

**Operational Procedures and Techniques for the
Artificial Infestation of *Lampsilis higginsii***

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The Higgins Eye Pearly Mussel (*Lampsilis higginsii*), a once widespread mussel of the upper Midwest, has most recently become the subject of federal and state restoration efforts. Federally endangered since 1976 this mussel has suffered noted reductions in population density and range throughout most of its former habitats. Environmental factors such as habitat changes and invasive species continue to reduce known populations and further threaten this species with extirpation in many areas. One strategy to enhance threatened populations of this species, or expand the fragmented range of this once widespread mussel is through the use of artificial propagation. The artificial infestation techniques and suitable host relationships of many North American freshwater mussels were established early in the 20th century. Specific work with *L. higginsii* can be found as early as the 1910's (Surber, 1913), with work continuing through the early decades of that century. (Wilson, 1916, Coker et al, 1921). Strong research was discontinued until the 1980's when several papers looked specifically for host suitability and transformation rates. (Sylvester et al, 1984, Waller & Holland-Bartels, 1988). Further advancement in infestation techniques have continued during the last century and have led to a degree of consistency in laboratory and hatchery trials. This operating procedure is based on techniques supported by the literature as well as personal communication with biologists and researchers currently practicing in the field.

Host Fish

Several species of Centrarchids as well as Percids are known host for *L. higginsii*. Largemouth Bass, (*Micropterus salmoides*) Smallmouth Bass, (*Micropterus dolomieu*), Walleye, (*Stizostedion vitreum*) and Sauger (*Stizostedion canadense*) are all suitable host for this species, with some variability in transformation rates (Waller & Holland-Bartels, 1988). Species utilized at Genoa NFH include Largemouth Bass, Smallmouth Bass, and Walleye. Success rate for transformations at the facility have ranged as low as 22% for small walleye up to 88% for fingerling largemouth bass.

Condition of host fish must be considered before use as a potential host. Fish weakened or physically compromised during culture, capture, transport or enumeration should not be selected for inoculation due to the low probability of subsequent survival after parasitism. All known techniques to reduce stress during the fishes time in captivity should be observed. Factors such as temperature, light levels, overcrowding, and excessive or rough handling all contribute to a cumulative stress response in host fish and should be managed to keep stresses to a minimum. Fish should be held in temperatures well within their optimum growth range whenever possible. Light levels should be reduced to as much as feasibly possible. Water quality parameters such as dissolved oxygen, CO₂, pH, and ammonia should be kept well within species tolerances. Fish showing overt signs of physical trauma or significant abrasions should not be selected as a host due to the likelihood that a subsequent secondary infection may develop resulting in the loss of the fish as well as its glochidia complement. Transformation times vary in relation to temperature and it is feasible that host fish may carry their glochidia infestation from several weeks up to multiple months. For this reason fish health concerns are of the utmost importance and should be considered one of the critical factors for any successful operation.

Donor Mussel Selection and Care

Selection of donor female *L. higginsii* for glochidia harvest should be undertaken with care and forethought. Improper handling or care while in captivity can have negative impacts on survivability of donor mussels as well as glochidia attachment rates. Only qualified biologists with extensive field experience should identify potential donor mussels as to species. The potential for inexperienced personnel selecting similar species of lampsiline mussels, (*L. siliquoidea*, *L. cardium*, *A. ligamentina*) does exist, this may be especially true in the light of recent discoveries of *L. siliquoidea* “look alikes”.

Selection of donor female *L. higginsii* should be based initially on those individuals actively engaged in mantle display activities. Selection of such individuals should minimize the number of animals that must be examined internally for gravidity. This pre-selection may not be practicable in areas of high water turbidity in which case all female *L. higginsii* may be examined until needed numbers of donors are collected.

Storage and transportation of *L. higginsii* should always be carried out in water from the site of collection whenever possible. Temperature should be monitored and not allowed to vary significantly from initial collection values. Small amounts of non-chlorinated ice may be added during transport to maintain consistent temperatures. Aeration should be provided to maintain O₂ saturation at or near 100%. Successful transportation of gravid females has been carried out utilizing a suitable sized insulated container containing 3"-4" river sand with a small external air pump maintaining saturation near 100%.

Caution should be taken to maintain transported animals in as stable environment as possible. Large fluctuations in temperature, physical shocks, and degrading water quality parameters can all induce abortion of glochidia. While *L. higginsii* have proven in transportation events to not be as sensitive as some Amblemine species, loss of glochidia has been observed in gravid individuals. Those responsible for maintenance of animals at holding facilities, isolation units, or short-term stockpiling sites should keep environmental parameters as stable as practicable. Changing water quality parameters such as those caused by utilizing various water sources, or moving animals between water sources repeatedly has been shown to induce spontaneous abortion of gravid Unionids. Small fluctuations in pH can be very stressful to mussels and should always be considered a factor when transferring mussels from one water supply to another. Temperature gradients between holding waters should be kept to a minimum. When large differences exist in temperature between holding environments a tempering process should be used to bring the transportation or donor water to within 3°C before transfer. Rate of tempering should not exceed 2°C over a 5 minute period. All prescribed procedures and precautions should be observed to prevent the inadvertent transfer of any life stages of *Dreissena polymorpha* between any waters. Operational procedures utilized by this facility are covered in the stations Zebra Mussel Control Protocol.

L. higginsii is bradyctictic in nature and is known to produce viable glochidia during May and September (Baker, 1928). Collection and transportation of donor mussels is similar during the spring and fall with no significant differences recognized. Potential differences to infection

operations to consider are time of excystment for juvenile mussels and availability of proper donor fish hosts. Lower water temperatures during the fall and early winter will lengthen the time required for larval mussels to mature and excyst. This is only true in open water systems or strategies using natural environs, (cages, free ranging fish). Caution should be taken in manipulating the temperature significantly in fall incubations from the natural riverine environment if plans call for reintroduction of resultant juveniles into said natural environment during winter months. Food availability and selection, as well as temperature stressors may inhibit growth and survival of juvenile mussels that are exposed to significant temporal shifts in natural life cycle rhythm. Current propagation trials and techniques have not shown the ability to culture the juvenile life stage of this organism in an artificial environment for extended time periods.

Field Infestation Techniques

Operational procedures for field infestation of host fishes by *L. higginsii* are similar to those utilized in more controlled environs such as those found in the laboratory or hatchery setting. Equipment requirements are similar and are composed of materials familiar to most hatchery or fishery management wet laboratories. A complete infestation kit can be contained in a single 75L tote, (see Appendix I). Differences in field and lab operations do exist in regard to nature of operational environment, host fish, and scale of infestations.

Field inoculations face many challenges not present in more controlled exercises. Factors such as weather, river conditions, and lack of services all present unique challenges to field personnel. Prior planning and contingency plan development are imperative to successful operations and should not be underestimated. Another challenge faced by field operations is the availability of suitable host fish. Unless propagated fishes are to be used for inoculation in the field, most fish will be collected from the area in which the exercise is being conducted. This presents several challenges not encountered in the laboratory environment. The number, species composition, current parasite load, and general health status of wild fish will not be known until just prior to infestation events. Long term collection efforts, to minimize the impacts of some of these unknowns are not recommended due to detrimental effects captivity can have on wild fishes. Collection efforts should be limited to no more than 48 hours prior to infestation events for smaller fish, (>225mm) and should stop at least 18-24 hours before inoculation. Injuries to potential host fish sustained during capture events should be ascertainable within 18-24 hours post collection. This period should give personnel the opportunity to select those fish most likely to carry a glochidial cohort to transformation. As stated earlier, only those fish that have the most likely possibility of survival after inoculation should be used as hosts. Although not practical for large infestation events, prior parasite load of host fish should be considered when making selections for use in inoculation events. It has been suspected that prior exposure to glochidial as well as copepod infestations may adversely effect attachment rates of glochidia (Howard 1914, Reuling 1919). This relationship has not been established for all species of freshwater Unionids, and has not been demonstrated in *L. higginsii*, but it is not suggested to utilize those host fish exhibiting infestations of glochidia or copepods.

Two methods of transporting glochidia for use in field infestations are available. The glochidia may be removed from donor mussels and transported in cool oxygenated water for immediate use at the inoculation site, (within 24 hrs.) or the donor mussel may be transported to the site of inoculation for glochidial harvest immediately preceding infestation. Both methods have advantages and disadvantages and every event should be evaluated as to what method optimizes survival of glochidia as well as donor female mussels. Glochidia have been harvested and held at moderate temperatures, (8-12°C) for over 24 hours without significant loss of viability. The remote collection of glochidia from natural environments for use in distant research laboratories has been successfully demonstrated by many investigators during the past quarter century. Pre-harvesting of glochidia is a viable option if there are expressed concerns over the ability to transport donor mussels between sites or if donor mussel survivability is in question. The availability of qualified personnel for the harvesting of glochidia may also be of concern if the inoculation site is remote or experienced personnel are not available on site to safely harvest larvae without undo injury to donor animals. Disadvantages to pre-collection of glochidia may be realized when vagaries of field operations present delays in scheduling or other factors out of control of project personnel. Inclement weather, personnel, loss or unknown status of host fish, and random events can all lead to less than optimum conditions in which to carry out operations. Pre-collection of larval mussels may not be the most prudent method to ensure a successful inoculation if the possibility of delays or cancellation exist. Unlike laboratory trials the exact number of host fish, as well as their condition may not be known until just prior to the infestation event. The ability to predict the required amount of viable glochidia for use during any trial is made more difficult by time and distance. The potential to over harvest glochidia from donor populations is a possibility and may lead to the “wasting” of thousands of larval *L. higginsi*. Given the preceding rationales it is this facilities policy to endorse the transfer of gravid donor mussels to onsite infestations whenever practicable. This facilities past experiences with mussel transfers, as well as our understanding of the challenges presented during field work, have guided this decision.

The infestation process is similar to laboratory procedures and follows established methodologies for artificial inoculation of Unionidae glochidia as described by many authors (Levevre and Curtis 1910, Young 1911). Differences in procedure relate primarily to scale of individual inoculations. Whereas in more controlled environments, (lab, hatchery) smaller inoculation baths with fewer host fish per bath are the typical practice, field operations tend to inoculate larger numbers of host fish in fewer glochidial baths. Disadvantages to larger inoculation episodes include less control of infestation, less consistency in infestation rates among hosts, and a larger probability of glochidial attachment to biological contamination (slime, feces) or non-gill areas. Advantages are limited generally to less manpower requirements and speed of infestation project. Managers and biologists must weigh lack of control/efficiency factors against the realities of field operations. A stepwise guide to general inoculation procedures is outlined in the following outline:

1. Prepare and sort potential host fish for inoculation procedure by size/species if possible. It may be advantageous to subject fish to 5000-7500ppm NaCl dip 15 minutes prior to inoculation to remove accumulated slime and debris from gill surfaces.

2. Open donor mussels with reverse pliers **slowly**. Do not open more than 20mm for medium/large individuals, less for smaller mussels. Opening mussels too quickly or overly wide may crack valves or rip abductor muscles. Prop valves open with rubber or cork stopper or similar object. Caution should be observed to not damage internal organs, labial palps or gill structures.

3. Inspect outer gill structure for presence of stored glochidia. Marsupium should appear swollen and dark in color in *L. higginsi* when gravid. Perforate margin of marsupium with sterile hypodermic syringe, (20 ga.) across several tubules. Insert needle through perforations dorsally, being careful not to damage gill structures within marsupium. Express glochidia by flushing gently into clean basin or other shallow container. Use water from containers or holding units containing donor mussels to prevent ion imbalances that may cause premature closure in glochidia. After first small sample of glochidia are removed test for viability prior to removing significant quantities. Testing entails placing a small subsample of glochidia in a petri dish and introducing several grains of NaCl to the area within the field of view while under adequate magnification. Mature glochidia appear well formed with sharp C shape discernable. Upon introduction of NaCl viable glochidia should “snap” shut quickly and consistently across the field as the salt concentration reach critical levels. A slow reaction, lethargic or incomplete closure or inconsistency of sample are all indicative of non-viable or immature glochidia. If sample appears composed of viable/mature glochidia continue harvest of perforated tubules. Repeat across glochidia bearing tubules until required numbers are harvested or tubules empty. Be as non-invasive as possible and do as little damage as circumstances allow.

4. After adequate numbers of glochidia are harvested to satisfy both numerical as well as genetic considerations of project, donor mussels should be returned to holding facilities supplied with high quality water at or near saturation. Facilities should be shaded, maintained at consistent temperatures, and not subject to physical shocks or movements.

5. Infestation containers should be of adequate size to accommodate host fish in a free swimming condition. Density index's used at Genoa NFH range approximately 700 g/L for 200 mm fingerling walleyes and 300 g/L for 115 mm largemouth bass. Densities this high are stressful to temperate fishes even with adequate aeration and should not be maintained for extended periods of time. Infestation containers will be equipped with aeration in the form of compressed air or oxygen. Paddle type or other mechanical aeration equipment do not provide the necessary lift to keep the glochidia in suspension without negative physical effects to the larvae. Air delivery systems should be kept simple and not as large as to provide dead spots below head, (flat micropore stones allow to settling around bases).

6. Glochidial mixture should be gently homogenized and subsampled to determine approximate number of larvae/ml. This can be done quickly and efficiently with the use of a plankton wheel and dissecting scope in a matter of minutes. Once a confident number has been established a test infestation may be carried out. A formula that Genoa NFH has used in the past to ensure adequate infestation is an inoculation rate of ~40-50% for introduced glochidia in a 5 minute bath when carried out at the above prescribed concentrations. Upon completion of a satisfactory test, infestation adjustments can be made in amounts of glochidial aliquot per liter.

7. Calculated amount of glochidial mixture should be introduced to infestation container prior to introduction of fish if possible. Introducing fish prior to glochidial sample can lead to the release of excess mucus and feces into the water which will reduce the efficiency of attachment for larval mussels. The release of undilute glochidial samples onto the host fish can also lead to over infestations in some individuals while reducing the larval load of others. Consistency of inoculation is important if host fish survival as well as numerical evaluation are a priority.

8. Host fish should be checked every minute for infestation rate or as often as practicable to ensure over infestations are not occurring, as well as to ensure adequate rates are being achieved. Rates of attachment of 100-150 for 115 mm largemouth bass or 200 mm walleye have not proven excessive in hatchery trials. Reaction to infestation is a relation of several factors including size, fish health condition of host, rate of infestation and environmental factors. Fish larger than those described can support larger glochidial cohorts with smaller or stressed fishes the inverse. A general guide is to restrict attachment rates to an area less than 5% of the total gill area of the host. This allows for adequate respiration/encystment sites without seriously compromising the fish.

9. Once infestation rates are achieved fish should be removed from container and placed (gently) in a holding area that is provided with high quality water, well within the preferred tolerances of the host fish species. Fish should either be immediately released or held for a minimum of 24 hours to allow complete encystment of attached glochidia. Rough or multiple handlings have the potential to dislodge newly attached larvae and should be kept to a minimum. If immediate release is the option of choice, sample of each inoculation event should be reserved. These samples should be allowed to survive for a minimum of 24 hours but preferable 72 hours to more accurately predict actual attachment rates of released fishes. Samples should be preserved in 90% ethanol for later analysis.

10. Upon completion of project donor mussels should be returned to initial collection sites after an adequate genetic sample is collected from each female harvested. Samples should be preserved in 90% ethanol and labeled sufficiently.

Appendix 1.

Materials required for field inoculations

Dissecting Microscope or similar magnifying device
Reversing pliers
Petri Dishes
Syringes
20 ga. needles
Wash bottles
Rubber stoppers
Air Pump
Shallow harvesting dish
10 ml pipettes
Inoculation container(s)
Plankton wheel
Small dip nets
Fish sample bottles
90% ethanol
NaCl

Selected References

- Baker, F.C. 1928. The Freshwater Molluska of Wisconsin. Wisconsin Geological and Natural History Survey Bulletin 70: 1-495.
- Coker, Robert E., Shira, A.F., Clark, Howard W., and Arthur D. Howard. 1921. Natural history and propagation of freshwater mussels. Bulletin of the U.S. Bureau of Fisheries [Document 893] **37**: 75-181
- Howard, Arthur D. 1914. Experiments in propagation of fresh-water mussels of the *Quadrula* group. Report of the Commissioner of Fisheries for the Fiscal Year 1913, Appendix 4 [Document 801]: 1-52; 6 pls.
- Lefevre, George and Winterton C. Curtis. 1910. Experiments in the artificial propagation of fresh-water mussels. Bulletin of the U.S. Bureau of Fisheries [document 671] **28**: 651-626
- Reuling, F.H. 1919. Acquired immunity to an animal parasite. Journal of Infectious Diseases **24**:337-346
- Surber, Thaddeus. 1913. Notes on the natural hosts of fresh-water mussels. Bulletin of the U.S. Bureau of Fisheries [Document 778] **32**:101-115; pls. 29-31
- Sylvester, Joseph R., Holland, Leslie E., and Thomas K. Kammer. 1984. Observations on burrowing rates and comments on host specificity in the endangered mussel *Lampsilis higginsii*. Journal of Freshwater Ecology **2**: 555-559
- Waller, Diane L. and Leslie E. Holland-Bartels. 1988. Fish hosts for glochidia of the endangered freshwater mussel *Lampsilis higginsii* Lea (Bivalva: Unionidae). Malacological Review **21**: 119-122.
- Wilson, Charles B. 1916. Copepod parasites of fresh-water fishes and their economic relations to mussel glochidia. Bulletin of the U.S. Bureau of Fisheries [Document 824] **34**: 331-374; pls. 60-74.
- Young, Daisy. 1911. The implantation of the glochidium on the fish. University of Missouri Bulletin, Science Series **2**: 1-16, 3 pls.