



Abstract

Freshwater mussels of the family Unionidae are native to the Eastern U.S. often found in rivers and small tributaries. They have a complex life cycle which requires a fish host for the development of larvae into juveniles. The larval mussel form, called glochidia, are released into the water by the process of dispersal which is the use of a lure to attract fish. Many of the fish hosts are unknown, but this study attempts to determine possible fish hosts for the Paper Pondshell (Utterbackia imbecillis) using three different fish species and twelve individuals. The glochidia were extracted from the mussel using a hypodermic needle and flushed over the gills of each fish. The fish were left in tanks for three weeks with water changes every other day. The tanks were siphoned through a 150-micrometer sieve and all contents were examined under a microscope to identify any juveniles. Results suggest that Specklebelly Sunfish, Bluegill, and Hybrid Bluegill are all confirmed hosts for the Paper Pondshell. Further experiments should be conducted to further confirm these hosts as well as identify the most effective fish host species. Finding host fish for the Paper Pondshell is important for efficient conservation strategies.

Introduction

Freshwater mussels of the family Unionidae have a complex life cycle which requires a fish host for the development of larvae into juveniles. The veliger larvae, glochidia, are stored in the gill pouch of a female mussel. Female mussels have evolved so the gill pouch storing the glochidia resembles a minnow or worm, called a lure, to entice a host fish. When the host fish bites down on the lure, the membrane of the lure is broken causing the glochidia to be released into the mouth of the fish and the glochidia then attach to the gills.

The glochidia stay attached to the gills of a suitable fish host for several weeks. During this time, the glochidia envelope into a cyst on the gills to metamorphosize. After developing from glochidia to juvenile mussels for several weeks, the newly developed juvenile mussels fall off the gills into the sediment.





Figure 1: Paper Pondshell (*Utterbackia imbecillis*)

Figure 2: Freshwater mussel lifecycle

The mussel reproduction cycle can be replicated in a lab setting, where glochidia are extracted from gravid female mussels and flushed over the gills of fish. The juveniles are raised into adults and later released into the wild. Mussel populations have been negatively impacted due to anthropogenic activity in freshwater ecosystems, so this process is important to the conservation of freshwater mussels to help bolster wild populations (Haag 2003).

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Methods

Gravid female mussels from Kentucky Fish and Wildlife in Frankfort, Kentucky were used to extract glochidia. The glochidia was flushed from the gills of the Utterbackia imbecillis using a hypodermic needle and filtered through a 150 μ m net until a concentrated sample was obtained.



Figure 3: Flushing glochidia out of Utterbackia imbecilis.



Figure 4: Infecting the gills of each fish with glochidia.

Twelve individuals within the *Lepomis* family were acquired from Jones Fish Hatchery for infection. Before infecting the fish, they were anesthetized approximately 1-2 minute under ms222. The operculum was then held open to efficiently flush 0.25mL of glochidia (approximately 437 individuals) on the gills. To avoid potential damage to both sides of the fish's gills, we only flushed one side.



Figure 8: Juvenile Utterbackia imbecilis.



Figure 7: Image of *Lepomis spp*.

The twelve fish were monitored to determine glochidia attachment over the course of two to three weeks. The tanks were siphoned each day to inspect any detritus for juveniles that had fallen off the fish host. The number of juveniles off each host was recorded and the tanks were monitored until no juveniles were found for three days in a row.

Results

During the study, the tanks were siphoned every day and the contents were observed under a dissecting scope. The goal was to find and count any juvenile mussels. Table 1 shows the species used, the date that the fish were infected, if attachment occurred, how long the attachment lasted, the time span it took for juveniles to finish falling off, and the total number of juveniles found off each fish. The results of the experiment show that the fish-host and mussel interaction occurred on all three species, Lepomis spp., Lepomis cyanellus x Lepomis machrochirus, and Lepomis macrochirus. Based on the collected data, we can confirm three host species for Utterbackia *imbecillis*.





Figure 5: Individual tank set up.



Glochidia under microscope

Tank	Species	Infecto

Tank	Species	Infected	Last day of Dropping	Attachment	Days of Juvenile Dropping	Total Number of Juveniles
1	Lepomis spp.	6/21/2021	7/2/2021	Y	4	20
2	Lepomis spp.	6/21/2021	7/2/2021	Y	4	23
3	Lepomis spp.	6/21/2021	7/2/2021	Y	4	11
4	Lepomis spp.	6/21/2021	7/2/2021	Y	4	9
5	Lepomis cyanellus x Lepomis macrochirus	6/21/2021	7/2/2021	Y	1	2
6	Lepomis cyanellus x Lepomis macrochirus	6/21/2021	7/6/2021	Y	5	29
7	Lepomis cyanellus x Lepomis macrochirus	6/21/2021	7/2/2021	Y	4	26
8	Lepomis cyanellus x Lepomis macrochirus	6/21/2021	7/2/2021	Y	4	26
9	Lepomis macrochirus	6/21/2021	7/2/2021	Y	4	14
10	Lepomis macrochirus	6/21/2021	7/2/2021	Y	4	18
11	Lepomis macrochirus	6/21/2021	7/2/2021	Y	4	23
12	Lepomis macrochirus	6/21/2021	7/1/2021	Y	3	20

Table 1: Results of experiment. Showing the species used, date the fish were infested, if attachment of glochidia occurred, how long attachment lasted, the time span it took for juveniles to fall off, and the total number of juveniles found fallen from each fish.

All the fish used in the experiment dropped at least two live, juvenile mussels. The average number of live, juvenile mussels dropped per fish was 18.4 mussels. The Lepomis cyanellus x Lepomis macrochirus hybrid had the greatest output of live juveniles when compared to *Lepomis spp.* and Lepomis macrochirus. One Lepomis cyanellus x Lepomis macrochirus hybrid, though, only dropped two live juveniles over a period of one day, while all the other fish dropped a minimum of nine juvenile mussels over a period of 3-5 days. Despite this outlier included in the data, the Lepomis cyanellus x Lepomis macrochirus hybrid still produced the most juvenile mussels.

The most efficient production method of juvenile paper pondhsell mussels would be to use the *Lepomis cyanellus x Lepomis macrochirus* hybrid as the fish host, though results indicate that the Lepomis spp. and Lepomis macrochirus are viable options as fish hosts for the paper pondhsell as well. Identifying the fish host requirements of freshwater mussels will help lay the foundation for their conservation by providing an efficient reproduction method.



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Results cont.

Conclusion

Literature Cited

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