Culture of *Anodonta cygnea* freshwater mussels under a combined "in vitro"/open flow system

M. Lopes-Lima [1,2], Lima,P[1,2], A. Rocha[1], J. Machado[1,2]
[1]Centro Interdisciplinar de Investigação Marinha e Ambiental – Universidade do Porto
[2]Instituto de Ciências Biomédicas de Abel Salazar – Universidade do Porto.

Introduction

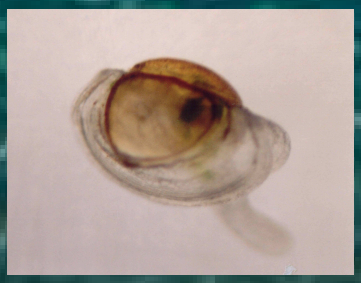
The number of freshwater mussels in the world has been gradually reduced due to pollution and sedimentation and some species have nearly become extinct. Therefore, an urgent plan is needed to protect these animals. The decline of *A. cygnea* has been noticed in Portugal where it occurs only at the Mira region lagoons. In this area, the decline in the bivalve populations highlights the need to carry out ecological studies and culture methods required for the development of a better management system for these species.

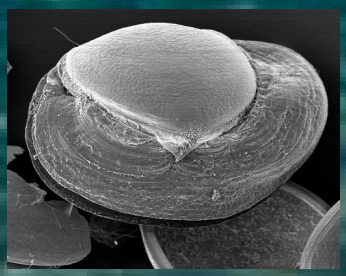
Mussels are generally cultured under a controlled process by imitating their natural habitat. Because the young juveniles are small and transparent, they are difficult to find in nature thus limiting studies on early stages. On the other hand, the microalgae combination from the lagoon constitute an important and delicate natural dietary factor due to the filtration and digestion processes from the bivalve juvenile stages.

So, following a semi-controlled natural system we proposed to breed and transform larva into juveniles based on in vitro culture followed by their maintenance in tanks with lagoon sediment and water in an open flow system.

Material and Methods

Glochidia were cultured in artificial medium based on the modifications of Uthaiwan *et al.* (2001, 2002) and Lima *et al.* (2006). As a source of protein, this medium contained fish plasma (1.0/ ml of *Cyprinus carpio*) mixture with M199 (2.0 m/l) and antibiotics and antimycotic (0.5/ml). In this medium were incubated 50–100 glochidia/ dish. Glochidia dishes were placed inside an incubator with a constant supply of 5% CO_2, 195% room air and 50% humidity at 23ºC. Foot extension outside the shell indicated complete transformation into juveniles.





On the other hand, a hundred early juveniles *A. cygnea*, from the controlled infestation were cultured in an open circuit of PVC tanks measuring 80× 50× 40 cm with lagoon sediment and a 100 L/h water flow of at the aquaculture station in Mira. The juvenile growth were monitored during a three year period.

Discussion and Results

The optimum maintenance conditions of juvenile populations of freshwater mussels, as for example *Anodonta cygnea,* are a major requirement for the successful reproduction of these organisms. Although the larvae metamorphosis has been successfully accomplished under both *in vitro* and infestation methods, the juvenile phase is still a very critical age not easily overcome, mainly in the laboratory. Eventually, there is some natural environmental or nutritional factor or even a combination of factors which may be relevant for the regular survival and growth of freshwater juveniles in the wild. The objective of the present work was to propose a simple model design for juvenile culture based on a combined in vitro and semi-natural systems, based on previous studies. In fact, this model is supported by two main published results and personal data where it was possible to conclude that: a) the larval metamorphosis phase is easier to accomplish in large quantities, faster, more functional and better controlled when processed in the medium M119+fish plasma/horse serum mixture with an adequate temperature; b) the juvenile survival and growth had succeeded under an open flow, semi-controlled system with lagoon water renewal.

Conclusions

Under artificial culture was possible to obtain a larval survival of 35%, whereas the proportion undergoing metamorphosis was 61%. However, the posterior juvenile culture in the laboratory failed completely. The correct combination of natural factors seems to be the main reason of this failure. Moreover, a high level of survival (85%) of young *A. cygnea*, from infested fish, was observed during a 3-year culture period under the semi controlled system with natural lagoon water and sediment. So, an appropriate design for future culture should be to breed juveniles obtained from *in vitro* culture, according our methodology, followed by their maintenance in tanks with lagoon sediment and water in an open flow system. This will offer the possibility to obtain young adults in a large scale.

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