DIET OF THE ORANGEBELLY DARTER, *ETHEOSTOMA RADIOSUM,* AMONG TRIBUTARIES OF THE LOWER MOUNTAIN FORK RIVER

By

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Abstract: Previous studies, in the Blue and Glover Rivers of Oklahoma, revealed that the endemic orangebelly darters, *Etheostoma radiosum,* are selective feeders with diets consisting primarily of aquatic insect larvae and dominated by fly larvae. In this study, orangebelly darters were collected from tributaries of the Lower Mountain Fork River, below Broken Bow Dam, with backpack electrofishing equipment in February and April 2015. One hundred and forty-one darters were captured from five tributaries and stomach contents were examined to determine benthic macroinvertebrate prey use. Standard length of darters was compared to determine if size differed among tributaries. Non-insect food items were grouped by order, while insect food items were identified to family. Prey composition was compared among tributaries. A total of 11 food types were found, with isopods being the most frequently consumed organism. Other common food items included aquatic insects in the families Heptageniidae, Chironomidae, Perlidae and Simuliidae (in order of abundance). Although no significant difference was found for darter lengths, a significant difference for consumed isopods was found among the tributaries. Darters appeared to be generalist feeders on aquatic macroinvertebrates in most tributaries and utilized different prey than previously reported. Additionally, for the first time, Acanthocephalan (Spiny-head worm) parasites were found in the stomachs of 17 of the orangebelly darters (Bee branch = 12, Beaver = 3, Rough Branch = 2). Studies such as this improve knowledge of freshwater biodiversity, ecology, and conservation and highlight differences in diet among populations of small fish inhabiting headwater tributaries and main channels in southeastern Oklahoma.

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CHAPTER I

REVIEW OF LITERATURE

Biodiversity and Freshwater Ecosystems

Biodiversity is the measurement of the species composition of an ecosystem (Mace et al. 2012) and plays an important role in maintaining ecosystem function (Hooper et al. 2005). Biodiversity can be affected by disturbances, climate change and alterations in resource availability, and such changes influence the speed, extent, and direction of ecosystem processes including nutrient cycling, biomass production and carbon capture (Diaz et al. 2005). Ecosystem processes support services, which benefits humans (Mace et al. 2012). Ecosystem services include air and water purification, climate regulation, and soil formation (Christensen et al. 1996). Higher ecosystem function is often associated with higher biodiversity (Balvanera et al. 2012). Thus, humans are often dependent upon ecosystem benefits derived from an ecosystem rich in biodiversity (Chapin et al. 2000).

Human alteration of ecosystems reduces the diversity of flora and fauna that inhabit these ecosystems globally (Vitousek et al. 1997). Human induced impacts such as: hydrologic alteration, pollution, habitat loss and fragmentation, introduction of exotic species, land conversion land and deforestation, alter stream ecosystems and reduce biodiversity (Dudgeon et al. 2006; Heino et al. 2009; Revenga et al. 2005; Resh et al. 1988).

In the last two millennia, human activities have caused extinction rates to occur at an abnormally high rate (Pimm et al. 1995; Vitousek et al. 1997; Chapin et al. 2000). Freshwater ecosystems are more limited in distribution and often more degraded than terrestrial ecosystems (WRI 2000) and consequently, a disproportionate number of extinctions have occurred to aquatic species (Jenkins 2003).

Despite constituting less than 0.008% of the volume of all water on the planet (Balian et al. 2010), freshwater ecosystems often have high proportions of endemic species, relatively high species diversity (Revenga et al. 2005) and host a number of unique populations (Dudgeon et al. 2006). Although freshwater ecosystems are important to maintaining ecosystem services, there is a deficiency in the amount of data on the abundance, diversity and distribution of freshwater species worldwide (Revenga and Kura 2003). Freshwater species including fish (Fausch et al. 1990), crustaceans (Pieri 2012), aquatic insects (Rosenberg and Resh 1993) and even aquatic vegetation (Kłosowski 1985; Penning et al. 2008) are good biological indicators of the environmental condition of aquatic ecosystems.

Freshwater ecosystems are so diverse in part because of the unique characteristics of flowing water. Precipitation in the form of rain or snow adds water that flows downhill because of gravity, joining other waters and spreading as the gradient becomes reduced. The “river continuum concept” (Vannote et al. 1980) characterizes the physical and biotic changes that occur from a headwater spring to when a river meets the ocean.

Headwater tributaries are essential to the conservation of biological diversity within stream ecosystems because they provide watershed protection, function as source populations for many species and serve as important spawning and rearing areas, while providing additional food sources for species residing in the mainstem of a stream (Meyer et al.2007). Headwater streams often contain unique species (Paller 1994) and small springs and spring runs contain endemic species found only in limited ranges (Hubbs 1995). Thus, small tributaries increase the biological diversity of stream systems by making habitats available for multiple species and providing a biological link between upstream and downstream areas (Meyer et al. 2007). Headwater streams are often more strongly influenced by terrestrial allochthonous inputs (Vannote et al. 1980), including plant matter and terrestrial invertebrates (Nakano et. al 1999).

Smaller streams are more strongly impacted by riparian vegetation that not only supplies large amounts of allochthonous detritus to the system, but also increases shading, thereby decreasing primary (autochthonous) production (Vannote et al 1980). For example, Fisher and Likens (1973) found that tree leaves accounted for 99% of the energy input headwater streams. In contrast, larger streams have less riparian canopy cover resulting in decreased terrestrial organic input and increased sunlight reaching the stream, allowing for an increase in primary production by means of photosynthesis (Vannote et al. 1980).

The “serial discontinuity concept” (Ward & Stanford 1983) describes how anthropogenic interruptions, such as dams, cause a disruption of the river continuum that begins at the point of the disturbance and recovers as the downstream distance from the disturbance increases. Dams are common structures in stream systems and may alter discharge patterns, downstream water temperature regimes, hydraulic characteristics, substrate composition, and channel morphology of streams (Gebrekiros 2016). These alterations change river ecosystems both upstream and downstream of the impoundment and threaten aquatic biodiversity (Bunn and Arthington 2002; Johnson and Harp 2005; Pringle et al. 2000).

Broken Bow Dam

The Lower Mountain Fork River (LMFR) is located in McCurtain County, in southeastern Oklahoma. The construction of Broken Bow dam to provide flood control, water supply, hydroelectric power, recreation, and conservation of fish and wildlife was completed in 1968 (Eley et al. 1981). This impoundment changed the Mountain Fork River by converting lotic habitat to lentic above the dam and altering temperature and the frequency and duration of stream flows below the dam (Harper 1994). The flow of the LMFR below Broken Bow dam fluctuates as a result of hydropower generation and the temperature of tailrace area of the river is impacted by the cold hypolimnetic discharge from Broken Bow Lake (Harper 1994). It is well-documented that flow regime alterations and hypolimnetic releases impact native fish and macroinvertebrate communities (Edwards 1978; Hilsenhoff 1971; Marchant 1989; Quist and Schultz 2014; Taylor et al. 2014) and these impacts are evident in the LMFR. After completion of the dam and releases of hypolimnetic waters, less than half of the 83 native fish species remained (Harper 1994). However, the release of cold waters from Broken Bow Dam allowed the establishment of a put-and-take trout fishery in Oklahoma (Harper 1994).

There is a shortage of studies assessing the impacts of environmental change on fish species that do not have recreational or commercial value (Comte et al. 2013), despite the significant role they play in nutrient cycling of freshwater ecosystems (Vanni 2002). Darters perform an important role in freshwater ecosystem services and processes by transferring energy and nutrients from allochthonous organic matter to other species in the stream community via consumption of benthic macroinvertebrates (Adamson and Wissing 1977). Etnier (1997) lists over 100 species of *Etheostoma* that are considered at risk of extinction because of altered flow, small range, exotics and point source and nonpoint source pollution. In a community fish survey of the tributaries of the LMFR, Long et al (2016) found the smaller tributary streams to be dominated by the orangebelly darter *Etheostoma radiosum*.

Darters

Etheostoma are small (< 10 cm SL) freshwater fish, native to North America (Bailey and Etnier 1988). Most darters are benthic species that inhabit rubble and gravel riffles i.e., in streams with moderate flow (Lachner et al. 1950). Most darters lack a swim bladder and occupy the bottom of streams where they forage for benthic invertebrates (Page and Burr 1991). Benthic insectivores are among the most abundant fishes in small temperate streams (Matthews 1990; Paller 1994) and within small streams, darters are one of the most significant consumers of benthic organisms (Small 1975). Darters are opportunistic predators consuming mostly insect larvae in the orders Diptera, Ephemeroptera, and Plecoptera (Martin 1984).

The orangebelly darter, *Etheostoma radiosum* (Hubbs and Black 1941) is endemic to southwest Arkansas and southeast Oklahoma above the Fall Line (Retzer et al. 1986). Endemic organisms are species whose distribution is restricted to a specific region and the number of endemic species is often used to characterize the biological distinctiveness of a region (Meyer et al. 2007). The range of *E. radiosum* extends from the Ouachita River of southwestern Arkansas westward to the Blue River system of Johnston and Bryan counties in southcentral Oklahoma (Echelle et al. 1975; Moore and Rigney 1952) and in small tributaries of the Washita River in Marshall and Bryan counties, Oklahoma (Matthews et al. 1986; Matthews and Gelwick 1988). Three subspecies of *E. radiosum* have been described: *E. radiosum radiosum* (Ouachita and Little rivers, Arkansas and Oklahoma, and LMFR and Washita River, Oklahoma), *E. r. paludosum* (Kiamichi and Boggy rivers, Oklahoma), and *E. r. cyanorum* (Blue River) (Moore and Rigney 1952).

The orangebelly darter is found in main river channels, small streams and tributaries in drainages within its range and field surveys reveal that it is one of the first fish to invade temporary streams (Echelle et. al 1975). It is most-often associated with clear, flowing, rocky-bottomed raceways and riffle areas of streams and is rarely found in the turbid, sluggish waters of lower mainstream areas (Scalet 1973). Despite being characterized as an early invader of streams, Scalet (1973) found that adults display very little movement within the stream. This lack of movement may contribute to the small distributional range of this species. Burkhead et al. (1997) reports that fish that have small ranges are more susceptible to threats, such as loss of habitat, and suggest that limited range size is the main cause of endangerment. Within tributaries of main river channels, gene flow may be limited between orangebelly darter populations (Echelle et. al 1975; Moore and Rigney 1952).

Orangebelly darters actively feed on moving food items, avoiding immobile items (Scalet 1972). Two previous studies examined the diets of orangebelly darters and found Diptera to be the most common item (Jones and Maughan 1989, Scalet 1972). Both studies reported that Ephemeroptera, Plecoptera and Trichoptera were eaten less frequently.

Diet Analysis

Feeding habit analyses are necessary to comprehend the trophic dynamics and interactions of a lotic ecosystem (Cummings 1974; Deus and Petrere-Junior 2003; Garvey et al.1998). Therefore, a quantitative assessment of food habits is a vital aspect of fisheries management and conservation of endemic species (Chipps and Garvey 2007). Diet is regarded as a species characteristic that incorporates ecological components such as behavior, condition, habitat use, energy intake and interspecific, as well as, intraspecific interactions (Chipps and Garvey 2007; Raffaelli 2007). Thus, diet studies are important to evaluate the significance of species within an ecosystem (Pouilly et al.2006). Stomach content analysis provides a measurable assessment of feeding habitat in addition to an interpretation of feeding patterns (Zacharia and Abdurahiman2010). The feeding ecology of *E. radiosum* has thus far only been reported for the Blue River, OK (Scalet 1972) and the Glover River, OK (Jones and Maughan 1989) that are larger rivers. It is important to assess whether orangebelly darters inhabiting smaller streams exhibit a different feeding ecology from what has been previously reported.

Objectives

The objective of this project was to assess orangebelly darter diet from five small tributaries of the Lower Mountain Fork River. The hypothesis tested was that the diets of orangebelly darters is consistent among tributaries that connect to the Lower Mountain Fork River.

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CHAPTER II

DIET OF THE ORANGEBELLY DARTER, *ETHEOSTOMA RADIOSUM* AMONG TRIBUTARIES OF THE LOWER MOUNTAIN FORK RIVER

Etheostoma, is the most diverse genus of freshwater fishes in North America, and have many endemic species that are threatened or endangered. They are generally small fishes that lack a swim bladder and take their common name from the behavior of darting along the bottoms of streams. Most species are adapted to cool flowing waters with rocky bottoms where they forage for benthic invertebrates (Page and Burr 1991).

The orangebelly darter, *Etheostoma radiosum*, is endemic to southwest Arkansas and southeast Oklahoma above the Fall Line (Retzer et al. 1986) where it is known from drainages associated with three river systems: the Ouachita River of southwestern Arkansas, the Blue River system in southcentral Oklahoma (Moore and Rigney 1952; Echelle et al. 1975) and the tributaries of the Washita River in southcentral Oklahoma (Mathews and Gelwick 1988). The orangebelly darter is a small fish (<10 cm in length) that is common in main river channels, small streams, and tributaries in drainages within its range (Scalet 1973).

Field surveys indicate that orangebelly darters are one of the first fish to invade temporary streams (Echelle et. al 1975). Diet studies for this species (Jones and Maughan 1989; Scalet 1973), have been conducted in the Blue and Glover rivers, in Oklahoma, and found adults to consume primarily aquatic dipteran larvae. Adult diets differed from juvenile diets and both adult and juvenile darters fed selectively as the frequency of prey shifted among seasons. Both existing studies were conducted on orangebelly darters living in large (third or fourth order) streams and to date no studies have examined the diet of this species living in small tributaries despite their documented colonization of these systems. Therefore, I investigated the feeding habits of *E. radiosum* residing in five tributaries of the Lower Mountain Fork River (LMFR) and performed a quantitative analysis of stomach contents to determine the most frequently consumed prey, analyze feeding patterns, and determine if consumed prey differed among tributaries. I hypothesized that the diets of the darters would be similar among these tributaries.

MATERIAL AND METHODS

Study Site

The Lower Mountain Fork River (LMFR) below Broken Bow Dam, in southeastern Oklahoma receives hypolimnetic discharge of cold water from Broken Bow Lake. In 1989, 19.3 km of the LMFR became a designated tailwater trout fishery (Harper 1994). Many small tributaries, ranging from intermittent to third order, discharge into the designated trout area. The tributaries sampled in this study ranged from 1.67 to 5.12 meters in width, 0.12 to 0.27 meters in depth and 2.68 to 3.53 kilometers in length (Long et al. 2016).

Fish Sampling

Orangebelly darters were collected as part of a larger community survey project to determine the presence of wild juvenile rainbow trout in the tributaries of the LMFR (Long et al. 2016). Surveys were conducted in seven tributaries: Beaver, Bee Branch, Cooper, Unnamed, Fish Fry, Horsepen and Rough Branch (Fig 1.) and each site was sampled once in February 2015 and then again in April 2015. Darters were captured via backpack electrofishing for 30 minutes of on-power time. At each site, sampling was accomplished by traveling upstream from just above the mouth of the tributary. Fish collected during these surveys were combined for both sampling dates for each tributary. A total of 153 orangebelly darters were collected from tributaries within the study site and all fish were preserved in 4% formalin. Less than 10 darters were collected from Cooper and Fish Fry, and thus, samples from these tributaries were removed from further analyses. Out of the remaining five tributaries 141 *E. radiosum* were analyzed to determine food habitats.

Diet Analysis

In the laboratory, the standard length of darters was measured to the nearest millimeter and the stomach was removed for prey identification. Food items were viewed under a stereoscopic dissecting microscope and each stomach was assessed separately. For stomachs with masses of partially digested food items, the heads of prey items were identified, and each head was counted as one food item. Food items were identified to lowest practical taxon using keys by Merritt et al. (2008) and Smith (2001). Non-insect prey types were grouped by order or category; insect prey types were organized by family. Darters with no food in their stomach were categorized as empty and those with prey too digested to identify were classified as such.

Statistical Analysis

Total abundance of prey types and prey types by tributary were expressed as percentages of the total number of organisms found in all darters for that tributary. A Canonical Correspondence Analysis (CCA) was conducted in PC-ORD 6.0 to investigate differences in prey types by tributary. CCA is a type of multivariate analysis used to investigate the relationships between biological assemblages of species and their environment. The primary outcome of CCA is an ordination diagram (ter Braak and Verdonschot 1995).

Sigma Stat software was used to test for differences in darter length and diet among tributaries. ANOVA ($p < 0.5$) was used to analyze darter length followed by a Tukey test. Darter length was tested to ensure that differences in diet were not a result of differences in darter size. A Kruskal-Wallis analysis of variance ($p < 0.05$) was used to analyze prey items by tributary due to non-normally distributed data and followed by a Dunn's test when significance was detected.

Diet patterns of orangebelly darters were evaluated using a graphical representation of prey composition developed by Amundsen et al. (1996). Information regarding feeding strategy, prey importance and niche breadth can be acquired by plotting the distribution of points along the diagonals and axes of the graph (Fig. 2). The Amundsen method plots specific-prey abundance (P_i) against frequency of occurrence (F_i), using the equations:

$$P_i = (\sum S_i / \sum S_{ti}) \text{ x } 100 \qquad (1)$$

$$F_i = (N_i / N) \text{ x } 100 \qquad (2)$$

Where P_i is the prey-specific abundance of prey i, S_i represents the quantity of prey item i in stomachs, S_{ti} is the total prey quantity in darters that contain prey i in their stomach, F_i is the frequency of occurrence of prey type i, N_i equals the number of darters with prey item i in their stomach, and N is the total number of darters with stomach contents.

Parasite Analysis

During the diet analysis, spiny head worm parasites (Acanthocephala) were found some of the darter stomachs. Because the majority of infected darters were found in Bee Branch, statistical analysis were conducted only on this tributary. A t-test was utilized to determine if a there was significant difference in darter size, using SL, between infected and non-infected fish. The average number of items consumed by infected and non-infected fish as well as the frequency of empty stomachs were compared to test if infected fish consumed more or less food than non-infected fish. Lastly, the number of isopods and amphipods consumed by darters was investigated because these organisms serve as intermediate hosts for Acanthocephalans.

RESULTS

The average standard length (SL) of all orangebelly darters was 48.74 (±0.67 SE) mm. The maximum SL of darters in this study was 70 mm (Horsepen) and the minimum SL was 21 mm (Bee Branch). Orangebelly darters in Horsepen had the largest average SL with a mean of 52.7 (± 1.5 SE) mm and the smallest average in the Unnamed tributary with a mean of 44 (± 1.3) mm. ANOVA revealed a difference in darter SL between Horsepen and the Unnamed tributary ($p = 0.019$) and Horsepen and Bee Branch ($p = 0.020$).

Of the 141 orangebelly darters analyzed, 116 darters had food in their stomachs, 7 darters had stomach contents too digested to identify (Beaver = 1, Bee Branch = 2, Horsepen = 1, Rough Branch = 3), and 18 darters had empty stomachs (Beaver = 2, Unnamed = 1, Bee Branch = 8, Horsepen = 4, Rough Branch = 3). A total of 664 total prey items were identified and classified into 13 prey types: Isopoda, Amphipoda, Heptageniidae, Baetidae, Perlidae, Simuliidae, Chironomidae, Hydropsychidae, Hydroptilidae, Elmidae, Ostracoda, Eggs, and Unknown. Isopods were the most frequently consumed organism (37%), followed by Chironomidae larvae (17%) (Table 2). Isopods were also the most abundantly consumed organism throughout all sites (Fig. 3).

Results of the Kruskal-Wallis analysis showed a significant difference in the number of isopods consumed among the tributaries ($p = < 0.001$) and Dunn's post hoc indicated differences were between the Unnamed tributary and Bee Branch, Beaver and Rough Branch in addition to, Horsepen and Bee Branch and Horsepen and Rough Branch (Fig. 4). There was also a significant difference in number of Heptageniidae ($p = <0.001$) consumed between Horsepen and Beaver Creek and Horsepen and the Unnamed Tributary (Fig. 5) and a significant difference in the amount of Perlidae ($p = < 0.001$) consumed between Rough Branch and Beaver, Horsepen and Bee Branch (Fig. 6). There was no significant difference in the amount of Chironomidae and Simuliidae consumed among the tributaries (Fig 7, Fig. 8).

The first and second axes of the CCA (Fig 9) were the most influential gradients representing variation of consumed prey among sites. The first axis explained 4.0% of the variation in prey items consumed among the tributaries (eigenvalue = 0.191). The second axis explained an additional 3.7% of the variation (eigenvalue = 0.176). The third CCA axis was not plotted because it accounted for the less variation (2.2%). The total explained variance of the CCA bi-plot was only 7.6%. The cluster of sites at the center of the ordination diagram indicates very little variation of consumed prey among sites and consumed prey variability among the tributaries could be better explained by variables other than tributary

Frequency of occurrence, plotted against prey-specific abundance was used to evaluate feeding strategy (specialized versus general) and prey importance (dominant versus rare) (Amundsen et al 1996). Prey-specific abundance plots indicated a generalized feeding pattern, for the most part, for all tributaries (Fig. 10, Fig.11, Fig. 12, Fig. 13, Fig. 14). In Horsepen and the Unnamed tributary (Fig. 10 and Fig 11), orangebelly darters demonstrated a high frequency of consumption of isopods (Horsepen = 95% and Unnamed = 100%) (Table 2).

<u>Parasites</u>

Acanthocephalan (Spiny-head worms) parasites were found in the stomachs of 17 of the orangebelly darters (Bee Branch = 12, Beaver = 3, Rough Branch = 2). There was no significant difference between the SL of orangebelly darters with and without Acanthocephalan parasites in Bee Branch (t-test, $\alpha = 0.05$, $p = 0.65$). The average number of food items consumed was 3.25

for darters with Acanthocephalan and 2 without; however, the results were not significantly different (t-test, $\alpha = 0.05$, $p = 0.15$). The frequency of empty stomachs for infected fish was 25% and the frequency of empty stomachs for uninfected fish was 13%. The number of isopods consumed in Bee Branch was 15 for all darters examined and 2 for darters with Acanthocephalan (both isopods were consumed by one fish, the other 7 fish consumed 0 isopods) and no amphipods were found in stomachs of fish from Bee Branch (Table 3).

DISCUSION

Based on gut content analysis, orangebelly darters inhabiting the sampled tributaries of the LMFR consumed a variety of organisms and appeared to be opportunistic feeders. These findings are inconsistent with previous studies that report orangebelly darters having specialized diets (Scalet 1973; Jones and Maughn 1989)

Significant differences in the use of one or more diet items for orangebelly darters occurred among all tributaries, except for Horsepen and the Unnamed tributary (Table 4). Significant differences in the consumption of isopods occurred among more tributaries than the consumption of other diet items It is unknown why prey use varied among tributaries in this study. Significant differences were detected for orangebelly darter SL in Horsepen and the Unnamed tributary, yet no significant differences in diet items occurred between these two tributaries. A significant difference in SL also existed between Horsepen and Bee Branch, which differed in the number of isopods consumed. Because SL only varied between two tributaries for one diet item, fish size can be ruled out as a variable to explain differences in diet among tributaries, but other variables not examined in this study could explain the variation. Gillette (2012) determined that selection of prey items varied greatly among riffles in orangethroat darters examined in southern

Oklahoma and this variation was explained by habitat differences. Habitat variables could be important aspect to add to a diet study.

Across all tributaries of the LMFR, orangebelly darters exhibit different feeding patterns than those previously examined by Scalet (1972) and Jones and Maughan (1989), who found the main food source to be dipteran larvae. In my study, Chironomidae (midge larvae) were consumed at a frequency of 17%, and Simuliidae (blackfly larvae) 12% (total 29%). In contrast, orangebelly darters from the Blue River, consumed Chironomidae at a frequency of 48% and Simuliidae at 5.6% (total 53.6%). In the Glover River dipterans were consumed at a frequency of 60% for darters collected during spring. In the tributaries of the LMFR, isopods were consumed with the greatest frequency (37%) and were the most abundantly consumed organism. While the previous studies do not report consumption of isopods by orangebelly darters, they also do not report finding isopods in benthic macroinvertebrate samples. Therefore, differences in diet could be the result of stream temperature as the Blue and Glover Rivers are much larger and warmer streams than the tributaries of the LMFR. Isopods are adapted to coldwater and could survive in the tributaries of the LMFR but not in larger warmwater rivers. In another coldwater habitat the Little Red River, the isopod *Lirceus* sp. represented 51% of the abundance of downstream benthic invertebrate samples and 71% of the prey consumed by trout (Johnson et al. 2007).

<u>Parasites</u>

The adult Acanthocephalans found in the darter stomachs are specialized intestinal parasites of vertebrates. Spiny-head worms possess an anterior proboscis covered with spiny hooks to attach to the stomach wall of their host organism and acquire nutrition by ingesting the previously digested food of the host organism directly through their body surface (Melhorn 2008). Adults attach to the intestinal wall of their final hosts and fully embryonated eggs are passed with the host's feces. Intermediate hosts include isopods, amphipods, or other arthropods that are infected when they consume Acanthocephalan eggs. Inside the intermediate host's intestine, the larva hatches and enters the body cavity and the final hosts such as fish are infected by consuming intermediate hosts (Melhorn 2008). Infected fish will exhibit reductions in energy efficiency, altered metabolism emaciation, lethargy and even death in some cases (Woo 2006). Acanthocephalan were found most frequently in Bee Branch, but orangebelly darters consumed the least number of isopods and no amphipods (Table 1, Fig. 15), both intermediate hosts of acanthocephalan, in Bee Branch. Orangebelly darters residing in Bee Branch were also smaller on average. There was a significant difference in SL between darters in Bee Branch and Horsepen. Horsepen had the largest mean SL, 52.7 ($\pm$ 1.5 SE), while Bee Branch had the second smallest mean SL, 45.5 ($\pm$ 1.5 SE). Differences in fish size could be due to high number of Acanthocephalan infected fish in Bee Branch.

Previously, acanthocephalans have not been found in orangebelly darters, but Oetinger and Buckner (1976) report acanthocephalans in the Stippled Darter, *Etheostoma punctulatum* from Northeastern Oklahoma. During a life history study of orangebelly darters, Scalet (1971) found and identified other parasites but not acanthocephalans. The most frequently observed parasite was *Illinobdella moorei*, a piscicolid leech, while *Crepidostomum cooperi,* a digenetic trematode and *Uvulifer ambloplitis*, the black-spot or black-grub were also found.

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Table 1. Number of orangebelly darters, total number of prey items and percent composition of diet by tributary

	Beaver		Bee Branch		Unnamed		Horsepen		Rough Branch		Total	
Orangebelly Darters	**26**		**37**		**16**		**25**		**37**		**141**	
Isopoda	19	20.88%	15	19.48%	74	51.05%	67	59.29%	73	30.29%	248	37.35%
Amphipoda	7	7.69%	0	0.00%	0	0.00%	6	5.31%	3	1.24%	16	2.41%
Heptageniidae	21	23.08%	26	33.77%	7	4.83%	1	0.88%	22	9.13%	77	11.60%
Baetidae	0	0.00%	0	0.00%	0	0.00%	0	0.00%	3	1.24%	3	0.45%
Perlidae	1	1.10%	4	5.19%	7	4.83%	1	0.88%	72	29.88%	82	12.35%
Simuliidae	28	30.77%	1	1.30%	28	19.31%	3	2.65%	22	9.13%	82	12.35%
Chironomidae	10	10.99%	19	24.68%	23	15.86%	32	28.32%	31	12.86%	115	17.32%
Hydropsychidae	2	0.22%	7	9.09%	2	1.38%	0	0.00%	2	0.83%	13	1.96%
Hydroptilidae	1	1.10%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	1	0.15%
Elmidae	0	0.00%	0	0.00%	0	0.00%	2	1.77%	0	0.00%	2	0.30%
Ostracoda	0	0.00%	0	0.00%	2	1.38%	0	0.00%	0	0.00%	2	0.30%
Eggs	1	1.10%	2	2.60%	0	0.00%	0	0.00%	9	3.73%	12	1.81%
Unknown	1	1.10%	3	3.90%	2	1.38%	1	0.88%	4	1.66%	11	1.66%

Table 2. Specific prey abundance (P_i) and frequency of occurrence (F_i) of prey types by tributary.

	Beaver		Bee Branch		Unnamed		Horsepen		Rough Branch	
	P_i	F_i	P_i	F_i	P_i	F_i	P_i	F_i	P_i	F_i
Isopoda	21.1	52.2	19.5	18.5	47.8	100	57	95	30.3	46.9
Amphipoda	7.8	4.3	0	0	0	0	7	10	1.2	9.4
Heptageniidae	23.3	65.2	33.8	63	5.1	33.3	1.2	5	9.1	37.5
Baetidae	0	0	0	0	0	0	0	0	1.2	6.3
Perlidae	1.1	4.3	5.2	11.1	5.1	13.3	1.2	5	29.9	50
Simuliidae	31.1	26.1	1.3	3.7	20.6	26.7	3.5	10	9.1	9.4
Chironomidae	11.1	21.7	24.7	40.7	16.9	66.7	26.7	30	12.9	43.8
Hydropsychidae	2.2	8.7	9.1	14.8	1.5	6.7	0	0	0.8	6.3
Hydroptilidae	1.1	4.3	0	0	0	0	0	0	0	0
Elmidae	0	0	0	0	0	0	2.3	5	0	0
Ostracoda	0	0	0	0	1.5	6.7	0	0	0	0
Eggs	1.1	4.3	2.6	3.7	0	0	0	0	3.7	12.5
Unknown	0	0	3.4	7.4	1.5	6.7	1.2	5	1.7	6.3

Table 3. The amount of Acanthocephalan in the gut, the SL and the number of consumed isopods and amphipods in infected orangebelly darters.

Site	SL	Isopoda	Amphipoda	Acanthocephalan
Beaver	34	1	0	1
Beaver	42	1	0	1
Beaver	54	1	0	2
Bee Branch	39	2	0	3
Bee Branch	41	0	0	1
Bee Branch	43	0	0	5
Bee Branch	43	0	0	1
Bee Branch	45	0	0	7
Bee Branch	45	0	0	3
Bee Branch	45	0	0	1
Bee Branch	47	0	0	1
Bee Branch	49	0	0	7
Bee Branch	50	0	0	1
Bee Branch	53	0	0	2
Bee Branch	56	0	0	7
Rough Branch	47	0	0	1
Rough Branch	51	0	0	3

Table 4. Kruskal-Wallis results **-** significant differences in the use of diet items for orangebelly darters among tributaries.

	Beaver	Horsepen	Rough Branch	Bee Branch	Unnamed
Beaver					
Horsepen	Isopoda, Heptageniidae				
Rough Branch	Perlidae	Perlidae			
Bee Branch	Heptageniidae	Isopoda	Perlidae		
Unnamed	Isopoda		Isopoda	Isopoda	

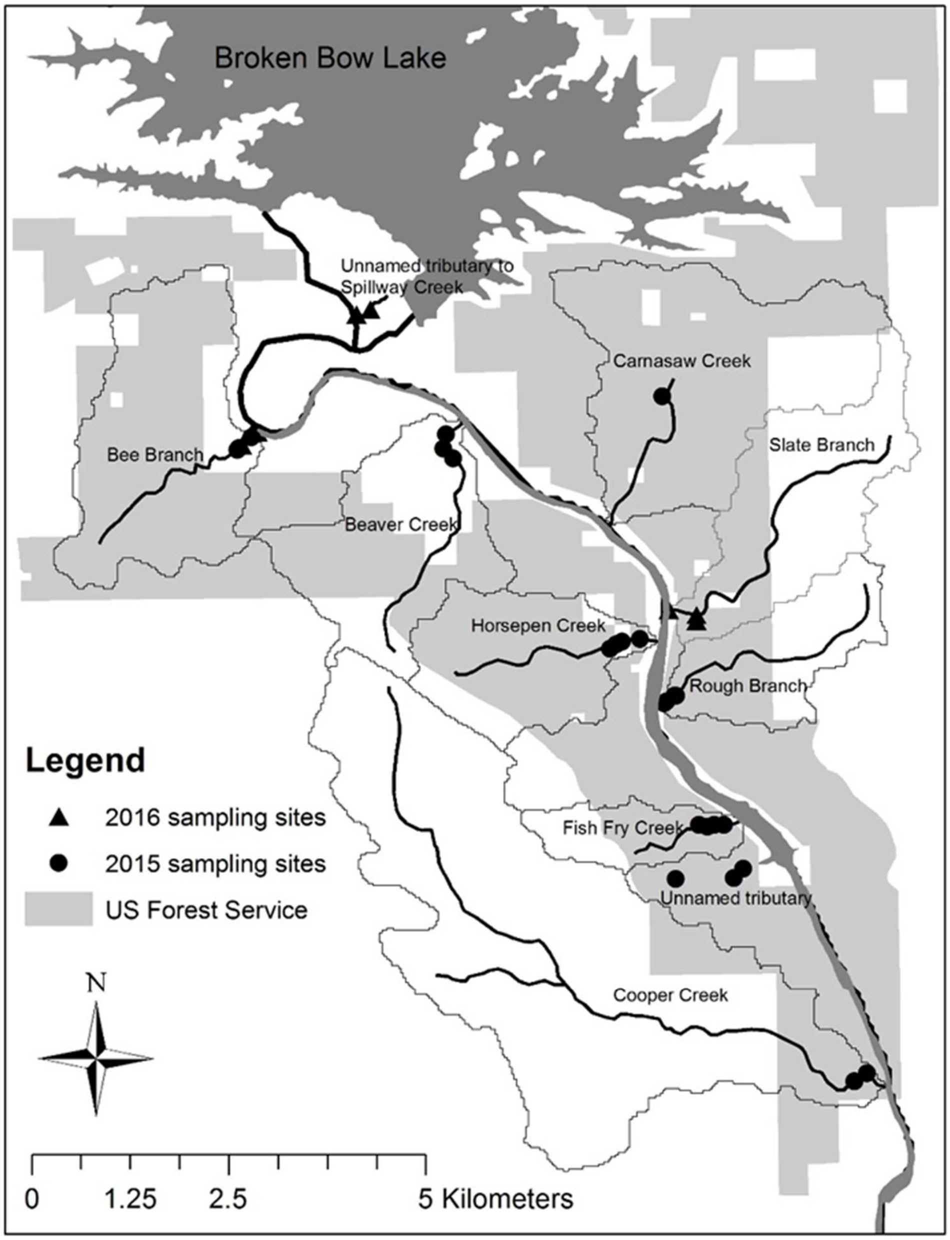

Figure 1. Sample sites for the tributaries of the Lower Mountain Fork River below Broken Bow Dam (Long et al. 2016

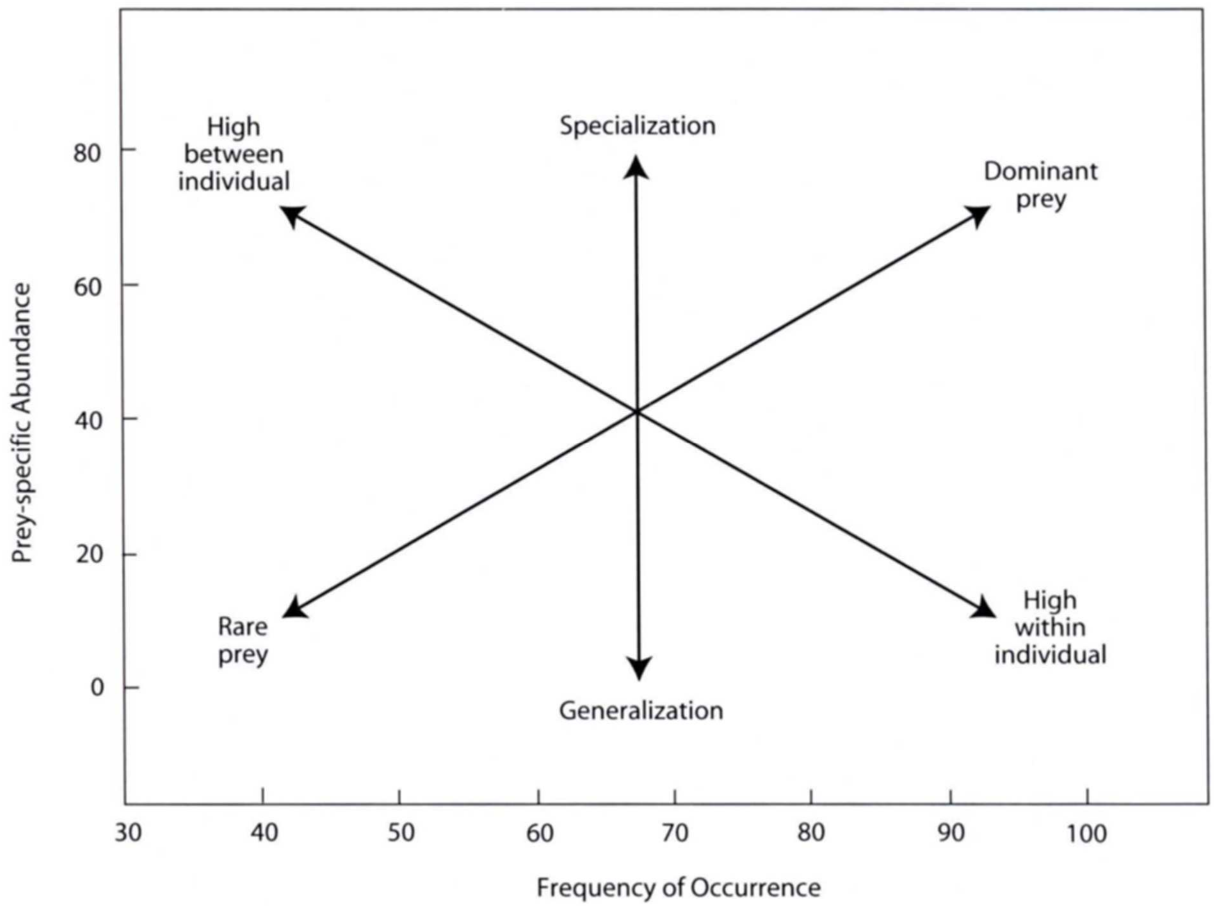

Figure 2. Graphical model that represents feeding strategy and relative prey importance based on the distribution of individual prey types. Prey-specific abundance is calculated from predators that contain prey i and is plotted against frequency of occurrence for each prey. Points located in the upper left of the plot indicate specialization; prey are ingested by a few individuals. Points located in the lower right convey generalization; prey items that are consumed occasionally by most individuals (Amundsen et al. 1996).

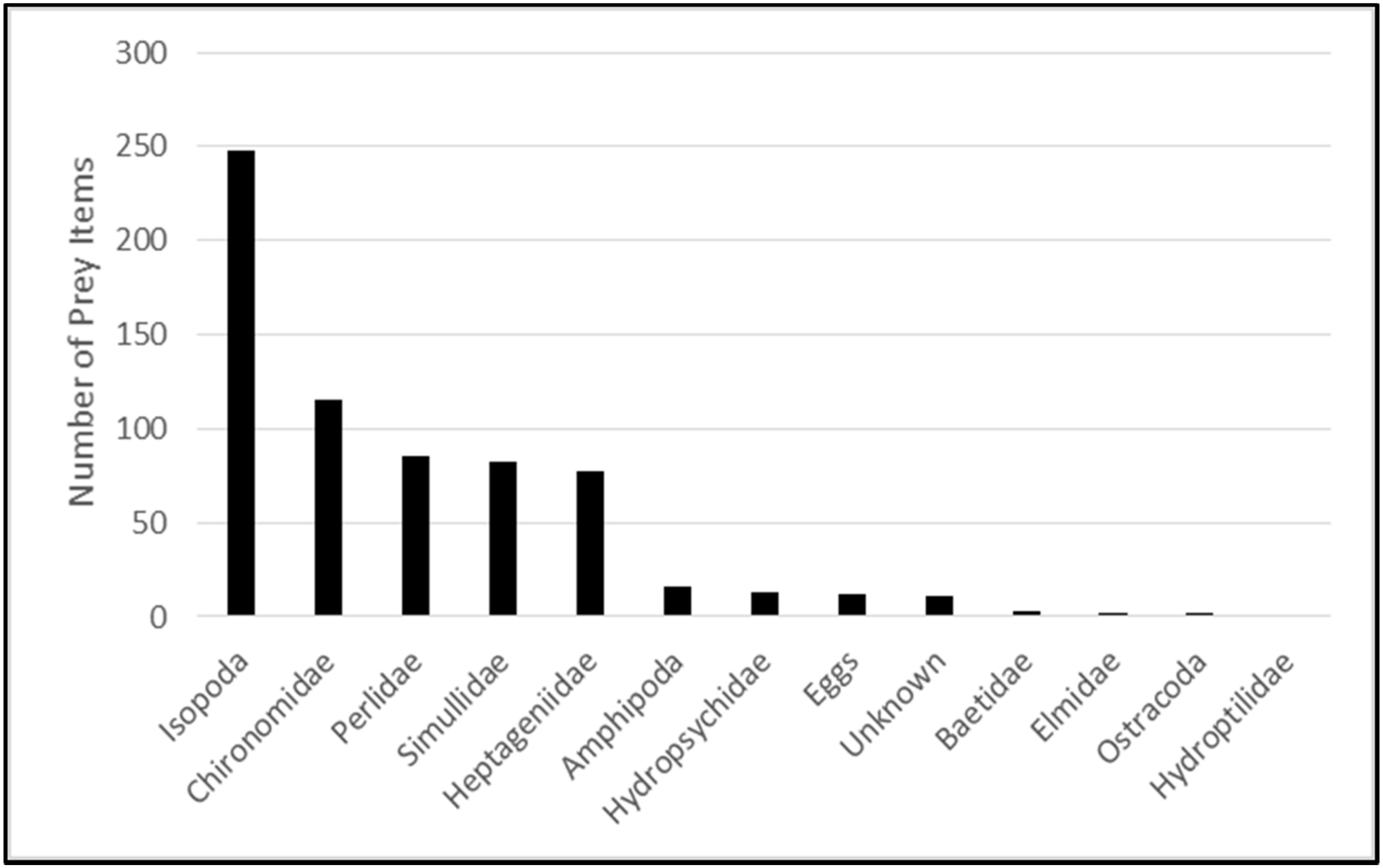

Figure 3. Total number of prey items consumed by orangebelly darters, *Etheostoma radiosum*

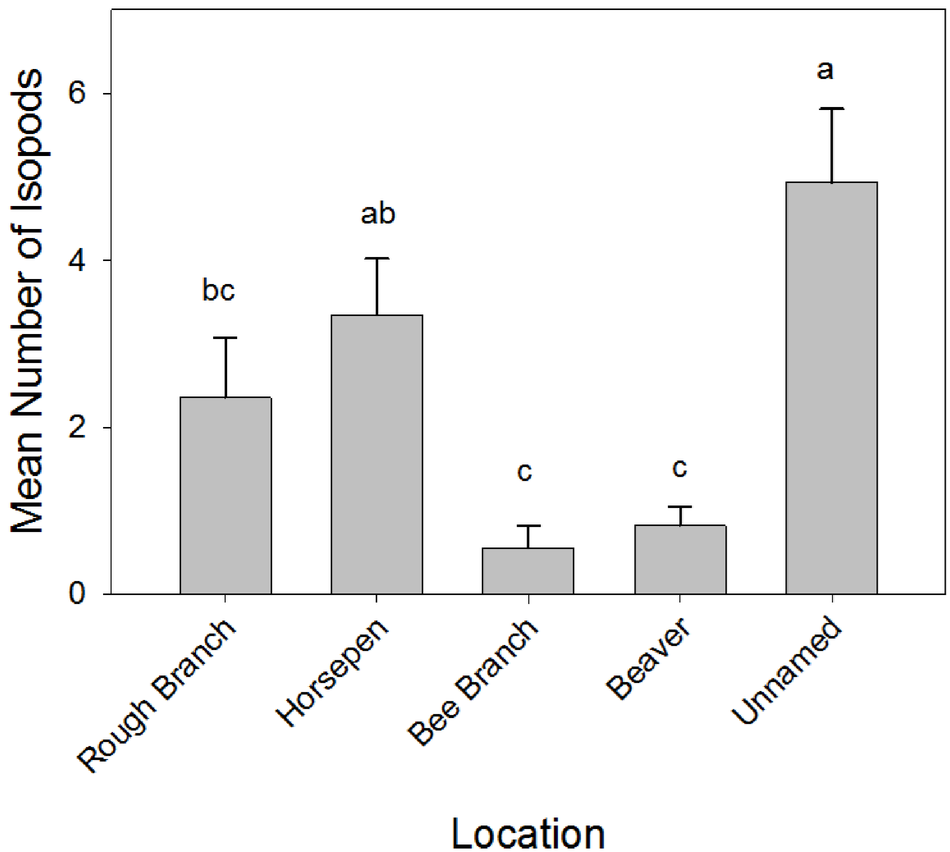

Figure 4. Graph of Kruskal-Wallis results - mean number of isopods by tributary of the Lower Mountain Fork River.

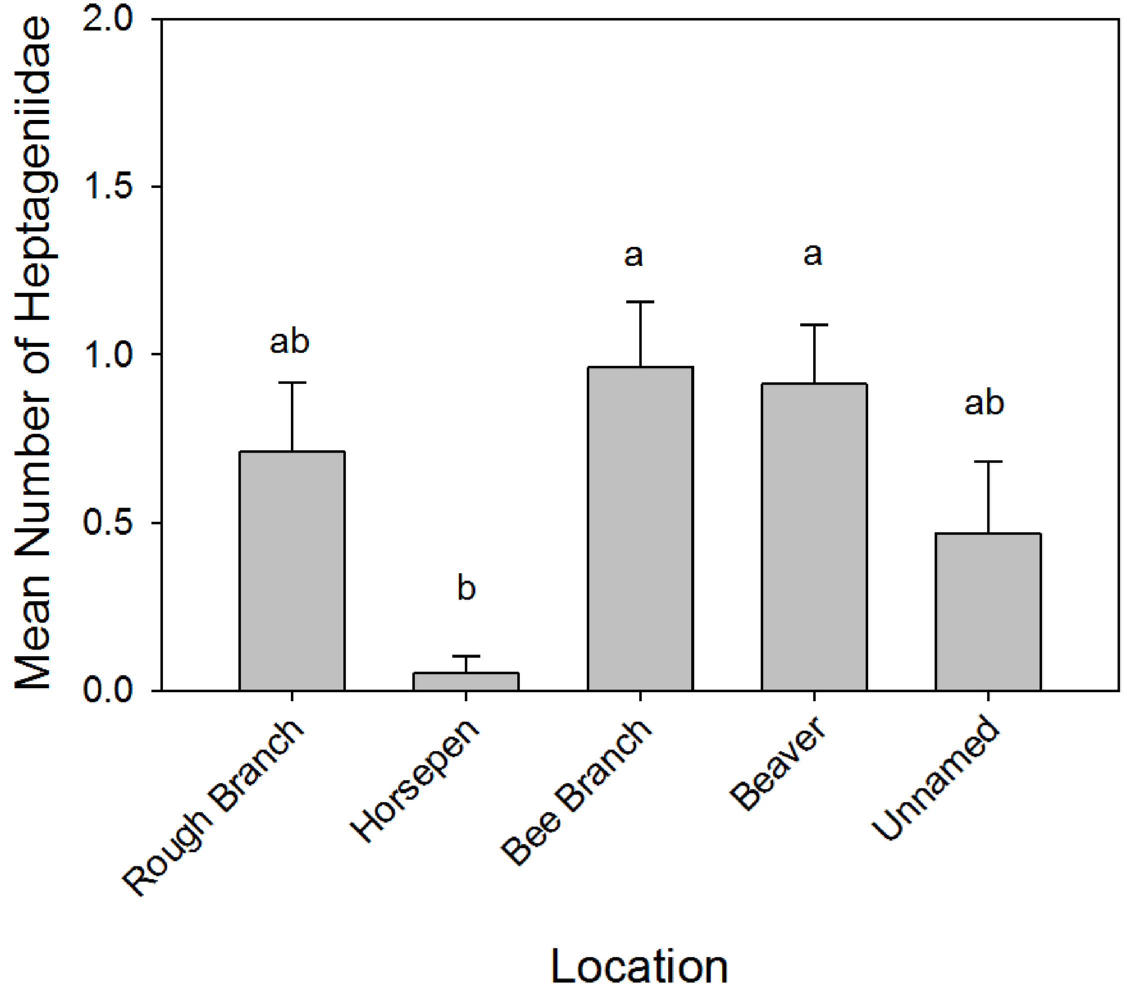

Figure 5. Graph of Kruskal-Wallis results - mean number of Heptageniidae by tributary of the Lower Mountain Fork River

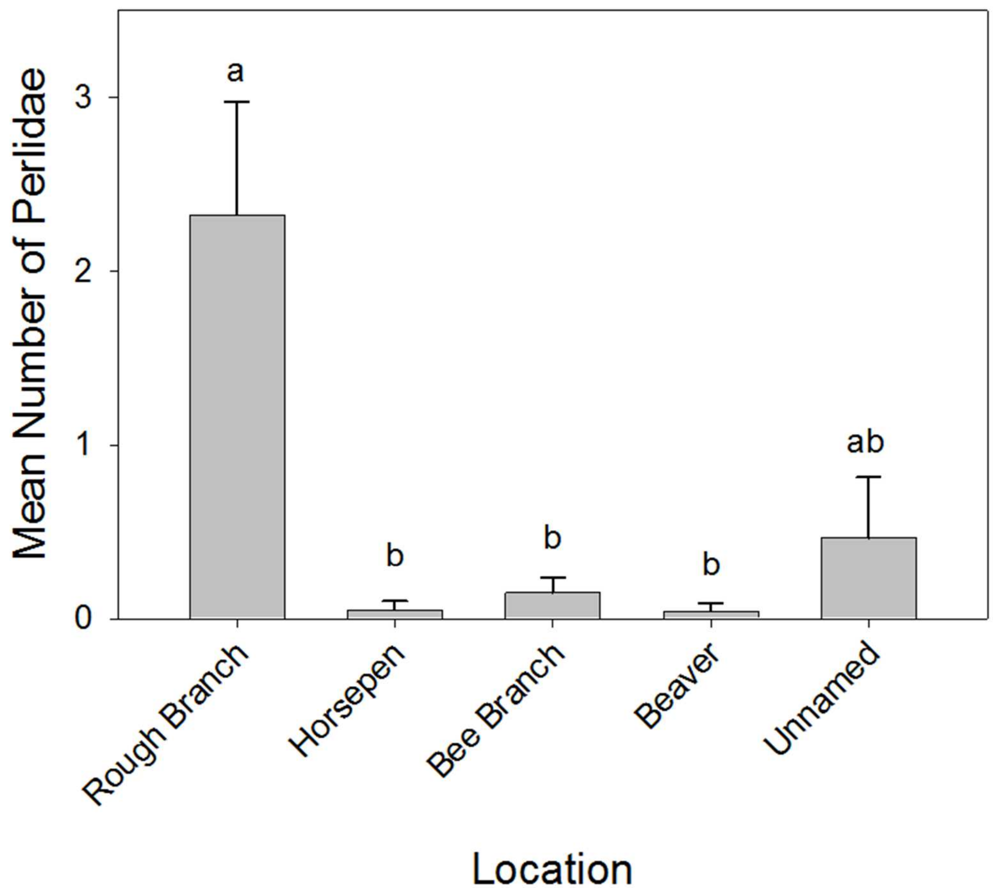

Figure 6. Graph of Kruskal-Wallis results - mean number of Perlidae by tributary of the Lower Mountain Fork River

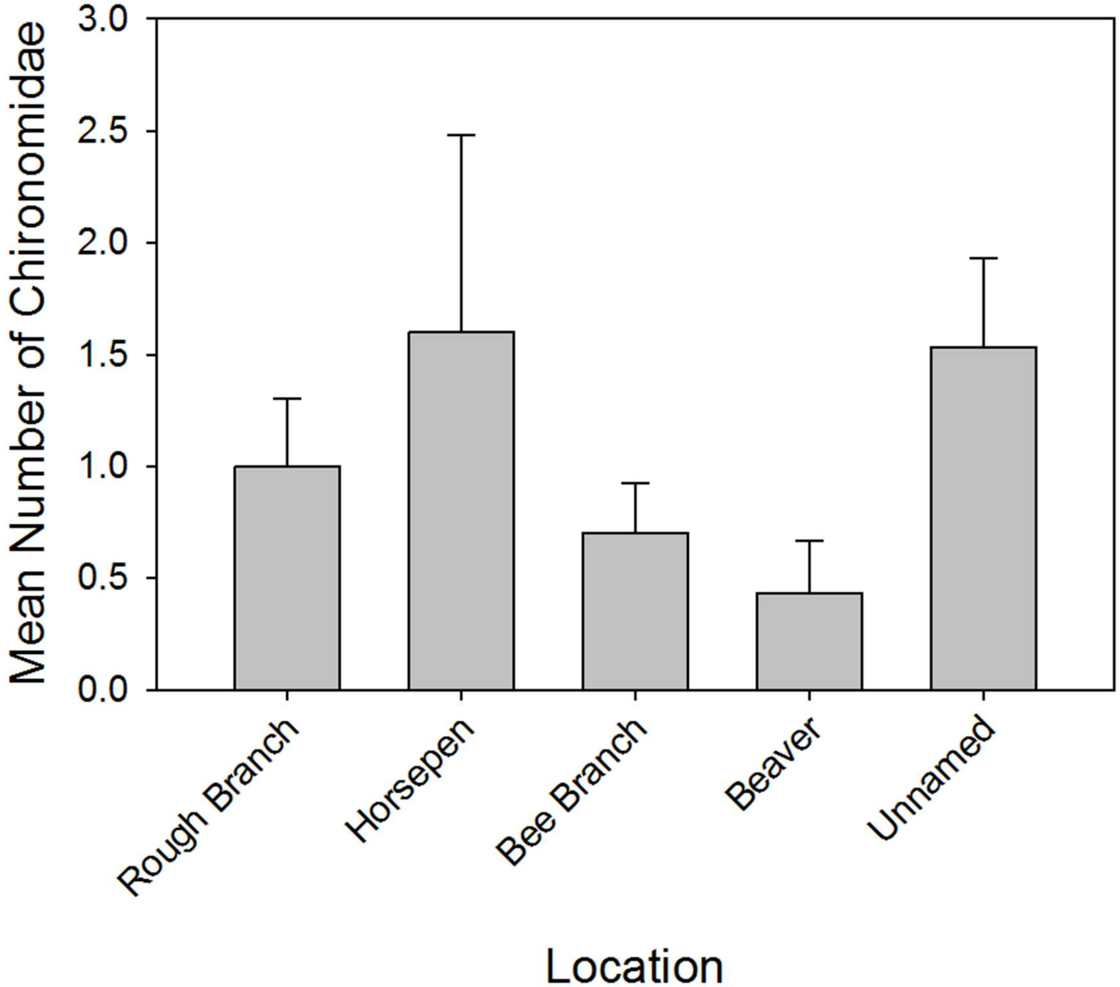

Figure 7. Graph of Kruskal-Wallis results - mean number of Chironomidae by tributary of the Lower Mountain Fork River

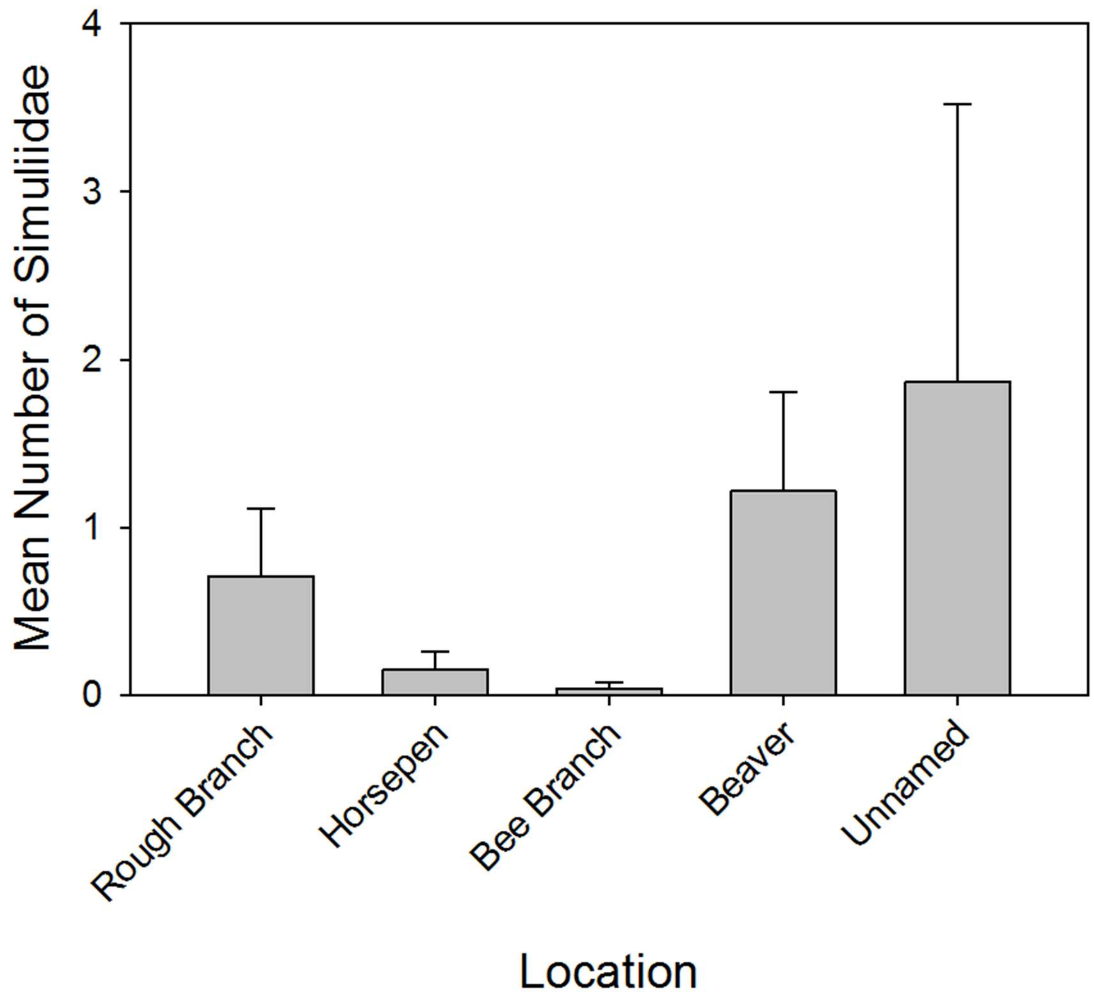

Figure 8. Graph of Kruskal-Wallis results - mean number of Simuliidae by tributary of the Lower Mountain Fork River

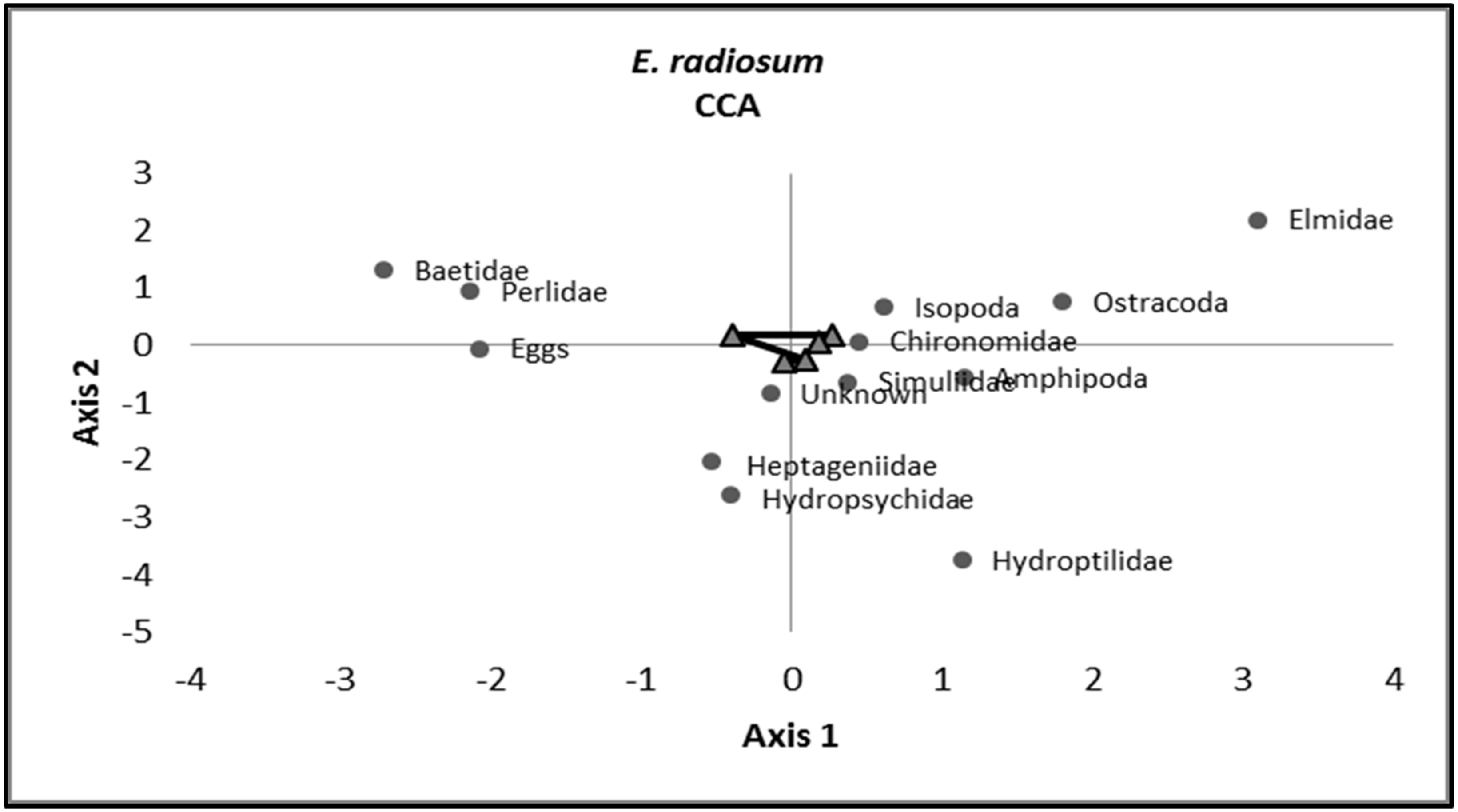

Figure 9. Bi-plot of the first two canonical correspondence analysis axes relating prey items consumed by orangebelly darters, *Etheostoma radiosum,* to tributaries of the Lower Mountain Fork River. Solid circles represent prey item scores and triangles represent tributaries.

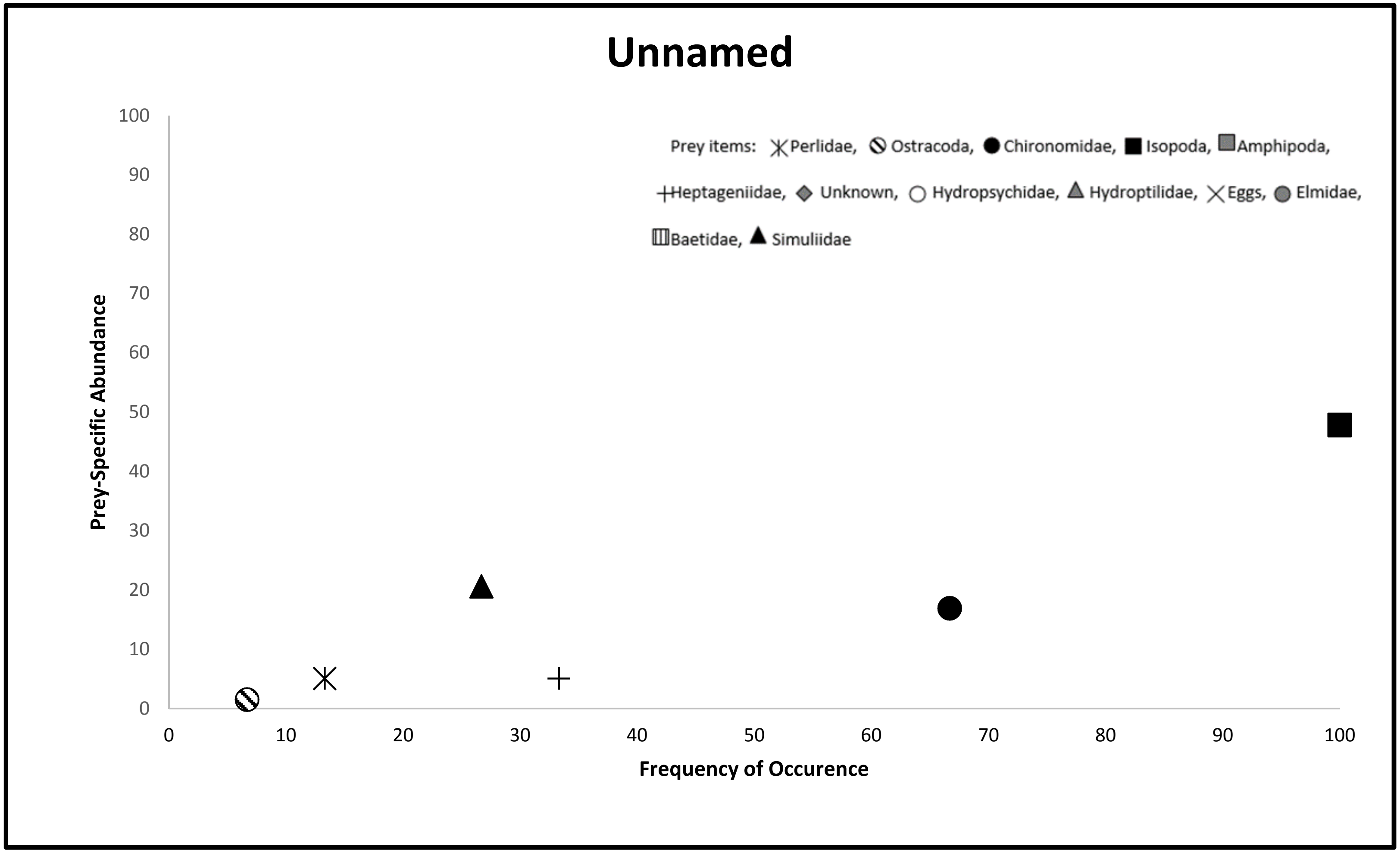

Figure 10. Plot of prey-specific abundance against frequency of occurrence for Unnamed tributary (N = 15).

*Amphipoda, Baetidae, Hydroptilidae, Elmidae and Eggs prey items did not occur in darter stomachs in Unnamed tributary

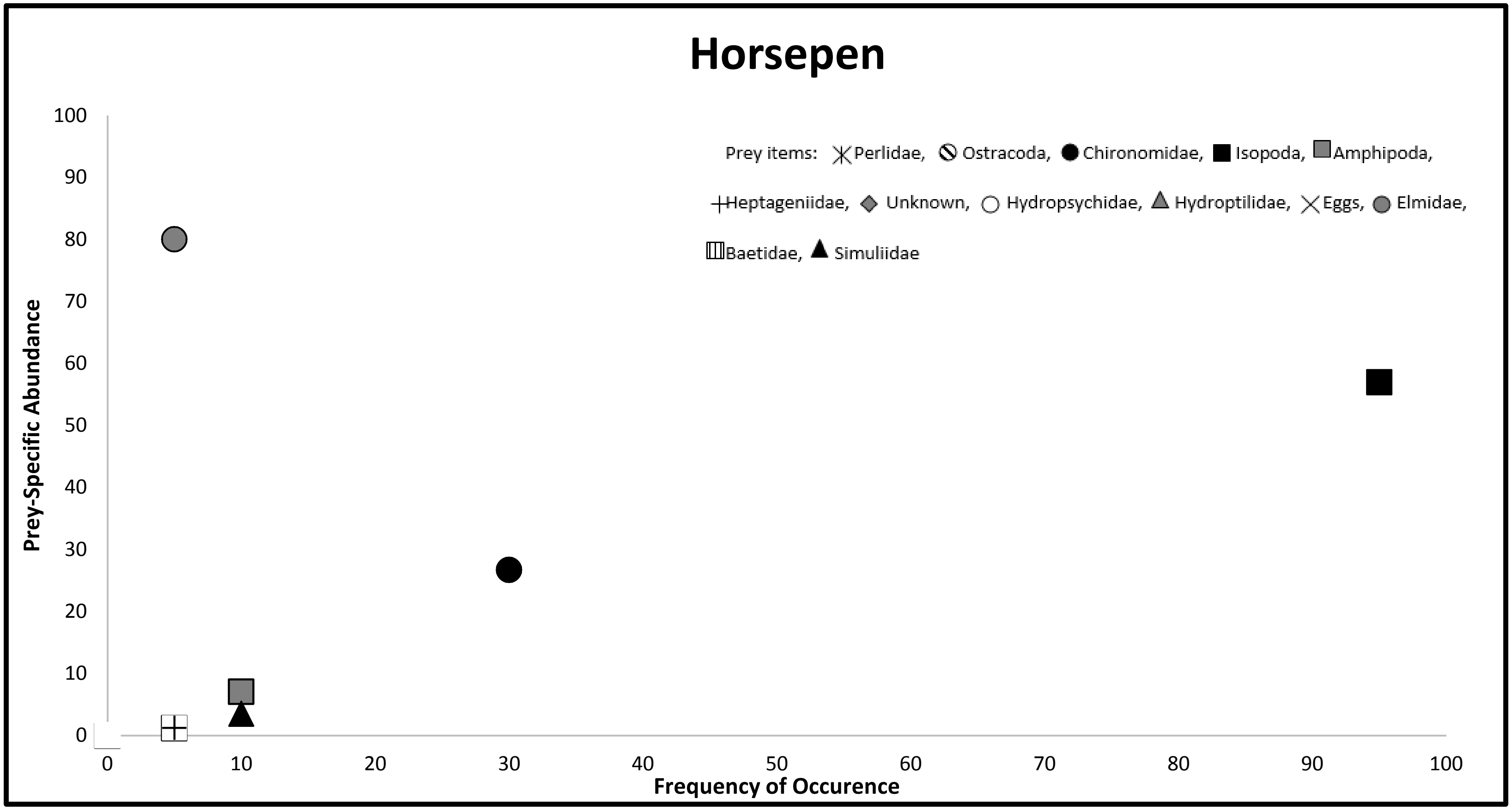

Figure 11. Plot of prey-specific abundance against frequency of occurrence for Horsepen Creek (N = 20).

*Baetidae, Hydropsychidae, Hydroptilidae, Ostracoda and Eggs prey items did not occur in darter stomachs in Horsepen Creek

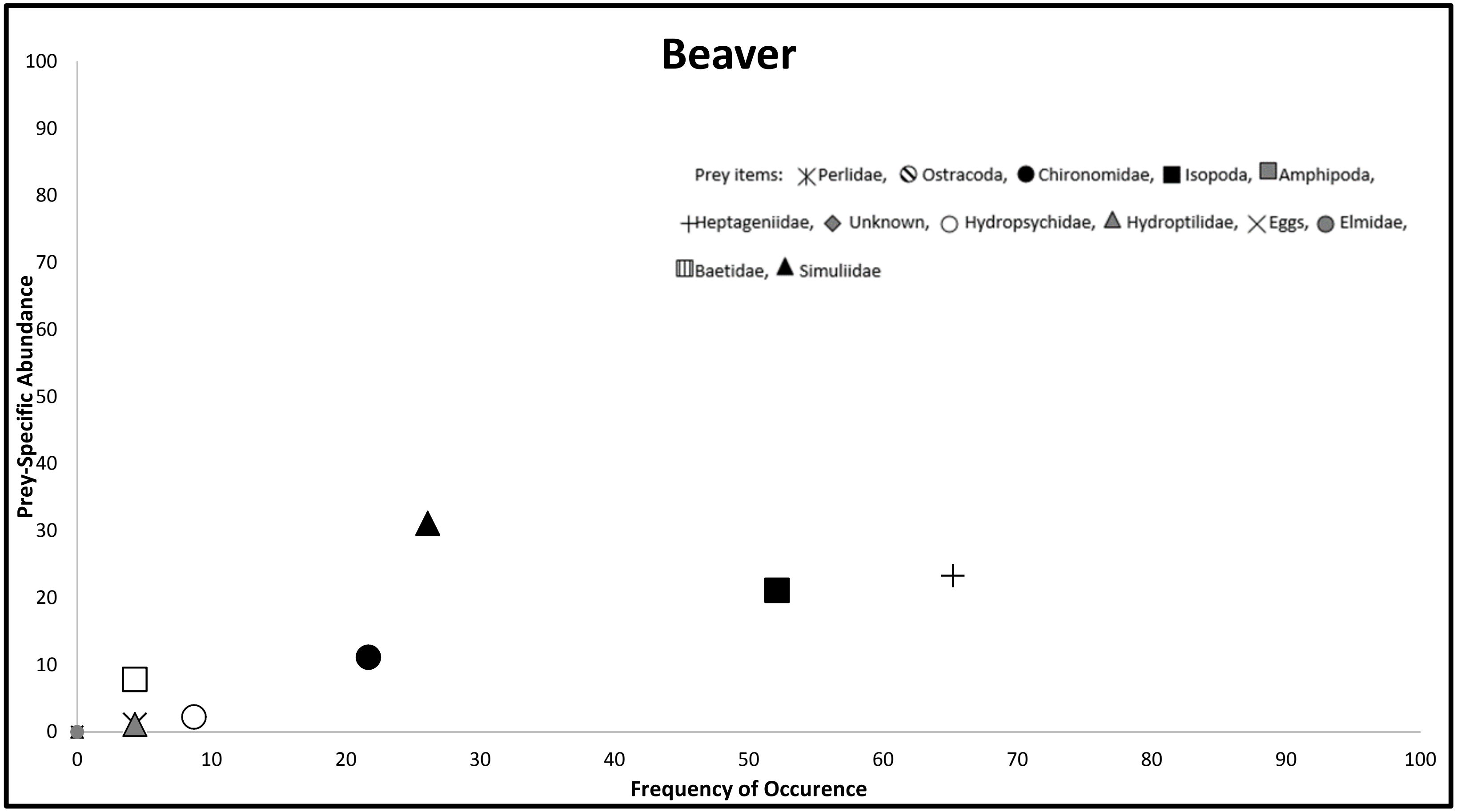

Figure 12. Plot of prey-specific abundance against frequency of occurrence for Beaver Creek (N = 23).

* Baetidae, Elmidae, Ostracoda and Unnknown prey items did not occur in darter stomachs in Beaver Creek

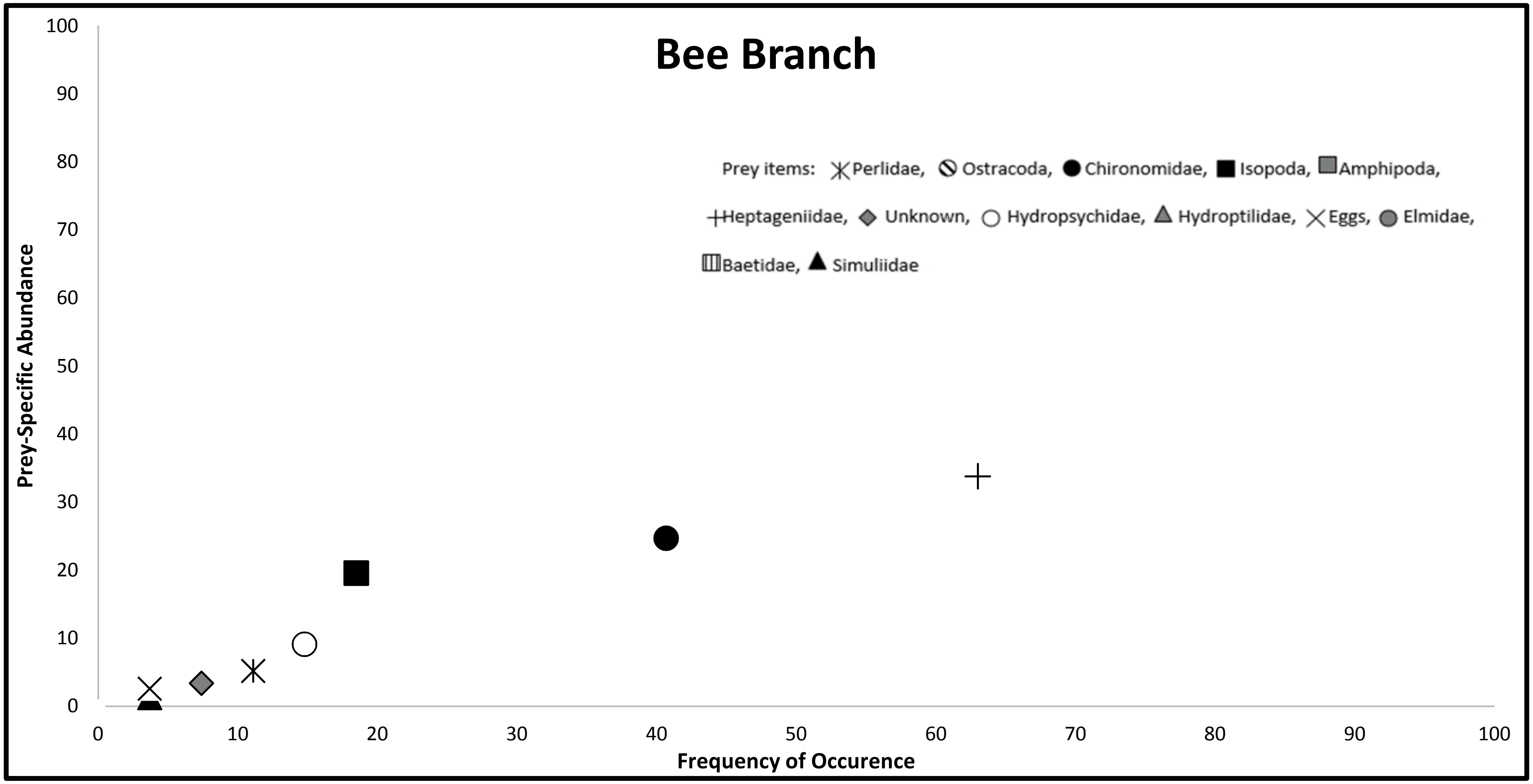

Figure 13. Plot of prey-specific abundance against frequency of occurrence for Bee Branch (N = 27).

*Amphipoda, Baetidae, Hydroptilidae, Elmidae and Ostracoda prey items did not occur in darter stomachs in Bee Branch

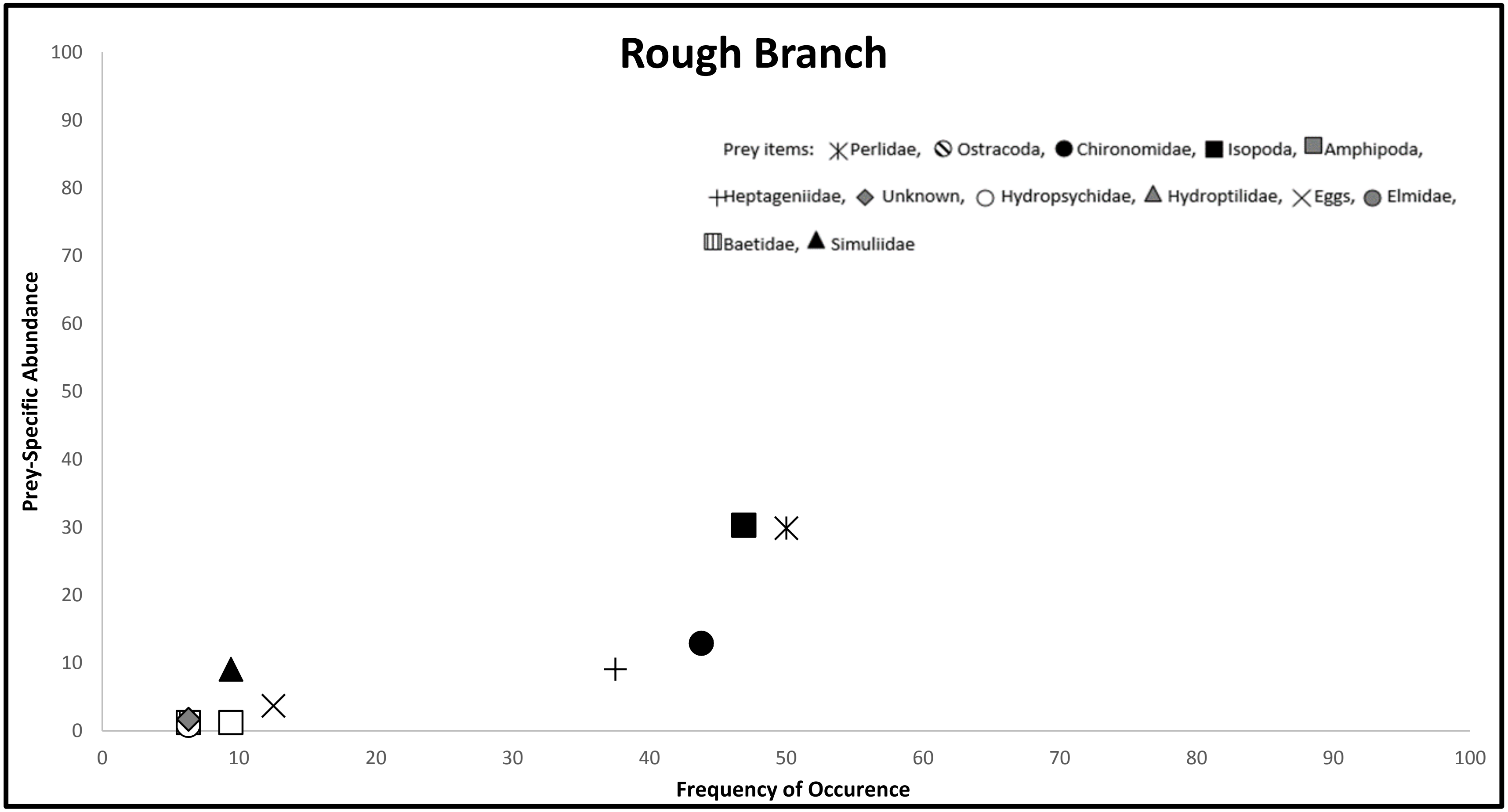

Figure 14. Plot of prey-specific abundance against frequency of occurrence for Rough Branch (N = 32).

*Hydroptilidae, Elmidae and Ostracoda prey items did not occur in darter stomachs in Rough Branch

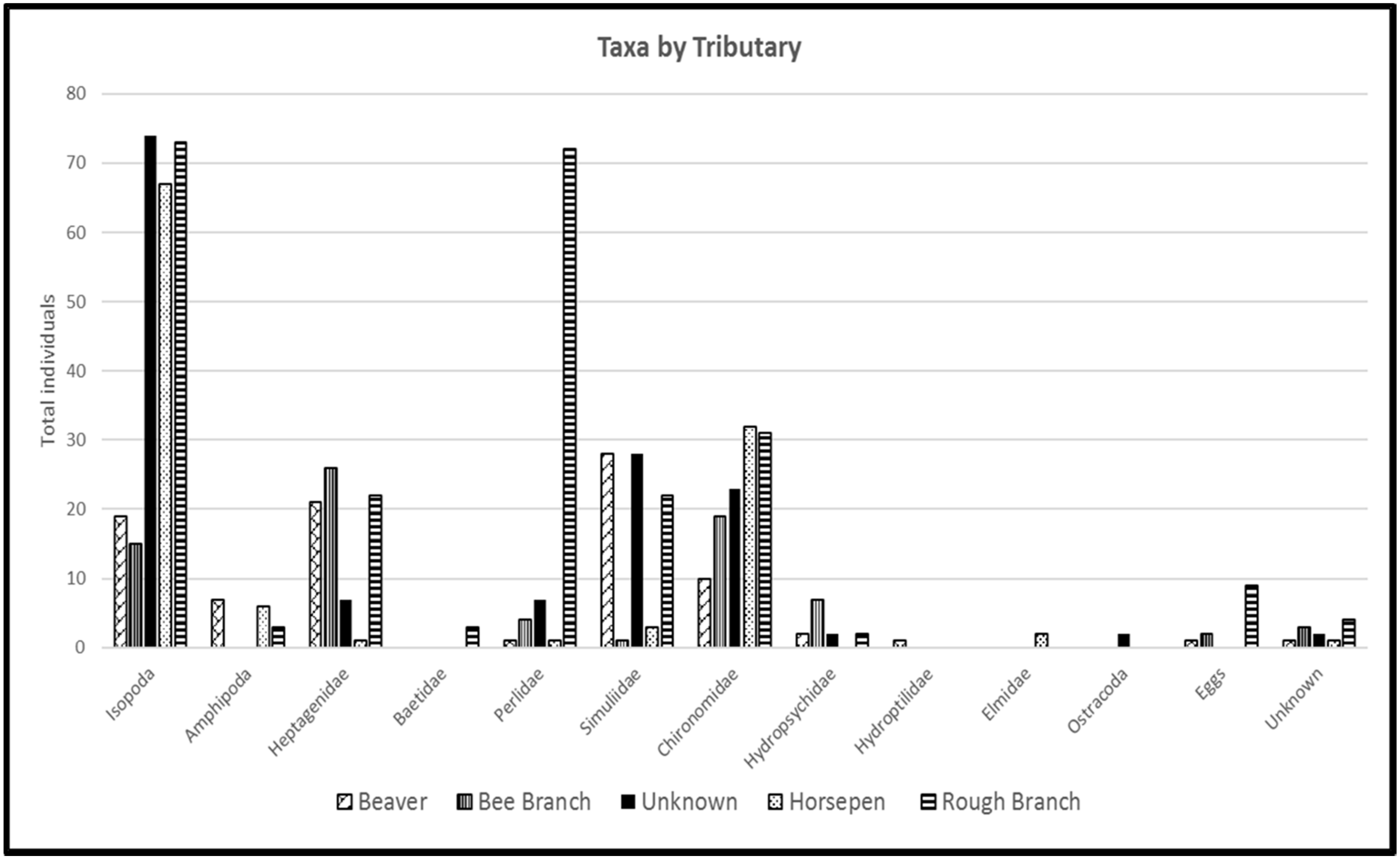

Figure 15. Total number of prey items consumed by orangebelly darters, *Etheostoma radiosum,* by tributary

VITA

Melissa Reed

Candidate for the Degree of

Master of Science

Thesis: DIET OF THE ORANGEBELLY DARTER, *ETHEOSTOMA RADIOSUM,* AMONG TRIBUTARIES OF THE LOWER MOUNTAIN FORK RIVER

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