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Technical improvements of a rearing system for the culture of decapod crustacean larvae, with emphasis on marine ornamental species

Ricardo Calado [a,b,*], Tânia Pimentel [c], António Vitorino [d], Gisela Dionísio [a], Maria Teresa Dinis [a]

[a] CCMAR/Universidade do Algarve, Campus de Gambelas, 8000-117 Faro, Portugal
[b] CESAM/Universidade de Aveiro, Departamento de Biologia, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal
[c] Rua da Venezuela N° 33, 4°Dto, 1500-618 Lisboa, Portugal
[d] Oceanário de Lisboa S.A., Esplanada D. Carlos I — Doca dos Olivais, 1990-005 Lisboa, Portugal

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ABSTRACT

The present study compares the efficiency of cylindrico-spherical (CST) and cylindrico-conical tanks (CCT) to culture the larvae of decapod crustaceans, with emphasis to marine ornamental species, and describes a new filter system to flush uneaten preys. The ornamental shrimps *Lysmata debelius*, *Lysmata seticaudata* and *Stenopus hispidus*, the ornamental crab *Stenorhynchus seticornis* and the ornamental hermit crab *Clibanarius erythropus* were used as case studies. The two types of tanks displayed different water circulation patterns, with the inflowing water in CST being pushed towards the spherical bottom and vertical tank walls, in a gentle upwelling motion, while in CCT the inflowing water was abruptly pushed towards the surface at the center of the tank. In comparison to the "traditional" system, the average time required to replace the mesh screens to flush uneaten preys was inferior when employing the new filter system (30 and 5 s per tank, respectively). The average survival to metamorphosis (±standard error) recorded for *L. seticaudata* was higher in CST (97.25±0.50%) than in CCT (94.25±0.96%), with a higher percentage of *L. debelius* larvae in CST also being able to metamorphose (33.75±4.77%), when compared to those in CCT (6.50±3.79%). *S. hispidus* larvae displayed higher survival to the fifth zoeal stage when raised in CST (93.25±2.99%) than when cultured in CCT (66.50±4.20%). A higher number of *C. erythropus* were able to successfully occupy gastropod shells and metamorphose in CST (80.25±5.12%) than in CCT (4.25±2.22%). *S. seticornis* cultured in CST displayed higher survival to metamorphosis than those cultured in CCT (76.25±3.40% and 16.00±2.58%, respectively). A higher percentage of *L. seticaudata* and *L. debelius* larvae at the last zoeal stage displayed intact fifth pereiopods in CST (96.02±3.40% and 88.15±8.70%, respectively), when compared to those raised in CCT (83.95±3.86% and 36.54±3.10%, respectively). The percentage of *S. hispidus* larvae displaying an undamaged rostrum and dorsal abdominal spine was higher in CST than in CCT (95.71±4.19% and 61.84±2.50%, respectively). Along with the new filter system, CST appear as a feasible option for the larviculture of decapods, namely marine ornamental species (shrimps, crabs and hermit crabs), allowing a better use of inert diets and minimizing the risks of larval mortality due to tangling damage, cannibalism and the action of opportunistic pathogens over damaged larvae.

1. Introduction

The larviculture of marine decapod crustaceans is still regarded as one of the major bottlenecks impairing the production of several commercially important species. The most striking example in this search for suitable larviculture protocols is that of spiny lobsters, with researchers agreeing on the importance of employing suitable larval rearing systems that may minimize the mechanical damage commonly induced to the frail phyllosoma (Kittaka, 2000). Several culture systems have already been developed with various degrees of success, with the most promising ones being commonly inspired on Greve's (1968) upwelling "planktonkreisel" (Illingworth et al., 1997; Kittaka, 2000; Sekine et al., 2000; Matsuda and Takenouchi, 2005).

In this way, it was not surprising to verify that when researchers addressing the larviculture of marine ornamental decapods faced similar problems to those displayed by the culture of spiny lobsters (e.g.: frail larval appendages and long larval periods) (Calado et al., 2003a,b), they have also turned their attention to Greve's "planktonkreisel". In fact, Calado et al. (2003c) achieved considerable success in research and commercial scale culture of several ornamental decapod species using a "planktonkreisel" type system. The main design and operation features of the larval rearing system described by Calado et al. (2003c) were the upwelling water inflow in each rearing tank and the daily replacement of

* Corresponding author. CESAM/Universidade de Aveiro, Departamento de Biologia, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal. Tel.: +351 91 629 1417.
E-mail address: rjcalado@hotmail.com (R. Calado).

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the mesh screens (inspired on Illingworth's et al. (1997) culture system) that allowed "24 hours old" *Artemia* to be flushed and replaced by newly enriched metanauplii. Despite the simplicity of the operation procedures of this larval rearing system, some of the steps required for the daily replacement of the mesh screens can become fairly time consuming when operating systems with a large number of rearing tanks. Additionally, the daily manipulation of the inflow valve of each tank (and consequent change of optimal flow rate), the risk of loosing larvae due to the potential clogging of the thin mesh filter and overflow, as well as during the daily displacement of the water level with the 800 mL beaker (larvae were sometimes stuck in the culture tank wall) urged the need to technically improve the rearing system.

Further testing of the research scale cylindrico-conical tanks using the larger and more delicate larvae of the fire shrimp *Lysmata debelius* Bruce, 1983 and the cleaner shrimp *Lysmata amboinensis* De Man, 1888 revealed that latter stages commonly displayed their fifth pair of pereiopods damaged. Rufino and Jones (2001) suggested that these frail appendages may play an important role on the "looping" behavior of *Lysmata* larvae employed during the capture of dietary preys. These suggestions legitimate the hypothesis that *Lysmata* larvae displaying damaged pereiopods may be more vulnerable to nutritional stress due to poorer feeding performances. In addition, damaged larvae are also more exposed to the action of opportunistic pathogens, a major constraint to the larviculture of decapod larvae (e.g. Smith et al., 2003; Bourne et al., 2004, 2007).

Penha-Lopes et al. (2005) also reported how Calado's et al. (2003c) system promoted suboptimal survival during the settlement period of the majoid ornamental crab *Mithraculus forceps* (A. Milne Edwards, 1875), in comparison to an alternative system described by Tunberg and Creswell (1988). According to Penha-Lopes et al. (2005), the main reason for the poorer performances of Calado's et al. (2003c) system was the agonistic interaction recorded among the megalopae aggregated in the deepest region of the tank's conical bottom.

The use of cylindrico-conical tanks also presented some limitations to the culture of hermit crabs. One of the problems is related to the fact of the last larval stage of hermit crabs (the glaucothoe or megalopa) commonly requiring a suitable gastropod shell to metamorphose (Harms, 1992; Harvey, 1996). However, the pronounced slope of the tank conical bottom does not allow researchers to suitably stock shells inside culture tanks, since they invariably slide towards the vertex. Additionally, the upwelling current in the vertex of the cone impairs the megalopae to properly explore the shells, commonly promoting a delay in metamorphosis and ultimately the death of cultured larvae.

The present work compares the efficiency of cylindrico-spherical and cylindrico-conical fiberglass tanks to raise decapod crustacean larvae, using marine ornamental species as case studies, and tests a new filter system to flush uneaten prey.

2. Materials and methods

2.1. Larval rearing system

2.1.1. Cylindrico-spherical tanks with new filter system to flush uneaten prey

White cylindrico-spherical fiberglass tanks (Fig. 1) were designed and manufactured with a diameter of 0.35 m, 0. 35 m of total height, a spherical bottom at 0.22 m from the top and having an approximate volume of 20 L. Round Nitex™ mesh screens (90 mm and 200 mm diameter with 500 µm and 150 µm mesh size, respectively) were mounted 0.1 m from the water surface and attached to a 25 mm diameter outlet equipped with a polyvinyl chloride (PVC) T, placed 50 mm from the top of the tank. No drain valve was fitted in the base of the tanks to prevent the disturbance of the upwelling water flow.

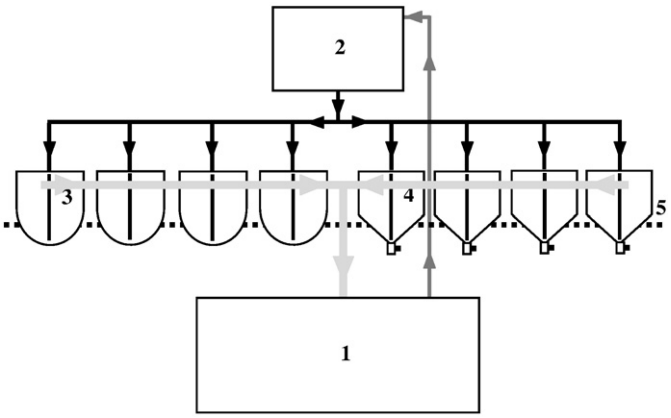

Fig. 1. Schematic representation of the experimental larviculture system: 1) 200 L sump filled with bioballs and housing a protein skimmer, 40 W ultra-violet filter, a heating/cooling system and a submersible pump rated at 5800 L h^{-1}; 2) 50 L header tank; 3) cylindrico-spherical 20 L larviculture tank; 4) cylindrico-conical 20 L larviculture tank; 5) perforated plate supporting the larviculture tanks. Dark grey lines represent inflowing water from the sump to the header tank. Black lines represent inflowing water from the header tank to the larviculture tanks. Light grey lines represent outflowing water from larviculture tanks to the sump.

2.1.2. Cylindrico-conical tanks with traditional filter system to flush uneaten prey

White cylindrico-conical fiberglass tanks (Fig. 1) were designed and manufactured with a diameter of 0.35 m, 0. 35 m of total height, a conical bottom at 0.25 m from the top, an approximate volume of 20 L and a 12 mm diameter drain valve was fitted to the base. A round Nitex™ mesh screen (90 mm diameter with 500 µm or 150 µm mesh size, depending if the tanks were being flushed from uneaten preys or not) was mounted 0.1 m from the water surface and attached to a 25 mm diameter outlet, placed 50 mm from the top of the tank (for further details see Calado et al., 2003c).

2.1.3. Filtration system of larval culture tanks

Cylindrico-spherical tanks (CST) and cylindrico-conical tanks (CCT) were connected in parallel to a recirculation system composed of a 200 L sump, filled with bioballs (to assure biological filtration) and a protein skimmer (Fig. 1). Water was pumped from the sump to a 50 L header tank with a pump rated at 5800 L h^{-1} and passed through a 40 W ultra-violet filter. The water was distributed to the culture tanks through 20 mm PVC pipes connected to the header tank. Each culture tank had a 16 mm diameter inlet positioned in the deepest point of the rearing tank and was equipped with two valves: the first used to regulate the water flow and the second used to stop the water flow when tank cleaning was necessary (without interfering with the optimized water flow regulated by the first valve). The rearing tanks water passed through the mesh screens and was returned to the sump through a 40 mm diameter PVC pipe equipped with a 100 µm mesh size basket that collected all flushed uneaten prey.

2.2. Water dynamics inside larval rearing tanks

In order to identify the prevailing water flow inside the different larval rearing tanks, a liquid blue colored food-grade dye (containing FD&C Blue Dye No.1, E133 — Brilliant Blue) (manufactured by Globo, Portugal) was added to four CST and four CCT only holding seawater and its prevailing dispersal pattern was recorded until it was completely diluted. The dye was added at a rate of 1 drop s^{-1} to inflowing water entering the bottom of CST and CCT. The tests were performed in CST and CCT equipped with the mesh screens employed to flush uneaten preys, in order to assure that the water flows monitored were as similar as possible to those occurring when the larviculture tanks are being operated.