An effect of the invasive Round Goby (*Neogobius melanostomus*) on the recruitment of unionid mussel Species at Risk (Bivalvia: Unionidae)

by

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ABSTRACT

An effect of the invasive Round Goby (*Neogobius melanostomus*) on the recruitment of unionid mussel Species at Risk (Bivalvia: Unionidae)

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I investigated whether *Neogobius melanostomus,* an invader of biodiversity "hot-spots" in Ontario facilitates or inhibits unionid mussel recruitment by serving as a host or as a sink for their parasitic larvae (glochidia). Infestation and metamorphosis rates of four mussel Species at Risk (*Epioblasma torulosa rangiana, Epioblasma triquetra, Lampsilis fasciola, and Villosa iris*) and one common species (*Actinonaias ligamentina*) on *N. melanostomus* were compared to rates on known hosts in the laboratory. All species successfully infested *N. melanostomus,* but only *E. triquetra, V. iris,* and *A. ligamentina* successfully metamorphosed, albeit at low rates. *Neogobius melanostomus* collected from areas of unionid occurrence in the Grand and Sydenham rivers exhibited body burdens of 39.4% and 5.1%, respectively. Analyses indicate that *N. melanostomus* serves more as a sink for glochidia than as a host for unionids, thereby limiting recruitment, which is a novel way by which *N. melanostomus* is affecting native mussel species.

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Table of Contents

INTRODUCTION 1

MATERIALS AND METHODS.....10

Assessment of Infestation and Metamorphosis on *N. melanostomus* in the Laboratory10

Mussel Collection.....11

Fish Collection.....12

Experimental Infestations.....13

Aquatic Habitat Units.....15

Assessment of Natural Infestation of *N. melanostomus* in the Field 15

Statistical Analyses 19

Assessment of Infestation and Metamorphosis on *N. melanostomus* in the Laboratory 19

Assessment of Natural Infestation of *N. melanostomus* in the Field.....20

Modelled contribution of *N. melanostomus* to Unionid Recruitment 23

RESULTS 24

Assessment of Infestation and Metamorphosis on *N. melanostomus* in the Laboratory25

Assessment of Natural Infestation of *N. melanostomus* in the Field 29

Modelled contribution of *N. melanostomus* to Unionid Recruitment.....31

DISCUSSION.....33

LITERATURE CITED 44

Appendix A – Biology of the unionid species used in infestation experiments 84

Appendix B – Dimensions of female unionids used for infestation experiments, and the viability of their glochidia.....87

Appendix C – Unionid mussel and fish pairings, and total lengths of experimental fish.....88

Appendix D – Power Analysis for Laboratory Experiments 90

Appendix E – Relative abundances of unionid mussel species at sites of interest (based on surveys by Metcalfe-Smith et al. 2000; Metcalfe-Smith et al. 2007) 91

List of Tables

Table 1. Biological and conservation aspects of the unionid species examined in the present study. 55

Table 2. Species and common names of fish species and the sites from which they were collected. 56

Table 3. Temperature in the experimental AHAB units in 2010 and 2011 (mean ± S.D). 56

Table 4. Permits for animal collection and utilization to Professor J.D. Ackerman..... 57

Table 5. Number of *N. melanostomus* collected by site in the Sydenham and Grand rivers by DFO in 2010. A-E corresponds to most upstream site to most downstream site (see Figures 3 and 4). 58

Table 6. Summary of species included in Discriminant Function Analysis model. 59

Table 7. Classification results for the Grand River DFA Model, which includes only those unionid species observed live since 1970 (source: Metcalfe-Smith et al. 2000) 61

Table 8. Classification results for the Sydenham River DFA model, which includes all species historically observed in the river (Metcalfe-Smith et al. 2007). 63

Table 9. Infestation and metamorphosis rates, and number of juvenile mussels produced per fish (mean ± S.E.) for all laboratory infestation experiments. 65

Table 10. Classifications of glochidia of unknown species collected from the Grand River using the DFA model 66

Table 11. Classifications of glochidia of unknown species collected from the Sydenham River sites using the DFA model. 68

Table 12. Species-specific values for the model of glochidia diluted vs. juvenile mussels produced (all values correspond to the Sydenham River). Sources: McNichols et al. 2007, Schwalb et al. 2010, COSEWIC, present study. 70

List of Figures

Figure 1. Unionid mussel life cycle. The cycle begins when a male mussel releases sperm into the water column. These are taken in by the female and fertilize her eggs, which develop into parasitic glochidia larvae. The glochidia are released by the female and attach to a host fish, where they develop into juvenile mussels. These juvenile mussels eventually drop off the host fish to become free-living, and the cycle continues (drawing by Tamy Rodella).....71

Figure 2. Fish and mussel experimental pairings. Orientation of tanks is not representative of actual setup as the pairings were randomly assigned to AHAB units. 72

Figure 3. Locations of *N. melanostomus* collection sites in the Grand River (location of inset map is approximate).....73

Figure 4. Locations of *N. melanostomus* collection sites in the Sydenham River (location of inset map is approximate). 74

Figure 5. Infestation Rates (mean ± SE) for all mussel species and all host types (primary host, marginal host and Round Goby, *N. melanostomus*); see Table 5 for list of primary hosts.

"n.s." indicates non-significant differences; different letters above two host types within a mussel species indicate significant differences.....75

Figure 6. Metamorphosis Rates (mean ± SE) for all mussel species and all host types (primary host, marginal host and Round Goby, *N. melanostomus*); see Table 5 for list of primary hosts. "n.s." indicates non-significant differences; different letters above two host types within a mussel species indicate significant differences. 75

Figure 7. Number of juvenile mussels produced per fish (mean ± SE) for all mussel species and all host types (primary host, marginal host and Round Goby, *N. melanostomus*); see Table 5 for list of primary hosts. Different letters above two host types within a mussel species indicate significant differences.....76

Figure 8. Proportion of *N. melanostomus* displaying body burdens of glochidia in the Grand and Sydenham rivers; weighted by number of fish at each site. Boxes indicate the spread of the proportions of fish with body burdens by site; horizontal lines within the boxes indicate medians; whiskers indicate the smallest and largest values; and outliers are represented by open circles. 77

Figure 9. Frequency of glochidia per *N. melanostomus*, for (a) Sydenham and (b) Grand rivers. 78

Figure 10. Distribution of total lengths of *N. melanostomus* from the Grand and Sydenham rivers. Boxes indicate the spread of total lengths; horizontal lines within the boxes indicate medians; whiskers indicate the smallest and largest values; and outliers are represented by open circles. Note: fish missing tails (n = 2) were not included.....79

Figure 11. Scatterplot of *N. melanostomus* (from Sydenham and Grand rivers) total lengths and body burden status (1 = present; 0 = absent). 80

Figure 12. Lengths and heights (mean) of glochidia for which raw values were not available, and raw length and height values for glochidia collected in the present study; there is little overlap between these two groups, with the exception, perhaps, of *T. parvum* (Sources: Clarke, 1981; Watters et al. 2009). 81

Figure 13. Modeled contribution of *N. melanostomus* to juvenile mussel production and glochidial "dilution" for *A. ligamentina* using equations 2 and 3. Slope varies with metamorphosis rate. Ratio of glochidia loss to juvenile mussel production (i.e., D_g/J) for *N. melanostomus* = 3.37. Ratio of glochidia loss to juvenile mussel production for *C. bairdi* = 8.35.....82

Figure 14. Modeled contribution of *N. melanostomus* to juvenile mussel production and glochidial "dilution", for *V. iris* using equations 2 and 3. Slope varies with metamorphosis rate. Ratio of glochidia loss to juvenile mussel production (i.e., D_g/J) for *N. melanostomus* = 394.99. Ratio of glochidia loss to juvenile mussel production for *C. bairdi* = 2.18.....82

Figure 15. Modeled contribution of *N. melanostomus* to juvenile mussel production and glochidial "dilution", for *E. triquetra* using equations 2 and 3. Slope varies with metamorphosis rate. Ratio of glochidia loss to juvenile mussel production (i.e., D_g/J) for *N. melanostomus* = 6.41. Ratio of glochidia loss to juvenile mussel production for *C. bairdi* = 2.68.....83

INTRODUCTION

The Laurentian Great Lakes watershed, which contains approximately 20% of the world's surface freshwater, is one of the most important freshwater commercial fisheries in the world and has experienced many disturbances over the last century (Munawar et al. 2005). Reductions in water quality and changes in food web structure in this system have been attributed to land use changes, agriculture, the removal of wetlands (Crosbie and Chow-Fraser 1999), pollution and eutrophication, the creation of dams limiting fish passage, toxin and contaminant accumulation, and the introduction of invasive species (Munawar et al. 2005, Dextrase and Mandrak 2006). Invasive species present one of the greatest threats to the Great Lakes and freshwater systems in general because of their impacts on all trophic levels (Munawar et al. 2005). The introduction of a new species can dramatically alter food web dynamics (e.g., Vander Zanden et al. 1999, Munawar et al. 2005), introduce pathogens, lead to hybridization with native species (Taylor et al. 1984), cause significant modifications to habitat (Crooks 2002), and is perhaps an important cause of species extinctions (e.g., Fritts and Rodda 1998, Wilcove et al. 1998). Many attempts have been made to identify factors that contribute to a successful invader, and although progress has been made (e.g., Kolar and Lodge 2001, Kolar and Lodge 2002, Kulhanek et al. 2011), it remains difficult to predict which exotic species may threaten an ecosystem and furthermore, what effects they may have.

Dreissenid mussels (zebra mussel, *Dreissena polymorpha* and quagga mussel, *Dreissena rostriformis bugensis*) are examples of species that invaded the Laurentian Great

Lakes in the mid-1980s (Hebert et al. 1989) and have impacted the ecosystem. These effects include altering nutrient cycling and dominating benthic biomass in some areas (Munawar et al. 2005). In addition, they have negative effects on native freshwater mussels in the family Unionidae, an already imperilled group (Williams et al. 1992), by interfering with their locomotion and burrowing, out-competing them for food, and preventing them from closing their valves, thus causing their death (Mackie 1991). Unionid mussels were largely extirpated from the Great Lakes and Lake St. Clair, where dreissenid species are now abundant (e.g., Schloesser et al. 2006), but remain in some refugia, including many of the lakes' tributaries, which are largely devoid of dreissenids (Poos et al. 2010).

The discovery of the Round Goby (*Neogobius melanostomus*) in the St. Clair River in the early 1990s (Jude et al. 1992) presented an additional threat to already declining unionid mussel populations (Poos et al. 2010). *Neogobius melanostomus* possesses many of the characteristics of a successful invader as suggested by Ehrlich (1989), including a large native range, high abundance in its native range (Jude et al. 1992), high dispersal ability, short generation times (Corkum et al. 2004), populations with high heterozygosity (Bronnenhuber et al. 2011), and an ability to tolerate wide ranges of various abiotic factors (e.g., salinity, temperature, and oxygen; Marsden et al. 1996). *N. melanostomus* has expanded its distribution to include all five Great Lakes and by 2008, individuals had been observed in the lower reaches of the Sydenham, Grand, Thames and Ausable rivers of Ontario. These rivers are considered Species at Risk "hot-spots" because of the diversity of at-risk species present (Poos et al. 2010), including many of the unionid species that were extirpated from the Great Lakes by dreissenid mussels (e.g., Nalepa et al. 1996, Schloesser et al. 2006).

The potential effects of the invasion of *N. melanostomus* on unionid mussels in these riverine environments were reviewed by Poos et al. (2010). These effects are wide-ranging and include competition with native fishes, predation of both fish and unionid mussels, and disruption of the unionid mussel life cycle by out-competing the host fishes that are required for their larval development (Poos et al. 2010). The predicted effects were based on evidence from a number of studies. For example, French and Jude (2001) found a significant overlap in diet between *N. melanostomus* and other small benthic fishes in the St. Clair River, including Logperch (*Percina caprodes*) and Rainbow Darter (*Etheostoma caeruleum*). *Neogobius melanostomus* is also ecologically similar to the Mottled Sculpin (*Cottus bairdi*) and populations of *C. bairdi* have declined dramatically since the invasion (Jude et al. 1995, Janssen and Jude 2001). Furthermore, *N. melanostomus* prey on the eggs and young of native Smallmouth Bass (*Micropterus dolomieu*) when adults are distracted from nest-guarding, such as during catch-and-release fishing (Steinhart et al. 2004). In North America, *N. melanostomus* consume bivalves including sphaeriids and dreissenids (Jude et al. 1995), as well as aquatic insects and zooplankton, such as *Daphnia* spp. (Carman et al. 2006). Furthermore, gut content analysis of *N. melanostomus* collected from the Sydenham River in 2010 indicated that two fish had each ingested one juvenile unionid mussel (M. Poos, DFO, pers. comm. 2011), which demonstrates that native unionids may be affected by the direct effects of predation by *N. melanostomus*. One effect not considered by Poos et al. (2010), however, is whether native mussels may use *N. melanostomus* as a host to complete their life cycle.

An understanding of the unionid life history is pertinent to these issues. Unionids have an obligate parasitic stage, the glochidium, which requires the use of host fish to

facilitate metamorphosis into free-living juvenile mussels (Barnhart et al. 2008). This characteristic likely evolved from the typical planktonic veliger larva in the Mesozoic era due to the selective advantage of upstream dispersal in watersheds (Watters 2001). The reliance of unionid mussels on host fishes is thought to facilitate dispersal of these otherwise relatively sedentary animals, rather than the provision of nutrients (Barnhart et al. 2008). Once the female mussel's eggs are fertilized by the sperm released into the water column by males, they are brooded in her gills and eventually develop into glochidia, which are released in response to a variety of cues, depending on the species (reviewed in Barnhart et al. 2008). Glochidia clamp to the gill, skin or fin tissue of the fish and there is a migration of the fish tissue around the glochidium, leading to the formation of a cyst (Arey 1921). Once metamorphosis occurs, juvenile mussels excyst and drop off their host and then begin to live independently in stream sediments (Barnhart et al. 2008) (Figure 1). It is at these early life history stages that mortality is greatest due to the challenges of both encountering a suitable host and then subsequently undergoing metamorphosis and dropping off in a suitable habitat (Howard and Anson 1922, Neves and Widlak 1988).

There are both host-generalist and host-specialist species of mussel (Barnhart et al. 2008). Host generalists broadcast their glochidia into the water, or release packages of glochidia called conglutinates, and may rely on several host fishes (Barnhart et al. 2008). An example of a host generalist is the Mucket, *Actinonaias ligamentina*, which is thought to use at least 17 fish species as hosts in the laboratory and field (Watters et al. 2009). Host specialists rely primarily on a small number of host fish species and may have elaborate species-specific strategies of host attraction (Barnhart et al. 2008). An example of a host specialist is the Snuffbox, *Epioblasma triquetra,* which relies almost exclusively on the

Logperch, *Percina caprodes*, as a host for its glochidia in nature (Schwalb et al. 2010). There is a trend whereby host specialists are more likely to be at risk of extinction than host generalists, although it does not hold for all unionid species (Strayer 2008). The number of confirmed hosts for a given species, and therefore the designation as a host specialist or a host generalist, depends on how many host identification studies have been conducted and the data are far from complete. Interestingly, Schwalb et al. (2011) found that host fish dispersal distance also influences conservation status This diversity of reproductive strategies has implications for understanding the conservation status of unionids, and for determining the focus of conservation efforts.

Both glochidial infestation and metamorphosis must occur for a fish to be considered a host for a given mussel species. Primary hosts provide high infestation rates (proportion of glochidia that attach to the host) and high metamorphosis rates (proportion of attached glochidia that metamorphose into juvenile mussels) for a given mussel species, whereas marginal hosts provide lower infestation and metamorphosis rates for a given mussel species (McNichols et al. 2011). For example, *E. triquetra* is thought to rely almost exclusively on *P. caprodes* as a host in nature (Schwalb et al. 2010), but its glochidia will transform at lower rates on other fish species in the laboratory (McNichols 2007). Marginal hosts can play an important role, particularly when primary hosts for a given species are rare. For instance, populations of the Northern Riffleshell (*Epioblasma torulosa rangiana*) in the Ausable and Sydenham rivers are thought to reproduce only on marginal hosts, because their primary host, the Iowa Darter (*Etheostoma exile*), is no longer thought to be present (McNichols et al. 2011).

Age and previous exposure of fish to glochidia can also influence the host-parasite dynamics. Mussel infestation and metamorphosis rates are higher on younger fish, regardless of whether they had been infested with glochidia previously (Bauer 1987, as cited in Strayer 2008) and host fishes that have already been exposed to unionid glochidia are less susceptible to subsequent infestations due to a heightened immunological response (e.g., Dodd et al. 2005). Such a response can also provide cross-resistance as was noted in the case of the Largemouth Bass (*Micropterus salmoides*) exposed to the glochidia of different but related species (Dodd et al. 2005).

Unionid mussels have several important ecological roles in freshwater systems (Vaughn et al. 2008). They are considered ecosystem engineers due to the creation and modification of habitat that results from their suspension feeding and burrowing activities (Gutierrez et al. 2003). Unionid shells provide habitat for other aquatic organisms in the form of hard substrate to which they can attach, and also provide refuge in the form of protection from abiotic stressors such as extreme temperatures and high flows, as well as from predators (Gutierrez et al. 2003). Furthermore, the habitat heterogeneity provided by mollusc shells facilitates the maintenance and creation of biodiversity in an area (Gutierrez et al. 2003) and it is has been theorized that biodiversity increases the resiliency of an ecosystem to disturbance (Wilson 1994, Chapin et al. 2000). Unionids clear particulates, including contaminants, from the water column through their suspension feeding activities. Being long-lived and relatively sedentary organisms (Schwalb and Pusch 2007), their presence is often indicative of good water quality (Farris and Van Hassel 2007, Cope et al. 2008). They are also considered sentinel species in the case of poor condition and are thus designated as the “canaries” of aquatic ecosystems (Watters et al. 2009).

Perhaps as a result of this sensitivity, 13 of the 41 unionid species in Ontario are considered Species at Risk (SAR), meaning they have been assessed as Endangered, Threatened or of Special Concern by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC, accessed July 2012). Some of the reasons for their SAR status are: siltation and water pollution (Bogan 1993); habitat modification and destruction (Bogan 2008); near extirpation from the Great Lakes due to *Dreissenid* species (Strayer 1999); the challenges of encountering a suitable host that will facilitate glochidial metamorphosis and subsequent excystment in a location that is conducive to the survival of juvenile mussels (Howard and Anson 1922; Neves and Widlak 1988); as well as the susceptibility of their early life stages to environmental contaminants including ammonia (Newton et al. 2003) and heavy metals (Naimo 1995).

The potential role of *N. melanostomus* as a host fish for unionids or a sink for their glochidia remains to be determined. A sink, here, is defined as something to which glochidia attach, but which facilitates little or no metamorphosis, as compared to native fishes. *Neogobius melanostomus* was found to be a potential host for unionids in nature when an individual collected in the St. Clair River exhibited glochidial parasitism on its gills (Muzzal et al. 1995). *Neogobius melanostomus* has been observed to facilitate the metamorphosis of glochidia from several unionid species in the laboratory, including: Fatmucket (*Lampsilis siloquoidea*); Broken-rays mussel (*L. reeveiana*); Black Sandshell (*Ligumia recta*) (Barnhart and Baird 2000); Rayed Bean (*Villosa fabalis*); Kidneyshell (*Ptychobranchus fasciolaris*) (McNichols and Ackerman, unpublished); and Giant Floater (*Pyganadon grandis*) (Watters et al. 2005). However, only a small number of juveniles were produced in all cases, with the exception of *L. reeveiana*, which exhibited a 31% metamorphosis rate on *N. melanostomus*

(Barnhart and Baird 2000). Given these results and the high projected relative abundance of *N. melanostomus*, it is possible that *N. melanostomus* may either facilitate the recruitment of unionid mussels by serving as a host fish, or limit successful unionid recruitment by hosting glochidia through initial attachment but not facilitating metamorphosis, as has been observed for other non-host species (Jansen et al. 2001).

This thesis will examine the ability of unionid mussels to use *N. melanostomus* as a host in controlled infestation experiments in the laboratory and in an evaluation of natural glochidial infestations on *N. melanostomus* captured in the field. Specifically, the recruitment success of four mussel SAR ((1) *Epioblasma torulosa rangiana* (Lea, 1838), (2) *Epioblasma triquetra* (Rafinesque, 1820), (3) *Lampsilis fasciola* Rafinesque, 1820, and (4) *Villosa iris* (Lea, 1829)), and one common mussel species (*Actinonaias ligamentina* (Lamarck, 1819)) on *N. melanostomus* was compared to known primary and marginal hosts in controlled laboratory experiments (Table 1). Descriptions of each unionid species used in the present study can be found in Appendix A. The mussel SAR were chosen because they are known to occur within the current distribution of *N. melanostomus* and their hosts are thought to be in competition with *N. melanostomus*. A common species was examined because it may be less sensitive to laboratory manipulation than at-risk species, and may utilize a greater number of host fishes (Strayer 2008, Schwalb et al. 2011). The Mottled Sculpin (*Cottus bairdi*), which is a marginal host for all five mussel species and used as such in the present study, is considered to be ecologically similar to, and therefore likely to compete with, *N. melanostomus* (Jude et al. 1995, Janssen and Jude 2001). Naturally occurring glochidial infestations (i.e., body burdens) were assessed through the examination of *N. melanostomus* collected from several sites in the Sydenham and Grand rivers by

Fisheries and Oceans Canada. Body burden differs from infestation rate, in that the exposure rate is unknown. The results of this study will contribute to knowledge regarding the effects of species introductions, help predict the effects of invasions elsewhere, as well as inform the management of invasions and imperilled unionid mussels.

Research Question:

Does *N. melanostomus* serve as a host for unionid mussels?

Research Hypothesis:

Neogobius melanostomus serves as a host for unionid mussel SAR through facilitation of successful glochidial infestation and subsequent metamorphosis of large numbers of free-living juvenile mussels, and as a result, may help to propagate unionid SAR. Alternatively, it may serve as a sink for glochidia, whereby glochidia attach but do not metamorphose (or metamorphose at extremely low rates), and thereby reduce unionid SAR recruitment.

Predictions:

If *N. melanostomus* serves as a host for unionid mussel SAR, then it will facilitate successful infestation and metamorphosis of juvenile mussels at rates similar to those of known hosts. Furthermore, *N. melanostomus* collected from areas of unionid occurrence will exhibit body burdens of glochidia.

If *N. melanostomus* serves as a sink for glochidia, metamorphosis in the laboratory will be low relative to other hosts, and individuals collected in nature will exhibit encysted glochidia.

If *N. melanostomus* serves as neither a sink nor a host for unionid glochidia, then laboratory infestation and metamorphosis will not occur, and *N. melanostomus* collected in nature will not exhibit encysted glochidia.

MATERIALS AND METHODS

Assessment of Infestation and Metamorphosis on N. melanostomus *in the Laboratory*

To address the question of whether *N. melanostomus* is able to serve as a host by facilitating successful infestation and metamorphosis of unionid glochidia, laboratory experiments were conducted using the following null hypotheses:

(1) H_0: Infestation rates of unionid SAR and common species on *N. melanostomus* will be equal to rates on reported primary and marginal host fish.

H_A: Infestation rates of unionid SAR and common species on *N. melanostomus* will differ from rates on reported primary and marginal host fish.

(2) H_0: Metamorphosis rates of unionid SAR and common species on *N. melanostomus* will be equal to rates on reported primary and marginal host fish.

H_A: Metamorphosis rates of unionid SAR and common species on *N. melanostomus* will differ from rates on reported primary and marginal host fish.

Mussel Collection

Gravid females of each mussel species were collected from areas of known occurrence within successfully reproducing mussel populations in the Sydenham, Grand and Thames rivers, in Ontario, Canada. In the highly turbid Sydenham River, mussels were collected via racooning, whereby field workers dug gently in the sediment with their hands to find mussels. In the Grand and Thames rivers, which have very clear waters with visible bottom sediments, viewing boxes and polarized sunglasses were used to search for desired species, and mussels were collected by hand. These techniques do not harm the mussels or their glochidia (McNichols et al. 2011).

Collection took place during known periods of gravidity (Table 1), which were checked in the field every two weeks for each mussel species. For a given species, three females of similarly-size were collected at the same time and from the same location to account for potential spatial and temporal effects. Once collected, the female mussels were transported to the Hagen Aqualab at the University of Guelph in a cooler (Mobicool X25 DC-HC) containing river water, with an air stone.

Mussels were acclimated to 16-18°C at the Hagen Aqualab, University of Guelph, and then moved to a circular flow-through tank containing well water at ~11°C (to inhibit release

of glochidia), and fed an algal diet (2×10^8 cells/L of commercial algal diet; Nanno 3600 and shellfish diet, Reed Mariculture Inc., Campbell, CA, USA) three times per week. When the infestations were complete, the mussels were returned to their collection site as soon as possible.

Fish Collection

Potential host fishes were collected from bodies of water that did not contain the mussel species of interest to reduce the likelihood of acquired immunity. Young-of-the-year (YOY) fish were collected whenever possible to reduce the likelihood of immunological responses. YOY status was assessed based on colouration and/or size, depending on the species (Holm et al. 2009). Fish were collected primarily by seine-netting, although angling, backpack and boat electro-fishing methods were also used. Collection sites for each species are presented in Table 2.

Fish were transported to the Hagen Aqualab in buckets filled with water from the collection site and an air stone. Upon arrival, in 2010, fish were acclimated to water temperatures of 16-18 °C then used for experiments (Table 3 for detailed information). However, due to a high mortality rate of fishes in the laboratory in 2010, all fishes in 2011 were treated with Melafix (active ingredient: tea tree oil; exposure concentration ~ 0.125 mL/L) for three days and quarantined in covered, aerated buckets containing well water for at least one week prior to the infestation experiment. Collection methodology, as well as animal

care and maintenance were approved by the appropriate organizations (permit numbers are provided in Table 4).

Experimental Infestations

Immediately prior to infestation, half the marsupial gill of each female mussel was flushed with water to obtain glochidia. The viability of a subsample of ~ 100 glochidia from each female was assessed through observation of the proportion that reacted to NaCl exposure by closing their valves (Huebner and Pynnonen 1992, ASTM 2005), and viabilities were recorded (Appendix B). Viability was tested to identify potential differences between female mussels, and to ensure that infestation was possible. The glochidia were divided into three approximately equal portions using a modified Motodo plankton splitter. The concentrations were determined by counting the glochidia in 4 × 3.0 mL subsamples, averaging these, dividing by three to determine the quantity in 1.0 mL and multiplying by the total volume of the sample. Each portion of glochidia was assigned randomly, using a random number table or drawing from a hat, to infest one of the three types of host fish species: (1) a known primary host of the mussel species of interest; (2) a known marginal host, *C. bairdi*, and (3) *N. melanostomus* (Figure 2). The assigned portion of glochidia was kept in suspension in a 1.0 L tank using an air stone. Differing quantities of glochidia were obtained from each mussel species, so the volume of water used across species was adjusted to maintain a uniform concentration of ~5000 glochidia/L. The infestation containers were kept dark using black plastic bags to reduce the stress on the fish, and maintained for one hour (McNichols et al. 2011). The fish were then placed in their assigned Aquatic Habitat

(AHAB) unit. All infestations for a given mussel species were undertaken within ~ 15 min in order to keep all experimental conditions consistent.

Each replicate (tank in a different AHAB) contained four conspecific fish of approximately equal mean total length (TL) (to reduce the effects of fish size) (Figure 2). Centrarchid species (*Ambloplites rupestris, Micropterus dolomieu,* and *Micropterus salmoides*) were fed bloodworms three times per week and crayfish (pincers removed) one to three times per week depending on availability. *Micropterus salmoides* were also fed smelt (intended for human consumption, Metro Ontario, Inc.) on a weekly basis. All other species (*E. exile*, *C. bairdi*, *N. melanostomus,* and *P. caprodes*) were fed 4.0 mL per tank of a bloodworm: brine shrimp (4:1) mixture, three times per week.

Twice per week after infestation, flow in a given tank was increased manually to flush any juvenile mussels and excysted glochidia into recovery caps (100 μm mesh) placed at the outflow of each tank. In addition, ½ - ⅓ of the tank water was siphoned through a similar 100 μm sieve to recover juvenile mussels and glochidia, and to remove fish waste on a weekly basis. These recovery caps and sieves were inspected visually for glochidia and juvenile mussels, which were removed and counted. Once the caps remained devoid of juvenile mussels for one week, the individual fish were removed from the tank, anaesthetized using adequate Tricaine Methanesulfonate (MS-222) to sedate the fish (~23 mg/L), and their gills were inspected manually. If no glochidia were visible, the experiment was terminated, and fish were euthanized in an MS-222 solution (~100 mg/L) and dissected to confirm the absence of encysted glochidia.

Aquatic Habitat Units

The three recirculating AHABs were temperature-controlled at 18-20 °C using 200 μm-filtered well water. Water quality was maintained using an aerobic bacterial filter comprised of gravel to remove nitrogenous compounds, and an activated carbon filter to remove free radicals and metals. Temperatures of each AHAB unit in 2010 and 2011 were measured on a weekly basis using a multi-parameter metre (650 MDS YSI sonde unit: YSI incorporated, Yellow Springs, OH, USA), and average readings are provided in Table 3. Dissolved Oxygen (DO) and pH were also measured on a weekly basis with the YSI sonde. Ammonia, NH_3, in each AHAB, was tested using a DR/890 Colorimeter (HACH Company, Loveland, CO, U.S.A.) on a monthly basis.

Assessment of Natural Infestation of N. melanostomus *in the Field*

N. melanostomus collected from the lower Grand River and the East branch of the Sydenham River were examined in order to address the question of whether *N. melanostomus* encounter unionid glochidia in nature and become infested.

The habitat in the three sections of the Grand River (Upper, Middle, and Lower) are reasonably distinct. The lower section between Brantford and Port Maitland is slow-flowing, deep, and has a substrate composed of clay and mud (Kidd 1973). *Neogobius melanostomus* were collected in the lower main stem between Middleport and Haldimand, Ontario (Figure 3). Thirty-two (32) species of unionid occurred historically in the Grand River (Metcalfe-

Smith et al. 2000) and 24 of these were collected live during surveys conducted in 1997-1998, which involved a 4.5 person hour (ph) search per site using waders, viewing boxes and polarized sunglasses, (Metcalfe-Smith et al. 2000). These sites roughly correspond to locations where *N. melanostomus* were collected by Fisheries and Oceans Canada (DFO) in the present study. It is important to note that this survey is the most recent quantitative survey conducted in those areas (T. Morris, DFO, pers. comm.).

The Sydenham River is separated into North and East branches. The East branch, where *N. melanostomus* were collected, is approximately 100 km in length (Figure 4), has a high diversity of habitats, and good quality mussel habitat overall, when compared to the North branch. The river as a whole supports a high diversity of aquatic organisms, at one time supporting ~80 fish species and 34 unionid mussel species (Metcalfe-Smith et al. 2007). Currently, the system has very high levels of silt and turbidity, which is likely due to the abundance of agricultural activities (85% of land use) in the watershed (Staton et al. 2003). Thirty out of the 34 species that occurred historically in the Sydenham River were collected live during surveys conducted in the river in 1997-1998 (Metcalfe-Smith et al. 2007). The five sites from which *N. melanostomus* were collected in the present study (Florence, Brick Road, Croton, Upstream of Dawn Mills, and Dawn Mills) were sampled quantitatively by Environment Canada using 1.0 m^2 quadrats (400 m^2 total per site), in the summers of 1999, 2002 and 2003 (with the exception of Upstream of Dawn Mills, which was not sampled for unionid mussels; Metcalfe-Smith et al. 2007).

N. melanostomus were collected via seining and electro-fishing or via trawling (depending on the river) then geo-referenced, fixed in formalin, and preserved in ethanol by

Fisheries and Oceans Canada (DFO) from known areas of unionid occurrence. These consisted of five sites in the Sydenham River (August 16-27, 2010), and 11 sites in the Grand River (June-July, 2010) (Table 5). Specimens were then assessed for body burdens using the following null hypotheses:

(3) H_0: *N. melanostomus* collected from areas of unionid occurrence will not have body burdens of glochidia.

H_A: *N. melanostomus* collected from areas of unionid occurrence will have body burdens of glochidia.

(4) H_0: The body burdens of glochidia of *N. melanostomus* will be consistent with the abundance of unionid species at the site where they were collected.

H_A: The body burdens of glochidia of *N. melanostomus* will not be consistent with the abundance of unionid species at the site where they were collected.

All *N. melanostomus* specimens collected from the Sydenham River were examined (n = 79 at five sites), however it was not possible to examine all specimens from the Grand River (n = 838), particularly specimens < 2.0 cm fork length because the small gill size limited dissection and did not appear to be large enough to accommodate glochidial encystment the majority of species (pers. obs.). To facilitate comparison between the two rivers, and because all specimens from a given site in the Sydenham River were examined, all

specimens within each of four sites in the Grand River were examined as well (Figure 3). These Grand River sites were chosen because low numbers of fish were collected there (fewer than 70), and as a whole they were representative of the stretch of river from which *N. melanostomus* were collected.

Total length, fork length and head width (defined as the point at which the greatest width was observed over the operculum) were determined for all *N. melanostomus* individuals to account for size differences in body burdens. Fish were examined with a 32× dissecting microscope (Wild Canada Limited, Ottawa, ON) and their bodies, including fins, were assessed visually for glochidial encystment. The gills were removed and examined using a dissecting microscope with a polarizer attachment. All glochidia were excised from the gills and photographed using a 3.34 MP CoolPix 995 digital camera (Nikon, Japan) with a wire of known diameter (300 μm) to determine the length, height and hinge length of each glochidium via image analysis (ImageJ 1.38× image analysis software, US National Institutes of Health).

Statistical Analyses

Assessment of Infestation and Metamorphosis on N. melanostomus in the Laboratory

The infestation rate (R_I) was calculated for a given mussel on a given group of fish as the proportion of glochidia that successfully attached to the fish (G_A) from the total number used to infest the fish (G_T; i.e., $R_I = G_A / G_T$; McNichols et al. 2011). The metamorphosis rate (R_M) was calculated for a given mussel on a given group of fish, and is equal to the proportion of glochidia that successfully metamorphosed into juvenile mussels (G_M) from the number that initially attached to the fish (G_A; i.e., $R_M = G_M / G_A$; McNichols et al. 2011). Glochidia encysted on fishes that died prior to the end of the experiment (determined though post-mortem dissection of the fish) were included in G_A for determining R_I, but were excluded from G_A for the determination of R_M.

To investigate the effectiveness of *N. melanostomus* as a host relative to known primary and marginal hosts, comparisons of the following measures were made: (1) infestation and (2) metamorphosis rates, as well as (3) the number of juveniles produced per fish. If glochidia from different females differed greatly in their viabilities (Huebner and Pynnonen 1992, ASTM 2005), the data were compared using a Two-Way Main Effects Analysis of Variance (ANOVA), with "fish species" and "female mussel" as the fixed effects. If glochidial viabilities were similar among females, a One-Way ANOVA was conducted, with "fish species" as the fixed effect. The assumption of normality was tested using a Shapiro-Wilks test, and homogeneity of variance was tested with Levene's test (Zar 1999). Data were "arcsine square root" or "log + 1" transformed to meet the assumptions of

normality and homogeneity of variance, but it was not always possible for these to be met. In cases where the assumption of normality was not met, but homogeneity of variance was met, non-parametric Kruskall-Wallis tests were conducted (Zar 1999). If differences between treatments were observed, a post-hoc Tukey test was conducted to assess which treatments differed (Zar 1999). Statistica v6.0 (Statsoft, Tulsa, OK, USA) was used for statistical tests.

Assessment of Natural Infestation of N. melanostomus *in the Field*

The proportion of fish displaying body burdens at each site, and the number of glochidia on each fish, were determined. The number of encysted glochidia was analysed with respect to site, species of glochidia, and total body length of the fish to determine whether these factors affect glochidial infestation in nature. Relative abundance of species of glochidia found attached to the gills of *N. melanostomus* at each site were compared to relative abundances of unionid species based on surveys conducted in 1997-1998 by Metcalfe-Smith et al. (2000, 2007), to determine whether infestation of *N. melanostomus* by unionids may differ among species (Appendix C).

All statistical evaluations of field data were conducted using SPSS v.19 (IBM, Armonk, NY, USA).

Proportion of Fish with Body Burdens

To assess potential differences in the proportion of fish with body burdens between rivers, an independent samples *t*-test was conducted. Comparisons were made using values of the proportion of fish with body burdens at the site, weighted by the number of fish at the site. To achieve normality and heterogeneity of variance, site-specific values of proportion of fish with body burdens were "arcsine square root" transformed. To assess the potential relationship between size and body burden status (i.e, presence or absence of encysted glochidia), a logistic regression was conducted using total length as the predictor and body burden status as the dependent variable (i.e., 0 = no body burden, 1 = body burden).

Identification of glochidia on gills of N. melanostomus

To determine what species of unionid glochidia were attached to the gills of *N. melanostomus*, a Discriminant Function Analysis was conducted (DFA; Quinn and Keough 2009). Specifically, river-specific DFA models were created using the measurements of height, hinge length and length of glochidia from 30 species of unionid (Table 6), which had been compiled in an atlas of glochidia images and dimensions developed in the Ackerman Laboratory at the University of Guelph (Unionid Glochidia Atlas of Ontario 2012). This atlas contains information from the reference collections of the Ackerman Laboratory at the University of Guelph and Fisheries and Oceans Canada in Burlington, ON, as well as data from collections in the United States, and information from Watters et al. (2009) and Clarke (1981). Measurements of the glochidia of five species were not available (*Fusconaia flava*, *Pleurobema sintoxia*, *Quadrula quadrula*, *Toxolasma parvum*, and *Truncilla donaciformis).*

It was therefore not possible to assess statistically whether glochidia from the present study belonged to those species.

The measurements of glochidia from known species were used to build the DFA models (and log-transformed to more closely meet the assumptions of the DFA), which were used to classify the glochidia of unknown species obtained from the fish gills to species-level. Two models were created: one for each of the Grand and Sydenham rivers. For each river, ~70% of observations were randomly selected to create discriminant functions (n_{train}), and the remaining 30% (n_{test}) were used to test the model (Table 7 and Table 8). Additionally, a jackknife procedure was used, in which the observation being classified is omitted from the model calculation, thus removing an inherent bias in the classification procedure (Quinn and Keough 2002). The assumption of equal population covariance matrices was tested for both Discriminant Function models (Grand and Sydenham Rivers). It was rejected in both instances; however, this test is very sensitive to large sample sizes (Quinn and Keough, 2009). Moreover, when "separate groups" covariance was used, the model changed very little, and this is thought to indicate that the assumptions have, more or less, been met (source: SPSS Help).

The Grand River model included only those mussel species that have been observed alive in the river or its tributaries since 1970 (Metcalfe-Smith et al. 2000; n = 19; Table 7); slightly more than three quarters (77.6%) of glochidia observations used to test this model were correctly classified. Significant differences between group means were detected for glochidia height, hinge length and glochidia length (Wilks' lambda, $W = 0.035$, $p < 0.001$; $W = 0.039$, $p < 0.001$; $W = 0.029$, $p < 001$, respectively, $df = 18,1057$), which indicates that the

model is effective at distinguishing between groups (species). The null hypothesis of equal population covariance matrices was rejected (Box test, M = 1794, $F_{108,3891} = 14.36$, $p < 0.001$).

The Sydenham River model included all species that have been observed historically in the Sydenham River and for which measurements were available (Metcalfe-Smith et al. 2007; n = 28) (Table 8); just under three quarters (70.8%) of glochidia observations used to test this model were correctly classified. Significant differences among group means were detected for glochidia height, hinge length and glochidia length (Wilks' lambda = W = 0.042, $p < 0.001$; W = 0.036, $p < 0.001$; W = 0.032, p < 001, respectively, df = 27,1492), which indicates that the model is effective at distinguishing between groups (species). The null hypothesis of equal population covariance matrices was rejected (Box test, M = 1907, $F_{156,7083} = 10.81$, $p < 0.001$).

Modelled contribution of N. melanostomus *to Unionid Recruitment*

A mathematical model was used to assess the potential role of *N. melanostomus* as a host fish for unionids or a sink for their glochidia based on the abundance (U) and fecundity (f) of unionids, the encounter rate of glochidia and hosts (R_e), and the measured laboratory infestation (R_I) and metamorphosis rates (R_M) obtained from the present study. In this case, the natural infestation on *N. melanostomus*, I_N, is given by,

$$I_N = U \times f \times R_e \times R_I \quad (1)$$

where *f* is the number of glochidia per female (from Schwalb et al. 2010, McNichols 2007 or estimated (i.e., *V. iris*)) and R_e is from Schwalb et al. (2010). The number of juvenile unionid mussels produced by *N. melanostomus, J*, is given by,

$$J = I_N \times R_M \times N$$

$$= (U \times f \times R_e \times R_I) \times R_m \times N \quad (2)$$

where *N* is the relative abundance of *N. melanostomus* to known hosts. By extension, the number of glochidia lost from potential recruitment, D_g, is given by,

$$D_g = I_N \times (1\text{-}R_M) \times N$$

$$= (U \times f \times R_e \times R_I) \times (1 - R_M) \times N \quad (3)$$

The only difference between equations 2 and 3 for D_g and *J* relates to R_M. Thus, the higher the R_M for a given species of unionid on *N. melanostomus*, the more juvenile mussels would be produced and the fewer glochidia would be lost, or "diluted' from the population. The ratio of glochidia loss to juvenile mussel production (D_g : *J*) for *N. melanostomus* was then compared to the ratio determined from models run for *C. bairdi*, the marginal host, to assess the role of *N. melanostomus* as a marginal host or as a sink for glochidia.

RESULTS

Assessment of Infestation and Metamorphosis on N. melanostomus *in the Laboratory*

In general, infestation rates were highest on the primary host, followed by the marginal host and *N. melanostomus* (Figure 5). Similarly, metamorphosis rates were generally highest on the primary host, followed by the marginal host and *N. melanostomus* (Figure 6). This pattern was also seen in juvenile mussel production, which was highest on the primary host, followed by the marginal host and *N. melanostomus* (Figure 7). There was considerable variation in each of these values, and a large amount of variation in the number of glochidia that successfully attached to an individual fish of a given species, based on dissections of fishes that died prior to completion of the experiments. The mean infestation and metamorphosis rates and the number of juveniles produced, for all mussel species × host type combinations are presented in Table 9.

Actinonaias ligamentina – Infestation rates were found to differ significantly among fish species using a non-parametric test (Kruskall-Wallis; $\chi^2 = 6.50$, df = 2, p = 0.039), which was used because the dataset was not normal, despite "arcsine square root" transformation (Shapiro-Wilk; W = 0.765, df = 9, p = 0.008). Specifically, rates were found to be significantly higher on the primary host (*M. salmoides*) than on either the marginal host (*C. bairdi*) or *N. melanostomus*. Metamorphosis rates differed significantly among fish species for *A. ligamentina* (One-Way ANOVA; $F_{2,4} = 8.00$, p = 0.040), with the highest rates observed on the primary host, followed by the marginal host and *N. melanostomus.* A post-hoc Tukey test indicated significant differences between the primary and marginal hosts (p = 0.044), marginally significant differences between *N. melanostomus* and the primary host (p = 0.064), but no differences between *N. melanostomus* and the marginal host (p = 0.74).

Juvenile mussel production did not differ significantly among fish species for *A. ligamentina* using a non-parametric test (Kruskall-Wallis; $\chi^2 = 4.23$, df = 2, p = 0.12), which was conducted because the data were not normal despite transformation (Shapiro-Wilk; W = 0.70, df = 7, p = 0.004). However, this may be the result of reduced power due to fish mortality and high variability within groups (Appendix E). It is relevant to note that significant differences were detected under ANOVA ($F_{2,4} = 177$, p = 0.0001) and significant pairwise differences were found between *N. melanostomus* and the primary host (p = 0.0004) and between the marginal and primary hosts (p = 0.0004), but not between *N. melanostomus* and the marginal host (p = 0.69). Ten juvenile *A. ligamentina* were produced on *N. melanostomus*.

Epioblasma torulosa rangiana – Infestation rates did not differ significantly among fish species for *E. t. rangiana* (One-Way ANOVA; $F_{2,6} = 3.06$, p = 0.12). Metamorphosis rates were marginally significantly different among fish species for *E. t. rangiana* (One-Way ANOVA; $F_{2,6} = 4.03$, p = 0.078). The highest rates observed on the marginal host (*C. bairdi*), followed by the primary host (*E. exile*), and *N. melanostomus.*

Juvenile mussel production was significantly different among fish species for *E. t. rangiana* (One-Way ANOVA; $F_{2,6} = 5.196$, p = 0.049), with the highest production on the marginal host, followed by the primary host and *N. melanostomus.* Significant pairwise difference were found between *N. melanostomus* and the marginal host (*C. bairdi*) (p = 0.042), but not between the primary host (*E. exile*) and the marginal host (p = 0.24) or between the primary host (*E. exile*) and *N. melanostomus* (p = 0.40). No juvenile *E. t. rangiana* were produced on *N. melanostomus*.

Epioblasma triquetra – Infestation rates did not differ significantly among fish species for *E. triquetra* (One-Way ANOVA; $F_{2,6}$ = 3.11, p = 0.12). Metamorphosis rates did not differ significantly among fish species for *E. triquetra* (One-Way ANOVA; $F_{2,6}$ = 1.37, p = 0.32), however the highest rates were observed on the primary host (*P. caprodes*), followed by the marginal host (*C. bairdi*) and then *N. melanostomus.*

Juvenile mussel production differed significantly among fish species for *E. triquetra* using a non-parametric test (Kruskall-Wallis; χ^2 = 6.01, df = 2, p = 0.05), which was used because the data set was not normal, despite "log + 1" transformation (Shapiro-Wilk; W = 0.82, df = 9, p = 0.031). Significant differences were found between the primary host and *N. melanostomus* (p = 0.050). Four juvenile *E. triquetra* were produced on *N. melanostomus.*

Lampsilis fasciola – Infestation rates were not found to be significantly different among fish species for *L. fasciola* (One-Way ANOVA; $F_{2,6}$ = 2.84, p = 0.14). Metamorphosis rates were significantly different among fish species for *L. fasciola* (One-Way ANOVA; $F_{2,5}$ = 13.22, p = 0.010), with the highest rates observed on the primary host *(M. dolomieu)*, followed by the marginal host (*C. bairdi*), and then *N. melanostomus.* A significant pairwise difference was found between *N. melanostomus* and the primary host (p = 0.009), and a marginally significant difference between the primary and marginal hosts (P = 0.056) but not between *N. melanostomus* and the marginal host (p = 0.16).

Juvenile mussel production differed significantly among fish species for *L. fasciola* (One-Way ANOVA; $F_{2,5}$ = 32.24, p = 0.001), with the highest production on the primary host, followed by the marginal host. A post-hoc Tukey test indicated significant pairwise

differences between the primary host and *N. melanostomus* ($p = 0.001$) and between the primary host and the marginal host ($p = 0.005$), but not between the marginal host and *N. melanostomus* ($p = 0.14$). However, no juvenile *L. fasciola* were produced on *N. melanostomus*.

Villosa iris – Differences in the viability of glochidia among females were only observed for *V. iris*, so Two-Way ANOVAs were conducted when possible. Infestation rates did not differ significantly among fish species or female mussels for *V. iris* (Two-Way ANOVA; $F_{2,4} = 3.24$, $p = 0.15$; $F_{2,4} = 0.31$, $p = 0.75$, respectively). To assess differences in metamorphosis rates and the number of juvenile mussels produced per fish, One-Way ANOVAs were conducted instead of Two-Way ANOVAs because there were not enough replicates per female due to fish mortality. Metamorphosis rates did not differ significantly among fish species (One-Way ANOVA; $F_{2,4} = 1.49$, $p = 0.33$).

The number of juvenile mussels produced per fish, however, differed significantly among hosts for *V. iris* (One-Way ANOVA; $F_{2,4} = 34.03$, $p = 0.003$) with the highest production observed on the primary host (*A. rupestris*), followed by the marginal host (*C. bairdi*) and *N. melanostomus*. There were significant pairwise differences between *N. melanostomus* and the primary host ($p = 0.003$) and between the primary and marginal hosts ($p = 0.014$), and between *N. melanostomus* and the marginal host ($p = 0.04$). One juvenile *V. iris* was produced on *N. melanostomus.* Unfortunately, there was high mortality of *N. melanostomus* with attached glochidia (0-19 per fish) prior to the completion of the experiment, so this may have affected the results.

Assessment of Natural Infestation of N. melanostomus *in the Field*

Body burdens of glochidia of *N. melanostomus* were observed, and these differed between the Grand and Sydenham Rivers. Of a total of 206 *N. melanostomus* examined, 50 of the 127 fish (i.e., 39.3 %) from the Grand River and 4 of the 79 fish (i.e., 5.1 %) from the Sydenham River had glochidia attached to their gills (Figure 8). Individual fish from the Grand River exhibited body burdens of up to 30 glochidia/fish, whereas fish from the Sydenham had body burdens of up to 6 glochidia/fish (Figure 9). Glochidia were only found attached to gills.

Values of the proportion of fish with body burdens at each site were arcsine square root transformed to achieve normality and heterogeneity of variance. Statistical comparisons were made using data weighted by the sample size at the site (i.e., proportion of fish with body burdens at the site, weighted by number of fish at the site). In general, a higher proportion of fish from the Grand River had body burdens of glochidia than fish from the Sydenham River (Figure 8; Table 5). A significantly higher proportion of fish from the Grand River (median = 0. 28) were found to have body burdens compared to fish from the Sydenham River (median = 0.00) by site (non-parametric median test; χ^2= 34.88, df = 1, p < 0.001, N = 206), because normality could not be achieved.

In order to determine whether these differences may have been due to differences in fish size, fish size was compared between rivers using a Mann-Whitney U test and a logistic regression was conducted using total length as the predictor variable and body burden status as the outcome variable (0 = no body burden; 1 = body burden). Although fish from the

Grand River were significantly smaller (total length) than those from the Sydenham River (Mann-Whitney U = 6112, p = 0.003, N = 204; two individuals were excluded from the analysis because they were missing tails; Figure 10), the results of the logistic regression indicated no relationship between total length and body burden status (Nagelkerke's R^2 = 0.109). Furthermore, a plot of the values showed no apparent relationship (Figure 11).

Identification of glochidia on gills of N. melanostomus

Neogobius melanostomus from two of the four sites in the Grand River had body burdens of glochidia. Sixteen glochidia encysted on *N. melanostomus* from site GR-d, and 174 glochidia encysted on *N. melanostomus* from site GR-a were successfully removed and measured (Figure 3; Table 5). The glochidia removed from *N. melanostomus* individuals collected at site GR-d (n = 16) and site GR-a (n = 174) were classified according to the model as: site GR-d: *V. iris* (n = 1), *L. fragilis* (n = 1), *A. plicata* (n = 14); site GR-a: *A. ligamentina* (n = 8), *E. dilatata* (n = 20), *O. reflexa* (n = 32), *A. viridis* (n =1), *L. fragilis* (n = 1), *A. plicata* (n = 111), and *L. compressa* (n = 1) (Table 10). Note that not all glochidia found on *N. melanostomus* were identified to species.

The majority of the glochidia in both sites in the Grand River were classified as *A. plicata*. These species and abundances were not consistent with the relative abundances of unionid species at 97-10, the site most closely associated with GR-d, or 97-4, the site most closely associated with GR-a (Metcalfe-Smith et al. 2000; Appendix D). Interestingly, as many as four species of glochidia were observed on a given fish according to the Grand River DFA model.

Neogobius melanostomus from three of the five Sydenham River sites had encysted glochidia. A total of four glochidia were successfully removed and measured. Given the limited sample size, it is difficult to determine whether the relative abundances correspond to those of unionid species at the sites where the fish were collected (Appendix D). The classifications of the four glochidia from the three sites (Florence (42.655677, -82.008247), Brick Road (42.636484, -82.01992) and Upstream of Dawn Mills (42.608695, -82.122231)) using the DFA model are as follows: *A. plicata* (n = 1), *E. t. rangiana* (n = 1), *L. recta* (n = 1), and *O. reflexa* (n = 1) (Table 11). As mentioned, it is impossible to determine with certainty whether these classifications are correct, due to the small number of observations.

As mentioned earlier, it was not possible to include the dimensions of five species of glochidia (*Fusconaia flava*, *Pleurobema sintoxia*, *Quadrula quadrula*, *Toxolasma parvum*, and *Truncilla donaciformis*) in either the Grand River or the Sydenham River DFA models, because raw glochidial dimensions were not available. However, it is unlikely that very many (if any) of the glochidia observed on *N. melanostomus* in the Grand River belonged to one of these five species, with the possible exception of *T. parvum.* This is because a plot of the mean lengths and heights of the glochidia (Figure 12; Clarke 1981, Watters 2009) showed very little overlap with those of the glochidia from the present study, or they did not appear to be a match because of differing shapes (e.g., size, relative dimensions). The fact that 77.6% of glochidia observations used to create the Grand River model were correctly classified indicates a potential error rate of just over 20%.

Modelled contribution of N. melanostomus *to Unionid Recruitment*

The mathematical model described in equations 1 – 3 was applied to *A. ligamentina*, *V. iris*, and *E. triquetra* (the species for which metamorphosis on *N. melanostomus* was observed) using species-specific parameter values (Table 12) for equations 2 and 3. The results were plotted as a function of the relative abundance of *N. melanostomus* to suitable hosts in the system (*G*; i.e., a proportion between 0 and 1.0). The ratio of the slopes of D_g (glochidia lost from potential recruitment) vs. *G* and *J* (juvenile mussels produced) vs. *G* curves provides an indication of the relative rates of increase in glochidial loss vs. juvenile mussel production associated with increased relative abundance of *N. melanostomus*. Results indicate that the loss of glochidia increases ~3.37× faster than juvenile *A. ligamentina* production (Figure 13); ~395× faster for *V. iris* (Figure 14), and ~6.41× faster for *E. triquetra* (Figure 15). Error bars are not included in figures to improve visual clarity; however, it should be noted that as metamorphosis rate increases, the slope of D_g would decrease and that of *J* would increase.

The $D_g : J$ ratios were also determined for the three aforementioned unionid species using the marginal host, *C.bairdi*. In two of the three cases, the $D_g : J$ ratios were lower on *C. bairdi* compared to *N. melanostomus* (i.e., 2.18 vs. 395, respectively on *V. iris*; and 2.68 vs. 6.41 on *E. triquetra*. However the trend was reversed on *A. ligamentina,* where the $D_g : J$ ratio was higher for *C. bairdi* than for *N. melanostomus (*i.e., 8.35 vs. 3.37, respectively). This may have been due in part to the fact that very few *C. bairdi* survived until the end of the metamorphosis period for *A. ligamentina*. It was not possible to compare $D_g : J$ ratios of *C. bairdi* and *N. melanostomus* in the cases of *E. t. rangiana* and *L. fasciola*, as none of the 164 and 1827 glochidia that infested *N. melanostomus*, respectively underwent successful

metamorphosis. These instances would thus represent a loss of glochidia from the mussel population without any contribution to recruitment.

DISCUSSION

The results of the present study suggest a novel way in which *N. melanostomus* affect unionid mussels. Specifically, relatively high laboratory infestation rates and the occurrence of body burdens of glochidia in nature, combined with relatively low metamorphosis rates in the laboratory support the hypothesis that *N. melanostomus* are a sink for unionid glochidia.

In the laboratory, *N. melanostomus* may appear to be equivalent to a marginal host for unionid glochidia of *V. iris, E. triquetra,* and *A. ligamentina*. Infestation rates on *N. melanostomus* were similar to those on known primary and marginal hosts for most mussel species; however, metamorphosis rates and juvenile mussel production were generally higher on the primary hosts examined. This suggests that although initial attachment of glochidia to *N. melanostomus* occurs, glochidia are not able to metamorphose effectively on them relative to reported primary or marginal hosts. This initial attachment is not surprising, given that glochidia have been observed to attach to non-host fish (Jansen et al. 2001) as well as to non-living objects such as paper and polythene (Wood 1974). However, long-term attachment and encystment requires some set of species-specific, chemical cues (Wood 1974). The primary hosts examined were more effective hosts than *N. melanostomus* in all respects, given that metamorphosis rates were generally higher on the primary hosts than on the marginal hosts or *N. melanostomus*, and the number of juvenile mussels produced per fish was higher on the primary and marginal hosts than on *N. melanostomus*.

The results of the laboratory infestation experiments for primary and marginal hosts are consistent with the results of previous experiments that examined the same mussel-host combinations, indicating that the experiments were conducted appropriately. For example, the infestation and metamorphosis rates of *E. t. rangiana* on *E. exile* were similar to rates observed by McNichols et al. 2011 (6.0 ± 1% and 44 ± 9 %, respectively), as were the rates of *L. fasciola* on *M. dolomieu* (i.e., 5 ± 1% and 82 ± 8%, respectively, McNichols et al. 2011). The highly variable number of encysted glochidia on an individual fish, for a given species combination (mussel species × fish species) is also consistent with previous findings. For example, Riusech and Barnhart (2000) found a high degree of variation in metamorphosis rates between individual fish of a given species (0-85%) on a *Venustaconcha* spp., which was not related to variation in size or condition of fish.

In the present study, the marginal host, *C. bairdi* served as a better host for *E. t. rangiana* than the reported primary host, *E. exile* (based on metamorphosis rates and juvenile mussel production), which was contrary to expectations. In the study by McNichols et al. (2011), upon which these host designations were based, metamorphosis rates were higher on *E. exile* than on *C. bairdi*, albeit not significantly. This could have been due to differences in size between the two fish species in the present experiment: *C. bairdi* individuals (mean total length ± S.D. = 8.29 ± 1.1 cm) were much larger overall than *E. exile* individuals (4.76 ± 0.32 cm), and body size is assumed to be positively correlated with gill size (Woolnough, 2002).

In cases where metamorphosis rates of a given species on *N. melanostomus* and on its primary host did not differ, this may have been due to a lack of statistical power as opposed to evidence that *N. melanostomus* serves as an effective host for unionid glochidia. This is

supported by a graphical representation of the data (Figure 6), as well as by tests of observed power (Appendix E).

Because much of the data collected in the present study violated assumptions of normality and homogeneity of variance, some of the null hypotheses were examined using non-parametric tests. As a result, there was a loss of statistical power and potentially the ability to detect differences that did, in fact, exist (Type II Error). The existence of non-normal and heteroscedastic data is common in ecology; however its occurrence may have been exacerbated in the present study by the small number of replicates and high mortality of experimental fishes. Unfortunately, it was not possible to have greater than three true replicates due to the laboratory set-up (i.e., 3 AHAB units), and although measures were taken in 2011 to reduce fish mortality (e.g., quarantine of fish, treatment with tea tree oil), this did not completely eliminate the need for non-parametric tests.

The laboratory experiments permitted a quantitative assessment of which unionid species are able to use *N. melanostomus* as a host, and as such provide us with valuable information about potential mussel-host fish relationships. However, they do not inform us regarding what is occurring in nature; for example, whether *N. melanostomus* encounter gravid unionids, and if so, whether the unionid glochidia are able to successfully infest *N. melanostomus*. For this reason, field collections provided information on whether *N. melanostomus* actually encounter and become infested by unionid glochidia in the field. Together, the laboratory experiments and field collections enable a more complete picture of how the *N. melanostomus* invasion might affect unionid mussels.

Neogobius melanostomus collected from the Grand and Sydenham rivers, which are occupied by a high diversity of unionid mussels, were found to exhibit glochidial body burdens. The proportion of fish displaying these body burdens differed between rivers; 39.4% of fish collected from the Grand River vs. 5.1% of fish collected from the Sydenham River. These differences between rivers as well as among sites could be related to differences in the size of fish, physical habitat characteristics of the sites, and/or local abundance of gravid female mussels. However it is difficult to assess the relative contribution of the latter two factors, as neither mussel surveys nor habitat surveys were conducted at the time of collection. Size differences may have been an important factor, as *N. melanostomus* collected from the Grand River were smaller (in terms of total length) than those collected from the Sydenham River, and were also more likely to exhibit body burdens of glochidia (Figure 8 and Figure 10); however, the results of the logistic regression suggest that size and body burden status are not related. The difference could also be due to differences in discharges (discharge (m^3/s) = cross-sectional area (m^2) × velocity in (m/s)) between the two rivers. Dispersal distance of glochidia is positively correlated with velocity (Schwalb et al. 2010), which should increase the likelihood that glochidia will encounter a host, at least for unionid species that broadcast their larvae. These findings are consistent with the present results, as the proportion of fish collected from the slow-flowing Sydenham River (5.17 to 19.6 m^3/s; mean = 11.7 m^3/s annually at Florence) that displayed body burdens was much smaller than the proportion of fish collected from the fast-flowing Grand River (26.4 to 95.5 m^3/s; mean = 57.8 m^3/s annually at Brantford) (Water Office, Environment Canada, 1984-2010 and 1913-2010, respectively). However, it should be noted that the concentration of glochidia in each river at the time of *N. melanostomus* collection is not known.

This is the first study, to my knowledge, that has specifically assessed the natural glochidial infestation of *N. melanostomus* in North America. Based on the results of the Discriminant Function Analysis for the Grand River sites, between 70 and 80% of the glochidia used to test the model were correctly classified, indicating a potential error rate of between 20 and 30%. Understanding which unionid species were encysted on *N. melanostomus* may provide insight as to how *N. melanostomus* became infested. Specifically, it provides insight as to whether *N. melanostomus* interacted directly with unionid mussels in the benthos, or whether they became infested through incidental contact with glochidia in the water column. In the present study, the majority of glochidia that were found encysted on *N. melanostomus* were classified as *A. ligamentina*, *E. dilatata*, *O. reflexa*, *and A. plicata*. *A. ligamentina*, one of the species examined in the laboratory experiments, is a host generalist that broadcasts its glochidia into the water column (Watters et al. 2009), and the fact that the glochidia of this species encountered *N. melanostomus* is not surprising. *Elliptio dilatata* is also a common species in Ontario, but not very common in the Lower Grand River (Metcalfe-Smith et al. 2000); it may also be a host generalist (Watters et al. 2009). Conversely, *Obliquaria reflexa* is a rare species in Ontario, and is thought to be a host specialist (Watters 1999). *Obliquaria reflexa* uses conglutinates to distribute its glochidia (Watters et al. 2009), and it is plausible that *N. melanostomus* may have encountered conglutinates and mistaken them for prey items, and thus become infested with glochidia. *Amblema plicata* is a fairly common species that is thought to be a host generalist, being able to use fish in multiple families as hosts, and glochidia are released in the form of fragile conglutinates that break apart easily (Watters et al. 2009), so it is not surprising that its glochidia might be infesting *N. melanostomus*. Knowledge of which species are infesting *N. melanostomus* in nature provides insight into what mechanisms might promote infestation.

Neogobius melanostomus collected from the Grand River were not encysted with glochidia at rates consistent with the relative abundance of unionids at nearby sites (Appendix D). However, it is difficult to interpret this observation for two important reasons. Firstly, it is not known whether all of the unionid species present at the site were gravid at or slightly before the time of fish collection. Secondly, it is possible that *N. melanostomus* may have become infested at a more downstream site, closer to the mouth of the river, before they moved upstream to their collection site. Even so, these results suggest that some unionid species may be better able to utilize *N. melanostomus* as a host, and therefore the role of *N. melanostomus* as a host or as a sink may depend on the mussel species. It was not possible to determine whether the glochidia found on *N. melanostomus* in the field would have metamorphosed (because of the collection method). This presents several possibilities for future research. First, laboratory experiments could be conducted to infest *N. melanostomus* with the glochidia of *A. plicata, E. dilatata,* and *O. reflexa,* which were found in large numbers on *N. melanostomus,* according to the DFA classifications. This would allow one to determine whether *N. melanostomus* may be serving as a host for these species in nature (i.e., whether metamorphosis is successful). Second, *N. melanostomus* could be collected from known areas of unionid occurrences, examined for body burden and if present, brought back to the lab and monitored for juvenile mussel excystment. Following excystment, juvenile mussels could potentially be identified to species, which would allow confirmation of the species classifications.

Several studies have examined naturally occurring glochidial infestation of known hosts in the field, or "body burden". For example, glochidial body burden of Smallmouth Bass (*M. dolomieu*), the primary host of *L. fasciola,* has been assessed in the Grand River.

Approximately 34% of fish were found to have been infested with glochidia of *L. fasciola* (the species for which it is a primary host), or that of another *Lampsilis* species (it is difficult to distinguish between species of this genera based on glochidial morphology alone; Morris and Granados, DFO, unpublished). A similar study in Maine found body burdens of approximately 40% on White Perch (*Morone americana*), one of the primary hosts of the Tidewater Mucket (*Leptodea ochracea)* and Yellow Lampmussel (*Lampsilis cariosa),* both of which are listed as Threatened (Kneeland and Rhymer 2008). In another study, known hosts for unionid SAR including *P. caprodes* and *A. rupestris*, collected by backpack electrofishing in Sydenham River in July 2007, had body burdens of 46-71% (D. Woolnough, CMU, unpub. data). It is evident that body burdens of known hosts in nature are comparable to those of *N. melanostomus* collected from the Grand River in the present study (i.e., 39.4% body burden).

The similar rates of body burdens of *N. melanostomus* in the Grand River and those of known hosts in the field demonstrate that glochidia encounter *N. melanostomus* in nature, either at the time of release from the female mussel, or post-release, either in the water column or on the river bottom. However, the results of the laboratory experiments suggest that despite initial attachment to *N. melanostomus,* these glochidia have a low probability of successfully metamorphosing into juvenile mussels (at least for the species examined in the present study). Furthermore, relatively high infestation rates (laboratory) and high body burdens (field), coupled with low metamorphosis rates (field), along with the results of the mathematical model suggest that *N. melanostomus* may serve as a sink for the glochidia of some species, whereby they "collect" glochidia, but promote little to no metamorphosis into juvenile mussels. In this way, they may 'dilute' the pool of effective hosts for unionid SAR

where they co-occur. If or when *N. melanostomus* achieve high population densities, this could interfere with the normal reproductive cycle of unionids, even if known primary hosts are present. By extension, through this novel indirect effect, the impact of the *N. melanostomus* invasion into SAR hot-spots on unionid mussels may be more significant than initially predicted by Poos et al. (2010).

It has taken time for substantial invasion of *N. melanostomus* in the Grand and Sydenham Rivers to occur (e.g., the first reported capture in an area with species at risk occurred in 2004; Poos et al. 2010). This is consistent with the concept of biotic resistance (Elton, 1958), which suggests that highly biodiverse regions are protected against invading species (Elton 1958). However, *N. melanostomus* are now well-established. Large numbers of individuals have been found upstream of the dams at Dunnville and Caledonia in the Grand River, but it is not clear whether this is unaided movement, or the result of accidental transportation. This upstream movement by *N. melanostomus* has the potential to affect unionid populations in another way. Specifically, it is possible that a small number of juvenile unionid mussels could excyst from *N. melanostomus* during the "spread" phase of the invasion and thus increase their range through 'hitch-hiking'. Increased dispersal by host fish would be beneficial (Barnhart 2008) as many species have limited areas of occurrence (e.g., Schwalb et al 2011). Such an occurrence could be investigated by examining the association between range expansions of both groups.

The results of a simple mathematical model of the relative contribution of *N. melanostomus* to juvenile mussel production versus the loss of glochidia (Figure 13, Figure 14, and Figure 15) were largely dependent on the metamorphosis rate of unionid glochidia on

N. melanostomus. In this model, both glochidia loss and juvenile mussel production increase as the proportion of *N. melanostomus* increase, but a higher metamorphosis rate leads to more juvenile mussel production. In this study, *A. ligamentina,* which had the highest metamorphosis rates on *N. melanostomus* in the laboratory, was nonetheless predicted to lose glochidia at 3.37 times the rate of juvenile mussel production (e.g., $D_g : J$ ratio) on this introduced species. Moreover, the $D_g : J$ ratio was higher for *N. melanostomus* than the marginal host, *C. bairdi,* for two of the three unionid species whose juveniles developed on *N. melanostomus,* which indicates that glochidial loss was greater on the introduced versus the marginal host. The opposite trend, which was observed for *A. ligamentina,* may have been the result of high mortality rates among *C. bairdi* during the metamorphosis period of *A. ligamentina.* Regardless, the high $D_g : J$ ratios reported above and the lack of juvenile development of *E. t. rangiana* and *L. fasciola* on *N. melanostomus* despite successful infestation, indicates that *N. melanostomus* serves as a sink of glochidia for the majority of the species examined in this study.

Unfortunately, the model does not account for predation or competition with natural host fishes of unionid mussels by *N. melanostomus,* which could result in *N. melanostomus* – mediated reproduction as the sole source of juvenile mussel production for some species. Moreover, the relative abundance of marginal hosts like *C. bairdi* may decrease as a result of such competition, which would skew the $D_g : J$ ratios reported above.

The results of the present study demonstrate an important ecological effect of the *N. melanostomus* invasion of the biologically diverse tributaries of the lower Laurentian Great Lakes that has not been considered previously. This system in which unionid glochidia are

intercepted by an invasive host, *N. melanostomus*, and subsequently metamorphose at very low rates or not at all, is analogous to that of native plants whose pollen is transported to incompatible alien hosts (via a variety of potential vectors) and essentially wasted. Unfortunately, several studies examining this type of relationship have found decreased reproductive success of native plants after the introduction of an invader that "competes" with native species for pollen (e.g., Bjerknes et al. 2007). The occurrence of a similar recruitment failure in unionid mussels as a result of the *N. melanostomus* invasion is certainly possible.

The introduction of a new species into an ecosystem has the potential to result in many unanticipated effects due to the high variability in the rates of spread and types of impacts and species interactions (e.g., Melbourne and Hastings 2009). The invasion of the Round Goby, *N. melanostomus*, into the Great Lakes in 1990 provides an interesting case study to investigate these impacts. The results of this study indicate that *N. melanostomus* may serve as marginal hosts for unionid mussel SAR in nature; however, they are likely acting more as a sink for glochidia, whereby they prevent glochidia from reaching their intended hosts. This has negative implications for unionid species that exhibit high rates of infestation and poor metamorphosis on *N. melanostomus*, particularly those species whose populations are limited to areas with large populations of this invasive fish (e.g., riverine refugia of the Great Lakes). A thorough understanding of the effects of an invasion on an ecosystem can help to predict the effects of its invasion elsewhere, at least in terms of type and direction (Kulhanek et al. 2011). These predictions, in turn, dictate how much effort will be directed towards halting the invasion and spread of a new species, and in a more general sense, contribute to our understanding of the ecology of invasions.

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Table 1. Biological and conservation aspects of the unionid species examined in the present study.

Species	**Gravidity Period***	**Collection Site (present study)**	**Known Host Family***	**COSEWIC Designation****
SAR (Species at Risk)				
Northern Riffleshell (*Epioblasma torulosa rangiana)*	August-October	Sydenham River at Florence	Percidae (Perch and Darters)	Endangered
Snuffbox (*Epioblasma triquetra)*	August-October	Sydenham River at Dawn Mills	Percidae (Perch and Darters)	Endangered
Wavyrayed lampmussel *(Lampsilis fasciola)*	May-September	Thames River at Thamesford	Centrarchidae (Sunfish and Bass)	Special Concern
Rainbow (*Villosa iris*)	May-June	Thames River at Thamesford	Percidae (Perch and Darters); Centrarchidae (Sunfish and Bass)	Endangered
Common Species				
Mucket (*Actinonaias ligamentina*)	May, August-October	Sydenham River at Florence	Centrarchidae (Sunfish and Bass)	n/a

*Sources: Watters et al. 2009; McNichols and Ackerman unpublished.

**Source: http://www.cosewic.gc.ca/

Table 2. Species and common names of fish species and the sites from which they were collected.

Species	Collection Site
Round Goby *(Neogobius melanostomus)*	Grindstone Creek, Royal Botanical Gardens, Burlington, ON (43.292607,-79.88376); LaSalle Park, Lake Ontario, Hamilton, ON (43.300119,-79.844525)
Mottled Sculpin *(Cottus bairdi)*	Speed River at Woodlawn Rd. East in Guelph, ON (43.569198,-80.271653)
Largemouth Bass *(Micropterus salmoides)*	Spencer Creek, Dundas, ON (43.2639, -79.946)
Logperch *(Percina caprodes)*	Spencer Creek, Dundas, ON (43.2639, -79.946)
Iowa Darter (*Etheostoma exile*)	Monastery Creek, Kitchener, ON (43.47302, -80.60796)
Rock Bass (*Ambloplites rupestris*)	"Correctional Ponds" (43.5567, -80.2128); Eramosa River, Guelph, ON (43.6064, -80.15589)
Smallmouth Bass (*Micropterus dolomieu*)	Niagara River, Niagara Falls, ON (43.053587, -79.022491)

Table 3. Temperature in the experimental AHAB units in 2010 and 2011 (mean ± S.D).

Year	AHAB 1	AHAB 2	AHAB 3
2010	19.04 ± 0.24 °C	19.86 ± 0.27 °C	20.05 ± 0.18 °C
2011	18.22 ± 0.013 °C	19.15 ± 0.63 °C	18.88 ± 0.64 °C

Table 4. Permits for animal collection and utilization to Professor J.D. Ackerman

Permit Type	**2010**	**2011**
University of Guelph Animal Utilization Protocol (AUP)	# 08RO91	# 08RO91
SECT 73 SARA C&A	10-012	11-020
S.17(2)(b) ESA, 2007	SR-B-004-10	AY-B-028-11
License to Collect Fish for Scientific Purposes	1056870	1056870

Table 5. Number of *N. melanostomus* collected by site in the Sydenham and Grand rivers by DFO in 2010. A-E corresponds to most upstream site to most downstream site (see Figures 3 and 4).

River	Site	Site Name	Coordinates	Total # Fish Collected	# Fish Dissected	# Fish with Body Burden	# Glochidia/ Fish
Grand	a	GR-8	43.087610, -80.040690	67	55	48	1-25
	b	GR-2	43.039180, -79.906460	29	15	0	n/a
	c	GR-1	42.966440, -79.881620	31	31	0	n/a
	d	GR-7	42.958570, -79.869990	27	26	2	1-30
Sydenham	A	33-366 Florence	42.655677, -82.008247	10	10	1	1
	B	38-366 Brick Road	42.636484, -82.01992	7	7	2	2-6
	C	34-366 Cider Mills	42.619307, -82.076912	12	12	0	n/a
	D	39-366 Upstream of Dawn Mills	42.608695, -82.122231	14	14	1	2
	E	35-366 Dawn Mills	42.600609, -82.12635	36	36	0	n/a

Table 6. Summary of species included in Discriminant Function Analysis model.

No.	Species	mean shell height (µm)	mean hinge length (µm)	mean shell length (µm)	n_{total}
1	Mucket, *Actinonaias ligamentina*	244.65	105.24	210.17	166
2	Elktoe, *Alasmidonta marginata*	379.66	140.23	324.82	109
3	Slippershell, *Alasmidonta viridis* [1]	250.29	251.14	306.86	7
4	Threeridge, *Amblema plicata* [2]	221.22	136.96	209.29	20
5	Cylindrical Papershell, *Anodontoides ferussacianus* [1]	323.86	234.29	323.00	7
6	Purple Wartyback, *Cyclonaias tuberculata* [3]	325.05	123.88	264.35	13
7	Spike, *Elliptio dilatata*	239.13	158.06	240.04	128
8	Northern Riffleshell, *Epioblasma torulosa rangiana*	231.53	179.32	249.81	47
9	Snuffbox, *Epioblasma triquetra*	209.88	141.76	211.23	40
10	Plain Pocketbook, *Lampsilis cardium*	280.33	110.46	239.66	225
11	Wavyrayed lampmussel, *Lampsilis fasciola*	300.40	116.62	247.88	222
12	Fatmucket, *Lampsilis siloquoidea*	277.72	118.40	237.95	143
13	White Heelsplitter, *Lasmigona complanata* [1]	300.17	201.00	292.83	6
14	Creek Heelsplitter, *Lasmigona compressa* [1]	285.80	233.60	322.60	5
15	Flutedshell, *Lasmigona costata*	383.36	250.06	348.99	315
16	Fragile Papershell, *Leptodea fragilis* [2]	86.07	37.06	70.66	38
17	Eastern Pondmussel, *Ligumia nasuta*	273.14	142.06	235.95	47

18	Black Sandshell, *Ligumia recta*	268.59	113.73	219.08	50
19	Threehorn Wartyback, *Obliquaria reflexa*	233.42	128.40	224.89	31
20	Round Hickorynut, *Obovaria subrotunda*	228.87	88.66	185.30	97
21	Pink Heelsplitter, *Potamilus alatus*	405.65	118.89	239.81	20
22	Kidneyshell, *Ptychobranchus fasciolaris*	201.39	87.2962	176.10	33
23	Giant Floater, *Pyganadon grandis*	369.46	257.51	359.15	30
24	Pimpleback, *Quadrula pustulosa* [4]	276.36	90.93	218.43	14
25	Mudpuppy Mussel, *Simpsonaias ambigua* [1]	260.83	167.83	254.83	6
26	Creeper, *Strophitus undulatus*	305.43	294.91	366.77	207
27	Deertoe, *Truncilla truncata*	67.29	30.85	56.04	8
28	Paper Pondshell, *Utterbackia imbecillis* [1]	301.86	247.43	304.86	7
29	Rayed Bean, *Villosa fabalis*	191.08	76.57	156.89	132
30	Rainbow, *Villosa iris*	303.80	115.62	234.76	44

n_{total} refers to the total number of individual glochidia used to create each model

[1] Hoggarth, 1999

[2] A. Ford, U.S. Fish and Wildlife Service, pers. comm., June 2012

[3] M.C. Barnhart, Missouri State University, pers. comm., June 2012

[4] M. Hove, Missouri State University, pers. comm., June 2012

Table 7. Classification results for the Grand River DFA Model, which includes only those unionid species observed live since 1970 (source: Metcalfe-Smith et al. 2000)

	Species	**n_{train}**	**n_{test}**	**% correct**	**Misclassified as (%)**
1	Mucket, *A. ligamentina*	110	56	57.1	*L. recta* (14.3), *O. reflexa* (8.9), *A. plicata* (19.6)
2	Elktoe, *A. marginata*	80	29	100	n/a
3	Slippershell, *A. viridis*	5	2	100	n/a
4	Threeridge, *A. plicata*	13	7	85.7	*O. reflexa* (14.3)
5	Cylindrical Papershell, *A. ferussacianus*	4	3	100	n/a
7	Spike, *E. dilatata*	83	45	64.4	*O. reflexa* (2.2), *L. siloquoidea* (2.2), *A. plicata* (28.9), *L. compressa* (2.2)
10	Plain Pocketbook, *L. cardium*	155	70	77.1	*P. alatus* (1.4), *L. siloquoidea* (8.6), *A. ligamentina* (2.9), *L. recta* (2.9), *V. iris* (5.7), *Q. pustulosa* (1.4)
12	Fatmucket, *L. siloquoidea*	103	40	30.0	*A. ligamentina* (2.5), *L. recta* (5.0), *V. iris* (7.5), *O. reflexa* (7.5), *L. cardium* (47.5)
14	Creek Heelsplitter, *L. compressa*	4	1	100	n/a
15	Flutedshell, *L. costata*	224	91	85.7	*A. ferussacianus* (3.3), *P. grandis* (11.0)
16	Fragile Papershell, *L. fragilis*	23	15	100	n/a
18	Black Sandshell, *L. recta*	36	14	64.3	*A. ligamentina* (7.1), *L. siloquoidea* (28.6)
19	Threehorn Wartyback, *O. reflexa*	22	9	88.9	*A. plicata* (11.1)

21	Pink Heelsplitter, *P. alatus*	14	5	100	n/a
23	Giant Floater, *P. grandis*	20	10	70.0	*L. costata* (20.0), *A. ferussacianus* (10.0)
24	Pimpleback, *Q. pustulosa*	10	4	100	n/a
26	Creeper, *S. undulatus*	135	72	97.2	*A. ferussacianus* (2.8)
27	Deertoe, *T. truncata*	4	4	100	n/a
30	Rainbow, *V. iris*	31	13	92.3	*L. cardium* (7.7)

Table 8. Classification results for the Sydenham River DFA model, which includes all species historically observed in the river (Metcalfe-Smith et al. 2007).

	Species	**n_{train}**	**n_{test}**	**% correct**	**Misclassified as (%)**
1	Mucket, *A. ligamentina*	114	52	67.3	*O. subrotunda* (5.8), *L. recta* (7.7), *O. reflexa* (5.8), *A. plicata* (13.5)
2	Elktoe, *A. marginata*	77	32	100	--
3	Slippershell, *A. viridis*	3	4	100	--
4	Threeeridge, *A. plicata*	13	7	71.4	*E. triquetra* (14.3), *E. dilatata* (14.3)
5	Cylindrical Papershell, *A. ferussacianus*	4	4	100	--
6	Purple Wartyback, *C. tuberculata*	10	3	100	--
7	Spike, *E. dilatata*	86	42	50.0	*A. plicata* (19.0), *O. reflexa* (2.4), *E. t. rangiana* (11.9), *S. ambigua* (16.7*)*
8	Northern Riffleshell, *E. t. rangiana*	35	12	33.3	*E. triquetra* (16.7), *E. dilatata* (25.0), *A. plicata* (8.3), *L. compressa* (8.3), *S. ambigua* (8.3)
9	Snuffbox, *E. triquetra*	26	14	42.9	*A. plicata*, *O. reflexa* (7.1), *E. dilatata* (14.3), *A. ligamentina* (7.1)
10	Plain Pocketbook, *L. cardium*	154	71	80.3	*A. ligamentina* (2.8), *L. recta* (1.4), *L. fasciola* (5.6), *L. siloquoidea* (8.5), *L. complanata* (1.4)
11	Wavyrayed Lampmussel, *L. fasciola*	169	53	45.3	*C. tuberculata* (30.2), *L. siloquoidea* (1.9), *L. cardium* (3.8), *O. reflexa* (1.9), *V. iris* (1.9), *L. recta* (11.3*)*, *A. ligamentina* (3.8)
12	Fatmucket, *L. siloquoidea*	97	46	19.6	*A. ligamentina* (2.2), *E. triquetra (2.2)*, *L. recta (4.3)*, *E. dilatata* (4.3), *V. iris* (2.2), *O. reflexa* (8.7*)*,

					L. cardium (52.2), *L. fasciola* (4.3), *S. ambigua* (2.2)
13	White Heelsplitter, *L. complanata*	4	2	100	--
14	Creek Heelsplitter, *L. compressa*	4	1	100	--
15	Flutedshell, *L. costata*	224	91	82.4	*P. grandis* (15.4), *A. ferussacianus* (2.2)
16	Fragile Papershell, *L. fragilis*	27	11	100	--
19	Threehorn Wartyback, *O. reflexa*	21	10	90	*A. plicata* (10.0)
20	Round Hickorynut, *O. subrotunda*	70	27	70.4	*A. ligamentina* (7.4), *P. fasciolaris* (18.5), *V. fabalis* (3.7)
21	Pink Heelsplitter, *P. alatus*	13	7	100	--
22	Kidneyshell, *P. fasciolaris*	22	11	36.4	*O. subrotunda* (18.2), *A. ligamentina* (9.1), *V. fabalis* (36.4)
23	Giant Floater, *P. grandis*	22	8	75	*L. costata* (25.0)
24	Pimpleback, *Q. pustulosa*	8	6	100	--
25	Mudpuppy Mussel, *S. ambigua*	3	3	100	--
26	Creeper, *S. undulatus*	137	70	94.3	*L. compressa* (1.4), *U. imbecillis* (4.3)
27	Deertoe, *T. truncata*	7	1	100	--
28	Paper Pondshell, *U. imbecillis*	5	2	100	--
29	Rayed Bean, *V. fabalis*	93	39	87.2	*O. subrotunda* (2.6), *P. fasciolaris* (10.3)
30	Rainbow, *V. iris*	36	8	62.5	*L. cardium* (12.5), *L. fasciola* (25.0)

Table 9. Infestation and metamorphosis rates, and number of juvenile mussels produced per fish (mean ± S.E.) for all laboratory infestation experiments.

MUSSEL SPECIES	HOST TYPE	MEAN INFESTATION RATE ± S.E. (%)	MEAN METAMORPHOSIS RATE ± S.E. (%)	MEAN NO. JUVENILE MUSSELS/FISH ± S.E.
A. ligamentina	PRIMARY *(M. dolomieu)*	13.1 ± 2.54	75 ± 13.5	236.67 ± 16.33
	MARGINAL (*C. bairdi*)	0.15 ± 0.044	10.7 ± 10.7	0.5 ± 0.5
	N. melanostomus	0.479 ± 0.17	22.9 ± 10.1	0.86 ± 0.27
E. t. rangiana	PRIMARY (*E. exile*)	2.07 ± 0.85	5.69 ± 4.76	1.33 ± 1.21
	MARGINAL (*C. bairdi*)	4.28 ± 0.75	14.2 ± 7.57	3.83 ± 1.71
	N. melanostomus	2.13 ± 0.31	0	0
E. triquetra	PRIMARY *(P. caprodes)*	12.5 ± 2.79	49.7 ± 10.3	72.33 ± 4.64
	MARGINAL (*C. bairdi*)	3.35 ± 2.65	27.2 ± 21.2	4.42 ± 3.22
	N. melanostomus	4.99 ± 2.87	13.5 ± 13.3	0.33 ± 0.17
L. fasciola	PRIMARY *(M. salmoides)*	19.4 ± 1.47	51 ± 15.5	247.75 ± 168.25
	MARGINAL (*C. bairdi*)	3.96 ± 1.13	9.91 ± 7.44	3.75 ± 1.98
	N. melanostomus	12.5 ± 7.72	0	0
V. iris	PRIMARY (*A. rupestris*)	4.27 ± 1.48	42.3 ± 9.23	78.75 ± 24.25
	MARGINAL (*C. bairdi*)	1.2 ± 0.39	31.4 ± 27.2	6.78 ± 2.75
	N. melanostomus	1.57 ± 0.354	0.253 ± 0.253	0.5 ± 0.17

Table 10. Classifications of glochidia of unknown species collected from the Grand River using the DFA model

	Species	**GR site 7**	**GR site 8**
3	Mucket, *A. ligamentina*	--	8
13	Elktoe, *A. marginata*	--	--
21	Slippershell, *A. viridis*	--	1
24	Threeeridge, *A. plicata*	14	111
20	Cylindrical Papershell, *A. ferussacianus*	--	--
25	Purple Wartyback, *C. tuberculata*	x	x
9	Spike, *E. dilatata*	--	20
5	Northern Riffleshell, *E. t. rangiana*	x	x
4	Snuffbox, *E. triquetra*	x	x
15	Plain Pocketbook, *L. cardium*	--	--
16	Wavy-rayed Lampmussel, *L. fasciola*	x	x
17	Fatmucket, *L. siloquoidea*	--	--
22	White Heelsplitter, *L. complanata*	--	--
26	Creek Heelsplitter, *L. compressa*	1	1
19	Flutedshell, *L. costata*	--	--
23	Fragile Papershell, *L. fragilis*	1	1
8	Eastern Pondmussel, *E. nasuta*	x	x
7	Black Sandshell, *L. recta*	--	--
12	Threehorn Wartyback, *O. reflexa*	--	32
2	Round Hickorynut, *O. subrotunda*	x	x
1	Pink Heelsplitter, *P. alatus*	--	--
6	Kidneyshell, *P. fasciolaris*	x	x
14	Giant Floater, *P. grandis*	--	--
29	Pimpleback, *Quadrula pustulosa*	--	--
18	Creeper, *S. undulatus*	--	--

28	Deertoe, *T. truncata*	--	--
27	Paper Pondshell, *U. imbecillis*	x	x
10	Rayed Bean, *V. fabalis*	x	x
11	Rainbow, *V. iris*	1	

"—" indicates that no glochidia were classified as a given species; "x" indicates that the species was excluded from the model

Table 11. Classifications of glochidia of unknown species collected from the Sydenham River sites using the DFA model.

		Sites		
	Species	**Upstream of Dawn Mills**	**Brick Road**	**Florence**
3	Mucket, *A. ligamentina*	--	--	--
13	Elktoe, *A. marginata*	--	--	--
21	Slippershell, *A. viridis*	--	--	--
24	Threeridge, *A. plicata*	--	1	--
20	Cylindrical Papershell, *A. ferussacianus*	--	--	--
25	Purple Wartyback, *C. tuberculata*	--	--	--
9	Spike, *E. dilatata*	--	--	--
5	Northern Riffleshell, *E. t. rangiana*	--	--	1
4	Snuffbox, *E. triquetra*	--	--	--
15	Plain Pocketbook, *L. cardium*	--	--	--
16	WRLM, *L. fasciola*	--	--	--
17	Fatmucket, *L. siloquoidea*	--	--	--
22	White Heelsplitter, *L. complanata*	--	--	--
26	Creek Heelsplitter, *L. compressa*	--	--	--
19	Flutedshell, *L. costata*	--	--	--
23	Fragile Papershell, *L. fragilis*	--	--	--
8	Eastern Pondmussel, *E. nasuta*	--	--	--

7	Black Sandshell, *L. recta*	1	--	--
12	Threehorn Wartyback, *O. reflexa*	1	--	--
2	Round Hickorynut, *O. subrotunda*	--	--	--
1	Pink Heelsplitter, *P. alatus*	--	--	--
6	Kidneyshell, *P. fasciolaris*	--	--	--
14	Giant Floater, *P. grandis*	--	--	--
29	Pimpleback, *Quadrula pustulosa*	--	--	--
30	Mudpuppy Mussel, *S. ambigua*	--	--	--
18	Creeper, *S. undulatus*	--	--	--
28	Deertoe, *Truncilla truncata*			
27	Paper Pondshell, *U. imbecillis*	--	--	--
10	Rayed Bean, *V. fabalis*	--	--	--
11	Rainbow, *V. iris*	--	--	--

"—" indicates that no glochidia were classified as a given species

Table 12. Species-specific values for the model of glochidia diluted vs. juvenile mussels produced (all values correspond to the Sydenham River). Sources: McNichols et al. 2007, Schwalb et al. 2010, COSEWIC, present study.

	Parameter	Species		
		E. triquetra	***V. iris***	***A. ligamentina***
	Occupied area (percent occupied × total area) (m^2)	25,000	630,000	1,650,000
	Density of mussels (#/m^2)	0.09	0.03	0.69
	Number of mussels (density × area occupied)	2,250	18,900	1,142,343
	U = Number of females (if unknown, assume 1:1)	1,125 (2.5:1 male:female)	4,725 (assumed 1:1)	571,171 (assumed 1:1)
	f = fecundity (glochidia/female)	17,580	~25,000 (estimate)	1,000,000
	R_e (encounter rate) = (10^{-2} – 10^{-4})	0.001	0.001	0.001
N. melanostomus	Infestation rate (%) per tank (attached glochidia/total glochidia)	4.98	1.57	0.48
	R_I, infestation rate (%) (as above, but per fish)	1.25	0.522	0.12
	R_M metamorphosis rate (%)	13.5	0.253	23.0
C. bairdi	Infestation rate (%) per tank (attached glochidia/total glochidia)	3.35	1.20	0.15
	R_I, infestation rate (%) (as above, but per fish)	8.38	0.4 4	0. 0375
	R_M metamorphosis rate (%)	27.18	31.4	10.71

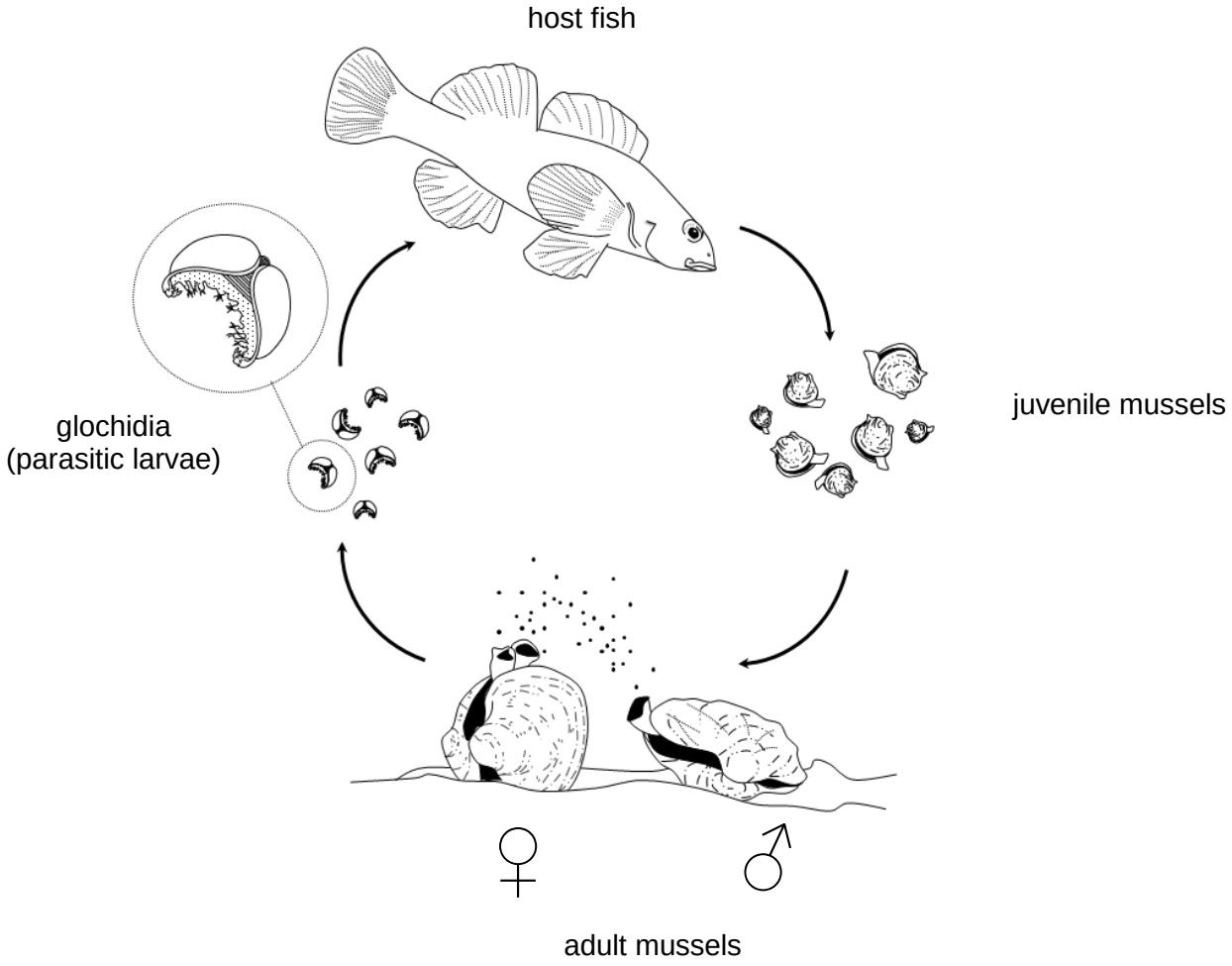


Figure 1. Unionid mussel life cycle. The cycle begins when a male mussel releases sperm into the water column. These are taken in by the female and fertilize her eggs, which develop into parasitic glochidia larvae. The glochidia are released by the female and attach to a host fish, where they develop into juvenile mussels. These juvenile mussels eventually drop off the host fish to become free-living, and the cycle continues (drawing by Tamy Rodella).

Figure 2. Fish and mussel experimental pairings. Orientation of tanks is not representative of actual setup as the pairings were randomly assigned to AHAB units.

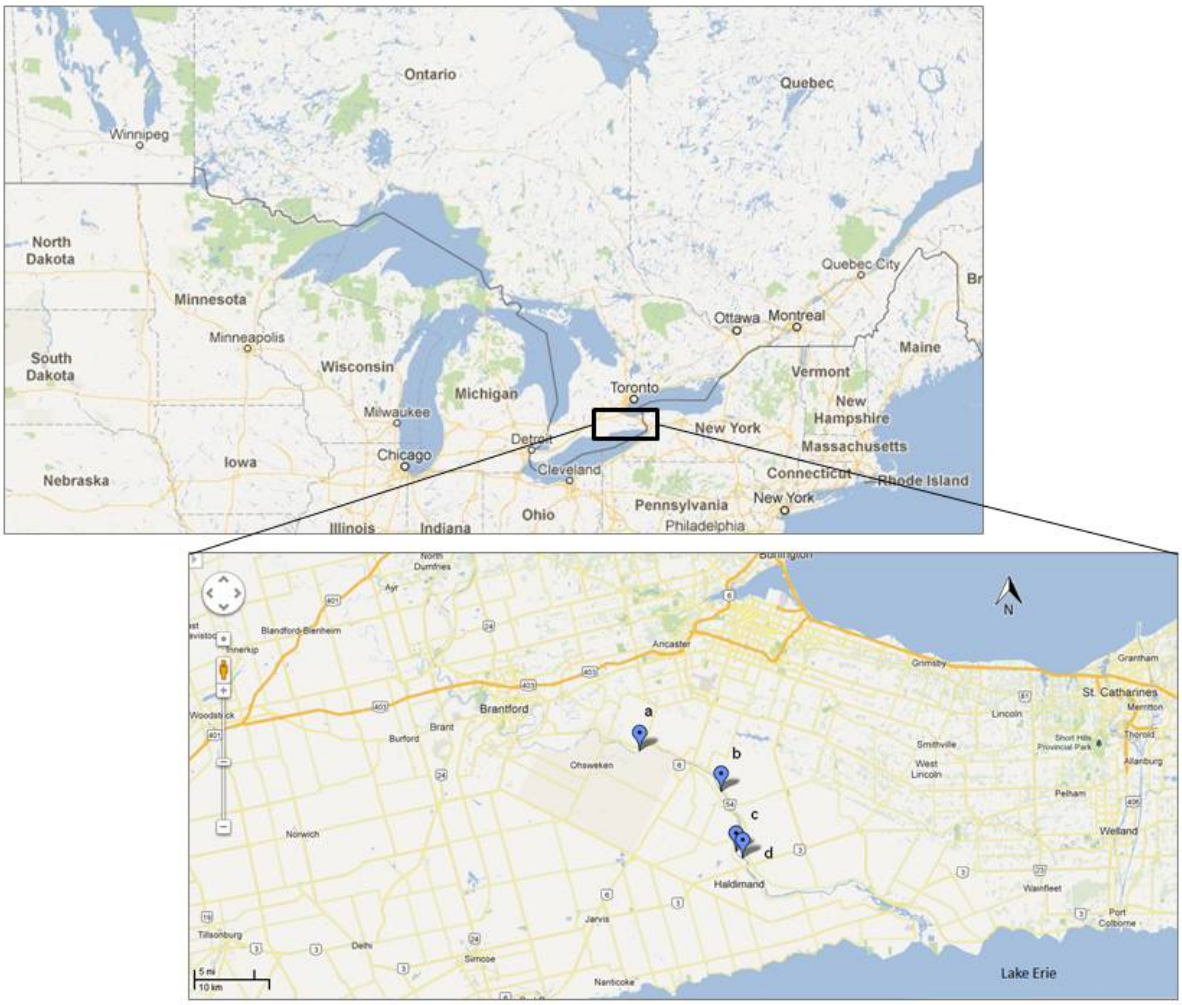


Figure 3. Locations of *N. melanostomus* collection sites in the Grand River (location of inset map is approximate).

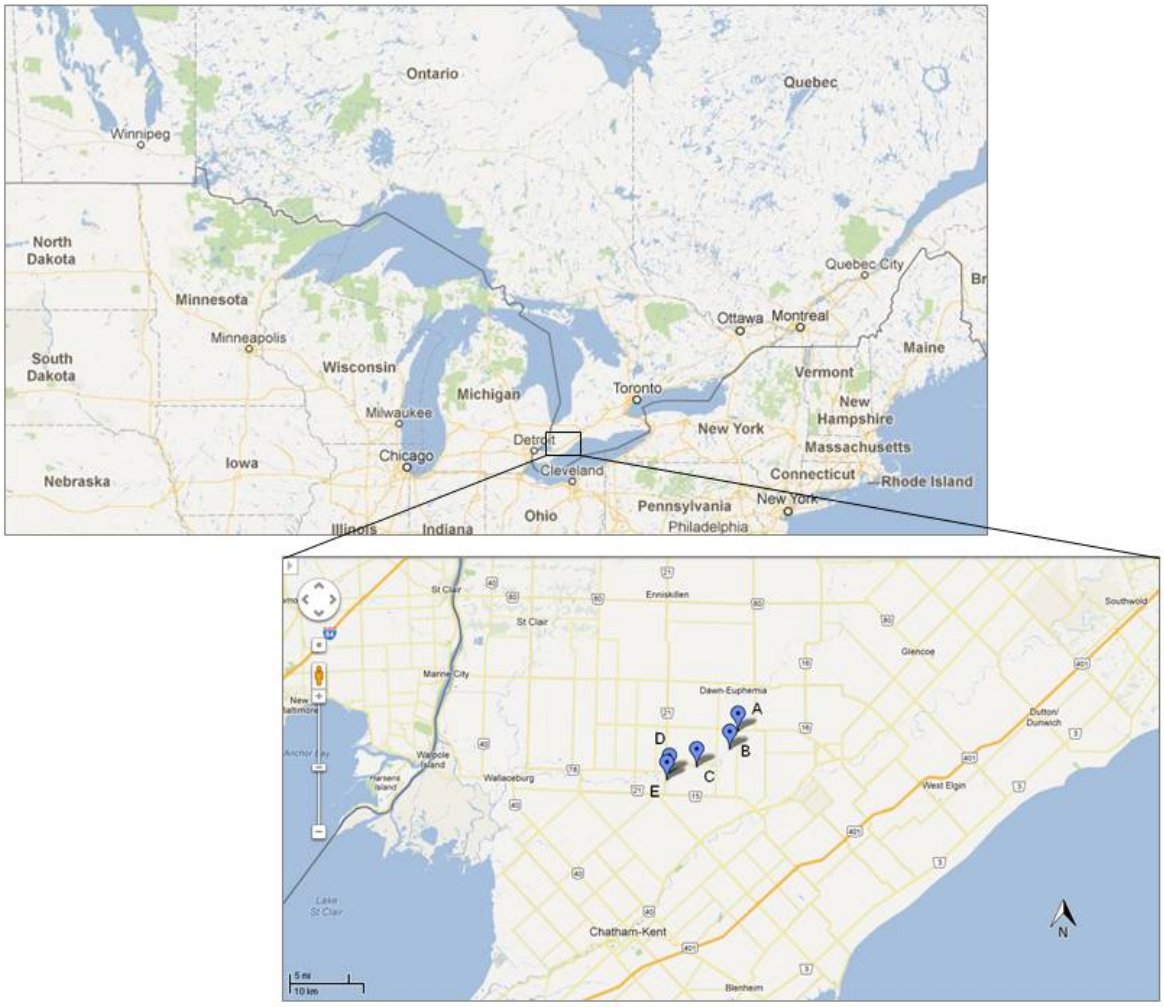


Figure 4. Locations of *N. melanostomus* collection sites in the Sydenham River (location of inset map is approximate).

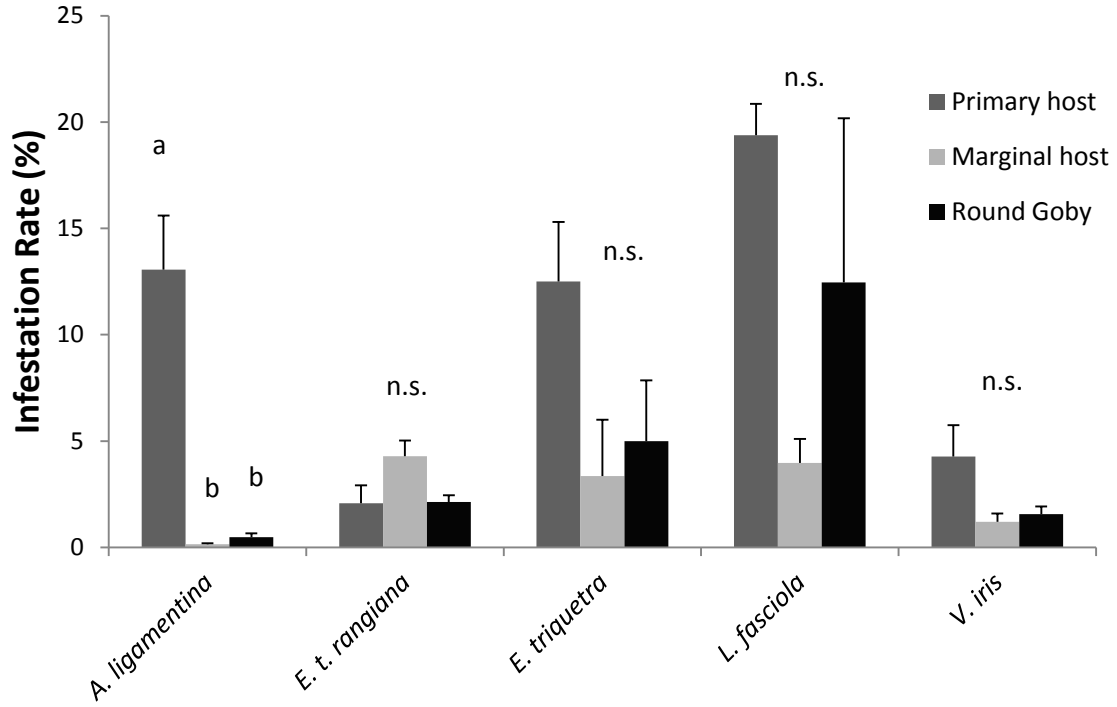


Figure 5. Infestation Rates (mean ± SE) for all mussel species and all host types (primary host, marginal host and Round Goby, *N. melanostomus*); see Table 5 for list of primary hosts. "n.s." indicates non-significant differences; different letters above two host types within a mussel species indicate significant differences.

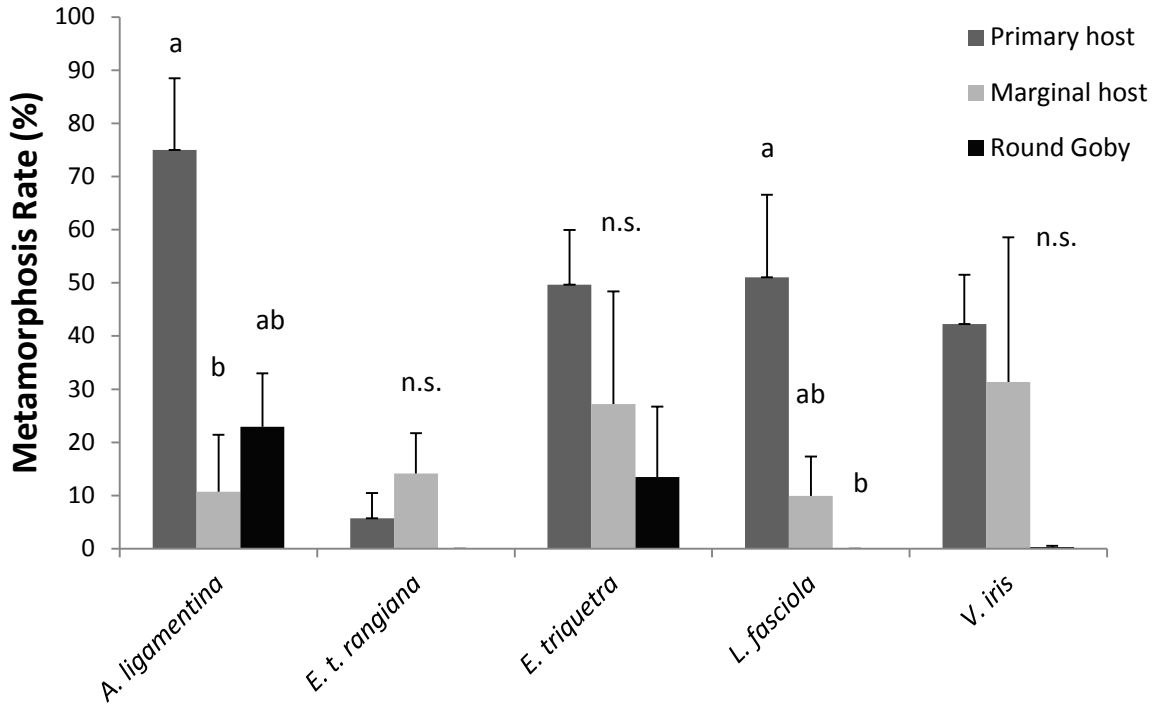


Figure 6. Metamorphosis Rates (mean ± SE) for all mussel species and all host types (primary host, marginal host and Round Goby, *N. melanostomus*); see Table 5 for list of primary hosts. "n.s." indicates non-significant differences; different letters above two host types within a mussel species indicate significant differences.

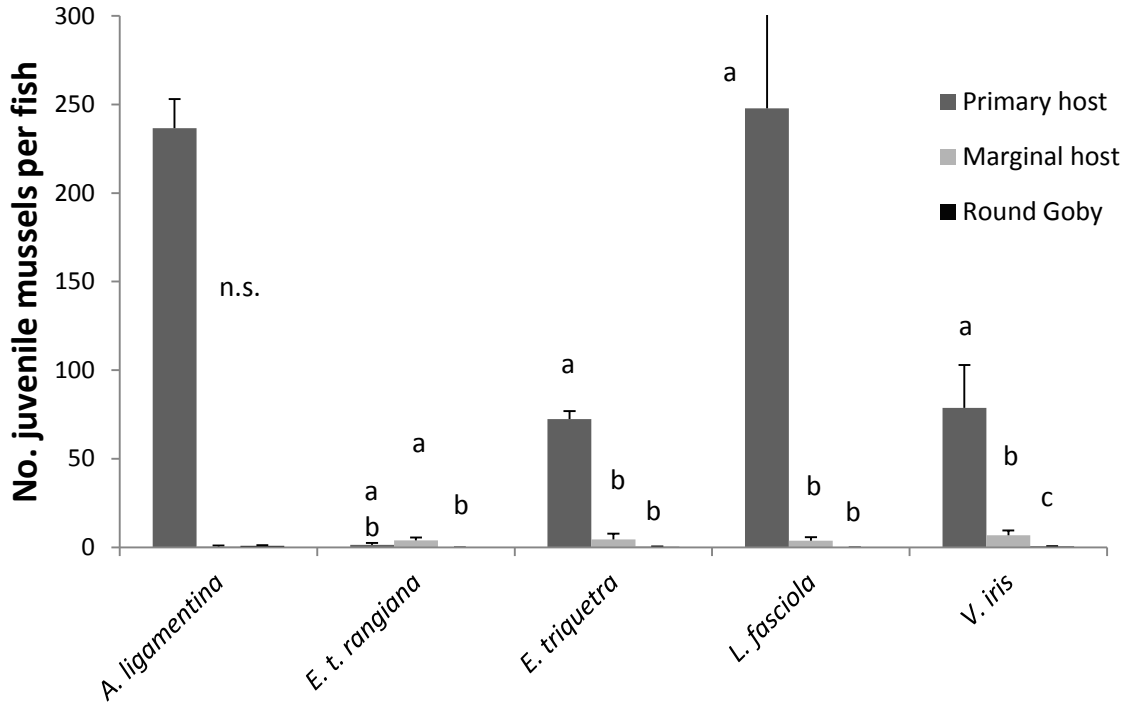


Figure 7. Number of juvenile mussels produced per fish (mean ± SE) for all mussel species and all host types (primary host, marginal host and Round Goby, *N. melanostomus*); see Table 5 for list of primary hosts. Different letters above two host types within a mussel species indicate significant differences.

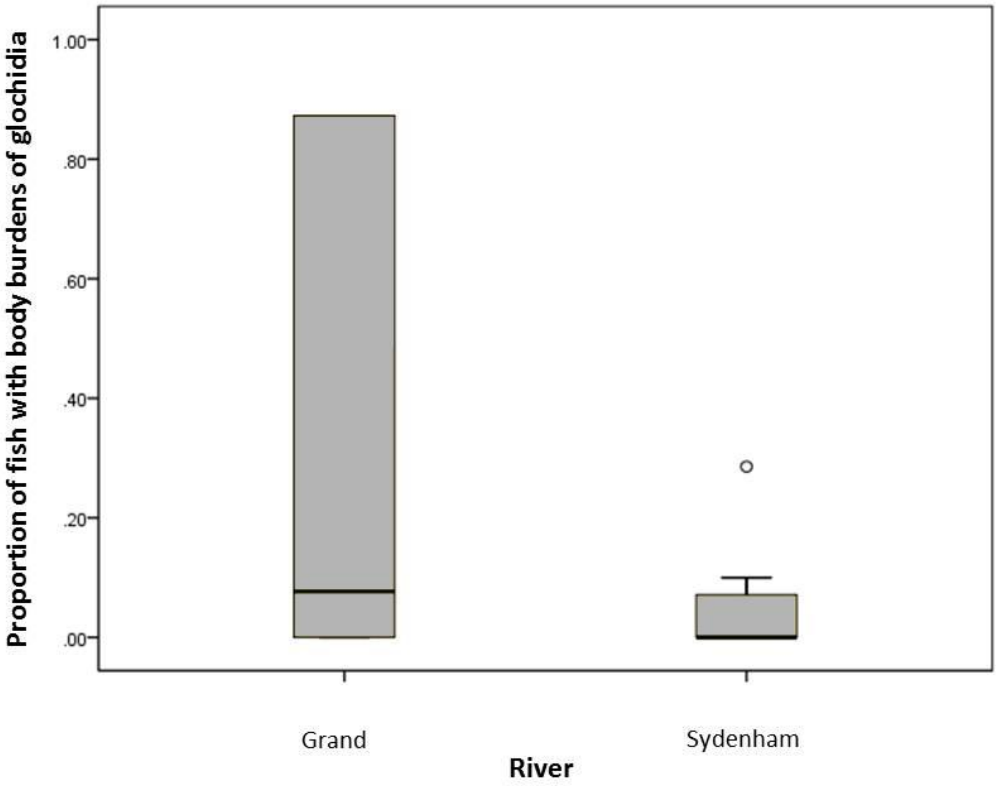
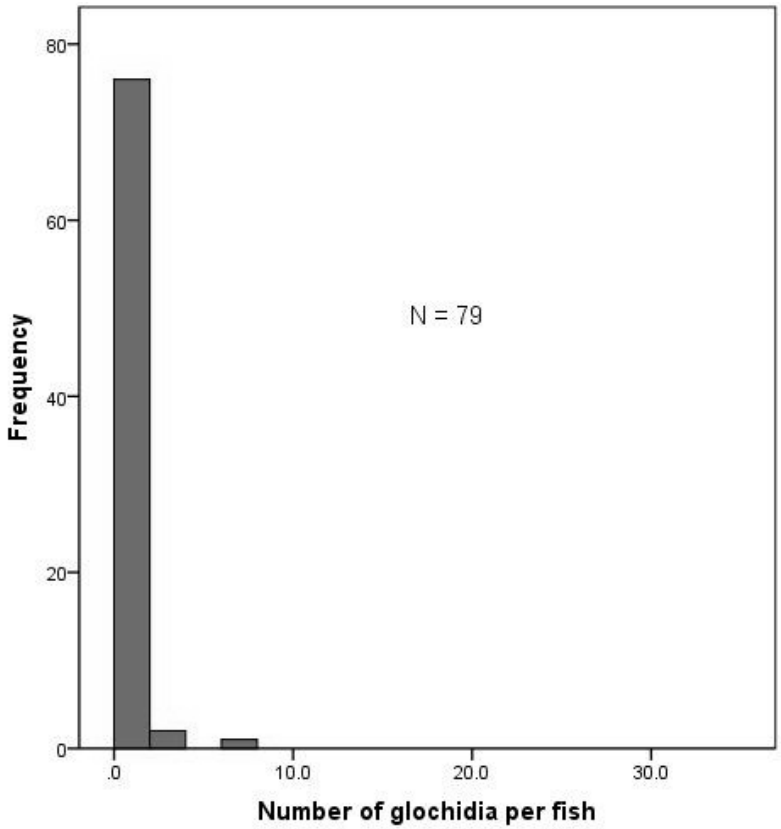


Figure 8. Proportion of *N. melanostomus* displaying body burdens of glochidia in the Grand and Sydenham rivers; weighted by number of fish at each site. Boxes indicate the spread of the proportions of fish with body burdens by site; horizontal lines within the boxes indicate medians; whiskers indicate the smallest and largest values; and outliers are represented by open circles.

a)



b)

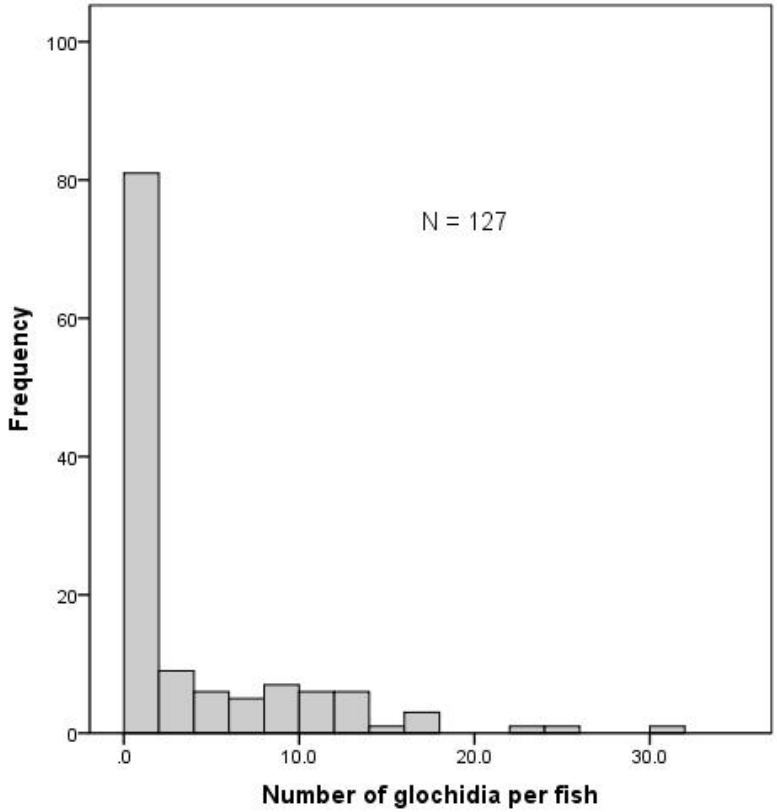


Figure 9. Frequency of glochidia per *N. melanostomus*, for (a) Sydenham and (b) Grand rivers.

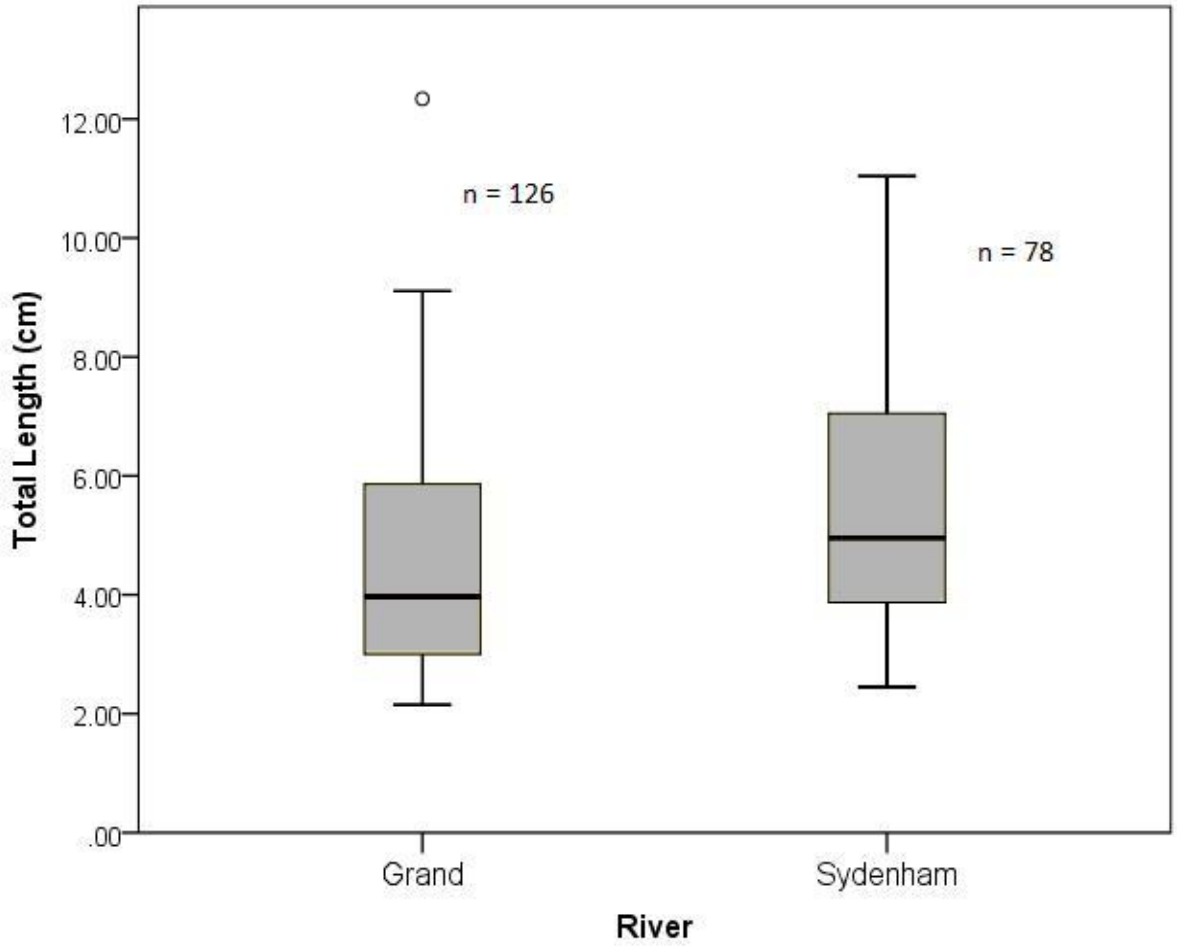


Figure 10. Distribution of total lengths of *N. melanostomus* from the Grand and Sydenham rivers. Boxes indicate the spread of total lengths; horizontal lines within the boxes indicate medians; whiskers indicate the smallest and largest values; and outliers are represented by open circles. Note: fish missing tails (n = 2) were not included.

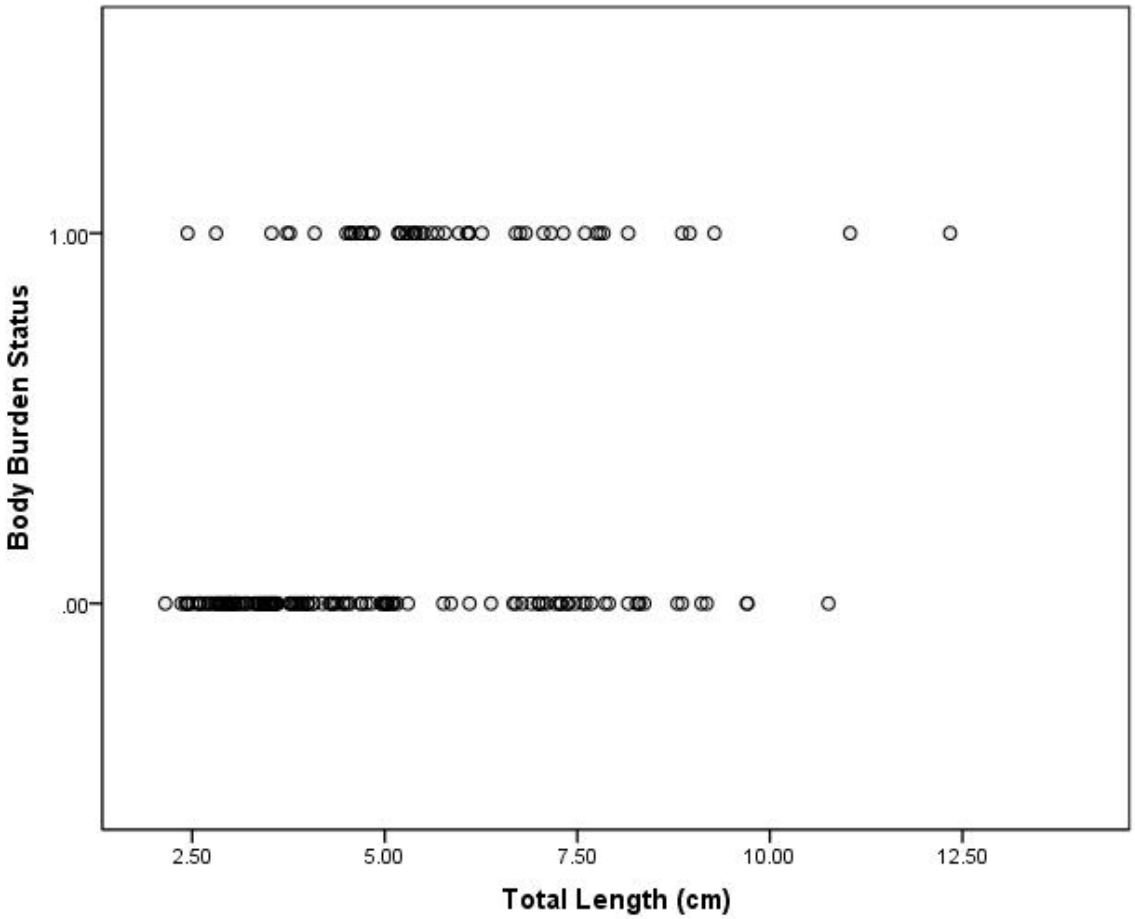


Figure 11. Scatterplot of *N. melanostomus* (from Sydenham and Grand rivers) total lengths and body burden status (1 = present; 0 = absent).

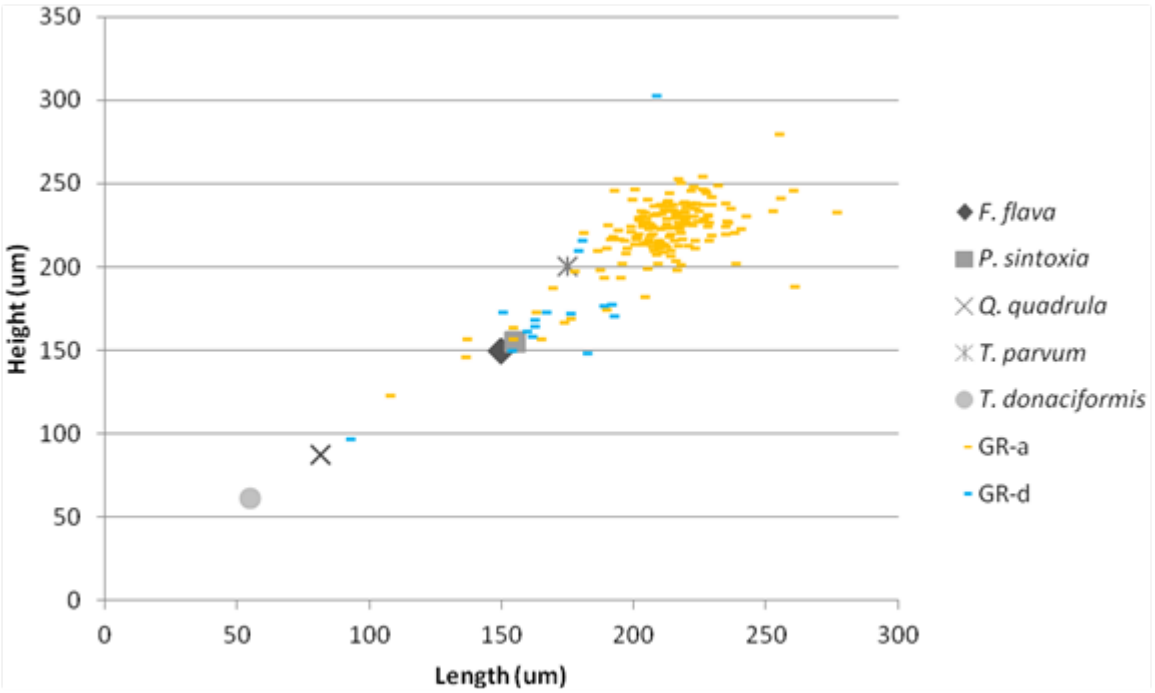


Figure 12. Lengths and heights (mean) of glochidia for which raw values were not available, and raw length and height values for glochidia collected in the present study; there is little overlap between these two groups, with the exception, perhaps, of *T. parvum* (Sources: Clarke, 1981; Watters et al. 2009).

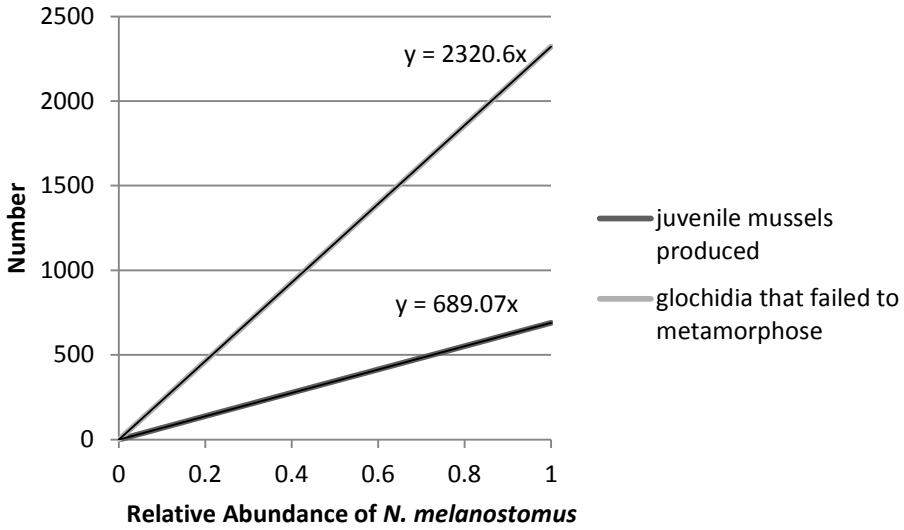


Figure 13. Modeled contribution of *N. melanostomus* to juvenile mussel production and glochidial "dilution" for *A. ligamentina* using equations 2 and 3. Slope varies with metamorphosis rate. Ratio of glochidia loss to juvenile mussel production (i.e., D_g/J) for *N. melanostomus* = 3.37. Ratio of glochidia loss to juvenile mussel production for *C. bairdi* = 8.35.

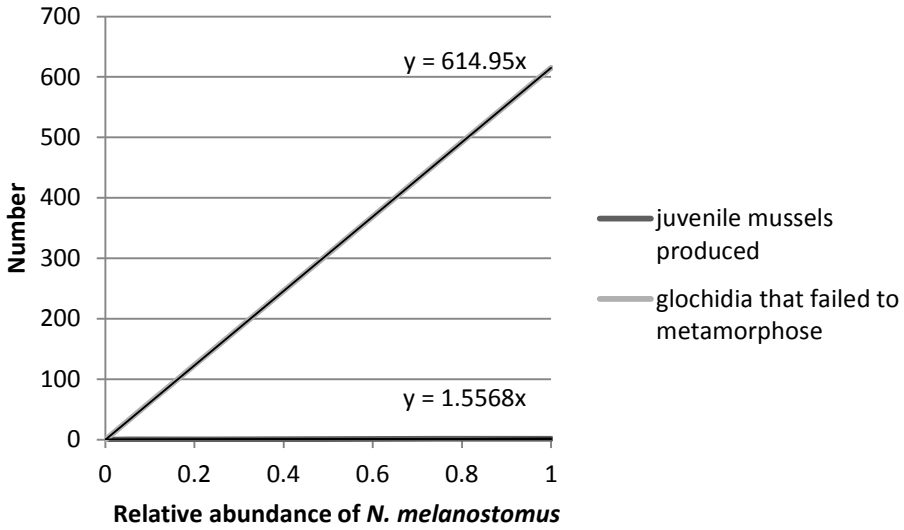


Figure 14. Modeled contribution of *N. melanostomus* to juvenile mussel production and glochidial "dilution", for *V. iris* using equations 2 and 3. Slope varies with metamorphosis rate. Ratio of glochidia loss to juvenile mussel production (i.e., D_g/J) for *N. melanostomus* = 394.99. Ratio of glochidia loss to juvenile mussel production for *C. bairdi* = 2.18**.**

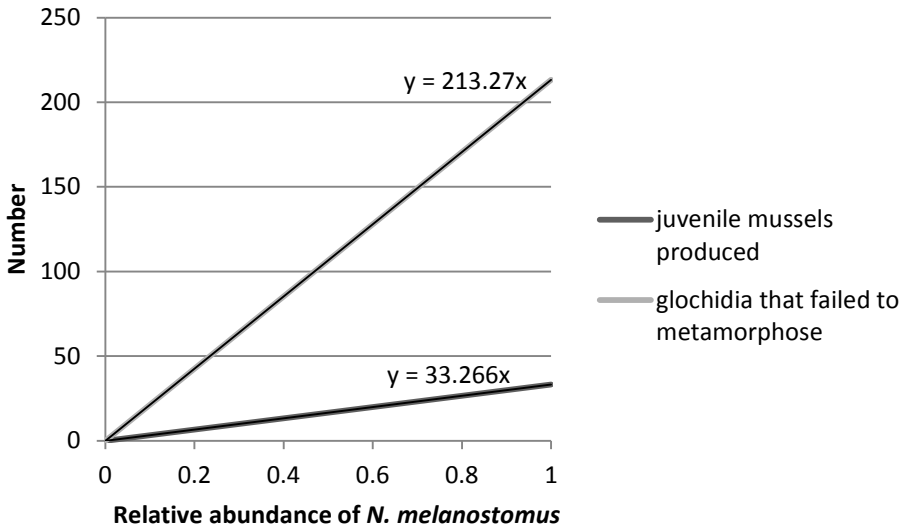


Figure 15. Modeled contribution of *N. melanostomus* to juvenile mussel production and glochidial "dilution", for *E. triquetra* using equations 2 and 3. Slope varies with metamorphosis rate. Ratio of glochidia loss to juvenile mussel production (i.e., D_g/J) for *N. melanostomus* = 6.41. Ratio of glochidia loss to juvenile mussel production for *C. bairdi* = 2.68**.**

Appendix A – Biology of the unionid species used in infestation experiments

Species: Northern Riffleshell *(Epioblasma torulosa rangiana)*

COSEWIC assessment: Endangered (2010)

Geographic Distribution: Populations of the Northern Riffleshell, *E. t. rangiana* in Canada are thought to be limited to portions of the Ausable and Sydenham Rivers in Southern Ontario; the latter population represents one of three known reproducing populations in North America, the species having been lost from 95% of its former range (Staton et al. 2003). Habitat Preferences: This species tends to be found in riffles with high dissolved oxygen and sandy or rocky substrate (Clarke 1981).

Reproductive Biology: McNichols et al. (2011) confirmed the Mottled Sculpin (*C. bairdi*) and the Iowa darter (*E. exile*) as primary hosts for *E. t. rangiana* in Canada, with *E. exile* exhibiting non-significantly higher metamorphosis rates than *C. bairdi*. *E. exile* is thought to have been extirpated from the Sydenham River, and while *C. bairdi* does occur here, it is at low abundances and not at sites of *E. t. rangiana* occurrence in 2003; this may be a main cause of declining population numbers (McNichols et al. 2011).

Species: Snuffbox *(Epioblasma triquetra)*

COSEWIC assessment: Endangered (2011)

Geographic Distribution: Reproducing populations of the Snuffbox, *E. triquetra*, are thought to be limited to the East Sydenham River and possibly the Ausable River. There are thought to be only 50 remaining reproducing populations in North America.

Habitat Preferences: Like its congener, *E. t. rangiana*, it is generally found in clear, fast flowing water with few suspended solids (COSEWIC, 2010).

Reproductive Biology: *E. triquetra* exhibit an interesting strategy of host capture, in which a gravid female closes her valves on the head of the potential host before releasing her glochidia. This species is a host specialist, thought to primarily use *P. caprodes* as a host (Schwalb et al. 2010), likely because its head and snout are strong enough to accommodate being held by valve closure (Barnhart et al. 2008). However, it is also able to complete its life cycle on other fish species in a laboratory setting (McNichols and Ackerman, unpublished data), and likely in nature as well (Zanatta and Wilson 2011).

Species: Rainbow Mussel *(Villosa iris)*

COSEWIC assessment: Endangered (2006)

Geographic Distribution: *V. iris* is widely distributed in Southern Ontario, with populations in the Ausable, Thames, Sydenham and Grand Rivers. Its largest known Canadian population occurs in the Maitland River (COSEWIC 2006). It has been lost from much of its former range including Lake Erie, Lake St. Clair, and the Detroit and Niagara Rivers, due largely to dreissenid mussel invasion.

Habitat Preferences: *V. iris* is most abundant in highly oxygenated riffles in clear river reaches, but does occasionally occur in mud or among boulders (COSEWIC, 2006).

Reproductive Biology: A variety of reproductive hosts have been identified for *V. iris* in the United States (Watters et al. 2009), indicating that it may be a host generalist, however of the species that occur in Canada, *A. rupestris* may be the primary host for this mussel (McNichols and Ackerman, unpublished).

Species: Wavyrayed Lampmussel *(Lampsilis fasciola)*

COSEWIC assessment: Endangered (1999); Special Concern (2010)

Geographic Distribution: *L. fasciola* is widely distributed in Southern Ontario, with populations in the Maitland, Ausable, Thames and the upper portions of the Grand River, where it is thought to be most abundant (COSEWIC, 2010). It is also found in rivers to the south, within the states of Ohio and Mississippi. It is thought to have been extirpated from the Sydenham River, and few, if any, live individuals are thought to remain in the Great Lakes, largely due to dreissenid biofouling (COSEWIC, 2010).

Habitat Preferences: This species is found in clear, fast flowing waters with stable substrates (COSEWIC, 2010).

Reproductive Biology: Several species have been identified as hosts for *L. fasciola* through experimental infestations, including *Micropterus salmoides, M. dolomieu,* and *Cottus bairdi*, but *M. dolomieu* is thought to be the primary host (McNichols et al. 2011).

Species: Mucket *(Actinonaias ligamentina)*

COSEWIC assessment: Not Assessed

Geographic Distribution: It is widespread and common in Ontario, and also in the United States (Metcalfe-Smith et al. 2005, Watters et al. 2009).

Habitat Preferences: *A. ligamentina* appears to prefer substrate composed of cobble and sand, within large rivers.

Reproductive Biology: *A ligamentina* is thought to be a host generalist, with many observed hosts in the Centrarchidae family (Bass and Sunfishes) (Watters and O'Dee 1998, Watters et al. 2009). Host screening experiments in 2007 at the University of Guelph identified *M. salmoides* and *Lepomis macrochirus* as primary hosts for Ontario populations of *A. ligamentina* (McNichols and Ackerman, unpublished data).

Appendix B – Dimensions of female unionids used for infestation experiments, and the viability of their glochidia

Table B1. Female mussel dimensions and respective glochidial viabilities based on a subsample of ~100 glochidia for all species examined

Species	Female	Size (cm)			Viability of Glochidia
		Length	Height	Width	
Snuffbox (*E. triquetra*)	1	3.60	2.09	2.16	99.3%
	2	3.80	1.95	2.22	95.9%
	3	3.84	2.26	2.12	98.7%
Mucket (*A. ligamentina*)	1	15.00	5.85	9.06	93.3%
	2	14.82	6.00	8.58	98.0%
	3	14.44	6.10	9.09	100%
Rainbow (*V. iris*)	1	4.36	2.39	1.36	62.5%
	2	4.03	2.14	1.15	70.7%
	3	4.60	2.44	1.31	54.2%
Northern Riffleshell (*E. t. rangiana*)	1	4.35	3.32	2.26	98.5%
	2	4.07	2.83	1.91	96.0%
	3	4.38	3.05	2.11	93.1%
Wavyrayed Lampmussel (*L. fasciola*)	1	6.10	4.75	3.04	87.1%
	2	6.57	5.00	2.93	87.0%
	3	6.40	4.97	3.03	95.5%

Appendix C – Unionid mussel and fish pairings, and total lengths of experimental fish

Table C1. Mean total length of experimental fishes and tank assignments

Mussel Species	AHAB	Fish Species	Mean Total Length ± S.D. (cm) (sample size)
Snuffbox (*E. triquetra*)	1	Logperch (*P. caprodes*)	8.81 ± 1.16 (n = 4)
	1	Mottled Sculpin (*C. bairdi*)	7.14 ± 0.96 (n = 4)
	1	Round Goby (*N. melanostomus)*	8.21 ± 1.02 (n = 4)
	2	Logperch (*P. caprodes*)	8.69 ± 0.81 (n = 4)
	2	Mottled Sculpin (*C. bairdi*)	7.70 ± 0.91 (n = 4)
	2	Round Goby **(***N. melanostomus)*	7.53 ± 1.09 (n = 4)
	3	Logperch (*P. caprodes*)	9.57 ± 0.67 (n = 4)
	3	Mottled Sculpin (*C. bairdi*)	7.23 ± 0.24 (n = 4)
	3	Round Goby **(***N. melanostomus)*	7.40 ± 1.04 (n = 4)
Mucket (*A. ligamentina*)	1	Largemouth Bass (*M. salmoides*)	8.02 ± 0.40 (n = 3)
	1	Mottled Sculpin (*C. bairdi*)	7.60 ± 0.83 (n = 4)
	1	Round Goby (*N. melanostomus)*	7.85 ± 0.45 (n = 4)
	2	Largemouth Bass (*M. salmoides*)	7.89 ± 0.34 (n = 4)
	2	Mottled Sculpin (*C. bairdi*)	7.14 ± 0.34 (n = 4)
	2	Round Goby (*N. melanostomus**)*	7.78 ± 1.64 (n = 4)
	3	Largemouth Bass (*M. salmoides*)	8.17 ± 1.08 (n = 4)
	3	Mottled Sculpin (*C. bairdi*)	8.89 ± 1.22 (n = 4)
	3	Round Goby **(***N. melanostomus**)*	6.70 ± 0.57 (n = 4)
Rainbow (*V. iris*)	1	Rock Bass (*A. rupestris*)	9.87 ± 1.84 (n = 3)
	1	Mottled Sculpin (*C. Bairdi*)	7.41 ± 0.30 (n = 3)
	1	Round Goby **(***N. melanostomus**)*	9.21 ± 0.30 (n = 3)
	2	Rock Bass (*A. rupestris*)	10.16 ± 2.94 (n = 3)
	2	Mottled Sculpin (*C. bairdi*)	8.08 ± 1.61 (n = 3)
	2	Round Goby (*N. melanostomus**)*	8.28 ± 0.46 (n = 3)
	3	Rock Bass (*A. rupestris*)	12.18 ± 1.61 (n = 3)
	3	Mottled Sculpin (*C. bairdi*)	8.25 ± 0.27 (n = 3)

	3	Round Goby (*N. melanostomus)*	8.22 ± 0.21 (n = 3)
Northern Riffleshell (*E. t. rangiana*)	1	Iowa Darter (*E. exile*)	5.08 ± 0.72 (n = 4)
	1	Mottled Sculpin (*C. bairdi*)	8.61 ± 1.81 (n = 4)
	1	Round Goby (*N. melanostomus)*	7.96 ± 0.48 (n = 4)
	2	Iowa Darter (*E. exile*)	4.70 ± 0.06 (n = 4)
	2	Mottled Sculpin (*C. bairdi*)	8.25 ± 0.64 (n = 4)
	2	Round Goby (*N. melanostomus)*	7.23 ± 0.89 (n = 4)
	3	Iowa Darter (*E. exile*)	4.50 ± 0.19 (n = 4)
	3	Mottled Sculpin (*C. bairdi*)	8.00 ± 0.85 (n = 4)
	3	Round Goby (*N. melanostomus)*	8.37 ± 0.99 (n = 3)
Wavyrayed Lampmussel (*L. fasciola*)	1	Smallmouth Bass (*M. dolomieu*)	9.68 ± 0.83 (n = 4)
	1	Mottled Sculpin (*C. bairdi*)	9.29 ± 1.19 (n = 4)
	1	Round Goby (*N. melanostomus)*	7.72 ± 1.20 (n = 4)
	2	Smallmouth Bass (*M. dolomieu*)	9.95 ± 0.47 (n = 4)
	2	Mottled Sculpin (*C. bairdi*)	8.56 ± 0.48 (n = 4)
	2	Round Goby (*N. melanostomus)*	8.03 ± 2.04 (n = 4)
	3	Smallmouth Bass (*M. dolomieu*)	10.42 ± 0.46 (n = 4)
	3	Mottled Sculpin (*C. bairdi*)	8.85 ± 0.49 (n = 4)
	3	Round Goby (*N. melanostomus)*	8.41 ± 1.90 (n = 4)

Appendix D – Power Analysis for Laboratory Experiments

	Observed Power*		
Species	Infestation Rate	Metamorphosis Rate	Number of Juvenile Mussels Produced
Snuffbox (*E. triquetra*)	0.314	0.235	1.00
Mucket (*A. ligamentina*)	0.997	0.510	1.00
Rainbow (*V. iris*)	0.424	0.179	0.997
Northern Riffleshell (*E. t. rangiana*)	0.387	0.489	0.557
Wavyrayed Lampmussel (*L. fasciola*)	0.278	0.926	0.999

$$\text{Power} \propto \frac{ES\sqrt{n}}{\sigma}$$

where ES = effect size, n = sample size, σ = variance

(source: Quinn and Kehoe 2002)

Power analysis was conducted using SPSS v.19 (IBM, Armonk, NY, USA).

Appendix E – Relative abundances of unionid mussel species at sites of interest (based on surveys by Metcalfe-Smith et al. 2000; Metcalfe-Smith et al. 2007)

Table D1. Relative abundances of unionid mussel species at Florence, Brick Road, Croton and Dawn Mills sites in the Sydenham River; from Metcalfe et al. 2007.

	Relative Abundance (%)			
Species	**Florence**	**Brick Road**	**Croton**	**Dawn Mills**
A. ligamentina	13.4	11	6.1	17.4
A. marginata	4.6	3.3	4.1	4.7
A. viridis	×	×	×	×
A. plicata	4.2	2.8	4	7.2
A. ferussacianus	×	×	×	×
C. tuberculata	17.5	36.7	48.4	14
E. dilatata	2.2	7.2	3.3	0.9
E. t. rangiana	1.7	0.6	0.3	
E. triquetra	0.9	0.4	0.1	0.4
F. flava	2.2	1.4	3.1	1.3
L. cardium	0.3	0.4	0.4	×
L. fasciola	×	×	×	×
L. siliquoidea	×	×	×	×
L. complanata	3.2	0.5	1.4	4.3
L. compressa	×	×	×	×
L. costata	18.9	8.4	10.1	9.4
L. fragilis	2.6	3.9	2.6	11.1
L. recta	2.2	1.4	1.3	0.9
O. reflexa	×	×	0.1	×
O. subrotunda	×	×	×	×
P. sintoxia	0.3	1.2	0.4	1.3
P. alatus	1.5	1	0.3	3.4
P. fasciolaris	1	1.4	1.4	5.5
P. grandis	×	0.1	0.1	×
Q. pustulosa	0.4	0.9	0.9	4.3

Q. quadrula	2.4	2.3	6	2.6
S. ambigua	0.1	0.4	0.3	×
S. undulatus	×	0.2	×	×
T. parvum	×	×	×	×
T. donaciformis	×	×	×	3
T. truncata	0.4	0.2	0.4	6.8
U. imbecillis	×	×	×	×
V. fabalis	20.1	14.3	5	1.3
V. iris	×	×	×	×

“×” indicates that species was not found at a given site

Table E2. Relative abundances of unionid mussel species at sites in the Grand River that correspond to approximate sites of *N. melanostomus* collection in the present study; from Metcalfe et al. 2000.

	Relative Abundance (%)				
	Original site	**97 - 4**	**97 - 5**	**97 - 6**	**97 - 10**
Species	**Site in present study**	**GR-a**	**GR-b**	**GR-b**	**GR- c, GR- d**
A. ligamentina		×	4.1	×	×
A. marginata		×	14.3	×	×
A viridis		×	×	×	×
A. plicata		×	×	1.4	×
A. ferussacianus		×	×	×	×
E. dilatata		×	×	×	×
F. flava		×	2.0	1.4	×
L. fasciola		×	×	×	×
L. cardium		12.5	2.0	×	×
L. siloquoidea		×	×	4.1	×
L. compressa		×	2.0	×	×
L. costata		25	30.6	9.6	×
L. fragilis		25	6.1	4.1	16.7
L. nasuta		×	×	×	×
L. recta		25	12.2	1.4	×
O. reflexa		×	×	1.4	×
O. subrotunda		×	×	×	×
P. sintoxia		×	×	1.4	×
P. alatus		12.5	4.1	1.4	×
P. fasciolaris		×	×	×	×
P. grandis		×	×	1.4	×
Q. pustulosa		×	6.1	30.1	25
Q. quadrula		×	×	×	8.3
S. undulatus		×	6.1	×	×
T. parvum		×	×	×	×
T. donaciformis		×	×	×	×

T. truncata	×	10.2	42.5	50
U. imbecillis	×	×	×	×
V. iris	×	×	×	×

"×" indicates that species was not found at a given site