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EVALUATION OF HOST FISHES FOR THE BROOK FLOATER (ALASMIDONTA VARICOSA) FROM POPULATIONS IN MASSACHUSETTS AND MAINE, USA

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Order of Authors:	Ayla J. Skorupa
	Allison H. Roy
	Peter D. Hazelton
	David Perkins
	Timothy Warren
Corresponding Author:	Ayla Skorupa, M.S. University of Massachusetts Amherst Amherst, MA UNITED STATES
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REGULAR ARTICLE

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EVALUATION OF HOST FISHES FOR THE BROOK FLOATER (*ALASMIDONTA VARICOSA*) FROM POPULATIONS IN MASSACHUSETTS AND MAINE, USA

Ayla J. Skorupa[1*], Allison H. Roy[2], Peter D. Hazelton[3], David Perkins[4], and Timothy Warren[4]

[1] *Massachusetts Cooperative Fish and Wildlife Research Unit, Department of Environmental Conservation, University of Massachusetts, Amherst, MA 01003 USA*

[2] *U.S. Geological Survey, Massachusetts Cooperative Fish and Wildlife Research Unit, Department of Environmental Conservation, University of Massachusetts, Amherst, MA 01003 USA*

[3]*Warnell School of Forestry and Natural Resources, University of Georgia, Athens, GA 30602 USA*

[4]*U.S. Fish and Wildlife Service, Richard Cronin Aquatic Resource Center, Sunderland, MA 01375 USA*

*Corresponding Author: askorupa@umass.edu

ABSTRACT

The Brook Floater (*Alasmidonta varicosa*) mussel is globally vulnerable and has disappeared from much of its historical range. Information on Brook Floater host fish use is needed for ecological and conservation purposes, but previous laboratory studies provide conflicting results. We evaluated host fish use by Brook Floater from populations in Massachusetts and Maine, USA. We conducted three experiments using a total of 10 fish species from six families, and we estimated glochidial attachment rate and juvenile metamorphosis rate. Across fish species, attachment ranged from 51.0–84.6% and metamorphosis ranged from 4.9–80.9%. Fish species and inoculation density (viable glochidia/mL) only weakly predicted attachment, and the number of glochidia that attached to fish did not affect metamorphosis rate. Juvenile metamorphosis was successful on all fish species tested, supporting evidence that Brook Floater is a host generalist. Fish species was an important factor in predicting metamorphosis rates in all experiments. The highest metamorphosis was on Slimy Sculpin (*Cottus cognatus*) (80.9% ± 2.6 SD) and Brook Trout (*Salvelinus fontinalis*) (71.6%), but metamorphosis on Brook Trout varied according to source and was lowest on hatchery-raised fish (12.8% ± 0.3 SD). These data contribute to our understanding of the life history of Brook Floater by identifying potential host fishes, and our results can inform propagation efforts for this species in the northeastern USA.

KEY WORDS - *Alasmidonta varicosa*, Brook Floater, glochidia, host fish, host generalist, propagation

INTRODUCTION

Captive propagation of freshwater mussels is an important tool to support the conservation and restoration of imperiled species (FMCS 2016; Cowie et al. 2017; Strayer et al. 2019). Captive propagation typically requires the identification of suitable host fishes that can facilitate the development of parasitic mussel larvae (glochidia). Glochidia of a particular mussel species often can parasitize multiple fish species, but fishes vary in suitability (Riusech and Barnhart 2000; McNichols et al. 2011), and host use can vary across geographic regions (Douda et al. 2014). Cost-effective propagation requires the identification of host fishes that consistently produce large numbers of juvenile mussels, and knowledge of host use has other important applications for conservation and understanding of mussel ecology (Barnhart et al. 2008; Douda et al. 2014). Consequently, the identification of host fishes is considered a research priority (Ferreira-Rodríguez et al. 2019).

The Brook Floater (*Alasmidonta varicosa*) occurs in Atlantic Coast rivers of North America from Georgia to New Brunswick and Nova Scotia, but it has disappeared from much of its former range and is considered vulnerable (NatureServe 2011). The largest declines have occurred in the central part of its range from Virginia to New Hampshire, and eight of eleven northeastern US states designate Brook Floater as critically imperiled (NatureServe 2011). Captive propagation is proposed as a tool to recover and restore Brook Floater populations in the northeastern US, and identification of host fishes is needed to support these efforts (Roy et al. 2022).

Two previous laboratory studies of Brook Floater host use identified 20 suitable host fish species, characterizing it as a host generalist (Eads et al. 2007; Wicklow et al. 2017). In North Carolina, Brook Floater glochidia metamorphosed on nine of 13 fish species tested, but measures

of metamorphosis rate were not provided, and host use was inconsistent between experiments (Eads et al. 2007). In New Hampshire, Brook Floater glochidia metamorphosed on 12 of 17 fish species tested, but these experiments were conducted with low inoculation densities (< 41 glochidia/fish) and few individuals of each fish species (1–5), leaving questions about which fishes are robust hosts and suitable for large-scale propagation (Wicklow et al. 2017). Furthermore, suitable hosts differed between the two studies: Margined Madtom (*Noturus insignis*) and Tessellated Darter (*Etheostoma olmstedi*) were suitable hosts in New Hampshire but not in North Carolina, and Redbreast Sunfish (*Lepomis auritus*) was a suitable host in North Carolina but not in New Hampshire (Eads et al. 2007; Wicklow et al. 2017). Additional information about Brook Floater host use is needed to inform propagation efforts and other conservation and ecological questions.

We evaluated host fish use in the laboratory for Brook Floater from populations in Massachusetts and Maine. We estimated glochidial attachment and juvenile metamorphosis rates on 10 fish species across three different experiments. We evaluated how well attachment and metamorphosis rates were predicted by inoculation density, density of glochidia on fish, and fish species. Finally, we compare our results with other studies of Brook Floater host use and discuss considerations for selecting the most effective hosts for propagation of Brook Floater in the northeastern US.

METHODS

We conducted three laboratory experiments in which we tested various combinations of potential hosts under different conditions (see subsequent description of each experiment). All

experiments were conducted at the U.S. Fish and Wildlife Service's Richard Cronin Aquatic Resource Center (CARC) in Sunderland, Massachusetts.

Host Fish Collection

Fish species and numbers varied by experiment based on our ability to collect fishes in the wild in early spring and on fish availability at hatcheries. We obtained salmonids from the following fish hatcheries: Nashua National Fish Hatchery, Nashua, New Hampshire (Atlantic Salmon, *Salmo salar*); Silvio O. Conte Anadromous Fish Research Laboratory, Turners Falls, Massachusetts (Brook Trout, *Salvelinus fontinalis*); Massachusetts Division of Fisheries and Wildlife, Sandwich, Massachusetts (Brook Trout; Brown Trout, *Salmo trutta*; Rainbow Trout, *Oncorhynchus mykiss*). We collected all other fishes by seining and backpack electrofishing in the Fall River, Massachusetts (Slimy Sculpin, *Cottus cognatus*; Longnose Dace, *Rhinichthys cataractae*; Blacknose Dace, *Rhinichthys atratulus*; White Sucker, *Catostomus commersonii*) or the Connecticut River, Massachusetts (Banded Killifish, *Fundulus diaphanous*; Bluegill, *Lepomis macrochirus*). We collected fishes from river sections where mussels were absent or rare to avoid removing potential hosts and to reduce the chances that fishes had immunity from prior exposure to glochidia (O'Connell and Neves 1999; Rogers and Dimock 2003). We maintained fishes in aquaria and fed them black worms until the start of experiments.

Mussel Broodstock Collection and Glochidia Extraction

We collected Brook Floater broodstock from streams by snorkeling. We collected one gravid mussel from the Nissitissit River in Middlesex County, Massachusetts, in March 2017 (Experiment 1); three gravid mussels from Wesserunsett Stream in Somerset County, Maine, in

April 2017 (Experiment 2); two gravid mussels from the West Branch Farmington River in Berkshire County, Massachusetts, and three gravid mussels from Wesserunsett Stream in October 2018 (Experiment 3). We transported mussels to the laboratory individually in aerated 3.7-L glass jars of water in a cooler. We maintained mussels in an environmental chamber at CARC at a temperature similar to stream temperatures at the time of broodstock collection (~5°C) to inhibit glochidia release. We conducted experiments within six weeks of broodstock collection. Immediately before extraction of glochidia for the experiments, we acclimated broodstock to 10°C, an approximate temperature at which glochidia are released in the wild (about 14°C, Wicklow et al. 2017).

We extracted glochidia for Experiments 1 and 2 by puncturing one or both gills with a 1-mL syringe and sterilized 18-gauge needle and flushing glochidia from the gills with water into a beaker. In Experiment 3, we used aquaria to immerse mussels in a water bath with serotonin (23 mg/L) for 2–3 hours (Eads et al. 2010; Patterson et al. 2018) to induce the release of glochidia and avoid gill trauma associated with gill punctures. Glochidia from the serotonin bath were collected on a 150-µm screen and then resuspended in water in a beaker.

We determined glochidia viability for each mussel by evenly suspending glochidia in a 1000-mL beaker and collecting five 200-µL subsamples with a pipette. We placed all five subsamples together in a petri dish, added a sodium chloride (NaCl) solution, and under a dissecting microscope counted the number of open and closed glochidia before and after exposure to NaCl. We calculated glochidia viability as:

$$\text{Glochidia viability} = \frac{(\text{No. open glochidia - No. open glochidia after NaCl})}{\text{No. total glochidia}} X\ 100$$

Glochidia viability across all broodstock individuals was 88–93%; based on consistently high viability we used all broodstock in the experiments (see Hove et al. 2000). For each experiment,

we combined glochidia from all broodstock, evenly suspended the glochidia, and then divided the total volume into equal stock solutions for each replicate inoculation based on target inoculation densities (see subsequent). We decanted water in each stock solution until there was only enough water to suspend glochidia in a petri dish then photographed the petri dish containing the glochidia with a digital camera and macro lens (5D Mark 3S camera, 100 mm f2.8/L Macro IS USM Lens, Canon U.S.A. Inc., Huntington, New York). Photographing allowed us to count glochidia added to each inoculation bath, resulting in a more accurate quantification of glochidia than volumetric estimates alone; these numbers were used to calculate attachment rates.

Experiment 1

In Experiment 1, we tested the host suitability of three fish species: Slimy Sculpin, Longnose Dace, and Atlantic Salmon. We inoculated Slimy Sculpin (mean length = 72 mm ± 5.0 SD) and Longnose Dace (87 mm ± 7.0) by placing six individuals of each species in 200 mL of water in a McDonald-type hatching jar (similar to those produced by Global Aquaculture Supply Co., Sioux Falls, South Dakota; hereafter, McDonald jar). Our target inoculation density was 200 glochidia/fish; however, counts of glochidia in photographs indicated true inoculation densities of 121 glochidia/fish (3.64 viable glochidia/mL; Table 1) for Slimy Sculpin and 150 glochidia/fish (4.50 viable glochidia/mL; Table 1) for Longnose Dace. Air injected into the bottom of the McDonald jars suspended the glochidia, facilitating glochidia contact with fishes. We exposed fishes for 20 minutes, removed the fish, and then filtered the water over a 150-µm mesh sieve to collect unattached glochidia. We counted the number of unattached glochidia and subtracted this number from the estimated number of glochidia in the inoculation bath to

estimate the attachment rate (the percentage of viable inoculated glochidia that attached to each fish; Table 2).

Atlantic Salmon (mean length = 180 mm ± 1.0 SD) were too large for the McDonald jars; therefore, we pipetted glochidia directly onto the gills of two individuals. Before inoculating fish, we photographed the petri dish containing the glochidia that we pipetted onto the gills of each fish. We anesthetized fish with tricaine methanesulfonate (MS 222) and pipetted the entire glochidia stock solution onto the left or right gills to obtain a target inoculation density of 300 glochidia/fish. We conducted the inoculation over a tray to collect unattached glochidia, and we counted glochidia in the tray to estimate the number of glochidia that attached to each fish by subtracting the number counted in the tray from the number counted in the photographs (Table 1).

After inoculation, we placed Slimy Sculpin and Longnose Dace in 3-L Aquatic Habitat (AHAB) tanks (Pentair Aquatic Ecosystems, Apopka, Florida), for a total of three tanks/species (two individuals/tank). We placed individual Atlantic Salmon in separate 9-L AHAB tanks. We inspected the contents of each tank every 1–3 days, beginning the day after inoculation. We collected sloughed glochidia or juveniles by increasing the flow in the AHAB tanks for 10 minutes and collecting flushed material on a 150-µm filter. We placed sloughed glochidia or juveniles from each tank and collection event in a petri dish and counted glochidia and juveniles under a dissecting microscope. Starting day seven post-inoculation, most juveniles exhibited a foot and two adductor muscles but lacked movement; thus, we left material in petri dishes overnight at room temperature (~18°C) and inspected it the next morning. Mussels that exhibited foot movement the next morning were considered metamorphosed juvenile mussels, and all other individuals were considered sloughed glochidia. We estimated the metamorphosis rate of

attached glochidia by dividing the total number of live juveniles recovered from tanks by the total number of glochidia collected from tanks (Rogers et al. 2001).

If no juveniles were collected after five days, we inspected a subsample of fish and if no glochidia were attached we terminated the experiment. We sacrificed all fishes at the completion of all experiments and inspected the fishes under a compound microscope for remaining glochidia. The duration of the experiments was 37– 40 days. Using room-controlled temperature we slowly increased the water temperature in the AHAB tanks from 13°C to 19°C (average rate = 1°C/day for 6 days) to facilitate glochidia metamorphosis. The initial AHAB temperature (13°C) was chosen to reduce thermal stress during transfer of glochidia and fishes from the holding and inoculation chambers. We measured dissolved oxygen in a subset of the AHAB tanks every three days with a YSI Professional Plus multiparameter water quality meter (Xylem, Inc., Yellow Springs, Ohio); dissolved oxygen was >7.0 mg/L for all measurements.

Experiment 2

In Experiment 2, we retested Slimy Sculpin (mean length = 72 mm ± 10 SD) and Longnose Dace (72 mm ± 11) using different individuals than in Experiment 1 and tested five new fish species: Blacknose Dace (mean length = 67 mm ± 7 SD), Banded Killifish (75 mm ± 9), Bluegill (77 mm ± 2), White Sucker (122 mm ± 5), and Brook Trout (375 mm ± 109). We inoculated 12 individuals each of Longnose Dace, Blacknose Dace and Banded Killifish, with each species divided into two replicate inoculations in separate McDonald jars with six individuals/jar. We inoculated six Slimy Sculpin together in a single McDonald jar. We inoculated three White Sucker and four Bluegill, with each species in a single McDonald jar. Water volume in all McDonald jars was 250 mL (50 mL higher than in Experiment 1).

Inoculation methods and duration in McDonald jars were as described for Experiment 1 using a McDonald jar.

Our target inoculation densities were 250 glochidia/fish for Longnose Dace, Blacknose Dace, Banded Killifish, and Slimy Sculpin; 300/fish for White Sucker; and 200/fish for Bluegill. Photographic counts indicated that inoculation densities differed slightly from our targets (Table 1). For example, replicate inoculations for Longnose Dace contained 1,284 viable glochidia (214 glochidia/fish; 5.14 viable glochidia/mL; Table 1) and 1,338 viable glochidia (223 viable glochidia/fish; 5.36 viable glochidia/mL; Table 1).

We inoculated Brook Trout together in a single bucket with 23 fish in 4000 mL of water. We exposed fish for 20 minutes, removed the fish, and then filtered the water over a 150-µm mesh sieve to collect unattached glochidia. Our target inoculation density was 1,000 glochidia/fish, but photographic counts indicated a density of 743 glochidia/fish (4.27 viable glochidia/mL).

After inoculations, we separated fishes into AHAB tanks that consisted of three 3-L tanks for Blacknose Dace (4 fish/tank), Longnose Dace (4 fish/tank), Banded Killifish (4 fish/tank), Slimy Sculpin (2 fish/tank), and White Sucker (1 fish/tank). We placed Bluegill (2 fish/tank) into two replicate 3-L tanks and Brook Trout into one 260-L circular tank.

We collected glochidia and juvenile mussels from AHAB tanks following methods described for Experiment 1. We collected glochidia and juveniles from the Brook Trout tank by siphoning 60 L of water from the tank bottom with a 2-cm hose every 1–3 days; we collected siphoned material on a 150-µm filter. We estimated metamorphosis rate and measured dissolved oxygen as described for Experiment 1. Experiment 2 ended on days 24–34.

205 **Experiment 3**

206 In Experiment 3, we tested new individuals of Brook Trout (mean length = 145 mm ± 13
207 SD), Rainbow Trout (146 mm ± 11), and Brown Trout (140 mm ± 10). We inoculated fishes
208 with glochidia following methods described for Brook Trout in Experiment 2, except that we
209 inoculated each fish species in three replicate inoculation baths, each with 15 individuals. Our
210 target inoculation density was 200 glochidia/fish, but photographic counts indicated densities of
211 200–315 glochidia/fish (0.75–1.18 viable glochidia/mL; Table 1). We calculated glochidia
212 attachment rate as in Experiments 1 and 2.

213 After inoculations, we transferred fishes from each bath into a 113-L circular tank with
214 flow-through well water; we used three replicate tanks for each species, each containing 15
215 individuals. Unlike in Experiments 1 and 2, for Experiment 3 we kept all fish from each replicate
216 inoculation bath in the same holding tank throughout the experiment, which allowed us to
217 examine the relationship between attachment rate and metamorphosis rate. We increased the tank
218 temperature from 15°C to 18°C using a heater (average increase = 1°C/day). We inspected tanks
219 for glochidia and juveniles as described for Brook Trout in Experiment 2. We estimated
220 metamorphosis rate as described for Experiment 1 and measured dissolved oxygen daily.
221 Experiment 3 lasted 25 days.

222

223 **Data Analysis**

224 We created sets of generalized linear models (GLM) to assess how well attachment rate
225 (Experiment 3) and metamorphosis rate (Experiment 1 and 3) were predicted by various factors.
226 We did not assess metamorphosis rate for Experiment 2 because of high fish mortality resulting
227 in insufficient replication for analysis. For Experiment 1, we created a model to assess how well

metamorphosis rate was predicted by host species (fixed factor). We excluded Atlantic Salmon from these models because of insufficient replication. For Experiment 3, we compared models to assess how well attachment rate was predicted by host species and inoculation density (number of viable glochidia/mL in the inoculation bath) individually, and when both factors were modeled together as an additive term (Table 3). For Experiment 3, we also created models to assess how well metamorphosis rate was predicted by host species and attachment rate, individually and together. For this model, we expressed attachment rate as the number of glochidia attached to the fish.

For each experiment, we created a separate model for each factor or combination of factors and included a null model (a model with no explanatory factors; Table 3). We fit all models with a logit link function and a quasi-binomial error structure; this error structure accounted for overdispersion that resulted from clustering in the data. We evaluated models by fitting them twice: we first extracted the log-likelihood from the binomial model, and then we extracted the dispersion parameter from the quasi model to calculate the likelihood; these were used to calculate a quasi-corrected Akaike Information Criterion (qAIC) (Bolker 2021). We calculated explained deviance by subtracting the residual deviance from the null deviance and dividing by the null deviance (Zuur et al. 2015). We selected the best model as the most parsimonious model with high explained deviance and low qAIC (Burnham and Anderson 2004; Wagenmakers and Farrell 2004). We contrasted marginal means using 95% confidence intervals to compare fixed factors in models, and we back-transformed standard error intervals from the logit scale using package ‘emmeans’ (Length et al. 2022; R package version 1.6.0.). All data analyses and models were created in R v4.0.2 software package (R Core Team 2020, Vienna, Austria).

RESULTS

Experiment 1

Glochidia attachment rate was high for all fish species (range = 78.1–84.0%, Table 2). For Slimy Sculpin and Longnose Dace, most sloughed glochidia appeared within five days of inoculation (Fig. 1). For Atlantic Salmon, large numbers of sloughed glochidia appeared within the first five days, but this was followed by another peak shortly before juveniles began to appear on day 15 (Fig. 1).

Mean metamorphosis rate of attached glochidia varied by host species and was highest for Slimy Sculpin (80.9% ± 2.6 SD), followed by Atlantic Salmon (35.2% ± 13.7) and Longnose Dace (29.1% ± 21.9) (Table 2). Metamorphosis rate was similar across the three replicates for Slimy Sculpin, but it varied for Atlantic Salmon and Longnose Dace (Fig. 2). Production of juveniles on Slimy Sculpin and Longnose Dace began on days 17 and 15, respectively, and Slimy Sculpin peaked on day 24; production of juveniles on Longnose Dace did not indicate a clear peak (Fig. 1). Juvenile production on Atlantic Salmon began on day 15 but appeared to occur over a more protracted period with no distinct peaks.

Fish species was a good predictor of metamorphosis rate. When comparing modeled probability of metamorphosis using 95% confidence intervals among fish species, Slimy Sculpin had a higher probability (0.81; 95% confidence interval = 0.57–0.93) than Longnose Dace (0.22; 95% confidence interval = 0.09–0.43) ($P < 0.05$); this model explained 79.5% of the deviance.

Experiment 2

Attachment rate varied among fish species (Table 2). The lowest attachment rate of glochidia was on Bluegill (51.0%) and the highest was on Brook Trout (80.3%), with the other species having attachment rates of 61.1–77.6%. Sloughed glochidia appeared mostly in the first five days after inoculation for all species except for Brook Trout, which sloughed glochidia until day 10 (Fig. 3).

Metamorphosis rate varied greatly among fish species and was highest for Brook Trout (71.6%) and Slimy Sculpin (72.6% ± 5.2 SD) and lowest for Bluegill (4.9%) (Table 2). Metamorphosis rate was similar across the three replicates for Slimy Sculpin, but it varied among replicates for all other species (Fig. 2). Production of juvenile mussels began on days 10–13 for all species except Bluegill, from which one juvenile appeared on day 24. Production of juvenile mussels peaked on day 11 for Brook Trout and on days 20 and 21 for Slimy Sculpin and Banded Killifish. Juvenile production from fish species that had a low metamorphosis rate (i.e., Longnose Dace, Blacknose Dace, White Sucker) did not display conspicuous peaks (Fig. 3), and Bluegill produced only a single juvenile.

Experiment 3

Attachment rate was similarly high among the three trout species (83.2–84.6%, Table 2). Sloughed glochidia appeared mostly before day 11 for Brook Trout and Brown Trout and before day 7 for Rainbow Trout (Fig. 4).

Metamorphosis rate varied widely among species and was highest for Brown Trout and lowest for Rainbow Trout (Table 2), but metamorphosis was similar among replicates for all three species (Fig. 2). Production of juvenile mussels began on days 11–12 for all three species

and peaked on day 12 for Brook Trout and days 14–16 for Brown Trout and Rainbow Trout (Fig. 4).

The top model for predicting glochidia attachment included host species + inoculation density and explained 54.9% of the deviance (Table 3). In the top model, contrasts among attachment rates for host species did not differ ($P > 0.05$), and inoculation density alone was only a marginally significant factor ($P = 0.07$). The model including only host species explained 8.3% of the deviance, and the model including only inoculation density explained 28.2% of the deviance. Overall, models with host species + inoculation density and inoculation density alone were within two qAIC units of the null model, and thus models were not considered strong predictors of glochidia attachment.

The top model for predicting glochidia metamorphosis contained host species only, explained 98.7% of the deviance, and had the lowest qAIC (Table 3). Brown Trout had the highest probability of metamorphosis (0.62 ± 0.02 SD), followed by Brook Trout (0.13 ± 0.02; $P < 0.001$) and Rainbow Trout (0.06 ± 0.01; $P < 0.001$).

DISCUSSION

In our experiments, Brook Floater metamorphosed on all 10 fish species tested, which represented six fish families. Our study was the first to observe metamorphosis on Banded Killifish and the first to test salmonids. Our results support previous categorizations of the Brook Floater as a host generalist (Eads et al. 2007; Wicklow et al. 2017; Table 4). The hooked glochidia of the tribe Anodontini may contribute to their ability to use multiple host species by allowing them to attach to skin, fins, and gills (Bauer 1994; Barnhart et al. 2008). High attachment rates (51.0–84.6% in our experiments) may offset their passive host infection strategy

in which females produce glochidia in mucus strands to entangle potential hosts (Wicklow et al. 2017). Host generalists are largely restricted to the tribe Anodontini; adults of most mussel species in other tribes have specialized adaptations to lure a particular host species or feeding guild, and their glochidia attach mainly to fish gills (Haag 2012).

Slimy Sculpin had the highest glochidia metamorphosis rate, similar to a previous study of Brook Floater host use in New Hampshire (Wicklow et al. 2017; Table 4). Fishes from the family Cottidae are potential hosts for other *Alasmidonta* including the Slippershell (*Alasmidonta viridis*; Zale and Neves 1982), Dwarf Wedgemussel (*Alasmidonta heterodon*; Michaelson and Neves 1995; White et al. 2017), and Elktoe (*Alasmidonta marginata*; Bloodsworth et al. 2013).

Our results about the relative suitability as hosts of other fishes varied in their agreement with the results of previous studies. Longnose Dace was a better host in New Hampshire (51% metamorphosis; Wicklow et al. 2017) than in our study (29.1% and 24.5% in Experiments 1 and 2, respectively). Metamorphosis on White Sucker was similar in our study and in New Hampshire (22.3%, and 26%, respectively; Wicklow et al. 2017). Blacknose Dace supported glochidia metamorphosis in all three studies, but the metamorphosis rate was low (6%) in New Hampshire (Wicklow et al. 2017) and North Carolina (four juveniles produced; Eads et al. 2007, metamorphosis rate not reported) but higher in our study (23.4%). Cutlip Minnow (*Exoglossum maxillingua*) may be a host to test in future experiments since we commonly observed this species at one of our broodstock collection sites.

The most conspicuous difference in host use in our study and previous studies involved Bluegill. Bluegill produced the highest number of juveniles of any fish species tested in North Carolina in one experiment (184 juveniles produced; Eads et al. 2007, metamorphosis rate not reported), but in another North Carolina experiment Bluegill produced no juveniles (Eads et al.

2007) and it produced only one juvenile in our study. Wicklow et al. (2017) did not test Bluegill. The poor production of juveniles on Bluegill in our study may have been due to high fish mortality, warranting additional tests on Bluegill in Massachusetts.

Variability in metamorphosis rate in our study may be explained by the source of broodstock and the timing of broodstock collection. Glochidia from genetically distinct populations of the same mussel species may vary in their ability to metamorphose on host fishes (evaluated through glochidial retention in the first 96 hours; Douda et al. 2014). Because of the small extant Brook Floater populations in Massachusetts, we were unable to collect all mussel broodstock from one location. Genetic differences between the three populations from which we obtained broodstock, and how they might influence host use, are unknown. Genetic information is also critical for informing decisions on where to collect broodstock for propagation to maintain genetic integrity during population augmentation (Jones et al. 2006; McMurray and Roe 2017; Lane et al. 2019). Finally, for Experiment 3, we collected glochidia from broodstock in the fall (October) instead of the spring, as in Experiment 1 (March) and Experiment 2 (April). It is unknown if the length of time that glochidia were brooded by the female mussel affected metamorphosis rate.

The source of host fish also may explain variability in metamorphosis rates between experiments. Brook Trout in Experiment 2 were a mix of wild F1 and F2 generations, whereas Brook Trout in Experiment 3 originated from a domesticated Sandwich strain raised in outdoor raceways at a hatchery; the two experiments resulted in vastly different rates of metamorphosis (71.6% in Experiment 2 vs 12.8% in Experiment 3). The Brook Trout Sandwich strain is registered with the National Fish Strain Registry and was developed at a state fish hatchery in Montague, Massachusetts from wild fish (Kincaid et al. 2002; Annett et al. 2012). If stocked

hatchery-strain trout displace wild-strain fish, the overall recruitment rate of Brook Floater could decrease because hatchery-raised fish can act as glochidia sinks (Salonen et al. 2016). Further assessment of differences in attachment and metamorphosis rates among fishes of different origins may expand our understanding of mussel-host relationships and provide important information for propagation programs.

Lastly, inoculation density can affect the metamorphosis rate. In the Paper Pondshell (*Utterbackia imbecillis*), higher inoculation densities (2,000-8,000 glochidia/L vs 1,000/L) resulted in higher mean metamorphosis rates (79.9% vs. 48.8%); this was attributed to increased host plasma cortisol levels and decreased fish immunity (Dubansky et al. 2011). However, another study found no relationship between inoculation densities (1,000, 4,000, and 8,000 glochidia/L) and metamorphosis rate for the Fatmucket (*Lampsilis siliquoidea*; Douda et al. 2018). In our Experiment 3, the number of glochidia that attached to fishes was not a good predictor of metamorphosis rate; rather, fish species was the most important factor in predicting Brook Floater metamorphosis. Similarly, we did not see an effect of inoculation density on glochidia attachment, although the narrow range we tested (0.75–1.18 viable glochidia/mL) limited our ability to evaluate density. Host fish species were not important in predicting glochidia attachment (only tested in Experiment 3); this is unsurprising because we tested species with relatively similar morphologies within the same family (Salmonidae). Host species may have a greater effect on glochidia attachment when testing fishes across families with varied morphologies.

Laboratory host studies are important for affirming fish species as physiological hosts (i.e., that can facilitate glochidia metamorphosis), but they do not confirm them as ecological hosts that are important in nature (Levine et al. 2012). To serve as a host in the wild, the habitat

of the fish and mussel must overlap and the mussels' mode of glochidia transfer must be compatible with the fishes' feeding or movement behavior (Barnhart et al. 2008). The only host for Brook Floater confirmed by both laboratory and field studies is the Margined Madtom in New Hampshire; glochidia were found on this species in the wild, and wild fish brought into the laboratory produced juveniles (Wicklow et al. 2017). However, the Margined Madtom is not native north of Connecticut (Page and Burr 1991) and is thought to have been introduced to New Hampshire in the 1930s (Hartel et al. 2002), indicating that Brook Floater glochidia can use non-native fish species as hosts in the wild. Brook Floater glochidia were found attached to Ninespine Stickleback (*Pungitius pungitius*) in New Brunswick, Canada, but glochidia inoculations in a laboratory are needed to confirm whether this fish can produce juveniles (Beaudet 2006 *in* Department of Fisheries and Oceans Canada 2016).

Cost-effective captive propagation requires selecting a host species that produces consistently high metamorphosis rates yet is easily procured in large numbers and maintained in captivity. Slimy Sculpin produced the highest metamorphosis rates in our study, but obtaining sculpins is dependent on suitable conditions for collection in streams, and these conditions may not coincide with availability of mussel broodstock. Furthermore, removing large numbers of sculpins from the wild may negatively affect those populations. Hatchery-reared Brook Trout from wild F1 and F2 generations produced a metamorphosis rate nearly as high as Slimy Sculpin (Experiment 2). The ability to easily procure large numbers of hatchery-reared Brook Trout could make them a cost-effective choice for large-scale propagation of Brook Floater in the northeastern U.S.; however, care must be taken to select hatchery strains that produce high metamorphosis. Brown Trout also produced relatively high metamorphosis rates, but they produced copious mucus and shed scales that entangled juvenile Brook Floater, which increased

411 the time needed to harvest juveniles. Furthermore, use of a non-native host species like Brown
412 Trout presents a potential for undesirable hatchery selection. These considerations highlight the
413 need to evaluate various fish species, sources, and other factors when selecting an optimal host
414 fish for captive mussel propagation.
415
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566 **FIGURE LEGENDS**

567

568 **Figure 1.** Number of sloughed glochidia or juvenile mussels produced by Brook Floater
569 (*Alasmidonta varicosa*) in Experiment 1. Data points and bars represent the mean and standard
570 deviation, respectively, among replicate fish holding chambers on each day standardized by the
571 number of fish in each chamber.

572 **Figure 2.** Juvenile metamorphosis rate (number of juveniles/number of glochidia) of Brook
573 Floater (*Alasmidonta varicosa*) on fishes in three experiments. Replicates refer to individual fish
574 holding chambers. Numbers above each bar refer to the number of fish in each chamber that
575 survived (left number) out of the initial number inoculated (right number).

576 **Figure 3.** Number of sloughed glochidia or juvenile mussels produced by Brook Floater in
577 Experiment 2. Data points and bars represent the mean and standard deviation, respectively,
578 among replicate fish holding chambers on each day standardized by the number of fish in each
579 chamber.

580 **Figure 4.** Number of sloughed glochidia or juvenile mussels produced by Brook Floater in
581 Experiment 3. Data points and bars represent the mean and standard deviation, respectively,
582 among fish holding chambers on each day standardized by the number of fish in each chamber.

Table 1. Inoculation methods for three host identification experiments for Brook Floater (*Alasmidonta varicosa*). Fish species without entries under "Replicate" were held in a single chamber. Water volume is the volume of the inoculation bath. The stock solution represents the glochidia solution used to inoculate fishes. The target inoculation density was determined volumetrically. The actual inoculation density and stock solution glochidia density were determined later by counting glochidia in photographs of the inoculation stock to which fishes were exposed (see text). Scientific names for fishes are in Table 4.

Species	Replicate	Inoculation method	Water volume (mL)	Stock solution glochidial density (viable glochidia/mL)	Target inoculation density (glochidia/fish)	Actual inoculation density (glochidia/fish)
Experiment 1						
Slimy Sculpin		McDonald	200	3.64	200	121
Longnose Dace		McDonald	200	4.50	200	150
Atlantic Salmon		Direct	n/a	n/a	300	326
Experiment 2						
Slimy Sculpin		McDonald	250	5.73	250	239
Longnose Dace	A	McDonald	250	5.14	250	214
	B	McDonald	250	5.36	250	223
Blacknose Dace	A	McDonald	250	4.72	250	197
	B	McDonald	250	4.45	250	185
Banded Killifish	A	McDonald	250	4.55	250	190
	B	McDonald	250	4.80	250	200
White Sucker		McDonald	250	2.82	300	235
Bluegill		McDonald	250	1.97	200	123
Brook Trout		Bucket	4000	4.27	1000	743
Experiment 3						
Brook Trout	A	Bucket	4000	1.01	200	270
	B	Bucket	4000	0.75	200	200
	C	Bucket	4000	0.81	200	217
Brown Trout	A	Bucket	4000	0.93	200	247
	B	Bucket	4000	0.87	200	232
	C	Bucket	4000	1.18	200	315
Rainbow Trout	A	Bucket	4000	1.12	200	299
	B	Bucket	4000	0.84	200	224
	C	Bucket	4000	0.90	200	241

Table 2. Glochidia attachment rates and juvenile metamorphosis rates of Brook Floater (*Alasmidonta varicosa*) on fishes in three experiments. Attachment rate is the percentage of inoculated glochidia that attached to fishes. Metamorphosis rate is the percentage of attached glochidia that metamorphosed into juvenile mussels. Average juveniles/fish is based on the daily number of juveniles produced/the number of fish surviving, summed across experimental days. Mean values and SD were calculated only from replicate chambers in which fishes survived to produce juvenile mussels (see Fig. 2). Scientific names for fishes are in Table 4.

Experi-ment	Fish species	% Attachment		% Metamorphosis		Avg. juveniles/ fish	No. fish inoculated	No. fish survivors
		Mean	SD	Mean	SD			
1	Slimy Sculpin	79.7		80.9	2.6	203	6	6
1	Longnose Dace	84.0		29.1	21.9	67	6	6
1	Atlantic Salmon	78.1		35.2	13.7	69	2	1
2	Longnose Dace	61.1		24.5	6.7	70	12	4
2	Blacknose Dace	77.6		16.9	9.1	9	12	1
2	Banded Killifish	64.1		43.0	34.2	44	12	4
2	Slimy Sculpin	75.1		72.6	5.2	301	6	5
2	White Sucker	64.7		22.3	12.9	23	3	3
2	Bluegill	51.0		4.9		1	4	1
2	Brook Trout	80.3		71.6		342	23	23
3	Brook Trout	83.2	2.3	12.8	0.3	67	45	45
3	Brown Trout	84.6	0.4	62.1	6.7	316	45	45
3	Rainbow Trout	83.5	4.7	5.7	0.4	31	45	45

Table 3. Results of generalized linear models (GLMs) assessing factors that predict Brook Floater glochidia attachment and juvenile metamorphosis rates in Experiment 3. Inoculation density is the number of viable glochidia/mL to which fishes were exposed (see Table 1). Attachment is the estimated number of glochidia attached/fish calculated as the chamber-wide attachment rate divided by the number of fish in the chamber. The top models are in bold.

Model	Δquasi-AIC	Explained deviance (%)	df
Attachment			
Inoculation density	0	28.2	2
Host species + Inoculation density	**1.0**	**54.9**	**4**
Null	1.1	0.0	1
Host species	4.2	8.3	3
Metamorphosis			
Host species	**0**	**98.7**	**3**
Host species + Attachment	1.5	98.8	4
Attachment	433.1	7.2	2
Null	465.4	0	1

Table 4. Summary of glochidia metamorphosis of Brook Floater observed on fishes in three studies.

Fish species		Metamorphosis		
Family, common name	Scientific name	Yes	No	Study
Ictaluridae				
Brown Bullhead	*Ameiurus nebulosus*	■		Wicklow et al. 2017
Catostomidae				
White Sucker	*Catostomus commersonii*	■		this study, Wicklow et al. 2017
White Sucker (adult)	*Catostomus commersonii*		■	Wicklow et al. 2017
Centrarchidae				
Bluegill	*Lepomis macrochirus*	■	■	Eads et al. 2007*, this study
Largemouth Bass	*Micropterus salmoides*		■	Wicklow et al. 2017
Mixed Sunfish	*Lepomis spp.*	■		Eads et al. 2007
Pumpkinseed	*Lepomis gibbosus*	■		Wicklow et al. 2017
Redbreast Sunfish	*Lepomis auritus*	■		Eads et al. 2007
Redbreast Sunfish	*Lepomis auritus*		■	Wicklow et al. 2017
Smallmouth Bass	*Micropterus dolomieu*		■	Wicklow et al. 2017
Cottidae				
Mottled Sculpin	*Cottus bairdii*	■		Eads et al. 2007
Slimy Sculpin	*Cottus cognatus*	■		this study, Wicklow et al. 2017
Cyprinidae				
Blacknose Dace	*Rhinichthys atratulus*	■		Eads et al. 2007, this study, Wicklow et al. 2017
Common Carp	*Cyprinus carpio*		■	Wicklow et al. 2017
Common Shiner	*Luxilus cornutus*	■		Wicklow et al. 2017
Fallfish	*Semotilus corporalis*	■		Wicklow et al. 2017
Golden Shiner	*Notemigonus crysoleucas*	■		Wicklow et al. 2017
Highfin Shiner	*Notropis altipinnis*		■	Eads et al. 2007
Longnose Dace	*Rhinichthys cataractae*	■		this study, Wicklow et al. 2007
White Shiner	*Luxilus albeolus*	■		Eads et al. 2007
Whitemouth Shiner	*Notropis alborus*		■	Eads et al. 2007
Fundulidae				
Banded Killifish	*Fundulus diaphanus*	■		this study
Ictaluridae				
Margined Madtom	*Noturus insignis*		■	Eads et al. 2007
Margined Madtom	*Noturus insignis*	■		Wicklow et al. 2017
Percidae				
Fantail Darter	*Etheostoma flabellare*	■		Eads et al. 2007
Johnny Darter	*Etheostoma nigrum*	■		Eads et al. 2007
Piedmont Darter	*Percina crassa*	■		Eads et al. 2007
Roanoke Darter	*Percina roanoka*	■		Eads et al. 2007
Tessellated Darter	*Etheostoma olmstedi*		■	Eads et al. 2007
Tessellated Darter	*Etheostoma olmstedi*	■		Wicklow et al. 2017
Yellow Perch	*Perca flavescens*	■		Wicklow et al. 2017

Salmonidae			
Atlantic Salmon	*Salmo salar*		this study
Brook Trout	*Salvelinus fontinalis*		this study
Brown Trout	*Salmo trutta*		this study
Rainbow Trout	*Oncorhynchus mykiss*		this study

* Eads et al. 2007 found conflicting results from two host trials including Bluegill

Figure 1

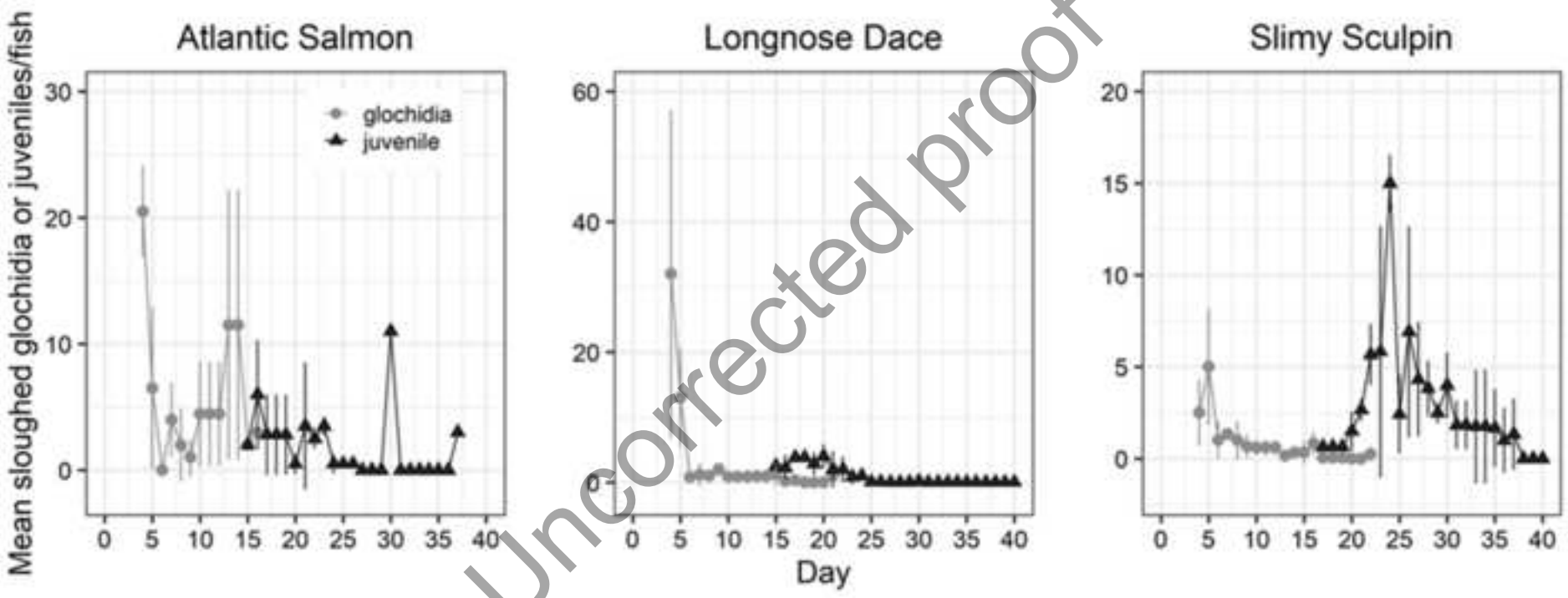

Figure 2

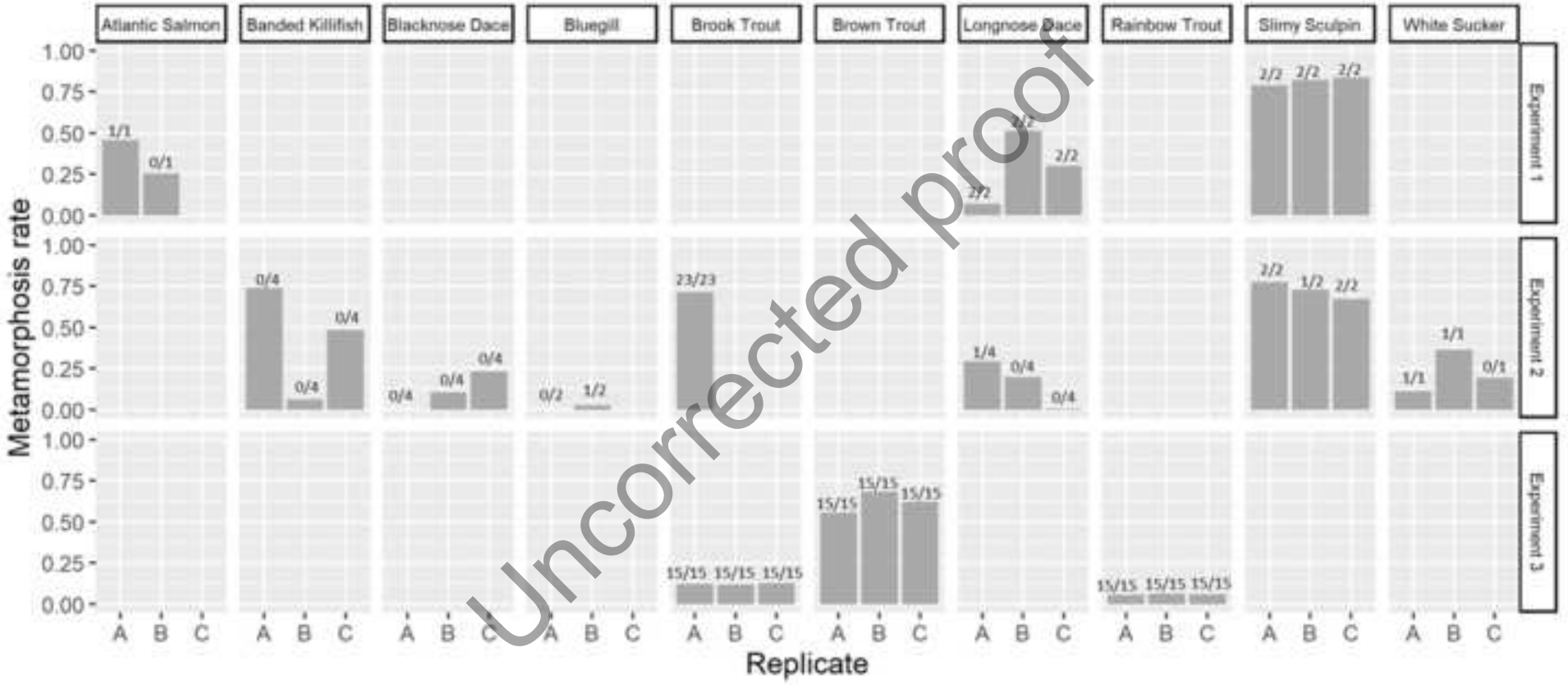

Figure 3

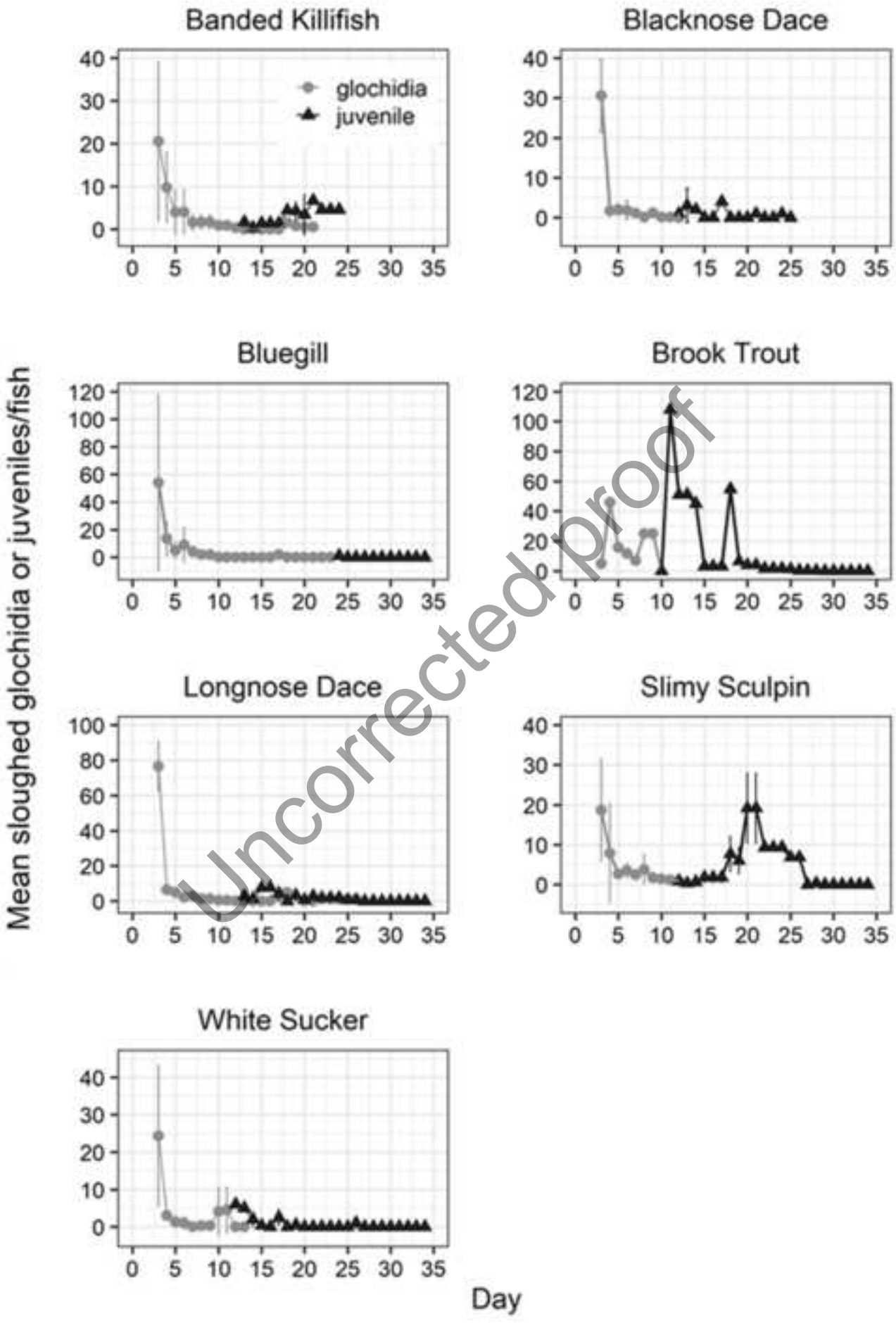

Figure 4