Effect of betel leaf extract (*Piper Betle* L.) toward hatching and survival rate of sea bass (*Lates calcarifer*) larvae

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Abstract. One of the factors that causes the hatchability of sea bass (*Lates calcarifer*) eggs to be low is fungal attack. One natural ingredient that can prevent fungal attacks on fish eggs is betel leaves (*Piper betle* L.) because they have antifungal compounds. The aim of this research was to determine the effect of giving betel leaf extract on the hatchability of eggs and the survival of sea bass larvae. This research was carried out from 21 February to 3 March 2024 at the Ujung Batee Brackish Water Aquaculture Fisheries Center. The method used in this research was a Complete Random Design (CRD) with 5 treatment levels and 4 replications. The concentrations of betle leaf extract used included treatment A (control), treatment B (1.40 ml/L), treatment C (1.50 ml/L), treatment D (1.60 ml/L) and treatment E (1.70 ml/L). Based on the ANOVA test that has been carried out, it can be concluded that the addition of betel leaf extract has a significant effect on the hatchability of sea bass eggs and the survival of sea bass larvae. Treatment C (1.50 ml/L) was the best treatment in this study, which resulted in a hatchability percentage of 89.50%, with a survival rate of 84.50%.

1 Introduction

Sea bass (*Lates calcarifer*) is a fish that has high economic value and is in great demand by local people and people abroad [1]. Sea bass (*Lates calcarifer*) cultivation has become a commercial cultivation business that needs to be developed. This happens because it grows relatively fast, is easy to maintain and has high adaptability to environmental changes, making sea bass (*Lates calcarifer*) relatively easy for people to cultivate, both on a small and large scale [2]. The success of developing sea bass cultivation is largely determined by the provision of broodstock and eggs of good quality and quantity. One of the factors that influences the success of aquaculture is the high hatchability and survival of larvae so that the availability of sea bass larvae is always available at all times [3].

The availability of sea bass seeds often faces problems, namely the presence of fungal attacks that attack fish eggs, both unfertilized eggs and fertilized eggs, resulting in egg hatchability and larval survival [4]. One of the factors that causes low hatchability of eggs is fungal attack. Natural products have the potential to be developed into effective antifungal agents due to their diverse bioactive compounds and environmentally friendly nature [5]. One of the efforts made to overcome the problem of high egg hatching failure caused by

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fungi is by adding plant extracts that have the potential to inhibit fungal growth, namely betle leaves. Betel leaves are a natural ingredient that has the potential to have anti-fungal and bacterial properties, because chemically betel leaves contain essential oils which can act as antimicrobials [6].

The chemical contents of betel leaves include essential oils, flavonoids, saponins and tannins. Green betel leaves have an essential oil content of 4.2% [7]. Many studies have been carried out regarding the administration of betel leaf extract (*Piper betle* L) on the hatchability of fish eggs. [8] reported that administering betel leaf extract at a dose of 1.50 ml/L of water resulted in an egg hatching rate of 84.33% for gourami fish. [9] also reported that administering betel leaf extract at a dose of 1.50 ml/L of water resulted in an egg hatching rate of 85.45% for milkfish. However, research related to giving betel leaf extract to increase the hatching rate of sea bass eggs has never been carried out. Therefore, researchers want to conduct research on administering betel leaf extract to increase the hatchability of sea bass eggs. The aim of this research was to determine the effect of giving betel leaf extract on the hatchability of eggs and the survival of sea bass larvae.

2 Methodology

2.1 Time and place

This research was carried out from February 21 to March 3 2024 at the Ujung Batee Aceh Besar Brackish Water Aquaculture Fisheries Center, Aceh Province.

2.2 Maitenance and feeding

The research method used was a Completely Randomized Design (CRD) consisting of five treatments with four replications. The manipulated factor was the addition of betel leaf extract. Determination of the dose used as a treatment refers to the dose modification used by [7]. The treatments given to the fish were, A = betel leaf extract at a dose of 1.40 ml/L, B = betel leaf extract at a dose of 1.50 ml/L, C = betel leaf extract at a dose of 1.60 ml/L and D = betel leaf extract at a dose of 1.70 ml/L. Sea bass were reared based on the guidelines of the Institutional Animal Care and Use Committee (IACUC, 2018) and have ethical approval 029/KEH/SKE/IX/2022 from the animal ethic committee of the School of Veterinary and Biomedical, IPB University.

2.3 Research Parameters

Meanwhile, the data analysed were *Hatching rate* (HR) and *Survival rate* (SR) which were maintained for 12 days. Observations of egg development and water quality parameters were analysed descriptively maintained for 12 days.

2.3.1 Hatching rate (HR)

Hatchability is the number of eggs that hatch. According to [10] hatchability can be calculated using the following formula:

$$HR = \frac{\text{Number of eggs hatched (tail)}}{\text{total number of eggs}} \times 100\ \% \qquad (1)$$

2.3.2 Survival rate (SR)

Survival is the ratio of the number of fishes that survive from the beginning to the end of the study. The survival value can be calculated using the formula according to [11] as follows:

$$SR=\frac{Nt}{No}\times 100\% \quad (2)$$

2.3.3 Water quality

The water quality parameters measured in this study were temperature (C), degree of acidity (pH), dissolved oxygen (DO), and salinity (ppt).

3 Results and discussion

3.1 Result

Based on the results of the ANOVA test, it showed that the administration of betel leaf extract (*Piper betle* L.) had a significant effect ($P<0.05$) on the hatchability of eggs and the survival of white snapper larvae that were reared until 12 days of age. BNJ further test results showed that treatment C (1.50 ml/L) was significantly different from treatment A (control) and treatment B (1.40 ml), but not significantly different from treatment D (1.60 ml/L) and treatment E (1.70 ml/L). Table 1 shows the data of Observation results on egg hatchability and larvae survival.

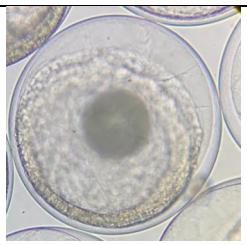
Table 1. Observation results on egg hatchability and larvae survival.

Treatment	Parameters	
	Hatching rate (%)	**Survival rate (%)**
A (control)	73.75 ± 2.62^{a}	68 ± 3.55^{a}
B (1,40 ml)	80.50 ± 4.04^{ab}	75.50 ± 4.04^{ab}
C (1,50 ml)	89.50 ± 4.40^{c}	84.50 ± 4.20^{c}
D (1,60 ml)	85.50 ± 2.08^{bc}	82 ± 2.58^{bc}
E (1,70 ml)	84 ± 2.94^{bc}	79.25 ± 3.86^{bc}

Note: The average value in the same column with a different superscript shows that it is significantly different and the value with the same superscript in the column shows that it is not significantly different. The ± sign indicates standard deviation.

The results of the development of sea bass (*Lates calcarifer*) eggs soaked using betel leaf extract (*Piper betle* L) can be seen in Table 2.

Table 2. Observations on the development of sea bass eggs.

Figure og Egg Development	Development Phase	Achievement of the Hour Phase	Information
	Morula	3	The formation that occurs after the fertilization phase, then the egg cell undergoes division
	Blastula	6	Formation of the blastosul layer
	Gastrula	9	The phase of egg yolk closure
	Organogenesis	13	The embryo begins to mature, marked by the formation of a head and tail
	Egg hatches	15	The process of movement of the embryo out of its shell

The results of water quality measurements during the research included measurements of temperature, pH and DO. During research, it shows that the water quality is still relatively good. The range values for these parameters are presented in table 3.

Table 3. Range of Water Quality Parameter Measurements

Treatment	Water Quality Range			
	Temperature (°C)	pH	DO (mg/l)	Salinity (ppt)
Treatment A (control)	28.4-29.2	7.2-8	5.1-5.5	23-27
Treatment B (1.40 ml)	28.7-30.0	7.3-8	4.9-5.6	24-26
Treatment C (1.50 ml)	28.6-29.9	6.75-7.9	4.8-5.8	23-26
Treatment D (1.60 ml)	28.4-29.1	7.5-8	4.9-5.6	23-26
Treatment E (1.70 ml)	28.6-29.4	7.5-7.8	4.8-5.7	22-27

3.2 Discussion

Treatment C (1.50 ml/L) was the best concentration to increase the hatchability of sea bass (*Lates calcarifer*) eggs, namely 89.50%. This is also in line with research conducted [9] with the best dose, namely 1.50 ml/L for the use of betel leaf extract for the hatching of milkfish eggs. Healthy fish eggs can be seen based on their colour. Good quality sea bass (*Lates calcarifer*) eggs have the characteristics of floating in the water, are transparent and round, while poor quality eggs have the characteristics of being milky white and sinking in the water [3]. [12] stated that there are flavonoids and essential oils in betel leaves which function as antimicrobials, these compounds bind microtubule proteins in cells and disrupt the function of mitotic spindles, thus protecting fish eggs from fungal attacks. Meanwhile, the low hatchability of sea bass (*Lates calcarifer*) eggs in treatment A (control), namely 73.75%, is thought to have occurred because the eggs were not treated with soaking with betel leaf extract, which resulted in no protection of the eggs against fungal attacks. Fungi that live and stick to the outer layer of the egg will hinder the egg hatching process and can even cause the egg to fail to hatch or die.

Soaking the eggs with a dose of 1.50 ml/L of water in treatment C gave the best results for the hatchability of sea bass eggs. It is thought that the fish still survive because the sea bass has natural body resistance, and it is also thought that it is because of the active ingredient from betel leaf extract which can increase the fish's immunity. This is confirmed by [13] who stated that betel leaves contain flavonoids, alkaloids, polyphenolic compounds, tannins and essential oils. These compounds have antibacterial properties and can also increase immunity in fish. Based on research on the survival rate, the highest was produced in eggs given betel leaf extract with a concentration of 1.50 ml/L with an average survival rate of 84.50%, followed by a concentration of 1.60 ml/L, a concentration of 1.70 ml/L and a concentration of 1.40 ml/L with an average of 82%, 79.25% and 75.50% and eggs that were not given betel leaf extract with an average of 68%. The survival value tended to increase with the increase in the dose of betel leaf extract, however at a dose of 1.70 ml/L the survival value decreased, this is thought to be because the dose of betel leaf extract was relatively high, thereby increasing tannin compounds, which are capable of softening the chorion layer. It is suspected that if the tannin compound is too high it will cause the larvae to hatch early and experience physical defects, so that within a few days of rearing the larvae will die.

The development phases of an egg into a larva start from the cell division (*cleavage*) phase of the morula, blastula, gastrula, organogenesis and the egg hatching into a larva [14] The results of observations of the development of sea bass (*Lates calcarifer*) eggs when the eggs were observed under a microscope with a magnification of 10x40µ, it was found that

the development of the eggs had entered the morula phase, because the cell division phase had passed.

The morula phase is the phase where the blastomeres that are formed will condense at the anima pole to form two cell layers [14]. This phase lasts for 3 hours. The morula stage is the end of the cleavage phase which will then be continued with the stage of embryonic organ formation. The white snapper embryo continues to undergo cell division until it enters the blastula phase. In this phase the blastomeres divide into smaller ones, so that in the morula stage the blastomeres which were originally solid will form an empty space called the blastosule [14]. This phase lasts for 3 hours. The gastrula phase is characterized by the blastoderma covering almost the entire yolk, the part of the yolk that is not covered is called the blastopore. In this phase, the rotating movement of the cells can be seen, the organs of the sea bass begin to form in this phase [15]. This phase lasts for 3 hours. Organogenesis is the formation of organs. In line with the process of embryo formation or embryogenesis, the process of forming the embryo's body organs, which is called organogenesis, occurs. Organogenesis takes place after the gastrula stage. Organs formed from neural tissue include the brain, eyes, the inside of the food digestive system with its glands and also some of the endocrine glands. The formation of all body organs is almost complete when the egg hatches [14]. This phase lasts 4 hours. Hatching is the final moment of the incubation period as a result of several processes so that the embryo emerges from its shell. Hatching occurs due to mechanical and enzymatic work. Mechanical work is hatching which occurs because the embryo often changes position due to lack of space in its shell. Meanwhile, enzymatic hatching is caused by enzymes released by the endodermal glands in the pharyngeal region of the embryo [16]. This phase lasts 2 hours. Water quality parameters are important in the process of egg hatching and larval rearing. This is in accordance with the statement of [3] who said that water quality parameters are external factors that influence fish reproduction and egg hatching. The water quality parameters observed during the research were salinity, temperature, pH and dissolved oxygen. The average salinity value obtained during the research was 24.8, temperature 28.90C, pH 7.7 and DO 5.2 mg/l. In this study, egg hatching took 15 hours. The pH value obtained in this study is in accordance with the statement [17] who said that a good pH for hatching fish eggs ranges from 7-9. And the DO value is in accordance [18] which says that dissolved oxygen is good for hatching fish eggs, namely >5 mg/L.

4 Conclusion

The effect of giving betel leaf extract (*Piper betle* L.) had a significant effect on egg hatchability and survival of sea bass (*Lates calcarifer*) larvae. The highest hatchability and survival of sea bass larvae were in treatment C with a concentration of 1.50 ml/L of water, namely 89.50% and 84.50%.

References

1. Sari, M., Hatta, M., Permana, A., Pengaruh ketinggian air dalam pemeliharaan larva ikan hias botia (Chromobotia macracanthus, Bleeker). Acta Aquat. **1(1)**, 24–30 (2014)
2. Jaya, B., Agustriani, F., Isnaini, Growth rate and survival rate of white sea bass seed (Lates calcarifer, Bloch) with different feeding. Maspari J. **5(1)**, 56-63 (2013)

3. Ulfani, R., Cut, N.D., Hasanuddin, Incubation of white sea bass (Lates calcarifer) eggs using a funnel system with different stocking densities. Unsyiah Mar. Fish. Stud. Sci. J. **3(1)**, 135-142 (2018)
4. Juniyanto, N.M., Atmoko, D., Kadari, Fatchudin, M.F., Rearing of white sea bass larvae (Lates calcarifer) in low salinity. Eng. J. BBPBL Batam **11**, 45-47 (2014)
5. C. Bulfon, D. Volpatti, M. Galeotti, Current research on the use of plant-derived products in farmed fish. Aquac. Res. **46(3)**, 513–551 (2015)
6. Fanitalya, Sudirman, A.A., Damayanti, Effect of betel leaf extract on fungal infections in gouramy fish eggs (Osphronemus gouramy). Unram Fish. J. **1(1)**, (2012)
7. Riezki, D.W., Pujiastuti, W.P., Ermawati, T., Differences in antibacterial effectiveness between red betel leaf extract (Piper crocatum) and green betel leaf extract (Piper betle L.) against Porphyromonas gingivalis. Stomatogenetic J. **10(1)**, 1-5 (2013)
8. Ghofur, M., Sugihartono, M., Thomas, R., Effectiveness of giving betel leaf extract (Piper betle L.) on the hatching of gourami fish (Osphronemus gouramy) eggs. Batanghari Univ. Jambi Sci. J. **14(1)**, 37-44 (2014)
9. Susanti, E., Yuniarti, T., Nugroho, R.A., Effect of betel leaf extract (Piper betle L.) with different doses on the hatchability of milkfish (Chanos chanos) eggs. J. Trop. Aquac. Sci., eISSN:2621-0525 (2022)
10. Sabrina, Suminto, Rachmawati, D., Performance of gonad maturity, fecundity, and hatching rates by providing a combination of natural feeds to parent tiger prawns (Penaeus monodon Fab.). J. Aquac. Manag. Technol. **3(3)**, 1-7 (2014)
11. Hidayat, D., Sasanti, A.D., Yulisman, Survival, growth, and feed efficiency of snakehead fish (Channa striata) fed with feed made from goldfish snail (Pomacea sp.) flour. Indones. Swamp Aquac. J. **1(2)**, 161-172 (2013)
12. Novizal, Successful hatchability of Siamese catfish (Pangasius hypophthalmus) eggs soaked with betel leaf extract (Piper betle L.). J. River Lake Aquac. **4(1)**, 28-34 (2019)
13. Shahida, I.E.A., Sarjito, S.B., Prayitno, A., Mariana, Effect of red betel leaf extract (Piper crocatum) on the blood profile and survival of goldfish (Cyprinus carpio) infected with Aeromonas hydrophila bacteria. J. Aquac. Manag. Technol. **2(4)**, 94-107 (2013)
14. Ardhardiansyah, S., Ujang, Y., Ayi, Embryogenesis and larval characteristics of crossing male Siamese patin fish (Pangasius hypophthalmus) with female baung fish (Hemibagrus nemurus). J. Fish. Mar. Aff. **7(2)**, 17-27 (2017)
15. Wahyuningtias, I., Diantar, R., Arifin, O.Z., The effect of temperature on the development of eggs and larvae of tambakan fish (Helostoma temminckii). e-J. Eng. Technol. Budid. Water. **4**, 439–448 (2015)
16. Violita, V., Muslim, M., Fitrani, Degree of hatching and length of hatching time for betok fish (Anabas testudinaeus) embryos incubated in media with different pH. JIPK J. Ilm. Fish. Mar. **11**, 21-27 (2019)
17. Hardianti, Q., Rusliadi, Mulyadi, Effect of feeding made with different composition on growth and survival of seeds of barramundi (Lates calcarifer, Bloch). Stud. Online J. **3(2)**, 1-10 (2016)
18. Windarto, S., Hastuti, S., Subandiyono, S., Nugroho, R.A., Sarjito, S., Growth performance of white sea bass (Lates calcarifer Bloch, 1790) cultivated with the floating net cage system (KJA). Trop. Aquac. Sci. **3(1)**, 56–60 (2019).