

# Diet and trophic niche overlap of native and nonnative fishes in the Gila River, USA: implications for native fish conservation

Pilger TJ, Gido KB, Propst DL. Diet and trophic niche overlap of native and nonnative fishes in the Gila River, USA: implications for native fish conservation.

Ecology of Freshwater Fish 2010: 19: 300–321. © 2010 John Wiley & Sons A/S

**Abstract** – The upper Gila River basin is one of the few unimpounded drainage basins west of the Continental Divide, and as such is a stronghold for endemic fishes in the region. Nevertheless, multiple nonindigenous fishes potentially threaten the persistence of native fishes, and little is known of the trophic ecology of either native or nonnative fishes in this system. Gut contents and stable isotopes ( $^{13}\text{C}$  and  $^{15}\text{N}$ ) were used to identify trophic relationships, trophic niche overlap and evaluate potential interactions among native and nonnative fishes. Both native and nonnative fishes fed across multiple trophic levels. In general, adult native suckers had lower  $^{15}\text{N}$  signatures and consumed more algae and detritus than smaller native fish, including juvenile suckers. Adult nonnative smallmouth bass (*Micropterus dolomieu*), yellow bullhead (*Ameiurus natalis*) and two species of trout preyed on small-bodied fishes and predaceous aquatic invertebrates leading to significantly higher trophic positions than small and large-bodied native fishes. Thus, the presence of these nonnative fishes extended community food-chain lengths by foraging at higher trophic levels. Although predation on juvenile native fishes might threaten persistence of native fishes, the high degree of omnivory suggests that impacts of nonnative predators may be lessened and dependent on environmental variability.

**T. J. Pilger<sup>1</sup>, K. B. Gido<sup>1</sup>,  
D. L. Propst<sup>2</sup>**

<sup>1</sup>Division of Biology, Kansas State University, Manhattan, KS, USA, <sup>2</sup>Conservation Services Division, New Mexico Department of Game and Fish, Santa Fe, NM, USA

**Key words:** diet analysis; introduced species; stable isotope analysis; apex predator

T. J. Pilger, Department of Biology, University of New Mexico, MSC03 2020, 1 University of New Mexico, Albuquerque, NM 87131-0001, USA; e-mail: tjpilger@unm.edu

Accepted for publication March 6, 2010

## Introduction

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, the introduction of smallmouth bass *Micropterus dolomieu* and rock bass *Ambloplites rupestris* coincided with a reduction in the mean trophic position native lake trout *Salvelinus namaycush*, presumably through competitive displacement (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).

In warm water streams with natural flow regimes, interactions among native and nonnative fishes are thought to be negative, although, the extent and magnitude of such interactions is equivocal. 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 contrast, nonnative predators (smallmouth bass, northern pike *Esox lucius*, and channel catfish *Ictalurus punctatus*) have been implicated in the decline of small-bodied native fishes in the Green and Yampa rivers, Colorado (Tyus & Beard 1990; Tyus & Nikirk 1990; Tyus & Saunders 2000; Johnson et al. 2008). Similarly, 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 these invasions 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 and lack of physical habitat modification, persistence of native fishes has been compromised by the establishment of nonnative fishes (Propst et al. 2008). The chronic presence of nonnative fishes, especially predators, combined with natural drought cycles led to declines in abundances and occurrences of native fishes. Propst et al. (2008) inferred negative trophic interactions between native and nonnative fishes using correlative evidence, yet little was known about shared resource use among species. Thus, our primary objective was to characterize trophic linkages among native and nonnative fishes in the upper Gila River. We analysed diets from gut contents that provided a direct characterization of resource use over short temporal scales (<24 h) and stable isotopes ( $^{13}\text{C}$  and  $^{15}\text{N}$ ) of dorsal muscle which integrates energy pathways over longer timescales (weeks to months). Isotopic signatures of dorsal muscle reflect integrated diet over long time periods because of slow isotopic turnover rates in muscle tissue (Dalerum & Angerbjörn 2005; Martinez del Rio et al. 2009). Ratios of naturally occurring  $^{13}\text{C}$  to  $^{12}\text{C}$  represent source pools of organic carbon (Peterson & Fry 1987) or habitat use (Gido et al. 2006). Increasing  $^{15}\text{N}$  relative to  $^{14}\text{N}$  is typically associated with trophic level (Peterson & Fry 1987; Peterson 1999). Specific goals of our study were to: (i) characterize the food

webs in six reaches of the Gila River that varied in abundance of nonnative predators, (ii) quantify resource overlap among native and nonnative fishes and (iii) quantify the effects of nonnative predators on native food webs based on differences in food web structure across sites and habitats with varying densities of nonnative predators. Diet studies of nonnative predators from other systems (Marsh & Douglas 1997; Robertson & Winemiller 2001) suggest they had to be preying on small-bodied fishes resulting in higher  $\delta^{15}\text{N}$  signatures (i.e., higher trophic positions) than native fishes. In addition, the presence of nonnative predators can reduce  $\delta^{15}\text{N}$  signatures of native fishes, presumably by reducing trophic position of natives (Marks et al. 2009). The presence of nonnatives also might decrease the range in  $\delta^{13}\text{C}$  of native fishes if nonnatives constrain the diets of natives or cause shifts in habitat use. Understanding these trophic linkages and interactions among native and nonnative fishes will help inform 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 (Fig. 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 Chihuahuan 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 4828 km<sup>2</sup>. The upper Gila River sites were located on each of the three forks of the Gila River. The West Fork (1737 m elevation) and Middle Fork (1733 m) sites were upstream of their confluence and the Heart Bar site (1725 m) was located ~2 km below their confluence. The East Fork site (1876 m) was approximately 37 km upstream of the confluence with the mainstem Gila River. The upper Gila sites have watersheds that are almost completely within federal lands, including the Gila and Aldo Leopold National Wildernesses, and are minimally altered by dispersed livestock grazing in the East Fork Gila River drainage. Each upper Gila tributary flows mostly through canyons and narrow valleys. The San Francisco River site (1433 m) 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

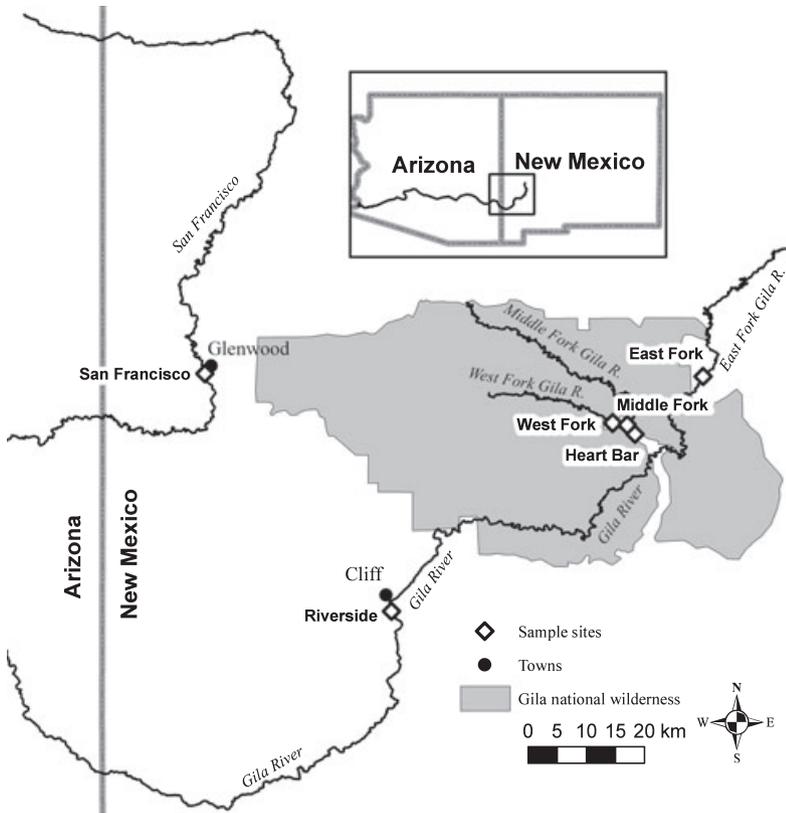


Fig. 1. Study area in the upper Gila River basin in southwest New Mexico, USA. Locations of sample sites are indicated by diamonds.

mainstem (1359 m) was near Cliff, New Mexico, 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 (Table 1). Large-bodied species were headwater chub *Gila nigra*, Sonora sucker *Catostomus insignis*, desert sucker *Pantosteus clarki*, yellow bullhead, rainbow trout *Oncorhynchus mykiss*, brown trout *Salmo trutta* and smallmouth bass. Small-bodied species (i.e., species with maximum total length <100 mm) were longfin dace *Agosia chrysogaster*, spikedace *Meda fulgida*, speckled dace *Rhinichthys osculus*, loach minnow *Tiaroga cobitis*, red shiner and western mosquitofish *Gambusia affinis*, and were considered a single group dominated by age-1 individuals based on length-frequency histograms.

Fishes, invertebrates and basal energy sources for stable isotope analysis were collected from pool and riffle complexes (hereafter called macrohabitats) within the six sample sites in June to July 2007 and 2008 using

a combination of seining (4.6 m × 1.2 m seine with 3.2 mm mesh) and electrofishing (Smith-Root Model LR24 backpack shocker, Smith-Root, Inc., Vancouver, WA). One to five macrohabitats were sampled per site, depending on availability. Macrohabitats were sampled until an appropriate representation of the species and size classes present 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 fishes >150 mm were removed and preserved in 10% formalin, except for adult headwater chub, for which a modified gastric lavage technique was used to extract gut contents. A 60-cc syringe with a 30-cm long piece of flexible tubing (3 mm, outside diameter) filled with water and inserted down the oesophagus flushed stomach contents, which were captured in a 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 Leptoheptidae), Trichoptera (Hydropsychidae), Megaloptera (Corydalidae) and Diptera (Tabanidae) were sorted and separated into containers of freshwater overnight to allow gut evacuation (Jardine et al. 2005).

Table 1. Biology and conservation status of native and nonnative fishes in the Gila River, New Mexico. Maximum sizes and age-classes estimated from length-frequency histograms (unpublished data).

	Max size (mm TL)	Trophic role	Conservation status	Age-classes (mm TL)		
				Juvenile	Sub-adult	Adult
Native species						
<i>Agosia chrysogaster</i>	76	Omnivore				
<i>Gila nigra</i>	302	Invertivore/piscivore	SE	<70	70–150	>150
<i>Meda fulgida</i>	76	Invertivore	T			
<i>Rhinichthys osculus</i>	89	Invertivore				
<i>Tiaroga cobitis</i>	85	Invertivore	T			
<i>Catostomus insignis</i>	610	Omnivore		<100	100–160	>160
<i>Pantosteus clarki</i>	411	Invertivore switching to algivore		<100	100–160	>160
Nonnative species						
<i>Cyprinella lutrensis</i>	70	Invertivore/detritivore				
<i>Ameiurus natalis</i>	340	Invertivore switching to piscivore		<75	75–130	>130
<i>Ictalurus punctatus</i>	578	Invertivore switching to piscivore				
<i>Pylodictis olivaris</i>	750	Invertivore switching to piscivore				
<i>Oncorhynchus mykiss</i>	386	Invertivore switching to piscivore		<80	80–140	>140
<i>Salmo trutta</i>	405	Invertivore switching to piscivore		<80	80–140	>140
<i>Gambusia affinis</i>	61	Invertivore				
<i>Lepomis cyanellus</i>	117	Invertivore switching to piscivore				
<i>Micropterus dolomieu</i>	414	Invertivore switching to piscivore		<80	80–185	>185

Species trophic information from Sublette et al. (1990). Conservation status codes are, SE, listed as endangered by the state of New Mexico and T, federally listed as threatened.

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 characterised 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 the natural log of dry mass of diet items to the natural log of the grid area for 148 individuals of native and nonnative fishes (slope = 0.033  $r^2 = 0.410$ ,  $P < 0.001$ ). 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 100× 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  $\delta^{15}\text{N}$  than other tissues and small amounts of inorganic carbonates (Pinnegar & Polunin 1999). Dorsal muscle also has relatively low lipid content compared to other tissues (e.g., liver), eliminating the need for lipid extraction (Sotiropoulos et al. 2004; Ingram et al. 2007). Muscle tissue was taken from a maximum of five individuals for small-bodied species and each 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). Microscopic examination suggested 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 homogenised using a mortar and pestle. Powdered samples were analyzed for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  with a ThermoFinnigan Delta Plus mass spectrometer (Thermo Fisher Scientific, Inc., Waltham, MA) with a CE 1110 elemental analyser 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:

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

where  $R = {}^{15}\text{N}/{}^{14}\text{N}$  or  ${}^{13}\text{C}/{}^{12}\text{C}$ . The  $\delta^{13}\text{C}$  values for all organisms were corrected for lipids using C:N

ratios for animals and %C for plants following Post et al. (2007), which account for materials with variable lipid contents. The Post et al. (2007) lipid correction is a linear model based on C:N ratios and is a species-independent approach to lipid correction (Mintenbeck et al. 2008). Overall, there was little variability among tissue samples from the same species collected at each site; mean coefficient of variation ( $CV_{13C}$ ) =  $3.7 \pm 2.7\%$  and ( $CV_{15N}$ ) =  $5.1 \pm 4.4\%$ .

Data analysis

*Characterizing stream food webs*

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

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

where  $TP_{\text{diet}}$  = the trophic position of a species weighted by the proportion of ingested material of the *i*th prey item ( $V_i$ ), and  $T_i$  = trophic level 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 levels 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 2). Because trophic level can vary greatly within macroinvertebrate taxonomic groups, we assigned trophic levels 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 standardised at each site to the  $\delta^{15}N$  signature of a primary consumer following the equation of Cabana & Rasmussen (1996):

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

We chose  $\delta^{15}N$  values of ephemeropterans as the baseline because they were abundant at all sites, they were frequently found in the diets of all fish species, and their  $\delta^{15}N$  was similar to other dominant primary consumers (e.g., chironomids). A potential bias with using this group is the relatively rapid turnover rates, which might increase the variability in  $\delta^{15}N$  values. Baseline  $\delta^{15}N$  was calculated for each site as the mean  $\delta^{15}N$  of common ephemeropteran families, primarily Baetidae and Heptageniidae. Linear regression was used to test the strength of the relationship between diet and stable isotope derived measures of trophic position, and help interpret discrepancies between these methods that represent diet over different temporal scales.

*Resource overlap*

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

Prey category	Code	Estimated trophic level	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, Ostracoda, Copepoda
Ephemeroptera	EPH	2.0	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 Simuliid larvae
Tipulidae	TIP	2.5	
Tabanidae	TAB	3.0	
Terrestrial invertebrates	TER	2.5	Orthoptera, Hymenoptera, Unknown winged invertebrates
Fish	FISH	3.0	

Table 2. Categories of prey taxa and their estimated trophic level used for calculating trophic positions based on the diets of native and nonnative fishes in the Gila River, New Mexico, 2007–2008.

Estimated trophic levels of prey categories are based on the dominant functional feeding group in each category (*sensu* Merritt & Cummins 1996). Prey categories and codes are used in principal coordinates analysis (Fig. 5).

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). Native large-bodied fishes included adults and sub-adults of native suckers and headwater chub (Table 3). Native small-bodied fishes included native small-bodied minnows, juvenile headwater chub and juvenile suckers. Non-native 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

Table 3. Overall mean  $\pm$  standard deviation (combined sites and years) of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  signatures for native and nonnative fishes, macroinvertebrates and basal carbon sources in Gila River food webs, New Mexico, 2007–2008.

Species	Number of individuals	Average $\delta^{13}\text{C}$ (‰)	Average $\delta^{15}\text{N}$ (‰)	C:N range
Native small-bodied				
<i>Agosia chrysogaster</i>	50	$-24.9 \pm 1.8$	$10.1 \pm 1.1$	2.49–6.45
Juvenile <i>Gila nigra</i>	7	$-24.1 \pm 1.4$	$8.8 \pm 0.6$	3.13–4.65
<i>Meda fulgida</i>	19	$-25.4 \pm 1.9$	$10.3 \pm 1.6$	3.33–4.12
<i>Rhinichthys osculus</i>	38	$-26.9 \pm 2$	$8.8 \pm 0.8$	2.4–5.87
<i>Tiaroga cobitis</i>	12	$-28.1 \pm 0.9$	$10.6 \pm 1$	3.65–4.43
Juvenile <i>Catostomus insignis</i>	82	$-24.5 \pm 1.9$	$9.3 \pm 1$	2.21–5.38
Juvenile <i>Pantosteus clarki</i>	54	$-25.6 \pm 2.3$	$8.3 \pm 1.2$	2.35–6.54
Native large-bodied				
Sub-adult <i>Gila nigra</i>	12	$-23.7 \pm 0.7$	$8.7 \pm 0.9$	3.24–3.24
Adult <i>Gila nigra</i>	15	$-23.8 \pm 1.6$	$9.2 \pm 1.1$	3.12–3.12
Sub-adult <i>Catostomus insignis</i>	34	$-24.6 \pm 2.2$	$9.1 \pm 1.5$	2.34–2.34
Adult <i>Catostomus insignis</i>	164	$-24.3 \pm 1.5$	$9 \pm 1.1$	2.15–2.15
Sub-adult <i>Pantosteus clarki</i>	28	$-24.8 \pm 2.6$	$8.2 \pm 1.4$	2.92–2.92
Adult <i>Pantosteus clarki</i>	50	$-24.2 \pm 2.2$	$8.5 \pm 1$	2.38–2.38
Nonnative small-bodied				
<i>Cyprinella lutrensis</i>	4	$-24.9 \pm 1.5$	$9.7 \pm 0.3$	1.91–3.37
Juvenile <i>Ameiurus natalis</i>	2	$-26.4 \pm 0.2$	$10.6 \pm 0.7$	3.43–4.17
<i>Gambusia affinis</i>	22	$-23.3 \pm 1.7$	$10.3 \pm 1.5$	3.18–4.32
Juvenile <i>Micropterus dolomieu</i>	6	$-24.6 \pm 1.5$	$9.4 \pm 2$	3.23–3.73
Nonnative Large-bodied				
Sub-adult <i>Ameiurus natalis</i>	3	$-24.5 \pm 1.5$	$10.8 \pm 1.6$	3.54–3.69
Adult <i>Ameiurus natalis</i>	41	$-23.3 \pm 1.3$	$10.3 \pm 1$	2.5–5.64
<i>Ictalurus punctatus</i>	3	$-26.6 \pm 1$	$11 \pm 0.6$	3.71–3.91
<i>Pylodictis olivaris</i>	2	$-26 \pm 0.8$	$10.9 \pm 2.3$	3.88–3.98
<i>Oncorhynchus mykiss</i>	23	$-22.8 \pm 2.2$	$9.3 \pm 0.6$	2.95–4.87
Sub-adult <i>Salmo trutta</i>	5	$-24.9 \pm 1.5$	$8.1 \pm 0.5$	2.72–4.11
Adult <i>Salmo trutta</i>	28	$-23.4 \pm 1.5$	$9.1 \pm 0.7$	3.01–3.89
<i>Lepomis cyanellus</i>	6	$-21.7 \pm 0.3$	$12.1 \pm 1.1$	3.06–4.07
Sub-adult <i>Micropterus dolomieu</i>	19	$-24.5 \pm 1.4$	$10.8 \pm 1.2$	3.1–3.99
Adult <i>Micropterus dolomieu</i>	18	$-23.3 \pm 1.4$	$11.7 \pm 1.2$	3.54–3.69
Macroinvertebrates				
Herbivorous invertebrates	137	$-27.2 \pm 3$	$5.4 \pm 1.7$	2.48–15.69
Predaceous invertebrates	114	$-26.4 \pm 2.4$	$6.5 \pm 1.5$	2.37–7.44
<i>Orconectes virilis</i>	12	$-24.9 \pm 1.3$	$7.9 \pm 0.8$	2.26–3.76
Basal resources				
Filamentous algae	34	$-27.4 \pm 7.1$	$2.8 \pm 1.8$	6.89–18.28
Single-celled algae	16	$-24.3 \pm 2.3$	$3.3 \pm 1.8$	n/a
Grass	17	$-26.9 \pm 3.4$	$2.4 \pm 2.2$	11.97–59.28
Emergent macrophytes	7	$-22.9 \pm 2.2$	$5 \pm 1.5$	7.59–20.40
Willow	17	$-27.8 \pm 1.3$	$1.4 \pm 1.6$	10.56–44.76
Detritus	17	$-27.6 \pm 1.6$	$-0.1 \pm 2.5$	16.65–56.76
FPOM	15	$-28.4 \pm 6.9$	$4.8 \pm 1.6$	n/a

Fish species are grouped by size-groups used in analyses of resource overlap.

the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures of species/age-classes, native and nonnative size-groups were independent factors, and site and year were co-variables. Box's test of equality of covariance matrices and Levene's test of equality of error variances were run to determine how well the dependent variables conform to the assumptions of the general linear model. *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, IL, USA).

#### *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 diets of native fishes across sites, as represented by the PCoA of the percent similarity matrix described above. CAP is a direct ordination technique, similar to canonical correspondence analysis (CCA) or redundancy analysis (RDA), that evaluates variation in a multivariate data set constrained by linear relationships with measured environmental gradients; in this case nonnative predator density. Nonnative predator density across sites 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 were used to test for differences in trophic ecology of native fishes associated with the presence of nonnative predators. We used the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  ranges of native fishes at each macrohabitat 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  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  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  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  ranges in macrohabitats across sites where nonnative predators were present and where they were absent. Separate MANOVAS tested between sites with presence or 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, and all age-groups of 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  $\delta$  units for solutions which were examined at 2% increments. We report both mean and range of each source contribution because 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 source contributions for yellow bullhead, rainbow trout or brown trout at the Heart Bar site from the available sources.

## Results

### Characterising stream food webs

Diets of 996 individuals representing 7 native and 9 nonnative species were analysed from the 6 sites. Native small-bodied fishes were primarily insectivorous (Fig. 2; Appendix A). Ephemeropteran larvae made up the largest percent volume of small-bodied native fishes diet (range 12.8–53.8% of diet per species/age-class), but chironomid, and simuliid larvae were generally the most frequently consumed items (31.0–79.0% of individuals). Adult Sonora sucker and desert sucker were omnivores consuming algae/detritus (16.0–74.0% of volume), as well as ephemeropteran, chironomid and simuliid larvae (33.0–91.0% 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 subadults (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 headwater chub 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 (Fig. 2; Appendix A). Nonnative trout consumed a wide variety of benthic invertebrates as well as terrestrial invertebrates. On average, the diets of adult nonnative fishes were comprised of 25% fish, although this was highly variable among species (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

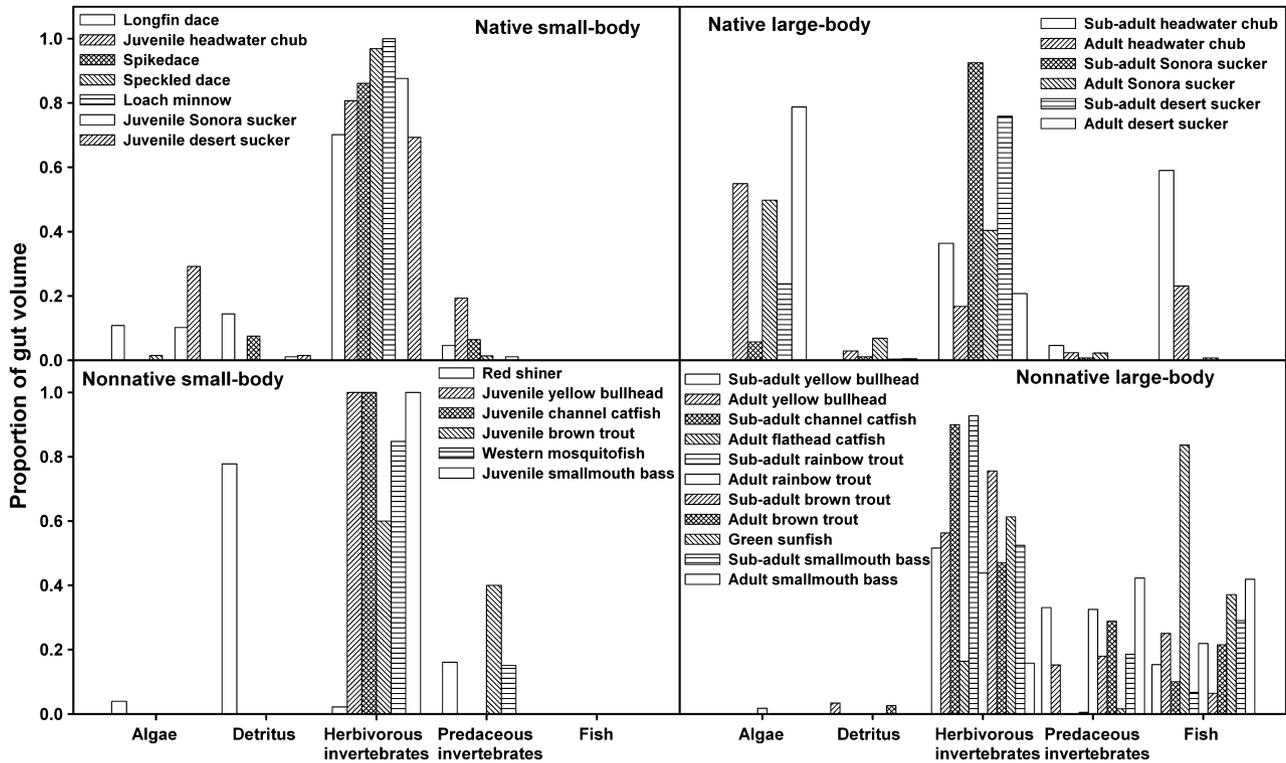


Fig. 2. Diets of native and nonnative fishes collected from the upper Gila River basin, June and July of 2007 and 2008. All individuals per species/age-class (indicated by fill style) were pooled to determine proportion of gut volume. Invertebrate prey was classified as herbivorous invertebrates or predaceous invertebrates.

found in the stomach of an adult yellow bullhead. Smallmouth bass had the greatest percentage of empty guts (44%) followed by yellow bullhead (22%), whereas rainbow trout and brown trout had low percentages of empty guts (4% for both species). Nonnative red shiner ( $N = 6$ ), and western mosquitofish ( $N = 53$ ) fed primarily on algae, detritus and ephemeropteran larvae.

Mean  $\delta^{13}\text{C}$  of potential basal resources (Table 3) was used to evaluate their relative importance in the food chain of fishes; the grand mean  $\delta^{13}\text{C}$  of all fish was  $-24.6\text{‰}$  (range  $-28.1$  to  $-21.7\text{‰}$ ). Mean  $\delta^{13}\text{C}$  of single-celled algae ( $-24.3\text{‰}$ ) was most closely aligned with fishes, suggesting this was a major carbon source in the food chain. Other sources, except emergent macrophytes (mean  $\delta^{13}\text{C}$   $-22.9\text{‰}$ ), were generally depleted relative to fishes. Filamentous algae and FPOM generally depleted relative to fish, but their values were highly variable, suggesting their importance as a basal resource may vary across sites and sample periods. Mean  $\delta^{13}\text{C}$  of herbivorous ( $-27.2\text{‰}$ ) and predaceous ( $-26.4\text{‰}$ ) invertebrates was depleted relative to fishes (Table 3). However, these differences were largely because of two samples (West Fork 2007 and Riverside 2008) in which the macroinvertebrates were  $\sim 2\text{‰}$  depleted relative to fishes. Macroinvertebrate  $\delta^{13}\text{C}$  aligned closely with fishes in samples from other sites and years.

Although there was notable variability in  $\delta^{15}\text{N}$  samples, the mean  $\delta^{15}\text{N}$  of potential basal resources was  $\sim 3\text{‰}$  more depleted than macroinvertebrates, and macroinvertebrates were about  $\sim 3\text{‰}$  more depleted than fishes. Mean  $\delta^{15}\text{N}$  of predaceous invertebrates overlapped with the most depleted fishes and herbivorous invertebrates were about  $1\text{‰}$  lower than predaceous invertebrates.

Tissue samples from 787 fishes were analysed for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures. When present, riffle-dwelling fishes (speckled dace and loach minnow) had the most depleted  $\delta^{13}\text{C}$  values ( $-27.4$  to  $-24.1\text{‰}$ ) compared with other fishes (Table 3 and Fig. 3). Nonnative adult and sub-adult yellow bullhead, channel catfish, flathead catfish, smallmouth bass, rainbow trout and brown trout generally had more enriched  $\delta^{15}\text{N}$  values ( $11.2$ – $14.8\text{‰}$ ) than native fishes. Although fish  $\delta^{13}\text{C}$  at most sites appeared to be centered among the highly variable  $\delta^{13}\text{C}$  of basal resources, at Middle Fork, Heart Bar and West Fork, fish  $\delta^{13}\text{C}$  was greater than basal resources. Overall, the mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of fish species/age-classes, invertebrates and basal resources was highly variable among sites.

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  $< 1$  (Fig. 4, slope =  $0.54$ ,  $95\%$  CI =  $0.39$ – $0.78$ ) and generally reflected higher trophic position assignment

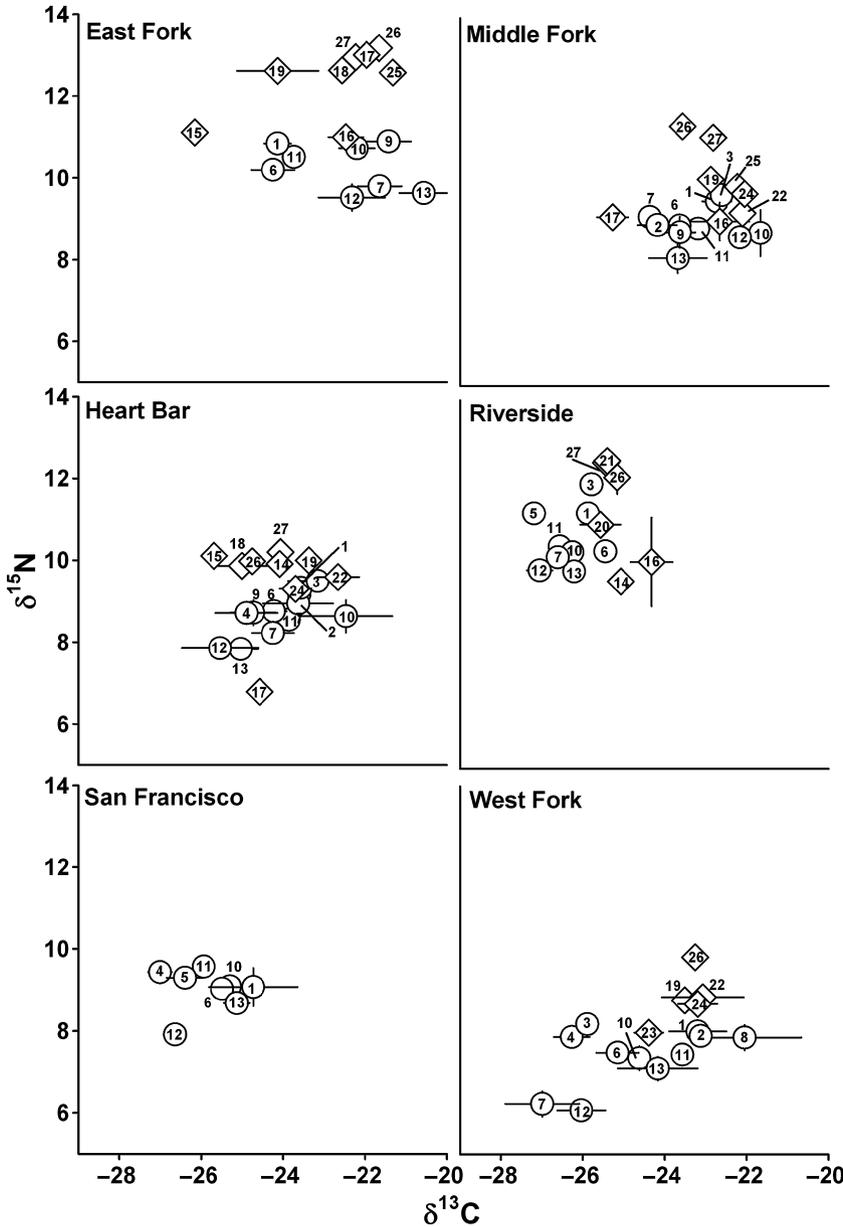


Fig. 3. Mean  $\delta^{13}\text{C}$   $\delta^{15}\text{N}$  values for native fishes (circles) and nonnative fishes (diamonds) for East Fork, Middle Fork, Heart Bar, Riverside, San Francisco, and West Fork sites sampled in 2007 and 2008. Error bars for each symbol represent standard error of the mean isotope signature for each species/age-class. Species codes are: 1-*A. chrysogaster*, 2-Juvenile *G. nigra*, 3-*M. fulgida*, 4-*R. osculus*, 5-*T. cobitis*, 6-Juvenile *C. insignis*, 7-Juvenile *P. clarki*, 8 & 9-Sub-adult and adult *G. nigra* (respectively), 10 & 11-Sub-adult and adult *C. insignis*, 12 & 13-Sub adult and adult *P. clarki*, 14-*C. lutrensis*, 15-Juvenile *A. natalis*, 16-*G. affinis*, 17-Juvenile *M. salmoides*, 18 & 19-Sub-adult and adult *A. natalis*, 20-*I. punctatus*, 21-*P. olivaris*, 22-*O. mykiss*, 23 & 24-Sub-adult and adult *S. trutta*, 25-*L. cyanellus*, 26 & 27-Sub-adult and adult *M. salmoides*. Basal resources and invertebrates were not included for clarity.

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, both indicate that adult and sub-adult nonnative predators had greater trophic positions than native species.

Resource overlap

Overlap in diet across sites, species age-classes, and years was summarized by PCoA (Fig. 5). The stress

value associated with the first two PCoA axes (18.7%) suggests this ordination is a reasonable representation of the variability in diet. Inclusion of a third axis (not shown) further reduced stress (12.3%), but only represented variability in native fish diets, algivores versus invertivores. 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, coleopterans and ephemeropterans. Most nonnative fishes had negative second axis scores and were associated with terrestrial and predaceous invertebrates (e.g., hellgrammites, belostomatids and naucorids) and fish.

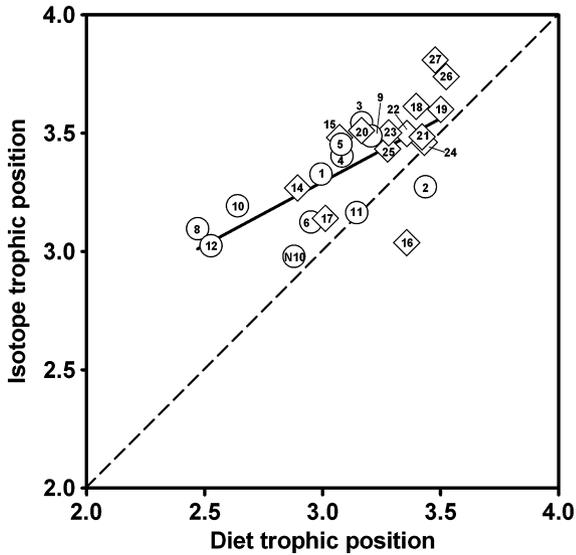


Fig. 4. The relationship of mean trophic positions calculated using diet data and stable isotope analysis of native (circles) and nonnative fishes (diamonds). Dashed line indicates a 1:1 relationship. The solid line indicates the line of best fit (slope = 0.54,  $r^2 = 0.49$ ). See Fig. 3 caption for species codes.

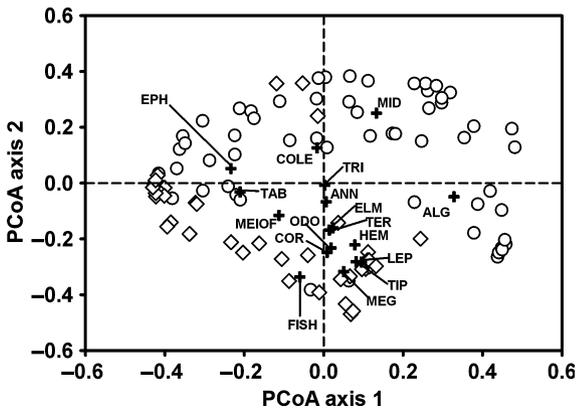


Fig. 5. Principal coordinates analysis of native (circles) and nonnative (diamonds) fishes' diets at each of the six sample sites in the upper Gila River basin during 2007 and 2008. Symbols are the scores for the combined diet of individuals per species and age-class. Species names not included for clarity. Crosses are the weighted average scores of diet items. Axis one was positively associated with algae (ALG) and negatively associated with Epemeroptera (EPH) and Tabanidae (TAB). Axis two was positively associated with midges (MID) and negatively associated with Megaloptera larvae (MEG) and fish (FISH). See Table 2 for diet codes.

Discriminant function analysis identified three distinct groups: native fishes, NNL and NNS (Fig. 6). Of the four pre-specified groups, NS and NNL were the most distinctly separated 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

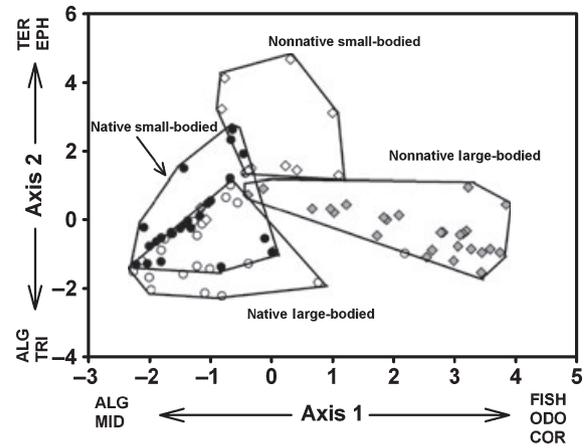


Fig. 6. Discriminant function analysis of diets of native and nonnative fishes from the Gila River, New Mexico collected June-July 2007 and 2008. Native (circles) and nonnative (diamonds) fish species/age-classes are plotted on the first two axes discriminating among size-groups based on proportions of diet items found in gut contents of each species/age-class at each site (see Table 3 for species in each size-group). Polygons represent the four size-groups of native and nonnative fishes.

simuliids. The second axis explained 25% of variation among samples and contrasted fish that consumed algae and trichopteran larvae with those that consumed terrestrial invertebrates and ephemeropterans. 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%), except for one headwater chub which was classified as NNL, and NNS were classified more often as NS (46%) than they were correctly classified (36%).

Error variance of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  were homogenous across size-groups of native and nonnative fishes (Levene's test,  $\delta^{15}\text{N}$   $F_{3,133} = 0.557$ ,  $P = 0.644$ ;  $\delta^{13}\text{C}$   $F_{3,133} = 0.288$ ,  $P = 0.834$ ) and the observed covariance across groups was similar (Box's  $M = 9.118$ ,  $P = 0.458$ ), so no data transformations were necessary. Overall, there was little variability in mean fish  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  between years (MANCOVA,  $N = 137$ ;  $\delta^{15}\text{N}$   $F_{1,131} = 2.58$ ,  $P = 0.110$ ,  $\delta^{13}\text{C}$   $F_{1,131} = 1.69$ ,  $P = 0.196$ ), yet there was significant variation in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  among the five sites where nonnative fishes were present ( $\delta^{15}\text{N}$   $F_{1,131} = 50.08$ ,  $P < 0.001$ ;  $\delta^{13}\text{C}$   $F_{1,131} = 39.85$ ,  $P < 0.001$ ). Although this test indicated groups had different  $\delta^{13}\text{C}$  signatures ( $F_{3,131} = 3.40$ ,  $P = 0.020$ ), the only significant ( $P = 0.018$ ) *post hoc* comparisons between groups was that between NS (estimated marginal mean =  $-25.1\text{‰}$ ) and NNL ( $-23.9\text{‰}$ ). There were differences among groups in  $\delta^{15}\text{N}$  signatures ( $F_{3,131} = 12.94$ ,  $P < 0.001$ ). Nonnative large-bodied fishes were the most enriched in  $\delta^{15}\text{N}$  (estimated

marginal mean = 10.4‰) and were higher than NL (8.7‰,  $P < 0.001$ ) and NS (9.4‰,  $P = 0.002$ ). Non-native 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, nonnative large-bodied fishes were the most distinct group having the most enriched  $\delta^{15}\text{N}$  signatures and having a diet comprised primarily of predaceous aquatic invertebrates and fish. Second, the degree of overlap of diet and stable isotopes was high among native large-bodied, native small-bodied and nonnative small-bodied fishes, but large-bodied native fishes tended to feed at lower trophic positions.

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  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  ranges or mean trophic position of native fishes, except in macrohabitats where nonnative trout were present where natives had a greater mean trophic position than in the absence of nonnative trout (rainbow trout  $F_{1,34} = 9.83$ ,  $P = 0.004$ ; brown trout  $F_{1,34} = 10.26$ ,  $P = 0.003$ ). This pattern was contrary to our prediction of nonnative causing a reduction in trophic position of natives. Correlation analysis between trophic positions of native fishes and nonnative predator density ranged from -0.59 to 0.05 (Table 4), but these relationships were not significant (all  $P > 0.1$ ).

IsoSource model estimates based on  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  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‰: 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, models suggested that predaceous invertebrates were likely energy sources for rainbow and brown trout at Middle Fork (53–84 and 46–84%, respectively), but according to the model estimates, these trout purportedly 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

A main conclusion from this research was that both native and nonnative fishes fed at multiple trophic levels. However, native fishes fed on lower trophic levels (algae/detritus and invertebrates) than nonnatives, which fed on invertebrates, predaceous invertebrates and fish. Native headwater chub were an exception, as they were occasionally piscivorous and although we did not detect predaceous invertebrates in their diet, these diet items have been recorded in the diet of its congener, roundtail chub *Gila robusta*, in downstream reaches of the Gila River (Schreiber & Minckley 1981). For the other native fishes, our results were consistent with those of Schreiber & Minckley (1981) who studied the diets of a similar native fish assemblage in Aravaipa Creek, Arizona and found that most native fishes fed on ephemeropteran, chironomid and simuliid larvae. Although differences in omnivory between native and nonnative fishes may alleviate competition for resources, both groups overlapped in their feeding on common invertebrates (ephemeropterans, chironomids and simuliids). Limitations of these resources may force increased reliance on other trophic levels.

Differences in mean trophic positions of native and nonnatives were related to ontogenetic diet shifts in these species. Suckers shifted from a higher trophic

Table 4. 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.

	East Fork	Middle Fork	Heart Bar	Riverside	San Francisco	West Fork	Pearson
Predator density (#/m <sup>2</sup> )	0.04	0.076	0.0058	0.0035	0.0013	0.02	
<i>A. chrysogaster</i>	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 <i>C. insignis</i>	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 <i>C. insignis</i>	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 <i>C. insignis</i>	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 <i>P. clarki</i>	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 <i>P. clarki</i>	2.7 ± 0.17	2.9 ± 0.19	3.0 ± 0.06	3.1 ± 0.2		3.1 ± 0.12	-0.54
Adult <i>P. clarki</i>	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

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.

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 ephemeropteran 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. DFA provided evidence that diets of nonnative small-bodied fishes and all age-groups of native fishes overlapped. Thus, the presence of both juvenile and adult nonnative species could pose both a competitive and predation threat to native fishes (Meffe 1985; Mills et al. 2004). Having access to both diet and stable isotope data provided an approach to quantifying resource overlap that integrated information from both short and long temporal scales (Vander Zanden et al. 1997).

Estimates of trophic position from stable isotope analyses were generally greater than those calculated from stomach contents for fishes in feeding at low trophic levels. Two scenarios might explain our observations: (i) omnivorous fishes with large amounts of algae and detritus in their diet were disproportionately assimilating animal tissue (Ahlgren 1990; Evans-White et al. 2001) or (ii) herbivorous fishes had 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 was related to feeding habits or trophic fractionation is unknown. Regardless, the relationship between both methods was strongly correlated and 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 the feeding ecology of native fishes. On an average, fish comprised 25% of the diet of nonnative predators, but this may underestimate fish predation because nonnative predators consistently were more enriched in  $^{15}\text{N}$  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 with the large and recalcitrant exoskeletons of macroinvertebrates. Nearly half (44%) of smallmouth bass and about a fourth (22%) of yellow bullhead had empty guts, suggesting their predation effects on native fishes may be greater than we observed with gut contents alone. 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. Furthermore, constrained analysis of principal coordinates failed to provide significant evidence that nonnative predator density affected the diets of native fishes. 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 and showed some disparity with the diet data. 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. Yet, our estimates from mixing models indicated juvenile suckers provided minimal contributions to the diet of nonnative predators at most 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).

The six study sites differed in long-term densities of nonnative predators (Propst et al. 2008), and as such, food webs (e.g., food chain lengths) were variable among sites. Potential factors that might influence densities of nonnative predators are stream size and elevation. For example, speckled dace was most common at high elevation, narrow sites such as Middle Fork, Heart Bar and West Fork sites, yet appeared to be replaced by loach minnow downstream at Riverside. Alternatively, water temperature potentially played a role in affecting fish assemblages. There was a substantial difference in water temperature ( $\sim 5$  C in summer) between Middle Fork and West Fork, despite their close proximity (unpublished data). In the warmer Middle Fork, the nonnative assemblage was comprised of smallmouth bass and yellow bullhead, whereas in West Fork, which was colder, nonnative trout were more prevalent. Such differences in physical characteristics likely played a role in the success of nonnatives and their potential impact on the native fish assemblages.

#### Conservation implications

Despite the low level of hydrologic modification to the upper Gila River, 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 nonnatives to be apex predators of Gila River drainage food webs that were preying on native fishes, providing a potential explanation for the instability of native fishes at some sites (Propst et al. 2008). Nonnative apex predators also appear to be using resources that could be underutilized by the native fish assemblage (i.e. predaceous invertebrates), yet any indirect effects (e.g. alleviating predation of native fish larvae by predaceous macroinvertebrates, Horn et al. 1994) on native fishes remains unknown. 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 had been crossed (Rinne & Miller 2006). Despite the declining trends in native fish abundances in the upper Gila River (Rinne & Miller 2006; Propst et al. 2008), the apparent coexistence of native and nonnative fishes suggests the threshold has not been reached, but may be imminent.

### Acknowledgements

Field and laboratory assistance was provided by S.M. Coleman, N.R. Franssen, A. Marek, J.A. Monzingo, J. Morrison, Y.M. Paroz, K. Schroeder, D. Soliz, M. Tobin, B. Senger and J.E. Whitney. Discussions with D.J. Hoeinghaus were helpful in preparing the manuscript. Suggestions by B. Hugueny and two anonymous reviewers were helpful in the organisation of the manuscript. This project was partially funded by Division of Biology, Kansas State University, New Mexico Department of Game and Fish, and U. S. Bureau of Reclamation. Collecting permits were graciously provided by New Mexico Department of

Game and Fish. All vertebrate animals were treated according to Institutional Animal Care and Use Committee permit # 2564.

### References

- Ahlgren, M.O. 1990. Nutritional significance of facultative detritivory to the juvenile white sucker (*Catostomus commersoni*). *Canadian Journal of Fisheries and Aquatic Sciences* 47: 49–54.
- Baxter, C.V., Fausch, K.D., Murakami, M. & Chapman, P.L. 2004. Fish invasion restructures stream and forest food webs by interrupting reciprocal prey subsidies. *Ecology* 85: 2656.
- Bestgen, K.R., Beyers, D.W., Rice, J.A. & Haines, G.B. 2006. Factors affecting recruitment of young Colorado pikeminnow: synthesis of predation experiments, field studies, and individual-based modeling. *Transactions of the American Fisheries Society* 135: 1722–1742.
- Bohn, T. & Amundsen, P.A. 2001. The competitive edge of an invading specialist. *Ecology* 82: 2150–2163.
- Bowen, S.H. 1996. Quantitative description of the diet. In: Murphy, B.R. & Willis, D.W., eds *Fisheries techniques*. Bethesda, Maryland: American Fisheries Society, pp. 513–529.
- Brown, D.E., ed. 1982. *Biotic communities: southwestern United States and northwestern Mexico*. *Desert Plants* 4: 1–342.
- Bryan, S.D., Robinson, A.T. & Sweetser, M.G. 2002. Behavioral responses of a small native fish to multiple introduced predators. *Environmental Biology of Fishes* 63: 49.
- Cabana, G. & Rasmussen, J.B. 1996. Comparison of aquatic food chains using nitrogen isotopes. *Proceedings of the National Academy of Sciences of the United States of America* 93: 10844–10847.
- Dalerum, F. & Angerbjörn, A. 2005. Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. *Oecologia* 144: 647–658.
- Douglas, M.E., Marsh, P.C. & Minckley, W.L. 1994. Indigenous fishes of western North America and the hypothesis of competitive displacement: *Meda fulgida* (Cyprinidae) as a case study. *Copeia* 1994: 9–19.
- Eby, L.A., Fagan, W.F. & Minckley, W.L. 2003. Variability and dynamics of a desert stream community. *Ecological Applications* 13: 1566–1579.
- Evans-White, M., Dodds, W.K., Gray, L.J. & Fritz, K.M. 2001. A comparison of the trophic ecology of the crayfishes (*Orconectes nais* (Faxon) and *Orconectes neglectus* (Faxon)) and the central stoneroller minnow (*Camptostoma anomalum* (Rafinesque)): omnivory in a tallgrass prairie stream. *Hydrobiologia* 462: 131–144.
- Flecker, A.S. & Townsend, C.R. 1994. Community-wide consequences of trout introduction in New Zealand streams. *Ecological Applications* 4: 798–807.
- Gido, K.B., Franssen, N.R. & Propst, D.L. 2006. Spatial Variation in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  Isotopes in the San Juan River, New Mexico and Utah: implications for the conservation of native fishes. *Environmental Biology of Fishes* 75: 197–207.
- Hamilton, S.K., Sippel, S.J. & Bunn, S.E. 2005. Separation of algae from detritus for stable isotope or ecological stoichiometry studies using density fractionation in colloidal silica. *Limnology and Oceanography: Methods* 3: 149–157.

- Horn, J.J., Marsh, P.C., Mueller, G. & Burke, T. 1994. Predation by odonate nymphs on larval razorback suckers (*Xyrauchen texanus*) under laboratory conditions. *The Southwestern Naturalist* 39: 371–374.
- Ingram, T., Matthews, B., Harrod, C., Stephens, T., Grey, J., Markel, R. & Mazumder, A. 2007. Lipid extraction has little effect on the  $\delta^{15}\text{N}$  of aquatic consumers. *Limnology and Oceanography: Methods* 5: 338–343.
- Jardine, T.D., Curry, R.A., Heard, K.S. & Cunjak, R.A. 2005. High fidelity: isotopic relationship between stream invertebrates and their gut contents. *Journal of North American Benthological Society* 24: 290–299.
- Johnson, B.M., Martinez, P.J., Hawkins, J.A. & Bestgen, K.R. 2008. Ranking predatory threats by nonnative fishes in the Yampa River, Colorado, via bioenergetics modeling. *North American Journal of Fisheries Management* 28: 1941–1953.
- Kiesecker, J.M. & Blaustein, A.R. 1998. Effects of introduced bullfrogs and smallmouth bass on microhabitat use, growth, and survival of native red-legged frogs (*Rana aurora*). *Conservation Biology* 12: 776–787.
- Marks, J.C., Haden, G.A., O'Neill, M. & Pace, C. 2009. Effects of flow restoration and exotic species removal on recovery of native fish: lessons from a dam decommissioning. *Restoration Ecology* doi: 10.1111/j.1526-100x.2009.00574.x.
- Marsh, P.C. & Douglas, M.E. 1997. Predation by introduced fishes on endangered humpback chub and other native species in the Little Colorado River, Arizona. *Transactions of the American Fisheries Society* 126: 343–346.
- Martinez del Rio, C., Wolf, N., Carleton, S.A. & Gannes, L.Z. 2009. Isotopic ecology ten years after a call for more laboratory experiments. *Biological Reviews* 84: 91–111.
- McCutchan Jr, J.H., Lewis Jr, W.M., Kendall, C. & McGrath, C.C. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos* 102: 378–390.
- Meffe, G.K. 1985. Predation and species replacement in American Southwestern fishes: a case study. *The Southwestern Naturalist* 30: 173–187.
- Merritt, R.W. & Cummins, K.W. 1996. *An introduction to the aquatic insects of North America*. Dubuque, Iowa: Kendall Hunt.
- Mill, A.C., Pinnegar, J.K. & Polunin, N.V.C. 2007. Explaining isotope trophic-step fractionation: why herbivorous fish are different. *Functional Ecology* 21: 1137–1145.
- Mills, M.D., Rader, R.B. & Belk, M.C. 2004. Complex interactions between native and invasive fish: the simultaneous effects of multiple negative interactions. *Oecologia* 141: 713–721.
- Mintenbeck, K., Brey, T., Jacob, U., Knust, R. & Struck, U. 2008. How to account for the lipid effect on carbon stable-isotope ratio ( $\delta^{13}\text{C}$ ): sample treatment effects and model bias. *Journal of Fish Biology* 72: 815–830.
- Moyle, P.B. & Light, T. 1996. Biological invasions of fresh water: empirical rules and assembly theory. *Biological Conservation* 78: 149–161.
- Moyle, P.B., Crain, P.K., Whitener, K. & Mount, J.F. 2003. Alien fishes in natural streams: fish distribution, assemblage structure, and conservation in the Cosumnes River, California, USA. *Environmental Biology of Fishes* 68: 143–162.
- Nystrom, P., Svensson, O., Lardner, B., Bronmark, C. & Greloni, W. 2001. The influence of multiple introduced predators on a littoral pond community. *Ecology* 82: 1023–1039.
- Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Simpson, G.L., Solymos, P., Henry, M., Stevens, H. & Wagner, H. 2008. *vegan: Community ecology package*. R version 1.15-1. Available at: <http://cran.r-project.org>.
- Olden, J.O., Poff, N.L. & Bestgen, K.R. 2006. Life-history strategies predict fish invasions and extirpations in the Colorado River Basin. *Ecological Monographs* 76: 25–40.
- Parker, I.M., Simberloff, D., Lonsdale, W.M., Goodell, K., Wonham, M., Kareiva, P.M., Williamson, M.H., Von Holle, B., Moyle, P.B. & Byers, J.E. 1999. Impact: toward a framework for understanding the ecological effects of invaders. *Biological Invasions* 1: 3–19.
- Peterson, B.J. 1999. Stable isotopes as tracers of organic matter input and transfer in benthic food webs: a review. *Acta Oecologia* 20: 479–487.
- Peterson, B.J. & Fry, B. 1987. Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics* 18: 293–320.
- Phillips, D.L. & Gregg, J.W. 2003. Source partitioning using stable isotopes: coping with too many sources. *Oecologia* 136: 261–269.
- Pinnegar, J.K. & Polunin, N.V.C. 1999. Differential fractionation of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  among fish tissues: implications for the study of trophic interactions. *Functional Ecology* 13: 225–231.
- Post, D.M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* 83: 703–718.
- Post, D.M., Layman, C.A., Arrington, D.A., Takimoto, G., Quattrochi, J. & Montaña, C.G. 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecologia* 152: 179–189.
- Propst, D.L., Gido, K.B. & Stefferud, J.A. 2008. Natural flow regimes, nonnative fishes, and native fish persistence in arid-land river systems. *Ecological Applications* 18: 1236–1252.
- R Development Core Team. 2008. *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical computing. Version 2.8.1. Available at: <http://www.R-project.org>.
- Rinne, J.N. & Miller, D. 2006. Hydrology, geomorphology and management: implications for sustainability of native southwestern fishes. *Reviews in Fisheries Science* 14: 91–110.
- Roberts, D.W. 2007. *labdsv: Ordination and multivariate analysis for ecology*. R version 1.3-1. Available at: <http://ecology.msu.montana.edu/labdsv/R>.
- Robertson, M.S. & Winemiller, K.O. 2001. Diet and growth of smallmouth bass in the Devil's River, Texas. *The Southwestern Naturalist* 46: 216–221.
- Ross, S.T. 1991. Mechanisms structuring stream fish assemblages: are there lessons from introduced species? *Environmental Biology of Fishes* 30: 359–368.
- Schooley, J.D., Karam, A.P., Kesner, B.R., Marsh, P.C., Pacey, C.A. & Thornbrugh, D.J. 2008. Detection of larval remains after consumption by fishes. *Transactions of the American Fisheries Society* 137: 1044–1049.
- Schreiber, D.C. & Minckley, W.L. 1981. Feeding interrelations of native fishes in a Sonoran Desert stream. *Great Basin Naturalist* 41: 409–426.

- Sotiropoulos, M.A., Tonn, W.M. & Wassenaar, L.I. 2004. Effects of lipid extraction on stable carbon and nitrogen isotope analyses of fish tissues: Potential consequences for food web studies. *Ecology of Freshwater Fish* 13: 155–160.
- Sublette, J.E., Hatch, M.D. & Sublette, M.F. 1990. *The fishes of New Mexico*. Albuquerque, USA: University of New Mexico Press.
- Taniguchi, Y., Fausch, K.D. & Nakano, S. 2002. Size-structured interactions between native and introduced species: can intraguild predation facilitate invasion by stream salmonids? *Biological Invasions* 4: 223–233.
- Tyus, H.M. & Beard, J.M. 1990. *Esox lucius* (Esocidae) and *Stizostedion vitreum* (Percidae) in the Green River basin, Colorado and Utah. *Great Basin Naturalist* 50: 33–39.
- Tyus, H.M. & Nikirk, N.J. 1990. Abundance, growth, and diet of channel catfish, *Ictalurus punctatus*, in the Green and Yampa rivers, Colorado and Utah. *The Southwestern Naturalist* 35: 188–198.
- Tyus, H.M. & Saunders, J.F. III 2000. Nonnative fish control and endangered fish recovery: lessons from the Colorado River. *Fisheries* 25: 17–24.
- Vander Zanden, M.J., Cabana, G. & Rasmussen, J.B. 1997. Comparing trophic position of freshwater fish calculated using stable nitrogen isotope ratios ( $\delta^{15}\text{N}$ ) and literature dietary data. *Canadian Journal of Fisheries and Aquatic Sciences* 54: 1142–1158.
- Vander Zanden, M.J., Casselman, J.M. & Rasmussen, J.B. 1999. Stable isotope evidence for the food web consequences of species invasions in lakes. *Nature* 401: 464–467.

## Appendix

### A: Feeding habits of native and nonnative fishes in the Gila River, USA

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.

Diet item	<i>Agosia chryso-gaster</i> (71)		<i>Cyprinella lutrensis</i> (6)		Juvenile <i>Gila nigra</i> (10)		Sub-adult <i>G. nigra</i> (11)	
	% 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

Prey item	Adult <i>G. nigra</i> (22)		<i>Meda fulgida</i> (22)		<i>Rhinichthys osculus</i> (40)		<i>Tiaroga cobitis</i> (14)	
	% 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

Appendix A. (Continued).

Prey item	Adult <i>G. nigra</i> (22)		<i>Meda fulgida</i> (22)		<i>Rhinichthys osculus</i> (40)		<i>Tiaroga cobitis</i> (14)	
	% Occurrence	% Volume	% Occurrence	% Volume	% Occurrence	% Volume	% Occurrence	% Volume
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

Prey item	Juvenile <i>Catostomus insignis</i> (93)		Sub-adult <i>C. insignis</i> (35)		Adult <i>C. insignis</i> (27)	
	% 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

Prey item	Age-0 <i>Catostomids</i> (129)		Juvenile <i>Pantosteus clarki</i> (56)		Sub-adult <i>P. clarki</i> (26)		Adult <i>P. clarki</i> (12)	
	% 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

Prey item	Age-0 Catostomids (129)		Juvenile <i>Pantosteus clarki</i> (56)		Sub-adult <i>P. clarki</i> (26)		Adult <i>P. clarki</i> (12)	
	% Occurrence	% Volume	% Occurrence	% Volume	% Occurrence	% Volume	% Occurrence	% Volume
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

Prey item	Juvenile <i>Ameiurus natalis</i> (4)		Sub-adult <i>A. natalis</i> (13)		Adult <i>A. natalis</i> (101)	
	% 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 ( <i>Orconectes virilis</i> )	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

Appendix A. (Continued)

Prey item	<i>Ictalurus punctatus</i> (3)		<i>Pylodictis olivaris</i> (2)	
	% 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

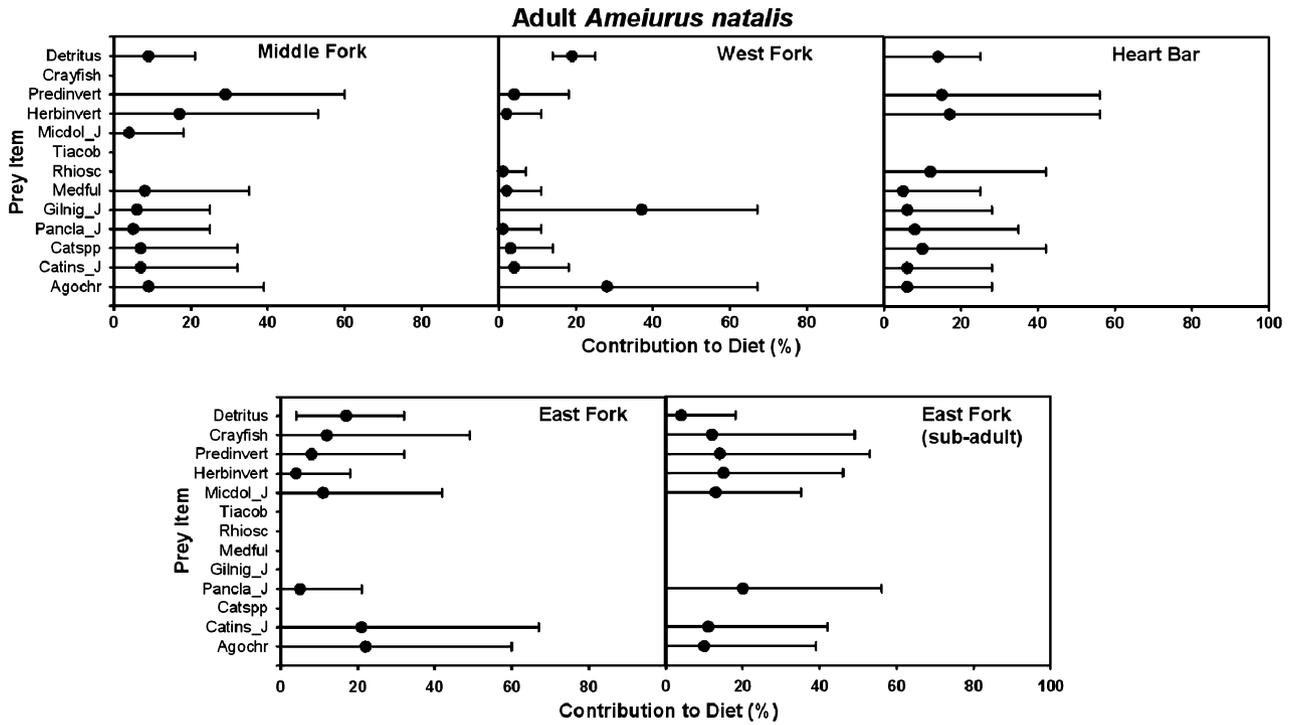
Prey item	<i>Pylodictis olivaris</i> (2)		Sub-adult <i>Oncorhynchus mykiss</i> (3)		Adult <i>O. mykiss</i> (66)	
	% Occurrence	% Volume	% Occurrence	% Volume	% Occurrence	% Volume
Ephemeroptera	100.0	15.7	100.0	61.2	45.0	9.6
Odonata (undetermined taxa)	0.0	0.0	0.0	0.0	8.0	0.2
Anisoptera	0.0	0.0	0.0	0.0	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

Prey item	Sub-adult <i>Salmo trutta</i> (7)		Adult <i>S. trutta</i> (102)		<i>Gambusia affinis</i> (48)	
	% 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

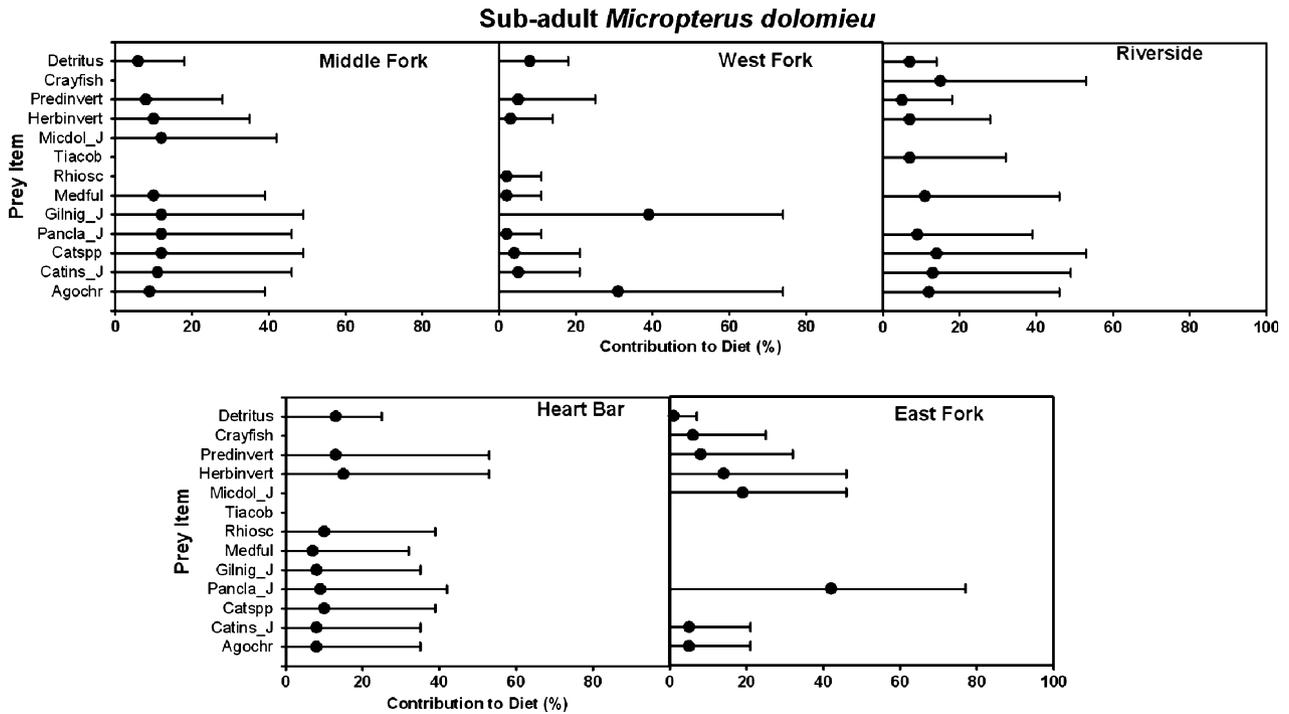
Prey item	Sub-adult <i>Salmo trutta</i> (7)		Adult <i>S. trutta</i> (102)		<i>Gambusia affinis</i> (48)	
	% Occurrence	% Volume	% Occurrence	% Volume	% Occurrence	% Volume
Veliidae	14.0	0.5	2.0	0.1	0.0	0.0
Megaloptera	0.0	0.0	13.0	5.8	0.0	0.0
Trichoptera (undetermined family)	0.0	0.0	24.0	0.9	4.0	0.6
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

Prey item	<i>Lepomis cyanellus</i> (8)		Juvenile <i>Micropterus dolomieu</i> (12)		Sub-adult <i>M. dolomieu</i> (29)		Adult <i>M. dolomieu</i> (12)	
	% 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 ( <i>Orconectes virilis</i> )	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

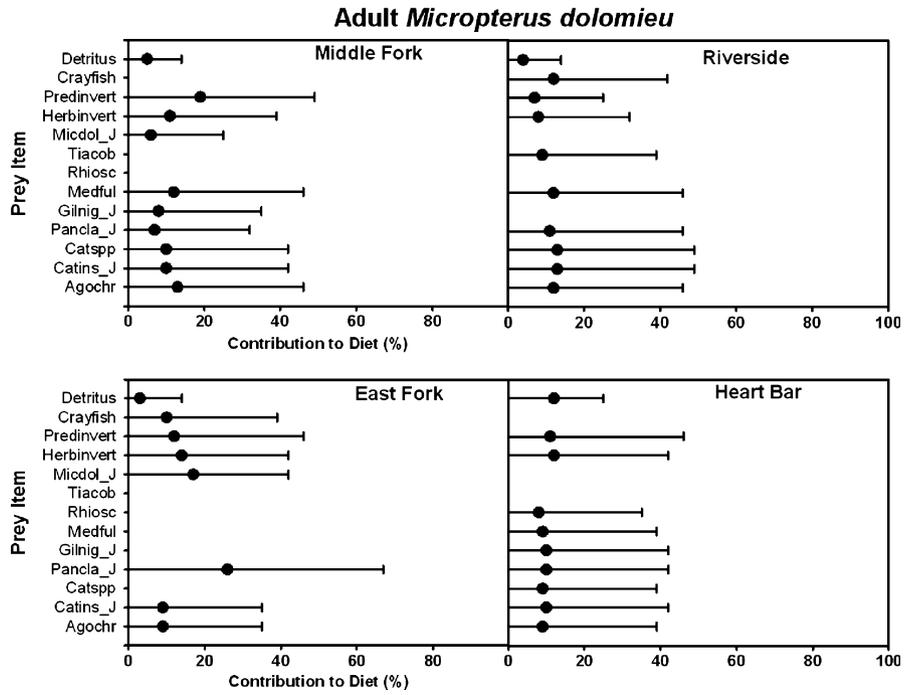
B: Results of nonnative predator IsoSource modelling



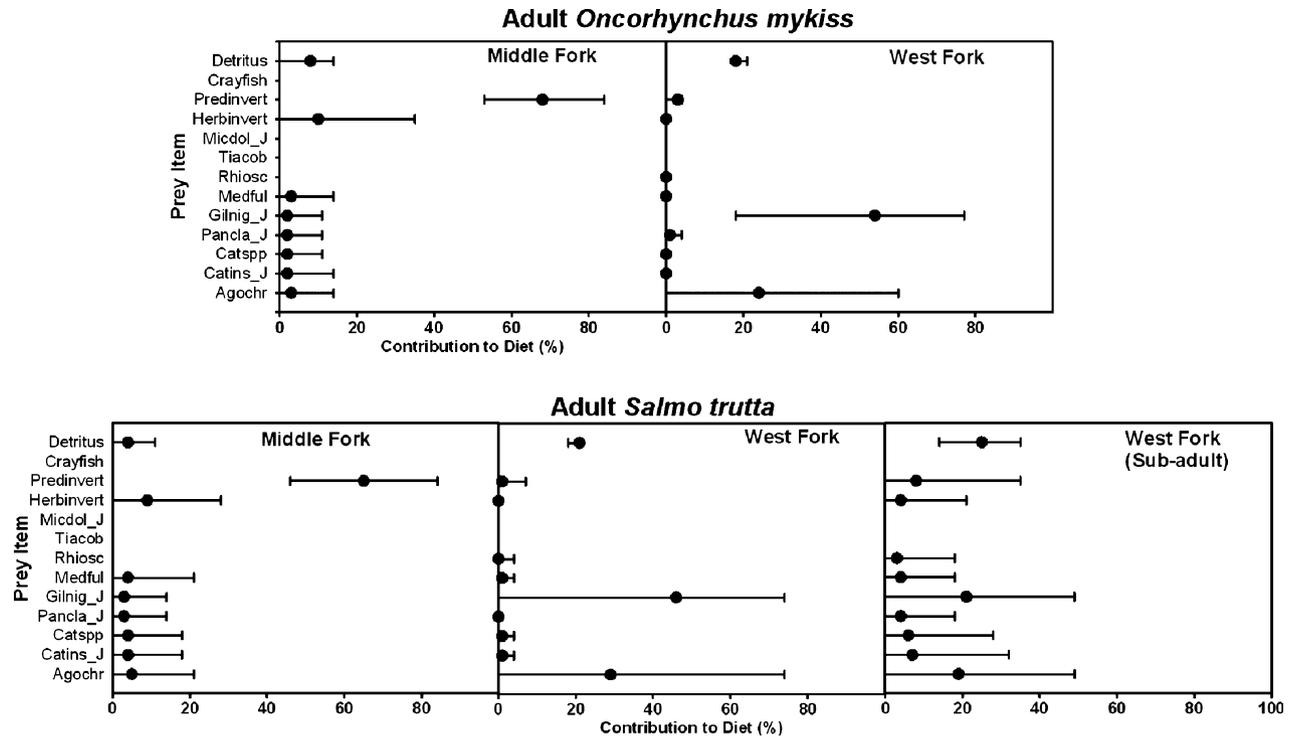
Results of IsoSource modeling for C and N isotopic signatures of adult and sub-adult yellow bullhead collected from West Fork, Middle Fork, Heart Bar, and East Fork reaches in the upper Gila River basin. Points represent the mean percent contribution of a prey item (y-axis) to the diet of the predator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



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 (y-axis) to the diet of the predator (x-axis) and error bars are the 1st to 99th percentiles.

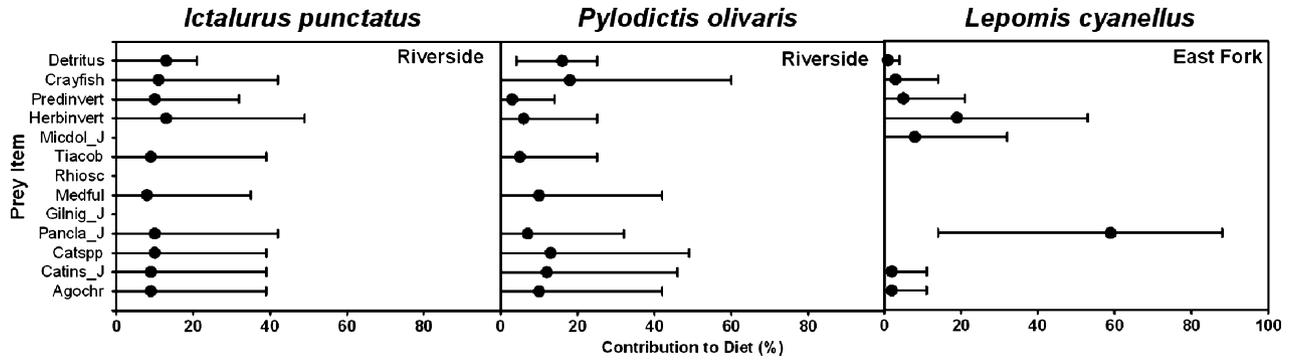


Results of IsoSource modeling for C and N isotopic signatures of adult smallmouth bass collected from Middle Fork, Riverside, Heart Bar, and East Fork reaches in the upper Gila River basin. Points represent the mean percent contribution of a prey item (y-axis) to the diet of the predator (x-axis) and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



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 (y-axis) to the diet of the predator (x-axis) and error bars are the 1st to 99th percentiles.

Gila River niche overlap



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 (y-axis) to the diet of the predator (x-axis) and error bars are the 1st to 99th percentiles.