A PROCESS OF CULTURING MUD POLYCHAETE *Marphysa iloiloensis*

TECHNICAL FIELD

The invention relates to the process for larval rearing of mud polychaete, *Marphysa iloiloensis*, using optimized regimes for larval food preference, light condition requirement, and rearing water management.

BACKGROUND

The worldwide demand for polychaetes from sea anglers and the aquaculture industry has led to the massive collection from the wild. Thus, the natural population started to decline through the years. In aquaculture, polychaetes are used as a supplemental diet for crustacean broodstock (Luis and Passos, 1995; Meunpol et al., 2005). Mud polychaete, *Marphysa iloiloensis*, is a newly-discovered, indigenous species found in the mangrove mudflats and brackish water fishponds of Iloilo, Philippines. It is named after the province of Iloilo, Panay Island, in the Western Visayas region, Philippines (Glasby et al., 2019). Polychaete species under the family Nereididae are commonly cultured; however, nereid species die right after spawning, hence, sustainability is questionable. *M. iloiloensis*, on the other hand, has a desirable characteristic for aquaculture since they can spawn many times throughout their lifetime. Sexual maturity is usually attained after 4 months (from trochophore to adult stage). In the wild, spawning peaks of this species start from April to July when seawater temperature becomes warmer. Gelatinous egg masses (jelly cocoons) containing polychaete eggs emerge from the sediment surface during spawning. Since this is a new species, larval rearing which includes preferred light condition, food preference, and rearing water management has not been optimized.

In the natural environment, this species is abundant in the mangrove areas where exposure to sunlight is limited by the mangrove leaves. Mangrove mudflat is an organic-rich environment and home to the different species of algae, bacteria, zooplankton, and other microorganisms. These organisms serve as food for benthic invertebrates, particularly polychaetes. Polychaete larvae consume microalgae, bacteria, and other small animals present in the muddy sediment. *M. iloiloensis* can also be found in an organic-rich brackish water fishponds. Organic matter which is composed mainly of unconsumed feeds and fecal wastes accumulates in the pond bottom.

In most aquatic species, the early life stage is considered as a critical stage of hatchery production. As such, a successful larval rearing of mud polychaete *M. iloiloensis* requires optimal culture conditions to improve survival and growth. Suitable food items and settlement substrate throughout the larval stage of polychaete are important factors to be considered. In addition, these food items should mimic the natural diet of mud polychaete. In this invention, bioflocs are used as food and settlement substrate of the polychaete larvae. Bioflocs are aggregates of algae, zooplankton, bacterial biomass, and organic material produced under aerobic condition (Crab et al., 2012). The use of bioflocs in the rearing of benthic invertebrates, particularly lugworm is found under patent number KR1020180078757 wherein biofloc water is supplied in the larval rearing tanks using a recirculating system (Kim & Kim, 2018). In the present invention, bioflocs are being produced in a separate tank and bioflocs are collected from the said tank, washed with freshwater, and stocked in the larval rearing tanks. A well-aerated, UV-treated seawater is recirculated in the rearing tanks such that clean and oxygen-rich seawater is supplied to the larvae. Furthermore, bioflocs are washed using freshwater to kill potential predators (particularly copepods) prior to stocking in the larval rearing tanks. When bioflocs are not washed with freshwater, other organisms present in the bioflocs could predate or induce stress to the polychaete larvae. In another patent (EP3005866), the invention relates to a closed recirculating aquaponics system using biofloc technology (Kim et al., 2016); however, this is quite different from the present invention. In said patent, bioflocs are generated from the organic feces of aquatic species, and biofloc water is purified and used as culture water for growing aquatic species. Bioflocs are directly used as a nutrient source of plants in the aquaponic system and purified water is supplied to aquatic species.

The aquaculture of mud polychaete, particularly the larval rearing of *M. iloiloensis* has not been established. Thus, a culture process for this species that aims to increase its survival and growth is of paramount importance. It is therefore the object of the present invention to provide such a culture process.

References:

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SUMMARY OF THE INVENTION

The present invention establishes the culture process of *M. iloiloensis* to improve the survival and growth of the larvae in captivity. This invention details the culture procedures for the successful rearing of mud polychaete larvae using optimized regimes for natural food, light condition, and rearing water management. The preferred light condition for the larval rearing of the trochophore larvae (1 DAH, day after hatching) to early juveniles (30 DAH) ranged from 0 to 50 lux. Bioflocs is the preferred food item and settlement substrate of the larvae. Bioflocs are produced using aquatic feeds 30 and sugarcane molasses at C/N ratio of 20 to 40 in a well-aerated system. Aerated, UV-treated seawater with 27-30 ppt is used in supplying the larval rearing tanks following a dripping method. This method of water delivery is used to provide enough dissolved oxygen for the larvae.

The first objective of this invention is to determine the preferred light condition of the larvae to improve its survival and growth.

The second objective of this invention is to determine the preferred natural food and settlement substrate of the larvae.

The third objective of this invention is to establish the production of bioflocs in indoor tank and preparations involve prior to feeding to the larvae.

The fourth objective of this invention is to establish the use of UV-treated seawater in supplying the larval rearing tanks.

The establishment of this culture process is an important prerequisite to the successful production of *M. iloiloensis* in captivity.

DETAILED DESCRIPTION

The present invention is a culture process for culturing the benthic invertebrates, mud polychaete *Marphysa iloiloensis*.

Mud polychaete, *M. iloiloensis*, is a newly-discovered indigenous species found in the mangrove mudflats and brackish water fishponds of Iloilo, Philippines. The overexploitation of polychaetes in the wild resulted in a drastic decline of the natural population. Polychaetes are important in aquaculture as a supplemental diet to improve the reproduction of crustacean broodstock. The culture of native polychaete species with high spawning rate and longevity is important to ensure steady supply and sustainability. Moreover, aquaculture of this species under biosecure condition is critical for a disease-free polychaete fed to cultured shrimps. Thus, the establishment of a culture process for this species is important.

In the present invention, the larval rearing set-up should be located in a shady area away from direct sunlight during daytime, at 0 to 50 lux. The present invention can be further supported by the experiment below:

A 3 x 3 factorial design plus the control (without light) was employed to determine the influence of light intensity and photoperiod on survival and growth of polychaete larvae. In each experimental room except the control, illumination was provided by incandescent bulbs (Philips LED bulb, cool daylight). Experimental cups were subjected to one of 3 light treatments: (1) 50 lux; (2) 600 lux; or

(3) 1500 lux. These 3 light combinations were tested simultaneously under each of three photoperiods (3:21 h light/dark; 6:18 h light/dark; 12:12 h light/dark). There were 3 replicates of each of the treatments. Each replicate cup was filled with 200 ml UV- treated seawater containing 15 ± 0.25 trochophore larvae. Aeration was not provided throughout the experiment while water change was done every other day (25-50% of water was drawn in each replicate cup) and renewed with fresh UV-treated seawater. Results showed that polychaetes in 0 lux treatment had the highest survival (94 ± 6%) among other light treatments followed by treatments 50 lux, 6 h light:18 h dark (82 ± 5%); 50 lux, 3 h light:21 h dark (81 ±11%); and 50 lux, 12 h light:12 h dark (69 ± 18%). Among light treatments, only those polychaetes under 3 h light:21 h dark survived. The number of segments of the surviving polychaetes was the same (18 ± 0.24 segments) except those in 1500 lux (3 h light:21 h dark) treatment (16 ± 0.23 segments). Graphical presentation of the result is shown in Figure 1.

Jelly cocoons are collected from the polychaete breeder tanks and checked under the microscope for egg viability after 24 hours of collection. After checking, trochophore larvae are stocked in the larval rearing tanks. Bioflocs of 10-20 g, were stocked in the rearing tanks 2 days after stocking. Prior to stocking, bioflocs were collected from the biofloc tank using a plankton net (90-um mesh). Bioflocs are then soaked in freshwater to kill other organisms that could potentially disturb the development of the larvae, thereafter, rinsed using seawater. The present invention can further be explained by this experiment:

The suitability of bioflocs as polychaete larval feed was evaluated and compared to other natural food items such as *Navicula* sp., *Amphora* sp., *Navicula* sp. + *Amphora* sp., and mix diatoms. Results showed that on the 7th day sampling no polychaete larvae were found in all 4 microalgae treatments and it was assumed that all had died as no remnants were found in the experimental containers. On the other hand, in the biofloc treatment, 60% of the polychaetes survived and 5 segments formed in their body after 7 days. Bioflocs improved the survival and growth of the polychaete larvae. The lack of aeration in the rearing water can be one of the possible reasons why all polychaete larvae did not survive in all diatom treatments. The decomposition of diatoms resulted in low dissolved oxygen level of the water caused by high bacterial oxygen demand during the breakdown process. On the other hand, bioflocs are dominated by bacterial biomass which are the end products of the decomposing aquatic feeds and sugarcane molasses under aerobic condition. In this case, the decomposition process is skipped and water quality under biofloc treatment was not polluted.

Bioflocs are produced in a separate tank through a combination of aquatic feeds and sugarcane molasses at C/N ratio of 20-40 (Fig. 2). The present invention can be further explained as follows: One-ton capacity circular fiberglass tanks was designed for biofloc production by installing four water pumps at the center and eight surrounding air diffusers to maintain continuous water movement and suspension of decaying matter. About 100 g of feed mill sweepings with 40.26% crude protein (nitrogen source) and 100 g sugarcane molasses (carbon source) which contain 87% nitrogen-free extracts were added to the biofloc tank (C/N ratio of 20-40) once a week. Biofloc water was agitated 3 times a day. The daily measured water quality parameters of the biofloc system stayed within the range: 3.5-5.8 mg/L dissolved oxygen, 27-30 °C temperature, 25-35 ppt salinity, and 7.5-8.5 pH. The formation of microbial flocs showed an increase in the total suspended solids (300-700 mg/L) in the biofloc system.

Another result of the experiment showed that polychaete larvae grown in biofloc without light exposure had 83.29 ± 3.79% survival with 21± 1 number of segments after 30 days of larval rearing.

In the present invention, rearing water which is an aerated, UV-treated seawater of 27-30 ppt, is recirculated in the larval rearing tanks. Seawater from a tank reservoir is supplied in the rearing tanks using a dripping method. Since aeration is not provided, this method agitates the water column. Seawater from the rearing tanks overflows in the drain pipe and directed to the reservoir tank and the cycle continues.