SUGGESTIONS FOR IMPROVING JUVENILE MUSSEL PROPAGATION SUCCESS

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Abstract

A well designed protocol is important for propagation of juvenile mussels. We conduct our studies in flow-through and static aquaria. Fish are generally infest fish with glochidia by placing fishes in a bath with several thousand glochidia under vigorous aeration. Aquaria are siphoned and siphonate checked for presence of juveniles one to three times a week. We have increased the survivorship of hosts and encapsulated juvenile mussel production by: (1) scrutinizing host-fish welfare just before and during juvenile mussel excystment, (2) conducting studies in flow-through aquaria, and (3) keeping tanks free of excess food. We have improved juvenile mussel recovery by: (1) holding small host fishes in suspended nets, (2) recording presence of pre-metamorphosed juveniles, (3) processing and washing the siphonate, and (4) increasing the number of individuals infested.

Introduction

- Many techniques developed in host suitability studies are applicable to juvenile mussel propagation.
- Procedures developed in the late 1800's and early 1900's (Brann 1878 cited in Watters 1994, Lefevre and Curtis 1912) have been refined into a standard protocol to obtain rare and common juvenile mussels from fish artificially infested with glochidia (*e.g.* Zale & Neves 1982).
- However, the rationale for operational details and considerations important in recovering juvenile mussels are not commonly discussed.
- The objective of this paper is to describe the rationale behind techniques developed for host suitability trials that are applicable to propagation of juvenile freshwater mussels.

Methods

- When conducting host suitability trials, our procedure generally follows the protocol described in Zale and Neves (1982).
- Test fish are held in holding aquaria (40 L or 400 L) at least 20 d prior to glochidia infestation, at room temperature (18-23° C).
- Hold gravid mussels in beakers in aquaria until glochidia released naturally.
- To determine the health of collected glochidia, a subsample is exposed to a 0.1-1‰ solution of sodium chloride. Glochidia are used if ≥ 70% of the sampled individuals close their valves upon exposure to salt.
- Fish are infested with glochidia by one of two techniques. Most fish species are exposed to glochidia by placing them in a vigorously aerated 1-3 L bath containing several hundred to several thousand glochidia. Fish are exposed to glochidia for 15 minutes to 24 hours, depending on the time required for approximately 5 - 50 glochidia to attach to the fish's gill lamellae. When the number of glochidia is limited or when test fish had fine gill rakers, fishes were infested by directly pipetting glochidia onto one set of fish gill holobranchs. After direct infestation, fish are held out of the water for 10 to 20 seconds to allow time for glochidia to attach to lamellae. Two to four hours after infestation, we check gills with a dissecting microscope and fins by naked eye to determine if infestation is successful.
- Infested fish are held in aquaria at 18° C± 3° C and fed one to three times per week. Fathead minnows are given to piscivorous fish once a week and removed from aquaria 5-10 minutes after introduction to minimize the possibility of their consuming juvenile mussels lying on the aquarium floor.
- Hold small test fishes (*e.g.*, cyprinids, etheostomids, catostomids, *etc.*) in suspended nets to prevent them from eating juvenile mussels.
- Aquaria are siphoned and siphonate checked for presence of glochidia and juveniles one - three times per week. After three consecutive searches fail to reveal a glochidium or juvenile mussel, a fish is anesthetized and searched for attached glochidia. If an attached glochidium is found, the fish is revived and the experiment continued. If a glochidium is not found the other fish are checked until either an attached glochidium is found, or glochidia are no longer attached to the fish.
- A mussel is considered to have reached the juvenile stage when foot movement, or paired adductor muscles and valve closure are observed.

Results and Discussion

Encapsulated juvenile

The following recommendations for conducting host suitability studies applicable to juvenile mussel propagation are a compilation of experiences gained by Mark Hove at Virginia Polytechnic Institute and State University and refined by the authors at the University of Minnesota, and from articles published on glochidia transformation studies.

Considerations for conducting host suitability tests
A well-designed protocol is important for propagation of juvenile mussels. We have found the following points important in successfully recovery of juvenile mussels:

Initiation of study
To avoid testing fish that have partial or full immunity to glochidial infestation of the mussel species under investigation, collect fish from: (1) lakes or streams without any mussel species, (2) waters with mussel species in a different genus from the mussel species of interest, or (3) streams and lakes without the mussel species you plan to study. Although some fishes repeatedly facilitate transformation of glochidia (Arey 1932, Lux 1993), and there is evidence that glochidial transformation rates are greater for fishes co-occuring with mussels than for fishes outside of the watershed (Rasnick and Barnhart 1998), immunity in the host has been shown to build with repeated exposures, thus lowering juvenile mussel transformation rates (Reuling 1919, Arey 1932, Bauer and Vogel 1987, O'Connell 1991, Watters 1996b). Additional work is needed to determine the effect acquired immunity plays in the glochidial transformation success.

Holding facilities
A well-designed testing facility can improve the recovery of juvenile mussels from infested fishes. Fish are put under stress when they are handled, inoculated with glochidia, and moved to a holding tank. Following basic fish husbandry techniques is important (Piper *et al.* 1983). The following practices have improved both survivorship of test fish and juvenile mussel recovery in our studies:
- o If possible, conduct tests in labeled aquaria with a single-pass or recirculating water replacement system. Adequate aeration is also important. Water temperature can influence the production of juvenile mussels (Yeager and Saylor 1995). Sound fish husbandry practices are especially important near the excystment period. We have observed that fishes may be more susceptible to secondary diseases (*e.g.* "Ich" or *Ichthyophthirius multifiliis*) at this time. Prior to juvenile excystment there may be an increase in the number of sloughed glochidia, or pre-metamorphosed juveniles (Kitchel 1985), found in the tank.
- o Feed fish high quality food to satiation and remove it promptly at cessation of feeding. Juvenile mussels will occupy clumps of debris that can form quickly as fungus grows on uneaten food. This hampers counting and collection of the juveniles. Larger fishes can be fed minnows purchased from a bait store. It is important to remove uneaten minnows soon after the larger fish have stopped feeding. Not only could juvenile mussels excyst from naturally infested feeder minnows, but the minnows may consume juvenile mussels lying on the aquarium floor (Hove 1996).
- o Hold catostomids, cyprinids, and other small fishes in suspended nets. In early trials we recovered fewer glochidia and juveniles from the floors of aquaria holding catostomids and cyprinids. We assumed that these fishes were consuming these small life stages. We now hold our small and sucker-mouthed test fishes in 1/16" mesh, plastic nets. The nets are suspended approximately 10 cm above the bottom of the aquarium to facilitate siphon-cleaning of the tank floor (Fig. 1). Suspended nets are superior to floor nets, in that the latter are difficult to siphon under and test fish frequently get trapped beneath the net while the tank is being siphoned. Note that Tedan and Hoeh (1986) have developed an ingenious test chamber that has a screen floor but avoids need for siphoning to recover juvenile mussels. Although we have not used this test chamber, an advantage of this design is rapid collection of settled juveniles through a drain in the bottom of the chamber.

Inoculation of hosts
Consider the following points when selecting between two techniques for inoculation of fish:
- o Application of glochidia with a pipette. Two advantages of this technique are: (1) it generally ensures that glochidia will attach to the test fish, and (2) it may be the only option for infesting fishes that can avoid glochidial infestation during respiration (*i.e.* - those fishes with fine gill rakers or small mouth openings). Two disadvantages of this technique are: (1) it is much more time-consuming and effort-intensive than placing fish in a suspension of glochidia, and (2) it is more stressful to the fishes.
- o Application of glochidia through placement of fish in a glochidial suspension. This technique allows the researcher to expose many fishes to glochidia in a short period of time. Its pitfalls are: (1) that glochidia may attach at extremely low levels to test fish or not at all, especially if water in the exposure tank has a large amount of fish slime or other organic suspensions; and (2) that large fish may feed on small fish during the glochidial exposure period.

Inoculation of hosts (continued)
Consider the following points when inoculating potential hosts:
- o Check test fish for natural glochidial infestation before inoculation.
- o Make sure temperature of holding aquaria for fishes and glochidia are same prior to inoculation.
- o Avoid mortalities of test fish due to excessive glochidial infestation by lightly infesting the fish (e.g. 5-10 glochidia for darters and small minnows, 50-100 glochidia for fish 15-25 cm in length). If the objective is to produce large numbers of juvenile mussels, we recommend increasing the number of infested fish rather than increasing the infestation rate on individual fish.

Juvenile collection
The following points should be considered when collecting juvenile mussels:
- o Before feeding fish, siphon tanks to remove juvenile mussels and again after feeding fish brine shrimp to prevent fungal growth on uneaten food.
- o Brass, 8" diameter, standard testing sieves are useful for collection of juvenile mussels. Use sieves with 20 – 200 µm mesh depending on the size of the juveniles you expect to recover. The wide bottom of these sieves allows a light build-up of detritus without clogging to the point of water overflow. Cheaper sieves can be constructed by gluing fine-mesh plastic netting to a section of PVC pipe.
- o Clean the sieve after each use by rinsing with water and wiping with a cloth to avoid accidental transfer of juvenile mussels from one trial to another. Hooked larval mussels are especially prone to attaching their teeth in the sieve's mesh.
- o To facilitate effective collection of juvenile mussels during tank siphoning we recommend constructing an apparatus to hold the outflow end of the siphon just above the collection sieve (Fig 3). Dedicating a free hand to this effort will distract the collector from efficiently siphoning the tank floor and may result in siphonate running onto the floor instead of through the sieve. Inserting an in line filter into the siphon hose is also an effective way to collect juvenile mussels (Rasnick and Barnhart 1998).
- o Consider the fish species ability to withstand handling stress, and gill excess when selecting protocol to recover juvenile mussels. Standard protocol for obtaining juveniles from intensively cultured fishes involves regular removal of fish waste or excysted juveniles from the aquarium floor and examination of fishes for attached glochidia (Yokley 1972, Zale and Neves 1982, Yeager and Saylor 1995). However, frequent handling of some fishes (*e.g.* malnourished or large fish, certain herrings, shiners, silversides, and sculpins) may lead to disease outbreaks. Also, access to the gills of some fishes (*e.g.* lampreys, and some catostomids and cyprinids) may be difficult and searching for attached glochidia on these species may be particularly stressful. When testing these fishes, increase the number of individuals tested to compensate for expected handling mortality, or minimize gill inspection.

Searching of siphonate for juvenile mussels
Juvenile mussels may be more effectively removed from siphonate material after considering the following suggestions:
- o A gridded petri dish, fine dissecting probe, and glass pipette are effective tools for isolation, manipulation, and collection of individual juvenile mussels. We have found that juvenile mussels and glochidia are more likely to adhere to plastic pipettes than to glass ones.
- o To reduce siphonate processing time: (1) agitate the siphonate container to suspend contents, (2) allow glochidia to settle, and (3) decant remaining suspended material (Figure 3). This technique, however, is not effective for separating small glochidia (*e.g. Cumberlandia, Tritogonia, Quadrula, Truncilla, Leptodea*) from other suspended organics due to similar settling rates.
- o To improve observation of larval mussels under a microscope use fiber optic light guides or cross-polarized light. Direct light with fiber optic guides onto the *sides* of a petri dish rather than from above to provide a greater contrast between the transparent mussel valves and black microscope stage (Figure 4). Cross-polarized light is also an effective way to visualize transparent, prismatic objects (Johnson 1995, Watters 1996a).
- o Juvenile mussels may be retrieved from test fish from natural infestations. We recommend storing these mussels in ethanol for future identification. Recent developments in molecular biology will likely allow species identification of these small animals in the near future (White *et al.* 1994).

Study conclusion
Consider the following suggestions when concluding your study:
- o Report collection locations of mussels & fishes used in study, deposit voucher specimens at a museum
- o Report water temperature and glochidial transformation period. Water temperature influences juvenile mussel production and transformation rates (Neves *et al.* 1985, Yeager and Saylor 1995).

Conclusion

We have refined a systematic and easily replicated protocol for propagating juvenile mussels.

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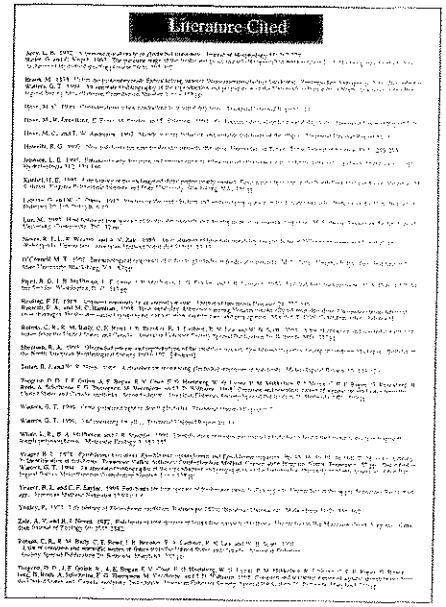

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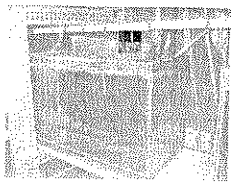

Figure 1. Small fish held in suspended net to prevent them from eating juvenile mussels.

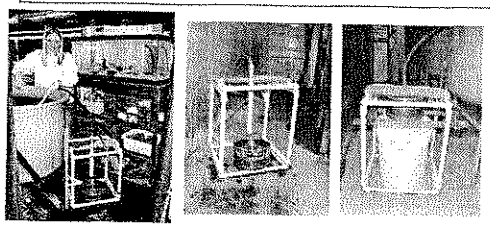

Figure 2. To facilitate effective collection of juvenile mussels during tank siphoning we recommend constructing an apparatus to hold the outflow end of the siphon just above the collection sieve.

Figure 3. To reduce siphonate processing time: (1) agitate the siphonate container to suspend contents, (2) allow glochidia to settle, and (3) decant remaining suspended material.

Figure 4. Direct light with fiber optic guides onto the *sides* of a petri dish *rather than* from above to provide a greater contrast between the transparent mussel valves and black microscope stage.

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