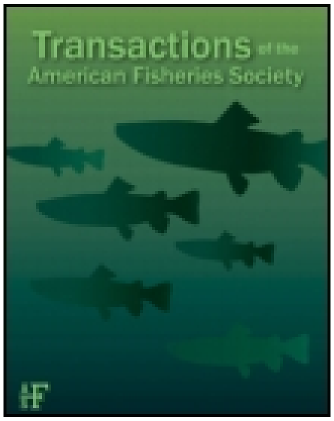

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High Diet Overlap between Native Small-Bodied Fishes and Nonnative Fathead Minnow in the Colorado River, Grand Canyon, Arizona

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ARTICLE

High Diet Overlap between Native Small-Bodied Fishes and Nonnative Fathead Minnow in the Colorado River, Grand Canyon, Arizona

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Abstract

River regulation may mediate the interactions among native and nonnative species, potentially favoring nonnative species and contributing to the decline of native populations. We examined food resource use and diet overlap among small-bodied fishes in the Grand Canyon section of the Colorado River as a first step in evaluating potential resource competition. We compared the diets of the predominant small-bodied fishes (native Speckled Dace *Rhinichthys osculus*, juvenile Flannelmouth Sucker *Catostomus latipinnis*, and juvenile Bluehead Sucker *C. discobolus*, and nonnative Fathead Minnow *Pimephales promelas*) across seasons at four sites downstream of Glen Canyon Dam using nonmetric multidimensional scaling and Schoener's similarity index. The diets of these fishes included diatoms, amorphous detritus, aquatic invertebrates (especially simuliid and chironomid larvae), terrestrial invertebrates, and terrestrial vegetation. Diets varied with season and were affected by high turbidity. Fish consumed more amorphous detritus and terrestrial vegetation during the summer monsoon season (July–September), when turbidity was higher. The diets of all species overlapped, but there was large variation in the degree of overlap. The diets of juvenile suckers and Fathead Minnows were most similar, while Speckled Dace had relatively distinct diets. The differences took the form of higher proportions of diatoms and amorphous detritus in the diets of Bluehead Suckers and Fathead Minnows and higher proportions of simuliids and chironomids in those of Speckled Dace. If food resources are or become limiting, diet overlap suggests

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that competition may occur among native and nonnative species, which could have implications for the population dynamics of these fishes and for the management of the Colorado River ecosystem in Grand Canyon.

Streams and rivers have experienced unprecedented rates of invasion by nonnative species worldwide (Rahel 2002; Strayer 2010). In the United States, over 530 fish species have been introduced (Fuller et al. 1999), and most of the watersheds in North America have at least one nonnative fish species present (Gido and Brown 1999). Nonnative species have been implicated in the decline of native fishes in rivers and streams across the United States (Strayer 2010) and may negatively affect native species in many ways, including predation, competition, hybridization, and the spread of diseases and parasites (Allan and Flecker 1993; Strayer 2010; Cucherousset and Olden 2011).

Flow regulation also affects most river networks globally (Palmer et al. 2008) and large dams have altered the flow regime of virtually all large rivers in North America (Poff et al. 2007). Flow regulation can mediate the interactions among native and nonnative fishes (Olden et al. 2006; Gido and Propst 2012) by altering habitat, physiochemical conditions, and community attributes. Dams not only change the temporal dynamics and magnitude of discharge but also shift temperature, sediment, and organic matter regimes (Ward and Stanford 1979; Poff et al. 2007), and the availability of suitable habitats (Bain et al. 1988; Scheidegger and Bain 1995). In addition, flow regulation changes the composition and production of organisms that serve as food resources for fish (e.g., Osmundson et al. 2002; Robinson and Uehlinger 2008; Cross et al. 2011, 2013; Wellard Kelly et al. 2013). These alterations modify the interactions among fishes, potentially favoring nonnative species and contributing to the decline of native species (Olden et al. 2006; Sabo et al. 2010).

The predominance of nonnative fishes and changes in the availability of food resources associated with flow regulation may amplify competition in large, regulated rivers. Such competition could be especially important if it affects early life history stages, because these often represent bottlenecks in survivorship that limit fish population sizes (e.g., Kennedy et al. 2008). The early life stages of different fishes may compete with one another for habitat or food (e.g., Fausch and White 1986), and in some cases these juveniles may compete with adults of small-bodied species (e.g., Zimmerman and Vondracek 2006). In addition, small-bodied fishes are ecologically important because they provide trophic linkages to larger piscivorous fish and may influence the dynamics of the algae and invertebrates that constitute their prey (Power 1990). However, the ecology of juvenile and small-bodied fishes, especially nongame species, in the context of regulated rivers has not been thoroughly studied (but see Markle and Dunsmoor 2007). In particular, the feeding habits of small-bodied fishes have not been investigated and the potential for exploitative interspecific competition is not well known. Assessment of competition is challenging because there may be large variation in diets over time as a result of ontogeny as well as among habitats and across seasons with dynamic food resource availability and foraging conditions. Thus, evaluating the potential for competition based on diet overlap may require comprehensive assessment of fish diets across both space and time. The dearth of such investigations for small fish in large regulated rivers limits our understanding of how flow regulation affects the interactions among native and nonnative fishes.

The mere presence of a nonnative species does not necessarily mean that competition with native species will occur. Interactions among native and nonnative fishes can be modulated by several factors, including habitat use and prey availability. Exploitative competition only occurs when two or more species overlap spatially and temporally in habitat and when they share a limiting resource (Connell 1983; Fausch 1998). Two species with similar ecological requirements may coexist with a high degree of habitat and diet overlap if these shared resources are abundant. However, if resources become limiting, two species may partition the resources to avoid competition; thus, nonoverlap in resource use may indicate past competition (Connell 1980).

The Colorado River in Grand Canyon is highly regulated (Topping et al. 2003) and the native fish populations in this river are an important conservation concern (Bureau of Reclamation 2011). In addition to flow regulation, nonnative fish introductions have been implicated in the loss and decline of native fishes in Grand Canyon (Minckley 1991; Minckley et al. 2003). By some estimates, as many as 23 nonnative fish taxa are established in Grand Canyon, while only 4 of 8 native species persist (Minckley et al. 2003; Gloss and Coggins 2005). Glen Canyon Dam has altered the physical and thermal regimes of the Colorado River in Grand Canyon, and these changes have modulated the relative availability of organic matter and prey taxa for higher trophic levels (Stevens et al. 1997; Cross et al. 2013). Such changes have the potential to influence competitive interactions among fishes, including interactions among native and nonnative species.

Interactions among native and nonnative species have been studied in Grand Canyon, but these studies have been mainly directed toward quantifying predation rates of large-bodied nonnatives such as Rainbow Trout *Oncorhynchus mykiss* (e.g., Yard et al. 2011). However, small-bodied nonnative fishes (e.g., Fathead Minnow *Pimephales promelas*, Plains Killifish

Fundulus zebrinus, and Red Shiner *Cyprinella lutrensis*) may pose a threat to small-bodied native species (Minckley 1991), such as Speckled Dace *Rhinichthys osculus* and juveniles of species like Flannelmouth Sucker *Catostomus latipinnis*, Bluehead Sucker *C. discobolus*, and Humpback Chub *Gila cypha*. For example, Fathead Minnows were the most abundant nonnative small-bodied fish in the river during the time of this study (Donner 2011), but dietary overlap between them and native species in the main-stem Colorado River in Grand Canyon has never been assessed.

We studied the trophic ecology of small-bodied fishes in Grand Canyon, with a focus on investigating the potential for exploitative competition among native and nonnative species. Competition is often measured directly using pairwise exclusions or introductions or inferred from niche overlap (Schoener 1983). Because of the remoteness of Grand Canyon and its status as a national park, we examined diet overlap as a first step in the process of evaluating whether competition is likely. Specifically, we examined the trophic ecology of the predominant small-bodied fishes across seasons and flow regimes. We predicted that the diets of small-bodied fishes would change among seasons, especially in the monsoon season when the river is more turbid. In addition, we predicted that allochthonous carbon sources (e.g., amorphous detritus and terrestrial vegetation) would represent a higher proportion of fish diets during turbid conditions. Based on diet studies on these species in different systems, we also predicted that the diets of Bluehead Suckers, Flannelmouth Suckers, and Fathead Minnows would overlap but that the diets of these species would overlap less with the diets of the invertivorous Speckled Dace (Childs et al. 1998; Ptacek et al. 2005; Rees et al. 2005).

METHODS

Study organisms, study sites, and sample collection.—In this study we focused on four predominant taxa: juvenile Bluehead Suckers, juvenile Flannelmouth Suckers, Speckled Dace, and Fathead Minnows. These species comprised over 90% of total small-bodied fish production across all sites during 2007 and 2008 (Donner 2011). To test our predictions, we compared the diets of these fishes among seasons at four sites downstream of Glen Canyon Dam. The Colorado River in Marble Canyon and Grand Canyon is bounded upstream by Glen Canyon Dam and downstream by Lake Mead, the reservoir formed by Hoover Dam. We sampled four sites seasonally (September 2006, April 2007, July 2007, September 2007, January 2008, and September 2008) along a 262-km reach downstream of Glen Canyon Dam (Figure 1). The sites were selected to encompass longitudinal gradients and to bracket major tributaries. In September 2008 dam operations were restricted to steady flows; discharge for all other sampling dates fluctuated daily due to hydroelectric power generation. Typically,

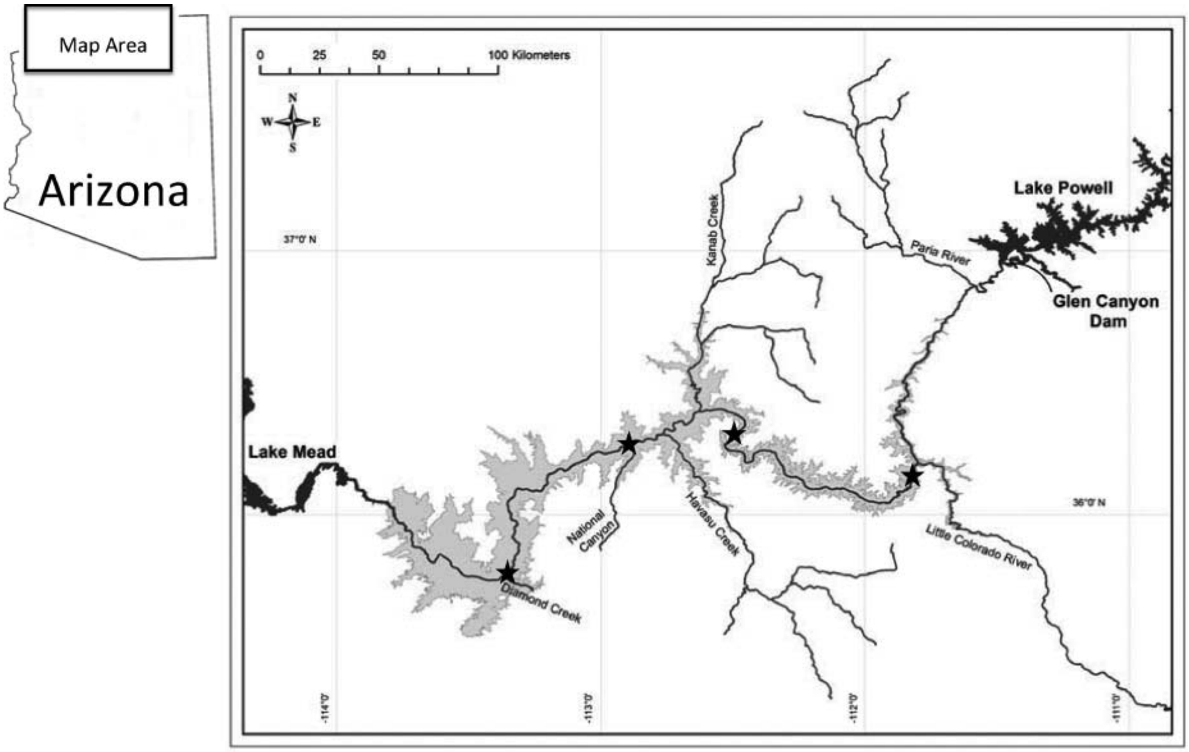

FIGURE 1. Map of the Colorado River and its tributaries in Grand Canyon. Four sites —125, 229, 291, and 387 river kilometers downstream from Glen Canyon Dam—were sampled (stars).

the sample sizes from each site for each sample date were small (<10 individuals/species) due to low abundances of native species. We used the combination of four sites and seasons to represent the diets of these fishes across a range of conditions in Grand Canyon, but the few sites and limited sample sizes were not adequate for among-site comparisons.

Up to 10 individuals of each species were collected at each site from backwater and nearshore main-stem habitats by seining and electroshocking. Backwater habitats and sandy nearshore habitats in the main stem were sampled during the day by seining (0.32-cm mesh). The time of day when sampling occurred ranged from midmorning to midafternoon. To collect small-bodied fishes from other nearshore habitats, we used boat-based electrofishing conducted at slow speeds (5–10 s per meter of shoreline) during nighttime. We used aluminum-hulled boats (4.9-m length) with 5,000-W generators and Coffelt Mark XXII control units (DC pulse current, 250–400 W, 13–25 A). Fish were handled according to the U.S. Geological Survey's Grand Canyon Monitoring and Research Center protocol (Persons et al. 2013) and the Idaho State University Institutional Animal Care and Use Committee (IACUC) protocol number 6261007. Whole specimens were preserved in the field in 70% ethanol for subsequent gut content analysis. We defined small-bodied fish as any specimen smaller than 150 mm total length (TL), regardless of life stage. Bluehead Suckers and Flannelmouth Suckers of this size are juveniles in the Colorado River in Grand Canyon (Robinson and Childs 2001; Walters et al. 2012). Native Speckled Dace have a maximum length of 110 mm TL (Moyle 2002; Lovich 2005), and nonnative Fathead Minnows have a maximum length near 100 mm TL (Tyus and Saunders 2000; Moyle 2002; Gloss and Coggins 2005).

Gut content analysis.—We analyzed gut contents following Rybczynski et al. (2008). Gut contents were removed from the anterior portion of the gut to the first U-bend of cyprinids and juvenile catostomids because these families lack true stomachs (Rybczynski et al. 2008). Gut contents were placed into petri dishes and visually sorted into coarse and fine fractions and examined under a microscope. The coarse fraction comprised macroinvertebrates and large pieces of plant material that were too large to place on a slide; the fine fraction comprised diatoms, amorphous detritus, plant material, and some invertebrate body parts. Amorphous detritus is mainly unidentifiable organic matter, which may have been derived from algae or terrestrial vegetation, potentially including bacteria, bacterial secretions, algae, fungi, sediment particles, and detrital fragments. Gut contents were placed in glycerin and spread to an approximately even thickness. The area of the gut contents, the fine fraction, and each food item in the coarse fraction were outlined digitally using a stereo microscope with 7.5–50× magnification and image analysis software (ImagePro Plus; Media Cybernetics, Bethesda, Maryland. and Leica Application Suite; Leica Microsystems Ltd., Heerbrugg, Switzerland). The software calculated the area of each resulting polygon, and the proportions were calculated by dividing the area of each food category by the total area of the gut contents. The fine fraction was suspended in 20 mL reverse osmosis water. Between 1 and 5 mL of the mixture was filtered onto 0.45-μm gridded Metricel membrane filters (Pall Corp.; Ann Arbor, Michigan) and preserved on slides with immersion oil type B for further examination. The relative proportions of each food category in the fine fraction were calculated based on the relative area measurements using a compound microscope at 100–400× magnification, depending on the particle density on the slide and the aforementioned image analysis software. At least 10 fields of view along random transects on the slide were examined, and a minimum of 50 total particles were identified and measured. The proportions of the items in the fine fraction derived from the measurements of area were applied to the proportion of total fines in the overall gut contents.

Because many standard gut content analytic methods are based on mass or volume rather than area (Hynes 1950; Hellawell and Abel 1971), the relative proportions of all food categories were also measured in terms of ash-free dry mass (AFDM). The AFDM of individual coarse diet categories was measured, and that of total fines was measured by suspending the fine fraction in 20 mL reverse osmosis water and filtering a known quantity (15–19 mL) onto a glass microfiber filter (nominal pore size of 0.7 μm; Whatman Ltd., Maidstone, United Kingdom). Gut contents were dried in a drying oven at 60°C for 48 h and then combusted at 500°C for 2 h in a muffle furnace. The remaining 1–5 mL of the suspended fine fraction was used for slide preparation, as described above, to obtain the proportions of individual fine fraction categories (diatoms, amorphous detritus, etc.). The areal proportions of each fine fraction category were used to estimate the mass represented by each category in the fine fraction. Both methods were applied to the first 290 guts analyzed (encompassing all species and sites) out of 569 total specimens. For each sampling date, proportional contribution of each of the predominant food categories (consistently greater than 10% in the diets) calculated by mass was highly correlated with the same measures calculated by area measurements alone. Correlation coefficients exceeded 0.5 for all seasons and diet items, and most (34 out of 41) correlation coefficients were greater than 0.8 (Seegert 2010). One-way analysis of similarity (ANOSIM) corroborated that there were no differences between methods for Bluehead Suckers (1-way ANOSIM; $R < 0.01$, $P = 0.39$), Flannelmouth Suckers ($R = 0.01$; $P = 0.11$), Speckled Dace ($R < -0.01$, $P = 0.65$), or Fathead Minnows ($R < 0.01$, $P = 0.20$). We used areal measurements for subsequent analysis, which reflects the entire sample of 569 specimens analyzed.

Data analysis.—To test the prediction that fish diets vary among seasons, we compared the relative contribution of each food resource to the diet of each fish species among seasons (April 2007–January 2008) using Bray—Curtis similarity matrices of square-root-transformed proportional data and one-way ANOSIM with 1,000 permutations (PRIMER version 6; PRIMER-E Ltd., Plymouth, United Kingdom). ANOSIM tests were also used to evaluate differences between the mass and areal-based methods used to analyze our samples and diet overlap among species. ANOSIM provides a test statistic R that ranges from -1 to $+1$ in value and that is based on the rank similarities of samples within versus among a priori groups. An R value of 1 indicates that the diets of all samples within a group are more similar to each other than to the diets of samples in any other group; an R value of -1 indicates that the diets of all samples within a group are more similar to the diets of samples in other groups than to each other, and an R value of 0 indicates completely random grouping. The significance of R is tested through a permutation test that randomly assigns samples to groups. The significance level is the percentage (out of 1,000 permutations) of simulated values that are greater than the observed R value (Warwick et al. 1990). If the R value was not significant or was close to zero, we considered this result to be consistent with no difference among groups.

To examine the effect of turbidity on diets, we used continuous estimates of the suspended sediment concentration near each of our collection sites (http://www.gcmrc.gov/discharge_qw_sediment/stations/GCDAMP) to develop a metric describing the turbidity of the river prior to sample collection. Primary production rates in the Colorado River in Grand Canyon approach zero when sediment concentrations are above approximately 316 mg/L (Hall et al. 2010). The number of observations in the 30 d prior to sampling (when silt concentrations were higher than 316 mg/L and when gross primary production was likely minimal) provides a metric of biologically relevant turbidity levels (MT; Wellard Kelly et al. 2013). Suspended sediment measurements were made every 15 min for 30 d, so MT ranges from 0 to 2,880. Small MT values correspond to low turbidity and presumably high rates of gross primary production in the weeks prior to our fish collections. The opposite is true for large MT values. We conducted a quantile regression analysis with a logit transformation to test whether there were relationships between the percent of the diet accounted for by each predominant food resource and MT across the entire distribution of data, pooled across fish species (quantiles 0.05–0.95). Quantile regression is similar to ordinary-least-squares regression except that instead of conditioning the mean of the diet on MT, quantile regression conditions any chosen quantile of diet on MT (Cade and Noon 2003). This method is best used when the mechanistic effect of MT on diet does not hold for all cases (Cade and Noon 2003), thus creating a wedge-shaped relation between the x and y variables. The logit transformation is used when conducting quantile regressions with a bounded y variable, such as percent of a diet (Bottai et al. 2010). We tested the hypothesis that the slope of each regression was 0 (no relationship between the percentage of a given diet item and MT). We used an inverted rank method to generate 95% confidence intervals for the slope at each quantile. Quantile regressions were performed using the quantreg package in R (Koenker 2011). Relationships were considered significant if the 95% confidence intervals did not include zero (i.e., the slopes were not zero).

Diet overlap.—We evaluated the extent of interspecific diet overlap using two methods. First, we used Schoener's similarity index (Schoener 1970):

$$C = 1 - 1/2\left(\sum_{i=1,\ldots,n} |P_{x,i} - P_{y,i}|\right) \tag{1}$$

where $P_{x,i}$ and $P_{y,i}$ are the proportions of food category i in the diets of species x and species y, respectively. Index values range from 0 (no overlap) to 1 (complete overlap). The variation in index values was calculated using bootstrap analysis, resampling individual fish diets from the data set with replacement 10,000 times. From each resampling, group mean diets for each species and sampling date were calculated and used to calculate the index for each species pair. Confidence intervals for the index values were developed using the 2.5 and 97.5 percentiles of the bootstrapped values of the diet proportions. Index values >0.60 typically indicate substantial overlap (Wallace 1981; Muth and Snyder 1995; Christiansen et al. 2012), whereas values <0.40 indicate substantial differences (Childs et al. 1998). However, others have used values ≥0.50 to indicate substantial overlap (e.g., Hartman and Brandt 1995). Here, we considered overlap substantial if the lower bound of the confidence interval was >0.50 (similar to Hartman and Brandt 1995; Grabowska and Grabowski 2005).

We also assessed the patterns of diet overlap across species and seasons using Bray–Curtis similarity matrices of square-root-transformed proportional data and nonmetric multidimensional scaling (NMDS; PRIMER version 6). PRIMER chooses the best two-dimensional ordination that minimizes stress, a measure of goodness of fit to the resemblance matrix. Stress ranges from 0 to 1 and any value less than 0.15 indicates a good fit. To complement the NMDS analysis, statistical differences among species were identified using one-way ANOSIM with 1,000 permutations (PRIMER version 6).

RESULTS

The diets of 569 juvenile Bluehead and Flannelmouth suckers, Speckled Dace, and Fathead Minnows largely contained diatoms, terrestrial vegetation, amorphous detritus, aquatic invertebrates (especially simuliids [particularly the blackfly *Simulium arcticum*] and chironomids). Other aquatic macroinvertebrates were also consistently present in diets (scuds *Gammarus lacustris*, Trichopterans, Hemipterans, Coleopterans, and other Dipteran species), albeit at lower proportions than simuliids and chironomids. The diets also contained terrestrial invertebrates, including Hemipterans and

Hymenopterans, though in lower quantities than aquatic invertebrates.

Bluehead Suckers ate mostly diatoms, amorphous detritus, and to a lesser extent, chironomids, simuliids, and terrestrial vegetation (Figure 2). Flannelmouth Sucker diets also included diatoms, amorphous detritus, terrestrial vegetation, and aquatic insects (Figure 2). Speckled Dace were predominantly invertivorous (Figure 2), with aquatic insects such as chironomids and simuliids composing a majority of their diets. Nonnative Fathead Minnows ate mainly diatoms, amorphous detritus, and terrestrial vegetation (Figure 2). The resources consumed were consistent across seasons, although there was temporal variation in the relative proportions of the resources found in diets (1-way ANOSIM; see Supplementary Table S.1 in the online version of this article).

Fish consumed fewer diatoms and more allochthonous carbon (i.e., amorphous detritus and terrestrial vegetation) when MT was high (Figure 3; Table S.2). The proportions of amorphous detritus, terrestrial vegetation, and aquatic invertebrates other than chironomids and simuliids in the diets of small-bodied fish were related to high MT values for the mid to upper quantiles (Figure 3; Table S.2). In contrast, the proportions of diatoms, chironomids, and simuliids in small-bodied fish diets were related to low MT values for the mid to upper quantiles (Figure 3; Table S.2). The slopes for all diet items were not different from zero for the lower quantiles (Figure 3; Table S.2). These patterns were similar when each species, pooled by date, was analyzed separately with the exception of Bluehead Suckers, likely due to the high variability and/or low sample size for this species.

Across all seasons, we observed the strongest and most consistent diet overlap between both sucker species and Fathead Minnows (Table 1). There was, however, variation in the degree of overlap among seasons. Schoener's similarity index showed that Fathead Minnow diets consistently overlapped with those of both sucker species on all dates, but the overlap was only considered substantial (lower CI > 0.50) for some sampling dates (Table 1). Bluehead Sucker diets generally overlapped with Flannelmouth Sucker diets and were substantially overlapping in January and September 2008. The diets of Speckled Dace did not typically overlap with the diets of Bluehead Suckers, Flannelmouth Suckers, or Fathead Minnows; the exception was in September 2007, when they overlapped with the diets of all other species. Speckled Dace diets also overlapped with Flannelmouth Sucker diets in July 2007 (Table 1). When the samples from all dates were pooled, diet overlap among species generally remained high. In fact, in comparisons between (1) Speckled Dace and Flannelmouth Suckers and (2) Bluehead Suckers and Fathead Minnows, diet overlap was greater when samples were pooled than they were on any single sample date (Table 1).

The results from the NMDS analysis also indicated diet overlap among Fathead Minnows, Bluehead Suckers, and Flannelmouth Suckers as well as that the degree of diet overlap varied by season (Figure 4). However, in all seasons Speckled Dace diets only overlapped slightly with Bluehead

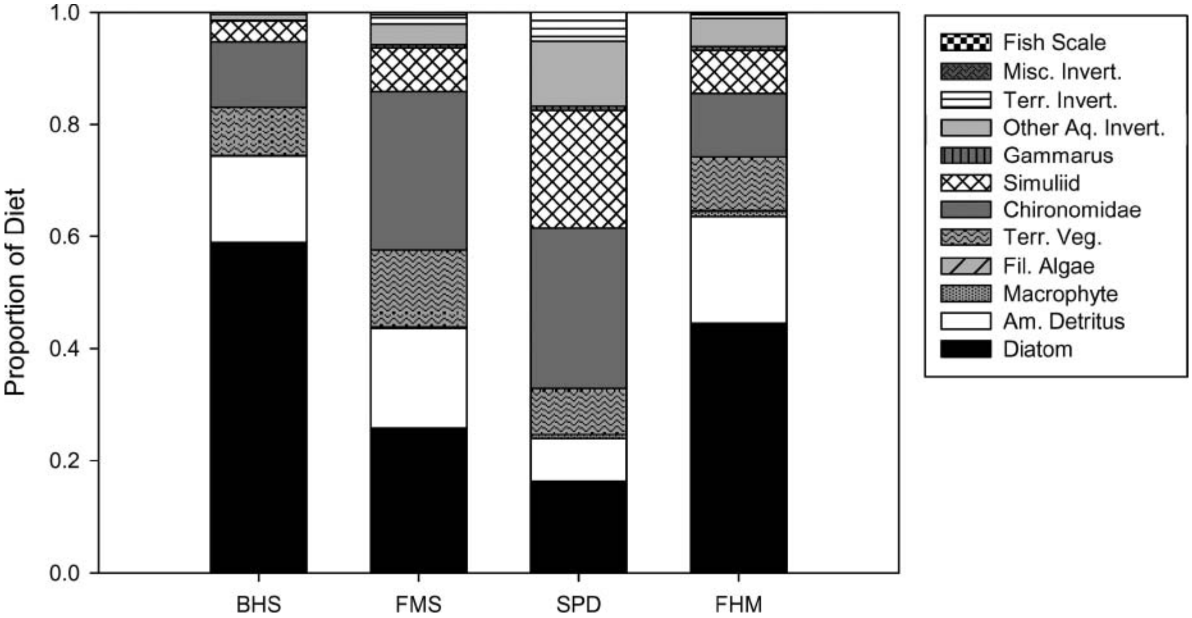

FIGURE 2. Diet composition for juvenile Bluehead Suckers (BHS; $n = 62$), juvenile Flannelmouth Suckers (FMS; $n = 129$), Speckled Dace (SPD; $n = 194$), and Fathead Minnows (FHM; $n = 184$) averaged across all sampling dates (September 2006; April, June, and September 2007; and January and September 2008). Diet items are diatoms, amorphous detritus, macrophytes, filamentous algae, terrestrial vegetation, Chironomidae, Simuliidae, *Gammarus* spp., other aquatic invertebrates, terrestrial invertebrates, miscellaneous (i.e., unidentifiable) invertebrates, and fish scales.

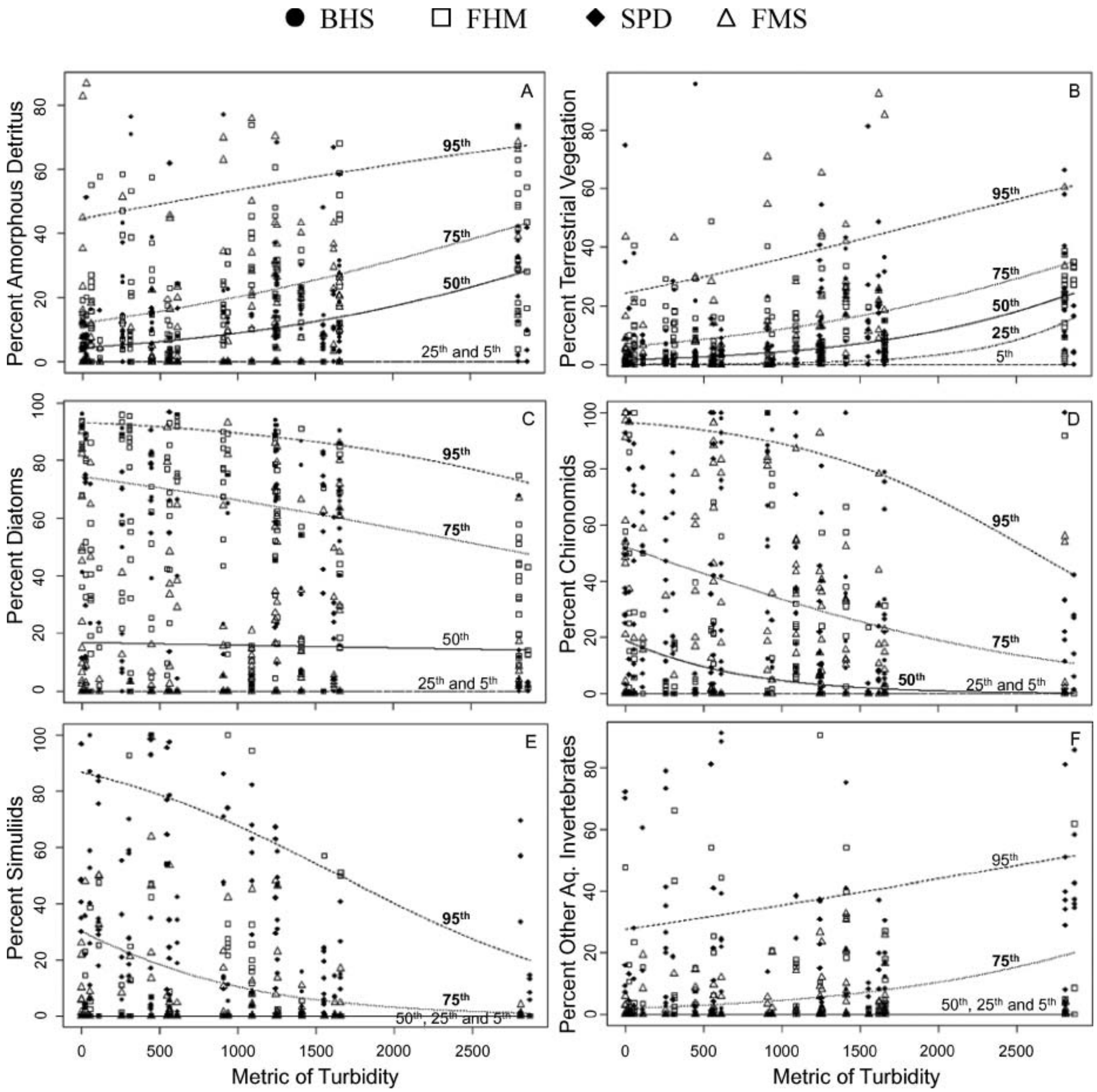

FIGURE 3. Quantile regressions of the percentages of Bluehead Sucker, Fathead Minnow, Speckled Dace, and Flannelmouth Sucker diets consisting of **(A)** amorphous detritus, **(B)** terrestrial vegetation, **(C)** diatoms, **(D)** chironomids, **(E)** simuliids, and **(F)** other aquatic invertebrates versus turbidity. Regression lines shown for the 5th, 25th, 75th, and 95th quantiles. The labels for lines whose slopes are significantly different than zero (i.e., whose confidence intervals do not include zero) are bolded. See appendix 2 for slopes and intercepts.

Sucker and Fathead Minnow diets (ANOSIM; $0.3 < R < 0.5$). In contrast, Speckled Dace diets overlapped substantially with Flannelmouth Sucker diets in September 2008 ($R = 0.15$, $P < 0.01$), but less so in April 2007 and January 2008 ($R =$ 0.27 and 0.38, respectively; $P < 0.01$). When there was evidence of substantial differences in diets among species, these differences were generally associated with higher proportions of invertebrates in Speckled Dace (and sometimes Flannelmouth Sucker) diets and higher proportions of diatoms and amorphous detritus in Bluehead Sucker and Fathead Minnow diets (similarity percentage [SIMPER] analysis; Table S.3).

DISCUSSION

Our study represents a comprehensive diet analysis of native and nonnative small-bodied fishes in the highly regulated section of the Colorado River in Grand Canyon. The diet composition for all of the species that we investigated changed seasonally and was strongly related to turbidity. The food resource use by these fishes in Grand Canyon is generally similar to their resource use in other parts of the Colorado River basin (i.e., Muth and Snyder 1995; Childs et al. 1998; Gido et al. 2006). We also demonstrated that there is a large degree of diet overlap, particularly among juvenile Bluehead Suckers,

TABLE 1. Schoener's similarity matrix for all species combinations for each sampling date and all dates combined. Species are Bluehead Sucker (BHS), Flannelmouth Sucker (FMS), Speckled Dace (SPD), and Fathead Minnow (FHM). The values given are the bootstrapped (10,000 replications) mean index values and the 95% confidence intervals (2.5th and 97.5th percentiles). Scores for which the lower bound of the 95% confidence interval is greater than 0.500 (indicating substantial overlap) are in bold italics.

		BHS		FMS		FHM	
Species	Date	Mean	95% CI	Mean	95% CI	Mean	95% CI
FMS	Sep 2006	0.62	0.49–0.78				
	Apr 2007	0.63	0.33–0.80				
	Jul 2007	0.51	0.20–0.77				
	Sep. 2007	0.56	0.39–0.73				
	Jan 2008	***0.74***	***0.54–0.89***				
	Sep 2008	***0.70***	***0.58–0.82***				
	All dates	***0.67***	***0.58–0.76***				
SPD	Sep 2006	0.56	0.48–0.64	***0.72***	***0.57–0.86***		
	Apr 2007	0.69	0.36–0.86	0.63	0.48–0.77		
	Jul 2007	0.54	0.10–0.78	0.58	0.39–0.77		
	Sep 2007	***0.69***	***0.53–0.85***	***0.69***	***0.55–0.82***		
	Jan 2008	***0.76***	***0.56–0.90***	***0.76***	***0.63–0.88***		
	Sep 2008	***0.80***	***0.71–0.90***	***0.79***	***0.65–0.91***		
	All dates	***0.83***	***0.76–0.90***	***0.76***	***0.70–0.83***		
FHM	Sep 2006	0.40	0.28–0.53	0.60	0.44–0.76	0.56	0.41–0.73
	Apr 2007	0.433	0.12–0.62	0.58	0.42–0.72	0.41	0.26–0.57
	Jul 2007	0.41	0.15–0.63	***0.72***	***0.58–0.84***	0.50	0.33–0.68
	Sep 2007	***0.74***	***0.60–0.87***	***0.63***	***0.51–0.75***	***0.79***	***0.66–0.90***
	Jan 2008	0.55	0.32–0.78	0.51	0.35–0.68	0.44	0.31–0.59
	Sep 2008	0.42	0.29–0.55	0.64	0.49–0.78	0.48	0.35–0.62
	All dates	0.49	0.40–0.58	***0.73***	***0.66–0.79***	***0.58***	***0.51–0.65***

juvenile Flannelmouth Suckers, and Fathead Minnows. Our results suggest that if food resources become limiting, competition may occur between native and nonnative small-bodied fishes in Grand Canyon.

Diet composition changed seasonally and was strongly related to turbidity, which is driven by monsoonal rain and tributary flooding. In the Colorado River in Grand Canyon, as in many large rivers, river regulation is superimposed upon diet composition and the influence of turbidity. Flow regulation alters the composition and production of organisms that serve as food resources for fish and can also affect seasonal patterns of food resource availability that may be important for fish (Osmundson et al. 2002). We found that the diets of small-bodied fishes in Grand Canyon varied, in part as a function of turbidity. This finding is consistent with the results of a large number of studies that have demonstrated the strong effect that turbidity has on fish feeding behavior (e.g., Abrahams and Kattenfeld 1997; Utne-Palm 2002; Yard et al. 2011). The importance of turbidity to fish diets could apply to other large rivers as well, especially those that are punctuated by seasonal changes in turbidity. Regulated rivers now dominate the landscape, and we demonstrate that changing suspended sediment regimes could influence fish diets in these rivers. Although our study focused on the influence of turbidity over long time scales (i.e., months), short-term variations in turbidity may also be important and further investigation of these effects could improve our understanding of the trophic ecology of small-bodied fishes in regulated rivers.

Invertebrate diets in Grand Canyon also vary with turbidity, and this variation may in turn influence prey availability for small-bodied fishes (Wellard Kelly et al. 2013). Invertebrates such as chironomids, simuliids, and *Gammarus* consumed more diatoms in clear-water seasons and more amorphous detritus and terrestrial organic matter in seasons when the river was more turbid. We observed similar patterns in fish diets, and these seasonal patterns may be due to the greater availability of organic matter or the fact that turbidity makes it harder for fish to detect and consume scarce invertebrate prey items. The composition of particulate organic matter in the Colorado River is seasonally variable (Shannon et al. 1996), and allochthonous (terrestrially sourced) carbon dominates the Colorado River during monsoon flooding in its tributaries in late summer and

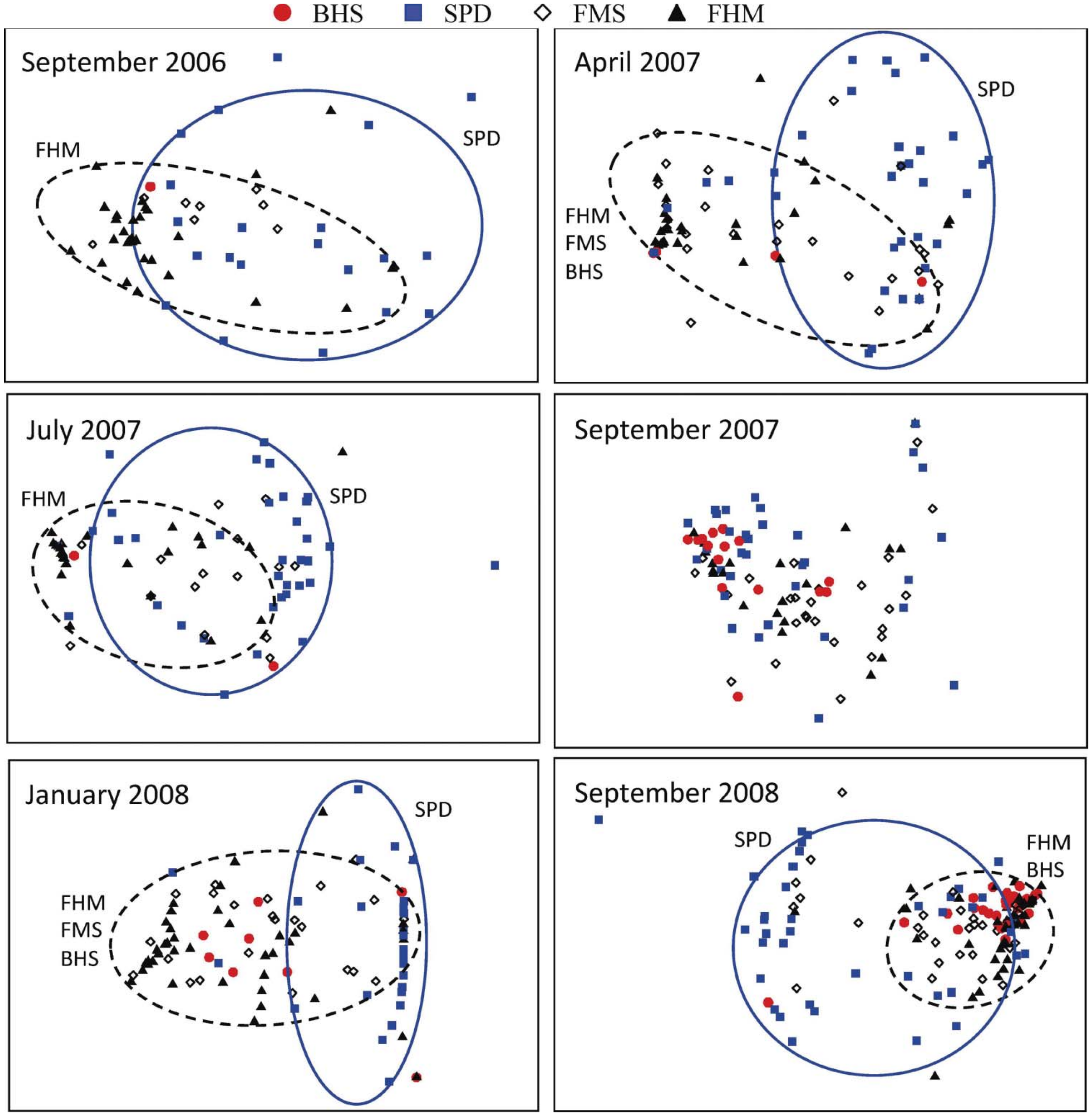

FIGURE 4. Seasonal changes in diet overlap among species derived from nonmetric multidimensional scaling based on the proportional diet composition of all species in all habitats on each sampling date (September 2006 [stress = 0.14], April 2007 [stress = 0.12], July 2007 [stress = 0.10], September 2007 [stress = 0.14], January 2008 [stress = 0.11], and September 2008 [stress = 0.11]). The ovals encompass >80% of the specimens of each species for which there were significant differences in diet (ANOSIM; $R > 0.200$ and $P < 0.05$). [Color figure available online.]

early fall (July 15–September 30; T. Kennedy et al., unpublished data), when turbidity in the main stem increases and primary production declines (Hall et al. 2010). Invertebrate production in the Colorado River was largely derived from primary production (Cross et al. 2013; Wellard Kelly et al. 2013). Thus, the net energy intake of fish may be substantially higher during (or immediately following) clear-water conditions.

Exploration of the use of food resources by nonnative and native species is a first step toward assessing the potential exploitative competition among nonnative invaders and resident fishes. Exploitative competition can only occur if the species overlap spatially and they share a limiting resource (Connell 1983; Fausch 1998). If the shared resources are plentiful, dietary overlap will not imply that competition is occurring. However, if these resources are (or become) limiting, owing to either reduced availability or the increased population size of one species, competition may influence the populations of the affected species or force them to partition resources (Connell 1980). Most species showed a higher degree of dietary overlap when the data for all dates were pooled than they did on any given date, which may indicate that these species partition resources seasonally.

A high degree of variability in population size coupled with limited resources may have consequences for degree of competition among small-bodied fish species. For example, Fathead Minnow populations can fluctuate among years in Grand Canyon, as do those of other nonnative small-bodied fishes (e.g., Red Shiners or Plains Killifish, which were not as abundant as the study species during this study [Donner 2011]). In addition, competition may increase when population sizes increase even if resource availability remains constant. Reciprocally, the changing relative availability of resources could increase competition if population sizes remain similar. Although not examined as a part of this study, low primary production and low invertebrate secondary production and diversity in Grand Canyon (Hall et al. 2010; Cross et al. 2013) suggest that food resources, particularly high-quality invertebrate prey items, are in limited supply. More research and continued monitoring of fish populations, food resources consumed, and food availability may provide information on the changing trophic interactions among these fishes.

Based on our diet overlap analysis, we suggest that there is potential for competition between nonnative and native small-bodied fishes in Grand Canyon if food resources become limiting. Competition with and predation by nonnative fishes have been implicated in the mid-twentieth-century declines and disappearances of some native fishes (Minckley 1991; Minckley et al. 2003). In addition, flow regulation downstream of Glen Canyon Dam creates a new context in which native species interact with each other and with nonnative species. Continued monitoring of invertebrates, other organic matter resources, and small-bodied fish will be important for interpreting the dynamics of fish populations and informing adaptive management of the Colorado River in Grand Canyon (Cross et al. 2011, 2013). In addition, our work adds to the growing body of literature that suggests human modification of physical regimes may synergistically interact with species invasions to alter the global trajectory of native fish populations.

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