Developing coral trout, *Plectropomus leopardus* larviculture technology: application of floating skimmer

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Abstract. Coral trout *Plectropomus leopardus* seed production has not been established well compared to other popular groupers such as tiger and hybrid grouper. Thus, the research has been focusing on the protocol construction of larviculture to improve survival and productivity. This study aimed to evaluate the application of floating skimmer to encourage swim bladder inflation in order to increase survival. Coral trout eggs were stocked in 5,000 L concrete tanks. Rotifer fed started on Day 2 (D2) afternoon and copepods nauplii on D4. Oil was dropped on the rearing water surface from D1 to D5 for without application of floating skimmer (control) and to D3 for with the application. A floating skimmer was installed on the surface of rearing water from D3 afternoons to D10. The survival rate was determined on D50. The results showed that application floating skimmer had a significantly higher survival rate (6.18±1.4%) than without application (5.28±1.64%) (p<0.05). Observation under a microscope confirmed that swim bladder inflation first noticed on D4. The application of floating skimmer promoted a higher swim bladder inflation rate than without skimmer. From an economical aspect, application of skimmer in coral trout larviculture resulted in higher profit.

1. Introduction

Coral trout, *Plectropomus leopardus* is one of exported marine commodity of Indonesia which its aquaculture production has not been established well, particularly compared to other popular groupers commodity such as tiger and hybrid grouper. A report by [1] displayed that coral trout, particularly *P. leopardus* larva culture has still very limited in Asia.

Seed production of coral trout has been tried in Bali (IMRAFE) and resulted in increasing survival rate in the hatchery. Development of coral trout larviculture protocol had resulted in survival 1.7% in 2016 by emphasizing on feeding management and environment manipulation, for instance photoperiod, live feed enrichment, application of copepods as reported by Kusumawati et al [2] and increased to 2.35% in 2017 by evaluating the optimum density of *Nannochloropsis* sp. (200,000 cells/mL) as green water as reported by Asih et al (in press) [3]. However, the results had not been feasible for a mass-scale production; hence the protocol was required to be improved to increase survival rate.

Mortality occurs on early stage of fish larvae mostly due to two factors: surface tension related death (STRD) and sinking syndrome related death (SSRD) [4]. Sinking syndrome occurs when the buoyancy of the larvae was not sufficient and most likely during the night time. The buoyancy of larvae was determined by larvae density and swim bladder inflation [4,5]. Swim bladder inflation was
reported to be critical in development of abnormalities and survival of marine fish larvae [4,5,6]. Larvae at some point at their stage swim to the surface and gulp the air to initiate swim bladder inflation [5,7]. Environment such as photo period, light intensity [7], aeration flow rate [5] and oil on the water surface [8,9] were reported to affect succeed of the inflation.

Oil on the water surface is believed to hindrance the process of swim bladder inflation by trapping the weak larvae when gulping the air. A study by Kawabe & Kimura [8] reported that the succeed of swim bladder inflation in blacktip grouper was significantly higher when the oil was removed from the surface. However common protocols of grouper larval rearing, fish oil was dropped into the surface of rearing water from D1-D5 in order to reduce surface tension so that mortality due to STRD could be avoided [10,11]. Hence, there supposed to be a window time when the oil on the surface was thinned out to reduce mortality due to STRD or SSRD.

Floating skimmer has been known to efficiently remove oil on blacktip grouper [8] and humpback grouper larviculture [6]. Removing oil on the surface by application of floating skimmer was also conducted in Australian bass, mulloway and yellowtail kingfish larviculture [9]. This study aimed to evaluate the application of floating skimmer in coral trout, *Plectropomus leopardus* larviculture to encourage swim bladder inflation in expect to increase the survival.

### 2. Materials and methods

#### 2.1. Protocol of larval rearing

Coral trout larval rearing was conducted as the protocol established by Kusumawati *et al* [2] with a modification on the density of *green water* as reported by Asih *et al* (in press) [3]. In brief, coral trout eggs were stoked in concrete tanks 5,000 L with a density of 10 eggs/L. The tanks were covered by transparent plastic to stabilize temperature, particularly during the night. *Nannochloropsis* sp. was added in the rearing water as green water on Day 2 (D2)-D35 as well as rotifers was given to the larvae on D2 afternoon onwards. Formulated feed, *Artemia* and Mysids were fed on D12, D20 and D40, respectively. To maintain water quality, siphoning was performed started on D12 onwards. Water exchange was conducted gradually to 100% from D10. Protocol of coral trout larval rearing was illustrated at Table 1.

| Days after hatching | 2 | 3 | 4 | 7 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 20 | 25 | 30 | 40 |
|---------------------|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|
| *Feed Management*   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |
| *Nannochloropsis* sp.|   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |
| Rotifer             |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |
| Nauplii copepod     |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |
| Formulated feed     |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |
| *Artemia*           |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |
| Mysids              |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |
| *Water Management*  |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |
| Water exchange 5-10%|   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |
| Water exchange 10-25%|   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |
| Water exchange ≥25% |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |
| gradually to 100%   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |
| Siphoning           |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |

Rotifers as first live feed was enriched using high-DHA based commercial enrichment, vitamin C (0.13 g/L) and taurine (25 mg/L). Larval rearing water was sterilized using UV before egg stocking and for water exchange. At this study, floating skimmer was installed on D3-D10 on the water surface and without skimmer was used as a control. Fish oil was dropped on the water surface from D1-D3 for
the treatment tanks, while from D1-D5 for the control tanks. Larval rearing was conducted in triplicates.

2.2. Parameters observation
Larvae morphology was observed under microscope at early stages of larvae (D2-D7) until the skin of larvae was scattered by pigment and hardly to be observed to determine the percentage of swim bladder inflation. Hatching rate was calculated by sampling and survival rate was determined by grading and counting all of the juveniles on D50. Survival rate was calculated by the number of hatched larvae in the tanks. At the end of the research, juveniles were checked for the swim bladder by dissection as well as for the spinal deformities. Water quality parameters i.e. temperature, pH, light intensity, salinity and dissolve oxygen (DO) were checked regularly at interval days in the morning (8-9 am). Total length of coral trout larvae was measured on a 5-day interval. Profit analysis was calculated based on the production cost and price of coral trout juvenile in 2020. We reported profit analysis in IDR only.

3. Results and discussion
3.1. Swim bladder inflation and survival
Observation of swim bladder inflation in coral trout larvae showed that percentage of larvae with inflated swim bladder reared in installed floating skimmer tanks was higher than of larvae reared in no-skimmer tanks (Figure 1). Swim bladder inflation was first observed on D4, which its percentage of larvae with inflated swim bladder 87.09% for skimmer treatment and 58.06% for no-skimmer. The percentage increased to 96.87% one day later for the skimmer and to 87.87% for no-skimmer. This result was in accordance with a study on humpback grouper. Installation of skimmer on D6 in humpback grouper larval rearing resulted in higher percentage of swim bladder inflation (5.7%) than installation on D15 (3.1%) and without installation of skimmer (1.1%) [6].

As our observation at the previous research of coral trout, we found that lots of larvae were dead on the surface on D4 morning. It is high likely that coral trout larvae started swimming to the surface to gulp air at the night before (D3). The installation of skimmer mostly was adjusted with the time of larvae initiate swim bladder inflation. In Australian bass, mulloway and yellowtail kingfish, floating skimmer was installed on D4, D4 and D2 respectively [9].

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Observation of the rearing water found that the oil on the surface was cleared out on D7 when the floating skimmer was installed, and at D10 or more when not installed. However, the oil collected inside the skimmer should be removed regularly to ensure its function to remove surface oil.

Figure 1. Percentage of coral trout larvae with inflated swim bladder (%) on D4 and D5 (A) (n=10). Survival rate of coral trout reared with and without application of floating skimmer (B).

Application of floating skimmer was demonstrated to increase survival rate by around 17%. Survival rate of coral trout larvae D50 reared with application of floating skimmer was significantly higher 6.18 ± 1.40 % than without application of skimmer 5.28 ± 1.64 % (p<0.05). Hatching rate of
coral trout larvae in this study was 70.6 ± 5.0 %). It is believed that this result was influenced by swim bladder inflation. Even though on D5 the percentage of swim bladder in the treatment no-skimmer surged to 87.87%, the mortality due to sinking syndrome could occur the night before since sufficient buoyancy might not be met. Observation of swim bladder on juvenile D50 showed that all of the samples (n=10), both reared with or without application of skimmer, had full inflated swim bladders. There were also no spinal deformities found in the D50 juvenile resulted from both treatments. This might indicate that all of the larvae which survived during metamorphosis had succeeded in swim bladder inflation.

A study by Ismi et al [6] on humpback grouper, *Cromileptes altivelis* larval rearing showed that application of floating skimmer resulted in higher survival. However, the absence of swim bladder does not necessary resulted in mortality. Had the larvae survived, it potentially resulted in spinal deformities which mostly kyphosis and lordosis due to undeveloped swim bladder [6]. Deformities were rarely found in coral trout larviculture, particularly spinal deformities. This was also reported by Aslianti et al [12] who observed spinal deformities on coral trout by staining.

### 3.2. Larvae morphology and growth

Observation on the larvae morphology found that the formation of swim bladder was first noticed as a shiny little bubble under the front vertebrae on D4 (Figure 2). Digestive tract, mouth gap and eyes were formed on D3 when the larvae started to prey on rotifers, which was in accordance as reported by Sudewi et al [13]. Pigmentation on the skin was observed on D3 onwards under the vertebrate.

![Figure 2. Morphology of coral trout larvae D3 (A), D5 (B) and D7 (C) (40x). A black arrow on the middle picture shows swim bladder formation.](image)

Total length growth of larvae was illustrated on the graph below (Figure 3). Total length of D1 were identical between skimmer (2.08 ± 0.05 mm) and no-skimmer (2.03±0.08 mm). The growth was gradually increase approximately 0.16 mm/day (skimmer) and 0.18 mm/day (no-skimmer), from D1 to D20. Then there was a surge of growth to 0.78 mm/day (skimmer) and 0.80 mm/day (no-skimmer) from D20-30. Total length growth was slower from D30-D50 with the rate of 0.43 mm/day (skimmer) and 0.34 mm/day (no-skimmer). Total length of D30 larvae of skimmer and no-skimmer treatments were 13.12 ± 1.76 and 13.65 ± 1.60 mm, respectively. At the end of larval rearing (D50), total length of both treatments was similar. Averages of total length of coral trout D50 reared in tanks applied with skimmer and no-skimmer were 21.70 ± 2.32 and 20.53 ± 3.07 mm, respectively.

The results showed that the installation of swim bladder did not differ the growth of larvae. It means that installation of skimmer did not interrupt larvae in preying the feed. Coral trout is known to have slower growth than other groupers. A study by Kusumawati et al [2] on coral trout found that the growth rate of coral trout larvae up to D30 was approximately 0.27-0.31 mm/day, with total length on D30 was 12-14 mm.
Figure 3. Total length growth of coral trout, *Plectropomus leopardus* larvae reared in tanks applied with floating skimmer and without skimmer (n=20).

Total length of coral trout larvae on D1 was relatively smaller than other groupers larvae such as tiger grouper *Epinephelus fuscoguttatus*, marble groper *E. polyphekadion* and hybrid between those two groupers (‘cantik’ grouper) [11]. Total length growth of tiger grouper was noticeably faster than of coral trout grouper. On D45, tiger grouper larvae had completely metamorphosed into juveniles and reached total length 3.24 ± 0.55 cm, hence enable to be graded [11]. While total length of coral trout on D50 was 2.17 ± 0.23 cm.

3.3. Water Quality
Water quality was observed regularly to ensure that larvae reared in suitable environment (Table 2). During larval rearing, both treatments water had similar quality. Temperature, light intensity, salinity and dissolve oxygen were in the recommended range for standard operational grouper larviculture [10].

| Water Quality       | Treatments                        |
|---------------------|-----------------------------------|
|                     | No-Skimmer | Skimmer          |
| Temperature (°C)    | 28.52 ± 0.60 | 28.54 ± 0.65     |
| pH                  | 8.02 ± 0.36  | 8.01 ± 0.30      |
| Light intensity (lux)| 121-588    | 120-577          |
| Salinity (ppt)      | 31-34       | 31-34            |
| Dissolve oxygen (mg/L)| 4.96 ± 0.41 | 4.87 ± 0.41      |

3.4. Profit Analysis
Profit analysis was illustrated for three tanks rearing with the number of eggs 50,000 eggs/tank. Profit was only analysed based on the production costs, electricity bill and technician salary per batch, with duration of two months from eggs stocking to harvest (Table 3). Price of coral trout seed was IDR 2,000 per cm and the market size was 3 cm.
Table 3. Profit analysis of coral trout *P. leopardus* larviculture from eggs stocking to harvest (3 cm size of fingerling)

| Production Cost         | Quantity | Unit Price (x IDR 1,000) | Total Price (x IDR 1,000) |
|-------------------------|----------|--------------------------|---------------------------|
| Eggs                    | 150,000 eggs | 10                       | 1,500                     |
| *Nannochloropsis*       | 1 bag    | 1,300                    | 1,300                     |
| Rotifers                | 150 packs | 20                       | 3,000                     |
| Copepods                | 1 package | 600                      | 600                       |
| Artemia                 | 3 cans    | 750                      | 2,250                     |
| Mysis                   | 350 bags  | 10                       | 3,500                     |
| Artificial feed         | 1 package | 3,783                    | 3,783                     |
| Fertilizers             | 1 package | 1,000                    | 1,000                     |
| *Electricity bill*      | 2 months  | 750                      | 1,500                     |
| Technician salary       | 2 months  | 1,000                    | 2,000                     |
| Total                   |           |                          | **20,733**                |

**SR**

- Skimmer: 6.18%  
- Numbers of fingerlings D50: 105,000 x 6.18% = 6,489
- No-skimmer: 5.28%  
- Numbers of fingerlings D50: 105,000 x 5.28% = 5,544

**Revenue**

- Skimmer: total harvested fingerlings 85%*6,489 = 5,516
  - Total revenue 5,516*IDR 6,000 = **IDR 33,093,900**
- No-skimmer: total harvested fingerlings 85%*5,544 = 4,712
  - Total revenue 4,712*IDR 6,000 = **IDR 28,274,400**

**Profit**

- Skimmer: IDR 33,093,900 – IDR 20,733,000 = **IDR 12,360,900**
- No-skimmer: IDR 28,274,400 – IDR 20,733,000 = **IDR 7,841,400**

a HR 70%, numbers of hatched larvae: 105,000 larvae

b Survival from D50 to 3 cm size was approximately 85%. Price of 3 cm fingerling was IDR 6,000.

Profit analysis showed that application of skimmer increased the profit of coral trout larviculture by 57.6%. The cost of skimmer production was IDR 100,000 and it could be utilized for many rearing cycles. Compared to other groupers fingerling price, coral trout fingerling price is considerably higher. Tiger grouper, marbled grouper and hybrid grouper between tiger x marble grouper were valued IDR 1,000 per fingerling (size 3 cm) [11]. However, the duration of larviculture of coral trout is also longer, which also should be put into consideration when calculating profit analysis for a bigger scale production.

4. Conclusion

Application of floating skimmer promoted higher survival on larviculture of coral trout, *Plectropomus leopardus* due to higher chance to initiate swim bladder inflation at early stage of larvae. Application of floating of skimmer did not differ the growth of larvae. From an economical aspect, application of floating skimmer in the coral trout larviculture increased its value of profit.

5. References

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