



Performance of Growth, Survival Rate, and Behavioral in Seabass (*Lates calcarifer*) Seed Production with *Artemia* Enrichment using Sardinella Oil

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Abstract

Essential nutrients for seabass (*Lates calcarifer*) growth can be derived from the live feed *Artemia* sp. *Artemia* sp. is reported to contain protein levels between 40 and 55%, carbohydrate levels between 15 and 20%, and a lipid content of around 0.4%. Enrichment of *Artemia* sp. is necessary to improve its nutritional value and promote seabass larval growth. Sardinella fish oil is one option for enriching *Artemia* sp. This study aims to determine the optimal dosage of Sardinella oil for *Artemia* sp. Enrichment to achieve optimal growth and survival rates in seabass (*Lates calcarifer*) larvae. The study employed a completely randomized design (CRD) with five treatments and three replicates. The treatments were: unenriched *Artemia* (K-), *Artemia* + 0.9 g/l Squalene Fish Oil (K+), *Artemia* + 1 ml Sardinella Oil (A), *Artemia* + 1 ml Sardinella + 1 ml Egg Yolk (B), and *Artemia* + 0.5 ml Sardinella Oil + 0.5 ml Egg Yolk (C). The highest absolute weight was observed in treatments A and C, both recording 2.02 g (± 0.20 g and ± 0.09 g, respectively). Regarding absolute length, treatments A and C also showed the highest values, measuring 1.89 cm (± 0.17 g) and 1.90 cm (± 0.17 g), respectively. The highest survival rate (SR) was likewise found in treatments A (34.67% $\pm$ 2.63%) and C (38.08% $\pm$ 3.89%). Based on the tested treatments, the optimal dose for seabass growth and survival (SR) was determined to be treatment C, which included 0.5 ml of Sardinella oil and 0.5 ml of egg yolk.

INTRODUCTION

The persistent market demand for seabass requires the establishment of a reliable supply of hatchery-produced seedstock. The production of this seedstock, however, faces a number of challenges (Halim *et al.*, 2022). One of the primary causes of problems in seabass (*Lates calcarifer*) seed production is the provision of

low-quality feed. Feed is fundamental for sustaining growth and survival, particularly in intensive farming practices where it is required as the main source of energy (Rahadiyani *et al.*, 2014). A crucial source of nutrients for seabass (*L. calcarifer*) larvae is derived from the live feed *Artemia* sp. The nutritional composition of *Artemia* sp.

Typically includes protein levels ranging from 40 to 55%, carbohydrate levels between 15 and 20%, and a lipid content of approximately 0.4% (Maulana, 2017). Nevertheless, the limited content of essential fatty acids in *Artemia* necessitates supplementation (Khasanah *et al.*, 2012).

Artemia sp. lacking essential fatty acids exhibits reduced nutritional value and is therefore detrimental when used as a food source (Sulistiyono *et al.*, 2016). Various studies have been conducted on *Artemia* enrichment using essential fatty acids (EFAs), demonstrating improved growth performance in, among others, Russian sturgeon (*Acipenser gueldenstaedtii*) larvae (Kamaszewski *et al.*, 2014). The use of different enrichment products is capable of modifying the biochemical composition of *Artemia*. Enrichment of *Artemia* with commercial products for 24 hours resulted in significant differences in lipid and protein levels compared to newly hatched *Artemia* (nauplii) (Novelli *et al.*, 2016). In Indonesia, particularly Bali, Sardinella oil is a potential source of essential fatty acids for broader utilization due to its high content of EPA and DHA, as well as omega-3 fatty acids, to enhance fish growth and development (Watanabe, 1988; Dewi *et al.*, 2020). The application of Sardinella oil as a dietary supplement in aquaculture feeds has been explored, with a particular focus on silver rasbora (*Rasbora argyrotaenia*) during both the juvenile and larval development phases (Agustin *et al.*, 2020; Ayunda *et al.*, 2020; Dewi *et al.*, 2020; Marini *et al.*, 2020).

The enrichment of *Artemia* sp. as a natural feed using Sardinella oil also showed positive growth effects in silver rasbora larvae (Yuniar *et al.*, 2023). Enrichment of *Artemia* sp. using Sardinella oil still has the potential to be developed for other marine fish larvae, namely seabass. Research related to the enrichment of *Artemia* sp. using fatty acids in seabass has indeed been carried out, namely using squalene with an EPA content of 4.05% and a DHA content of 1.23% (Pridona *et al.*, 2016). Analysis of Sardinella oil revealed EPA and DHA concentrations of 8.97% and 6.56%, respectively (Maulana *et*

al., 2014). Based on the above description, it is evident that studies concerning the enrichment of *Artemia* sp. with Sardinella oil in seabass (*L. calcarifer*) larvae have not been performed and present a promising avenue for future investigation. This study aims to determine the optimal dosage for enriching *Artemia* sp. using Sardinella oil to achieve good growth and survival rates in seabass (*L. calcarifer*) larvae.

METHODOLOGY

Ethical Approval

This research was conducted without causing harm or mistreatment to any animals. The study ensured proper animal care, following optimal guidelines.

Place and Time

The research was conducted in October – December 2024 at the Polytechnic of Marine and Fisheries Jembrana.

Research Materials

The tools needed in this research were experimental aquarium, plankton net with mesh size 150 and diameter 15 cm (locally produced, Indonesia), plankton net with mesh size 80 and diameter 15 cm (locally produced, Indonesia), refractometer (Vivaria, PT. Aneka Akuatika Makmur, Indonesia), LED tube lamp (Meval, PT. Mitra Amertha Sejahtera, Indonesia), round plastic jar (Shinpo, PT. Surya Utama Putra, Indonesia), air stone (Vivaria, PT. Aneka Akuatika Makmur, Indonesia), airstone weight (Vivaria, PT. Aneka Akuatika Makmur, Indonesia), and water pump (Shimizu, PT. Pedoman Karya, Indonesia). The materials needed in this research were seabass seeds, *Artemia* sp. (*Artemia* EG, INVE Aquaculture, Belgium), squalene fish oil (Nutrimax, PT Suryaprana Nutrisindo, Indonesia), and egg yolks.

Research Design

The method used was an experimental method with a completely randomized design (CRD) consisting of five treatments and three replications. The treatments were:

unenriched *Artemia* (K-), *Artemia* + 0.9 g/l Squalene Fish Oil (K+), *Artemia* + 1 ml Sardinella Oil (A), *Artemia* + 1 ml Sardinella + 1 ml Egg Yolk (B), and *Artemia* + 0.5 ml Sardinella Oil + 0.5 ml Egg Yolk (C). The objective of the experiment is to examine the possibility of causal relationships by administering one or more treatment conditions to one or more experimental groups and comparing the outcomes with

one or more control groups that were not subjected to the treatment. Water quality parameters are measured using instruments such as a Thermometer for measuring water temperature, pH meter for measuring pH, a Refractometer for measuring salinity, DO meter for measuring dissolved oxygen in water, and a Test Kit for measuring nitrite and ammonium.

Table 1. Parameter, Tools, and Sampling Technique.

Parameter	Tools	Sampling Technique
Temperature	Thermometer	Insitu
pH	pH meter	Exsitu
Salinity	Refractometer	Exsitu
Dissolved Oxygen	DO Meter	Exsitu
Nitrite	Test Kit	Exsitu
Ammonium	Test Kit	Exsitu

Work Procedure

Observed Variables

Absolute Weight

According to Haetami *et al.* (2023), absolute weight is determined by the formula below.

$$G = W_t - W_o$$

Description:

G = Absolute Weight (g)

W_t = Final average weight of the fish (g)

W_o = Initial average weight of the fish (g)

Absolute Length

According to Haetami *et al.* (2023), absolute length is determined by the formula below.

$$G = P_t - P_o$$

Description:

G = Absolute Length (cm)

P_t = Final Average length of the fish (cm)

P_o = Initial average length of the fish (cm)

Specific Growth Rate (SGR)

According to Hartono *et al.* (2022), the specific growth rate is determined by the formula below:

$$SGR = \frac{\ln W_t - \ln W_o}{t} \times 100\%$$

Description:

SGR = Specific Growth Rate (SGR)

W_o = Final average weight of the fish (g)

W_t = Initial average weight of the fish (g)

T = Rearing period

Survival Rate (SR)

According to Hartono *et al.* (2022), the survival rate is determined by the formula below:

$$SR = \frac{\text{Abundance of live fish (ind)}}{\text{Total number of mortalities (ind)}} \times 100\%$$

Proximate Analysis

All treatments were subjected to proximate analysis. The tested proximate parameters include protein content, lipid content, moisture content, and Ash Content. Proximate analysis obtained in Unit Pelaksana Teknis Pengendalian dan Pengujian Mutu Hasil Perikanan (PPMHP) Banyuwangi.

Experimental Aquarium Preparation

The containers used in this study were aquariums disinfected using potassium permanganate (KMnO₄) at a concentration of 10 ppm applied to all internal surfaces for 10–15 minutes. The aquariums were then scrubbed with a brush and rinsed with fresh water. Each aquarium was subsequently filled with 180 liters of seawater and equipped with an aeration system.

Preparation of Test Fish Larvae

The seabass (*L. calcarifer*) larvae were 20 days post-hatch, measuring 0.8 – 1.2 cm in length. The larvae originated from captive-bred white snapper broodstock and were obtained from local fish farmers in the Gerokgak Buleleng region.

Enrichment of *Artemia* sp.

Artemia sp. culture was conducted by preparing the culture media in plastic containers using 1 liter of freshwater and 2 liters of seawater. The salinity used for *Artemia* sp. culture was 30 ppt. Five grams of *Artemia* sp. cysts were cultured for 24 hours. The hatched *Artemia* sp. were filtered using a 60 µm plankton net to prevent the shells from mixing with the newly hatched *Artemia* nauplii. The *Artemia* sp. nauplii were enriched according to the treatments for 1 hour. For the treatments involving egg yolk and lemuru fish oil, these were first mixed thoroughly.

Rearing of Test Fish Larvae

Seabass (*L. calcarifer*) larvae were reared for 15 days at a stocking density of 2 individuals/liter, including feeding with enriched *Artemia* sp. nauplii according to the treatments. *Artemia* sp. were fed four times daily at 09:00, 12:00, 16:00, and 21:00 WITA (Central Indonesian Time) to

satiation, with an estimated density of 5 *Artemia* sp. nauplii/ml.

Sampling

Sampling was conducted every 5 days, encompassing survival rate and growth parameters, by randomly selecting 10 seabass per aquarium.

Data Analysis

Survival and growth data were analyzed using Analysis of Variance (ANOVA) statistical test with SPSS 25.0 and followed by a post-hoc test using W-Tukey's Honest Significant Difference (HSD) test with a 95% confidence level (alpha = 0.05). Water quality parameter data were analyzed descriptively in the form of tables and graphs.

RESULTS AND DISCUSSIONS

Water Quality

Water quality significantly influences the growth of cultivated biota. Good water quality that meets aquaculture standards will support optimal growth. Conversely, poor water quality can cause stress, resulting in stunted growth due to decreased appetite. Therefore, in aquaculture, it is crucial to maintain the carrying capacity of the environment to avoid crop failure (Farabi and Latuconsina, 2023). Water quality data during the research were presented in the following table.

Table 2. Water Quality parameters during the experiment.

Parameter	Treatments					References
	K(-)	K(+)	A	B	C	
Temperature (°C)	28,5-33,7	28,4-33,7	28,4-33,7	28,5-33,7	28,5-33,7	28-32*
pH	8,1-7,5	7,5-8,1	7,5-8,07	7,6-8,1	7,5-8,1	7-8,5*
Salinity (ppt)	30-32	30-32	30-32	30-32	30-32	>27*
DO (mg/l)	4,3-5,17	4,2-5,03	4,2-5	4,5-4,83	4,3-4,6	>4*
Nitrite (mg/l)	0-0,1	0-0,1	0-0,1	0-0,1	0-0,1	0,08**
Ammonium (mg/l)	0,15-0,25	0,15-0,25	0,15-0,25	0,15-0,25	0,15-0,25	max 1*

Sources: *SNI 6145.4-2014, **Yaqin *et al.* (2018).

Water quality measurement data indicate that water quality remained within optimum conditions for most parameters. Water quality measurements in this study were conducted at the time of sampling.

Temperature is a factor that influences the dissolved oxygen levels in the culture media. Low temperatures increase dissolved oxygen and enhance the metabolic rate of aquatic organisms, and can determine which aquatic

organisms can survive in the rearing media (Ngho *et al.*, 2015).

Dissolved oxygen is a crucial component for survival through respiration, maintaining health, and bacterial activity in decomposing fish metabolic waste. Dissolved oxygen levels impact oxygen availability for respiration and the formation of toxins in the water (nitrite and ammonia). Low dissolved oxygen affects fish growth, as it can lead to stress, hypoxia, decreased swimming activity, and weakened immunity to diseases (Fitriawati and Utami, 2023). Salinity influences fish growth due to its relation to osmoregulation activity. Low salinity makes it difficult for larvae to osmoregulate, potentially causing mortality. Conversely, excessively high salinity makes it difficult for

larvae to adapt. Therefore, salinity stability in a water body should be maintained (Putri and Kurniawan, 2023). Nitrite levels in this study ranged from 0-0.1 mg/L. This is still within the optimal range for seabass, as the optimal range for seabass is 0.08 mg/L.

Proximate Analysis of Treatments

Proximate analysis is a method used to determine the nutrient and energy content of feedstuffs (Isharyudono *et al.*, 2019). This study analyzed the proximate composition of each treatment. The proximate analysis results obtained Unit Pelaksana Teknis Pengendalian dan Pengujian Mutu Hasil Perikanan (PPMHP) Banyuwangi can be seen in Table 1.

Table 3. Results of Proximate Analysis on the Treatments.

Analysis	Treatments				
	K(-)	K(+)	A	B	C
Protein Content	9.76%	10.37%	9.33%	9.61%	9.68%
Lipid Content	0.25%	0.17%	0.07%	0.12%	0.17%
Moisture Content	81.39%	82.29%	83.34%	82.72%	82.38%
Ash Content	1.83%	1.51%	1.73%	1.80%	1.88%

Results of the proximate analysis showed the highest protein content in treatment K(+) at 10.37%. The lowest protein content was found in treatment A at 9.33%. The highest lipid content was found in treatment K(-) at 0.17%. The lowest lipid content was found in treatment A at 0.07%. The highest moisture content was found in treatment A at 83.34%. The lowest moisture content was found in treatment K(-) at 81.39%. The highest ash content was found in treatment C at 1.88%, and the lowest ash content was found in treatment A at 1.73%. Based on the data above, it is known that *Sardinella* produces oil that is frequently used for nutritional enrichment in feed. Fish oil is extracted from the fish's fatty tissue. The main components of fish oil are omega-3 fatty acids, particularly EPA and DHA. These compounds possess potent anti-inflammatory properties. The presence of omega-3s in fish depends on their diet. Fish that consume microalgae, such as

phytoplankton, will accumulate omega-3s in their bodies. Examples include herring and sardines. The fatty acid profile of fish oil is dominated by long-chain polyunsaturated fatty acids, including EPA and DHA, which play crucial roles in various bodily functions (Hasan *et al.*, 2021).

Length and Weight Growth of Seabass

The growth of white snapper was assessed based on length and weight data obtained through sampling. Sampling was conducted three times during the study.

Absolute Weight

Absolute weight gain was calculated as the difference between the initial and final weights of snapper fry during the rearing period (Mahardhika *et al.*, 2017). The absolute weight data obtained during the study are shown in Figure 1.

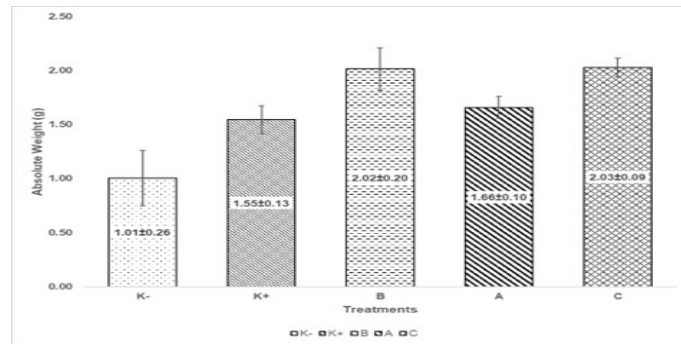


Figure 1. Absolute Weight.

Figure 1 shows that treatment K- was significantly different from all other treatments ($P < 0.05$). The treatments with the highest absolute weight gain were treatment A at 2.02 g (± 0.20 g) and treatment C at 2.02 g (± 0.09 g). This indicates that the addition of lemuru fish oil to *Artemia* sp. can increase the absolute weight gain of snapper fish. *Sardinella lemuru* fish oil, as a source of fatty acids (lipids), contains high energy used for

growth (Yuniar *et al.*, 2023).

Absolute Length

Absolute length was calculated by determining the difference between the final length of the fish at the end of the rearing period and the initial length of the fish at the beginning of the rearing period. The absolute length of the white snapper during the study is presented in Figure 2.

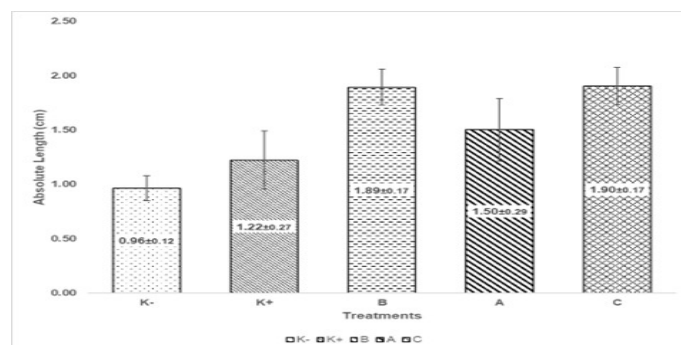


Figure 2. Absolute Length.

Figure 2 shows that treatment K- was significantly different from treatments A and B but not significantly different from treatments K(+) and B, with the highest absolute length observed in treatments A at 1.89 cm (± 0.17 g) and C at 1.90 cm (± 0.17 g). As the research progressed, the absolute length of the fish increased, especially in those given fish oil enrichment. According to

Lestari *et al.* (2021), feed consumption rate influences the growth and development of each individual as well as the biomass at the end of the rearing period, which is related to the optimization of larval growth.

Based on observations using Inskam 315-W Digital Camera, the following images show the total length of seabass during a two-week rearing period.

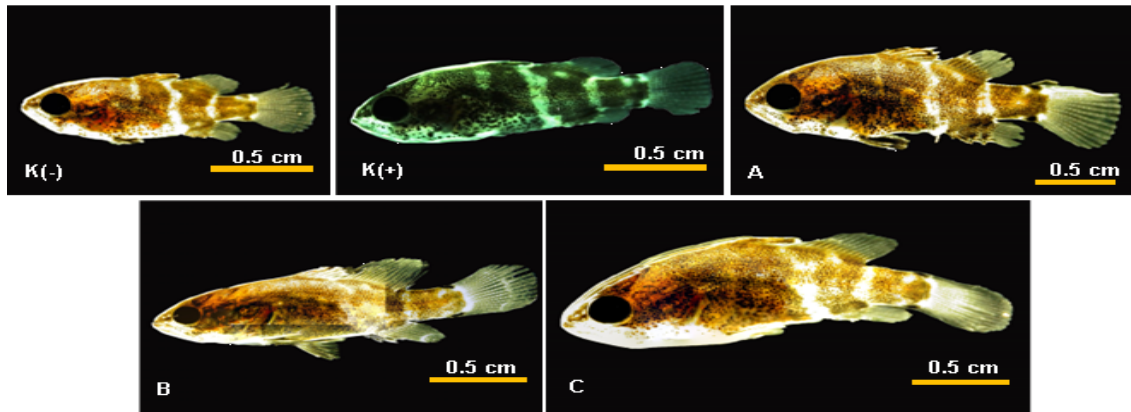


Figure 3. Total length of seabass.

Survival Rate (SR)

Survival rate is a metric employed as a parameter to assess the success of a fish

cultivation activity (Imani *et al.*, 2021). The SR obtained during the rearing period is presented in Figure 4.

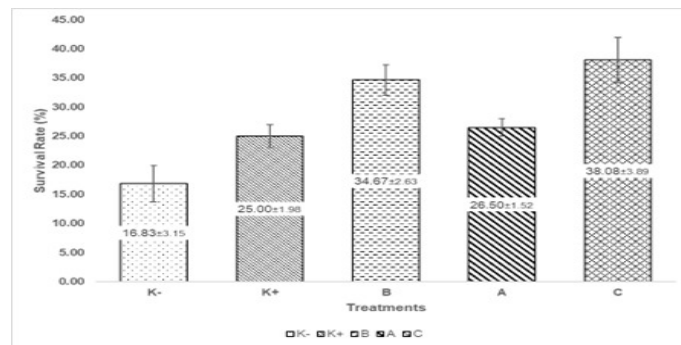


Figure 4. Survival Rate.

Figure 4 shows that treatment K- was significantly different from all other treatments ($P < 0.05$), with the highest SR treatment observed in treatment A at 34.67% ($\pm 2.63\%$) and in treatment C at 38.08% ($\pm 3.89\%$). The administration of lemuru fish oil in feed can enhance metabolism and optimize fat utilization in the body. The content of unsaturated fatty acids in lemuru fish oil can be used as an

additional source of nutrients for the body (Andhikawati *et al.*, 2020).

Specific Growth Rate (SGR)

The growth performance of seabass can also be evaluated by its daily growth rate or Specific Growth Rate (SGR) (Fitriawati and Utami, 2023). The SGR data during the research are presented in Figure 5 below.

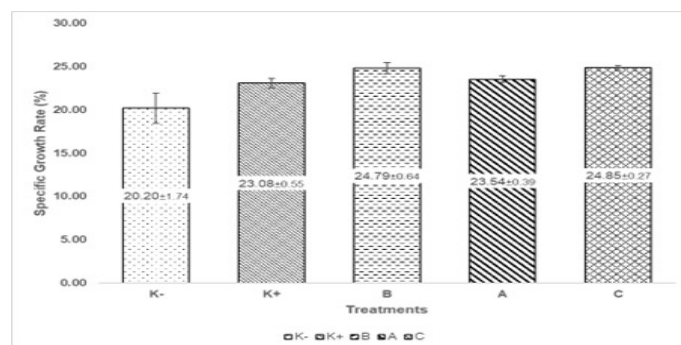


Figure 5. Specific Growth Rate (SGR).

Figure 5 shows that the K- treatment was significantly different from all other treatments, while the K(+) treatment was not significantly different from treatments A, B, and C. The highest SGR values were found in treatment A, 24.79% ($\pm 0.64\%$), and C, 24.85% ($\pm 0.27\%$). The increased growth rate during the rearing period can be influenced by the fish's ability to digest and utilize feed for weight and length gain. Fish

can grow from food consumption, and growth can only occur if the energy needed for body maintenance and other functions is fulfilled (Syahailatua *et al.*, 2017).

Seabass Seeds Behaviour

The behavior of seabass seeds during the rearing period is presented in Table 2 below.

Table 3. Behavioral observation results of Seabass (*Lates calcarifer*) seed.

Number	Behavior	Treatments	Day of Observation		
			1 to 5	6 to 10	11 to 15
1	Feeding Response	K(-)	++	++	+++
		K(+)	++	+++	+++
		A	+++	+++	+++
		B	++	+++	+++
		C	+++	+++	+++
2	Behavioral Response to Swimming	K(-)	++	+++	+++
		K(+)	++	+++	+++
		A	+++	+++	+++
		B	++	+++	+++
		C	+++	+++	+++
3	Grouped response	K(-)	++	++	++
		K(+)	++	++	++
		A	+++	+++	++
		B	+++	+++	++
		C	+++	+++	++
4	Reflex Responses in Fish	K(-)	++	++	++
		K(+)	++	+++	+++
		A	+++	+++	+++
		B	+++	+++	+++
		C	+++	+++	+++

Description:

- (-) = No response was observed in less than 20% of the tested fish
- (+) = Low response in 20-50% of the tested fish
- (++) = Moderate response was observed in 50–70% of the tested fish
- (+++)= High response rate > 70% of the tested fish

Based on the results of behavioral observations, it was found that seabass seeds experienced an increased response. This was evident from the response to recognizing feed, namely in treatments K-, K+, and B, which showed an increase in response from moderate to high at the end of the rearing period. Conversely, treatments A and C already showed a high response from the beginning to the end of the rearing period. The natural feed in the

form of *Artemia*, which was given, could attract the attention of snapper fish seeds to devour it, resulting in a higher feed recognition response. This is in line with the statement of Sahputra *et al.* (2017) that snapper fish, which are generally carnivorous, prefer natural feed compared to artificial feed. In addition, Sardinella oil, which contains EPA, DHA, and omega-3 fatty acids as energy sources, can function in fish development (Dewi *et al.*, 2020).

Regarding swimming behavior, the response also showed an increasing trend from the beginning of the rearing period for treatments K(-), K(+), and B, changing from a moderate to a high response. Meanwhile, treatments A and C already exhibited a high response from the start of the rearing period. The schooling behavior of the barramundi fry showed a moderate response from the beginning to the end of the rearing period in treatments K(-) and K(+), while treatments A, B, and C experienced a decrease in schooling response from high to moderate between the 11th and 15th days of observation. This schooling behavior in barramundi fry was observed around the aeration and inlet pipes. This behavior is consistent with the statement by Hidayat *et al.* (2023), which stated that young barramundi exhibit schooling behavior and are more active compared to adult barramundi, which tend to remain stationary throughout the day and only move when prey is present.

Reflexive behavior of snapper fish seeds under treatments A, B, and C exhibited a high response from the beginning of rearing, while the K(-) treatment showed a moderate response from the start, and the K(+) treatment experienced an increase in response from moderate to high on the 6th day of observation. The administration of natural *Artemia* feed enriched with lemuru oil demonstrated a high response. This suggests that *Artemia* oil enrichment can minimize fish stress tolerance factors (Jalali *et al.*, 2008).

CONCLUSION

The treatments with the highest absolute weight were found in treatment A at 2.02 g (± 0.20 g) and treatment C at 2.02 g (± 0.09 g), and the treatments with the highest absolute length were found in treatment A at 1.89 cm (± 0.17 cm) and treatment C at 1.90 cm (± 0.17 cm). The treatment with the highest SR (assuming SR refers to a specific measured variable, clarify if otherwise) was observed in treatment A at 34.67% ($\pm 2.63\%$) and in

treatment C at 38.08% ($\pm 3.89\%$). The optimal dose for growth and survival rate of seabass was obtained in treatment C with 0.5 ml of Lemuru fish oil + 0.5 ml of egg yolk.

CONFLICT OF INTEREST

There is no conflict interest in this manuscript between all authors upon writing and publishing this manuscript.

AUTHOR CONTRIBUTION

The contribution of each author is as follows: Andina Chairun Nisa, Ilham, Annisa Khairani Aras, Liga Insani, Diklawati Jatayu, Desy Febrianti, and I Gede Rezza Mahendra collected data, measured, and wrote on this manuscript.

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