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The Use of Copepods to Improve Juveniles Production of Coral Trout *Plectropomus leopardus* (Lacepède, 1802)

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Abstract: Copepods are highly nutritious of live feed for marine fish larvae. The application of using copepods tested to improve coral trout juvenile production. This study purposed to determine the effect of copepods on the growth and survival rates of coral trout larvae. Two different kinds of live feeds were tested: (A) Rotifers and (B) Rotifers + Copepods. The results indicated that at 20 days old, larvae on B have better growth (6.40±0.67 mm) than those on A (5.92±0.76 mm) (P>0.05). Lengths of dorsal fin and ventral fin on B (4.49±0.68 mm; 3.44±0.51 mm) were longer than those on A (3.95±1.05 mm; 3.06±0.84 mm) (P>0.05). Larvae on B consumed more live feeds than on A. The survival rate on B (1.26±0.41%) was higher than those on A (0.33±0.30%) (P<0.01). In summary, copepods have positive impact to survival rate and tendency to better growth of coral trout larvae and could improve the juvenile production.

Key words: Copepods · Coral trout · Larvae · Survival rate

INTRODUCTION

Coral trout *Plectropomus leopardus* is one of highly economical value of tropical marine fish. The market price and demand of coral trout are quite high over the world, theferore the exploitation level of this species tends to increase and this fact contributes to the decreasing population of coral trout [1]. The cultivation of coral trout has been implemented to prevent it's over exploitation and to conserve its existence in the nature.

There are several factors that influence the success of marine fish juvenile production. Feed is one of the key successes during larval rearing period. In marine aquaculture, rotifers *Brachionus* sp. [2] and artemia [3] are two kind of live feeds commonly used. Although the use of those kinds of live feeds, the survival rate of grouper larvae is still low [4]. Rotifers and artemia lack of nutritional requirements of marine fish larvae especially for High Unsaturated Fatty Acid (HUFA) as essential fatty acids for marine fish larvae, so they are needed to be enriched with ingredients containing HUFA before feeding to larvae [5].

Copepods, another kind of zooplankton, have high nutrition value because consist of some vitamins, amino acids and fatty acids [6], mainly docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) as essential fatty acids for supporting better growth of marine fish larvae [7]. So, copepods seem to be an alternative live feed for coral trout larvae.

In the wild, copepods have an important role in an ecosystem food chain [8]. Copepods are the kinds of zooplankton that mainly consumed by several marine fish larvae [9-14]. In cultivation scale, copepods are the dominant live feed preferenced by some marine fish larvae [15-17].

Copepods also have some variations in size and stages during their life cycle. This fact is an advantage for first feeding of marine fish larvae because the larvae can choose the size of live feeds in according to their size of mouth width. The copepod size in nauplii stages range between 100 to 150 µm [18].

Because of those superiorities, copepod is being developed in aquaculture as a live feed for some species of marine fish larvae [19]. Based on those facts, it is necesarry to set up a research experimental design to obtain some of empirical data's about the influence of copepods to larval growth and juvenile production of coral trout.

During this time, some of the studies related to coral trout larvae in cultivation scale have been documented [20, 21] and the study related to feeding of coral trout larvae was still limited [22, 23]. To our knowledge, the

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study on the impact of copepods as a live feed for coral trout was not reported yet. Therefore, the study to determine the effect of copepod on the growth and survival rate of coral trout larvae was carried out. The results of this study expected to become one of the basic information for the improvement of coral trout cultivation.

MATERIALS AND METHODS

Larval Rearing: The study conducted during May until July 2012 in Institute for Mariculture Research and Development located in northern part of Bali, Indonesia. Larval rearing was carried out in semi outdoor hatchery using 6.000 L concrete tanks which equipped with aeration system to supply dissolved oxygen.

The coral trout eggs obtained from natural spawning of domesticated broodstocks that kept in outdoor concrete tanks. Suitable eggs was selected by visible and microscopic observation. Only good quality of eggs, indicated by transparent and floating eggs, were used in this study. The eggs density was 15 eggs/L. The hatching rate of egg was counted when larvae have already hatched.

Phytoplankton *Nannochloropsis ocullata* was added into the larval rearing tanks at 2 days after hatching (DAH). Rotifers *Brachionus* sp. was given as live feed started from 3 to 30 DAH. The initial density of rotifer was 3-5 ind./mL. The density of rotifers enhanced according to increasing age of larvae. Artificial feed and artemia was given started at 8 and 25 DAH, respectively.

In this study, two kinds of live feed treatment were implemented: (A) Rotifers and (B) Rotifers + Copepods. Copepods were given as larvae feeding started at 3 DAH, four times in a week. The number of copepods depended on the availability.

Collecting Copepod: The wild copepods which used in this study were obtained from brackish water pond located approximately 10 km from the study site. Copepods were collected during the night time by using light as an attractant to copepods. Copepods were obtained by pumping water pond into a plot sized 1 x 1 m that was surrounded by planktonet 120 μm mesh size. The harvesting of copepods was done after a large amount of copepods collected.

Larval Growth: The larval growth was observed at 1, 5, 10, 15 and 20 DAH larvae. Total length, length of dorsal fin and ventral fin were the parameters measured from larval growth. A total of 15 larval samples were taken at each age from each treatment. Larvae samples were placed on a single concave object glass and then larvae measured by using micrometer in stereoscopic microscope.

Number of Copepods and Rotifers in Larval Digestive Tract: The observation was performed at 1, 5, 10, 15 and 20 DAH larvae. Larvae samples were placed on single concave object glass, afterwards the digestive tract of larvae were dissected. Copepods and rotifers were known from their shells and their number was counted. The observation was done by stereoscopic microscope.

Larval Survival Rate: Survival rate is the percentage of the number of juveniles at the end of the study compared to the number of hatched larvae. The harvested juveniles were separated by their total length and divided into three size groups i.e. big, medium and small. Furthermore the juveniles which already grouped were counted totally. The total length of juveniles was measured by taking 10 samples randomly from each size groups.

Proximate Analysis, Fatty Acids and β Caroten of Copepods: Copepod samples were screened by using planktonet 60 μm mesh size and then the sample was dried by frezeedryer. Analysis of proximate, fatty acids and β caroten were carried out at Gadjah Mada University, Yogyakarta, Indonesia.

Identification the Clasification Copepods: Copepod samples preserved with 4% buffered formalin. The identification process was carried out at the Laboratory of Zoology, Indonesian Institute of Science. The identification based on morphological characterization of samples.

Statistic Analysis: Statistical analysis of t test was carried out to data of total length, dorsal and ventral fin length and survival rate to determine the significant difference between treatments.

RESULTS

Larval Growth: The study indicated that coral trout larvae on both treatments have similar pattern of total length from 1 until 15 DAH. However, total length of 20 DAH larvae was 6.40±0.67 mm (B) tended bigger than 5.92±0.76 mm (A) but not significantly difference ($P>0.05$) (Fig. 1).

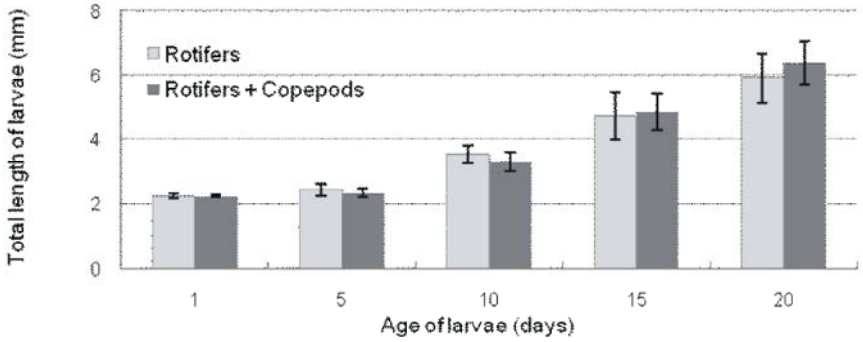

Fig. 1: Total length of coral trout larvae fed (A) rotifers and (B) rotifers + copepod

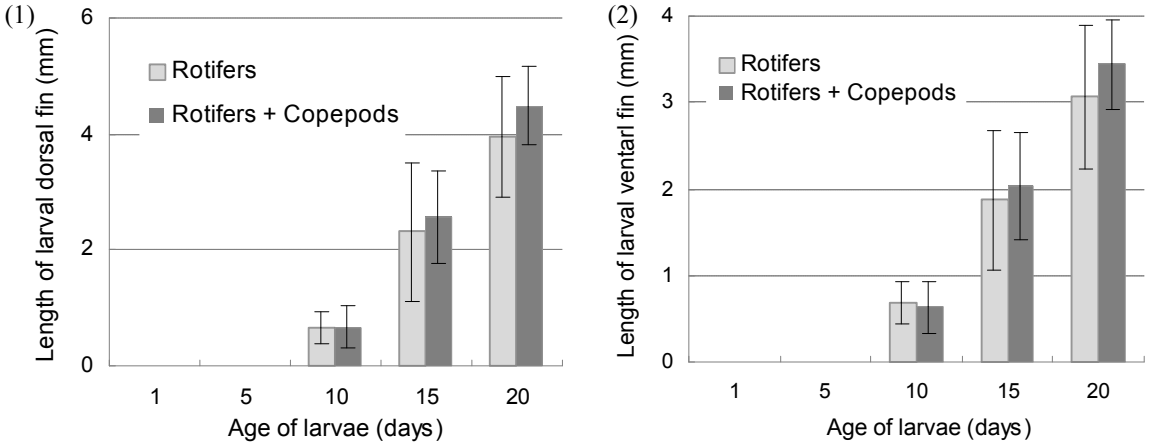

Fig. 2: Dorsal fin length (1) and ventral fin length (2) of coral trout larvae fed with (A) rotifers and (B) rotifers + copepod

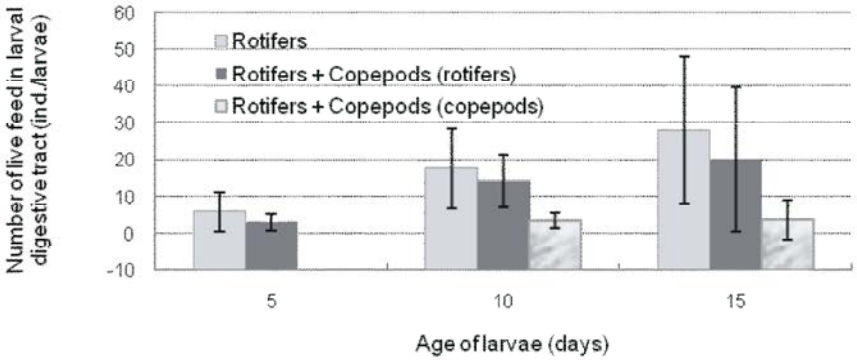

Fig. 3: Number of live feeds in digestive tract of coral trout larvae fed with (A) rotifers and (B) rotifers + copepod

Dorsal fins were detected at 10 DAH larvae (Fig. 2.1). Dorsal fin length on 10 DAH was similar on two treatments, i.e; 0.67±0.28 mm (A) and 0.67±0.37 mm (B). However, dorsal fin length of 15 and 20 DAH larvae on (B) tends to be longer than on (A) but not significantly difference (P>0.05).

In addition to dorsal fin, ventral fin was also detected as shown in Fig. 2.2. Length of ventral fin on 10, 15 and 20 DAH larvae (A) were 0.69±0.23 mm; 1.87±0.81 mm and 3.06±0.84 mm, respectively, while (B) were 0.64±0.30 mm; 2.04±0.61 mm and 3.44±0.51 mm, respectively. The result indicated that larval ventral fin on (B) tends to longer than on (A) but not significantly difference (P>0.05).

Number of Copepods and Rotifers in Larval Digestive Tract: The observation on larval digestive tract indicated that number of rotifers consumed by 5 DAH larvae on (A) and (B) were 5.80±5.25 ind./larvae and 3.00±2.26 ind./larvae, respectively (Fig. 3).

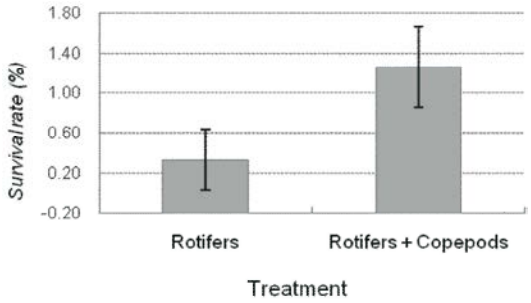

Fig. 4: Survival rate of coral trout larvae fed with (A) rotifers and (B) rotifers + copepod

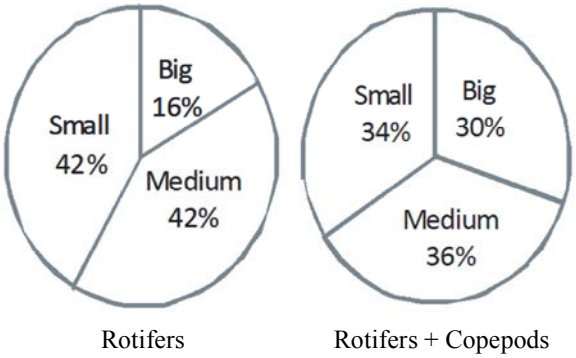

Fig. 5: Size distribution of coral trout juveniles from different kind of live feed on larval stage (A) rotifers and (B) rotifers + copepods

The result indicated that 5 DAH larvae on (B) consumed only rotifers, eventhough copepods were added into the larval rearing tanks. Copepods were found in larval digestive tract on (B) started at 10 DAH. The number of live feeds at 10 DAH were 17.60±10.80 ind. rotifers/larva on (A), while on (B) were 14.20±7.26 ind. rotifers/larva and 3.50±2.12 ind. copepods/larva.

Larval Survival Rate and Size Distribution: Larval survival rate on (A) was 0.33±0.30%, while on (B) was 1.26±0.41% and strongly significant difference ($P<0.01$) (Fig. 4). Based on their total length, the harvested juveniles were divided into 3 size groups, i.e. small (1.2-1.9 cm), medium (2.0-2.7 cm) and large (2.8-3.5 cm), respectively. The number of juveniles belonged to small, medium and large group on (A) were 42.0%; 42.67% and 16.67%, respectively, while on (B) were 34.17%; 36.68% and 30.15%, respectively (Fig. 5). The result indicated that juveniles on (B) were bigger, while on (A) were smaller. Besides of bigger size, the juveniles from (B) was also had better pigmentation because the color was more red than on (A). The juveniles from (A) had the same color as the wild ones.

Analysis of Proximate, Fatty Acids and Β Caroten of Copepods: Proximate analysis of copepods indicated that copepods consist of protein 54.08%, fat 6.01%, ash 21.00%, water 4.38%, EPA 8.06%, DHA 27.33% and β karoten 7954.85 µg/100 g sampel.

Identification the Copepods: Identification of copepods indicated that *Acartia sinjiensis* Mori, 1940 and *Oithona brevicomis* Giesbrecht, 1891 were two dominant copepods which used in the study.

DISCUSSION

Total length is one of commonly parameter which used to measure larval growth. However, because of belong to family Serranidae, coral trout larvae also have others specific parameters i.e. dorsal and ventral fin that will elongated and then will shortened afterwards disappeared completely [24]. For grouper, the fins will elongated at 7 DAH and will shortened at 30 DAH then disappeared completely when the larvae completely metamorphosed to be juveniles [25]. Slower growth of larval fin than normally can be assumed as slower growth of larvae. Thus the growth of larval fin could be used as an indicator for the growth of coral trout larvae. In this study, coral trout larvae which fed rotifers and copepods tend to have longer total length and fins until 20 DAH, indicated that those larvae tend to have better growth.

Larval growth can also be used as an indicator of the utilization of feed. From the dissection of larval digestive tract known that larvae which fed 2 kinds of live feed consumed more live feeds. Number of rotifers which consumed by larvae on (B) were fewer than those on (A). This is assumed because of larvae on (B) fed by rotifers and copepods, so the larvae could consume not only rotifers but also copepods. Number of copepods which consumed by larvae were fewer than rotifers because the density of copepods in the larvae rearing tanks was lower than those of rotifers. The density of copepods wasn't always the same and the number couldn't be determined because the copepods are harvested from the wild, so the availability of copepods was fully depend on their abundance in the wild. Even though the density of copepods was low but naturally the larvae have natural ability to change their behavior by increasing swimming activity to explore their prey [26].

The copepods that used during this study were obtained from the wild because mass culture of copepods couldn't produce copepods in high densities yet [27-29]. During this time, production of copepods was still limited on research scale in laboratorium [30-32].

This fact impacts the production of copepods from the cultivation is very expensive. On the other hand, harvesting copepods from the wild is more cheap economically [33]. The use of wild copepods also performed for Atlantic halibut fish *Hippoglossus hippoglossus* [34].

The kinds of species and the size of copepods in this experiment were varied due to their abundance .in the wild. It would be affected to larval digestive tract analysis because some of the copepods found broken, so it was difficult to observe and count the exact number of total copepods. Only the copepods which can still be identified were counted in this study. Consequently, the observation couldn't exhibit the real data because chances are the number of copepods consumed by the larvae is much more than could be observed.

The study also indicated that the survival rate of coral trout larvae which fed combination rotifers and copepods was higher than those fed only rotifers. Feeding marine fish larvae with only one kind of live feed isn't sufficient to support larval growth [35]. Juvenile production of coral trout in our institute during 2012 perfomed that 91% of total production derived from larvae fed rotifers and copepods, while 9% from larvae fed only rotifers. Combination feeding of rotifers and copepods also could support better growth and higher survival rate of some marine fish larvae [21, 36-38].

Copepod seems also to affect better pigmentation of coral trout juveniles which produced. Larvae which fed rotifers and copepods have more red color than those fed only rotifers. Red color juveniles have higher selling price because the color as same as wild juveniles, so It is more profitable economically. Copepods also have the same impact to normal pigmentation of Atlantic halibut fish *H. hippoglossus* [39]. The more red color due to copepods have the high level of β carotene or astaxanthin because not all of the copepods have β carotene [6].

Proximate analysis indicated that protein level in copepods was more than 50%. It is higher than the protein requirement for grouper which range 40-50% [40]. The higher protein means better nutritional value for larvae. Copepods also have high eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3). EPA and DHA are essential fatty acids for normal growth and better survival rate of marine fish larvae [41, 42] and as the energy source for larvae [43]. High level of DHA in copepods due to copepods could accumulate DHA from their feeds and keep them inside their body [44]. Because of their DHA content are high, copepods may improve better growth of larvae [45, 46].

This fact indicates that copepods are high quality of feed for the marine fish larvae. The high level of HUFA in copepods make copepods is very potential as live feed for larvae, particularly for grouper larvae [4]. Therefore, coral trout larvae which fed rotifers and copepods have better growth and higher survival rate than those which fed only rotifers.

Acartia sinjiensis Mori, 1940 and *Oithona brevicomis* Giesbrecht, 1891 are the commonly copepods spesies which found during this study. It might be the blooming time for those two kinds of copepods in the wild. The cultivation of *Acartia sinjiensis* has been developed but still on a limited scale cultivation currently [47]. *Oithona* sp. distributed widely in almost all of Indonesia water and dominates more than 70% of zooplankton during the rainy season in South China waters [48].

It could be concluded that copepods influenced the growth, survival rate and pigmentation of coral trout larvae. The same impact of feeding copepods also happened to *Amphiprion clarkii* [49]. and Halibut *H. hippoglossus* [39]. In summary, this study indicated that copepods have positive impact to higher survival rate, better pigmentation and tendency to better growth of coral trout larvae. Based on this result, copepods are very recommended as the live feed and furthermore should be improved in mass culture [4, 5, 50]. In the future, the quantity and quality of coral trout juvenile production are expected to increase by using copepods and rotifers as the live feed.

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