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Assessment of Macroinvertebrate Communities and Food Availability for the Larval Eastern Hellbender Salamander (Cryptobranchus alleganiensis) in Northern Georgia

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ASSESSMENT OF MACROINVERTEBRATE COMMUNITIES AND FOOD AVAILABILITY FOR THE LARVAL EASTERN HELLBENDER SALAMANDER (*CRYPTOBRANCHUS ALLEGANIENSIS*) IN NORTHERN GEORGIA

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ABSTRACT

Macroinvertebrates are indicators of stream health and environmental change, and form complex communities in aquatic ecosystems. In addition to their role as indicators of stream health, they also provide a valuable food source for iuvenile salamanders. including the Eastern Hellbender manv (Cryptobranchus alleganiensis), a species of special conservation concern in Georgia. Therefore, macroinvertebrate diversity is not only an essential indicator of stream health, but also provides additional information on food availability for larval hellbender salamanders, a largely unknown life history stage for the species. We sampled during July of 2016, and report on the Ephemeroptera, Plecoptera, and Trichoptera (EPT) richness and diversity of macroinvertebrate communities in Georgia Appalachian streams that were concomitantly monitored for Eastern Hellbender populations. Over 1,200 macroinvertebrates were collected and identified from four streams in Georgia's Toccoa River basin. These specimens were keyed out to the lowest taxonomic level possible in order to provide a macroinvertebrate profile for streams with stable, healthy hellbender populations with confirmed gilled hellbender larvae. Macroinvertebrate communities comprised over 29 genera across a wide range of functional feeding groups, with biotic indexes indicating both high diversity and high water quality. EPT richness index and percent EPT were also extraordinarily high across all sampled streams (15-21 and 66.1-89.5%, respectively). This macroinvertebrate profile suggests that the larval Eastern Hellbender salamander populations in these streams have diverse macroinvertebrate prey species available to them. This research provides insight into the association of larval Eastern Hellbender populations with macroinvertebrate communities, and can be used by conservation managers to inform preservation of the natural integrity of Appalachian streams.

Keywords: freshwater ecology, macroinvertebrates, Cryptobranchidae, salamander diet, stream health

INTRODUCTION

Anthropogenic habitat loss and degradation are major threats to freshwater ecosystem biodiversity (Dudgeon et al. 2006). Amphibians are among the most imperiled stream taxa, and are often considered important indicator species characterized by increased sensitivity to environmental change due to their highly permeable skin and reliance on subcutaneous respiration (Stuart et al. 2004; Welsh and Ollivier 1998). Stream salamanders which occur in the Appalachian regions form community assemblages which are integral to ecosystem processes (Davic and Welsh 2004; Keitzer and Goforth 2012).

Like salamanders, macroinvertebrates are also considered indicators of stream health. Among the most important indicator species in these regions are the diverse taxa of macroinvertebrates, integral for overall stream health, and presumed to be the primary food source available for larval salamanders (Hecht et al. 2017; Walsh 2005; Schultheis and Batzer 2005). Larval hellbenders readily consume food by the fifth month (Smith 1907), which includes primarily invertebrate prey from the aquatic insect orders Ephemeroptera, Megaloptera, Trichoptera, and Diptera (Hecht et al. 2017; Pitt et al. 2006). Benthic macroinvertebrate community structure (relative abundance, taxa richness, etc.) provides a direct indicator of anthropogenic environmental stressors due to aquatic insect sensitivity or pollution tolerance (Klemm et al. 2003; Kodani 2018). For these reasons, fully aquatic larval salamander habitat requirements (in stream microhabitat) and their diet (macroinvertebrate communities) represent ideal model systems for studying anthropogenic pollution and environmental disturbance (Lowe and Bulger 2002; Raphael et al. 2002).

Eastern Hellbender salamanders are completely aquatic and found in many streams ranging from Missouri across the eastern United States to New York (Petranka 1998). Several of these hellbender populations have been documented to suffer from low juvenile recruitment (Burgmeier et al. 2011; Wheeler et al. 2003). Proposed reasons for these declines include siltation, water pollution due to industry and agriculture, and illegal collection (Nickerson and Briggler 2007; Wheeler et al. 2003). Larval and juvenile age classes can be difficult to detect due to their cryptic nature, small size, and use of microhabitat substratum (Gillespie 2010), resulting in considerable gaps in our knowledge of larval life history of this unique species (Foster et al. 2009).

In Georgia, the Eastern Hellbender is primarily found in clear, cool flowing streams of the northern portion of the state including the Toccoa River basin (Petranka 1998; Humphries 2005). While recent monitoring efforts for this unique fully aquatic salamander have furthered our understanding of stable, healthy, adult, Eastern Hellbender populations in Georgia, there remains a paucity of research on the diet and habitat requirements (water quality and in-stream habitat) of early life history stages (gilled larvae under 2 years). Hence, in the case of Eastern Hellbenders, a candidate species for listing under the Endangered Species Act (USFW 2011), there is a critical need for assessing the diet (food availability) of early life history stages (juveniles, or gilled larvae) in order to properly assess appropriate conservation management strategies and monitoring. However, to date there have been no studies to empirically characterize the macroinvertebrate communities of streams that contain larval Hellbenders in Georgia, presumably one of the more stable populations throughout the species' geographic range. Therefore, the goal of this study was to quantify and characterize the food availability and macroinvertebrate community assemblage for larval Eastern Hellbender streams based on Ephemeroptera, Plecoptera, and Trichoptera (EPT) richness, pollution tolerance indices, and functional feeding groups. Our study design is limited to macroinvertebrate communities of only healthy, stable populations of Eastern Hellbenders with confirmed, successful recruitment (presence of juveniles and gilled larvae). Furthermore, these findings provide the first quantitative assessment of macroinvertebrate prey availability and concomitant water quality attributes of larval hellbender-occupied streams across northern Georgia.

MATERIALS AND METHODS

Study Sites

Field locations were chosen in coordination with the Georgia Department of Natural Resources with sampling occurring in stream reaches with known occupancy (individuals present) of younger age classes of Eastern Hellbenders (S.U. unpublished data). Samples were collected from four streams in the Toccoa River watershed (TC1, TC2, TC3, and TC4) between July 11th and 12th 2016. Specific stream names are being withheld according to Georgia Department of Natural Resources to protect these populations from potential illegal collection. For each stream sampled we categorized the reach surveyed as either riffle or run habitat. For each river, Surber samplers were used to collect macroinvertebrates, using a list of random numbers to select random sites within each stream. Macroinvertebrates for each stream were collected from a total of 20 Surber sample locations (10 Surber samples from riffle habitat and 10 Surber samples from a run habitat). For each site, we standardized our placement of insect collection and sampled \sim 3 riffles and 3 runs each consisting of \sim 50 m in length and pooled samples for each stream into either riffle or run. We sampled both riffles (which contain the majority of aquatic insects) and runs, since Eastern Hellbenders are typically found in runs (Freake and DePerno 2017). Surber samplers resemble standard D-nets but also allow for quantitative collection of aquatic insects by sampling a defined area (0.3 m²). Moreover, the use of Surber samplers may allow for detection of more taxa including cryptic insect species (Storey et al. 1991). For each Surber sample, we gently scraped all surfaces of substrate and stream bed to prevent damage of macroinvertebrates. We initially sorted samples in the field and picked macroinvertebrates from the net using spring steel forceps with additional bucket washes to ensure collection of all insects. We then placed all collected macroinvertebrates in 95% ethanol for storage.

In addition to these biotic surveys, we characterized water quality environmental variables including temperature, pH, percent dissolved oxygen, percent specific conductivity, and percent total dissolved solids. We used an HM Digital TDS tester for total dissolved solids, and standard LaMotte water quality test kit for remaining water quality measurements. Additional characterization of the physical stream habitat within survey locations was completed by estimating substratum composition for each stream by measuring 100 randomly selected bed particles measured with a gravelometer (Wolman 1954).

Laboratory Component

The laboratory component of this research project involved the use of dichotomous keys to enumerate and identify macroinvertebrate assemblages using a dissecting microscope at Wingate University. Initial sorting of the samples based on morphology was followed by further identification based on the dichotomous key of Merrit et al. (2008), which was used to sort organisms down to genus level. These identifications were conducted by the first author (S.K.) and confirmed by author (S.U.).

Data Analysis

In order to assess diversity and overall water quality of the sampled streams, several indices were selected. The organisms were then categorized by family and those identified as either Ephemeroptera (E), Plecoptera (P), or Trichoptera (T) were used to calculate the total percent EPT overall and for each riffle and run stream (TC1, TC2, TC3, and TC4). This calculation consisted of finding the sum of EPT classified individuals in each set and dividing this number into the total number of specimens collected for the respective Toccoa River watershed stream.

We calculated the Shannon diversity index of streams with a score from one to five, and further examined overall water quality using the Hilsenhoff biotic index (Hilsenhoff 1987). The Hilsenhoff biotic index bases scores for each organism on the overall tolerance of the organismal family, calculating a final score between one and ten with the highest quality having a score of one. To further relate our macroinvertebrate community tolerances to other published studies, we used the Georgia benthic macroinvertebrate index, specifically designed for Georgia streams to determine water quality scores for each stream as well as for individual organisms to assess local water quality scores within Georgia at a local state wide scale modified from Helms et al. 2009 and Georgia DNR (2007). Diversity estimates were confined to aquatic insects, members of the class Insecta to relate findings to potential larval hellbender diet and more readily examine overall indicators of stream health. Moreover, according to one of the few published studies on larval salamander diet in our geographic area, (Eurycea cirrigera, southern two-lined salamanders), stomach contents primarily include aquatic insects (Barrett et al. 2012). We report on total numbers of other organisms (crayfish, salamanders, etc.) sampled in Surber samples. Furthermore, organisms identified were divided based on their functional feeding group for each of the streams to compare and contrast the presence of organisms filling various niches between sample sites. The typing of organisms into their functional groups was determined from the appendix of the same key used in identification (Merrit et al. 2008).

RESULTS

In total across all four streams known to contain healthy populations of larval hellbenders, over 1,200 individuals were identified across 25 families, not including organisms outside of class Insecta (Table I). Other organisms were also listed for each stream if they were found in Surber samplers. For TC1, these included 8 crayfish of the genus Cambarus, 12 sculpins of the genus Cottus, and 2 salamanders of the genus Eurycea; in TC2, 1 sculpin (Cottus) was captured, as well as 1 salamander (Eurycea) and 1 snail of the genus Leptotoxis. In TC3, 2 crayfish (Cambarus) were captured, as well as 1 snail (Leptotoxis); TC4 had the highest number of organisms outside of the class Insecta, with 3 crayfish (Cambarus), 4 sculpins (Cottus), 3 salamanders (Eurycea), and 13 snails (Leptotoxis). No Eastern Hellbender salamanders were captured in Surber samplers across all streams. In total, 1,246 macroinvertebrates were successfully keyed out with no subsampling, comprising 1,013 in riffles and 233 in runs across all Georgia streams. The majority of insects collected include representatives from families Hydropsychidae and Philopotamidae in the order Trichoptera, followed by families Perlidae and Leuctridae in the order Plecoptera (Figure 1). Additionally, all four streams exhibited not only healthy, diverse scores based on the Shannon diversity index (Table II), but also high percent EPT values (Table III) and Hilsenhoff biotic index scores. Notably, TC2 had a percent EPT of 93.4% in run habitat and just over 89% overall. These high scores were closely followed by TC1, with an overall percent EPT of 85.3%. TC2 was the only stream in which EPT was higher in the runs than in the riffles. Additionally, all of the streams scored in the highest quality tier of the Hilsenhoff biotic index, indicating high quality based on the organisms living in each stream. Furthermore, it is notable that TC3 had a very high percentage of shredder feeding group organisms as compared to the other streams, with a score of 23.44%; the next highest percent was 13.91% in TC4. TC1 had the highest percent of filterers, at 43.85%; TC2 and TC4 had the highest percent of predator species, at 35.70% (Table IV). Overall, most insects were categorized as filterers (Figure 2). Macroinvertebrates from Georgia hellbender streams exhibited a range of both Georgia BMI tolerance values and functional feeding groups (Appendix).

Water chemistry data collected for each stream (dissolved oxygen, water temperature, pH, and total dissolved solids) was similar across streams. TC3 had the highest total dissolved solids. Specifically, TC1 had a dissolved oxygen percent of 89.1, water temperature of 20 °C, pH of 7.1, and TDS of 6.8 mg/L. TC2 exhibited a dissolved oxygen percent of 91.2, water temperature of 19.2 °C, pH of 6.6, and TDS of 9.1 mg/L. TC3 values were 89.5% dissolved oxygen, 21 °C, 7.5 pH, and 10 mg/L TDS. TC4 had a dissolved oxygen of 93.5%, temperature of 19.3 °C, 7.4 pH, and 8.9 mg/L TDS. Wolman pebble count median and mean substrate size for streams was similar across all four streams and ranged from 38.5 mm median, 48.6 mm mean for TC3, to 45 mm median, 61.7 mm mean for TC4.

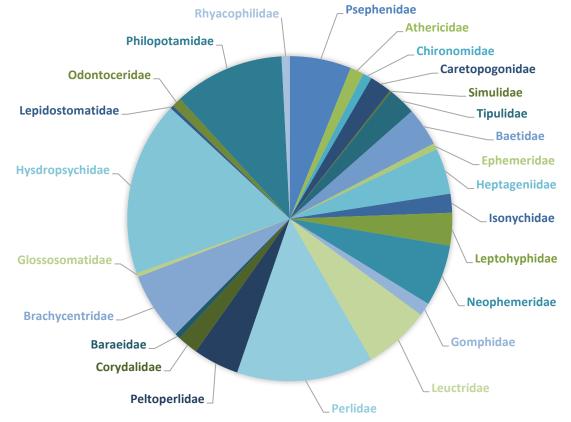


Figure 1. Insect family groups found in the Toccoa basin (across all streams TC1, TC2, TC3, and TC4; combined riffles and runs). Data represent field collections from July 2016.

Order:Family	Genus	TC1	TC2	TC3	TC4	Total
Coleoptera						
Psephinidae	Psephenus	8	2	25	40	75
Diptera	-					
Athericidae	Atherix	3	-	-	14	17
Chironomidae	Paramerina	5	2	1	3	11
Ceratopogonidae	Dasyhelea	_	18	3	6	27
Simuliidae	Greniera	1	-	1	-	2
Tipulidae	Tipula	7	9	9	9	34
Empemeroptera	-					
Baetidae	Baetis	13	15	14	5	47
Ephemeridae	Ephemera	-	1	2	5	8
Heptageniidae	<i>Maccafertium</i>	13	11	20	12	56
Isonychidae	Isonychia	2	7	9	5	23
Leptohyphidae	Homoleptohyphes	4	6	-	3	13
	Leptohyphes	3	24	-	-	27
Neophemeridae	Neophemera	22	38	7	8	75
Odonata	•		Ū	,		, 0
Gomphidae	Progomphus	-	-	7	9	16
Plecoptera						
Leuctridae	Leuctra	16	27	19	19	81
Perlidae	Beloneuria	15	10	1	47	73
	Perlesta	49	34	10	1	94
Peltoperlidae	Tallaperla	-	8	1	38	47
-	Viehoperlaada	6	2	-	1	9
Megaloptera	-					
Corydalidae	Corydalus	10	3	4	25	42
Trichoptera						
Baraeidae	Baraea	3	3	1	-	7
Brachycentridae	Brachycentrus	38	3	19	15	75
·	Micrasema	-	1	4	3	8
Glossosomatidae	Glossosoma	3	1	-	1	5
Hydropsychidae	Hydropsyche	78	87	30	18	213
Lepidostomatidae	Lepidostoma	2	1	1	-	4
Odontoceridae	Pseudogoerasingularis	-	1	-	11	12
Philopotamidae	Dolophilodes	47	30	-	58	135
Rhyacophilidae	Rhyacophila	4	4	1	1	10
Total:	÷	352	348	189	35 7	1246

Table I. Insects found in the Toccoa basin (streams TC1, TC2, TC3, and TC4; combined riffles and runs), and their relative richness in each stream (keyed out to genus). Data represent collections from July 2016.

Stream	Shannon Diversity Index	Hilsenhoff Biotic Index	Average Index
TC1	2.614	2.56	2.587
TC2	2.623	3.31	2.967
TC ₃	2.648	2.88	2.764
TC4	2.848	2.56	2.704

Table II. Scoring of the surveyed streams based on three biotic indices, including the Shannon diversity index, the Hilsenhoff biotic index, and the average index for aquatic insects found in the stream from July 2016

Table III. Stream quality using percent EPT, including percent Ephemeroptera (E), percent Plecoptera (P), and percent Trichoptera (T) separately to indicate differences

Stream	%Ephemeroptera	%Plecoptera	%Trichoptera	% EPT- Riffle	% EPT- Run	% EPT- Stream	EPT Richness Index	
TC1	15.2	23.8	46.8	88.4	70.8	85.0	17	
TC2	29.1	23.1	37.3	88.4	93.4	89.5	21	
TC3	33.3	16.2	29.2	77.8	59.7	72.4	15	
TC4	16.5	28.1	28.1	70.9	40.1	66.1	18	

Table IV. Functional groups based on percent of the total number of collected species

Functional					
Group	TC1	TC2	TC3	TC4	
% shredders	6.68	18.37	17.71	18.37	
% scrapers	6.42	3.99	23.44	13.91	
% filterers	43.85	23.88	26.04	23.88	
% gatherers	12.03	7.09	14.06	7.09	
% predators	20.10	35.70	17.19	35.70	

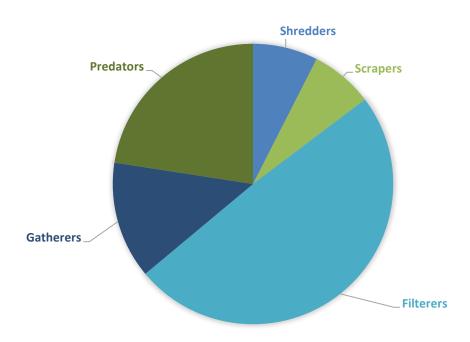


Figure 2. Insects found in the Toccoa basin (across all streams TC1, TC2, TC3, and TC4; combined riffles and runs). Data represent field collections from July 2016.

DISCUSSION

This study reports on the first characterization of macroinvertebrate communities present and thus food available to larval Eastern Hellbender salamanders. The results in this study are consistent with the original expectation that these streams, each with known successful recruitment of juveniles, i.e. occupied by gilled larval hellbenders, would have both high diversity and high stream quality indicated by their macroinvertebrate communities. The results were even better than anticipated, as in some of the streams, TC2, for example, exhibited an extremely high percent EPT, especially in the runs where larvae are commonly encountered. The high percent EPT of Eastern Hellbender occupied streams (66.1-89.5; Table III) in the Toccoa watershed is notably a higher percentage than reported for similar sized streams in geographic proximity to our study streams, where it ranges from 13 to a maximum of 61 %EPT (Nuckols and Roghair 2005). This is of particular significance as larval hellbender streams are characterized by a high presence of EPT organisms. This observation could indicate a high quality, abundant food source for larvae in these streams. Other studies in geographic proximity to our study location also yielded a large number of Ephemeroptera and Plecoptera (Rowe et al. 2017), while other studies have found high EPT richness in areas with largely forested cover and riparian buffer (Roy et al. 2003). Moreover, we found a diversity of aquatic insects within each stream based on sampling only ~2 m² of habitat (10 Surber samples in runs and 10 Surber samples in riffles) per stream. This indicates streams containing hellbender larvae have diverse food available to them within even a relatively small confined area (~1,200 macroinvertebrates per ~8 m² total across streams). Therefore, we conclude that high quality macroinvertebrates coincide with known populations of larval Eastern Hellbender salamanders in Georgia.

One possible reason for the extraordinarily high water quality we observed in TC2 and other streams could be the land use in the 1 km buffer zone around the stream, which we observed to be almost entirely forested. Similar conditions are found in nearly all of the streams, with the land surrounding the study sites being predominately forested with some small portion used for agriculture. The exception to this is TC3, which has a higher percentage of agriculture as compared to forest in its riparian zone based on our observations near the sample location. One potential area where this becomes evident in our results is the high percentage of scraper functional feeding group of organisms for TC3. These organisms are known to scrape algae off of rocks and other hard surfaces, so their abundance may indicate a prevalence of algae growth resulting from exposure to fertilizers. Additionally, TC3 had the highest total dissolved solids and concomitantly the lowest percent of predator species. This may be an indicator of high abundance in other functional feeding groups as they consume other macroinvertebrates as prey. Therefore, their absence could indicate a lower abundance of prey organisms in TC3 than in the other streams sampled in this study. This is confirmed in Table I, as the total number of organisms collected (189) was much lower than in the other streams (~350). However, larval hellbenders have been found downstream of this location (S.U.), therefore these slightly reduced macroinvertebrate densities could indicate that this site may need further monitoring. Lastly, our diversity and stream quality characterizations for TC3 could be a result of TC3 having the lowest sample size (n = 189 vs. ~350 in others). The observation of high macroinvertebrate diversity found on primarily forested land (Kodani 2018) seems to indicate that one potential requirement for a reproductively successful hellbender population (larval diet) is an adequate buffer zone within the watershed, allowing for the proper balance of nutrients entering the stream in order to support a diverse macroinvertebrate community.

Another point of interest in our study is that the Shannon diversity index score of TC4 is the highest of the streams, yet its percent EPT is lowest and Hilsenhoff biotic index is similar to the others. This could indicate that a broader range of organisms were captured in TC4, but that many of these organisms were also intolerant to pollution. We suspect when sampling the run habitat, there were less aquatic insects overall, possibly due to TC4 being more of a high gradient stream with seasonably high flow decreasing some macroinvertebrate abundances in runs. However, percent EPT of stream is highest for TC1 and TC2, 85.0 and 89.5%, respectively. Notably, both TC1 and TC2 both contain consistently high densities of Eastern Hellbenders, with TC4 characterized by higher gradient, patchy concentrations of larval Eastern Hellbenders (S.U. and T.F. unpublished). TC3, with the lowest EPT richness index of 15 (Table III) and in close proximity to agricultural runoff, contains fewer overall observations of larvae compared to the other streams (S.U. and T.F. unpublished). Furthermore, it is important to note that all of the streams received an average diversity score according to the Shannon diversity index, but an "excellent" Hilsenhoff score. This could indicate that the streams all have adequate diversity and an overwhelming majority of organisms present in hellbender streams are intolerant of organic pollutants. Moreover, all streams had high percentages of dissolved oxygen, and exhibited a range in temperature and Wolman pebble counts, with TC3 having the highest total dissolved solids of 10 mg/L TDS. While our study largely reports on the aquatic insects available to larval hellbenders, we note the presence of crayfish, small sculpin, other salamanders, and snails may provide

additional food items. We recommend future studies incorporate these taxa in biodiversity estimates in larval hellbender streams.

Based on the results of our study, we recommend further sampling of potential prey items of larval Eastern Hellbenders with direct sampling of stomach contents to elucidate prev items consumed in Georgia. Our study lacks comparison to streams which are known to contain either low, moderate, or declining populations of Eastern Hellbenders, and thus low levels of larvae. Further studies could incorporate sampling across varying densities of hellbender populations, and assess ecological relationships between densities of larvae and macroinvertebrates. It is likely there are complex ecological relationships between macroinvertebrate communities and juvenile salamanders which utilize them for food. Therefore, conservation of Eastern Hellbender populations should incorporate assessment of macroinvertebrate communities as indicators of prey available to larval salamanders, which are important for recruitment into populations for long term viability. Moreover, we recommend further comparisons of sites which lack recruitment into local populations, or where larval hellbenders are typically not found, to examine macroinvertebrate communities in declining sites, perhaps across the range of the species. Due to our small sample size (four streams), generalizations regarding macroinvertebrate communities and Eastern Hellbender larvae should be interpreted with caution, as hellbender larvae densities may be correlated with other factors including overall stream quality and shelter habitat, and larvae may rely on multiple sources of prey for their dietary needs. Finally, wildlife managers should continue to monitor aquatic insect communities in these watersheds to detect future land use changes or any threats to overall stream water quality to the Toccoa River watershed of Georgia.

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APPENDIX

The Georgia benthic macroinvertebrate index value (GA-BMI) for each organism, using the average of all species scores within a genus. Omitted cells (-) have no GA tolerance values.

¥•	C		Functional Feeding
Order:Family	Genus G	A-BMI Tolerance Value	Group
Coleoptera			
Psephinidae	Psephenus	2.50	Scrapers
Diptera			
Athericidae	Atherix	2.10	Predators
Chironomidae	Paramerina	2.80	Predators
Ceratopogonidae	Dasyhelea	6.65	Gatherers
Simuliidae	Greniera	3.30	Filterers
Tipulidae	Tipula	7.70	Shredders
Empemeroptera			
Baetidae	Baetis	5.88	Gatherers
Ephemeridae	Ephemera	2.20	Gatherers
Heptageniidae	Maccafertium	3.74	Scrapers
Isonychidae	Isonychia	3.80	Predators
Leptohyphidae	Homoleptohyphes		Gatherers
1 11	Leptohyphes	-	Gatherers
Neophemeridae	Neophemera	-	Gatherers
Odonata	•		
Gomphidae	Progomphus	8.70	Predators
Plecoptera	· -		
Leuctridae	Leuctra	0.70	Shredders
Perlidae	Beloneuria	0.00	Predators
	Perlesta	2.45	Predators
Peltoperlidae	Tallaperla	1.40	Shredders
•	Viehoperlaada	1.40	Shredders
Megaloptera	•		
Corydalidae	Corydalus	5.60	Predators
Trichoptera	v		
Baraeidae	Baraea	-	Gatherers
Brachycentridae	Brachycentrus	1.47	Filterers
•	Micrasema	0.30	Shredders
Glossosomatidae	Glossosoma	1.29	Scrapers
Hydropsychidae	Hydropsyche	4.16	Filterers
Lepidostomatidae	Lepidostoma	1.00	Shredders
Odontoceridae	Pseudogoerasingu		Predators
Philopotamidae	Dolophilodes	1.00	Filterers
Rhyacophilidae	Rhyacophila	0.80	Predators