HOST FISH SUITABILITY TESTS AND EFFECTS OF INVASIVE MOLLUSKS FOR
SNUFFBOX, *EPIOBLASMA TRIQUETRA*, FROM LAURENTIAN GREAT LAKES BASINS

Mandi L. Caldwell

A thesis submitted in partial fulfillment of
the requirements for the degree of
Master of Science

Department of Biology

Central Michigan University
Mount Pleasant, Michigan
August 2014

ABSTRACT

HOST FISH SUITABILITY TESTS AND EFFECTS OF INVASIVE MOLLUSKS FOR
SNUFFBOX, *EPIOBLASMA TRIQUETRA*, FROM LAURENTIAN GREAT LAKES BASINS.

by Mandi L. Caldwell

Freshwater mussel populations have declined, in part, from changes in host communities
and an increase in invasive species. With an increasing number of State and Federally listed
mussels, host fish suitability information and understanding how invasive species influences host
and mussel populations can help direct management practices. This study tested host fish origin
impacts on *Epioblasma triquetra* transformation into juvenile mussels. Recent research shows
that, *Lampsilis cardium* and *Lasmigona costata,* exhibit two genetically distinct populations in
lower Michigan: the Lake Erie basin and the lakes Michigan and Huron basins. The objectives
of our study were two fold, first, to quantitatively determine if *Percina caprodes* and *Percina
maculata* from the lake Michigan, Erie and Huron basins are equally suitable hosts for *E.
triquetra.* Secondly, this study examined the relationship between the Federally Endangered
Epioblasma triquetra and invasive mollusks. Host fish suitability tests were conducted in Fall
2012, Spring 2013, and Fall 2013 with *P. caprodes* and *P. maculata*. *Percina caprodes* was re-
confirmed to be a successful host and *P. maculata* was also determined to be a successful host
for the first time in the Great Lakes region. Results suggest no differences in juvenile
transformation with allopatric and sympatric fish and mussel pairings based on Great Lakes
basin origin; therefore, transformation success may not parallel differences in genetic structure.
In addition, results suggest seasonal differences in the developmental stages of *E. triquetra*
juveniles between spring and fall. We found that invasive mollusk abundance ($p = 0.021$, $r_s = -
0.316$) and biomass ($p = 0.008$, $r_s = -0.357$) can have significant relationships on *E. triquetra*
length. In addition, relationships with *Corbicula fluminea* and dreissenids on *E. triquetra*

differed in the two study rivers (Flat and Clinton). We found a significant negative relationship with dreissenid abundance ($p = 0.001$, $r_s = -0.434$) and biomass ($p \ll 0.001$, $r_s = -0.465$) with *E. triquetra* at the Clinton River location which had a density of dreissenids of 15 m^{-2}. This relationship was not present in the Flat River where dreissenid density was 2 m^{-2}. Locations with higher invasive species presence had lower juvenile transformation. Knowing the most effective way to optimize juvenile transformations as well as *E. triquetra* interactions with invasive species can make reintroductions and therefore conservation of *E. triquetra* more successful. These data will help in developing recovery strategies for *E. triquetra* in the Laurentian Great Lakes.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

SNUFFBOX (*EPIOBLASMA TRIQUETRA*) HOST FISH SUITABILITY TESTS USING FISH
FROM MULTIPLE GREAT LAKES BASINS

Introduction

With the ever increasing presence of human degradation, extinction rates of all organisms

have increased 100 to 1000 times since pre-human times (Pimm et al. 1995) and some scientist

believe humans are responsible for the next mass extinction (Barnosky et al. 2011). One group

of organisms hit hardest by human impacts is the native freshwater mussels (Family: Unionidae).

Currently, 72% of freshwater mussels in North America are either endangered, threatened, or

need protection predominately due to human actions (Haag 2012, Vaughn 2012). With over 300

species of freshwater mussels in North America, they encompass a variety of different guilds

(e.g., host use, brood time, habitat use).

Some freshwater mussels are more vulnerable to extinction; for example, smaller species,

species that are long term brooders may be more vulnerable to extinction than others (Strayer

2008, Haag 2012). However, all species are important to the aquatic community. Freshwater

mussels are important to aquatic ecosystems because they stabilize stream beds, oxygenate the

sediments and filter large portions of the water column (Haag 2012). Being a dominant presence

in the benthic community, making up the majority of zoobenthic biomass, freshwater mussels

can filter 10 to 100% of the water column per day (Haag 2012). Additional aquatic ecosystem

benefits include altering benthic communities through biodepositing faeces and pseduofaeces,

and adding nutrients to the water from excretion, all which play an important role in water

quality (Vaughn and Hakenkamp 2001). Changes in water quality can be identified while

studying freshwater mussel shells and tissue (Williams et al. 1993) because freshwater mussels

have long lifespans that accumulate various toxic substances that allow scientists to determine stream health (Cope et al. 2008).

In addition to providing aquatic ecosystem services, freshwater mussels also provide a link to terrestrial systems because mammals such as river otters, raccoons, and minks use them as a food source (Haag 2012). Along with being a food source for terrestrial organisms, freshwater mussels carry out important ecosystem processes. Sites with higher unionid richness lead to an increase in aquatic invertebrate emergence rates (Allen et al. 2012). An example of this linking the aquatic environment to the terrestrial ecosystems is that increasing number of aquatic invertebrates has been determined to increase the abundance of terrestrial spiders at a site (Allen et al. 2012). Freshwater mussels play a key role in food webs since food webs are held together by small interactions that tend to get overlooked (McCann et al. 1998, Allen et al. 2012). It is important for conservationists to focus on maintaining the small interactions that keep the system stable (McCann et al. 1998). High biodiversity has been found to help an ecosystem be more resilient to disturbances (Walker 1995).

The life cycle of a freshwater mussel involves a parasitic stage on fish which is highly susceptible to these disturbances (Strayer 1999, McMahon 2002). Some freshwater mussels require specific host fish, an obligate ectoparasitic relationship, for glochidia development (Strayer et al. 1999, Strayer 2008). There are two types of freshwater mussels involving host fish use; generalist and specialists. Generalists are able to metamorphose on a wide range of fish families and species. Contrastingly, specialists can only transform successfully on a limited range of fish species or in some cases some species can only transform on one fish species (Haag 2012). Glochidia attach to the fish's gills until they develop into juveniles (Strayer 2008). The glochidia attached to the host fish uptakes nutrients from the fish until fully developed, before

2

excising (Barnhart et al. 2008, Strayer 2008). In order for the juvenile to survive, it must land in suitable habitat and burrow itself into the substrate (Strayer 2008). The likelihood that all glochidia survive to become juveniles and land in suitable habitat is highly unlikely; therefore, freshwater mussels produce large numbers of glochidia [up to millions of glochidia (Bauer and Wachtler 2001)] and have adapted strategies to attract their host fish (Barnhart et al. 2008). If a mussel population is not surviving it could be because the disappearance, shift, decline or change in host or host community (Newton et al. 2008). Freshwater mussel distribution is dependent on host fish (Watters 1992, Strayer 2008, Haag 2012).

The snuffbox, *Epioblasma triquetra* (Rafinesque 1820; (Family Unionidae)*,* is a Federally endangered native freshwater mussel and had developed a unique way to capture its host (Barnhart et al. 2008). Gravid females, *E. triquetra,* gape to attract and capture (i.e., trap) a host fish. When a fish approaches and inserts its rostrum into the valve gape, *E. triquetra* clamps its valves onto the fish and pumps glochidia onto the gills of the trapped fish (Barnhart et al. 2008). *Epioblasma triquetra* host fish are limited because not all fish can survive being captured. *Percina caprodes* (logperch; Rafinesque 1818)*,* has a thick skull that prevents *E. triquetra* from crushing its head making it a suitable host (Barnhart et al. 2008). Zanatta and Wilson (2011) found that there is a covarying pattern of genetic differentiation between *P. caprodes* and *E. triquetra* in the United States and Canada. This finding suggests that this combination of mussel and host share a history and a coevolutionary relationship (Zanatta and Wilson 2011) and could explain why the *P. caprodes* is a productive host. Laboratory host fish testing for freshwater mussels is currently the most successful way to determine potential host fish in nature. Laboratory host fish tests have found a variety of potential hosts for *E. triquetra*, *Cottus hypselurus* (Ozark sculpin; Robins and Robison 1985), *Cottus baileyi* (black sculpin;

Robins 1961) *Fundulus olivaceus* (blackspotted topminnow; Storer 1845), *Cottus bairdii* (mottled sculpin; Girard 1850), *Cottus carolinae* (banded sculpin; Gill 1861), *Etheostoma caeruleum* (rainbow darter; Storer 1845), *Etheostoma exile* (Iowa darter; Girard 1859), *P. maculata* (blackside darter; Girard 1859) and *P. caprodes* (Yeager and Saylor 1995, Hillegrass and Hove 1997, Barnhart et al. 1998, Jones and Neves 2000, Watters et al. 2005, US Fish and Wildlife Service 2012). Although numerous studies have been conducted on *E. triquetra,* few have been conducted in the Great Lakes region;The University of Guelph has conducted *E. triquetra* host fish suitability studies concluding that *P. caprodes* is a successful host and that the invasive *Neogobius melanostomus* (Pallas 1814) is a confirmed but inefficient host (Castanon et al. 2011).

Epioblasma triquetra* were used for this study to increase the understanding of the host fish and freshwater mussel relationship since this is unknown for Michigan rivers. For this study, *P. caprodes* and *P. maculata* were tested because they are available in the local basins and have been found to be successful hosts in previous basins (Hillegrass and Hove 1997, Barnhart et al. 1998). Host fish tests were performed on *P. caprodes*, *P. maculata* and *E. triquetra* from the lower Great Lakes basins. *Lampsilis cardium* (Rafinesque 1820) and *Lasmigona costata* (Rafinesque 1820), from the lakes Huron and Michigan drainages were found to be indistinguishable genetically, but distinct from the Lake Erie drainage (Bergner 2013). The distinct populations within Michigan are likely due to post-glacial colonization of the central Great Lakes by freshwater mussels and their host fish (Bergner 2013). Although *E. triquetra* were not tested by Bergner (2013) a related study in Ontario did show that an *E. triquetra* in the Lake Huron drainage (Ausable River, Ontario) was genetically distinct from a population in the Sydenham River (Lake St. Clair drainage) (Gabraith et al. In review). Because *E. triquetra* has a

similar mode of dispersal, requirement of a host fish, and distribution to the species assessed by Bergner (2013) it would be expected to have a similar genetic pattern. Furthermore, *E. triquetra* and its host *Percina caprodes* have been determined to share similar genetic patterns (Zanatta and Wilson 2011).

Host fish testing has been conducted using various methods to determine suitable fish for specific freshwater mussels and understand the host-parasite relationship (Zale and Neves 1982, Allen et al. 2007, Lima et al. 2012). Host fish suitability tests have been conducted using different methods depending on the objective of the study. One objective of host fish testing involves considering the origin of fish and using mussels from a different area. The majority of host fish suitability tests use allopatric fish and freshwater mussel populations with the objective of avoiding using fish that have developed immunity to glochidia or are currently infested with glochidia (Zale and Neves 1982, Haag and Warren 1997, Jones et al. 2004, Dodd et al. 2005, Jones et al. 2006, Taeubert et al. 2012).

In addition to allopatric host fish studies, there has been some research on sympatric fish and mussel communities and their compatibility. Taeubert et al. (2010) tested different salmonid strains that were from areas in and outside of the freshwater pearl mussel, *Margaritifera margaritifera* (Linnaeus 1758)*,* distribution. They found the trout strain from *M. margaritifera* home location was the most successful (Taeubert et al. 2010). Another study, found that co-adaption of host fish and mussels may contribute to the success of laboratory rearing juveniles; therefore, fish that live in the same stream with mussels may be more productive host fish (Rogers et al. 2001). Rogers et al. (2001) used fish and mussels from various river drainages (Tennessee River and Atlantic slope drainage), in comparison to this study that looks at Great Lakes basins (approximately 521,000 km^2) which is a larger scale. In addition, Rogers et al.

(2001) study was focused in an unglaciated region; therefore, this study is an older system and has had longer to allow populations to differ genetically.

With native freshwater mussel populations in decline, it is imperative that we have effective and empirically driven propagation programs. There has been an increase in propagation efforts throughout North America; however, not many studies have been conducted on the endangered *E. triquetra* in the Great Lakes basins. There are two objectives for this study focusing on how to maximize successful propagation efforts. The first objective of is to quantitatively determine host fish suitability of *E. triquetra* with *P. caprodes* and *P. maculata* based on individuals (fish and mussels) from multiple Great Lakes basins. Understanding the relationship between fish and freshwater mussels is important for conservation purposes; knowing the best combinations to produce the most juveniles can help with introducing more propagated freshwater mussels back into the streams and also understanding the reason for potential declines in nature. Experimental host fish tests were conducted to determine how successful *P. caprodes* and *P. maculata* are as host fish based on rivers from basins of lakes Michigan, Erie, and Huron. It is hypothesized that fish from the same basin and hypothesized genetic population (Bergner 2013) as the freshwater mussel will be most compatible (i.e., the most successful in producing juveniles). It is expected that fish from the same community as *E. triquetra* will have more successful transformations of glochidia to juveniles than fish from other basins (Rogers et al. 2001, Taeubert et al. 2012). Therefore, it can be predicted that *P. caprodes* and *P. maculata* from the rivers from the Lake Erie basin will produce more juveniles with *E. triquetra* from the rivers from Lake Erie basin than from fish from the rivers from the Lake Huron or Lake Michigan basins. Fish from the rivers of Lake Huron and Michigan basins will also be predicted to be more successful with *E. triquetra* from the Michigan basin. In addition,

for our second objective, we address which fish is the most suitable host, *P. caprodes* or *P. maculata* and hypothesize that *P. caprodes* will be the most successful host because of their close coevolutionary relationship (Zanatta and Wilson 2011). In addition, the phylogenetic relationship between *P. caprodes* and *P. maculata* suggests that these species are not closely related (Near 2002) adding further support for our hypothesis.

Methods

Sampling Sites

Epioblasma triquetra, *Percina caprodes,* and *Percina maculata* were collected from rivers in the lower peninsula of Michigan Fall 2012, Spring 2013, and Fall 2013; all *E. triquetra* collected were gravid. Throughout this study, the origin of fish and mussel are referenced with their basin origin, this refers to the inland rivers of that particular basin. For example, *E. triquetra* from the Michigan basin, refers to when *E. triquetra* were collected from the inland rivers within the Lake Michigan basin, not the actual lake. Female *E. triquetra* were collected from the Flat River of the Michigan basin (42.928092˚N, -85.338081˚W) and from the Clinton River of the Lake Erie basin (42.62825˚N, -83.395886˚W). These are two of the 10 known remaining US populations in Michigan; there are no known populations of *E. triquetra* extant in the Lake Huron basin (US Fish and Wildlife Service 2012). Fish were collected from the Michigan [Flat River (42.92599˚N, -85.35418˚W) and Maple Rapids River (43.14088˚N, -84.59924˚W)], Erie [Huron River (42.56182˚N, -83.50249˚W) and Wolf Creek (41.91300˚N, -84.06477˚W)] and Huron basins [Salt River (43.69206 ˚N, -84.54799 ˚W), Chippewa River (43.60916 ˚N, -84.78219 ˚W) and Cass River (43.32549 ˚N, -83.73886 ˚W)].

Quantitative host fish determination

During the summer of 2012 and 2013 fish were collected from the inland rivers of three basins, Erie, Michigan and Huron, with a Smith-Root L-24 backpack electrofisher and a seine. From each basin the goal was to collect ten *P. maculata* and ten *P. caprodes*. *Percina maculata* and *P. caprodes* were selected as possible hosts based on past laboratory experiments that documented *E. triquetra* glochidia metamorphosis (Hillegrass and Hove 1997, Ohio State University (OSU) Division of Molluscs 2009, US Fish and Wildlife Service 2012). In addition, fish species were chosen due to availability in the rivers of Lake Erie, Michigan and Huron basin and benthic dwelling habitat preferences of fish making them more likely to interact with *E. triquetra*. Fish were held in individual Aquatic Habitat (AHAB, Apopka, Florida) tanks (3L and 10L based on fish size) for the experiment. Fish were held in tanks for up to 90 days prior to the experiment.

Gravid female *E. triquetra* were collected using viewers and collection baskets from the Michigan (Flat River) and Erie (Clinton River) basins, from sites established from quantitative excavation sampling (Strayer and Smith 2003) in 2009-2010 (Bergner 2013). *Epioblasma triquetra* were transported to Central Michigan University host fish laboratory. Glochidia were extracted from the gravid mussels by flushing the marsupial gills with deionized water using a syringe (Zale and Neves 1982, Allen et al. 2007). A subsample of the glochidia from each female was tested for viability using 24% NaCl solution. If at least 80% of the glochidia shut in the salt water they were considered viable (Lefevre and Curtis 1912) and were used in the experiment within minutes of extraction. A subset of glochidia from each mussel was counted under a dissection microscope and only glochidia from mussels that had over 150 glochidia mL^{-1} were used for the experiment. The glochidia were separated into 6 subsamples for each Great

8

Lakes basin for each fish, with a total of 12 treatments (Table 1). For the Fall 2012, Spring

2013, and Fall 2013 experiments, multiple gravid female mussels were used from each basin.

Spring 2013 had above average high stream flows reducing the number of gravid females

collected from the Flat River.

Table I.1. Treatments used for host fish suitability test. *Epioblasma triquetra* glochidia from the inland rivers of Michigan or Erie basin were used to infest potential host fish; F1, F2, and F3 = number of individual fish used for each treatment. F1= Fall 2012, S2= Spring 2013, F3= Fall 2013.

E. triquetra Origin (Basin)	Fish Origin	Fish Species	Code
Michigan	Michigan	*Percina caprodes*	F1=7, F3=11
		Percina maculata	F1=13, F3=11
	Erie	*Percina caprodes*	F1=13, F3=11
		Percina maculata	F1=3, F3=11
	Huron	*Percina caprodes*	F1=8, F3=10
		Percina maculata	F1=11, F3=11
Erie	Michigan	*Percina caprodes*	F1=6, S2=3, F3=10
		Percina maculata	F1=13, S2=5, F3=10
	Erie	*Percina caprodes*	F1=16, S2=5, F3=10
		Percina maculata	F1=3, S2=5, F3=10
	Huron	*Percina caprodes*	F1=9, S2=5, F3=8
		Percina maculata	F1=11, S2=5, F3=8

After the host fish were separated by basin; half of the fish were used for the Erie basin

E. triquetra treatments and half was used for the Michigan basin *E. triquetra* treatments (Table

1). All fish subsamples for each treatment were placed in an aerated bucket with 5000 mL of

tank water. The glochidia that were placed in a petri dish were rinsed with tank water to ensure

the whole sample went in the bucket; the amount of water used to rinse the petri dish was

recorded for each sample. The fish were exposed to the glochidia for 60 minutes in the bucket

and observed periodically for any signs of lethargy (Zale and Neves 1982). Three samples of 40

mL of water were collected after the end of the experiment from the aerated bucket while

9

agitating the water (and non-attached glochidia) that remained. All glochidia were counted from each of the three 40 mL samples to determine how many glochidia did not attach to fish and then were stored in 90% ethanol for future studies. The three 40 mL samples were then averaged to represent the average glochidia left in each infestation bucket. After the infestation, each fish was placed in an individual AHAB tank. A catcher, made with PVC pipe affixed with 125 µm mesh, was attached to each tank to catch glochidia or juvenile *E. triquetra* that drop off the fish. At the end of the experiment (i.e., at least 40 days or 10 days without juveniles excising off fish beyond the initial 40 days), the AHAB tanks of each fish were put through a 125 µm mesh to collect all glochidia and juveniles that remain in the tank.

Each day catchers were removed and examined with a Leica EZ4 dissection microscope. The number of glochidia and juveniles were counted and recorded daily. This experiment was repeated three times (Fall 2012, Spring 2013, and Fall 2013). Infestation intensities were determined by the total juveniles and glochidia that had attached to each fish (Crane et al. 2011). Host fish experiments were analyzed using univariate statistics; a two-way ANOVA was used to determine the relationships between percent transformation (juveniles/ [glochidia attached + juveniles]) for each fish and mussel pairing to determine any relationships. A Shapiro-Wilk test was used to determine normality and a Bartlett Test of Homogeneity of Variances was used to evaluate variance. Type III two-way ANOVAs were used whenever there was an interaction between the fish and mussel basins, if no interaction was determined then Type II two-way ANOVAs were used followed by a Tukey's post hoc test to distinguish the significant differences (McKillup 2006, Zar 2010). In an attempt to standardize for the varying rates of glochidia infestation concentrations each year, standardized transformation success (juveniles/ initial glochidia in each treatment bucket) for each pairing was calculated and analyzed using a

two-way ANOVA. In addition, Spearman's rank tests were performed to determine if fish length

is correlated with transformation success and fish length and infestation intensity.

Results

Gravid *E. triquetra* were found in the Clinton River during June, August, and October

and gravid *E. triquetra* were found in the Flat River during July, August, and October. For Fall

2012 and Fall 2013 experiments, 3 gravid female mussels were used for each basin. Spring 2013

was extremely rainy, so we were unable to collect gravid females from the Flat River because of

high water. Therefore, for Spring 2013, only 2 gravid females were found and used for the

experiment from the Clinton River in the Erie basin.   For the experiment, there were twelve

treatments (six *P. caprodes* treatments and six *P. maculata* treatments), except for Spring 2013

which had only six treatments (Table 1). Although our goal was to infest equal numbers of fish

for each treatment, the samples sizes varied for each basin due to availability of fish, thus we

were not able to meet our goal of 10 individuals in each treatment resulting in unequal sample

sizes.

Host fish suitability

In order to determine if fish basin origin or *E. triquetra* basin origin or a consideration of

the two impacts the success of propagation, first, we had to determine if *P. caprodes* and *P.*

maculata were hosts for *E. triquetra* in the Great Lakes inland rivers of Michigan. *Epioblasma*

triquetra glochidia encysted and metamorphosed on both fish species that were tested. A total of

844 metamorphosed *E. triquetra* juveniles or an average of 6.39 fish^{-1} excysted from *P.*

carprodes 13 to 65 days after the infestation (peaking at day 25 for Fall 2012, day 29 for Fall

2013, and day 20 for Spring 2013) from the Fall 2012, 2013 and Spring 2013 experiments with

11

33.4% overall transformation success (Table 2 and 3). A total of 130 *E. triquetra* juveniles or

1.01 fish^{-1} excysted from *P. maculata* 13 to 45 days after infestation (peaking at day 25 for Fall

2012, day 21 for Fall 2013, and day 20 for Spring 2013) from the Fall 2012, 2013 and Spring

2013 experiments with 9.9% overall transformation success (Table 2 and 3).

Epioblasma triquetra development and seasonal differences

For all experiments, the majority of untransformed glochidia (approximately 65.5%)

excised within the first two days of the initial infestation and continued to excise for 28 days

(Table 2). For Fall 2012 and Fall 2013 experiments for both fish species there was a period of

developing juveniles dropping off from day 13 to day 22 (Table 2). Juveniles that excised, but

were not fully developed (lacking a dark spot of a developing heart, developed tissue but no foot

movement) were considered "developing juveniles"; however, we do not consider them

glochidia because the shells are thicker and tissues were beginning to form. The developing

juvenile or transition stage is the intermediate stage between glochidia and full function juveniles

and to our knowledge it the first time this stage and timing has been observed and quantified.

For the Spring 2013 experiment with both fish species there was no developing juvenile stage;

fully transformed juveniles began to drop off starting day 13 and majority finished dropping off

after day 28 (Table 2). Fall 2012 and 2013 experiments began to produce fully developed

juveniles around day 20 and lasted to approximately day 40 (Table 2). However, 10% (6 fish) of

P. caprodes each produced one juvenile between day 45 to day 65.

Table I.2. Infestation timeline from initial infestation day to 45 days (juveniles continued to drop off until day 65 but less than 5% of experimental fish produced juveniles after 45 days) post infestation for *Epioblasma triquetra* using *Percina caprodes* (LP) and *Percina maculata* (BSD) for experiments conducted in Fall 2012, Spring 2013, and Fall 2013 on species from the Flat and Clinton rivers, Michigan. The timeline displays the drop off periods of glochidia, developing juveniles, and fully developed juveniles. ▢ =>0-4.9%, ▨ =5-9.9%, ▨ =10-24.9%, ▨ =25-49.9%, and ■ =50% or greater. Percentages represent the proportion of fish that glochidia, developing juveniles, or fully developed juveniles excise off on the day (see text for definitions of developing juveniles versus fully developed juveniles)

Infestation Results Fall 2012, Spring 2013, Fall 2013

Days	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
Spring 2013 Glochidia LP	
Fall 2012 Glochidia LP	
Fall 2013 Glochidia LP	
Spring 2013 Glochidia BSD	
Fall 2012 Glochidia BSD	
Fall 2013 Glochidia BSD	
Spring 2013 Developing LP	
Fall 2012 Developing LP	
Fall 2013 Developing LP	
Spring 2013 Developing BSD	
Fall 2012 Developing BSD	
Fall 2013 Developing BSD	
Spring 2013 Juveniles LP	
Fall 2012 Juveniles LP	
Fall 2013 Juveniles LP	
Spring 2013 Juveniles BSD	
Fall 2012 Juveniles BSD	
Fall 2013 Juveniles BSD	

13

Table I.3. *Epioblasma triquetra* host fish suitability test results for Fall 2012, Spring 2013 and Fall 2013 results for *Percina caprodes* and *Percina maculata.*

Experiment	Host Fish	Juveniles	Number of Fish	*E. triquetra* juveniles produced per fish	Percent transformation
Fall 2012	*P. caprodes*	462	59	7.83	37.4%
	P. maculata	39	54	0.72	5.5%
Spring 2013	*P. caprodes*	253	13	19.46	35.8%
	P. maculata	12	14	0.80	7.4%
Fall 2013	*P. caprodes*	129	60	2.15	26.9%
	P. maculata	79	61	1.30	16.7%
Average	***P. caprodes***	**281**	**44**	**6.39**	**33.4%**
	P. maculata	**43.3**	**43**	**1.01**	**9.9%**

Host fish suitability *Epioblasma triquetra* basin and fish basin interactions

For Fall 2012 *P. maculata* experiments, there was no difference between fish basin origin or *E. triquetra* basin origin on percent transformation of *E. triquetra* to the juvenile stage (Table I.4). For *P. caprodes* in Fall 2012, a difference was found with percent transformation and *E. triquetra* basin origin ($\chi^2 = 15.5$, $df = 1$, $p << 0.001$). Indicating there was a difference between the inland river sites for the Lake Michigan basin *E. triquetra* and the inland river sites for the Lake Erie basin *E. triquetra* (Figure I.1, Table I.4).

Table I.4. Two-way ANOVA results for host fish suitability tests comparing fish (*Percina maculata* and *Percina caprodes*) and *Epioblasma triquetra* basin origins with transformation success and standardized transformation success for Fall 2012 and Fall 2013 experiments. Basin origins refer to the inland rivers of those basins. * = $p < 0.10 – 0.05$ and ** = $p < 0.05$.

Time of Experiment	Type of Comparison	*Percina maculata*		*Percina caprodes*	
		Transformation Success (probability)	Standardized Transformation	Transformation Success	Standardized Transformation
		P	P	P	P
Fall 2012	Fish Basin	0.360	0.078*	0.139	0.385
	Mussel Basin	0.090*	0.099*	<<0.001**	<<0.001**
	Fish x Mussel Basin	0.462	0.215	0.434	0.075*
Fall 2013	Fish Basin	0.256	0.021**	0.148	<<0.001**
	Mussel Basin	0.035**	0.051*	0.426	0.024**
	Fish x Mussel Basin	0.641	0.283	0.836	0.863
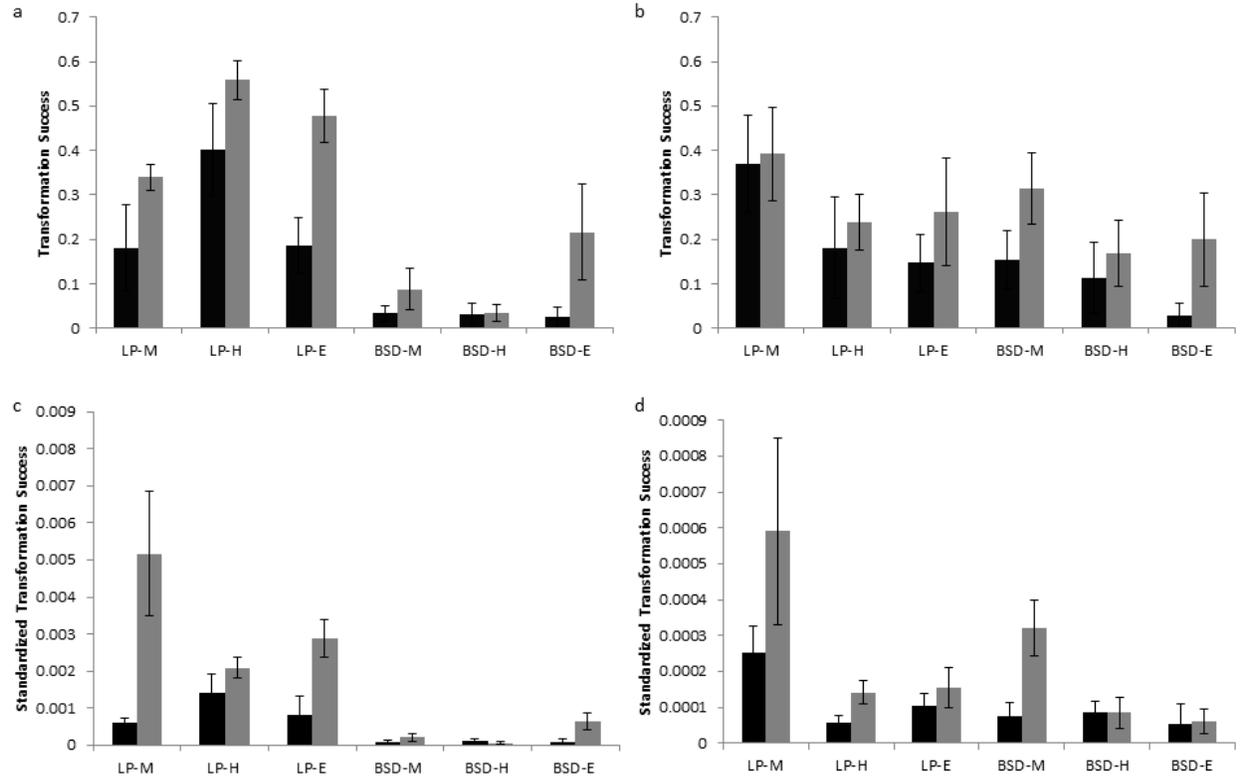
Figure I.1. Transformation success (juveniles/ [juveniles + glochidia]) for a) Fall 2012 experiment and b) Fall 2013 experiment and standardized transformation success for c) Fall 2012 experiment and d) Fall 2013 organized by *Epioblasma triquetra* basin and fish basins that are from inland waters within the Erie, Michigan or Huron basin. Black = Erie basin *E. triquetra* origin and gray = Michigan basin *E. triquetra* origin. BSD = *Percina maculata,* LP = *Percina caprodes*, M = Michigan, H = Huron, E = Erie origin. For example, LP-M is *P. caprodes* from the Michigan basin. Bars represent standard error.

For *P. maculata*, host fish suitability tests for Fall 2013, there was a difference in transformation rates for *E. triquetra* basin origin (χ^2= 4.45, *df* = 1, *p* = 0.035) with Erie basin *E. triquetra* being less successful in juvenile transformation success compared to Michigan basin *E. triquetra*. Results for *P. caprodes* suggest that there was no difference between fish or *E. triquetra* basin origins and transformation success (Figure I.1).

In addition, Spring 2013 host fish experiments were compared to Fall 2012 and Fall 2013. Since we were only able to run the experiment with *E. triquetra* from the Erie basin in Spring 2013, a two-way ANOVA was run to compare transformation success with fish basin and

time of experiment. For *P. maculata*, there was no significant difference between fish basin and time of experiment with transformation success. A Type III two-way ANOVA was run on the *P. caprodes* transformation success data for all three experiments that used *E. triquetra* from the Erie Basin. There was a significant interaction with the fish basin and year in relationship to transformation success ($\chi^2 = 11.4$, $df = 4$, $p = 0.023$). However, from the large numbers of possible interactions, no significant relationships were determined from the Tukey's post hoc test.

Standardized transformation success

When infesting fish with glochidia from *E. triquetra*, glochidia infestation concentrations were calculated for each fish basin origin and *E. triquetra* origin experiment. Glochidia infestation concentrations for each experiment varied. All glochidia were used resulting in unequal glochidia concentrations for infestation because of the extreme imperilment of *E. triquetra*. In an attempt to standardize for the varying rates of glochidia infestation concentrations each year (Figure I.2), standardized transformation success for each pairing of fish basin and mussel basin was calculated (see Methods) and analyzed using a two-way ANOVA. Type II two-way ANOVAs were conducted for Fall 2012 experiments. For *P. caprodes*, a difference was found with standardized transformation success ($\chi^2 = 16.9$, $df = 1$, $p \ll 0.001$) and the *E. triquetra* basins, with Michigan basin treatments being the most successful. However, for *P. caprodes* in Fall 2012, there was no difference found with standardized transformation success and fish basins ($\chi^2 = 1.91$, $df = 2$, $p = 0.385$). For *P. maculat* for Fall 2012, there was no difference found between, fish basin origin or *E. triquetra* basin origin.
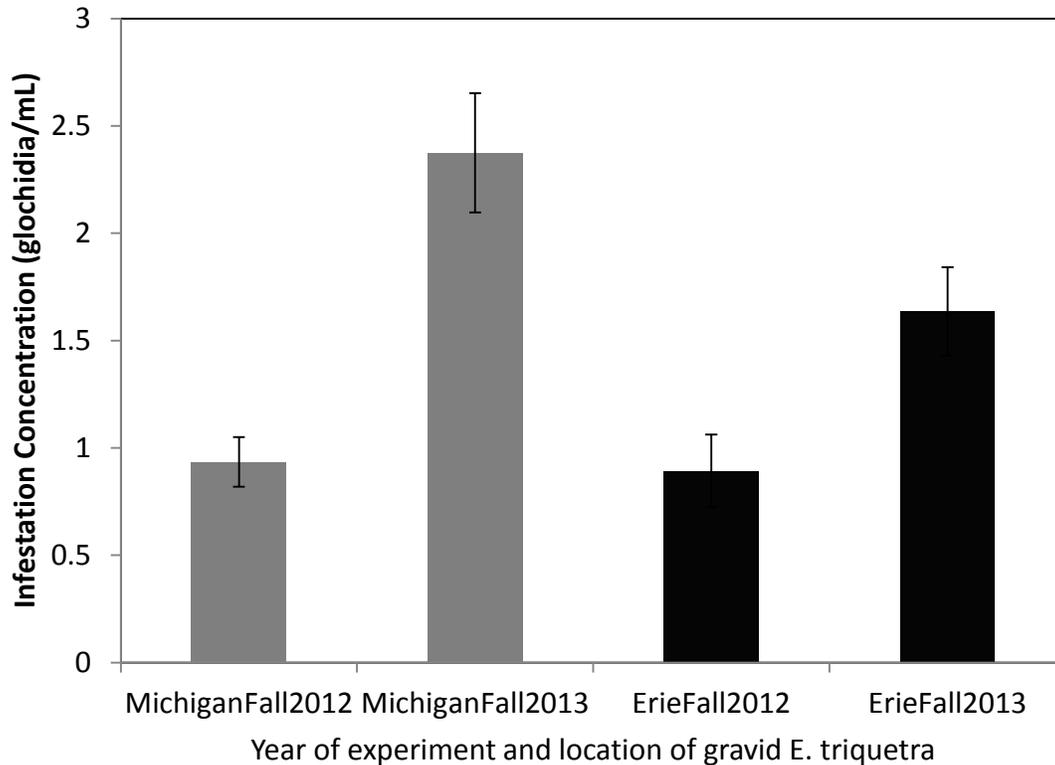
Figure I.2. Glochidia infestation calculation (glochidia mL^{-1}) *Epioblasma triquetra* originating from the inland rivers within the Michigan basin (gray) and *E. triquetra* originating from the inland rivers with the Erie basin *E. triquetra* (black) that each experiment was infested with for Fall 2012 and Fall 2013. Bars represent standard error.

For Fall 2013, Type II two-way ANOVAs were used to analyze results because there were no significant differences between the interactions of fish and mussel basin origins. *Percina caprodes* standardized transformation success was significantly different for fish (χ^2= 12.1, *df* = 2, *p* = 0.001) and *E. triquetra* basins origin (χ^2= 5.09, *df* = 1, *p* = 0.021). Lake Erie and Lake Michigan fish basins along with Lake Michigan and Lake Huron fish basins were significantly different from each other with Lake Michigan fish being the most successful (Figure I.1). In addition, *E. triquetra* origin was different with Lake Michigan origin glochidia having the most success. *Percina maculata* standardized transformation success was different for fish (χ^2= 7.76, *df* = 2, *p* = 0.021) and *E. triquetra* basin (χ^2= 3.81, *df* = 1, *p* = 0.051).

18

Epioblasma triquetra from the Lake Michigan basin were more successful transforming than Lake Erie basin *E. triquetra* (Figure I.1).

Spring 2013 host fish experiments standardized transformation successes were compared to Fall 2012 and Fall 2013. For *P. maculata*, there were no differences between fish basin and time of experiment with transformation success. A Type III two-way ANOVA was run on the *P. caprodes* transformation success for all three experiments that used *E. triquetra* from the Lake Erie Basin inland rivers. There was an interaction with the fish basin and year in relationship to transformation success (χ^2 = 14.4, df = 4, p = 0.006). A significant relationship ($p \ll 0.001$) was found between *P. caprodes* from the Lake Huron basin from Spring 2013 and *P. caprodes* from the Lake Erie basin from Spring 2013, *P. caprodes* from the Lake Erie basin from Spring 2013 and *P. caprodes* from the Lake Michigan basin from Spring 2013, *P. caprodes* from the Lake Erie basin from Spring 2013 and *P. caprodes* from the Lake Erie basin from Fall 2013, *P. caprodes* from Spring 2013 and *P. caprodes* from the Lake Michigan basin from Fall 2012, *P. caprodes* from Lake Erie basin from Spring 2013 and *P. caprodes* from the Lake Huron basin for Fall 2012, and *P. caprodes* from the Lake Erie basin from Spring 2013 and *P. caprodes* from the Lake Erie basin from Fall 2012. In addition, weaker relationships were found between *P. caprodes* from the Lake Michigan basin from Spring 2013 and *P. caprodes* from the Lake Erie basin from the Spring 2013 basin. Also, there was a difference with year and standardized transformation success, with Fall 2012 and Spring 2013 (p = 0.014) and Fall 2013 and Spring 2013 (p = 0.004) different from each other.

Host fish suitability and fish length

     A Mann-Whitney U test found that *P. maculata* (76 ± 0.895 mm) used for the experiment were smaller than *P. caprodes* (103 ± 1.097 mm) (W = 14155, $p \ll 0.001$). Positive relationships were found with fish length for both *P. caprodes* and *P. maculata* and transformation success (r_s = 0.450, $p \ll 0.001$) (Figure I.3). In addition, a positive relationship was found between *P. caprodes* length and infestation intensity (r_s = 0.294, p = 0.001) (Figure I.4). A marginally significant positive relationship was found between *P. maculata* length and infestation intensity (r_s = 0.163, p = 0.077). Lastly, there was a positive relationship with transformation success and infestation intensity (r_s = 0.173, p = 0.007) (Figure I.5).
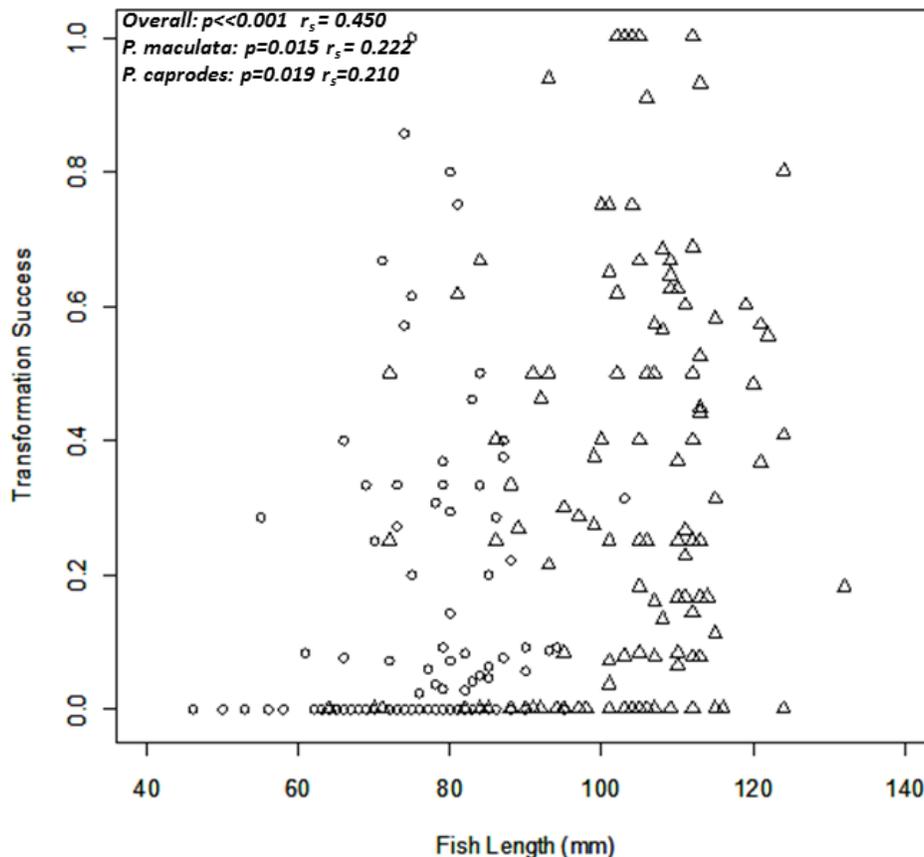
Figure I.3. Fish length of *Percina caprodes* (triangles) and *Percina maculata* (circles) for all experiments (Fall 2012, 2013 and Spring 2013) and transformation success relationship (juveniles/ [glochidia + juveniles]).
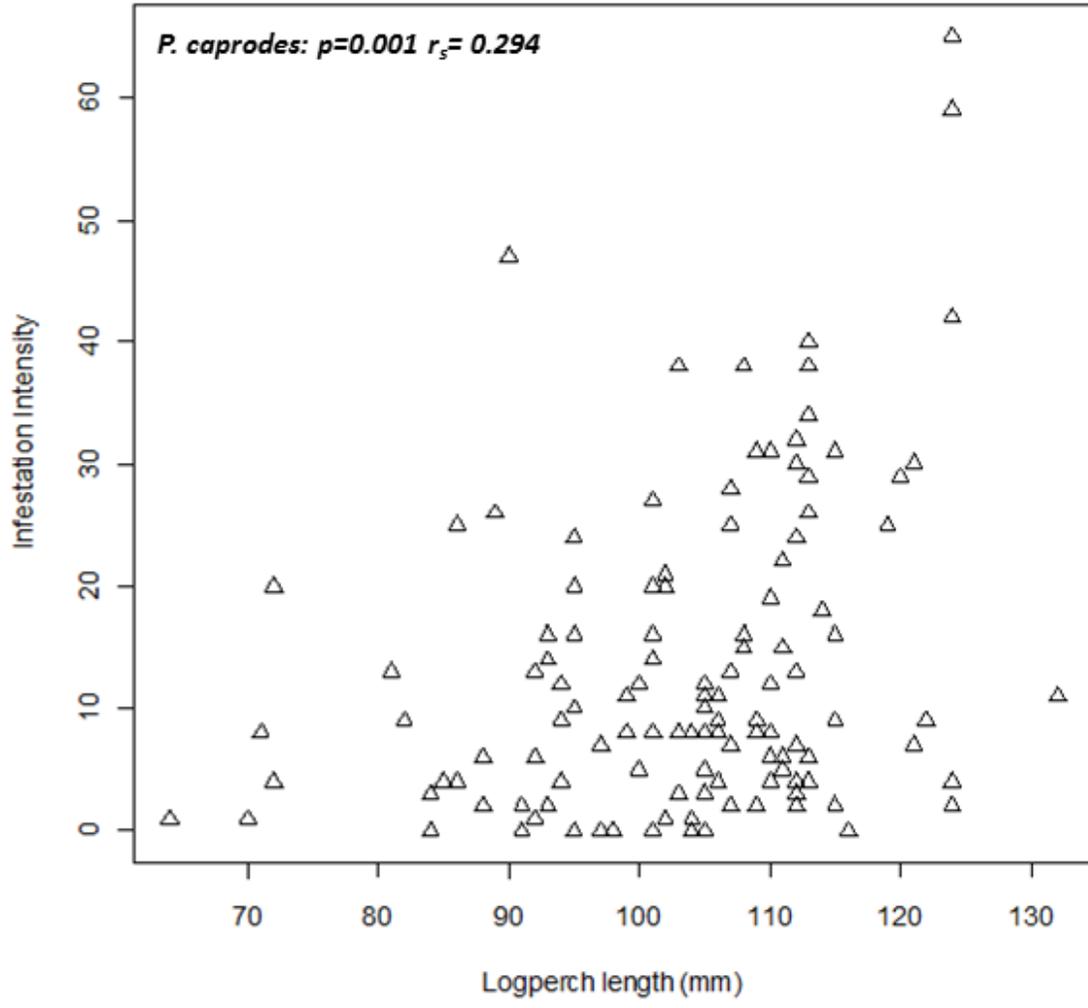
Figure I.4. *Percina caprodes* (logperch) length and infestation intensity (glochidia + juveniles) relationship from all host fish suitability test (Fall 2012, 2013 and Spring 2013).

21

Plot annotations:
- Overall: p=0.007 $r_s = 0.173$
- P. maculata: p=0.732 $r_s = 0.032$
- P. caprodes: p<0.001 $r_s = 0.315$

Y-axis: Transformation Success
X-axis: Infestation Intensity
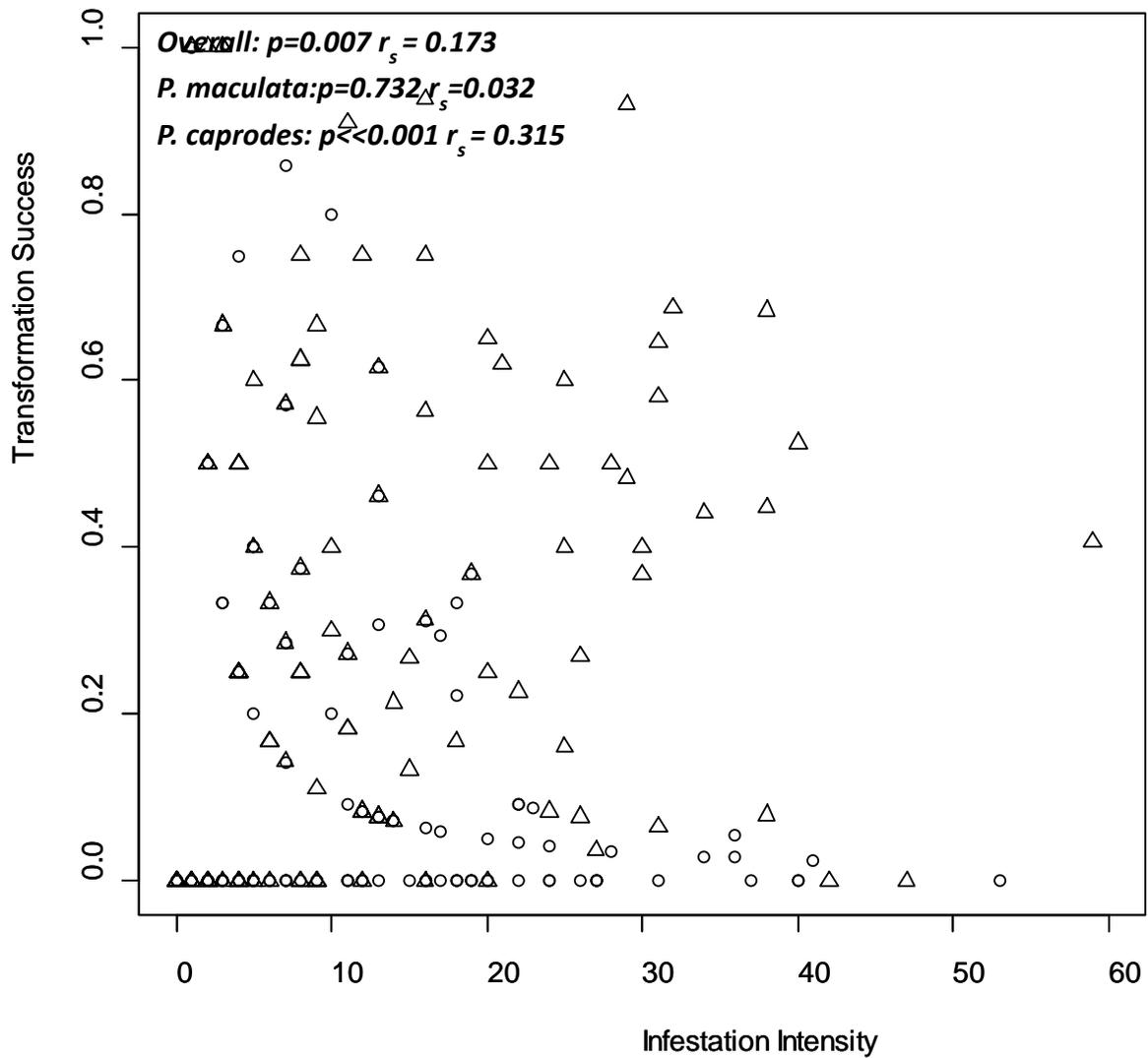
Figure I.5. Infestation intensity (glochidia + juveniles) and transformation success (juveniles/ [glochidia + juveniles]) for all *Epioblasma triquetra* host fish suitability experiments (Fall 2012, 2013 and Spring 2013), *Percina caprodes* (triangles) and *Percina maculata* (circles).

Discussion

Host fish suitability tests for all three experiments (Fall 2012, Spring 2013, and Fall 2013) determined that *Percina maculata* and *Percina caprodes* are successful hosts for *Epioblasma triquetra* located in rivers of Lake Erie and Lake Michigan basins. Our studies for *P. caprodes* are consistent with results from past research (Hillegrass and Hove 1997, Barnhart et al. 1998). This is the first time that *P. maculata* confirmed as a successful host for *E. triquetra* in the Great Lakes region. This also supports previous research that *P. caprodes* is a successful host in the Great Lakes region (Castanon et al. 2011). Our study found that *P. caprodes* is a much more successful host (33.4 ± 2.52 % transformation success rate) compared with *P. maculata* (9.9 ± 1.71 % transformation success rate).

The variation in success between fish species could be attributed to the biology of the host fish species and their history with *E. triquetra*. *Epioblasma triquetra* capture host fish by snapping its valves on the rostrum of the fish and then pumping glochidia onto the gills of the fish (Barnhart et al. 2008). *Percina caprodes* are able to endure being captured because of their thick skull that prevents them from being crushed (Barnhart et al. 2008), whereas this adaptation for *P. maculata* has never been documented and *P. maculata* has a smaller skull making this species less suitable of a host in nature since they are less likely to survive the trapping that *E. triquetra* appear to use during their infestation. The behavior of *P. maculata* is also unusual for a darter species, spending the majority of its time at mid-depth, rather than at the benthos, making it less likely to encounter a gravid *E. triquetra* (Scott and Crossman 1973). This suggests that although *P. maculata* successfully transformed *E. triquetra* glochidia, this interaction may not be common in nature. Confirmation of *P. maculata* as a host in this study suggests that *P. maculata*

23

could be an important secondary host and may be important for sustaining *E. triquetra* populations especially in areas with reduced or small populations of *P. caprodes*.

In addition to *P. caprodes* being less resilient to stress caused by the infection strategy of *E. triquetra*, research suggests they share a coevolutionary relationship. Zanatta and Wilson (2011) suggest that *P. caprodes* and *E. triquetra* demonstrate a covarying pattern of genetic differentiation in the central basin of North America. This suggests that the *E. triquetra* and *P. caprodes* relationship may have a coevolutionary history (Zanatta and Wilson 2011). Therefore, *P. caprodes* may be a more successful host than *P. maculata* because *E. triquetra* have evolved adaptations to successfully transform specifically on *P. caprodes*. It is hypothesized that *P. maculata* would not have a coevolutionary relationship with *E. triquetra* because of its limited success as a host for *E. triquetra* and that *P. maculata* is not as closely related to *P. caprodes* when compared to other *Percina* species (Near 2002). However, a potential coevolutionary relationship between *P. maculata* and *E. triquetra* has not been investigated. Knowing the most successful host fish species for an endangered species is imperative for successful conservation efforts.

While *P. maculata* may not seem to be the most successful host, it may due to the length of the fish rather than the species. Our experimental fish lengths varied from nature with *P. maculata* average length being 58 mm and *P. caprodes* average length being 89 mm (Scott and Crossman 1973). Although overall experimental fish length varied from the natural environment, *P. maculata* are still smaller than *P. caprodes* as observed in our experiment. It has been suggested that larger fish may be more successful host because of the increased gill surface area (Cyr and Pace 1993, Taeubert et al. 2010), therefore the larger the fish the more successful the host. Our study found that as fish length increases for both *P. maculata* and *P.*

caprodes, transformation success also increases. Therefore, although *P. maculata* was not as successful in juvenile transformation as *P. caprodes*, this may be due to the average size of *P. maculata* in our experiment being statistically smaller in size. However, previous host fish suitability research did not find a significant relationship with length and transformation success (Riusech and Barnhart 1998). More research needs to be conducted in order to fully understand the relationship between fish length and transformation success.

In addition to fish length influencing juvenile transformation success, fish body size can be used as a predictor of home range of fish (Woolnough et al. 2009). Larger fish have larger home ranges (Woolnough et al. 2009). Therefore, in addition to *P. caprodes* successfully transforming more juvenile, *P. caprodes* could possibly aid in long distance dispersal of *E. triquetra* juveniles. The loss of a primary host for any freshwater mussel species could be detrimental for a population even if it can transform on multiple host (Martel and Lauzon-Guay 2005).

Seasonal variation in juvenile transformation was revealed in this study, by conducting host fish suitability tests in the fall and spring. Standardized host fish transformation success revealed that transformation success was significantly different with metamorphosis being accelerated for the *P. caprodes* experiments in Spring 2013 in comparison to Fall 2012 and 2013. Furthermore, juvenile development differed in Spring 2013 without any developing or transitional stage juveniles being found and shorter infestation durations in comparison to Fall experiments. *Epioblasma triquetra* are considered long-term brooders that brood glochidia from September to May (Roe 2010). Long-term brooders can also be referred to as bradytictic; however, this term is considered obsolete because not all mussels fit easily into this or the tachytictic (short-term brooder) category, but are more along a continuum (Haag 2012). Longer-

25

term brooders often brood mature glochidia over the winter and release glochidia into the water column as a whole within a small timeframe in the spring and summer (Haag 2012). The lack of a developing or transition stage for juvenile transformation in the spring compared to the fall experiments suggests that glochidia may have a chance to develop more over winter making them quick to fully transform on the fish. It is unknown if *E. triquetra* utilize host fish in the fall when they are less developed or in the spring in nature. However, our results suggest that *E. triquetra* has a large timeframe involving when to release glochidia (see Results). Our research suggests when collecting gravid *E. triquetra* for conservation efforts it would be best to collect them at the end of their brooding season around June, but early enough that all gravid *E. triquetra* have not encountered a host, in order to have the most successful transformation rates from the shortest period of time.

We determined that the origin of host fish and freshwater mussels does not play an important role in producing the most *E. triquetra* juveniles. With host fish testing becoming increasingly important for understanding and conserving freshwater mussels it is imperative that we determine the best strategies possible to ensure the most successful transformation rates. It is imperative to determine the best strategy, even if unnatural, to sustain declining populations in nature. We expected to observe fish from the same genetic population as glochidia (sympatric combinations) to be more successful transforming glochidia to juveniles than fish from other basins (Rogers et al. 2001, Taeubert et al. 2012). Therefore, since there are two genetically distinct freshwater mussel populations in Michigan with mussels from the lakes Huron and Michigan drainages being indistinguishable genetically, but distinct from the Lake Erie drainage, expected sympatric fish and mussel pairings to be most successful in comparison to allopatric fish and mussel pairings (Bergner 2013). Our results do not demonstrate that genetic structure

among freshwater mussel population plays a role in juvenile transformation success. These results contradict recent research that found distinct relationships with transformation success and fish-mussel pairings (Douda et al. Accepted) suggesting that maybe the *E. triquetra* populations in Lake Erie basins and Lake Michigan/Huron basins have not had enough time to evolve distinct genetic differences. It would be interesting to expand the study beyond Michigan and conduct host fish suitability tests with mussels and fish from different biogeographic regions (faunal provinces), for example from the central highlands (Ozark Mountains) and the Cumberland plateau (Upper Tennessee River) and Great Lakes, to see if results would reflect Douda et al. (in press) results. In addition, it would be interesting to conduct this experiment using a more common species and instead of various basins, use sympatric and allopatric fish and freshwater mussels pairings within the same basin.

Two methods were used to analyze these data, transformation success (glochidia that attached to fish gills transformation success rate) and standardized transformation success (total glochidia transformation success rate including glochidia that did not attach onto fish gills), and neither method shows consistent results supporting sympatric or allopatric genetic population pairings of fish and mussel as the most productive juvenile transformation combination. For the most successful propagation efforts, our results demonstrate the most important factor in propagation success (i.e., number of juveniles) is the origin of the freshwater mussel. Standardized transformation success, which looks at juvenile transformation and takes into account that glochidia did not attach to fish gills evenly was used in an attempt to standardize the variation of glochidia concentration infestations. Standardized transformation success results suggest that mussel basin origin is the most significant factor in juvenile propagation; however, for the Fall 2013 experiment fish basin is also important. The lack of successful transformations

27

for Fall 2013 experiment may be due to decrease attachment rates to fish gills. The large number

of glochidia that do not attach onto the fishes gills may be due to immunity (Dodd et al. 2005).

However, our results suggest that allopatric fish and mussel pairings or sympatric fish and

mussel pairing combinations do not matter for juvenile production; therefore, immunity is

unlikely to be a factor in transformation success suggesting additional factors may have

contributed to decreased transformation success in Fall 2013. Overall, our results suggest that

freshwater mussel basin origin in comparison to fish basin origin and the combination of

freshwater mussel and fish origin are the most significant drivers in transformation success and

standardized transformation success, with *E. triquetra* originating from the Michigan basin

inland river being more successful for all experiments except for the transformation success for

P. caprodes from Fall 2013.

Epioblasma triquetra glochidia originating from the Lake Michigan basin had higher

rates of metamorphosis success than *E. triquetra* originating from the Lake Erie basin origin

glochidia. If apparently unrelated to genetic structure, a reason for the markedly higher

transformation success by mussels originating from the Lake Michigan drainage in comparison

to the Lake Erie drainage may have to do with the mussels' condition as a result of their

surrounding habitat. Lake Erie freshwater mussel populations have been declining since the

1960s from increased pollution and habitat degradation (Stevens and Neilson 1989, Nalepa et al.

1991, Bryan et al. 2013). Lake Erie populations continued to decline with the introduction of

dreissenids in 1986 (Schloesser and Nalepa 1994, Schloesser et al. 1996, Bryan et al. 2013).

River water quality and aquatic communities from the Lake Erie basin are a reflection of Lake

Erie's current condition. Decline in Lake Erie freshwater mussel populations suggests that the

habitat quality of basin may play an important role in propagation success. Clinton River

freshwater mussel community has been on a continual decline from an initial 26 species in 1978 to only 14 species in 2004 (Francis and Haas 2006). This apparent decline in unionid richness is suggested to be due to high flow instability (Francis and Haas 2006). In addition, the Clinton River is considered to be a degraded river due to the large number of point sources that introduced large amounts of pollution into the river. Although the majority of pollution sources have been found and are now regulated, chemicals still persist in the river's sediment (Francis and Haas 2006). The Flat River, a tributary of the Grand River, has been thought to be impacted by large amounts of agriculture, but this has not been linked back to freshwater mussel communities (Badra and Goforth 2003).

Infesting fish with glochidia for propagation is a common method for determining successful host fish combinations. However, it is difficult to infest each treatment with the same concentration of glochidia for each experiment; especially with extremely rare species. For our study, we documented and reported the concentration of glochidia each experimental treatment was infested with unlike other host fish suitability tests (Rogers et al. 2001, Allen et al. 2007, Taeubert et al. 2010, Lellis et al. 2013). Fall 2013 fish were infested with much higher concentrations than Fall 2012. The standardized transformation success for Fall 2013 juvenile transformation was much less successful in comparison to Fall 2012. It may be possible that infestations at high concentrations can have a negative impact on glochidia attachment rates to fish gills. Over infestation has been known to cause increased stress, decrease weight, gill damage and fish mortality (Dodd et al. 2005, Kaiser 2005, Howerth and Keller 2006, Crane et al. 2011). The increased infestation intensities may have masked any patterns expected to see with fish and freshwater mussel combinations, therefore, it is important when doing future studies to account for glochidial concentration when conducting host fish tests.

With over 70% of North American species either endangered, threatened or in need of protection it is important to have the most effective propagation strategies in action (Vaughn 2012). *Epioblasma triquetra* populations are continually facing new threats and currently are only present in 79 out of the 210 streams they were historically present in throughout United States, representing a 62 percent drop in distribution (US Fish and Wildlife Service 2012). Within these 79 streams, some populations only consist of a few individuals that may not be viable much longer decreasing distribution even further. Our study found that glochidia from the inland rivers of the Lake Erie basin were less successful in having successful transformations into juveniles in comparison glochidia from the inland rivers of the Lake Michigan basin. This suggests the Clinton River *E. triquetra* population may need more conservation efforts and reproduction complications may be a reason for *E. triquetra* decline in the Clinton River. Revision of *E. triquetra* conservation plans are needed to help restore viable populations and reestablish populations that have been extirpated (National Native Mussel Conservation Committee 1998).

The results of our host fish suitability tests for *E. triquetra* can be used towards advancing propagation efforts. Reintroduction efforts are constantly being built on increased knowledge of endangered species (Neves 2004). From our study, we suggest that when options are available, use larger *P. caprodes* as a host fish because it has the highest transformation rates. In addition, our study suggests freshwater mussels coming from healthier basins (e.g., Lake Michigan and Lake Huron basins instead of the Lake Erie basin) may produce more viable glochidia and juveniles. Therefore, instead of investing time in finding combinations, it may be better to focus efforts on healthiest source populations for propagation efforts. However, our study suggests the Clinton River snuffbox population is in decline; therefore, habitat restoration

and *E. triquetra* conservation efforts should be directed towards rebuilding this population. So although the Clinton River may not be the best source for *E. triquetra* to be used for propagation, it is still an important *E. triquetra* population in Michigan and should be protected.

The objective of this study was to quantitatively determine host fish suitability of *E. triquetra* with the *P. caprodes* and *P. maculata* based on individuals (fish and mussels) from multiple Great Lake basins. We determined both species were hosts for *E. triquetra* in Great Lakes basin and that origin of host fish and mussel does not contribute to the overall success of *E. triquetra* propagation. While conducting host fish suitability tests for *E. triquetra* we were able to confirm *P. caprodes* and *P. maculata* as successful hosts. In addition, seasonal variation in juvenile development was documented suggesting more developed glochidia are available in the spring. Lastly, host fish size influences transformation success of *E. triquetra* juveniles; therefore, the larger the host fish, the more successful it will be for propagation. Overall these findings should be used to help guide conservation efforts and ignite new research

CHAPTER II

INVASIVE MOLLUSK IMPACTS ON NATIVE SNUFFBOX (*EPIOBLASMA TRIQUETRA*)

Introduction

Human presence has caused the extinction rate of all organisms to increase by 100 to 1000 times since pre-human times (Barnosky et al. 2011). Introduction of invasive species by humans have major impacts on the world's biodiversity (Vitousek et al. 1997). Furthermore, natural resource management and ecological studies often deal with disturbances such as invasive species and their influence on the ecosystem. The disruptions caused by human introductions of invasive species have negative effects on the economy and the environment; therefore, it is imperative that we begin to understand the consequences of human actions (Lodge 1993). For example, it costs the Great Lakes region approximately $3 billion annually to mitigate the biofouling of water intakes from dreissenids and they have been known to decrease biodiversity therefore impacting the overall ecosystem (Lodge 1993), with an overall $5 billion economic impact to industries, businesses, and communities (Chakraborti et al. 2013). Increases in invasive species have caused a decrease in abundance of many Great Lakes species (Strayer 1999).

Introductions of invasive species have major repercussions to the global biodiversity and ecological communities (Lodge 1993). The introduction of invasive species has contributed to the declining unionid populations (Lucy et al. 2014) fundamentally changing the base of the ecosystem at this lower trophic level (Lucy et al. 2014). Asian clam, *Corbicula fluminea* (O. F. Müller 1774)*,* is an invasive mollusk that has invaded the Great Lakes region. *Corbicula,* native to southeast Asia, were introduced on purpose in the west coast of North America in the 1900s as a food source (Britton and Morton 1979) and have managed to spread quickly to a significant

portion of lakes, ponds, and streams throughout the United States due to continual and ongoing introductions (Counts 1986, Vaughn and Spooner 2006a, Nedeau 2008). *Corbicula* burrow in the sediment and filter feed on suspended material (Hakenkamp et al. 2001) and differ from unionids in a few distinct characteristics. *Corbicula* are smaller, short lived (living only 1 to 5 years), grow quickly (reaching maturity around 3 to 6 months), and produce multiple cohorts each year making them good invaders (Prezant and Chalermwat 1984, McMahon and Bogan 2001). *Corbicula* disperse downstream by releasing juveniles that float or adults can float down with long mucous threads (Prezant and Chalermwat 1984). They are also able to disperse upstream by crawling (Atkinson et al. 2011).

The appearance of *Corbicula* in the United States coincides with mussel declines (Haag and Williams 2013). *Corbicula* negatively affects survival of juvenile unionids. They compete for food, space and disturb the sediment displacing juveniles from preferred habitat to downstream locations (Vaughn and Spooner 2006a). Also, *Corbicula* siphon sperm, newly metamorphosed juveniles and glochidia, and decaying *Corbicula* have produced levels of ammonia that kill juvenile unionids (Yeager et al. 2000). In addition to having a large impact on the survival of juveniles, *Corbicula* filter at rates significantly higher than native unionid communities and may limit the abundance of planktonic food available for native species (Vaughn and Hakenkamp 2001). Belenger et al. (1990) found that with increasing *Corbicula* densities there was a decrease in growth of *Elliptio* species (Belanger et al. 1990). Lastly, *Corbicula*, when present, often occur at densities much higher than unionids (Vaughn and Hakenkamp 2001).

Although there seem to be a lot of negative consequences of *Corbicula* on native mussels, it is not clear if these consequences are a result of *Corbicula* presence or if *Corbicula*

take advantage of weak declining populations and show up as the population is already in trouble (McMahon and Bogan 2001, Haag 2012). *Corbicula* are often present among diverse, healthy unionid communities (Haag 2012) which infers that it may be possible that *Corbicula* and unionids may occupy different niches (Vaughn and Spooner 2006a)**.**

In addition to *Corbicula*, zebra mussels, *Dreissena polymorpha* (Pallas 1771)*,* and quagga mussels, *Dreissena rostriformis bugensis* (Andrusov 1897), are invasive species that are responsible for a massive decrease in unionid populations in North America. Introduced in the mid-1980's, *D. polymorpha* was accidently transported to the Laurentian Great Lakes from the ballast water of ships that traveled across the Atlantic into Lake St. Clair (Metcalfe-Smith et al. 2005). In 1990, *D. rostriformis bugensis* appeared in Lake Ontario near Niagara-on-the-Lake, Ontario but were originally misidentified as *D. polymorpha* (Benson 2013). From these events, the invaders have spread quickly throughout a large segment of eastern North America {Metcalfe-Smith, 2005 #86;Nalepa, 2001 #216} after colonizing the Great Lakes in the late 1980's (Hebert et al. 1989).

One of the main reasons dreissenids are successful invaders is their life cycle. Dreissenids have a unique life cycle that helps aid in their rapid expansion across the Great Lakes. Dreissenid larvae are microscopic organisms that eat small plankton and develop while they are suspended in the water (Strayer 1999). Development for a dreissenid varies based on food availability but can take as little as one week to develop completely (Strayer 1999). Dreissenid larvae are microscopic organisms that attach to solid objects (e.g., rocks, walls, human structures and unionid shells) using byssal threads (Strayer 1999). The average life span of a dreissenid is 1.5 to 2 years (Mackie 1991). Dreissenids preferentially colonize unionid shells rather than the walls of canals and were able to colonize and extirpate all the living

unionids within four years in the Soulanges Canal, PQ Canada (Ricciardi et al. 1996). In the

Hudson River, dreissenids decreased phytoplankton biomass by 85 % (Caraco et al. 1997) which

takes away available food for unionids, negatively impacting unionid reproduction and survival

rates (Strayer 1999). Unionid movement, respiration and feeding are all hindered activities due

to dreissenids attaching to their shells (Ricciardi et al. 1996). The unionids have to then carry

dreissenid mass hindering how far and fast they can move and decreasing native mussels'

reproductive ability (Van Appledorn and Bach 2007). Ricciardi et al. (1996) found that unionids

with more than their own weight of dreissenids attached to them die off and most of the energy

expended from competing with dreissenids makes them more susceptible to harsh winter

conditions. Dreissenids have led to major declines of unionids in the Great Lakes Region

(Strayer 1999) and are considered the ultimate stressor of native unionids when their densities

exceed 1000 m^{-2} on an individual unionid (Ricciardi et al. 1995).

With all these potential impacts of invasive mollusks to unionid populations and their

hosts it is important we understand the ecological interactions of these organisms. The

increasing presence of invasive species in the Great Lakes region has caused a decrease in many

native species (Strayer 1999). There is an abundance of evidence that shows unionids are

struggling with dreissenids hindering their movement, respiration, reproduction and survival

(Ricciardi et al. 1996, Caraco et al. 1997, Strayer 1999, Van Appledorn and Bach 2007).

Corbicula abundance can impact unionid abundance and negatively impact juveniles (Vaughn

and Spooner 2006a), however, the impact of *Corbicula* on unionids, especially on the highly

imperiled species, is still unclear (Haag 2012). Competition for habitat and resources are

potential reasons for the struggle to coexist. Studies have investigated the impact of dreissenids

and *Corbicula* on unionids but not many studies have looked at the relationship of invasive

35

species biomass or how the invasive species community (concurrent *Corbicula* and dreissenid effects) can impact the life of an endangered unionid.

This study examined the influence of invasive mollusks on *E. triquetra.* We hypothesized that there will be a negative relationship reflected in *E. triquetra* length and invasive mollusk abundance and biomass because previous studies find declines in unionid abundance with the presence of invasive species (Ricciardi et al. 1996, Strayer 1999, Vaughn and Spooner 2006a). In addition, invasive mollusk data and unionid community relationships were analyzed. We hypothesized that there would be a negative relationship between invasive mollusk presence and unionid community density and diversity because dreissenids prefer to colonize on freshwater mussels than other substrate (Ricciardi et al. 1996). Lastly, biotic parameters were analyzed relative to invasive species to determine whether there were differences between sex and fecundity of *E. triquetra*. We hypothesized that there would be a difference between sexes and fecundity; with female *E. triquetra* and gravid *E. triquetra* localized habitat preferentially colonized by invasive species because these are the most vulnerable freshwater mussels since they sit at the substrate surface and are expending energy resources to attract a host.

Methods

Invasive mollusks co-occurring with *Epioblasma triquetra*

While gravid *E. triquetra* were collected for the host fish experiment {Caldwell, 2014 #199}, invasive mollusk species data was collected using a 0.5 m x 0.5 m quadrat to determine what invasive and native mollusks are found surrounding individual *E. triquetra* (Strayer and Smith 2003). While surveying for gravid female *E. triquetra*, any *E. triquetra* found was

36

measured (mm), labeled using a dissecting probe, photographed, and the sex was identified. In addition, when *E. triquetra* (male or female) were found during the survey, a flag was placed in the substrate to mark its location along with a 0.5 m x 0.5 m quadrat around the flag. The females were checked for gravidity. If there were dreissenids attached to the *E. triquetra*, they were removed and weighed using a Pesola Light Line (10g and 500g) spring scale (g). The quadrat was surveyed for any additional freshwater mussels (live or shells) and any species found were recorded and measured. The quadrat was excavated to approximately 50 mm of sediment and all live and dead *Corbicula* and dreissenids were collected and saved for analysis. The *Corbicula* and dreissenids were counted and weighed (g). At each site, the GPS coordinates and water temperature were collected.

Analysis

Spearman's rank tests were performed with data collected from the quadrats surrounding *E. triquetra* to determine if there was a relationship between the number of *Corbicula* and the length of *E. triquetra* (Belanger et al. 1990). In addition to *Corbicula,* data were also collected to determine if there is a relationship with number of dreissenids and *E. triquetra* length. Also, *Corbicula* and dreissenid data were combined for total invasive mollusk data and Spearman's rank tests were performed to determine if there was any correlation. In addition all invasive mollusk and *E. triquetra* relationships were analyzed by gender of *E. triquetra.* A Mann-Whitney U test was conducted to see if there was a difference in mussel communities at the Flat and Clinton Rivers (*E. triquetra* densities and native mussel community abundance and richness). Mann-Whitney U tests were used to determine if female or male surroundings or gravid and non-gravid female surroundings differ. All statistical tests were performed using R software.

Results

Flat and Clinton River Site Summary

$Epioblasma$ $triquetra$ density at the Flat River is significantly greater (density $= 0.46$ m^{-2})

than the Clinton River (density $= 0.28$ m^{-2}, W$= 2050$, $p = 0.047$) (Bergner 2013). In addition, the

unionid richness (W $= 793$, $p << 0.001$) and abundance (W $= 999$, $p << 0.001$) was significantly

higher at the Flat River in comparison to the Clinton River (Bergner 2013). Unionid richness

and abundance at the Flat River were 3.37 m^{-2} and 5.69 m^{-2}. Unionid richness and abundance at

the Clinton River were 1.74 m^{-2} and 2.67 m^{-2}. 18 female and 17 male *E. triquetra* were found at

the Flat River and 30 female and 41 male *E. triquetra* were found at the Clinton River.

Two sites were sampled for invasive and native mollusks surrounding *E. triquetra*; the

Flat and Clinton rivers. The Flat River site is a braided stream channel that is located less than 1

km below a dam and the land use surrounding the area is less developed. The Clinton River

sampling location runs under a bridge and the site is located next to local suburban businesses.

The average densities of invasive mollusk and surroundings from both sites vary. Mean density

of *Corbicula* and dreissenids at the Flat River was 205 and 2 m^{-2} respectively. Mean density of

Corbicula and dreissenid at the Clinton River was 213 and 15 m^{-2}. At the Clinton River the

dreissenid presence, size and fouling in 2013 was less severe, with smaller and fewer individuals

attaching to *E. triquetra* in comparison to Fall 2012.

Invasive mollusk relationship on *Epioblasma triquetra*

The Spearman's rank test showed there was a relationship between invasive mollusk

abundance and biomass with the length (mm) of *E. triquetra* from both sampling locations. The

Spearman's rank test revealed a negative relationship with total invasive mollusk abundance and

E. triquetra length for both sites combined ($r_s = -0.253$, $p = 0.016$) and for the Clinton River site ($r_s = -0.316$, $p = 0.021$) and the relationship for the Flat River was similar but not as strong ($r_s = -0.306$, $p = 0.062$) (Figure I.1a). The Spearman's rank test revealed a positive relationship with total invasive mollusk biomass and *E. triquetra* length in the Clinton River ($r_s = 0.357$, $p = 0.009$), however, there was no relationship shown for both sites combined ($r_s = -0.135$, $p = 0.206$) or the Flat River site ($r_s = -0.151$, $p = 0.381$) (Figure I.1b).
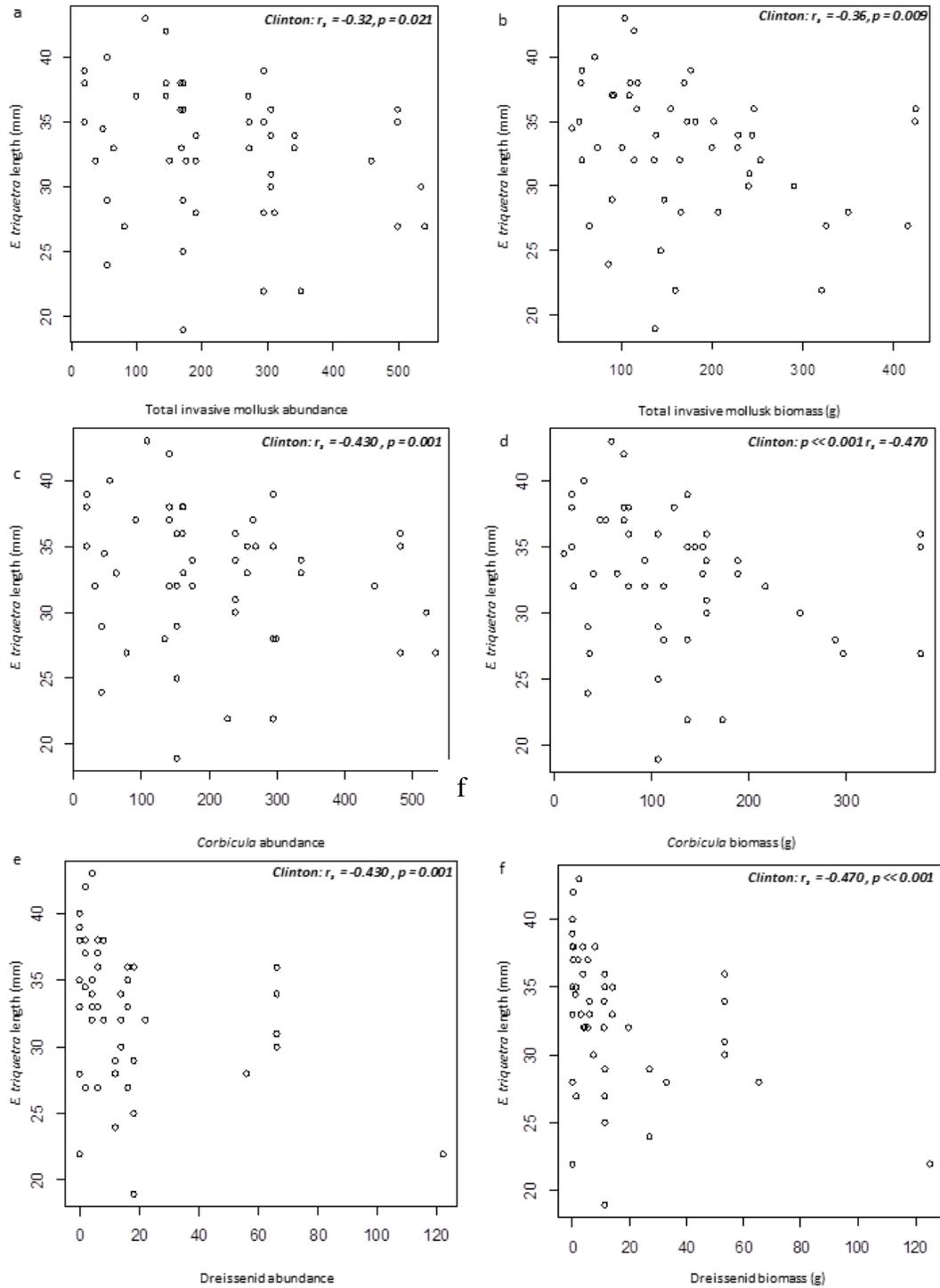
Figure II.1. Spearman's rank test correlation between invasive mollusk densities (m^{-2}) from the Clinton River (circle) (triangle) and *Epioblasma triquetra* length (mm). Relationships are a) Total invasive abundance (*Corbicula* and dreissenid abundance b) Total invasive biomass (*Corbicula* + dreissenid biomass (g)) c) *Corbicula* abundance d) *Corbicula* biomass (g) e) Dreissenid abundance f) Dreissenid biomass (g) and *E. triquetra* length (mm) for all figures.
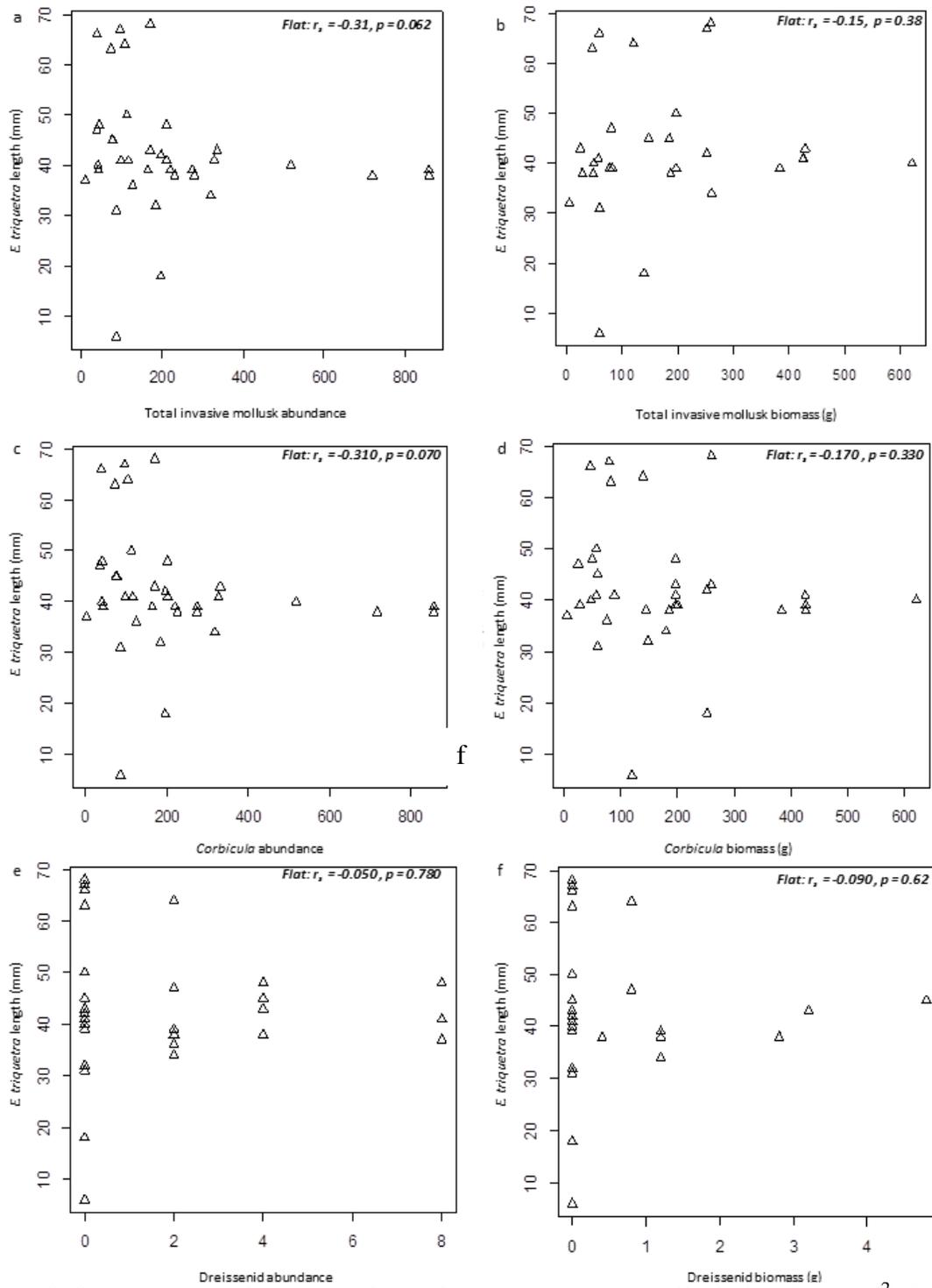
Figure II.2. Spearman's rank test correlation between invasive mollusk densities (m^{-2}) from the Flat River (triangle) and *Epioblasma triquetra* length (mm). Relationships are a) Total invasive abundance (*Corbicula* and dreissenid abundance b) Total invasive biomass (*Corbicula* + dreissenid biomass (g)) c) *Corbicula* abundance d) *Corbicula* biomass (g) e) Dreissenid abundance f) Dreissenid biomass (g) and *E. triquetra* length (mm) for all figures.

41

Invasive mollusk weight and abundance and their influence on *E. triquetra* length were separated by species; *Corbicula* and dreissenids. Spearman's rank test revealed no relationship between *Corbicula* abundance and *E. triquetra* length, however, the negative relationship at the Flat River ($r_s = -0.311$, $p = 0.065$) and both sites combined ($r_s = -0.194$, $p = 0.066$) are approaching statistical significance (Figure II.2c). In addition, there was a negative relationship with *Corbicula* biomass and *E. triquetra* length for the Clinton River ($r_s = -0.307$, $p = 0.026$) but there was no relationship for the Flat River ($r_s = -0.167$, $p = 0.330$) or both sites combined ($r_s = -0.074$, $p = 0.490$) (Figure II.1d). Lastly, data revealed a positive significant relationship between *E. triquetra* length and *Corbicula* average weight per quadrat for both sites combined ($r_s = 0.230$, $p = 0.029$), but not in tests for Clinton River ($r_s = -0.207$, $p = 0.137$) or Flat River ($r_s = 0.226$, $p = 0.185$).

The Spearman's rank test revealed a negative relationship with dreissenid abundance and *E. triquetra* length at both sites ($r_s = -0.470$, $p << 0.001$) and the Clinton River site ($r_s = -0.434$, $p = 0.001$) but not at the Flat River ($r_s = -0.049$, $p = 0.779$) (Figure II.1e and II.2e). The Spearman's rank test revealed a negative relationship with dreissenid biomass and *E. triquetra* length at both sites ($r_s = -0.437$, $p << 0.001$) and the Clinton River site ($r_s = -0.465$, $p << 0.001$) but not at the Flat River ($r_s = -0.086$, $p = 0.618$) (Figure II.1f and II.2f). *Epioblasma triquetra* gender did not play a role in the strength of the relationship between invasive mollusks abundance and biomass with *E. triquetra* length.

In addition, Spearman's rank test was performed on invasive mollusk abundance and biomass and *E. triquetra* density and only found two positive significant relationships between *Corbicula* biomass at the Flat River ($r_s = 0.446$, $p = 0.011$) and total invasive species biomass at the Flat River ($r_s = 0.358$, $p = 0.044$) (Table II.1).

Table II.1. Spearman's rank test results demonstrating the relationship between invasive mollusks, unionid communities, and *E. triquetra* for Flat and Clinton river data. * = $p < 0.10 – 0.05$ and ** = $p < 0.05$, r_s = the strength and direction of the relationship, S= S statistic.

Relationship	Flat River			Clinton River		
	S	p	r_s	S	p	r_s
Total invasive mollusk biomass vs. *E.triquetra* density	3500	0.044**	0.358	3965	0.904	0.023
Total invasive mollusk abundance vs. *E. triquetra* density	4674	0.434	0.143	3881	0.820	0.044
Corbicula abundance vs. *E.triquetra* density	3892	0.111	0.286	3812	0.753	0.061
Corbicula biomass vs. *E.triquetra* density	3023	0.011**	0.445	4051	0.604	0.099
Dreissenid abundance vs. *E.triquetra* density	4950	0.614	0.926	4347	0.863	0.0339
Dreissenid biomass vs. *E. triquetra* density	5047	0.684	0.075	4314	0.833	0.040
Total invasive abundance vs. unionid diversity	6096	0.207	0.215	21394	0.326	0.137
Total invasive biomass vs. unionid diversity	5070	0.038**	0.347	20346	0.197	0.179
Total invasive abundance vs. unionid abundance	5714	0.118	0.264	19523	0.125	0.212
Total invasive biomass vs. unionid abundance	4868	0.024**	0.373	18381	0.061*	0.258
Corbicula abundance vs. unionid abundance	6314	0.274	0.187	23254	0.657	0.062
Total mollusk abundance (include native species) vs. *E. triquetra* length	9904	0.104	-0.274	32206	0.030**	-0.298
Unionid abundance v. *E triquetra* length	6206	0.258	0.193	27880	0.376	-0.124

43

Invasive mollusk relationship with unionid community

The Spearman's rank test revealed a positive relationship between unionid abundance and richness with invasive mollusk biomass for both sites combined (diversity ($r_s = 0.204$, $p = 0.055$), abundance ($r_s = 0.234$, $p = 0.027$)) and the Flat River (diversity ($r_s = 0.425$, $p = 0.010$), abundance ($r_s = 0.530$, $p = 0.001$)) (Table II.1). Unionid abundance and invasive mollusk also had a positive significant relationship with the Flat River ($r_s = 0.322$, $p = 0.056$) and both sites combined ($r_s = 0.206$, $p = 0.053$), but not the Clinton River ($r_s = 0.137$, $p = 0.328$) (Table II.1).

There is a negative relationship between total mollusk abundance (unionid, *Corbicula* and dreissenid abundance) and *E. triquetra* length at the Clinton River ($r_s = -0.300$, $p = 0.030$) and both sites ($r_s = -0.236$, $p = 0.026$) (Table II.1). The Flat River results also suggest a relationship between total mollusk abundance and *E. triquetra* length, however, it was not significant ($r_s = -0.275$, $p = 0.105$) (Table II.1). Lastly, the relationship between unionid abundance and *E. triquetra* length was tested but no significant relationship was found (Table II.1).

Invasive mollusk relationship with *Epioblasma triquetra* gender and fecundity

Epioblasma triquetra were found gravid in the Clinton and Flat rivers throughout the field season from June to October {Caldwell, 2014 #199}. Significant or nearly significant differences were found with males and female lengths and their immediate surroundings (0.25 m x 0.25 m quadrat) at the Clinton River sampling site with dreissenid abundance (W = -223, $p = 0.016$) (Figure II.3a), dreissenid biomass (W = 249, $p = 0.052$) (Figure II.3b) and *Corbicula* biomass (W = 250, $p = 0.056$) with males having more invasive mollusks in their immediate surroundings. In addition, there is a difference in gravid females and non-gravid females surroundings in the Clinton River in dreissenid abundances (W = 151, $p = 0.011$) (Figure 3c) and

dreissenid biomass (W = 173, p = 0.0004) (Figure II.3d) with non-gravid females being

surrounded by more dreissenids. No significant relationships were found with gender

([*Corbicula* biomass (W = 174, p = 0.487) and abundance (W = 170, p = 0.586)] and [dreissenid

biomass (W = 144, p = 0.742) and (W = 143, p = 0.726)]) or gravidity ([*Corbicula* biomass (W

= 22, p = 0.204) and abundance (W=22, p = 0.206)] and [dreissenid biomass (W=27, p = 0.372)

and abundance (W = 26, p = 0.285)]) and invasive species presence at the Flat River.
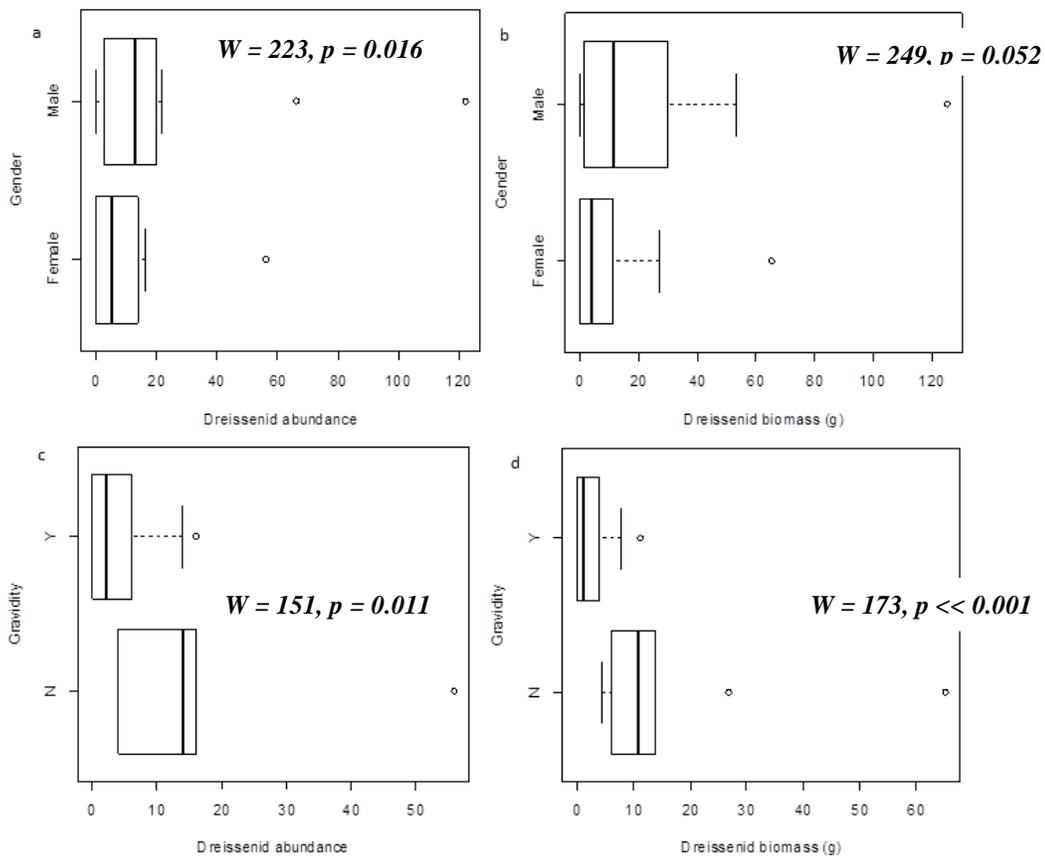
Figure II.3. Boxplots of dreissenid abundance and biomass in relationship to *Epioblasma triquetra* sex and fecundity. Mann-Whitney's U test showed differences in male and female *E. triquetra* surroundings (0.5 m x 0.5 m quadrat) (a and b) and gravid and non-gravid female *E. triquetra* surroundings (0.5 m x 0.5 m quadrat) (c and d). a) Dreissenid abundance for the Clinton River of female and male surroundings. b) Dreissenid biomass (g) for the Clinton River of female and male surroundings. c) Dreissenid abundance for the Clinton River of gravid and non-gravid female surroundings. d) Dreissenid biomass (g) for the Clinton River of gravid and non-gravid female surroundings. Boxplots display the distribution of data using box and whiskers. The box shows the 25th to 75th percentile with mean as the center line within the box.

45

The whiskers display the minimum and maximum invasive species data within the quadrat with an exception to the outliers at the top of each box.

<div align="center">Discussion</div>

Despite common perception, the relationship between *E. triquetra* and invasive mollusks presence was not always negative. With two sampling locations consisting of various invasive mollusk densities, this provided an opportunity to consider the strength in relationship as invasive species presence increases on *E. triquetra* length. The Flat River had higher *E. triquetra* density, unionid richness, and abundance in comparison to the Clinton River. In addition, dreissenid presence in the Flat River consisted of fewer individuals and lower biomass in comparison to dreissenid presence in the Clinton River. Before interpreting *E. triquetra* relationships with dreissenid presence, reasons for the variation in dreissenid density at sampling sites need to be understood.

Healthy ecosystems, with high species richness, diversity, and abundance, may be key to surviving invaders. Biodiversity in a system can be important in influencing the resilience of a system (Shea and Chesson 2002). Haag and Williams (2013) suggest that unionid abundance could help unionids endure the impacts from the invasive species. In addition, other ecosystems have been found to have similar relationships with biodiversity and invasive species. For example, kelp forests are much more resilient to disturbances or invasive species with higher biodiversity (Steneck et al. 2002) and in plant systems high species diversity can help increase invasion resistance (Shea and Chesson 2002). Therefore, although we do not see many relationships with *E. triquetra* and invasive species in the Flat River and see many negative relationships with invasive species and *E. triquetra* within the Clinton River, this could be due to the fact that the higher unionid richness, *E. triquetra* abundance, and *E. triquetra* densities (Bergner 2013) are making the ecosystem at the Flat River more resilient to changes induced by

invasive species. The Flat River might support a more robust molluscan fauna (native and invasive species) due to more conducive habitat.

Corbicula interaction with *Epioblasma triquetra*

   Corbicula had a dominant presence at both the Flat and Clinton rivers. Some invasive species, such as *Corbicula*, are very prevalent in aquatic ecosystems throughout the United States (Counts 1986, Vaughn and Spooner 2006a, Nedeau 2008). Although, *Corbicula* has been well researched on distribution and life history characteristics; the impact on unionid populations is not certain (Vaughn and Spooner 2006a). For example, *Corbicula* appeared in the same time period as unionid populations began to decline (Haag 2012), however, it is not clear if the decline of unionids is a cause of the arrival of *Corbicula* or if *Corbicula* was taking advantage of a new niche opening as unionid populations began to decline (Haag 2012). In addition, *Corbicula* are often present alongside healthy unionid communities (Haag 2012). Vaughn and Spooner (2006a) suggest this uncertainty is due to the various spatial scale experiments being tested. Our analysis was taken at the same small spatial scale of 0.25 m^2 and results conflict with Vaughn and Spooner's (2006a) study. Vaughn and Spooner (2006a) demonstrated that at small spatial scales, there is a negative relationship with *Corbicula* abundance and unionid abundance because unionids outcompete *Corbicula* for space and resources. Our study did not find any relationship between unionid abundance and *Corbicula* abundance (Table II.1). However, a positive relationship was found with *Corbicula* biomass and *E. triquetra* density suggesting that at small spatial scales *Corbicula* can have a positive relationship on unionid densities.

   Our results are consistent with other research, suggesting the relationship between unionids, particularly *E. triquetra*, and *Corbicula* is not clear (Vaughn and Spooner 2006a). The results are unclear involving *E. triquetra* length and *Corbicula* abundance and biomass. For

example, at the Flat River, there was a marginally negative relationship with length and *Corbicula* abundance and at the Clinton there was a negative relationship with *E. triquetra* length and *Corbicula* biomass. Overall, *Corbicula* seem to be negatively impacting *E. triquetra*, however, *Corbicula* is positively related to *E. triquetra* density at the Flat River making the results for the *Corbicula* study hard to interpret. Perhaps this relationship suggests that *Corbicula* can have negative impacts on *E. triquetra* because they are competing for resources but the quadrats with larger *E. triquetra* densities may have more suitable habitat which causes more *Corbicula* to live in the same area. With all this uncertainty involving *Corbicula* and considering its prevalence throughout the United States, more laboratory experiments should be conducted to try to explain the true relationship between *Corbicula* and unionids.

Dreissenid interaction with *Epioblasma triquetra*

Historically, the presence of dreissenids within an aquatic ecosystem is considered to be detrimental for unionids. The impact of dreissenid presence on *E. triquetra* in both sites is not consistent; however, a negative relationship is present at the Clinton River. Since the presence of dreissenids in the Great Lakes, there has been a decrease in unionid populations (Strayer 1999, Strayer and Malcom 2014); however, since the 1990s, dreissenid survival has decreased from 50% to 1% per year, which may allow for remaining freshwater mussels to recover. Dreissenid presence in our lotic sampling locations is not as severe as lentic systems likely because dreissenid attachment is lower in lotic systems from there being more available substrate to attach to (Ricciardi et al. 1995) which can have higher negative impacts on unionids (Strayer and Malcom 2007). Therefore, dreissenid presence at the Flat and Clinton rivers may not be detrimental to the unionid populations; however, dreissenids may be having more subtle impacts on the unionids, rather than causing mortality.

According to the US Fish and Wildlife Service (2012), *E. triquetra* populations in the Upper Great Lakes Sub-Basin are not in known distress, whereas in the Lower Great Lakes Sub-Basin, the *E. triquetra* population has been under stress in Lake Erie even prior to dreissenid arrival (Schloesser and Nalepa 1994). But, following the dreissenid invasion, populations of *E. triquetra* began a major decline and populations from Lake St. Clair and the Detroit River were eliminated (Nalepa and Gauvin 1988, Schloesser et al. 1998). The population of *E. triquetra* in the Flat and Grand rivers in Michigan are recruiting but the population trend is unknown, in comparison to the Clinton River population that is recruiting but is on a downward trend (US Fish and Wildlife Service 2012). Our research suggests that *E. triquetra* population at the Clinton River is being negatively impacted more, especially from dreissenids than the *E. triquetra* at the Flat River.

Dreissenid densities varied for the Flat River and Clinton River; the Clinton River has higher dreissenid density compared to the Flat River. With high dreissenid density at the Clinton River, a negative relationship was found between dreissenid abundance and biomass and *E. triquetra* length. However, there was no relationship with dreissenid abundance or biomass and *E. triquetra* length at the Flat River. Dreissenid introductions originated from the Erie basin, so dreissenid impact may not be as large because of the distance from introduced location (Schloesser and Nalepa 1994, Schloesser et al. 1996, Bryan et al. 2013). The Flat River had very low dreissenid presence suggesting dreissenids may not negatively impact unionids until there is a larger presence within a location similar to the conditions at the Clinton River. Ricciardi et al. (1995) suggests that the density of dreissenids can predict colonization (or infestation) on unionids. Therefore, the more dreissenids present, the more likely they will colonize on unionids and have direct negative influences on the species. Lucy et al. (2013) suggest that 100% of

unionids are infested with dreissenids when dreissenids exists at densities of 200 m^{-2} or more. Past research also suggests unionids and dreissenids can coexist without negative impacts when dreissenid distribution is scattered and the population is low (only fouling 1% of the unionid population) (Nichols and Amberg 1999). At the Clinton River, dreissenids are much more dense with more *E. triquetra* fouled in comparison to the Flat River. In contrast, Strayer and Malcom (2013) suggest that exploitation competition may be harming unionids more than fouling. Unionid body condition is more impacted by overall river wide filtration rate of dreissenid than direct contact from dreissenids. In addition, some species are more vulnerable to dreissenid presence than others, such as *E. triquetra* tribe, Lampsilini, and Anodontini in comparison to Amblemines and Pleurobemines (Haag et al. 1993, Strayer 1999). So, although the Flat River and Clinton River study sites did not have extreme dreissenid densities and the majority of unionids found were not fouled, *E. triquetra* could still be vulnerable to dreissenid invasions. In addition, even though dreissenids and *E. triquetra* have been documented co-occurring since their arrival in North America, we believe this is the first time their effects have been quantified directly for a highly imperiled species. Our research suggests that dreissenid impact threshold for unionids may be lower than previously assumed.

Seasonal variation in dreissenid presence

The severity of dreissenid presence and fouling on *E. triquetra* varied each sampling season (Fall 2012 and Fall 2013) at the Clinton River. The dreissenid presence, size and fouling in 2013 was less severe, with smaller and fewer individuals attaching to *E. triquetra* in comparison to Fall 2012. This change may allow unionids to recover from higher invasive presence the following year. Studies on the effects of dreissenids on unionids have demonstrated evidence of cycles in dreissenid presence (Lucy et al. 2014, Strayer and Malcom 2014). The

small sized dreissenids (i.e., young) attached to *E. triquetra* for Fall 2013 support the evidence that the dreissenid populations have cycles or that the dreissenid population at the Clinton River is decreasing. Dreissenid populations do well when the majority of the dreissenids are young and adult presence is not substantial (Strayer and Malcom 2014). Younger dreissenid presence can eventually lead to a more stable dreissenid population consisting of dreissenids that are less than one year old (Strayer and Malcom 2014). The Flat River unionid community is thriving in the presence of a relatively small dreissenid infestation. However, dreissenid presence is very low at the Flat River, suggesting that dreissenids have stabilized and unionids and dreissenids now live in equilibrium (Lucy et al. 2014). In addition, Clinton River and Flat River dreissenid colonization and fouling of unionids is much lower in comparison to the Great Lakes especially because lotic systems in general have lower dreissenid colonization rates because there is more habitat to attach to in lotic systems (Ricciardi et al. 1995).

Overall invasive mollusk relationship with *Epioblasma triquetra*

Overall, both locations had negative relationships with total invasive mollusk abundance and *E. triquetra* length; however, the Flat River relationship is not as strong. Combining *Corbicula* and dreissenid presence addressed the question if the relationships demonstrated were species specific or if an increase in competitors creates a negative relationship. Although the Clinton River *E. triquetra* length was not always negatively impacted by invasive mollusk abundance; overall, the combined total mollusk abundance can have a negative relationship on the *E. triquetra*. At the Flat River, *E. triquetra* length was marginally related to invasive species abundance but overall there was no truly significant relationship between *Corbicula* or dreissenid abundance. Also, there was a negative relationship with total invasive mollusk biomass and *E. triquetra* length at the Clinton River. Suggesting that at the Clinton River, not the Flat River, as

the biomass of invasive mollusk increased it was negatively influencing *E. triquetra.* The biomass of dreissenid attachment has been determined to play a larger role than the number of dreissenids that attach to a unionid (Lucy et al. 2014). Generally, the relationship between total invasive species biomass and abundance is not completely clear, but the larger amounts or weights of invasive species may have a negative impact on body condition on unionid species. Larger dreissenids can have more impact to unionids than smaller, younger dreissenids (Strayer and Malcom 2014). The strongest and most consistent relationships with this study were with dreissenids at the Clinton River, suggesting that although *Corbicula* may have negative influences on *E. triquetra,* the relationship is not as understandable. Dreissenid presence is considered to be the biggest threat to the unionids in the Great Lakes (Strayer 1999), whereas *Corbicula* presence has not consistently been determined to be a direct threat to unionids (Strayer 1999, Vaughn and Spooner 2006a).

Invasive mollusks and unionid densities

Densities of unionids relationship with invasive species could help explain if competition is a driving factor or if niche effects are potentially explaining the relationships seen within our analysis. Overall, the only significant relationship between *E. triquetra* density and invasive mollusks is at the Flat River with the total invasive mollusk biomass and *Corbicula* biomass. As invasive species biomass increases, the density of *E. triquetra* also increases suggesting that the invasive mollusk presence at the Flat River may not be detrimental especially when dreissenid presence is low. We may be able to consider the biomass of invasive species as the current condition of the organism; the larger invasive species are (or more biomass) suggests that invasive species are thriving in their current environment. Therefore, suggesting the immediate vicinity of suitable habitat has enough resources for *E. triquetra* to be less impacted from

competition. Since this relationship is only seen at the Flat River, it may suggest that there are

enough nutrients or habitat for everyone to survive without too much competition. Round gobies

(*Neogobius melanostomus*: Pallas 1814), known predators of dreissenids, are present at the Flat

River and it is possible that they may be containing the dreissenid population (Naddafi and

Rudstam 2014). The absence of round gobies in the Clinton River is hypothesized to be due to

high predator abundance which can limit their distribution (D. Jude, University of Michigan

2008). In addition, although it has been reported that dreissenids are the ultimate stressor for the

unionids, once dreissenid populations begin to stabilize, coexistence between unionids and

dreissenids is possible (Lucy et al. 2014, Strayer and Malcom 2014).

The availability of resources may be influencing invasive species relationships with

unionid communities. A positive relationship between total invasive species biomass and

unionid diversity was found at the Flat River. In addition, a positive relationship was found with

total invasive species biomass and unionid abundance for the Flat and Clinton River. These

results may suggest that invasive species can be positively related to unionid species and that the

presence of invasive species may be related to the quality of the habitat or the availability of

resources. Unionids are often distributed patchily based on resources and often live and thrive in

suitable habitats {Vaughn, 2006 #132;Newton, 2008 #52}. Vaughn and Spooner (2006) study

found that aquatic invertebrate densities were correlated with unionid density. Therefore, the

Flat River may have positive relationships with biomass of *Corbicula* because unionids are

providing good environments for other species. In addition, healthy unionid communities may

allow for higher invertebrate densities because they are able to contribute to sediment nutrients.

Dreissenid presence in relation to gender and fecundity

The dreissenids surrounding male and female *E. triquetra* and gravid female and non-gravid female *E. triquetra* differed at the Clinton River with males and non-gravid females having more dreissenids in their surroundings. Dreissenid attachment on unionids can hinder unionid feeding, movement and respiration which can decrease their reproductive ability (Ricciardi et al. 1996, Van Appledorn and Bach 2007). Few studies focus on the impact that dreissenids can have by just being present in a system, however, Strayer and Malcom (2013) suggest that simple presence of dreissenids increases competition and negatively impacts unionid condition. While few *E. triquetra* were colonized by dreissenids, they may still have endured negative relationships from competition. One defense unionids have against dreissenid colonization is the ability to survive by burying themselves under the substrate (Nichols and Wilcox 1997). Unionids that are able to bury themselves under soft substrate can rid themselves of dreissenids making themselves less vulnerable to invasive species (Lucy et al. 2014). However, although females can bury themselves in the substrate, they must spend a large portion of their time on top of the sediment in order to attract a host which is required in order to reproduce, making them more vulnerable to dreissenids. *E. triquetra* is a long term brooder and is reported to brood from September to May each year (Ortmann 1912, US Fish and Wildlife Service 2012), but while surveying, *E. triquetra* were found gravid from June through October {Caldwell, 2014 #199}. In order to reproduce, gravid female *E. triquetra* make themselves more vulnerable to dreissenid attachment for over half the year while they attempt to attract and trap a host (Barnhart et al. 2008). In addition, females put forth more energy towards reproduction effort (Haag 2013) resulting in having less energy for dealing with competition from other organisms. It has been hypothesized that female unionids are more susceptible to dreissenid

infestation than males (Haag et al. 1993, Schloesser et al. 1996). Therefore, higher dreissenid presence surrounding males and non-gravid females in the Clinton River may be a result from higher mortality of gravid *E. triquetra* from too much competition.

Implications for management and conservation

Our research investigated at the condition of *E. triquetra* populations at the Flat River and Clinton River, Michigan. We found that the Flat River *E. triquetra* population is not clearly impacted by the invasive mollusk communities using parameters we quantified, but the Clinton River *E. triquetra* show signs of being negatively impacted by invasive mollusks (specifically with dreissenids). Our study suggests that conservation efforts should focus on the Clinton River *E. triquetra* population because it appears to currently be in decline and not responding well to the drastic changes due to invasive species present in their habitat (US Fish and Wildlife Service 2012). In addition, our research has found that there may be a threshold in the ability of *E. triquetra* to successfully compete with dreissenid infestation. Our results found that even at low densities of 15 dreissenids m^{-2}, *E. triquetra* can show negative responses in reduced length. Overall, the Genus *Epioblasma* is more vulnerable to dreissenids because they are smaller, have few hosts, and are considered long-term brooders (Mackie et al. 2000). In addition, *Epioblasma* is the most endangered Genus of all North American freshwater mussels, with 14 of the 25 species extinct it is imperative we attempt to salvage all surviving species (Williams et al. 1993, Roe 2010). With a large majority of the *Epioblasma* extinct, it is important to gather and understand the history and current status of all surviving members of *Epioblasma* to understand and conserve this Genus. With *Epioblasma* being one of the most sensitive Genera within the unionids, as *Epioblasma* begin to decline the decline of *E. triquetra* is a red flag that other species may follow.

Epioblasma triquetra was the last species of the remaining extant members of the Genus *Epioblasma* to be listed as endangered under the US Endangered Species Act. *Epioblasma triquetra* populations continue to decline in the United States. Currently, *E. triquetra* is only present in 79 out of the 210 streams that they were historically present in representing a 62 percent drop in distribution (US Fish and Wildlife Service 2012). Within these 79 locations, some populations only consist of a few individuals that may not be viable much longer decreasing distribution even further. For example, the Clinton River *E. triquetra* population is currently in decline, which may be due to low success in glochidia to juvenile transformations {Caldwell, 2014 #199}. *E. triquetra* populations are in need of successful conservation plans immediately to help restore viable populations and reestablish populations that have died off.

Invasive species have had major impacts on unionid populations, with this study highlighting impacts on highly imperiled *E. triquetra*, and it is imperative that we gather as much information on their interactions with invasive species to help guide conservation efforts (Haag and Williams 2013). Our study suggests that more diverse, dense, and healthy unionid communities may be more resilient and show fewer impacts from invasive species. However, our study suggests that *Corbicula* is not as serious of an invader even at the high densities found in the Great Lakes basins studied. Though, our research did not show clear results necessitating additional research to rule out *Corbicula* as a threat to unionid communities. Whereas, the intensity of dreissenid impact increases as densities of dreissenids rise (Ricciardi et al. 1995); therefore, although a location may have dreissenids present, the density of those dreissenids may be too low, similar to conditions at the Flat River, to cause harm to unionid communities. These discoveries could help guide efforts toward more effective unionid conservation plans that mitigate the negative impacts of invasive species on unionids.

REFERENCES

Allen, D. C., B. E. Sietman, D. E. Kelner, M. C. Hove, J. E. Kurth, J. M. Davis, J. L. Weiss, and D. J. Hornbach. 2007. Early life-history and conservation status of *Venustaconcha ellipisformis* (Bivalvia: Unionidae) in Minnesota. American Midland Naturalist 157:74-91.

Allen, D. C., C. C. Vaughn, J. F. Kelly, J. T. Cooper, and M. H. Engel. 2012. Bottom-up biodiversity effects increase resource subsidy flux between ecosystems. Ecology 93(10):2165-2174.

Atkinson, C. L., M. R. First, A. P. Covich, S. P. Opsahl, and S. W. Golladay. 2011. Suspended material availability and filtration-biodeposition processes performed by a native and invasive bivalve species in streams. Hydrobiologia 667(1):191-204.

Badra, P. J., and R. R. Goforth. 2003. Freshwater mussel surveys of Great Lakes Tributary Rivers in Michigan. Michigan Department of Environmental Quality, Lansing, MI.44pp.

Barnhart, M. C., F. Riusech, and M. Bairs. 1998. Hosts of the salamander mussel (*Simpsonaias ambigua*) and snuffbox (*Epioblasma triquetra*) from the Meramec River system, Missouri. Triannual Unionid Report 16:34.

Barnhart, M. C., W. R. Haag, and W. N. Roston. 2008. Adaptations to host infection and larval parasitism in Unionoida. Journal of the North American Benthological Society 27:370-394.

Barnosky, A. D., N. Matzke, S. Tomiya, G. O. U. Wogan, B. Swartz, T. B. Quental, C. Marshall, J. L. McGuire, E. L. Lindsey, K. C. Maguire, B. Mersey, and E. A. Ferrer. 2011. Has the Earth's sixth mass extinction already arrived? Nature 471(7336):51-57.

Bauer, G., and K. Wachtler. 2001. Ecology and evolution of the freshwater mussels Unionoida. Springer, New York, NY.

Belanger, T. V., C. G. Annis, and D. D. Vanepps. 1990. Growth-rates of the Asiatic clam, *Corbicula fluminea*, in the Upper and Middle St. Johns River, Florida. Nautilus 104(1):4-9.

Benson, A. J., editor. 2013. Chronological history of zebra and quagga mussels (Dreissenidae) in North America, 1998-2010. CRC Press, New York, NY.

Bergner, J. L. 2013. Does scale matter? Genetic structuring and species assemblages of unionids in centreal Great Lakes tributaries. MS Thesis. Central Michigan University, Mount Pleasant, MI. 92pp.

Britton, J. C., and B. Morton. 1979. *Corbicula* in North America: The evidence reviewed and evaluated. Texas Christian University Research Foundation, Fort Worth, USA.

Bryan, N. J., C. V. Florence, T. D. Crail, and D. L. Moorhead. 2013. Freshwater mussel community response to warm water discharge in western Lake Erie. Journal of Great Lakes Research 39(3):449-454.

Caldwell, M. L. 2014. Host fish suitability tests and effects of invasive mollusks for snuffbox, *Epioblasma triquetra*, from Laurentian Great Lakes basins. MS Thesis. Central Michigan University, Mount Pleasant, MI. 82pp.

Caraco, N. F., J. J. Cole, P. A. Raymond, D. L. Strayer, M. L. Pace, S. E. G. Findlay, and D. T. Fischer. 1997. Zebra mussel invasion in a large, turbid river: Phytoplankton response to increased grazing. Ecology 78(2):588-602.

Castanon, R., M. Tremblay, K. McNichols, G. L. Mackie, and J. D. Ackerman. 2011. Investigating research gaps for the recovery of unionid mussel species at risk in Canada. Report for Species at Risk Committee/ Comité sur les espèces en péril and the Canadian Wildlife Federation.16pp.

Chakraborti, R. K., S. Madon, J. Kaur, and D. Gabel, editors. 2013. Management and control of dreissenid mussels in water infrastructure facilities of the southwestern United States. CRC Press, New York, NY.

Cope, W. G., R. B. Bringolf, D. B. Buchwalter, T. J. Newton, C. G. Ingersoll, N. Wang, T. Augspurger, F. J. Dwyer, M. C. Barnhart, R. J. Neves, and E. Hammer. 2008. Differential exposure, duration, and sensitivity of unionoidean bivalve life stages to environmental contaminants. Journal of the North American Benthological Society 27(2):451-462.

Counts, C. L. 1986. The zoogeography and history of the invasion of the United States by *Corbicula fluminea* (Bivalvia: Corbiculidae). American Malacological Bulletin, Special Edition 2:7-39.

Crane, A. L., A. K. Fritts, A. Mathis, J. C. Lisek, and M. C. Barnhart. 2011. Do gill parasites influence the foraging and antipredator behaviour of rainbow darters, *Etheostoma caeruleum*? Animal Behaviour 82(4):817-823.

Cyr, H., and M. L. Pace. 1993. Allometric theory: Extrapolations from individuals to communities. Ecology 74(4):1234-1245.

Dodd, B. J., M. C. Barnhart, C. L. Rogers-Lowery, T. B. Fobian, and R. V. Dimock. 2005. Cross-resistance of largemouth bass to glochidia of unionid mussels. Journal of Parasitology 91(5):1064-1072.

Douda, K., J. Sell, L. Kubikova-Pelakova, P. Horky, A. Kaczmarczyk, and M. Mioduchowska. Accepted. Host compatibility as a critical factor in management unit recognition: Population-level differences in mussel-fish relationships. Journal of Applied Ecology. 33pp.

Francis, J. T., and R. C. Haas. 2006. Clinton River assessment. Michigan Department of Natural Resources: Fisheries Division, Lansing, Michigan. 97pp.

Gabraith, H. S., D. T. Zanatta, and C. C. Wilson. In review. Comparative analysis of riverscape genetic structure in rare, threatened and common freshwater mussels. Conservation Genetics. 10 pp.

Haag, W. R. 2012. North American freshwater mussels: natural history, ecology, and conservation. Cambridge University Press, New York, NY.

Haag, W. R. 2013. The role of fecundity and reproductive effort in defining life-history strategies of North American freshwater mussels. Biological Reviews 88(3):745-766.

Haag, W. R., and J. D. Williams. 2013. Biodiversity on the brink: an assessment of conservation strategies for North American freshwater mussels. Hydrobiologia 1:1-16.

Haag, W. R., and M. L. Warren. 1997. Host fishes and reproductive biology of 6 freshwater mussel species from the Mobile Basin, USA. Journal of the North American Benthological Society 16(3):576-585.

Haag, W. R., D. J. Berg, D. W. Garton, and J. L. Farris. 1993. Reduced survival and fitness in native bivalves in response to fouling by the introduced zebra mussel (*Dreissena polymorpha*) in the western Lake Erie. Canadian Journal of Fisheries and Aquatic Sciences 50(1):13-19.

Hakenkamp, C. C., S. G. Ribblett, M. A. Palmer, C. M. Swan, J. W. Reid, and M. R. Goodison. 2001. The impact of an introduced bivalve (*Corbicula fluminea*) on the benthos of a sandy stream. Freshwater Biology 46(4):491-501.

Hebert, P. D. N., B. W. Muncaster, and G. L. Mackie. 1989. Ecological and genetic studies on *Dreissena polymorpha* (Pallas): A new mollusk in the Great Lakes. Canadian Journal of Fisheries and Aquatic Sciences 46(9):1587-1591.

Hillegrass, K. R., and M. C. Hove. 1997. Suitable fish host for glochidia for three freshwater mussels: strange floater, ellipse, and snuffbox. Triannual Unionid Report 13:25.

Howerth, E. W., and A. E. Keller. 2006. Experimentally induced glochidiosis in smallmouth bass (*Micropterus dolomieu*). Veterinary Pathology 43(6):1004-1008.

Jones, J. W., and R. J. Neves. 2000. Annual progress report for 1999: life history and artificial culture of endangered mussels. Tennesse Wildlife Resource Agency, Nashville. 66pp.

Jones, J. W., R. J. Neves, A. Ahlstedt, and E. M. Hallerman. 2006. A holistic approach to taxonomic evaluation of two closely related endangered freshwater mussel species, the oyster mussel *Epioblasma capsaeformis* and tan riffleshell *Epioblasma florentina walkeri* (Bivalvia: Unionidae). Journal of Molluscan Studies 72:267-283.

Jones, J. W., R. J. Neves, S. A. Ahlstedt, and R. A. Mair. 2004. Life history and propagation of the endangered dromedary pearlymussel (*Dromus dromas*) (Bivalvia: Unionidae). Journal of the North American Benthological Society 23(3):515-525.

Kaiser, B. E. 2005. The effects of glochidiosis on fish respiration. Missouri State University 1:1-50.

Lefevre, G., and W. C. Curtis. 1912. Studies on the reproduction of artificial propagation of freshwater mussels. U.S. Bureau of Fisheries Bulletin 30:105-209.

Lellis, W. A., B. S. White, J. C. Cole, C. S. Johnson, J. L. Devers, E. V. Gray, and H. S. Galbraith. 2013. Newly Documented Host Fishes for the Eastern Elliptio Mussel, *Elliptio complanata*. Journal of Fish and Wildlife Management 4(1):75-85.

Lima, P., M. L. Lima, U. Kovitvadhi, S. Kovitvadhi, C. Owen, and J. Machado. 2012. A review on the "in vitro" culture of freshwater mussels (Unionoida). Hydrobiologia 691(1):21-33.

Lodge, D. M. 1993. Biological invasions- Lessons for ecology. Trends in Ecology & Evolution 8(4):133-137.

Lucy, E. L., L. E. Burlakova, A. Y. Karatayev, S. E. Mastitsky, and D. T. Zanatta. 2014. Zebra mussel impacts on unionids: A syntheis of trends in North America and Europe. Pages 623-646 *in* T. F. Nalepa, and D. W. Schloesser, editors. Quagga and zebra mussels; Biology, impacts, and control., volume 2. CRC Press, New York, NY.

Mackie, G. L. 1991. Biology of the exotic zebra mussel, *Dreissena polymorpha,* in relation to native bivalves and its potenital impact in Lake St. Clair. Hydrobiologia 219:251-268.

Mackie, G. L., D. T. Zanatta, J. L. Metcalfe-Smith, J. Di Maio, and S. K. Staton. 2000. Toward developing strategies for re-habilitating/re-establishing Unionidae populations in southwestern Ontario. Final Report to the Endangered Species Recovery Fund.

Martel, A. L., and J. S. Lauzon-Guay. 2005. Distribution and density of glochidia of the freshwater mussel *Anodonta kennerlyi* on fish hosts in lakes of the temperate rain forest of Vancouver Island. Canadian Journal of Zoology-Revue Canadienne De Zoologie 83(3):419-431.

McCann, K., A. Hastings, and G. R. Huxel. 1998. Weak trophic interactions and the balance of nature. Nature 395(6704):794-798.

McKillup, S. 2006. Statistics explained: An introductory guide for life scientists. Cambridge University Press, Cambridge, UK.

McMahon, R. F. 2002. Evolutionary and physiological adaptations of aquatic invasive animals: r selection versus resistance. Canadian Journal of Fisheries and Aquatic Sciences 59(7):1235-1244.

McMahon, R. F., and A. E. Bogan. 2001. Mollusca: Bivalvia. Academic Press, San Diego, California, USA.

Metcalfe-Smith, J. L., A. ManKenzie, I. Carmichael, and D. McGoldrick. 2005. Photo field guide to the freshwater mussels of Ontario. St. Thomas Field Naturalist Club Incorporated, St. Thomas.

Naddafi, R., and L. G. Rudstam. 2014. Predation on invasive zebra mussel, *Dreissena polymorpha*, by pumpkinseed sunfish, rusty crayfish, and round goby. Hydrobiologia 721(1):107-115.

Nalepa, T. F., and J. M. Gauvin. 1988. Distribution, abundance and biomass of freshwater mussels (Bivalvia: Unionidae) in Lake St. Clair. Journal of Great Lakes Research 14:411-419.

Nalepa, T. F., B. A. Manny, J. C. Roth, S. C. Mozley, and D. W. Schloesser. 1991. Long-term decline in freshwater mussels (Bivalvia, Unionidae) of the western basin of Lake Erie. Journal of Great Lakes Research 17(2):214-219.

Nalepa, T. F., D. W. Schloesser, S. A. Pothoven, D. W. Hondorp, D. L. Fanslow, M. L. Tuchman, and G. W. Fleischer. 2001. First finding of the amphipod *Echinogammarus ischnus* and the mussel *Dreissena bugensis* in Lake Michigan. Journal of Great Lakes Research 27(3):384-391.

National Native Mussel Conservation Committee. 1998. National strategy for the conservation of native freshwater mussels. Journal of Shellfish Research 17(5):1419-1428.

Near, T. J. 2002. Phylogenetic relationships of *Percina* (Percidae: Etheostomatinae). Copeia (1):1-14.

Nedeau, E. J. 2008. Freshwater mussels and the Connecticut River Watershed. Connecticut River Watershed Council, Greenfield, Massachusetts, USA.

Neves, R. J. 2004. Propagation of endangered freshwater mussels in North America. Journal of Conchology 3:69-80.

Newton, T. J., D. A. Woolnough, and D. L. Strayer. 2008. Using landscape ecology to understand and manage freshwater mussel populations. Journal of the North American Benthological Society 27(2):424-439.

Nichols, S. J., and D. A. Wilcox. 1997. Burrowing saves Lake Erie clams. Nature 389(6654):921-921.

Nichols, S. J., and J. Amberg. 1999. Co-existence of zebra mussels and freshwater unionids: Populations dynamics of *Leptodea fragilis* in a coastal wetland infested with zebra mussels. Canadian Journal of Zoology 77:423-432.

Ohio State University (OSU) Division of Molluscs. 2009. Unionid host-parasite database.

Ortmann, A. E. 1912. Notes upon the families and genera of the najades. Annals of the Carnegie Musuem 8(2):222-365.

Pimm, S. L., G. J. Russell, J. L. Gittleman, and T. M. Brooks. 1995. The future of biodiversity. Science 269(5222):347-350.

Prezant, R. S., and K. Chalermwat. 1984. Flotation of the bivalve *Corbicula fluminea* as a means of dispersal. Science 225(4669):1491-1493.

Ricciardi, A., F. G. Whoriskey, and J. B. Rasmussen. 1995. Predicting the intensity and impact of *Dreissena* infestation on native unionid bivalves from *Dreissena* field density. Canadian Journal of Fisheries and Aquatic Sciences 52(7):1449-1461.

Ricciardi, A., F. G. Whoriskey, and J. B. Rasmussen. 1996. Impact of the Dreissena invasion on native unionid bivalves in the upper St Lawrence River. Canadian Journal of Fisheries and Aquatic Sciences 53(6):1434-1444.

Riusech, F. A., and M. C. Barnhart. 1998. Host suitability and utilization in *Venustaconcha ellipsiformis* and *Venustaconcha pleasii* (Bivalvia: Unionidae) from the Ozark Plateaus. Proceedings of the Conservation, Captive Care, and Propagation of Freshwater Mussels Symposium:83-91.

Roe, K. J. 2010. Conservation Assessment: The snuffbox, *Epioblasma triquetra* (Rafinesque, 1820). Saint Louis University:1-14.

Rogers, S. O., B. T. Watson, and R. J. Neves. 2001. Life history and population biology of the endangered tan riffleshell (*Epioblasma florentina walkeri*) (Bivalvia: Unionidae). Journal of the North American Benthological Society 20(4):582-594.

Schloesser, D. W., and T. F. Nalepa. 1994. Dramtic decline of unionid bivalves in offshore waters of western Lake Erie after infestation by the zebra mussel, *Dreissena polymorpha.* Canadian Journal of Fisheries and Aquatic Sciences 51(10):2234-2242.

Schloesser, D. W., R. D. Smithee, G. D. Longdon, and W. P. Kovalak. 1998. Zebra mussel induced mortality of unionids in firm substrata of western Lake Erie and a habitat for survival. American Malacological Bulletin 14:67-74.

Schloesser, D. W., T. F. Nalepa, and G. L. Mackie. 1996. Zebra mussel infestation of unionid bivalves (Unionidae) in North America. American Zoologist 36(3):300-310.

Schloesser, D. W., T. F. Nalepa, and G. L. Mackie. 1996. Zebra mussel infestation of unionid bivalves (Unionidae) in North America. American Zoologist 36(3):300-310.

Scott, W. B., and E. J. Crossman. 1973. Freshwater fishes of Canada, volume 184. Fisheries Reasearch Board of Canada, Ottawa, Canada.

Shea, K., and P. Chesson. 2002. Community ecology theory as a framework for biological invasions. Trends in Ecology & Evolution 17(4):170-176.

Steneck, R. S., M. H. Graham, B. J. Bourque, D. Corbett, J. M. Erlandson, J. A. Estes, and M. J. Tegner. 2002. Kelp forest ecosystems: biodiversity, stability, resilience and future. Environmental Conservation 29(4):436-459.

Stevens, R. J. J., and M. A. Neilson. 1989. Interlake and intralake distributions of trace organic contaminants in surface waters of the Great Lakes. Journal of Great Lakes Research 15(3):377-393.

Strayer, D. L. 1999. Effects of alien species on freshwater mollusks in North America. Journal of the North American Benthological Society 18(1):74-98.

Strayer, D. L. 2008. Freshwater mussel ecology: A multifactor approach to distribution and abundance, volume 1. University California Press, Berkeley, CA.

Strayer, D. L., and D. R. Smith. 2003. A guide to sampling freshwater mussel populations. American Fisheries Society, Bethesda, MD.

Strayer, D. L., and H. M. Malcom. 2007. Effects of zebra mussels (Dreissena polymorpha) on native bivalves: the beginning of the end or the end of the beginning? Journal of the North American Benthological Society 26(1):111-122.

Strayer, D. L., and H. M. Malcom. 2014. Long-term change in the Hudson's River's bivalve populations: A history of multiple invasions (and recovery?). Pages 71-81 *in* T. F. Nalepa, and D. W. Schloesser, editors. Quagga and zebra mussels: biology, impacts and control, volume 2. CRC Press, New York, NY.

Strayer, D. L., N. F. Caraco, J. J. Cole, S. Findlay, and M. L. Pace. 1999. Transformation of freshwater ecosystems by bivalves: A case study of zebra mussels in the Hudson River. Bioscience 49(1):19-27.

Taeubert, J. E., B. Gum, and J. Geist. 2012. Host-specificity of the endangered thick-shelled river mussel (*Unio crassus,* Philipsson 1788) and implications for conservation. Aquatic Conservation-Marine and Freshwater Ecosystems 22(1):36-46.

Taeubert, J. E., M. Denic, B. Gum, M. Lange, and J. Geist. 2010. Suitability of different salmonid strains as hosts for the endangered freshwater pearl mussel (*Margaritifera margaritifera L.*). Aquatic Conservation-Marine and Freshwater Ecosystems 20(7):728-734.

US Fish and Wildlife Service. 2012. Endangered and threatened wildlife and plants; Determination of endangered status for the Rayed Bean and Snuffbox Mussels throughout their ranges. Pages 67552-67583 *in* D. o. F. a. W. Service, editor. U.S. Fish and Wildlife Service, Columbus. 34pp.

Van Appledorn, M., and C. E. Bach. 2007. Effects of zebra mussels (Dreissena polymorpha) on mobility of three native mollusk species. American Midland Naturalist 158(2):329-337.

Vaughn, C. C. 2012. Life history traits and abundance can predict local colonisation and extinction rates of freshwater mussels. Freshwater Biology 57(5):982-992.

Vaughn, C. C., and C. C. Hakenkamp. 2001. The functional role of burrowing bivalves in freshwater ecosystems. Freshwater Biology 46(11):1431-1446.

Vaughn, C. C., and D. E. Spooner. 2006a. Scale-dependent associations between native freshwater mussels and invasive *Corbicula*. Hydrobiologia 568:331-339.

Vaughn, C. C., and D. E. Spooner. 2006b. Unionid mussels influence macroinvertebrate assemblage structure in streams. Journal of the North American Benthological Society 25(3):691-700.

Vitousek, P. M., C. M. D'Antonio, L. L. Loope, M. Rejmanek, and R. Westbrooks. 1997. Introduced species: A significant component of human-caused global change. New Zealand Journal of Ecology 21(1):1-16.

Walker, B. 1995. Conserving biological diversity through ecosystem resilience. Conservation Biology 9(4):747-752.

Watters, G. T. 1992. Unionids, fishes, and the species-area curve. Journal of Biogeography 19(5):481-490.

Watters, G. T., T. Menker, K. Kuehnl, and S. Thomas. 2005. Host identifications or confirmations. Ellipsaria 7(2):11-12.

Williams, J. D., M. L. Warren, K. S. Cummings, J. L. Harris, and R. J. Neves. 1993. Conservation status of freshwater mussels of the United States and Canada. Fisheries 18(9):6-22.

Woolnough, D. A., J. A. Downing, and T. J. Newton. 2009. Fish movement and habitat use depends on water body size and shape. Ecology of Freshwater Fish 18(1):83-91.

Yeager, B. L., and C. F. Saylor. 1995. Fish host for four species of freshwater mussels (Pelecypoda, Unionidae) in the upper Tennessee river drainage. American Midland Naturalist 133(1):1-6.

Yeager, M. M., D. S. Cherry, and R. J. Neves, editors. 2000. Competitive interactions between early life stages of *Villosa iris* (Bivalvia: Unionidae) and adult Asian clams (*Corbicula flumniea*), volume 274. Ohio Biological Survey Columbus, Ohio.

Zale, A. V., and R. J. Neves. 1982. Fish hosts of 4 species of lampsiline mussels (Mollusca, Unionidae) in big Moccasin Creek, Virginia Canadian Journal of Zoology-Revue Canadienne De Zoologie 60(11):2535-2542.

Zanatta, D. T., and C. C. Wilson. 2011. Testing congruency of geographic and genetic
population structure for a freshwater mussel (Bivalvia: Unionoida) and its host fish.
Biological Journal of the Linnean Society 102(3):669-685.

Zar, J. H. 2010. Biostatistical analysis: Fifth edition 5th edition. Pearson Education, Inc., Upper
Saddle River, NJ.