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RESEARCH PAPER

Flood pulse trophic dynamics of larval fishes in a restored arid-land, river-floodplain, Middle Rio Grande, Los Lunas, New Mexico

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Abstract Rio Grande water is intensively managed and regulated by international and interstate compacts, Native American treaties, local water rights, and federal, state, and local agencies. Legislation and engineering projects in the early twentieth century brought about water impoundment projects and channelization of the Rio Grande which led to the eventual loss of floodplain habitats. In particular, current water management practices in the Middle Rio Grande (MRG) have altered the natural flood regime altering the riparian community and floodplain dynamics which may be causing the demise of many fish species by altering food web processes. The Rio Grande silvery minnow (*Hybognathus amarus*), a federally endangered species, has been classified as an herbivore, detritivore, or carnivore. During low flow conditions *H. amarus* is primarily an algivore; however, during flood conditions, hydrodynamic scouring reduces or eliminates benthic algal food sources. The objective of this study was to identify and characterize food resources and trophic interactions for *H. amarus* on a restored floodplain during an extended flood-pulse release from reservoirs using stable isotope analyses ($\delta^{13}C$ and $\delta^{15}N$) and paleolimnology techniques. Results from stable isotope ratios indicate that *H. amarus* obtained carbon primarily from chironomids while aquatic invertebrates (including chironomids) obtained their carbon from macrophytes. Results from the GLIMMIX procedure indicate that the range of isotopic signatures for prey items was much broader at parallel habitats (i.e. floodplain flow parallel to main stem flow) than perpendicular (i.e. floodplain flow perpendicular to main stem flow) or leeward habitats (i.e. leeward sides of island where flow was near zero) indicating a wider selection of food resources. This study suggests that increased duration of floodplain inundation in the MRG provides vital habitats for spawning, nursery, and recruitment of threatened and endangered fish species. A combination of allochthonous and autochthonous resources best describes the nutrient and energy transfers for the Los Lunas, NM restored floodplain.

Keywords *Hybognathus amarus* · Floodplain · Flood pulse · Stable isotopes

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Introduction

Most of the native fish fauna in the southwestern U.S. is listed as threatened or endangered under the Endangered Species Act, largely as a consequence of water withdrawal, impoundment, and flow stabilization (Williams et al. 1989; Poff et al. 1997). Few large alluvial rivers of the southwestern U.S. have been

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studied and documented as well as the MRG in particular, hydraulic, topographic, photogrammetric, and sediment data have been collection by federal and state agencies that have tracked changes in the river since 1895 (Richard 2001). Little is known about resource use by Rio Grande ichthyofauna on floodplains, however, Junk et al. (1989) suggested that floodplain inundation via annual flood pulse enhances fish recruitment by providing suitable environmental conditions, abundant food, and nursery habitat for larvae.

The present study was intended to reveal floodplain resource use by Rio Grande ichthyofauna during a long-term, artificially induced flood pulse in the Middle Rio Grande (MRG) of New Mexico. The MRG is defined as the reach from Cochiti Dam to the headwaters of Elephant Butte Reservoir in southern NM, a distance of 280 km. The MRG is generally characterized as slightly sinuous with straight, meandering, and braided reaches with a gravel riverbed downstream from Cochiti Dam and a shifting sandbed in the lower reaches (Crawford et al. 1993). Water storage practices have altered flows in the MRG and reduced the riparian forest to the area between levees (Crawford et al. 1996; Molles et al. 1998). This has left much of the floodplain disconnected and abandoned through degradation and aggradation of the channel bed (Valett et al. 2005; Massong et al. 2006). Channelization and regulation of the MRG has virtually eliminated overbank flooding throughout its length in New Mexico (Molles et al. 1998). Flow regulation has led to the loss of connectivity between the river and floodplains in the MRG and has shifted the flood regime to longer inter-flood intervals (Valett et al. 2005). Most MRG floodplains remain isolated from flooding and very few are still regularly inundated by the flood pulse (Valett et al. 2005). The importance of the timing and duration of these floods on the floodplain modulate water temperatures appropriate for spawning of native fish species and may dictate the strength of biotic responses to the flood (Boulton and Lloyd 1992; Galat et al. 1998; Sparks et al. 1998; King et al. 2003).

As a result of Dam operations, the MRG no longer has a seasonal flood regime; rather it has a 'naturalized flood regime' (Bayley 1995) in the form of short, 1–5 days, annual releases from Cochiti Reservoir (Cowley et al. 2006) that try and mimic the historic flood pulse. The most significant ecological effect of Cochiti Dam was to retain sediment and diminish the historic flood regime by taking the peaks off of some the high discharges and increasing duration of lower flows (Crawford et al. 1993). Consequently, sediment starvation caused by the retention Dam has accelerated the incising of the channel in the Cochiti and Albuquerque reaches (Crawford et al. 1993; Dahm et al. 2003). Presently, the riverbanks along the MRG are generally 1.2–1.5 m above the river surface and the incision of the river channel makes it very unlikely that controlled discharges from Cochiti Dam will overtop the riverbanks under present reservoir management practices (Crawford et al. 1993).

The present study took place during a "wet" year where snowpack in northern New Mexico Mountains was higher than normal (NOAA 2007). In anticipation of a higher spring runoff, federal agencies began releasing water on 1 April 2005 and continued for approximately 100 days. The flood pulse release increased discharge from baseflow of 11.33 m^3 s^{-1} to a maximum of 198 m^3 s^{-1} from 1 April to 2 June and gradually decreased to baseflow level by 23 July. The spring 2005 flood pulse provided us the first opportunity to investigate resource use by Rio Grande ichthyofauna on a restored floodplain during a flood of greater magnitude and duration than any flood pulse since 1993.

The Rio Grande silvery minnow (*Hybognathus amarus*) was historically the most abundant fish in the Rio Grande and Pecos River occupying approximately 3,800 river km from Colorado to the Gulf of Mexico (Bestgen and Platania 1991). Water impoundment and diversion have reduced the historic range of *H. amarus* to a 280 km (174 mi) stretch of river that runs from Cochiti Dam to the headwaters of Elephant Butte Reservoir, approximately seven percent (7 %) of its former range. *H. amarus* is no longer present in the Cochiti reach north of the Angostura diversion Dam (Torres 2007) thus decreasing its range to 141 km, or approximately 3.7 %. Historically, *H. amarus* relied on the floodplain for spawning, however little information is available with respect to trophic interactions during flood events in the MRG.

Stable isotopes

The advent of stable isotope analyses ($\delta^{13}C$ and $\delta^{15}N$) has allowed researchers to identify food sources (DeNiro and Epstein 1978; Vander Zanden and

Rasmussen 2001). Previous studies found that the $^{13}C/^{12}C$ ratio of an organism should reflect that of its food source (DeNiro and Epstein 1978; Hamilton et al. 1992; Thorp et al. 1998; Vander Zanden and Rasmussen 1999, 2001; Jardine et al. 2005) and could be used to trace the flow of energy through the ecosystem as long as the sources have distinct isotopic signatures and are collected in close proximity to the consumers (Thorp et al. 1998). The advantage of stable isotope analyses is that it provides a time-integrated measure of assimilated prey, rather than a list of prey items encountered in the diet at the time of collection (Thorp et al. 1998; Vander Zanden and Rasmussen 1999; Finlay 2001; Herwig et al. 2004).

Trophic fractionation

Trophic fractionation (Δ) is the change of isotope ratios that accompanies digestion and incorporation of consumed matter (Peterson and Fry 1987). Animals typically possess more of the heavier isotopes ^{13}C or ^{15}N than their food (DeNiro and Epstein 1981) and stepwise enrichment occurs with each trophic level in food webs/chains (Minigawa and Wada 1984). Trophic fractionation reported in the literature for $\delta^{13}C$ is approximately 0.48 ‰ and a presumed constant trophic fractionation of $^{15}N/^{14}N$ between predator and prey (usually 2.5–3.4 ‰) for diet–muscle tissue differences allows inferences to be made about feeding interactions and trophic level in food web studies (Minigawa and Wada 1984; Peterson and Fry 1987; Vander Zanden and Rasmussen 1999, 2001).

An important issue for recovery of *H. amarus* populations in the MRG may be food availability, quality, and quantity, particularly during floods. Because feeding habits of *H. amarus* are poorly known (Cowley 2003), it is difficult to assess if changes in the food base have contributed to the decline of the population over time (Cowley 2002). During low flow conditions *H. amarus* is primarily an algivore/herbivore (Magaña 2009; Propst 1999) as suggested by its coiled gut and gut content analysis; however little is known about food resources during flood conditions because hydrodynamic scouring reduces or eliminates known food resources forcing *H. amarus* to forage for other food sources. The 2005 flood pulse in the MRG provided a rare opportunity to investigate resource use on a restored floodplain during flood conditions since hydrodynamic scouring reduces or eliminates benthic food sources in the river proper. This led us to our question; do *H. amarus* shift and adapt different foraging strategies during low flow periods versus high flow periods? The objective of this study was to collect periphyton, aquatic macroinvertebrates, and larval fish and employ stable isotope analyses to identify and track the flow of carbon sources supporting *H. amarus* during a long-term flood pulse on a MRG restored floodplain and verify using paleolimnology techniques.

Methods

In 2001, the U.S. Fish and Wildlife Service (USFWS 2001) concluded that current reservoir management practices in the MRG would likely jeopardize the continued existence of *H. amarus* and developed a habitat rehabilitation plan for the river. Funded through the Middle Rio Grande Endangered Species Act Collaborative Program, the Los Lunas Habitat Restoration Project was initiated in 2002 and is located at (N 34°45′55.1055″ W 106°44′3.2556″) in Los Lunas, NM (Fig. 1). It restored an approximately 1,800 × 100 m area of floodplain (approximately 16 hectares). The goals were to produce floodplain inundation at a wide range of flows from less than 30 up to 70 $m^3\ s^{-1}$ (Slaugh 2003). Discharge data for this study was taken from USGS gage station (08330000) at Central Bridge in Albuquerque, NM. The project was designed to widen the active river channel and improve adjacent riparian habitats by removing burned and nonnative vegetation. Over 50,000 m^3 of material within the former floodplain was moved to produce a heterogeneous topography. Sites were vegetated by the U.S. Army Corps of Engineers (COE) using seed, potted shrubs, or cottonwood (*Populus deltoids*) and willow poles (*Salix exigua* Nutt). Other floodplain modifications included a network of variable depth side and transverse channels designed to aid in egg retention and provide shallow water/low velocity rearing habitat for *H. amarus* and other MRG ichthyofauna (U.S. Bureau of Reclamation 2007).

After two and a half years of monthly monitoring of floodplain hydrology allowed me to identify three distinct habitat types related to flow within the floodplain. These habitats were defined by flow orientation: (1) perpendicular to main stem flow

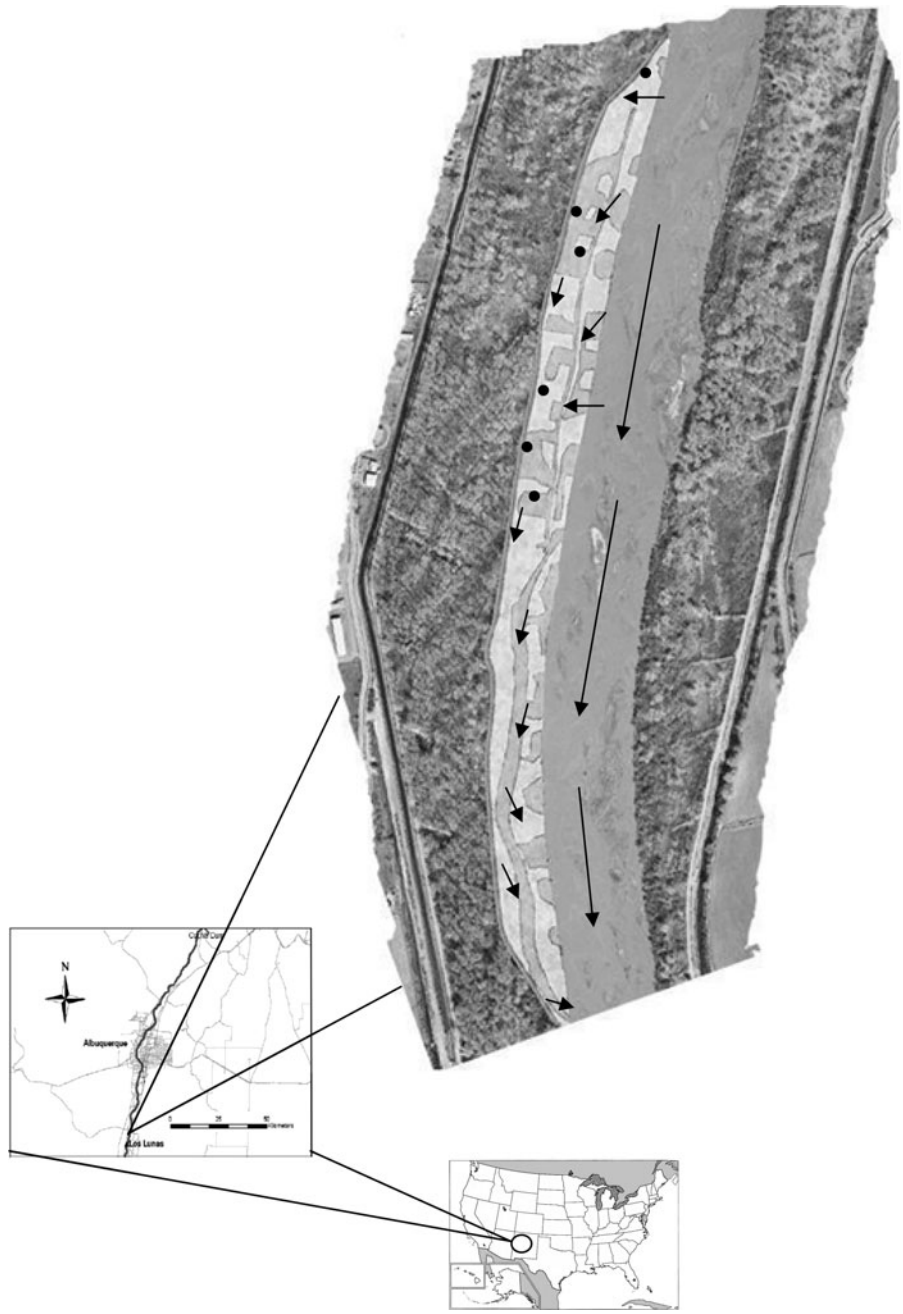

Fig. 1 Middle Rio Grande, New Mexico. Cochiti Dam to Los Lunas, NM. *Inset* Los Lunas Habitat Restoration Project. *Light gray area* (*center-left*) is the restored floodplain showing areas of inundation at >71 and <71 $m^3\ s^{-1}$. *Light gray color* in *center* of picture is the Rio Grande (flowing south). *Dark gray* areas adjacent to river are riparian area. *Black lines* on either side of photo are diversion canals bordered by levees. *Dots* indicate locations of light traps on the floodplain numbered 1–6 north to south. *Arrows* indicate direction of flow on floodplain

(0.06 m s^{-1} vs. main channel 0.8 m s^{-1}), (2) parallel to main stem flow ($\sim$0.11 m s^{-1}), and (3) leeward sides of island where flow was near zero (<0.01 m s^{-1}). I selected three sample sites, one located in each habitat type in the northern half of the floodplain and three sample sites in the southern half of the floodplain. The habitat types were used for larval fish light trap locations as point measurements.

Bayley (1995) reported that annual primary and secondary production in many temperate river systems may depend more on mechanisms occurring during declining flows as opposed to those occurring when the water is rising, therefore; this study was initiated at peak discharge (198 m^3 s^{-1}, 24 May 2005) (Fig. 2) and continued for 44 days during the descending limb of the hydrograph.

Larval fish light traps (Aquatic Research Instruments Inc. 2007, ARII) illuminated with a chemical light stick (Cyalume, Omniglow Corp) were deployed at each of the six sampling sites. All habitats contained native and nonnative terrestrial vegetation with sand/silt substrate. No light trap controls were used as reference sites in the main channel margin since *H. amarus* is rarely collected in deep, high velocity water (Dudley and Platania 1997).

Light traps were deployed overnight, once a week at dusk and retrieved at dawn on 24 May, 1 June, 8, 14, 21, and 28 at six permanent habitat locations during the 44 days of the descending limb of the hydrograph. Light traps were anchored to a metal fence post in water less than 1 m deep (0.39 mean ± 1.9 SE). Upon retrieval, larval fishes were removed from cod-end of the light trap and placed into 250 mL polycarbonate bottles filled with ice water. Alka-Seltzer® tablets were added to euthanize fish via CO_2 narcosis (Wall 1993). Euthanized fish were placed in 5 % buffered formalin for 48 h, transferred to 35 % ethanol for 7 days, and transferred to 70 % ethanol for long-term preservation (Wall 1993; Pease et al. 2006). All larval

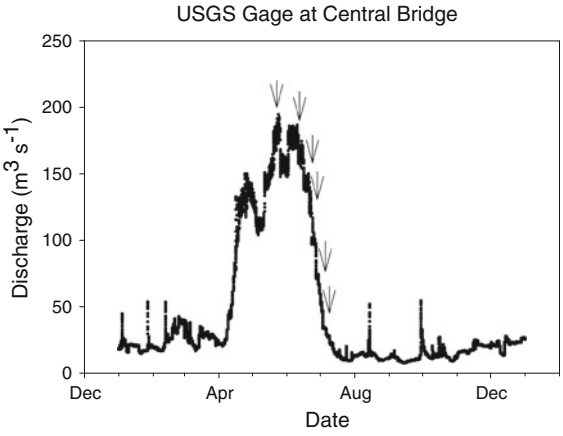

Fig. 2 Hydrograph for the Middle Rio Grande at Central Ave. Bridge, Albuquerque, New Mexico in 2005. *Arrows* indicate approximate sampling dates during descending limb of the hydrograph

fishes were identified and measured for length prior to processing for gut contents or stable isotope analyses.

A subset of *H. amarus* (n = 25, SL 4.6–19.8 mm) were utilized for making microscope slides of gut contents required for diatom identification (Julius et al. 1997). Guts were excised from larger specimens and smaller specimens were used whole. Fish specimens/guts were placed into 20 mL 30 % hydrogen peroxide (H_2O_2) and heated for 30 min. After cooling, 20 mL 70 % nitric acid (NHO_3) was added and heated for 30 min to eliminate organic matter. Digested samples were transferred to 50 mL conical, graduated centrifuge tubes and filled with deionized water (DI) and centrifuged at 1,500 rpm for 10 min. Supernatant was aspirated down to 10 mL, filled to 25 mL with DI, shaken, and filled to 50 mL with DI and centrifuged. The process was repeated seven times until a circumneutral pH was achieved. Permanent slides were prepared from homogenized 1 mL aliquots of samples and micropipetted onto a 22 mm × 22 mm coverslip, illuminated with a 100 W incandescent bulb and allowed to dry. Diatom frustules were identified using keys and descriptions by Krammer and Lange-Bertalot (1999).

Stable carbon and nitrogen isotope ratios (δ13C and δ15 N) were determined to identify intraspecific variance for a subset of all fish species (n = 80) and aquatic macroinvertebrates (n = 122). Samples were dried in a constant temperature oven (Yamato Scientific American Inc. model DKN 810) at 70 °C for 48 h, weighed, and packed into 5 × 9 mm tin capsule. Samples were sent to the UC Davis Stable Isotope Facility for stable isotope analyses ($\delta^{13}C$ and $\delta^{15}N$). Analyses were performed on a Europa Hydra 20/20 continuous flow mass spectrometer. Carbon and nitrogen isotope ratios, defined as parts-per-thousand (per mille or ‰) are the difference between the sample and Pee Dee Belemnite (PDB) standard or atmospheric N_2 (Peterson and Fry 1987).

Deployed larval fish light traps also collected benthic macroinvertebrates concurrently with larval fish. The unique design (ARII) of the larval fish light trap enabled the capture two trophic levels simultaneously. Upon retrieval, invertebrates were also removed from traps and placed into 70 % ethanol. Invertebrates were examined using a Zeiss Stemi 2000-C at 6.5–45× magnification, identified to family or genus, enumerated, and categorized according to Merritt and Cummins (1996). Individual specimens

were acid digested and/or prepared for examination of their gut contents similar to fish. Smaller specimens (e.g. chironomids) were pooled together (five individuals) and digested. Guts were not excised from larger specimens since body tissue and gut contents are highly correlated (Jardine et al. 2005).

Repeated attempts to collect periphyton during the flood pulse proved unsuccessful due to scouring flow and elevated turbidity (Fisher et al. 1982; Rempel et al. 1999). Subsequently, periphyton samples were collected in December 2005 and January 2006 according to USGS National Water Quality Assessment protocol (Moulton et al. 2002) and transported to USDA Forest Service, Rocky Mountain Research Station RMRS for processing. Periphyton samples were digested and prepared for microscopic examination in the same manner as fish and aquatic invertebrate specimens.

During each week of the descending hydrograph water samples (60 mL) were collected during larval fish light trap retrieval and placed on ice, delivered to the University of New Mexico, and analyzed for dissolved nitrate (NO_3–N), soluble reactive phosphorus (PO_4–P) and ammonium (NH_4–N). A multiprobe meter (YSI 556, Yellow Springs Inc.) was used to measure water parameters; temperature, conductivity, dissolved oxygen, percent saturation dissolved oxygen, and pH. Depth was measured to the nearest tenth of a meter using a stadia rod (Crain Enterprises Inc. model # 90370). Water velocity was measured at six–tenths total depth using a flow pressure sensor (Marsh-McBirney Model 2000). Light quanta ($\mu mol\ m^2\ s^{-1}$) were measured at 100 and 170 mm depth using a Li-Cor quantum meter (Li-Cor Biosciences model Li-1000 and a 4π quantum sensor model Li-193SA).

Statistical analyses

Since the experimental design consisted of weekly sampling at six larval fish light trap locations in three habitat types with various associated environmental parameters we employed a negative binomial general linear mixed model with repeated measures (SAS ver. 9.3, GLIMMIX procedure, SAS Institute Inc., Cary, NC) to determine the relationship between fish, stable isotope signatures, environmental variables, and light trap location.

A canonical correspondence analysis (CCA) was employed to visually demonstrate placement of species and stations along environmental gradients reflected onto two dimensions. The CCA is a multivariate analysis technique that directly relates community composition to known variation in the environment (Ter Braak 1986). The CCA biplots suggest environmental gradients where the length of the vector corresponds to the relative rate of change in the environmental gradient. The more a pair of vectors tend to point in the same direction, the more correlated the gradients described by the vectors are. Conversely, vectors pointing in opposite directions from each other suggest negatively correlated gradients. Pairs of vectors in the biplots that approach orthogonality or are perpendicular to each other, suggest independent or uncorrelated environmental gradients. Examples of this would be pH versus NO_3 and pH versus NH_4. Perpendicular projections of species along each vector suggest where species lie along that particular gradient. For example, *Pimephales promelas* abundance has a moderately positive correlation with the pH gradient while *Cyprinus carpio* abundance has a negative correlation with pH. Placement of stations along environmental gradients is interpreted similarly.

Two similarity indices were employed for analyses, the Chao–Jaccard index, weighted by abundance (Chao et al. 2005) and were used for assessing compositional similarity of assemblages based on the presence/absence of species in paired assemblages. The second (dis)similarity index (Dyer 1978) was designed for data sets which involve both multiple species and multiple environmental variables. This (dis)similarity index provides a versatile and convenient tool for quantitatively comparing the species composition of one (1) multispecies sample with another (Dyer 1978).

Results

During the sample dates, deployed larval fish light traps captured 394 individual fish representing four species from the Family Cyprinidae: (*P. promelas*, n = 228, 59 %), *H. amarus*, n = 123, 32 %), *Cyprinella lutrensis*, n = 27, 7 %) and *C. carpio*, n = 16, 2 %) (Fig. 3). The highest captures were obtained in leeward habitats (46 %) followed by perpendicular habitats (33 %) and parallel habitats (21 %). Results from the general linear mixed model (GLIMMIX) among the environmental variables and light trap

locations indicated that only depth and velocity were significantly different. Comparisons showed that depth at LT4 was significantly higher compared to LT2 ($p = 0.02$), and LT3 ($p = 0.003$). Water velocity was higher at LT2 and LT5 and were significantly different from LT1 ($p = 0.04$), LT3 and LT6 ($p = 0.02$). Phosphorus was the only nutrient found to be significantly different between north habitats and south habitats with concentrations highest at LT3 ($p < 0.05$).

The results from the CCA show that environmental variables NH_4 (ammonium), DO (dissolved oxygen), and PO_4 (phosphorus) had a strong positive correlation while NO_3 was negatively correlated to these three variables. The variable pH was not correlated to any other environmental variable. Observed correlations for abundance of fish species and variables NH_4, DO, and PO_4 were moderately positively correlated to *P. promelas* and *C. lutrensis*, neutral to *H. amarus* and negatively correlated to *C. carpio*. The variable NO_3 was moderately positively correlated to *C. carpio*, slightly positively correlated to *P. promelas*, neutral with *H. amarus*, and moderately negatively correlated to *C. lutrensis* (Table 1; Fig. 4).

Results from Chao–Jaccard index indicated that relative contribution to fish assemblage, weighted on abundance, was highest for *P. promelas* at 64 % followed by *H. amarus* 33 %. Results from (dis)similarity analysis (Dyer 1978) reveal that species composition among habitats, dates, and environmental variables was highest for *H. amarus* at 42 % followed by *P. promelas* 40 %. *C. lutrensis* and *C. carpio* represented 9.5 and 8.5 % (Table 2).

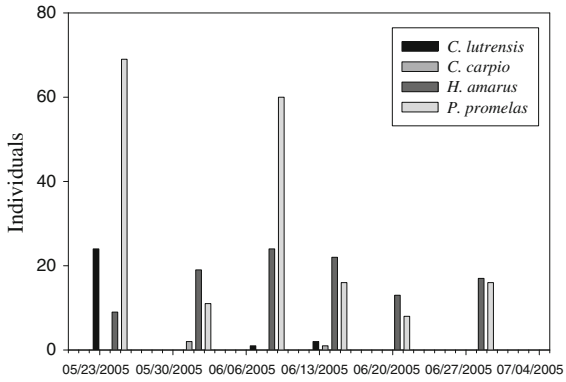

Fig. 3 Larval fish captures at Los Lunas restoration site during sampling period

Fish gut contents

Acid digested gut contents of *H. amarus* (n = 25) were processed to a final volume of 50 mL. Microscopic examination (1,000×) of gut content microscope slides revealed that each 1 mL aliquot yielded between 0 and 8 diatom valves per fish (3.33 mean ± 0.48 SE). A diatom is composed of two over-lapping halves (similar to a Petri dish) called valves. Eighteen (72 %) of the microscope slides contained no diatom valves indicating that diatoms were either not a significant food source or were not available during the flood pulse. Gut contents of *H. amarus* larvae collected in 2004 at the Los Lunas site revealed that diatoms were a main component of their diet. A total of 13 genera and 15 species of diatoms were identified from 2004 *H. amarus* larvae (Table 3).

Fish stable isotopes

Formalin fixation and ethanol preservation affect carbon and nitrogen isotopic signatures in fish tissue in a predictable manner (Edwards et al. 2002). Carbon isotope ratios from white muscle tissue are depleted by 1.1 ‰ and nitrogen isotope ratios are enriched by 0.5 ‰ when averaged across Rio Grande ichthyofauna (Edwards et al. 2002). Results for carbon isotope ratios for larval fish were corrected by adding 1.1 ‰ to observed $\delta^{13}C$ and by subtracting 0.5 ‰ from observed $\delta^{15}N$. The mean isotopic data showed high intraspecific variability in $\delta^{13}C$ up to 6.51 ‰ for *H. amarus* (−24.9 to −18.4 ‰). *C. lutrensis* had the narrowest range of $\delta^{13}C$ at 3.9 ‰ (−21.5 to −17.6 ‰). *P. promelas* (−22.46 to −18.05 ‰) and *C. carpio* (−23.42 to −17.44 ‰) had $\delta^{13}C$ values intermediate to *H. amarus* and *C. lutrensis*. *H. amarus* captured in parallel habitats revealed two distinct isotopic groups (Fig. 5a); one group fed on periphyton (−18 to −16.5 ‰), while the other consumed aquatic macroinvertebrates (−23 to −20.5 ‰). *H. amarus* captured in parallel habitats had a wider range of $\delta^{13}C$ values (−22.65 to −16.45 ‰) than perpendicular or leeward habitats ($p = 0.02$). Watson et al. (2009) reported that "small and medium fish consumed a greater variety of foods compared to large fish, suggesting perhaps a more generalist feeding behavior in small and medium fish". Perpendicular and leeward locations of the floodplain had similar $\delta^{13}C$ values (−23.97 to −18.57 ‰), but were less enriched in $\delta^{13}C$

Table 1 CCA maximum values for species, site, and environmental parameters

Site	*Cyp-lut*	*Hyb-ama*	*Pim-pro*	*Cyp-car*	%DO	pH	NO_3	PO_4	NH4
1	1.00	6.00	43.00	0.00	77.58	8.17	148.25	65.50	20.00
2	0.00	3.00	1.50	1.00	82.54	8.09	132.40	62.40	32.00
3	24.00	7.33	22.75	0.00	85.02	8.17	112.83	88.17	63.33
4	0.00	2.67	3.50	1.00	82.77	8.08	154.67	89.67	40.00
5	1.00	15.00	2.00	1.50	76.48	8.01	120.25	59.50	42.50
6	0.00	3.50	1.50	7.00	77.88	7.96	153.20	61.60	30.00

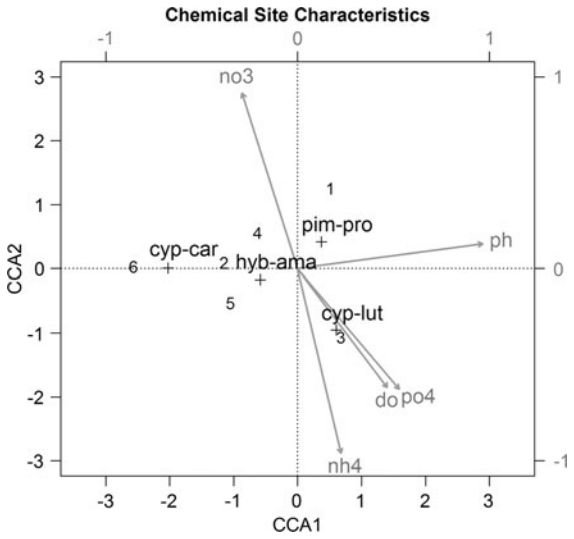

Fig. 4 CCA biplot for chemical site characteristics. cyp_car = *Cyprinus carpio*, hyb_ama = *Hybognathus amarus*, pim_pro = *Pimephales promelas*, and cyp_lut = *Cyprinella lutrensis*, NO_3 = nitrate, NH_4 = ammonium, DO = dissolved oxygen, and PO_4 = phosphate

than parallel habitats ($p = 0.03$). *H. amarus* larvae captured in leeward locations of the floodplain had the narrowest range of $\delta^{15}N$ values (10.41–7.66 ‰) followed by perpendicular locations (11.71–6.65 ‰). *H. amarus* captured in parallel locations had the widest range of $\delta^{15}N$ values (14.12–7.43 ‰) suggesting a broader selection of food sources than other habitats than either perpendicular or leeward habitats (Fig. 5b).

Aquatic invertebrates

Eleven orders, 14 families, and 17 genera (n = 1,311) of aquatic macroinvertebrates were captured during the May–June, 2005 sampling period. Results from Chao–Jaccard similarity, weighted by abundance, revealed that relative contribution to the macroinvertebrate community was highest for Corixidae at 37 % followed by Chironomidae and *Baetis* at 28 and 25 %, respectively. Results from the (dis)similarity index (Dyer 1978) showed that species composition among habitats, dates, and environmental variables was highest for Corixidae at 29 % with *Baetis* and Chironomidae representing 19 and 14 %, respectively (Table 4). These three families comprised 82 % of the benthic invertebrate community during the entire sampling period. During the first four sampling dates, the aquatic invertebrate community was dominated by collector/gatherers (45–80 %) followed by predators, which comprised approximately (15–50 %) of the population.

Table 2 Captured cyprinid fishes at the restored Los Lunas floodplain

Species	n	%	Chao–Jaccard contribution (%)	Similarity (%)
P. promelas	228	59	64	40.7
H. amarus	123	32	33.3	42.1
C. lutrensis	27	7	3.6	6.4
C. carpio	16	2	0.1	7.4
	394	100		

Genus, species, quantity, and similarity coefficients for larval fish collected during sampling period (24 May–28 June, 2005)

Table 3 USFWS Rio Grande silvery minnow (*H. amarus*) rescue/salvage

Years	*H. amarus* rescued/salvaged
2001	240
2002	3,662
2003	173
2004	12,865
2005	626,444
2006	62,889

The year 2005 is bold to indicate flood and sampling year

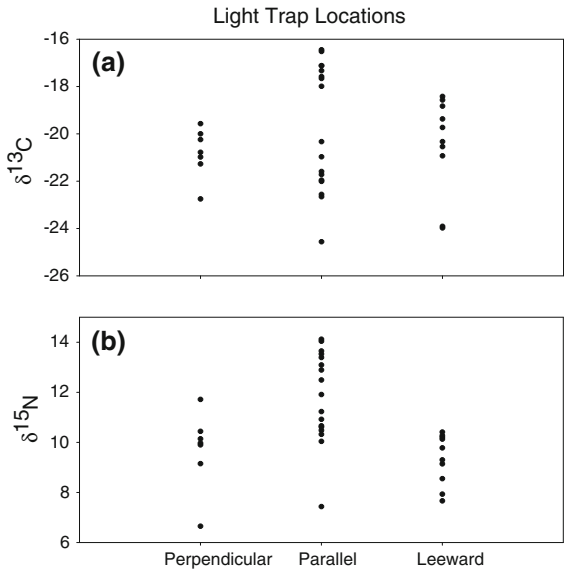

Fig. 5 **a** Parallel habitats were significant different from perpendicular and leeward habitats ($p = 0.03$ and $p = 0.02$, respectively). $\delta^{13}C$ values for *H. amarus* captured in perpendicular, parallel, and leeward locations were not significantly different ($p = 0.99$). Two distinct groups are identifiable at the parallel habitats. One group fed upon algae (−18 to −16 ‰) while the other group consumed macroinvertebrates (−23 to −20 ‰). **b** *H. amarus* captured in leeward locations of the floodplain had the narrowest range of $\delta^{15}N$ values (10.41–7.66 ‰) followed by perpendicular locations (11.71–6.65 ‰). *H. amarus* captured in parallel locations had the widest range of $\delta^{15}N$ values (14.12–7.43 ‰)

Predators (80–100 %) dominated the community structure during the final 3 weeks of the sampling period (Fig. 6).

Invertebrate gut content analysis

Acid digested gut contents of aquatic invertebrates (n = 35) were processed to a final volume of 50 mL. Microscopic examination (1,000×) of gut content of the gastropod, Physidae *Physella* sp., revealed that gut contents consisted entirely of diatom valves of *Cocconeis placentula*. These results agree with other studies that have demonstrated invertebrates will selectively consume/assimilate predominantly one food type (DeNiro and Epstein 1978). The only other aquatic invertebrate identified to consume diatoms was the scraper, Heptageniidae *Heptogenia* sp., which was found to consume the diatoms *Navicula capitotoradiata* and *Gyrosigma acuminate*.

Table 4 Results for relative contribution of Chao–Jaccard and similarity indices for aquatic invertebrates collected during sampling period

Order/family/genus	Chao–Jaccard (%)	Similarity (%)
Hemiptera/Corixidae/ Graptocorixa	37.41	28.55
Diptera/Chronimidae	27.98	14.37
Ephemeroptera/Baetidae/Baetis	24.76	18.55
Ephemeroptera/Baetidae/ Acentrella	5.32	5.93
Tricoptera/Leptoceridae/ Nectopsyche	1.72	6.26
Ephemeroptera/Heptagenaidae/ Heptogenia	1.21	3.45
Copepoda	0.76	3.67
Ephemeroptera/Baetidae/ Centroptilum	0.67	2.58
Odonata/Gomphidae/Gomphus	0.12	3.66
Diptera/Chronimidae/pupae	0.03	0.68
Tricoptera/Pupae	0	0.59
Tricoptera/Hydropsycheidae/ Hydropsyche	0	1.37
Odonata/Gomphidae/Stylurus	0	2.74
Odonata/Gomphidae/ Ophigomphus	0	0.88
Odonata/Gomphidae/ Erpetogomphus	0	0.69
Odonata/Coengrionidae/ Enallagma	0	0.95
Hemiptera/Simulidae	0	0.13
Hemiptera/Simulidae/Pupae	0	0
Gastropoda/Physidae/Physella	0	0
Ephemeroptera/Leptohyphidae/ Tricorythodes	0	0.05
Ephemeroptera/Isonychiidae/ Isonychia	0	0.48
Ephemeroptera/Caenidae/ Brachycerus	0	0.29
Ephemeroptera/Baetidae	0	3.81
Diptera/Chronimidae/Midge	0	0.16
Coleoptera/Dytiscidae/ Hydroporous	0	0.16

The Chao–Jaccard, is a Jaccard coefficient weighted by abundance and percent similarity was used for quantitatively comparing the species composition of a multi-species sample with another

Aquatic invertebrate stable isotopes

In the present study, the $\delta^{13}C$ and $\delta^{15}N$ signatures of aquatic macroinvertebrates (n = 122) were highly

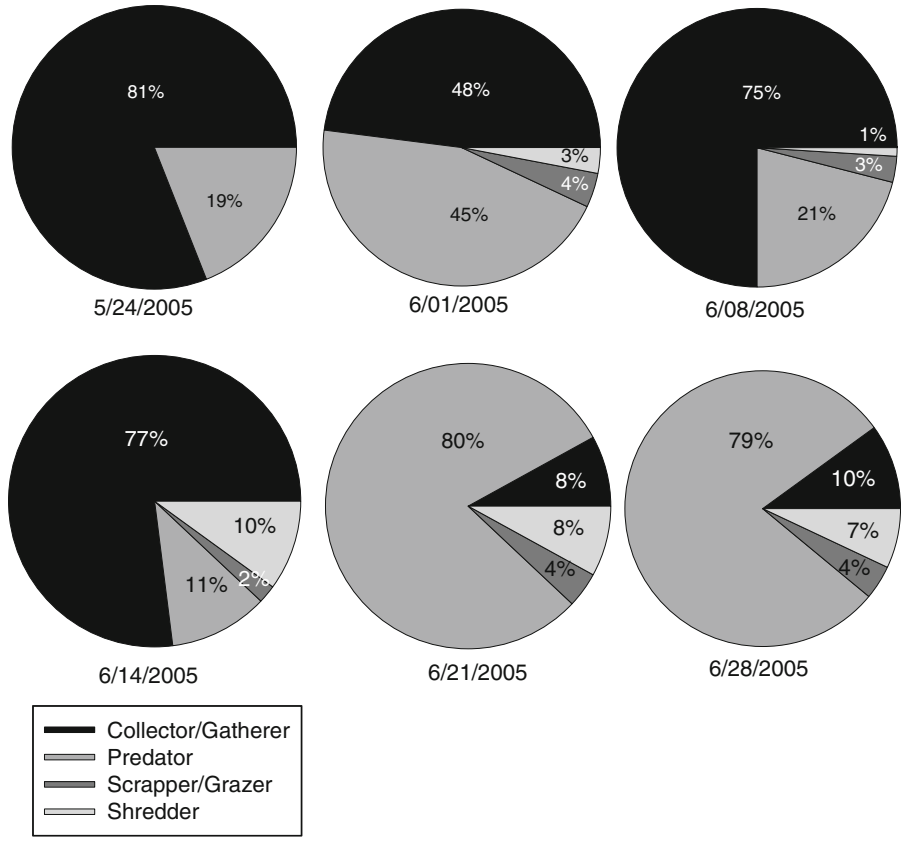

Fig. 6 Change in aquatic invertebrate community composition during the six sampling dates

variable; however, their isotopic values were consistently less enriched in carbon and nitrogen than fish species. Chironomids and *Graptocorixa sp*. displayed the widest range in $\delta^{13}C$ (−26.89 to −18.93 and −27.1 to −16.6 ‰ respectively), while filterer/collectors displayed the narrowest range in $\delta^{13}C$ and $\delta^{15}N$ values (−24.52 to −23.92 and 5.05–6.55 ‰ respectively). The $\delta^{13}C$ values for predatory insects were very uniform (−22.89 to −22.17) indicating selectivity.

Graphing stable isotope values for MRG invertebrates and fishes shows the ideal step-wise increase from one trophic level to the next (Fig. 7). Results indicate two possible food sources for *H. amarus* given that they fall within the expected trophic fractionation range. The two candidates were chironomids which had a $\Delta\ ^{13}C$ of 0.48 ‰ and a $\Delta\ ^{15}N$ of 2.78 ‰ and predators 0.76 and 2.21 ‰ respectively. These values were closest to the mean trophic fractionation values reported by Vander Zanden and Rasmussen (1999) and Herwig et al. (2004). Trophic fractionation (Δ) of possible food sources for *H. amarus* were calculated by subtracting measured $\delta^{13}C$ and $\delta^{15}N$ values of prey from measured *H. amarus* δ values (Cabana and Rasmussen 1996; Post 2002).

Discussion

The above average snowfall in 2004/2005 and subsequent spring runoff allowed for the unique opportunity to investigate resource use by MRG ichthyofauna during a long-term flood pulse since the last overbank flood occurred in 1993 (Taylor et al. 1999). Results from stable isotope analyses and paleolimnology techniques confirm that *H. amarus* shifts foraging strategies and isotopic values reflect the food resource consumed during varying hydrodynamic conditions. The observed stable isotope results show the closest association between energy sources and fish were aquatic invertebrates. Average isotopic values for aquatic invertebrates were consistently less enriched in $\delta^{13}C$ and $\delta^{15}N$ than fish indicating aquatic

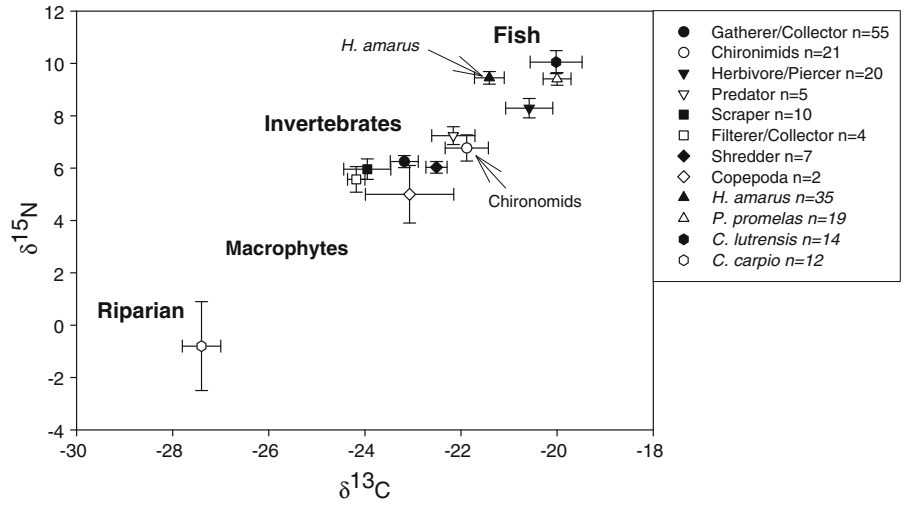

Fig. 7 Mean and SE of stable isotope results for Middle Rio Grande fish, invertebrates, and riparian vegetation. *Arrows* point to predator (*H. amarus* 2005) and prey (chironomids)

invertebrates as a possible food source for MRG cyprinid fishes.

The $\delta^{13}C$ isotopic signatures from other studies reveal wide variability. Fish $\delta^{13}C$ values in North American temperate river ranged from −31 to −26 ‰ (Rosenfeld and Roff 1992) and −26 to −23 ‰ (Thorp et al. 1998). Temperate lake fish $\delta^{13}C$ values ranged from −31 to −26.8 (Keough et al. 1996). Los Lunas *H. amarus* $\delta^{13}C$ values from this study were less depleted in $\delta^{13}C$ compared with *H. amarus* from Bosque del Apache in southern New Mexico reported by Pease et al. (2006) (−22.54 to −18.5 and −24.9 to −18.4 ‰ respectively). While it is not unusual for isotopic signatures to vary from river to river and lake to lake it is surprising to find wide variability in $\delta^{13}C$ values of *H. amarus* specimens that were separated by less than 100 m. Intraspecific variability in $\delta^{13}C$ was likely due to discharge and/or habitat type.

There are two food items reported here as possible candidates (chironomids and predators) as food sources for *H. amarus* given that they fall within the range. The $\Delta\ \delta^{13}C$ for chironomids to *H. amarus* (0.48 ‰) is similar to the overall $\Delta\ ^{13}C$ mean value (0.47 ‰) reported by Vander Zanden and Rasmussen (1999). *H. amarus* possess a small, toothless, subterminal mouth, and are morphologically incapable of consuming large invertebrates that possess protruding appendages such as Odonates. Results from stable isotope analysis indicate that chironomids are a likely food source for *H. amarus*. Chironomids are small, worm-like organisms that can easily be consumed whole by *H. amarus* and are ubiquitous and abundant food source found in the MRG (Weibell 2007). There is anecdotal information of *H. amarus* consuming macroinvertebrate appendages, but this is probably due to incidental ingestion (Watson et al. 2009).

Despite the fact that I was limited to 1 year of data from the present study, the results combined with previous USFWS Rescue and Salvage Operations (Smith 2001; Smith and Munoz 2002; Smith and Basham 2003) provide further evidence that *H. amarus* has evolved a life-history strategy that enables the species to quickly colonize, spawn, and grow in recently inundated areas (sensu Bayley 1995). The inundation of this former floodplain increased recruitment of *H. amarus* as well as other ichthyofauna in the MRG during 2005 contradicting King et al. (2003) that few studies have recorded larvae or juveniles using temporary floodplain habitats. Others question whether fish in arid-zone rivers are adapted to take advantage of irregular flooding, and whether such floods promote fish production and floodplain energy subsidies (Bayley 1995, Balcombe et al. 2007). The results from the Los Lunas floodplain provide ample evidence that *H. amarus* as well as other MRG ichthyofauna do make use of temporary floodplains where habitats are shallow with lower water velocities. The results of this study follow the predictions of the Flood Pulse Concept (Junk et al. 1989) which described floods as "the principal driving force

responsible for the existence, productivity, and interactions of the major biota in river-floodplain systems". *H. amarus* and other ichthyofauna in the MRG use the flood pulse as an environmental cue for spawning during the rising limb of the hydrograph, but since flood pulses in the MRG are too short lived (1–5 days) (M. Porter (USBOR), personal communication) they are likely not favorable for successful spawning for MRG species. The 2005 flood pulse release provided for additional floodplain habitats within the MRG. The increased amplitude (2.8 vs. 150 m^3 s^{-1}) and duration (1–5 vs. 100 days) of the flood pulse (Fig. 8) provided more nursery habitat for riverine fauna which translated to a substantial increase in animal biomass (Fig. 9).

The channelization of the Rio Grande has reduced or eliminated most backwaters, edge areas, and slow-water refugia which are vital habitat for benthic algae and nursery habitat for MRG ichthyofauna. It is our suggestion that the duration and amplitude of the "naturalized flow" regime be increased by federal and local management to provide low velocity, shallow nursery habitats to provide sufficient time to grow to the juvenile stage thereby increasing their likelihood of reaching sexual maturity. This study reveals the importance of floodplains to the MRG ichthyofauna by providing shallow, low-flow habitats for spawning, nurseries, and recruitment. As stated in Junk et al. (1989) the lateral transfer of energy from the floodplain to the river is important to regulated rivers like the MRG.

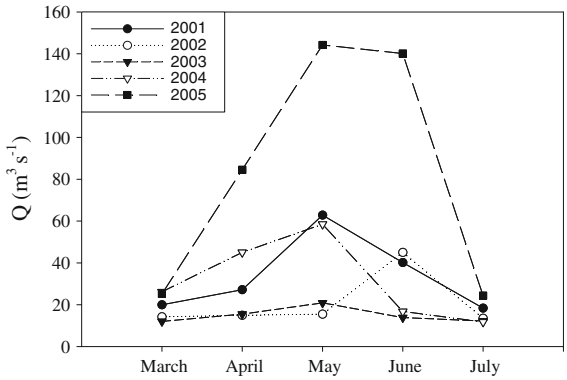

Fig. 8 Monthly discharge during flood pulse releases on MRG for the preceding four years. The year 2005 shows a much longer duration and magnitude of flood pulse compared to previous years. Sampling began on 24 May 2005 and continued during descending limb of hydrograph

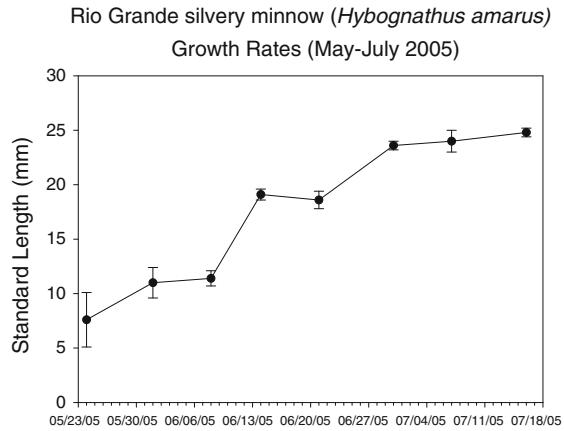

Fig. 9 Growth rate of *H. amarus* during sampling period

Generally, the stable isotope data from the Los Lunas site is irrefutable that *H. amarus* uses both direct autochthonous and allochthonous carbon sources during flood pulses. It is clear that terrestrial C_3 plants are a likely food source for aquatic invertebrates when algae are limited. This is similar to the conclusions of Araujo-Lima et al. (1986). The step-wise increase from riparian leaves (Tibbets 2005) to macroinvertebrates is too great both in $\delta^{13}C$ and $\delta^{15}N$, and therefore it is likely that macrophytes with a $\delta^{13}C$ value of approximately −26.5 to −24.5 ‰ and a $\delta^{15}N$ value of approximately 2–3.5 ‰ (Edwards and Turner 2003) probably provide carbon to aquatic macroinvertebrates. The Flood Pulse Concept (Junk et al. 1989) adequately addresses food web structure for this restored arid-land floodplain. It seems likely that a combination of an autochthonous/allochthonous resource theories (Junk et al. 1989; Thorp and Delong 1994) best describes the nutrient and energy transfers for the Los Lunas restored floodplain. In lieu of periphyton data, I propose that in a light-limited system like the MRG, indirect leaf litter input from riparian zone compensates for the decreased autochthonous primary production during floods. These conclusions are based on stable isotope analyses of potential sources of organic matter and various functional feeding groups of invertebrates and fish consumers. The results show a larger role for allochthonous inputs during the flood pulse in 2005 than other studies (Araujo-Lima et al. 1986; Hamilton et al. 1992; Hamilton and Lewis 1992; Thorp and Delong 2002), which emphasizes the primary role of autotrophic production in large rivers.

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