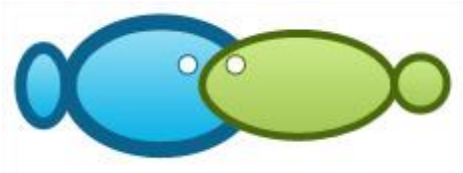

Study on eggs through larval development of yellowfin tuna, *Thunnus albacares*, based on tropical temperature condition

[1]Tri H. Prihadi, [1]Gunawan Gunawan, [1]John H. Hutapea, [1]Gusti N. Permana, [2]Deni Radona, [1]Lies E. Hadie, [1]Adang Saputra, [1]Taukhid Taukhid, [1]Yohanna R. Widyastuti, [1]Endhay K. M. Kontara, [1]Tutik Kadarini, [2]Amran R. Syam, [1]Ediwarman Ediwarman, [1]Brata Pantjara, [1]Titin Kurniasih, [1]Yuani Mundayana, [3]Irin I. Kusmini

[1] Research Center for Fisheries, National Research and Innovation Agency, Cibinong Science Center, Indonesia; [2] Research Center for Conservation of Marine and Inland Water Resources, National Research and Innovation Agency, Cibinong Science Center, Indonesia; [3] Research Center for Applied Zoology, National Research and Innovation Agency, Cibinong Science Center, Indonesia. Corresponding author: D. Radona, deniradona_kkp@yahoo.com

Abstract. Yellowfin tuna, *Thunnus albacares*, is a cosmopolitan fish species inhabiting tropical and subtropical waters in the Atlantic, Pacific, and Indian Oceans. The study aimed to enhance the survival and development of *T. albacares* larvae under Indonesia tropical temperature conditions. Six concrete tanks, each with dimensions of 3 m x 2 m x 1 m (total capacity of 6 m^3), were filled with 5 m^3 of seawater and 100,000 eggs per pack (equivalent to 20 eggs L^{-1}). The experimental treatments involved subjecting the rearing tanks to varied temperatures. There were two treatments labeled A and B, with three replicates for each treatment. Treatment A was conducted at room temperature of 29±0.5°C, while treatment B was conducted at a temperature of 30±0.5°C. The ACT-1 program was used to capture larval pictures. T-tests were employed to examine the outcomes. Based on the research results obtained after the experiment, the larvae treated with treatment A exhibited a survival rate of 0.83±0.01%. By comparison, treatment B exhibited a mean value of 0.58±0.02%. The outcome was not statistically significant, as indicated by a P-value of 0.08 (P>0.05). Total length under the treatments A and B was of 28.43±3.16 mm and 23.25±1.97 mm, respectively. The difference between the two treatments was statistically significant at a level of 0.0004 (P<0.05). The growth rate and survival of yellowfin tuna larvae are greatly influenced by stable temperatures.
Key Words: larvae, live feed, temperature, yellowfin tuna.

Introduction. The implementation of a ban on tuna exploitation and the imposition of restrictions on catch areas (Ruiz-Jarabo et al 2022; Satrioajie et al 2018; Tidd et al 2018) have led to a significant increase in the demand for farmed tuna in both domestic and export markets (Hidayati et al 2018; Kantun et al 2014). Various nations, such as Japan and Panama (Jelić Mrčelić et al 2023), have undertaken endeavors to establish tuna aquaculture. Certain tuna species exhibit low larval survival rates, resulting in a limited number of individuals reaching the juvenile stage (Aung et al 2020; Ruiz-Jarabo et al 2022). Locating juvenile tuna for cultivation in floating net cages is challenging (Matsumoto et al 2011; Okada et al 2021). The larval phase of certain marine species, such as tuna, is crucial and often experiences elevated death rates, as indicated by studies conducted by Gunawan et al (2018), Russo et al (2022), Hall et al (2019), Downie et al (2020), and Arevalo et al (2023).

Larval mortality in tuna fish occurs during the later stages of larval development, specifically when the larvae are more than ten days old and about to undergo metamorphosis

into juveniles. This issue has been observed in various countries with tuna fish hatcheries, as documented by Tridjoko et al (2022), Gunawan et al (2018), Blanco et al (2017), Kjørsvik et al (2007), and Nakagawa et al (2011). The mortality at this early stage is linked to the water quality or surface tension. The suboptimal growth and survival rate of tuna larvae are influenced by factors such as their limited predatory skills (Shiroza et al 2021), their propensity to sink with age, their capacity to locate fish larvae as a food source, and various environmental factors, all of which collectively contribute to their diminished growth and survival rate (Partridge 2013). Reglero et al (2014) found that providing bluefin tuna larvae with fish larvae as food can increase their longevity and speed up their growth. Moreover, as stated by Reglero et al (2014), tuna larvae undergo a transition from being planktivorous to being piscivorous throughout their larval stage. This means that they rely on fish larvae as their primary source of food to sustain their existence and promote growth.

Three main elements can be categorized as primary restrictions leading to higher death rates in the rearing of tuna larvae. Initially, a significant number of them perish within the initial ten-day period due to factors such as spoiled eggs, insufficient nourishment, and fluctuations in temperature, light, and water quality parameters including flow, oxygen levels, ammonia, nitrite content, and osmotic pressure from the surrounding water (Okada et al 2014). In a study conducted by Miyashita et al (2001), it was shown that the growth rate of tuna larvae is influenced by the size of their meal. Based on their research, the larvae undergo a moderate level of growth in the first ten days. Nevertheless, a larger feed quantity is positively associated with a future accelerated growth rate. This stage is usually referred to as the "large prey-fast growth" phase. The second element emerges around 20 days after birth due to increased levels of cannibalism among the larvae (Reglero et al 2014). Another factor to consider is the attainment of the juvenile period with a near-perfect swimming proficiency. Nevertheless, young deaths often occur due to impacts with walls or by leaping out of the tanks in which they are being reared (Kadota et al 2016).

Temperature fluctuations often occur when producing yellowfin tuna (*Thunnus albacares*) larvae in the hatchery, particularly during diurnal and nocturnal cycles. Temperature fluctuations commonly arise during the early phases of larval development, when there is no water exchange, and during the beginning of water changes, when the proportion is still less than 20%. Once the juvenile stage is completed, when water changes surpass 100%, temperature variations tend to stabilize. To tackle the problem of larval mortality during the initial stages caused by temperature and its variations, it is imperative to research the optimal temperature for the survival of yellowfin tuna larvae. To achieve this objective, it is beneficial to integrate an automated thermostat into the tanks utilized for larval rearing. Consequently, it is anticipated that the temperature in the larval tanks will remain rather constant. This study investigated the impact of temperature changes on the viability of *T. albacares* larvae in experimental conditions.

Material and Methods

Location of the research. The larval rearing research was carried out at the Center for Marine Aquaculture Research and Fisheries Extension (BBRBLPP) in Gondol, Bali, from March to October 2019.

Preparation of rearing tanks. The larval rearing process involved the use of 3 m x 2 m x 1 m concrete tanks, each with a volume of 6 m^3. There was a total of six tanks, each filled with 5 m^3 of saltwater (33-34 ppt) and containing 100,000 eggs, resulting in a concentration of 20 eggs L^{-1}. Each tank was equipped with an oxygen installation (aeration) to maintain a stable oxygen concentration in the water used for larval development, as with well as a mechanism for changing the water. Treatment A room temperature (28±0.5°C) and Treatment B involved the installation of a heater with an automated thermostat, which was set to a temperature of 30±0.5°C. Each treatment were conducted with 3 replicates. Floating cage net yielded fecund

yellowfin tuna eggs that were subsequently moved to six concrete tanks with dimensions of 3×2×1 m^3.

Larva rearing. The treatments for the effects of temperature on the rearing of yellowfin tuna larvae used two treatments: The room temperature is maintained at 30±0.5°C. Before being placed in the rearing tanks, the eggs of *T. albacares* were examined under a microscope to determine the fertilization rate. The floating cage net successfully yielded fertile *T. albacares* eggs through the natural spawning of the raised broodstocks. Eggs were stocked in six concrete tanks (volume 6 m^3) equipped with an aeration system and a water change system. The cleaned larval tanks were then filled with seawater that had passed through a sandy filter and a 5 m^3 filter bag. Subsequently, the fertile eggs were moved to the tanks specifically designed for larval rearing and were placed at a density of 100,000 eggs per tray, equivalent to 20 eggs L^{-1}. Larval rearing was carried out for 26 days until they reached juvenile size. The treatments tested were at different temperatures in the rearing tanks. The feeding management of yellowfin tuna larvae rearing is shown in Table 1.

Table 1

Feeding behavior in *Thunnus albacares* larval rearing

Live feed	*Days after hatching*												
	2	*4*	*6*	*8*	*10*	*12*	*14*	*16*	*18*	*20*	*22*	*24*	*26*
Nannochloropis sp.													
Rotifers													
Copepod nauplii													
Grouper larvae													
Milkfish larvae													
Milkfish juvenile													

The phytoplankton *Nannochloropsis oculata* is added to the rearing tanks when the larvae are two days to 17 days old. The phytoplankton serves as food for the rotifers and as well as a natural shading method in the larval-rearing water. Rotifers (*Brachionus rotindiformis*) was the first food in the afternoon when the larvae were two days old (H-2) at an initial density of 10 ind mL^{-1}. Rotifers were fed twice daily, in the morning and evening, depending on the feed remaining in the rearing tank. As the larvae grow, the amount of rotifers fed increases. Yellowfin tuna larvae were fed with newly hatched grouper larvae (*Plectropomus leopardus*) at the age of nine days (H-9). On the twelfth day (H-12), the yellowfin tuna larvae were fed with newly hatched milkfish larvae as live food. The size of newly hatched milkfish larvae was larger than newly hatched grouper larvae. Newly hatched milkfish larvae will open their mouths at the age of 12 days (H-12) to 17 days (H-17). All treatments received the same feeding model for both grouper larvae and newly hatched milkfish larvae.

Additionally, starting at day 18 (D-18), yellowfin tuna larvae were nourished with milkfish juveniles until they reached 26 days of age and grew to a size of 2.5-3 cm, at which point they were considered juveniles and ready for harvesting. We adjusted the feeding of larvae and juveniles to the density of tuna larvae in the rearing tanks.

Water quality. For larval rearing, the water is filtered using a sandy filter to ensure that the tanks always have the best possible quality. This technique aims to eliminate small particles found in seawater and eradicate sources of disease in seawater. The maintenance of water quality follows the criteria specified in Figure 2, starting from the larval rearing stage until the juvenile size is attained. Before administering fish oil and cleaning the tank bottom, we reviewed the literature to determine which water management methods were best for raising yellowfin tuna larvae (Dharma et al 2023; Honryo et al 2014; Stuart et al 2016; Vdastein et

al 2018). Fish oil to the top of the water in the raising tank was applied twice daily from the time the larvae emerged until they were four days old. The fat or mucus the hatching larvae release from the aeration system must be diverted and collected in a glass container. The purpose of providing fish oil is to reduce the death rate of larvae on the water's surface due to surface tension. Water alterations began at a rate of 5% when the larvae reached the age of 5 days (D-5) and increased to juvenile. Cleaning the tanks' bottom using pipetting began at 17 days (D-17) when the larvae had reached this stage. Tuna larvae between D-8 and D-15 typically prefer staying toward the bottom of the water. During this stage of development, when cleaning the bathtub, the larvae will be inadvertently drawn into the cleaning process. The observed water quality data comprised temperature, oxygen levels, pH, ammonia concentration, and nitrite measurements (Table 2).

Table 2

Water management, tank bottom cleaning and fish oil feeding in *Thunnus albacares* larvae rearing

Treatments	*Days after hatching*												
	2	*4*	*6*	*8*	*10*	*12*	*14*	*16*	*18*	*20*	*22*	*24*	*26*
Fish oil													
Water change (%)		5			10			20			50		100
Tank bottom cleaning													

Larval development. The parameters observed in the study were larval growth, larval ability in terms of feed predation (stomach content), morphological development, and survival. Traces were taken from larvae aged D-1, D-4, D-7, and D-10 to determine how many lived. Sampling of larvae at D-1 was done to determine the level of hatchability of eggs. Sampling was carried out in the morning because the larvae were not yet active. Meanwhile, sampling at D-4, D-7, and D-10 occurred at 9:00 p.m., when the larvae began to move less actively taking pictures of larvae with the ACT-1 program. The diameter, oil globule, and larval growth (total and standard length) of yellowfin tuna eggs were determined using the WinRoof V-5.0 program after photographing them with the ACT-1 program. Another parameter calculated at the end of the study was the survival rate (SR).

Data analysis. The obtained growth data were analyzed using regression to determine the relationship between growth rates (total and standard length) and larval age. At the end of the study, the overall juvenile survival and growth rate were calculated using a t-test.

Results and Discussion

Diameter and hatching rate of eggs. In this study, the average egg diameter of *T. albacares* and the oil globules diameter used in experiments were as follows: 894±16 µm and 216±5 µm, respectively, in Experiment I, 959+18 µm and oil globules of 224±5 µm, respectively, in Experiment II, and 935±13 µm and 224±5 µm, respectively, in Experiment III. When compared to yellowfin tuna raised in the hatchery of the Inter-American Tropical Tuna Commission's Achotines Laboratory in Panama, the egg diameter and oil globule sizes in this study were slightly smaller, with egg diameter ranging from 0.85–1.13 mm and oil globule diameter 0.22 mm (Buentello et al 2011).

Wexler et al (2003) and Hutapea (2007) found that *T. albacares* eggs have a uniform diameter of 1 mm, while bluefin tuna eggs have an average diameter of 989 µm and 970 µm, respectively. Based on these findings, the diameter of eggs from *T. albacares* spawning in Indonesia is slightly smaller than previously reported (Buentello et al 2011; Hutapea 2007). The age of the broodstock and environmental conditions, particularly the water temperature,

which was greater than in Japan and Panama, may cause this discrepancy. The study found that tuna eggs had a hatching rate of 63.96±5.86% in Experiment I (78.57±1.78% in Experiment II, and 72.36±5.21% in Experiment III, as shown in Figure 1.

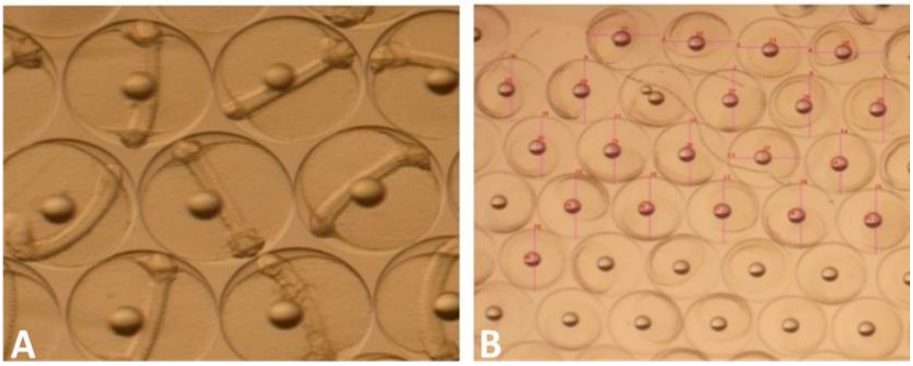

Figure 1. *Thunnus albacares* eggs (A), Measurement of egg diameter and oil globule with Win Roof program (B).

Larval development. The three experiments in this study revealed that yellowfin tuna larvae grew slowly at first but quickly after D-13. The yellowfin tuna larvae could consume newly hatched "sunu" grouper and milkfish larvae larger than the previous feed (rotifer and copepod nauplii). Figure 2 shows the overall length increase of yellowfin tuna larvae (both total and standard length).

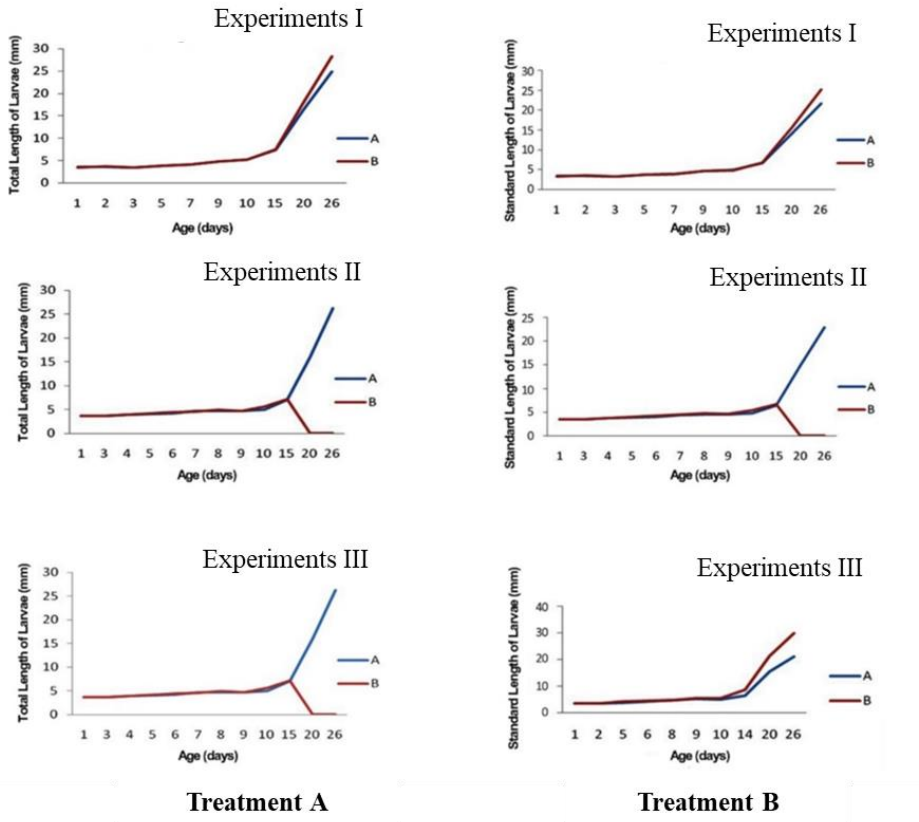

Figure 2. *Thunnus albacares* larvae growth in experiments I (A), II (B), and III (C) Total length and standard length.

One day-old *T. albacares* larvae (D-1) in Experiment I showed that treatment A had a total body length of 3.60±0.05 mm and a standard length of 3.49±0.05 mm, and treatment B had a total length of 3.53±0.15 mm and a standard length of 3.41±0.13 mm. In Experiment II, treatment A had a total body length of 3.69±0.08 mm and a standard length of 3.54±0.07 mm, and treatment B had a total length of 3.67±0.10 mm and a standard length of 3.51±0.09 mm. In Experiment III, treatment A had a total body length of 3.67±0.10 mm and a standard length of 3.54±0.10 mm, and treatment B had a total length of 3.61±0.12 mm and a standard length of 3.47±0.11 mm. The *T. albacares* larvae measured 3.34±0.08 mm in total length and 3.16±0.07 mm in all during D-1 in this study, which is larger than the D1 larvae measured 3.24+0.11 mm in total length and 3.14+0.11 mm in all during (Aoki et al 2020) and 3.38±0.07 mm in total length and 3.26±0.07 mm in all during (Kwan et al 2019). This condition is due to the larger egg diameter used in this study compared to previous years (Aoki et al 2020; Hutapea 2007; Hutapea et al 2020; Kaji et al 1999; Kwan et al 2019; Tamura & Takagi 2009).

According to the observations of larval growth (total length and standard length) in both treatments of the three experiments, larval growth in treatment B is more significant than in treatment A (room temperature). This condition is due to treatment B's higher maintenance water temperature than in the other treatment. Higher temperatures increase the predation of fish larvae on the feed given. The higher content of larval stomach contents in treatment B also evidences this condition. While alive, the *T. albacares* larvae actively pursued the natural prey feed (rotifer and copepod nauplii). Similarly, when *T. albacares* larvae were given live food in the form of grouper and milkfish larvae, they would actively pursue prey. After D-16, both treatments showed a significant increase in growth because the larvae had begun to develop into juveniles.

Larval growth in Experiment I, from D-1 to harvest (D-26), was not statistically significantly different. This was because the water temperatures in treatments A and B were not significantly different (<1^{o}C), which caused insignificant differences in the larval predation on food among the two treatments. In Experiment II, the larvae in treatment B on D16 dropped, so the larval growth data in treatment B only reached D15. When egg stocking was conducted in Experiment II, the water temperature in the rearing tanks was 27.5^{o}C. In D-1 age larvae, the temperature was 30^{o}C in treatment B. As a result of the difference in water temperature in the rearing tanks with a high-temperature difference (2.5^{o}C), D-2 larvae in treatment B suffered high mortality. In Experiment III, when the eggs were laid, the water temperature in the rearing tanks was 27.5^{o}C. Based on experience from research in Experiment II, for treatment B to reach a temperature of 30^{o}C, the water temperature was gradually increased by 0.5^{o}C on the thermostat. With this method, no larval deaths occurred due to high-temperature differences. In Experiment III, larval growth in treatment B was better than larval growth in treatment A. Statistical analysis also showed a significant difference between the two treatments, with a $P=0.0004$ ($P<0.05$).

The larval body length growth at D-16 increased due to the larvae consuming milkfish larvae. Observations of tuna larvae's stomach contents at D-12 revealed the presence of food in the form of newly hatched larvae in most *T. albacares* larvae across all treatments. The results of this study showed that the growth of *T. albacares* larvae at D-20 reared in these two treatments was higher compared to the study conducted by Kobayashi et al (2015) on the same species and the same age in Panama, which had a total body length of 10.40±0.70 mm and a standard length of 9.30±0.60 mm (Table 3). *T. albacares* larvae in both treatments had a total body length of approximately 17 mm, comparable to Atlantic bluefin tuna larvae at D-20 (Yúfera et al 2014).

Table 3

Summary of growth pattern of *Thunnus albacares* larvae during the study

Experiment I	*Treatments*	
	A	*B*
Number of eggs (egg)	100,000	100,000
Egg diameter (μm)	894±17	894±16
Oil globule diameter (μm)	216±16	216±14
HR (%)	63.96±5.66	63.95±4.16
Total larva length D-1 (mm)	3.67±0.16	3.68±0.06
Standard larva length D-1 (mm)	3.53±0.25	3.54±0.05
Juvenile length at D-26 (mm)	28.81±2.60[b]	24.19±1.40[a]
SR (%)	0.79±0.06[b]	0.68±0.05[a]
Experiment II		
Number of eggs (egg)	100,000	100,000
Egg diameter (μm)	952±1.70	953±1.08
Oil globule diameter (μm)	224±0.50	224±0. 51
HR (%)	78.57±1.78	78.55±1.12
Total larva length D-1 (mm)	3.50±0.15	3.45±0.25
Standard larva length D-1 (mm)	3.38±0.14	3.29±0.24
Juvenile length at D-26 (mm)	27.31±2.59[b]	23.62±1.69[a]
SR (%)	0.83±0.01[b]	0.58±0.02[a]
Experiment III		
Number of eggs (egg)	100,000	100,000
Egg diameter (μm)	935±12	935±13
Oil globule diameter (μm)	225±51	224±65
HR (%)	73.16±5.21	72.36±5.21
Total larva length D-1 (mm)	3.86±0.12	3.66±0.09
Standard larva length D-1 (mm)	3.54±0.15	3.54±0.19
Juvenile length at D-26 (mm)	29.17±4.30[b]	21.94±2.81[a]
SR (%)	0.82±0.02[b]	0.34±0.09[a]

Survival rate. The juvenile *T. albacares* fish will be transferred to the nursery tanks. The average juvenile survival rate was very low in each Experiment, with treatment A in Experiment I being 0.79±0.06%, treatment B being 0.68±0.05%, treatment A in Experiment II being 0.83±0.01%, and treatment B being 0.58±0.02%, and treatment A in Experiment III being 0.82±0.02% and treatment B being 0.34±0.09%, as shown in Figure 3. The result showed that the average total length of tuna juvenile after 26 days after hatching at room temperature (29±0.5°C) was 28.43±3.16 mm, significantly different ($p<0.05$) from juvenile reared at a temperature of 30°C (23.25±1.97 mm). Survival rate at room temperature was 0.81±0.03%, significantly different ($p<0.05$) from juveniles reared at a temperature of 30°C with 0.53±0.05. Room temperature was better for rearing of juvenile tuna. The low survival rate is thought to be due to cannibalism and wall hitting of the rearing tank when the larvae approach the change to juveniles until they become juveniles. *T. albacares* larval cannibalism is a result of the nature of *T. albacares* larvae, which change from planktivorous to piscivorous in the larval stage when fish larvae are required as food to maintain life and growth. Cannibalization of larvae and juveniles occurs due to size variation. Larger larvae or juveniles will eat smaller larvae. However, larvae that are relatively the same size are only bitten but cannot be swallowed. Mortality from hitting the wall after becoming a juvenile occurs when chasing the live milkfish fry given as food. The milkfish fry in the tuna larvae rearing tanks tend to swim at the edge of the tanks. However, among the treatments, juveniles produced

by treatment A had a better survival rate than those produced by treatment B, although statistical analysis revealed no significance.

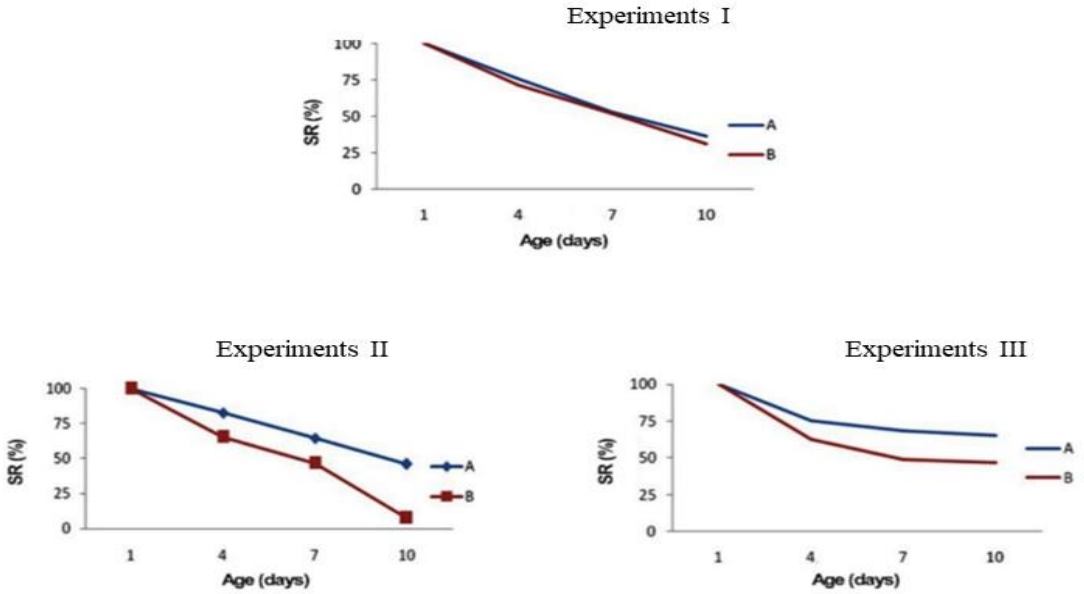

Figure 3. *Thunnus albacares* larvae survival rate in experiments A and B with 3 replications.

Gut content. Rotifers and nauplii copepods are used as initial feed from yellowfin tuna larvae. This method's efficiency is revealed when the larvae are examined for their digestive tract. The contents of the larval gastrointestinal tract D-3 was examined. In the digestive tract of *T. albacares* larvae, rotifers, and copepods, naupli multiply with age. As copepod feed consumption increases and some tuna larvae begin to become piscivorous or prey on newly hatched tuna larvae given to H-9, according to Gunawan et al (2020) and Gunawan & Hutapea (2020), the number of rotifers in the larval digestive tract begins to decline after H-11 (Figure 4, 5, and 6). However, in the third study, larval predation on natural food was quite effective. As *T. albacares* larvae age, the number of rotifers and nauplii copepods in their digestive tract also increases. The gut content of the larvae, after dissecting and examining under a microscope, is shown in Figure 7.

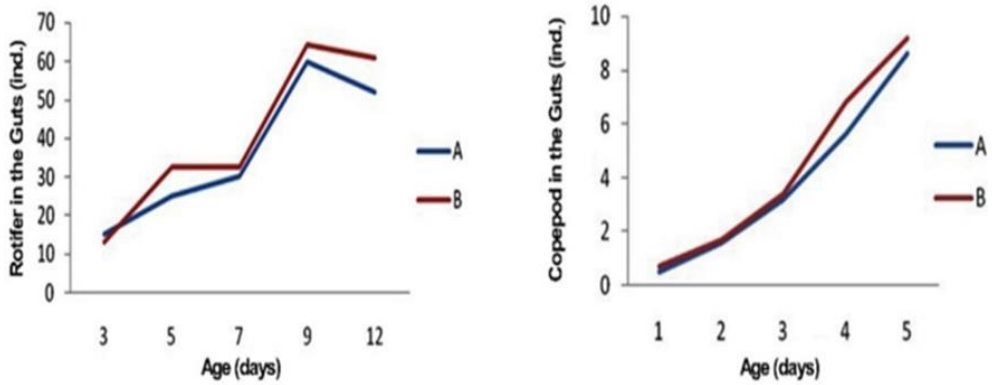

Figure 4. Amount rotifers and copepod naupli in the guts of *Thunnus albacares* larvae in Experiment 1.

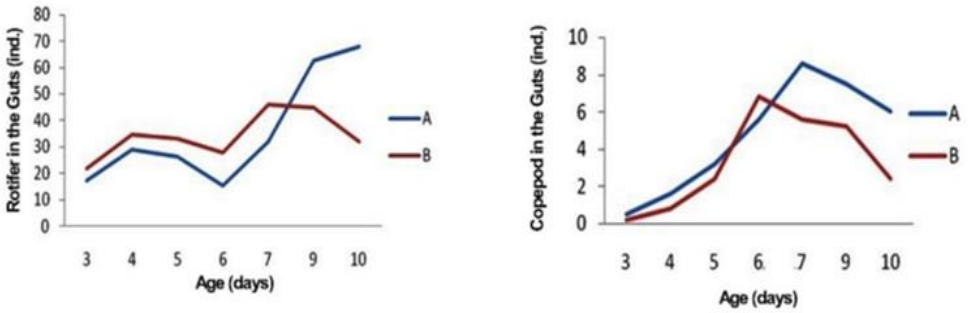

Figure 5. Amount rotifers and copepod naupli in the guts of *Thunnus albacares* larvae in experiment II (Observation times vary).

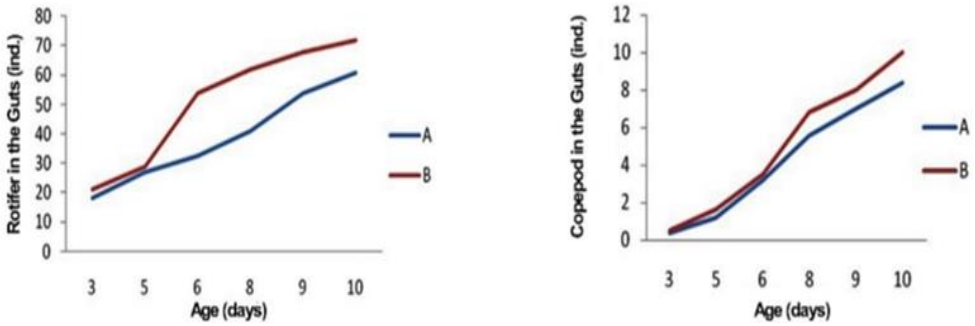

Figure 6. Amount rotifers and copepod nauplii in the guts of *Thunnus albacares* larvae in Experiment III (Observation times vary).

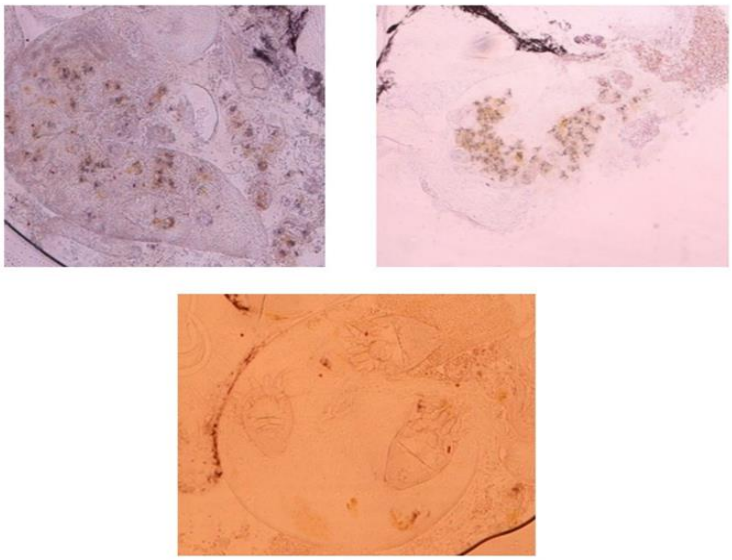

Figure 7. Gut contents of larvae *Thunnus albacares* during the study.

Water quality. Initially, the highest water temperature occured at noon because when the larvae were 2-4 days old there was no water change. When the larvae were D-5 days old, water changes started at a rate of 5%. As the larvae got older, the water temperature stayed the same, and the rate of water changes went up. The temperature range in the larval rearing medium is still within normal limits for the life of marine fish larvae in general, including *T. albacares* larvae. Measurements of dissolved oxygen (DO) levels in the water used to rear

larvae yellowfin tuna revealed that they tended to be reasonably constant throughout treatments, ranging from 5.00 to 8.00 mg L^{-1}, with saturation values between 78 and 103%. DO values in this study are still within the limits of quality standards for the growth of *T. albacares* larvae. The DO limit established by the quality standard of seawater for marine biota (aquaculture) is >5 mg L^{-1} (Bergamasco et al 2021; Wexler et al 2011). The high DO value in the rearing tanks of both treatments is due to the addition of aeration in the tanks and the water circulation system during the larval rearing period.

The limits of ammonia in the larval rearing media of the two treatments ranged from 0.60-2.60 mg L^{-1} and was not very different. This ammonia content could be reduced after an increase in water changes. The ammonia level in the larval rearing water exceeds the normal limit established by the seawater ammonia quality standard for marine biota (aquaculture) of 0.3 mg L^{-1}. The main source of ammonia in the larval rearing water is thought to be uneaten feed, larval feces, and fertilizer residues contained in the *Nannochloropsis* culture, which are fed into the larval tanks daily. The nitrite concentration in the rearing tanks of yellowfin tuna larvae during this study tended to increase steadily from 0.04 mg L^{-1}, at the beginning of the study, to values above 0.5 mg L^{-1}. It is likely that with the addition of natural food and the provision of fish larvae and milkfish juveniles as feed for tuna larvae and juveniles, all these organisms contribute to the production of ammonia, which is further nitrified by bacteria. During the study, the pH range in the media used to raise the larvae dropped from 8.2 at the start to below 8 by the end (Figure 8). However, the pH became more stable with the temperature increase.

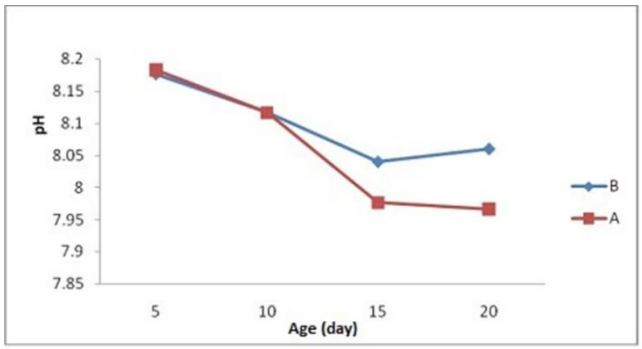

Figure 8. Relationship between pH and age of *Thunnus albacares* larvae.

During the study, a good water quality management was practiced, and the marine water conditions were generally stable. Thus, there was never an excessively high bacterial bloom. The total was between 5.5-24.0x103 CFU mL^{-1}. The total bacteria in the larval rearing water is still within the normal range, where total bacteria in waters range from 10^3 to 10^6 CFU mL^{-1}.

Conclusions. The growth of *T. albacares* larvae is influenced by temperature; the optimal temperature for larval growth is obtained in treatment A (29±0.5°C). The survival rates of *T. albacares* were 0.81±0.03% in treatment A and 0.53±0.05% in treatment B. Cannibalism and collision of *T. albacares* larvae with the wall were the causes of high mortality or very low survival (<1%).

Conflict of interest. The authors declare no conflict of interest.

References

Aoki Y., Jusup M., Nieblas A. E., Bonhommeau S., Kiyofuji H., Kitagawa T., 2020 Early-life ontogenetic developments drive tuna ecology and evolution. Journal of Marine Systems 206:103307.

Arevalo E., Cabral H. N., Villeneuve B., Possémé C., Lepage M., 2023 Fish larvae dynamics in temperate estuaries : A review on processes, patterns and factors that determine recruitment. Fish and Fisheries 2022:466–487.
Aung N. W., Yamazaki W., Hasegawa T., Higuchi K., Takashi T., Gen K., Sumida T., Hagiwara A., Sakakura Y., 2020 Effect of tank shape on survival and growth of Pacific bluefin tuna *Thunnus orientalis* larvae. Aquaculture 524:735283.
Bergamasco A., Nguyen H. Q., Caruso G., Xing Q., Carol E., 2021 Advances in water quality monitoring and assessment in marine and coastal regions. Water 13(14):2–5.
Blanco E., Reglero P., Ortega A., de la Gándara F., Fiksen Ø., Folkvord A., 2017 The effects of light, darkness and intermittent feeding on the growth and survival of reared Atlantic bonito and Atlantic bluefin tuna larvae. Aquaculture 479:233–239.
Buentello J. A., Pohlenz C., Margulies D., Scholey V. P., Wexler J. B., Tovar-Ramírez D., Neill W. H., Hinojosa-Baltazar P., Delbert M. G., 2011 A preliminary study of digestive enzyme activities and amino acid composition of early juvenile yellowfin tuna (*Thunnus albacares*). Aquaculture 312(1):205–211.
Dharma T. S., Setiadi A., Gunawan G., Hutapea J. H., Zafran Z., 2023 The nursery of yellowfin tuna seeds (*Thunnus albacares*) by increasing light intensity in the rear control system. IOP Conference Series: Earth and Environmental Science 1137:012026.
Downie A. T., Illing B., Faria A. M., Rummer J. L., 2020 Swimming performance of marine fish larvae: review of a universal trait under ecological and environmental pressure. Reviews in Fish Biology and Fisheries 30(1):93–108.
Gunawan G., Hutapea J. H., Setiadi A., Mahardika K., 2018 Development of the digestive tract and system in the larvae of the yellowfin tuna, *Thunnus albacares* (Bonnaterre, 1788). Journal of Aquaculture Research 13(4):309–316.
Gunawan G., Hutapea J. H., 2020 The difference in the initial time of feeding fish larvae as feed in the maintenance of yellowfin tuna larvae Thunnus albacares. JFMR-Journal of Fisheries and Marine Research 4(1):135–143.
Hall A. E., Vitale L., Kingsford M. J., 2019 Planktonic larval duration, early growth, and the influence of dietary input on the otolith microstructure of *Scolopsis bilineatus* (Nemipteridae). Environmental Biology of Fishes 102(4):541–552.
Hidayati D., Herlambang R., Jadid N., Sa'Adah N. N., Maulidina N., Nurhayati A. P. D., 2018 Potential of yellowfin tuna catch in East Java-Indian Ocean based on length frequency and age distribution. Journal of Physics: Conference Series 1040:012007.
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(6):1–9.
Hutapea J. H., 2007 Embryo development of yellowfin tuna (*Thunnus albacares*) at different incubation temperature. Indonesian Aquaculture Journal 2(2):99–105.
Hutapea R. Y. F., Mardiah R. S., Arkham M. N., Sari R. P., Syaputra W. N., 2020 Operation study and construction of purse seine at KM Marwah Lampulo Aceh Utara. Coastal and Ocean Journal (COJ) 4(1):47–56.
Jelić Mrčelić G., Nerlović V., Slišković M., Zubak Čižmek I., 2023 An overview of Atlantic bluefin tuna farming sustainability in the Mediterranean with special regards to the Republic of Croatia. Sustainability 15(4):2976.
Kadota T., Takashi T., Oka M., Higuchi K., Gen K., Tanaka Y., Sawaguchi S., Mushiake K., 2016 Prevalence of collision death in 2-year-old Pacific bluefin tuna, *Thunnus orientalis*, reared in land-based tanks for broodstock management. Aquaculture 454:252–256.
Kaji T., Tanaka M., Oka M., Takeuchi H., Ohsumi S., Teruya K., Hirokawa J., 1999 Growth and morphological development of laboratory-reared yellowfin tuna *Thunnus albacares* larvae and early juveniles, with special emphasis on the digestive system. Fisheries Science 65(5):700–707.
Kantun W., Mallawa A., Rapi N. L., 2014 Comparison of the structure size yellowfin tuna

(*Thunnus albacares*) between caught at fad deep sea and shallow waters at Makassar Strait. Jurnal IPTEKS PSP 1(2):112–128.
Kjørsvik E., Pittman K., Pavlov D. A., 2007 From fertilisation to the end of metamorphosis-functional development. Culture of Cold-Water Marine Fish 204–278.
Kobayasi T., Honryo T., Agawa Y., Sawada Y., Tapia I., Macias K. A., Cano A., Scholey V. P., Margulies D., Yagishita N., 2015 Gonadogenesis and slow proliferation of germ cells in juveniles of cultured yellowfin tuna, *Thunnus albacares*. Reproductive Biology 15(2):106-112.
Kwan G. T., Wexler J. B., Wegner N. C., Tresguerres M., 2019 Ontogenetic changes in cutaneous and branchial ionocytes and morphology in yellowfin tuna (*Thunnus albacares*) larvae. Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology 189(1):81–95.
Matsumoto T., Okada T., Sawada Y., Ishibashi Y., 2011 Changes in the scotopic vision of juvenile Pacific bluefin tuna (*Thunnus orientalis*) with growth. Fish Physiology and Biochemistry 37(3):693–700.
Miyashita S., Sawada Y., Okada T., Murata O., Kumai H., 2001 Morphological development and growth of laboratory-reared larval and juvenile *Thunnus thynnus* (Pisces: Scombridae). Fishery Bulletin 99(4):601–616.
Nakagawa Y., Kurata M., Sawada Y., Sakamoto W., Miyashita S., 2011 Enhancement of survival rate of Pacific bluefin tuna (*Thunnus orientalis*) larvae by aeration control in rearing tank. Aquatic Living Resources 24(4):403–410.
Okada T., Honryo T., Kawahara M., Takahashi I., Murayama K., Ishibashi Y., 2021 Appropriate size for transportation to sea cages for juvenile Pacific bluefin tuna *Thunnus orientalis* (Temminck and Schlegel). Aquaculture Research 52(3):1282–1290.
Okada T., Honryo T., Sawada Y., Agawa Y., Miyashita S., Ishibashi Y., 2014 The cause of death of juvenile Pacific bluefin tuna (*Thunnus orientalis*) reared in sea net cages. Aquacultural Engineering 59:23–25.
Partridge G. J., 2013 Closed-cycle hatchery production of tuna. In: Advances in aquaculture hatchery technology. Woodhead Publishing Limited, pp. 457–497.
Reglero P., Ortega A., Blanco E., Fiksen Ø., Viguri F. J., de la Gándara F., Seoka M., Folkvord A., 2014 Size-related differences in growth and survival in piscivorous fish larvae fed different prey types. Aquaculture 433:94–101.
Ruiz-Jarabo I., Laiz-Carrión R., Ortega A., de la Gándara F., Quintanilla J. M., Mancera J. M., 2022 Survival of Atlantic bluefin tuna (*Thunnus thynnus*) larvae hatched at different salinity and pH conditions. Aquaculture 560:738457.
Russo S., Torri M., Patti B., Musco M., Masullo T., Di Natale M. V., Sarà G., Cuttitta A., 2022 Environmental conditions along tuna larval dispersion: Insights on the spawning habitat and impact on their development stages. Water 14(10):1568.
Satrioajie W. N., Suyadi S., Syahailatua A., Wouthuyzen S., 2018 The importance of the Banda Sea for tuna conservation area: A review of studies on the biology and the ecology of tuna. IOP Conference Series: Earth and Environmental Science 184(1):1–11.
Shiroza A., Malca E., Lamkin J. T., Gerard T., Landry M. R., Stukel M. R., Laiz-Carrión R., Swalethorp R., 2021 Active prey selection in developing larvae of Atlantic bluefin tuna (*Thunnus thynnus*) in spawning grounds of the Gulf of Mexico. Journal of Plankton Research 44(5):728–746.
Stuart K., Rotman F., Drawbridge M., 2016 Methods of microbial control in marine fish larval rearing: clay-based turbidity and passive larval transfer. Aquaculture Research 47(8):2470–2480.
Tamura Y., Takagi T., 2009 Morphological features and functions of bluefin tuna change with growth. Fisheries Science 75(3):567–575.
Tidd A., Blanchard J. L., Kell L., Watson R. A., 2018 Predicting global tuna vulnerabilities with spatial, economic, biological and climatic considerations. Scientific Reports 8(1):1–12.
Tridjoko T., Setiadi A., Gunawan G., Hutapea J. H., 2022 Spawning productivity of yellowfin

tuna broodstock in floating net cage. Journal of Fisheries and Marine Sciences 2(2):112-123.
Vdastein O., Attramadal K. J. K., Bakke I., Forberg T., Olsen Y., Verdegem M., Giatsis C., Skjermo J., Aasen I. M., Gatesoupe F. J., Dierckens K., Sorgeloos P., Bossier P., 2018 Managing the microbial community of marine fish larvae: A holistic perspective for larviculture. Frontiers in Microbiology 9:1–51.
Wexler J. B., Margulies D., Scholey V. P., 2011 Temperature and dissolved oxygen requirements for survival of yellowfin tuna, *Thunnus albacares*, larvae. Journal of Experimental Marine Biology and Ecology 404(1–2):63–72.
Wexler J. B., Scholey V. P., Olson R. J., Margulies D., Nakazawa A., Suter J. M., 2003 Tank culture of yellowfin tuna, *Thunnus albacares*: Developing a spawning population for research purposes. Aquaculture 220(1–4):327–353.
Yúfera M., Ortiz-Delgado J. B., Hoffman T., Siguero I., Urup B., Sarasquete C., 2014 Organogenesis of digestive system, visual system and other structures in Atlantic bluefin tuna (*Thunnus thynnus*) larvae reared with copepods in mesocosm system. Aquaculture 426–427:126–137.

Received: 13 August 2024. Accepted: 09 December 2024. Published online: 17 January 2025.
Authors:
Tri Heru Prihadi, Research Center for Fisheries, National Research and Innovation Agency, Cibinong Science Center 16915, Indonesia, e-mail: triheru_p@yahoo.com
Gunawan Gunawan, Research Center for Fisheries, National Research and Innovation Agency, Cibinong Science Center 16915, Indonesia, e-mail: gunawan9505@gmail.com
John Harianto Hutapea, Research Center for Fisheries, National Research and Innovation Agency, Cibinong Science Center 16915, Indonesia, e-mail: hutapeaharianto@gmail.com
Gusti Ngurah Permana, Research Center for Fisheries, National Research and Innovation Agency, Cibinong Science Center 16915, Indonesia, e-mail: igus022@brin.go.id
Deni Radona, Research Center for Conservation of Marine and Inland Water Resources, National Research and Innovation Agency, Cibinong Science Center 16915, Indonesia, e-mail: deniradona_kkp@yahoo.com
Lies Emmawati Hadie, Research Center for Fisheries, National Research and Innovation Agency, Cibinong Science Center 16915, Indonesia, e-mail: lies005@brin.go.id
Adang Saputra, Research Center for Fisheries, National Research and Innovation Agency, Cibinong Science Center 16915, Indonesia, e-mail: adan004@brin.go.id
Taukhid Taukhid, Research Center for Fisheries, National Research and Innovation Agency, Cibinong Science Center 16915, Indonesia, e-mail: tauk001@brin.go.id
Endhay Kusnendar Kontara, Research Center for Fisheries, National Research and Innovation Agency, Cibinong Science Center 16915, Indonesia, e-mail: endh001@brin.go.id
Tutik Kadarini, Research Center for Fisheries, National Research and Innovation Agency, Cibinong Science Center 16915, Indonesia, e-mail: tutikdarso@gmail.com
Yohanna Retnaning Widyastuti, Research Center for Fisheries, National Research and Innovation Agency, Cibinong Science Center 16915, Indonesia, e-mail: yohanna.retnaning.widyastuti@brin.go.id
Amran Ronny Syam, Research Center for Conservation of Marine and Inland Water Resources, National Research and Innovation Agency, Cibinong Science Center 16915, Indonesia, e-mail: amransyam62@gmail.com
Ediwarman Ediwarman, Research Center for Fisheries, National Research and Innovation Agency, Cibinong Science Center 16915, Indonesia, e-mail: ediwarman@brin.go.id
Brata Pantjara, Research Center for Fisheries, National Research and Innovation Agency, Cibinong Science Center 16915, Indonesia, e-mail: bpantjara@yahoo.com
Titin Kurniasih, Research Center for Fisheries, National Research and Innovation Agency, Cibinong Science Center 16915, Indonesia, e-mail: titin.kurniasih@brin.go.id
Yuani Mundayana, Research Center for Fisheries, National Research and Innovation Agency, Cibinong Science Center 16915, Indonesia, e-mail: yuan003@brin.go.id
Irin Iriana Kusmini, Research Center for Applied Zoology, National Research and Innovation Agency, Cibinong Science Center 16915, Indonesia, e-mail: iriniriana@gmail.com

How to cite this article:
Prihadi T. H., Gunawan G., Hutapea J. H., Permana G. N., Radona D., Hadie L. E., Saputra A., Taukhid T., Widyastuti Y. R., Kontara E. K. M., Kadarini T., Syam A. R., Ediwarman E., Pantjara B., Kurniasih T., Mundayana Y., Kusmini I. I., 2025 Study on eggs through larval development of yellowfin tuna, *Thunnus albacares*, based on tropical temperature condition. AACL Bioflux 18(1):67-79.