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Captive Breeding of the Critically Endangered Wild *Betta burdigala* Under Laboratory Conditions

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ABSTRACT

Betta burdigala, a species endemic to Bangka Island and listed as Critically Endangered by the International Union for Conservation of Nature (IUCN), has been successfully bred under controlled laboratory conditions. This study provides detailed insights into its captive breeding, highlighting key parameters essential for *ex situ* conservation and the development of sustainable breeding protocols. The research involved breeding trials, bubble nest formation observations, hatching rate (HR) assessments, larval monitoring, initial feeding evaluations, fry rearing, and survival rate (SR) analysis. On average, females measuring 2.45 ± 0.07cm in total length produced 61.50 ± 4.95 eggs, from which 60.00 ± 4.24 larvae hatched, resulting in a high HR of 97.00 ± 1.41%. Larval development and yolk sac absorption were tracked from 0 to 4 days post-hatching, after which larvae were fed *Moina* sp. for up to 50 days. In the initial trial, 30.50 ± 3.54 larvae survived to day 50, yielding an SR of 50.50 ± 9.19%. These findings offer valuable baseline data for the conservation and early life stage management of *B. burdigala*, supporting future recovery programs. Further research is recommended to examine the long-term impacts of captivity on behavior, physiology, and phenotypic traits.

INTRODUCTION

Bangka Island, as part of the Bangka Belitung Archipelago, is located in the Sunda Shelf region—a low-lying geological area that was once integrated into mainland Southeast Asia **(Nazran *et al.*, 2025)**. This position gives Bangka Island a distinctive biogeographic history and favors the establishment of biodiversity, including unique freshwater fish communities **(Syarif *et al.*, 2023a; Hasan *et al.*, 2024; Islamy *et al.*, 2025a)**. *Encheloclarias taeptenerus* **(Valen *et al.*, 2024a)**, *Parosphromenus deissneri* **(Valen *et al.*, 2025a)** and *Betta burdigala* **(Syarif *et al.*, 2025a)** are among the many endemic fish species found in the island's acidic and blackwater habitats. Geographical isolation and extreme environmental conditions have led to high specialization in these species, but also increased their vulnerability to ecological disturbances, making habitat protection essential for conserving Bangka's unique biodiversity **(Hasan *et al.*, 2023; Kusumah *et al.*, 2023; Syarif *et al.*, 2023b; Pramono *et al.*, 2025)**.

B. burdigala, a member of the *Betta coccina* complex, is the only species of this group known to be endemic to Bangka Island, Indonesia **(Valen *et al.*,2023b; Syarif *et al.*, 2025a)**. It is ecologically closely associated with blackwater peat swamp ecosystems, which are characterized by high acidity, dense vegetation, and low dissolved oxygen levels **(Hui *et al.*, 2005)**. As a specialist of this environment, *B. burdigala* exhibits distinctive morphological and physiological adaptations, including the presence of a labyrinth organ—an additional respiratory structure that enables direct oxygen uptake from atmospheric air. This adaptation is vital for its survival in oxygen-poor waters **(Tate *et al.*, 2017; Nur *et al.*, 2022)**.

Currently, the natural habitat of *B. burdigala* is undergoing severe degradation due to land conversion for oil palm plantations, tin mining, pollution, and unsustainable harvesting. These threats have led to its classification as Critically Endangered (CR) on the IUCN Red List, indicating a high risk of extinction in the wild **(Syarif *et al.*, 2025a)**.

In response to these threats, *ex-situ* conservation breeding programs have been established to rear *B. burdigala* in captivity under controlled conditions. These efforts help preserve the genetic diversity of endangered species and establish population reserves that can support future reintroduction into restored habitats **(Andrews *et al.*, 1994; Priyadi *et al.*, 2024)**. Additionally, captive breeding programs have the potential to raise public awareness and serve as educational platforms, thereby fostering long-term support for conservation initiatives **(Buckley *et al.*, 2024; Budi *et al.*, 2024)**.

This study aimed to develop a spawning protocol for *B. burdigala* under controlled laboratory conditions, and evaluate the reproductive success, larval growth rates, and the genetic stability of the captive-bred population. The findings are intended to provide a scientific foundation for the development of sustainable *ex-situ* conservation strategies. Future research should focus on assessing population genetic diversity **(Insani *et al.*, 2022; Valen *et al.*, 2024)**, evaluating reintroduction potential, restoring peat swamp habitats **(Terzano *et al.*, 2023)** In addition, further research should integrate dynamic population models with locally grounded, community-based conservation approaches **(Hamilton *et al.*, 2023; Hasan *et al.*, 2023)**.

MATERIALS AND METHODS

1. Broodstock collection site

The *Betta burdigala* broodstock used in this study was obtained from a natural habitat within the peat swamp ecosystem of Bikang Village, South Bangka Regency, Bangka Belitung Islands Province, Indonesia (2°54'20.9" N; 106°27'50.4" E) (Fig. 1).

Fig. 1. Broodstock collection site of *B. burdigala* (Red Square)

2. Habitat description

The habitat at the collection site is characterized by shallow blackwater with typical peat swamp characteristics, including low pH, high organic matter content, and complex microhabitat structures composed of leaf litter, tree roots, and submerged twigs, which provide natural shelter and spawning sites (Fig. 2).

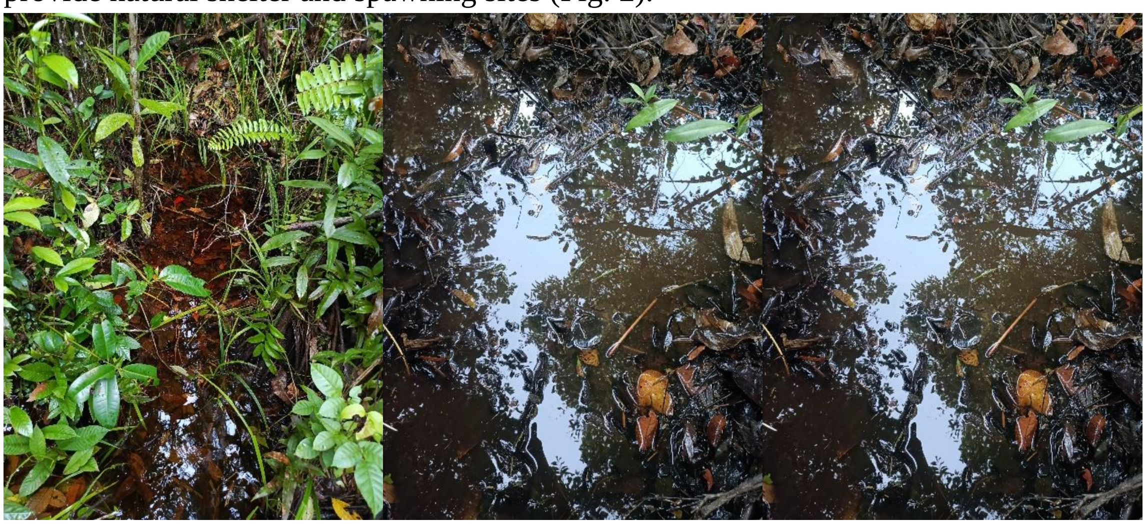
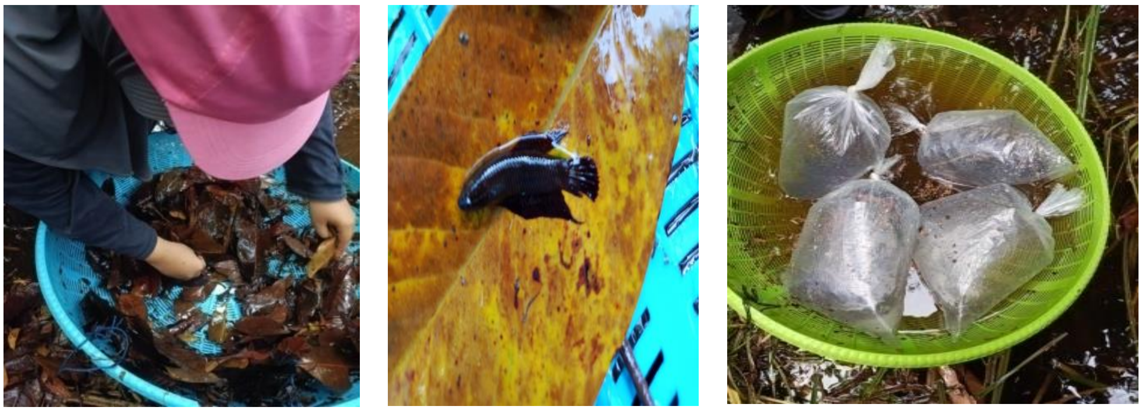


Fig. 2. Habitat *B. burdigala*

3. Broodstock collection

A total of 30 adult *B. burdigala* were collected from the site, comprising 15 males and 15 females. Collection was performed manually using traditional fishing gear (serving hood), with a minimum disturbance to preserve the physiological condition of the fish. Individuals were selected in the field based on gonadal maturity and secondary morphological characteristics. Mature males were identified by intensified body coloration, and extended fin development, whereas mature females exhibited prominent genital spots and a rounded abdomen. Following selection, each broodstock pair was individually packed in plastic bags containing habitat water enriched with leaf litter as a tranquilizing agent, maintaining a water-to-air volume ratio of 1:3. The bags were then placed in styrofoam boxes to ensure temperature stability during transport. Transportation to the Aquaculture Laboratory at the University of Bangka Belitung took approximately three hours by land vehicle. All individuals survived the journey and were subsequently acclimatized and maintained in an aquarium system under strictly controlled physicochemical water conditions.



a. Manual collection **b. Male specimen** **a. Packing for transport**

Fig. 3. Specimen collection

4. Fish acclimatization and rearing conditions

Wild-caught *Betta burdigala* individuals underwent an acclimatization process to adjust to the controlled laboratory environment. Initial adaptation consisted of floating the plastic bags with fish on the aquarium surface for 10–15 minutes to gradually equalize temperature. Once temperature equilibrium was achieved, the fish were transferred to separate rearing tanks according to sex.

Each breeding pair was housed individually in a 14 × 14 × 20cm container furnished with substrates such as wood roots, leaf litter and small pots to provide shelter. The rearing medium was darkened by the addition of ketapang (*Terminalia catappa*) leaves to replicate peat swamp water conditions and coarse salt, and methylene blue solution was added to reduce the risk of pathogen infection during the acclimation period. The resulting water parameters were 28- 28.5°C for temperature, pH of 4-5, and 3-4 for dissolved oxygen. Water depth was maintained at 5-7cm to facilitate atmospheric respiration via the labyrinth organ.

The first feeding occurred on the second day post-capture. During the initial week of acclimatization, fish were fed 2–4 mosquito larvae per individual per day to support physiological adaptation to the new nutrient source. After one week, the diet was expanded to include both mosquito larvae and *Artemia* nauplii, which were provided ad libitum.

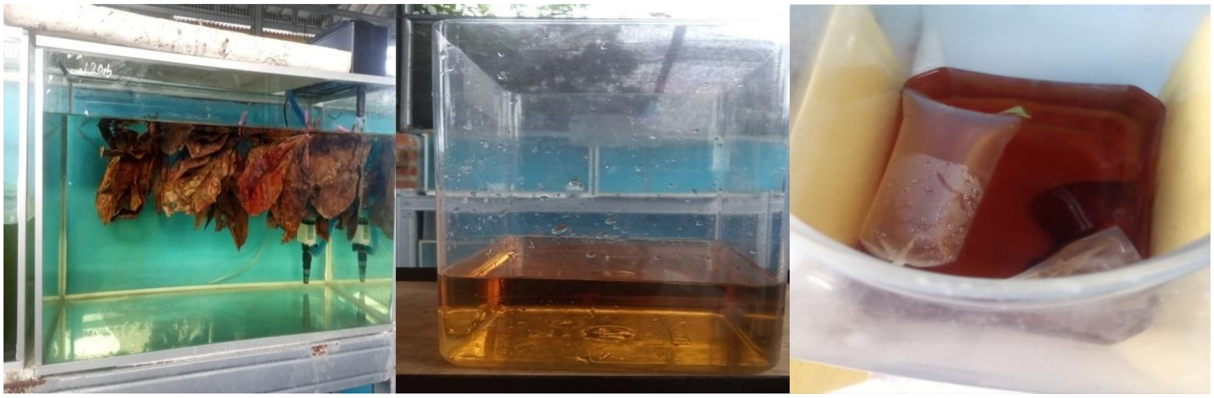


Fig. 4. Fish acclimatization

5. Broodstock selection and spawning

Spawning of the endemic *Betta burdigala* was conducted using a 1:1 male-to-female ratio. The process took place in individual containers measuring 14 × 14 × 20cm. Sex identification was based on morphological characteristics, with males exhibiting brighter body coloration and longer anal, dorsal, and caudal fins compared to females. For this study, two breeding pairs were selected from the wild-caught broodstock. Body length measurements were conducted to assess initial morphometric characteristics related to physiological readiness and broodstock selection. Standard length (measured from the tip of the snout to the base of the caudal fin) was recorded using a digital caliper with a precision of 0.01cm.

Spawning was initiated by introducing a male into the container in the morning to allow environmental adaptation, followed by the addition of the female in the afternoon. The spawning period lasted 2 to 4 days. During this time, human activity near the containers was minimized to reduce visual disturbance and stress to the fish.

The addition of the female stimulates reproduction behavior in the male, who then initiates the breeding process by constructing a bubble nest. Bubble nest formation serves as an indicator of spawning readiness and functions as a storage site for fertilized eggs. The presence of a bubble nest was used as a key initial parameter to assess the likelihood of successful spawning, as this structure is critical for egg incubation and male parental care. When the female is ready to spawn, the male embraces her body to stimulate egg release. He then collects the eggs and deposits them into the bubble nest. Spawning is considered complete when the female moves away from the male and the nest. At this stage, the female should be promptly removed from the spawning container to prevent egg predation or interference with the male's parental care.

6. Hatching rate

Hatchability refers to the proportion of eggs that successfully hatch, commonly expressed as the hatching rate. Egg hatchability is influenced by internal factors such as the quality of eggs and sperm. The hatching rate in this study was calculated using the formula as follows:

$$\text{HR (\%)} = \frac{Number\ of\ larvae\ (tail)}{number\ of\ eegs} \times 100$$

7. Larval rearing and first feeding

The number of live larvae was defined as the count of individuals that successfully hatched from spawned eggs. Manual counting was conducted when the larvae were 1–2 weeks old, a stage at which the fry had developed sufficiently for visual observation. To facilitate counting, larvae were transferred from the spawning container to a clean, pre-prepared water container. This observation aimed to assess the effectiveness of different live food types on reproductive success, particularly the survival rate. Three natural food types were used during broodstock rearing – mosquito larvae, *Daphnia magna*, and *Moina* sp. Survival rate (SR) was calculated based on the following formula:

$$\text{SR (\%)} = \frac{fish\ number\ day{-}t\ (fish)}{fish\ number\ day{-}0\ (fish)} \times 100\%$$

8. Data analysis and presentation

The effects of the treatments on the number of live larvae and the percentage of spawning success were analyzed using analysis of variance (ANOVA). When significant differences were detected, the analysis was followed by a Least Significant Difference (LSD) test at a 5% significance level (α = 0.05), following the method of **Steel and Torrie (1991)**. Supporting data on water quality parameters were analyzed descriptively to characterize the environmental conditions during the study. All data were processed using Microsoft Excel 2010 and are presented in the form of tables, graphs, and figures to facilitate interpretation of the results.

RESULTS

1. Breeding pair selection

Betta burdigala exhibits clear sexual dimorphism in both morphology and behavior. Phenotypically, males display a darker, more vivid red body coloration with striking patterns, making them visually distinct from females (Fig. 5). Their ventral, anal, and caudal fins are relatively longer and larger, serving as prominent visual cues during reproductive and territorial behaviors. Males also engage in pronounced flaring behavior, characterized by the maximal extension of fins and body inflation, which functions as a display of visual aggression toward both males and females.

In contrast, females exhibit paler, more uniform body coloration, lacking the prominent patterns observed in males. Their ventral, anal, and caudal fins are shorter and more rounded. While females may also display flaring behavior during interactions with males, its intensity is generally lower compared to that of males.

a. Male **b. Female**

Fig. 5. The male and female broodstock

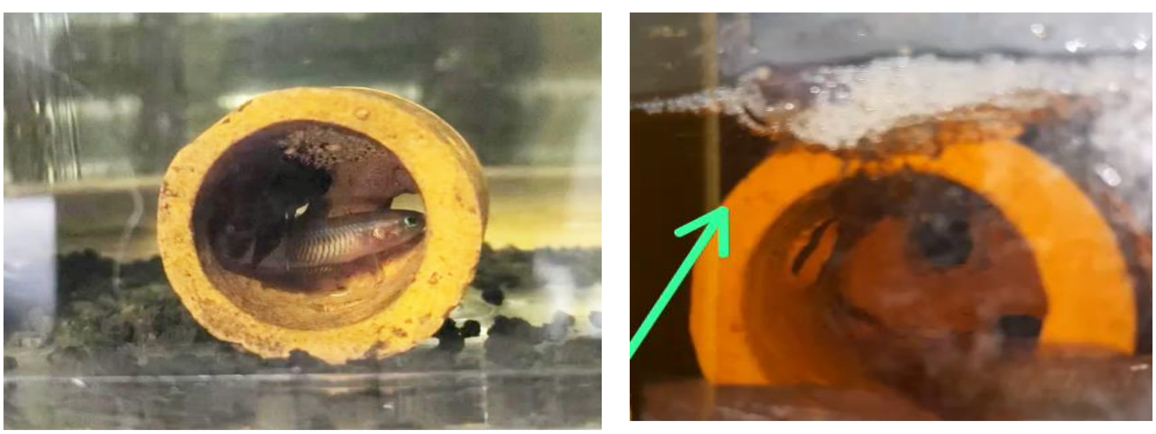
The average body length of the female broodstock was 2.45 ±0.07cm, with a minimum of 2.4cm and a maximum of 2.5cm. The relatively low variation in body size indicates a high degree of homogeneity among the individuals selected and falls within the typical size range for *B. burdigala*, one of the smallest species in the *B. coccina* complex. Body length measurement results are presented in Table (1).

Table 1. *B. burdigala* length measurements

Specimen	Total length (cm)
Female 1	2.4
Female 2	2.5
Mean ±SD	2.45 ±0.07

2. Spawning

The males from both breeding pairs successfully created bubble nests (Fig. 6). Nest formation by males occurred on days 2 to 3 after the female was introduced into the spawning container.



a. **b.**

Fig. 6. Bubble nests formed during spawning trials: (a) Pair 1; (b) Pair 2. Both nests were observed between days 2 and 3 after male–female introduction

3. Hatching

Observations on the hatching success of *B. burdigala* eggs revealed consistently high rates across both replicates. Spawning under controlled conditions resulted in an average hatching rate of 96% per breeding pair. Detailed results are presented in Table (2).

Table 2. Hatching rate results of *B. burdigala*

Specimen	Total length (cm)	Number off eggs	Number of hatched larvae	Hatching rate (%)
Female 1	2.4	58	57	98
Female 2	2.5	65	63	96
Mean ±SD	2.45 ±0.07	61.5 ±4.95	60.0 ±4.24	97.0 ±1.41

These results demonstrate that the applied spawning system is highly effective in promoting fertilization and egg hatching. The highest hatching rate, 98.00%, was recorded in the first replicate (Fig. 7). This success likely reflects the high quality of the broodstock, the functionality of the bubble nest, and the stability of environmental conditions during incubation. Additionally, the elevated hatching rate suggests successful adaptation of the broodstock to the controlled rearing environment.

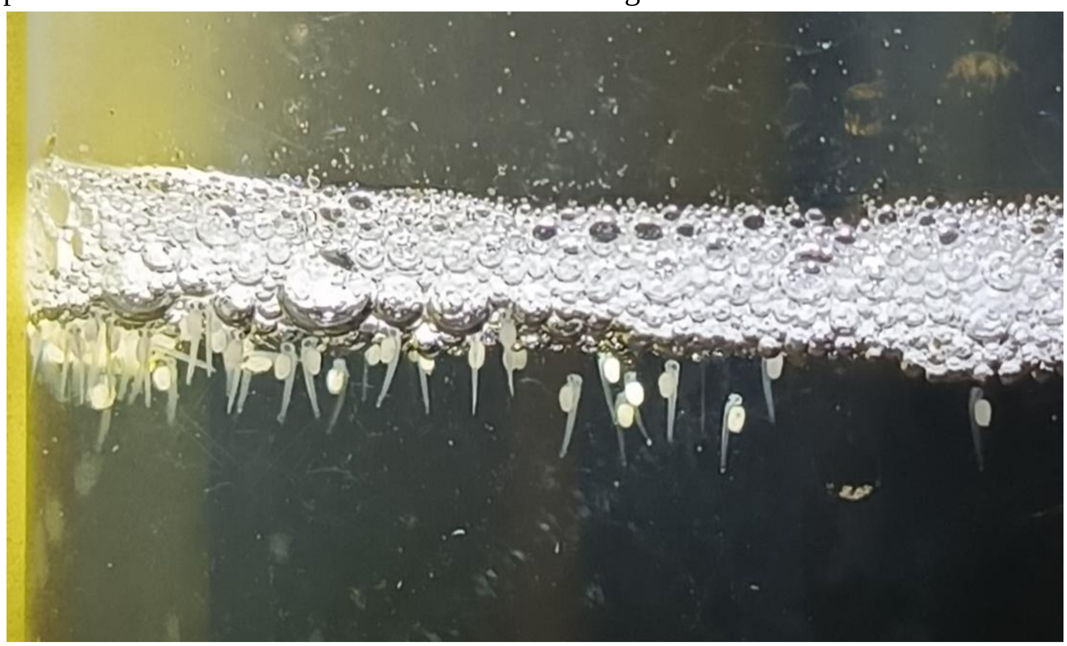


Fig. 7. Early larval stage 1 dph (days post-hatching), size 3.5mm

4. Larval rearing

A total of 57 and 63 larvae were reared in two glass aquaria measuring 14 × 14 × 20cm. Prior to the first feeding, larvae were examined under a microscope to assess their health and readiness for external feeding (Fig. 3). The larvae were reared until 50 days of age (Fig. 4). The primary parameter observed was survival rate (SR), as detailed in Table (3).

Table 3. Survival rate of *B. burdigala*.

Specimen	Total length (cm)	Number of hatched larvae	Number of specimens at 50 days	Survival rate at 50 days (%)
Female 1	2.4	57	33	58
Female 2	2.5	63	28	44
Mean ±SD	2.45 ±0.07	60.0 ±4.24	30.5 ±3.54	50.5 ±9.19

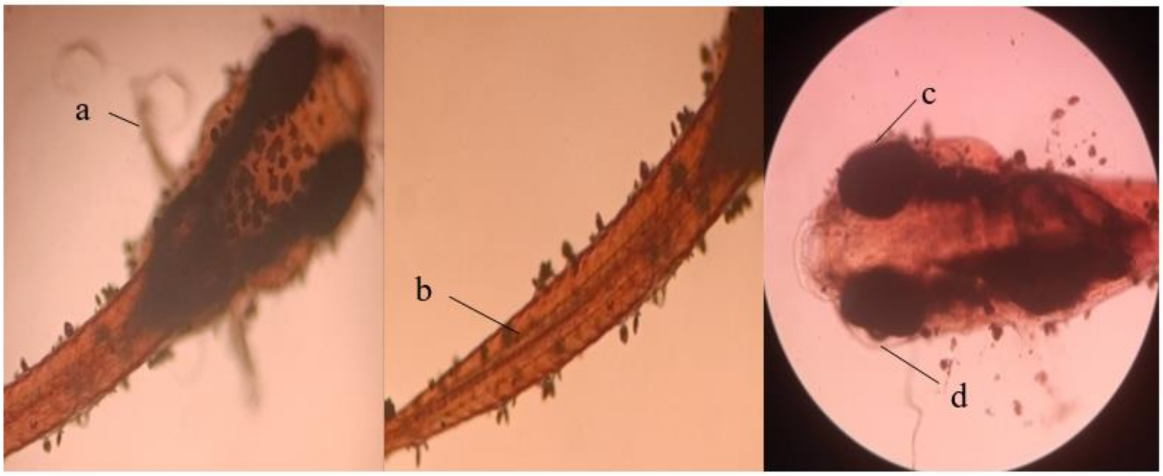


Fig. 8. *B. burdigala* larvae (a) Pectoral fin; (b) Body skeleton; (c) Eye pigment; (d) Optic vesicle

Fig. 9. Grow-out phase of *B. burdigala* larvae in laboratory

Length development of F1 specimens was measured at 20, 30, 40, and 50 days post-hatching (Table 4).

Table 4. F1 specimen length measurements

Age	20 Days (cm)	30 Days (cm)	40 Days (cm)	50 Days (cm)
Size	0.9	1.28	1.58	1.83

DISCUSSION

Betta burdigala has previously been bred by hobbyists, but this study presents the first scientifically documented captive breeding under controlled laboratory conditions. Our two experimental replicates, supplemented by observational trials, provide key biological and environmental data essential for developing standardized ex-situ conservation protocols for this critically endangered species **(Mestanza-Ramón *et al.*, 2020; Cabrita *et al.*, 2022)**.

In comparing reproductive output, the egg numbers and larval survival rates of *B. burdigala* align closely with those reported for other similarly sized wild *Betta* species that also build bubble nests. For example, *Betta channoides*, an endangered species of comparable size, produced an average of 30.67 ± 9.23 larvae per spawning under laboratory conditions **(Permana *et al.*, 2023)**, closely matching our observed larval yields of 57 and 63 larvae (up to 4 days old) for *B. burdigala*. Similarly, *Betta rubra*—which is notably larger (approximately twice the length of *B. burdigala*)—produced an average of 73.67 ± 7.09 eggs but showed lower hatching rates (46.67 ± 5.77%) and larval counts (~34.33 ± 5.13) in captivity **(Priyadi *et al.*, 2024)**. The smaller clutch size and higher hatching success of *B. burdigala* reflect species-specific reproductive strategies likely linked to body size and ecological niche specialization. These comparisons highlight that *B. burdigala*'s reproductive parameters are consistent with expectations for a small-bodied *Betta* species with bubble nesting behavior, supporting the validity of our breeding protocol.

The conservation status of *B. burdigala* as Critically Endangered is driven by rapid habitat loss due to oil palm plantations and open-pit tin mining **(Valen *et al.*, 2023b; Syarif *et al.*, 2025a)**. With the destruction of its type locality and the potential loss of remaining habitats within a decade, the species faces imminent risk of extinction. This underscores the importance of combining *in-situ* conservation with robust *ex-situ* efforts **(Budi *et al.*, 2025)**.

To ensure the long-term viability and survival of *B. burdigala*, an integrated conservation approach is essential. In addition to in-situ strategies, *ex-situ* conservation plays a critical role, particularly considering the rapid loss of the species' natural habitats **(Kumar *et al.*, 2024; Priyadi *et al.*, 2025)**. A captive breeding program conducted at the laboratory of Universitas Bangka Belitung has demonstrated significant success in maintaining *B. burdigala* populations outside their native environment. Beyond achieving larval production, the program has recorded a survival rate of up to 57% at 50 days post-hatching, indicating strong potential to support long-term conservation efforts.

Our captive breeding program at Universitas Bangka Belitung has demonstrated promising results, with a larval survival rate of up to 57% at 50 days post-hatching. This indicates the potential of *ex-situ* breeding to sustain populations beyond their natural habitats **(Putnam *et al.*, 2023; Mayer & Pšenička, 2024)**. Importantly, approximately half of the captive-bred individuals have been reintroduced into their native habitats in a staged manner, with ongoing monitoring to evaluate survival, adaptation, and ecological impact. These efforts aim to mitigate extinction risk by reinforcing wild populations and maintaining ecosystem functions **(Jachowski *et al.*, 2016; Esquivel-Muelbert *et al.*, 2018)**.

During the breeding trials of *B. burdigala*, the observed water quality parameters included a temperature range of 28– 28.5°C, pH levels between 4 and 5, and dissolved oxygen (DO) concentrations of 3– 4mg/ L. These conditions are considered suitable for the reproductive performance of *Betta* species, with the temperature range supporting optimal gonadal maturation, bubble nest formation, and successful embryonic development **(Priyadi *et al.*, 2025)**. The acidic pH closely reflects the natural conditions of *B. burdigala*'s native peat swamp ecosystems, indicating that the experimental environment effectively mimicked the species' ecological requirements **(Hui & Ng 2005; Valen *et al.*, 2023b; Syarif *et al.*, 2025a)**. Despite the relatively low DO levels, *B. burdigala*, as a labyrinth fish, is physiologically adapted to hypoxic environments through the utilization of atmospheric oxygen **(Alton *et al.*, 2013)**. Consequently, the water quality parameters maintained throughout the study likely played a critical role in facilitating successful spawning and enhancing early larval survival under *ex-situ* conditions.

Looking ahead, integrating ecological, genetic, and socio-economic research is critical for comprehensive conservation planning **(Hasan *et al.*, 2023; Valen *et al.*, 2024; Syarif *et al.*, 2025b)**. Given the complex ecological threats faced by *Betta burdigala*, future studies should combine ecological **(Ndobe *et al.*, 2022; Nurjirana *et al.*, 2022; Hasan *et al.*, 2023)**, genetic **(Syarif *et al.*, 2023; Robin *et al.*, 2023; Valen *et al.*, 2024)**, and socio-economic perspectives. Molecular tools such as mitochondrial DNA and microsatellite analyses are crucial for monitoring genetic diversity and preventing inbreeding depression **(Hasan *et al.*, 2021; Valen *et al.*, 2022; Hafidah *et al.*, 2024; Romdon *et al.*, 2024)**.

Moreover, emerging technologies like environmental DNA (eDNA) sampling **(Nakamichi *et al.*, 2023)**, automated monitoring systems, and GIS-based habitat modeling offer powerful means to enhance population and habitat assessments **(Hasan & Izzul 2020; Serdiati *et al.*, 2023)**. This multi-disciplinary approach will strengthen evidence-based conservation strategies tailored to the unique challenges facing *B. burdigala* and other endemic *Betta* species.

CONCLUSION

This study concludes that *Betta burdigala* exhibits strong potential for captive breeding. Females with an average length of 2.45cm produced approximately 61 eggs, with a mean hatching rate of 97.0 ± 1.41%. The survival rate at 50 days post-hatching reached 50.5 ± 9.19%. During the first four days after hatching, larvae relied on yolk reserves, after which they were fed natural live food (*Moina* sp.). Future research should investigate the effects of varying initial feeding regimes on larval growth and survival, as well as optimize water quality and spawning temperature conditions. Additionally, population genetic analyses are essential to support conservation efforts. Long-term studies on reproductive behavior and ecological adaptation in natural habitats are also necessary to ensure the species' sustainable management and conservation.

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