FOOD WEBS OF FISHES IN FLOODPLAIN LAKES

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MASTER OF SCIENCE

BY

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Introduction

Floodplain lakes are biodiversity hotspots of large river systems (Pyron et al., 2014). Many riverine species use these waterbodies for refugia during high flow and to take advantage of available habitat when connected to the main river (Sullivan and Watzin 2009). Floodplain lakes can support a greater diversity of fishes than the main river channel (Winemiller et al. 2000) and function as source pools for recruitment of large river fishes, some of which are socioeconomically important (Zeug and Winemiller 2007). Globally, these habitats are impaired owing to anthropogenic effects of human activities such as agriculture and development (Sullivan and Watzin 2009).

The lower Wabash River floodplain is impaired by many anthropogenic factors including dam construction and operation (i.e., altered flow regimes), agricultural activities, urban point source pollution, and the introduction of invasive fishes such as bigheaded carps (*Hypophthalmichthys spp.*) (Broadway et al. 2015). Agricultural management practices are likely the most influential anthropogenic impact on the lower Wabash River floodplain lakes, as much of the watershed land is used for row crop agriculture (Gammon 1998). Dembrowski and Miranda (2012) determined that there was a negative association between the percent of watershed land in agriculture and fish biodiversity in floodplain lakes of the Mississippi River

floodplain. Row crop agriculture alters the hydrology of rivers by the rapid draining of fields through drainage tiles, and increases in nutrient loading with fertilizer runoff (Broadway et al. 2015). Another potential influence of row crop agricultural practices is the export of terrestrially-derived organic matter (i.e., food source) to aquatic food webs. The input of terrestrial (allochthonous) organic matter from agriculture may modify food webs and fish assemblages in floodplain lakes.

The recognition of the importance of floodplain lakes to river systems has led to many restoration projects to be initiated to alleviate the effects of anthropogenic-caused impairment. The primary components of the restoration of temperate floodplain lakes are: reestablishment of lost hydrologic connectivity to the main river, limiting the input of excess sediment and nutrients from the watershed, reconstruction of historic fish assemblages, and the removal/management of invasive fishes (Obolewski et al. 2016; VanMiddlesworth et al. 2017; Obolewski et al. 2018). Reestablishment of lost hydrologic connectivity to the river can result in a large influx of invasive carps [bigheaded carps, common carp (*Cyprinus carpio*), and grass carp (*Ctenopharyngodon idella*)] (VanMiddlesworth et al. 2017). Biomanipulation is a technique often employed where a robust population of native piscivorous fishes is established to suppress the population of invasive fishes through direct predation or by applying top-down pressure that reduces the abundance of the invasive fishes' food sources (VanMiddlesworth et al. 2017). To effectively employ this technique, it is necessary for the food web to be well understood by managers. It is important to enact management decisions that will achieve the greatest negative effect on invasive fish populations. Identification of the basal food sources supporting the food web allows managers to determine which actions will be most effective at suppressing the populations of invasive fishes.

The relative importance of allochthonous versus autochthonous source contributions to the food webs of riverine systems has been widely debated, with multiple hypotheses for these dynamics (Thorpe and Bowes 2017). A relatively new technique, amino acid compound-specific isotope analysis (AA-CSIA), offers investigators conclusive answers. AA-CSIA offers greater precision and flexibility than bulk isotope analysis and can be used to determine food sources in an aquatic ecosystem (Thorp and Bowes 2017). Our objective was to determine the ultimate sources of carbon in food webs of floodplain lakes of the lower Wabash River using AA-CSIA of carbon sources in fishes. This information will contribute to our understanding of the carbon basis of large river food webs, which has been of interest to ecologists for decades.

Methods

Study Site

We collected fishes in three floodplain lakes in the Lower Wabash River region from July 16-17, 2020 (Figure 1). The lakes were selected based on reliability of boat access, and sufficient volume to contain fishes. The lakes vary in size, depth, and connectivity to the main stem river. Mackey lake was the largest (1.19 km^2), deepest (2 m max depth), and maintains a permanent connection to the main stem river. Greathouse and Ribeyre lakes are annually connected to the main stem river during periods of high water, maximum depth in both is 1 m. Greathouse was the smallest of the study lakes (0.41 km^2) and Ribeyre was intermediate size (0.92 km^2). Land use in the watershed is primarily row crop agriculture (Gammon 1998).

Fish Collection

Fishes were collected via boat electrofishing (60 Hz DC, Midwest Boat Management, Inc.), with a focus on sampling all available habitats in each lake (Ball State University IACUC

#126193). Four electrofishing runs of 15 min were performed for each lake. Fish were identified

to species and assigned to one of three trophic feeding guilds: piscivore, invertivore, or

planktivore. In each lake, a dorsal muscle tissue sample was taken from three individuals of a

single species from each trophic guild (Table 1). Piscivores were represented by shortnose gar

(*Lepisosteus platostomus*) in Greathouse and Ribeyre lakes and longnose gar (*Lepisosteus*

osseus) in Mackey lake, invertivores were represented by freshwater drum (*Aplodinotus*

grunniens), and planktivores were represented by silver carp (*Hypophthalmichthys molitrix*).

Processing and Isotope Analysis of Samples

Fish tissue samples were dried in a benchtop drying oven at 60˚C for 48hr, homogenized

using a mortar and pestle, and packaged in individual glass vials. Samples were sent to the

Louisiana State University Stable Isotope Ecology Laboratory for amino acid compound specific

isotope analysis (AA-CSIA) of carbon. Isotope analysis was performed using the chloroformate-

based method for ecological studies described in Walsh et al (2014). $\delta^{13}C$ signatures for five

non-essential amino acids (alanine, aspartic acid, glutamic acid, glycine, and proline) and five

essential amino acids (isoleucine, leucine, phenylalanine, threonine, and valine) were analyzed

(Table 2). Essential amino acids are classified as such because they cannot be synthesized by

consumers and must come from the diet. $\delta^{13}C$ signatures for essential amino acids remain

unchanged by trophic level, therefore the change in $\delta^{13}C$ of the consumer from $\delta^{13}C$ of the diet

($\Delta^{13}C_{C-D}$) should be near 0‰ (Whiteman et al. 2019). This analysis facilitates the construction of

links between food sources and consumers (Thorp and Bowes 2017).

$\delta^{13}C_{AA}$ data for potential food sources were provided by Emily Arsenault (University of

Kansas). Fourteen food sources, representing algae (*Chlorella* sp*.,* diatoms*,* and *Spirogyra* sp.),

aquatic macrophytes (*Hydrilla* sp*.*), cyanobacteria (*Spirulina* sp.), C_3 terrestrial plants (*Salix* spp.

and *Pinus* sp.), and C4 terrestrial plants (*Zea* sp. and *Panicum* sp.), were analyzed for $\delta^{13}C_{AA}$ (Table 3). These food sources were selected because they commonly occur in freshwater ecosystems and represent likely food sources. Samples of diatoms, *Spirogyra* sp., *Hydrilla* sp., *Salix* sp., *Pinus* sp., *Zea* sp., and *Panicum* sp. were collected in situ, while samples of *Chlorella* sp. and *Spirulina* sp. were obtained from commercial suppliers (E. Arsenault, pers. comm.). Food source samples were prepared in the same manner as tissue samples, with the exception that they were homogenized using a Wig-L-Bug mixer/amalgamator. Food source samples were also analyzed at the Louisiana State University Isotope Ecology Laboratory.

Analysis of Food Source Contributions

Amino acids were classified as essential or non-essential according to Whiteman et al. (2019). Essential amino acid $\delta^{13}C$ values were used to characterize the isotope signatures of food sources. Non-essential amino acids were not used in this study because trophic modification can occur in animals for non-essential amino acids. Essential amino acid $\delta^{13}C$ fingerprints of producers can be used to trace the $\delta^{13}C$ of consumers back to the ultimate source of carbon in the food web (Larsen et al. 2013).

Food source $\delta^{13}C$ values were normalized to their respective sample means prior to statistical analysis (Larsen et al., 2020). Principal components analysis (PCA) was used to identify producer food groups based on normalized $\delta^{13}C$ values and linear discriminate analysis (LDA) was used to determine food source groups. A cross-one-out cross validation approach was used to calculate the probability of membership in source groups. Five source groups were identified: algae, cyanobacteria, aquatic macrophytes, C3 terrestrial plants, and C4 terrestrial plants. A Pillai's trace multivariate analysis of variance (MANOVA) was used to test the null

hypothesis that there was no difference in group membership. All statistical analyses were performed in R version 4.0.3 (R Development Core Team 2020).

The relative contributions of essential amino acids to consumers were quantified using Bayesian mixing models with the MixSIAR package in R (Stock and Semmens 2016). MixSIAR is a framework that allow the user to create and run mixing models that use biotracer data to estimate the proportional contribution of sources to consumers. We assumed that all food sources were potentially present in diets and had the potential to constitute 100% of the diet for the consumers. Small non-zero trophic discrimination factors (0.01 +/- 0.01) were used to represent the minimal trophic fractionation that occurs between consumer and diet with essential amino acids (McMahon et al. 2016). Consumer species were treated in the model as a factor that was nested within the factor of site. The factors of site and species were treated as fixed effects because each factor had few levels. To achieve convergence of the model, the "very long" option for run length was chosen.

Results

Principal components analysis of normalized $\delta^{13}C_{EAA}$ values demonstrated that food sources formed groups composed of taxonomically related individuals (Figure 2). The first PCA axis explained 57.5% of the variation and separated autochthonous food sources from allochthonous food sources. The second PCA axis explained 21.1% of the variation and separated cyanobacteria from all other sources and diatoms from other algae. Food sources were classified into their respective groups without error by linear discriminant analysis (Figure 3). The first linear discriminant axis (LD1) explained 68.2% of the between group variation, while the second linear discriminant axis (LD2) explained 19.6% of the variation. The variation between food source groups was best explained by four essential amino acids: Ile (0.96), Leu (-

0.74), Phe (0.97), and Val (- 0.87) loaded strongly onto LD1, while Thr (- 0.06) explained little of the between food source group variation. MANOVA of food source groups showed a significant difference among food source groups (Pillai's trace = 2.478, $F_{(20,132)}$ = 10.74, P = 0.0001).

The diets of the three trophic feeding guilds, based on their EAA composition, primarily contained carbon from algae (Figures 4-6, Table 4). The diet of silver carp across all sites was composed of 94.4 – 96.7% carbon from algae. The remaining food source groups comprised no more than 2.3% of the diet (Figure 4). The diet of gar across all sites was composed of 63.3 – 79.2% carbon from algae. The remaining food source groups comprised no more than 11.5% of the diet, except cyanobacteria, which comprised 30.6% of carbon in the diet of gar in Greathouse lake (Figure 5). The diet of freshwater drum across all sites was composed of 60.3 – 71.0% carbon from algae. C4 terrestrial plants comprised 28.6% and 28.4% of the carbon in diets in Mackey and Ribeyre lakes, respectively. The remaining food source groups comprised no more than 12% of the diet carbon sources (Figure 6).

Discussion

Our results indicated that sources of carbon for the food webs of floodplain lakes on the lower Wabash River were autochthonous, and primarily algae. Aquatic macrophytes and C3 terrestrial plants contributed negligible amounts to the diets of all three consumer groups, indicating that neither were an important source of carbon for these lakes. The diet of silver carp was solely dependent on algae as the ultimate source of carbon. Cyanobacteria and C4 terrestrial plant were secondary contributors to the diets of gar and freshwater drum, although the degree of contribution varied across the lakes sampled. Other recent AA-CSIA studies in the Upper

Mississippi River and Lower Ohio River also found that algae was the primary basal food source in both systems (Thorpe and Bowes 2017; Bowes et al., 2019).

Silver carp are planktivorous filter feeders that are passively selective in their consumption of plankton (Pongruktham et al., 2010; Minder and Pyron 2017). Minder and Pyron (2017) found silver carp in the Wabash River to selectively feed on cyanobacteria in the spring based on gut content analysis. We did not find cyanobacteria were a component of carbon in silver carp tissues, likely because cyanobacteria contain a digestion-resistant mucilaginous coating that results in passage through the digestive tracts of silver carp undamaged (Vörös et al., 1997). A significant limitation of gut content analysis is that it only provides a glimpse at what the individual has recently consumed and does not provide information about assimilation, as opposed to stable isotope techniques which represent an individual's assimilated diet over a more long-term temporal scale (Davis et al. 2012). Gut content analysis of silver carp in the summer revealed selective consumption of diatoms and chlorophyta (Minder and Pyron, 2017), which are represented in the "algae" food source group in this study. Freshwater drum in these three lakes likely primarily consume dominant insect taxa as a main food source. The contribution of carbon from algae, cyanobacteria, and C4 plants to freshwater drum carbon are likely from invertebrates that consumed these producers. Jacquemin et al. (2013) used stomach analyses for freshwater drum in the Wabash River and found dietary specialization. Freshwater drum < 500 mm standard length specialized in consuming diptera, trichoptera, and ephemeroptera (Jacquemin et al., 2013). All individuals sampled in our study were < 500 mm (Table 1). The dietary contribution of carbon to our piscivorous gar was likely from the consumption of a variety of invertivorous fishes. Both long and shortnose gar are primarily opportunistic piscivores, with no species-specific prey specialization (Holloway 1954; Sutton et al., 2009).

This study appears to be the first to use AA-CSIA techniques to trace carbon from producers to consumers in temperate floodplain lakes. All three lakes in this study are annually reconnected to the main stem Wabash River during the spring high-water period (Mackey Lake maintains a year-round connection at its southern terminus), allowing exchange of nutrients, source materials, and consumers (M. Pyron, pers. obser.). The majority of riverine food web studies used bulk tissue stable isotope techniques (Thorp et al. 1998; Bunn et al. 2003; Delong and Thorp 2006; Zeug and Winemiller 2008; Medeiros and Arthington 2011), rather than AA-CSIA in which isotopic signatures represent the long-term average of source contributions and are not affected by spatial or temporal variations (Thorp and Bowes 2017). Isotopic signatures of consumers remain consistent whether samples are collected during periods of high-water connectivity or low-water hydrologic isolation when using AA-CSIA. Seasonal variation in diets of consumers can lead to different results from bulk-tissue stable isotope analyses (and gun content analysis) depending on when and where sampling occurred (Hladyz et al. 2012), likely resulting in conflicting results for carbon sources in riverine food webs. For example, Delong and Thorp (2006) found that algae represented the primary source of carbon to primary and secondary consumers in the upper Mississippi river during summer low-flow conditions. However, Zeug and Winemiller (2008) found that C_3 terrestrial plants represented the primary carbon source for consumers in the main channel and floodplain of the Brazos River, Texas during high-flow flooding conditions.

The current cost of AA-CSIA is quite high due to the labor-intensive nature of the analysis and the limited number of laboratories with the necessary equipment to perform the analysis (Thorp and Bowes 2017), which could be prohibitive to some studies. In many cases, it may be more sensible for researchers, especially those with limited budgets, to use more cost-

effective bulk isotope techniques. AA-CSIA is most appropriate when it is important to investigators to know, with great precision, the ultimate sources of consumers' dietary carbon. AA-CSIA is an exciting new technology that has the potential to definitively answer many ecological questions; with its increased use and advancements in technology the cost of analysis will likely decline.

The purpose of this study was to determine the basal food sources for floodplain lake food webs. To expand on this work, it would be of value to apply the methods used in this study to the main-stem Wabash River. This would allow for comparisons to be made between food webs of each habitat in the river-floodplain system. We also recommend gut-content analysis for the species surveyed and surveys of the invertebrate and prey fish communities with an accompanying AA-CSIA. A gut-content analysis would reveal the consumed diets of our studied species in addition to the ultimate sources of carbon in their diets, as was examined in this study. Gut content analysis provides little information about assimilation of consumed items (Davis et al. 2012), which is why gut-content analysis in concert with AA-CSIA would be preferred. An AA-CSIA of invertebrate and prey fish communities would illustrate the pathways of carbon from producers to primary, secondary, and tertiary consumers. This would provide a more detailed understanding of the food webs, beyond the ultimate sources of carbon, of these floodplain lakes.

The results of this study demonstrated that the basal sources of carbon in temperate floodplain lake food webs are primarily autochthonous, not allochthonous. This is in concurrence with the findings of recent AA-CSIA studies (Thorpe and Bowes 2017; Bowes et al., 2019) and earlier bulk isotope studies (Thorp et al., 1998; Bunn et al., 2003; Delong and Thorp, 2006). . Food webs are complex processes that are influenced by many abiotic and biotic factors

(Winemiller 2005); therefore, food webs may be affected in unexpected ways by management decisions if not well understood. Management and restoration of river floodplain systems requires an understanding of the trophic pathways that support their food webs (Zeug and Winemiller, 2008). This is especially true for the control of invasive fishes during floodplain lake restoration projects. A well-informed understanding of the tropic pathways supporting the food web will allow managers to identify potential pinch points in the flow of energy from producers to invasive fishes, facilitating the suppression of invasive fish populations by reducing their food sources. This demonstration of the importance of autochthonous carbon will contribute to better management of river floodplain ecosystems by providing further evidence of the importance of autochthonous carbon to large river floodplain food webs.

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a large-river food web. Ecology 89:1733-1743.

Figures and Tables

Figure 1. Locations of the three study sites on the lower Wabash River in southwest Indiana, USA. Thin white lines represent state boundaries. Mackey Lake (37°48'34.32"N 88° 4'52.92"W) Greathouse Lake (37°55'55.42"N 88° 3'53.53"W) Ribeyre Lake (38° 5'25.02"N 87°59'8.77"W)
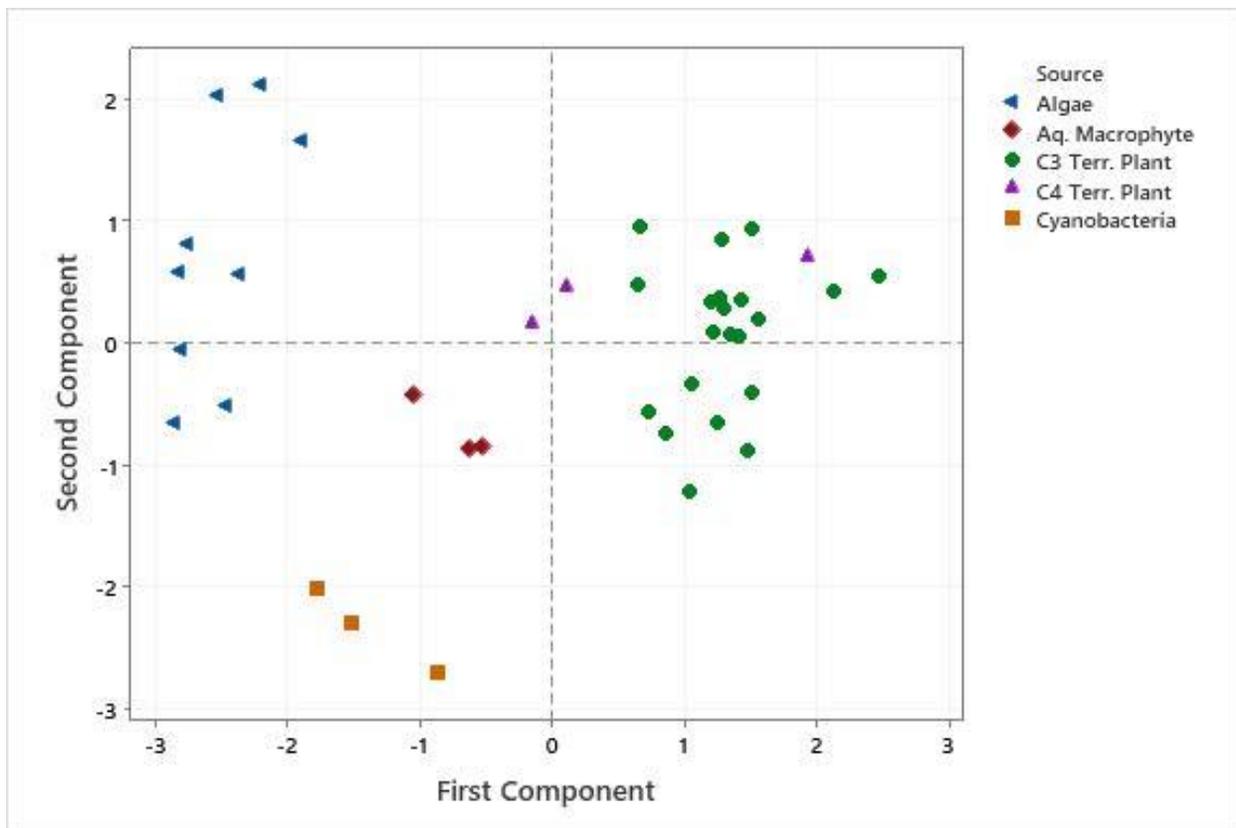
Figure 2. Principal Components Analysis of food source $\delta^{13}C$ values. The first principal component explained 57.5% of the variation and the second component explained 21.1% of the variation.
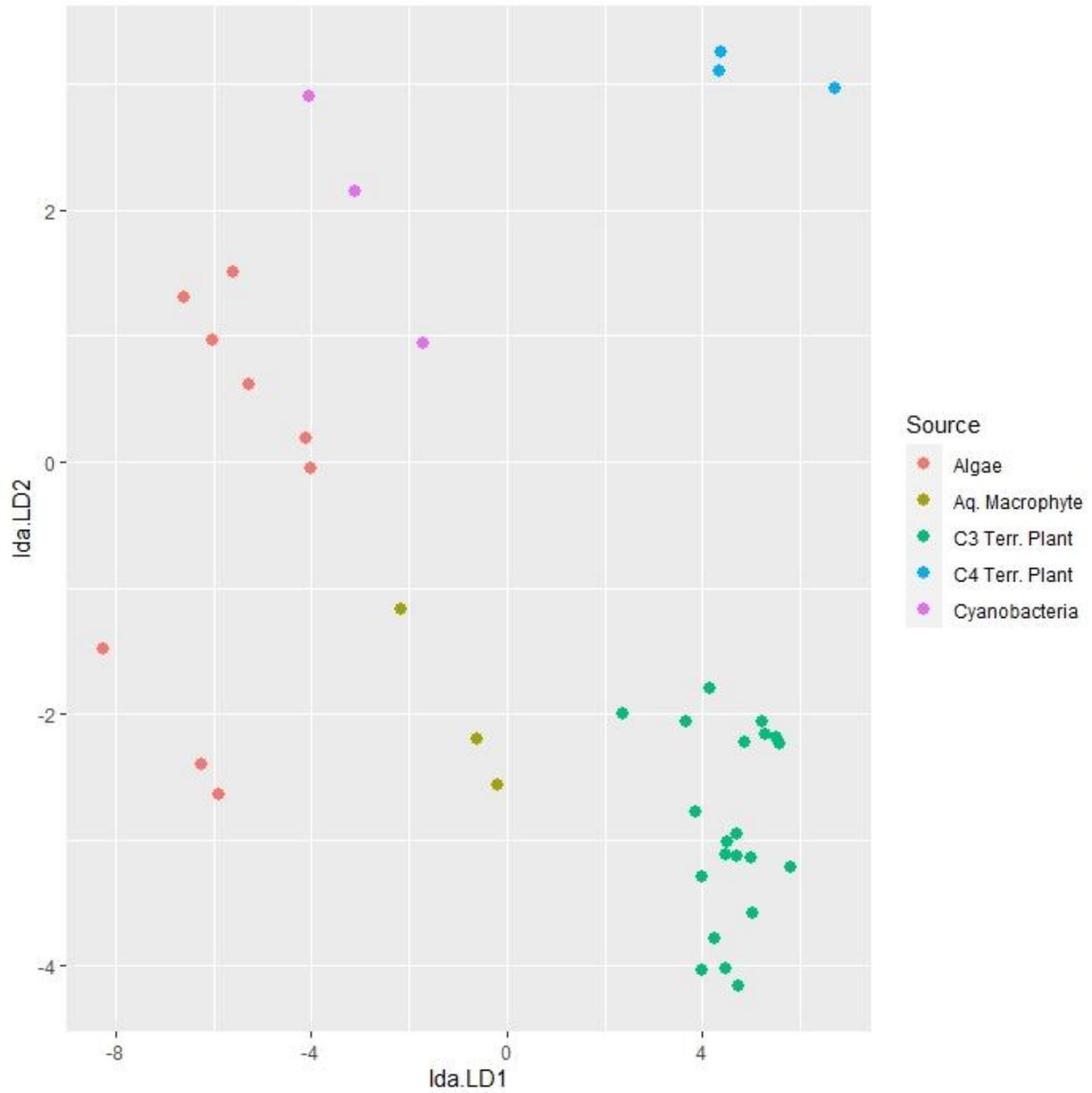
Figure 3. Linear discriminant analysis of food source group classification. LD1 explained 68.2% of the variance between groups and LD2 explained 19.6% of the variance.
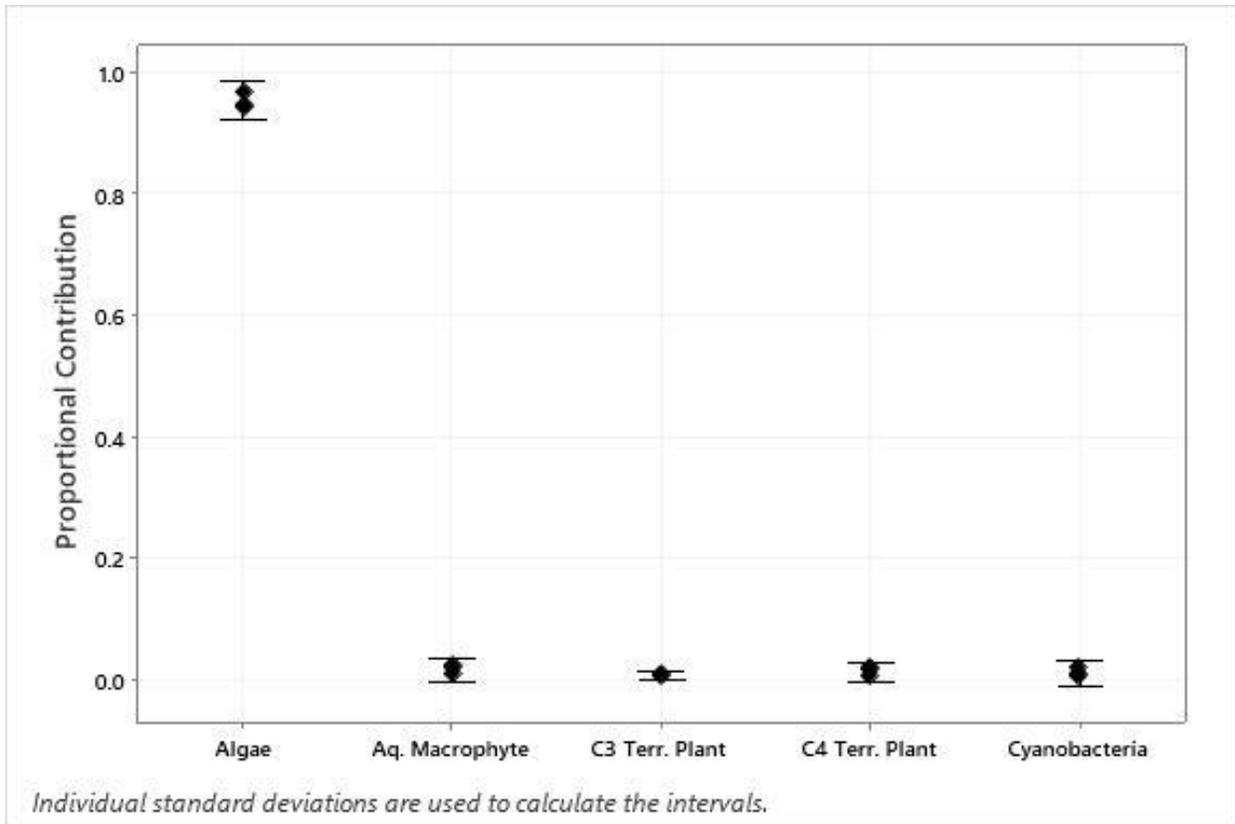
Figure 4. Mean proportional contributions of five food source groups to the diet of silver carp. Bars represent the 95% CI of the mean proportional contributions.
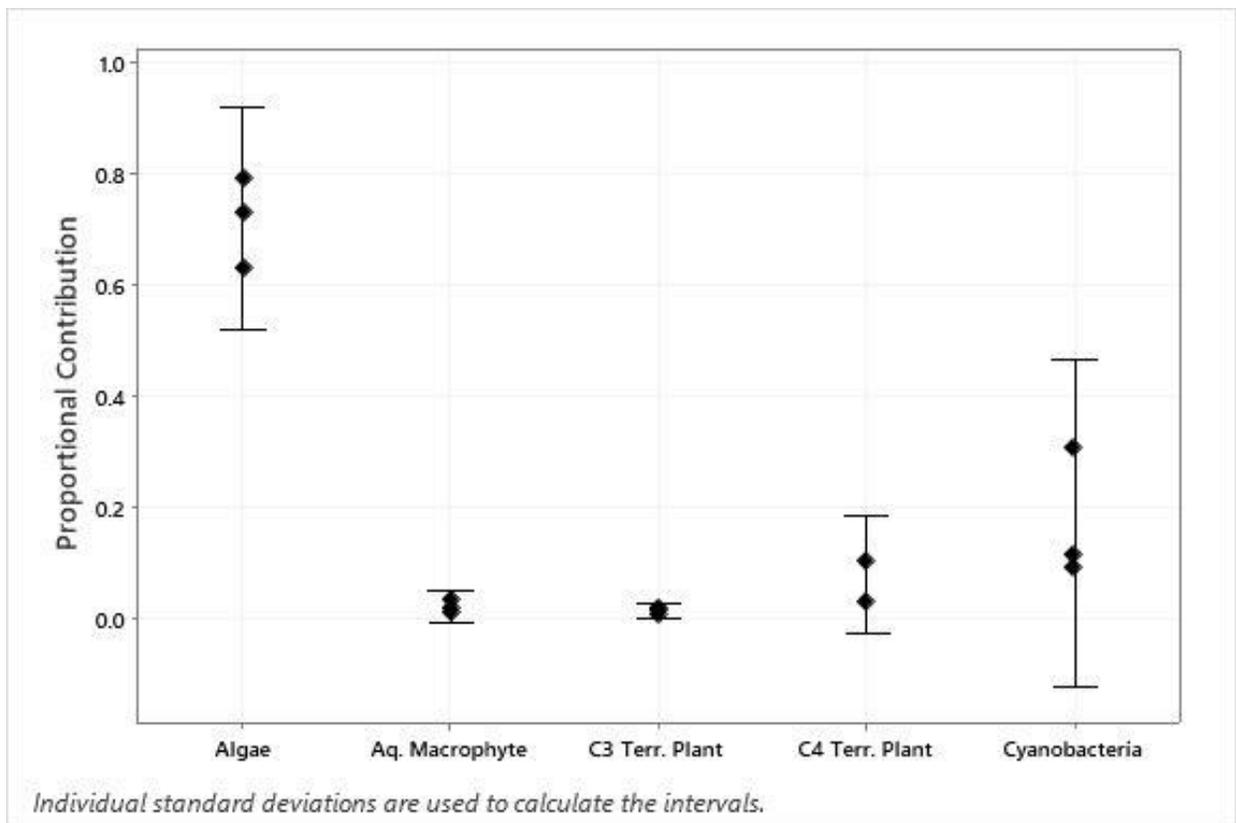
Figure 5. Mean proportional contributions of five food source groups to the diet of gar. Bars represent the 95% CI of the mean proportional contributions.
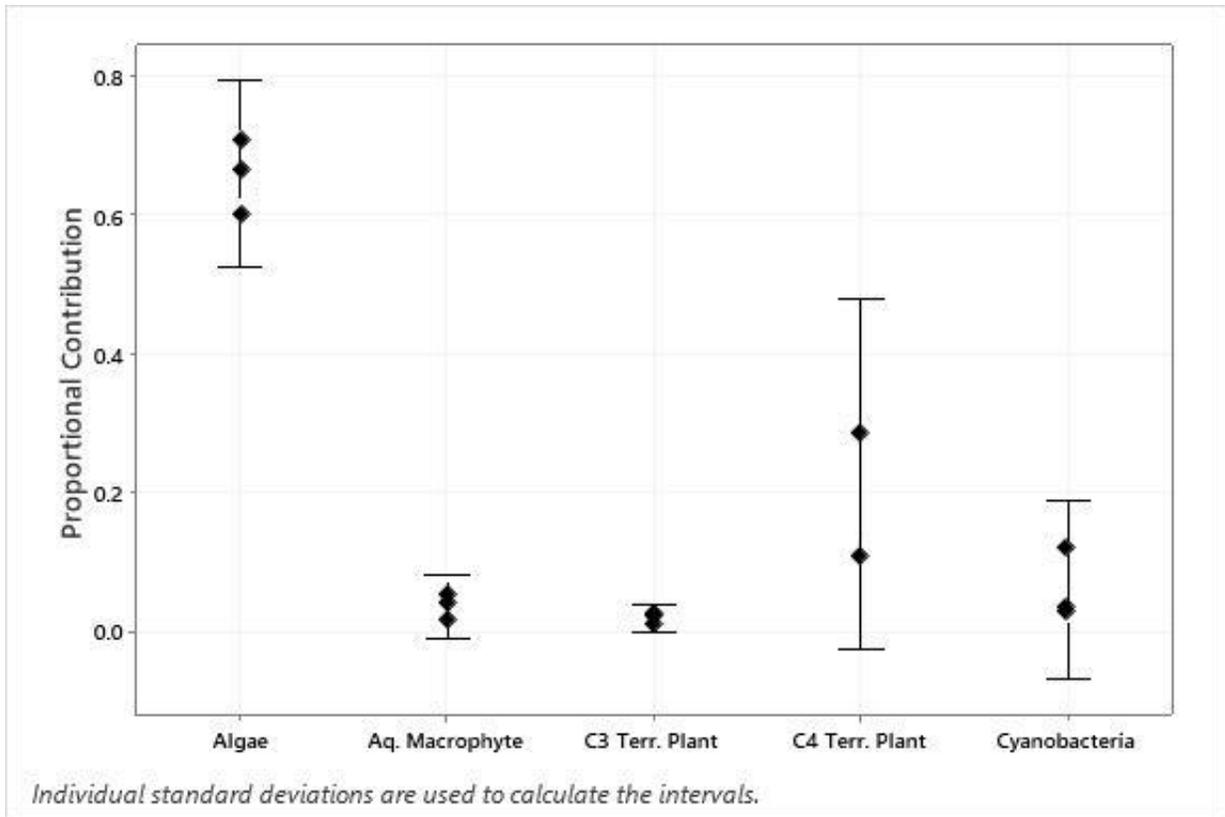
Figure 6. Mean proportional contributions of five food source groups to the diet of freshwater drum. Bars represent the 95% CI of the mean proportional contributions.

Table 1. Body size and collection site of consumer species sampled.

Site	Total Length (mm)			
	Shortnose Gar	Freshwater Drum	Silver Carp	Longnose Gar
Greathouse	460	199	620	*N/A*
	529	186	820	*N/A*
	625	211	605	*N/A*
Ribeyre	520	296	580	*N/A*
	495	380	570	*N/A*
	445	330	590	*N/A*
Mackey	*N/A*	296	710	567
	N/A	380	605	604
	N/A	330	710	671

Table 2. Mean (± SD) δ13C values of five essential amino acids for consumers.

Species	Essential Amino Acids				
	Ile	Leu	Phe	Thr	Val
Shortnose Gar	-27.47 ± 0.93	-33.95 ± 0.83	-35.40 ± 1.02	-18.82 ± 1.62	-31.45 ± 1.27
Longnose Gar	-26.30 ± 0.50	-34.57 ± 0.57	-35.13 ± 0.49	-19.93 ± 0.06	-31.70 ± 1.04
Freshwater Drum	-25.80 ± 2.04	-32.38 ± 1.56	-33.20 ± 2.01	-16.67 ± 1.37	-30.42 ± 1.57
Silver Carp	-30.11 ± 1.05	-37.29 ± 0.56	-38.38 ± 0.76	-22.80 ± 1.01	-35.77 ± 0.88

Table 3. Mean (± SD) δ13C values of five essential amino acids for food source groups.

Source Group	Essential Amino Acid				
	Ile	Leu	Phe	Thr	Val
Algae	-29.69 ± 4.52	-37.66 ± 5.28	-35.14 ± 3.83	-21.97 ± 5.49	-32.00 ± 3.84
Chlorella sp.					
Diatoms					
Spirogyra sp.					
Cyanobacteria	-24.00 ± 0.82	-29.23 ± 0.76	-24.80 ± 0.10	-10.33 ± 0.50	-25.43 ± 1.66
Spirulina sp.					
Aq. Macrophyte	-28.27 ± 0.55	-35.63 ± 0.51	-31.23 ± 0.25	-17.93 ± 0.85	-33.57 ± 1.14
Hydrilla sp.					
C4 Terrestrial	-11.57 ± 1.54	-22.23 ± 0.31	-16.27 ± 0.76	0.23 ± 4.42	-18.23 ± 0.76
Zea sp.					
Panicum sp.					
C3 Terrestrial	-24.91 ± 1.08	-36.07 ± 1.46	-27.77 ± 1.90	-13.9 ± 1.66	-32.30 ± 1.81
Pinus sp.					
Pinus sp.					
Salix sp.					
Salix sp.					
Salix sp.					
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Salix sp.					

Table 4. Mean proportional contribution (± SD) of δ13C from food source groups to consumers, as estimated by MixSIAR modeling.

Species	Source Group				
	Algae	Aq. Macrophyte	C3 Terrestrial	C4 Terrestrial	Cyanobacteria
Gar	0.718 ± 0.080	0.021 ± 0.011	0.013 ± 0.006	0.078 ± 0.042	0.170 ± 0.118
Freshwater Drum	0.659 ± 0.054	0.035 ± 0.018	0.018 ± 0.008	0.226 ± 0.102	0.060 ± 0.052
Silver Carp	0.953 ± 0.012	0.017 ± 0.008	0.007 ± 0.003	0.013 ± 0.006	0.011 ± 0.008