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An upwelling system for culturing common octopus paralarvae and its combined effect with supplying natural zooplankton on paralarval survival and growth

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ABSTRACT

Despite a growing interest in common octopus for aquaculture, massive mortalities during the planktonic paralarval stage still hamper production. Prior to die-off during rearing trials for paralarvae, it could be observed that the food-capturing paralarvae tended to be carried downward in a current induced by the aeration system, eventually depositing the animals on the tank bottom. The aim of this study was to investigate the effect of upwelling water-flow, that might support paralarval swimming and floating, on paralarval survival and growth. A new artificial upwelling rearing system using a strong downward flow in the center to the bottom of the tank was developed. The effects of the new system in combination with supplying natural zooplankton (mainly decapod crustacean zoeae) or *Artemia* were evaluated using three different broods hatched from three mothers (broods A, B, and C). As food items for zooplankton, n-3 highly unsaturated fatty acid (n-3 HUFA)-fortified rotifers and digestible microalgae *Nannochloropsis oculata* were supplemented. The hatchling dry weight was significantly different among broods, showing heavier weight in order of broods A > C > B. Use of the upwelling rearing system significantly improved the rate of paralarval survival as compared with using the standard system, and paralarval growth was significantly faster when reared in the upwelling system with zooplankton supplementation. Paralarvae in the upwelling + zooplankton group kept high survival rates over 80% until 18 days after hatching (DAH). The survival rate of the tank of brood A could achieve 80.8% at 20 DAH, however, those of broods B and C dropped to 8.2% and 54.4% at 20 DAH, respectively, presumably due to combined effect of small hatchling size and insufficient food supply. The octopuses of broods A and C reached benthic stage by 25 and 23 DAH, with survival rates of 51.0% and 45.3%, respectively. The overall results suggest that an inadequate water-flow environment in combination with poor food quality and quantity are major causes of paralarval mortality of the common octopus. For culturing paralarvae, the combined use of an upwelling rearing system and supplying decapod crustacean zoeae supplemented with n-3 HUFA fortified rotifers and digestible *N. oculata* was recommended.

1. Introduction

Traditionally, the common octopus have been grouped into one species of *Octopus vulgaris*, which is distributed in shallow temperate waters in the Atlantic, Mediterranean, and western North Pacific oceans (Kaneko et al., 2011; Sasaki, 1929; Warnkle et al., 2004). However, recent researches have suggested that the group inhabiting the western North Pacific Ocean can be recognized as the East Asian common octopus *Octopus sinensis* distinct from *O. vulgaris* based on genetic and male morphometric characteristics (Amor et al., 2017; Gleadall, 2016), though the *O. sinensis* has yet been classified as "taxon inquirendum" by the World register of Marine Species (WoRMS Editorial Board, 2018) and has been included in *O. vulgaris* in the FAO statistics (FAO, 2017). The world-capture fisheries production of the common octopus *O. vulgaris* species complex including *O. sinensis* and *O. vulgaris* reached > 100,000 t (t) in the late 1960s, but had declined to 33,965 t by 2015 owing to overharvesting (FAO, 2017). The common octopus has excellent potential for aquaculture because of rapid growth and a high commercial value (Vaz-Pires et al., 2004); therefore, attempts to develop technologies for the artificial production of juveniles (i.e., paralarval rearing) have been undertaken since the 1960s (Iglesias et al., 2007; Itami et al., 1963). However, a reliable rearing technology has

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not yet been established as massive mortalities and poor growth commonly occur during the planktonic paralarval period (Iglesias et al., 2007).

It has long been suggested that inappropriate food supplies are a major cause of paralarval mortality (Garrido et al., 2016; Iglesias et al., 2007). In their natural habitat, the common octopus paralarvae are known as specialist predators on decapod crustacean larvae (Olmos-Pérez et al., 2017; Roura et al., 2012). Indeed, paralarvae reared with a supply of decapod crustacean zoeae often exhibited better survival rates and faster growth as compared with *Artemia*-fed paralarvae under laboratory conditions (Garrido et al., 2016; Iglesias et al., 2007, 2014). Many studies have investigated the effects of various nutrients, such as fatty acids, amino acids, vitamins and other elements, on paralarval survival and growth by using *Artemia* of differing nutritional status or by comparing the results of feeding with *Artemia* or decapod crustacean zoeae (Garrido et al., 2016; Guinot et al., 2013; Hamasaki and Takeuchi, 2000, 2001; Hamasaki et al., 1991; Iglesias et al., 2007, 2014; Kurihara et al., 2006; Navarro and Villanueva, 2000, 2003; Roo et al., 2017; Seixas et al., 2010; Vaciano et al., 2011; Varó et al., 2017; Villanueva and Bustamante, 2006; Villanueva et al., 2004, 2009). Although these studies succeeded in identifying the beneficial effects of several nutrients, such as marine lecithin, high protein content and docosahexaenoic acid (DHA), the crucial nutrients needed to prevent paralarval mass mortality have not yet been identified. Furthermore, even in cases when decapod crustacean zoeae have been supplied as an adequate food for the common octopus paralarvae, few of the studies succeeded in producing benthic juveniles: the survival rates from hatching to settlement have fluctuated considerably, ranging from 0%–31.5% (Carrasco et al., 2006; Garrido et al., 2016; Iglesias et al., 2004; Villanueva, 1995). These results to date suggest other underlying causes of paralarval mortality besides an inadequate food supply.

It has been well known that the common octopus paralarvae are poor swimmers, because they rely predominantly on pulsed-jet swimming produced by contraction of the mantle cavity and lack physical features for active swimming or balanced floating, such as fins, swimbladder, or cuttlebone (Villanueva et al., 1996). Generally, a paralarval rearing tank equips an aeration system to provide oxygen into the rearing water (e.g., Okumura et al., 2005a), and the aeration generates downward water current along the wall of the cylindrical tank (Sakakura et al., 2006, 2007). During the rearing trials for paralarvae, it could be observed that the food-capturing paralarvae tended to be swept to the bottom by this downward current, hence their natural swimming and feeding were disturbed (Dan, personal observation). This suggests the possibility that the water-flow condition is an important factor affecting the paralarval survival and growth.

To support paralarval swimming and floating, the upwelling water-flow appears to be suitable, because it may help paralarval suspension in the water column. Many studies have demonstrated advantages of upwelling system in culturing planktonic larvae of many marine species (Calado et al., 2003; Greve, 1968; Illingworth et al., 1997; Kittaka, 1997; Okamura et al., 2009). There are two previous works conducting the paralarval rearing of octopus species with the upwelling system (Carrasco et al., 2006; Kolkovski et al., 2015). Although these systems employed an upward or radial water inlet from multiple small points at the bottom of the tank, they have not achieved stable survival rate at an acceptable level. In these systems, the water-flow induced via small holes might restrict the flow strength and direction, resulting in the insufficient water-flow conditions for paralarval suspension in the water column.

Within this context, a new rearing system that could induce a steady upwelling water current was developed for culturing octopus paralarvae, and the aim of this study was to evaluate the effects of this system in combination with supplying natural zooplankton (mainly decapod crustacean zoeae) or *Artemia* on paralarval survival and growth of the common octopus.

2. Material and methods

2.1. Broodstock and hatchlings

Six adult females of the common octopus with body weight of 1327 ± 188 g were collected from local fisheries using octopus traps, on 25 August 2016, in the central area of the Seto Inland Sea, off Hiroshima, Japan (34° 20 N, 133°14 E). Considering the geographical location where females were collected, the octopus species in the present study might be considered *O. sinensis*. The females were reared in a cylindrical fiberglass tank (3.3 kL, diameter 2384 mm, depth 1000 mm) with a flow-through water system and a sufficient aeration. They were fed frozen shrimp *Trachysalambria curvirostris* and clams *Ruditapes philippinarum* ad libitum once a day. Twelve shelters (entrance diameter 120 mm, depth 285 mm; Sunpoly Co. Ltd., Yamaguchi, Japan) were settled in the tank. Male was not stocked into the tank, because most matured females have already received spermatophores from males in this area in August. Three of the females started spawning in their shelters, on August 30 (brood A), August 31 (brood B), and September 2 (brood C) and were transferred into 500-L cylindrical polyethylene tanks and maintained individually. The tanks were cleaned daily by siphoning, and paralarvae hatched successfully; the hatchlings were daily released into the sea excluding those used for the experiment. Three thousands of newly hatched paralarvae of each brood, that hatched on September 21 (brood A), September 22 (brood B), and September 23 (brood C), were used for the rearing experiment (total 9000 animals). Dry weight of the hatchlings was determined by weighing individual animals (15 per brood); the paralarvae were first blotted on filter paper, and then dried at 100 °C for 24 h. Before taking measurements, the animals were anesthetized in ethanol at a concentration of 20 mL L^{-1} (Escánez et al., 2017; Fiorito et al., 2015). The average water temperature and salinity during broodstock management and the egg incubation periods were 27.1 ± 0.9 °C and 32.9 ± 0.6 ppt.

2.2. Effects of an upwelling rearing system and supplying natural zooplankton on paralarval survival and growth

To evaluate the effects of the new upwelling rearing system combined with supplying natural zooplankton on paralarval survival and growth, we prepared three treatment groups: 1) aeration + *Artemia* supply, 2) upwelling + *Artemia* supply, and 3) upwelling + natural zooplankton supply. For each treatment group, a thousand individuals of newly hatched paralarvae of each brood (broods A, B, and C) were reared separately in each tank (total 9 tanks: 3 treatment groups × 3 broods, 9000 paralarvae) until 20 DAH, while different broods were treated as replicates.

In the aeration group, paralarvae were reared in a standard rearing system (e.g., Okumura et al., 2005a) using a 500-L cylindrical polyethylene tank (internal diameter 1170 mm, height 770 mm) (Fig. 1). Aeration was provided via a silicon tube (internal × external diameters: 4 × 6 mm) and an airstone (diameter 50 mm) at a rate of 350 mL min^{-1}. Sand-filtered seawater was added continuously at a rate of 347 mL min^{-1} (500 L day^{-1}) and the rearing water flowed out via a filter (diameter 110 mm, length 630 mm, mesh size 276 μm) and a siphon hose (internal diameter 15 mm). A titanium heater (500 W, TH1–05, Tanaka Sanjiro Co. Ltd., Fukuoka, Japan) was anchored in the filter and connected to a thermostat (Rei-Sea TC-100; Iwaki Co. Ltd., Tokyo, Japan). In this system, aeration brings water toward the surface where it diffuses horizontally toward the cylindrical wall of the tank, and then a steady downward flow occurs along the tank wall toward the bottom.

The upwelling rearing system was set up in two 500-L cylindrical polyethylene tanks (same as the standard rearing system), one was the paralarval culture tank and the other was a server tank, and both tanks contained seawater (volume 500 L + 500 L, total volume 1 kL) (Fig. 2).