Mapping Embryogenesis in the Early Phases of Seabass (*Lates calcarifer*) Eggs on Different Salinities

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Abstract

Embryogenesis is a critical stage in the development of fish eggs, as it determines the successful hatching and survival of larvae. Understanding the effects of salinity on embryogenesis is crucial for optimizing hatchery practices and improving the production of fish larvae, such as the seabass. The purpose of this study was to map the stages of embryonic development against various salinities in order to identify the ideal salinity for seabass egg hatching. Eggs from natural spawning in a maintenance bath were used in this investigation. Four containers with salinity treatments (20 ppt, 25 ppt, 30 ppt, and 35 ppt) are used to lay fertilized eggs. The findings demonstrated that, in comparison to 20 ppt and 25 ppt treatments, eggs at 30 ppt and 35 ppt treatments generated faster embryonic development stages. Different incubation salinities have a highly substantial effect on the hatching speed of seabass eggs. Further testing revealed that the eggs hatched in 14 h and 40 min, which had the fastest seabass roe hatching time (35 ppt), and 15 h and 20 min for the 20 ppt salinity treatment. The 35 ppt salinity treatment had the highest hatchability rate (80.67%), while the 20 ppt salinity treatment had the lowest percentage (71.78%). It can be concluded that a salinity of 35 ppt provides a good embryo development response where there are no embryos that fail to develop, have the shortest hatching times of 14 h and 40 min, and produce the highest hatchability of eggs compared to other salinities.

Keywords: *embryogenesis, hatching, early life, salinity, seabass*

Introduction

The development of fish eggs into new individuals (embryogenesis) starts from the phase of cleavage (cell division) of the morula, blastula (formation of blastoderm), gastrula (closure of the yolk sac), and organogenesis until the embryo hatches and comes out of the eggshell (Ardhardiansyah *et al.*, 2017; Yang *et al.,* 2020). The quality and development of eggs are influenced by internal factors such as parent genes, hormones, feed, maintenance techniques and water quality as well as external factors in the form of environmental parameters such as temperature, dissolved oxygen light, and salinity (Reading *et al*., 2018; Al Khaziri *et al.,* 2019; Melianawati and Slamet, 2019; Amir *et al*., 2021; Perdana *et al.,* 2022; Mottaa *et al.,* 2023).

Good quality seabass eggs (*Lates calcarifer*) float in water and are transparent and round, whereas eggs of poor quality are milky white and sink in water (Ulfani *et al.,* 2018; Wirasakti *et al.,* 2021). Although seabass is a euryhaline fish, eggs and larvae of seabass can only live at salinities of 22–40 (Schipp *et al.,* 2007). Salinity was defined as the weight in grams of all solids dissolved in kilograms of seawater. The salinity value is expressed in g/kg, which is generally expressed in ‰ or ppt, which stands for parts per thousand. Approximately. The salinity of seawater is approximately 0.14 ‰ lower than its actual salt content.

Previous studies have shown that the hatching time of seabass eggs at a salinity of 5 ppt lasted an average of 18.40 h and at a salinity of 40 ppt, the average hatching time was 16.69 h. At higher salinity, embryonic development of eggs is faster (Kumaresan, 2011). Higher salinity can speed up the hatching time of the egg and affect the metabolic processes in the egg, such that the embryo in the shell will move more intensively. The higher the salinity, the greater the calcium content (Ca^{+}), which accelerates the hatching time of eggs because calcium accelerates the formation and hardening of eggshells (chorion), so that eggs break more easily (Wang *et al.,* 2006: Zafran *et al*., 2017; Skorupa *et al*., 2022).

Large sea bass live in brackish water and migrate to seawater for gonad maturation and spawning. The larvae of white and juvenile snappers enter brackish water through the tide of seawater. Seabass eggs are exposed to various environmental conditions, such as salinity, during their life cycle, especially in the early stages. The development of eggs is strongly influenced by salinity, which plays a vital role in egg buoyancy, osmoregulation, egg incubation time, and hatchability (Kumaresan 2011;

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Zafran *et al*., 2017; Noval *et al*., 2019). The goal of the present study is to investigate the effects of varying salinity levels on the development, survival rates, and physiological reactions of seabass embryos. By examining the unique impact of salinity—which has received less attention—this goal closes the knowledge gap, especially with regard to seabass. Therefore, this effect might be beneficial for larval production (Terence *et al.,* 2021; Liajian *et al.,* 2022).

Materials and Methods

This study was conducted at the Takalar Brackish Water Aquaculture Fisheries Center at Takalar Regency, South Sulawesi. The research container used was in the form of a glass jar of four pieces for observation of embryonic development and a glass jar of 12 pieces for observation of hatchability of eggs, each of which was provided with aeration equipment. The study began with sterilization of all tools by washing with soap. Subsequently, the jars were filled with seawater each 1.5 L, the salinity of which was arranged. The salinity of seawater was lowered from 35 ppt to 20, 25, and 30 ppt by adding fresh water.

Seabass eggs were taken from the hatchery division using a net measuring 400 µm. The eggs were stored in a research container containing 150 eggs each. Then, the jar was aerated. During the observation process, eggs were collected using a drip pipette and petri dish, and their development was observed every 60 min until hatching using a microscope. The data were captured in the form of photographs of egg development. As supporting data, water quality parameters such as temperature, pH, and dissolved oxygen were measured at the beginning and end of this experimental.

The phase of embryo development and the time it takes for the egg to reach the hatching stage within a time span of 60 min for each observation until the egg hatches. The hatching speed was determined by recording the time at which the egg was placed in the research container and the time at which the egg hatched. The percentage hatchability of seabass eggs was calculated as the percentage of eggs hatched into larvae from fertilized eggs. Data analysis, Embryo development phase data obtained in this study were tabulated in the form of images and tables, and analyzed descriptively. Data on hatching speed and hatchability of eggs were processed using a non-parametric analysis of variance. The W-Tukey test is used to compare group averages after an ANOVA, accounting for potential false positives. The Observational data were presented in Table 1.

Result and Discussion

Effect of salinity differences on embryonic development

The response of embryo development at salinity differs with a time span of 1 h each observation and enlargement 40 times are shown in Table 1. Based on observations of the development of seabass eggs in the first hour, all treatments started from cleavage phase 2 and continued until entering the morula phase at 4 o'clock and the blastula phase at the 5th hr. In this phase, the eggs in the 35 ppt treatment entered the blastula phase earlier than those in the other treatments. The gastrula phase at the 6th h. During organogenesis, the embryo forms parts of the body, including of the ovarian tail, somit, heart, eyes, head, body, yolk, crystals, and melanophores.

At a salinity of 20 ppt, the 12th and 13th hour of embryos failed to develop into the next phase, which is thought to be due to the embryo's inability to tolerate salinity. In this situation, the egg experiences turgor (increased pressure in the egg) or plasmolysis (shrinking of the egg due to the discharge of fluid from the egg into the medium) if the change in salinity exceeds the tolerance limit acceptable to the egg (Diana *et al*., 2017; Konovalov *et al.,* 2018; Santisathitkul *et al*., 2020).

The effects of salinity on seabass roe embryos can be direct or indirect. Salinity can directly affect the level of osmotic work because of the difference in osmolarity between the egg fluid (cytoplasm) and the perivitelline fluid, as well as its external medium, water absorption power, egg-type weight, and the hardening process of the chorionic membrane. Indirect salinity affects eggs (embryos) by affecting the availability of dissolved oxygen in water (Kumaresan, 2011; Truong *et al*., 2020; Rahi *et al.,* 2021).

Effect of salinity on hatching speed

The results of the observation of the hatching time of seabass eggs are shown in Figure 1. From the data obtained, it can be seen that the treatment with a salinity of 20 ppt had the longest hatching time of 15 h 20 min, whereas the treatment with 35 ppt had the fastest hatching time of 14 h 40 min. It can be concluded that the higher the salinity, the faster the time it takes for eggs to hatch. Previous studies have shown that egg hatching at a salinity of 40 ppt takes approximately 16.69 h, and that at a salinity of 5 ppt takes 18.40 h. This suggests that there is a relationship between salinity and egg hatching speed (Kumaresan, 2011; Wade *et al*., 2020; Terence *et al.,* 2021).

Table 1. The response of embryo development at salinity differs with a time span of 1 h each observation and enlargement 40 times

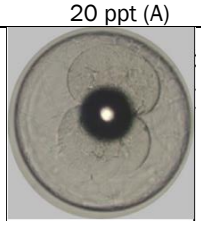
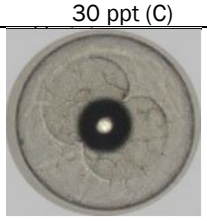
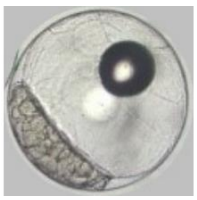
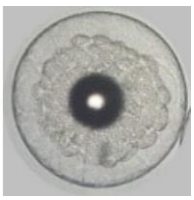
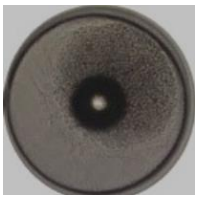
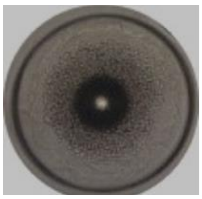
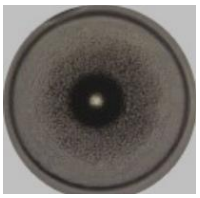
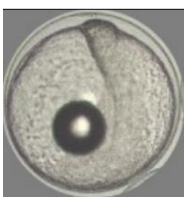
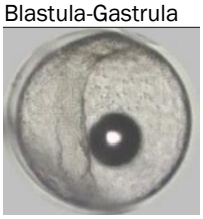
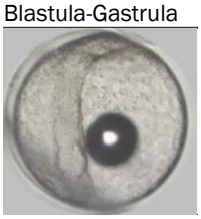
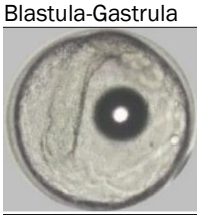
Time	Salinity treatment (Time span of 60 min per observation)			
	20 ppt (A)	25 ppt (B)	30 ppt (C)	35 ppt (D)
1	 Cleavage	 Cleavage	 Cleavage	 Cleavage
2	 Cleavage	 Cleavage	 Cleavage	 Cleavage
3	 Morula	 Morula	 Morula	 Morula
4	 Morula	 Morula	 Morula	 Morula
5	 Morula	 Morula	 Morula	 Morula
6	 Blastula-Gastrula	 Blastula-Gastrula	 Blastula-Gastrula	 Blastula-Gastrula
7	 Organogenesis	 Organogenesis	 Organogenesis	 Organogenesis

Table 1. The response of embryo development at salinity differs with a time span of 1 h each observation and enlargement 40 times (continue)

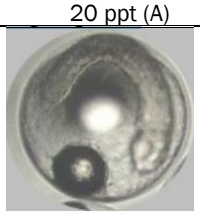
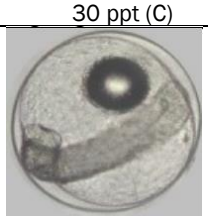
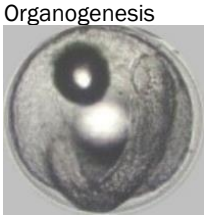
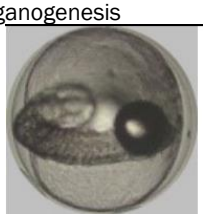
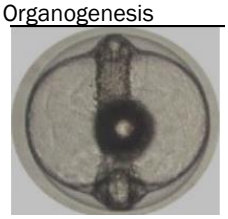
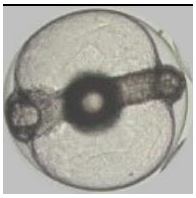
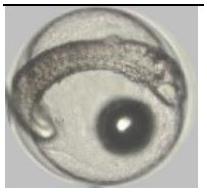
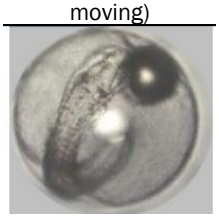
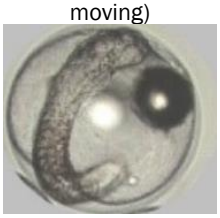
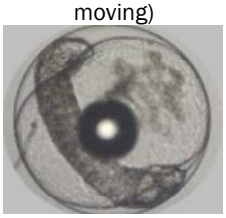
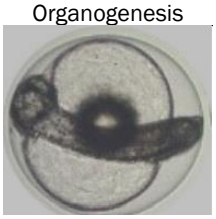
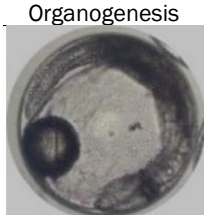
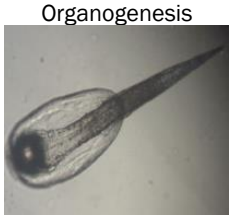
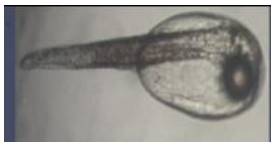
Time	Salinity treatment (Time span of 60 min per observation)			
	20 ppt (A)	25 ppt (B)	30 ppt (C)	35 ppt (D)
8	 Organogenesis	 Organogenesis	 Organogenesis	 Organogenesis
9	 Organogenesis	 Organogenesis	 Organogenesis	 Organogenesis
10	 Organogenesis	 Organogenesis	 Organogenesis (Start moving)	 Organogenesis (Start moving)
11	 Organogenesis (Start moving)	 Organogenesis (Start moving)	 Organogenesis	 Organogenesis
12	 Organogenesis	 Organogenesis	 Organogenesis	 Organogenesis
13	 Organogenesis	 Organogenesis	 Organogenesis	 Organogenesis
14	 Organogenesis	 Organogenesis	 Hatching eggs	 Hatching eggs

Table 1. The response of embryo development at salinity differs with a time span of 1 h each observation and enlargement 40 times (continue)

Time	Salinity treatment (Time span of 60 min per observation)			
	20 ppt (A)	25 ppt (B)	30 ppt (C)	35 ppt (D)
15	 Hatching eggs	 Hatching eggs		

Hatching speed also affects embryonic deformities. At the 12th h of salinity 20, the embryo failed to develop (deformities). This result is in line with those of previous studies, which stated that there tends to be deformity in marine fish eggs incubated at low salinities. Deformities usually occur in the spine and tail, and are not fully formed or bent. (Nguang *et al*., 2012; Tanjung *et al*., 2019). In addition, research on fugu fish shows that the salinity of egg incubation, which is not optimal, in addition to related deformities and death, can also affect the speed at which eggs hatch. Deformity and death are also caused by the use of energy for osmoregulation, resulting in negative effects on embryonic development (Yang and Chen, 2006; Nguang *et al*., 2012; Pinto *et al.,* 2021).

According to a study by Nguang *et al*., 2012, hatching speed is influenced by intraspecific salinity variations in the hatching time of fish eggs depending on other environmental variables (e.g., oxygen availability, temperature, and light). However, the salinity was not a major factor. Slow embryonic development is believed to be caused by indirect consequences of embryo morphological and physiological deformities due to differences in salinity and not by direct effects on the hatching glands (Hoar and Randall, 1988; Mottaa *et al.,* 2023).

The difference in the length of hatching time was likely due to an increase in chloride cell content in the seabass eggs. If fish eggs are at high salinity, the yolk sac membrane content becomes complex in response to changes in salinity. These chloride cells control osmoregulation and increase the activity of Na^+/K^+ - ATPase during salt exchange to improve tolerance. Chloride cells also caused the liquid in the fish eggs to become thicker and closer to the fluid concentration in the hatching medium. Thus, the energy used for osmoregulation and other processes inside the egg decreases and the remaining energy can be used for the growth of fish eggs (Mubarokah *et al.,* 2014; Pinto *et al.,* 2021).

Effect of salinity on eggs hatchability

The results of the observation of the hatchability of seabass eggs are shown in Table 2. The hatching rate (HR) was calculated by comparing the number of larvae hatched from the eggs to the number of fertilized eggs. Hatchability of seabass eggs was calculated 18 h after fertilization. The ANOVA results showed that the salinity treatment of different hatching media had a significant effect ($P<0.05$) on the hatchability of seabass eggs. Continued with further tests, W-Tuckey showed that the treatment with a salinity of 20 ppt was significantly different from the treatment with 35 ppt. However, it was not significantly different from that of the 25 and 30 ppt treatments.

From the hatchability percentage, it was found that the average hatchability for all the treatments was relatively high. The salinity of 35 ppt was the highest, with a value of 80.67%, whereas hatchability of seabass eggs was found in the treatment with a salinity of 20 ppt, with a value of 71.78%. The high hatchability of seabass eggs is thought to be due to the euryhaline nature of the eggs that follow their mothers. Seabass eggs can develop in a salinity range of 22–40 ppt (Schipp *et al.,* 2007; Roy *et al*., 2022). Previous studies have shown that mullet fish eggs have optimal hatchability at 35 ppt, and continue to fall at higher salinities. Hatchability is allegedly due to the failure of the eggs to osmoregulate. It can be concluded that there is a relationship between salinity and egg hatchability of eggs (Tanjung *et al.,* 2019; Yang *et al.,* 2020). Research on Leopard Grouper has shown that eggs with the highest hatchability are at a salinity of 32 ppt, and the eggs that hatch at eight ppt and 56 ppt are deformed and die when hatching (Gracia *et al.,* 2004). Salinity exerts an influence that supports the process of hatching eggs, with the same osmoregulation of eggs as in the environment. Providing energy that has been used for the adjustment of environmental conditions in eggs can be optimized to help the development of eggs and the resistance of the eggs themselves (Heltonika, 2014; Pinto *et al.,* 2021). Environmental factors such as temperature, salinity, and water quality significantly affected the number of hatched larvae. The differences in hatching rates were due to the different osmoregulatory processes and temperatures in each

Table 2. The results of the observation of the hatchability of seabass eggs

Treatment	Treatment Hatching Speed (Time)
A (20 ppt)	71.78 ± 4.07[a]
B (25 ppt)	72.00 ± 4.67[ab]
C (30 ppt)	76.22 ± 1.68[ab]
D (35 ppt)	80.67 ± 1.67[b]

Note: Different letters in the same column indicate significant effects (P< 0.05).

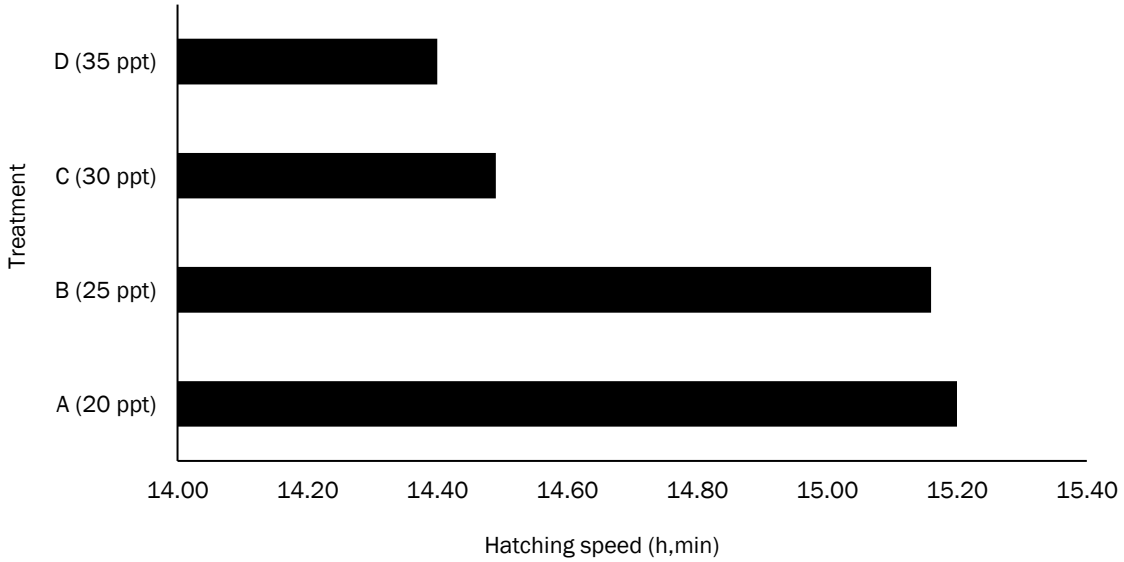


Figure 1. The hatching time of seabass eggs

treatment. Salinity is one of the factors that can affect the hatching rate of eggs (Pinto *et al.,* 2021; Roy *et al*., 2022; Zoli *et al*., 2023).

Conclusion

A salinity of 35 ppt provides a good embryo development response where there are no embryos that fail to develop, have the shortest hatching times of 14 h and 40 min, and produce the highest hatchability of eggs compared to other salinities.

Acknowledgement

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