

# Effects of temperature on duration of viability for glochidia of freshwater mussels (*Bivalvia: Unionidae*)

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**Abstract:** Glochidia from gravid females of *Villosa iris* (Lea, 1829) and *Actinonaias pectorosa* (Conrad, 1834) were extracted from marsupia and tested for viability over several days. Glochidia that were flushed from the gill and those retained in the excised gill marsupium were tested at three holding temperatures: 0°, 10°, and 25°C. Viability was tested by exposing glochidia to a sodium chloride solution, and then confirmed by infesting known host fish with glochidia at 1 week and 2 weeks post-extraction from the female mussel. Results indicate that extracted glochidia remained viable for significantly longer than excised intra-marsupial glochidia. There was no significant difference in viability of glochidia between 0° and 10°C in extracted treatments ( $p > 0.05$ ). Extracted glochidia of *V. iris* maintained >75% viability for 180, 192, and 46 hr at 0°, 10°, and 25°C, respectively. Similarly, glochidia extracted from *A. pectorosa* maintained >75% viability for 345, 310, and 108 hr at 0°, 10°, and 25°C, respectively. Long-term viability of glochidia would promote downstream dispersal and has practical applications in captive propagation.

**Key Words:** Unionidae, freshwater mussels, *Villosa*, *Actinonaias*, glochidia viability

Freshwater mussels have a complex reproductive cycle involving an obligate parasitic larval stage. The cycle begins by the release of sperm by males into the water column, which are subsequently siphoned by females of the same species to fertilize the eggs. Fertilized eggs are then brooded in the gill marsupium until they reach the larval stage (glochidium). Fully developed glochidia are released for intended attachment to the gills or fins of a suitable host fish. The duration of encystment is dependent on species and water temperatures. After 2-6 weeks of encystment, the glochidia transform to juvenile mussels and drop to the substratum to begin the free-living juvenile stage.

The viability of glochidia after release and prior to attachment has been reported only as anecdotal comments (Murphy, 1942; Matteson, 1948; Tedla and Fernando, 1969). Although these studies indicate that longevity of extracted glochidia may exceed 1 week, the methods and percent viability are inadequately defined and inconclusive. The purpose of this study is to determine longevity of glochidia at three water temperatures, conducted with glochidia retained within the excised gill and glochidia extracted from the gill marsupium.

## METHODS

The viability of glochidia over time was tested at three water temperatures and for two modes of exposure.

Temperature treatments were conducted in a low temperature incubator (Fisher Scientific Model 307) at 25°C, a refrigerator at 10°C, and a cooler filled with ice at near 0°C. Modes of exposure included an extracted treatment, referring to glochidia that were flushed from the gill marsupium; and an intra-marsupial treatment, in which the entire marsupial gill was excised and samples of glochidia were extracted at designated intervals. These trials were conducted twice; first using the rainbow mussel, *Villosa iris* (Lea, 1829), and then with the pheasantshell, *Actinonaias pectorosa* (Conrad, 1834). Both species are long-term brooders (bradytictic), with fully developed glochidia in gravid females from fall through spring (Parmalee and Bogan, 1998).

Eighteen gravid specimens of *Villosa iris* from Indian Creek, Tazewell County, Virginia, were collected in April 1999 and held in a Living Stream (Frigid Units, Toledo, OH) at 13°C at the Virginia Tech Aquaculture Center. The Living Stream contained a 50:50 mixture of well water and dechlorinated town water (Blacksburg, Virginia) to achieve a hardness of approximately 250 mg/l. This mixed water was used in all experiments. Mussels were held for 24 h and then transported to the laboratory for testing. In the laboratory, each gravid mussel was opened by cutting posterior and anterior adductor mussels; 1 gill was excised intact and placed in a petri dish with 5 cm of water from the Living Stream (13°C). This was

adequate water to immerse the gill, and to allow subsamples of glochidia to be extracted. The second gill was punctured with a large bore (20 gauge) needle and syringe, and glochidia were gently flushed with water into a petri dish. A subsample of glochidia from each dish was tested to confirm viability using a saturated solution of sodium chloride (Lefevre and Curtis, 1910; Zale and Neves, 1982). Live glochidia, normally in an open (gaping) position, will clamp shut when exposed to saline solution. Once viability was determined, dishes containing glochidia were randomly assigned to a temperature treatment (0°, 10°, and 25°C). Six replicate dishes were used for each of the three temperature treatments and for both extracted and intra-marsupial glochidia treatments, for a total of 36 dishes.

A second trial was conducted in January 2000, using *Actinonaias pectorosa* collected from the Middle Fork Holston River, Washington County, Virginia. This second trial was conducted in an identical fashion to the first, except that only extracted glochidia were tested. Eighteen gravid *A. pectorosa* were collected, and held in the Living Stream at 13°C prior to glochidial extraction. Since the intra-marsupial treatment was not replicated, sacrifice of the animal was not necessary. Glochidia were extracted by gently prying apart the valves, inserting the large bore needle into the water tube of one of the gills, and flushing it with water. Glochidia were collected in a petri dish, tested for viability (as described above), and randomly assigned to a temperature treatment. Six replicates of each temperature treatment were tested, for a total of 18 dishes.

Samples of glochidia from both species were tested for viability at 0, 0.5, 1, 3, 6, 12, 18, 24 h and at 6 h intervals thereafter, until 96 h post-extraction. To ensure an adequate number of glochidia for the duration of the experiment, the sampling interval was increased from 6 h to 12 h after 96 h. Observations continued for 320 h and 474 h for *Villosa iris* and *Actinonaias pectorosa*, respectively. At each observation interval, a subsample of approximately 75 to 100 glochidia was removed from each of the extracted glochidia treatments using a Pasteur pipette and then placed on a clean petri dish. Glochidia from the intra-marsupial treatment were extracted by inserting a large bore needle into the water tube and gently sucking out a small sample at each observation interval. Glochidia from all water tubes were assumed to be mature. From the subsample, an initial count of 25 glochidia was made, noting any closed individuals. A saturated solution of sodium chloride then was added, and after 10 s, the 25 glochidia were recounted to records the number of open and closed individuals. Individuals remaining open after the addition of sodium chloride were considered to be functionally dead, as were individuals closed before the addition of sodium chloride. These individuals may not have been dead; however, a closed or unresponsive glochidium is incapable of attaching

to host fish (Jacobson *et al.*, 1997).

To confirm the viability of glochidia after 1 week and 2 weeks post-extraction, a sample of glochidia from two randomly selected replicates of both the 10° and 0°C extracted treatments was used to infest known host fish and to confirm attachment to gills and metamorphosis. Glochidia from the 25°C trial and the within-gill treatment were not tested, as they were all dead at 1 week of testing. For the *Villosa iris* infestations, 8 rock bass (*Ambloplites rupestris*) collected from the New River (Pulaski County, Virginia) were infested. For *Actinonaias pectorosa*, 24 banded sculpin (*Cottus carolinae*) from the North Fork Roanoke River (Montgomery County, Virginia) were used. All fish were collected using a backpack electroshocker (Smith Root model 15-D). Zale and Neves (1982) identified rock bass to be a successful host for *V. iris*, and banded sculpin were confirmed as a host for *A. pectorosa* by J. Layzer (pers. comm., Tennessee Tech). Glochidia were pipetted directly onto the gills of the fish. Two fish were infested with glochidia from each of the treatments used, and fish from each treatment were held in separate 38 l tanks. The gills of the infested fish were examined on days 1, 2, and 3 post-infestation to ensure encystment of glochidia. Average water temperature in the tanks for the *V. iris* infestations was 24°C; temperature was maintained at 21°C for *A. pectorosa*. Once encystment was confirmed, the bottom of each tank was siphoned beginning eight days post-infestation, and the siphonate was passed through a 105 µm sieve to collect newly metamorphosed juveniles or sloughed glochidia.

Survival of glochidia was non-normal, non-continuous, and binary (alive vs. dead); therefore, data were modeled by logistic regression analysis (PROC GENMOD; SAS Institute, 1988). No correlation was necessary, as new glochidia were used at each sampling interval. Goodness of fit was evaluated using a deviance test (SAS Institute, 1988). Pair-wise differences between treatments were assessed using scaled Wald tests. The scale parameter increases the calculated Z statistic, thereby making probability estimates more conservative to compensate for any potential lack of fit to the model (DSKALE option, PROC GENMOD). The level of significance for all statistical tests was set at  $\alpha = 0.05$ .

## RESULTS

Survival of *Villosa iris* glochidia differed significantly between extracted and intra-marsupial treatments (Table 1). For both species, significant differences occurred between the 25°C and the 0°C treatments, and also between the 25°C and 10°C treatments (Table 1). However, there was no significant difference between the survival of extracted glochidia held at 0° and 10°C for either species.

**Table 1.** Number of hours that viability of glochidia remained above 75%. Times within a row followed by different letters were significantly different ( $p < 0.05$ ; Wald Z test).

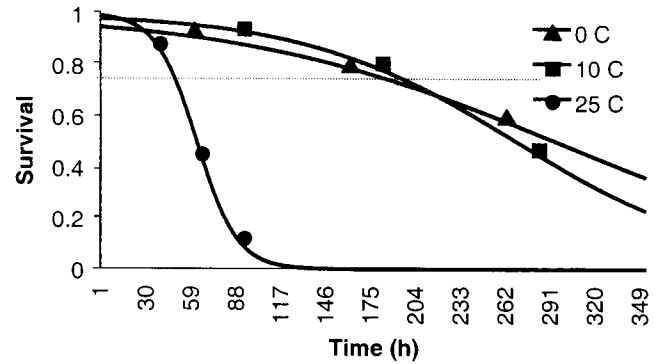
	Holding temperature (°C)		
	0°	10°	25°
Trials with glochidia			
<i>Villosa iris</i> (within gill)	41 <sup>a</sup>	98 <sup>b</sup>	28 <sup>c</sup>
<i>V. iris</i> (extracted)	180 <sup>a</sup>	192 <sup>a</sup>	46 <sup>b</sup>
<i>Actinonaias pectorosa</i> (extracted)	345 <sup>a</sup>	310 <sup>a</sup>	108 <sup>b</sup>

The viabilities of glochidia over time for *Villosa iris* and *Actinonaias pectorosa* were similar and consistent. In all cases, the 0° and 10°C treatments had similar trends, and greatly outlived glochidia in the 25°C treatments (Table 1). For comparison of treatments and species, we selected 75% viability as a lower threshold. The 75% viable cutoff is a somewhat arbitrary value; however, we consider it to be a practical threshold when using glochidia for infestations, where further mortality of glochidia would negate any benefits of *in situ* extractions. Extracted glochidia of *V. iris* maintained >75% viability for 180, 192, and 46 hr at 0°, 10°, and 25°C, respectively (Fig. 1). The intra-marsupial treatment maintained >75% viability for a shorter period; namely, 41, 98, and 28 hr at 0°, 10°, and 25°C, respectively (Fig. 2). After approximately 24 h, gill tissue and glochidia of the intra-marsupial treatments exhibited extensive bacterial growth and tissue decay. This decay and the reduced period of glochidial viability compelled us to omit the intra-marsupial treatment during our *A. pectorosa* trials. Viability of extracted glochidia for *A. pectorosa* remained >75% for 345 hr at 0°C, 310 hr at 10°C, and 108 hr at 25°C (Fig. 3).

Verification of the viability of glochidia was confirmed through subsequent host fish infestations. Glochidia transformed to juveniles in all *Villosa iris* treatments, from 13 days to 18 days post-infestation, and for *Actinonaias pectorosa* from 16 days to 23 days post-infestation.

**DISCUSSION**

Our experiments demonstrate that the duration of viability of glochidia is considerably longer than document-



**Fig. 1.** Model-generated survivorship curves for the extracted glochidia of *Villosa iris*. Dashed line indicates 75% viability.

ed in previous studies (Table 2). Results of these studies indicate that there is some variability in longevity among species, but all tested species maintain some level of viability for at least one week when held at cool temperatures. Tedla and Fernando (1969) tested glochidia of *Lampsilis radiata siliquoidea* (Barnes, 1823) and reported that few were able to infest host fish after 9 days at 10°C; none survived more than 24 h at 25°C. Murphy (1942) tested *Margaritifera falcata* (Gould, 1850) held at 11.2°C, and reported that some glochidia remained viable after 11 days. Matteson (1948) reported that extracted glochidia of *Elliptio complanata* (Lightfoot, 1786) remained alive after seven days when held at 4.5°C. Although these studies were not designed specifically to address life span of glochidia, they seem to indicate considerable variation among species. Differences in longevity and viability of glochidia in these studies emphasize the likely variability among species and its correlation with water temperatures. The longevity of viable glochidia differed greatly between the 2 species and 3 temperatures tested in our study. Extracted *Actinonaias pectorosa* glochidia remained viable almost twice as long as extracted glochidia of *Villosa iris* at the same temperatures, and both species showed significant differences in viability over time between the 0° and 10°C treatments and the 25°C treatment. Both species used in this

**Table 2.** Comparison of longevity of glochidia between previous studies and this study.

Mussel Species	Duration of viability (days)	Temperature (°C)	Viability (%)	Citation
<i>Margaritifera falcata</i>	11	11.2	not reported	Murphy (1942)
<i>Elliptio complanata</i>	7	4.5	not reported	Matteson (1948)
<i>Lampsilis r. siliquoidea</i>	9	10	1.2	Tedla and Fernando (1969)
<i>Villosa iris</i>	7.5	0	75	This study
	8	10		
<i>Actinonaias pectorosa</i>	14.4	0	75	This study
	12.9		10	

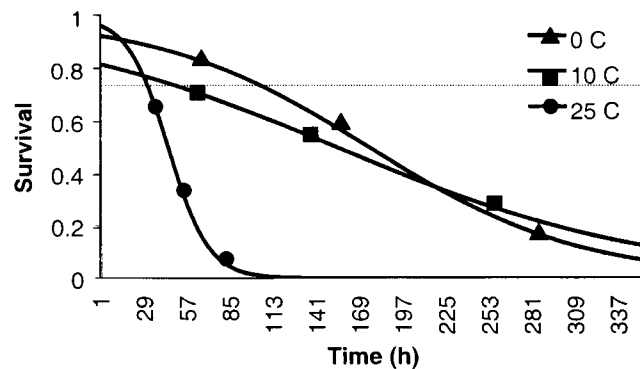


Fig. 2. Model-generated survivorship curves for the glochidia of *Villosa iris* retained within the gill. Dashed line indicates 75% viability.

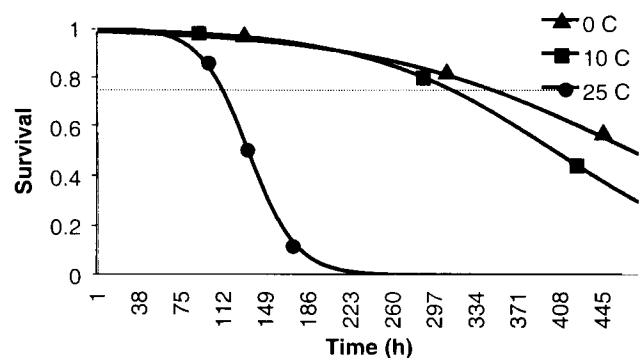


Fig. 3. Model-generated survivorship curves for the extracted glochidia of *Actinonaias pectorosa*. Dashed line indicates 75% viability.

study are bradytictic, with glochidia overwintering in the gills of a female mussel for up to six months. It is possible that tachytictic species (summer brooders) may have a shorter duration of viability.

The long-term viability of extracted glochidia has obvious implications for captive propagation. Production of juvenile mussels typically involves the collection of gravid females from the wild, and then transport to the laboratory where glochidia are extracted and used to infest host fishes. In light of our findings, it is not essential that gravid female mussels be transported from the collection site, as glochidia can be flushed from the gills by hypodermic syringe at streamside, returning only the extracted glochidia to the laboratory. This is particularly applicable to federally listed species, where handling stress, distance of travel, and other factors may jeopardize the health and survival of individuals (Chen *et al.*, 2001). If glochidia are extracted and transported in cooled water (0-10°C), they can remain viable for infestation for several days without reduction in attachment success. Removing glochidia on site eliminates transport stress to the gravid mussel and the need for a return trip to release specimens. It should be possible also to chill and

ship extracted glochidia to a propagation facility, such that infestations can occur at a distant laboratory or propagation facility. The time that glochidia remain viable also is useful when a gravid mussel aborts glochidia after removal from the substratum, during transport, or in captivity. As long as the mature glochidia are collected and held at cool temperatures, they can be used over an extended period to infest host fish.

Long-term viability of glochidia also is important from a life history perspective. Previously, longevity of expelled glochidia was thought to be approximately three days, providing limited opportunity for downstream drift, dispersal, and incidental contact with proximate fishes (Neves and Widlak, 1988). However, if glochidia remain viable for up to two weeks in cool water (spring or fall), there is a greater opportunity for dispersal of glochidia between suitable shoals where mussel aggregations and host fish typically occur. Greater distances of dispersal to contact host fishes would thereby extend the range and potential for genetic mixing between localized demes. These greater dispersal distances would be especially important for long-term brooders that release glochidia in spring when flow is high and water temperatures are cool. Further testing of viability of glochidia of other species, particularly tachytictic species, is needed to confirm lengthy periods of viability and the adaptive significance of this life stage to the reproductive success and genetic integrity of mussel populations.

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