TROPHIC RELATIONSHIPS AMONG COLORADO PIKEMINNOW (*PTYCHOCHEILUS LUCIUS*) AND ITS PREY IN THE SAN JUAN RIVER

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EXECUTIVE SUMMARY

Trophic relationships among Colorado pikeminnow and their prey were examined using stable isotopes and a series of field and mesocosm experiments in 2003, 2004, and 2005. Stable isotopes of nitrogen and carbon indicated that age-1 Colorado pikeminnow (range 90-110 mm TL) primarily fed on chironomids during Spring and Summer. This was likely because of suitable sizes of native and nonnative prey were limited, as this age-class of Colorado pikeminnow only had access to young-of-the-year and very small age-1 prey species. In field mesocosms stocked with both age-0 flannelmouth sucker (*Catostomus latipinnis*) and age-1 red shiner (*Cyprinella lutrensis*), we found that age-1 Colorado pikeminnow (range 97-127 mm TL) preferentially foraged on the smaller, age-0 flannelmouth sucker. In addition, consumption rates for age-0 flannelmouth suckers and age-0 speckled dace (*Rhinichthys osculus*) were 3 – 10 times greater than for larger age-1 red shiner, when each species was the only option. Field enclosures in 2004 and 2005 using age-1 Colorado pikeminnow also demonstrated a significant preference for smaller age-0 flannelmouth suckers over age-1 red shiners. In 2005, field enclosures stocked with age-2 Colorado pikeminnow (range 160-250 mm TL) that were allowed to prey on age-1 flannelmouth sucker and age-1 red shiner showed the opposite pattern, with significantly higher consumption rates for the smaller age-1 red shiner. Based on these experiments, size of prey species is likely the driving factor in prey selection by Colorado pikeminnow. Finally, ^{15}N labeled prey successfully verified observed consumption rates in field experiments. Our data suggest critical limitations of native and nonnative prey availability for early age classes of Colorado pikeminnow, primarily based on gape limitation. Moreover, we provide essential quantitative data that can be

used to model prey availability and recruitment success for Colorado pikeminnow in the San Juan River. Because age-0 native fishes (e.g., flannelmouth sucker, bluehead sucker and speckled dace) appear in the San Juan River up to two months earlier that nonnative fishes (e.g., red shiner and fathead minnow), preservation of the natural predator-prey linkages in this system may be critical for the maintenance of a naturally reproducing population of Colorado pikeminnow.

INTRODUCTION

An essential element of restoration or recovery of an endangered species, such as Colorado pikeminnow (*Ptychocheilus lucius*), is a thorough understanding of the relative importance of factors that have contributed to its decline. Previous studies have demonstrated that altered flow regimes, habitat modification, range fragmentation, and establishment of nonnative species have contributed to the imperiled status of Colorado pikeminnow (e.g., Holden and Wick 1992, U.S. Fish and Wildlife Service 1990, Platania et al. 1991). To enhance survival of this species, efforts have been made to remove or ameliorate factors identified as causing its decline. For example, increased flows can potentially be used to increase the availability of spawning and rearing habitats. In addition, stocking hatchery-reared fish is a necessary step towards recovery of Colorado pikeminnow in the San Juan River where a self-sustaining population was most likely extirpated. However, these restoration efforts implicitly assume there is an adequate prey base for all life stages of Colorado pikeminnow, and currently there is no evidence to indicate successful recruitment to adults (Ryden 2003). Thus, considering the drastic changes in the prey assemblages of this system through the extirpation of some natives and introduction of nonnatives, it is possible prey availability might limit recruitment of Colorado pikeminnow in the San Juan River.

Introduction and establishment of nonnative fish species can both cause the decline of native fish species (via competitive interactions or predation by nonnatives) and the addition of potential prey items for native predators. For Colorado pikeminnow, it seems logical that small-bodied cyprinids (e.g., red shiner and fathead minnow) would be an abundant prey resource. Alternatively, these nonnatives may be better adapted to escape predation than native prey species because they evolved in eastern North American systems with a greater diversity of predators. In addition, both the timing and abundance of prey species availability can influence their potential as a prey resource for predators (Cushing 1990). In the San Juan River, most nonnative fishes require warmer temperatures to spawn than native fishes. Although nonnative fishes may seem a viable prey for Colorado pikeminnow because of their small body size and numerical abundance in many habitats, the appearance of YOY nonnatives may occur after a critical period of resource need for Colorado pikeminnow. The effects of highly abundant small-bodied nonnative fishes on recruitment of Colorado pikeminnow in the San Juan River are currently unknown. To assess these effects, we explored the following questions. Has the introduction of nonnative species increased, decreased, or had no effect on the forage base of Colorado pikeminnow? And, how important is prey assemblage composition in regulating Colorado pikeminnow foraging success?

The overarching goal of this three-year study was to *assess the capacity of the current San Juan River prey base to maintain a viable Colorado pikeminnow population*. Specific objectives/goals of the study were:

1) Characterize the prey base of Colorado pikeminnow and linkages with lower trophic levels by determining stable isotope signatures ($\delta^{15}N$ and $\delta^{13}C$) of the biotic assemblages in the San Juan River for six geomorphic reaches of the river (Farmington to Lake Powell).
2) Quantify prey suitability, trophic relationships, and caloric content of lower trophic groups for use in bioenergetics models.

3) Determine if Colorado pikeminnow use nonnative prey as efficiently as native prey by conducting foraging experiments in field enclosures in secondary channels of the San Juan River and artificial streams located at the Konza Prairie Biological Station (KPBS), Kansas.

4) Quantify the use of specific prey items by Colorado pikeminnow by using ^{15}N labeled prey species in field enclosure experiments.

To address these four objectives, we conducted a series of field and laboratory experiments between 2003 and 2005. Stable isotopes were first used to characterize trophic interactions of fishes in the San Juan River. Laboratory mesocosm experiments were used to identify habitat use and gape limitations of young Colorado pikeminnow. Finally, field mesocosms and enclosures were used to test for prey preference and estimate consumption rates of native and nonnative prey species.

METHODS

Stable isotope signatures and caloric content

To establish baseline data on carbon and nitrogen isotope signatures of the fish assemblage in the San Juan River, samples of fishes and potential prey items were collected and analyzed from six geomorphic reaches of the San Juan River from Farmington to Lake Powell between July 7 and 13, 2003 (Figure 1). This information allowed us to characterize trophic position of each species in the assemblage and helped develop hypotheses on prey items of native (Colorado pikeminnow) and nonnative (channel catfish) predators (Gido et al. in press). These data also provided essential information on naturally occurring levels of N isotopes to compare with the experiments described below.

The ratio of ^{15}N: ^{14}N has been used as an indicator of trophic position and the ratio of ^{13}C: ^{12}C as an indicator of carbon source. Because consumers are consistently ^{15}N enriched (3.4 ± 0.3 ‰) compared to their prey (Cabana and Rasmussen 1994), higher trophic levels will be ^{15}N enriched. However, stable carbon isotopes (13C:12C or δ^{13}C) show less fractionation between predators and prey (0.8 ± 0.28 ‰; Vander Zanden and Rasmussen 1999) and can be used to assess the ultimate source of carbon to organisms because terrestrial and aquatic primary producers often have different ratios of carbon isotopes (Rounick and Winterbourn 1986). In addition, ^{13}C accumulates in algae in different ratios depending on water velocity. Algae growing in higher water velocity will be more depleted in ^{13}C versus algae growing in low velocity habitats (Finlay et al. 1990). Because these levels are relatively conserved with increasing trophic position, δ^{13}C in fishes can be a good indicator of habitat use (i.e. low vs. high velocity habitats). In addition, we analyzed energy density (i.e., calories/g) and C:N ratios for each potential prey item as a measure of prey quality.

Traditionally, white muscle has been the preferred tissue for stable isotope analysis because it has lower variability in δ^{15}N compared to other tissues, and does not require acidification to remove inorganic carbonates (Pinnegar and Polunin 1999). However, removing muscle tissue from endangered fishes for stable isotope analysis is problematic, therefore fin tissue has been suggested as an alternative because of its statistical similarity in stable isotope ratios with muscle tissue (Jardine et al. 2005).

Small-bodied fishes (< 100 mm) were collected whole. For fish > 100 mm TL, tissue plugs and/or fin clips were taken from each individual. At each site, three replicate tissue samples were taken from each trophic item. Tissue samples from fishes and other organisms from lower trophic levels were frozen in the field and brought to the laboratory, thawed, dried at 50°C for 48hr and ground to a powder with a mortar and pestle. Algae were separated from FPOM by centrifuging FPOM in colloidal silica as described by Hamilton and Lewis (1992). Ground samples were analyzed in the Stable Isotope Mass Spectrometry Laboratory (SIMSL) in the Division of Biology at Kansas State University (KSU) using a ThermoFinnigan Delta Plus mass spectrometer. Stable isotope ratios were calculated in the standard notation:

$$\delta^{15}N = [{}^{15}N/{}^{14}N_{sample}/{}^{15}N/{}^{14}N_{standard}) - 1 \times 1000$$

$$\delta^{13}C = [{}^{13}C/{}^{12}C_{sample}/{}^{13}C/{}^{12}C_{standard}) - 1 \times 1000$$

Values were expressed on a per mil (‰) basis. Because carbonates are known to bias isotope ratios of carbon, a separate aliquot was taken from organic matter (non-fish) samples, acidified to remove carbonates and then analyzed for carbon isotope ratios as described above.

Caloric content of fishes, invertebrates, and plant material was measured with a Parr semi-microbomb calorimeter. Individual fish, or composite samples of invertebrates were used for these analyses. All samples were homogenized, pressed into a pellet and combusted in the calorimeter.

Gape limitation of age-1 Colorado pikeminnow

The gape limitation of young Colorado pikeminnow was estimated to assess physical limitations to prey consumption. Five individuals of two prey species (i.e. flannelmouth sucker or red shiner) of similar sizes (+/- 2.5 mm) were stocked in indoor circular mesocosms (1.5 m diameter, 0.3 m depth) with sand substrate. Lack of cover reduced the ability of prey to avoid predation, thus limiting certain predator avoidance behavior. Age-1 Colorado pikeminnow of known sizes were starved for three days and then allowed to forage for 24 hrs in each trial. The ratio of prey: predator length for consumed and unconsumed prey was recorded for each trial. The largest prey species to predator length ratio consumed was estimated as the maximum gape limitation of Colorado pikeminnow for each species and was assumed to be constant across the range of predator sizes. In addition, the gape limitation of Colorado pikeminnow for red shiner, flannelmouth sucker, and speckled dace was estimated from body depth to length ratio of prey species and the length to gape ratio of Colorado pikeminnow from Portz and Tyus (2004).

Habitat use of age-1 Colorado pikeminnow and potential prey

Because high turbidity in the San Juan River precluded visual assessment of water column position, we evaluated microhabitat use by age-1 Colorado pikeminnow and potential prey in the experimental stream facility located at Konza Prairie Biological Station (KPBS) during Autumn 2004. Experimental units are described in Knight and Gido (2005). Each experimental unit was 6 m^2 and consisted of two pool/riffle sequences (12 m in total length) and water was circulated using an electronic trolling motor. Substrate in these streams was sand. Fifteen age-0 flannelmouth sucker (range 24-64 mm TL), 15 age-1 red shiner (range 23-56 mm TL) and a single age-1 Colorado pikeminnow (Range 100-117 mm TL) were stocked into each experimental unit (n=6). Fishes were observed twice daily (morning, afternoon) for three days and vertical position of fishes in each stream unit was recorded.

Prey selectivity: Mesocosms and age-1 Colorado pikeminnow

Field mesocosms were used during July 2004 to evaluate the prey selectivity of age-1 (90 – 120 mm SL) Colorado pikeminnow in ten circular mesocosms (1.2 m diameter, 0.25 m depth). Mesocosms were filled with a thin layer of river sand, placed at the waters edge and covered with shade cloth to prevent over-heating. Rather than attempt to offer pikeminnow native and nonnative prey of the same lengths, we selected random individuals from those available in the river at the time of the experiments. Prey species (i.e. red shiner, flannelmouth sucker, speckled dace) were collected during July 2004. In the first series of experiments, we tested the prey selectivity by Colorado pikeminnow by placing pairs of native (five age-0 flannelmouth sucker or five age-0 speckled dace) and nonnative (five age-1 red shiner) fishes in each mesocosm with one age-1 Colorado pikeminnow. Colorado pikeminnow were allowed to forage for 24 hours. By measuring lengths of all prey at the beginning and end of each trial, we could identify length of consumed prey. In addition, we quantified consumption rates of these three prey species by placing 10 individuals of each prey species with one age-1 Colorado pikeminnow. As above, remaining prey were counted and sizes of consumed and non-consumed individuals was recorded. Differences in red shiner, flannelmouth sucker, and speckled dace sizes that were made available to Colorado pikeminnow were assessed using t-tests. Differences in consumption rate of Colorado pikeminnow on each prey were assessed using t-tests when prey were stocked in pairs and ANOVA when species were stocked singly.

Field enclosures using age-1 Colorado pikeminnow

Field enclosures were constructed in a secondary channel of the San Juan River during July 2004 and 2005. In 2004, 6 m^2 enclosures (n = 6) were constructed using 3mm steel mesh. For these experiments the river bank served as one side of the enclosure. In 2005, 6 m^2 enclosures were constructed using nylon mesh (4mm) boxes stretched over PVC frames and placed in low velocity habitats in a secondary channel. This design allowed us to move enclosures and more efficiently remove predator and prey species at the end of the experiment. Lengths of prey were measured and individuals were randomly stocked in species pairs (i.e. 15 age-1 red shiner and 15 age-0 flannelmouth sucker) and allowed to acclimate for 6 to 24 hrs. Rather than attempt to offer pikeminnow native and nonnative prey of the same lengths, we selected random individuals from those available at the time of the experiments. Age-1 Colorado pikeminnow were stocked and allowed to forage for 3-6 days. Predator and prey were removed by seining and backpack electrofishing in 2004 and seining in 2005. Differences in stocked red shiner and flannelmouth sucker sizes were assessed using t-tests. Difference in consumption rates of Colorado pikeminnow on red shiner and flannelmouth sucker were assessed using t-tests.

Field enclosures using age-2 Colorado pikeminnow

Nylon mesh box enclosures as described above were used to assess consumption of prey species by age-2 Colorado pikeminnow in July 2005. Age-1 flannelmouth sucker and age-1 red shiner were collected on site at the time of the experiments and randomly stocked into enclosures in species pairs [i.e. (15 age-1 flannelmouth sucker and 15 age-1 red shiner) or (15 age-1 speckled dace and 15 age-1 red shiner)]. Prey were allowed to acclimate for 8-24 hrs after which an age-2 Colorado pikeminnow was stocked and allowed to forage for 3 days. Predator and prey were removed by seining and enclosures were picked up to ensure all fishes were removed. Sizes of all stocked and consumed fishes were recorded. Differences in stocked red shiner, flannelmouth sucker, and speckled dace sizes were assessed using t-tests. Differences in consumption rate of Colorado pikeminnow on each prey species were assessed using t-tests.

Growth of Colorado pikeminnow and gape limitation during summer 2004 and 2005

Growth in length of Colorado pikeminnow in the San Juan River were estimated from individuals captured throughout the summers of 2004 and 2005. Based on our estimates of maximum prey:predator length ratios, we evaluated the maximum size of prey (e.g., red shiner, flannelmouth sucker and speckled dace) available to predation by age-1 Colorado pikeminnow through the summers of 2004 and 2005.

Quantification of prey items using ^{15}N label

In addition to the field enclosures, we also evaluated the effectiveness of labeling prey species with ^{15}N to verify the number of prey consumed by a predator. The impetus for this study was to eliminate other sources of prey loss in field enclosures (e.g., escapement, predation by crayfish or raccoons) and to evaluate this method as a potential tool for studying consumption in larger scale enclosures where tracking the number of prey consumed is more difficult. If the $\delta^{15}N$ value of a prey item and $\delta^{15}N$ value of predator pre- and post-consumption are known, we can calculate the number of prey consumed using the following equation:

$$\text{Number of prey consumed} = \frac{\Delta\delta^{15}N_{pred}}{(\text{Assim rate}_{^{15}N})(g^{15}N/\text{prey})}$$

Where $\Delta\delta^{15}N_{pred}$ is the change of $\delta^{15}N$ in the predator pre- and post-consumption of labeled prey, Assim rate$_{^{15}N}$ is the predator assimilation efficiency, and $g^{15}N$/prey the mass of ^{15}N in each prey item. As a pilot study we grew algae in three different concentrations of ^{15}N (Low, Medium, High) and allowed bluntnose minnow (*Pimephales notatus*) to forage on the labeled algae for one week. After one week largemouth bass (*Micropterus salmoides*) were fed bluntnose minnow at a rate of one minnow per day for up to four weeks. The $\delta^{15}N$ values of both bluntnose minnow and largemouth bass were measured before the experiment and after each week of the experiment.

The field test of this method during the summers of 2004 and 2005 included prey fishes (flannelmouth sucker and red shiner) that were fed ^{15}N enriched algae and used in enclosure studies. Only one species was labeled per trial. Tissue was taken from prey fishes and Colorado pikeminnow before (baseline ^{15}N) and after each trial to assess enrichment of ^{15}N by the predator.

MODIFICATIONS FROM ORIGINAL PROPOSAL (Submitted on 30 March 2003)

Artificial stream experiments

We originally proposed to monitor the behavior of three native species (speckled dace, flannelmouth sucker, and roundtail chub) and two nonnative species (red shiner and fathead minnow) before and after the introduction of a caged pikeminnow into experimental streams. In these experiments we proposed to measure habitat use noting location in water column (surface, bottom, etc.), mesohabitat (pool or riffle), proximity to caged predator, and activities (e.g., feeding, swimming, resting). Unfortunately, many of our Colorado pikeminnow that were to be used for these experiments were diseased. By the time they were healthy, it was too cold to run experiments outdoors. Although we obtained some data on habitat use (see Fig. 7), we focused our efforts on predator efficiency and gape limitations across a range of different sized prey.

Field experiments

We had originally proposed to use sub-adult pikeminnow (200 to 350 mm TL) stocked into ca.100-m reaches in a secondary channel of the San Juan River to measure prey selectivity and consumption. However, in 2004 only age-1 Colorado pikeminnow (< 100 mm TL) were available and in 2005 only age-2 Colorado pikeminnow (150 – 250 mm TL) were available. This resulted in a shift of focus to smaller fish, which called for a reduction in the size of the enclosures, as these small fish would be harder to monitor in a large enclosure and more likely to escape. Thus, we opted for smaller, more controlled experiments.

RESULTS

Stable isotope signatures of aquatic organisms and detritus in the San Juan River

Fishes and organic matter were sampled for stable isotopes and caloric content from one main channel site in six geomorphic reaches and one secondary channel site in reaches 3, 4 and 5 (Figure 1). Isotopic signature and caloric content of fishes and lower trophic level items were analyzed across all sites and the mean ± 1 S.E. was calculated for each species or trophic group (Appendix 1). Each site was sampled to collect as many species as possible, however, not all fishes or invertebrates were encountered at each site in sufficient numbers for stable isotope or caloric analysis. The $\delta^{15}N$ and $\delta^{13}C$ values for each trophic item were plotted by geomorphic reach to evaluate food web structure and make comparisons across reaches (Figure 2). All flannelmouth and bluehead suckers were juveniles unless otherwise indicated and other small-bodied species analyzed were adults. In general, we identified three distinct trophic levels in each reach; fishes, invertebrates and algae/detritus. Algae and fine particulate organic matter were not included in Figure 2 because their $\delta^{15}N$ and $\delta^{13}C$ were very depleted and their inclusion obscured the relationships among fishes and invertebrates. Among invertebrates, chironomids were the most ^{13}C enriched and ephemeroptera the most depleted in ^{13}C. In reach 4, ten juvenile Colorado pikeminnow (<100mm) were captured in 2004 and a fin clip was taken from each individual. Based on this analysis, it appears that age-1 flannelmouth and age-1 Colorado pikeminnow overlap considerably in their trophic position. Mean $\delta^{15}N$ and $\delta^{13}C$ values for common fishes and other trophic items across reaches suggested a high degree of overlap in trophic position and resource base of small-bodied fishes in the San Juan River (Figure 3). All fishes sampled appeared to be secondary consumers, and with the exception of speckled dace, chironomids appeared to be the primary food source. Energy density and C:N ratio varied by species and lower trophic items (Figure 4). Bluehead sucker, speckled dace, and red shiner had the highest caloric content, nearly 20% higher calories/g than fathead minnow and flannelmouth sucker. Algae and FPOM were considerably lower in energy content with algae being more energy dense than FPOM. The mean C:N ratio was greatest in algae and FPOM (16.4:1), second highest in invertebrates (5.2:1) and least in fishes (3.4:1).

Gape limitation of age-1 Colorado pikeminnow

At the beginning of the growing season, age-1 Colorado pikeminnow were smaller in 2005 than 2004 (Figure 5). Although growth rates were similar between the two years, age-1 Colorado pikeminnow in 2005 were consistently smaller than in 2004. Larger age-1 Colorado pikeminnow individuals will not be as gape limited as smaller individuals, suggesting consumable prey were larger in 2004. Calculation of gape limitation, based on Portz and Tyus's gape equations and prey body morphology, indicated a Colorado pikeminnow will only be able to consume a red shiner that is 26% of its body length and smaller, flannelmouth sucker 40% of its length and smaller, and speckled dace 38% of its length and smaller. The gape limitation from our experiments showed Colorado pikeminnow could consume red shiner less than 37% and flannelmouth sucker less than 43% of their total length. The maximum physical gape limitation of Colorado pikeminnow preying on speckled dace was not tested, but was assumed to be similar to flannelmouth sucker due to similar body morphologies. Based on predator growth data and gape limitations for each prey species, the maximum length of consumable prey can be estimated through the growing season (Figure 6).

Habitat use of age-1 Colorado pikeminnow and potential prey

Habitat use observations from experimental stream mesocosms on the KPBS provided additional insight to Colorado pikeminnow foraging and a potential explanation for why they might show a greater predilection to consume native prey (regardless of size considerations). On average, Colorado pikeminnow occupied the lower 1/3 of the water column 82% of the time (Figure 7). This pattern of habitat use was more similar to flannelmouth sucker, which occupied this habitat 60% of the time than red shiner, which always occurred in the middle of the water column. Although habitat use of speckled dace and bluehead sucker were not investigated, both species are also thought to use benthic habitats.

Prey selectivity by Colorado pikeminnow

Mesocosms and age-1 Colorado pikeminnow

In mesocosm experiments, age-1 Colorado pikeminnow were allowed to select between five flannelmouth sucker or five speckled dace and five red shiner during the same trial. When red shiner and flannelmouth sucker were stocked together, Colorado pikeminnow predominately selected the smaller flannelmouth sucker ($t = -6.39$, $P < 0.001$; Figure 8). When five speckled dace and five red shiner were stocked together, speckled dace were also consumed at a higher rate ($t = -6.20$, $P < 0.001$; Figure 8). In addition, when presented only one prey species [i.e., 10 native (flannelmouth or speckled dace) or 10 nonnative (red shiner) prey], consumption rates for the native prey was 10 –30 times greater than for nonnative prey ($F_{2,20} = 63.8$, $P < 0.001$; Figure 9). Because prey individuals were collected at the time of the experiment, stocked age-1 red shiner were larger than stocked age-0 flannelmouth sucker ($t = 17.07$, $P < 0.001$) and age-0 speckled dace ($t = 28.7$, $P < 0.001$). All prey species and predator lengths are presented in Appendix 2.

Field enclosures using age-1 Colorado pikeminnow

Results from prey preference trials using field enclosures verified the patterns described in mesocosm experiments. In this case, each enclosure was stocked with native (15 flannelmouth sucker) and nonnative (15 red shiner) prey. Stocked age-1 red shiner were larger than stocked age-0 flannelmouth sucker ($t = 4.7$, $P < 0.001$). We recovered significantly fewer flannelmouth sucker than red shiner from field enclosures ($t = 2.98$, $P = 0.007$), lending support to our hypothesis that Colorado pikeminnow preferred smaller YOY native prey species over the larger age-1 nonnative red shiner (Figure 10). All prey species and predator lengths are presented in Appendix 2.

Field enclosures using age-2 Colorado pikeminnow

In July 2005, age-2 Colorado pikeminnow preferentially foraged on age-1 red shiner when stocked with age-1 flannelmouth sucker ($t = -4.30$, $P < 0.001$) or age-1 speckled dace ($t = -5.17$, $P = 0.002$; Figure 11). Stocked age-1 red shiner were smaller than stocked age-1 flannelmouth sucker ($t = -37.97$, $P <0.001$) and age-1 speckled dace ($t = -14.60$, $P < 0.001$). These results suggest simple size selectivity, rather than preference for native species. All prey species and predator lengths are presented in Appendix 2.

Prey size selectivity by Colorado pikeminnow

To evaluate size dependent consumption rates, we compared the average size of available and consumed prey for each predation experiment. In mesocosm experiments the size of speckled dace consumed was not different from available sizes ($t = -0.101$, $P = 0.92$), but for both flannelmouth sucker ($t = 3.25$, $P = 0.001$) and red shiner ($t = 3.66$, $P < 0.001$), consumed prey were on average smaller than those available (Figure 12). Moreover, mean size of available red shiner was larger than flannelmouth sucker or speckled dace. Thus, a greater consumption rate of natives apparently was because of their smaller size relative to nonnatives. When an exponential decay curve ($y = ae^{-bx}$) is fitted to consumption rates verses prey:predator length ratio for mesocosm and gape limitation experiments, there is a significant decline in consumption rate with increased prey:predator length ratio for age-1 ($a = 15.2$, $b = 7.71$, $R^2 = 0.25$, $P < 0.001$; Figure 13) and age-2 Colorado pikeminnow ($a = 3.53$, $b = 4.53$, $R^2 = 0.20$, $P = 0.003$; Figure 14).

Quantification of prey items using ^{15}N label

In addition to assessing the feasibility of enclosures to quantify predation by Colorado pikeminnow, we also investigated the feasibility of labeling prey species with ^{15}N to estimate consumption rates of the predator. Bluntnose minnow were shown to readily assimilate the ^{15}N label, which accumulated in their tissues through the duration of the trial (Figure 15). After 2 weeks of consuming labeled bluntnose minnows, there was more than an order of magnitude enrichment of ^{15}N in the largemouth bass. These results demonstrated that we could detect consumption of labeled prey items by a predator, and thus quantify numbers of prey consumed.

The predator enrichment experiment during summer 2004 only provided limited data because only three of six Colorado pikeminnow survived the duration of the trial. However, the surviving individuals did show an enrichment of ^{15}N from labeled prey confirming the consumption of individuals not lost to other means (e.g. escape, crawfish, raccoons etc.) than predation by Colorado pikeminnow. The difference between our predicted and observed consumption of prey ranged from 0.1 – 2.3 individuals. Based on these results, the use of stable isotopes may be useful in large scale enclosures where recovery of all prey is difficult.

DISCUSSION

The research described above provides quantitative data that can be used to evaluate the prey quantity and quality for juvenile Colorado pikeminnow in the San Juan River. Given the current failure of Colorado pikeminnow to recruit to adults in this system, such data are critical to develop hypotheses on factors that limit or impede recovery of this species. For example, our results provided evidence that invertebrate prey (e.g. chironomids) are the primary food base for gape-limited age-1 Colorado pikeminnow until young-of-year (YOY) fishes become available during summer. Diet analysis has also supported the importance of chironomidae larvae as food items for juvenile Colorado pikeminnow (Vanicek and Kramer 1969; Muth and Snyder 1995). Assuming fish prey are necessary to sustain optimal growth, the timing of occurrence and abundance of YOY native fishes are potentially critical influences on age-1 Colorado pikeminnow survival.

There is a wealth of literature on lentic systems to suggest ontogenic dietary shifts from invertebrate to fish prey is critical for growth of gape-limited juvenile piscivores (e.g. Garvey and Stein 1998; Persson and Bronmark 2002). The consensus of these studies suggests a switch to higher quality food items is necessary to reduce competition for invertebrates and growth out of periods of when gape dimensions constrain food selection. Moreover, as piscivorous fishes grow, their efficiency at consuming small invertebrate prey decreases (Persson and Bronmark 2002). Colorado pikeminnow as small as 73 mm TL have been observed to be piscivorous (Muth and Snyder 1995) and fish greater than 200 mm TL are thought to be completely piscivorous (Vanicek and Kramer 1969). However, the size at which piscivory becomes important within this range (73 – 200 mm TL) is potentially critical. For example, a switch to piscivory not only relaxes gape limitations by increased predator size resulting from more consumption of fish prey, but increased growth could potentially increase over-winter survival (Garvey et al. 1998). This is consistent with studies by Carlson and Kaeding (1991), which reported larger age-0 Colorado pikeminnow had a higher likelihood of over-winter survival. Results of these studies highlight the importance of understanding how growth and condition of young Colorado pikeminnow influences recruitment to reproductive adults.

Introduced prey species can have avoidance behavior or life history traits that make them unavailable to native predators (e.g., Nesler and Bergersen 1991, Noble 1986). In the San Juan River, the hypothesis that nonnative prey are more adept at avoiding predation by Colorado pikeminnow than native prey is confounded by differences in size distribution of prey throughout summer months. Thus, greater consumption rates of YOY native prey by age-1 Colorado pikeminnow in our experiments could simply be attributed to a preference for smaller prey items. This hypothesis was supported by a general preference for smaller individuals (e.g., greater consumption rates) when age-1 Colorado pikeminnow were only allowed to forage on a single prey species. Moreover, our field experiments in 2005, in which age-2 Colorado pikeminnnow consumed smaller age-1 red shiner at a higher rate than larger age-1 natives, further supports a size-selectivity hypothesis. We conclude from these experiments that nonnative prey (i.e., red shiner) are a viable prey resource, but only if they do not exceed the gape limitation of Colorado pikeminnow.

Our data also suggest the shift to picscivory is related to the size distribution of available prey. Because native and nonnative fishes have different preferred spawning temperatures, piscivory on different species may vary considerably through the summer. This has important implications for management of this system and the limitations that nonnative fishes impose on the recruitment of Colorado pikeminnow. It is clear from our studies that because of gape limitation, mainly YOY native fishes are available for consumption by age-1 Colorado pikeminnow during late spring and early summer. The spawning chronology of native and nonnative prey is dependent on a variety of factors, including timing of discharge and water temperature. It will be important to evaluate the link between prey density and timing of occurrence with growth and survival of juvenile Colorado pikeminnow, as proposed for the second phase of this research.

The original proposal to evaluate trophic relations of Colorado pikeminnow focused on age-2+ individuals. We proposed to use large field enclosures, necessary to accommodate comparatively large (>250 mm TL) study organisms, and to conduct intensive studies in experimental stream mesocosms. However, only age-1 Colorado pikeminnow were available in 2004 and changes in holding facilities were necessary to accommodate changes in study organism size. Fish health issues and size of available study organisms precluded timely completion of several experimental stream trials and necessitated modification of experimental stream study trials. Although experiments using age-2 Colorado pikeminnow (as detailed in our original study proposal) are still needed to answer important questions regarding predator efficiency and prey selectivity, the experiments conducted with age-1 individuals provided relevant information for an equally, if not more, critical life stage of Colorado pikeminnow in the San Juan River. This shift in focus resulted in an improved understanding of the dynamics between native and nonnative prey fishes and highlighted the importance of a shift to piscivory by age-1 Colorado pikeminnow.

How does this research fit into the current San Juan Recovery and Implementation Program? We suggest the data from this study provide critical information to help structure conservation efforts in the San Juan River that relate to 1) control of nonnative species, 2) influence of flow manipulations on prey base, 3) recommendations for stocking programs:

Control of nonnative species – Our data suggest YOY native flannelmouth sucker, bluehead sucker and speckled dace are available for gape limited age-1 Colorado pikeminnow well before YOY nonnative fishes become available. If adult nonnative fishes (i.e., red shiner and fathead minnow) compete for resources with (Gido and Propst 1999) or potentially prey on these natives (Brandenburg and Gido 1999), this would indirectly reduce prey availability for Colorado pikeminnow. Only when nonnative fishes spawn or when Colorado pikeminnow are large enough to consume adult nonnatives, are these fishes an available prey resource. Future studies should consider the negative effects of nonnative fishes on the abundance of YOY native fishes, and the potential consequences of these interactions on prey availability for Colorado pikeminnow.

Influence of flow manipulations on prey base – Flow manipulations can benefit native fishes by structuring river habitats to increase the availability of spawning (e.g., clean substrates) and rearing habitats (e.g., backwaters) (Bliesner and Lamarra 2002). Increased spring discharge also is positively correlated to abundance of native fishes, whereas increased duration of low flows during summer is negatively related to native fish abundance (Propst and Gido 2004). This pattern is likely linked, at least in part, to changes in temperature in that increased discharge during spring and summer is related to cooler water temperatures that favor native species (e.g., Marchetti and Moyle 2001). Based on these studies, it is likely discharge manipulations could be an effective tool to manage prey availability for Colorado pikeminnow.

Recommendations for stocking programs – Stocking Colorado pikeminnow is necessary to restore a naturally reproducing population in the San Juan River. However, timing of stocking and size of stocked individuals could strongly influence the success of these efforts. For example, stocking larger individuals will reduce gape limitations and potentially increase prey availability. Additionally, stocking individuals during optimal periods when prey are most abundant could increase growth and over-winter survival. Although availability of fish prey is only one of many possible limitations to recruitment of Colorado pikeminnow, our research clearly identifies this as a potential limiting factor. Given the low success of stocked Colorado pikeminnow, future studies should evaluate role of prey quality and quantity in limiting recovery of Colorado pikeminnow.

CONCLUSIONS

Stable isotope analysis of organic matter, invertebrates, and fishes in the San Juan River revealed age-1 Colorado pikeminnow feed mainly on chironomids during Spring and early Summer. Consumption of invertebrates by young Colorado pikeminnow is consistent with previous research from the Green River, Colorado and Utah (Vanicek and Kramer 1969). Mesocosms and field enclosures stocked with prey collected from the San Juan River in June and July demonstrated that as they shift from an invertebrate-dominated to fish dominated diet, age-1 Colorado pikeminnow preferentially consumed smaller age-0 native prey over larger age-1 nonnative red shiner. However, using age-2 Colorado pikeminnow in field enclosures stocked with age-1 native prey (i.e. flannelmouth sucker or speckled dace) and nonnative age-1 red shiner, Colorado pikeminnow preferentially foraged on the smaller age-1 red shiner. Based on these findings, prey size appears to be a major factor controlling the availability of prey for Colorado pikeminnow.

A goal of the San Juan Recovery and Implementation Program is to sustain a naturally recruiting population of Colorado pikeminnow. Even if stocking efforts ultimately increase the number of mature adults in the San Juan River, the offspring of those fish will need to recruit into the population. Thus, identifying critical life stages and potential limiting factors during each stage is essential for restoration of a self-sustaining population of Colorado pikeminnow in the San Juan River. The combined use of stable isotopes to identify food web pathways and field experiments has provided insight into the potential prey limitations for juvenile Colorado pikeminnow. Future studies that use empirical information to evaluate how prey availability varies across years and with discharge to influence recruitment success and relative growth of Colorado pikeminnow are necessary to develop and refine augmentation efforts.

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Appendix 1. Mean (± SE) for percent carbon, percent nitrogen, C:N ratio, isotope signature and energy density from fishes, invertebrates, algae, benthic organic matter and seston collected from the San Juan River in July 2003.

Item	n	%C	%N	C:N ratio	$\delta^{13}C$ (‰)	$\delta\ ^{15}N$ (‰)	n (Caloric)	Calories/g dry mass
Fishes								
Ptychocheilus lucius	3	--------	--------	--------	-21.89 (0.20)	12.96 (0.57)	--------	--------
Cyprinella lutrensis	17	46.84 (4.34)	13.36 (0.52)	3.51:1	-22.41 (0.44)	12.24 (1.12)	7	4923.2 (522.1)
Pimephales promelas	16	46.74 (3.34)	13.76 (0.48)	3.40:1	-21.80 (0.70)	12.97 (1.56)	5	4286.1 (219.8)
Rhinichthys osculus	14	49.08 (3.48)	11.63 (0.93)	4.22:1	-24.08 (0.66)	13.65 (0.99)	6	5576.9 (81.7)
Catostomus discobolus	6	43.60 (5.27)	13.58 (0.22)	3.21:1	-21.73 (0.71)	13.22 (0.72)	1	5564.6
Catostomus latipinnis	24	45.33 (3.69)	13.84 (0.39)	3.28:1	-21.55 (0.58)	12.74 (1.43)	9	4308.7 (450.6)
Ictalurus punctatus	8	41.01 (5.15)	13.78 (0.23)	2.98:1	-22.64 (0.65)	13.61 (0.92)	--------	--------
Micropterus salmoides	10	44.11 (3.12)	13.89 (0.25)	3.18:1	-22.85 (0.97)	13.44 (1.66)	5	4196.4 (56.3)
Invertebrates								
Chironomidae	6	43.50 (4.8)	9.36 (1.1)	4.65:1	-22.73 (0.68)	8.88 (2.69)	3	3601.6 (156.3)
Ephemeroptera	6	46.26 (8.1)	7.81 (1.25)	5.92:1	-25.43 (1.39)	9.07 (1.48)	3	4480.7 (417.1)
Trichoptera	4	51.99 (4.1)	9.01 (0.74)	5.77:1	-24.40 (0.82)	8.86 (1.94)	3	4790.2 (55.3)
Odonata	5	46.09 (5.17)	10.04 (0.54)	4.59:1	-23.91 (0.95)	10.08 (1.11)	1	4732.7
Algae and Organic matter								
Filamentous algae	6	25.32 (7.29)	1.04 (0.23)	24.3:1	-29.15 (0.08)	7.28 (1.63)	3	2828.3 (337.3)
FPOM (total)	9	7.04 (2.27)	0.83 (0.28)	8.48:1	-25.23 (0.77)	6.64 (2.0)	--------	--------
FPOM (light fraction)	9	3.45 (2.62)	0.25 (0.05)	13.80:1	-23.73 (1.09)	6.75 (1.97)	3	2149.7 (7.2)
MPOM	9	39.02 (9.98)	1.37 (0.50)	28.48:1	-26.90 (1.82)	1.10 (2.44)	--------	--------
CPOM	9	41.33 (7.37)	1.35 (0.53)	30.60:1	-26.87 (1.52)	1.34 (1.29)	--------	--------
Suspended OM > 0.45 um	9	3.16 (0.71)	0.54 (0.22)	5.85:1	-27.17 (1.01)	7.39 (1.96)	--------	--------

Appendix 2. Summary of prey and Colorado pikeminnow sizes for available and consumed individuals from 2004 and 2005 experiments.

	Ptychocheilus lucius	*Cyprinella lutrensis*		*Catostomus latipinnis*		*Rhinichthys osculus*	
Experiment	Mean size TL (mm) (range)	Mean size TL (mm) (range)		Mean size TL (mm) (range)		Mean size TL (mm) (range)	
		available	consumed	available	consumed	available	consumed
Mesocosms	110 (97-127)	44.1 (30-52)	38.5 (33-45)	35.2 (24-60)	31.6 (21-41)	28.6 (19-40)	28.7 (19-35)
Field enclosure age-1 CPM	118 (107-138)	41.6 (29-52)	38.2 (29-44)	37.9 (23-59)	32.1 (23-40)	n/a	n/a
Field enclosure age-2 CPM	199 (160-251)	47.4 (27-74)	40.9 (27-47)	78.7 (49-119)	64.7 (49-77)	58.7 (37-86)	48.8 (37-59)

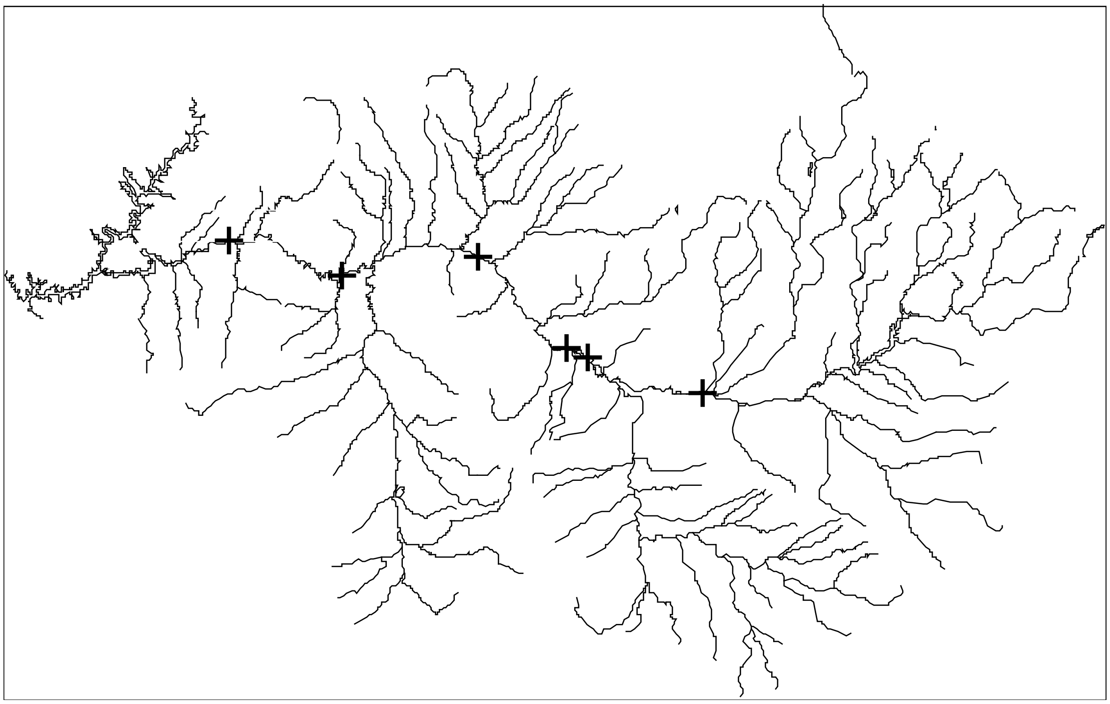

Figure 1. Location of sample sites in six geomorphic reaches of the San Juan River sampled between July 7 and 13, 2003. The primary channel was sampled at each site and secondary channels were sampled at reach 3, 4, and 5.

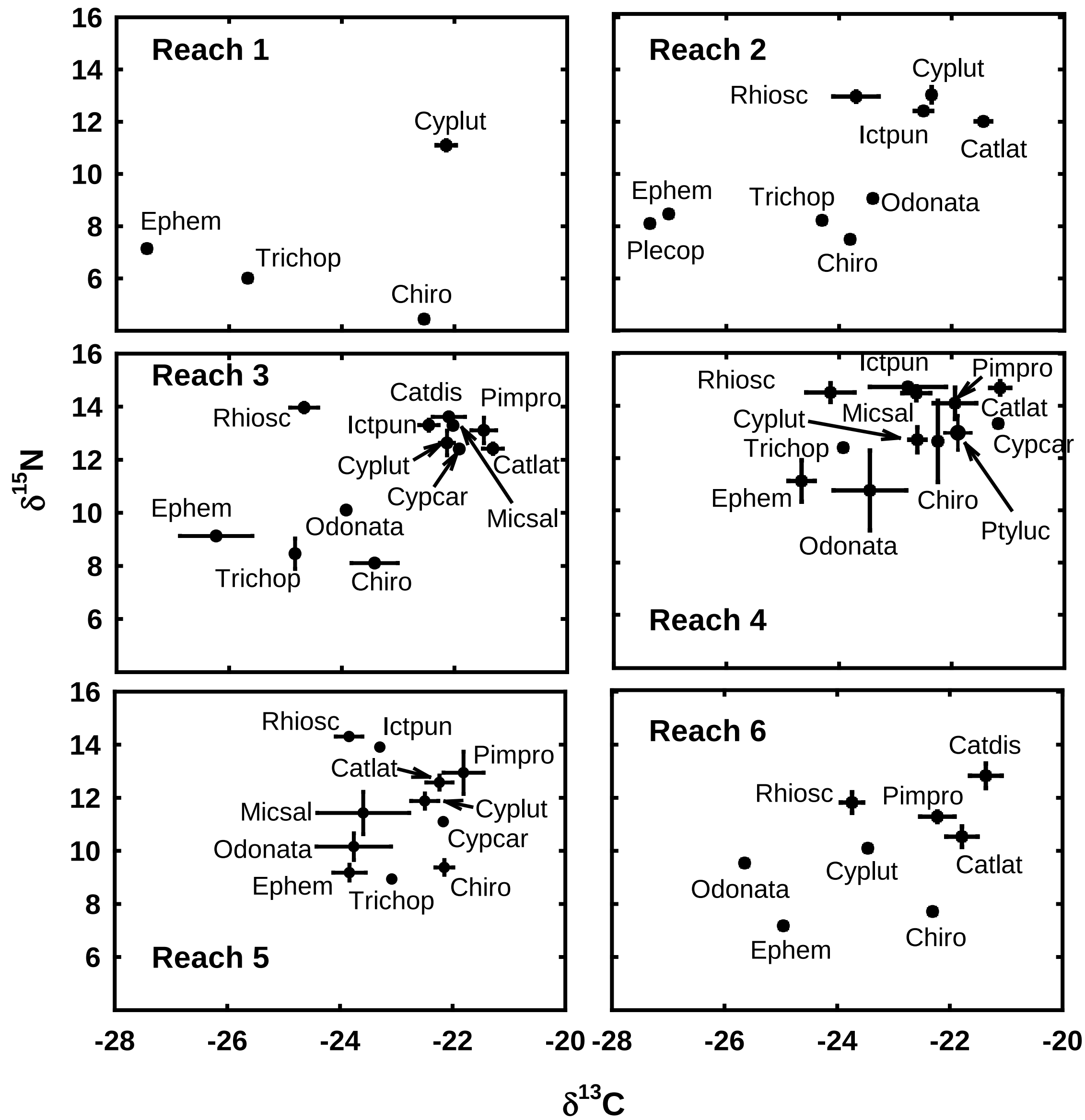

Figure 2. Mean (± 1 SE) isotopic values of fishes and invertebrates at each reach sampled. Items with similar values indicate similar positions in the food web.

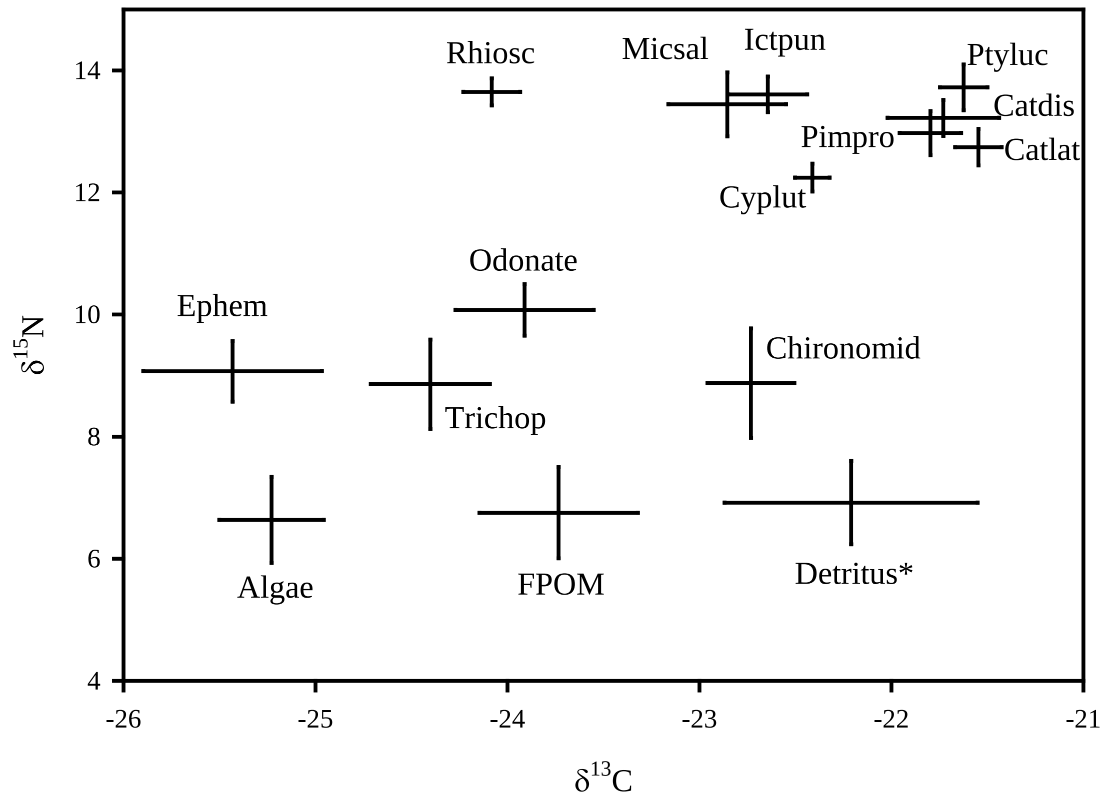

Figure 3. Mean (± 1 SE) isotopic values from all reaches of algae, FPOM, and detritus occupy the lowest trophic level based on $\delta^{15}N$ values. Detritus* indicates assumed isotope values for detritus based on differences between composite samples of algae and detritus (i.e., FPOM) and those of only the algal fraction of these samples.

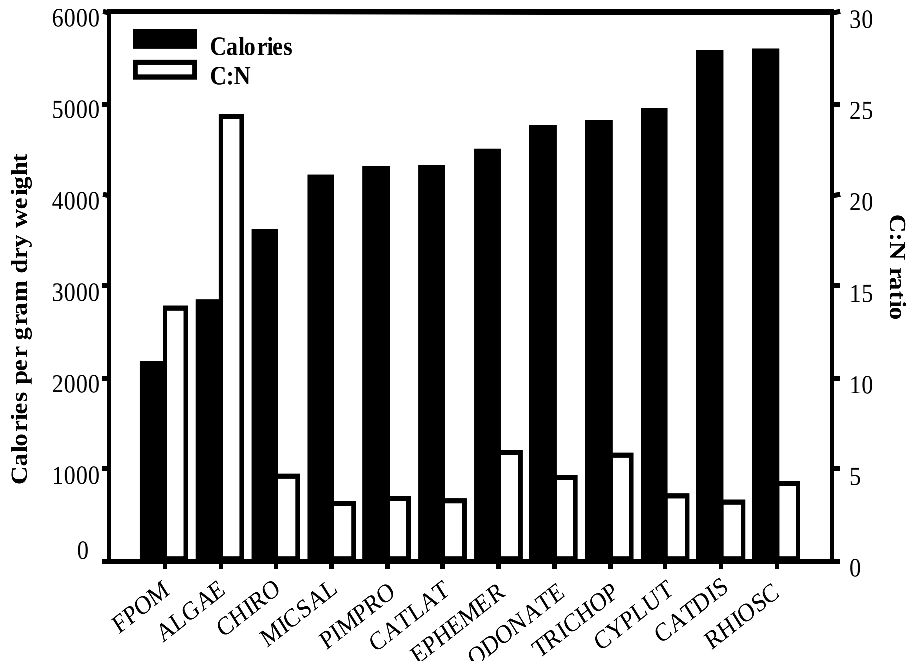

Figure 4. Mean energy density (cal/g) and C:N ratio of fishes and lower trophic level items from the 6 geomorphic reaches sampled.

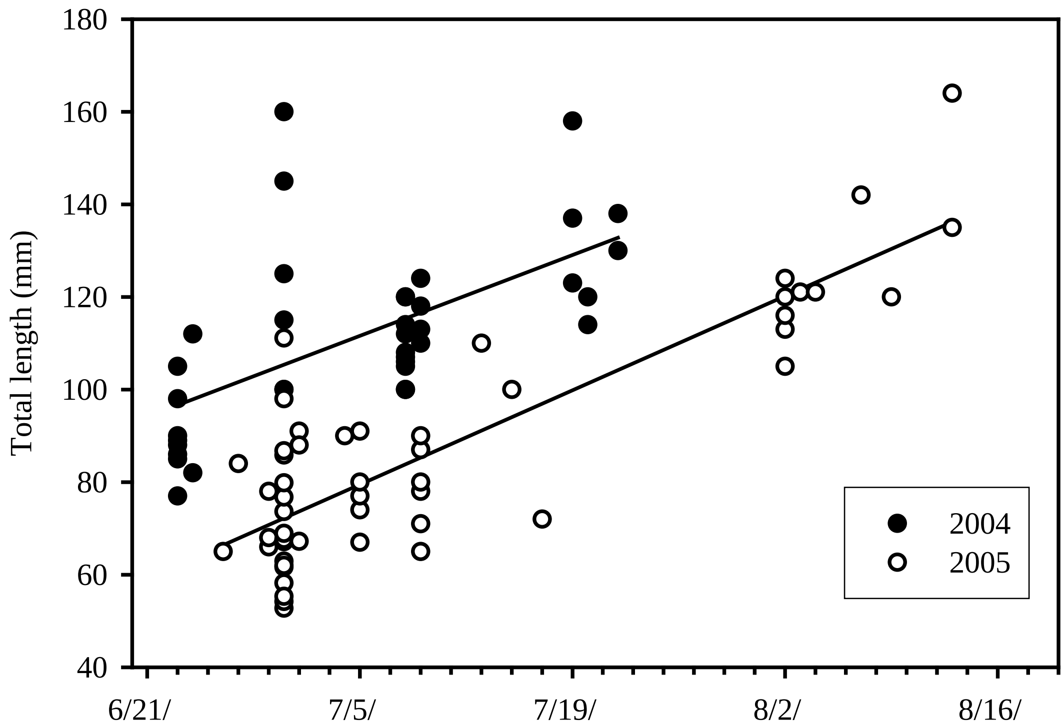

Figure 5. Length of juvenile Colorado pikeminnow captured in the San Juan River between 23 June and 20 August 2004 and 2005.

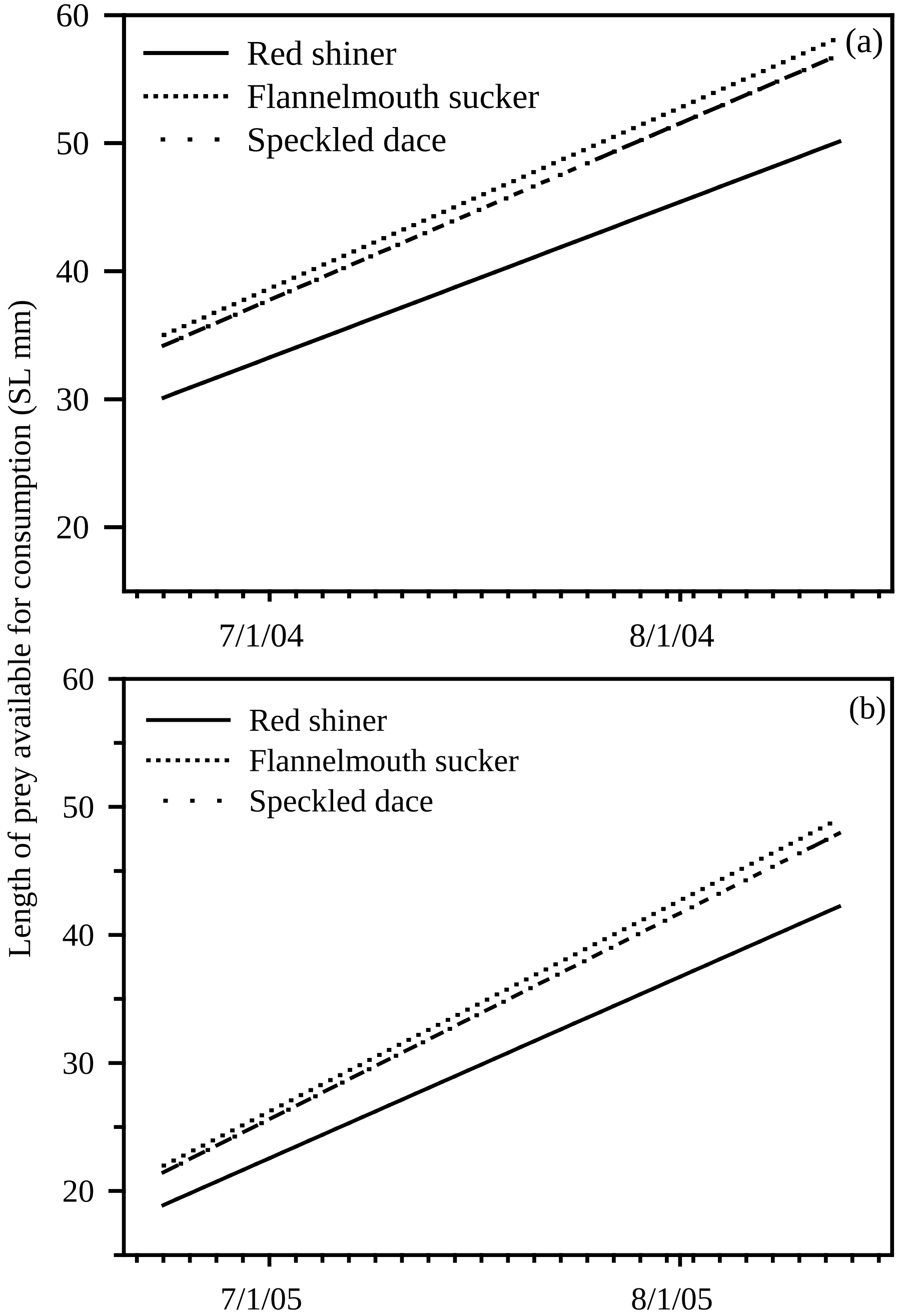

Figure 6. Maximum size of red shiner, flannelmouth sucker, and speckled dace consumable based on gape limitation for age-1 Colorado pikeminnow during summer 2004 (a) and 2005 (b).

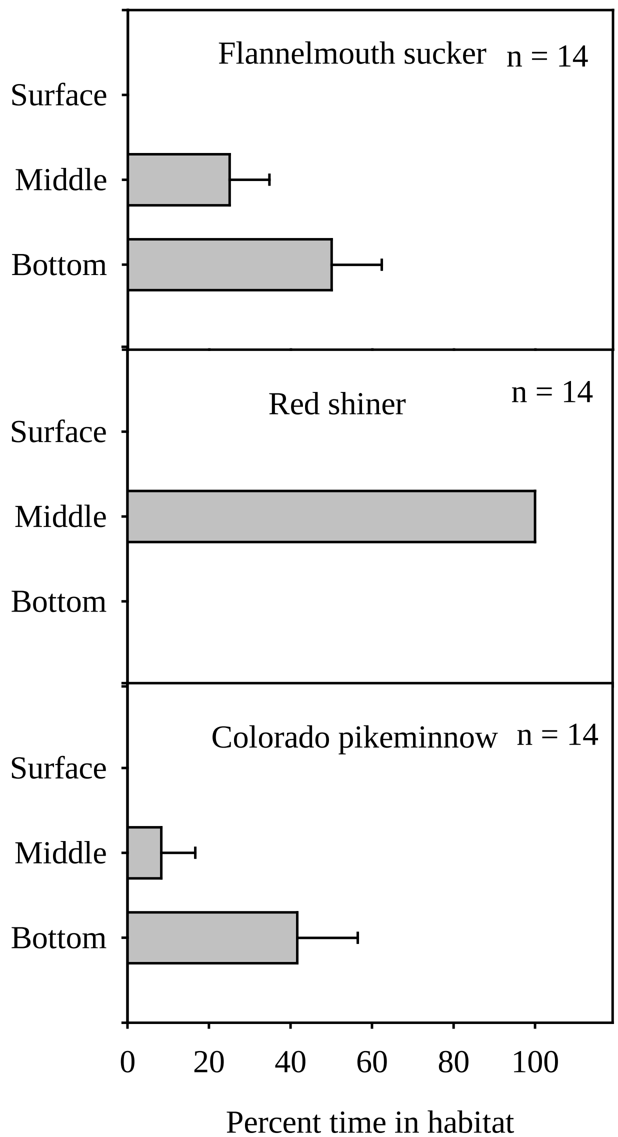

Figure 7. Mean (+1 SE) percent of time YOY flannelmouth sucker, age-1 red shiner and age-1 Colorado pikeminnow were observed in habitats in experimental streams at Konza Prairie Biological Station, KS.

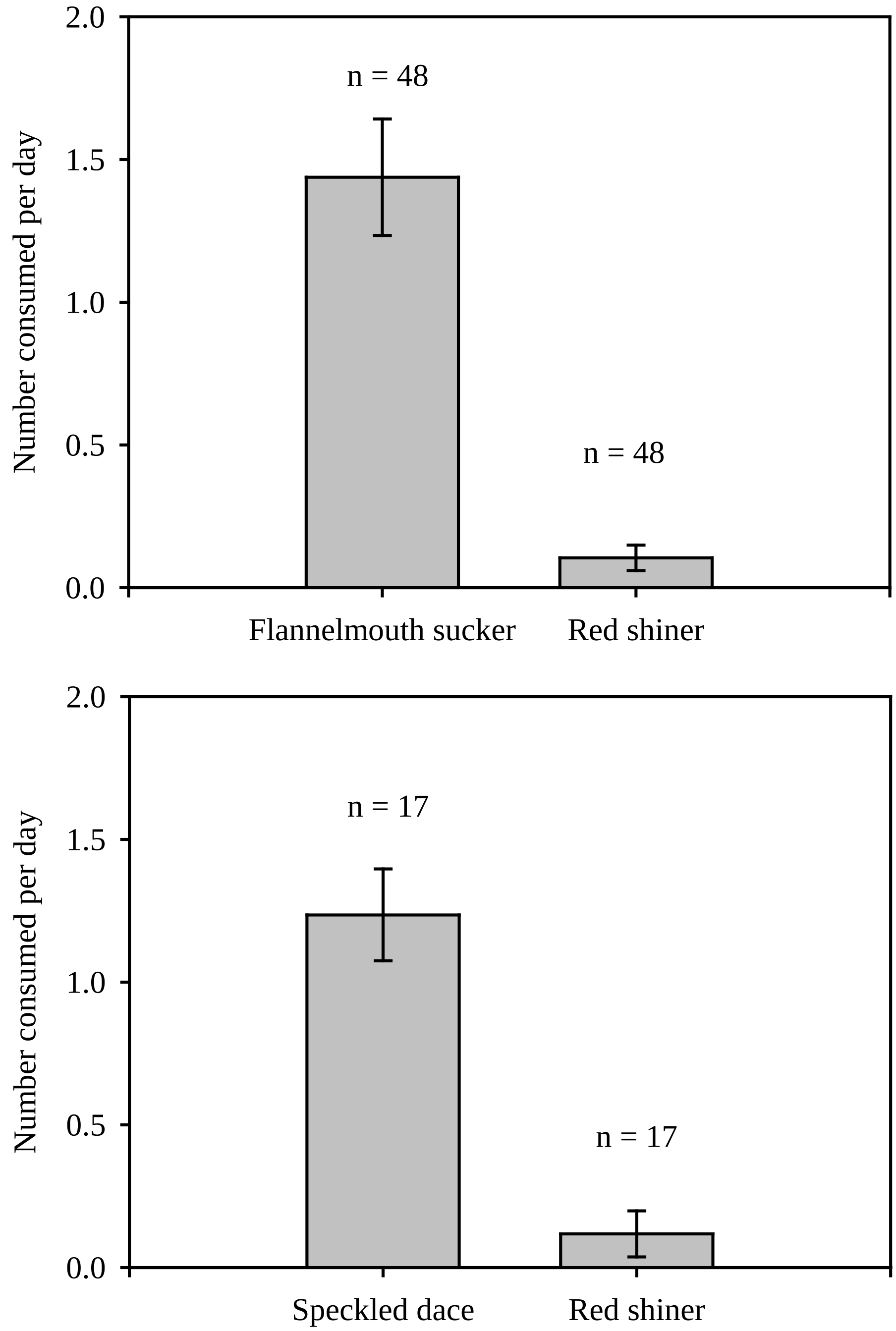

Figure 8. Mean (± 1 SE) consumption rate of native and nonnative prey individuals by age-1 Colorado pikeminnow from mesocosms on the San Juan River during summer 2004. Five individuals of each prey species were stocked into each mesocosm and one age-1 Colorado pikeminnow was allowed to forage for 24 hrs.

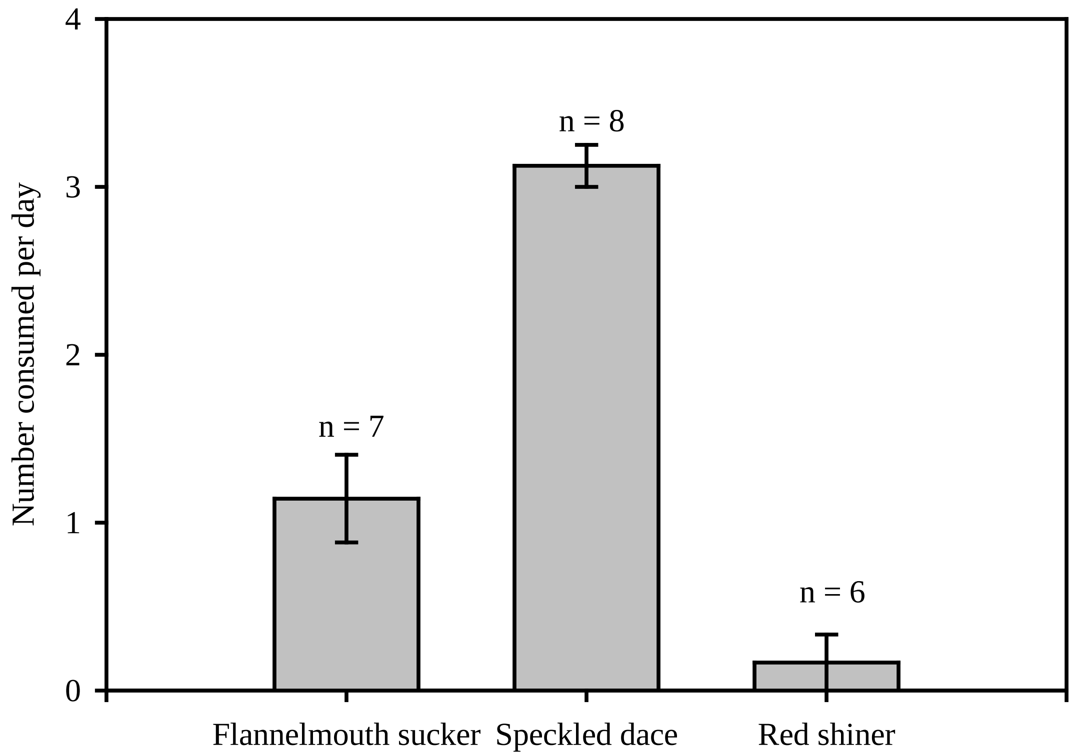

Figure 9. Mean (± 1 SE) consumption rate of three prey fishes by Colorado pikeminnow stocked in mesocosms in the San Juan River during summer 2004. In each trial, five individuals of the same species was stocked with one age-1 pikeminnow.

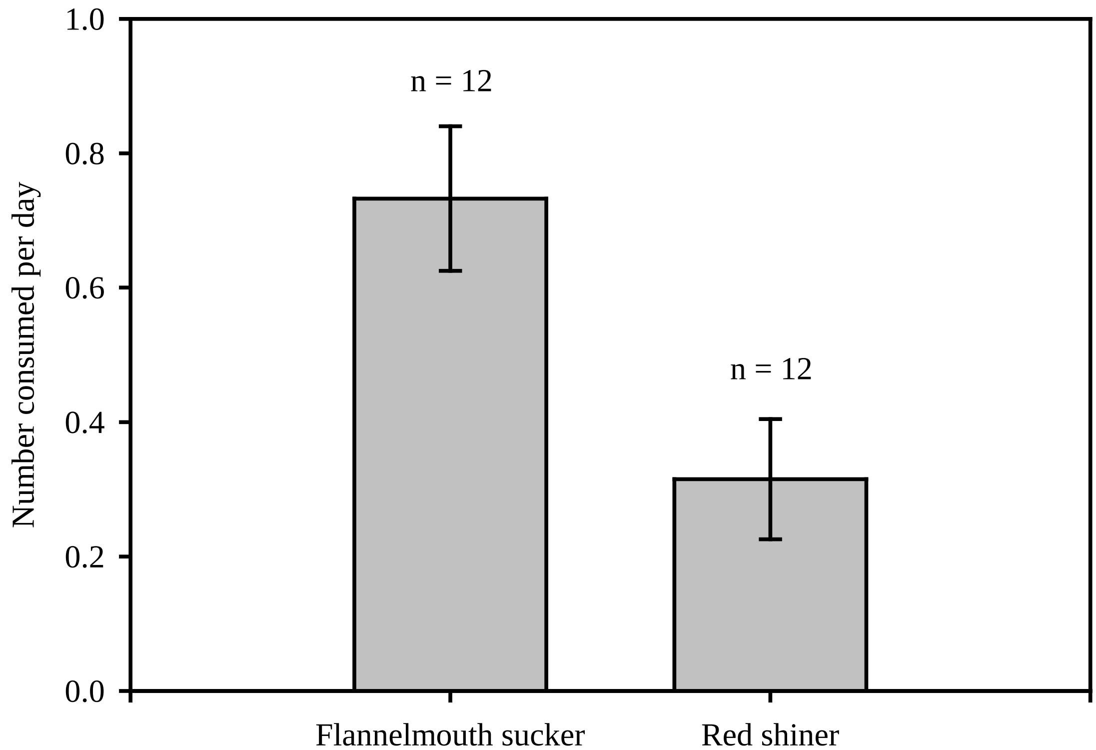

Figure 10. Mean (± 1 SE) consumption rates of YOY flannelmouth sucker and age-1 red shiner by age-1 Colorado pikeminnow stocked in field enclosures in the San Juan River during July 2004 and 2005.

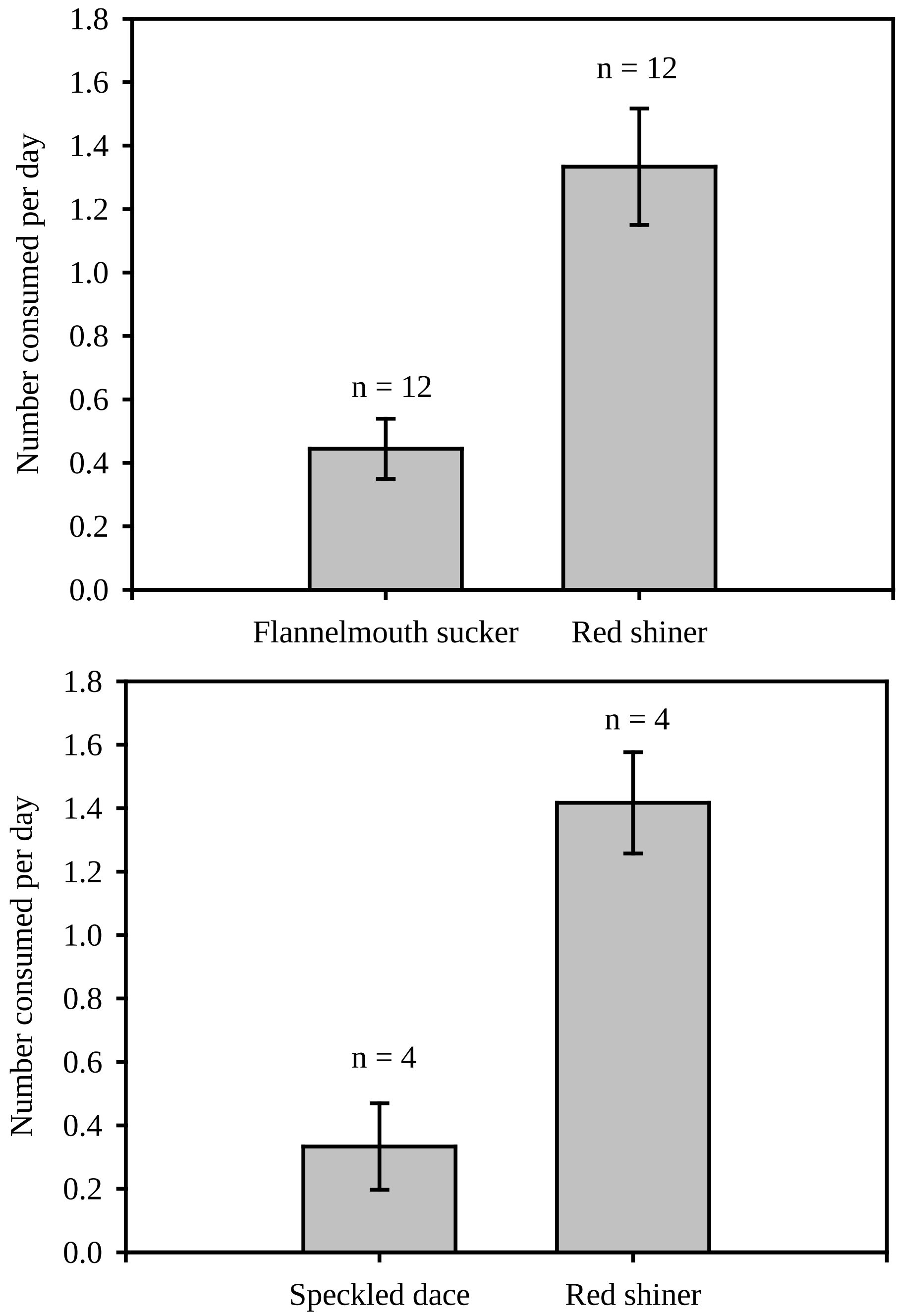

Figure 11. Mean (± 1 SE) consumption rates of age-1 flannelmouth sucker and age-1 red shiner by age-2 Colorado pikeminnow stocked in field enclosures in the San Juan River during July 2005.

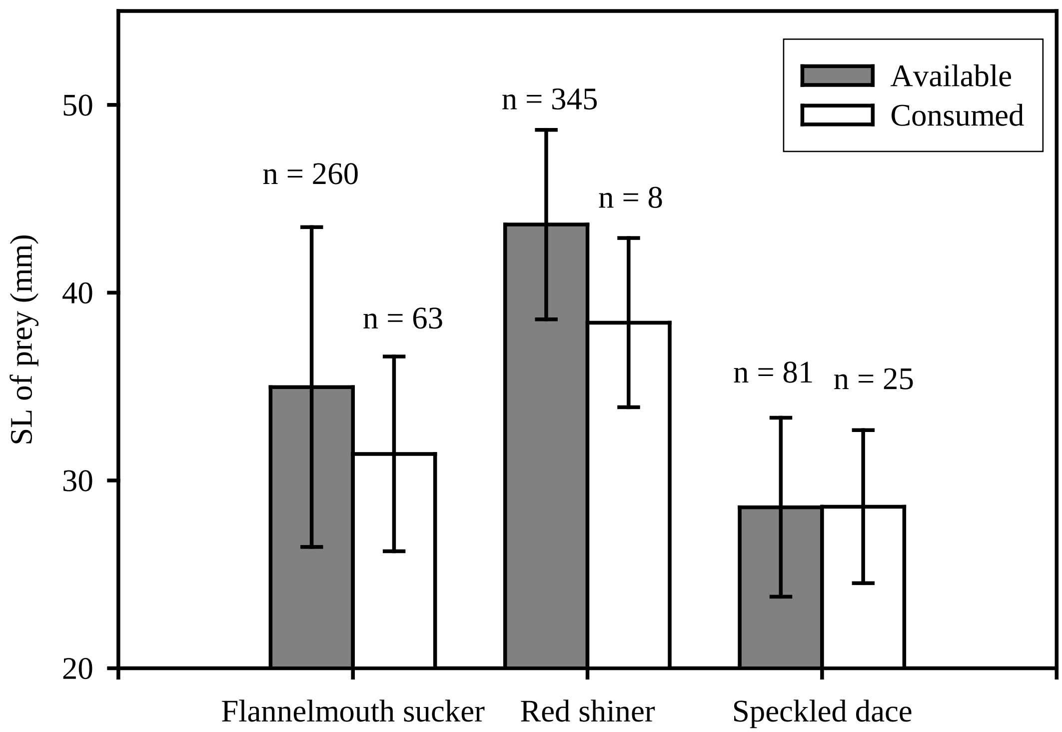

Figure 12. Mean (± 1 SD) length of prey available versus those consumed from mesocosm experiments.

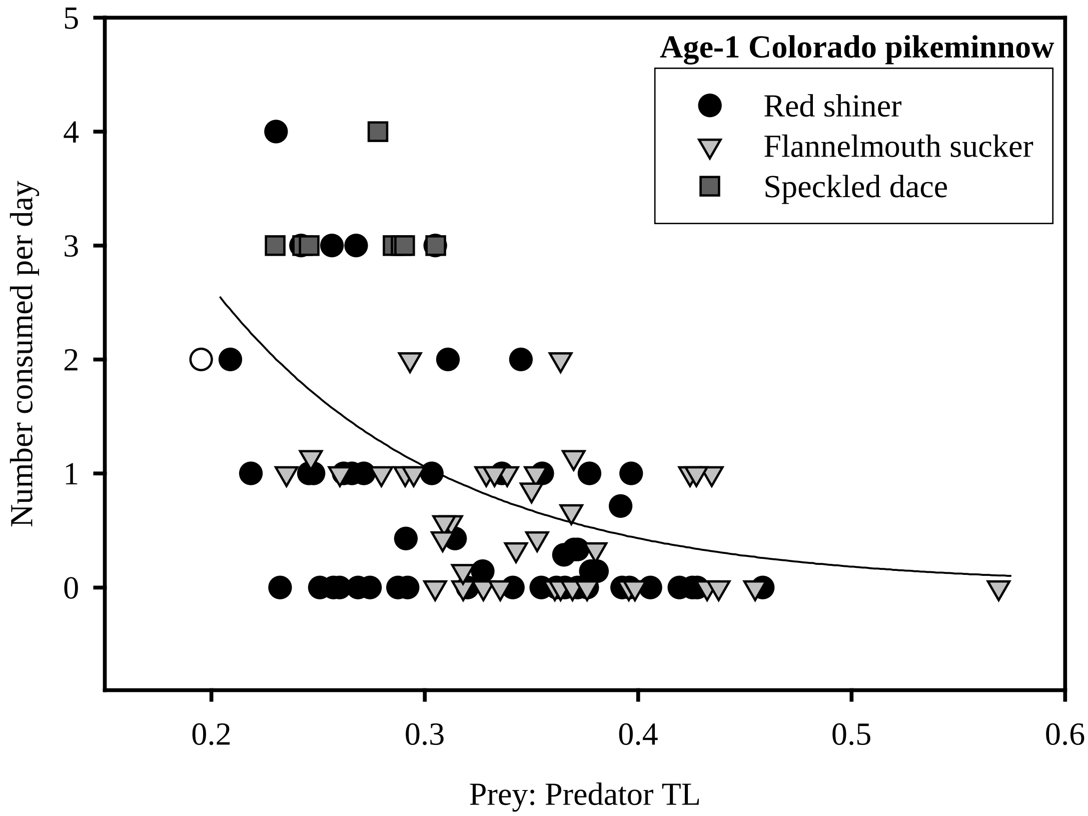

Figure 13. Prey:predator length ratio available and consumption rate of age-1 red shiner, YOY flannelmouth sucker and age-0 speckled dace by age-1 Colorado pikeminnow.

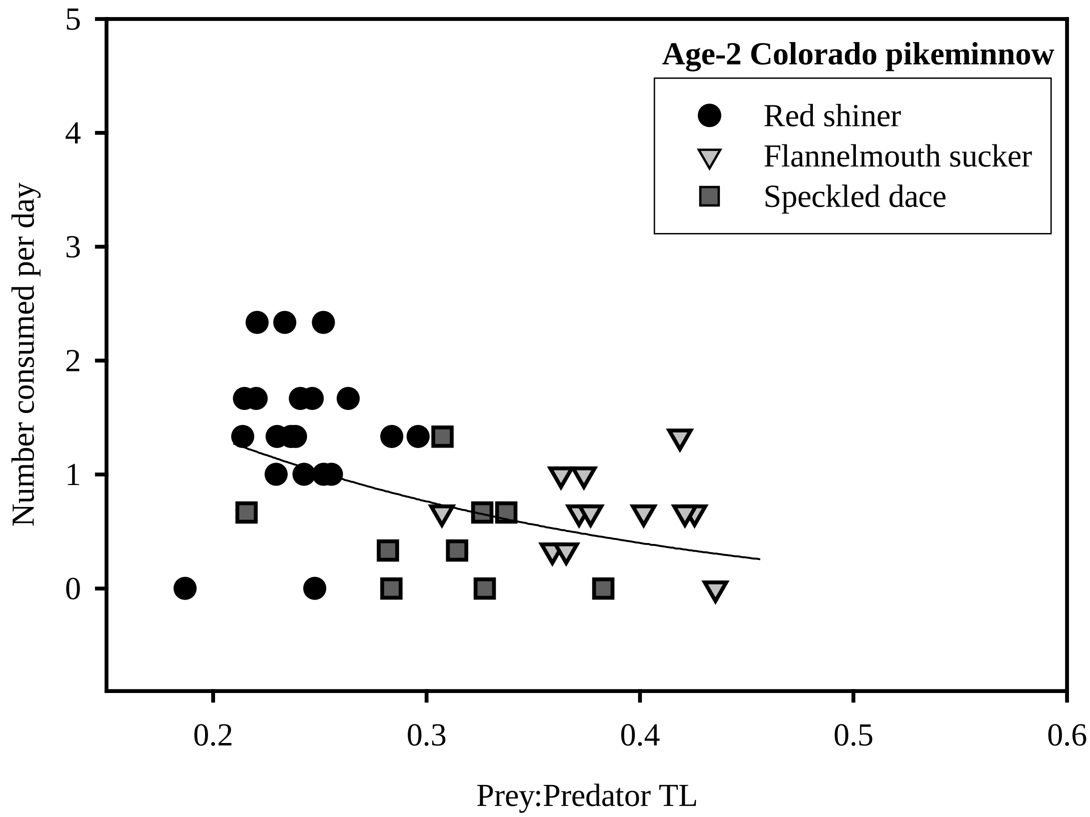

Figure 14. Prey:predator length ratio available and consumption rate of age-1 red shiner, YOY flannelmouth sucker and age-1 speckled dace by age-2 Colorado pikeminnow.

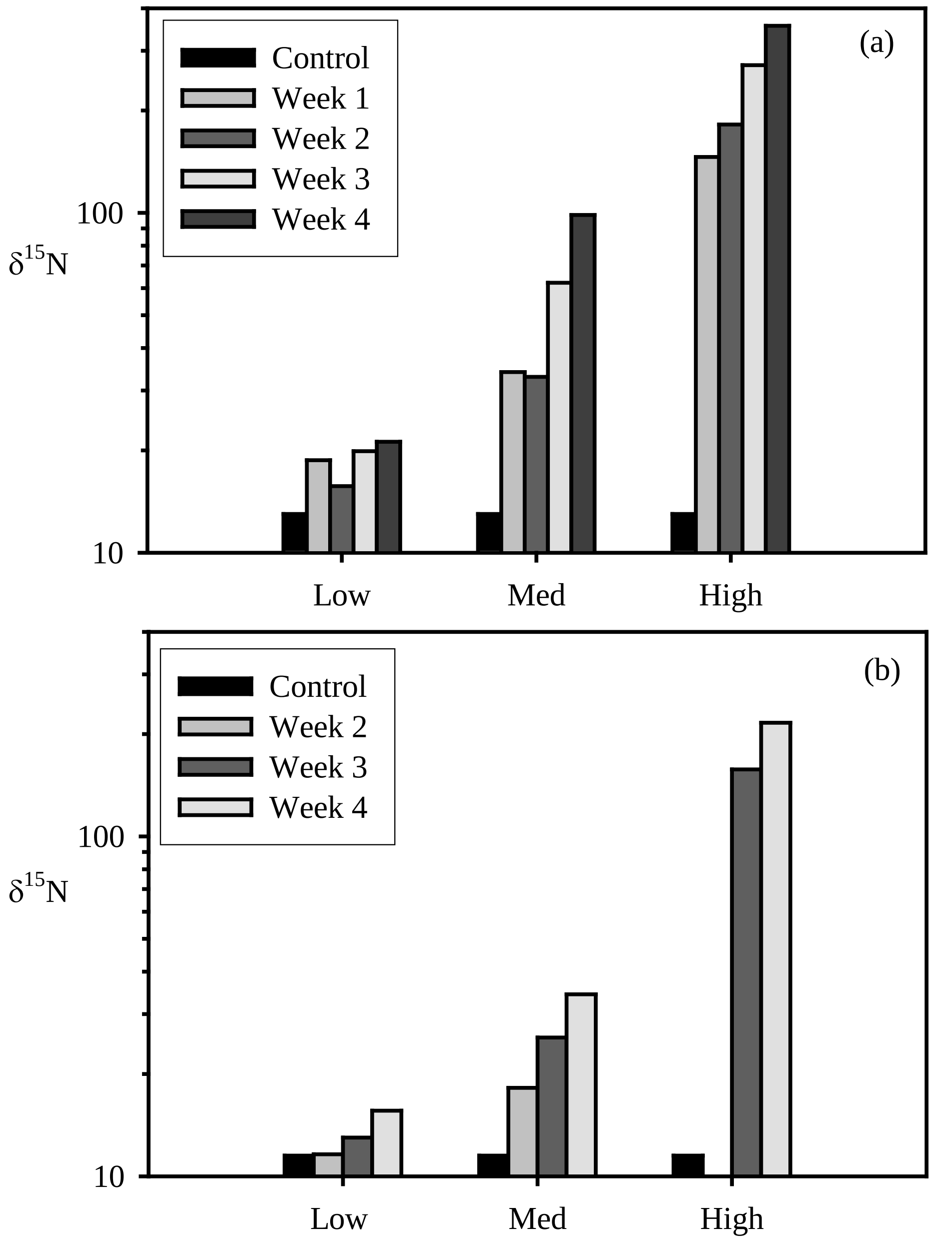

Figure 15. ^{15}N enrichment of *Pimephales notatus* (a) fed ^{15}N enriched algae and ^{15}N enrichment of *Micropterus salmoides* fed enriched *Pimephales notatus* (b).