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Copepod growth populations *(Acartia* sp.) in outdoor mass culture tanks: Exploring natural feed potentials for sustainable aquaculture

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ABSTRACT

Larvae feed plays an important role in aquaculture activities. It helps the fish to pass through their initial life during the larvae stage after egg hatching. Copepods are zooplankton which has a high potential to be developed as a natural feed for fish fry. Hence, this study was aimed at figuring out a technique for mass culturing of copepod in outdoor culture tanks. Copepods were cultured for six days in a culture tank, starting from the nauplii phase up to the adult phase. The life cycle of the cultured copepod started with nauplii (phase N1-N6), copepodite (phase C1-C5), and the adult stage. The copepod seeds cultured were under nauplii phase (N1-N2). After being cultured for 6 days and harvested daily for 16 days (under two different sampling technique), this study obtained the total number of 25,470 copepods using first harvesting technique and 136,730 copepods using the second harvesting technique. The obtained number consisted of copepods from all the three life cycles. Results of this study showed a potential of this copepods culture method in producing sustainable stock of fish larvae feed.

Key words: *Animal feed, Aquaculture, Copepods, Harvest, Outdoor mass culture*

Introduction

Aquaculture activities in both marine and freshwater require sustainable seeds, both in quantity and quality. Naturally, marine fish larvae will consume zooplankton to support early growth, especially in the nauplii and copepodite phase copepods (Llopiz, 2013; Robert *et al.*, 2014). Therefore, natural food is given during the seed phase because it has good nutritional content and size that matches the mouth of fish larvae (Wahyuningsih, 2009).

Two of the commonly used feeds in aquaculture activities are rotifer and artemia. Nevertheless, there are several challenges in the field of aquaculture in using both rotifer and artemia. The nutritional value of rotifers, in general, is not yet ideal for the devel-

opment of marine fish larvae because of the still low content of essential fatty acids (Castel *et al*., 2003). Similarly, the application of artemia is optimal if combined with Rotifera (Randazzo *et al.,* 2018). If there is a shortage in the availability of phytoplankton, the rotifer will have a high rate of dying due to the limited content of nutrient (Esron and Sukendi, 2015).

Therefore, a natural food as an alternative option with high nutritional value and relatively more practical in terms of culturing is highly required. In accordance with this statement, it is necessary to develop natural food with copepod so that it is able to meet the needs of marine fish larvae and several reports mention the application of copepod can be done on the entire life cycle both nauplii to adult (Evjemo *et al.,* 2003; Llopiz, 2013; Robert *et al*., 2014; Perumal *et al.,* 2009).

To date, the copepods are known to consist of ten orders in which the Harpacticoida, Cyclopoida, and Calanoida are the three most commonly utilized in the production of natural feed (Huys and Boxshall, 1991). Harpacticoida and Calanoida were the most dominant orders found in waters because they have high adaptability to various environmental conditions. This ability provides a great potential for them to be used as natural feed (Kusmiyati *et al*., 2002). The types of copepods that can be cultured as natural food include *Acartia* sp. from the order of Calanoida, *Macrosetella* sp. from the order of Harpacticoida, and *Oithona* sp. from the Cyclopoida order.

Indonesian waters have various types of copepods (Mulyadi, 2003). This rich biodiversity makes aquaculture in Indonesia a potential sector with huge chances to find a substitute for Artemia which is currently also increasing in price. Copepodsare rich in protein, fat, essential amino acids which can accelerate growth, increase disease resistance and brighten the color of shrimp and fish (Stottrup, 2003). Copepod protein content ranges from 24% - 82%. This protein content is greater than rotifer's which is around 28-63% of its dry weight (Kusmiyati *et al*., 2002). According to Sargent *et al*., (1997), copepods in the copepodite and adult phases contain immunostimulant substances, attractants and some digestive enzymes that play an important role in supporting the nutritional needs of fish larvae. The initial phase of fish larvae only has yolk as food reserves hence when the food reserves are exhausted fish larvae must begin feeding with the consumption of natural food from its surroundings since they lack a full digestive system. The administration of copepods can increase the growth and digestibility of fish larvae (Karlsen *et al*. 2015). They are better digested than Artemia during the initial larvae phase (Lavens and Sorgeloos, 1996). This is supported by the results of the analysis of the stomach content of cod catches that show that there are various copepod species from various developmental stages being the most important food for cod larvae in their natural habitat (Wiborg, 1948).

Toledo *et al.* (1999) reported that the EPA (eicosapentaenoic acid)as essential fatty acid content in copepods and rotifers was almost the same with 9.25% and 8.26% respectively. However, the DHA (docosahexaenoic acid) content of copepods was much higher than rotifer, which is 24.41% against 0.17%, respectively. Therefore, the DHA/EPA value in the copepod is higher than rotifer. The high value of DHA/EPA can help the growth, increase the survival rate and reduce the occurrence of abnormalities in the fish larvae (El Kertaoui *et al*., 2019). In addition, copepods are detritivores in nature, which means copepods eat the remnants of fish feed, fish droppings, and some bacteria in the aquaculture ecosystem (Poulsen *et al*., 2011). The presence of copepods can help to control water quality by eating un-eaten feed which can cause bacterial overload in fish ponds (Stottrup and Norsker, 1997).

Copepod culture techniques in aquaculture are one of the important aspects that should be mastered byfarmers (Kline and Laidley, 2015), but the use of copepods as natural feed is still limited due to the lack of information on the mass culture methods. The development and mastery of the copepod culture method essential to support the sustainability of the fish industry, therefore this research was aimed to analyze the outdoor mass production of copepods in order to provide a sustainable feedstock for fish larvae. The result of this study will contribute greatly in providing alternative feedstock for fish larvae and portray the effectiveness of simple outdoor mass production of copepods in terms of its produced masses.

Materials and Methods

Research Period and Location

The study was conducted at the Research Center of Marine Aquaculture and Fisheries Counseling,

Gondol, Buleleng Regency, Bali. All researchstage was carried out for a complete one month from January to February 2019.

Copepod mass culture analysis

The copepod mass culture conducted in this study followed the method from Kline and Ladley (2015). This culture stage included several activities, namely seed sampling, seed density calculation, seed culturing and maintaining for 6 or until adulthood at 9 days.

Seed sampling

Seed samples were taken from the previous copepod culture of *Acartia* sp. in 1000 L of tanks. The *Acartia* sp. was filtered with plankton net. The previous culture was carried out for 8 days and harvested on the 9th day.Harvesting results obtained were still mixed with dirt and other organisms therefore a stratified filtration was performed to separate them. In this stratified filter, the coarse filter was placed at the very top to hold mosquito larvae, mosses and other impurities. The second filter used plankton net of 275 µm to obtain an adult phase copepod and the third filter used a 120 µm plankton net to get a copepodite phase of the copepods. The last filter used plankton net with a 45 µm net to obtain the nauplii phase. Nauplii copepods were then put into a container and filled up with seawater to 10 Land an aerator was used as the oxygen source. The retained seeds on 45 µm net was then observed under a microscope and the range of nauplii phase was confirmed as N1 to N3.

Calculation of seed density

About 20 mL sample of the Nauplii copepods phase was taken from the aerated container. The sample was transferred into a microplate with 6 plates then observed under a microscope. The density of the copepods was calculated manually. The total density was calculated based on the sum of all the observed density on each plate.

Copepod mass culture in the outdoor tank

The obtained Nauplii phase of the copepods with density of 51,000 individuals/L were cultured in a 1,000 L culture tank with installed bottom aeration system. The culture was given a mixture of PS-P feed (Commercial feed, CP Prima) with nutritional value Protein (38-42%); Lipid (4-6%); Fiber (2-3%); Water content (9-10%) and fish meal on a ratio of 2:1. Mixed feed (PS-P and fish meal) was given every morning with a dosage of 0.003 g/L.

PS-P pellet feed was not only used as a feed by copepods in the culture tank, but also used by phytoplankton as a source of nutrients to grow. The presence of phytoplankton in the culture of the copepods occurred naturally. The phytoplankton also served as a food source for the copepod. This was related to the nature of the copepod as a detritivore that can eat a variety of food sources as long as the food has a smaller size than their mouth (Turk *et al.* 1982). Copepod mass culture was carried out for 6 days, the growth of copepods (changes in age/ stage) was observed every day from the nauplii stage to adulthoodusing a microscope. In addition, the physical condition of culture media(culture water) was also observed. The physical conditions of the culture media observation including temperature, salinity, and sunlight intensity.

Copepods harvesting

Harvesting of copepods was also carried outdaily in the morning around 06.00 - 08.00. This was due to the fact that copepods are negative phototaxis in the morning and positive phototaxis at night. At 06.00 - 08.00, the light intensity is not too high so many copepods still migrate to the surface area in search offood. The daily harvesting method was by taking 50% of the total volume of mass culture and substitute the removed volume with seawater into the mass culture tank until it reached the initial mass culture volume. Total harvesting of adult copepods was carried on the 7th day after no significant grow on adult copepods was obtained. Two different harvesting method was applied to analyze the total obtained copepods differences. First harvesting method was draining from the surface of the tank (conducted on day 1 to day 7) while the second harvesting method was draining from the bottom of the tank (conducted on day 8 to day 16). Total harvesting was conducted at day 7 and day 16.

Results and Discussion

Calculation of the seed density

The obtained nauplii phase copepods after stratified filtration was observed for its density under microscope. The results of the density calculation were obtained as:

- The first hole (Lb1) = 23 individuals,

• The second hole (Lb2) = 21 individuals,
• Third hole (Lb3) = 17 individuals,
• The fourth hole (Lb4) = 12 individuals,
• The fifth hole (Lb5) = 10 individuals,
• The sixth hole (Lb6) = 19 individuals.

Total individuals = Lb1 + Lb2 + Lb3 + Lb4 + Lb5 + Lb6 = 102 individuals/ 20 mL

Seed density =5,100 individual/L

A total of 10 L nauplii phase copepods were cultivated into the 1,000 L mass production tank, resulting in the average of 510 individuals/L copepods inside the tank.

Copepods harvesting

The data on 50% daily harvesting and total harvest on the 7th day are presented on Table 1.

Table 1. Density of copepods daily harvesting (individual/L)

Harvest day	Nauplii	Copepodite	Adult	Total
1	450	30	0	480
2	1,020	10	10	1,040
3	1,480	80	40	1,600
4	2,710	1,370	120	4,200
5	4,200	1,370	120	5,690
6	10,400	1,840	150	12,390
7	1,110	400	0	1,510
8	1,060	450	0	1,510
9	8,140	3,460	940	12,540
10	18,470	220	220	18,910
11	15,980	180	230	16,390
12	18,080	100	50	18,230
13	17,280	250	80	17,610
14	14,520	1,580	420	16,520
15	14,080	1,200	960	16,240
16	20,200	300	10	20,510

Onthe 6th day, the nauplii phase reached the adult stage and were confirmed through microscopic observation. The number of nauplii stages inside tank reached up to 1,110 individuals/ Lduring the harvesting period on the 7th day. Second total harvesting was conducted on the 16th day, considering the initial second phase starting on the 8th day.

Turk *et al.,* (1982) reported that the presence of excess waste products and food deposits will trigger the growth of contaminants and cause mass death of copepods. But in this culture, phytoplankton was dissolved during daily harvest, so there was no residual deposition on the bottom of the tub. Among the two different techniques. The first technique obtained a lower number of copepods than the second technique. The total number of copepods in the first harvest technique from day 1 to day 7 were 480 individuals; 1,040 individuals; 160 individuals; 4,200 individuals; 5,690 individuals; 12,390 individuals; and 1,510 individuals, respectively. For the second harvesting technique, obtained copepods starting from the day 8 to day 16were 1,510 individuals; 10,810 individuals; 18,910 individuals; 16,390 individuals; 18,230 individuals; 17,610 individuals; 16,520 individuals; 16,240 individuals; and 20,510 individuals.

The increasement of individuals throughout day 1 to day 5 were not significantly different, but on the 6th day there was a significant increase. Incidentally, it was rained on the previous day. Rain is one of the environmental factors that can affect the culture production. According to Lavens and Sorgeloos (1996), environmental conditions can cause changes in several significant parameters during cultivation. The rain was quite heavy and lasted a long time hence this could be the reason for the significant increment in harvesting compared to the other day. According to Dussart and Defaye (2001), copepods have negative phototoaxic properties. During the rain, the sunlight was covered by thick clouds which lowered the intensity of the light, so that copepods migrated to the surface to look for food thus will affect the overall growth. The overall environmental condition during mass cultivation are presented on Table 3.

The amount of harvest on the second cycle which on the 8th to the 16thday was stable, following the trend of linear increasement. This shows that the technique was good to maintain the copepod life

Table 3. Measurement of environmental parameters (Presented data are the range during the whole research period)

Environmental Parameters	Morning (08.00)	Afternoon (13.00)	Evening (16.00)
Temperature (°C)	26 –33	30 – 35	26 - 35
Salinity (ppt)	30 - 32	28 – 34	27 – 32
Light intensity (Lux)	3,000 – 73.300	6,600– 83,600	400– 29,400

cycle. The number of copepods was quite high until the 16th day. However, there were some possible factors that cause a decrease at the end. Phytoplankton growth began to occur such that the sediment at the bottom of the tub increased. The *Acartia* sp. species is a species that hatches itseggs at the bottom of the tub. Therefore, by having increased sedimentation at the bottom of the tub, the eggs can get mixed with sediments and caused many eggs to die before hatching (Turk *et al*., 1982).

Further Research Direction

For further studies, it is suggested that the age of nauplii copepods used is homogenous to give a higher precision data in terms of growth. In addition, research on feed formulations for copepods will be very interesting in order to ascertain optimum feed formulation and to observe higher yield of copepodsin harvesting.

Conclusion

To summarize, this study found that copepod life cycle started with nauplii (N1-N6 phase), copepodite (C1-C5 phase), and adults. Nauplii (N1-N2) stage was successfully cultured as a copepod seeds in this study. After being cultured for 6 days and harvested daily for 16 days (under two different sampling technique), this study obtained the total number of 25,470 copepodsusing first harvesting technique and 136,730 copepods using the second harvesting technique. With these findings, this study suggest that second sampling technique was more reliable in providing higher number of copepods from the same cultivation tank. This study also successfully proven that copepods can be cultured locally in outdoor mass cultivation setting tank and can be used as natural feed for the sustainable aquaculture.

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