

Article

Reproductive Ecology and Early-Life Morphological Development of Krabi Mouth-Brooding Fighting Fish *Betta simplex* Kottelat, 1994 (Actinopterygii: Osphronemidae)

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Abstract

The Krabi mouth-brooding fighting fish, *Betta simplex* Kottelat, 1994, is a critically endangered and endemic fish species in Krabi province, Southern Thailand. Little information is available on its reproductive ecology and early developmental morphology, which are essential for studying its conservation. Generally, *B. simplex* is considered an adaptable animal that can tolerate lower alkalinity and higher hardness compared to its natural environment conditions. In this study, wild broodstocks of *B. simplex* were collected from the reported type localities and bred in captivity under laboratory conditions for size-series collection. Some biological aspects of *B. simplex* in its natural environmental conditions were determined. We found that its flaring and mating behavior was similar to those of bubble-nesting fighting fish but did not involve bubble-nest building. The fertilized eggs and pre-flexion larvae were nurtured in the mouth cavity of parental males within 11–12 (mode = 11) days after fertilization (DAF). The first-release offspring developed to the post-flexion stage with a body size of 4.39 ± 0.01 mm of standard length (SL; $n = 6$) and then to the juvenile stage within 30 days after release with 11.72 ± 0.62 mm SL ($n = 4$). Thus, we propose the following linear regression equation for growth prediction by age (DAF) and body size (SL; mm): $\text{age} = 0.2425 \text{ SL} + 1.7036$ ($r^2 = 0.9549$). The findings of this study will deepen our knowledge of the reproduction and ontogeny of *B. simplex* and contribute to its future conservation and management.

Keywords: conservative breeding; fish larva; ontogeny; stage transformation; wild betta



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1. Introduction

The Krabi mouth-brooding fighting fish, *Betta simplex* Kottelat, 1994, is a critically endangered and endemic species restricted to Krabi province in Southern Thailand. It usually lives in small lakes and creeks connected to limestone rivers with dense shoreline vegetation. It is distinguished from the other species of its species group, the *B. picta* group, by a combination of a dark broad subdistal stripe along the anal fin and ventral part of the caudal fin, a rounded end tip of the anal fin, and fewer anal fin rays (22–23 vs. 24–26) [1–3]. Currently, fighting fish aquaculturists recognize two *B. simplex* morphs, namely ‘type I,’ which has characters similar to the original description, and ‘type II,’ which contains green

or blue turquoise spots scattered around the caudal and the black band at the distal tip of the pelvic fin. However, these two morphs are still recognized as the same species [4].

B. simplex has been known since its original description in 1994. However, essential biological data, especially on its reproductive biology (the optimal conditions for its living in both natural and breeding environments) and reproductive biology, such as the number of offspring per crop, age, and stage transformation, are still lacking. Currently, it has a threatened status because of the decrease in its natural population caused by commercial collecting, aquarium trades, and habitat alteration by agricultural and anthropogenic activities. The Union for Conservation of Nature and Natural Resources (IUCN) had recently classified *B. simplex* as a critically endangered species [5]. In addition, according to the Government Gazette of Thailand, the Department of Fisheries of Thailand has banned the export of this fish species from the Kingdom of Thailand [6].

In this study, we aimed to determine the environmental conditions of *B. simplex* in its natural habitat compared to those species in captivity. Some fish samples were caught and kept in broodstocks to study their morphological development. Furthermore, captive fish were studied for several biological aspects, including mating and parental care behavior, fecundity, growth, morphology, and stage transformation. The findings of this study will deepen our knowledge of the reproduction and ontogeny of *B. simplex* as well as support the future conservation and management of its natural populations and ornamental fish breeding.

2. Materials and Methods

2.1. Study Area, Habitat Observation, and Broodstock Collection

Broodstocks were taken from a small lake (called Sra-Keaw) with the geographic coordinates of 8°10'7.45" N and 98°48'27.67" E and a connected creek (called Klong Sra-Keaw) that receives overflow water from Lake Sra-Keaw, located in the KaoThong subdistrict of the Muang district in Krabi province [7] (Figure 1). Therefore, we expected to observe *Betta simplex* close to its original type specimen locality, according to Kottelat, 1994. Broodstocks were collected using a dip net and 'bung kee,' a local plastic basket with a cockle shell-like shape. Various environmental parameters were recorded at both the sampling sites and breeding capacities in the morning (8:00–10:00 am) with five replications. Dissolved oxygen (DO) content, water temperature, conductivity, and salinity were measured in situ by using a DO meter (YSI Pro 2030—Fondriest Environmental, Inc., Fairborn, OH, USA). Turbidity was measured using a portable turbidity meter (Lamotte Model, 2020—Lamotte Company, Chestertown, MD, USA). While the water quality parameters required a laboratory, a 1 L water sample was collected and analyzed as follows: Hardness, alkalinity, ammonia–nitrogen, nitrite–nitrogen, and orthophosphate–phosphorus were analyzed according to [8,9]. Nitrate–nitrogen was analyzed using the cadmium reduction method. This study was performed during the early rainy season (July 2021), which is the expected period before fish spawning. Mature fish were selected for broodstock and transported to the Laboratory of Ichthyology and Aquatic Ecology, Department of Fishery Biology, Kasetsart University.

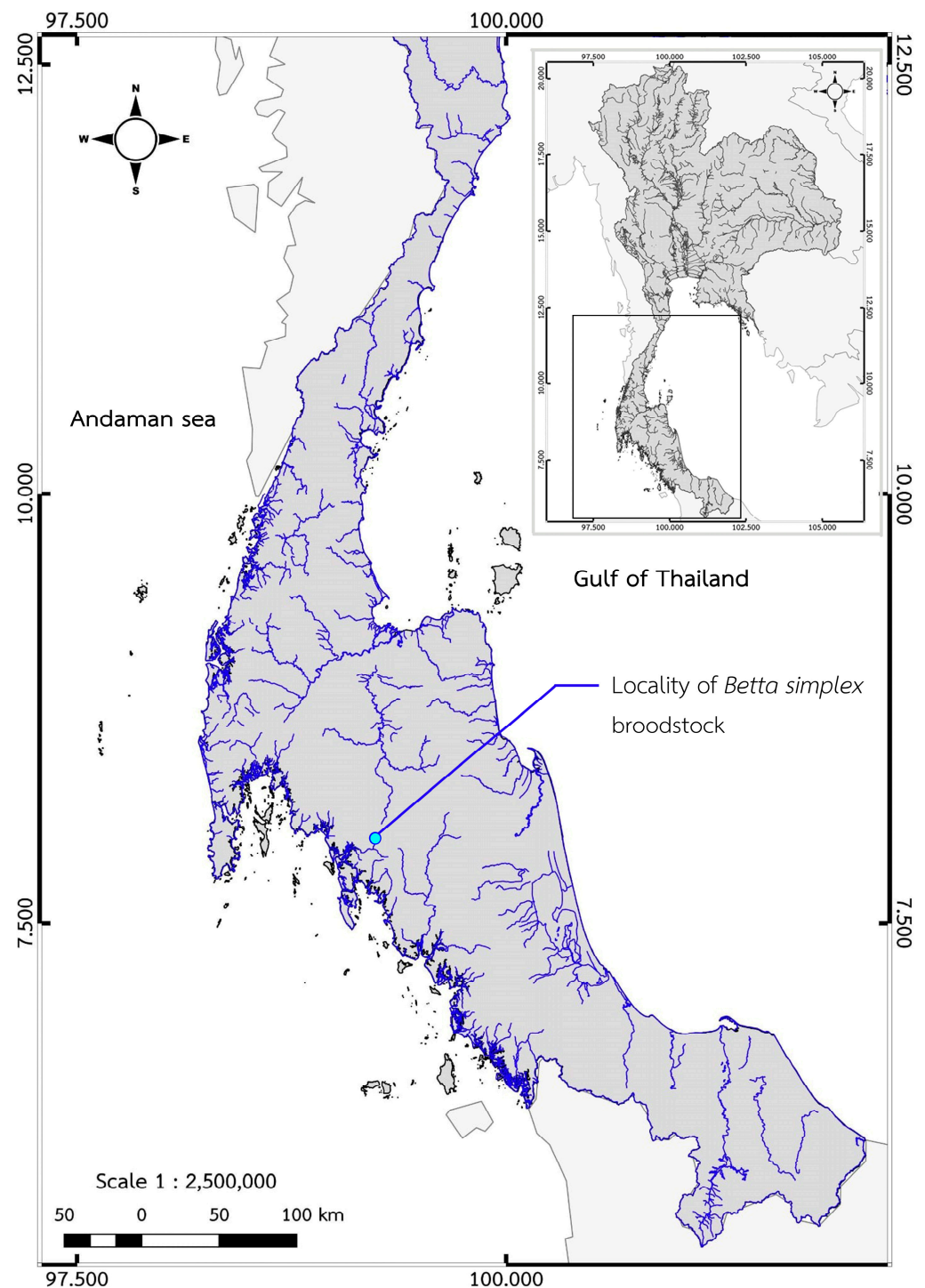


Figure 1. Location of the observation area and broodstock locality of *Betta simplex* in Thailand.

2.2. Broodstock Collection and Breeding

Five broodstock couples (2.5–3.5 mm SL) were acclimatized and kept in three glass tanks of dimensions 30 cm × 90 cm × 30 cm, containing 68 L of rearing water. They were provided with submerged plants (*Anubias* spp.) 2 cm in diameter and PVC pipes 10 cm in length as shelters and fed twice daily with adult brine shrimp (*Artemia salina*) and grindal worms (*Enchytraeus buchholzi*). Fully mature broodstocks (4th gonadal stage) mated during the early night, and parental males were noticed the next morning. Parental males can be easily distinguished from the normal males as they bulge out their buccal membrane (the anteroventral side of the operculum), stop eating, and hide under the vegetation or in PVC

pipes. Therefore, we used this clue to assume that the first fertilizing day was the evening of the day before the parental males were observed. Fertilized eggs gradually developed and were expected to hatch within the next 7–8 days. During this period, interference with the parental males was avoided to prevent the premature release or devouring of their brooding eggs. After hatching, the mouth-brooding males were isolated to avoid cannibalism. After being released from their fathers, all offspring were fed water fleas (*Moina macrocopa*) and brine shrimp nauplii. The living and reproductive behaviors of the fish, as well as water quality, were recorded throughout this study.

2.3. Specimen Size-Series Collection

Larval fish were sampled according to [10], who required the collection of at least 20 specimens from a series of different age classes. However, we could not obtain the recommended number of specimens because our broodstocks had low fecundity and survival rates during each nursing period. Thus, we collected 3–5 larval fish samples per size series. At this stage, an 11-period size series comprises offspring at 0, 3, 6, 9, 12, 15, 20, 25, 30, 35, and 40 days after release (DAR). The specimens were anesthetized by using a $200\text{ }\mu\text{g}\cdot\text{L}^{-1}$ eugenol solution before being preserved in 4% neutralized formaldehyde and deposited in the Kasetsart University Museum of Fisheries (KUMF).

2.4. Specimen Examination and Data Analysis

Developmental stages were classified according to [11]. Morphological measurements were performed according to [12] and were recorded to the nearest 0.01 mm (Mitutoyo, Kawasaki, Japan, 150 mm range ± 0.01 mm) using a digital vernier caliper. The measured morphological parameters included notochord length (NL), standard length (SL), head length (HL), head depth (HD), head width (HW), eye diameter (ED), pre-anal length (PAL), pre-dorsal length (PDL), and body depth (BD) (Figure 2A,B). In this study, the first stage of *B. simplex* offspring release was the post-flexion stage and development to the juvenile stage. Therefore, the SL parameter was used to record growth rates at different ages, from the newly mouth-released to the post-flexion and juvenile stages. At this stage, the age of the offspring was counted on the day after fertilization (DAF) and the DAR. Age and size data were analyzed using the correlation coefficient (r^2) and a simulated equation.

Specimens from each developmental stage were measured for four different body parameters (head length, body depth, pre-dorsal length, and pre-anal length). The measurements were performed by photographing the specimens with a scale bar mark under a stereomicroscope. Body dimensions are expressed as a percentage of the standard length (%SL). Additionally, three head dimensions (head depth, head width, and eye diameter) were measured and expressed as a percentage of head length (%HL). Myomeres were counted in post-flexion specimens that had transparent bodies, and fin rays were counted in juvenile specimens that had completely developed fins. The water quality and specimen body dimensions were expressed as means and standard deviations. Meristic features, such as myomeres and fin ray counts, were reported as modes with minimal and maximal values. Melanophore (pigmentation) patterns were recorded as ‘punctate spots’ and ‘stellate spots,’ according to [13], whereas dark pigments on head regions were recorded as “bands,” longitudinal dark lines on the sides of the body as “stripes,” and the rounded dark band present at the base of the caudal fin as the “caudal spot,” according to [14] (Figure 2C). Water qualities of broodstock and rearing habitats were expressed by mean and standard deviation, and the differences between each parameter were compared using a *t*-test statistic, which was performed by the Jamovi program, version 2.7.14.

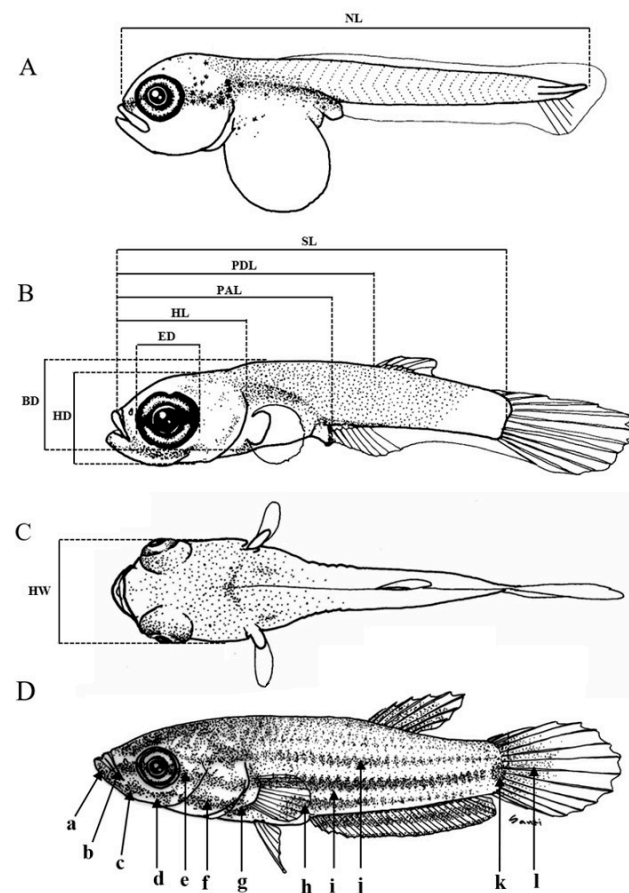


Figure 2. General illustration of mouth-brooding fighting fish yolk-sac larvae (A), post-flexion larvae (B,C), and juveniles (D) showing morphometric characters and dark pigmentation. Abbreviations of body dimensions: NL = notochord length; SL = standard length; BD = body depth; HL = head length; HD = head depth; HW = head width; ED = eye diameter; PAL = pre-anal length; PDL = pre-dorsal length. Abbreviations for pigmentation patterns: a = chin band; b = pre-orbital band; c = first sub-orbital band; d = second sub-orbital band; e = post-orbital band; f = second post-orbital band; g = pectoral fin base band; h = ventral stripe; i = central stripe; j = dorsal stripe; k = caudal spot; l = scattered spots on the caudal fin.

3. Results

3.1. Natural Habitat and Rearing Environmental Conditions

From field observations, *Betta simplex* broodstocks were collected from Lake Sra-Keaw and Klong Sra-Keaw creeks. The lake has a rounded sinkhole of 20 m in diameter. The shoreline is covered with floating plants (*Hydrilla verticillata*), water lilies (*Nymphaea lotus*), Para grass (*Brachiaria mutica*), and some perennial plants. The lake water was blue and clear in the dry season, but it was red-brown and turbid in the rainy season (observation period) (Figure 3a,b), especially in the littoral area. In addition, the lake water had high DO content with a mean $\pm$ standard deviation of $30.43 \pm 1.16 \text{ mg}\cdot\text{L}^{-1}$ and high hardness and alkalinity of 203 ± 6 and $243 \pm 4 \text{ mg}\cdot\text{L}^{-1}$ as CaCO_3 , respectively. However, under natural habitat vs. captivity conditions, the rearing water had significantly low DO content (7.50 ± 0.30 vs. $26.70 \pm 0.26 \text{ mg}\cdot\text{L}^{-1}$; p -value < 0.001), alkalinity (243 ± 4 vs. $31 \pm 2 \text{ mg}\cdot\text{L}^{-1}$ as CaCO_3 ; p -value < 0.001), and hardness (203 ± 6 vs. $69 \pm 24 \text{ mg}\cdot\text{L}^{-1}$ as CaCO_3 ; p -value < 0.001), except for orthophosphate–phosphorus, which showed no significant difference (0.0203 ± 0.0048 vs. $0.0178 \pm 0.0008 \text{ mg}\cdot\text{L}^{-1}$; p -value < 0.310). The other water qualities compared between the broodstock habitat and the rearing habitat are shown in Table 1. Other fish species found in the same habitat were croaking gourami

(*Trichopsis vittata*), blue panchax (*Aplocheilichthys panchax*), harlequin rasbora (*Trigonostigma espei*), and two-spotted barbs (*Barbodes binotatus*).

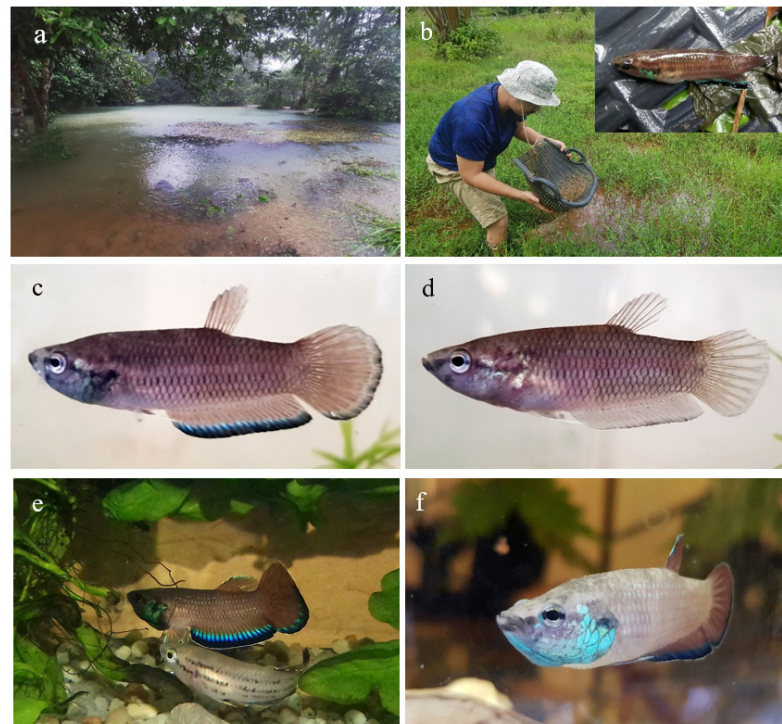


Figure 3. Environmental conditions of broodstock natural habitat (a), sampling site (b), mature male (c), mature female (d), mating behavior (photo by Sutawut Sainuy) (e), and incubating offspring male (f) of *Betta simplex* broodstock.

Table 1. Water qualities (mean $\pm$ standard deviation) comparing the broodstock habitat in the early rainy season with the rearing in captivity of *Betta simplex*.

Water Qualities	Natural Habitat	Rearing Captivities	<i>p</i> -Value
Water temperature ($^{\circ}\text{C}$)	30.43 ± 1.16	26.70 ± 0.26	<0.001 *
Dissolved oxygen ($\text{mg}\cdot\text{L}^{-1}$)	7.50 ± 0.30	6.73 ± 0.14	<0.001 *
Turbidity (NTU)	50.6 ± 1.6	118.67 ± 6.11	<0.001 *
pH	6.5 ± 0.2	7.12 ± 0.11	<0.001 *
Salinity (ppt)	0.1	0.1	1.000
Conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$)	155 ± 6	301 ± 10	<0.001 *
Hardness ($\text{mg}\cdot\text{L}^{-1}$ as CaCO_3)	203 ± 6	69 ± 24	0.008 *
Alkalinity ($\text{mg}\cdot\text{L}^{-1}$ as CaCO_3)	243 ± 4	31 ± 2	<0.001 *
Ammonia–nitrogen ($\text{mg}\cdot\text{L}^{-1}$)	0.1938 ± 0.0058	0.2313 ± 0.0029	<0.001 *
Nitrite–nitrogen ($\text{mg}\cdot\text{L}^{-1}$)	0.0035 ± 0.0006	0.0137 ± 0.0032	0.008 *
Orthophosphate–phosphorus ($\text{mg}\cdot\text{L}^{-1}$)	0.0203 ± 0.0048	0.0178 ± 0.0008	0.310

Remark: * indicates significant differences between groups at $\alpha = 0.05$ ($p < 0.05$).

3.2. Mating Behavior and Parental Care

After broodstocks were acclimatized to the laboratory conditions and sexually developed to mature stages within 2 months, the fish were ready to breed and the males could be distinguished from females by darker black stripes on the marginal edge of the anal fin, thicker caudal fin, and shinier body color, especially the metallic green at the chin and ventral region of the operculum, which were characterized by a pale-yellow bulging belly and an ovipositor in front of the anal fin (Figure 3c,d). Broodstock mating was observed during the mid-to-late rainy season (September to October 2021). Furthermore, 4 of a total of 5 males successfully mated, or 80% successfully bred, and continued to perform parental care of their offspring until the completion of this size-series study. We found that

the mating behavior of the fish was similar to that of bubble nesters. The couple would separate from the school and hide near the provided submerged plants and PVC pipes. The male courts the female by flaring his fins and wrapping his body around her abdomen until she releases the eggs, at which point he injects sperm to fertilize them. Then the male gathers and carries the fertilized eggs that have fallen to the bottom and incubates them in its mouth cavity. The parental males were easily identified by their separation from schools to hide under the provided submerged plants and PVC pipes (Figure 3e,f). Fertilized eggs developed safely in the mouth cavity of males. Offspring were expected to hatch at 7 DAF. Fortunately, one parental male accidentally released some of its yolk-sac larvae on this day (Figure 4a). Normally, parental males released their offspring within 11–12 DAF, mostly at 11 ($n = 3$) DAF. Fecundity, which refers to the number of the first-released offspring, was 63 ± 13 offspring per crop ($n = 4$). Each crop has 45, 61, 71, and 75 offspring per crop, respectively. The lowest fecundity was found at 12 DAF in parental males, with 45 offspring per crop.

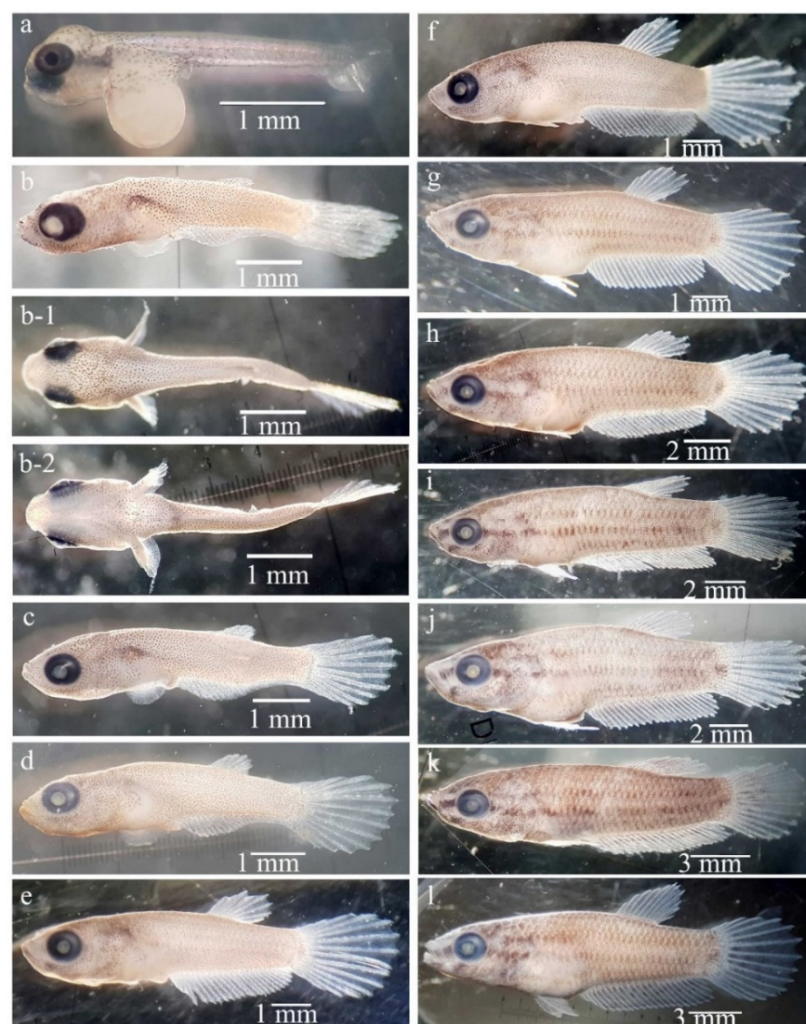


Figure 4. Morphological and pigmentation features of the immature stages of *Betta simplex* (KUMF 7071): (a) 7 DAF yolk-sac larva (parental mouth phase) (NL = 3.18 mm); (b) 11 DAF post-flexion larva (SL = 4.39 mm)—(b-1) dorsal view and (b-2) ventral view; (c) 14 DAF post-flexion larva (SL = 4.86 mm); (d) 17 DAF post-flexion larva (SL = 5.00 mm); (e) 20 DAF flexion larva (SL = 7.07 mm); (f) 23 DAF post-flexion larva (SL = 8.15 mm); (g) 26 DAF post-flexion larva (SL = 8.56 mm); (h) 31 DAF post-flexion larva (SL = 8.92 mm); (i) 36 DAF post-flexion larva (SL = 9.39 mm); (j) 41 DAF juvenile (SL = 11.68 mm); (k) 46 DAF juvenile (SL = 13.18 mm); (l) 51 DAF juvenile (SL = 19.17 mm).

3.3. Growth

One successful offspring litter with the most developed and the highest number of offspring was selected for the developmental study. A 12-specimen series and a total of 43 specimens (including the 7 DAF early-release offspring) were deposited as a museum reference collection under catalog number KUMF 7071. During the parental mouth phase, the 7 DAF specimens had a body size of 3.18 ± 0.00 mm NL ($n = 3$). When released from the parental males at 11 DAF, the length of the offspring was 4.39 ± 0.01 mm SL ($n = 6$). The fish developed to the juvenile stage at 30 DAF with 11.72 ± 0.62 mm SL ($n = 4$) (Table 2). The linear regression equation between age (DAF) and body size (mm SL) was estimated to be $\text{age} = 0.2425 \text{ SL} + 1.7036$ ($r^2 = 0.9549$), as shown in Figure 5. This equation was estimated using an optimized dataset ranging from 11 to 46 DAF because it provided the highest developmental progress in r^2 .

Table 2. Age (DAF or DAR), developmental stage, and body size (mm NL or mm SL) of rearing *Betta simplex*.

DAF	DAR	Developmental Stage (Represented in Figure 4)	Body Size (Number of Specimens, n)
7	-	Yolk sac (Figure 4a)	3.18 ± 0.00 mm NL ($n = 3$)
11	0	Post-flexion (Figure 4b)	4.39 ± 0.01 mm SL ($n = 6$)
14	3	Post-flexion (Figure 4c)	4.87 ± 0.02 mm SL ($n = 4$)
17	6	Post-flexion (Figure 4d)	4.99 ± 0.02 mm SL ($n = 3$)
20	9	Post-flexion (Figure 4e)	7.07 ± 0.04 mm SL ($n = 3$)
23	12	Post-flexion (Figure 4f)	8.17 ± 0.05 mm SL ($n = 3$)
26	15	Post-flexion (Figure 4g)	8.59 ± 0.09 mm SL ($n = 3$)
31	20	Post-flexion (Figure 4h)	8.90 ± 0.45 mm SL ($n = 3$)
36	25	Post-flexion (Figure 4i)	9.39 ± 0.53 mm SL ($n = 3$)
41	30	Juvenile (Figure 4j)	11.72 ± 0.62 mm SL ($n = 4$)
46	35	Juvenile (Figure 4k)	13.20 ± 0.74 mm SL ($n = 4$)
51	40	Juvenile (Figure 4l)	19.30 ± 0.97 mm SL ($n = 4$)

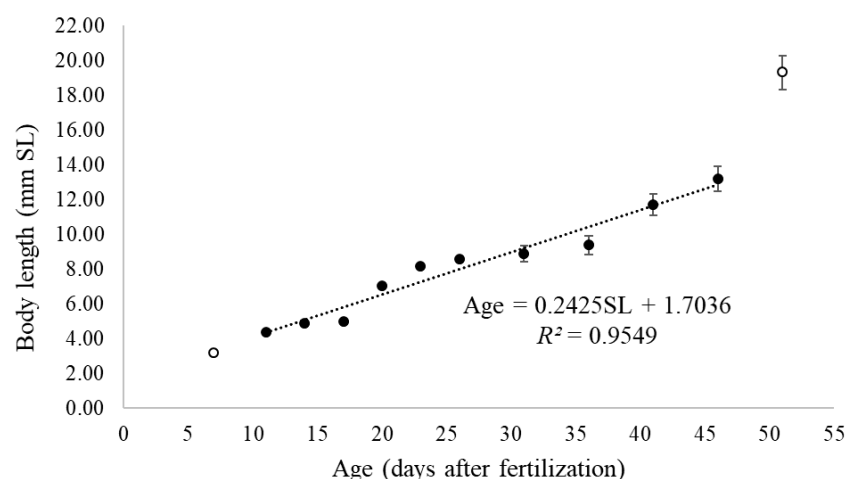


Figure 5. Relationship between age (DAF) and body size (mm SL) for the early-life stage of *Betta simplex*. Black circles indicate the optimized data for the simulated equation. White circles indicate the unoptimized data and are not input for the simulated equation.

3.4. Morphological Description

We described the morphology and produced illustrations of the offspring-release phase comprising the post-flexion and juvenile stages. Furthermore, the yolk-sac stage during the parental mouth phase was described in this series to complete early development as much as possible. Based on the KUMF 7071 referent specimen series, the total number of

myomeres examined from 10 post-larval specimens ranged from 29 to 30 (mode = 29, $n = 9$), which comprised 10–11 (mode = 10, $n = 9$) pre-anal and 19 ($n = 9$) post-anal myomeres. The number of fin rays examined from 12 juvenile specimens included 9–10 (mode = 9, $n = 10$) dorsal fin rays, 11–12 (mode = 12, $n = 11$) pectoral fin rays, 1 spinous-pelvic and 5 ($n = 12$) soft-pelvic fin rays, 2 spinous-anal and 20–22 (mode = 21, $n = 8$) soft-anal fin rays, and 12–13 (mode = 13, $n = 10$) principle caudal fin rays.

At 7 DAF, the yolk-sac stage (parental mouth phase) (3.18 mm NL; Figure 4a) had an oblong and slightly compressed body (BD = 19.68% of NL), small and rounded head (HL = 21.28% of NL), and large, oval-shaped eyes with complete pigmentation (ED = 55.55% of HL). The sagged-down yolk sac contained no oil droplets. The anus opened at the anterior part of the body, with PAL being 44.68% of NL. The fins had no undeveloped rays. The stellate and punctate spots were scattered throughout the anterior half of the body, especially in the post-orbital and gut areas.

At 11 DAF, the post-flexion stage (first offspring release) (4.39 mm SL; Figure 4b) had an oblong (BD = 21.73% of SL), slightly rounded body compressed in the caudal part, large and slightly depressed head (HL = 34.67% of SL; HW = 108.11% of HD), and large and slightly rounded eyes (ED = 46.30% of HL). The gut coiled, and the anus opened at the posterior half of the body (PAL = 56.00% of SL). The pectoral, dorsal, anal, and caudal fin rays begin to develop at the anterior end of or above the fin lobes (PDL = 69.33% of SL). The fading-head and body pigmentation reached the caudal peduncle area.

At 14 and 17 DAF, in the post-flexion stage (4.86, 5.00 mm SL; Figure 4c,d), fin rays were more developed, light pigmentation covered the entire body, but dense pigmentation was found in the sub-orbital and upper-gut areas. At 20 DAF, in the post-flexion stage (7.07 mm SL; Figure 4e), the pectoral, dorsal, anal, and caudal fin rays developed completely, and the dorsal, central, and ventral stripes appeared faintly. At 23, 26, 31, and 36 DAF, the post-flexion stage (8.15, 8.56, 8.92, and 9.39 mm SL; Figure 4f–i) had a pelvic bud and clearly defined pre-orbital, first sub-orbital, and post-orbital bands and caudal spots. Finally, at 41, 46, and 51 DAF, in the juvenile stage (11.68, 13.18, and 19.17 mm SL; Figure 4j–l), pelvic fin rays developed completely, and faded stripes appeared along the upper and submarginal edges of the anal fin.

4. Discussion

The results of the water quality analysis indicated that *Betta simplex* lives in well-oxygenated, highly alkaline, and hard-water conditions and is adaptable to different rearing conditions. In this study, *B. simplex* adapted well to significantly changed alkalinity (243 ± 4 vs. 31 ± 2 mg Ca^{+2} L^{-1}) and hardness conditions (203 ± 6 vs. 69 ± 24 mg Ca^{+2} L^{-1}). However, the high alkalinity and hardness conditions did not exceed the maximum level of tolerance in freshwater animals, according to [15]. Although *B. simplex*, like other air-breathing fishes, can live and adapt well to a wide range of environmental conditions [16], it still needs to live in a favorable environment and have a sufficient food supply.

In this study, the mating behavior of *B. simplex* was usually observed during the early night. The mating couples remained isolated and usually hidden under the aquatic vegetative or bottom substrates. They mated like bubble nesters but did not build bubble nests. It takes longer to reach the embryonic phase (egg phase) at 7 DAF in *B. simplex* than in wild bubble nesters at approximately 1.5 DAF, according to [17]. It has been locally reported previously that *B. simplex* and other mouth-brooding fighting fish can breed in captivity. In a previous study, one or more broodstock couples were introduced to a cement tank provided with vegetative shelter and feed and reared until they had spawned. However, the spawning behavior and reproductive information were not reported. The number of first-release offspring of *B. simplex* was lower than that of bubble nesters (63 ± 13

vs. 274–454 offspring per crop) according to [14]. We assumed that the lower number of offspring of *B. simplex*, which represented mouth-brooders, would be compensated by their intensive parental care to ensure the success of their offspring's survival, which agreed with the fish reproductive strategies of [11,18]. Additionally, we noticed that one of the four parental males with a more extended period of offspring brooding (12 DAF) had the lowest number of offspring at the first release (45 offspring per crop). This was caused by the limited space in the mouth cavity, and slower development occurred due to the lower ambient water temperature condition [19,20].

The first-hatched larva of *B. simplex* differed in appearance from those of bubble-nester fighting fishes and other higher teleosts according to [11,14]. *B. simplex* has a large, sagged-down yolk sac and completely dark eye pigmentation. After hatching, the offspring is still carried in the mouth cavity of the parental male until 11–12 DAF (mostly 11) and reaches the post-flexion stage with a body size of 4.39 ± 0.01 mm SL ($n = 6$). *B. simplex* has a rounded head with a head width of 108.11% HD, while bubble nesters have a compressed head at the same developmental stage. In addition, melanophore pigmentation comprises randomized scattered spots on the head and body of *B. simplex*, whereas bubble nesters have condensed melanophore pigmentation at the head bands and body stripes [10,14].

The simulated equations based on the growth theory of [21] were used for the age estimation of *B. simplex* (Figure 5). Although [21] described an asymptotic growth trend for fishes, we observed a slow and steady growth rate in *B. simplex* larvae in our study. We, therefore, inferred that the simulated equation exhibits a linear growth trend, where the Y-intercept predicts the size at the first release, and the slope predicts the growth rate. This equation estimates the age of the pre-flexion larvae to the juvenile stage for *B. simplex* reared in captivity or collected from the wild. However, the equation was limited by the range of the simulated data, and the environmental conditions of the larvae were therefore optimized for larval specimens at 11 and 45 DAF.

Understanding early-life histories is essential for fish breeding. It is useful for effective fish-rearing planning. This study described the morphological description of the *Betta simplex* for significant development stages such as the yolk-sac, pre-flexion, and juvenile stages, which help aquaculturists' decisions due to rearing protocol. This includes determining the initial feeding time for larvae that have absorbed the yolk sac. The first feeding should be performed with high essential fatty acid feeding, such as docosahexaenoic acid (DHA) and highly unsaturated fatty acids (HUFAs) [22,23]. The enrichment technique using *Artemia* containing DHA and HUFA is suggested to improve the survival rate and growth of the early-feeding teleost fishes [24,25].

Starting the first feeding by *artemia* nauplii for the *B. simplex* offspring should be accomplished on the day after release. At this stage, the result indicated that it had completely absorbed the yolk sac and developed its mouth. However, the muscles and fins are still not fully developed. Therefore, the optimal size and density of the diet for the newly released offspring should be considered for catching and feeding [26,27]. Then, after the young fish grows and transforms, especially in the pre-flexion to post-flexion stage and the post-flexion to juvenile stage, the feeding and water quality control must be optimized. This is necessary because different stages of fish exhibit different living, feeding, and energy-utilizing habits. Therefore, adequate feeding and appropriate age are beneficial for their health and growth. Additionally, it also reduces water quality problems caused by overfeeding [28].

Overall, this information would enhance *B. simplex* breeding and produce high-potential offspring for ornamental fish and conservation purposes. An adequate and high-quality fingerling supply would reduce hunting from the wild. Additionally, the fish

habitat protection from environmental degradation and human threats must be considerable to be carried out simultaneously [29,30].

5. Conclusions

Betta simplex can successfully live, spawn, and perform offspring care in rearing environments with different alkalinity and hardness conditions. Its offspring first hatch within 7 DAF, develop to the post-flexion stage, and are then released from the mouth of a parental male within 11 DAF with a fecundity of 63 ± 13 offspring per crop. It differs from bubble-nesting fighting fish by a large sagged-down yolk sac and completely dark eye pigmentation in the yolk-sac stage, a rounded head, and randomized scattered spots of melanophore pigmentation on the head and body in the post-flexion stage. It reaches the juvenile stage at 30 DAR. The optimized age class can be predicted using the linear regression relationship between age and body size between 11 and 45 DAF.

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Data Availability Statement: *Betta simplex* referent specimen collection was deposited at Kasetsart University Museum of Fisheries, Faculty of Fisheries, Kasetsart University, Chatuchak, Bangkok, Thailand (<https://fish.ku.ac.th/en/node/345> (accessed on 18 August 2025)).

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Abbreviations

The following abbreviations are used in this manuscript:

BD	Body depth
DAF	Days after fertilization
DAR	Days after release
ED	Eye diameter
HD	Head depth
HL	Head length
HW	Head width
KUMF	Kasetsart University Museum of Fisheries
NL	Notochord length
PAL	Pre-anal length
PDL	Pre-dorsal length
SL	Standard length

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