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THE HATCHING OF JAVA COMBTAIL (*Belontia hasselti*) EGGS IN MEDIA WITH DIFFERENT pH LEVELS

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ABSTRACT

The production of java combtail fry still relies heavily on wild catches, necessitating the development of semi-natural breeding techniques. One important factor influencing hatching success is the medium's pH. The pH value influences the activity of the enzyme chorionase, which softens the chorion, making the eggshell softer and facilitating hatching. This study aims to determine the optimal pH for the Java combtail hatching medium. The research method employed a completely randomized design (CRD) with 5 treatments and 3 replicates. The pH treatments for hatching were: medium pH 4±0.3 (P1), pH 5±0.3 (P2), pH 6±0.3 (P3), pH 7±0.3 (P4), and pH 8±0.3 (P5). The results showed that different pH levels in the hatching medium significantly affected hatching percentage, hatching duration, fry survival, fry length growth, and absolute fry weight. However, they had no significant effect on the percentage of larval abnormalities. P4 was the best treatment, yielding the highest hatching percentage of 90.00±0.82%, a hatching duration of 27.33±0.14 hours, and a larval survival rate of 96.19±2.43%, no larval abnormalities, absolute length growth of 4.94±0.05 mm, and absolute weight growth of 0.042±0.0010 g. This is likely because a pH of 7 (P4) represents the optimal condition supporting chorionase enzyme activity, thereby accelerating chorion softening and enhancing hatching success. Water quality during the study remained within the tolerance range for hatching and rearing of mudskipper fry. The research results serve as a reference for mudskipper fish farmers.

Keywords: Chorionase; Java Combtail; Hatching; pH

INTRODUCTION

The java combtail (*Belontia hasselti*) belongs to the gourami family (Osphronemidae). Several other names, such as *kakapar, klopar, and selincah*, also know this species. The java combtail is a food fish with significant economic value in Sumatra and Kalimantan, priced at Rp20,000.00 to Rp30,000.00 per kg. This fish species also has potential as an ornamental fish commodity, with prices ranging from Rp5,000.00 to Rp10,000.00 per fish (Muslim *et al*., 2020). Market demand for *selincah* remains quite high; however, its aquaculture has not yet developed optimally, so the majority of the fish supply still comes from wild-caught sources. This situation makes the sustainability of its population in natural waters vulnerable if exploitation continues to increase without being balanced by adequate management and aquaculture efforts (Tanbiyaskur *et al*., 2023). To date, aquaculturists have not been able to produce java combtail fry independently and sustainably, so the supply of fry for aquaculture activities still depends on wild catches (Yonarta *et al*., 2023). One step that can be taken to increase java combtail production is to develop sustainable aquaculture to address the decline in its population.

The egg hatching process in mudskippers is a key indicator of the success of aquaculture operations. Egg hatching is carried out to supply mudskipper fry. Based on research by Yonarta *et al.* (2023), the use of gonadotropin hormone at a dose of 0.5 mL/kg resulted in a fecundity of 2,677 eggs, an egg fertilization rate of 75%, and a hatching rate of 93%. The success of egg hatching is influenced by several factors, including the quality of the broodstock, the quality of feed provided to the broodstock, metabolic processes during fish egg development, and the conditions of the spawning and hatching media (Triwardani *et al*., 2022). Acidity (pH) is a critical factor affecting the fish egg hatching process, as pH influences the activity of the chorionase enzyme, which plays a role in softening the chorion to facilitate the larvae's emergence from the eggshell; suboptimal pH can inhibit this enzyme's activity and reduce hatching success (Putra *et al*., 2020).

Acidity (pH) is one of the main factors influencing the development and hatching success of mudskipper eggs. An inappropriate pH value, as a water quality parameter, can inhibit chorionase activity, an enzyme that softens the chorion, making the eggshell thinner and more pliable (Korwin, 2012). Chorionase functions optimally within a pH range of 7.1–9.6. Although the enzyme's operational range is relatively broad, many studies indicate that neutral pH (around 7) often provides the most stable conditions for embryo development and hatching enzyme activity, resulting in higher hatching rates and larval survival compared to conditions that are too acidic or alkaline (Marimuthu *et al*., 2019). Based on research by Violita *et al*. (2019), *betok* fish require a medium with a pH of 7±0.2 and can achieve a hatching rate of 86%. In red tilapia, the percentage of hatching eggs can reach 96% at a pH of 7±0.2 (Astuti, 2018), while snakehead fish at the same pH yield a hatching rate of 83.67% with a survival rate of 84% (Altiara *et al*., 2016). Research by Sinaga (2022) indicates that sepatung fish hatching is also optimal at a pH of 7±0.2, with a survival rate of 96%. *Betok* fish (*Anabas testudineus*) exhibited an egg hatching rate

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of 86% with a larval survival rate of approximately 88% in a medium with a pH of 7±0.2 (Violita *et al*., 2019).

The java combtail (*Belontia hasselti*) is a freshwater fish commonly found in swamp and peatland waters across Southeast Asia, including Sumatra and Kalimantan. These habitats are typically characterized by dark-colored water, high levels of organic matter, and a tendency toward acidity due to humic acid content resulting from vegetation decomposition (Pratama *et al*., 2023). This fish is adapted to the relatively calm swamp environment and its distinctive water quality, suggesting that pH plays a significant role in physiological processes, including embryonic development and egg hatching. However, the optimal pH range for Java combtail egg hatching remains unclear. The objective of this study is to determine the optimal pH for Java combtail hatching.

RESEARCH METHOD

Time and Location of the Study

This study was conducted at the Basic Fisheries Laboratory and the Fisheries Products Microbiology and Biotechnology Laboratory, Department of Fisheries, Faculty of Agriculture, Universitas Sriwijaya, from December 2023 to January 2024. This study used a completely randomized design (CRD) consisting of five treatments and three replicates. The pH treatments applied were based on the study by Violita *et al*. (2019), namely P1 (pH 4±0.3), P2 (pH 5±0.3), P3 (pH 6±0.3), P4 (pH 7±0.3), and P5 (pH 8±0.3).

Materials and Equipment

The equipment used in this study included a 30x30x30 cm^3 aquarium, a 60x40x40 cm^3 plastic *box*, a dissolved oxygen (DO) meter, a pH meter, a thermometer, a microscope, a syringe, an aerator, an aeration system, a filter, a vernier caliper, an analytical balance, a scale, beakers, and a spectrophotometer. The materials used in this study were adult java combtail measuring 10±1 cm, Ovaprim®, NaOH solution, H_2SO_4 solution, potassium permanganate, 0.9% NaCl solution, *Artemia* sp., distilled water, standard solutions, $MnSO_4$, Chlorox, and phenate.

Preparation of Spawning and Hatching Containers

The hatching and spawning containers consisted of aquariums and plastic boxes that were first sterilized using 20 mg $L^{-1}$of potassium permanganate for 24 hours, after which the aquariums were rinsed with clean water (Sinaga, 2022). Each aquarium was set up according to the predetermined treatment. The plastic spawning boxes were filled with water to a depth of 20 cm and equipped with an aerator. The hatching aquariums were each filled with 10 L of water and labeled to indicate each treatment. The water pH in the egg hatching containers was adjusted prior to the parent injection process by adding H_2SO_4 to lower the pH or NaOH to raise the pH until the desired treatment pH was reached (Violita *et al*., 2019). Thereafter, pH measurements were taken every 4 hours to ensure stability.

Java Combtail Spawning

Java combtail broodstock spawning is carried out using a semi-natural method, which begins with broodstock stimulation followed by natural spawning (Yuatiati *et al*., 2015). Fishermen caught the java combtail broodstock used from Gelumbang Village. Before spawning, the broodstock is first reared for two weeks, followed by a selection process. According to Muchlisin (2017), broodstock that have reached gonadal maturity can generally be identified through morphological and physiological characteristics. Male broodstock usually have a relatively slimmer body and, when stripped, will release sperm.

In contrast, female broodstock are characterized by an enlarged, soft abdomen and, when stripped, release mature eggs. The injection of gonadotropin hormones into male and female broodstock is intended to stimulate spawning. The injection is administered once intramuscularly in the right back at a dose of 0.5 mL kg^{-1} for each male and female broodstock. After injection, both broodstocks are placed together in a single spawning tank.

Egg Hatching

The egg density used in this study was 10 eggs per L. The eggs used were selected, fertilized eggs. The java combtail eggs were then placed in the respective hatching media prepared according to the treatment, and their development was observed until hatching (Muslim *et al*., 2018). According to Selviana (2024), fertilized *selincah* eggs are transparent yellow, while unfertilized eggs are white. The next step was to monitor the eggs until hatching was complete. Dead eggs were immediately removed using a dropper to avoid disturbing or damaging the live eggs. The hatching period for java combtail eggs lasted approximately 31 hours.

Larvae Rearing

The hatched java combtail fry were reared in a 30×30×30 cm^3 aquarium with a water depth of 11 cm for two weeks. Once the larvae's yolk sac reserves were depleted, they were fed *Artemia* sp. for 14 days using the *ad libitum* method (Sari *et al*., 2015). Feed was provided four times daily at 08:00, 11:00, 14:00, and 17:00 WIB.

Data Analysis

Data on hatching percentage, egg incubation duration, percentage of fry abnormalities, fry survival rate, and fry growth in terms of length and absolute weight were analyzed using analysis of variance (ANOVA) at a 95% confidence interval. If the analysis results indicated significant differences, the least significant difference (LSD) test was conducted. Water quality data were processed descriptively and supported by the literature.

RESULTS AND DISCUSSION

Hatching Percentage and Hatching Duration

Data on the hatching percentage and duration of java combtail eggs at various pH levels are presented in Table 1. The results of the BNT test at α = 0.05 indicate that the hatching percentage of java combtail eggs in treatment P4 (pH 7 ± 0.3) was significantly higher than in P3 (pH 6 ± 0.3), P2 (pH 5 ± 0.3), and P1 (pH 4 ± 0.3), but was not significantly different from P5 (pH 8 ± 0.3). A similar pattern was observed for hatching duration: treatment P4 had a significantly shorter duration than P3, P2, and P1, but was not significantly different from P5.

Table 1. Hatching Percentage and Hatching Duration of Java Combtail Eggs

Treatment	Hatching percentage BNTα0.05 = 3.67	Hatching Duration BNTα0.05 = 0.61
P1	73.67 ± 2.62[a]	29.51 ± 0.30 [a]
P2	77.00 ± 0.82 [ab]	28.75 ± 0.10 [b]
P3	78.00 ± 1.63 [b]	28.55 ± 0.48 [b]
P4	90.00 ± 0.82[c]	27.33 ± 0.14 [c]
P5	87.00 ± 1.63 [c]	27.63 ± 0.17 [c]

Note: Numbers in the same column followed by different superscript letters indicate significantly different results in the BNT test with a 95% confidence interval.

The hatching rate and duration of hatching for java combtail eggs were highest in the pH 7±0.3 treatment (P4), at 90±0.82% over 27.33±0.14 hours. This is likely because the pH in that treatment fell within the optimal range for Java combtail egg hatching. Although the java combtail naturally inhabits swamp waters that tend to be acidic, such conditions are not always optimal for embryonic development. During the embryonic phase, the ability to adapt to environmental pH is still limited; therefore, the eggs require a more stable environment, such as a pH close to neutral, to support embryogenesis and improve hatching rates (Astuti *et al*., 2023). The high hatching percentage in the optimal pH treatment indicates that embryonic development proceeds normally, resulting in hatched larvae with better yolk energy reserves and the ability to utilize them effectively for early growth. Korwin (2012) stated that pH greatly affects the activity of the enzyme chorionase, which is most effective at pH 7.1-9.6. Treatments P4 (pH 7±0.3) and P5 (pH 8±0.3) exhibited faster hatching times compared to the other treatments, as within the pH range of 7.1-9.6, the endodermal glands produce chorionase in the embryo's pharyngeal region, which functions to break down the chorionic layer composed of pseudokeratin, rendering it softer.

A study by Violita *et al*. (2019) on *betok* fish reported a 86% hatching rate over 14 hours at pH 7±0.2. This is believed to be because the pH range of 7±0.3 to 8±0.3 is optimal for stimulating chorionase activity. This enzyme plays a role in softening the egg's chorion, thereby accelerating the hatching process. The chorionase enzyme, or hatching enzyme, is a protease secreted by hatching gland cells in fish embryos and functions to degrade the egg's chorion layer, thereby allowing the larva to emerge from the eggshell during the hatching process (Kawaguchi *et al*., 2017). The lowest hatching percentage for Java combtail eggs was observed in treatment P1 (73.67%), with a hatching time of 29.51 hours. This condition is suspected to have occurred because the pH of the hatching medium was outside the optimal range, destabilizing chorionase activity and potentially hindering hatching. pH is an environmental factor that influences embryonic development and fish egg hatchability (Astuti *et al*., 2023). Hatching failure can also be triggered by suboptimal pH conditions, which disrupt the balance of the embryonic development process (Altiara *et al*., 2016). Additionally, a pH that is too acidic prevents enzymes from functioning optimally, causing the embryo to struggle to break the shell and resulting in a longer hatching time compared to other treatments.

Survival and Abnormalities of Java Combtail Larvae

The average survival and abnormalities of java combtail fryduring 14 days of rearing at different pH levels are presented in Table 2. The results of the BNT test $_{\alpha=0.05}$showed that the survival rate of java combtail larvae in the pH 7±0.3 treatment (P4) was significantly higher than in the other treatments. The highest survival rate of java combtail fry was observed in P4 (pH 7±0.3), at 96.19±2.43%. Research by Sari *et al*. (2016) showed that the survival rate of sepat fish at pH 6 reached 65%. Meanwhile, Yonarta *et al*. (2023) stated that the survival rate of java combtail was 80.8% within the pH range of 5.2–6.65. The results of this study indicate a higher survival rate compared to previous studies. These conditions are likely due to the treatment used being within a pH range suitable for survival and not disrupting the health of the java combtail larvae. Fish survival is greatly influenced by rearing environmental conditions, particularly water quality and proper feeding, as both factors play a crucial role in supporting metabolism, growth, and fish health during the cultivation process (Septian *et al*., 2024).

Table 2. Survival and Abnormalities of Java combtail Larvae

Treatment	Survival rate (%) BNTα0.05 = 7.41	Percentage of Abnormal Larvae
P1	77.14 ± 5.35 [a]	0.00±0.00
P2	85.71 ± 2.33 [bc]	0.67±0.94
P3	87.62 ± 3.75 [c]	0.00±0.00
P4	96.19 ± 2.43[d]	0.00±0.00
P5	78.57±1.17[ab]	0.00±0.00

Note: Numbers in the same column followed by different *superscript* letters indicate significantly different results in the BNT test with a 95% confidence interval.

Fry survival rates were lowest at a pH of 4±0.3 (P1), at 77.14±5.35%. This is believed to be due to the larvae experiencing stress resulting from their still-vulnerable immune systems and the influence of their surrounding environment. An environment with a pH that is too acidic or too alkaline can trigger stress in larvae and lead to death. Surbakti (2015) stated that a suboptimal pH range can cause stress in fish, leading to physiological disturbances and ultimately death. pH levels significantly affect fish survival, as low pH can reduce appetite, weaken digestive enzyme activity, and inhibit respiration by causing mucus to clump on the gills (Pratama & Mukti, 2015).

Analysis of variance indicated that hatching Java combtail eggs in media with different pH levels did not affect the incidence of abnormal Java combtail fry (Table 2). The highest percentage of abnormal Java combtail fry was observed in treatment P2 (pH 5±0.3), at 0.67±0.94%, while no abnormal fry were found in the other treatments. The results of this study show lower values than those reported by Altiara *et al*. (2016), who reported a percentage of abnormal larvae at pH 5±0.3 of 1.23%. In this study, Java combtail fry exhibiting abnormalities showed spine and tail deformities characterized by curvature. Fish eggs or embryos with developmental disorders can produce abnormal fry characterized by various morphological abnormalities, such as spinal or tail curvature (*spinal curvature*), yolk-sac *edema*, and other developmental abnormalities that can affect the larvae's swimming ability and survival (Hossain *et al*., 2021).

Length and Absolute Weight Growth of Java Combtail Fry

The average length and absolute weight growth of Java combtail fry over 14 days are presented in Table 3. The BNT results α=0.05indicate that the length growth and absolute weight of the java combtail fry in the pH 7±0.3 treatment (P4) were significantly higher than those in the pH 8±0.3 treatment (P5), the pH 5±0.3 treatment (P2), and the pH 4±0.3 treatment (P1), but were not significantly different from the pH 6±0.3 treatment (P3). The highest average length and absolute weight growth of java combtail fry was observed in P4 at 4.94±0.05 mm with a weight of 0.042±0.0010 g. The results of this study indicate that pH treatments not only affect the hatching percentage and hatching duration of java combtail eggs, but also the hatching percentage and hatching duration of Java combtail eggs. However, they are also associated with the growth of the resulting fry. In Weda's (2023) study and this study, during a 14-day rearing period at pH 6.6, the fish weighed only 0.038 g. However, they were 7.78 mm long. It is suspected that differences in pH may affect the fish's appetite, leading to stunted growth and increased stress. The lowest value was obtained at P1, at 4.53 ± 0.09 mm, with a weight of 0.02 ± 0.0005 g.

Table 3. Average Length and Absolute Weight Growth of Java Combtail Fry

Treatment	Absolute length (mm) BNTα0.05 = 0.13	Absolute weight (g) BNTα0.05 = 0.005
P1	4.53 ± 0.09[a]	0.023 ± 0.0005[a]
P2	4.71 ± 0.04[b]	0.027 ± 0.0014[a]
P3	4.90 ± 0.03[c]	0.039 ± 0.0017[b]
P4	4.94 ± 0.05 [c]	0.042 ± 0.0010[b]
P5	4.75 ± 0.06 [b]	0.027 ± 0.0042 [a]

Note: Numbers in the same column followed by different superscript letters indicate significantly different results in the BNT test with a 95% confidence interval.

The low pH in treatment P1 resulted in a lower hatching rate and longer hatching time, followed by lower fry length and weight growth compared to the other treatments. This is believed to be caused by the Fry limited nutritional capacity to maintain digestive balance, unstable water quality conditions, and the fact that the feed provided consisted solely of the natural food *Artemia* sp. Pratama & Mukti (2015) state that pH affects fish growth because under low pH conditions, appetite decreases, thereby weakening digestive enzyme activity and inhibiting respiration due to mucus clumping on the gills. If the pH falls outside the optimal range, fish growth will be stunted, and they will become more susceptible to bacterial and parasitic infections. Conversely, a pH above the optimal range can also disrupt fish growth (Usman *et al.,* 2022).

Water Quality

The water quality data observed during the study, namely, temperature, dissolved oxygen, and ammonia, are presented in Table 4. Water quality is a critical factor in the success of egg hatching, fry survival, and fish fry growth. The water-quality data in Table 4 indicate that these conditions remain suitable for rearing java combtail fry. This is in line with Government Regulation of the Republic of Indonesia No. 82 of 2001, which states that the appropriate temperature range for freshwater fish farming is approximately 25–32°C. The temperatures recorded during the study, namely 27.0–30.2°C, remain within a suitable range for rearing java combtail fry.

Table 4. Water Quality in the Rearing of Java combtail Fry

Treatment	Temperature (°C)	Dissolved Oxygen (mg/L)	Ammonia (mg/L)
P1	27.0–29.9	3.80–4.60	0.030–0.146
P2	27.0–29.9	3.90–4.50	0.038–0.182
P3	27.0–30.2	3.90–4.70	0.049–0.146
P4	27.1–29.9	3.80–4.60	0.041–0.182
P5	27.3–29.8	3.90–4.80	0.043–0.122

The dissolved oxygen range for fish farming is >3 mg/L (Riadhi *et al*., 2017). The dissolved oxygen levels recorded during this study were 3.80–4.80 mg/L, which is sufficient for rearing java combtail larvae. Dissolved oxygen levels that are too low or too high also affect the survival of java combtail larvae and can cause the fry to experience stres.

Ammonia levels suitable for fish farming are generally below 1 mg/L. During the study, ammonia concentrations ranged from 0.030 to 0.182 mg/L, which is still within the tolerance limit and within the optimal range. Ammonia concentrations toxic to fish generally exceed 1.5 mg/L (Wahyuningsih & Gitarama, 2020). An increase in ammonia concentration above the water's tolerance limit can be toxic to fish, as it can disrupt metabolic processes, reduce growth and appetite, and increase physiological stress, ultimately leading to fish mortality (Li *et al.,* 2016).

CONCLUSION

A pH value of 7±0.3 yielded good results, namely a hatching rate of 90.00±0.82% with a hatching time of 27.33±0.14 hours, a survival rate of 96.19±2.43%, absolute length growth of 4.94±0.05 mm with an absolute weight of 0.046±0.0010 g, and no abnormalities were observed in the java combtail fry.

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