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# Resource utilization by the Rio Grande silvery minnow at the Los Lunas Silvery Minnow Refugium

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## Introduction

Gut content analysis of fish and larval invertebrates has been widely used for many purposes, including monitoring water quality (Rosati et al. 2003), analyzing selective feeding patterns and resource use (Mittelbach et al. 1992, Peterson et al. 1998), illustrating algal taxa susceptibility to grazing (Peterson 1987, Edlund and Francis 1999), and determining diet shifts with life history development (Alverson et al. 2001). Additionally, food preference studies are often verified with gut analysis (Steinman 1996).

While gut content analyses are extremely useful, there are some limitations to interpretation of gut contents. For example, there can be disproportionately higher numbers of some food items (diatoms with silica cell walls, insects with sclerotized body parts) that are more likely to pass through the gut; other algal taxa and invertebrates have cell walls composed of less durable substances, such as cellulose, which are more easily digested and often unidentifiable in the gut (Gelwick and Matthews 2006). This is often remedied by comparing gut contents with the biotic assemblages in the system.

Research indicates that the Rio Grande Silvery Minnow (*Hybognathus amarus*, RGSM), like many grazers, has an omnivorous diet composed of benthic algae and invertebrates/crustaceans. The genus *Hybognathus* has pharyngeal teeth and pharyngeal taste buds, which may allow the fish to selectively filter diatoms (Hlohowskyj et al. 1989). However, it is likely that RGSM do not selectively graze only diatoms, but may also get nutrition from other periphyton and other invertebrates and crustaceans. Stable isotope analysis has shown that larval and juvenile fish in the Middle Rio Grande consume benthic algae as part of their diet during high flow periods in spring (Pease et al. 2006). Furthermore, gut content analysis of adult RGSM shows a dominance of diatom frustules, with some green algae and cyanobacteria in the guts (Shirey et al. 2008, Watson et al. 2009). Recent work investigating the gut content analysis of hatchery-raised Year-0 RGSM showed a diverse diet that included 60% formulated feed as well as 40% diatoms, filamentous algae and smaller percentages of insects and crustaceans (Watson et al. 2009). Seasonal shifts in algal and invertebrate communities may be reflected in the diet of the RGSM. In general, diatoms are generally considered to be a superior food source with high lipid content, while cyanobacteria are less palatable (Steinman 1996). Additionally, shifts in diatom species composition can result in changes of overall lipid content (Sicko-Goad and Andresen 1991). Therefore, shifts in algal community composition can affect food quality and quantity for grazers.

The Los Lunas Silvery Minnow Refugium is a facility designed to house RGSM. The facility is designed with concrete runs combined with overbank flooding into pools and vegetated habitats. Preliminary surveys from September 2010 show algal communities dominated by diatoms with some green algae and cyanobacteria (Aquatic Consulting and Testing Inc., D. Tave, pers. comm.). From the same surveys, the macroinvertebrates were primarily snails and *Chironomus*

midge larvae; the zooplankton community was dominated by rotifers with few other zooplankton taxa.

We conducted gut content analysis and benthic algal and invertebrate assemblages in the naturalized outdoor refugium. The RGSM in the refugium are not fed formulated feed, compared to hatchery fish; therefore, the results of the proposed gut content analysis and natural substrate analysis will be more representative of RGSM diets in the main channel of the Rio Grande. We used higher resolution taxonomy for algae and invertebrates to better understand feeding preference strategy and location of feeding within the refugium. Finally, the combination of lentic and lotic habitats sampled for natural substrates will better reflect the main channel geomorphology.

The questions proposed in this study are:

1. **Does the RGSM have selective feeding habits in the refugium?**

   By comparing RGSM gut contents to periphyton and invertebrate assemblages available from natural substrates in the refugium, we will be able to determine if the RGSM is selectively grazing on certain types of algae and invertebrates.

2. **Are there temporal changes in diet of RGSM?**

   Monthly sampling of gut contents and substrates in the refugium should determine the seasonal changes in the benthic communities in the facility.

## Methods

The refugium was first flooded on 28 February and 1 March 2011 and drained 5 June 2011 for another experiment. After draining, there was no standing water in the refugium, although sediment in pools was damp, until it was re-flooded (13 June 2011). Ammonium polyphosphate fertilizer (11%N:37%P:0%K) was added in 50 mL amounts on 14 June, 17 June, 4 July and 100 mg on 9 August and 7 September.

### General water quality

Monthly sampling was conducted in the refugium four times from July to October 2011: 26 July, 26 August, 20 September and 18 October (Figure 1).

Physical and chemical measurements were taken from two sites (one pool and one run habitat) (Figure 1). Water depth and a brief habitat description were recorded at each site. Water temperature (°C), specific conductance (µS/cm), pH, dissolved oxygen (mg/L, %), and salinity (ppt) were measured using a multiparameter water quality meter (YSI Model 556 MPS).

Turbidity (NTU) (as a surrogate for light attenuation) was measured using a portable turbidity meter (La Motte 2020e). Water samples were collected in replication (n = 3) from the water column in the run and pool, returned to UNM and filtered using a 47-mm diameter Millipore membrane filter (0.45 µm pore size) and a Swinnex filter apparatus and syringe.

***Nutrient analysis***

Anions were analyzed at the University of New Mexico Biology Annex Analytical Laboratory. $PO_4$-P (µg/L), $NO_3$-N (µg/L), $Cl^-$ (mg/L), $Br^-$ (µg/L), and $SO_4$ (mg/L) were analyzed using a Dionex DX-100 Ion Chromatograph, using Chromeleon 6.60 software. $NH_4$-N (µg/L) was analyzed using a colorimetric spectrophotometric method (AWWA 1998).

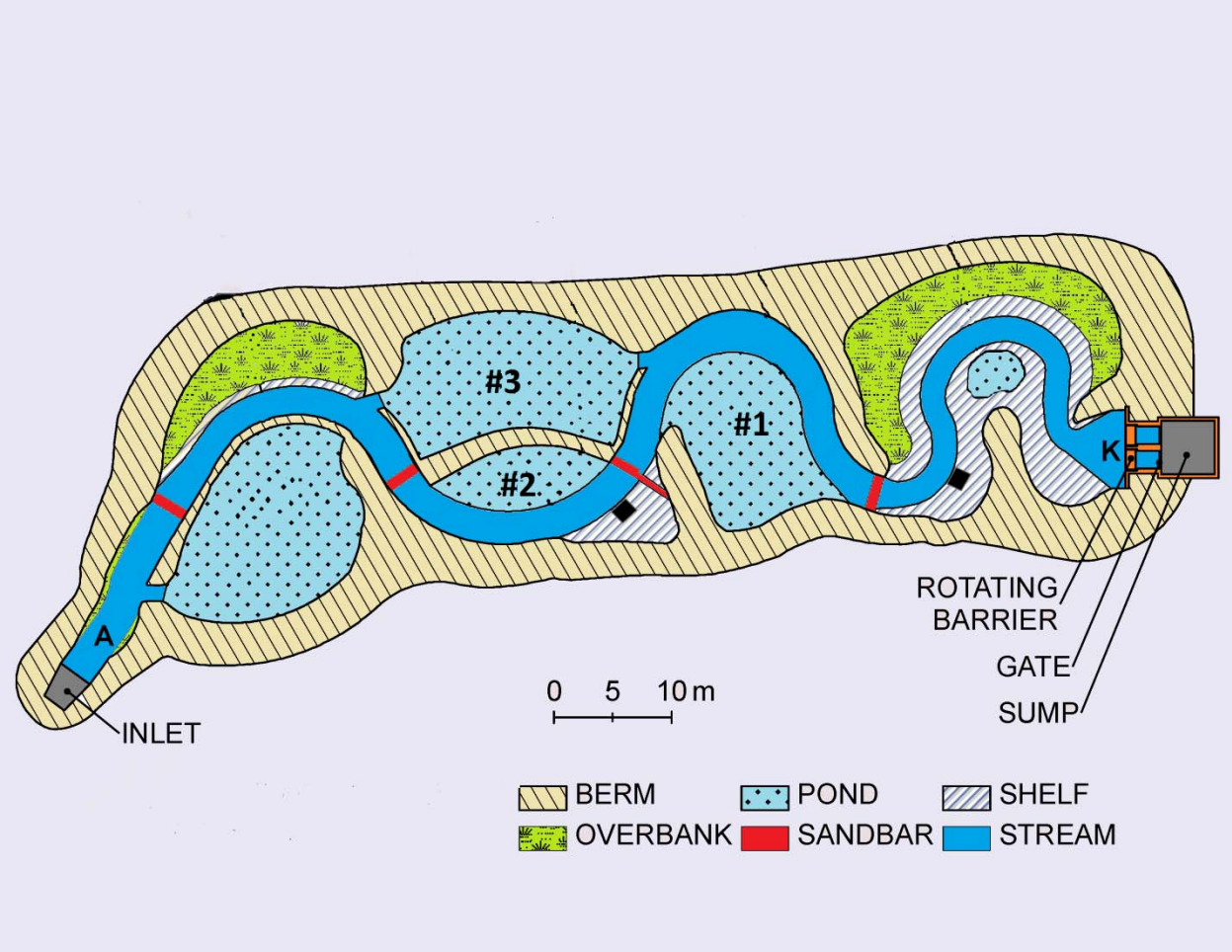



**Figure 1: Plan view of the Los Lunas Silvery Minnow Refugium. The run habitat is the solid dark blue section that flows from point A to point K. Pools # 1 (Pond 4), 2 (Pond 3), and 3 (Pond 2) respectively were used in this study (modified fromHutson et al. 2012).**

## Food resource sampling from natural substrates

### *Periphyton*

Benthic periphyton was quantitatively sampled from four habitats (epipelic habitats from pools, epilithic habitat from rocks, epiphytic samples from the submerged macrophytes, and samples from the water column in the refugium) (Figure 1). Three replicate epipelic samples were collected from each subsite using a 0.5-cm core made from a modified 60 mL syringe. Epilithic samples were sampled from the concrete raceway using a modified Loeb sampler (Loeb 1981). For epiphytic samples, submerged macrophytes were collected into plastic bags then returned to the laboratory where samples were scraped from the surface of the macrophytes. Dimensions of the macrophytes stems were recorded to later be used to calculate surface area of the stems. Ten liters of water were filtered from the water column using a plankton net (10 µm mesh). All samples were preserved in 10% formalin. Samples were standardized to 20 mL (water column = 10 mL).

Densities of filamentous cyanobacteria, unicellular green algae, and diatoms were determined using a Palmer-Maloney counting chamber at 425× magnification (brightfield optics) on a Zeiss Universal research microscope. Diatoms were enumerated (as "diatoms") and soft-bodied taxa were identified to genus and enumerated along one or more transects until ~200 live cells/units are recorded. Some cyanobacterial filaments that lack cell differentiation were counted in 10 micrometer lengths (one length = one unit) (Lowe and Laliberte 2006). In samples with extremely low cell densities, a maximum of 10 transects were examined.

To determine diatom species, 3 mL aliquots from each sample were processed using 30% hydrogen peroxide to oxidize organic material. These samples were rinsed seven times with distilled water to remove oxidation by-products. Processed samples were evaporated onto coverslips and mounted to microscope slides with Z-Rax mounting medium, making permanent slides. Specimens along transects were examined under oil immersion at 1250× magnification using brightfield optics. Three hundred valves were enumerated and identified to species-level along transect(s) from each sample. In samples with extremely low diatom densities, counting ceased after 10 transects. Identification of taxa was based on taxonomic literature including work from the southwestern United States (Czarnecki and Blinn 1978, Czarnecki et al. 1981). A database of digital images of Rio Grande diatoms was utilized for identification verification as well as diatom slides accessioned at the Museum of Southwestern Biology. Additionally, digital images have been recorded of all taxa found at the LLSMR.

### *Invertebrates*

Invertebrate fauna were collected from the water column and the same three substrates as periphyton samples.

To collect pelagic microcrustaceans, 10 L of water was passed through a 10-µm plankton net. Microcrustacea collected in the net were immediately preserved in 10% formalin.

Instead of sampling directly from the concrete surface in the runs, concrete pavers were used as quantitative sampling units. Collecting macroinvertebrates from smooth concrete surfaces is difficult to do quantitatively because it is difficult to contain a discrete area without the organisms escaping (and thereby causing an underestimation of organism density). The pavers were placed *in situ* in run habitats three weeks before the first sampling (7 July 2011), allowing time for invertebrate fauna from the surrounding area to colonize the pavers before sampling began. Pavers were collected as benthic run samples at monthly intervals without replacement (three pavers each month). Samples were collected by quickly lifting the paver out of the water and into a white sorting tray. Macroinvertebrates were removed from the entire surface of the paver with forceps, and then the pavers were scrubbed with a nail brush and rinsed with distilled water to remove smaller invertebrate fauna. Macroinvertebrates were not removed from the underside, as this did not simulate the concrete surface of the refugium. The paver was removed from the sorting tray, and then the sample was rinsed into a plastic bag and immediately preserved with formalin. In the laboratory, samples were rinsed through a fine sieve (47 µm mesh) then stored in ethanol (70% v/v).

In pool habitats with stones and sediment (rather than concrete) a mini Surber sampler was used to collect macroinvertebrates. The Surber sampler provided a sampling area of ~40 $cm^2$. Samples were collected by placing the Surber sampler with the open face of the net upstream, and then agitating the sediment within the sample area for 30 seconds. Invertebrates were collected in the 500- µm Nitex® net and then preserved in formalin.

Macrophytes were collected for epiphytic samples. Three to four stems (~15 cm length) were removed and placed into a plastic bag. In the laboratory, samples were examined under a dissecting microscope; invertebrates were manually removed and immediately preserved in ethanol. Dimensions of the macrophytes stems were recorded to later be used to calculate surface area of the stems.

In the laboratory, invertebrate fauna were sorted and identified to the lowest practical level using a dissecting microscope. Generally, microfauna were identified to order (Smith 2001) and macroinvertebrates were identified to family or genus (Kupferberg 1997).

Densities of organisms were extremely high in run (paver) and pool (kicknet) samples, so subsampling methods were adopted to decrease processing time. Run samples were split into twelve parts using a tackle-box subsampler (designed and built by Burdett) and pool samples were split into at least four parts using a Motodo subsampler (Motoda 1985). Pool samples contained heavy sediment, so a hypersaturated solution of Epsom salts ($MgSO_4$) was used to separate less dense organic matter from dense sediment (Biggs et al. 1998). For both pool and run samples, subsamples were randomLy selected to be sorted. A minimum of 500 individuals was counted from subsamples and then total abundance was calculated for the whole sample. The whole sample was also checked for large, rare invertebrates (Vinson and Hawkins 1996).

### Gut content analysis

Twenty-five RGSM were collected by Refugium employees during the monthly sampling (total for the study = 100). The fish were euthanized with an overdose of MS-222 to prevent gut evacuation. Fish were stored in 10% formalin and transported back to the laboratory at UNM for dissection.

For each fish, standard length was recorded. From at least 10 fish/month, the alimentary canal was removed from the esophagus to the anus; total gut length was measured as well as an estimate of filled gut length. Fish were dissected until ten fish were recorded with full guts. The gut was opened and all contents were removed. Macroinvertebrates and zooplankton were identified following methods described above. Invertebrate gut contents were recorded in two ways. Firstly, each occurrence of each type of organism was recorded (e.g. ostracod, chironomid larva). Secondly, the total volume of gut contents was estimated as number of squares covered on a grid (1 mm × 1 mm). Invertebrate gut contents were stored in formalin, while the gut and any remaining gut contents were stored together in formalin for periphyton analysis.

Following invertebrate identification and enumeration, periphyton was analyzed from the same gut samples using the Palmer-Maloney method described above for genus-level identification. For diatom species-level identification, the gut was boiled in 30% hydrogen peroxide in 10 mL centrifuge tubes to remove organic material. The rest of the diatom processing method was as described above. These methods are similar to other studies that have examined gut contents of fish for diatoms and other algae (Rosati et al. 2003).

### Data analysis

The benthic samples are reported as relative abundances and in units/sample area. However, the algal and invertebrate data are reported as relative abundances because of the difficulty in quantifying sample size. T-tests and nested ANOVAS were utilized to determine differences among habitats, prey items, and seasonality (Gelwick and Matthews 2006). Comparisons between natural substrates and gut content were calculated using the Ivlev's index of electivity (Ivlev 1961) which was used to determine feeding preferences. To analyze whether the fish were selective in their feeding habits, relative abundances of all prey items collected from the guts were compared to food items collected from benthic samples using Ivlev's index: $E = (R_i - P_i)/(R_i + P_i)$, where $_i$ = type of food, $R_i$ = relative abundance of food in diet, and $P_i$ = relative abundance of food in benthic samples. This index of electivity calculated a value between 1.0 and -1.0 for each food item. Values near 1.0 indicate a favored food item while a number close to -1.0 indicated an avoided food item.

Non-metric multidimensional scaling (MDS) was used to visually examine the multivariate assemblage data and compare among sample events and habitat types (Clarke and Gorley 2006). MDS is an ordination plot, based on a matrix of similarities between samples (Clarke 1993). The stress level indicates how well the two-dimensional plot represents the relationships among

assemblages and ranges from zero to one. Stress levels less than 0.2 are preferable (McCune and Grace 2002).

Analysis of Similarities (ANOSIM, in PRIMER) was used to examine statistical differences among multivariate assemblage data (Clarke 1994). ANOSIM conducts similar tests to an ANOVA, but uses non-parametric (randomization-based) methods to examine difference in taxonomic density and composition among sample units (e.g. sample event or habitat type). Several statistics are produced. (1) Global R describes the overall difference among all sample units (i.e., the degree of 'clumpiness' within sample units). When Global R = 0, there is no difference among sample units and the assemblages completely overlap, whereas when Global R = 1, there is no overlap among assemblages. (2) R statistics are produced from pairwise tests. R statistics indicate the degree of clumpiness between individual sample units (e.g., comparing assemblages from pool habitats to those from run habitats). (3) P values are produced for each test, indicating the significance level for both the global test and the pairwise tests.

## Results

### General water quality

Water quality parameters measured *in situ* differed very little over the four sample months and were very similar in the pool and run habitats (Table 1). Water temperature decreased seasonally over time, whereas salinity increased slightly. Turbidity was relatively low in the first three sample months and relatively high in October, although this small increase probably had no effect on the biotic assemblage. Levels of dissolved oxygen (DO), specific conductivity, pH, and flow velocity differed very little over the four sample months.

#### *Nutrient analysis*

Levels of nutrients differed very little between pool and run habitats or over the four survey months (Table 2). While some statistically significant differences were detected among sampling events (Table 3), these differences were small and not biologically significant and did not vary among habitats. Bromide concentrations, an indicator of groundwater inputs, remained stable throughout the sampling period. Notably, $PO_4$ and $NH_4$ were both below detection limits in all samples.

**Table 1. Summary of physiochemical parameters collected in the field each month.**

| | habitat | July | August | September | October |
|---|---|---|---|---|---|
| Water temperature (°C) | run | 25.78 | 24.70 | 19.57 | 12.72 |
| | pool | 25.96 | 24.67 | 19.46 | 12.82 |
| Salinity (ppt) | run | 0.29 | 0.32 | 0.37 | 0.37 |
| | pool | 0.29 | 0.32 | 0.37 | 0.37 |
| Turbidity (NTU) | run | 0.93 | 0.30 | 0.74 | 4.14 |
| | pool | 0.70 | 1.06 | 1.59 | 4.19 |
| DO (%) | run | 107.0 | 98.1 | 109.0 | 103.8 |
| | pool | 93.6 | 82.8 | 79.0 | 103.4 |
| DO ($mgL^{-1}$) | run | 8.67 | 8.86 | 9.95 | 10.96 |
| | pool | 7.62 | 6.94 | 7.24 | 10.84 |
| Specific conductivity ($\mu Scm^{-1}$) | run | 603 | 659 | 751 | 578 |
| | pool | 608 | 654 | 752 | 578 |
| pH | run | 8.80 | 8.80 | 9.00 | 8.64 |
| | pool | 8.83 | 8.79 | 9.01 | 8.63 |
| Flow velocity ($ms^{-1}$) | run | 0.01 | 0.05 | no data | 0.01 |
| | pool | 0.00 | 0.00 | 0.00 | 0.00 |

**Table 2. Summary of nutrient analyses from water samples collected in pool and run habitats at LLSMR, July-October 2011.**

| | | July | August | September | October |
|---|---|---|---|---|---|
| Br ($mgL^{-1}$) | run | 0.7 ± 0.0 | 0.7 ± 0.0 | 0.6 ± 0.0 | 0.7 ± 0.0 |
| | pool | 0.7 ± 0.0 | 0.7 ± 0.1 | 0.6 ± 0.0 | 0.6 ± 0.0 |
| $SO_4$ ($mgL^{-1}$) | run | 127.3 ± 0.4 | 146.0 ± 3.8 | 213.1 ± 1.6 | 173.5 ± 0.6 |
| | pool | 130.2 ± 0.8 | 146.5 ± 1.2 | 215.3 ± 0.2 | 173.9 ± 0.6 |
| Cl ($mgL^{-1}$) | run | 18.3 ± 0.1 | 23.1 ± 1.4 | 29.3 ± 0.8 | 35.8 ± 0.1 |
| | pool | 18.7 ± 0.2 | 23.4 ± 0.8 | 30.0 ± 0.1 | 36.0 ± 0.2 |
| $NO_3$ ($mgL^{-1}$) | run | 0.7 ± 0.0 | 0.7 ± 0.0 | 0.7 ± 0.0 | 0.5 ± 0.2 |
| | pool | 0.8 ± 0.2 | 0.7 ± 0.0 | 0.7 ± 0.0 | 0.4 ± 0.2 |
| $PO_4$ ($mgL^{-1}$) | run | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | pool | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| $NH_4$ ($\mu L^{-1}$) | run | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | pool | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |

**Table 3. Results from nested analysis of variance of all nutrients measured from water samples, testing for differences among sample events (July – October) and habitat types (run, pool). Note that $PO_4$ and $NH_4$ were below detection limits in all samples.**

| | Sample Event | | | Habitat (Sample Event) | | |
|---|---|---|---|---|---|---|
| | Wald χ-Square | df | P | Wald χ -Square | df | P |
| Br | 14.4 | 3 | **0.002** | 11.2 | 4 | **0.024** |
| $SO_4$ | 5047.5 | 3 | **0.000** | 4.2 | 4 | 0.384 |
| Cl | 1266.9 | 3 | **0.000** | 1.5 | 4 | 0.835 |
| $NO_3$ | 9.6 | 3 | **0.022** | 1.4 | 4 | 0.836 |
| $PO_4$ | -- | -- | -- | -- | -- | -- |
| $NH_4$ | -- | -- | -- | -- | -- | -- |

## Periphyton analyses

Eighty-four diatom taxa and 27 soft algal taxa (mostly green algae and a few cyanobacteria) were identified and enumerated from benthic pools, run, and macrophyte habitats as well as the water column. In the run collections, densities of diatoms increase over the four-month sampling period, while densities were variable in the pools and on the macrophytes and declined in the water column over the season (Figure 2). Taxa richness is consistent over the four-month sampling period with numbers ranging from 20-30 diatom taxa and 10-15 soft algal taxa in a given habitat (Figure 3). The soft algae community contained multiple taxa from the Chlorophyta (green algae) including diverse unicellular desmids (~5 taxa from *Cosmarium* spp.) and unicellular *Elakatothrix* and *Ankistrodesmus*, filamentous greens (*Oedogonium* and *Zygnema*) and multicellular colonial green algae (e.g., *Oocystis*) and small abundances of two filamentous cyanobacteria genera, *Lyngbya* and *Anabaena* (Appendix 1). Soft-bodied taxa are estimated to represent and average of ~66% of the algal cell abundances. Diatom taxa that are dominant include *Epithemia sorex* (7.5% mean across sampling periods and habitats, with some samples at 23%) and *Rhopalodia gibba* (2.7%) which both contain nitrogen-fixing endosymbionts, characteristic of naturally occurring low-nitrogen conditions in the southwest U.S. The most common diatom was *Nitzschia perminuta* which was found 8.9% mean across sampling periods and habitats. Other dominant taxa included mobile *Navicula cryptocephala*, and *Cymbella pusilla* (Appendix 1). The remaining top 10 taxa include *Achnanthidium minutissimum (*upright-growing species), *Fragilaria capucina* var. *mesolepta* (upright-growing), *Gomphonema gracile* (upright-growing), *Nitzschia amphibia* (upright-growing species) and *Staurosira construens* (sand-dwelling).

Taxonomic richness was significantly different among sampling periods and among habitats; the water column has much lower diversity but also a different assemblage as well (Table 4). Notably, the diatom abundances were maintained through the sampling periods at relatively high cell densities and there were few significant differences among habitats. The one taxon noted to

have significant differences, *Cymbella pusilla*, which was significantly lower in July. This taxon is associated with water of high mineral content, including moderate salinity. Other taxa were noted to have similar trends, reflecting the establishment of a stable algae community in the refugium.

The MDS shows that the algal communities were variable among habitats and seasons (Figure 3). There were no significant differences among survey months (ANOSIM: Global R = 0.00, p = 0.480) but there were differences among habitats (ANOSIM: Global R = 0.354, p = 0.001). Algal communities were most similar in run and macrophyte samples, whereas pool samples were different. *Anabaena*, a filamentous cyanobacterium, tended to be lower in numbers in July, especially in the macrophytes and run samples. *Elakatothrix*, a unicellular green alga, was also lower in numbers in July as were the two common filamentous green algae, *Oedogonium* and *Zygnema*.

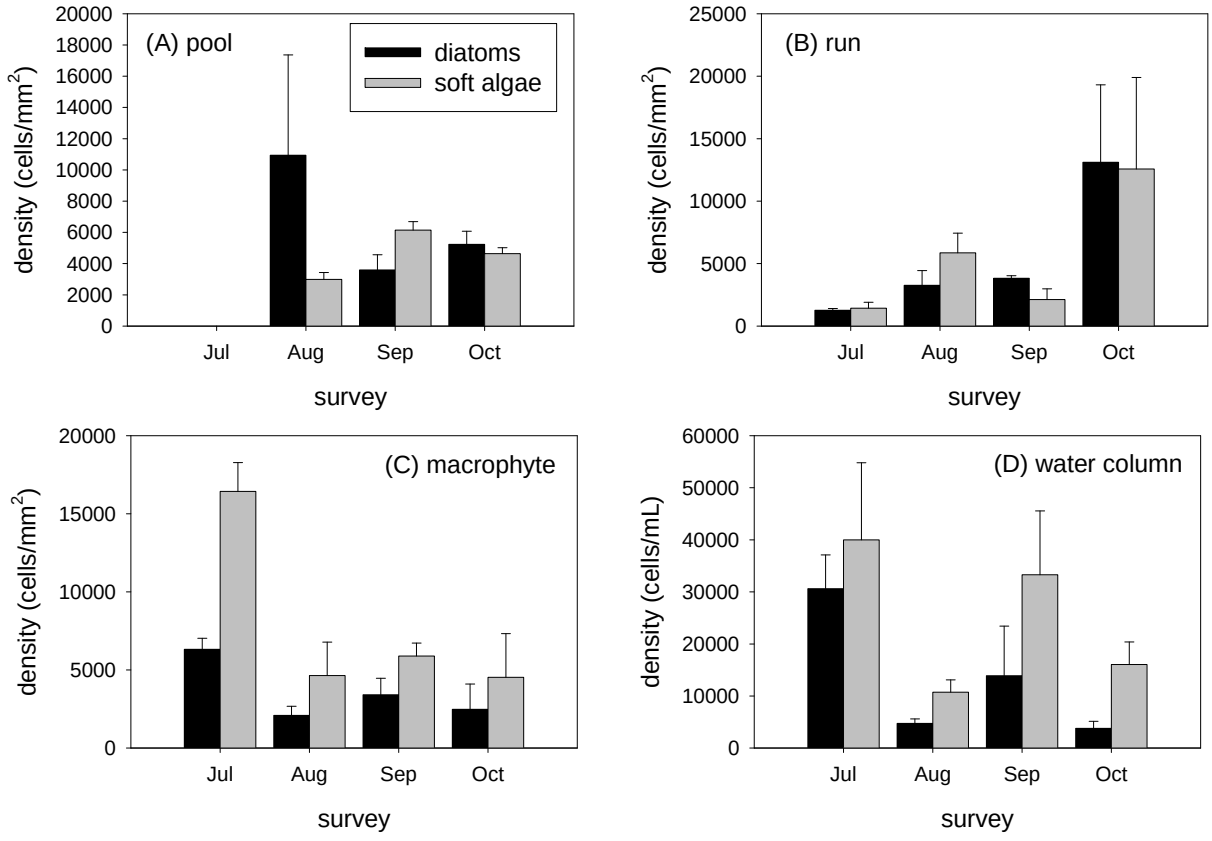



**Figure 2. Summary of abundances of algae collected during monthly surveys in each of the four habitats. Total density (#individuals/mm$^2$ or cells/mL) (mean + s.d.) measured for diatoms and soft algae in each habitat type (pool, run, macrophyte, and water column). Note that samples from pool, run, and macrophyte habitats are scaled to surface area (mm$^2$) whereas samples from the water column are scaled to volume (mL).**

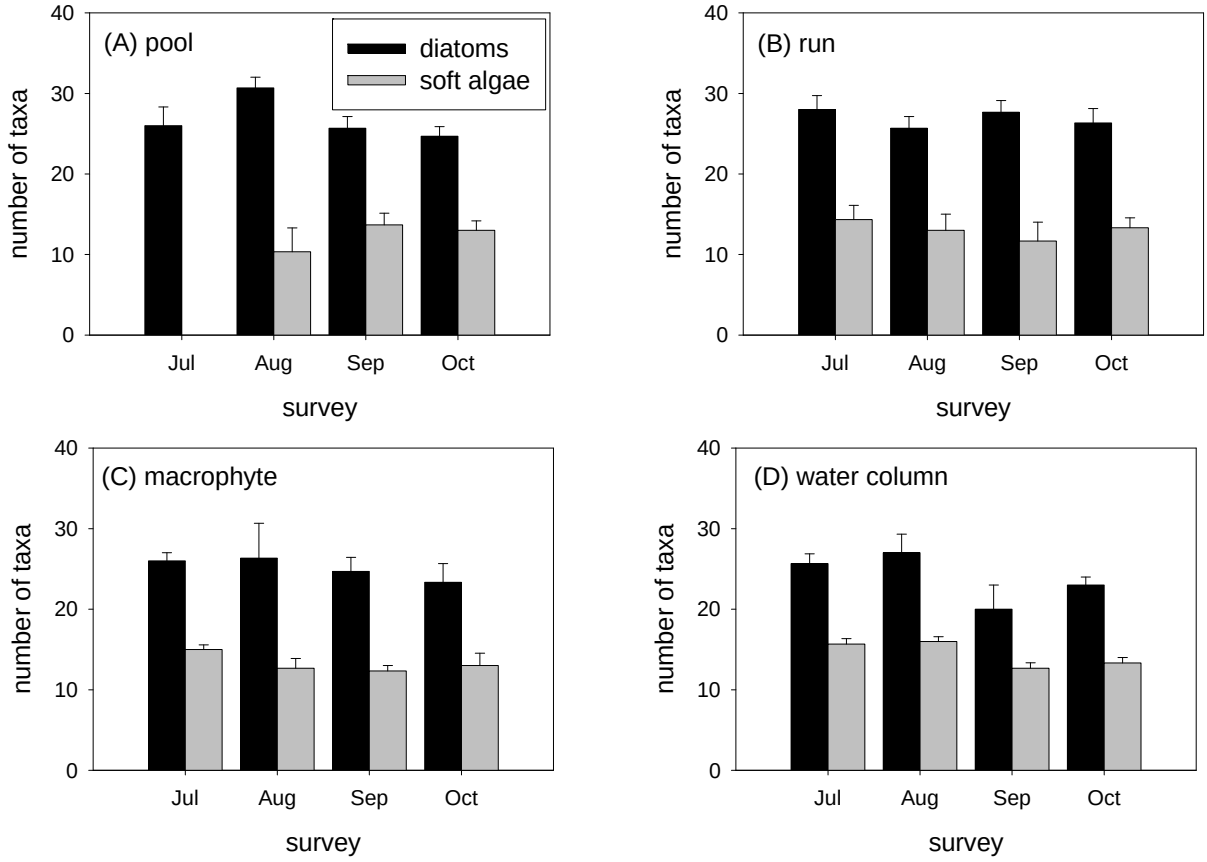



**Figure 3. Summary of algal taxa richness collected during monthly surveys in each of the four habitats. Taxonomic richness (#taxa/mm$^2$ or cells/mL) (mean + s.d.) measured for diatoms and soft algae in each habitat type (pool, run, macrophyte, water column).**

**Table 4. Results from nested analysis of variance of main diatom and soft algae taxa, testing for differences among sample events (July – October) and habitat types (pool, run, macrophyte, water column). Significant results, P>0.05, bold font.**

| | Sample Event | | | Habitat (Sample Event) | | |
|---|---|---|---|---|---|---|
| | Wald $\chi$-Square | df | P | Wald $\chi$-Square | df | P |
| **all algae** | | | | | | |
| Total abundance* | 0.3 | 3 | 0.968 | 5.1 | 11 | 0.927 |
| Taxonomic richness | 153.9 | 3 | **0.000** | 150.4 | 11 | **0.000** |
| **diatoms only** | | | | | | |
| Total abundance* | 0.3 | 3 | 0.961 | 4.7 | 11 | 0.944 |
| Taxonomic richness | 81.2 | 3 | **0.000** | 170.8 | 12 | **0.000** |
| *Achnanthidium minutissimum** | 1.1 | 3 | 0.782 | 8.1 | 11 | 0.705 |
| *Cymbella pusilla** | 8.0 | 3 | **0.045** | 6.6 | 11 | 0.828 |
| *Epithemia sorex** | 1.0 | 3 | 0.793 | 4.5 | 11 | 0.953 |
| *Gomphonema gracile** | 2.0 | 3 | 0.581 | 7.6 | 11 | 0.750 |
| *Navicula cryptocephala** | 0.5 | 3 | 0.914 | 12.7 | 11 | 0.316 |
| *Nitzschia perminuta** | 1.2 | 3 | 0.753 | 6.2 | 11 | 0.860 |
| *Rhopalodia gibba** | 0.2 | 3 | 0.972 | 4.3 | 11 | 0.962 |
| **soft algae only** | | | | | | |
| Total abundance* | 0.4 | 3 | 0.950 | 7.0 | 11 | 0.800 |
| Taxonomic richness | 33.4 | 3 | **0.000** | 57.9 | 11 | **0.000** |
| *Anabaena** | 13.9 | 3 | **0.003** | 16.6 | 11 | 0.121 |
| *Cosmarium* sp. 1* | 1.8 | 3 | 0.623 | 3.9 | 11 | 0.973 |
| *Cosmarium* sp. 2* | 2.9 | 3 | 0.410 | 10.0 | 11 | 0.531 |
| *Cosmarium* sp. 3* | 0.6 | 3 | 0.897 | 13.4 | 11 | 0.267 |
| *Elakatothrix gelatinosa** | 12.6 | 3 | **0.006** | 17.2 | 11 | 0.101 |
| *Oedogonium** | 0.8 | 3 | 0.852 | 28.7 | 11 | **0.002** |
| *Oocytis** | 4.5 | 3 | 0.214 | 10.1 | 11 | 0.523 |
| Sp. 1 green balls* | 0.9 | 3 | 0.820 | 13.0 | 11 | 0.292 |
| *Zygnema** | 6.9 | 3 | 0.076 | 35.0 | 11 | **0.000** |

*indicates variables that were log-transformed [$x' = \log_{10}(x+1)$] prior to analysis

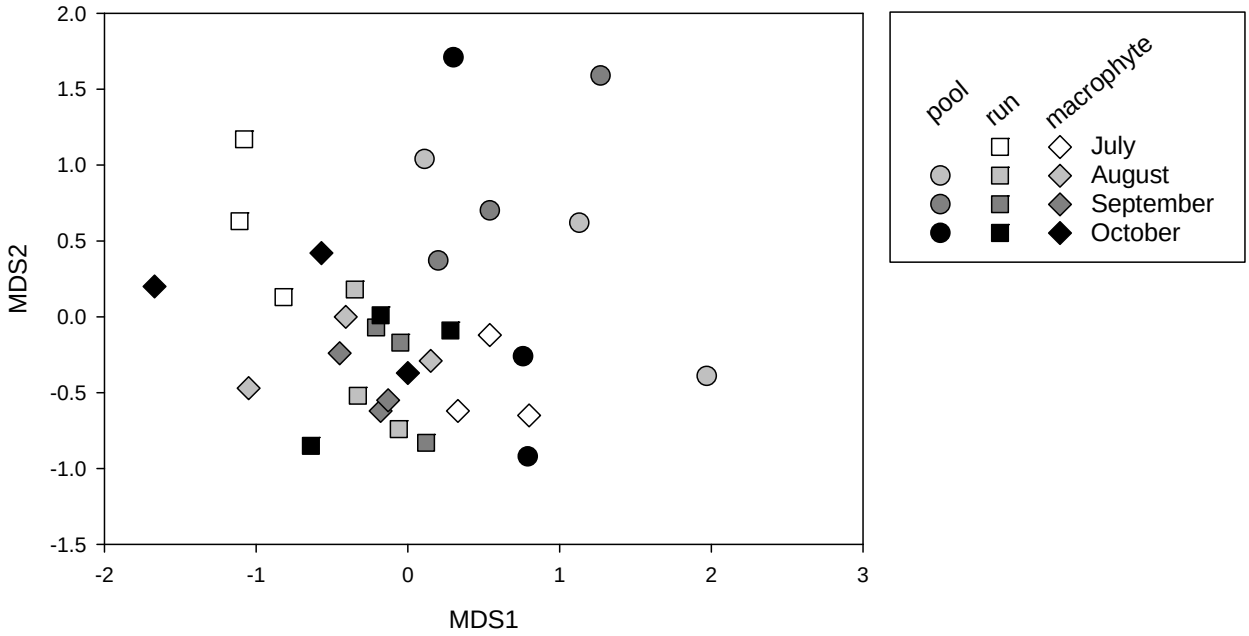



**Figure 4. Non-metric multi-dimensional scaling (MDS) plot demonstrating similarity among diatom collected from pool, run, macrophyte, and water column habitats at the four sampling times. Stress = 0.19. Because the units are different, water column samples were not included in the analysis.**

### Invertebrate analyses

The invertebrate assemblage collected from benthic pool and run habitats, from macrophytes and from the water column consisted of 24 identifiable aquatic taxa and four terrestrial taxa (Figure 5; Appendix 2). Ostracods were the dominant organism in all habitats. Chironomids and ceratopogonids (both midges) were also abundant. Densities of nearly all taxa differed significantly among habitats within sample events but not among sample events (Table 5; Appendix 3). Relatively low densities of all organisms were collected from macrophytes and the water column compared to the benthic habitats. Densities were generally higher in the earlier sample months (July and August) than the later sample months (September and October).

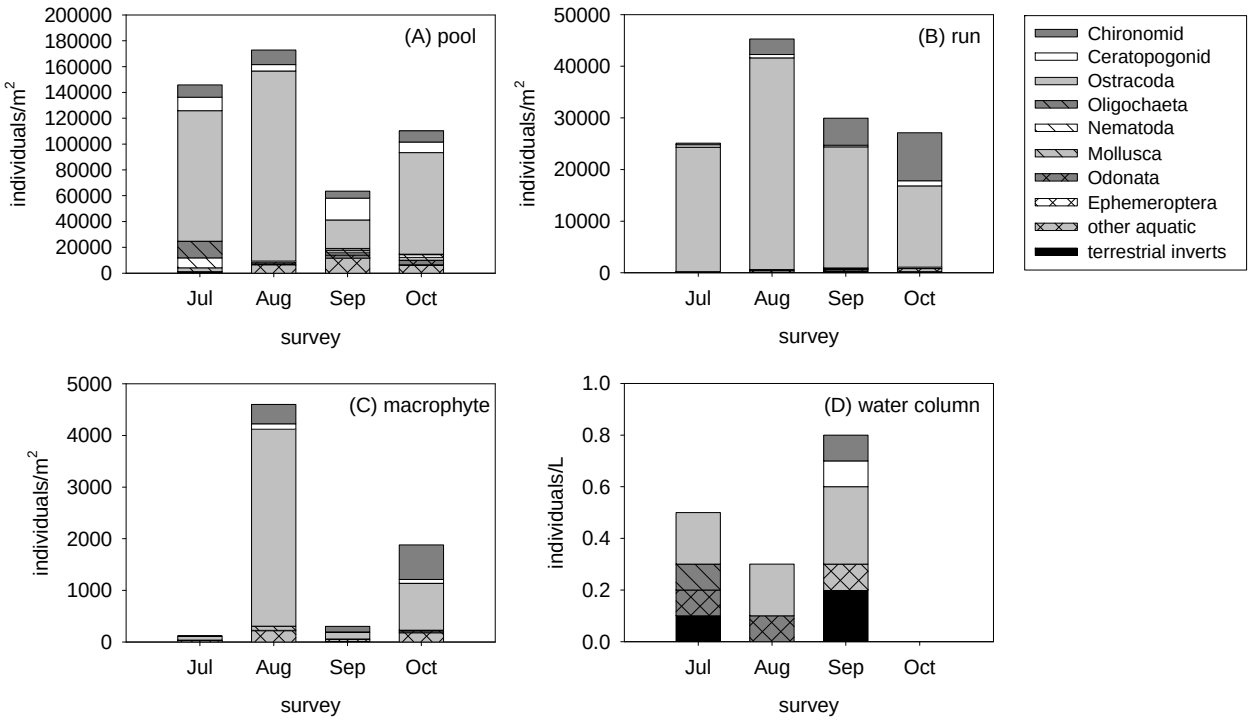



**Figure 5. Summary of abundances of invertebrate fauna collected during monthly surveys (July-October) in each of the four habitats. For more details of invertebrate abundances, refer to Appendix 3. Note that samples from pool, run and macrophyte habitats are scaled to surface area ($m^2$) whereas samples from the water column are scaled to volume (L).**

**Table 5. Results from nested analysis of variance of main invertebrate groups, testing for differences among sample events (July – October) and habitat types (pool, run, macrophyte, water column).**

| | Sample Event | | | Habitat (Sample Event) | | |
|---|---|---|---|---|---|---|
| | **Wald χ-Square** | **df** | **P** | **Wald χ-Square** | **df** | **P** |
| Total abundance* | 2.2 | 3 | 0.532 | **154.0** | **12** | **0.000** |
| Taxonomic richness | **34.5** | **3** | **0.000** | **844.2** | **12** | **0.000** |
| Chironomidae* | 5.8 | 3 | 0.122 | **92.7** | **12** | **0.000** |
| Ceratopogonidae * | 1.4 | 3 | 0.698 | **86.5** | **12** | **0.000** |
| Ostracoda* | 3.7 | 3 | 0.299 | **147.9** | **12** | **0.000** |
| Oligochaeta * | 4.6 | 3 | 0.199 | **29.6** | **12** | **0.003** |
| Nematoda* | 1.1 | 3 | 0.788 | **70.5** | **12** | **0.000** |
| Mollusca * | 3.6 | 3 | 0.311 | **37.2** | **12** | **0.000** |
| Odonata * | 3.1 | 3 | 0.369 | **48.0** | **12** | **0.000** |
| Ephemeroptera * | 1.1 | 3 | 0.772 | 16.8 | 12 | 0.159 |
| Other aquatic fauna* | **10.2** | **3** | **0.017** | **55.5** | **12** | **0.000** |
| Terrestrial invertebrate fauna* | 0.8 | 3 | 0.845 | 20.6 | 12 | 0.057 |

*indicates variables that were log-transformed [$x'=\log_{10}(x+1)$] prior to analysis

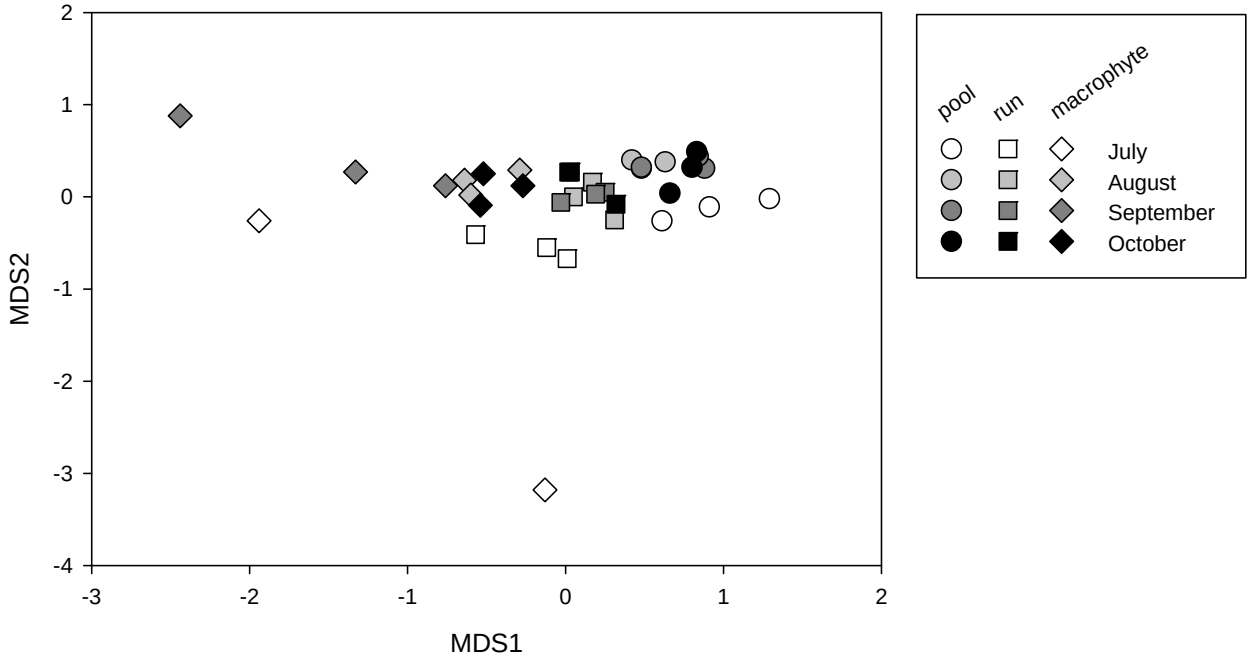



**Figure 6. Non-metric multidimensional scaling (MDS) plot demonstrating similarity among invertebrate assemblages collected from pool, run, and macrophyte habitats at the four sampling times. Stress = 0.10. Note that the water column assemblage is not included in this analysis because of extremely low densities and different units.**

The invertebrate assemblage in the refugium differed spatially among habitat types within sample events (ANOSIM: Global R = 0.587, p = 0.001) but not temporally among sample events (ANOSIM: Global R = -0.089, p = 0.973). The MDS plot indicates that assemblages collected in pool, run and macrophyte habitats also differed – generally, assemblage composition from run habitats was intermediate to those of macrophyte and pool habitats (Figure 6). Assemblages collected in July were different to those collected in the other months but they were highly variable.

Common invertebrate taxa were found in all habitats, including Ostracoda, Diptera (e.g. Chironomidae, Ceratopogonidae, Tipulidae) and gastropod snails (mainly *Physa*). However, assemblages in pools were more diverse (mean number of taxa ± s.e. = 11 ± 0.8) than assemblages on macrophytes (mean number of taxa ± s.e. = 4 ± 0.7). Several taxonomic groups were present in benthic habitats but absent from macrophytes (Ephemeroptera, Coleoptera, Oligochaeta, Nematoda) or occurred infrequently (Odonata, Hemiptera, Trichoptera).

## Gut content analyses

Across the four sampling events, 57 fish were dissected and examined for gut content analysis. In July and August, all of the fish that were examined (n = 10 for each month) had some items in their guts and the guts were relatively full compared to later samples. In July, 66.3% of the gut tract in each fish was filled on average. In August, 78.2% of the gut tract was filled on average. By comparison, nine of the twelve fish examined from September samples had items in their guts (75% of fish). Of those nine fish, 24.4% of the gut tract was filled on average. Only seven of the 25 fish examined from October samples had items in their guts (28% of fish) and only 14.4% of the gut tract was filled on average in those seven fish.

### *Diatom flora in the guts*

Diatoms in the guts were classified into three different growth forms that are differentially affected by grazers (Steinman 1996). The majority of the diatom taxa (and densities) are represented by mobile, but prostrate, taxa including *Epithemia* and *Rhopalodia*. Several of the upright taxa, which should be grazed preferentially (actively or passively), have a number of IEI scores that showed preferential consumption compared to the environmental densities of the same taxa (Table 6). A few diatoms were noted as planktonic; we would expect the consumption of planktonic diatoms to be lower because their habitat, the water column, is not generally grazed by benthic feeders. Finally, many of the prostrate and attached taxa were described as less preferred, which may be an indicator of food preference or growth habit; prostrate growth forms are less likely to be grazed.

In July (Figure 7), a number of taxa preferred by the minnow including a diatom, *Epithemia sorex,* and two desmids *Cosmarium* taxa, although the preferences were not strong. The diatom *Nitzschia perminuta* was moderately avoided. In August, the same taxa were preferred as the July samples, although the preferences are more pronounced. *Nitzschia perminuta* and a green alga, *Elakatothrix,* were more common in the environment, compared to the guts (Figure 8). In September, *Epithemia sorex* continues to be preferred by the RGSM, and the green alga *Ankistrodesmus* also becomes more common in the guts. *Elakatothrix* and the filamentous green alga *Zygnema* were avoided (Figure 9). In October, *Epithemia* continued to be commonly consumed while a number of taxa are less preferred (*Nitzschia perminuta*, *Cosmarium* sp. 5, *Elakatothrix,* and *Zygnema* (Figure 10). Overall, algal taxa densities decreased during the last month of sampling in October.

**Table 6: Summary of algae by growth from and preference found in Refugium samples and fish guts for each of the four sample months. Ivlev's (1961) Electivity Index is given for each taxon. Values near 1.0 indicate a favored food item while a number close to -1.0 indicated an avoided food item. Bolded numbers are > 0.000. This table continues over three pages.**

| Growth form | Taxon | Jul | Aug | Sep | Oct |
|---|---|---|---|---|---|
| **Generally preferred** | | | | | |
| Upright | *Synedra ulna* | **0.667** | **0.629** | **0.077** | **0.207** |
| Detached and prostrate | *Ankistrodesmus spiralis* | **0.161** | **0.774** | **0.981** | **0.580** |
| | *Cosmarium* sp. 1 | **0.282** | **0.160** | **0.116** | **0.100** |
| | Green taxon-in 4 cells | ---- | **0.450** | ---- | **0.863** |
| Mobile and prostrate | *Epithemia sorex* | **0.594** | **0.465** | **0.611** | **0.528** |
| | *Rhopalodia gibba* | **0.150** | **0.163** | **0.276** | **0.356** |
| **Shifting preferences** | | | | | |
| Detached and prostrate | *Chroococcus* | -1.000 | -1.000 | -0.049 | **0.645** |
| | *Cosmarium* sp. 3 | -0.300 | **0.317** | **0.062** | -1.000 |
| | *Cosmarium* sp. 5 | **0.118** | **0.075** | -0.015 | -0.149 |
| | *Merismopedia* | -0.241 | **0.171** | **0.068** | **0.083** |
| | *Oocytis* | -0.461 | -0.026 | -0.443 | **0.050** |
| | *Peridinium* | -0.113 | -0.733 | **0.035** | -1.000 |
| | *Scenedesmus* | -1.000 | -1.000 | -1.000 | **0.698** |
| | Sp. 1 (green balls) | -0.240 | -0.026 | **0.112** | **0.200** |
| | Sp. 2 (balls in mucilage) | -1.000 | -0.176 | -0.509 | **0.006** |
| | *Tetrahedron* | **0.284** | -0.494 | **0.167** | -1.000 |
| Filamentous | *Lyngbya* (wide) | **0.891** | -0.289 | -1.000 | -1.000 |
| | *Oedogonium* | **0.041** | -0.036 | **0.087** | **0.697** |
| | *Zygnema* | **0.078** | -0.604 | -0.200 | -1.000 |
| Upright | *Achnanthidium minutissimum* | -0.489 | **0.411** | -0.128 | **0.240** |
| | *Fragilaria capucina* var. *mesolepta* | -1.000 | -0.250 | **0.108** | -0.941 |
| | *Fragilaria* cf. *vaucheriae* | ---- | -1.000 | 1.000 | ---- |
| | *Fragilaria delicatissima* var. *angustissima* | -0.048 | **0.197** | -0.049 | **0.532** |
| | *Fragilaria vaucheriae* | -1.000 | **1.000** | -1.000 | -1.000 |
| | *Gomphonema clavatum* | **0.716** | **0.295** | -0.260 | -1.000 |
| | *Gomphonema gracile* | **0.573** | **0.087** | -0.310 | **0.002** |
| | *Gomphonema lagenula* | -0.076 | -0.724 | **0.416** | **0.201** |
| | *Gomphonema parvulum* | -1.000 | **0.134** | -0.515 | -1.000 |
| Mobile and prostrate | *Amphora* sp. 1 | **0.621** | **0.010** | -1.000 | -1.000 |
| | *Anomoeoneis sphaerophora* | **0.089** | -1.000 | -1.000 | -1.000 |
| | *Epithemia adnata* | -1.000 | **0.499** | **0.268** | **0.305** |
| | *Epithemia adnata* (w/ cap ends) | **0.800** | **0.447** | -1.000 | -1.000 |

| | | | | | |
|---|---|---|---|---|---|
| | *Fallacia pygmaea* | -1.000 | ---- | ---- | ---- |
| | *Mastogloia elliptica* | ---- | **0.732** | -1.000 | -1.000 |
| | *Navicula cryptotenella* | -0.269 | **0.136** | -1.000 | -1.000 |
| | *Navicula recens* | **0.488** | -1.000 | -1.000 | **0.152** |
| | *Nitzschia amphibia* | -0.777 | **0.012** | -0.151 | -0.210 |
| | *Nitzschia clausii* | -1.000 | **1.000** | ---- | ---- |
| | *Nitzschia linearis* | -1.000 | -1.000 | **1.000** | ---- |
| | *Nitzschia palea* | **0.684** | -0.415 | **0.362** | -1.000 |
| | *Nitzschia perminuta* | ---- | ---- | **1.000** | **1.000** |
| | *Pinnularia viridis* | -0.605 | -0.228 | **0.174** | -1.000 |
| | *Pseudostaurosira brevistriata* | -1.000 | **0.830** | **0.762** | -1.000 |
| | *Rhopalodia gibberula* | -1.000 | **0.243** | -1.000 | -1.000 |
| | *Tryblionella hungarica (*not undulate) | **1.000** | ---- | -1.000 | ---- |
| **Generally unpreferred** | | | | | |
| Detached and prostrate | *Closterium* | -1.000 | -1.000 | -0.486 | -0.198 |
| | *Cosmarium* sp .2 | -1.000 | -1.000 | ---- | -1.000 |
| | *Cosmarium* sp. 4 | -1.000 | -1.000 | -1.000 | -1.000 |
| | *Elakatothrix gelatinosa* | -0.179 | -0.609 | -0.462 | -0.721 |
| | *Gleotrichia* | -1.000 | -1.000 | -1.000 | -1.000 |
| | *Merismopedia* (larger cells) | -0.237 | -1.000 | -0.666 | -1.000 |
| | *Pediastrum* | -1.000 | ---- | ---- | ---- |
| Filamentous | *Phacus* | ---- | ---- | ---- | 1.000 |
| | *Anabaena* | -0.611 | -1.000 | -0.834 | -1.000 |
| | *Lyngbya* (narrow) | ---- | -1.000 | -1.000 | ---- |
| Mobile and prostrate | *Spirogyra* | -1.000 | -1.000 | -1.000 | ---- |
| | *Achnanthes linearis* | -1.000 | -0.067 | -0.837 | -1.000 |
| | *Achnanthidium exiguum* | -1.000 | -1.000 | -1.000 | -1.000 |
| | *Adlafia muscora* | ---- | -1.000 | ---- | ---- |
| | *Amphora pediculus* | -1.000 | -1.000 | ---- | ---- |
| | *Amphora veneta* | -1.000 | -1.000 | -1.000 | -1.000 |
| | *Caloneis bacillum* | ---- | -0.890 | -1.000 | -1.000 |
| | *Cocconeis placentula* | -0.768 | -1.000 | -1.000 | -1.000 |
| | *Craticula ambigua* | -1.000 | -1.000 | -1.000 | -1.000 |
| | *Craticula cuspidata* | -1.000 | -1.000 | -1.000 | -0.069 |
| | *Craticula cuspidata* (wide ends) | -1.000 | -1.000 | ---- | ---- |
| | *Cymbella pusilla* | -0.674 | -0.926 | -0.689 | -0.292 |
| | *Encyonema minutum* | -1.000 | ---- | ---- | ---- |
| | *Hantzschia* sp. | -1.000 | -0.206 | ---- | ---- |
| | *Luticola muticoides* | -1.000 | ---- | ---- | ---- |
| | *Navicula capitatoradiata* | ---- | -1.000 | ---- | -1.000 |
| | *Navicula cryptocephala* | -0.487 | -0.450 | -0.713 | -0.659 |

| | | | | | |
|---|---|---|---|---|---|
| | *Navicula minuscula* | -1.000 | ---- | -1.000 | -1.000 |
| | *Navicula radiosa* | ---- | ---- | -1.000 | ---- |
| | *Navicula rostellata* | ---- | -1.000 | ---- | -1.000 |
| | *Navicula schroeteri* | -1.000 | ---- | ---- | ---- |
| | *Navicula* sp. 1 | -1.000 | -1.000 | -1.000 | -1.000 |
| | *Navicula* sp. 2 | -1.000 | -1.000 | ---- | ---- |
| | *Navicula* sp. 3 | -1.000 | -1.000 | ---- | ---- |
| | *Navicula* sp. 4 | ---- | ---- | ---- | -1.000 |
| | *Navicula veneta* | -0.432 | -0.724 | -0.669 | -1.000 |
| | *Nitzschia acicularis* | -1.000 | ---- | -1.000 | -1.000 |
| | *Nitzschia perminuta* | -0.190 | -0.270 | -0.670 | -0.457 |
| | *Nitzschia gracilis* | -0.223 | -0.220 | -1.000 | **0.355** |
| | *Nitzschia inconspicua* | ---- | ---- | -1.000 | -1.000 |
| | *Pinnularia* sp. 1 | -1.000 | ---- | ---- | ---- |
| | *Planothidium lanceolatum* | ---- | -1.000 | ---- | ---- |
| | *Rhoicosphenia abbreviata* | ---- | ---- | ---- | -1.000 |
| | *Sellaphora pupula* | -1.000 | -0.839 | -1.000 | -0.714 |
| | *Stauroneis anceps* | ---- | ---- | -1.000 | -1.000 |
| | *Staurosira* cf. *construens* | -1.000 | ---- | -1.000 | ---- |
| | *Staurosira construens* | -1.000 | -1.000 | -1.000 | -1.000 |
| | *Staurosira construens* var. *venter* | -1.000 | -1.000 | -0.782 | -1.000 |
| | *Staurosira* sp. | -1.000 | ---- | ---- | ---- |
| | *Staurosirella pinnata* | -1.000 | ---- | -1.000 | ---- |
| | *Tryblionella hungarica* (undulate) | -1.000 | -0.077 | ---- | -1.000 |
| Planktonic | *Cyclotella stelligera* | -1.000 | -0.840 | -0.174 | -1.000 |
| | *Diatoma moniliformis* | -1.000 | -1.000 | ---- | -1.000 |
| | *Diatoma oval* | -1.000 | ---- | ---- | ---- |
| | *Diatoma vulgare* | -1.000 | ---- | ---- | ---- |
| | *Stephanodiscus hantzschii* | -1.000 | -1.000 | -1.000 | -1.000 |
| Upright | *Achnanthidium* sp. (long valve) | ---- | -1.000 | -1.000 | -1.000 |
| | *Gomphoneis* cf. *herculeana* | -1.000 | -1.000 | -1.000 | -1.000 |
| | *Gomphonema* cf. *gracile* | ---- | -1.000 | -1.000 | ---- |
| | *Gomphonema gracile*-small (23-26 L) | -0.449 | -0.306 | -0.592 | -1.000 |
| | *Gomphonema olivaceum* | -1.000 | ---- | ---- | ---- |
| | *Gomphonema pumilum* | -1.000 | -1.000 | ---- | -1.000 |
| | *Gomphonema rhombicum* | ---- | ---- | -1.000 | ---- |
| | *Synedra rumpens* var. *familaris* | -1.000 | ---- | ---- | ---- |
| | *Synedra* sp. 1 | -0.663 | -1.000 | -1.000 | -1.000 |
| | *Synedra* with narrow ends | -1.000 | -0.324 | -0.725 | -0.136 |

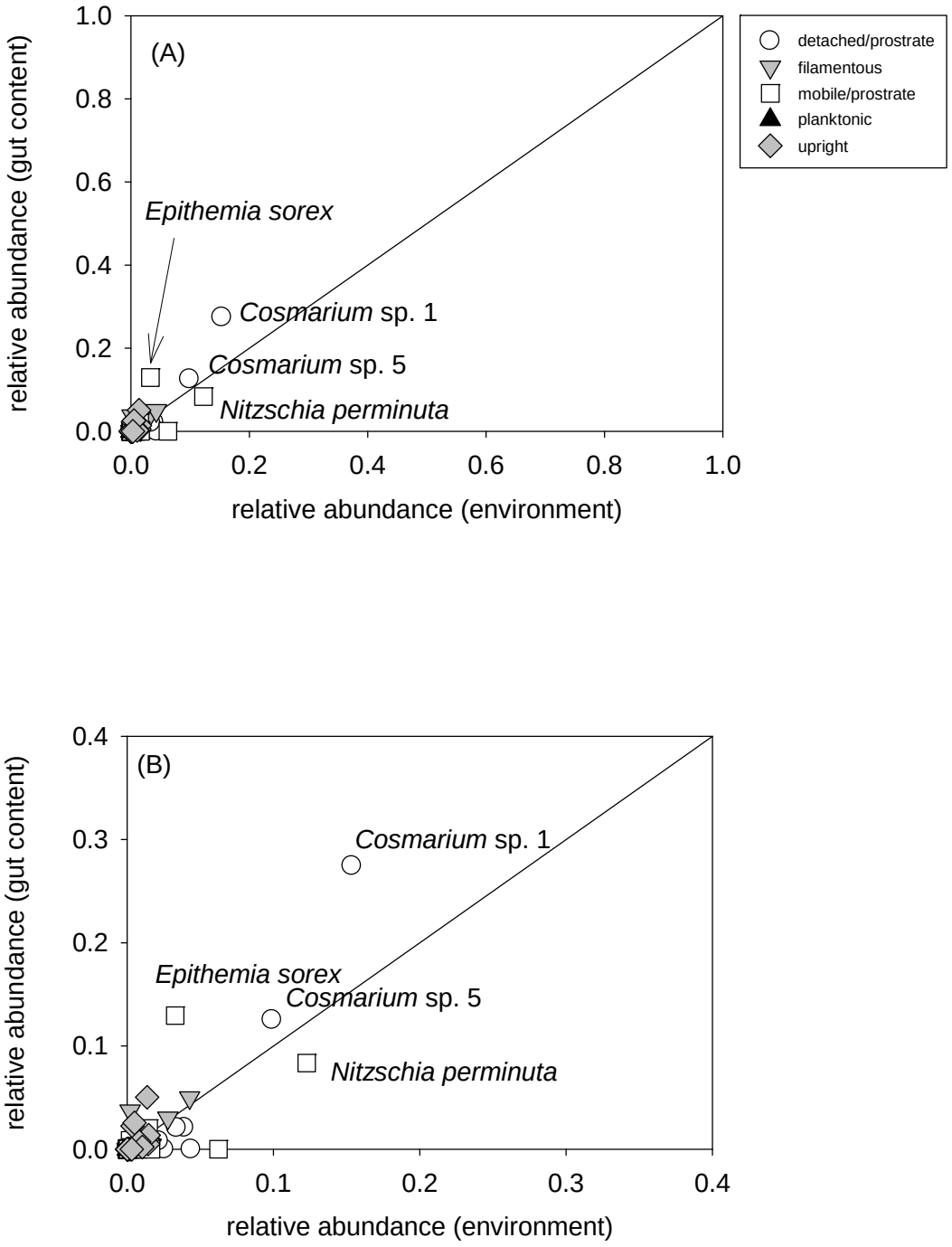



**Figure 7. Relative abundance of major algal groups in the Refugium environment and in fish gut contents in July. The diagonal line indicates 1:1 – taxa above the line are preferentially selected whereas those below the line are not. (A) All taxa. (B) Taxa with low relative abundances.**

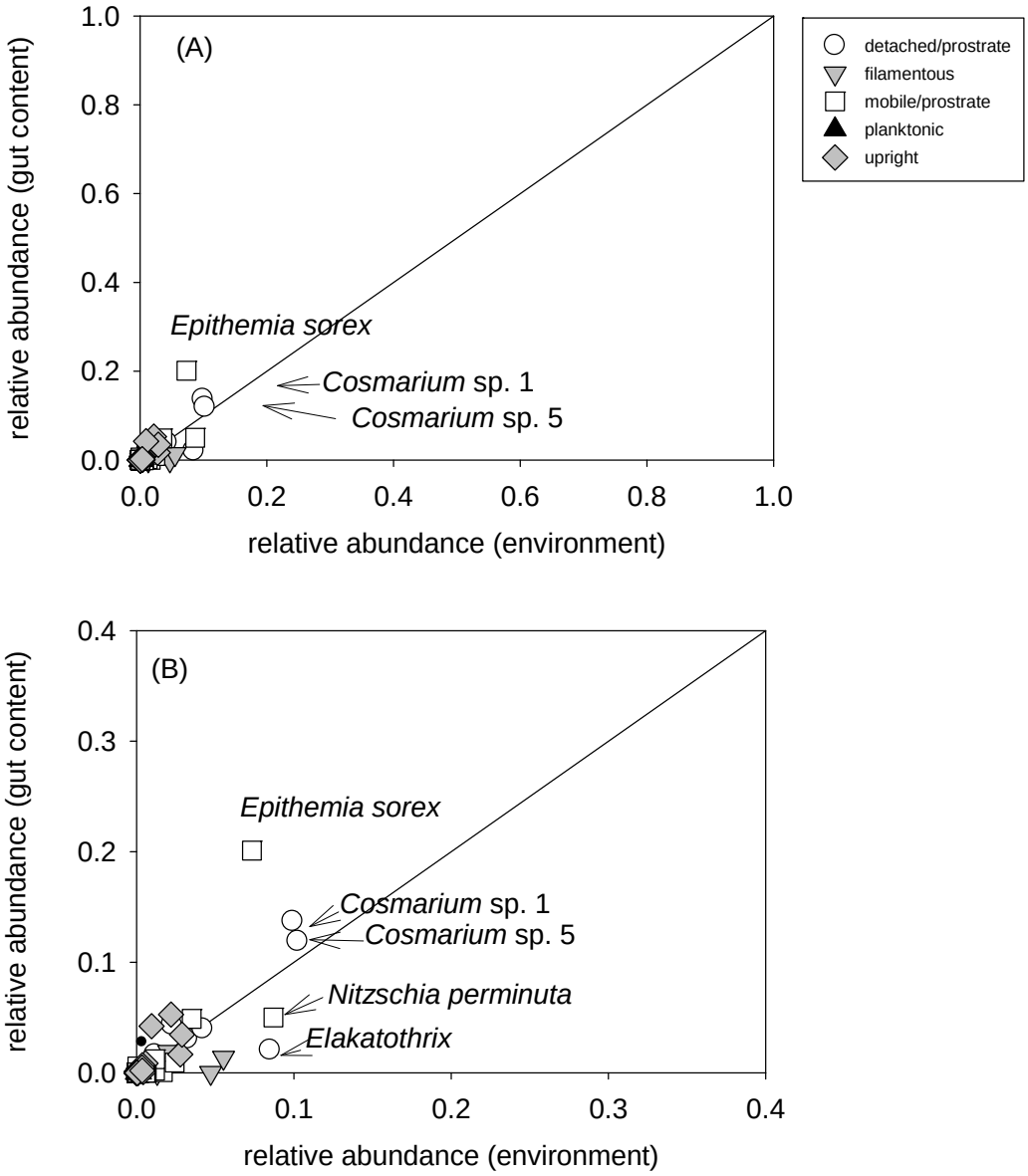
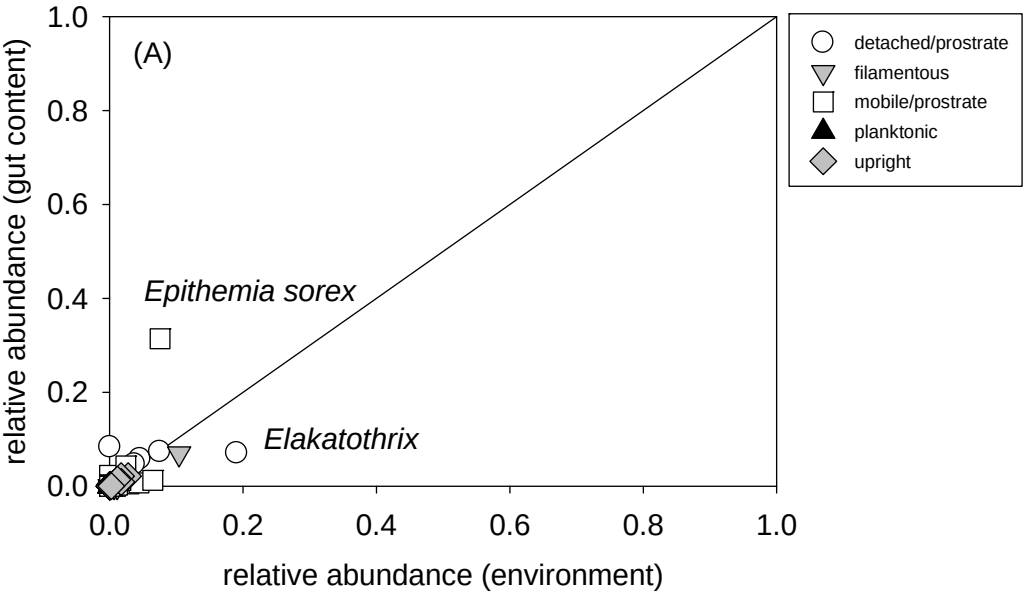



**Figure 8. Relative abundance of major algal taxa in the Refugium environment and in fish gut contents in August. The diagonal line indicates 1:1 – taxa above the line are preferentially selected whereas those below the line are not. (A) All taxa. (B) Taxa with low relative abundances.**



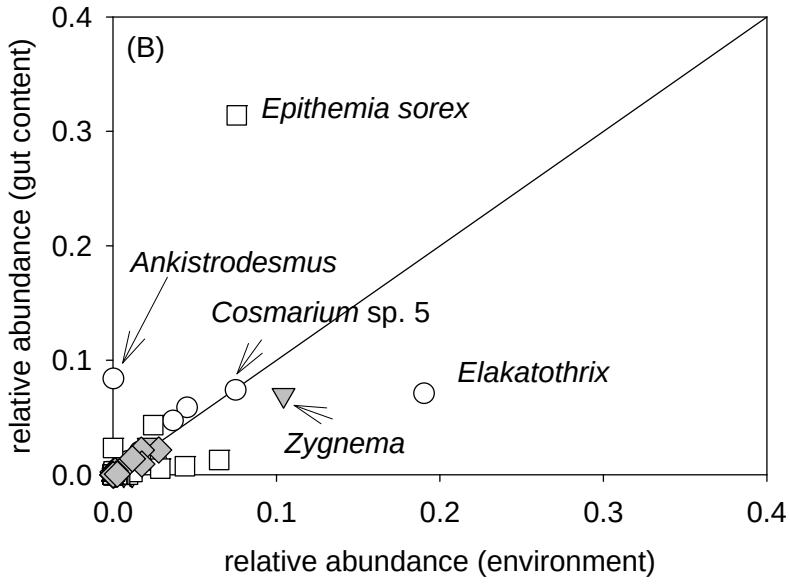




**Figure 9. Relative abundance of algal taxa in the Refugium environment and in fish gut contents in September. The diagonal line indicates 1:1 – taxa above the line are preferentially selected whereas those below the line are not. (A) All taxa. (B) Taxa with low relative abundances.**

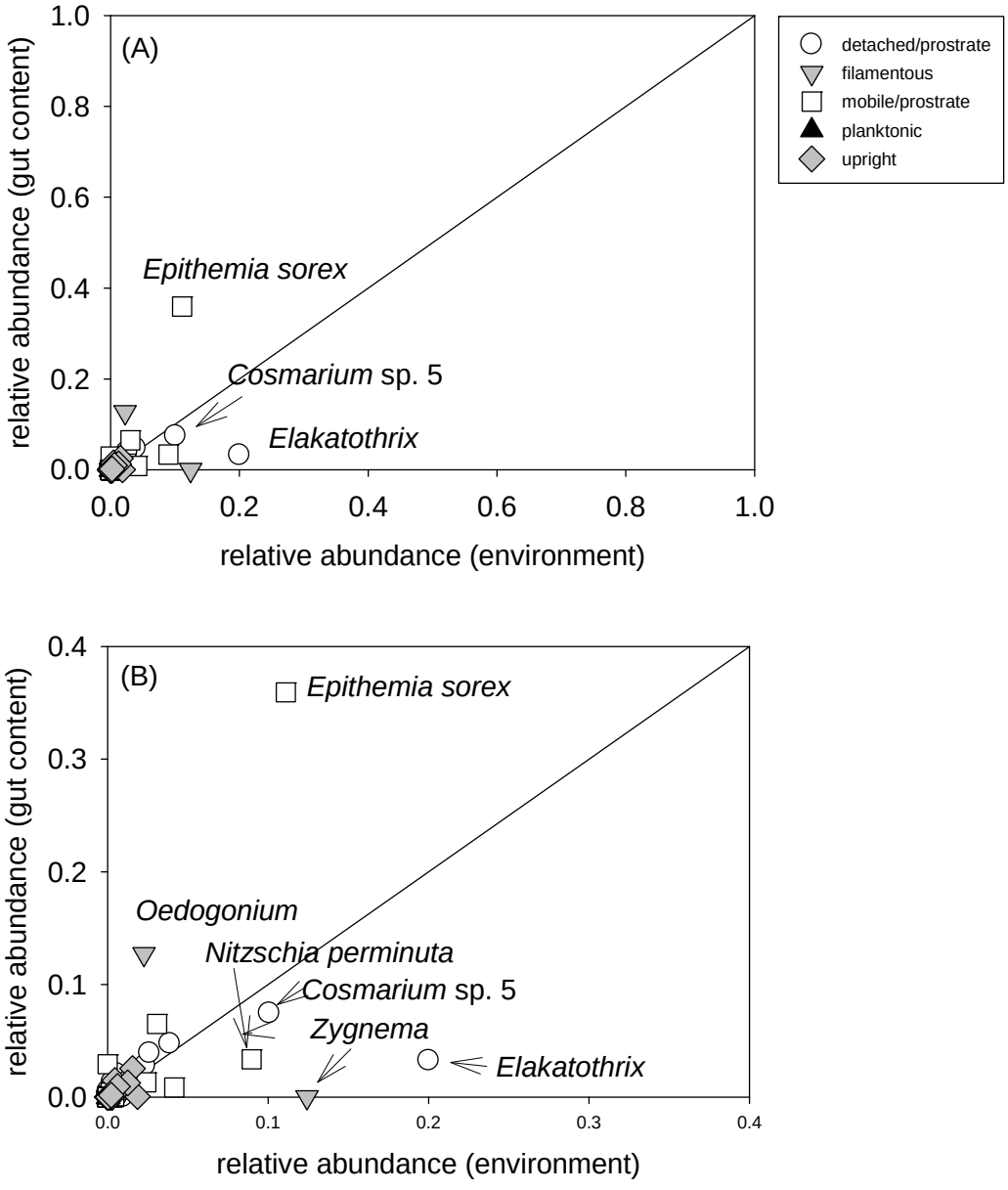



**Figure 10. Relative abundance of major algal taxa in the Refugium environment and in fish gut contents in October. The diagonal line indicates 1:1 – taxa above the line are preferentially selected whereas those below the line are not. (A) All taxa. (B) Taxa with low relative abundances.**

### *Invertebrate fauna in the guts*

Invertebrate items found in the gut were classified into 22 different categories (Table 7). Diptera (true flies) and ostracods were the most common taxa in the gut contents, but other insects also occurred. The taxonomic composition of fish guts varied over time (Figure 11-Figure 14).
In July, ostracods were dominant in both the environment and the guts. Chironomids and ceratopogonids were also common, but were rarely consumed by fish. Ivlev's Electivity Index (Table 7) indicates that fish were preferentially consuming some of the less common dipterans (Simuliidae, Tipulidae, Tabanidae), mayflies (Ephemeroptera) and nematodes. However, the high positive Electivity Index for these taxa is an artifact of low abundances in the environment. Observations during sample processing indicate that these organisms were not the most common food item – ostracods were found in nine of the ten guts from July.

In August, ostracods were still a major food source and were found in six of the ten guts. A variety of fly taxa were found as well (Tipulidae, Chironomidae, Simuliidae), but only in one or two guts. Interestingly, an adult midge was also found in one of the guts – probably a chironomid or ceratopogonid. Ivlev's Electivity Index indicates that ostracods were not being preferentially selected by fish in August. Ostracods were extremely abundant at this time (Figure 5) and were clearly a major invertebrate component of the fish diet.

By September, adult midges were found in five of the 12 guts examined and had become one of the main food sources. The abundance of ostracods had declined and subsequently ostracods had become a less important food source for fish. The diet of fish appears to be more diverse (Figure 13) but guts were obviously less full.

In October, many guts were empty of invertebrate items. Unidentifiable insect parts were the most common item found in guts (in five of the seven fish). Other items occurred only once or twice, including ostracods. Invertebrate abundances (like the algal abundances) had generally declined since the peak in September, but still seemed to be in high enough densities to provide adequate food resources?

**Table 7. Summary of invertebrate groups found in Refugium samples and fish guts for each of the four sample months. Ivlev's (1961) Electivity Index is given for each group. Values near 1.0 indicate a favored food item while a number close to -1.0 indicated an avoided food item. N/A indicates groups that were found in guts but were not classified in Refugium samples. Bolded numbers are > 0.000.**

| group | taxon | Jul | Aug | Sep | Oct | overall |
|---|---|---|---|---|---|---|
| **generally preferred** | | | | | | |
| Diptera | Tipulidae | **1.000** | **0.957** | **0.792** | **-1.000** | **0.788** |
| | Diptera - pupa | **-0.824** | **-1.000** | **0.992** | **1.000** | **0.810** |
| | Diptera - adult | **1.000** | **1.000** | **1.000** | **1.000** | **1.000** |
| | Simuliidae | **1.000** | **1.000** | -- | -- | 1.000 |
| Terrestrial | Terrestrial mite | **0.626** | **0.990** | **0.346** | **-1.000** | **0.367** |
| **shifting preferences** | | | | | | |
| Aquatic insect | Ephemeroptera | **1.000** | **0.487** | **-1.000** | -- | **0.300** |
| | Corixidae | **-0.768** | -- | **0.992** | -- | **0.697** |
| | Insect parts | N/A | N/A | N/A | N/A | N/A |
| microcrustaceans | Ostracoda | **0.177** | **-0.484** | **-0.798** | **0.881** | -0.270 |
| | Cladocera | **0.183** | **-1.000** | **0.874** | **0.991** | **0.182** |
| | Copepoda | **--** | **0.802** | **-1.000** | **--** | **0.311** |
| Other aquatic | Nematoda | **1.000** | **-1.000** | **-1.000** | **-1.000** | -0.682 |
| **generally unpreferred** | | | | | | |
| Diptera | Tabanidae | **-1.000** | -- | -- | -- | -1.000 |
| | Ceratopogonidae | **-0.853** | **-1.000** | **-1.000** | **-0.268** | -0.467 |
| | Chironomidae | **-0.825** | **-0.315** | **0.235** | **-0.479** | -0.386 |
| Aquatic insect | Odonata | **-1.000** | **-1.000** | **-1.000** | **-1.000** | -1.000 |
| | Other insect | **-1.000** | **-1.000** | **-1.000** | -- | -1.000 |
| Other aquatic | Oligochaeta | **--** | **-1.000** | **-1.000** | **-1.000** | -1.000 |
| | snails | **-1.000** | **-1.000** | **-1.000** | -- | -1.000 |
| Terrestrial | Terrestrial invertebrates | **-1.000** | -- | **-1.000** | **-1.000** | -1.000 |
| **not recorded in benthos** | | | | | | |
| Other aquatic | shell scraps | N/A | N/A | N/A | N/A | N/A |
| | eggs | N/A | N/A | N/A | N/A | N/A |

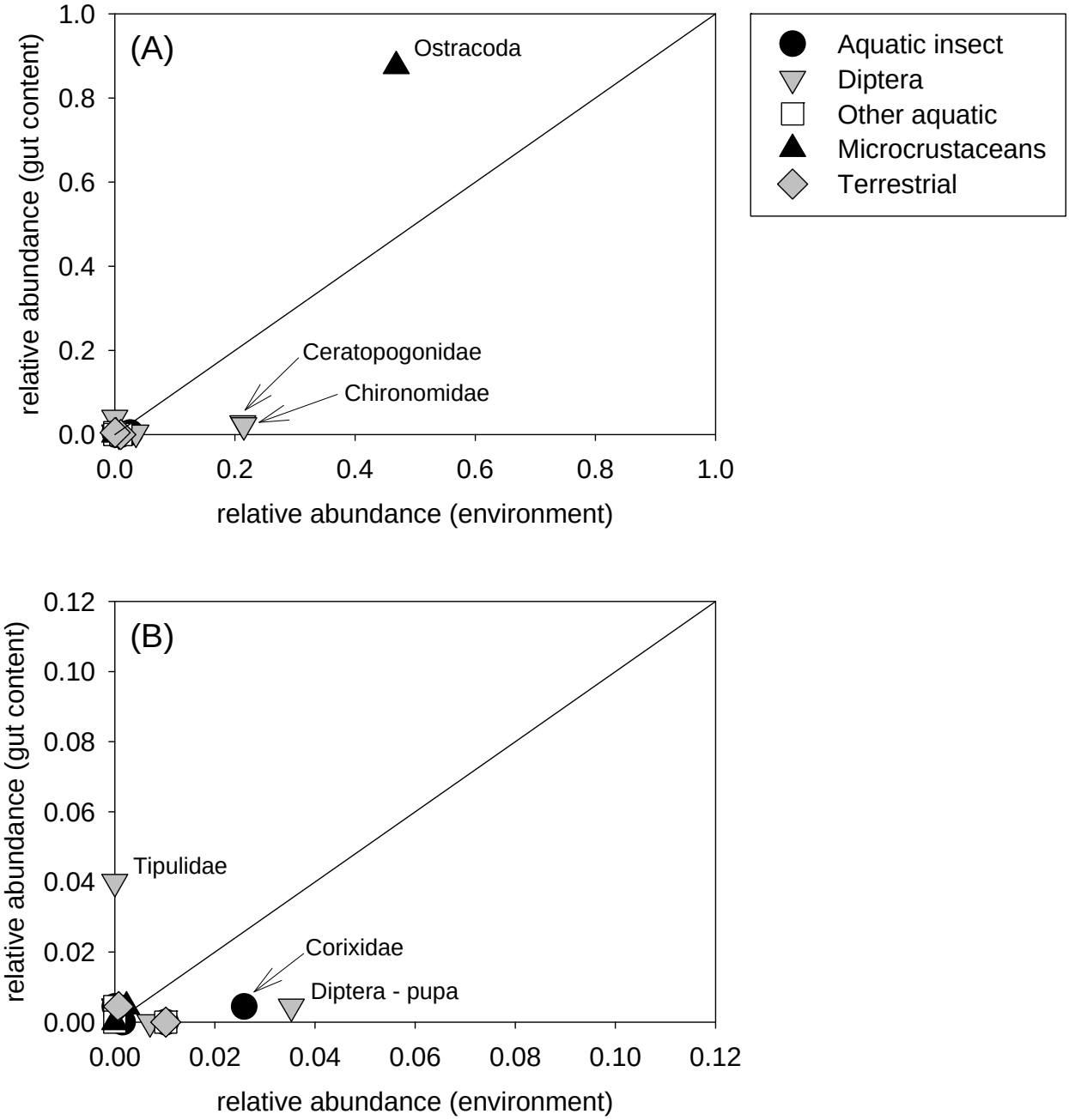



**Figure 11. Relative abundance of major taxonomic groups in the Refugium environment and in fish gut contents in July. The diagonal line indicates 1:1 – taxa above the line are preferentially selected whereas those below the line are not. (A) All taxa. (B) Taxa with low relative abundances.**

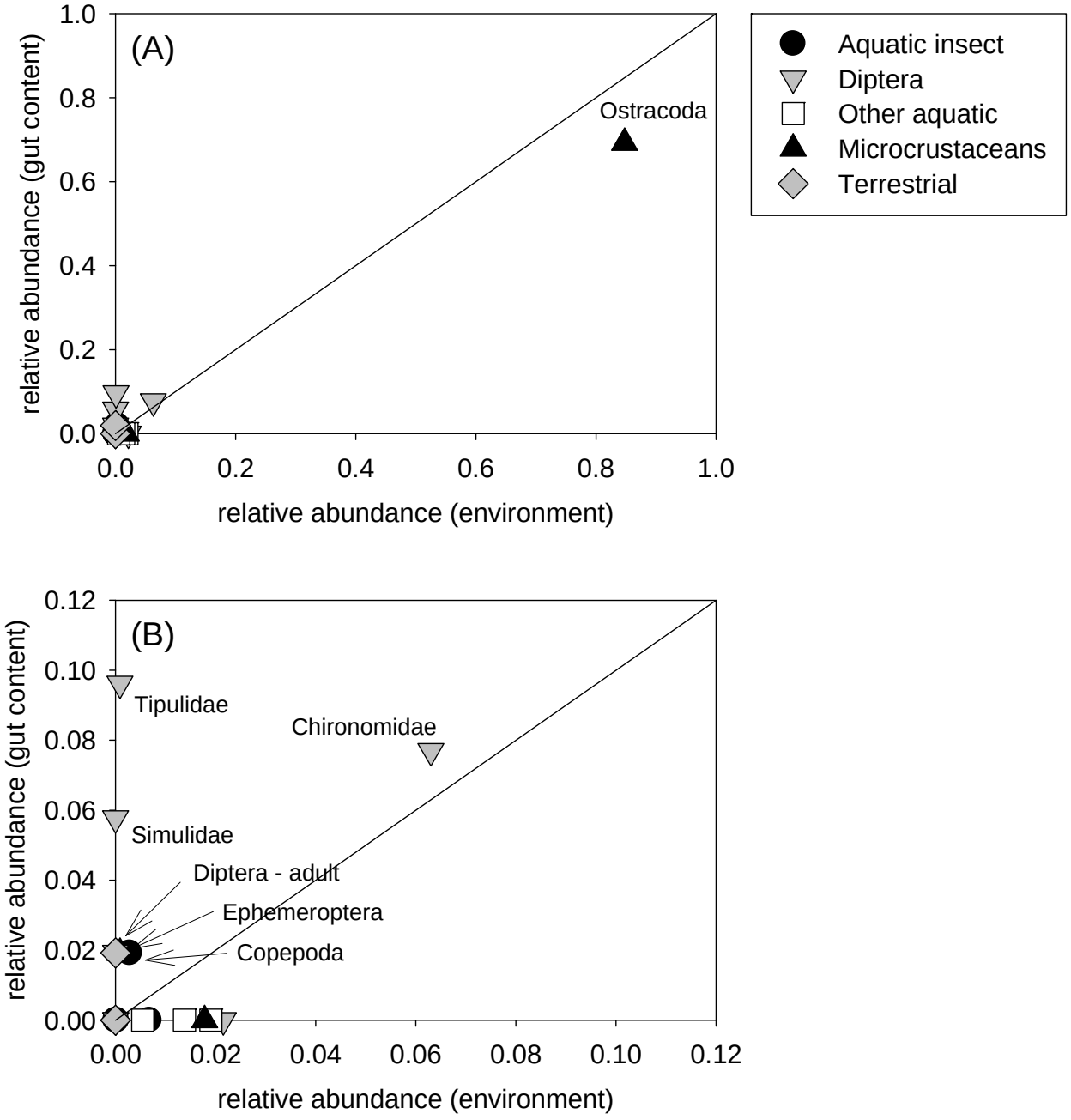



**Figure 12. Relative abundance of major taxonomic groups in the Refugium environment and in fish gut contents in August. The diagonal line indicates 1:1 – taxa above the line are preferentially selected whereas those below the line are not. (A) All taxa. (B) Taxa with low relative abundances.**

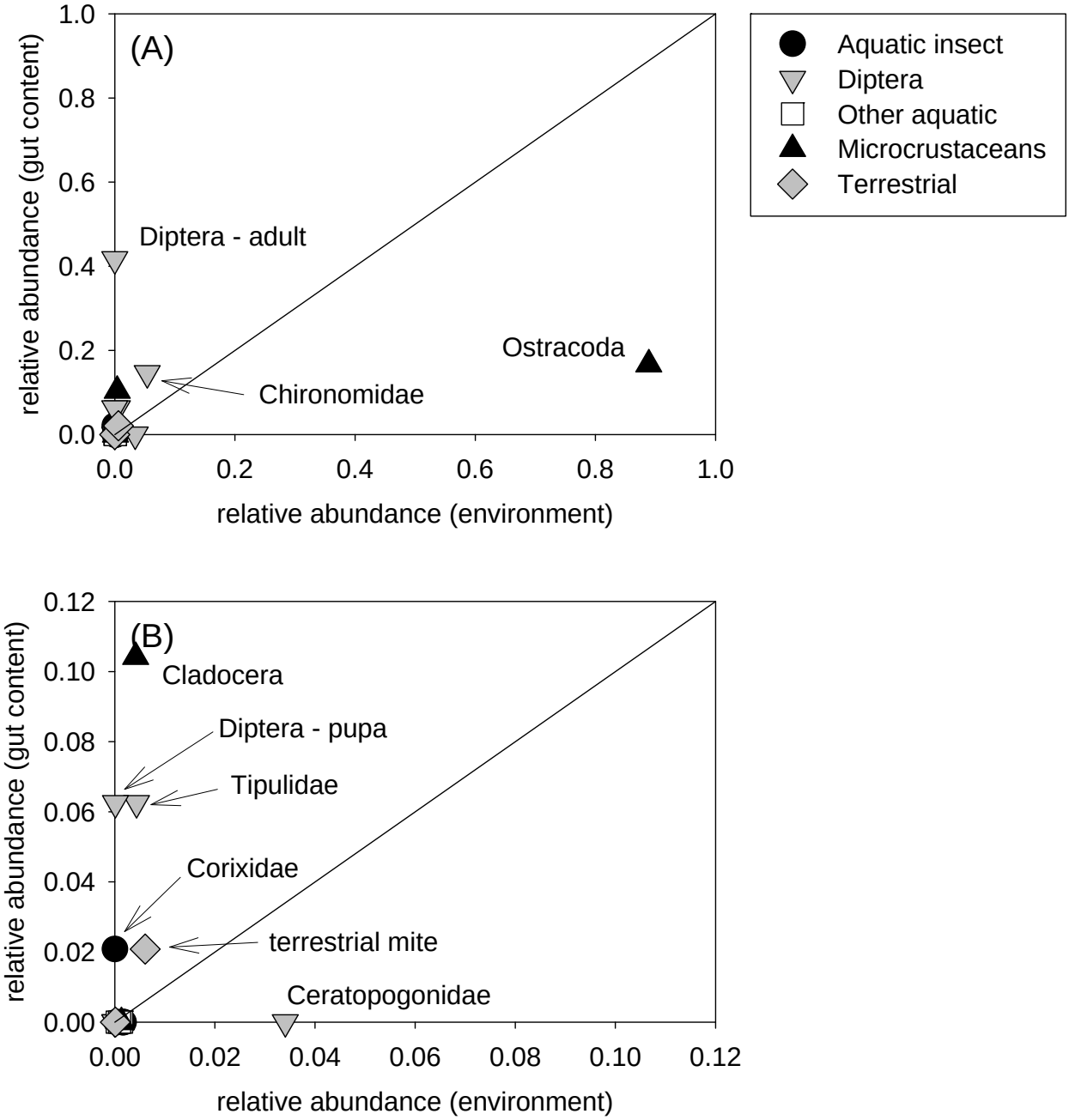



**Figure 13. Relative abundance of major taxonomic groups in the Refugium environment and in fish gut contents in September. The diagonal line indicates 1:1 – taxa above the line are preferentially selected whereas those below the line are not. (A) All taxa. (B) Taxa with low relative abundances.**

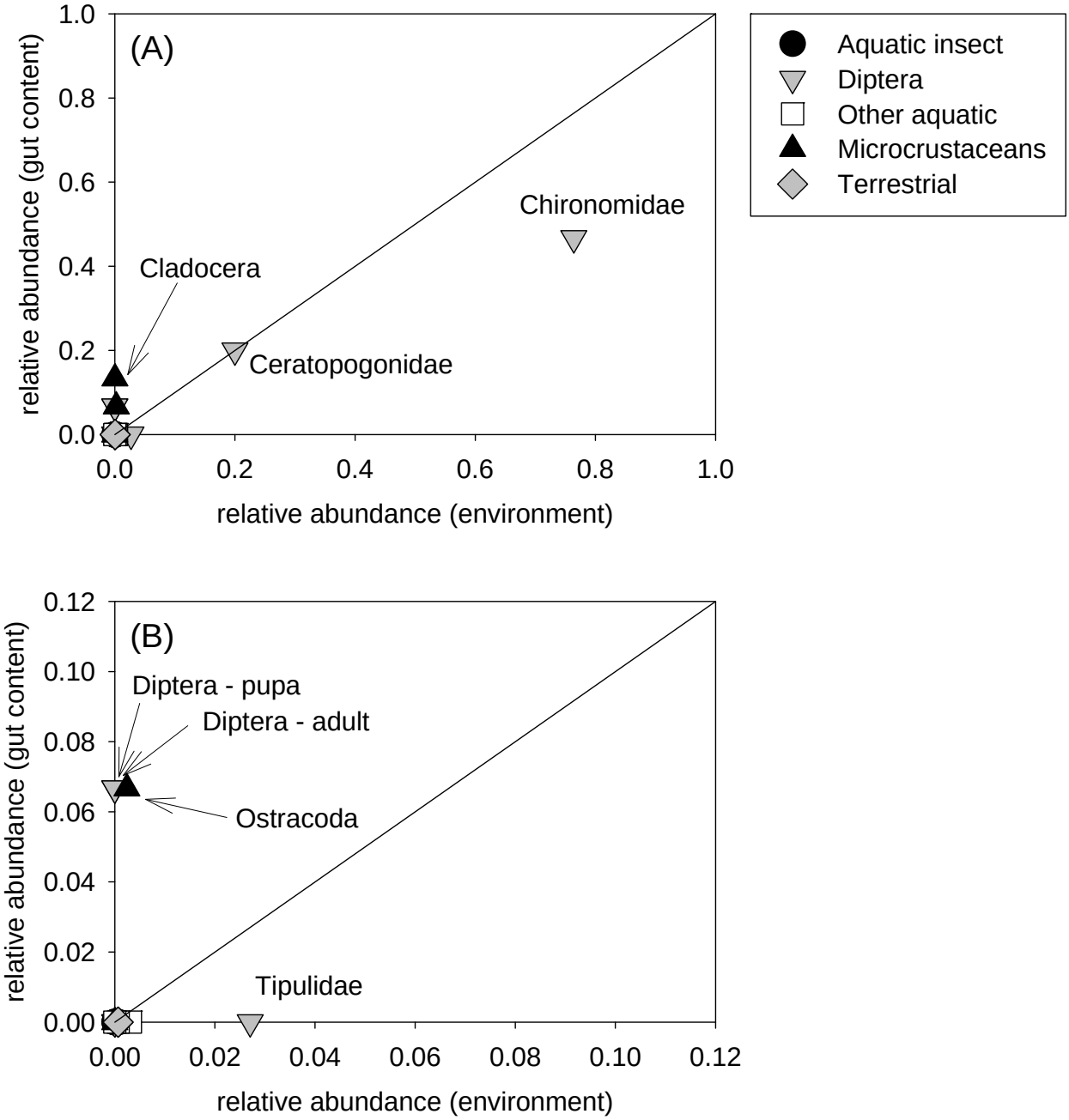



**Figure 14. Relative abundance of major taxonomic groups in the Refugium environment and in fish gut contents in October. The diagonal line indicates 1:1 – taxa above the line are preferentially selected whereas those below the line are not. (A) All taxa. (B) Taxa with low relative abundances.**

## Discussion and Conclusions

This study had two questions proposed:

1. **Do the RGSM have selective feeding habits in the refugium?**

The benthic habitat sampling has demonstrated that the densities of food resources (algal and invertebrate) are variable but overall stable, except for the water column densities of both diatoms and invertebrates.

There is some evidence of selective feeding in the refugium, particularly in the early months. If the minnows were not selectively feeding, the ratio of prey items in the guts and the environment should be equal. However, the IEI results show a number of algal and invertebrate taxa are preferentially eaten by the minnow. The diatom *Epithemia sorex*, ostracods and dipterans seem to be important food resources. In contrast, many of the other diatom taxa and green algae were only recorded in the environment and not in gut contents. However, the absence of some diatom taxa in gut content samples may be related to sampling and dissolution/physical damage to the cell walls, needed for taxonomic identification.

2. **Are there temporal changes in the diet of the larval RGSM?**

Among the sample months, there were clear differences in the types of food items occurring in gut content of the RGSM. The fish guts examined from the two earlier months (July and August) were full of algae and invertebrates, and the diet items were dominated by a few key taxa. However, in the later months a high proportion of guts were empty of invertebrates and nearly the same number lacked algal contents as well. The presence of empty guts in some of the fish is not unusual; empty guts in the RGSM have be noted in previous studies (Watson et al. 2009). Additionally, the diversity of invertebrate food items was higher in the earlier months than in the later months. It is noted that the October benthic samples showed moderate densities of diatoms and invertebrates in all habitats, except the water column – densities that are comparable to the three earlier months (Figure 2, Figure 5).

We saw no evidence of toxic algal blooms, although we did note blooms of filamentous green *Cladophora* in the later months of the study (not surprising in warmer months with the addition of nutrients via fertilizer). Two cyanobacteria were common in the samples from guts and benthic samples. *Lyngbya* is not a cyanobacterium known for producing phytotoxins (Komárek et al. 2003) and was recorded in small numbers. *Anabaena* can cause algal blooms but it was recorded in very small numbers in only a few samples. In terms of algal palatability, the high densities of diatoms provided the highest quality food resources for fish with lipids as energy storage products (Feminella and Hawkins 1995). Green algae are regarded as intermediate food resources while cyanobacteria can often be less palatable because of gelatinous coatings and possible toxins (Dodds and Whiles 2010). The dominance of both diatoms and green algae, with some cyanobacteria, should provide appropriate food resources for the RGSM.

Although the concentrations of some nutrients differ significantly among sampling events, most differences are not biologically significant. There may be some variation in nutrients levels based on evaporative water loss and well water additions to the refugium facility, especially anions related to groundwater (e.g., Br, Cl) (D. Van Horn, pers. comm.). This system is typical of other water bodies in New Mexico. As is typical in low impacted (i.e., low nutrient inputs) streams in New Mexico, the diatom assemblage was dominated by *Epithemia* and *Rhopalodia*, which have nitrogen-fixing cyanobacterial endosymbionts (Floener and Bothe 1980). Having an endosymbiont with the ability to fix atmospheric $N_2$ allows the host organisms to survive in environments with low nitrate concentrations.

The next year of sampling will help to understand the variability in algal and invertebrate taxa and cell densities seasonally and by habitat. Both types of food items (algae and invertebrates) displayed different patterns in habitat colonization and response to grazing. An additional year of seasonal information will add to a robust data set which helps to understand resource utilization of the silvery minnow in the refugium.

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## Appendix 1: Preliminary taxonomic list of algal flora in benthic and guts samples

**Diatoms**

Achnanthes linearis
Achnanthidium exiguum
Achnanthidium minutissimum
Achnanthidium sp. (long valve)
Adlafia muscora
Amphora pediculus
Amphora sp. 1
Amphora veneta
Anomoeoneis sphaerophora
Caloneis bacillum
Cocconeis placentula
Craticula ambigua
Craticula cuspidata
Craticula cuspidata (wide ends)
Cyclotella stelligera
Cymbella pusilla
Diatoma moniliformis
Diatoma oval
Diatoma vulgare
Encyonema minutum
Epithemia adnata
Epithemia adnata (w/ cap ends)
Epithemia sorex
Fallacia pygmaea
Fragilaria capucina var. mesolepta
Fragilaria cf. vaucheriae
Fragilaria delicatissima var. angustissima
Fragilaria vaucheriae
Gomphoneis cf. herculeana
Gomphonema cf. gracile (coarser puncts)
Gomphonema clavatum
Gomphonema gracile
Gomphonema gracile-small (23-26 L)
Gomphonema lagenula
Gomphonema olivaceum
Gomphonema parvulum
Gomphonema pumilum
Gomphonema rhombicum
Hantzschia sp.
Luticola muticoides

Mastogloia elliptica
Navicula capitatoradiata
Navicula cf. symmetrica
Navicula cf. tripunctata
Navicula cryptocephala
Navicula cryptotenella
Navicula minuscula
Navicula radiosa
Navicula recens
Navicula rostellata
Navicula schroeteri
Navicula sp. 1
Navicula sp. 2
Navicula sp. 3
Navicula sp. 4
Navicula veneta
Nitzschia acicularis
Nitzschia amphibia
Nitzschia perminuta
Nitzschia clausii
Nitzschia gracilis
Nitzschia inconspicua
Nitzschia linearis
Nitzschia palea
*Nitzschia perminuta*
Pinnularia sp. 1
Pinnularia viridis
Planothidium lanceolatum
Pseudostaurosira brevistriata
Rhoicosphenia abbreviata
Rhopalodia gibba
Rhopalodia gibberula
Sellaphora pupula
Stauroneis anceps
Staurosira cf. construens
Staurosira construens
Staurosira construens var. venter
Staurosira sp.
Staurosirella pinnata
Stephanodiscus hantzschii
Synedra rumpens var. familaris
Synedra sp. 1

Synedra ulna
Synedra with narrow ends
Tryblionella hungarica (not undulate)
Tryblionella hungarica (undulate)

**Green algae**

Ankistrodesmus spiralis
Closterium
Cosmarium sp. 1
Cosmarium sp. 2
Cosmarium sp. 3
Cosmarium sp. 4
Cosmarium sp. 5
Elakatothrix gelatinosa
Gleotrichia
Green-in 4 celled colonies
Merismopedia
Merismopedia (larger cells)
Oocytis
Pediastrum
Peridinium
Phacus
Scenedesmus
Sp. 1 (green balls)
Sp. 2 (balls in mucilage)
Tetrahedron
Oedogonium
Spirogyra
Zygnema

**Cyanobacteria**

*Anabaena*
*Chroococcus*
*Lyngbya*

## Appendix 2: Preliminary taxonomic list of invertebrate fauna

| Phylum | Subphylum | Class | Subclass | Order | Family | Genus | notes |
|---|---|---|---|---|---|---|---|
| **AQUATIC** | | | | | | | |
| Annelida | | Clitellata | Oligochaeta | | | | RTU_1* |
| Annelida | | Clitellata | Oligochaeta | | | | RTU_2 |
| Annelida | | Clitellata | Oligochaeta | | | | RTU_3 |
| Mollusca | | Gastropoda | | | Lymnaeidae | *Pseudosuccinea* | |
| Mollusca | | Gastropoda | | | Physidae | *Physa* | |
| Mollusca | | Gastropoda | | | Planorbidae | | |
| Mollusca | | Gastropoda | | | Planorbidae | *Vorticifex* | |
| Nematoda | | | | | | | |
| Arthropoda | Crustacea | Branchiopoda | Phyllopoda | Cladocera | | | |
| Arthropoda | Crustacea | Maxillopoda | Copepoda | Cyclopoida | | | |
| Arthropoda | Crustacea | Ostracoda | | | | | |
| Arthropoda | Chelicerata | Arachnida | | | Hydrachnidae | | |
| Arthropoda | Hexapoda | Insecta | | Odonata | Aeshnidae | | nymph |
| Arthropoda | Hexapoda | Insecta | | Odonata | Coenagrionidae | | nymph |
| Arthropoda | Hexapoda | Insecta | | Odonata | Coenagrionidae | | nymph |
| Arthropoda | Hexapoda | Insecta | | Odonata | Gomphidae | | nymph |
| Arthropoda | Hexapoda | Insecta | | Odonata | Corduliidae | | nymph |
| Arthropoda | Hexapoda | Insecta | | Odonata | Libelluidae | *Libellula* | nymph |
| Arthropoda | Hexapoda | Insecta | | Trichoptera | Hydroptilidae | | pupae |
| Arthropoda | Hexapoda | Insecta | | Ephemeroptera | Baetidae | | nymph |
| Arthropoda | Hexapoda | Insecta | | Ephemeroptera | Baetidae | *Americabaetis* | nymph |
| Arthropoda | Hexapoda | Insecta | | Ephemeroptera | Baetidae | *Heterocloen* | nymph |
| Arthropoda | Hexapoda | Insecta | | Hemiptera | Corixidae | | nymph |
| Arthropoda | Hexapoda | Insecta | | Hemiptera | Gerridae | | nymph |
| Arthropoda | Hexapoda | Insecta | | Coleoptera | Dytiscidae | | adult |
| Arthropoda | Hexapoda | Insecta | | Coleoptera | Dytiscidae | *Hydaticus* | larvae |
| Arthropoda | Hexapoda | Insecta | | Coleoptera | Hydrophilidae | | adult |
| Arthropoda | Hexapoda | Insecta | | Coleoptera | Hydrophilidae | *Berosus* | larvae |
| Arthropoda | Hexapoda | Insecta | | Coleoptera | Hydrophilidae | | larvae |
| Arthropoda | Hexapoda | Insecta | | Diptera | Ceratopogonidae | | larvae and pupae |
| Arthropoda | Hexapoda | Insecta | | Diptera | Chironomidae | | larvae and pupae |
| Arthropoda | Hexapoda | Insecta | | Diptera | Tabanidae | | larvae |
| Arthropoda | Hexapoda | Insecta | | Diptera | Tipulidae | | larvae |
| **TERRESTRIAL** | | | | | | | |
| Arthropoda | | Arachnida | Acari | | | | |
| | | Hymenoptera | | | Formicidae | | |
| | | Thysanoptera | | | | | |
| | | unidentified | | | | | |

*RTU: Recognizable Taxonomic Unit (Beattie and Oliver 1994)

## Appendix 3: Summary of invertebrate fauna density

*Note that some taxa occur in very low densities and appear to be absent from samples due to the number of decimal places used in the tables.*

**Table A3-1: Density of invertebrate fauna (individuals/m$^2$) collected from pool samples (mean ± se).**

| | July | | | August | | | September | | | October | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| total abundance | 48577.8 | ± | 14117.8 | 57614.8 | ± | 5230.1 | 21170.4 | ± | 3689.5 | 36725.9 | ± | 1964.5 |
| taxonomic richness | 10.3 | ± | 1.9 | 9.3 | ± | 0.9 | 12.3 | ± | 1.7 | 12.0 | ± | 1.7 |
| Oligochaeta (RTU 1) | 1792.6 | ± | 1277.9 | 0.0 | ± | 0.0 | 163.0 | ± | 141.3 | 88.9 | ± | 88.9 |
| Oligochaeta (RTU 2) | 1881.5 | ± | 1881.5 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| Oligochaeta (RTU 3) | 607.4 | ± | 607.4 | 0.0 | ± | 0.0 | 385.2 | ± | 385.2 | 29.6 | ± | 29.6 |
| Lymnaeidae | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 29.6 | ± | 29.6 |
| Physidae | 651.9 | ± | 544.5 | 0.0 | ± | 0.0 | 370.4 | ± | 286.1 | 266.7 | ± | 223.7 |
| Planorbidae | 325.9 | ± | 145.9 | 0.0 | ± | 0.0 | 192.6 | ± | 131.7 | 444.4 | ± | 444.4 |
| Nematoda | 2563.0 | ± | 926.7 | 400.0 | ± | 133.3 | 548.1 | ± | 64.6 | 696.3 | ± | 221.2 |
| Cladocera | 0.0 | ± | 0.0 | 770.4 | ± | 291.8 | 1377.8 | ± | 706.0 | 888.9 | ± | 336.5 |
| Cyclopoida | 59.3 | ± | 59.3 | 44.4 | ± | 25.7 | 44.4 | ± | 44.4 | 14.8 | ± | 14.8 |
| Ostracoda | 33718.5 | ± | 16500.1 | 49081.5 | ± | 5191.6 | 7377.8 | ± | 2279.4 | 26251.9 | ± | 2608.5 |
| Hydrachnidae | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 14.8 | ± | 14.8 | 0.0 | ± | 0.0 |
| Aeshnidae | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 14.8 | ± | 14.8 |
| Coenagrionidae | 74.1 | ± | 39.2 | 177.8 | ± | 25.7 | 459.3 | ± | 282.6 | 370.4 | ± | 97.1 |
| Gomphidae | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| Corduliidae | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| Libelluidae | 29.6 | ± | 14.8 | 207.4 | ± | 165.0 | 370.4 | ± | 14.8 | 740.7 | ± | 397.8 |
| Hydroptilidae | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 74.1 | ± | 53.4 | 29.6 | ± | 14.8 |
| Baetidae | 74.1 | ± | 39.2 | 163.0 | ± | 82.5 | 29.6 | ± | 29.6 | 148.1 | ± | 106.8 |
| Corixidae | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| Gerridae | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| Dytiscidae (adult) | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| Hydaticus (larvae) | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| Hydrophilidae (adult) | 14.8 | ± | 14.8 | 0.0 | ± | 0.0 | 14.8 | ± | 14.8 | 0.0 | ± | 0.0 |
| Berosus (larvae) | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| Ceratopogonidae | 3481.5 | ± | 1324.7 | 1688.9 | ± | 102.6 | 5629.6 | ± | 3262.5 | 2755.6 | ± | 445.2 |
| Chironomidae | 3155.6 | ± | 836.6 | 3763.0 | ± | 406.0 | 1807.4 | ± | 258.3 | 2859.3 | ± | 218.2 |
| Tabanidae | 133.3 | ± | 133.3 | 14.8 | ± | 14.8 | 0.0 | ± | 0.0 | 14.8 | ± | 14.8 |
| Tipulidae | 0.0 | ± | 0.0 | 1303.7 | ± | 412.4 | 2311.1 | ± | 1050.2 | 1081.5 | ± | 754.4 |
| Acari | 14.8 | ± | 14.8 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| Formicidae | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| Thysanoptera | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| unidentified (terrestrial) | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |

**Table A3-2: Density of invertebrate fauna (individuals/m$^2$) collected from run samples (mean ± se).**

| | July | | | August | | | September | | | October | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| total abundance | 8367.2 | ± | 2773.1 | 15084.8 | ± | 5556.0 | 9981.5 | ± | 979.2 | 9031.9 | ± | 1549.7 |
| taxonomic richness | 8.0 | ± | 2.3 | 10.7 | ± | 0.3 | 12.0 | ± | 0.6 | 9.7 | ± | 2.7 |
| Oligochaeta (RTU 1) | 19.3 | ± | 11.2 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| Oligochaeta (RTU 2) | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| Oligochaeta (RTU 3) | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| Lymnaeidae | 1.9 | ± | 1.9 | 0.0 | ± | 0.0 | 3.7 | ± | 3.7 | 0.0 | ± | 0.0 |
| Physidae | 5.6 | ± | 3.2 | 5.6 | ± | 5.6 | 11.1 | ± | 5.6 | 15.2 | ± | 15.2 |
| Planorbidae | 6.9 | ± | 3.5 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 1.9 | ± | 1.9 |
| Nematoda | 6.3 | ± | 6.3 | 0.0 | ± | 0.0 | 46.3 | ± | 35.8 | 0.0 | ± | 0.0 |
| Cladocera | 0.0 | ± | 0.0 | 60.0 | ± | 13.9 | 61.1 | ± | 14.7 | 43.0 | ± | 12.9 |
| Cyclopoida | 7.4 | ± | 7.4 | 35.6 | ± | 17.4 | 0.0 | ± | 0.0 | 8.9 | ± | 8.9 |
| Ostracoda | 8032.8 | ± | 2683.3 | 13655.6 | ± | 5061.0 | 7822.2 | ± | 655.6 | 5244.4 | ± | 1213.3 |
| Hydrachnidae | 0.0 | ± | 0.0 | 2.2 | ± | 2.2 | 5.6 | ± | 5.6 | 0.0 | ± | 0.0 |
| Aeshnidae | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 1.9 | ± | 1.9 | 0.0 | ± | 0.0 |
| Coenagrionidae | 3.2 | ± | 3.2 | 25.9 | ± | 16.1 | 11.1 | ± | 3.2 | 4.4 | ± | 4.4 |
| Gomphidae | 1.9 | ± | 1.9 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| Corduliidae | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 4.4 | ± | 4.4 |
| Libelluidae | 0.0 | ± | 0.0 | 14.8 | ± | 1.9 | 5.6 | ± | 0.0 | 41.5 | ± | 7.8 |
| Hydroptilidae | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| Baetidae | 0.0 | ± | 0.0 | 24.1 | ± | 9.3 | 11.1 | ± | 6.4 | 26.7 | ± | 26.7 |
| Corixidae | 0.0 | ± | 0.0 | 1.9 | ± | 1.9 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| Gerridae | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 1.9 | ± | 1.9 | 0.0 | ± | 0.0 |
| Dytiscidae (adult) | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 4.4 | ± | 4.4 |
| Hydaticus (larvae) | 0.0 | ± | 0.0 | 1.9 | ± | 1.9 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| Hydrophilidae (adult) | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| Berosus (larvae) | 0.0 | ± | 0.0 | 1.9 | ± | 1.9 | 1.9 | ± | 1.9 | 4.4 | ± | 4.4 |
| Ceratopogonidae | 156.1 | ± | 50.4 | 217.0 | ± | 84.7 | 90.7 | ± | 32.8 | 321.5 | ± | 79.3 |
| Chironomidae | 107.1 | ± | 49.7 | 999.6 | ± | 470.1 | 1759.3 | ± | 277.4 | 3102.2 | ± | 392.8 |
| Tabanidae | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| Tipulidae | 0.0 | ± | 0.0 | 5.6 | ± | 3.2 | 25.9 | ± | 14.8 | 140.7 | ± | 42.3 |
| Acari | 13.8 | ± | 6.9 | 33.3 | ± | 19.2 | 122.2 | ± | 5.6 | 68.1 | ± | 14.1 |
| Formicidae | 1.9 | ± | 1.9 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| Thysanoptera | 3.2 | ± | 3.2 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| unidentified (terrestrial) | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |

**Table A3-3: Density of invertebrate fauna (individuals/m$^2$) collected from macrophyte samples (mean ± se).**

| | July | | | August | | | September | | | October | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| total abundance | 77.6 | ± | 47.6 | 3065.8 | ± | 1756.3 | 202.5 | ± | 121.6 | 1253.2 | ± | 303.3 |
| taxonomic richness | 1.7 | ± | 0.9 | 5.3 | ± | 0.7 | 4.3 | ± | 1.2 | 6.3 | ± | 1.2 |
| Oligochaeta (RTU 1) | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| Oligochaeta (RTU 2) | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| Oligochaeta (RTU 3) | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| Lymnaeidae | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| Physidae | 1.7 | ± | 1.7 | 16.5 | ± | 16.5 | 0.6 | ± | 0.6 | 4.2 | ± | 4.2 |
| Planorbidae | 0.0 | ± | 0.0 | 38.0 | ± | 30.9 | 0.0 | ± | 0.0 | 4.1 | ± | 4.1 |
| Nematoda | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| Cladocera | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 12.6 | ± | 12.6 |
| Cyclopoida | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| Ostracoda | 53.0 | ± | 53.0 | 2543.3 | ± | 1531.2 | 87.9 | ± | 53.3 | 605.5 | ± | 177.4 |
| Hydrachnidae | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| Aeshnidae | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| Coenagrionidae | 2.8 | ± | 2.8 | 5.0 | ± | 5.0 | 3.4 | ± | 3.4 | 24.9 | ± | 14.5 |
| Gomphidae | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| Corduliidae | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| Libelluidae | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| Hydroptilidae | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 8.1 | ± | 8.1 |
| Baetidae | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| Corixidae | 18.4 | ± | 18.4 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| Gerridae | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| Dytiscidae (adult) | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| Hydaticus (larvae) | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| Hydrophilidae (adult) | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| Berosus (larvae) | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| Ceratopogonidae | 0.0 | ± | 0.0 | 70.0 | ± | 39.4 | 4.0 | ± | 3.1 | 50.5 | ± | 22.1 |
| Chironomidae | 1.7 | ± | 1.7 | 248.5 | ± | 49.9 | 73.6 | ± | 50.9 | 445.4 | ± | 136.4 |
| Tabanidae | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| Tipulidae | 0.0 | ± | 0.0 | 144.5 | ± | 113.9 | 25.2 | ± | 13.0 | 93.9 | ± | 41.9 |
| Acari | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 4.1 | ± | 4.1 |
| Formicidae | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| Thysanoptera | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 |
| unidentified (terrestrial) | 0.0 | ± | 0.0 | 0.0 | ± | 0.0 | 7.9 | ± | 7.9 | 0.0 | ± | 0.0 |

**Table A3-4: Density of invertebrate fauna (individuals/L) collected from water column samples (mean ± se).**

| | July | August | September | October |
|---|---|---|---|---|
| total abundance | 0.2 ± 0.0 | 0.1 ± 0.1 | 0.3 ± 0.0 | 0.0 ± 0.0 |
| taxonomic richness | 1.7 ± 0.3 | 0.7 ± 0.3 | 2.3 ± 0.3 | 0.0 ± 0.0 |
| Oligochaeta (RTU 1) | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Oligochaeta (RTU 2) | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Oligochaeta (RTU 3) | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Lymnaeidae | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Physidae | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Planorbidae | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Nematoda | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Cladocera | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Cyclopoida | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Ostracoda | 0.1 ± 0.0 | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.0 ± 0.0 |
| Hydrachnidae | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Aeshnidae | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Coenagrionidae | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Gomphidae | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Corduliidae | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Libelluidae | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Hydroptilidae | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Baetidae | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Corixidae | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Gerridae | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Dytiscidae (adult) | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Hydaticus (larvae) | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Hydrophilidae (adult) | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Berosus (larvae) | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Ceratopogonidae | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Chironomidae | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Tabanidae | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Tipulidae | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Acari | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Formicidae | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Thysanoptera | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| unidentified (terrestrial) | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |