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OPTIMUM DENSITY OF *Nannochloropsis* sp. FOR MASS LARVAL REARING OF CORAL TROUT, *Plectropomus leopardus* (Lacepède, 1802)

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ABSTRACT

Coral trout, *Plectropomus leopardus* (Lacepède, 1802)is a visual feeder. Turbidity caused by phytoplankton or clay particle in the water will affect the visual foraging of coral trout larvae. Addition of *Nannochloropsis* sp. has been included in standard operational procedure for marine fish larval rearing as green water. However, the density of *Nannochlorposis* sp. in coral trout larval rearing system has not been evaluated. This study aimed to evaluate the optimal of *Nannochloropsis* sp. required for rearing of coral trout larvae. *Nannochloropsis* sp. was given to two days old larvae (D-2), with the densities of 2 x 10^5, 4 x 10^5, and 6 x 10^5cell/mL. After 50 days rearing period (D-50), evaluation on the average size and total harvest were recorded. The results showed that the density of 2 x 10^5 cell/mL *Nannochloropsis* sp. was the best in survival rate (2.35 ± 1.05%) than other densities, but they were not significantly different (P>0.05) than those of 4 x 10^5 cell/mL (1.67 ± 0.70%) and 6 x 10^5 cell/mL (1.26 ± 1.05%). The lower densities, 2 x 10^5 and 4 x 10^5cell/mL, were dominated by more than 50% of > 2.7 cm sized juvenile. Histological analysis of fish eyes supported that the two lower densities produced dominant cone shape as the receptor cells in the retina observed. From an economical aspect, addition of 2 x 10^5 cells/mL resulted on the higher profit, hence optimum density of *Nannochloropsis* sp. added in coral trout larval rearing on a mass scale was 2 x 10^5 cells/mL.

KEYWORDS: coral trout; larval rearing; *Nannochloropsis* sp.; turbidity

INTRODUCTION

Appropriate feeding and water managements have pivotal roles in the larval rearing of coral trout, *Plectropomus leopardus*. The first critical stage was at the first feeding when the egg yolk had been absorbed completely, thus larvae should obtain the energy source out of their bodies to survive. The first feeding time for coral trout larvae is on day-3 when their egg yolks have been absorbed (Qu *et al*., 2012) and the mouth gap has opened completely (Sudewi *et al*., 2020). It was reported by Kusumawati *et al.* (2019) that feeding of live feed containing high fatty acid resulted in better survival for coral trout larvae. High fatty acid source could be obtained from *Nannochloropsis* sp. (Rebollosso-Fuenters *et al*., 2011), which is also utilized as green water in the larval rearing.

Coral trout is a visual feeder fish (Yoseda *et al*., 2008), which deeply relies on their vision to capture the prey. The right amount of light intensity is required for the fish to enable distinguishing the prey and its surrounding. Research results of Andamari *et al*. (2007) regarding light intensities (0-1,200 lux) for coral trout larval rearing showed range of light intensity from 900 to 1,200 resulted the highest survival by the end of larval rearing. This result was accordance with Yoseda *et al*. (2008) who reported that coral trout at 5 days after hatching (DAH) had better survival when rearing in levels of light intensity 1,000-3,000 lux than of 0-500 lux.

Light entering into water will be absorbed or scattered by particles (Sandstrom, 1999; Utne-Palm, 2002; Wellington *et al*., 2010), known as turbidity. Level of turbidity affects the vision of fish and its behavior in foraging (Cutts & Batty, 2005; Utne-Palm, 2002). According to Cutts & Batty (2005), level of

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turbidity was highly correlated to the density of phytoplankton in the water. Effect of turbidity depends on the development stage of the fish, since the dominancy of rod or cone cells which are photoreceptor cells in the eyes determine the function of vision. Cone cells work better in higher light intensity than rod cells and vice versa (Sandstrom, 1999).

Optimum levels of turbidity increase the vision of fish larvae to detect the prey and provide a safety sense from predator during foraging (Utne-Palm, 2002). Wellington *et al*. (2010) argued that low level of turbidity will benefit larvae by increasing contrast between live feed (zooplankton) and its surrounding. This arguably will improve the ability of fish to detect and capture the zooplankton. Survival of fish increases with the increasing of feeding success. However, in the larval rearing of humpback grouper, density of phytoplankton 5 x 10^5 cells/mL resulted in the higher survival compared to those reared in lower density of phytoplankton 3 x 10^5 and 1 x 10^5 cells/mL (Ismi *et al*., 2012). The function of phytoplankton as green water in the larval rearing water may explain of those results. Adding shading with green water might increase the foraging activity of the fish in looking for preys (Li *et al*., 2013). Different species might require different optimum density of phytoplankton in their environment. Hence, the objective of the research is to evaluate the optimum amount of addition of phytoplankton in coral trout, *Plectropomus leopardus* larval rearing water.

MATERIALS AND METHODS

Larval Rearing

This study was conducted in a closed hatchery using concrete yellow tanks with volume of 5000 L. Tanks were covered with transparent plastic to stabilize water temperature during the night. Approximately 50,000 eggs were stocked into each larval tank. Standard protocol in rearing coral larval trout was given on Table 1.

In the current study, a modification was created by treating the system with different densities of *Nannochloropsis* sp. given.

Experiment

Experiment was conducted at Gondol Research Insitute for Mariculture from March to December 2017. Plankton used was obtained from mass-cultured as much as 60 L, which was counted every day for the density and added with frozen concentrate (Nanno3600®) for the remaining amount. Larval rearing water was treated with different of *Nannochloropsis* sp. densities 2 x 10^5, 4 x 10^5, and 6 x 10^5 cells/mL. Each density treatment was repeated three times using the tanks of 5,000 L. *Nannochloropsis* sp. was given from two days old larvae (D-2) to D-30, once a day using two aeration hoses. Total of nine tanks were used for the larval rearing with number of initial larvae was approximately 31,000 larvae which was counted from hatching rate of eggs (62%).

The number of rotifers in the larval gut was counted by squishing the larval stomach from D-3 to D-8 when the stomach started to harden. Growth of larvae was also observed on interval days, every two days from D-1—D-10, and every five days from D-10—D-40. Both observations were performed under microscope and as many as 10 fish were observed for each sample. Survival rate, size diversity and percentage of deformities were calculated at harvest, which was executed manually by sorting the fish based on their size and performance (deformed or normal).

Histology observation, particularly on the eyes development of the fish was focused on the dominancy of rod or cone cells. Levels of turbidity, pH, ammonium (NH_4), and nitrite (NO_2) were checked at 2-day interval up to D-20, and at 5-day interval afterwards up to D-40.

RESULTS AND DISCUSSION

Survival and Size Variation

Addition of *Nannochloropsis* sp. in coral trout larval rearing with a density of 2 x 10^5 cells/mL resulted higher survival (2.35 ± 1.05%) but not significantly different ($P > 0.05$) compared to those reared in densities of 4 x 10^5 cells/mL (1.67 ± 0.70%) and 6 x 10^5 cells/mL (1.26 ± 1.05%). However, the survival was still highly fluctuated based on the value of standard deviation. This contrasted study of humpack grouper larval rearing, in which the survival tent to increase with the increasing of *Nannochloropsis* sp. density (Ismi *et al*., 2012).

At the end of the experiment (D-50), observation was conducted by harvesting all of the fish to count survival, percentage of deformities, and variation of the grade (size). Deformities such as lordosis, scoliosis, twisted jaws, or shorten operculum were hardly detected at this stage. The percentage of size variation was presented in Table 2.

Addition of 600,000 cells/mL of *Nannocloropsis* sp. resulted higher percentage of variation than those of two other treatments. Addition of 2 x 10^5 cells/mL resulted in homogenous size (> 2.7 cm) of the juvenile around 52.3%, while addition of 4 x 10^5 cells/mL resulted more homogenous size (> 2.7 cm) around 63.5%. This might be due to the light and turbidity

Table 1. Feeding and water exchange management in coral trout, *Plectropomus leopardus* larval rearing

	Days															
	2	3	4	7	9	10	11	12	13	14	15	16	17	20	25	30	40
Feeding management																	
Nannochloropsis sp.																	
Rotifers																	
Nauplii copepods																	
Formulated feed																	
Nauplii *Artemia*																	
Mysids																	
Water exchange management																	
5%-10%																	
10%-25%																	
≥ 25% increased gradually up to 100%																	
Siphoning																	

Tabel 2. Size variation of coral trout seed resulted from larval rearing with densities of *Nannochloropsis* sp. 2 x 10^5; 4 x 10^5; and 6 x 10^5 cells/mL

Addition of *Nannochloropsis* sp. (cells/mL)	**Large (> 2.7 cm) (%)**	**Medium (2.5-2.7 cm) (%)**	**Small (< 2.5 cm) (%)**
2 x 10^5	52.3	20.2	27.5
4 x 10^5	63.5	13.05	23.45
6 x 10^5	31.4	37.5	31.1

conditions in the larval rearing water of those two treatments provide better condition to forage. Hence fish were more active and easier to capture preys, which encourage the similar growth of fish in the same population.

Numbers of Rotifer in The Fish Gut and Fish Growth

Observation in the fish gut to understand the amount of rotifers captured and eaten by the larvae showed that addition of *Nannochloropsis* sp. 4 x 10^5 cells/mL as greenwater in the water resulted in frequently higher incidence of rotifer consumption than addition of *Nannochloropsis* sp. 2 x 10^5 and 6 x 10^5 cells/mL (Figure 1) during the early feeding. However, all of the treatments showed positive results in which fish was able to capture rotifer. The success of fish to capture rotifers during the first feeding was pivotal to ensure the survival of the fish since yolk sac of coral trout as endogenous source of energy was absorbed 3 days after hatching (Qu *et al*., 2012), which means that the larvae should be able to obtain exogenous energy from rotifers to maintain metabolism function.

However, even though the addition of 4 x 10^5 cells/mL had higher rate of rotifer consumption, the growth of all the treatments were similar (Figure 2) during the rearing. At the end of the experiment, addition of 4 x 10^5 cell/mL resulted in a slight bigger growth than the others. This was in accordance with the variation size data above. Total length of coral trout juvenile on D-40 was approximately 14-15 mm and on D-50 could reach 2.7 cm.

Turbidity levels showed consistently increased with the increasing of *Nannochloropsis* sp. additions in the larval rearing water. Addition of *Nannochloropsis* sp. at 6 x 10^5 cells/mL resulted in the highest turbidity level followed by 4 x 10^5 and 2 x 10^5 cells/mL, respectively (Figure 3). Turbidity level was highly related to light intensity penetrated to rearing water and affected to vision of larvae to detect the prey. Turbidity levels determine the contrast between preys and the surrounding, thus it immensely affects the

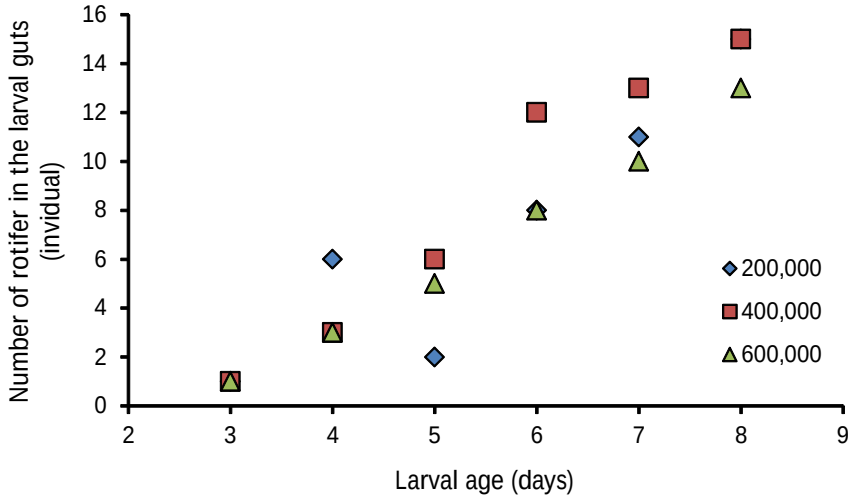

Figure 1. Numbers of rotifers (individual) observed in the fish gut on day-3 to day-8.

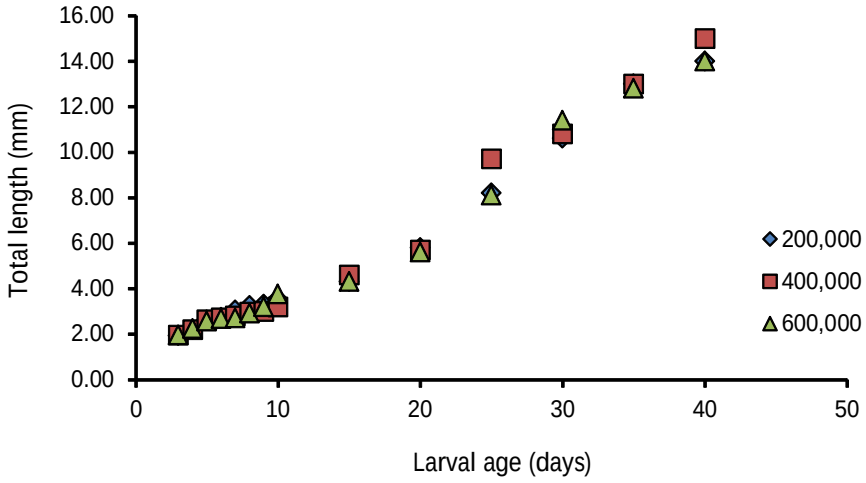

Figure 2. Total length of fish during larval rearing up to day-40.

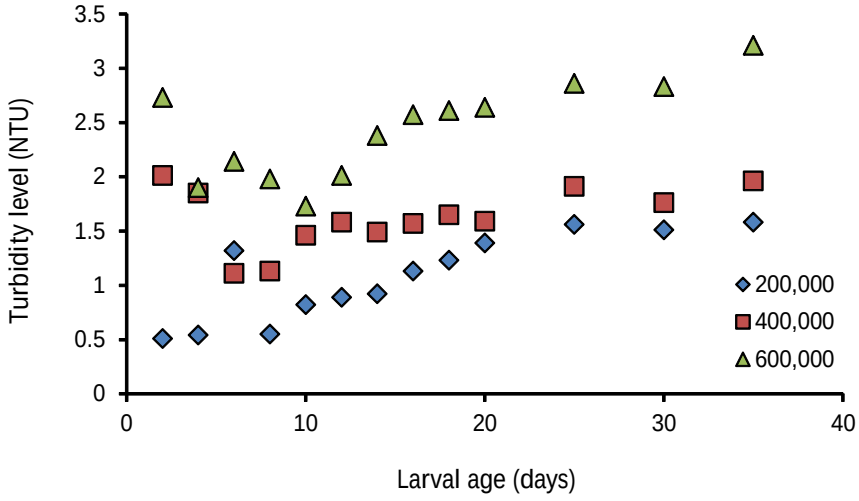

Figure 3. Turbidity level (NTU) of larval rearing water.

ability of fish to detect the preys accurately. Study conducted by Cutts & Batty (2005) found that turbidity level due to low density of phytoplankton (1.79 NTU) resulted in more contrast between *Artemia* and the background. Hence the success rate of fish detects and capture *Artemia* increased due to the contrast. This may also explain fish with addition of 4 x 10^5 cells/mL had higher growth and higher percentage of large size of juvenile at the end of the experiment since the larvae capture *Artemia* better during the rearing.

Eye Histology

Eye histology was observed to find out the development of the coral trout larvae and its vision ability in the light or dark condition. The results showed that eye of the fish had been completely developed on D-3, showed by complete structure and pigmentation (Figure 4). This indicates that coral trout larvae started to be actively foraging and preying since D-3.

Results of histology also revealed that eye of coral trout larvae was dominated by cone cells as the receptor cells than by rod cells (Figure 5). Ratio of cone and rod cells (Table 3) ranged from 1.3 to 3.8, which means that the number of cone cells were higher than the number of rod cells during larval stage of coral trout. This signified that coral trout larvae required relatively high light intensity in order to see and differentiate colors. Cone cells are the photoreceptor cells which work better in light condition, while rod cells work better in low light intensity or dark (Kjorsvik *et al*., 2004; Monk J. *et al*., 2006; Villamizar *et al*., 2011). According to Monk J. *et al*. (2006), pelagic larvae of marine fish tend to have higher number of cone cells than rod cells. The number of rod cells increases as the development of the fish, thus fish will be adapted to the darker environment as they grow.

Water Quality

During the rearing, generally ammonium (NH_4) levels in the rearing water increased considerably after D-6 and reached highest level on D-10 around 1 ppm (Figure 6). This was expected since water exchange and siphoning had not been initiated until D-10. However, level of ammonium 1 ppm was considered unfavorable for some grouper larvae. Sugama *et al*. (2012)

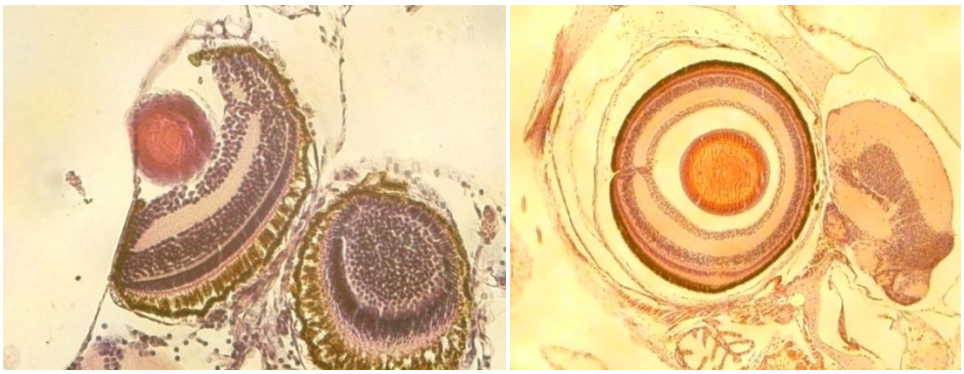

Figure 4. Eye histology of coral trout, *Plectropomus leopardus* larvae day-3 (left) and day-20 (right) (400x).

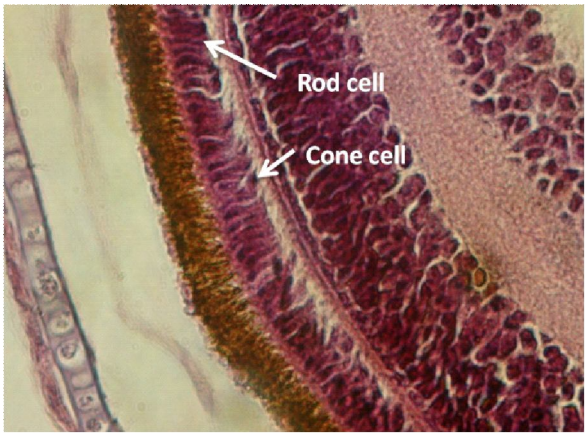

Figure 5. Cone and rod cells of coral trout, *Plectropomus leopardus* eye (1,000x).

Table 3. Ratio of cone and rod cells in the eye of coral trout larvae per 100 μm

Larval age (days)	Addition of *Nannochloropsis* sp. (cells/mL)		
	2×10^5	4×10^5	6×10^5
3	2.6	1.6	1.6
5	2.3	3.5	1.7
10	2.3	2.1	1.8
15	2.0	3.8	1.6
20	1.5	1.3	2.6

recommended maintaining level of ammonia in tiger grouper larval rearing below 0.1 ppm. Feces, dead rotifer and phytoplankton were accumulated in the water since the beginning of the larval rearing. It led to the loading of organic matter which converted to ammonia. After water exchange on D-10 and siphoning on D-12, the ammonium levels were noticeably dropped to 0 ppm. This pattern of ammonia levels was in accordance with the research report of Kusumawati *et al*. (2019) on coral trout larval rearing with similar protocol which ammonia level peaked on day 10-15 with levels of 1.5-2.5 ppm and decreased significantly after siphoning.

In general, higher density of phytoplankton added in the rearing water resulted in the increasing levels of ammonium. Dead phytoplankton added the amount of organic matter in the water. In this experiment, not only cultured *Nannochloropsis* sp. added in the larval rearing but also paste/concentrated *Nannochloropsis* sp. which tends to drop in the bottom after 4-5 days.

In contrast with the ammonia levels pattern in the rearing water, increasing of nitrite (NO_2) was only detected after D-14 and rise gradually up to D-40 (Figure 7). Higher levels of nitrite were resulted from higher density of *Nannochloropsis* sp. added in the water. Nitrite is notoriously toxic to fish, however each species has different toxic levels. A study conducted on gila trout *Oncorhynchus gilae* showed that 10 ppm of nitrite was able to kill the fish after 96 hours (Fuller *et al*., 2003). While Parra & Yufera (1999) reported that larvae of gilt head seabream could tolerate nitrite level of 200 ppm after 24 hours. High levels of nitrite exposure with relatively long duration might influence the survival of coral trout and there should be a further investigation.

While levels of oxygen during rearing ranged from 4.5 to 6.4 ppm. Research result on larval rearing reported by Kusumawati (2019) showed that DO levels during coral trout larval rearing were above 5 to 7 ppm. Based on the ammonia and nitrite data which were considerably higher than recommended (Sugama *et al*., 2012), this concern should be addressed in the future research which might lead to increasing of survival rate.

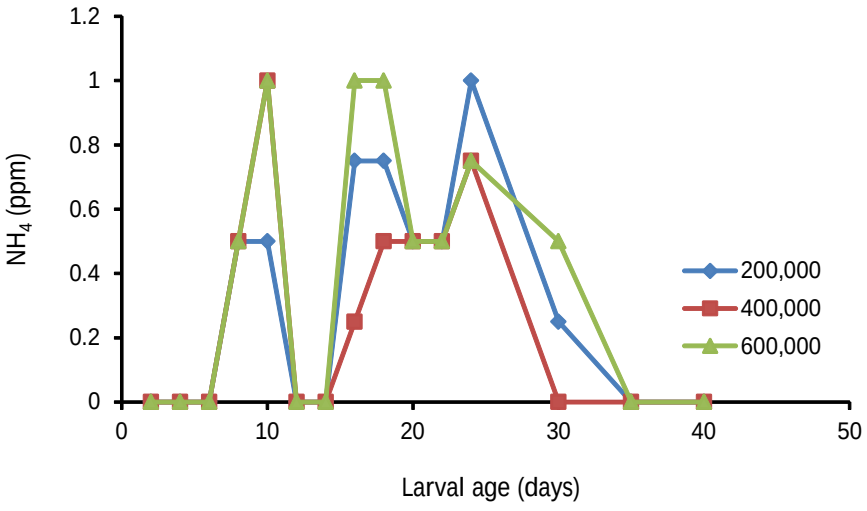

Figure 6. Ammonium (NH_4) levels (ppm) during coral trout larval rearing.

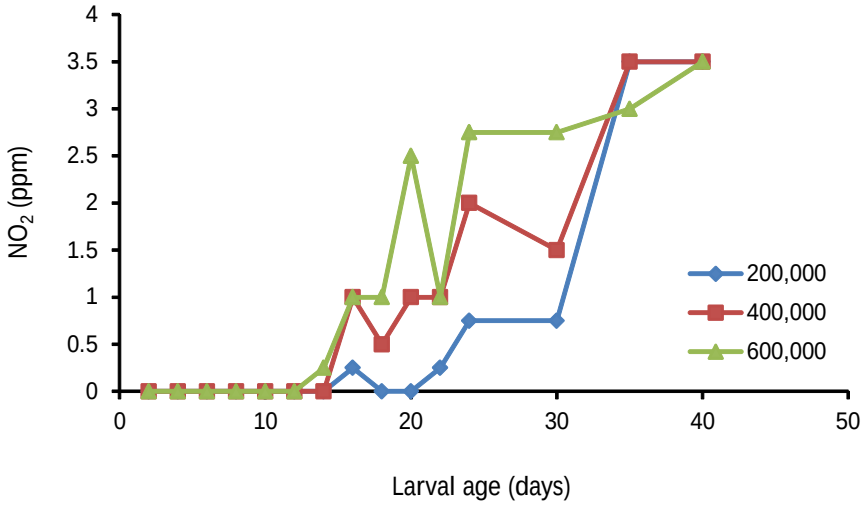

Figure 7. Nitrite (NO_2) levels (ppm) during coral trout larval rearing.

Profit Analyses

Since the survival among treatments were not significantly different. An economical factor was considered to determine the optimum density needed for larval rearing. Profit of mass scale larval rearing of coral trout was calculated based on the cost of *Nannochloropsis* sp. concentrate added in the water, feed cost and the revenue of the fingerlings harvested on D-50, since those were the differential factors in determining the profit in this study. Feed cost of larval rearing was IDR 1,135/fish. Revenue was calculated on the price of fish based on the size (refers to Table 2). The cost of *Nannochloropsis* sp. concentrate was IDR 1,100/mL and the price of the fingerlings was based on its size (large: IDR 5,400; medium: IDR 4,860; and small: IDR 4,500) (Tabel 4).

CONCLUSION

Addition of *Nannochloropsis* sp. at density of 2 x 10^5 cells/mL would be suitable in the larval rearing of coral trout, *Plectropomus leopardus*. Coral trout larva's retina was dominated by cone cells instead of rod cells, thus their vision was better in lighter condition than darker condition. Hence, addition of high density of *Nannochloropsis* sp. hindered the ability of the larvae to detect prey. However, right amount of green water density was necessary to stimulate foraging activity and improve vision of the larvae. Also addition of 2 x 10^5 cells/mL would be more favorable from an economical aspect for production of coral trout due to its value of profit (IDR 3,777 per fish).

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Table 4. The cost and revenue of a mass scale coral trout, *Plectropomus leopardus* larval rearing added with different densities of *Nannochloropsis* sp. values presents as IDR

	Density of *Nannochloropsis* sp. added in the larval rearing water (cells/mL)		
	2 x 10^5	**4 x 10^5**	**6 x 10^5**
Nannochloropsis sp. concentrate cost	95.7	287.1	861
Feed cost	826.641	587.443	411.562
Revenue	3,674,131	2,649,837	1,783,614
Profit	2,751,790	1,775,294	511.052
Profit per fish	3.777	3.429	1.409

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