DIET AND MESOHABITAT ASSOCIATIONS OF THE THREATENED SAN MARCOS SALAMANDER (EURYCEA NANA)

THESIS

Presented to the Graduate Council of
Texas State University-San Marcos
in Partial Fulfillment
of the Requirements

for the Degree

Master of SCIENCE

by

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San Marcos, Texas
May 2010

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ACKNOWLDEGEMENTS

This project may have my name on it, but without the help of my major advisor, Dr. Weston H. Nowlin and the support of my family and friends there would have been no way that I would have been able to accomplish this task. I particularly would like to thank Dr. Nowlin for his patience and support during my time at Texas State during my graduate career and my undergraduate career. Furthermore, I would like to thank Dr. Bonner for the continued help that he has given me over the years. With their guidance I have been able to learn my limitations and surpass them. In addition, I have enjoyed getting to know Joe Fries and Dr. Alexander over the last five years. During my time with them I have acquired many skills and techniques that are now in my "toolbox". I would also like to take this time to thank the other graduate students that have steered me through some of the hard times and the good times; you know who you are.

Additionally without the support and advice that I received from Carol Ramirez (my mother), my Tia Judy, and Peter H. Diaz Sr. I would have been lost. During my time at Texas State I also met the newest addition to the Diaz family, my love, Kirsten Sheyne Collins. Over the last 4 years we have come to understand each other and love one another. In addition, we are expecting our first child, sometime in July. Thank you Honey for always standing by me and allowing me to float back and forth between my work and us.

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The Endangered Species Act was created to aid in the conservation and protection of species under threat of extinction through all or part of their range. Data regarding habitat associations and dietary needs are required for the efficient recovery and maintenance of endangered or threatened species populations. The San Marcos salamander (*Eurycea nana*) is a spring-associated organism that exhibits a geographic range limited to the headwaters of the San Marcos River in central Texas, USA. The USFWS and the state of Texas currently list the SMS as threatened and its designated critical habitat includes the headwaters (Spring Lake) and the first 50 m of the river. The present study determined mesohabitat associations and the trophic ecology of the San

Marcos salamander in its critical habitat. San Marcos salamander habitat associations were determined over a 1-year period and it was determined that San Marcos salamanders are associated with mesohabitats containing cobble and gravel substrates with coverage of *Amblystegium* and filamentous algae. In addition, these mesohabitats account for about 14% of the area within the designated critical habitat. To examine the trophic ecology of the San Marcos salamander, gut contents were collected from salamanders and invertebrate samples from the lake and river were collected. Dietary analyses suggest that the San Marcos salamanders in Spring Lake and the San Marcos River are generalist predators of aquatic invertebrates and the composition of their diets closely follows temporal changes in the invertebrate community. I conclude that due to the generalist and flexible diet of the San Marcos salamander, conservation and recovery issues related to the diet and food availability is likely to be a less substantial issue than mesohabitat availability and quality.

CHAPTER I

DIET AND MESOHABITAT ASSOCIATIONS OF THE THREATENED SAN MARCOS SALAMANDER (EURYCEA NANA)

INTRODUCTION

The Endangered Species Act (ESA) was created to aid in the conservation and protection of species under threat of extinction (16 U.S.C. 1531 et seq.). In addition to the ESA, a document deemed "recovery plan" is used to promote the conservation of endangered or threatened species by providing biological data and threats associated with each species (Hoekstra et al. 2002), and serves a guideline to recover and conserve listed species (United States Fish and Wildlife Service (USFWS) 1995). As of March 2010, of the 614 animals and 750 plant species listed as threatened or endangered, only 22 species have been recovered (USFWS 2001c; Hoekstra et al. 2002). Although funding for recovery plans is limited, it is imperative to determine and maximize efforts to remove species from the list (Lawler et al. 2002). In particular, understanding the ecology of threatened or endangered species is critical to preserve and manage these populations (Tumlinson et al. 1999). Thus, data regarding critical biological information, such as habitat associations and dietary needs should be added to existing recovery plans to augment more efficient recovery (Harvey et al. 2002; Seminoff et al. 2002).

Many threatened or endangered aquatic species are associated with spring ecosystems (Sada et al. 2005). The abundance and distribution of spring-associated organisms frequently depend on the physical and biological stability of their environment

(Holsinger and Longley 1980; Humphreys 2006). Spring-fed aquatic ecosystems generally display consistent hydrologic and physiochemical characteristics (Roca and Baltanas 1993), and ecologists have hypothesized that this constancy has led to local adaptation of fauna in spring-influenced ecosystems (Hubbs 1995). Currently, many spring-associated species experience habitat degradation from human activities, including decreased flows due to ground water extraction and introduction of non-native species (Bowles et al. 2006) and these stresses have led to extirpation of native spring-associated species (Strayer 2006). The San Marcos salamander (SMS), *Eurycea nana*, is a spring-associated organism with a range limited to the headwaters of the San Marcos River in central Texas, USA (Chippindale et al. 1998). The USFWS and the state of Texas currently list the SMS as threatened (USDI 1980), and the designated critical habitat of the SMS includes the headwaters of the river (Spring Lake) and the first 50 m of the river below a dam at the end of Spring Lake (USFW 1996) – this area is their entire range. Critical habitat has the physical and biological features required for the conservation of a listed species (ESA 1973).

The SMS was described by Bishop (1941) and its ecology has been the focus of numerous studies. Tupa and Davis (1976), Nelson (1993), and Lucas (2006) examined SMS population sizes in the headwater region of the San Marcos River. Tupa and Davis (1976) additionally conducted observations on habitat associations of the SMS within the upper headwaters of the San Marcos River and concluded that SMS were most commonly observed within the aquatic moss *Amblystegium* sp. along the 40-m area on the northwestern side of Spring Lake, typically in close proximity to spring openings. Their findings subsequently led other researchers to examine underlying factors for these habitat associations in Spring Lake and the upper San Marcos River. Berkhouse and Fries (1995) noted that SMS became quite active at temperatures >29°C and that elevated thermal regimes (> 35° C) caused ecological mortality in juveniles and adults. This suggests that SMS exhibit thermal tolerances linked to the spring-influenced cooler waters of the upper San Marcos River. Additionally, in a laboratory study, Fries (2002) determined that SMS prefer relatively slow flowing conditions (1 cm/s). These results suggest that SMS exhibit physiological constraints characteristic of spring-associated organisms given in Sada et al. (2005) and that SMS are likely to prefer habitats within the upper San Marcos River which have numerous spring openings. Although field observations and lab studies have suggested specific mesohabitats SMS may be associated with, there has been no systematic and quantitative examination of mesohabitat associations of SMS in its natural habitat.

There are limited data on trophic ecology of SMS in their natural habitat and these are qualitative in nature and not specific as to prey selectivity or preferences. Tupa and Davis (1976) examined stomach contents of SMS in Spring Lake and reported that Chironomidae larvae and amphipods constituted a majority of stomach contents. Studies examining diets of other species of *Eurycea* generally have concluded that they are generalist predators of aquatic invertebrates (Pentraka 1984; Muenz et al 2008). For example, *E. bislineata* is a generalist predator consuming larval chironomids, isopods, larval mayflies, and dytiscid larvae (Pentraka 1984). *Eurycea cirrigera* also is largely a generalist predator, but exhibited some preference for tanypodin chironomids (Muenz et al 2008). It is unknown whether SMS displays preference or avoidance for prey in its natural habitat or, if found, prey preference varies spatially. Currently, the San Marcos

National Fish Hatchery and Technology Center (SMNFHTC), USFWS, can successfully rear the SMS in captivity, but information on preferred food items may allow for improved breeding efforts and stewardship of captive populations of the SMS.

The objectives of my study are to determine the mesohabitat associations and the trophic ecology of the SMS, in the upper San Marcos River. This information will allow for better stewardship of the SMS in its natural habitat, improved maintenance of captive SMS populations, and add important biological data to the SMS recovery plan. I hypothesize that SMS will exhibit habitat preferences for areas around spring openings with the presence of *Amblystegium*. In addition, I hypothesize that SMS are generalist invertebrate predators, but I do not specifically predict that SMS display preference or avoidance for specific prey items found within their natural habitat.

MATERIALS AND METHODS

Study Site

The upper San Marcos River, including Spring Lake, is located in Hays County, central Texas. The lake and upper river are almost exclusively supported by groundwater spring flows from more than 200 individual spring openings which arise from the upper portion of the Spring Arm of the lake (Spring Arm of the lake, Zones 1-3,Figure 1) and flow from the San Antonio portion of the Edwards Aquifer. In contrast, the Slough Arm (Figure 1) is largely fed by surface runoff from the ephemerally flowing Sink Creek. Water flows down the lake and over two dams to the upper San Marcos River. Damming was done in 1849 to power a gristmill; the impoundment, Spring Lake, did not exist prior to this and early descriptions of the headwaters describe it as a stream which on average was approximately 20 m wide and 1 m deep (McClintock 1846; Kimmel 2006).

Study Design

I divided the salamanders' natural habitat into four zones, based upon hydrology, to examine mesohabitat associations and trophic ecology of the SMS (Figure 1). The first three zones are within Spring Lake. Zone 1 encompasses the first 240 m of the spring arm of Spring Lake and contains the highest density of spring openings. Zone 2 extends 180 m to where the Slough Arm of the lake converges with the Spring Arm of the lake. Zone 3 encompasses the remaining 150 m of the lake and contains relatively few spring openings. Zone 4 begins at the dam at the outflow of the lake and ends 50 m downstream. Thus, all four zones examined in this study encompass the SMS entire critical habitat. I did not include the Slough Arm of the lake in my sampling design because its hydrology and water quality are quite different from the Spring Arm of the lake and SMS have never been observed in the Slough Arm portion of the lake.

Assessment of Mesohabitat Associations

Each zone was surveyed for SMS mesohabitat associations five times (May, July, August, November, and December) in 2008. Mesohabitat associations were assessed using a modified random sampling design nested within each of the four sampling zones (Nielson et al 1983). In addition, all samples were divided equally between day and night to determine if diel foraging differences existed in SMS. I define mesohabitat as visually distinct habitats, following Pardo and Armitage (1997). For example, relatively small areas (several square meters) with clearly differing vegetation types and benthic substrates would be classified as different mesohabitats. Transects were run every 15 m in Spring Lake and the upper San Marcos River below Spring Lake dam, and all transects were perpendicular to the lake and river channel. Using pairs of SCUBA divers, a 1-m^2 quadrat was placed on the substrate at randomly generated points along each transect in each zone. I examined an initial set of 50 quadrats throughout the entire Spring Arm and the first 50 m of the San Marcos River below Spring Lake dam in May 2008. Data from this preliminary mesohabitat analysis indicated that SMS were only found along the remnant river channel within Spring Lake. Therefore, the remaining quadrats were taken from the remnant river channel within the Spring Arm; the remnant river channel runs though Zones 1, 2, intermittently through Zone 3, and is the total area of Zone 4.

After delineating the remnant river channel, I attempted to ensure equal sampling effort among zones by basing the number of quadrats sampled within each zone on the surface area of each zone; this was done so that approximately the same proportional area inside each zone was sampled. For each quadrat, macrophytes were identified to genus, and the percent cover of each macrophyte species and dominant substrate type were visually estimated. Substrate types were classified using a modified Wentworth classification scheme (Cummins 1962, Nielsen and Johnson 1989) and included boulder, cobble, gravel, sand, and mud/silt mix. At each quadrat, I noted the presence of spring openings and measured water depth (m). Dissolved oxygen (DO; mg/L), pH, temperature (ºC), conductivity (μS/cm), and salinity (ppt) also were recorded at each quadrat using a YSI sonde (Survey 4 sonde). Immediately after recording these data, an active search for SMS by the pair of SCUBA divers was conducted inside each quadrat for a 5-minute period by turning over rocks and debris. While searching, any SMS inside the quadrat were quickly captured using aquarium nets. San Marcos salamanders captured during the mesohabitat association surveys were used for diet analysis (see Diet and Prey Electivity below).

Diet and Prey Electivity

After mesohabitat characteristics were determined and SMS had been removed from each quadrat, invertebrate samples were taken to enable calculation of prey electivity. To sample invertebrates inside the 1-m^2 quadrat, a 0.5-m x 0.5-m modified dip net was placed in a downstream position in a corner of the quadrat. The substrate inside the sub-sampled area was agitated continuously for 2 minutes towards the net opening to collect invertebrates, similar to the method used for a Hess sampler. All invertebrates were preserved in 95% ethanol and identified to the lowest practical taxon (Thorp and Covich 2001; Merritt and Cummins 2009).

Traditionally, diet composition and prey electivites of salamanders, have been determined by sacrificing individuals and examining gut contents (Burton 1976; Gunzberger 1999). Given the listed status of the SMS, I made an attempt to prevent the sacrifice of individuals for gut content analysis and examined the efficiency of a gastric lavage technique used for other species (Legler 1977, Legler and Sullivan 1979, Culp et al. 1987, Forbes and Lupus 1993) on hatchery-reared SMS at the SMNFHTC. This took place before any field sampling occurred. Unfortunately, I determined that gastric lavage was ineffective at collecting gut contents of SMS. Therefore SMS were sacrificed to obtain gut contents.

During the mesohabitat assessment, a total of 104 SMS were collected from quadrats within Spring Lake. Federal and state collection permits allowed me to sacrifice a total of 150 SMS so additional quadrats were sampled in probable SMS habitat to collect an additional 46 individuals for gut content analysis. Data from these additional non-randomized quadrats were not included in mesohabitat association analyses.

Upon removal from a quadrat, each individual SMS was pithed and placed into a vial containing 95% ethanol. Length (snout to tail and snout to vent) and gender of all SMS were recorded. The entire digestive tract, a non-convoluted straight tube, was removed. Prey items were collected, and identified to the lowest practical taxonomic level (Thorp and Covich 2001; Merritt and Cummins 2009). In general, prey items from guts were intact because SMS is an engulfing predator that does not greatly masticate prey items (Pers. Obs.). In addition, I observed limited deterioration of hard structures (e.g., exoskeletons, shells) as they moved from foregut to hindgut of SMS.

Data Analyses

Mesohabitat Analysis

Available mesohabitat types in SMS natural habitat were determined by principle component analysis (PCA) using the software package R. In the PCA analysis, the abiotic characteristics of each quadrat including pH, DO, conductivity, and temperature were included, along with percentage cover of all vegetation types and the dominant substrate type. All data were z-transformed prior to analysis. Canonical correspondence analysis (CCA) was used to examine mesohabitat associations of SMS using Canoco for Windows version 4.5. For the CCA, I excluded several abiotic variables, including DO, conductivity, temperature, and pH. This was primarily done to prevent an inverted matrix in the CCA (McCune and Grace 2002), but there also were substantial ecological reasons, which supported their exclusion, in order to ascertain mesohabitats associations. Although these variables varied significantly ($\alpha \leq 0.05$) among zones (all Kruskal-Wallis tests $p \leq 0.001$), the magnitude of differences in DO (range = 5.65-7.87 mg/L), temperature (range = 21.68-22.50 ºC), conductivity (range = 0.57-0.58 μS/cm), and pH (7.43-7.67) among zones (Table A-1) were in reality not that large and great enough to influence the abundance and distribution of SMS. Abiotic variables in CCA analysis were dominant substrate type and percent cover of all vegetation types while biotic variables were densities of aquatic invertebrates and salamanders for each quadrat. To determine the significance of the CCA model, Monte Carlo permutations were done (999 permutations).

Diet Analysis

To examine the diet of SMS, Kruskal-Wallis Tests were conducted to compare the percent composition of the various macroinvertebrate groups collected in the guts of SMS of different sexes, and SMS collected in the day versus in the night. To examine if SMS diet differed temporally, I compared the percent composition of the various macroinvertebrate groups in SMS guts in summer (May, July, and August dates combined) to individuals collected in the winter (November and December dates combined). These analyses were conducted only for the top five taxa found in the diet and the environment: *Hyalella* sp., *Stenocypris* cf. *major*, *Cypria opthalmica*, *Chironomini*, *Chimarra* sp., Chydoridae. In several cases, the number of prey items in SMS guts collected on a given date was too small to analyze (≤10 SMS). All percentages were arcsin-transformed prior to analysis. In addition, the percent composition and frequency of occurrence for the diet of SMS were determined following Murphy and Willis (1996).

To examine electivity of prey items, Strauss's linear index (L_o) was calculated, using the equation $L_0 = r_i - p_i$, where r_i is the relative abundance of each prey item in the digestive tract and p_i is the relative abundance of each prey item in the community (Strauss 1979; Strauss 1982). Strauss index values range from +1 to –1, with values close to +1 indicating high selectivity, values close to –1 indicating avoidance, and values close to zero indicating no selection. This index was selected because of its ability to accommodate dissimilar sample sizes in the gut and in the community (Strauss 1979), and has been used by other studies to analyze prey electivity of other salamander species (e.g., Muenz et al. 2008).

RESULTS

Mesohabitat Associations of SMS

In Zones 1 and 2, the remnant river channel is pronounced and created a corridor of sand, gravel, cobble, and boulders with numerous spring openings running the length of these zones. However, in Zone 3, mud and silt comprised the majority of the area, with relatively small patches of gravel associated with the remnant river channel running down the length of the zone. In Zone 4, the habitat changes from more lentic to lotic in nature with habitat dominated by large boulders and cobble with no obvious spring openings. Principle component analysis revealed distinct separation between quadrats sampled in portions of the natural habitat (Figure 2). PCA axis I and II (PC I and PC II) cumulatively explained 25% of the variation among sites. PC I clearly separated sites sampled in Spring Lake from those in the San Marcos River below Spring Lake dam. Negative loadings along PC I were DO (-0.43), temperature (-0.38), the macrophyte

Hydrocaudal sp. (-0.21) and gravel (-0.21), whereas positive loadings along PC I were water depth (0.36), presence of spring openings (0.21), the macrophyte *Myriophyllum* sp. (0.17), and *Amblystegium* (0.15). Although PC I displayed the gradient from the lake to the river, PC II described a gradient of habitats within Spring Lake. The negative loadings along PC II were mud and silt (-0.52), with the macrophytes *Ceratophyllum* sp. (0.31), *Sagittaria* sp. (-0.20), and conductivity (-0.15), to positive loadings of *Amblystegium* (0.39), the presence of spring openings (0.31), cobble (0.25), and algae (0.24). PC II exhibits a gradient of mud and silt and *Ceratophyllum* to *Amblystegium* and cobble with the presence of spring openings. The positive loadings on PC II are indicative with the habitats found largely throughout Zone 1 and the remnant river channel in Zones 2 and 3.

Due to the obvious separation between sampling sites in the lake and the river in the PCA, I assessed the mesohabitat associations in the CCA for the first three zones (i.e., Spring Lake) and the fourth zone (i.e., the river below Spring Lake dam) separately. The first two axes of the CCA accounted for 21% (p = 0.006) of the variation in the Spring Lake community (Figure 3a and b). Of the 21% explained by the model, canonical axis (CA) I explained 39% (p = 0.040), and CA II explained an additional 24% of the total variance in community composition. CA I described an environmental gradient of gravel (0.46), filamentous algae (0.31), *Amblystegium* (0.30) and boulder (0.29) mesohabitats, to mesohabitats composed of mud and silt (-0.66), the macrophytes *Cabomba* sp., (-0.71) and *Sagittaria* (-0.12) (note: bi-plot scores presented). The gradient along CA I separated specific mesohabitats with cobble, gravel, and *Amblystegium* from the majority of mesohabitat within Spring Lake. The environmental gradient along CA II displayed positive loadings of *Myriophyllum* (0.43), *Cabomba* (0.39), gravel (0.22), and spring openings (0.18), while the opposite end of this gradient was represented by meoshabitats composed of *Ceratophyllum* (-0.57), boulder (-0.27), mud and silt (-0.18), and *Sagittaria* (-0.15). CA II represented a longitudinal gradient of mesohabitats along the remnant river channel, ranging from mesohabitats in the remnant river channel in Zones 1, which have more spring openings to mesohabitats farther down in the river channel characterized by boulders and filamentous algae.

There were 133 SMS observed within Spring Lake, with 95 in Zone 1, 34 in Zone 2, and four in Zone 3. On the CCA species bi-plot (Figure 3b), SMS occur in the upper right quadrant of the species plot, indicating that SMS is associated with mesohabitats composed of gravel, cobble, spring openings, and *Amblystegium*. This mesohabitat type is mainly found through Zone 1 and sporadically along the remnant river channel in Zone 2. In addition, four macroinvertebrates (*Stenocypris* cf. *major*, Chydoridae, Turbellaria and *Elimnia* sp.) are associated with this same mesohabitat type. On CA II, the gastropods *Elimnia*, Hydrobidae, and *Gyralus* sp. were associated with mesohabitats containing *Myriophyllum*, *Cabomba*, and gravel. *Palaemonetes* sp. and *Tricorythodes* sp. were associated with mesohabitats containing *Ceratophyllum* and boulder. *Hyalella* sp., the most abundant species in Spring Lake, was near the origin of the CCA species bi-plot, indicating its cosmopolitan distribution across mesohabitats in the lake.

The CCA from the upper San Marcos River below Spring Lake dam explained 55% of the variation in community composition and distribution in Zone 4 ($p = 0.010$) (Figure 4a and b). CA I explained 36% ($p = 0.010$) of the total variance, while CA II explained another 22%. CA I described a gradient of mesohabitats containing

Hydrocaudal sp. (0.67), *Amblystegium* (0.47), cobble (0.48, and *Zizania texana* (0.45) to mesohabitats composed of *Hygrophila* sp. (-0.61), mud and silt (-0.55), *Potamogetan* sp. (-0.48), and *Vallisneria* sp. (-0.20). Positive loadings on CA II were *Amblystegium* (0.82), cobble (0.15), mud and silt (0.9), filamentous algae (0.7) and negative loadings along this axis were *Z. texana* (-0.37), *Heteranthera* sp. (-0.21), gravel (-0.17), and sand (-0.16).

A total of 73 SMS were observed in the upper San Marcos River below Spring Lake dam, and were associated with mesohabitats consisting of cobble, *Amblystegium*, filamentous algae, and relatively low macrophyte cover (Figure 4b). Invertebrate taxa co-occurring with SMS were Turbellaria (flatworms) and *Chimarra* (caddisflies). Similar to my findings in Spring Lake, *Hyalella* ordinated near the center of the biplot in multivariate space, indicating its cosmopolitan distribution across mesohabitats.

SMS Diet and Prey Electivity

A total of 101 SMS from Spring Lake were used for diet analysis (Zone 1 = 62; Zone 2 = 37; Zone 3 = 2) consisting of 26 female, 55 male, and 20 juveniles that ranged in size from 11-79 mm. A total of 493 prey items were identified from the digestive tracts of all SMS from Spring Lake with five SMS having empty digestive tracts. A total of 56 SMS were captured in the San Marcos River below Spring Lake dam (Zone 4), consisting of 19 females, 32 males, and 5 juveniles, with individuals ranging in size from 11-76 mm. A total of 177 prey items were removed from the guts and four individuals had empty guts.

In Spring Lake, a total of 68,336 aquatic macroinvertebrates were collected from 100 quadrats, representing 35 families and 53 genera. *Hyalella* made up 62% of the invertebrate community sampled, followed by the ostrocad, *Stenocypris* cf. *major* (10%), and the dipteran tribe Chironomini (6.64%) (Figure 5a). The invertebrate community demonstrated temporal shifts in Spring Lake for the taxa *Hyalella* ($p < 0.001$), Chironomini ($p < 0.001$), and Orthocladiinae ($p < 0.001$) (Table 1). For the analysis in the river, a total of 10,208 aquatic invertebrates were enumerated. The macroinvertebrate community in the headwaters of the San Marcos River below Spring Lake dam was more diverse than in Spring Lake, having 50 families and 88 genera present. An endemic species of concern (Bowles and Arsuffi 1992) was found exclusively in Zone 4, *Oxyelophila* sp. (Lepidoptera), and this species is only found in Texas and considered rare (Merritt and Cummins 2009). Out of the 45 quadrats sampled, *Hyalella* made up 43%, followed by *Stenocypris* cf. *major* (16%) and *Tricorythodes* sp. (11%) (Figure 5b). There were no significant temporal changes in invertebrate community of the San Marcos River below Spring Lake dam ($p \geq 0.100$; Table 2).

Hyalella were the most frequently occurring taxa in Spring Lake SMS guts (83%), followed by *Stenocypris* cf. *major* (70%), *Cyria opthalmica* (ostrocoda; 49%), and Chironomini (16%) (Figure 6a). Collectively, these taxa composed 84% of the diet for SMS in the lake. The percent composition of the prey items in the diet of SMS closely tracked the frequency of occurrence data (Figure 6b). I detected no significant differences in percent composition of these four invertebrate taxa when I compared SMS captured at night versus day ($p \geq 0.416$) or salamander sexes ($p \geq 0.134$) (Table 3). However, there were significant differences between temporal trends in the percent composition of *Hyalella* ($p < 0.001$), *Stenocypris* cf. *major* ($p = 0.002$), and Chironomini ($p < 0.001$) in the diets of Spring Lake SMS. Additionally, the percent composition of these prey items did not significantly vary within SMS size (total length) (Figure A-1). For the riverine SMS, the ostrocad *Stenocypris* cf. *major* (48%), the caddisfly *Chimarra* (37%), *Hyalella* (26%) and *Cypria opthalmica* (19%) constituted the majority (66%) of their diet (Figure 7a). The percent composition by number again followed the frequency of occurrence data (Figure 7b). For the four invertebrate taxa there were no significant differences in their composition for the diet of SMS collected during the day versus night ($p \geq 0.063$). However, there was a significant seasonal change in the abundance of *Hyalella* in the diet of riverine SMS, with a reduced abundance in the winter months ($p = 0.047$; Table 4). However, it is notable that Gastropods from five different genera made up about 10% of the diet for the SMS that lived below Spring Lake dam in the San Marcos River. Again, percent consumption of prey items was not significant when examined with total length of SMS within the river (Figure A-2).

Strauss's linear index (L_o) indicated there was no apparent electivity or avoidance of prey items in Spring Lake SMS (Table 5). SMS actually showed a slightly negative selection for *Hyalella,* presumably due to the high percentage of this group in all the invertebrate samples ($L_o = -0.08$; $n = 70$). *Stenocypris* cf. *major* had the highest electivity ($L_o = 0.18$; $n = 43$) followed by *Cypria opthalmica* ($L_o = 0.11$; $n = 37$). When temporal changes in the percentage of macroinvertebrate samples are examined with percentages of diet, the shifts in diet appear to be loosely dictated by the community of invertebrates present (Figure 8). Similar to the lake SMS, the riverine SMS showed no significant electivity (i.e., L_0 near zero) for any of the invertebrate taxa (Table 6). Again, as in the lake, temporal changes in the diet composition loosely followed the temporal changes of invertebrate community (Figure 9).

DISCUSSION

San Marcos Salamander Mesohabitat Associations

I observed strong and specific mesohabitat associations of SMS throughout Spring Lake and the river area. SMS generally were found in mesohabitats containing cobble and gravel substrates with coverage of *Amblystegium* and filamentous algae. Association of SMS with cobble and gravel substrates is not entirely unexpected because other species of aquatic *Eurycea* also are associated with these types of substrates (Tumlinson et al. 1990; Bowles et al. 2006). Rock and gravel substrates may create spatial refuges for *Eurycea* spp. (Dundee 1958; Tumlinson et al. 1990) from potential predators such as fish (Barr and Babbitt 2002) and crayfish (Tumlinson et al. 1990; Bowles et al. 2006). *Eurycea* are susceptible to fish predation and sufficient presence of predators may lead to their elimination from habitats (Barr and Babbitt 2002). Crayfish frequently co-occur with aquatic salamanders and may consume *Eurycea* (Tupa and Davis 1976); however, Bowles et al. (2006) determined habitat selection by a closely related salamander species, the Jollyville salamander, *E. tonkawae,* was not influenced by the presence of crayfish.

SMS found within Spring Lake displayed clear associations with mesohabitats composed of gravel, cobble, *Amblystegium,* filamentous algae, and the presence of spring openings. A longitudinal gradient of these mesohabitats occurs within the lake with highest SMS densities in Zone 1, and lowest densities in Zone 3. These mesohabitats were almost exclusively associated with the clearly defined areas of the remnant river channel within the lake. Within the remnant river channel, two main mesohabitat types occur that SMS associated with, the first being gravel, cobble, and *Amblystegium*, and the second with boulder, cobble, and filamentous algae. The closely related *E. tonkawae*, which also occurs in central Texas, also associates with large rocks and sites that were spring-influenced (Bowles et al. 2006). San Marcos salamander associations for these specific mesohabitats in Spring Lake are likely an externality of the conditions in which the SMS first evolved as a species. Prior to impoundment in 1849 the headwaters of the San Marcos River (San Marcos Springs) was largely lotic in nature (McClintock 1846; Kimmel 2006) and past evidence of this condition is still clearly observable in the lake today (i.e., the remnant river channel). The results of this study clearly indicate that, although SMS are found in Spring Lake, they are associated with specific mesohabitats within the lake, which are more representative of lotic conditions.

Results of the mesohabitat analysis in Spring Lake indicate that SMS exhibit a restricted distribution within their designated critical habitat. Preliminary sampling indicated that their distribution was strongly associated with the area in and around the remnant river channel in Spring Lake (see Methods). However, mesohabitats in the remnant river channel constitute a small fraction of the critical habitat within Spring Lake. After completion of my mesohabitat association data collection, I sampled transects, lengthened to encompass the entire breadth of the lake, for additional mesohabitat types. I sampled a total of 566 1-m^2 quadrats to estimate the aerial portions of different mesohabitats within the Spring Arm of the lake. Mesohabitats inside these quadrats were classified according to dominant substrate type (see Methods) and whether low growing (e.g., *Amblystegium* and filamentous algae) or long-stemmed macrophytes were present. Based upon these surveys, I estimated that about 14% of the area within the Spring Arm of the lake would be considered probable mesohabitats which would could be associated with the presence of SMS (i.e., habitats composed of cobble, gravel or boulder substrates, the presence of *Amblystegium* and attached filamentous algae). These findings clearly indicate that although all of Spring Lake is considered critical habitat for the SMS, the actual mesohabitats they probably occupy in the lake is a much smaller portion of the critical habitat.

Much like the lake populations of SMS, riverine SMS were associated with cobble substrates. However, riverine SMS were not as strongly associated with *Amblystegium* and were more frequently found in mesohabitats containing no or low macrophyte abundance (>85% of SMS observed). In addition, my results indicate that SMS were only found in the first 20 m of the 50-m riverine reach in the critical habitat. This, coupled with similar findings in the lake, indicates that SMS populations likely are limited to a small fraction of the area designated as their critical habitat.

San Marcos Salamander Trophic Ecology

Results from this study suggest that the SMS in Spring Lake and the San Marcos River below Spring Lake dam are generalist predators of aquatic invertebrates. Prey electivities indicate that SMS consumed prey items that were the most common in the environment. For example, *Hyalella* is ubiquitous in the lake and the river and on average comprised 26% of the diet for SMS in both environments. Consequently, the cosmopolitan distribution and high abundance of *Hyalella* in both systems led to an electivity value of this prey item to be close to zero. Generalist predator behavior observed in the SMS is consistent with other *Eurycea* throughout the southeastern U.S. (McMillan and Semlitsch 1980; Petranka 1984; Tumlinson et al. 1990; Muenz et. al

2008). In addition, my study found little evidence that diel variation, sex, or salamander size had a substantial influence on the diet composition or prey electivities of SMS. Petranka (1984) found that the prey composition in the guts of *Eurycea bislineata* did not differ between day and night. In conclusion, SMS was an opportunistic generalist predator, showing little electivity for specific prey taxa while primarily consuming benthic prey dwelling in the salamanders' habitat, invertebrates such as ostracads and amphipods which have poor swimming abilities (Thorp and Covich 2001).

I observed that temporal changes in the proportion of prey items in SMS diets closely tracked the temporal changes in the abundance of invertebrate communities. For example, Spring Lake SMS consumed more chironomids in July and August, which coincided with the highest abundance and largest size prior to emergence of this tribe in the community (see Figure 8). Burton (1976) and Caldwell and Houtcooper (1973) similarly found that chironomid larvae composed a greater proportion of the diet of *Eurycea bislineata* during summer when chironomid larval densities are greatest.

Results from my study also indicate that foraging activity and success of prey consumption may partially depend upon visual cues from prey items. SMS are thought to be most active at night (i.e., a largely nocturnal activity pattern) (Thaker et al. 2006). Predominantly nocturnal activity would suggest that visual forging cues would be less important for foraging success in SMS. However, evidence from my study does not entirely support this hypothesis. Although the composition of prey items in the guts of SMS did not significantly differ between SMS captured in the day and night, the overall number of prey items found in SMS guts did significantly differ (t-test; day average = 4.65; night average = 3.54; p = 0.03); SMS captured during the day had a significantly greater number of prey items in their guts than those captured at night. Petranka (1998) stated that *E. cirrigeria* mainly uses visual cues to forage for prey, which may explain the difference between day versus the night analysis for the overall number of prey items in the guts of SMS.

Although diets of SMS in the lake and river were largely similar, there were some notable differences between the diets of SMS in the two habitats due to the inherent abiotic conditions present in each system. For example, in the lake, caddisflies adapted to lentic conditions (*Oxytheria* sp.; Wiggins 1996) were consumed; while in the river they consumed *Chimarra*, *Smicridea* sp., and *Protoptila* sp., that are more associated with a lotic environment (Merritt and Cummins 2009). In addition, I observed differences in the genera of mayflies consumed by SMS in the two systems: the mayfly,*Tricorythodes* sp. was consumed in the lake, and the lotic taxa, *Leptohypes* sp., was consumed in the river.

Conservation Implications

I found that SMS has a distribution limited to a small portion of its designated critical habitat. Critical habitats and the requirements of species associated with these are not well understood because operational definitions, conservation approaches, and the amount of information needed to identify critical habitat are poorly elucidated (Rosenfeld and Hatfield 2006). Rosenfeld and Hatfield (2006) identify the critical information required for the identification and maintenance of critical habitats and include information on organism life histories, the amount of available habitat, the specific recovery targets, if there are habitat-abundance relationships, and the actual amount of habitat required to meet recovery goals. For the SMS, the critical habitat area has been identified, but this study indicates that the SMS uses only a small fraction of this area

(<15%) and that historical anthropogenic alterations to the ecosystem have likely modified the type and proportions of mesohabitats in the historical range of the SMS.

The current distribution of SMS in Spring Lake shows a ‘ghost of disturbance past’ and that its current spatial distribution is likely influenced by anthropogenic pressures (e.g., see Knight and Arthington 2008). Indeed, the creation of the dam in 1849, while undoubtedly adding more wetted area to the San Marcos River headwaters, likely caused the loss of some mesohabitat for SMS though alteration of flow rates and increased siltation. I propose that the successful maintenance and recovery of SMS should include the specific limited mesohabitats inside their critical habitat area.

The SMS is a generalist invertebrate predator and the results from my study indicate that the current population shows no evidence of specialized co-evolved feeding relationships with specific invertebrate taxa. Currently, the USFWS feeds hatchery-reared SMS a diet composed of *Hyalella*, snails, miscellaneous zooplankton, and blood worms. I hypothesize that the hatchery diet is likely adequate and should not present any major issues associated with the captive breeding efforts. In addition, the opportunistic and flexible diet of the SMS indicates that it preys largely upon invertebrate taxa which are found throughout their critical habitat area such as amphipods, ostrocads, and chironomids which are tolerant of a broad array of environmental conditions (Thorp and Covich 2001). Thus, it is likely that conservation and recovery issues related to the diet and food availability for SMS should be less of a concern than issues related to mesohabitat availability.

The conservation and recovery of threatened and endangered species is a large concern and has been the focus of research and management efforts. In North America, a substantial portion of aquatic species are under threat of extinction (Fausch et al. 2002). Here, I show that undertaking research with the goal of understanding basic ecological aspects of a threatened species is a highly productive approach to the maintenance and recovery of that species. Understanding linkages between local or small scale processes (e.g., human effects across the entire geographic range of a species) are critical for setting and meeting recovery and restoration targets (Bond and Lake 2003). Thus, future studies examining the abundance and distribution of imperiled taxa should focus on examining population and ecological interactions at multiple spatial scales (Fausch et al. 2002; Knight and Arthington 2008).

Table 1. Kruskal-Wallis results for Spring Lake showing invertebrate samples analyzed by sample period (May, July, and August combined versus November and December combined). An asterisk notes significant values p-values.

Spring Lake Taxa	Summer Average	Winter Average	p-values
Hyalella	55	72	<0.0001*
Stenocypris	10	7.4	0.1830
Cypria	2.2	1.9	0.5667
Chironomini	8.9	1.1	<0.0001*
Orthocladiinae	3.1	0.3	<0.0001*
Chydoridae	2.2	1.8	0.2857

Table 2. Kruskal-Wallis results for San Marcos River below Spring Lake dam showing invertebrate samples analyzed temporally (May, July and August to November and December). An asterisk notes significant values p-values.

San Marcos River Taxa	Summer Average	Winter Average	p-value
Hyalella	36	25	0.1007
Chimarra	1.1	5.1	0.1378
Cypria	0.1	0.7	0.2997
Stenocypris	9.8	11	0.3293

Table 3. Kruskal-Wallis results for Spring Lake, with diet samples analyzed temporally (May, July and August to November and December). Male and female and day and night samples were examined as a total. An asterisk notes significant values p-values.

Spring Lake Taxa	Average	Average	p-value
Hyalella Diet Seasonally	21	51	0.0001*
Hyalella Diet Day and Night	40	36	0.4405
Hyalella Male and Female	35	51	0.4957
Stenocypris Diet Seasonally	29	17	0.0024*
Stenocypris Diet Day and Night	22	16	0.4160
Stenocypris Male and Female	24	13	0.1348
Cypria Diet Seasonally	14	11	0.1796
Cypria Diet Day and Night	13	12	0.8056
Chironomini Diet Seasonally	11	1.0	<0.0001*
Chironomini Diet Day and Night	4.4	5.6	0.7028
Chironomini Male and Female	5.8	2.6	0.1665

Table 4. Kruskal-Wallis results for San Marcos River below Spring Lake dam, with diet samples analyzed temporally (May, July and August to November and December). Male and female and day and night samples were examined as a total. An asterisk notes significant values p-values.

San Marcos River Taxa	Average (%)	Average (%)	p-value
Hyalella Diet Seasonally	22	5.2	0.0478*
Hyalella Diet Day and Night	18	4.1	0.0639
Chimarra Diet Seasonally	11	10	0.0743
Chimarra Diet Day and Night	20	22	0.9615
Cypria Diet Seasonally	13	3.7	0.0698
Cypria Diet Day and Night	7.9	7.1	0.8276
Stenocypris Diet Seasonally	31	19	0.0991
Stenocypris Diet Day and Night	26	18	0.3150

Table 5. Strauss Electivity Scores (*L_o*) for Spring Lake showing maximum and minimum values with the number of SMS that consumed a specific prey item (n).

Prey Items	L_o	Maximum	Minimum	n
Hyalella	-0.08	0.51	-0.90	70
Stenocypris	0.18	0.97	0.05	43
Cypria	0.11	0.97	0.04	37
Orthocladiinae	0.009	0.072	0.09	3
A. limosa	0.01	0.99	0.33	3
Tricorythodes	0.02	1	0.06	4
Chironomini	0.02	0.56	-0.27	17

Table 6. Strauss Electivity Scores (L_o) for the San Marcos River below Spring Lake dam showing maximum and minimum values with the number of SMS that consumed a specific prey item (n).

Prey Items	L_o	Maximum	Minimum	n
Hyalella	0.05	0.48	-0.40	14
Stenocypris	0.20	0.96	-0.0028	27
Cypria	0.07	0.75	0.16	11
Orthocladiinae	0.04	1	0.25	3
Smicridea	0.02	0.96	0.23	3
Chimarra	0.17	1	-0.26	21
Turberellia	0.02	0.091	-0.29	6

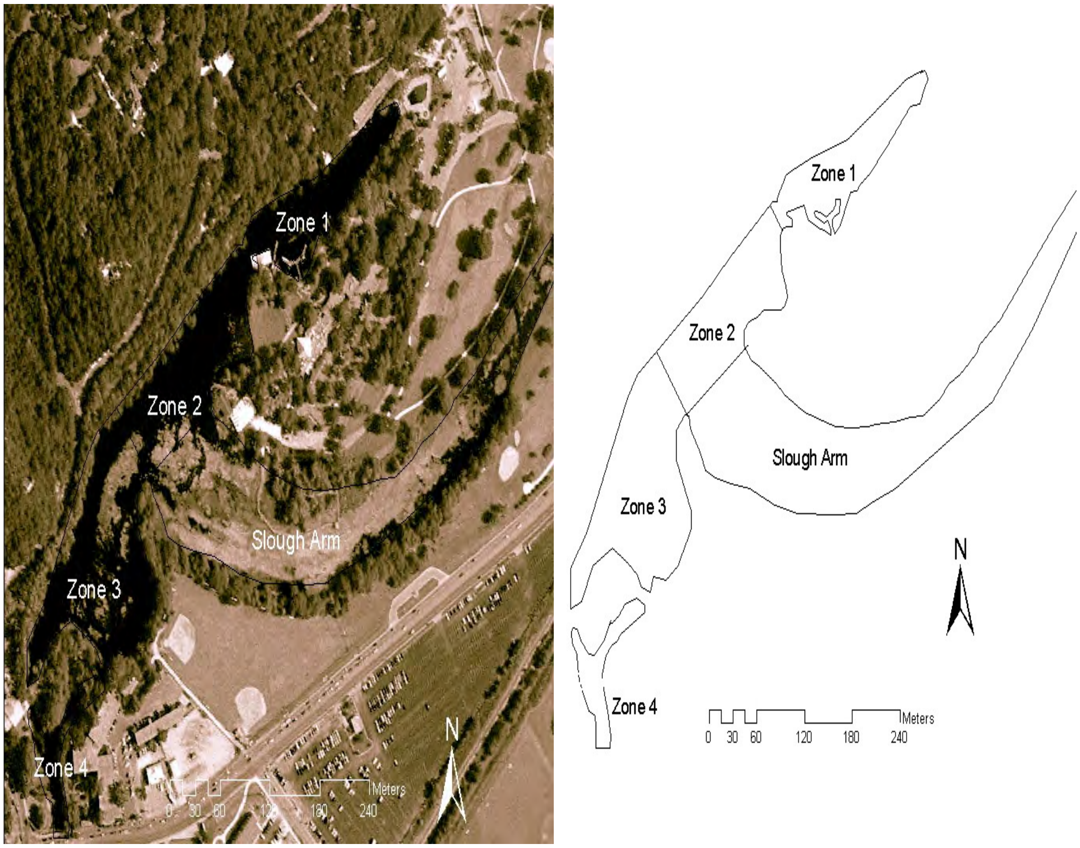

Figure 1. Spring Lake (Zones 1-3) and the upper San Marcos River below Spring Lake dam (Zone 4)

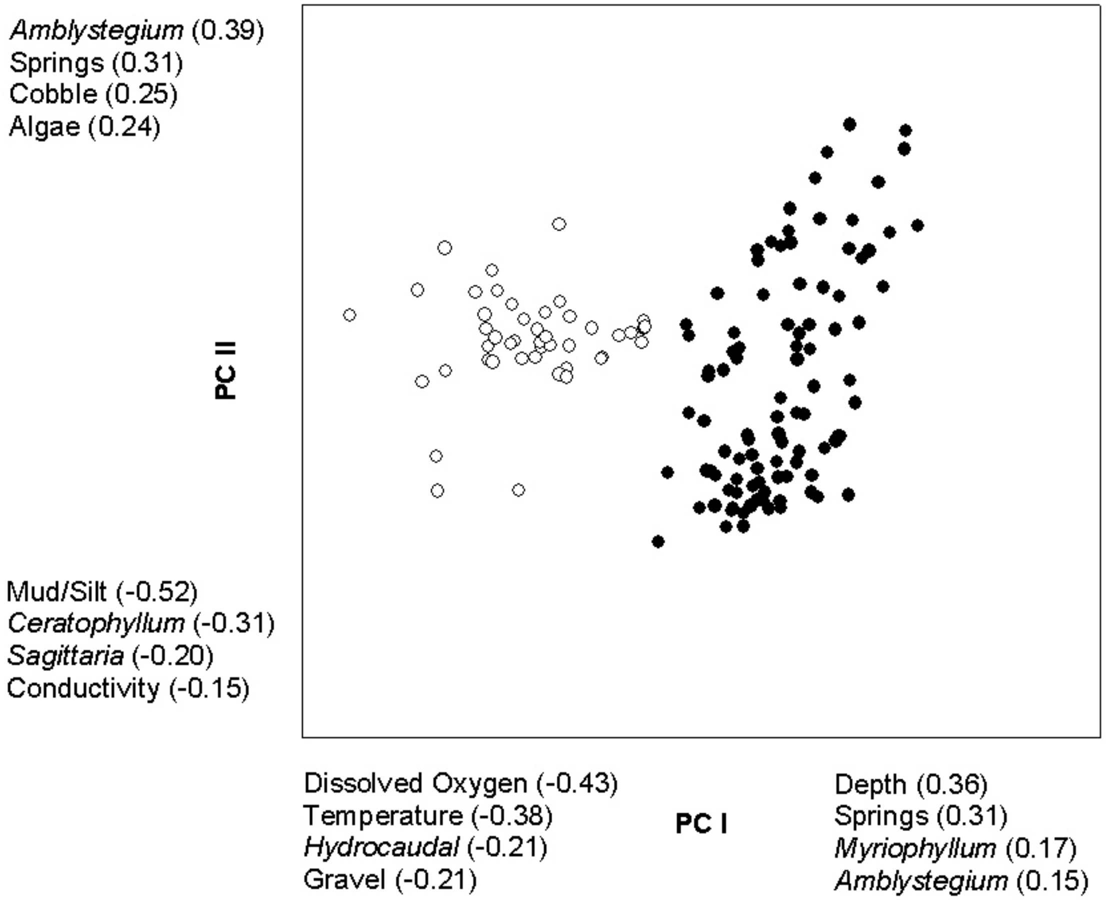

Figure 2. Results of PCA of critical habitat for Spring Lake (●) and the San Marcos River below Spring Lake dam (○) showing biplot scores

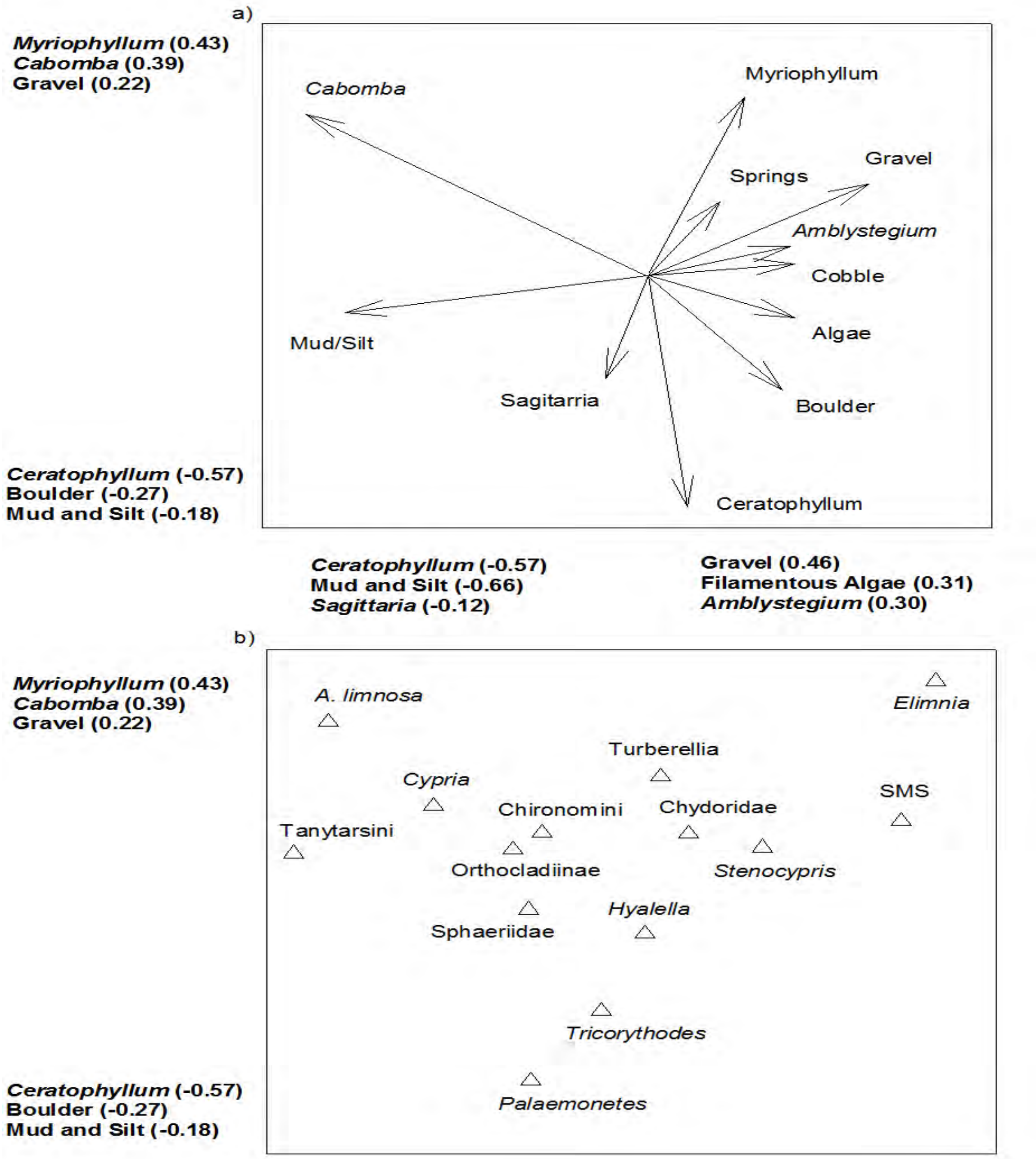

Figure 3. Results of CCA of physical characteristics for Spring Lake with loadings (a) and species biplot (b)

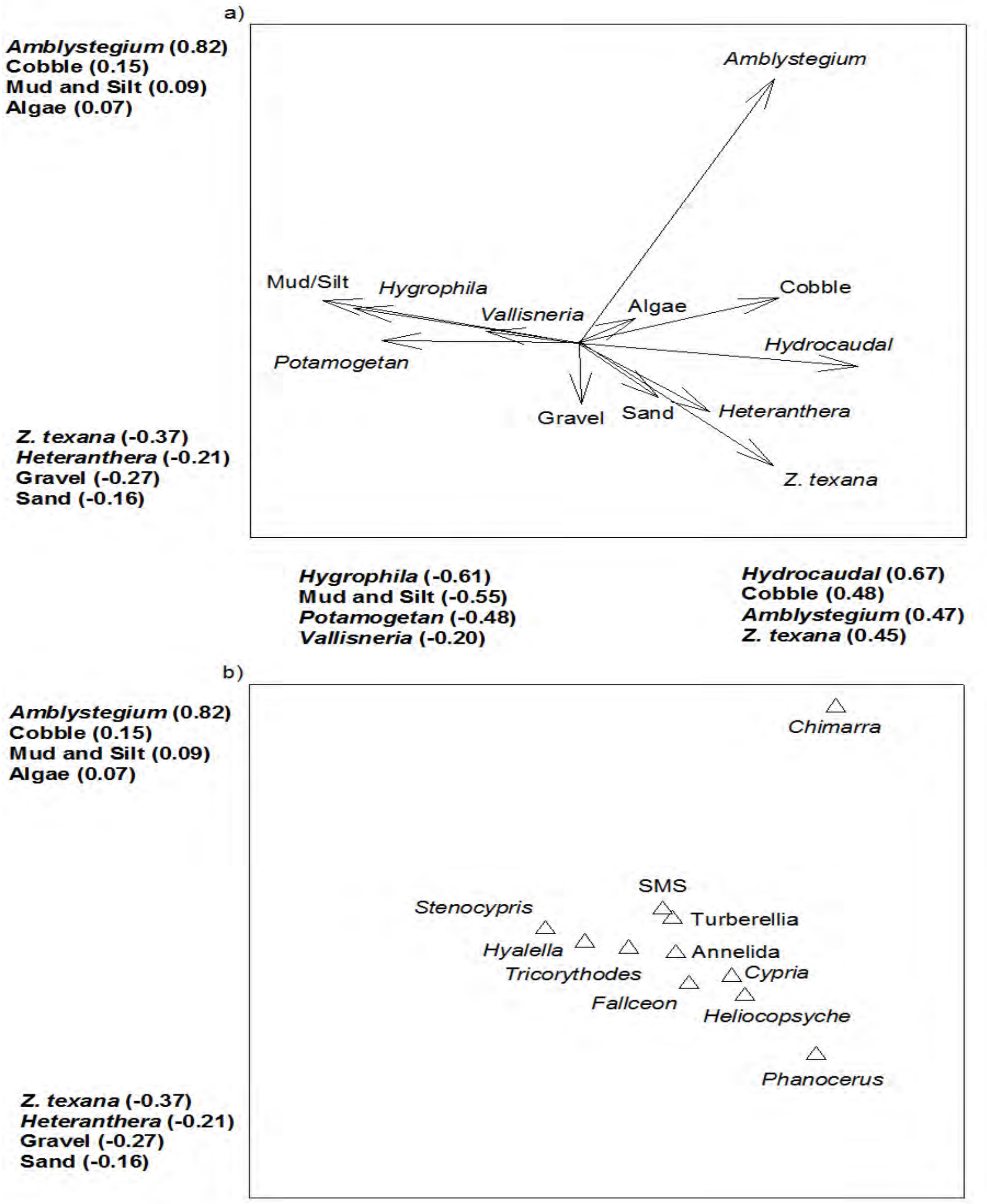

Figure 4. CCA of physical characteristics for the San Marcos River below Spring Lake dam with loadings (a) and species bi-plot (b)

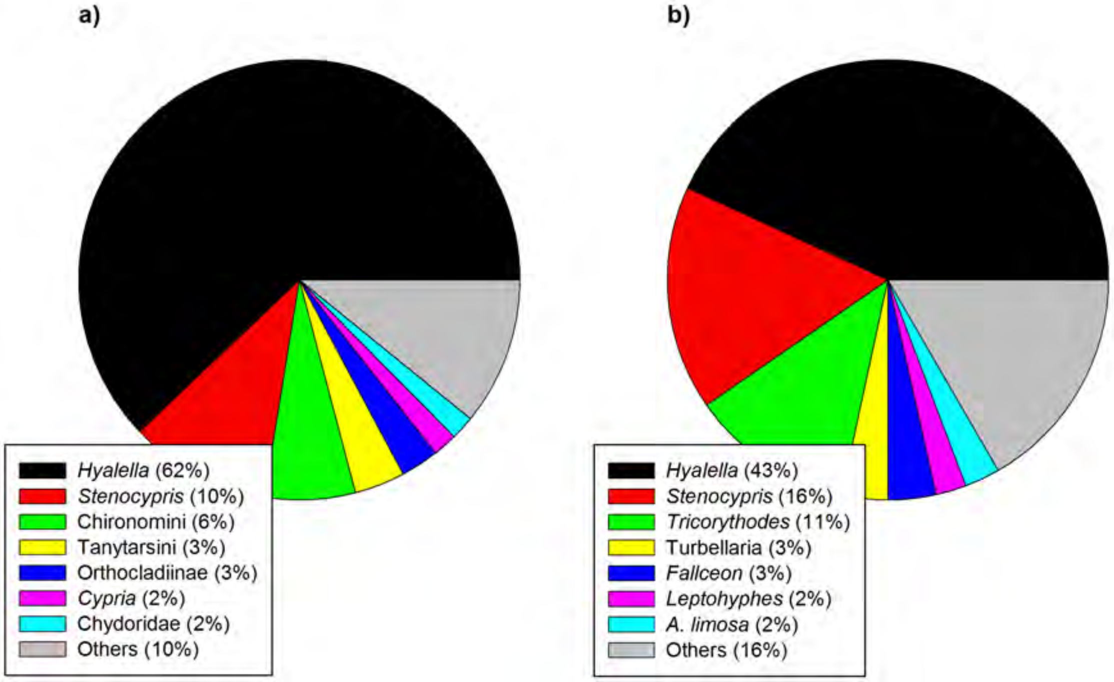

Figure 5. Percentages of the total invertebrates collected from Spring Lake (a) and the San Marcos River below Spring Lake dam (b) showing the most abundant taxa during habitat survey to be used in evaluation of SMS diet

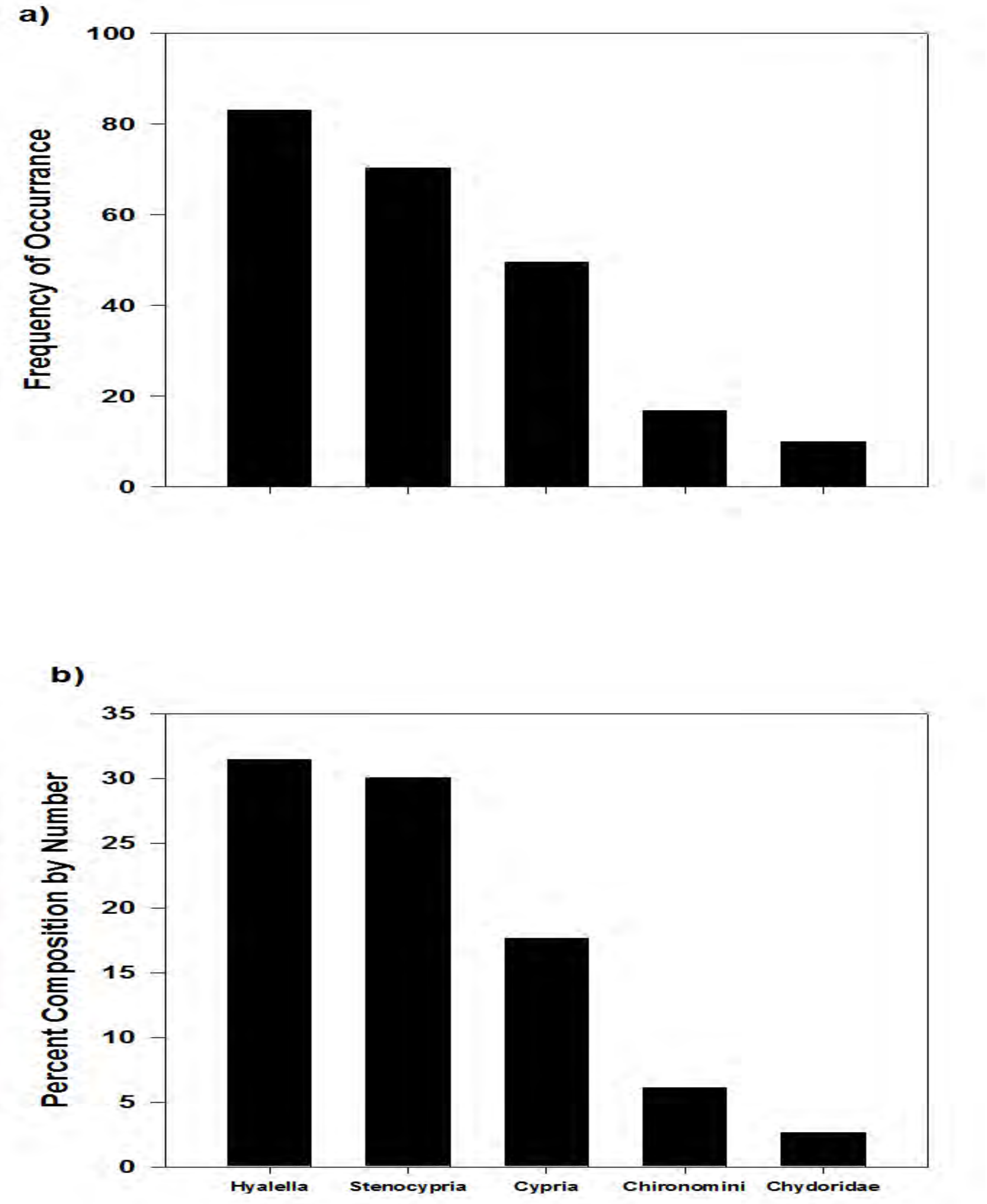

Figure 6. Frequency of occurrence (a) and percent composition (b) in the diet of San Marcos salamanders in Spring Lake

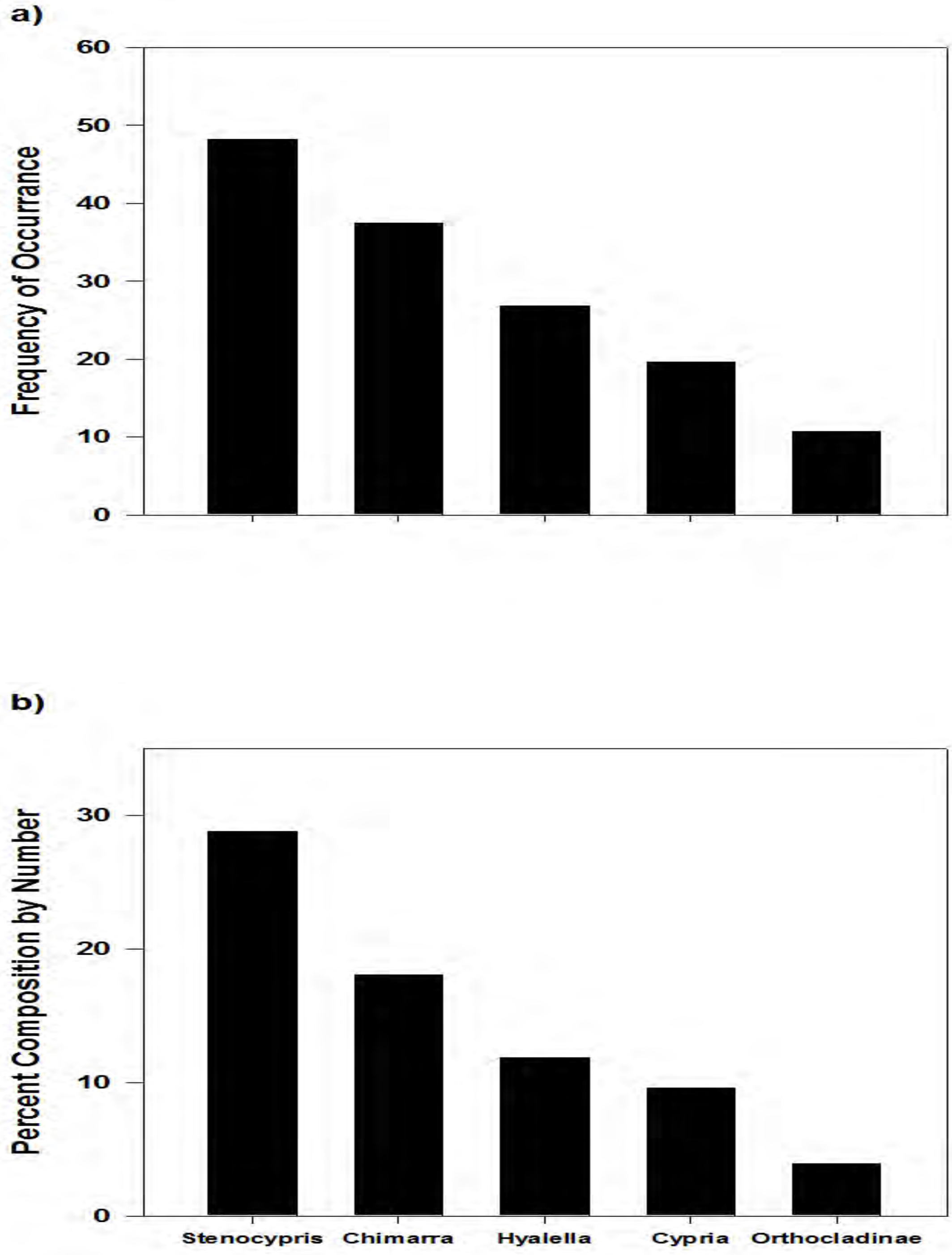

Figure 7. Frequency of occurrence (a) and percent composition (b) in the diet of San Marcos salamanders in the San Marcos River below Spring Lake dam

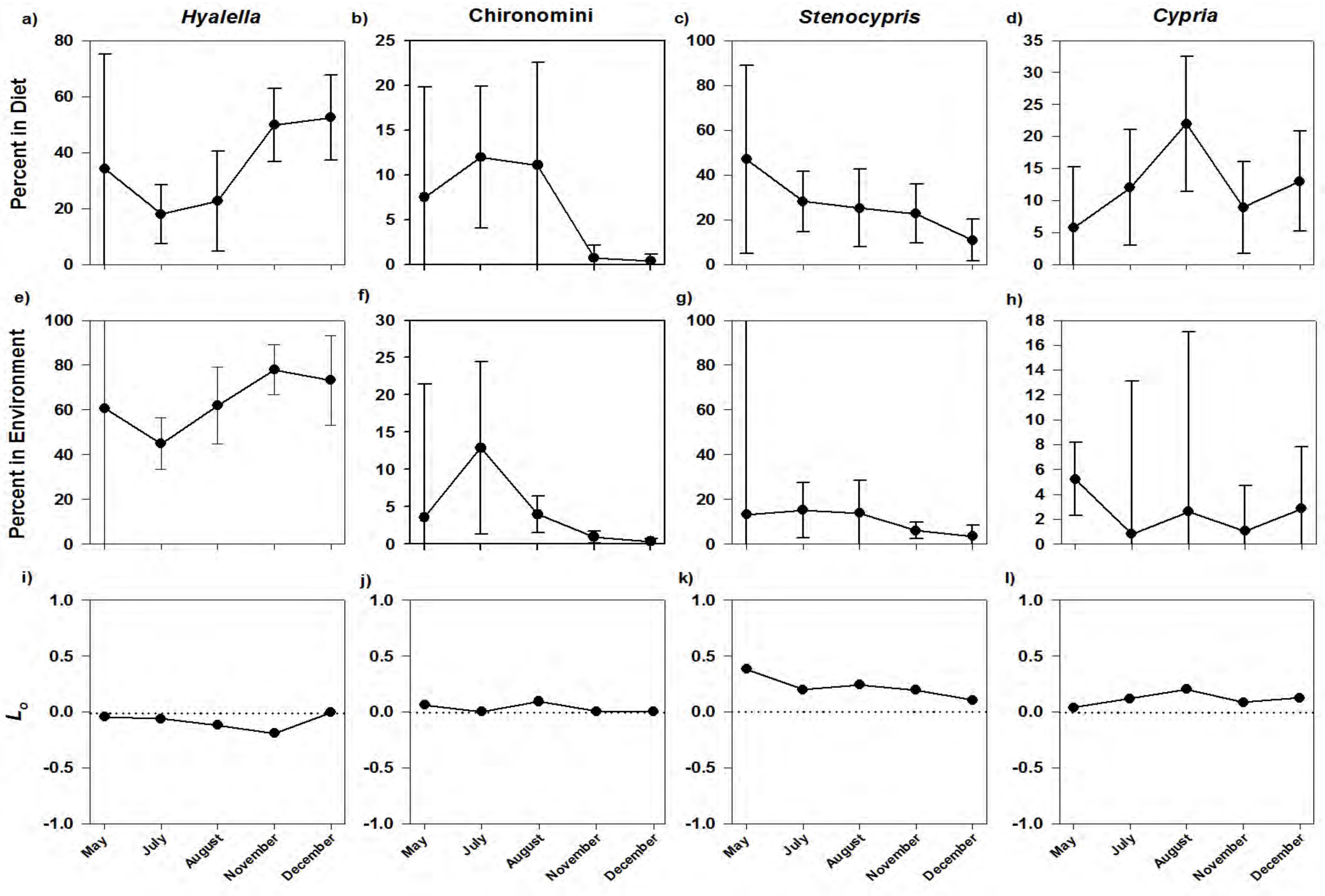

Figure 8. Percent composition in the diet (a-d), environment (e-h), and linear electivity (i-l) of *Hyalella*, Chironomini, *Stenocypris,* and *Cypria* in Spring Lake

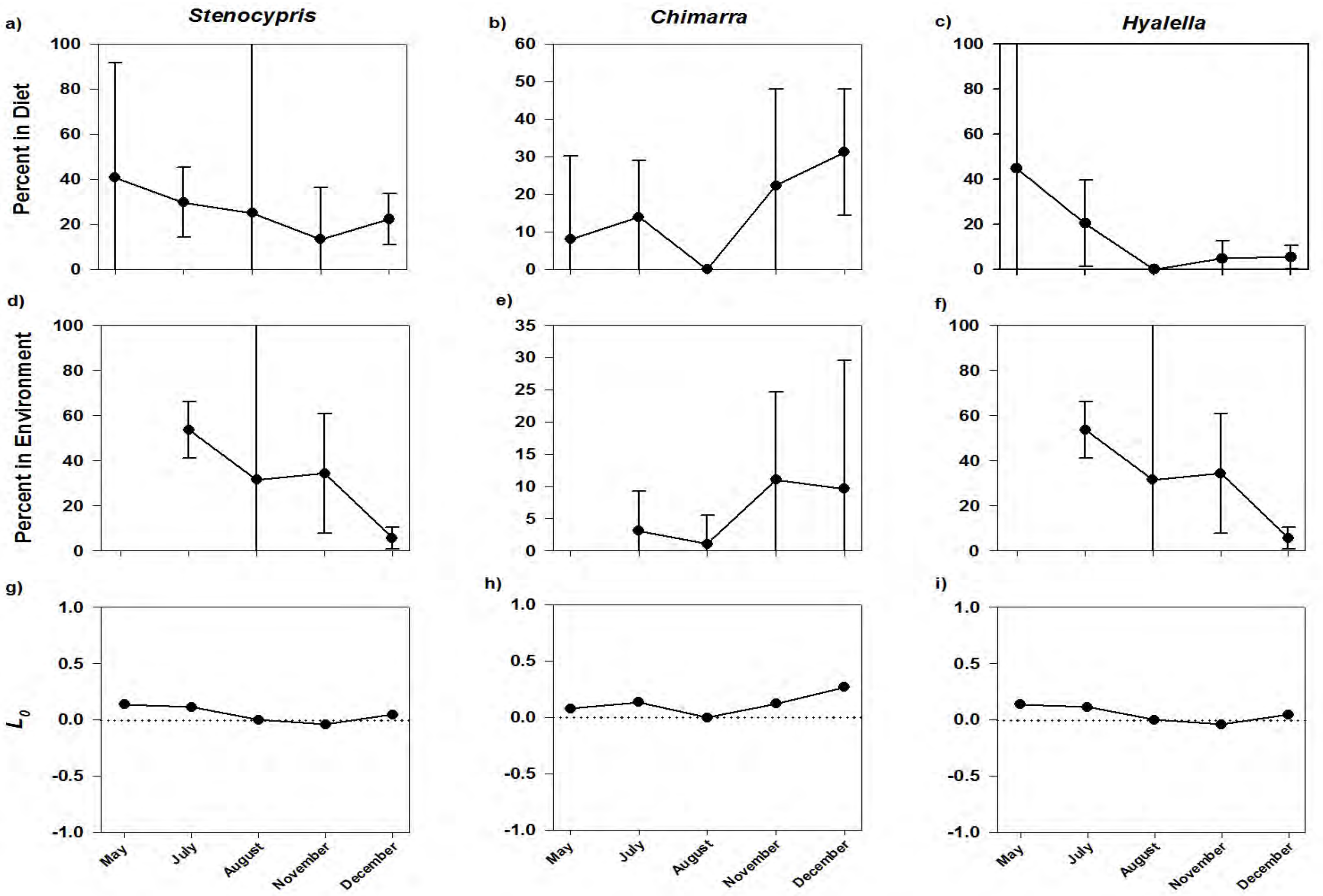

Figure 9. Percent composition in the diet (a-c), environment (d-f), and linear electivity (g-i) for *Stenocypris, Chimarra, and Hyalella* in the San Marcos River below Spring Lake dam

Appendix

Table A-1. Results from the Kruskal-Wallis tests by zone for water chemistry data in the critical habitat with mean

Zone	D.O. (mg/L)	Temperature (C°)	Conductivity (µS/cm)	pH
1	5.65	21.68	0.571	7.67
2	5.85	21.94	0.580	7.63
3	6.40	22.08	0.582	7.43
4	7.87	22.50	0.579	7.55
p	<0.001	<0.001	<0.001	<0.001

TableA-2. Aquatic invertebrates collected from benthic samplin during the study.

Order / sub-order	Family	Sub-Family	Genus
Ephemeroptera	Isonychiidae		*Isonychia sicca*
"	Ephemeridae		*Hexagenia bilineata*
"	"		*Tricorythodes*
"	"		*Leptohyphes*
"	"		*Vacupernius packeri*
"	Leptophlebiidae		*Thraulodes gonzalesi*
"	Baetidae		*Baetis*
"	"		*Baetodes*
"	"		*Callibaetis pictus*
"	"		*C. floridanus*
"	"		*Camelobaetidius variabilis*
"	"		*Fallceon quilleri*
Odonata/Zygoptera	Coenagrionidae		*Argia*
"	"		*Enallagma*
"	"		*Ischnura*
"	"		*Telebasis salva*
"	Calopterygidae		*Hetaerina vulnerata*
Odonata/Anisoptera	Libelullidae		*Brechymorhoga mendax*
"	"		*Erythemis*
"	Aeshnidae		*Anax*
"	Gomphidae		*Hagenius brevistylus*
"	Cordulidae		*Macromia*
Trichoptera	Leptoceridae		*Oecitis*
"	"		*Nectopsyche*
"	Heliocopsychidae		*Helicopsyche piroa*
"	Hydropsychidae		*Smicridea fasciatella*
"	"		*Cheumatopsyche*
"	Hydroptilidae		*Hydroptila*
"	"		*Oxyethira*
"	"		*Ochrotrichia*
"	"		*Leucotrichia saritia*
"	Glossosomatidae		*Protoptila*
"	Hydrobiosidae		*Atopsyche erigia*
"	Polycentropodidae		*Cernotina*
"	"		*Polycentropus*
"	Philopotamidae		*Chimarra*
Lepidoptera	Crambidae		*Paraponyx*
"	"		*Petrophila*

Table a-2—Continued

Order / sub-order	Family	Sub-Family	Genus
"	"		*Oxyelophelia*
Coleoptera	Elmidae		*Hexacylloepus ferrugineus*
"	"		*Phanocerus clavicornis*
"	"		*Stenelmis*
"	"		*Macrelmis*
"	"		*Microcylloepus pusillus*
"	"		*Neoelmis caesa*
"	Dryopidae		*Helichus*
"	"		*Postelichus*
"	Hydrophilidae		*Enochrus*
"	Psephenidae		*Psephenus*
Hemiptera	Naucoridae		*Ambrysus*
"	"		*Cryphocricos*
"	"		*Limnocoris*
"	Gerridae		*Metrobates*
"	Veliidae		*Rhagovelia*
Diptera	Empididae		*Hemerodromia*
"	Stratiomyidae		*Caloparyphus*
"	Ceratopogonidae		*Probezzia*
"	"		*Ceratopogon*
"	"		Culicoides
	"		*Sphaeromias*
"	Chironomidae	Chironomini	*Axarus*
"	"	"	*Xestochironomus*
"	"	"	*Dicrotendipes*
"	"	"	*Chironomus*
"	"	"	*Microchironomus*
"	"	Orthocladinae	*Rheocricotopus*
"	"	"	*Psectrocladius*
"	"	"	*Cricotopus*
"	"	Tanypodinae	*Ablabesmyia*
"	"	"	*Labrundinia*
"	"	"	*Procladius*
"	"	Tanytarsini	*Rheotanytarsini*
"	Culicidae		*Anopheles*
"	Athericidae		*Atherix*
"	Simuliidae		*Simulium*
"	Tabanidae		*Tabanus*

Table A-2—Continued

Order / sub-order	Family	Sub-Family	Genus
"	Tipulidae		
"	Rhagionidae		*Chrysopilus*
Decapoda	Palaemonidae		*Palaemonetes*
"	Cambaridae		
Anomopoda	Chydoridae		
Amphipoda	Hyalellidae		*Hyalella*
"	Crangonictidae		*Crangonyx*
Mesogastropoda	Thiaridae		*Melanoides*
"	"		*Tarebia*
"	Hydrobiidae		*Amnicola limosa*
"	"		*Pyrgophorus spinosus*
"	Pleuroceridae		Elimia
Limnophila	Planorbidae		*Gyralus*
"	"		*Helisoma*
"	Physidae		
"	Ancylidae		*Hebetancylus*
Veneroida	Corbiculida		
"	Sphaeriidae		
Ostrocada			*Darwinula stevensoni*
"			*Cypria ophtalmica*
"			*Chlamydotheca texasiensis*
"			*Stenocypris cf. major*
"			*Physocypria kreapelini*
"			*Pseudocandona cf. stagnalis*
"			*Candona cf. neglecta*
"			*Typhlocypris sp.*
"			*Bradleycypris oblique*
"			*Eucypris cf. pigra*

- -

Table A-3. SMS prey items in critical habitat by count and frequency of occurrence (%), for each system, excluding unknowns.

Taxa		Spring Lake Count	Spring Lake Frequency	San Marcos River below Spring Lake dam Count	San Marcos River below Spring Lake dam Frequency
Baetidae	*Fallceon*	0	0	1	0.99
"	*Callibaetis*	1	0.99	0	0
Leptohyphidae	*Leptohyphes*	0	0	5	7.14
"	*Tricorythodes*	5	4.95	0	0
Naucoridae	*Ambrysus*	0	0	3	5.36
Elmidae	*Microcylloepus*	0	0	2	3.57
"	*Macrelmis*	0	0	2	3.57
"	*Stenelmis*	2	1.98	0	0
Heliocopsychidae	*Heliocopsyche*	0	0	2	3.57
Hydropsychidae	*Smicridea*	0	0	3	5.36
Philopotamidae	*Chimarra*	0	0	32	37.50
Hydroptilidae	*Oxytheria*	2	0.99	0	0
Glossosomatidae	*Protoptila*	0	0	1	1.79
Chironomidae	Pupa	0	0	1	1.79
"	Orthocladiinae	5	7.92	7	10.71
"	Pseudochironomini	7	4.95	0	0
"	Chironomini	30	16.83	0	0
"	Tanypodinae	5	2.97	0	0
"	Tanytarsini	0	0	1	1.79
Simuliidae	*Simulium*	0	0	2	3.57
Asellidae	*Lirceolus*	2	1.98	0	0
Cirolanidae	*Cirolanides*	1	0.99	0	0
Hyalellidae	*Hyalella*	155	83.17	21	26.79
Chydoridae		13	9.90	1	1.79
Copepoda		5	6.93	3	3.57
Ostrocada	*Cypria opthalmica*	87	49.50	17	19.64
"	*Stenocypris* cf. *major*	148	70.30	51	48.21
"	*Darwinula stevensoni*	3	1.98	0	0
Physidae		2	1.98	0	0
Planorbidae	*Gyralus*	0	0	2	3.57
Pleuroceridae	*Elimnia*	6	4.95	0	0
Thiridae	*Tarebia*	0	0	3	3.57

Table A-3— Contiunued Taxa		Spring Lake Count	Spring Lake Frequency	San Marcos River below Spring Lake dam Count	San Marcos River below Spring Lake dam Frequency
Hydrobiidae	*Pyrgophorus spinosus*	0	0	4	5.36
“	*Amnicola limnosa*	3	8.91	6	10.71
“	Phreatic A	1	0.99	0	0
Ancylidae	*Hebatancylus*	0	0	2	3.57
Hydracarina		2	1.98	0	0
Turberellia		3	8.91	6	10.71
Fish larva		2	0.99	0	0
Seed		1	0.99	0	0
Unknown		2	2	0	0

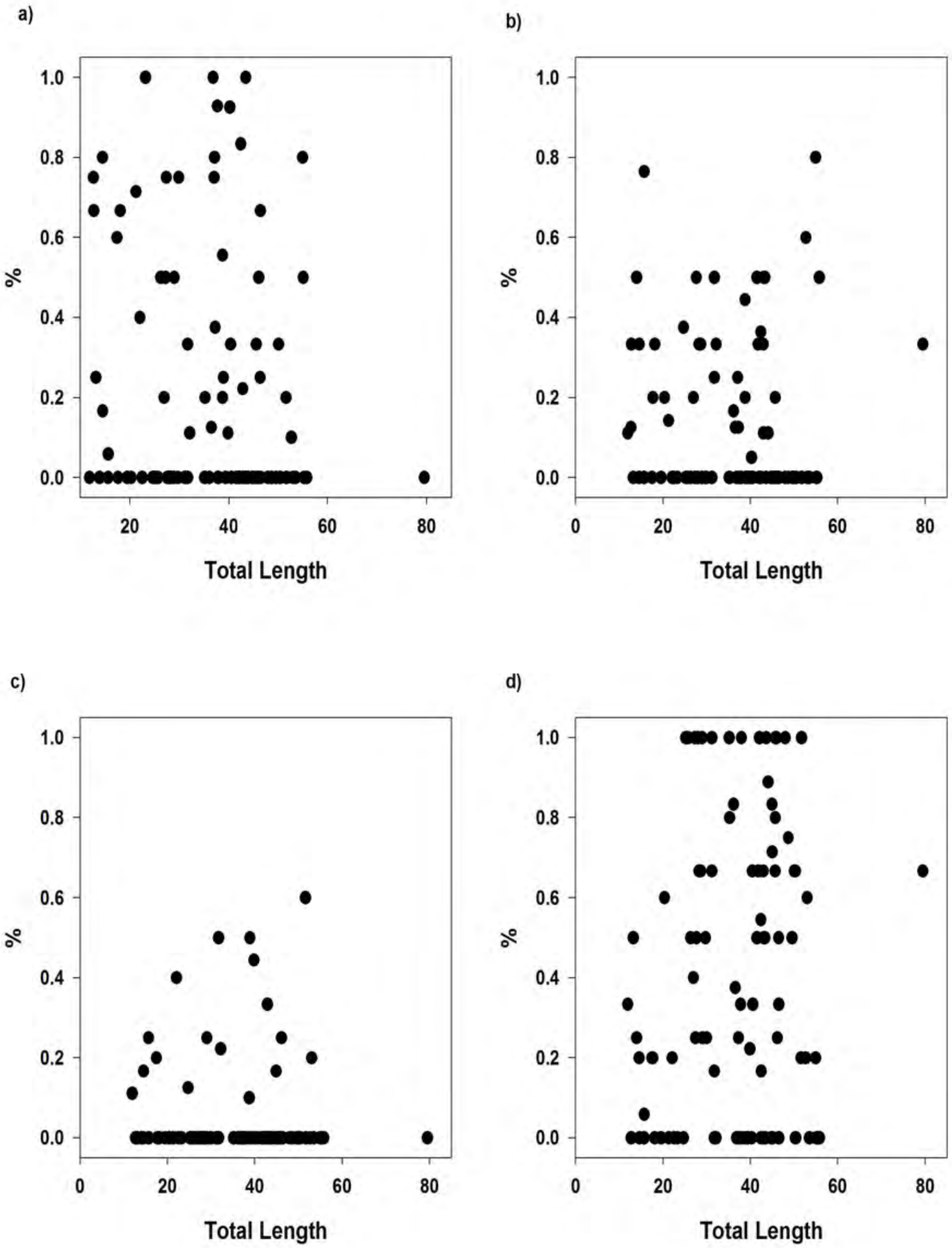

Figure A-1. Total length of SMS and percentage of diet for prey items (a) *Stenocypris* cf. *major*, (b) *Cypria*, (c) Chironomini and (d) *Hyalella* in Spring Lake

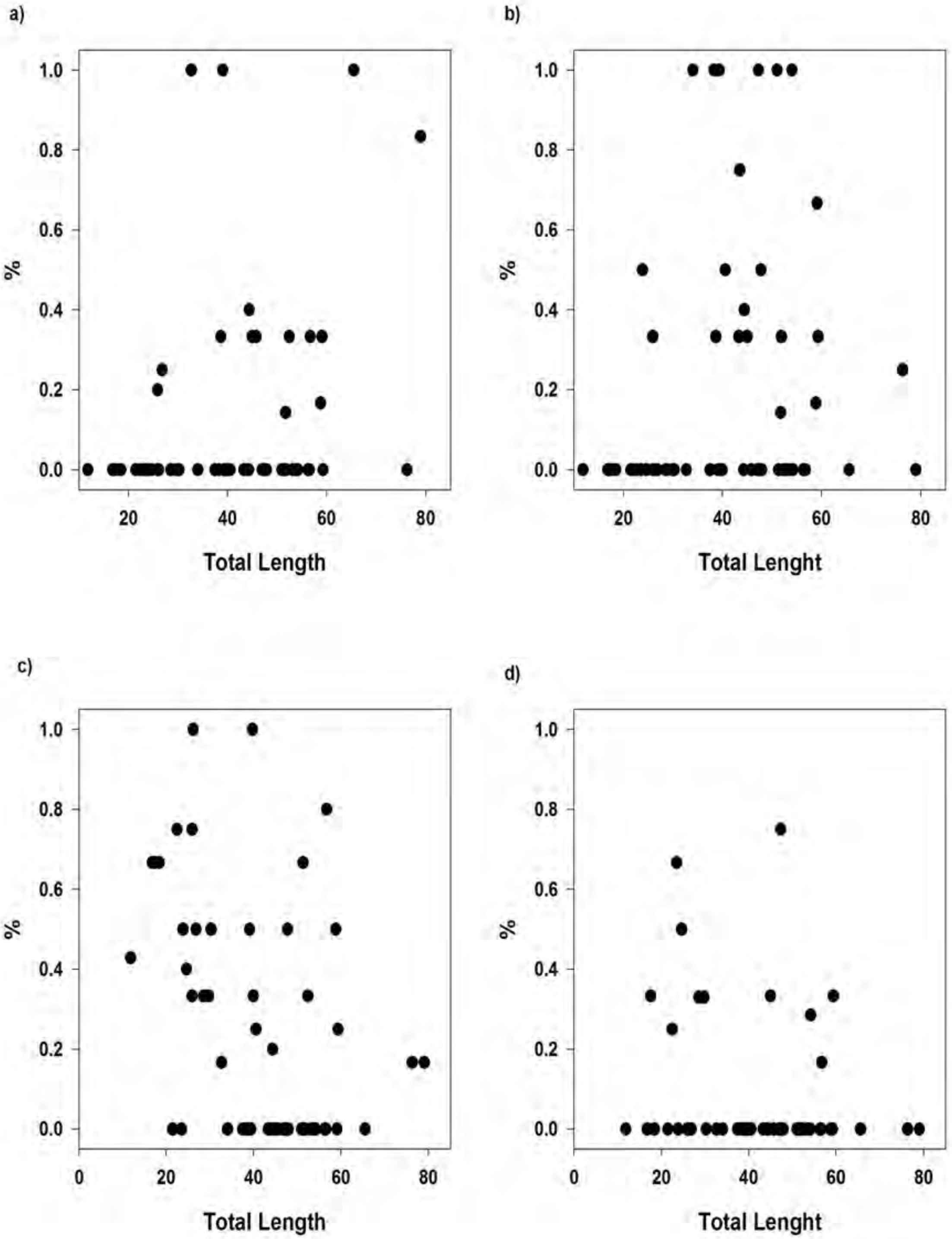

Figure A-2. Total length of SMS and percentage of diet for prey items (a) *Hyalella*, (b) *Chimarra*, (c) *Stenocypris cf. major* and (d) *Cypria* in the San Marcos River below Spring Lake dam

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