TESTING TROPHIC GUILD CLASSIFICATIONS IN TEMPERATE RIVER FISH COMMUNITIES USING STABLE ISOTOPES

Gabe M. Madel

A thesis submitted in partial fulfillment of
the requirements for the degree of
Master of Science

Department of Biology

Central Michigan University
Mount Pleasant, Michigan
February, 2014

ACKNOWLEDGEMENTS

I would like to thank my committee members for their guidance and support throughout my project. I would specifically like to thank my advisors Dr. Brent Murry and Dr. Tracy Galarowicz for providing the encouragement and expertise I needed to complete this project. This work was supported by grants from Central Michigan University, the Great Lakes Fisheries Trust, and the Saginaw Bay Watershed Initiative Network. For assistance with field work I thank Clarence Fullard, Jacob Stoller, Mel Haas, Janine Lavavic, Kyle Broadway, and Lindsay Adams. I would also like to thank Dr. Kevin Pangle for his valuable assistance with the statistical program R. Thank you to my parents for their continued encouragement and support throughout my career.

ABSTRACT

TESTING TROPHIC GUILD CLASSIFICATIONS IN TEMPERATE RIVER FISH COMMUNITIES USING STABLE ISOTOPES

by Gabe Madel

Trophic guilds have provided important advancement in fish community ecology and are used widely by managers to evaluate the condition of aquatic systems, investigate the response of fish assemblages to habitat alterations and invasions of non-native species, and determine the mechanisms structuring fish assemblages. While trophic guilds are widely used and provide a powerful tool for managing fish assemblages, the majority of trophic guild classifications are based on diet analyses and remain untested for most aquatic ecosystems. Tissue samples were collected from 354 individual fish comprising 22 species in five temperate river food webs and analyzed for $\delta^{13}C$ and $\delta^{15}N$. Stable isotope ratios were used to test literary trophic guild classifications for these temperate river food webs. Multivariate statistical analyses of the stable isotope ratios revealed a lack of trophic guild structure in these temperate river fish assemblages. We observed extensive trophic overlap among fish species previously defined in to separate trophic guilds. The data suggest that individuals show no evidence of dietary specialization and that, at least during the summer season, temperate riverine fishes in the five temperate river food webs are largely opportunistic. Our results indicate a lack of support for trophic guild classification in five temperate river food webs which is in contrast to research in tropical and sub-tropical fish communities and reinforce the need for further evaluation of trophic guild classifications in other aquatic food webs.

TABLE OF CONTENTS

LIST OF TABLES

TABLE PAGE

LIST OF FIGURES

FIGURE PAGE

TESTING TROPHIC GUILD CLASSIFICATIONS IN TEMPERATE RIVER FISH COMMUNITIES USING STABLE ISOTOPES

Introduction

Food webs describe the feeding interactions between species in a community and can be used to depict the flow of energy and nutrients throughout the system (Lawton 1989; Pimm et al. 1991). Analysis of food webs often provides transformative insight into ecosystem function and dynamics and forms the basis of modern ecology (Elton 1927; Lindeman 1942; MacArthur 1955). Understanding the feeding relationships that exist in a food web is a fundamentally important component of evaluating food web structure. If the feeding interactions that exist between species can be discerned, the trophic structure can be identified and used to compare to other food webs to answer questions about food web functioning (Jepsen and Winemiller 2002; Cattin et al. 2004).

A variety of methods are used to evaluate food web relationships including diet analyses, stable isotope analyses, and assigning individuals to trophic guilds (Jennings et al. 2002; Jepsen and Winemiller 2002; Franssen and Gido 2006; Frimpong and Angermeier 2010). Unfortunately, the trophic pathways that compose food webs are difficult to identify because conventional techniques such as diet analysis provide only a small temporal window into resource use by consumers (Vander Zanden et al. 1998; Jennings et al. 2002; Post 2002). In light of these problems, stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) provide information about feeding relationships that integrate all trophic pathways leading to an organism over a longer temporal period (Peterson and Fry 1987; Kling 1992; Cabana and Rasmussen 1994; Vander Zanden et al. 1998; Jennings et al. 2002; Post 2002). Stable isotope ratios identify trophic interactions and track the movement of energy through the food web (Peterson and Fry

1987; Vander Zanden et al. 1998; Post 2002). $\delta^{15}N$ are enriched in a stepwise manner by approximately 3.4 ‰ in a predator's tissue compared to their prey, thus providing an accurate method of estimating trophic position (Vander Zanden et al. 1998; Post 2002). Many primary producers in aquatic systems have distinct $\delta^{13}C$ which can be used to identify each unique producer (Peterson and Fry 1987). The $\delta^{13}C$ of consumers are similar to the food sources they ingest so $\delta^{13}C$ can provide estimates of the dietary sources of aquatic consumers (DeNiro and Epstein 1978).

A less common way of examining food web structure is the use of trophic guilds (Franssen and Gido 2006). Guilds represent a functional unit that links fish communities to individual based characteristics, which are independent of taxonomic classifications (Noble et al. 2007). Trophic guilds are assigned to species based on information about their diet and functional roles (Franssen and Gido 2006; Zambrano et al. 2006). Functional analyses such as trophic guild membership are an important component of comparative ecology for fluvial fish and macroinvertebrate communities (Vannote et al. 1980; Karr et al. 1986; Poff and Allen 1995). Trophic guilds are used in community ecology to investigate the response of fish communities to habitat alterations, invasions of non-native species, and the mechanisms structuring fish assemblages (Poff and Allan 1995; Zambrano et al. 2006; Gido and Franssen 2007, Frimpong and Angermeier 2010). Trophic guilds have been the foundation of Indices of Biotic Integrity (IBIs) as well as other metrics used to evaluate the health and condition of aquatic systems (Noble et al. 2007).

While trophic guilds have provided important advancements in fish community ecology, the majority of these classifications are based on diet analyses, which only take into account recently ingested food items and do not differentiate between diet items that are assimilated

versus consumed (Post 2002; Cabana and Rasmussen 1994). There can be considerable variation in the diet of fish as available food items are affected by diurnal, seasonal, and habitat fluctuations (Poff and Allan 1995; Frimpong and Angermeier 2010). Fish may also feed opportunistically, and many species undergo ontogenetic diet shifts (Poff and Allan 1995; Jackson et al. 2001; Hoeinghaus et al. 2007). The majority of fish species examined change their trophic ecology during ontogeny, consuming plankton as larval fish to larger invertebrates as juveniles before reaching their full diet range as adults (Matthews 1998; Jennings 2002).

Ontogenetic diet shifts and body size need to be taken into account when classifying fish into trophic guilds as fish can shift trophic guilds as they undergo ontogeny. Most trophic ontogeny studies, however, have focused on species that are piscivorous as adults and are recreationally important game species. There is a lack of research examining trophic ecology of non-game, non-piscivorous species. All of the factors influencing the diet of fluvial fish species have the potential to introduce bias into trophic classifications based on diet analyses. Since trophic guilds are used to analyze food web structure and function of fish communities, there is a need for validation of these classifications across species and aquatic systems (Jepsen and Winemiller 2002; Franssen and Gido 2006; Burress et al. 2012).

River systems are dynamic in nature with fluctuating environmental conditions that are driven to a large extent by hydrology (Matthews 1998; Taylor and Warren 2001). Hydrologic variation affects the availability of habitat and food for fish and may be the most dominant abiotic factor structuring fluvial fish communities (Poff and Ward 1990; Poff and Allan 1995; Matthews 1998; Taylor and Warren 2001). For example, fish may move into the floodplain in temperate rivers during flood events to forage on food resources that are unavailable during lower flows (Matthews 1998), and fish may become restricted to individual pools due to reduced

habitat connectivity during drought conditions (Taylor and Warren 2001). While extreme, these examples demonstrate how trophic links in lotic food webs can be heavily influenced by seasonal hydrology. The seasonal variation in trophic pathways, the versatility of feeding by fish species, and ontogenetic diet shifts result in fish assemblages that often display variable trophic pathways in temperate rivers (Poff and Allan 1995; Matthews 1998; Fisher et al. 2001; Jackson et al. 2001).

Temperate rivers have a relatively high diversity of species that occupy a wide range of habitat types and display a variety of feeding modes. Feeding strategies of fluvial fish species are linked to the hydrologic stability (Poff and Allan 1995; Jackson et al. 2001; Taylor and Warren 2001; Franssen and Gido 2006). In river systems with fairly stable hydrology, fish species are often resource specialists (Poff and Allan 1995, Jepsen and Winemiller 2002; Hoeinghaus et al. 2007). If the hydrology is more variable, there are usually more resource generalists (Poff and Allen 1995; Taylor and Warren 2001; Jepsen and Winemiller 2002). The temperate rivers sampled in this study experience large variation in flow throughout the year. For example, the Shiawassee River, Michigan, had an average maximum flow of 25,000 cubic feet per second from 2009 to 2012 while the average minimum flow was 58 cubic feet per second. The Cass and Flint Rivers, Michigan, have similar flow regimes with large seasonal variation in flow. Since these temperate rivers have variable hydrologic conditions, many fish species may act as resource generalists by feeding opportunistically, which could result in extensive trophic overlap among species and individuals and poor differentiation of trophic guilds.

Stable isotope signatures of fish species have rarely been used to examine trophic guild structure of fish communities in temperate rivers with the exception of a study by Franssen and

Gido (2006), which tested trophic classifications of small bodied fish in streams in the Midwestern United States using $\delta^{15}N$ values. Trophic position did not differ among algivore/detritivores, omnivores, and invertivores, failing to support trophic classifications made using literary references (Franssen and Gido 2006). The results from Franssen and Gido (2006) and a lack of parallel research in temperate systems highlights the need for future studies testing trophic guild classifications across a suite of aquatic systems.

Carbon and nitrogen stable isotope data were collected and analyzed from five temperate river food webs to test multiple hypotheses. The first hypothesis was that fish species show differential clustering in $\delta^{13}C$ and $\delta^{15}N$ space suggesting differential energy acquisition pathways consistent with a food web composed of distinct trophic guilds. The null hypothesis was that due to large trophic overlap (in $\delta^{13}C$ and $\delta^{15}N$ space) between individuals within and among species, stable isotope ratios would not support trophic guild classifications. The second hypothesis (which hinges on acceptance of the first hypothesis) was that stable isotope classifications of individuals into trophic guilds would be consistent with literature based classifications of individuals into trophic guilds.

To test the hypothesis that fish species in our temperate rivers would show differential stable isotope clustering, we developed two objectives. The first objective was to allow the stable isotope data to show any patterns that may exist in the food web that would support distinct trophic guilds. Bi-plots of standardized $\delta^{13}C$ and $\delta^{15}N$ data from individual fish were used to look for natural groups (separation of individuals into groups in isotopic space) that support trophic guilds. If groups naturally differentiated in the bi-plots, distinct trophic guilds would be supported and the groups could then be analyzed to see if the individual fish matched literary trophic guild classifications. The second objective was to classify individual fish into

trophic guilds based on primary literature and test these classifications with the standardized isotope data. This method forces individual fish into trophic guilds because classifications were based on the literature instead of the isotope ratios but it allows us to test the literary classifications and has been used in studies in tropical and subtropical rivers (Jepsen and Winemiller 2002; Burress et al. 2012). We used stable isotope data to test whether trophic guild classifications (based primarily on diet data) are valid in these temperate river food webs. In order to classify individuals into trophic guilds using literary references, a meta-analysis of the existing literature was conducted to identify which guilds species are classified into. Ontogenetic diet shifts were also incorporated as individuals from the same species can be classified into multiple guilds depending on body size (Mittelbach and Persson 1998).

Methods

Study Sites

Our study sites were located in three tributaries of the Saginaw River: the Shiawassee, Cass, and Flint Rivers in the Saginaw Bay watershed, Lake Huron (Figure 1). Stable isotope samples were collected from five unique river reaches and food webs. Two reaches were sampled on the Shiawassee River: one downstream of the rock ramp and one upstream of the rock ramp in Chesaning, Michigan. Prior to implementation of the rock ramp in 2009, the upstream and downstream reaches had been separated since the construction of the Chesaning Dam in 1863. Due to this long term loss of connectivity, we treated these reaches as separate food webs. Two reaches were also sampled on the Cass River: one reach was located below the dam in Frankenmuth, and the second reach was upstream of the dam. There is no connectivity between the fish assemblages in the two reaches due to the Frankenmuth Dam, thus we considered the two reaches separate food webs. The last reach sampled was on a free-flowing

reach of the Flint River. The downstream reaches of the Cass and Shiawassee Rivers and the entire Flint River had direct uninhibited connections with Saginaw Bay and Lake Huron; the two upstream reaches did not.

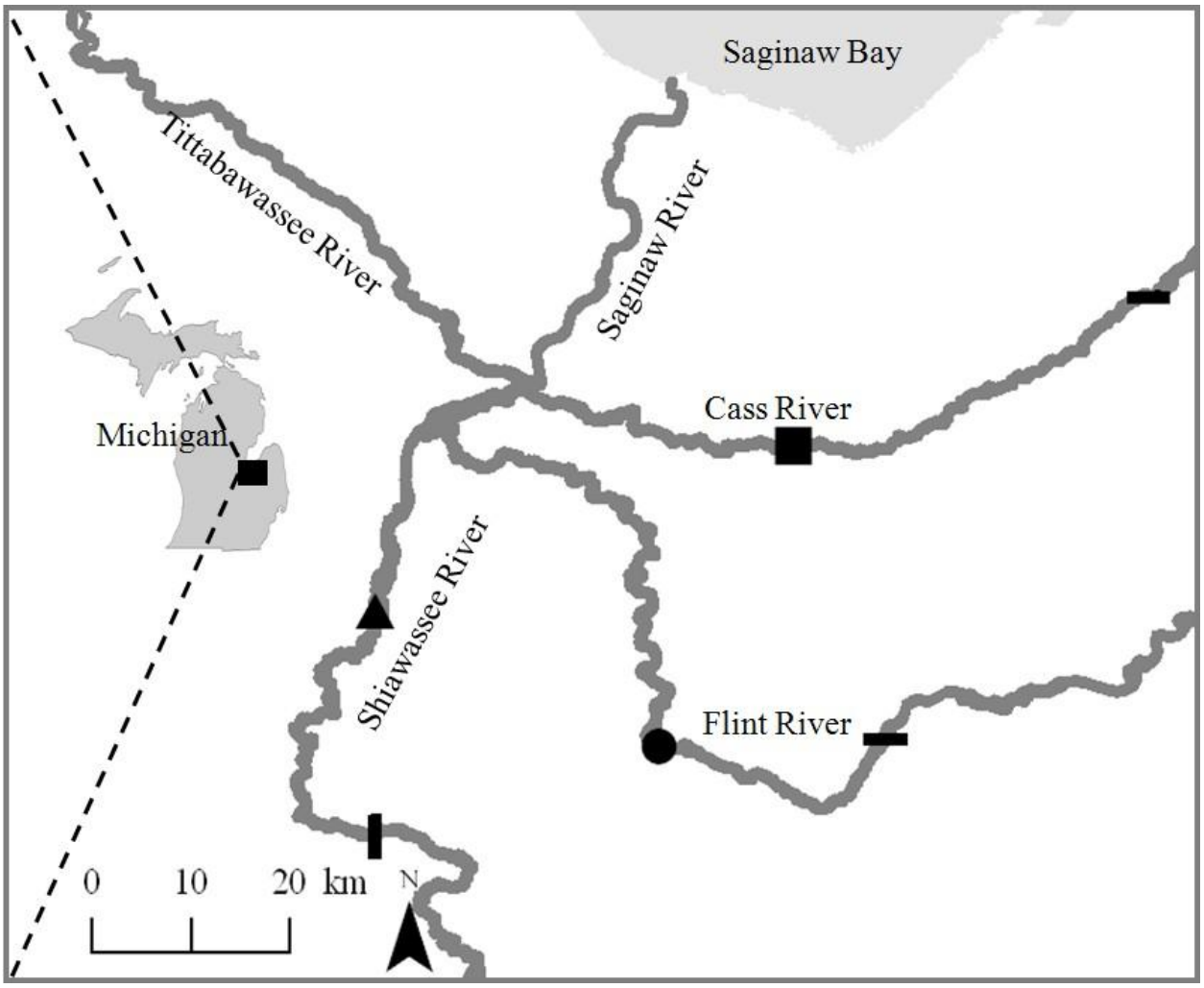

Figure 1. Map of the Saginaw River, Michigan Tributaries. The triangle represents the location of the rock ramp installed on the Shiawassee River, the circle represents the approximate sampling locations on the Flint River, and the square represents the dam in Frankenmuth, Michigan on the Cass River. Rectangles represent the first upstream barriers to fish passage on the Shiawassee and Flint rivers, and the second fish barrier on the Cass River. Map courtesy of Dr. D. Woolnough.

Stable Isotope Tissue Collection

Fish species representative of the different trophic guilds present in each river were selected for stable isotope analysis. Stable isotope samples were collected from 354 individual fish comprising 22 species (Table 1). Fish for stable isotope analysis were collected by electro-fishing or hook and line sampling in August and September, 2012, in order to encompass the primary growing season. Total length (mm) of each fish was measured, and a small section of skinless white muscle tissue was filleted from the dorsal caudal peduncle region. Tissue samples were collected from three discrete size classes; 64-128 mm, 192-256mm, and >384 mm. All samples were immediately frozen and stored until samples were freeze dried. Gilled snails and mussels (*Actinonaias ligamentina*, *Lampsilis siliquoidea*, and *Dreissena polymorpha*) were collected by hand to use as an isotopic baseline. Non-lethal methods were used to sample all unionids by taking a small tissue clip from the mantle of each individual. Primary consumers (snails and mussels) provided baseline isotopic values, which allowed us to standardize data to overcome inter-site differences in ambient isotopic variation in available carbon and nitrogen. Such standardizations can be used to estimate the trophic position of other organisms in the food web and compare across food webs (Post 2002). Tail muscle tissue from *Orconectes rusticus* (rusty crayfish) and a conglomerate macroinvertebrate sample were also collected to use in the stable isotope analysis of each food web. Macroinvertebrates were captured using a kicknet, and rusty crayfish were caught during electro-fishing. Conglomerate macroinvertebrate samples consisted primarily of species from the Tricoptera (caddisfly) and Ephemeroptera (mayfly) orders in the Cass River upstream and Flint River reaches along with species from the Psephenidae (water penny) family in the Cass River downstream and Shiawassee River downstream and upstream reaches and species from the Elmidae (riffle beetle) family in the Cass

River downstream reach. All tissue samples were freeze-dried and then weighed (500-700 µg) in tin cups in preparation for stable isotope analysis. The conglomerate samples were ground to a fine powder, freeze-dried, and weighed (700-1000 µg) in tin cups in preparation for stable isotope analysis. Carbon and nitrogen stable isotope ratios were measured using a Thermo Finnigan Delta^{Plus} mass-spectrometer (Thermo Finnigan, San Jose, CA, USA) coupled with a Costech elemental analyzer at the Great Lakes Institute for Environmental Research at the University of Windsor, Ontario, Canada.

Table 1. Summary of the different species that stable isotope samples were collected from in each food web. The data in the species rows represents the total length (mm) range sampled for that species in that reach and N is the number sampled of that species. The letter designations beneath the common name for each species are codes for the references used to classify each species into a trophic guild. A = Poff and Allen (1995), B = Taylor and Warren (2001), C = Gido and Franssen (2007), D = www.fishbase.org, E = ww.fishtraits.info Frimpong and Angermeir (2009).

Species	**Common Name**	**Shiawassee Down Stream**	**Shiawasee Up Stream**	**Cass Down Stream**	**Cass Up Stream**	**Flint**
Benthic Invertivores						
Moxostoma duquesnii	black redhorse	-	-	-	199-400	-
N	D, E				(10)	
Cyprinus carpio	common carp	585-636	225-803	-	-	545-675
N	A ,C, D, E	(2)	(3)			(5)
Aplodinotus grunniens	freshwater drum	-	-	91-121	-	-
N	A, D, E			(5)		
Moxostoma erythrurum	golden redhorse	376-382	192-439	239-252	-	-
N	A, D, E	(4)	(5)	(4)		
Moxostoma valenciennesi	greater redhorse	-	-	349-510	-	-
N	A, D, E			(5)		
Etheostoma blennioides	greenside darter	-	-	-	79-86	78-85
N	B, D, E				(5)	(2)

Percina caprodes	logperch	-	-	86-105	-	114-132
N	A, B, D, E			(5)		
Hypentelium nigricans	northern hog sucker	88-295	120-357	194-239	56-215	-
N	A, B, D, E	(10)	(7)	(5)	(11)	
Etheostoma caeruleum	rainbow darter	-	37-61	-	-	-
N	A, D, E		(6)			
Moxostoma macrolepidotum	shorthead redhorse	-	-	360-418	-	-
N	A, D, E			(4)		
General						
Invertivores						
Lepomis macrochirus	bluegill	-	-	-	89-109	-
N	A, B, D, E				(5)	
Notropis atherinoides	emerald shiner	69-85	71-87	-	-	-
N	A, C, D, E	(5)	(5)			
Dorosoma cepedianum	gizzard shad	146-153	-	79-106	-	-
N	A, D, E	(4)		(5)		
Nocomis micropogon	river chub	65-116	78-133	-	-	-
N	D, E	(5)	(5)			
Ambloplites rupestris	rock bass	65-100	66-100	76-100	72-100	95-100
N	A, D, E	(4)	(5)	(3)	(1)	(2)
Neogobius melanostomus	round goby	66-100	50-50	37-106	-	43-112
N	D, E	(5)	(1)	(12)		(11)
Cyprinella spiloptera	spotfin shiner	70-87	74-85	63-76	63-82	73-93
N	A, D, E	(5)	(5)	(5)	(5)	(5)
Piscivores						
Ambloplites rupestris	rock bass	100-201	100-198	100-205	100-235	100-219
N	A, D, E	(5)	(4)	(8)	(8)	(8)
Micropterus dolomieu	smallmouth bass	81-417	91-435	82-454	66-416	75-432
N	A, B, D ,E	(12)	(16)	(15)	(15)	(15)
Omnivores						
Ictalurus	channel	93-482	98-243	57-622	-	72-77

punctatus	catfish					
N	A, C, D, E	(6)	(5)	(9)		(2)
Noturus flavus	stonecat	67-246	53-90	-	-	-
N	A, D, E	(10)	(3)			
Ameiurus natalis	yellow bullhead	-	223-224	-	-	230-246
N	A, B, D, E		(2)			(3)

Stable Isotope Analyses

Carbon and nitrogen isotope ratios derived from the tissue samples from individual fish were used in the stable isotope analysis. Stable isotope ratios are expressed in the delta (δ) notation as parts per thousand (‰) difference from a known standard:

$$\delta^{13}C \text{ or } \delta^{15}N = [(R_{sample} - R_{standard}) / R_{standard}] \times 1000$$

where R is the ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$ (Peterson and Fry 1987). Results are reported relative to international standards: Vienna Pee Dee Belemnite for carbon, and atmospheric nitrogen for nitrogen (Peterson and Fry 1987).

Lipids are depleted in ^{13}C relative to carbohydrates or proteins, and lipid content can vary among organisms such as different fish species, which could bias stable isotope analyses that utilize $\delta^{13}C$ (Post et al. 2007). Post (2002) recommends correcting for lipids when samples exceed a C:N ratio of 5 and organisms differ in lipid content. While other studies have shown that lipid extraction had no discernible effect on the interpretation of food web structure of fish communities (Murry et al. 2006), we conservatively corrected for lipids since we had samples with C: N ratios that exceeded 5 and samples were taken from an assortment of organisms that varied in lipid content. The $\delta^{13}C$ of all samples were normalized for lipid content using the equation for aquatic organism provided by Post et al. (2007):

$$\delta^{13}C_{normalized} = \delta^{13}C_{untreated} - 3.32 + 0.99 \times C:N$$

where $\delta^{13}C_{untreated}$ is the untreated carbon isotope ratio of each sample and C:N is the carbon to nitrogen ratio for each sample. The equation provides an estimate of $\delta^{13}C$ for each sample that is normalized for lipid content (Post et al. 2007).

Trophic positions (TP) of individuals were calculated using the model developed by Post (2002):

$$TP = \lambda + (\delta^{15}N_{secondary\ consumer} - \delta^{15}N_{base})/\Delta_n,$$

where λ is the TP of the organism used to estimate the $\delta^{15}N_{base}$, $\delta^{15}N_{secondary\ consumer}$ is the $\delta^{15}N$ signature of the consumer measured directly in the stable isotope analysis, and Δ_n is the $\delta^{15}N$ enrichment per trophic level. A 3.4 ‰ enrichment per trophic level of $\delta^{15}N$ was used as it is a widely accepted value of $\delta^{15}N$ trophic fractionation in aquatic food webs (Vander Zanden and Rasmussen 2001; Post 2002). Gilled snails were used as the $\delta^{15}N_{base}$ because they have an isotopic signature similar to detritus and periphyton which is the base of the benthic food web and they integrate temporal variation in $\delta^{15}N$ (Vander Zanden and Rasmussen 1999; Post 2002). Converting $\delta^{15}N$ of consumers to TP is a method of standardization which allows for comparisons across aquatic systems since variation in $\delta^{15}N$ can occur at the base of the food web where consumers obtain their nitrogen (Zohary et al. 1994; Cabana and Rasmussen 1996; Vander Zanden and Rasmussen 1999; Post 2002). TP was also estimated using the two source model (gilled snails, unionids) developed by Post (2002), and the estimates from both models were compared using a paired t-test. Trophic positions calculated for all individuals did not differ ($P = 0.28$), so the one source model was used.

The proportion of carbon in each consumer that is derived from the benthic food web was estimated using the two-end-member-mixing model developed by Post (2002):

$$\alpha = (\delta^{13}C_{sc} - \delta^{13}C_{suspended}) / (\delta^{13}C_{benthic} - \delta^{13}C_{suspended})$$

where α is the proportion of carbon in the consumer derived from the base of the benthic food web, $\delta^{13}C_{sc}$ is the carbon isotope ratio of the secondary consumer, $\delta^{13}C_{suspended}$ is the carbon isotope ratio of the primary consumer from the suspended food web, and $\delta^{13}C_{benthic}$ is the carbon isotope ratio of the primary consumer from the benthic food web. Gilled snails were used as the primary consumer of the benthic food web, and unionids were used as the primary producers of the suspended food web. The benthic food web is primarily supported by autochthonous production (periphyton) while the suspended food web is composed of allochthonous food sources (drifting particulate matter) transported from upstream (Doi 2009). The two-end-member mixing model transforms $\delta^{13}C$ values into dietary proportions of different isotopic sources (Post 2002). Dietary proportions are often more ecologically meaningful than raw $\delta^{13}C$ values and allow comparisons between food webs that are similarly defined (Newsome et al. 2007). These transformed values can also be used to calculate metrics such as "isotopic niche", which are independent of the actual $\delta^{13}C$ values and make comparisons between aquatic systems (Newsome et al. 2007). Dietary proportions of individuals may exceed 1 in instances when secondary consumers are obtaining carbon from a source that is more enriched in ^{13}C than the sources used for the base of each food web.

Literary Trophic Guild Classifications

Individual fish were classified into trophic guilds based on primary literature (Poff and Allan 1995; Taylor and Warren 2001; Gido and Franssen 2007; www.fishbase.org; www.fishtraits.info Frimpong and Angermeir 2009). Both the fishtraits and fishbase databases synthesize available literature and databases on fish species, making the data available to researchers in an accessible format. The other three studies (Poff and Allan 1995; Taylor and Warren 2001; Gido and Franssen 2007) classify fluvial fish species that are present in the study

rivers into trophic guilds and helped guide the classifications. Species were classified into four main trophic guilds: benthic invertivores, general invertivores, omnivores, and piscivores. Only one species present in the study rivers would have been classified as an herbivore (central stoneroller *Campostoma anomalum*), but it was not abundant enough to be used in the stable isotope analyses.

Data Analysis

The standardized dietary proportion and trophic position data for individual fish were plotted as x-y data to detect natural patterns that may exist in the data. Individuals were not classified into predefined trophic guilds to allow the data to show naturally occurring groups that differed based only on their observed trophic ecology (defined by stable isotope signatures). If groups did separate based on the standardized isotope data, there would be strong support for trophic guilds in these temperate fish communities. $\delta^{13}C$ and $\delta^{15}N$ data from each food web were plotted as x-y data to examine the position species occupy in isotopic space as well as food web structure (Appendix A, Figures 1-5). Bi-plots of the standardized isotope data were constructed for each reach as well as an analysis where individuals from all reaches were combined to determine if patterns exist at a larger watershed scale when all tributaries were combined.

Once each individual fish was classified into a trophic guild, the standardized trophic position, dietary proportion data, body length, and river reach were used as variables in a discriminant function analysis (DFA) to determine if these data could be used to reclassify individuals into the predefined trophic guild. A canonical correlation analysis is performed by the DFA, which produces canonical functions providing the most discrimination among groups and classified individuals into groups (Manly et al. 2004). The DFA uses the cross validation technique jackknifing to determine which trophic guild each individual had the highest

probability of being placed in. Canonical variates were also generated for each individual and these scores were plotted as x-y data to examine if patterns in the data supported distinct trophic guilds.

Patterns in the stable isotope data of the fish assemblages from each reach as well as the combined data from all reaches were examined with Nonmetric Multidimensional Scaling (NMDS). Trophic position, dietary proportions (α), and total body length were used as variables in each NMDS. The number of dimensions used in each NMDS equalled three, and the stress value with only three dimensions was acceptable (stress values < 0.05). The NMDS operated through an iterative process by seeking to improve the goodness of fit (stress) of the regression of ordination distances against the original distance matrix (Zuur et al. 2007). Bray-Curtis was used as the distance measure in all NMDS analyses.

Standardized stable isotope data among trophic groups were compared with Analyses of Variance (ANOVAs). All statistical tests were conducted with the R v3.01 statistical package (R Development Core 2013). DFAs were run using the MASS package (Venables and Ripley 2002). All NMDS analyses were run using the vegan package v2.0-8 (Oksanen et al., 2013)

Results

Trophic position and proportion benthic carbon were used in comparisons between trophic guilds from all five food webs combined. Trophic position and proportion benthic carbon did not significantly different among any trophic guilds in any of the five food webs (Table 2). Mean trophic position of all fish species and trophic guilds are reported in Table 3. With the exception of piscivores, trophic guilds from the Flint River had the highest mean trophic positions when compared to the other reaches. Piscivores from the Cass River

downstream reach had the highest mean trophic position, and the other three trophic guilds had the second highest mean trophic position of all reaches.

Table 2. ANOVA results for between trophic guild comparisons of trophic position and proportion benthic carbon with all five food webs combined.

Variables	r^2	F	P	df
Trophic Position	0.54	2.41	0.39	3, 353
Proportion Benthic Carbon	0.33	1.56	0.43	3, 353

Table 3. Mean trophic position of fish species from all five reaches. SE is the standard error of the mean trophic position for each fish species. Fish species are organized by trophic guild and the average trophic position for each guild is reported. Individual rockbass (*Ambloplites rupestris*) were classified into both the General Invertivore and Piscivore trophic guilds based on their body size (Mittelbach and Persson 1998).

Species	**River and Site**									
	Shiawasee				Cass				Flint	
	Downstream		Upstream		Downstream		Upstream			
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Benthic Invertivores										
Moxostoma duquesnii	-	-	-	-	-	-	2.86	0.02	-	-
Cyprinus carpio	2.47	0.13	2.63	0.17	-	-	-	-	3.1	0.04
Aplodinotus grunniens	-	-	-	-	2.97	0.09	-	-	-	-
Moxostoma erythrurum	2.82	0.03	2.79	0.04	2.82	0.05	-	-	-	-
Moxostoma valenciennesi	-	-	-	-	2.97	0.06	-	-	-	-
Etheostoma blennioides	-	-	-	-	-	-	3.05	0.07	3.13	0.01
Percina caprodes	-	-	-	-	2.87	0.02	-	-	2.90	0.08
Hypentelium nigricans	2.93	0.03	2.80	0.02	3.01	0.03	2.57	0.06	-	-
Etheostoma caeruleum	-	-	2.97	0.02	-	-	-	-	-	-
Moxostoma macrolepidotum	-	-	-	-	2.92	0.02	-	-	-	-
Average	2.74		2.80		2.93		2.83		3.04	
General										

Invertivores										
Lepomis macrochirus	-	-	-	-	-	-	2.83	0.06	-	-
Notropis atherinoides	2.90	0.09	2.64	0.16	-	-	-	-	-	-
Dorosoma cepedianum	2.49	0.06	-	-	-	-	-	-	-	-
Nocomis micropogon	3.05	0.05	2.98	0.04	-	-	-	-	-	-
Ambloplites rupestris	3.22	0.05	3.08	0.040	3.30	0.041	2.89	0.049	3.43	0.031
Neogobius melanostomus	2.99	0.043	2.92	0	3.01	0.043	-	-	3.14	0.035
Cyprinella spiloptera	3.17	0.109	2.92	0.056	2.83	0.264	2.45	0.036	2.85	0.151
Average	2.97		2.91		3.05		2.61		3.14	
Piscivores										
Ambloplites rupestris	3.01	0.058	3.00	0.040	3.24	0.041	2.89	0.049	3.14	0.031
Micropterus dolomieu	3.26	0.061	3.08	0.036	3.31	0.030	2.95	0.064	3.40	0.094
Average	3.14		3.04		3.28		2.92		3.27	
Omnivores										
Ictalurus punctatus	3.05	0.127	2.88	0.079	3.00	0.085	-	-	2.91	0.185
Noturus flavus	2.76	0.087	2.93	0.008	-	-	-	-	-	-
Ameiurus natalis	-	-	2.40	0.061	-	-	-	-	3.30	0.046
Average	2.91		2.74		3.00				3.11	

The bi-plots created using the standardized trophic position and dietary proportion data showed extensive trophic overlap and no clear separation between individuals resulting in a lack of support for trophic guilds in the individual river reaches or with all food webs combined (Figure 2). There was no natural separation of individuals into groups that would support distinct trophic guilds. Individual fish were tightly clustered around one another, and the few

outliers that separated from the majority of individuals were from a variety of species and trophic guilds based on literary classifications (i.e., no clear patterns were evident).

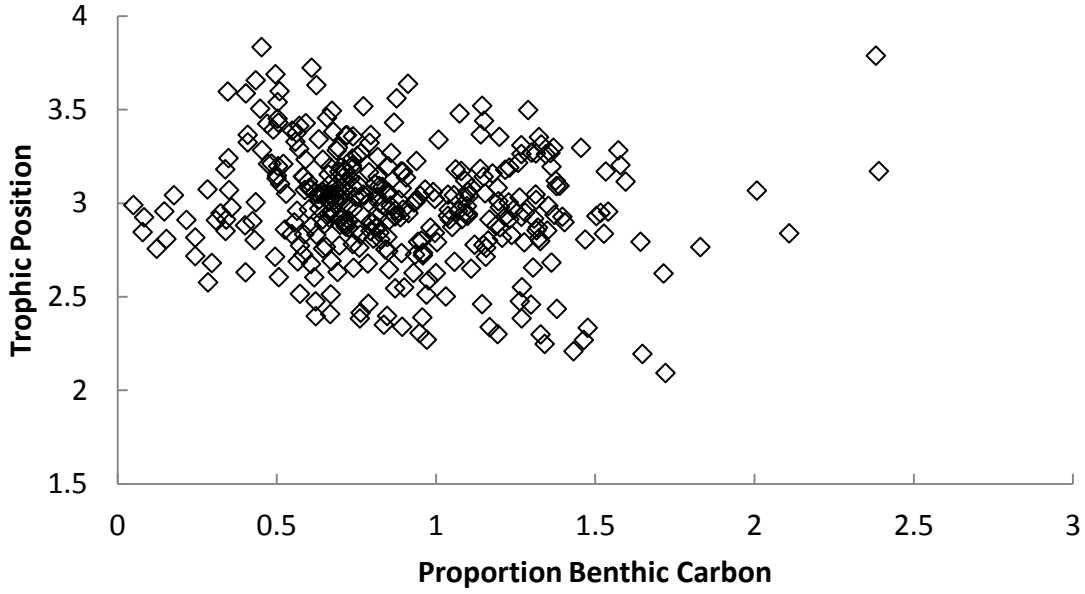

Figure 2. Bi-plot of standardized stable isotope data of all individuals from every reach combined. Proportion Benthic carbon is the standardization of the $\delta^{13}C$ and estimates energy source (i.e., proportion derived from benthic algae sources) and trophic position is the standardization of the $\delta^{15}N$. Each point represents an individual fish (n = 354).

The ordination plot for the NMDS for all reaches combined did not reveal divergent grouping patterns (Figure 3). The patterns that did emerge in the NMDS were strongly driven by body length of individual fish (correlation coefficient for body length and NMDS1 = 0.87). This is likely due to field sampling methods where individual fish from 3 discrete size classes were primarily targeted. For example, the majority of the general invertivores sampled were in the smallest size class due to their relatively small body size as adults; this pattern can be seen in the ordination plot. When body length was not included as a variable in the NMDS, these patterns failed to emerge and the ordination plot more closely resembled the bi-plot of standardized isotope data. This held true for the individual reaches as distinct groups did not emerge in these NMDS analyses, and the only patterns were heavily influenced by body length.

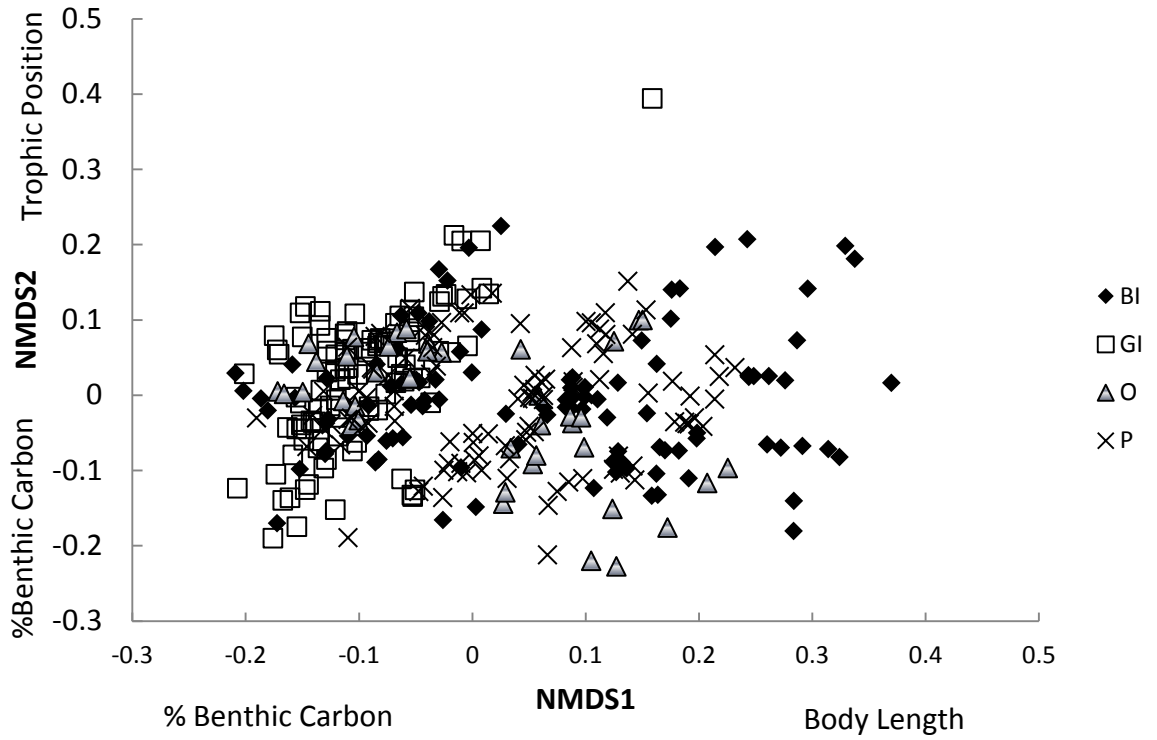

Figure 3. Ordination plot of NMDS axes. Explanatory variables included in the NMDS were proportion benthic carbon, trophic position, and body length (mm). The variables are placed one each axis in accordance with their correlation coefficient with that axis, which represents their relationship with that axis. Data are shape coded by literature-based trophic groups where: BI=Benthic Invertivore, GI=General Invertivore, O=Omnivore, and P=Piscivore

Discriminate function analysis (DFA) with all five food webs combined was used to examine the variables that could discriminate between trophic guilds. Proportion benthic carbon, trophic position, body length, and river reach were initially used as variables. River reach was not significant ($F = 2.36$, $P = 0.07$) and was removed. We subsequently ran the DFA with and without body length to assess how the variable influenced trophic guild discrimination. After excluding river reach, a DFA was run with the variables trophic position (TP), proportion benthic carbon, and body length. There were significant differences between the guild centroids ($F = 22.05$, $P < 0.0001$, df = 9, 847), and all variables were significant (TP $F = 27.40$, $P < 0.0001$, df = 3, 350; proportion benthic carbon $F = 3.44$, $P = 0.02$, df = 3, 350; body length $F = 33.95$, P

<0.0001, df = 3,350). Only the first two canonical functions were significant (Canonical Function 1 F = 22.05, $P < 0.0001$, df = 9,847; Canonical Function 2 F = 21.60, $P < 0.0001$, df = 4, 698), and they described 55.12% and 44.12% of the variation between guilds, respectively. The last DFA was run with only TP and proportion benthic carbon as the variables. There were significant differences between the group centroids (F = 16.35, P <0.0001, df = 6, 698), and both variables were significant (TP F = 27.4, $P < 0.0001$, df = 3, 350; proportion benthic carbon F = 3.44, $P = 0.02$, df = 3, 350). The first two canonical functions were significant (Canonical Function 1 F = 16.35, $P < 0.0001$, df = 6, 698; Canonical Function 2 F = 3.4, $P < 0.0001$, df = 2, 350) describing 93.4% and 6.6% of the variation between guilds, respectively.

Classification rates for each DFA were relatively low (Tables 4 and 5). Total classifications success for the DFA with body length included was 55.6%, whereas classification success without body length was only 41.4%. Classifcation success was 95.2% for general invertivores when body length was included. Individuals in the general invertivore guild were only present in the smallest body size class so body length was the primary variable driving discrimination between general invertivores and other trophic guilds, resulting in the high classification success for this guild. The expectation was that general invertivores and benthic invertivores would have a high degree of trophic overlap due to their similarities in diet items being assimilated; however, body length and proportion benthic carbon provided more differentiation between the two guilds than predicted. Classification success was lower for the other three trophic guilds in these analyses, all of which had individuals in all three size classes. Body length was unable to provide as much discrimination between these guilds since individuals from these three guilds spanned all three size classes. Piscivores had the second highest classification rate (59.3%). Misclassified piscivores were classified as general

invertivores 26.9% of the time and benthic invertivores 13.9% of the time. Benthic invertivores had a classification success of 55.3%; the majority of benthic invertivores that were misclassified were classified as general invertivores (36.9%). Omnivores had the lowest classification success (12.5%) which could be driven by their diverse diet. Omnivores feed on a variety of food sources, which span a large range of $\delta^{13}C$ or $\delta^{15}N$, resulting in isotopic signatures that overlap with the other guilds.

In the DFA without body length, general invertivores had a lower classification success (23.3%). Individuals were misclassified as benthic invertivores 38.8% of the time and piscivores 37.9% of the time, indicating large isotopic overlap with these guilds. When body length was removed, the DFA was less successful at discriminating between general invertivores and the other guilds. Piscivores had the highest classification success (72.2%), and benthic invertivores had the second highest success (69.9%). The majority of piscivores that were misclassified were classified as benthic invertivores (24.1%), and 16.5% of the misclassified benthic invertivores were classified as piscivores. This implies isotopic overlap between piscivores and benthic invertivores. This was not anticipated since piscivores were expected to feed at higher trophic levels than individuals from the other guilds. Omnivores again had extremely low classification rates which are likely driven by their diverse diet.

Table 4. DFA classification summary of individuals into trophic guilds with body length, trophic position, and proportion benthic carbon as variables. Each row is the number of individuals from that trophic guild the DFA classified into each trophic guild.

Trophic Guild	Benthic Invertivore	General Invertivore	Omnivore	Piscivore	Total
Benthic Invertivore	57	38	1	7	103
% classification	55.34	36.89	0.97	6.8	
General Invertivore	3	98	0	2	103
% classification	2.91	95.15	0	1.94	
Omnivore	12	19	5	4	40
% classification	30	47.5	12.5	10	
Piscivore	15	29	0	64	108
% classification	13.89	26.85	0	59.26	

Table 5. DFA classification summary of individuals into trophic guilds with trophic position and proportion benthic carbon as variables (body length not included). Each row is the number of individuals from that trophic guild the DFA classified into each trophic guild.

TG	Benthic Invertivore	General Invertivore	Omnivore	Piscivore	Total
Benthic Invertivore	72	14	0	17	103
% Classification	69.9	13.59	0	16.5	100
General Invertivore	40	24	0	39	103
% Classification	38.83	23.3	0	37.86	100
Omnivore	17	7	0	16	40
% Classification	42.5	17.5	0	40	100
Piscivore	26	4	0	78	108
% Classification	24.07	3.7	0	72.22	100

Discussion

We observed extensive trophic overlap among fish species previously defined as separate trophic guilds. Our data collected at the end of summer effectively summarize the primary growing season (Hesslein et al. 1993; Sakano et al. 2005; Quevedo et al. 2009) in these systems (June, July, and August) and show no evidence of dietary specialization among species, strongly suggesting that, at least during the summer season, temperate riverine fishes in the five temperate river food webs examined are largely opportunistic. Stable isotope signatures of individual fish did not support literary trophic guild classifications (Poff and Allan 1995; Taylor and Warren 2001; Gido and Franssen 2007; www.fishbase.org; www.fishtraits.info Frimpong and Angermeir 2009) and revealed a lack of trophic guild structure. In contrast, examination of trophic guild structure of fluvial fish communities in tropical and subtropical rivers found a high degree of dietary specialization among fish species and strong trophic guild structure (Jepsen and Winemiller 2002; Burress et al. 2012). Fish species in tropical and subtropical rivers act as resource specialists, while opportunistic feeding appears to be the foraging strategy of most individuals during the summer growth period in the temperate rivers we studied.

Stable isotope bi-plots are used extensively by food web ecologists to examine food web structure (Layman et al. 2007). The standardized bi-plots constructed with all five food webs combined showed large isotopic overlap between species with no separation of individuals into groups. Individuals of most species appear to display large variation in the carbon sources they are utilizing and an ability to feed at multiple trophic levels. If trophic guilds existed in these fish communities, natural separation of individuals into groups in these bi-plots would have occurred that support trophic guilds. Similarly, there were no differences in standardized stable isotope data between trophic guilds, implying large trophic overlap between predefined trophic guilds. This pattern was not observed in fish communities in other aquatic systems where

differences in stable isotope ratios existed between trophic guilds (Fisher et al. 2001; Grey 2001; Jepsen and Winemiller 2002; Burress et al. 2012).

Previous studies also used DFA to test if stable isotope ratios could classify individuals into predefined trophic guilds in tropical and subtropical rivers with high success (Jepsen and Winemiller 2002; Burress et al. 2012). Jepsen and Winemiller (2002) had classification rates greater than 70 % in all rivers and two of their rivers had classification rates of 86 and 99%. Burress et al. (2012) had a classification rate of 70%. Our classification success was 56% when body length was included as a variable and 44% when body length was not included, respectively. The low classification rates observed in the temperate river food webs we studied imply that there is large isotopic overlap among individuals and species, providing little support for trophic guild classifications based on diet analyses.

The results from this and past stable isotope analyses suggest differences in the mechanisms structuring fish communities in tropical and subtropical versus temperate rivers. Resource specialization by fish species appears to be the preferred strategy in tropical and subtropical rivers (Lowe-McConnell 1987; Winemiller 1990; Winemiller 1995; Jepsen and Winemiller 2002), while resource generalization is the strategy employed by the species in the temperate rivers we examined. The broad diets of fish species, the ability to opportunistically feed on a variety of diet items, are all factors that may contribute to the isotopic overlap of guilds in these temperate rivers.

Trophic guilds supply information about the functional niches of fish species (Power 1992; Gelwick et al. 1998; Mathews 1998; Gido and Franssen 2006), which managers can use to improve conservation and management of aquatic food webs. The functional niches fish species occupy may help foresee the effect invasive species will have on fish communities and which

invasive species could be successful (Moyle and Light 1996; Kolar and Lodge 2002; Gido and Franssen 2006). Trophic guilds have also been the foundation of Indices of Biotic Integrity as well as other metrics used to evaluate the health and condition of aquatic systems (Karr 1981; Noble et al. 2007). While trophic guilds play an important role in fish ecology, the classification of fish species into guilds remains largely untested in most aquatic systems (Gido and Franssen 2006). Stable isotope analyses provide a valid method of testing trophic guild classifications while also analyzing food web structure (Jepsen and Winemiller 2002; Franssen and Gido 2006; Burress et al. 2012). Our results indicate a lack of trophic guild structure in five temperate river fish communities and reinforce the need for further evaluation of trophic guild classifications in other aquatic food webs. Trophic classifications based only on literary references should be used with caution, especially in lotic food webs in temperate regions where opportunistic feeding is more the rule rather than the exception.

When the proportion of carbon assimilated from the benthic food web was calculated for individual fish using the model developed by Post (2002), a number of individuals had proportions that exceeded 1.00 (Figure 2). This could be caused by a few different factors. The first factor could be an unknown dietary source (that was not sampled) which is more enriched in $\delta^{13}C$ than the gilled snails and unionids that were used for the isotopic baseline. If fish are assimilating a dietary source more enriched in $\delta^{13}C$ than the end members used the model could predict that the proportion of carbon assimilated from the benthic food web exceeds 1 for those individuals. Previous studies have obtained proportions greater than 1 or less than 0 when using two source mixing models, however those proportions were adjusting by setting them at 0 or 1 (Bunn et al. 2003; Vander Zanden et al. 2003; Xu et al. 2011; Zhou et al. 2011). While this method has been used to deal with $\delta^{13}C$ of consumers that are more enriched or depleted than the

end members used in the model (Zhou et al. 2011), it may ignore the biological mechanisms responsible for consumer signatures outside of the baseline organisms. We believe that using the actual proportions calculated by the model incorporates these mechanisms into the analyses even if there is uncertainty about what the specific mechanisms. Gilled snails have an isotopic signature similar to detritus and periphyton which forms the base of the benthic food web, and unionids have an isotopic signature similar to seston which is the base of the suspended food web (Cabana and Rasmussen 1996; Post 2002; Xu et al. 2011). The benthic and suspended food webs are the two primary food webs available to fluvial fish species in temperate rivers (Vannote et al. 1980; Finlay 2001; Bunn et al. 2003), so gilled snails and mussels should represent the key energy sources utilized by fish in these food webs. The second factor could be temporal variation in the $\delta^{13}C$ signature of the gilled snails and unionids used as end members. If there is high temporal variation in the $\delta^{13}C$ signature of gilled snails or mussels it could bias the results of the model. There is considerable temporal variation in the $\delta^{13}C$ signature of primary producers in aquatic systems (Cabana and Rasmussen 1996; Post 2002; Finlay 2004; Xu et al. 2005), but previous research has shown that the isotopic signature of benthic snails and unionids has much lower temporal variance than primary producers (Cabana and Rasmussen 1996; Vander Zanden and Rasmussen 1999; Post 2002; Xu et al. 2011). Past research has found that snails and unionids are long lived primary consumers that provide an accurate representation of the benthic and suspended food web (Cabana and Rasmussen 1996; Vander Zanden and Rasmussen 1999; Post 2002; Xu et al. 2011). However, tissue turnover rates are correlated with body mass (Fry and Arnold, 1982; Peters 1983; Hesslein et al. 1993; Post 2002) and due to the smaller body mass of snails and unionids compared to the fish species sampled, it is possible there is a temporal disconnect between the primary consumers and fish that the model does not

account for. Fish have tissue turnover rates that can range from months to years (Hesslein et al. 1993) and due to their smaller body mass snails and mussels almost certainly have shorter turnover times (Post 2002). This has not posed problems in previous studies (Cabana and Rasmussen 1996; Vander Zanden and Rasmussen 1999; Post 2002; Xu et al. 2011), but certainly deserves attention in future research to determine if considerable variation in the $\delta^{13}C$ signature of the gilled snails and unionids exists on a small temporal scale in temperate rivers.

It is also highly likely that species migrating from Lake Huron into the study tributaries are influencing the mixing model. Each spring, a variety of species migrate from Lake Huron into all three of the tributaries sampled in this study. The spawning run includes a large number of Catostomidae species, *Sander vitreus,* and *Micropterus dolomieu* as well as other species that make annual spawning migrations. During the spawning migrations both eggs and larval fish could be readily available for resident fish to prey upon. If the lake-run individuals have a more enriched $\delta^{13}C$ signature than the resident fish, individuals who prey on the eggs and to a lesser extent the larval fish of the migratory adults may be more enriched in $\delta^{13}C$ (Vander Zanden et al. 1998). Tissue samples for stable isotope analyses were not collected from any of the migratory fish in the spring so we could not specifically test this hypothesis. However, large schools of gizzard shad (*Dorosoma cepedianum*) that migrated from Lake Huron were present in the Shiawassee River downstream food web in August, and tissue samples were collected for stable isotope analyses. Gizzard shad had a more enriched $\delta^{13}C$ signature than any other species in the food web (Figure 1, Appendix A), indicating that fish migrating from Lake Huron are more enriched in $\delta^{13}C$ than resident individuals. Gizzard shad were a seasonally important component of predator diets (Fullard 2014). The effect that seasonal spawning runs have on the food web

dynamics of the resident fish assemblages in tributaries of the Laurentian Great Lakes could be examined with stable isotopes and deserves further attention.

Stable isotope samples used in this study were collected during the end of the summer in order to reflect the resources assimilated by fish species over the summer months (primary growth period). This is likely the most optimal foraging period in these temperate rivers (Schlosser 1991; Matthews 1998), which may be reflected in the trophic structure of these fish communities. It is possible that the trophic structure may change during the winter months when resources may be less available. Further research that encompasses the seasonal and temporal changes these temperate rivers experience is needed in order to test if food web structure of these fluvial fish communities changes throughout the year.

LITERATURE CITED

Bunn, S. E., P.M. Davies, and M.Winning. 2003. Sources of organic carbon supporting the food web of an arid zone floodplain river. Freshwater Biology 48(4): 619-635.

Burress, E. D., A. Duarte, M. M. Gangloff, and L. Siefferman. 2012. Isotopic trophic guild structure of a diverse subtropical South American fish community. Ecology of Freshwater Fish 22(1):66-72.

Cabana, G., and J. B. Rasmussen. 1994. Modelling food chain structure and contaminant bioaccumulation using stable nitrogen isotopes. Nature 372(6503):255-257.

Cabana, G., and J. B. Rasmussen. 1996. Comparison of aquatic food chains using nitrogen isotopes. Proceedings of the National Academy of Sciences 93(20):10844-10847.

Cattin, M.-F., L.-F. Bersier, C. Banašek-Richter, R. Baltensperger, and J.-P. Gabriel. 2004. Phylogenetic constraints and adaptation explain food-web structure. Nature 427(6977):835-839.

Doi, H. 2009. Spatial patterns of autochthonous and allochthonous resources in aquatic food webs. Population Ecology 51(1):57-64.

DeNiro, M. J., and S. Epstein. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochimica et cosmochimica acta 42(5):495-506.

Finlay, J. C. 2001. Stable-carbon-isotope ratios of river biota: implications for energy flow in lotic food webs. Ecology 82(4): 1052-1064.

Finlay, J. C. 2004. Patterns and controls of lotic algal stable carbon isotope ratios. Limnology and Oceanography 49(3): 850-861.

Fisher, S., M. Brown, and D. Willis. 2001. Temporal food web variability in an upper Missouri River backwater: energy origination points and transfer mechanisms. Ecology of Freshwater Fish 10(3):154-167.

Franssen, N. R., and K. B. Gido. 2006. Use of stable isotopes to test literature-based trophic classifications of small-bodied stream fishes. The American Midland Naturalist 156(1):1-10.

Frimpong, E. A., and P. L. Angermeier. 2010. Trait-based approaches in the analysis of stream fish communities. American Fisheries Society Symposium 73:109-136.

Fry, B., and C. Arnold. 1982. Rapid 13C/12C turnover during growth of brown shrimp (Penaeus aztecus). Oecologia 54(2): 200-204

Garrison, L. P., and J. S. Link. 2000. Dietary guild structure of the fish community in the Northeast United States continental shelf ecosystem. Marine Ecology Progress Series 202:231-240.

Gelwick, F. P., M. S. Stock, and W. J. Matthews. 1997. Effects of fish, water depth, and predation risk on patch dynamics in a north-temperate river ecosystem. Oikos:382-398.

Gido, K., and N. Franssen. 2007. Invasion of stream fishes into low trophic positions. Ecology of Freshwater Fish 16(3):457-464.

Grey, J. 2001. Ontogeny and dietary specialization in brown trout (Salmo trutta L.) from Loch Ness, Scotland, examined using stable isotopes of carbon and nitrogen. Ecology of Freshwater Fish 10(3):168-176.

Hesslein, R. H., A. K. Hallard, and P. Ramlal. 1993. Replacement of sulfur, carbon, and nitrogen in tissue of growing broad whitefish (Coregonus nasus) in response to a change in diet traced by δ34S, δ13C, and δ15N. Canadian Journal of Fisheries and Aquatic Sciences 50(10): 2071-2076.

Hoeinghaus, D. J., K. O. Winemiller, and J. S. Birnbaum. 2007. Local and regional determinants of stream fish assemblage structure: inferences based on taxonomic vs. functional groups. Journal of Biogeography 34(2):324-338.

Jackson, D. A., P. R. Peres-Neto, and J. D. Olden. 2001. What controls who is where in freshwater fish communities the roles of biotic, abiotic, and spatial factors. Canadian Journal of Fisheries and Aquatic Sciences 58(1):157-170.

Jennings, S., J. K. Pinnegar, N. V. C. Polunin, and K. J. Warr. 2002. Linking size-based and trophic analyses of benthic community structure. Marine Ecology Progress Series 226:77-85.

Jepsen, D. B., and K. O. Winemiller. 2002. Structure of tropical river food webs revealed by stable isotope ratios. Oikos 96(1):46-55.

Karr, J. R. 1981. Assessment of biotic integrity using fish communities. Fisheries 6(6):21-27.

Karr, J. R., K. D. Fausch, P. L. Angermeier, P. R. Yant, and I. J. Schlosser. 1986. Assessing biological integrity in running waters. A method and its rationale. Illinois Natural History Survey, Champaign, Special Publication 5.

Kling, G. W., B. Fry, and W. J. O'Brien. 1992. Stable isotopes and planktonic trophic structure in arctic lakes. Ecology:561-566.

Kolar, C. S., and D. M. Lodge. 2002. Ecological predictions and risk assessment for alien fishes in North America. Science 298(5596):1233-1236.

Lawton, J. 1989. Food webs.

Layman, C. A., D. A. Arrington, C. G. Montaña, and D. M. Post. 2007. Can stable isotope ratios provide for community-wide measures of trophic structure? Ecology 88(1):42-48.

Lindeman, R. L. 1942. The trophic-dynamic aspect of ecology. Ecology 23(4):399-417.

Lowe-Mac Connell, R. H., and R. Lowe-McConnell. 1987. Ecological studies in tropical fish communities. Cambridge University Press.

Manly, B. F., L.L. McDonald, D.L. Thomas, T.L. McDonald, and W.P. Erickson. 2004. Discriminant function analysis. Pages 171-178 *in* Resource selection by animals: statistical design and analysis for field studies. Springer Netherlands.

Moyle, P. B., and T. Light. 1996. Fish invasions in California: do abiotic factors determine success? Ecology 77(6):1666-1670.

MacArthur, R. 1955. Fluctuations of animal populations and a measure of community stability. Ecology 36(3):533-536.

Matthews, W. J. 1998. Patterns in freshwater fish ecology. Springer.

Mittelbach, G. G., and L. Persson. 1998. The ontogeny of piscivory and its ecological consequences. Canadian Journal of Fisheries and Aquatic Sciences 55(6):1454-1465.

Murry, B. A., J. M. Farrell, M. A. Teece, and P. M. Smyntek. 2006. Effect of lipid extraction on the interpretation of fish community trophic relationships determined by stable carbon and nitrogen isotopes. Canadian Journal of Fisheries and Aquatic Sciences 63(10):2167-2172.

Newsome, S. D., C. Martinez del Rio, S. Bearhop, and D. L. Phillips. 2007. A niche for isotopic ecology. Frontiers in Ecology and the Environment 5(8):429-436.

Noble, R., I. Cowx, D. Goffaux, and P. Kestemont. 2007. Assessing the health of European rivers using functional ecological guilds of fish communities: standardizing species classification and approaches to metric selection. Fisheries Management and Ecology 14(6):381-392.

Oksanen, J., R. Kindt, P. Legendre, B. O'Hara, M.H.H. Stevens, and M.J. Oksanen. 2007. The vegan package. Community ecology package. Disponível em: http://www. R-project. org. Acesso em 10(01):2008.

Peters, R. H. 1983. The ecological implications of body size. Cambridge University Press, Cambridge.

Peterson, B. J., and B. Fry. 1987. Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics 18:293-320.

Pimm, S. L., J. H. Lawton, and J. E. Cohen. 1991. Food web patterns and their consequences. Nature 350(6320):669-674.

Poff, N. L., and J. D. Allan. 1995. Functional organization of stream fish assemblages in relation to hydrological variability. Ecology 76(2):606-627.

Post, D. M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83(3):703-718.

Post, D. M., and C.A Layman, D.A. Arrington, G. Takimoto, J. Quattrochi, and C.G. Montana. 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia 152(1):179-189.

Power, M. E. 1992. Top-down and bottom-up forces in food webs: do plants have primacy. Ecology 73(3):733-746.

Power, M. E., and W. E. Dietrich. 2002. Food webs in river networks. Ecological Research 17(4):451-471.

Ripley, B., K. Hornik, A. Gebhardt, and D. Firth. 2011. MASS: support functions and datasets for Venables and Ripley's MASS. R package version7.3-16.

Sakano, H., E. Fujiwara, S.,Nohara, and H. Ueda. 2005. Estimation of nitrogen stable isotope turnover rate of Oncorhynchus nerka. Environmental Biology of Fishes 72(1): 13-18.

Schlosser, I. J. 1991. Stream fish ecology: a landscape perspective. BioScience: 704-712.

Taylor, C. M., and M. L. Warren Jr. 2001. Dynamics in species composition of stream fish assemblages: environmental variability and nested subsets. Ecology 82(8):2320-2330.

Vander Zanden, M. J., Chandra, S., Allen, B. C., Reuter, J. E., & Goldman, C. R. (2003). Historical food web structure and restoration of native aquatic communities in the Lake Tahoe (California–Nevada) basin. Ecosystems 6(3): 274-288.

Vander Zanden, M. J., and J. B. Rasmussen. 2001. Variation in δ15N and δ13C trophic fractionation: implications for aquatic food web studies. Limnology and Oceanography 46(8):2061-2066.

Vander Zanden, M. J., M. Hulshof, M. S. Ridgway, and J. B. Rasmussen. 1998. Application of stable isotope techniques to trophic studies of age-0 smallmouth bass. Transactions of the American Fisheries Society 127(5):729-739.

Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell, and C. E. Cushing. 1980. The

river continuum concept. Canadian Journal of Fisheries and Aquatic Sciences 37(1):130-137.

Winemiller, K. O. 1990. Spatial and temporal variation in tropical fish trophic networks. Ecological monographs 60(3):331-367.

Winemiller, K. O. 1995. The structural and functional aspects of fish diversity. Bulletin Francais de la Peche et de la Pisciculture 68.

Xu J., P. Xie, M. Zhang, H. Yang. 2005. Variation in stable isotope signatures of seston and a zooplanktivorous fish in a eutrophic Chinese lake. Hydrobiologia 541:215–220

Xu, J., M. Zhang, and P. Xie. 2011. Sympatric variability of isotopic baselines influences modeling of fish trophic patterns. Limnology 12(2): 107-115.

Zambrano, L., M. R. Perrow, C. D. Sayer, M. L. Tomlinson, and T. A. Davidson. 2006. Relationships between fish feeding guild and trophic structure in English lowland shallow lakes subject to anthropogenic influence: implications for lake restoration. Aquatic Ecology 40(3):391-405.

Zhou, Q., P. Xie, J. Xu, X. Liang, J. Qin, T. Cao, and F. Chen. 2011. Seasonal trophic shift of littoral consumers in eutrophic Lake Taihu (China) revealed by a two-Source mixing model. The Scientific World Journal 11: 1442-1454.

Zohary, T., J. Erez, M. Gophen, I. Berman-Frank, and M. Stiller. 1994. Seasonality of stable carbon isotopes within the pelagic food web of Lake Kinneret. Limnology and Oceanography 39(5):1030-1043.

Zuur, A. F., E. N. Ieno, and G.M. Smith. 2007. Analysing ecological data. Springer, New York.

Appendices

APPENDIX A

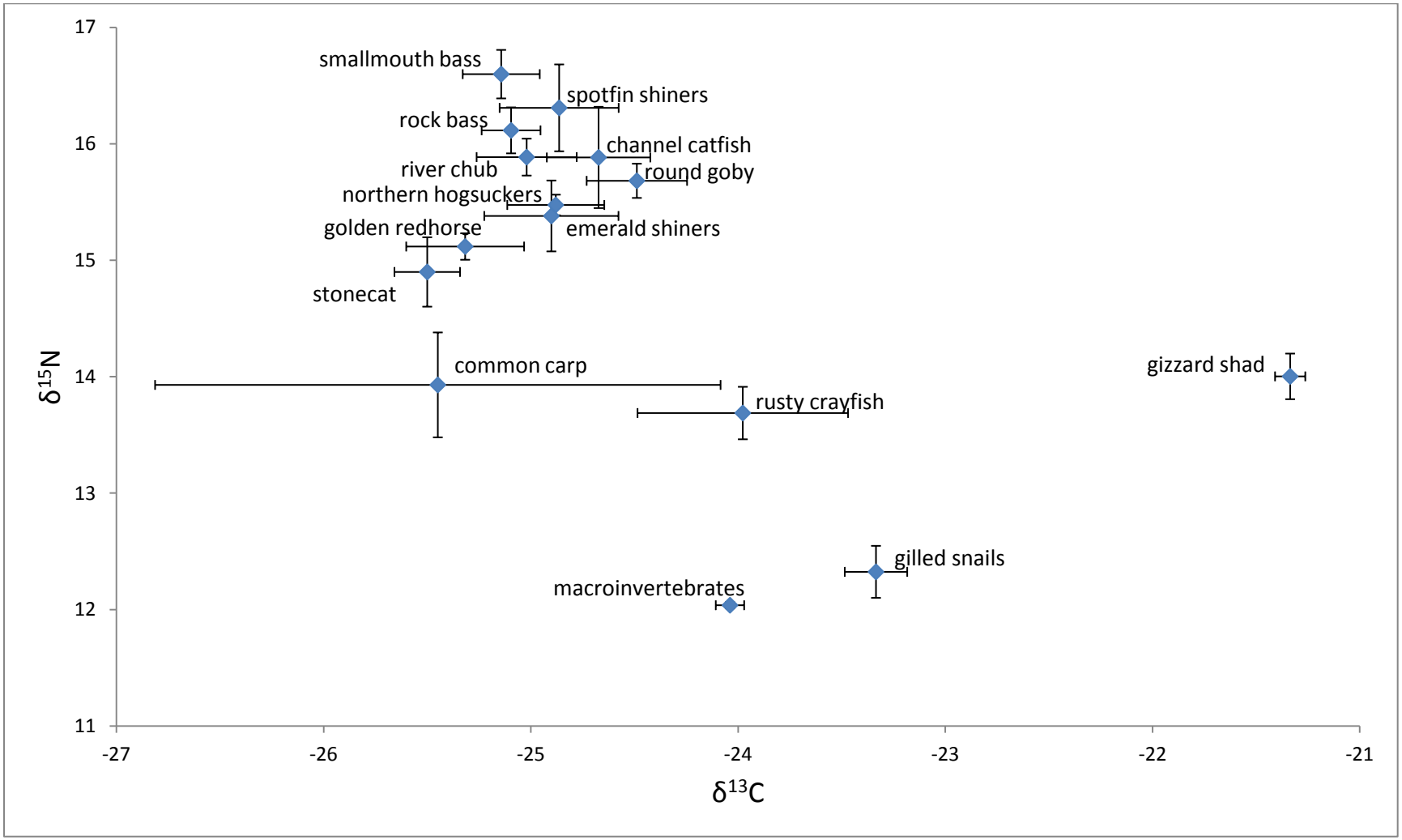

Figure 1. Stable isotope bi-plot of $\delta^{13}C$ and $\delta^{15}N$ for the Shiawassee River downstream food web. Each diamond is the mean position of that species in isotopic space. Error bars represent the standard error of each species.

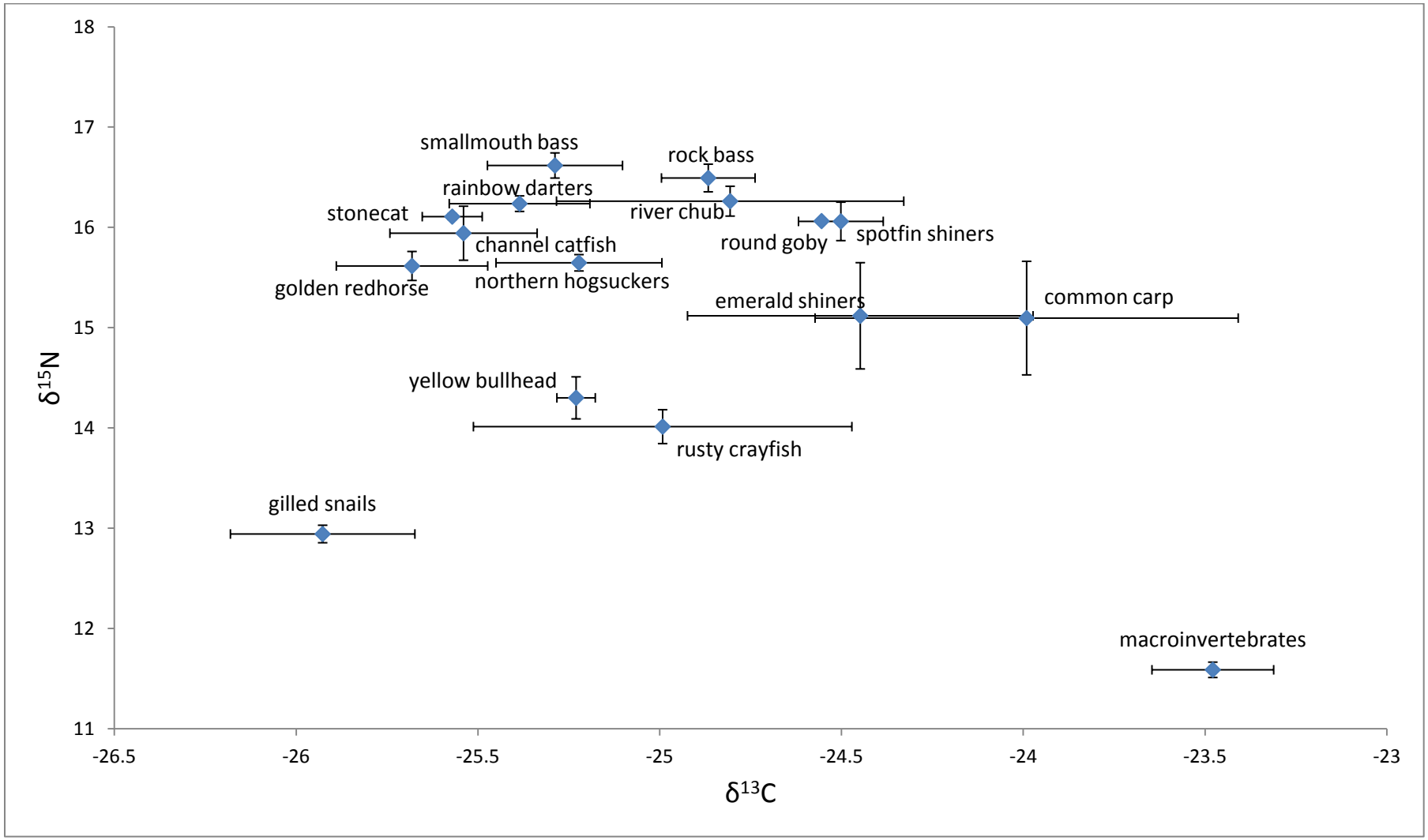

Figure 2. Stable isotope bi-plot of $\delta^{13}C$ and $\delta^{15}N$ for the Shiawassee River upstream food web. Each diamond is the mean position of that species in isotopic space. Error bars represent the standard error for each species.

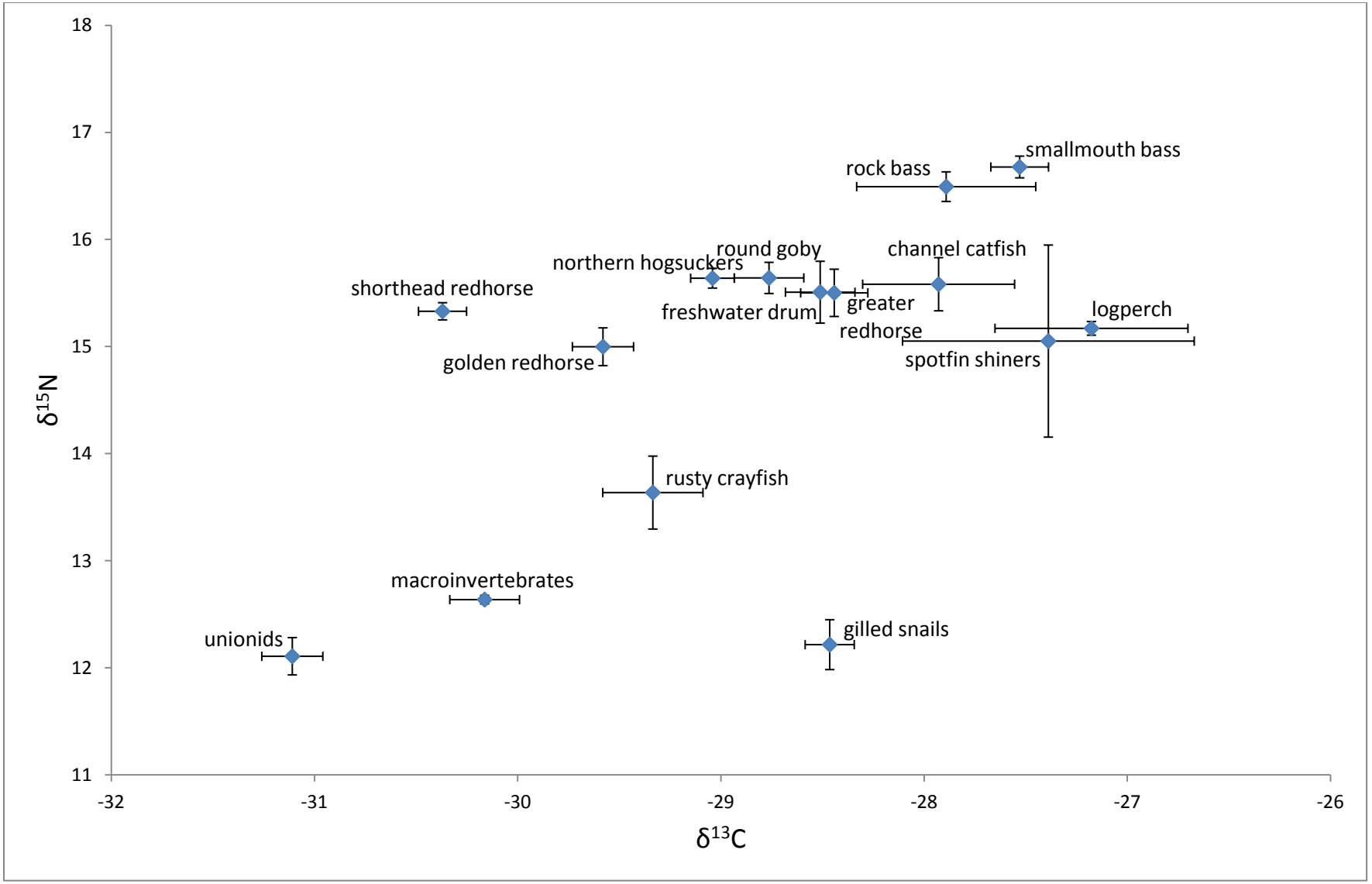

Figure 3. Stable isotope bi-plot of $\delta^{13}C$ and $\delta^{15}N$ for the Cass River downstream food web. Each diamond is the mean position of that species in isotopic space. Error bars represent the standard error for each species.

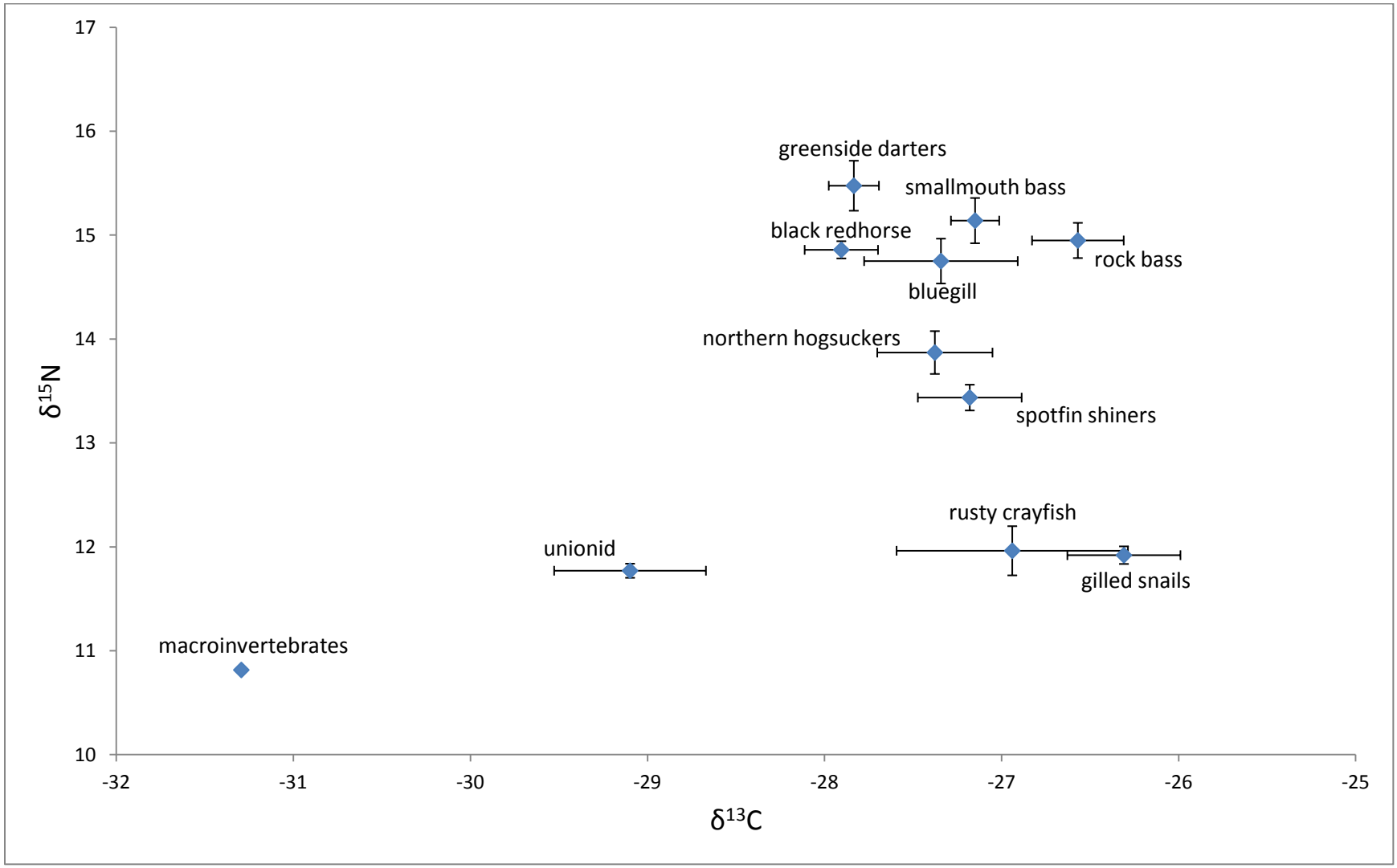

Figure 4. Stable isotope bi-plot of $\delta^{13}C$ and $\delta^{15}N$ for the Cass River upstream food web. Each diamond is the mean position of that species in isotopic space. Error bars represent the standard error for each species.

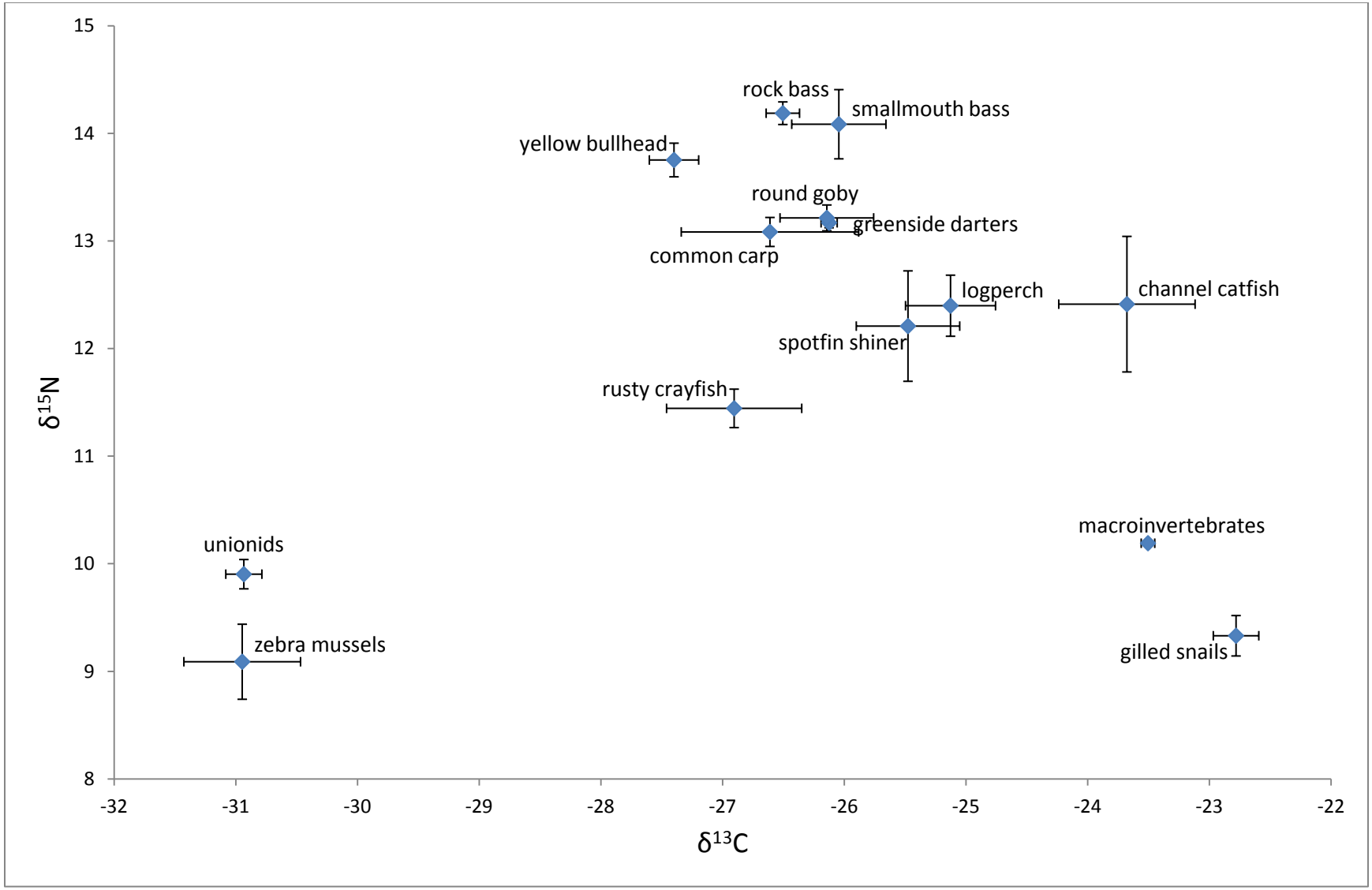

Figure 4. Stable isotope bi-plot of $\delta^{13}C$ and $\delta^{15}N$ for the Flint River food web. Each diamond represents the mean position of that species in isotopic space. Error bars represent the standard error for each species.