



Faculty of Resource Science and Technology

**Checklist of Wild *Betta* Fish from Sarawak and Captive Breeding
Protocols**

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Checklist of Wild *Betta* Fish from Sarawak and Captive Breeding Protocols

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DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.



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ABSTRACT

The genus *Betta*, (commonly known as fighting fish) includes a diverse group of freshwater fishes with significant ecological and economic roles. However, scientific knowledge of wild *Betta* species in Sarawak remains limited, with most studies focusing on hybrid species for the ornamental trade. This study aimed to update the checklist of wild *Betta* in Sarawak and establish captive breeding protocols for selected native species. A total of 72 specimens were collected from seven locations across Sarawak. There were 7 species of wild *Betta* had been recorded namely, *B. ibanorum*, *B. brownorum*, *B. lehi*, *B. midas*, *B. taeniata*, *B. akarensis* and *B. macrostoma*. Morphometric comparisons showed that males were generally larger and heavier than females, except in *B. ibanorum*, where no significant sexual dimorphism was observed. Successful captive breeding was achieved for *B. ibanorum*, *B. macrostoma*, *B. taeniata* and *B. brownorum*, with documentation of mating behaviour, larval stages and growth performance. These findings provide a foundational reference for future conservation programs and sustainable aquaculture efforts involving Sarawak's indigenous *Betta* species.

Keywords: *Betta*, breeding protocol, larval development

Senarai Semak Spesies Ikan Betta Liar dari Sarawak dan Protokol Pembiakan dalam Kurungan

ABSTRAK

Genus Betta (dikenali sebagai ikan laga) merangkumi kumpulan ikan air tawar yang pelbagai dan mempunyai peranan penting dari segi ekologi dan ekonomi. Walau bagaimanapun, pengetahuan saintifik mengenai spesies Betta liar di Sarawak masih terhad, kerana kebanyakan kajian terdahulu lebih tertumpu kepada spesies hibrid untuk tujuan perdagangan hiasan. Kajian ini dijalankan bagi mengemas kini senarai semak spesies Betta liar di Sarawak serta membangunkan protokol pembiakan dalam kurungan bagi spesies asli terpilih. Sebanyak 72 spesimen telah dikumpul dari tujuh lokasi di seluruh Sarawak. Tujuh spesies telah dikenal pasti iaitu B. ibanorum, B. brownorum, B. lehi, B. midas, B. taeniata, B. akarensis dan B. macrostoma. Perbandingan morfometrik menunjukkan bahawa jantan secara umumnya lebih besar dan berat berbanding betina, kecuali bagi B. ibanorum yang tidak menunjukkan dimorfisme seksual yang ketara. Pembiakan dalam kurungan berjaya dilaksanakan bagi B. ibanorum, B. macrostoma, B. taeniata dan B. brownorum, dengan dokumentasi tingkah laku pembiakan, peringkat larva dan prestasi tumbesaran. Penemuan ini menyediakan asas penting bagi program pemuliharaan masa hadapan dan usaha akuakultur lestari yang melibatkan spesies Betta asli dari Sarawak.

Kata kunci: *ikan laga, protokol pembiakan, tumbesaran larva*

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LIST OF ABBREVIATIONS

°C	Degree Celsius
pH	Potential of hydrogen
mg/L	Milligram per liter
NTU	Nephelometric Turbidity Unit
DO	Dissolved Oxygen
dH	Degree of Hardness
SR	Survival Rate
cm	Centimeter
mm	Millimeter
g	Gram
LG	Length Gain
ADLG	Average Daily Length Gain

CHAPTER 1

INTRODUCTION

1.1 Study Background

The genus *Betta*, mostly known as the fighting fishes, is a small freshwater fish adapted to the swampy, stagnant-water pool and hill stream environment (Kamal et al., 2020). According to Fishbase (2025), there are a total of 83 species of *Betta* fish distributed globally. In Malaysia, 34 species were reported, of which seven species were indigenous species of Sarawak (Kamal et al., 2020). However, based on the IUCN Red List, five out of seven *Betta* species were classified as vulnerable, while the other two species were classified as data deficient (IUCN, 2020). There are two reproductive strategies for *Betta* fish, which are bubble nesting and mouthbrooding (Panijpan et al., 2020).

Fighting fish are among the most popular and sought-after freshwater ornamental fish in the world. The ornamental fish business is worth between USD 15 and USD 30 billion each year, with over a billion wild-caught or captive-bred fish from over 6,500 species sold in over 100 countries across the world (Teletchea, 2019). Despite the wide range of freshwater species available in the ornamental fish trade, certain species, such as the *B. splendens*, which originated in the Mekong River, are highly popular among aquarium hobbyists. Due to the coloration, form, economic and commercial worth of its body and fins, this species is one of the most well-known and popular ornamental fish (Sipaúba-Tavares et al., 2016). Native *Betta* species, on the other hand, were rarely traded, and the data were limited to fish hobbyists on the internet and social media.

Captive breeding refers to the breeding and nurturing of wildlife under controlled conditions, facilitated by humans (Waples, 2013). Fish and other aquatic populations have been bred in captivity for thousands of years. Many researchers have attempted captive breeding of ornamental fish. Saha et al. (2017) conducted captive breeding on an indigenous ornamental fish of West Bengal, which was the *Trigocaster lallius*. There was also captive breeding of the honey gourami done by Mitra et al. (2006). There were a few studies involving the captive breeding of wild *Betta* fish (Permana et al., 2024; Priyadi et al., 2024), but none of them were about the captive breeding of indigenous *Betta* fish from Sarawak. Even though there is information on the species description and the current number of indigenous *Betta* species in Sarawak (Kamal et al., 2020; Tan & Ng, 2005), information on captive breeding of the *Betta* species is still insufficient and anecdotal, based primarily on information exchanged between hobbyists on web pages (Puello-Cruz et al., 2010).

1.2 Problem Statement

Despite the rich diversity of *Betta* species in Sarawak, scientific knowledge on their distribution, ecology, and reproductive biology remains scarce. Most existing research has concentrated on hybrid or commercial species such as *B. splendens*, while studies on indigenous wild species are underrepresented. This gap has left limited baseline information on the occurrence of native *Betta* across Sarawak and little documentation of their reproductive strategies under controlled conditions. Without such data, efforts to conserve these vulnerable species are constrained, particularly as they face increasing threats from habitat degradation, overharvesting, and environmental changes. Moreover, standardized captive breeding protocols for Sarawak's native *Betta* have not been established, limiting

both conservation initiatives and sustainable aquaculture development. Therefore, this study addresses these gaps by compiling an updated checklist of wild *Betta* species in Sarawak, conducting morphometric and ecological assessments, and documenting captive breeding protocols that can serve as a foundation for conservation and management strategies.

1.3 Objectives

The research aimed to conduct captive breeding of wild *Betta* fish from Sarawak. This can be achieved through the following set of objectives:

- i. To provide a checklist for wild *Betta* in Sarawak
- ii. To develop a breeding protocol for wild *Betta* in captivity

CHAPTER 2

LITERATURE REVIEW

2.1 *Betta* Fish

The *Betta* genus, which includes small freshwater fighting fish known popularly as "ikan laga," belongs to the Osphronemidae family, which is believed to have originated in Southeast Asia and thrives in a variety of aquatic conditions. According to Lichak et al. (2022), numerous species found in the *Betta* genus are native to certain regions of Southeast Asia. Among fish hobbyists, *Betta* fish are among the most sought-after decorative fish due to their value in fish trading. Since the 1970s, fish farming has been more and more popular worldwide where at the moment, over 1 billion individual fish are traded annually (Evers et al., 2019). Rahman and Matthew (2021) stated that *Betta* fish have long been favoured as decorative fish because of their striking colour schemes, low setup needs and the distinctive way that the male *Betta* asserts his authority by flaring his fin and operculum in the face of danger. Throughout history, male *B. splendens* have been chosen for paired-staged bouts in Southeast Asia (Ramos & Gonçalves, 2019). The *Betta* fish is highly marketable due to its stunning body colour and fins, which enable it to be entered into national and international competitions (Widodo et al., 2023). The primary market for this species is now the ornamental hobby, therefore modern commercial *Betta* are very different from these early fighting fish and have been selected for vivid colours and complex fins. *B. splendens* are well-liked as ornamental fish in home aquariums because of their intricate fins and vivid colours (Watson et al., 2019).

2.1.1 Taxonomic Classification

The genus *Betta*, which belongs to the Osphronemidae family, is a diverse group of freshwater fish known as fighting fish and several species are native to Sarawak. Tan and Ng (2005) provided the keys to identifying the *Betta* species groups in Sarawak. *B. ibaanorum* and *B. akarensis* both belong to the *B. akarensis* group. Meanwhile, *B. brownorum* and *B. rutilans* belong to the *B. coccina* group. *B. lehi* and *Betta taeniata* belong to the *B. pugnax* group and *B. picta* group, respectively. Lastly, *B. macrostoma* belongs to *B. unimaculata* group.

2.1.2 Species Distribution

According to Fishbase (2025), 83 species of *Betta* fish have been described worldwide and each one has its special traits and adaptations. Most wild *Betta* species are located in Southeast Asia, where they live in a variety of aquatic habitats. The habitats of most *Betta* species are restricted to specific localities and their distributions are uneven. According to Chan (2015), *Betta* fish can be found from clearwater hill streams, freshwater swamps and peat swamp forests to the harsh man-made environment of canals and rice fields. Numerous factors such as geographic location, environmental conditions and human activity, affect the spread of the *Betta* species. Nur et al. (2022) stated that habitat disruptions caused by land conversion, climate change, highways and settlements affect the distribution of *Betta* fishes.

Several ecological and environmental factors greatly influence the distribution of wild *Betta* species (Brand et al., 2021; Kwon et al., 2022; Nur et al., 2022). Febriani et al. (2020) stated that *Betta* fish prefer environments with an abundance of aquatic plants, which

helps shield them from fish-eating birds. These environments should also have temperature ranges between 24°C and 30°C, a pH range of 6.5 to 7.5 and a level of dissolved mineral content or hardness between 8.5 and 10 dH.

In its natural habitat, the water temperature is often high, with low pH and dissolved oxygen. Due to their labyrinth organ (pharyngeal diverticulum) the most important characteristic in the Anabantids group, this species can survive in water with oxygen concentrations as low as 0 to 2 ppm, whereas other fish die at this concentration (Pleeging & Moons, 2017). Alton et al. (2013) stated that the pharyngeal diverticulum allows the consumption of oxygen from the air. This labyrinth organ is also responsible for producing air bubbles during nest building, where the female deposits her eggs (Pleeging & Moons, 2017). The distribution of *Betta* species is also significantly influenced by the structural complexity of habitats, which includes factors like substrate type, density of vegetation and the existence of shelters. According to Ramos et al. (2025), the fighting fish were found in various marshy habitats with thick herbaceous plants, ranging from still water areas in muddy flats to narrow canals near a lake. Substrata like leaf litter and submerged roots offer feeding possibilities, while dense vegetation offers hiding places and breeding grounds. *Betta* populations are more densely populated in areas where food is abundant. This is further supported by Comte and Olden (2018), who stated that a variety of ecological processes, including feeding, evasion, exploration, migration and reproduction, are linked to dispersal and these processes have an impact on the distribution of *Betta* fish.

2.1.2.1 Southeast Asia

The freshwater habitats of Southeast Asia are the primary distribution areas for wild *Betta* species. According to Abidin et al. (2022), the natural habitats of *Betta* fish can be found in several Southeast Asian countries such as Thailand, Malaysia, Brunei Darussalam, Singapore, Vietnam and Indonesia. In addition to these, wild *Betta* species are also naturally distributed in Cambodia and Laos, with Borneo (part of East Malaysia and Indonesia) being particularly rich in endemic species (Panijpan et al., 2020). Panijpan et al. (2020) also stated that there are no confirmed records of wild *Betta* populations in Myanmar or the Philippines and despite the term “Pla Kat Jeen” or “Chinese fighting fish,” China has no indigenous *Betta* species, but it refers to the fish’s elaborate fins, which resemble traditional Chinese opera costumes. These species inhabit a wide variety of environments, including marshes, rice paddies, peat forests, and slowly flowing rivers and streams. Every species has a preference for particular environmental circumstances, which affects how widely distributed they are.

There are numerous well-known *Betta* species found in Thailand. The Siamese fighting fish, *B. splendens*, is indigenous to central and eastern Thailand (Orós et al., 2022). This species lives in areas with dense vegetation and shallow, stagnant waters. Other species found in northeastern and southern Thailand, respectively, include *Betta smaragdina* and *Betta imbellis*. According to Laopichienpong et al. (2021), Northeastern Thailand is home to Mekong fighting fish (*B. smaragdina*). In addition, Southern Thailand is the native environment of *B. imbellis* (Ahmad et al., 2020).

Meanwhile, in Indonesia, there are many habitats for the *Betta* species within its large archipelago. On Sumatra Island, peat swamps and forest streams are frequent habitats for species like *Betta foerschi* and *Betta picta*. According to Syarif et al. (2023), wild *B. foerschi* can be found in the inland waters of Sumatra's Belitung Islands, where there is peat swamp forest and very acidic blackwater. *B. picta*, often called Spotted *Betta* or Java Fighting Fish, is a mouth-brooding species which are exclusively found on the island of Java, specifically in West Java and the northern region of Central Java (Hasan et al., 2023). Certain species live in blackwater rivers and streams in Kalimantan such as *Betta channoides* and *Betta rutilans*. Priyadi et al. (2021) stated that East Kalimantan is the home to the native and endemic snakehead fish, *B. channoides*. Furthermore, *B. rutilans* frequently inhabit the blackwater rivers in Kalimantan (Fahmi et al., 2020; Putri et al., 2021).

Southern Vietnam is home to several *Betta* species, including *B. splendens* and *B. smaragdina*. These fish usually inhabit slow-moving or stagnant waterways such as canals, ponds and rice paddies. For this fish, the profusion of aquatic vegetation provides vital cover and breeding grounds. Several *Betta* species are also found in Cambodia and Laos, though less is known about their precise ranges. There are several freshwater habitats where *B. splendens* and *B. smaragdina* can be found, including marshes, floodplains and slow-moving streams. These species can find adequate habitats in the Mekong River and its tributaries.

2.1.2.2 Malaysia

Malaysia's diverse habitats, including peat swamps and hill streams, support a rich variety of *Betta* species, making the country an important center for *Betta* biodiversity and evolution (Ding et al., 2025). Among them is *Betta persephone*, the Malaysian black

fighting fish, which is critically endangered and found only in the Ayer Hitam Peat Swamp Forest Reserve in Johor (Chan, 2015; Rafiq et al., 2021). Malaysia is also home to other wild *Betta* species, including *Betta livida*, which is found in the Klang Valley (Rahman & Matthew, 2021) and *Betta nuluhon*, a recently described species from western Sabah (Kamal et al., 2020). In Malaysia, 34 species of *Betta* have been described and reported (Schindler & Schmidt, 2008; Tan & Ng, 2005; Tan, 2009; Tan & Ahmad, 2018).

2.1.2.3 Sarawak

Sarawak is a Malaysian state known for its distinctive ecosystems and abundant wildlife. Different *Betta* species find perfect circumstances in the region's varied ecosystems, which include peat woods, rivers, streams and swamps. There are specifically seven species of *Betta* fish recorded in Sarawak which are *B. akarensis* (Regan, 1910), *B. brownorum* (Witte & Schimdt, 1992), *B. ibanorum* (Tan & Ng, 2004), *B. lehi* (Tan & Ng, 2005), *B. macrostoma* (Regan, 1910), *B. taeniata* (Regan, 1910) and *B. midas* (Tan, 2009). Many *Betta* species, each adapted to distinct habitats within the state of Sarawak, are supported by the state's distinctive geographic and environmental circumstances.

2.1.3 IUCN Status

The IUCN identifies overexploitation, habitat loss and degradation, pollution, by-catch, and human disturbance as the primary human-induced causes. According to (IUCN, 2025), a total of 72 *Betta* species were recorded from South and Southeast Asia, with 26.39% being data deficient, 23.61% endangered, 18.06% vulnerable, 15.28% critically endangered, 15.28% least concern and 1.39% near threatened.

In Malaysia, a total of 28 *Betta* species were recorded, of which 25% data deficient, 28.57% endangered, 21.43% vulnerable, 3.57% critically endangered, and 21.43% least concern (IUCN, 2025).

Five out of seven *Betta* species in Sarawak were classified as vulnerable, while the other two species were lacking in data (IUCN, 2025). According to the IUCN, a species is considered ‘Vulnerable’ when it faces a high risk of extinction in the wild. The IUCN uses several criteria, including population size, rate of decline, and geographic range to evaluate vulnerability. If a species meets certain thresholds indicating a high risk of extinction, it may be designated as vulnerable. Meanwhile, a species is considered ‘Data Deficient’ when there is a lack of information to conduct a direct or indirect assessment of its risk of extinction based on its distribution.

2.1.4 Ecology and Diet

Betta fish live in shallow, slow-moving environments like rice paddies, floodplains, and canals (Watson et al., 2019). These habitats are characterized by variable environmental conditions and high concentrations of organic materials, including humic substances (Hemmatzadeh et al., 2024). Syarif et al. (2021) stated that the brown colour of waters can be caused by high levels of dissolved organic substances in the form of humic acid and its derivatives which can be caused by the organic plant material turning into peat. Due to the frequently low oxygen levels in these habitats, they have evolved a special labyrinth organ that enables them to breathe in ambient air (Alton et al., 2013; Tate et al., 2017).

In their natural habitats, *Betta* fish exhibit carnivorous feeding behaviour, primarily consuming small aquatic invertebrates such as insect larvae, worms and zooplankton

(Sipaúba-Tavares et al., 2016). In captive conditions, *Betta* fish perform best on a protein-rich diet consisting of live foods (*Artemia* nauplii), formulated feeds or a combination of both. Research indicates that feeding a mixed diet of live and formulated foods results in optimal growth and survival rates (Murray et al., 2024).

2.1.5 Morphology

B. akarensis is a paternal oralbrooder which exhibits pre- and postorbital stripes (the latter sometimes faint or interrupted), a chin bar and a lanceolate caudal fin with elongated median rays in mature males (Tan & Ng, 2005). Mature males typically show transverse caudal bars and may bear greenish to bluish iridescent body scales, while opercular scales are generally non-iridescent, with faint greenish-gold tones occasionally present but often lost in captivity. Female *B. akarensis* has a less iridescent body and has no caudal-fin transverse bars.

B. brownorum can be distinguished by a large iridescent green lateral blotch in both males and females (Tan & Ng, 2005). They have a reddish-magenta body colour and practice a bubble-nesting reproductive strategy. As for *B. ibanorum*, it has a relatively slender body, with a broadly lanceolate caudal fin, pointed dorsal and anal fins and filamentous pelvic fins (Tan & Ng, 2005). Tan & Ng (2005) also stated that mature male *B. ibanorum* show iridescent green to blue scales on the posterior body and transverse bars on the dorsal and caudal fins, whereas females have reduced iridescence and lack caudal bars. Juveniles display a distinct midlateral stripe with a caudal peduncle spot, while all specimens possess a narrow chin bar and an opercle with little to no patterning.

On the other hand, *B. lehi* has a stocky body with a pointed head, lanceolate caudal fin, pointed dorsal and anal fins, and filamentous pelvic fins with white tips. According to the description by Tan & Ng (2005), mature males exhibit faint blue iridescence on the anal and caudal fins, green opercular sheen and interracial membranes tinged blue when alive, though these colours are lost upon preservation. In contrast, females and juveniles are less brightly coloured, with shorter fins, two dark opercular marks beneath the postorbital stripe and a narrow chin bar can be identified by the green iridescent scales on the opercle and the body. *B. macrostoma* is slender-bodied with a relatively large head, rounded caudal, dorsal, and anal fins, and falcate pelvic fins bearing a short filamentous ray. Mature males are bright red with a red opercular blotch bordered by black, a dorsal ocellus, red anal fin edged in black, caudal fin with transverse red and black bars, and pelvic fins tipped white. Females and juveniles display two dark midlateral stripes and a caudal peduncle spot, which remain distinct in preserved material, while males lose most red coloration and generally have a red body colour with transverse black and red bands on the caudal fin (Tan & Ng, 2005).

B. taeniata shows relatively weak sexual dimorphism, with males exhibiting more intense coloration that diminishes under stress. (Tan & Ng, 2005) stated that both sexes possess blue-green iridescent opercular scales, with unpaired fins bordered distally in dark to iridescent blue, especially pronounced in males during display. Males also have reddish-brown fins with white-tipped pelvic filaments, while females bear fainter colours and occasionally dark fin markings. Finally, *B. midas* can be distinguished by its blunt head, relatively thick, elongated body, rounded pectoral fins, filamentous pelvic fins with white tips and a lanceolate caudal fin bearing projecting median rays. The dorsal and anal fins are

pointed with extended posterior rays and the caudal peduncle is notably deep. Both sexes appear externally similar. The body is brown dorsally with lighter ventral coloration, speckled with gold iridescence, and the opercle shows a greenish-gold sheen. Distinct pre- and postorbital stripes and a black lower lip are present, while a chin bar is absent. Fins are generally brown, with dorsal and caudal fins marked by transverse bars and the pectorals are often tinged yellow with the brown colour of their dorsal side, along with a lighter brown colour speckled with gold iridescence on their ventral side. The description of *B. midas* followed Tan (2009).

2.1.6 Threats to Freshwater Fishes in Malaysia

Freshwater fish species in Malaysia are subjected to various critical threats, with habitat loss and degradation identified as the most pressing issues. These impacts are primarily driven by deforestation, rapid urbanization, construction of dams and the expansion of agricultural activities which contribute to the alteration or destruction of natural freshwater ecosystems (Chong et al., 2010; Jamaluddin et al., 2022; Ng et al., 2017). Pollution, particularly the emerging issue of microplastic contamination, has become a growing concern as recent studies have reported elevated concentrations of microplastics in riverine fish which raising serious implications for fish health and the overall integrity of freshwater ecosystems (Yamen et al., 2024). The introduction of non-native and invasive species, primarily driven by the aquarium trade and aquaculture, poses a significant threat to native fish biodiversity. These species can outcompete, prey upon, or hybridize with indigenous species, often leading to population declines and localized extinctions (Saba et al., 2020a; Saba et al., 2020b; Vythalingam et al., 2022). Overfishing and unsustainable

harvesting methods further intensify population declines, particularly affecting species with limited distributions or those of high economic importance (Chong et al., 2010). Ng et al. (2017) and Saba et al. (2021) also stated that the deterioration of water quality due to agricultural runoff, industrial discharge and urban pollution poses a significant threat to fish health and can adversely affect their reproductive success.

2.1.6.1 Threats to Wild *Betta* in Malaysia and Sarawak

Wild *Betta* fish in Malaysia and Sarawak face numerous important challenges, most of which are caused by human activity. The primary threats to *Betta*'s survival stem from habitat loss and degradation caused by land encroachment for oil palm plantations, illegal logging and mining which lead to the destruction and fragmentation of critical peat swamp forests and blackwater ecosystems (Chan, 2015; Rafiq et al., 2021). Rafiq et al. (2021) further stated that the water pollution resulting from agricultural runoff, industrial effluents and forest fires further deteriorates these fragile ecosystems which rendering them unsuitable for sustaining *Betta* populations. In addition, the overexploitation issue, either for the ornamental fish trade or local use, puts extra pressure on the already declining *Betta* population (Rahman & Matthew, 2021). The introduction of non-native species and the proliferation of exotic fish can also disrupt local ecosystems and pose significant threats to endemic *Betta* species through competition and potential hybridization (Ramadani et al., 2024).

2.2 *Betta* Fish Reproductive Strategy

Betta species are classified into two types which are the bubble nesters and the mouthbrooders, based on the modality of paternal care for fertilized eggs and newly hatched fry (Panijpan et al., 2017).

In bubble-nesting species, Panijpan et al. (2020) stated that the nest-building male parent creates a nest of sticky bubbles, resembling a 3D convex lens on the water's surface or beneath aquatic plant leaves to deposit fertilized eggs and absorb atmospheric oxygen. According to Kang & Lee (2010), the floating nest built by the male *Betta* is coated with glycoprotein-rich mucus produced by a specialized pharyngeal organ. Nugroho et al. (2020) stated that the female and male *Betta* fish interacted with each other first (fin flaring by the male and distinctive swimming patterns) before the spawning began underneath the nest. The female and male *Betta* fish entangle with each other, where the female will produce the eggs and the male will secrete sperm (Nugroho et al., 2020). Nugroho et al. (2020) also stated that the eggs sink after fertilization and the male gathers them into his mouth as soon as possible and gently nestles each egg within the bubble nest. The male guards the nest and provides parental care to the eggs and newly hatched fry until they reach the free-swimming stage (Panijpan et al., 2020). Normally, the eggs hatch in 24-36 hours depending on the temperature and other external factors. For two to three days or until the fry is strong enough to swim on its own, the male tends to the newly hatched fry and keeps watch over the nest. After the eggs hatch, the male parent attempts to retrieve some of the falling fry back into the nest (Panijpan et al., 2020).

Mouthbrooding is another reproductive method found in a number of *Betta* species. Unlike bubble nesters, mouthbrooders keep their eggs safe and healthy as they place the fry in the mouth of the male fish. Paternal mouthbrooding is a parental care approach in which the male incubates eggs or larvae in his buccal cavity, protecting them from both biotic and abiotic stresses (Abecia et al., 2022). Mouthbrooding *Betta* fish use elaborate displays of courtship to attract a partner, just like bubble nesters do. To attract the female, the male frequently swims in elaborate patterns and vivid colours. After the female agrees, spawning takes place, usually in a remote part of their environment. The male externally fertilizes the eggs that the female releases during spawning. The male gathers the eggs in his mouth as soon as they are fertilized. In certain species, the female could help collect the eggs before the male incubates them. Throughout the whole incubation period, the male keeps the fertilized eggs in his buccal cavity or mouth. According to Abecia et al. (2022), mouthbrooding requires a significant investment from the parent as the brooder is often unable to feed or is severely restricted. The duration of this time may vary between 7 and 14 days, contingent upon the species and surrounding circumstances. The male provides ideal conditions for egg development throughout this period in a protected setting. When the eggs hatch, the fry stay in the mouth of the male until they are mature enough to swim on their own. Periodically, the male lets the fry go so they can feed but he retrieves them promptly if there is any threat. This conduct persists until the fry can survive on their own. The male parent still holds their young fry in their mouth before releasing them to swim freely (Panijpan et al., 2020).

2.3 Captive Breeding

Captive breeding is an important conservation method for preserving or increasing populations of species at risk of extinction owing to habitat loss, overharvesting or environmental changes. Captive breeding of endangered species can avert extinction, boost long-term survival rates and reduce extinction risk (Wakchaure & Ganguly, 2016). According to Priyadi et al. (2024), captive breeding serves as a crucial conservation strategy for endangered species like *Betta rubra*, enabling population survival despite the degradation or loss of their natural habitats. Captive-bred individuals can be utilized to supplement or reintroduce wild populations, contributing to the recovery of both population size and genetic diversity (Budi et al., 2024).

In addition to serving conservation goals, captive breeding provides insights into the genetic diversity and research of the species. Based on Buckley et al. (2024), a well-managed breeding programs play a vital role in maintaining genetic variation which is critical for the long-term survival and adaptability of species. Budi et al. (2024) stated that captive breeding provides valuable insights into fish reproduction, growth and early developmental stages, supporting the development of more effective management and conservation strategies. Captive breeding can help to save endangered species by providing a safe and secure setting for breeding, reintroduction and scientific research (Leus, 2011).

CHAPTER 3

MATERIALS AND METHODS

3.1 Overview

Figure 3.1 shows the materials and methods overview used in this study.

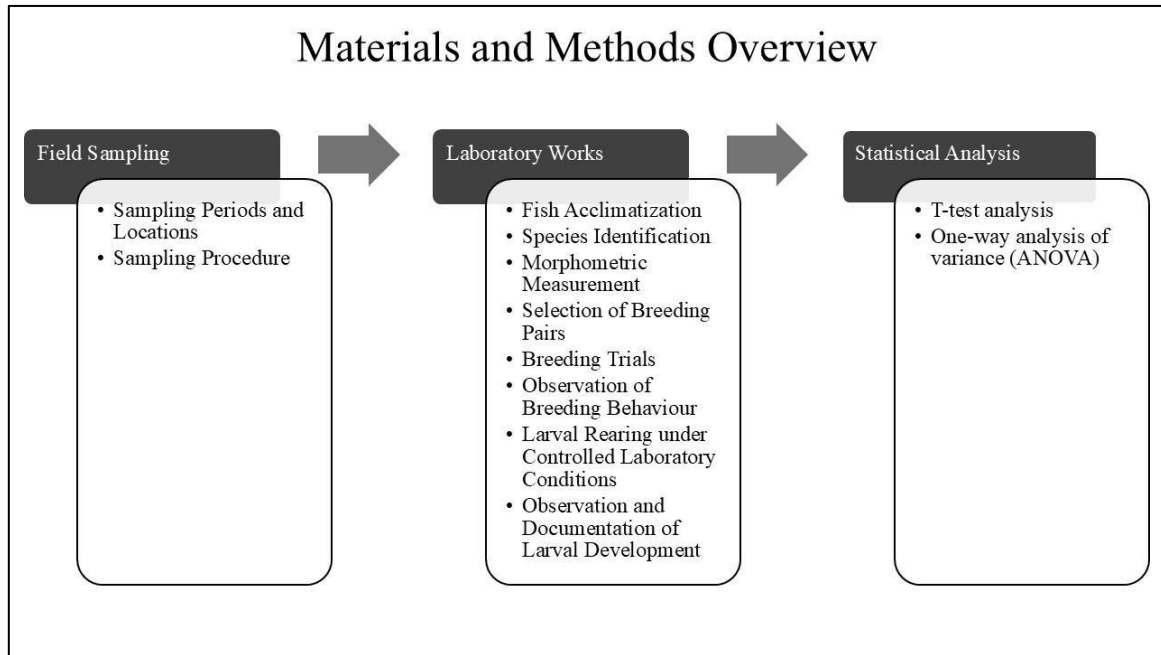


Figure 3.1: Materials and Methods Overview

3.2 Field Sampling

3.2.1 Sampling Periods and Locations

Sampling locations were selected based on (Tan & Ng, 2005). The sampling covered divisions from the southern to the northern parts of Sarawak. The study was conducted from March 2022 until July 2023 at seven sampling sites within Sarawak, namely Kuching (1), Lundu (2), Serian (1), Sibul (2) and Miri (1) (Figure 3.2). Table 3.1 shows the GPS

coordinates and the characteristics of all the sampling sites while Figure 3.3 shows the habitats of the *Betta* fish.



Figure 3.2: Map of Sarawak showing the sampling sites (Tan, 2009; Tan & Ng, 2005)

Table 3.1: GPS coordinates and characteristics of the *Betta* fish sampling sites

District	Sampling Site(s)	GPS coordinate	Locality	Description of General Habitat	Collection Date
Kuching	1	01°34'45.8" N 110°15'51.7" E	Peat swamps in Matang area located near Musang King Durian plantation.	The waters are brownish with muddy substrates	30 March 2022
Lundu	2	01°28'41.3" N 109°54'56.3" E	Stream near the Lundu main highway	The waters are clear with muddy substrates	24 May 2022
		01°49'47.2" N 109°43'02.0" E	Stream located by the Sematan road	The waters are brownish with gravel substrates	24 May 2022
Serian	1	01°09'36.1" N 110°29'07.7" E	Stream located near Kampung Lanchang	The waters are clear with gravel substrates	24 July 2022
Sibu	2	02°21'42.3" N 111°56'55.0" E	Irrigation canal by Kemuyang road	The waters are brownish with muddy substrates.	03 March 2023
		02°10'05.3" N 112°00'40.9" E	A tributary near Durin bridge	The waters are clear with muddy substrates	04 March 2023
Miri	1	04°12'08.51" N 114°25'43.65" E	Peat swamp near Rumah Gadong in Marudi	The waters are clear with gravel substrates	29 March 2023



Figure 3.3: Habitat of *Betta* fish: a) Kuching, b) Lundu, c) Sematan, d) Serian, e) Sibul (A), f) Sibul (B), g) Miri

3.2.2 Sampling Procedure

Samples in Matang, Sematan, Serian and Marudi areas were collected using scoop nets while samples in Lundu and Sibu waters were collected using fishing rods. Different methods were employed to transport the fish back to the Faculty of Resource Science and Technology Laboratory (FSTS), UNIMAS. During the field sampling in Sibu which was conducted for 3 days and 2 nights, two portable aquariums were brought along to house the collected samples. The aquariums were continuously aerated using portable air pumps to ensure their survival throughout the trip. For samples collected in Miri, the fish were carefully packed and sent back to UNIMAS using a reliable courier bus service as the samples could not be hand-carried on the return flight from Miri Airport to Kuching International Airport. Appropriate handling and aeration were ensured to minimize stress and mortality of the collected samples. In other sampling areas which were closer to UNIMAS, the collected samples were kept in labelled plastic bags with the habitat waters and transported directly to FSTS, UNIMAS.

The water quality parameters recorded during this study include temperature (°C), pH value, dissolved oxygen (mg/L) and turbidity (NTU). For each parameter, three replicates ($n = 3$) were taken per site to ensure reliability and representativeness of the data. The mean values were then used for statistical analysis. The temperature and pH value of the habitat were recorded using a pH meter (Extech SDL100) and the dissolved oxygen and turbidity were recorded using a DO meter (Extech DO210) and a turbidity meter (Extech TB400), respectively. The general habitat of the sites was observed and recorded. The location was also photographed and videographed for future reference.

3.3 Laboratory Works

3.3.1 Fish Acclimatization

Prior to using the fish samples in further studies, a 14-day confinement period was used to evaluate the health of the fish samples during the quarantine and adaptation periods. The fish samples were acclimated to the captivity unit by progressively changing the water temperature which was accomplished by letting each plastic bag float in the rearing medium for around ten minutes. The samples of male and female fish were raised in different aquariums. To mimic the *Betta* fish's native soft, brown water habitat, the water in the aquariums was treated with dried catappa leaves. These leaves are known to release humic substances and tannins which acidify the water and give it a brownish hue, closely mimicking the physicochemical conditions of peat swamp environments, the natural habitat of *Betta* species. Catappa leaves contribute to maintaining optimal pH levels and adequate dissolved oxygen concentrations and supporting the *Betta* fish's health (Zukflee et al., 2024). The aquarium's water and catappa leaves were replaced every 7 days. The fish were fed twice daily at 0900 hours and 1600 hours until satiation with grindal worms (*Enchytraeus buchholzi*) during the adaptation phase. The 14-day adaptation period did not affect the health of any of the fish samples. The fish acclimatization was conducted following the Permana et al. (2024) method on the captive breeding of *Betta rubra* from Indonesia.

3.3.2 Species Identification

The taxonomic status was identified based on the morphological and meristic characteristics, following Tan & Ng (2005) and Kamal et al. (2020).

3.3.3 Morphometric Measurement

The fish samples were measured while alive using a ruler ($\pm 0.1\text{cm}$) and weighed using a digital balance (A&D FY-300, made in Japan) with an accuracy of $\pm 0.01\text{g}$. The measurements include standard length (cm), total length (cm), and body weight (g) of the samples. In cases where samples were measured post-mortem, this was indicated accordingly in the figure captions.

3.3.4 Selection of Breeding Pairs

The broodstock were chosen for breeding based on their mature traits, physical health and lack of physical disabilities. Mature male can be identified by their more vivid body colours.

The selected male and female pairs were placed in cubic breeding tanks (approximately $30\text{cm} \times 30\text{cm} \times 30\text{cm}$) with catappa leaves to maintain ideal conditions for the pairs to breed.

3.3.5 Breeding Trials

The breeding trials followed a pairwise method adapted from *Betta channoides* breeding studies by Permana et al. (2024) with modifications based on preliminary observations. A total of 31 breeding tanks ($30\text{ cm} \times 30\text{ cm} \times 30\text{ cm}$) were used, each housing a single male-female pair per species.

Each species had at least one replicate per pair, with the total number of pairs as follows: *B. brownorum* (n = 4 pairs), *B. ibanorum* (n = 6 pairs), *B. lehi* (n = 4 pairs), *B. midas* (n = 3 pairs), *B. taeniata* (n = 5 pairs), *B. akarensis* (n = 3 pairs), and *B. macrostoma* (n = 6 pairs).

Breeding trials were conducted according to the samples' breeding strategies which were mouthbrooding and bubble-nesting. Each pair with fully developed males and females was placed in the breeding tanks in addition to dried catappa leaves to obtain the soft brown water condition which was favourable for spawning. A polystyrene cup cut in half was placed in each of the bubble-nester breeding tanks. The fish behaviour during this process was observed and recorded.

3.3.6 Observation of Breeding Behaviour

The fish behaviour during breeding was photographed using a mobile phone for documentation. Observations were conducted continuously during the active spawning phase, which was identified by courtship displays such as chasing, flaring, bubble nest construction (for bubble-nesters), or mouthbrooding behaviour (for mouthbrooders). Each observation session lasted for 30 minutes per pair, and key behaviours were photographed or recorded for documentation and later analysis.

3.3.7 Larval Rearing under Controlled Laboratory Conditions

The larvae were reared under controlled laboratory conditions. Water temperature was maintained at 26–28°C, with a 12:12 light-dark photoperiod. Dissolved oxygen levels were sustained using gentle aeration and pH ranged between 6.5 and 6.8. Partial water changes (30%) were conducted every 3 days to maintain optimal water quality.

3.3.8 Observation and Documentation of Larval Development

Upon completion of the breeding trials, the number of successfully fertilised eggs and hatched larvae was observed. The total length (mm) of the larvae for each was measured

using a stereomicroscope (OLYMPUS SZX7) connected to a computer with an accuracy of $\pm 0.01\text{mm}$.

3.4 Statistical Analysis

All statistical analyses were performed using SPSS Statistics. Descriptive statistics, including means and standard errors (SE), were calculated for all morphometric measurements and water quality data.

3.4.1 T-test analysis

An independent samples t-test was conducted to compare total length (TL) and body weight (BW) between male and female individuals within each *Betta* species to assess intraspecific size variation between sexes.

3.4.2 One-way Analysis of Variance (ANOVA)

One-way Analysis of Variance (ANOVA) was used to compare morphometric measurements (TL and BW) across all *Betta* species sampled to determine interspecific differences. Additionally, ANOVA was also applied to assess variations in water quality parameters (temperature, pH, dissolved oxygen and turbidity) between the different sampling sites or habitats of the species. Where statistically significant differences were found ($p < 0.05$), Tukey's post hoc test was employed to determine pairwise differences among groups. Before analysis, all data were tested for normality and homogeneity of variances to confirm suitability for parametric testing. Statistical significance was accepted at $p < 0.05$.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Sample Collection and Species Identification

A total of 72 samples of *Betta* fish were collected from 7 sampling sites in Sarawak (Matang, Lundu, Sematan, Serian, Sibü (A), Sibü (B) and Maruidi), comprising seven species which were *B. ibanorum*, *B. brownorum*, *B. lehi*, *B. midas*, *B. taeniata*, *B. akarensis* and *B. macrostoma* (Figure 4.1).

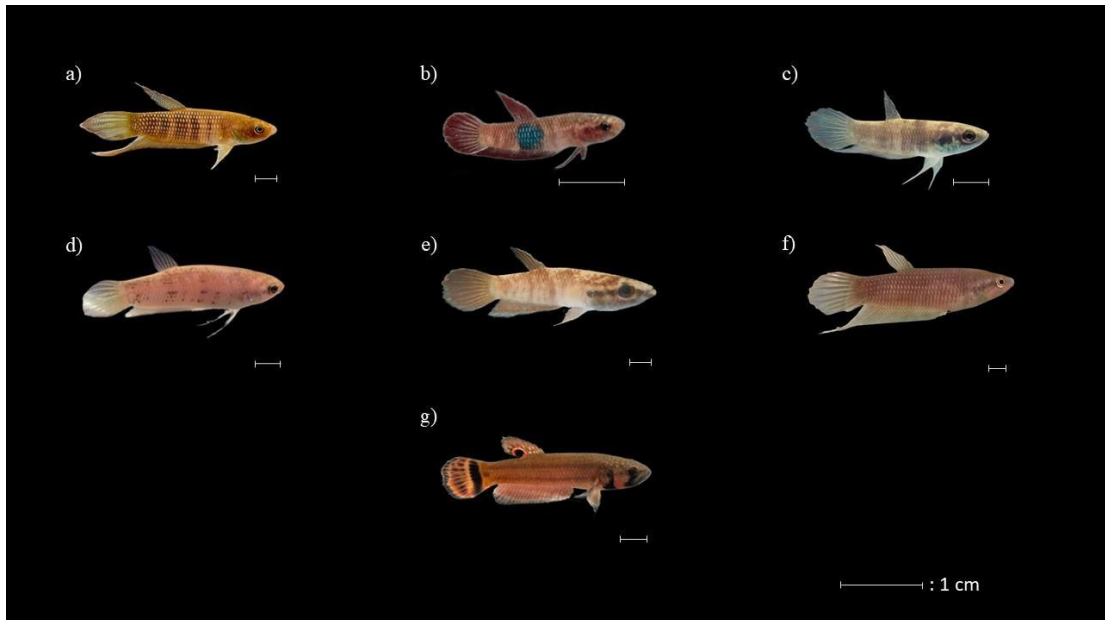


Figure 4.1: *Betta* species from Sarawak waters, a) *B. ibanorum*, b) *B. brownorum*, c) *B. lehi*, d) *B. midas*, e) *B. taeniata*, f) *B. akarensis*, g) *B. macrostoma*

4.1.1 *Betta brownorum*

A total of ten individuals were caught during the field sampling, consisting of four males and six females. Figure 4.2 shows a live specimen of *B. brownorum*. *B. brownorum*

were identified by the reddish magenta body colouration with a large iridescent green spot on the side of its body. The identification followed the description from Tan and Ng (2005).

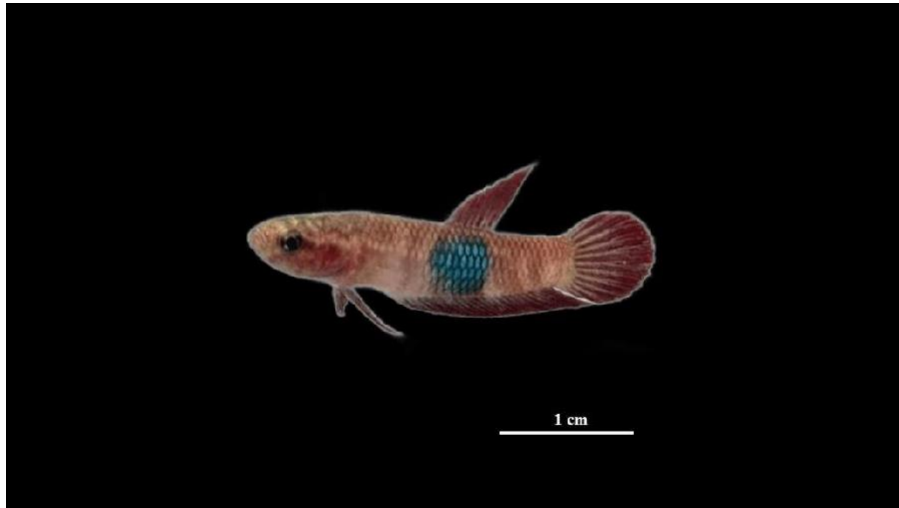


Figure 4.2: Live specimen of *B. brownorum*

4.1.2 *Betta ibanorum*

Thirteen individuals (seven males, six females) of *B. ibanorum* were successfully caught during the field sampling. The body colouration of *B. ibanorum* appears to be brownish and it displays iridescent green to blue colouration on the posterior body scales. The identification followed the description in Tan & Ng (2005). Figure 4.3 shows the live specimen of *B. ibanorum*



Figure 4.3: Live specimen of *B. ibanorum*

4.1.3 *Betta lehi*

Eleven individuals of *B. lehi* were caught during the field sampling at Lundu waters. Out of eleven individuals, four were males and the other seven were females. *B. lehi* were distinguished by the greenish-blue colourations along their operculum and posterior area. This observation is consistent with the identification by Tan & Ng (2005), who reported that mature male *B. lehi* exhibit an iridescent light blue margin on the anal fin and, to a lesser extent, on the caudal fin when alive. Figure 4.4 illustrates the live specimen of *B. lehi*.



Figure 4.4: Live specimen of *B. lehi*

4.1.4 *Betta midas*

A total of seven individuals of *B. midas* (three males, four females) were caught during the field samplings at Sematan waters. *B. midas* were identified by the brown dorsal body and head with a lighter brown at the ventral area. It also exhibits gold iridescent scales at the ventral area and blotches of dark brown behind the eye. The observation is in line with the identification conducted by Tan (2009). Figure 4.5 illustrates the live specimen of *B. midas*.



Figure 4.5: Live specimen of *B. midas*

4.1.5 *Betta taeniata*

Ten individuals of *B. taeniata* were caught, comprising five males and five females during the field sampling at Serian waters. Observation revealed that *B. taeniata* exhibits iridescent blue to green colourations on the opercle scales. The dorsal, anal and caudal fins are edged with a distinct blue iridescent colour. The observation matched the description of *B. taeniata* by (Tan & Ng, 2005). Figure 4.6 shows the live specimen of *B. taeniata*.



Figure 4.6: Live specimen of *B. taeniata*

4.1.6 *Betta akarensis*

Five males and three females of *B. akarensis* were caught during the field sampling at Sibu waters. Observation showed that *B. akarensis* exhibits blue to green colouration on the posterior half of its body scales with a prominent iridescent opercle scale. A white-tipped pelvic fin was also observed along with faint transverse bars on the caudal fin. The observation was consistent with the identification of *B. akarensis* conducted by Tan & Ng (2005). Figure 4.7 shows the live specimen of *B. akarensis*.



Figure 4.7: Live specimen of *B. akarensis*

4.1.7 *Betta macrostoma*

Thirteen individuals of *B. macrostoma* were caught during the field sampling at Marudi waters. Seven out of thirteen individuals were males while the rest were females. Observation revealed that the body of *B. macrostoma* is bright red. It also displays a red opercle blotch surrounded by black and a visible black ocellus on the dorsal fin. There are transverse black and red bars on the caudal fin, along with black edges on the anal fin. The observation is in line with the identification of *B. macrostoma* conducted by (Tan & Ng, 2005). Figure 4.8 illustrates the live specimen of *B. macrostoma*.



Figure 4.8: Live specimen of *B. macrostoma*

4.2 Environmental Parameters Across Sampling Sites

Table 4.1 summarises the water quality readings across all sampling sites. The temperature readings across all sampling sites ranged from 25.00 °C to 28.60 °C, meanwhile the dissolved oxygen readings ranged from 0.80 mg/L to 7.88 mg/L. The pH value ranged from 5.27 to 7.87 and the water turbidity readings across sampling sites ranged from 0.58 NTU to 56.83 NTU.

Table 4.1: Water quality across each sampling site

Sampling Site	Temperature (°C) Mean±SE (Ranges)	Dissolved Oxygen (mg/L) Mean±SE (Ranges)	pH value Mean±SE (Ranges)	Water Turbidity (NTU) Mean±SE (Ranges)
Matang	26.37±0.37 (25.90-27.10)	6.20±0.21 (5.80-6.50)	5.30±0.02 (5.27-5.34)	16.30±6.84 (7.49-29.77)
Lundu	27.50±0.61 (26.50-28.60)	6.20±0.21 (5.80-6.50)	5.30±0.02 (5.27-5.34)	16.30±6.84 (7.49-29.77)
Sematan	27.70±0.36 (27.20-28.40)	1.00±1.52 (0.80-1.00)	5.37±0.01 (5.36-5.39)	45.90±5.47 (39.77-56.83)
Serian	25.57±0.18 (25.00-26.30)	6.65±0.07 (6.40-6.90)	7.74±0.04 (7.59-7.87)	6.71±0.40 (5.92-7.23)
Jalan Kemuyang	26.06±0.25 (25.60-26.48)	5.06±1.43 (3.16-7.88)	7.18±0.07 (7.05-7.28)	2.17±0.65 (0.58-2.89)
Durin Bridge	26.74±0.22 (26.30-27.03)	3.77±0.29 (3.21-4.18)	6.90±0.08 (6.76-7.03)	2.09±0.65 (0.81-2.96)
Marudi	25.38±0.16 (25.20-25.70)	6.63±0.03 (6.59-6.66)	6.36±0.04 (6.32-6.40)	8.54±1.75 (6.19-11.95)

4.2.1 Temperature (°C)

Table 4.2 depicts the mean, standard deviation and standard error of water temperature (°C) recorded across different sampling sites. The mean temperature ranged from 25.38 ± 0.16 °C (Marudi) to 27.70 ± 0.36 °C (Sematan). Among the sites, the Sematan site shows the highest mean temperature (27.70 ± 0.36 °C), followed closely by the Lundu site (27.50 ± 0.61 °C). In contrast, Marudi (25.38 ± 0.16 °C) and Serian (25.57 ± 0.38 °C) show the lowest mean temperature values. The standard deviations were generally low, indicating minimal variation in readings across replicates within each site.

Table 4.2: Mean, standard deviation, and standard error of water temperature (°C)

Sites	Mean	Std. Deviation	Std. Error
Matang	26.37	0.64	0.37
Durin Bridge	26.74	0.39	0.22
Kemuyang Road	26.06	0.44	0.25
Lundu	27.50	1.05	0.61
Marudi	25.38	0.28	0.16
Sematan	27.70	0.62	0.36
Serian	25.57	0.66	0.38

The observation suggests spatial differences in thermal conditions between the sampling sites, warranting further statistical analysis using one-way ANOVA and Tukey post hoc test. One-way ANOVA revealed a statistically significant difference in mean temperature across sites, $F = 6.086$, $p = 0.003$. This indicates that at least one site differs significantly from the others in terms of water temperature. The Tukey post hoc test results revealed that Sematan and Lundu recorded significantly higher mean temperatures compared to Serian and Marudi ($p < 0.05$). Results also revealed that there were no significant differences detected between Matang, Kemuyang Road and Durin Bridge. These three sampling sites formed an intermediate group. Based on the post hoc grouping, sites were assigned Tukey labels to reflect statistical similarities and differences. Sites sharing the same letter are not significantly different, while those with different letters are significantly different ($p < 0.05$). These groupings are visualized in Figure 4.9.

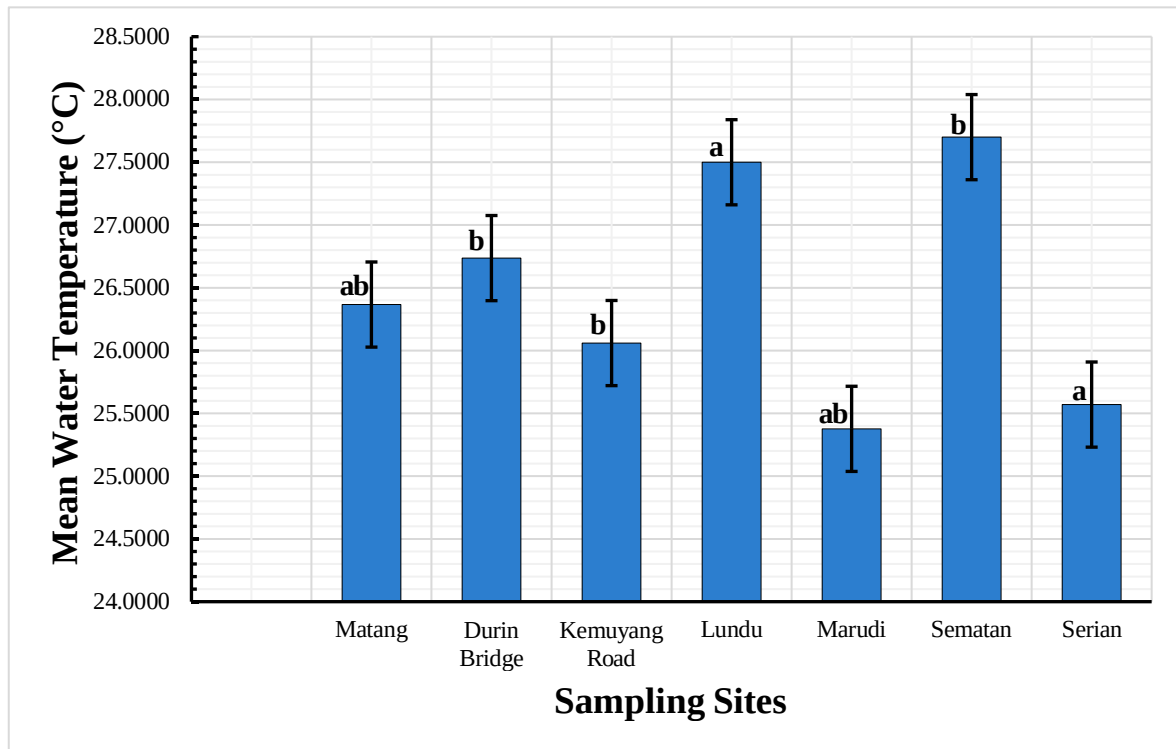


Figure 4.9: Mean water temperature (°C) across sampling sites. Error bars represent ± 1 standard error of the mean ($n = 3$). Bars not sharing the same letters are significantly different at $p < 0.05$

These results affirm that water temperature significantly differs among sampling sites ($p < 0.05$), underscoring the presence of distinct microthermal conditions that may influence metabolic rates, reproductive success, or species distribution patterns in *Betta* populations inhabiting these varied freshwater habitats. According to (Rezende & Carter, 2024), water temperatures influence the distributions of many fish species. Elevated temperatures may significantly impact early developmental stages by reducing the dispersal range of larvae, thereby increasing the likelihood of population fragmentation and genetic isolation (Raventos et al., 2021). A study conducted by Nur et al. (2022) on the distribution of *Betta* species in Aceh also suggested that water temperature influences the distribution of *Betta*

fish. Collectively, these studies emphasise how temperature acts as a driving force in shaping species distribution, especially through its influence on larval development and population connectivity.

4.2.2 Dissolved Oxygen (mg/L)

The mean dissolved oxygen (DO) concentrations across the seven sampling sites ranged from 1.00 ± 0.12 mg/L in Sematan to 6.65 ± 0.14 mg/L in Serian, as shown in Table 4.3. Relatively low DO levels were recorded in Sematan and Lundu, while Serian and Marudi exhibited higher oxygen concentrations, exceeding 6.5 mg/L. These findings suggest variation in oxygen availability between sites, potentially reflecting differences in hydrological flow, organic matter input or vegetation cover.

Table 4.3: Mean, standard deviation, and standard error of dissolved oxygen (mg/L)

Sites	Mean	Standard Deviation (SD)	Standard Error (SE)
Matang	6.20	0.36	0.21
Durin Bridge	3.79	0.47	0.27
Kemuyang Road	5.06	2.49	1.44
Lundu	3.60	0.70	0.40
Marudi	6.63	0.04	0.02
Sematan	1.00	0.20	0.12
Serian	6.65	0.25	0.14

One-way ANOVA analysis revealed a statistically significant difference in mean dissolved oxygen (DO) concentrations among the seven sampling sites ($F = 12.564$, $p < 0.001$). This indicates that at least one site differs significantly from the others in terms of DO levels. Tukey's post hoc test revealed distinct groupings among the sampling sites with respect to mean dissolved oxygen (DO) concentrations. Sematan recorded the lowest mean DO and was significantly different from most other sites, forming an independent group (a). Although Lundu and Durin Bridge were on the lower end of DO values, they overlapped statistically with both the low and intermediate groups and were thus assigned to group ab. Kemuyang Road and Matang exhibited moderate DO levels and were statistically similar to both mid and high DO sites, falling into group bc. Meanwhile, Serian and Marudi consistently showed the highest DO concentrations and were statistically distinct from the lower sites, forming group c. Figure 4.10 illustrates the mean dissolved oxygen (DO) concentration (mg/L) across seven sampling sites, with error bars representing standard errors.

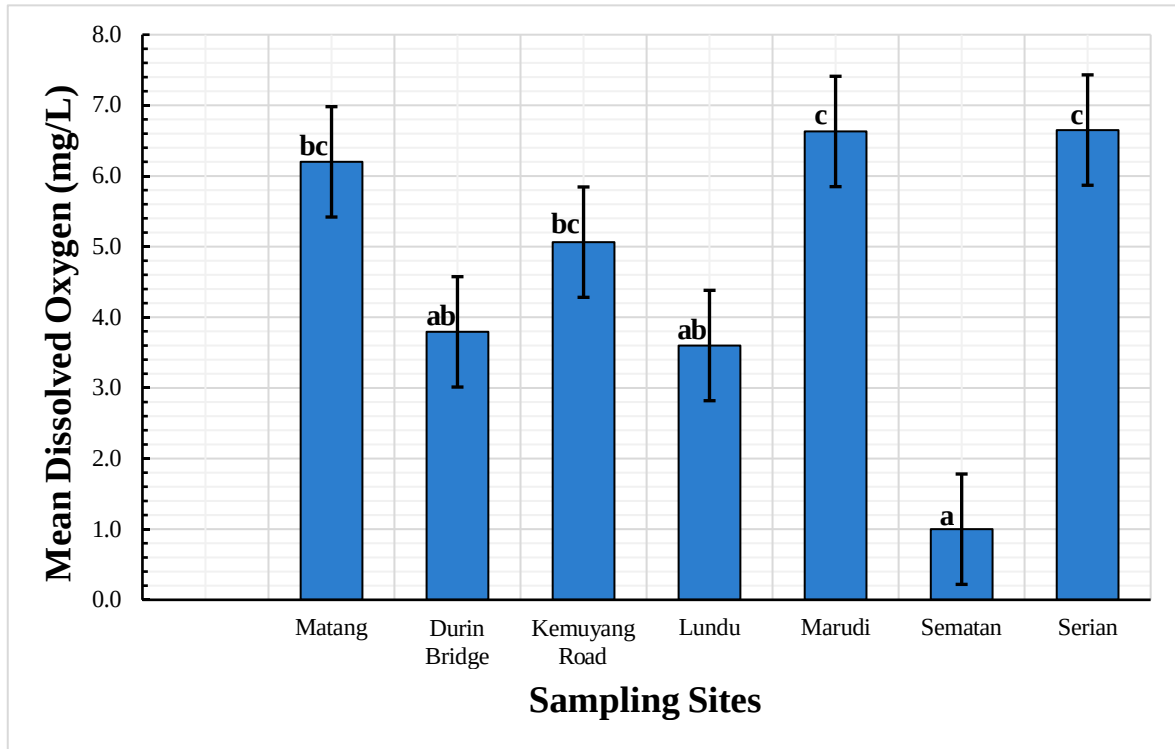


Figure 4.10: Mean dissolved oxygen concentration (mg/L) across sampling sites (± 1 SE). Bars that do not share the same letters are significantly different ($p < 0.05$; Tukey)

The results indicate noticeable variation in DO levels among sites, ranging from as low as 1.00 mg/L at Sematan to as high as 6.65 mg/L at Serian. These spatial differences suggest the presence of localized environmental or hydrological conditions that may influence oxygen availability in their habitats. Jane et al. (2021) stated that warmer temperatures reduce dissolved oxygen levels and promote thermal stratification, which limits oxygen mixing between surface and deeper layers, ultimately causing oxygen depletion in deeper waters of lakes and reservoirs. Stream flow, water depth and hydrological connectivity influence oxygen renewal where low flow or drought reduces oxygen input and promotes stagnation while high flow enhances oxygenation through mixing and atmospheric exchange

(Abdul-Aziz & Gebreslase, 2023). Another factor that can influence the dissolved oxygen in the *Betta* fish habitats is the organic matter inputs. Piatka et al. (2021) reported that elevated nutrient and organic matter inputs from runoff, pollution or internal cycling raise biological oxygen demand which leads to oxygen depletion, particularly during algal blooms or following organic pollution events.

4.2.3 pH value

The water pH levels across the seven sampling sites demonstrated noticeable variation. The pH values ranged from acidic to slightly alkaline conditions, with the lowest mean recorded at Matang (5.30 ± 0.02) and the highest at Serian (7.74 ± 0.08) as shown in Table 4.4. Other sampling sites showed intermediate mean values (Sematan (5.37), Lundu (6.03), Marudi (6.36), Durin Bridge (6.90), and Kemuyang Road (7.18)).

Table 4.4: Means, standard deviation and standard error across all sampling sites.

Sites	Mean	Standard Deviation (SD)	Standard Error (SE)
Matang	5.30	0.04	0.02
Durin Bridge	6.90	0.14	0.08
Kemuyang Road	7.18	0.12	0.07
Lundu	6.03	0.46	0.27
Marudi	6.36	0.04	0.02
Sematan	5.37	0.02	0.01
Serian	7.74	0.14	0.08

A one-way ANOVA test revealed a statistically significant difference in water pH among the sampling sites ($F = 65.841$, $p < 0.001$). Tukey's post hoc test revealed several significant pairwise differences. For instance, pH at Matang was significantly lower than at Serian, Kemuyang Road, Durin Bridge, Lundu, and Marudi ($p < 0.05$). Similarly, Serian exhibited significantly higher pH than most other sites except Kemuyang Road. The sites could be divided into five distinct groups. Figure 4.11 shows the mean pH values ($\pm$ SE) for each sampling site with the grouping. The error bars represent the standard error of the mean.

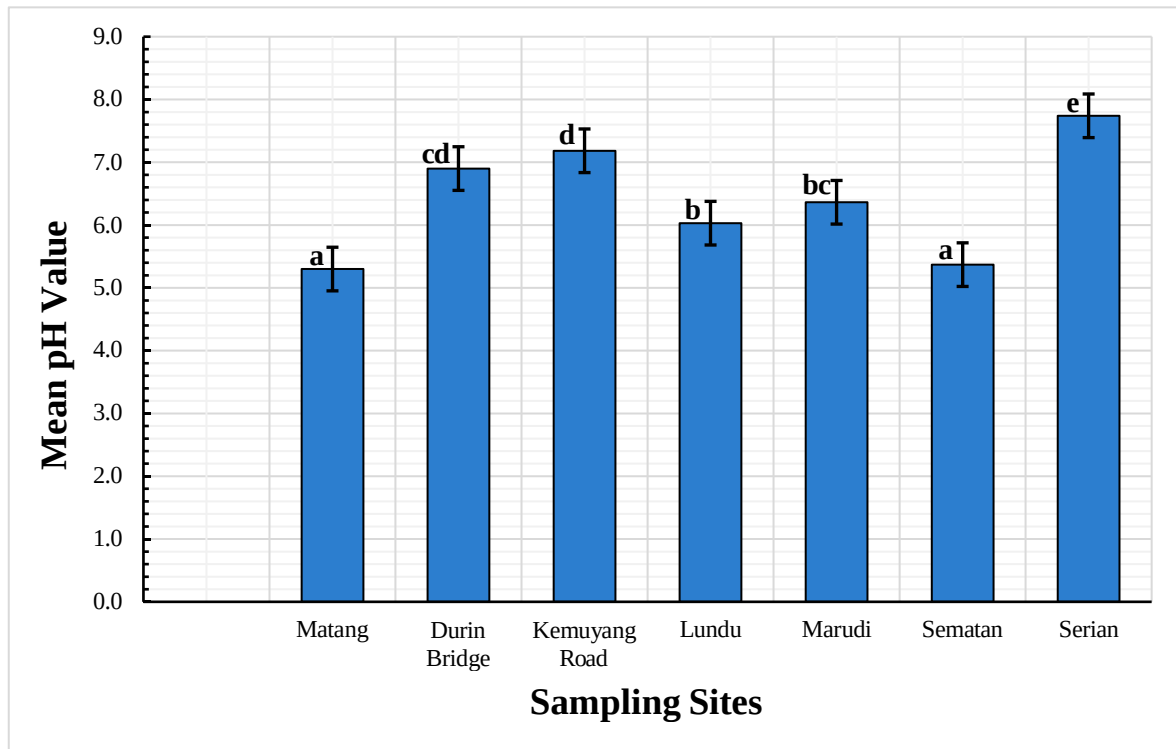


Figure 4.11: Mean ph value across sampling sites (± 1 SE). Bars that do not share the same letters are significantly different ($p < 0.05$; Tukey)

These results suggest that environmental factors such as surrounding vegetation, water flow, and anthropogenic influence might be contributing to the observed differences in water pH across the sampling sites. The significantly low pH at Matang and Sematan could indicate the presence of peat swamp or humic substances, which are commonly associated with acidic conditions. Omarjee et al. (2021) stated that vegetation type and coverage influence water pH by altering organic matter input, soil interactions, and buffering capacity, with ecosystems like peatlands potentially shifting pH levels away from those determined by underlying geology, particularly in geologically sensitive areas. Pollutants, nutrients and altered runoff from urbanization, agriculture and industry can disrupt natural

pH balance which often raises pH levels in agricultural and urban areas (alkalinization) while simultaneously destabilizing equilibrium through pollution and land use changes (Akhtar et al., 2021).

4.2.4 Turbidity (NTU)

Table 4.5 presents the turbidity values across the seven sampling sites. The highest mean turbidity was observed at Sematan (45.90 ± 5.48), followed by Matang (16.30 ± 6.84) and Marudi (8.54 ± 1.75). In contrast, the lowest mean turbidity was recorded at Kemuyang Road (1.45 ± 0.73), followed closely by Durin Bridge (2.09 ± 0.65) and Lundu (3.35 ± 0.39).

Table 4.5: Mean, standard deviation and standard error for turbidity (NTU)

Sites	Mean	Standard Deviation (SD)	Standard Error (SE)
Matang	16.30	11.85	6.84
Durin Bridge	2.09	1.13	0.65
Kemuyang Road	1.45	1.26	0.73
Lundu	3.35	0.68	0.39
Marudi	8.54	3.02	1.75
Sematan	45.90	9.49	5.48
Serian	6.71	0.70	0.40

The ANOVA results revealed a statistically significant difference in mean turbidity across the locations, $F = 21.452$, $p < 0.001$, indicating that at least one site differed markedly in turbidity compared to the others. Tukey's post hoc test identified that Sematan exhibited significantly higher turbidity values (45.90 NTU) than all other sites ($p < 0.05$) and thus was classified into a separate homogeneous subset (Group B). Conversely, the remaining six sites, which were Kemuyang Road, Durin Bridge, Lundu, Serian, Marudi, and Matang showed no statistically significant differences among themselves and were collectively grouped under Group A. Figure 4.12 shows the mean turbidity (NTU) across seven sampling sites in Sarawak with their grouping.

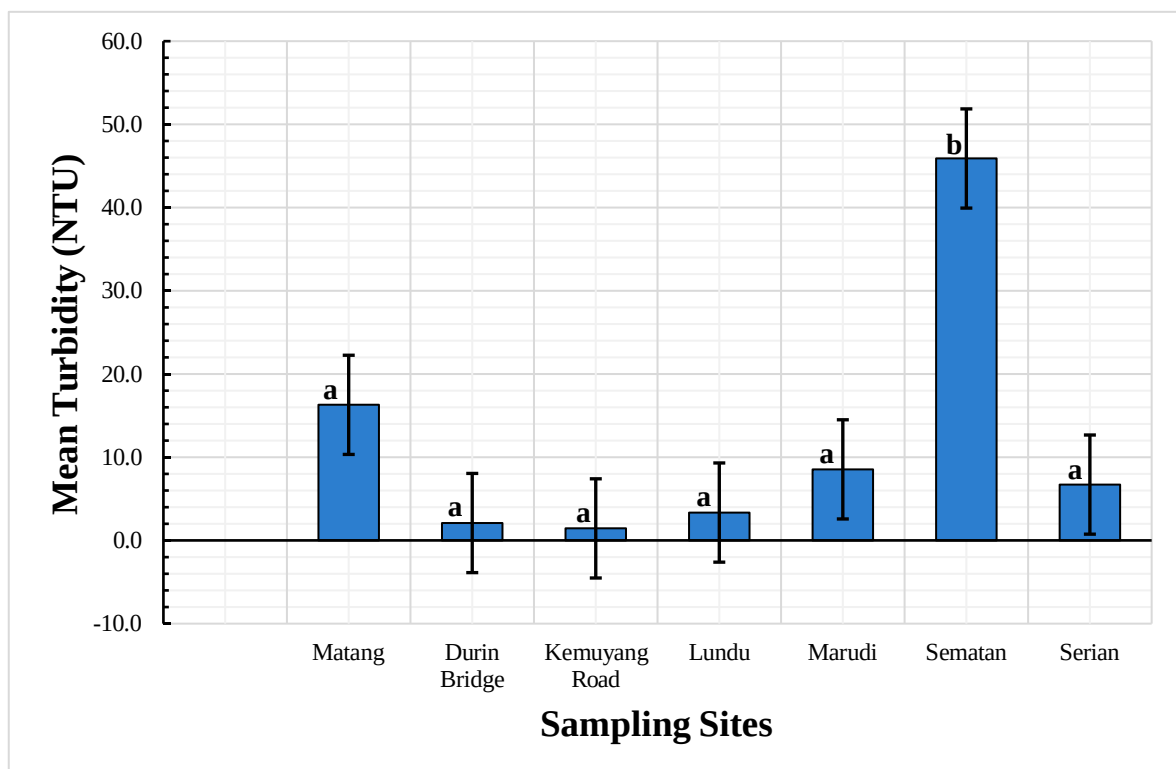


Figure 4.12: Mean turbidity (NTU) across sampling sites (± 1 SE). Bars that do not share the same letters are significantly different ($p < 0.05$; Tukey)

These findings underscore that turbidity conditions in Sematan are uniquely elevated, potentially reflecting site-specific environmental influences such as sediment input, runoff, or localized anthropogenic activities. This elevated turbidity could have implications for *Betta* fish visual feeding, gill function or reproductive success in the area. Research conducted by Zulfahmi et al. (2022) on the effect of turbidity on *Betta* fish proved that increased turbidity, particularly from sources such as palm oil, has been shown to significantly impair *Betta* fish feeding efficiency, reduce their foraging area and suppress aggressive behaviour by limiting visual prey detection. Although direct studies on gill function in *Betta* species under turbid conditions are limited, high turbidity is widely recognized to induce physiological stress in fish, often resulting in gill irritation or clogging (Zulfahmi et al., 2024).

4.3 Morphometric Measurement

Table 4.6 summarizes the morphometric characteristics (total length (TL), standard length (SL), and body weight (BW)) of male and female individuals across the sampled *Betta* species. Notable size differences were observed between sexes within species, suggesting the presence of sexual dimorphism, a common phenomenon in many fish taxa driven by ecological, behavioural or reproductive pressures.

Table 4.6: Morphometric measurement of male and female broodstock

Species	Sex	SL (cm) Mean±SE (Range)	TL (cm) Mean±SE (Range)	BW (g) Mean±SE (Range)	Number of Samples (N)
<i>B. brownorum</i>	Male	2.18±0.03 (2.10-2.20)	2.78±0.10 (2.70-2.90)	0.17±0.00 (0.16-0.18)	4
	Female	1.78±0.03 (1.70-1.90)	2.12±0.12 (2.00-2.30)	0.13±0.02 (0.08-0.18)	6
<i>B. ibanorum</i>	Male	6.60±0.54 (4.40-8.50)	8.89±0.79 (5.60-11.00)	7.03±1.52 (1.61-11.74)	7
	Female	6.03±0.57 (4.60-8.00)	7.67±0.86 (5.60-10.50)	5.85±1.41 (1.96-11.32)	6
<i>B. lehi</i>	Male	5.63±0.32 (4.80-6.30)	7.23±0.31 (6.50-8.00)	4.64±0.64 (3.06-6.02)	4
	Female	4.97±0.18 (4.50-5.90)	6.44±0.24 (5.80-7.50)	2.95±0.28 (1.97-3.94)	7
<i>B. midas</i>	Male	5.70±0.55 (5.10-6.80)	6.77±0.52 (5.90-7.70)	4.89±1.27 (3.60-7.44)	3
	Female	5.23±0.41 (4.30-6.20)	6.13±0.54 (5.00-7.10)	4.78±1.37 (3.26-8.90)	4
<i>B. taeniata</i>	Male	4.78±0.24 (4.40-5.70)	6.16±0.18 (5.80-6.80)	2.32±0.18 (1.92-2.93)	5
	Female	4.44±0.14 (4.00-4.80)	5.82±0.18 (5.40-6.30)	2.01±0.18 (1.64-2.47)	5
<i>B. akarensis</i>	Male	7.10±0.22 (6.50-7.70)	9.62±0.38 (8.90-10.60)	7.63±1.03 (4.46-10.67)	5
	Female	6.57±0.27 (6.30-7.10)	8.50±0.26 (8.10-9.00)	4.57±0.49 (3.78-5.48)	3
<i>B. macrostoma</i>	Male	6.23±0.25 (4.90-6.90)	7.80±0.32 (6.10-8.60)	4.17±0.49 (1.94-5.68)	7
	Female	5.68±0.30 (4.40-6.70)	7.25±0.37 (5.90-8.70)	3.55±0.45 (2.57-5.62)	6

4.3.1 T-test results

4.3.1.1 *B. brownorum*

The independent samples t-test revealed a statistically significant difference in total length (TL) between male and female *B. brownorum* ($t = 9.32$, $df = 8$, $p < 0.001$), with males exhibiting a higher mean TL (2.78 ± 0.10 cm) compared to females (2.12 ± 0.12 cm). The Levene's test indicated homogeneity of variances ($F = 0.143$, $p = 0.715$), validating the assumption for equal variances. On the other hand, the independent samples t-test did not reveal a statistically significant difference in body weight (BW) between male and female *B. brownorum* ($t = 1.86$, $df = 8$, $p = 0.101$), although males showed a higher average BW (0.168 ± 0.010 g) compared to females (0.132 ± 0.037 g). The result indicates that differences in total length between males and females are not a primary driver of sexual dimorphism in *B. brownorum*. Nomano and Kutsukake (2022) stated that in *B. brownorum*, sexual dimorphism is more strongly associated with the display and social use of ornamental traits, such as side blotches, than with differences in body size.

4.3.1.2 *B. ibanorum*

The independent samples t-test conducted to compare the total length (TL) between male and female *B. ibanorum* revealed no statistically significant difference between sexes. Males exhibited a higher mean TL (8.89 ± 0.79 cm) compared to females (7.67 ± 0.86 cm). However, this difference was not statistically significant ($t = 1.05$, $df = 11$, $p = 0.316$), indicating that the observed variation in TL could be attributed to random sampling variation rather than a true sex-based morphological difference. While males appeared slightly larger on average, the overlap in standard deviations suggests considerable variability within each

group. The independent samples t-test also demonstrated no statistically significant difference in body weight (BW) between male and female *B. ibanorum* ($t = 0.564$, $df = 11$, $p = 0.584$). Males exhibited a higher mean BW of 8.89 ± 2.10 g, while females had a mean BW of 7.71 ± 2.12 g, reflecting a modest difference of 1.18 g. However, this difference was not significant at the $\alpha = 0.05$ level. The absence of statistically significant differences in total length (TL) and body weight (BW) between male and female *B. ibanorum* observed in this study is consistent with the species' classification within the pugnax group, which comprises primarily male mouthbrooders. As described by Tan and Ng (2005), members of this group, including *B. ibanorum*, typically exhibit limited sexual size dimorphism, with males and females showing overlapping morphological traits. This trend may be attributed to the energetic constraints associated with paternal mouthbrooding, which may reduce the development of pronounced secondary sexual characteristics commonly observed in bubble-nesting congeners.

4.3.1.3 *B. lehi*

The independent samples t-test indicated that there was no statistically significant difference in the total length (TL) between male and female *B. lehi* individuals ($t = 1.99$, $df = 9$, $p = 0.077$). Males exhibited a slightly higher mean TL than females, with a mean difference of 0.79 cm. However, the p-value exceeded the conventional alpha level of 0.05, suggesting that the observed variation in TL may be due to random chance rather than actual sexual dimorphism. The independent samples t-test also revealed no statistically significant difference in body weight (BW) between male and female *B. lehi* specimens ($t = 2.82$, $df = 9$, $p = 0.020$, equal variances assumed). Although the males exhibited a notably higher mean body weight ($M = 2.37 \pm 0.36$ g) compared to the females ($M = 0.68 \pm 0.06$ g), the observed

difference did not reach statistical significance at the 0.05 level when assuming unequal variances ($p = 0.070$). *B. lehi* is also in the pugnax group as stated in Tan & Ng (2005), hence, it exhibits limited sexual dimorphism. However, this result may also be influenced by the small sample size and variability in male body weights, as reflected by the wider confidence interval of the mean difference (95% CI: -0.22 to 3.60). Morita and Sato (2025) stated that limited sample sizes in studies may only offer partial or tentative support for hypotheses concerning sexual dimorphism.

4.3.1.4 *B. midas*

The independent samples t-test conducted on total length (TL) measurements between male and female *B. midas* revealed no statistically significant difference ($t = 0.829$, $df = 5$, $p = 0.445$). The mean TL difference between sexes was 0.64 cm, with males showing a slightly larger TL. Similarly, for body weight (BW), the t-test result also indicated no significant difference between males and females ($t = 0.058$, $df = 5$, $p = 0.956$), with a minimal mean difference of 0.11 g. These findings suggest that *B. midas* may exhibit limited or non-distinct sexual dimorphism in terms of both size and body mass. Tan (2009) stated that the differences in body size between males and females of *B. midas* are often subtle and males typically exhibit more pronounced features related to their mouthbrooding role. The absence of significant differences may also be attributed to the small sample size, which limits the statistical power of the analysis and warrants cautious interpretation.

4.3.1.5 *B. taeniata*

The independent samples t-test conducted to compare total length (TL) between male and female *B. taeniata* did not yield a statistically significant difference ($t = 1.32$, $df = 8$, p

= 0.222). Males exhibited a higher mean TL (5.22 ± 0.26 cm) relative to females (4.88 ± 0.25 cm), with a mean difference of 0.34 cm. Despite the observable difference in means, the p-value exceeds the 0.05 significance threshold, indicating that the variation is not statistically meaningful. In addition, the independent samples t-test conducted to compare body weight (BW) between male and female *B. taeniata* also revealed no statistically significant difference ($t = 1.23$, $df = 8$, $p = 0.253$). Males exhibited a marginally higher mean BW (3.29 ± 0.17 g) compared to females (2.98 ± 0.15 g), with a mean difference of 0.31 g. The subtle size differences observed between male and female *B. taeniata* in this study are consistent with the species' classification under the picta group, where all members are male oral-brooders. In such lineages, sexual dimorphism is typically expressed through differences in fin morphology and ornamental traits rather than body size (Tan & Ng, 2005).

4.3.1.6 *B. akarensis*

The independent samples t-test conducted to assess differences in total length (TL) between male and female *B. akarensis* revealed no statistically significant difference ($t = 2.41$, $df = 6$, $p = 0.052$). Males exhibited a higher mean TL (5.99 ± 0.45 cm) compared to females (4.87 ± 0.47 cm), resulting in a mean difference of 1.12 cm. Although the mean TL of males was notably higher, the p-value slightly exceeded the conventional significance threshold ($p > 0.05$), suggesting that the observed sexual dimorphism in TL is not statistically confirmed. The marginal p-value, however, may imply a potential trend, and further validation with larger sample sizes could help clarify this difference.

An independent samples t-test indicated a statistically significant difference in body weight (BW) between male and female *B. akarensis* ($t = 2.69$, $df = 5.484$, $p = 0.039$). Males

had a higher mean BW (6.59 ± 0.97 g) than females (3.53 ± 1.03 g), resulting in a mean difference of 3.06 g. This observed sexual dimorphism in body mass may reflect the distinct parental roles observed in mouthbrooding species, where males often carry developing eggs in their buccal cavity. Increased body mass in males could facilitate prolonged incubation and enhanced endurance during mouthbrooding (Kolm, 2002). Fasya and Mufidah (2022) stated that in the *Betta* species, fecundity is strongly influenced by broodstock body weight, showing a high positive correlation. However, due to the limited sample size, further investigation with a larger cohort is warranted to confirm these findings.

4.3.1.7 *B. macrostoma*

The independent samples t-test conducted to compare total length (TL) between male and female *B. macrostoma* showed no statistically significant difference ($t = 1.14$, $df = 11$, $p = 0.279$). Males had a higher mean TL (7.93 ± 0.53 cm) than females (7.38 ± 0.49 cm), with a mean difference of 0.55 cm. Despite a visible size difference, the p-value exceeded the 0.05 threshold, suggesting that this difference is not statistically significant. This result indicates the absence of strong sexual dimorphism in TL for *B. macrostoma* within the sampled broodstock. Similarly, for body weight (BW), no statistically significant difference was detected between sexes ($t = 0.94$, $df = 10.999$, $p = 0.368$). Males showed a slightly higher average weight (7.53 ± 0.67 g) compared to females (6.91 ± 0.66 g), with a mean difference of 0.62 g. These findings support the conclusion that no significant sexual dimorphism in BW exists for *B. macrostoma* in the present study.

According to Tan and Ng (2005), *B. macrostoma* is a paternal mouthbrooder from the unimaculata group which exhibits sexual dimorphism primarily through cranial and

buccal adaptations related to brooding rather than in overall size. This corresponds with the non-significant differences in total length and body weight observed in this study. However, the absence of significant differences may also be attributed to the small sample size, which limits the statistical power of the analysis and warrants cautious interpretation.

4.3.2 One-way ANOVA Results

Table 4.7 shows the morphometric measurements of all *Betta* species caught across all the sampling sites in Sarawak. *B. akarensis* showed the highest mean total length (9.20 ± 0.32). However, in terms of body weight, *B. ibanorum* showed the highest mean body weight (6.47 ± 1.01). On the other hand, *B. brownorum* showed the lowest mean value in both total length (2.38 ± 0.11) and body weight (0.15 ± 0.01).

Table 4.7: Morphometric measurements of all *Betta* species caught

Species	SL (cm) Mean $\pm$ S E (Range)	TL (cm) Mean $\pm$ S E (Range)	BW (g) Mean $\pm$ S E (Range)	Number of Samples (N)
<i>B. brownorum</i>	1.94 ± 0.07 (1.70-2.20)	2.38 ± 0.11 (2.00-2.90)	0.15 ± 0.01 (0.16-0.18)	10
<i>B. ibanorum</i>	6.34 ± 0.37 (4.40-8.50)	8.32 ± 0.58 (5.60-11.00)	6.47 ± 1.01 (1.61-11.74)	13
<i>B. lehi</i>	5.21 ± 0.18 (4.50-6.30)	6.73 ± 0.21 (5.80-8.00)	3.56 ± 0.38 (1.97-6.02)	11
<i>B. midas</i>	5.43 ± 0.32 (4.30-6.80)	6.40 ± 0.37 (5.00-7.70)	4.83 ± 0.88 (3.26-7.44)	7
<i>B. taeniata</i>	4.61 ± 0.14 (4.00-5.70)	5.99 ± 0.13 (5.40-6.80)	2.17 ± 0.13 (1.64-2.93)	10
<i>B. akarensis</i>	6.90 ± 0.19 (6.30-7.70)	9.20 ± 0.32 (8.10-10.60)	6.49 ± 0.85 (3.78-10.67)	8
<i>B. macrostoma</i>	5.98 ± 0.20 (4.40-6.90)	7.55 ± 0.24 (5.90-8.70)	3.88 ± 0.33 (1.94-5.68)	13

A one-way ANOVA was conducted to compare the total length (TL) across seven different *Betta* species. The results revealed a statistically significant difference in TL among species ($F = 38.071$, $df = 6$, $p < 0.001$), indicating that at least one species differed significantly in mean TL from the others. Subsequent post hoc comparisons using the Tukey post hoc test further identified distinct interspecific differences. *B. brownorum* exhibited the shortest mean TL (2.38 cm), significantly smaller than all other species ($p < 0.001$), followed by *B. taeniata* (5.99 cm), *B. midas* (6.40 cm), *B. lehi* (6.73 cm), *B. macrostoma* (7.55 cm), *B. ibanorum* (8.32 cm), and *B. akarensis* (9.20 cm). The largest TL was recorded in *B. akarensis*, which significantly differed from most other species except *B. ibanorum*. The homogeneous subsets table grouped species into overlapping clusters, reflecting varying degrees of similarity. Notably, *B. brownorum* formed a distinct subset, while *B. taeniata*, *B. midas*, and *B. lehi* clustered into intermediate groups. In contrast, *B. macrostoma*, *B. ibanorum*, and *B. akarensis* exhibited higher TLs, with *B. akarensis* forming the uppermost group. Figure 4.13 illustrates the mean total length (cm) of all the *Betta* fish caught.

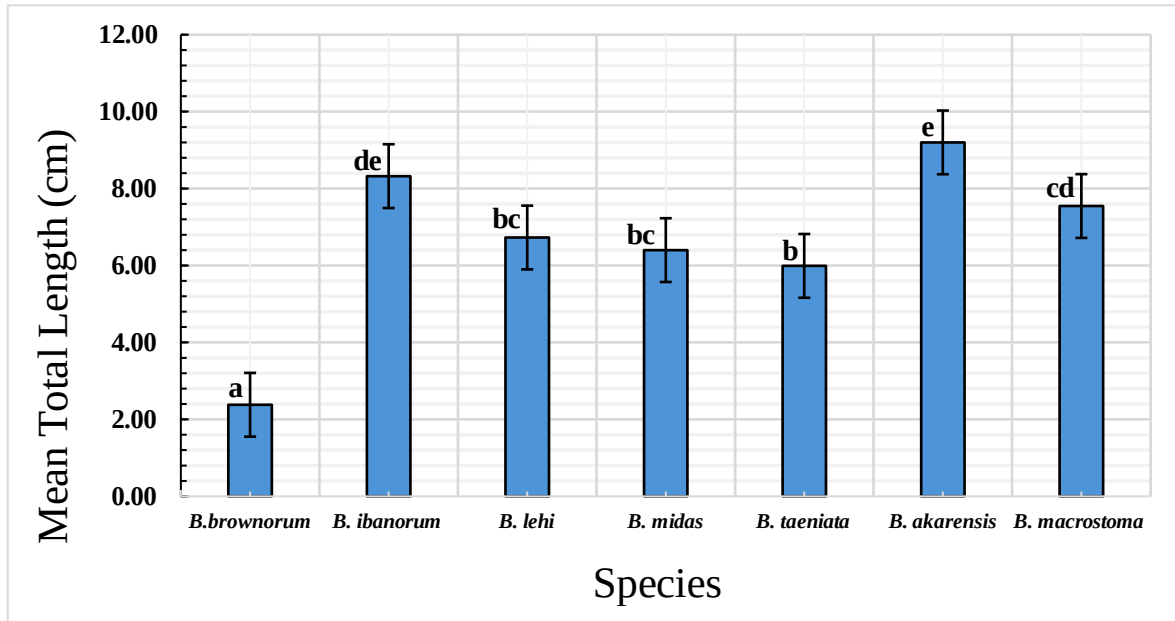


Figure 4.13: Mean total length (cm) of *Betta* fish caught across all *Betta* species in Sarawak

On the other hand, one-way ANOVA on differences in body weight (BW) across seven *Betta* species revealed a statistically significant variation in BW among species ($F = 12.911$, $df = 6$, $p < 0.001$), suggesting that at least one species differed significantly in mean BW from the others. Post hoc comparisons using the Tukey post hoc test provided further insights into interspecific distinctions. The smallest mean BW was recorded in *B. brownorum* (1.46 g), which significantly differed from *B. akarensis* (6.49 g), *B. ibanorum* (6.49 g) and *B. macrostoma* (3.88 g). Meanwhile, *B. midas* (4.83 g) also exhibited significantly greater BW compared to *B. brownorum*. The heaviest species were *B. ibanorum* and *B. akarensis*, which did not differ significantly from each other ($p = 1.000$). The homogeneous subsets table identified three primary groupings. *B. brownorum* was placed alone in the lowest BW subset. Intermediate weights were shared by *B. taeniata* (2.17 g), *B.*

lehi (3.56 g), and *B. macrostoma* (3.88 g). The highest-weight group consisted of *B. midas*, *B. akarensis*, and *B. ibanorum*, with overlapping subset memberships for the latter two. Figure 4.14 presents the mean body weight of all *Betta* fish caught.

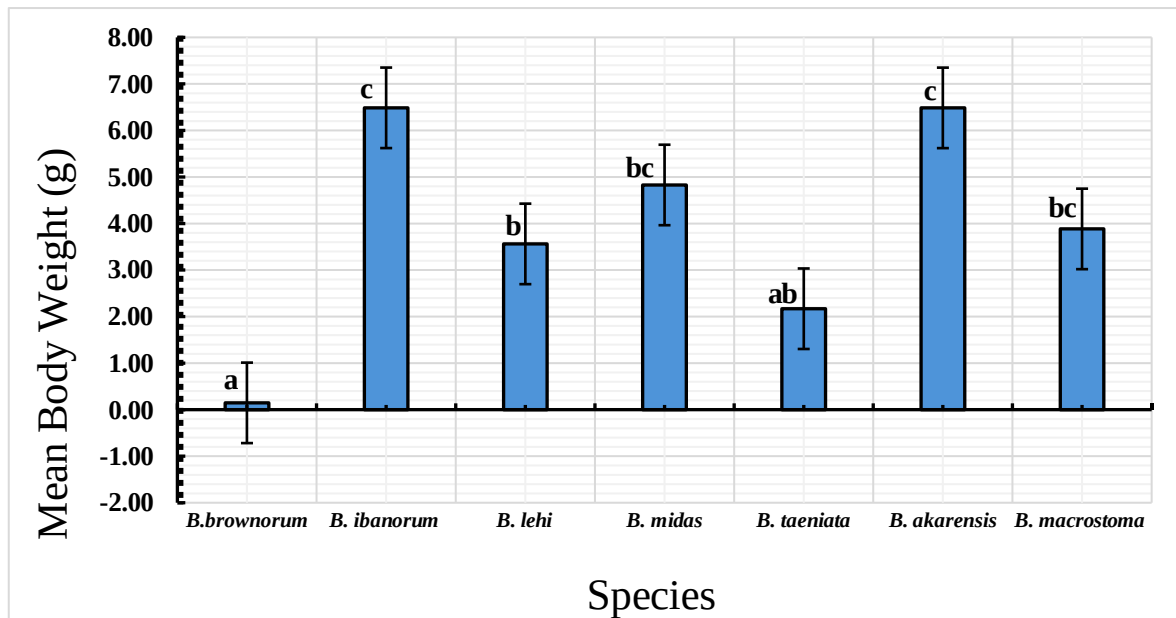


Figure 4.14: Mean body weight (g) of *Betta* fish caught across all *Betta* species in Sarawak

These interspecific differences in BW reinforce patterns of morphological disparity observed in TL, and may reflect species-specific ecological roles, habitat adaptations, or reproductive strategies. According to NengTias et al. (2021), growth rate and body condition are largely driven by the quantity and quality of food, which are regulated by environmental factors. Hence, the differences in body size and weight of all the *Betta* fish may be influenced by the availability of food in their habitats. The differences may also be influenced by the water type in their habitats. Fish in lentic waters, such as *Betta* habitats, typically develop a shorter, deeper body form compared to the more elongated form seen in lotic environments,

reflecting habitat-driven differences in swimming behaviour and energetic demands (Li et al., 2023).

4.4 Breeding trials

The pair availability was checked for each species to proceed with the breeding experiment. The breeding tanks were set up according to their breeding method. According to Tan & Ng (2005), *B. brownorum* is a bubble-nester while the others are mouthbrooders.

4.4.1 Mouth brooder

Figure 4.15 illustrates the standardized breeding protocol for mouthbrooding *Betta* species conducted under laboratory conditions. The protocol comprises five sequential phases, beginning with the sampling of wild *Betta* specimens from their native habitats (Stage 1). This is followed by conditioning and pairing of broodstock (Stage 2), which spans approximately two weeks to ensure acclimatization and readiness for reproduction.

During Stage 3, spawning behaviour and oral incubation are observed, typically lasting around one week. The male undertakes mouthbrooding duties by incubating fertilized eggs within the buccal cavity. This is followed by hatching of eggs (Stage 4), which occurs approximately four weeks after incubation. Finally, in Stage 5, larval rearing is initiated under controlled laboratory conditions, commencing two days post-hatching to ensure optimal survival and growth.

Mouthbrooder's breeding protocol

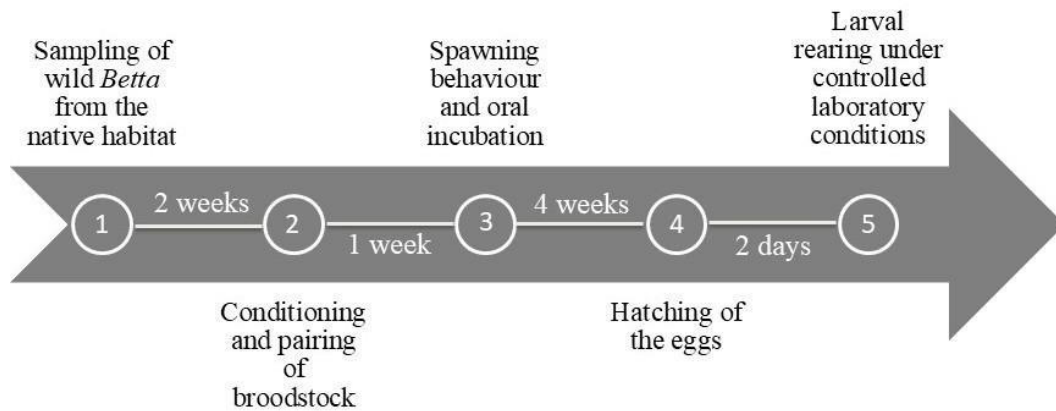


Figure 4.15: Captive breeding protocols for mouthbrooding *Betta* fish

4.4.1.1 *Betta ibanorum*

4.4.1.1.1 Breeding Protocol

(A) Sampling of wild *B. ibanorum* from the native habitat

B. ibanorum were collected from the peat swamps in the Matang area (01°34'45.8" N, 110°15'51.7" E) on 30th March 2022.

(B) Conditioning and pairing of broodstock

Pairing was done after two weeks of fish being caught. Broodstock selection was done based on their healthy physical characteristics, the absence of any physical disabilities and maturity characteristics. Mature males can be identified by their prominent iridescence, particularly on the posterior half of the body scales with distinct green to blue hues

accompanied by transverse bars on the dorsal and caudal fins while females display less iridescence and typically lack caudal transverse bars (Tan & Ng, 2005). Adult males are typically larger than females, with proportionally longer fins and broader heads. Figure 4.16 illustrates the comparison of male and female *B. ibanorum*.

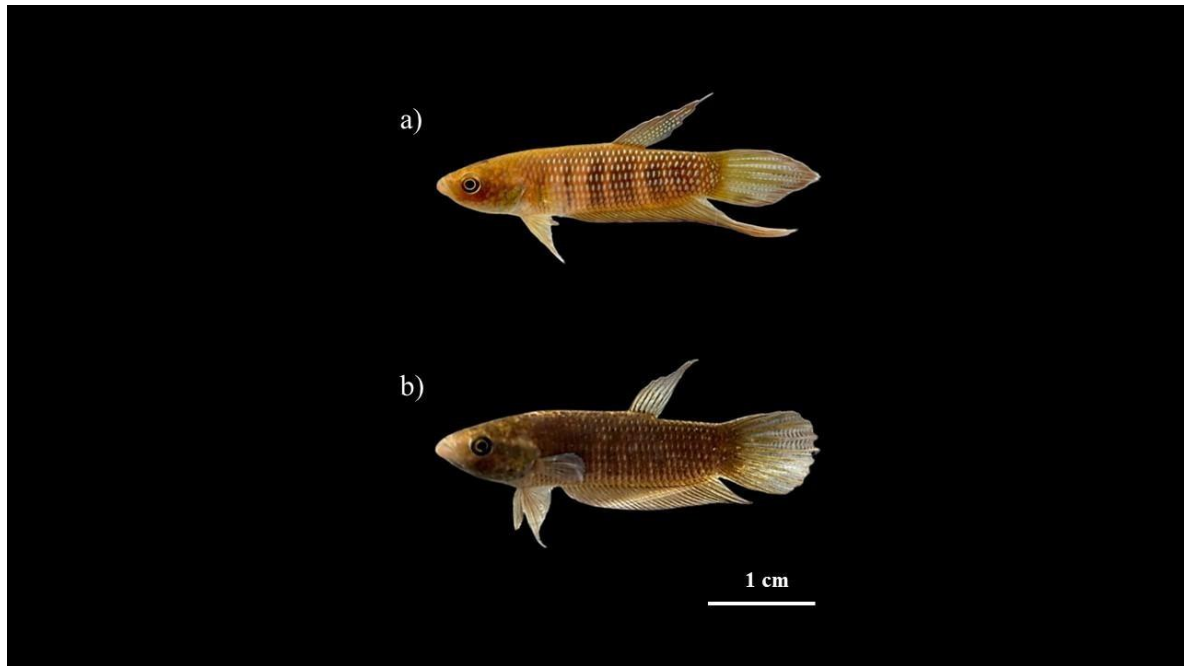


Figure 4.16: Comparison between male and female *B. ibanorum*, a) Male *B. ibanorum*, b) Female *B. ibanorum*

(C) Spawning behaviour and oral incubation

After one week of being placed in the breeding tank, spawning occurred. During the spawning phase, male *B. ibanorum* and female *B. ibanorum* were noticed to be entangled around each other (Figure 4.17) and undergo external fertilisation where the male released sperm to fertilise the eggs. After that, the male picked up the deposited eggs (Figure 4.18)

and mouthbrooded. This process was repeated until all the eggs were released. After the spawning process was completed, the female *B. ibanorum* was removed from the breeding tank and the male was left to incubate the eggs in its mouth (Figure 4.19) until they hatched.

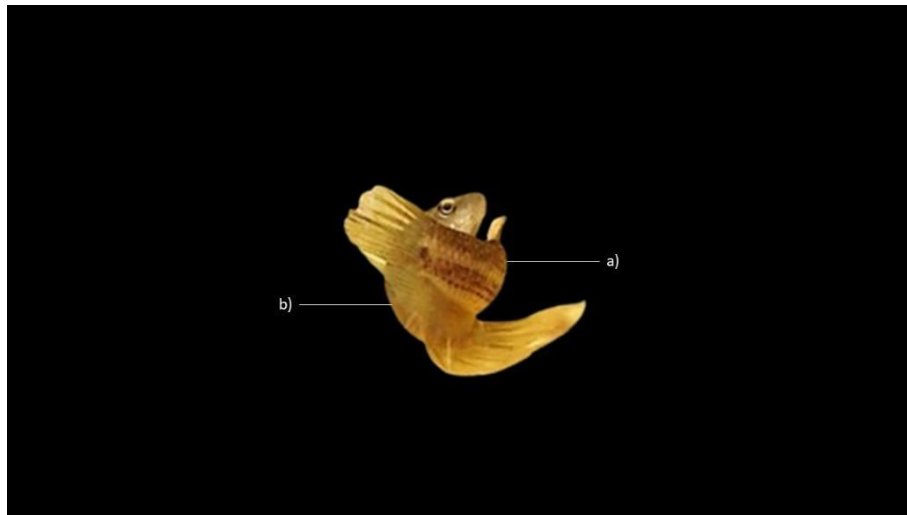


Figure 4.17: Female *B. ibanorum* (a) and male *B. ibanorum* (b) were noticed to be entangled around each other

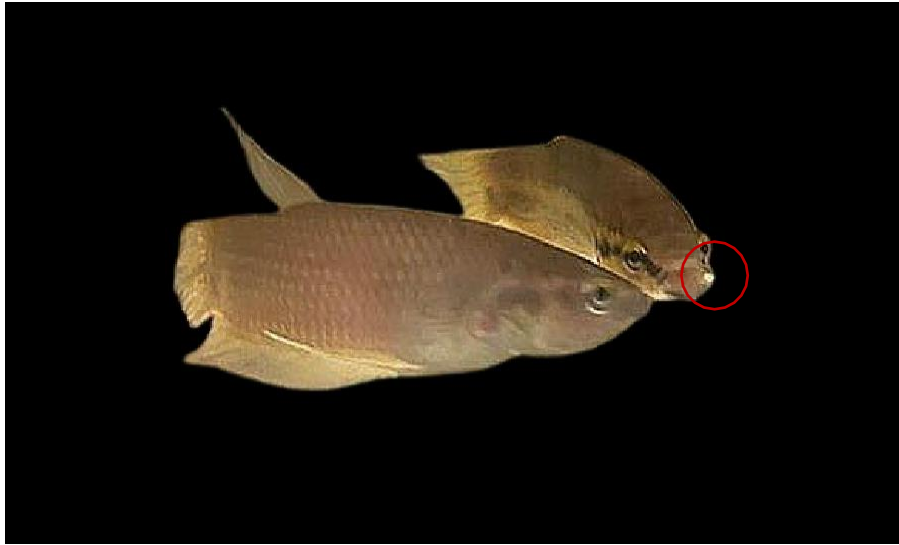


Figure 4.18: Male *B. ibanorum* attempted to pick up the deposited eggs (circled)

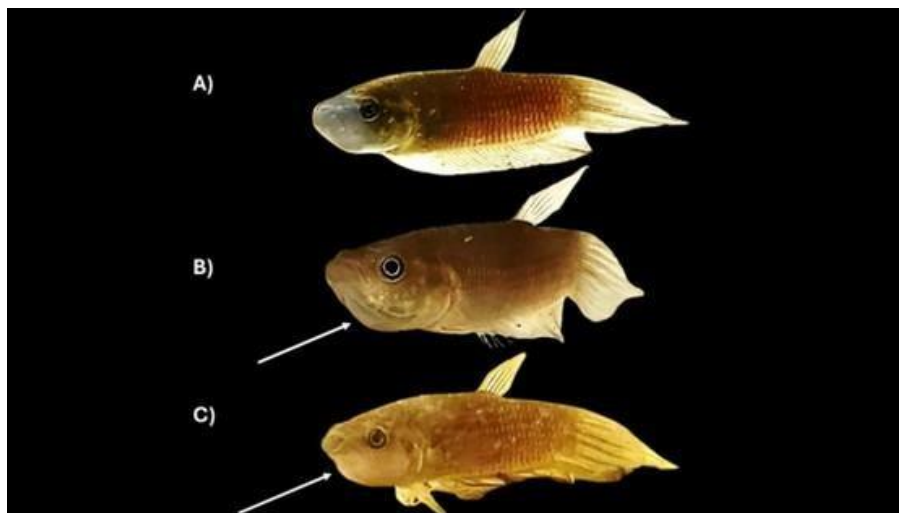


Figure 4.19: Mouthbrooding of male *B. ibanorum*. A) *B. ibanorum* in normal condition. B) *B. ibanorum*'s jaw appears to expand during mouthbrooding. C) Male *B. ibanorum* are ready to release the eggs

(D) Hatching of the eggs

Based on the observation, the male *B. ibanorum* released the fully hatched eggs after four weeks. Once the fry could swim and forage freely, they were isolated from the male *B. ibanorum* (Figure 4.20).



Figure 4.20: Fry isolated from broodstock

4.4.1.1.2 Larval Development

The larval development of *B. ibanorum* was observed under controlled laboratory conditions and is illustrated in Figure 4.21. The development was characterized by six distinguishable stages based on external morphology, pigmentation, yolk absorption, and fin differentiation.

At the newly hatched stage (A), the larvae exhibited large yolk sacs, undeveloped fins and minimal pigmentation. The eyes were not yet fully developed and swimming was limited. By the early yolk-sac stage (B), eye pigmentation began to appear and the yolk sac slightly reduced in size. Larval elongation became more evident, and the initial development

of fin structures could be observed.

In the late yolk-sac stage (C), the yolk reserves continued to diminish, and the pigmentation of the body became more pronounced. The larvae also displayed increased mobility. At the flexion stage (D), the upward bending of the notochord indicated the beginning of caudal fin formation. Pectoral and dorsal fins were visibly forming, and the larval body took on a more defined shape.

The post-flexion stage (E) was marked by fully developed fin rays and near-complete yolk absorption. The larvae began to display active swimming and improved pigmentation. Finally, in the pre-juvenile stage (F), the larvae resembled juvenile fish in external morphology. The body was slender, the fins were completely formed, and pigmentation was evenly distributed along the body and head. Figure 4.22 presents a scientific drawing that further complements the photographic documentation of *B. ibanorum* larval development.

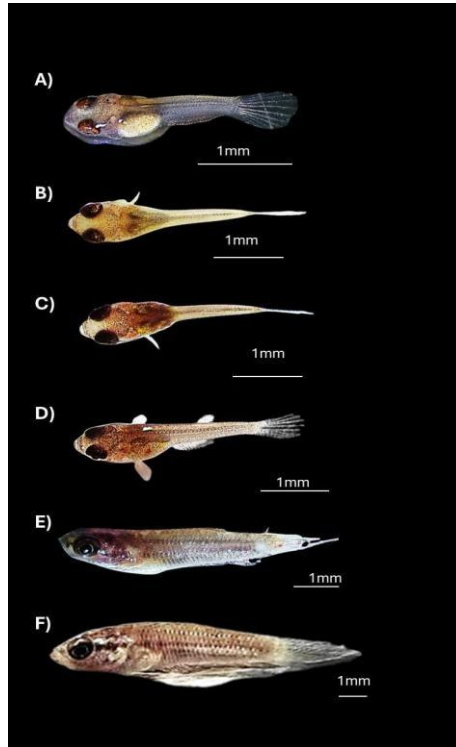


Figure 4.21: *B. ibanorum* larvae under microscope: A) Day 7 (2.5X), B) Day 14 (2.5X), C) Day 21 (2.5X), D) Day 28 (2.5X), E) Day 120 (preserved, 0.8X), F) Day 150 (preserved, 2.5X)

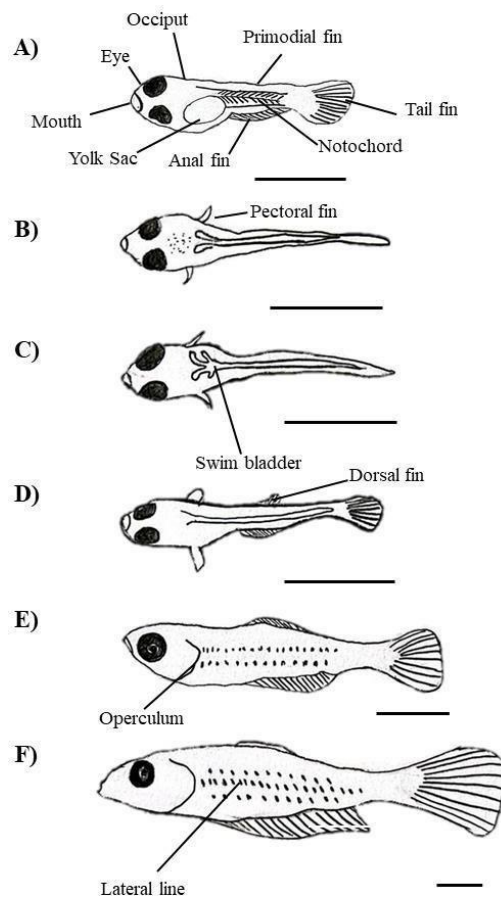


Figure 4.22: Scientific drawings of *B. ibanorum* larvae: A) Day 7, B) Day 14, C) Day 21, D) Day 28, E) Day 120, F) Day 150

The growth measurements of *B. ibanorum* larvae exhibited a consistent and significant increase over the 150-day observation period. This progressive growth, starting from an average total length of 6.49 mm on Day 7 and reaching 35.17 mm by Day 150, was confirmed through repeated measurements of 10 individual larvae per time point (Figure 4.23). This highlights the species' substantial growth potential under controlled laboratory conditions.

The standard deviation (SD) values indicate the consistency of measurements, with the lowest SD observed on Day 21 (0.03), suggesting minimal variation in larval size at this stage. Conversely, the highest SD on Day 150 (0.39) indicates increased variability in growth, which may be attributed to individual growth differences among the larvae as they approached maturity, possible genetic variation or environmental factors (Britz, 2017).

Throughout the rearing period, the feeding regime involved an initial use of Grindal worms, followed by a gradual transition to artificial feed as the larvae matured. While the early growth phase (Day 7–21) showed minimal variability, suggesting that feeding had a negligible influence during this stage and the increased variability observed in later stages could potentially be influenced by this change in feed. However, based on the consistent early trends and low standard deviation, feed variation was unlikely to be the primary factor influencing overall growth trends.

The observed developmental stages and growth trajectory of *B. ibanorum* were found to align closely with findings from previous studies on *B. rubra* (Priyadi et al., 2024), another small-bodied mouthbrooding *Betta* species. These similarities suggest that continuous growth under controlled rearing conditions may be a conserved trait within this group. However, specific features such as the early formation of the operculum and the late-stage development of the lateral line (Stage F) may reflect evolutionary adaptations to the unique microhabitats of Northwestern Borneo, where *B. ibanorum* is endemic. The formation of the lateral line in Stage F highlights the importance of sensory adaptations in predator detection and social interactions (Mogdans, 2019).

Table 4.8 shows the length gain, average length gain and the survival rate of the *B. ibanorum*'s larvae. The survival rate of *B. ibanorum* larvae was observed to be 25.50%. This relatively low percentage is likely influenced by multiple interrelated factors, notably the

transition in feeding method from live grindal worms to artificial feed. Grindal worms, widely used in aquaculture due to their high protein content (11.6%), soft body, motility and ease of cultivation, are highly suitable for early-stage larvae due to their digestibility and nutritional value (Zulkifle et al., 2021). In contrast, artificial feed may not be readily accepted by young larvae, leading to poor nutrient uptake. Uneaten artificial feed can further deteriorate water quality by increasing organic load, creating a stressful environment and making larvae more vulnerable to disease and mortality (Munguti et al., 2020).

The observed survival rate of *B. ibanorum*'s larvae was markedly lower than survival rates reported for other *Betta* species in captivity such as *B. channoides* (Permana et al., 2024) and *B. rubra* (Priyadi et al., 2024). This discrepancy may be attributed to species-specific sensitivities as *B. ibanorum* is a wild mouthbrooding species endemic to the acidic blackwater habitats of Northwestern Borneo and may be more vulnerable to stress under captive conditions.

While key parameters such as temperature (26–28°C) and pH (6.5–7.0) were maintained within acceptable ranges, unmonitored factors like ammonia accumulation or low dissolved oxygen are common risks during feed transitions which may have exacerbated mortality. Furthermore, unlike highly domesticated species such as *B. splendens*, wild *B. ibanorum* may require more tailored husbandry practices to thrive.

Despite the lower survival rate, these findings provide valuable insight into the species' growth and rearing challenges, underscoring the need for refined, species-specific protocols. Future breeding programs should consider prolonged use of live feeds, improved water quality monitoring, and gradual feed transitions to enhance larval survival and support conservation initiatives. Understanding the growth patterns and factors influencing growth

variability can aid in developing more effective breeding strategies and conservation measures for this species (Gicquel et al., 2020).

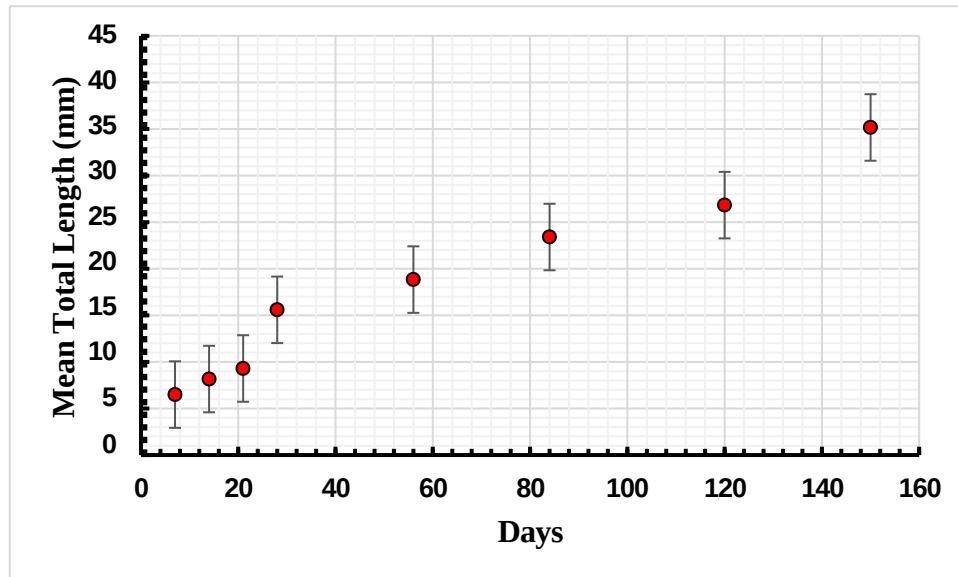


Figure 4.23: Growth of *B. ibanorum*'s larvae (total length)

Table 4.8: LG, ADLG and SR of *B. ibanorum*'s larvae

Parameter	Result
Length Gain (LG)	28.68mm
Average Daily Length Gain (ADLG)	0.20mm
Survival Rate (SR)	25.50%

4.4.1.2 *Betta macrostoma*

4.4.1.2.1 Breeding Protocol

(A) Sampling of wild *B. macrostoma* from the native habitat

B. macrostoma were collected from the peat swamp near Rumah Gadong in Marudi, Miri Division (04°12'08.51" N 114°25'43.65" E) on 29th March 2023.

(B) Conditioning and pairing of broodstock

Pairing was done upon two weeks of fish being collected. Broodstock selection was done based on their healthy physical characteristics, the absence of any physical disabilities and maturity characteristics. Mature male displays a broader head, elongated fins, and distinct transverse black bands on the caudal fin, which are absent in females. Additionally, the male exhibits more pronounced iridescent coloration, especially on the posterior body and fin membranes, whereas the female shows duller coloration with less visible iridescence. Figure 4.24 illustrates the comparison of male and female *B. macrostoma*.

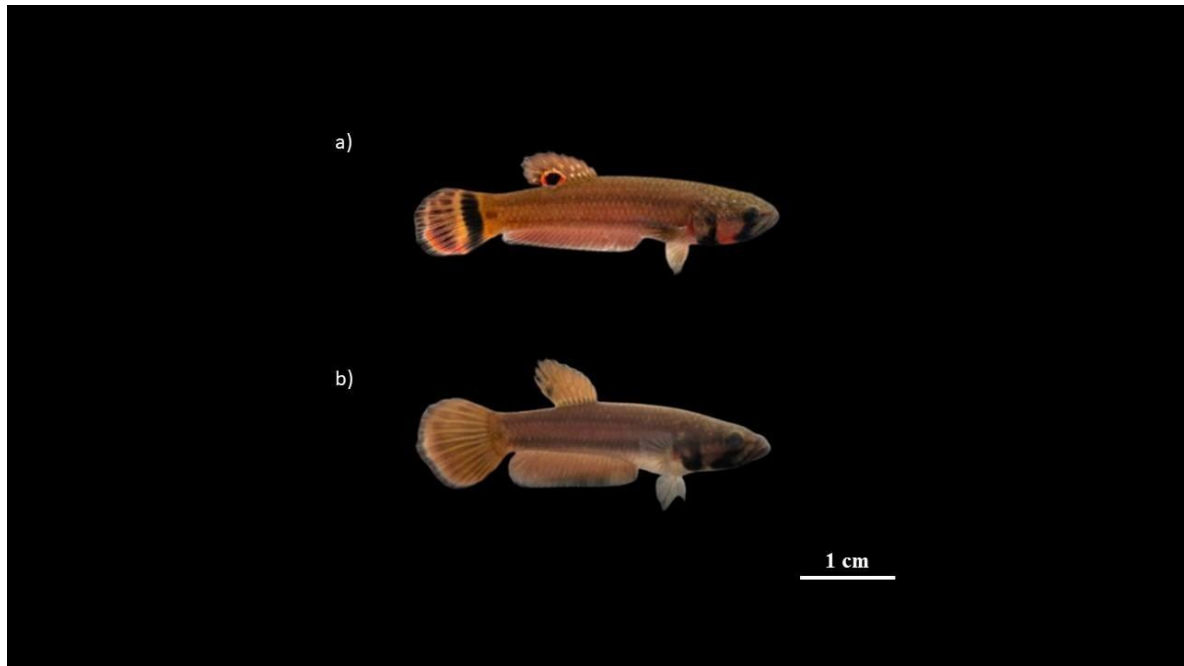


Figure 4.24: Comparison between male and female *B. macrostoma*. a) Male *B. macrostoma*, b) Female *B. macrostoma*

(C) Spawning behaviour and oral incubation

During the spawning phase, male *B. macrostoma* wrapped his body around the female *B. macrostoma* (Figure 4.25) and the female deposited eggs. Simultaneously, the male releases sperm, which fertilize the eggs externally as they are being released into the water. After that, the male picked up the deposited eggs (Figure 4.26) and mouthbrooded. This process was repeated until all the eggs were released. After the spawning process was completed, the female *B. macrostoma* was removed from the breeding tank and the male was left to incubate the eggs in its mouth (Figure 4.27) until they hatched.

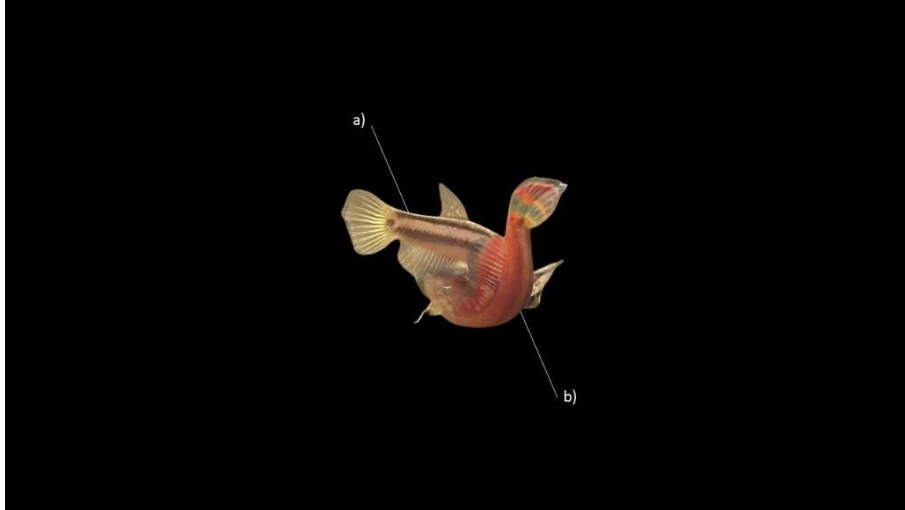


Figure 4.25: Female *B. macrostoma* (a) and male *B. macrostoma* (b) were noticed to be entangled around each other

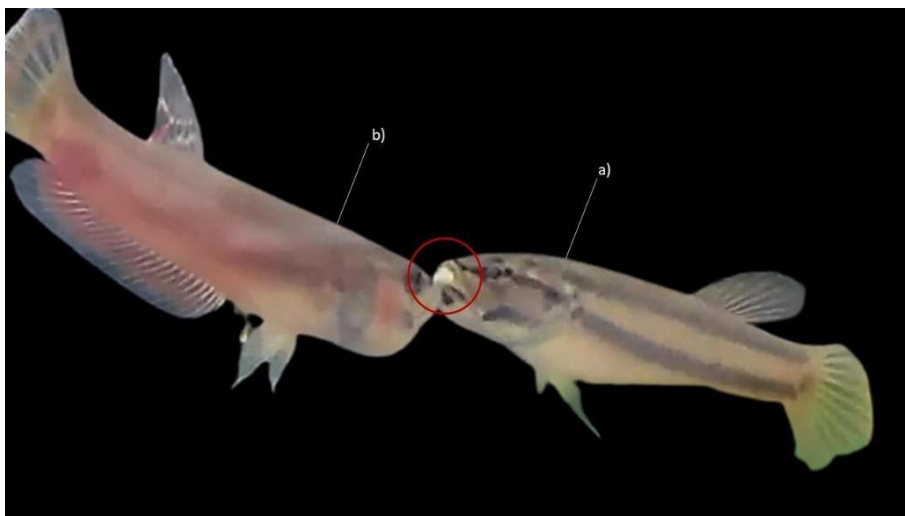


Figure 4.26: Male *B. macrostoma* (b) attempted to pick up the deposited eggs (circled)



Figure 4.27: Mouthbrooding of *B. macrostoma*; a) *B. macrostoma* in normal condition, b) *B. macrostoma* during mouthbrooding

(D) Hatching of the eggs

Based on the observation, the male *B. macrostoma* released the fully hatched eggs after four weeks. Once the fry was able to swim and forage freely, they were isolated from the male *B. macrostoma*.

4.4.1.2.2 Larval Development

The documentation of *Betta macrostoma* larval development was carried out using a stereomicroscope (OLYMPUS SZX7) connected to a computer. Figure 4.28 presents a photographic series of seven distinct developmental stages (labeled A–G), capturing the morphological changes from hatching to the juvenile phase.

In Stage A, the larvae appear newly hatched, characterized by a rounded head, prominent yolk sac and underdeveloped fins. Progressing to Stage B, the yolk sac begins to shrink and slight elongation of the body is observed. At Stage C, pigmentation starts to appear on the dorsal side, and the fins exhibit early signs of differentiation.

By Stage D, the yolk is nearly fully absorbed, the eyes are more developed, and the pectoral fins show increased mobility. In Stage E, further elongation of the body and enhanced pigmentation are evident, with fin rays beginning to separate. Stage F marks the emergence of defined body stripes, particularly along the lateral line, and the caudal and dorsal fins become more pronounced.

In the final observed stage, Stage G, the larvae closely resemble juvenile fish, with fully formed fins establishing pigmentation patterns and notable development of operculum and fin spines. The appearance of well-defined lateral stripes and fin morphology in this stage indicates the transition from larval to juvenile stage, signifying readiness for active swimming and exogenous feeding.

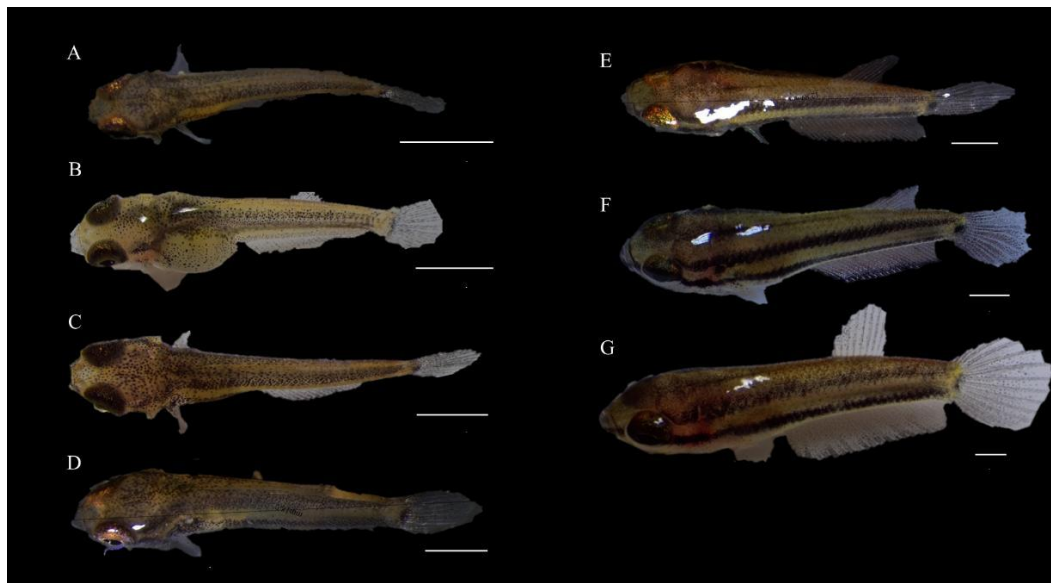


Figure 4.28: Documentation of larvae *B. macrostoma*. A) Newly hatched *B. macrostoma*. B) Flexion stage of *B. macrostoma*. C) Post-flexion stage of *B. macrostoma*. D) End of the post-flexion stage. E) Pre-juvenile stage. G) Juvenile stage

Figure 4.29 illustrates the larval growth trajectory of *B. macrostoma* over a 120-day post-hatching (DAH) period, based on total length (mm) measurements. The larvae exhibited

a steady and progressive increase in total length, beginning at 6.94 mm on Day 1 and reaching 14.64 mm by Day 120, indicating substantial somatic growth under the given rearing conditions.

The early growth phase (Days 1–30) showed a relatively gradual increase in length, suggesting a typical post-hatching acclimatization period. Notable acceleration in growth was observed from Day 60 onwards, indicating a transition to more rapid developmental phases, possibly associated with enhanced feeding efficiency or improved digestive capability. This growth pattern is consistent with developmental trends reported in other *Betta* species, such as *B. rubra* (Priyadi et al., 2024), which also exhibited prolonged growth periods and similar morphometric trends.

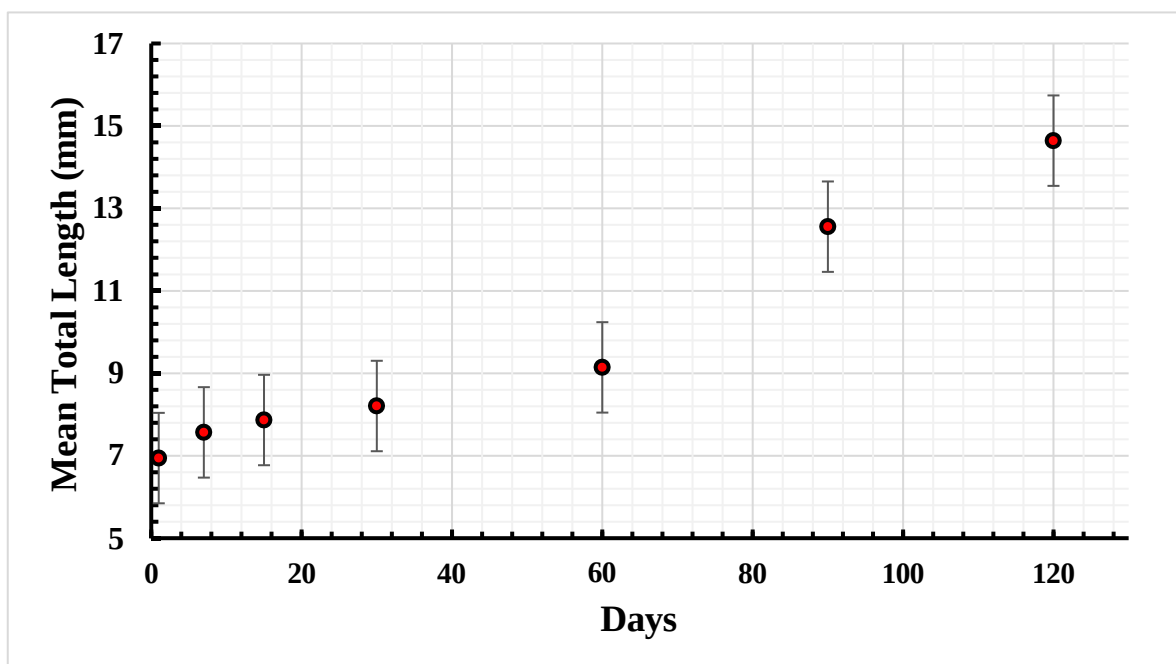


Figure 4.29: Growth performance of *B. macrostoma*'s larvae (total length)

4.4.1.3 *Betta taeniata*

4.4.1.3.1 Breeding Protocol

(A) Sampling of wild *B. taeniata* from the native habitat

B. taeniata were collected from the stream located near Kampung Lanchang in Serian at Serian Division (01°09'36.1" N 110°29'07.7" E) on 24th July 2022.

(B) Conditioning and pairing of broodstock

Broodstock selection was done based on their healthy physical characteristics, the absence of any physical disabilities and maturity characteristics. Mature males display iridescent blue-green coloration on the opercle region and exhibit a brownish-red colouration on the unpaired fins, with distinct dark margins and a light iridescent edge, particularly along the anal and caudal fins. These features are especially prominent during display or antagonistic behaviour. In contrast, mature females exhibit fainter fin margins, lack prominent opercular iridescence and show darker markings or spots on the unpaired fins. Figure 4.30 illustrates the comparison of male and female *B. taeniata*.



Figure 4.30: Comparison between male and female *B.taeniata*. a) Male *B. taeniata* b) Female *B. taeniata*

(C) Courtship and mouthbrooding

Although photographic evidence is lacking, the spawning behaviour of *Betta taeniata* is believed to involve a characteristic spawning embrace in which the male wraps around the female during oviposition. The female releases eggs during this embrace, and the male simultaneously releases sperm, enabling external fertilisation of the eggs. Following fertilisation, the male collects the eggs into his mouth for incubation. This spawning embrace and egg transfer process is repeated several times until all eggs have been deposited and collected. The male then orally incubates the fertilised eggs in his buccal cavity (Figure 4.31) until hatching occurs.



Figure 4.31: Male *B. taeniata* (circled) incubated the eggs in its mouth

(D) Hatching of eggs

Based on the observation, the male *B. taeniata* released the fully hatched eggs after four weeks. Once the fry were able to swim and forage freely, they were isolated from the male *B. taeniata*.

4.4.1.3.2 Larval Development

The developmental stages of *B. taeniata*'s larvae were documented under a stereomicroscope (OLYMPUS SZX7) connected to a computer. Figure 4.32 illustrates the progressive morphological changes from Day 1 to Day 15 after hatching. In Stage A, the larvae exhibit a relatively underdeveloped fin structure and a translucent body. By Stage B, slight pigmentation and fin extension become visible. Subsequent stages (C–E) show a gradual development of caudal and dorsal fins, increased pigmentation, and enhanced body proportions which indicate continuous morphological maturation.

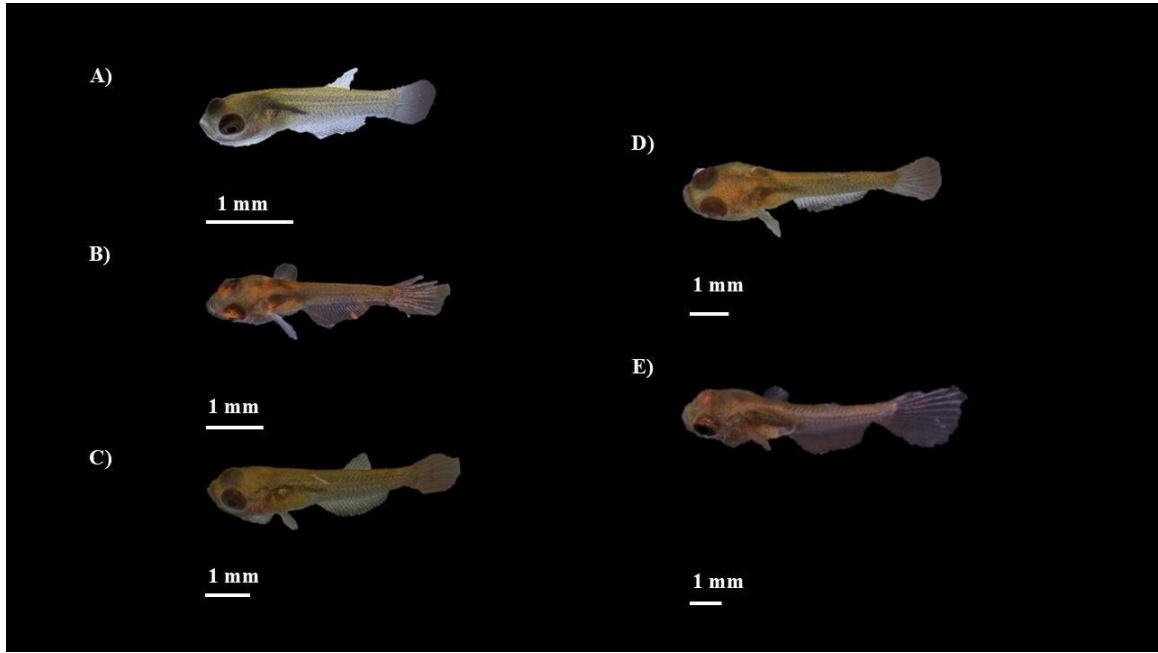


Figure 4.32: Documentation of larvae *B. taeniata*: A) Newly hatching of *B. taeniata*, B) Flexion stage, C) End of flexion stage, D) Post-flexion stage, E) End of Post-flexion stage

Figure 4.33 presents the mean total length (TL) of the larvae which increased from 7.02 mm on Day 1 to 8.07 mm on Day 15, signifying a modest but steady growth trend. Compared to *B. macrostoma* and *B. ibanorum*, the growth rate of *B. taeniata* appears slower, potentially due to species-specific metabolic rates or physiological constraints.

These findings are consistent with previous observations on *B. rubra* by Priyadi et al. (2024), which also demonstrated gradual and consistent growth patterns under well-controlled rearing conditions.

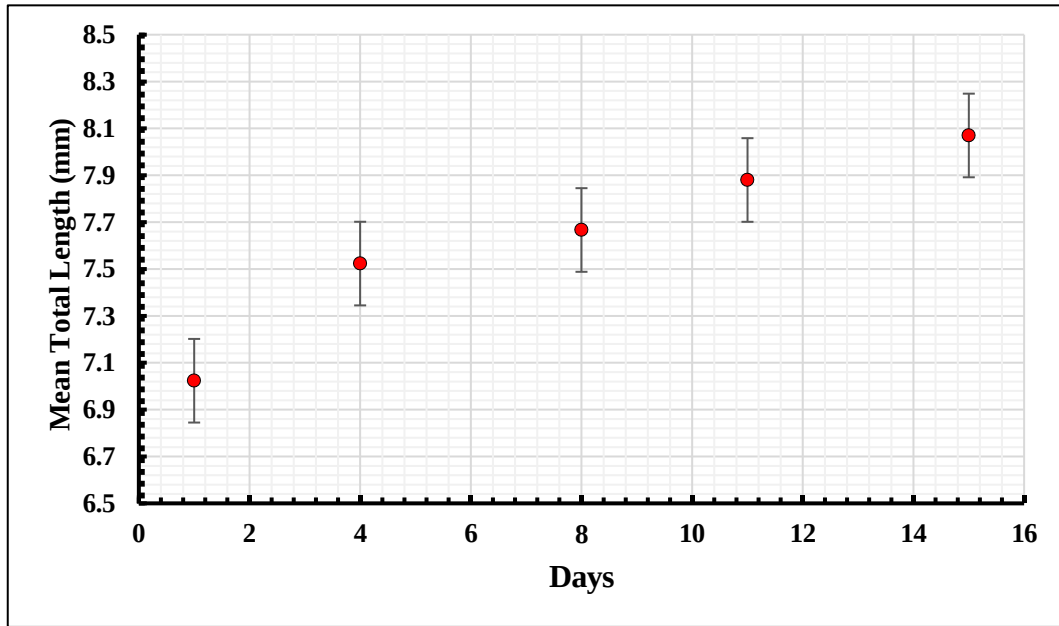


Figure 4.33: Growth performance of *B. taeniata*'s larvae (total length)

4.4.2 Bubble Nester

Figure 4.34 illustrates the standardized breeding protocol implemented for bubble-nesting *Betta* species under controlled laboratory conditions. The process is initiated by sampling wild *Betta* specimens from their native habitats (Stage 1), followed by a conditioning and pairing period of approximately 2 weeks to ensure sexual maturity and compatibility of the broodstock (Stage 2).

Subsequently, bubble nest construction by the male marks the onset of spawning behaviour (Stage 3), typically observed within one week. The presence of fertilized eggs (Stage 4) is monitored over 3–4 days until embryonic development progresses. Egg hatching (Stage 5) occurs within two days, after which the larvae are transferred for larval rearing under controlled laboratory conditions (Stage 6).

Bubble-nester's breeding protocol

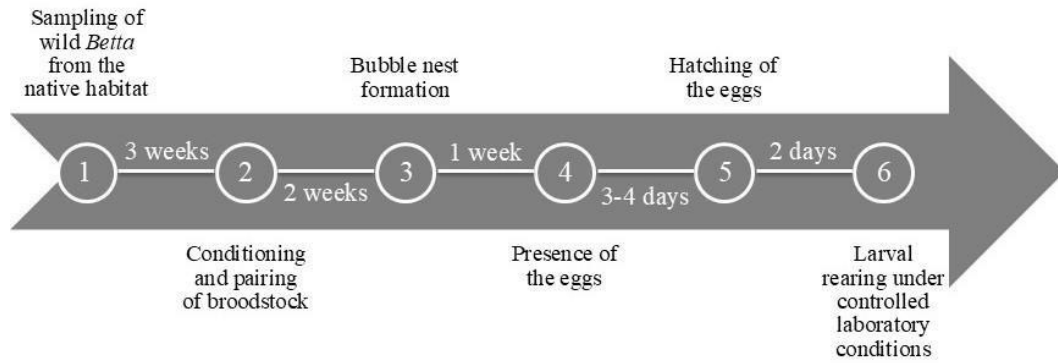


Figure 4.34: Captive breeding protocol for bubble-nest *Betta* species

4.4.2.1 *Betta brownorum*

4.4.2.1.1 Breeding Protocol

(A) Sampling of wild *B. brownorum* from the native habitat

B. brownorum (Figure 4.35) were collected from peat swamps located at Matang area near Musang King Durian farm (01°34'45.8" N 110°15'51.7" E) on 25th March 2024.



Figure 4.35: *B. brownorum* sample collected from the wild habitat

(B) Conditioning and pairing of broodstock

Pairing was done after three weeks of fish being caught. Broodstock selection was done based on their healthy physical characteristics, the absence of any physical disabilities and maturity characteristics. In both male and female *B. brownorum*, a prominent iridescent green lateral blotch is visible. However, the male exhibits a more intensely pigmented reddish-magenta body colour and elongated pelvic fins with a falcate shape and a white filamentous tip, which is less developed in the female. Figure 4.36 shows the comparison between male and female *B. brownorum*. Figure 4.37 also presents the male *B. brownorum* while the female was shown in Figure 4.38

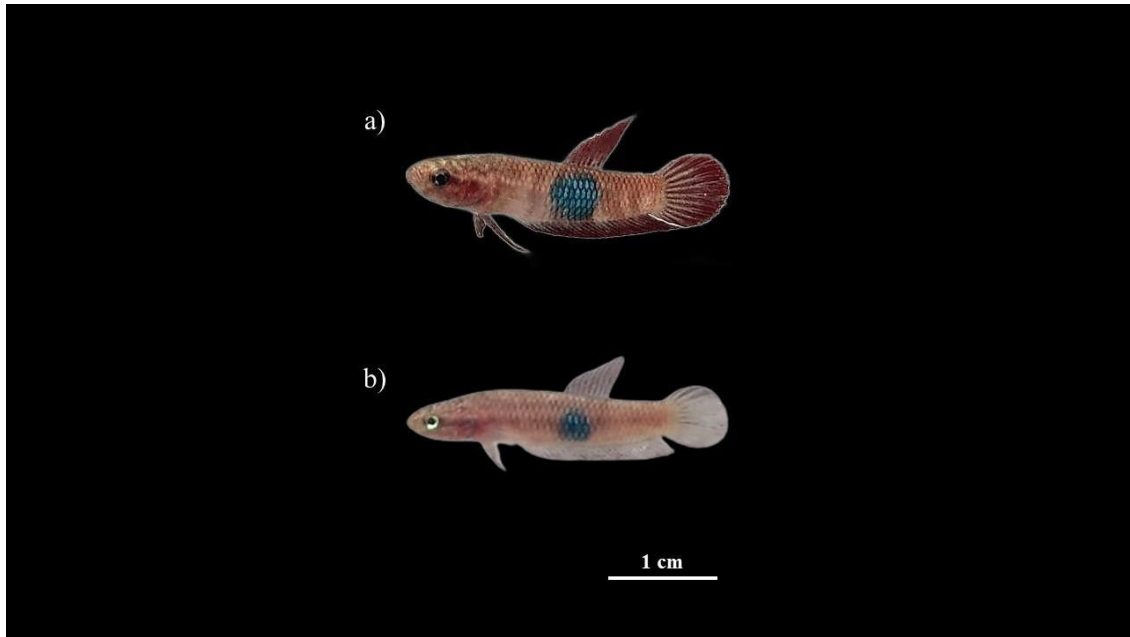


Figure 4.36: Comparison between adult male and female *B. brownorum*, a) Male *B. brownorum*, b) Female *B. brownorum*

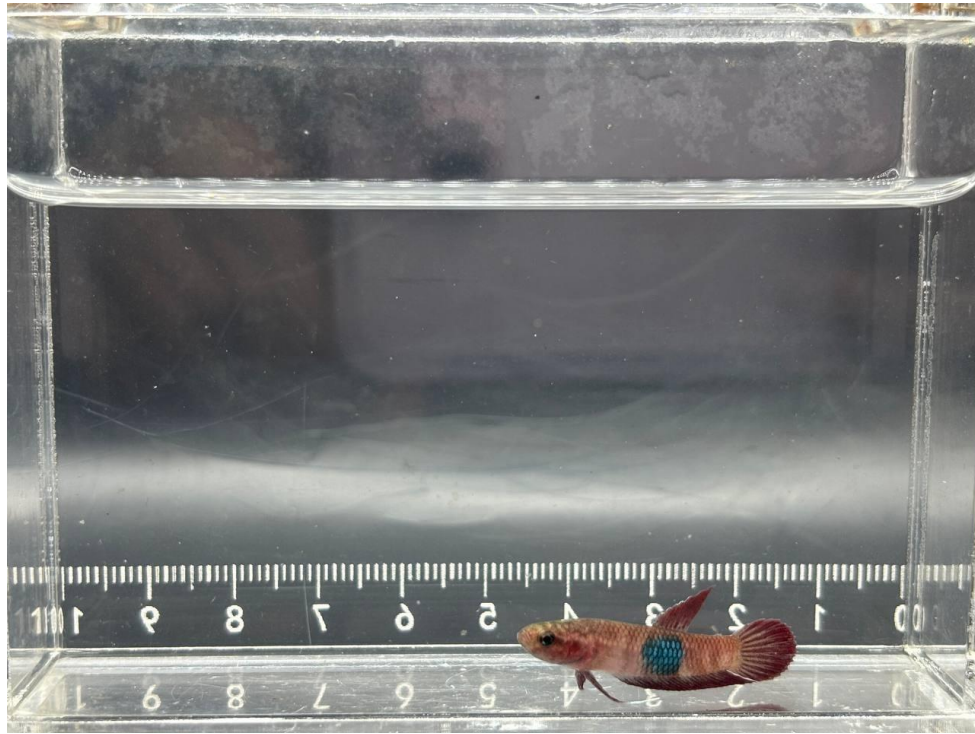


Figure 4.37: Male *B. brownorum*

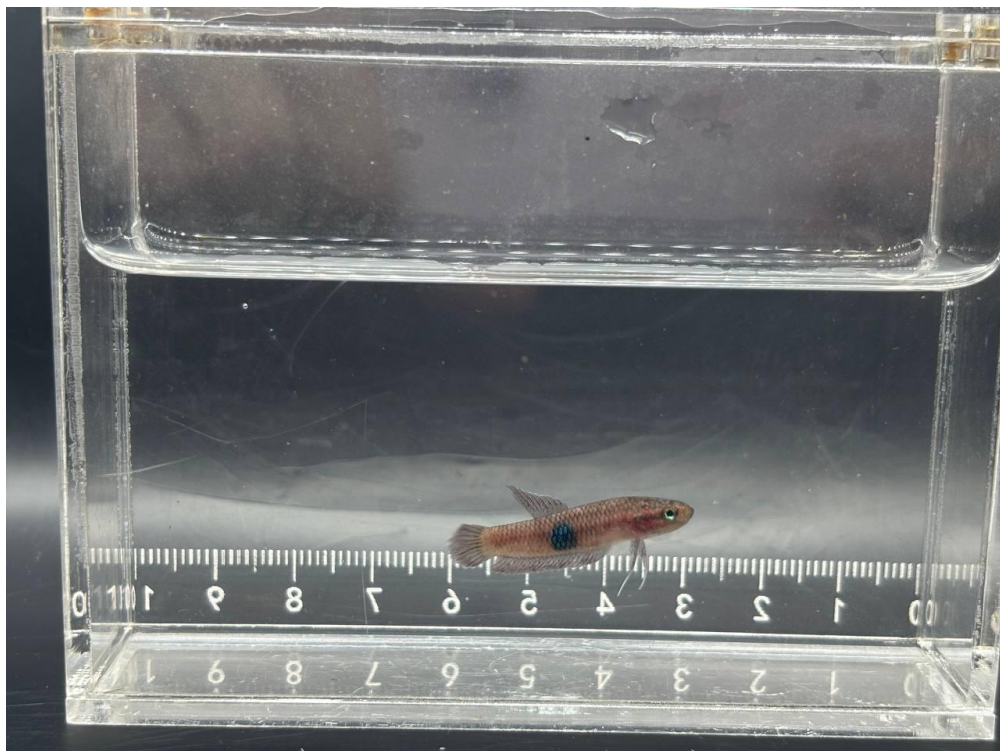


Figure 4.38: Female *B. brownorum*

(C) Bubble-nest formation

The breeding protocol continued with the male *B. brownorum* constructing a bubble nest. The bubble nests were built at the water surface near the polystyrene cup as shown in Figure 4.39. Spawning occurred after the bubble nest construction was completed. In the process of spawning, the male and female become entangled with one another. Male *B. brownorum* released sperm to fertilize the eggs released by the female during the embrace as shown in Figure 4.40.



Figure 4.39: Bubble-nest construction near the polystyrene cup



Figure 4.40: Male and female *B. brownorum* entangled around each other

(D) Presence of eggs

After the spawning process, it was noticed that the male *B. brownorum* placed the eggs at the bubble-nest and guarded the eggs. Figure 4.41 illustrates male *B. brownorum* placed and guarding the eggs at the bubble-nest.



Figure 4.41: Male *B. brownorum* placed and guarded the eggs at the bubble-nest

(E) Hatching of eggs

Based on the observation, the eggs that were placed at the bubble-nest began to hatch after three to four days. The hatched larvae were noticed to be hanging at the bubble nest (Figure 4.42). The broodstock were removed from the breeding tank right after the larvae hatched to avoid the larvae being eaten by the broodstock. Figure 4.43 shows the newly hatched larvae of *B. brownorum*.



Figure 4.42: Hatched larvae hanging at the bubble nest (circled)



Figure 4.43: Newly hatched larvae were noticed on top of the polystyrene. (circled)

4.4.2.1.2 Larval Development

The early development of *B. brownorum* larvae was shown in Figure 4.44 along with the scientific drawing of the larvae in Figure 4.45. On Day 1, observations revealed that the larvae still retained and consumed their yolk sacs, which served as their primary source of nutrition, as supported by Permana et al. (2024) and Riddle and Hu (2021). This is a critical stage as the yolk provides essential nutrients required for the initial development of the larvae, which supports their growth until they can transition to external food sources (*Artemia* nauplii), as evidenced by the increment in total length by the third day.

By the third day, the larvae had transitioned to external feeding, by consuming *Artemia* nauplii. This shift to an external food source supports continued growth and development.

The retention of yolk sacs on the first day is a typical characteristic of *B. brownorum* larvae, indicating a developmental phase where endogenous resources are crucial for sustaining early growth. The introduction of *Artemia* nauplii on the third day provides a rich source of nutrients, further promoting larval growth and development as suggested by Policar et al. (2007).

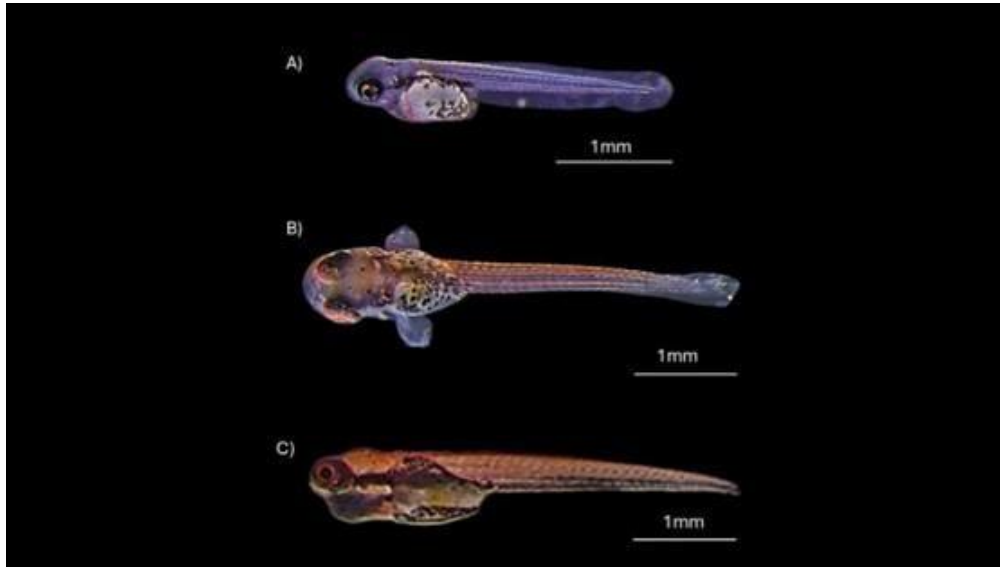


Figure 4.44: The newly hatched *B. brownorum* was observed under the microscope. A) Larvae *B. brownorum* Day 1 under microscope (2.5X). B) Top view of larvae *B. brownorum* Day 3 under microscope (1.5X). C) Side view of larvae *B. brownorum* Day 3 under microscope (1.5X).

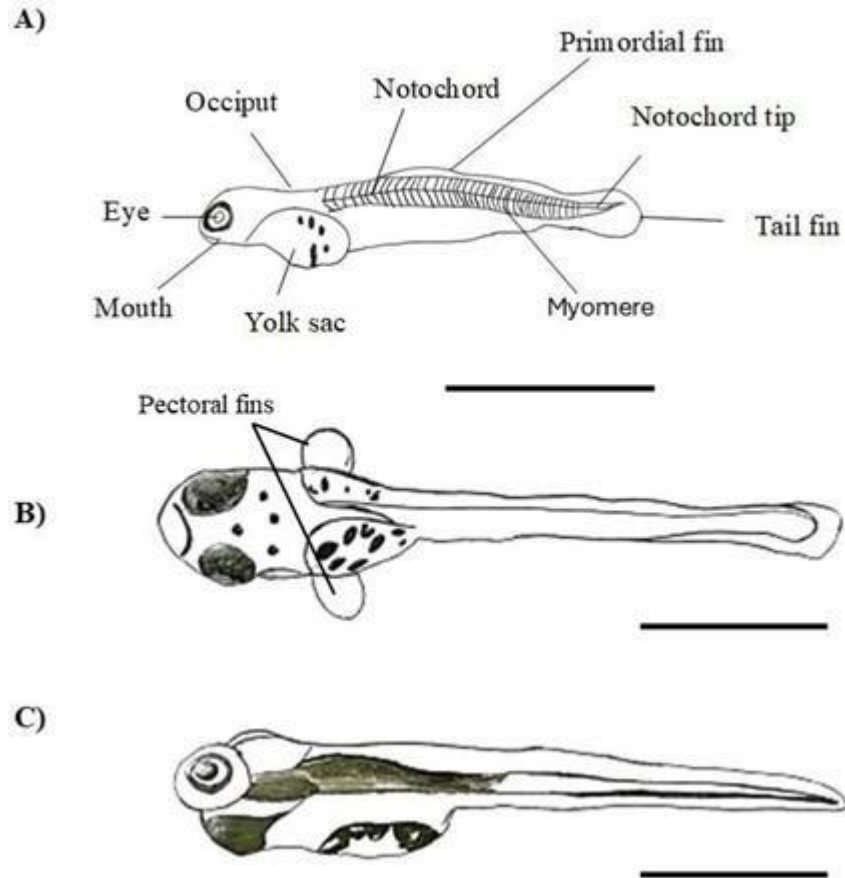


Figure 4.45: The scientific drawing of newly hatched *B. brownorum*. A) Larvae *B. brownorum* Day 1. B) Top view of larvae *B. brownorum* Day 3. C) Side view of larvae *B. brownorum* Day 3

Figure 4.46 illustrates the total length of *B. brownorum* larvae from Day 1 to Day 3 post-hatching. A notable increase in length was observed, from 3.38 mm to 4.42 mm, coinciding with the transition from endogenous yolk feeding to exogenous feeding using *Artemia* nauplii. However, larval observations could only be conducted for three days due to high mortality beyond this period.

This limited survival is likely attributed to suboptimal water quality conditions associated with the use of *Artemia*, a brackish water species commonly used in aquaculture. When introduced into a freshwater system, *Artemia* nauplii may contribute to increased organic

waste due to uneaten feed and rapid decomposition, leading to elevated ammonia levels and reduced dissolved oxygen which are harmful to sensitive larval stages.

Studies have shown that maintaining optimal water quality is critical during the early development of freshwater fish larvae. The deterioration of water conditions in this study likely caused stress, reduced feeding efficiency, and ultimately mortality among the *B. brownorum* larvae. To address this, future protocols may consider freshwater live feeds such as *Moina* or *Daphnia*, which are better suited to the larvae's aquatic environment. These feeds are not only nutritionally suitable but also help maintain water quality due to their motility and slower decomposition rate. Implementing freshwater live feeds may enhance larval survival and allow for longer monitoring periods in subsequent studies.

Another contributing factor to the early mortality of *B. brownorum* larvae may be the stress induced by handling and transferring the parental fish. In bubble-nesting *Betta* species, the male is responsible for constructing the nest and tending to the fertilized eggs and larvae. Disturbances caused by poor transfer techniques, such as abrupt netting or relocation of either parent, may damage the integrity of the bubble nest, dislodge the eggs, or trigger stress-induced filial cannibalism. These outcomes can compromise larval survival and development during the critical early post-hatching period.

According to Matsumoto et al. (2018), filial cannibalism which is the act of a parent consuming its own offspring, is an adaptive reproductive strategy observed in various animal species, including *B. brownorum*. This behaviour, although once considered maladaptive, is now understood as a mechanism to optimize reproductive success under environmental or physiological stress. Factors such as limited resources, poor water quality, or the energetic cost of caring for large broods may induce the parent to selectively consume part of the offspring, potentially increasing the survival chances of the remaining few.

In this study, the possibility that parental disturbance during transfer led to nest damage or induced cannibalism cannot be ruled out. Such disturbances, coupled with environmental stressors, likely contributed to the short observation period and high mortality observed in the larvae. These findings underscore the importance of minimizing handling stress during breeding experiments involving delicate species, such as *B. brownorum*, and reinforce the need for optimized transfer protocols to support successful larval development.

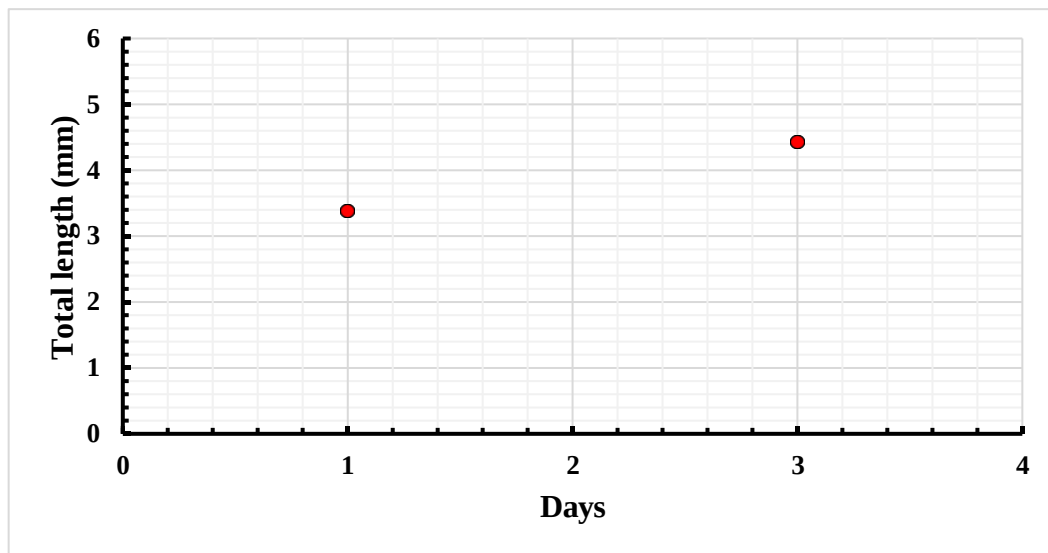


Figure 4.46: Growth performance of *B. brownorum*'s larvae (total length)

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study successfully documented a checklist of seven wild *Betta* species from Sarawak and developed captive breeding protocols for four of them which were *B. ibanorum*, *B. macrostoma*, *B. taeniata*, and *B. brownorum*. Species-specific breeding behaviour was observed and recorded, including paternal mouthbrooding and bubble nesting strategies. Morphometric analyses revealed species- and sex-based variations in body size, while larval development data indicated consistent growth under controlled conditions. Environmental parameters such as water temperature, pH, and dissolved oxygen levels were found to vary across habitats and may influence the natural distribution of these species. The study contributes essential empirical data to support ex-situ conservation, fill gaps in reproductive biology, and encourage future efforts in the preservation and sustainable use of native *Betta* populations. Continued research and habitat protection are crucial for ensuring the long-term survival of these ecologically and economically valuable freshwater fishes.

5.2 Recommendations

Based on the findings of this study, it is recommended that future research expand the sampling coverage to other regions in Sarawak to uncover potentially undocumented *Betta* species and improve the completeness of species distribution data. Long-term captive breeding trials should be continued and supported with genetic monitoring to maintain healthy, diverse broodstock populations and prevent inbreeding. Standardisation of environmental parameters such as temperature, pH, and photoperiod is also encouraged to

enhance breeding success and larval survival. Additionally, the food for the larvae during captive breeding should be changed from *Artemia* nauplii to *Daphnia* or *Moina* to increase the survival rate of the larvae.

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APPENDICES



Mr Nasir Jumat (left) assisted in sampling at Matang site



Dr Syafiq (left) and Armeen(right) packing the collected fish in plastic bags



Mr Nasir Jumat (front) and Armeen (back) conducted sampling at Sematan site



Mr Nasir Jumat sampling *B. akarensis* using scoop net at Jalan Kemuyang, Sibul



Lab assistant, Mr Nazri fishing *B. akarensis* at Durin Bridge, Sibul



Mr Nasir Jumat conducted sampling at Serian



Local guider from Miri showing way to get to the sampling site



Jeffery conducted water quality reading at Marudi, Miri

