# *Anodonta* Propagation Studies: Determination of Mussel Sexual Maturity and Glochidia Release Agents

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ABSTRACT

The objectives of this study were to develop a non-destructive technique for assessing maturity in *Anodonta sp.* and induced gonadal release, both of them basic knowledge for larviculture process. Gonad condition of different sized individuals was assessed through dissection and microscopal examination. Glochidial release was tested using the following agents on standardized size (5.5 - 7 cm) mussels: a) KCL (0.5 Mol.) injection in the mantel cavity at 8 different concentrations (0.5, 0.1, 0.15, 0.20, 0.25, 1.0, 1.5, 2.0 ml/ind.; b) Oxytocin injection in the mantle cavity at 6 different concentrations (0.05, 0.1, 0.15, 0.2, 0.25, and 0.3 ml/ind.); c0 Immersion in alkalinized hydrogen peroxide solution (170 mg/l); d) Thermal shock (38-40°) at 8 different exposure times (10, 15, 20, 30, 35, 40, 45, 60 min); e) Punctures in the abductor muscle. Ripeness assessment without killing the animal was achieved by opening mussel valves with forceps and observing marsupia coloration. White was associated to unfertilized ova; brownish gray to immature glochida and reddish brown to mature glochidia. Glochidia release agent tests showed that oxytocin injection at 0.15 - 0.20 ml/ind. was the best release agent, followed by punctures at the abductor muscle. Other agents failed to induce glochidia release or resulted in mature and immature glochidia release surrounded by a gelatinous matrix, which restrained glochidia movement and hence subsequent fish infestation.

## INTRODUCTION

Information regarding induced reproduction in freshwater bivalves is actually limited to one report on gamete release in zebra mussels (*Dreissena polymorpha*) via serotonin injection. In unionids the reported method for obtaining glochidia was excision of the marsupia from gravid female mussels. This contrasts with the large amount of information available for marine bivalves.

The objectives of the research were to determine sexual maturity in *Anodonta* and to develop a technique to induce its reproduction.

## MATERIAL AND METHODS

### Gravid Mussel Determination

In order to perform experiments on induced reproduction, a stock of gravid females were needed. Due to the lack of information regarding methods to ascertain reproductive stage in freshwater bivalves a technique for teh

determination of mussel reproductive stage, eventually without killing the animal, was required. Observations and dissections of large numbers of individuals were made under microscope and stereoscope in order relate the stage of development of the glochidia with any peculiar characteristic of the soft parts or shell of the mussel.

**Inducing Agent**

Because there is a lack of information regarding techniques for induced glochidia in unionids, procedures already documented for marine bivalves were used as a starting point. However, these data refer to gametes, not glochidia release. Different agents of varied nature (chemical, physical) were employed on standardized size (5.5 - 7 cm). Each test was performed in quintuplicate using 2L aquaria (1 mussel / aquarium).

Potassium chloride injection. RC1 was injected in the mantle cavity with a syringe, following the methods of Iwata (1951). Eight different concentrations (0.5, 0.1, 0.15, 0.20, 0.25, 1.0, 1.5, and 2.0 ml/mussel) were tested.

Alkalinized hydrogen peroxide. Water pH was raised to 9.1. Hydrogen peroxide was added immediately at 170 mg/l and each mussel introduced into this solution, following Morse *et al.* (1977).

Hydrogen peroxide to mussels acclimated to pH 9.1. This procedure was a modification of the previous one, since it was postulated that mussels needed to adjust to the pH change. Each mussel was introduced to an aquarium with the pH already adjusted to 9.1 and acclimated to the new pH. The amount of time for acclimation was adjusted to 1 hour considering that the initial pH was between 8.5 to 8.7. Hydrogen peroxide was added lately in the same proportion as the previos experiment.

Oxytocin injection. Oxytocin was injected in teh mantle cavity at 6 different concentrations (0.05, 0.1, 0.15, 0.2, 0.25 and 0.3 ml/mussel.). This muscular physiology modifier was chosen among others because of its ease of acquisition in local markets.

Thermal shock. Water was heated to 38-40°C on each aquarium with a 500 W thermostat. One mussel was placed immediately. Temperature was recorded with a thermometer. Eight (8) different intervals (10, 15, 20, 30, 35, 40, 45, 60 min.) were teseted. After the procedure was finished the mussels were removed and placed in individual aquaria at ambient temperature (29.5°C ± 1).

Punctures (physical trauma). Punctures at the abductor muscle were performed with a sterile needle.

## RESULTS AND DISCUSSION

**Gravid Mussel Determination**

Using a microscope and stereocope plus dissections a simple technique for the determination of mussel ripeness, without killing the animal, was developed.

The procedure consisted of opening the mussel valves with a forceps and observing the color of the marsupia. White color indicated unfertilized ova. Brownish gray color indicated immature glochidia and reddish brown color indicated mature glochidia. The latter was the ideal condition for spawning. This technique allowed the selection of only gravid animals with a higher degree of maturity for the inducing agent experiments and subsequent experiments which required mature mussels.

Results suggest that sexual maturity in *A. grandis* could be reached at an approximate length of 5 cm. (before 1 year).

**Proper Inducing Agent**

A summary of the effects of the different inducing agents is shown in Table 8. Potassium chloride showed negative results in all tests. Negative results for this agent had been previously reported in the giant clam, *Tridacna gigas* (Gwyther and Munro, 1981).

Alkalinized hydrogen peroxide caused an almost immediate eight valve closure, preventing the normal filtration activity. Mussel previously acclimated to pH 9.1 and then submerged in hydrogen peroxide exhibited the same response as those in the previous test.

In the case of oxytocin, dosages of 0.1 ml or less did not cause appreciable reaction. Positive results were observed between 0.15 and 0.20 ml oxytocin/mussel (4-10 µl/g), evidenced by a massive release of mature glochidia. Higher dosages caused tight valve closure with no filtration activity or glochidia release. The mechanism of action for oxytocin is dissimilar to effects of the widely used serotonin (not tested). Oxitocin is a muscular stimulant (Goodman, 1986) while serotonin produces muscle relaxation and increases epithlial activity in bivalves (Gardiner *et al.*, 1991).

All thermal shocks under 60 minutes resulted in a very reduced number of glochidia being released. Thermal shocks greater than 60 minutes induced release of glochidia in higher numbers. However, they were invariably accompanied by a mucous matrix that contained great amounts of immature glochidia (abortion). This matrix restricted the free movement of the mature glochidia, hence making infestation impossible.

Punctures showed positive but not satisfactory results since the amount of glochidia was minimal (avg = 1920/ml) compared to oxytocin (avg = 18,000/ml). A combination punctures / oxytocin (0.15-0.20 ml/mussel) proved to be no better than oxytocin alone (avg = 19,350/ml).

## CONCLUSION

Mussel ripeness could be determined by opening the valves of the mussel and observing the color of the marsupia. Mussels ready for glochidia release showed a reddish brown marsupia.

Oxytocin injection in the mantle cavity at 0.15 - 0.20 ml for 5.5 - 7 cm mussel. (= 4- - 10 µl/g) produced the best results of all inducing agents tested. This paper is the first report on the use of this chemical for bivalve spawning purposes.