Level K Consultant's Report

Glochidial hosts of the pondmussel (*Ligumia subrostrata*)

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Introduction

The pondmussel (*Ligumia subrostrata*) is uncommon in Minnesota, living only in streams in the far southwest corner of the state. Pondmussel conservation efforts could be improved with a greater understanding of its life history requirements. Most North American freshwater mussels must attach as larvae (glochidia) to a fish to facilitate their metamorphosis into a juvenile. Preliminary studies have shown that *L. subrostrata* can metamorphose on centrarchids in the laboratory (Hove 1995, Watters *et al.*, 2007). However, previous host suitability analyses have been limited in the number of species covered and additional work is needed to determine if *L. subrostrata* glochidia will metamorphose on other Minnesota fishes. Additionally, field observations are needed to determine which laboratory-determined suitable hosts are natural hosts. The objectives of this study were: (1) identify juvenile mussels collected from naturally infested Rock River fishes living with *L. subrostrata*, and (2) conduct laboratory host suitability trials with *L. subrostrata* glochidia to identify new suitable host fish species or confirm previously identified potential hosts.

Methods

We followed standard methods to recover glochidia and juvenile mussels from naturally infested fishes (Hove *et al.*, 2016a). Fishes were collected from the Rock River near Pipestone, MN in June 2016 and on October 24, 2016, held in species-specific aquaria between 20-22 °C, and young mussels released by these fishes were preserved in ethanol. Glochidial shell outline and dimensions were measured using scanning electron microscopy, and compared with glochidia morphometrics of known species also collected from the Rock River study site.

We conducted glochidia host suitability trials using standard methods (Zale and Neves 1982, Hove *et al.,* 2000). The gravid *Ligumia subrostrata* used for these trials was collected from the Rock River near Pipestone, MN. Test fishes were collected using a seine, trap net, angling, or electrofishing equipment from Minnesota streams and rivers. Host suitability trials were conducted at the UMN Wet Laboratory in St. Paul. Test subjects were held in aquaria (40 L or 400 L) at least 14 d prior to glochidia infestation, at temperatures between 22-23 °C. Glochidia were obtained by puncturing and flushing the marsupia of the *L. subrostrata*. To determine glochidia health we exposed a subsample to a 0.1-1% NaCl solution. If ≥ 70% of the glochidia closed their valves upon exposure to salt, the rest of the glochidia were used for host tests. After completion of experiments, we returned the female mussel to the MN DNR. Fish and adult mussel identifications were based on Becker (1983) and Sietman (2003), respectively. Fish and mussel nomenclature follows Robins *et al.*, (1991) and Williams *et al.*, (2008), respectively.

Results

Several juvenile mussels were recovered from spring-collected Rock River fishes, while only glochidia were sloughed by fishes caught during fall 2016. *Ligumia subrostrata* and *Toxolasma parvum*, the only Lampsilines known to occur in the upper Rock River, had different sized glochidia. *Ligumia subrostrata* glochidia had shell heights ≥260 µ while *T. parvum* glochidia shell height was <220 µ (Table 1). Additionally *L. subrostrata* had relatively high, narrow glochidial shells compared to *T. parvum* glochidia. Juvenile *L. subrostrata* and *T. parvum* were released by naturally infested bluegill, green sunfish, and orange-spotted sunfish collected during June 2016 (Table 1, Figure 1). Several fish species were collected from the

Rock River during October 2016 but only a few glochidia were collected from bluntnose minnow, white sucker, and troutperch (Table 2).

Laboratory host suitability trials with *Ligumia subrostrata* glochidia showed that a variety of fishes are potential hosts. Three centrarchids, bluegill, pumpkinseed, and largemouth bass, facilitated *L. subrostrata* metamorphosis, as did northern pike (Table 3). Pumpkinseed facilitated the highest percentage of glochidia metamorphosis. No juveniles were recovered from smallmouth bass or from four other fish species.

Discussion

This study adds to the growing body of literature showing that *Ligumia subrostrata* will metamorphose on fishes other than centrarchids. Early studies showed that various sunfishes were either naturally infested with *L. subrostrata* or supported glochidia metamorphosis in the laboratory (Lefevre and Curtis 1912, Stern and Felder 1978). This study confirms that *Lepomis* spp. are naturally infested with *L. subrostrata*, and showed these glochidia metamorphosed as well. Glochidia host suitability trials from this study and work conducted earlier in the year revealed that several non-centrarchid fishes are potential hosts for *L. subrostrata* (Hove et al., 2016b). Additional research is needed to determine the breadth of fish species that serve as *L. subrostrata* potential hosts and as hosts under natural conditions.

Recovering juvenile lilliput (*Toxolasma parvum*) from naturally infested Rock River fishes was an unanticipated but welcome finding during this project. *Lepomis* spp. releasing juvenile *T. parvum* is consistent with studies that show *Lepomis* facilitate *T. parvum* glochidial metamorphosis in the laboratory (Hove 1995, Watters *et al.*, 2007), and naturally infest *Lepomis* (Wilson 1916).

Acknowledgements

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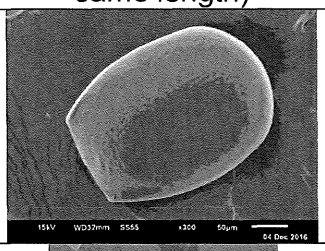
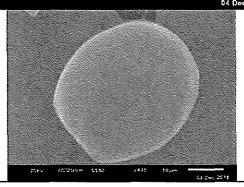
Table 1. Glochidial shell dimensions of Rock River mussels. (N=number of glochidia measured)

Species (N)	Height ± 1 s.d. (µ)	Length ± 1 s.d. (µ)	Hinge length± 1 s.d. (µ)	Scanning electron micrograph (scale bars approximately same length)
Ligumia subrostrata (20)	275±9	212±6	128±5	
Toxolasma parvum (50)	197±6	173±6	96±6	

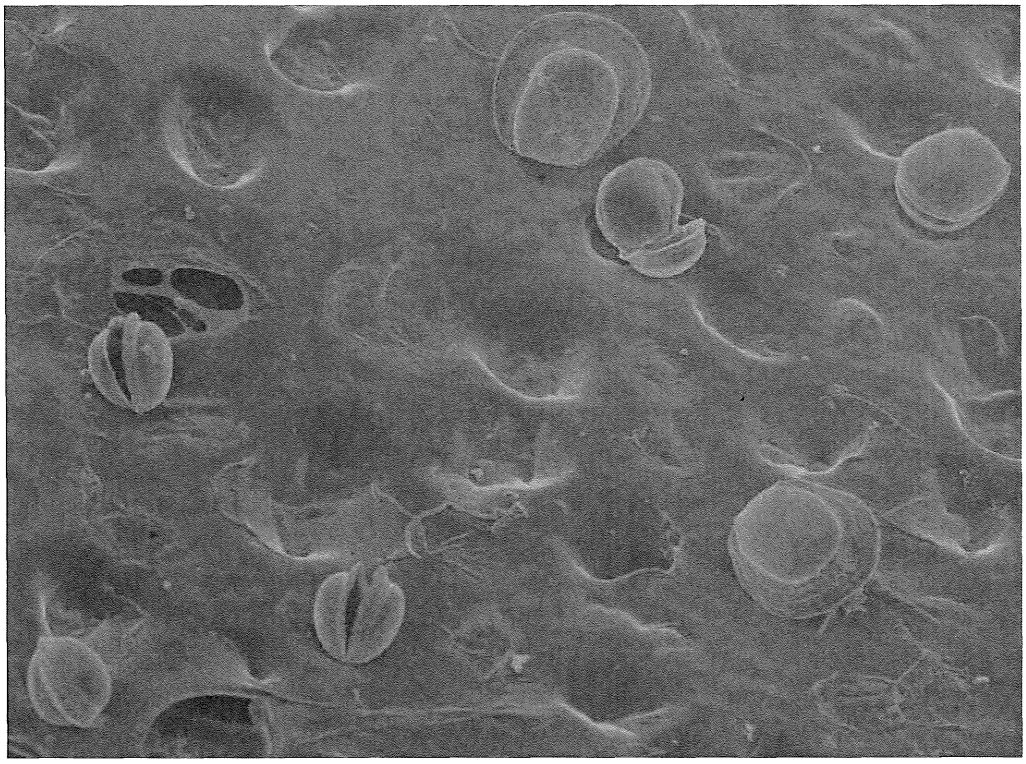


Figure 1. Juvenile *L. subrostrata* and *T. parvum* collected from Rock River fishes during June 2016.

Table 2. Juvenile mussels and glochidia released by naturally infested Rock River fishes. Species were identified by measuring glochidial valve height, where *Ligumia subrostrata* >260µ and *T. parvum* ≤220µ. (N=number of fish held)

Fish species (N)	*L. subrostrata* juveniles	*T. parvum* juveniles	*L. subrostrata* glochidia	*T. parvum* glochidia	Unknown glochidia
Collected during June 2016					
Lepomis cyanellus (6)	7	4	0	3	
L. humilus (9)	26	17	0	0	
L. macrochirus (2)	23	55	1	0	
Collected on October 24, 2016					
Campostoma anomalum (41)	0	0			0
Cyprinella lutrensis (4)	0	0			0
Luxilus cornutus (9)	0	0			0
Notropis dorsalis (5)	0	0			0
Notropis stramineus (41)					
Pimephales notatus (31)	0	0			1
Semotilus atromaculatus (36)	0	0			0
Catostomus commersoni (13)	0	0			1
Moxostoma macrolepidotum (14)	0	0			0
Ameiurus melas (2)	0	0			0
Noturus gyrinus (11)	0	0			0
Fundulus sciadicus (8)	0	0			0
Culaea inconstans (2)	0	0			0
Percopsis omiscomaycus (1)	0	0			6

Lepomis cyanellus (36)	0	0			0
Lepomis humilus (4)	0	0			0
Micropterus salmoides (1)	0	0			0
Etheostoma exile (1)	0	0			0
Etheostoma nigrum (17)	0	0			0
Percina maculata (6)	0	0			0

Table 3. *Ligumia subrostrata* host suitability trials.

Fish species	No. of individuals inoculated	No. of survivors	Glochidia/ juvenile recovery period (days)	No. of juveniles recovered	No. of glochidia and juveniles recovered	Percent meta-morphosis
black bullhead	6	6	8	0		
yellow bullhead	2	1	8	0		
tadpole madtom	11	11	8	0		
northern pike	1	1	12-15	25	45	56%
bluegill	14	9	15-22	56	112	50%*
pumpkinseed	11	10	12-22	136	169	80%*
largemouth bass	17	16	12-19	390	1155	34%
smallmouth bass	5	2	15	0		
walleye	2	2	15	0		

* Fish still releasing juveniles at the writing of this report.