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Artificial Fertilization Techniques in Bronze Featherback (*Notopterus notopterus*): First Report and Preliminary Findings

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ABSTRACT

The Java-native bronze featherback (Belida Jawa), *Notopterus notopterus*, has been designated as a protected species under limited protection status by the Decree of the Minister of Maritime Affairs and Fisheries of Indonesia No. 83 of 2024, with captive breeding efforts undertaken for conservation and aquaculture to meet increasing consumer demand. This study provides a preliminary evaluation of artificial fertilization techniques in *N. notopterus*, focusing on hormone-induced spawning using Ovaprim™. The objective was to determine the effectiveness of hormone injection, optimal egg stripping timing, and hatching success under controlled water quality conditions. Two female broodstock received different dosages of Ovaprim™, and successful egg stripping was conducted after a 41-hour latency period. Fertilization rates of 30% and 11.23% were observed for the first and second females, respectively; however, hatching success remained extremely low at 0.52% for the first female, with no larvae hatching from the second. Despite maintaining water quality parameters such as temperature, pH, conductivity, and total dissolved solids within acceptable limits, the poor hatching rate suggests that additional factors, including ammonia concentration and dissolved oxygen levels, may have impacted embryonic development. This study establishes a foundational basis for artificial reproduction in *N. notopterus*, emphasizing the need for further research to optimize hormone protocols, sperm viability, and environmental conditions. These findings contribute to the advancement of aquaculture strategies for the conservation and sustainable management of this ecologically important species.

Keywords: aquaculture, development, environmental, Ovaprim™, spawning

INTRODUCTION

The bronze featherback (*Notopterus notopterus*) is a freshwater fish species known for its unique morphology and ecological significance in Southeast Asian aquatic ecosystems. This species exhibits a complex reproductive biology characterized by gradual spawning, where mature oocytes are released over an extended period rather than all at once (Muslim *et al.*, 2024). On the other hand, increasing pressures on natural populations due to habitat degradation and overfishing of this fish may lead to a decline in its wild populations. The dominance of smaller total length in captured bronze featherback, with an spawning potential ratio (SPR) value of 5%, indicates a threat to population sustainability, which requires an SPR value greater than 30%, achievable if the exploited fish reach a total length of 80 cm (Warsa *et al.*, 2018).

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Additionally, the Java-native bronze featherback (Belida Jawa) has been designated as a protected species under limited protection status, as outlined in the Decree of the Minister of Maritime Affairs and Fisheries of the Republic of Indonesia No. 83 of 2024. Captive breeding has been conducted for this species as part of conservation efforts and aquaculture practices to meet the growing consumer demand (Yanwirsal *et al.*, 2017).

Several efforts to breed bronze featherback in captivity have been successfully conducted, including spontaneous spawning methods (Setijaningsih *et al.*, 2018; Srivastava *et al.*, 2010; Yanwirsal *et al.*, 2017); however, artificial fertilization has not yet been attempted. The advantages of artificial fertilization over spontaneous spawning methods include enhanced control over reproductive processes (Kucharczyk *et al.*, 2024), year-round breeding opportunities (Andriani *et al.*, 2023), the ability to select superior breeding stock (Kucharczyk *et al.*, 2024), reduced risk of disease transmission (Andriani *et al.*, 2023), and opportunities for research and development (Zamri *et al.*, 2022).

Artificial fertilization techniques have been widely studied in various fish species, with hormone-induced spawning being a common method to enhance reproductive success in captivity (Zamri *et al.*, 2022). The use of hormonal treatments, such as Ovaprim™, has been shown to effectively induce ovulation and spawning in several fish species (Budi *et al.*, 2024, 2023, 2020; Warni *et al.*, 2024), including those within the Notopteridae family (Setijaningsih *et al.*, 2018; Srivastava *et al.*, 2010). However, the specific application of these techniques to bronze featherback, such as hormone administration, gamete stripping, and fertilization, remains underexplored.

This study aims to evaluate the effectiveness of hormone injection techniques for artificial fertilization in bronze featherback and to assess the subsequent hatching success under controlled water quality conditions. This preliminary study will provide valuable insights into the reproductive biology of bronze featherback and contribute to the development of effective aquaculture practices for this species. By establishing a foundation for artificial spawning techniques, this research seeks to enhance the sustainability of bronze featherback populations and support conservation efforts in their natural habitats.

MATERIALS AND METHODS

Broodstock Selection

The broodstock of bronze featherback used in this study were obtained from Rawa Pening, Sumurup Hamlet, Asinan Village, Bawen District, Semarang Regency; and were selected based on their maturity (Figure 1.). On October 4, 2024, two mature female broodstock were chosen based on their swollen, soft abdomens, which signified they were ready for ovulation. The weight of the females was recorded at 188 g and 106 g. Additionally, three male broodstock with mature gonads were selected, identified by the reddish coloration of their genital papillae and the ease with which semen could be expressed upon abdominal pressure.

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Figure 1. Mature bronze featherback (*N. notopterus*) broodstock; a) lateral view of female, b) ventral view of female, c) male (red circle indicates male genitalia).

Table 1. Female broodstock weight, hormone dosage, injection times, and water quality during hormonal induction for bronze featherback (*N. notopterus*) propagation using intramuscular Ovaprim™ injections.

Female	Weight (g)	Ovaprim™ doses (ml)		Injection time (PM)		Water quality			
		First injection	Second injection	First injection	Second injection	Temperature (°C)	pH	Conctivity (mS)	Total dissolved solid (ppm)
1	188	0.05	0.10	12.00	10.00	27.6-28.0	6.3-7.1	0.08-0.09	46-50
2	106	0.03	0.07	12.00	10.00				

Hormone Injection

On October 5, 2024, hormone injections were administered to the selected mature female broodstock to induce spawning; male were not induced. The hormone Ovaprim™ was used, and the dosage was calculated based on the body weight of the females. Two distinct dosages were used, with 0.8 mL/kg administered to the first female weighing 188 g, and 1.0 mL/kg given to the second female, which weighed 106 g (modified from Setijaningsing *et al.*, 2018). Specifically, the dosage calculations resulted in 0.15 mL of hormone for the first female and 0.10 mL for the second.

The hormone injections were delivered intramuscularly in two stages. The first injection, accounting for 30% of the total dosage, was administered to the left dorsal muscle, while the second injection, comprising the remaining 70%, was given to the right dorsal muscle (Figure 2). The interval between the two injections was set at 10 hours to optimize the spawning induction process. All data related to the hormone injections, including the female broodstock weight, hormone dosage, injection times, and water quality during hormonal induction, are presented in Table 1.

Stripping and Fertilization

Stripping was performed on October 6, 2024, following an assessment of the latent period after hormone injection. The first attempt to strip the fish began 12 hours after the second injection, but no eggs were released at that time. It wasn't until 41 hours later that stripping was successful, although the eggs exhibited signs of hydration, indicating that ovulation had been delayed.

For egg collection, two different types of containers were used: a wide tray and a bowl (Figure 3). The wide tray allowed the eggs to be distributed more evenly, preventing them from clumping together, while the bowl, with its narrow surface area, caused the sticky eggs to cluster.

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Sperm from the male broodstock was used to fertilize the eggs in both the tray and bowl. The dry method was employed for fertilization, where sperm was first spread over the eggs before water was added to activate the sperm.

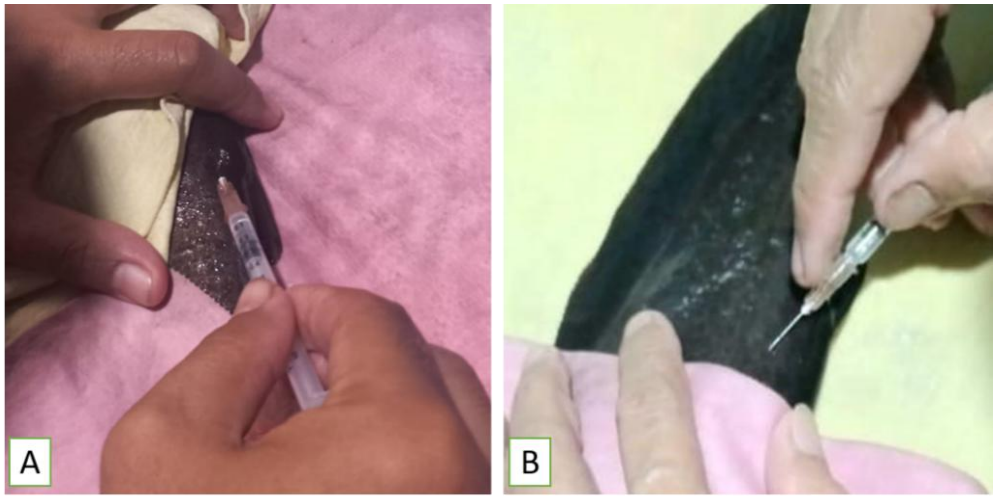

Figure 2. Hormone injection for propagation induction in bronze featherback (*N. notopterus*) administered intramuscularly; a) first injection on the left dorsal side, b) second injection on the right dorsal side.

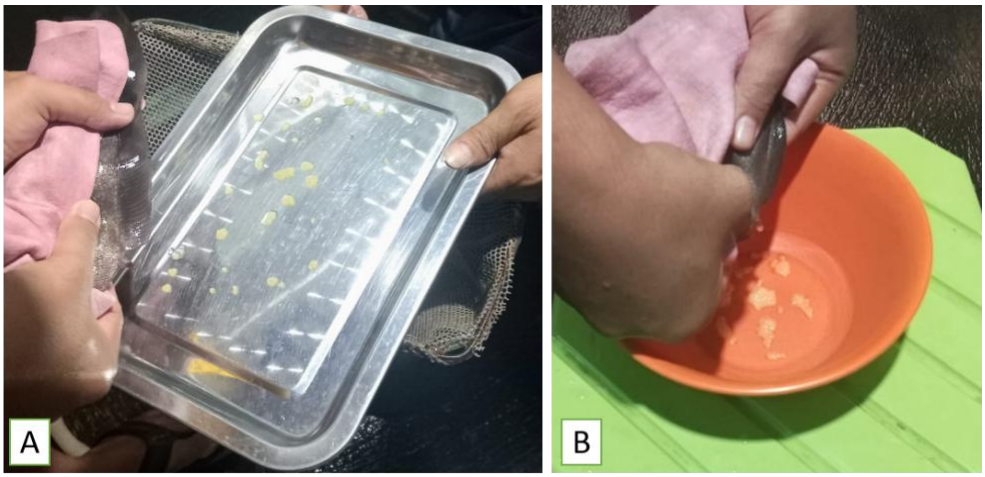

Figure 3. Stripping of bronze featherback (*N. notopterus*) to release eggs, using two types of egg collection containers; a) tray with a wide surface area, b) bowl with a narrow surface area.

Table 2. Latency period and condition of bronze featherback (*N. notopterus*) during induction, observed through egg stripping.

Date	Latency period (h after second injection)	Fish condition
6 October 2024	12	No significant development observed in the abdominal and genital areas
6 October 2024	13	The abdomen was more swollen and softer, but no eggs were released when stripping was applied.
6 October 2024	14	The condition was the same as in the second check, leading to the preliminary conclusion that ovulation was delayed, and the fish was quarantined for further evaluation.
7 October 2024	41	After trying to stripping them again, the abdomen feels softer, and the eggs were successfully released; however, the condition of the eggs were somewhat watery, indicating hydration.

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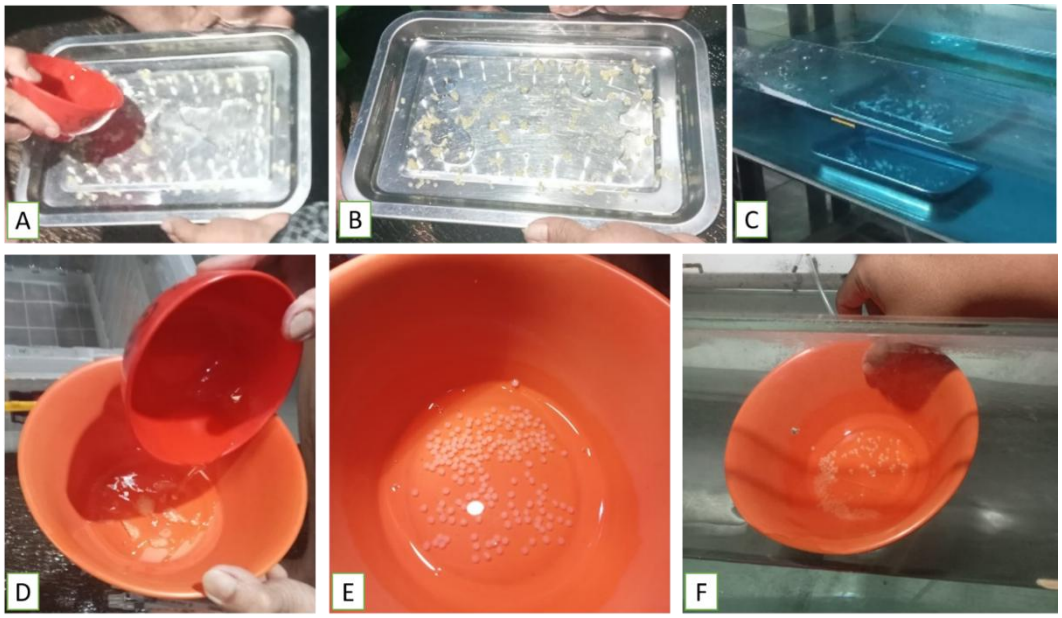

Figure 4. Fertilization process of bronze featherback (*N. notopterus*) eggs to incubation in aquaria; A-C: fertilization to incubation of eggs from the first female using a tray, D-E: fertilization to incubation of eggs from the second female using a bowl.

The containers were gently shaken for 2-3 minutes to ensure uniform fertilization, after which the excess water was removed. The fertilized eggs were then transferred to aquaria for incubation and further development (Figure 4).

Egg Incubation and Water Quality Monitoring

The eggs were incubated in aquaria under controlled environmental conditions to monitor the success of fertilization and hatching. Throughout the incubation period, several key water quality parameters were closely recorded to ensure optimal conditions for egg development. The water temperature, pH, conductivity, and total dissolved solids (TDS) were 27.2-27.6°C, 7.2-7.4, 0.08-0.13 mS, and 48-76 ppm.

Daily observations were conducted to track the development of the eggs. Fertilization and hatching rates were assessed through visual inspection, with fertilized eggs identified by changes in appearance and successful hatching determined by the emergence of larvae. These observations provided valuable data on the effectiveness of the spawning techniques used in the study.

Data Collection and Analysis

Data on egg weight, the total number of eggs, and the average egg size were collected for each broodstock. The fertilization and hatching rates were calculated for both female broodstock, and the duration of egg incubation was recorded. Egg development was documented using daily observations, with specific attention to the timing of hatching.

Fertilization rate was determined as the percentage of fertilized eggs in relation to the total number of stripped eggs, and the hatching rate was calculated as the percentage of hatched eggs in relation to the total number of fertilized eggs. Additionally, water quality data were collected during the incubation period to ensure that the conditions remained within the tolerable range for successful egg development.

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Table 3. Female broodstock weight, number of males used, total number of eggs produced, fertilization rate, and hatching rate in the artificial spawning of bronze featherback (*N. notopterus*).

Female	Weight (g)	Males used (fish)	Number of eggs	Hatching rate (%)	Fertilization rate (%)
1	188	2	382	30	0.52
2	106	1	187	11,23	0

Table 4. Water quality during the incubation of bronze featherback (*N. notopterus*) eggs from artificial fertilization.

Parameters	Range
Temperature (°C)	27,2 - 27,6
pH	7,2 - 7,4
Conductivity (mS)	0,08 – 0,13
Total dissolved solid (ppm)	48 - 76

RESULTS AND DISCUSSIONS

Latency Period and Egg Stripping

The latency period and the condition of the bronze featherback during the induction process were monitored through egg stripping (Table 2). Egg stripping was first attempted 12 hours after the second hormone injection on October 6, 2024, but no significant changes were observed in the abdominal and genital areas of the broodstock. After 13 hours, the abdomen was more swollen and softer, but no eggs were released when stripping was applied. At the 14-hour mark, the condition remained unchanged, leading to a preliminary conclusion that ovulation was delayed, and the broodstock was quarantined for further observation.

After 41 hours, on October 7, 2024, a second attempt at egg stripping was successful. The abdomen felt softer, and eggs were successfully released. However, the eggs appeared somewhat watery, indicating that hydration had occurred, which may have impacted their viability.

Fertilization and Hatching Rates

The artificial spawning trials produced varying results in terms of fertilization and hatching success (Table 3). The first female, weighing 188 g, produced 382 eggs after being paired with two males. The fertilization rate was 30%, and the hatching rate reached 0.52%, with larvae hatching approximately 4 days, 8 hours, and 47 minutes after fertilization (Figure 5). In contrast, the second female, weighing 106 g, produced 187 eggs after being paired with one male. However, this batch experienced a fertilization rate of 11.23% and no successful hatching. The difference in female weights influenced the number of eggs produced, as larger females generally have greater reproductive capacity. These weight variations were due to the availability of mature females at the time of treatment.

Water Quality During Incubation

Water quality was closely monitored during the incubation period to ensure optimal conditions for egg development (Table 4). The temperature during incubation ranged from 27.2°C to 27.6°C, with a pH level maintained between 7.2 and 7.4. Conductivity was kept between 0.08 and 0.13 mS, and the Total Dissolved Solids (TDS) ranged from 48 to 76 ppm. These parameters remained within acceptable ranges for Bronze Featherback egg incubation.

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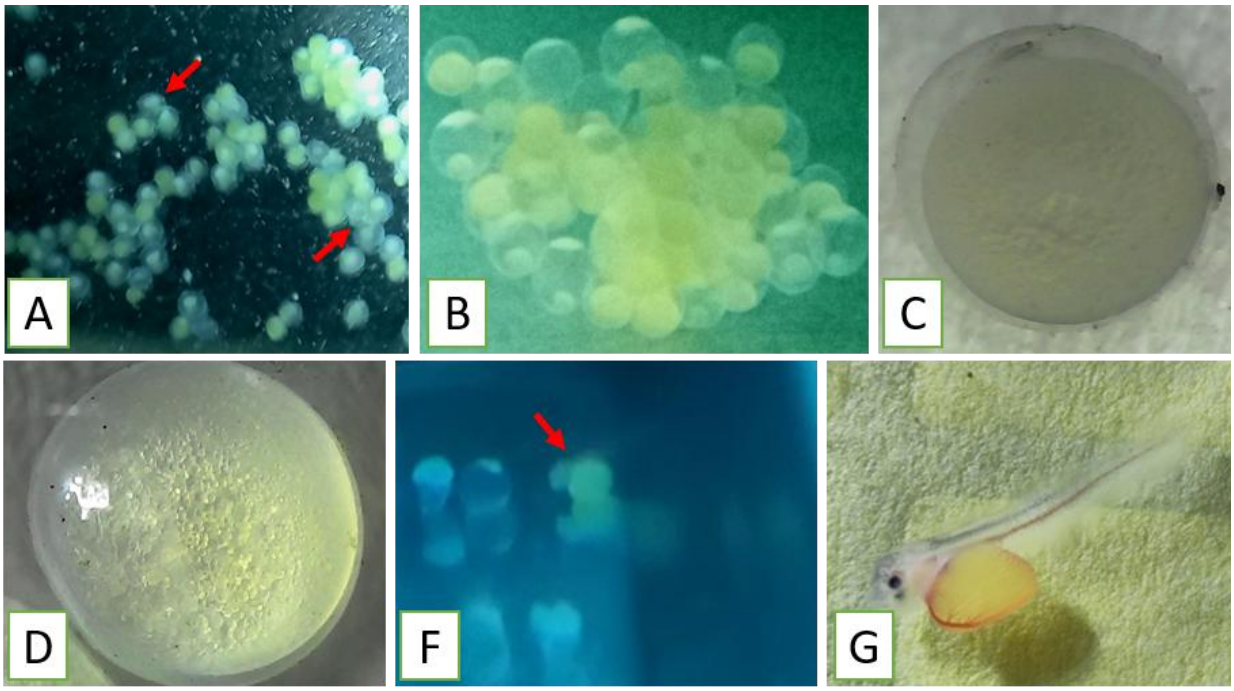

Figure 5. Development of bronze featherback (*N. notopterus*) eggs from artificial fertilization until hatching. A) 1-day-old eggs after fertilization, with the red arrow indicating unfertilized eggs. B) 2-day-old eggs after fertilization. C) 3-day-old eggs after fertilization. D) 4-day-old eggs after fertilization. E) Eggs hatched into larvae (red arrow) 4 days, 8 hours, and 47 minutes after fertilization. F) Hatched larvae (red arrow). G) 2-day-old larvae after hatching.

Discussion

The effectiveness of artificial fertilization techniques in bronze featherback through hormone injection and hatching success are evaluated in this preliminary study. The findings provide crucial insights into the reproductive biology of this species and highlight the potential for advancing aquaculture practices aimed at conservation and population sustainability.

The results indicate that hormone-induced spawning can be effectively applied to bronze featherback, although the process exhibited variability in response. The latency period observed after hormone injections suggests that the timing and dosage of Ovaprim™ that contains salmon gonadotropin releasing hormone analogue (sGnRH-a) and dopamine antagonist (DA) could significantly influence ovulation (Sahoo *et al.*, 2008). The successful egg stripping occurring 41 hours post-injection demonstrates the importance of careful monitoring during the induction process. Notably, previous research on *N. chitala* using human chorionic gonadotropin (HCG) and Ovaprim reported a shorter latency period of 13–15 hours post-injection (Setijaningsih *et al.*, 2018). The prolonged latency observed in *N. notopterus* in this study may be influenced by species-specific physiological differences, environmental conditions, or hormonal responsiveness. This suggests that while delayed ovulation may not necessarily indicate an abnormal response, further investigation is needed to optimize hormone administration protocols. Future studies should explore adjustments in dosage, alternative hormonal treatments, or environmental factors that could enhance the ovulatory response and reduce variability in spawning success.

The fertilization and hatching rates achieved in this study were relatively low, particularly for the second female broodstock. The first female produced a total of 382 eggs,

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achieving a fertilization rate of 30% and a hatching rate of 0.52%, while the second female produced only 187 eggs, with a fertilization rate of 11.23% and no successful hatching. These results align with the complexities of artificial fertilization in fish species, where factors such as egg quality, sperm viability, and environmental conditions can significantly impact reproductive success (Sahoo *et al.*, 2008).

Compared to previous studies, Setijaningsih *et al.* (2018) reported higher reproductive performance in *N. chitala* using broodstock weighing over 1 kg. In hormone-induced spawning, they obtained two spawning females with egg diameters of 3.1 ± 0.3 mm, fecundity ranging from 282 to 907 eggs, fertilization rates of 21%–40%, hatching rates of 56%–75%, and larval survival of 30%–50%. Meanwhile, in natural spawning, one female produced 1,616 eggs with a fertilization rate of 86.7% and an egg diameter of 3.5 ± 0.3 mm.

Compared to these findings, the lower fertilization and hatching rates in *N. notopterus* in this study may be attributed to the smaller broodstock sizes (106 g and 188 g), which were considerably below the broodstock sizes used in *Notoptera chitala*. The size of the parent fish is known to influence reproductive capacity, with larger females generally producing more eggs with better quality and a higher likelihood of successful fertilization and hatching. Additionally, the observed hydration of eggs in this study suggests potential suboptimal conditions or timing during the stripping process (Adawiyah *et al.*, 2019; Budi *et al.*, 2023; Ningrum *et al.*, 2019). This underscores the need for further investigation into optimizing broodstock size, hormone administration protocols, and spawning conditions to improve reproductive success in *N. notopterus*.

Water quality parameters during the incubation phase remained within acceptable ranges for bronze featherback egg development, suggesting that the controlled environment effectively supported the incubation process. However, despite this, the low hatching rate observed in this study indicates that other water quality factors, such as ammonia levels and dissolved oxygen, may have played a role in limiting hatching success. Therefore, continued monitoring and adjustment of these parameters are critical for future studies. Specifically, ammonia and dissolved oxygen levels should be closely observed and controlled, as these can significantly affect embryonic development and survival (Chen *et al.*, 2012; De Leão Serafini *et al.*, 2009; Dmitrieva, 2015). The successful observation of larval emergence approximately 4 days, 8 hours, and 47 minutes post-fertilization is encouraging, yet highlights the importance of thorough documentation of developmental stages to identify potential bottlenecks in the process.

Overall, this study establishes a foundational understanding of artificial fertilization techniques for bronze featherback and indicates the feasibility of hormone-induced spawning as a viable method for captive breeding. Future research should focus on refining these techniques by experimenting with different hormone protocols, enhancing sperm collection methods, and evaluating the influence of environmental factors on egg viability. The long-term goal of these efforts is to contribute to the conservation of bronze featherback populations in their natural habitats while meeting the increasing demand for this species in aquaculture.

The findings from this preliminary study lay the groundwork for further exploration of artificial reproduction techniques in bronze featherback. By addressing the challenges

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identified, such as delayed ovulation and low fertilization rates, we can enhance breeding success and contribute to the sustainable management of this ecologically significant species.

CONCLUSION

This preliminary study demonstrated the feasibility of hormone-induced spawning in bronze featherback using Ovaprim™, although the timing and administration of the hormone significantly influenced ovulation success. The artificial fertilization trials resulted in low fertilization and hatching rates, with 30% fertilization and 0.52% hatching for one female, while the second female achieved an 11.23% fertilization rate with no hatching success. These findings highlight the need for refined techniques, particularly in hormone protocols, sperm viability, and timing of egg stripping. The successful observation of larval emergence is promising, indicating the potential for improvement through adjustments to hormone injection protocols, stripping timing, and environmental controls.

AUTHORS' CONTRIBUTIONS

Each author's contribution is as follows: DD, AF, MA, and DY collected the data research, prepared the manuscript, and designed tables and graphs. DD, AH, DL, FN, CRM and YM; processing data, writing manuscripts, and proofreading manuscripts. All authors contributed to the final manuscript.

CONFLICT OF INTEREST

All authors declare that they have no conflict of interest.

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