DIET AND ENERGY PATHWAY PERTURBATIONS OF ROCK BASS AND SMALLMOUTH BASS IN THREE ROUND GOBY INVADED GREAT LAKES TRIBUTARIES

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This is dedicated to a

revengeful catfish

who resides in the Cass River

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ABSTRACT

DIET AND ENERGY PATHWAY PERTURBATIONS OF ROCK BASS AND SMALLMOUTH BASS IN THREE ROUND GOBY INVADED GREAT LAKES TRIBUTARIES

By Clarence Fullard

The Round Goby (*Neogobius melanostomus*) is a small (<150mm) benthic invasive fish now common to the Laurentian Great Lakes region. Although well studied in lakes, less research has investigated how the secondary invasion of the Round Goby into Great Lakes tributaries is changing riverine food webs. Previous studies have found elevated predation of Round Goby by Smallmouth Bass (*Micropterus dolomieu*) in many areas of the Great Lakes where Round Goby are common. This study used stable isotope and stomach content analyses of two lotic predators, Smallmouth Bass and Rock Bass (*Ambloplites rupestris*) to determine the impacts of Round Goby on native stream food webs in three Michigan rivers. Stable isotope ratios of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) provided a time-integrated approach to estimating the trophic diversity of aquatic ecosystems, and combined with high resolution gut content data, accurate depictions of consumer feeding behaviors is possible. I found that Round Goby abundance was positively related to consumption of Round Goby by both Smallmouth Bass and Rock Bass. Smallmouth Bass and Rock Bass trophic position was positively related to Round Goby abundance. Round Goby have become an important part of the diet and energy pathways leading to Smallmouth Bass and Rock Bass in Saginaw River tributaries. Understanding the ecological impacts of invasive species such as the Round Goby to native food web functioning is fundamental to the management of our aquatic resources, as changes to the trophic structure of a food web can have cascading and unanticipated effects elsewhere in the ecosystem.

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INTRODUCTION

Aquatic habitat and biodiversity throughout the Laurentian Great Lakes and their tributaries have suffered during the last two centuries from the cumulative effects of habitat degradation and non-native species invasions (International Joint Commission 1987, Mills et al. 1993, Charlebois et al. 2001, Fielder 2002). Within the state of Michigan, which borders four of the five Great Lakes, over 2,500 impoundments exist; each of which alters and degrades aquatic habitat for native biota (American Society of Civil Engineers Michigan Section ASCEMS, 2009). Flowing through the lower peninsula of Michigan, the Saginaw River and its tributaries are major inputs to Saginaw Bay, Lake Huron (Figure 1). Impoundments block upstream migration of fish in 72% of the Saginaw Bay tributaries and negatively affect upstream species richness and abundance by fragmenting habitat and limiting connectivity (Fielder and Baker 2004, Guenther and Spacie 2006, McLaughlin et al. 2006, Stoller 2013). In addition to habitat degradation, the Great Lakes have suffered perturbations to their aquatic food webs from non-native species invasions (Mills et al. 1993, Munawar et al. 2005). Since the 1800’s, 136 non-native organisms have been established in the Great Lakes through intentional and accidental introductions, including at least 24 non-native fish species (Mills et al. 1993). The Round Goby (*Neogobius melanostomus*) is one such species that has invaded the Great Lakes ecosystem and has caused serious ecological harm (Steinhart et al. 2004a, Kornis et al. 2012). The combined effects of non-native species invasions and habitat fragmentation have resulted in the decline of many recreational and commercial fisheries, as well as many ecologically important non-game native fish populations (French and Jude 2001, Fielder 2002, Fielder and Baker 2004). Though we have a strong understanding of how fragmentation and invasive organisms have affected individual species and assemblages (French and Jude 2001, Steinhart et al. 2004b, Burroughs et al. 2010), we know relatively little about how these perturbations influence food web relationships and what the consequences of such impacts are, especially in small tributaries to the Great Lakes (Campbell and Tiegs 2012).

The Round Goby is a small (<150mm) freshwater benthic fish native to the Black and Caspian Seas (Kornis et al. 2012). Since their introduction into the St. Clair River in 1990, they have spread throughout each of the Great Lakes and many of their tributaries, including several within the Saginaw River watershed (Bronnenhuber et al. 2001, Phillips et al. 2003, Kornis and Vander Zanden 2010, Lynch and Mensinger 2012, CMU unpublished data). Round Goby are present in varying densities within the Flint, Cass, and Shiawassee Rivers, all of which are tributaries to Saginaw Bay (Jude et al. 2010, CMU unpublished data). They consume a variety of abundant food sources, including dreissenid mussels, benthic invertebrates, fish eggs, and small fishes (Ray and Corkum 1997, French and Jude 2001, Steinhart et al. 2004a, Ng 2008, Brush et al. 2012, CMU unpublished data). In addition to having abundant food sources, Round Goby spawn prolifically, reproducing up to six times per year (Charlebois 2001, Corkum et al. 2004). Because of their exceptional fecundity and competitiveness for resources, Round Goby have successfully exploited much of the habitat in areas they occur to the demise of native aquatic food webs (Bronnenhuber et al. 2011, Hall 2011). French and Jude (2001) found that Round Goby have caused declines of many native benthic fishes, including sculpins (*Cottus* spp.) and darters (*Etheostoma* spp., *Percina* spp.) through competition and exclusion. High densities of Round Goby impact not only fish communities, but also benthic macroinvertebrates and native unionid mussels through predation and host fish displacement, respectively (Phillips et al. 2003, Barton et al. 2005, Poos et al. 2010). Despite these negative impacts, the presence of Round Goby has provided some native piscivores, such as Smallmouth Bass (*Micropterus dolomieu*) and Rock Bass (*Ambloplites rupestris),* with an abundant prey source (Ng 2008, Kornis et al. 2012).

Trophic interactions and energy pathways between Smallmouth Bass, Rock Bass, Round Goby, and other prey are not well understood. Adult Smallmouth Bass diets typically consist of fish, insects, and crayfish (Cambaridae), although their exact dietary habits depend on location and prey availability (Dauwalter and Fisher 2008). Smallmouth Bass prey heavily on Round Goby in lakes and near-shore areas where they coexist (Ng 2008, Kwon et al. 2009), to the extent that the Round Goby invasion has enabled Smallmouth Bass in Lake Erie to switch to piscivory at smaller sizes because of the increased availability of prey (Steinhart et al. 2004b). Rock Bass have similar prey preferences as Smallmouth Bass and are present in high numbers within Saginaw Bay tributaries; however, less is known about their diets or their use of Round Goby as prey (Probst et al. 1984, Paterson et al. 2006, Kornis et al. 2012). Round Goby have similar dietary habits as native benthic prey fish, but they are also known to prey on eggs and smaller fish, including other Round Goby (French and Jude 2001). The effects of the Round Goby invasion to top predators has been studied in lakes and near-shore areas (Steinhart et al. 2004a, 2004b, Ng 2008, Kwon et al. 2009), but little research has specifically addressed the impacts to riverine top predators following the secondary invasion of the Round Goby (Phillips et al. 2003, Kornis and Vander Zanden 2010, Poos et al. 2010, Campbell and Tiegs 2012). Understanding the dietary habits of the two most abundant predators is crucial to understanding the food web structure of mid-order rivers such as these Saginaw River tributaries.

The use of stable isotope analysis to detect the impacts of invasive organisms to native ecosystems is emerging as one the most temporally integrative and cost effective methods of trophic food web analysis (Cucherousset et al. 2012). Vander Zanden et al. (1999) examined the effects of non-native piscivorous fish on lentic food webs using stable isotope analysis and determined that changes in the food web structure resulting from the introduction of non-native piscivores caused a decrease in the food chain length of the native top predators. Extending this concept, this study uses traditional diet examination (Kamler and Pope 2001, Hakala and Johnson 2004, Kapuscinski et al. 2012), assemblage surveys, and stable isotope analysis (Post 2002) to explore the changes in the energy pathways leading to Smallmouth Bass and Rock Bass in three Saginaw River tributaries resulting from an invasive species perturbation.

Combined stable isotope and stomach content analysis provides the best view of the food web structure in an aquatic ecosystem (Post 2002). Stomach content analysis enables direct observation and quantification of seasonal changes in dietary habits (French and Jude 2011, Kapuscinski et al. 2012) by providing conclusive predator-prey linkages and insights into foraging habits (Paterson and Drouillard 2006), though it reveals only coarse temporal scale dietary information and occasional omission of important diet items can occur (Carreon-Martinez et al. 2011). Stable isotopes of carbon and nitrogen are increasingly used in aquatic ecology to determine time integrated relationships between species. Trophic positions within food webs can be calculated based on the typical 3.4‰ enrichment of the nitrogen stable isotope ratio ($\delta^{15}N$) between food chain links (Hansson et al. 1997, Vander Zanden et al. 1999, Post et al. 2000, Finlay 2001, Vander Zanden and Rasmussen 2001, Post 2002, Grey 2006, Roth et al. 2006, Cucherousset et al. 2012); however, it is limited to identifying relative measures of food web connectivity and does not reveal direct food web links (Fry 1991, Anderson and Cabana 2007, Layman et al. 2007). Carbon stable isotope ratios ($\delta^{13}C$) reflect the origin of various food sources within food webs, which can vary within aquatic systems from pelagic, littoral, and terrestrial (Post 2002). Using these two stable isotope signatures, consumers and sources (i.e., prey) can be plotted in two-dimensional isotope space which describe a consumer's isotopic niche (Jackson et al. 2011). Advantages to coupling stable isotope and stomach content analysis include the ability to observe long-term trends in dietary habits, identify sources of productivity, and to provide highly descriptive accounts of seasonal changes in feeding habits and produce direct food web linkages (Post 2002, Jardine et al. 2005, Paterson et al. 2006). This study uses stable isotopes and diet data to detect and characterize the changes in the Smallmouth Bass and Rock Bass food chains within Round Goby invaded streams by comparing them with an un-invaded, fragmented stretch of river.

Round Goby are found throughout four of the five sites in this study, though in varying abundances. They are not present upstream of the Frankenmuth Dam on the Cass River (CMU unpublished data). This variation in distribution is a result of habitat fragmentation and angler-aided introduction (Michigan Department of Natural Resources, personal communication), and presents a prime opportunity to study the effects of the invasion's impact to the riverine food web through a gradient of Round Goby abundances. The overall goal of this study was to evaluate the impacts of the Round Goby invasion on Smallmouth Bass and Rock Bass diets. I hypothesized that each of these top predators would increase their consumption of Round Goby with increasing Round Goby abundances. Furthermore, I predicted that if Round Goby predation increased with increasing Round Goby abundances, consumption would be reflected in predator trophic positions (i.e., higher abundances of Round Goby = higher Round Goby predation = higher predator trophic position). This increase in predator trophic position may be attributed to the increase in piscivory and decrease in consumption of crayfish as well as the elevated nitrogen isotope ratio ($\delta^{15}N$) of Round Goby. By sampling each river stretch throughout the summer 2012 using fish assemblage surveys, stomach contents, and stable isotope analysis, I investigated the following: (1) How and to what extent do Smallmouth Bass and Rock Bass use Round Goby as prey in each system, and how does that predation vary throughout the growing season? (2) Do feeding patterns (generalist versus specialist) and diet overlap differ with varying abundances of Round Goby? (3) Does Round Goby presence in Smallmouth Bass and Rock Bass diets correlate with Round Goby relative abundance? (5) Does the trophic position of Smallmouth Bass and Rock Bass differ among sites with varying densities of Round Goby? This insight into the Smallmouth Bass and Rock Bass food webs can lead to a better understanding of the complexities that exist within aquatic ecosystems, and can help direct management objectives that intend to maintain or improve recreational fisheries and preserve the health and integrity of native fish biodiversity (Rasmussen et al. 1990, Vander Zanden and Rasmussen 1996).

METHODS

Study Area

This project examined three tributaries to the Saginaw River (Figure 1): the Cass River, Shiawassee River, and Flint River, each of which exist within our zone of study as impounded (low-head dam), partially impounded (rock ramp), and free flowing, respectively. The Chesaning rock ramp on the Shiawassee River is 72km upstream of the mouth of the Saginaw River and is approximately 123m long and 58m wide with a 3% overall slope. This rock ramp is designed to allow fish migration; however, the success of this structure's ability to allow passage of fish is still being studied (CMU unpublished data). The Frankenmuth dam on the Cass River is 66km upstream of the mouth of the Saginaw River and is approximately 73m wide and 4.5m tall. The impoundments on the Cass and Shiawassee Rivers have resulted in limited upstream fish migration for native and non-native fishes, including the Round Goby. The study site in the Flint River is 104km upstream of the mouth of the Saginaw River and has full connectivity throughout our study area; the first dam on the Flint River is 73km upstream of the focal point of this study. Sites upstream of impoundments on the Cass River and Shiawassee River were located at or above the first upstream riffle of the dam pool to avoid influence from the lentic fish assemblages that exist within them. Sites were located within 8km upstream and downstream of the dam and rock ramp on the Cass River and Shiawassee River, respectively. Flint River sites were located at a similar distance upstream of the Saginaw River as the Cass River and Shiawassee River sites. Habitat conditions were assessed in each river and are presented in Table 1. Pebble counts were done by using methods outlined by Kondolf and Li (1992).

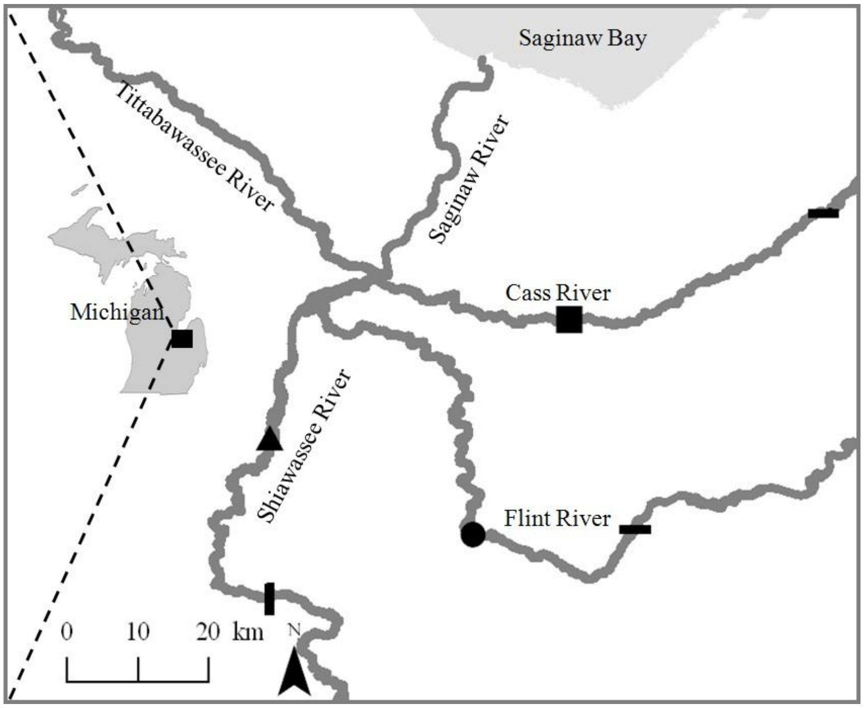

Figure 1. Cass River (dam, square), Shiawassee River (rock ramp, triangle), and Flint River (free-flowing, circle) study sites are shown. Shiawassee River and Cass River study sites are located upstream and downstream of their markers. All rivers flow into the Saginaw River, Michigan, USA. Map courtesy of D. Woolnough.

Table 1. Habitat characteristics of Cass, Shiawassee, and Flint River fish assemblage sites. Mean (±SE) of wetted width, depth, and velocity are shown. Representative substrate type was assessed using a pebble count and was ranked on following scale (Kondolf and Li 1992): 1 = organic, 2 = clay, 3 = silt, 4 = sand, 5 = very fine gravel, 6 = fine gravel, 7= medium gravel, 8 = coarse gravel, 9 = very coarse gravel, 10 = small cobble, 11 = large cobble, 12 = small boulder, 13 = medium boulder. All streams are tributaries to the Saginaw River, Michigan, USA.

River	Position	Mean width (m)	Mean depth (cm)	Mean velocity (m/sec)	Median particle
Cass	Upstream	37.8 (2.3)	38.4 (2.3)	0.06 (0.01)	7
	Downstream	28.2 (1.8)	27.4 (1.2)	0.14 (0.01)	6
Shiawassee	Upstream	32.5 (1.3)	34.2 (1.3)	0.24 (0.01)	8
	Downstream	24.6 (1.9)	42.9 (1.6)	0.25 (0.01)	5
Flint		37.2 (2.7)	42.4 (2.3)	0.22 (0.01)	8

Stream Fish Abundances

To determine relative abundances of each species within each stretch, fish assemblage surveys of each of the five study sites were completed during summer 2012. We used a variable distance sampling scheme to select three ~150m sites above and below barriers on the Shiawassee and Cass Rivers. We also selected three ~150m sites on the Flint River. All surveys were completed using wading based barge electrofishing equipment with pulsed DC current produced by a Smith-Root ® 2.5 gpp and two anode probes (Smith Root, Inc.). We electrofished upstream transects within each site, and 3-5 transects were completed to span the river's entire width. Electrofishing surveys were performed once a month during June, July, and August 2012 at all sites, with the exception of the July sites on the Flint, which were not sampled due to high water levels. All fish encountered during sampling were captured, identified to species, measured [total length (TL), nearest millimeter], and enumerated. Unidentifiable fishes were euthanized in the field by preserving in ethanol and identified in the laboratory. Relative abundances of Smallmouth Bass, Rock Bass, and Round Goby were calculated as fish captured per hour of electrofishing at each site (CPUE; #/hr).

Stomach Content Analysis

Each site was sampled once per month during June, July, and August 2012 for stomach contents. Fish were collected from ~500m stretches within each of the five sites. Sites were selected for their close proximity to assemblage sampling sites, though they were outside of assemblage sites to avoid re-shocking assemblage sites between surveys. Fish were captured by electrofishing for 1500-3000 seconds at each site. After capture, fish were placed in a floating holding pen to await diet sampling, and Round Goby and other small fish were separated from large fish to avoid predation while in captivity. Collections were limited to 50-60 fish >80mm to limit digestion time before stomach sampling. Sampling events began post-dawn (0600-0730) to capture morning feeding habits.

Stomach contents were collected from Smallmouth Bass and Rock Bass >80mm using a non-lethal gastric lavage technique (Kamler and Pope 2001, Kapuscinski et al. 2012). A pressurized garden sprayer with slight nozzle modification that allowed easy insertion into the gut was used to collect samples. Stomachs were flushed with river water and collected in a fine mesh-lined funnel. Stomach contents were flushed into the funnel for 15-30s per fish, depending on body size and stomach fullness (Kapuscinski et al. 2012). Stomachs were massaged to aid in the regurgitation of gut contents during flushing. Once stomachs were empty, fish were released and contents were rinsed from the funnel using 95% ethanol and stored in scintillation vials or glass jars. Validation of this technique was performed on 15 fish by sacrificing and dissecting to determine lavage effectiveness; the technique proved to be 100% effective. Those fish were kept on ice for less than eight hours, stomach contents were removed by dissection, and contents were preserved as detailed above.

Diet items were examined under a dissecting microscope and separated by category, lightly blotted with a paper towel, and weighed to the nearest 0.001g. Specimens of the same category were counted and then weighed together. To aid in the identification of partially or mostly digested fishes, cliethra bones were used to identify fishes to species level (Traynor et al. 2010). Diagnostic body parts were used to identify other prey as needed. All categories for individual fish were quantified as percent composition by weight (Murphy and Willis 1996).

Prey was recorded to species level when possible for fish and to order or higher for benthic invertebrates and terrestrial insects. Crayfish were occasionally identified to species in diets when possible, however rusty crayfish (*Orconectes rusticus*) were the only crayfish present in these rivers based on field observations during summer 2012. Broad prey categories based on taxa >1% in all diets were created to ease presentation of diet for some analyses. High resolution diet data was used to compare diet overlap between predators.

To examine the association between abundance of Round Goby prey and consumption of Round Goby by predators, I used least squares linear regression to compare the proportion (W_i) of Round Goby in predator diets with Round Goby CPUE (#/hr) from each diet and assemblage site during June, July, and August 2012. Because of continuous changes in fish recruitment and migration, data were assumed to be independent. Data were transformed to meet assumptions of normality.

Diet overlap (C_{xy}) between two species can reveal similarities or differences in feeding habits, which can show how two species react to different prey sources. The diet overlap of Smallmouth Bass and Rock Bass was determined at each site during June, July, and August (n=15). Unknown and identifiable categories were removed prior to analysis, as unknown categories were not comprised of the same items and are uninformative when looking at diet overlap. Diet overlap was calculated using the Schoener's index (Schoener 1970, Murphy and Willis 1996):

$$C_{xy} = 1 - 0.5\left(\sum |p_{xi} - p_{yi}|\right)$$

where C_{xy} is the overlap of species x and y, P_{xi} is the proportion of food category i in the diet of species x, and P_{yi} is the proportion of food category i in the diet of species y. Values range from 0 to 1, with 0 indicating no overlap and 1 indicating complete overlap. Past studies have commonly found biologically significant diet overlap when the Schoener's index exceeded 0.60 (Wallace 1981, French and Jude 2001). This index has been used extensively in fisheries research (Wallace 1981, Beck et al. 1998, French and Jude 2001, Frey et al. 2003) and is preferable to other indices because it is capable of using percent composition by weight, which is the most valuable measurement if correlation to prey caloric importance is a priority in the study (Chipps and Garvey 2007). Schoener's index helps examine the differences in feeding patterns between predators; however, it does not reveal any information about competition between species because prey may or may not be limiting for one or both of the predators (Murphy and Willis 1996).

A graphical diet approach was also used to assess feeding strategy (specialized versus generalized) of Smallmouth Bass and Rock Bass (Figure 2). To build graphical diet bi-plots, I used the Amusnden et al. (1996) modification to the Costello (1990) method which plots the prey-specific abundance of each prey taxa with the frequency of occurrence of that same prey in the diet. Plots were created for each site/month combination for each predator (5 sites x 3 months x 2 species = 60 plots total). The prey-specific abundance (P_i) was calculated as

$$P_i = \left(\sum S_i \,/ \sum S_{ti}\right) *\ 100$$

where P_i is the prey-specific abundance of prey taxa i, S_i is the total mass of prey i consumed, and S_{ti} is the total mass of all prey taxa in the stomachs of only those predators that consumed prey taxa i. Frequency of occurrence (O_i) was calculated as

$$O_i = \frac{J_i}{P}$$

where J_i is the number of fish containing prey i and P is the number of fish with food in their stomachs. High between-phenotype and within-phenotype niche width can be assessed, rare and dominant prey items can be identified, and specialized and generalized feeding patterns can be noted. When prey taxa fall in the area of high between-phenotype niche width, seldom eaten prey constitute large proportions of the predator's diet. When prey taxa fall in the area of high within-phenotype niche width, prey are consumed by most predators, though the prey's contribution to total diet mass is low (e.g., grazed upon by many fish but not the major diet source by mass). Opportunistic feeding occurs when prey are found in low frequencies (i.e., the left side of the biplot). Uncommon and unidentifiable benthic invertebrate prey taxa and all terrestrial taxa were combined into single categories (other aquatic benthic invertebrates, Ob; terrestrial, Te).

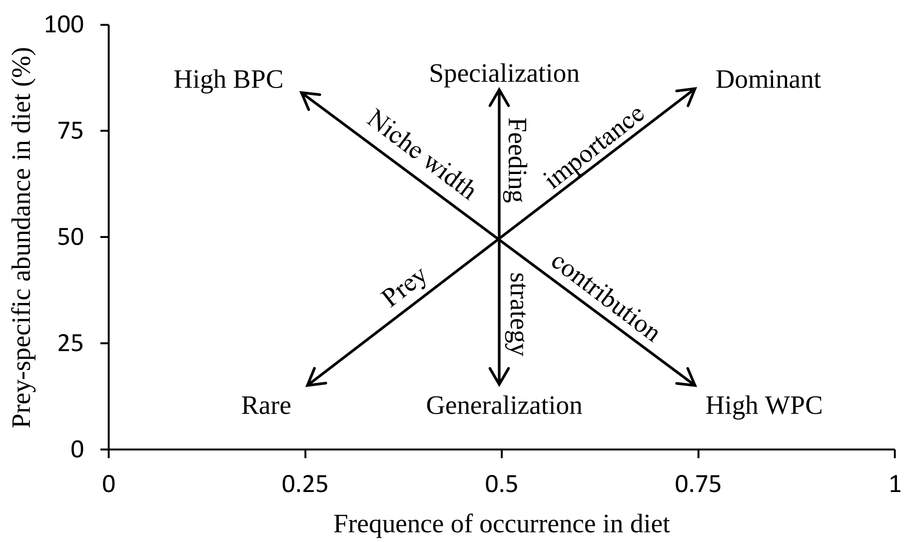

Figure 2. Costello graphical model of feeding strategy, prey importance, and niche variation based on the biplot of frequency of occurrence and prey-specific abundance of different prey types (BPC = between-phenotype component; WPC = within-phenotype component). All fish were captured during 2012 in tributaries to the Saginaw River, Michigan, USA.

Stable Isotope Analysis

Stable isotope analysis for determining food web relationships used $\delta^{15}N$ and $\delta^{13}C$ obtained from consumer and source tissues (Vander Zanden and Rasmussen 1999, Post 2002). Fish samples were collected at each site using the electrofishing methods described above or by hook and line. Smallmouth Bass and Rock Bass were separated into three size classes: 64-128mm (small), 192-256mm (medium), and >384mm (large). Smallmouth Bass occurred in all size classes. Rock Bass occurred only in the small and medium size classes. Round Goby were collected in a single size class (37-112mm). Three to five individuals per species per size class were collected in each stretch. Fish were euthanized upon capture, frozen, and dorsal muscle tissue was later removed through dissection and stored at $0^{0}C$. Rusty crayfish and prey fishes were also collected from each site, frozen, and were analyzed individually using muscle tissue. Baseline food web samples were taken from each site to determine the primary consumer $\delta^{15}N$ signatures (Post 2002). Aquatic gilled snails (*Prosobranchia* spp., long-lived grazers) were selected as the baseline food web organisms and were collected by removing them individually from rocks or vegetation at our fish sampling locations. Fifteen to 30 snails were collected at each site and then frozen. Conglomerate macroinvertebrate samples were also collected from each site using a kick seine.

All fish tissue was freeze-dried, crushed, and portioned into 500-700ug samples. Snails and crayfish specimens were freeze-dried, crushed, and portioned into 700-1000ug samples. Snails were separated into three equal subsamples per site. Each snail’s visceral mass was removed and added to the subsample. The portions were then freeze-dried and packed into tin capsules (4 x 6 mm; Costech Analytical Technologies, Inc.) and placed in individually labeled

96-well trays for carbon and nitrogen isotope analysis at the Great Lakes Institute for Environmental Research (GLIER) at the University of Windsor using their elemental analyzer (Costech) coupled with an isotope ratio mass spectrometer (Thermo Delta V) (EA-IRMS). The stable isotope ratio ($\delta^{15}N$ or $\delta^{13}C$ =[($R^{sample}/R^{standard}$)-1] x1000), where R is the ratio $^{15}N/^{14}N$ or $^{13}C/^{12}C$, is reported for each sample in delta notation (δ). Delta notation represents a ratio of ratios, which is expressed as permil (‰), or parts per thousand difference from the standard. Nitrogen results were standardized against atmospheric N_2 and carbon against CO_2 in PeeDee Belemnite limestone. Delta values (δ) are strongly related to % heavy isotope, so higher delta values (or less negative values) represent higher enrichment of the heavy isotope in a sample (Fry 2006). $\delta^{15}N$ values can be converted into estimates of trophic position by interpreting the $\delta^{15}N$ of consumers relative to baseline $\delta^{15}N$ values, which is based on the assumed 3.4‰ (SD = 1‰) fractionation between tropic levels in aquatic systems (Cabana and Rasmussen 1996, Post et al. 2000, Post 2002). Consumer trophic position was calculated using the formula:

$$\text{trophic position}_{consumer} = ((\delta^{15}N_{consumer} - \delta^{15}N_{baseline})/3.4) + 2$$

where the +2 represents the trophic position of the primary consumer (i.e., trophic position 2; Vander Zanden et al. 1999). The mean trophic fractionation of $\delta^{13}C$ was assumed to be 0.4‰ (SD = 1.3‰; Post 2002).

Since lipids are ^{13}C depleted relative to protein in muscle tissue ($\Delta\delta^{13}C$), it is necessary to normalize for lipids in situations where C:N ratios in aquatic organisms are > 3.5 (Murry et al. 2006, Post et al. 2007). Lipids can be chemically removed prior to sample analysis or mathematical normalization can be used to simplify sample processing as well as preserve the integrity of the sample for ^{15}N processing (Murry et al. 2006, Sweeting et al. 2006). C:N ratios are a good predictor of lipid content in animals, though not in plants (Post et al. 2007). Since 34% of the isotope samples used in this study had a C:N ratio of > 3.5, thus potentially having high lipid contents and high inter-sample variability, mathematical normalization was used for all samples (outlined in Post et al. 2007), where:

$$\delta^{13}\mathrm{C}_{\mathrm{normalized}} = \delta^{13}\mathrm{C}_{\mathrm{untreated}} + \Delta\delta^{13}\mathrm{C}$$

$$\delta^{13}\mathrm{C}_{\mathrm{normalized}} = \delta^{13}\mathrm{C}_{\mathrm{untreated}} - 3.32 + 0.99 \times \mathrm{C{:}\,N}.$$

$\delta^{13}C_{normalized}$ was used for subsequent analysis and will be referred to simply as $\delta^{13}C$.

$\delta^{13}C$ and $\delta^{15}N$ signatures from consumers and sources were used to create bi-plots of isotopic niche space (Figure 3). These bi-plots were used to infer feeding relationships between predators and prey within each system. To delineate the niche width of consumers, carbon and nitrogen isotope signatures of each individual within each system were used to calculate both convex hull areas (TA; Laymen et al. 2007) and standard ellipse areas (SEA; Jackson et al. 2011). Figure 3 shows a typical bi-plot with ellipses and convex hulls delineated. Convex hulls were used only to graphically examine the extent of the consumer-based polygon and the relationship with the standard ellipse.

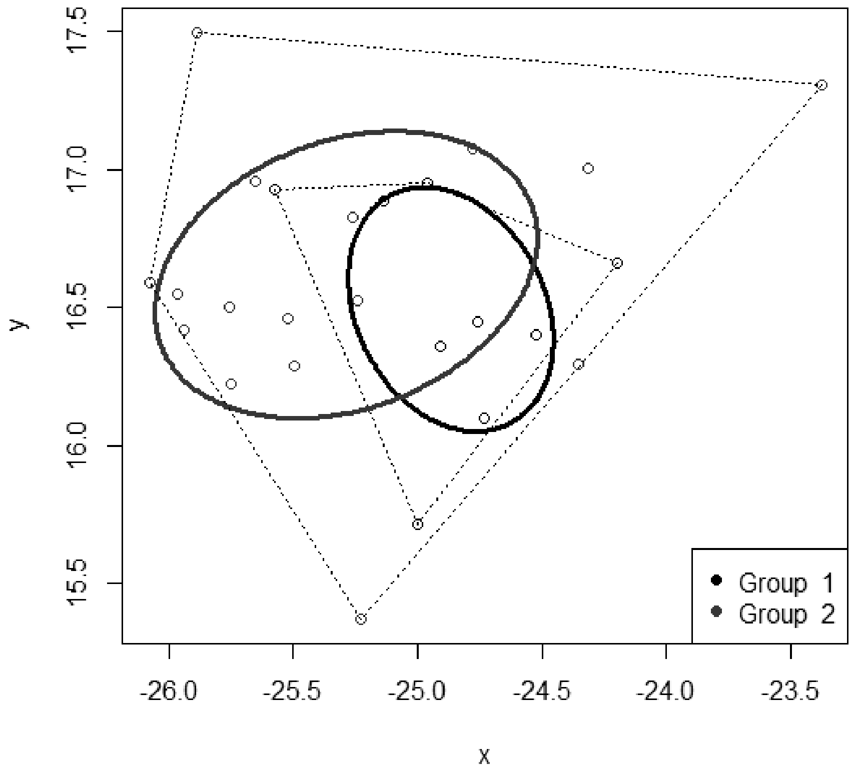

Figure 3. Example of convex hulls and standard ellipses for two groups within a bi-lot. The area of overlap can be calculated for either convex hulls or standard ellipses and represents the niche space overlap between two consumers. Convex hulls encompass 100% of the stable isotope data, whereas standard ellipses were calculated using 40% of the stable isotope data.

Convex hulls were created by connecting individuals within the isotope space and constructing polygons, and the area within that polygon was called niche space. This method is prone to sample size bias since the consumers with larger sample sizes tend to have larger convex hull areas (Podani 2009). The use of standard ellipses as a measure of niche width using the SIBER function in SIAR (Parnell et al. 2010, Parnell and Jackson 2011) uses a small sample size correction and is insensitive to sample size bias. Standard ellipses containing 40% of the isotope data were created using a Bayesian approach which returned estimates of ellipse area and the uncertainty associated with sampling bias, which was used to quantitatively compare different ellipse areas (Jackson et al. 2011). Proportion of total available niche space (delinated by standard ellipses) in overlap for both species was calculated as:

$$\text{Proportion overlap} = \frac{\text{area of overlap} * 2}{\text{Rock Bass SEA} + \text{Smallmouth Bass SEA}}$$

and proportion of predator X’s niche space area in overlap with predator Y was calculated as:

$$\text{Proportion of predator X SEA in overlap} = \frac{\text{area of overlap}}{\text{predator X SEA}}$$

RESULTS

Assemblage

Sampling efforts during the summer growing season of 2012 in the Cass, Shiawassee, and Flint Rivers yielded a total 50,393 fish comprised of 60 different species. Three exotic aquarium trade fishes (one *Hypostomus plecostomus* and two Cichlids) were found in the Shiawassee River but were removed before data summary and analysis. The top six most abundant species found across rivers were Rock Bass (14.1%), Mimic Shiners (*Notropis volucellus*) (11.2%), Emerald Shiners (*Notropis atherinoides*) (10.8%), Spotfin Shiners (*Cyprinella spiloptera*) (9.6%), Round Goby (7.4%), and Smallmouth Bass (6.7%). Species richness varied by site location, with the highest richness found in the Cass River downstream site and lowest found in the Shiawassee River upstream site (Table 2).

Table 2. Number of species captured upstream and downstream of impoundments and for all sites combined during summer 2012. All fish were sampled in tributaries to the Saginaw River, Michigan, USA.

	Upstream	Downstream	River Total
Cass	43	51	54
Shiawassee	36	40	44
Flint	-	-	38

Rock Bass were most abundant in the Cass River upstream site, followed by the Shiawassee River upstream, Flint River, Shiawassee River downstream, and Cass River downstream sites, respectively (Table 3). Smallmouth Bass abundances were highest in the Shiawassee River upstream and Flint River. Round Goby were most abundant in the Cass River downstream, followed by the Flint River, Shiawassee River downstream, and Shiawassee River upstream sites, respectively (Table 3). Round Goby were absent in the Cass River upstream stretch.

Table 3. Summary of mean (±SE) Round Goby, Rock Bass, and Smallmouth Bass CPUE (#/hr). July assemblage surveys were not performed (marked as x) on the Flint due to poor weather. All fish were sampled in tributaries to the Saginaw River, Michigan, USA.

		CPUE (#/hr) per river				
Species	Month	Cass downstream	Cass upstream	Shiawassee downstream	Shiawassee upstream	Flint
Round Goby	June	199 (69)	0	20 (9)	4 (2)	99 (12)
	July	192 (117)	0	19 (9)	1 (.7)	x
	August	145 (28)	0	19 (7)	1 (.1)	119 (45)
	Mean	177 (34)	0	19 (4)	2 (1)	109 (21)
Rock Bass	June	58 (17)	217 (51)	76 (12)	139 (20)	98 (12)
	July	65 (4)	181 (8)	58 (2)	100 (31)	x
	August	74 (16)	205 (79)	71 (13)	158 (43)	151 (54)
	Mean	65 (8)	203 (31)	68 (6)	132 (18)	125 (31)
Smallmouth Bass	June	20 (6)	11 (1)	5 (1)	23 (8)	34 (18)
	July	34 (10)	55 (42)	70 (59)	149 (39)	x
	August	48 (20)	49 (22)	37 (14)	77 (31)	124 (32)
	Mean	34 (8)	36 (13)	37 (20)	83 (23)	79 (26)

Diet

During summer 2012, 367 Smallmouth Bass and 486 Rock Bass stomachs were examined, and 21% (n=77) and 6% (n=31) were empty, respectively. Complete diet information is presented in Appendix tables A1-A2. A positive relationship existed between percent Smallmouth Bass empty stomachs and Round Goby CPUE (r^2=0.49, P=0.02; Figure 4). Percent empty Rock Bass stomachs was not related to Round Goby CPUE (r^2=0.06, P=0.48). Smallmouth Bass sampled for diets ranged in length from 80-472mm (n=367; mean=230mm, SE=3.9). Piscivory by Smallmouth Bass was first observed at 85mm (second smallest Smallmouth Bass sampled, smallest = 80mm), though only fish above 130mm were observed with Round Goby in their stomachs. Rock Bass sampled for diets ranged in length from 53-242mm (n=486; mean=145mm, SE=1.8). Piscivory by Rock Bass on Round Goby was first observed at 87mm. Within all rivers and months combined, 36 Rock Bass and 53 Smallmouth Bass stomachs contained Round Goby as prey. Round Goby predation is summarized in Figure 5. No relationship was found between Smallmouth Bass or Rock Bass total length and percent Round Goby in diet.

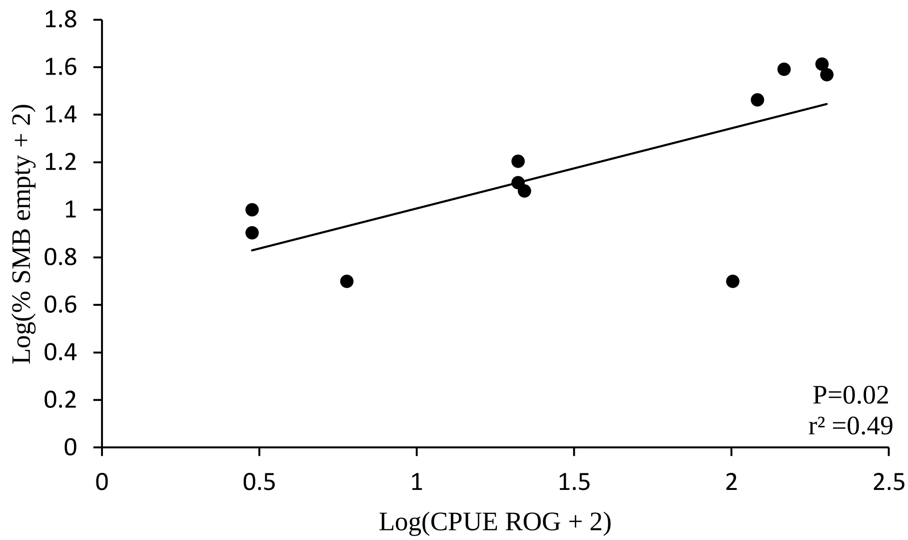

Figure 4. Relationship between percent empty Smallmouth Bass (SMB) empty stomachs and Round Goby (ROG) catch per unit effort (CPUE; #/hr). Data are transformed to satisfy assumptions of normality. One data point from the Cass River upstream site was used. Flint River July data is missing due to flooding events that prevented assemblage surveys. All fish were sampled in tributaries to the Saginaw River, Michigan, USA.

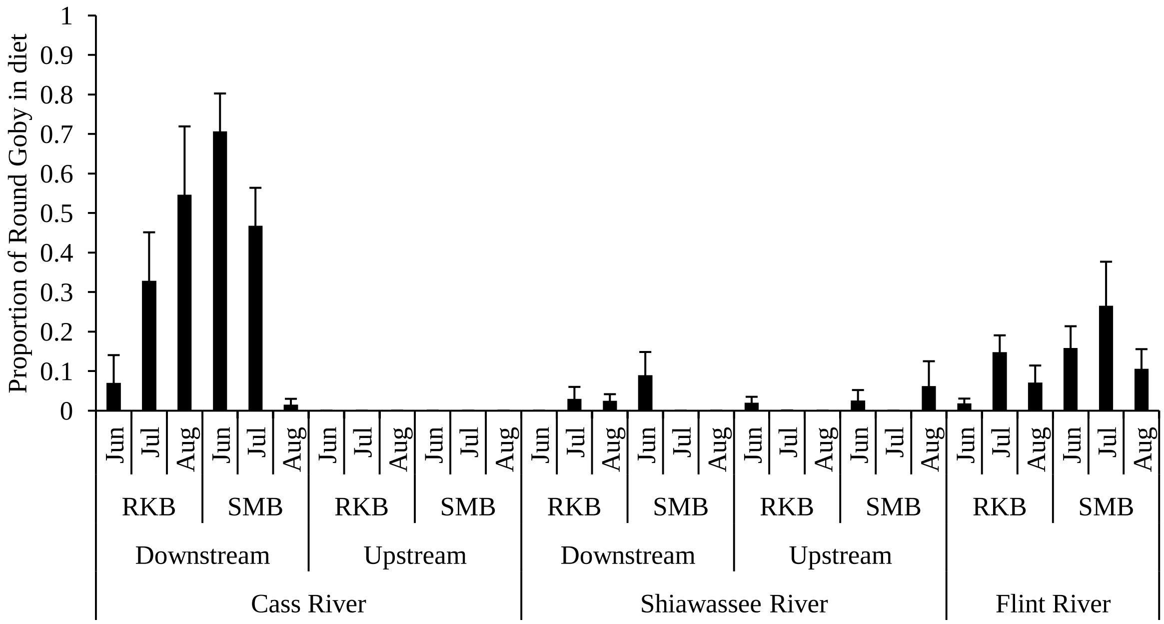

Figure 5. Proportion of Round Goby within Smallmouth Bass and Rock Bass diets (±SE). Proportions were assessed using wet weights of gut contents. RKB = Rock Bass, SMB = Smallmouth Bass. All fish were sampled in tributaries to the Saginaw River, Michigan, USA.

Diet: Cass River Upstream

Smallmouth Bass and Rock Bass diets were much less piscivorous in the upstream Cass River than other systems in this study. Diets at this site showed the highest overlap of all rivers within this study (Figure 9). No Round Goby were present in the Cass River upstream stretch and no Round Goby predation occurred. Diets for Smallmouth Bass and Rock Bass in the upstream Cass River were both dominated by crayfish. Crayfish were the dominant prey taxa for each predator during all months while Cyprinids and Greenside Darters both were found in the high between-phenotype area in June for Smallmouth Bass (Figure 6). In August, ephemeroptera was found in the high between-phenotype area for Smallmouth Bass. The conglomerate aquatic benthic invertebrate category was in the high between-phenotype area (i.e., occurred rarely in diets, though was high in proportion when it did occur) for Rock Bass in June, driven by a single fish with a diet comprised mostly of unidentified aquatic benthic invertebrates. All other prey items (benthic invertebrates and some terrestrial insects) for both predators were found in the rare prey areas of the diagrams (Figure 6).

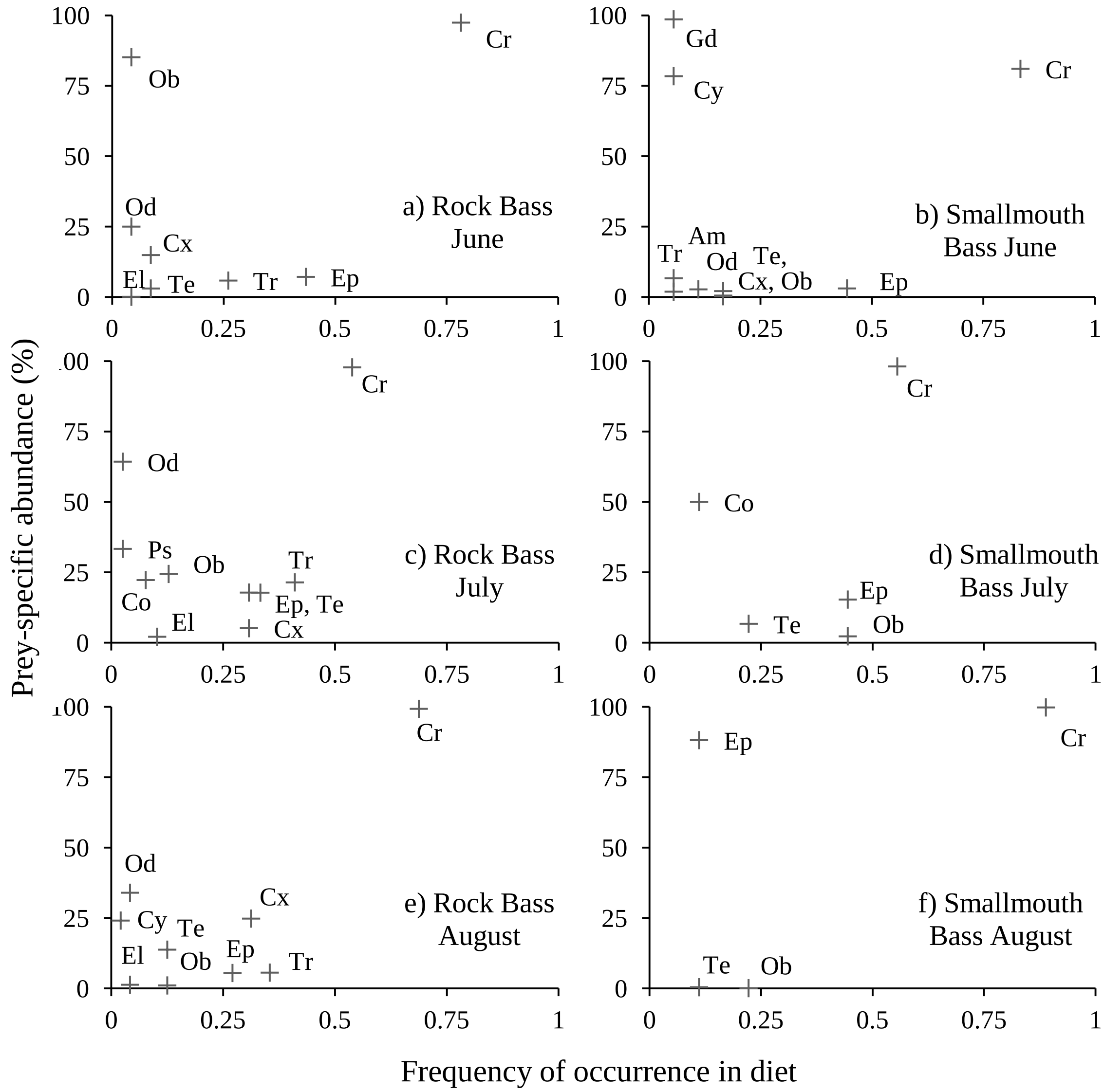

Figure 6. Costello diagrams (prey-specific abundance versus frequency of occurrence) for Smallmouth Bass (b, d, f) and Rock Bass (a, c, e) in the upstream Cass River study site during June (a, b), July (c, d) and August (e, f). Prey are: Am = amphipod, Co = coleoptera, Cr = crayfish, Cy = Cyprinids, El = elmidae, Ep = ephemeroptera, Gd = Greenside Darter, Gs = Gizzard Shad, Ob = other aquatic benthic invertebrates, Od = odonata, Ps = psephenidae, Rg = Round Goby, Te = terrestrial insects.

Diet: Cass River Downstream

Piscivory was greatest in the Cass River downstream site for both species, and Round Goby were seasonally important to both species. No diet overlap was found during any month between Smallmouth Bass and Rock Bass (Figure 9). Smallmouth Bass consumption of Round Goby (mean; ±SE) was high during June (0.71) and July (0.47) in the Cass River downstream site, though it decreased substantially in August (0.02) during which time predation of Emerald Shiners (0.17) and Gizzard Shad (0.28) substantially increased (Figures 7, 8). Decreasing Round Goby predation by Smallmouth Bass in August occurred during a period of high increase in Emerald Shiner abundance. Though the assemblage data does not reflect this increase in Gizzard Shad abundance, but large schools of Gizzard Shad were noted during electrofishing efforts for diet samples. Mean CPUE (#/hr; ±SE) of Emerald Shiners was: June = 20 (20; 0.0), July (12.0; 21.6), and August (524.0; 277.0). The Costello plots revealed that the Round Goby moved horizontally across the bi-plot from being specialized prey taxa in June to being high between-phenotype component prey taxa in August (Figure 8). By August all Smallmouth Bass prey was consumed in the specialized to high between-phenotype range, with no dominant prey types and only a few rare benthic aquatic invertebrates. Smallmouth Bass crayfish consumption was consistently low during all months.

Rock Bass consumption of Round Goby increased during the summer months in the Cass River downstream site, showing the opposite trend to that of the Smallmouth Bass population (Figure 7). Mean proportion of Round Goby in Rock Bass gut contents was low during June (0.07), but increased during July (0.12) and peaked in August (0.17). During June, consumption of crayfish by Rock Bass was highest (0.63) and decreased during July (0.30) and August (0.11) as Round Goby predation increased. There was a tradeoff between Round Goby predation and Rock Bass predation from June-August in the Cass River downstream site. The Costello plots showed that crayfish left the dominant prey zone and became high between-phenotype prey by August. Round Goby did the opposite and eventually became the dominant prey taxa by August (Figure 8). Other fish and terrestrial and benthic invertebrates were consumed in small amounts and were fed upon opportunistically (Appendix A1, A2).

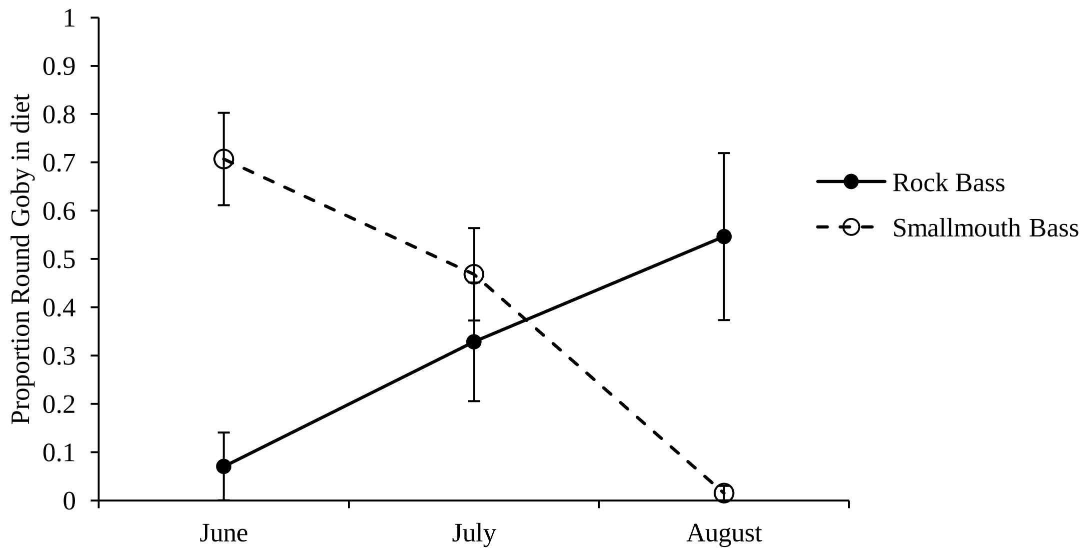

Figure 7. Mean (±SE) proportion Round Goby in Smallmouth Bass (dashed line) and Rock Bass (solid line) diets during summer diet sampling in the Cass River downstream site in 2012.

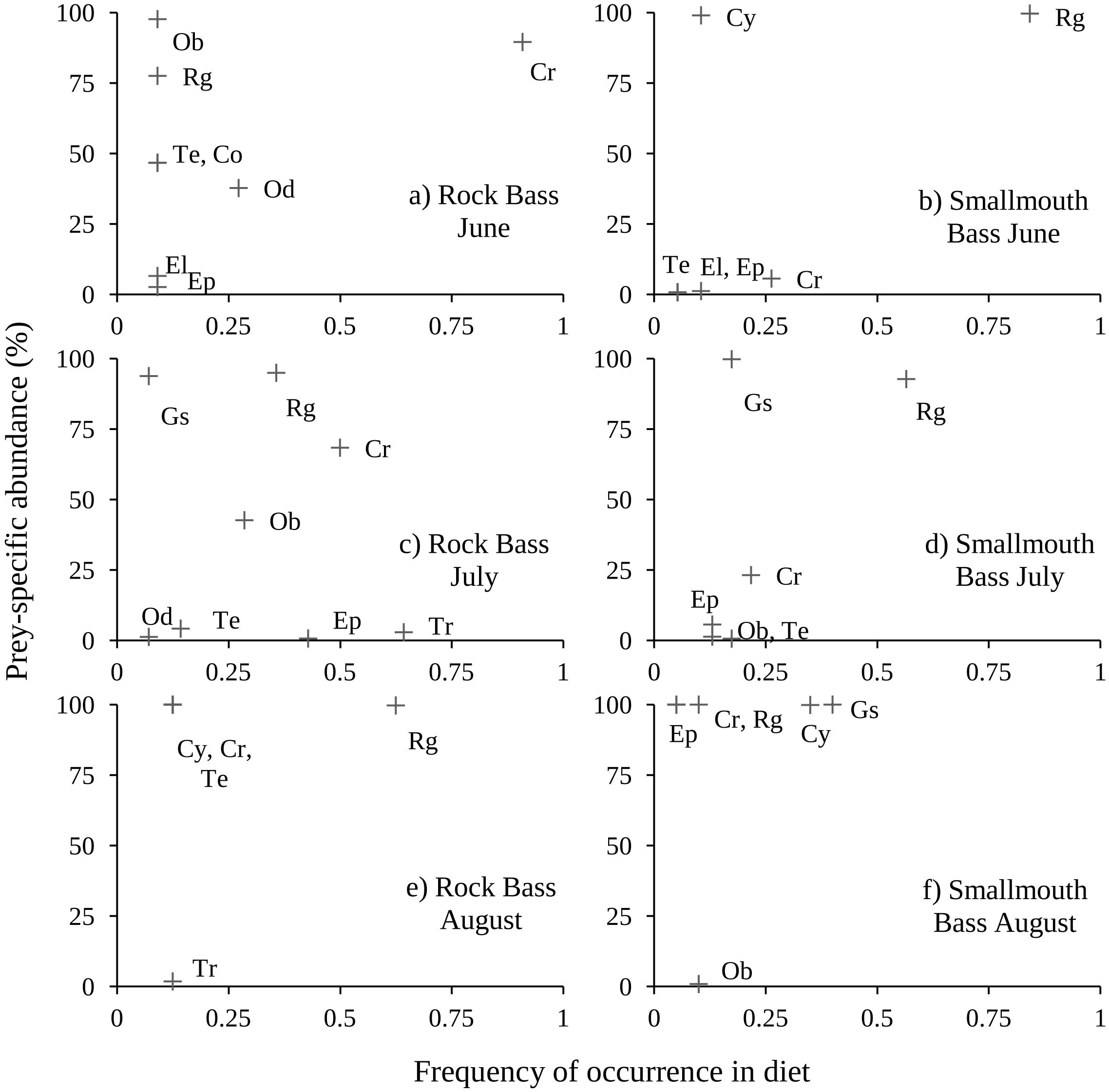

Figure 8. Costello diagrams (prey-specific abundance versus frequency of occurrence) for Smallmouth Bass (b, d, f) and Rock Bass (a, c, e) in the downstream Cass River study site during June (a, b), July (c, d) and August (e, f). Co = coleoptera, Prey are: Cr = crayfish, Cy = Cyprinid, El = elmidae, Ep = ephemeroptera, Gs = Gizzard Shad, Ob = other aquatic benthic invertebrates, Od = odonata, Rg = Round Goby, Te = terrestrial insects.

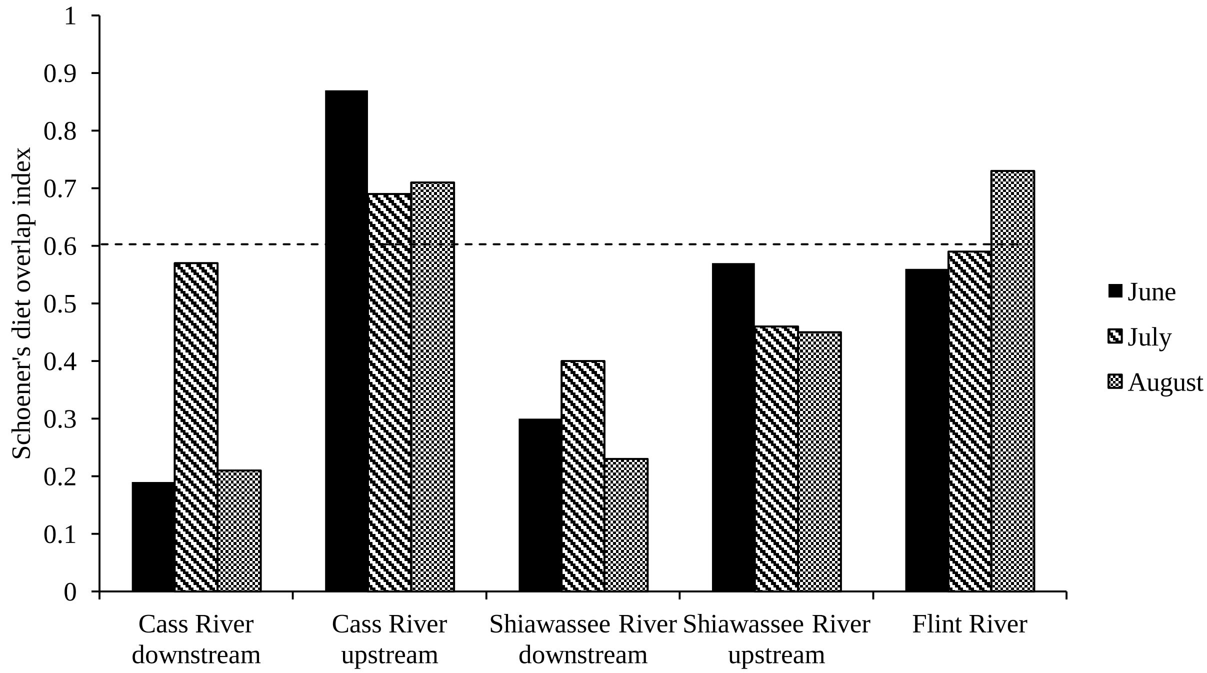

Figure 9. Schoener's diet overlap index comparing Smallmouth Bass and Rock Bass for each site and month. Area above horizontal dashed line indicates biologically significant overlap (>0.60). UST = upstream, DST = downstream. All fish were sampled during 2012 in tributaries to the Saginaw River, Michigan, USA

Diet: Shiawassee River Upstream

Smallmouth Bass and Rock Bass diets were highly variable and opportunistic in the Shiawassee River upstream site. No diet overlap was found during any month in the upstream Shiawassee River (Figure 9). In the upstream Shiawassee River, Round Goby were consumed by Smallmouth Bass in June (0.03) and August (0.06) and did not make up a large proportion of their diets. Round Goby were consumed by Rock Bass in June and July and were also not a large proportion of their diet (0.03, <0.01, respectively). During June in the upstream Shiawassee River, Smallmouth Bass consumed Bluegill (*Lepomis macrochirus)*, Bluntnose Minnow (*pimephales notatus*), Stonecat Madtom (*Noturus flavus*), and Round Goby as high between-phenotype prey, and ephemeroptera in the high within-phenotype prey area (Figure 10).

Smallmouth Bass in July in the upstream site consumed crayfish in the high between-phenotype component area and Cyprinids and conglomerate benthic invertebrates in the specialized area; all other prey that month was rare to generalized. In August in the upstream site, Smallmouth Bass consumed Round Goby, Smallmouth Bass, crayfish, and odonata in the high between-phenotype area and all other prey were found in the rare to generalized categories. Rock Bass in the upstream Shiawassee River in June consumed psephenidae and Round Goby in the high between-phenotype component area and crayfish and other prey items were fed on more opportunistically in the rare area. In July, Rock bass consumed tipulidae, Cyprinids, and crayfish in the high between-phenotype area, trichoptera in the high within-phenotype component area, and crayfish and Bluegill, Round Goby, and other prey in the rare area. In July, Rock Bass continued to feed on trichoptera in the high within-phenotype area, specialized on crayfish, and all other prey (no fish) was rarely consumed.

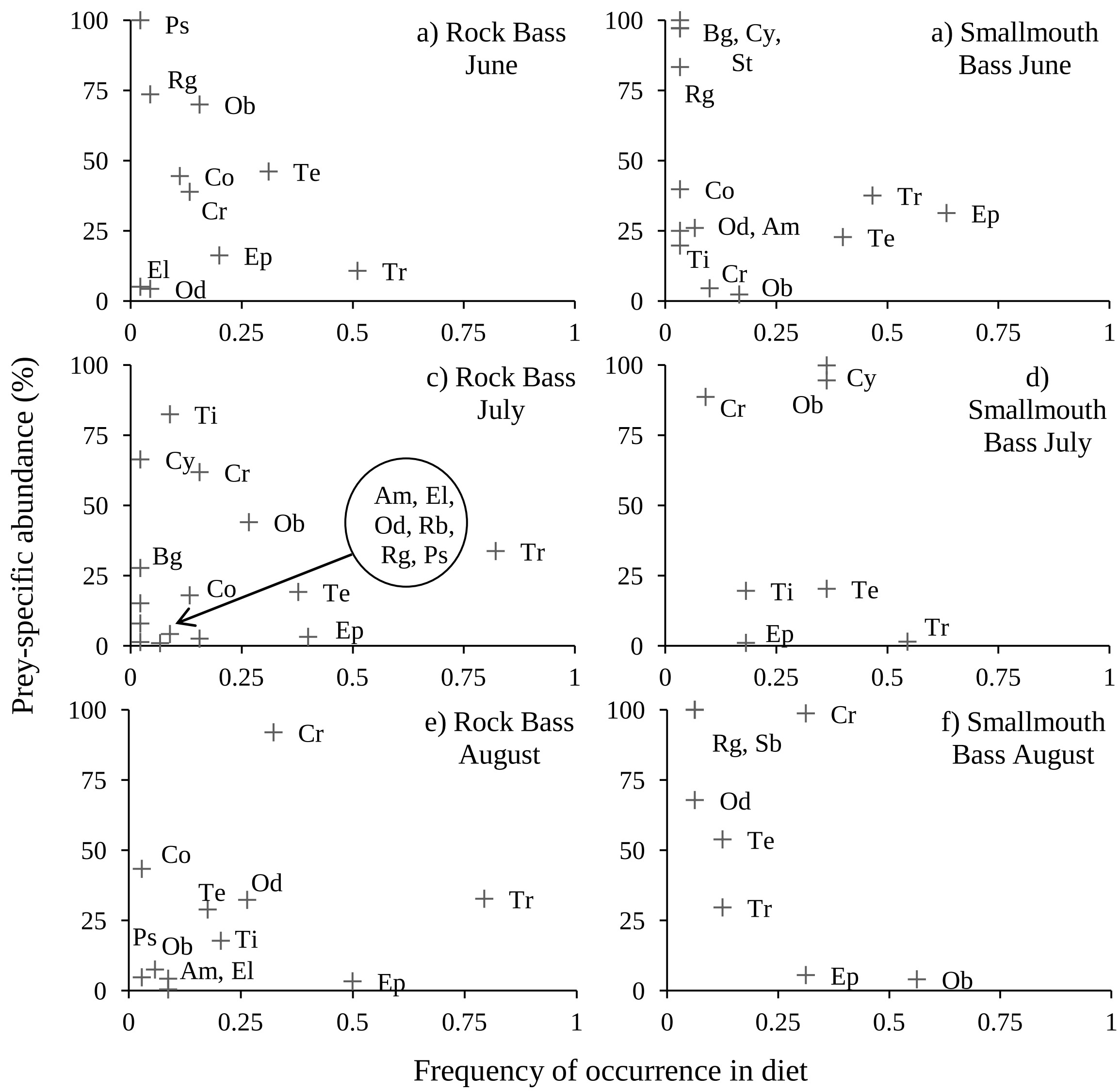

Figure 6. Costello diagrams (prey-specific abundance versus frequency of occurrence) for Smallmouth Bass (b, d, f) and Rock Bass (a, c, e) in the upstream Shiawassee River study site during June (a, b), July (c, d) and August (e, f). Prey are: Am = amphipod, Bg = Blue Gill, Co = coleopterans, Cr = crayfish, Cy = Cyprinids, El = elmidae, Ep = ephemeroptera, Gd = Greenside Darter, Gs = Gizzard Shad, Ob = other aquatic benthic invertebrates, Od = odonata, Ps = psephenidae, Rb = Rock Bass, Rg = Round Goby, St = Stonecat Madtom, Te = terrestrial insects, Ti = tipulidae, Tr = tricoptera.

Diet: Shiawassee River Downstream

Shiawassee River diets were diverse for both Smallmouth Bass and Rock Bass during all months. No diet overlap was found during any month in the downstream Shiawassee River (Figure 9). Round Goby were only consumed in June in the downstream Shiawassee River by Smallmouth Bass and did not make up a large proportion of their diets (0.09). Round Goby were consumed by Rock Bass in July (0.03) and August (0.03) and were also not a large proportion of their diets. Downstream Shiawassee River Smallmouth Bass consumed Cyprinids in the high between-phenotype area in June, consumed crayfish opportunistically, and other prey taxa were rarely consumed (Figure 11). For Smallmouth Bass in July, Crayfish were the only dominant prey item. Smallmouth Bass in July fed on trichoptera in the high within-phenotype area, generalized on ephemeroptera, and rarely fed on terrestrial insects. Crayfish, Cyprinids (Emerald Shiner and Spotfin Shiner (*Cyprinella spiloptera*)), ephemeroptera, and other benthic invertebrates were the only prey consumed in the downstream Shiawassee River by Smallmouth Bass in August and were all found in the high between-phenotype area. Rock Bass specialized on trichoptera in June, consumed *trichoptera* in the high within-phenotype component area and Round Goby in the high between-phenotype area in July, and specialized on crayfish in August. All other prey items were rare. Round Goby were not consumed by Smallmouth Bass in July or August and were not consumed by Rock Bass in June in the downstream Shiawassee River site.

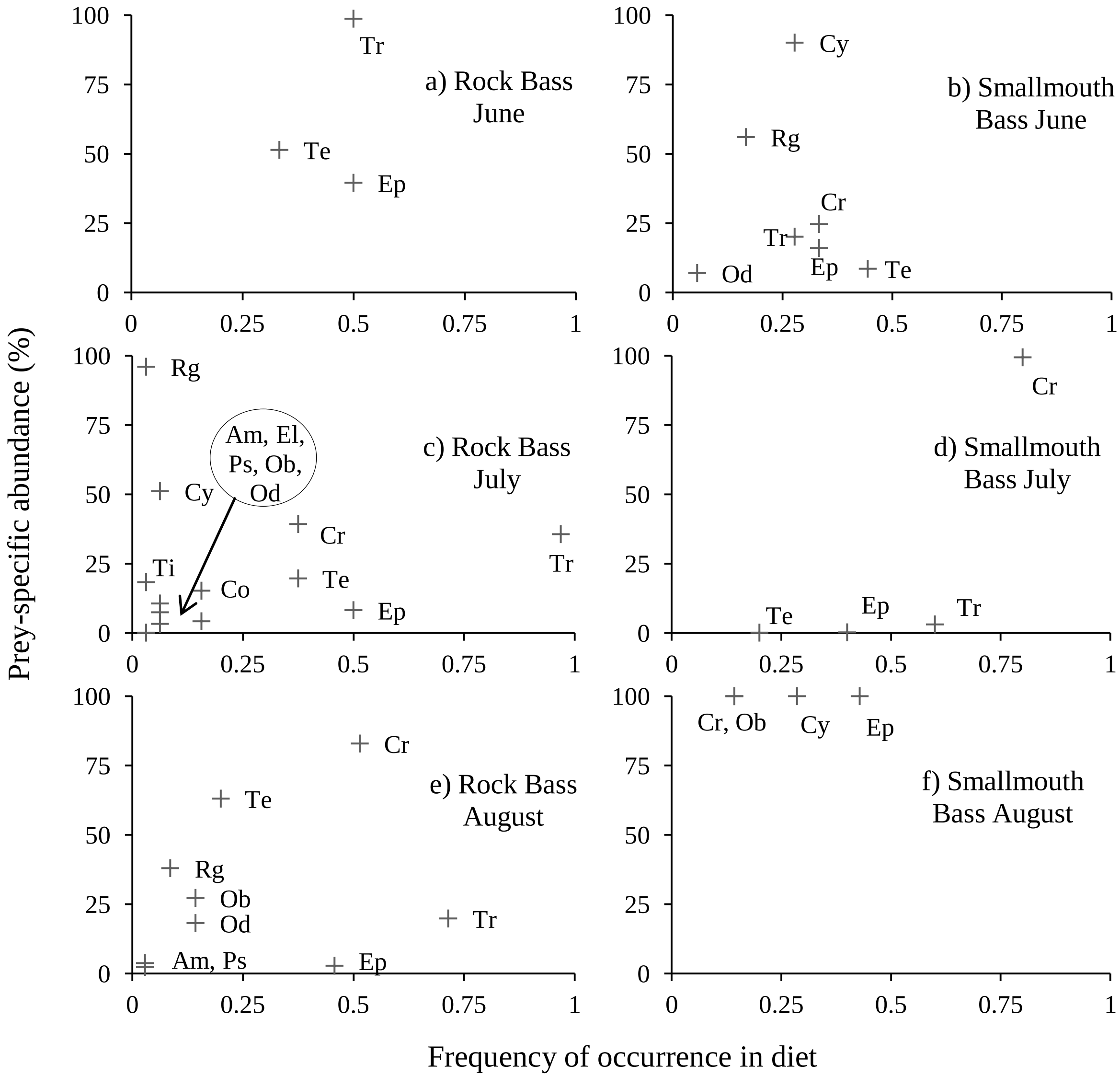

Figure 11. Costello diagrams (prey-specific abundance versus frequency of occurrence) for Smallmouth Bass (b, d, f) and Rock Bass (a, c, e) in the downstream Shiawassee River study site during June (a, b), July (c, d) and August (e, f). Prey are: Am = amphipod, Co = coleoptera, Cr = crayfish, Cy = Cyprinids, El = elmidae, Ep = ephemeroptera, Gd = Greenside Darter, Gs = Gizzard Shad, Ob = other aquatic benthic invertebrates, Od = odonata, Ps = psephenidae, Rg = Round Goby, Te = terrestrial insects.

Diet: Flint River

Smallmouth Bass and Rock Bass diets had similar prey taxa during all months in the Flint Rivera and diets overlapped in August (Figure 9). Round Goby were consumed by Smallmouth Bass in June (0.21), July (0.04), and August (0.12). Round Goby were also consumed by Rock Bass in June (0.02), July (0.15), and August (.07). During the summer, crayfish were more important to Rock Bass than Smallmouth Bass and Round Goby were more important to Smallmouth Bass than Rock Bass. The dominant prey of Rock Bass during all three months in the Flint River was crayfish (Figure 12). Trichoptera were consumed in the high within-phenotype component area during June for both predators during June. Round Goby were consumed opportunistically in all months by Smallmouth Bass and in July and August by Rock Bass. Smallmouth Bass specialized on crayfish in June, and crayfish and Cyprinids (Spotfin Shiner) during July. Smallmouth Bass fed on Cyprinids (Creek Chub (*Semotilus atromaculatus*)), Gizzard Shad, gerridae, and Round Goby in the high between-phenotype area and consumed crayfish as the dominant prey in August. Ephemeroptera and trichoptera were grazed on in the high within-phenotype niche area by Smallmouth Bass in June. Rock Bass consumed Gizzard shad in the high between-phenotype area during July. All other taxa found in gut contents were rare or fed on opportunistically by both predators.

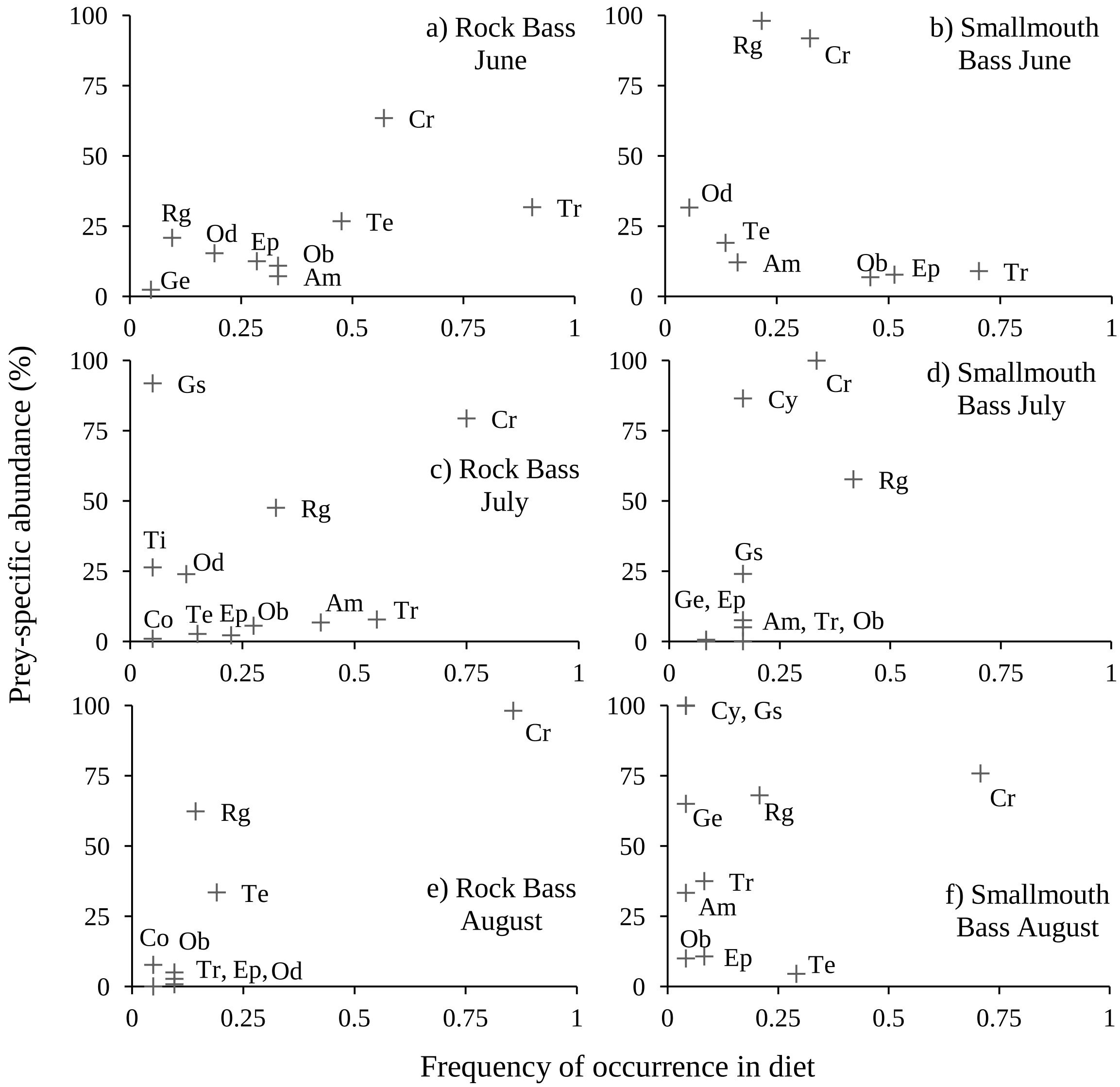

Figure 12. Costello diagrams (prey-specific abundance versus frequency of occurrence) for Smallmouth Bass (b, d, f) and Rock Bass (a, c, e) in the Flint River study site during June (a, b), July (c, d) and August (e, f). Prey are: Am = amphipod, Co = coleoptera, Cr = crayfish, Cy = Cyprinids, El = elmidae, Ep = ephemeroptera, Ge = Gerridae, Gd = Greenside Darter, Gs = Gizzard Shad, Ob = other aquatic benthic invertebrates, Od = odonata, Ps = psephenidae, Rg = Round Goby, Te = terrestrial insects, Ti = tipulidae, Tr = trichoptera.

The proportion of Round Goby in diets of Smallmouth Bass and Rock Bass increased as Round Goby abundance increased (Figure 13). The Flint River July data was not available because of flood conditions, and only one of the three Cass River upstream sites was used because the number of Round Goby present each month was constant at 0/hr.

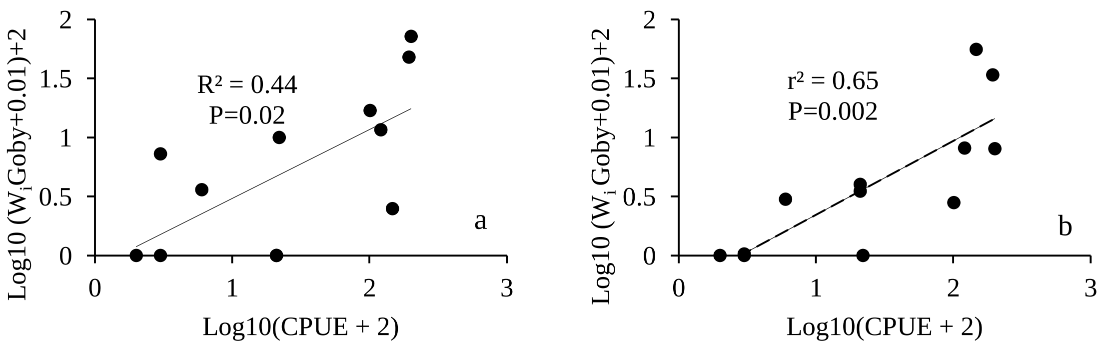

Figure 7. Relationships between proportion (W_i) Round Goby in Smallmouth Bass (a) and Rock Bass (b) diets and Round Goby abundance. Solid line represents the best fit, least squares regression line (Smallmouth Bass P=0.02, Rock Bass P=0.002). Transformations were made to achieve data normality and are indicated on the axes. All fish were sampled during 2012 in tributaries to the Saginaw River, Michigan, USA.

Stable Isotopes

Stable isotopes of predator, prey, and baseline organisms were sampled during August-October 2012 in the Cass, Shiawassee, and Flint Rivers. In all rivers, 73 Smallmouth Bass, 49 Rock Bass, 29 Round Goby, 29 crayfish, 18 Spotfin Shiners, 9 Gizzard Shad, and 5 Emerald Shiners were collected for analysis along with 8 conglomerate gilled snail samples and 13 conglomerate macroinvertebrate samples.

Gilled snails were the only baseline organism that was captured at all five sites and were used as the baseline organism to standardize trophic position across sites (Vander Zanden et al. 1999). Other baseline organisms were used to visually compare carbon sources in the isotope bi-plots (Appendix A3-A5). Trophic positions, sample sizes, and mean lengths of predators and main prey items are summarized in Table 4. No relationships were found between total length and trophic position or total length and $\delta^{13}C$ for Smallmouth Bass, Rock Bass, or Round Goby, so all size classes were combined (Figure 14). Size ranges of fishes used for calculating trophic position were similar to the size ranges used for diet analysis.

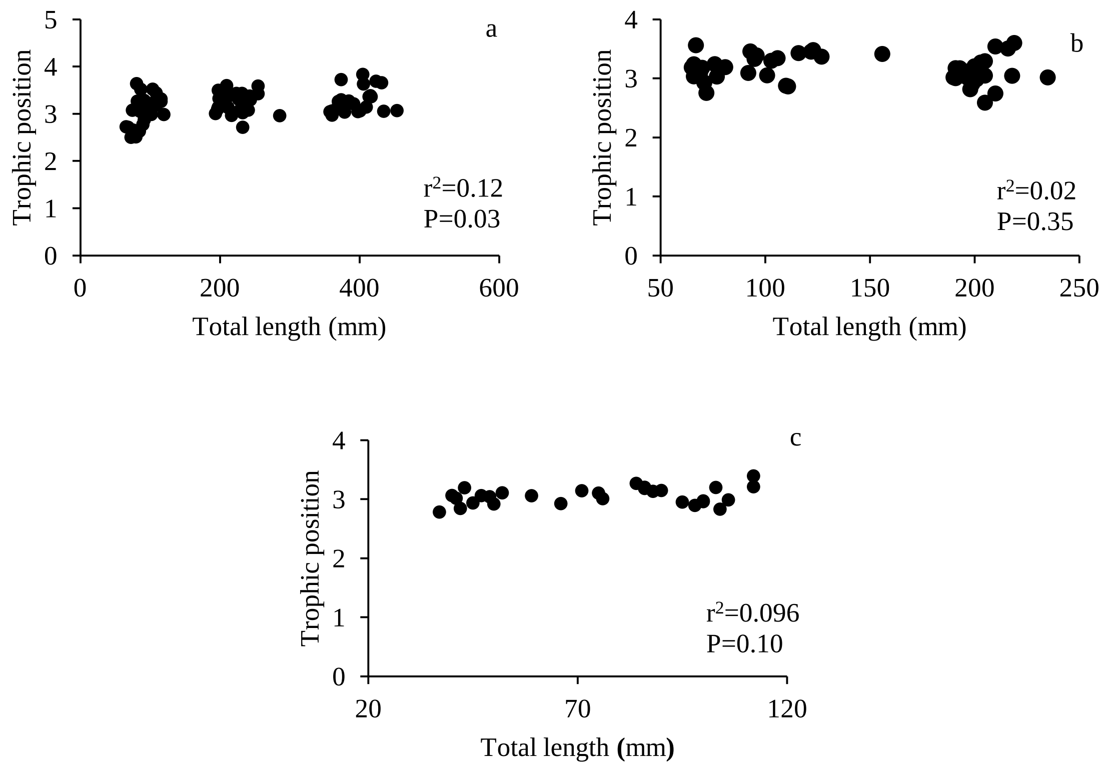

Figure 14. Relationships between trophic position and total length (mm) of Smallmouth Bass (a) and Rock Bass (b) and Round Goby (c). Data represent all systems and reveal no relationships between fish size and continuous trophic position.

Smallmouth Bass and Rock Bass mean trophic position increased with Round Goby CPUE (P=0.02, P=0.034, respectively; Figure15). Smallmouth Bass mean trophic position increased with proportion (W_t) of Round Goby in Smallmouth Bass diet (P=0.045; Figure 15). No significant relationship was found between Rock Bass trophic position and proportion (W_t) of

Round Goby in Rock Bass diet (P=0.09; Figure 15). Crayfish and Round Goby $\delta^{15}N$ differed in the downstream Cass River (T-test; P=0.003), downstream Shiawassee River (P<0.001), and the Flint River (P<0.001). Only a single Round Goby was collected in the upstream Shiawassee River, so no comparison of means was available, though the trophic position of that single round goby was higher than the mean crayfish trophic position in that system. No Round Goby were present in the upstream Cass River.

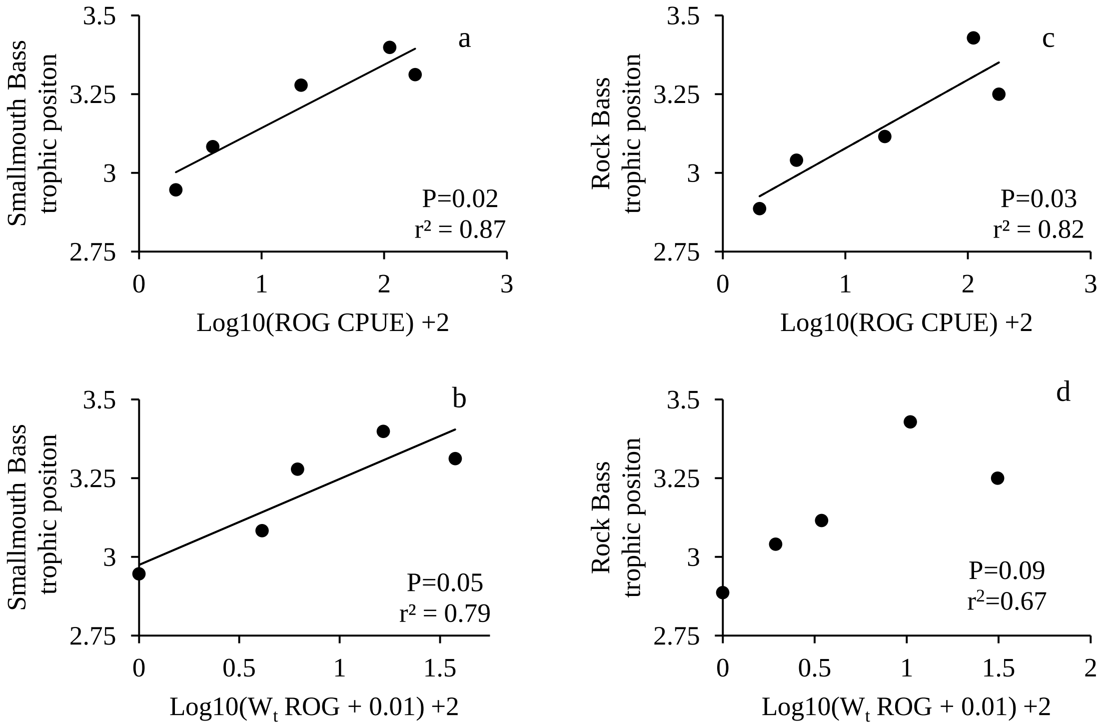

Figure 15. Relationships between Smallmouth Bass (a) and Rock Bass (c) trophic position and Round Goby (ROG) abundance were strongly correlated and significant. Relationships between Smallmouth Bass (b) and Rock Bass (d) trophic position and proportion (W_t) Round Goby in diets were significant for Smallmouth Bass (b), but not for Rock Bass (d). Data transformations were done to satisfy assumptions of normality and are indicated. Best fit, least squares regression lines are shown where significant. All data were collected in Saginaw River tributaries, Michigan, USA.

Table 4. Summary of sample size, mean length (±SE), and mean (±SE) stable isotope-based estimates of trophic position for Rock Bass and Smallmouth Bass and their most common prey types, *O. rusticus* and Round Goby. Missing values for Round Goby in the upstream Cass River site are indicated by x and indicate no presence of Round Goby at that site. All data were collected during August-October 2012 electrofishing events in Saginaw River tributaries, Michigan, USA.

		O. rusticus			Round Goby			Rock Bass			Smallmouth Bass		
River	Location	N	Mean carapace length (mm)	Trophic position (±SE)	N	Mean TL (mm)	Trophic position (±SE)	N	Mean TL (mm)	Trophic position (±SE)	N	Mean TL (mm)	Trophic position (±SE)
Cass	Downstream	5	26 (1.7)	2.42 (0.17)	12	73 (8.2)	3.01 (0.05)	11	145 (16.2)	3.26 (0.03)	15	239 (31.8)	3.31 (0.02)
	Upstream	3	21 (2.4)	2.01 (0.08)	x	x	x	9	162 (20.7)	2.89 (0.04)	15	229 (34.6)	2.95 (0.04)
Shiawassee	Downstream	5	31 (1.7)	2.40 (0.12)	5	82 (7.1)	2.99 (0.04)	10	133 (20.9)	3.12 (0.04)	12	197 (33.7)	3.26 (0.03)
	Upstream	11	23 (1.3)	2.31 (0.16)	1	50	2.92	9	125 (21.7)	3.04 (0.03)	16	241 (30.6)	3.08 (0.02)
Flint		5	29 (2.4)	2.62 (0.08)	11	71 (8.1)	3.14 (0.04)	10	154 (16.8)	3.43 (0.02)	15	224 (35.6)	3.40 (0.06)

Standard ellipse areas (i.e., niche space) were calculated for all predators at each site using the SIBER function in SIAR (summarized in Table 5). Proportion of total niche space available to both predators in overlap ranged from 0.18 to 0.44. Smallmouth Bass niche area was largest at all sites except Cass River downstream. No trend existed between Schoener's overlap index and niche overlap based on standard ellipse area. Highest niche overlap based on standard ellipses was in the upstream Shiawassee River and the highest Schoener's overlap index value was in the upstream Cass River.

Table 5.Summary of standard ellipse area (SEA) and standard ellipse overlap of Smallmouth Bass and Rock Bass. Asterisks (*) represent significant differences between predator ellipse areas ($\alpha = 0.05$). Proportion overlap = (area of overlap * 2) / (total area Rock Bass + total area Smallmouth Bass).

		Standard Ellipse Area		Area of Overlap / Total Predator Area		
River	Location	Smallmouth Bass	Rock Bass	Smallmouth Bass	Rock Bass	Proportion Overlap
Cass	Downstream	.44*	2.06*	0.82	0.18	0.29
	Upstream	1.42	0.39	0.21	0.76	0.33
Shiawassee	Downstream	0.98	0.78	0.27	0.35	0.30
	Upstream	1.21	0.55	0.32	0.71	0.44
Flint		3.76*	0.37*	0.10	1.00	0.18

DISCUSSION

Round Goby have altered the diet and energy pathways leading to Smallmouth Bass and Rock Bass in Saginaw River tributaries. Increasing Round Goby abundances in these systems led to increasing Round Goby consumption and lengthened food chains. Predator diets in the Cass River below and above the Frankenmuth dam are very different from each other, a result of the high levels of Round Goby consumption by both Smallmouth Bass and Rock Bass in the downstream, highly invaded reach. Feeding strategies differed across the gradient of Round Goby abundances. Where Round Gobies were highest in abundance, they tended to be the dominant prey item for both predators. At moderate abundances they were preyed on opportunistically. Only in the systems with very low Round Goby abundance were they relatively unimportant to predator diets. This is a similar pattern to other work that has shown Round Goby importance to Smallmouth Bass and Rock Bass in lakes (Corkum et al. 2004, Steinhart et al. 2004, Campbell et al. 2009, Ng et al. 2009) and large river systems (Jude et al. 1995). This is the first description of Round Goby impacts to either predator's diet in mid-order river systems. This is important, as Smallmouth Bass and Rock Bass tend to be the most abundant piscivores in these systems and may be the most affected fish species resulting from the Round Goby invasion (e.g., higher contaminant biomagnifications, altered growth rates, increased spread of disease).

Though Round Goby were important to Smallmouth Bass and Rock Bass diets, that importance was seasonal. Diets differed between and within each predator throughout the summer growing season of 2012, as evidenced by the gut content data and resulting diet overlap indices. Though biologically significant diet overlap was found during 4 out of the 15 sampling periods, four other sites were nearly at the 0.60 level and thus had high overlap. The major diet taxa (Round Goby, Cyprinids, crayfish, ephemeroptera, trichoptera) were found in most predator's stomachs, though the degree to which they were consumed varied, as did the feeding strategy used to consume them. Seasonal changes in prey abundances have a strong influence on dietary habits of both predators, as documented by the increase in Gizzard Shad and Emerald Shiner presence in gut contents and seasonal changes in prey taxa importance. The August Emerald Shiner abundance increase was likely attributed to recruitment to sizes and/or life stages that made them attractive and vulnerable as prey to predator, and the increase in gizzard shad abundance, though not reflected in the assemblage surveys, was a result of their potodromous behavior (Miller 1960).

Variations in feeding strategies were described using Costello diagrams, which plot the frequency of occurrence versus prey-specific abundance (mass) of each prey tax. Examining these diagrams revealed that Round Goby were the dominant prey items for both predators in the downstream Cass River (highest Round Goby abundance) but during different months. The upstream Cass River is currently unaffected by the Round Goby invasion, and predators within that system both consume crayfish as their primary, dominant food source from June-August. This evidence clearly shows that Round Gobies have altered the diet and energy pathways for Smallmouth Bass and Rock Bass. Although the upstream fish assemblage is altered by the presence of a low-head dam (Stoller 2013), the dietary habits of Smallmouth Bass and Rock Bass in that system are likely an artifact of what Smallmouth Bass and Rock Bass diets were like in un-invaded rivers during stable, low-flow conditions. It therefore seems probable that once this low-head dam is breached in Fall 2014 (US Fish & Wildlife Service personal communication) and Round Goby and migratory fishes are able to pass upstream and become established, predator diets will shift to include more piscivory and reliance on invasive prey like the other four systems in this study.

Flint River diets showed moderate Round Goby predation, though they were not the dominant prey items as they were in the downstream Cass River. Predator populations in the Flint River tended to consume Round Goby less frequently than the downstream Cass River, though when they were consumed they were the majority diet item (high between-phenotype contribution). Similar trends were found in the upstream and downstream Shiawassee River, though much lower and less dominant consumption of Round Goby was documented there. It is unclear why Round Goby predation differed between Cass River downstream and the Flint River, as both Rivers have relatively high Round Goby abundances. Though not available in this study, information on prey selectivity may help to clarify this behavior in the future.

By combining assemblage data with gut content and stable isotope analysis, this study was better able to explore how Round Goby have become an integrated part of the Cass, Shiawassee, and Flint River aquatic food webs. Regressing predator diet proportions consisting of Round Goby with predator trophic position and Round Goby CPUE revealed positive relationships. This is likely indicative of the higher trophic position of Round Goby relative to crayfish and other benthic prey that is typically consumed by predators in areas of low Round Goby abundance (e.g., Cass upstream, Shiawassee River upstream and downstream). The positive relationship found between Round Goby abundance and diet proportion consisting of Round Goby shows that as Round Goby become more abundant, predators are more likely to use them as prey and become more trophically elevated.

By examining the bi-plot of the un-transformed Smallmouth Bass and Rock Bass diet proportion Round Goby versus Round Goby abundance, it seems that predators are assuming a type III functional response to the novel prey (Figure 22; Holllings 1959, 1965, 1966), though further investigation and a larger sample size is needed to fully evaluate this relationship. Round Goby abundances in rivers tends to decrease after initially peaking during occupation of novel areas (Kornis et al. 2012), so if abundances fall to lower levels and feeding habits of predators revert to native prey it could mean that moderate abundances of Round Goby may not trigger high levels of predation, thus food chain length and trophic biomaginfication of contaminants may stabilize to pre-invasion levels if Round Goby abundances drop back into the range of low predation. Further research is needed to fully explore this relationship.

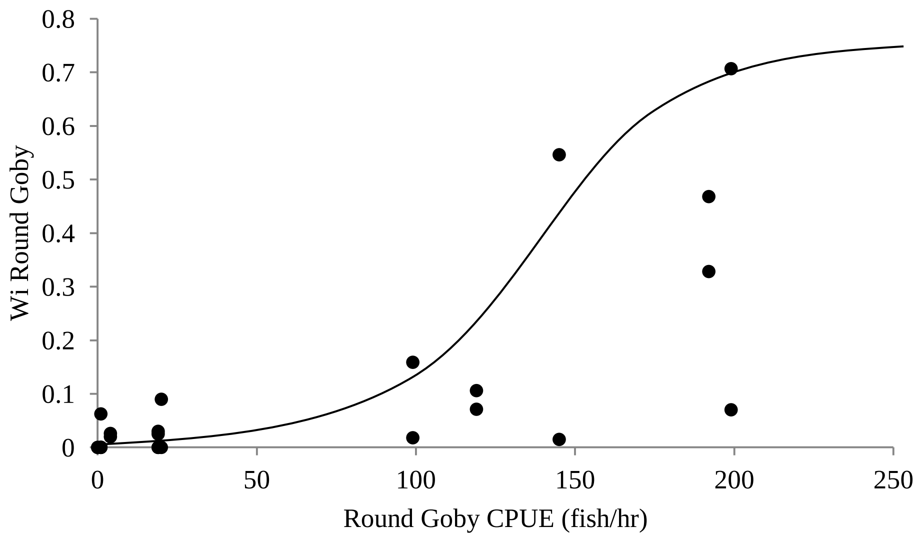

Figure 16. Un-transformed diet proportion Round Goby (Wi) and Round Goby abundance data. Theoretical type III functional response curve is overlaid on the bi-plot.

Gut content data are subject to bias resulting from sampling time, season, temperature, and other temporal changes (Murphy and Willis 1996). For this reason stable isotope analysis can be used to reveal feeding relationships that gut content data may miss (Cabana and Rasmussen 1996). In lentic environments, there is typically a more depleted $\delta^{13}C$ signature in the deeper, pelagic zones and less depleted $\delta^{13}C$ signature in the littoral zones, which allows discrimination of food sources in consumers using isotope mixing models (Post 2002). Stable isotope analysis that aims to distinguish between sources in mid to small-order rivers is more difficult because lotic systems are more dynamic (i.e., seasonal energy sources are highly prone to change due to the dynamic riverine conditions from floods, drought, etc.) and have a higher riparian-to-surface area relationship than do lakes, all of which contribute to carbon input into the stream. Vander Zanden and Rasmussen (1999) used a simple two source mixing model to distinguish between two food sources, one of which was highly affected by an invasive species. Though it would have been desirable to do so, this study was unable to distinguish such changes because of the high diversity in feeding behavior exhibited by various populations of predators and our inability to capture all available carbon sources. Examining the $\delta^{13}C$ and $\delta^{15}N$ biplots shows that there are sources of carbon that contribute to the consumers in this system that aren't reflected in the baseline organisms (consumers lay outside of the furthest outside producers on the x axis). Because of this, the most advanced Bayesian mixing models could not be used to examine source contributions to consumer diets (i.e., relative proportions of prey to Smallmouth Bass and Rock Bass diets). Future studies should aim to capture as many baseline and prey sources as possible, including prey that are only seasonally available such as migratory fishes or early hatching aquatic macroinvertebrates (e.g., *plecoptera*). Mixing models assume that consumers are in equilibrium with their prey, and the variability in predator diets revealed by this study show that that assumption is likely violated. Tissue turnover in fish can take months (Harvey et al. 2002), so isotopic signatures derived from prey may not be revealed in predators for months after consumption. Time series studies of stable isotopes may be the best method for capturing spatial and temporally integrated diet data in small and mid-order rivers.

Isotope space bi-plots were used in this study to examine the spatial and temporally integrated feeding habits of both predators. This study was able to delineate the isotopic niche space that each predator existed within by using standard ellipses that encompass the isotope data. Larger niche areas translate into more generalist feeding strategies, as increasing spread in isotope space indicates consumption of multiple food sources and higher between-phenotype component feeding strategies (Bearhop et al. 2004). The isotopic niche area of Smallmouth Bass tended to be larger than that of Rock Bass in four of the five sites, indicating a larger diet breadth. High diet differentiation was revealed in the Cass River downstream site, which matched the gut content analysis. However, there were no other clear trends or associations between the ellipse areas, ellipse overlap, and the diet or assemblage information. Again, this is likely an artifact of a dynamic environment with highly variable carbon sources, though the information still clearly shows that each predator have diets that overlap from 18-44%.

Energy densities of Round Goby are variable and depend on their environments and available prey (Johnson et al. 2005, Ruetz et al. 2009). Ruetz et al. 2009 found that Round Goby living in Great Lakes tributaries tend to have higher energy densities than those living in the Great Lakes proper, as those residing in the tributaries rely less on dreissenid mussels as prey. Dreissenid mussels are present in varying abundances throughout the study areas, with the highest abundances existing within the stretch of the Flint River under study and much lower densities in the other two river systems (CMU personal observation), though even the highest densities of dreissenid mussels in the river are unlike the densities found in the Great Lakes (CMU personal observation). Carmen et al. (2006) found that Round Goby in the Flint River thrive in the absence of dreissenid mussels and have a diverse feeding capacity. It is probable that predators take advantage of the high abundances of non-native prey because of lower energetic costs associated with pursuing and capturing other energy dense prey (Kornis et al. 2012). The percentage of Smallmouth Bass empty stomachs was positively associated with Round Goby CPUE, possibly because increasing Round Goby abundance (and associated predation) created less need to feed as often to maintain metabolic activity. Since a positive relationship existed between Round Goby abundance and Round Goby predation, it is likely that the energy-dense prey cause changes in predator growth rates and contaminant biomagnification. Further research in this area is needed.

It is important to understand how invasive species impact native aquatic food webs, and how those changes can have cascading effects that modify the feeding behavior, growth, and contaminant levels in recreationally important fisheries. This study has shown that Round Goby have become an important part of the aquatic food webs that they have invaded, though that importance is seasonal and depends on the abundance at which they exist. Like many other systems, it is probable that Round Goby have displaced native prey and caused irreversible changes to the aquatic food web (Kornis et al. 2012). Understanding these effects and the impacts they have to biodiversity and ecosystem functioning is a key component to making wise conservation decisions. Disruptions to trophic interactions in native ecosystems can have cascading effects and cause changes to basic food web functioning, the aquatic-terrestrial interface, contaminant loading in top predators, and species extirpations. Understanding trophic changes in aquatic systems is an integral part of the conservation of aquatic resources in our Great Lakes tribuatries.

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APPENDIX

Table A1. Summary of Smallmouth Bass diets in Saginaw River tributaries from the 2012 sampling effort. Diets are shown as percent composition by weight. Number sampled (N), percent of sample with empty guts (% empty) and mean length (TL; mm) are shown. Values displayed as 0.0 are > 0 but less than 0.1.

	Cass DST			Cass UST			Shiawassee DST			Shiawassee UST			Flint		
SMB	Jun	Jul	Aug	Jun	Jul	Aug	Jun	Jul	Aug	Jun	Jul	Aug	Jun	Jul	Aug
N	34	44	46	18	9	14	21	7	9	33	12	17	38	21	44
% empty stomachs	35	39	37	0	0	21	10	14	11	3	8	6	3	38	27
Mean length (mm)	205	229	225	239	236	233	248	248	208	218	256	184	237	265	237
Aquatic															
Crayfish	5.6	8.1	4.4	67.2	51.8	63.9	10.6	47.3	12.4	3.2	8.1	26.5	12.8	29.1	41.3
Detritus	9.4	11.2	23.1	4.1	8.5	9.7	15.0	31.2	22.7	16.8	8.8	10.0	20.8	3.5	23.9
ROG	70.7	46.8	1.5				9.0			2.6		6.3	15.9	26.5	10.6
Cyprinid	4.5		3.4	4.4			2.5				2.5				
Spotfin Shiner							8.3				18.2			13.0	
Stonecat										3.0					
Greenside Darter				5.4											
Emerald Shiner			17.0				10.2		12.5		9.1				
Mimic Shiner			3.4												
Common Shiner									12.5						
Creek Chub															3.1
Bluntnose Minnow										3.0					
Smallmouth Bass												5.9			
Gizzard Shad		13.6	27.6											8.3	3.1
Blue Gill										3.1					
Rock Bass															
UID Fish	4.6	11.0	11.3	1.2			4.7	14.9	21.1	3.6	8.1	4.4	5.8		8.4
Trichoptera				0.4			10.5	6.3		13.4	2.8	1.8	16.2	0.0	1.9
Ephemeroptera	0.0	0.3	0.4	8.8	22.6	8.0	7.5	0.3	12.6	20.5	0.2	14.7	7.3	0.0	0.7
Plecoptera		0.0		0.1											
Odonata				0.1			0.4			1.4		4.2	0.9		
UID Benthic		0.0	0.0	0.0	12.2	9.1			6.3	0.6	23.3	25.4	2.9		0.0
Asellus															
Amphipoda				1.6						0.2			2.4	1.3	1.0
Elmidae	0.1														
Corixidae				1.2											
Gerridae														0.0	0.2
Gastropoda		0.4													
Simulidae													9.8		
Sphaeriidae										0.2			0.0	0.1	
Corydalidae															
Notonectidae															
Chironomidae					1.4										
Oligochaetes														4.2	
Tipulidae										0.4	6.2				
Psephenidae															
Hirudinea															
Coleptera larvae							0.0			0.1			0.6		
Corbicula fluminea											0.0				
Ranatra					0.0										
Terrestrial															
UID Terrestrial	0.0	3.7		0.1	0.6		16.1			9.3	10.9	0.8	1.3		2.4
Odonata										0.0					
Bee/wasp						0.0		0.0		3.2					0.3
Coccinellidae															
Simuliidae															
Coleoptera					2.9					1.9					
Gastropoda															
Lepidoptera							5.3								
Oligochate															
Araneae		3.7													
Ant										0.2					
Unknown	5.0	1.2	7.9	5.4		9.3				13.3	2.0		3.4	13.8	3.1

Table A2. Summary of Smallmouth Bass diets in Saginaw River tributaries from the 2012 sampling effort. Diets are shown as percent composition by wieght. Number sampled (N), percent of sample with empty guts (% empty) and mean length (TL; mm) are shown. Values displayed as 0.0 are > 0 but less than 0.1.

	Cass DST			Cass UST			Shiawassee DST			Shiawassee UST			Flint		
RKB	Jun	Jul	Aug	Jun	Jul	Aug	Jun	Jul	Aug	Jun	Jul	Aug	Jun	Jul	Aug
N	13	14	12	29	40	50	9	34	36	80	46	36	21	41	25
% empty stomachs	15	0	25	6	3	4	11	6	0	11	0	3	0	2	12
Mean length (mm)	188	156	145	183	139	158	123	142	139	120	144	144	145	149	176
Aquatic															
Crayfish	62.8	30.2	11.1	67.2	49.0	62.4		13.7	30.0	5.6	9.0	19.2	28.1	53.3	71.9
Detritus	2.8		0.7	3.8	0.5	6.8	12.5	7.8	3.0	3.3	5.6	20.5	6.9	7.1	5.9
ROG	7.0	32.8	54.6					3.0	2.5	2.0	0.0		1.8	14.8	7.1
Cyprinid						0.5		2.2			1.4				
Spotfin Shiner															
Stonecat															
Greenside Darter															
Emerald Shiner								0.8							
Mimic Shiner			11.1												
Common Shiner															
Creek Chub															
Bluntnose Minnow															
Smallmouth Bass															
Gizzard Shad		5.1												3.7	
Blue Gill											0.5				
Rock Bass											0.2				
UID Fish	3.6	5.0			0.1	0.7		2.9	4.1		4.3			0.1	4.5
Trichoptera		15.2	0.2	3.1	17.1	11.2	17.0	43.7	37.6	6.5	41.8	34.3	33.7	7.0	0.0
Ephemeroptera	0.2	3.3		10.5	11.0	5.2	5.0	7.1	4.0	2.1	2.4	3.3	1.6	0.8	0.2
Plecoptera															
Odonata	7.0	0.1		0.3	1.6	1.0		0.5	2.4	1.4	0.4	6.6	6.1	2.3	0.7
UID Benthic		0.4		3.7	0.6	0.6		1.1	1.8	6.4	9.1	0.8	3.0	1.8	0.1
Asellus															0.3
Amphipoda								0.2	0.1		0.1	0.3	2.3	4.7	
Elmidae	0.5			0.0	0.3	0.0		0.0		0.1	0.3	0.0			
Corixidae				0.9	5.8	6.7									
Gerridae													0.1		
Gastropoda	7.6	0.6						0.6	2.4						
Simulidae								0.1			0.1		0.8		
Sphaeriidae														0.0	
Corydalidae		6.7			0.9									1.1	
Notonectidae					0.0										
Chironomidae					0.4				0.1				0.1		
Oligochaetes															
Tipulidae								0.6			5.0	7.8		1.2	
Psephenidae					0.9			0.6	0.1	0.3	0.9	0.1			
Hirudinea										0.5	0.5			0.2	
Coleptera larvae								0.1		0.1	1.3		0.0		
Corbicula fluminea															
Ranatra															
Terrestrial															
UID Terrestrial		0.5	11.1	0.2	6.8	1.4	14.3	8.2	9.0	3.5	5.3	0.2	11.0	0.9	0.1
Odonata						1.7						2.9	0.4		4.5
Bee/wasp						0.9				0.2					
Coccinellidae						0.4					0.0				
Simuliidae															4.2
Coleoptera	3.7				2.6			2.4		4.5	3.8	2.2		0.0	0.4
Gastropoda											0.0				
Lepidoptera															
Oligochate					0.1			0.1							
Araneae									1.6				0.0		
Ant															
Unknown	4.7	0.1	11.1	10.2	2.2	0.6	51.2	4.4	1.5	63.6	7.8	1.8	4.1	1.1	

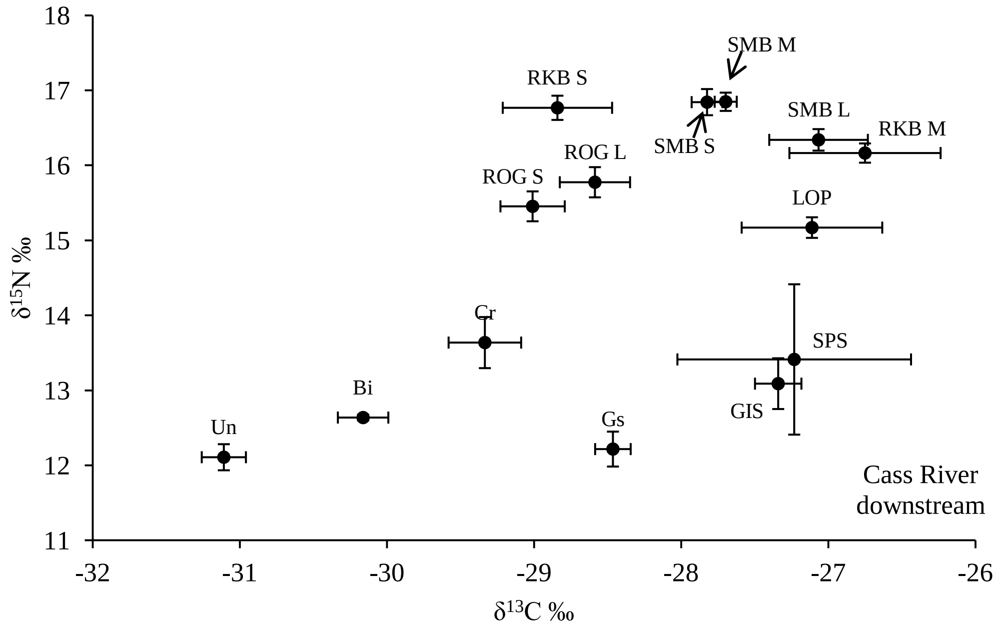

Figure A1. Average carbon and nitrogen stable isotope signatures of the aquatic food web in the downstream Cass River. Error bars for both $\delta^{13}C$ and $\delta^{15}N$ indicate ±1 SE. Un = unionid mussel, Bi = benthic invertebrates, Cr = crayfish, Gs = gastropods, GIS = Gizzard Shad, SPS = Spotfin Shiner, LOP = Log Perch, ROG S = Round Goby small, ROG L = Round Goby Large, RKB S = Rock Bass small, RKB M = Rock Bass medium, SMB S = Smallmouth Bass small, SMB M = Smallmouth Bass medium, and SMB L = Smallmouth Bass large.

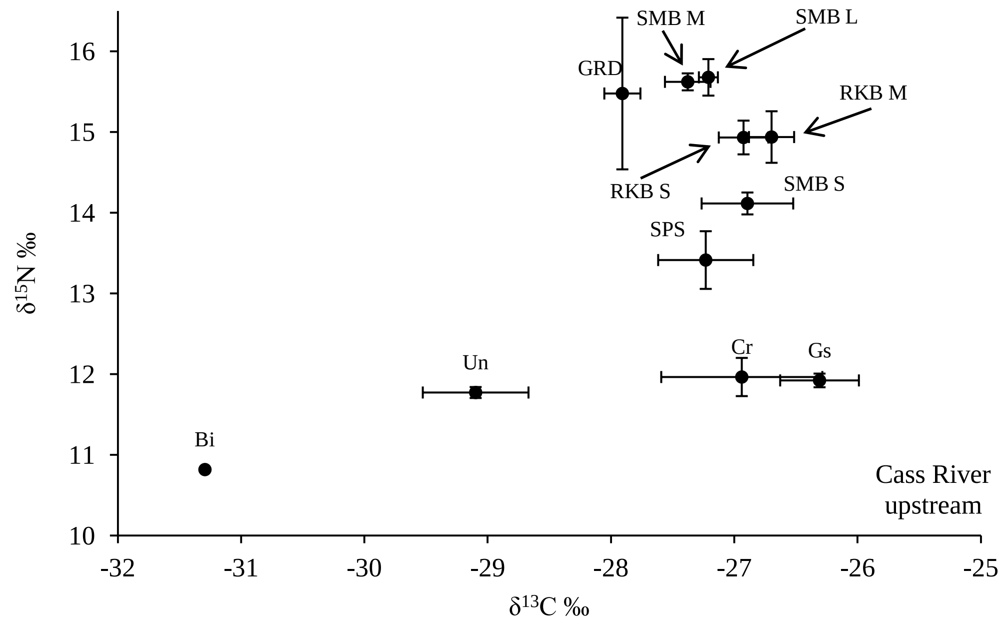

Figure A2. Average carbon and nitrogen stable isotope signatures of the aquatic food web in the upstream Cass River. Error bars for both $\delta^{13}C$ and $\delta^{15}N$ indicate ±1 SE. Un = unionid mussel, Bi = benthic invertebrates, Cr = crayfish, Gs = gastropods, GRD = Greenside Darter, SPS = Spotfin Shiner, RKB S = Rock Bass small, RKB M = Rock Bass medium, SMB S = Smallmouth Bass small, SMB M = Smallmouth Bass medium, and SMB L = Smallmouth Bass large.

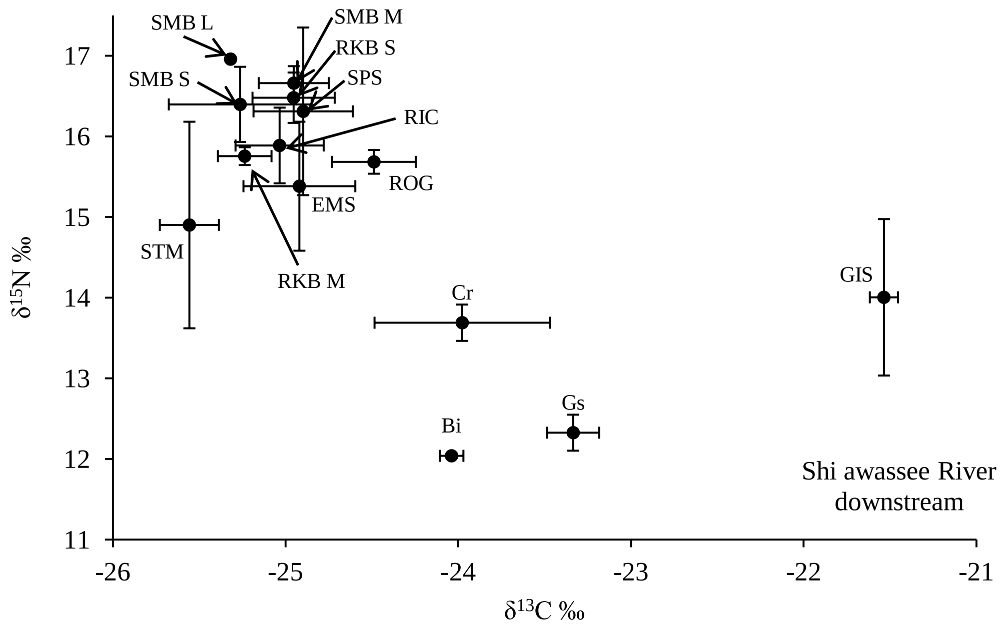

Figure A3. Average carbon and nitrogen stable isotope signatures of the aquatic food web in the downstream Shiawassee River. Error bars for both δ^{13}C and δ^{15}N indicate ±1 SE. Un = unionid mussel, Bi = benthic invertebrates, Cr = crayfish, GIS = Gizzard Shad, Gs = gastropods, EMS = Emerald Shiner, RIC = River Chub, RKB S = Rock Bass small, RKB M = Rock Bass medium, ROG = Round Goby, SMB S = Smallmouth Bass small, SMB M = Smallmouth Bass medium, SMB L = Smallmouth Bass large, SPS = Spotfin Shiner, and STM = Stonecat Madtom.

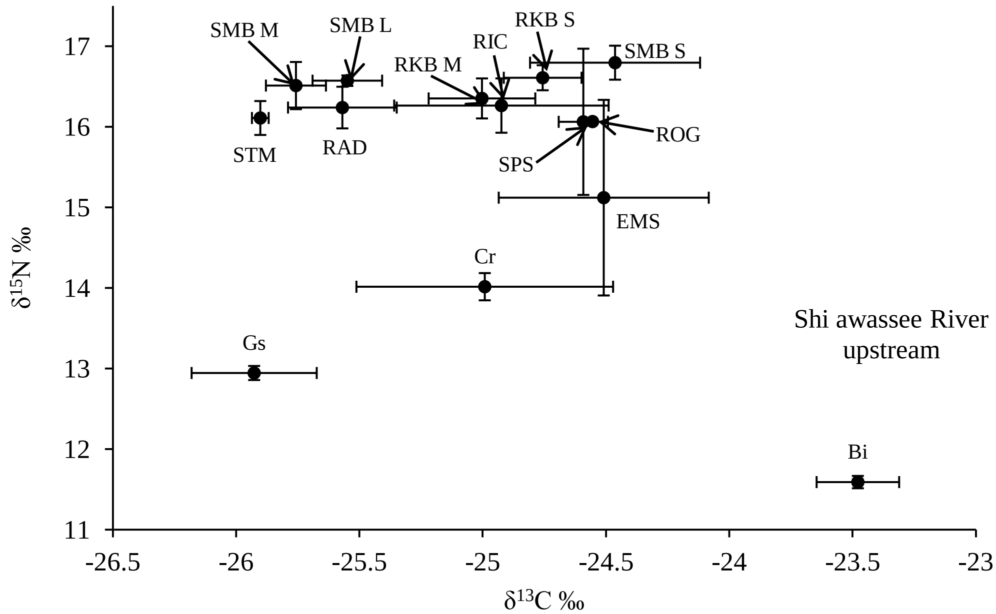

Figure A4. Average carbon and nitrogen stable isotope signatures of the aquatic food web in the downstream Shiawassee River. Error bars for both δ^{13}C and δ^{15}N indicate ±1 SE. Un = unionid mussel, Bi = benthic invertebrates, Cr = crayfish, Gs = gastropods, EMS = Emerald Shiner, RAD = Rainbow Darter, RIC = River Chub, RKB S = Rock Bass small, RKB M = Rock Bass medium, ROG = Round Goby, SMB S = Smallmouth Bass small, SMB M = Smallmouth Bass medium, SMB L = Smallmouth Bass large, SPS = Spotfin Shiner, and STM = Stonecat Madtom.

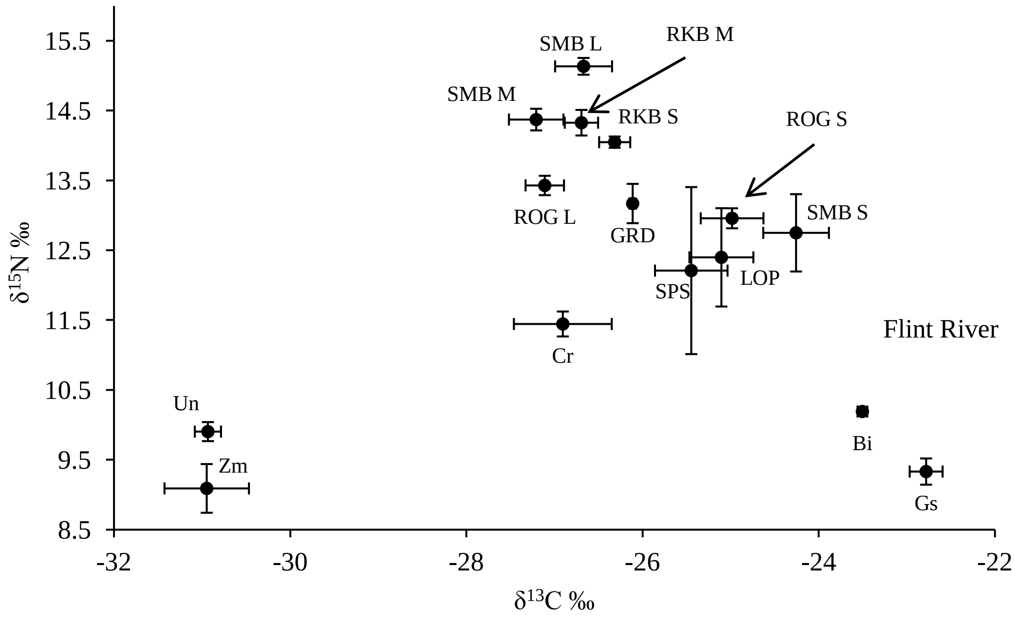

Figure A5. Average carbon and nitrogen stable isotope signatures of the aquatic food web in the downstream Shiawassee River. Error bars for both $\delta^{13}C$ and $\delta^{15}N$ indicate ±1 SE. Un = unionid mussel, Bi = benthic invertebrates, Cr = crayfish, GRD = Greenside Darter, Gs = gastropods, EMS = Emerald Shiner, LOP = Logperch, RKB S = Rock Bass small, RKB M = Rock Bass medium, ROG = Round Goby, SMB S = Smallmouth Bass small, SMB M = Smallmouth Bass medium, SMB L = Smallmouth Bass large, SPS = Spotfin Shiner, and Zm = zebra mussel.