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FIRST GENERATION REPRODUCTIVE PERFORMANCE AND SECOND GENERATION LARVAL PRODUCTION ON THE DOMESTICATED TINFOIL BARB, *Barbonymus schwanenfeldii* (BLEEKER, 1854)

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ABSTRACT

Tinfoil barb, *Barbonymus schwanenfeldii* (Bleeker, 1854), is a potential freshwater fish for Indonesian aquaculture. Before widely used, the candidate needs to be evaluated. This research aimed to evaluate the reproductive performance of the first generation (G1) and larval performance of the second generation (G2) of domesticated tinfoil barb. A total of 68 female and 24 male broodstocks were selected for the artificial breeding program. The gonadal maturity test was carried out by canulating the eggs every month. Before spawning, GnRHa hormone was injected into the dorsal area (0.5 mL/kg for female and 0.2 mL/kg for male). Fecundity, fertility rate, hatching rate, embryogenesis, and larvae ontogeny were recorded. The results showed that the first matured G1 males of tinfoil barb were at the standard length of 16.01 ± 1.18 cm, while females at 15.79 ± 1.23 cm. The mature broodstock indicated by the gonad maturity stage III and IV confirming higher estradiol concentration (above 400 pg mL^{-1}). The fecundity of two mature broodstock-sized of 217.2 g and 197.3 g were 12,495 and 15,782 eggs, respectively. The spawning season of G1 tinfoil barb was in October and November (rainy seasons). The fertilized eggs latency time was 10 hours 44 minutes at 25°C and hatched after 23 hours 7 minutes. The fertility rate was 96.96 % and the hatching rate was 95.16 %. The survival rate of G2 normal larvae was 100 % at three days of the rearing period. The G2 larvae production in this experiment provides an excellent opportunity for fish diversification both for aquaculture and restocking.

KEYWORDS: Aquaculture; breeding; domestication; reprodution; tinfoil barb

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INTRODUCTION

Tinfoil barb, *Barbonymus schwanenfeldii* (Bleeker, 1854), is a species of freshwater fish native to Thailand, Indochina, Malaysia, and Indonesia (Kottelat *et al*., 1993). In Indonesia, tinfoil barb (TB), locally known as "tengadak" or "lampam" is an economically important species, mostly caught and cultivated by communities in Java, Sumatra, and Kalimantan (Gustiano *et al*., 2021). In nature, this species is found in clean open waters such as lakes, large and small rivers as well as in canals and ditches. Currently, TB is one of the fish that makes a significant contribution to the production of Indonesian inland fisheries (Gustiano *et al*., 2021). Based on commodity analysis, TB is classified as a potential freshwater fish species for aquaculture in Indonesia (Kurniawan *et al.*, 2021). It was reported that the first-time artificial spawning of TB was carried out artificially using a combination of the hormone human chorionic gonadotrophin (HCG) and carp pituitary extract (CPE) (Bailey & Cole, 1999) and spawning using the hormone ovaprim (Epasinghe *et al*., 2016). However, there have been no reports regarding breeding programs through the domestication process. To carry out an optimal spawning and breeding program, a complete domestication process is required (Teletchea & Fontaine, 2014; Lorenzen *et al*., 2012). Domesticated species have different reproductive characteristics from their ancestors because humans have changed them to suit the new environment or habitat (Balon, 2004).

In Indonesia, the domestication of TB began with characterizing the genotype, morphological, and biological reproduction of 3 populations, Sumatra, Java, and Kalimantan (Kusmini *et al*., 2018; Radona *et al*., 2016a,b), followed by a series of performance evaluations to have the best populations for the domestication program (Radona *et al*., 2016a,b; Radona *et al*., 2018; Kusmini *et al*., 2021). After several generations, investigations of breeding performance showed populations from Kalimantan had the highest growth performance compared to other populations for specific growth rates, survival rates, and feed conversion ratios (Kusmini *et al*., 2015). At a later stage, domestication testing was carried out to evaluate the performance of the Kalimantan population generation obtained to determine the domestication strategy to be carried out (Kurniawan *et al*., 2021). In the progress of domestication of TB, the promising candidate showed that the mature broodstock was indicated in the weight of 150 to 400 g for females and 200 to 400 g for males (Kusmini *et al.*, 2021), within the size range of the adult wild Barbonymous species (Efizono *et al*., 2021). The broodstocks reached their gonad maturity level by spawning twice a year. While in its natural habitat, the spawning period of TB occurred twice in 15 months, commonly during the rainy season (Isa *et al*., 2012). At present, the second generation of domesticated TB has been produced at the research station. To be commercialized and widely accepted, it is imperative to test the acceptable commercial performance before being officially released. The second generation (G2) results from mass spawning with a ratio of 1:1 (one male fertilizes one female) from the previous generation of parents, which were selected based on fast growth. This research aimed to evaluate the first-generation reproductive performance of domesticated tinfoil barb (G1) and their second-generation offspring (G2).

MATERIALS AND METHODS

Broodstock maturation and maturity test

This research was conducted in the experimental pond of the Research Institute for Freshwater Aquaculture and Fishery Extension (RIFAFE), Bogor, Indonesia from March to November 2020. The broodstock used in this study was first-generation tinfoil barb fish (G-1) collected from Kalimantan. A total of 68 females (170.50 ± 34.30 g) and 24 males (156.40 ± 24.91g) broodstock were reared in a rectangular pond with a stocking density of 3 fish m^{-2}. Fish were fed 4% of their biomass with commercial pellets containing 31-33% protein, twice a day at 08.00 and 16.00 hours. The broodstock maturity stage was examined periodically every month based on the morphology, and sexual secondary characters of the female and male fish. During observation, eggs were cannulated with a catheter (sterile tube for endometrial biopsy from Laboratoire C.C.D REF: 1103000) 2.75 mm in diameter for observation. Measurement of egg diameter (n=50) was conducted using an Olympus BX 43 microscope with a camera (Toupcam UCMOS05100KPA) equipped with an ocular micrometer (4x10 magnification).

The gonadal maturity stages were determined based on the egg morphology, diameter, and nucleus position. The cell nucleus in a mature egg will shift to the side of the egg. To confirm the maturity stage, the estradiol level analysis was also performed. For estradiol analysis, blood samples were obtained from each fish. Blood was taken from caudal artery as much as 1 mL using a 3 mL syringe given heparin sodium after broodstocks were treated with anesthetic solution (Ocean Free, 5 mL/L) for five minutes. The blood was then stored in the 1.5 mL heparinized tube. The blood samples were centrifuged for 10 minutes using a microcentrifuge (Heraeus Biofuge Pico, 10,000 rpm) to obtain the blood plasma. The samples of blood plasma were stored in the freezer (-20°C) for

further analysis. The concentration of estradiol from each sample was detected using Estradiol ELISA kit (DRG Instruments GmbH, Germany) (Barrero *et al*., 2007) and microplate photometer (Biosan HiPo MPP-96, Latvia).

Artificial fertilization

Artificial breeding of the TB was carried out based on the induced breeding method using Gonadotropin-Releasing Hormone analog (GnRHa) (Kusmini *et al*., 2020). GnRHa was injected into the dorsal area of the fish (dosage: 0.5 mL kg^{-1} for females and 0.2 mL kg^{-1} for males). The latency time of broodstock was evaluated after 12 hours of injection. Before collecting eggs and sperm, broodstocks were anesthetized using the anesthetic solution (Ocean Free, 5 mL/L). The eggs dan sperms were released based on the stripping method and mixed into a media containing NaCl solution (0.9 %) (Figure 1). The fertilized eggs were incubated in the rectangular tank filled with clear water. The hatched larvae were reared in rectangular tanks (2 tons in size, filled with 1 ton of water) and fed using microalgae and chicken egg yolk (*ad-libitum*) after three days of hatching. The microalgae used were mixed from the Chlorophyceae and Bacillariophyceae classes, consisting of *Asterionella*, *Tetraselmis sp*., *Hydrodictyo*, *Scenedesmus*, *Volvox*, and *Chlorella* with an abundance of around >500 ind/L. Larval embryogenesis was observed to determine the early development of larvae. Water quality was measured periodically during the study including temperature, dissolved oxygen using a DO meter (Lutron DO-5510, pH using a pH meter (Lutron BPH-231), and nitrate using a Nitrate test kit (Merck 1.11170.0001).

Embryogenesis of larvae was observed using a stereomicroscope equipped with a camera to determine the development of larvae (morula, blastula, gastrula, and organogenesis stage).

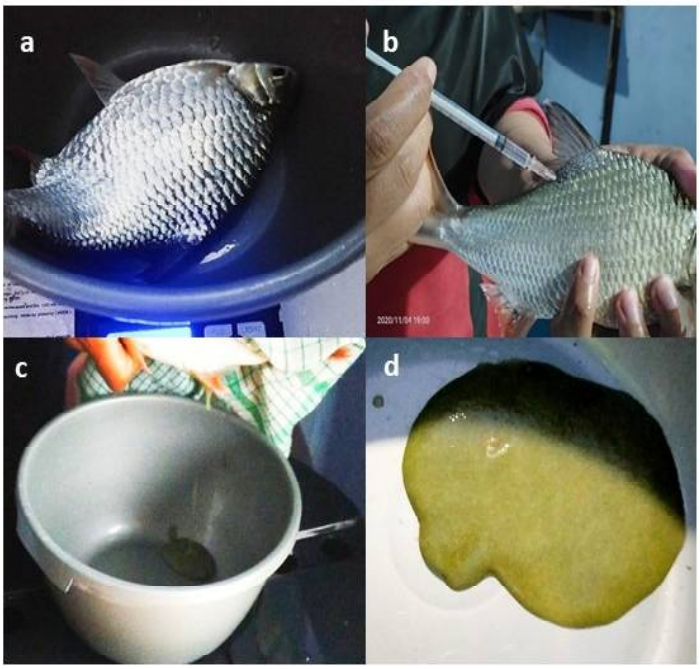

Figure 1. Artificial breeding activities of tinfoil barb *Barbonymus schwanenfeldii*.
(a. weighing; b. Injecting hormone; c. stripping; and d. collecting eggs from stripping)

Data analysis

Reproductive data collected were total weight, standard length of fish, gonadal maturity index, egg diameter, and concentration of the estradiol hormone. Fertilization was observed after 2 hours of egg and sperm mixing. Fertilized eggs will be clear and transparent, while unfertilized eggs will be white. Larvae survival was observed after yolk-sac absorption was complete (2-3 days). The reproductive biology parameters observed were measured using Radona *et al*. (2020) formula for the striped snakehead study. Data were analyzed descriptively using tables and graphs to figure out the reproductive characters of tinfoil barb broodstocks.

RESULTS AND DISCUSSION

Based on the selection of 68 females and 24 males, the broodstock used was ten females and seven males with mature gonads. Ten females with body weight (BW) of 170.5 ± 34.3 g, 17.5 ± 1.00 cm SL, and 22.6

± 1.60 cm TL, and seven males with BW of 156.43 ± 24.91 g, 16.64 ± 0.85 cm SL, 21.43 ± 1.10 cm TL were used in this study (Table 1). The female and male broodstock used did not show significant differences for BW, SL, and TL ($p < 0.05$).

The gonadal maturity stage (GMS) for 1 year of observation is shown in Fig. 2. The GMS has a distribution of stage I at 40% in July, stage II at 35%, and stage III at 25 % from January to June. Two broods, 20%, reached stage IV in November indicated by enlarged and rounded abdomen, rough body scales, the reddish urogenital colour, and 1.0 mm eggs diameter. This study shows that in general, the female parent has an average size larger than the male. The phenomenon of male fish having a smaller size and gonads that mature faster was also reported by previous studies on the genus Barbanymous (Efizono *et al*., 2021). This research also shows that TB can mature throughout the year, but spawning occurs in the rainy season in November. The spawning pattern of TB is categorized as total spawning which releases mature eggs from the ovaries in one reproductive cycle and then matures again in the next breeding season. There was no different sexual ratio between males and females of TB in the three native habitats observed (Dewantoro *et al*., 2011). In the dry season, generally, the fish caught have the level of gonad maturity in stages I and II, but in the rainy season the level of gonad maturity are spread from stages I to IV. The gonad maturity index, fecundity, and egg diameter of TB explained that species would spawn throughout the year with the maximum spawning activity during the rainy season, from October to November. Thus, the results in the present study confirm previous reports. The broods still have their natural spawning behavior.

Table 1. Body weight, standard length and total length of female and male parents

No.	Female			Male		
	Body weight (g)	Standard length (cm)	Total length (cm)	Body weight (g)	Standard length (cm)	Total length (cm)
1	168.6	16.0	20.6	180.8	16.5	22
2	170.1	18.0	23.0	115.4	15.5	20
3	228.6	17.5	25.0	177.1	16.5	20
4	190.6	18.0	23.0	178.2	17.5	22
5	161.8	18.0	23.0	152.7	16.5	21
6	150.4	17.0	21.5	1574	18.0	23
7	128.1	17.0	21.5	1334	16.0	21
8	118.9	16.0	20.0	-	-	-
9	174.0	18.0	23.5	-	-	-
10	123.6	19.0	24.5	-	-	-
Mean ± SD	170.5 ± 34.30	17.5 ± 1.00	22.6 ± 1.60	156.4 ± 24.91	16.4 ± 0.85	21.4 ± 1.10

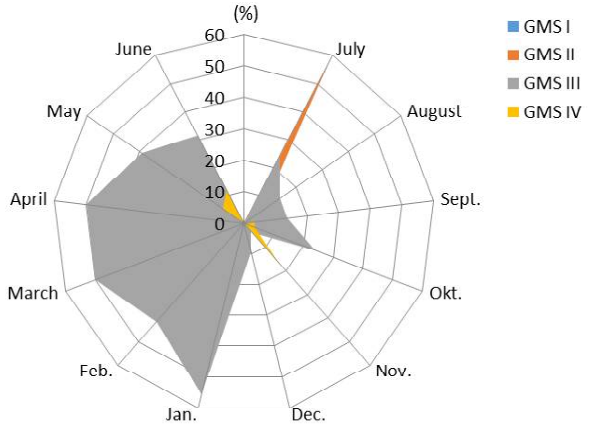

Figure 2. Reproduction cycles on the first generation of TB *B. schwanenfeldii* from July 2020 to June 2021.

Observation of estradiol hormone cooncentration was carried out from December 2020 to May 2021. The estradiol level was higher at GMS III and IV compared to those of the lower levels, about 400 pg/mL (Fig. 3). While the present study showed that the latency time of TB occurred in 10 hours 44 minutes at an incubated temperature of 25°C and hatched after 23 hours 7 minutes. Fecundity ranged from 12,495 and 15,782 eggs, respectively (Table 2).

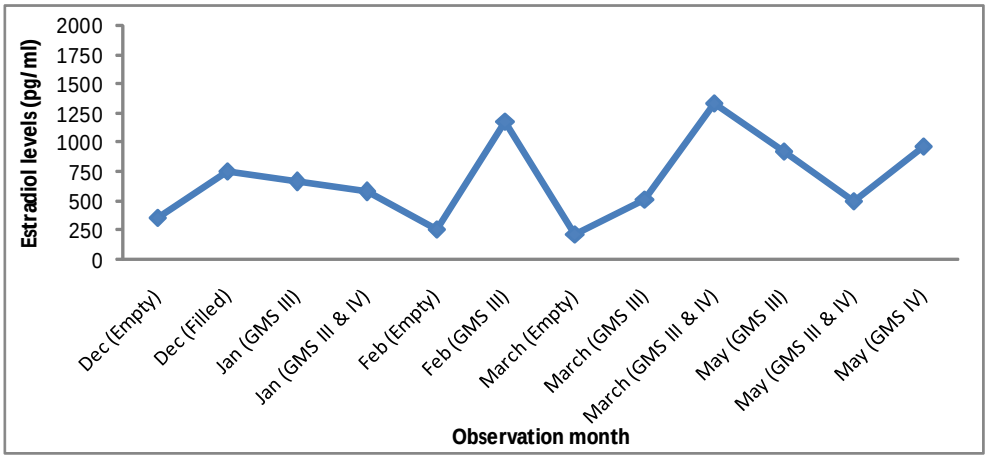

Figure 3. Estradiol level on the first generation of tinfoil barb *Barbonymus schwanenfeldii* from December 2020 to May 2021.

Table 2. Length, body and gonad weight of broods, gonadal somatic index, and fecundity

No of broods	Standard length (cm)	Total length (cm)	Body weight (g)	Gonad weight (g)	Gonadal somatic index (%)	Fecundity (eggs)
1	18.0	23.6	217.2	5.3	2.4	12,495
2	15.5	21.4	197.3	8.7	4.1	15,782

The estradiol hormone trend showed that estradiol levels of the fish increased in line with the increase in egg diameter. In May, the gonadal maturity stage of the female broodstocks commonly reached stages III and IV. However, they were unable to spawn with semi-artificial spawning methods that were introduced in this study. Similar results were also found in the domestication of other cyprinids species (Gustiano *et al*., 2013). The development of gonad maturity is one of the most important stages of reproductive development in fish reproduction. Gonadal maturation is strongly associated with photoperiod and temperature changes throughout the year (Campos-Mendoza *et al*., 2004). Steroid hormones, such as testosterone, estradiol-17â, 20b-dihydroxy-4-pregnant-3-one, and 11-ketotestosterone play an important role in the development and gonadal maturation of both male and female fish (Lubzens *et al*., 2010; Schulz *et al*., 2010). In addition, environmental factors can also stimulate the production of some steroid hormones. Gonad development will be disrupted if one of the hormones is not sufficiently available in the fish's body. Fish will produce mature eggs and sperm after completing gonad maturation.

In the present study, three female broodstocks (20%) were identified as showing full maturity (GMS IV) in November which was indicated by an enlarged and rounded abdomens, rough body scales, and the reddish urogenital color. The males also showing full maturity as the semen was easily released during the stripping method. This is in accordance with the results conducted by Dewantoro *et al*. (2018) that identified the spawning season of the tinfoil barb at Kapuas River that occurred in October-November. The diameter of eggs of GMS III and IV was found more than 1.0 mm, while the egg diameter GMS II below was commonly found to be smaller than 1.0 mm. Evaluation of GMS III and IV observed based on eggs diameter were ranging from 0.9–1.0 mm (Kusmini *et al*., 2018). The fecundity of the first generation of tinfoil barb broodstock (Weight: 197.3 and 217.2 g) ranged between 12,495 and 15,782 eggs, respectively (Table 2). The fecundity of tinfoil barb collected from Sarolangun and Anjong, West Borneo ranged between 20,168 to 232,040 eggs with the gonad weight ranged from 9.0 to 45.5 g (Kusmini *et al*., 2018). The average fecundity of TB caught from Musi River, Sumatra was 5,096 eggs. The results were slightly lower than

the fecundity of the species collected from different areas (Kusmini *et al*., 2018). The fecundity of TB from the three sampling locations in Borneo ranged between 1,289 and 48,648 eggs (Dewantoro *et al*., 2011).

In general, fecundity can be affected by several factors including spawning frequency, broodstock protection, egg size, environmental conditions, and population density. Variation in the number of fish eggs in different fish species can be triggered by variation in fish size, total length, and body weight. However, in other cases, fish weight and length do not guarantee a high level of fecundity, it was more determined by the value of the gonadal somatic index (GSI) which is mainly used as an indicator to determine the maturity of the gonads quantitatively.

The first generation of tinfoil barb reached maturation in 18 months. The standard length, and mean body weight were 16.01 ± 1.18 cm, 21.35 ± 1.42 cm, and weight of 156.64 ± 31.90 g, respectively. The females had a standard length of 15.79 ± 1.23 cm, a total length of 21.62 ± 1.37 cm, and a body weight of 138.15 ± 30.75 g. The wild tinfoil barb collected from Musi River, Sumatra had their first-time gonad maturation at the length of 182 mm (male) and 156 mm (female). The first-time gonad maturation is different among fish species, and it would also be different even in similar species inhabiting different habitats.

Investigation on the reproductive performance of the second generation broodstock (G2) through the artificial breeding method showed that the broodstocks with a fertility rate (FR) of 99.72% have a hatching rate (HR) of about 95.29% (Table 3). The high fertility rate in fish reproduction can be supported by excellent spermatozoa and eggs quality (Lismawati *et al*., 2016). Formulated feed supplemented using *Spirulina* 3% / kg and turmeric 3% / kg, combined with Oodev injection (0.50 mL/kg) improved the reproduction performance of the TB outside of the spawning season (Lestari *et al*., 2016). This treatment also generates results 2.2 times faster than other Oodev treatments (0.25 mL/kg) and control over 4-14 rearing periods. The recorded embryogenesis phases showed that the development of fertilized eggs, blastomere, blastula, gastrula, neurula, organogenesis, and larvae is in accordance (Fig. 4) by Kusmini *et al*. (2015).

Table 3. Reproduction of Tinfoil barb *Barbonymus schwanenfeldii*

weight gonad (g)	Egg number	unfertilized eggs (number)	fertilized eggs (%)	unhatched eggs (number)	Hatching rate (%)	Survival rate (%) at day 3
0.11	362	11	96.96	17	95.16	100

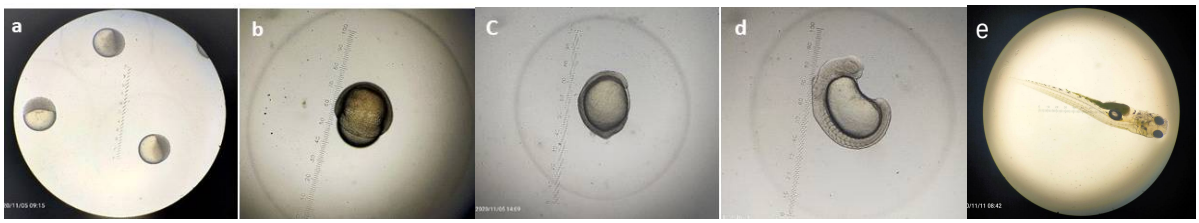

Figure 4. Development of tinfoil barb embryo *Barbonymus schwanenfeldii* (a. blastula, b. gastrula, c. organogenesis, d. embryo and e. larvae at day 3).

Feeding newly hatched larvae with live microalgae enabled them to present 100% survival rate up to three days old. The microalgae composition was identified from Chlorophyceae and Bacillariophyceae with an abundance of > 500 ind/L, consisting of six species including *Asterionella* sp., *Tetraselmis* sp., *Hydrodictyo* sp., *Scenedesmus* sp., *Volvox* sp., and *Chlorella* sp (Fig. 5 and 6). The microalgae were observed using a compound microscope with a magnification of 100 times. Two groups of zooplankton were identified in this study, namely *Rotifera* and *Nitzschia* (abundance > 10 ind/L). Bacillariophyceae

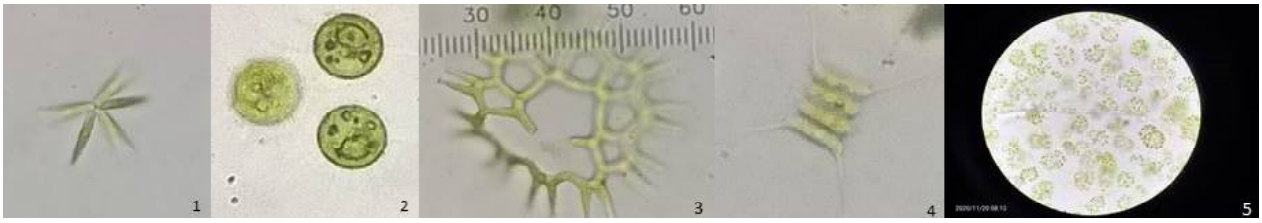

Figure 5. Microalgae used as life food for the tinfoil barb *Barbonymus schwanenfeldii* larvae (1. *Asterionella*; 2. *Tetraselmis* sp.; 3. *Hydrodictyo*; 4. *Scenedesmus*; 5. *Volvox* and *Chlorella*).

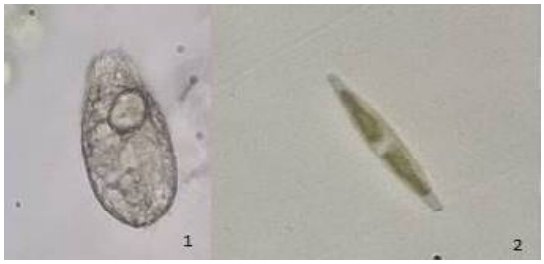

Figure 6. Zooplankton used as life food for the tinfoil barb *Barbonymus schwanenfeldii* larvae: 1). *Rotifer*; 2). *Nitzschia.*

and zooplankton were commonly consumed as live food for the cyprinid larvae (Pratiwi *et al*., 2011). Bacillariophyceae utilization decreases as the larvae stage increases. In contrast, Chlorophyceae are consumed in small amounts at the beginning of larvae rearing period, but it increases with increasing larval age. Water quality during the study was optimum for larval growth referring to Boyd (2012). The ranges of temperature, DO, pH, and nitrate were 27.1-27.5°C, 7.3-7.6 mg/L, 5.8-7.1, and < 0.5 mg/L, respectively.

CONCLUSION

There was no significant difference in the size of the adult female parent compared to the male parent for BB, SL, and TL. The tinfoil barb matures throughout the year and spawns in the rainy season in November. The spawning pattern is categorized as total spawning which releases mature eggs from the ovaries in one reproductive cycle and then matures again in the next breeding season. The estradiol trend showed that concentration is higher in line with the increase in egg diameter. The mature broodstock was indicated by gonadal maturity stage (GMS) III and IV confirming higher estradiol concentration (above 400 pg/mL). The fecundity of the mature confirmed the previous similar study. The latency time of fertilized eggs was 10 hours 44 minutes occurred at temperature of 25°C and larvae hatched after 23 hours 7 minutes. The fertility rate was observed at about 96.96% and the hatching rate was 95.16%. The survival rate of G2 larvae was 100% at three days of the rearing period. The first success of G2 larvae production in this experiment provides an excellent opportunity for the diversification of cultured fish species both for aquaculture and restocking purposes.

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