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Egg Incubation and Quality of Yellowfin Tuna (*Thunnus albacares*) Larvae at Different Salinity

Inkubasi Telur Dan Kualitas Larva Ikan Tuna Sirip Kuning (*Thunnus albacares*) Pada Salinitas Yang Berbeda

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ABSTRACT

This study aims to determine the proper salinity of sea water for egg incubation and maintenance of early yellowfin tuna larvae (Thunnus albacares). This research was conducted in November - December 2017 at Large Marine Research Center and Fisheries Extension of Gondol, Bali. This study used an experimental method with complete randomized design with 4 treatments and 3 replications, namely treatment (A) 29 ppt, (B) 31 ppt, (C) 33 ppt, (D) 35 ppt. Data were analyzed using analysis of variance (ANOVA) with a real level of 5%. The results showed that different salinity during the incubation and maintenance period of yellow fin tuna larvae did not have a significant effect on incubation time, hatchability and total larval length, but had a significant effect on the level of abnormalities of the larvae. Salinity of 35 ppt, with incubation time spent 20 hours 43 minutes, showed egg hatchability of 49.89%, lowest abnormality of 16.03%, and total larval length of 2.82 mm. The conclusion of this study is that salinity for the egg incubation period and maintenance of yellow fin tuna larvae are still in the range 33-35 ppt

ABSTRAK

Penelitian ini bertujuan untuk mengetahui salinitas air laut yang tepat untuk inkubasi telur dan pemeliharaan awal larva ikan tuna sirip kuning (*Thunnus albacares*). Penelitian ini dilaksanakan pada bulan November – Desember 2017 di Balai Besar Riset Budidaya Laut dan Penyuluhan Perikanan (BBRLPP) Gondol, Bali. Penelitian ini menggunakan metode eksperimental dengan Rancangan Acak Lengkap (RAL) dengan 4 perlakuan dan 3 kali ulangan, yaitu perlakuan (A) 29 ppt, (B) 31 ppt, (C) 33 ppt, (D) 35 ppt. Data dianalisis menggunakan analisis sidik ragam (ANOVA) dengan taraf nyata 5%. Hasil penelitian menunjukkan bahwa salinitas yang berbeda pada masa inkubasi dan pemeliharaan larva ikan tuna sirip kuning tidak memberikan pengaruh secara nyata pada lama waktu inkubasi, daya tetas dan panjang total larva aka tetapi memberikan pengaruh nyata (F-hitung > F-tabel 5%) terhadap tingkat abnormalitas larva. Salinitas 35 ppt dengan lama waktu inkubasi telur menghabiskan waktu selama 20 jam 43 menit,

menunjukkan daya tetas telur sebesar 49,89%, tingkat abnormalitas terendah sebesar 16,03%, dan panjang total larva 2,82 mm. Kesimpulan dari penelitian ini adalah salinitas untuk masa inkubasi telur dan pemeliharaan larva ikan tuna sirip kuning masih dalam kaisaran 33-35 ppt.

Kata Kunci	Thunnus albacares, salinitas, inkubasi telur		
Keywords	Thunnus albacares, salinity, egg incubation		
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INTRODUCTION

Tuna (*Thunnus* sp.) is one of Indonesia's mainstay export commodities. Tuna fisheries make a huge contribution to fisheries development in Indonesia. Tuna cultivation is starting to be developed because every year the demand for tuna continues to increase. Since 2007, tuna catches in Indonesia reached 136,655 tonnes and in 2008 Indonesia was able to export 6,821 tonnes of tuna in fresh and frozen form to Japan, Hong Kong and America (Andamari et al., 2012). When Indonesia experiences an economic crisis, the export value of tuna and skipjack commodities will increase by 9.1% and the volume of demand will increase by 17.8% (Ediyanto, 2017).

Yellowfin tuna (Thunnus albacares) is a member of the Family Scombridae, which is included in the group of large tuna fish, while the small ones include tuna, skipjack and skipjack (Faizah, 2010). The development of tuna cultivation in the world today is focused on several types of species including specific bluefin tuna (*Thunnus orientalis*), southern bluefin tuna (*Thunnus maccoyii*) and yellowfin tuna (*Thunnus albacares*) (Hutapea et al., 2010). Yellowfin tuna is a fast swimming fish and lives in groups, especially when looking for food (Nuraini et al., 2013). Currently, tuna cultivation businesses still rely on seeds from nature in the form of juvenile tuna caught by fishermen (Hutapea et al., 2017).

According to Sunyoto and Mustahal (2000), egg incubation aims to create conditions so that embryo development takes place well so that quality larvae are obtained. Salinity is one of the water qualities that affects eggs and larvae. In hatchery cultivation activities, the main problem faced is environmental factors, including salinity, which can affect the balance of the osmoregulation process, egg hatching and larval survival rates (Heltonika, 2014).

Salinity will affect energy utilization, because more protein will be stored to be used as energy in maintaining the balance of body salts and a decrease in salinity will affect the egg rearing medium, causing the eggs to shrink, which will cause the fluid inside the egg to move out (Hadid *et al.*, 2014).

Until now, there has not been much research on egg incubation and initial larval rearing at different salinities. However, Kim's (2015) research shows that a salinity of 38 ppt has a good influence on the hatching and survival of tuna larvae, and Hutapea's (2007) research on yellowfin tuna embryo development shows that a salinity of 33 ppt is the ideal salinity for embryo development. and egg hatching.

Based on the description above, it is necessary to carry out further studies regarding salinity adjustments during egg incubation and initial rearing of yellowfin tuna (*Thunnus*

albacares) larvae and their impact on abnormalities and length growth of larvae during observation.

This research was conducted to determine the appropriate seawater salinity for egg incubation and the quality of yellowfin tuna (*Thunnus albacares*) larvae. And the benefits of the results of this research are expected to: (1) Become a contribution to knowledge for students, researchers, cultivators and the outside community in general (2) become a reference in regulating appropriate salinity for incubation of eggs and initial maintenance of yellowfin tuna larvae (*T. Albacares*). And (3) become a reference in improving the quality of yellowfin tuna products even better.

METHODS

This research was carried out for 45 days from November to December 2017 at the Center for Mariculture Research and Fisheries Extension (BBRLPP) Gondol, Bali.

The tools used in this research were a 50 liter volume tank, a 2 liter volume small tank, a 5 ton fiber tub, an aerator, a refractometer, a Do meter, a pH meter, a petri dish, a pipette, a digital microscope, a bucket and stationery. The materials used in this research were yellowfin tuna eggs (*Thunnus albacares*), natural food (*Nannochloropsis* sp), sea water, fresh water and pure salt.

Yellowfin tuna (*T. albacares*) eggs are harvested at night at the main cage of BBRLPP Gondol, Bali. Eggs that have been harvested will be filtered using a multi-level filter with a size of 1000 μ m to filter out copepods and other dirt that enters when harvesting the eggs and 400 μ m only to separate the eggs from the dirt. Each container is filled with 300 eggs. This research used different salinity test factors, each test factor consisting of 4 treatments and 3 replicates. Treatments were tested at salinity 29‰(A), salinity 31‰(B), salinity 33‰(C) and salinity 35‰(D). Each experimental unit was then arranged in a Completely Randomized Design (CRD), with tank maintenance media volumes of 2 liters and 50 liters.

Data Analysis

Water quality; temperature, pH and DO, hatchability; HR= Number of larvae hatched/number of eggs laid × 100%., percentage of abnormality; Abnormality = Number of Abnormal larvae/total number of larvae × 100%, and the larval length obtained is interpreted in the form of graphs and tables. Data analysis used analysis of variance (ANOVA) in the costat sin plus program with a significance level of 5%. If there is a significant treatment response, data analysis continues using the Least Significant Difference (BNT) test at the error level 5%.

RESULT AND DISCUSSION

Water Quality

The results of water quality measurements during the research showed that the temperature of the water medium was at 27.8 0C - 27.9 0C, with the dissolved oxygen (DO) content and the degree of acidity (pH) of the water each at 6.42 – 7. 94 mg/l and 8.37 – 8.50. Based on the results of the diversity analysis, the temperature, DO and pH of the water during the study on average were not significantly different between treatments (F-count < F-table 5%). The data obtained shows that salinity levels between 29-35 ppt do not affect the quality of the supporting water.

Water quality is an important factor in the egg incubation process. Water quality is an important factor in the egg incubation process and fish larvae rearing, where good water quality will support the development of egg embryos and optimum survival of fish larvae.

Table 1.Water quality

Parameter		Results			Optimal Range
Temperature (⁰ C)	27,86	27,86	27,86	27,83	27-28 (Hutapea, 2007)
Salinity (º/₀₀)	29	31	33	35	33 – 34 (Hutapea, 2007)
рН	8,5	8,4	8,4	8,4	7,6 – 8,4 (Hutapea, 2010)
DO (mg/ <i>l</i>)	7,26	7,06	7,53	6,96	5 – 8 (Ulfani <i>et al.</i> , 2018)

According to Hutagalung (2016), high dissolved oxygen content will increase egg growth, egg hatching and early survival of fish larvae, while low dissolved oxygen concentration will affect the health of fish larvae because oxygen is needed in the metabolic process and movement activities of organisms (I'tishom, 2008). The dissolved oxygen content in this study still supports the hatching and maintenance of yellowfin tuna larvae. The dissolved oxygen level in the 35 ppt treatment was lower than in all treatments, allegedly because in this treatment the aeration stone was blocked so that oxygen delivery in this treatment was uneven. Oxygen levels for fisheries purposes should not be less than 5 mg/l, and dissolved oxygen levels of less than 2 mg/l will result in fish death (Effendi, 2000 in Hadid et al., 2014). The pH value in this study was still within the tolerance limits for hatching and rearing yellowfin tuna. In general, the pH value of seawater has a distinctive characteristic, namely above 8 (Hutapea, 2010). The temperature range in this study is still within the optimal range for hatching and rearing yellowfin tuna, where in Hutapea's (2007) research, yellowfin tuna eggs hatched for 18 hours 55 minutes at a temperature of 27 - 28 0C. The quality of the rearing media during observations of egg incubation and initial rearing of yellowfin tuna larvae was still relatively good. Therefore, the water quality parameters in this study did not have a significant influence on the diversity of the parameters measured.

Egg Hatching Time

The results of observations of the length of time required for eggs to hatch during the research showed that treatment D (29 ppt) showed the fastest hatching time, namely with an average time of 20 hours 43 minutes. The next sequence is treatment C (33 ppt), treatment B (31 ppt) and treatment A with an average time of 21 hours 2 minutes, 21 hours 5 minutes and 21 hours 8 minutes (Table 2). This data shows that at a salinity level of 29-35 ppt, yellowfin tuna eggs will hatch more quickly as salinity increases.

To determine the effect of different salinities on the hatching time of Yellowfin Tuna, a diversity analysis was carried out. The results of the diversity analysis (Appendix 1) show that the salinity levels tested gave a response to egg hatching time that was not significantly different (F-count < F-table 5%). The fastest hatching time for yellowfin tuna eggs is determined by a salinity of 35 ppt, while the longest hatching period is determined by a salinity of 29 ppt. This data shows that at a salinity level of 29 ppt to 35 ppt, the hatching time for yellowfin tuna eggs will be faster with increasing salinity. This data also proves that the embryonic metabolic processes in eggs and embryos will move more actively with increasing salinity, so that hatching also occurs more quickly. This was also stated by Hadid et al. (2014), that the higher the salinity, the faster the egg hatching time and it affects the metabolic process of the embryonic egg in the shell so that it becomes more intensive. Meanwhile, at low salinity, egg hatching time will spread, it is thought that salinity is also one of the water qualities that influences hatching, which makes fish larvae have a high survival rate. Energy use for osmoregulation can be suppressed if the organisms kept live in an isotonic medium. If the difference in osmolarity of a treatment is very different then the fish will need a lot of energy to adapt (Mubarokah et al., 2014). Table 2. Egg Hatching Time Interval (*hatching rate*)

Salinity	Time
29 ⁰ / ₀₀ ±1 (A)	21 hours 8 minutes
$31 {}^{0}/_{00} \pm 1 (B)$	21 hours 5 minutes
33 ⁰ / ₀₀ ±1 (C)	21 hours 2 minutes
35 º/oo±1 (D)	20 hours 43 minutes

Data source: Primary data processed

Hatchability of Eggs

Based on the research results, the hatching rate value obtained in treatment C (33 ppt) was 51.56%, then obtained in treatment D (35 ppt) was 49.89%, treatment B (31 ppt) was 45.22% and treatment A (29 ppt), resulting in a hatching rate value of 44.00%. The graph of the hatching rate value for each treatment is shown in Figure 5. The relationship between salinity and egg hatchability can be explained in the form of an equation using regression analysis, namely y = -0.181x2 + 12.78x - 175.2 with a coefficient of determination (R2) value of 0.143. Judging from the coefficient of determination, the effect of salinity on the hatchability of yellowfin tuna eggs is only 14.3%. Meanwhile, 83.7% was influenced by other external factors.

The results of the diversity analysis on the hatching rate values showed that the influence of different salinities did not have a significant effect (F-count < F-table 5%) on the hatchability of Yellowfin Tuna eggs. Based on these results, salinity for the hatchability of yellowfin tuna eggs is still optimal at 29-35 ppt.

Salinity is one of the factors that influences the hatchability of fish eggs and will influence the osmoregulation process of fish eggs. When stored in a medium with low salinity, marine fish eggs will shrink because the fluid inside the egg will move outward (Hadid et al., 2008). From the results of research that has been carried out, the highest egg hatchability is at a salinity of 33 ppt, while the lowest egg hatchability is at a salinity of 29 ppt. This data shows that the higher the salinity, the higher the egg hatchability until it reaches the optimum point. Salinity that is higher than the optimum point has the opposite effect where egg hatchability appears to decrease. This is thought to be because at low salinity the egg releases fluid from the inside and shrinks so that when it is about to hatch the egg is damaged because the fluid in its body has run out.

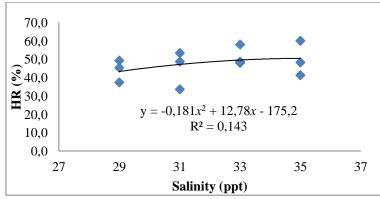


Figure 1. Graph of the relationship between salinity and the Hatching rate (HR) value of Yellowfin Tuna

The hatchability of yellowfin tuna eggs showed an earlier decline at salinities below 33 ppt although with a lower difference. As reported by Watanabe & Kuo (1985) in Jalaludin (2014), the ability of eggs to hatch is actually the same at all salinities but death occurs after some time after hatching. Egg quality is also thought to influence egg

hatchability. As stated by Melianawati et al. (2010), that the hatchability of eggs in each treatment can be influenced by several internal factors, for example egg quality and external factors such as temperature, salinity, pH and dissolved oxygen. And to maintain the quality of yellowfin tuna eggs, the eggs have been filtered before they are used in research with the aim of eliminating the presence of dirt and parasites attached to the eggs so as not to reduce the quality of the eggs during their development period.

Larval Abnormalities

The results of observations of larval abnormalities showed that the level of larval abnormalities decreased as the salinity value of the hatching media increased. The lowest level of abnormality was in treatment D (35 ppt), namely 16.03%. Sequentially higher levels of abnormalities were achieved by treatment C (33 ppt) at 43.44%, treatment B (31 ppt) at 57.79% and treatment A (29 ppt) at 58.26%. The graphic results of the level of larval abnormalities can be seen in Figure 2.

The relationship between salinity and larval abnormalities can be explained using regression analysis y = -1.683x2 + 100.6x - 1446 with a coefficient of determination (R2) of 0.749. The results of this research show that at a salinity level of 29-35 ppt, the higher the salinity level, the lower the abnormality value or the quality of larvae produced. The morphological appearance of abnormal larvae and normal larvae can be seen in Figure 2.

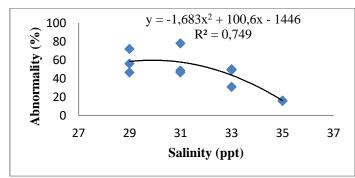


Figure 2. Graph of the relationship between salinity and larval abnormalities

The results of the diversity analysis showed that different salinity treatments had a significant effect (F-count > F-table 5%) on the level of abnormality in Yellowfin Tuna larvae (Appendix 3). Based on the results of further test analysis (Appendix 3), the lowest level of larval abnormalities in this study (treatment D) showed results that were not significantly different from treatment C but were significantly different from treatments A and B. Based on the results of this analysis, the level of salinity that produced larvae normal (low abnormality) is a salinity level of 33-35 ppt.

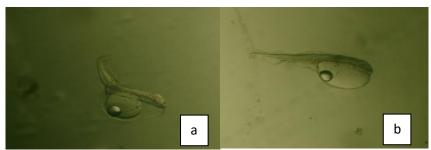


Figure 3. Morphological appearance of abnormal larvae and normal larvae. a. Abnormal (Crooked) larvae, b. Normal Larvae (Straight)

The differences in experimental salinity showed the influence of high levels of abnormalities in yellowfin tuna larvae at low salinity and low levels of abnormalities in high salinity treatments. Larvae that had hatched at low salinity showed higher body shape abnormality values. This is thought to be due to the incubation process being too long so that the growth of the embryo in the egg is also less than perfect and results in abnormal larvae. Abnormalities in yellowfin tuna larvae cause their body organs to not develop properly. It can be clearly seen from the shape of the body, whether the tail is curved or the bones of the body are curved downwards (Figure 7). Abnormal larvae also have a shorter life span compared to normal larvae. This was stated by Melianawati et al. (2010), that larvae which successfully hatch and whose bodies are abnormal and crooked may be influenced by the incubation period, where an incubation period that is too long results in the growth of the embryo in the egg being too long and less than perfect so that the embryo becomes abnormal. Larvae whose body shape is abnormal cannot survive. The research results of Hakim et al., (2008) also reported that the growth and survival rate of goldfish decreased at high salinity (15 ppt) due to high abnormality values.

Total Length of Larvae

Length of yellowfin tuna larvae after the larvae are 2 days old. The larval length measured is the total length. The results of measuring the total body length of larvae showed that larvae in treatment D (35 ppt) showed the highest average value, namely 2,822.80 μ m. The next highest order was in treatment C (33 ppt) at 2,772.05 μ m., treatment A (29 ppt) at 2,691.78 μ m. and treatment B (31 ppt) was 2,624.93 μ m. The total length of the larva was measured starting from the tip of the mouth to the tip of the tail of the larva (Fig 4).

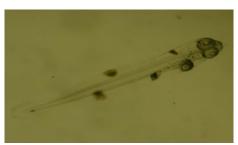


Figure 4. Measurement of the total length of yellowfin tuna larvae

The relationship between salinity level and larval length can be explained in the form of a quadratic regression equation y = 7.350x2 - 443.4x + 9353 and based on analysis of the coefficient of determination (R2) of 0.015 (Figure 5). The graph in this image explains that only 0.15% of the influence of salinity on the total length of larvae and that increasing salinity will be followed by an increase in the body length of yellowfin tuna larvae. Even though the larval length values show differences, the results of the diversity analysis show that the salinity level does not have a significantly different effect (F-count < F-table 5%) on the length of yellowfin tuna larvae.

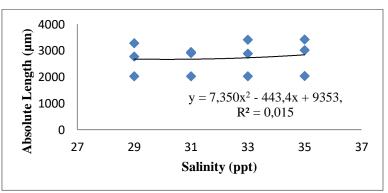


Figure 5. Graph of the relationship between salinity and growth in length of Yellowfin Tuna larvae

Yellowfin tuna larvae that were reared for 2 days showed development and growth in length. A salinity of 35 ppt produced larvae with the highest total body length value among all treatments, while a salinity of 31 ppt showed the lowest total body length. The results of the diversity analysis showed that larval length in all treatments did not differ significantly (F-count < F-table 5%), meaning that the salinity of all treatments still supported the growth of yellowfin tuna larvae. This is also supported by Hutapea (2010) that water salinity is between 32-34 ppt, still suitable for the growth of yellowfin tuna unless the salinity is 29 ppt.

Salinity has an influence on the fish's body metabolism to determine the ion balance in its body. This was stated by Jalaludin (2014), that salinity has a big influence on fish tuna metabolism, because it determines body balance. In this case NaCl, the largest compound of salinity determines the flow of substances to and from cells. Salt conditions that are too high in the media outside the cell will cause the cell to become dehydrated due to the release of water outside the cell.

The process of larval development (Figure 6) and the formation of body organs continues to progress, followed by the absorption of yellow as the main source of nutrition before the larvae are given external food. Gut formation begins to become clearly visible in newly hatched larvae within a few hours. As the egg yolk shrinks, intestinal pigmentation, eye pigmentation, ventral, dorsal, head and caudal sides also begin to develop. After the egg yolk has been completely absorbed, the pigmentation of the organs, including the intestines, eyes, caudal side, ventral side, head side, caudal side, is clearly visible.

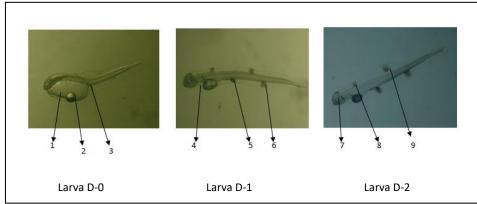


Figure 6. Larval Development. (1) Egg yolk (2) oil droplets (3) heart (4) Muscles (5)Anus Pigmentation (6) Dorsal Fin Pigmentation (7) Eye Pigmentation (8) FinPigmentation From Side Of Head (9) Fin Pigmentation In Mid Body

According to Usman et al. (2003), when the egg yolk is completely absorbed, the larva's mouth is generally open. The eyelids have opened during the process of absorbing the egg yolk, but the eye pigment is still not visible. Differences in the speed of egg yolk absorption occur due to differences in egg yolk volume and other external environmental influences (temperature, salinity and dissolved oxygen). And continued by Hutapea et al., (2010) that larval pigmentation occurs at the larval stage before the larvae hatch. This egg yolk pigment is called melanopor which is found in three parts of the body and the black pigment on the wall of the egg yolk and the pigment itself is used as a means of camouflage from predator attacks.

CONCLUSSION AND SUGGESTION

From the results of the research that has been carried out, it can be concluded that different salinities have a significant effect on larval abnormality, and the salinity for egg incubation in yellowfin tuna eggs still ranges between 33-35 ppt.

Suggestions that can be given from the results of this research are that it is recommended for researchers and farmers to maintain salinity stability during egg incubation and rearing of yellowfin tuna larvae so that it can support the hatching and survival of the larvae, and for further research to better control the development of egg embryos each time. hours and observing the rate of egg yolk absorption.

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