HOST FISH FOR THE SNUFFBOX MUSSEL (*EPIOBLASMA TRIQUETRA)*
by Mark Ledford 2004

Abstract:
Minnesota's snuffbox mussel (*Epioblasma triquetra*) is on the verge of becoming a federally endangered species. Mussel larvae (glochidia) usually attach to a host fish native to Minnesota rivers to complete their metamorphosis. I tested the host suitability of eleven different fish species, all of which are native to either the St. Croix or the Mississippi rivers. Over a period of two months, I collected aquarium siphonate from the 11 different fish species that had been infested with sunffbox glochidia and counted the number of glochidia that had no tissue, and glochidia that had tissue. The presence of tissue implied there was a cell mass. I also counted the number of juvenile mussels from siphonate. My project identified the logperch (*Percina caprodes*) as a suitable host fish for the snuffbox, both of which are native to the St. Croix and Mississippi rivers. I also possibly identified the developmental stages of glochidia associated with the lifecycle in the snuffbox. The glochidia that had no tissue decreased over time up until 21 days, after which they were no longer found in the siphonate. The number of glochidia with tissue stopped falling off the fish after the initial infestation, then peaked in the siphonate just when juveniles started to show up. I also established that the period of development from infestation to juvenile stage was 30 days or more for the snuffbox when hosted by the logperch.

Introduction:
The Snuffbox *(Epioblasma triquetra)* is a freshwater mussel that is on the verge of becoming a federally listed endangered species in the state of Minnesota. Possible reasons for the Snuffbox mussel being endangered in the Minnesota rivers are: pollution, impoundments (dams, weirs), muskrat and raccoon predation, zebra mussels, channelization, and loss of host fish species *(1)*. Very little is known about the ecology and behavior of juvenile mussels due to their complex life cycle and difficulty of collecting in the field, which is due to the fact that they are transparent for the first two months of their life cycle *(2)*. The goal of my project was to identify host fish species for the Snuffbox mussel in the St. Croix and Mississippi Rivers in Minnesota. The survival of the Snuffbox mussel in Minnesota depends greatly on finding host fish species to make it easier to raise these mussels in a laboratory environment, so they can be reintroduced into their natural habitats.

Previous studies done in Missouri, Ohio, and Tennessee showed the following fish species were suitable hosts for the Snuffbox mussel: Logperch *(Percina caprodes)*, Banded Sculpin (*Cotus carolinae)*, Ozark Sculpin *(Cottus hypselurus)*, and Blackspotted Topminnow (*Fundulus olivaceus) (2)*. In an earlier study done in Missouri, natural glochidial infections (traces of glochidia found on gills of fish) had been found on the Hornyhead Chub fish species, which is a good indication that the fish species is a suitable host *(3*).

My hypothesis in this project was that the categories of "glochidia with tissue" and "glochidia without tissue" depict levels of maturity in the mussel. I also hypothesized that glochidia with tissue grown were further along in their development to becoming juvenile mussels than glochidia without tissue. Tissue implies there is a cell mass inside the glochidia, which may have come from this development. All literature searches that I did, produced no information regarding this topic. My University advisor also has never seen literature with this information recorded, nor has he researched this topic. In this project, I used the fish species listed in Table 1.

Table 1: Fish Species Used in Project

Common Name	Genus Species
Iowa Darter	*Etheostoma exile*
Fathead minnow	*Pimephales promelas*
Yellow Perch	*Perca flavescens*
Hornyhead Chub	*Nocomis biguttatus Kirtland*
Blue Gill	*Lepomis macrochirus*
Walleye	*Stizostedion vitreum*
Rainbow Darter	*Etheostoma caeruleum*
Black Bullhead	*Ictalurus melas*
Brook Stickleback	*Culaea inconstans*
Central minnow	*Campostoma anomalum*
Logperch	*Percina caprodes*

In the paper that follows, I will show that from these fish, only the Logperch fish proved to be a suitable host fish for the Snuffbox mussel in the St. Croix and Mississippi Rivers. I will also present data that suggest possible developmental stages of glochidia.

Background

The Snuffbox mussel is found in rivers all over the Midwest. In Minnesota, the Snuffbox mussel lives in the upper parts of the St. Croix and Mississippi Rivers and in clear, gravel riffles *(4)*. It is listed as threatened in Ohio, rare in Missouri, and endangered in Illinois, Indiana, Wisconsin, Michigan, and Minnesota (*5)*. To be listed as an endangered species means the species is on the verge of extinction throughout all or a significant portion of its range.

The key characteristics of the Snuffbox include a triangular shell, relatively thick for its size, with a sharply defined, and broadly expanded posterior slope, with yellow or yellowish green with green rays, blotches, or chevron markings *(6)*.

Similar species to the Snuffbox mussel are the Elktoe *(Alasmidonta viridis),* Deertoe *(Truncilla truncata),* and Fawnsfoot *(Truncilla donaciformis)* mussels. Since these mussel species are related to the Snuffbox mussel, fish that serve as hosts for it might be host fish for the Snuffbox species. But, based on a previous study in Missouri, none of the host fish for the Elktoe, Deertoe, and Fawnsfoot mussels were found to be host fish for the Snuffbox mussel *(6)*.

The life cycle of the Snuffbox and most freshwater mussels begins when the female mussel is siphoning water where the eggs are fertilized by sperm drawn in by the siphoning process. Once fertilized, the eggs develop to the larval stage inside the mouth of the female. To increase chances of the glochidia making contact with a fish host, most females "go fishing." By displaying specially adapted tissues that look like fish prey, the females try to lure fish to swim near them. Sensing a fish nearby, the female releases the glochidia into the water through minute pores in her marsupial gills *(7)*.

All freshwater mussels exhibit a parasitic phase where the glochidia of the mussels attach to the gills or fins of the host fish. The glochidia attach to the gills of the host fish, develop into a juvenile, and then release to begin their adult life as free-living juveniles in the substrate. This period can take anywhere from a couple of days to several weeks, but it depends on the host fish and the mussel species. Generally, it takes the Logperch one to four weeks to meatamorphasize from a glochidium to a juvenile *(7)*.

Once the parasitic phase is over, the juveniles release from the host fish and drop to the bottom of the stream or the river bottom. The best conditions for juvenile survival at this point in their life cycle are when they settle into depositional areas behind boulders or rocks where there are swift currents or along the banks of these rivers *(8)*.

Very little is known about the ecology and behavior of glochidia, because they are transparent for up to two months *(9)*. There has never been a study done in Minnesota on the Snuffbox mussel. Therefore there has never been a host fish identified for the Snuffbox mussel in Minnesota. The identification of a host fish and information about the life cycle of glochidia found in this study may enable the Snuffbox mussles to be rasied in laboratories for reintroduction into Minnesota Rivers.

Experimental Methods
I collected ten different fish species (see Table I) from the upper St. Croix and Mississippi Rivers by electro-fishing (refer to Table 1 for species collected). The number of each species varied by the number caught. I purchased the remaining species, the Central minnow *(Campostoma anomalum),* from the Crystal Bait and Tackle shop. Each of these fish species were held in suspended nets in individual tanks with a constant flow of water 18-23 °C and a constant air supply. Next I obtained gravid female Snuffbox mussels from Macalester College scuba divers who retrieved them from the St. Croix River in St. Croix, Minnesota. The graduate students tried to find Snuffbox mussels in the Mississippi, but were unsuccessful. They were only able to retrieve one gravid female Snuffbox mussel from the St. Croix, but that mussel released sufficient glochidia for this project.

I put the gravid mussel into a beaker that was submerged in an aquarium. Once the mussel released its glochidia, I removed approximately 30 glochidia from the beaker and tested their maturity level with NaCl (s). I judged the sample of glochidia to be mature enough for infestation when 70% or more reacted to the NaCl by closing their mouths. If the glochidia were not yet mature, I waited for two days for the glochidia to mature. I separated the sample of glochidia tested with NaCl (s) from the rest of the glochidia and did not use those glochidia for the project.

Once the glochidia had matured, I infested all test fish by pipetting glochidia onto the gills of each fish. To do this, I put the pipet directly into the marsupial gland of the mussel, pipetted out the glochidia, and transferred the glochidia onto the gills of the fish. After direct infestation, I held the fish out of water for 10-20 seconds to allow the glochidia to attach to the gills.

Once all fish were infested, I fed the larger fish with small minnows over a ten-minute span until the fish stopped eating. For the smaller fish, I fed them 5-10 mL of small invertebrate bloodworms from Crystal Bait and Tackle shop two to three times a week. I siphoned the tanks before and after feeding periods and checked the siphonate for glochidia and juvenile mussels, using a compound microscope.

I recorded the number of glochidia with tissue, glochidia without tissue, and the number of juveniles present in the siphonate for each fish type. The glochidia without tissue looked like small clear mussels, while the glochidia with tissue appear to be in the same form but were whitish in color. The juveniles looked like the glochidia with tissue, but after 10-15 seconds of observation, their mouths closed if they were juveniles. I placed each group of juveniles (all from the same fish species) into small-labeled vials, one for each fish species that released juveniles. After three consecutive siphonates that did not contain glochidia or juveniles, I checked the gills of the fish to determine if glochidia were still attached, and if I did not find any glochidia, I ended that portion of the study. If I did find glochidia or juveniles attached to the gills of the fish, I continued to siphon the tanks in the same intervals as I had done before until all glochidia had detached from the host fish.

Before beginning this project I determined that if one to four juveniles were produced by a fish species, that the species would serve as a marginal host species. If there were five or more juveniles produced by a test fish I identified it as a definite host fish.

Results:

Table 2 shows that the Logperch hosted a total of 84 juvenile mussels, giving a positive study for the Logperch. No other fish produced juveniles. The percent of glochidia without tissue ranged from 15%-100%. The percent of glochidia with tissue ranged from 0%-85%. The study done on the Iowa Darter was incomplete, because one fish of that species dies before the study was completed.

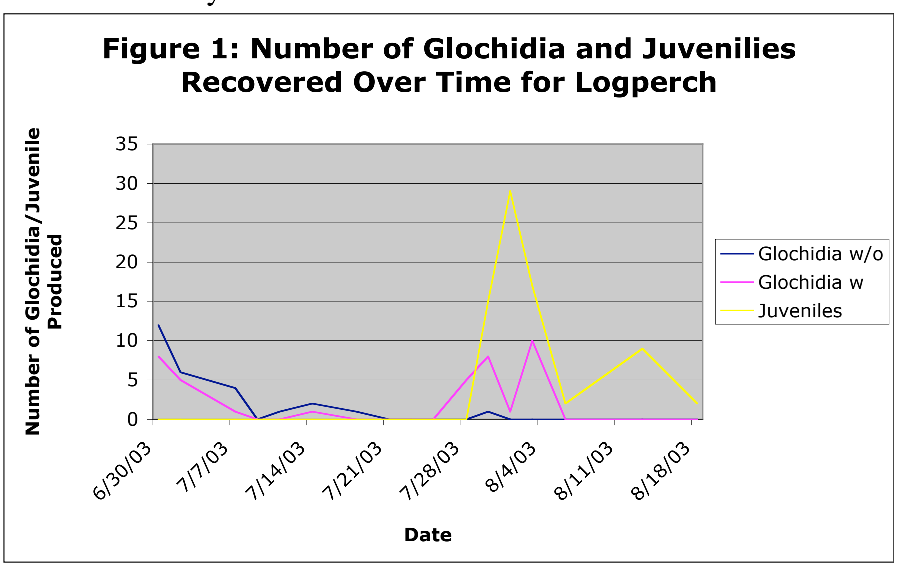
Table 2: Number of glochidia and juveniles found.

Name Of Fish	Initial # of Fish	Mortality Rate of Fish	Num ǀ Percent Glochidia w/o tissue		Num ǀ Percent Glochidia w/ tissue		Juveniles	Status
Iowa Darter	1	100%	0	0%	0	0%	0	Inconclusive
Fathead Minnow	15	67%	180	64%	100	36%	0	Negative
Yellow Perch	4	25%	8	15%	44	85%	0	Negative

Hornyhead Chub	6	33%	8	100%	0	0%	0	Negative
Logperch	9	44%	27	19%	29	21%	84	Definite
Bluegill	7	0%	11	73%	4	27%	0	Negative
Walleye	4	0%	8	42%	11	58%	0	Negative
Rainbow Darter	2	0%	77	64%	44	36%	0	Negative
Black Bullhead	1	0%	27	63%	16	37%	0	Negative
Brook Sticklebrook	1	0%	2	40%	3	60%	0	Negative
Central Minnow	4	0%	38	70%	16	30%	0	Negative

Key Inconclusive: all fish of that species died
Negative: no juveniles were produced
Positive: juveniles were produced

Figure 1 shows the number of glochidia with tissue, without tissue, and the number of juveniles found in the siphonate over the duration of study for the Logperch, which was the only definite host fish for the Snuffbox mussel. The first siphonate was collected three days after infestation, and at that time the siphonate showed 12 glochidia without tissue and 8 with tissue. From the period from July 2nd to July 28th, the number of glochidia that fell off the gills of the fish was almost zero. On the 30th of July, the first juvenile mussels appeared in the siphonate. From July 30th to August 3rd , the number of juveniles found in the siphonate peaked with the highest number being 29 juveniles. At the same time that juveniles appeared, glochidia with tissue appeared and follow the same trend as the juveniles did, but the juveniles did continue to appear after the glochidia with tissue ceased to appear. The number of glochidia without tissue was essentially zero after the first three days after infestation.



Discussion:
The purpose of this project was to find a suitable host fish species in the St. Croix and Mississippi Rivers for the Snuffbox mussel and to verify the Logperch fish as a suitable host for the Snuffbox. I tested the host suitability of eleven different fish species, all of

which are native to either the St. Croix or the Mississippi Rivers in Minnesota. I determined that the Logperch was a host fish for the Snuffbox mussel, because 84 juvenile mussels were produced.

My hypothesis was that the categories of glochidia with tissue and glochidia without tissue are developmental stages of the mussel. I hypothesized that glochidia with tissue were further along in their development to becoming juvenile mussels. Based on the results obtained in Figure 1, it seems my hypothesis may be correct. It appears that while glochidia are in the mouth of the Logperch, they have already developed into glochidia with and without tissue, with the number without tissue being greater. This may be the result of some glochidia dying during the laboratory infestation, but the ones that survived stayed on the gills of fish as they developed. At the same time the juveniles were produced, there were no glochidia without tissue in the siphonate, but there were glochidia with tissue that dropped off at the same time the juveniles did. This seems to show that glochidia without tissue develop into glochidia with tissue, which then develop into juveniles. Furthermore it seems as though the period of development from infestation to juvenile stage is 30 days or more for the Logperch, evidence by the fact that glochidia with tissue fell off in the same period as the juveniles after infestation, and were no longer seen. Essentially there were no glochcidia without tissue found after 21 days after infestation. Problems with the study resulted from not having the same number of fish in each species sample size, the biomasses of the fish species used were different, and so statistical analysis could not be done.

Ideas for further studies could be done on the developmental stages of glochidia using the Logperch as a host fish in order to determine whether the period of development from infestation to the juvenile stage is really 30 days or more. To do so, a larger sample size of mussels used for infestation is needed. Further investigation could be done using a large enough sample size of the Logperch to investigate the developmental stages of the glochidia by actually looking at the glochidia while they are on the gills of the fish. Another study could be done on the Iowa Darter species to see if that fish serves as a suitable host. Although my results have led me to determine that the growth of tissue in glochidia is an indicator of development, future studies could be done on why there are glochidia with and without tissue produced on fish that do not produce juveniles. A larger sample size of each species would be needed, so biochemical testing could be done to see if the host fish exhibits some type of defense mechanism and if there is any anti-body response to the glochidia in some fish species.

Sources Cited

1. Fresh Water Mussels (2003); see http://www.thamesriver.on.ca/Species_at_Risk/mussels.htm.
2. Mesocosm Scale Freshwater Mussel Rearing (2002); see http://www.fred.net/acepoff/mussel/mussel.html.
3. Department of Evolution, Ecology & Organismal Biology: Division of Mollusks 2003); see http://www.biosci.ohio-state.edu/~molluscs/OSUM2/index.htm.
4. Fishes of Minnesota (2002); see http://www.gen.umn.edu/faculty_staff/hatch/fishes/iowa_darter.htm.

5. Epioblasma Triquetra (2002); see web4.msue.msu.edu/mnf/abstract/aquatics.html
6. Minnesota Department of Natural Resources (2003); see http://www.dnr.state.mn.us.
7. Freshwater Mussels of the Midwest - Epioblasma triquetra (2003); see http://www.inhs.uiuc.edu/chf/pub/mussel_man/page162_3.html.
8. Discover America's Hidden Treasure: Fresh Water Mussels (2003); see http://news.fws.gov/mussels.html.
9. A. Zale, R. Neves, *Can. J. Zool.* **60,** 2535-2542 (1982)

Bibliography

- A. Zale, R. Neves, *Can. J. Zool.* **60,** 2535-2542 (1982).
- Department of Evolution, Ecology & Organismal Biology: Division of Mollusks 2003); see http://www.biosci.ohio-state.edu/~molluscs/OSUM2/index.htm.
- Discover America's Hidden Treasure: Fresh Water Mussels (2003); see http://news.fws.gov/mussels.html.
- Effect of Invasive Fish Species on Freshwater and Estuarine Fishes in Midwest (2001); see http://www.sgnis.org/publicat/moyle.htm.
- Epioblasma Triquetra (2002); see web4.msue.msu.edu/mnf/abstract/aquatics.html
- Minnesota Department of Natural Resources (2003); see http://www.dnr.state.mn.us.
- Field Guide to Freshwater Mussels of the Midwest (2000); see http://www.inhs.uiuc.edu/chf/pub/mussel_man/cover.html.
- Fishes of Minnesota (2002); see http://www.gen.umn.edu/faculty_staff/hatch/fishes/iowa_darter.htm.
- Fishing for Answers: Hosting Freshwater Mussel Populations in Lake (1999); see http://www.benthos.org/meeting/nabs2000/nabstracts2000.cfm/id/830.
- F. O'Berin, R. Neves, M. Steg, *American Malacological Bulletin.* **14**, 165-171 (1998).
- Freshwater Mussels (2003); see http://www.thamesriver.on.ca/Species_at_Risk/mussels.htm.
- Freshwater Mussel Genera of the World (1998); see http://www.inhs.uiuc.edu/~ksc/MusselGeneraMP.html.
- Freshwater Mussels of the Midwest - Epioblasma triquetra (2003); see http://www.inhs.uiuc.edu/chf/pub/mussel_man/page162_3.html.
- J. Thorp, A. Covich, *Ecology and Classification of North American Freshwater Invertebrates*. (Academic Press, San Diego, 1991), pp. 315-400.
- Mesocosm Scale Freshwater Mussel Rearing (2002); see http://www.fred.net/acepoff/mussel/mussel.html.
- Mussels (1999); see http://www.bio.umass.edu/biology/conn.river/fwmussel.html.
- Species at Risk- Mussels- (2002); see http://www.thamesriver.on.ca/Species_at_Risk/mussels.htm