DOI: http://dx.doi.org/10.15578/iaj.21.1.2026.35-42 *Indonesian Aquaculture Journal, 21 (1), 2026, 35-42*

Available online at: http://ejournal-balitbang.kkp.go.id/index.php/iaj

GASTROINTESTINAL ENZYME ACTIVITY OF YELLOWFIN TUNA LARVAE (*Thunnus albacares*)

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(Received: February 12, 2026; Final revision: June 4, 2026; Accepted: June 4, 2026)

ABSTRACT

Knowledge of morphological development, digestive system, and digestive enzymes in larvae is key to developing optimal larval and seedling maintenance strategies. The purpose of this study was to determine the digestive system and digestive enzyme activity in yellowfin tuna (Thunnus albacares) larvae cultivated in hatcheries. Yellowfin tuna eggs were spread in concrete tanks measuring 3x2x1.2 m (volume 6 m³), with 90,000 eggs per tank. The analysis of digestive enzymes in yellowfin tuna larvae in this study included protease, amylase, and lipase enzymes. Digestive enzyme analysis method using a spectrophotometer with a wavelength of 340–560 nm. Enzyme analysis was performed on tuna eggs and larvae at stages D-1, D-4, D-7, D-10, D-15, and D-20. The results showed that the digestive enzyme activity of yellowfin tuna (protease, amylase, and lipase) was detected while still in the egg stage and was also detected in D-1 larvae. The emergence of digestive enzymes is triggered by internal mechanisms related to the development of the digestive system. The presence of all digestive enzymes (protease, amylase, and lipase) in yellowfin tuna larvae indicates the importance of these enzymes in early development.

KEYWORDS: enzymes; larvae; *Thunnus albacares;* tuna

INTRODUCTION

The world's main tuna production still comes from wild catches, even though tuna farming has been successful in several countries such as Japan, Latin America, Europe, and Australia. The yield of tuna farming in floating nets is still very small compared to wild catches. Tuna farming begins with the capture of seeds/juveniles or adult fish from the wild using nets. Tuna farming has been carried out in several countries such as Japan, Australia, several Mediterranean countries (such as Spain, Italy, Morocco, Portugal, Malta, Croatia, and Turkey), Mexico (Lecomte *et al*., 2017, Benetti *et al*., 2016, Gandara *et al*., 2016) and Indonesia has also made efforts to grow tuna. However, the survival rate of tuna larvae to reach the juvenile stage remains low for most tuna species. The larval phase of several marine fish, including tuna, is a highly critical phase (Patridge, 2009; Hutapea *et al*., 2015; Kjorsvik *et al*., 2004; Nakagawa *et al*., 2011). It has even been found that the survival and growth of bluefin tuna larvae can only be improved if fish larvae are added as feed (Reglero *et al*., 2014). Furthermore, it is said that the nature of tuna larvae changes from planktivorous to piscivorous in the larval stage, where fish larvae must be present as feed in order to maintain their survival and growth. In addition, juvenile tuna mortality occurs after transfer to offshore net cages due to stress related to environmental fluctuations (Tsuda *et al*., 2012, Ishibashi *et al*., 2009).

The successful development of the digestive system is very important for the survival and growth of tuna larvae (Kjorsvik *et al*., 2004). Knowledge of the morphological development, digestive system, and digestive enzymes of larvae is key to developing optimal larval and seedling maintenance strategies. Knowledge of morphological development and the

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digestive system can help identify limiting factors in larval rearing and reduce mortality in larval rearing. The ability of fish to utilize nutrients depends on the activity of digestive enzymes located along the digestive tract, such as enzymes from the stomach, exocrine pancreas, and intestines, but also includes the transport and absorption of nutrients by intestinal cells (Clark *et al.*, 1985).

Enzymatic studies are the first step in identifying and implementing new alternative feeds for marine fish larvae, and are an important source of information for determining when the digestive system of larvae is functionally ready to digest and assimilate artificial feed (Sarasquete *et al.*, 1995). The successful development of the digestive system is very important for the survival and growth of fish larvae because an efficient digestive system allows fish to capture, digest, and absorb food (Kjorsvik *et al.*, 2004).

Research on the digestive system and digestive enzymes of yellowfin tuna larvae in Indonesia has never been conducted. Research on digestive enzymes in tuna eggs and juveniles has been conducted in Panama, with juvenile tuna sizes ranging from 23 mm to 34 mm (Buentello *et al.*, 2011). However, no research information on the digestive system and digestive enzymes of yellowfin tuna larvae has been obtained. Therefore, it is necessary to conduct research related to the digestive system and digestive enzyme activity of yellowfin tuna larvae from aquaculture.

The objective of this study is to determine the digestive system and digestive enzyme activity in yellowfin tuna (*Thunnus albacares*) larvae from hatchery cultivation.

MATERIALS AND METHODS

Research Time and Place

This research was conducted at the tuna hatchery and laboratory of the Center for Marine Aquaculture Research and Development (BBPPBL), Gondol, Penyabangan Village, Gerokgak District, Buleleng Regency, Bali.

Larvae Maintenance Yellowfin tuna (*Thunnus albacares*) eggs from natural spawning in floating net cages were harvested, selected, disinfected, counted, and then randomly sampled volumetrically, which were then observed under a microscope to determine the fertilization rate, embryo development, and normality. After determining the number of fertile and good eggs, the yellowfin tuna eggs are spread in a 3x2x1.2 m concrete tank (volume 6 m^3), 90,000 eggs per tank. The rearing tank is equipped with an aeration system to maintain oxygen content and a seawater pipe for the water exchange system. The feeding management for yellowfin tuna larvae until they reach juvenile stage is shown in Figure 1 and Table 1.

The water quality in the larval rearing tank can be maintained by using seawater that has undergone a sand filtration process. This process aims to remove fine particles in seawater and also to eliminate sources of disease in seawater. Then, to maintain the water quality in the larval rearing tank in optimal condition, water quality management is carried out as described.

Air changes are carried out gradually using a flow-through system to maintain optimal air quality without stressing the larvae due to currents or drastic

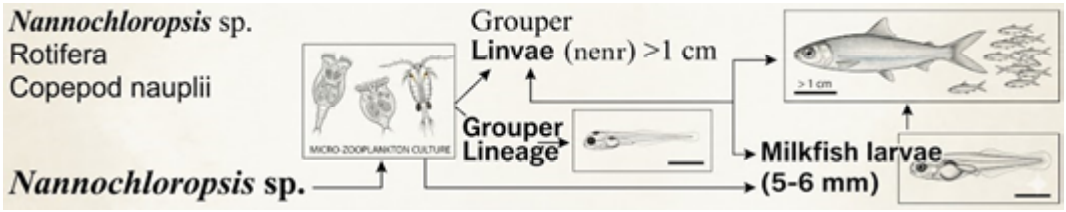

Figure 1. Description of the feed given to yellowfin Tuna (*Thunnus albacares*) according to its size.

Table 1. Feeding pattern for rearing yellowfin tuna (*Thunnus albacares*) larvae

Feed	*Days after hatching* 1 2 3 8 12 15 20 25 30
Nannochloropsis sp.	----------
Rotifera	----------
Copepod nauplii	----------
Grouper larvae (3-4 mm)	----------
Milkfish larvae (5-6 mm)	----------
Milkfish fry (nener) >1 cm	----------

temperature changes. The percentage of air changes is adjusted according to the age of the larvae (D-0 = days after hatching). Initial Phase (D-0 to D-7): Usually there are no air changes (0%). The tank is filled to capacity from the start and only given very weak aeration to prevent damage to the eggs and newly hatched larvae. Initial Feeding Phase (D-8 to D-10): Air changes are minimal, ranging from 10% – 20% per day. The air flow is kept very slow. Development Phase (D-12 to D-15): As feed consumption (rotifers) increases, air changes are increased to 30% – 50% per day. Advanced Phase (D-25 and above until metamorphosis): The organic load from leftover feed (microfeed) increases. Air changes are intensively increased to 100% – 200% per day using a flow-through system with constant flow for 24 hours (Table 2). Cleaning by siphoning usually begins between D-20 and D-25 (performed routinely once or twice daily, usually in the morning and evening before or after feeding).

Digestive Enzyme Analysis of Larvae

The analysis of digestive enzymes in yellowfin tuna larvae in this study included protease, amylase, and lipase enzymes. The digestive enzyme analysis was performed according to the Bergmeyer & Grassl (1983) method using a spectrophotometer with a wavelength of 340–560 nm. Enzyme analysis was performed on tuna eggs and larvae at D-1, D-4, D-7, D-10, D-15, and D-20. The amount of larval samples required for enzyme analysis was considered when performing periodic analysis. The selection oflarval ages for analysis was as follows: D-1 when the larvae still utilized food from endogenous sources, D-4 after the larvae began to consume food from exogenous sources in the form of rotifers, D-7 when the larvae consumed rotifers in large quantities, D-10 when the larvae begin to be fed newly hatched fish larvae, D-15 when the larvae consume a large amount of newly hatched larvae, and D-20 when they become juveniles. Protease enzyme activity was determined by measuring the enzyme's ability to hydrolyze protein, resulting in the release of tyrosine.

The measurement was carried out using casein substrate and tyrosine as a standard using a spectrophotometer (Suryanti, 2002). Amylase enzyme activity is determined by measuring the enzyme's ability to hydrolyze starch solution to release reducing sugars. The reducing sugars produced are measured using the Shaffer Hartman method and *automatic analysis* Bochringer Mannheim-amylase PNP (Suryanti, 2002).

Lipase enzyme activity is measured using triolein substrate. The released fatty acids will form fatty acid salts that precipitate, and these salts are then measured turbidimetrically at a wavelength of 340 nm (Suryanti, 2002). Protease, amylase, and lipase enzyme activities were each expressed in units of enzyme activity/mL sample/minute (Affandi *et al.*, 1994).

RESULTS AND DISCUSSION

Morphological development

From the observations, the average diameter of the yellowfin tuna eggs used in this study was 872 ± 18 µm and the oil globules were 207 ± 7 µm (Figure 2). When yellowfin tuna larvae first hatched (D-1), they had a transparent body shape, their eyes were not yet formed/open, and their mouths were also not yet open. The abdomen still contained a large yolk sac as a food reserve (Figure 2). This yolk sac was the only *endogenous* food source for yellowfin tuna larvae until they opened their mouths and were able to utilize *exogenous* food sources.

Yellowfin tuna larvae D-4 begin to show black pigmentation on the abdomen, which continues until the larvae enter the juvenile stage. At D-4, the larvae begin toactively search for prey. Yellowfin tuna larvae at D-4 actively search for external food sources because their eyes and mouths are fully open. In D-4 larvae, the yolk sac and oil droplets are depleted, and the stomach is filled with rotifers that can be consumed by the larvae. Pigmentation on the top of the head begins to appear at the D-7 larval stage, and

Table 2. Water management providing fish oil and cleaning the bottom of the tank in the yellowfin (*Thunnus albacares*) larvae rearing

Treatments	*Days after hatching* 1 2 3 8 10 12 15 20 25
Fish oil	----------
Water changes	5%-10%--20%---50%--100--- >100%
Tank bottom cleaning	----------

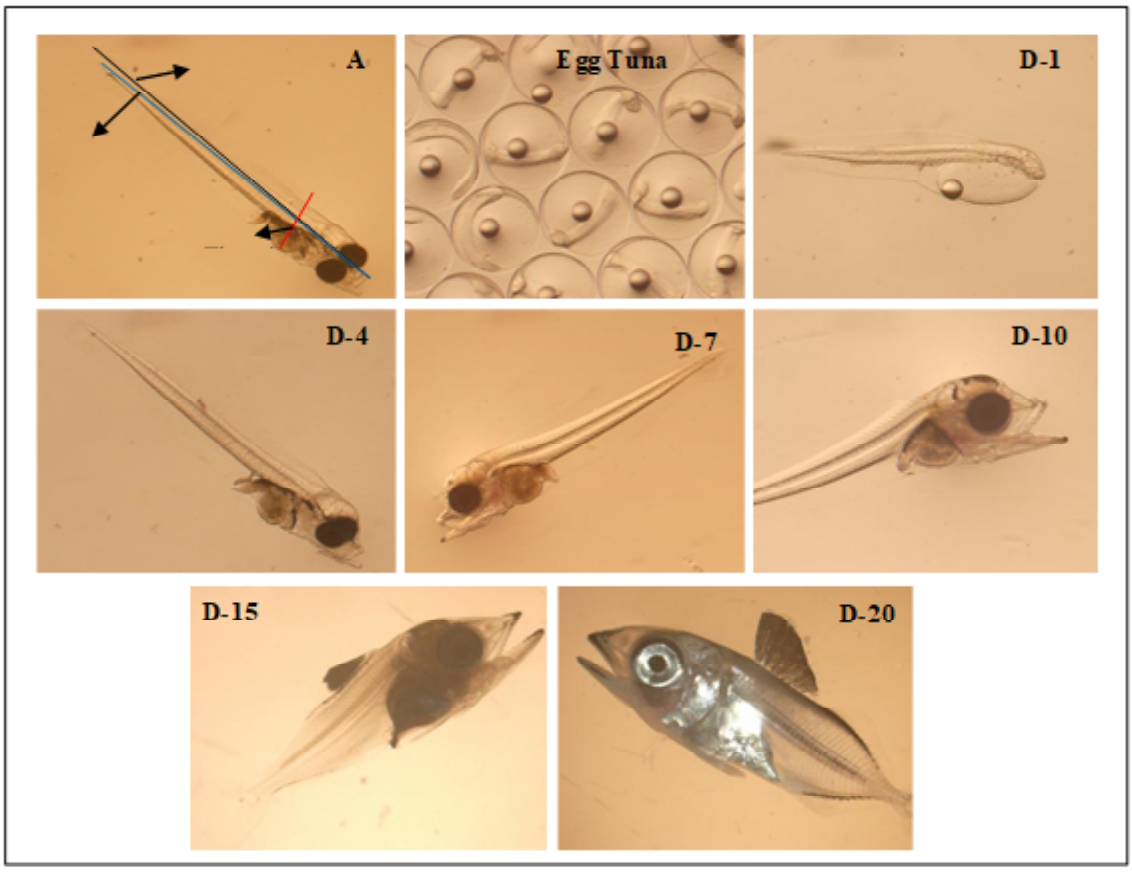

Figure 2. Image pictures of yellowfin tuna eggs and larvae using the ACT-1 program, Nikon SMZ100 microscope and EclipseE600 40X and measurements using the Win Roof program.

this pigmentation becomes more visible as the larvae age. At D-7, teeth also begin to grow on the upper jaw, and this tooth growth becomes more visible as the larvae age. By D-10, the larvae's teeth are fully grown, indicating that cannibalism begins to appear at this stage. To reduce cannibalism, feed larger than the previous feed (rotifers and artemia nauplii) is required, in the form of newly hatched fish (grouper larvae).

The tail fins of yellowfin tuna larvae are fully developed at D-15, with a tail fin shape that is split like that of an adult fish. At this stage, the larvae can swim freely around the rearing tank to search for food and obtain oxygen. At D-20, yellowfin tuna larvae have transformed into juveniles (physically resembling adult fish), with both the first and second *dorsal* fins fully grown and beginning to elongate. Similarly, *the pectoral fins* and *pelvic fins* have also grown completely, and the tail has developed fully with a small, strong base, characteristic of fast-swimming fish.

Growth of Yellowfin Tuna Larvae

One-day-old T. albacares larvae (D-1) have a total body length of 2.50 ± 0.05 mm to 15.55 ± 0.08 mm and a body height of 0.59 ± 0.05 mm to 4.74 ± 0.08 mm which is smaller than D1 larvae, which measure 3.24 ± 0.11 mm in total length and 3.14 ± 0.11 mm in overall length (Aoki *et al*., 2020) and 3.38 ± 0.07 mm in total length and 3.26 ± 0.07 mm in overall length (Kwan *et al*., 2019). Observation of larval growth (total length and standard length), *T. albacares* larvae actively pursue natural prey (rotifera and copepod nauplii). Similarly, when fed live food such as grouper and milkfish larvae, they actively pursue prey. After day 16, both treatments showed significant growth increases as the larvae began to develop into juveniles.

Larval body length growth on day 16 increased due to the larvae consuming milkfish larvae. Observations of the gut contents of tuna larvae on day 12 revealed the presence of newly hatched larvae in most T. albacares larvae in all treatments. Yellowfin tuna larval growth (total length, standard length, and body height) is shown Figure 3. At the beginning of cultivation, yellowfin tuna larval growth was very slow, but this larval growth accelerated after day 13. This rapid growth after day 13 was due to the yellowfin tuna larvae consuming newly hatched larvae (tuna and milkfish larvae) which were larger than their previous feed (rotifera and artemia nauplii). Larval Growth (mm) Larval Growth (mm). The results of this study

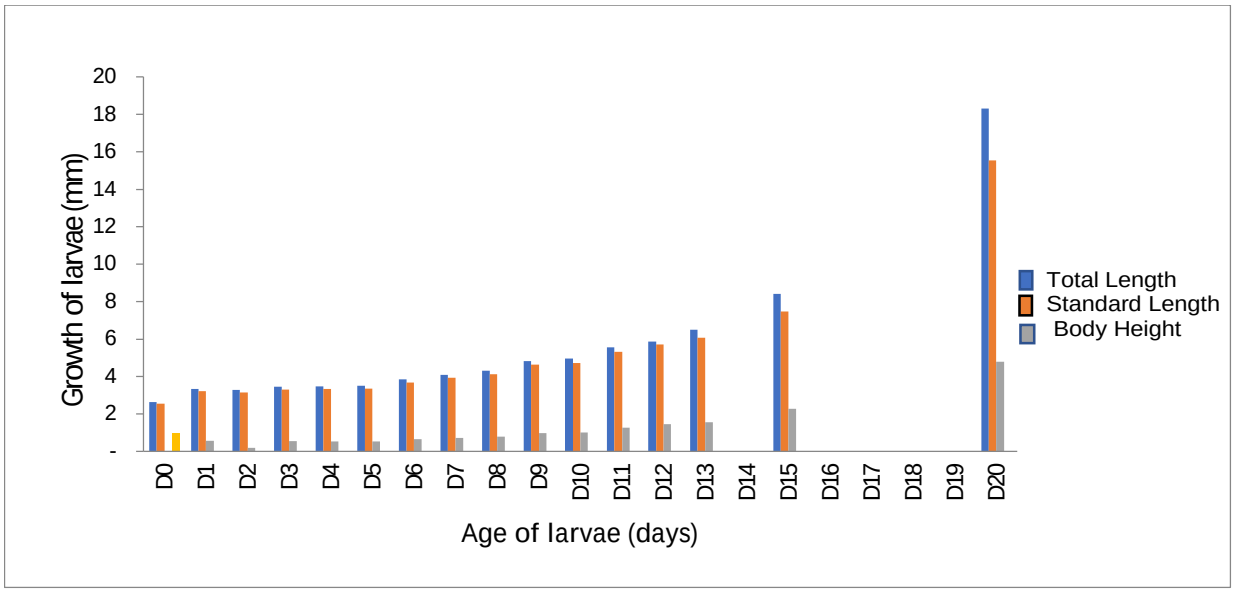

Figure 3. Daily growth of total length, standard length, and body height of yellowfin tuna larvae.

indicate that the growth of T. albacares larvae on the 20th day maintained in these two treatments was higher compared to the study conducted by Kobayashi *et al.* (2015) on the same species and 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. T. albacares larvae in both treatments had a total body length of approximately 17 mm, comparable to Atlantic bluefin tuna larvae on the 20th day (Yúfera *et al.*, 2014).

Digestive Enzyme Activity of Yellowfin Tuna Larvae

The digestive enzyme activity of yellowfin tuna larvae (protease, amylase, and lipase) was detected while still in the egg stage and was also detected in D-1 larvae (Figure 4). In yellowfin tuna eggs studied by Buentello *et al.* (2011), protease, amylase, and lipase enzyme activity was also detected. The level of enzyme activity fluctuated from the egg stage to the D-20 larvae stage for protease, amylase, and lipase enzymes. The same thing also occurred in the larvae of the *Gnathadon speciosus* fish species (Aslianti & Afifah, 2012), *Ompok bimaculatus* (Bloch) butter catfish (Pradhan *et al.,* 2013), *Seriola lalandi* yellowtail kingfish (Chen *et al.*, 2006), *Oreochromis mossambicus* tilapia (Lo *et al.*, 2006), *Chanos chanos* Forsskal milkfish (Aslianti *et al.*, 2014), *Atractoscion nobilis* white seabass (Galaviz *et al.*, 2011), *Huso huso* beluga (Asgari *et al.*, 2013), sobaity sea bream *Sparidentex hasta* (Nazemroaya *et al.*, 2015), flatfish *Solea senegalensis,* Kaup 1858 (Ribeiro *et al.*, 1999), sea louse *Labrus* (Hansen *et al.*, 2013).

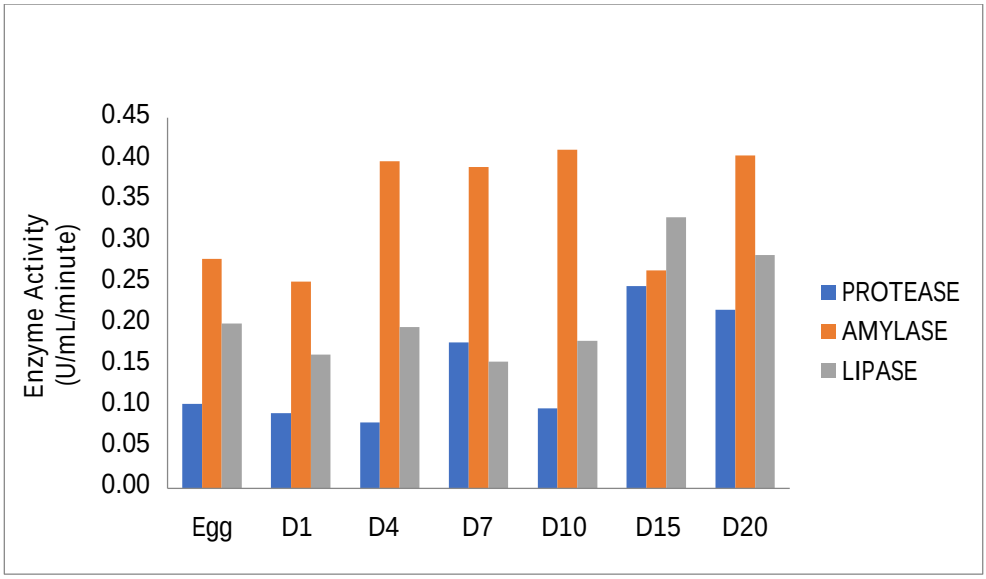

Figure 4. Protease, amylase, and lipase enzyme activity in yellowfin tuna eggs and larvae.

The presence of all digestive enzymes (protease, amylase, and lipase) in yellowfin tuna larvae indicates the importance of these enzymes in earlydevelopment. According to Nazemroaya (2015), qualitative and quantitative evaluation of digestive enzymes serves as an indicator of food acceptance by larvae, digestive capacity in relation to the type of food offered, and the development and potential survival of larvae. Protease enzyme activity was detected in yellowfin tuna eggs, as well as in D-1 larvae. According to Chen *et al.* (2006), most digestive enzymes are present before larvae obtain food from exogenous sources, and the development of these enzymes determines the time required to digest exogenous food. Furthermore, it is said that the emergence of digestive enzymes is triggered by internal mechanisms related to the development of the digestive system, not by food intake.

Fluctuations in the activity of certain enzymes cover the period of morphological differentiation of in the digestive tract and related glands. After the formation of the stomach glands, the digestive system becomes functional.The protease enzyme activity in yellowfin tuna eggs in this study was 0.1028 U/mL/minute. The protease enzyme activity in yellowfin tuna eggs in this study was higher than the total protease enzyme activity reported by Buentello *et al.* (2011) in yellowfin tuna eggs in Panama, which was 0.0216 U/mL/minute. Feeding the tunabroodstock with flying fish and squid at BBPPBL-Gondol resulted in slightly higher protease enzyme activity in tuna eggs at BBPPBL-Gondol compared to yellowfin tuna eggs in Panama, where the broodstock was fed lemuru fish and squid.

Protease enzyme activity decreased slightly in D-1 larvae, although not significantly, with an enzyme activity of 0.0913 U/mL/minute. At D-1, the larvae only utilized the yolk sac as an endogenous food source, as they were not yet able to obtain food from exogenous sources because their digestive tract, from the mouth, esophagus, and intestines to the anus, was not yet developed. The same thing also occurred in milkfish (*Chanos chanos* Forsskal) larvae, which were kept in a culture medium with molasses added. Protease enzyme activity was also detected in D-1 larvae, but the level decreased until D-3 compared to the protease enzyme in milkfish eggs (Aslianti *et al.*, 2014).

In Sobaity sea bream *Sparidentex hasta* larvae, protease enzyme activity was not detected until the larvae were fourteen days old (Nazemroaya *et al.,* 2015). In D-4 larvae, protease enzyme levels also decreased to 0.0799 U/mL/minute even though the larvae were already able to feed on exogenous sources in the form of rotifers, as indicated by the presence of rotifers in the larvae's stomachs at D-4. An increase in protease levels in yellowfin tuna larvae occurred at D-7 with a level of 0.1770 U/mL/minute, but protease enzyme activity at D-10 decreased again to a level of 0.0970 U/mL/minute after the larvae were fed artemia nauplii starting at D-8. A significant increase in protease enzyme activity occurred in D-15 larvae with a protease enzyme level of 0.2455 U/mL/minute after yellowfin tuna larvae were fed newly hatched milkfish larvae starting on D-12. ase enzyme activity was relatively stable in D-20 larvae with a protease enzyme level of 0.2169 U/mL/minute. Protease enzyme activity in D-20 larvae in this study, with a total length of 18 mm, was much lower than the total protease enzyme activity in juvenile yellowfin tuna in Panama, which measured 2.3 mm and had an activity of 1.587 U/mL/minute. juveniles measuring 2.5 mm had protease enzyme activity of 1.739 U/mL/minute, and juveniles measuring 3.4 mm had protease enzyme activity of 2.923 U/mL/minute (Buentello *et al.*, 2011).

The amylase enzyme activity in yellowfin tuna eggs in this study was 0.2787 U/mL/minute, which was much lower than the results of Buentello *et al.* (2011) on the same species, which was 2.262 U/mL/minute. In D-1 tuna larvae, amylase enzyme activity was also detected at 0.2508 U/mL/minute, and this amylase enzyme activity increased significantly in D-4 larvae to 0.3971 U/mL/minute. This amylase enzyme activity was stable in D-7 and D-10 larvae, with amylase enzyme activity levels of 0.3902 U/mL/minute in D-7 and 0.4111 U/mL/minute in D-10. The amylase enzyme activity of yellowfin tuna larvae decreased again at D-15 with a level of 0.2647 U/mL/minute and increased again when the yellowfin tuna larvae were twenty days old (D-20) with a level of 0.4041 U/mL/minute. The amylase enzyme activity in D-20 larvae in this study, with a total length of 18 mm, was much lower than the amylase enzyme activity in juvenile yellowfin tuna in Panama, which measured 2.3 mm and had a level of 4.183 U/mL/minute. juveniles measuring 2.5 mm had amylase enzyme activity of 11.692 U/mL/minute, and juveniles measuring 3.4 mm had amylase enzyme activity of 2.860 U/mL/minute (Buentello *et al.*, 2011).

Similar to protease and amylase enzyme activity, lipase enzyme activity has also been detected in the eggs and larvae of yellowfin tuna D -1. Lipase enzyme activity in yellowfin tuna eggs is relatively stable compared to that in yellowfin tuna larvae D-1, D-4, D-7, and D-10. Lipase enzyme activity in yellowfin tuna eggs was 0.200 U/mL/minute, which is almost the same as lipase enzyme activity in yellowfin tuna eggs in Panama, which was 0.272 U/mL/minute (Buentello *et*

al., 2011). Lipase enzyme activity in D-1 larvae was 0.163 U/mL/minute, in D-4 larvae it was 0.196 U/mL/minute, in D-7 larvae it was 0.154 U/mL/minute, and in D-10 larvae, lipase enzyme activity in yellowfin tuna larvae was 0.179 U/mL/minute. Lipase enzyme activity in yellowfin tuna larvae increased significantly in D-15 larvae with lipase enzyme activity of 0.329 U/mL/minute, and its activity decreased slightly in D-20 larvae with lipase enzyme activity of 0.283 U/mL/minute. The lipase enzyme activity in D-20 larvae in this study, with a total length of 18 mm, was much lower than the lipase enzyme activity in juvenile yellowfin tuna in Panama, which measured 23 mm and had an activity of 0.963 U/mL/minute. Juveniles measuring 25 mm had lipase enzyme activity of 1.289 U/mL/min, and juveniles measuring 34 mm had lipase enzyme activity of 2.688 U/mL/min (Buentello *et al*., 2011).

CONCLUSION

All digestive enzymes (protease, amylase, and lipase) in yellowfin tuna larvae indicates the importance of these enzymes in early development. The activity of digestive enzymes in yellowfin tuna larvae (protease, amylase, and lipase) was detected while still in the egg stage and was also detected in D-1 larvae. The emergence of digestive enzymes is triggered by internal mechanisms related to the development of then digestive system.

ACKNOWLEDGEMENTS

This research was made possible by funding from the Indonesian Ministry of Marine Affairs and Fisheries (KKP). We also express our gratitude to Dr. Akio Nakazawa (Chief Expert of the OFCF) for his assistance in providing input on the implementation of this research. We also extend our gratitude to the technicians at the yellowfin tuna hatchery project who assisted in the implementation of this research.

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