PARTNERSHIP FOR THE DELAWARE ESTUARY
Science Group

Glochidia Extraction and Assessment

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Freshwater Mussel Tagging, Release, and Monitoring

Partnership for the Delaware Estuary (PDE) Freshwater Mussel Recovery Program Method
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Description

This Freshwater Mussel Recovery Program Method (FMRPM) describes the general conduct of glochidia extraction and assessment. A gravid mussel contains glochidia (freshwater mussel larvae) which may be extracted from the female in a few methods. Methods vary in disturbance to the mussel and least invasive methods are preferred. Prior to use in propagation efforts, extracted glochidia may be assessed for the percentage of viable larvae. This is done to estimate the number of larvae needed for desired infestation rates and track the efficiency of propagation.

Summary of Approach

The general approach is to determine preferred method of glochidia extraction based on project goals. Glochidia are extracted and assessed to determine the total number of glochidia extracted and the percentage that are viable.

Equipment

- Datasheet
- Pen
- Clipboard
- Syringe
- Lab Pure Water
- Reverse Pliers
- Corks (assorted sizes)
- Beakers (assorted volumes)
- Squirt Bottle
- Hand Towel
- 0.5-5 mL Pipettor
- 0.5mL Pipette Tips
- Petri Dishes
- Tally Counter
- Table Salt
- Dissecting Microscope

Procedures

1. Glochidia Extraction

1.1 Syringe Method

To extract glochidia using the syringe method, trained personnel must gently pry open the mussel. This is performed by holding the mussel by the dorsal end and finding a small opening at the ventral end of the mussel either towards the anterior or posterior end. Gently slide the reverse pliers into the opening between the valves. Holding the mussel with a towel will help with grip if wet. Apply pressure on the pliers and continue to push the ends of the pliers into the mussel to maintain a solid grip. Care should be exercised to avoid opening the mussel to the point of damaging muscle tissue. When the mussel shell is opened enough to clearly observe the inside tissues, place a cork between the valves to serve as a wedge while taking pressure off the pliers. Locate the inflated gills and insert a syringe into the gills gently moving back and forth while pushing water into the water tubes. Flush glochidia out of the water tubes of the gills

into a beaker or other large container filled halfway with lab pure water. Use a squirt bottle to flush out any glochidia on the mussel tissue. When extraction is complete, insert pliers gently and apply pressure to easily remove the cork. When cork is removed, slowly release pressure on pliers and allow mussel to close valves.

1.2 Warming Method

To extract glochidia using the warming method, remove the mussel from its temperature controlled environment and place into a container with its ambient water. Let the water approach room temperature. Mussels should release glochidia within a few hours but may take up to a day. If glochidia or conglutinates are not expelled, resort to alternative methods.

2. Glochidia Assessment

Once extracted, glochidia may be enumerated and assessed for their viability. This step provides critical data to inform propagation efforts and is strongly recommended.

2.1 Enumeration

To count the total number of glochidia, fill the container with glochidia to a known volume. Use a turkey baster or other tool to evenly mix and suspend glochidia into the water column. Simultaneously sample the water with a pipettor (e.g. 1 ml aliquot). Dispense the sample onto a petri dish. For accuracy, adjust sample volume so they contain 20-30 glochidia. Repeat for a total of five samples. Count all glochidia within each sample and determine the average count (AC) per sample. Follow the equation below to estimate the total number of glochidia extracted where T = the original volume of the container and S = the volume of one aliquot.

$$Total\ Glochidia\ Estimate = AC * \frac{T}{S}$$

2.2 Viability Test

To determine the percentage of viable glochidia, perform a salt test. Refer to 3.1 for sampling methods. Dispense five samples onto a petri dish. For each sample, count all glochidia (G) and closed glochidia (C). Glochidia may ‘snap’ closed for a brief moment but eventually open, these do not count towards closed glochidia. After counting each sample, add a few grains of table salt to each sample and wait until glochidia finish snapping closed and no glochidia are moving. Count the number of closed glochidia again (C’) for each sample. Use the following equation to determine the percent of viable glochidia in one sample.

$$Percent\ Viable\ Glochidia = \frac{C' - C}{G}$$

Average five samples to determine the average percent of viable glochidia and multiply by the total glochidia estimate to determine the total estimate of viable glochidia.