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MACROINVERTEBRATE RESPONSE TO ZEBRA MUSSEL (DREISSENA POLYMORPHA) COLONIZATION OF STREAM SUBSTRATES

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MACROINVERTEBRATE RESPONSE TO ZEBRA MUSSEL (DREISSENA POLYMORPHA) COLONIZATION OF STREAM SUBSTRATES

By

Alyson Alissa Olesen

A THESIS

Submitted to
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ABSTRACT

MACROINVERTEBRATE RESPONSE TO ZEBRA MUSSEL (DREISSENA POLYMORPHA) COLONIZATION OF STREAM SUBSTRATES

By

Alyson Alissa Olesen

Zebra mussels (*Dreissena polymorpha*) quickly spread throughout the Great Lakes soon after being introduced to the region. They are now abundant in many inland lakes, lake-outlet streams, and large rivers. The effects of dreissenid colonization on native aquatic communities have been extensively studied in lake habitats. However, unintentional spreading of dreissenids will undoubtedly continue in other aquatic ecosystems, and it is important to understand how lotic taxa may respond. I conducted an in-stream study of native stream macroinvertebrate responses to simulated zebra mussel colonization using shells adhered to ceramic tiles and a laboratory experiment to determine if a prevalent taxon in the stream samples, hydropsychid larvae (Trichoptera), exhibited substrate preference in the presence of live vs. dead zebra mussels. My results suggest that only a few macroinvertebrate taxa exhibited positive response to simulated zebra mussel colonization in streams. However, there was a strong positive response by hydropsychid larvae to live zebra mussels in the laboratory experiments. This suggests that benthic communities of small streams are likely to become altered in response to spreading of dreissenids into these ecosystems. Given that such macroinvertebrate responses have led to significant food web changes in lentic ecosystems, there is a great need for management plans to both prevent and respond to future colonization in lotic ecosystems.

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Thanks to Oaklawn Hospital, Binder Park Zoo, and the Kellogg Experimental Forest for allowing me to conduct my field research on their properties. In addition, I thank Reuben Goforth for his help with developing and executing the project and Janice Sloan, Holly Campbell, Jen Pellegrini, and Fred Simmons who helped collect or glue zebra mussels. I also thank Eric Benbow and Rich Merritt for their guidance as my graduate committee members.

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INTRODUCTION

Zebra mussels (*Dreissena polymorpha*), native to the Ponto-Caspian region, first appeared in North America in Lake St. Clair (USA, Canada) in the mid 1980s (Hebert et al. 1989). They quickly spread throughout the rest of the Great Lakes, causing economic damage and ecological changes throughout the basin (Stewart et al. 1998a, Perry et al. 2000). These changes result from the zebra mussel's ability to attach to almost anything sessile; they have essentially become the substrate in many places in the Great Lakes, requiring expensive removal for many industrial and recreational applications. They have also displaced a pelagic driven food web to a benthic environment (Kuhns and Berg 1999, Lowe and Pillsbury 1995, Stewart and Haynes 1994). Mills et al. (2003) referred to this shift to a benthic based food web as benthification and suggests that it has the potential to make benthic habitats more susceptible to the establishment of other invasive species and to change the structure of native benthic macroinvertebrate communities (Botts and Patterson 1996, Haynes et al. 1999, Haynes et al. 2005).

One of the most commonly observed ecological consequences of zebra mussel colonization is an increase in benthic species richness and biomass in response to increasing benthic primary production. Several factors contribute to this increased biomass, including the interstitial habitats and refugia provided by their shells, pseudofecal deposition (i.e., particles filtered but not digested), and water currents generated by their siphons which some macroinvertebrates take advantage of for feeding (Ratti and Barton 2003, Ricciardi et al. 1997, Nalepa et al. 2003, Mayer et al. 2001, Haynes et al. 2005). Shell complexity increases habitat complexity that provides refugia from predators and wave action, and it also traps phytoplankton and nutrients (Nitrogen and Phosphorus) that would otherwise be swept away (Haynes et al. 1999, Stewart and Haynes 1994, Botts and Patterson 1996). Zebra mussel feces and pseudofeces provide additional food for benthic organisms (Vanderploeg et al. 2002, Greenwood et al. 2001). Zebra mussels also increase water clarity by siphoning nutrients and phytoplankton from the water column (Mayer et al. 2001, Lowe and Pillsbury 1995, Stewart and Haynes 1994). Greater water clarity allows deeper light penetration, promoting benthic algal growth and concomitant increases in grazer abundance. However, despite these positive influences, zebra mussels also have negative effects on the through competitive displacement (Ricciardi et al. 1997, Stewart et al. 1998a). These responses by the benthos have been reported based largely on observations in the Great Lakes, although the changes are not restricted to these ecosystems.

Following colonization of the Great Lakes, zebra mussels have become prevalent in many inland lakes, especially in the Great Lakes region, where boaters readily move from lake-to-lake carrying veligers (larval zebra mussels) on boat hulls, in bilges, and in live wells. As in the Great Lakes, these mussels attach to any available substrates in inland lakes, including aquatic macrophytes, and can dominate the benthic community (Horvath et al. 1999). Zebra mussels have also increased water clarity in small lakes, which has increased macrophyte growth and benthic invertebrate densities (Mayer et al. 2001). As zebra mussels have successfully colonized isolated inland lakes, their colonization of lotic systems is not unexpected.

Zebra mussels have entered large rivers in the United States, such as the Ohio, Hudson, Huron, St. Lawrence, and Mississippi Rivers, as well as smaller lake-outlet streams. The river and outlet stream zebra mussel populations are often established first by adults that are carried in by stream flow or attached to floating debris (Horvath et al. 1996, Horvath et al. 1999, Perry et al. 2000). The mussels have not reached densities in rivers comparable to those observed in lakes, perhaps because the moving river water creates high mortality for veligers or decreases the ability of veligers to settle out and colonize substrates (Horvath and Lamberti 1997). However, colonization of lotic habitats has occurred nonetheless, suggesting the potential for continued spread into both large river and smaller stream ecosystems.

Zebra mussels are often found just downstream from lake outlets because of an increasing probability for veliger mortality as water flow and turbulence increase. However, Horvath and Lamberti (1997) suggested that macrophytes are an important method for zebra mussel dispersal in rivers and streams. Adult zebra mussels attached to macrophytes that are dislodged from the benthos or cut up by boat traffic can be carried into outflowing streams. These adults may have a greater chance of colonizing the stream compared to veligers when the macrophyte they are attached to becomes snagged in downstream areas. Adult zebra mussels can then detach their byssal threads from the macrophytes and move to more permanent stream substrates (Horvath and Lamberti 1997). This suggests that there is a good likelihood for zebra mussels to become established in smaller streams as they continue to spread.

There is a good potential for zebra mussels in lotic systems to induce different responses by benthic invertebrates compared to benthic invertebrates of lakes. First, the taxonomic composition of lentic communities is different from lotic communities. In addition, zebra mussels enhance transfer of nutrients from phytoplankton nutrients to benthic primary production (Greenwood et al. 2001). Finally, nutrient rich feces or pseudofeces may be swept away by river currents (Horvath et al. 1999). Therefore, habitat complexity, rather than the changes in trophic structure, may be the primary driver for changes in macroinvertebrate abundance when the zebra mussels are present in lotic ecosystems (Botts and Patterson 1996, Horvath et al. 1999, Stewart et al. 1998b).

While some evidence of response by benthic organisms in larger rivers exists (e.g. Greenwood et. al. 2001, Strayer et. al. 1998, Haynes et. al. 1999, the extent to which zebra mussels will colonize and influence smaller stream ecosystems is poorly understood. There have been a few studies of macroinvertebrate response in streams that already have zebra mussels (e.g., Greenwood et. al. 2001), but the potential effects of zebra mussels have not been determined in headwater streams where they are not yet found. Macroinvertebrate communities are already benthic driven in these systems, so the effects zebra mussels will have on the community are unknown. However, it is likely that benthic communities in small streams will respond largely to increased habitat complexity rather than to displaced nutrients to the benthos (Botts and Patterson 1996).

Among the benthic taxa in southern Michigan headwater streams, collector-filterers are a group that may be expected to exhibit a response to the structural complexity of zebra mussels. Organisms in the collector-filterer group are suspension feeders and filter fine particulate organic matter (FPOM) from the water column (Merritt and Cummins 1996). Hydropsychid caddisfly larvae are found in high abundances on rocks in flowing streams (Mackay and Waters 1986) and belong to the collector-filterer functional feeding group (Wiggins 1996). They build fixed silken nets to capture drifting organic particles caught in the stream current (Fairchild and Holomuzki 2002). Hydropsychid larvae were of particular interest in this study because they were thought to benefit from zebra mussel colonization for both the habitat complexity onto which they can build their nets and the siphoning action that could have increased their access to food particles.

It is important to consider the ways in which stream macroinvertebrate communities might respond to zebra mussel colonization for proactive management. By studying the effects and responses prior to widespread colonization, we may know what to expect when zebra mussels do colonize small stream ecosystems, thus enabling the proactive development of management plans. To address the potential management needs, my objective was to observe aquatic insect larvae (i.e., changes in densities and taxa richness) to the simulated presence of zebra mussels in headwater streams that have not yet been colonized by dreissenids. I therefore used a combination of *in situ* and laboratory experiments to quantify responses of stream benthic invertebrates to the presence of zebra mussels to test several hypotheses related to benthic macroinvertebrate responses in headwater streams.

Hypotheses

In situ Experiment

H_1) Benthic macroinvertebrate densities and taxa richness will be greater on zebra mussel shell tiles compared to natural substrate samples in southern Michigan streams.

H_2) Collector-filterer densities will be greater on zebra mussel shell tiles compared to natural substrate samples in southern Michigan streams.

Laboratory Experiment

H_3) Hydropsychid caddisfly larvae density will be:

a) greater on zebra mussel shell tiles vs. natural substrate tiles

b) greater on live zebra mussel tiles vs. natural substrate tiles

c) greater on live zebra mussel tiles vs. zebra mussel shell tiles

METHODS

In situ Experiment

Three headwater streams (Bear Creek, Calhoun Co., MI; Harper Creek, Calhoun Co., MI; and Augusta Creek, Kalamazoo Co., MI) that have not been colonized by zebra mussels were used for *in situ* experiments and for collection of hydropsychid larvae for lab experiments. All of these streams are tributaries of the Kalamazoo River in southwestern Michigan and have similar riparian characteristics (i.e., moderate to high riparian canopy cover, etc.). The substrate in each stream was sandy with gravel and some cobble.

Zebra mussels collected in Lake Lansing (Ingham Co., MI) were emptied and their shells were bleached using a 10% Chlorox® solution (The Chlorox Co., Oakland, CA). The valves were glued closed (Henkel LocTite® Control™ Gel Super Glue, Loctite Henkel Consumer Adhesive, Inc., Avon, OH) so that aquatic insect larvae could not use the insides of the shells as habitat. A single layer of zebra mussel shells was glued to terra cotta tiles (232 cm^2) using PL® polyurethane adhesive (Henkel, Mentor, OH). Eighty-one zebra mussel shells were glued to each tile to achieve a density of 3521.7 individuals/m^2. The density of zebra mussel shells on each tile was based on previous studies on zebra mussel densities in North America (e.g., Horvath et. al. 1996 and Ricciardi et. al. 1995), this experimental density was expected to elicit response by benthic macroinvertebrates. The orientation of the zebra mussels on the tiles was random. On 13 July 2006, nine tiles were placed in the run habitat of each of the three streams to simulate the habitat complexity created by zebra mussel colonization. Three tiles that had been covered with naturally occurring substrate adhered with Gorilla Glue® (The Gorilla Glue Co., Cincinnati, OH) were also placed in each stream at this time.

All tiles were retrieved on 29 September 2006, after an approximate incubation period of 10 weeks. The incubation period was based on sample considerations and time constraints for sampling. During tile retrieval, a D-frame benthic sampling net (0.5 mm mesh size) was placed immediately downstream of each tile to catch any larvae that became dislodged. All macroinvertebrates were removed from the upper surface of the tiles and placed in 70% ETOH for later laboratory processing. Representative community composition for naturally occurring substrates in the study streams was also determined at the time the tiles were retrieved by sampling randomly selected areas of natural substrate using a 232 cm^2 custom sample template. The steel template had an open top and two solid sides. The template was placed on the stream bottom so that the sides were parallel to the current and field crew members used their hands to disturb the substrate within the template area. Dislodged macroinvertebrates were carried via stream flow into a D-frame net placed directly downstream.

Sample Processing

Each sample was sorted and the macroinvertebrates were identified using a dissecting microscope. The aquatic insects were identified to the genus level, with the exception of chironomids which were identified to the family level, using keys from Merritt and Cummins (1996). Other macroinvertebrates (e.g., leeches, gastropods, limpets, oligochaetes) were identified to the lowest practical taxonomic level based on time and resources. Mean total densities, functional feeding group densities, and individual taxa densities were calculated as the number of individuals present per square centimeter (no. individuals/cm^2) and then converted to the number of individuals present per square meter (no. individuals/m^2) for comparisons to other literature. Individual taxa identified from the *in situ* experiment were assigned to appropriate functional feeding groups (FFG) according to Merritt and Cummins (1996) and Smith (2001). FFGs reflect the feeding mechanism of each taxon and provided a means by which rare taxa not included in the individual taxa analyses because they were considered rare could be included as part of the analyses. Placing taxa into FFGs also provided information on the benthic macroinvertebrate community structure in the streams. Taxa richness for each site was also determined as the numbers of individual taxa identified in each sample.

Statistics

Statistical tests were conducted to determine whether benthic macroinvertebrates responded to the introduction of zebra mussel shell tiles (ZMST) at several levels. The treatments were split between 1) ZMST vs. benthic samples to test for shell effects on macroinvertebrate colonization and 2) benthic samples vs. natural substrate tiles to test for tile effects for the tile used as a substratum for gluing the zebra mussel shells. A two-factor Analysis of Variance (ANOVA) was initially used to determine if mean total densities of macroinvertebrates and mean taxa richness measures were significantly different between treatments in the *in situ* experiment (α=0.05) (Version 15.0 2006, SPSS, Chicago). Multivariate Analysis of Variance (MANOVA) tests were initially used to test the hypotheses that the FFGs and individual benthic taxa densities differed significantly between the ZMST and benthic samples in the *in situ* experiments (α=0.05) (Version 15.0 2006, SPSS, Chicago). The factors in this test were stream and treatment, and the response variables were total densities of benthic macroinvertebrate taxa, FFG densities, and individual taxa densities observed on both the ZMST and in the benthic samples. All invertebrates collected were included in the overall density and FFG analyses. However, only taxa present in at least 20% of the samples taken from each stream were included in the individual taxa analysis to exclude rare taxa that may have skewed the results. Repeated efforts to normalize the data using transformations were unsuccessful (e.g., log(x+1) and square root(x+1)). Thus, non-parametric statistical methods were used in lieu of ANOVA and MANOVA to analyze the field study data.

The first level of statistics performed tested for a stream-level effect. Kruskal-Wallace (K-W) tests were used as a non-parametric equivalent of a two-way ANOVA (α=0.05) (Version 15.0 2006, SPSS, Chicago). In cases where K-W indicated a significant difference among all streams, the Mann-Whitney (M-W) test was used to determine significant differences for each pair of streams in lieu of a parametric post-hoc test (α=0.05). Lastly, densities found to be non statistically different between a stream pair were tested for differences between treatments using K-W tests (α=0.05). K-W tests were used to determine statistical differences of mean total densities between treatments in each stream (α=0.05).

Laboratory Experiment

Three recirculating-flow laboratory aquaria (110 L) were used to observe the response of hydropsychid caddisfly larvae to simulated colonization of zebra mussels. Each aquarium was painted black on three sides using Quick Color Spray Enamel (ROC Sales, Inc., Vernon Hills, IL). During the experiments, the side left as a window was covered with construction paper so light could only enter from the top of the aquarium. One end of the aquarium was raised using plexiglass so that water would flow in a downstream direction. The aquaria were filled with water so that the shallow end was under approximately 5 cm of deionized water. An aquarium power head (AquaClear® 3000, Rolf C. Hagen Corp., Mansfield, MA) with a flow rate of 757 L/h was placed in the sump to pump water up through PVC pipes and down to the shallow end where it was returned via a spray bar (Figure 1). This simulated stream flow over the shallow end of the aquarium. The aquaria were used to house three experimental treatments that provided resident hydropsychids with contrasting substrates for colonization. Terra cotta tiles (232 cm^2) were covered with the appropriate substrate using Stick Fast™ (Tech Marketing Inc., Peachtree City, GA) instant adhesive in the same density as the *in situ* experiment (3521.7 individuals/m^2). They were placed on the bottom of the shallow side in each of the three aquariums. The three experimental treatments used were: a) half zebra mussels, half natural substrate, b) half zebra mussels, half empty zebra mussel shells, and c) half empty zebra mussel shells, half natural substrate.

The hydropsychid caddisfly larvae used in the laboratory were collected in Bear Creek (Calhoun Co., MI) and were allowed to acclimate to laboratory temperatures for a minimum of 48 h before the experimental trials were conducted. The number of hydropsychids used was based on field observations from natural substrate samples in the three study streams (i.e., 3 individuals/m^2). Twelve hydropsychids were placed in the center of the four tiles in each aquarium and left to settle for 1 h before the water flow from the pump was turned on. Once the water flow was initiated, the hydropsychids were left to colonize the substrate for 48 h. At the end of the 48 h trial, the water flow was turned off and each tile was removed. The densities of hydropsychids on all tiles were calculated to determine whether the hydropsychids colonized the substrates differently. Any mortality was noted and a trial was rerun if more than two hydropsychids were dead at the end of an experimental trial. The trials were run twice, for a total of six replications per treatment pair. A one-way ANOVA was used to determine whether hydropsychid densities varied among the substrate treatments in the laboratory experiments (α=0.05) (SPSS 2006).

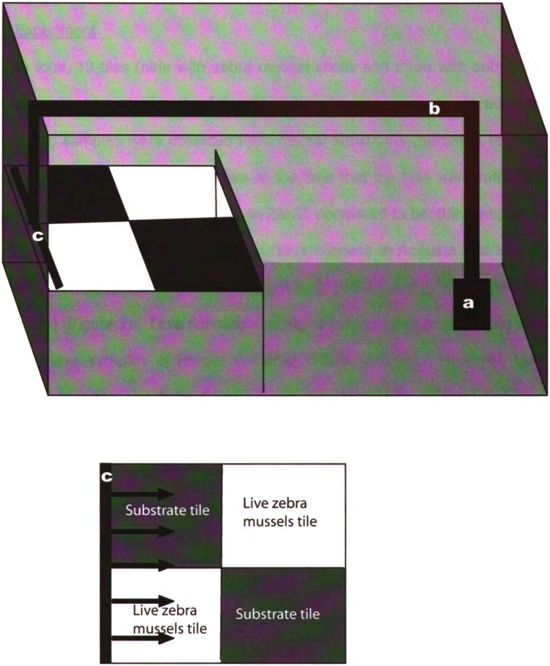


Figure 1. Diagram of lab experiment design and tile orientation. The power head (a) pumped water through the PVC pipe (b) and out through a spray bar (c) to allow the water out in a unidirectional flow (indicated by arrows) across the tiles.

RESULTS

In situ Experiment

In total, 12 tiles (nine with zebra mussel shells and three with substrate) were set and retrieved in each of the three experimental streams. In addition, nine benthic samples were collected from natural substrates (hereafter referred to as benthic samples) in each stream at the time that the tiles were retrieved. Taxa richness was significantly higher on ZMST compared to benthic samples in Bear Creek (χ^2=5.55, p=0.02). However, taxa richness in Augusta and Harper Creeks was similar between treatments (χ^2=0.72, p=0.40 and χ^2=3.32, p=0.07, respectively) (Figure 2). Taxa richness was significantly higher on substrate tiles than in benthic samples in Harper Creek (χ^2=4.29, p=0.04). However, taxa richness in Augusta and Bear Creeks were not statistically different between treatments (χ^2=2.21, p=0.14 and χ^2=0.93, p=0.33, respectively).

The overall mean macroinvertebrate densities on ZMST in each stream were as follows: 1020.1 individuals/m^2 (Augusta Creek), 6374.5 individuals/m^2 (Harper Creek), and 450.2 individuals/m^2 (Bear Creek) (Figure 3). The overall mean macroinvertebrate densities in benthic samples in each of the streams were as follows: 1230.8 individuals/m^2 (Augusta Creek), 3486.6 individuals/m^2 (Harper Creek), and 205.9 individuals/m^2 (Bear Creek) (Figure 3). The most common taxa across all streams included amphipods, elmids, oligochaetes, and hydropsychids. These taxa were present in at least 20% of the samples taken from each stream (Appendix A).

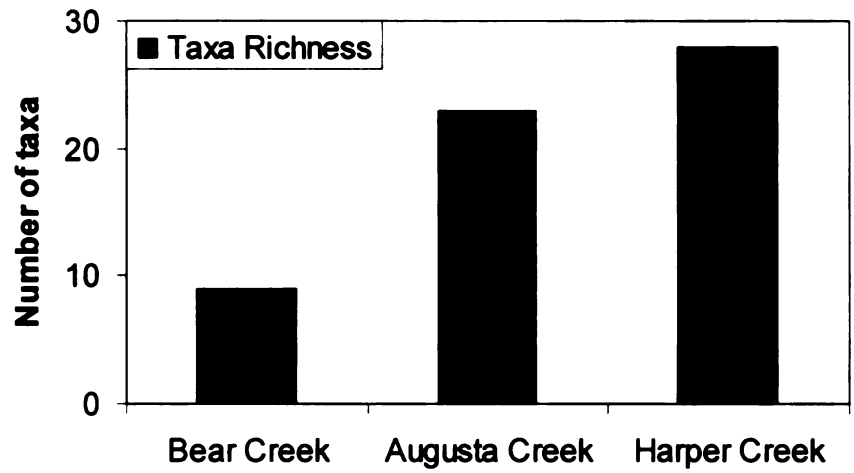


Figure 2. Taxa richness found in all samples in each experimental stream.

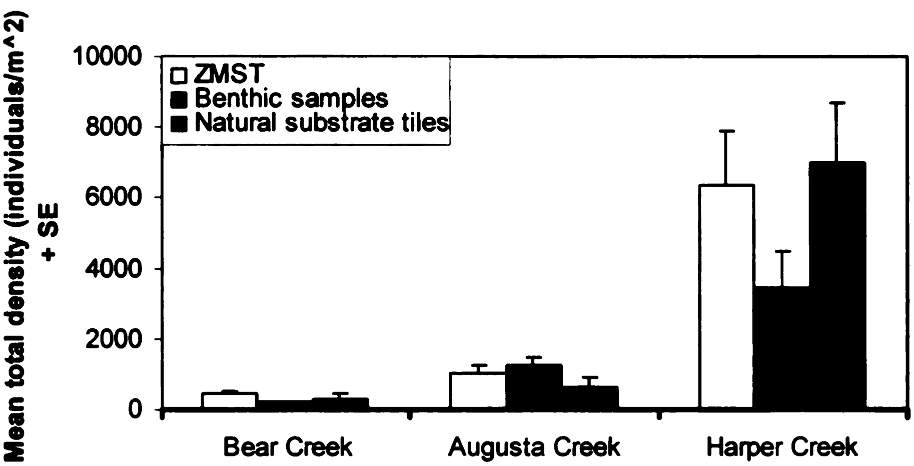


Figure 3. Mean total densities (+ 1 SE) of macroinvertebrates (individuals/m^2) in each of the three experimental streams by treatment.

ZMST vs. benthic samples

Mean total densities of macroinvertebrates were not statistically different in Augusta and Harper Creeks between ZMST and in benthic samples (χ^2=0.24, p=0.63 and χ^2=1.03, p=0.31, respectively). However, the mean total densities of macroinvertebrates were significantly higher on ZMST than in the benthic samples in Bear Creek (χ^2=7.58, p=0.006) (Figure 3).

The collector-filterer group was the only functional feeding group with densities that were statistically similar among all three streams (Table 1, Figure 4). Scraper densities were statistically similar between Augusta and Harper Creeks (Table 1), and shredder densities were statistically similar between Augusta and Bear Creeks (Table 1, Figure 4). No FFG groups were statistically similar between Harper and Bear Creeks (Table 1).

K-W analyses were conducted to determine whether FFG densities were different between ZMST and benthic samples based on the schedule of statistically similar streams presented in Table 1. The results of this test indicated that collector-filterer, scraper, and shredder densities were not statistically different between the ZMST and benthic samples (χ^2=0.36, p=0.55; χ^2=0.17, p=0.68; χ^2=0.14, p=0.71, respectively) (Figure 4).

Table 1. Functional feeding groups with densities found to be statistically similar between streams using Kruskal-Wallace (χ^2 value, p-value) for among stream comparisons and Mann-Whitney (Z-value, p-value) for pairwise comparisons (zebra mussel shell tiles vs. benthic samples). Streams are represented by (A) Augusta Creek, (B) Bear Creek, and (H) Harper Creek. Collector-gatherer and predator densities were not statistically similar between any of the stream pairs.

Functional Feeding Group	Streams	Test value	p-value
Collector-filterer	**A&H&B**	**χ^2=4.76**	**0.09**
Scraper	**A&H**	**Z= -0.56**	**0.58**
Shredder	**A&B**	**Z= -0.02**	**0.99**

The hydropsychid caddisfly group was the only taxon with densities that were statistically similar among all three streams. All remaining taxa group densities analyzed were based on comparisons between only two of the three experimental streams according to the schedule provided in Table 2.

Table 2. Taxa with densities found to be statistically similar between streams using Kruskal-Wallace (χ^2 value, p-value) for among stream comparisons and Mann-Whitney (Z-value, p-value) for pairwise comparisons (zebra mussel shell tiles vs. benthic samples). Streams are represented by (A) Augusta Creek, (B) Bear Creek, and (H) Harper Creek.

Class	Subclass	Order	Family	Streams	Test value	p-value
Insecta		Coleoptera	Elmidae	A&H, H&B	Z= -1.53, Z= -1.51	0.13, 0.13
		Ephemeroptera	Caenida	A&H	Z= -1.08	0.28
			Heptageniidae	A&H	Z= -1.1	0.27
		Trichoptera	Hydropsychidae	A&H&B	χ^2=3.66	0.16
			Helicopsychidae	H&B	Z= -1.78	0.07
			Leptoceridae	A&B	Z= 0.00	1.00
Crustacea		Amphipoda	Gammaridae	A&B	Z= -0.88	0.38
Gastropoda				A&B	Z= -1.43	0.15
Clitellata	Hirudinea			A&B	Z= 0.00	1.00

Table 3. Taxa found to be statistically similar between streams using Kruskal-Wallace for among stream comparisons and Mann-Whitney for pairwise comparisons and their statistical significance between the treatments zebra mussel shell tiles vs. benthic samples (χ^2 value, p-value). Streams are represented by (A) Augusta Creek, (B) Bear Creek, and (H) Harper Creek.

Class	Subclass	Order	Family	Streams	χ^2	p
Insecta		Coleoptera	Elmidae	A&H, H&B	0.43, 3.73	0.51, 0.05
		Ephemeroptera	Caenida	A&H	1.68	0.19
			Heptageniidae	A&H	9.21	0.002
		Trichoptera	Hydropsychidae	A&H&B	0.84	0.36
			Helicopsychidae	H&B	0.35	0.55
			Leptoceridae	A&B	0.00	1.00
Crustacea		Amphipoda	Gammaridae	A&B	0.78	0.38
Gastropoda				A&B	0.002	0.97
Clitellata	Hirudinea			A&B	0.00	1.00

Densities of elmid beetle larvae were statistically similar between 1) Augusta and Harper Creeks (Table 2) and 2) Harper and Bear Creeks (Table 2). Elmid densities were not statistically different between ZMST and benthic samples in Augusta and Harper Creek (Table 3) but were statically higher on ZMST compared to benthic samples in Harper and Bear Creeks (Table 3, Figure

5). Caenid mayfly densities were similar between Augusta and Harper Creeks (Table 2), but their densities were not statistically different between ZMST and benthic samples (Table 3, Figure 5). Heptageniid mayfly densities were similar between Augusta and Harper Creeks (Table 2), and their densities were statistically higher on ZMST vs. benthic samples (Table 3, Figure 5). Hydropsychid densities were similar between all streams (Table 2), but they were not statistically different between ZMST and benthic samples (Table 3, Figure 5). Helicopsychid densities were statistically similar between Harper Creek and Bear Creek (Table 2), and were not statistically different between the treatments in these streams (Table 3). Leptocerid densities were statistically similar between Augusta and Bear Creeks (Table 2), and they not statistically different between ZMST and benthic samples (Table 3). Amphipod, gastropod, and leech densities were also statistically similar between Augusta Creek and Bear Creek (Table 2), and the densities of all three of these taxa were not statistically different between ZMST and benthic samples (Table 3).

Benthic samples vs. substrate tiles

Tests to determine whether the use of the tiles as a substrate for simulating zebra mussel colonization influenced macroinvertebrate substrate preference in the streams were based on nine benthic samples and three substrate covered tiles from each stream. All the streams had statistically similar mean total densities in benthic samples although they were statistically similar on the substrate tiles in Augusta and Bear Creeks only (χ^2=1.92, p=0.17 and χ^2=0.009, p=0.93, respectively) and the treatments were statistically higher on substrate tiles compared to benthic samples in Harper Creek (χ^2=3.769, p=0.052) (Figure 3).

The collector-filterer group was the only FFG with densities that were statistically similar among all three streams (Table 4). Scraper densities were statistically similar between Augusta and Harper Creeks (Table 4) and shredder densities were similar between Augusta and Bear Creeks (Table 4, Figure 4). No FFG groups were statistically similar between Harper and Bear Creeks (Table 4).

K-W analyses were conducted to determine whether FFG densities were different between benthic samples and substrate tiles based on the schedule of statistically similar streams presented in Table 4. Collector-filterer, scraper, and shredder densities were not statistically different between the treatments (χ^2=0.06, p=0.81; χ^2=0.16, p=0.69; and χ^2=2.82, p=0.09, respectively).

Table 4. Functional Feeding groups with densities found to be statistically similar between streams using Kruskal-Wallace (χ^2 value, p-value) for among stream comparisons and Mann-Whitney (Z-value, p-value) for pairwise comparisons (benthic samples vs. substrate tiles). Streams are represented by (A) Augusta Creek, (B) Bear Creek, and (H) Harper Creek. Collector-gatherers and predators were not statistically similar between any of the stream pairs.

Functional Feeding Group	Streams	Test value	p-value
Collector-filterer	A&H&B	χ^2= 4.63	0.10
Scraper	A&H	Z= -0.67	0.51
Shredder	A&B	Z= -0.06	0.95

Initial tests to determine whether resident taxa densities were different among streams indicated that only densities of hydropsychid caddisflies and heptageniid mayflies were statistically similar among all streams (Table 5). All remaining taxa group densities analyzed were based on comparisons between only two of the three experimental streams according to the schedule provided in

Table 5. However, no taxa had similar densities between Harper Creek and Bear Creek (Table 5).

Densities of elmid beetle larvae were statistically similar between Augusta and Harper Creeks (Table 5) and were also not significantly different between benthic samples and substrate tiles (Table 6, Figure 5). Caenid mayfly densities were similar between Augusta and Harper Creeks (Table 5), but their densities were not significantly different between benthic samples and substrate tiles (Table 6, Figure 5). Heptageniid mayfly densities were similar among all streams, but they were not statistically different between the treatments (Table 6, Figure 5). Diptera pupae densities were statistically similar between Augusta Creek and Bear Creek (Table 5), but were not statistically different between benthic samples and substrate tiles (Table 6, Figure 5). Hydropsychid densities were similar among all streams, but they were not statistically different between benthic samples and substrate tiles (Table 6, Figure 5). Leptocerid densities were statistically similar between Augusta and Bear Creeks (Table 5), and they were not statistically different between benthic samples and substrate tiles (Table 6). Amphipod, gastropod, leech, and oligochaete densities were also statistically similar between Augusta Creek and Bear Creek (Table 5) and the densities of these taxa were not statistically different between benthic samples and substrate tiles (Table 6).

Table 5. Taxa found to be statistically similar between streams using Kruskal-Wallace (χ^2 value, p-value) for among stream comparisons and Mann-Whitney (Z-value, p-value) for pairwise comparisons (benthic samples vs. substrate tiles). Streams are represented by (A) Augusta Creek, (B) Bear Creek, and (H) Harper Creek.

Class	Subclass	Order	Family	Streams	Test value	p-value
Insecta		Coleoptera	Elmidae	A&H	Z= -1.65	0.1
		Ephemeroptera	Caenida	A&H	Z= -0.56	0.58
			Heptageniidae	A&H&B	χ^2= 5.8	0.06
		Diptera (pupae)		A&B	Z= -1.45	0.19
		Trichoptera	Hydropsychidae	A&H&B	χ^2= 0.96	0.62
			Leptoceridae	A&B	Z= 0.00	1.00
Crustacea		Amphipoda	Gammaridae	A&B	Z= -0.79	0.43
Gastropoda				A&B	Z= -1.00	0.32
Clitellata	Hirudinea			A&B	Z= 0.00	1.00
	Oligochaeta			A&B	Z= -1.40	0.16

Table 6. Taxa found to be statistically similar between streams using Kruskal-Wallace for among stream comparisons and Mann-Whitney for pairwise comparisons and their statistical significance between the treatments benthic samples vs. substrate tiles (χ^2 value, p-value). Streams are represented by (A) Augusta Creek, (B) Bear Creek, and (H) Harper Creek.

Class	Subclass	Order	Family	Streams	χ^2	p-value
Insecta		Coleoptera	Elmidae	A&H	0.09	0.76
		Ephemeroptera	Caenida	A&H	0.07	0.79
			Heptageniidae	A&H&B	0.001	0.98
		Diptera (pupae)		A&B	0.68	0.40
		Trichoptera	Hydropsychidae	A&H&B	0.22	0.64
			Leptoceridae	A&B	0.00	1.00
Crustacea		Amphipoda	Gammaridae	A&B	0.32	0.57
Gastropoda				A&B	0.33	0.56
Clitellata	Hirudinea			A&B	0.00	1.00
	Oligochaeta			A&B	2.74	0.1

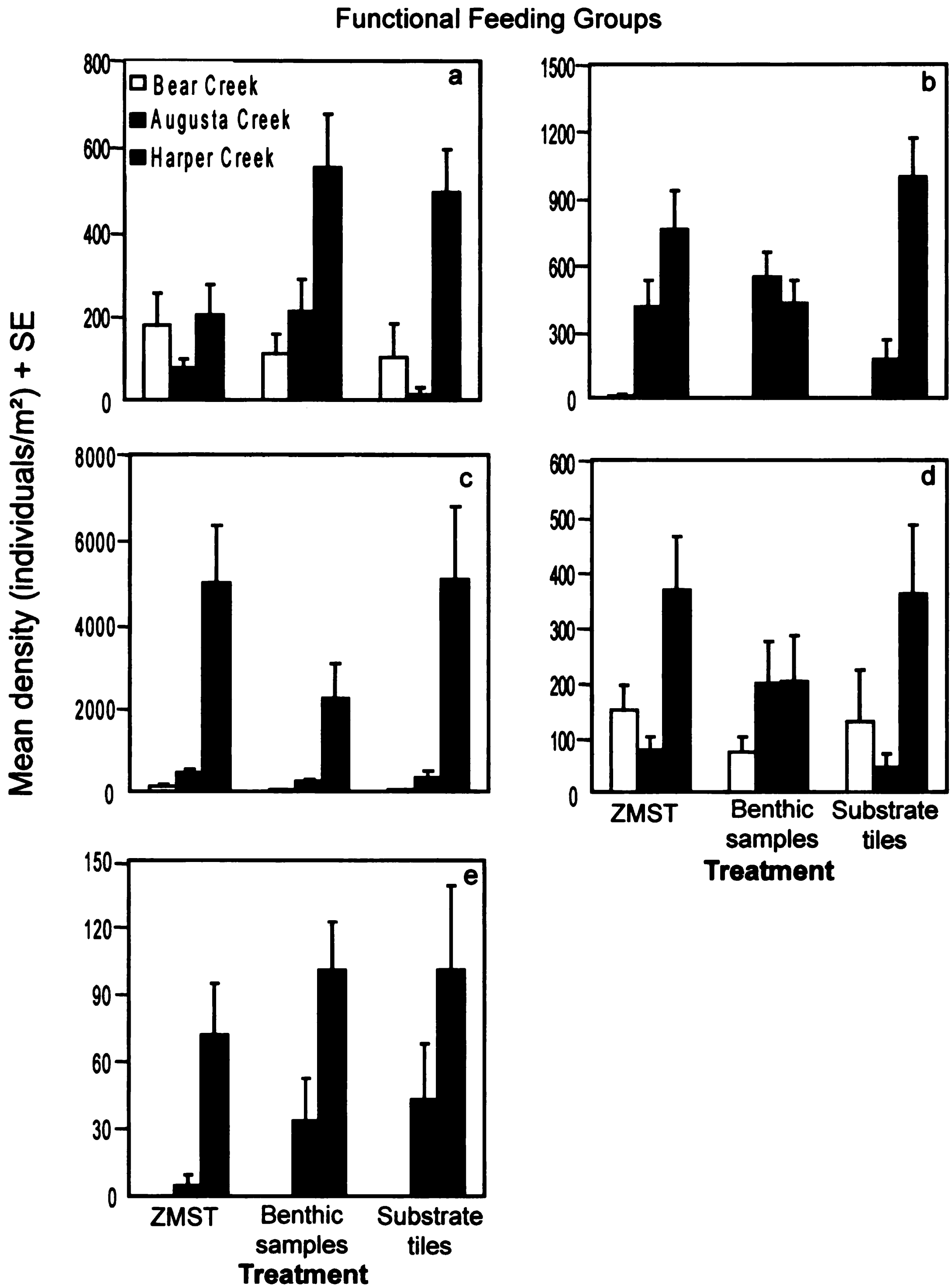


Figure 4. Mean total densities (+ SE) of functional feeding groups for each treatment in each stream, including: a) Shredders; b) Scrapers; c) Collectors-gatherers; d) Collectors-filterers; e) Predators. Mean total density values used in graphs are means taken from the raw data, but statistical tests were based on Kruskal-Wallace tests according to mean rank. ZMST= zebra mussel shell tiles.

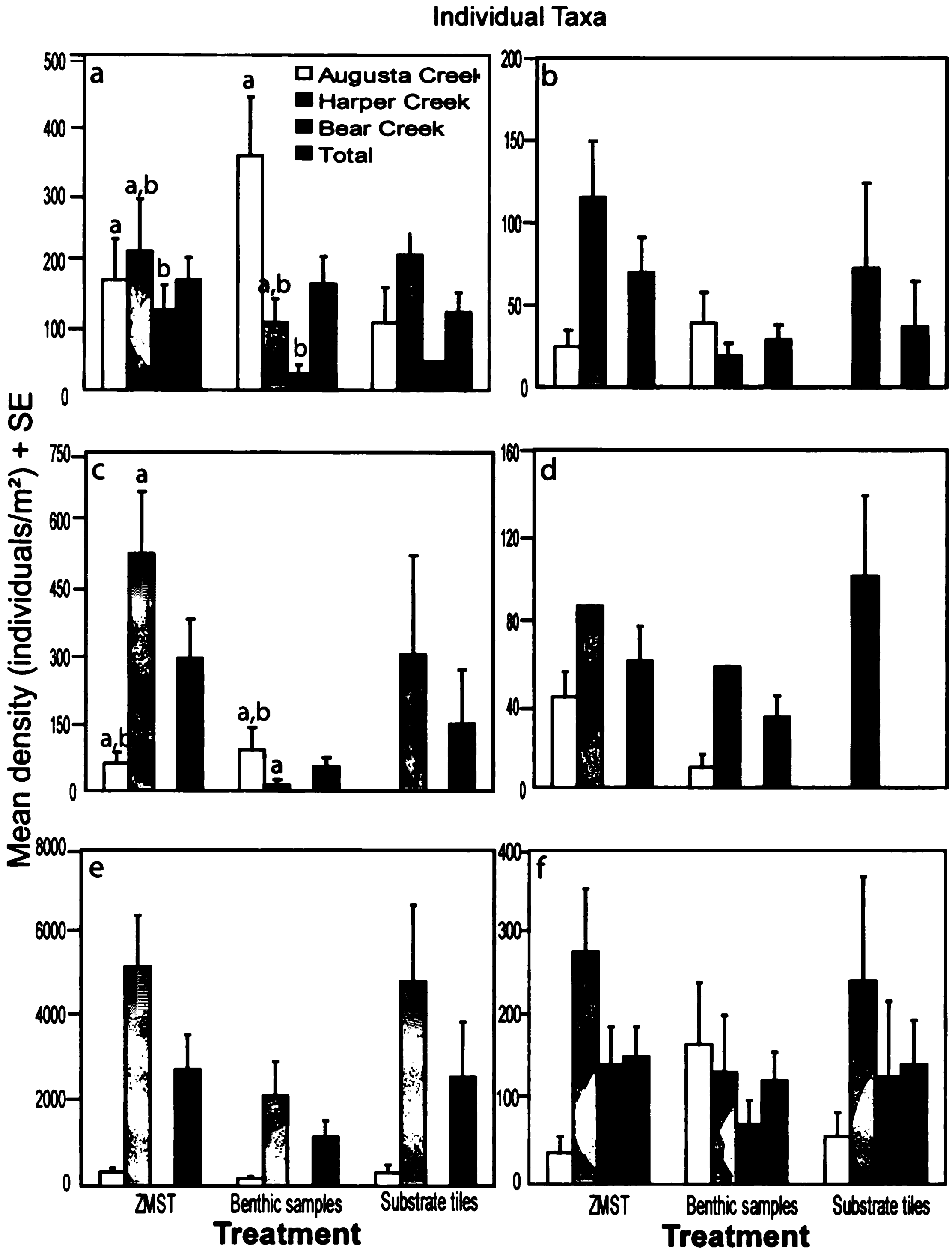


Figure 5. Mean total densities (+ SE) for individual benthic taxa for each treatment in each stream, including: a) elmidae; b) caenidae; c) heptageniidae; d) diptera pupae; e) chironomidae; f) hydropsychidae. Statistically similar treatments are denoted by the same letters. Mean total density values used in graphs are means taken from the raw data, but statistical tests were based on Kruskal-Wallace tests according to rank. ZMST = zebra mussel shell tiles.

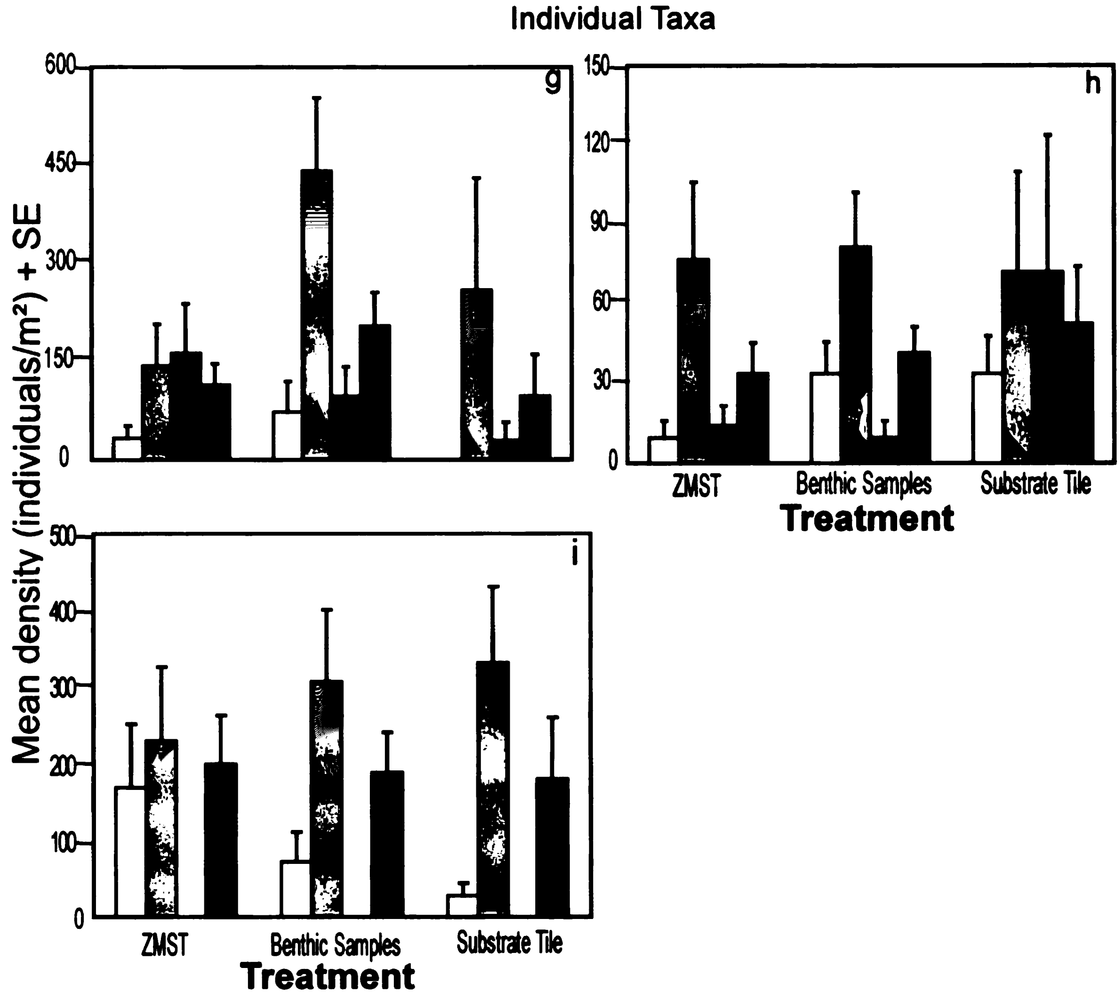


Figure 5 (cont'd). g) oligochaete; h) gammaridae; i) gastropoda (limpet).

Lab Experiment

Analyses of differences in hydropsychid caddisfly larvae densities among live zebra mussels, zebra mussel shells, and simulated natural substrates were based on data collected from three trials with each experimental comparison replicated twice. The first comparison tested to detect potential differences in hydropsychid densities between ZMST similar to those used in the *in situ* experiment and simulated natural substrate. Based on this analysis, there was no significant difference in hydropsychid densities between ZMST and the natural substrate (F=1.11, p=0.31, df=1) (Figure 6).

There was no significant difference in hydropsychid caddisfly densities on the live zebra mussel tiles vs. the simulated natural substrate (F=0.66, p=0.43, df=1) (Figure 6). However, there was a significant interaction between treatment and tile effects (i.e., upstream vs. downstream placement) (F=5.92, p=0.03, df=1). There was no significant difference in hydropsychid densities when an ANOVA was conducted separately for both the upstream tiles (F=3.25, p=0.10, df=1) and the downstream tiles (F=1.00, p=0.34, df=1). There was also an interaction between the trial and tile effects (F=9.50, p=0.007, df=1). Based on separate ANOVAs for each factor, there was no significant difference in hydropsychid densities between the treatments in the first trial (F=0.39, p=0.55, df=1). There was also no significant difference in hydropsychid densities between the treatments in the second trial (F=1.65, p=0.23, df=1).

The final experimental trial tested for potential differences in hydropsychid larvae densities between live zebra mussel tiles and ZMST. The results of this experiment indicated that hydropsychid caddisfly densities were significantly higher on live zebra mussel tiles versus ZMST ($F=5.51$, $p=0.03$, $df=1$) (Figure 6).

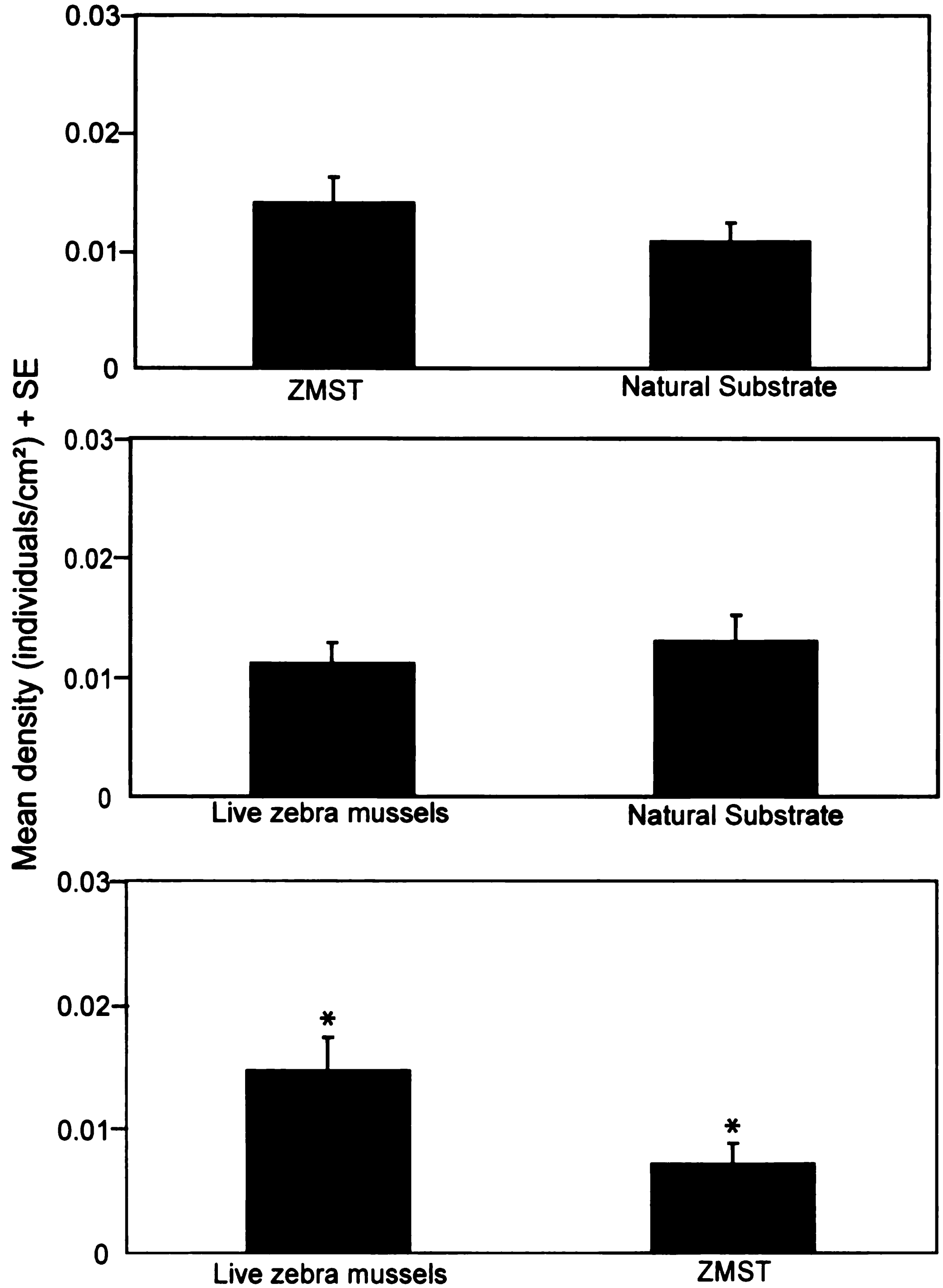


Figure 6. Mean total densities (+ SE) of hydropsychid larvae for each treatment in the laboratory experiment. *Densities were significantly different between treatments ($p<0.05$). ZMST=zebra mussel shell tiles.

DISCUSSION

The encroachment of dreissenids in North American freshwater ecosystems is of great concern. Many studies have shown changes in benthic macroinvertebrate community structure after the colonization of zebra mussels in the Great Lakes. These studies have had contrasting results; some report that densities of some benthic taxa increase in response to zebra mussel colonization, while the densities of other benthic taxa decrease. Taken as a whole, these results suggest that overall biomass and abundance of aquatic invertebrates generally increase due to abiotic and/or biotic effects of zebra mussels in the Great Lakes (Botts and Patterson 1996, Haynes et al. 1999, Haynes et al. 2005, Kuhns and Berg 1999, Mayer et al. 2002, Reed et al. 2004, Stewart and Haynes 1994, Stewart et al. 1998a, 1998b).

Studies of zebra mussel colonization in riverine and stream systems have not been as common to date. Among the few existing studies, some have shown that abiotic factors are more important than biotic factors in streams (Stewart et al. 1998b, Horvath et al. 1999), while others show that biotic factors are just as important (Greenwood et al. 2001). One component of the present study (the *in situ* experiment) accounted for abiotic factors through the increased habitat complexity provided by simulated zebra mussel colonization. The other component of the study (the laboratory experiment) accounted for both biotic and abiotic factors through the use of live zebra mussels. In both cases, the results of this study provide some evidence to suggest that both biotic and abiotic factors likely play roles in stream macroinvertebrate responses to zebra mussel colonization.

In situ Experiment

Although each stream was chosen because of similar substrate and riparian characteristics, it was apparent the benthic communities of the streams were not as similar as initially expected after analyses were completed. For example, overall benthic invertebrate densities were different between each stream; with Harper Creek mean total densities much higher than the other two streams. Chironomids were particularly abundant in the Harper Creek samples, likely resulting from nutrient inputs from a zoo located upstream of the study site. In contrast, Bear Creek mean total densities were much lower than the other two streams, likely as a result of accumulated organic materials during tile incubation. Agricultural fields located upstream of the site may have also provided greater sediment loads to this stream compared to Harper and Augusta Creeks. Bear Creek, however, had significantly higher overall macroinvertebrate densities on ZMST than in benthic samples. Oligochaetes, elmid larvae, and hydropsychid larvae are three taxa that had much higher densities on ZMST than in the benthic samples, which may explain the difference. These taxa may be responding to the complexity created by the ZMST or the sediment that accumulated on the tiles in Bear Creek. In contrast, Augusta Creek was located in a forest, but did have agricultural and residential land upstream from the site; however, the site used for the *in situ* experiment was much farther downstream of these land uses compared to sites in the other two streams. Given that the landscape contexts of the three experimental streams likely influenced the results of the *in situ* experiment, the results presented herein should be interpreted with caution. Regardless, the results are expected to provide a general indication of stream benthic macroinvertebrate community responses to zebra mussel colonization in a multi-land use landscape.

Only two FFGs were represented in Bear Creek – collector-filterers and shredders. The presence of collector-filterers may be explained by the nutrient inputs and sediment input from the agricultural lands upstream, and the presence of shredders suggests that the stream has sufficient allochthonous inputs to provide support for this FFG. All FFGs were present in Augusta Creek; possibly due to the absence of inputs from agricultural or residential land-uses immediately upstream from the site increasing functional diversity in processing organic matter. Similarly, all FFGs were present in Harper Creek, but with an overwhelming majority of collector-gatherers, especially chironomids. This is likely due to inputs from a zoo upstream as previously discussed. The absence of three of the FFGs (scrapers, collector-gatherers, and predators) in Bear Creek once again suggests the experimental streams were dissimilar enough that the landscape context of each stream was likely to be a significant factor in influencing the community structure of the streams, thus making experimental comparisons using all the streams as replicates difficult and often impossible.

The general lack of responses by stream benthic taxa to the introduction of increased habitat complexity provided by the ZMST was surprising. Timing of the experiment may have underestimated overall benthic macroinvertebrate response. However, this study was performed during the summer to avoid spring and fall spate events that were expected to damage the experimental tiles. Taxa such as amphipods, oligochaetes, and chironomids have been shown to have greater responses to abiotic factors than biotic factors in lake systems (Botts and Patterson 1996). Such abiotic factors (e.g., increased habitat complexity created by zebra mussel shells) are thought to be the most important factors that influence changes in benthic macroinvertebrate communities in the rocky substrates of Lake Erie (Stewart et al. 1998b). However, increasing habitat heterogeneity does not necessarily increase species richness (Wise and Molles 1979) or abundance, and the effect of zebra mussel colonization in streams has been shown to be positive or negative depending on the characteristics of the stream (Strayer et al. 1998, Haynes et al. 1999). The results of the *in situ* experiment appear to concur with this in that the densities of only two taxa (elmid larvae and heptageniid mayflies) were significantly different between ZMST and benthic sample treatments. In this case, the densities of both taxa were significantly higher on ZMST than in benthic samples. However, most other taxa, including some groups that have shown responses to zebra mussel structure in lentic habitats, did not exhibit a response to the increased habitat complexity provided by the ZMST. This suggests that the increased structural complexity of zebra mussel shells may not be a strong driver of benthic taxa in streams. Other studies have increased habitat complexity by manipulating structures and have demonstrated changes in stream benthic macroinvertebrate communities (e.g., Courtmanch 1984, Stewart et. al. 1998b, Obernborfer et. al. 1984). However, the abiotic effect of zebra mussels may have been lessened in some cases in which it appeared that the zebra mussels helped to trap inorganic substrate particles, thus decreasing available habitat complexity. Studies in streams with lower sediment loads may indicate different responses by benthic macroinvertebrates. Use of live zebra mussels in the *in situ* experiments may have lessened the accumulation of sediments on the tiles by their filtering action reducing the settling of inorganic and organic materials. However, the use of live zebra mussels for this experiment was not an option for obvious reasons.

The increased densities of heptageniid mayfly nymphs on ZMST compared to benthic samples be because the heptageniids responded to the areas of exposed tile between the shells rather than the habitat complexity created by the shells. Although no tile effect was found when comparing benthic samples to substrate tiles, the tile covered with natural substrate did not have flat exposed areas comparable to those provided on the ZMST. Heptageniids are clingers and are often found on and under loose cobble and boulders (Merritt and Cummins 1996), which provide flatter surfaces than those created by zebra mussels. Heptageniids were also observed to be on the bottom sides of the tiles when they were retrieved, and although these individuals were not included in the samples, their presence suggests the preference of heptageniids for the flat surfaces provided by the tiles. While these exposed flat surfaces may have influenced heptageniid colonization, accumulated inorganic sediments on many of the ZMST limited the amount of exposed tile on many of the ZMST similar to the substrate tiles, suggesting that the heptageniids may have been relying heavily on zebra mussel shell surfaces vs. exposed areas of tile. The use of zebra mussel shells by heptageniids requires a more detailed behavioral study that was beyond the scope of the present study.

Elmid beetle larvae also exhibited increased densities on ZMST compared to benthic samples. Elmids, like heptageniids, are clingers and they are often found in erosional and depositional areas (Merritt and Cummins 1996). However, unlike the heptageniids, no elmids were observed to be clinging to the bottom sides of the tiles during retrieval. Three possibilities exist to explain the increased density of elmids on the ZMST: 1) elmids may be responding to the inorganic sediments that accumulated on the ZMST during the incubation of the tiles in the streams, 2) elmids may be clinging to the zebra mussel shell, and/or 3) elmids may be clinging to exposed tile. The use of zebra mussel shells by elmids also requires a more detailed behavioral study.

The absence of biotic factors in the *in situ* experiment may have also resulted in the general lack of response by stream benthic macroinvertebrates. Biotic factors, such as higher food quality from the deposition of feces and pseudofeces, have been found to be very important influencing factors on post-zebra mussel colonization benthic macroinvertebrate communities (Ricciardi et al. 1997, Stewart et al. 1998a, Greenwood et al. 2001, Mayer et al. 2002). While some studies (Stewart et al. 1998b, Horvath et al. 1999) have found abiotic factors to cause increases in macroinvertebrate density in zebra mussel colonizations versus benthic samples, other studies have shown contrasting results because feces and pseudofeces are likely to wash downstream in lotic ecosystems. For example, a study by Horvath et al. (1999), which had both zebra mussel shells and live zebra mussel treatments, found no significant difference in macroinvertebrate densities between the two treatments in their stream. They suggested that this was because no accumulation of feces or pseudofeces occurred as a result of stream flow. In contrast, a study by Greenwood et al. (2001) found that macroinvertebrate densities, especially gastropods and amphipods, increased in the presence of zebra mussels because of the higher food quality of their feces and pseudofeces in lotic systems. Another study found that the only benthic macroinvertebrate to exhibit significant differences in density on areas of zebra mussel colonization were native unionoid mollusks, which declined (Strayer et al. 1998). The *in situ* experiment focused only on abiotic factors influencing macroinvertebrate density changes on simulated zebra mussel colonization due to my inability to use live zebra mussels as part of *the in situ* experiment. While the interstitial spaces did allow for organic debris to fall out and collect on the tiles, this type of organic debris may not have been of sufficient nutritional value to generate the kinds of responses that zebra mussel feces and pseudofeces elicit.

Densities of zebra mussels have been reported to be 400,000 mussels/m^2 in Lake Erie (MacIssac et al. 1991), 60,000 mussels/m^2 near the headwaters of the Rhine River in Europe (Cleven and Frenzel 1993), and 100 mussels/m^2 in the St. Joseph River in southwestern Michigan (Horvath et al. 1996). It has been suggested that zebra mussels must exceed a density of 1000 mussels/m^2 to significantly affect unionid clams in the St. Lawrence River (Ricciardi et al. 1995).

In this study, a simulated density of 3521.7 individuals/m² was used to test the effects of zebra mussel colonization on benthic densities. While this density has not been proven to be sufficient to generate a response by stream macroinvertebrates, it was nonetheless expected to have had an effect on the macroinvertebrates in the streams. Additional studies should be performed to consider the threshold density in which macroinvertebrates are affected by zebra mussel colonization.

Lab Experiment

The laboratory experiment tested the potential effects of both biotic and abiotic factors on hydropsychid larvae substrate preferences because both live zebra mussel tiles and ZMST treatments were used. Hydropsychid larvae prefer stable substrates, such as large stones or logs, in high flow areas and tend to avoid settling in less stable substrates such as fine gravel (Fairchild and Holomuzki 2002). Further, they prefer habitats that are structurally complex to provide refuge from predators, materials useful in retreat construction, and easy access to foraging (Fairchild and Holomuzki 2002). Hydropsychid caddisfly larvae also prefer to inhabit areas of higher turbulence, such as notches in rocks and boulders, because it increases the efficiency of prey being caught in their nets (Osborne and Herricks 1987, Hart and Finelli 1999). Hydropsychid larvae are also territorial and the increased habitat complexity provided by zebra mussel colonization may allow greater numbers of hydropsychids to coexist. In general, as velocity and/or food concentration increases, the distance in territorial caddisflies decreases (Matczak and Mackay 1990). Thus, hydropsychids could respond positively to both the increases in microturbulence and/or the potential increase in food availability resulting from zebra mussel colonization, and the simulated colonization by zebra mussels was expected to influence all of these requirements in the laboratory experiment.

Changes in substrate complexity (i.e., interstitial spaces and notches) change the microhabitat characteristics (Osborne and Herricks 1987). The changes in substrate and microvelocity zebra mussels provide via interstitial spaces may influence responses of some taxa to prefer to inhabit zebra mussel colonies. Filter-feeders, such as hydropsychids, are commonly found in areas with high velocity (Hart and Finelli 1999). In this study, the interstitial spaces among the zebra mussels would increase the turbulence. The hydropsychid larvae inhabited tiles with live zebra mussels rather than dead zebra mussels but not necessarily in other treatments. The results, however, would most likely be more prominent in natural zebra mussel colonies in streams because natural colony densities, mussel positions, and clumping were not replicated on the tiles.

SUMMARY

Although there was limited response by macroinvertebrates in the *in situ* experiment based on overall densities, functional feeding group assignments, or individual taxa to the simulated colonization of zebra mussels, the laboratory experimental results suggest that live zebra mussels may more strongly influence macroinvertebrates in streams. In the presence of both abiotic and biotic factors provided by live zebra mussels, a representative taxon (i.e., hydropsychid caddisfly larvae) chose to inhabit substrate with live zebra mussels rather than substrate with dead zebra mussels. Quagga mussels (*Dreissena bugensis*) are a close relative of the zebra mussels. They are also native to the Ponto-Caspian area and have been recently found spreading in the Great Lakes region. Similar results are expected in response to quagga mussel invasion of streams. Additional studies are needed to determine the responses of benthic macroinvertebrates to the colonization of dreissenids in different habitats and in streams with different characteristics. In addition, the specific behavioral responses of certain taxa (i.e., heptageniids, elmids, and hydropsychids) may provide greater insight into the potential influences of zebra mussel colonization on stream benthos. Regardless, my study suggests at least some level of response by headwater stream benthic macroinvertebrates, indicating a need for the development of management plans to address future introductions into headwater streams. Further, based on the differences in benthic community structure among the three streams used in my study, it is likely that such management approaches will need to be adaptive depending on stream characteristics, from local riparian conditions to larger scale basin land use.

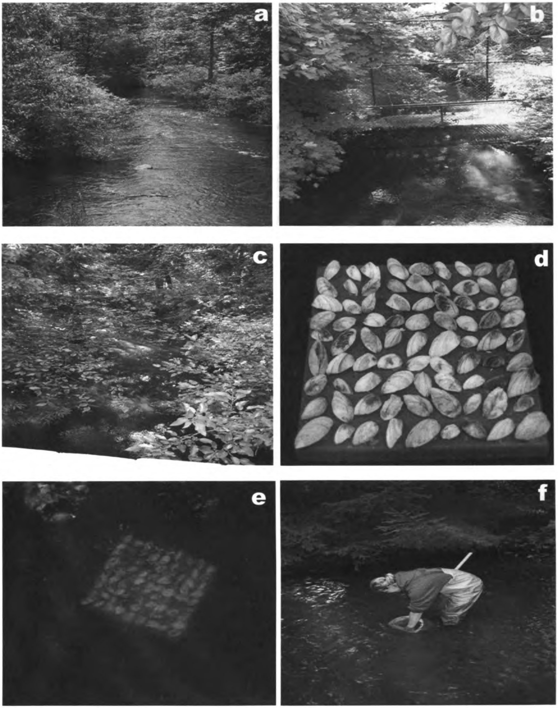
Appendix A. Taxa identified, stream, and functional feeding group assignment. Taxon in bold represent those taxa present inmore than 20% of the samples for each stream.

Taxonomic Group							Stream			
Phylum	Class	Subclass	Order	Suborder	Family	Genus	Augusta Creek	Harper Creek	Bear Creek	Functional Group
Arthropoda	Insecta		**Coleoptera**		Elmidae	***Duberaphia***	X		X	collector-gatherer
					Elmidae	***Optioservus***	X	X	X	scraper
					Psephenidae	*Ectopria*	X			scraper
					Psephenidae	*Psephenus*	X			scraper
			Diptera		Ceratopogonidae	*Probezzia*	X	X		predator
					Chironomidae		X	X	X	collector-gatherer
					Stratiomyidae	*Myxosargus*		X		collector-gatherer
					Tabanidae	*Silvius*		X		predator
					Tipulidae	*Tipula*	X			shredder
					Tipulidae	*Antocha*	X			collector-gatherer
			Lepidoptera		Pyralidae	*Crambus*			X	shredder
			Megaloptera		Sialidae	*Sialis*		X		predator
			Trichoptera		Brachycentridae	*Brachycentrus*			X	collector-gatherer
					Helicopsychidae	*Helicopsyche*	X	X		scraper
					Hydropsychidae	***Cheumatopsyche***	X	X	X	collector-filterer
					Hydropsychidae	***Macrostemum***		X		collector-filterer
					Lepidostomatidae	*Lepidostoma*	X			shredder
					Leptoceridae	*Nectopsyche*		X		shredder
					Limnephilidae		X			shredder
					Polycentropodidae	*Cymellus*		X		collector-filterer

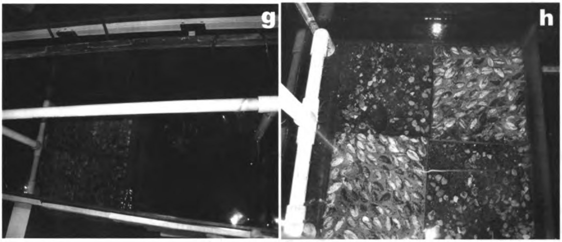
Appendix A (cont'd). Taxa identified, stream, and functional feeding group assignment. Taxon in bold represent those taxa present inmore than 20% of the samples for each stream.

Taxanomic Group							Stream			
Phylum	**Class**	**Subclass**	**Order**	**Suborder**	**Family**	**Genus**	**Augusta Creek**	**Harper Creek**	**Bear Creek**	**Functional Group**
Arthropoda	Insecta		Ephemeroptera		Baetidae		X			collector-gatherer
					Baetiscidae	*Baetisca*	X			collector-gatherer
					Caenidae	*Caenis*	X	X		collector-gatherer
					Heptageniidae	*Stenenoma*	X			scraper
					Heptageniidae	*Stentacron*	X	X		scraper
					Leptophlebiidae			X	X	collector-gatherer
			Odonata		Coenagrionidae	*Amphiagrion*		X		predator
					Gomphidae	*Erpetogomphus*	X			predator
					Gomphidae	*Hagenius*	X			predator
					Gomphidae	*Ophiosomphus*	X			predator
			Plecoptera		Pteronarcyidae	*Pteronarys*	X			shredder
Nematoda								X		predator
Mollusca	Bivalvia						X	X		collector-filterer
	Gastropoda						X	X		scraper
Annelida	Clitellata	Hirudinea						X		predator
		Oligochaeta					X	X	X	shredder
Arthropoda	Crustacea		Amphipoda		**Gammaridae**	***Gammarus***	X	X	X	shredder
			Isopoda		Asellidae	*Caecidotea*	X			shredder
			Decapoda				X			shredder
	Arachnida			**Hydracarina**			X	X		predator

Appendix B. In situ experiment photos (a-f) and laboratory experiment photos (g-h). a) Augusta Creek; b) Harper Creek; c) Bear Creek; d) zebra mussel shell tile (ZMST); e) ZMST incutbating in stream; f) tile retrieval; g) laboratory experiment design; h) laboratory tile orientation.



Appendix B (continued). g) laboratory experiment design; h) laboratory tile orientation.



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