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entitled

Spatial Correlation and Facilitation Between *Dreissena* and *Hexagenia*: Possible Food-Web Disruption?

by

Kristen M. DeVanna

Submitted to the Graduate Faculty as partial fulfillment of the requirements for the

Doctor of Philosophy Degree in Biology

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An Abstract of

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Understanding what factors drive an organism's population fluctuations in time and space can be very difficult in complex ecosystems due to changing environmental conditions and issues with spatial scale. In this dissertation, I examined the effect of soft-sediment *Dreissena* (*D. polymorpha* and *D. rostriformis bugensis*) clusters, an invasive ecosystem engineer, on a native burrowing mayfly (*Hexagenia limbata* and *H. rigida)*. Specifically, at the small-scale, I examined the effect of *Dreissena* clusters on *Hexagenia*: 1) habitat preference, 2) availability as prey, and 3) behavior during changing abiotic (low oxygen) and biotic (presence of a predator) conditions. At the large scale I examined: 1) the spatial association of *Hexagenia* and *Dreissena* and 2) the abiotic and biotic factors influencing *Hexagenia* spatial distribution and temporal fluctuations. I conducted experiments, spatial mapping and statistics, and generalized additive models to examine these objectives. *Hexagenia* were found to prefer sediment covered with both live and artificial clusters over bare sediment, likely due to decreased fish predation when under *Dreissena* clusters in turbid conditions. *Hexagenia* also received benefit from

Dreissena clusters during hypoxic conditions; *Hexagenia* were able to exit anoxic burrows to seek well-oxygenated waters while staying within the *Dreissena* cluster as refuge from predation. At the large scale, *Hexagenia* density was not related to *Dreissena* density, however *Hexagenia* were more likely to occur where *Dreissena* were also present. Similarly, *Dreissena* density was not a good predictor of *Hexagenia* density in western Lake Erie, but abiotic factors, such as percent silt, organic carbon, depth, and distance from western shore did significantly influence *Hexagenia* distribution. Sites with high average *Hexagenia* density were clustered near the western shoreline of Lake Erie and exhibited a two-year density cycle, likely a density-dependent regulation. Overall, abiotic factors appear to be the diving force behind *Hexagenia* spatial distribution, temporal fluctuations a result of a density-dependent population regulation, and biotic factors likely regulate small-scale habitat preference and behavior of *Hexagenia*. What is important is that *Dreissena* presence and resulting habitat alterations are not inhibiting *Hexagenia* presence and *Hexagenia* are maintaining sustainable population levels in areas with high densities of dreissenids in western Lake Erie.

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First of all, I am grateful to my advisor, Dr. Christine Mayer for helping to develop as better scientist, independent thinker, and person in general. I also greatly appreciate all of the guidance and constructive comments from my committee: Dr. Jonathan Bossenbroek, Dr. Thomas Bridgeman, Dr. Lynda Corkum, and Dr. Ken Krieger. I would like to thank the University of Toledo Department of Environmental Sciences and Lake Erie Center for support throughout the course of this study. I would also like to thank the Mayer, Bossenbroek, and Bridgeman labs for help with experiments, handling fish, data analyses, manuscript revisions. I also appreciate Betsy Bodamer Scarbro, Amanda Haponski, Rachel Kuhaneck, Jennifer Sieracki, Audra Croskey, Patty Armenio, and Colleen Wellington for being great friends who have kept me sane throughout this process. I would like to acknowledge my family: Tim, Cheryl, Courtney, and Ryan, who have been very supportive throughout these past few years both emotionally and financially. I would especially like to thank Courtney for dealing with me through my qualifying exams; I know I was difficult. I would also like to thank Anthony Fussell, who I am very lucky to have and makes me very happy lady everyday. Lastly, I would like to thank my Grandpa and Grandma Limmer for feeding me, taking care of me when I am sick, but most of all, just for being my home away from home.

Table of Contents

List of Tables

List of Figures

Preface

Chapter 2 of this dissertation contains an experiment, conducted in my master's thesis, DeVanna (2006). This experiment from my MS work served as the catalyst for further experiments conducted as part of this dissertation and was therefore combined with the dissertation work in a recent publication. This publication in the Journal Freshwater Biology has been included here as Chapter 2 for completeness and clarity. In compliance with regulations of the University of Toledo Graduate College the chapter is an exact replication of the published work. Complete citation is provided. Chapter 3 of this dissertation is not yet published. Chapter 4 is in has been revised based on the editors comments for publication in the book: 2nd edition of Zebra Mussels: Biology, Impacts and Control, edited by T.F. Nalepa and D.W. Schloesser. This chapter also contains the material in Chapter 2 (DeVanna et al. 2011) that originated in my MS thesis (DeVanna 2006) and a small subset of the experiment discussed in Chapter 3. Chapter 5 is not published elsewhere.

Chapter 1

Introduction

Invasive species can have profound effects on ecosystem function. Consequences may be intensified when the invader is an ecosystem engineer, meaning that it causes physical state changes in biotic or abiotic materials (Jones et al. 1994 and 1997). Understanding how invasive ecosystem engineers affect the distribution and behavior of native organisms is challenging at a large spatial scale. Alone, population fluctuations of organisms are difficult to understand and predict in large, dynamic ecosystems, and attributing mechanism to an invasive species is especially challenging. Another issue of concern when examining spatial trends is that factors influencing a species' spatial distribution occurs at multiple spatial scales; biotic or abiotic processes that are important at one scale are not always predictive at a different scale (Turner et al. 1989; Wiens 1989). Therefore, in this dissertation, I examined the effects of two invasive species that are also ecosystem engineers, *Dreissena polymorpha* (Pallas 1771) and *D. rostriformis bugensis* (Andrusov 1897), on the habitat selection and behavior of native burrowing mayflies, *Hexagenia limbata* (Serville 1829) and *H. rigida* (McDunnough 1924), at different spatial scales. Small-scale experiments are useful for establishing mechanisms for an observed relationship, whereas large-scale spatial analyses and quantitative approaches are useful in observing spatial patterns and then relating process to observed patterns. In my dissertation, I combined both small and large-scale approaches to gain an understanding of the relationship between *Hexagenia* and *Dreissena*.

Hexagenia population densities across their range have fluctuated widely, and decreases in *Hexagenia* density has been attributed to increased pollution and hypoxia (eg., Krieger 1996; Howmiller and Beeton 1971; Clady 1975) because *Hexagenia* are sensitive to low oxygen concentrations (Winter et al. 1996). *Hexagenia* were historically abundant in the western basin of Lake Erie, but populations declined to near extirpation in the 1950's during times of eutrophic conditions (Britt 1955, Nebeker 1972, Gerlofsma et al. 1998). Although *Hexagenia* were nearly absent from offshore sites in Lake Erie during the 1960's through 1980's, populations did still exist in the Detroit River mouth (Krieger et al. 1996), Lake Erie shoreline areas (Corkum et al. 1997), as well as in Lake St. Clair (Schloesser et al. 1991). Increases in abundance and recolonization throughout western Lake Erie have been documented since the early 1990's (Krieger et al. 1996, Schloesser et al. 2000, Schloesser and Nalepa 2001) and many biologists have been optimistic about the recolonization because *Hexagenia* are large benthic organisms and an additional food source to many economically important fish species in Lake Erie, such as yellow perch (*Perca flavescens*, Mitchill 1814; Hayward and Margraf 1987; Schaeffer et al. 2000). However, populations continue to show spatial and temporal variability linked to anthropogenic and environmental factors, such as short-term periods of hypoxia (Winter et al. 1996, Schloesser and Nalepa 2001, Bridgeman et al. 2006).

Dreissena polymorpha (zebra mussels) and *D. rostriformis bugensis* (quagga mussels) are an example of two invasive species in the Laurentian Great lakes that can function as ecosystem engineers (Karatayev et al. 2002; Zhu et al. 2006) by modifying available bottom habitat. *D. polymorpha* on hard substrates have been shown to support enhanced numbers of benthic invertebrates (e.g. Silver Botts et al. 1996; Ricciardi et al. 1997; Mayer et al. 2002) due to increased habitat complexity and protection from predation (Gonzalez and Downing 1999; Mayer et al. 2001; Beekey et al. 2004a). However, quagga mussels, first recorded in North American in 1989 in Lake Erie (Mills *et al.*, 1996), have become the dominant dreissenid species in the Laurentian Great Lakes and are the main coloniser of soft sediments (Roe and MacIsaac 1997; Patterson et al. 2005; Wilson et al. 2006). Quagga mussels have increased from 20% of the dreissenid population in the western basin of Lake Erie in 1998 to 80% in 2001 (Stoeckmann 2003). The change from primarily hard substrate colonies in the Great Lakes (dominated by zebra mussels) to soft substrates colonies (dominated by quagga mussels) can be expected to affect the infaunal benthic community, such as *Hexagenia*, and higher trophic levels. In this dissertation, I examine the effects of quagga mussel-dominated clusters on soft sediment, which fundamentally change the soft bottom habitat of lakes to a more spatially complex, hard-cluster-covered substrate, on native *Hexagenia* habitat preference, availability to fish, spatial and temporal variability, and behavior to changing biotic and abiotic conditions.

Experiments conducted for my M.S. degree show the unexpected result that *Hexagenia* strongly select for habitat covered with live *Dreissena* clusters. I had originally hypothesized that the structure of *Dreissena* clusters would decrease the abundance or survival of bioturbators including burrowing mayflies, which has been shown previously (Freeman et al. 2011; Osterling et al. 2007), but this may be an effect of the experimental tank or the scale and duration of study. *Dreissena* affect *Hexagenia* at a small spatial scale in a variety of way including: 1) modifying their habitat by the addition of shells to soft sediment 2) providing refuge from predation, 3) adding food resources by means of *Dreissena* feces and pseudofeces, and 4) increasing the flow of well-oxygenated pelagic water to areas close to the cluster via *Dreissena* filter feeding. At a larger scale, physical factors, such as sediment type, water movement, and oxygen availability may be more important than small-scale factors in structuring both *Dreissena* and *Hexagenia* distributions. Thus, the spatial association between *Hexagenia* and *Dreissena* may differ depending on the scale at which they are observed. Quantifying the spatial relationships between these two important species at multiple scales may help in understanding what mechanisms, biotic or abiotic, are structuring their distributions.

The continued spread of *Dreissena* onto soft sediments and the preference of *Hexagenia* for sediment beneath *Dreissena* clusters may reduce fish foraging success on *Hexagenia* during high oxygen conditions. *Dreissena* clusters have been found to provide invertebrates a refuge from predation (Gonzalez and Downing 1999; Mayer et al. 2001; Beekey et al. 2004a), and this is a potential mechanistic explanation for the observed relationship. Even though *Dreissena* may facilitate *Hexagenia* growth and survival, they may reduce *Hexagenia* connectivity to native fish, benthic-pelagic coupling, and food-web function in Lake Erie. However, based on their feeding strategies, fish species may be affected differently by the preference of *Hexagenia* for sediment beneath *Dreissena* clusters. For example, yellow perch may be greatly impacted by the presence of *Hexagenia* underneath *Dreissena* clusters because they do not commonly feed on *Dreissena* and, as opportunistic feeders may not search below the clusters for food. Alternatively, round gobies (*Neogobius melanostomus*, Pallas 1814) may be more able to find *Hexagenia* because they are primarily benthic feeders, readily dig in the sediment for food, and consume dreissenid clusters (Ray and Corkum 1997; Carman et al. 2006). Together, these data, observations, and previous studies suggest that although *Hexagenia* may utilize and benefit from *Dreissena* colonized sediments, fish may have decreased access to *Hexagenia* beneath mussel clusters.

Alternatively, the western basin of Lake Erie can undergo short periods of hypoxia (Bridgeman et al. 2006) and the presence of *Dreissena* may affect *Hexagenia* behavior since oxygen concentration beneath *Dreissena* clusters is already low (Burks et al. 2002; Beekey et al. 2004b). Therefore hypoxic events may force *Hexagenia* to leave their burrows in bare sediment or sediment beneath dreissenids to seek well-oxygenated water. Exiting a burrow or other refuge leaves *Hexagenia* very vulnerable to predation and may create an opportunistic event for fish feeding on *Hexagenia*, as fish have been found to swim into lethally hypoxic waters when food is abundant (Rahel and Nutzman 1994). When presented with multiple stresses, *Hexagenia*, which are oxygen sensitive and highly vulnerable to predation, may remain in low oxygen waters longer when a predator is present, similar to other mayfly species (Kolar and Rahel 1993). Therefore, *Hexagenia* preference for *Dreissena*-covered sediments may impede fish consumption under high oxygen conditions, but short-term hypoxia in western Lake Erie may alter *Hexagenia* behavior making them vulnerable to fish predation.

Goal: The overall goal of this dissertation is to examine how the habitat modifications created by *Dreissena*, a non-indigenous ecosystem engineer, affect native *Hexagenia* habitat preference, spatial and temporal variability, and behavior in response to changing abiotic and biotic factors. I will closely examine two distinct mechanisms that may affect whether or not *Hexagenia* select *Dreissena*-colonized habitat: 1) hypoxia and 2) risk of predation by fish. My approach includes small-scale in-lab experiments as well as large-scale mapping and statistical modeling using long-term data sets.

Objectives:

1: A) Do *Dreissena* clusters reduce fish predation on *Hexagenia*? B) Is this effect altered based on the fish's feeding style?

2: A) Does *Hexagenia* habitat selection change in the presence of a predator and low oxygen? B) How does the presence of *Dreissena* affect *Hexagenia* behavior in the presence of a predator and low oxygen conditions?

3: A) Do *Hexagenia* and *Dreissena* co-occur in the western basin of Lake Erie? B) Are *Hexagenia* and *Dreissena* spatially correlated in the western basin of Lake Erie?

4) A) What abiotic and biotic factors are important in determining *Hexagenia* density and spatial distribution? B) Do *Hexagenia* show temporal trends at a given location over time?

Chapter 2

Invasive Ecosystem Engineers on Soft Sediment Change the Habitat Preferences of Native Mayflies and their Availability to Predators

DeVanna, K.M., P.M. Armenio, C.A. Barrett, and C.M. Mayer. (2011) Invasive ecosystem engineers change the habitat preferences of native mayflies and their availability to predators. *Freshwater Biology*, **56**, 2448-2458.

2.1 Summary

1. Dreissenid mussels (quagga mussels, *Dreissena bugensis*, and zebra mussels, *D. polymorpha*) are invasive species that function as ecosystem engineers in the Laurentian Great Lakes. *Dreissena* are increasingly abundant on silt, sand, and other soft substrates; by altering benthic habitat, these mussels can alter benthic community structure.
2. We used laboratory mesocosm experiments to examine the effects of soft-sediment *Dreissena* clusters on the habitat preference of *Hexagenia*, a native burrowing mayfly that is an important food source to fish. We conducted three experiments to test if *Hexagenia*: 1) select for bare sediment, soft sediment covered with live *Dreissena* (added structure and food resources), or soft sediment with clusters made of empty *Dreissena* shells (added structure only), 2) prefer a specific density of live *Dreissena*

on soft sediment, and 3) select for or avoid sediment with an accumulation of empty *Dreissena* shells.

3. Contrary to initial expectations, we found that *Hexagenia* selected for sediment covered with live *Dreissena* clusters, followed by empty *Dreissena* shells clusters, and lastly what was previously thought to be the preferred habitat, bare sediment. Not only did *Hexagenia* prefer *Dreissena*-covered sediment, but they also preferred high densities of *Dreissena*.
4. We also experimentally tested the effects of *Dreissena*-covered soft sediment on the availability of *Hexagenia* to fish. We had three treatment levels representing three distinct habitat types: 1) bare sediment (no *Dreissena*) treatment in which water was turbid due to mayfly activity, 2) *Dreissena*-covered sediment treatment in which water was clear due to *Dreissena* filtration, and 3) *Dreissena*-covered sediment with added turbidity. We found that in low light conditions, similar to many locations where both organisms are found to co-occur, both yellow perch and round goby consumption of *Hexagenia* significantly decreased when *Dreissena* covered the bottom sediment.
5. These results suggest that by choosing *Dreissena*-covered habitat, *Hexagenia* receive protection from fish predation in turbid/low-light systems. However, protection from predation cannot be the only reason *Hexagenia* select *Dreissena*-covered sediments, as *Hexagenia* selected for live clusters more often than empty clusters, and may be a result of additional food resources.

2.2 Introduction

Invasive species can significantly impact ecosystem function. Consequences may be intensified when the invader is an ecosystem engineer, meaning that it causes physical state changes in biotic or abiotic materials (Jones, Lawton, & Shachak, 1994, 1997). Two invasive ecosystem engineers, zebra mussels (*Dreissena polymorpha*, Pallas 1771) and quagga mussels (*D. rostriformis bugensis*, Andrusov 1897) both alter benthic habitat in a variety of large, permanent, aquatic ecosystems (Karatayev, Burlakova & Padilla, 2002). The initial invasion by zebra mussels in North America involved clusters colonising bedrock and other hard surfaces and increasing habitat complexity on hard substrate. Subsequently, both zebra and quagga mussels have been spreading to soft substrates in many invaded lakes (Berkman *et al*., 1998; Bially & MacIsaac, 2000). However, quagga mussels, first recorded in North America in 1989 in Lake Erie (Mills *et al*., 1996), have become the dominant dreissenid species in the Laurentian Great Lakes and are the main coloniser of soft sediments (Roe & MacIsaac, 1997; Patterson, Ciborowski & Barton, 2005; Wilson, Howell & Jackson, 2006), increasing from 20% of the dreissenid population in the western basin of Lake Erie in 1998 to 80% in 2001 (Stoeckmann, 2003). In this paper, we focus on the effects of quagga mussel-dominated clusters on soft sediment, which fundamentally change the soft bottom habitat of lakes to a more spatially complex, hard-cluster-covered substrate. The change from primarily hard substrate colonies in the Great Lakes (dominated by zebra mussels) to soft substrates colonies (dominated by quagga mussels) can be expected to affect the infaunal benthic community and higher trophic levels.

Dreissena on hard substrates have been shown to increase local abundance and diversity of macroinvertebrates because of: 1) habitat complexity, 2) increased food resources from *Dreissena* faeces and pseudofaeces (eg., Silver Botts, Patterson, & Schloesser, 1996; Ricciardi, Whoriskey, & Rasmussen, 1997; Stewart, Miner, & Lowe, 1998) and 3) decreased fish predation (Gonzalez & Downing, 1999; Mayer *et al*., 2001; Beekey, McCabe, & Marsden, 2004a). Quagga mussel colonies on soft sediment fundamentally shift habitat type (bare sediment to hard substrate), and infaunal invertebrates are likely to respond to added structure differently than hard substrate dwelling invertebrates. Few infaunal species have been shown to increase in the presence of soft sediment *Dreissena* clusters (Bially & MacIsaac 2000; Beekey *et al.* 2004b), while others have declined (Beekey *et al*., 2004b) including the dramatic decrease of infaunal filter feeders (Strayer *et al*., 1999; Nalepa *et al*., 2003; Nalepa, Fanslow, & Messick, 2005). We focussed on native *Hexagenia* spp. (*H. limbata*, Serville 1829 and *H. rigida*, McDunnough 1924), burrowing mayflies important to fish and to ecosystem function, whose preferred habitat type has always been perceived as bare sediment (Freeman, 1999; Schloesser & Nalepa, 2001; Wang, Tessier, & Hare, 2001).

Hexagenia became rare in the Great Lakes during the 1950s during eutrophic conditions (Britt, 1955; Nebeker, 1972; Gerlofsma & Ciborowski, 1998), but their recent recolonisation of Lake Erie provides an additional food source to many economically important fish species, such as yellow perch (*Perca flavescens*, Mitchill 1814) (Hayward & Margraf, 1987; Schaeffer, Diana, & Haas, 2000). *Dreissena* clusters on soft sediments may reduce the consumption rate of fish feeding on benthic prey that are protected in the interstitial spaces of mussel clusters (Gonzalez & Downing, 1999; Mayer *et al*., 2001;

Beekey *et al*., 2004a); however, the density of mayflies inhabiting mussel clusters may be higher than on bare soft sediments, which could compensate for the reduced rate of consumption. Further, a reduction in consumption rate may differ between fish species with different feeding strategies, and we examined the effects of *Dreissena* clusters on consumption of *Hexagenia* by two fish types, a visual feeder, the yellow perch, and a primarily benthic feeder adapted to low light, the round goby (*Neogobius melanostomus*, Pallas 1814). Fish such as yellow perch that feed visually (Diehl, 1988), may have greatly reduced consumption because *Dreissena* clusters add structure (Gonzalez & Downing, 1999; Mayer *et al*., 2001). Alternately, *Hexagenia* are bioturbators, meaning they mix the sediment through feeding, respiration, and burrowing activities (Bartsch, Cope, & Rada, 1999), resulting in high turbidity at the sediment-water interface (Bachteram, Mazurek, & Ciborowski, 2005) and *Dreissena* filtering is likely to reduce turbidity. In contrast, fish such as the invasive round goby, may be more able to find *Hexagenia* despite the presence of *Dreissena* because they are primarily benthic feeders, feed in low light (Dubs & Corkum, 1996), and also consume dreissenid clusters (Ray & Corkum, 1997; Carman, Janssen, & Berg, 2006). While multiple factors (*e.g.* prey density, abiotic conditions) will ultimately determine the quantity of benthic prey transferred to higher trophic levels, the addition of structure to previously soft sediment and increased water clarity are both substantial habitat alterations that are likely to affect the amount of biomass consumed by fish.

The direction and strength of interactions between *Dreissena* and *Hexagenia* are important, as they may change ecosystem processes at the sediment-water interface in addition to affecting the flow of benthic energy to fish. In this study we tested experimentally the ecological interactions between invasive *Dreissena* and native *Hexagenia* on soft sediment, examining both habitat preference and availability of *Hexagenia* to fish. Specifically, we hypothesised that, unlike many invertebrates on hard substrates that congregate in *Dreissena* clusters, *Hexagenia* will avoid this habitat. Our first objective was therefore to examine burrowing mayfly habitat preference with respect to *Dreissena* presence on soft sediments. To assess this, we conducted three separate habitat preference experiments: 1) *Habitat type selection* - tested whether burrowing mayflies select for bare sediment (no structure), empty *Dreissena* clusters (structure only), or live *Dreissena*-colonized habitat (structure and increased food resources) for two types of western Lake Erie sediment, 2) *Dreissena-density selection* – examined how *Hexagenia* respond across a gradient of increasing *Dreissena* densities, and 3) *Effect of accumulated shells* - tested whether burrowing mayflies would avoid sediment with a build up of empty *Dreissena* shells, a phenomenon that has been observed in many *Dreissena*-colonized lakes. Our second objective was to assess the effects of *Dreissena*-colonized sediment on the availability of *Hexagenia* to fish and we hypothesised that, like other hard substrates, the presence of *Dreissena* will reduce fish consumption of *Hexagenia*. We conducted an experiment to test the effects of *Dreissena*-covered sediments, examining both added structure and changes in water clarity, on fish consumption of *Hexagenia* by two different fish species, yellow perch and round gobies, which vary in feeding strategy. Our expectation was that *Dreissena* will act strongly as ecosystem engineers on soft substrate where they cause a switch in habitat type. The direction of these effects may differ from what has been observed previously for zebra mussels on hard substrate habitats.

2.3 Methods

2.3.1 Habitat preference experiments

To examine the possible association between burrowing mayflies and *Dreissena*, laboratory mesocosm experiments were conducted at the University of Toledo's Lake Erie Center. All experiments were run indoors at room temperature along a set of large windows to allow for natural light cycles. *Dreissena* and mayflies were collected from western Lake Erie. *Dreissena* were collected from soft substrates, and age-one burrowing mayfly nymphs (> 10 mm) were collected to decrease risk of emergence during the experiment, and *H. limbata* and *H. rigida* were collected at their natural occurring proportions. Quagga mussels dominated *Dreissena* clusters collected, however zebra mussels were present in small numbers. Three separate experiments were conducted: 1) habitat type selection, including differences in habitat selection between coarse nearshore sediment and very fine offshore sediment, 2) *Dreissena* density selection, and 3) effect of accumulated *Dreissena* shells. All mesocosms in these experiments were filled with 6 cm of either nearshore (41.6885 W, 83.4250 N) or offshore (41.7976 W, 83.3136 N) Lake Erie sediment that was first sieved through 1.0 mm mesh. The three experiments (habitat type selection, *Dreissena* density selection and effects of accumulated shells) ran for different lengths of time, but all trials within an experiment ran for the same length of time and no statistical comparisons were made between the three experiments.

2.3.1.1 Experiment 1 - Habitat type selection: We tested whether burrowing mayflies selected for or avoided *Dreissena* clusters on soft sediment. Experimental mesocosms (circular plastic tubs; 41 cm diameter and 43 cm height) filled with 6 cm of nearshore sediment were separated into three equal “pie-slice” shaped sections (0.046 m^2) using metal dividers and three different habitat types were created: 1) bare sediment, 2) live *Dreissena* clusters, and 3) empty *Dreissena* clusters. Live and empty *Dreissena* cluster treatments contained approximately 250 individuals (5434 m^{-2}; Patterson *et al.*, 2005). We created empty *Dreissena* clusters by gluing clean shells together with non-toxic glue and adding five 1 g lead weights to each cluster. To ensure the weights did not influence results of the experiment, lead weights were added to all treatments. Metal dividers were removed after habitat types were in place.

The experiment was conducted using five densities of burrowing mayflies that fell within the range seen in western Lake Erie (0 to 2000 m^{-2}; Krieger, 1999): 5 individuals (~ 100 m^{-2}), 9 (~ 200 m^{-2}), 18 (~ 400 m^{-2}), 36 (~ 800 m^{-2}), and 54 (~ 1200 m^{-2}). Each mayfly density treatment was replicated three times (N=15). Mayflies were added to the centre of the mesocosm at the water surface and allowed to select between the habitat types. One replicate of each density was run at the same time and mesocosms were placed in a straight line in random order. After 48 hours, metal dividers were again pushed into the sediment between habitat types, water was siphoned, sediment from each habitat was removed and sieved through 250µm nitex mesh, and mayflies in each habitat type were counted.

The habitat selection experiment was repeated with offshore Lake Erie sediment from a site where burrowing mayflies were very abundant (41.7976 W, 83.3136 N) to test if sediment type had an effect. Experiments were run as described above with three densities of burrowing mayflies: 9 individuals (~200 m^{-2}), 18 (~400 m^{-2}), and 36 (~800 m^{-2}). Each density was replicated three times (N=9).

2.3.1.2 Experiment 2 - Dreissena density selection: The design for experiment 2 and selection of treatment conditions was based, in part, on results from experiment 1. We manipulated the density of *Dreissena* to better understand if burrowing mayflies prefer a specific percent coverage of live *Dreissena* clusters. Each rectangular mesocosm (80 cm x 30 cm) was split into four equal sections containing a mixture of offshore and nearshore western Lake Erie sediment covered with 0%, 25%, 50% or 100% live *Dreissena* clusters. Coverage was estimated by covering a template of the appropriate area with shells. An intermediate density of mayflies, ~ 400 m^{-2}, was used in this experiment. Four replicates were conducted (N=4); each replicate consisted of all four *Dreissena*-coverage habitat types, and the arrangement of habitats within the mesocosms was randomly determined for each replicate. Metal dividers were placed into the sediment between habitat types after 64 hours. Mayflies were removed and counted as described in the mayfly habitat selection experiment above.

2.3.1.3 Experiment 3 - Effect of accumulated shells: The final habitat selection experiment examined the effect of accumulated *Dreissena* shell fragments on mayfly habitat preference. *Dreissena* shells and shell fragments accumulate in colonized lakes and may also affect the habitat preference of burrowing animals. Experimental mesocosms (circular plastic tubs; 41 cm diameter and 43 cm height) were divided into two sections, each 0.069 m^2. Both sections were filled with nearshore Lake Erie sediment and one section had 515 g of empty *Dreissena* shells mixed in. The shells used in each trial were from a single ponar collected in western Lake Erie and therefore represented the natural density and size distribution of shell fragments. Mayflies were added at an intermediate density of ~ 400 m^{-2}. Five replicates were run for 60 hours (N=5); trials were run sequentially, and mayflies were removed and counted as above.

2.3.1.4 Data Analysis: The percentage of total number of burrowing mayflies in each habitat type was arcsin square root transformed to help achieve a normal distribution (Zar, 1999) for all 3 experiments. For experiment 1, all habitat type selection data (nearshore and offshore sediment experiments) were analyzed using a split plot ANOVA model (SAS 9.1, α =0.05) followed by a Tukey multiple comparison test when appropriate, with initial mayfly density as a main plot factor, habitat type as a subplot factor, and their interaction (Potvin, 2001). A split-plot model was used because each experimental mesocosm was split into three different habitat types and treatments were applied to different scales; habitat type was applied to one third of the mesocosm (subplot) while mayfly density was applied to the full mesocosm (main plot). Experiment 2, the *Dreissena* density selection experiment, was analysed using a one-way ANOVA, followed by a Tukey multiple comparison test. Lastly experiment 3, the effect of accumulated empty shells, was analysed using a two-sample, two-tailed, t-test (SAS 9.1, α=0.05).

2.3.2 Fish foraging experiments

To assess the effects of *Hexagenia* habitat choice on their availability to fish as a food resource, we conducted mesocosm experiments measuring number of *Hexagenia* consumed by fish in different habitats. We had three treatment levels representing three distinct habitat types: 1) bare sediment (no *Dreissena*) treatment in which water was turbid due to mayfly bioturbation, 2) *Dreissena*-covered sediment treatment in which water was clear due to *Dreissena* filtration, and 3) *Dreissena*-covered sediment with added turbidity (*Dreissena* + turbidity). The *Dreissena* + turbidity treatment was included in order to assess the effects of *Dreissena* and water clarity separately. *Hexagenia* bioturbation in the bare sediment treatments without *Dreissena* increased turbidity levels (200 – 400 NTU) and reduced light levels (average 0.308 μE m^{-2} s^{-1}), while *Dreissena* filtering in the *Dreissena*-covered sediment treatment resulted in decreased turbidity and increased light levels (average 0.873 μE m^{-2} s^{-1}). We attempted to create a clear water treatment with no *Dreissena*, but could not achieve this condition.

To establish the *Dreissena* + turbidity treatment, two large tanks were filled with dechlorinated water and nearshore lake sediment (same sediment as used on bottom of experimental tanks) until turbidity levels reached 400 NTU. The highly turbid water from each tub was pumped into two experimental mesocosms to keep bottom light levels similar to the bare sediment treatment (~0.300 μE m^{-2} s^{-1}). The flow of water pumped into experimental mesocosms was slow to minimise disturbance and a small tube was inserted to the top of the mesocosm to allow overflow water to return to the turbid water tanks. Light readings were taken at the start of the experiment, end of day 1, beginning of day 2, and at the conclusion of the experiment (beginning of day 3). Light levels were not statistically different in the bare (mean and standard deviation; 0.31 ± 0.32 μE m^{-2} s^{-1}) and *Dreissena* + turbidity (0.13 ± 0.08 μE m^{-2} s^{-1}) treatments at the end of experimentation, while light levels in both treatments were significantly lower than that in the *Dreissena*-covered sediment treatment (0.87 ± 0.35 μE m^{-2} s^{-1}) (ANOVA $F_{2,40}$ = 29.87, p<0.0001, Tukey p<0.05). All other aspects of the *Dreissena* + turbidity treatment were kept the same as the bare and *Dreissena*-covered sediment treatments described below.

Each experimental mesocosm (circular plastic tubs of diameter 34.3 cm and height 43 cm) included 6 cm of sieved (1 mm mesh) nearshore western Lake Erie sediment, 18 *Hexagenia* (~200 *Hexagenia* m^{-2}, a common density in Lake Erie; Krieger, 1999) and one fish. Each treatment was replicated 10 times (N=10), totalling ten individual yellow perch (total length 7.0-13.0 cm) and ten round gobies (total length 6.0-9.8 cm). Treatments with *Dreissena* had 15 000 individuals m^{-2}, a density observed on soft sediments (Patterson *et al*., 2005). As above, *Dreissena* and mayflies were collected from western Lake Erie. Prior to the experiment, sediment, *Hexagenia*, and *Dreissena* (if applicable) were added to establish the correct habitat type and a mesh screen was placed above the sediment surface to restrict fish access to *Hexagenia* or *Dreissena*. Four mesocosms for the same fish species were run simultaneously and treatments were randomly assigned to mesocosms. Individual fish were placed in the experimental mesocosms above the mesh 24 hours prior to experimentation to allow for acclimation to surroundings and standardization of hunger levels. The mesh was then removed and fish were allowed to feed for 24 hours. After 24 hours, light levels were recorded, fish were removed, and the number of prey consumed was determined by sieving the mesocosm sediment through 250 µm nitex mesh and counting the remaining *Hexagenia*. When only a head or tail end of a *Hexagenia* was left, we counted that as 0.5 eaten in our total numbers consumed. To assess our error in recovering *Hexagenia*, trials (3 per treatment) with no fish present were run at the same time as the fish foraging experiment to measure the number of *Hexagenia* recovered at the end of the experiment without loss to predation.

The effect of *Dreissena*-covered sediment and turbidity on consumption of *Hexagenia* was tested separately for yellow perch and round gobies by comparing the number of *Hexagenia* consumed across the three habitat types using a one-way non-parametric Kruskal-Wallis test (SAS 9.1, $\alpha=0.05$). The Kruskal-Wallis test was followed by a Nemenyi test, a non-parametric multiple comparisons test, which is an analog to a Tukey's test (Zar, 1999). We used non-parametric statistics because the response variable (number eaten) is a count variable and there were many low values resulting in a non-normal distribution.

2.4 Results

2.4.1 Habitat selection experiments

In experiment 1, habitat type selection, burrowing mayflies were most often located in the live *Dreissena* habitat for both sediment types (Fig. 1). In nearshore sediment, mayfly density differed among all three habitat types (Split-plot ANOVA: $F_{24,20} = 95.17$, $p<0.0001$, Tukey: $p<0.05$). The live *Dreissena* habitat had the highest percentage of mayflies, followed by empty *Dreissena*, then bare sediment. There was a significant interaction in percentage of mayflies in each habitat type based on mayfly density (Split-plot ANOVA: *nearshore habitat*density*: $F_{24,20} = 4.86$, $p<0.0001$), showing that the percentage of mayflies selecting each habitat type changes with mayfly density. In offshore sediment, there were also significantly more burrowing mayflies in the live *Dreissena* habitat (Split-plot ANOVA: $F_{14,12} = 6.85$, $p = 0.0104$, Tukey $p<0.05$), but no difference occurred between the empty *Dreissena* and bare sediment habitats. There was no significant interaction in percentage of mayflies in each habitat type based on mayfly density (Split-plot ANOVA: *offshore habitat*density*: $F_{14,12} = 0.52$, $p = 0.7213$).

For experiment 2, *Dreissena* density selection, mayfly habitat preference differed significantly with percent *Dreissena* coverage (Figure 2; ANOVA: $F_{3,12} = 14.54$, $p = 0.0003$). Densities in the 0% and 25% *Dreissena* coverage habitats were significantly lower than in the 50% and 100% *Dreissena* coverage habitats (Tukey: $p<0.05$). Our third experiment, the effect of shell accumulations, showed that mayflies did not show a preference between un-altered sediment and sediment mixed with accumulated empty *Dreissena* shells (mean = 43% and 57% of mayflies added, respectively) (T-test: $t_{0.05,8} = 1.71$, $p = 0.127$).

2.4.2 Fish foraging experiments

The control tanks used to estimate error in retrieving *Hexagenia* in the absence of predation, showed that the average error was very low and not significantly different between treatments (Kruskal-Wallis: $\chi^2_2=2.67$, $p=0.2636$; mean number of *Hexagenia* not counted: bare=0.0, *Dreissena*=0.33, *Dreissena* + turbidity=0.60) and therefore was not included in the subsequent analyses. The presence of different bottom habitat types (*Dreissena*-covered sediment and bare sediment) did affect the consumption of *Hexagenia* by both yellow perch and round gobies (Fig. 3; Kruskal-Wallis: yellow perch $\chi^2_2=12.44$, $p=0.0020$; round gobies $\chi^2_2=10.27$, $p=0.0059$). Overall, the presence of *Dreissena* only reduced yellow perch and round goby consumption of *Hexagenia* when water was turbid (200-400 NTU) and light levels were low (~0.300 $\mu E\ m^{-2}\ s^{-1}$) (Fig. 3).

Yellow perch did not consume fewer *Hexagenia* when *Dreissena* were present without added turbidity (Nemenyi: $q_{0.05, \infty, 3}$=0.23, p>0.50), but consumed fewer *Hexagenia* in the *Dreissena* + turbidity treatment than in the bare (Nemenyi: $q_{0.05, \infty, 3}$=4.40, 0.01>p>0.005) and *Dreissena* only treatments (Nemenyi: $q_{0.05, \infty, 3}$=4.17, 0.01>p>0.005; Fig. 3). Similarly, round gobies were not affected by the presence of *Dreissena*-covered sediment in clear water, as predation of *Hexagenia* was relatively high (Nemenyi: $q_{0.05, \infty, 3}$=1.13 p>0.50). However, presence of *Dreissena* reduced consumption when light levels were kept low due to high turbidity. Round gobies in the bare sediment treatment consumed more *Hexagenia* than in the *Dreissena*-covered sediment with added turbidity treatment (Nemenyi: $q_{0.05, \infty, 3}$=4.31 0.01>p>0.005; Fig. 3). Both species of fish therefore consumed fewer *Hexagenia* only when *Dreissena*-covered sediment and low light (~0.300 µE m^{-2} s^{-1}) were present together, and the magnitude of effects between fish species were also similar.

2.5 Discussion

2.5.1 Habitat selection experiments

We hypothesised that burrowing mayflies would avoid the structure created by *Dreissena* clusters, as has been suggested previously (Freeman, 1999; Beekey *et al.*, 2004b; Osterling *et al.*, 2007). However, our habitat choice experiments showed that burrowing mayflies consistently and strongly preferred sediments covered by live *Dreissena* clusters over empty clusters and bare sediment, regardless of the type of sediment used (coarse nearshore vs. fine offshore) (Figs 1, 2). We were often able to observe where the entrances to burrows were positioned; they were frequently directly beneath clusters, suggesting that the mayflies were burrowing directly under clusters, not seeking cluster margins. This occurred despite the fact that *Dreissena* clusters can decrease water quality and oxygen concentration beneath them (Burks *et al*., 2002; Beekey *et al*., 2004b). Bare sediment, typically thought to be the habitat of burrowing mayflies, (Freeman, 1999; Schloesser & Nalepa, 2001; Wang *et al*., 2001), was the least selected habitat type (Figs 1, 2). Selecting *Dreissena*-covered habitat may not be beneficial over long time spans as *Hexagenia* survival has been found to be lowest in mesocosms with *Dreissena* (Freeman, 1999; Osterling *et al*., 2007), but this may be an effect of the mesocosm, as mayfly larvae residing in clusters are densely aggregated and may compete for food. As a result, the strength of habitat preference in natural situations is also likely to depend on food availability and needs to be evaluated in a natural lake system. Our results suggest that burrowing mayflies can alter their behaviour to take advantage of increased habitat complexity created by the mussels. The observed preference of burrowing mayflies for *Dreissena* clusters could impact the spatial distribution of burrowing mayflies if they select for "low-quality" sediment covered with *Dreissena* over "high-quality" bare sediment.

Epifaunal invertebrates living in interstital spaces of hard substrate mussel clusters have been shown to occur both in equal densities in live and empty *Dreissena* cluster habitats (Gonzalez & Downing, 1999; Silver Botts *et al*., 1996), and similarly to our experiment, prefer live mussel clusters over empty ones (Stewart *et al.*, 1998; Ricciardi *et al.*, 1997). Further, burrowing mayflies in our study selected equally for high levels of live *Dreissena* spatial coverage (Fig. 2), and are unlike epifaunal invertebrates that show a linearly increasing response to *Dreissena* density (Mayer *et al*., 2002). The build up of empty *Dreissena* shells did not affect *Hexagenia* habitat choice as expected, suggesting that this material does not create difficulties in burrowing and, although not significant, we did find more *Hexagenia* in the accumulated *Dreissena* shell habitat.

The provision of structure is evidently not the only mechanism affecting mayfly selection for *Dreissena* habitat because burrowing mayflies preferred live *Dreissena* to empty clusters (Fig. 1). Burrowing mayflies may be responding to the food resource represented by mussel faeces and pseudofaeces, similarly to other invertebrates (Stewart *et al*., 1998; Roditi, Strayer, & Findlay, 1997). Alternatively, *Dreissena* are very efficient filter feeders (Kryger & Riisgard, 1988) and may increase the flow of well-oxygenated water above the clusters. Therefore, although water within and below *Dreissena* clusters has been shown to have lower dissolved oxygen and water quality (Burks *et al*., 2002; Beekey *et al*., 2004b), water just above the cluster may still be well oxygenated, and *Dreissena* may direct highly oxygenated microcurrents into their burrows.

While removing mayflies from the habitat choice experiments, we observed that some mayflies occupied empty *Dreissena* shells or space just below a live *Dreissena* and were not actually burrowing into the sediment. Furthermore, mayflies maintained in the laboratory sought shelter near *Dreissena* shells and dug very shallow burrows. Bioturbation is vital to benthic community structure because it influences sediment properties (Levinton, 1995; Solan *et al*., 2004), nutrient and contaminant fluxes at the sediment-water interface (Matisoff & Wang, 1998; Bartsch *et al*., 1999; Chaffin & Kane, 2010), and may influence species richness and diversity (Widdicombe *et al*., 2000). Therefore, the effect of *Dreissena* clusters on soft sediment may not only alter infaunal invertebrate community density and diversity, but more surprising, may change the behaviour of native ecosystem engineers, resulting in changes in bioturbation activity and in the ability of fish to detect *Hexagenia* as prey.

2.5.2 Fish foraging experiments

Increased habitat complexity may not have been the only reason *Hexagenia* chose *Dreissena* clusters, but in high turbidity habitats, *Dreissena* on soft sediment do afford *Hexagenia* some protection from predators. In treatments with *Dreissena* present, decreased water clarity affected yellow perch and round goby consumption of *Hexagenia* equally when at a density of 200 m^{-2}. Light conditions, turbidity and structural complexity can have large impacts on the foraging of visually oriented fish (eg. Diehl, 1988; Miner & Stein, 1993; Utne-Palm, 2002). There are other potential effects of increased turbidity, such as clogging gills and interfering with respiration, but the visual affects of turbidity have been shown to be most important (Wellington *et al*. 2010). Turbid conditions are common in lakes where *Hexagenia* and *Dreissena* co-occur. One example is the western basin of Lake Erie, where bottom light measurements vary widely based on season and weather, but are frequently near zero, resulting in the photic zone not reaching maximum depth, even in very shallow areas (T. Bridgeman, unpublished data). Moreover, *Hexagenia* can create turbid plumes at the sediment-water interface through their bioturbating activity. A common density of 400 *Hexagenia*/m^2 has been found to resuspend sediment at a rate of 12 $g/m^2/h$ (Bachteram *et al*., 2005), which is more than can be filtered out by dense clusters of *Dreissena* (Bachteram *et al*., 2005). Therefore fish may not only be experiencing low light, but spikes of turbidity near the sediment-water interface, making finding prey more difficult.

Contrary to our original hypothesis, yellow perch and round gobies showed similar reduction in consumption of *Hexagenia* when water clarity was low. Round gobies have a good lateral line system and feed efficiently in low or no light (Dubs & Corkum, 1996). However, the lateral line may not be effective when benthic prey are found in structurally complex habitat. As a result, round gobies may rely more on visual foraging when *Dreissena* are present and may explain why like yellow perch, they consumed lower numbers of *Hexagenia* with low water clarity and *Dreissena* covered sediment. Therefore, even if fish are able to feed in very low light conditions, the added structure and filter-feeding activities of *Dreissena* may impede these abilities.

We found that *Dreissena* presence did not reduce fish prey consumption when the mussel's filtration was allowed to increase water clarity. Similarly, several species of fish (Beekey *et al*. 2004a) and yellow perch (Cobb & Watzin 2002) did not show reduced consumption of non-burrowing benthic prey with patchy coverage of zebra mussels on sandy substrate where water clarity was likely high. Zebra mussel clusters on hard substrates have been shown to decrease fish consumption of benthic prey even with high water clarity (Gonzalez & Downing, 1999; Mayer *et al*., 2001; Dieterich, Mörtl, & Eckmann, 2004). However, in the complex lake habitat, increased water clarity partially compensates for the negative effect of increased structure on yellow perch prey consumption (Mayer *et al*., 2001). *Hexagenia* burrowing activity and sediment resuspension prevented the creation of high water clarity + bare sediment treatment, but this situation is unlikely to occur in lakes where *Dreissena* are absent due to *Hexagenia* sediment preference. The net effect of *Dreissena* on trophic transfer of mayflies to fish will also depend on changes in mayfly density in mussel colonized and other habitats. In many instances increased prey density results in increased consumption, but yellow perch show no such increasing relationship for benthic prey in laboratory experiments or long-term data from Oneida Lake (Mayer *et al*., 2001). Consequently, the possible reduction of mayfly-derived energy available to fish associated with *Dreissena* will likely depend on the level of water clearing near the sediment water interface. In locations where sediment resuspension or thick algal blooms are prominent, the effect is likely to be more severe.

In conclusion, the current range expansion of dreissenid mussels onto soft sediments and consequent ecosystem engineering effects resulting in changes to available habitat can be expected to alter the already vulnerable benthos of temperate lakes. The observed habitat selection by burrowing mayflies for *Dreissena* clusters may impact their spatial distribution and possibly also that of other benthos with potential cascading effects to higher trophic levels and overall ecosystem functioning. We have shown that *Dreissena* clusters on soft-sediment have similar effects on *Hexagenia* susceptibility to predation by yellow perch and round goby; *Dreissena* presence only decreased consumption in high turbidity/low light conditions. Given the water clarity of areas such as the western basin of Lake Erie, *Hexagenia* may not be as readily exploited by fish as a food source. Also, *Hexagenia* densities under *Dreissena* clusters may increase due to their habitat preference, thereby making them more available to fish. However, in western Lake Erie, *Dreissena* and *Hexagenia* densities are not positively correlated (D. Schloesser, unpublished data). Research should continue to look at the relationship between *Hexagenia* and *Dreissena* in lake systems, to see if selection for *Dreissena* habitat is affecting the distribution and abundance of this dominant benthic organism.

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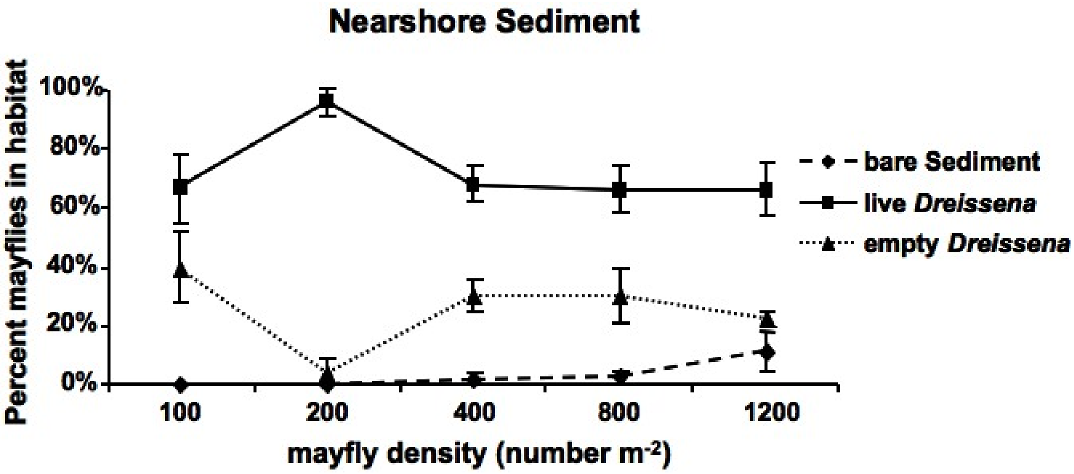


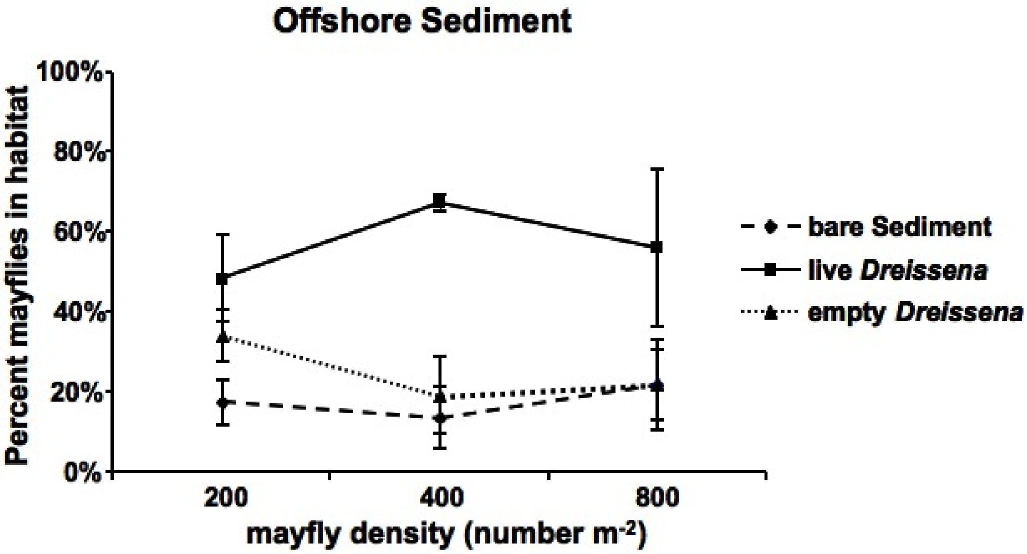


Figure 2-1: Mean (± 1 standard error) percent of total number of burrowing mayflies found in each habitat type (bare sediment, live mussel clusters and empty mussel clusters) in laboratory habitat preference experiments. Experiments were conducted on two different sediment types, nearshore (coarse) and offshore (fine), across a range of burrowing mayfly densities.

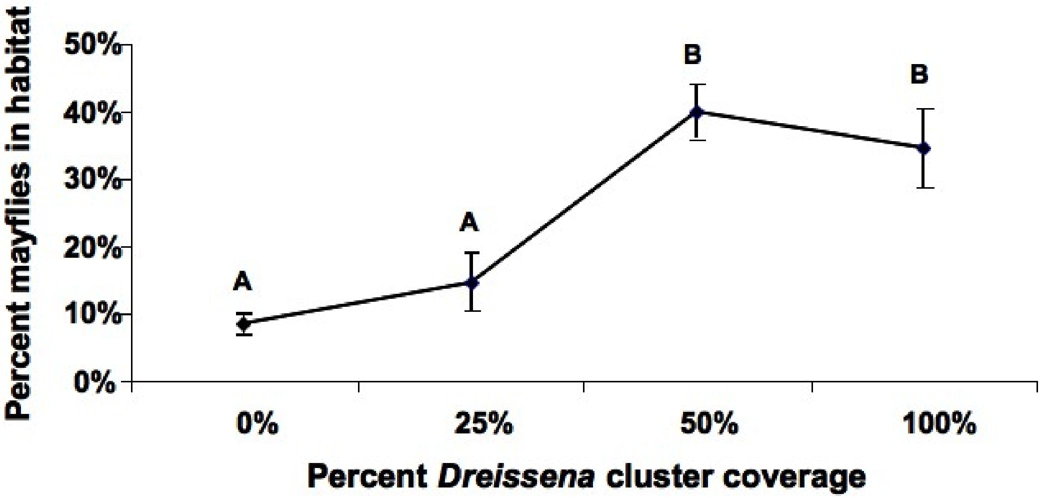


Figure 2-2: Mean (± 1 standard error) percent of total number of burrowing mayflies found in each habitat, each with varying percentages of *Dreissena* spatial coverage, in laboratory habitat preference experiments. Letters represent statistically significant differences based on the Tukey multiple comparison test (α=0.05).

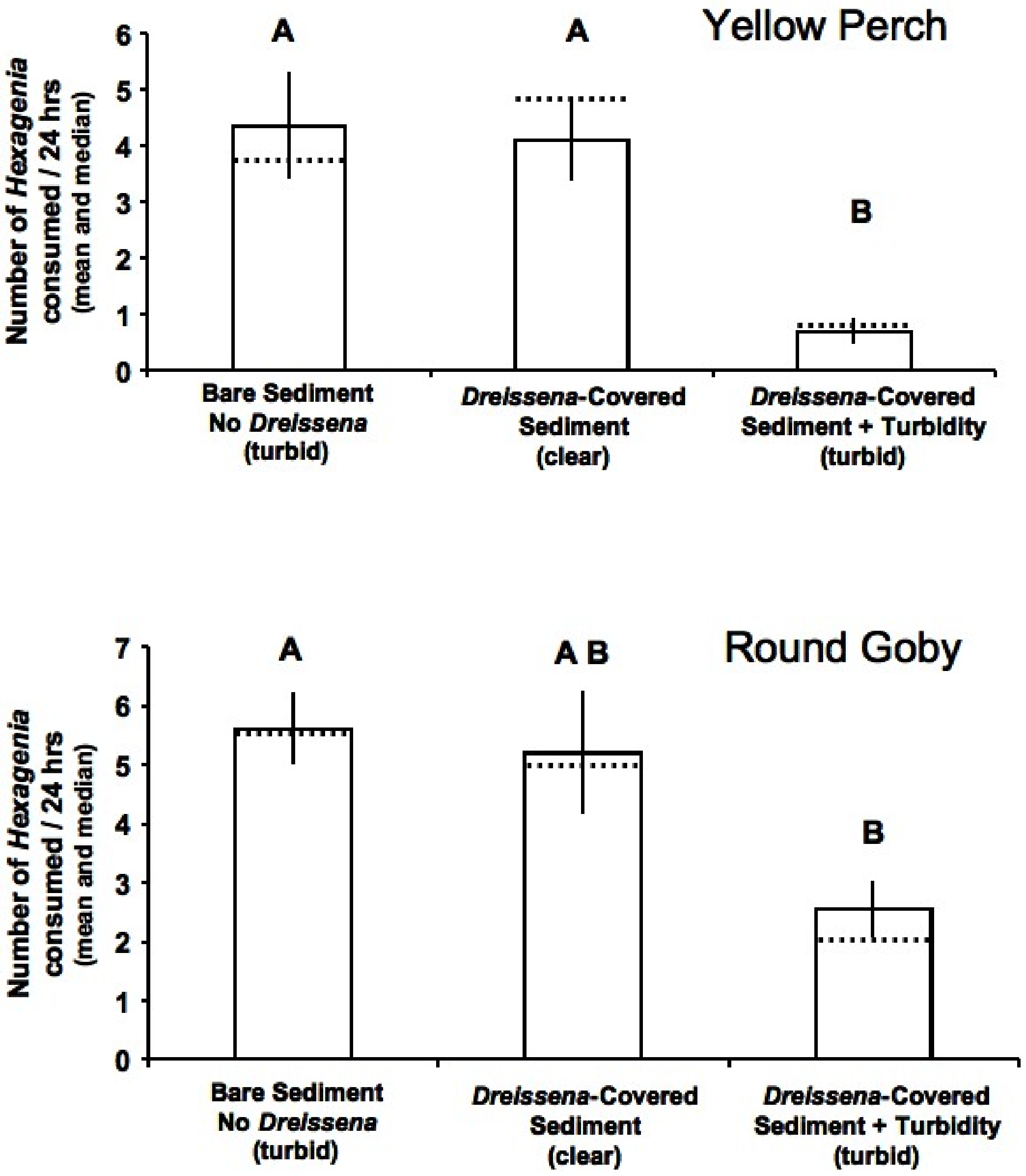


Figure 2-3: Number of *Hexagenia* consumed by yellow perch and round gobies in a 24-hour period for three different habitat treatments. Vertical lines represent ± 1 standard error and dashed lines represent median values. Letters represent statistically significant differences based on the Nemenyi's non-parametric multiple comparison test (α=0.05).

Chapter 3

Effects of an Invasive Ecosystem Engineer on Native Burrowing Mayfly Behavior during Changing Abiotic and Biotic Conditions

3.1 Abstract

The habitat modifications created by invasive ecosystem engineers may affect the behavior of native organisms because the relative risks and benefits of a particular location may be altered by the changing habitat conditions. In this study I examined the effects of an invasive ecosystem engineer, *Dreissena* sp., which changes available soft sediment to a hard, structurally-complex habitat, on the behavior of a native burrowing mayfly that is also an ecosystem engineer, *Hexagenia* sp. in the presence of changing biotic (predator presence) and abiotic (oxygen concentration) conditions. To closely examine how *Dreissena* presence affects *Hexagenia* habitat choice and behavior in the presence of a predation threat and low oxygen concentrations, thin, clear viewing chambers were constructed and *Hexagenia* were observed at high and low oxygen concentrations, with a fish predator either present or absent, in three habitat types: 1) bare sediment, 2) live dreissenid mussels (representing added structure and lower oxygen beneath the cluster), and 3) artificial dreissenid clusters (representing added structure only). *Hexagenia* preferred habitat altered by invasive *Dreissena* due to increased habitat

complexity, and this selection was stronger when a predation threat was present, but was not altered by decreasing oxygen conditions. *Hexagenia* came out of their burrows more often when in habitat with added structure, but remained within the *Dreissena* cluster and therefore were less vulnerable to predation. The ecosystem engineering effects of *Dreissena* may be creating a habitat for native *Hexagenia* that is energetically more profitable than bare sediment, and selection for this habitat may alter the trade-off between low oxygen and predation risk, since *Hexagenia* in *Dreissena* clusters can leave the anoxic sediment but stay sheltered in the dreissenid cluster decreasing their predation risk.

3.2 Introduction

The physical habitat alterations created by invasive ecosystem engineers are likely to have cascading effects to native organisms (Jones et al. 1994 and 1997; Crooks 2002). Many invasive species are known to have dramatic effects on native communities; however, when the invader is also an ecosystem engineers these consequences can be intensified. Not only does the native community now have to cope with possible increased predation and/or competition, but also changes in the physical surroundings of the habitat (Vitousek 1990; Crooks 2002; Cuddington and Hastings 2004). For example, invasive North American beavers (*Castor canadensis*) decreased tree canopy cover and seedling abundance and composition in sub-Antarctic landscapes in southern South America (Anderson et al. 2006) and invasive earthworms reduced availability of nutrients, plant richness, and plant abundance in northern hardwood forests (Hale 2005 and 2006). The habitat modifications created by invasive ecosystem engineers may not only alter habitat for native species, but also change the behavior of native organisms that are also ecosystem engineers. Interestingly, Gribben et al. (2009) showed that an invasive alga indirectly increased community diversity by altering the ecosystem engineering behavior of a native clam. In this study we examine the effects of an invasive ecosystem engineer on the behavior of a native ecosystem engineer, a burrowing mayfly, in the presence of changing biotic (predator presence) and abiotic (oxygen concentration) conditions.

Periodic disturbances, leading to unfavorable, changing conditions, are situations in which an organism's behavior may vary due to a trade-off in the relative risks and benefits associated with the changing environmental circumstances. Many ecological studies have focused on the trade-offs between foraging rate and increased predation risk (e.g., Dill and Fraser 1984; Lima et al. 1985; Werner and Hall 1988). In this study, I am interested in examining behavioral trade-offs that occur during disturbances to a system, both biotic and abiotic. Organisms can remain in a habitat that is profitable in some way, *e.g.* low risk of predation, during a disturbance, but may die if hazardous conditions persist too long. The alternative is to move to a new location that may expose the organism to unfavorable interspecific interactions (Kolar and Rahel 1993).

Aquatic organisms increasingly face such a trade-off during hypoxic conditions (low oxygen, ≤ 2 mg O_2/l; Diaz 2001). Worldwide the occurrence, strength, and duration of hypoxic events are increasing (Diaz 2001; Baustian and Rabalais 2009; Vanderploeg et al. 2009). Fish leave hypoxic waters limiting their access to preferred prey and temperatures (Roberts et al. 2009), bottom-dwelling copepods migrate towards oxygenated conditions (Tinson and Laybourn-Parry 1985), and zooplankton that normally migrate daily to avoid spatial overlap with fish cease migration leading to increased predation pressure (Vanderploeg et al. 2009). Also, organisms respond differently to hypoxia depending on their mobility, sensitivity to low oxygen, and vulnerability to predation. For example, when fish are absent, benthic invertebrates seek refuge in areas of higher oxygen concentration; however, with fish present, taxa highly vulnerable to predation remain in the hypoxic benthic environment and endure low oxygen for a longer time period (Kolar and Rahel 1993). Therefore, a trade-off occurs between low oxygen concentrations and increased vulnerability to predation, which can affect the distribution, abundance, and availability of prey organisms to predators and this tradeoff may be affected by invasive species that modify habitat.

In many North American systems, *Dreissena* sp. (*Dreissena polymorpha* (zebra mussels) and *D. rostriformis bugensis* (quagga mussels)) are invasive ecosystem engineers (Karatayev et al. 2002) and increase numbers of benthic organisms, due to increased habitat complexity (e.g. Silver Botts et al. 1996; DeVanna et al. 2011; Mayer et al. 2002) and resulting protection from predation (Gonzalez and Downing 1999; Mayer et al. 2001; Beekey et al. 2004a). However, these clusters can also decrease oxygen levels and water quality beneath their clusters (Burks et al. 2002; Beekey et al. 2004b), which may have negative consequences to oxygen sensitive species, such as burrowing mayflies (*Hexagenia limbata* and *H. rigida*). These ecosystem engineers modify habitat in multiple ways (ie. refuge from predation in the same place as low oxygen), and therefore the behavior of native organisms may change because the relative risks and benefits of a particular location have been altered. I examined the effect of *Dreissena* sp. on the behavior and habitat preference of a native ecosystem engineer, *Hexagenia* sp., when in the presence of a fish predator and low oxygen conditions.

Hexagenia are found in many North American lakes and streams, however populations across their range have fluctuated widely, and decreases in *Hexagenia* abundance have been attributed to increased pollution and hypoxia (e.g., Britt 1955; Clady and Hutchinson 1975; Krieger 1996) due to the sensitivity of *Hexagenia* to low oxygen (Winter et al. 1996). *Hexagenia* dig u-shaped burrows and change the habitat due to their bioturbating activities, meaning they mix the sediment through feeding, respiration, and burrowing activities (Bartsch et al. 1999). *Hexagenia* are also a valuable food source to many important fish species due to their large size in comparison to other benthic prey (Hayward and Margraf 1987; Schaeffer et al. 2000). On soft sediments in normoxic conditions, *Hexagenia* have been shown to select for *Dreissena* colonized habitat, at least partially, because they experience decreased predation by fish when water clarity is low (DeVanna et al. 2011). However, since oxygen concentration beneath *Dreissena* clusters is already low (Beekey et al. 2004a), hypoxic events may force *Hexagenia* to leave preferred habitat beneath dreissenids to seek well-oxygenated waters more often than when they are burrowed in bare sediment. Exiting a burrow or other refuge leaves *Hexagenia* vulnerable to predation, even in low oxygen conditions, since fish have been found to swim into lethally hypoxic waters when food is abundant (Rahel and Nutzman 1994). Therefore, when presented with multiple stresses, *Hexagenia* may remain in low oxygen waters longer when a predator is present, similar to other mayfly species (Kolar and Rahel 1993). Since this species is highly vulnerable to both low oxygen and predation, it is a valuable model organism for the study of behavioral trade-offs.

In order to examine the effects of an invasive ecosystem engineer on the behavior of a native ecosystem engineer, burrowing mayflies, in the presence of changing abiotic and biotic conditions, I conducted small-scale experiments in thin, clear chambers that allowed observations of behavior to address two questions: a) Does *Hexagenia* habitat selection change in the presence of a predator and low oxygen? and b) How does *Hexagenia* habitat choice affect their behavior in the presence of a predator and low oxygen conditions?

3.3 Methods

To closely examine how *Dreissena* presence affects *Hexagenia* habitat choice and behavior in the presence of a predator and low oxygen concentrations, viewing chambers were constructed, and all pairwise comparisons of habitat type were made; meaning each chamber had two of the three habitat types examined: 1) bare sediment, 2) sediment covered with live dreissenid mussels (representing added structure and lower oxygen beneath the cluster), and 3) artificial dreissenid clusters (representing added structure only). I had two levels of fish predation risk (fish present vs. absent); resulting in two treatments replicated 5 times (N=10) for each pair of habitat types; the total number of trials was 30. Each trial ran for 28 hours in a dark, room-temperature laboratory, and each chamber was observed four times, twice in high oxygen conditions and then twice in hypoxic conditions. Oxygen concentration and temperature was measured near the water's surface using a YSI 5000 table-top dissolved oxygen meter during all four observaton periods. Five viewing chambers were run simultaneously and treatments were randomly assigned to chambers.

The viewing chambers (25.4cm x 1.90cm x 25.4cm) were constructed of acrylic sheets and filled with 6 cm of nearshore (41.6885 W, 83.4250 N) Lake Erie sediment that was first sieved through 1.0 mm mesh. *Dreissena* and mayflies were collected from western Lake Erie. *Dreissena*, mostly quagga mussels, were collected from soft substrates, and age-one burrowing mayfly nymphs (> 10 mm) were collected to decrease risk of emergence during the experiment. To establish habitat types, a thin metal sheet was placed in the chamber dividing it into two equal sections, and the metal was removed before addition of fish and *Hexagenia*. All chambers were bubbled with forced air to keep oxygen levels high until low oxygen time periods began, as described below. For both artificial and live *Dreissena* cluster habitat types, a density of 3400 *Dreissena*/m^2 was added to half of the viewing chamber, a density commonly seen in western Lake Erie (Patterson et al. 2005). I created artificial *Dreissena* shells by gluing empty, clean shells together with non-toxic glue, and each shell was punctured with a small hole to allow it to sink.

In treatments with fish present I added a single age-1 yellow perch (total length; 10-12 cm) one hour prior to *Hexagenia* addition. All chambers, regardless of fish treatment, had a permeable plastic barrier hung 10 cm from the top of the chamber to allow the fish an area to swim, yet prevent consumption of *Hexagenia*. Yellow perch were not allowed to function as predators due to the size of the chambers. After fish acclimated, six *Hexagenia*, representing a density of 1400/m^2, were released at the center of the chamber and watched carefully so that initial habitat selection could be recorded (initial, high oxygen, time=0). Initial habitat selection was recorded when a mayfly began actively burrowing in a habitat. The viewing chambers were started one at a time and observed for 15 minutes each. Chambers were undisturbed for 24 hours after initial data was recorded to allow *Hexagenia* to acclimate to the surroundings. After the 24 hour acclimation period, each chamber was again observed under high oxygen conditions for 15 minutes (post-acclimation, high oxygen, time = 24hr). To examine *Hexagenia* habitat selection, the number of *Hexagenia* in each habitat type was recorded, although I could not locate some of the *Hexagenia*. I also documented the habitat location and number of *Hexagenia* that were not burrowed in the sediment (i.e. all body parts were out of the sediment). For *Hexagenia* out of their burrow in the live or artifical *Dreissena* habitat, their location relative to the cluster was also examined. *Hexagenia* were recorded as either below the cluster (between the soft sediment and bottom of cluster), within the cluster, or above the cluster (fully exposed).

After all chambers were observed post-acclimation, fish were removed, the oxygen level in each chamber was lowered, and chambers were observed one at a time for 15 minutes immediately following hypoxic condition (hypoxia onset, low oxygen, time = 25hr). Oxygen was lowered by removing the forced air and bubbling nitrogen gas until oxygen levels reached between 10% and 15% saturation. The chambers were then covered with clear plastic film to prevent oxygen exchange from the atmosphere to the chambers. The chambers stayed covered, keeping oxygen levels low for 3 hours after hypoxia onset, afterwhich all chambers were observed again, individually, for 15 minutes (3 hours of hypoxia, low oxygen, time = 28hr). Again, for both time periods, I measured the number of *Hexagenia* in each habitat type, habitat location and number of *Hexagenia* that were not burrowed in the sediment, and for *Hexagenia* out of their burrow in the live or artifical *Dreissena* habitat, their location relative to the cluster was also recorded (below, in, or above the cluster).

All data were analyzed using a 2 x 3 repeated-measures split-plot ANOVA model using SAS 9.1, with 2 levels of predator (absent and present), 3 habitat types (bare, artificial *Dreissena*, and live *Dreissena*), across time periods. Habitat selection was analyzed across all four time periods (initial, post-acclimation, hypoxia onset, and 3 hours post-hypoxia), while the number and location of *Hexagenia* out of burrows were analyzed for the final three time periods (post-acclimation, hypoxia onset, and 3 hours post-hypoxia) because all *Hexagenia* were initially not burrowed. A split-plot model was used because each experimental unit was split into two different habitat types and treatments were applied to different scales (Potvin 2001). The presence of a predator was applied to the whole chamber (main plot factor), whereas each habitat type was applied to only one-half of the chamber (subplot factor). When appropriate, ANOVA's were followed by a Tukey multiple comparison test. The proportion of *Hexagenia* located in each habitat for all four time periods was calculated as the number of *Hexagenia* recorded in each habitat type divided by 6, the total number of *Hexagenia* added to the chamber. I also recorded the number of *Hexagenia* that I could not find during each time period. Data on the number and location of exposed mayflies was analyzed as a proportion of *Hexagenia* known to be in each habitat during that time period; *Hexagenia* in unknown locations were excluded from this calculation. All proportion data were arcsine square root transformed to help achieve a normal distribution (Zar 1999).

3.4 Results

Hexagenia were found to select for structured habitat over bare sediment, and *Hexagenia* selected equally for live and artificial *Dreissena* habitats (Table 3.1a, Figure 3-1). Initial habitat selection was recorded for all *Hexagenia* because they were individually added to the chambers; however, during the post-acclimation and hypoxia onset time periods many *Hexagenia* could not be found and were either burrowed or not visible within a *Dreissena* cluster (Figure 3-1). During the final time period, 3 hours of hypoxia, many *Hexagenia* exited their burrows and could be observed and their location documented (Figure 3-1). As a result of my inability to see some mayflies when they were burrowed, there was a significant effect of time period on habitat selection (Table 3.1a), with more *Hexagenia* observed initially than all other time periods, and more *Hexagenia* observed during the final time period, 3 hours post hypoxia, than the middle two periods, post-acclimation and hypoxia onset (Figure 3-1). There was no main effect of predator presence, but there was a significant predator by habitat interaction (Table 3.1), suggesting that the proportion of *Hexagenia* selecting each habitat type changes with the presence of a predator; more *Hexagenia* were in the live *Dreissena* cluster habitat when a predator was present (Figure 3-1b).

The number of *Hexagenia* located above the sediment was significantly different between the habitat types and across time periods (Table 3.1b). The presence of a predator had no affect on the number of *Hexagenia* out of their burrows (Table 3.1b). More *Hexagenia* were out of their burrows in the structured habitat than on bare sediment, but there was no difference between live and artificial clusters (Figure 3-2). The duration of hypoxia also affected the number of *Hexagenia* out of their burrows;

more *Hexagenia* were fully out of the sediment 3 hours after hypoxia than during the high-oxygen post-acclimation and hypoxia onset time periods (Figure 3-2). Although more *Hexagenia* were out of the sediment in the structured habitat, many were still not fully exposed because they were below or within the structure of the cluster. Unlike *Hexagenia* out of their burrow on bare sediment, which are fully exposed, *Hexagenia* can be out of the sediment but still sheltered when in a *Dreissena*-covered habitat. The proportion of *Hexagenia* above the cluster and fully exposed to predation increased with duration of hypoxia, more *Hexagenia* were fully exposed after 3 hours of hypoxia than the other time periods (Table 3.1c, Figure 3-3). There was also a significant predator by time interaction, meaning that the effect of the predator differs through time, and I observed a sharp increase in the proportion of *Hexagenia* fully exposed 3 hours after hypoxia when a predator was present (Table 3.1c, Figure 3-3).

3.5 Discussion

The habitat preference of *Hexagenia* did not vary due to the trade-off in relative risks and benefits associated with the changing abiotic conditions. *Hexagenia* selected for habitats with added structure created by *Dreissena* shells equally, both live and artificial clusters, over bare sediment (Figure 3-1). Studies of some invertebrates living in hard substrate dreissenid clusters have also shown no preference between live and artificial clusters (Silver Botts et al. 1996; Gonzalez and Downing 1999), suggesting that increased habitat complexity is driving the observed relationship. However, *Hexagenia* in larger scale experiments have been shown to prefer live *Dreissena* clusters to artificial clusters (DeVanna et al. 2011). Therefore, differences between live and artificial dreissenid clusters, such as additional food resources (Roditi et al. 1997) and increased flow of pelagic water above the cluster, may not play a role in habitat selection at this smaller scale. Contrary to my expectations, changing oxygen concentration during the study did not impact *Hexagenia* habitat preference. Therefore, although oxygen concentrations have been shown to already be low beneath *Dreissena* clusters (Burks et al. 2002; Beekey et al. 2004b), this did not affect *Hexagenia*'s habitat preference. The evaluation of the trade-offs between inhabiting sediment beneath *Dreissena* clusters and low oxygen concentrations, suggests that the benefits received from *Dreissena*-covered sediment outweigh the possible negative effects from more decreased oxygen concentrations in areas of live *Dreissena* clusters.

Similar to other invertebrates vulnerable to predation, *Hexagenia* may be selecting for habitat with increased complexity as a refuge from predators, since fish consumption of invertebrates, including *Hexagenia*, has been shown to decrease when in *Dreissena* cluster habitat (Gonzalez and Downing 1999; Mayer et al. 2001; DeVanna et al. 2011). *Hexagenia* selected for complex habitat with a lower risk of predation even when a fish predator was not present. This is different from studies on the trade-off between foraging and predation risk, where organisms have been shown to spend more time in a refuge when predators are present (Fraser and Huntingford 1986), and switch from feeding in areas high in food resources to less optimal habitats only when a predation threat is present (Caldwell 1986; Ferguson et al. 1988; Lima and Dill 1990). My results suggest that this avoidance behavior, selecting for structured habitat, has low or no costs to *Hexagenia* feeding and development (Feltmate and Williams 1991; Peckarsky et al. 1993; Huryn and Chivers 1999), and even when a predator is not sensed, it is beneficial to select for structured habitat. Also, *Hexagenia* are large in comparison to other benthic prey and easily preyed upon by fish, so selecting a habitat with a lower risk of predation may be beneficial even when a predator is not an immediate threat. However, when fish were present, *Hexagenia* did select for live clusters more often than in the fishless treatment (Table 3.1a; Figure 3-1). Therefore, although at a small scale *Hexagenia* did not prefer live *Dreissena* to artificial clusters, this data still suggests that *Hexagenia* are receiving other benefits, such as added food resources from live *Dreissena* feces and pseudofeces (Roditi et al. 1997).

The increased habitat complexity created by *Dreissena* on soft sediment also alters the behavior of native *Hexagenia*, as *Hexagenia* were found to come out of the sediment and not burrow more often when in the structured habitats (Figure 3-2). Under high oxygen conditions, almost all *Hexagenia* in the bare sediment habitat were burrowed in the sediment; however, in the structured habitat, both artificial and live clusters, many *Hexagenia* were found completely out of the sediment and this behavior was not affected by the presence of a predator (Figure 3-2; Table 3.1). *Hexagenia* in dreissenid clusters may be taking advantage of increased protection from predation, and at the same time receive increased food resources from *Dreissena* feces, detritus, and bacteria in the interstitial spaces of the cluster (Izvekova and Ivova-Katchanova 1972; Roditi et al. 1997; Lohner et al. 2007). But, the number of *Hexagenia* out of their burrow in the live cluster habitat was not greater than in the artificial clusters, so added food resources is not the only explanation for this observed relationship. *Hexagenia* expend energy to burrow and must almost continuously bioirrigate by pumping well-oxygenated water through their burrow to avoid anoxic conditions (Wang et al. 2001; Gallon et al. 2008). *Hexagenia* in

Dreissena clusters do not expend energy by digging or bioirrigating because they are no longer burrowed in anoxic sediments. If water above the *Dreissena* clusters is well oxygenated, their filter-feeding activity will likely maintain high oxygen levels within the clusters. Therefore, in high oxygen conditions, the ecosystem engineering effects of *Dreissena* may be creating a habitat for native *Hexagenia* that is energetically more profitable than bare sediment, which was previously presumed to be the preferred habitat of burrowing mayflies, while decreasing their risk to predation.

During low oxygen conditions, the proportion of mayflies that moved out of their burrow increased in all habitat types with increasing duration of hypoxia (Figure 3-2). Similarly the mayfly *Callibaetis montanus* increases activity when oxygen declines, unlike other invertebrates which all decreased activity during hypoxia in the same study (Kolar and Rahel 1993). Mayflies may increase activity during low oxygen due to the additional risks of remaining in a burrow during such an event, risks that non-burrowing invertebrates do not encounter. In this experiment, *Hexagenia* burrowed in bare sediment endured low oxygen conditions and stayed less active longer than *Hexagenia* in the structured habitat (Figure 3-2) likely because they would be fully exposed to predators. However, the risk of remaining in anoxic sediment includes exposure to released toxic substances such as hydrogen sulfide and ammonium, which are otherwise readily oxidized (Gray et al. 2002). *Hexagenia* are sensitive to these substances (Oseid and Smith 1975; Wang and Chapman 1999), therefore toxic gasses along with low oxygen may create an uninhabitable environment in the sediment for *Hexagenia*. Although *Hexagenia* leaving their burrows makes them very vulnerable to predation when no other structure is available, it may be a less risky option than enduring lethal conditions.

Selection for dreissenid habitat may alter the trade-off between withstanding low oxygen or exiting burrows and increasing predation risk. Even though more *Hexagenia* came out of the sediment in *Dreissena* clusters and they exited their burrows sooner (Figure 3-2), they were in the structured and therefore protective environment of the cluster and likely did not experience high levels of toxins meaning that the cluster habitat was likely more advantageous in terms of both physiology and avoiding predation.

The presence of a predator did not affect the proportion of *Hexagenia* exiting their burrows unlike other mayfly species that decrease activity and reduce exits from benthic refugia during hypoxia when a fish is present (Rahel and Kolar 1990; Kolar and Rahel 1993). Three hours after hypoxia, more *Hexagenia* were above the *Dreissena* cluster, and therefore fully exposed to predation when the predation threat was present (Figure 3-3). This was contrary to my expectation that *Hexagenia* would stay more protected if a predator was present. I do not have data between initial hypoxia and 3 hours of hypoxia, and during that time *Hexagenia* may have stayed more protected in or below the cluster and withstood lower oxygen conditions when the predator was present, causing them to seek higher oxygen concentrations to decrease their risk of direct mortality from hypoxia. Also, when subjected to multiple stressors, such as low oxygen and predator presence, the stress of these factors can be added or multiplied resulting in increased consequences that are greater than the individual stressors alone (Folt et al. 1999). Therefore, when exposed to multiple stressors, both low oxygen and a predation threat, *Hexagenia* may experience increased stress levels than treatments with only low oxygen. This could lead to increased respiration by *Hexagenia* forcing them to come above the clusters sooner to seek high oxygen with a predator was present. Overall, *Hexagenia* may be so sensitive to low oxygen concentrations that it is less risky to increase their susceptibility to predation, because the probability of mortality could be lower than withstanding long periods of low oxygen.

In conclusion, *Hexagenia* continue to prefer habitat altered by invasive *Dreissena* due to increased habitat complexity. Although this added structure completely changes the bottom habitat from soft-sediment to hard clusters, *Hexagenia* may benefit by choosing this habitat during changing biotic and abiotic conditions. The ecosystem engineering alterations by *Dreissena* have been shown to affect the trade-off between the amount of time *Hexagenia* stay burrowed and withstand low oxygen, or leave their burrow and increase their vulnerability to predation. *Hexagenia* selection for *Dreissena* habitat may be energetically more profitable, and allow *Hexagenia* to come out of the sediment during low oxygen conditions, while staying in a refuge from predators (Mayer et al. 2001; Beekey et al. 2004; DeVanna et al. 2011). Therefore, invasive *Dreissena* on soft sediment are not only affecting *Hexagenia* habitat preference, but also their behavior and the risk and benefits of changing environmental conditions.

Table 3.1: Repeated measures, split-plot analysis of varience table with 2 levels of predator (absent and present; applied to the whole chamber), 3 habitat types (bare, artificial *Dreissena*, and live *Dreissena*; applied to half of the chamber), across four time periods differing in oxygen concentrations. Response variables were: a) the proportion of *Hexagenia* in each habitat type, b) the proportion of *Hexagenia* in each habitat type out of their burrows, and 3) the proportion of *Hexagenia* in the structured habitats that are fully exposed above the cluster.

A. Habitat Selection

Effect	Numerator df	Denominator df	F-value	P-value
predator	1	54	0.23	0.6317
time period	**3**	**168**	**52.67**	**<0.0001**
habitat	**2**	**54**	**19.33**	**<0.0001**
habitat*predator	**2**	**54**	**3.26**	**0.0459**
habitat*time period	6	168	1.06	0.3864
predator*time period	3	168	0.73	0.5349

B. Out of Burrow

Effect	Numerator df	Denominator df	F-value	P-value
predator	1	54	0.62	0.4352
time period	**2**	**111**	**25.12**	**<0.0001**
habitat	**2**	**54**	**8.09**	**0.0008**
habitat*predator	2	54	1.55	0.2225
habitat*time period	4	111	0.87	0.4844
predator*time period	2	111	0.20	0.8157

C. Full Exposure

Effect	Numerator df	Denominator df	F-value	P-value
predator	1	36	3.04	0.0899
time period	**2**	**74**	**18.38**	**<0.0001**
habitat	2	36	0.07	0.7983
habitat*predator	2	36	0.25	0.6222
habitat*time period	4	74	0.51	0.6028
predator*time period	**2**	**74**	**5.03**	**0.0089**

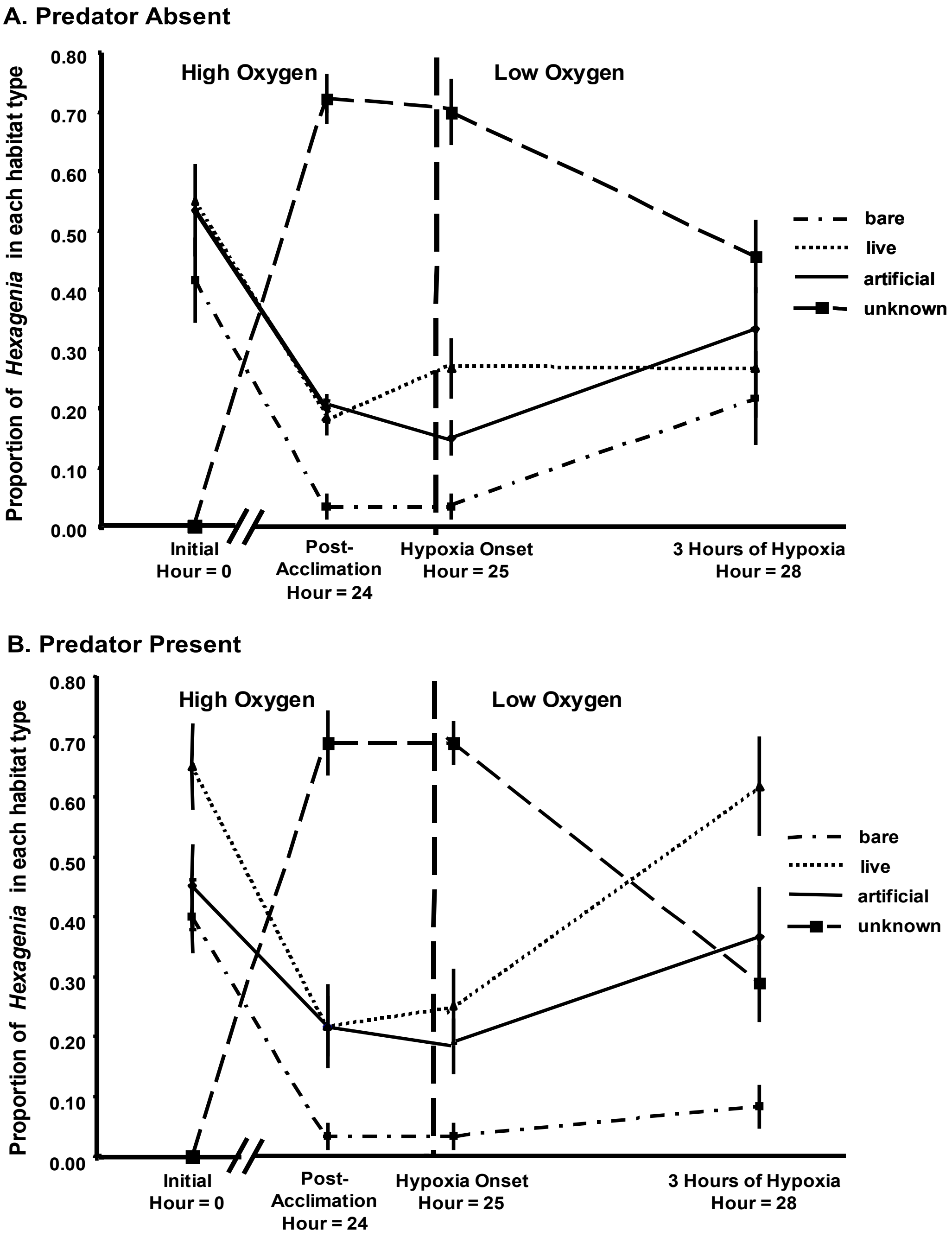
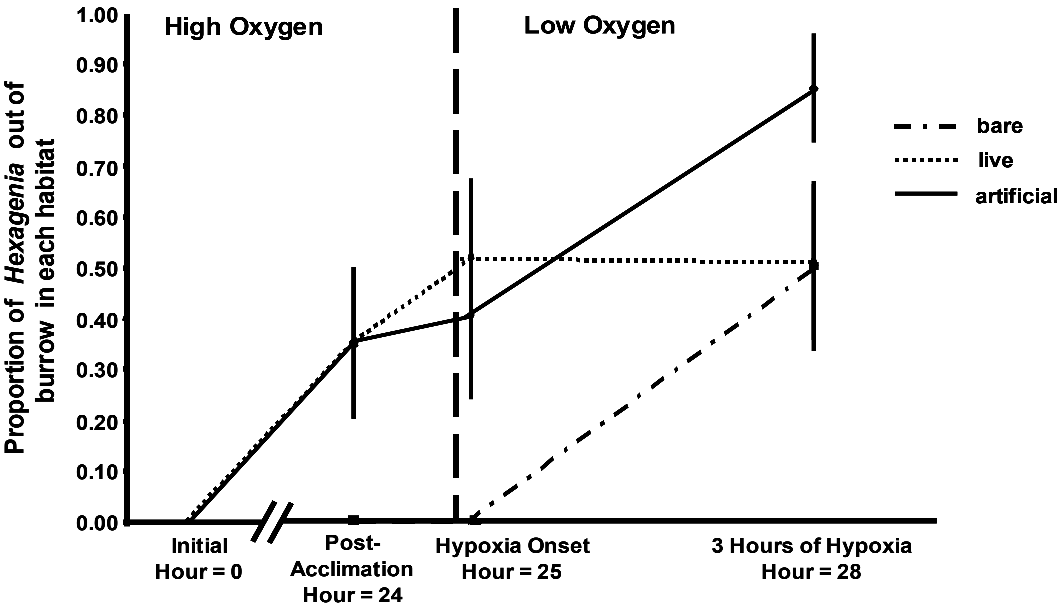


Figure 3-1: Mean proportion (± 1 standard error) of the total number of *Hexagenia* found in each habitat type (bare sediment, live mussel clusters and artificial mussel clusters) across four time periods differing in oxygen concentration: 1) initial, hour = 0, 2) post acclimation, hour =24, 3) hypoxia onset, hour = 25, and 4) 3 hours of hypoxia, hour = 28. The mean proportion of *Hexagenia* not found during each time period was also graphed.

A. Predator Absent



B. Predator Present

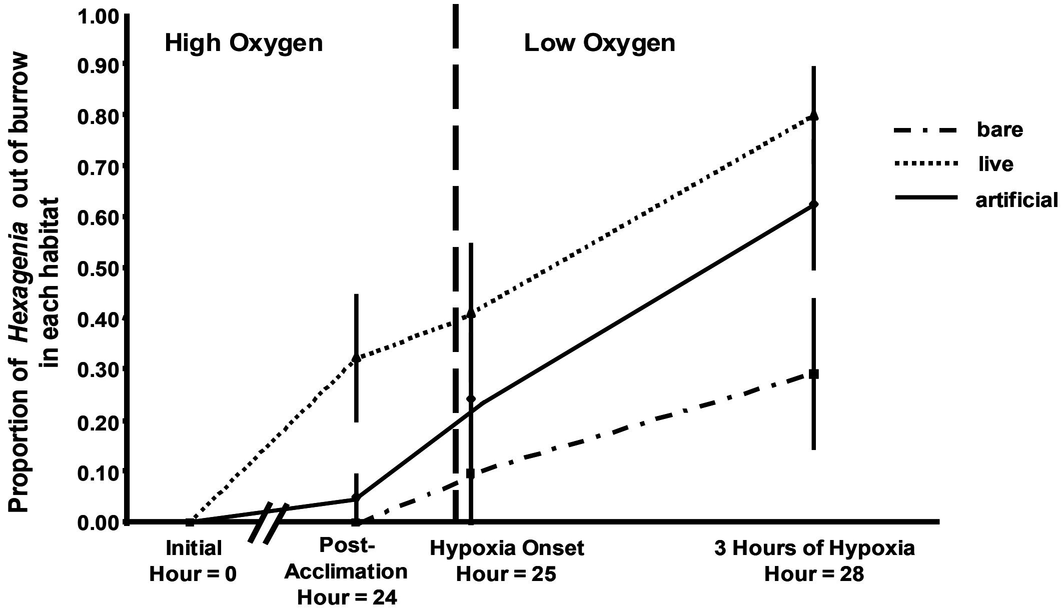


Figure 3-2: Mean proportion (± 1 standard error) of the number of *Hexagenia* in each habitat type completely out of their burrow across the four time periods differing in oxygen concentration: 1) initial, hour = 0, 2) post-acclimation, hour =24, 3) hypoxia onset, hour = 25, and 4) 3 hours of hypoxia, hour = 28.

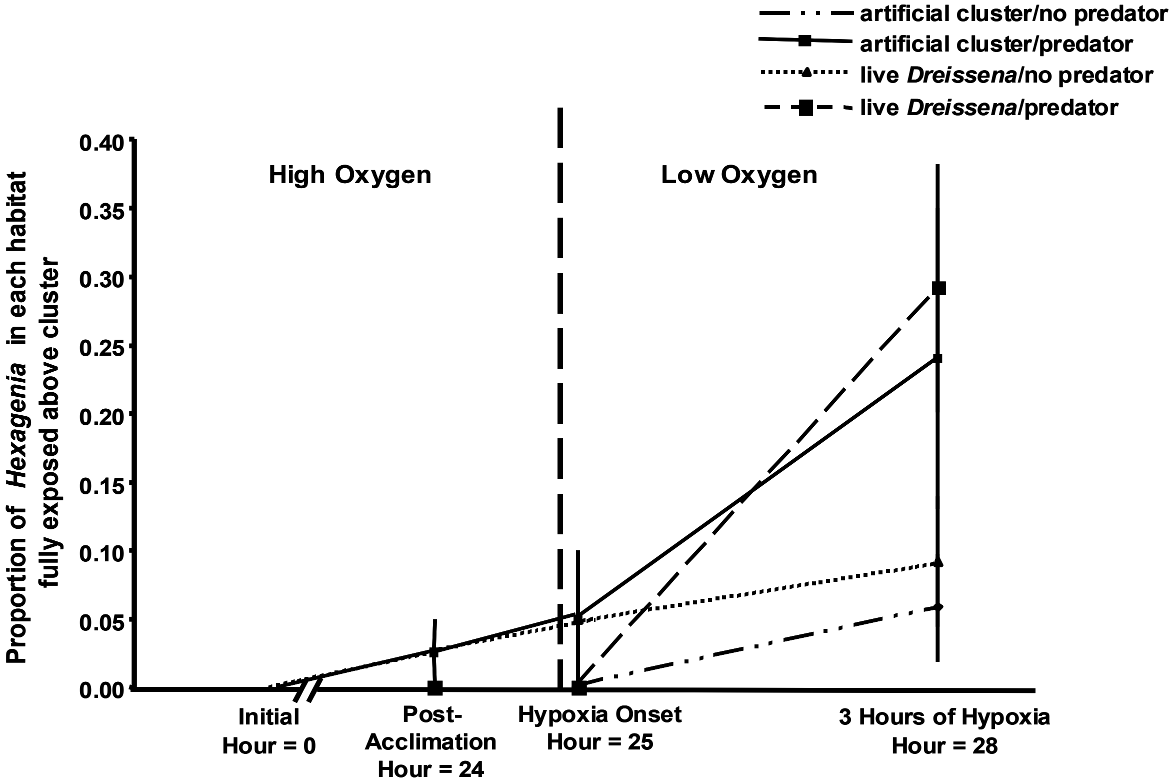


Figure 3-3: Mean proportion (± 1 standard error) of the number of *Hexagenia* in the structured habitat, live and artificial *Dreissena* clusters, completely above the clustered habitat across the four time periods differing in oxygen concentration: 1) initial, hour = 0, 2) post-acclimation, hour =24, 3) hypoxia onset, hour = 25, and 4) 3 hours of hypoxia, hour = 28.

Chapter 4

Scale-Dependent Interactions between Soft-Sediment *Dreissena* Clusters and Native Burrowing Mayflies in Western Lake Erie: Effects of an Invasive Ecosystem Engineer

DeVanna, K.M., D.W. Schloesser, J.M. Bossenbroek, and C.M. Mayer. Scale-dependent effects of soft-sediment *Dreissena* clusters on *Hexagenia* in western Lake Erie (in revision for 2nd edition of Zebra Mussels: Biology, Impacts and Control. Edited by T.F. Nalepa and D.W. Schloesser)

4.1 Abstract

An invasive ecosystem engineer, *Dreissena rostriformis bugensis* (the quagga mussel), is changing the bottom habitat of lakes by covering soft sediment with hard clusters. This shift in available habitat type is likely to affect infaunal invertebrates, such as burrowing mayflies (*Hexagenia* spp.). I examined the effects of soft-sediment dreissenid clusters on *Hexagenia* in both small-scale habitat choice experiments and large-scale spatial analyses across western Lake Erie. I have found that when given a choice of habitat types: live *Dreissena*, artificial *Dreissena*, or bare sediment, *Hexagenia* strongly select for live dreissenid cluster habitat. In viewing chamber microcosms,

Hexagenia also selected for live *Dreissena* covered sediment, and this selection is stronger in the presence of a fish predator. At the western basin-wide scale, the presence of *Dreissena* does not inhibit *Hexagenia* presence and *Hexagenia* are more likely to be present where *Dreissena* are also present, but there is no spatial cross-correlation between the densities of the two species. *Hexagenia* at sites without *Dreissena* can achieve very high densities, but are highly variable, whereas at sites with *Dreissena*, *Hexagenia* are present at a lower mean density, but are less variable. These findings show that at a small spatial scale *Hexagenia* prefer *Dreissena*-covered sediment, but at a large-scale are not selecting for or avoiding *Dreissena* on soft sediment, suggesting that other mechanisms are determining *Hexagenia* distribution in western Lake Erie.

4.2 Introduction

Dreissena polymorpha (zebra mussels) and *D. rostriformis bugensis* (quagga mussels) have changed the Great Lakes ecosystem in many ways as a result of their effects as ecosystem engineers (Karatayev et al. 2002; Zhu et al. 2006). Ecosystem engineers are organisms that alter the availability of resources to other species by creating physical state changes in biotic or abiotic resources (Jones et al. 1994 and 1997). *D. polymorpha* were first to be introduced to the Great Lakes and colonized primarily hard substrates where they increased invertebrate densities by importing food to the bottom (eg., Silver Botts et al. 1996; Stewart et al. 1998; Gonzalez and Downing 1999) and increasing structural complexity, leading to decreased fish predation on invertebrates within druses (Gonzalez and Downing 1999; Mayer et al. 2001; Beekey et al. 2004a). However, quagga mussels, first recorded in North American in 1989 in Lake Erie (Mills et al. 1993), were initially found in deeper, cooler waters, are replacing zebra mussels (Mills et al. 1999), and have become the dominant dreissenid species in most areas of the Great Lakes (Stoeckmann 2003). Quagga mussels are capable of inhabiting soft substrates where their colonies fundamentally shift habitat type because they cap sediment and create a hard, structurally complex substrate, which will likely influence the infaunal (sediment-dwelling) benthic invertebrate community (Dermott and Kerec 1997; Bially and MacIsaac 2000; Freeman et al. 2011). The effects of quagga mussels on native benthic invertebrates are likely to differ from those of zebra mussels because quagga mussels tend to be found on soft sediment and will therefore interact with different guilds of native organisms. In this study, I focused on the effects of soft-sediment *Dreissena* clusters on *Hexagenia* spp. (*H. limbata* and *H. rigida*), a native infaunal mayfly species important to fish and ecosystem function. My objective was to examine the spatial association on soft-sediment between invasive *Dreissena* and native *Hexagenia* at differing spatial scales.

Hexagenia were formerly abundant in the areas of the Great Lakes and other inland lakes, but populations declined to near extirpation in the 1950's (e.g., Nebeker 1972; Winter et al. 1996; Gerlofsma and Ciborowski 1998). Increases in abundance and recolonization in western Lake Erie have been documented since the early 1990s (Krieger et al. 1996; Schloesser et al. 2000; Schloesser and Nalepa 2001) and many have been optimistic because *Hexagenia* are a valuable food source to many economically important fish species in Lake Erie (Hayward and Margraf 1987; Schaeffer et al. 2000). *Hexagenia* have been shown to prefer soft sediment colonized by *Dreissena* in small-scale laboratory experiments (DeVanna et al. 2011) similar to the way that epifaunal invertebrates respond to *Dreissena* colonies on hard substrate. However, *Hexagenia* has additionally been shown to select for live *Dreissena* clusters over artificial ones, suggesting that structure and resulting protection from predation, is not the only reason *Hexagenia* select this habitat (DeVanna et al. 2011). Burrowing animals are already protected from predation and therefore may respond differently to the threat of predation than invertebrates living on the sediment surface. Although much is known about the effects of hard substrate clusters on epifaunal invertebrates, burrowing invertebrates may respond very differently to soft-sediment *Dreissena* clusters due to their presence beneath the sediment.

Factors influencing a species' spatial distribution occur at multiple spatial scales; biotic or abiotic processes that are important at one scale are not always predictive at a different scale (Turner et al. 1989; Wiens 1989). For example, Least Flycatchers (*Empidonax minimus*) negatively influence the distribution of American Redstart (*Setophaga ruticilla*) at a small spatial scale, but are positively associated at a larger regional scale (Wiens 1989). Similarly, Graf et al. (2005) found that environmental variables that significantly explained capercaillie (*Tetrao urogallus*) distribution at a small scale differed from significant variables at a larger scale. *Dreissena* affect *Hexagenia* at a small spatial scale in a variety of way including: 1) modifying their habitat by the addition of shells to soft sediment 2) refuge from predation, 3) the addition of food resources by means of *Dreissena* feces and pseudofeces, and 4) the increased flow of well-oxygenated pelagic water to areas close to the cluster via *Dreissena* filter feeding. Whereas at a larger scale, physical processes, such as sediment type, water movement, and oxygen availability may be more important in structuring both *Dreissena* and *Hexagenia* distributions. Thus the spatial association between *Hexagenia* and *Dreissena* may differ depending on what scale they are observed, as the choice of scale can directly affect the outcome of the study and comparability between studies (Rahbek 2005). Quantifying the spatial relationship between these two important species at multiple scales may help in understanding what mechanisms are structuring their distributions.

I first examined *Hexagenia* habitat preference with respect to *Dreissena* presence on soft sediments at a small scale. I conducted two separate laboratory habitat preference experiments: 1.) *Viewing chamber experiments* - examined the effect of a predator on the habitat preference of *Hexagenia* 2.) *Habitat type selection* - tested whether *Hexagenia* select for *Dreissena*-colonized habitat, artificial *Dreissena* clusters, or bare sediment. Secondly, in order to assess this relationship at multiple scales, I conducted large scale spatial analyses of *Dreissena* and *Hexagenia* at 30 sites sampled over 10 years in the western basin of Lake Erie. I examined both the co-occurrence and density relationship of *Dreissena* and *Hexagenia*, as well as the spatial autocorrelation and cross-correlation of *Dreissena* and *Hexagenia* across western Lake Erie.

4.3 Methods

4.3.1 Experiments

To examine the possible association between *Hexagenia* and *Dreissena*, laboratory experiments were conducted at the University of Toledo's Lake Erie Center at different scales: 1) viewing chamber experiments (a small subset from chapter 3 of this dissertation) and 2) habitat type selection (data from DeVanna et al. 2011 and can also be found in chapter 2 of this dissertation). All experimental units in these experiments were filled with 6 cm of nearshore (41.6885 W, 83.4250 N) Lake Erie sediment that was first sieved through 1.0 mm mesh. *Dreissena* and *Hexagenia* were collected from western Lake Erie. *Dreissena* were collected from soft substrates, and age-one *Hexagenia* nymphs (> 10 mm) were collected to decrease risk of emergence during the experiment.

4.3.1.1 Viewing Chambers - *Hexagenia* burrowing behavior was examined at a very small spatial scale using viewing chambers, these chambers limited the *Hexagenia* to a choice between single patches of two habitat types and allowed us to make some detailed observations of behavior. *Hexagenia* were given a choice of bare sediment and *Dreissena*-cluster covered sediment in the presence (N=5) and absence (N=5) of a predator. The viewing chambers (25.4cm x 1.90cm x 25.4cm; Figure 4-1) were constructed of acrylic sheets and filled with Lake Erie sediment as described above. To establish habitat types (bare sediment and *Dreissena*-covered sediment), a thin metal sheet was placed in the chamber dividing it into two equal sections, which was removed before addition of fish and *Hexagenia*. For the live *Dreissena* cluster habitat, a density of 3400 *Dreissena*/m^2 (Patterson et al. 2005) was added to half of the microcosm.

In treatments with fish present I added a single age-1 yellow perch (*Perca flavescens*) one hour prior to *Hexagenia* addition. All arenas, regardless of fish treatment, had a plastic, permeable barrier hung 10 cm from the top of the chamber to allow the fish an area to swim, yet prevent consumption of *Hexagenia*. Yellow perch were not allowed to function as predators due to the size of the chambers. After fish acclimated, six *Hexagenia*, representing a density of 1400/m^2 (Krieger 1999), were released at the center of the chamber and watched carefully so that initial habitat selection could be recorded. Initial habitat selection was recorded when a mayfly began actively burrowing in a habitat. The arenas were started one at a time and observed for 15 minutes each. Five viewing chambers were run at the same time and treatments were randomly assigned to chambers.

4.3.1.2 - Habitat type selection: I tested whether *Hexagenia* selected for or avoided *Dreissena* clusters on soft sediment. Experimental tanks (circular plastic tubs; 41 cm diameter and 43 cm height) allowed enough room for *Hexagenia* to select among multiple clusters of *Dreissena* and were large relative to the body size of the *Hexagenia*. Tubs were filled with nearshore sediment and were separated into three equal “pie-slice” shaped sections (0.046 m^2) using metal dividers to create three different habitat types: 1) bare sediment, 2) live *Dreissena* clusters, 3) and artificial *Dreissena* clusters. Live and artificial *Dreissena* treatments contained approximately 250 individuals (5434 m^{-2}; Patterson et al. 2005). I created artificial *Dreissena* clusters by gluing empty, clean shells together with non-toxic glue and adding five 1 g lead weights to each cluster. To ensure the weights did not affect the experiment, lead weights were added to all treatments. Metal dividers were removed after habitat types were in place.

The experiment was conducted using 5 densities of *Hexagenia* that fell within the range seen in western Lake Erie (0 to 2000 m^{-2}; Krieger 1999): 5 individuals (~ 100 m^{-2}), 9 (~ 200 m^{-2}), 18 (~ 400 m^{-2}), 36 (~ 800 m^{-2}), and 54 (~ 1200 m^{-2}). Each *Hexagenia* density was replicated three times (N=15). *Hexagenia* were added to the center of the tank at the water’s surface and allowed to select habitat types. One replicate of each density was run at the same time and tanks were placed in a straight line in random order. After 48 hours, metal dividers were again pushed into the sediment between habitat types, water was siphoned, sediment from each habitat was removed and sieved through 250µm mesh, and *Hexagenia* were counted.

To analyze the results of both experiments, the percentage of *Hexagenia* in each habitat type was arcsin square root transformed to help achieve a normal distribution (Zar 1999). Data were analyzed using a split plot analysis of variance (ANOVA) model (SAS 9.1, $\alpha = 0.05$), because each experimental unit was split into different habitat types and treatments were applied to different scales (Potvin 2001). For the habitat selection experiment, *Hexagenia* density was applied to the whole experimental unit (main plot factor), whereas each habitat type was applied to only one-third of the experimental unit (sub-plot factor). In the viewing chamber experiment, predators were applied to the whole chamber (main-plot factor), but habitat type was applied to only half of the chamber (sub-plot factor). When appropriate, split-plot ANOVA's were followed by a Tukey multiple comparison test.

4.3.2 Large Scale Spatial Analyses

In order to assess the large-scale spatial association of *Dreissena* and *Hexagenia*, densities were sampled in 1999-2009 across the western basin of Lake Erie (Figure 4-2). In each year 30 sites were sampled, though there were data missing from 7 sites in 1999, 11 in 2008, and 16 in 2009, and a larger sampling effort was conducted at 59 sites in 2003. Both taxa were sampled at the same time, using three standard Ponar grabs (0.048m^2 opening) per site. Collection and enumeration methods can be found in Schloesser et al. (1991).

I determined if there was a relationship between the co-occurrence and density of *Hexagenia* and *Driessena*. First, to test the null hypothesis that the presence *Hexagenia* is independent of the presence of *Dreissena* I: A) conducted a Chi-Square test of independence and B) examined the probability of occurrence (McCarthy 2007) and proportion of sites containing *Dreissena, Hexagenia*, or both species. Secondly, I examined the Pearson correlation (r) between *Dreissena* and *Hexagenia* densities at each site for all available data. Thirdly, I examined the spatial autocorrelation for both taxa and the cross-correlation between *Hexagenia* and *Dreissena* densities in western Lake Erie for all available data using Moran's correlation coefficient (I). Moran's I is an extension of Pearson's product moment correlation, however because I assume points close to one another will be more similar, weights are given to each pair of points, with large values given to points close to one another and points further away having smaller weights (Reich et al. 1994; Kalkhan and Stohlgren 2000). When examining the spatial autocorrelation of a species or cross-correlation between species, values of I range from -1 to +1; with values close to +1 indicate clustering, -1 dispersion, and values near zero suggest randomness (Reich et al. 1994; Kalkhan and Stohlgren 2000). The spatial autocorrelation of each taxa and the cross-correlation between taxa (Moran's I) was plotted for the range of distances between points, split into 10 equal distance classes (R, version 2.13.0), with correlations at a distance of zero representing the same site across years.

4.4 Results

4.4.1 Experiments

Hexagenia selected live *Dreissena* clusters over bare sediment in both small-scale experiments (Figures 4-3 and 4-4). *Hexagenia* selected *Dreissena* cluster habitat over bare sediment in the viewing chamber experiments (Split-plot ANOVA: $F_{3,36} = 11.44$, $p=0.0017$, Figure4-4). There was no significant effect for the presence of a predator (Split-plot ANOVA: $F_{3,36} = 0.84$, $p=0.4408$, Figure 4-4), meaning *Hexagenia* did not select clusters more or less when a predator was present. Similarly, in the *Hexagenia* habitat type selection experiment, the percentage of *Hexagenia* differed among all three habitat types (Split-plot ANOVA: $F_{24,20} = 95.17$, $p<0.0001$, Tukey: $p<0.05$, Figure 4-3). The live *Dreissena* habitat had the highest percentage of *Hexagenia*, followed by artificial *Dreissena*, and lastly bare sediment. There was a significant interaction in percentage of *Hexagenia* in each habitat type based on *Hexagenia* density (Split-plot ANOVA: *habitat*density*: $F_{24,20} = 4.86$, $p<0.0001$, Figure 4-3), showing that the percentage of *Hexagenia* selecting each habitat type changes with *Hexagenia* density.

4.4.2 Large Scale Spatial Analyses

At the basin-wide scale, the presence of *Hexagenia* was related to *Dreissena* presence (Chi-square, $\chi^2=7.51$, $p=0.006$; Table 4.1). Of the 334 observations examined, 64.7% had both *Hexagenia* and *Dreissena* present; whereas 23.3% had only *Hexagenia*, 6.3% had only *Dreissena*, and 5.7% had neither taxa (Table 4.1). Overall the probability of finding *Hexagenia* at any site was 0.88; the probability of obtaining *Hexagenia* at a site with *Dreissena* present was 0.91, while the probability of *Hexagenia* at a site without *Dreissena* was 0.80. *Hexagenia* presence was more likely at sites where *Dreissena* were also present, but their density was slightly lower at these sites. *Hexagenia* at sites without *Dreissena* can achieve very high densities (>1500/m^2), but had very high variability (standard deviation = 483.8) and a mean density of 384/m^2 (Figure 4-5). However, at sites where *Dreissena* are present, *Hexagenia* mean density was 270/m^2 and variability was lower (standard deviation = 341.2) than when *Dreissena* were absent (Figure 4-5). Even though the mean density of *Hexagenia* was lower when *Dreissena* are present, it is within the range rated "excellent" in the Lake Erie Index of Biotic Integrity (Krieger 2004).

There was no significant linear correlation between the densities of *Hexagenia* and *Dreissena* in the western basin of Lake Erie (p = 0.9381, r = -0.0043, Figure 4-5). Similarly, no spatial cross-correlation between *Hexagenia* and *Dreissena* was found in the western basin of Lake Erie using a spatial cross-correlogram (Figure 4-6c). Also, there was no spatial autocorrelation for either *Dreissena* or *Hexagenia* (Figure 4-6a and 4-6b), meaning both species were distributed randomly across the western basin of Lake Erie. For *Dreissena*, across all distances, the greatest correlation (Moran's I) was 0.06, which is very low, suggesting a random distribution (Figure 4-6a). *Hexagenia* showed a slight correlation at a distance of zero (same site across years, I=0.30), however from site to site in the western basin no spatial autocorrelation was shown (Figure 4-6b).

4.5 Discussion

4.5.1 Experiments

At a small spatial scale, the ecosystem engineering effects of *Dreissena* clusters promote *Hexagenia* selection for this habitat over soft-sediment. My experiments showed that *Hexagenia* consistently preferred sediments covered by live *Dreissena* clusters over other habitat in both small scale and a larger scale experiments (Figures 4-3 and 4-4). Bare sediment, which is typically the habitat of *Hexagenia*, was the least selected habitat type (Figures 4-3 and 4-4). Selecting *Dreissena*-covered habitat may not be beneficial over long time spans as *Hexagenia* survival has been found to be lowest in tanks with *Dreissena* (Osterling et al. 2007; Freeman et al. 2011), but this may be an effect of the experimental tank, as mayfly larvae residing in clusters are densely aggregated and may compete for food. As a result, the strength of habitat preference in natural situations is also likely to depend on food availability and needs to be evaluated in a natural lake system.

The addition of structure was not the only mechanism affecting *Hexagenia* selection for *Dreissena* habitat, as *Hexagenia* preferred live *Dreissena* to artificial clusters. Live *Dreissena* change available habitat not only by their physical structure, which is simulated by the artificial clusters, but they also filter feed, respire, and excrete feces and pseudofeces. Most epifaunal invertebrates living in interstitial spaces of mussel clusters located on hard substrates have been shown to occur in equal densities in live and artificial *Dreissena* habitats (Silver Botts et al. 1996; Gonzalez and Downing 1999). However, comparable to my experiment, some species prefer live mussel clusters over artificial ones (Ricciardi et al.1997; Stewart et al. 1998). Therefore, *Hexagenia* may be responding to the added food resource (Roditi et al. 1997), similar to other detritivores, such as snails, tubificid oligochaetes, and some chironomids (Stewart et al. 1998). Alternatively, *Dreissena* are very efficient filter feeders (Kryger and Riisgard 1988) and may increase the flow of well-oxygenated water above the clusters. Although water below *Dreissena* clusters has lower dissolved oxygen and water quality (Burks et al. 2002; Beekey et al. 2004b), water above the cluster may still be well oxygenated and filtering by *Dreissena* may direct well-oxygenated water near *Hexagenia* burrows. Therefore, the localized habitat alterations due to the presence of *Dreissena* are increasing *Hexagenia* selection for this habitat type, analogous to their effects on other benthic invertebrates.

Dreissena-covered habitat was the preferred habitat for *Hexagenia* when a predator was present; however, contrary to my expectation, this preference was not stronger than in the fishless treatment (Figure 4-4). This is consistent with the results from the habitat selection experiment, which suggests that *Hexagenia* are choosing live *Dreissena* clusters for reasons other than protection from predation. Also, in the viewing chamber experiments *Hexagenia* densities were high (1200/m^2) to ensure I could observe the behavior of at least a few *Hexagenia*. This may have resulted in a saturated area of *Hexagenia* under *Dreissena* that minimized the possibility of more *Hexagenia* using the *Dreissena* habitat.

Although *Hexagenia* are not selecting for *Dreissena*-covered habitat primarily for protection from predation, since they selected for live clusters more often than artificial ones, *Hexagenia* are consumed at lower levels when beneath clusters under turbid conditions. *Dreissena*-clusters reduce the ability of fish to eat *Hexagenia* in turbid conditions, but there was no decrease when *Dreissena* were present in low turbidity/high light conditions (Figure 4-7; DeVanna et al. 2011). Light conditions, turbidity, and structural complexity can have large impacts on the foraging of visually oriented fish (eg. Diehl 1988; Miner and Stein 1993; Utne-Palm 2002). Although I observed a decrease in burrowing activity by *Hexagenia* when beneath *Dreissena* clusters, which may result in lower amounts of resuspended sediment particles; areas where *Hexagenia* and *Dreissena* co-occur, such as the western basin of Lake Erie, remain turbid due to resuspension of very fine bottom sediment particles and river inputs. Zebra mussel clusters on hard substrates have been shown to decrease fish consumption of benthic prey even when turbidity is low and bottom light levels are high (Gonzalez and Downing 1999; Mayer et al. 2001; Dieterich et al. 2004), whereas under similar bottom light conditions on soft substrates, fish may not be as affected by the presence of *Dreissena* clusters (DeVanna et al. 2011).

4.5.2 Large Scale Spatial Analyses

The ecosystem-engineering effects of *Dreissena* alter soft-sediment habitat at a local scale, but at a lake wide scale many other processes play a role in determining *Hexagenia* distribution and each life stage is influenced by different factors (Corkum et al. 2006; Corkum 2010). *Hexagenia* eggs are deposited at the surface of the water (Corkum 2010); planktonic eggs are likely to be highly influenced by large-scale physical processes such as wind and currents (Corkum et al. 2006). Once the eggs settle, substrate type and oxygen levels play a part in determining if the eggs will hatch and *Hexagenia* begin to burrow in the sediment. My small-scale experiments only examine the life stage after *Hexagenia* hatch and are able to move and select habitat type, which is when the localized ecosystem engineering effects of *Dreissena* are important factors. The large-scale spatial analyses allow us to look at the overall distribution of *Hexagenia* and *Dreissena*, which incorporates the influence of many biological and physical processes that affect different life stages of *Hexagenia*.

Hexagenia and *Dreissena* were found to co-occur at the majority of the sites sampled in western Lake Erie, suggesting that *Dreissena* do not inhibit *Hexagenia* presence. Not only do *Hexagenia* co-occur with *Dreissena*, they are more likely to occur where *Dreissena* are present (Table 4.1). This suggests that *Hexagenia* even at a large scale are positively related to *Dreissena* presence. This may be due to *Hexagenia* selection for sediment covered with live *Dreissena* clusters, as shown in the small-scale experiments (Figures 4-3 and 4-4), but it is unknown how far an individual *Hexagenia* will actively select for a habitat. Also, *Dreissena* filtering may create currents above their druses, which increase the number of *Hexagenia* eggs that settle near *Dreissena*-covered sediment. It is also possible that physical processes, such as currents, take planktonic *Dreissena* veligers and *Hexageina* eggs to similar locations. A combination of small-scale habitat selection, along with large-scale physical processes may determine the spatial relationship between these two dominant benthic species.

Although *Hexagenia* presence is positively associated with *Dreissena*, the densities of both taxa are not correlated (Figures 4-5 and 4-6c). When *Dreissena* are absent or at low numbers the density of *Hexagenia* has a large range, *Hexagenia* can reach very high densities, but there is also a high proportion of sites without *Hexagenia*.

This suggests that while *Dreissena* may decrease the upper limit of *Hexagenia* density, the habitat alterations by *Dreissena* are promoting *Hexagenia* selection for this habitat. The Lake Erie Index of Biotic Integrity suggests a three-year moving average of *Hexagenia* density between 201-300 as "excellent", and densities greater than 400 as "imperiled" (Krieger 2004). High densities of *Hexagenia* suggest abundant food resources and likely over-enrichment of the lake. It has been suggested that these dense patches, due to high amounts of organic matter, can decrease oxygen levels to a point where *Hexagenia* can no longer survive (Krieger 2004).

When *Dreissena* are present, *Hexagenia* densities are slightly lower, but much less variable. Also, the proportion of sites without *Hexagenia* is very low; in only 8% of the sites with *Dreissena* were *Hexagenia* not found, compared to 20% of the sites without *Dreissena*. *Hexagenia* may not be able to reach high densities when *Dreissena* are present due to oxygen availability constraints, since oxygen beneath *Dreissena* clusters has been shown to be low (Burks et al. 2002; Beekey et al. 2004b). Also, unlike epifaunal invertebrates that show a linearly increasing response to *Dreissena* density (Mayer et al. 2002), *Hexagenia* do not show a trend with increasing *Dreissena* density (Figure 4-5). When *Dreissena* density increases there is an increase in habitat complexity and surface area available to epifaunal invertebrates living on top of the cluster (e.g., Silver Botts et al. 1996; Stewart et al. 1998; Gonzalez and Downing 1999), but this is different for sediment dwelling invertebrates, where the available sediment habitat surface area does not change with increasing *Dreissena* density. *Hexagenia* are more likely to be found in areas with *Dreissena*, and although *Hexagenia* are not reaching densities greater than 1000/m^2 when co-occurring with *Dreissena*, *Dreissena* may restrain

Hexagenia to population levels more in accordance with what is seen as a "healthy" *Hexagenia* density.

Hexagenia and *Dreissena* densities in western Lake Erie appear to be highly spatially and temporally variable. At the spatial scale of the western basin of Lake Erie (Figure 4-2), both *Hexagenia* and *Dreissena* populations are distributed randomly (Figure 4-6). These random spatial distributions may be due to the fact that both species have a planktonic early life history stage, both *Dreissena* veligers and *Hexagenia* eggs. Planktonic larvae act as passive particles and are at the mercy of the currents until they settle out of the water column (Hannan 1984; Jackson 1986). Although both taxa have planktonic stages, water currents in lakes are highly variable (Beletsky et al. 1999), and if the two species are not in the water column at the same time or have different settling rates, they may be distributed very differently. Once out of the water column, it is unknown how far either *Dreissena* or *Hexagenia* can select for suitable habitat, but this would be expected to be a short distance due to mobility of the organisms and susceptibility to predation. For *Hexagenia*, there is a weak positive correlation of density at a distance of zero (Figure 4-6b), which is a correlation at the same site through time. This suggests that *Hexagenia* densities at a number of sites are consistent through time, representing either sites that always have *Hexagenia* or sites that are inhabitable, maybe due to sediment type, and never have *Hexagenia* present. I examined spatial autocorrelation and cross-correlation across all years of data, therefore temporal variations in the data were masked, however the correlograms were also run for individual years and yielded similar results. At larger spatial scales, many factors are influencing both *Hexagenia* and *Dreissena* spatial distributions and densities, and more work needs to be conducted examining these factors.

In conclusion, the current range expansion of dreissenid mussels onto soft sediments and the observed small-scale habitat selection by burrowing mayflies for *Dreissena* clusters may have potential cascading effects to higher trophic levels and overall ecosystem functioning. At the western basin-wide scale, the presence of *Dreissena* does not inhibit *Hexagenia* presence and *Hexagenia* are more likely to be present where *Dreissena* are also present. *Hexagenia* at sites without *Dreissena* can achieve very high densities, but are highly variable, whereas at sites with *Dreissena*, *Hexagenia* are present at a lower mean density, but are less variable. What is important is that *Dreissena* presence and resulting habitat alterations are not inhibiting *Hexagenia* presence and *Hexagenia* are maintaining sustainable population levels in areas with high densities of dreissenids in western Lake Erie.

Table 4.1**:** Chi-square contingency table showing number of sites (N=334) in western Lake Erie collected between 1999 and 2009 with both *Hexagenia* and *Dreissena* present and/or absent.

		Hexagenia		
		Present	Absent	Total
Dreissena	Present	216	21	237
	Absent	78	19	97
	Total	294	40	334

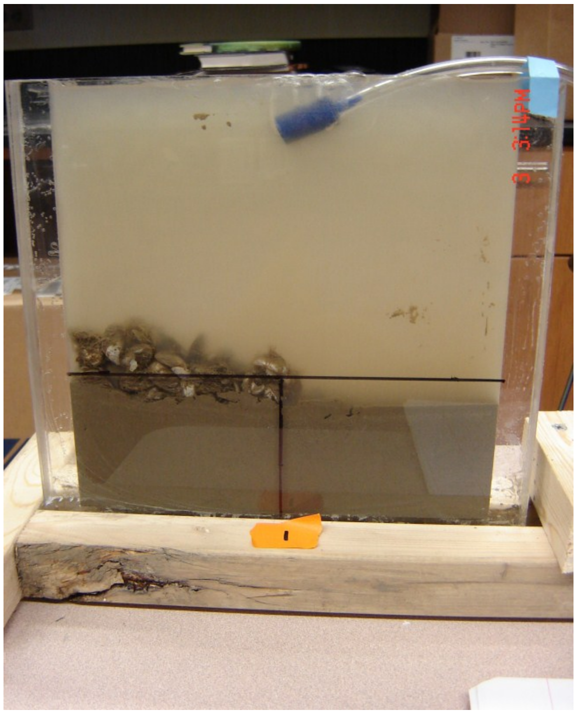


Figure 4-1: Viewing chamber constructed of acrylic sheets and filled with Lake Erie sediment split into two habitat types (bare sediment and live *Dreissena* clusters).

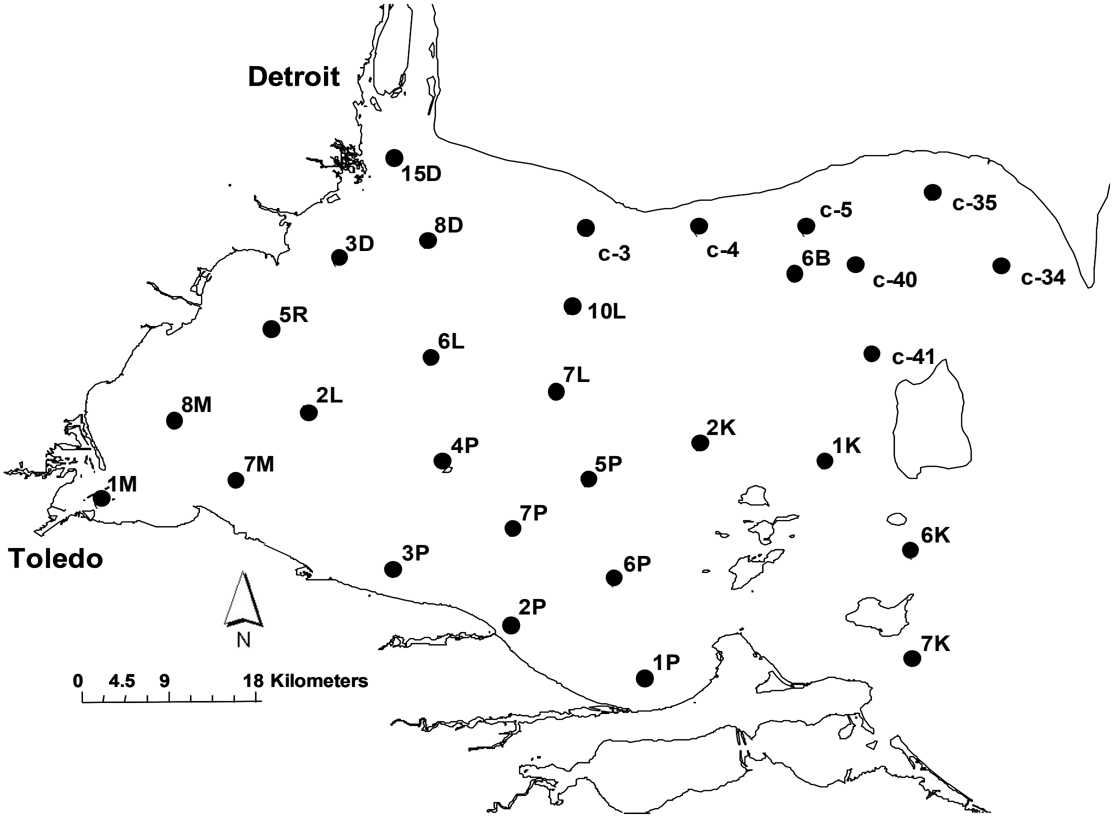


Figure 4-2: Location of 30 sites sampled in 1999-2009 for *Hexagenia* and *Dreissena* densities in the western basin of Lake Erie.

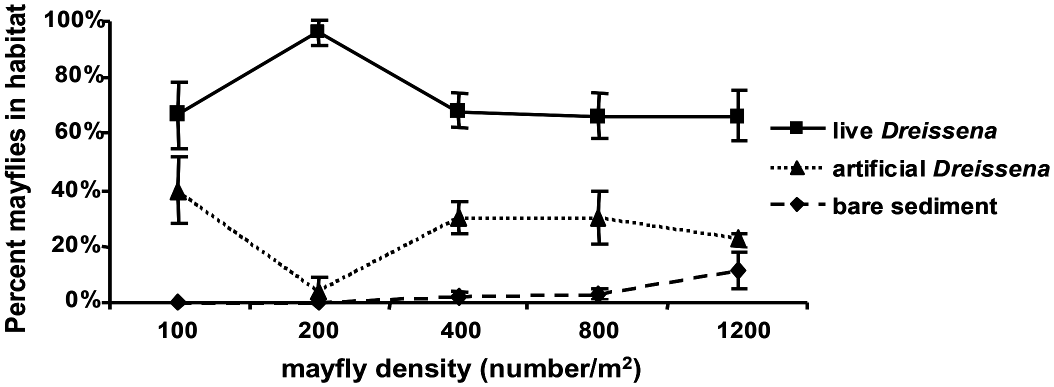


Figure 4-3: Mean percent of total number of *Hexagenia* found in each habitat type, bare sediment, live mussel clusters, and artificial mussel clusters in laboratory habitat preference experiments across a range of *Hexagenia* densities. Bars represent ± 1 standard error (as in dissertation chapter 2, DeVanna 2006, and DeVanna et al. 2011).

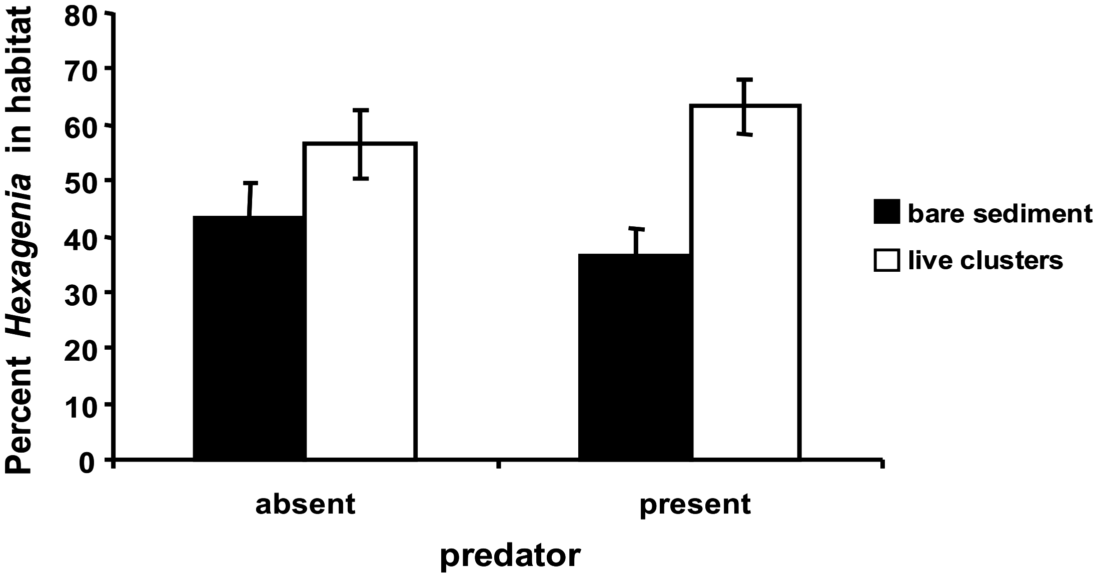


Figure 4-4: Mean percent of total number of *Hexagenia* found in each habitat type, bare sediment or live mussel clusters, in the both the presence and absence of a fish predator. Bars represent ± 1 standard error. (Data also shown in dissertation chapter 3)

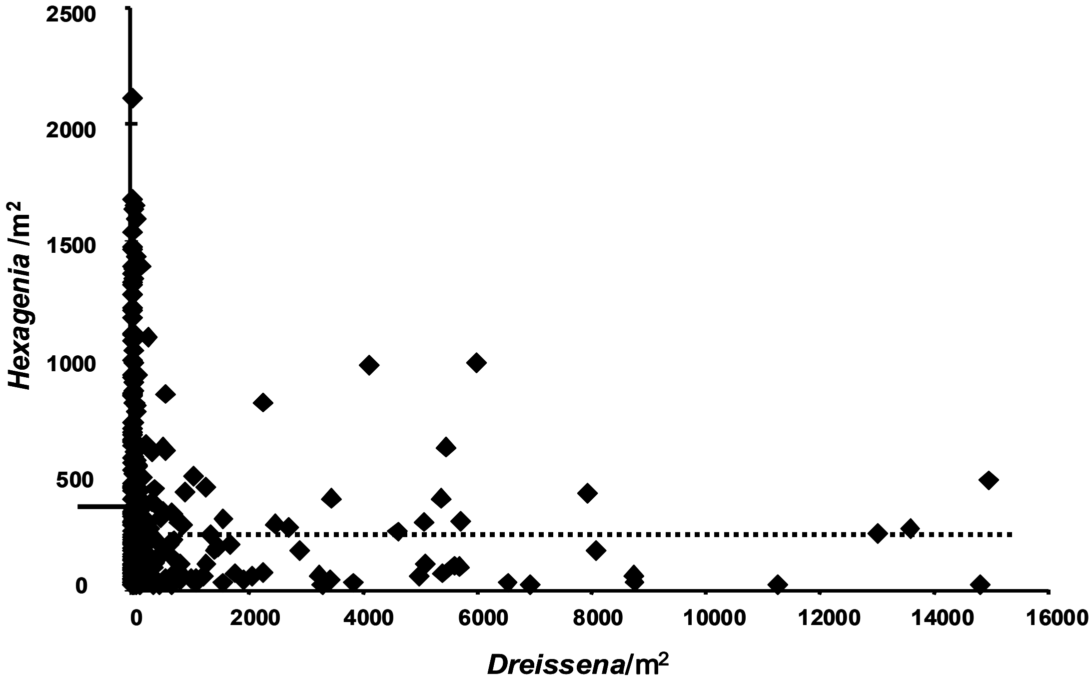


Figure 4-5: Scatter plot of *Hexagenia* and *Dreissena* densities at all sampling location and years. Each point represents the density of *Hexagenia* and *Dreissena* at a given site, sampled at the same time. Solid line represents mean *Hexagenia* density when *Dreissena* are absent (384/m^2) and dotted line is the mean density when *Dreissena* are present (270/m^2).

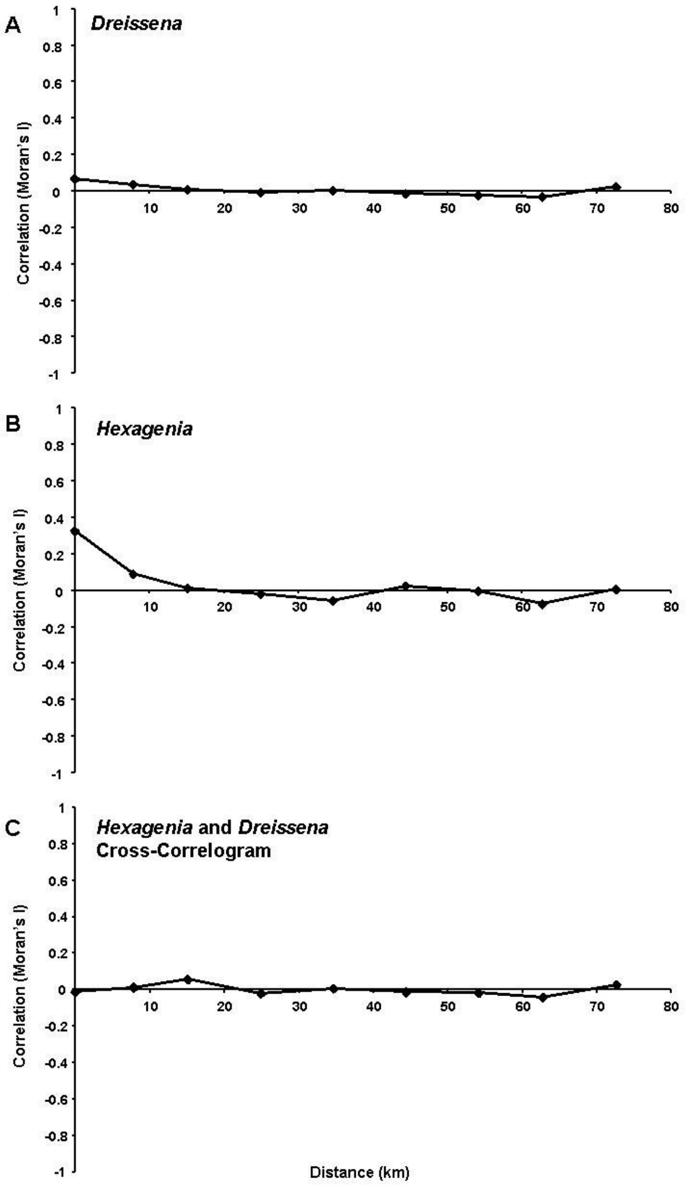


Figure 4-6: Correlograms representing spatial autocorrelation (Moran's I) of a) *Dreissena*, b) *Hexagenia,* and c) cross-correlation between *Hexagenia* and *Dreissena* for ten distance classes spanning all sampling points for all years in western Lake Erie. Correlations at a distance of zero represent the same sampling location across years. Moran's I values range from -1 to +1; with values close to +1 indicate clustering, -1 dispersion, and values near zero suggest randomness.

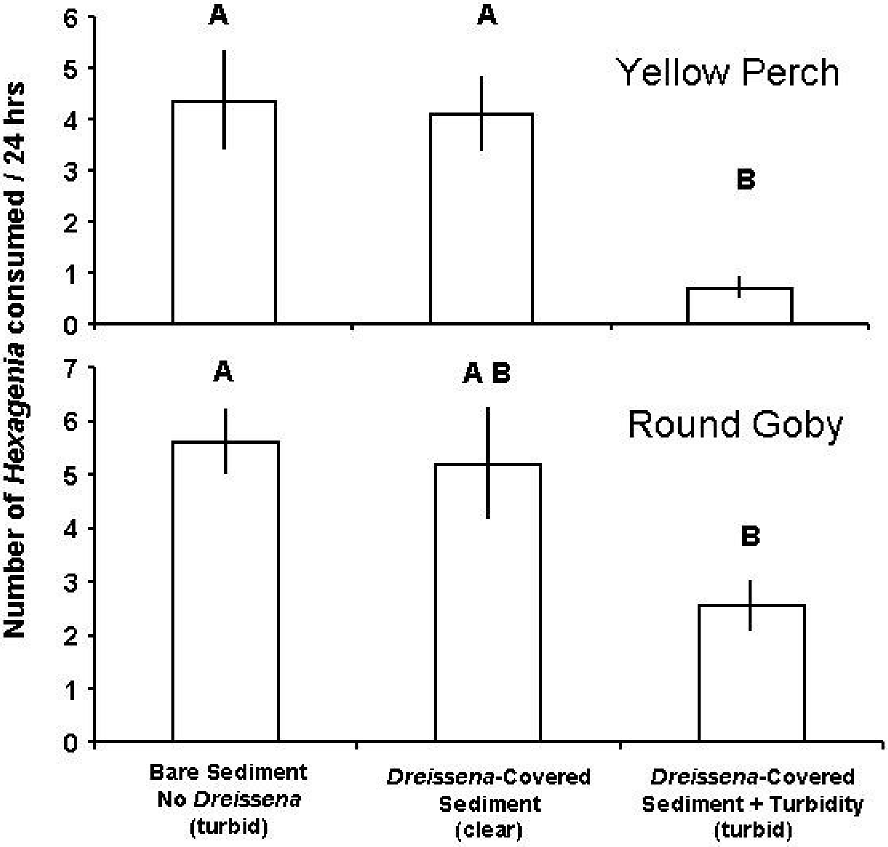


Figure 4-7: Number of *Hexagenia* consumed by a) yellow perch and b) round gobies in a 24-hour period for three different habitat treatments. Bars represent ± 1 standard error. Different letters represent statistically significant habitats based on the Nemenyi's non-parametric multiple comparison test ($\alpha=0.05$) (same as in dissertation chapter 2, DeVanna 2006, and DeVanna et al. 2011).

Chapter 5

Spatial and Temporal Density Fluctuations in Native Burrowing Mayflies: An Examination of Abiotic and Biotic Factors

5.1 Abstract

Understanding what factors drive an organism's population fluctuations in time and space can be very difficult in complex ecosystems. Therefore, in order to understand processes behind observed patterns in these complex systems, long-term, spatially broad data sets are needed along with appropriate quantitative approaches to examine changes in many abiotic and biotic factors in relation to the organism of concern. In this study, I used generalized additive models to understand the abiotic (percent silt, organic carbon, depth, and distance from western shore) and biotic factors (*Dreissena* density) affecting native *Hexagenia* sp. (burrowing mayflies; *H. limbata* and *H. rigida*) populations in western Lake Erie using ten years of data (1999-2008). *Hexagenia*, and indicator of a healthy ecosystem, became rare in the Great Lakes during the 1950s during eutrophic conditions, have recently recolonized Lake Erie, and continue to show fluctuations in their distribution and densities. I found that abiotic factors were most important in predicting *Hexagenia* density across western Lake Erie. *Hexagenia* density was negatively related to increasing percent silt and organic carbon content, greater at

shallower depths further from the shoreline, and high-density sites were clustered near the western shoreline of Lake Erie. Surprisingly, *Dreissena* density was not a good predictor of *Hexagenia* density in western Lake Erie. I did find that temporal dynamics differed for sites with low, intermediate, or high *Hexagenia* densities. Low-density sites were very stable and did not fluctuate much from year-to-year, whereas intermediate sites did fluctuate, but had no pattern. High-density sites revealed a two-year cycle of high and low densities, suggesting a density-dependent regulation of population densities. Therefore, abiotic factors play a large role in determining *Hexagenia* densities in western Lake Erie, and although *Hexagenia* are known to be highly variable, this variability may not be all due to changing environmental conditions, but rather a density-dependent regulation of the *Hexagenia* population.

5.2 Introduction

Population fluctuations are difficult to understand and predict in large, dynamic ecosystems. In some cases, there are clear mechanistic explanations for observed population level changes in organisms, such as non-native sea lamprey (*Petromyzon marinus*) causing declines in native lake trout (*Salvelinus namaycush*; eg. Holey et al. 1995) and the population declines of atlantic cod (*Gadus morhua*) and lake sturgeon (*Acipenser fulvescens*) due to overfishing (eg. Jackson et al. 2001a; Allen et al. 2005). On the other hand, ecosystems are very complex and many times there is no "smoking gun" explanation for observed trends. In the Laurentian Great Lakes, the population level changes of many organisms, such as decreases in *Diporeia* density (Nalepa et al., 1998, 2003, and 2009) and lake whitefish (*Coregonus clupeaformis*) size and condition

(Madenjian et al., 2002; Pothoven and Madenjian, 2008), are most certainly confounded by many environmental and ecological factors. Another issue of concern when examining large spatial trends is that factors influencing a species' spatial distribution occurs at multiple spatial scales; biotic or abiotic processes that are important at one scale are not always predictive at a different scale (Turner et al. 1989; Wiens 1989; Collingham et al. 2000), and the choice of scale can directly affect the outcome of the study and comparability between studies (Rahbek 2005). Therefore, in order to understand processes behind observed patterns in these complex systems, long-term, spatially broad data sets are needed along with appropriate quantitative approaches to examine changes in many abiotic and biotic factors in relation to the organism of concern. In this study, I used a modeling approach to understand the abiotic and biotic factors affecting native *Hexagenia* sp. (burrowing mayflies; *H. limbata* and *H. rigida*) populations in western Lake Erie.

Hexagenia are the dominant benthic marcoinvertebrate in many North American lakes, owing in part to their large size in comparison to other benthic fauna (Hayward and Margraf 1987) and their ability to reach high population densities (Rasmussen 1988; Krieger et al. 1996; Schloesser et al. 2000). Fish diets comprised of *Hexagenia* have been associated with increased growth rates in yellow perch (Hayward and Margraf 1987) and drum (Swedberg 1968). *Hexagenia* are also bioturbators, meaning they mix the sediment through feeding, respiration, and burrowing activities (Bartsch et al. 1999; Bachteram et al. 2005), which is vital to benthic community structure because it influences sediment properties (Levinton 1995; Solan et al. 2004), nutrient and contaminant fluxes at the sediment-water interface (Matisoff and Wang 1998; Bartsch et al. 1999; Chaffin and Kane 2010), and may increase species richness and diversity (Widdecombe et al. 2000). Therefore, *Hexagenia* are an ecologically important link in many temperate freshwater systems, but their populations across North America have been fluctuating widely and *Hexagenia* are currently extirpated from many systems where they used to be abundant, such as Oneida Lake (Clady 1975), Green Bay (Lake Michigan), and Saginaw Bay (Lake Huron) (Howmiller and Beeton 1971). In order to understand mechanisms driving the variable spatial distribution and temporal fluctuations of *Hexagenia*, I examined their spatial and temporal distribution and constructed generalized additive models (GAMs) to evaluate important underlying biotic and abiotic factors influencing their density.

Hexagenia population fluctuations and local extirpations may be due to periods of increased eutrophication and resulting low benthic oxygen concentrations (eg. Britt 1955; Krieger et al. 1996; Gerlofsma and Ciborowski 1998) since *Hexagenia* are sensitive to low oxygen conditions (Reynoldson et al. 1989; Winter et al. 1996), and the occurrence, strength, and duration of hypoxia is increasing worldwide (low oxygen, ≤ 2 mg O_2/l; Diaz 2001; Baustian and Rabalais 2009; Vanderploeg et al. 2009). Based on the effects to benthic communities, dissolved oxygen concentration has been shown to be the single most ecologically important environmental variable to marine aquatic ecosystems as a result of its drastic changes in such a short amount of time (Diaz and Rosenberg 1995; Rabalais et al. 2002). Many shallow systems where *Hexagenia* have historically been abundant, such as the western basin of Lake Erie, can experience short-term stratification due to high productivity. As a result, these systems have experienced periods of hypoxia that can lead to increased mortality and temporal fluctuations in *Hexagenia* density

(Bridgeman et al. 2006) or may stratify long enough to suffocate entire *Hexagenia* populations (Howmiller and Beeton 1971; Clady 1975). Rasmussen (1988) examined *Hexagenia* presence/absence and biomass in 12 Quebec lakes in terms of variables that are thought to be related to sediment oxygen demand and oxygen supply to the sediments, since *Hexagenia* are known to be oxygen sensitive. Sites with *Hexagenia* present were found to have higher sediment oxygen-reduction potential, secchi disk transparency, slope, exposure, and lower sediment-water content, chlorophyll *a* concentration, and plant biomass (Rasmussen 1988). *Hexagenia* biomass was best predicted in a linear model with significant contributions form chlorophyll concentration (-), plant biomass/plant height (-), sediment-water content (-), and water depth (-) (Rasmussen 1988). Therefore, based on these findings, Rasmussen (1988) concluded that the dramatic declines of *Hexagenia* in lacustrine habitats are likely the result of eutrophication.

In this study, we used variables similar to Rasmussen (1988) to predict *Hexagenia* density, such as percent silt and water depth, as well as variables that were not included the earlier study. Similar to Rasmussen (1988), we used depth as a predictor of *Hexagenia* distribution because it can be used as a surrogate for strength and duration of hypoxic events that can create high *Hexagenia* mortality in a relatively short period of time. Areas such as the central basin of Lake Erie that are deep and productive enough to stratify yearly and become hypoxic are consistently devoid of *Hexagenia* (Reynoldson and Hamilton 1993); conversely, very shallow lakes, such as Lake St. Clair that do not stratify have maintained consistently high populations (Schloesser et al. 1991). The depth of the western basin of Lake Erie, our study area, is intermediate to the two above systems, and can experience short-term hypoxia events that affect *Hexagenia* survival (Bridgeman et al. 2006).

Hexagenia dig u-shaped burrows in soft-sediment and pump through well-oxygenated water to maintain high oxygen concentrations (Wang et al. 2001; Gallon et al. 2008). They feed on available bacteria and detritus within the burrow (Dermott 1980). As a result, sediment type is important in determining *Hexagenia* distribution. Hard substrates are inhabitable by *Hexagenia*, as they cannot dig burrows and would therefore be readily consumed by fish. On the other hand very fine substrates may be too unstable to maintain a burrow, and *Hexagenia* have previously been shown to be present more often in soft-substrates with lower sediment-water content (Rasmussen 1988). Sediment organic carbon can be used as a measure for the amount of food available to *Hexagenia*. However, excessive food resources, suggesting over enrichment of the lake, can create a density-dependent regulation of the population, where the dense patches of *Hexagenia* can decrease oxygen levels to a point where *Hexagenia* can no longer survive (Krieger 2004). Sediment quality may be a good predictor of where *Hexagenia*, once hatched, will be able to survive, and may help determine where hypoxic events will occur leading to temporal fluctuations in *Hexagenia* density.

In addition to abiotic properties of the water body (hypoxia, depth, and sediment) that influence *Hexagenia* density, a location's distance from adult mating swarms is also likely important. Sub-adult and adult *Hexagenia* mayflies are weak fliers; once *Hexagenia* emerge from the water they are carried by wind to shore, and the location of adult swarms can be predicted by onshore wind speed (Corkum et al. 2006). After mating, female *Hexagenia* are again carried by wind to the water, where the eggs are deposited at the surface (Corkum 2010); planktonic eggs are likely to be highly influenced by large-scale physical processes such as wind and currents (Corkum et al. 2006) and have been found at least 4 km from shore (Corkum 2010). Once the eggs settle, *Hexagenia* most likely do not travel far due to their high vulnerability to predation, therefore substrate type and oxygen levels play a part in determining if the eggs will hatch and *Hexagenia* begin to burrow in the sediment. The location of *Hexagenia* eggs deposited into the water likely depends the location of adult swarms and direction of prevailing winds and may have a large effect on where *Hexagenia* will occur within a water body.

A biotic factor likely to affect *Hexagenia* is the invasive ecosystem engineers, zebra mussels (*Dreissena polymorpha*) and quagga mussels (*D. rostriformis bugensis*), which have significantly altered the bottom habitat of many large temperate aquatic systems where *Hexagenia* are also located (Karatayev et al. 2002). The initial invasion by zebra mussels in North America involved clusters colonising bedrock and other hard surfaces and increasing habitat complexity on hard substrate. Subsequently, both zebra and quagga mussels have been spreading to soft substrates in many invaded lakes (Berkman et al. 1998; Bially and MacIsaac 2000). However, quagga mussels, first recorded in North American in 1989 in Lake Erie (Mills et al. 1996), have become the dominant dreissenid species in the Laurentian Great Lakes and are the main colonizer of soft sediments (Roe and MacIsaac 1997; Patterson et al. 2005; Wilson et al. 2006), and are likely to affect *Hexagenia* distribution and abundance. *Hexagenia* have been shown to select for *Dreissena* colonized habitat (DeVanna et al. 2011) and in the western basin of Lake Erie are more likely to occur where *Dreissena* are also present (DeVanna et al. *in* *review*), but long-term survival in this habitat may be decreased (Freeman et al. 2011). Therefore, selection of *Hexagenia* for *Dreissena* clusters could impact their spatial distribution, as they select for "low-quality" sediment covered with *Dreissena* over "higher-quality" bare sediment.

In this study, I examined spatial and temporal trends of *Hexagenia* in western Lake Erie, which is a valuable model system due to available long-term data sets on many abiotic and biotic factors and a spatially and temporally variable *Hexagenia* population. *Hexagenia* became rare in the Great Lakes during the 1950s during eutrophic conditions (Britt 1955; Nebeker 1972; Gerlofsma and Ciborowski 1998), have recently recolonized Lake Erie (Krieger et al. 1996; Corkum et al. 1997; Schloesser et al. 2000), and continue to show fluctuations in their distribution and densities (Schloesser et al. 2001; Bridgeman et al. 2006; Corkum 2010). I used generalized additive models (GAMs) and statistical analyses to understand which abiotic (depth, percent silt, organic carbon, and distance from shore of prevailing winds) and biotic (*Dreissena* density) factors are important in determining *Hexagenia* density. I also visually examined spatial patterns and temporal trends of *Hexagenia* density across ten years of data for western Lake Erie.

5.3 Methods

5.3.1 Data

In order to assess possible factors influencing *Hexagenia* density and distribution in western Lake Erie, I examined one biological factor, *Dreissena* density, and four physical factors, percent silt, organic carbon content, depth, and distance from the western shoreline of Lake Erie. *Hexagenia* and *Dreissena* densities were sampled in 1999-2009 across the western basin of Lake Erie (Figure 5-1). In each year 30 sites were sampled, though there were data missing from seven sites in 1999, 11 in 2008, and 16 in 2009, and a larger sampling effort was conducted at 59 sites in 2003. Both taxa were sampled at the same time, using three standard Ponar grabs ($0.048 m^2$ opening) per site. Collection and enumeration methods can be found in Schloesser et al. (1991).

Sediment data, percent silt and organic carbon (mg/kg), were retrieved for Lake Erie from the Great Lakes Sediment Database (Environment Canada). Individual data points for percent silt and organic carbon were plotted in ArcGIS 10 (ESRI 2010) and predictive surfaces were created using kriging. Kriging analysis utilizes the assumption of spatial auto-correlation between individual known points to predict values in areas not directly measured (Johnston et al. 2003). For each sampling site with known *Hexagenia* densities (Figure 5-1), the estimate of percent silt and organic carbon from the predicted surface was extrapolated. A bathymetry map of Lake Erie from the Great Lakes Information Network (GLIN) was added to the map in ArcGIS 10 (ESRI 2010) and used to find the depth (m) at each sampling location. The western shoreline was defined from the southwestern edge of the Detroit River mouth to the northwestern tip of the Maumee River mouth. The shortest strait line distance (km) from the western shoreline to each sampling site was measured and recorded in ArcGIS 10 (ESRI 2010).

5.3.2 Spatial Distribution

Factors affecting spatial distribution of *Hexagenia* were examined by testing a suite of *a priori* candidate models, each including one or more of the biological and physical factors described above. Ten candidate models were developed, along with a global full model including all parameters (Table 5.1). Each model included the overall mean (β_0), the predictor variables listed (Table 5.1), and error (normally distributed, mean=0, variance = σ^2). Due to the large amount of low values, I transformed (log + 1) both *Hexagenia* and *Dreissena* densities to help achieve a more normal distribution. Instead of using general linear models, for this study I used a Generalized Additive Model (GAM) approach (Hastie and Tibshirani 1986, Wood 2004). GAM's are useful when dealing with non-parametric, non-linear data. GAM's fit small linear regression lines to the data and then combines them, creating a smoothed surface, rather than forcing a single linear trend through non-linear data (Faraway 2006). I evaluated my candidate models by using the GAM function in R (v. 2.13.1), specifying a Gaussian family with a logistic link function, and the GAM function fit a smoothing spline to all of the factors included in the candidate models. The models were then compared using AIC to identify the model with the greatest amount of deviance explained, and thus the best fit to the data (Burnham and Anderson 2002). AIC values also include a trade-off between model fit and complexity, and will choose the most parsimonious model with the greatest fit, and will only select a more complex model if it has a sufficiently greater fit to the data (Burnham and Anderson 2002). The Δ AIC values (the difference between the best-fit model and any other model) were used to compare the relative fit of the candidate models, where a difference > 2 is considered a meaningful difference in the fit of the models (Hilborn and Mangel 1997).

The environmental factors thought to affect *Hexagenia* densities across western Lake Erie were also examined by averaging densities at each site across all years, and I categorized the average densities as low, intermediate, or high. The extra sites collected in 2003 were not included in this analysis, as there was only one data point for each site. The density categories were based on the Lake Erie Index of Biotic Integrity, which uses three year running averages of *Hexagenia* densities as a biological indicator (Krieger 2004). *Hexagenia* densities less than 30/m^2 are considered "poor" and between 30-100/m^2 is listed as "fair" (Krieger 2004), therefore average densities less than 100/m^2 were placed in the "low" density category (seven sites). Average *Hexagenia* densities between 100 and 400/m^2 are considered "good to excellent" and were placed in the "intermediate" density category (14 sites), and densities greater than 400/m^2 are considered "imperiled" and were placed in the "high" density category (nine sites). These average *Hexagenia* density categories were mapped using ArcGIS 10 (ESRI 2010) and examined. For each predictive factor in the best-fit model of *Hexagenia* density in the western basin of Lake Erie, I conducted a one-factor ANOVA comparing the mean for each factor across all three *Hexagenia* density categories using SAS 9.1. Tukey multi-comparison tests were conducted for all significant ANOVA models. I also conducted a two-tailed t-test comparing the densities of *Hexagenia* east and west of an apparent density threshold boundary.

5.3.3 Temporal Dynamics

To examine the year-to-year variation in *Hexagenia* densities across western Lake Erie, I plotted *Hexagenia* density at each site across time. I examined the temporal trends individually for each density category, to evaluate if trends differ based on the average density at that site. I also selected and plotted four high-density sites (8D, c-3, 5R, and

3D) through time to more closely examine temporal trends. The extra sites collected in 2003 were not included in this analysis, as there was no time series for these sites.

5.4 Results

5.4.1 Spatial Distribution

Hexagenia densities in western Lake Erie were best explained using candidate model 10, which included smoothed factors for all physical variables; percent silt, organic carbon, depth, and distance from western shoreline (Table 5.1). There were two models that did not different from one another; the full model, including all possible parameters, was not different from model 10 with a ΔAIC value of only 1 (Table 5.1). However, model 10 was chosen as the best model because it was the most parsimonious and had a greater percentage of deviance explained. The addition of *Dreissena* density to the model did not have a great impact on the fit of the model, which is not surprising given that *Dreissena* density alone (Model 1) only explained 0.15% of the deviance (Table 5.1). All other models had much greater AIC values, suggesting they did not explain much of the variability in the data. When all parameters were run individually in the first five candidate models, percent silt had the lowest AIC value, followed by organic carbon, distance from western shore (which had the greatest percent deviance explained), depth, and lastly *Dreissena* density.

The smoothed relationship for percent silt in the GAM appeared to have a negative relationship with *Hexagenia* density; greater densities of *Hexagenia* occurred with lower percent silt (Figure 5-2a) however the percent silt in western Lake Erie only ranged from 30 to 50 percent. Similarly, when percent silt was compared across the three

Hexagenia density categories, sites with high densities of *Hexagenia* had significantly lower percent silt than sites with intermediate or low *Hexagenia* average densities (Figure 5-3a, ANOVA; $F_{2,284}$=24.70, p<0.001). Organic carbon was significantly greater at sites with low densities of *Hexagenia* than high or intermediate sites (Figure 5-3b, ANOVA; $F_{2,284}$=8.52, p=0.003), and the smoothing relationship revealed a very slight negative trend (Figure 5-2b). Sites with intermediate densities of *Hexagenia* were significantly deeper than those with either high or low densities of *Hexagenia* (Figure 5-3c, ANOVA; $F_{2,\,284}$=12.16, p<0.001). The relationship of *Hexagenia* and depth may have a threshold effect; sites too shallow or too deep may decrease average *Hexagenia* density (Figure 5-2c).

The distance from the western shoreline of Lake Erie to each sampling site appeared to have a strong relationship with *Hexagenia* density (Figures 5-1, 5-2d, and 5-3d). The smoothed relationship of *Hexagenia* density to distance from western shoreline revealed a sharp threshold around 23 km from the shoreline (Figure 5-2d). Sites within 23 km of the shoreline had much higher densities of *Hexagenia* (Figure 5-2d). Similarly, distance from shore differed significantly among all three density categories; sites with high densities of *Hexagenia* were found close to the western shoreline of Lake Erie, intermediate sites were the furthest from the shoreline and low sites were in the middle (Figure 5-3d, ANOVA; $F_{2,\,284}$=34.84, p<0.001). When the sampling sites, divided into density categories, were examined spatially in western Lake Erie, again there appeared to be a threshold boundary around 23 km from the western shoreline (Figure 5-1). Seven of the nine high-density sites were within 23 km of the western shore and only 2 fell outside this boundary. Also, the mean number of *Hexagenia* west of the threshold (468

Hexagenia/m^2) was significantly greater than the density of *Hexagenia* east of the threshold (214/m^2 *Hexagenia*/m^2; T-test, t_{28}=2.96, p=0.006). Also, when the spatial location of the density categories was examined, sites with very low densities of *Hexagenia* appeared to be in areas with fast moving water, or very close to the shorelines subjected to wave-action. Intermediate density sites are clustered further out in the center of the western basin of Lake Erie, but further from the shoreline.

5.4.2 Temporal Dynamics

Temporal trends of *Hexagenia* density differed between the three density categories (Figure 5-4). Sites with low densities of *Hexagenia* appeared to be consistently low, with only minor year-to-year fluctuations (Figure 5-4a). Intermediate *Hexagenia* density sites did vary from year-to-year, however no overall trend was observed for these sites (Figure 5-4b). Sites with high densities of *Hexagenia* did show a strong temporal 2-year cycle (Figures 5-4c and 5-5). For the high-density sites, if one year had a high density of *Hexagenia*, it was likely that the density decreased the next year, and the cycle continued throughout the dataset (Figures 5-4c and 5-5). Overall, 2001 and 2005 appeared to have higher densities of *Hexagenia* at many sites in the intermediate and high-density categories, and 2005 was also a relatively high-density year for many low-density sites.

5.5 Discussion

5.5.1. Spatial Distribution

Understanding and predicting what drives the spatial pattern of organisms and the mechanisms behind their population fluctuations in large, complex landscapes is difficult (Turner 2005) and varies with the spatial scale of the study (Wiens 1989). In many cases, there is not a single driver that is underlying the spatial distribution, but rather multiple factors that interact with one another and are difficult to disentangle (Turner 2005). Soft-sediment benthic organisms are known to have patchy, highly dynamic distributions (Morrisey et al. 1992), and using large, long-term data sets may help determining the biotic and abiotic factors underlying their distributions. Abiotic factors have been shown to be the determining factor in many organisms spatial distribution, including zooplankton (eg. Arnott and Vanni 1993; Laprise and Dodson 1994; Dejen et al. 2004), fish (eg. Blaber and Blaber 1980; Jackson et al. 2001b), and macroinvertebrates (eg. Kneib 1984; Lodge et al. 1987; Corkum and Ciborowski 1988). At the "within-water-body" scale, the physical environment has been shown to be the most influential in determining an organism's distribution, whereas biotic variables have been shown to have the most impact at a microhabitat scale (Crowl and Schnell 1990). Similarly, in this study, all abiotic factors, percent silt, organic carbon, depth, and distance from western shoreline were most important in predicting *Hexagenia* density across western Lake Erie, and no single factor explained much variance, rather the combination of multiple abiotic factors.

Percent silt and organic carbon both had negative relationships with *Hexagenia* density (Figures 5-2a, 5-3a, 5-2b, and 5-3b). Percent silt was the single factor that had the lowest AIC value, suggesting it has a strong relationship with *Hexagenia*. Sediments with high percent silt may be too fine for *Hexagenia* burrows causing them to be unstable, and similarly Rasmussen (1988) found *Hexagenia* were present more often in areas with lower sediment-water content. Organic carbon content, a measure of food available to *Hexagenia*, was highest at sites where *Hexagenia* were at low densities (Figures 5-2b and 5-3b), and suggests that food is not a limiting factor throughout the western basin of Lake Erie. Rather, areas with organic carbon content also have high levels of decomposition, leading to increased sediment-oxygen demand, which in turn may create pockets of anoxic sediments harmful to *Hexagenia*. Similarly, *Hexagenia* have been shown to occur in lakes with lower water column chlorophyll concentrations (Rasmussen 1988), indicative of less productive lakes, which would have less organic carbon on the bottom of the lake. One issue with both of these factors is the small range of values across the spatial scale I examined, and *Hexagenia* were found to be present across the entire range percent silt and organic matter in western Lake Erie. Regardless, a pattern of decreasing *Hexagenia* density with increasing percent silt and organic carbon was observed even within the narrow range of observed values, suggesting that there is a range of optimal sediment properties that can begin to determine their distribution.

Overall, the western basin of Lake Erie is very shallow (average depth 7.4 m), and does not form a true summer hypolimnion, which is a benefit to oxygen-sensitive *Hexagenia* (Bridgeman et al. 2006). However *Hexagenia* did show a depth preference, with intermediate densities at the deepest sites, and both high and low densities at more shallow sites (Figures 5-2c and 5-3c). Although western Lake Erie does not fully stratify during the summer, deeper sites may undergo temporary stratification after a period of warm calm days, creating a hypoxic layer of water above the bottom sediments (Bridgeman et al. 2006; Corkum 2010). Therefore, intermediate densities of *Hexagenia* may be found at deeper sites due to this infrequent disturbance. Many low-density sites are located around the shoreline of the basin (Figure 5-1), and at these sites wave-action may disturb the burrows, interfere with eggs settling to the bottom, or may erode away fine sediment, creating an area too coarse for *Hexagenia* establishment. Similarly, a study of *Hexagenia* distribution found highest densities of *Hexagenia* at 5-7m depth, with lower densities at both greater depths due to hypoxic conditions and shallower depths when exposed to wave action (Swanson 1967). Two different abiotic processes may be contributing to the relationship of *Hexagenia* density to depth; hypoxia at sites deep enough to undergo short-term stratification and wave action at shallow shoreline sites may be limiting *Hexagenia* distribution and densities in western Lake Erie.

Hexagenia are slow, weak fliers, and likely not able to fly much faster than the wind speed, therefore they are carried in the direction of the prevailing wind (Compton 2002). I observed a significant cluster of high *Hexagenia* densities near the western shoreline of Lake Erie (Figures 5-1, 5-2d, and 5-3d) where many adults swarm (Masteller and Obert 2000), and distance from the western shore explained the most variation in the model. Female adults from these locations of dense *Hexagenia* swarms flying to deposit eggs at the water's surface may be more likely carried east, in the direction of prevailing winds. The sites with high densities of *Hexagenia* are clustered within 23 km of the western shoreline, and this distance may represent the maximum distance *Hexagenia* can fly into the basin and deposit eggs plus the distance eggs can travel in the water column. Planktonic larvae act as passive particles and are at the mercy of the currents until they settle out of the water column (Hannan 1984; Jackson 1986). *Hexagenia* eggs have been shown to occur at equal densities up to 4 km from the shoreline (Corkum 2010) and my data suggest they are distributed at high densities even further from the shore. Also, sites near the western shoreline may have high densities due to two major river inputs, the Detroit and Maumee Rivers. *Hexagenia* may also be depositing eggs in the rivers, which are then moved into the lake. The Detroit River has very fast moving, high volumes of water entering Lake Erie (Kaiser 1985); therefore this water may take *Hexagenia* further into the basin before settling out. The site in the mouth of the Detroit River had very low densities of *Hexagenia*, probably a result of eggs being flushed out from the fast moving river water (Figure 5-1). The Maumee River has much slower flow, but is highly productive and turbid (Moorhead et al. 2008), as a result populations can exist in the mouth of the Maumee River and eggs may not travel as far until they settle out (Figure 5-1). The western edge of Lake Erie appears to be a hot spot for *Hexagenia*, likely due to many physical processes, such as wind direction, speed, and water current, which are hard to unravel due to the complex hydrodynamics of the lake (Beletsky et al. 1999).

Even though at a small-scale *Hexagenia* have been shown to select for soft-sediment covered with *Dreissena* clusters (DeVanna et al. 2011), *Dreissena* density was not a good predictor of *Hexagenia* density in western Lake Erie (Table 5.1). This may be since in western Lake Erie, *Hexagenia* and *Dreissena* densities are not positively correlated, but the two taxa have been shown to co-occur, and *Hexagenia* are more likely to be found where *Dreissena* are located (DeVanna et al. *in review*). Although the dominance of physical factors determining *Hexagenia* distribution at the water-body scale is not unlikely (Crowl and Schnell 1990), there may be other biotic factors influencing *Hexagenia* distribution. Chrionomid larvae have been shown to consume *Hexagenia* eggs (Plant et al. 2003), and chironomids are highly abundant in many areas of western Lake Erie (Stewart et al. 1998; Plant et al. 2003), and therefore may decrease *Hexagenia* populations. Also, *Hexagenia* are an important prey item for many fish species (Hayward and Margraf 1987; Schaeffer et al. 2000; Tyson and Knight 2001), and fish may be regulating *Hexagenia* densities in areas with good habitat and large fish populations. As a result, in this study physical factors had a large impact on the overall spatial distribution of *Hexagenia*, but at a smaller scale, biotic factors likely impact *Hexagenia* spatial and temporal variation in density.

5.5.2 Temporal Dynamics

Examining *Hexagenia* densities over ten years revealed temporal trends, which varied based on average density at the site (Figures 5-4 and 5-5). Low-density sites remained low throughout the ten years of sampling (Figure 5-4a). Abiotic properties specific to these locations likely limit their densities. As mentioned above, many are located along the shoreline (Figure 5-1) and *Hexagenia* may be disrupted by wave action (Swanson 1967) or may not have high numbers of eggs settle in those locations. Regardless, whatever factor is playing a role at the low-density sites, it is consistently keeping populations at these sites low and likely does not vary from year to year. Intermediate *Hexagenia* density sites do vary from year to year, but no pattern exists across ten years examined (Figure 5-4b). Intermediate density sites are mainly located at deeper depths in the eastern portion of the western basin (Figure 5-1) and may be experiencing temporal fluctuations in density due to pockets of hypoxic conditions during to short-term stratification (Bridgeman et al. 2006). At these low and intermediate density sites, temporal fluctuations, or lack thereof, appear to be due to abiotic factors independent of *Hexagneia* density.

A two-year cycle of extremely high *Hexagenia* densities, followed the next year by low densities, is occurring only at high-density *Hexagenia* sites in western Lake Erie (Figures 5-4c and 5-5). This is suggestive of density-dependent regulation of the population, and is commonly observed in aquatic communities (Pekarsky 1979; Rosillon 1989; Van Buskirk and Smith 1991), although it is likely that abiotic factors are the underlying density-independent determinant of the high *Hexagenia* densities (Rosillon 1989; David et al. 1997). It has been suggested that very high densities of *Hexagenia* consume so much oxygen that they can create their own pockets of hypoxia above the sediment, resulting in high localized mortality of *Hexagenia* (Krieger 2004). Due to the high productivity of western Lake Erie (eg. Moorhead et al. 2008), it is unlikely that food is a limiting resource. Most *Hexagenia* are burrowed in the lake sediment as larvae for almost 2 years (Corkum 2010). After which winged sub-adults emerge from the water, molt one last time into sexually mature adults, mate, and then females deposit their eggs after only being adults for a day or two. These eggs quickly fall to the bottom sediment, where the previous years' *Hexagenia* cohort is located. Therefore, if a high density of *Hexagenia* is already located at that site, the later *Hexagenia* instars may consume the newly deposited eggs or the newly hatched instars may compete for space. Many high-density sites fluctuate in sync with one another (Figure 5-5), whereas other sites appear to have a longer decreasing *Hexagenia* density trend, followed by a large spike in density (Figure 5-4). *Hexagenia* densities in western Lake Erie are known to be highly variable (Krieger et al. 1996; Schloesser et al. 2000; Bridgeman et al. 2006), but this variability may not be all due to changing environmental conditions, but rather a density-dependent regulation of the *Hexagenia* population.

In conclusion, *Hexagenia* populations in western Lake Erie may be structured at the large scale by multiple abiotic properties, but at a smaller scale may still be regulated by biotic interactions and I must take into account the spatial scale of my study when understanding what regulates the distribution. Again, Lake Erie is a highly dynamic system, and similar to many studies, I was unable to find a single factor that was the underlying determinant of their distribution, but rather a complex combination of factors that are difficult to disentangle and affect *Hexagenia* populations differently depending on the spatial scale examined. If this study were extended to include all of Lake Erie, I would likely find a range of optimal values for these factors for *Hexagenia* and would include areas where *Hexagenia* are continuously absent, such as bedrock, or areas that completely stratify during the summer such as central Lake Erie (Reynoldson and Hamilton 1993). This may help in understanding the full impact that these abiotic factors have in regulating *Hexagenia* distribution. Wind and lake hydrodynamics may play a large role in the location of adult swarms (Corkum et al. 2006) and where eggs are deposited and settle to the lake bottom, and more work needs to be done to examine their impact on the distribution of *Hexagenia*. I did find that *Hexagenia* at high densities show a strong two-year cycle, indicative of a density-dependent regulation of the population, and may account for some of the temporal variation that has been observed in their population. Overall, *Hexagenia* are an important benthic invertebrate used as a biological indicator with a complex life cycle, in which different stages are impacted by different factors. Therefore, understanding what affects *Hexagenia* population size and distribution in imperative to understanding how changes in their densities are related to possible changing environmental conditions, such as increases in eutrophication.

Table 5.1: Model structure for the candidate models predicting *Hexagenia* density and their respective AIC and ΔAIC values. Model number is indicated on the left, including the full global model. An "X" denotes factors included in each model. Bolded cells indicate the model with the lowest AIC value, and therefore the best-fit model. All factors in these models were smoothed, and *Hexagenia* and *Dreissena* densities were log+1 transformed.

Model	*Dreissena* density	Percent Silt	Organic Carbon	Depth	Distance from west shoreline	AIC	ΔAIC	Deviance Explained
1	X					1295	149	0.157%
2		X				1216	70	18.6%
3			X			1236	90	12.7%
4				X		1257	111	16.7%
5					X	1240	94	20.8%
6		X	X			1176	30	33.1%
7				X	X	1213	67	31.9%
8	X	X	X			1176	30	33.8%
9	X			X	X	1215	69	31.9%
10		**X**	**X**	**X**	**X**	**1146**	**0**	**41.6%**
Full	X	X	X	X	X	1147	1	41.4%

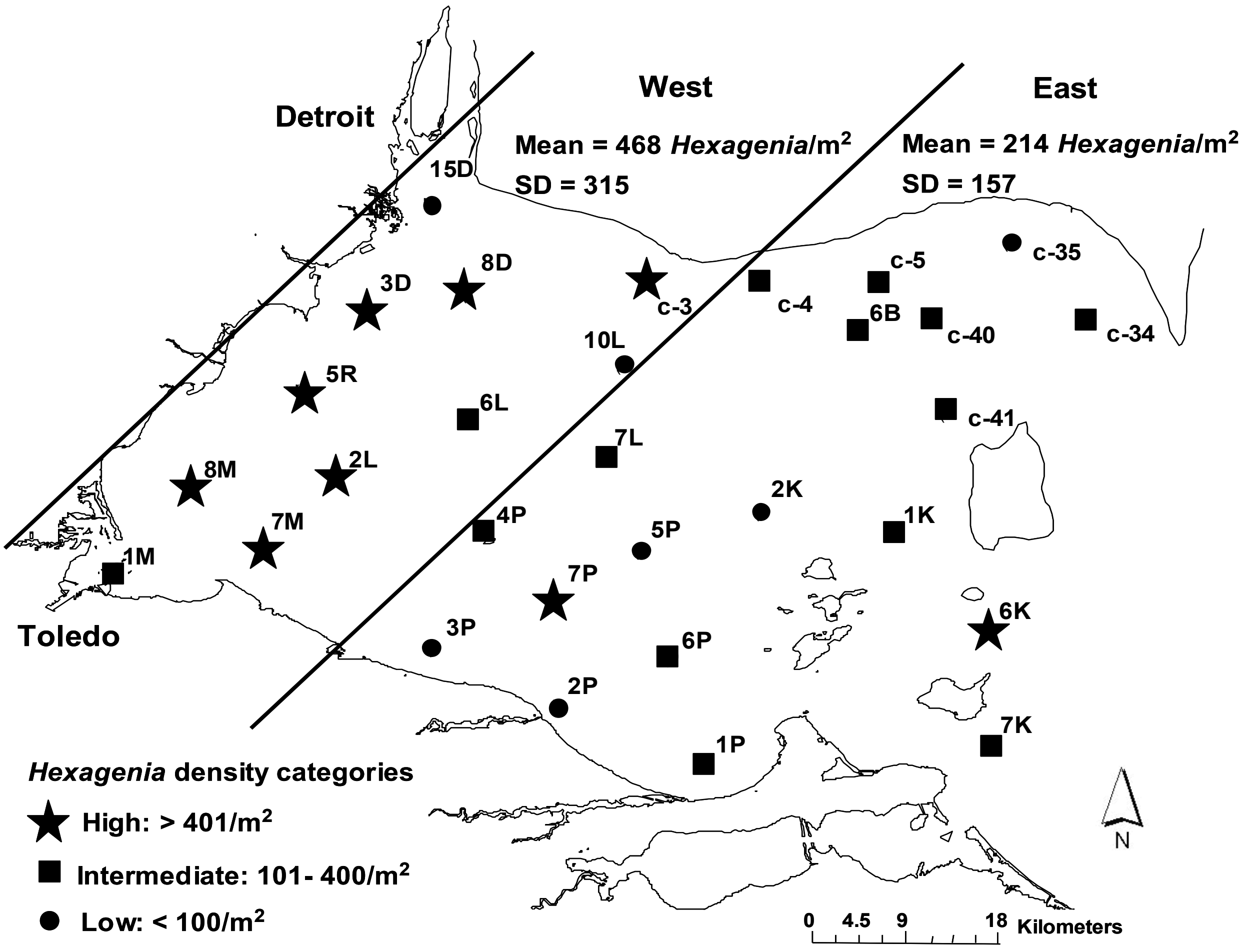


Figure 5-1: Location of 30 sites sampled in 1999-2009 for *Hexagenia* and *Dreissena* densities in the western basin of Lake Erie. Symbols indicate different categories of average *Hexagenia* density, circle = low density (<100/m^2), square = intermediate density (101-400/m^2), and stars represent high density sites (> 401/m^2). The two solid parallel lines represent the boundary of high density sites near the western shoreline, the lines are 23 km apart. Average *Hexagenia* density west of the boundary is 468/m^2 and the standard deviation (sd) = 315, the average Hexagenia density east of the boundary is 214/m^2 and the sd=157.

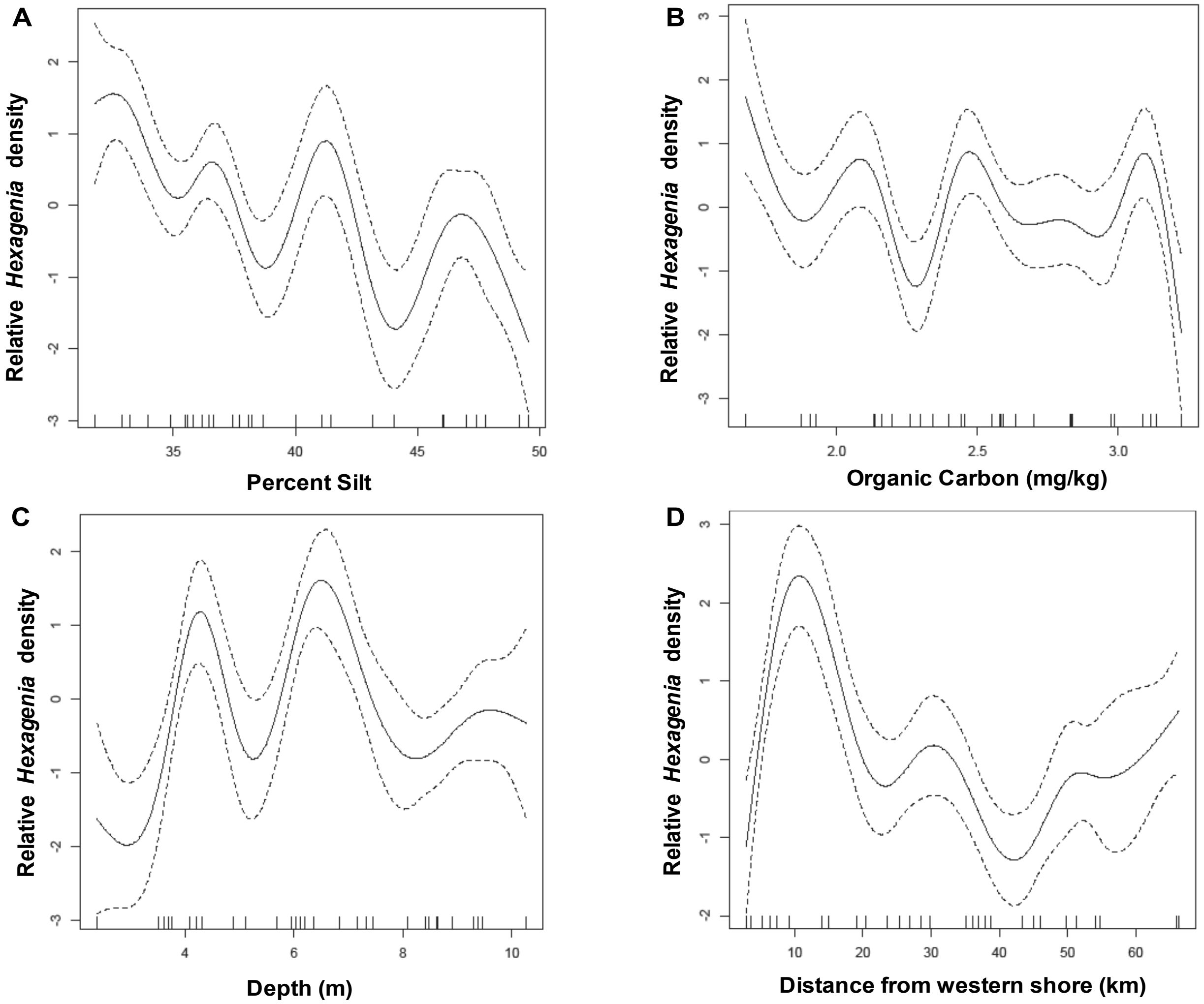


Figure 5-2: Graphs of the smoothing functions for all included factors in the best-fit candidate model predicting *Hexagenia* density. Factors include: a) percent silt, b) organic carbon (mg/kg), c) depth (m), and d) distance from western shoreline (km), showing the shape of the relationship for these factors for *Hexagenia* density. The y-axis is the relative *Hexagenia* density, 0.0 is the mean for the data. The dotted lines are the 95% confidence intervals.

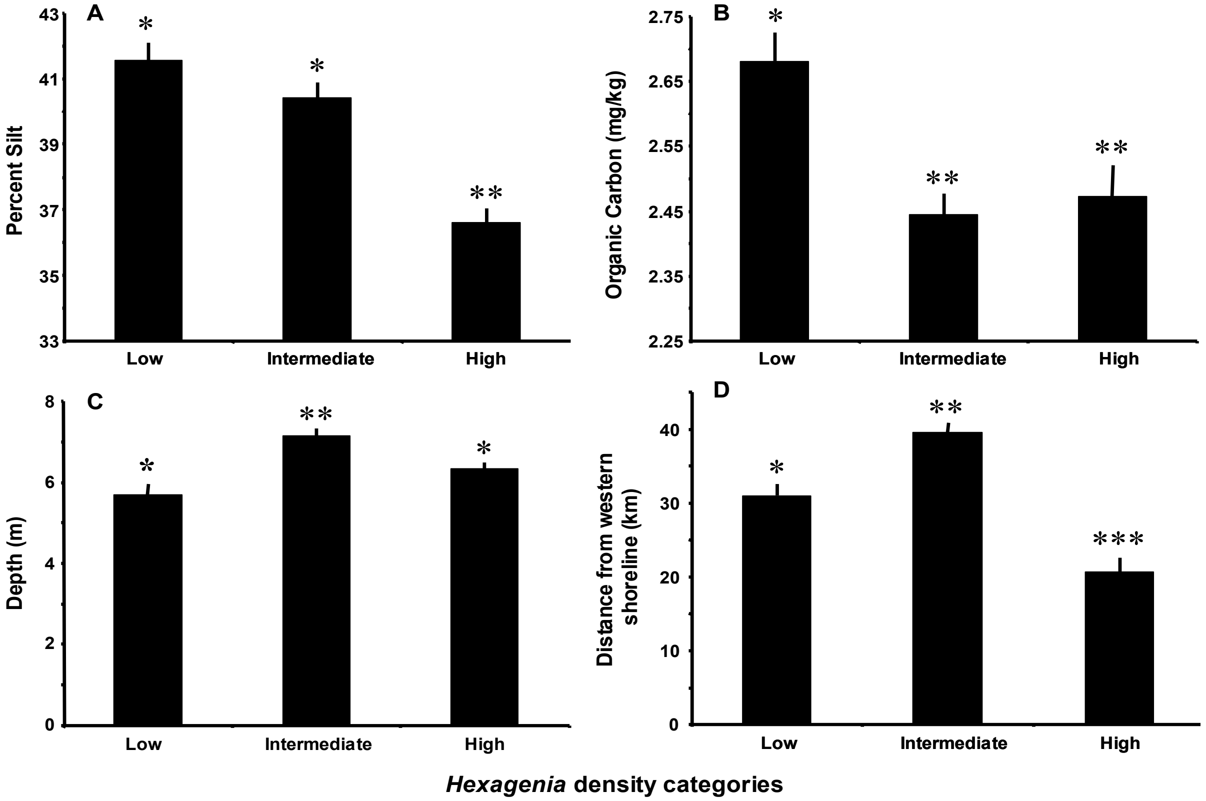


Figure 5-3: Mean (± 1 standard error) for all factors included in the best-fit candidate model: a) percent silt, b) organic carbon (mg/kg), c) depth (m), and d) distance from western shoreline (km). Each factor is divided compared across three *Hexagenia* density categories: low <100/m^2, intermediate 101-400/m^2, and high >401/m^2.

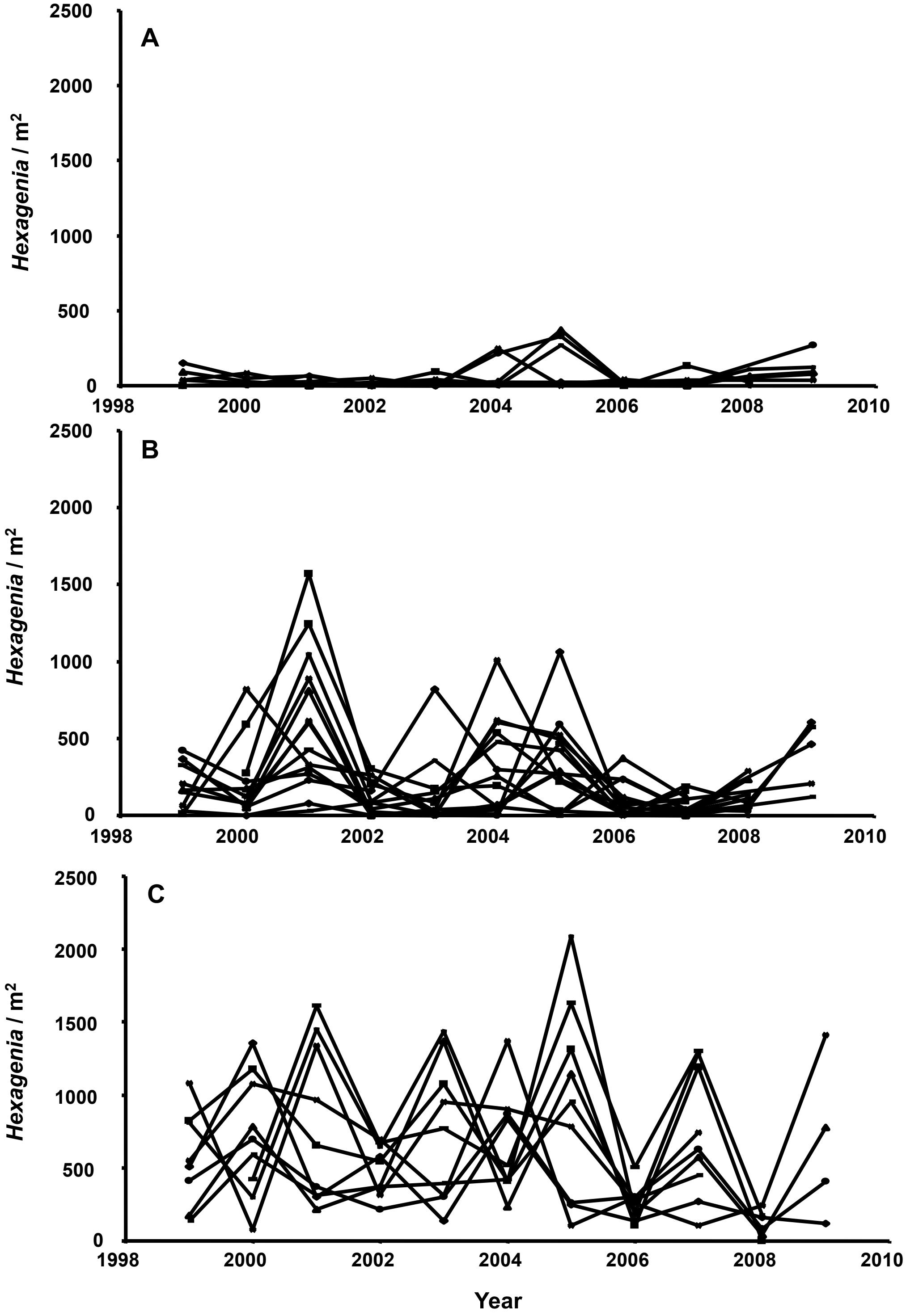


Figure 5-4: *Hexagenia* densities (number/m^2) plotted across all available years, 1999-2009, for each site. Graphs are split into three *Hexagenia* density categories: a) low <100/m^2, b) intermediate 101-400/m^2, and c) high >401/m^2.

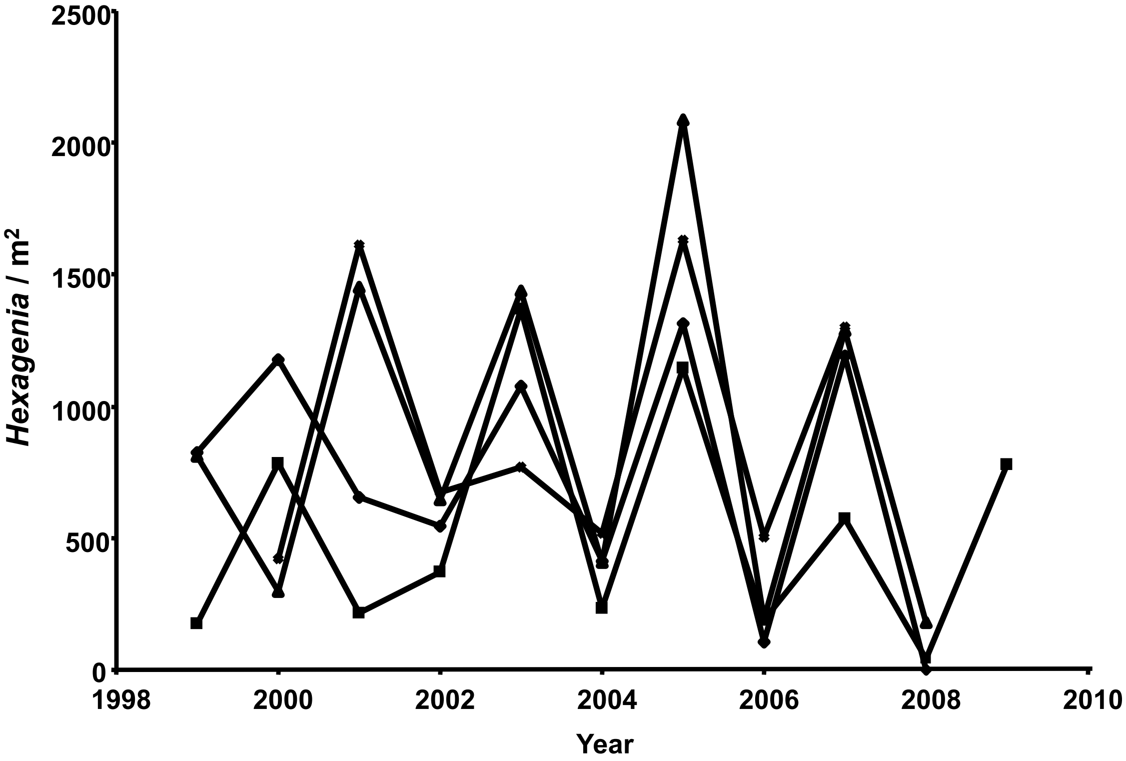


Figure 5-5: *Hexagenia* density (number/m^2) plotted from 1999-2009 for four sites in the high-density (>401/m^2) category.

Chapter 6

Discussion

The habitat alterations of *Dreissena* (*D. polymorpha*, Pallas 1771 and *D. rostriformis* bugensis, Andrusov 1897) have large impacts on *Hexagenia* (*H. limbata*, Serville 1829 and *H. rigida*, McDunnough 1924), but these impacts are also largely influenced by the spatial-scale to which they are examined. At a small spatial scale, the ecosystem engineering effects of *Dreissena* clusters promoted *Hexagenia* selection for this habitat over soft-sediment. Experiments conducted for my masters degree, showed that *Hexagenia* consistently preferred sediments covered by live *Dreissena* clusters (Figures 2-3 and 2-4). Bare sediment, which is typically the habitat of *Hexagenia*, was the least selected habitat type (Figs 2-3 and 2-4). The addition of structure however, was not the only mechanism affecting *Hexagenia* selection for *Dreissena* habitat, as *Hexagenia* preferred live *Dreissena* to artificial clusters. Live *Dreissena* change available habitat not only by their physical structure, but they also filter feed, respire, and excrete feces and pseudofeces. Therefore, *Hexagenia* may be responding to the added food resource (Roditi et al.1997), similar to other detritivores such as snails, tubificid oligochaetes, and some chironomids (Stewart et al. 1998). Alternatively, *Dreissena* are very efficient filter feeders (Kryger and Riisgard 1988) and may increase the flow of well-oxygenated water above the clusters. Therefore, the localized habitat alterations due to the presence of *Dreissena* are increasing *Hexagenia* selection for this habitat type, analogous to *Dreissena* effects on other benthic invertebrates.

Although *Hexagenia* are not selecting for *Dreissena*-covered habitat primarily for protection from predation, since they selected for live clusters more often than artificial ones, *Hexagenia* were consumed at lower levels when beneath clusters under turbid conditions (Figures 2-3 and 4-7). Light conditions, turbidity, and structural complexity can have large impacts on the foraging of visually oriented fish (eg., Diehl 1988; Miner and Stein 1993; Utne-Palm 2002). Zebra mussel clusters on hard substrates have been shown to decrease fish consumption of benthic prey even when turbidity is low and bottom light levels are high (Gonzalez and Downing 1999; Mayer et al. 2001; Dieterich et al. 2004), whereas under similar bottom light conditions on soft substrates, fish may not be as affected by the presence of *Dreissena* clusters.

At a very small scale, *Hexagenia* selected for habitats with added structure created by *Dreissena* shells equally, both live and artificial clusters, over bare sediment (Figure 3-1). This is different from *Hexagenia* in larger scale experiments that selected for live *Dreissena* clusters over artificial ones, and suggests that at this scale habitat complexity is driving the observed relationship. Contrary to my expectations, changing oxygen concentration and the presence of a predator did not impact *Hexagenia* habitat preference. This suggests that the avoidance behavior, selecting for structured habitat, has low or no costs to *Hexagenia* feeding and development (Feltmate and Williams, 1991; Peckarsky et al. 1993; Huryn and Chivers, 1999), and even when a predator is not sensed it is beneficial to select for structured habitat.

The burrowing behavior of *Hexagenia* differed depending on their habitat choice and was impacted by both changing abiotic (oxygen) and biotic conditions (presence of a predator). *Hexagenia* came out of the sediment more often when in the structured habitats (Figure 3-2). *Hexagenia* use energy to burrow and must almost continuously bioirrigate to maintain high oxygen conditions (Wang et al. 2001; Gallon et al. 2008). In sediment beneath *Dreissena* clusters *Hexagenia* may not be expending as much energy because they are no longer burrowed, but rather most often in interstitial spaces of the cluster. During low oxygen conditions, the proportion of *Hexagenia* that left their burrow increased with increasing duration of hypoxia (Figure 3-2). *Hexagenia* burrowed in bare sediment stayed less active and endured low oxygen conditions longer than *Hexagenia* in the structured habitat (Figure 3-2), likely because they would be fully exposed to predators. Therefore, in high oxygen conditions, the ecosystem engineering effects of *Dreissena* may be creating a habitat for native *Hexagenia* that is energetically more profitable than bare sediment. In low oxygen conditions, *Hexagenia* were more often out of their burrows and likely did not experience high levels of toxins released from anoxic sediment, meaning that the cluster habitat was more advantageous in terms of both physiology and avoiding predation.

The ecosystem-engineering effects of *Dreissena* alter soft-sediment habitat at a local scale, but at a lake-wide scale many other processes play a role in determining *Hexagenia* distribution and each life stage is influenced by different factors (Corkum et al. 2006; Corkum 2010). The large-scale spatial analyses allowed me to look at the overall distribution of *Hexagenia* and *Dreissena*, which incorporates the influence of many biological and physical processes that affect *Hexagenia*. At the basin-wide scale, the physical environment has been shown to be the most influential in determining an organism's distribution, whereas biotic variables have been shown to have the most impact at a microhabitat scale (Crowl and Schnell 1990). Similarly, in my study, percent silt, organic carbon, depth, and distance from western shoreline, which are all abiotic factors, were most important in predicting *Hexagenia* density across western Lake Erie.

Sediment with high percent silt may be too fine for *Hexagenia* burrows causing them to be unstable, and may be why *Hexagenia* densities were low in these areas (Figures 5-2a and 5-3a). Organic carbon content, a measure of food available to *Hexagenia*, was highest at sites where *Hexagenia* were at low densities (Figures 5-2b and 5-3b), and suggests that food is not a limiting factor throughout the western basin of Lake Erie. Rather, areas with organic carbon content also have high levels of decomposition, leading to increased sediment-oxygen demand, which in turn may create pockets of anoxic sediments harmful to *Hexagenia*. *Hexagenia* did show a depth preference, with intermediate densities at the deepest sites, and both high and low densities at more shallow sites (Figures 5-2c and 5-3c). Although western Lake Erie does not fully stratify during the summer, deep sites may undergo temporary stratification after a period of warm calm days, creating a hypoxic layer of water above the bottom sediments (Bridgeman et al. 2006; Corkum 2010). Therefore, intermediate densities of *Hexagenia* may be found at deeper sites due to this infrequent disturbance. I also observed a significant cluster of high *Hexagenia* densities near the western shoreline of Lake Erie (Figures 5-1, 5-2d, and 5-3d), where many adults swarm (Masteller and Obert 2000). Female adults from locations of dense *Hexagenia* swarms may be more likely carried east, in the direction of prevailing winds before depositing eggs. The sites with high densities of *Hexagenia* are clustered within 25 km of the western shoreline, and this distance may represent the maximum distance *Hexagenia* can fly into the basin and deposit eggs in addition to the distance eggs travel through the water column.

Even though *Hexagenia* have been shown to select for soft-sediment covered with *Dreissena* clusters at a small-scale, *Dreissena* density was not a good predictor of *Hexagenia* density in western Lake Erie (Table 5.1). This may be since *Hexagenia* and *Dreissena* densities were not positively correlated in western Lake Erie (Figures 4-5 and 4-6c); however, *Hexagenia* were more likely to occur where *Dreissena* are present (Table 4.1). When *Dreissena* were present, *Hexagenia* densities were slightly lower, but much less variable. Also, the proportion of sites without *Hexagenia* was very low; only 8% of the sites with *Dreissena* were absent of *Hexagenia*, compared to 20% of the sites without *Dreissena*. *Hexagenia* were more likely to be found in areas with *Dreissena*, and although *Hexagenia* were not reaching densities greater than 1000/m^2 when co-occurring with *Dreissena*, *Dreissena* may restrain *Hexagenia* to population levels more in accordance with what is seen as a “healthy” *Hexagenia* density (Lake Erie Commission, 2004).

Hexagenia and *Dreissena* densities in western Lake Erie appeared to be highly variable, both spatially and temporally. At the spatial scale of the western basin of Lake Erie (Figure 4-2), both *Hexagenia* and *Dreissena* populations were distributed randomly (Figure 4-6). These random spatial distributions may be due to the fact that both species have a planktonic early life history stage (*Dreissena* veligers and *Hexagenia* eggs) and planktonic larvae act as passive particles and are at the mercy of the currents until they settle out of the water column (Hannan 1984; Jackson 1986). For *Hexagenia*, there was a weak positive correlation of density at a distance of zero (Figure 4-6b), which represents a correlation at the same site through time. This suggests that *Hexagenia* densities at a number of sites were not temporally variable, and may represent the very stable low-density *Hexagenia* sites (Figure 5-4). This is different from sites with high *Hexagenia* densities, where many sites exhibited a two-year cycle (Figures 5-4c and 5-5), suggestive of density-dependent regulation of the population. Very high densities of *Hexagenia* may consume so much oxygen that they can create their own pockets of hypoxia above the sediment, resulting in localized mortality of *Hexagenia* (Krieger 2004). After adult *Hexagenia* deposit their eggs, they quickly fall to the bottom sediment, where the previous years' *Hexagenia* cohort is located. Therefore, if a high density of *Hexagenia* was already located at that site, the later *Hexagenia* instars may consume the newly deposited eggs or the newly hatched instars may compete for space. *Hexagenia* densities in western Lake Erie are known to be highly variable (Krieger et al. 1996; Schloesser et al. 2000; Bridgeman et al. 2006), but this variability may not be all due to changing environmental conditions, but rather a density-dependent regulation of the *Hexagenia* population.

In conclusion, the current range expansion of dreissenid mussels onto soft sediments and the observed small-scale habitat selection by burrowing mayflies for *Dreissena* clusters may have potential cascading effects to higher trophic levels and overall ecosystem functioning. At a small-scale, *Hexagenia* may be incurring benefits from choosing habitat beneath *Dreissena*, such as increased food resources and protection from predation during high and low oxygen conditions. At the western basin-wide scale, *Hexagenia* were more likely to be present where *Dreissena* are also present. However, abiotic factors appear to be the diving force behind *Hexagenia's* spatial distribution, and temporal fluctuations may be a result of a density-dependent regulation of the population. What is important is that *Dreissena* presence and resulting habitat alterations are not inhibiting *Hexagenia* presence, and *Hexagenia* are maintaining sustainable population levels in areas with high densities of dreissenids in western Lake Erie.

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