our data were focused in warmer periods when fish productivity is greatest in the UMR (Delong and Thorp 2006), and at least some north temperate zone fish lose weight in other seasons (for example, Bowes and others 2014). Consequently, we contend that autochthonous carbon is the primary, ultimate food source during periods of maximum fish productivity, but terrestrial plants may supplement metabolic processes throughout the year and possibly be the primary source of carbon sustaining them in months of slow to negative growth. Finally, we did not examine fish collected when the floodscape was immersed following spring snow melt (a short period of inundation in the UMR and LOR compared to rainfall induced flooding in tropical rainforest rivers), and thus cannot definitively conclude that decaying terrestrial vegetation was not the primary carbon source at that time (cf. conclusions of the Flood Pulse Concept in Junk and others 1989). However, conclusions for lentic systems (as discussed in Brett and others, unpublished) and our results showing a low dietary contribution of C_3 aquatic macrophytes in the riverscape of the UMR and LOR would suggest that autochthonous carbon from algae is still the dominant source during the flood pulse periods.

Based on the chemical data discussed previously, we contend that conclusions on the importance of allochthonous carbon in the riverscape of large river food webs by the older but still frequently cited River Continuum Concept (RCC: Vannote and others 1980) and Flood Pulse Concept (FPC: Junk and others 1989) were largely incorrect, while predictions of the newer River Wave Concept (RWC; Humphries and others 2014) currently lack sufficient support and rely in part on accuracy of the RCC and FPC models. All three theories ignored that terrestrial carbon is largely recalcitrant by the time it reaches a large river from upstream or via the floodscape and that in tropical systems this carbon may be composed of toxic or unpalatable compounds (compare Brett and others unpublished).

Although two of these models have received vast (RCC) to moderate (FPC) attention over the last few decades, the RWC is relatively new and not yet thoroughly analyzed (for example, Roach and Winemiller 2015). Future analyses of the RWC should evaluate: (a) the importance of allochthonous carbon during the model's wave crest period when instream primary production is low, consumer reproduction is absent, and somatic growth is minimal or negative; and (b) the implications of the model's graphical ''sine wave'' for consistency and predictability of relevant phenomena versus the often high noise-to-signal ratio and stochastic elements in aquatic food webs.

In contrast, AA-CSIA results of the current study and biochemically based evidence discussed by Brett and others (unpublished) are entirely consistent with predictions of the Riverine Productivity Model (RPM; Thorp and Delong 1994, 2002) and applicable tenets in the Riverine Ecosystem Synthesis (RES; Thorp and others 2006, 2008).

A caveat to our conclusions is that all ecological models should be evaluated in light of the universal admonition in the preface of the Riverine Ecosystem Synthesis book (Thorp and others 2008, p. 12) that ''Theories should be viewed as formed of unfired clay. They need a lot of shaping and remolding before they accurately model the real world, and sometimes you need to toss them out and start again.'' This is especially pertinent for both old and new models that we teach in our classrooms and use to frame government environmental policy.

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