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Aquaculture

Maintenance of *Octopus vulgaris* Type II paralarvae in an estuarine area

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ABSTRACT - We assessed the survival of paralarvae kept in a floating wooden box attached to an oyster extensive cultivation system with no extra food supply. A total of 7700 newly hatched paralarvae were maintained in a 10.5-L floating box (7 cm height × 30 cm width × 50 cm length) covered with a 180-µm mesh net for 14 days with no extra food supply. Skin damages and tentacle deformities were observed in 43% of the paralarvae at 14 days after hatching (DAH). The survival rate was 64.7% at 7 DAH and 42.8% at 14 DAH. The floating box is a promising structure for culturing *O. vulgaris* paralarvae in an extensive system.

Key Words: aquaculture, larviculture, mariculture, mollusc

Introduction

The common octopus, *Octopus* cf. *vulgaris* (Cuvier, 1797), is one of most important commercial fishery resources worldwide. The *Octopus* cf. *vulgaris* comprises a complex of cryptic species with unknown distribution limits within temperate and tropical seas (Vidal et al., 2014). In Brazilian waters, the *Octopus* cf. *vulgaris* occurs along the coast, mainly distributed in South and Southeast regions (Jereb et al., 2016). A newly study suggested that the Brazilian *Octopus vulgaris* is morphologically similar but genetically distinct from *vulgaris*-like species (the *O. vulgaris* species complex) and the so-called *Octopus vulgaris* Type II (Amor et al., 2017).

The *Octopus vulgaris* species complex is a promising candidate for diversifying the aquaculture industry due to its global market, high commercial value, and animal performance potential such as direct embryological development, short life cycle, rapid growth, and elevated feed conversion (Vaz-Pires et al., 2004).

The major hindrance to the expansion of the *Octopus* cf. *vulgaris* culture, however, is the intense mortality observed during the first life stages of the species. The lack of a standardized culture system and the absence of a balanced diet that fulfills the paralarval nutritional requirements are among the main factors contributing to such mortalities (Iglesias and Fuentes, 2014). In this context, commercial culture of *O.* cf. *vulgaris* has been performed by growing wild-captured subadults using bycatch, in suspended cages in the sea, as currently performed in Galicia coast (northwest Spain) (García-García et al., 2014). Nevertheless, such technique is unsustainable, and efforts should concentrate on the development of a rearing protocol for the planktonic paralarval phase of the species, avoiding overfishing of natural populations. Promising octopus larviculture results were obtained in laboratory conditions, as summarized in Vidal et al. (2014). However, better survival and growth performance are still required to scale-up the activity to a commercial level.

In recent years, the *Octopus vulgaris* Type II (Brazilian *Octopus vulgaris*) has revealed its potential as a new species for mariculture in Brazil. Besides the performance characteristics, this species presents a still-growing demand as a food source and attractive price market. A current study suggested that Brazilian *O. vulgaris* ongrowing in artisanal farming system using low-cost techniques is a potential and innovative activity to mollusk farmers in southern Brazil (Bastos et al., 2014). One of the key factors to provide the basis for this commercial culture is to ensure an appropriate paralarval supply. Nonetheless, the larviculture is still limited to experimental levels (Iglesias et al., 2007).

Laboratory conditions usually provide a better production control and minimize the risks of outbreaks in the larviculture of aquatic organisms. However, the

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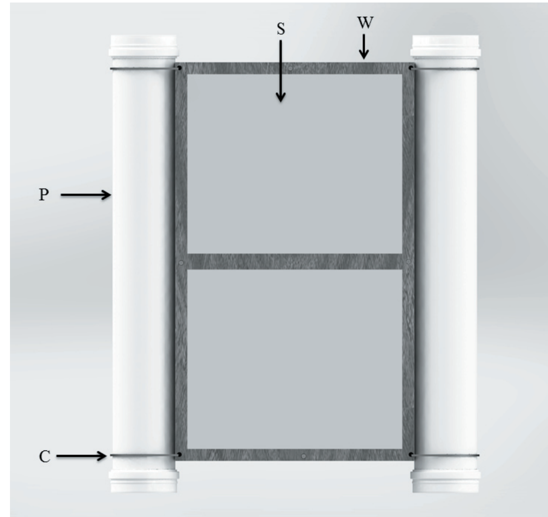
maintenance of animals on their natural habitat could be an alternative to reduce costs. The main advantage of such an extensive culture system is the use of existing water bodies, implying that both the initial capital investment and operational costs are considerably lower than culturing in laboratory. Thus, our study aimed to assess the maintenance of *O. vulgaris* Type II paralarvae in a floating structure attached to an oyster culture system in southern Brazil.

Material and Methods

The study case was performed in a mollusk farming area in Sambaqui Beach, North Bay Florianópolis, Santa Catarina, Brazil (27°28'30" S and 48°33'40" W). The Sambaqui area faces west and is characterized by intermediate water circulation within 2.5-3.5 m depth, muddy bottom, and predominant North wind (Ferreira et al., 2006). Paralarvae were obtained from a breeding female kept in a suspended cage attached to an extensive culture system of oyster *Crassostrea gigas* (Thunberg, 1793). At the beginning of spawning, the female was isolated in a small tank until paralarvae hatching. A wooden floating box, of 10.5-L volume (70 cm height × 30 cm width × 50 cm length), was manufactured using a 180-µm mesh net (Figure 1A), wood pieces, PVC tubes, and four stainless steel screws (Figure 1B). The floating box was previously tested in laboratory under simulated natural turbulence conditions and no paralarval escapes were recorded.

A total of 7700 newly hatched *O. vulgaris*, with total length of 1.81±0.87 mm (mean ± standard deviation) (Figure 2), were stocked in a fully submerged wooden box (733.3 paralarvae L^{-1}) attached to the same oyster longline used for the cage holding the breeding female. The paralarvae were reared under extensive culture system, with no extra food supply for 14 days, from November 3rd to 17th, 2010, under natural photoperiod (approximately 12 h light and 12 h dark). The net mesh size (180 µm) determined the minimum prey sizes retained in the box. At 7 and 14 days after hatching (DAH), the box was opened to check for the presence of potential predators and prey, paralarval survival, and skin or tentacle deformities. Survival rate was estimated by counting the number of paralarvae in five 2-L samples. To check skin damages or tentacle deformities, 10 paralarvae of each sample were observed through microscope. Cages were cleaned every two days by brushing the outer surface of the net box to avoid clogging and fouling organisms. The temperature and salinity of the surface of the water were monitored daily.

A



S: 180-µm mesh net; P: PVC tube Ø 75mm; W: wooden box; C: nylon cable.

B

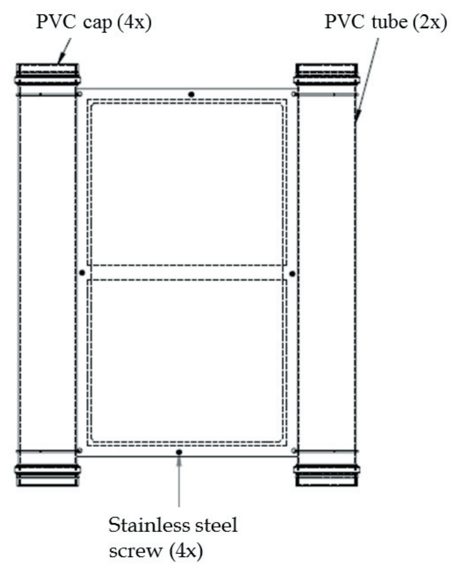


Figure 1 - Floating wooden box for paralarval culture (A) and top view of the box (B).

Figure 2 - Hatching *Octopus vulgaris* Type II paralarvae.

Results

The survival rates of the paralarvae was 64.7% at 7 DAH and 42.8% at 14 DAH (Table 1). At 7 DAH, the floating box was opened and no preys were visually detected, whereas at 14 DAH, 152 crab zoeas of *Callinectes* sp. were found.

The paralarvae showed good tentacle and sucker formation and their chromatophores were well developed with dark red staining pattern on the dorsal mantle (Figure 3). However, skin damages and tentacle deformations were observed in 30% of the paralarvae observed at 7 DAH and 43% at 14 DAH.

Temperature was kept at 23.6±0.9 °C and salinity at 34.5±0.5 psu (mean±SD). These values are within the recommended range to ensure survival and growth of the common octopus paralarvae, since they closely resemble those observed in the species natural environment (Boyle, 1991).

Discussion

The extensive system used in the present study and the handmade floating box demonstrated promising results with survival rates of 42.8% at 14 DAH in springtime. Our results were superior to the 20% survival at 15 DAH previously reported by Seixas et al. (2010) under controlled conditions for the *Octopus vulgaris*. Nonetheless, laboratory can provide more stable conditions as reported by De Wolf et al. (2011), who successfully reared *O. vulgaris* from 0 (zero) to 160 DAH in controlled conditions.

Table 1 - Survival rate of *Octopus vulgaris* Type II paralarvae ketp in a wooden box during 14 days after hatching in an extensive system, in an estuarine área

Days after hatching	Density of paralarvae (n)	Survival rate (%)
0	7700	100
7	4982	64.70
14	3297	42.80

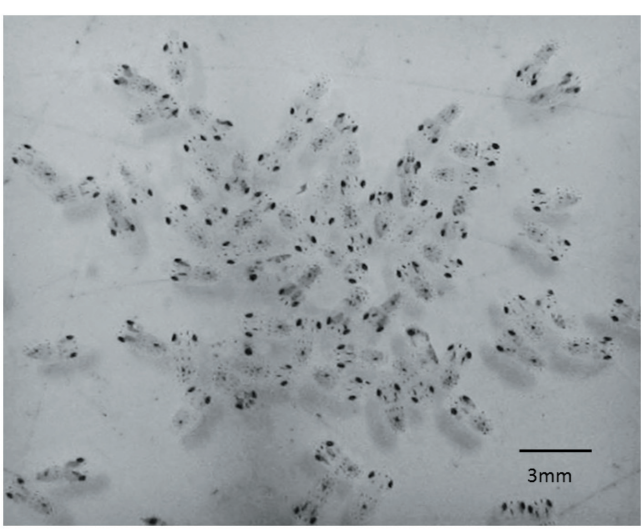


Figure 3 - *Octopus vulgaris* paralarvae maintained in a wooden box at seven days after hatching.

Massive paralarvae mortalities usually takes place within the transition period from endogenous (yolk) to exogenous feeding (prey), normally associated with the inability of paralarvae to tolerate even short periods of starvation due to their high metabolism at this stage (Vidal et al., 2002b; 2006). In experimental rearing of lolignids, the yolk reserve of paralarvae is completely absorbed in the first two DAH at 16 °C (Vidal et al., 2002b) and at the same temperature, the paralarvae did not survive longer than four DAH (Vidal et al., 2006). In the conditions of the present study, with no external feed supply and high temperature (22-24 °C), it is likely that the paralarvae fed natural feed after the period of endogenous feeding, contributing to their development up to 14 DAH and the survival rate.

In this context, we propose that the *Octopus vulgaris* Type II paralarvae possibly fed the crab zoea, since they were detected and available inside the box. The decapods are essential as prey for paralarvae mainly because their lipid composition, such as phospholipids, cholesterol, and long-chain polyunsaturated fatty acids (PUFA), are abundant in marine crustaceans (Villanueva et al., 2017). The lipids and PUFA play an important role in cephalopod metabolism and development, particularly in the fast growth of the species and in the early stages of life (Navarro and Villanueva, 2003; Navarro et al., 2014). Additionally, cephalopod paralarvae require feed rich in copper (Villanueva and Bustamante, 2006), which is probably related to the hemocyanin requirements for oxygen transport, typical of crustaceans and mollusks (Villanueva et al., 2017). Once more, marine crustaceans are an essential item in the diet composition of octopuses. Despite the scarce knowledge on the diet composition of wild octopus paralarvae, it is known that decapod crustacean larvae, including crabs, are natural prey of planktonic *O. vulgaris* paralarvae and other cephalopod species (Villanueva and Norman, 2008). Recent studies using molecular techniques suggested that wild *O. vulgaris* paralarvae in Galician waters (Northwest Spain) strongly preferred decapod crustaceans as feed at least in the earlier stage of life cycle (Roura et al., 2012; 2016). Furthermore, a new study using metagenomic approaches revealed that *O. vulgaris* paralarvae fed a wide variety of decapod species in which crabs were the most abundant group detected (Olmos-Perez et al., 2017). Thus, these findings reinforce our suggestion that the paralarvae might feed the crab zoeas. This suggestion is also supported by captive studies in which increased survival rates were obtained when decapod zoea were used as sole diet or complemented with *Artemia* as live prey in paralarval rearing (Itami et al., 1963; Iglesias and Fuentes, 2014). The complete life cycle of *O. vulgaris* was closed for the first time in 2001 using a co-feeding regime of spider crab zoea (Iglesias et al., 2004). In a short time, Carrasco et al. (2006) also completed the life cycle of this species using crab zoea and *Artemia* as feed. Besides, the first protocol to rearing *Octopus vulgaris* published by Iglesias et al. (2014) recommended the use of crab zoea to increase growth and improve quality of the paralarvae in terms of biochemical composition, until 30 DAH. Hence, in our study, we believe that the decapods established inside the box might serve as feed to the *O. vulgaris* Type II paralarvae, contributing to their development and survival until 14 DAH.

Additionally, the ropes used in the oyster and mussel longlines provide shelter and accumulate food not only for decapod crustaceans, as reported by Macedo et al. (2012), but also for a great variety and abundance of species that compound the zooplankton.

Recent studies reveal that wild *O. vulgaris* paralarvae in Galicia coast (Northwest Spain) prey not only decapod species (Roura et al., 2016), but copepods and cladoceran in abundance, and also frequently other groups such as euphausiids, amphipods, echinoderms, hydroids, fish larvae, and mollusks such as bivalves and gastropods (Olmos-Perez et al., 2017). Interestingly, many of these diverse taxonomic groups are also abundant in the zooplankton community on the coast of Santa Catarina (Resgalla Jr. et al., 2011; Domingos-Nunes and Resgalla Jr. et al., 2012). These findings suggest that particularly in the coast of Santa Catarina, the mussel and oyster farms may be an interesting site to culture *O. vulgaris* Type II as they possibly provide potential prey for the planktonic paralarvae. Since there is no information about the feeding habit of wild *O. vulgaris* Type II paralarvae, studies on the diet composition of these wild paralarvae using modern technologies as molecular tools or stable isotopes can help us understand the diet preference and nutritional requirements of the paralarvae at least in the planktonic phase.

Once prey is available, other important aspect is to ensure adequate paralarval swimming and allow good interactions between paralarvae and their prey. During the planktonic phase, paralarvae swim near-surface water and use forward, backward, and lateral swimming as type of displacement (Villanueva and Norman, 2008). In laboratory conditions, at 30 DAH (octopus total mean length of 7.4 mm), the maximum predator-prey distance at which the paralarvae notice the prey was 15.5 mm (Villanueva et al., 1996). Thus, in our study, the height of the box (70 mm) was probably sufficient to enable paralarvae and live prey encounters, improving the efficiency of prey-predator interactions up to 14 DAH. Since feed was available, it is possible that the prey-predator relationship was successfully improved by the box design. Mesh size should be chosen in a way to allow retention of live feed besides avoiding paralarvae escapes. In this study, 180 μm seemed to be the appropriate mesh size to rear octopus paralarvae from 0 to 14 DAH, since no escapes were observed and potential live feed were detected inside the box.

Overall, the paralarvae were well developed, with good tentacle and suckers and seemed to be capable to feed and swim. The skin damages and tentacle deformations observed are probably associated with an unsuitable design of the floating box. The surface of the box may have become slightly coarsened, which, associated with the tidal and hydrodynamic forces acting in the study area, may have influenced the swimming conditions of paralarvae, making them collide against the box walls and, thus, damaging skin and deforming tentacles. Vidal et al. (2002a) recommended keeping paralarvae protected from the contact with tank walls and bottom to reduce the occurrence of skin lesions. Further studies should rethink the floating box material and design to allow paralarvae to have a more natural swimming and avoid skin damages.

In practical terms, the advantages of the floating box are the low cost and ease to be manually fixed and removed from the longline structure, which make it accessible and used by artisanal or small-scale mollusk farmers. Nevertheless, the floating box should be designed in a way to allow easy cleaning, handling, and net replacement to avoid clogging and reduction in efficiency.

Conclusions

The present study provides the first results on the development of an extensive rearing technology for *Octopus vulgaris* Type II paralarvae. The floating box is a promising structure for culturing *O. vulgaris* Type II paralarvae during the first weeks after hatching with no extra food supply, in an estuarine area.

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