CRYOPRESERVATION OF BLUE MUSSEL (*Mytilus galloprovincialis*) TROCHOPHORE LARVAE AND LARVAL REARING DEVELOPMENT

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INTRODUCTION

Due to the economic importance of the mussel *Mytilus galloprovincialis* in Spanish aquaculture, there is a high interest in developing advance breeding methodologies, ensure seed production and garantee the supply of biological material all-year-round, reducing the risk associated with environmental events that might be a source of economic risk for this sector. The aim of this study was the long-term viability study of cryopreserved larvae of the bivalve *Mytilus galloprovincialis*.

RESULTS

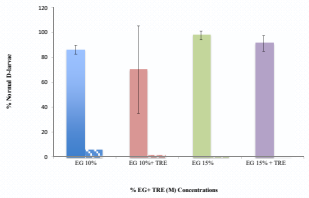


Figure 1. Effect of different concentrations of cryoprotective agent EG on the percentage of cryopreserved D-72h (solid) and D- 48h (pattern) normal larvae. All data has been normalized to controls. Mean ± SD.

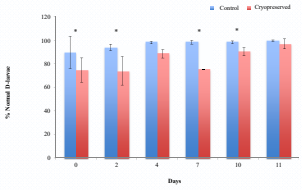


Figure 2. Percentage of normal D larvae 48h after the trochophore larvae cryopreservation. Asterisks indicate statistical differences $P < 0.05$. Mean ± SD

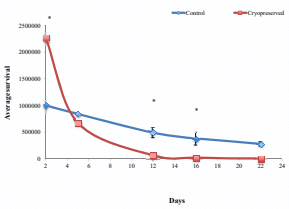


Figure 3. Average survival (± SD) of *Mytilus galloprovincialis* larvae after cryopreservation during a 22 day long larval rearing. Asterisks indicate statistical differences $P < 0.05$.

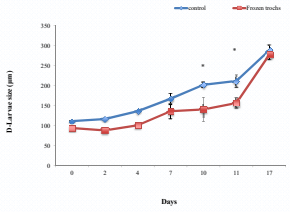


Figure 4. Average growth (µm) during larval rearing from control and cryopreserved larvae developed from trochophore larvae. Asterisks indicate statistical differences $P < 0.05$. Mean ± SD

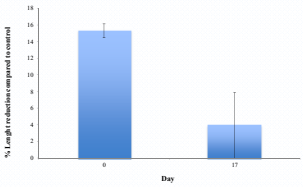


Figure 5. Size difference of the cryopreserved larvae normalized to the control for the first and last day of the larval rearing. Mean ±SD

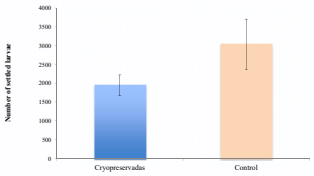
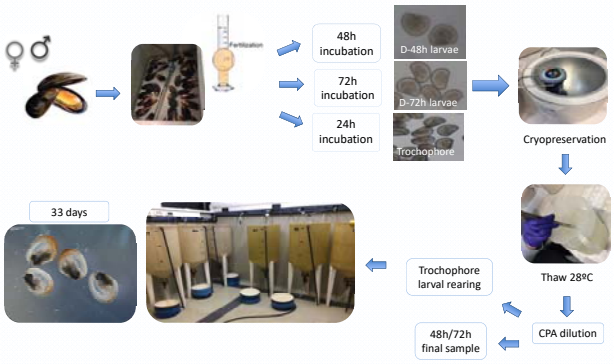


Figure 6. Larvae settlement post larval rearing. Mean ± SD

CONCLUSIONS

1. The percentage of normal D larvae was different depending on the cryopreserved larval stage selected. This was higher for cryopreserved larvae D-72h compared to larvae D-48h and also trochophores (Fig.1)
2. No significant differences were found between using EG 10-15%(v/v) alone or alongside a non-permeating cryoprotecting agent as trehalose (Fig.1)
3. The normality of D larvae developing from the cryopreserved trochophore larvae was lower than the control during the first days (Fig.2)
4. For the same cryoprotecting agent concentration, the normal D larvae results show a greater tolerance of the trochophore larvae, followed by the larvae D-72 h and D-48h.
5. Cryopreserved trochophore larvae survival was 0.17% by day 22 of larval rearing (Fig.3)
6. The D-larvae size difference normalized to control was 15% on day 0 and reduced to 4% by day 17 (Fig. 4-5)
7. The settlement of the cryopreserved larvae was a 36% lower than the control larvae (Fig.6)



MATERIALS & METHODS

During the natural breeding season mature mussels were collected in Vigo (Spain) and were spawned by thermal cycling. For the different cryopreservation studies gametes of several males and females were pooled. The results of each experiment were always compared with controls.

D-48h larvae cryopreservation & D-72h larvae cryopreservation :
Evaluation of the cryoprotecting agent Ethylene Glycol (EG) effects at different concentrations, with or without the addition of 0.4 M trehalose. Addition/dilution was in a single step at 18ºC ±2 for both 48 and 72 hour old D-shaped larvae. Larvae were incubated 24 hours post exposure to cryoprotecting agents and fed with microalgae 30 min. prior the fixation with formaline 10%. Survival was determined under microscope analysis as larvae showing stomach content.

Trochophore larvae cryopreservation :
Trochophore larvae were cryopreserved using 10% EG+0.4 M trehalose (Paredes et *al.* 2013). Addition/dilution was in a single step at 18ºC ±2. Larval rearing development and subsequent post-freezing study of survival, growth and settlement.

References:
[1] Paredes, E., Adams, S. L., Tervit, H. R., Smith, J. F., McGowan, L. T., Gale, S. L., ... & Watts, E. (2012). Cryopreservation of Greenshell™ mussel (*Perna canaliculus*) trochophore larvae. Cryobiology, *65*(3), 256-262.
[2] Paredes, E., Bellas, J., & Adams, S. L. (2013). Comparative cryopreservation study of trochophore larvae from two species of bivalves: Pacific oyster (*Crassostrea gigas*) and Blue mussel (*Mytilus galloprovincialis*). Cryobiology, *67*(3), 274-279.

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