HOST FISH AND LIFE CYCLE OF THE ROUND PIGTOE MUSSEL (*PLEUROBEMA COCCINEUM*)

by Meggie Marzec 2004

Abstract

Once distributed throughout many Minnesota rivers, the round pigtoe (*Pleurobema coccineum)* has been listed as threatened with danger of becoming endangered in Minnesota. Early life history research is needed to better manage this species. The purpose of my project was to identify host fish for the round pigtoe and identify larval stages of development. Suitable hosts identified in my study can be applied in propagating juveniles for re-establishing round pigtoe populations in their former range. Fish and round pigtoe were collected from the St. Croix River. Fish were infested with mussel larvae and kept in species-specific aquaria. Aquaria were siphoned three times a week and siphonate checked for glochidia or juvenile mussels that had fallen off the potential host fish. I found spotfin shiner, common shiner, and hornyhead chub are suitable host species for the round pigtoe. Creek chub, green sunfish, and golden shiner are marginal suitable hosts. Further studies are needed to determine if they are suitable hosts. I may have discovered a new stage in the life cycle of the round pigtoe. My data suggest that this mussel transforms from glochidia that do not have tissue to glochidia with an additional tissue mass before becoming juveniles. Descriptive anatomical research is needed to better describe the development of round pigtoe larvae.

Introduction

Mussels serve a biological importance by cleaning streams and rivers. Large quantities of heavy metals, pesticides and agricultural residues in the water accumulate in the tissues of the mussel's body and can be potentially toxic to not only the mussels but their predators (1). Sudden die off of mussels should trigger concern for the quality and health of a river system and may adversely affect other wildlife in river systems since mussels serve as an important source of food for many animals such as muskrats and minks (2).

Freshwater mussels have a unique reproductive cycle. Once females receive sperm from males and embryos develop, they are considered gravid. They hold glochidia (larva) in their gills and several species package their glochidia in packets called conglutinates. Females release their glochidia near potential host fish. If the glochidia do not attach to a host, they generally die within 1-2 days. If the fish is a suitable host species, the glochidia attach to the gills of the fish and begin to metamorphose into juveniles (3)(4). Most glochidia metamorphose into juvenile mussels in 6-8 weeks. At this point, the juveniles drop off the gills of host fish and settle to the river bottom. If juvenile mussels drop off host fish onto sand or gravel suitable for growth, they grow into mature adult mussels. If the glochidia fall off fish before they can mature into juveniles, they do not survive (3).

To successfully attach to fish, glochidia act as parasites; the only fish that can be hosts for glochidia are ones whose immune systems do not kill glochidia. Glochidia that fall off from fish before they mature into juveniles have been observed in two forms: some glochidia have an additional tissue mass that is visible with a light microscope, these are considered to be glochidia with tissue. Glochidia that do not have this additional tissue mass are defined as glochidia without tissue (Appendix A). My hypothesis is that these forms are developmental stages where glochidia without tissue mature into glochidia with tissue before metamorphosing into juveniles.

A previous study done in St. Louis, Missouri showed the spotfin shiner, northern redbelly dace, and bluntnose minnow are suitable host fish for the round pigtoe mussel (6)(7). No studies have been done on round pigtoe mussels in more northern waters, and no studies have been done to determine if glochidia without tissue and with tissue are developmental stages in the life cycle of the round pigtoe. The objectives of my project were to first identify suitable host fish for the round pigtoe glochidia and to determine if glochidia without and with tissue are developmental stages.

If the host fish for the round pigtoe mussel and more about the life cycle were known then it may be possible to raise this mussel in the laboratory so that it can be reintroduced to native habitats in the upper St. Croix River in Minnesota. Information gained from my study could also be applied to other closely related species of mussels from the St. Croix River. There are nine species of federally endangered Pigtoe mussels (see Appendix B) that this study could benefit (8). Information from this study could also be applied to the five other species of endangered mussels (see Appendix C) listed in the state of Minnesota that are in the same subfamily, Ambleminae, as the round pigtoe (5).

Procedure

I began by collecting potential host fish (see Appendix D) from the upper St. Croix River in Taylor's Falls, Minnesota, by electrofishing and seining. I identified and separated the fish into tanks by species, suspending the smaller fish in nets. I kept the temperature of the tanks at 18-23 °C, set in-take and out-take tubes so water flowed constantly through the tanks and added an airstone so there would be a constant source of air.

Next, I collected gravid female round pigtoe mussels from the St. Croix River at Taylors Falls, Minnesota. Divers from Macalester College retrieved brooding females, and I brought them to the University of Minnesota. I kept female mussels in individual beakers in 40 L flow-through, aerated aquaria at 18-23°C. After the female mussels released their glochidia, I tested the maturity of the glochidia by exposing a small sample to solid sodium chloride (NaCl) on a microscope slide. If more than 70% of the glochidia closed with addition of NaCl to their environment, I knew the remaining glochidia were mature enough for infestation.

I infested fish by placing them in a bath of glochida. Fish were placed a 3-L container and aerated with five airstones. Several thousand round pigtoe glochidia were then added to the bath. The fishes respired rapidly due to the stress of being contained in a small area, making it easier for the glochida to attach to the gills. I exposed the fish to glochida for 15 minutes, at which point I removed one fish of each species and checked the gills under a microscope for attached glochidia. When I saw glochida had attached to the fish, I placed all individuals of a single species in one aquarium. I held different species in different aquaria.

I siphoned each aquarium three times each week for six weeks and inspected the siphonate under a microscope to record the number of glochidia without tissue, glochidia with tissue, and juvenile mussels. I considered the mussels to be juveniles if there was visible foot movement. I separated the juvenile mussels from the siphonate and stored them in separate glass vials, according to fish species. After siphoning aquaria, I fed the fish frozen bloodworms. Smaller fish were held in suspended nets in their aquaria so they would not eat juvenile mussels or glochidia from the bottom of their tanks.

When three consecutive siphonate checks did not show any glochidia or juveniles, I checked the fish to see if any glochidia were still attached to their gills. I continued recording data until glochidia were no longer attached to the fish.

Before beginning the project, I decided, based on a conventional standard, that if the study produced between one and four juveniles I would consider the fish to be a marginal sutiable host and would need further studies to determine if it was in fact a host fish. If there were five or more juveniles found, I would classify the fish as a suitable host fish.

Results
After concluding my study, I found that the blacknose dace, goldfish, mimic shiner, largemouth bass, smallmouth bass, and bluntnose minnow studies were negative (see Appendix E), because they did not produce juveniles. The golden shiner, creek chub, and green sunfish were marginal suitable host fish because less than five juveniles were found in their siphonate. The spotfin shiner, common shiner, and hornyhead chub yielded positive results, more than five juveniles were found in their siphonate.

Figure 1 shows the number of glochidia without tissue in the siphonate of the spotfin shiner decreased over time as the number of glochidia with tissue and juveniles increased. The number of glochidia without tissue in the siphonate peaked at 9 days, glochida with tissue peaked at 23 days, and juveniles peaked at 25 days. The spotfin shiner produced 10 juveniles total and is a suitable host fish for the Round Pigtoe.

Figure 1 - Number of Glochidia and Juveniles Produced Over Time for Spotfin Shiner

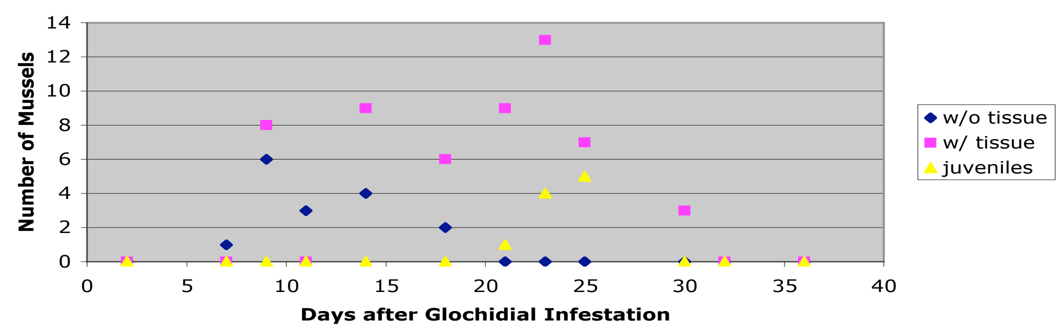
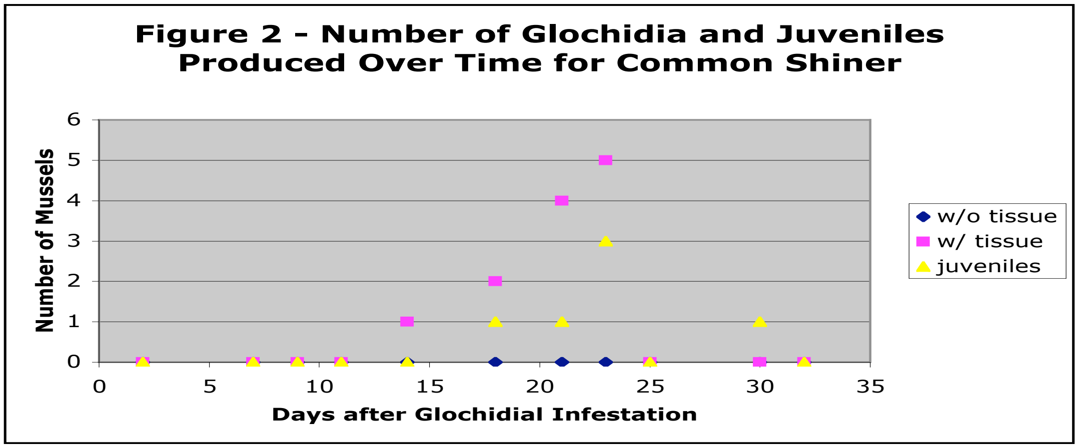


Figure 2 shows that in the siphonate from the common shiner, no glochidia without tissue were found. The number of glochidia with tissue and the number of juveniles both peaked at 23 days. The common shiner produced 6 juveniles total so it is a suitable host fish for the round pigtoe.



In Figure 3, the number of glochidia without tissue recovered from hornyhead chubs in the siphonate peaked at 9 days. The number of glochidia with tissue in the siphonate peaked at 21 days, and the number of juveniles peaked at 23 days. Figure 3 shows a general downward trend of glochidia without tissue overtime and an upward trend of glochidia with tissue and juveniles. The hornyhead chub produced 5 juveniles total and so is a suitable host fish for the round pigtoe.

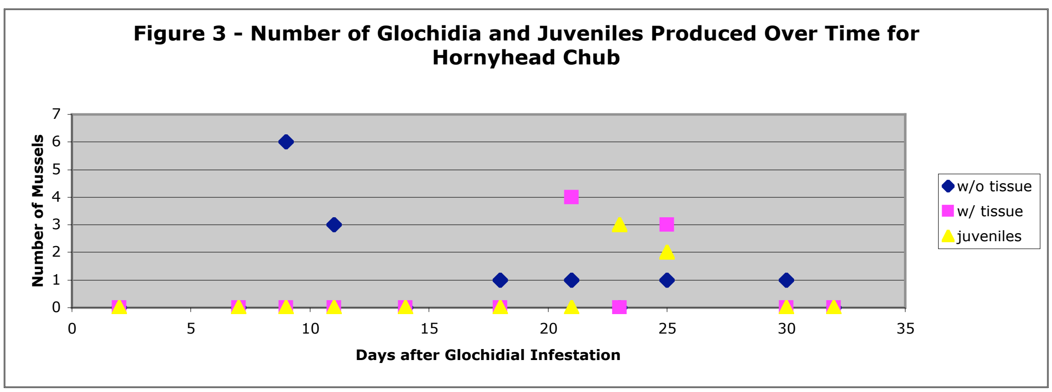


Figure 4 shows that the number of glochidia without tissue that fell off the golden shiner peaked at 21 days. The number of juveniles peaked at 23 days, and the number of glochidia with tissue peaked at 30 days. The golden shiner produced 3 juveniles total and is a marginal host fish for the round pigtoe.

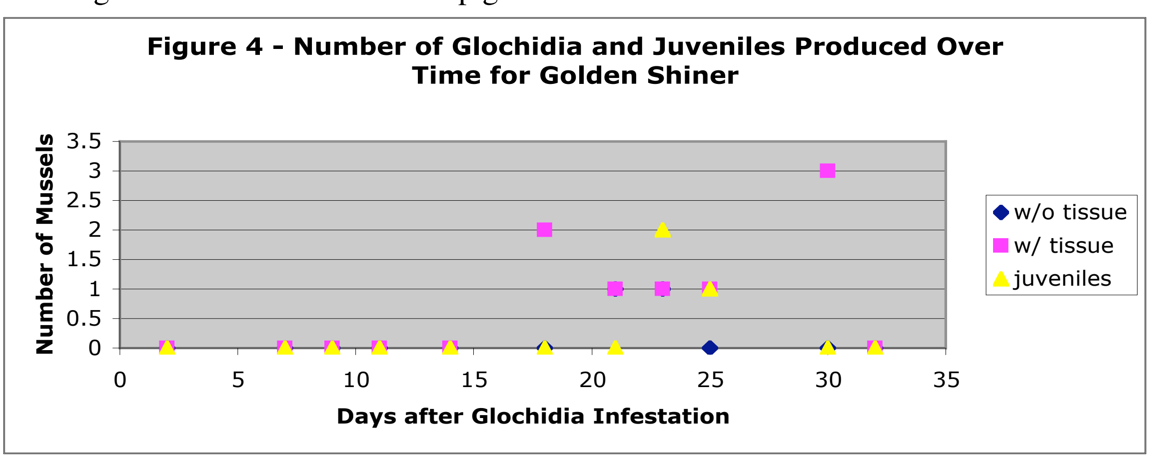
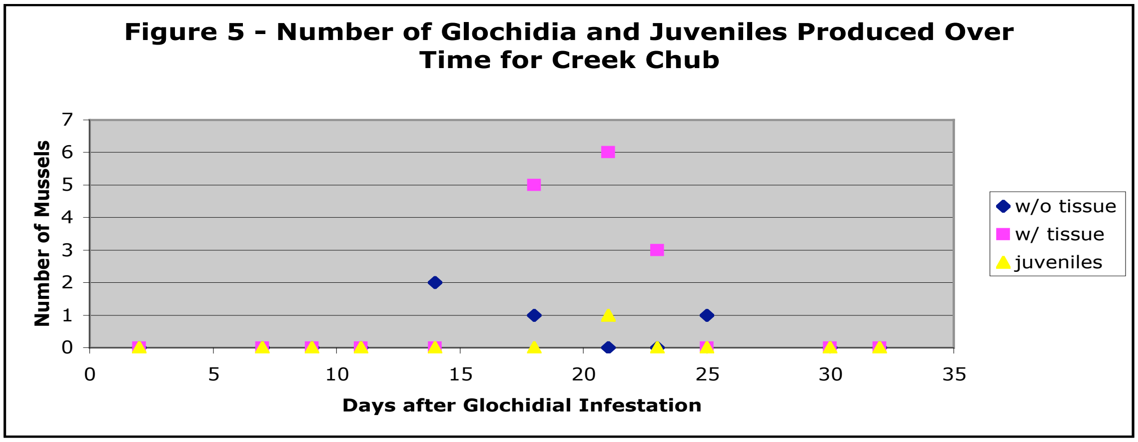


Figure 5 shows the number of glochidia without tissue from the creek chub peaked at 14 days. The number of glochidia with tissue and the number of juveniles peaked at 21 days. The creek chub produced 1 juvenile and so it is a marginal host for the round pigtoe.



In the siphonate of the Green Sunfish, no glochidia without or with tissue were found and only one juvenile was recovered.

Discussion

I found the spotfin shiner, common shiner, and hornyhead chub are suitable host fish for the round pigtoe. I determined the golden shiner and the creek chub are marginal suitable hosts for the round pigtoe, while the green sunfish is probably not a marginal host fish because no glochidia without or with tissue were found and only one juvenile was recovered. To determine the potential ability of the golden shiner, creek chub and the green sunfish to be host fish for the round pigtoe, this study should be repeated for these species. For another possible study, fish that are in the same genera as the definite host fish could be tested to determine their ability to host the round pigtoe.

Glochidia without tissue and glochidia with tissue may be stages in the life cycle of the round pigtoe. Data suggest that the number of glochidia without tissue produced by host fish decreases after the initial infestation while the number of glochidia with tissue increases. In all species, the number of glochidia with tissue increased as the number of juveniles also increased. In all the studies that involved suitable host fish where glochidia was found the number of glochidia without tissue in the siphonates peaked around 13 days before the number of glochidia with tissue. Glochidia with tissue peaked two days before the juveniles in two studies and on the same day in the third. There are not enough data for me to confidently say that glochidia without tissue and with tissue are developmental stages in the life cycle of the round pigtoe, but I believe after further studies I may be able to prove that these are stages in the mussel's life cycle. Because of the lack of information found on this stage in the life cycle of the round pigtoe this new information would be very important to any future study involving freshwater mussels.

For future studies, this project could be repeated with a larger number of fish for each fish species. Instead of waiting for the glochidia to drop off the fish into the siphonates, the fish gills could be examined under a dissecting microscope for glochidia every few days and the siphonate checked for juveniles. This project could also be repeated using a different mussel species to verify the life cycle results represent all mussels. In future studies, it would also be valuable to count the number of glochidia without and with tissue in a small sample of the glochidia used for infestation before the fish were infested to see which glochidia attach better to potential host fish. Monitoring the mussel once it is gravid and recording when the mussel naturally releases its glochidia could also be a valuable study. A histological study that would provide insight on larval anatomical development could possibly prove that glochidia without and with tissue are developmental stages. Another possibility to explore would be that glochidia without and with tissue are not developmental stages, but when the glochidia is found without tissue it has had its tissue mass broken down by the immune system of the fish or consumed by other organisms.

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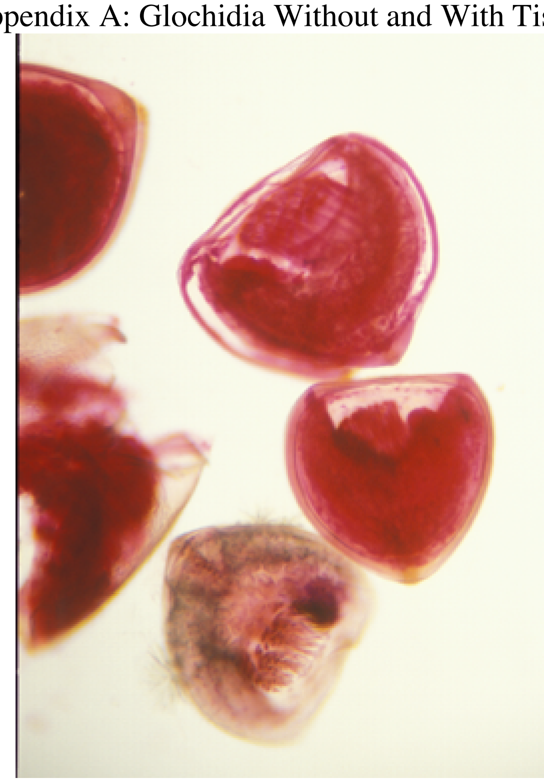
Appendix A: Glochidia Without and With Tissue



Appendix B: Federally Endangered Pigtoe Mussels, Genus and Species

Common Name	Scientific Name
Cumberland Pigtoe	*Pleurobema gibberum*
Dark Pigtoe	*Pleurobema firvum*
Finerayed Pigtoe	*Fusconaia cuneolus*
Flat Pigtoe	*Pluerobema marshalli*
Heavy Pigtoe	*Pleurobema taitianum*
Oval Pigtoe	*Pleurobema pyriforme*
Rough Pigtoe	*Pleurobema plenum*
Shiny Pigtoe	*Fusconaia cor*
Southern Pigtoe	*Pleurobema georgianum*

Appendix C: Endangered Minnesota Mussels in the Subfamily Ambleminae

Common Name	Scientific Name
Elephant Ear	*Ellipto crassidens*
Ebonyshell	Fusconaia ebena
Sheepnose	*Plethobasus cyphyus*
Winged Mapleleaf	*Quadrula fragosa*
Wartyback	*Quadrula nodulata*

Appendix D: Common and Scientific Names of the Fish used in the Study:

Common Name	Scientific Name
Blacknose Dace	*Rhinichthys atratulus*
Bluntnose Minnow	*Pimephales notatus*
Common Shiner	*Luxilus cornutus*
Creek Chub	*Semotilus atromaculatus*
Golden Shiner	*Notemigonus crysoleucas*
Goldfish	*Carassius auratus auratus*
Green Sunfish	*Lepomis cyanellus*
Hornyhead Chub	*Nocomis biguttatus*
Largemouth Bass	*Micropterus salmoides*
Mimic Shiner	*Notropis volucellus*
Smallmouth Bass	*Micropterus dolomieui*
Spotfin Shiner	*Cypinella spiloptera*

Appendix E: Total Juveniles Found in Siphonates

Name of Fish	# of Fish	Juveniles	Status
Blacknose Dace	11	0	Negative
Goldfish	1	0	Negative
Spotfin Shiner	12	10	Positive
Common Shiner	12	6	Positive
Golden Shiner	3	3	Marginal
Mimic Shiner	20	0	Negative
Hornyhead Chub	14	5	Positive

Large Mouth Bass	4	0	Negative
Small Mouth Bass	2	0	Negative
Creek Chub	3	1	Marginal
Bluntnose Minnow	7	0	Negative
Green Sunfish	4	1	Marginal