FOOD WEB STRUCTURE AND VARIATION IN THE GILA RIVER, USA

by

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Abstract

The upper Gila River basin in southwest New Mexico, USA is one of the few unimpounded drainage basins in North America and is a stronghold for the unique and endemic fishes west of the Continental Divide. Multiple non-indigenous fishes have been introduced to the Gila River and are a potential threat to native fishes, yet very little is known of the trophic ecology of the native and nonnative fishes. We used diet and stable isotopes collected from native and nonnative fishes to identify their trophic relationships and evaluate potential interactions in the upper Gila River basin during June-July, 2007 and 2008. Diet and stable isotope data indicated aquatic invertebrates were the primary food for both native and nonnative fishes. Native large-bodied fishes were mainly algivore/detritivores and native small-bodied fishes were primarily insectivores. Small-bodied nonnative fishes fed on detritus and aquatic invertebrates. Nonnative predators preyed on small-bodied fishes and predaceous aquatic invertebrates and had higher trophic positions than all native fishes. Although nonnative predators did not rely exclusively on native fishes as prey, their presence extended community food-chain lengths, and the combined predation on juvenile native fishes by multiple apex predators may threaten persistence of native fishes. The lack of concise evidence for negative effects suggested that impacts of nonnative predators were more subtle and confirmed the underlying complexity of a relatively simple community

The extensive database on feeding relations of Gila River fishes allowed us to further understand how energy moves through ecosystems. Specifically, the goal of chapter two was to characterize variation in fish-community food web structure within and among study reaches on the Gila River using ^{13}C and ^{15}N stable isotopes. We hypothesized that food web structure would reflect variation in fish community structure, resource availability and environmental conditions across habitats. Food web structure in isotope bi-plot space was estimated using community-wide measures of trophic structure, mean trophic position, and food-chain length. Permutational multivariate analysis of variance indicated that indices of food web structure were more variable among than within reaches and this pattern was primarily associated with variation in trophicl area occupied by taxa in isotope bi-plot space and mean trophic position of those taxa. Variation in food web structure was significantly associated with fish species richness across macrohabitats but was weakly associated with abiotic reach-scale factors. Variation in food web

structure was concordant with variation in fish community composition and suggested that factors influencing the distribution of fishes also influence food web structure.

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CHAPTER 1 - Food web structure and interactions in the Gila River: implications for native fish conservation[1]

ABSTRACT

Diet and stable isotopes of native and nonnative fishes were used to identify trophic relationships and evaluate potential interactions among native and nonnative fishes in the upper Gila River basin during June-July, 2007 and 2008. These data indicated aquatic invertebrates were a common food for both native and nonnative fishes and that both were highly omnivorous. Native large-bodied fishes were mainly algivore/detritivores and native small-bodied fishes were primarily insectivores. Small-bodied nonnative fishes fed on detritus and aquatic invertebrates. Nonnative predators preyed on small-bodied fishes and predaceous aquatic invertebrates and had significantly higher trophic positions than small and large-bodied native fishes. Although nonnative predators did not rely exclusively on native fishes as prey, their presence extended community food-chain lengths. The combined predation on juvenile native fishes by multiple apex predators might threaten persistence of native fishes. However, the high degree of omnivory suggested that impacts of nonnative predators may be more subtle and dependent on environmental variability.

INTRODUCTION

Freshwater ecosystems are becoming increasingly threatened by human activities and the ability to manage these systems is limited by an incomplete understanding of the effects of anthropogenic stressors (Naiman & Turner 2000). Human-induced habitat modifications and establishment of nonnative species have been implicated as a major cause for declines of native freshwater fishes of North America (Minckley & Deacon 1991; Jelks et al. 2008). Species introductions are facilitated by anthropogenic alterations of freshwater systems, so assessing the effects of nonnative species is likely confounded by habitat modifications such as channelization or impoundment (Bunn & Arthington 2002). Unfortunately, there are few unaltered systems in

[1] This chapter has been formatted for submission to the journal Ecology of Freshwater Fish

which to evaluate interactions among native and nonnative fishes, which are critical for separately identifying the relative importance of these stressors.

Negative interactions among native and nonnative fishes are common in aquatic systems, and include displacement through competitive interactions (Douglas et al. 1994; Flecker & Townsend 1994; Taniguchi et al. 2002) and effects of predators (Ross 1991; Bryan et al. 2002). In particular, the introduction of nonnative predators can drastically alter food web interactions, and, by extension, ecosystem functioning of native communities. For example, introduced smallmouth bass *Micropterus dolomieu* and rock bass *Ambloplites rupestris* displaced native lake trout *Salvelinus namaycush* thereby decreasing the mean trophic position of lake trout (Vander Zanden et al. 1999). Invasion-mediated shifts in the trophic niche of native fishes can also result in trophic cascades (Flecker & Townsend 1994; Bohn & Amundsen 2001) or affect reciprocal subsidies between streams and riparian forests (Baxter et al. 2004). Despite the evidence for negative interactions among native and nonnative fishes, many invasions of lotic systems have few observed effects on native species (Moyle & Light 1996), and understanding the context in which nonnative species become harmful is essential for predicting their effects on native communities (Parker et al. 1999).

Much evidence for negative interactions among native and nonnative fishes comes from cold water systems (Fausch et al. 2001), but a few, sometimes contrasting, examples exist for warm water streams. Eby et al. (2003) observed persistence of native species despite the presence of multiple nonnative fishes, such as red shiner *Cyprinella lutrensis*, yellow bullhead *Ameiurus natalis*, and green sunfish *Lepomis cyanellus*, in Aravaipa Creek, Arizona. In the Green and Yampa rivers, Colorado, nonnative predators (smallmouth bass *Micropterus dolomieu*, northern pike *Esox lucius*, and channel catfish *Ictalurus punctatus*) have been implicated in the decline of small-bodied native fishes (Tyus & Beard 1990; Tyus & Nikirk 1990; Tyus & Saunders 2000; Johnson et al. 2008). In the Cosumnes River, California, introduction of green sunfish, largemouth bass *Micropterus salmoides*, and redeye bass *Micropterus coosae*, are likely responsible for the decline and extirpation of native fishes (Moyle et al. 2003). Unfortunately, potentially complex interactions, such as size-dependent effects of introduced fishes (Mills et al. 2004), make predicting the consequences of invasion difficult because many assemblages have multiple nonnative species that increase the complexity of community food web interactions (Kiesecker & Blaustein 1998; Nystrom et al. 2001).

The upper Gila River basin in southwest New Mexico provided an opportunity to characterize the role of nonnative fishes in the food web of an arid-land stream with relatively low human influence. Land use in the upper forested watershed is mostly restricted to low-impact outdoor recreation, dispersed livestock grazing, and sparse human settlement. Downstream portions of the basin have been moderately influenced by humans (minimal water diversion, livestock grazing, and scattered human settlements). Despite its relatively natural flow regime, declines in abundances and occurrences of native fishes coincided with establishment of nonnative fishes (Propst et al. 2008). Thus, our primary objective was to characterize trophic linkages among native and nonnative fishes in the upper Gila River. We analyzed diets from stomach contents which provided a direct characterization of resource use over short temporal scales (<24 h), and stable isotopes (^{13}C and ^{15}N) to evaluate energy pathways integrated over longer timescales. Specific goals were to 1) characterize the food webs in different reaches of the Gila River that vary in abundance of nonnative predators, 2) quantify resource overlap among native and nonnative fishes, and 3) quantify the effects of nonnative predators on native food webs. Understanding these trophic linkages and relations among native and nonnative fishes will be helpful in making management decisions for the highly endangered fauna of the Gila River basin.

STUDY AREA

We sampled sites on four major tributaries and the mainstem Gila River (Figure 1.1). The Upper Gila River (West, Middle, and East forks) originates in the Mogollon Mountains of southwestern New Mexico and flows in a westerly direction into Arizona. The San Francisco River begins in eastern Arizona, flows into New Mexico continuing back into Arizona to join the Gila River near Clifton, Arizona. Riparian vegetation ranges from fir and aspen at high elevations to Chihuahua desert scrub at lower elevations (Brown 1982). Study sites on the Gila and San Francisco rivers matched long-term fish community monitoring sites (see Propst et al. 2008) and represented a gradient of stream sizes with catchment areas of 295 to 4,828 km^2. The Upper Gila River sites (East Fork, Middle Fork, West Fork, and Heart Bar) have watersheds that are almost completely within federal lands, including the Gila and Aldo Leopold National Wildernesses, and were almost entirely undisturbed except for dispersed livestock grazing in the East Fork Gila River drainage. The San Francisco River site was near the village of Glenwood,

downstream of a broad valley used for livestock grazing and irrigated agriculture, and was approximately 1.3 km upstream of an irrigation diversion. The site on the Gila River mainstem was near Cliff, New Mexico and was 12 km downstream of irrigation diversions and had seasonal livestock grazing in the riparian corridor.

METHODS

Sampling methods

Large-bodied fishes(i.e., species whose maximum total length exceed 100 mm) were categorized into three age-classes (juvenile, sub-adult, and adult) based on length-frequency histograms (unpublished data) to incorporate ontogenetic shifts in resource use. Large-bodied species were headwater chub *Gila nigra* (<70 mm, 70 to 150 mm, > 150 mm), Sonora sucker *Catostomus insignis* (<100 mm, 100 to 160 mm, >160 mm), and desert sucker *Pantosteus clarki* (<100 mm, 100 to 160 mm, >160 mm), yellow bullhead (<75 mm, 75 to 130 mm, >130 mm), rainbow trout *Oncorhynchus mykiss*, and brown trout *Salmo trutta* (<80 mm, 80 to 140 mm, >140 mm), and smallmouth bass (<80 mm, 80 to 185 mm, >185). Small-bodied species (i.e., species with maximum total length <100mm) were longfin dace *Agosia chrysogaster*, spikedace *Meda fulgida*, speckled dace *Rhinichthys osculus*, loach minnow *Tiaroga cobitis*, red shiner, and western mosquitofish *Gambusia affinis,* were considered a single group dominated by age-1 individuals.

Fishes, invertebrates, and basal energy sources for stable isotope analysis were collected from the six sample sites in June-July 2007 and 2008. Fishes were collected from one to five pool and riffle complexes using a combination of seining (4.6 m X 1.2 m seine with 3.2 mm mesh) and electrofishing (Smith-Root Model LR24 backpack shocker). Each habitat complex was sampled intensively until no additional fish species were collected. A maximum of five individuals were collected to represent species and size-classes present at sites. A 5 mm diameter biopsy punch was used to extract dorsal muscle from individuals > 150 mm and individuals <150 mm were collected whole. Alimentary canals of Sonora sucker, desert sucker, and all nonnative fishes were removed and preserved in 10% formalin. A modified gastric lavage technique was used to extract gut contents of adult headwater chub. A 60 cc syringe with a 30 cm long piece of flexible tubing (3 mm, outside diameter) was filled with water and inserted down the esophagus to flush stomach contents, which were captured in sealable plastic bag and

preserved in 10% formalin. Fishes < 150 mm were placed on ice and later frozen for isotope tissue samples and diet. Aquatic invertebrates were sampled from multiple habitats within each site using kick nets and by scrubbing rocks. Numerically dominant invertebrate groups, Ephemeroptera (Baetidae, Heptageniidae, and Leptohyphidae), Trichoptera (Hydropsychidae), Megaloptera (Corydalidae) and Diptera (Tabanidae) were sorted and separated into containers of freshwater overnight to allow gut evacuation (Jardine et al. 2005). Basal energy sources were collected from each site and included small detritus (< 30 mm) from debris piles in pools and low velocity habitats, filamentous algae (when present), dominant bank vegetation (primarily willow and grass), and emergent vegetation. Fine particulate organic matter (FPOM) was scraped from substrates into a sealable plastic bag. All isotope samples were kept on ice until they could be stored in a freezer (-20 °C).

We characterized the diet of native and nonnative fishes collected for isotopic analysis and additional nonnative fishes collected from a nonnative removal study near the Heart Bar site to compare diet with stable isotope signatures. Diet was quantified from contents of the anterior portion of the gut to the first bend of the digestive tract (Bowen 1996). Gut contents were spread on a clear petri dish placed over a 1.8 mm grid and the area of each item was recorded. The area covered by each diet item was assumed to be proportional to its dry weight. We validated this assumption by comparing dry weight of diet items to grid area in a subset of samples ($n = 148$, $r^2 = 0.581$, $P < 0.001$). Gut contents were identified taxonomically for animals (order and family for invertebrates, family for fish, if identification possible) or classified as filamentous algae or detritus, which included aquatic and terrestrial derived plant material. If gut contents included fine particulate organic matter (e.g., diatoms), area was measured as above, then a subsample of that material was viewed at 100X magnification using a compound microscope. The percentage of organic matter (primarily diatoms) in the subsample was estimated under the microscope and this percentage was extrapolated to the entire sample to yield the estimated area for the entire contents.

Dorsal muscle was used to measure stable isotope signatures because it has lower variability in $\delta^{15}N$ than other tissues, acidification to remove inorganic carbonates is not necessary (Pinnegar & Polunin 1999), and it does not require lipid extraction because of relatively low lipid content compared to other tissues (Sotiropoulos et al. 2004; Ingram et al. 2007). Muscle tissue was taken from a maximum of five individuals for small-bodied species

and five individuals per age-class for large-bodied species. Light and heavy fractions of FPOM were separated by centrifuging in colloidal silica as described by Hamilton et al. (2005). The light fraction was primarily single-celled algae, whereas the heavy fraction was primarily composed of detritus. All FPOM samples were acidified to remove inorganic carbonates. Isotope samples were dried for 48 h at a constant temperature (60 °C) then homogenized using a mortar and pestle. Powdered samples were analyzed for $\delta^{15}N$ and $\delta^{13}C$ with a ThermoFinnigan Delta Plus mass spectrometer with a CE 1110 elemental analyzer and Conflo II interface in continuous flow mode (CF-IRMS) in the Stable Isotope Mass Spectrometry Laboratory (SIMSL) at Kansas State University. Stable isotope ratios were expressed as parts per thousand (‰) and calculated in the standard notation:

$$\delta X = [(R_{sample} / R_{standard}) - 1]*1000$$

where $R = {}^{15}N/{}^{14}N$ or ${}^{13}C/{}^{12}C$. The $\delta^{13}C$ values for all organisms were corrected for lipids using C:N ratios for animals and %C for plants following Post et al. (2007) because the organisms of interest in reconstructing the food webs likely had variable lipid contents. Overall, there was little variability among tissue samples from the same species collected at each site; mean coefficient of variation (CV_{13C}) = 3.7 ± 2.7% and (CV_{15N}) = 5.1 ± 4.4%.

Data analysis

Characterizing the stream food web

Diet data were used to estimate trophic position for species and age-classes at each site following the formula:

$$TP_{diet} = \Sigma(V_i \cdot T_i) + 1$$

where TP_{diet} = the trophic position of a species weighted by V_i = proportion of ingested material of the *i*th prey item, and T_i = trophic position of the *i*th prey item (*sensu* Vander Zanden et al. 1997). We calculated the relative percentage of ingested material from each prey item for species and age-classes by site using the area of each prey item. Trophic positions of prey items were assigned by major taxonomic groups ranging from algae and detritus (trophic level 1.0) to predaceous invertebrates and fish (trophic level 3.0; Table 1.1). Because trophic position can vary greatly within macroinvertebrate taxonomic groups, we assigned trophic positions based on the functional group (sensu Merritt & Cummins 1996) of the majority of members of the group (e.g., filterers = 2.0 or predators = 3.0).

Trophic positions of fishes based on $\delta^{15}N$ values were standardized at each site to the $\delta^{15}N$ signature of a primary consumer following the equation of Cabana and Rasmussen (1996):

$$TP_{isotope} = [(\delta^{15}N_{fish} - \delta^{15}N_{ephem})/3.4] + 2$$

We chose $\delta^{15}N$ values of Ephemeropterans as the baseline because they were abundant at all sites and their $\delta^{15}N$ was similar to other dominant primary consumers where collected (e.g., chironomids). We used linear regression to evaluate the relationship between diet and stable isotope derived measures of trophic position. Linear regression helped interpret discrepancies among these methods that provide inferences over different temporal scales.

To compare diets of native and nonnative fishes we calculated percent similarity in diet among species/age-classes and across sites based on the percentage of ingested material of each prey item. Principal coordinates analysis (PCoA) was used to ordinate samples based on the matrix of similarities to visualize differences in diet among species/age-classes. Calculations for similarity and PCoA were performed in R (R Development Core Team 2008) using the labdsv package (Roberts 2007).

Resource overlap

To assess resource overlap among native and nonnative fishes, species were categorized into four size-groups: native large-bodied (NL), native small-bodied (NS), nonnative large-bodied (NNL), and nonnative small-bodied fishes (NNS; Table 2). Native large-bodied fishes included adults and sub-adults of native suckers and headwater chub. Native small-bodied fishes included native small-bodied minnows, juvenile headwater chub and juvenile suckers. Nonnative trout, adult and sub-adult sunfish and bass, and adult and sub-adult catfish were grouped as NNL. Juveniles of these nonnative fishes along with red shiner and western mosquitofish were grouped as NNS. Although these size-groups precluded analysis of overlap at the species level, grouping was consistent with ecological and life-history traits of species (see data in Olden et al. 2006), and allowed for greater statistical power when comparing groups of native and nonnative fishes. We used discriminant function analysis (DFA) with leave-one-out cross validation to evaluate our ability to classify species/age-classes into one of the four size-groups of native and nonnative fishes based on percentages of prey items found in gut contents of species/age-classes across our sites. The DFA also allowed us to evaluate the similarity of species diets and identify prey items used by different size-groups of fishes. In addition, we used multivariate analysis of covariance (MANCOVA) to assess differences in isotopic signatures

among size-groups of native and nonnative fishes. Dependent variables were the $\delta^{13}C$ and $\delta^{15}N$ signatures of species/age-classes, native and nonnative size-groups were independent factors, and site and year were co-variables. *Post hoc* comparisons were made using separate ANOVAs. MANCOVA and DFA calculations were performed using SPSS for Windows (version 11.0.1, SPSS Inc., Chicago, Illinois).

Effects of nonnative predators on native food webs

A constrained analysis of principal coordinates (CAP) was used to evaluate the relationship between log (x+1) density of nonnative predators (i.e., large-bodied nonnative fishes) and variation in the relative percentages of prey items in the diets of native fishes across sites. Nonnative predator density was based on long-term data from Propst et al. (2008). Constrained analysis of principal coordinates was performed in R using the vegan package (Oksanen et al. 2008)

Stable isotopes also allowed us to test for shifts in trophic ecology of native fishes in the presence of nonnative predators. We used the $\delta^{13}C$ and $\delta^{15}N$ ranges of native fishes at each pool/riffle complex and the mean trophic positions of native fishes combined to evaluate a potential trophic shift in the feeding ecology of native fishes in the presence of nonnative predators. If nonnative predators constrain native fish diets to low quality food (i.e., algae and detritus), we would expect a decrease in $\delta^{13}C$ and $\delta^{15}N$ ranges of native fishes as well as a decrease in native fish mean trophic position. We used MANOVA to test for differences in mean native trophic position and $\delta^{13}C$ and $\delta^{15}N$ ranges in pool/riffle complexes across sites where nonnative predator fishes were present versus where absent. Separate MANOVAs were run to test between presence and absence of yellow bullhead, smallmouth bass, rainbow trout, brown trout, or any nonnative predator. *Post hoc* ANOVAs were used to test for univariate differences in response variables. In addition, we used correlation analysis to test for an association between nonnative predator density and trophic positions of longfin dace, Sonora sucker, and desert sucker (only native species occurring at five or more sites were used in the analysis).

The extent to which nonnative predators consume fish and other resources was evaluated with the IsoSource routine (Phillips & Gregg 2003). To satisfy isotopic mass balance of consumers, sources were corrected for trophic fractionations of nitrogen (3.4‰ per trophic level; Post 2002) and carbon (0.5‰ per trophic level; McCutchan et al. 2003) prior to inclusion in the

model. Because isotopic signatures of sources were naturally variable, we allowed a mass balance tolerance of 0.5 δ units for solutions which were examined at 2% increments. We report both mean and range of each source contribution as the mean alone does not represent the true contribution (Phillips & Gregg 2003). Despite our efforts to collect isotope data from as many sources as possible, IsoSource could not estimate contributions for yellow bullhead, rainbow trout or brown trout at the Heart Bar site from the available sources.

RESULTS

Characterizing the stream food web

Diets of 996 individuals representing seven native and nine nonnative species were analyzed from the six sites. Native small-bodied fishes were primarily insectivorous (Figure 1.2A; Appendix A). Ephemeroptera nymphs made up the largest percent volume of small-bodied native fishes diet (range 12.8 to 53.8% of diet per species/age-class), but chironomid, and simuliid larvae were generally the most frequently consumed items (31.0 to 79.0% of individuals). Adult Sonora sucker and desert sucker were omnivores consuming algae/detritus (16.0 to 74.0% of volume), as well as Ephemeroptera, chironomid, and simuliid larvae (33.0 to 91.0 % of individuals), but in low volume. Headwater chub was the only native species found to be piscivorous. Fish were found in guts of adults (18.0% of individuals, 19.7% of volume) and sub-adults (27.0% of individuals, 53.8% of volume), but algae was frequently found (55.0% of individuals) and was a large percentage (46.8% of volume) of adult diets.

Nonnative species consumed a greater diversity of invertebrates and more fish than native species. In addition, nonnative fishes preyed on predaceous invertebrates and terrestrial invertebrates more frequently than native fishes (Figure 1.2B; Appendix A). Nonnative trout consumed a wide variety of benthic invertebrates as well as terrestrial invertebrates. On average, the diets of nonnative predators were comprised of 25% fish, although this was highly variable (yellow bullhead-12%, channel catfish-6%, green sunfish-31%, smallmouth bass-23%, rainbow trout-8%, flathead catfish *Pylodictis olivaris*-84%, and brown trout-10%). Of the fish prey, 64% were suckers, 6% were minnows, 29% were unknown fish, and one age-0 smallmouth bass was found in the stomach of an adult yellow bullhead. Nonnative red shiner (n = 6), and western mosquitofish (n = 53) fed primarily on algae, detritus and Ephemeroptera.

The $\delta^{13}C$ of filamentous algae was highly variable across sites (-35.5 to -17.7‰) and often did not overlap with invertebrates or fish (Figures 3-5). In contrast, the light fraction of FPOM, which was predominately algae, had similar $\delta^{13}C$ to fish and invertebrates (-29.1 to -23.4‰) and also had $\delta^{15}N$ signatures somewhat depleted to herbivorous invertebrates (1.8 to 6.5‰). Large detritus collected in streams had less variable $\delta^{13}C$ signatures than filamentous algae (-29.1 to -26.0‰), but had variable and depleted $\delta^{15}N$ signatures (-3.4 to 3.3‰). The heavy fraction of FPOM was more enriched in $\delta^{15}N$ than large detritus (1.4 to 6.2 ‰), but was similar in $\delta^{13}C$ to invertebrates and fish (-27.2 to -21.2‰). Stream bank vegetation, which was mainly C3 plants, had variable $\delta^{13}C$ values (-29.2 to -20.9‰) that were generally more depleted than fish $\delta^{13}C$ values. The $\delta^{15}N$ of primary producers and detritus was highly variable within and among sites (-3.0 to -6.4‰). Aquatic invertebrates had similar $\delta^{13}C$ values as fishes, but had depleted $\delta^{15}N$ values, which was consistent with the predominance of invertebrates in the diet of fishes. The $\delta^{15}N$ of predaceous invertebrates overlapped with the most depleted fishes and herbivorous invertebrates were about 1‰ lower than predaceous invertebrates.

Tissue samples from 787 fishes were analyzed for $\delta^{13}C$ and $\delta^{15}N$ signatures. When present, riffle-dwelling fish (speckled dace and loach minnow) had the most depleted $\delta^{13}C$ values (-27.4 to -24.1‰) compared to other fishes (Table 1.2). Nonnative adult and sub-adult yellow bullhead, channel catfish, flathead catfish, smallmouth bass, rainbow trout, and brown trout generally had more enriched $\delta^{15}N$ values (11.2 to 14.8‰) than native fishes.

There was a significant relationship between diet- and isotope-based calculations of trophic position ($r^2 = 0.49$, $P < 0.001$). The slope of this relationship was < 1 (Figure 1.6, slope 95% CI = 0.39 – 0.78) and generally reflected higher trophic position assignment based on stable isotopes for species feeding at lower trophic positions. Western mosquitofish and juvenile headwater chub diet-estimated trophic positions, however, were higher than isotopic-estimated trophic positions. These two species had high percentages of detritus and algae along with variable percentages of invertebrates (Appendix A) in their diet. Removal of these species increased the explanatory power of this relationship ($r^2 = 0.62$). Regardless of method, adult and sub-adult nonnative predators had greater trophic positions than native species.

Variation in diet across sites, species age-classes, and years was summarized by PCoA (Figure 1.7). Species/age-class scores plotted on the first two axes showed a strong fit to the matrix representing percent similarity of diet among species and age-classes (Mantel R = 0.72, *P*

< 0.001). Native fish with a high percent of algae in their diet had high first axis scores. Other native and nonnative fishes had intermediate to low first axis scores associated with invertebrates. The greatest separation of native and nonnative fishes was observed along the second axis in which positive scores were associated with chironomid larvae, simuliid larvae, Coleoptera and Ephemeroptera. Most nonnative fishes had negative second axis scores and were associated with terrestrial and predaceous invertebrates (e.g., hellgrammites, belostomatids, and naucorids) and fish.

Resource overlap

Discriminant function analysis produced three distinct groups: native fishes, NNL, and NNS (Figure 1.8). Of the four pre-specified groups, NS and NNL were the most distinctly separate groups along the first axis. The first axis explained 71% of the variation among samples and contrasted species and age-classes that consumed fish, predaceous invertebrates and corixids with those that consumed algae and larval chironomids and simuliids. The second axis explained 25% of variation among samples and contrasted fish that consumed algae and Ttrichoptera larvae with those that consumed terrestrial invertebrates and Ephemeroptera. Leave-one-out cross validation of models correctly classified 61% of species/age-classes and was most accurate at predicting NNL (74%) and NS (70%). Native large-bodied fishes were classified as NS equally as often as they were correctly classified (46%), and NNS were classified more often as NS (46%) than they were correctly classified (36%).

Overall, there was little variability in mean fish $\delta^{13}C$ and $\delta^{15}N$ between years (MANCOVA, n = 137; $\delta^{15}N$ $F_{1,131} = 2.58$, $P = 0.110$, $\delta^{13}C$ $F_{1,131} = 1.69$, $P = 0.196$), yet there was significant variation in $\delta^{13}C$ and $\delta^{15}N$ among the five sites where nonnative fishes were present ($\delta^{15}N$ $F_{1,131} = 50.08$, $P < 0.001$; $\delta^{13}C$ $F_{1,131} = 39.85$, $P < 0.001$). Although this test indicated groups had different $\delta^{13}C$ signatures ($F_{3,131} = 3.40$, $P = 0.020$), all comparisons between groups had similar $\delta^{13}C$ values ($P > 0.15$ for all comparisons), except that between NS (estimated marginal mean = -25.1‰) and NNL (-23.9‰, $P = 0.018$). However, the difference between these groups was minimal and we did not consider it to be biologically significant. There were differences among groups in $\delta^{15}N$ signatures ($F_{3,131} = 12.94$, $P <0.001$). Nonnative large-bodied fishes were the most enriched in $\delta^{15}N$ (estimated marginal mean = 10.4‰) and were higher than NL (8.7‰, $P <0.001$) and NS (9.4‰, $P = 0.002$). Nonnative small-bodied

fishes (9.8‰) were more enriched than NL (P =0.027), but were not different from the other two groups ($P > 0.957$ for both comparisons).

Combined, the analyses indicated two general results. First, the greatest degree of overlap of diet and stable isotopes occurred among native large-bodied, native small-bodied, and nonnative small-bodied fishes. Second, nonnative large-bodied fishes were the most distinct group having the most enriched $\delta^{15}N$ signatures and having a diet comprised primarily of predaceous aquatic invertebrates and fish.

Effects of nonnative predators on native food webs

Constrained analysis of principal coordinates indicated that nonnative predator density was not associated with diet of native fishes (pseudo-F = 1.02, P = 0.42). Presence or absence of a nonnative predators did not affect $\delta^{13}C$ and $\delta^{15}N$ ranges or mean trophic position of native fishes, except mean native trophic position in pool/riffle complexes where nonnative trout were present was greater than in the absence of nonnative trout (rainbow trout $F_{1,34}$ = 9.83, P = 0.004; brown trout $F_{1,34}$ = 10.26, P = 0.003). Correlation analysis between trophic positions of native fishes and nonnative predator density ranged from -0.59 to 0.05 (Table 1.3), but these relationships were not significant (all $P > 0.1$).

IsoSource model estimates based on $\delta^{13}C$ and $\delta^{15}N$ did not support a constrained energy source for nonnative predators. Rather, estimates of resource use of prey items were highly variable (Appendix B). For example, predaceous invertebrates likely contributed the most to isotope signatures of yellow bullhead at Middle Fork (1-99 percentile: 0 – 60%), whereas the greatest contribution to yellow bullhead at West Fork came from longfin dace and juvenile headwater chub (0 – 67% for both) and at East Fork from longfin dace and juvenile Sonora sucker (0 – 60% and 0 – 67% respectively). Similarly, predaceous invertebrates made high contributions to rainbow trout and brown trout at Middle Fork (53 – 84% and 46-84% respectively), but according to the model estimates, these trout preyed mainly on headwater chub at West Fork. Detritus was generally not an important source for nonnative predators; however, it was for yellow bullhead at Middle Fork and East Fork, and to flathead catfish at Riverside.

DISCUSSION

Moyle and Light (1996) hypothesized that the most successful fish invasions in unaltered streams could be explained by the invaders' trophic ecology. They predicted that top predators

and omnivore/detritivores would be the most successful invaders because of abundant food supplies during the establishment and integration phases of invasion. Data from the Gila River food web partially supported this prediction in that predatory fishes that feed on fishes and predatory macroinvertebrates were the most abundant invaders in this system.

Diet and stable isotope analyses from our study sites in the Gila River basin provided evidence of a four trophic level food web when NNL were present compared to a three trophic level food web when these nonnative predators were absent. Diets of all NNL partly comprised fish, but yellow bullhead and smallmouth bass consumed notable percentages of predaceous aquatic invertebrates, whereas rainbow trout and brown trout also consumed terrestrial invertebrates, and emergent aquatic invertebrates. In general, native fishes were secondary consumers with some exceptions; headwater chub occasionally was piscivorous and although we did not detect predaceous invertebrates in the diet of headwater chub, these diet items have been recorded in the diet of roundtail chub *Gila robusta*, a closely related species present in downstream reaches of the Gila River (Schreiber & Minckley 1981) and in other populations (Quist et al. 2006). Adult Sonora and desert suckers were omnivores that fed on algae, detritus, and herbivorous invertebrates. Native small-bodied fishes were mostly invertivorous, but longfin dace and juvenile Sonora and desert suckers consumed some algae and detritus. Results from stomach contents were consistent with those of Schreiber and Minckley (1981) who studied the diets of native fishes in Aravaipa Creek, Arizona and found that most native fishes fed on Ephemeroptera nymphs, chironomid larvae, and simuliid larvae, whereas longfin dace and desert sucker had substantial percentages of filamentous algae in their diets in addition to invertebrates.

Ontogenetic diet shifts associated with life history of fishes differed among native and nonnative fishes. Suckers shifted from a higher trophic position as juveniles, primarily consuming insects, to a lower trophic position as adults, consuming more algae and detritus. In contrast, NNL had low trophic positions as juveniles (feeding primarily on Ephemeroptera and chironomid larvae) and increasing trophic position with body size (adults were piscivorous and also fed on predaceous invertebrates). Therefore, nonnative small-bodied fishes, including juvenile NNL, were more likely to overlap with native fishes while sub-adult and adult nonnative fishes were capable of preying upon small-bodied native fishes.

Estimates of trophic position from stable isotope analyses were generally greater than those calculated from stomach contents for low-trophic level fishes. Two scenarios might

explain our observations 1) omnivorous fishes with large amounts of algae and detritus in their diet are disproportionally assimilating animal tissue (Ahlgren 1990; Evans-White et al. 2001), or 2) herbivorous fishes have lower trophic fractionation than the assumed 3.4‰ resulting in inflated isotopic trophic positions (Mill et al. 2007). Whether the discrepancy between diet and stable isotopes is related to feeding habits or trophic fractionation is unknown. Although there was much variability in diet and isotope trophic position, the relationship between both methods was strongly correlated. The concordance between diet and stable isotopes validates the use of stable isotopes as a means to estimate trophic dynamics in this system.

Our data did not directly quantify the effect of nonnative predators on populations of native fishes. On average, fish comprised 25% of the diet of nonnative predators, but this may underestimate fish predation because nonnative predators consistently were more enriched in ^{15}N and had higher trophic positions than native fishes. That fish did not make up a large percent of nonnative large-bodied fish diets was not surprising because soft, small-bodied fishes can be quickly digested (Schooley et al. 2008) compared to the large and recalcitrant exoskeletons of macroinvertebrates. We also found that nonnative fishes consumed large, predaceous invertebrates, which were not found in the diet of native fishes and may have contributed to high trophic position of nonnative predators. Stable isotope mixing models of nonnative predators did not conclusively indicate predation on native fishes because nonnative predators could have assimilated material from a broad range of sources including fish and predaceous invertebrates. The primary prey fish from diet analysis was juvenile and age -0 suckers, which were the most abundant small-bodied fish under 50 mm at all sites. Predation on this age-class of native fishes by nonnative predators has been implicated in the decline of native fishes in other portions of the Colorado River basin (Marsh & Douglas 1997; Bestgen et al. 2006). Whereas nonnative fishes in the Gila River basin likely have negative effects on native fish populations, consumption of large, predaceous invertebrates may alleviate some of their demands on native fishes or potentially release larval native fish from these predaceous invertebrates (Horn et al. 1994)

Conservation implications

Despite the low level of anthropogenic disturbances to the Upper Gila River watershed, native species ranges have declined in the presence of nonnative fishes (Propst et al. 2008). The establishment of nonnative predators poses serious threats to recruitment of native fishes

elsewhere in the Colorado River basin (Bestgen et al. 2006; Johnson et al. 2008) and probably poses similar threats to Gila River basin native fish assemblages. We found nonnative predators to be apex predators of Gila River drainage food webs that were preying on native fishes, providing a mechanistic explanation for the negative effects of nonnative fishes. The generalist feeding strategy of small-bodied nonnative fishes could further affect native fishes through competition, especially if there is a high degree of overlap in habitat use. Mitigating these effects through removal and preclusion of nonnative predators and competitors, if feasible, may be necessary for conservation of native fishes in these pristine habitats.

Although native fishes have persisted with nonnative fishes at some sites in the upper Gila River basin for decades, species interactions are likely to vary across the basin (Propst et al. 2008). Negative interactions also are likely to vary seasonally, with some periods when nonnative fishes are more detrimental to native fishes than others. For example, predation of young fishes could be severe in late spring after spawning, or competition could be major factor in late spring-early summer (June and July) when flows are generally low and fish densities are highest. Understanding the factors responsible for the apparent short-term (<100 years) coexistence of native and nonnative fishes will help determine management strategies to maintain the tenuous balance between native and nonnative fishes in the upper Gila River drainage. In the upper Verde River, Arizona, native fishes have declined precipitously since the mid 1990s, clearly indicating a stressor threshold has been crossed (Rinne & Miller 2006). In the upper Gila River, the apparent coexistence of native and nonnative fishes suggests the threshold has not been reached. The declining trends in native fish abundances and occurrences (Rinne & Miller 2006; Propst et al. 2008) may be reversible if resource managers remain vigilant in their conservation efforts.

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TABLES AND FIGURES

Table 1.1 Categories of prey taxa and their estimated trophic position used for calculating trophic positions based on the diets of native and nonnative fishes in the Gila River, New Mexico, 2007-2008. Estimated trophic positions of prey categories are based on the dominant functional feeding group in each category (*sensu* Merrit and Cummins 1996). Prey categories and codes are used in principal coordinates analysis (Fig. 6).

Prey Category	Code	Estimated Trophic Position	Includes
Algae	ALG	1.0	Filamentous algae
Detritus	DET	1.0	Plant material, Amorphous detritus
Annelida	ANN	2.0	Oligochaeta
Meiofauna	MEIOF	2.5	Cladocera, Ostrocoda, Copepoda
Ephemeroptera	EPH	2.5	Baetidae, Heptageniidae, Isonychiidae
Odonata	ODO	3.0	
Hemiptera	HEM	2.5	Belostomatidae, Naucoridae
Corixidae	COR	3.0	
Megaloptera	MEG	3.0	
Trichoptera	TRI	2.0	
Lepidoptera	LEP	2.0	
Coleoptera	COL	2.5	Carabidae, Dytiscidae, Gyrinidae, Haliplidae,
Elmidae	ELM	2.0	Adult and larvae
Midge	MID	2.5	Chironomid and Simulliid larvae
Tipulidae	TIP	2.5	
Tabanidae	TAB	3.0	
Terrestrial Invertebrates	TER	2.5	Orthoptera, Hymenoptera, Unkown winged invertebrates
Fish	FISH	3.0	

Table 1.2 Overall mean ± standard deviation (combined sites and years) of $\delta^{15}N$ and $\delta^{13}C$ signatures for native and nonnative fishes, macroinvertebrates and basal carbon sources in Gila River food webs, New Mexico, 2007-2008. Fish species are grouped by size-groups used in analyses of resource overlap. Species codes are used in Figures 2-4.

Species	Species code	Number of individuals	Average $\delta^{13}C$ (‰)	Average $\delta^{15}N$ (‰)
Native Small-bodied				
Agosia chrysogaster	1	50	-24.9 ± 1.8	10.1 ± 1.1
Juvenile *Gila nigra*	2	7	-24.1 ± 1.4	8.8 ± 0.6
Meda fulgida	3	19	-25.4 ± 1.9	10.3 ± 1.6
Rhinichthys osculus	4	38	-26.9 ± 2	8.8 ± 0.8
Tiaroga cobitis	5	12	-28.1 ± 0.9	10.6 ± 1
Juvenile *Catostomus insignis*	6	82	-24.5 ± 1.9	9.3 ± 1
Juvenile *Pantosteus clarki*	7	54	-25.6 ± 2.3	8.3 ± 1.2
Native Large-bodied				
Sub-adult *Gila nigra*	9	12	-23.7 ± 0.7	8.7 ± 0.9
Adult *Gila nigra*	8	15	-23.8 ± 1.6	9.2 ± 1.1
Sub-adult *Catostomus insignis*	11	34	-24.6 ± 2.2	9.1 ± 1.5
Adult *Catostomus insignis*	10	164	-24.3 ± 1.5	9 ± 1.1
Sub-adult *Pantosteus clarki*	13	28	-24.8 ± 2.6	8.2 ± 1.4
Adult *Pantosteus clarki*	12	50	-24.2 ± 2.2	8.5 ± 1
Nonnative Small-bodied				
Cyprinella lutrensis	14	4	-24.9 ± 1.5	9.7 ± 0.3
Juvenile *Ameiurus natalis*	15	2	-26.4 ± 0.2	10.6 ± 0.7
Gambusia affinis	16	22	-23.3 ± 1.7	10.3 ± 1.5
Juvenile *Micropterus dolomieu*	17	6	-24.6 ± 1.5	9.4 ± 2
Nonnative Large-bodied				
Sub-adult *Ameiurus natalis*	19	3	-24.5 ± 1.5	10.8 ± 1.6
Adult *Ameiurus natalis*	18	41	-23.3 ± 1.3	10.3 ± 1
Ictalurus punctatus	20	3	-26.6 ± 1	11 ± 0.6
Pylodictis olivaris	21	2	-26 ± 0.8	10.9 ± 2.3
Oncorhynchus mykiss	22	23	-22.8 ± 2.2	9.3 ± 0.6
Sub-adult *Salmo trutta*	24	5	-24.9 ± 1.5	8.1 ± 0.5
Adult *Salmo trutta*	23	28	-23.4 ± 1.5	9.1 ± 0.7
Lepomis cyanellus	25	6	-21.7 ± 0.3	12.1 ± 1.1
Sub-adult *Micropterus dolomieu*	27	19	-24.5 ± 1.4	10.8 ± 1.2
Adult *Micropterus dolomieu*	26	18	-23.3 ± 1.4	11.7 ± 1.2
Macroinvertebrates				
Herbivorous invertebrates	M1	137	-27.2 ± 3	5.4 ± 1.7
Predaceous invertebrates	M2	114	-26.4 ± 2.4	6.5 ± 1.5
Orconectes virilis	M3	12	-24.9 ± 1.3	7.9 ± 0.8

Table 1.2 Continued

Species	Species code	Number of individuals	Average $\delta^{13}C$ (‰)	Average $\delta^{15}N$ (‰)
Basal Resources				
Filamentous algae	A1	34	-27.4 ± 7.1	2.8 ± 1.8
Single-celled algae	A2	16	-24.3 ± 2.3	3.3 ± 1.8
Grass	V1	17	-26.9 ± 3.4	2.4 ± 2.2
Emergent macrophytes	V2	7	-22.9 ± 2.2	5 ± 1.5
Willow	V3	17	-27.8 ± 1.3	1.4 ± 1.6
Detritus	D1	17	-27.6 ± 1.6	-0.1 ± 2.5
FPOM	D2	15	-28.4 ± 6.9	4.8 ± 1.6

Table 1.3 Mean ± SD of trophic position for *A. chrysogaster* and age-classes of *C. insignis* and *P. clarki* in the Gila River, New Mexico, 2007-2008. Nonnative predator density for each site is based on long-term monitoring at the six sites (Propst et al. 2008). Pearson product-moment correlation coefficient is for average trophic position at each site separated by years.

	East Fork	Middle Fork	Heart Bar	Riverside	San Francisco	West Fork	Pearson
Predator Density (#/m^2)	0.04	0.076	0.0058	0.0035	0.0013	0.02	
A. chrysogaster	3.2 ± 0.07	3.2 ± 0.28	3.4 ± 0.13	3.5 ± 0.12	3.1 ± 0.27	3.6 ± 0.04	-0.37
Juvenile *C. insignis*	3.0 ± 0.18	3.0 ± 0.13	3.2 ± 0.2	3.3 ± 0.07	3.1 ± 0.12	3.5 ± 0.22	-0.49
Sub-adult *C. insignis*	3.0 ± 0.04	3.0 ± 0.24	3.2 ± 0.23	3.3 ± 0.23	3.1 ± 0.08	3.4 ± 0.05	-0.12
Adult *C. insignis*	3.1 ± 0.15	2.9 ± 0.14	3.2 ± 0.16	3.2 ± 0.04	3.3 ± 0.15	3.5 ± 0.21	-0.59
Juvenile *P. clarki*	2.8 ± 0.08	3.0 ± 0.04	3.1 ± 0.18	3.3 ± 0.13	2.9 ± 0.27	3.2 ± 0.18	0.05
Sub-adult *P. clarki*	2.7 ± 0.17	2.9 ± 0.19	3.0 ± 0.06	3.1 ± 0.2		3.1 ± 0.12	-0.54
Adult *P. clarki*	2.8 ± 0.21	2.7 ± 0.25	3.00 ± 0.18	3.0 ± 0.18	3.1 ± 0.21	3.4 ± 0.19	-0.43

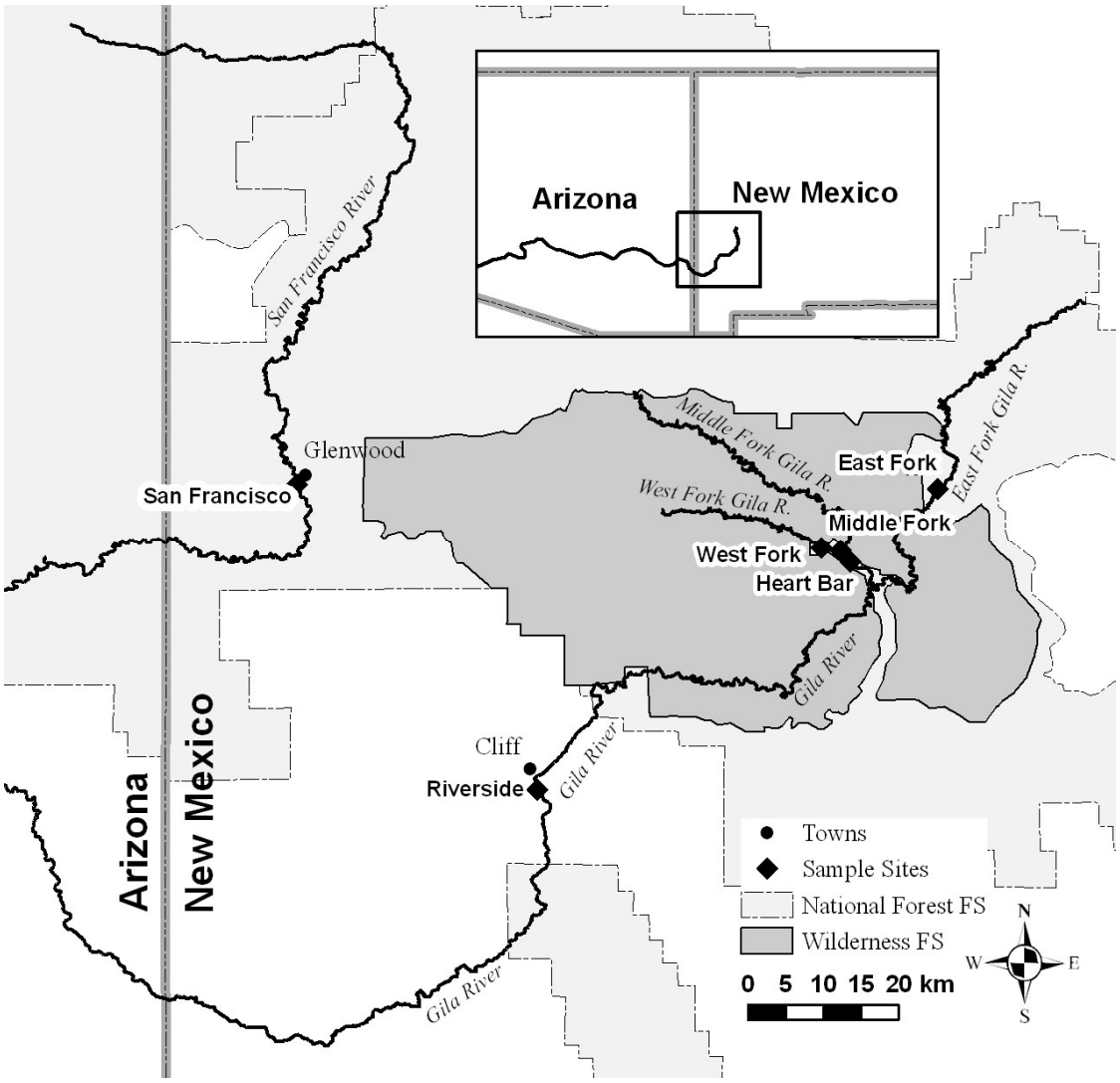

Figure 1.1 Study area in the Upper Gila River basin in southwest New Mexico, USA. Locations of sample sites are indicated by black diamonds.

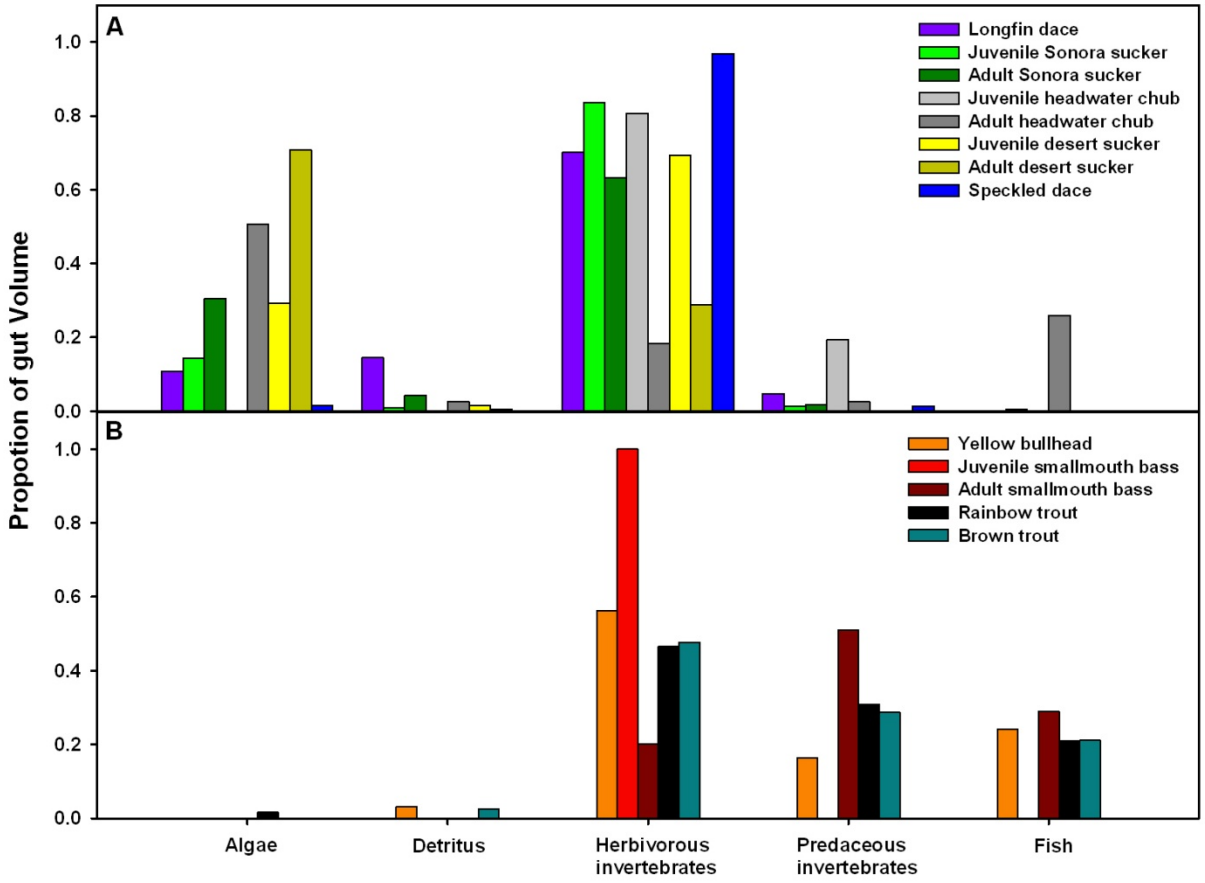

Figure 1.2 Native (A) and nonnative (B) fish diets collected from the upper Gila River basin, 2007 and 2008. All individuals per species/age-class (indicated by color) were pooled to determine proportion of gut volume. Invertebrate prey were grouped as herbivorous invertebrates and predaceous invertebrates.

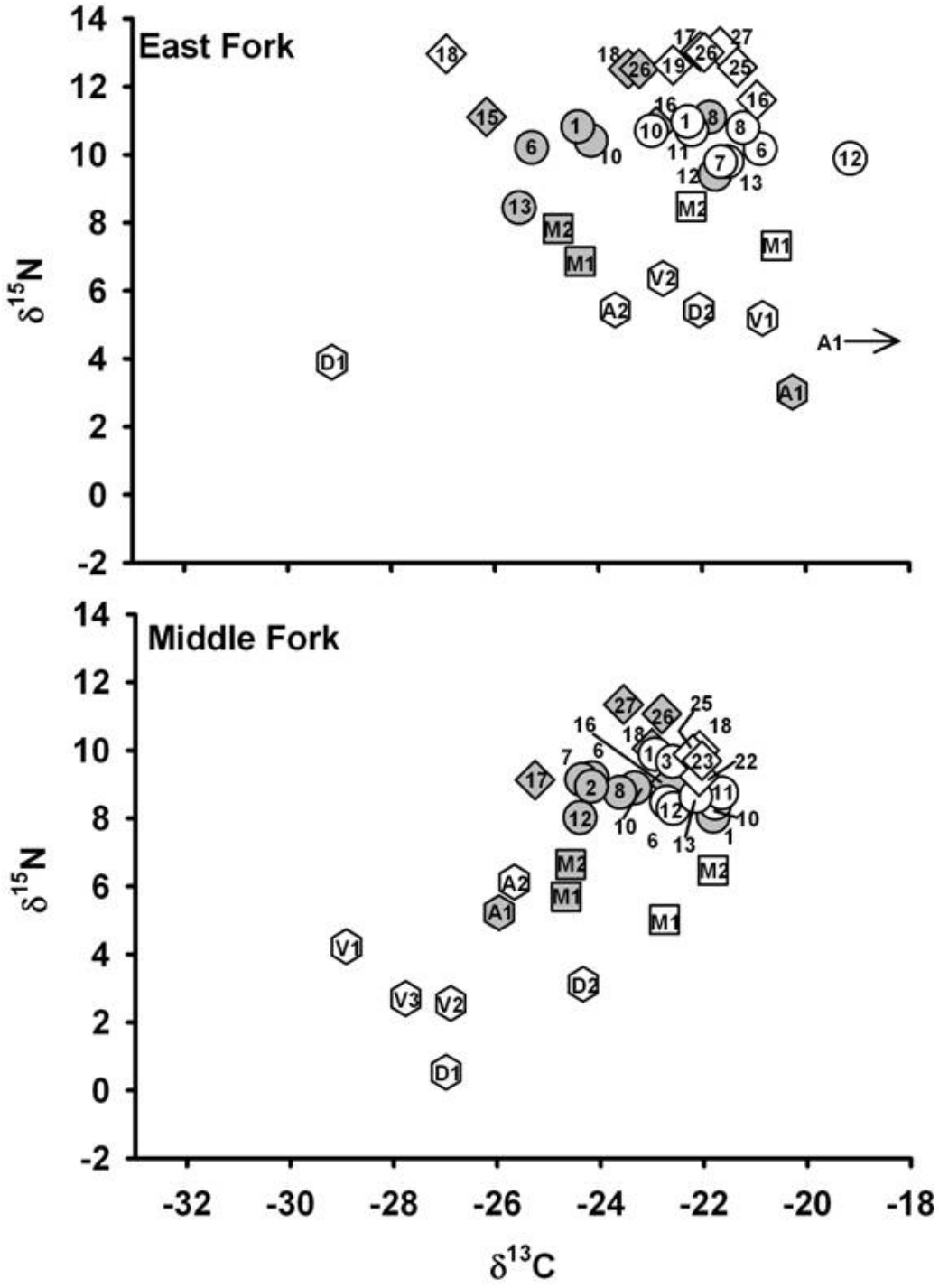

Figure 1.3 Mean $\delta^{13}C$ $\delta^{15}N$ values for native fishes (circles) and nonnative fishes (diamonds), invertebrates (squares), and basal energy sources (hexagons) for the East Fork and Middle Fork sites sampled in 2007 (filled circles) and 2008 (open circles). Standard deviations for each mean not included for clarity. See Table 2 for species codes.

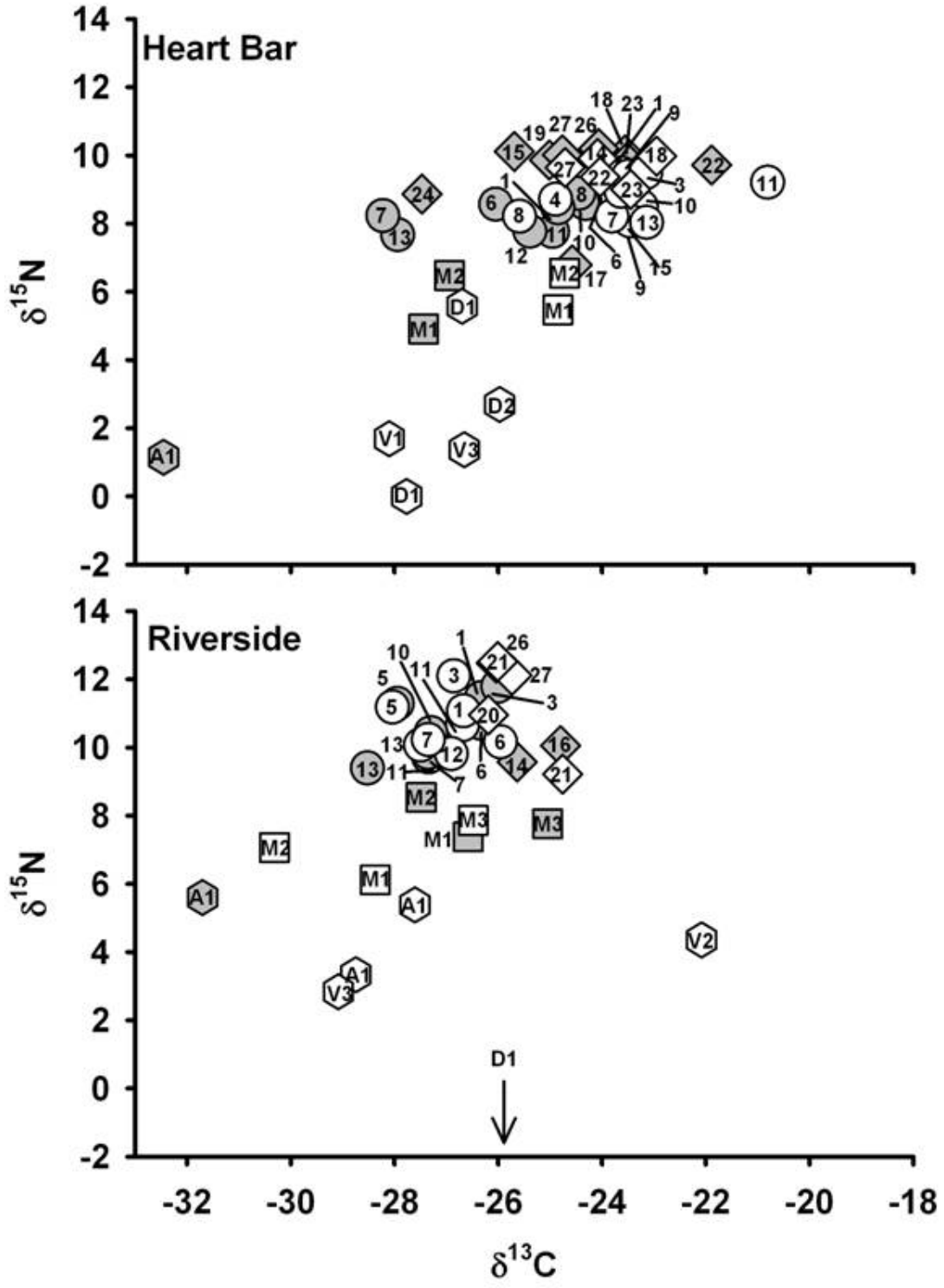

Figure 1.4 Mean $\delta^{13}C$ $\delta^{15}N$ values for native fishes (circles) and nonnative fishes (diamonds), invertebrates (squares), and basal energy sources (hexagons) for the Heart Bar and Riverside sites sampled in 2007 (filled circles) and 2008 (open circles). Standard deviations for each mean not included for clarity. See Table 2 for species codes.

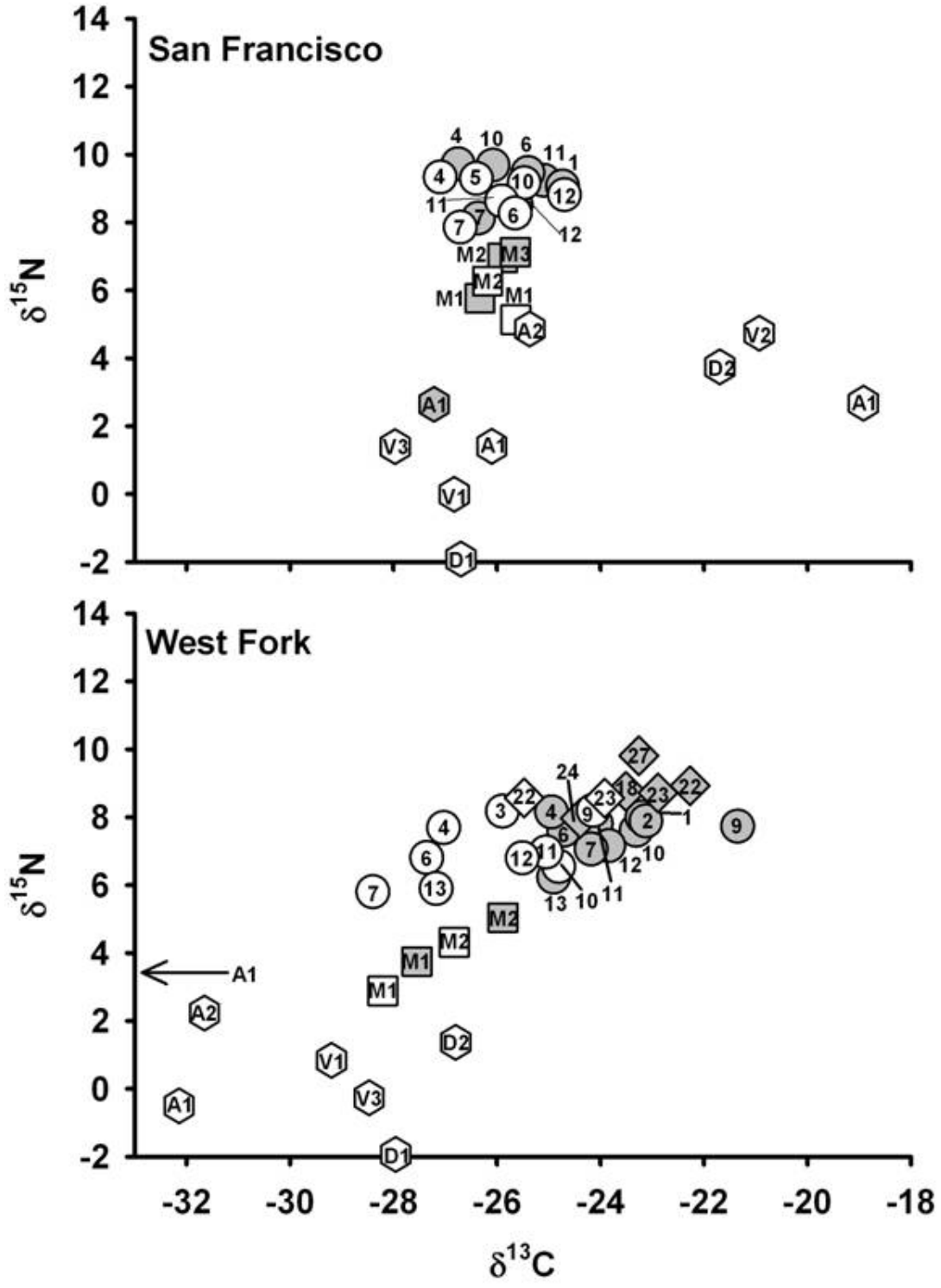

Figure 1.5 Mean $\delta^{13}C$ $\delta^{15}N$ values for native fishes (circles) and nonnative fishes (diamonds), invertebrates (squares), and basal energy sources (hexagons) for the San Francisco and West Fork sites sampled in 2007 (filled circles) and 2008 (open circles). Standard deviations for each mean not included for clarity. See Table 2 for species codes.

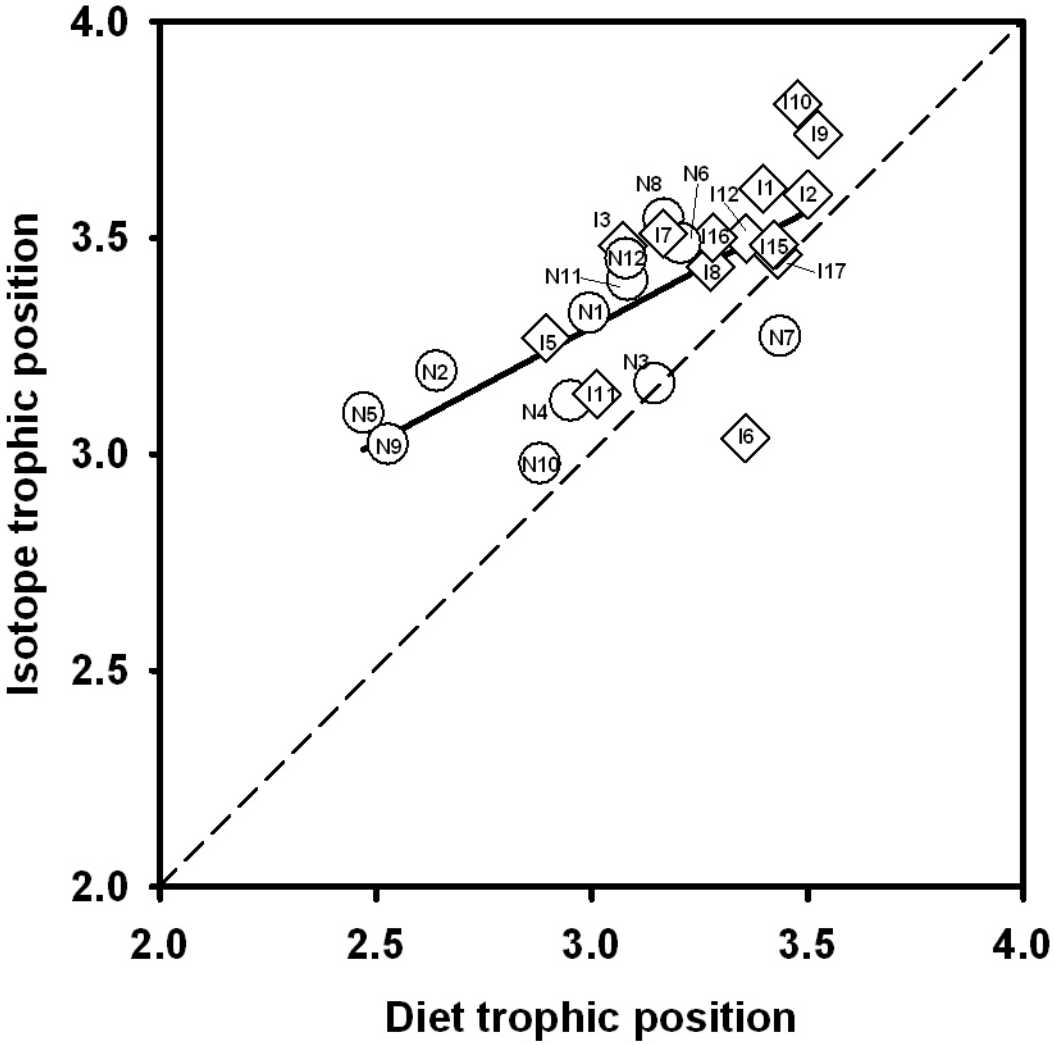

Figure 1.6 The relationship of mean trophic positions calculated using diet data and stable isotope analysis of native (circles) and nonnative fishes (diamonds). Dashed line indicates a 1:1 relationship. See Table 2 for species codes.

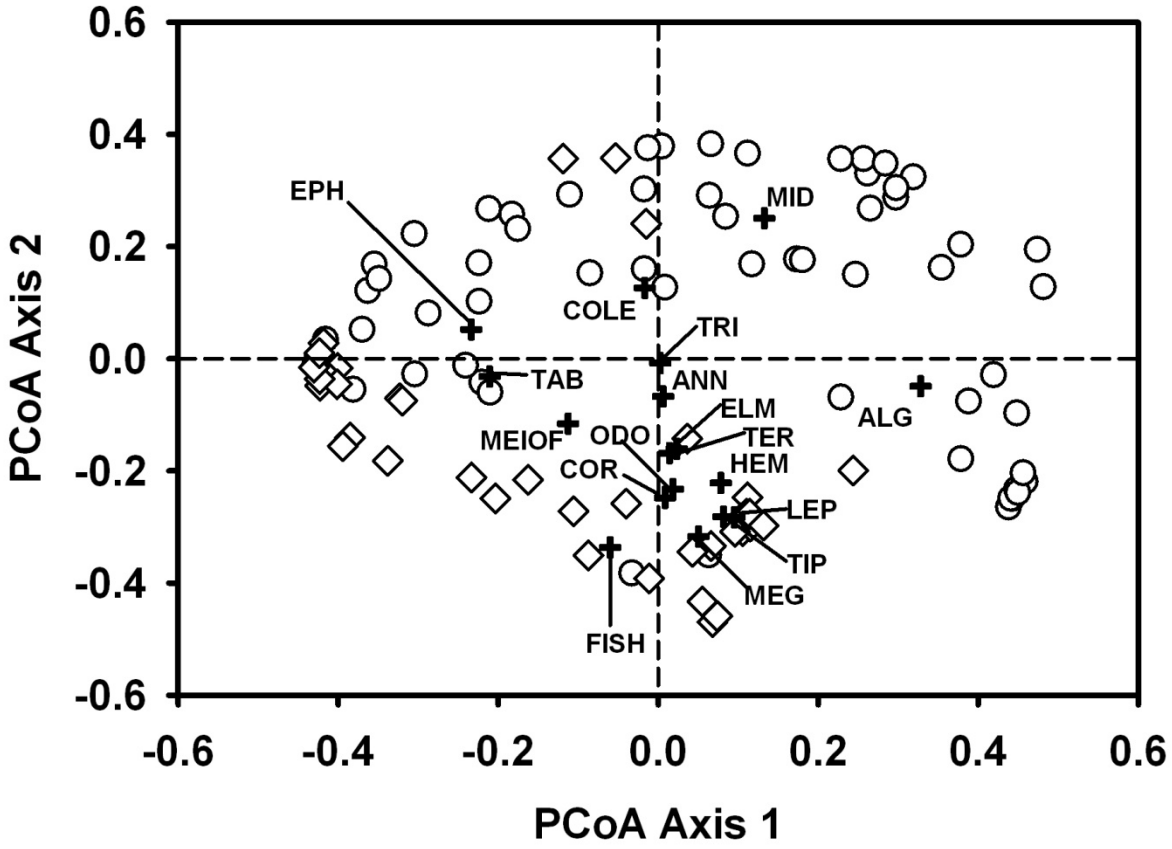

Figure 1.7 Principal coordinates analysis of native (circles) and nonnative (diamonds) fishes' diets at each of the six sample sites in the upper Gila River basin during 2007 and 2008. Symbols are the scores for the combined diet of individuals per species and age-class. Species names not included for clarity. Crosses are the weighted average scores of diet items. See Table 1 for diet codes.

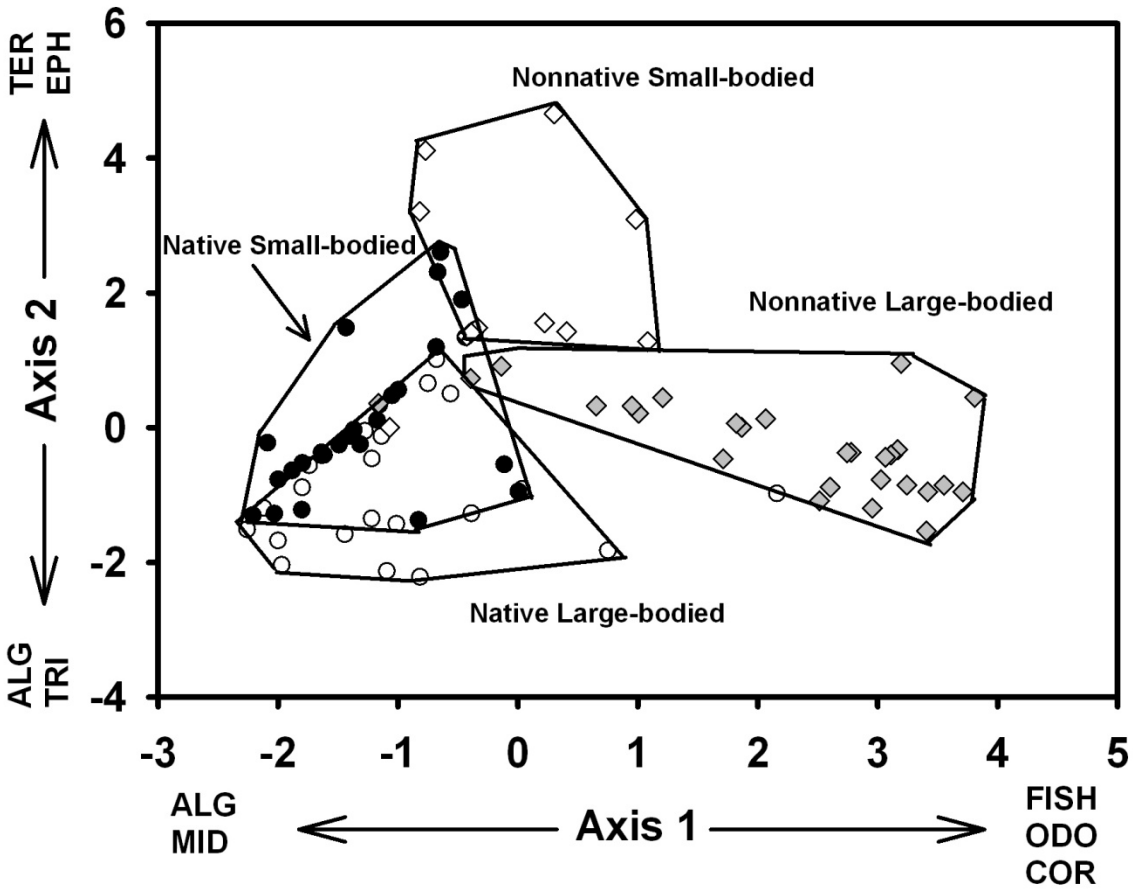

Figure 1.8 Plot of native (circles) and nonnative (diamonds) fish species/age-classes on the first two axes derived from a discriminant function analysis to classify species by diet (see Table 2 for species considered to be in each size-group). Polygons represent size-groups of native and nonnative fishes.

CHAPTER 2 - Variability in food web structure across spatial and temporal scales in an arid-land river system

ABSTRACT

Understanding how food web structure changes with spatial scale, resource availability, and community properties such as species richness is critical for understanding how energy moves through ecosystems. The goal of this chapter was to characterize variation in fish assemblage food web structure within and among study reaches on the Gila River using ^{13}C and ^{15}N stable isotopes. We hypothesized that food web structure would reflect variation in fish assemblages, resource availability and environmental conditions across habitats. Food web structure in isotope bi-plot space was estimated using community-wide measures of trophic structure, mean trophic position, and food-chain length. Permutational multivariate analysis of variance indicated that indices of food web structure were more variable among than within reaches and this pattern was primarily associated with variation in total area occupied by taxa in bi-plot space and mean trophic position of those taxa. Variation in food web structure was significantly associated with fish species richness across macrohabitats but was weakly associated with abiotic reach-scale factors. Concordance between food web structure and fish community composition suggests that factors influencing the distribution of fishes also influence food web structure.

INTRODUCTION

Community ecologists are challenged with discerning spatial and/or temporal patterns at one scale and associating them with mechanisms that can occur at entirely different scales (Levin 1992). Deriving these associations and mechanisms is particularly important in descriptions of food web dynamics if researchers are interested in relating food web properties at different scales to community dynamics and ecosystem function (Pimm et al. 1991, Pimm 2002). Syntheses of published food web studies identified structural patterns could be invariant to increasing numbers of species, such as short food-chains (typically less than 5 trophic levels), a constant number of links per species, the product of the number of species and web connectance (a fraction of possible links to all potential links), and ratios of top, intermediate and basal species (Briand and Cohen 1984, Cohen and Briand 1984, Briand and Cohen 1987, Sugihara et al. 1989, Pimm et al.

1991, Schoenly and Cohen 1991). In contrast, studies consisting of greater numbers of species and more resolved food webs found such structural properties to be scale dependent (Winemiller 1990, Polis 1991, Havens 1992, Martinez 1993, 1994, Martinez and Lawton 1995). There is currently no consensus on the ability to predict how food web properties will vary across space and time and with community properties.

Quantifying variability in food webs across space and time can aid in developing a mechanistic understanding of community dynamics within a landscape. Holt and Hoopes (2005) propose a metacommunity concept for food webs, in which variability in food web structure across space and time (i.e., shifting food web structure) could be a result of resource heterogeneity across a landscape and/or spatiotemporal population dynamics. For example, in heterogeneous landscapes, if variability in food web structure is high over large areas, this could be a result of shifting food web structure that is maintained by alternative communities that sort out along environmental gradients (i.e., species sorting perspective). Alternatively, if communities have high immigration rates, then low spatial variability in food web structure results from consistent food web topology (i.e., mass effects perspective). If patches are similar in resource availability, then spatial variability in food web structure is a result of local extinction and colonization (i.e., patch dynamic perspective). These conditions have implications for understanding species interactions, as interactions are likely to be strong under species sorting or patch dynamics models where competitive ability or predators regulate food web structure. Under mass effects models, weak interactions are expected because species with high dispersal could migrate to patches with fewer competitors or predators. Thus, evaluating spatial and temporal variation in food web structure could provide a better understanding of the myriad of factors regulating communities.

Previous studies of scaling properties of food webs focused primarily on topological structure such as linkage, connectance, and ratios of top, intermediate, and basal species based on diet analysis (e.g., Cohen and Briand 1984, Martinez and Lawton 1995). The advent of stable isotope analysis in ecosystem studies provides a tool for studying topological properties of food webs that incorporate longer temporal scales and prey-specific assimilation than observation of gut contents could provide. The commonly used stable isotopes ^{13}C and ^{15}N can quantify species-level feeding habits of a consumer of interest, such as relative trophic positions (Vander Zanden et al. 1997, Post 2002a), relative contributions of different prey to consumer (Vander

Zanden and Vadeboncoeur 2002, Phillips and Gregg 2003), species niche shifts (Persson and Hansson 1999, Post 2003), and diet variability of species (Bearhop et al. 2004, Matthews and Mazumder 2004). Stable isotope analysis has recently been used to quantify community-level food web structure such as food-chain length (Hoeinghaus et al. 2008, Walters and Post 2008) and community-wide metrics for $\delta^{13}C$ and $\delta^{15}N$ bi-plot space (Layman et al. 2007).

Understanding how food webs vary across space and time in stream networks must consider how organization of stream communities result from local and regional processes. Consistent patterns in species-area relationships of freshwater fish communities have been observed for various ecosystems (Angermeier and Schlosser 1989, Matthews and Robison 1998). Communities in riverine systems also show consistent longitudinal patterns in species turnover and changes in community composition across basins (Schlosser 1982, Oberdorff et al. 1993). Recurrent patterns in aquatic communities across broad spatial scales suggest similar patterns should be observed in food web structure. At local scales, variability in fish community structure can result from variable habitats and interspecific interactions among fishes (Taylor 1996, Taniguchi and Nakano 2000), but it is not clear how changes in local conditions influence food web structure among reaches and macrohabitats.

The goal of this study is to characterize variation in food web structure within and among study reaches on the Gila River using ^{13}C and ^{15}N stable isotopes, and to relate variation in fish assemblage food web structure with biotic and abiotic characteristics of study reaches. We hypothesized that food web structure would reflect variation in fish assemblage structure, resource availability and environmental conditions across reaches. Understanding variability in food web structure is of particular interest in the Gila River because it has been invaded by several apex predators (Chapter 1) that may have negative consequence on the native fish community (Propst et al. 2008).

METHODS

Study reaches

The upper Gila River originates in the mountains of southwest New Mexico, USA, and flows approximately 224 km to the New Mexico-Arizona border (Figure 2.1). The San Francisco River rises in eastern Arizona and flows east into New Mexico for about 122 km before turning west into Arizona to converge with the Gila River. Headwater reaches are canyon

bound, whereas mainstem reaches flow alternately through canyons and broad floodplains. Riparian vegetation for most reaches consists of grasses, forbs, small willows (*Salix* sp.) cottonwoods (*Populus* sp.) and Arizona alder (*Alnus oblongifolia*) (Brown 1982). Anthropogenic impacts in the upper catchment are limited to low impact outdoor recreation, minimal livestock grazing, and dispersed human settlement. Mainstem valley reaches are somewhat more impacted by minimal water diversion, livestock grazing, increased human settlement.

Fish assemblage food web data were taken from five reaches throughout the basin that matched long-term fish community monitoring sites (Propst et al. 2008). Within each study reach three to five macrohabitats, which consisted a pool and the immediate upstream riffle, were chosen. Three macrohabitats were chosen at East Fork, Middle Fork, West Fork, and San Francisco, and five were chosen on the mainstem below the confluence of the West and Middle Fork, on the Heart Bar Ranch Wildlife Area.

Sampling methods

Fishes and Ephemeroptera nymphs (necessary for baseline calculations of food web metrics; Vander Zanden and Rasmussen 1999) for stable isotope analysis were collected from the five study reaches in June-July, 2007 and 2008. Fishes were collected from each macrohabitat using a combination of seining (4.6 m X 1.2 m seine with 3.2 mm mesh) and electrofishing (Smith-Root Model LR24 backpack shocker). A maximum of five individuals from each species of small-bodied fishes were collected (i.e., species with maximum total length <100mm) and each age-class of large-bodied fishes. Large-bodied fishes (i.e., species whose maximum total length exceed 100 mm) were separated into three age-classes (juvenile, sub-adult, adult) based on length-frequency data to incorporate ontogenetic variability in isotope signatures. A 5 mm biopsy punch was used to extract dorsal muscle from individuals > 150 mm and individuals <150 mm were collected whole. Ephemeropterans were sampled from riffles in each pool/riffle complex using kick nets and by scrubbing rocks and were left in containers of freshwater overnight to allow gut evacuation (Jardine et al. 2005). Tissue samples were kept on ice in the field until they could be stored in a freezer (-20 °C).

Macroinvertebrate assemblages in each macrohabitat were sampled by collecting three samples from riffles using a surber sampler (0.93 m^2) and three from pools using a core sampler

(0.73 m^2). Macroinvertebrate samples were preserved in 10% formalin in the field and separated from detritus and sediment under a dissecting microscope.

Laboratory procedures

Dorsal muscle was used to measure stable isotope signatures because it has lower variability in $\delta^{15}N$ than other tissues, acidification to remove inorganic carbonates is not necessary(Pinnegar and Polunin 1999), and does not require lipid extraction because of relatively low lipid content compared to other tissues (Sotiropoulos et al. 2004, Ingram et al. 2007). Scales and epidermal tissue was removed from muscle samples collected in the field then rinsed with deionized water. Dorsal muscle was excised from individuals collected whole and rinsed. Ephemeropterans were rinsed and examined under a stereomicroscope to verify field identification. Isotope samples were dried for 48 h at a constant temperature (60 °C) then homogenized using a mortar and pestle. Powdered samples were analyzed for $\delta^{15}N$ and $\delta^{13}C$ with a ThermoFinnigan Delta Plus mass spectrometer with a CE 1110 elemental analyzer and Conflo II interface in continuous flow mode (CF-IRMS) in the Stable Isotope Mass Spectrometry Laboratory (SIMSL) at Kansas State University.

Macroinvertebrates were identified to family following Merrit and Cummins (1996). Individuals were counted and body lengths were measured to the nearest 1 mm on a petri dish placed over 1 mm grid paper. Biomass was estimated from length-mass power equations (Benke et al. 1999).

Food web metrics

Average $\delta^{13}C$ and $\delta^{15}N$ signatures for each species/age-class of fishes were converted to community-wide metrics of food web structure. Following Layman et al. (2007), we used the ranges of $\delta^{13}C$ and $\delta^{15}N$, trophic area (i.e., the area of a convex hull encompassing all species in $\delta^{13}C$ and $\delta^{15}N$ bi-plot space), and the mean Euclidean distance of each species to the $\delta^{13}C$ and $\delta^{15}N$ centroid to provide measures of community-wide trophic diversity. Mean Euclidean distance to each species' nearest neighbor and standard deviation of the mean nearest neighbor distance are measures of species packing in the $\delta^{13}C$ and $\delta^{15}N$ bi-plot and reflect the extent of trophic redundancy. In addition, mean trophic position and food-chain length were calculated to provide measures of vertical food web structure. Trophic positions of fishes based on $\delta^{15}N$

values were standardized at each reach to the $\delta^{15}N$ signature of a primary consumer following the equation of Cabana and Rasmussen (1996):

$$TP_{isotope} = [(\delta^{15}N_{fish} - \delta^{15}N_{ephem})/3.4] + 2$$

We chose $\delta^{15}N$ values of Ephemeropterans as the baseline because they were abundant at all reaches and their $\delta^{15}N$ was similar to other dominant primary consumers where collected (e.g., chironomids). Food-chain length (the number of trophic transfers from the base to the top of a food web) is a measure of vertical food web structure (Post 2002a) and can be estimated from the maximum isotopic derived trophic position from each reach (Post et al. 2000, Post 2002b). Food web metrics were calculated for each macrohabitat for 2007 and 2008 separately.

Statistical analyses

We constructed resemblance matrices representing variability in food web metrics, fish assemblage composition and macroinvertebrate composition among macrohabitats to describe spatial and temporal variation in fish assemblage food web structure and potentially influencing factors. Variation in food web indices was characterized using principal components analysis (PCA), based on a correlations matrix of $\log_{10}$ transformed indices across sites. Variability in fish and macroinvertebrate assemblages was examined using principal coordinates analysis (PCoA). Jaccard's index was used to characterize fish assemblage similarity based on presence/absence and a Bray-Curtis index was used to characterize similarity of macroinvertebrate assemblages based on $\log_{10}$ transformed biomass. Permutational multivariate analysis of variance (pMANOVA, Anderson 2001) was used to partition variation within these three data sets. Permutational MANOVA is a method for analysis of multiple response variables in the form of distance matrices based on linear predictors or covariates. A permutational MANOVA uses a pseudo-*F* ratios derived from permutations to test for significance. Because pseudo-*F* values represent the ratio of variation among treatments (i.e., reaches or years) to within treatments (i.e., among macrohabitats within reaches), we also used this as a means to partition variation attributed to the different spatial scales of measure.

We also tested for concordance among these three ordinations using a Procrustes rotation analysis (Peres-Neto and Jackson 2001), which tests if the dispersion of samples along the first two ordination axes is different from random. The Procrustes analysis rotates a matrix to maximum similarity with a target matrix minimizing the sums of squares between corresponding

points in both matrices, concordance is measured with a correlation-like statistic (m_{12}). Data were permuted 999 times to estimate the significance of the Procrustes statistic. Significant concordance among matrices would indicate similar factors driving patterns of variation in the three data sets across samples.

We compiled abiotic variables at the reach scale and biotic variables at the macrohabitat scale to investigate their relationship with variation in food web structure. Reach-scale variables were elevation and link magnitude, compiled from a GIS, and identical for all macrohabitats within a reach. Biotic variables within each macrohabitat were fish species richness, fish assemblage composition (i.e., the first two axes of the fish community PCoA), total macroinvertebrate biomass, and macroinvertebrate composition (i.e., the first two axes of the macroinvertebrate community PCoA). We tested for association between food web structure and abiotic reach level variables and biotic macrohabitat-level variables using partial redundancy analysis with year as a covariable. Significance of variables was tested using a permutational ANOVA. All analyses were performed in R (R Development Core Team 2008) using labdsv (Roberts 2007) and vegan (Oksanen et al. 2007) packages.

RESULTS

Spatial and temporal variation in food web structure, fish, and macroinvertebrate communities

Principal components analysis summarized the majority of the variation (76%) in fish assemblage food web structure on the first two axes (Figure 2.2). All community-wide metrics were negatively correlated with the first principal component, but trophic area and carbon range had the strongest negative loadings. Sites with negative axis 1 scores such as East Fork 2008 had large trophic areas and carbon ranges (3.6 to 13.6 and 2.1 to 7.3‰ respectively), whereas those with positive axis 1 scores such as San Francisco 2007 had small trophic area and carbon ranges (0.2 to 1.3 and CR 0.8 to 3.5‰, respectively). In general, there was substantial overlap among reaches and between years on the first axis. Mean trophic position and food-chain length were negatively associated with the second axis and there appeared to be less overlap among sites on this axis. For example, macrohabitats in the West Fork reach during 2007 had higher mean trophic position (range 3.5 to 3.7) and longer food-chain length (3.8 to 4.1) than East Fork 2007 (3.1 to 3.2, 3.4 to 3.9, respectively). Permutational MANOVA indicated that variability in food

web structure among reaches was > 2X than within reaches (pseudo-F = 2.56, Table 1), but spatial variability in food web metrics was dependent on year of sampling (significant reach x year interaction; pseudo-F = 2.39, P = 0.035).

Principal coordinates analysis and pMANOVA (Table 2.1) also revealed that variation in fishes (Figure 2.3) and macroinvertebrates (Figure 2.4) was greater among reaches than within reaches and between years. Fish assemblages were > 3X more variable among reaches than within reaches (pseudo F = 3.33) and total macroinvertebrate biomass was > 5X more variable across sites than within sites (pseudo-F = 5.08).

Correlates of food web structure

Procrustes analyses revealed variable levels of synchrony in the structure of food webs, fish communities, and macroinvertebrate communities. Variation in food web measures across samples was weakly associated with fish community structure (m_{12} = 0.311, P = 0.068) but not with macroinvertebrate community structure (m_{12} = 0.077, P = 0.962). Concordance between the food web measures and fish communities appeared to be driven by macrohabitats in West Fork 2007, 2008, and Heart Bar 2007 which had high mean trophic positions and longer food chains. Speckled dace and nonnative trout, a strict invertivore and nonnative predators, respectively were present in these macrohabitats. Macrohabitats with low mean trophic position and short food chains, which were associated with the presence of smallmouth bass and western mosquitofish (i.e., macrohabitats in the East Fork and Middle Fork reaches).

Spatial and temporal variation in fish community structure was strongly associated with variation in macroinvertebrate community structure (m_{12} = 0.567, P < 0.001). Macrohabitats where few nonnative predators were present (i.e., low Axis 2 scores in Figure 2.3), had macroinvertebrate communities with relatively high biomass of Pyralidae larvae (Lepidoptera), Plecoptera nymphs, Chironomidae larvae and pupae (Diptera), and ostracods (i.e., high Axis 2 scores in Figure 2.4). Reaches were several nonnative predators were present (i.e., high Axis 2 scores in Figure 2.3) had macroinvertebrate communities with relatively high biomass of Gomphidae (Odonata) Psephenidae (Coleoptera), Naucoridae (Hemiptera), Isonychiidae (Ephemeroptera), and Polycentropodidae (Trichoptera) (i.e., low Axis 2 scores in Figure 2.4).

Partial RDA suggested a marginally significant relationship between food web structure and predictor variables after controlling for annual variability ($F_{8,22}$ = 1.91, P = 0.06; Figure 2.5);

a pattern that was primarily driven by increasing trophic area with fish species richness ($r2$ = 0.30, $F_{1,32}$ = 13.66, P = 0.001; Figure 2.6). Although food-chain length was not indicated as a significant measure of food web structure by the RDA, univariate analysis indicated that food-chain length was longer and more variable when nonnative predators were present than absent (t = 3.885, one-tailed test $P < 0.001$).

DISCUSSION

Fish assemblage food web structure and fish and macroinvertebrate assemblages and were more variable among reaches than within reaches in the upper Gila River basin. Study reaches that had higher fish species richness tended to have higher carbon ranges and convex hull area (East Fork 2007, 2008, and Heart Bar 2007). Further, in reaches where speckled dace (strict invertivore) and nonnative trout (invertivore/piscivores) were present (West Fork 2008, 2008, and Heart Bar 2007) food web members had higher mean trophic positions and food-chain lengths were longer compared to reaches dominated by native suckers (algivore/detritivores; San Francisco 2007 and 2008). Although macroinvertebrate communities showed the same patterns of greater variability among reaches as food web structure and fish communities, variation in food web measures was not concordant with macroinvertebrates communities.

Variability in fish and macroinvertebrate assemblages were strongly concordant suggesting there may be a similar suite of environmental constraints on these communities operating at the reach scale. However, other studies have reported that fish and macroinvertebrate assemblages respond to different environmental gradients. For example, Williams et al. (2003) found fish assemblages to respond to environmental variability unique to individual basins, whereas variation in macroinvertebrate assemblages was attributed to large-scale environmental gradients independent of individual basins. Adult macroinvertebrates, capable of dispersing across reaches, can choose breeding sites based on the environmental conditions at stream macrohabitats (Huryn et al. 2008). Variability in fish assemblages across reaches also can occur from interspecific interactions within macrohabitats (e.g., Taylor 1996, Taniguchi and Nakano 2000), but there was little evidence from our study to support this conclusion.

Although our analyses indicated food web structure, fish assemblages, and macroinvertebrate assemblages were more variable across study reaches, food web structure was

also variable among macrohabitats within reaches. Differences in habitat depth and structure (i.e., presence of macrophytes or large woody debris) among macrohabitats could allow different macrohabitats within the same reach to harbor different fish assemblages. In addition, biotic interactions within macrohabitats could lead to different fish assemblages in adjacent macrohabitats (e.g., Power et al. 1985). Although we attempted to select macrohabitats with similar size and depth, stream reaches are inherently heterogeneous and this was not always logistically possible. Although we did return to the same locations during both years of the study, changes in macrohabitat characteristics did occur in some instances. We found fish and macroinvertebrate composition to vary among macrohabitats and between years, although, macroinvertebrate composition was less variable than food webs and fish communities. Parsons et al. (2003) found macroinvertebrate assemblages to vary among riffles within the same reach, yet within reach variability was minimal when considering variability at larger spatial scales.

Spatial variation in food web measures was primarily associated with differences in convex hull area across macrohabitats and reaches, and this variability was partly attributed to fish species richness; convex hull area increased proportionately with species richness (Figure 6). Increased convex hull area would be expected to increase with increased number of species if additional species occupied different trophic levels (increased nitrogen range) or used additional resources (increased carbon range), i.e., increased trophic diversity. Alternatively, additional species would not increase convex hull area if their feeding ecology was redundant with other members of the community. Our results suggest that an understanding of factors driving species richness at both the macrohabitat and reach scale might provide insight into variation in food web structure.

Several hypotheses have been set forth to explain variation in food chain length. The "productive-space hypothesis" predicts food-chain length should increase proportionally with total ecosystem productivity (the product of ecosystem size and per unit productivity; Schoener 1989). Studies in temperate lakes, however, found no effect of lake productivity on food chain length, but instead found food-chain length to increase with increasing lake size (Vander Zanden et al. 1999, Post et al. 2000). In South American rivers, food-chain length was associated with hydrogeomorphology and impoundments (Hoeinghaus et al. 2008). In this study, macroinvertebrate biomass had no effect on food-chain length, nor did the surrogate for watershed size, link magnitude. The introduction of an apex predator should, by definition,

increase food chain length, as was observed in macrohabitats with nonnative predators. A clear driver of food chain length in the upper Gila River is the introduction of nonnative predators. Food-chain length can affect community structure (Pace et al. 1999) and ecosystem function (Schindler et al. 1997, Duffy et al. 2005), therefore the introduction of nonnative predators in the Gila River could indirectly affect community structure and ecosystem effects.

Food webs are not static entities, but are highly variable in space and time. Stable isotopes incorporate assimilation of diet items over medium time scales (weeks to months) and, depending on species movement, over broad areas. Stable isotopes could be masking fine grained variation in ingested prey items from different time periods and different macrohabitats. Understanding how species interact within the food web would require a more detailed account of daily feeding habits. For example, stable isotope analysis and mixing models could be used to identify the resources used by two species. However, if these species were partitioning the resource on a diurnal basis, this would be missed by the isotope analysis. Similarly, understanding the effects of food web structure on ecosystem function would benefit from detailed analyses of the roles of food web constituents.

A central issue to community ecology is linking food web structure with community dynamics and ecosystem function. Our results suggest variability in food webs among study reaches is likely the result of heterogeneous distribution of fishes in the upper Gila River. Spatial variability may result from colonization-extinction dynamics among reaches (patch-dynamics) or differences in environmental conditions (species-sorting). High degree of spatial variability suggests mass-effects are not as important in structuring communities and food webs in the Gila River, albeit spatial variation was weak in some cases. Distinguishing between these processes will require further investigation regarding fishes' dispersal abilities among reaches and evaluating the characteristics of reaches that promote coexistence of species.

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TABLES AND FIGURES

Table 2.1 Permutational multivariate analysis of variance for fish community food web structure, fish community, and macroinvertebrate community ordinations in the upper Gila River, USA, 2007-2008.

Source	*df*	*MS*	*Pseudo-F*	*P*
Food web structure				
Reach	4	0.648	2.558	0.025
Year	1	0.242	0.954	0.373
R * Y	4	0.606	2.392	0.035
Residual	24	0.253		
Total	33			
Fish assemblage presence/absence				
Reach	4	0.688	3.329	<0.001
Year	1	0.601	2.912	0.005
R * Y	4	0.242	1.172	0.252
Residual	24	0.207		
Total	33			
Macroinvertebrate community composition				
Reach	4	0.210	5.077	< 0.001
Year	1	0.589	14.217	< 0.001
R * Y	4	0.118	2.844	< 0.001
Residual	23	0.041		
Total	32			

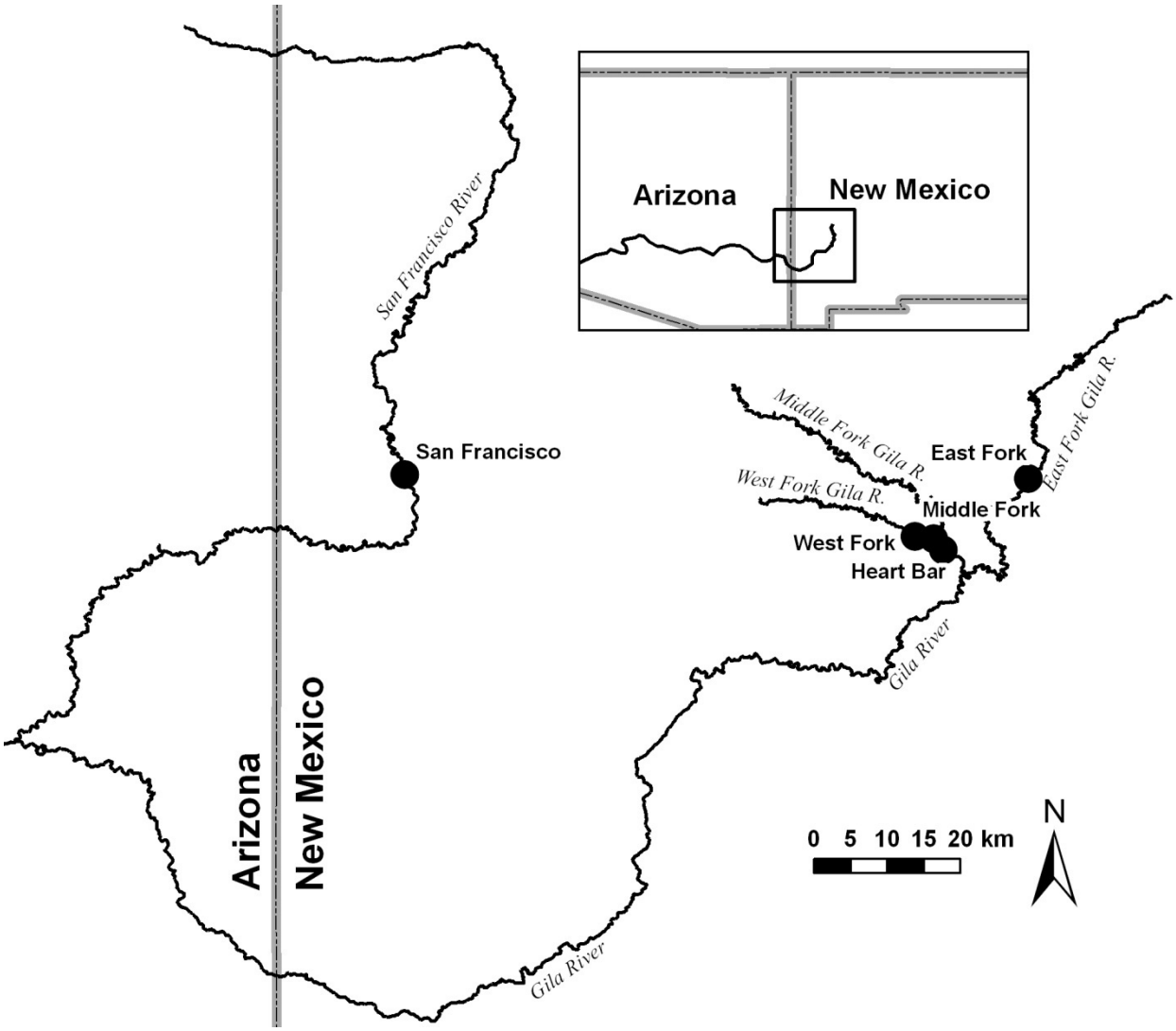

Figure 2.1 Study area in the upper Gila River basin in southwest New Mexico, USA. Locations of sample reaches are indicated by black circles.

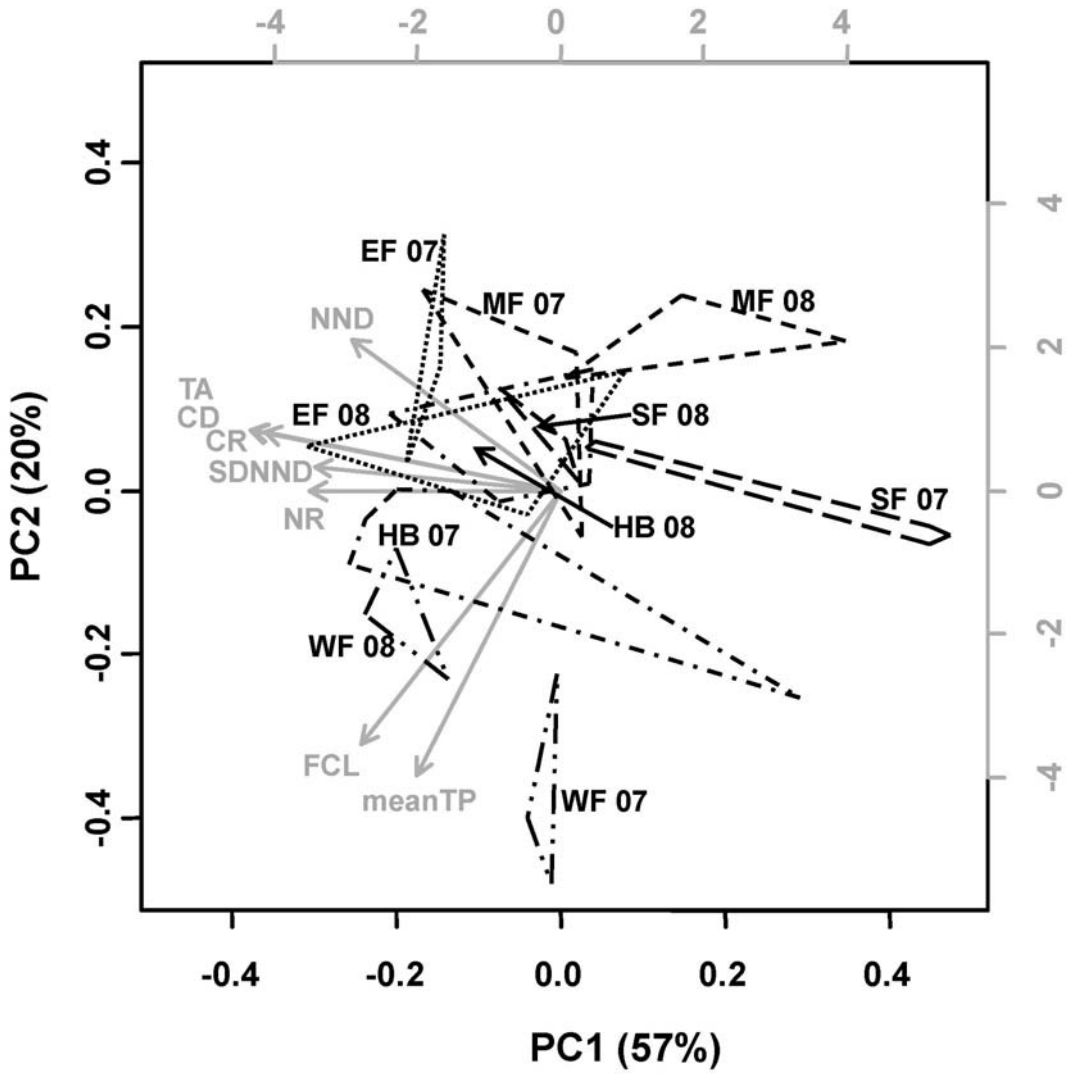

Figure 2.2 Principal components analysis based on a correlations matrix evaluating spatial and temporal variation in fish community food web structure in the upper Gila River, USA, 2007-2008. Community food web metrics of food web structure are were convex hull area (TA), $\delta^{13}C$ range of all species (CR), $\delta^{15}N$ range of all species (NR), mean distance to centroid (CD), mean nearest neighbor distance (NND), standard deviation of NND (SDNND), mean trophic position (meanTP) and food-chain length (FCL). Macrohabitats within study reaches are delineated by line style; dotted for East Fork, short dash for Middle Fork, dot-dash for Heart Bar, long dash for San Francisco, and dot-dot-dash for West Fork.

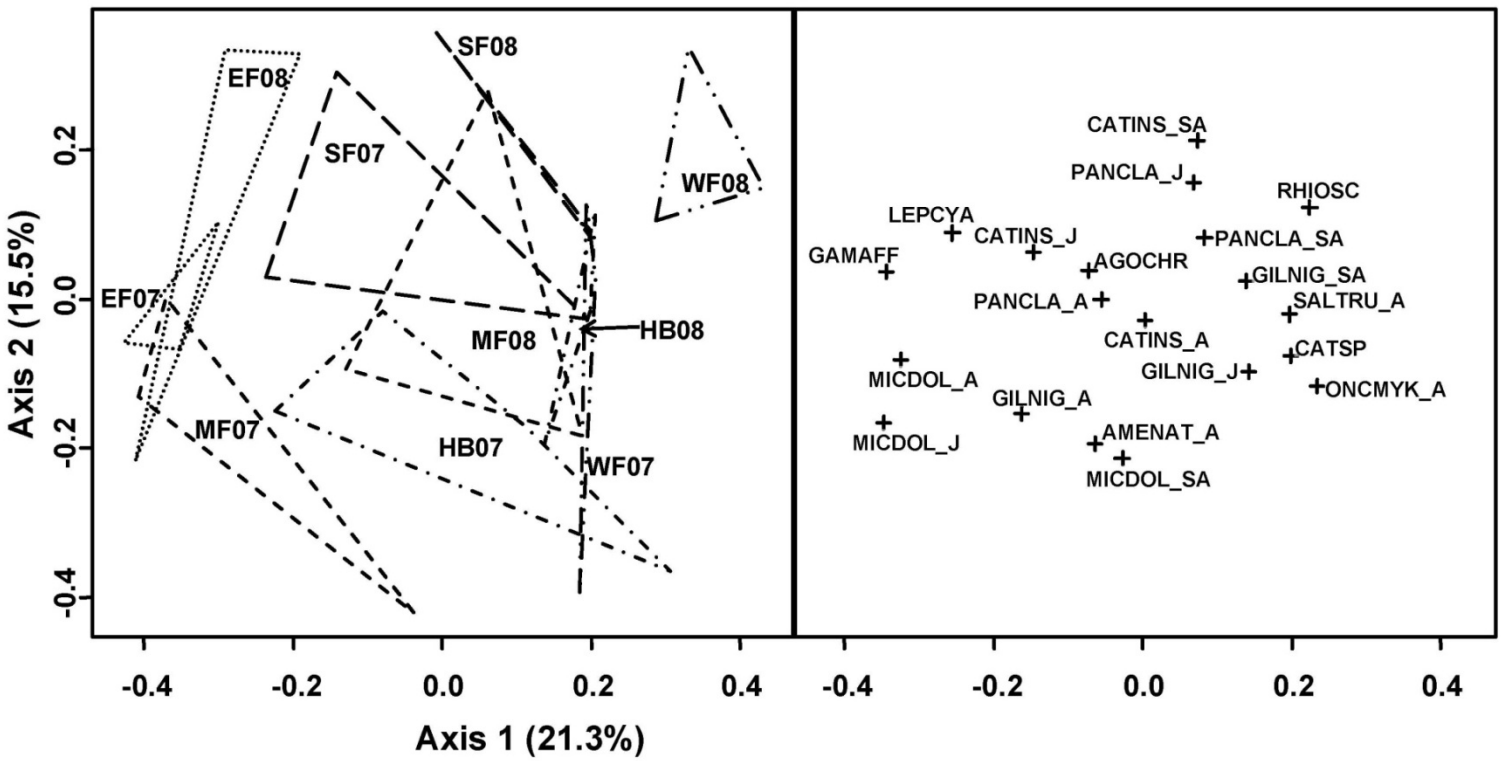

Figure 2.3 Principal coordinates analysis evaluating spatial and temporal variation in fish community composition at study reaches in the upper Gila River, USA, 2007-2008. Macrohabitats (left panel) are outlined by study reach and year (see Figure 2.2 for delineations). Fish species scores (right panel) are abbreviated according to the following key: AGOCHR = *Agosia chrysogaster*, GILNIG = *Gila nigra*, RHIOSC = *Rhinichthys osculus*, CATSP = larval catostomids, CATINS = *Catostomus insignis*, PANCLA = *Pantosteus clarki*, AMENAT = *Ameiurus natalis*, GAMAFF = *Gambusia affinis*, ONCMYK = *Oncorhynchus mykiss*, SALTRU = *Salmo trutta*, LEPCYA = *Lepomis cyanellus*, MICDOL = *Micropterus dolomieu*. Species codes are followed by age-class if species was assigned to an age-class.

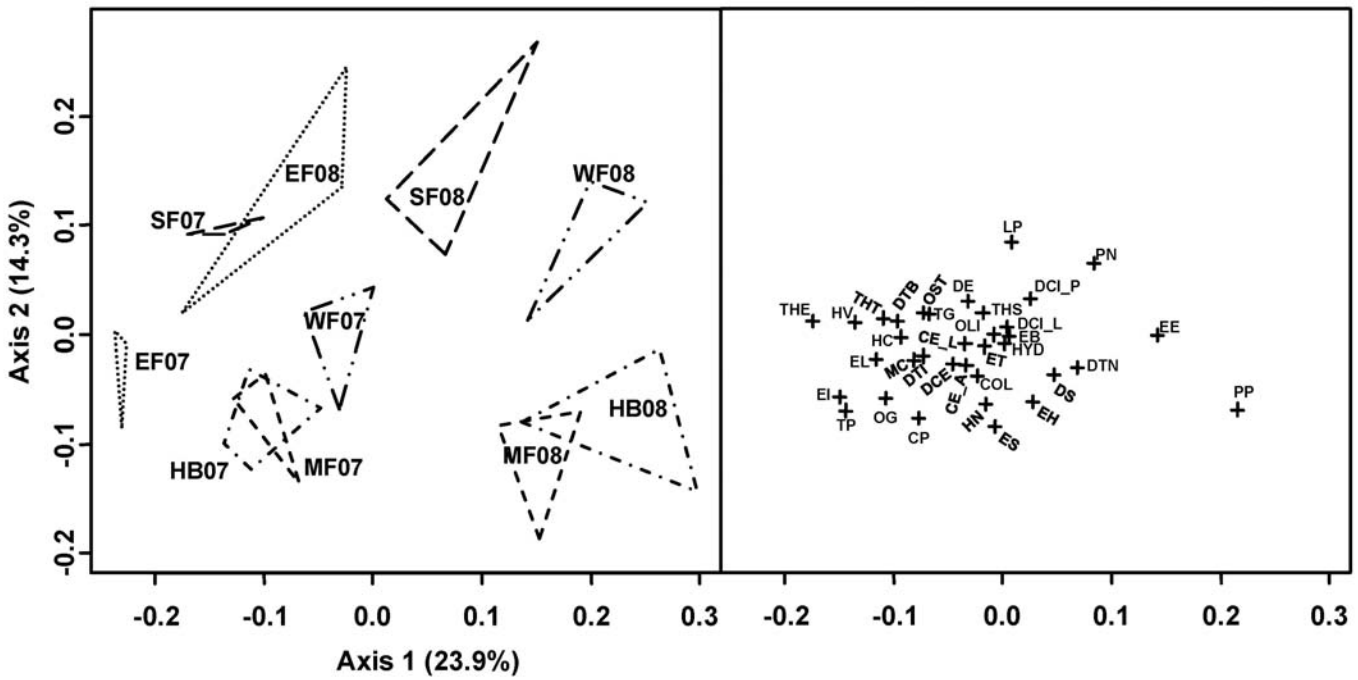

Figure 2.4 Principal coordinates analysis evaluating spatial and temporal variation in macroinvertebrate community composition at study reaches in the upper Gila River, USA, 2007-2008. Macrohabitats (left panel) are outlined by study reach and year (see Figure 2.2 for delineations). Macroinvertebrate taxa scores (right panel) are coded according to the following key: COL = Collembola, EB = Ephemeroptera Baetidae, EE = Ephemerellidae, EH = Heptageniidae, EI = Isonychiidae, ET = Leptohyphidae, EL= Leptophlebiidae, ES = Siphlonuridae, OG = Odonata Gomphidae, PN = Plecoptera Nemouridae, PP = Perlodidae, HC = Hemiptera Corixidae, HN = Naucoridae, HV = Veliidae, MC = Megaloptera Corydalidae, TG = Trichoptera Glossossomatidae, THE = Helicopsychidae, THS = Hydropsychidae, THT = Hydroptilidae, TP = Polycentropodidae, LP = Leptoceridae Pyralidae, CE_L = larval Elmidae, CE_A = Coleoptera adult Elmidae, CP = Psephenidae, DCE = Diptera Ceratopogonidae, DCI_L = larval Chironomidae, DCI_P = pupae Chironomidae, DE = Empididae, DS = Simuliidae, DTB = Tabanidae, DTN = Tanyderidae, DTI = Tipulidae, OST = Ostracoda, HYD = Hydracarina, OLI = Oligochaeta.

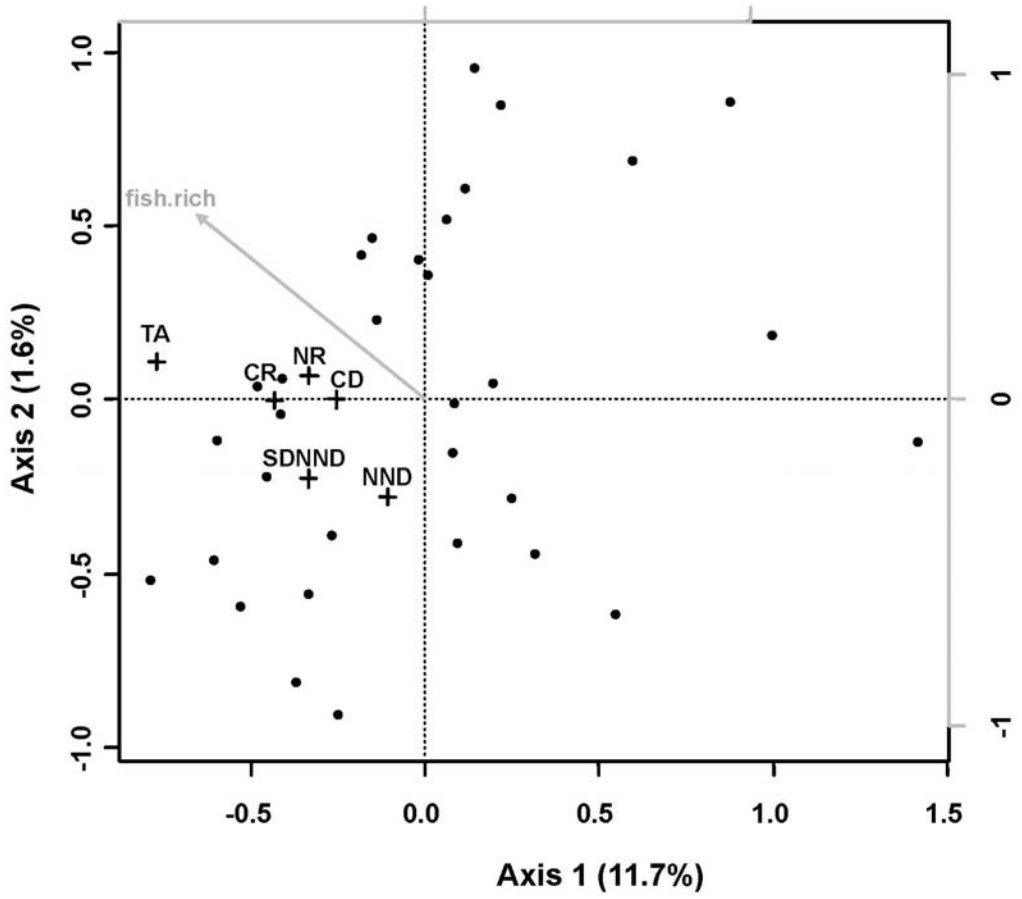

Figure 2.5 Redundancy analysis of fish community food web structure in the upper Gila River, USA, 2007-2008. Measures of food web structure for each macrohabitat (points) are constrained by biotic habitat-scale factors, fish species richness (fish.rich), fish community composition (the first two axes of the fish PCoA,), macroinvertebrate biomass, and macroinvertebrate community composition (the first two axes of macroinvertebrate PCoA,). In addition reach-scale factors were elevation and link magnitude. Constraining factors and their scaling are indicated by gray shading, but nonsignificant factors are not shown.

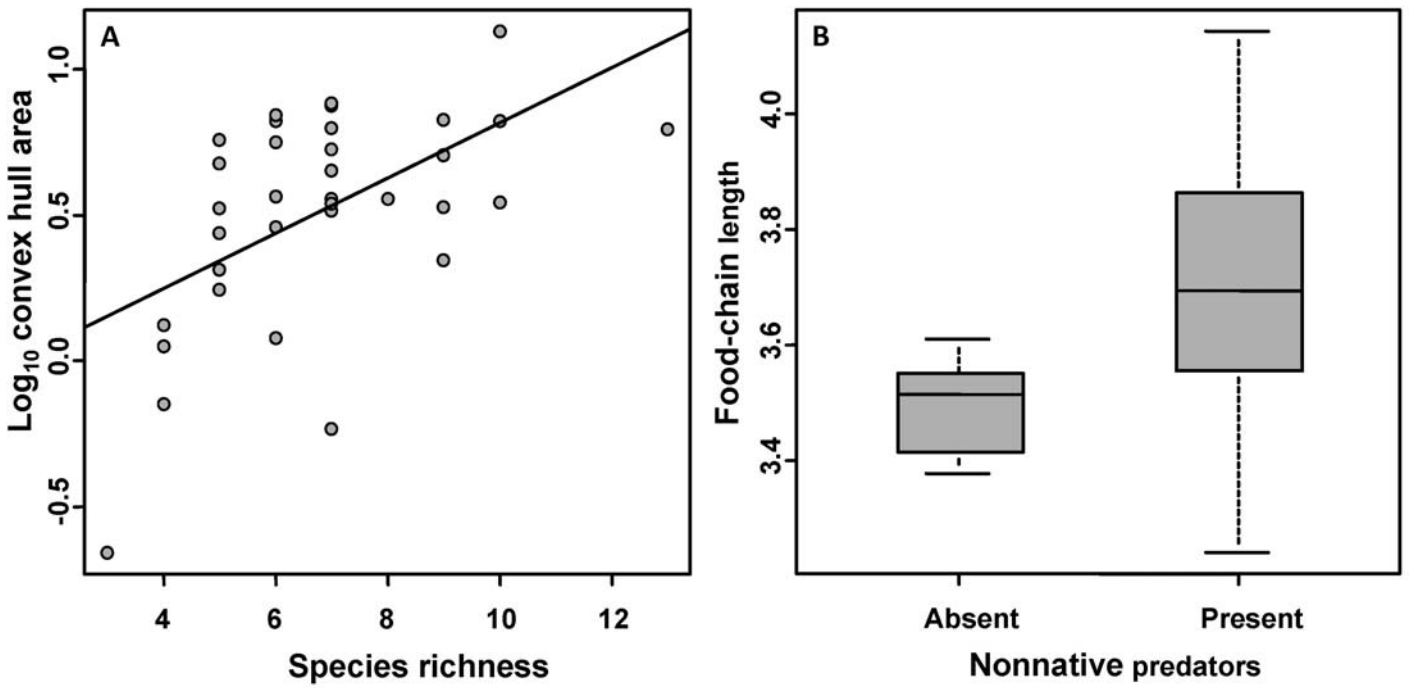

Figure 2.6 Relationships of food web structure measures in the upper Gila River, USA 2007-2008. Panel A is the relationship between convex hull area (log_{10} transformed) and fish species richness. The circle size indicates the number of introduced species present in the macrohabitat. Panel B is a boxplot showing increased food-chain length in macrohabitats where nonnative predators are present.

Appendix A - Feeding habits of native and nonnative fishes in the Gila River

Table A.1 Feeding habits of native and nonnative fishes collected at six sites in the upper Gila River basin, New Mexico, during 2007 and 2008. Diets of large-bodied fishes are separated into three age-classes (see text for sizes ranges of each class). Percent volume of diet items is relative to the total area of all diet items for each species/age-class. Numbers of individuals sampled are given in parentheses.

	Agosia chrysogaster (71)		*Cyprinella lutrensis* (6)		Juvenile *Gila nigra* (10)		Sub-adult *G. nigra* (11)	
Diet item	% Occurrence	% Volume	% Occurrence	% Volume	% Occurrence	% Volume	% Occurrence	% Volume
Ephemeroptera	39.0	24.5	0.0	0.0	20.0	12.8	55.0	18.5
Corixidae	1.0	1.5	0.0	0.0	0.0	0.0	0.0	0.0
Veliidae	0.0	0.0	17.0	3.7	0.0	0.0	0.0	0.0
Trichoptera (undetermined family)	15.0	2.7	0.0	0.0	0.0	0.0	18.0	0.6
Hydropsychidae	0.0	0.0	0.0	0.0	0.0	0.0	9.0	4.5
Diptera (undetermined family)	8.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0
Ceratopogonidae	0.0	0.0	0.0	0.0	20.0	1.9	0.0	0.0
Chironomidae	31.0	2.9	0.0	0.0	60.0	40.4	18.0	0.4
Simuliidae	7.0	6.4	0.0	0.0	20.0	0.9	9.0	0.1
Benthic Inverts (undetermined taxa)	23.0	8.5	33.0	4.0	20.0	30.6	9.0	1.8
Terrestrial	6.0	3.8	17.0	1.4	30.0	13.4	9.0	4.2
Cladocera	1.0	1.7	0.0	0.0	0.0	0.0	0.0	0.0
Copepoda	0.0	0.0	0.0	0.0	0.0	0.0	27.0	9.0
Hydracarina	1.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Snail	0.0	0.0	17.0	0.7	0.0	0.0	0.0	0.0
Fish	0.0	0.0	0.0	0.0	0.0	0.0	27.0	53.8
Algae	28.0	16.8	17.0	1.3	0.0	0.0	0.0	0.0
Amorphous detritus	25.0	20.9	83.0	64.1	0.0	0.0	9.0	7.2
Detritus	11.0	6.6	67.0	24.9	0.0	0.0	0.0	0.0
Undetermined taxa	4.0	2.5	0.0	0.0	0.0	0.0	0.0	0.0

Table A.1 Continued

	Adult *G.nigra* (22)		*Meda fulgida* (22)		*Rhinichthys osculus* (40)		*Tiaroga cobitis* (14)	
Prey item	% Occurrence	% Volume	% Occurrence	% Volume	% Occurrence	% Volume	% Occurrence	% Volume
Ephemeroptera	64.0	7.2	68.0	35.3	85.0	53.8	79.0	42.4
Corixidae	27.0	4.6	0.0	0.0	0.0	0.0	0.0	0.0
Megaloptera	5.0	1.5	0.0	0.0	0.0	0.0	0.0	0.0
Trichoptera (undetermined family)	36.0	1.2	14.0	1.7	13.0	1.1	21.0	6.0
Hydropsychidae	0.0	0.0	0.0	0.0	5.0	3.9	57.0	34.0
Hydroptilidae	5.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Elmidae	9.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0
Diptera (undetermined family)	0.0	0.0	0.0	0.0	3.0	0.1	0.0	0.0
Ceratopogonidae	0.0	0.0	0.0	0.0	5.0	0.1	0.0	0.0
Chironomidae	32.0	0.2	59.0	30.8	60.0	3.9	36.0	11.4
Simuliidae	14.0	0.1	50.0	3.2	28.0	9.6	7.0	0.1
Tabanidae	0.0	0.0	0.0	0.0	5.0	1.5	0.0	0.0
Benthic Inverts (undetermined taxa)	27.0	4.4	14.0	14.6	13.0	2.0	7.0	2.4
Terrestrial	18.0	0.5	18.0	5.3	0.0	0.0	0.0	0.0
Hydracarina	14.0	0.0	0.0	0.0	3.0	0.0	0.0	0.0
Oligochaeta	5.0	0.0	0.0	0.0	8.0	0.5	0.0	0.0
Snail	9.0	0.6	0.0	0.0	0.0	0.0	0.0	0.0
Fish	18.0	19.7	0.0	0.0	0.0	0.0	0.0	0.0
Algae	55.0	46.8	0.0	0.0	5.0	1.4	0.0	0.0
Amorphous detritus	36.0	5.4	14.0	3.1	30.0	21.6	14.0	3.6
Detritus	23.0	2.4	18.0	6.2	5.0	0.2	0.0	0.0
Undetermined taxa	9.0	5.0	0.0	0.0	3.0	0.3	0.0	0.0

Table A.1 Continued

	Juvenile *Catostomus insignis* (93)		Sub-adult *C. insignis* (35)		Adult *C. insignis* (27)	
Prey item	% Occurrence	% Volume	% Occurrence	% Volume	% Occurrence	% Volume
Collembola	0.0	0.0	3.0	0.0	0.0	0.0
Ephemeroptera	61.0	34.0	80.0	21.4	56.0	8.8
Anisoptera	2.0	0.1	3.0	0.0	4.0	0.1
Zygoptera	0.0	0.0	0.0	0.0	4.0	1.3
Naucoridae	0.0	0.0	3.0	0.2	0.0	0.0
Trichoptera (undetermined family)	15.0	1.2	34.0	2.5	52.0	2.7
Hydropsychidae	3.0	0.1	6.0	1.2	7.0	0.1
Hydroptilidae	2.0	0.2	9.0	0.3	0.0	0.0
Dytiscidae	0.0	0.0	0.0	0.0	7.0	0.1
Elmidae	11.0	0.3	17.0	0.3	30.0	0.1
Gyrinidae	0.0	0.0	0.0	0.0	4.0	0.0
Haliplidae	0.0	0.0	3.0	0.0	0.0	0.0
Diptera (undetermined family)	1.0	0.1	0.0	0.0	7.0	0.1
Ceratopogonidae	4.0	0.1	3.0	0.0	19.0	0.2
Chironomidae	76.0	41.5	91.0	50.1	81.0	10.4
Simuliidae	18.0	1.2	31.0	1.5	19.0	0.2
Tabanidae	2.0	0.1	3.0	0.2	7.0	0.0
Tipulidae	0.0	0.0	0.0	0.0	7.0	0.1
Benthic Inverts (undetermined taxa)	10.0	0.9	9.0	0.2	4.0	0.0
Terrestrial	4.0	0.9	9.0	0.1	4.0	0.0
Cladocera	4.0	1.3	0.0	0.0	0.0	0.0
Copepoda	2.0	0.0	0.0	0.0	0.0	0.0
Ostracoda	5.0	0.4	0.0	0.0	0.0	0.0
Hydracarina	22.0	0.2	26.0	0.1	22.0	0.1
Oligochaeta	1.0	0.1	0.0	0.0	41.0	1.4
Planaria	0.0	0.0	0.0	0.0	37.0	4.5
Bivalve	0.0	0.0	3.0	0.0	0.0	0.0
Snail	2.0	0.0	3.0	0.0	0.0	0.0
Fish	0.0	0.0	0.0	0.0	4.0	0.5
Algae	34.0	9.3	20.0	4.8	63.0	35.2
Amorphous detritus	23.0	7.0	43.0	16.0	63.0	29.1
Detritus	4.0	1.0	9.0	0.9	22.0	4.8

Table A.1 Continued

	Age-0 Catostomids (129)		Juvenile *Pantosteus clarki* (56)		Sub-adult *P. clarki* (26)		Adult *P. clarki* (12)	
Prey item	% Occurrence	% Volume	% Occurrence	% Volume	% Occurrence	% Volume	% Occurrence	% Volume
Ephemeroptera	50.0	27.1	61.0	17.2	58.0	25.2	33.0	1.5
Trichoptera (undetermined family)	5.0	0.3	5.0	0.4	15.0	1.7	0.0	0.0
Hydropsychidae	2.0	1.8	0.0	0.0	0.0	0.0	17.0	0.4
Hydroptilidae	0.0	0.0	0.0	0.0	4.0	0.3	8.0	0.2
Lepidoptera	0.0	0.0	2.0	0.1	4.0	0.1	0.0	0.0
Elmidae	0.0	0.0	5.0	0.3	8.0	0.2	8.0	0.0
Diptera (undetermined family)	2.0	0.6	0.0	0.0	0.0	0.0	0.0	0.0
Ceratopogonidae	3.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0
Chironomidae	79.0	28.5	70.0	43.9	54.0	18.4	83.0	9.3
Simuliidae	19.0	2.4	27.0	5.3	46.0	7.6	8.0	0.1
Benthic Inverts (undetermined taxa)	5.0	1.8	0.0	0.0	0.0	0.0	0.0	0.0
Terrestrial	2.0	0.6	2.0	0.0	0.0	0.0	0.0	0.0
Cladocera	3.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0
Copepoda	2.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Hydracarina	7.0	0.3	7.0	0.1	0.0	0.0	0.0	0.0
Oligochaeta	0.0	0.0	0.0	0.0	4.0	0.1	17.0	0.2
Planaria	0.0	0.0	0.0	0.0	0.0	0.0	25.0	8.6
Algae	34.0	29.7	43.0	28.3	31.0	16.8	75.0	77.4
Amorphous detritus	11.0	5.8	14.0	2.8	46.0	29.4	25.0	1.8
Detritus	0.0	0.0	4.0	1.4	4.0	0.3	17.0	0.5
Undetermined taxa	2.0	0.4	2.0	0.1	0.0	0.0	0.0	0.0

Table A.1 Continued

	Juvenile *Ameiurus natalis* (4)		Sub-adult *A. natalis* (13)		Adult *A. natalis* (101)	
Prey item	% Occurrence	% Volume	% Occurrence	% Volume	% Occurrence	% Volume
Ephemeroptera	50.0	9.0	38.0	29.4	31.0	21.4
Odonata (undetermined taxa)	0.0	0.0	8.0	0.2	3.0	0.1
Anisoptera	0.0	0.0	8.0	2.8	2.0	0.6
Zygoptera	0.0	0.0	0.0	0.0	2.0	0.4
Plecoptera	0.0	0.0	0.0	0.0	4.0	0.4
Hemiptera (undetermined family)	0.0	0.0	0.0	0.0	4.0	0.2
Belostomatidae	0.0	0.0	8.0	12.6	4.0	2.0
Corixidae	0.0	0.0	8.0	1.0	14.0	1.1
Naucoridae	0.0	0.0	0.0	0.0	2.0	0.1
Veliidae	0.0	0.0	0.0	0.0	1.0	0.0
Megaloptera	0.0	0.0	0.0	0.0	3.0	1.1
Trichoptera (undetermined family)	0.0	0.0	0.0	0.0	17.0	1.2
Hydropsychidae	25.0	0.2	0.0	0.0	10.0	0.8
Lepidoptera	0.0	0.0	0.0	0.0	5.0	0.9
Dytiscidae	0.0	0.0	0.0	0.0	9.0	0.4
Elmidae	25.0	0.3	0.0	0.0	5.0	0.1
Gyrinidae	0.0	0.0	8.0	0.1	9.0	0.2
Diptera (undetermined family)	0.0	0.0	0.0	0.0	3.0	0.1
Ceratopogonidae	0.0	0.0	0.0	0.0	1.0	0.0
Chironomidae	50.0	0.1	0.0	0.0	15.0	0.3
Simuliidae	0.0	0.0	8.0	0.2	11.0	0.2
Tabanidae	0.0	0.0	8.0	4.7	2.0	0.2
Tipulidae	0.0	0.0	0.0	0.0	7.0	2.3
Benthic Inverts (undetermined taxa)	50.0	2.4	23.0	10.4	28.0	21.6
Terrestrial	25.0	4.0	0.0	0.0	16.0	3.3
Ostracoda	0.0	0.0	0.0	0.0	1.0	0.2
Decapoda (*Orconectes virilis*)	25.0	5.3	0.0	0.0	0.0	0.0
Amphipoda	0.0	0.0	8.0	0.8	1.0	0.0
Hydracarina	0.0	0.0	0.0	0.0	6.0	0.0
Oligochaeta	0.0	0.0	0.0	0.0	4.0	3.0
Planaria	0.0	0.0	0.0	0.0	1.0	0.0
Snail	25.0	2.8	15.0	0.4	21.0	1.6
Fish	0.0	0.0	15.0	9.5	21.0	14.8
Amorphous detritus	75.0	54.8	31.0	12.2	32.0	15.0
Detritus	50.0	21.2	0.0	0.0	11.0	2.4
Undetermined taxa	0.0	0.0	8.0	15.8	5.0	4.0

Table A.1 Continued

	Ictalurus punctatus (3)		*Pylodictis olivaris* (2)	
Prey item	% Occurrence	% Volume	% Occurrence	% Volume
Ephemeroptera	100.0	66.0	100.0	15.7
Hydropsychidae	33.0	1.6	0.0	0.0
Chironomidae	100.0	21.4	50.0	0.7
Simuliidae	67.0	0.8	0.0	0.0
Oligochaeta	67.0	4.1	0.0	0.0
Fish	33.0	6.2	50.0	83.6

Table A.1 Continued

	Pylodictis olivaris (2)		Sub-adult *Oncorhynchus mykiss* (3)		Adult *O. mykiss* (66)	
Prey item	% Occurrence	% Volume	% Occurrence	% Volume	% Occurrence	% Volume
Ephemeroptera	100.0	15.7	100.0	61.2	45.0	9.6
Odonata (undetermined taxa)	0.0	0.0	0.0	0.0	8.0	0.2
Anisoptera	0.0	0.0	0.0	0.0	2.0	0.4
Zygoptera	0.0	0.0	0.0	0.0	9.0	0.4
Plecoptera	0.0	0.0	0.0	0.0	2.0	0.1
Hemiptera (undetermined family)	0.0	0.0	0.0	0.0	8.0	0.2
Belostomatidae	0.0	0.0	0.0	0.0	2.0	0.0
Corixidae	0.0	0.0	0.0	0.0	21.0	0.8
Gerridae	0.0	0.0	0.0	0.0	5.0	0.7
Naucoridae	0.0	0.0	0.0	0.0	11.0	0.6
Notonectidae	0.0	0.0	0.0	0.0	2.0	0.0
Veliidae	0.0	0.0	0.0	0.0	9.0	0.2
Megaloptera	0.0	0.0	0.0	0.0	18.0	2.3
Trichoptera (undetermined family)	0.0	0.0	33.0	0.3	29.0	1.4
Hydropsychidae	0.0	0.0	33.0	3.9	45.0	1.5
Lepidoptera	0.0	0.0	0.0	0.0	6.0	0.3
Dytiscidae	0.0	0.0	0.0	0.0	6.0	0.1
Elmidae	0.0	0.0	0.0	0.0	15.0	0.8
Hydrophilidae	0.0	0.0	0.0	0.0	2.0	0.0
Diptera (undetermined family)	0.0	0.0	33.0	1.6	8.0	0.3
Ceratopogonidae	0.0	0.0	0.0	0.0	0.0	0.0
Chironomidae	50.0	0.7	33.0	0.5	12.0	0.1
Simuliidae	0.0	0.0	33.0	0.9	9.0	0.1
Tabanidae	0.0	0.0	0.0	0.0	5.0	0.1
Tipulidae	0.0	0.0	0.0	0.0	2.0	0.5
Benthic Inverts (undetermined taxa)	0.0	0.0	33.0	8.6	73.0	55.9
Terrestrial	0.0	0.0	33.0	0.4	56.0	6.4
Hydracarina	0.0	0.0	33.0	0.1	12.0	0.0
Oligochaeta	0.0	0.0	0.0	0.0	2.0	0.5
Snail	0.0	0.0	0.0	0.0	5.0	0.0
Fish	50.0	83.6	33.0	4.9	18.0	7.9
Algae	0.0	0.0	0.0	0.0	2.0	0.6
Amorphous detritus	0.0	0.0	0.0	0.0	18.0	3.7
Undetermined taxa	0.0	0.0	33.0	17.7	3.0	4.0

Table A.1 Continued

	Sub-adult *Salmo trutta* (7)		Adult *S. trutta* (102)		*Gambusia affinis* (48)	
Prey item	% Occurrence	% Volume	% Occurrence	% Volume	% Occurrence	% Volume
Ephemeroptera	86.0	23.2	64.0	23.8	50.0	34.3
Odonata (undetermined taxa)	14.0	0.2	12.0	3.0	0.0	0.0
Anisoptera	0.0	0.0	7.0	2.2	0.0	0.0
Zygoptera	29.0	9.1	4.0	0.7	0.0	0.0
Plecoptera	0.0	0.0	4.0	0.1	0.0	0.0
Hemiptera (undetermined family)	0.0	0.0	2.0	0.6	4.0	2.8
Belostomatidae	0.0	0.0	1.0	0.1	0.0	0.0
Corixidae	29.0	5.5	11.0	3.3	8.0	3.7
Gerridae	0.0	0.0	13.0	0.8	0.0	0.0
Naucoridae	0.0	0.0	4.0	0.4	0.0	0.0
Notonectidae	0.0	0.0	2.0	0.1	0.0	0.0
Veliidae	14.0	0.5	2.0	0.1	0.0	0.0
Megaloptera	0.0	0.0	13.0	5.8	0.0	0.0
Trichoptera (undetermined family)	0.0	0.0	24.0	0.9	4.0	0.6
Hydropsychidae	0.0	0.0	24.0	1.0	0.0	0.0
Lepidoptera	0.0	0.0	2.0	0.2	0.0	0.0
Dytiscidae	14.0	3.3	4.0	0.5	0.0	0.0
Elmidae	0.0	0.0	16.0	1.0	0.0	0.0
Gyrinidae	0.0	0.0	1.0	0.0	0.0	0.0
Diptera (undetermined family)	14.0	26.7	9.0	0.6	0.0	0.0
Ceratopogonidae	14.0	0.2	2.0	0.0	0.0	0.0
Chironomidae	14.0	0.2	14.0	2.3	15.0	0.8
Simuliidae	29.0	0.5	10.0	0.1	6.0	0.8
Tabanidae	0.0	0.0	1.0	0.1	0.0	0.0
Tipulidae	0.0	0.0	4.0	0.3	0.0	0.0
Benthic Inverts (undetermined taxa)	57.0	14.8	38.0	24.5	17.0	11.2
Terrestrial	14.0	0.5	50.0	6.2	19.0	9.7
Cladocera	0.0	0.0	0.0	0.0	4.0	0.6
Hydracarina	0.0	0.0	6.0	0.0	0.0	0.0
Oligochaeta	0.0	0.0	1.0	0.0	2.0	0.3
Bivalve	0.0	0.0	0.0	0.0	4.0	3.5
Snail	0.0	0.0	2.0	0.0	27.0	10.6
Fish	14.0	4.8	20.0	15.4	0.0	0.0
Algae	0.0	0.0	1.0	0.0	4.0	0.3
Amorphous detritus	29.0	10.5	10.0	1.2	27.0	21.0
Detritus	0.0	0.0	10.0	1.8	0.0	0.0
Undetermined taxa	0.0	0.0	10.0	2.7	0.0	0.0

Table A.1 Continued

	Lepomis cyanellus (8)		Juvenile *Micropterus dolomieu* (12)		Sub-adult *M. dolomieu* (29)		Adult *M. dolomieu* (12)	
Prey item	% Occurrence	% Volume	% Occurrence	% Volume	% Occurrence	% Volume	% Occurrence	% Volume
Ephemeroptera	63.0	18.8	100.0	67.7	55.0	33.3	29.0	6.6
Odonata (undetermined taxa)	0.0	0.0	0.0	0.0	0.0	0.0	8.0	2.1
Anisoptera	0.0	0.0	0.0	0.0	7.0	11.5	13.0	3.2
Zygoptera	0.0	0.0	0.0	0.0	3.0	1.2	8.0	2.3
Belostomatidae	0.0	0.0	0.0	0.0	0.0	0.0	4.0	4.6
Corixidae	38.0	28.9	17.0	6.2	7.0	0.5	17.0	1.0
Naucoridae	0.0	0.0	0.0	0.0	0.0	0.0	8.0	0.0
Veliidae	0.0	0.0	0.0	0.0	0.0	0.0	4.0	0.0
Megaloptera	0.0	0.0	0.0	0.0	0.0	0.0	17.0	12.3
Trichoptera (undetermined family)	13.0	3.4	0.0	0.0	3.0	0.1	8.0	0.0
Hydropsychidae	0.0	0.0	8.0	2.5	7.0	1.0	8.0	0.8
Lepidoptera	0.0	0.0	0.0	0.0	0.0	0.0	4.0	0.1
Chironomidae	0.0	0.0	17.0	0.2	21.0	0.9	8.0	0.0
Simuliidae	0.0	0.0	17.0	0.5	3.0	0.1	4.0	0.0
Benthic Inverts (undetermined taxa)	25.0	13.4	0.0	0.0	10.0	3.4	29.0	14.1
Terrestrial	13.0	1.3	8.0	0.5	0.0	0.0	0.0	0.0
Decapoda (*Orconectes virilis*)	0.0	0.0	0.0	0.0	0.0	0.0	4.0	24.6
Fish	25.0	30.9	8.0	22.4	31.0	34.5	38.0	22.8
Amorphous detritus	13.0	3.4	0.0	0.0	10.0	13.5	8.0	5.3

Appendix B - Results of nonnative predator IsoSource Modeling

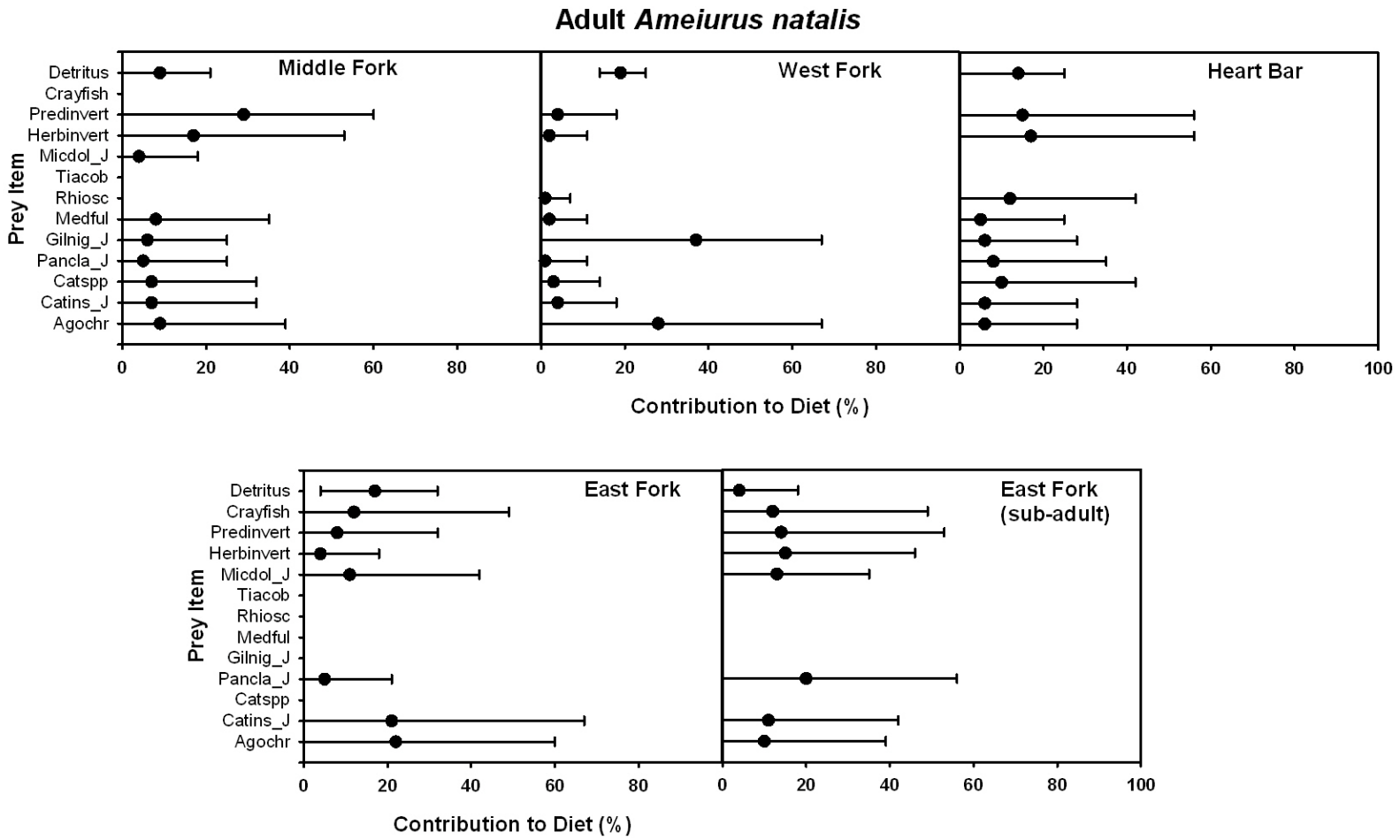

Figure B.1 Results of IsoSource modeling for C and N isotopic signatures of adult and sub-adult yellow bullhead collected from West Fork, Middle Fork, Heart Bar, and East Fork reaches in the upper Gila River basin. Points represent the mean percent contribution of a prey item to the diet of the predator and error bars are the 1st to 99th percentiles.

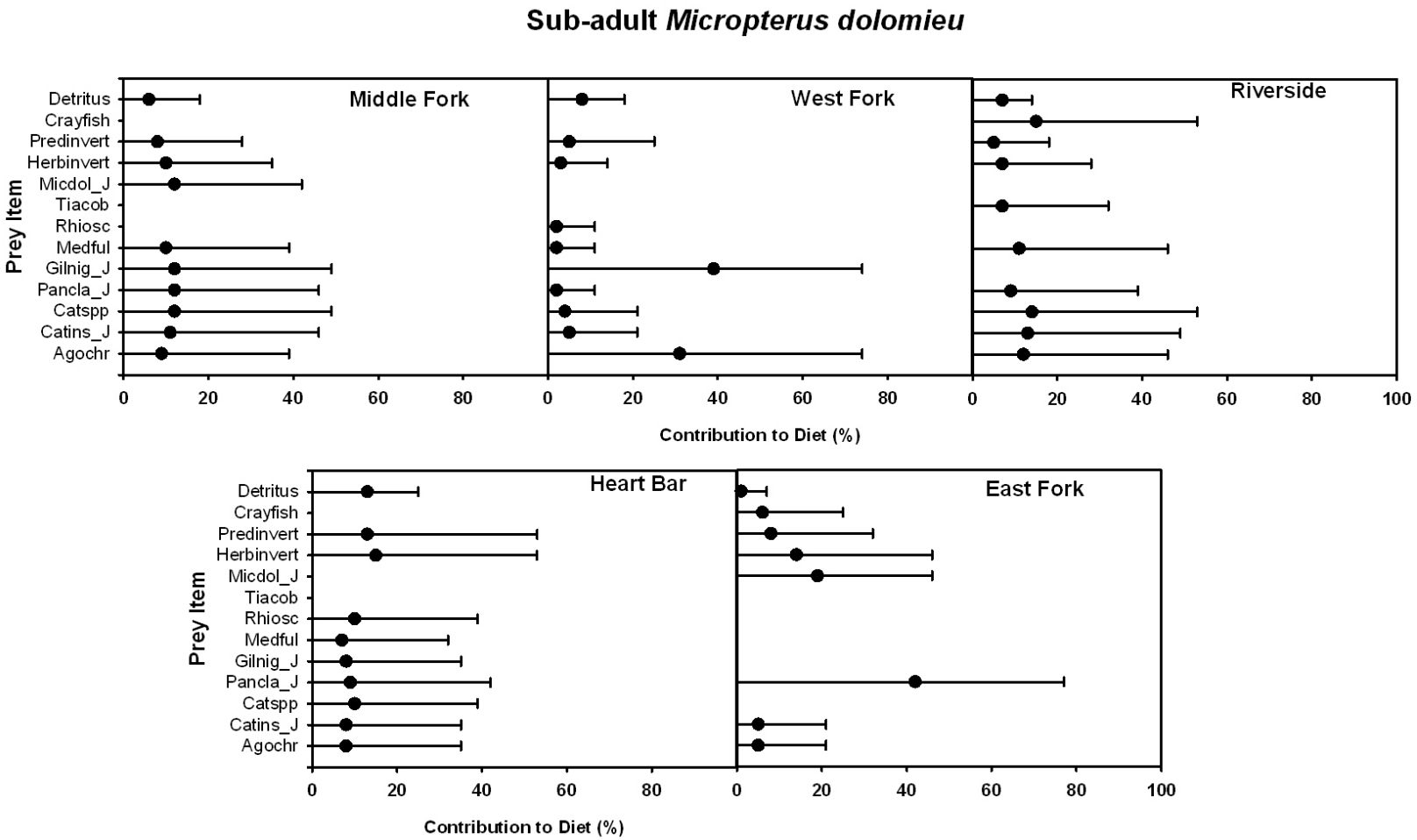

Figure B.2 Results of IsoSource modeling for C and N isotopic signatures of sub-adult smallmouth bass collected from Middle Fork, West Fork, Riverside, Heart Bar, and East Fork reaches in the upper Gila River basin. Points represent the mean percent contribution of a prey item to the diet of the predator and error bars are the 1st to 99th percentiles.

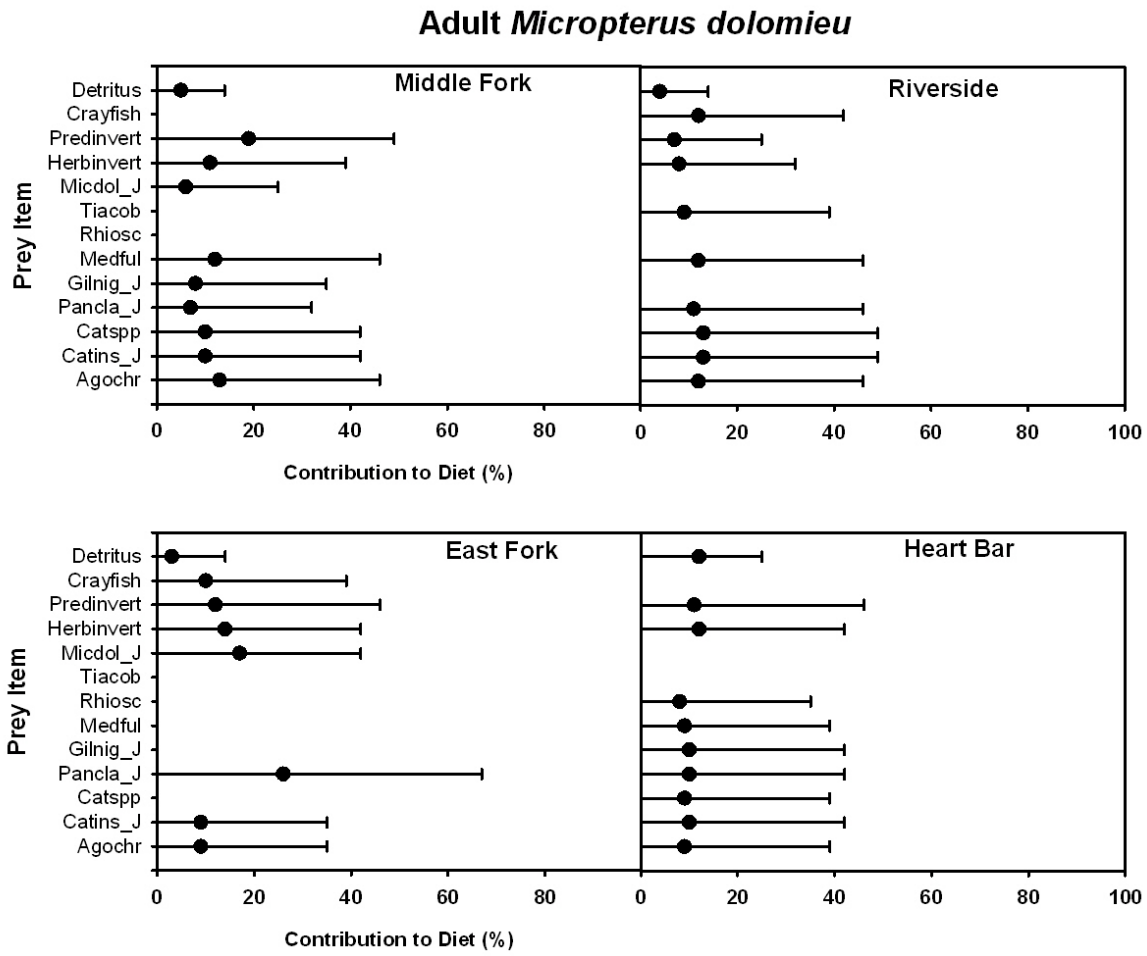

Figure B.3 Results of IsoSource modeling for C and N isotopic signatures of adult smallmouth bass collected from Middle Fork, Riverside, Heart Bar, and East Fork reaches in the upper Gila River basin. Points represent the mean percent contribution of a prey item to the diet of the predator and error bars are the 1st to 99th percentiles.

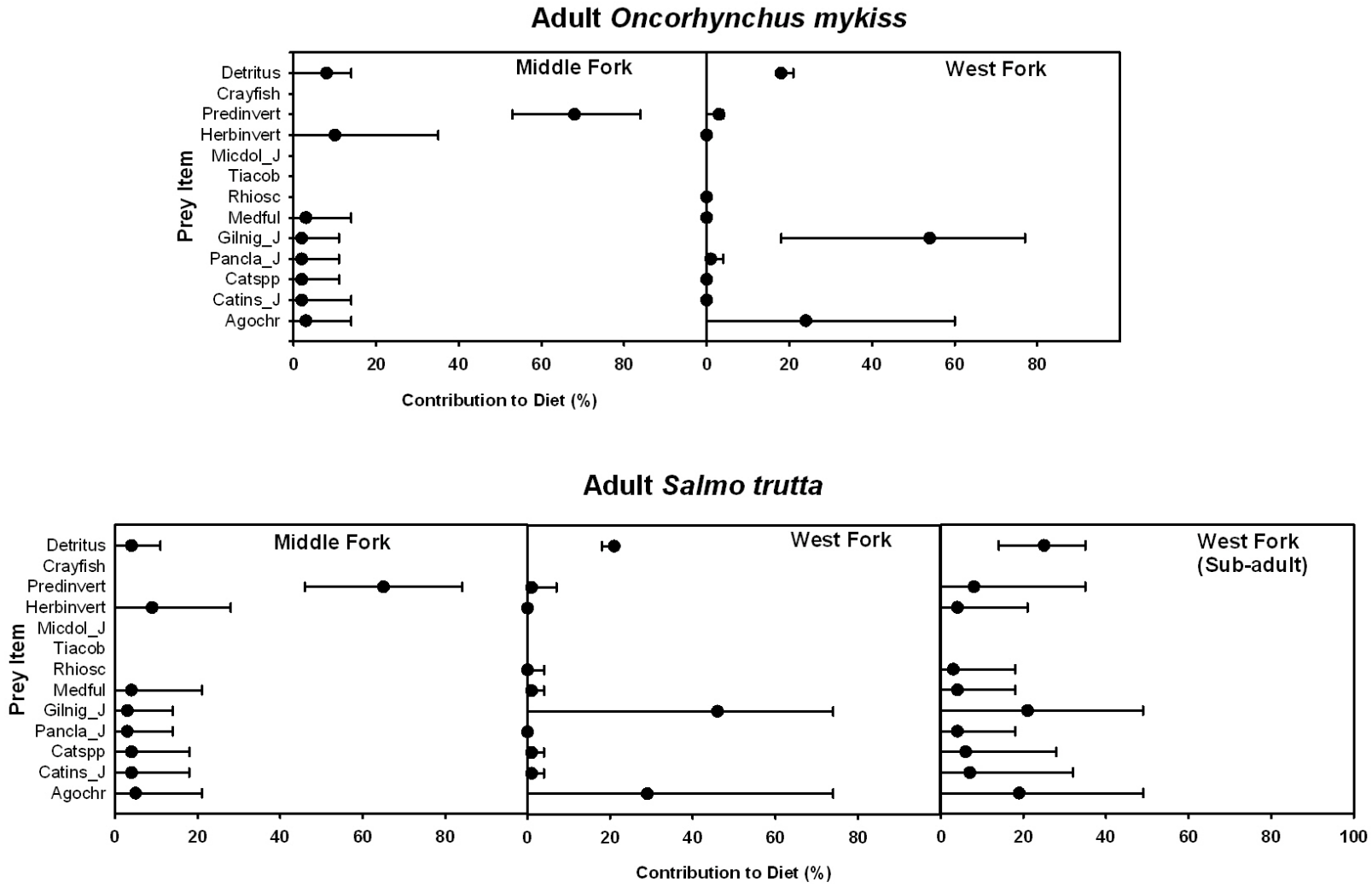

Figure B.4 Results of IsoSource modeling for C and N isotopic signatures of adult and sub-adult rainbow and brown trout collected from West Fork and Middle Fork reaches in the upper Gila River basin. Points represent the mean percent contribution of a prey item to the diet of the predator and error bars are the 1st to 99th percentiles.

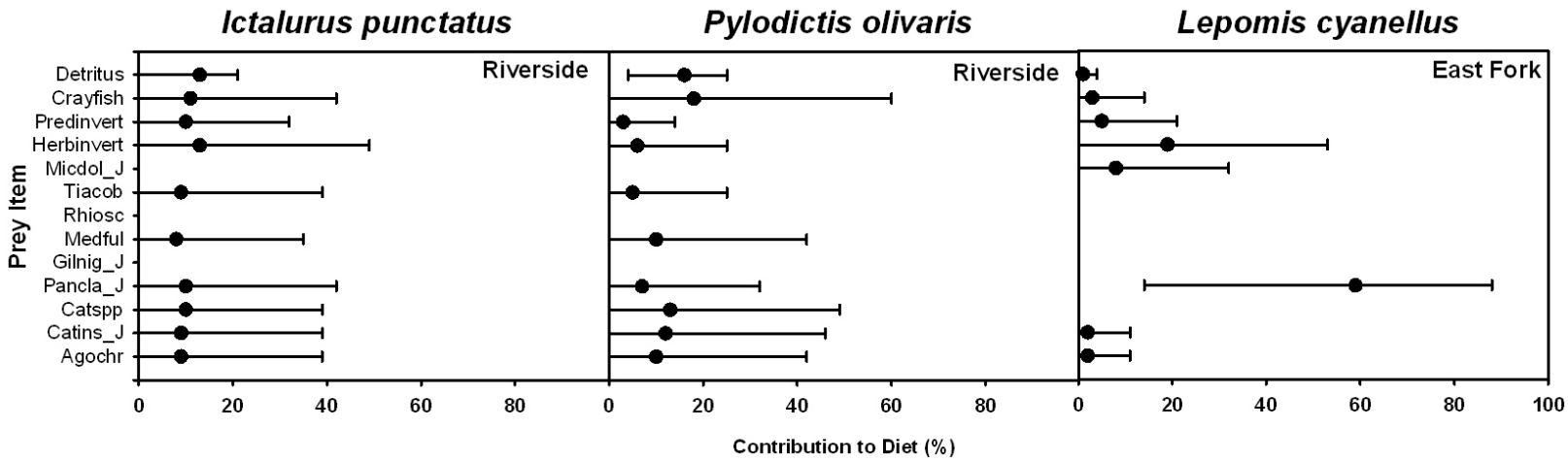

Figure B.5 Results of IsoSource modeling for C and N isotopic signatures of adult channel catfish, flathead catfish, and green sunfish collected from Riverside and East Fork reaches in the upper Gila River basin. Points represent the mean percent contribution of a prey item to the diet of the predator and error bars are the 1st to 99th percentiles.