A multi-basin approach determines variability in host fish suitability for unionids in tributaries of the Laurentian Great Lakes

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SUMMARY

1. Freshwater mussel populations have declined, in part, from changes in host communities. However, it is unknown if fish from adjacent catchments could be used to augment mussel populations in the Great Lakes inland rivers, and if so, whether this association would be impacted by known genetic structure in mussels and fish.
2. This study tested how host fish origin (i.e. catchment) impacts the transformation of the endangered unionid *Epioblasma triquetra* from larval into juvenile life stages while concurrently considering potentially genetically distinct populations of mussels and hosts.
3. We quantitatively determined if *Percina caprodes* and *Percina maculata* from the Lake Michigan, Erie and Huron basins are laboratory-successful hosts for *E. triquetra*. Experiments were performed in autumn and spring to document any seasonal effects on transformation.
4. *Percina caprodes* was reconfirmed to be a successful host for *E. triquetra*, and for the first time, *P. maculata* was also determined to be a successful host 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.
5. Knowing the most efficient strategy to optimise juvenile transformations can make reintroductions, augmentation and overall conservation efforts of *E. triquetra* successful. These data will help in developing recovery strategies for *E. triquetra* in the Laurentian Great Lakes by understanding variation in host use and nuances in this host–parasite relationship.

Keywords: endangered species, *Epioblasma triquetra*, freshwater mussels, parasitic relationship, *Percina*

Introduction

With the ever increasing human impacts on biodiversity, extinction rates of all organisms have increased 100–1000 times since pre-human times (Pimm *et al.*, 1995); one group of organisms most impacted by human disturbances are freshwater mussels (order: Unionoida; unionids). Currently, 72% of freshwater mussels in North America are considered endangered, threatened or need protection, predominately as a result of human activities (Haag, 2012; Vaughn, 2012). With over 300 species of freshwater mussels in North America, they encompass a variety of different guilds including variable host use, brood time and habitat use.

Freshwater mussels are important to aquatic ecosystems because they stabilise stream beds, oxygenate the sediments, and filter large portions of the water column (Haag, 2012). Often dominating the biomass of the benthic community, freshwater mussels can filter up to 100% of the water column per day (Haag, 2012). Additional aquatic ecosystem benefits include altering benthic communities through biodepositing faeces and pseudofaeces, and adding nutrients to the water from excretion, all which play an important role in water quality and

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ecosystem function (Vaughn & Hakenkamp, 2001; Atkinson & Vaughn, 2015). High biodiversity has been found to help an ecosystem to be more resilient to disturbances and sustain a variety of ecosystem functions (Walker, 1995; Vaughn, 2010).

The life cycle of a freshwater mussel involves a parasitic stage on fish which is highly susceptible to disturbances (Strayer, 1999; Mcmahon, 2002). Freshwater mussels require a host fish for development of their larval stage (glochidia), an obligate ectoparasitic relationship (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 number of fish species (Haag, 2012), sometimes a single species or clade of fish. Glochidia typically attach to the gills of a fish forming an encystment until they develop into juveniles (Strayer, 2008). The glochidia attached to the host fish uptakes nutrients from the fish until fully developed, undergoing a metamorphosis to a free-living juvenile mussel, and excising (Barnhart, Haag & Roston, 2008; Strayer, 2008; Denic, Taeubert & Geist, 2015). In order for the juvenile mussel to survive, it must settle in a suitable habitat and burrow itself into the substrate (Strayer, 2008). Survivorship for glochidia to the juvenile stage is low; therefore, freshwater mussels produce large numbers of glochidia and have adapted strategies to attract their host fish (Barnhart *et al.*, 2008). If a mussel population is in decline or not successfully recruiting juveniles, it could be a result of the disappearance, shift, decline or change in host or host community (Newton, Woolnough & Strayer, 2008). Freshwater mussel distribution and dispersal is dependent on host fish (Watters, 1992; Strayer, 2008; Haag, 2012).

Host fish testing has been conducted using various methods to determine suitable fish for specific freshwater mussels and understand the host–parasite relationship (Zale & Neves, 1982; Allen *et al.*, 2007; Douda, Horký & Bílý, 2012; Lima *et al.*, 2012; Taeubert, Gum & Geist, 2012a, 2013; Taeubert *et al.*, 2012b). It is recognised that laboratory methods do not replicate natural processes; however, it is a first step to understanding what may happen naturally in a controlled setting. 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 of the geographical source of fish and mussels. 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 & Neves, 1982; Haag & Warren, 1997; Jones *et al.*, 2004, 2006; Dodd *et al.*, 2005; Hoftyzer *et al.*, 2008; Taeubert *et al.*, 2012a,b). In addition to allopatric host fish studies, there has been some research on the compatibility of sympatric fish and mussel populations. For example, Taeubert *et al.* (2010) tested transformation success of *Margaritifera margaritifera* (Margaritiferidae) glochidia on different salmonid strains from areas within and outside of the distribution of *M. margaritifera*. They found the trout strain sympatric to *M. margaritifera* populations successfully transformed the highest proportion of glochidia (Taeubert *et al.*, 2010). Also, it has been 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 produce more juveniles per fish (Rogers, Watson & Neves, 2001). The study by Rogers *et al.* (2001) focused on an unglaciated region of North America; therefore, an older system than our study that populations have had a longer to adapt to changes in host fish assemblages. There have been other studies that use mussels and fish species sampled from different river basins to compare and assess their host compatibility (e.g. Serb & Barnhart, 2008; Taeubert *et al.*, 2010; Österling & Larsen, 2013; Douda *et al.*, 2014); however, this study is unique due to the rarity of the mussel, the ecology of the hosts tested, the large glaciated catchments where this study was conducted, and the variety of treatment combinations used (i.e. locations of mussels and host from the Laurentian Great Lakes basin).

With many native freshwater mussel populations in decline, it is imperative that we have effective and empirically driven conservation programmes. There has been an increase in propagation and augmentation efforts throughout North America; however, not many studies have been conducted on the endangered *Epioblasma triquetra* (Unionidae) in the Great Lakes basins (COSEWIC, 2011; US Fish and Wildlife Service, 2012). This study had three objectives: (i) to quantify which fish is the most laboratory-successful host. We hypothesise that *Percina caprodes* (Percidae) will be the most successful host relative to *Percina maculata* because of their close coevolutionary relationship (Zanatta & Wilson, 2011). In addition, the relatively distant phylogenetic relationship between *P. caprodes* and *P. maculata* suggests that these species are not closely related (Near, 2002) adding further support for our hypothesis. (ii) To analyse host fish suitability of *E. triquetra* with *P. caprodes* and *P. maculata* from multiple Great Lakes basins. It is expected that fish from the same

basin as *E. triquetra* will have more successful transformations of glochidia to juveniles than fish from other basins (Rogers *et al*., 2001; Taeubert *et al*., 2012a,b). (iii) To test seasonal differences in juvenile transformation success of *E. triquetra*.

Methods

Target species

Epioblasma triquetra is listed as endangered under the United States *Endangered Species Act* and in Canada under the S*pecies at Risk Act*. *Epioblasma triquetra* is a native freshwater mussel with a unique adaptation for capturing and parasitising its host with glochidia (Barnhart *et al*., 2008). Gravid *E. triquetra* females 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). *Percina caprodes* has a sturdier skull that prevents *E. triquetra* from crushing its head making it a suitable host (Barnhart *et al*., 2008); it is possible that not all fish survive being captured. Zanatta & Wilson (2011) found that there is a covarying pattern of genetic differentiation between *P. caprodes* and *E. triquetra* in the United States and Canada. Laboratory host fish tests have found a variety of potential hosts for *E. triquetra*: *Cottus hypselurus*, *C. baileyi*, *C. bairdii*, *C. carolinae* (Cottidae), *Fundulus olivaceus* (Fundulidae), *Etheostoma caeruleum*, *E. exile* (Percidae), *P. maculata* and *P. caprodes* (Yeager & Saylor, 1995; Hillegrass & Hove, 1997; Barnhart, Riusech & Bairs, 1998; Jones & 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 with only *P. caprodes* being confirmed as a laboratory host for Lake Erie drainage *E. triquetra* populations (Castanon *et al*., 2011). We do not know of any studies that have considered multibasin interactions and multiple host species in the Great Lakes basin. 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 & Hove, 1997; Barnhart *et al*., 1998).

Sampling sites

Epioblasma triquetra, *P. caprodes* and *P. maculata* were collected from rivers in the lower peninsula of Michigan Fall 2012 (F1), Spring 2013 (S1) and Fall 2013 (F2); all *E. triquetra* collected were gravid. Throughout this study, the origin of fish and mussel are referenced with their basin origin, refering to the inland rivers within that particular basin (e.g. *E. triquetra* from the Michigan basin refers *E. triquetra* collected from inland rivers within Lake Michigan basin, not from Lake Michigan). 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) (Fig. 1). 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). Due to the extreme rarity of *E. triquetra*, the federal permit limited the number of mussels allowed in the experiments. We understand that genetic variation is limited by the numbers of females used (e.g. host compatibility genetic variation, potential maternal effects; Douda *et al*., 2014); however, we were unable to address that in these experiments.

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 F1 and F2, three gravid female mussels were used for each basin. Due to high waters during S1, only 2 gravid females were found in the Clinton River (Erie basin). For the experiment, there were 12 treatments (six *P. caprodes* treatments and six *P. maculata* treatments), except for S1 which had only six treatments (Table 1). Although our goal was to infest equal num-

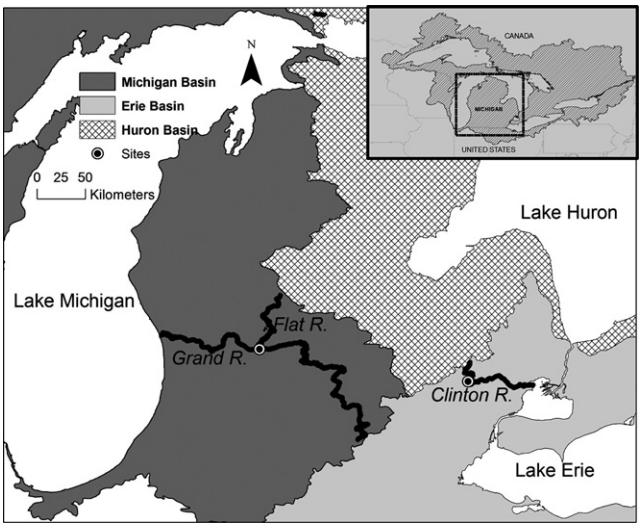

Fig. 1 *Epioblasma triquetra* collection locations [Flat River (west) and Clinton River (east)] used for this study. Collection sites indicated by circles. Inset is key map; hatched area is the Laurentian Great Lakes catchment.

Table 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 (*Percina caprodes* and *P. maculata*); Replicates = number of fish used for each experimental treatment (F1 = Fall 2012, F2 = Fall 2013 and S1 = Spring 2013).

E. triquetra origin (basin)	Fish origin	Fish species	Replicates
Michigan	Michigan	*P. caprodes*	F1 = 7, F2 = 11
		P. maculata	F1 = 13, F2 = 11
	Erie	*P. caprodes*	F1 = 13, F2 = 11
		P. maculata	F1 = 3, F2 = 11
	Huron	*P. caprodes*	F1 = 8, F2 = 10
		P. maculata	F1 = 11, F2 = 11
Erie	Michigan	*P. caprodes*	F1 = 6, S1 = 3, F2 = 10
		P. maculata	F1 = 13, S1 = 5, F2 = 10
	Erie	*P. caprodes*	F1 = 16, S1 = 5, F2 = 10
		P. maculata	F1 = 3, S1 = 5, F2 = 10
	Huron	*P. caprodes*	F1 = 9, S1 = 5, F2 = 8
		P. maculata	F1 = 11, S1 = 5, F2 = 8

bers of fish for each treatment, the samples sizes varied for each basin due to availability of fish.

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 10 *P. maculata* and 10 *P. caprodes*. All fish used were not young of year and were likely <4 years old based on this being the maximum lifespan of both species (Scott & Crossman, 1973); exact ageing was not performed but not assumed to be a factor as seen in Taeubert *et al.* (2013) as the hosts for *M. margaritifera* can live twice as long *as P. caprodes* and *P. maculata*. Fish were held in individual Aquatic Habitat (AHAB, Apopka, FL) tanks (3 and 10 L based on fish size) for the experiment. Fish were held in tanks for at least 1 week to up to 90 days prior to the experiment limiting the possibility of other unionid transformations occurring on the fish during this experiment. All glochidia and juveniles that were quantified during these experiments resembled the morphological structure of *E. triquetra*, which is quite unique in the family Unionidae (i.e. much more spherical and taller in height than other unionids). Length of time fish were kept varied due to collection logistics. In addition to length of time fish were held in tanks, juveniles that excised off of the fish resembled the morphological structure of *E. triquetra* which is unique from other unionids.

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 & Smith, 2003) in 2009–2010 (Bergner, 2013). Glochidia were extracted from the gravid mussels by flushing the marsupial gills with deionised water (Zale & 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 NaCl solution, they were considered viable (Lefevre & Curtis, 1912) and were used in the experiments within minutes of extraction. Glochidia used in this experiment had an average viability of 99.7% (±0.83). 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 six subsamples for each Great Lakes basin for each fish, with a total of 12 treatments (Table 1). The average concentration of glochidia for the infestation baths was 1.40 glochidia mL^{-1} (±0.71). For the F1, S1 and F1 experiments, multiple gravid female mussels were used from each basin. S1 had above average high stream flows reducing the number of gravid females collected from the Flat River (Michigan basin).

After the fish were separated by basin, half of the fish were used for the Erie basin *E. triquetra* treatments and half were 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 fish were exposed to the glochidia for 60 min and observed for signs of lethargy (Zale & Neves, 1982). Three replicates of 40 mL of water were collected after experiment, and all glochidia were counted from these replicates to quantify glochidia that did not attach to fish. After the infestation, each fish was placed in an individual AHAB tank. Water for these tanks was continuously monitored for pH, conductivity and temperature which were set at 8.0, 750 μS and 16 °C, respectively; that were water parameters found in resident rivers as recommended in Taeubert, El-Nobi & Geist (2014). Each tank had a separate aeration tube. Tanks had a flow-through system with a 10% exchange of fresh water daily. Water chemistry was adjusted immediately

if chemistry deviated from initial settings. A catcher, made with 2.54 cm (1 inch) PVC pipe affixed with 125-μm mesh, was attached to each outflow of each individual tank with wire to catch glochidia or juvenile *E. triquetra* that drop off the fish. To count glochidia and juveniles, catchers were removed and examined daily with a Leica EZ4 (Leica Microsystems, Buffalo Grove) dissection microscope. Juveniles that excised, but were not fully developed (lacking a dark spot of a developing heart and/or developed tissue but no foot movement); we considered them to be at a transitional stage of juvenile development. 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), fish were killed and the water from each tank was sieved through 125-μm mesh to collect any glochidia and juveniles that remained.

Infestation intensities were determined by the total juveniles and glochidia that had attached to each fish (Crane *et al*., 2011). Host fish experiments were analysed 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, and 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 standardise for the varying rates of glochidia infestation concentrations each year, standardised transformation success (juveniles/glochidia introduced to 5000 mL bucket for each treatment) for each pairing was calculated and analysed using a two-way ANOVA. In addition, Mann–Whitney *U* test was performed to determine whether there were differences in the fish lengths. Spearman's rank tests were performed to determine if fish length is correlated with transformation success and fish length and infestation intensity. Type III two-way ANOVAs were used to determine seasonality differences.

Results

Host fish suitability: host fish differences

Epioblasma triquetra glochidia encysted and metamorphosed on both fish species that were tested. Eight hundred forty-four ($\bar{x}$ = 6.39 per fish) metamorphosed *E. triquetra* juveniles excysted from *P. caprodes* 13–65 days after the infestation (peaking at day 25 for F1, day 29 for F2 and day 20 for S1; Table 2) with 33.4 ± 2.52% overall transformation (Table 3). One hundred thirty ($\bar{x}$ = 1.01 per fish) *E. triquetra* juveniles excysted from *P. maculata* 13–45 days after infestation (peaking at day 25 for F1, day 21 for F2 and day 20 for S1; Table 2) with 9.9 ± 1.71% overall transformation (Table 3).

Standardised transformation success. Glochidia infestation concentrations for each experiment varied (Fig. 2). However, due to the extreme imperilment of *E. triquetra,* all glochidia were used. There was a positive relationship between transformation success and infestation intensity (r_s = 0.173, P = 0.007) (Fig. 3a). In an attempt to standardise for the varying rates of glochidia infestation concentrations each experiment (Fig. 2), standardised transformation success for each pairing of fish basin and mussel basin was calculated (see Methods). For F1 *P. caprodes*, a difference was found with standardised transformation success (Type II two-way ANOVA; χ^2 = 16.9, df = 1, $P \ll 0.001$) and the *E. triquetra* basins, with Michigan basin treatments being the most successful (Fig. 4c). However, there was no difference found with standardised transformation success and fish basins (χ^2 = 1.91, df = 2, P = 0.385) (Fig. 4d). For F1 *P. maculata* experiments, there were no differences found between fish basin origin or *E. triquetra* basin origin.

For F2 *Percina caprodes* experiments, standardised transformation success was significantly different for fish (Type II two-way ANOVA; χ^2 = 12.1, df = 2, P = 0.001) and *E. triquetra* basins origin (Type II two-way ANOVA; 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 (Fig. 4d). In addition, *E. triquetra* origin was different with Lake Michigan origin glochidia having the most success. *Percina maculata* standardised transformation success was different for fish (Type II two-way ANOVA; χ^2 = 7.76, df = 2, P = 0.021) and *E. triquetra* basin (Type II two-way ANOVA; χ^2 = 3.81, df = 1, P = 0.051). *Epioblasma triquetra* from the Lake Michigan basin were more successful transforming than Lake Erie basin *E. triquetra* (Fig. 4c and d).

Host fish suitability and fish length. Percina maculata (76 ± 0.895 mm) were smaller than *P. caprodes* (103 ± 1.097 mm) (W = 14155, $P \ll 0.001$) and lengths varied from those reported in literature (Scott & Crossman, 1973; Hugg, 1996). *Percina maculata* had an average length of

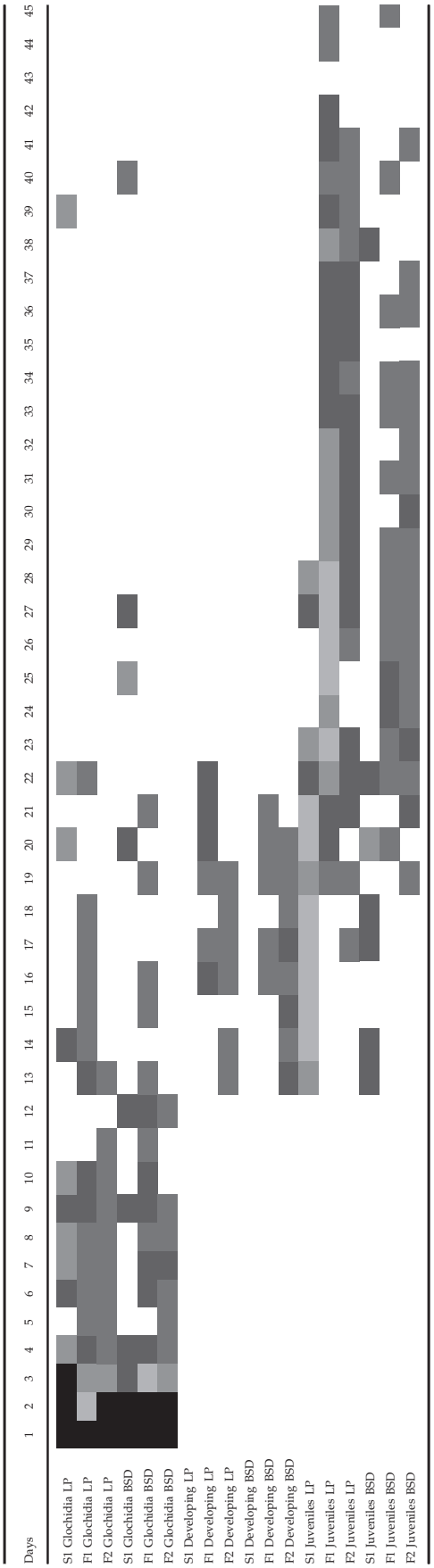

Table 2 Infestation timeline from initial infestation day to 45 days (juveniles continued to drop off until day 65 but less than 5% of experimental fish produced juveniles after 45 days) after infestation for *Epioblasma triquetra* using *Percina caprodes* (LP) and *Percina maculata* (BSD) for experiments conducted in F1, F2 and S1 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).

58 mm (Scott & Crossman, 1973; Hugg, 1996), and *P. caprodes* average length in the literature varied and was reported as 89 mm (Scott & Crossman, 1973) and a common length of 125 mm (Hugg, 1996). A positive relationship was found between *P. caprodes* length and infestation intensity (r_s = 0.294, P = 0.001) (Fig. 3b). A marginally significant positive relationship was found between *P. maculata* length and infestation intensity (r_s = 0.163, P = 0.077). In addition, positive relationships were found with fish length for both *P. caprodes* and *P. maculata* and transformation success (r_s = 0.450, $P \ll 0.001$) (Fig. 3c).

Host fish suitability: mussel-basin and fish-basin interactions

For F1 *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 4). For *P. caprodes* in F1, a difference was found with percent transformation and *E. triquetra* basin origin (χ^2 = 15.5, df = 1, $P << 0.001$), indicating there were transformation differences between Lake Michigan basin *E. triquetra* and the Lake Erie basin *E. triquetra* (Fig. 4a, Table 4).

For *P. maculata*, host fish suitability tests for F2, 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 with Michigan basin *E. triquetra*. F2 results for *P. caprodes* suggest that there was no difference between fish or *E. triquetra* basin origins and transformation success (Fig. 4b).

Seasonal differences in transformation success

For *P. maculata*, there was no significant difference between fish basin and time of experiment with transformation success (Type III two-way ANOVA; χ^2 = 1.13, df = 4, P = 0.889). There was a significant interaction with the fish basin and year in relationship to transformation success among all *E. triquetra* and *P. caprodes* experiments (Type III two-way ANOVA; χ^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.

S1 host fish experiments standardised transformation successes were compared to F1 and F2. For *P. maculata*, there were no differences between fish basin and time of experiment with standardised transformation success. For *P. caprodes*, there was an interaction with the fish basin and year in relationship to standardised transfor-

mation success (Type III two-way ANOVA; $\chi^2 = 14.4$, $df = 4$, $P = 0.006$). In S1, a significant relationship ($P << 0.001$) was found between *P. caprodes* from the Lake Huron and Lake Erie basins and from the Lake Erie basin and Lake Michigan basin. Differences were also observed from in the *P. caprodes* from the Lake Erie basin from S1 and from the Lake Erie basin from F2, *P. caprodes* from S1 and from the Lake Michigan basin from F1, *P. caprodes* from Lake Erie basin from S1 and from the Lake Huron basin for F1, and *P. caprodes* from the Lake Erie basin from S1 and *P. caprodes* from the Lake Erie basin from F1. In addition, weaker relationships were found between *P. caprodes* from the Lake Michigan basin from S1 and *P. caprodes* from the Lake Erie basin from the S1 basin. Also, there was a difference with year and standardised transformation success, with F1 and S1 ($P = 0.014$) and F2 and S1 ($P = 0.004$).

For all experiments, the majority of untransformed glochidia (65.5%) excised within the first 2 days of the experiment and continued to excise for 28 days (Table 2). For F1 and F2 experiments for both fish species, there was a period of developing juveniles dropping off from day 13 to day 22 (Table 2). For the S1 experiment with both fish species, there was no transitional stage; fully transformed juveniles began to drop off starting day 13 and majority finished dropping off after day 28 (Table 2). F1 and F2 experiments began to produce fully developed juveniles around day 20 and lasted to approximately day 40 (Table 2). However, 10% of *P. caprodes* (6 fish) each had one juvenile excise between day 45 and day 65.

Discussion

Host fish suitability

Host fish suitability tests for all three experiments (F1, S1, F2) determined that *P. maculata* and *P. caprodes* are both laboratory-successful hosts for *E. triquetra* from rivers in the Lake Erie and Lake Michigan basins. Our studies for *P. caprodes* are consistent with results from past research from outside of the region (Hillegrass & Hove, 1997; Barnhart *et al*., 1998) and from one river in the Lake St. Clair/Erie region (Castanon *et al*., 2011). This is the first time that *P. maculata* was confirmed as a successful host for *E. triquetra* in the Great Lakes region. Our study found that, as a host, *P. caprodes* is able to transform a greater proportion of glochidia to juveniles when compared with *P. maculata*.

For this study, *P. caprodes* and *P. maculata* were tested because of their presence and relative abundance in the local basins and their success as hosts in other basins (Hillegrass & Hove, 1997; Barnhart *et al*., 1998). Freshwater mussels from the Huron and Michigan drainages were found to be indistinguishable genetically, as but distinct from the Lake Erie drainage (Bergner, 2013). Although *E. triquetra* were not tested by Bergner (2013), a related study in Ontario did show that *E. triquetra* in the Lake Huron drainage (Ausable River, Ontario) was genetically distinct from a population in the Sydenham River (Lake St. Clair/Lake Erie drainage) that is in the same Great Lakes drainage as the Clinton River (Galbraith, Zanatta & Wilson, 2015). 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, *P. caprodes*, have been determined to share similar genetic patterns (Zanatta & Wilson, 2011).

The variation in success between fish species could be attributed to the biology of the host fish species and their interaction with *E. triquetra*. *Epioblasma triquetra* capture host fish by closing its valves on the rostrum of the host and then pumping glochidia onto the gills of the fish (Barnhart *et al*., 2008). *Percina caprodes* have been shown to endure being captured because of their sturdy skull that prevents them from being crushed (Barnhart *et al*., 2008), whereas this adaptation for *P. maculata* has

Table 3 *Epioblasma triquetra* host fish suitability test results for F1, F2 and S1 results for *Percina caprodes* and *Percina maculata*.

Experiment	Host fish	Juveniles	Number of fish	*E. triquetra* juveniles produced per fish	Percent transformation
F1	*P. caprodes*	462	59	7.83 ± 9.16	37.4
	P. maculata	39	54	0.72 ± 1.34	5.5
S1	*P. caprodes*	253	13	19.46 ± 34.51	35.8
	P. maculata	12	14	0.80 ± 1.42	7.4
F2	*P. caprodes*	129	60	2.15 ± 3.85	26.9
	P. maculata	79	61	1.30 ± 2.00	16.7
Average	*P. caprodes*	281	44	6.39	33.4
	P. maculata	43	43	1.01	9.9

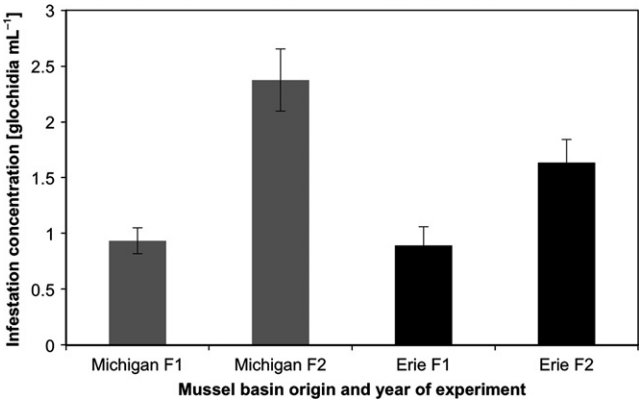

Fig. 2 Glochidia infestation calculation (glochidia mL^{-1}) *Epioblasma triquetra* originating from the inland rivers within the Michigan basin (grey) and *E. triquetra* originating from the inland rivers with the Erie basin *E. triquetra* (black) that each experiment was infested with for F1 and F2. Bars represent standard error.

never been documented. However, a less sturdy skull would make *P. maculata* a less suitable host in nature due to an increased chance of mortality as result of trapping by *E. triquetra*. The behaviour 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* under natural conditions (Scott & 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* could be an important 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 more resilient to stress caused by the infection strategy of *E. triquetra*, research suggests that they share a close coevolutionary relationship (Zanatta & 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*. Despite the critical role that hosts play in the life history of unionoid

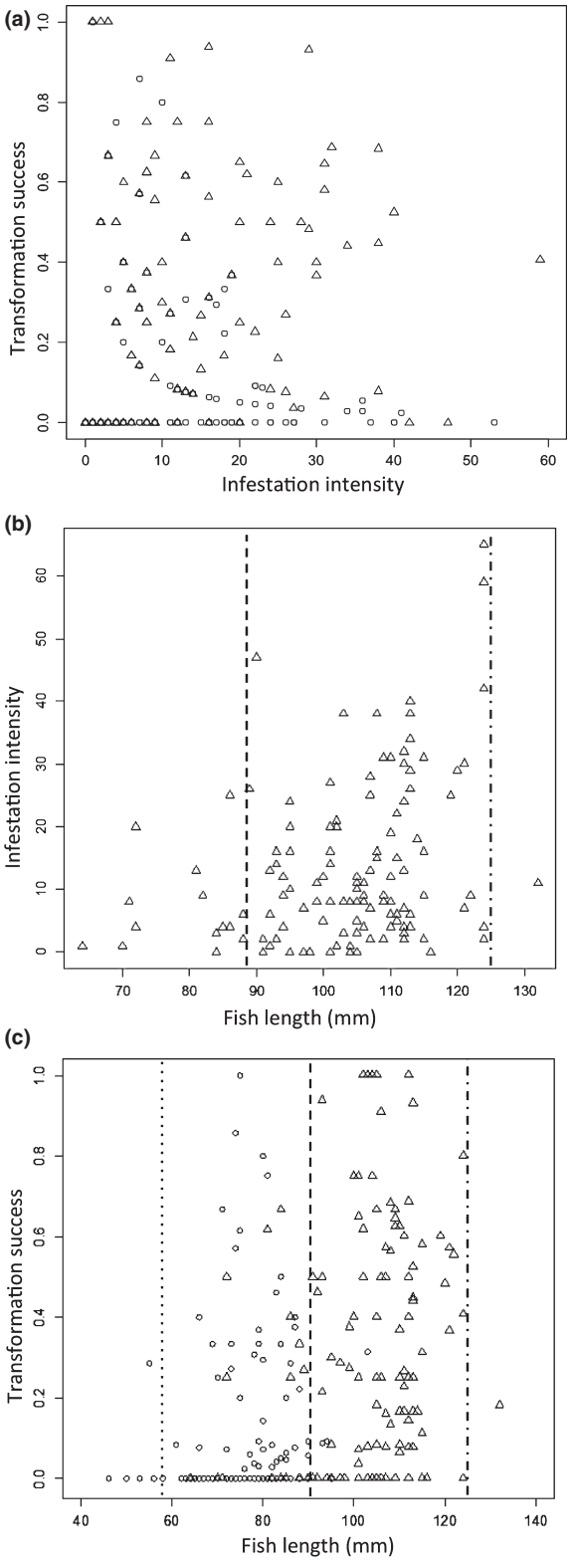

Fig. 3 Transformation success and infestation intensity during host experiments. (a) Infestation intensity (glochidia + juveniles) and transformation success (juveniles/ [glochidia + juveniles]) for all Epioblasma triquetra host fish suitability experiments (F1, F2, and S1), *Percina caprodes* and *P. maculata*, (b) *Percina caprodes* length and infestation intensity (glochidia + juveniles) relationship from all host fish suitability tests (F1, F2, and S1), (c) fish length of *P. caprodes* and *P. maculata* for all experiments (F1, F2, and S1) and transformation success relationship (juveniles/ [glochidia + juveniles]). Circles=*P. maculata*, Triangles= *P. caprodes*. Vertical lines indicate: dotted- *P. maculata* common size (Scott & Crossman, 1973 and Hugg, 1996); dashed *P. caprodes* common size (Scott & Crossman, 1973), dashed-dotted *P. caprodes* common size (Hugg, 1996).

Table 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 standardised transformation success for Fall 2012 and Fall 2013 experiments. Basin origins refer to inland rivers within those basins.

		Transformation success	Standardised transformation	Transformation success	Standardised transformation
		Percina maculata		*Percina caprodes*	
Time of experiment	Type of comparison	*P*	*P*	*P*	*P*
F1	Fish basin	0.360	0.078	0.139	0.385
	Mussel basin	0.090	0.099	<<0.001***	<<0.001***
	Fish × Mussel basin	0.462	0.215	0.434	0.075
F2	Fish basin	0.256	0.021*	0.148	<<0.001***
	Mussel basin	0.035*	0.051	0.426	0.024*
	Fish × Mussel basin	0.641	0.283	0.836	0.863

*$P < 0.05$.
***$P < 0.001$.

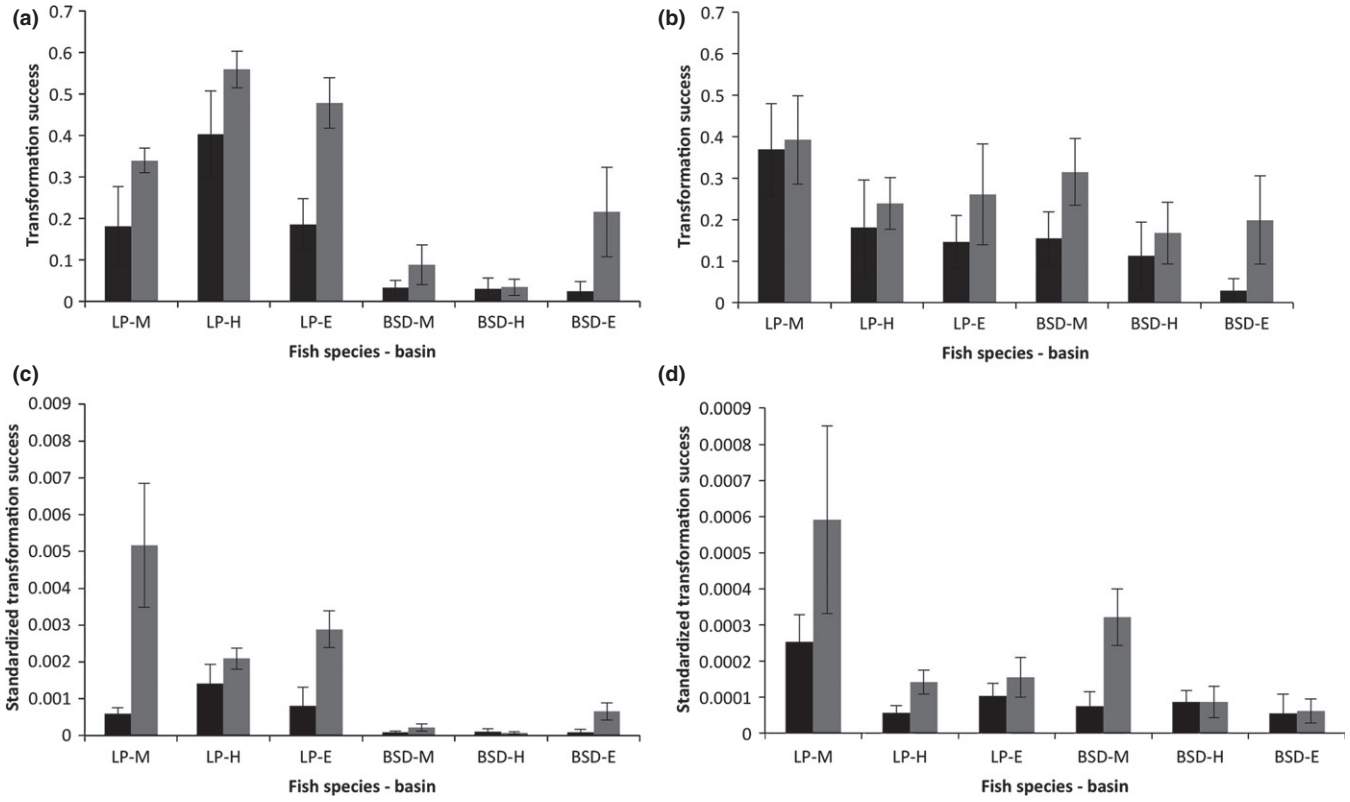

Fig. 4 Transformation success [juveniles/(juveniles + glochidia)] for (a) F1 experiment and (b) F2 experiment and standardised transformation success for (c) F1 experiment and (d) F2 organised by *Epioblasma triquetra* basin and fish basins that are from inland waters within the Erie, Michigan or Huron basin. Black = Erie basin *E. triquetra* origin and grey = 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.

mussels, this intuitive covarying genetic pattern may not always be the case for all unionoids and their hosts as demonstrated by Geist & Kuehn (2008).

While our data suggest that *P. maculata* may not be the most successful host relative to *P. caprodes*, this may be the result of body size of the fish used rather than

the species. Although overall lengths of fish used varied from those reported in nature, *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 & Pace, 1993; Taeubert *et al*., 2010). Our study found that as fish length increased for both *P. maculata* and *P. caprodes*, transformation success also increased. Therefore, although *P. maculata* was not as successful in juvenile transformation as *P. caprodes*, this may be due to the average length of *P. maculata* in our experiment being smaller. This conflicts with previous host fish suitability research that did not find a significant relationship with length and transformation success (Riusech & Barnhart, 1998). In addition to fish length influencing juvenile transformation success, fish body size can be used as a predictor of home range of fish (Woolnough, Downing & Newton, 2009); larger fish have larger home ranges (Woolnough *et al*., 2009). Therefore, in addition to higher rates of metamorphosis of *E. triquetra* glochidia, parasitism of *P. caprodes* could also result in longer dispersal distance 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 hosts (Martel & Lauzon-Guay, 2005). If *P. maculata* is being used by *E. triquetra* as a host in natural systems, it is likely that movement among locations could be limited due to the smaller home range of the smaller host species, therefore limiting gene flow. More research needs to be conducted to fully understand the relationship between fish length, transformation success and host movement.

Host fish suitability mussel-basin and fish-basin interactions

We determined that the origin of host fish and freshwater mussels does not play an important role in rates of transformation success for *E. triquetra* in the Great Lakes. With host fish testing becoming increasingly important for understanding and conserving freshwater mussels, it is imperative that we determine (i) the best practices to ensure high rates of transformation and (ii) the best strategies for sustaining populations. 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*., 2012a,b). Therefore, since there are at least two genetically distinct freshwater mussel populations in the Great Lakes with mussels from the 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; Galbraith *et al*., 2015). Our results do not support that genetic structure among freshwater mussel population plays a role in juvenile transformation success. These results contradict recent research that found clear relationships with transformation success and fish–mussel pairings (Douda *et al*., 2014), suggesting that genetic populations of *E. triquetra* in Lake Erie basins and Lake Michigan/Huron basins (Galbraith *et al*., 2015) have not been isolated long enough to evolve genetic differences and that the genetic differentiation is neutral with respect to differences in host fish use.

Two methods were used to analyse these data, transformation success and standardised transformation success. Neither method showed consistent results supporting sympatric or allopatric genetic population pairings of fish and mussel as the most productive juvenile transformation outcome. Our results demonstrate that the most important factor in propagation success (i.e. number of juveniles) is the origin of the freshwater mussel. Standardised transformation success results suggest that mussel basin origin is the most significant factor in juvenile propagation; however, for the F2 experiment, fish basin of origin is also important. The low number of successful transformations for F2 experiment may be due to decrease attachment rates to fish gills. The large number of glochidia that do not attach onto the gills of exposed fish 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 F2. 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, with *E. triquetra* originating from the Lake Michigan basin being more successful for all experiments except for the transformation success for *P. caprodes* from F2.

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. A reason for the markedly higher transformation success by mussels originating from the Lake Michigan drainage in comparison to the Lake Erie drainage may be related to the mussels' condition as a

result of their habitat quality. Lake Erie freshwater mussel populations have been declining since the 1960s resulting from pollution and habitat degradation (Stevens & Neilson, 1989; Nalepa *et al.*, 1991; Bryan *et al.*, 2013; Zanatta *et al.*, 2015). Lake Erie populations declined precipitously with the introduction of dreissenids in 1986 (Schloesser & Nalepa, 1994; Schloesser, Nalepa & Mackie, 1996; Bryan *et al.*, 2013; Zanatta *et al.*, 2015). Decline in the freshwater mussel populations of Lake Erie suggests that the habitat quality in the basin may play a role in propagation success. The freshwater mussel assemblage in the Clinton River has been in decline from an initial 26 species in 1978 to only 14 species in 2004 (Francis & Haas, 2006; Morowski, James & Hunter, 2009). This apparent decline in richness is suggested to be due to flow instability (Francis & Haas, 2006). Also, the Clinton River is considered to be degraded due to point source pollution in the river. Although the majority of the inputs have been found, chemicals persist in the river (Francis & Haas, 2006). The Flat River is impacted by large amounts of agriculture, but in this catchment agriculture has not been definitively linked back to changes in mussel assemblages (Badra & 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); fish were infested during the F2 experiment with much higher concentrations than F1. The standardised transformation success for F2 was much less successful in comparison to F1. 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, gill damage, decreased weight, negative influences on critical swimming speeds and mortality of fish (Dodd *et al.*, 2005; Kaiser, 2005; Howerth & Keller, 2006; Crane *et al.*, 2011; Taeubert & Geist, 2013). 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 concentrations of glochidia throughout the stages of experiment as we demonstrated in these experiments.

Seasonal differences in transformation success

Seasonal variation in juvenile transformation was revealed in this study. Standardised host fish transformation success revealed that transformation success was significantly different with metamorphosis being accelerated for the *P. caprodes* experiments in spring in comparison to both fall experiments. Furthermore, juvenile development differed in spring without any 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 (Watters, Hoggarth & Stansbery, 2009). However, more recently, it has been concluded that there is a brooding continuum; longer-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 transitional juvenile form in the spring compared to the autumn suggests that glochidia may have a chance to develop more over winter making them quick to fully transform on the fish, falling along this brooding continuum and potentially not a short-term or long-term brooder. However, adding to previous studies, our results suggest that *E. triquetra* can release glochidia over a long-time period (e.g. spring through autumn). Our data suggest that *E. triquetra* could have the ability to use a variable brooding strategy, depending on the availability of hosts. Also, when collecting gravid *E. triquetra* for conservation efforts and in order to metamorphose the largest numbers of fully developed juveniles, 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.

Management implications

The results of our host fish suitability tests for *E. triquetra* can be used to advance propagation practices. Reintroduction efforts are constantly being built on increased knowledge of endangered species (Neves, 2004). From our study, we suggest that when available, larger *P. caprodes* (e.g. >100 mm) should be used for propagation of *E. triquetra* because larger fish produce the highest transformation rates. In addition, our study suggests brood stock mussels coming from streams with better water quality and fewer threats 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 also suggests that *E. triquetra* in the Clinton River may be in decline; therefore, habitat restoration and *E. triquetra* conservation efforts should be directed towards rebuilding this population. So, although there are streams that may not be the best source for *E. triquetra* to be used for propagation for the entire Great Lakes basin, they are still important for the overall Great Lakes population of *E. triquetra* and should be protected.

We determined that both *P. caprodes* and *P. maculata* were laboratory-successful hosts for *E. triquetra* in Great Lakes basin. It also appears that host fish body size influences transformation success; for *E. triquetra*, the larger the host fish, the more successful it will be for juvenile transformation. The suitability of *E. triquetra* with the *P. caprodes* and *P. maculata* based on individuals (fish and mussels) from multiple catchments in the Great Lakes was also analysed, with mussel origin being found as the only important factor. Finally, we documented seasonal variation in juvenile transformation success in *E. triquetra* by observing quicker transformation rates in the spring, suggesting more mature glochidia are available for infesting hosts in the spring. Overall, these findings should be used to help guide conservation efforts and stimulate research in other regions.

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