

## Research Article



Check for updates

OPEN ACCESS

# Green Water Improves Growth and Clear Water Improves Swim Bladder Inflation Rates in Asian seabass, *Lates calcarifer* Larval Rearing System

Jhon Harianto Hutapea, Gunawan Gunawan, Ananto Setiadi, IG Ngurah Permana, Sari Budi Moria Sembiring\*, Ahmad Muzaki, Haryanti Haryanti

National Research and Innovation Agency, Research Center for Fishery, Cibinong Science Center, Bogor 16912, Indonesia

## ARTICLE INFO

### Article history:

Received September 27, 2025

Received in revised form August 28, 2026

Accepted October 1, 2026

Available Online October 20, 2026

### KEYWORDS:

digestive enzymes,  
endogenous probiotics,  
growth,  
larvae-juvenile,  
*Lates calcarifer*

## ABSTRACT

Asian seabass (*Lates calcarifer*) is suitable for industrial-scale development due to its high economic value worldwide. In Indonesia, hatchery juvenile production and aquaculture technology for Asian seabass are established; however, production often struggles to supply high-quality juveniles at the right size and time. Size variation due to uninflated swim bladders in larvae is a major factor causing slow growth in their cultivation. Two treatments, the green water system and clear water system (GWS and CWS), were tested to reduce size variation in the produced juveniles, with observed parameters including survival, growth rate, and enzyme activity. The results of a 20-day experiment showed that the juvenile mean standard length ( $8.85 \pm 0.54$  mm) and body weight ( $14.0 \pm 5.0$  mg) in GWS were higher, along with high gene expression of trypsin ( $>10$ -fold) and lipase ( $\pm 10$ -fold). In contrast, CWS had a lower final mean standard length ( $7.98 \pm 0.62$  mm) and body weight ( $10.0 \pm 3.0$  mg), with low trypsin and lipase gene expression but high amylase gene expression ( $>20$ -fold). The swim bladder inflation rate was not significantly different between GWS and CWS ( $p > 0.05$ ). Endogenous probiotics are more noticeable and can support better growth and survival rates in the green water system by activating key enzymes such as trypsin and lipase. The green water system (GWS) provided a better substrate for the probiotic, which ultimately has a better effect on the development and growth of larvae by activating the main enzymes, such as trypsin and lipase.



Copyright (c) 2026 @author(s).

## 1. Introduction

Size variation among Asian seabass juveniles produced in hatcheries in Indonesia is a major problem in subsequent cultivation activities and is suspected to be caused by uninflated swim bladders from the larval stage onward (Sawada *et al.* 2021). Juveniles with uninflated swim bladders tend to grow slowly and suffer high mortality when exposed to diseases in the culture system (Boglione *et al.* 2001). Uninflated swim bladders (failure to fill air into the swim bladders) are associated with a surface film layer formed by

the larvae's mucus, feed given, or phytoplankton added in the larval rearing tank (Kawabe & Kohno 2009), which prevents the larvae from engulfing air directly from the water surface (Schwebel *et al.* 2018).

Two approaches reduce variation in juvenile size by increasing the success of swimbladder inflation: the Green Water System (GWS) and the Clear Water System (CWS). GWS has advantages in maintaining the quality of rotifers in the tanks (Reitan *et al.* 1997; Wang *et al.* 2019) by accumulating algae from the water (Romero-Romero and Yúfera 2012), serving as a natural shade against sunlight penetration, and, to some extent, can stabilize water quality (Liao *et al.* 2001; Ismi *et al.* 2012; Hutapea *et al.* 2020). The weakness of GWS is that it produces a thicker biofilm

\* Corresponding Author

E-mail Address: moriasembiring@gmail.com

layer and more organic material at the bottom of the tank. On the other hand, CWS has been proven effective in temperate zones (Izquierdo *et al.* 2006; Cremen *et al.* 2007) because the formed biofilm layer is thin and the organic material at the bottom of the tank is lower, but the rotifers' quality is reduced very fast (Romero and Yúfera 2012). However, applying CWS in tropical areas tends to cause a faster decline in rotifer quality, especially because the enrichment time is relatively short (4-5 hours). The enrichment materials can become toxic for longer periods at high ambient temperatures. Furthermore, larval distribution tends to be patchy near the water surface in CWS.

Water quality management is very important for the success of larval rearing, considering that there are no water changes during the first 5 days of larval rearing (stagnant), followed by a gradual increase as larvae grow older, to maintain water quality. Considering that in this study there are additional organic materials, either as natural feed, artificial feed, phytoplankton, and feces from the reared larvae, so, to ensure good water quality and improve larval health, endogenous and specific probiotics were added during the study. It has been proven that the application of probiotics in aquaculture can stabilize water quality and also improve fish health (Sarioglu *et al.* 2012; Mahdhi *et al.* 2012; Assan *et al.* 2022) and increase fish larvae's digestive enzyme activity (Tovar *et al.* 2022).

The three main digestive enzymes in marine finfish larvae are trypsin in processing food (Rønnestad *et al.* 2013), which is related to predation activity (Suzer *et al.* 2007a; Mata-Sotres *et al.* 2016), lipase for fat digestion, and amylase to transform complex carbohydrates into simpler sugars (Assan *et al.* 2022). For this study, three selected species of endogenous probiotics isolated from the digestive tract of wild-caught Asian seabass were *Bacillus subtilis* strain KB-4, *Enterobacter hormaechei* strain KC-1, and *Bacillus* sp. strain KL-5 (Haryanti *et al.* 2019), with the ability to hydrolyze carbohydrates, protein, and fats; they are not antagonistic among strains, and are not pathogenic to Asian seabass fish larvae. The specific probiotics *Alteromonas* sp. BY-9 is also added to suppress specific pathogens bacteria in the bottom and water media (Haryanti *et al.* 2014).

This study was designed to see the impact of CWS and GWS on larval environmental conditions, the ability of larvae to catch prey which will ultimately support the growth and survival of larvae to juvenile and swim bladder inflation rate.

## 2. Materials and Methods

### 2.1. Research Design

Larval rearing was conducted with two treatments: (A) Green water system (GWS) and (B) Clear water system (CWS), with three replications and a completely randomized design. Data from this experiment were presented in tables and figures, and we used t-tests for data requiring further analysis. *Nannochloropsis* sp. at  $0.45\text{--}0.50 \times 10^6$  cells mL<sup>-1</sup> was added to each GWS tank. To each CWS tank unit, Rotifeed- a commercial rotifer enrichment made of microalgae was added at 2.5 g day<sup>-1</sup> tank<sup>-1</sup>. Light penetration into GWS treatment media decreased due to the addition of *Nannochloropsis* sp. So, to adjust the similar light intensity in both treatments, a net shading (orchid sheet) was set up on top of each CWS tank, and the light intensity at the water surface measured at 12.00–13.00 ranged from 450 to 600 lux.

The current study was carried out at the Scientific Conservation Region for Marine Organisms, National Research and Innovation Agency, Gondol-Bali, Indonesia.

### 2.2. Larval Rearing of Asian seabass Under Green & Clear Water System

Asian seabass broodstocks were reared in a circular concrete tank and spawned naturally. Eggs were harvested, selected, and disinfected in a 50 ppm Iodine solution for 5 minutes (0.75 mL Iodine in 15 L filtered seawater). One thousand eggs L<sup>-1</sup> were then incubated in three 200 L transparent cylindrical fiberglass tanks, equipped with three aeration systems at  $40 \pm 14$  mL sec<sup>-1</sup> and flow-through seawater at  $15 \pm 3$  mL sec<sup>-1</sup>. Six rectangular concrete tanks,  $2.80 \times 2.45 \times 1.00$  m<sup>3</sup>, filled with 4 m<sup>3</sup> seawater, were used as larval rearing tanks. Newly hatched larvae (D0, 0 days after hatching) from the incubation tank were transferred into each tank at a density of 20 larvae L<sup>-1</sup>. Four fish oil capsules were dripped onto the water surface of the larval rearing tank from D0 to D4 to reduce the surface tension. Fish oil will push the film layer to the tank side, making it easier to scoop. Water exchange was performed at 5% day<sup>-1</sup> for D7–D9, increasing to 10% day<sup>-1</sup> for D10–D14, then 15% day<sup>-1</sup> for D15–D17, and 20% day<sup>-1</sup> for D18–D20. Detritus siphoning from the bottom of the tanks commenced daily from D15 to the end of the experiment (Table 1).

Table 1. Water management during larval rearing of Asian seabass (*Lates calcarifer*) larval

| Figure 1: Water management during larval rearing of African seabass ( <i>Lates niloticus</i> ), larval |                     |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |
|--------------------------------------------------------------------------------------------------------|---------------------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|
| Water                                                                                                  | Days after hatching |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |
| exchange rate                                                                                          | 0                   | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| 5% day <sup>-1</sup>                                                                                   |                     |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |
| 10% day <sup>-1</sup>                                                                                  |                     |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |
| 15% day <sup>-1</sup>                                                                                  |                     |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |
| 20% day <sup>-1</sup>                                                                                  |                     |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |
| Siphoning                                                                                              |                     |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |

Water quality during the experiment was maintained under optimum conditions, such as dissolved oxygen >4.0 mg L<sup>-1</sup>, salinity 33-34 ppt, pH >7.5, Ammonium <2.0 mg L<sup>-1</sup>, nitrite <0.01 mg L<sup>-1</sup>, and water temperature 28.7-30.1°C.

Larval sampling was conducted using three different methods. First, samples for digestive enzymatic analysis were taken daily from 09.00 to 10.00 in the morning, with 80–100 larvae from D1 to D10 and 40–50 larvae from D11 to D20. Second, sampled at night on D4 and D7 to calculate survival and swim bladder inflation rates, collecting three points per tank. The sampling equipment was a PVC pipe, 2 inches in diameter and 120 cm long, equipped with a valve and a rope at one end. It was closed by pulling the rope. Third, because larvae being highly active at night which not be possible to collect using PVC pipe, and to minimize the stress without reducing the minimum sample weight required, samples for D10 (n = 30), D15 (n = 20), and D20 (n = 10) were taken randomly by scooping during the day (14.00–15.00) for measuring growth rate, observation of swim bladder inflation and enzyme analysis. Final survival and swim bladder rate in each tank were calculated based on sampling and the total number of juveniles at the end of the study. Larval growth was obtained from measuring and weighing of D1 to D10 (n = 30), D15 (n = 20), and D20 (n = 10). Larvae and swim bladder condition were observed under a Binocular Microscope (Nikon Eclipse E600 & Nikon SMZ1000) at 20–40x magnification, connected to a Camera (Nikon Digital Camera-DXm1200F) and Image analyzer (WinROOF). Weighing was conducted using a Sartorius Balance (Shimadzu LIBROR AEL-200:0.0001 g accuracy).

### 2.3. Feed and Feeding Schedule

The GWS and CWS treatments were carried out by adding *Nannochloropsis* sp. and Rotifeed consecutively during the experiment (Table 2).

Rotifer started from D2 with a density of five individuals mL<sup>-1</sup> and increased to 10 individuals mL<sup>-1</sup> according to larval growth. When larval predation on rotifers increased rapidly (D6), weaning to artificial feed

began (D7). The size of the commercial feed given is 200–400 µm for D7–D12 and 400–800 µm for D12–D20 larvae. Feeding with nauplii of *Artemia* started at D14, when larvae were accustomed to artificial feed. The number of *Artemia nauplii* given was as much as 100–125 larvae<sup>-1</sup> day<sup>-1</sup>. All types of feed (rotifer, artificial feed, and *Artemia nauplii*) were given until the end of the study (D20). Rotifers were enriched with *Nannochloropsis* sp. for 4–5 hours to feed larvae D2–D5. When the predation of larvae on rotifers increased rapidly (D6), rotifers were enriched with commercial enrichments consisting of Taurine 40 g, Algamac 10 g, and vitamin C 10 g, dissolved in 150 mL of fresh water, and mixed using a mixer for about 10 minutes. The enrichment mixture was then put into a 200 L *Nannochloropsis* sp. tank, and 100–150 million rotifers were put into it and enriched for 4–5 hours. The same commercial enrichment was also applied to *Artemia nauplii*: 4–5 million in a 200 L container, depending on the daily needs of the larvae. Before and after enrichment, rotifers or *Artemia nauplii* must be cleaned by slowly flowing seawater into the plankton net containing rotifers or *Artemia nauplii*. The feeding ratio of rotifers and *Artemia nauplii* decreased with larval age, while the commercial feed ratio increased.

### 2.4. Probiotic Application

During the larval rearing, endogenous probiotics (*Bacillus subtilis* strain KB-4, *Enterobacter hormaechei* strain KC-1, and *Bacillus* sp. strain KL-5) and specific probiotics (*Alteromonas* sp. BY-9) are administered to each tank. Endogenous probiotic administration begins at D2 with 400 mL of a mixture of 3 strains (pure culture density of 1 × 10<sup>6</sup> CFU mL<sup>-1</sup>) to each tank, followed by 400 mL of specific probiotics at D3 and D4 at the same pure culture density. Again, at D5, endogenous probiotics are followed by specific probiotics at D6 and D7, and so on until the end of the experiment. *Vibrio* sp. density was counted periodically to determine the effectiveness of probiotic administration and to maintain optimum probiotic conditions in the larval-rearing media.

[illegible]

*Vibrio* sp. density (total bacteria  $<10^4$  CFU mL<sup>-1</sup> and *Luminouce* *Vibrio* mostly undetected, Table 4) and provided a reasonable survival rate for larvae.

### 3.3. Survival Rate and Swim Bladder Development

There was no significant difference in the survival rates of larvae from initial to the end of the experiment between the GWS treatment and the CWS treatment ( $p>0.05$ ) (Table 5). Swim bladder inflation started at D4 and continued until the end of the study. It is clear that in

the beginning, the swim bladder inflation rate of larvae in the CWS treatment was significantly higher compared to larvae in the GWS treatment, but as the larvae grew bigger and the initial inflation continued, it showed no more significant difference between CWS and GWS.

### 3.4. Larval Growth Under the Green and Clear Water System

Growth in standard length and body weight of larvae in GWS significantly higher started from D15 and D10 onwards consecutively compared to CWS treatment (Table 6).

Table 4. Bacterial density is suppressed by the application of probiotics (endogenous and specific probiotics) in the larval rearing of Asian seabass, *Lates calcarifer* larvae, in both CWS systems and in GWS systems

| Age (day) | Parameter                      | Treatment                   |                             |
|-----------|--------------------------------|-----------------------------|-----------------------------|
|           |                                | CWS (CFU mL <sup>-1</sup> ) | GWS (CFU mL <sup>-1</sup> ) |
| 1         | Total bacteria                 | $7.0 \pm 1.0 \times 10^3$   | $6.6 \pm 0.2 \times 10^3$   |
|           | <i>Luminouce</i> <i>Vibrio</i> | Undetected                  | Undetected                  |
| 5         | Total bacteria                 | $0.7 \pm 0.3 \times 10^2$   | $1.21 \pm 0.37 \times 10^2$ |
|           | <i>Luminouce</i> <i>Vibrio</i> | Undetected                  | Undetected                  |
| 12        | Total bacteria                 | $0.81 \pm 0.58 \times 10^2$ | $1.2 \pm 1.4 \times 10^2$   |
|           | <i>Luminouce</i> <i>Vibrio</i> | Undetected                  | Undetected                  |
| 15        | Total bacteria                 | $1.6 \pm 0.8 \times 10^3$   | $1.7 \pm 0.5 \times 10^3$   |
|           | <i>Luminouce</i> <i>Vibrio</i> | 27±29                       | 40±26                       |
| 20        | Total bacteria                 | $5.3 \pm 2.0 \times 10^2$   | $6.6 \pm 3.8 \times 10^2$   |
|           | <i>Luminouce</i> <i>Vibrio</i> | 23±15                       | 23±32                       |

Table 5. Survival rate and the ratio of Asian seabass, *Lates calcarifer* larvae with inflated swim bladder on Green dan Clear water system treatments

| Larva age (day) | Survival rate (%) |                  | Inflated swim bladder (%) |                   |
|-----------------|-------------------|------------------|---------------------------|-------------------|
|                 | GWS               | CWS              | GWS                       | CWS               |
| 0               | 100               | 100              | 0                         | 0                 |
| 4               | $56.3 \pm 3.6^a$  | $64.3 \pm 4.3^a$ | $22.2 \pm 15.0^a$         | $46.3 \pm 9.0^b$  |
| 7               | $55.9 \pm 1.2^a$  | $62.5 \pm 4.3^a$ | $30.0 \pm 8.0^a$          | $48.3 \pm 3.9^b$  |
| 10              | nd                | nd               | $21.5 \pm 10.8^a$         | $40.9 \pm 27.3^b$ |
| 20              | $30.8 \pm 15.7^a$ | $31.4 \pm 4.4^a$ | $45.2 \pm 1.7^a$          | $58.1 \pm 11.5^a$ |

Values in the same row followed by different superscript letters are significantly different ( $p<0.05$ )

Table 6. Standard length, body weight, and specific growth rate (SGR) of Asian seabass, *Lates calcarifer*, larvae from hatching (D0) to the end of the experiment (D20)

| Larva age (day) | Larval rearing system |                   |                    |                    |
|-----------------|-----------------------|-------------------|--------------------|--------------------|
|                 | Standard length (mm)  |                   | Body weight (mg)   |                    |
|                 | GWS                   | CWS               | GWS                | CWS                |
| 0               | $2.03 \pm 0.07^a$     | $2.03 \pm 0.07^a$ | $0.05 \pm 0.00^a$  | $0.05 \pm 0.00^a$  |
| 4               | $3.33 \pm 0.06^a$     | $3.20 \pm 0.05^a$ | $0.10 \pm 0.00^a$  | $0.09 \pm 0.00^a$  |
| 7               | $3.92 \pm 0.02^a$     | $3.78 \pm 0.36^a$ | $0.30 \pm 0.00^a$  | $0.29 \pm 0.00^a$  |
| 10              | $5.80 \pm 0.47^a$     | $5.84 \pm 0.46^a$ | $1.66 \pm 0.02^a$  | $1.17 \pm 0.02^b$  |
| 15              | $7.06 \pm 0.54^a$     | $6.71 \pm 0.46^b$ | $5.68 \pm 0.85^a$  | $4.26 \pm 0.55^b$  |
| 20              | $8.85 \pm 0.54^a$     | $7.98 \pm 0.62^b$ | $14.20 \pm 5.00^a$ | $10.01 \pm 2.00^b$ |
| SGR (%/day)     | 7.36                  | 6.84              | 28.24              | 26.50              |

Values in the same row followed by different superscript letters are significantly different ( $p<0.05$ ).

### 3.5. Patterns of Enzymatic Function Under the Green and Clear Water System

The concentration of trypsin, lipase, and amylase enzymes of D1 larvae was set as a denominator to express enzyme folds from larval to juvenile in Asian seabass (Figure 1). Lipase and amylase expression at D3 increased slightly in both GWS and CWS treatments, while trypsin in CWS increased up to 20 times compared with D1.

The expression of the trypsin enzyme of larvae from D3 increased remarkably to D7, followed by a very sharp decrease to D11 at the GWS treatment, but the opposite happened at the CWS treatment. From D13 onward, the trypsin pattern was the same in both treatments, but expression was higher in CWS.

The lipase expression pattern in both treatments was similar, increasing early and then decreasing from the

middle of the larval stage through the juvenile stage. The peak also occurred earlier (D9) and was higher (50-fold) in the GWS than in the CWS (D13, 20-fold).

The expression pattern of amylase increased from the beginning, decreased from D7-D11 for CWS and D9-D13 for GWS, and fluctuated from the metamorphosis stage towards the juvenile stage in both treatments. The peak expression occurred earlier and higher (on D5 by 16 folds and D13 by 37 folds) in CWS compared to the GWS treatment (on D9 by 32 folds and D15 by 15 folds).

### 4. Discussion

Higher initial feeding (D2) success in the GWS is related to the addition of *Nannochloropsis* sp.; when the sun shines, the dark inner wall tank provides optimal feed

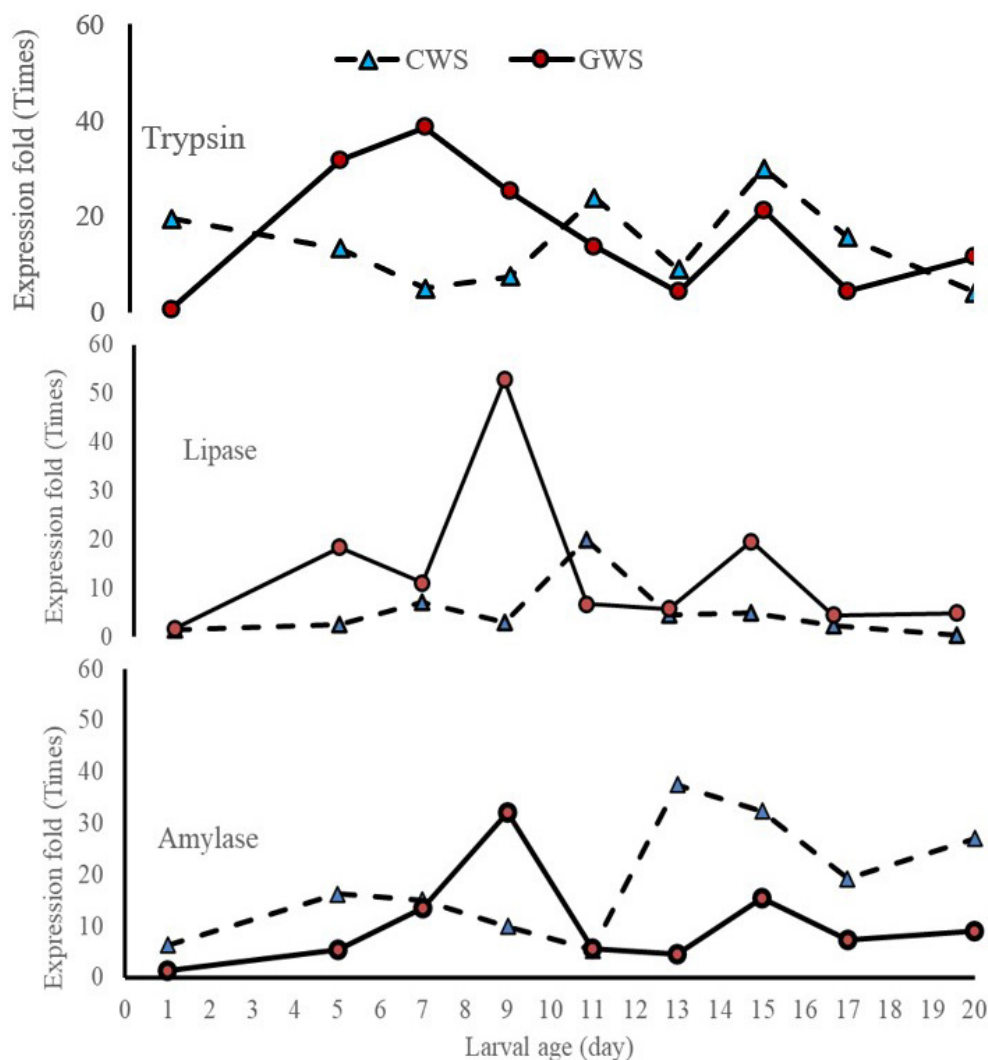


Figure 1. Patterns of trypsin, lipase and amylase enzyme expression of Asian seabass, *Lates calcarifer* larvae to the juvenile stage under green and clear water system treatment (X: Day after hatching Larvae, Y: Expression fold)

contrast and chemical stimulation (Shaw *et al.* 2006), making larvae easier to see and catch the rotifers. In contrast, when the inner wall tank was the same color, commercial microalgae powder added to the CWS larval tank did not provide optimal feed contrast, resulting in low initial feeding success, and the number of rotifers in the larvae's digestive tract was also low. This study found that the green water system is still better than a clear one in promoting feeding response, as has also been reported (Degidio *et al.* 2017; Pereira-Davison & Callan 2017). Optimizing feeding in GWS provides greater growth in total length and body weight than CWS, and this difference becomes more pronounced as larval age increases.

Adding endogenous probiotics that are known and associated with this Asian seabass species is very helpful in the digestive system and immunity of the larvae by improving larval digestive bacteria. Together with specific probiotics, this maintains the health of the larval-rearing environment (Haryanti *et al.* 2019) and improves nutrient utilization and food digestion (Afrilasari *et al.* 2016). Furthermore, when probiotics were applied in repeated dosing, their beneficial effects prolonged (Sohn *et al.* 2016), as shown in this experiment where total bacteria and *Luminouce* *Vibrio* controlled in very low colony-forming units. With the same probiotic administration in the CWS, the growth of larvae is lower, indicating that the microbial flour substrate to support the growth of probiotics in the larval rearing tank is still lacking compared with live microorganisms. This study also found that, to some extent, delay of initial feeding or late feeding, as shown in the CWS treatment, does not affect the larvae's survival, but it does affect growth, as reported elsewhere (Kailasam *et al.* 2007; Stuart *et al.* 2011; Chen & Zeng 2021).

The higher percentage of inflated swim bladders of larvae in CWS at the early stage is mostly due to the weak surface tension or thinner oil film layer formed at the water surface, or, according to Sawada *et al.* (2021), a cleaner air-water interface. It is easier for the larvae to engulf air directly from the surface water to initiate air filling into the swim bladder. In contrast, in GWS, adding *Nannochloropsis* sp. daily increases surface tension and makes it more difficult for larvae to engulf air directly from the surface water. According to Honryo *et al.* (2014), adding organisms, enrichment ingredients, or feed residues can produce the surface film layer. However, at the end of the study, juvenile swimbladder inflation did not differ significantly among treatments ( $p > 0.05$ ), suggesting that Asian seabass larvae have a longer period of air filling in the swimbladder. Swimbladder inflation

success determines the quality of the fish to be cultivated, because larvae with an inflated swim bladder show normal development and better growth (Martin-Robichaud & Peterson 2008). In contrast, an uninflated swim bladder causes atrophy and deformity, eventually leading to low larval growth and survival rate (Schwebel *et al.* 2018).

Early larval survival and growth depend heavily on natural feed, which provides exogenous enzymes that stimulate enzyme secretion or directly contribute to digestion in fish larvae (Sarvi *et al.* 2010). Meanwhile, digestive enzyme activity can indicate larvae acceptance of the feed (Suzer *et al.* 2007a; Khoa *et al.* 2019). All three enzymes (trypsin, lipase and amylase) exhibited an earlier peak in GWS. Trypsin is the most essential enzyme for the digestive capacity of early-stage larvae (Rønnestad *et al.* 2013). The very low trypsin enzyme expression in D3 larvae in the GWS treatment is also found by Gamberoni *et al.* (2021), for fast-growing fish species, characterized by a trend of low trypsin activity at the beginning due to the accelerated development of the stomach. In contrast, trypsin in the CWS treatment was relatively high, coinciding with low initial feeding success (D2) at 11% and an average of  $0.2 \pm 0.6$  rotifers larvae<sup>-1</sup> in the larval digestive tract. Low feeding success is due to low feed contrast, as happen in CWS treatment (Moyano *et al.* 1996; Suzer *et al.* 2007b; Infante *et al.* 2008). In this condition, larvae require more energy to survive and get the feed, which causes earlier trypsin activation by utilizing endogenous proteins. As larvae age, endogenous nutrients are depleted, and trypsin in the CWS treatment also decreases. The highest trypsin enzyme expression value in GWS treatment occurred in D7, proving that the feed digestibility and the nutritional conditions of fish larvae are better, as found by Khoa *et al.* (2019). In contrast to larvae in CWS treatment, the highest trypsin enzyme expression occurs on D15 or much slower, and this indicates that the nutritional conditions of fish larvae have just recovered. Thus, after the endogenous nutrients are absorbed, larvae in GWS treatment can hydrolyze proteins much earlier than those in CWS treatment, producing better nutritional conditions and growth.

An increase in lipase expression in earlier-stage larvae in GWS indicates that the larvae's ability to prey on feed, hydrolyze, and store lipids continues to increase (Rønnestad *et al.* 2013; Srichanun *et al.* 2013) and decreases when the exhaustion of endogenous nutrients, and short fasting periods, enter a change in food type (Cara *et al.* 2003). Conversely, at the same larval stage, lipase expression was low in CWS due to

the exhaustion of endogenous nutrients in early-stage larvae. Lipase expression rises again at D9 in GWS and D11 in CWS when the larvae can utilize artificial feed. Lipase expression in GWS treatment was much higher than in CWS treatment, suggesting that larvae in GWS begin synthesizing fat earlier and at higher levels than in CWS (Navarro-Guillén *et al.* 2015; Murashita *et al.* 2018).

The high amylase enzyme in earlier-stage larvae does not mean that larvae could digest carbohydrates from the natural feed, but it is derived from internal gene expression programs (Kim *et al.* 2001). It suggests that larvae can properly synthesize endogenous carbohydrates. The peak of amylase enzyme expression at D9 in GWS occurs earlier than in CWS treatment at D13, indicating an increased ability to synthesize carbohydrates. Since artificial feeding began on D7, larvae in the GWS treatment needed only two days to respond, whereas larvae in the CWS treatment needed six days. The decrease in amylase expression in the subsequent stage suggests that the larvae have entered the carnivorous phase (Ringø *et al.* 2007), and as the further development of the digestive system (Kim *et al.* 2001; P'erez *et al.* 2020), shown at D13 in GWS treatment larvae and delayed to D17 in CWS treatment.

In conclusion, the specific growth rate of larvae to juveniles in the GWS (SL: 7.36% day<sup>-1</sup>; BW: 28.24% day<sup>-1</sup>) is better than in the CWS treatment (SL: 6.84% day<sup>-1</sup>; BW: 26.50% day<sup>-1</sup>). In other words, adding *Nannochloropsis* sp. still plays a vital role in increasing the success of initial feeding and promoting better growth. Further research on evaluating the density of *Nannochloropsis* sp. needs to be conducted, and a better mechanism for cleaning the oil film layer from the water surface must be improved.

## Conflict of Interest

The authors declare that they have no conflict of interest in this article.

## Acknowledgements

This research is funded by the Ministry of Marine Affairs and Fisheries, Republic of Indonesia (DIPA. 032.12.403827/2021). The authors thanked the Asian seabass hatchery technicians who had worked hard to implement this experiment at the Institute for Mariculture Research and Fisheries Extension, Bali, Indonesia.

## References

- Afrilasari, W., Widanarni, Meryandini, A., 2016. Effect of probiotic *Bacillus megaterium* PTB 1.4 on the population of intestinal microflora, digestive enzyme activity and the growth of catfish (*Clarias* sp.). *HAYATI J. Biosci.* 23, 168–172. <https://doi.org/10.4308/hjb.23.4.168>
- Assan, D., Kuebutornye, F.K.A., Mustapha, U.F., Hlordzi, V., Abarike, E.D., 2022. Effects of probiotics on digestive enzymes of fish (finfish and shellfish); status and prospects: a mini-review. *Comparative Biochemistry and Physiology. Part B.* 257, 110653. <https://doi.org/10.1016/j.cbpb.2021.110653>
- Baer, A., Langdon, C.J., Mills, S., Schulz, C., Hamre, K., 2008. Particle size preference, gut filling and evacuation rates of the rotifer *Brachionus* “Cayman” using polystyrene latex beads. *Aquaculture.* 282, 75–82. <https://doi.org/10.1016/j.aquaculture.2008.06.020>
- Boglione, C., Gagliardi, G., Scardi, M., Cataudella, S., 2001. Skeletal descriptors and quality assessment in larvae and post-larvae of wild-caught and hatchery-reared gilthead sea bream (*Sparus aurata* L. 1758). *Aquaculture.* 192, 1–22. [https://doi.org/10.1016/S0044-8486\(00\)00446-4](https://doi.org/10.1016/S0044-8486(00)00446-4)
- Cara, J.B., Moyano, F.J., Cardenas, S., Ferna, C., Fernandes-Diaz, C., Yufera, M., 2003. Assessment of digestive enzyme activities during larval development of white bream. *Journal of Fish. Biology.* 63, 1, 48–58. <https://doi.org/10.1046/j.1095-8649.2003.00120.x>
- Chen, J.Y., Zeng, C., 2012. The effects of live prey and green water on the early larval rearing of orchid dotyback *Pseudochromis fridmani*. *Aquaculture.* 543, 737008. <http://dx.doi.org/10.1016/j.aquaculture.2021.737008>
- Cremen, M.C.M., Martinez-Goss, M.R., Corre Jr, V.L., Azanza, R.V., 2007. Phytoplankton bloom in commercial shrimp ponds using green-water technology. *J. Appl. Phycol.* 19, 615–624. <http://doi.org/10.1007/s10811-007-9210-7>
- Degidio, J.M.L.A., Yanong, R.P.E., Ohs, C.L., Watson, C.A., Cassiano, E.J., Barden, K., 2017. First feeding parameters of the milletseed butterflyfish *Chaetodon miliaris*. *Aquac. Res.* 49, 2, 1087–1094. <https://doi.org/10.1111/are.13558>
- Gamberoni, P., Yúfera, M., Sigüero, I., Gilannejad, N., Martínez-Rodríguez, G., Navarro-Guillén, C., 2021. Ontogeny and diurnal patterns of molecular gene expression and activity of digestive enzymes in developing greater amberjack. *Aquaculture.* 534, 736330. <https://doi.org/10.1016/j.aquaculture.2020.736330>
- Haryanti, Muzaki, A., Sembiring, S.B.M., Fahrudin, Permana, I.G.N., Wardana, I.K., 2014. The effect of probiotics on immunity improvement in the fry and spawner production of Pacific white shrimp *Litopenaeus vannamei*. *Indonesian Aquaculture Journal.* 9, 2, 133–146.
- Haryanti, Wibawa, G.S., Sembiring, S.B.M., Permana, I.G.N., Muzaki, A., Fahrudin, Sugama, K., 2019. The role of probiotics in larval and juvenile rearing of Asian sea bass (*Lates calcarifer*). Technical report IMRAFE, Gondol, Bali

- Honryo, T., Tanaka, T., Guillen, A., Wexler, J.B., Cano, A., Margulies, D., Scholey, V.P., Stein, M.S., Sawada, Y., 2014. Effect of water surface condition on survival, growth and swim bladder inflation of yellowfin tuna, *Thunnus albacares* (Temminck and Schlegel), larvae. *Aquaculture Research*. 47, 46, 1832–840. <http://dx.doi.org/10.1111/are.12641>
- Hutapea, J.H., Gunawan, Setiadi, A., 2020. Providing *Nannochloropsis* sp. for yellowfin tuna (*Thunnus albacares*) larval rearing system. In: *Proceedings of the National Seminar of AUP Polytechnics, Indonesia*. Jakarta: Lentera Mina. pp. 101–112.
- Infante, J.L.Z., Gisbert, E., Sarasquete, C., Navarro, I., Gutiérrez, J., Cahu, C.L., 2008. *Ontogeny and Physiology of the Digestive System of Marine Fish Larvae*. IBH Publishing Co. Pvt. Ltd, Oxford. <http://doi.org/10.1201/b10749-8>
- Ismi, S., Asih, Y.N., Slamet, B., Suwiry, K., 2012. The effect of *Nannochloropsis* sp. density in the larval rearing of mouse grouper (*Cromileptes altivelis*) in hatchery. *Journal of Aquaculture Research*. 3, 407–419. <https://doi.org/10.15578/jra.7.3.2012>
- Izquierdo, M., Forster, I., Divakaran, D., Conquest, L., Decamp, O., Tacon, A., 2006. Effect of green and clear water and lipid source on survival, growth and biochemical composition of Pacific white shrimp *Litopenaeus vannamei*. *Aquaculture Nutrition*. 12, 192–202. <https://doi.org/10.1111/j.1365-2095.2006.00385.x>
- Kailasam, M., Thirunavukkarasu, A.R., Selvaraj, S., Stali, P., 2007. Effect of delayed initial feeding on growth and survival of Asian sea bass *Lates calcarifer* (Bloch) larvae. *Aquaculture*. 271, 298–306. <http://doi.org/10.1016/j.aquaculture.2007.05.005>
- Kawabe, K., Kohno, H., 2009. Morphological development of larval and juvenile blacktip grouper, *Epinephelus fasciatus*. *Aqua. Fish. Sci.* 75, 1239–1251. <https://doi.org/10.1007/s12562-009-0128-7>
- Khoa, T.N.D., Waqalevu, V., Honda, A., Shiozaki, K., Kotani, T., 2019. Early ontogenetic development, digestive enzymatic activity and gene expression in red sea bream (*Pagrus major*). *Aquaculture*. 512, 734283. <https://doi.org/10.1016/j.aquaculture.2019.734283>
- Kim, B.G., Divakaran, S., Brown, C.L., Ostrowski, A.C., 2001. Comparative digestive enzyme ontogeny in two marine larval fishes: Pacific threadfin (*Polydactylus sexfilis*) and bluefin trevally (*Caranx melampygus*). *Fish Physiology and Biochemistry*. 24, 225–241. <https://doi.org/10.1023/A:1014054431627>
- Liao, I.C., Su, M.H., Chang, E.Y., 2001. Techniques in finfish larviculture in Taiwan. *Aquaculture*. 200, 1–31. [https://doi.org/10.1016/S0044-8486\(01\)00692-5](https://doi.org/10.1016/S0044-8486(01)00692-5)
- Mahdhi, A., Kamoun, F., Messina, C., Santulli, A., Bakhrouf, A., 2012. Probiotic properties of *Brevibacillus brevis* and its influence on sea bass (*Dicentrarchus labrax*) larval rearing. *African Journal of Microbiology Research*. 6, 6487–6495. <http://dx.doi.org/10.5897/AJMR12.1201>
- Martin-Robichaud, D.J., Peterson, R.H., 2008. Effects of light intensity, tank colour and photoperiod on swimbladder inflation success in larval striped bass, *Morone saxatilis* (Walbaum). *Aquaculture Research*. 29, 539–547. <http://doi.org/10.1046/j.1365-2109.1998.00234.x>
- Mata-Sotres, J.A., Moyano, F.J., Martínez-Rodríguez, G., Yúfera, M., 2016. Daily rhythms of digestive enzyme activity and gene expression in gilthead seabream (*Sparus aurata*) during ontogeny. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*. 197, 43–51. <https://doi.org/10.1016/j.cbpa.2016.03.010>
- Moyano, F.J., Díaz, M., Alarcón, F.J., Sarasquete, M.C., 1996. Characterization of digestive enzyme activity during larval development of gilthead seabream (*Sparus aurata*). *Fish Physiol. Biochem.* 15, 121–30. <https://doi.org/10.1007/bf01875591>
- Murashita, K., Matsunari, H., Furuita, H., Rønnestad, I., Oku, H., Yamamoto, T., 2018. Effects of dietary soybean meal on the digestive physiology of red seabream *Pagrus major*. *Aquaculture*. 493, 219–228. <https://doi.org/10.1016/j.aquaculture.2018.05.005>
- Navarro-Guillén, C., Moyano, F.J., Yúfera, M., 2015. Diel food intake and digestive enzyme production patterns in *Solea senegalensis* larvae. *Aquaculture*. 435, 33–42. <https://doi.org/10.1016/j.aquaculture.2014.09.017>
- Pereira-Davison, E., Callan, C.K., 2017. Effects of photoperiod, light intensity, turbidity and prey density on feed incidence and survival in first feeding yellow tang (*Zebrasoma flavescens*) (Bennett). *Aquac. Res.* 49, 890–899. <https://doi.org/10.1111/are.13535>
- Pérez, J.A., Papadaki, I.E., Papandroulakis, N., Cruces, L., Cotou, L.E., Gisbert, E., Lorenzo, A., Mylonas, C.C., Rodríguez, C., 2020. The ontogeny of greater amberjack digestive system under different rearing conditions: a histological and enzymatic approach. *Aquacult Nutrition*. 26, 1908–1925. <https://doi.org/10.1111/anu.13128>
- Reitan, K.I., Rainuzzo, J.R., Øie, G., Olsen, Y.A., 1997. A review of the nutritional effects of algae in marine fish larvae. *Aquaculture*. 155, 207–221. [https://doi.org/10.1016/S0044-8486\(97\)00118-X](https://doi.org/10.1016/S0044-8486(97)00118-X)
- Romero-Romero, S., Yúfera, M., 2012. Contribution of gut content to the nutritional value of *Brachionus plicatilis* used as prey in larviculture. *Aquaculture*. 364–365, 124–129. <https://doi.org/10.1016/j.aquaculture.2012.08.011>
- Rønnestad, I., Yúfera, M., Ueberschar, B., Ribeiro, L., Saele, O., Boglione, C., 2013. Feeding behaviour and digestive physiology in larval fish: current knowledge, and gaps and bottlenecks in research. *Rev. Aquac.* 5, S59–S98. <https://doi.org/10.1111/raq.12010>
- Sarioglu, O.F., Suluyayla, R., Tekinay, T., 2012. Heterotrophic ammonium removal by a novel hatchery isolate *Acinetobacter calcoaceticus* STB. *International Biodeterioration & Biodegradation*. 71, 67–71. <https://doi.org/10.1016/j.ibiod.2012.04.012>
- Sarvi, B., Matinfar, A., Mahmoudzadeh, H., Eskandary, G.R., 2010. Replacing rotifers with a microparticle diet from first feeding in yellowfin seabream, *Acanthopagrus latus* (Houttuyn), larvae. *Aquaculture Research*. 41, 1614–1621. <http://doi.org/10.1111/j.1365-2109.2010.02492.x>

- Sawada, Y., Mizushima, Y., Sera, T., Honryo, T., Kurata, M., Agawa, Y., 2021. Requirement of access to an air–water interface for initial swim bladder inflation and ontogenetic and day–night change of body-specific gravity in Japanese amberjack, *Seriola quinqueradiata* larvae. *Aquaculture Research*. 52, 6485–6496. <https://doi.org/10.1111/are.15516>
- Schwebel, L.N., Stuart, K., Lowery, M.S., Wegner, N.C., 2018. Swim bladder inflation failure affects energy allocation, growth, and feed conversion of California Yellowtail (*Seriola dorsalis*) in aquaculture. *Aquaculture*. 497, 117–124. <https://doi.org/10.1016/j.aquaculture.2018.07.050>
- Shaw, G.W., Punkhust, P.M., Battaglene, S.C., 2006. Effect of turbidity, prey density and culture history on prey consumption by greenback flounder *Rhombosolea tapirina* larvae. *Aquaculture*. 253, 447–460. <https://doi.org/10.1016/j.aquaculture.2005.09.025>
- Sohn, S., Lundgren, K.M., Tammi, K., Karim, M., Smolowitz, R., Nelson, D.R., Gómez Chiarri, M., 2016. Probiotic strains for disease management in hatchery larviculture of the eastern oyster *Crassostrea virginica*. *Shellfish Res*. 35, 307–317. <https://doi.org/10.2983/035.035.0205>
- Srichanun, M., Tantikitti, C., Utarabhand, P., Kortner, T.M., 2013. Gene expression and activity of digestive enzymes during the larval development of Asian seabass (*Lates calcarifer*). *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*. 165, 1–9. <https://doi.org/10.1016/j.cbpb.2013.02.005>
- Stuart, K.R., Drawbridge, M., 2011. The effect of light intensity and green water on survival and growth of cultured larval California yellowtail (*Seriola lalandi*). *Aquaculture*. 321, 152–156. <http://doi.org/10.1016/j.aquaculture.2011.08.023>
- Suzer, C., Kamaci, H.O., Coban, D., Saka, S., Firat, F., Oscara, B., Oscara, A., 2007a. Digestive enzyme activity of the red porgy (*Pagrus pagrus* L.) during larval development under culture conditions. *Aquaculture Research*. 38, 1778–1785. <https://doi.org/10.1111/J.1365-2109.2007.01841.X>
- Suzer, C., Aktülün, S., Çoban, D., Kamacı, H.O., Saka, S., Firat, K., Albaz, A., 2007b. Digestive enzyme activities in larvae of sharpnose seabream (*Diplodus puntazzo*). *Comparative Biochemistry and Physiology Part A*. 148, 470–477. <https://doi.org/10.1016/j.cbpa.2007.06.418>
- Tovar, D., Zambonino, J., Cahu, C., Gatesoupe, F.J., Va'zquez-Jua'rez, R., Le'sel, R., 2002. Effect of live yeast incorporation in compound diet on digestive enzyme activity in sea bass (*Dicentrarchus labrax*) larvae. *Aquaculture*. 204, 113–123. [https://doi.org/10.1016/S0044-8486\(01\)00650-0](https://doi.org/10.1016/S0044-8486(01)00650-0)
- Wang, J., Shu, X., Wang, W., 2019. Micro-elemental retention in rotifers and their trophic transfer to marine fish larvae: influences of green algae enrichment. *Aquaculture*. 499, 374–380. <https://doi.org/10.1016/j.aquaculture.2018.09.066>