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STABLE ISOTOPE SIGNATURES OF NATIVE AND NONNATIVE FISHES IN UPPER COLORADO RIVER BACKWATERS AND PONDS

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ABSTRACT—Naturally-occurring stable isotopes of carbon (^{13}C) and nitrogen (^{15}N) were analyzed to address questions about trophic interactions among native and nonnative fishes in the upper Colorado River basin, and to begin to evaluate the discreteness of floodplain pond fish assemblages. Specifically, 2 hypotheses were evaluated: 1) can stable isotope analysis be used to establish trophic relationships among native and nonnative fishes, and 2) can stable isotope signatures be used as a naturally-occurring marker to identify river fishes that have migrated from floodplain ponds? Nitrogen isotope ratios showed that at a particular location, either in ponds or backwaters, centrarchids were usually the top predators in each system. In one backwater, $\delta^{15}N$ and $\delta^{13}C$ signatures of the fish assemblage ranged about 4‰. The number of trophic levels represented was limited to about 2, and variation in carbon sources appeared to be great. The native flannelmouth sucker, *Catostomus latipinnis*, was most distinct with a relatively low $\delta^{15}N$ and high $\delta^{13}C$ signature. Stable isotope signatures appeared to have utility as a naturally occurring marker for tracking influx of nonnative fishes to the Colorado River from ponds. Whereas pond signatures were not consistently depleted or enriched relative to those from backwaters, mean stable isotope signatures were more different among ponds than among backwater sites. Further, within particular species, some ponds had carbon or nitrogen signatures that were quite different from those at backwater sites. Stable isotope analysis appears to have promise for studying trophic relationships and movement patterns of native and nonnative fishes in the upper Colorado River basin.

RESUMEN—Isótopos estables de carbón (^{13}C) y nitrógeno (^{15}N) que ocurren naturalmente fueron analizados para responder a preguntas acerca de interacciones tróficas entre pescados nativos y no-nativos en la parte alta de la cuenca del Colorado River, y para empezar a evaluar la autonomía de los grupos de peces de estanques en los planos de inundación. Dos hipótesis fueron evaluadas: 1) ¿puede usarse el análisis de isótopos estables para establecer relaciones tróficas entre peces nativos y no-nativos, y 2) ¿pueden usarse la firmas de los isótopos estables como un marcador que ocurre naturalmente para identificar peces de río que han emigrado de los estanques de los planos de inundación? Las proporciones del isótopo de nitrógeno mostraron que, en un lugar en particular, tanto los estanques como en los remansos de río, centrárquidos fueron usualmente los depredadores superiores en cada sistema. En un remanso de río, firmas de $\delta^{15}N$ y $\delta^{13}C$ del grupo de peces varía cerca de 4‰. El número de niveles tróficos representado se limitó a cerca de 2, y la variación en las fuentes de carbón pareció ser grande. El matalote nativo, *Catostomus latipinnis*, fue más distinto con una firma de $\delta^{15}N$ relativamente baja y $\delta^{13}C$ alta. Firmas de isótopos estables parecieron tener una utilidad como un marcador natural para rastrear el influjo de peces no-nativos al Colorado River desde estanques. Mientras que las firmas de los estanques no fueron consistentemente mermadas o enriquecidas en relación a las de remansos, promedios de firmas de isótopos estables fueron más diferentes entre estanques que entre remansos. Más aún, entre especies particulares, algunos estanques tuvieron firmas de carbón o nitrógeno que fueron muy diferentes de las de los remansos. El análisis de isótopos estables parece ser bueno para el estudio de relaciones tróficas y patrones de desplazamiento de peces nativos y no-nativos en la parte alta de la cuenca del Colorado River.

Competition and predation are presumed to be important interactions between native and nonnative fishes in the Colorado River (Martinez et al. 1994; Osmundson and Burnham, 1998; Tyus and Saunders, 2000). However, documenting these interactions has proven diffi-

cult (Tyus, 1991). Traditional diet studies are limited by the short time (<1 day) a meal is present and identifiable in the gut. This necessitates collecting multiple samples at frequent intervals to characterize the diet over a time period. Such intensive sampling may pose unacceptable risks of incidental injury or mortality when threatened and endangered species are present. Alternative approaches for studying trophic interactions are desirable.

A second persistent question within the Upper Colorado River Endangered Fish Recovery Program, and one with implications for state agencies charged with sport fishery management in the region, is to what extent do floodplain ponds serve as sources of nonnative fishes to the river? Some nonnative species may have difficulty reproducing in the mainstem Colorado River. However, these species may reproduce successfully in lentic habitat provided by abundant floodplain ponds along the river, which are frequently managed for nonnative, warmwater sport fisheries (Tyus and Saunders, 2000). Thus, the ponds could be important periodic sources of nonnative fish to the river during flood events that temporarily connect the ponds with the river. The importance of these ponds as sources of nonnative fish to the river has been debated (Tyus and Saunders, 2000) but remains largely unsubstantiated.

Stable isotope analysis has potential for improving our understanding of both of these questions. We set out to evaluate two hypotheses: 1) can stable isotope analysis be used to establish trophic relationships among native and nonnative fishes, and 2) can stable isotope signatures be used as a naturally-occurring marker to identify the origin (ponds or river) of river fishes?

Characteristic transformations in isotopic signatures during plant photosynthesis and during assimilation by animals of plant and animal tissue make it possible to elucidate trophic relationships from isotope signatures (Peterson and Fry, 1987; Lajtha and Michener, 1994; Vander Zanden et al., 1998). Typically, metabolic fractionation results in the $\delta^{15}N$ signature of a consumer being enriched (i.e., more ^{15}N) by about 3 to 4‰ over its diet (Vander Zanden and Rasmussen, 1996) leading to a progressively higher $\delta^{15}N$ signature at higher trophic levels within the food web. The stable carbon isotope shows little fractionation (ca. 1‰ enrichment) between consumer and food, and thus provides a useful tracer to determine the main sources of carbon (and energy) to the consumers (Peterson and Fry, 1987; Lajtha and Michener, 1994). The isotopic signature of an organism is a function of the signature of its diet and the amount of growth on that diet. Stable isotope signatures typically integrate the diet on a time scale of months and serve as relatively long-term indicators of trophic relationships (Hesslein et al., 1993; Vander Zanden et al., 1998).

Site-specific isotopic signatures also can tell the geographic history of migrating animals (Hesslein et al., 1991; Hansson et al., 1997; Kline et al., 1998). If a consumer emigrates from one habitat (i.e., food web) into another of differing baseline isotopic signatures or food sources, then that consumer will exhibit a unique isotopic signature reflective of the source food web for a period of months until assimilation and growth on the new diet dilutes the source signature. Thus, stable isotopes may be useful for assessing the degree to which ponds contribute nonnative fishes to the upper Colorado River.

METHODS AND MATERIALS—*Study Area*—The Colorado River in the study reach was composed primarily of run and riffle habitat with a bed of cobble and gravel. The banks and adjacent floodplain of the river were composed of silt and sand with some bank segments modified by levees or rip-rap. Riparian and floodplain vegetation consisted of thickets of nonnative tamarisk, *Tamarisk chinensis,* and Russian olive, *Eleagnus angustifolia,* and native sandbar willow, *Salix exigua,* and cottonwood, *Populus deltoids* (Pitlick and Van Steeter, 1998). Backwater habitats were ephemeral, low velocity embayments that formed along the river's shore, downstream of islands or at the mouths of secondary channels in braided reaches. Backwaters were small (<0.3 ha) but they are considered key habitats for recruitment of native fishes, particularly the endangered Colorado pikeminnow, *Ptychocheilus lucius* (Van Steeter and Pitlick, 1998). Maximum depth of the backwaters was <2 m, with silt and sand substrate. No aquatic macrophytes were present.

Ponds sampled during this study were all <4 ha and were located within the 100-year floodplain of the Colorado River, an area designated as critical habitat for the Colorado pikeminnow and the endangered razorback sucker, *Xyrauchen texanus* (Federal Register, Part III, 50 CFR Part 17, Department of the Interior, Fish and Wildlife Service, Washing-

TABLE 1—Sites where fishes were sampled for stable isotope analysis during 1997 to 1998 in upper Colorado River backwaters and floodplain ponds.

Code	Site name	Dates sampled	Location (latitude/longitude; hddd.ddddd°)
Colorado River backwaters			
LC	Labor Camp	29 Sep 1998	N 39.09039 W 108.39638
CO	Connected Lakes	22 Apr 1998	N 39.08111 W 108.60020
RR	Redlands Reach	2–3 Oct 1997	N 39.10293 W 108.65767
HB	Horsethief Canyon	21 Apr 1998, 17 Nov 1998	N 39.16946 W 108.79648
Floodplain ponds			
RP	Rifle Pond (rest area)	08 Oct 1998	N 39.52332 W 107.78772
PP	Parachute Pond	06 Oct 1998	N 39.44565 W 108.04647
WA	Wildlife area Pond	29 Sept 1998	N 39.05913 W 108.48098
DP	Dike Road Pond	16 Dec 1998	N 39.07464 W 108.59155
HP	Horsethief Canyon Ponds	11–20 Nov 1998	N 39.16929 W 108.79754
SS	South Skippers Pond	19–24 Nov 1998	N 39.16139 W 108.77770

ton, D.C.). Ponds were mostly gravel pit depressions with a maximum depth of <10 m, with sand and silt substrate, and varying quantities of aquatic plants (mainly *Chara* and *Potamogeton*) depending on depth, slope, and water clarity. These ponds may be permanently, seasonally, or periodically connected to the river by ditches or overland flow during high flow events.

Sampling and Analyses—We sampled at 4 backwaters and 6 floodplain ponds in 1997 and 1998 (Table 1) along a 142 km reach of the upper Colorado River in western Colorado (Fig. 1). One site (pond, CO) was sampled only in spring; another site (backwater, HB) was sampled in spring and fall. All other pond and backwater sites were sampled in fall (Table 1).

We collected 15 species of fish (Table 2) by electrofishing, seining, or trap netting. We retained selected specimens spanning the size range encountered in the various habitat types. We used MS-222 to anesthetize large native fish or to euthanize nonnative and small native fishes. Fish were weighed and measured and a sample of dorsal muscle (0.5–1.0 cm^3) was removed from each specimen and frozen in the field. Dorsal muscle is the preferred tissue for fish trophodynamics studies (Cabana and Rasmussen, 1994; Pinnegar and Polunin, 1999). For native fishes larger than 120 mm, a plug of dorsal muscle was removed with a 5-mm biopsy punch. A new punch was used for each sample and the fish was treated with 10% povodone-iodine solution before being released.

We dried tissue samples at 60°C for 24 h and ground them to a flour-like consistency with a mortar and pestle. A 1 to 2 mg sample, representing one fish, was placed in a tin cup and processed for $\delta^{13}C$ and $\delta^{15}N$ ratios in a VG Isochrom mass spectrometer with Carlo-Erba NA1500 elemental analyzer at the Colorado State University Natural Resources Ecology Laboratory. The quantity measured in stable isotope analysis, δ, is the relative difference between isotope ratios of the sample and a standard:

$$\delta(‰) = \left[\frac{R_{sa} - R_{std}}{R_{std}}\right] \cdot 10^3$$

where: $\delta(‰)$ is the per mil difference (parts per thousand), R_{sa} is the ratio of heavy to light isotopes (isotope ratio) in the sample, and R_{std} is the isotope ratio of a standard. We used Pee Dee Belemnite and atmospheric air as primary standards (Lajtha and Michener, 1994) for carbon and nitrogen, respectively. The mass spectrometer was calibrated at the start of each session and after approximately every 10 samples using a glycine standard. Mean coefficient of variation (*CV*) among replicate tissue samples analyzed on different days and by different operators was 1.2%.

RESULTS—We analyzed 107 fish samples from backwater sites (Table 3) and 53 samples from pond sites (Table 4) for stable isotope signatures. A two sample *t*-test, corrected for multiple comparisons, did not show differences between April and November $\delta^{13}C$ or $\delta^{15}N$ values for largemouth bass (*Micropterus salmoides*; $P > 0.11$, April $n = 4$, November $n = 2$) or for green sunfish (*Lepomis cyanellus*; $P > 0.019$, April $n = 7$, November $n = 8$) at HB, so we combined data from spring and fall samples at this location.

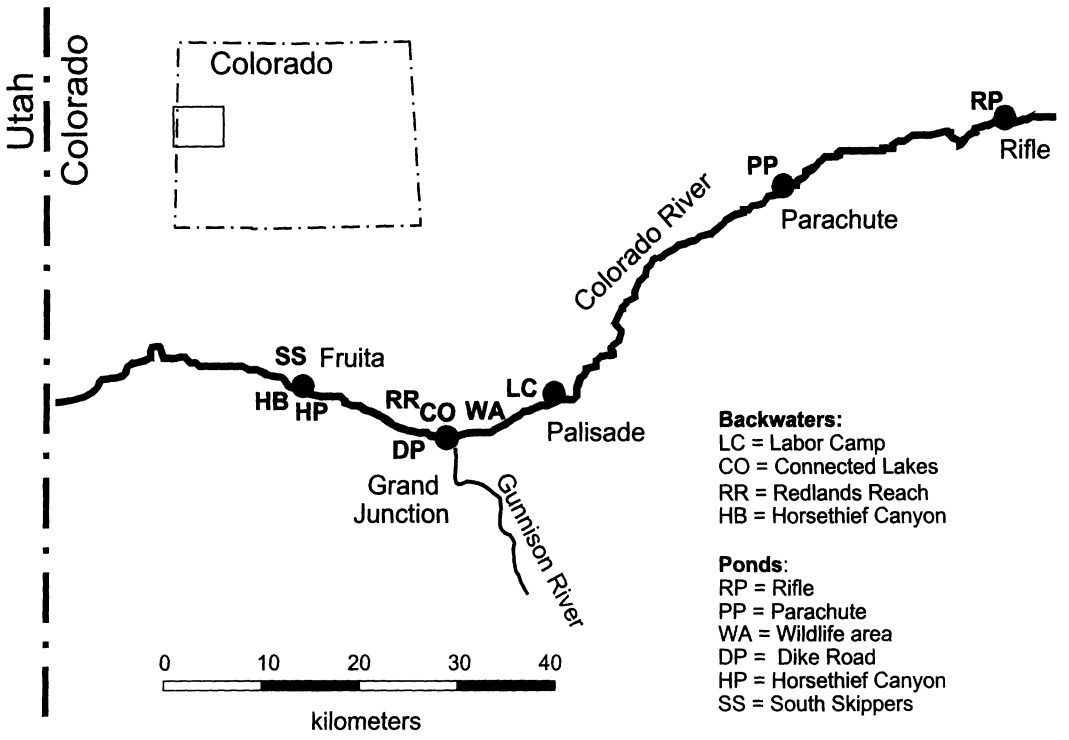

FIG. 1—Map of the upper Colorado River in western Colorado showing backwater and pond sites where fish were sampled for stable isotope analysis during 1997 to 1998, and major municipalities (black dots).

Largemouth bass $\delta^{13}C$ and $\delta^{15}N$ values increased with fish length ($r > 0.6$) at 5 of 5 and 3 of 5 sites, respectively (Figs. 2, 3); however, in many cases sample size was too low for correlation coefficients to be statistically significant. There was no evidence that isotopic signatures varied with fish length in the 3 catostomids or green sunfish. Size ranges of bluegill (*Lepomis macrochirus*), western mosquitofish (*Gambusia affinis*), and cyprinids (except common carp, *Cyprinus carpio*) were too limited (range < 100mm) to assess whether signatures varied with fish length (Fig. 4). A few green sunfish and catostomid samples from within a site differed by 5 to 15‰ for either $\delta^{13}C$ or $\delta^{15}N$ (Figs. 2, 3). Intraspecific variation in $\delta^{13}C$ and $\delta^{15}N$ (Tables 3, 4) was similar at backwater sites (mean $CV = 6.8\%$ and 7.7%, respectively). Intraspecific variation in $\delta^{13}C$ was lower than in $\delta^{15}N$ at pond habitats (mean = 3.2% and 10.4%, respectively). There were relatively large differences in species-specific signatures across ponds for bluegill, largemouth bass, and green sunfish (Fig. 5), but co-occurring species appeared to be more similar to each other within a pond than were signatures of a particular species among ponds. For example, signatures of bluegill, largemouth bass, and green sunfish were similar within HP pond and within WA pond, but large differences in the signatures of these species existed across all 6 ponds.

Largemouth bass $\delta^{15}N$ signatures were usually higher in backwaters than in ponds (Fig. 6a). Carbon signatures were quite consistent within backwater sites, and either higher or lower in ponds. The lowest $\delta^{13}C$ values for largemouth bass occurred at the most upstream ponds and highest $\delta^{13}C$ values were found in the ponds farthest downstream in the study area. With the exception of WA pond, which had unusually high $\delta^{15}N$ values, there was some indication that green sunfish might have had higher $\delta^{15}N$ values in backwaters than in ponds (Fig. 6b). Carbon signatures of green sunfish did not show the longitudinal trend observed with largemouth bass.

Trophic relationships were most apparent at the Redlands Reach (RR) backwater site because both sample size and number of species

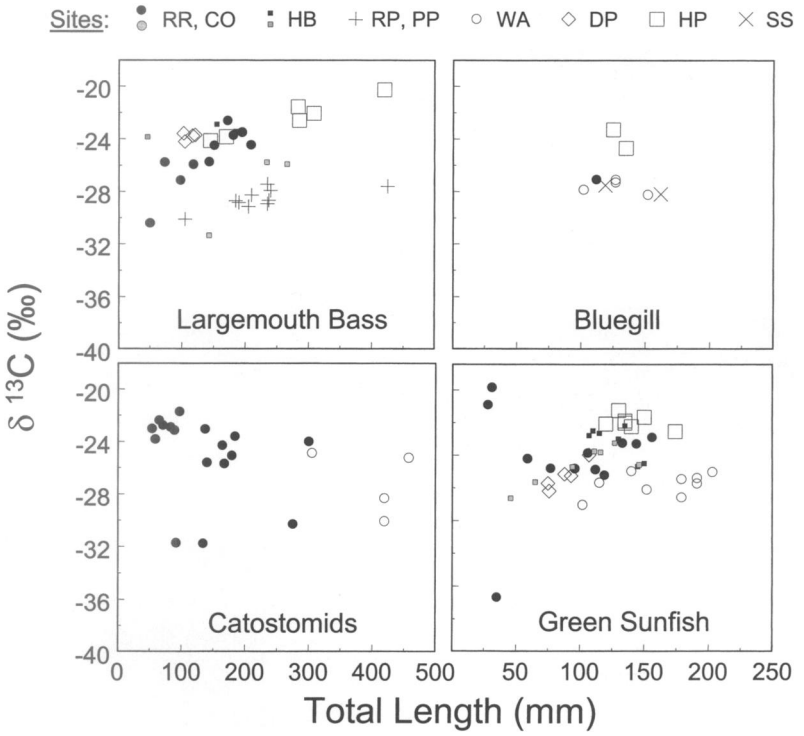

FIG. 2—Stable carbon ($\delta^{13}C$) isotope signatures as a function of length of largemouth bass, bluegill, 3 catostomids (bluehead sucker, flannelmouth sucker, white sucker), and green sunfish sampled at backwater (closed symbols; black denotes samples from October, gray denotes samples from April) and pond (open symbols or + and x) sites in the upper Colorado River, 1997 to 1998. See Table 1 for site names and sampling dates.

sampled was greatest (Fig. 7). Mean nitrogen signatures were highest for largemouth bass, black crappie (*Pomoxis nigromaculatus*), and green sunfish. Nitrogen signatures of the remaining 12 species sampled were consistently lower than for these 3 nonnative centrarchids. Average carbon signatures were variable but the 3 native species present (speckled dace, *Rhinichthys osculus;* bluehead sucker, *Catostomus discobolus*; flannelmouth sucker) showed signatures that were among the highest for all species at the site.

DISCUSSION—Nitrogen isotope analysis showed that within a particular location, either in ponds or in backwaters, nonnative centrarchids were usually the top predators in each system. Although $\delta^{15}N$ is a reliable indicator of trophic position (Peterson and Fry, 1987; Vander Zanden and Rasmussen, 1996; Vander Zanden et al., 1997), caution is warranted when inferring trophic differences by comparing nitrogen signatures across locations. Within a particular food web, the highest $\delta^{15}N$ values will normally be found in the top predators, but there can be high variation in $\delta^{15}N$ values of nutrient inputs depending on whether the source nitrogen is from natural or anthropogenic sources (Lajtha and Michener, 1994). For example, some fertilizers, septic tank waste, and animal waste typically have much higher $\delta^{15}N$ values than natural soil N or N in atmospheric deposition (Kendall, 1998; Fry, 1999). Elevated baseline $\delta^{15}N$ values in contaminated systems result in elevated consumer $\delta^{15}N$ values at each trophic level.

The extremely high (>30‰) $\delta^{15}N$ signatures of fishes from the WA pond would make these fish highly distinctive if they immigrated to the river and were subsequently captured for stable isotope analysis. Signatures at this pond are well above the expected range for

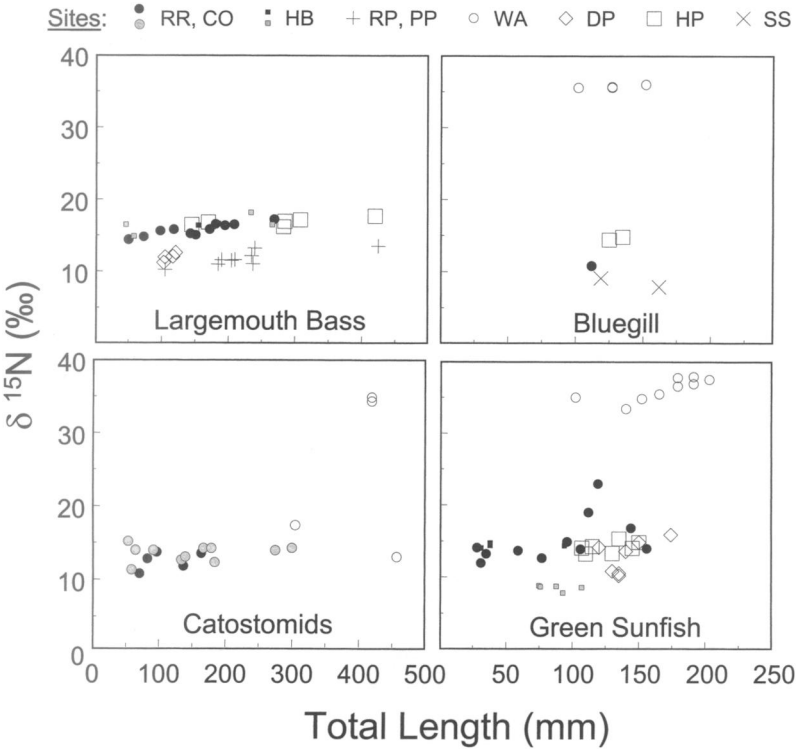

FIG. 3—Nitrogen ($\delta^{15}N$) signatures as a function of length of largemouth bass, bluegill, 3 catostomids (bluehead sucker, flannelmouth sucker, white sucker), and green sunfish sampled at backwater (closed symbols; black denotes samples from October, gray denotes samples from April) and pond (open symbols or + and x) sites in the upper Colorado River, 1997 to 1998. See Table 1 for site names and sampling dates.

TABLE 2—Fish species collected for stable isotope analysis in upper Colorado River backwaters and floodplain ponds, 1997 to 1998. (N) = native.

Code	Common name	Scientific name
BBH	Black bullhead	*Ameiurus melas*
BCR	Black crappie	*Pomoxis nigromaculatus*
BGL	Bluegill	*Lepomis macrochirus*
BHS	Bluehead sucker (N)	*Catostomus discobolus*
CCP	Common carp	*Cyprinus carpio*
FHM	Fathead minnow	*Pimephales promelas*
FMS	Flannelmouth sucker (N)	*Catostomus latipinnis*
GSF	Green sunfish	*Lepomis cyanellus*
LMB	Largemouth bass	*Micropterus salmoides*
MSQ	Western mosquitofish	*Gambusia affinis*
RSH	Red shiner	*Cyprinella lutrensis*
SPD	Speckled dace (N)	*Rhinichthys osculus*
SSH	Sand shiner	*Notropis stramineus*
WHS	White sucker	*Catostomus commersoni*
YLP	Yellow perch	*Perca flavescens*

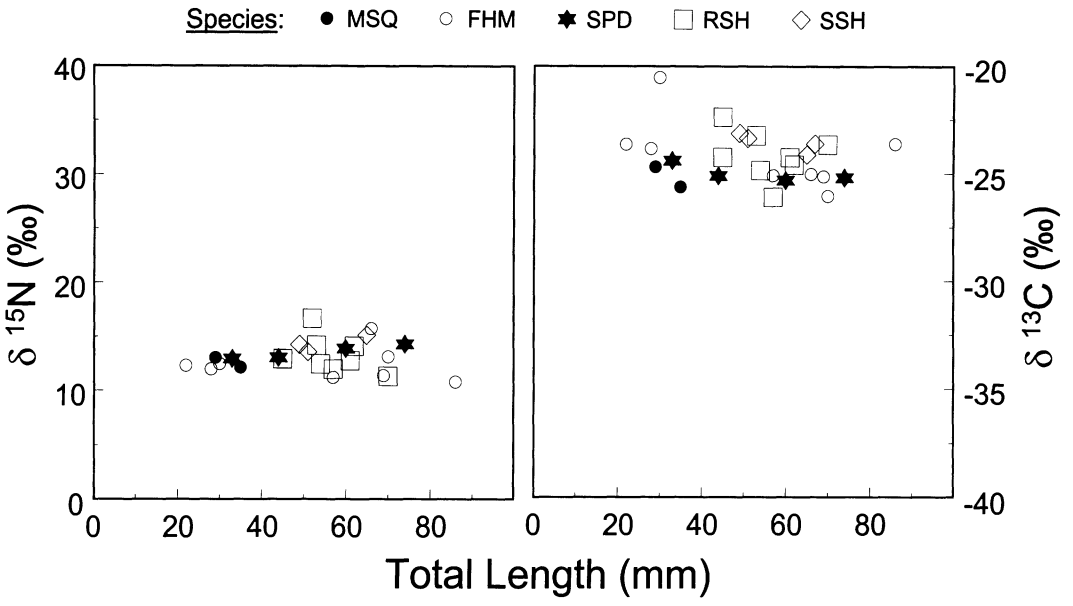

FIG. 4—Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) signatures as a function of total length of 4 cyprinids (fathead minnow, red shiner, sand shiner, speckled dace) and western mosquitofish sampled at backwater sites in the upper Colorado River, 1997 to 1998. See Table 1 for site names and sampling dates.

TABLE 3—Mean carbon and nitrogen isotope signatures (‰), standard deviation (*SD*), sample size (*n*), and coefficient of variation (*CV*, %) of species sampled in 4 river backwaters on the upper Colorado River during 1997 to 1998. See Table 2 for species names and Fig. 1 for site locations.

		$\delta^{13}C$				$\delta^{15}N$			
Species	Site	Mean	*SD*	*n*	*CV*	Mean	*SD*	*n*	*CV*
BBH	RR	−25.25	0.80	5	3.2	13.43	1.32	5	9.8
BCR	RR	−25.63	1.58	3	6.2	15.41	0.67	3	4.4
BGL	RR	−27.07	—	1	—	10.80	—	1	—
BHS	RR	−23.73	—	1	—	13.64	0.47	2	3.4
CCP	RR	−26.37	2.39	7	9.1	13.18	1.95	7	14.8
FHM	RR	−24.11	1.68	8	7.0	12.36	1.54	8	12.5
FMS	RR	−22.94	0.92	5	4.0	12.54	1.22	5	9.7
GSF	LC	−26.09	—	1	—	18.96	—	1	—
	CO	−33.36	2.66	3	8.0	14.36	0.30	4	2.1
	RR	−25.74	3.87	10	15.0	15.10	3.16	9	20.9
	HB	−24.56	1.30	14	5.3	14.38	0.78	12	5.4
LMB	CO	−25.58	1.70	2	6.6	15.70	0.02	2	0.13
	RR	−25.38	2.22	10	8.8	15.75	0.86	11	5.5
	HB	−25.54	3.10	6	12.1	16.51	1.06	6	6.4
MSQ	RR	−25.14	0.66	2	2.6	12.57	0.64	2	5.1
RSH	RR	−24.15	1.11	8	4.6	13.25	1.57	9	11.8
SPD	RR	−25.00	0.41	4	1.6	13.52	0.66	4	4.9
SSH	RR	−23.56	0.42	4	1.8	14.29	0.76	3	5.3
WHS	RR	−25.84	3.44	12	13.3	13.56	1.11	11	8.2
YLP	RR	−24.10	—	1	—	13.37	—	1	—

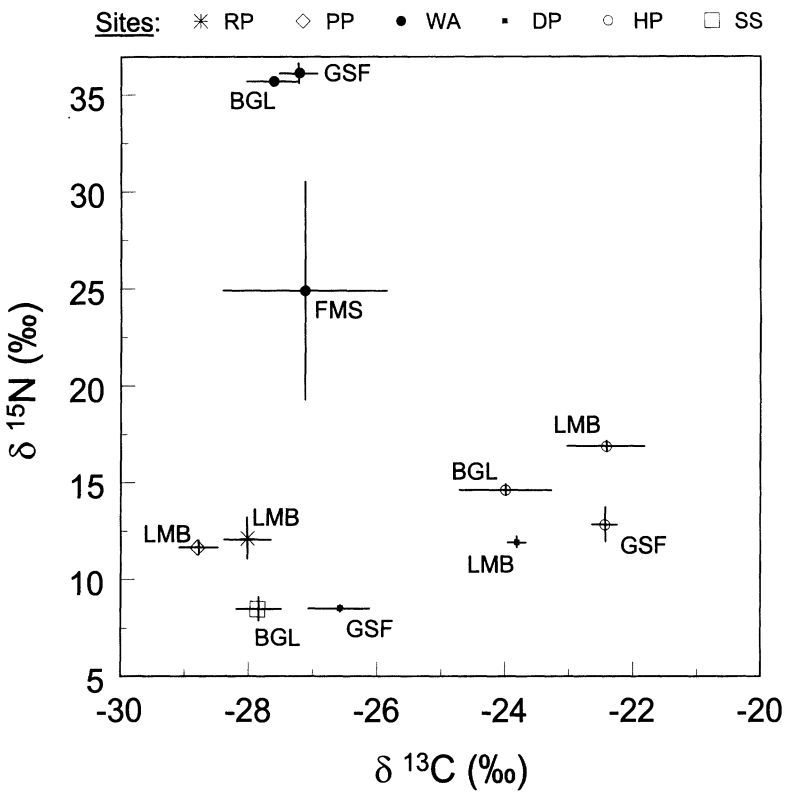

FIG. 5—Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) signatures ($\pm SE$) of largemouth bass (LMB), green sunfish (GSF), bluegill (BGL), and flannelmouth suckers (FMS) sampled at 6 pond sites (see Table 1 for site names) in the upper Colorado River, 1997 to 1998.

TABLE 4—Mean carbon and nitrogen isotope signatures (‰), standard deviation (*SD*), sample size (*n*), and coefficient of variation (*CV*, %) of species sampled in 5 floodplain ponds on the upper Colorado River during 1997 to 1998. See Table 2 for species names and Fig. 1 for sites.

		$\delta^{13}C$				$\delta^{15}N$			
Species	Site	Mean	*SD*	*n*	*CV*	Mean	*SD*	*n*	*CV*
BGL	WA	−27.62	0.52	4	1.9	35.69	0.21	4	0.59
	SS	−27.86	0.48	2	1.7	8.48	0.85	2	10.0
	HP	−24.00	0.10	2	0.42	14.61	0.30	2	2.0
FMS	WA	−27.13	2.50	4	9.2	24.89	11.3	4	45.4
GSF	DP	−26.58	1.03	5	3.9	8.53	0.40	5	4.7
	HP	−22.44	0.51	7	2.3	12.82	2.29	7	17.9
	WA	−27.22	0.86	9	3.2	36.13	1.55	9	4.3
LMB	DP	−23.82	0.28	4	1.2	11.93	0.57	4	4.8
	HP	−22.42	1.45	6	6.5	16.88	0.53	6	3.1
	PP	−28.80	0.77	7	2.7	11.65	1.02	7	8.8
	RP	−28.02	0.62	3	2.2	12.12	1.53	2	12.6

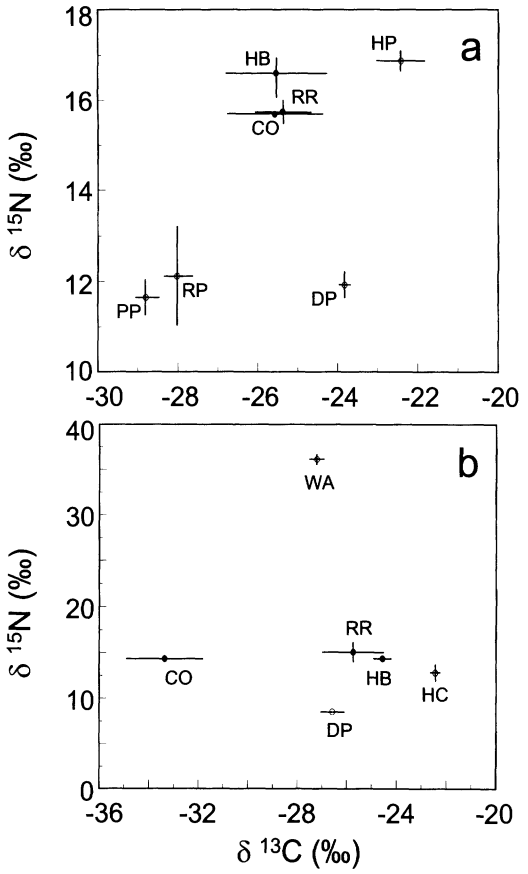

FIG. 6—Mean carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) signatures ($\pm SE$) of (a) largemouth bass sampled at 3 backwater (open circles) and 4 pond (closed circles) sites and (b) green sunfish sampled at 3 backwater (open circles) and 3 pond (closed circles) sites, in the upper Colorado River, 1997 to 1998.

nitrogen in precipitation, natural soils, or fertilized croplands. Rather, the $\delta^{15}N$ values may be indicative of either concentrated animal waste or septic tank inputs to the WA pond, or they may suggest anoxic conditions that allowed strong fractionation by bacteria during denitrification (Macko and Ostrom, 1994).

Within the RR backwater, where taxonomic diversity of our samples was greatest, both $\delta^{15}N$ and $\delta^{13}C$ signatures of the fish assemblage ranged about 4‰. Thus, the number of fish trophic levels represented was limited to about 2, and variation in carbon sources was considerable. The native flannelmouth sucker was most distinct with a relatively low $\delta^{15}N$ and the highest $\delta^{13}C$ signature. Angradi (1994) found that terrestrial vegetation had a higher $\delta^{13}C$ signature than did algae in lower Colorado River and tributary food webs, and terrestrial insects had higher $\delta^{13}C$ signatures than did zooplankton or aquatic macroinvertebrates. France (1995*a*) and Vander Zanden and Rasmussen (1999) found considerable separation in $\delta^{13}C$ signatures of autotrophic food sources and of consumers between pelagic and littoral habitats of lentic systems. Attached algae was significantly ^{13}C-enriched relative to planktonic algae.

If these patterns in signatures of food sources exist in the upper Colorado River then we would hypothesize that RR backwater fishes with enriched $\delta^{13}C$ values may be more reliant on either allochthonous inputs (plant or animal) from the terrestrial environment or on benthic food sources, whereas ^{13}C-depleted fishes may depend more on pelagic autochthonous energy sources. Although debate continues as to the discriminatory power of $\delta^{13}C$ in studying carbon pathways (France, 1995*b*, 1996; Doucett et al., 1996), additional isotopic data on primary producers would help determine the importance of food resource partitioning among species as an explanation for the range in carbon signatures of fishes in backwater sites.

At the RR backwater, largemouth bass, black crappie, and green sunfish appeared to be at least partially piscivorous and the system's top predators. Because the carbon signatures of red shiner (*Cyprinella lutrensis*), sand shiner (*Notropis stramineus*), fathead minnow (*Pimephales promelas*), yellow perch (*Perca flavescens*), bluehead sucker and flannelmouth sucker were considerably higher than the centrarchid piscivores, it is unlikely that these fishes were important prey for centrarchids. Rather, carbon signatures of bluegill, speckled dace, western mosquitofish, black bullhead (*Ameiurus melas*), carp, and white sucker (*Catostomus commersoni*) suggested these species were more likely prey for piscivorous centrarchids.

Stable isotopes appear to have utility as a naturally occurring marker for tracking influx of nonnative fishes to the Colorado River from ponds. Although pond signatures were not consistently depleted or enriched relative to those from river backwaters, we did find that mean stable isotope signatures were more different among ponds than among backwater

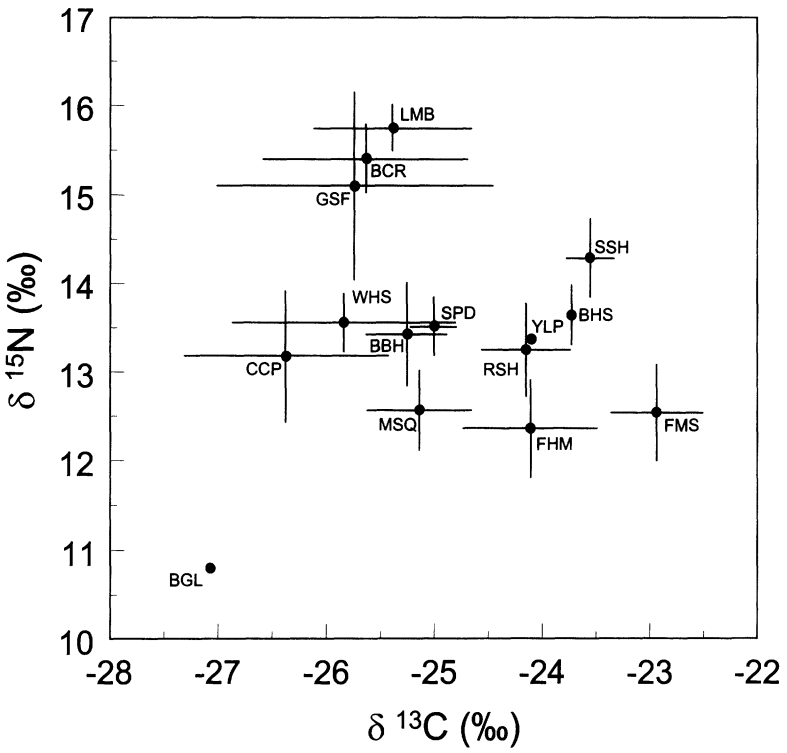

FIG. 7—Mean carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) signatures ($\pm SE$) of 15 species of fish (abbreviations in Table 2) sampled at the RR backwater in the upper Colorado River, 1997 to 1998. Sample size was 1 for bluegill and yellow perch (Table 3).

sites. Further, within particular species, some ponds had carbon or nitrogen signatures that were quite different from those at backwater sites. This suggests more discrete food sources and baseline $\delta^{13}C$ and $\delta^{15}N$ signatures within ponds. There appeared to be little spatial coherence between ponds and nearby backwater sites, suggesting localized processes and baseline signatures of carbon and nitrogen inputs in ponds, and that backwater sites are more intimately connected to the river and its homogenizing influences on trophic structure and carbon inputs. However, occasional signatures far outside the usual signatures for a species at a site (e.g., green sunfish at RR backwater or flannelmouth suckers at WA pond; Tables 3 and 4) suggest some mixing between ponds and backwaters.

One application of this isotope approach involves sampling nonnative fishes from suspect ponds and nearby river sites for isotope analysis prior to expected mixing events (e.g., high spring flows, irrigation releases). Another sample of these species could be obtained from the river after an event to determine relative abundance of fishes with pond versus river signatures. Because whole-body isotope signatures change relatively slowly (e.g., on the order of months), it may be practical to obtain baseline samples in spring and freeze them without analyzing for isotopes, which is expensive. Then, if a mixing event occurred, pre- and post-mixing samples could be analyzed for isotope signatures to assess the contribution of pond fish to the river. This approach would be much less time-consuming than using physical marks because the isotope signature is naturally-occurring and only a sample of fish needs to be captured and examined. However, individual variation in isotope signatures would necessitate a power analysis to determine sample sizes required to make strong inferences about immigration of fishes from particular ponds to the river.

In conclusion, stable isotope analysis is a promising approach for studying trophic rela-

tionships and movement patterns of native and nonnative fishes in the upper Colorado River. Because muscle tissue samples can be removed without sacrificing fish, and because stable isotope signatures integrate feeding and distributional history of a fish over a multi-month period the approach is a desirable method to study ecology of threatened and endangered fishes with minimal sampling and injury. Temporal variation in food sources and in isotopic signatures of those food sources complicates trophic interpretations. Overlap of isotopic signatures among native and nonnative fishes may suggest reliance on a common resource base, but makes it difficult to assess which species are more important as prey for piscivorous fishes. Still, we derived some general insights into trophic structure of ponds and backwaters with little destructive sampling, and we demonstrated that stable isotopes provide a practical naturally-occurring marker to track origins and movements of nonnative fishes in the upper Colorado Basin.

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