Loss of reproductive output caused by an invasive species

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We investigated whether Neogobius melanostomus, an invader of biodiversity ‘hot-spots’ in the Laurentian Great Lakes region, facilitates or inhibits unionid mussel recruitment by serving as a host or sink for their parasitic larvae (glochidia). Infestation and metamorphosis rates of four mussel species with at-risk (conservation) status (Epioblasma torulosa rangiana, Epioblasma triquetra, Lampsilis fasciola and Villosa iris) and one common species (Actinonaias ligamentina) on N. melanostomus were compared with rates on known primary and marginal hosts in the laboratory. All species successfully infested N. melanostomus, but only E. triquetra, V. iris and A. ligamentina successfully metamorphosed into juveniles, albeit at very low rates well below those seen on even the marginal hosts. Neogobius melanostomus collected from areas of unionid occurrence in the Grand and Sydenham rivers (Ontario, Canada) exhibited glochidial infection rates of 39.4% and 5.1%, respectively, with up to 30 glochidia representing as many as six unionid species per fish. A mathematical model suggests that N. melanostomus serve more as a sink for glochidia than as a host for unionids, thereby limiting recruitment success. This represents a novel method by which an invasive species affects a native species.

1. Introduction

The Laurentian Great Lakes have experienced a number of threats and impacts including those from invasive dreissenid mussels in the late 1980s [1,2], which extirpated native unionid mussels by interfering with their locomotion and burrowing and preventing them from closing their valves [3]. Although unionids remain in some tributary and embayment refugia of the Great Lakes [4–6], 15 of 41 unionid species in Ontario have been assessed as at risk for extinction or extirpation by the Committee...
on the Status of Endangered Wildlife in Canada [6]. Several large rivers in southwestern Ontario are
considered species conservation ‘hot-spots’ [5] because of the high diversity of unionids present. The
recent expansion of the round goby (Neogobius melanostomus) into the lower reaches of these rivers is
cause for concern [6,7] because of the potential for predation on juvenile unionids and competition
with and/or predation on native host fishes required by unionids for their larval development [6,8,9].
However, such assessments have not considered whether unionid mussels can use N. melanostomus as
hosts to complete their life cycle and mitigate these hypothesized impacts.

Unionids have an obligate parasitic larval stage (glochidium), which requires a vertebrate host
(usually a fish) to facilitate metamorphosis into free-living juvenile mussels [10,11]. Among unionids,
there are host generalists (able to use many host fishes) and host specialists (only use a few host fish
species) and many species have evolved elaborate species-specific strategies of host attraction [9]. For
a fish to be considered a host, both glochidial infection and metamorphosis must occur; primary hosts
have high infestation rates (intensity of infection or the proportion of glochidia that attach) and high
metamorphosis rates (proportion of attached glochidia that metamorphose into juveniles), whereas
marginal hosts provide lower rates, particularly metamorphosis rates, for a given mussel species [11].
Neogobius melanostomus may be a marginal host for several unionid species in the laboratory [12] (J.D.A.
2010, personal communication) and glochidial parasitism was reported for a single individual in the field
[13]. Given the capacity for high post-invasion abundance of N. melanostomus [14], N. melanostomus may
either facilitate the recruitment of unionid mussels as a host fish or limit successful unionid recruitment
by removing large proportions of glochidia through initial attachment followed by low or unsuccessful
metamorphosis (i.e. serving as a sink for glochidia) as observed for non-host species [15]. This may
contribute to the biotic homogenization of the region, which can decrease the availability of native host
fishes to mussels [16]. The role of N. melanostomus as a host for unionids or as a sink for their glochidia
remains to be determined.

The purpose of this study is to determine whether N. melanostomus serve as hosts for unionid mussels
or as sinks for their glochidia. As hosts, they should facilitate glochidial infection and metamorphosis
of large numbers of juvenile mussels, and thus propagate unionids. As sinks, glochidia should attach but
not metamorphose (or do so at low rates), thereby reducing unionid mussel recruitment. We investigated
the host quality of N. melanostomus in the laboratory using controlled infection experiments, and in the
field where natural glochidia infections of N. melanostomus were evaluated to determine the abundance
and species of glochidia.

2. Material and methods

2.1. Assessment of infection and metamorphosis on Neogobius melanostomus in the laboratory

Gravid female Epioblasma torulosa rangiana (Lea 1838) (an endangered species), and Actinonaias
ligamentina ( Lamarck 1819) (a common species) were collected from the Sydenham River at Florence,
Ontario (42.655677, −82.008247) and Epioblasma triquetra (Rafinesque 1820) (an endangered species) were
collected at Dawn Mills, Ontario (42.600609, −82.12635). Lampsis fasciola Rafinesque 1820 (a species
of special concern) and Villosa iris (Lea 1829) (an endangered species) were collected from the Thames
River at Thamesford, Ontario (43.05601, −80.99274). Three females of each species and similar size were
transported in aerated coolers to the Hagen Aqualab (University of Guelph) where they were acclimated
to 16–18°C and moved to a circular flow-through tank containing well water at approximately 11°C.
Mussels were fed an algal diet (2 × 10⁸ cells l⁻¹ of Nanno 3600 and shellfish diet, Reed Mariculture,
Campbell, CA, USA) three times per week.

Young-of-the-year fish, whenever possible, were collected from water bodies in Guelph, Burlington,
Dundas, and Niagara Falls, Ontario that did not contain the mussel species of interest (i.e. to reduce
immunological responses due to previous glochidial encystment [17]). Species included N. melanostomus
(Pallas, 1814), Cottus bairdii Girard, 1850, a known marginal host for all mussel species and ecologically
similar to N. melanostomus [9,18], and primary fish hosts of the respective mussel species (Micropterus
salmoides (Lacepède, 1802) for A. ligamentina; Etheostoma exile (Girard, 1859) for E. t. rangiana; Percina
caprodes (Rafinesque, 1818) for E. triquetra; Micropterus dolomieu Lacepède, 1802 for L. fasciola and
Ambloplites rupestris (Rafinesque, 1817) for V. iris). Fish were transported in aerated buckets and
acclimated to 16–18°C prior to use in experiments (2010) or were treated with Melafix (Mars Fishcare,
Chalfont, USA; 0.125 ml l⁻¹ tea tree oil) for 3 days and quarantined for at least one week prior to
experiments (2011). Fish were maintained on bloodworms, crayfish, brine shrimp and/or smelt (Metro
Ontario, Inc.) as appropriate several times weekly.
Laboratory infections, which followed the techniques in [11], provided an opportunity to evaluate the capacity for successful host-glochidia infection among fish species in a controlled manner regardless of the mechanism used by a particular mussel species (e.g. broadcasting, host attraction, direct contact) [9]; it was not designed to estimate infection rates by drifting glochidia rather it is closer to that of host attraction and direct contact strategies given the concentration of glochidia used. Briefly, glochidia from half the gill of each female mussel were assessed for viability [19,20] before being divided into three portions and used to infest four conspecific fish of similar total length (TL) at a concentration of ca 5000 glochidia l\(^{-1}\) in an aerated 1.0 l tank for 1 h in the dark. Each group of four fish from a specific mussel–fish infestation (i.e. replicate) was placed in one of three Aquatic Habitat (AHAB) units (Pentair Aquatic Habitats, Apopka, USA) operated at 18–20°C using 200 µm-filtered well water. All juvenile mussels and excysted glochidia were removed and counted after being flushed into 100 µm mesh caps located in each AHAB tank (twice weekly) or after \(\frac{4}{7}\) of the water volume of each tank was siphoned through a 100 µm sieve (weekly). Once the recovery caps were devoid of juveniles for seven consecutive days, fish were inspected for glochidial encystment under tricaine methanesulfonate (MS-222; approx. 23 mg l\(^{-1}\)), and if none were found, they were euthanized (MS-222; approx. 100 mg l\(^{-1}\)) and dissected to confirm the absence of encysted glochidia.

2.2. Assessment of natural infection of *Neogobius melanostomus* in the field

*Neogobius melanostomus* were collected, geo-referenced, fixed in formalin and preserved in ethanol from the lower Grand River (between Middleport and Haldimand, Ontario; 43.087610, –80.040690 to 42.958570, –79.869990; June–July 2010) and the East Sydenham River (42.655677, –82.008247 to 42.600609, –82.12635; 16–27 August 2010). The former is slow flowing, deep, and has a substrate composed of clay and mud [20], and the latter is low gradient with a high diversity of habitats (including gravel and sand), exhibits distinct riffles and pools and high turbidity likely resulting from agricultural activities (85% of land use) [21]. A total of 127 *N. melanostomus* from the Grand River (\(N = 127\)) and 79 from the Sydenham River were measured for TL, the body and fins were examined for glochidial encystment under a 32\(\times\) dissecting microscope (Nikon SMZ-2 T, Nikon Japan), and glochidial encystment on excised gills was determined using cross polarization. All glochidia were photographed (3.34MP CoolPix 995 Nikon, Japan) and the length, height and hinge length of each glochidium were measured via image analysis (ImageJ 1.38\(\times\), US National Institutes of Health).

2.3. Statistical analyses

2.3.1. Assessment of infection and metamorphosis on *Neogobius melanostomus* in the laboratory

The infestation rate (\(R_I\)) was calculated as the proportion of glochidia that attached to the fish (\(G_A\)) from the number used to infest the fish (\(G_I\)) (\(R_I = G_A / G_I\)) [11], whereas the metamorphosis rate (\(R_M\)) was calculated as the proportion of glochidia that metamorphosed into juvenile mussels (\(G_M\)) from \(G_A\) (\(R_M = G_M / G_A\)) [11]. Glochidia encysted on fishes that died prior to the end of the experiment were included in \(G_A\) for determining \(R_I\), but were excluded from \(G_A\) for the determination of \(R_M\).

One-way analysis of variance (ANOVA) was used to compare \(R_I\), \(R_M\) and the number of juveniles produced per fish, respectively, with 'fish species' as the fixed effect. A two-way main effects ANOVA, with 'fish species' and 'female mussel' as the fixed effects, was used if the viability of glochidia differed among female unionids [18]. Assumption of normality (Shapiro–Wilks test) and homogeneity of variance (Levene's test) were examined and data were ‘arc sine square root’ or \(\log + 1\) transformed as necessary [22]. A non-parametric Kruskall–Wallis test was used if the data were not normal but satisfied homoscedasticity [22]. Significant pairwise treatments were examined using post-hoc Tukey tests [22]. STATISTICA v. 6.0 (Statsoft, Tulsa, OK, USA) was used for all analyses.

An independent samples \(t\)-test was used to compare the proportion of fish infected with glochidia (i.e. prevalence of infection) between rivers. A logistic regression was conducted using TL as the predictor and glochidia infection (i.e. 0 = not infected, 1 = glochidia infected) as the dependent variable. All analyses were undertaken using SPSS v. 19 (IBM, Armonk, NY, USA).

2.3.2. Identification of glochidia from the field-collected fish

Glochidia were assigned to species via discriminant function analysis (DFA) [23] using river specific models based on glochidia length, width and hinge length data collected for all 36 species of unionids found in these rivers (\(\mu = 83 \pm 16\) (mean \pm s.e.) glochidia/species) [24]. Classification success was 78 \pm 4%
and 75 ± 4% for the Grand and Sydenham rivers, respectively, including five species within two genera that were particularly difficult to resolve [24].

2.4. Modelled contribution of Neogobius melanostomus to unionid recruitment

A model was used to assess the role of *N. melanostomus* as a host fish for unionids versus a sink for their glochidia using the abundance (*U*) and fecundity (*f*; the number of glochidia per female [25,26] or estimated (i.e. *V. iris*)) of unionids, the encounter rate between glochidia and hosts (*R_e*; from [27]), and the *R_f* and *R_M* measured in this study. The infection (*I_N*) on *N. melanostomus* is given by

\[ I_N = U \times f \times R_e \times R_I, \]

whereas the number of juvenile unionid mussels produced (*J*) is given by

\[ J = I_N \times R_M \times N \\
= (U \times f \times R_e \times R_I) \times R_M \times N, \]

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

\[ D_g = I_N \times (1 - R_M) \times N \\
= (U \times f \times R_e \times R_I) \times (1 - R_M) \times N. \]

The difference between equations (2.2) and (2.3) is *R_M* whereby a higher *R_M* leads to more juveniles produced and fewer glochidia lost. The ratio of glochidia loss to juvenile mussel production (*D_g / J*) for *N. melanostomus* was compared to model results for the primary as well as the marginal host (*C. bairdii*). Although the model is based on parameters derived from controlled laboratory experiments that do not incorporate specific host–fish interactions [9] nor the behaviour of *N. melanostomus*, it does provide an opportunity to evaluate *D_g / J*.

3. Results

3.1. Infection and metamorphosis on Neogobius melanostomus in the laboratory

Infestation (*R_I*) and metamorphosis rates (*R_M*) were generally highest on the primary host, followed by the marginal host and then *N. melanostomus* (figure 1a,b). This pattern was also seen in juvenile mussel production (figure 1c). Considerable variation in each of these values was observed including the number of glochidia that successfully attached to individual fish, which was revealed by dissections of fishes that died prior to completion of the experiments (mortality rate for primary hosts: 33 ± 16%; *C. bairdii*: 2 ± 2% and *N. melanostomus*: 20 ± 10%).

3.1.1. Actinonaias ligamentina

Infestation rates differed significantly among fish species (Kruskal–Wallis; \( \chi^2 = 6.50, p = 0.039 \)) and were higher on the primary host (*M. salmoides*) versus the marginal host (*C. bairdii*; *p = 0.034*). Metamorphosis rates also differed (ANOVA *F*₂,₄ = 8.00, *p = 0.040*) with the highest rates on the primary host versus marginal hosts (post-hoc Tukey test, *p = 0.044*) and *N. melanostomus* (*p = 0.064*). Juvenile production did not differ significantly (\( \chi^2 = 4.23, p = 0.12 \)), but this was likely the result of reduced power due to fish mortality as differences were detected under ANOVA (*F*₂,₄ = 177, *p = 0.0001*). Ten juvenile *A. ligamentina* were produced on *N. melanostomus*.

3.1.2. Epioblasmatorulosarangiana

Infestation rates did not differ significantly (*F*₂,₆ = 3.06, *p = 0.12*), but marginal differences in *R_M* were observed (*F*₂,₆ = 4.03, *p = 0.078*) with the highest rates on the marginal host (*C. bairdii*), followed by the primary host (*E. exile*) and *N. melanostomus*. Juvenile production differed significantly (*F*₂,₆ = 5.20, *p = 0.049*), with the highest production on the marginal host, followed by the primary host and *N. melanostomus*. Significant differences were found between the marginal host (*C. bairdii*) and *N. melanostomus* (*p = 0.042*). No juvenile *E. t. rangiana* were produced on *N. melanostomus*. 
3.1.3. *Epioblasma triquetra*

Infestation rates did not differ significantly ($F_{2,6} = 3.11$, $p = 0.12$), nor did $R_M$ ($F_{2,6} = 1.37$, $p = 0.32$); however, the highest rates were on the primary host (*P. caprodes*), followed by the marginal host (*C. bairdii*) and then *N. melanostomus*. Juvenile production was marginally significantly different ($\chi^2 = 6.01$, $p = 0.05$) with marginal differences between the primary host and *N. melanostomus* ($p = 0.05$). Four juvenile *E. triquetra* were produced on *N. melanostomus*.

3.1.4. *Lampsilis fasciola*

Infestation rates did not differ significantly ($F_{2,6} = 2.84$, $p = 0.14$), whereas $R_M$ did ($F_{2,5} = 13.2$, $p = 0.010$) with the highest rates on the primary host (*M. dolomieu*), followed by the marginal host (*C. bairdii*), and then *N. melanostomus*. Significant differences were found between the primary host and *N. melanostomus* ($p = 0.009$) and marginally with the marginal host ($p = 0.056$). Juvenile production differed significantly ($F_{2,5} = 32.2$, $p = 0.001$), with the highest production on the primary host, followed by the marginal host
and N. melanostomus. Differences were detected between the primary host and N. melanostomus \((p = 0.001)\) and the marginal host \((p = 0.005)\). No juvenile L. fasciola were produced on N. melanostomus.

3.1.5. *Villosa iris*

Significant differences in the viability of glochidia among female mussels were observed, so two-way ANOVAs were conducted. Infestation rates did not differ significantly among fish species or female mussels (fish factor: \(F_{2,4} = 3.24, p = 0.15\); mussel factor: \(F_{2,4} = 0.31, p = 0.75\)). One-way ANOVAs were conducted to assess \(R_M\) and the number of juvenile mussels produced per fish as fish mortality reduced the number of replicates per female. Metamorphosis rates did not differ significantly \((F_{2,4} = 1.49, p = 0.33)\), but the number of juveniles produced per fish did \((F_{2,4} = 34.0, p = 0.003)\) with the highest production on the primary host \((A. rupestris)\), followed by the marginal host \((C. bairdii)\) and N. melanostomus \((p < 0.04\) for all pairwise comparisons). One juvenile V. iris was produced on N. melanostomus. There was high mortality of N. melanostomus with attached glochidia \((0–19\) per fish).

3.2. Natural infection of *Neogobius melanostomus* in the field

There was a significantly higher prevalence of glochidia infection on N. melanostomus in the Grand versus the Sydenham river \((50/127 (39.3\%) vs 4/79 (5.1\%)) of fish, respectively; \(\chi^2 = 34.88, p < 0.001\), \(N = 206\) using a non-parametric median test following arcsine square root transformation; figure 2), and glochidia were only found attached to the gills. Individual fish also exhibited different intensity of glochidia infection (i.e. parasite burden), with up to 30 glochidia/fish in the Grand River versus up to 6 glochidia/fish in the Sydenham River (figure 2a,b). Although N. melanostomus from the Grand River were significantly smaller than those from the Sydenham River \((N. m. melanostomus)\), the highest production on the primary host \((A. rupestris)\), followed by the marginal host \((C. bairdii)\) and N. melanostomus \((p < 0.04\) for all pairwise comparisons). One juvenile V. iris was produced on N. melanostomus. There was high mortality of N. melanostomus with attached glochidia \((0–19\) per fish).

3.3. Modelled contribution of *Neogobius melanostomus* to unionid recruitment

The ratio of the slopes of glochidia lost from potential recruitment \((D_g)\) to the slope of juvenile mussels produced \((J)\) versus the relative abundance of N. melanostomus to known suitable fish hosts \((N;\) proportion between 0 and 1.0) was assessed using species-specific parameter values for E. triqueta, V. iris and A. ligamentina (electronic supplementary material, table S1). The loss of glochidia by N. melanostomus increased \(6.41 \times \) faster than juvenile production for E. triqueta (figure 3a), \(3.35 \times \) faster for A. ligamentina (figure 3b). These \(D_g: J\) ratios were always lowest for the primary hosts \((1.01, 1.36\) and 0.33, respectively, for E. triqueta, V. iris and A. ligamentina), were generally higher for the marginal host \((C. bairdii; 2.68, 2.18\) and 8.34, respectively, for E. triqueta, V. iris and A. ligamentina) and highest for N. melanostomus (figure 3b). The trend between the marginal host and N. melanostomus was reversed on A. ligamentina, likely due to the fact that very few C. bairdii survived until the end of the metamorphosis period, which limited the assessment of \(R_M\). In the case of E. t. rangiana and L. fasciola, none of the 164 and 1827 encysted glochidia, respectively, metamorphosed on N. melanostomus thus representing a complete loss of reproductive output.

4. Discussion

The results of this study reveal a novel way in which N. melanostomus affect unionid mussels. Specifically, relatively high laboratory infestation rates confirmed by the occurrence of glochidia infection on N. melanostomus in nature, combined with relatively low metamorphosis rates in the laboratory support.
the hypothesis that *N. melanostomus* are a sink for unionid glochidia. Model results indicate that *N. melanostomus* contribute more to the loss of reproductive potential in unionid mussels than to their recruitment. To the best of our knowledge, this represents a hitherto unknown manner by which an invasive species affects a native species. This system in which unionid glochidia are intercepted by an invasive host, and subsequently metamorphose at low rates or not at all, is analogous to that of native plants whose pollen is transported to incompatible invasive species and essentially wasted [26]. It differs from parasite spillback systems [28] because increased parasitism of invasive fish hosts represents a sink for glochidia and a loss to native mussel communities.

Generally, glochidial infestation rates on *N. melanostomus* were similar to those on known primary and marginal hosts; however, metamorphosis rates and juvenile mussel production were generally higher on primary hosts. Moreover, metamorphosis on *N. melanostomus* was only observed for three of the five unionid species examined (endangered *V. iris* and *E. triquetra*, and a common species, *A. ligamentina*). This indicates that although initial attachment of glochidia to *N. melanostomus* occurs, glochidia do not metamorphose at rates comparable to their primary or marginal hosts. Initial attachment is not surprising, given that glochidia attach to non-host fish [15] as well as to inanimate objects [29]. Longer term attachment and encystment, necessary for successful juvenile mussel production, requires some set of species-specific chemical cues [29] that did not occur for *N. melanostomus*.

The infestation rates on primary and marginal hosts are consistent with previous experiments that have examined these mussel-host fish using individuals from the northern limit of their respective species ranges [30] (K. Loftus 2014, Ontario Ministry of Natural Resources and Forestry, personal

Figure 2. Frequency distributions of glochidia infection on *Neogobius melanostomus* in the field. Glochidia per individual *N. melanostomus* in the (a) Sydenham and (b) Grand rivers, (c) total lengths of *N. melanostomus* with infections from both 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; two fish missing tails were not included) and (d) total lengths and the proportion of fish infected with glochidia (present or absent) of *N. melanostomus* collected from both rivers.
Glochidia infection rates of approximately 40% in the Grand River indicate that *N. melanostomus* encounter and become infected with glochidia at rates comparable to known hosts: infection rates of 34% have been reported on *M. dolomieu* by *L. fasciola* or another *Lampsilis* species (Morris and Granados 2010, Fisheries and Oceans Canada, personal communication); infection rates of 40% were found on *Morone americana*, which is a host of *Leptidea ochracea*, and *Lampsilis cariosa* [32]; and infection rates of 46–71% were found on *P. caprodes* and *A. rupestris*, which are known hosts of some rare unionid mussels (D. Woolnough 2011, Central Michigan University, personal communication). The reduced infection rates observed in the Sydenham River may be reflective of the recent invasion of this system by *N. melanostomus* as subsequent samples obtained in 2013 and 2014 have shown glochidia infection rates of 36% (29 of 79 fish) and 81% (50 of 61 fish) (T.J.M. 2015, personal communication).

Although the spatial and temporal nature of the unionid–*N. melanostomus* encounters is not known, the analysis of *N. melanostomus* from the Grand River provides some valuable information. The majority of encysted glochidia included *A. ligamentina*, *A. plicata*, *E. dilatata*, *E. triquetra* and *O. reflexa*. *Actinonaia ligamentina*, the common species examined in the laboratory experiments, is a host generalist that broadcasts its glochidia into the water column [33], so glochidia encounter with *N. melanostomus* is not surprising. *Ambloplites plicata* is also a host generalist [33] and is common. *Elliptio dilatata*, which is common in Ontario (S Rank = 5 [34]) and a host generalist [33], is not abundant in the lower Grand River where
The relative contribution of *N. melanostomus* to juvenile mussel production (*J*) versus glochidia loss (*D₈*) is determined primarily by the metamorphosis rate (*Rₘ*) of glochidia on *N. melanostomus* (equation (2.3)/(2.2)) given by

\[
\frac{D₈}{J} = \frac{1 - Rₘ}{Rₘ}.
\] (4.1)

Both *J* and *D₈* increase as the proportion of *N. melanostomus* increase, but higher *Rₘ* leads to more *J*. This model does not account for differences in host attraction strategies among unionids [9], nor predation or competition for conglutinates with natural host fishes by *N. melanostomus*, which could result in *N. melanostomus*-mediated recruitment being the sole source of *J* for some species whose hosts experience decline or are extirpated. *Neogobius melanostomus* may reduce the pool of effective hosts for unionids where they co-occur, interfere with the normal reproductive cycle of unionids when they achieve high population densities [16], and consequently have a greater impact on these biodiversity ‘hot-spots’ than was predicted [5]. Such indirect effects have been recently demonstrated in *Anadonta anatina* (a host generalist), which had lower metamorphosis on non-native fish hosts and lower success overall (i.e. 67–94% fewer fish species) as a result of ‘biotic homogenization’ [16]. Although there is error associated with our model, especially the error associated with estimate of *Rₘ* for relatively poor host species, and simplification of the mussel–host interactions in the laboratory, the implication of glochidia loss compared with primary hosts remains a fundamental finding, which will be exacerbated as *N. melanostomus* dominates the ecosystem in biodiversity hot-spots. It is relevant to note that significant differences in infection rates have been found between recently isolated mussel populations [36], and such differences may also occur between geographically distinct lineages of host fish as well as within lineages of mussels and their fish hosts [37]. Consequently, it is possible that the effects of invasive *N. melanostomus* on host–parasite relationships may vary spatially and temporally due to population-specific attributes arising from local adaptations and fine-scale coevolutionary dynamics.

The introduction of a new species into an ecosystem has the potential to cause many unanticipated effects as a result of the high variability in the rates of spread, types of impacts and species interactions [38]. The results of this study indicate that *N. melanostomus* are likely acting 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 infection and poor/no metamorphosis on *N. melanostomus* (equation (2.3)/(2.2)) given by

\[
\frac{D₈}{J} = \frac{1 - Rₘ}{Rₘ}.
\] (4.1)

Both *J* and *D₈* increase as the proportion of *N. melanostomus* increase, but higher *Rₘ* leads to more *J*. This model does not account for differences in host attraction strategies among unionids [9], nor predation or competition for conglutinates with natural host fishes by *N. melanostomus*, which could result in *N. melanostomus*-mediated recruitment being the sole source of *J* for some species whose hosts experience decline or are extirpated. *Neogobius melanostomus* may reduce the pool of effective hosts for unionids where they co-occur, interfere with the normal reproductive cycle of unionids when they achieve high population densities [16], and consequently have a greater impact on these biodiversity ‘hot-spots’ than was predicted [5]. Such indirect effects have been recently demonstrated in *Anadonta anatina* (a host generalist), which had lower metamorphosis on non-native fish hosts and lower success overall (i.e. 67–94% fewer fish species) as a result of ‘biotic homogenization’ [16]. Although there is error associated with our model, especially the error associated with estimate of *Rₘ* for relatively poor host species, and simplification of the mussel–host interactions in the laboratory, the implication of glochidia loss compared with primary hosts remains a fundamental finding, which will be exacerbated as *N. melanostomus* dominates the ecosystem in biodiversity hot-spots. It is relevant to note that significant differences in infection rates have been found between recently isolated mussel populations [36], and such differences may also occur between geographically distinct lineages of host fish as well as within lineages of mussels and their fish hosts [37]. Consequently, it is possible that the effects of invasive *N. melanostomus* on host–parasite relationships may vary spatially and temporally due to population-specific attributes arising from local adaptations and fine-scale coevolutionary dynamics.

The introduction of a new species into an ecosystem has the potential to cause many unanticipated effects as a result of the high variability in the rates of spread, types of impacts and species interactions [38]. The results of this study indicate that *N. melanostomus* are likely acting 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 infection and poor/no metamorphosis on *N. melanostomus*, particularly those species whose populations are limited geographically to areas with large populations of this invasive fish (e.g. riverine refugia of the Laurentian Great Lakes). A thorough understanding of the effects of a species invasion on an ecosystem can help to predict the effects of its invasion elsewhere, at least in terms of type and direction [39]. These predictions, in turn, dictate how much effort should be directed towards halting the invasion and spread of a new species, and more generally, contribute to our understanding of the ecology of invasions.

Ethics. Fish and mussels were collected under Licence to Collect Fish for Scientific Purposes OMNR permit 1056870 and cared for according to University of Guelph Animal Utilization Protocol 08RO91. All mussels were collected under Species at Risk Act, 2004 permit SECT 73 SARA C&A: 10-012 and 11-020 and Endangered Species Act, 2007 permit SR-B-004-10 and AY-B-028-11. Data accessibility. The supporting data are available on Dryad: http://dx.doi.org/10.5061/dryad.1kv63. Authors’ contributions. M.E.M.T., T.J.M. and J.D.A. designed the research; M.E.M.T. performed research; M.E.M.T. and J.D.A. wrote the paper. All authors gave final approval for publication. Competing interests. The authors have no competing interests. Funding. Support was provided by Fisheries and Oceans Canada (DFO), Ontario Ministry of Natural Resources (OMNR), Canadian Wildlife Federation (CWF) and Natural Sciences and Engineering Research Council of Canada (NSERC) to J.D.A. Acknowledgements. The authors would like to thank Dr Gerry Mackie, Dr Mike Nishizaki, Kelly McNichols-O’Rourke, Rosario Castañon, Amanda Conway, Anthony Merante, Bob Frank, Matt Cornish, John Schwintdt (Upper Thames Region Conservation Authority), Dr Mark Poesehe (University of Alberta) and Jason Barnucz and his field crew at DFO.
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