A Case for Classifying the Rio Grande Silvery Minnow (Hybognathus amarus) as an Omnivore

by

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DEDICATION

I dedicate this body of work to my children, Alexis and Emilio who have been my inspiration and motivation throughout my educational career.

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A case for classifying the Rio Grande silver minnow (*Hybognathus amarus*) as an omnivore

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ABSTRACT

The Rio Grande has been identified as one of the most endangered rivers in the United States by American Rivers. Water impoundment, water extraction, and point-source pollution have likely contributed to the decline of the federally endangered Rio Grande silvery minnow (*Hybognathus amarus*). The overall goal of this study was to locate, identify, and characterize food resources for *H. amarus* and the ichthyofauna of the Middle Rio Grande (MRG). After locating possible food resources (chapter 1) A single diatom cell was isolated from a mixed environmental collection and grown in monoculture. Unialgal cultures were used to assess *H. amarus* diatom preferences and conditioning response (chapter 2). An extended flood-pulse release from Cochiti Reservoir allowed me to investigate food resource usage during a flood event. Trophic interactions between fish, aquatic invertebrates, and periphyton were identified using stable isotope analyses ($\delta^{13}C$ and $\delta^{15}N$) and paleolimnology techniques (chapter three).

Results from chapter 1 suggest that point-source pollution from the Rio Rancho, NM and Albuquerque, NM wastewater treatment plants have influenced seasonal diatom distribution south of outfalls. Results also indicate that a mismatch exists between peak algal biomass (represented by chlorophyll content) and the historic *H. amarus* spawning period in the MRG. Decreased food availability during this critical life history stage when larval fish switch from endogenous to exogenous food sources may help explain why *H. amarus* populations have been in rapid decline since construction of Cochiti reservoir.

Results from chapter 2 indicate that *Nitzschia palea* and *N. paleaformis* were the overall preferred diatoms of *H. amarus* in the feeding trials. I have shown in this study that *H. amarus* can be conditioned to respond to food stimuli. Large-scale training of foraging skills is feasible, relatively simple, and inexpensive to initiate. Using social learning protocols to train *H. amarus* en masse prior to release and may help to increase their survivability in the wild.

H. amarus has been classified by various investigators as an herbivore, detritivore, or carnivore. During low flow conditions *H. amarus* is primarily an algivore as previously reported. However, during flood conditions, hydrodynamic scouring eliminates or reduces the benthic algal food source. Therefore, *H. amarus* makes use of other locally abundant food sources, primarily chironimids, during and immediately after floods. Results from this study suggest that *H. amarus* is an opportunistic feeder and should be classified as an omnivore.

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Introduction

The impetus for this dissertation study is the plight of the federally endangered Rio Grande silvery minnow (*Hybognathus amarus*) an endemic, small-bodied, cyprinid fish. *H. amarus* was historically the most abundant fish in the Rio Grande Basin, Pecos River drainage, and coastal drainages of Texas from the Brazos River drainage west to the Rio Grande Basin of New Mexico (Sublette et al. 1990). Unfortunately, *H. amarus* now occupies only 5% of its historic range (Bestgen and Platania 1991). Many probable causes for the decline of *H. amarus* populations over the past century have been identified including water impoundment, water diversion, drought, timber harvest, invasive or introduced fish species, and cattle grazing.

Riverine nutrient cycling and the effect on primary production in the Middle Rio Grande (MRG) have not been thoroughly investigated and remain undocumented. A variety of physical, chemical, and biological stimuli affect the potential environment for aquatic primary producers, which in turn affect potential food resources for *H. amarus*. Despite extensive recovery efforts one plausible cause for the species decline which has received little attention is seasonal nutrient availability and its relationship to food resources.

An essential issue for long-term recovery of *H. amarus* populations in the MRG may be food; including availability, quality, and quantity. The MRG is a vital river reach for *H. amarus*, but habitat has been lost primarily due to water impoundment and diversions. Wastewater discharge may be another factor influencing *H. amarus* food supplies. I investigated spatial and temporal diatom composition during 2005 in the MRG north and

south of Albuquerque, NM to locate and identify potential food resources for *H. amarus* (chapter one). *H. amarus* has been classified as an algivore (Shirey et al. 2007), an herbivore (Propst 1999) and carnivore (Pease et al. 2006). Minnows feed on diatoms, algae, larval insect exuvia, partially decayed organic matter, and plant material scraped from "ooze" in bottom sediment (Starrett 1950, Whitaker 1977). Etnier and Starnes (1993) and Ross (2001) suggested that adults of the genus *Hybognathus* are obligate herbivores because they lack a defined stomach and instead have a long, narrow and coiled alimentary tract. My objectives were to: 1) assess spatial and temporal nutrient distribution, 2) identify seasonal distribution and species composition of diatom communities, 3) evaluate effects of treated wastewater discharge on diatom communities, and 4) examine interactions between nutrient availability, the proliferation of diatoms, and the spawning and growing season of *H. amarus*.

Although the biology and feeding habits of *H. amarus* are poorly understood, some studies show that *H. amarus* consume diatoms (Shirey 2004 and Cowley et al. 2006). I investigated the diatom preference of *H. amarus* by examining food consumption (chapter two). *H. amarus* protolarvae, mesolarvae, and metalarvae used in feeding trials were provided by the Albuquerque, NM BioPark. Feeding trials were videotaped to assess food awareness, establish substrate preference (fine-grain sediment or coarse sand), reveal diatom preference among 15 diatom species, determine peak diatom sampling (tasting/feeding), and confirm *H. amarus* conditioning response. Multi-species periphyton samples were collected from the Middle Rio Grande and transported to the U.S.D.A. Forest Service, Rocky Mountain Research Station, Albuquerque, NM for

isolation and culturing. Diatoms have various growth forms, upright (erect); adhesive prostrate or gliding prostrate (Kawamura 2004). I successfully cultivated 15 unialgal cultures with varying growth forms for six diatom preference feeding trials.

In the southwestern U.S., virtually the entire native river fish fauna is listed as threatened under the Endangered Species Act, largely as a consequence of water withdrawal, flow stabilization, and exotic species proliferation (Poff et al. 1997). I investigated resource use by *H. amarus* in a restored floodplain during a prolonged (~100 days) flood pulse release from Cochiti reservoir (chapter three). Dams and diversion dams on the Rio Grande have fragmented the MRG into distinct reaches and arrested most overbank flooding in the MRG, with the last major flood occurring in 1941-1942 (Molles et al. 1998). Much of the floodplain has become abandoned through degradation of the channel bed and the building of levees (Massong et al. 2006).

I used stable isotope analyses and paleolimnology techniques to identify carbon sources of *H. amarus* in a restored floodplain. The advent of stable isotope analyses has allowed researchers to identify food sources and trophic position of organisms in aquatic habitats globally. However, stable isotope analyses ($\delta^{13}C$ and $\delta^{15}N$) have scarcely been used to study trophic interactions in the important rivers of the Southwest. Researchers found that the $^{13}C/^{12}C$ ratio of an organism should reflect that of its food source 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. In this study, stable isotope ratios were measured to identify food sources and characterize

trophic interactions between fishes and invertebrates in a restored floodplain. The primary goal of this study was to identify and analyze food resources in order to correctly classify the trophic position of *H. amarus*.

CHAPTER 1: Spatial and Temporal Distribution of Diatoms in the Middle Rio Grande, New Mexico, U.S.A.

Introduction

Seasonal diatom community composition was studied during 2005 in the Middle Rio Grande (MRG) north and south of Albuquerque, New Mexico. The objective was to locate and identify potential food resource changes for the federally endangered Rio Grande silvery minnow (*Hybognathus amarus*). Currently, *H. amarus* now only occupies approximately 5% of its historic range (Bestgen and Platania 1991) resulting in an endangered status listing by the U.S. Fish and Wildlife Service (1994). Many probable causes for the decline of *H. amarus* population numbers over the past century have been identified including water impoundment, water diversion, drought, timber harvest, invasive or introduced fish species, and cattle grazing. One plausible cause for the species decline, which has received little attention, is seasonal nutrient availability and its relationship to the spatial and temporal distribution of diatoms.

Minnows feed on diatoms, other algae, larval insect exuvia, and plant material scraped from "ooze" in bottom sediment (Starrett 1950, Whitaker 1977). Etnier and Starnes (1993) and Ross (2001) suggest that adults of the genus *Hybognathus* are obligate herbivores because they lack a defined stomach, have a black peritoneum, and a long, narrow, and coiled alimentary tract. Studies have shown that *H. amarus* consume diatoms as a food resource (Shirey 2004, Cowley et al. 2006, Magaña, Chapter 2, this publication). Shirey (2004) examined and quantified gut contents of *H. amarus*

specimens collected in 1874 near San Idelfonso, NM. Examination of those specimens indicated that *H. amarus* fed on 30 genera and 70 species of diatoms.

Algae have been identified as a food resource for *H. amarus*, however, primary production in the MRG is limited by turbidity, light attenuation, and nutrient concentrations (Vannote et al. 1980, Mulholland et al. 1995, Stelzer and Lamberti 2001, Dodds et al. 2002, Thorp and Delong 2002). Turbidity in the MRG is continually high (20-1200 NTU) (David Van Horn, University of New Mexico). Low light levels restrict algal growth to the shallow margins of the river and sand bars where light penetration is greater (Anderholm et al. 1995). Generally, periphyton in the MRG grows along river and sandbar margins, and this band of algae is a likely limiting primary food source for *H. amarus*.

The Rio Grande is influenced by non-point source pollution primarily from urban runoff and point source pollution from agriculture and wastewater treatment plants (WWTP) that create a nutrient gradient downstream of returns and outfalls. Moore and Anderholm (2002) linked an increase in nutrient concentration in the MRG to discharge from municipal WWTP, and Passell et al. (2005) reported that the chemical form of nitrogen discharged to the Rio Grande has shifted from ammonium and organic nitrogen to nitrite and nitrate during the years 1975-1999. Nitrate, ammonium, and soluble reactive phosphorus are often the primary limiting nutrients for aquatic primary productivity (Dodds et al. 2002). Testing hypotheses regarding correlations between nutrient availability and the effects of treated municipal wastewater discharged into the MRG and

interactions with primary producers and *H. amarus* are needed for a better understanding of *H amarus* survival.

Cushing (1990) theorized that the reason for variability in recruitment of oceanic pelagic fish was due to the match/mismatch hypothesis. The match/mismatch hypothesis consists of two parts: (1) fish in temperate waters spawn at a fixed time, and (2) the fish larvae are released during the spring or autumn peaks in plankton production cycle, when more food is available. In this study, I explore the usefulness of the match/mismatch hypothesis to the MRG and *H. amarus* to identify a potential match/mismatch between food availability and spawning periodicity.

My objectives were to 1) assess spatial and temporal nutrient availability in the MRG, 2) identify seasonal distribution and composition of diatom communities in the MRG, 3) evaluate the effects of treated wastewater discharge on diatom communities, and 4) examine interactions between nutrient availability, the proliferation of diatoms, and the historical spawning and growth period of *H. amarus*.

Methods

Study sites

Seasonal sampling in late winter (February 22), late spring (June 16), summer (August 8), and winter (December 15) was conducted at four MRG reaches (Angostura, Bernalillo, Rio Bravo, and Shirk). These sites were established as a subset of potential sites for the Middle Rio Grande, New Mexico (Fig. 1). The Angostura and Bernalillo sites are located approximately 35 and 21 km north of Albuquerque, NM, respectively. The

diversion dam at Angostura constrains the Rio Grande to less than 50 m in width. The floodplain is elevated ~3 m above the river surface on the west bank and does not receive overbank flooding. The river margin on the east bank runs along an alluvial island bordered with stream-side vegetation. The substrate consists of large cobble, gravel, and sand. At Bernalillo, the floodplain on the west bank is elevated approximately 1.5-2.0 m above the river surface. The floodplain here is ~120 m distant from the active channel. The substrate at this reach consists of sand and cobble. The Angostura and Bernalillo sites are minimally influenced by agricultural or wastewater pollutants.

The Rio Bravo site is located within Albuquerque city limits and 30 km downstream from the Bernalillo site. The Shirk site is located six km south of the Albuquerque wastewater treatment plant (AWWTP). These sites differ geomorphologically from the northern sites. The Rio Grande is much wider (~150 m), shallower, and the floodplain is elevated less than 1 m above the river surface. The river substrate consists of fine-grain sediment and coarse-grain sand. Both sites have riparian vegetation up to the river margin. Non-native salt cedar (*Tamarix ramosissima*) and Russian olive (*Elaeagnus angustifolia*) dominate the river margin at the Rio Bravo and Shirk site. To minimize influence of the AWWTP, the Rio Bravo reach terminated 50 m north of the outfall.

To maintain uniformity, sampling was conducted on the east bank of the river and each location was partitioned into five 100 m subreaches. Sampling was conducted using a modified version of the U.S. Geological Survey revised protocol for sampling algal, invertebrate, and fish communities as part of the National Water Quality Assessment

Program (Moulton et al. 2002). There are three possible types of quantitative algal sampling; richest-targeted habitat, depositional-targeted habitat (DTH), and phytoplankton (Moulton et al. 2002). I selected the DTH sampling method because the habitat targeted for sampling each reach is where fine-grained silt and coarse-grained sand provide substrate for epipelic and episammic diatoms.

Nutrients

In this paper, I report dissolved inorganic nitrogen concentrations as NO_3-N, nitrogen concentrations derived from ammonium as NH_4-N, and soluble reactive phosphorus as PO_4-P in accordance with standard methods (American Public Health Association 1998, Wetzel 2001). Water samples were collected during a range of flow regimes in the MRG (Fig. 2). Water for nutrient analyses were collected in triplicate using three connected, bottomless, five-gallon buckets placed at the river margin. Episammic and epipelic algal samples were collected within each bucket using a 100 mm x 15 mm Petri-dish and spatula (Moulton et al. 2002). Water and algal samples were placed on ice and transported to the U.S.D.A. Forest Service, Rocky Mountain Research Station, Albuquerque, NM. Water samples were analyzed at the University of New Mexico in the Department of Biology for dissolved nitrate (NO_3-N) and soluble reactive phosphorus (PO_4-P) on a Dionex-500 Ion Chromatograph (Dionex, Sunnyvale, CA) using standard method 4110 B (American Public Health Association 1998) and 300.1 (U.S. Environmental Protection Agency 1997) (detection limits of 0.003 and 0.05 mg/L, respectively). Ammonium (NH_4-N) was analyzed on a Technicon® Autoanalyzer using

automated phenate standard method 4500-NH3-G (American Public Health Association 1998) (detection limit of 0.01 mg/L).

Dissolved inorganic nitrogen: soluble reactive phosphorus ratios (DIN: SRP)

In this study, molar DIN was calculated by summing dissolved nitrate expressed as μg/L of nitrogen and dissolved ammonium expressed as μg/L of nitrogen for each sample and dividing by 14 μg/μmole to express DIN in molar units of nitrogen. Molar SRP was calculated by dividing the concentration of SRP expressed as μg/L of phosphorus by 31 μg/μmole to express SRP in molar units of phosphorus. Molar DIN was divided by molar SRP to yield a DIN: SRP ratio.

Chlorophyll a

Chlorophyll *a* concentration ($\mu g/cm^2$) was used as a proxy for algal biomass in each multi-species sample (Welschmeyer 1994). For each replicate, a 17x30 mm tube (227 mm^2) was inserted into algae/sediment collected in the Petri-dish allowing the tube to protrude ~12-13 mm above sediment. Three milliliters (ml) of deionized water (DI) was injected into the tube via syringe, withdrawn, and re-injected three times to suspend the top 2-3 mm of substrate. The slurry was vacuum filtered onto a Whatman filter (GF/C) at 250 mm Hg. Algal samples were processed for chlorophyll *a* using a cold-methanol and non-acidification extraction process (Welschmeyer 1994). This extraction method is specific for chl *a* and is not affected by the presence of chl *b* or *c*. Filtered algae were placed into a 16 x 100 mm test tube, 10 mL of 100% methanol added, capped with tin

foil, and placed in a freezer over-night. Chlorophyll *a* concentration from each sample was quantified fluorometrically (650-700nm) (Turner Designs Inc. model TD-700).

Diatom valve counts

For each replicate, a second subsample of sediment was collected for diatom valve counts in the same manner as chl *a*, and processed for permanent microscope slides consistent with Julius et al. (1997). For each one ml (4.5 mm^2) sample at least 300 valves or 10 transects were enumerated for sparse samples using Leica DMLB microscope with brightfield oil immersion optics (N.A = 1.32) at 1200x magnification. Mounted diatom valves were identified using keys and descriptions by Krammer and Lange-Bertalot (1999). Digital voucher photographs (Nikon CoolPix 995) were taken of all identified taxa.

Statistical Analyses

Data that did not meet the assumptions of normality were natural log transformed to produce normal distributions. Because of the variability in nutrient concentration, the means were adjusted to correct for imbalances and least-square means were adjusted for the effects of covariates and given equal weight when computing means. The least square means or natural log least square means were used for analyses. Statistical analyses for nutrients were performed using a general linear mixing model (SAS Glimmix procedure, SAS Institute Inc. Cary, NC). Because time trends were not smooth, interaction means were "sliced" by holding site or season constant. This allowed for inspection of pattern of seasonal differences for each site and pattern of variability among

sites. To maintain a Type I error for each set of subanalyses, "sliced" p-values were multiplied by four to perform a Bonferroni adjustment (Milliken and Johnson 1998).

A percent similarity index (Dyer 1976) was used to compare diatom community structure over time and space. This index provides a versatile and convenient tool for quantitatively comparing the species composition of one multi-species sample with another sample based on presence/absence. A Shannon diversity (richness and eveness) index was computed for sites above and below AWWTP and an additional Shannon index was computed for each season across sites.

Results

Dissolved nitrate (NO_3-N)

Dissolved inorganic nitrate concentrations decreased across all sites during a prolonged flood pulse (April-June 2005) and concentrations increased across all sites during summer. Mean concentrations of nitrate were significantly different between the northern and southern sites in the MRG. The highest NO_3-N concentrations were found at the Shirk site across all seasons (146-1485 μg/L). Mean NO_3-N concentrations were significantly different at Rio Bravo and Shirk for late winter compared to other sites ($p < 0.0001$). During late spring Angostura had significantly lower NO_3-N concentration than Shirk ($p = 0.0032$) (Fig. 3, Graph A). During summer, mean NO_3-N concentrations at Rio Bravo and Shirk were 225 ± 8.2 and 794.3 ± 397 μg/L, respectively. Water samples taken during summer at Shirk exhibited an order of magnitude increase in NO_3-N

concentration compared to samples taken at Rio Bravo (1465 ± 36 vs. 535 ± 36 μg/L, respectively).

Ammonium (NH_4-N)

Ammonium concentrations increased from late winter to late spring at sites south of Angostura during the flood pulse. Average ammonium concentrations were significantly different across all sites during late spring ($p < 0.0004$). The highest ammonium concentrations were measured at Shirk and Rio Bravo during late spring (270 and 164 μg/L, respectively). Ammonium concentrations dropped considerably in the summer and then increased in winter at all sites (Fig. 3, Graph B).

Soluble reactive phosphorus (PO_4-P)

Soluble reactive phosphorus concentrations fluctuated with flow, increasing from late spring to summer, and decreasing during winter (Fig. 3, Graph C). Mean soluble reactive phosphorus concentrations were significantly higher at Shirk for late winter, summer, and winter ($p < 0.004$). There were no other significant differences among reaches (Fig. 3, Graph C).

Dissolved Inorganic Nitrogen: Soluble Reactive Phosphorus Ratios (DIN:SRP)

Dissolved inorganic nitrogen to soluble reactive phosphorus ratios in the MRG varied throughout the year. DIN:SRP ratios ranged from 6.9 to 14.9 for Angostura and 5.3 to 52 for Bernalillo sites, respectively. Ratios were 8 to 14.4 for the Rio Bravo site and 4.4 to 11.9 for the Shirk site, respectively (Fig. 4).

Chlorophyll a ($\mu g/cm^2$)

Chlorophyll *a* concentrations varied throughout the year. From late winter to late spring, chl *a* content increased at all sites except Angostura, which stayed constant. Periphyton chl *a* at downstream sites was lower than upstream sites during late winter and summer (Fig. 5). Winter chl *a* at the northern-most site was higher than either of the impacted southern sites. Chl *a* content ranged from 4.4 to 12 $\mu g/cm^2$ during late winter, 9.0 to 22.7 $\mu g/cm^2$ during late spring, 19.3 to 50.3 $\mu g/cm^2$ during summer, and 12.6 to 69.9 $\mu g/cm^2$ during winter. Comparisons among sites for individual seasons revealed that only Angostura and Shirk displayed significant differences in chlorophyll content ($p = 0.028$). Chl *a* concentrations increased from late winter to late spring at all sites except Angostura. During late spring to summer chl *a* increased at all sites except at Rio Bravo. A decrease in chl *a* concentration was observed from summer to winter, except for Angostura, which showed an increase and Shirk, which remained constant.

Diatoms

Results for seasonal sampling rejected the null hypothesis that diatoms are equally distributed in the MRG. Thirty-five genera and 94 species were identified from MRG sampling sites (Table 1). Species richness fluctuated seasonally for late winter (42 spp), late spring (64 spp), summer (34 spp), and winter (55 spp), but were not significantly different. The dominant diatom species by season are listed in Table 2. During late winter, *Achnanthidium lanceolatum* was the most common taxon and contributed the highest percent (dis)similarity at 32%. During late spring and winter, *Fragilaria parasitica* was the most common taxon and contributed the highest percent (dis)similarity

at 16 and 21% respectively. *Rhopalodia gibba* var. *parallela* was the most common taxon and contributed the highest percent (dis)similarity during summer at 28% (Table 3).

Shannon Diversity Index

Results from Shannon diversity index computation revealed a difference between northern and southern sites (3.04 vs. 2.75, respectively). Late winter and winter diversity indices were identical (2.74), while the late spring index revealed the highest diversity (2.86) and summer revealed the lowest diversity index (1.9) (Table 4).

Discussion

Natural and anthropogenic factors affect the quality of surface and groundwater in the MRG (Moore and Anderholm 2002, Passell et al. 2005). Among the anthropogenic factors are irrigation, wastewater effluent, urban runoff, septic tank discharge, and leaching of fertilizers (Moore and Anderholm 2002). Many WWTPs are located along the Rio Grande, with the two largest at Rio Rancho and Albuquerque, New Mexico. During wet years, WWTP effluent contributes approximately 20% of river discharge in Albuquerque increasing to approximately 38% in dry years (Oelsner et al. 2007). The impact of the WWTP discharge is highlighted by the differences in nutrient concentrations above and below AWWTP outfalls. Results from water chemistry analyses revealed that nutrient concentrations were generally higher in NO_3-N and SRP downstream of WWTPs compared to upstream sites.

Large increases in nutrient concentrations downstream of the AWWTP are likely driven by the large volume of treated sewage effluent discharged to the Rio Grande (Moore and Anderholm 2002, Passell et al. 2005). Benthic diatoms can be used as a biological tool for monitoring point sources of nutrients (Patrick 1963). One of the first effects of pollution is to cause some pollution-tolerant algal species to become more abundant (Patrick and Hohn 1956, Dela Cruz et al. 2006), and these species changes can be used to determine the degree of pollution in a river (Patrick and Strawbridge 1963). Many studies have used diatoms to indicate changes in water quality due to point-source pollution in various parts of the world (Dela-Cruz et al. 2006, Duong et al. 2006, Fawzi et al. 2002, Juttner et al. 2003, Kwandras et al. 1998, Lobo et al. 1995, 2004, Maznah and Mansor 2002, Reavie and Smol 1998, Soininen and Kononen 2004, and Weilhoefer and Pan 2006).

Diatom species composition varied along the MRG with respect to pollution. *Fragilaria parasitica* and *Rhopalodia gibba* var. *parallela* were consistently the dominant diatom species downstream of the AWWTP from late spring to winter. These species are tolerant of eutrophic and alkaline conditions (Fore and Grafe 2002). Total inorganic nitrogen and phosphorus can be toxic to algae at concentrations >610 μg/L (Miltner and Rankin 1998, Dodds 2003) and may explain the decrease in chl *a* and diatom species richness at the Rio Bravo and Shirk sites during summer when NO_3-N and PO_4-P were highly elevated.

Over the course of this study, approximately 63% of dominant diatom species found at Angostura and Bernalillo were present in the guts of *H. amarus* collected in 1874 and reported by Shirey (2004), while only 25% of the dominant diatoms found at Rio Bravo and Shirk were present in *H. amarus* collected in 1874. This north to south difference may be indicative of nutrient shifts influenced by wastewater effluent (Dela Cruz et al. 2006). One of the dominant diatom species (*Nitzschia palea*) found in *H. amarus* collected in 1874 (Shirey 2004), and was present in high numbers only at the Angostura site. *Nitzschia palea* was also found at Shirk, but at a lower frequency compared to Angostura. The Shannon diversity index computation revealed a difference between northern and southern sites (3.04 vs. 2.75, respectively) potentially correlated with point source pollution from Rio Rancho and Albuquerque WWTPs.

One issue in the early life history of *H. amarus* that has not received much attention is food availability following yolk absorption. Failure to successfully feed at this time in the life cycle would likely result in significant mortality because a larva's energy reserves would quickly be exhausted (Pepin 2002). Hjort (1914) was the first to link feeding, larval survival and stock recruitment to food abundance during the transition of larvae from endogenous (yolk) to exogenous (plankton) feeding (Cowan and Shaw 2002). Hjort (1914) termed this the "critical period hypothesis," which states that larvae can only survive for only a brief period without food after their supply of yolk and oil globules are exhausted. When food availability is high, larvae survival would be high.

Cushing (1990) described a match/mismatch hypothesis that extended Hjort's (1914) critical period hypothesis suggesting that the degree of match and mismatch in the time of larval fish production and primary productivity could explain variability in fish stock recruitment in the North Atlantic. Cushing (1990) concluded that fish in temperate waters should release their larvae during the spring or autumn peaks in the production cycle, when more food is available. *Hybognathus amarus* spawn during high flows and yolk absorption would occur on the falling limb of the hydrograph.

In this study, I extend the match/mismatch hypothesis to the MRG and *H. amarus*. *H. amarus* spawn during the spring runoff; however, results of chl *a* content indicate that periphyton biomass remained low at the impacted sites for the entire year. Specifically, periphyton biomass is low during late spring, which corresponds to the critical period when fish larvae deplete their yolksac and are dependent on exogenous food resources. The length of time that a fish can survive without food is governed by the rate of energy expended and the amount of energy stored in the tissues (Fuiman 2002). When food is withheld from the larvae, they reach a point of no return at which starvation is irreversible (Fuiman 2002). The point of no return varies among species and is related to body size and temperature. At temperatures of 5-10^{o}, larvae may reach starvation after 20-35 days, but at 25-30^{o} it may only take four or five days (Fuiman 2002). During late spring, mean water temperature in the MRG was 17.9^{o} C and increased to 23^{o} C during summer. At water temperatures of 17.9^{o} C during the critical period, *H. amarus* larvae could possibly survive without food for 7-10 days before succumbing to irreversible starvation. Therefore, it is imperative to address the important issue of food limitation for

H. amarus during the critical transition in the life history between endogenous and exogenous food resources.

Conclusions

This study examined seasonal nutrient concentrations, chlorophyll content, diatom community composition, and the match/mismatch between algal abundance and the historic *H. amarus* spawning period at four sites along the Middle Rio Grande of New Mexico. The shift in low nutrient concentration for primary producers north of Albuquerque to excess nutrients for primary producers through the urbanized reach of the MRG are indicative of nutrient shifts influenced by wastewater effluent that have changed the diatom distribution in the MRG. Consistent with previous work (Moore and Anderholm 2002, Passell et al. 2005), these data indicate that wastewater was the largest source of inorganic nitrogen to the MRG.

Results from the present study indicate that a mismatch exists between peak algal biomass (represented by chlorophyll content) and the historic *H. amarus* spawning period in the MRG. Decreased food availability during this critical life history stage, when larval fish switch from endogenous to exogenous food sources, may help explain why *H. amarus* populations have been in rapid decline since construction of Cochiti Reservoir. Understanding interactions between nutrient availability, algal food resources, and *H. amarus* spawning are essential for sustaining *H. amarus* populations in the MRG for the future.

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Tables

Table 1. Diatoms identified from Middle Rio Grande at sampling sites during 2005 (n=35 genera, n=86 species).

Achnanthes brevipes
A. joursacense
A. kuelbsii
A. lavenderi
A. minutissima
A. petersenii
A. subsala
Achnanthidium lanceolatum
Amphora ovalis
A. pediculus
A. perpusilla
Asterionella formosa
Aulacoseira granulata
Caloneis amphisbaena
C. lewisii
C. silicula
Cocconeis placentula
Craticula cuspidata
Cyclotella meneghiniana
Cymatopleura eliptica
C. solea
Cymbella cistula
Diatoma vulgare
Diploneis ovalis
Encyonema minutum
Epithemia adnata
E. argus
E. sorex
Fragilaria brevistriata
Fragilaria capucina
F. construens
F. leptostauron
F. parasitica
F. pinnata
Gomphonema gracilis
G. parvulum
G. sarcophagus
G. truncatum
Gyrosigma acuminatum
G. attenuatum
Hantzschia amphioxys
Hippodota denticulata
Melosira varians
Navicula capitoradiata
N. cryptocephala
N. cryptotenella
N. elginensis
N. radiosa
N. tripartita
N. veneta
N. viridula
Neidium iridis
Nitzschia acicularis
N. amphibia
N. angustata
N. brebissonii
N. denticula
N. dissipata
Nitzschia epithemoides
N. gracilis
N. intermedia
N. palea
N. paleacaea
N. pumila
N. recta
N. sigma
N. siliqua
N. sinuata
N. vermicularis
Pinnularia divergens
P. intermedia
Pleurosira laevis
Reimeria sinuata
Rhoicosphenia curvata
Rhopalodia gibba v. parallela
R. gibberula
Sellaphora pupula
Stauroneis smithii
Staurosira pinnata
Stephanodiscus alpinus
Surirella angusta
S. linearis
S. ovalis
Synedra ulna
S. vaucheriae
Tryblionella constricta
T. tryblionella

Table 2. Dominant diatom taxa identified by site and season in the Middle Rio Grande, New Mexico.

Site	Season	Genus and Species	Relative Abundance
Angostura	Late Winter	*A. lanceolatum*	33
Bernalillo	Late Winter	*N. tripunctata*	35
Rio Bravo	Late Winter	*A. lanceolatum*	52
Shirk	Late Winter	*N. vermicularis*	25
Angostura	Late Spring	*F. brevistriata*	22
Bernalillo	Late Spring	*A. ovalis*	25
Rio Bravo	Late Spring	*F. parasitica*	29
Shirk	Late Spring	*F. parasitica*	29
Angostura	Summer	*G. parvulum*	31
Bernalillo	Summer	*N. intermedia*	100
Rio Bravo	Summer	*R. gibba v. parallela*	97
Shirk	Summer	*R. gibba v. parallela*	42
Angostura	Winter	*N. dissipata*	26
Bernalillo	Winter	*C. placentula*	26
Rio Bravo	Winter	*F. parasitica*	65
Shirk	Winter	*F. parasitica*	39

Table 3. Relative contribution to (dis)similarity of top ten diatom species for seasons across all sites.

Late winter (n = 42)	%	Summer (n = 34)	%
A. lanceolatum	32	*R. gibba v. parallela*	28
N. vermicularis	16	*N. palea*	14
N. gracilis	9	*N. intermedia*	13
A. minutissima	7	*G. parvulum*	12
S. pinnata	6	*C. placentula*	12
F. parasitica	6	*R. brebissonii*	7
N. palea	6	*S. puppula*	4
C. solea	5	*A. lanceolatum*	2
D. vulgare	3	*N. amphibia*	2
S. angusta	2	*F. pinnata*	1
Late Spring (n = 64)		Winter (n = 55)	
F. parasitica	16	*F. parasitica*	21
A. lanceolatum	13	*C. placentula*	17
A. granulata	11	*N. dissipata*	10
F. brevistriata	8	*N. palea*	8
C. placentula	6	*N. recta*	6
A. ovalis	5	*A. minutissima*	4
A. minutissima	4	*G. parvulum*	4
S. pinnata	4	*S. angusta*	34
A. formosa	3	*F. pinnata*	3
N. dissipata	3	*S. ovalis*	3

Table 4. Shannon diversity index for diatoms north and south of Albuquerque, New Mexico and Shannon seasonal diversity index in the Middle Rio Grande.

Variable	Northern sites	Southern sites	Late Winter	Late Spring	Summer	Winter
Number of spp	64	56	42	64	34	55
Diversity index	3.04	2.75	2.72	2.86	1.9	2.72
H_{max}	3.83	4.03	3.74	4.17	3.5	4.06

Figures

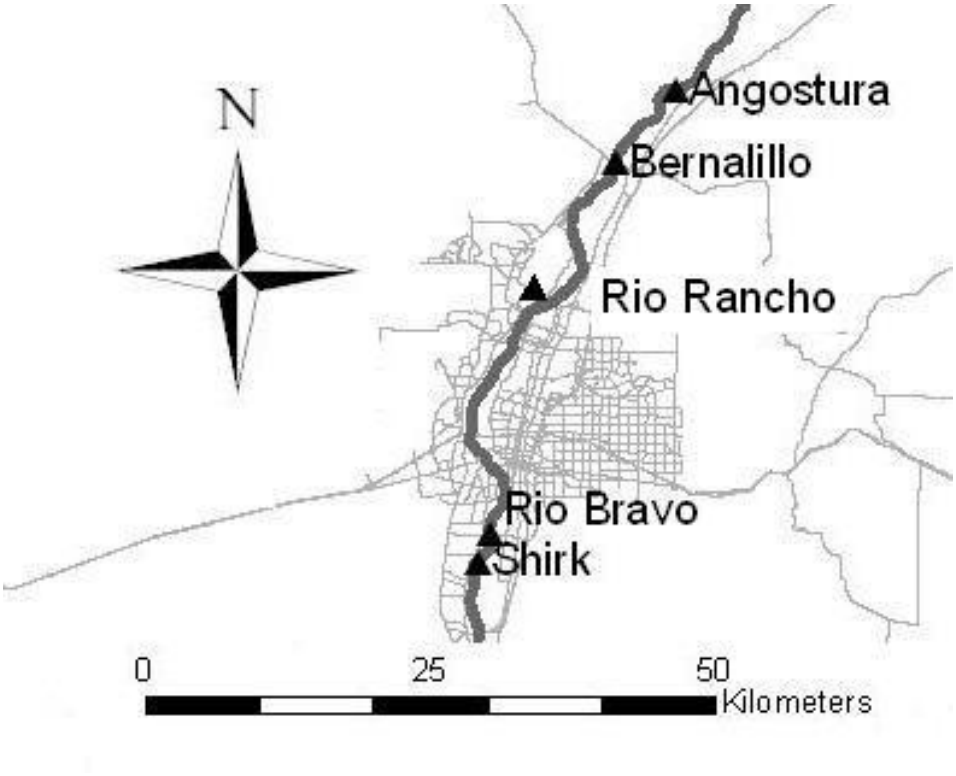

Fig. 1. Sampling sites north and south of Albuquerque, New Mexico along the Middle Rio Grande of Central New Mexico.

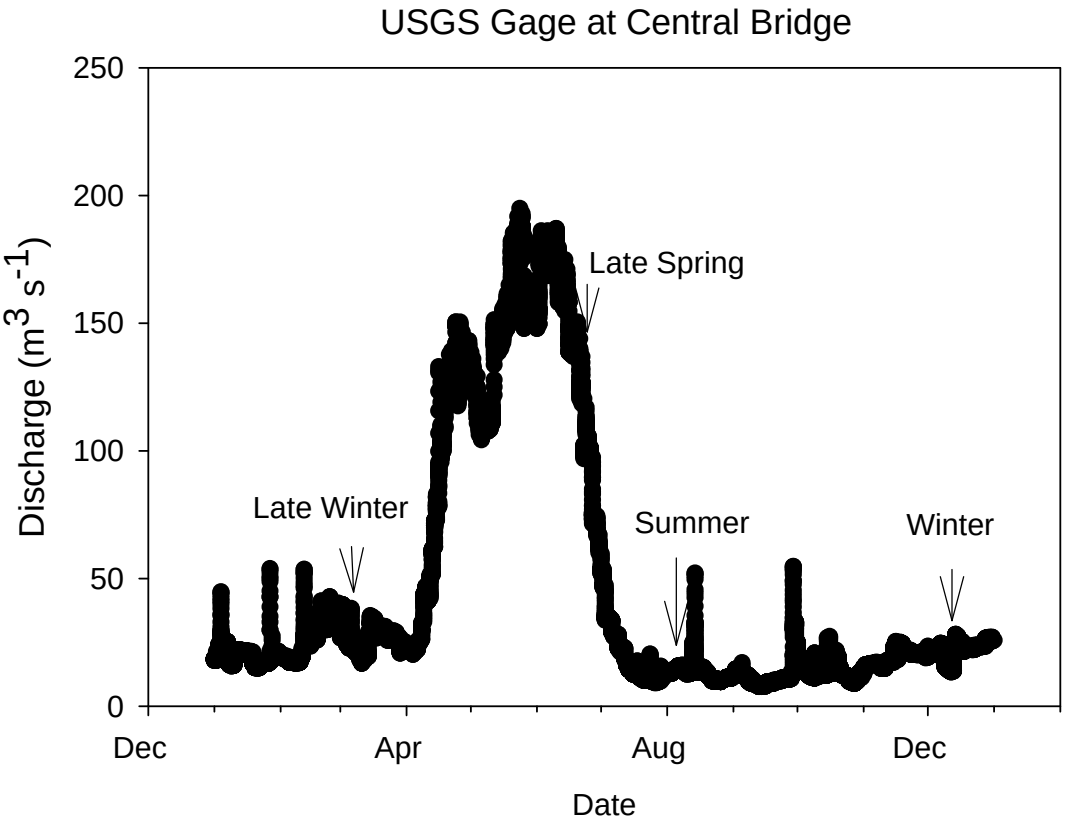

Fig. 2. Hydrograph for 2005 for the Middle Rio Grande with sampling dates noted on the figure.

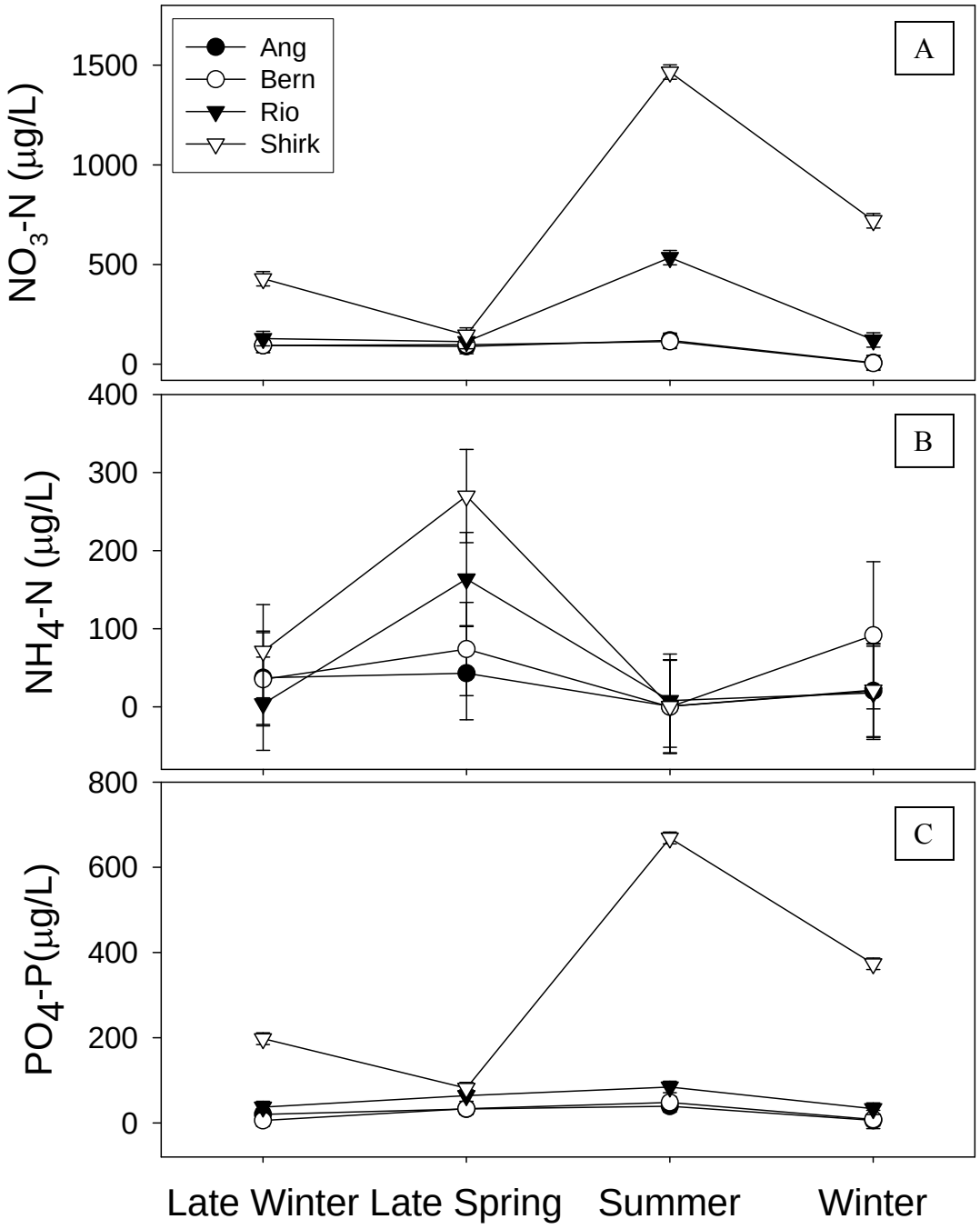

Fig. 3. Least-square means for nutrient concentration (µg/L) site by season. Middle Rio Grande, New Mexico. A: nitrate, B: ammonium, and C: soluble reactive phosphorus.

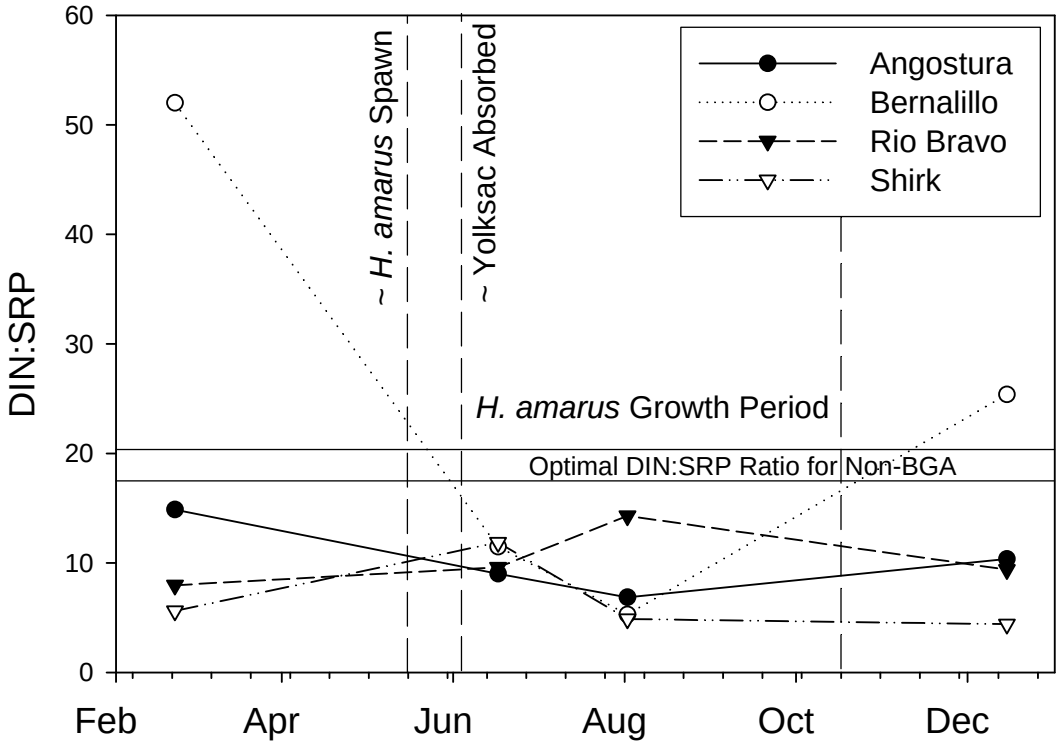

Fig. 4. Seasonal molar DIN: SRP ratios at sampling sites in the Middle Rio Grande with respect to *H. amarus* spawning and growth period. Open symbols are northern non-impacted sites and closed symbols are southern impacted sites.

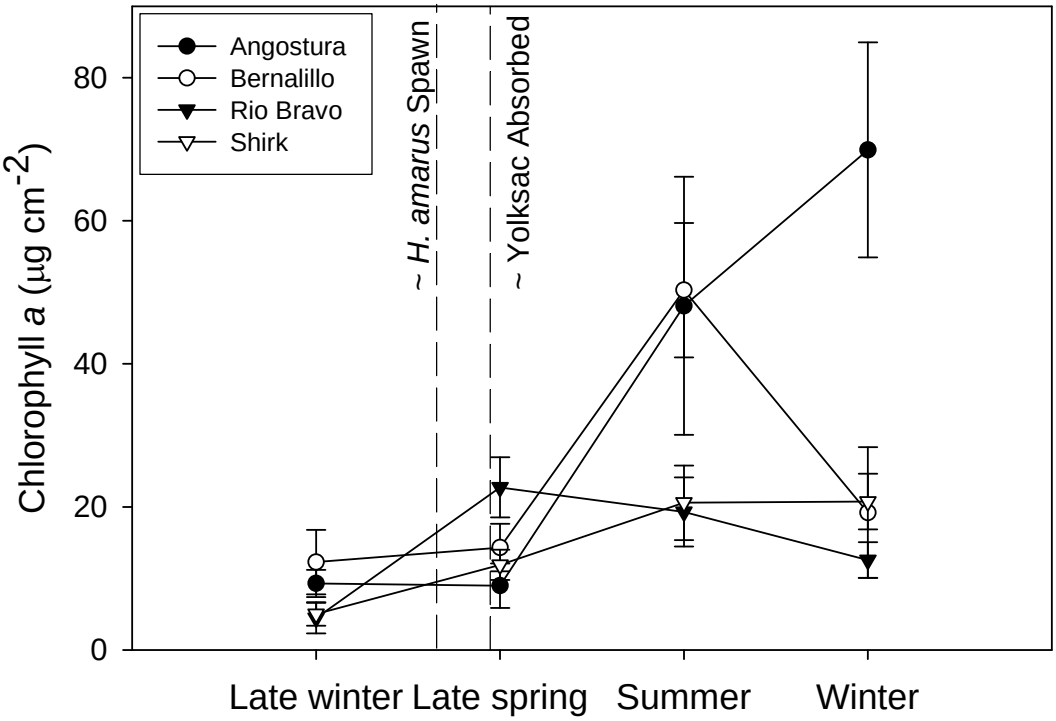

Fig. 5. Seasonal chlorophyll *a* concentrations (µg/cm^2) across sites, Middle Rio Grande, New Mexico with approximate *H. amarus* historical spawning period and yolk absorption superimposed.

CHAPTER 2: Diatom Preferences of the Rio Grande Silvery Minnow (*Hybognathus amarus*)

Introduction

Historically, the federally endangered Rio Grande silvery minnow (*Hybognathus amarus*) was historically the most abundant fish in the Rio Grande Basin (Bestgen and Platania 1991). *Hybognathus amarus* populations have been declining for over 100 years, but the exact causes have not been identified. Despite extensive recovery efforts, little research has been conducted on *H. amarus* food resources. Minnows are generalists and can forage on diatoms found in benthic and planktonic communities (Sray 1998). Other species of the genera *Hybognathus* feed on "diatoms, algae, larval insect exuvia, and plant material scraped from bottom sediments" (Whitaker 1977). Adults of the genus *Hybognathus* are thought to be obligate herbivores because they lack a defined stomach, have a black peritoneum, and have a long, narrow, and coiled alimentary tract (Etnier and Starnes, 1993 and Ross, 2001).

This anecdotal information led to a cooperative effort between New Mexico Cooperative Fish and Wildlife Service, U.S. Fish and Wildlife Service, and Dexter National Fish Hatchery and Technology Center conducted feeding trials with *H. amarus* and improved survivability of post-hatch (4-20 day) larvae decreasing the mortality rates to less than 1% by providing live food (*Artemia* naupulii) (Caldwell 2004). Growth rates of *H. amarus* also were improved by providing manufactured flake and pelleted feed (Caldwell 2004).

While these studies refined captive rearing methods, they did not address naturally occurring food resources. To investigate potential shifts in diet over time, Shirey (2004) quantified gut contents of historical *H. amarus* specimens collected in 1874 and 1978. Examination of the 1874 specimens indicates that *H. amarus* fed on 30 genera and 70 species of diatoms as well as cyanobacteria (*Anabaena* sp. and *Merismopedia* sp.), detritus, and pine pollen (Shirey 2004). A comparison of gut contents between *H. amarus* collected in 1874 and *H. amarus* collected in 1978 revealed that only 24 diatom taxa were common to both years. There was a 35% increase in number of diatom genera consumed by *H. amarus* in 1978 compared to *H. amarus* collected in 1874, however, there was a 34% decrease in the number of diatom species in consumed 1978.
In addition, 96% of the diatoms from 1874 *H. amarus* specimens were motile species, suggesting *H. amarus* foraged on soft, fine sediment substrate, consistent with the reported habitats for these diatoms (Shirey 2004).

In commercial aquaculture, feed tends to comprise 50% of the operating budget, therefore, the total fed expenditure depends on the conversion ratio of the organism and the cost of the feed (Landau 1992). To reduce costs, aquaculture facilities search for cheaper replacements for fishmeal and fish oil by using natural food resources such as algae. Investigators have studied diatom consumption by fish (Tang and Hwang 1996, Yang 2005) and abalone (Fukami et al. 1998, Siqueiros-Beltrones and Voltolina 2000). Tang and Hwang (1996) investigated milkfish (*Chanos chanos*) consumption of six genera of diatoms in brackish-water pond algae as food sources. They found that filamentous cyanobacteria and benthic diatoms were the most desirable food resources

for all age groups of *C. chanos*. It has also been shown that red abalone (*Haliotis sieboldii*) could be reared to release size solely on a culture of *Nitzschia* (species not identified) (Fukami et al. 1998). In addition, the red abalone, *Haliotis rufescens* has been shown to prefer specific diatoms as food resources (*Pinnularia biceps* var *minor* and *Navicula incerta*) (Siqueiros-Beltrones and Voltolina 2000).

This study is the first to visually record *H. amarus* feeding behavior using natural food sources (unialgal diatom cultures) on agar-amended substrates to elucidate feeding preferences. The objectives of this study include 1) the assessment of food awareness in two-week old protolarvae and three month old *H. amarus* metalarvae, 2) substrate preference, and 3) diatom preference of *H. amarus* among 15 diatom taxa. I recorded diatom contact time (tasting and feeding) during 30 minute feeding trials to determine peak sampling time and determine conditioning response (recognition/associative learning) of older, pre-conditioned *H. amarus* metalarvae.

Methods

Diatom Culturing

I collected periphyton samples from five sites located within the Middle Rio Grande (MRG) (Fig. 1). Samples were collected according to U.S. Geological Survey (USGS) National Water-Quality Assessment protocols (NAWQA) (Moulton et al. 2002). The NAWQA program provides an understanding of water-quality conditions with an emphasis on how those conditions may vary locally, regionally, and nationally whether

conditions are improving over time and how natural features and human activities affect those conditions.

Multi-species periphyton samples were transported to U.S. Department of Agriculture Forest Service, Rocky Mountain Research Station (RMRS) in Albuquerque, NM. Periphyton samples were washed into ½ dram glass vials with Bozniak community growth media (Bozniak 1969) and placed in environmental growth chambers (Sheldon Manufacturing Model 2015). Individual diatom cells were isolated consistent with the methods of Hoshaw and Rosowski (1969). A single 20 µL sample was placed onto a microscope slide and examined at 1000x magnification. Standard Pasteur pipettes (133 mm) were flamed and pulled to a thickness of 0.3 mm and the desired diatom was drawn up into the pipette via capillary action and deposited into a sterile drop of water. A new pipette was used to re-isolate the diatom and deposited into a new drop of water. The serial wash process was continued for six drops or until only the chosen diatom remained. The individual diatom was transferred to autoclaved 50 ml Erlenmeyer flasks containing approximately 3 mm of #30 silica sand as a substrate and 20 ml of Bozniak growth media. After visible algal growth was observed (40-60 days) individual flasks were examined. Samples with <5% (determined by cell counts) of non-target diatom species were saved as inoculum for feeding trials. Diatom cultures were processed and permanently mounted consistent with Julius et al. (1997) and microscopically identified to species using keys by Krammer and Lange-Bertalot (1999).

Substrate characteristics may influence diatom growth. To test for differences experimentally, I obtained fine-grained sediment and coarse-grain sand from the margins of the MRG from two sites (Shirk and Angostura, respectively, Figure 1). Sediment and sand were autoclaved and dried in a constant temperature oven (Yamato Scientific American Inc. Model DKN 810). Dried sediment was crushed, sieved (500μm), and stored at room temperature. River water was obtained from each sampling site, sequentially filtered (44, 25, 3 μm), amended with micronutrients and macronutrients (Bozniak 1969) and vacuum filtered with a Nalgene bottle-top filter (0.2 μm). Substrates for diatom culturing were prepared by heating 250 ml of Bozniak media in a 1000 ml beaker and adding 3.75 g of noble agar because Patrick and Wallace (1953) found that *Nitzschia linearis* grew better on agar. After boiling, an equal volume of fine-grained sediment or sand was added, mixed thoroughly, and poured into 100x15mm Petri dishes until half full, covered, and sealed with Parafilm (American Can Co.). Substrates were allowed to acclimate in environmental growth chambers for three days prior to inoculation. Substrates were inoculated with one ml of the diatom inoculum, resealed and allowed to grow until visible diatom growth was evident (40-60 days).

Feeding Trials

Eight 37.5 L aquaria were maintained at room temperature (23.3-26.5^{o}C) and six were used for feeding trials and two for water exchange. A total of six feeding trials were performed with six replicates for each trial. *Hybognathus amarus* (n = 130, 9.17 ± 0.34 mm Standard length) used in feeding trials were obtained from the *H. amarus* propagation facility at the Albuquerque BioPark, Albuquerque, New Mexico.

Hybognathus amarus were randomly selected and placed into one of six aquaria until each aquarium contained 10 fish. Three to six algal cultures were randomly selected and one puck 21 mm in diameter (346 mm^2) was removed from each Petri dish with a cork borer. Diatom pucks were randomly placed onto a Plexiglas table in a 2 x 3 configuration and placed into each aquarium. Diatom puck location was identical for replicate feeding trials. In total, fifteen diatom species were cultured and presented to *H. amarus* in various combinations during feeding trials (Table 1).

Feeding trials were designed for several objectives. I used recently hatched *H. amarus* mesolarvae (6.8-9.2 mm standard length) for feeding trials one and two to evaluate food awareness, peak diatom sampling determined from time spent in contact with each puck, and substrate and diatom preference. I define food awareness as the time required to locate and sample a diatom puck from the time of introduction. To facilitate documentation of *H. amarus* food awareness, the front panel of each aquarium was divided into equal quadrants and food stimuli were placed in quadrant III for each trial. Food awareness was determined from the quadrant location of each fish in five-minute intervals over the 30-minute. I also used pre-conditioned *H. amarus* of two ages from prior feeding trials to assess conditioning response to food stimuli, peak diatom sampling, and diatom preference. Conditioning response was reported as the time between the introductions of food stimuli to the time of first sampling. Feeding trials were digitally videotaped (Sony DCR-VX2100, 33 frames/sec) for 30 minutes each. Video was transferred to a computer where diatom and substrate preference was examined using video editing software (Cyberlink PowerDirector 4).

Statistical Analyses

Data that did not meet assumptions of normality were $\log_{10}$ transformed. To assess food awareness, I examined whether *H. amarus* were randomly distributed among quadrants or preferred one quadrant over another. A multi-response permutation procedure (MRPP) was conducted to test whether there were significant differences between two or more groups of sampling units. This analysis was conducted because total summed frequencies differed among tanks. The variables analyzed were the summed frequency of fish presence in each quadrant over time interval for feeding trials 1-3. Following the completion of a MRPP analysis, the principal components can be estimated from the data and the first two components plotted to provide a visual description of separation among feeding trials. Blocks are ignored for this analysis. Peak diatom sampling time was determined by summing total time recorded per diatom puck per five-minute interval and analyzed with randomized block design analysis of variance (ANOVA) where each tank was considered to be a statistical block. Successive five minute time intervals within each replicate trial were included in analyses and treated as repeated measures. Diatom and substrate preference was determined by recording the number of visits to each diatom puck and summing total sampling time recorded per diatom puck over 30 minutes. Diatom and substrate preferences were analyzed using randomized block rank test and Friedman's statistic testing. Conditioning response in experiment two was evaluated using multiple comparisons analysis (Dunnet 1980) based on control trials (non-conditioned). Significance was determined using an alpha = 0.05. Statistical analyses were performed with SAS statistical software (SAS Institute Inc. Cary, NC), MRPP and

PCA analyses performed according to Mielke and Berry (2001), and descriptive statistics were generated using MicroSoft Excel®.

Scanning Electron Microscopy

Micrographs of *H. amarus* mouthparts were taken using a JEOL 5800LV scanning electron microscope (SEM). The SEM is equipped with secondary electron, backscattered electron and cathodoluminscence detectors, a Link Analytical ultrathin-window Energy Dispersive X-ray (EDX) spectrometer and an Oxford Isis 300 X-ray analytical system. The SEM is a "dual mode' microscope, meaning that it can operate at both high vacuum (the conventional mode of operation) and in a low vacuum, to 1 Torr. The low vacuum mode allows imaging and analysis of uncoated and "wet" samples, such as biological samples or moist geological materials like sandstone and clay. The use of EDX analysis in the low vacuum provides a nondestructive analysis method for samples that could not otherwise be coated with conductive material such as gold-palladium alloy or carbon.

Because I did not investigate taste preference in *H. amarus* I looked at the growth form of diatom cultures. Diatom cultures were processed with a serial ethanol dilution (25%, 50%, 75%, and 100%). Each diatom puck was covered with ethanol in each dilution for one hour and then exchanged to the next highest concentration. After the 100% ethanol treatment the puck was transferred to Hexamethyldisilazane (HMDS) and left overnight. The puck was removed from HMDS and placed onto a 25 mm aluminum stub with carbon tape. The specimen was then sputter coated with approx 5-10 nm of gold/

paladium with a Fullam EMS-76M sputter coater. Observations were made in a JEOL 6060LV SEM at accelerating voltage of 15,000 kv and a working distance of 10 mm.

Results

Food Awareness and Peak Diatom Sampling

Results of MRPP and PCA indicate that the distribution of summed and proportion of summed frequencies among quadrants did not differ between trials one and two (non-conditioned fish), but did differ for trial three (conditioned fish) compared to either trial one or two. Activity in trial three was concentrated in quadrant III (food location), while activity was more dispersed in trials one and two ($p < 0.001$) (Fig. 2). During each feeding trial, maximum diatom sampling occurred at 15 minutes ($p < 0.001$) (Figs. 3a-b). The exceptions were feeding trial three (Fig. 3c), where sampling peaked between zero to five minutes, and feeding trial six (Fig. 3e), where sampling peaked at 10 minutes.

Substrate preference

Results of randomized block design ANOVA indicate no apparent variation associated with diatoms and fine-grained sediment or coarse-grained sand substrate ($p=0.26$). When looked at individually, each of the three *Nitzschia* species (*palea, paleacaea,* and *linearis*) used in feeding trials one and two were fed upon equally, regardless of substrate (Fig. 4).

Diatom preference

Friedman's rank test for feeding trial one indicate that *H. amarus* protolarvae preferred *Nitzschia palea* over *N. linearis* and *N. paleacaea* ($p<0.05$) (Fig. 3a). Sampling time by *H. amarus* of *N. palea* was 5.6 times greater for *N. palea* compared to *N. linearis* and 10 times more compared to *N. paleacaea*. Results for feeding trial two indicate that *H. amarus* protolarvae sampled *N. palea* two times more than *N. linearis* and four times more compared to *N. paleacaea* (Fig. 3b).

Results from feeding trial three using pre-conditioned *Hybognathus amarus* mesolarvae suggest that *Nitzschia palea* was preferred over other diatoms ($p<0.025$). Sampling time of *H. amarus* was 3.6 times greater for *N. palea* than *Synedra ulna*, 5.3 times greater than *Fragilaria crotonensis* and *Surirella angusta*, and 13 times more compared to *Cyclotella meneghiniana* (Fig. 3c). Results from feeding trial five indicate *H. amarus* mesolarvae preferred *Navicula veneta* over other diatoms. Sampling time for *Navicula veneta* was 3 times more than *Navicula sp*, 3.6 times more than *Nitzschia palea*, and 7 times more compared to *N. capitellata* (Fig. 3e).

Results from feeding trial four using non-conditioned *Hybognathus amarus* metalarvae indicate that *Nitzschia paleaformis* was preferred over other diatoms ($p<0.025$). Sampling time was 7.4 times greater for *N. paleaformis* than *Navicula venta*, 8.2 times greater than *Achnanthes suchlandtii*, 9.5 times greater than *N. palea*, and 14 times greater compared to *N. cf palea* (Fig. 3e). Results from feeding trial six indicate that *Nitzschia paleaformis* was preferred over other diatoms. Sampling time for *N. paleaformis* was 1.5

times greater than *Nitzschia cf intermedia*, 1.8 times greater than *N. palea*, 3.7 times greater than *Navicula venta*, 4.4 times greater than *N. cf palea*, and 6.2 times more compared to *Navicula molestiformis* (Fig. 3f).

In addition to recording the time spent sampling diatom pucks, I also recorded the number of visits to each diatom puck and calculated a mean number of visits per feeding trial (Fig. 5). Results of single factor ANOVA for number of visits to each diatom puck indicate significant difference between non-conditioned and pre-conditioned feeding trials ($p=0.016$). There was a 790% increase in the number of visits by pre-conditioned *H. amarus* between feeding trials two and three, and a 165% increase in visits by pre-conditioned *H. amarus* between feeding trials four and five. *H. amarus* in feeding trial six were older metalarvae when received at RMRS.

Conditioning response

Results from feeding trials one, two, and four indicate that non-conditioned fish were often not immediately attentive to the food stimuli presented. Mean time to first feeding in non-conditioned trials was 344 seconds ± 72 seconds. *H. amarus* used in feeding trials three and five were pre-conditioned from their use in feeding trials 2 and 4 and arrived at diatom pucks in 49 seconds ± 39 seconds ($p = 0.0014$) (Fig. 6).

Scanning Electron Microscope Micrographs

Inspection of *H. amarus* protolarvae (two weeks old, 6.8-9.2 mm SL) with a scanning electron microscopic revealed putative taste papillae developing on the tip of the

mandible (Fig. 7a). These similar structures have been identified as taste papillae of other fish (Reutter et al. 1974, Kortschal 1992, Gomahr et al. 1992). Microscopic examination of juvenile *H. amarus* (6 months old, 14.4 -18.8 mm SL) revealed that putative taste papillae were well developed along the mandible, premaxilla, inside of the mouth, and the ventral surface of the jaw (Fig. 7b). Examination of adult *H. amarus* (18 months old, 60 mm SL) showed that putative taste papillae had also developed on the tongue (Fig. 7c) and dorsal and ventral surface of the head (Fig. 7d). The SEM images of the diatom cultures reveal that the preferred diatoms exhibited an erect growth form while less preferred diatoms exhibited a prostrate growth form (Figs. 8a-d).

Discussion

Results from all feeding trials resulted in the rejection of the null hypothesis that *H. amarus* feed equally on these 15 diatoms from the MRG. The use of video playback has been an important tool to ethologists for the past few decades because it can provide important insights into feeding behavior, mate courtship, and visual receptor sensitivity (Kodric-Brown 1999, Nicoletto and Kodric-Brown, 1999, Rowland 1999). Video playback used in the present study allowed for the determination of food awareness, conditioning response, and diatom feeding selectivity of *H. amarus* in a laboratory setting. Feeding trial videos revealed that *H. amarus* moved quickly from diatom puck to diatom puck touching and tasting for no more than one second until selecting a preferred diatom species and commencing feeding.

During the course of this study, non-conditioned *H. amarus* were not attentive to food stimuli. Preconditioned *H. amarus*, however, showed a significant difference in time to first feeding, arriving at feeding pucks on average seven times quicker. Warburton (2003) found that learning can lead to significant improvements in foraging performance in only a few exposures. Pre-conditioning *H. amarus* used in feeding trials three and five led to improvements in foraging performance with only one 30 minute exposure. *H. amarus* metalarvae used in feeding trials four and six were older (3 months) than *H. amarus* protolarvae used in feeding trials one and two (two weeks old) indicating that age also may influence feeding time.

Nitzschia palea was presented to *H. amarus* in all feeding trials and was preferred in 50% of trials. *Nitzschia paleacaea* was present in feeding trials four and six and was the preferred diatom in these trials. The gut contents from *H. amarus* collected in 1874 revealed that *Nitzschia palea* and *N. paleacaea* were the 4th and 5th most dominant diatom taxa (Shirey 2004). The gut contents from *H. amarus* collected in 1978 revealed that the dominant diatom taxa were *Pseudostaurosira construens* var. *binodis* (34%), *Staurosirella pinnata* var. *subrotunda* (17%), *Staurosira construens* (13%), and *Staurosira construens* var. *venter* (12%). *Nitzschia palea* and *N. paleacaea* comprised less than 0.01% of taxa identified (Shirey 2004). The diatom taxa from the 1874 *H. amarus* specimens were primarily eutratrophic species which suggests eutrophic conditions at the site where they were collected. The diatom taxa in the 1978 *H. amarus* specimens were meso-eutraphentic suggesting slightly less eutrophic conditions.

Repeated attempts to culture all identified diatoms (38 genera and 120 species) collected from the MRG proved unsuccessful regardless of modifications to growth media. Seven genera and 15 diatom species were successfully cultured at RMRS. Nine species of the genera *Nitzschia* were cultured while only one species each of the other six genera was cultured. It is unclear why the *Nitzschia* species grew well, but not other genera.

Vision is the dominant sense of many fishes (Rowland 1999) and the sight of other fish feeding may be a cue. Other fish may rely upon auditory or chemical cues. Living in an aquatic environment that is often devoid of light has led fish to evolve and develop compensatory senses such as chemosensory and chemical signaling systems (Hara 1994). The Albuquerque reach of the Middle Rio Grande is highly turbid (20- 1200 nephelometric turbidity units, (D. Van Horn, personal communication, University of New Mexico) and light penetration is generally less than 50 cm.

In virtual darkness, fish are unlikely to depend on vision for foraging, but instead rely on their senses of smell, touch, and taste. Many research studies have investigated the olfactory and gustatory systems in fish (Gomahr et al. 1992, Kotrschal 2000, Boudriot and Reutter 2001, Kasumyan 2002, Kasumyan and Døving 2003, Dieterman and Galat 2005, Devitsina 2006) and have elucidated taste preferences of many fish species using free amino acids.

In fish, taste buds are not only within the oral cavity, pharynx, esophagus, and gills, but may also occur on the lips, barbells and fins, and over the entire body surface in many

species (Gomahr et al. 1992, Bouriot and Reutter 2001, Kasumyan and Døving 2003, Dieterman and Galat 2005, Devitsina 2006). Oral taste preferences for more than 20 fish species were highly species-specific as shown by Kasumyan and Døving (2003). *Salmo trutta* (brown trout) from three widely separated catchments in Russia were used to compare preferences and found that taste preferences were similar from all three populations (Kasumyan and Sidorov 1995). Taste inclination in fish, as in other vertebrates, are thought to be genetically determined (Kasumyan and Nikolaeva unpublished study). Kasumyan and Nikolaeva compared the taste inclination of wild female goldfish, male common carp, and hybrids produced from these two cyprinid species. Taste preferences were similar in carp and hybrids but differed between hybrids and wild goldfish. Genetic determination may help explain why multiple cohorts of *H. amarus* from 2005 preferred *N. palea* as they did in 1874.

I can only infer that *H. amarus* prefers the taste of *N. palea* and *N. paleaformis*. SEM images of the preferred diatoms, however, reveal that these diatom species could be preferred because of growth form. SEM images of *N. palea* and *N. paleaformis* reveal that these two species have an erect growth form (Fig. 12) compared to *N. molestiformis* and *Achnanthes suchlandtii* which have prostrate growth forms (Fig 13). In this study, diatoms with prostrate growth form are considered "poor" food quality because an aquatic grazer must deal with a relatively large portion of indigestible biogenic silica and agar/substrate to gain a proportionally small amount of organic matter. "Good" food quality is the opposite scenario with a proportionally small amount of biogenic silica and agar/substrate to relatively large organic matter content.

Conclusion

This investigation addressed the diatom preference of *H. amarus* for specific benthic diatoms found in the MRG. *Nitzschia palea* was the preferred diatom in three of the six feeding trials (50%). Unfortunately, *N. palea* is currently not abundant in the MRG (chapter one this dissertation).

Typically, 95% of all fish released from hatcheries die from predation or starvation in the first few weeks following release (Brown and Laland 2001). Hatchery-reared *H. amarus* could be trained en masse to recognize and feed on natural food resources (diatoms). Transfer of learned information via observation, between individuals is reliant on social learning processes (Laland et al. 2003). Using social learning protocols to train *H. amarus* en masse prior to release may help to increase their survival in the wild. This information can be advantageous to *H. amarus* propagation managers, as well as propagation managers of other threatened or endangered fish species.

I have shown that *H. amarus* can be conditioned to respond to food stimuli. Large-scale training of foraging skills is feasible, relatively simple, and inexpensive to initiate (Brown and Laland 2001). This study greatly increases the knowledge base concerning the feeding habits of *H. amarus* and elucidated some of the preferred diatoms available to the *H. amarus* in the Middle Rio Grande. Further studies are required to determine if *H. amarus* cues on taste or nutritive value of diatom species when selecting food sources.

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Tables

Table 1. Friedman's rank score (**bold**) for fifteen diatom species used in six individual feeding trials with six replicates each.

Feeding Trial #1	Feeding Trial #2	Feeding Trial #3	Feeding Trial #4	Feeding Trial #5	Feeding Trial #6
5.3 *Nitzschia palea* (sediment)	**5** *N. palea* (sand)	**4.7** *N. palea*	**1.6** *N. paleaeformis*	**3.5** *N. veneta*	**5.7** *N. paleaeformis*
4.2 *N. linearis* (sand)	**4.5** *N. linearis* (sediment)	**3** *Fragilaria crotonensis*	**-.08** *Navicula veneta*	**3** *Navicula sp*	**4.5** *N. cf intermedia*
4 *N. palea* (sand)	**3.3** *N. palea* (sediment)	**3** *Synedra ulna*	**-0.25** *Achnanthes suchlandtii*	**1.8** *N. palea*	**4.3** *N. palea*
3.2 *N. paleacaea* (sand)	**3.3** *N. paleacaea* (sediment)	**2.5** *Surirella angusta*	**-0.92** *N. palea*	**1.7** *N. capitellata*	**2.5** *N. cf palea*
2.3 *N. paleacaea* (sediment)	**3** *N. linearis* (sand)	**1.8** *Cyclotella meneghiniana*	**-2** *N. cf palea*		**2.2** *N. veneta*
2.1 *N. linearis* (sediment)	**1.8** *N. paleacaea* (sand)				**1.8** *N. molestiformis*

Figures

Fig. 1. Map of the Middle Rio Grande north and south of Albuquerque, New Mexico, showing the 5 sites where diatoms used in the feeding trials were collected.

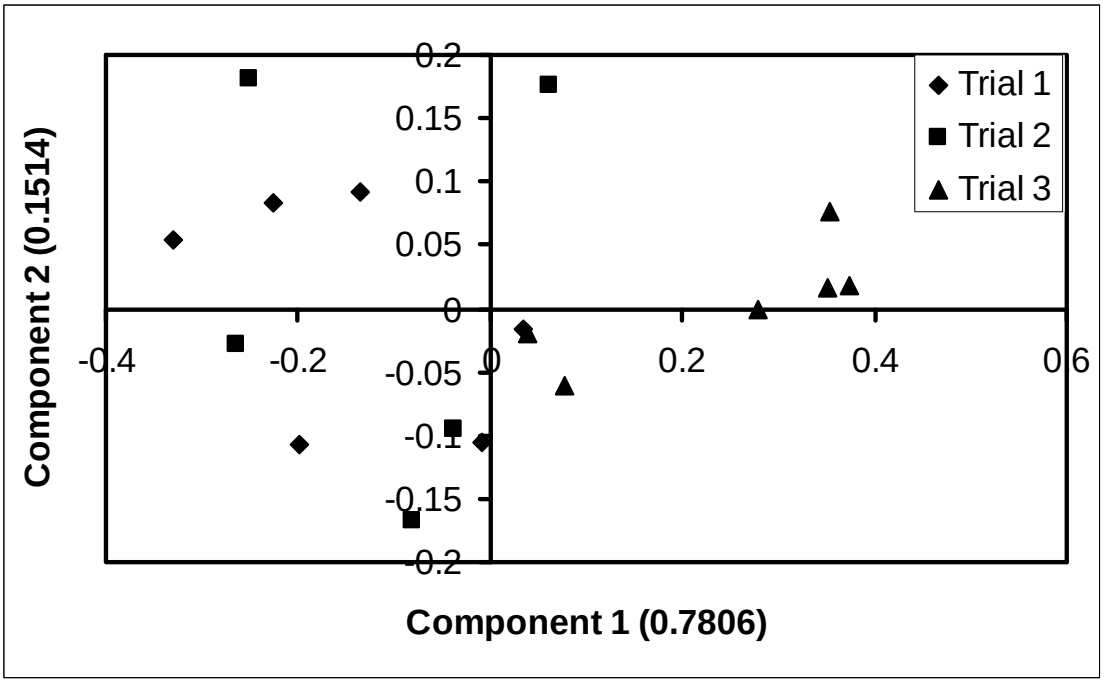


Fig. 2. Results of the principal components analysis for fish location among quadrants for feeding trials one, two, and three. Feeding trials one and two (non-conditioned fish) compared with feeding trial three (conditioned fish). Food was placed in quadrant 3 for all feeding trials. Seventy-eight percent of variation in fish location is explained by component one which is quadrant 3 compared with quadrants 1, 2, and 4. Fifteen percent of variation in fish location can be explained by component two, which is quadrant 1 and quadrant 2 versus quadrant 4. Activity in trial three was concentrated in quadrant III, while more dispersed in trials one and two.

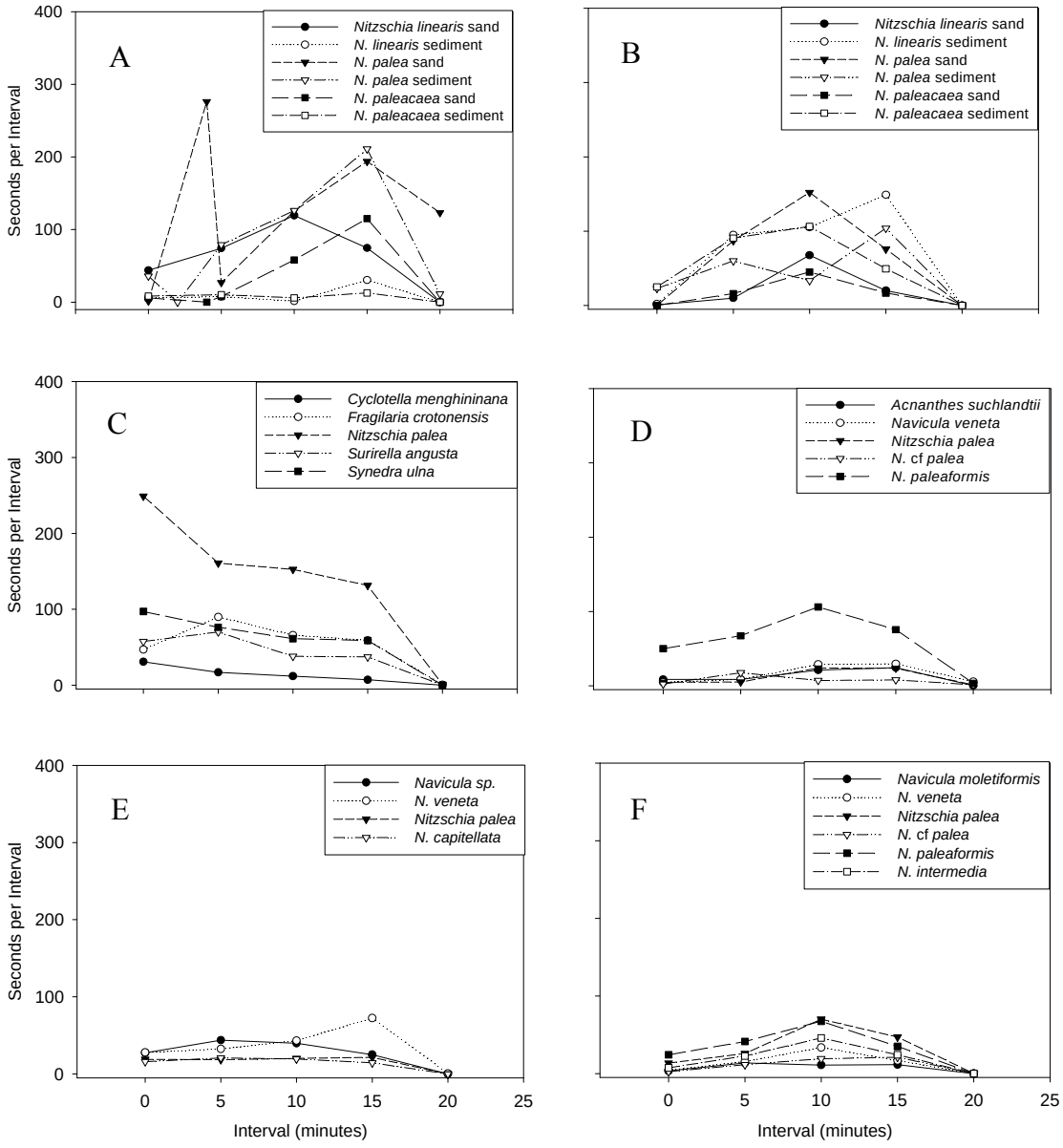


Fig. 3. Magnitude and direction of feeding trials for *H. amarus*. The x-axis is time intervals in five minute segments and y-axis is the number of seconds per five minute interval. Graphs A and B: feeding trials one and two. Food awareness results using non-conditioned *H. amarus* protolarvae. Graphs C and D: feeding trials three and five. Conditioning response results using pre-conditioned *H. amarus* mesolarvae. Graphs E and F: feeding trials four and six. Diatom preference results using non-conditioned *H. amarus* metalarvae.

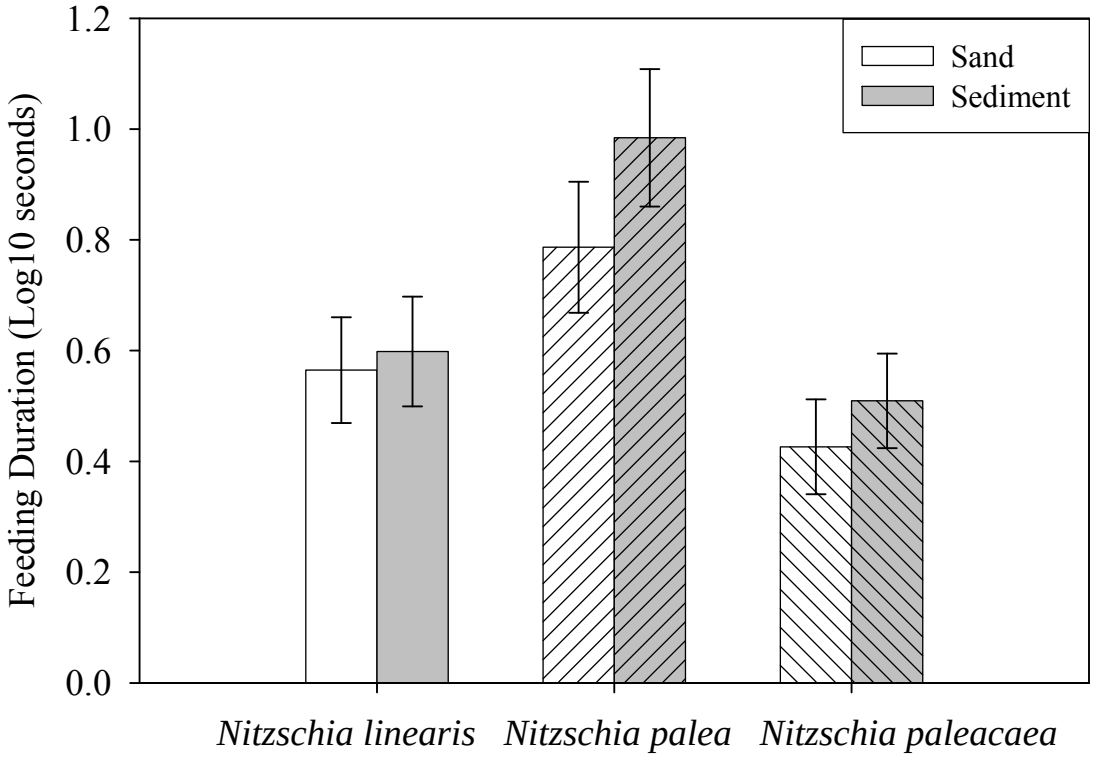


Fig. 4. Diatom preference on two different substrates amended with agar (coarse-grained sand versus fine-grained sediment) and growth media for feeding trials 1 and 2. No significant difference was observed ($p = 0.28$).

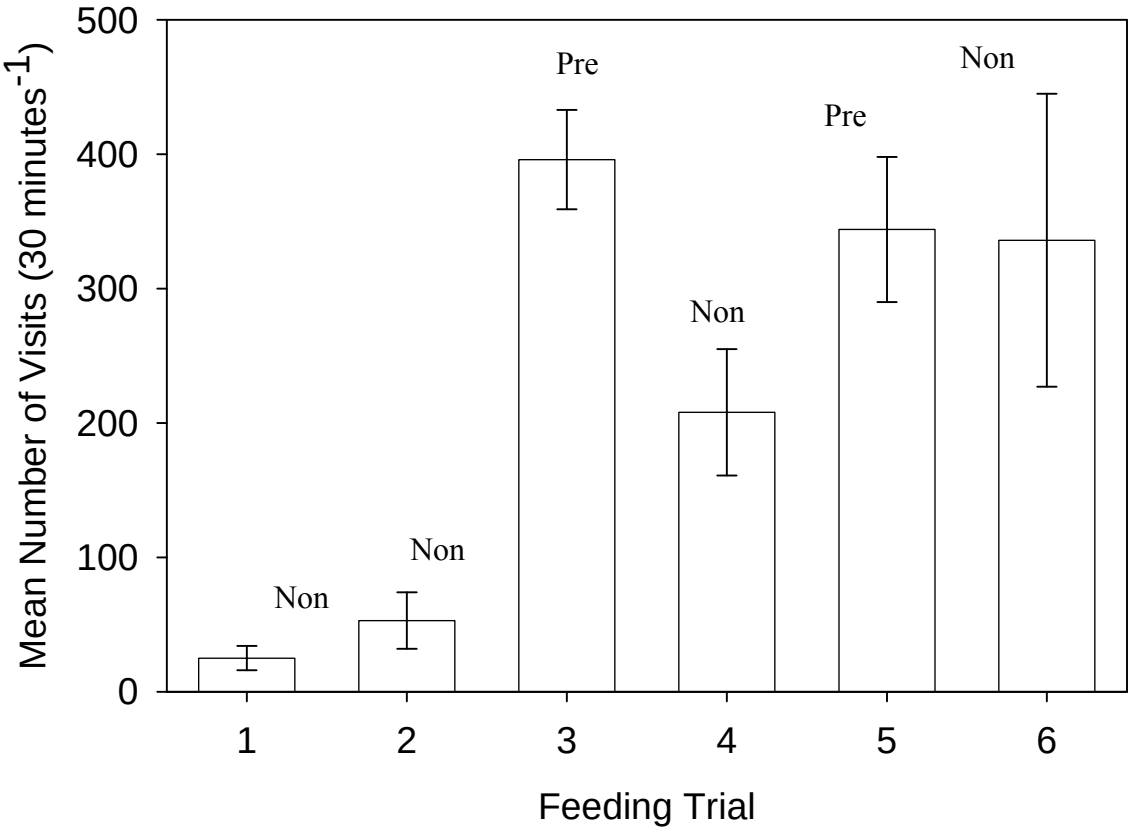


Fig. 5. Number of visits by *H. amarus* to diatom pucks over the course of 30 minute feeding trials. Feeding trials two and four non-conditioned (Non) *H. amarus* protolarvae were randomly selected for use as pre-conditioned fish in feeding trials three and five. There was a 790% increase from feeding trial 2 to 3 and a 165% increase from feeding trial 4 to five. *H. amarus* metalarvae used in feeding trial six were older than other *H. amarus*.

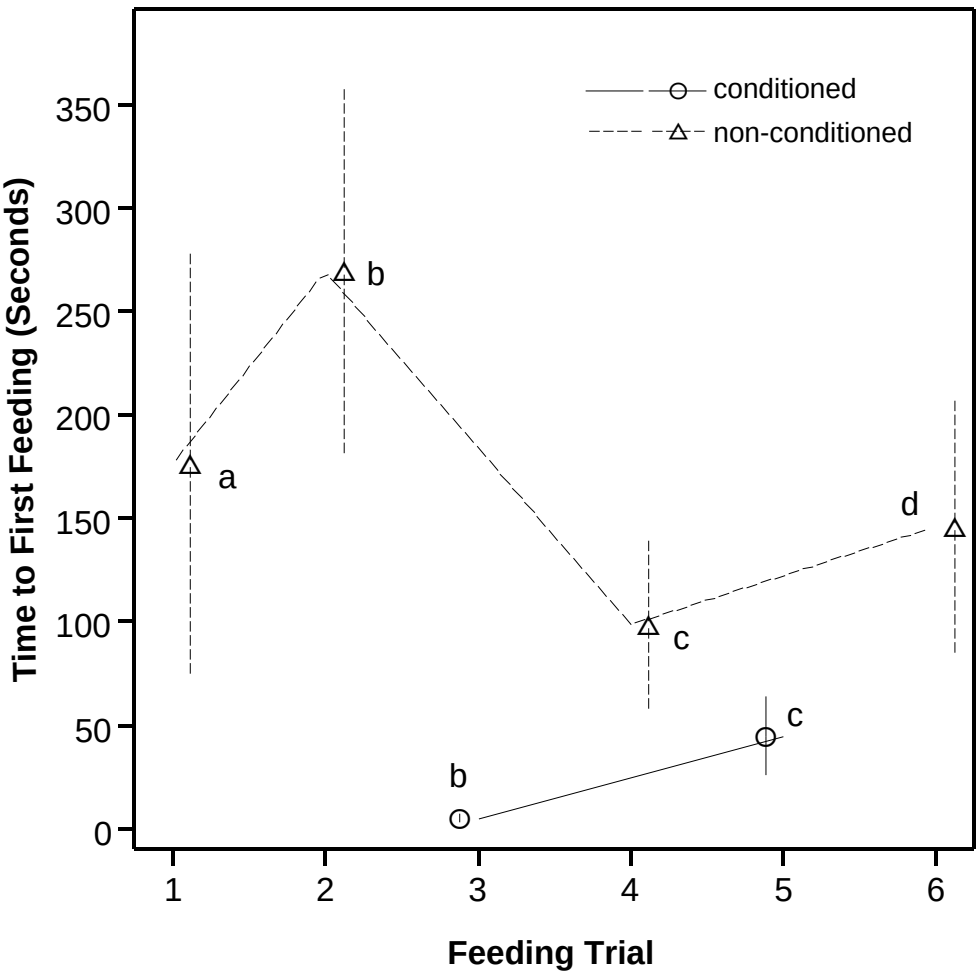
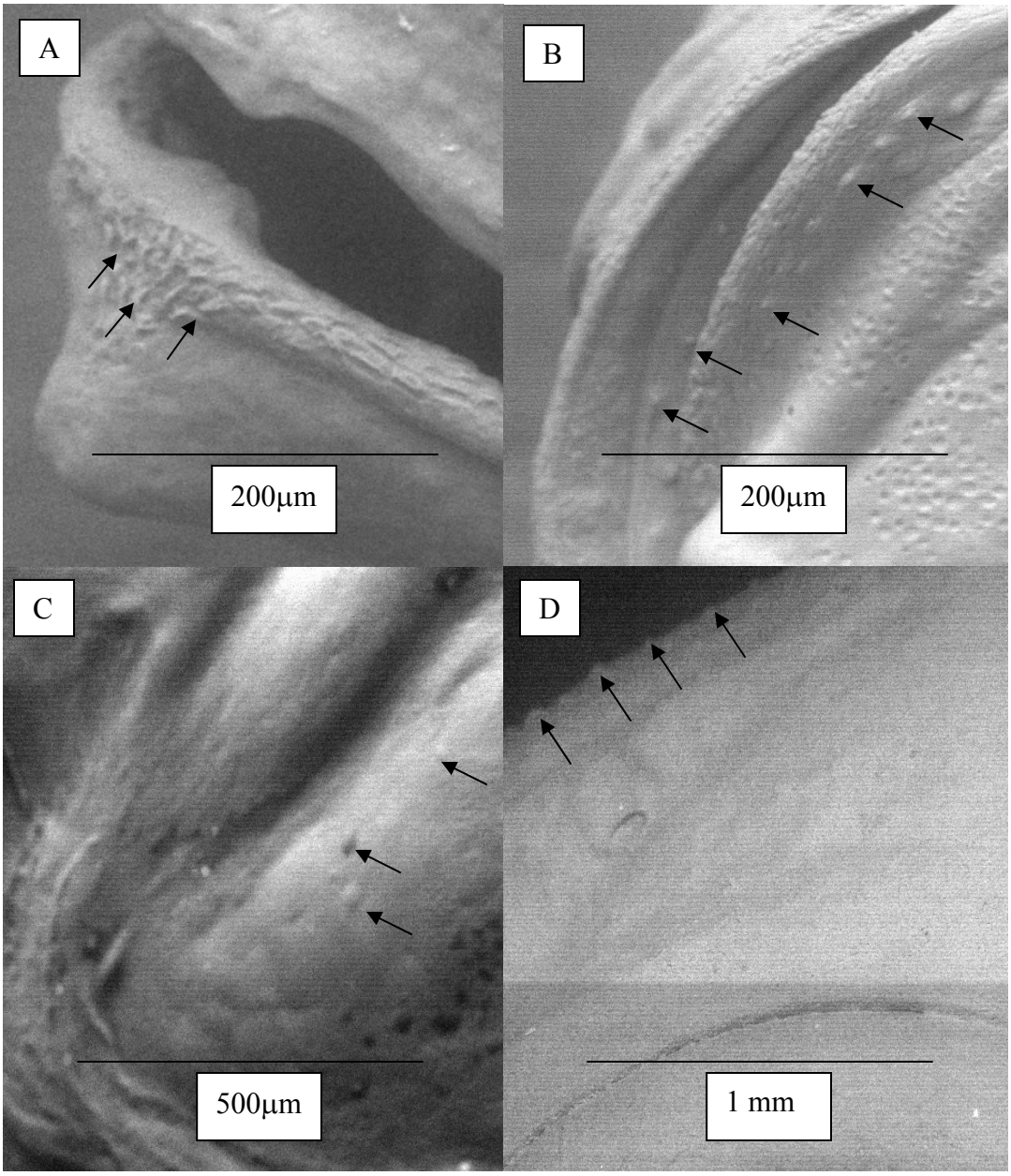


Fig. 6. Results of conditioning response of *H. amarus* to feeding pucks. Letters indicate fish cohorts used in feeding trials. For example, feeding trial one non-conditioned fish were designated as group *a* and mean time to first feeding recorded in seconds. For feeding trial two non-conditioned fish were designated as group *b* and were randomly selected for use as pre-conditioned fish in feeding trial three, and time to first feeding was recorded as conditioning response. Feeding trial four non-conditioned fish were designated as group *c* and were randomly selected for use as pre-conditioned fish in feeding trial five. Feeding trial six measured feeding response of older non-conditioned *H. amarus*.



Figs. 7a, b, c, and d. SEM micrographs (A) preserved two-week old *H. amarus*. Arrows point to developing putative taste papillae on mandible. (B) Preserved six-month old *H. amarus*. Arrows point to putative taste papillae inside mouth and on mandible. (C) Preserved 18 month old *H. amarus*. Arrows point to putative taste papillae inside mouth. (D) Preserved 18 month old *H. amarus*. Arrows point to putative taste papillae on ventral surface of head.

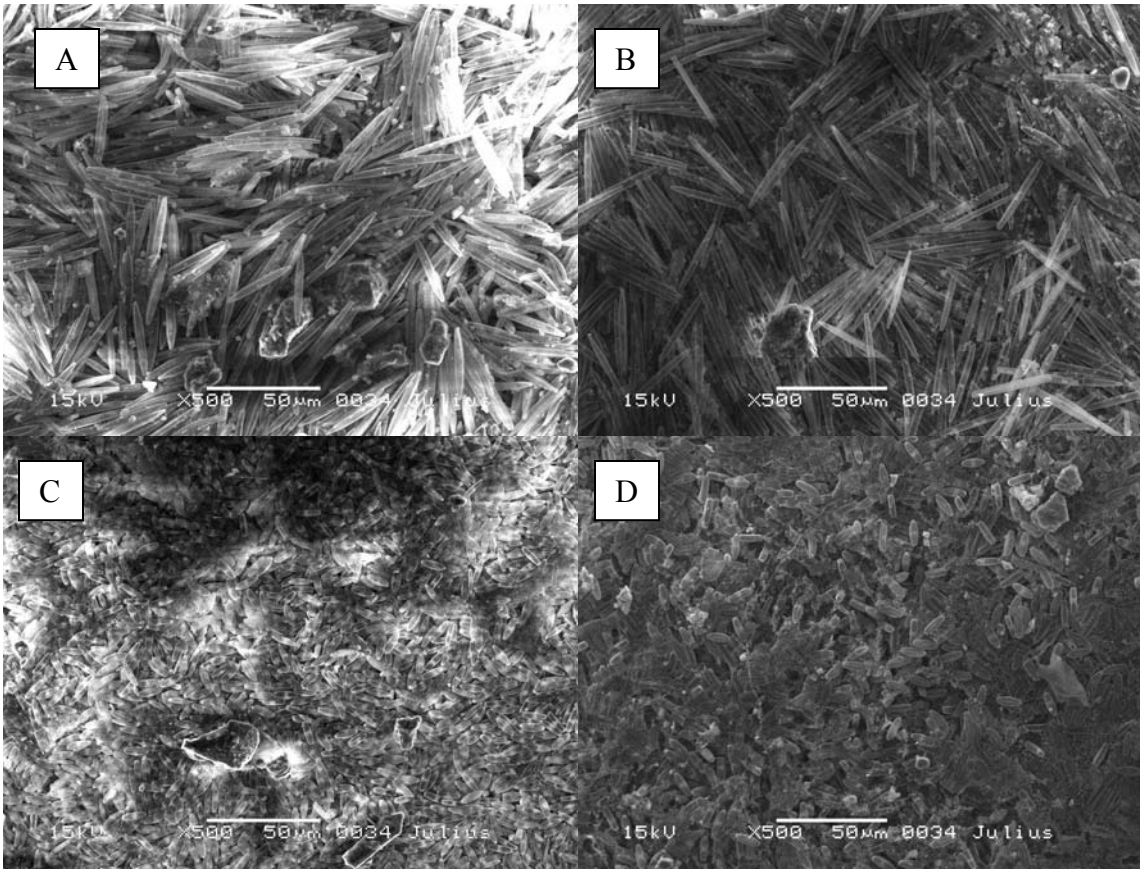


Fig. 8a, b, c, and d. Erect growth form of preferred diatoms *Nitzschia palea* (panel A) and *N. paleaformis* (panel B). Prostrate growth form of least preferred diatoms *Nitzschia molestiformis* (panel C) and *Achnanthes suchlandtii* (panel D).

CHAPTER 3: Flood pulse trophic dynamics in a restored arid-land river-floodplain

Introduction

Current views of the structure and function of large river ecosystems are based on the seminal paper by Lindeman (1942). Lindeman (1942) focused on pond "ooze" that is a mix of allochthonous and autochthonous carbon. Subsequently, Lindeman's paper became the foundation for future work concerning the dynamic flow of energy in plant and animal communities (Cook 1977). Trophic dynamic hypotheses (e.g. the River Continuum Concept (RCC) (Vannote et al. 1980), the Flood Pulse Concept (FPC) (Junk et al. 1989), and the Riverine Productivity Model (RMP) (Thorp and Delong 2002) have evolved over the past three decades and have advanced our understanding of the processes that regulate trophic structure in lotic aquatic ecosystems (Shurin et al. 2006). There are two schools of thought with respect to the carbon source in the trophic cascade. One perspective is that local autochthonous production supports large river food webs (Thorp and Delong 1994, Thorp and Delong 2002, Bunn et al. 2003). Alternatively, allochthonous production supports riverine food webs (Vannote et al. 1980, Ward and Stanford 1983, Junk et al. 1989).

The advent of stable isotope analyses has allowed researchers to identify food sources and trophic position of organisms in aquatic habitats. Investigators have found that the $^{13}C/^{12}C$ ratio of an organism should reflect the $^{13}C/^{12}C$ of its food source (DeNiro and Epstein 1978, Hamilton et al. 1992, Rosenfeld and Roff 1992, Thorp et al. 1998, Vander Zanden and Rasmussen 1999, Vander Zanden and Rasmussen 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).

Stable isotope analyses have an advantage over traditional techniques, such as gut content analysis, in that the $\delta^{13}C$ and $\delta^{15}N$ ratios of a consumer provide a time-integrated measure of assimilated prey, rather than a list of prey items encountered in the diet (Vander Zanden and Rasmussen 1999, Herwig et al. 2004). Consumers integrate prey $\delta^{13}C$ over a relatively long period of time (weeks to years) depending on body size and growth rate (Finlay 2001). Use of stable isotope techniques to quantify food web relationships requires *a priori* estimates of enrichment or depletion in $\delta^{15}N$ and $\delta^{13}C$ value between prey and predator known as trophic fractionation (hereafter $\Delta\delta^{15}N$ and $\Delta\delta^{13}C$) (Vander Zanden and Rasmussen 2001). Previous trophic fractionation studies have demonstrated that $\Delta\delta^{13}C$ from prey to predator can range from 0 to 1‰ (Peterson and Fry 1987) or ±1‰ (DeNiro and Epstein 1978). Vander Zanden and Rasmussen (2001) in their study of 20 temperate lakes reported an overall trophic fractionation value of approximately +0.47‰ from prey to predator.

Many aquatic stable isotope studies have been carried out in mesic temperate rivers (e.g. Eckblad et al. 1984, Rosenfeld and Roff 1992, Thorp et al. 1998, Herwig et al. 2004, Jardine et al. 2005) and temperate lakes (Keough et al. 1996, Vander Zanden and Rasmussen 1999, Vander Zanden and Rasmussen 2001, Carpenter et al. 2005). In contrast, arid lands of the southwestern United States that are plagued with droughts and variable flows (Molles and Dahm 1990) have not received as much attention with regards

to stable isotope analyses of river and floodplain foodwebs. In the highly regulated middle segment of the Rio Grande in central New Mexico, little information is available with respect to aquatic trophic interactions in the river and floodplains.

Water of the Rio Grande is intensively managed and regulated by international and interstate compacts, Native American treaties, local water rights, and Federal and local agencies (Crawford et al. 1996). Flooding of irrigated and inhabited lands in the first half of the 20th century prompted the Rio Grande Project (1950s-1970s) to create a series of large dams to control flooding and sedimentation (Lagasse 1980). The last major dam on the Rio Grande, Cochiti Dam, was completed in 1975 and regulates flow through the Middle Rio Grande (MRG) of central New Mexico. The most significant ecological effect of Cochiti Dam was to diminish the river's historic flooding regime (Crawford et al. 1996, Dahm et al. 2003).

Dams on the Rio Grande have arrested most overbank flooding in the MRG, with the last major flood occurring in 1941-1942 (Molles et al. 1998). Located between Cochiti and Elephant Butte Reservoirs are three major diversion dams built in the 1930s that fragment the MRG into distinct reaches (Fig. 1). Consequently, the active channel width has been decreasing since the 1930s (Makar et al. 2006). Flow and most riparian forests are confined to the area between levees (Crawford et al. 1996, Molles et al. 1998, Massong and Slaugh 2002), where much of the floodplain has become abandoned through degradation and aggradation of the channel bed (Massong et al. 2006).

In the southwestern U.S., virtually the entire native river fish fauna is listed as threatened or endangered under the Endangered Species Act, largely as a consequence of water withdrawal, flow stabilization, and exotic species proliferation (Poff et al. 1997). The federally endangered Rio Grande silvery minnow (*Hybognathus amarus*) is an endemic, small-bodied, cyprinid fish, which now occupies only 5% of its historic range (Bestgen and Platania 1991). *Hybognathus amarus* is described as primarily an herbivore, indicated by its elongated gastrointestinal tract (Propst 1999). It is believed that minnows feed on diatoms, other algae, larval insect exuvia, partially decayed organic matter, and plant material scraped from "ooze" in bottom sediment (Whitaker 1977). Because feeding habits are poorly known (Cowley 2003, Porter and Massong 2003), it is difficult to assess if changes in the food base have contributed to the decline of the *H. amarus* population.

Cushing (1990) described a match/mismatch hypothesis that extended Hjort's (1914) critical period hypothesis suggesting that the degree of match and mismatch in the time of larval fish production and primary productivity could explain variability in fish stock recruitment in the North Atlantic. Cushing (1990) concluded that fish in temperate waters should release their larvae during the spring or autumn peaks in the production cycle, when more food is available. Similarly, an important issue for recovery of *H. amarus* populations in the MRG may be food; including the availability, quality, and quantity of food sources for the minnow.

Existing literature describes *H. amarus* as a herbivore, however, hydrodynamic scouring

during floods all but eliminates the benthic periphyton community. As a result, the objective of this study was to identify food sources for *H. amarus* in a restored floodplain during a flood pulse in the Rio Grande using stable isotope analyses to trace carbon flow through the food web.

Methods

The study site, the Los Lunas, New Mexico (NM) Habitat Restoration Project, is located on the west bank of the Rio Grande adjacent to the Mid Valley Airpark, Los Lunas, NM (Fig. 1). Dimensions of the overbank area are approximately 1829 m along the existing riverbank with a uniform width of 107 m encompassing an area of approximately 16.2 hectares (Fig. 2). The site is bounded on the west by an earthen and rootwad berm approximately two meters high. The restoration area burned in a severe fire in April of 2000. Vegetation consisted of dry grasses, salt cedar, willows, and cottonwoods. The floodplain was constructed to have varied topography that produced inundation of the floodplain areas at flows of greater than or equal to 71 m^3 s^{-1} and to ensure some inundation at a wide range of flows less than 71 m^3 s^{-1}.

The study took place in 2005 during an unusually wet year. The snow pack in northern New Mexican Mountains was 195% of normal levels (NCDC 2005). Discharge data for this study were taken from a US Geological Survey (USGS) gage station nearest the study site (Fig. 3). The predicted discharge at Los Lunas was based on a linear regression model (r^2=0.73) of the Albuquerque and Bernardo, NM, USGS gages during a previous spring pulse-overbank event in 2003. Predicted discharge at Los Lunas was calculated at

between 19 and 29 m^3 s^{-1}.

The flood pulse release of 2005 lasted approximately 98 days. This study was initiated at peak discharge (~198 m^3s^{-1}), on 24 May 2005 during a prolonged hypolimnetic release from Cochiti Reservoir (8 April-17 July 2005) and continued during approximately the last 44 days of the descending limb of the hydrograph (Fig. 3).

Fish collection and processing

Light traps for larval fish were deployed during this study and followed the design of California Fisheries Management (Aquatic Research Instruments Inc., ARII). Aquatic Research Instruments Inc. traps are designed in a four-point star configuration with a central tube for chemical light stick illumination. When compared to traditional quatrefoil design of larval fish light traps, the ARII trap has a much larger chamber. The entrance is wider (30 cm vs. 10 cm) and slightly angled (25^o) towards the entrance slit (10 mm). The ARII light traps were effective in capturing larval fish and aquatic invertebrates concurrently.

The sampling design consisted of collecting fish larvae using six larval light traps at six sampling sites representing three habitat types. Sampling sites (slow-water habitats) were perpendicular to river flow (0.06 vs. main channel 0.80 m/s), side channel (~0.11 m/s), or leeward side of islands (~0.01 m/s). Light traps were deployed at dusk and retrieved at dawn on May 24, June 1, 8, 14, 21, and 28 at six permanent habitat locations. Light traps were anchored to a metal post in water less than 1 m deep (39 cm ± 1.9 SE). At dawn,

larval fish were removed from the cod-end of the trap and placed into 250 ml polycarbonate bottles of ice water. Alka-Seltzer® tablets were added to anesthetize fish via CO_2 narcosis (Wall 1993). Fish were then placed in 5% buffered formalin for 48 hrs, 35% ethanol for 7 days, and transferred to 70% ethanol for long-term preservation (Wall 1993, Pease et al. 2006).

In the laboratory, fish specimens were identified to species using Snyder (1976) and reference specimens from the Museum of Southwestern Biology (MSB) at the University of New Mexico (UNM). A subset of captured *H. amarus* (n = 25, SL 4.6-19.8 mm) were examined and guts were excised from larger specimens and small specimens used whole. Fish specimens/guts were placed into 30 % H_2O_2 and heated for 30 minutes. After cooling, HNO_3 (70%) was added and heated for 30 minutes. Digested samples were transferred to deionized water (DI) and centrifuged at 1,500 rpm for 10 minutes, the supernatant was aspirated down to 10 ml, filled to 25 ml with DI, shaken, and filled to 50 ml with DI. The centrifugation and rinsing process was repeated six times until a circumneutral pH was achieved. Permanent slides were prepared from 1 ml aliquots of sample and allowed to dry for 16-18 hours. Dried samples were heated and mounted onto a microscope slide with Naphrax® mounting media. Mounted diatom frustules were identified using keys and descriptions by Krammer and Lange-Bertlot (1999).

Aquatic Invertebrates

Using the ARII larval fish light traps I collected benthic aquatic invertebrates simultaneously with larval fish. All aquatic invertebrates were removed from light traps

at dawn and placed into 70% ethanol. Aquatic invertebrates were identified and enumerated by Dr. Jerry Jacobi (Jacobi and Associates). Specimens were categorized into functional feeding groups according to Merritt and Cummins (1996). The four functional feeding groups were designated as follows; collector/gatherers, predators, scrapers/grazers, and shredders. Larger individuals were digested and prepared for microscopic examination of gut contents as described above for fish specimens. Smaller specimens (e.g., chironomids) were pooled together (five individuals) and acid digested. Guts were not excised from specimens because Jardine et al. (2005) reported that stable isotope signatures of body tissue and gut contents were highly correlated ($r = 0.94$ and $r = 0.93$, respectively).

Diatoms

Repeated attempts to collect periphyton and other autochthonous components during the flood pulse were unsuccessful due to hydrodynamic scouring and elevated turbidity. Therefore, periphyton samples were collected at the study site in December 2005 and January 2006 according to USGS National Water Quality Assessment Protocols (Moulton et al. 2002) and transported to US Department of Agriculture (USDA), Forest Service, Rocky Mountain Research Station (RMRS) Albuquerque, NM for processing. Periphyton samples were acid digested and prepared for microscopic examination in the same manner as fish and aquatic invertebrate specimens.

Water Chemistry

I collected water samples (60 ml) contemporaneously with light trap deployments for water chemistry analysis. Samples were placed on ice and delivered to the UNM Biology Annex for processing. Water samples were analyzed for dissolved NO_3-N and soluble reactive phosphorus (PO_4-P) with a Dionex-500 Ion Chromatograph (Dionex, Sunnyvale, CA) EPA 300.1 standard method (Environmental Protection Agency 1997) (detection limits of 0.003 and 0.005 mg/L respectively). Ammonium (NH_4-N) was analyzed using a Technicon® two channel autoanalyzer using automated phenate standard method 4500-NH3-G (American Public Health Association 1998) and No. 98-70W (Technicon Industrial Methods 1973) (detection limit of 0.01 mg/L).

Environmental parameters

I used a multi-probe meter (YSI 556) to measure water quality parameters (temperature (^oC), conductivity (μSiemens cm^{-1}), dissolved oxygen (mg/L), % saturation dissolved oxygen (%DO), and pH adjacent to the light traps. I also recorded depth (cm), velocity (cm s^{-1}), and light quantum values (μmoles m^{-2} s^{-1}). Depth was measured using a stadia rod (Crain Enterprises Inc. model # 90370). Water velocity was measured at 0.6 times total depth using a flow pressure sensor (Marsh-McBirney Model 2000). Light was measured using a Li-Cor quantum meter (Li-Cor Biotechnologies model Li-Cor 1000 and 4π quantum sensor model Li-193SA).

Isotope analysis

Stable carbon and nitrogen isotope ratios were determined for a subset of fish muscle tissue (n=80), aquatic invertebrates (n=103) sampled during the study period, and algal (n=10) specimens collected later in the year. Fish, aquatic invertebrates, and algae were analyzed to identify intraspecific variance of isotope ratios. Samples were dried in a constant temperature oven (Yamato Scientific American Inc. model DKN 810) at 70^{o}C for 48 hrs, weighed, and packed into 5 x 9 mm tin capsules and analyzed at the University of California-Davis Stable Isotope Facility for multiple stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$). Isotopic analyses were performed on a Europa Hydra 20/20 continuous flow mass spectrometer with 0.1 ‰ reproducibility for carbon (CO_2) and 0.2 ‰ reproducibility for nitrogen (N_2). The $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios of samples were determined and data were reported as δ values relative to standard gases (i.e., $\delta^{13}C$ and $\delta^{15}N$, respectively) calibrated against PDB limestone carbonate (CO_2) and atmospheric nitrogen (N_2). In accordance with convention, carbon and nitrogen isotope ratios are expressed as parts-per-thousand (per mil, ‰) difference between the sample and PDB standard or atmospheric N_2 (Peterson and Fry 1987):

$\delta^{13}C$ or $\delta^{15}N = [(R_{sample} / R_{standard}) -1] * 1000$, where $R = {}^{13}C/{}^{12}C$ or $^{15}N/^{14}N$.

Statistical Analyses

Two similarity indices for fish and invertebrate data were employed to compare fish and invertebrate composition between trap locations. The Chao-Jaccard, is a Jaccard coefficient weighted by abundance (Chao et al. 2005) and percent similarity was used for quantitatively comparing the species composition of a multi-species sample with another.

I employed linear regression and analysis of variance (ANOVA) to determine the relationship among fish, invertebrates, and environmental parameters. A scatterplot was generated for $\delta^{15}N$ and $\delta^{13}C$ versus each candidate explanatory variable. A linear regression between $\delta^{15}N$ or $\delta^{13}C$ versus each explanatory variable was computed with functional feeding group (FFG) or fish species included as a class variable and as an interaction with the explanatory variable.

Results

Fish species diversity

A total of 394 fish larvae were collected from four genera of the Family Cyprinidae (*Pimephales*, *Hybognathus*, *Cyprinella* and *Cyprinus*) over the five-week sampling period (Table 1). The most abundant species was *P. promelas* comprising 57% of total fish captured with *H. amarus* comprising 32% of captures. *C. lutrensis* and *C. carpio* comprised 8% and 3% of captures, respectively.

Results from Chao-Jaccard similarity indices indicate that relative contribution to (dis)similarity index was highest in *P. promelas* at 64% followed by *H. amarus* at 33%. *Cyprinella lutrensis* and *C. carpio* played a lesser role (Table 1). Results from similarity analysis using percent similarity reveal that the relative contribution was highest in *H. amarus* at 42% followed by *P. promelas* at 40%. *Cyprinella lutrensis* and *C. carpio* represented 7 and 6%, respectively (Table 1).

Variation among light trap locations was minor and was of comparable magnitude to variation among dates. A linear regression model using nitrate concentrations as an independent variable explains over 50% ($r^2 = 0.58$, $p = 0.001$) of the variability in abundance of *H. amarus*. A second linear regression model using conductivity as an independent variable explains over 90% ($r^2 = 0.94$, $p = 0.029$) of the variability in abundance of *C. lutrensis*.

Gut content analysis

Gut contents of *H. amarus* (n=25) were processed to a final volume of 50 mL. Microscopic examination (1000x) of gut content slides revealed that each 1 ml sample yielded between 0-8 diatom valves per fish (mean 3.33, ± SE 0.48). Eighteen (72%) of the microscope slides contained no diatom valves in 2005. 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. The mean number of diatom valves per fish averaged 28.8 ± SE 11.4.

Aquatic Invertebrate diversity

Eleven orders, 14 families, and 17 genera (n=1294) of invertebrates were captured during the sampling period (Table 2). The Family Corixidae dominated the community composition (37%) followed by Baetidae (25%) and Chironomidae (20%). These three families comprised 82% of the benthic invertebrate community for the sampling period. Aquatic invertebrates were organized by functional feeding groups according to Merritt and Cummins (1996).

During the first four sampling dates the aquatic invertebrate community was dominated by collector/gatherers (Chironomidae, 48-81%) followed by predators (*Graptocorixa*, 11-45%) (Fig. 4). Predators dominated the community structure (Corixidae *Graptocorixa*, 79-80%) during the fifth and sixth week. Scrapers/grazers and shredders comprised a small portion of the aquatic invertebrate community during the study period (Heptogeneaidae *Heptogenia*, 2-4% and 1-10%, respectively).

Results from Chao-Jaccard similarity indices reveal that relative contribution was highest in Corixidae at 37% followed by Chironomidae and *Baetis* at 20 and 13%, respectively. The remainder of aquatic invertebrates did not make up a significant part of the community (Table 3). Results from the similarity analysis reveal that relative contribution was highest in Corixidae at 24% with *Baetis* and Chironomidae representing 19 and 14%, respectively (Table 3). Analysis results are similar between the two coefficients. A linear regression model using trap location as an independent variable explains over 60% of the variability in abundance of Baetidae *Baetis* ($r^2 = 0.64$). A linear regression model using trap location as an independent variable explains 48% of the variability in abundance of chironimids ($r^2 = 0.88$).

Gut content analysis

Gut contents of aquatic invertebrates (n=35) were processed to a final volume of 50 mL. Microscopic examination (1000x) of gut content slides revealed that only the scrapers *Physella* (Physidae) and *Heptogenia* (Heptogeniidae) contained diatom valves. *Physella*

fed primarily on the diatom *Cocconeis placentula* (Fig. 5) and *Heptogenia* fed on the diatoms *Nitzschia palea*, *N. linearis*, *Hanzschia virgata*, and *Craticula cuspidata*.

Water chemistry

Surface nutrient concentrations (NO_3-N, PO_4-P, and NH_4-N) varied during the sampling period (Fig. 6). During the first sampling date (24 May 2005) average nitrate concentration measured 116 μg/L and increased to a high of 226 μg/L on 28 June. Mean phosphate concentrations ranged between 45-65 μg/L from 24 May to 14 June and increased to 96 and 116 μg/L the last two sampling dates. Initial mean ammonium concentration measured 39 μg/L, and remained lower than nitrate concentrations (26-51mg/L) throughout the sampling period. There was an overall decrease in ammonium concentration through time.

Isotope Analysis

No significant statistical relationships were found between $\delta^{15}N$ or $\delta^{13}C$ values, water chemistry parameters (temperature, conductivity, DO, %DO, pH, depth, velocity, and nutrients), functional feeding groups and fish species.

Fish

Results for carbon isotope ratios for preserved larval fish were corrected by adding ~1.1‰ to measured $\delta^{13}C$ and by subtracting ~0.5‰ from measured $\delta^{15}N$ (Edwards et al. 2002). Corrected $\delta^{13}C$ values for fish (n = 80) ranged from -24.9 to –17.6‰ and $\delta^{15}N$ ranged from 6.7 to 12.5‰. Mean isotopic values are depicted in Fig. 8 and listed in

Table 4. The isotopic data showed high intraspecific variability in $\delta^{13}C$ up to 6.5‰ (-24.9 to -18.4‰) for *H. amarus*. *C. lutrensis* had the narrowest $\delta^{13}C$ range of 3.9‰ (-21.5 to -17.6‰). Ranges of *P. promelas* (-22.5 to -18.1‰) and *C. carpio* (-23.4 to -17.4‰) $\delta^{13}C$ values were intermediate to *H. amarus* and *C. lutrensis* (4.4‰ and 6‰, respectively).

H. amarus captured in perpendicular and leeward locations of the floodplain had similar $\delta^{13}C$ values (-23.5 to -19‰) while *H. amarus* captured off-channel had a wider range of $\delta^{13}C$ values (-25.5 to -17.5‰). *H. amarus* captured in 2004 during a drier than normal year at the Los Lunas site had similar low end $\delta^{13}C$ values, but more enriched high-end values (-22.6 to -16.5‰).

Results of $\delta^{13}C$ values for *H. amarus* compared with DO, pH, and NO_3-N values revealed two groups where levels were elevated (DO, 10.6 mg/L, pH, 9.2, and nitrate, 706 μg/L) (Fig. 8). One group was clustered between -17.8 to -16.2‰ and the other group was clustered between -22.5 to -22.8‰.

Trophic Fractionation

Possible food sources for *H. amarus* were calculated for isotopic fractionation (Δ) by subtracting observed $\delta^{13}C$ and $\delta^{15}N$ values of prey from δ values for *H. amarus* (Table 5). The closest prey items were chironomids and predatory invertebrates. When the differences were calculated, chironomids had a $\Delta\ \delta^{13}C$ and $\Delta\ \delta^{15}N$ of 0.5 and 2.8‰, respectively while odonates had $\Delta\ \delta^{13}C$ and $\Delta\ \delta^{15}N$ values of 0.8 and 2.2‰, respectively.

Invertebrates

The $\delta^{13}C$ and $\delta^{15}N$ values of primary consumers were highly variable, ranging from -27.1 to -16.6‰ and 1.09 to 13.32‰, respectively (Table 6). Chironomids and Corixidae displayed the widest range in $\delta^{13}C$ (7.9 and 10.5‰, respectively) and $\delta^{15}N$ (10.1 and 7.7‰, respectively). *Isonychia sp.* displayed the narrowest range in $\delta^{13}C$ and $\delta^{15}N$ values (0.6 and 1.5‰, respectively).

Algae

Only one multi-species algal sample was obtained concurrently with fish and invertebrate sampling and had $\delta^{13}C$ and $\delta^{15}N$ values of -19.5 and 1.2‰, respectively.

Periphyton samples (n = 5) collected in December 2005 had a $\delta^{13}C$ and $\delta^{15}N$ signatures of -17.5 and 3.4‰, respectively. January 2006 periphyton samples (n = 2) had a $\delta^{13}C$ signature of -16.6‰ and $\delta^{15}N$ signature of 5.9‰. Periphyton samples (n = 2) from February 2006 had a $\delta^{13}C$ and $\delta^{15}N$ signatures of -17.0 and 8.9‰, respectively.

Discussion

Delta ^{13}C isotopic signatures from other studies reveal wide variability for fish species in aquatic ecosystems. Values of $\delta^{13}C$ for river fish in temperate rivers of Ontario, and Quebec Canada, ranged from -31 to -23.8‰ (Rosenfeld and Roff 1992, Vander Zanden and Rasmussen 1999) and the Ohio River, Ohio, -26 to -23‰ (Thorp et al. 1998). The $\delta^{13}C$ values of fish from Lake Superior ranged from -31 to -26.8 (Keough et al. 1996). The $\delta^{13}C$ values for *H. amarus* from this study at Los Lunas were less depleted in $\delta^{13}C$ compared with *H. amarus* from Bosque del Apache in southern New Mexico (-22.5 to -

18.5 and -24.9 to -18.4‰, respectively) reported by Pease et al. (2006). 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. Intraspecific variability in $\delta^{13}C$ is food related and these differences tell us that food sources or proportion of food sources varied and was indirectly due to discharge and/or habitat type.

Hybognathus amarus captured in perpendicular and leeward locations in the floodplain had similar $\delta^{13}C$ values while *H. amarus* captured off-channel had a wider range of $\delta^{13}C$ values. This suggests that off-channel habitats offered a wider selection of food sources to the fish than within channel habitats. The $\delta^{13}C$ values and gut content analysis also confirmed that *H. amarus* collected in 2004 consumed more diatoms than *H. amarus* collected in 2005.

Comparing *H. amarus* $\delta^{13}C$ values to DO, pH, and NO_3-N values revealed two groups where environmental levels were elevated (DO, 10.6 mg/L, pH, 9.2, and nitrate, 706 µg/L) (Fig. 7)**.** One group was clustered between -17.8 to -16.2‰ indicating an algal diet while the other group was clustered between -22.5 to -22.8‰ indicating that aquatic invertebrates also comprise part of the diet.

Hansson et al. 1997 investigated three Baltic Sea areas influenced by ^{15}N-rich nutrient discharges from sewage treatment plants and found discharges significantly increased $\delta^{15}N$ values in the whole food web, from phytoplankton to piscivorous fish. Schlacher et al. (2005) investigated fish from three estuaries of which one received treated sewage,

one without licensed treated wastewater outfalls, but did have marinas and harbors, and another neither received discharges nor had suspected wastewater loads. Of the 19 fish species sampled, those from the impacted estuary had significantly elevated $\delta^{15}N$ values (up to 9.9‰). Schlacher et al. (2005) concluded that enrichment of $\delta^{15}N$ signatures in fish muscle tissue result from wastewater loading, and that fish-$\delta^{15}N$ is a suitable indicator of wastewater-N inputs. Delta ^{15}N values for *H. amarus* were more enriched in 2004 than 2005. Inspection of nutrient results for 2004 revealed that nitrate concentration was four times higher than in 2005 (589 vs. 177 μg/L), and may be due to the close proximity (< 1.6 km) of the Los Lunas wastewater treatment facility to the Los Lunas site.

The number of families and genera of aquatic invertebrates that were sampled may not be representative of aquatic invertebrates in the MRG because the sampling method (light traps for larval fish) may have introduced sampling bias for certain species of aquatic invertebrates. Sampling with a light trap would select for those aquatic invertebrates that are positively phototactic. Aquatic invertebrates that represented greater than 5% of total captures with the light traps were *Graptocorixa* sp. (Corixidae), chironomid spp. (Chironomidae), *Baetis* sp. (Baetidae), and *Centroptilum* sp. (Baetidae) (Table 2). A high percentage of the aquatic invertebrates captured were either predators or mayflies (Fig. 4). Quantitative sampling estimates for aquatic invertebrates for this reach of the Rio Grande are not available. Concurrent sampling with light traps and with quantitative benthic samplers for aquatic invertebrates is needed to address the degree of sampling bias from the light trap method.

Average isotopic values for aquatic invertebrates were consistently less enriched in $\delta^{13}C$ and $\delta^{15}N$ than fish indicating aquatic invertebrates as a possible food source for MRG cyprinid fishes. The $\delta^{13}C$ values for invertebrates were more depleted (–24.2 to –20.6‰) than periphyton (-19.7 to -14.4‰) suggesting that algae and diatoms were either not their primary diet or were not available during the flood pulse. My results show a larger role for allochthonous inputs during the flood pulse of 2005 than other studies (Araujo-Lima et al. 1986, Hamilton et al. 1992, Hamilton and Lewis 1992, and Thorp and Delong 2002). The $\delta^{13}C$ values for predatory insects were very uniform (-22.9 to -22.2), indicating selectivity in food source. Although shredders such as *Nectopsyche* are presumed to consume terrestrial leaves, they had isotopic values more ^{13}C-enriched (-22.51‰) than $\delta^{13}C$ values reported for leaves from dominant riparian plant species along the Middle Rio Grande, (-27.8 to -27‰) (Tibbets 2005).

The closest association between energy sources and fish was aquatic invertebrates. There are two food items reported here as possible candidates (chironomids and predators) as food sources for *H. amarus* as they fall within the expected range of likely food items for the fish. The $\Delta\ \delta^{13}C$ for chironomids to *H. amarus* (0.5‰) is the same as the overall $\Delta\ ^{13}C$ mean value (0.5‰) reported by Vander Zanden and Rasmussen (1999). *H. amarus* possess a small, toothless, sub-terminal mouth, and are morphologically incapable of consuming large invertebrates that possess protruding appendages such as odonates. Results from the stable isotope analyses indicate that chironomids are a likely food source for *H. amarus*. Chironomids are generally small and worm-like and it can be inferred that they can easily be consumed whole by *H. amarus*.

The abundance of *H. amarus* in the Rio Grande has drastically declined. The decline could be due in part to loss of a significant food source that other fish may have not been eating. Evidence provided by Shirey (2004) and Cowley et al. (2006) shows that *H. amarus* specimens collected in 1874 consumed diatoms. Channelization of the Rio Grande has reduced or eliminated most backwaters, edge areas, and slow-water refugia, which are typical habitat for benthic algae. The disturbance regimes of large river ecosystems impose different constraints on organisms in terms of intensity and duration of flooding (Rempel et al. 1999). Where the substrate is mobile or flood intensity is high enough to regularly produce substrate mobility, benthic algal biomass will be reduced (Rempel et al. 1999). Hydrodynamic scouring during floods typically results in periods where sources of benthic primary productivity are not available (Bunn et al. 2006). Unlike most large flowing rivers, phytoplankton are minimal in the MRG because turbidity is continuously high. Light penetration commonly attenuates to zero at 30-40 cm thereby largely eliminating possible benthic algal primary production during the flood pulse.

Measured $\delta^{13}C$ values for algae (-19.5‰) in the MRG were more enriched compared to those reported by Rounick et al. (1982) who reported $\delta^{13}C$ values of -33‰, and Peterson and Fry (1987), who reported $\delta^{13}C$ values for algae between -24 to -19‰. The $\delta^{13}C$ signature of periphyton during the sampling period was generally more ^{13}C-enriched (-19.5‰) compared to aquatic invertebrates (-24.1 to -20.5‰) or fish (-24.9 to –16.45‰). The $\delta^{13}C$ values for the benthic algae reported here are too enriched to serve as a primary

food source to most of the invertebrates collected in this study. The $\delta^{13}C$ values of benthic algae collected at Los Lunas later in the year (December 2005 and January 2006) were much more varied, but within the range of potential food sources for primary consumers (-22.3 to -15.8‰).

A single periphyton sample collected in June 2005 had a $\delta^{15}N$ signature of 1.2‰, which is indicative that the sample contained a high proportion of nitrogen-fixing cyanobacteria. Microscopic examination confirmed the dominance of the cyanobacteria *Oscillatoria* sp. in the sample. Delta ^{15}N values for benthic algae collected later in the year (December 2005 and January 2006) were extremely variable (2.14 to 4.3‰). Microscopic examination of field samples revealed that the cyanobacteria (*Oscillatoria* sp.) were the dominant algae in some samples but not all samples.

Conclusions

H. amarus has been classified by various investigators as an herbivore and detritivore (Propst 1999) or carnivore (Pease et al. 2006), but results from this study suggest that *H. amarus* is an opportunistic feeder and should be classified as an omnivore. During low flow conditions, *H. amarus* is primarily algivorous as previously reported by Shirey (2004) and Cowley (2006). However, during flood conditions, hydrodynamic scouring eliminates or reduces benthic algal food resources. Therefore, *H. amarus* makes use of other food sources, primarily chironomids, during and immediately after floods. Chironomids are an abundant food source found in the Middle Rio Grande (Weibell 2007).

Consumer $\delta^{13}C$ values integrate prey $\delta^{13}C$ over relatively long periods of time (weeks to years) depending on body size and growth rate (Rosenfeld and Roff 1992, Finlay 2001). Results from stable isotope analyses and gut content analyses from *H. amarus* at the Los Lunas site confirm that isotopic values reflect available food resources consumed during varying hydrodynamic conditions.

Current models of the structure and function of large river ecosystems are primarily theoretical and commonly suffer from lack of extensive field data (Thorp and Delong 1994). In particular, there are limited field data on the role of autochthonous and allochthonous food resources for river food webs in arid and semi-arid environments. The stable isotope data from the Los Lunas site on the Rio Grande are inconclusive with respect to either autochthonous or allochthonous carbon dependence as a food source for *H. amarus*. A mixture of autochthonous and allochthonous sources are indicated. It is also unclear whether terrestrial C_3 plants are an important food source for aquatic invertebrates in the absence of algae. The food web, however, appears to be supported by an unknown primary or secondary producer with a $\delta^{13}C$ value of approximately -26.5 to -24.5‰ and a $\delta^{15}N$ value of approximately 2-3.5‰.

Allochthonous resource theory (Junk et al. 1989) does not appear to fully describe nutrient and energy transfers for this restored arid-land floodplain. The FPC (Junk et al. 1989) stresses the importance of the flood pulse, which is the major force controlling food resources for biota in river-floodplains. I propose that in light-limited river

ecosystem like the MRG that indirect input from riparian zones during inundation from floods partially compensates for the lack of autochthonous primary production. Allochthonous primary production, particularly along shallow slow-velocity margins of the channel and floodplain, still represent an important component of the food web during the flood pulse in the Rio Grande.

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Tables

Table 1. Family, genus, species, quantity, and similarity coefficients for larval fish collected during sampling period.

Family	Genus	Species	n	%	Chao-Jaccard contribution (%)	Similarity (%)
Cyprinidae	*Pimephales*	*promelas*	228	59	64	40.7
Cyprinidae	*Hybognathus*	*amarus*	123	32	33.3	42.1
Cyprinidae	*Cyprinella*	*lutrensis*	27	7	2.6	6.4
Cyprinidae	*Cyprinus*	*carpio*	16	2	0.1	7.4
Total			394	100		

Table 2. Order, family, genus, number, and percent of capture of aquatic invertebrates.

Order	Family	Genus	n	%
Coleoptera	Dytiscidae	*Hydroporous*	1	0.2
Copepoda			3	0.2
Diptera	Chronomidae		254	20
Ephemeroptera	Baetidae	*Acentrella*	33	3
Ephemeroptera	Baetidae	*Baetis*	119	9
Ephemeroptera	Baetidae	*Centroptilum*	168	13
Ephemeroptera	Caenidae	*Brachycerus*	21	2
Ephemeroptera	Heptagenaidae	*Heptogenia*	7	0.2
Ephemeroptera	Isonychiidae	*Isonychia*	6	0.2
Ephemeroptera	Leptohyphidae	*Tricorythodes*	5	0.2
Gastropoda	Physidae	*Physella*	4	0.2
Hemiptera	Simulidae		6	0.2
Odonata	Coengrionidae	*Enallagma*	2	0.2
Odonata	Gomphidae	*Erpetogomphus*	5	0.2
Odonata	Gomphidae	*Gomphus*	18	1
Odonata	Gomphidae	*Ophigomphus*	10	1
Odonata	Gomphidae	*Stylurus*	4	0.2
Oligachaete			1	0.2
Hemiptera	Corixidae	*Graptocorixa*	479	37
Trichoptera	Hydropsycheidae	*Hydropsyche*	1	0.2
Trichoptera	Leptoceridae	*Nectopsyche*	130	10
Total			1277	100

Table 3. Results for relative contribution of Chao-Jaccard and similarity indices for aquatic invertebrates collected during sampling period. 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.

Order/Family/Genus	Chao-Jaccard (Dis)similarity Analysis contribution (%)	Similarity Analysis contribution (%)
Hemiptera/Corixidae/*Graptocorixa*	37	28.6
Diptera/Chronomidae	28	14.3
Ephemeroptera/Baetidae/*Baetis*	25	18.6
Ephemeroptera/Baetidae/*Acentrella*	5	5.9
Trichoptera/Leptoceridae/*Nectopsyche*	2	6.1
Ephemeroptera/Heptagenaidae/*Heptogenia*	1	3.4
Copepoda	1	3.6
Ephemeroptera/Baetidae/*Centroptilum*	0.7	2.6
Odonata/Gomphidae/*Gomphus*	0.1	3.5
Diptera/Chronimidae/pupae	0	0.7
Trichoptera/pupae	0	0.6
Trichoptera/Hydropsycheidae/*Hydropsyche*	0	1.3
Odonata/Gomphidae/*Stylurus*	0	2.5
Odonata/Gomphidae/*Ophigomphus*	0	0.9
Odonata/Gomphidae/*Erpetogomphus*	0	0.7
Odonata/Coengrionidae/*Enallagma*	0	01
Hemiptera/Simulidae	0	0.1
Hemiptera/Simulidae/pupae	0	0.1
Gastropoda/Physidae/*Physella*	0	0
Ephemeroptera/Leptohyphidae/*Tricorythodes*	0	0
Ephemeroptera/Isonychiidae/*Isonychia*	0	0.5
Ephemeroptera/Caenidae/*Brachycerus*	0	0.3
Diptera/Chronimidae/Midge	0	0.1
Coleoptera/Dytiscidae/Hydroporous	0	0.1

Table 4. Isotopic signatures and ranges for larval fish collected during sampling period.

Genus/Species	n	$\delta^{15}N$ (‰)	Range (‰)	$\delta^{13}C$ (‰)	Range (‰)
Hybognathus amarus	35	9.5 ± 0.2 SE	6.7 – 12.5	-21.4 ± 0.3 SE	-24.9 to -18.4
Pimephales promelas	19	9.4 ± 0.2 SE	7.3 – 11.2	-20 ± 0.3 SE	-22.5 to -18.1
Cyprinus carpio	12	10 ± 0.5 SE	7.7 – 12.7	-20.0 ± 0.5 SE	-23.3 to -18
Cyprinella lutrensis	14	11.2 ± 0.4 SE	8.9 – 13.9	-19.9 ± 0.3 SE	-21.5 to -17.6

Table 5. Possible food sources for *H. amarus*. Chironomid Δ for $\delta^{13}C$ and $\delta^{15}N$ were closest to values reported in the literature (Δ $\delta^{13}C$ of 2.5 to 3.4‰ and Δ $\delta^{15}N$ of 0.5‰). Previous trophic fractionation (Δ) studies have demonstrated that $\Delta\delta^{13}C$ from prey to predator can range from 0 to 1‰ (Peterson and Fry 1987) or ±1‰ (DeNiro and Epstein 1978). Vander Zanden and Rasmussen (2001) in their study of 20 temperate lakes reported an overall mean value for prey $\delta^{13}C$ and predator $\delta^{13}C$ shifts approximately +0.5‰.

Predator	Prey	Δ $\delta^{13}C$ (‰)	Δ $\delta^{15}N$ (‰)
H. amarus	Corixidae	-0.8	1.2
H. amarus	Chironomids	0.5	2.8
H. amarus	Predators	0.8	2.2
H. amarus	Shredders	1.1	3.4
H. amarus	Gatherer/Collectors	1.8	3.2
H. amarus	*Heptogenia*	2.6	3.5

Table 6. Order, family, genus, number, and mean stable isotope values (‰) and standard error for aquatic invertebrates collected during study period.

Order	Family	Genus	n	$\delta^{13}C$	SE ±	$\delta^{15}N$	SE ±
Coleoptera	Dytiscidae	*Hydroporous*	1	-22.7	0	8.2	0
Copepoda			3	-24.0	1.1	3.9	0.9
Diptera	Chronimidae		19	-21.9	0.5	6.8	0.5
Ephemeroptera	Baetidae	*Acentrella*	7	-22.9	0.8	6.5	0.9
Ephemeroptera	Baetidae	*Baetis*	21	-23.2	0.4	6.3	0.2
Ephemeroptera	Baetidae	*Centroptilum*	4	-23.6	0.3	5.8	0.4
Ephemeroptera	Heptagenaidae	*Heptogenia*	11	-24.0	0.5	6.0	0.4
Ephemeroptera	Isonychiidae	*Isonychia*	3	-24.2	0.2	5.7	0.5
Ephemeroptera	Leptohyphidae	*Tricorythodes*	1	-22.8	0	4.4	0
Hemiptera	Simulidae		1	-23.3	0	7.1	0
Odonata	Gomphidae	*Erpetogomphus*	4	-22.9	0	7.5	0
Odonata	Gomphidae	*Gomphus*	5	-20.4	0	7.5	0
Odonata	Gomphidae	*Ophigomphus*	8	-22.6	0	6.6	0
Odonata	Gomphidae	*Stylurus*	1	-22.2	0	6.3	0
Hemiptera	Corixidae	*Graptocorixa*	20	-21.8	0.2	9.2	0.4
Trichoptera	Leptoceridae	*Nectopsyche*	7	-22.5	0.2	6.7	0.7

Figures

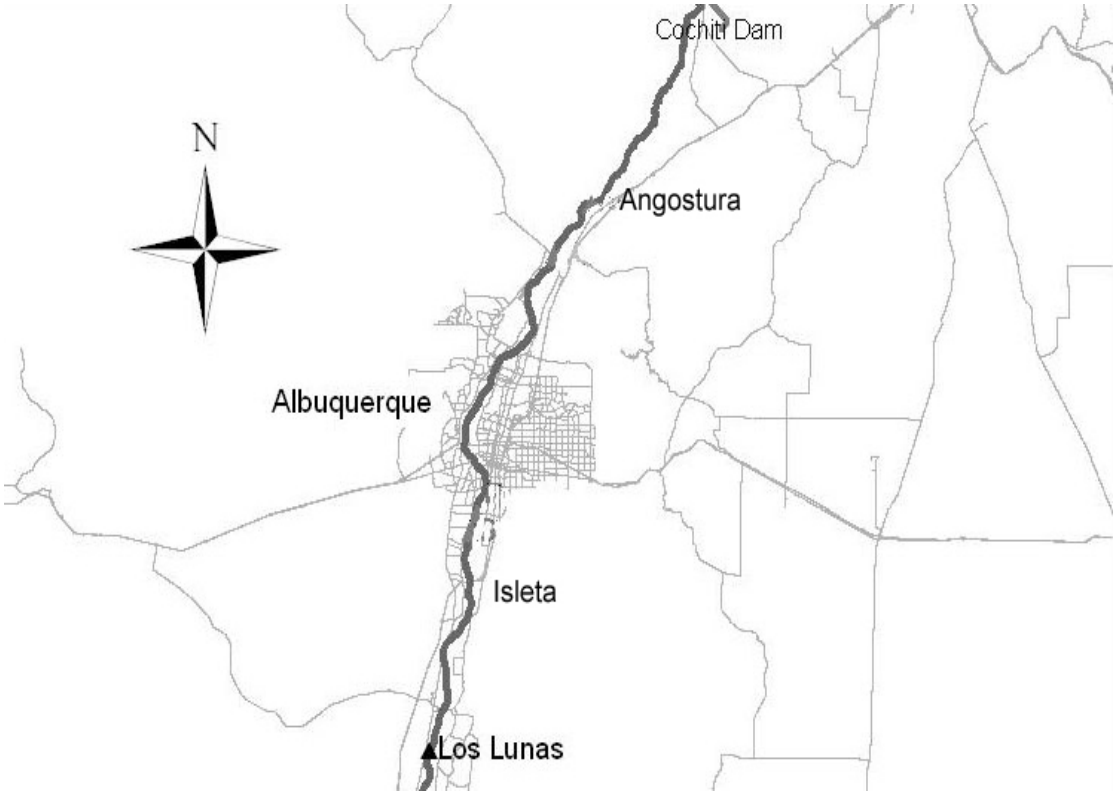

Fig. 1. Angostura and Isleta diversion dams and the Los Lunas site (triangle) on the Middle Rio Grande downstream of Cochiti Reservoir, NM.

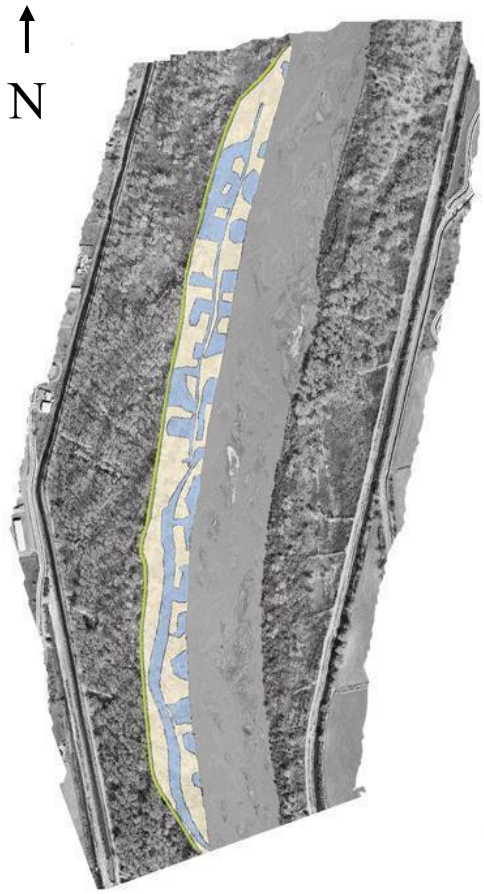

Fig. 2. Los Lunas Habitat Restoration Project. Floodplain dimensions; 1829 m x 107 m encompassing approximately 16 hectares. ArcGIS map courtesy of Dr. Michael Porter (US Bureau of Reclamation, Albuquerque, NM). Blue indicates created nursery habitat areas. Light brown indicates higher elevations.

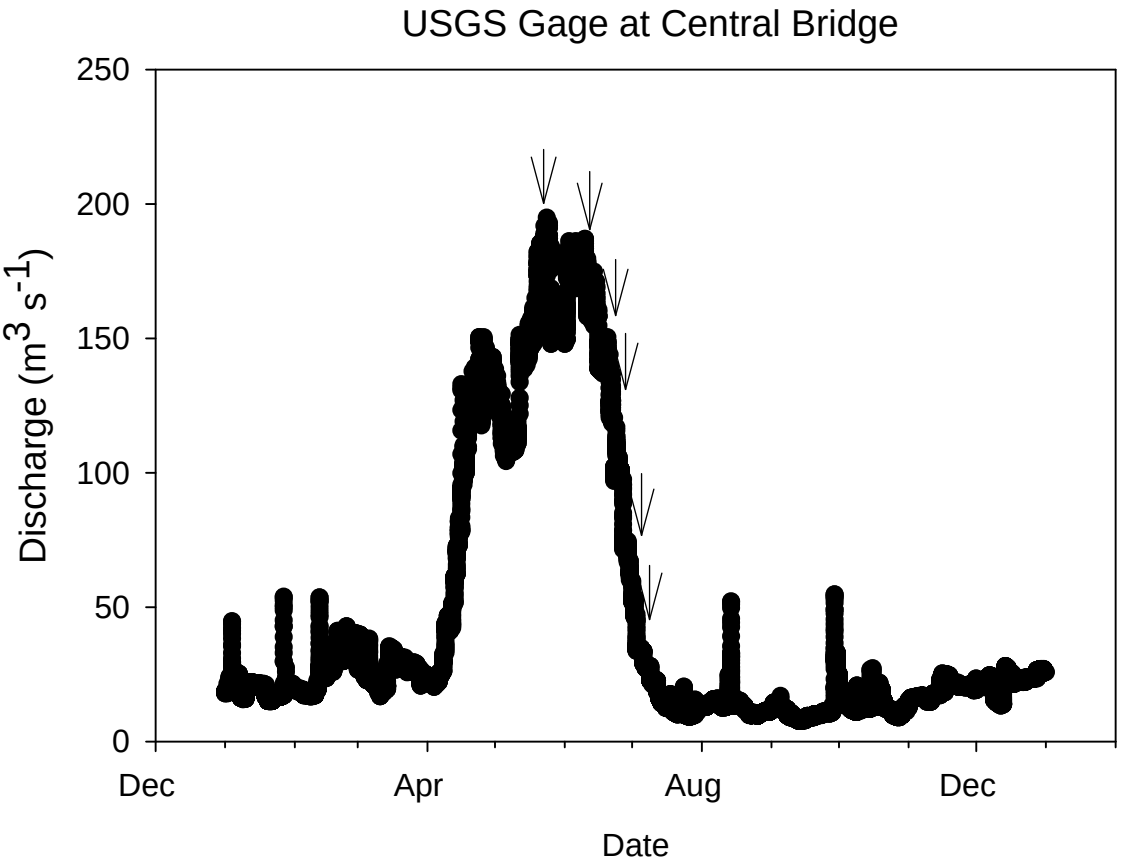

Fig. 3. Hydrograph for the Middle Rio Grande at Central Bridge, Albuquerque, New Mexico in 2005. Arrows indicate approximate sampling dates during descending limb of the hydrograph.

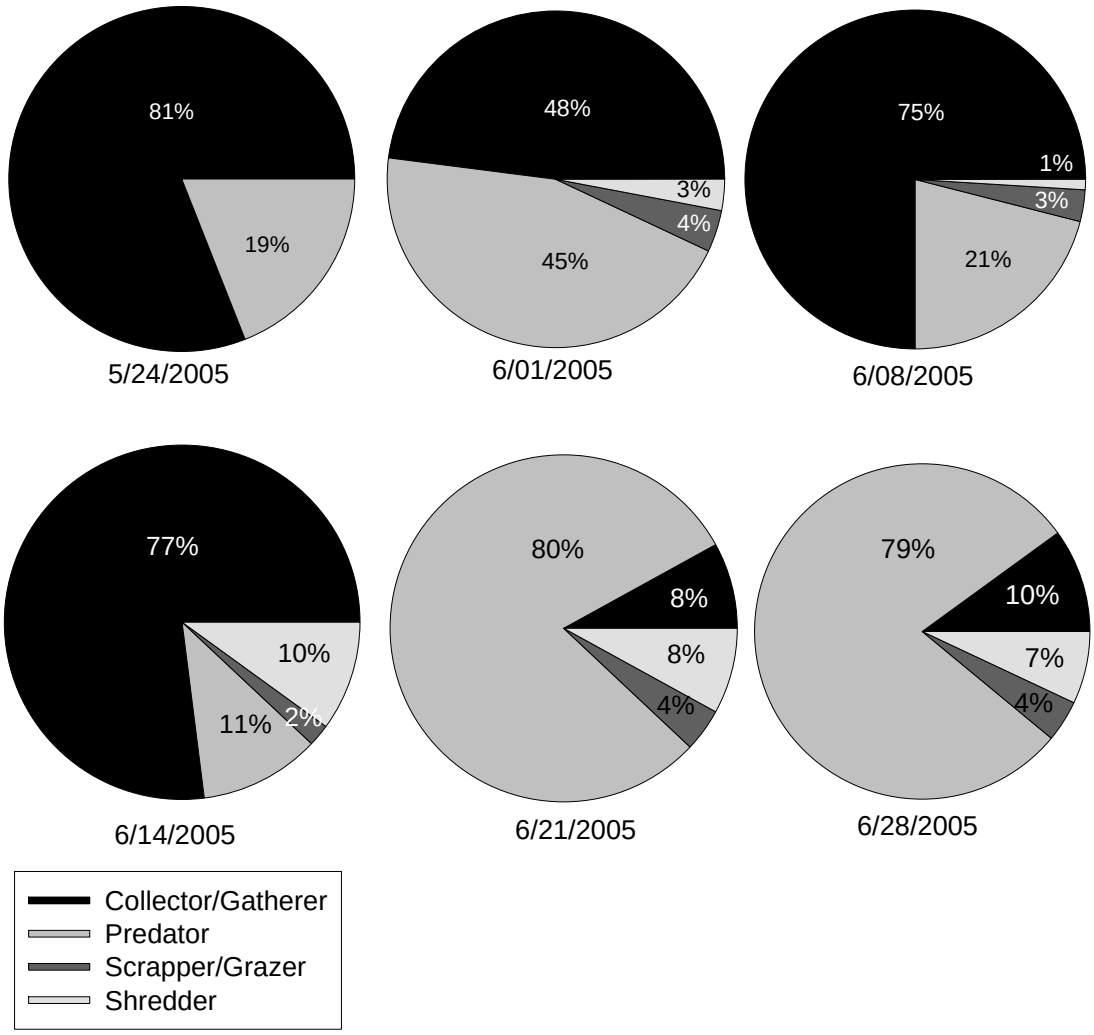

Fig. 4. Change in aquatic invertebrate community composition during the six sampling dates.

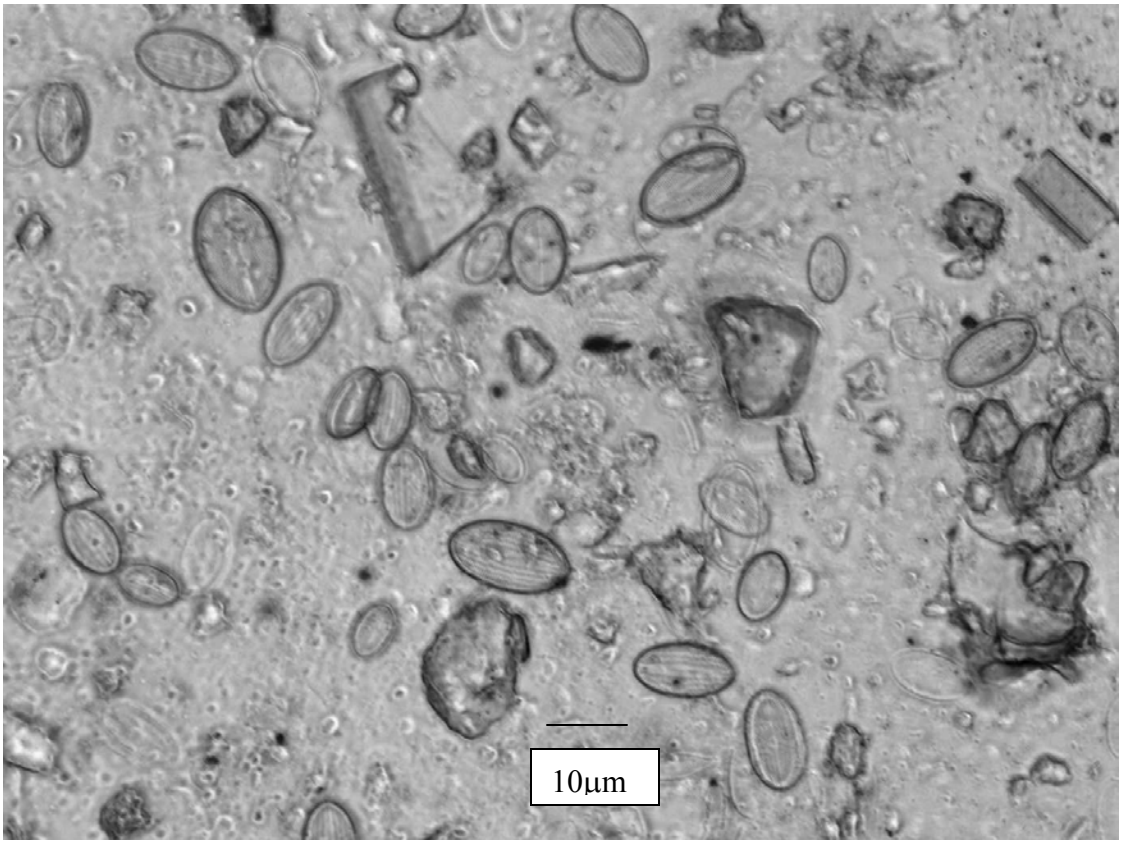

Fig. 5. Gut contents of Physidae (*Physella* sp.). The diatom *Cocconeis placentula* is the dominant diatom in the image.

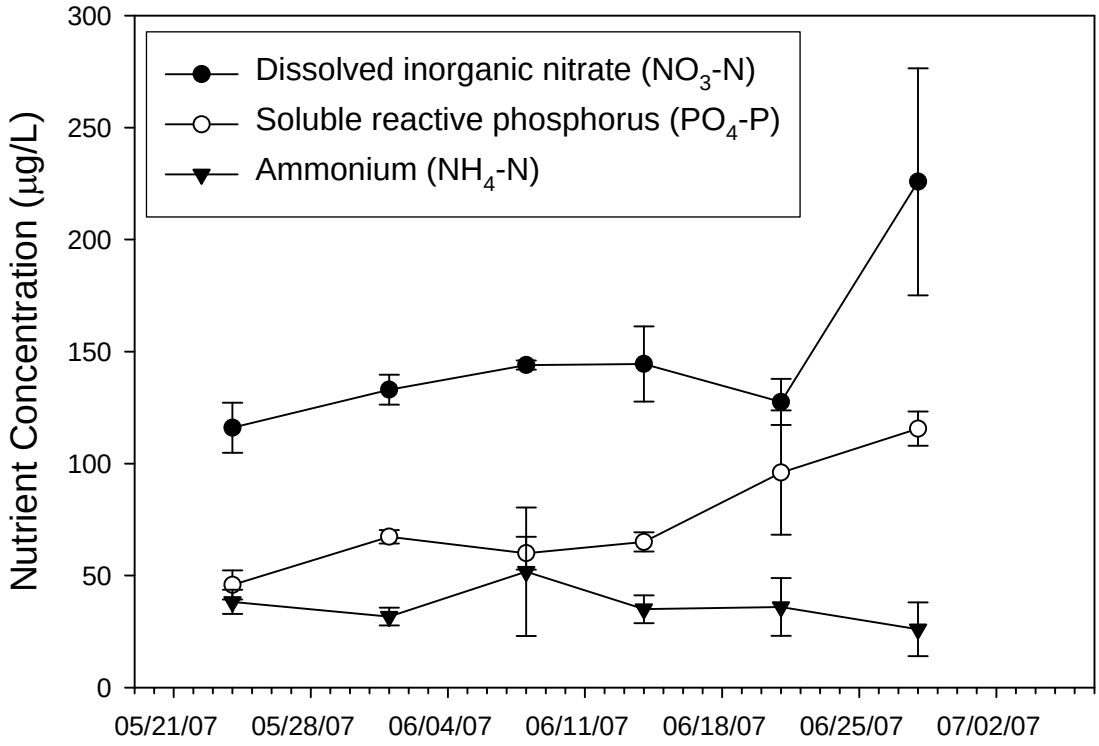

Fig. 6. Nutrient concentrations of the Los Lunas floodplain during the 2005 sampling period. NO_3-N and PO_4-P concentrations increased during the descending limb of the hydrograph, while NH_4-N concentrations decreased.

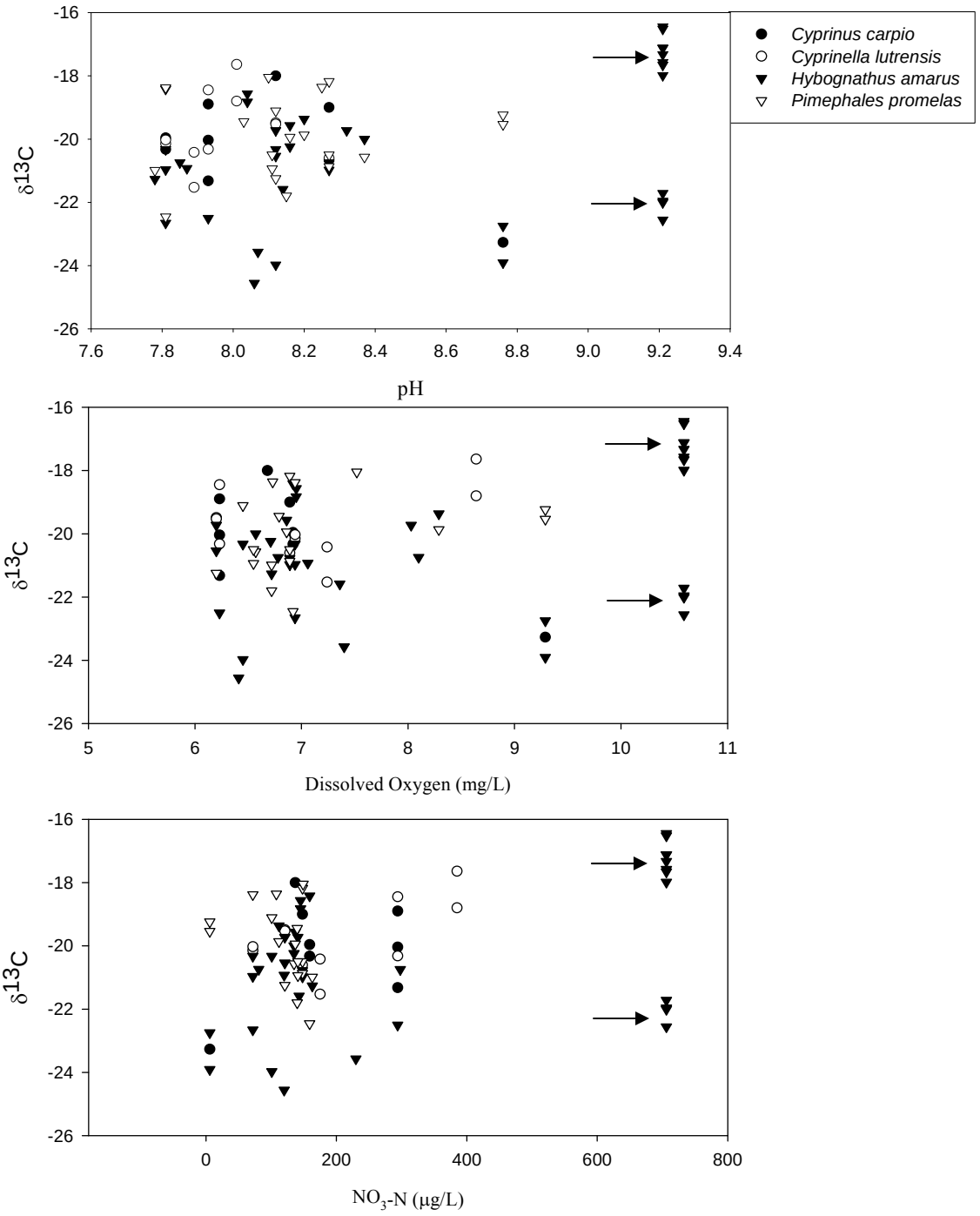

Fig. 7. Ichthyofauna $\delta^{13}C$ values graphed against environmental parameters (pH, DO, and NO_3-N). Two groups of *H. amarus* (arrows) are visible at the extreme end of each graph. Elevated DO and pH levels are indicative of high levels of algal growth, and $\delta^{13}C$ values confirm that algae comprise a major part of the diet. Delta ^{13}C values clustered around -22 indicating that aquatic invertebrates also comprise a part of the diet.

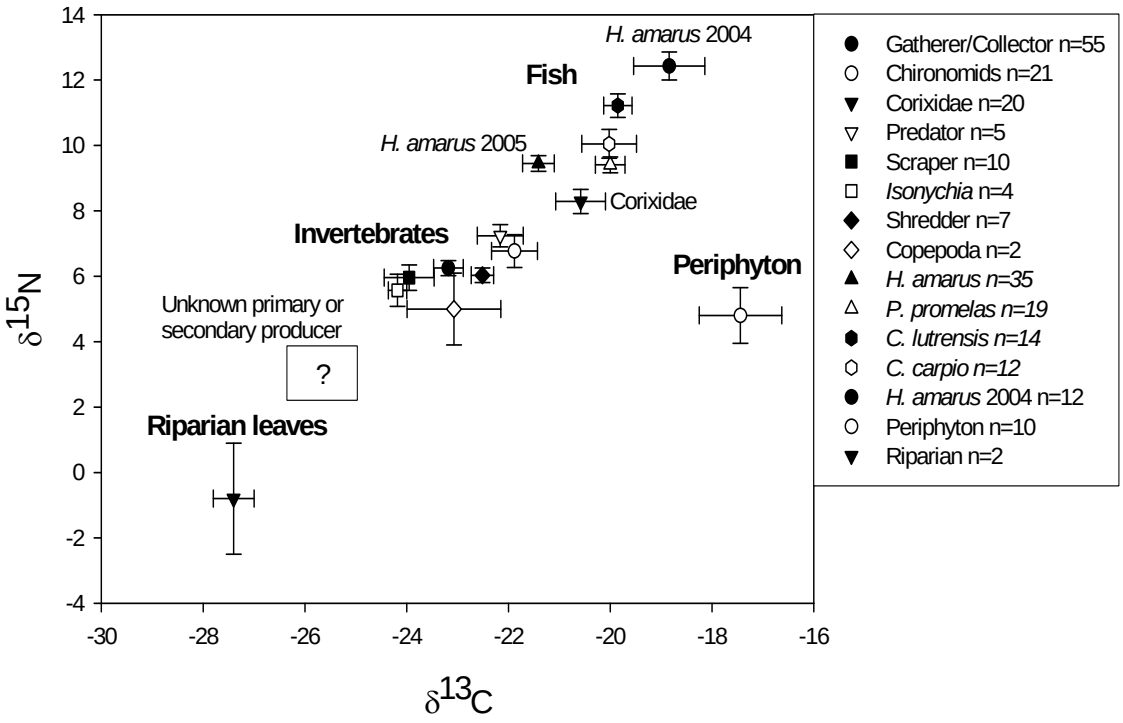

Fig. 8. Mean isotopic values for Los Lunas floodplain organisms. Trophic fractionation indicates that potential food sources for *H. amarus* are chironimids and predatory invertebrates (open symbols between fish and invertebrates). The food web also appears to be supported by an unknown C and N source with $\delta^{13}C$ values of approximately -26.5 to -24.5‰ and a $\delta^{15}N$ value of approximately 2-3.5‰.

Conclusion

Chapter one examined seasonal nutrient concentrations, chlorophyll *a* content, diatom species composition, and the match/mismatch between algal proliferation and historic *H. amarus* spawning period in Middle Rio Grande. A shift exists in nutrient limitation of primary producers north of Albuquerque to excess nutrients of primary producers through the urbanized reach of the MRG. These nutrient shifts influenced by wastewater effluent that have changed the diatom distribution and diversity in the MRG. The nutrients may not be optimal to support algal growth during *H. amarus* spawning. Results from this study indicate that a mismatch exists between peak algal biomass (represented by chlorophyll content) and the historic *H. amarus* spawning period. If this mismatch has occurred long-term it is likely to have led to the decline of *H. amarus*.

Chapter two addressed the preferences of *H. amarus* for diatoms and substrate. I also assessed food awareness and peak sampling time, and conditioning response. *Nitzschia palea* and *N. paleacae* were the preferred diatoms in the feeding trials regardless of substrate and may have been preferred because they exhibit an upright growth form. This growth form may benefit aquatic grazers because they can forage a relatively small portion of indigestible biogenic silica and agar/substrate to gain a proportionally large amount of organic matter. Protolarvae and mesolarvae were less attentive to food and took longer to reach peak sampling compared to older metalarvae. Results from feeding trials suggest that *H. amarus* can be conditioned to recognize food sources after a single 30 minute exposure to food.

Chapter three investigated food consumption of *H. amarus* using stable isotope analyses and paleolimnology to trace the flow of carbon from food to fish. Results from isotope analyses and gut content analyses suggest that during high-flow events *H. amarus* utilizes various food resources. Among the food sources identified were aquatic invertebrates, specifically chironimids, and periphyton. Results from this dissertation study require the reclassification of *H. amarus* as an omnivore and not an herbivore or carnivore as it has been classified by previous studies.