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Dietary Enhancement of Reproductive Performance in the Red Wine Fighting Fish *Betta burdigala* for Conservation Breeding Programs

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ABSTRACT

Betta burdigala, an endemic species of Bangka Island, is currently classified as Critically Endangered (CR) based on the IUCN Red List of Threatened Species due to the degradation of its native peat swamp habitats. Effective *ex-situ* conservation through controlled breeding is crucial to prevent extinction. This study evaluated the effects of dietary supplementation with vitamin E, vitamin C, and commercial ovulation stimulant on the reproductive performance of *B. burdigala*. Three breeding pairs, collected from Pergam and Bikang villages, were reared under controlled aquarium conditions. The broodstock were fed natural live feed *Daphnia magna* enriched with the supplements. Reproductive success was assessed based on egg hatching rate and larval survival. The ovulation stimulant group yielded the highest hatching rate (96.6%) and larval survival (84 larvae), followed by vitamin C (94.4%; 68 larvae) and vitamin E (91.7%; 55 larvae). Water quality parameters remained within optimal ranges: temperature at 28–31.9°C, pH 4.6–5.1, TDS of 8–10 ppm, and EC of 10–16 µS/cm, resembling natural peat swamp conditions. These results highlight the potential of dietary supplementation, particularly ovulation stimulants, to enhance reproductive output in *B. burdigala*, thereby supporting *ex-situ* conservation and reintroduction initiatives.

INTRODUCTION

Indonesia is recognized as one of the world's centers of freshwater fish biodiversity, hosting more than 1,200 native species distributed across its vast archipelagic river systems, lakes, and peat swamp ecosystems **(Hasan *et al.*, 2020; Valen *et al.*, 2022; Jatayu *et al.*, 2023)**. Many of these taxa are endemic, occurring only in limited regions such as Sumatra, Kalimantan, and Sulawesi, reflecting the country's complex biogeographic history and ecological diversity **(Valen *et al.*, 2022; Syarif *et al.*, 2023; Hasan *et al.*, 2024a; Pramono *et al.*, 2025)**. Endemic species such as *Betta burdigala* **(Valen *et al.*, 2023a)**, *Betta uberis* **(Syarif *et al.*, 2025a)**, and *Parosphromenus* *deissneri* **(Valen *et al.*, 2025)**, exemplify the unique evolutionary adaptations of Indonesia's freshwater fauna to specialized habitats like blackwater peat swamps **(Valen *et al.*, 2024; South *et al.*, 2025)**.

B. burdigala is an endemic fish species of Bangka Island, taxonomically classified under Kingdom Animalia, Phylum Chordata, Class Actinopterygii, Order Perciformes, Family Osphronemidae, and Genus *Betta* **(Kottelat, 2013)**. First described by **Kottelat and Ng (1994)**, this species belongs to the coccina group **(Valen *et al.*, 2023b; Syarif *et al.*, 2025b)**. Inhabiting blackwater peat swamp ecosystems rich in humic acids from decomposing organic matter, the presence of *B. burdigala* reflects the biodiversity of Bangka's aquatic habitats **(Said *et al.*, 2019)**. However, its survival is increasingly threatened by anthropogenic pressures, including logging, peat swamp forest conversion for industrial plantations, and tin mining activities that have led to severe habitat degradation **(Giam *et al.*, 2012; Hasan *et al.*, 2024b, c)**. As a result, the wild population of *B. burdigala* has declined sharply, leading to its classification as Critically Endangered (CR) by the IUCN b.

Currently, conservation efforts are necessary to prevent the extinction of this endemic species **(South *et al.*, 2025)**. One relevant strategy is *ex-situ* conservation through controlled breeding programs **(Priyadi *et al.*, 2021; Syarif *et al.*, 2025c)**. Nevertheless, there is no issue in maintaining *B. burdigala* outside its natural habitat. The reproductive success of fish is influenced by internal factors such as gonadal maturity, fecundity, broodstock age, and genetic factors. In addition, external factors such as environment and feed quality also play a role **(Arif *et al.*, 2022)**.

Nutritional interventions are considered essential in supporting gonadal maturation, enhancing gamete viability, and improving offspring quality through the provision of diets enriched with vitamin E and vitamin C **(Liu *et al.*, 2024)**. Vitamin E plays an important role in fish reproduction, and its requirement varies among species **(Palumbo *et al.*, 2025)**. A deficiency in vitamin E may lead to immature gonads, reduced egg hatching rates, and low fry survival **(Skibniewska *et al.*, 2024)**. In addition, the use of egg stimulants can accelerate and facilitate the spawning process. Egg stimulants have been proven to increase egg production in fish **(Ramadhan *et al.*, 2021)**. Thus, feed formulations based on vitamin E, vitamin C, and stimulants are expected to enhance the reproductive success of *B. burdigala* in conservation breeding programs.

This study focuses on enhancing gonadal maturation and reproduction of *B. burdigala* through dietary supplementation with vitamin E, vitamin C, and commercial ovulation stimulant. The results can contribute to the preservation of genetic diversity, the establishment of reserve populations, and the support of reintroduction into restored peat swamp habitats. They may also improve reproductive performance in *ex-situ* populations. Consequently, the sustainability of this endemic species can be ensured, and the scientific basis for long-term conservation planning can be strengthened **(Budi *et al.*, 2024)**.

MATERIALS AND METHODS

1. Broodstock collection site

The *B. burdigala* broodstock used as the research subjects were obtained from their natural habitat in the peat swamp ecosystems of Pergam Village and Bikang Village, South Bangka Regency, Bangka Belitung Islands Province, Indonesia.

2. Habitat description

The habitat at the sampling sites is characterized by shallow blackwater, which is a typical feature of peat swamp ecosystems. The aquatic conditions are marked by relatively low pH, abundant organic matter, and the presence of diverse microhabitat structures such as leaf litter layers, tree root networks, and submerged branches. This combination of natural elements not only provides optimal shelter for *B. burdigala* but also offers potential spawning areas, while reflecting the complexity of peat swamp ecosystems that support the survival of this endemic fish species.

Fig. 1. Natural habitat of *B. burdigala*

3. Broodstock treatment

This study employed three treatment types consisting of the addition of vitamin E, vitamin C, and commercial ovulation stimulant during the spawning process of *B. burdigala*. Each treatment involved one pair of broodstock comprising one male and one female, resulting in a total of three pairs with three males and three females. The selection of this broodstock number was based on considerations of treatment effectiveness, where each pair of fish was individually subjected to a specific dietary supplementation. This design allowed focused observation of the physiological and reproductive responses of each broodstock pair to different nutritional stimulations, while minimizing potential external variables that could affect spawning outcomes. Thus, this treatment arrangement is expected to provide a clearer understanding of the relative effectiveness of vitamin E, vitamin C, and commercial ovulation stimulant in supporting the reproductive success of *B. burdigala*.

Fig. 2. Collection of broodstock from catches

4. Fish acclimatization and rearing conditions

Wild caught *B. burdigala* individuals were acclimatized to the laboratory environment through an adaptation process. Plastic bags containing the fish were first floated on the aquarium surface for 10–15 minutes to equalize the temperature before transferring the fish into separate containers according to sex. Each broodstock pair was placed in a 14 × 14 × 20 cm container equipped with leaf litter substrate and a small pot as shelter. The rearing medium was supplemented with Indian almond leaves (*Terminalia catappa*) to mimic peat swamp water conditions, and methylene blue solution was added to reduce the risk of infection. During maintenance, water quality was maintained at 26°C, pH 5–6, dissolved oxygen at 4mg/ L, and a water depth of 7– 8cm to support respiration through the labyrinth organ. Feeding began on the second day with 6–7 mosquito larvae per fish per day. After one week, fish were additionally provided with cultured *Daphnia* sp. enriched with vitamin E, vitamin C, and commercial ovulation stimulant, which were supplied *ad libitum* as part of the nutritional treatment strategy.

Fig. 3. Fish acclimatization

5. Broodstock selection and spawning

The initial stage of *B. burdigala* spawning began with the selection of healthy broodstock that were gonadally mature and morphologically suitable for reproduction. A 1:1 male-to-female ratio was applied to maintain reproductive efficiency and to maximize spawning success. The spawning containers used measured 14 × 14 × 20 cm, which were considered ideal to provide sufficient movement space for both broodstock while also facilitating observation during the process.

Sex identification was conducted both in the field and in the laboratory. Males were characterized by brighter and more contrasting body coloration, along with relatively longer anal, dorsal, and caudal fins. In contrast, females exhibited duller coloration, a rounded abdomen, and a distinct genital papilla. From the wild-caught population, two pairs of broodstock were selected that best met the physiological criteria. To ensure reproductive readiness, morphometric measurements were taken by assessing standard length (from snout tip to caudal fin base). Measurements were performed using a digital calliper with 0.01cm precision to obtain accurate data that could be used as a reference for broodstock quality determination.

The spawning process began by introducing the male into the spawning container in the morning, allowing the male fish to acclimate to the environment and initiate territorial behavior. After several hours, in the afternoon, a female was introduced into the same container. The spawning period lasted for 2 to 4 days, during which human activity around the containers was minimized. This precaution was essential as *B. burdigala* is known to be highly sensitive to visual disturbances, making environmental tranquillity a critical factor for successful reproduction.

The presence of the female typically stimulated the male to exhibit reproductive behavior, notably the construction of a bubble nest. The nest served as a key indicator of spawning readiness and functioned as the site for depositing fertilized eggs. It provided mechanical protection against physical damage and supported incubation by maintaining oxygen stability around the eggs. The bubble nest also signalled the onset of male parental responsibility, as males played the dominant role in guarding and caring for the eggs until hatching.

When the female was ready, the male performed a distinctive courtship behavior by wrapping his body around the female's abdomen, stimulating egg release. After the eggs were expelled, the male immediately collected them with his mouth and placed them into the bubble nest. This sequence was repeated several times until the female finished releasing all the eggs. Spawning was considered complete when the female distanced herself from the nest and ceased active interaction with the male.

Following spawning, the female was promptly removed from the spawning container to prevent the risk of egg predation or disruption of the male's nest-guarding role. In the subsequent phase, all responsibility for guarding the nest and eggs was taken over by the male, who instinctively continued to add bubbles to the nest and ensured that the eggs remained in a safe position until hatching.

6. Hatching rate

The hatching rate represents the percentage of eggs that successfully hatch into viable larvae out of the total number of eggs produced. This parameter is commonly expressed as the hatching rate and serves as a key indicator in evaluating the success of the spawning process. Hatching success is influenced not only by environmental conditions but also by internal factors, particularly the quality of the eggs and sperm involved in fertilization.

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

7. Larval rearing and first feeding

The number of surviving larvae is defined as the total individuals that successfully hatched from the fertilized eggs. The counting process was carried out manually when the larvae were approximately 1–2 weeks old, at which the fry stage was sufficiently developed to be visually identified and directly observed. To facilitate counting, the larvae were first transferred from the spawning container to a new container filled with clean water that had been prepared beforehand to ensure more controlled conditions.

This observation aimed to evaluate the effect of natural feed, particularly *Daphnia magna* enriched with vitamins, on broodstock reproductive performance and larval survival rate. During broodstock rearing, only *Daphnia magna* was provided as the natural feed, which had been previously cultured with the addition of vitamin E, vitamin C, and commercial ovulation stimulant. This enrichment was intended to enhance the nutritional quality of the feed, thereby optimizing gonadal maturation, spawning success, and larval survival.

The survival rate (SR) was determined using the following formula:

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

8. Water quality monitoring

Water quality was monitored daily in the morning and afternoon by measuring temperature, pH, and dissolved oxygen (DO) using a digital device **(Islamy *et al*., 2024a)**. In addition, physical conditions such as water clarity and color were observed to detect any changes that could affect fish health. Partial water replacement was carried out in case of turbidity, and ketapang *(Terminalia catappa)* leaves were periodically added to maintain pH stability and suppress pathogen growth. This monitoring ensured that the environment remained optimal for the spawning process and larval survival.

9. Data analysis and presentation

This study employed treatments in the form of natural feed (*Daphnia magna*) enriched with vitamin E, vitamin C, and commercial ovulation stimulant to enhance nutritional quality in supporting the spawning process. The broodstock of *B. burdigala* used consisted of three females and three males, resulting in uniform spawning pairs.

The primary data analyzed included the number of viable larvae hatched and the spawning success rate for each treatment. In addition, supporting data on water quality parameters (temperature, pH, and dissolved oxygen) were analyzed descriptively to provide an overview of the rearing environment during the study. The results were presented in the form of tables, graphs, and figures to facilitate the interpretation of differences among treatments. All data processing was performed using Microsoft Excel 2010 software.

RESULTS

1. Selection of broodstock pair

B. burdigala exhibits clear sexual dimorphism, both morphologically and behaviourally. Males typically display a dark reddish body coloration with striking contrasting patterns, making them easily distinguishable from females. Their ventral, anal, and caudal fins are longer and wider, functioning as visual signals during reproductive activities as well as in territorial defence. In addition, males frequently perform flaring behavior, characterized by fully spreading their fins and expanding their bodies as a visual display of aggression toward other individuals.

In contrast, females possess a paler body coloration with relatively uniform patterns and no prominent markings. Their ventral, anal, and caudal fins are shorter and more rounded in shape. Females may also exhibit flaring when interacting with males, although the intensity is generally lower compared to that of males.

a. Female **b. Male**

Fig. 4. The male and female broodstock

The body length of female *B. burdigala* exhibited a high degree of uniformity, with mean values of 2.45 ± 0.07cm (range 2.4– 2.5cm) and 2.50 ± 0.05cm (range 2.45–2.55cm). These measurements remain within the normal size range for the species, which is recognized as one of the smallest members of the *B. coccina* group.

2. Spawning

During the spawning phase, the male fish from both pairs used in the study successfully constructed bubble nests (Fig. 5). Nest building activity was first observed on the second to third day after the female was introduced into the spawning container. The bubble nest not only serves as an indicator of male readiness to spawn but also functions as a storage site and protective structure for fertilized eggs. The successful formation of bubble nests indicates that the environmental conditions of the spawning container were sufficiently supportive, both in terms of water quality and fish comfort, thereby stimulating the expression of this natural behavior.

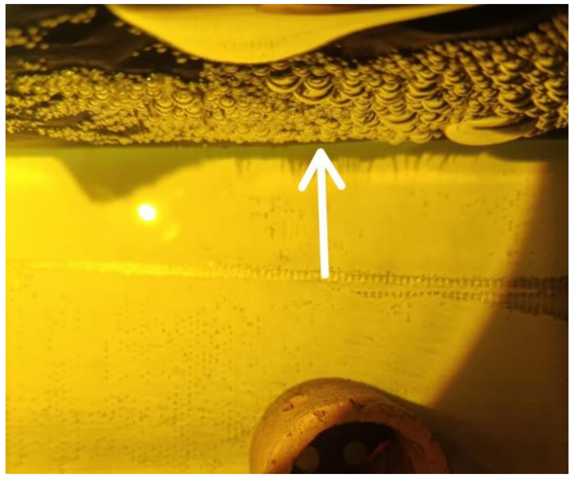

Fig. 5. Bubble nests formed during spawning trials

3. Hatching

The observations revealed variations in the hatching success of *B. burdigala* eggs across the nutritional treatments. The treatment supplemented with commercial ovulation stimulant produced the highest number of hatched larvae, totaling 84 individuals. This was followed by the vitamin C treatment with 68 individuals and the vitamin E treatment with 55 individuals. Differences in the number of hatched larvae were consistent with the variations in the total number of eggs produced in each treatment, as presented in Table (1).

Table 1. Hatching rate results of *B. burdigala*

Treatment	Number of Eggs (grains)	Number of Hatched Larvae (individuals)	Hatching Rate (%)
Vitamin E	60	55	91.7
Vitamin C	72	68	94.4
Commercial ovulation stimulant	87	84	96.6
Average	73 ± 13.1	69 ± 14.6	94.2 ± 2.5

The results of this study demonstrated that dietary supplementation had a significant effect on the hatching success of *B. burdigala* eggs. The treatment with commercial ovulation stimulant produced the highest outcome, with a hatching rate of 96.6% and the greatest number of hatched larvae (84 individuals). This finding suggests that the commercial ovulation stimulant effectively stimulates gonadal maturation and enhances gamete quality, thereby optimizing the fertilization process. The treatment supplemented with vitamin C also yielded relatively high results, with a hatching rate of 94.4% and 68 hatched larvae. Vitamin C plays a crucial role as an antioxidant that protects eggs from oxidative damage, thereby improving the viability of fertilized eggs.

In contrast, the vitamin E treatment produced the lowest outcome, with 55 hatched larvae and a hatching rate of 91.7%. Although still relatively high, this value was lower compared to the other treatments. While vitamin E is known to act as an antioxidant and contribute to gonadal maturation, its effectiveness in this study appeared to be lower than that of vitamin C and commercial ovulation stimulant. Overall, these findings confirm that commercial ovulation stimulant is more effective in supporting the reproductive success of *B. burdigala* compared to vitamin C and vitamin E. The observed differences among treatments were likely influenced by the distinct physiological functions of each supplement, the quality of broodstock, and the interaction with stable environmental conditions during rearing.

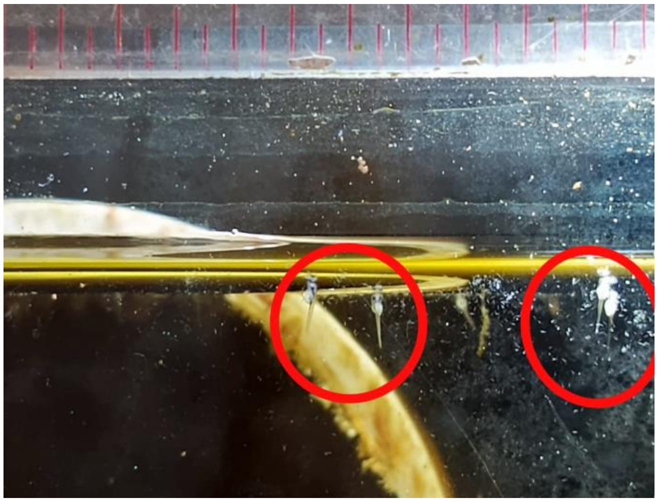

Fig. 6. Early larval stage 1

4. Larval rearing

Larval rearing was carried out in three glass aquaria, each corresponding to the respective treatment. The larvae from each treatment were fed with live feed enriched with vitamins and stimulants. Observations revealed differences in survival rates among the treatments. In the vitamin E treatment, the number of larvae that survived until the end of the rearing period was 55 individuals. The vitamin C treatment produced better results with 68 surviving larvae. Meanwhile, the treatment supplemented with commercial ovulation stimulant yielded the most optimal outcome, with 84 surviving larvae. These findings emphasize that the type of dietary enrichment provided had a significant effect on the larval survival rate (SR) during the rearing period. Thus, it can be concluded that commercial ovulation stimulant supplementation was more effective in supporting larval viability of *B. burdigala* compared to vitamin C and vitamin E.

DISCUSSION

This study represents the first scientific record of the spawning of *B. burdigala* under controlled laboratory conditions with the addition of dietary supplementation treatments in the form of Vitamin E, Vitamin C, and ccommercial ovulation stimulant. The results showed that all three treatments enhanced gonadal maturation and reproductive success, as indicated by the number of larvae hatched in each treatment: 55 larvae with Vitamin E, 68 larvae with Vitamin C, and the highest number, 84 larvae, with commercial ovulation stimulant. The commercial ovulation stimulant contains a complete set of vitamins, oligosaccharides, folic acid, and other nutrients. Meanwhile, the study by **Syarif *et al.* (2025c)** reported an average of 60 ± 4.24 hatched larvae of *B. burdigala* with a high hatching rate of 97.0 ± 1.41% under controlled conditions without dietary supplementation. This finding indicates that although both studies achieved high hatching rates, the inclusion of nutritional additives in the diet proved to quantitatively enhance larval productivity. Therefore, the nutritional approach applied in the present study demonstrates strong potential as an effective strategy to optimize spawning success and larval production in *ex situ* conservation programs for this endemic species.

In the context of reproductive comparison, the number of larvae produced in this study is relatively higher than that of *Betta channoides*, which was reported to produce an average of 30.67 ± 9.23 larvae per spawning **(Permana *et al.*, 2023)**. Meanwhile, the larger-sized *Betta rubra* can produce up to 73.67 ± 7.09 eggs, but its hatching rate is relatively low (46.67 ± 5.77%), resulting in an average of only 34.33 ± 5.13 larvae **(Priyadi *et al.*, 2024)**. These findings indicate that *B. burdigala* has a distinctive reproductive strategy, characterized by a relatively small number of eggs but high hatching success, thereby achieving reproductive efficiency in line with the characteristics of small bubble-nesting species.

Differences in results among treatments in this study illustrate the significant influence of nutritional supplementation on reproductive quality. Vitamin E functions as an antioxidant that protects germ cells from oxidative damage, supports gonadal development, and enhances sperm and oocyte viability. Vitamin C plays an important role in collagen synthesis and strengthens the broodstock's immune system **(Islamy *et al.*, 2024b; Serdiati *et al.*, 2024)**, thus stabilizing physiological conditions during reproduction. Meanwhile, commercial ovulation stimulant produced the highest response with the greatest number of larvae (84), likely due to its bioactive compounds stimulating gonadotropin release and accelerating oocyte maturation. These results confirm that nutritional supplementation can affect fish health **(Islamy *et al.*, 2024c)** and serve as an effective strategy to improve the success of *ex-situ* breeding programs.

Currently, *B. burdigala* is categorized as Critically Endangered due to peat swamp habitat degradation and pressure from oil palm plantation expansion and open-pit tin mining **(Valen *et al.*, 2023b; Syarif *et al.*, 2025a)**. The loss of the type locality and the serious threats to the remaining habitats place this species at emminent risk of extinction. Therefore, a combination of *in-situ* and *ex-situ* conservation strategies is crucial **(Budi *et al.*, 2025)**.

Artificial spawning programs at the Laboratory of Universitas Bangka Belitung have demonstrated success in maintaining *B. burdigala* populations outside their natural habitat. In addition to producing larvae, the program recorded a survival rate of up to 57% at 50 days post-hatching, showing significant potential to support long-term conservation **(Putnam *et al.*, 2023; Mayer & Pšenička, 2024)**. Some captive-bred individuals have even been gradually released back into their natural habitat, with continuous monitoring to evaluate survival rates, adaptation, and ecological impacts. These efforts are expected to reduce extinction risk by strengthening wild populations and maintaining ecosystem functions **(Esquivel-Muelbert *et al.*, 2018)**.

During the spawning trials, the recorded water quality parameters included a temperature of 28– 28.5°C, pH 4–5, and dissolved oxygen (DO) levels of 3– 4mg/ L. These conditions are suitable for *Betta* reproduction, as the temperature supports gonadal maturation, bubble nest construction, and embryo development **(Priyadi *et al.*, 2025; Syarif *et al.*, 2025c)**. The acidic pH values resemble the natural peat swamp habitat of *B. burdigala* **(Hui & Ng, 2005; Valen *et al.*, 2023b; Syarif *et al.*, 2025a)**. Although DO levels were relatively low, *B. burdigala*, as a labyrinth fish, can adapt to hypoxic environments by directly obtaining oxygen from the air **(Alton *et al.*, 2013)**. Therefore, the maintained water quality conditions during the study played a crucial role in supporting spawning success and larval survival in *ex-situ* environments.

Moreover, integrating ecological, genetic, and socio-economic research is essential in comprehensive conservation planning **(Hasan *et al.*, 2023; Valen *et al.*, 2024a; Syarif *et al.*, 2025a)**. Molecular analyses such as mitochondrial DNA and microsatellite markers are important to monitor genetic diversity and prevent inbreeding depression **(Hasan *et al.*, 2021; Hafidah *et al.*, 2024; Romdon *et al.*, 2024; Valen *et al.*, 2024b)**. Cutting-edge technologies such as environmental DNA (eDNA) sampling **(Nakamichi *et al.*, 2023; Nazran *et al.*, 2025)**, automated monitoring systems, and GIS-based habitat modeling **(Serdiati *et al.*, 2023)** also have the potential to strengthen evidence-based conservation strategies. This multidisciplinary approach will enhance the effectiveness of protecting *B. burdigala* and other endemic *Betta* species in facing increasingly complex ecological threats.

CONCLUSION

This study demonstrates that the spawning of *Betta burdigala* in laboratory conditions can be successfully achieved through dietary supplementation with vitamin E, vitamin C, and commercial ovulation stimulant. The commercial ovulation stimulant treatment produced the best results, with a hatching rate of 96.6% and 84 larvae, followed by vitamin C (94.4%; 68 larvae) and vitamin E (91.7%; 55 larvae). Water quality conditions resembling the natural peat swamp habitat also supported reproductive success. These findings confirm that nutritional supplementation, particularly commercial ovulation stimulant, is effective in enhancing the reproduction of *B. burdigala* and contributes to *ex-situ* conservation efforts and restocking in its natural habitat.

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