Annual Report 2012-2013

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 has indicated 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 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 conservation 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 are not fed formulated feed but rather allowed to feed on naturally occurring food items in the refugium; therefore, the results of the proposed gut content analysis and natural substrate analysis will be more representative of RGSM diets in 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 in the refugium are similar to the heterogeneous habitats available in the Rio Grande.

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

In 2012, the refugium was used to conduct a spawning study and, once that ended, to raise offspring that had been produced during the study. The refugium was flooded on 16 January 2012. Sand bag levees were constructed around the ponds, shelves, and overbank areas to confine the adults to the stream. Seven hundred and forty-five adults (Lot 10CSDX) were stocked on February 15, 2012. Water was added to the refugium three times to simulate the spring snow melt runoff in the Rio Grande and to cause flooding of the overbank areas of the refugium (Tave et al. 2011); water level was lowered at the end of each flood to pre-flood level. Overbank areas were flooded three times (24-29 April, 7-15 May, 22-29 May). Spawning was observed after the first and second flood. Fish collections for this project occurred after May 29.

Fertilizer was added to the refugium eight times between 14 March and 23 May (the spawning component), totaling 100 mL (0.91 L ha^{-1}) of 11-37-0 N-P-K liquid fertilizer and 11.35 kg (103.2 kg ha^{-1}) of alfalfa pellets. Beginning in June, the refugium was fertilized indirectly from three 737 L source tanks which were siphoned into the sump, the location where pumps drive water

back to the inlet for reuse (Figure 1). Source tanks were each fertilized with 25 mL 11-37-0 and 500 g alfalfa pellets and allowed to sit for 48-72 h before being siphoned into the sump. Between June 28 and September 9 sixty-two source tanks were siphoned into the sump (total water volume was 45,694 L); total fertilizer added to the refugium via the source tanks was 1.56 L 11-37-0 (15 L ha^{-1}) and 33 kg alfalfa pellets (300 kg ha^{-1}).

General water quality

Monthly sampling was conducted in the refugium four times from July to October 2012: 27 July, 17 August, 19 September and 17 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 (mS cm^{-1}), pH, dissolved oxygen (mg L^{-1}, %), 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). Flow velocity (m s^{-1}) was measured with a Marsh-McBirney Flo-mate 2000 meter. 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 Earth and Planetary Sciences 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).

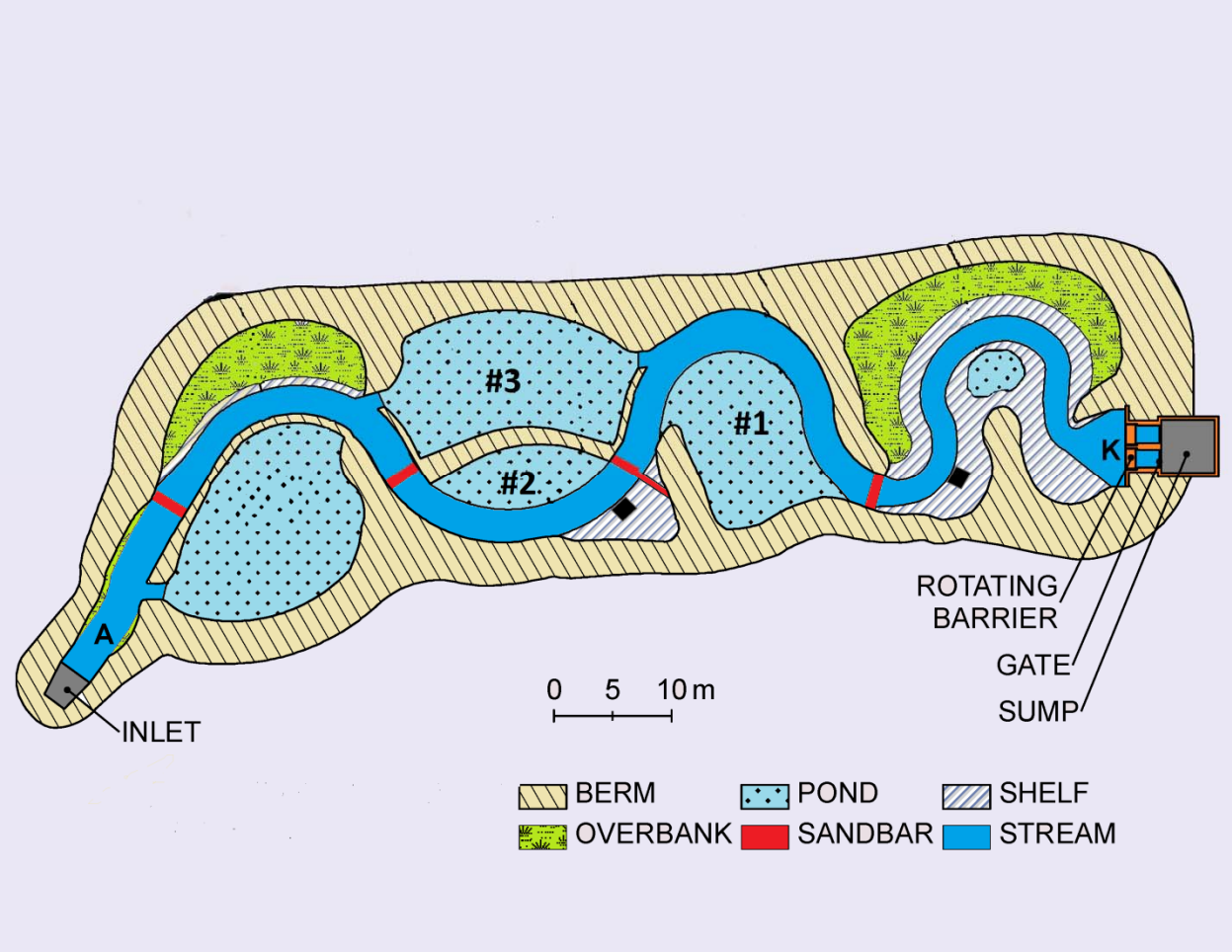

Figure 1: Plan view of the naturalized outdoor refugium at the Los Lunas Silvery Minnow Refugium. The run habitat is the solid dark blue section that flows from point A to point K. Pools # 1 (Pond 4), 2 (Pond 3), and 3 (Pond 2) respectively were used in this study (modified fromHutson et al. 2012).

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 refugium water column) (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 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 were 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 boiled with 30% hydrogen peroxide to oxidize organic material. These samples were rinsed seven times with distilled water to remove oxidation byproducts. 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). An image database 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 microcrustacea, 10 L of water was passed through a 10-µm plankton net. Microcrustacea collected in the net were 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, 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 sorting tray. Macroinvertebrates were removed from the entire exposed 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, 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 macrophyte 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 Motoda 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

RGSM were collected by refugium employees during the monthly sampling, up to a maximum of 25 (July, n = 25; August, n = 25; September, n = 15; October, n = 12). 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. First, 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 done as described above. These methods were similar to other studies that have examined gut contents of fish for diatoms and other algae (Rosati et al. 2003).

Data analysis

The algal and invertebrate benthic samples were reported as units/sample area while the samples from the gut analyses were reported as relative abundances because of the difficulty in quantifying sample size. T-tests and nested analysis of variance (ANOVA) were utilized to determine differences among habitats, prey items, and seasonality (Gelwick and Matthews 2006).

Analysis of Similarities (ANOSIM, in PRIMER) was used to examine statistical differences among multivariate assemblage data (Clarke and Warwick 1994). ANOSIM conducts similar tests to an ANOVA, but uses non-parametric (randomization-based) methods to examine differences 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.

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).

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.

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 higher 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 anions differed very little between pool and run habitats but some nutrients were significantly different over the four survey months (Table 2). While differences in some anions (e.g., sulfate and chloride) were statistically significant among sampling events (Table 3), these anions are generally not biologically significant. In contrast, nitrate is a biologically important (e.g., biological reactions, growth) and was elevated and significantly different among sampling periods, although the absolute seasonal difference in nitrate concentration in the pools was 0.5 mg L^{-1}. Notably, PO_4 and Br were below detection limits in all samples.

Table 1. Summary of physiochemical parameters collected in pool and run habitats at LLSMR, July-October 2012.

	habitat	July	August	September	October
Water temperature (°C)	run	24.28	23.68	17.62	14.29
	pool	24.35	23.75	17.62	14.28
Salinity (ppt)	run	0.79	0.81	0.94	0.92
	pool	0.79	0.81	0.94	0.92
Turbidity (NTU)	run		1.1	3.28	16.7
	pool		1.1	4.12	
DO (%)	run	108.2	104.2	110.3	108.6
	pool	108.7	107.8	109.1	109.9
DO (mgL^{-1})	run	9.02	8.77	10.47	11.08
	pool	9.06	9.09	10.33	11.18
Specific conductivity ($mScm^{-1}$)	run	1.543	1.562	1.58	1.437
	pool	1.546	1.524	1.576	1.437
pH	run	7.9	7.63	7.92	7.31
	pool	8.04	7.86	7.87	7.31
Flow velocity (ms^{-1})	run	0.02	0.06	0.04	0.01
	pool	0	0	0	0

Table 2. Summary of nutrient analyses from water samples collected in pool and run habitats at LLSMR, July-October 2012; n.d. = non detectable.

		June	July	August	September
NO_3 (mgL^{-1})	pool	4.7 ± 0.3	4.6 ± 0.1	4.4 ± 0.0	4.2 ± 0.1
	run	4.9 ± 0.3	4.6 ± 0.1	4.4 ± 0.0	5.2 ± 0.5
NH_4 (μgL^{-1})	pool	12.3 ± 0.8	10.8 ± 0.9	13.9 ± 1.6	6.4 ± 1.0
	run	11.3 ± 4.3	7.2 ± 0.3	10.3 ± 1.2	5.2 ± 0.3
SO_4 (mgL^{-1})	pool	208.6 ± 6.9	214.1 ± 11.6	280.8 ± 2.7	241.6 ± 0.3
	run	207.9 ± 8.4	207.5 ± 32.4	287.1 ± 5.8	240.4 ± 0.5
Cl (mgL^{-1})	pool	23.4 ± 0.8	22.2 ± 0.8	32.5 ± 0.1	37.1 ± 0.3
	run	33.2 ± 10.5	21.9 ± 2.0	32.8 ± 0.2	36.9 ± 0.2
PO_4 (mgL^{-1})	pool	n.d. ± n.d.	n.d. ± n.d.	n.d. ± n.d.	n.d. ± n.d.
	run	n.d. ± n.d.	n.d. ± n.d.	n.d. ± n.d.	n.d. ± n.d.
Br (mgL^{-1})	pool	n.d. ± n.d.	n.d. ± n.d.	n.d. ± n.d.	n.d. ± n.d.
	run	n.d. ± n.d.	n.d. ± n.d.	n.d. ± n.d.	n.d. ± n.d.

Table 3. Results from nested analysis of variance of all nutrients measured from water samples, testing for differences among sample events (July – October 2012) and habitat types (run, pool). Note that PO_4 and Br were below detection limits in all samples.

	Sample Event			Habitat (Sample Event)		
	Wald χ-Square	df	P	Wald χ -Square	df	P
NO_3	10.5	3	**0.015**	0.8	4	0.940
NH_4	0.5	3	0.913	0.2	4	0.996
SO_4	22372.3	3	**0.000**	127.4	4	**0.000**
Cl	736.6	3	**0.000**	144.6	4	**0.000**
PO_4	--	--	--	--	--	--
Br	--	--	--	--	--	--

Periphyton analyses

Sixty-two diatom taxa and 37 soft algal taxa (mostly green algae and cyanobacteria) were identified and enumerated from benthic pool, run, and macrophyte habitats as well as the water column (Appendix 1). The soft algae community contained multiple taxa from the Chlorophyta (green algae) including diverse unicellular desmids (3 *Cosmarium* spp. and 2 *Closterium* spp.), unicellular *Elakatothrix*, *Ankistrodesmus*, and *Actinatrum*, filamentous greens (*Oedogonium* and *Zygnema*), and multicellular colonial green algae (e.g., *Oocystis, Scenedesmus*). Filamentous cyanobacteria genera *Anabaena*, *Lyngbya*, and *Oscillatoria,* and two taxa of the colonial cyanobacterium *Chroococcus* were also recorded (Appendix 1). Overall, soft-bodied taxa were estimated to represent an average of 35.4% of the algal cell abundances. Diatom taxa that were common included *Epithemia sorex* (10.8% mean across sampling periods and habitats, with some samples as high as 25.7%) and *Rhopalodia gibba* (1.7% mean); these two genera contain nitrogen-fixing endosymbionts and are characteristic of the naturally occurring low-nitrogen conditions in the southwest U.S.A. The most common diatom was *Nitzschia perminuta* which was found 24.6% (average) (up to 56% in pools) across sampling periods and habitats (much higher density compared to 2011) (Bixby and Burdett 2013). Other common taxa were the mobile *Navicula cryptocephala* and *Nitzschia amphibia* (Appendix 1). The remaining common taxa included a number of upright-growing taxa (*Synedra ulna*, *Synedra rumpens* var. *familaris*, *Fragilaria vaucheriae,* and *Achnanthidium minutissimum*) and a planktonic taxon, *Stephanocyclus meneghiniana.*

All algal densities were significantly different by habitat but were not significantly different among sampling periods (Figure 2, Table 4). Algal densities (and diatoms specifically) were variable in the pool and water column samples while cell abundances increased on the macrophyte samples over the four-month sampling period (Figure 2). Densities of numerous diatom and soft alga taxa were significantly different among sampling periods and/or habitats (Table 4), compared to only one diatom taxon, *Navicymbula pusilla*, in 2011-2012. Some taxa

were in higher densities in the early summer (July, August) compared to later in the year, including diatom *Nitzschia amphibia*, a cyanobacterium *Lyngbya*, and green algae *Actinastrum* and *Oocystis.* Taxa in higher numbers in the late summer include diatoms *Navicula cryptocephala, Rhopalodia gibba*, and *Stephanocyclus meneghiniana* and a green alga *Closterium* sp. 1, which may reflect increased nitrate levels as a result of fertilization.

Taxonomic richness was significantly different among sampling periods and among habitats (Figure 3, Table 4); the water column has much lower diversity but also a different assemblage as well. Richness numbers ranged from 20-32 diatom taxa (mean = 22) with fewer taxa in the water column (6-24 taxa). There were 3-21 soft algal taxa (mean = 12) in a given habitat, including the water column (12-15 taxa).

The MDS shows that the algal communities (as all diatom and soft algal populations) were variable among habitats and seasons (Figure 4). There were no significant differences as an entire community among survey months (ANOSIM: Global R = -0.096, p = 0.689) but there were differences among habitats (ANOSIM: Global R = 0.743, p = 0.001); algal communities were most similar in pool and run samples, whereas macrophyte samples were different.

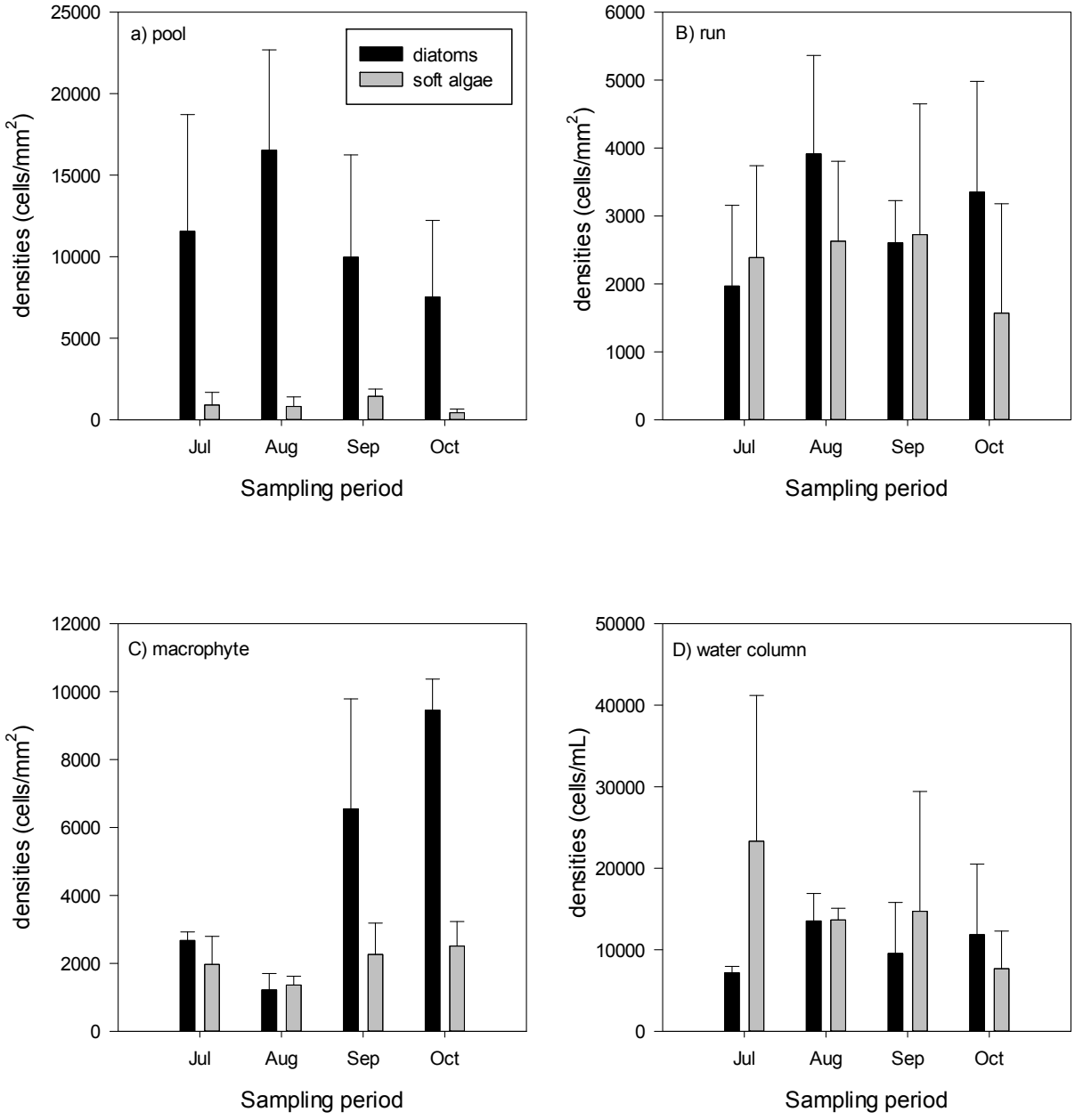

Figure 2. Summary of abundances of algae collected during monthly surveys in each of the four habitats. Total density (#individuals/mm^2 or cells/mL) (mean + s.e.) measured for diatoms and soft algae in each habitat type (pool, run, macrophyte, and water column). Note that samples from pool, run, and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

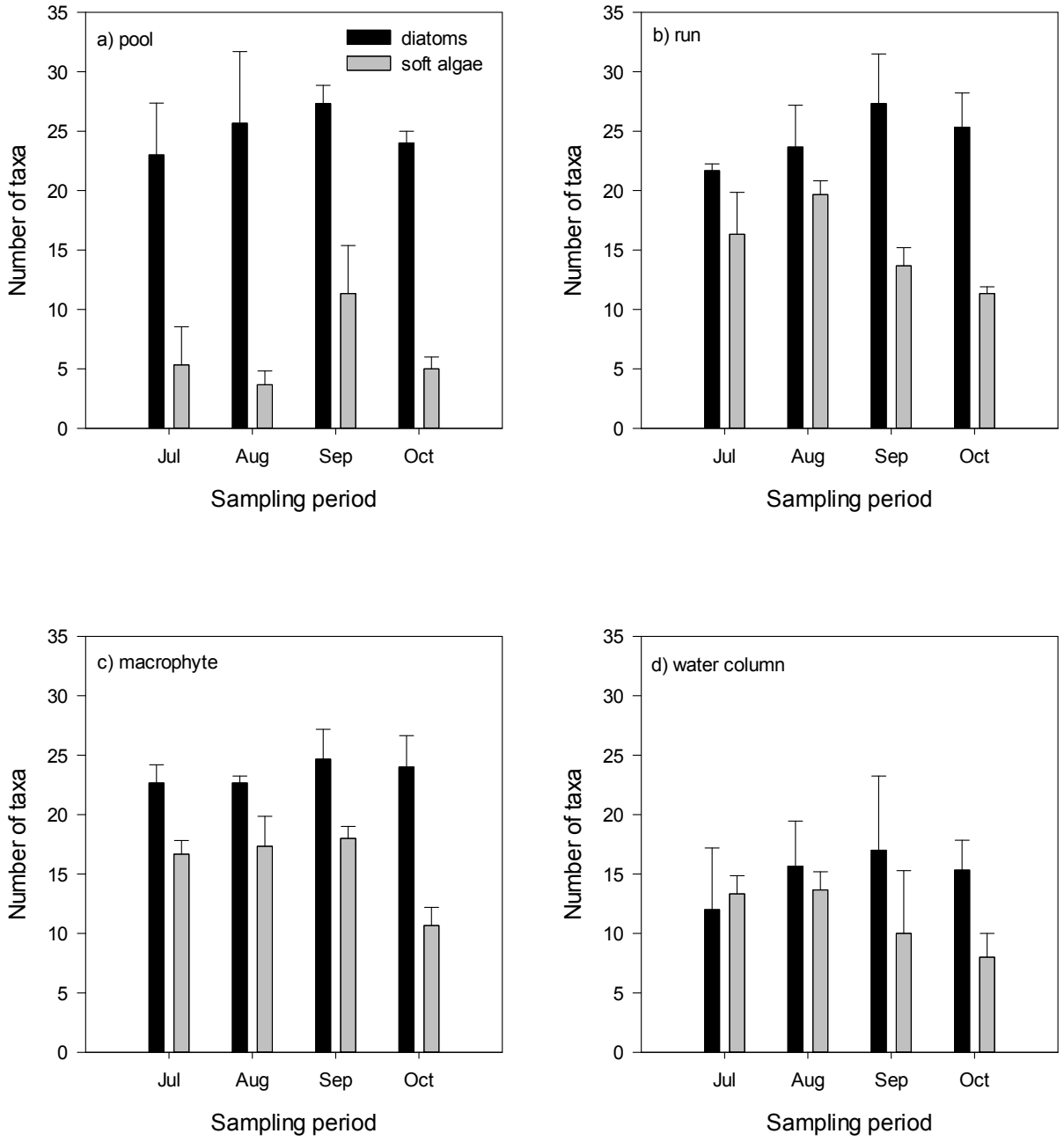

Figure 3. Summary of algal taxa richness collected during monthly surveys in each of the four habitats. Taxonomic richness (#taxa/mm^2 or cells/mL) (mean + s.e.) measured for diatoms and soft algae in each habitat type (pool, run, macrophyte, water column).

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 χ-Square	**df**	**P**	**Wald χ-Square**	**df**	**P**
All algae						
Total abundance*	0.2	3	0.975	113.3	12	**0.000**
Taxonomic richness	17.7	3	**0.000**	102.2	12	**0.000**
diatoms only						
Total abundance*	5.2	3	0.161	124.1	12	**0.000**
Taxonomic richness	13.1	3	**0.004**	99.0	12	**0.000**
*Achnanthidium minutissimum**	7.5	3	0.057	22.0	12	**0.038**
*Epithemia sorex**	1.9	3	0.587	19.8	12	0.071
*Fragilaria vaucheriae**	10.8	3	**0.013**	37.3	12	**0.000**
*Navicula cryptocephala**	16.7	3	**0.001**	88.4	12	**0.000**
*Nitzschia amphibia**	34.3	3	**0.000**	65.9	12	**0.000**
*Nitzschia perminuta**	6.4	3	0.094	148.7	12	**0.000**
*Rhopalodia gibba**	10.6	3	**0.014**	34.3	12	**0.001**
*Stephanocyclus meneghinana**	19.3	3	**0.000**	68.1	12	**0.000**
Synedra rumpens var. *familiaris**	40.0	3	**0.000**	39.4	12	**0.000**
soft algae only		3				
Total abundance*	9.3	3	**0.025**	190.7	12	**0.000**
Taxonomic richness	47.7	3	**0.000**	228.2	12	**0.000**
*Actinastrum**	272.8	3	**0.000**	301.1	12	**0.000**
*Anabaena**	4.9	3	0.180	29.6	12	**0.003**
Closterium sp. 1	56.1	3	**0.000**	36.4	12	**0.000**
Cosmarium sp. 5*	6.5	3	0.089	83.9	12	**0.000**
*Crucigenia**	11.0	3	**0.012**	18.4	12	0.103
*Elakatothrix gelatinosa**	12.0	3	**0.007**	7.2	12	**0.000**
*Lyngbya**	9.3	3	**0.025**	34.3	12	**0.001**
*Oedogonium**	11.0	3	**0.020**	72.5	12	**0.000**
*Oocystis**	10.7	3	**0.013**	52.8	12	**0.000**
*Oscillatoria**	7.0	3	0.071	44.0	12	**0.000**
*Scenedesmus**	4.5	3	0.216	37.2	12	**0.000**
*Zygnema**	6.3	3	0.100	74.4	12	**0.000**

*indicates variables that were log-transformed [x'=log10(x+1)] prior to analysis

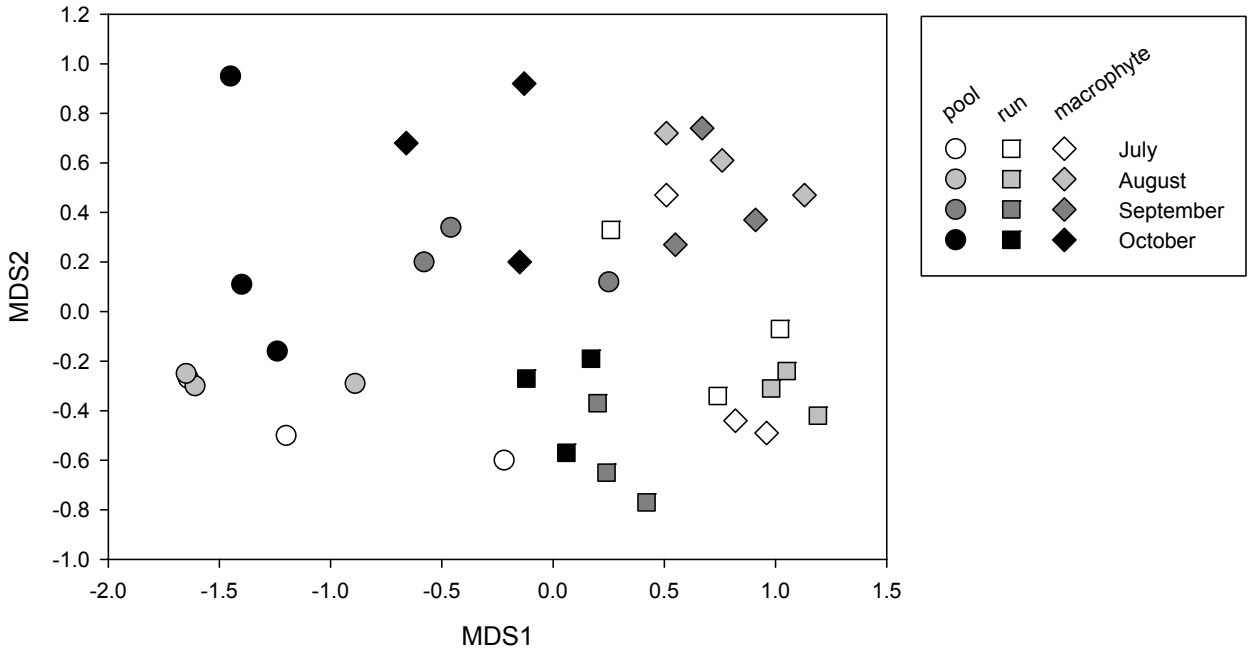

Figure 4. Non-metric multi-dimensional scaling (MDS) plot demonstrating similarity among diatom collected from pool, run, and macrophyte habitats at the four sampling times. Stress = 0.18. Because the units are different from the other samples, water column samples were not included in the analysis.

Invertebrate analyses

The invertebrate assemblage collected from pools, runs, macrophytes, and water column consisted of 52 identifiable aquatic taxa and four terrestrial taxa (Figure 5~~Figure 5~~; Appendix 2). This was more aquatic taxa than reported from 2011 surveys, partly because of higher taxonomic resolution of some groups. Ostracods were the dominant organism in all habitats. Chironomids and ceratopogonids (both midges) were also abundant. There were significant differences among sample events in taxonomic richness and the density of several taxa (chironomids, ceratopogonids, molluscs, mayflies, terrestrial fauna) and significant differences among habitats for most taxa (Table 5; Appendix 3). Densities were generally higher in the earlier sample months (July and August) than the later sample months (September and October). Relatively low densities of all organisms were collected from macrophytes and the water column compared to the benthic habitats.

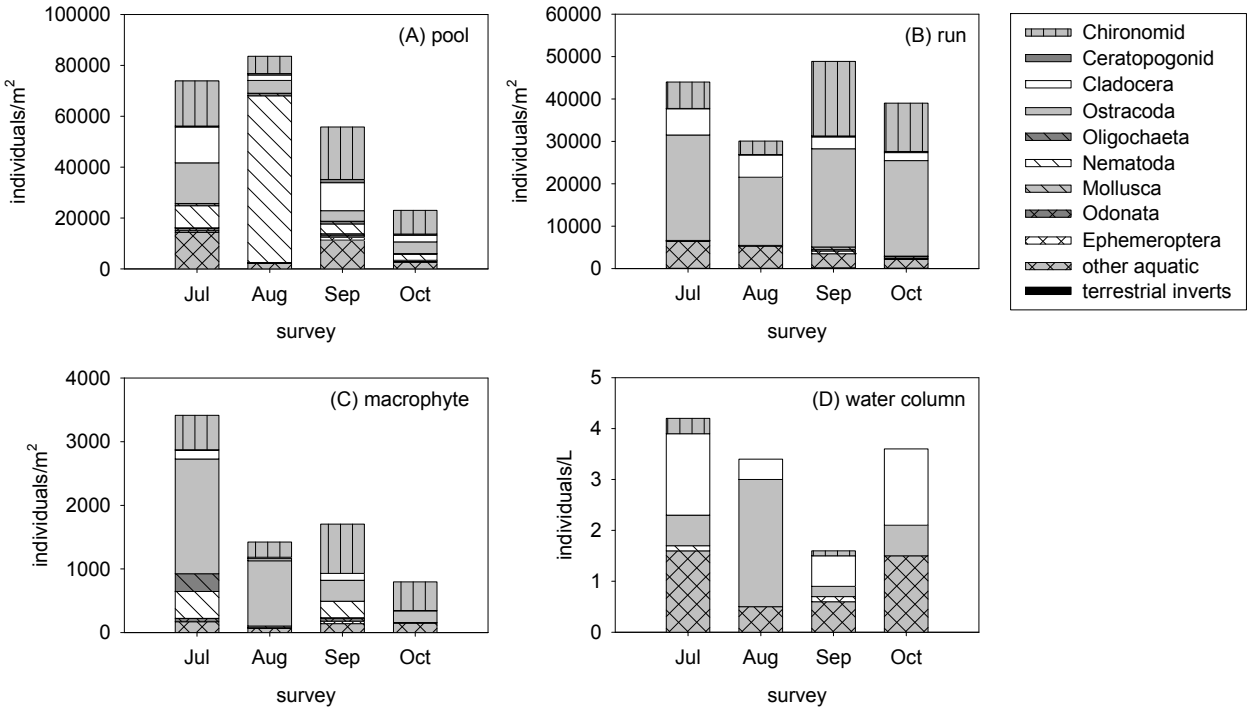

Figure 5. Summary of abundances of invertebrate fauna collected during monthly surveys (July-October) in each of the four habitats. For more details of invertebrate abundances, refer to Appendix 3. Note that samples from pool, run and macrophyte habitats are scaled to surface area (m^2) whereas samples 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). Significant results, P<0.05, bold font.

	Sample Event			Habitat (Sample Event)		
	Wald χ-Square	df	P	Wald χ-Square	df	P
Total abundance	3.8	3	0.279	**51.2**	**8**	**0.000**
Taxonomic richness	**26.7**	**3**	**0.000**	**69.2**	**8**	**0.000**
Chironomidae	**21.4**	**3**	**0.000**	**71.3**	**8**	**0.000**
Ceratopogonidae	**18.4**	**3**	**0.000**	**131.7**	**8**	**0.000**
Ostracoda	3.7	3	0.297	**46.1**	**8**	**0.000**
Cladocera	4.9	3	0.176	**17.4**	**8**	**0.026**
Oligochaeta	1.2	3	0.744	14.1	8	0.079
Nematoda	6.3	3	0.096	**20.2**	**8**	**0.010**
Mollusca	**14.7**	**3**	**0.002**	**18.0**	**8**	**0.021**
Odonata	6.5	3	0.088	**23.9**	**8**	**0.002**
Ephemeroptera	**8.6**	**3**	**0.036**	15.4	8	0.051
Other aquatic fauna	5.2	3	0.160	**18.9**	**8**	**0.015**
Terrestrial invertebrate fauna	**9.0**	**3**	**0.029**	**102.5**	**8**	**0.000**

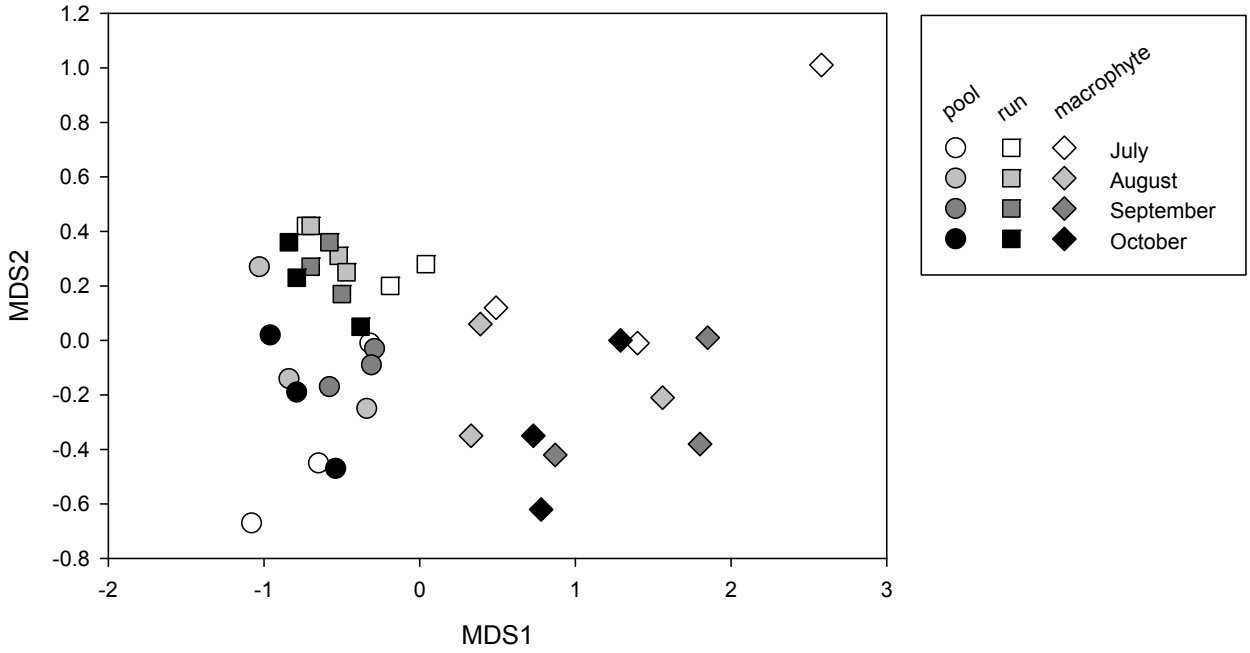

Figure 6. Non-metric multidimensional scaling (MDS) plot demonstrating similarity among invertebrate assemblages collected from pool, run, and macrophyte habitats at the four sampling times. Stress = 0.07. Note that the water column assemblage is not included in this analysis because of extremely low densities and different units.

The invertebrate assemblage in the refugium differed spatially among habitat types within sample events (ANOSIM: Global R = 0.706, p = 0.002) but did not differ temporally among sample events (ANOSIM: Global R = -0.110, p = 0.120). The MDS plot indicates that assemblages from run habitats were generally more similar to those collected from pool habitats than those from macrophyte habitats (Figure 6). Assemblages on macrophytes were more variable because of comparatively low densities.

Common invertebrate taxa were found in all habitats, including Cladocera, Ostracoda, Diptera (e.g., Chironomidae, Ceratopogonidae, Tipulidae) and gastropod snails (mainly *Physa*). However, the diversity of assemblages in pools (mean number of taxa ± s.e. = 11.8 ± 1.0) and runs (10.3 ± 0.8) was great than on macrophytes (6.1 ± 0.7) or in the water column (2.0 ± 0.3). 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, 40 RGSM were dissected and examined for gut content analysis. Weight and length of fish increased through time (Figure 7A). All of the fish that were examined (n = 10 for each month) had some items in their guts (Figure 7B). The guts were relatively full in the first three sampling events compared to October. On average, 87% of the gut tract was filled in July, 86% in August and 80% in September, compared to 64% in October.

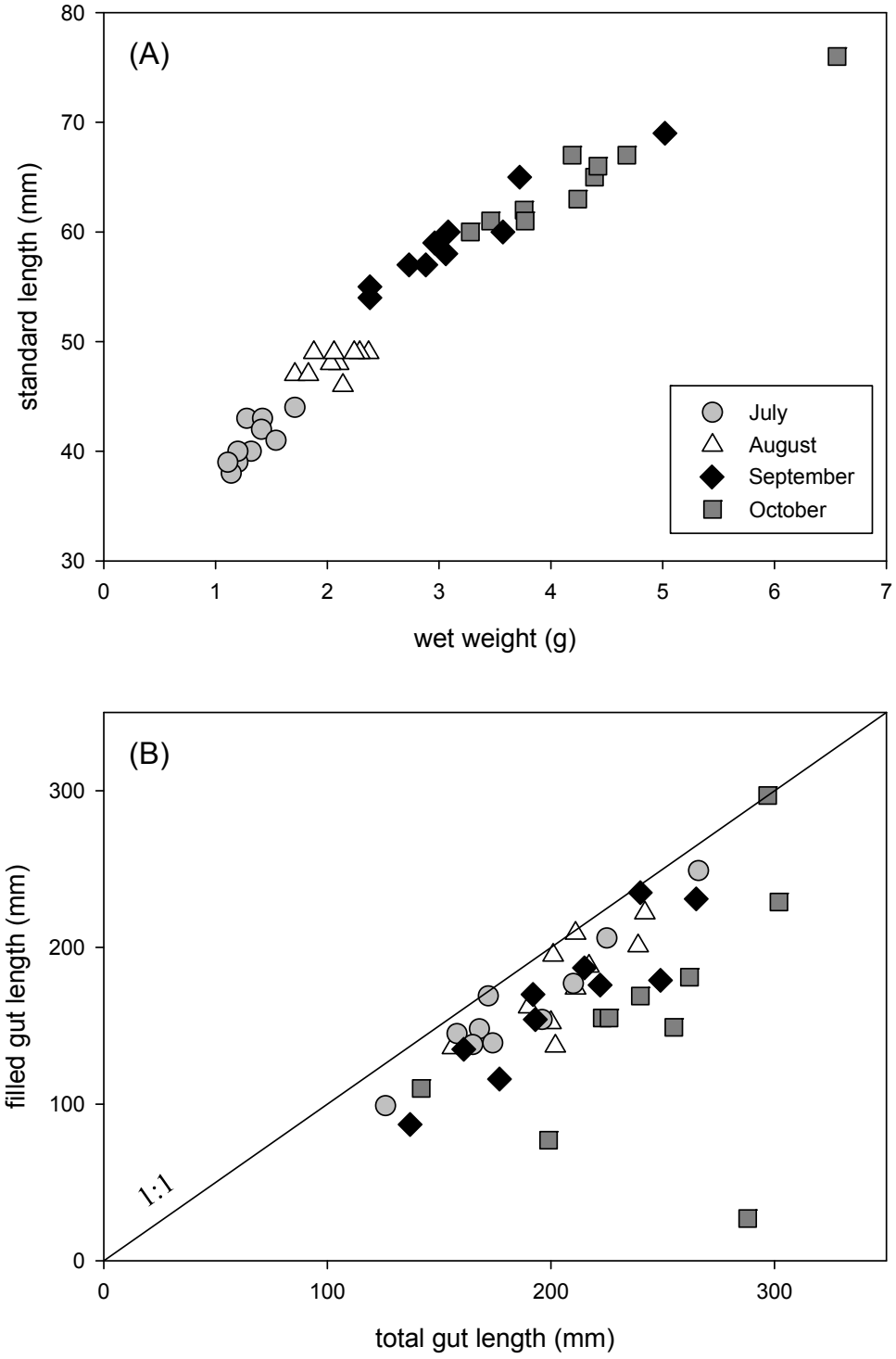

Figure 7. Information for fish used in gut content analysis. (A) Relationship between wet weight of fish and standard length, y=21.0 ln(x) + 34.6, R^2=0.967. (B) Relationship between total gut length and filled gut length; symbols closer to the 1:1 line indicate that the gut was full.

Diatom flora in the guts

Diatoms in RGSM guts were classified into five different growth forms that are differentially affected by grazers (Steinman 1996). The majority of the diatom taxa (and densities) are represented by detached or 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.

Both spatial and temporal seasonal patterns in algae from RGSM guts versus the environment are noted from the four sampling periods (Figure 8, Table 6). In July, a number of taxa preferred by the minnow including a diatom *Epithemia sorex* and *Selanastrum* sp., a planktonic green algae that may have settled on substrates being grazed (Figure 8). It should be noted that *Selanastrum* declined in overall numbers throughout the sampling period. The diatom *Nitzschia amphibia* was strongly avoided, while *Nitzschia perminuta* was neither consumed nor avoided relative to the population in the environment. In August, upright growing *Synedra ulna* and *Synedra rumpens* var. *familiaris* were preferentially consumed, while *Epithemia sorex* was neutral and *Nitzschia perminuta* was strongly avoided (Figure 8). The pattern for *Epithemia sorex* was similar in September, while *Nitzschia perminuta* was slightly more preferred, compared to earlier samples, as well as *Oocytis* sp., a colonial green alga that was associated with the benthic substrates. In October, *Epithemia sorex* continued to be commonly consumed, as well as *Cosmarium* sp. 1 and *Nitzschia amphibia* while *Nitzschia perminuta* was much less preferred compared to previous months.

Table 6. Summary of algae by growth from and preference found in refugium samples and RGSM 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. Bold numbers are > 0.000. This table continues over three pages.

Growth form		Jul	Aug	Sep	Oct
Generally preferred					
Detached and prostrate	*Closterium* sp. 2	**1.000**	**1.000**	**1.000**	**1.000**
	Cocconeis placentula	**1.000**	----	**1.000**	----
	Dinoflagellate	**0.625**	**0.923**	**0.892**	**1.000**
	Green alga sp. 3	**1.000**	**1.000**	**1.000**	**1.000**
	Green alga sp. 4	**1.000**	**1.000**	**1.000**	----
	Hantzschia virgata	**1.000**	**1.000**	----	----
	Staurosira construens	**1.000**	**1.000**	**1.000**	----
	Tetraedron	**1.000**	**0.709**	**1.000**	**0.256**
Filamentous	*Anabaena* cf.	**1.000**	**1.000**	**1.000**	**1.000**
	Cylindrospermum	**1.000**	**1.000**	**1.000**	----
	Mougeotia	**1.000**	**1.000**	**1.000**	**0.061**
	Zygnema	**0.031**	**0.236**	**0.339**	**0.599**
Mobile and prostrate	*Achnanthes linearis*	**1.000**	----	**1.000**	----
	Caloneis bacillum	**0.970**	**0.921**	**0.065**	**0.772**
	Green alga sp. 2	**0.438**	**0.416**	**0.540**	**1.000**
	Navicula cf. *symmetrica*	**0.947**	**0.899**	**0.481**	**0.560**
	Navicula cryptocephala	**0.113**	**0.446**	**0.082**	**0.302**
	Navicula sp. 5	**1.000**	**1.000**	----	----
	Navicymbula pusilla	**0.607**	**0.779**	**0.076**	**0.657**
	Nitzschia terrestris	----	**1.000**	----	----
	Pinnularia borealis	**1.000**	**1.000**	----	----
Planktonic	*Actinastrum*	**0.720**	**0.720**	----	----
	Diatoma moniliformis	**1.000**	**0.000**	----	----
	Green alga sp. 5	**1.000**	**1.000**	**1.000**	----
Upright	*Gomphonema* cf. *gracile*	**0.296**	**0.532**	**0.372**	**0.308**
Shifting preferences					
Detached and prostrate	*Chroococcus* sp.	-0.870	**----**	-0.572	**0.278**
	Chroococcus turgida	-0.314	**0.915**	-0.473	-1.000
	Closterium sp. 1	-0.381	**1.000**	**0.389**	**0.746**
	Colonial green	**0.623**	-0.282	-1.000	-1.000
	Cosmarium sp. 3	**0.713**	**0.134**	-0.079	**0.108**
	Cosmarium sp. 5	-0.389	**0.120**	-0.029	-0.145
	Green alga sp. 6	**1.000**	-1.000	----	----

	Oocytis	**0.154**	**0.550**	**0.715**	-0.581
	Peridinium	-1.000	----	**1.000**	----
	Phacus	**1.000**	-1.000	**1.000**	----
	Staurosira construens var. *venter*	**0.837**	**1.000**	-1.000	----
Filamentous	*Lyngbya*	**0.684**	**0.230**	-0.091	**0.341**
	Oedogonium	**0.798**	-0.423	**0.033**	-0.222
	Oscillatoria	-0.597	**0.365**	-0.509	-0.255
Mobile and prostrate	*Achnanthidium exiguum*	-0.414	-0.820	**0.301**	-0.286
	Adlafia muscora	**1.000**	**1.000**	-1.000	-1.000
	Amphora libyca	-0.117	**0.451**	-0.198	-0.117
	Amphora sp. 1	**0.160**	-0.245	**0.378**	**0.280**
	Anomoeoneis sphaerophora	**1.000**	**1.000**	-1.000	**0.342**
	Craticula ambigua	**1.000**	**1.000**	-1.000	-1.000
	Craticula cuspidata	**0.639**	**0.493**	-0.108	**0.524**
	Craticula sp.	**0.861**	**0.396**	-0.065	-1.000
	Diploneis elliptica	**1.000**	-0.651	**1.000**	-0.426
	Epithemia adnata	-0.506	**0.732**	0.019	**0.285**
	Epithemia cf. *adnata*	**1.000**	----	----	-1.000
	Navicula capitatoradiata	**1.000**	**1.000**	-1.000	----
	Navicula cryptotenella	**1.000**	----	**1.000**	-1.000
	Navicula veneta	**1.000**	**1.000**	-0.236	**1.000**
	Nitzschia acicularis	**1.000**	----	-1.000	-1.000
	Nitzschia amphibia	**0.665**	-0.183	**0.010**	-0.136
	Nitzschia clausii	**1.000**	----	**1.000**	-1.000
	Nitzschia gracilis	**1.000**	**1.000**	-0.747	**0.573**
	Nitzschia inconspicua	----	**1.000**	-1.000	-1.000
	Nitzschia linearis	-0.299	----	-0.039	**1.000**
	Nitzschia palea	**1.000**	**1.000**	-0.672	**1.000**
	Nitzschia perminuta	-0.064	**0.282**	-0.056	**0.351**
	Pinnularia viridis	**0.193**	**0.383**	-0.495	-0.302
	Pseudostaurosira brevistriata	**1.000**	----	-1.000	-1.000
	Rhopalodia gibba	-0.348	**0.144**	**0.197**	**0.003**
	Rhopalodia gibberula	**1.000**	**1.000**	-0.007	-0.387
	Scenedesmus (2 cell)	-0.219	-0.669	**1.000**	-1.000
	Scenedesmus (4 cell)	**0.092**	**0.040**	**0.100**	-0.416
	Tryblionella hungarica (no undulate margin)	**1.000**	----	----	-0.544
	Tryblionella hungarica (undulate margin)	**0.164**	-0.018	**0.166**	**0.257**
Planktonic	*Ankistrodesmus spiralis*	-1.000	**0.449**	**1.000**	----
	Crucigenia	**0.681**	-0.584	-0.433	**0.145**

	Elakatothrix gelatinosa	-0.126	**1.000**	**1.000**	----
	Green alga sp. 1	-0.498	**0.277**	**0.341**	-0.739
	Merismopedia	-0.077	**0.129**	-0.337	-0.429
	Selanastrum sp. 1	-0.779	**0.042**	----	**0.220**
	Selanastrum sp. 2	**1.000**	-0.860	----	-1.000
	Sellaphora pupula	**0.861**	**1.000**	-0.723	**1.000**
	Stephanocyclus meneghiniana	**0.037**	-0.173	-0.251	**0.195**
	Synura	**0.168**	**0.183**	-0.135	-0.241
Upright	*Fragilaria capucina* var. *mesolepta*	**1.000**	**1.000**	-0.577	-0.444
	Fragilaria delicatissima var. *angustissima*	-0.599	-0.435	**0.029**	-0.058
	Fragilaria vaucheriae	-0.472	-0.697	**0.035**	-0.374
	Gomphonema clavatum	**0.543**	**0.735**	**0.150**	-0.750
	Gomphonema gracile	-0.043	**0.243**	**0.157**	**0.184**
	Gomphonema lagenula	-0.320	-0.058	**0.681**	**0.168**
	Gomphonema parvulum	-0.547	-0.188	**0.513**	-0.447
	Gomphonema pumilum	**1.000**	**1.000**	----	-1.000
	Synedra rumpens	**0.620**	----	**1.000**	-1.000
	Synedra sp. 1	**1.000**	----	----	-1.000
	Synedra ulna	-0.654	-0.222	-0.081	**0.222**
Generally unpreferred					
Upright	*Achnanthidium minutissimum*	-0.729	-0.499	-0.273	-0.418
Detached and prostrate	*Cosmarium* sp. 1	-0.619	-0.203	-0.253	-0.367
Mobile and prostrate	*Epithemia sorex*	-0.491	-0.047	-0.154	-0.129
	Mastogloia elliptica	----	----	----	-1.000
	Navicula recens	----	1.000	----	----
	Navicula rostellata	----	----	1.000	----
Planktonic	*Pediastrum*	----	----	-1.000	----
Upright	*Synedra rumpens* var. *familiaris*	-0.706	-0.798	-0.099	-0.254
	Synedra rumpens var. *fragilarioides*	----	----	-1.000	-1.000

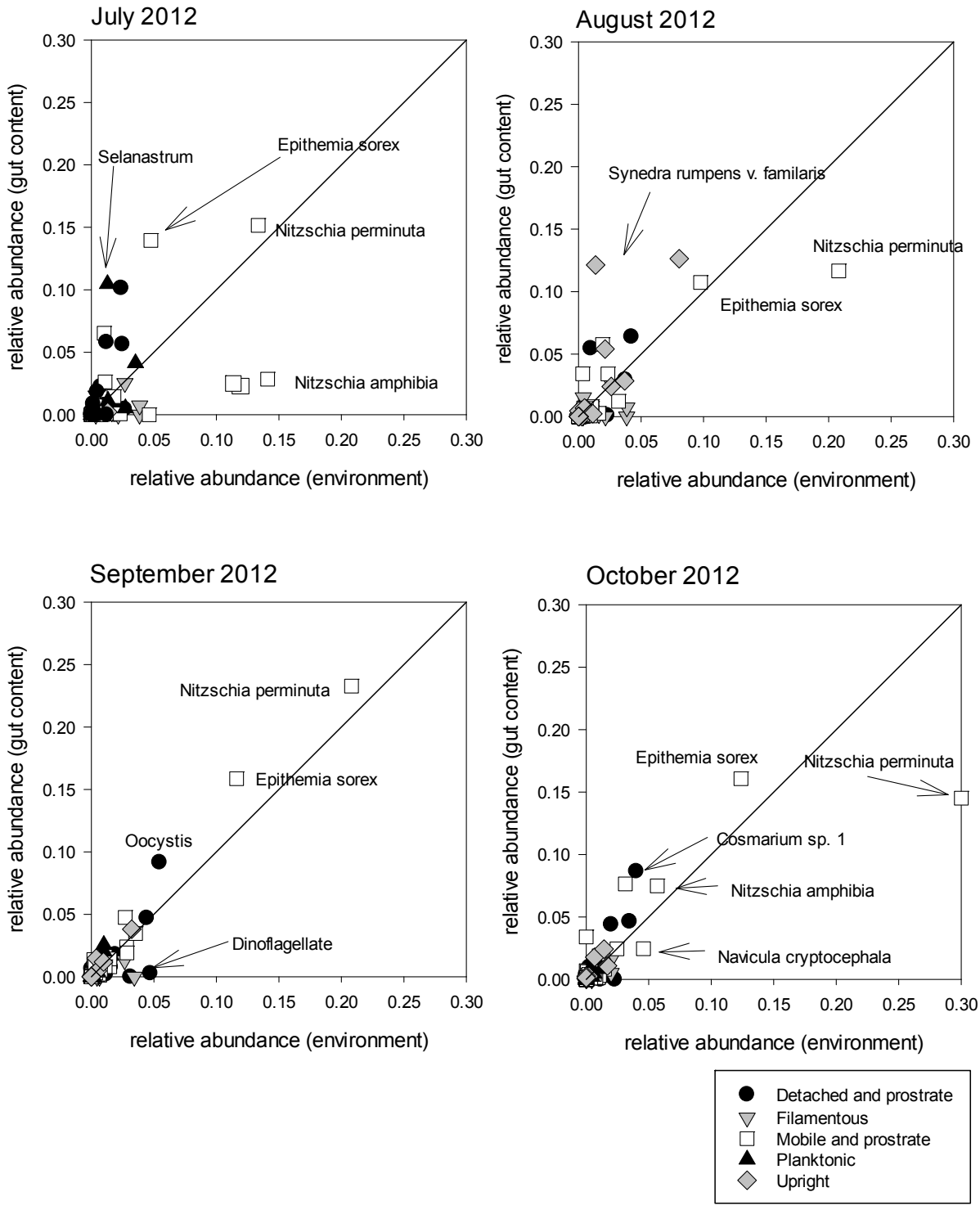

Figure 8. Relative abundance of major algal groups in the refugium environment and in RGSM gut contents in 4 sampling periods (A-D); a graph of mean relative abundances over the sampling period was not included. The diagonal line indicates 1:1 – taxa above the line are preferentially selected whereas those below the line are not.

Invertebrate fauna in the guts

Invertebrate items found in the gut were classified into 20 different categories, including identifiable animals and non-identifiable 'insect parts.' The number of different items gradually declined from July to October (Table). Ostracoda was the most common taxon in the guts in July and August (Figure 9~~Figure 9~~). Later in the season, Cladocera (September) and Simuliidae (October) were relatively abundant in the guts.

Results from the Ivlev's Electivity Index indicated that RGSM were preferentially consuming some of the less common dipterans (Simuliidae, Tipulidae, Tabanidae), mayflies (Ephemeroptera) and water boatmen (Hemiptera: Corixidae)(Table). However, the high positive Electivity Index for these taxa is an artifact of relatively low abundances in the environment compared to the more abundant taxa (e.g., ostracods and chironomids). Observations during sample processing indicate that these organisms were not the most common food item – ostracods were found in half of the guts throughout the study.

Chironomids were relatively abundant in the environment throughout the study but were rarely found in RGSM guts between July and September. Nematodes were also abundant at times, but never found in RGSM guts. Nematodes were found in RGSM guts in 2011 (Bixby and Burdett 2013); it is unclear why nematodes were not a preferred food source in 2012.

Adult midges were only found in one gut from a fish collected in September. By comparison, adult midges were identified as an important part of the fish diet in later samples collected in 2011 (Bixby and Burdett 2013).

Ivlev's electivity index only includes items that were identified in *both* environmental and gut samples. Because only invertebrate individuals were identified in the environmental samples, several important items were excluded from Ivlev's index. In particular, seeds (37%) and insect parts that could not be identified (15%) were frequently found in RGSM guts. Broken shells (3%, usually Ostracoda) and unidentifiable eggs (2%, likely Cladocera) were also found in RGSM guts regularly.

Table 7. Number of items collected from fish guts at each sample event. "All items" includes invertebrates, insect parts, eggs, seeds and mineral items. "Invertebrate" only includes identifiable invertebrate items that could be recorded from the environment.

	All items		Invertebrate	
	mean	se	mean	se
July	5.5	0.9	3.5	0.6
Aug	4.8	0.4	3.3	0.5
Sept	3.5	0.7	1.7	0.5
Oct	2.8	0.4	1.2	0.3

Table 8. Summary of invertebrate groups found in refugium samples and RGSM 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. "na" indicates groups that were not found in guts or in the environment in that sample month.

group	taxon	Jul	Aug	Sep	Oct
generally preferred					
Diptera	Diptera - pupa	na	1.000	1.000	-1.000
	Diptera - adult	na	na	1.000	na
	Simuliidae	Na	1.000	1.000	0.997
	Tipulidae	1.000	0.979	0.998	-1.000
Other aquatic	Corixidae	0.979	1.000	na	na
shifting preferences					
Other aquatic	Ostracoda	0.193	0.040	-0.860	-0.075
	Ephemeroptera	-1.000	0.730	-1.000	0.808
	Other aquatic insect	0.177	0.146	0.816	-1.000
	Odonata	-1.000	-1.000	-1.000	0.652
Terrestrial	Terrestrial mite	0.874	0.885	-1.000	-1.000
generally unpreferred					
Diptera	Chironomidae	0.135	-0.151	-0.288	-1.000
	Ceratopogonidae	0.072	-1.000	-1.000	-1.000
	Tabanidae	-1.000	na	na	na
Microcrustacea	Cladocera	-0.543	-0.667	-1.000	-1.000
	Copepoda	0.274	-1.000	na	-1.000
Other aquatic	Nematoda	-1.000	-1.000	-1.000	-1.000
	Oligochaeta	-1.000	-1.000	-1.000	-1.000
	snails	-1.000	-1.000	-1.000	-1.000
Terrestrial	Terrestrial inverts	-1.000	-1.000	na	na

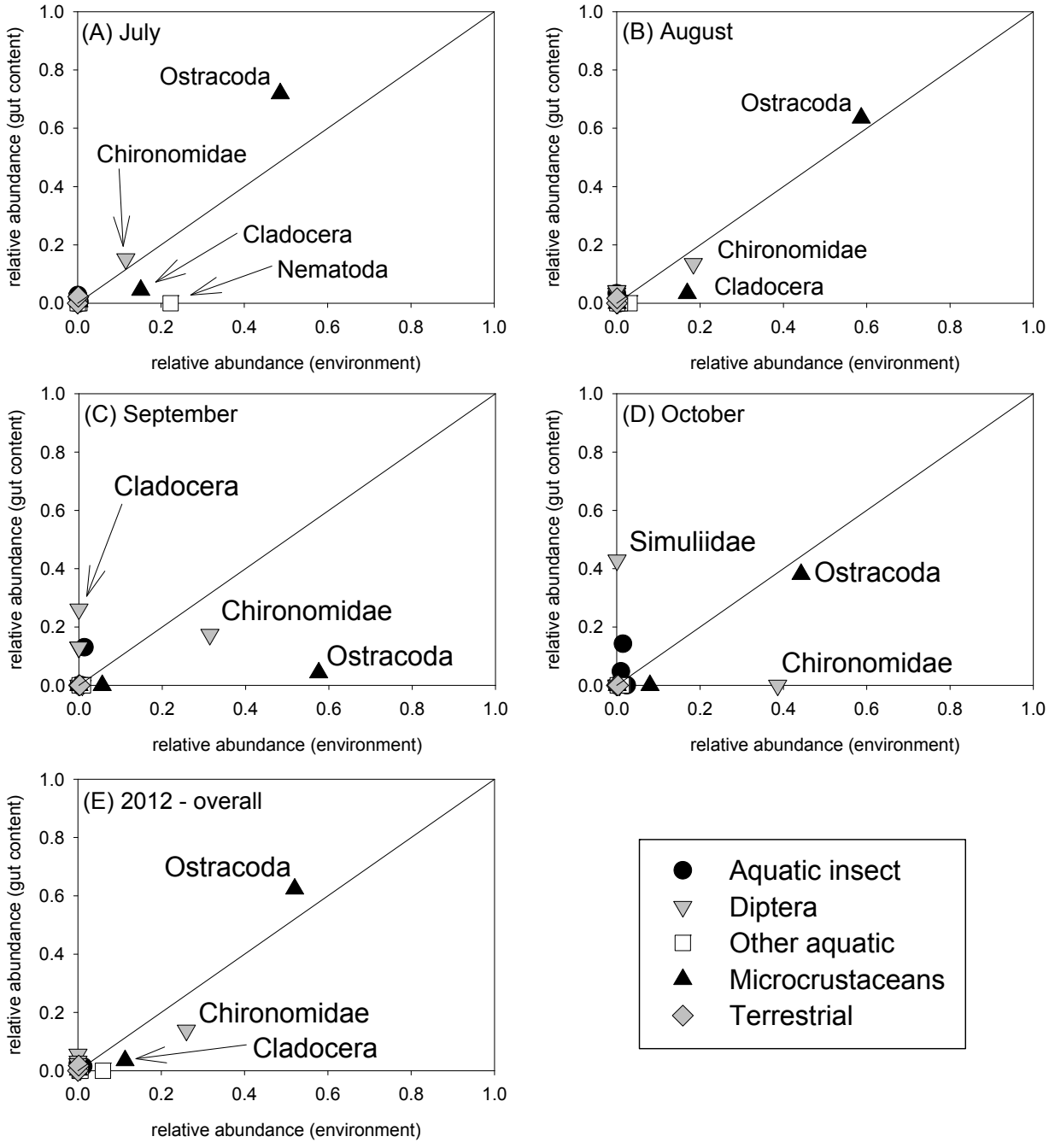

Figure 9. Relative abundance of major invertebrate groups in the refugium environment and in RGSM gut contents at each sampling event (A-D), and averaged across the study period (E). The diagonal line indicates 1:1 – taxa above the line are preferentially selected whereas those below the line are not.

Discussion and Conclusions

This study had two questions proposed:

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

The benthic habitat sampling demonstrated that the densities of food resources (algal and invertebrate) were 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. If RGSM 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. Characteristics including growth form (upright, prostrate, attached to a substrate), overall abundance, palatability, and mobility (ability to escape consumption) play a role in whether food items are consumed or avoided. The diatoms *Epithemia sorex* and *Nitzschia perminuta*, 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 RGSM 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, guts, in general, were less full although no guts were empty, compared to 2011 data (Bixby and Burdett, 2013). 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 including the water column – densities that are comparable to the three earlier months (Figure 2~~Figure 2~~, Figure 5~~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). A number of cyanobacteria taxa 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. The two more common cyanobacteria which cause toxic blooms (*Microcystis*, *Aphanizomenon*) were not collected. 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 the appropriate type of food resources for the RGSM (i.e., quality), although we are unable to assess in this study whether the quantities of these food resources were adequate for RGSM in this study and can make no conclusions on food production or food sufficiency in the refugium.

Most differences in anion concentrations were not biologically significant but nitrate, which is key for growth and fitness, differed significantly among sampling events (although the absolute difference in concentration was relatively small). Variation in nitrate 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.). There was also increased fertilization in 2012, compared to 2011, reflected by higher nitrate concentration [0.7 mgL^{-1} mean (2011), 4.6 mgL^{-1} mean (2012)] as a result of increased fertilization (Bixby and Burdett 2013). This increase in nitrate, in turn, is reflected in community shifts from taxa associated with low nutrient environments to higher nutrient environments. For example, from 2011 to 2012, there was a decrease in diatom taxa (*Epithemia* and *Rhopalodia)* that are typical of low-nitrogen water bodies in New Mexico. These taxa have nitrogen-fixing cyanobacterial endosymbionts with the ability to fix atmospheric nitrogen, allowing the host organisms to survive in environments with low nitrate concentrations (Floener and Bothe 1980).

Sampling in both 2011 and 2012 has allowed us to examine the seasonal variability of taxa richness and abundance through two seasons. Both types of food items (algae and invertebrates) displayed different patterns in habitat colonization and seasonality (Bixby and Burdett 2013). Total abundances for all algae and invertebrates by sampling period were not significantly different in both years, showing a consistent number of organisms at the refugium throughout the summer. In contrast, seasonality continued to be reflected as changes in taxa richness and responses of many individual algal and invert taxa among sampling periods with a strong, consistent seasonal component to the overall community structure, especially in 2012. There were notably less soft-bodied algae in 2012 compared to 2011 which might be related to fertilization practices in 2012.

Habitat differences continued to also partition organismal data with differences reflected in total abundances and taxa richness response. Macrophyte habitats were, in general, different, compared to other habitats. Increased fertilization in 2012 may also affect habitats disproportionately. Pools and macrophytes, with potentially increased organic material deposition and production, may experience greater impact of fertilization compared to the concrete runs sampled. Ultimately, these habitat differences in abundance and community structure support the importance of multiple habitats in the refugium to support diversity of food resources.

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Appendix 1: Taxonomic list of algal flora in benthic and guts samples

Bacillariophyceae (diatoms)

Achnanthes linearis
Achnanthidium exiguum
Achnanthidium minutissimum
Adlafia muscora
Amphora libyca
Amphora sp. 1
Anomoeoneis sphaerophora
Caloneis bacillum
Cocconeis placentula
Craticula ambigua
Craticula cuspidata
Craticula sp.
Diatoma moniliformis
Diploneis elliptica
Epithemia adnata
Epithemia cf. *adnata*
Epithemia sorex
Fragilaria capucina var. *mesolepta*
Fragilaria delicatissima var. *angustissima*
Fragilaria vaucheriae
Gomphonema cf. *gracile*
Gomphonema clavatum
Gomphonema gracile
Gomphonema lagenula
Gomphonema parvulum
Gomphonema pumilum
Hantzschia virgata
Mastogloia elliptica
Navicula capitatoradiata
Navicula cf. *symmetrica*
Navicula cryptocephala
Navicula cryptotenella
Navicula recens
Navicula rostellata
Navicula sp. 5
Navicula veneta
Navicymbula pusilla
Nitzschia acicularis
Nitzschia amphibia
Nitzschia clausii
Nitzschia gracilis
Nitzschia inconspicua
Nitzschia linearis
Nitzschia palea
Nitzschia perminuta
Nitzschia terrestris
Pinnularia borealis
Pinnularia viridis

Pseudostaurosira brevistriata
Rhopalodia gibba
Rhopalodia gibberula
Sellaphora pupula
Staurosira construens
Staurosira construens var. *venter*
Stephanocyclus meneghiniana
Synedra rumpens
Synedra rumpens var. *familiaris*
Synedra rumpens var. *fragilarioides*
Synedra sp. 1
Synedra ulna
Tryblionella hungarica (no undulate margin)
Tryblionella hungarica (undulate margin)

Chlorophyta (green algae)
Actinastrum
Ankistrodesmus spiralis
Closterium sp. 1
Closterium sp. 2
Colonial green
Cosmarium sp. 3
Cosmarium sp. 5
Crucigenia
Elakatothrix gelatinosa
Green alga sp. 1
Green alga sp. 2
Green alga sp. 3
Green alga sp. 4
Green alga sp. 5
Green alga sp. 6
Merismopedia
Mougeotia
Oedogonium
Oocytis
Pediastrum
Phacus
Scenedesmus (2 cell)
Scenedesmus (4 cell)
Selanastrum sp. 1
Selanastrum sp. 2
Tetraedron
Zygnema

Cyanobacteria (blue-green algae)
Anabaena cf.
Chroococcus sp.
Chroococcus turgida
Cylindrospermum
Lyngbya

Oscillatoria

Dinoflagellata (dinoflagellates
Dinoflagellate sp.
Peridinium

Synurophyceae (synurids)
Synura

Appendix 2: Preliminary taxonomic list of invertebrate fauna

Phylum	Subphylum	Class	Subclass	Order	Family	Genus	notes
AQUATIC							
Annelida		Clitellata	Oligochaeta				
Mollusca		Gastropoda			Lymnaeidae	*Pseudosuccinea*	
Mollusca		Gastropoda			Physidae	*Physa*	
Mollusca		Gastropoda			Planorbidae		
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	Aeshnidae	*Gynacantha*	nymph
Arthropoda	Hexapoda	Insecta		Odonata	Aeshnidae	*Oplonaeschna*	nymph
Arthropoda	Hexapoda	Insecta		Odonata	Aeshnidae	*Anax*	nymph
Arthropoda	Hexapoda	Insecta		Odonata	Coenagrionidae		nymph
Arthropoda	Hexapoda	Insecta		Odonata	Coenagrionidae	*Ischnura*	nymph
Arthropoda	Hexapoda	Insecta		Odonata	Coenagrionidae	*Telebasis*	nymph
Arthropoda	Hexapoda	Insecta		Odonata	(Anisoptera)		nymph
Arthropoda	Hexapoda	Insecta		Odonata	Corduliidae		nymph
Arthropoda	Hexapoda	Insecta		Odonata	Libelluidae		nymph
Arthropoda	Hexapoda	Insecta		Odonata	Libelluidae	*Libellula*	nymph
Arthropoda	Hexapoda	Insecta		Odonata	Libelluidae	*Pseudoleon*	nymph
Arthropoda	Hexapoda	Insecta		Odonata	Libelluidae	*Sympetrum*	nymph
Arthropoda	Hexapoda	Insecta		Odonata	Libelluidae	*Celithemus?*	nymph
Arthropoda	Hexapoda	Insecta		Odonata	Libelluidae	*Erythemis*	nymph

Appendix 2 continued

Phylum	Subphylum	Class	Subclass	Order	Family	Genus	notes
Arthropoda	Hexapoda	Insecta		Trichoptera	Hydroptilidae		nymph and pupae
Arthropoda	Hexapoda	Insecta		Trichoptera	Hydroptilidae	*Stactiobiella*	nymph
Arthropoda	Hexapoda	Insecta		Trichoptera	Hydroptilidae	*Oxyethira*	nymph
Arthropoda	Hexapoda	Insecta		Trichoptera	Hydroptilidae	*Ochrotrichia*	nymph
Arthropoda	Hexapoda	Insecta		Trichoptera	Leptoceridae		nymph and pupae
Arthropoda	Hexapoda	Insecta		Trichoptera	Hydropsychidae		nymph
Arthropoda	Hexapoda	Insecta		Trichoptera	Ecnomidae	*Austrotinodes?*	nymph
Arthropoda	Hexapoda	Insecta		Ephemeroptera	Baetidae		nymph
Arthropoda	Hexapoda	Insecta		Ephemeroptera	Baetidae	*Pseudocentroptiloides*	nymph
Arthropoda	Hexapoda	Insecta		Hemiptera	Corixidae		nymph
Arthropoda	Hexapoda	Insecta		Hemiptera	Gerridae		nymph
Arthropoda	Hexapoda	Insecta		Coleoptera	Dytiscidae		larvae and adult
Arthropoda	Hexapoda	Insecta		Coleoptera	Dytiscidae	*Hydaticus*	larvae
Arthropoda	Hexapoda	Insecta		Coleoptera	Hydrophilidae		adult
Arthropoda	Hexapoda	Insecta		Coleoptera	Hydrophilidae	*Berosus*	larvae
Arthropoda	Hexapoda	Insecta		Diptera	Ceratopogonidae		larvae and pupae
Arthropoda	Hexapoda	Insecta		Diptera	Chironomidae		larvae and pupae
Arthropoda	Hexapoda	Insecta		Diptera	Simulilidae		larvae
Arthropoda	Hexapoda	Insecta		Diptera	Tipulidae		larvae
Arthropoda	Hexapoda	Insecta		Diptera	Tabanidae		larvae
Arthropoda	Hexapoda	Insecta		Diptera	Tabanidae	*Tabanus*	larvae
Arthropoda	Hexapoda	Insecta		Diptera	Empididae		larvae
Arthropoda	Hexapoda	Insecta		Diptera			pupa
TERRESTRIAL							
Arthropoda		Arachnida	Acari				

Appendix 3: Summary of invertebrate fauna density

Note that only taxa occurring in each habitat are listed 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	20059.3	±	8264.6	27365.9	±	17604.8	15111.1	±	5182.5	6814.8	±	1396.4
taxonomic richness	10.7	±	2.3	9.3	±	0.7	13.7	±	1.2	8.3	±	0.9
Chironomidae	5896.3	±	2386.4	2254.8	±	635.8	6874.1	±	1936.8	3096.3	±	1199.8
Ceratopogonidae	133.3	±	67.9	195.6	±	67.3	414.8	±	82.5	177.8	±	51.3
Tipulidae	59.3	±	59.3	0.0	±	0.0	29.6	±	29.6	14.8	±	14.8
Tabanidae	0.0	±	0.0	14.8	±	14.8	0.0	±	0.0	0.0	±	0.0
Oligochaeta	296.3	±	232.8	334.8	±	312.9	355.6	±	311.1	88.9	±	67.9
Copepoda	133.3	±	88.9	225.2	±	53.9	192.6	±	170.9	0.0	±	0.0
Cladocera	4696.3	±	2826.1	711.1	±	235.2	3659.3	±	3059.5	859.3	±	103.7
Ostracoda	5318.5	±	3577.4	1685.9	±	517.8	1377.8	±	703.2	1525.9	±	286.1
Baetidae	207.4	±	53.4	29.6	±	14.8	385.2	±	276.8	103.7	±	82.5
Aeshnidae	0.0	±	0.0	0.0	±	0.0	14.8	±	14.8	0.0	±	0.0
Libellulidae	192.6	±	97.1	29.6	±	14.8	88.9	±	67.9	29.6	±	29.6
Coenagrionidae	133.3	±	133.3	44.4	±	0.0	177.8	±	111.8	74.1	±	53.4
Hydroptilidae	14.8	±	14.8	0.0	±	0.0	59.3	±	14.8	0.0	±	0.0
Leptoceridae	14.8	±	14.8	0.0	±	0.0	14.8	±	14.8	0.0	±	0.0
Dytiscidae	14.8	±	14.8	0.0	±	0.0	14.8	±	14.8	0.0	±	0.0
Nematoda	2903.7	±	1706.2	21840.0	±	16885.4	1288.9	±	911.9	800.0	±	398.4
Planorbidae	0.0	±	0.0	0.0	±	0.0	74.1	±	29.6	14.8	±	14.8
Physidae	29.6	±	29.6	0.0	±	0.0	74.1	±	14.8	29.6	±	29.6
Lymnaeidae	14.8	±	14.8	0.0	±	0.0	14.8	±	14.8	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	12577.8	±	3157.6	8270.4	±	4626.0	15343.0	±	4342.4	12377.8	±	1765.0
taxonomic richness	8.7	±	0.9	7.7	±	1.5	12.3	±	0.7	10.3	±	0.3
Chironomidae	2066.7	±	154.9	1051.9	±	488.4	5855.6	±	1166.6	3798.1	±	468.4
Ceratopogonidae	22.2	±	11.1	27.8	±	20.0	73.3	±	13.9	88.9	±	22.2
Simuliidae	0.0	±	0.0	0.0	±	0.0	11.1	±	11.1	0.0	±	0.0
Tipulidae	0.0	±	0.0	0.0	±	0.0	15.6	±	9.7	0.0	±	0.0
Oligochaeta	5.6	±	5.6	11.1	±	11.1	0.0	±	0.0	11.1	±	11.1
Copepoda	0.0	±	0.0	11.1	±	11.1	0.0	±	0.0	0.0	±	0.0
Cladocera	2072.2	±	43.4	1751.9	±	1507.5	940.0	±	785.5	616.7	±	492.8
Ostracoda	8272.2	±	3074.5	5355.6	±	2648.4	7711.1	±	3137.0	7520.4	±	1401.9
Baetidae	16.7	±	16.7	29.6	±	13.0	211.1	±	178.8	68.5	±	24.1
Aeshnidae	0.0	±	0.0	5.6	±	3.2	1.9	±	1.9	0.0	±	0.0
Libellulidae	5.6	±	3.2	1.9	±	1.9	94.8	±	45.3	16.7	±	8.5
Coenagrionidae	27.8	±	14.7	3.7	±	3.7	20.0	±	10.2	20.4	±	1.9
Anisoptera	0.0	±	0.0	0.0	±	0.0	11.1	±	11.1	0.0	±	0.0
Hydroptilidae	0.0	±	0.0	0.0	±	0.0	115.2	±	48.4	13.0	±	6.7
Hydropsychidae	0.0	±	0.0	0.0	±	0.0	33.3	±	33.3	0.0	±	0.0
Hydrophilidae	44.4	±	36.4	0.0	±	0.0	0.0	±	0.0	53.7	±	34.0
Nematoda	5.6	±	5.6	0.0	±	0.0	0.0	±	0.0	16.7	±	16.7
Planorbidae	5.6	±	5.6	0.0	±	0.0	24.4	±	12.4	27.8	±	20.0
Physidae	11.1	±	5.6	0.0	±	0.0	124.4	±	58.4	96.3	±	96.3
Lymnaeidae	0.0	±	0.0	0.0	±	0.0	42.2	±	29.9	0.0	±	0.0
Acari	16.7	±	9.6	18.5	±	9.8	57.8	±	8.9	29.6	±	19.6
Cicadellidae	5.6	±	5.6	1.9	±	1.9	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	464.4	±	345.3	1092.0	±	481.2	266.3	±	162.8	529.8	±	136.4
taxonomic richness	5.7	±	1.2	5.3	±	1.8	3.7	±	0.3	6.3	±	0.9
Chironomidae	80.2	±	68.2	180.0	±	81.8	149.7	±	110.0	258.0	±	132.8
Ceratopogonidae	7.0	±	7.0	2.1	±	2.1	0.0	±	0.0	0.0	±	0.0
Empidae	10.3	±	5.2	0.0	±	0.0	0.0	±	0.0	0.0	±	0.0
Diptera (pupa)	0.0	±	0.0	0.0	±	0.0	0.0	±	0.0	5.2	±	5.2
Oligochaeta	0.0	±	0.0	92.6	±	92.6	0.0	±	0.0	0.0	±	0.0
Cladocera	10.5	±	4.1	46.5	±	40.0	1.5	±	1.5	36.1	±	18.1
Ostracoda	342.3	±	269.0	601.1	±	271.0	61.0	±	25.1	110.1	±	25.3
Corixidae	1.8	±	1.8	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	12.0	±	4.6
Libellulidae	0.0	±	0.0	2.2	±	2.2	0.0	±	0.0	5.2	±	5.2
Coenagrionidae	1.3	±	1.3	15.3	±	9.7	1.0	±	1.0	12.0	±	4.6
Hydroptilidae	0.0	±	0.0	11.0	±	8.1	51.6	±	32.2	3.4	±	3.4
Hydrophilidae	1.8	±	1.8	0.0	±	0.0	0.0	±	0.0	0.0	±	0.0
Nematoda	8.0	±	5.6	141.0	±	141.0	0.0	±	0.0	86.0	±	86.0
Planorbidae	1.3	±	1.3	0.0	±	0.0	1.5	±	1.5	0.0	±	0.0
Physidae	0.0	±	0.0	0.0	±	0.0	0.0	±	0.0	1.7	±	1.7

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

	July	August	September	October
total abundance	0.87 ± 0.19	1.00 ± 0.17	0.33 ± 0.20	0.70 ± 0.50
taxonomic richness	2.67 ± 0.33	2.33 ± 0.33	1.67 ± 0.88	1.33 ± 0.33
Chironomidae	0.10 ± 0.06	0.00 ± 0.00	0.03 ± 0.03	0.00 ± 0.00
Cladocera	0.53 ± 0.20	0.13 ± 0.03	0.20 ± 0.15	0.50 ± 0.31
Ostracoda	0.20 ± 0.12	0.83 ± 0.20	0.07 ± 0.07	0.20 ± 0.20
Corixidae	0.00 ± 0.00	0.03 ± 0.03	0.00 ± 0.00	0.00 ± 0.00
Baetidae	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.03	0.00 ± 0.00
Nematoda	0.03 ± 0.03	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00