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Current and Historical Aquatic Food Webs of the Middle Rio Grande, NM

Melanie S. Edwards

CURRENT AND HISTORICAL AQUATIC FOOD WEBS OF THE MIDDLE RIO GRANDE, NEW MEXICO - EDWARDS

THE UNIVERSITY OF NEW MEXICO
ALBUQUERQUE, NEW MEXICO 87131

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Date

CURRENT AND HISTORICAL AQUATIC FOOD WEBS OF THE MIDDLE RIO GRANDE, NEW MEXICO

BY

MELANIE S. EDWARDS

B.S., Evolution, Ecology, and Organismal Biology, Ohio State University, 1999

DISSERTATION

Submitted in Partial Fulfillment of the
Requirements for the Degree of

Doctor of Philosophy
Biology

The University of New Mexico
Albuquerque, New Mexico

December 2006

DEDICATION

To my sweet, loving, funny, wonderful sister, Laura Ruth Edwards (8/29/62 – 12/10/05). Without your guidance and support throughout my life, I would not be who I am today. We all miss you terribly.

ACKNOWLEDGEMENTS

I would like to thank my committee chair, Dr. Thomas Turner, for guidance and encouragement on the long road of graduate school. Thanks also to the members of my graduate committee, Dr. Clifford Dahm, Dr. Manuel Molles, and Dr. Zachary Sharp.

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Special thanks to my husband, John Webb, for many hours of field help, and for unending support and encouragement.

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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. Biologically, the consequences of the massive anthropogenic alterations of this river have been substantial. The overall aim of this study was to characterize the ecological implications of these alterations in the aquatic food web of the middle Rio Grande (MRG) of New Mexico. Our approach was to analyze the stable isotope composition of current food web components and of historical museum preserved fish specimens. After determining that we could use stable isotope analyses of historical museum preserved fish specimens (chapter 1), we proceeded with our study on the current food web of the MRG. The results of this study allowed us to develop a food web model of the MRG. Regardless of sample site or season, autochthonous production was the dominant carbon source in our system. Fishes sampled from the MRG are not supported directly by primary producers, but instead by an intermediate step consisting of either macro- or micro-invertebrates. Omnivory is evident in the contemporary fish food web, as fishes used similar resources regardless of nominal dietary differences. In addition, we observed enhanced food web dependence on

autotrophic production at one of our sites (Bernardo), which may be linked to anthropogenic inputs of nutrients. In the final portion of this study, we characterized the historical food web, and found many similarities with our current-day food web results. Omnivory has dominated the fish food web for the past 60 years, and may be contributing to food web stability in the face of anthropogenic change. Overall, carbon and nitrogen stable isotope values of fish changed little over time. The exception to this is at our Bernardo site, which displays enhanced autotrophy in the later years, similar to our results in the current food web. The timing of the switch to autotrophy at Bernardo coincides with an expansion of the Albuquerque Wastewater Treatment Plant, which may be contributing soluble nutrients and driving eutrophication.

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INTRODUCTION

Human alteration of river ecosystems, and the resulting biological impacts, is an enormous concern worldwide. Our study is focused in the middle Rio Grande (MRG) of New Mexico, which has experienced numerous such impacts over the past several decades. The overall aim of the research that is presented here is to provide information on past and present conditions of the MRG aquatic food web, and to understand how anthropogenic alteration affects ecosystem processes. Our approach is to use stable isotope analyses of current and historical MRG aquatic food web components to understand the structure and function of the food web. In the first chapter of our study, we examined the effects of fixation and preservation procedures on the stable isotope composition of fish tissue. Chapter two characterized the current aquatic food web of the MRG by analyzing the stable isotope composition of food web components collected from four research sites within the basin. Finally, in chapter three, we analyzed fish specimens held in the Museum of Southwestern Biology and compared them to recently collected fishes to evaluate changes in ecosystem structure and function that have resulted from hydrological alteration in the system over the past 60 years. Information from the study was used to develop a model of the contemporary MRG aquatic food web, and to evaluate two types of changes potentially impacting the Rio Grande food web; (i) changes in carbon sources entering the food web due to changing hydrological regimes, and (ii) variation in nitrogen inputs from agriculture and human waste over time.

CHAPTER 1: SHORT- AND LONG-TERM EFFECTS OF FIXATION AND PRESERVATION ON STABLE ISOTOPE VALUES (δ13C, δ15N, δ34S) OF FLUID-PRESERVED MUSEUM SPECIMENS

Coauthors: TF Turner, ZD Sharp. Copeia 2002:1106-1112.

INTRODUCTION

Stable isotope chemistry is increasingly used by ecologists to quantify the nutrient flow and structure of food webs (Peterson and Fry 1987, Fry 1991, Angradi 1994, Vander Zanden et al. 1999). Nitrogen isotope ratios are used to determine the trophic position of food web components, and carbon isotopes are used to trace the flow of organic matter to organisms within food webs (Fry 1991). Sulfur isotopes are used less often, but have been used to quantify migration patterns (Hesslein et al. 1991), and have also been used in pollution studies (Zhao et al. 1998). Stable isotopes have also played an important role in understanding community-level responses to anthropogenic and natural disturbance (Vander Zanden et al. 1999).

One approach for quantifying changes in pre- and post-disturbance food webs is to compare the disturbed system to an unaltered reference system (Vander Zanden et al. 1999). The reference system is assumed to be identical to the pre-disturbance condition of the altered system. This assumption is unlikely to hold strictly true, because of variation in abiotic and biotic factors across systems. For many freshwater aquatic systems, like large rivers in the lower 48 United States, an undisturbed reference system does not exist (Benke 1990), indicating the need for an alternative approach.

Natural history museums offer a potential "reference system" because they are repositories of specimens collected prior to natural and/or anthropogenic disturbance. For

example, the Museum of Southwestern Biology at the University of New Mexico has fishes collected from the Rio Grande since 1937, prior to major alterations of flow regime in the river. If museum-preserved specimens retain their original isotopic compositions, then they should be useful for examining how the Rio Grande aquatic community has changed in response to increasing river regulation over the past 60 years. More generally, natural history museum holdings may provide researchers an alternative approach to studying the effects of natural and human induced disturbance on nutrient cycling and food webs in aquatic systems.

Concerns about the use of fluid-preserved specimens (such as fishes, amphibians, and reptiles) for stable isotope studies arise because of the near universal use of formalin (i.e., formaldehyde) for specimen fixation. Formalin fixation is known to alter carbon isotopic values of tissues (Hobson et al. 1997, Bosley and Wainwright 1999), but magnitude, direction, and specific causes of formalin-induced alteration remain poorly studied. Our aim was to determine how formalin changes the stable isotope composition of preserved fish tissues by comparing paired fresh-frozen tissues to tissues that were subjected to fixation and preservation protocols typically used in natural history museums. To examine the long-term effects of fluid preservation, we compared 12 to 15 year-old frozen specimens to preserved specimens taken in the same sample. Finally, we conducted a series of experiments to identify the mechanism by which formalin alters carbon, nitrogen, and sulfur isotopic values and to determine whether these alterations are predictable.

MATERIALS AND METHODS

Specimens deposited in natural history collections typically undergo a series of fixation, washing, and preservation steps to insure utility and durability for future research use. We used the following procedures to prepare field-caught fish specimens for experimental analysis. Specimens (either whole fish or tissue samples) were fixed in 10% buffered formalin for not less than ten days. Upon removal from formalin, specimens were washed in daily changes of distilled water for three to five days. Finally, specimens were placed in 35% ethanol for two weeks, and then placed in 70% ethanol for long-term preservation. We then conducted a series of experiments to determine how this procedure affected isotope values of preserved tissues compared to fresh-frozen tissues. Following all experimental treatments, samples were lyophilized prior to analysis of stable isotope ratio

Samples were packed in tin capsules and weighed for isotopic analysis. For carbon and nitrogen isotopic analysis, between 0.6 and 1.2 mg of lyophilized tissue was used for each specimen. For sulfur, between 3.0 and 5.0 mg of lyophilized tissue was used. Weighed samples, packed in tin capsules, were reacted in an elemental analyzer. The evolved CO_2, N_2, and SO_2 gases were analyzed on a Finnigan Mat Delta Plus isotope ratio mass spectrometer. Data are reported in parts per thousand (‰ or per mil) in delta (δ) notation. The delta value is computed using the following equation:

$$\delta^{13}C, \delta^{15}N \text{ , or } \delta^{34}S = [(R_{sample}/R_{standard}) - 1] \times 1000$$

where R is equal to $^{13}C/^{12}C$, $^{15}N/^{14}N$, or $^{34}S/^{32}S$ (McKinney et al. 1950, Peterson and Fry 1987). Delta values are reported relative to a standard. Standards are PeeDee belemnite limestone for carbon (VPDB), air for nitrogen, and Cañyon Diablo Troilite (CDT) for

sulfur. Reproducibility of standards for carbon and nitrogen was within 0.1‰, and for sulfur was within 0.2‰.

Experiment 1: Does duration of the formalin fixation step result in predictable changes in isotopic values? --- Although the formalin fixation step is not typically less than ten days, it can be substantially greater depending on factors such as processing backlogs sometimes encountered in museums. To understand how duration of the formalin fixation step affected isotopic signatures, we collected 20 longnose dace (Rhinichthys cataractae - a common minnow in the Rio Grande system), and returned them to the laboratory on ice (MSB 42375, SL = 37--88mm). A small portion of lateral muscle tissue was removed from each specimen and stored at -80 C. The remainder of each specimen was fixed in 10% buffered formalin. Three specimens (two for the 100 day and 130 day treatments) were haphazardly removed from formalin at thirty-day intervals beginning at 10 days and ending at 190 days (seven intervals total). Upon removal from formalin, specimens were subjected to washing and preservation steps (as described above).

Experiment 2: Does formalin fixation and long-term preservation in ethanol alter stable isotope signatures? --- We then sought to determine if formalin fixation and long-term preservation in ethanol affected isotopic values. We examined this question by obtaining 12 to 15 year-old frozen and fluid-preserved fish from the Illinois Natural History Survey Fish Collection (INHS) at the University of Illinois, Urbana-Champaign Campus, USA. When field collections were made, a subsample of fish from each species was frozen immediately in liquid nitrogen for biochemical analysis, and the remainder were fixed

and preserved as voucher specimens (L. Page, pers. comm.). Lateral muscle tissues were sampled from three frozen and three museum-preserved individuals in three species of darters (family Percidae); logperch (Percina caprodes, INHS 68905, SL = 54--87mm), Roanoke darter (Percina roanoka, INHS 64359, SL = 46--49mm), and Tippecanoe darter (Etheostoma tippecanoe, INHS 64017, SL = 24--29mm). Logperch were collected from Kosciusko County, Indiana, in 1985, Roanoke darters from Franklin County, Virginia, in 1988, and the Tippecanoe darters from Green County, Kentucky, in 1988.

Experiment 3: Does lipid extraction by formalin explain changes in carbon isotope values of preserved tissues? --- Lipids are extracted from tissues by formalin (Von Endt 1994, but see Gloutney and Hobson 1998), and ethanol (Bosley and Wainwright 1999). The stable carbon isotope composition of lipids frequently differs from other components of an organism (DeNiro and Epstein 1977), suggesting that lipid extraction by preservatives may alter isotopic values of fluid-preserved specimens. To determine if lipid extraction by formalin alters carbon isotope values, ten longnose dace were collected (MSB 46723, SL = 59--90mm), returned on ice, and stored in a –80 C freezer. Three portions of lateral muscle tissue were removed from each fish and treated as follows. One portion was frozen and kept at –80 C; a second portion was fixed and preserved according to the method described above; and a third portion was extracted with petroleum ether to remove lipids (Kerr et al. 1982, Dobush et al. 1985). Tissues were minced and reacted with petroleum ether for three hours, with changes of the solution at the end of each hour. Lipid-extracted samples were lyophilized and weighed. Samples were extracted a second time for another hour, lyophilized, and weighed again. Sample weight did not change

between the first and second extractions, suggesting that the maximum amount of lipids had been extracted. Frozen, preserved, and lipid-extracted tissues were lyophilized and analyzed for carbon isotopic composition. We used paired t-tests to assess the null hypothesis that the difference of frozen, lipid extracted, and formalin treated and ethanol preserved tissues did not differ from zero.

Experiment 4: Does addition of light carbon during formalin-fixation cause changes in carbon isotope values of fluid-preserved tissue? --- Previous work has shown that treating tissues in solutions containing carbon (e.g., formalin) alters carbon isotope composition of the tissue, presumably by isotope exchange (Hobson et al. 1997, Bosley and Wainwright 1999). Such alteration would be expected to occur only if the treatment solution had a $\delta^{13}C$ value that was out of equilibrium with the tissues. Moreover, the magnitude and direction of change in isotope ratios of preserved tissues should vary in relation to the difference in isotopic composition of formalin and tissues. To test this idea, we first determined the $\delta^{13}C$ values of different preparations of 10% buffered formalin obtained from three different manufacturers (Mallinckrodt, JT Baker, and EM Science). We then asked whether the magnitude of the shift observed in tissues varied with isotopic composition of formalin used to fix the tissue. Four samples of lateral white muscle tissues were taken from each of eight white suckers, *Castostomus commersoni* (MSB 46725, SL = 64--92mm). Three portions were fixed in different brands of buffered formalin, and the fourth frozen. We tabulated isotopic shifts by taking the difference of $\delta^{13}C$ and $\delta^{15}N$ values of formalin-fixed and frozen tissue samples. We then used single classification analysis of variance (ANOVA) and unplanned comparison

of means (Tukey-Kramer method) to test for differences in the magnitude of isotopic shifts among tissues fixed in different formalin preparations.

RESULTS

Experiment 1: Formalin fixation appeared to cause systematic decrease in the $\delta^{13}C$ values of preserved tissues compared to frozen tissues, but the magnitude of decrease was not correlated with time that tissues were treated with formalin ($r = 0.11$, $P = 0.66$; Figure 1). We observed a mean shift in the $\delta^{13}C$ values between preserved and frozen tissues of –2.0‰ that was consistent over time (Figure 2A). A smaller systematic shift was observed between $\delta^{15}N$ values. Mean difference of preserved and fresh tissues was 0.4‰ (Figure 2B), and the magnitude of enrichment was not correlated with time in formalin ($r = 0.07$, $P = 0.78$). Mean difference between preserved and frozen sulfur isotope values was -0.2‰ (SD = 0.77) and no systematic variation was observed, i.e., values were distributed about line of isotope ratio equality of $\delta^{34}S$ values of preserved and frozen tissues (Figure 2C).

Experiment 2: Specimens preserved and maintained over 12 to 15 years exhibited shifts in isotopic composition consistent with those observed in more recently fixed and preserved specimens in experiment 1. Mean shift in the $\delta^{13}C$ values was -0.8‰ (Figure 3A), about 1.2 ‰ less than mean shift observed in experiment 1. The mean shift in $\delta^{15}N$ values was 0.5‰ (Figure 3B), which was very similar to the shift observed in experiment 1. The mean shift in $\delta^{34}S$ values was 0.8‰ (SD = 0.53). For sulfur, individual values were distributed about the line of isotope ratio equality of fixed and frozen tissue as in experiment 1 (Figure 3C).

Experiment 3: The difference of lipid extracted and frozen tissues was not significantly different from zero in a paired t-test (mean difference = -0.058‰, $P_{[\text{two-tailed}]} = 0.54$) indicating that lipid extraction had very little effect on isotope ratios of white muscle tissues we studied. The difference of formalin-fixed tissue and frozen tissues was significantly different from zero (mean difference = -1.50‰, $P_{[\text{two-tailed}]} < 0.0001$).

Experiment 4: In general, 10% buffered formalin had much lower $\delta^{13}C$ values than fishes collected from the Rio Grande. $\delta^{13}C$ for Mallinckrodt formalin was -37.8‰, JT Baker formalin was -40.3‰, and EM Science formalin was –52.5‰. Larger shifts were observed in tissues fixed in lighter formalin (ANOVA, P = 0.008, df = 2, 21). Mean carbon isotope shift was greatest in EM Science formalin (mean = -0.5‰) followed by JT Baker formalin (mean = -0.3‰) and Mallinckrodt formalin (mean = -0.2‰). Mean values were significantly different from each other at $\alpha = 0.05$.

Formalin fixation may alter carbon isotopes in a species-specific way. When a second species, flathead chub (*Platygobio gracilis*, MSB 46726, SL = 38--101mm) was treated in formalin preparations with different isotopic composition, the magnitude of the shift was greater in lighter formalin when compared to white sucker. However, flathead chub tissues (n = 2) were more depleted (-1.2‰ on average) than white sucker tissues (-0.3‰ on average). We then treated tissues from three individuals each of four fish species in the same formalin and found that the magnitude of the shift appeared to differ among species (range -0.4‰ to -1.8‰). Correlation analysis of the magnitude of the shift

and body size yielded no significant relationships, indicating that the magnitude of isotopic shift is not related to body size differences among species.

There was no detectable nitrogen in the formalin. Additionally, fixation in different brands of formalin did not appear to affect the magnitude of ^{15}N enrichment observed in tissues (ANOVA, $P = 0.910$, df = 2, 21). The difference in $\delta^{15}N$ values between preserved and frozen tissues were nearly identical among formalin treatments (mean difference of 0.5‰ for all three brands of formalin).

Discussion

If natural history collections are to provide material for stable isotope studies, it is critical that we understand how museum practices affect isotope ratios of preserved specimens. Our study shows that for fluid-preserved specimens, stable isotope ratios of muscle tissues are altered primarily by formalin fixation of tissues. Variation in fixation time does not appear to alter isotope ratios, nor does long-term preservation and maintenance in a natural history collection setting. The nature of formalin-induced alteration of tissues differs among the isotopes we studied, but, in general, the magnitude of isotopic shifts appear to be small compared to natural fractionation processes that occur in ecological communities.

Of the three isotopes studied, formalin fixation alters carbon isotope ratios most. $\delta^{13}C$ values of preserved tissue were lowered by 1.1‰ on average when compared to frozen tissue across all experiments. It is unlikely that extraction of lipids by formalin or ethanol altered $\delta^{13}C$ values in preserved muscle tissues appreciably, probably because white muscle has relatively low lipid content (Pinnegar and Polunin 1999). Rather, our results support the hypothesis that formalin alters carbon isotope ratios in preserved

tissues either by exchanging light isotopes for heavy ones (Gearing 1991, Hobson et al. 1997), or by simple addition of light carbon from formalin (pers. comm. B. Fry). The magnitude of alteration of preserved tissues depends, at least in part, on the isotopic composition of formalin used for fixation. Any exchange or addition process appears to go to completion within 10 days, as longer time in formalin does not alter tissues further (Figure 1).

Because of observed differences among species we are hesitant to propose a universal correction factor based on these data. However, regression analysis (not shown) of $\delta^{13}C$ of fluid preserved tissues on paired frozen tissues suggested that the slope of the regression is less than one in all cases (after removing outliers in experiment one) and that the difference in $\delta^{13}C$ are well approximated by a linear model (r^2- values ranged from 0.80 to 0.95). If the magnitude of the shift were driven by carbon isotope values of formalin, then we would expect no shift when isotopic values of formalin and tissue were equal. This suggests that slope and intercept values could be calculated by forcing the linear equation through the $\delta^{13}C$ value of formalin. However, since formalin is discarded after use, the only possible way to determine the delta value of the formalin that was used to fix specimens would be to extract any remaining formalin from the preserved tissues. Future research will seek to uncover the nature of species-specific differences in carbon isotope exchange.

Mean shifts in carbon isotopes did not exceed –2.0‰ in any experiment. In many ecosystems, carbon sources differ by greater than 2‰. For example, C_3 and C_4 plants differ by approximately 14‰ (O'Leary 1988). Angradi (1994) found that lotic plant material (except *Oscillatoria*) differed from terrestrial plant material by at least 2‰.

Algae were found to have an average $\delta^{13}C$ value of approximately –33‰, while upland vegetation had an average $\delta^{13}C$ value of –23‰ (Angradi 1994). Fry and Sherr (1989) discuss other studies that have also found that phytoplankton and terrestrial vegetation differ by 7 to 17‰. Finlay (2001) found that in headwater streams, the mean $\delta^{13}C$ value of herbivore and epilithic algae was approximately -35‰, while riparian vegetation and CPOM had means of –29.3‰ and –28.2‰, respectively.

As long as carbon sources are isotopically different by more than 2‰, as they are in the above examples, a -2.0‰ shift in preserved specimens will not confound results. If different sources of carbon are isotopically distinct from one another, isotopic mixing models can be used to determine the importance of multiple sources of carbon (which would be important in the case of omnivory; Fry and Sherr 1989). An error of –2.0‰ should also not change the interpretation of these mixing models.

Formalin fixation and ethanol preservation led to nearly uniform increase in the $\delta^{15}N$ values of fluid-preserved tissues across all experiments (range 0.4‰ to 0.5‰). The magnitude of enrichment was independent of time in formalin, long-term storage in ethanol, extraction of lipids, or of isotopic composition of formalin. Our results suggest that it may be appropriate to simply subtract 0.5‰ from isotopic values of preserved material (Arrington and Winemiller 2002). However, even if values remain uncorrected, 0.5‰ enrichment is minor compared to the average shift between trophic levels of 3.4‰ (Vander Zanden and Rasmussen 1999). While it is true that the trophic structure of food webs is a continuum, and nitrogen isotopes are sometimes used to determine finer-scale trophic relationships (i.e., to distinguish between trophic level 3.0 and 3.5), even such small trophic level differences are associated with fairly large $\delta^{15}N$ differences. For

example, a difference of half a trophic level will be associated with a difference in $\delta^{15}N$ values of approximately 1.7‰ (assuming a 3.4‰ shift between trophic levels). Vander Zanden et al. (1999) found that the trophic position of native fishes in disturbed lakes differed from that of natives in undisturbed lakes by approximately 0.6 trophic levels. This trophic level difference is associated with a $\delta^{15}N$ difference of 2.04‰ (0.6 trophic levels multiplied by 3.4‰). Enriched $\delta^{15}N$ values of preserved tissue would not introduce significant error into the interpretation of nitrogen isotope results in these cases. However, determination of small-scale trophic relationships (such as differences of less than 0.2 trophic levels or 0.7‰), may require accounting for nitrogen enrichment of preserved tissue.

Observed shifts in sulfur isotope values in preserved tissues were small (absolute difference 0.6‰), and appeared to be haphazard and independent of any treatment we conducted. Differences in sulfer isotope ratios between frozen and preserved material will not affect data interpretation in many cases. Hesslein et al. (1991) determined that the $\delta^{34}S$ values of migrating fish in Travaillant Lake, Canada, ranged from –10.7 to –15.8‰, while those of year-round resident fish ranged from –8.2 to –10.2‰. The type of error we observed in the sulfur data of our preserved fish (generally less than 1‰ difference between preserved and frozen tissues) may cause some mixing of the delta values of individuals at the middle limit of these ranges (i.e., those with $\delta^{34}S$ values near –10.5‰). However, the variation that we observed between preserved and frozen tissues may not exceed the variation that would be observed between or within fishes from a system such as Travaillant Lake.

Natural history collections are extremely useful for documenting changes in species abundance and/or composition (Shaffer et al. 1998). Our data support the idea that preserved specimens can be used in a new way to answer important questions about changes in ecosystems. Our study shows that, in many cases, shifts in delta values of preserved tissues are small enough to be ignored, or can be corrected. This technique circumvents the uncertainty of reference systems, and allows us to better understand disturbed systems that have no undisturbed counterparts.

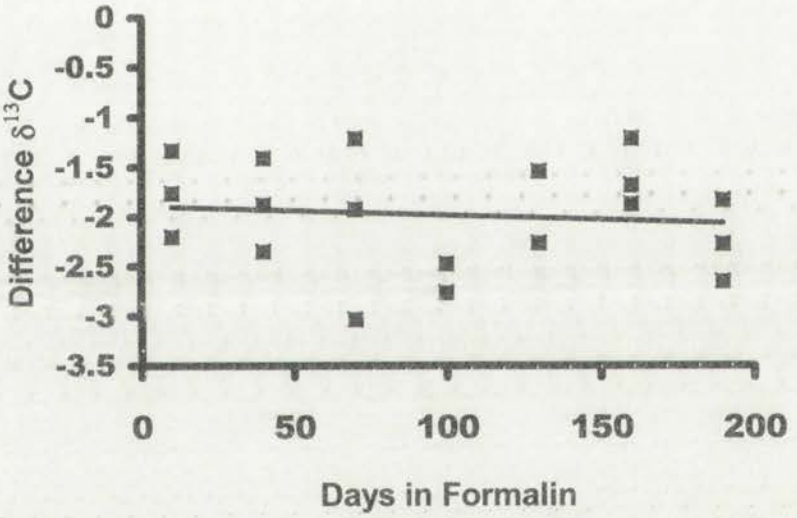
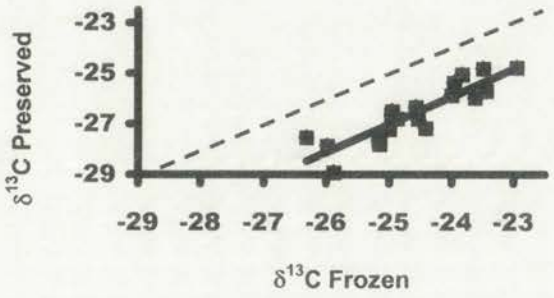


Figure 1. Duration of formalin fixation (days) plotted against the difference of δ13C values between fluid-preserved and frozen white muscle tissues. Correlation analysis indicated that difference of $\delta^{13}C$ values and the duration of the fixation step are independent. The dashed line represents the line of isotopic equality. The trend line is fitted by least-squares regression. Mean difference of $\delta^{13}C$ values between frozen and preserved tissues (-2.0‰). The reference for carbon is VPDB.

(A)



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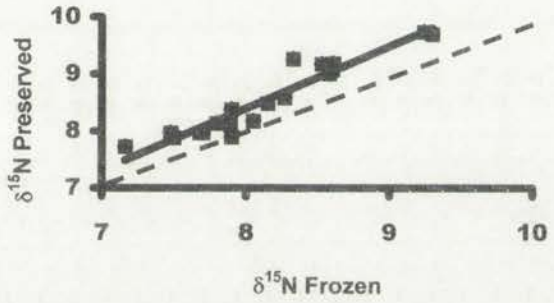


(C)

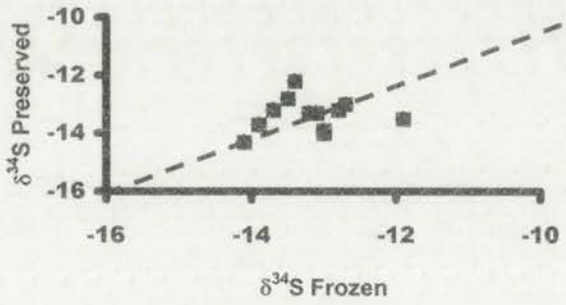
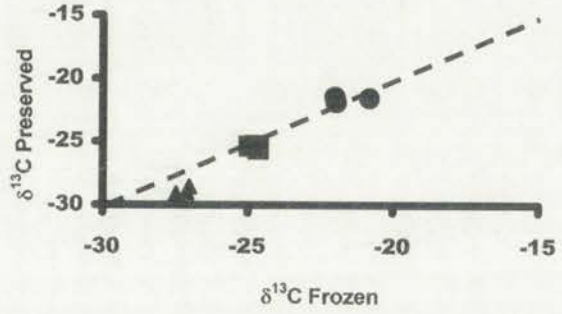


Figure 2. (A) δ13C , (B) δ15N , and (C) δ34S values of fluid preserved vs. frozen tissue. Dashed lines indicates equality of fluid preserved and frozen tissues. The dashed line represents the line of isotopic equality. Trend lines (solid) are fitted by least-squares regression. The reference for carbon is VPDB, for nitrogen is air, and for sulfur is CDT.

(A)



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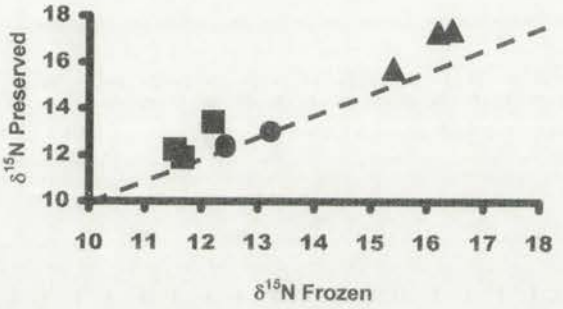

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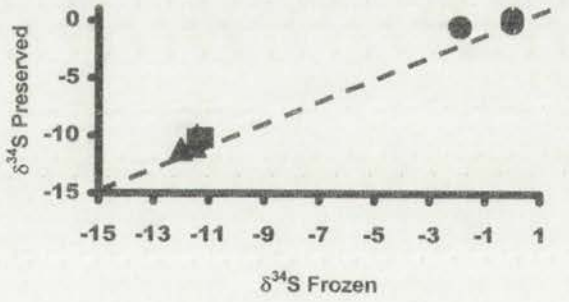

▲ *P. caprodes*
• *P. roanoka*
■ *E. tippicanoe*

Figure 3. (A) δ13C, (B) δ15N, and (C) δ34S values of 12 to 15 year old fluid-preserved vs. frozen tissues. Mean difference of $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$ values between preserved and frozen tissues for all three species were -0.8‰, 0.5‰, and 0.8‰, respectively. The dashed line represents the line of isotopic equality. The reference for carbon is VPDB, for nitrogen is air, and for sulfur is CDT.

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CHAPTER 2: THE CONTEMPORARY AQUATIC FOOD WEB OF THE MIDDLE RIO GRANDE, NM

INTRODUCTION

Rivers are among the most altered ecosystems in the world. Humans have substantially altered all of the large rivers in the contiguous 48 states except the Yellowstone River for purposes of hydropower or navigation (Benke 1990). Biological implications of river alterations are great; for example, the structure and function of fish and invertebrate communities below dams are often irreparably changed (Benke 1990). Despite the importance of rivers, and large-scale degradation of these ecosystems, little is known about how large rivers function and how they are impacted by human activities (Johnson et al. 1995). There is an urgent need for information on the impacts of anthropogenic activities on ecosystem function. River restoration activities are becoming more common, but it is unlikely that these restoration attempts will be fully successful without a better understanding of river ecosystem function. In order to effectively re-create ecosystem dynamics, an understanding of the river food web, including dominant carbon sources, are particularly critical in restoration.

Our study focused on the Rio Grande that flows through arid lands in Colorado, New Mexico, Texas, and Mexico in southwestern North America. Since 1993, The Rio Grande was identified as one of 10 most endangered river systems (identified as critically endangered in 4 out of 14 years) in the United States in annual assessments by American Rivers (http://www.americanrivers.org). Our aim was to provide information on sources of nutrients and cycling of nutrients in the contemporary Rio Grande aquatic food web, which is likely to have been strongly affected by intensive river regulation and water

extraction for human use. The study area is confined to a ~300 km reach between Cochiti dam in the north (upstream) and Elephant Butte dam in the south (downstream). This reach is commonly referred to as the middle Rio Grande (MRG) and historically was a braided, sinuous river with a broad floodplain (Crawford et al. 1993). Spring snowmelt and summer monsoon rains generated high river flows and allowed for overbank flooding. River-floodplain interactions were historically essential in maintaining the native riparian forest or 'bosque' (Molles et al. 1998, Ellis et al. 2002). At present, regulation of the river has severely limited overbank flooding, which has led to decreased recruitment of new native riparian vegetation and an over-accumulation of woody debris in the riparian zone (Molles et al. 1998). Floodplains in the Rio Grande may have historically served as nursery grounds for the fish community, and almost certainly contributed large amounts of organic material to the river in the form of riparian litter (Pease et al. 2006).

The framework for interpretation of Rio Grande food web data comes from four important conceptual models of river ecosystem function. The River Continuum Concept (RCC) (Vannote et al. 1980) predicts that large rivers will receive little energy input from the floodplain. Rather, upstream sources of fine particulate organic matter (FPOM) are predicted to be the dominate carbon source to high order rivers, with minimal input of organic material from riparian vegetation. The degree of autochthonous production is determined by the light attenuation associated with turbidity and/or depth. For large rivers like the MRG, the RCC predicts autochthonous production to be low, with a primary production to respiration ratio of less than one. The RCC was developed using results of research done mostly on low-order streams. Subsequent studies have shown

that the RCC may not apply to all large river ecosystems (Sedell et al. 1989). A contrasting perspective, the Flood Pulse Concept (FPC), emphasizes river-floodplain interactions as supplying important carbon for river food webs (Junk et al. 1989). The river and floodplain are linked by overbank flooding, whereby water, nutrients, and organisms pass back and forth between these compartments. The FPC may be particularly applicable to large floodplain dominated rivers, which are not well represented by the RCC.

Another perspective is presented in the Riverine Productivity Model (RPM), which suggests that autochthonous production in large rivers is more important that previously thought (Thorp and Delong 1994). Although riparian production tends to be plentiful in river systems, it is also mostly recalcitrant, and consumers tend to prefer the more labile autochthonous algal energy sources (Thorp and Delong 2002). In addition, upstream sources of FPOM tend to be recalcitrant, and therefore relatively unimportant (Thorp and Delong 1994). A similar model of "low-flow recruitment" predicts that low-velocity flow provides habitat conductive to autochthonous (i.e., algae) production (Humphries et al. 1999). The low-flow recruitment hypothesis emphasizes the importance of highly productive low velocity habitats that occur during times of low river discharge. In large arid-land rivers like the Rio Grande where low river discharges commonly occur, isolated pools, edges, and backwaters provide potential refugia for fishes. Algal production within these environments could be an important energy source to the aquatic food web (Figure 2 and 3) (Pease et al. 2006).

In one of the few studies of an arid zone river food web, Bunn et al. (2003) detailed the carbon sources supporting Cooper Creek in central Australia. Despite high

turbidity in Cooper Creek, benthic algae were the major carbon source to aquatic consumers. The floodplain of this creek is extensive, and yet provides little input to the food web. Similarly, Angradi (1994) found that algae formed the base of the aquatic food web in the Colorado River. In a study of the Ohio River, it was also found that autotrophic primary production was the main energy source for the aquatic food web (Thorp et al. 1995). Like Cooper Creek and the Colorado River, the Ohio River does not receive significant food web inputs from terrestrial vegetation. The importance of in-stream algal production in these food webs may be explained by the relatively labile nature of algal carbon sources compared to recalcitrant floodplain carbon sources (Thorp and Delong 2002). These studies demonstrate that neither the RCC nor the FPC necessarily apply to all large rivers. The RPM or the low-flow hypotheses appear to be better suited to explain the patterns observed in Cooper Creek and the Colorado and Ohio Rivers, at least during periods of low discharge (Thorp and Delong 2002, Humphries et al. 1999).

Our study characterizes the aquatic food web of the MRG, and investigates temporal and spatial (longitudinal) variability of patterns in the food web. A study of this kind has not been done in the region, and provides vital information to river managers and restoration planners. Many of the food web models described above are static, and therefore, are difficult to apply to our study system because it is a very dynamic system. However, previous stable isotope studies have shown that autochthonous algae can be an important source of carbon in the food web (Pease et al. 2006) during low flow periods in summer. Therefore, of the available food web models, the RPM or the "low-flow recruitment hypothesis" may be the best suited to describe the MRG system (Thorp and

Delong 1994, Humphries et al. 1999) during low flow periods. In this study, we used stable isotope analyses of food web components (i.e., inputs) to characterize the aquatic food web of the MRG to develop a seasonal picture of the sources and cycling of nutrients in this important arid-zone river ecosystem.

Background and Food Web Predictions

Historically, the extensive floodplain of the Rio Grande was inundated in springtime as a result of snowmelt (Crawford et al. 1993). Large scale intensive anthropogenic alteration of the MRG ecosystem began in the early 20th century. Dams and levees prevent or limit overbank flooding in the MRG, which decreases the input of floodplain organic matter to the river. Cochiti Dam was completed in 1975, and is one of the major structures affecting the MRG (Crawford et al. 1993). This flood control dam reduced wet season peak flows in the MRG, which would have historically produced overbank flooding (Figures 1, 2 and 3). Before the construction of regulation structures in the MRG, springtime snowmelt flows occasionally inundated the river floodplain, bringing a potentially important source of organic material into the river (Molles et al. 1998, Ellis et al. 2002). These anthropogenic alterations have had an enormous effect on the MRG ecosystem; for example, up to19 non-native fish species have been introduced and up to12 native species have been extirpated in various reaches of the Rio Grande over the past five decades (Sublette et al. 1990, Propst 1999).

The geomorphology of the MRG has changed dramatically over the past 60 years. Where the river was once broad, shallow, and winding, it is now channelized, relatively straight, and deeper than it was historically (Crawford et al. 1993). The water released from Cochiti Dam is cold and clear, which is a fundamental alteration of the historically

warm, sediment-loaded river condition (Figure 1 and Table 1). This clear water has effectively scoured the river bed, causing deep incision downstream of Cochiti Reservoir (Crawford et al. 1993). In the Albuquerque reach of the MRG, the river is confined to a relatively narrow, and deep, channel. Channelization is not an important process in the Bernardo region (south and downstream of Albuquerque), and there are sandbars and some shallow, backwater habitats. Further downstream, the Bosque del Apache National Wildlife Refuge is one of very few sites in the MRG that still regularly experiences managed overbank floods. These floods occur every few years, and are being explored as a possible tool for restoration of the riparian ecosystem (Molles et al. 1998).

Because the flood-pulse concept stresses the importance of river-floodplain interactions driven by lateral transport of materials into the channel by flood waters, this concept is unlikely to describe the MRG food web (Junk et al. 1989). Anthropogenic river alteration (via channelization, levees, and dams) has limited the extent and duration of overbank flooding, thereby limiting floodplain inputs into the river. Similarly, the River Continuum Concept is unlikely to apply to our study system, as it relies on an unfragmented river to provide downstream transport of fine particulate organic matter (FPOM) (Vannote et al. 1980). The reservoir associated with Cochiti Dam promotes settlement and limits transport of FPOM downstream.

Food web models that rely on instream production (e.g., RPM and "low flow recruitment" hypothesis) are more likely to describe the MRG aquatic food web (Thorp and Delong 1994; Humphries et al. 1999). Because floodplain contributions to the MRG food web are likely to be minimal (because of flow regulation and channelization), we predict that the present day food web will reflect more reliance on autochthonous carbon sources,

such as algae and macrophytes, than on floodplain derived materials. One study site, Bosque del Apache, still occasionally experiences overbank flooding, and we predict that the food web of this site will reflect more allochthonous input than the northern three sites. However, because previous studies have shown minimal floodplain inputs even in areas that experience managed floods, we predict that autochthonous carbon inputs will represent a higher percentage of consumer carbon than allochthonous sources at Bosque del Apache (Pease et al. 2006).

MATERIALS AND METHODS

Study Sites

Sampling was conducted at four sites in the MRG (Figure 1). The Cochiti site is the northernmost (upstream) site and is located just below Cochiti Dam. Hypolimnetic dam releases dominate flows at this site, making the river clear and cold. The substrate is composed of cobbles and gravel, with little sand or fine sediment. The Bernalillo site is near the town of Bernalillo, NM and is approximately 48 km downstream of the Cochiti site. River water temperature here is generally higher than Cochiti, and has a higher sediment load (see Table 1 for temperature information). Substrate in the Bernalillo reach of the MRG is a mix of sand and cobbles. The Bernardo site is approximately 93 km downstream of the Bernalillo site. The substrate here is predominately sand or silt, and river water is warm and turbid due largely to sediment inputs from tributaries. The Albuquerque Wastewater Treatment Plant discharges into the river north of Bernardo. A number of farms operate in the river floodplain between Albuquerque and Bernardo, and there is potential for nutrient input from irrigation return flows to the river. The southernmost site is at Bosque del Apache National Wildlife Refuge, and is

approximately 77 km downstream of the Bernardo site. This site most resembles historical river conditions: water is warm and turbid and the substrate is sand or silt. Increased sediment loading from two upstream tributaries (Rio Puerco and Rio Salado) has led to aggradation within the river channel at Bosque del Apache (Leopold et al. 1964, Crawford et al. 1993). Also, this site has a more expansive floodplain that is occasionally inundated by flood waters.

Collection of primary carbon sources and consumers

When possible, all MRG aquatic food web samples were collected from each field site during the following time periods: April 2002 (prior to high spring flows), June 2002 (historically, after spring flows), September 2002 (after summer rainy season). Sampling was done in spring, summer, and fall because this is a highly productive time of year, and substantial primary production inputs into the river food web should occur during this time. By collecting during the productive seasons, we hoped to capture important inputs of organic matter in the MRG aquatic food web. An attempt was made to sample broadly at each trophic level of the river food web from primary producers (e.g., riparian plants, algae) to top-predators (e.g., predatory invertebrates and fish) (Table 2).

Sample preparation and analysis

Following collection (field methods outlined in Table 2), samples were placed on ice for transport back to the laboratory. Plant samples, organic matter, particulate organic matter (POM), particulate organic matter in transport (POMT), and precipitated dissolved inorganic carbon (DIC) were desiccated in a drying oven (at 60 C) prior to analysis (details of field collection methods can be found in Table 2). Dried samples were ground to a homogeneous powder using a mortar and pestle. POM, POMT, and DIC samples

were scraped off the filter paper prior to grinding. Some samples were frozen in Liquid N_2 to aid in pulverizing harder plant materials (e.g., woody matter). Invertebrates were identified to order, dried, and ground to a homogeneous powder. Fish specimens were identified to species, measured for standard length and frozen at -20°C. White muscle tissue samples were removed from each fish specimen and lyophilized.

For fish and invertebrates, between 0.6 and 1.2 mg of tissue was used for stable isotope analysis. Approximately 1 mg of dried, ground tissue was analyzed for plant, POM, and DIC samples. Weighed samples were packed in tin capsules and then reacted in an elemental analyzer. Evolved CO_2 and N_2 gases were analyzed on a Finnigan Mat Delta Plus isotope ratio mass spectrometer. The only exception to this protocol was CO_2 gas samples, which were injected directly into the mass spectrometer for analysis.

Data are reported in parts per thousand (‰ or per mil) in delta (δ) notation. The delta value is computed using the following equation:

$$\delta^{13}C \text{ or } \delta^{15}N \text{ , } = [(R_{sample}/R_{standard}) - 1] \times 1000 \quad \text{(Eq. 1)}$$

where R is equal to $^{13}C/^{12}C$ or $^{15}N/^{14}N$ (McKinney et al. 1950, Peterson and Fry 1987). Delta values are reported relative to a standard. Standards are PeeDee belemnite limestone for carbon (VPDB) and air for nitrogen. Reproducibility of standards for carbon and nitrogen was within 0.1‰ (pers. comm., V. Atudorei, Stable Isotope Laboratory, University of New Mexico).

RESULTS

Primary Energy Sources

Carbon isotope signatures of primary energy sources exhibited a gradient of decreasing $\delta^{13}C$ values from DIC and POM (both FPOM and POMT) to algae and finally

to riparian organic material, with riparian materials being the most ^{13}C depleted (Table 3 and Figures 4 and 5). Dissolved inorganic carbon (DIC) samples had similar $\delta^{13}C$ values across sites and seasons, and were significantly ^{13}C enriched relative to other carbon sources in the MRG ($P_{[two\text{-}tailed]} = 9.4 \times 10^{-35}$) (average for all DIC samples = -9.1‰). Fine particulate organic matter (FPOM) samples became more ^{13}C enriched with time at each of the four sites (average FPOM for all samples = -15.2‰). For example, the $\delta^{13}C$ values of FPOM from Bernalillo were –15.2, -10.9, and –8.6‰ for the spring, summer, and fall, respectively. FPOM and DIC were ^{13}C enriched compared to all particulate organic matter in transport (POMT) samples (average for all POMT samples = -18.7‰). In addition, POMT samples had more negative $\delta^{13}C$ values in the summer, compared to in the spring and fall, at all sites except Cochiti.

Carbon isotope ratios of riparian vegetation were similar across seasons and sites, with $\delta^{13}C$ values ranging from –22.5 to –28.4‰ (average $\delta^{13}C$ value of –26.4‰) Algae was ^{13}C enriched relative to riparian vegetation across sites (average $\delta^{13}C$ value of –19.0‰ for all algae samples). Emergent macrophytes had $\delta^{13}C$ values close to those of riparian vegetation (average $\delta^{13}C$ value of –25.3‰ for all emergent macrophyte samples), whereas submerged macrophytes were isotopically closer to algae (average $\delta^{13}C$ value of –17.5‰ for all submerged macrophyte samples). The $\delta^{13}C$ values of woody debris were similar to that of riparian vegetation (average $\delta^{13}C$ value of -26.0 for all wood debris samples). Figure 5 shows $\delta^{13}C$ and $\delta^{15}N$ mean values for algae, submerged macrophytes, emergent macrophytes, and riparian plants, graphed by season. With the exception of algae, there was little temporal change in carbon or nitrogen isotope values. The $\delta^{15}N$

values for algae become slightly more negative (^{15}N depleted) with time. The $\delta^{13}C$ and $\delta^{15}N$ values of vegetation were not substantially different between study sites, with the exception of riparian vegetation, which had significantly more positive $\delta^{15}N$ values at Bosque del Apache than at any of the northern sites ($P_{[two\text{-}tailed]} = 7.8 \times 10^{-11}$) (Figure 6).

Nitrogen isotopic values of FPOM tended to decrease (become less positive) with time (spring through autumn) across all sites except Cochiti. Riparian vegetation from Bosque del Apache had high $\delta^{15}N$ values relative to the $\delta^{15}N$ values of samples taken from the other three field sites. Nitrogen isotopic values of algae tended to decrease with the passing seasons across all sites. No trend was observed in the $\delta^{15}N$ values of submerged or emergent macrophytes (Figures 4 and 5).

Consumers

Invertebrates and fishes had broadly overlapping carbon isotope values at all sites, ranging from –16.2 to –28.2‰ (Table 3). On average, carbon isotope signatures of fishes were closer to in-stream sources (algae and submerged macrophytes) rather than to riparian vegetation and emergent macrophytes. Fishes were also, on average, slightly ^{13}C enriched compared to invertebrates. $\delta^{13}C$ values of fishes increased (became more ^{13}C enriched) south from Cochiti to Bernardo, and then reverted back to a more negative signature at Bosque del Apache (Figure 6). Overall, there is no temporal trend in carbon isotopes signatures of fishes; spring, summer and fall mean $\delta^{13}C$ values were similar, and there was a high degree of overlap in carbon isotope signatures. Further, there was substantial overlap between species for $\delta^{13}C$, and considerable variation within single species displaying up to 6‰ spread in $\delta^{13}C$ values (Figure 9).

Nitrogen isotope values for invertebrates in the MRG were highly variable, and ranged from 2.5 to 13.5‰. Fish were ^{15}N enriched relative to invertebrates by 0 to 14.7‰, with an average enrichment across all samples of 4.4‰ (average $\delta^{15}N$ of 8.3 and 12.7‰ for invertebrates and fish, respectively). The trophic structure of the food web, as represented by $\delta^{15}N$ values, is shifted up from Bernalillo south to Bosque del Apache, with Cochiti $\delta^{15}N$ values more intermediate (close to Bernardo values, Figure 7). In addition, there is a trend of decreasing $\delta^{15}N$ values of fish temporally, with fall 2002 being most ^{15}N depleted. Like carbon isotope signatures, nitrogen values of fish exhibit substantial variability, and there was no clear trophic structure among species (Figure 9).

DISCUSSION

Our study used stable isotope analysis of MRG aquatic food web components to characterize the food web and to identify spatial and temporal (seasonal) patterns. Dominant sources of organic carbon into the food web could be broken into groups (algae/submerged macrophytes versus riparian vegetation/emergent macrophytes), and these were distinguishable from one another based on isotopic signatures (Edwards and Turner 2003). This allowed the relative importance of each source to be quantified. Fish from the all sites except Cochiti were dependant on in-stream sources of organic matter (algae/submerged macrophytes). However, fish were generally not directly feeding on algae, but instead on either invertebrates or microinvertebrate grazers. Isotopic signatures of fish samples exhibited little change temporally. However, substantial longitudinal changes were evident, possibly suggesting river eutrophication driven by human

wastewater and/or agricultural inputs, especially at sites downstream of the city of Albuquerque, NM.

Primary carbon sources

In freshwater environments, dissolved inorganic carbon (the primary carbon source for algae and submerged macrophytes) comes from several sources: carbonate rock weathering, respiration, and atmospheric CO_2 (O'Leary 1988, Peterson and Fry 1987). When CO_2 dissolves in water at neutral pH, most of it will form carbonic acid, which will rapidly dissociate to form bicarbonate (HCO_3^-). When CO_2 forms HCO_3^-, the heavy isotope of carbon (^{13}C) concentrates in the bicarbonate, which is then heavier than the remaining carbon dioxide. CO_2 that forms HCO_3^- can originate from the atmosphere, from respiration, or from $CaCO_3$ rock weathering. Bicarbonate formed from atmospheric CO_2 will have a δ value close to 0‰ (Fry and Sherr 1989). If HCO_3^- is formed from respired CO_2, its δ value will be closer to the value of organic material (for example, aquatic plants) (Barth and Veizer 1999). When respiration is prominent, the $\delta^{13}C$ value of dissolved inorganic carbon (DIC) can approach –20‰ (Peterson and Fry 1987). The $\delta^{13}C$ value of $CaCO_3$ rocks is variable, but Raven et al. (1982) reported that the carbonate rocks in their study system have δ values close to 0‰. Therefore, bicarbonate formed from $CaCO_3$ dissolution would have a δ value greater than 0. These processes, $CaCO_3$ rock weathering, respiration, and atmospheric CO_2 dissolution, occur as water is being transported in a catchment, altering the $\delta^{13}C$ value of DIC present in the ecosystem (Barth and Veizer 1999).

Bicarbonate makes up the majority of any DIC sample taken from most rivers (Wetzel 2001). Therefore, the $\delta^{13}C$ value of the total DIC present in the MRG will

depend on the proportional contributions of the sources discussed above to the total bicarbonate found in river water. The average $\delta^{13}C$ value for DIC in the middle Rio Grande was -9.1‰. If the $\delta^{13}C$ values of the sources of bicarbonate discussed above are assumed to be correct (0, 0, and -20‰ for air, $CaCO_3$, and respiration, respectively), approximately 45% of the DIC in our samples would be derived from respiration. The remaining 55% would then be equally from atmospheric and $CaCO_3$ sources. However, respiration inputs can have lower $\delta^{13}C$ values than 20‰, in which case the inputs of air and $CaCO_3$ would be higher.

Because the carbon isotope signature of fine particulate organic matter (FPOM) samples are similar to our algae and submerged macrophytes samples, these samples are likely composed of in-stream materials. Interestingly, the $\delta^{13}C$ values of FPOM samples increased seasonally at all sites (i.e., fall values higher than spring or summer). This may indicate an increasing input of either algae or submerged macrophytes over the growing season. Increased temperatures and decreased flows (relative to spring snowmelt peak flows) occur in the MRG during the fall, creating low flow habitat conducive to growth of these types of vegetation.

The average carbon isotope values for particulate matter in transport (POMT) samples were more negative than either FPOM or DIC. However, this average is skewed by the summer data for POMT from Bosque del Apache. The Bosque del Apache summer POMT $\delta^{13}C$ data were unusually negative, and may reflect terrestrial organic matter that enters the river from upstream tributaries (Rio Puerco and Rio Salado) (Table 3). If the outlier Bosque del Apache POMT data are ignored, a pattern emerges: Cochiti POMT samples have $\delta^{13}C$ values which indicate input from either riparian vegetation or

emergent macrophytes, whereas the three Southern sites have $\delta^{13}C$ values closer to in-stream carbon sources. This is somewhat counter-intuitive because Cochiti is the site closest to Cochiti Dam, and is in an area that is highly disconnected from the floodplain. In addition, this site has extensive filamentous algal growth. Because of these features, POMT $\delta^{13}C$ values might be expected to resemble in-stream carbon sources. Flow is high at the Cochiti site compared to flow at the southern three sites, and other studies have demonstrated a strong negative relationship between algal $\delta^{13}C$ values and flow velocity (Finlay et al. 1999). However, because the $\delta^{13}C$ values algae collected from Cochiti are not unusually negative (compared to the other 3 study sites), this relationship is unlikely to explain POMT isotope signatures at Cochiti. Cochiti POMT samples may instead represent autochthonous phytoplankton production contributed from the upstream reservoir. Downstream of Cochiti, POMT $\delta^{13}C$ signatures are close to those of the algae/submerged macrophyte grouping, indicating POMT downstream is likely composed primarily of autochthonous production.

Riparian vegetation collected in our study had highly variable carbon isotope signatures, ranging from –22.5 to –28.4‰. This variability may be influenced by several factors, including soil water, river flow, and precipitation. Angradi (1994) notes that plants growing in dry soil tend to be ^{13}C enriched compared to those growing in wet soil. Additionally, river flow and precipitation can affect $\delta^{13}C$ values of riparian vegetation. Leffler and Evans (1999) note that when river flow is less than 25 m^3s^{-1}, a linear relationship exists between riparian plant $\delta^{13}C$ values and river flow. Also, during times

of low flow (<25 m^3s^{-1}), there is a linear relationship between $\delta^{13}C$ values and precipitation (Leffler and Evans 1999).

The average $\delta^{13}C$ value of emergent macrophytes is similar to that of riparian vegetation across sites, suggesting utilization of the same carbon source (CO_2 from air). Submerged macrophytes are quite distinct from emergent macrophytes, and are similar isotopically to algae. This indicates that algae and submerged macrophytes both use carbon found in river water (DIC). We grouped data on the vegetation types discussed above based on carbon isotope values. The riparian vegetation/emergent macrophyte group was found to be significantly different than the algae/submerged macrophytes group (mean difference = -7.4‰, $P_{[\text{two-tailed}]} = 8.9 \times 10^{-14}$) (Edwards and Turner 2003).

Consumer stable isotope analysis

Because the $\delta^{13}C$ values of the algae/submerged macrophytes group were significantly different than the riparian plants/emergent macrophyte group, we can use $\delta^{13}C$ values of fishes to determine the relative importance of these two groupings as carbon sources to the fish food web of the middle Rio Grande. The following two source mixing model was used to determine the fraction of carbon derived from the two vegetation groupings (riparian vegetation/emergent macrophytes versus algae/submerged macrophytes) that was assimilated into fish tissue:

$$\%\ \text{C from algae/submerged macrophytes} = \left(\frac{\left(\delta^{13}C_{fish} - \delta^{13}C_{riparianveg\,/\,emergmacrophytes}\right)}{\left(\delta^{13}C_{algae\,/\,submacrophytes} - \delta^{13}C_{riparianveg\,/\,emergmacrophytes}\right)} \right) \times 100 \quad \text{(eq.2)}$$

Using this model, the percentages of dietary carbon obtained from algae and/or submerged macrophytes for all fishes from Cochiti, Bernalillo, and Bosque del Apache are: 47%, 70%, and 74%, respectively. The average carbon isotope value for fish from Bernardo was more positive than the average value for submerged macrophytes or algae, but is within the range of sample error for these in-stream carbon sources. This suggests that fish from Bernardo likely obtain near 100% of dietary carbon from algae and submerged macrophytes. It is worth noting that in-stream sources of organic matter transported from upstream in the form of POMT could also be providing carbon to the fish food web of the MRG. $\delta^{13}C$ signatures of POMT are indistinguishable from those of algae and submerged macrophytes, making it impossible to distinguish between on-site autochthonous production and autochthonous carbon transported from upstream.

The ^{15}N enrichment between algae and fish (from 6.0 to 9.1‰) at all sites suggests that fish do not rely directly on algae as a food source (Figure 7). ^{15}N enrichment between algae and invertebrates varies between sites, and ranges from 1.7 to 4.5‰. ^{15}N enrichment between invertebrates and fish ranges from 3.1 to 4.6‰, suggesting that these could serve as an intermediate step in the food web. Another explanation posited by Pease et al. (2006) is that microinvertebrate grazers (e.g., rotifers, cladocerans, early instars of chironomids) could serve as a step between algae and fish in the MRG food web. However, these microinvertebrates were not sampled for this study. $\delta^{15}N$ values of the invertebrates we sampled suggest a reliance on algae or submerged macrophytes as a food source (1.7 to 4.5‰ $\delta^{15}N$ increase between algae/submerged macrophytes and invertebrates).

Spatial and temporal patterns in the MRG aquatic food web

The locations of our study sites along the middle Rio Grande span a longitudinal gradient in discharge, riparian vegetation [cottonwood dominate upstream, tamarisk downstream], anthropogenic nutrient additions, turbidity, and temperature. In addition, we sampled across a six month time span, which should capture temporal patterns in the food web during an extremely productive time of the year in this temperate river system. Temporally, there appears to be little change. Primary producer carbon and nitrogen isotope values were variable but overlapping among spring, summer and fall seasons (Figure 5). However, the entire food web exhibited substantial longitudinal isotopic shifts between the sample sites (Figures 6 and 7). Carbon isotope signatures of fishes were similar in the northern two study sites and the southern most site (Cochiti, Bernalillo, and Bosque del Apache), but $\delta^{13}C$ values were significantly more positive at Bernardo, reflecting high dependence on in-stream carbon sources at the Bernardo site (ANOVA with post hoc comparison of means and Bonferroni adjustment for multiple tests, $df = 1$, $p < 0.001$, Figure 6). Carbon isotope signatures at Bernardo show little to no input from the floodplain, indicating enhanced autotrophy relative to the other study sites. Similarly, the Bosque del Apache food web isotopic signatures indicate little input from floodplain carbon sources, despite occasional overbank flooding at this site.

One reason for this switch to autochthonous production could be nutrient enrichment from the Albuquerque Wastewater Treatment Plant (AWTP). The effluent from this plant enters the river near Albuquerque, between the Bernalillo site and the Bernardo site. Previous research has shown a substantial input of organic nitrogen and dissolved inorganic nitrogen (DIN) from the AWTP, and this spike of nitrogen remains in

the river at Bernardo, and beyond (Passell et al. 2005). The large input of DIN could be driving the river towards eutrophy downstream of the AWTP, leading to a spike in in-stream production. Another factor that could possibly be driving increased autotrophy at Bernardo is agriculture. Agriculture is prevalent in the historic floodplain of the reach between Bernalillo and Bernardo (Passell et al. 2005). Irrigation return flows from agricultural fields could carry water with high nutrient loads into the river, causing eutrophication. It is possible that high turbidity at the Bosque del Apache site prevents in-stream production at levels observed at the Bernardo site, accounting for the drop in autochthonous input in the Bosque del Apache food web.

The trend of increasing $\delta^{15}N$ values of the entire aquatic food web from Bernalillo south to Bosque del Apache is depicted in Figure 7. Because nitrogen input from human waste can be quite ^{15}N enriched, input from the AWTP could be driving this downstream upward shift (Chapelle 2000). However, pollution into freshwater systems from agriculture can also increase the $\delta^{15}N$ value of the total nitrate present within the water (Harrington et al. 1998, Hebert and Wassenaar 2001). Therefore, $\delta^{15}N$ values of the food web do not allow us to distinguish between wastewater or agricultural inputs as possible driving factors of eutrophication at Bernardo. The $\delta^{15}N$ signatures of food web components of the two northern sites show similar trophic structures, with a ^{15}N enrichment occurring downstream of Bernalillo. This enrichment coincides with both wastewater and agricultural inputs in the river, and supports the idea of human activity driving trophic structure change in the food web.

Conclusion

Neither the River Continuum Concept nor the Flood Pulse Concept adequately explain the patterns we observed in our Rio Grande data (Vannote et al. 1980, Junk et al. 1989). Contrary to the predictions of these studies, in-stream production was the dominant carbon source to the aquatic food web of our study system. These results are similar to the results of isotopic food web studies in other arid land rivers (Bunn et al. 2003, Angradi 1994), and suggest that in these types of ecosystems, autochthonous production is more important than previously thought. The Riverine Productivity Hypothesis and the "low-flow recruitment" hypothesis are more applicable to the MRG (Thorp and Delong 1994, Humphries et al. 1999). Regardless of sample site or season, autochthonous production dominated in our system. Fishes sampled from the MRG are not supported directly by vegetation, but instead by an intermediate step consisting of either macro- or micro-invertebrates. Omnivory is evident in the fish food web; fishes used similar resources regardless of nominal dietary differences (Figure 8 and 9, and Table 3 for dietary information).

Previous research has shown that the upper Rio Grande (north of Albuquerque) is generally oligotrophic, with low ambient concentrations of nitrogen and total organic carbon (Passell et al. 2005). Compared to other rivers in the western US, the upper Rio Grande is depleted with respect to nutrients. Historically, this tendency towards oligotrophy likely continued south throughout the MRG. The Albuquerque Wastewater Treatment Plant underwent substantial expansion in 1961, and has had considerable impacts on the nutrient chemistry of the river below the treatment plant effluent (Passell et al. 2005). In addition, irrigation return flows from agricultural fields located between

Bernalillo and Bernardo are likely delivering additional nutrients to the river. Our data suggest a substantial impact of human activities, whether from wastewater effluent or agricultural practices, on the entire river food web, driving the system towards eutrophy and shifting the community $\delta^{15}N$ values.

Future directions in MRG food web research

Our research indicates that fish in the MRG do not directly feed on algae, but instead on invertebrates. Previous research has proposed that microinvertebrate grazers may represent the step between primary production and fish in the MRG food web (Pease et al. 2006). However, microinvertebrates were not collected for this study. Determining the stable isotope composition of microinvertebrates from the MRG could clarify the relative importance of these grazers in the fish food web of the MRG.

Future work on the MRG food web could help to clarify the relationship between human activities and enhanced autotrophy at the Bernardo site. For instance, if the isotopic signatures of effluent water entering the MRG from the AWTP were known, it may be possible to determine the relative inputs of this source into the Bernardo aquatic food web. Similarly, water from agriculture fields could be collected in irrigation return canals, and the isotopic composition of irrigation return flow may shed light on the influences of these nutrient inputs on the food web.

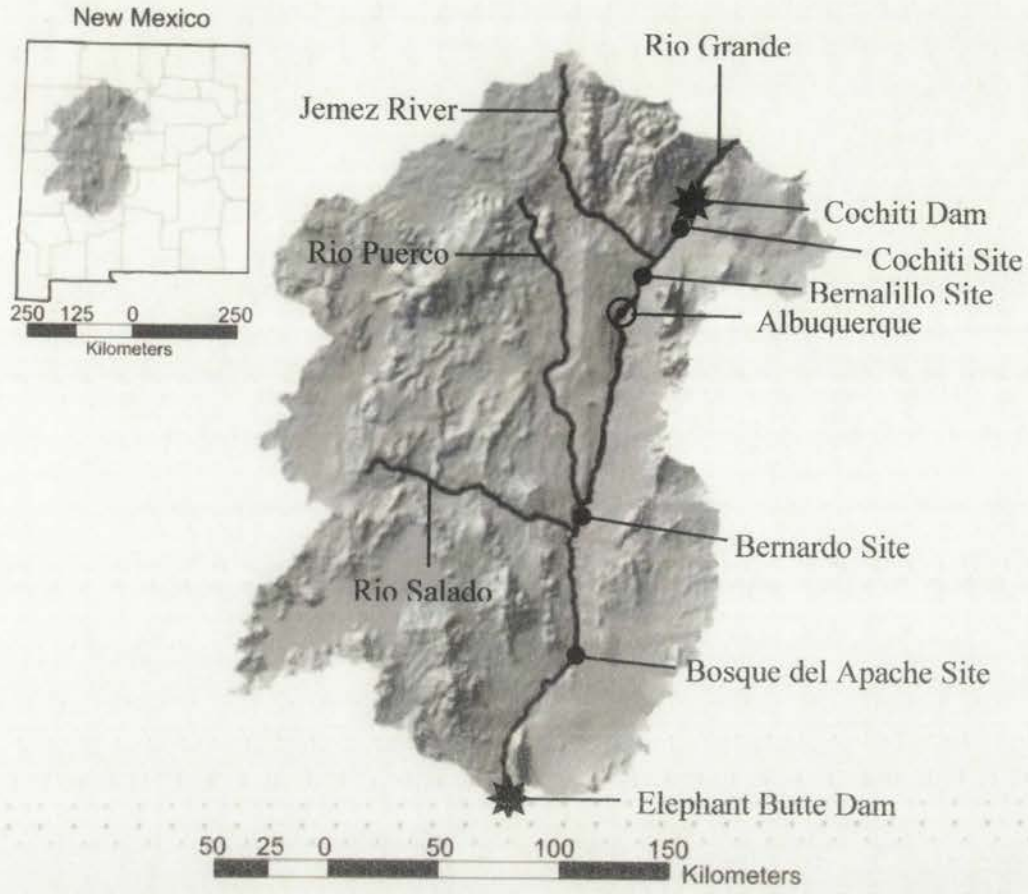

Figure 1. Map of the middle Rio Grande basin of New Mexico, which is the portion of the river between Cochiti Dam and Elephant Butte Dam.

Sampling sites and the major features and tributaries of the middle Rio Grande are indicated.

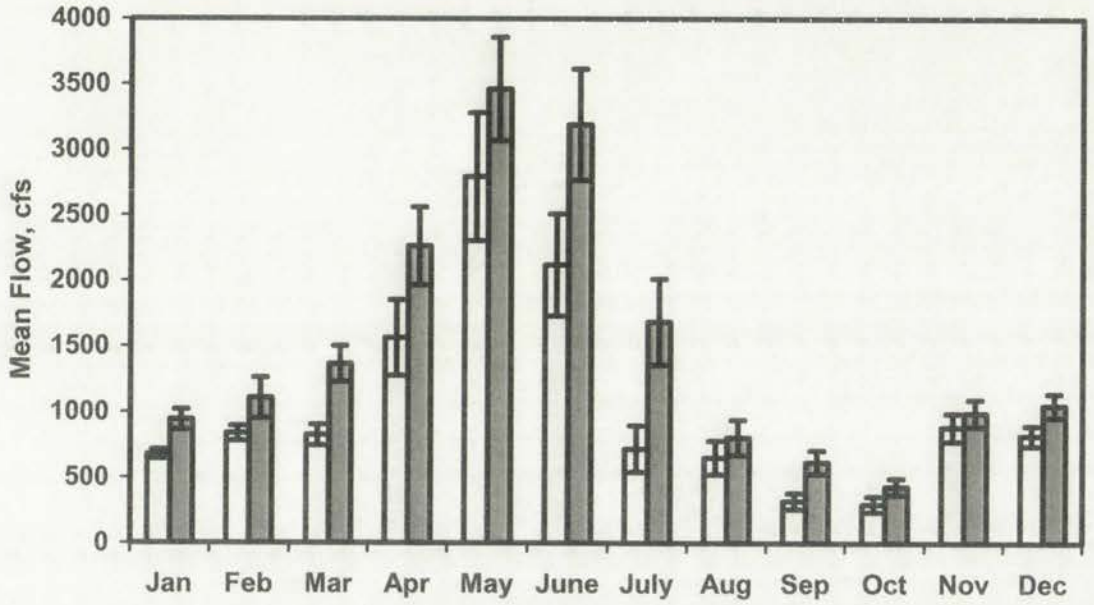

Figure 2. Mean monthly flow in the Rio Grande in Albuquerque.

Data obtained from the USGS website for station 08330000. Error bars represent one standard error. Unfilled bars represent flows before the installation of Cochiti Dam (completed in 1975), filled bars represent flows after Cochiti Dam was completed. This figure shows that the magnitude and variability of discharge was reduced substantially after Cochiti Dam was closed.

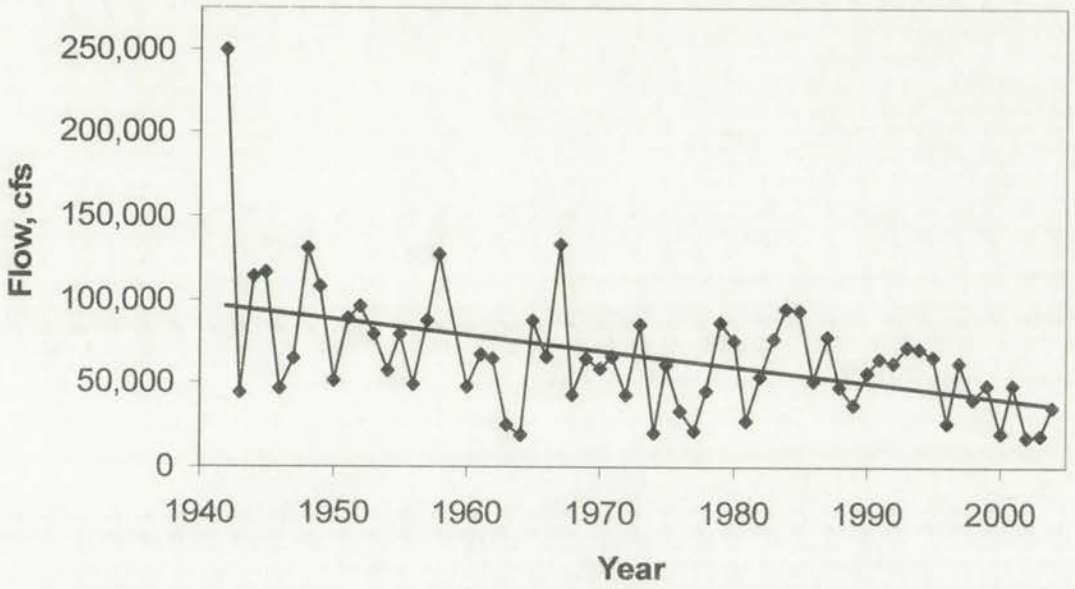

Figure 3. Yearly peak flows in the Rio Grande at Albuquerque with trend line shown.

Data obtained from the USGS website for station 08330000.

Table 1. River temperature at each sampling site, in degrees Celsius.

	Cochiti	Bernalillo	Bernardo	Bosque del Apache
Spring	13.2	13.3	25	18.8
Summer	19.8	22.9	29.8	*
Fall	18.9	21.8	24.6	20.1

Temperature data was recorded when fish were collected at sampling sites in each season. *The river at Bosque del Apache was dry in the summer of 2002, and temperature data could not be collected.

Table 2. Food web components collected from the MRG.

Item Collected	Method of Collection
Riparian Vegetation	Leaves were collected by hand from all dominant species.
Macrophytes	Dominant species were collected by hand
Algae	Benthic algae were colleced by hand from woody material or directly from the substrate
Woody debris	Pieces of debris were collected by hand.
Inorganic carbon	Dissolved Inorganic Carbon was precipitated from river water[1].
Particulate organic matter in transport	River water was filtered onto glass micro fiber filters[2].
Fine particulate organic matter	Particulate material collected by hand in area of deposition[3].
Invertebrates	Kick and drift nets were used[4].
Fish	Fish were collected using backpack electroshocking and seining[5].

[1]Dissolved inorganic carbon (DIC) was collected as an important carbon source to aquatic plants. DIC was precipitated from river water in a basic solution of $SrCl_2$ (Trujillo et al. 1987). Precipitated DIC was then filtered onto glass micro fiber filters. [2]Particulate organic matter in transport (POMT) was collected by collecting water from a flowing portion of the river, and filtering the particulate material onto glass micro fiber filter paper. [3]Particulate organic matter (POM) was collected by hand from an area of deposition, in which there was little flow and organic matter drops out of the water column. [4]A 500 micron drift net was placed in flowing water, and left for approximately 30 minutes, or until the collection container was clogged with debris. The contents were then sorted in the field, and any invertebrates were collected. A kick net was used in any edge and/or snag habitat present. Any invertebrates found were collected. [5]When the river was flowing sufficiently, backpack electroshocker was used to collect fish. If flows were low, simple seining was used. A variety of habitats were samples from each site, including fast flowing water, backwaters, edge and snag habitats.

Table 3. $\delta^{13}C$ and $\delta^{15}N$ values of food web components collected from each of 4 locations in the middle Rio Grande.

	Cochiti 2002						Bernalillo 2002					
	Spring		Summer		Fall		Spring		Summer		Fall	
Sample Type	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$
Primary Sources												
Algae	8.8	-19.8	8.6	-21.5	4.4	-19.9	6.8	-16.1	4.9	-28.5	2.2	-18.3
	-0	-0.8			-0.6	-2.6					-0.7	-2.6
DIC	-	-	-	-8.9	-	-7.9	-	-	-	-9.7	-	-8.4
				-0.1						-0.1		
POMT	-	-23	-	-23.4	-	-26.3	-	-17.4	-	-18.6	-	-16.3
												-0.3
FPOM	5.7	-21.6	7.5	-21.1	4	-17.7	4.5	-15.2	-	-10.9	2.9	-8.5
Riparian Vegetation	2.9	-26.7	2.7	-27.4	-1.6	-27.9	-1.1	-23.4	0.3	-27	1.2	-26
	-0.4	-0.7	-0.7	-0.5	-1.6	-0.7	-0.9	-0.7	-0.7	-0.8	-1	-2
Emergent Macrophytes	-	-	6.8	-26.9	4.2	-26.7	5.9	-27.3	3.2	-26.3	-	-
			-0.5	-0.3	-0.9	-1	-1.9	-0.5	-1.7	-1.8		
Submerged Macrophytes	-	-	8.4	-16.2	6.4	-20.4	3	-18.6	7.3	-16.8	4.6	-14.8
			-0.2	-0.6								
Woody Debris	3.1	-25.7	-0.9	-27.6	-	-	-0.4	-27.1	-0.7	-26.5	-0.9	-23.6
Secondary Consumers												
Basomatophora (C, SC, P)	-	-	8.7	-16.5	-	-	-	-	-	-	-	-
Diptera (SH, C, SC, P)	-	-	-	-	-	-	9.9	-25.9	7.9	-24.8	-	-
Ephemeroptera (SC)	-	-	8.9	-16.5	5.7	-24.5	9	-23.9	12.5	-26.5	2.5	-24.9
			-0.7	-0.2								
Hemiptera (C, SC, P)	-	-	8.3	-23.5	-	-	-	-	-	-	8.3	-32.2
Megaloptera (P)	-	-	-	-	-	-	-	-	-	-	5.8	-23.4
Odonata (P)	-	-	10.2	-23.1	-	-	-	-	-	-	6.2	-22.7
Trichoptera (SC, C, P)	-	-	8.8	-21.9	-	-	-	-	9	-25.1	5.3	-24.1
Fishes												
Catostomus commersoni	12.6	-23.7	13.8	-22.6	12	-21.8	-	-	11.2 (5)	-19.3 (5)	9.1	-21.8
(periphyton)	-0.2	-0.7	-0.1	-0.3	-0.3	-0.1			-0.3	-1.2	-0.5	-0.2
Rhinichthys cataractae	12.6	-23.3	13.1	-23.3	11.4	-21.8	-	-	11.9 (5)	-20.0 (5)	9.8	-22.6
(invertebrates)	-0.1	-0.1	-0.2	-0.2	-0.9	-0.3			-0.1	-0.3	-0.5	-0.3
Gambusia affinis	-	-	13.1	-21.9	-	-	-	-	-	-	-	-
(invertebrates, algae)			-0.6	-0.3								
Micropterus salmoides (piscivorous)	-	-	-	-	10.9	-20.4	-	-	-	-	-	-
Cyprinella lutrensis	-	-	-	-	-	-	11	-22.4	13.6 (5)	-19.6 (5)	-	-
(invertebrates)							-0.4	-0.3	-1.2	-0.9		
Pimephales promelas	-	-	-	-	-	-	9.7	-22.1	10.6 (5)	-19.7 (5)	-	-
(invertebrates)							-0.7	-0.1	-0.5	-1.2		
Platygobio gracilis	-	-	-	-	-	-	-	-	11.2 (5)	-19.7 (5)	8.5	-21.4
(invertebrates)									-0.2	-0.8		
Ictalurus punctatus	-	-	-	-	-	-	-	-	13.4 (3)	20.8 (3)	7.7	-23.1
(omnivore)									-0.2	-0.6		

Table 3 *Continued*

	Bernardo 2002						Bosque del Apache 2002					
	Spring		Summer		Fall		Spring		Summer		Fall	
Sample Type	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$
Primary Sources												
Algae	7.9	-18.8	7.5	-17.3	2.3	-14.3	-	-27.2	4.3	-20.1	2.2	-20.9
	-1.7	-2.2	-1.4	-1.2								
DIC	-	-	-	-	-	-9.7	-	-	-	-	-	-9.9
POMT	-	-15.4	-	-17.5	-	-14.2	-	-18.7	-	-28.2	-	-12.1
								0.5				1.1
FPOM	-	-	5.2	-14.8	4.6	-10.4	-	-	6.1	-19.7	4.2	-11.6
Riparian Vegetation	3.5	-26.3	3.7	-27.3	2.1	-28.4	7.1	-26.7	8.4	-26.5	7	-22.5
	-2.7	-0.5	-1.2	-0.4	-1.2	-0.5	1	0.2	0.9	1	0.5	2.6
Emergent Macrophytes	9.3	-24	9.8	-26.9	7.4	-12.3	8.8	-22.1	7.7	-27.7	-	-
							0.4	4.7				
Submerged Macrophytes	-	-	-	-	-	-	-	-	-	-	-	-
Woody Debris	3.6	-24.2	3.9	-27.2	-3	-25.4	8.5	-28.8	5.4	-29	-0.3	-20.6
Secondary Consumers												
Coleoptera	6	-25.9	-	-	-	-	6	-25.6	-	-	-	-
(SH, C, SC, P)	-1	-1.7										
Diptera	13.4	-19.5	-	-	-	-	10.7	-28.2	-	-	-	-
(SH, C, SC, P)	-1.4	-0.3										
Ephemeroptera	11.9	-18.1	-	-	-	-	12.4	-21.2	-	-	-	-
(SC)												
Hemiptera	-	-	11.4	-18.4	4.7	-20.2	11.3	-19.5	-	-	-	-
(C, SC, P)												
Hymenoptera	-	-	-	-	-	-	4.4	-25.7	-	-	-	-
(SC)												
Isopoda	1.5	-22.2	-	-	-	-	-	-	-	-	-	-
(*)												
Odonata	12.3	-20.4	-	-	-	-	12.3	-20.1	-	-	-	-
(P)												
Plecoptera	-	-	-	-	-	-	13	23.2	-	-	-	-
(SH, P)												
Fishes												
Gambusia affinis	14.4	-18.5	14.4	-16	12.4	-17.5	-	-	-	-	10.5	-21.7
(invertebrates, algae)	-0.6	-1	-0.2	-0.3	-0.2	-0.5						
Micropterus salmoides	-	-	-	-	-	-	-	-	-	-	-	-
(piscivorous)												
Cyprinella lutrensis	15.3	-17.2	14.4	-16.8	11	-17.8	15.7	-20.5	-	-	-	-
(invertebrates)	-0.1	-0.2	-0.2	-0.5	-0.3	-0.2	-0.2	-0.4				
Pimephales promelas	15.2	-16.6	-	-	12.5	-17.6	-	-	-	-	-	-
(invertebrates)	-0.9	-0.7			-1	-0.4						
Ictalurus punctatus	15.2	-18.1	-	-	-	-	-	-	-	-	13.8	-20.1
(omnivorous)											-0.3	-0.7
Cyprinus carpio	15.3	-16.4	13.6	-17	-	-	14.7	-18.7	-	-	-	-
(invertebrates)			-0.2	-0.3								
Carpiodes carpio	16.2	-16.8	-	-	13.1	-16.5	-	-	-	-	-	-
(detritus, algae)	-0.2	-0.2										
Ictalurus furcatus	-	-	-	-	12.4	-16.9	-	-	-	-	-	-
(omnivore)												
Pomoxis annularis												
(omnivore)	-	-	-	-	-	-	12.9	-22.6	-	-	-	-

Mean values (± 1 SE, n ≥ 3 samples) or individual values are presented (where n = 1). Shown below the fish scientific names is the presumed diet for each species (Sublette et al. 1990). Feeding groups indicated for invertebrates: SH = Shredder, C = Collector, SC = Scraper, P = Predator (Wetzel 2001). *No feeding group indicated for Isopoda because this is primarily a terrestrial taxa.

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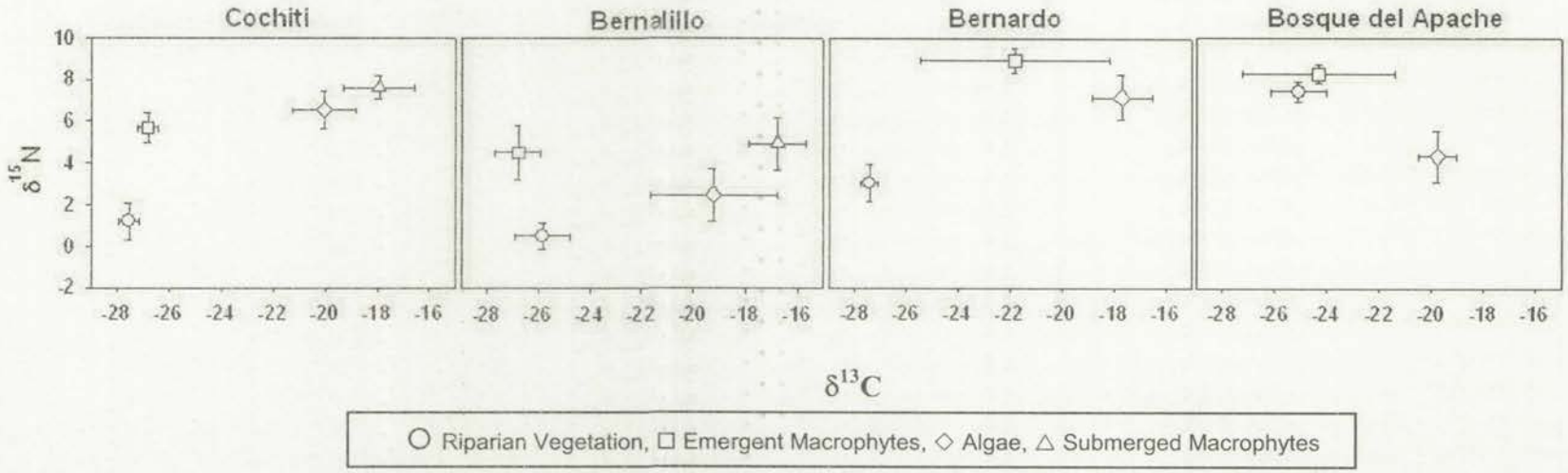


Figure 4. Mean $\delta^{13}C$ and $\delta^{15}N$ values of primary carbon sources in the middle Rio Grande, plotted by field site.

Symbols represent mean for all samples collected. Error bars represent one standard deviation.

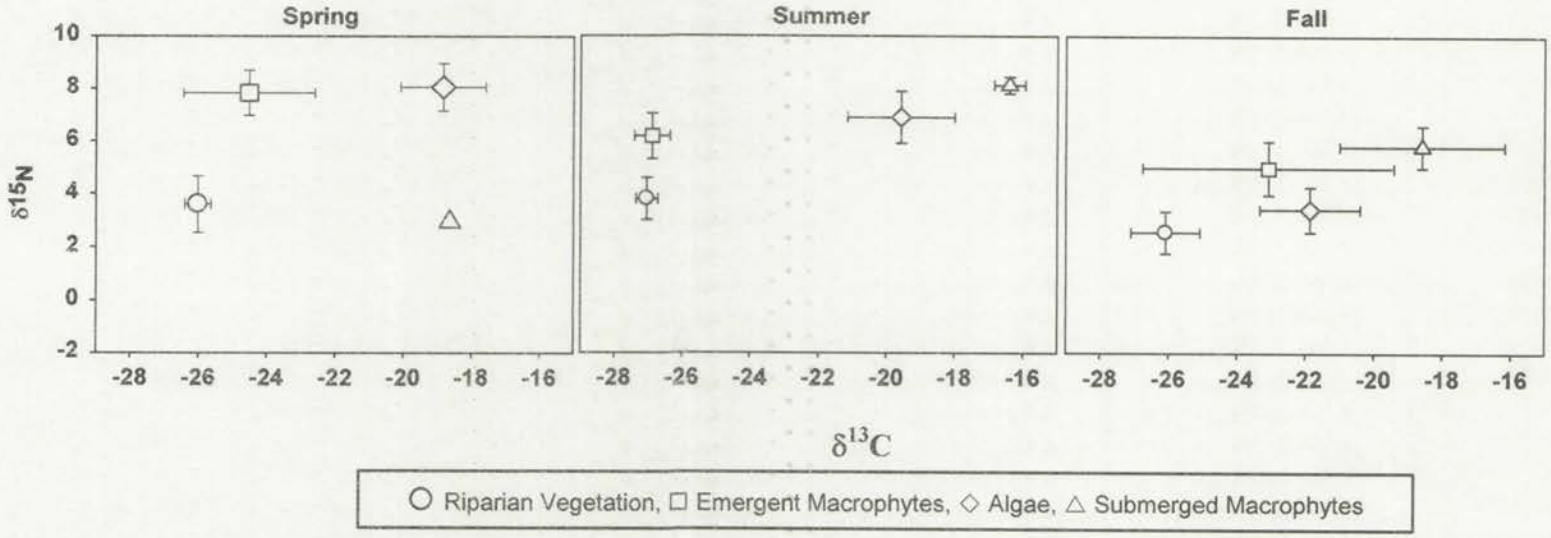


Figure 5. Mean $\delta^{13}C$ and $\delta^{15}N$ values of primary carbon sources in the middle Rio Grande, plotted by season.

Symbols represent mean for all samples collected in each season from all four field sites. Error bars represent one standard deviation.

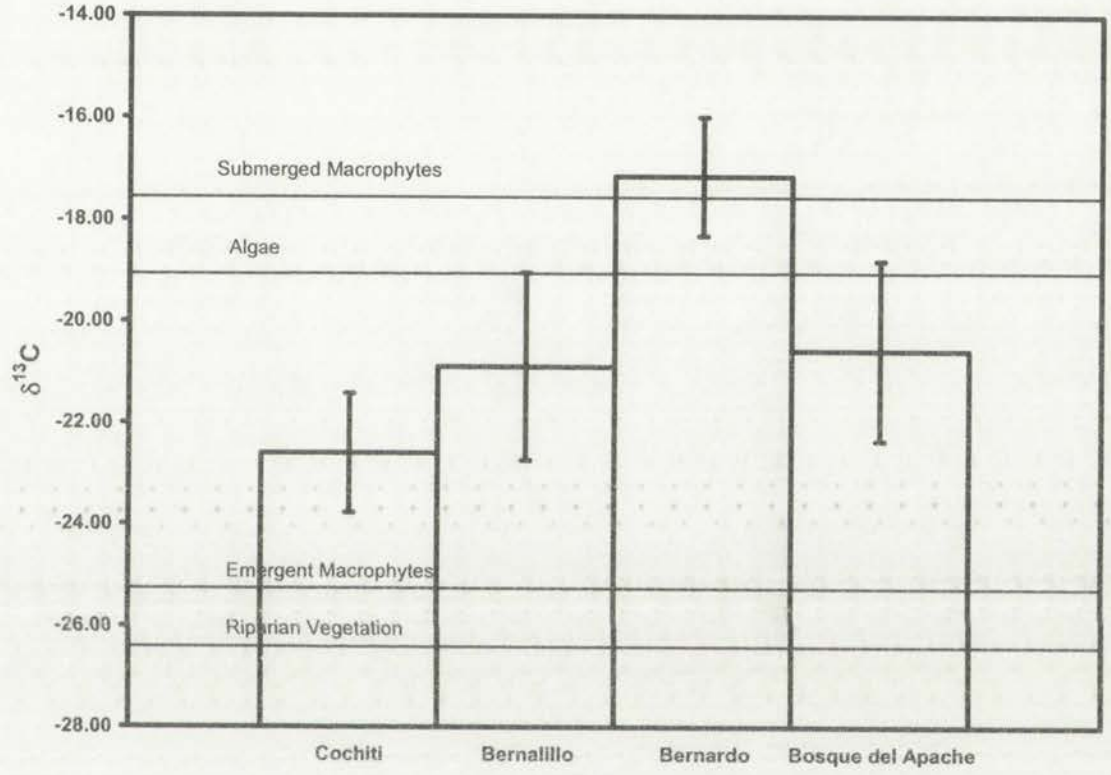


Figure 6. Average carbon isotope values for fishes collected in 2002 at each site (boxes).

Values shown are averages for samples collected from each site (averages for all seasons). Bars represent one standard deviation. Average values for the four vegetation classifications are also shown.

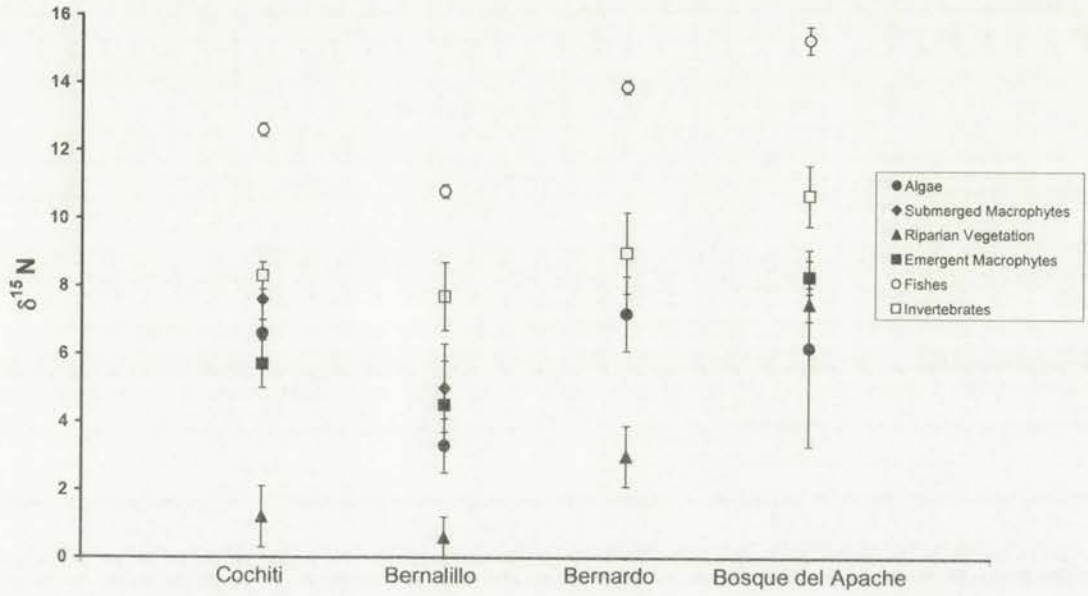


Figure 7. Average nitrogen isotope values for food web components collected from the middle Rio Grande during 2002.

Values shown are averages for samples collected from each site (averages for all seasons). Bars represent one standard error.

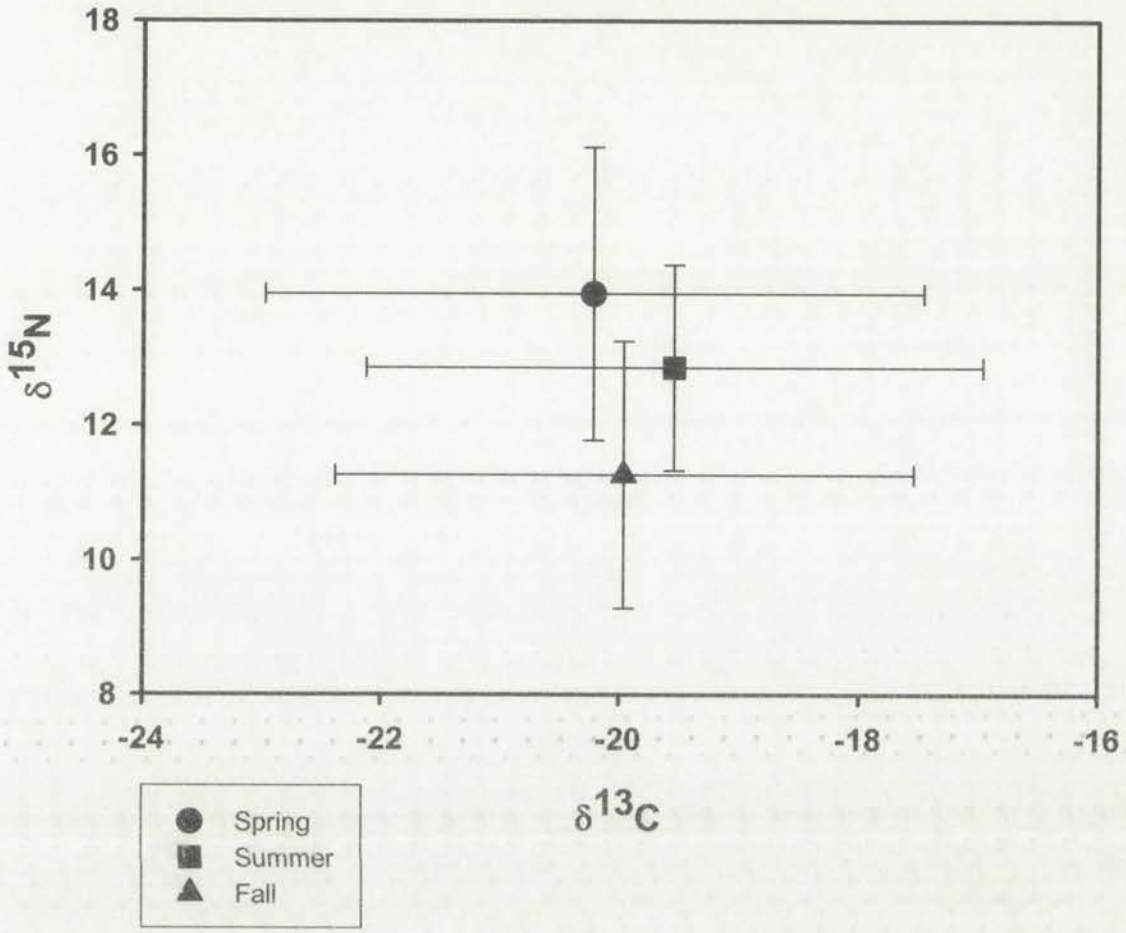
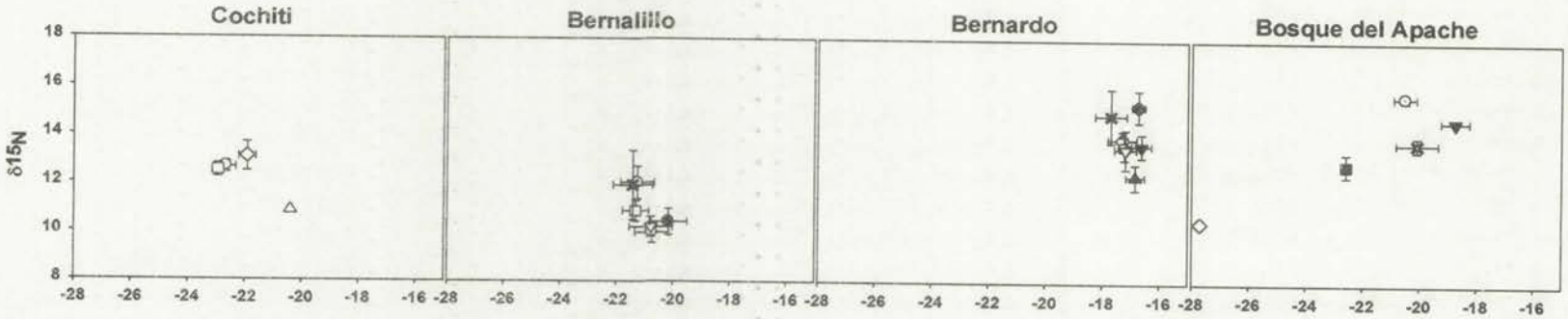


Figure 8. Mean $\delta^{13}C$ and $\delta^{15}N$ values of fishes collected from all sites in the spring, summer, and fall of 2002.

Error bars represent one standard deviation.



- *Catostomus commersoni*
- *Rhinichthys cataractae*
- *Gambusia affinis*
- *Micropterus salmodes*
- *Cyprinella lutrensis*
- *Pimephales promelas*
- *Platygobia gracilis*
- *Ictalurus punctatus*
- *Cyprinus carpio*
- *Ictalurus furcatus*
- *Carpiodes carpio*
- *Pomoxis annularis*

Figure 9. Mean $\delta^{13}C$ and $\delta^{15}N$ values of fishes collected in 2002, plotted by field site.

Error bars represent one standard deviation.

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CHAPTER 3: THE HISTORICAL AQUATIC FOOD WEB OF THE MIDDLE RIO GRANDE, NM

INTRODUCTION

The Rio Grande flows through arid lands in Colorado, New Mexico, Texas, and Mexico in the Southwestern North America, and is one of the most severely degraded rivers in the United States (one of ten most endangered river systems in the US in 2000, American Rivers). The middle Rio Grande (MRG) of New Mexico is a ~300 km reach of river flowing from Cochiti Dam to Elephant Butte Dam (Figure 1). The MRG supports the largest human population in New Mexico and, not surprisingly, the river is heavily regulated for flood control and to provide water supply for agriculture. This reach of river was historically dominated by highly variable flows, with flood pulses in the spring and summer (Crawford et al. 1993). River flows peaked in the spring and due to snowmelt, and in the late summer/fall with monsoonal precipitation. Extreme high flow events in the MRG were interspersed with years of devastating droughts with low or no river flows in portions of the reach (Figure 2) (Crawford et al. 1993). The river channel was braided and sinuous, and often shifted course. Stands of cottonwood and willow made up the riparian zone, along with wetlands and oxbow lakes. Roughly 17 native fish species (of 27 native fishes found in the Rio Grande in New Mexico as a whole) were present in the MRG, along with an abundance of aquatic invertebrates (Crawford et al. 1993, Sublette et al. 1990, Propst 1999).

The last century has brought major anthropogenic change to the middle Rio Grande valley. Beginning in the 1950s, the Corps of Engineers constructed major

structures to control the flow of the MRG, including dams, levees, and jetty jacks (Figure 2) (Crawford et al. 1993). These structures fundamentally changed the hydrology of the MRG, decreasing flow variability and virtually eliminating overbank flooding (Dahm et al. 2005). Cochiti Dam was completed in 1975, and reduced wet season peak flows that historically caused overbank flooding (Figure 2). Biologically, the river was severely impacted by these changes. The native MRG fish community is declining, and there have been many exotic fish introductions (Crawford et al. 1993, Sublette et al. 1990). The Rio Grande silvery minnow (*Hybognathus amarus*) was once one of the most abundant fish in the middle Rio Grande, but is now on the federal endangered species list, and only occurs in 5% of its historical range (Crawford et al. 1993).

The human population is growing at a rapid rate in the Rio Grande valley. There is increasing pressure on an already overallocated river ecosystem, and thus there is substantial urgency to improve the condition of the river through intensive management. There is great concern that the river ecosystem will continue to lose biodiversity, and that the structure and function of the river ecosystem will be irreparably changed if water extraction and diversion increases concomitantly with population growth. In principle, management of the river should protect and enhance the region's biological quality (species diversity and abundance) and ecosystem integrity (ability to recover following disturbance) (Crawford et al. 1993). It may also be possible to restore portions of the Rio Grande ecosystem; for example, Molles et al. (1998) discuss steps necessary for restoration of the Rio Grande riparian forest. In order to accomplish restoration goals, there is a "fundamental need to understand how the ecosystem worked in the past and

how it works now" (Crawford et al. 1993). The aim of our research is to provide information on prior conditions of the Rio Grande aquatic food web, and to understand how hydrologic alteration and species introductions affect Rio Grande ecosystem processes. An understanding of conditions prior to large-scale anthropogenic disturbance will help focus restoration and management efforts toward effective restoration to enhance abundance and biodiversity. For example, if the bosque provided the majority of nutrients to the historical food web, then management efforts should aggressively seek to restore the connection of the river channel and the bosque.

One approach for quantifying changes in pre- and post-disturbance food webs is to compare the disturbed system to an unaltered reference system (Vander Zanden et al. 1999). The reference system is assumed to be identical to the pre-disturbance condition of the altered system. For many freshwater aquatic systems, like large rivers in the lower 48 States, an undisturbed reference system does not exist (Benke 1990), indicating the need for an alternative approach. Our method compares stable isotope values of museum preserved fish specimens held in the Museum of Southwestern Biology to recently collected fishes to evaluate changes in ecosystem structure and function that have resulted from hydrological alteration in the system over the past 65 years. Previous research has demonstrated that preserved museum specimens retain reliable stable isotope information, and therefore can be used in food web studies (Edwards et al. 2002, Vander Zanden 2003, Arrington and Winemiller 2002). Because of this, we can use museum preserved fish collected from the MRG to construct a timeline of change in the aquatic food web.

Stable isotope chemistry is a well-studied and widely used approach to quantify nutrient flow and structure of food webs, and is a commonly used tool in freshwater ecology (e.g., Peterson and Fry 1987, Fry 1991, Fry 2006). Isotopic analysis has been used to identify omnivory in fish assemblages of tropical river food webs (Jepsen and Winemiller 2002), distinguish between allochthonous and autochthonous sources of organic matter into aquatic food webs (Bunn et al. 2003, Pease et al. 2006), and even to characterize anthropogenic nutrient inputs into freshwater systems (Harrington et al. 1998, McKinney et al. 2002). Stable isotopes have also played an important role in understanding community-level responses to anthropogenic and natural disturbance (Vander Zanden et al. 1999). Vander Zanden et al. (2003) and Chasar et al. (2005) demonstrated how historic preserved fish specimens can be used to quantify food web changes over time. In our study in the Rio Grande, we use historical fish specimens collected from the river over the past several decades to characterize food web changes.

Materials and Methods

Study Sites

Museum specimens were collected from four sites in the MRG (Figure 1). Sites were chosen because the Museum of Southwestern Biology at the University of New Mexico (UNM) has preserved fishes from each site, collected for many years before, during, and after hydrologic alteration of the river. Additionally, the sites are relatively evenly spaced along the MRG. The Cochiti site is northernmost (upstream) site and is located just below Cochiti Dam. Hypolimnetic dam releases have likely dominated flows at this site since the completion of Cochiti dam in 1975, making the river clear and cold.

The substrate is composed of cobbles and gravel, with little sand or fine sediment. The Bernalillo site is near the town of Bernalillo, NM and is approximately 48 km downstream of the Cochiti site. River water temperature here is generally higher than Cochiti, and has a higher sediment load. Substrate in the Bernalillo region of the MRG is a mix of sand and cobbles. The Bernardo site is approximately 93 km downstream of the Bernalillo site. The substrate here is predominately sand or silt, and river water is warm and turbid due to sediment inputs from tributaries. The most downstream (southern) site is at Bosque del Apache National Wildlife Refuge, and is approximately 77 km downstream of the Bernardo site. This site most resembles historical river conditions in that the water is warm and turbid and the substrate is sand or silt. Increased sediment loading from two upstream tributaries (Rio Puerco and Rio Salado) has led to aggradation within the river channel at Bosque del Apache (Leopold et al. 1964, Crawford et al. 1993). Also, this site has a more expansive floodplain that is occasionally inundated by flood waters.

Museum Specimens

The following materials from the Division of Fishes in the Museum of Southwestern Biology (MSB) were analyzed. This includes five specimens of each species listed below (if available) collected during each time frame from each of my four research sites.

Time frames: 1939 – 1956, 1970 – 1978, 1990 – 1995, and 1999 – 2000.

The time frames listed above were chosen because the MSB contains substantial holdings of fishes collected from the research sites during these years. Considerable river

alteration occurred in the past several decades, particularly in the late 1970s, with completion of a major mainstem reservoir (Cochiti Dam) in 1975. Analysis of fishes collected during these time frames should provide insight into food web effects of these alterations. The species listed are (or were historically) dominant in this portion of the river.

Species list: *Cyprinella lutrensis* (red shiner), *Platygobio gracilis* (flathead chub), *Carpiodes carpio* (carpsucker), *Pimephales promelas* (fathead minnow), *Rhinichthys cataractae* (longnose dace), *Hybognathus amarus* (silvery minnow), *Ictalurus punctatus* (channel catfish), *Gambusia affinis* (mosquito fish), *Catostomus commersoni* (white sucker), and the non-native *Cyprinus carpio* (common carp). Table 1 shows the presumed diets for each of these species. In general, the fish food web is thought to be composed of three trophic guilds: algae/detritus/diatom consumers, insectivores, and an intermediate omnivore step.

A small portion (5 – 10 mg) of lateral white muscle tissue was removed from the right side of each specimen and lyophilized. Approximately 1 mg of lyophilized fish tissue was used for stable isotope analysis. Weighed samples were packed in tin capsules and then reacted in an elemental analyzer. Evolved CO_2 and N_2 gases were analyzed on a Finnigan Mat Delta Plus isotope ratio mass spectrometer. Data are reported in parts per thousand (‰ or per mil) in delta (δ) notation. The delta value is computed using the following equation:

$$\delta^{13}C \text{ or } \delta^{15}N = [(R_{sample}/R_{standard}) - 1] \times 1000 \quad \text{(Eq. 1)}$$

where R is equal to $^{13}C/^{12}C$ or $^{15}N/^{14}N$ (McKinney et al. 1950, Peterson and Fry 1987). Delta values are reported relative to a standard. Standards are PeeDee belemnite limestone for carbon (VPDB) and air for nitrogen. Reproducibility of standards for carbon and nitrogen was within 0.1‰ (pers. comm., V. Atudorei, Stable Isotope Laboratory, University of New Mexico). Because formalin fixation lowers (makes more negative) the $\delta^{13}C$ values of preserved tissue by approximately 1.1‰ (Edwards et al. 2002), we corrected the isotope ratios of fishes by adding 1.1‰ to observed $\delta^{13}C$ values of preserved fish tissues. The alteration in $\delta^{15}N$ values caused by formalin fixation is minimal compared to the average shift between trophic levels (Edwards et al. 2002), and we did not correct $\delta^{15}N$ values of preserved fish. We then used single classification analysis of variance (ANOVA) and two-sample t-tests (assuming unequal variances) to test for differences in fish stable isotope values between seasons and sampling sites.

Background and predicted changes in stable isotope signatures in fishes

1. Carbon sources to the fish food web. Historically, the extensive floodplain of the Rio Grande was inundated in springtime as a result of snowmelt (Crawford et al. 1993). The placement of dams and levees has consistently reduced peak flows in the MRG (Figure 2). These high flows would have historically produced overbank flooding (Crawford et al. 1993, Molles et al. 1998, Ellis et al. 2002), bringing floodplain materials into the river with receding waters. Restriction of peak flows has substantially decreased the movement of floodwaters on to the floodplain, and thus restricted nutrient flow from decomposing riparian vegetation to the main channel. Because floodplain inundation has been virtually eliminated, carbon and nitrogen transported from upstream or produced in the channel

proper (e.g., algae) should provide the majority of nutrients for the food web in recent years, and inputs from riparian habitats should be minimal.

Carbon isotopes can be used to trace the inputs of carbon into an aquatic food web (Fry and Sherr 1989) and were used in this study to quantify the effects (discussed above) of hydrological regime alteration on the carbon inputs into the MRG fish food web. In the MRG, dominant sources of organic material into the food web can be statistically distinguished (algae/submerged macrophytes versus riparian vegetation/emergent macrophytes) based on isotopic signatures. For example, $\delta^{13}C$ values of the algae/submerged macrophytes group was found to be significantly more positive than $\delta^{13}C$ values of the riparian vegetation/emergent macrophytes group (Edwards et al. 2003, Edwards et al., in preparation). Because the present-day food web likely receives little floodplain input relative to historical conditions, we predicted that the $\delta^{13}C$ values of fish would be more positive (reflecting higher algal inputs) after the construction of Cochiti Dam.

2. Agriculture and Population Growth. Human population growth and associated activities are expected to affect stable isotope signatures through increased use of fertilizers and increased sewage and wastewater, detected by shifts in nitrogen signatures. Anthropogenic nitrogen inputs into freshwater systems from agriculture and human waste can increase the $\delta^{15}N$ value of the total organic nitrogen present within the water (Harrington et al. 1998, Hebert and Wassenaar 2001). This ^{15}N rich nitrogen would likely be incorporated into the food web by primary producers, and the elevated (more positive) $\delta^{15}N$ signatures perpetuated throughout the food web. We predicted that the $\delta^{15}N$ values

of fish collected downstream of major anthropogenic nitrogen inputs (e.g., wastewater treatment plant effluents, agricultural fields) will be higher (more positive) in recent years, when human population growth has increased the magnitude of these inputs.

RESULTS

Cochiti fish samples had $\delta^{15}N$ values that were significantly more positive than Bernalillo samples (all years) and Bernardo samples before 1961 ($P_{[\text{two-tailed}]} < 0.05$ for all comparisons), but were not significantly more positive than Bernardo samples after 1961 or Bosque del Apache samples (Figure 7). The $\delta^{15}N$ values of Bernardo fish samples taken after 1961 were significantly more positive than all samples except those from Cochiti ($P_{[\text{two-tailed}]} < 0.05$ for all comparisons).

Prior to 1961, fishes collected at the four study sites had indistinguishable $\delta^{13}C$ values (Figure 6). $\delta^{13}C$ values of fishes collected after 1961 were similar in the two upstream study sites and the most downstream site (Cochiti, Bernalillo, and Bosque del Apache), but $\delta^{13}C$ values of fishes collected after 1990 were significantly more positive (more ^{13}C enriched) at Bernardo (Figure 6) (mean difference between Bernardo fishes collected before and after 1961 = 2.5‰, $P_{[\text{two-tailed}]} = 5.6^{-7}$).

Little temporal change in fish carbon and nitrogen isotope signatures was observed over the museum collection period of record when all samples were combined (Figures 3 and 4). There was no significant difference between the mean $\delta^{15}N$ or $\delta^{13}C$ values of fish collected in the MRG prior to the completion of Cochiti dam (1975) and fish collected after dam installation ($p = 0.22$ and $p = 0.14$ for $\delta^{15}N$ and $\delta^{13}C$,

respectively). A scatter plot of $\delta^{15}N$ and $\delta^{13}C$ of fishes from all years shows substantial overlap (Figure 5). The fish food web was generally unstructured. In other words, nitrogen isotope ratios did not indicate strong trophic divergence among fish species.

DISCUSSION

Historically, the middle Rio Grande was a highly dynamic river that experienced large floods interspersed by severe droughts. The river channel freely migrated across the floodplain with varying river discharge (Crawford et al. 1993). Ephemeral ponds and wetlands and discontinuous groves of riparian vegetation dotted the river landscape. Regulation of the river has led to channelization, less flow variability, and little river-floodplain interaction (Crawford et al. 1993). Habitat variability has also decreased; for example, ephemeral floodplain habitat is now virtually non-existent in many areas of the MRG, excepting Bosque del Apache (Crawford et al. 1993, Molles et al. 1998). Our study endeavored to provide information on food web effects of these changes in the MRG, which will be useful in restoration planning in the basin.

Contrary to what might be expected based on presumed diets of MRG fishes (Table 1), our data revealed no trophic structure between fish species in the food web (Figure 5). Instead, we observed substantial overlap in $\delta^{15}N$ and $\delta^{13}C$ values within and between species, indicative of generalist feeding habits (Figure 5). This feeding strategy would allow Rio Grande fishes to take advantage of whatever transient food sources are available at a given time. In other words, we suggest that fishes of the Rio Grande are largely omnivorous and opportunistic in their feeding habits both in the past and in the present. Omnivory is common in aquatic food webs (Jepsen and Winemiller 2001,

Beaudoin et al. 2001, Holyoak and Sachdev 1998), and has been demonstrated in previous Rio Grande food web studies (Edwards et al., in preparation). In addition, omnivory has been identified as a stabilizing feature in communities; Fagan (1997) found that in experimental terrestrial arthropod communities, an increased degree of omnivory was correlated with increased resistance to major community disturbance. Our data suggest omnivory has been dominant in the MRG fish food web for at least 60 years. Omnivory and flexible feeding habits of these fishes could be contributing to food web stability and resistance to disturbance. This hypothesis is supported by the fact that there is no statistical difference between the $\delta^{15}N$ or $\delta^{13}C$ values of fishes collected (from all sites) before and after the installation of Cochiti Dam, which constituted a major anthropogenic ecosystem level disturbance in the river. High food web resistance to change in the face of large-scale disturbance has been found in other studies (Chasar et al. 2005), and flexible feeding habits of fish may be an important factor driving this stability (Fagan 1997).

We hypothesized that the aquatic food web of the MRG would have become more autotrophic over time and have proportionally lower contributions of carbon from riparian floodplain production due to decreasing river-floodplain interaction associated with regulation. The $\delta^{13}C$ values of all museum fish taken as a whole did not reflect this trend (Figure 3), but rather suggested that the majority of carbon for fish food webs was derived from autochthonous production over the last six decades. However, there were some interesting temporal and spatial trends revealed from historical analyses. For example, the $\delta^{13}C$ values of fishes collected from Bernardo in the later years (after 1961) were

significantly more positive (more ^{13}C enriched) than the $\delta^{13}C$ values of fish collected in the same years at the other three sites (significant difference between the $\delta^{13}C$ values of Bernardo fishes collected after 1961 and fishes collected from all other sites; $P_{[\text{two-tailed}]} < 0.05$ for all comparisons). Similar to previous findings of enhanced autotrophy in the contemporary food web of Bernardo (relative to other sites in the MRG) (Edwards et al. in preparation), the signatures of museum fish from Bernardo collected after 1961 suggest that there is little to no input from the floodplain (Figure 6).

The observed increase in autotrophy at Bernardo coincides with a major expansion of the Albuquerque Wastewater Treatment Plant (AWTP) in 1961. This plant discharges effluent into the Rio Grande south of the city of Albuquerque, between the Bernalillo and Bernardo sites. Previous research has shown a substantial input of organic nitrogen and dissolved inorganic nitrogen (DIN) from the AWTP, and this spike of increasing nitrogen remains in the river at Bernardo (Passell et al. 2005). The input of DIN could be driving the river towards eutrophy downstream of the AWTP, leading to a spike in in-stream production. It is possible that high turbidity at the Bosque del Apache site prevents in-stream production at levels observed at the Bernardo site (e.g., by limiting light penetration into the water), accounting for the drop in autochthonous input in the Bosque del Apache food web.

In the segment of river including Bernardo and 20 miles of river upstream (north), the extent of agricultural coverage in the historic MRG floodplain has increased over the past several decades (Crawford et al. 1993 and the land coverage maps therein). This could be contributing to the apparent eutrophication we observed at the Bernardo site.

However, because agricultural coverage has also increased between Bernardo and Bosque del Apache, and we did not observe increased autotrophy at our most downstream site, agriculture may not be the explanation for eutrophy at Bernardo; if agricultural inputs of nutrients were causing increasing autotrophy at Bernardo, we would expect this response at Bosque del Apache as well.

The $\delta^{15}N$ signatures of fish collected from Bernardo after 1961 were significantly more positive than the values for fish collected from all other sites (and years) except Cochiti (Figure 7). Nitrogen input from human waste can be quite ^{15}N enriched, meaning that input from the AWTP could be driving the upward shift in our nitrogen isotope data for Bernardo (Chapelle 2000). This supports the hypothesis of wastewater inputs from the AWTP driving increased autotrophy. Because pollution into freshwater systems from agriculture can also increase the $\delta^{15}N$ value of the total nitrate present within the water, we cannot distinguish between wastewater or agricultural inputs as possible driving factors of eutrophication (Harrington et al. 1998, Hebert and Wassenaar 2001).

The $\delta^{15}N$ signatures of fish collected from Cochiti were also enriched relative to fish from Bernalillo and from Bernardo prior to 1961 (Figure 7). Because the $\delta^{13}C$ signatures of Cochiti fish did not indicate enhanced autotrophy, nitrate pollution is not likely an important nitrogen source in the food web. Instead, a source of nitrogen with a relatively high $\delta^{15}N$ value may be supplying the food web at Cochiti. Cochiti Reservoir may contribute materials, possibly phytoplankton, to the Cochiti site. These materials may be impacting the $\delta^{15}N$ signatures of fish collected from the Cochiti site.

CONCLUSION

Contrary to our expectations, our study demonstrated little overall food web change in the MRG over the past several decades. The fish food web in this river appears to be resistant to change in the face of major anthropogenic ecosystem alterations. Omnivory and resistance to change could be a stabilizing force in this food web, allowing the fish to utilize changing food resources. This is not to say that the ecosystem is not impacted by anthropogenic changes; previous work has shown significant impacts in the MRG (Molles et al. 1998, Crawford et al. 1993, Ellis et al. 2002). Although we observed little overall change in the food web, our data did indicate enhanced autotrophy at Bernardo, which may be driven by anthropogenic inputs from the AWTP. Previous research in the MRG has also indicated possible eutrophication at Bernardo in recent years (Edwards et al. in preparation). Because the historic MRG was likely to have been generally oligotrophic (Passell et al. 2005, Edwards et al. in preparation), this recent tendency towards eutrophy at Bernardo is a significant finding. Future research could pinpoint the factor driving eutrophication at Bernardo, providing important information to river managers. Sampling the isotopic content of AWTP effluent would allow the relative inputs of this source into the Bernardo aquatic food web to be determined.

Our study demonstrated an overall dependence on autotrophic production for the majority of carbon entering the fish food web, and this type of production peaks during low flow periods, when drying pools, edge habitats, and backwaters are available for use by fish. However, Pease et al. (2006) demonstrated that fish utilize off channel habitat that is only available during high flow events, when floodplains are inundated. Therefore,

both high and low flow events are likely important to the fish food web of the MRG, and river managers should allow these higher flow events to occur. In addition, management of the MRG should continue to focus on increasing the extent of connected natural areas through ecological restoration, but should also quantify and address anthropogenic nutrient inputs into the river.

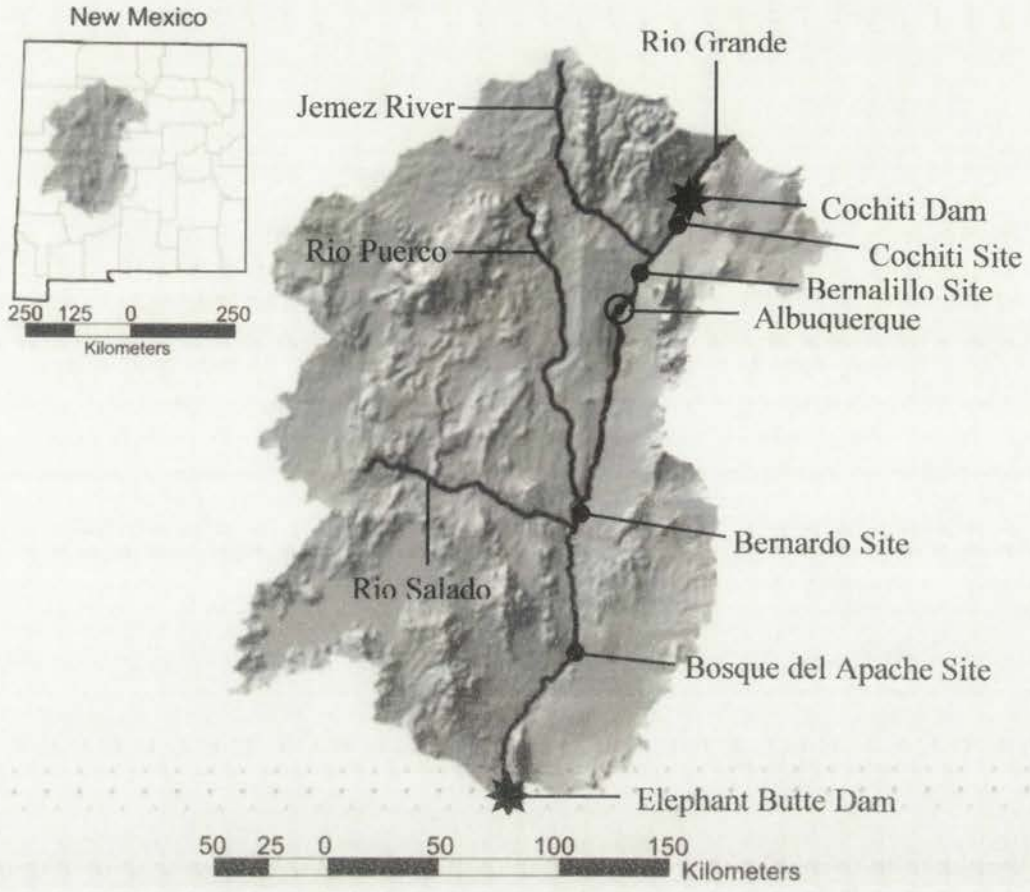

Figure 1. Map of the middle Rio Grande basin of New Mexico, which is the portion of the river between Cochiti Dam and Elephant Butte Dam.

Sampling sites and the major features and tributaries of the middle Rio Grande are indicated.

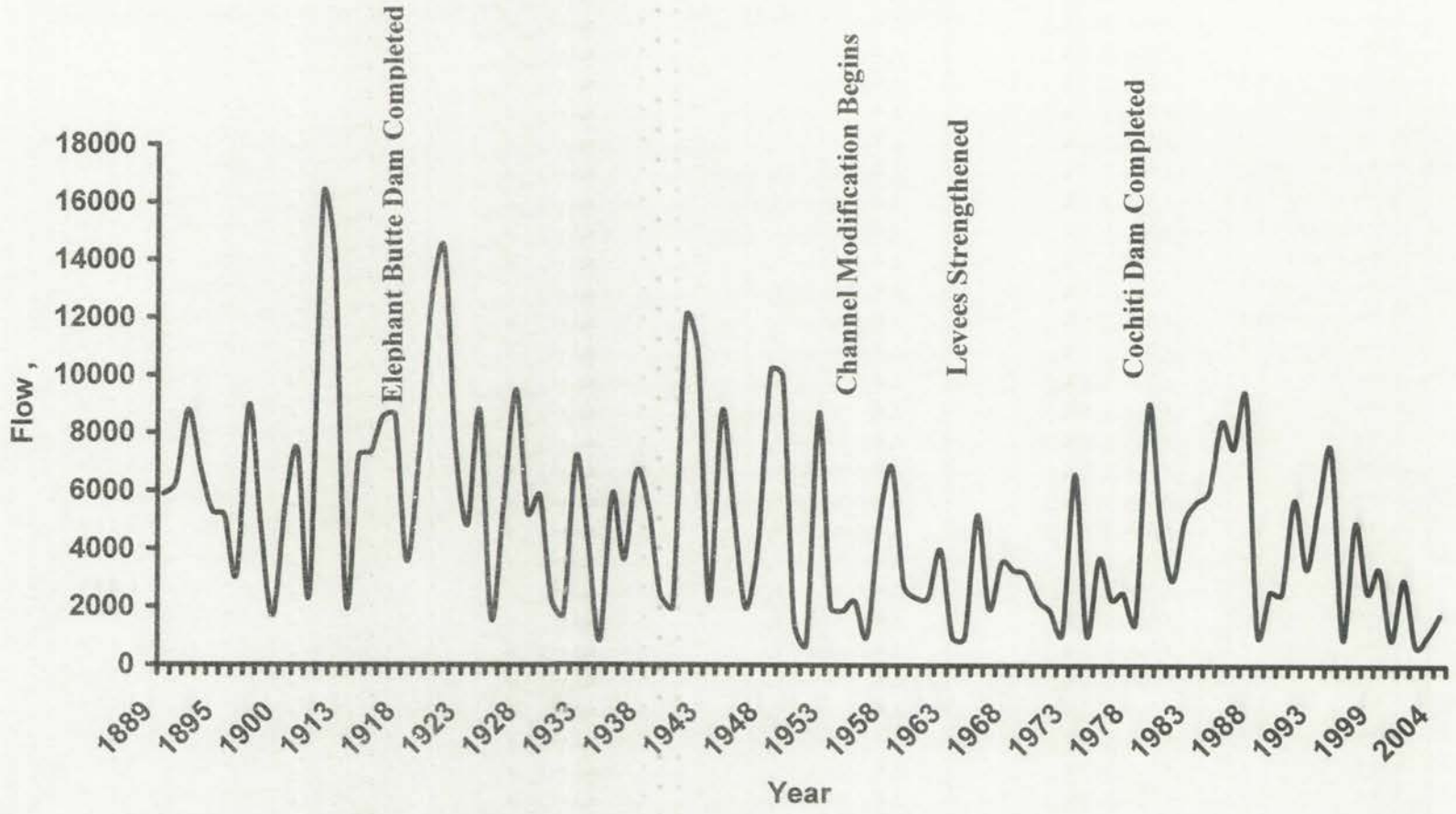

Figure 2. Yearly peak flows (in cubic feet per second) in the Rio Grande at Embudo, New Mexico.

Data obtained from the USGS website for station 08279500, located at latitude 36°12'20", longitude 105°57'51". A timeline for principle flood control and water storage structures in the middle Rio Grande is shown. Note: gauge is immediately upstream of the middle Rio Grande basin.

Table 1. Species sampled from the Museum of Southwestern Biology at the University of New Mexico.

Species	Presumed Diet - Sublette	Presumed Diet - Koster	Cochiti	Bernalillo	Bernardo	BDA
Carpiodes carpio	Detritus, algae	Invertebrates, detritus, algae	1947	1992, 2000	1978, 1992	1978, 1999
Catostomus commersoni	Periphyton	Omnivorous	1978, 1992			
Cyprinella lutrensis	Invertebrates	Invertebrates, algae	1978, 1993, 1999	1990, 2000	1956, 1978, 1995, 1999	1993
Cyprinus carpio	Invertebrates	Omnivorous	1942, 2000	1978, 1995	1939, 1978, 1994, 2000	1978, 1994
Gambusia affinis	Invertebrates, algae, diatoms	Invertebrates, algae	1942, 1978, 2000	1947, 2000	1939, 1978	1978
Hybognathus amarus	Algae	Algae	1947, 1978	1943, 1978, 1994	1939, 1978	1977, 1999
Ictalurus punctatus	Omnivorous	Omnivorous		2000	1992, 1999	
Pimephales promelas	Invertebrates	Invertebrates, algae	1942, 1992		1978, 1992, 2000	1978, 1992
Platygobio gracilis	Invertebrates	Omnivorous	1978, 1993	1978, 1995	1978	1977, 1993
Rhinichthys cataractae	Invertebrates	Invertebrates, algae	1978, 1994, 1999	1977, 1995		

Collection location, collection year, and presumed diet for each species is shown. Diet information was taken from Sublette et al. 1990, and Koster 1957.

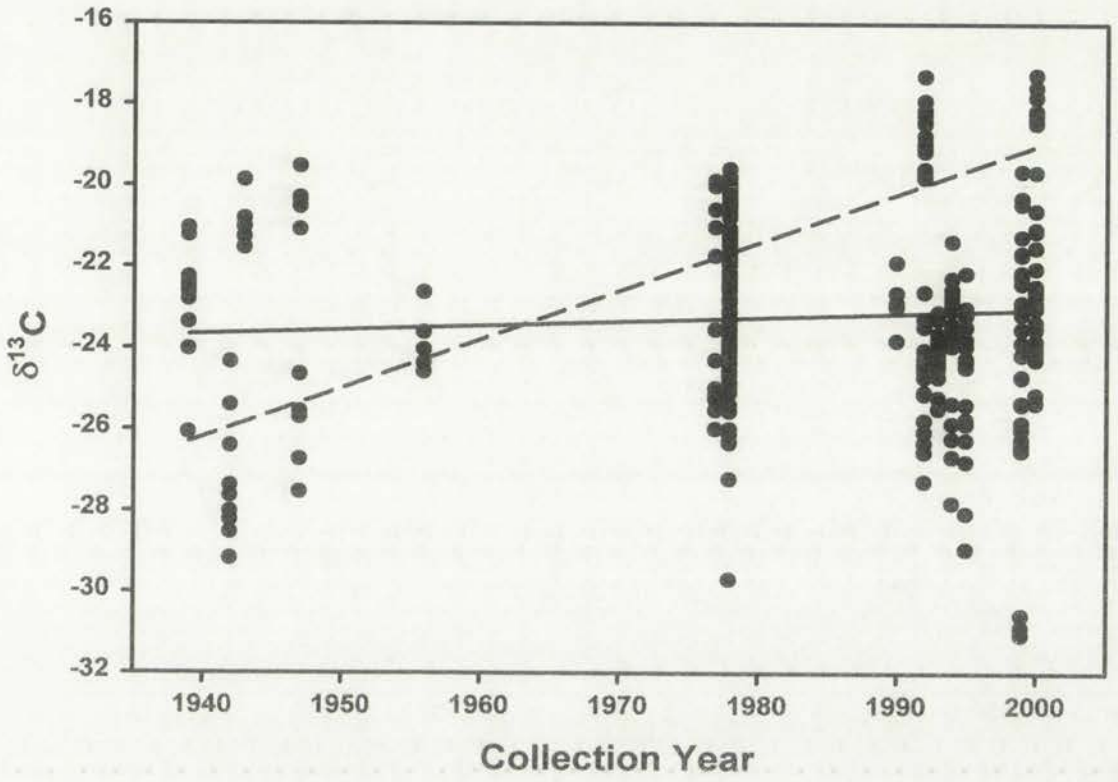

Figure 3. $\delta^{13}C$ values of all fishes sampled from the Museum of Southwestern Biology at UNM.

The solid line is a linear regression of all the museum fish data, and the dashed line indicates our hypothesized change in $\delta^{13}C$ values over time. Mean $\delta^{13}C$ for riparian production is -26 ‰ and mean $\delta^{13}C$ for algal (or submerged macrophyte) production is -19‰. We hypothesized that the proportion of carbon contributed to the food web by algal (instream) sources would increase over time as river regulation and fragmentation increased. Linear regression of observed data indicates that historical and present-day carbon sources are the same despite intensive regulation.

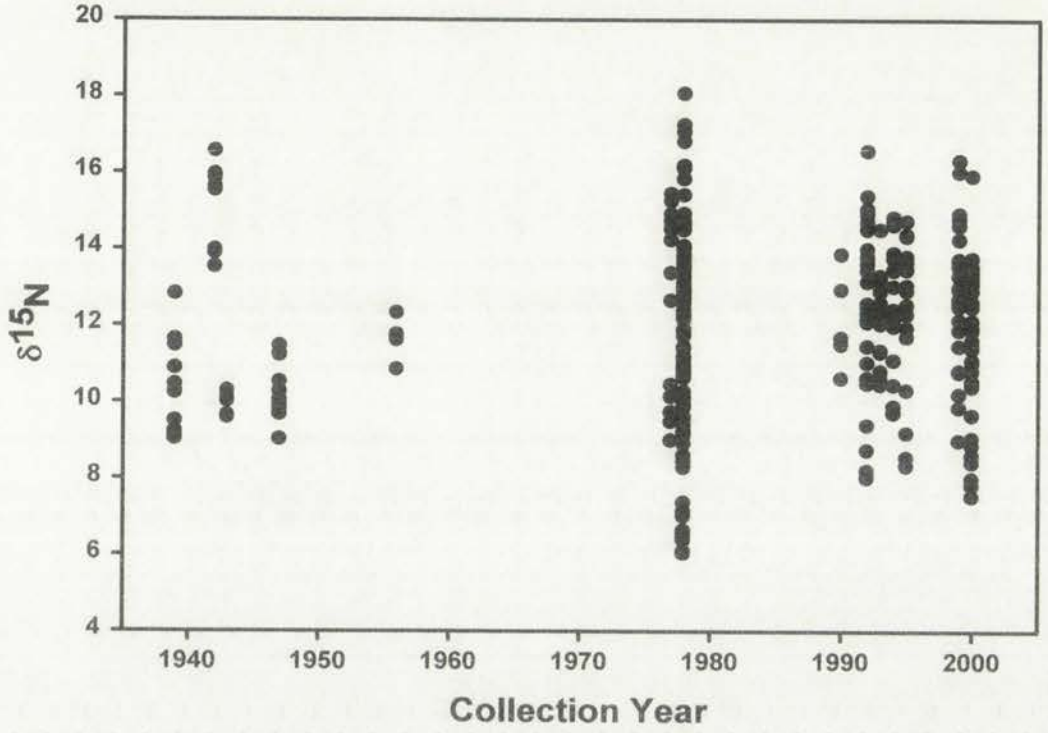

Figure 4. $\delta^{15}N$ values of all fish sampled from the Museum of Southwestern Biology at UNM.

The data taken as a whole, as shown above, do not indicate any trends.

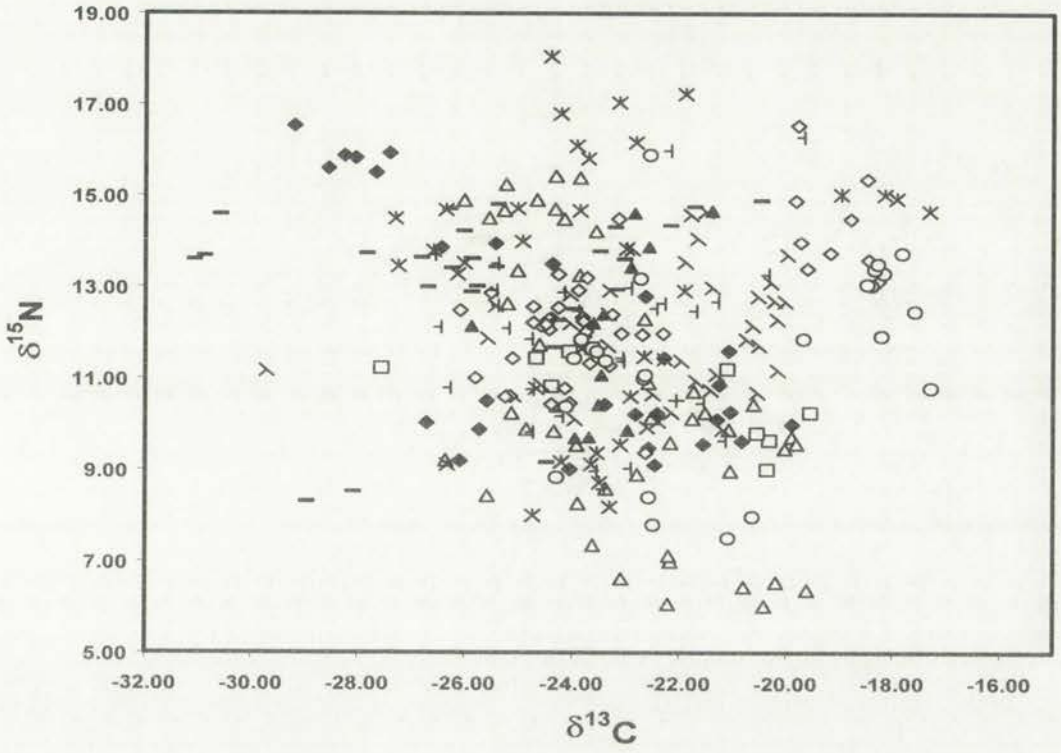

Figure 5. $\delta^{15}N$ and $\delta^{13}C$ values of all museum fish from all years.

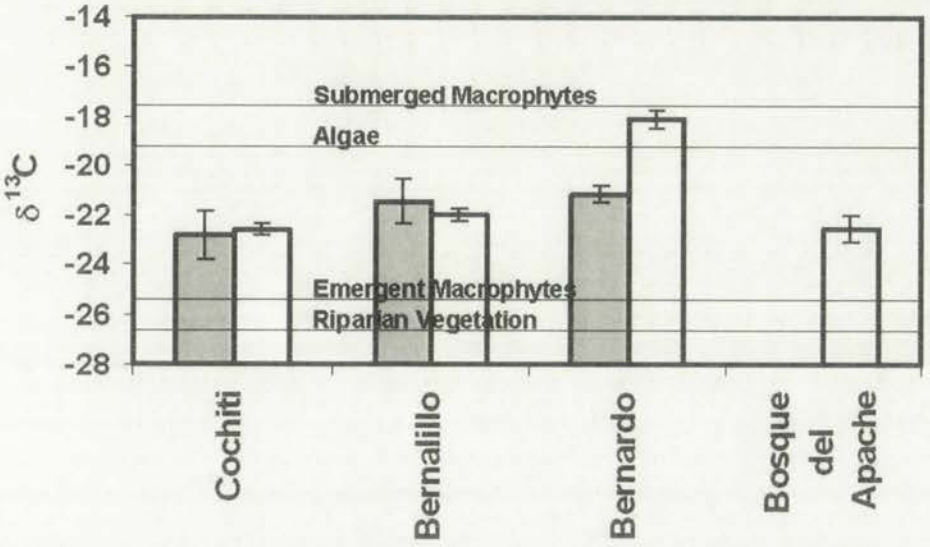

Figure 6. Columns indicate corrected mean $\delta^{13}C$ values of fish sampled from the MSB, before (shaded columns) and after (unshaded columns) a major expansion of the Albuquerque Wastewater Treatment Plant (AWTP) in 1961.

Bars represent one standard error. The mean values for submerged macrophytes, algae, emergent macrophytes, and riparian vegetation collected in the summer of 2002 (Edwards and Turner 2003; Edwards et al., in preparation).

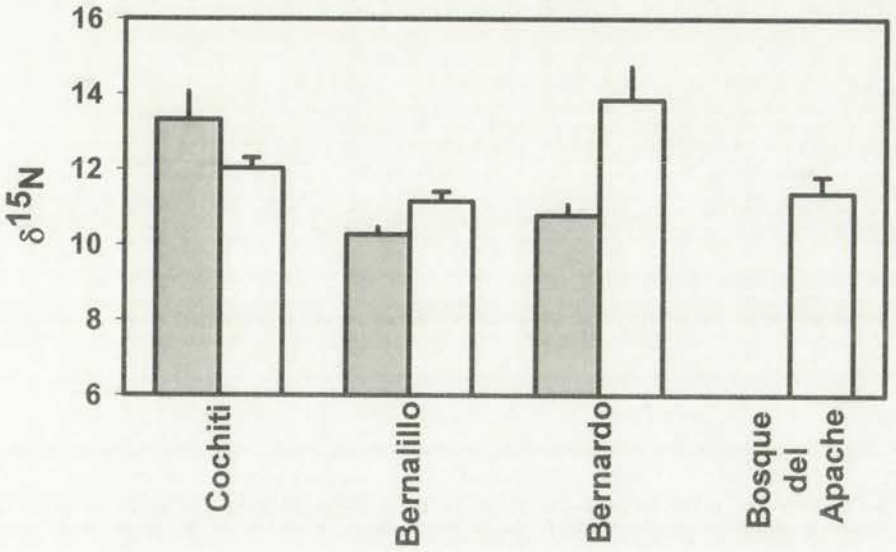

Figure 7. Columns indicate mean $\delta^{15}N$ values of fish sampled from the MSB, before (shaded columns) and after (unshaded columns) a major expansion of the Albuquerque Wastewater Treatment Plant (AWTP) in 1961.

Bars represent one standard error.

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SUMMARY AND CONCLUSION

The work presented here was used to characterize the contemporary and historic aquatic food web of the middle Rio Grande (MRG) of New Mexico, which has been severely altered by human activities. Because our approach was to use stable isotope analyses of historic fish specimens, the first part of this study examined the effects of fixation and preservation on the stable isotope composition of museum preserved fish tissues. It was found that formalin-induced changes in carbon, nitrogen, and sulfur isotope values of fish tissue were small compared to changes expected from natural fractionation processes that are of interest in ecosystem studies. Thus, specimens held by natural history museums offer an important source of pre-disturbance material for quantifying historical change in nutrient cycles and food webs in aquatic ecosystems.

In the second part of this research, we characterized the current aquatic food web of the MRG by analyzing the stable isotope composition of food web components collected from four research sites within the basin. We found that regardless of sample site or season, autochthonous production was the dominant carbon source system. Fishes sampled from the MRG are not supported directly by vegetation, but instead by an intermediate step consisting of either macro- or micro-invertebrates. Omnivory is evident in the fish food web; fishes used similar resources regardless of nominal dietary differences. Our data from the Bernardo site suggest a substantial impact of human activities, whether from wastewater effluent or agricultural practices, on the entire river food web, driving the system towards eutrophy and shifting the community $\delta^{15}N$ values.

The final portion of this research involved stable isotope analysis of museum preserved fish specimens collected from the MRG. Our findings coincide well with the results of the contemporary food web portion of the study. Omnivory appears to have been dominant in the fish food web of the MRG for the past 60 years, possibly contributing to food web stability in the face of anthropogenic change. Contrary to our predictions, the overall food web did not become more dependant on autochthonous production over time, but remained stable with respect to carbon and nitrogen isotopic signatures. As was seen in the contemporary food web, the Bernardo site was the exception to the lack of change observed at the remaining sites. The carbon and nitrogen isotopic signatures of fish from the Bernardo indicate that the food web became significantly more dependant on autochthonous production over time. This may be indicative of eutrophication driven by anthropogenic nutrient inputs from the agriculture or Albuquerque wastewater effluents.

APPENDIX A

Appendix A. Uncorrected $\delta^{13}C$ and $\delta^{15}N$ values of museum fish sampled from the Museum of Southwestern Biology at the University of New Mexico. Included in the table are the museum catalog numbers for each specimen, and the collection location and year.

MSB #	Species	Collection Location	Collection Year	$\delta^{15}N$	$\delta^{13}C$
1570-1	*Cyprinus carpio*	Cochiti	1942	15.595	-28.556
1570-2	*Cyprinus carpio*	Cochiti	1942	13.951	-25.424
327-1	*Gambusia affinis*	Cochiti	1942	15.88	-28.25
327-2	*Gambusia affinis*	Cochiti	1942	13.501	-24.37
679-1	*Pimephales promelas*	Cochiti	1942	15.931	-27.405
679-2	*Pimephales promelas*	Cochiti	1942	15.503	-27.661
679-3	*Pimephales promelas*	Cochiti	1942	16.532	-29.199
679-4	*Pimephales promelas*	Cochiti	1942	15.824	-28.042
679-5	*Pimephales promelas*	Cochiti	1942	13.868	-26.431
1132-1	*Hybognathus amarus*	Cochiti	1947	9.80	-20.55
1132-2	*Hybognathus amarus*	Cochiti	1947	11.20	-21.11
1132-3	*Hybognathus amarus*	Cochiti	1947	9.00	-20.36
3211-1	*Carpiodes carpio*	Cochiti	1947	10.243	-19.549
3211-2	*Carpiodes carpio*	Cochiti	1947	9.646	-20.322
4087-1	*Catostomus commersoni*	Cochiti	1978	10.79	-24.67
4087-2	*Catostomus commersoni*	Cochiti	1978	11.879	-25.564
4087-3	*Catostomus commersoni*	Cochiti	1978	10.61	-25.08
4087-4	*Catostomus commersoni*	Cochiti	1978	11.17	-29.71
4087-5	*Catostomus commersoni*	Cochiti	1978	10.86	-24.58
4487-2	*Hybognathus amarus*	Cochiti	1978	9.12	-23.65
4487-3	*Hybognathus amarus*	Cochiti	1978	9.114	-26.318
4487-4	*Hybognathus amarus*	Cochiti	1978	9.17	-24.208
4487-5	*Hybognathus amarus*	Cochiti	1978	9.37	-23.54
4489-1	*Platygobio gracilis*	Cochiti	1978	10.059	-22.401
4489-4	*Platygobio gracilis*	Cochiti	1978	9.544	-23.102
4601-1	*Cyprinella lutrensis*	Cochiti	1978	13.99	-24.921
4601-2	*Cyprinella lutrensis*	Cochiti	1978	13.525	-26.006
4601-3	*Cyprinella lutrensis*	Cochiti	1978	13.28	-26.13
4601-4	*Cyprinella lutrensis*	Cochiti	1978	12.588	-25.416
4601-5	*Cyprinella lutrensis*	Cochiti	1978	12.407	-23.321
4606-1	*Rhinichthys cataractae*	Cochiti	1978	18.047	-24.403
4606-2	*Rhinichthys cataractae*	Cochiti	1978	13.452	-27.237
4606-3	*Rhinichthys cataractae*	Cochiti	1978	17.04	-23.13
4606-4	*Rhinichthys cataractae*	Cochiti	1978	14.67	-23.86
4606-5	*Rhinichthys cataractae*	Cochiti	1978	14.72	-25.01

Appendix A *continued*.

MSB #	Species	Collection Location	Collection Year	$\delta^{15}N$	$\delta^{13}C$
4750-1	*Gambusia affinis*	Cochiti	1978	16.09	-23.93
4750-2	*Gambusia affinis*	Cochiti	1978	16.788	-24.222
4750-3	*Gambusia affinis*	Cochiti	1978	17.228	-21.89
4750-4	*Gambusia affinis*	Cochiti	1978	16.163	-22.815
4750-5	*Gambusia affinis*	Cochiti	1978	15.81	-23.71
11169-1	*Catostomus commersoni*	Cochiti	1992	12.488	-26.079
11169-2	*Catostomus commersoni*	Cochiti	1992	12.31	-24.39
11169-3	*Catostomus commersoni*	Cochiti	1992	12.147	-24.575
11169-4	*Catostomus commersoni*	Cochiti	1992	12.55	-24.26
11169-5	*Catostomus commersoni*	Cochiti	1992	12.069	-24.428
12434-1	*Pimephales promelas*	Cochiti	1992	11.007	-25.796
12434-2	*Pimephales promelas*	Cochiti	1992	9.37	-22.63
12434-3	*Pimephales promelas*	Cochiti	1992	10.46	-24.06
12434-4	*Pimephales promelas*	Cochiti	1992	10.607	-25.145
12434-5	*Pimephales promelas*	Cochiti	1992	11.44	-25.10
30081-1	*Cyprinella lutrensis*	Cochiti	1993	12.30	-24.43
30081-2	*Cyprinella lutrensis*	Cochiti	1993	11.281	-23.305
30081-3	*Cyprinella lutrensis*	Cochiti	1993	10.60	-25.23
30081-4	*Cyprinella lutrensis*	Cochiti	1993	10.43	-24.39
30081-5	*Cyprinella lutrensis*	Cochiti	1993	12.249	-23.802
30084-1	*Platygobio gracilis*	Cochiti	1993	12.87	-25.50
30084-3	*Platygobio gracilis*	Cochiti	1993	13.277	-24.264
30084-4	*Platygobio gracilis*	Cochiti	1993	12.18	-24.49
30084-5	*Platygobio gracilis*	Cochiti	1993	10.784	-24.141
18627-1	*Rhinichthys cataractae*	Cochiti	1994	14.81	-25.38
18627-2	*Rhinichthys cataractae*	Cochiti	1994	13.00	-26.69
18627-3	*Rhinichthys cataractae*	Cochiti	1994	13.419	-26.243
18627-4	*Rhinichthys cataractae*	Cochiti	1994	13.74	-27.83
18627-5	*Rhinichthys cataractae*	Cochiti	1994	12.139	-25.872
43223-1	*Rhinichthys cataractae*	Cochiti	1999	12.927	-25.391
43223-2	*Rhinichthys cataractae*	Cochiti	1999	13.707	-26.54
43223-3	*Rhinichthys cataractae*	Cochiti	1999	13.51	-25.36
43223-4	*Rhinichthys cataractae*	Cochiti	1999	12.474	-23.88
43223-5	*Rhinichthys cataractae*	Cochiti	1999	12.119	-26.437
43345-1	*Cyprinella lutrensis*	Cochiti	1999	10.79	-26.26
43345-2	*Cyprinella lutrensis*	Cochiti	1999	11.86	-24.74
43345-3	*Cyprinella lutrensis*	Cochiti	1999	9.83	-24.73
43345-4	*Cyprinella lutrensis*	Cochiti	1999	10.76	-24.71
43345-5	*Cyprinella lutrensis*	Cochiti	1999	10.18	-24.18

Appendix A *continued.*

MSB #	Species	Collection Location	Collection Year	$\delta^{15}N$	$\delta^{13}C$
45421-1	*Gambusia affinis*	Cochiti	2000	8.402	-22.569
45421-2	*Gambusia affinis*	Cochiti	2000	7.97	-20.63
45421-3	*Gambusia affinis*	Cochiti	2000	7.508	-21.088
45421-4	*Gambusia affinis*	Cochiti	2000	10.38	-24.12
45421-5	*Gambusia affinis*	Cochiti	2000	8.839	-24.294
48496-1	*Cyprinus carpio*	Cochiti	2000	7.80	-22.49
48496-2	*Cyprinus carpio*	Cochiti	2000	11.07	-22.64
1155-1	*Hybognathus nuchalis*	Bernalillo	1943	9.627	-20.834
1155-2	*Hybognathus nuchalis*	Bernalillo	1943	10.102	-21.293
1155-3	*Hybognathus nuchalis*	Bernalillo	1943	10.266	-21.044
1155-4	*Hybognathus nuchalis*	Bernalillo	1943	9.99	-19.89
1155-5	*Hybognathus nuchalis*	Bernalillo	1943	9.561	-21.555
365-1	*Gambusia affinis*	Bernalillo	1947	10.029	-26.755
365-2	*Gambusia affinis*	Bernalillo	1947	11.438	-24.668
365-3	*Gambusia affinis*	Bernalillo	1947	11.224	-27.559
365-4	*Gambusia affinis*	Bernalillo	1947	9.883	-25.725
365-5	*Gambusia affinis*	Bernalillo	1947	10.505	-25.586
4610-1	*Rhinichthys cataractae*	Bernalillo	1977	8.974	-21.046
4610-2	*Rhinichthys cataractae*	Bernalillo	1977	9.717	-19.895
4610-3	*Rhinichthys cataractae*	Bernalillo	1977	10.43	-20.61
4610-4	*Rhinichthys cataractae*	Bernalillo	1977	10.12	-21.75
4610-5	*Rhinichthys cataractae*	Bernalillo	1977	9.46	-20.03
4431-1	*Platygobio gracilis*	Bernalillo	1978	9.892	-24.846
4431-2	*Platygobio gracilis*	Bernalillo	1978	9.592	-22.165
4431-3	*Platygobio gracilis*	Bernalillo	1978	10.109	-23.956
4431-4	*Platygobio gracilis*	Bernalillo	1978	10.253	-22.145
4431-5	*Platygobio gracilis*	Bernalillo	1978	9.929	-22.605
4981-2	*Cyprinus carpio*	Bernalillo	1978	14.481	-24.156
4981-3	*Cyprinus carpio*	Bernalillo	1978	13.253	-23.87
4981-4	*Cyprinus carpio*	Bernalillo	1978	15.388	-23.864
4981-5	*Cyprinus carpio*	Bernalillo	1978	14.90	-24.66
4984-1	*Hybognathus amarus*	Bernalillo	1978	9.539	-23.927
4984-2	*Hybognathus amarus*	Bernalillo	1978	8.265	-23.901
4984-3	*Hybognathus amarus*	Bernalillo	1978	8.588	-23.372
4984-4	*Hybognathus amarus*	Bernalillo	1978	8.429	-25.576
4984-5	*Hybognathus amarus*	Bernalillo	1978	9.207	-26.35
9012-1	*Cyprinella lutrensis*	Bernalillo	1990	10.60	-22.91
9012-2	*Cyprinella lutrensis*	Bernalillo	1990	12.914	-21.904
9012-3	*Cyprinella lutrensis*	Bernalillo	1990	13.84	-22.99
9012-4	*Cyprinella lutrensis*	Bernalillo	1990	11.48	-22.66
9012-5	*Cyprinella lutrensis*	Bernalillo	1990	11.653	-23.833
11948-1	*Carpiodes carpio*	Bernalillo	1992	8.00	-24.73
11948-3	*Carpiodes carpio*	Bernalillo	1992	8.187	-23.296
11948-4	*Carpiodes carpio*	Bernalillo	1992	8.722	-23.492

Appendix A *continued.*

MSB #	Species	Collection Location	Collection Year	$\delta^{15}N$	$\delta^{13}C$
18878-1	*Hybognathus amarus*	Bernalillo	1994	11.07	-23.46
18878-2	*Hybognathus amarus*	Bernalillo	1994	9.863	-22.97
18878-3	*Hybognathus amarus*	Bernalillo	1994	10.42	-23.51
18878-4	*Hybognathus amarus*	Bernalillo	1994	9.714	-23.69
18878-5	*Hybognathus amarus*	Bernalillo	1994	9.69	-23.96
24963-1	*Platygobio gracilis*	Bernalillo	1995	11.68	-24.35
24963-2	*Platygobio gracilis*	Bernalillo	1995	12.272	-24.266
24963-3	*Platygobio gracilis*	Bernalillo	1995	12.89	-25.88
24963-4	*Platygobio gracilis*	Bernalillo	1995	11.915	-23.791
24963-5	*Platygobio gracilis*	Bernalillo	1995	10.29	-24.279
25001-1	*Rhinichthys cataractae*	Bernalillo	1995	13.02	-25.77
25001-2	*Rhinichthys cataractae*	Bernalillo	1995	13.44	-25.41
25001-4	*Rhinichthys cataractae*	Bernalillo	1995	13.64	-26.81
25001-5	*Rhinichthys cataractae*	Bernalillo	1995	14.71	-26.28
25009-1	*Cyprinus carpio*	Bernalillo	1995	12.52	-23.97
25009-2	*Cyprinus carpio*	Bernalillo	1995	8.522	-28.086
25009-3	*Cyprinus carpio*	Bernalillo	1995	9.166	-24.48
25009-4	*Cyprinus carpio*	Bernalillo	1995	8.31	-28.962
45144-1	*Cyprinella lutrensis*	Bernalillo	2000	12.88	-24.15
45144-2	*Cyprinella lutrensis*	Bernalillo	2000	12.591	-25.362
45144-3	*Cyprinella lutrensis*	Bernalillo	2000	12.097	-25.161
45144-5	*Cyprinella lutrensis*	Bernalillo	2000	11.37	-23.033
45434-1	*Carpiodes carpio*	Bernalillo	2000	9.64	-21.14
45434-2	*Carpiodes carpio*	Bernalillo	2000	9.029	-22.901
45434-4	*Carpiodes carpio*	Bernalillo	2000	8.58	-23.402
45434-5	*Carpiodes carpio*	Bernalillo	2000	10.453	-21.543
45436-1	*Ictalurus punctatus*	Bernalillo	2000	11.437	-23.974
45436-2	*Ictalurus punctatus*	Bernalillo	2000	11.59	-23.54
45436-3	*Ictalurus punctatus*	Bernalillo	2000	11.384	-23.38
45437-1	*Gambusia affinis*	Bernalillo	2000	11.85	-23.84
45437-2	*Gambusia affinis*	Bernalillo	2000	13.18	-22.73
45437-3	*Gambusia affinis*	Bernalillo	2000	10.52	-22.05
45437-4	*Gambusia affinis*	Bernalillo	2000	11.00	-22.67
45437-5	*Gambusia affinis*	Bernalillo	2000	15.89	-22.55
1103-1	*Gambusia affinis*	Bernardo	1939	12.797	-22.637
1103-2	*Gambusia affinis*	Bernardo	1939	11.605	-21.078
1103-5	*Gambusia affinis*	Bernardo	1939	11.447	-22.283
1151-1	*Hybognathus nuchalis*	Bernardo	1939	10.86	-21.252
1151-2	*Hybognathus nuchalis*	Bernardo	1939	10.236	-22.415
1151-3	*Hybognathus nuchalis*	Bernardo	1939	10.43	-23.39
1560-1	*Cyprinus carpio*	Bernardo	1939	9.103	-22.455
1560-2	*Cyprinus carpio*	Bernardo	1939	9.477	-22.576
1560-3	*Cyprinus carpio*	Bernardo	1939	9.01	-24.05
1560-4	*Cyprinus carpio*	Bernardo	1939	10.214	-22.822

Appendix A *continued.*

MSB #	Species	Collection Location	Collection Year	$\delta^{15}N$	$\delta^{13}C$
1560-5	*Cyprinus carpio*	Bernardo	1939	9.199	-26.09
1315-1	*Cyprinella lutrensis*	Bernardo	1956	11.657	-23.633
1315-2	*Cyprinella lutrensis*	Bernardo	1956	12.3	-22.656
1315-3	*Cyprinella lutrensis*	Bernardo	1956	11.737	-24.61
1315-4	*Cyprinella lutrensis*	Bernardo	1956	11.589	-24.058
1315-5	*Cyprinella lutrensis*	Bernardo	1956	10.824	-24.387
4337-3	*Cyprinella lutrensis*	Bernardo	1978	14.58	-21.79
4337-4	*Cyprinella lutrensis*	Bernardo	1978	12.988	-21.381
4337-5	*Cyprinella lutrensis*	Bernardo	1978	13.705	-19.976
4339-2	*Cyprinus carpio*	Bernardo	1978	11.18	-20.16
4339-3	*Cyprinus carpio*	Bernardo	1978	14.06	-21.66
4339-4	*Cyprinus carpio*	Bernardo	1978	12.69	-20.22
4340-2	*Carpiodes carpio*	Bernardo	1978	10.75	-21.41
4340-3	*Carpiodes carpio*	Bernardo	1978	10.94	-21.688
4340-4	*Carpiodes carpio*	Bernardo	1978	12.92	-23.30
4340-5	*Carpiodes carpio*	Bernardo	1978	13.55	-21.89
4986-1	*Hybognathus amarus*	Bernardo	1978	12.15	-20.63
4986-2	*Hybognathus amarus*	Bernardo	1978	11.386	-21.967
4986-3	*Hybognathus amarus*	Bernardo	1978	13.099	-20.292
4986-4	*Hybognathus amarus*	Bernardo	1978	12.792	-20.528
4986-5	*Hybognathus amarus*	Bernardo	1978	10.657	-22.495
5001-1	*Platygobio gracilis*	Bernardo	1978	12.683	-20.018
5001-2	*Platygobio gracilis*	Bernardo	1978	10.687	-20.541
5001-3	*Platygobio gracilis*	Bernardo	1978	11.854	-20.748
5001-4	*Platygobio gracilis*	Bernardo	1978	11.718	-20.515
5001-5	*Platygobio gracilis*	Bernardo	1978	11.42	-22.275
5013-2	*Pimephales promelas*	Bernardo	1978	12.29	-20.17
5013-3	*Pimephales promelas*	Bernardo	1978	11.09	-21.307
5013-4	*Pimephales promelas*	Bernardo	1978	9.886	-21.187
5038-1	*Gambusia affinis*	Bernardo	1978	11.716	-23.335
5038-2	*Gambusia affinis*	Bernardo	1978	12.20	-24.04
5038-3	*Gambusia affinis*	Bernardo	1978	12.833	-24.044
5038-4	*Gambusia affinis*	Bernardo	1978	13.825	-22.88
5038-5	*Gambusia affinis*	Bernardo	1978	14.65	-21.501
11462-1	*Carpiodes carpio*	Bernardo	1992	14.935	-17.927
11462-2	*Carpiodes carpio*	Bernardo	1992	14.66	-17.32
11462-3	*Carpiodes carpio*	Bernardo	1992	15.03	-18.97
11462-4	*Carpiodes carpio*	Bernardo	1992	15.36	-18.48
11462-5	*Carpiodes carpio*	Bernardo	1992	15.01	-18.15
11476-2	*Pimephales promelas*	Bernardo	1992	13.41	-19.61
11476-3	*Pimephales promelas*	Bernardo	1992	13.75	-19.17
11476-4	*Pimephales promelas*	Bernardo	1992	13.123	-18.273
11476-5	*Pimephales promelas*	Bernardo	1992	71.975	-19.121
11480-1	*Ictalurus punctatus*	Bernardo	1992	13.594	-18.453

Appendix A *continued.*

MSB #	Species	Collection Location	Collection Year	$\delta^{15}N$	$\delta^{13}C$
11480-2	*Ictalurus punctatus*	Bernardo	1992	14.487	-18.789
11480-3	*Ictalurus punctatus*	Bernardo	1992	14.885	-19.822
11480-4	*Ictalurus punctatus*	Bernardo	1992	13.978	-19.727
11480-5	*Ictalurus punctatus*	Bernardo	1992	16.54	-19.78
20503-1	*Cyprinus carpio*	Bernardo	1994	14.67	-21.39
20503-2	*Cyprinus carpio*	Bernardo	1994	13.876	-22.57
20503-3	*Cyprinus carpio*	Bernardo	1994	13.432	-22.909
20503-4	*Cyprinus carpio*	Bernardo	1994	14.61	-22.84
24841-1	*Cyprinella lutrensis*	Bernardo	1995	14.35	-22.17
24841-3	*Cyprinella lutrensis*	Bernardo	1995	13.78	-23.49
24841-4	*Cyprinella lutrensis*	Bernardo	1995	14.31	-23.20
24841-5	*Cyprinella lutrensis*	Bernardo	1995	13.61	-23.027
43273-1	*Cyprinella lutrensis*	Bernardo	1999	13.272	-20.328
43273-2	*Cyprinella lutrensis*	Bernardo	1999	8.971	-23.54
43273-3	*Cyprinella lutrensis*	Bernardo	1999	11.44	-23.05
43273-4	*Cyprinella lutrensis*	Bernardo	1999	12.48	-21.67
43406-1	*Ictalurus punctatus*	Bernardo	1999	12.64	-22.259
43406-2	*Ictalurus punctatus*	Bernardo	1999	12.676	-21.267
43406-4	*Ictalurus punctatus*	Bernardo	1999	12.53	-22.42
43406-5	*Ictalurus punctatus*	Bernardo	1999	12.98	-22.89
45237-1	*Pimephales promelas*	Bernardo	2000	13.737	-17.829
45237-2	*Pimephales promelas*	Bernardo	2000	13.034	-18.405
45237-3	*Pimephales promelas*	Bernardo	2000	13.052	-18.481
45237-4	*Pimephales promelas*	Bernardo	2000	13.504	-18.282
45237-5	*Pimephales promelas*	Bernardo	2000	13.385	-18.342
45385-1	*Cyprinus carpio*	Bernardo	2000	10.788	-17.286
45385-2	*Cyprinus carpio*	Bernardo	2000	11.86	-19.68
45385-3	*Cyprinus carpio*	Bernardo	2000	13.30	-18.17
45385-4	*Cyprinus carpio*	Bernardo	2000	12.462	-17.587
45385-5	*Cyprinus carpio*	Bernardo	2000	11.926	-18.213
4028-1	*Platygobio gracilis*	Bosque del Apache	1977	12.631	-25.194
4028-2	*Platygobio gracilis*	Bosque del Apache	1977	13.356	-25.009
4028-3	*Platygobio gracilis*	Bosque del Apache	1977	14.89	-26.01
4028-4	*Platygobio gracillis*	Bosque del Apache	1977	14.22	-23.56
4163-1	*Hybognathus amarus*	Bosque del Apache	1977	15.422	-24.311
4163-2	*Hybognathus amarus*	Bosque del Apache	1977	14.68	-25.26
4163-3	*Hybognathus amarus*	Bosque del Apache	1977	15.24	-25.23
4163-4	*Hybognathus amarus*	Bosque del Apache	1977	14.698	-24.316
4163-5	*Hybognathus amarus*	Bosque del Apache	1977	14.50	-25.54
4117-2	*Cyprinus carpio*	Bosque del Apache	1978	6.998	-22.166
4117-3	*Cyprinus carpio*	Bosque del Apache	1978	7.35	-23.62
4117-4	*Cyprinus carpio*	Bosque del Apache	1978	6.619	-23.085
4117-5	*Cyprinus carpio*	Bosque del Apache	1978	7.119	-22.197
4118-1	*Pimephales promelas*	Bosque del Apache	1978	6.37	-19.61

Appendix A *continued*.

MSB #	Species	Collection Location	Collection Year	$\delta^{15}N$	$\delta^{13}C$
4118-2	*Pimephales promelas*	Bosque del Apache	1978	6.019	-20.412
4118-3	*Pimephales promelas*	Bosque del Apache	1978	6.54	-20.19
4118-4	*Pimephales promelas*	Bosque del Apache	1978	6.437	-20.798
4118-5	*Pimephales promelas*	Bosque del Apache	1978	6.067	-22.208
4200-1	*Carpiodes carpio*	Bosque del Apache	1978	9.882	-21.075
4200-2	*Carpiodes carpio*	Bosque del Apache	1978	9.572	-19.805
4200-3	*Carpiodes carpio*	Bosque del Apache	1978	10.715	-21.728
4200-4	*Carpiodes carpio*	Bosque del Apache	1978	10.13	-22.53
4200-5	*Carpiodes carpio*	Bosque del Apache	1978	10.25	-21.52
5039-1	*Gambusia affinis*	Bosque del Apache	1978	8.895	-22.787
5039-2	*Gambusia affinis*	Bosque del Apache	1978	10.24	-25.12
5039-3	*Gambusia affinis*	Bosque del Apache	1978	9.842	-24.341
5039-5	*Gambusia affinis*	Bosque del Apache	1978	10.90	-22.58
10988-1	*Pimephales promelas*	Bosque del Apache	1992	14.49	-27.30
10988-2	*Pimephales promelas*	Bosque del Apache	1992	13.79	-26.578
10988-4	*Pimephales promelas*	Bosque del Apache	1992	14.69	-26.35
19115-1	*Cyprinella lutrensis*	Bosque del Apache	1993	12.399	-23.254
19115-2	*Cyprinella lutrensis*	Bosque del Apache	1993	13.2	-23.733
19115-4	*Cyprinella lutrensis*	Bosque del Apache	1993	14.49	-23.13
19115-5	*Cyprinella lutrensis*	Bosque del Apache	1993	12.93	-23.89
19119-1	*Platygobio gracilis*	Bosque del Apache	1993	12.335	-23.813
19119-2	*Platygobio gracilis*	Bosque del Apache	1993	11.328	-23.675
19119-3	*Platygobio gracilis*	Bosque del Apache	1993	12.219	-24.72
19119-4	*Platygobio gracilis*	Bosque del Apache	1993	12.566	-24.725
19119-5	*Cyprinella lutrensis*	Bosque del Apache	1993	12.02	-23.75
20575-1	*Cyprinus carpio*	Bosque del Apache	1994	11.982	-22.304
20575-2	*Cyprinus carpio*	Bosque del Apache	1994	11.959	-22.695
20575-3	*Cyprinus carpio*	Bosque del Apache	1994	12.40	-23.42
20575-4	*Cyprinus carpio*	Bosque del Apache	1994	12.207	-23.608
20575-5	*Cyprinus carpio*	Bosque del Apache	1994	11.98	-23.08
43326-1	*Carpiodes carpio*	Bosque del Apache	1999	13.603	-31.075
43326-2	*Carpiodes carpio*	Bosque del Apache	1999	14.226	-26.014
43326-3	*Carpiodes carpio*	Bosque del Apache	1999	13.62	-25.85
43326-4	*Carpiodes carpio*	Bosque del Apache	1999	13.68	-30.88
43326-5	*Carpiodes carpio*	Bosque del Apache	1999	14.59	-30.59
43450-1	*Hybognathus amarus*	Bosque del Apache	1999	16.29	-19.66
43450-2	*Hybognathus amarus*	Bosque del Apache	1999	14.76	-21.72
43450-3	*Hybognathus amarus*	Bosque del Apache	1999	15.986	-22.16
43450-4	*Hybognathus amarus*	Bosque del Apache	1999	14.897	-20.477
43450-5	*Hybognathus amarus*	Bosque del Apache	1999	12.95	-23.18