Movement into floodplain habitats by gizzard shad (*Dorosoma cepedianum*) revealed by dietary and stable isotope analyses

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Abstract Floodplain habitats have been inferred to provide a variety of functions for aquatic organisms, yet few studies have documented movement between channel and aquatic floodplain habitats. We exploited spatial variation in stable isotope ratios of gizzard shad (*Dorosoma cepedianum*) to document movement between floodplain lakes and the main river channel of the Brazos River, Texas, during a period of frequent hydrologic connectivity. Additionally, we examined stomach contents of shad to determine if ontogenetic diet shifts or faunal exchange best explained variation in isotopic ratios. Regression analysis indicated significant relationships between gizzard shad size and isotopic ratios in oxbow lakes, whereas these relationships were not significant for the main channel. Plots of individual fish in each habitat suggested that adult shad migrated into oxbow lakes during floods whereas juveniles assimilated material produced in oxbows. Some adults in oxbows had signatures similar to juveniles, and these individuals were probably long-term oxbow residents. The proportion of adults with a "river" signature was greater in the oxbow with the shortest flood recurrence interval where opportunities for faunal exchange were more frequent. Analysis of stomach contents indicated almost total overlap between adult and juvenile diets indicating that movement between habitats having different isotopic ratios of basal resources rather than ontogenetic dietary shifts best explained patterns of isotopic variation in Brazos River gizzard shad.

Keywords Brazos river · Faunal exchange · Oxbow lake · Reproduction · Texas

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Introduction

Off-channel habitats have been inferred to provide a variety of ecological functions for aquatic organisms in large rivers. High densities of larval and juvenile fishes in floodplain ponds and oxbow lakes indicate that these areas are important spawning and rearing areas for certain species (Sabo and Kelso 1991; Killgore and Baker 1996). Both main channel and off-channel habitats can support diverse fish assemb-

lages (Winemiller et al. 2000). Some species can survive and reproduce within either habitat; however, other species require multiple habitats to complete their life cycle (Schiemer 2000) or obtain growth and survival benefits from temporary residence in flood-plain habitats (Sommer et al. 2001). Thus, under-standing the functions of different habitat units for fish reproduction and recruitment in floodplain rivers is of great importance to maximize the effectiveness of habitat restoration and biodiversity conservation.

Tracking the movement of individuals between habitat types can be complicated by the large spatial scales over which faunal exchanges occur and the short duration of major dispersal events (Gillanders et al. 2003). Provided there is sufficient spatial variation in the isotopic ratios of production sources, stable isotope ratios of consumers provide a viable means for tracking faunal exchange among habitats (Hansson et al. 1997; Fry 2002; Cunjak et al. 2005). In large, spatially heterogeneous ecosystems such as floodplain rivers, stable isotopes can provide advantages over traditional mark-recapture methods in that all individ-uals in the population are marked by the consumption of material in a particular habitat. Using isotopes as a mark provides information on batches of fish but must be combined with telemetry or mark-recapture data to gain information on individual fish (Cunjak et al. 2005).

Here we use stable isotope ratios of carbon and nitrogen to examine movement of gizzard shad (*Dorosoma cepedianum*) between channel and flood-plain habitats in the Brazos River, Texas. Gizzard shad are a dominant species in fish assemblages of many large rivers throughout the eastern and mid-western United States; however, studies of their reproductive and trophic ecology have primarily focused on reservoirs where they have been exten-sively stocked as forage for sport fish. Larval gizzard shad feed almost exclusively on zooplankton, and between 25 mm and 35 mm they switch to filtering detritus, phytoplankton and zooplankton (Mundahl and Wissing 1987; Miranda and Gu 1998). In the Brazos River, Zeug and Winemiller (2007) found that gizzard shad reproduction coincided with high flows that connect the channel to off-channel oxbow lakes. Oxbow lakes support much higher densities of zooplankton (Winemiller et al. 2000) and appear to provide superior rearing areas for juvenile gizzard shad (Zeug and Winemiller 2008a).

Our goals for this study were to analyze variation in the isotopic composition of gizzard shad in the Brazos main channel and oxbow lakes relative to spatial variation in the proportion of different produc-tion sources supporting these populations previously identified by Zeug and Winemiller (2008b) to examine movement of adult and juvenile gizzard shad among habitats. We also examined stomach contents to determine if faunal exchange or ontoge-netic diet shifts best explained observed variation in isotopic signatures. We predicted a net movement of adult gizzard shad into oxbow lakes for reproduction, and a net movement of post-larval juveniles into the river channel.

Materials and methods

Study site

The Brazos River flows 1,485 km southeast across Texas through agricultural and forested lands before entering the northern Gulf of Mexico. Our study was conducted on the middle Brazos located in east-central Texas. In this region the river is a single-thread channel and oxbow lakes are common on the floodplain (Winemiller et al. 2000). Connections between oxbows and the main channel occur at irregular intervals and are determined by the elevation of natural levees that separate oxbows from the active channel and the magnitude of river discharge. Patterns of discharge in the middle Brazos are unpredictable both within and among years (Zeug and Winemiller 2008b). Sampling was conducted in two oxbow lakes with different flood recurrence intervals plus a 7 km reach of the main river channel. One oxbow, hereafter referred to as OXFREQ, is located 200 m from the channel and has a flood recurrence interval of 1.1 years. The other oxbow, located 1,200 m from the channel, has a flood recurrence interval of >2 years and is hereafter referred to as OXRARE. A previous study of these sites conducted during the same time period found that differences in baseline nitrogen values based on primary producers (seston, benthic algae and riparian and floodplain vegetation) were significantly different between habitats with the main channel having a significantly higher value than either oxbow lake (Zeug and Winemiller 2008b). Additional-ly, shad in the river channel assimilate greater

fractions of C$_4$ macrophytes resulting in heavier carbon signatures relative to shad in oxbow lakes (Zeug and Winemiller 2008b; Fig. 1). For additional study site details, see Zeug and Winemiller (2007) and Zeug and Winemiller (2008b).

Sample collection and processing

Gizzard shad were collected monthly from April 2004 to August 2004 from each habitat. A combination of beach seine hauls, overnight gill-net sets and electrofishing was used to obtain samples of juvenile and adult fish. For a complete description of fish collection methods see Zeug and Winemiller (2007). Shad were collected during all five collections in the two oxbow lakes and during three collections in the main channel. During each survey, three to four individuals were selected for isotopic analysis based on their representation of the minimum, maximum, and approximate mean of the size range collected. Fish were placed into plastic bags and transported on ice to the laboratory where they were frozen for later processing.

Individual fish were defrosted, measured to the nearest millimeter (standard length; SL) and weighed to the nearest 0.1 g. Samples of white muscle tissue were removed from the dorsal region of one side of each individual, rinsed with deionized water, and inspected to ensure samples did not contain tissues other than muscle. Tissue samples were placed into Petri dishes and dried at 60°C to a constant weight. Dried samples were homogenized by grinding them into a fine powder. Sub-samples were weighed to the nearest 0.01 mg, enclosed in Ultra-Pure tin capsules (Costech Analytical Technologies, Inc) and shipped to the Analytical Chemistry Laboratory, Institute of Ecology, University of Georgia, for analysis of isotope ratios.

Results from carbon and nitrogen analysis were quantified as deviations relative to isotopic standards (delta notation):

$$\delta^{13}\text{C or} * \delta^{15}\text{N} = \left[\left(R_{sample} / R_{standard} \right) - 1 \right] * 1000,$$

where R = ^{13}C/^{12}C or ^{15}N/^{14}N. The carbon isotope standard was Pee Dee Belemnite limestone, and atmospheric nitrogen was the nitrogen standard.

As part of a concurrent study, stomach contents were examined from gizzard shad collected in the two oxbow lakes. This study was focused on oxbows and did not analyze stomach contents from fish in the river channel. Gizzard shad that were not used for isotopic analysis were fixed in a 10% formalin solution for 48 h and then transferred to 70% ethanol for storage. In the laboratory, gizzard shad were separated into 25-mm size classes, and ten individuals from each size class were randomly selected for analysis. The anterior portion of the alimentary tract
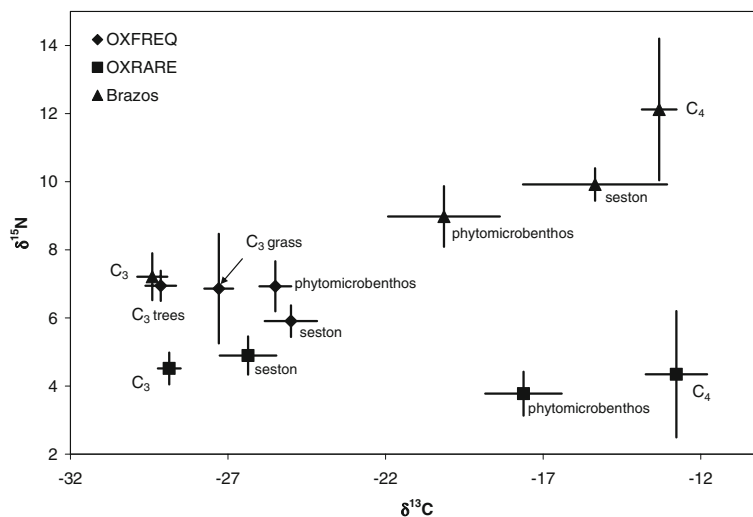
Fig. 1 Means and standard errors of carbon and nitrogen isotope ratios of primary production sources supporting shad population in the Brazos River, OXFREQ and OXRARE.

Sources: C$_3$ macrophytes, C$_4$ macrophytes, phytomicrobenthos (benthic algae and associated microorganisms), and seston

was removed, and the total volume of gut contents was determined by water displacement in a graduated cylinder. Gut contents were then homogenized, and a sub-sample was taken for identification of individual food items. Food items were identified to the lowest feasible taxonomic level, and volumes were determined using a modified glass slide, containing a rectangular well etched with a grid. Each square on the grid represented a volume of 0.0001 ml. Total volumes of each diet item in the sub-sample were then calculated based on the total volume of gut contents for each individual.

Data analysis

Bi-variate plots of carbon and nitrogen isotope ratios of individual fish in each habitat suggested that there were two distinct size cohorts in the population. These two size groups were used for all subsequent comparisons: fish <150 mm (juveniles) and fish >150 mm (adults). Trophic positions for each size class were calculated based on stomach contents using the method of Adams et al. (1983);

$$T_i = 1 + \sum_{j=1}^{n} \left(p_{ij} \right)$$

where T_j is the trophic position of species j and p_{ij} is the volumetric proportion of diet item i consumed by species j. This metric yields trophic position values in a continuum rather than discrete whole numbers. The difference in trophic positions between juveniles and adults in each habitat also was estimated from isotopic data using the equation;

$$\delta^{15}N_a - \delta^{15}N_j / 2.54$$

where $\delta^{15}N_a$ is the mean nitrogen ratio for adults, $\delta^{15}N_j$ is the mean ratio for juveniles and 2.54 is the value for trophic fractionation based on a meta-analysis of fractionation studies (Vanderklift and Ponsard 2003).

Diet overlap between juveniles and adults in oxbow lakes was calculated using Pianka's measure;

$$\phi_{jk} = \frac{\sum p_{ij} * p_{ik}}{\sqrt{\sum p_{ij}^2 \sum p_{ik}^2}}$$

where p_{ij} and p_{ik} are the volumetric proportions of item i in the diet of species j and k. Values for this measure range from 0–1 with 0 indicating no overlap and 1 indicating complete overlap.

Differences in the carbon and nitrogen isotope ratios of juveniles and adults in each habitat were tested using multi-response permutation procedures (MRPP). This technique compares the significance of *apriori* groupings in δ-space based on Euclidian distance. To test the null hypothesis of no relationship between gizzard shad standard length and carbon or nitrogen isotope ratios, we performed a series of linear regression analyses. Analyses were performed separately for each habitat to avoid bias resulting from significant spatial differences in baseline $\delta^{15}N$ values. Gizzard shad standard length was the independent variable and $\delta^{13}C$ or $\delta^{15}N$ was the dependent variable in each regression model. Regression analyses were conducted in SPSS (Version 11.0.1, SPSS Inc.) and MRPP were conducted in PC-ORD (Version 4; MJM Software).

Results and discussion

A total of 42 gizzard shad was analyzed for isotopic ratios and 102 individuals were examined for stomach contents. Gizzard shad used in isotopic and dietary analyses ranged in size from 46–338 mm and 63–342 mm, respectively. Bi-plots of juvenile and adult isotopic ratios in each habitat revealed that certain adults in oxbow lakes had isotopic signatures similar to gizzard shad in the river channel, whereas other adults had signatures similar to juveniles in oxbow lakes (Fig. 2). This pattern is consistent with the hypothesis that some adults moved into oxbow lakes during hydrologic connections with the main channel where baseline nitrogen values are significantly greater. Adult gizzard shad with isotopic signatures similar to juveniles may have been produced *in situ* or resided in oxbows for a sufficient period that their tissues had acquired the "oxbow" signature. This interpretation is supported by the presence of adults with intermediate isotopic signatures that may be in a transitional period. Additionally, greater proportions of adult gizzard shad with "river" signatures were present in the oxbow lake with the relatively short flood recurrence interval where opportunities for faunal exchange are more frequent (Fig. 2). During the study period, OXFREQ connected to the river on five occasions for 24 total
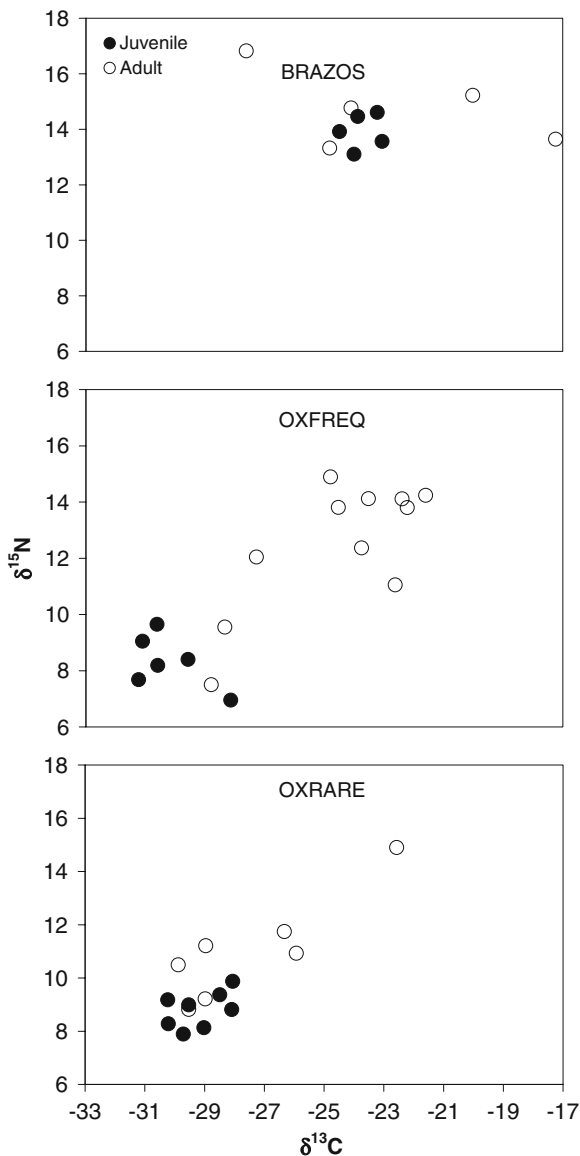
Fig. 2 Bi-plot of carbon and nitrogen isotope ratios of gizzard shad in the Brazos River channel and two oxbow lakes with different flood recurrence intervals

days, and OXRARE connected on three occasions for six total days (Zeug and Winemiller 2007). Juvenile and adult gizzard shad in the river channel had similar nitrogen ratios; however, carbon ratios were more variable in adults.

Analysis of sample groupings in δ-space yielded significant differences between juveniles in the river channel and both oxbow lakes whereas comparisons between oxbows were not significant (Table 1). Adult groupings were not significant between the main channel and OXFREQ where hydrologic connections are frequent, and significant differences were found between the main channel and OXRARE where floods are infrequent and of shorter duration. A comparison of adults between oxbows was signifi-cant; however the p-value approached the critical value and larger sample sizes could have altered this result (Table 1).

Ontogenetic diet shifts could provide an alternate explanation for the observed variation in isotopic ratios between juvenile and adult gizzard shad in oxbows. Previous studies have found that gizzard shad larvae feed almost exclusively on zooplankton and switch to a diet of detritus, phytoplankton and zooplankton at approximately 25 mm (Miranda and Gu 1998). Regres-sion analyses indicated there was a significant positive relationship between gizzard shad standard length and both $\delta^{13}C$ and $\delta^{15}N$ in oxbow lakes, whereas this relationship was not significant for gizzard shad in the river channel (Table 2). The positive slope for regression equations with $\delta^{15}N$ as the dependent variable was unexpected. This is because ontogenetic diet shifts in gizzard shad result in greater dietary proportions of materials with lower trophic positions (detritus and phytoplankton) as body size increases (Mundahl and Wissing 1987). Thus, we predicted the slope of the relationship would be negative if the pattern of variation in isotopic ratios were driven by

Table 1 Results of multi-response permutation procedures comparing juvenile and adult sample groupings between habitats

Comparison	Adults (>150 mm)		Juveniles (<150 mm)	
	A	P	A	P
OXFREQ×Brazos	0.016	0.258	0.710	<0.001
OXFREQ×OXRARE	0.100	0.047	0.060	0.086
Brazos×OXRARE	0.210	0.013	0.701	<0.001

Table 2 Results of linear regression analyses testing the relationship between shad standard length and isotopic ratios

Habitat	Isotope	R^2	F	P
OXFREQ	$\delta^{13}C$	0.50	14.755	0.002
	$\delta^{15}N$	0.46	12.908	0.003
OXRARE	$\delta^{13}C$	0.30	5.497	0.036
	$\delta^{15}N$	0.55	15.665	0.002
Brazos	$\delta^{13}C$	<0.01	0.003	0.957
	$\delta^{15}N$	0.16	1.497	0.256

diet shifts. Nitrogen and carbon ratios of gizzard shad are significantly heavier in the river channel relative to oxbow lakes, and faunal exchange could account for the positive relationship between gizzard shad standard length and both $\delta^{13}C$ and $\delta^{15}N$ values.

Schaus et al. (2002) found that post-larval gizzard shad switched between zooplankton and detritus dominated diets in association with annual changes in population biomass. When biomass was high, gizzard shad primarily consumed detritus whereas zooplankton was more abundant in shad diets when population biomass was low. In their analysis, shad feeding primarily on detritus showed greater trophic fractionation of nitrogen than zooplankton feeding fish. This could explain isotopic patterns in the current study. However, in our study, there was considerable variation in the isotopic ratios of shad of different sizes during the same time period. Thus, relationships between isotopic ratios and feeding strategy as a function of population standing stock seem unlikely. Sweeting et al. (2007a) found that trophic fractionation of carbon can decline with body mass and this could account for differences between adults and juveniles in the Brazos shad population. However, in a related study nitrogen fractionation was shown to be independent of body mass (Sweeting et al. 2007b) and significant relationships between size and $\delta^{15}N$ were observed (Table 1).

Direct examination of shad stomach contents in oxbow lakes revealed that the diets of both juveniles and adults were dominated by detritus. Diet overlap between juveniles and adults in both oxbow lakes was high (0.99), indicating that variation in the proportional consumption of diet items was not significant. Trophic positions of adults and juveniles calculated from dietary data reflected this similarity with a difference of 0.26 and 0.17 for shad in OXFREQ and OXRARE,

respectively. Differences in trophic position based on isotopic data were greater, with values of 1.64 and 0.88 in OXFREQ and OXRARE, respectively. In oxbow lakes, the large difference in isotope-based estimates combined with the small difference in diet-based estimates and high diet overlap suggest that isotope-based estimates do not reflect real differences in trophic level. Rather, they suggest that some adult shad had previously assimilated ^{15}N enriched material in the river and recently moved into oxbows. Stomach contents analysis was not conducted on shad collected from the river channel; however, the difference in the trophic positions of juveniles and adults based on isotopes was 0.32; a value similar to the juvenile-adult difference of oxbow fish based on T_i estimates from stomach contents.

The combination of isotopic and stomach contents analysis suggests that many adult shad may move from the main channel into oxbow habitats during periods of hydrologic connectivity. The flood pulse concept (Junk et al. 1989) predicts that fish should time reproduction to coincide with floods that afford opportunities to place offspring in productive floodplain habitats. Oxbow lakes on the Brazos floodplain have greater zooplankton densities than the main channel (Winemiller et al. 2000). Thus oxbows should provide good rearing habitat for larval shad that depend on zooplankton (Zeug and Winemiller 2008a). Most adult shad probably move into oxbows for reproduction. Zeug and Winemiller (2007) found that the probability of flooding was the strongest predictor of shad reproductive activity. Juvenile shad were collected in the main channel; however, abundances were significantly greater in oxbows (Zeug and Winemiller 2008a). Similarly, Sabo and Kelso (1991) found that larval shad occurred at highest densities in floodplain ponds of the Mississippi River.

Gillanders et al. (2003) suggested that documenting movement of juveniles into adult habitats is an essential component of evaluating the value of rearing areas. Although oxbows provide habitat for both juveniles and adults, we did not collect shad with "oxbow" signatures in the river channel. This may be partially attributed to the spatial and temporal scale of our study and low sample sizes from the main channel. Flood events during the 5-mo study occurred when the average size of juveniles was relatively small (<40 mm). Brazos River flooding is characterized by a rapid rise and fall in water level. In order for small shad to move from an oxbow to the river channel, they may have to be of a sufficient size and/ or the flood connection may have to be of a sufficient duration. Collections over longer time periods and larger sample sizes might reveal greater movement of individuals from oxbows to the river channel. Additionally, the much larger size of the river channel (7-km reach surveyed) relative to the two oxbow lakes may have reduced our ability to collect representative numbers of fish that had recently moved into the river.

Isotopic and dietary data provide evidence that oxbow lakes provide important spawning and nursery habitat for gizzard shad in the Brazos River. Alteration of river flows that reduce or eliminate overbank flooding could have a significant impact on recruitment and population abundance of this species (Bayley 1991). Juvenile shad are important food items for predators in this system (Robertson et al. 2008), and reductions in shad populations due to hydrologic modification could have direct and indirect effects on food web dynamics in both oxbow lakes and the main channel.

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