

Oocyte Development and Fecundity of Snakehead Murrel, *Channa striatus* (Bloch 1793) in Captivity

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Abstract

This study describes the different stages of ovarian development and estimate the fecundity in captive-raised snakehead murrel, *Channa striatus*. The fish (14.9±3.9 g) were fed twice daily until satiation from February to September 2010 with a commercial diet. The gonadosomatic index (GSI), gradually increased from the 1st to the 4th month, remained unchanged at the 5th until the 7th month, and then declined slightly by the 8th month. However, the hepatosomatic index (HSI) fluctuated as the feeding period progressed. In contrast, HSI increased from the 1st month and peaked by the 3rd month. However it declined sharply in the 4th month to a level lower than that in the first 3 months and remained consistently low thereafter. Interestingly, HSI sharply increased in the 8th month, when GSI declined. The absolute fecundity was found to be 33,949±3,388. Oocyte and egg diameters were found to be 1.21mm±0.07mm and 1.33mm±0.09 mm, respectively. Four different developmental stages were identified and discussed through histological examination: primary, yolk vesicle, vitellogenic and mature oocytes. The results demonstrated that captive-raised fingerling *C. striatus* came to sexual maturity after 8 months.

Introduction

Snakehead, *Channa striatus* (Bloch 1793), is a warm freshwater fish which is known as haruan in Malaysia. Its nutraceutical properties have already been studied as its flesh is claimed to reduce pain and anxiety in postoperative period and that its aqueous extract generates a safe and effective film for both incision and burn wounds (Baie and Sheikh 2000; Laila et al. 2011). These properties have been attributed to the abundance of certain amino and fatty acids in the flesh for collagen synthesis during wound healing (Saringat and Sheikh 2000).

Intensive breeding of *C. striatus* takes place during the rainy months. They breed in ditches, ponds and flooded paddy fields. Young shoal at the surface and are guarded by male parent, hiding below the surface water. In captivity, as soon as the male bends its body close to the female during mating, milt is released following the release of the eggs (Froese and Pauly 2011). Observation of (Alikunhi 1953) indicates that the smallest mature female specimen taken was 23.4 cm in length with ovaries in the 4th stage of development.

The reproductive biology of wild *C. striatus* has been detailed previously (Alikunhi 1953; Marimutu et al. 2007; Hussain and Latifa 2008). However, little is known regarding the reproductive biology of *C. striatus* in captivity. This paper describes the oocyte development and estimates the fecundity of *C. striatus* in captivity.

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Materials and Methods

Experimental fish, husbandry conditions and sampling

A total of 1,000 male and female *C. striatus* juveniles (14.9g±3.0 g), from an in-house breeding programme at the School of Biological Sciences, Universiti Sains Malaysia, were reared in two rectangular cement tanks (each of 3,600 m³ capacity) until sexual maturity. The tanks were equipped with oxygen supply via continuous aeration and constant water exchange in a flow-through system with the flow rate set at 2 Lmin⁻¹. A commercial diet containing 43.0%±1.0% crude protein and 9.18%±0.03% crude lipid was fed to the fish until satiation twice daily at (10 and 18 h). Monthly, 10 female fish were randomly sampled and weighed after anaesthetising with 10 ppm clove oil. Subsequently, a longitudinal ventral incision was made on each fish and the gonad was dissected for macroscopic and microscopic classifications.

Gonadosomatic (GSI) and hepatosomatic (HSI) indices

The ratio of ovary weight to body weight, GSI, was determined by weighing the whole fish and its ovary. GSI was calculated by using the formula described by Brooks et al. (1997).

$$GSI = \frac{\text{total ovary weight}}{\text{whole body weight}} \times 100$$

The ratio of liver weight to body weight (HSI) was determined based on the weights of the fish and its respective liver. HSI was calculated by the following formula:

$$HSI = \frac{\text{total liver weight}}{\text{whole body weight}} \times 100$$

Fecundity and oocyte diameter

Only mature fish were subjected to absolute fecundity calculation (Ahyaudin 1999). Absolute fecundity was determined using the gravimetric method described by Babiker and Ibrahim (1979). The mature fish were randomly sampled, transported to the laboratory, killed and after a longitudinal ventral incision, eviscerated and three sub-samples from middle, interior and posterior part of the ovary were placed in Gilson's fluid (Humason 1961) and shaken periodically to loosen the oocyte. The mean from three sub-samples were used to calculate absolute fecundity (number of oocyte female⁻¹) by counting the number of oocytes per subsample of ovary to the total ovary weight. Total numbers of oocytes (N) in both ovaries were calculated using the following formula described by Bagenal (1978).

$$N = \frac{Wt}{Ws} \times Ns$$

where: W_t = total weight of two ovaries, W_s = subsample weight, and N_s = number of counted oocytes in the subsample. The oocyte diameters of mature fish were measured based on the mean of the short and long axis of the oocytes using a light microscope equipped with a camera.

Spawning and measuring oocyte and egg diameters

Female fish with swollen, soft abdomen, reddish swollen vent and male with pointed genital papilla were selected, anaesthetised and injected intramuscularly with ovaprim at 0.5 ml.kg⁻¹ body weight. The brooders were distributed at a ratio of 1: 2 (female: male) into circular fiberglass tanks filled with de-chlorinated tap water to a depth of 30.0 cm for induced spawning (Hussain and Latifa 2008; Haniffah et al. 1996). The aquatic macrophyte, *Eichhorina crassipes* was placed in the breeding tanks which were covered with black meshed plastic sheets to provide a suitable hiding place for spawning. Spawning occurred 24 h up to 36 h after hormonal injection. Three egg batches (100 egg each) were collected and fixed in 4% formalin and transferred to the laboratory to calculate individual egg diameters using light microscope equipped with a camera.

Microscopic study

Ovary specimens (5 mm thick) from the middle of freshly dissected ovaries were fixed in buffered 4% formalin for 24 h and processed according to standard procedures (Vandyk and Pieterse 2008). Eight micron sections were prepared using a rotary microtome (A.O. Spencer, model Reicheit-Jurg 820, Leica, Germany) and stained as described in Wallace and Selman (1981). Histological slides were then examined under a compound light microscope and photographed using an attached digital camera (Olympus Xcam-Alpha, Germany). In the present study, the oocyte germ cells including the primary growth phase, yolk vesicle (cortical alveoli), vitellogenic and maturation stages were identified according to the method described by Gentek et al. (2009).

Length – weight relationship

To determine the length-weight relationship, 80 samples of *C. striatus* were studied. The total length of the fish was measured (to the nearest 0.5 cm) from the tip of the snout to the distal end of the caudal fin, whereas the fish was weighed (to the nearest 0.1 g), after drying off excess water on the body. The relationship between the length and weight of the fish in the given experimental population was analysed by measuring the weights and lengths of the fish samples taken at the time of the experiment. The relationship between length (l) and weight (y) typically takes the general form of $y = aL^b$.

Results

GSI and HSI

Table 1 shows descriptive statistic of specimens sampled monthly. The GSI increased sharply from February to May and reached a maximum value of 11.5 ± 0.7 in August followed by a notable decrease in September (10.4 ± 0.9). Conversely, the maximum HSI value was noted in April (1.4 ± 0.2), with a significant decline observed when the GSI value increased. The lowest HSI value was recorded in August (0.7 ± 0.1). However, HSI started to increase in September, the same time that GSI was noticed to decline (Fig. 1).

Table 1. Descriptive statistics of the specimens collected by month

Months	Fish weight (g)	Fish length (cm)	ovary weight (g)	Liver weight (g)	GSI (%)	HSI (%)
Feb	14.9 ± 3.9	11.7 ± 1.2	0.18 ± 0.1	0.16 ± 0.04	1.1 ± 0.6	1.1 ± 0.2
Mar	48.0 ± 6.5	17.5 ± 0.7	2.5 ± 0.5	0.59 ± 0.09	5.3 ± 0.8	1.2 ± 0.1
Apr	68.6 ± 7.3	19.2 ± 0.4	6.0 ± 0.6	1.0 ± 0.1	8.8 ± 0.4	1.4 ± 0.2
May	92.3 ± 7.0	21.3 ± 0.7	9.7 ± 1.2	0.94 ± 0.9	10.6 ± 1.2	1.0 ± 0.2
Jun	110.8 ± 5.5	22.5 ± 1.2	12.2 ± 1.3	1.2 ± 0.2	11.0 ± 1.1	1.1 ± 0.1
Jul	136.6 ± 9.8	24.1 ± 0.6	15.5 ± 3.7	1.3 ± 0.4	11.2 ± 2.1	0.98 ± 0.02
Aug	187.4 ± 4.8	26.0 ± 0.8	21.6 ± 1.3	1.34 ± 0.3	11.5 ± 1.3	0.71 ± 0.01
Sep	215.7 ± 13.8	28.0 ± 1.7	22.4 ± 2.5	2.1 ± 0.3	10.4 ± 1.1	1.0 ± 0.1

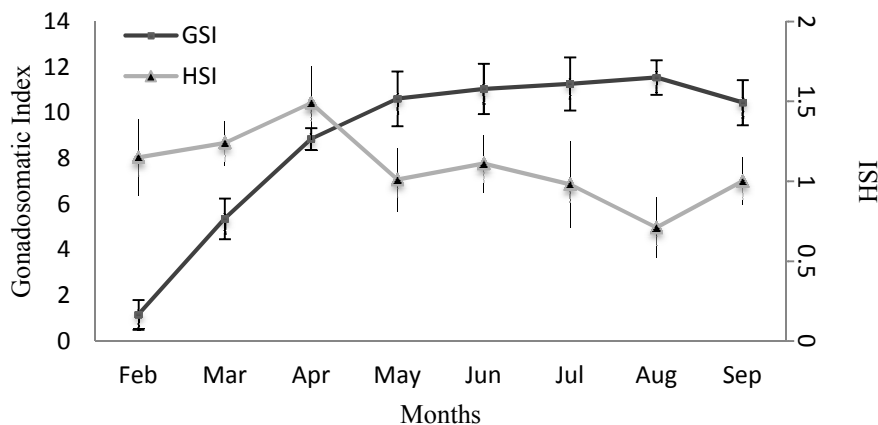


Fig. 1. Gonadosomatic index (GSI) and hepatosomatic index (HSI) changes in female *C. striatus* cultivated in captivity for 8 months.

Fecundity as well as oocyte and egg diameters

Table 2 shows the absolute fecundity as well as the oocyte, and egg diameters of mature female *C. striatus*. In the current study, the mean of absolute fecundity (number of oocytes per female) was $33,949 \pm 3,388$. The maximum and minimum absolute fecundity were found to be

41,068 and 28,332, respectively. Oocyte and egg diameters were 1.21 ± 0.07 mm and 1.33 ± 0.09 mm, respectively.

Table 2. Absolute fecundity, oocyte and egg diameters of mature *C. striatus* cultivated in captivity for 8 months.

	Fish weight (g)	fish length (cm)	Absolute fecundity	oocyte diameter (mm)	egg diameter (mm)
Mean	215.7±12.8	28.02±1.71	33939±3388	1.21±0.07	1.33±0.09
Range	197-245	25-31	28332-41069	1.03-1.46	1.04-1.57

Microscopic and macroscopic features of ovaries

Four main oocyte development stages were identified throughout the study according to Gentek et al. (2009). Figure 2 shows the frequency of occurrences (%) of gonadal stages during the 8-month study period. Between February and March, 91% and 9% of the oocytes in the fresh sampled ovaries, were in the primary oocyte stage and in the cortical alveoli stage, respectively (Fig. 2). The primary growth involves the period of oocyte development from meiotic chromatin-nucleus stage to the early cortical alveoli stage. In addition it is closely linked with the development of follicle layers surrounding the oocyte. During the primary growth stage, the oocyte increased in volume and diameter, and the nuclear/cytoplasmic ratio started to decrease. The nucleus became more spherical in shape than the oogonia with multiple nucleoli. Table 3 shows that the nucleus developed and that the nucleoli increased in number. In this stage, ovary was narrow, thin and transparent without irrigation (Table 4).

By April and May, the percentage of cortical alveoli stage (60%) and the emergence of vitellogenic (10%) increased, while percentage of primary oocyte stage decreased. Some oogonia and primary oocytes were also observed among the cortical alveoli oocytes (Fig. 2). This result indicates the asynchronous ovary development of the fish. The transition of the primary oocytes into the secondary growth phase starts with an accumulation of cortical alveoli. The appearance of cortical alveoli filling the periphery of the oocyte is an early event associated with oocyte enlargement. This stage is also termed primary vitellogenic. With time, the cortical alveoli increased in number and size, filling the oocyte cytoplasm and eventually displacing the oocyte periphery during the later stage of oocyte development because of centripetal accumulation of yolk protein. The end of this stage was categorized by the initiation movement of the nucleus to the animal pole (Table 3). Moreover, ovaries were elongated and only few oocytes were visible and faint blood vessels were present (Table 4).

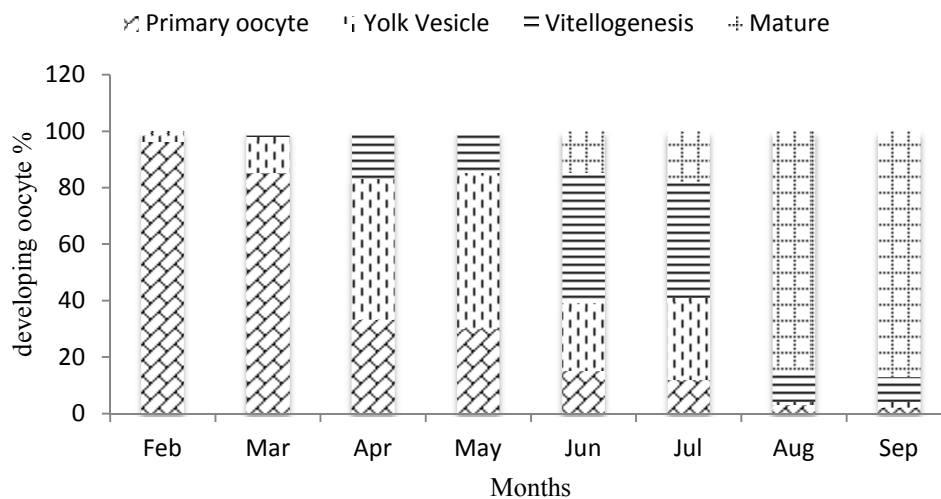


Fig. 2. Frequency of occurrences (%) of gonadal development stages for female *C. striatus* cultivated in captivity for 8 months.

In June and July, most of the oocytes were in the third phase (i.e the vitellogenic stage) and approximately 45% of the ovaries examined were in this developmental stage. However, some fully mature oocytes, yolk vesicles and primary oocytes were also observed (Fig. 2). This finding is attributed to the asynchronous nature of this fish. Vitellogenesis is the third step of oocyte maturation initiating with the incorporation of vitellogenin (VTG) proteins by the oocyte and their processing into yolk proteins ending with the passage of nucleus to the animal pole. At the end of this stage the oocytes are able to go through the maturation stage (Table 3). Furthermore, ovaries continued to enlarge in size. The oocytes were yellowish and blood vessels were not well developed (Table 4).

Table 3. Dominant stage of oocyte development of *C. striatus* over the 8 months study period

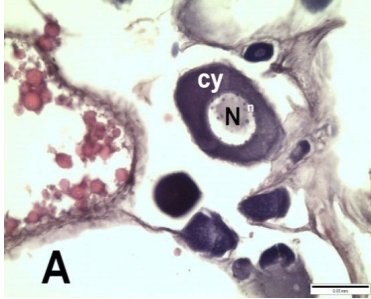
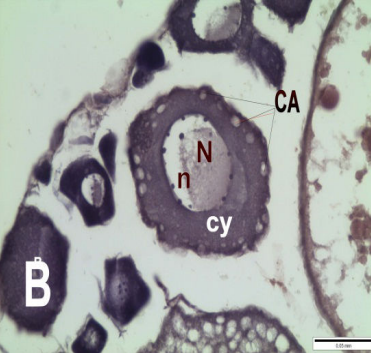

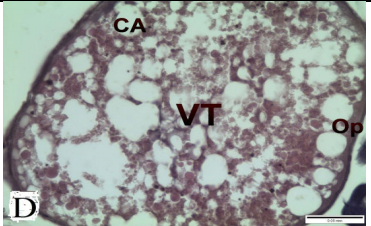


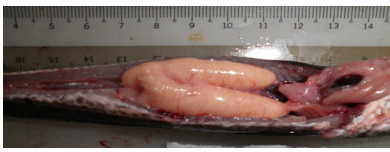
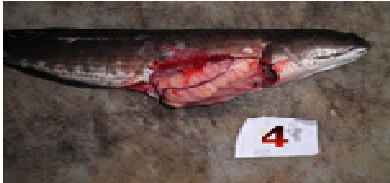
Oocyte Development Stage	Description	Histomorphology
Primary growth phase	Oocyte located within the germinal epithelium respectively with lightly eosinophilic to basophilic cytoplasm. Nucleus (N), nucleoli (n) and cytoplasm (cy). Bar=0.05 mm	
Yolk vesicle	In initiation of yolk vesicle stage oocyte breaks from the germinal epithelium; they are enveloped by a simple squamous follicular epithelium. Nucleoli appear at the peripheral of the nucleus (thin lines). Nucleus (N), nucleoli (n) and cortical alveoli. Bar= 0.05 mm	
Vitellogenesis	In the beginning of vitellogenesis, appearance of yolk and fat vacuoles in the ooplasm. Bar= 0.05mm	
Mature	Increasing of yolk vesicles which fill the entire ooplasm. Ooplasm (Op), Vitellogenin (VT) and cortical alveoli (CA). Bar= 0.05 mm	

Table 4. Description of the different stages of the ovarian development of *C. striatus* in captivity.

Stages	Description	External features
Immature	Ovary is narrow, thin and transparent without irrigation	
Initial maturation	Ovary is elongated and only few oocytes are visible. Faint blood vessels present	
Intermediate maturation	Ovary continues to enlarge in size. The oocyte is yellowish and blood vessels are not well developed.	
Mature	Ovary is large with a thin and transparent membrane. Oocytes are orange in colour, peripheral and central blood vessels are evident.	

After the vitellogenic stage, oocytes undergo maturation. This phase was observed in (85%) of the fish oocytes in August and September. In addition, all other oocyte phases could be found except the primary oocytes during these months (Fig. 2). At this time, the follicles appeared to attain a critical size, becoming capable of responding to maturation-inducing hormones. In the early maturation phase, the oocytes were observed to have large nuclei or germinal vesicles located centrally or halfway between the center and periphery (Table 3). Eventually, the oocytes reached the late maturation stage when the envelope of the germinal vesicle disintegrated and the nuclear content mixed with the surrounding cytoplasm (Table 3). At this stage, ovaries were large with a thin and transparent membrane. Oocytes were orange in colour, and peripheral and central blood vessels were evident (Table 4). It is the proper time for spawning after hormonal induction.

Length – weight relationship

The parameters of the length-weight relationship for the sampled fish are given in Fig. 3, together with the regression coefficient as well as the range and number of the specimens measured (n). The regression line derived from the data for *C. striatus* showed a power relationship between length and weight. The coefficient of determination was 0.97. This result suggests that approximately 97% of the variation in the fish length data could be explained by the weight of the fish. The value of the exponent b was calculated to be 3.06, whereas the correlation coefficient value r^2 was noted to be close to 1 (0.978).

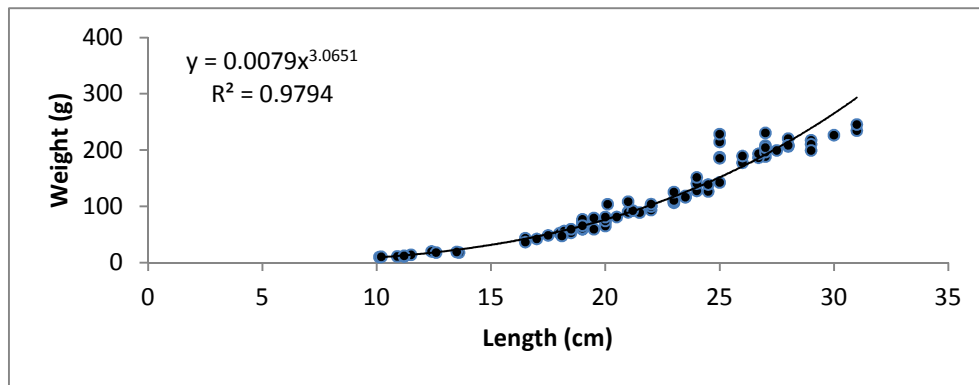


Fig. 3.Length–weight relationship in female *C. striatus* cultivated in captivity for 8 months

Discussion

Important and limiting concerns of aquaculture include fish reproduction, ovarian development and timing of maturation. In most fish species, the gonadal development and reproductive approach are often used to study physiological development of production systems. The process of oogenesis has often been split into four, five, six or eight stages. Four developmental stages have also been reported in other species including: *Sardina plichardus* (Walbuam 1792) (Nejedli et al. 2004), *Xiphas gladius* (Linnaeus 1758) (Lamas and Godinho 1996), and *Ophiocephalus striatus* (Bloch 1793) (Kilambi 1986).

To date a brief description of *C. striatus* ovarian development has been documented by Kilambi (1986). However, this was not supported by microscopic images of each developmental stage. The current study presents a detailed description of the ovarian developmental stages based on both the ovary histological analysis as well as external features (Table 2). The oocyte development in *C. striatus* was similar to most teleost fish (Aytekin and Yüce 2008; Chelemaal et al. 2009) and was divided into four main stages, based on the descriptions of Gentek et al. (2009). Over the 8-month period, oogonia initially multiplied and turned into primary oocytes, which then grew into follicles and created yolk vesicles which then progressed into vitellogenic stage. West (1990) described the nucleus to be large in the primary growth phase, with the nucleolus situated at the center of the nucleoplasm. Yolk vesicles are reported to be visible on the cytoplasm at the cortical alveolus phase. The dissolution of the nucleus membrane and the peripheral migration of the nucleus were noted to begin at the maturation of oocyte phase (West 1990). Similarly in the present study, all the reported observations above were also identified at the different stages mentioned, in a similar and consistent pattern. The monthly distribution of oocyte stages of *C. striatus* was reported based on the maximum number of advanced oocyte in the ovary sections (Fig. 2). The results showed the presence of all the oocyte developmental stages throughout the 8-month study period. In aquaculture, asynchronous ovaries have practical importance in establishing a breeding programme as it indicates a consistent supply of eggs to the hatcheries.

Ovarian morphology and GSI provide useful tools to indicate oocyte growth and maturation. In the present study, GSI of *C. striatus* continually increased regularly with each morphological stage over the 8 months experimental period corresponding with the final stages of oocyte maturation and did not change significantly thereafter, suggesting that spawning induction can be applied during the 8th month. The highest GSI value of 10.4% coincided with an average weight of 215.0 g at the end of the experiment. In contrast, in wild population, Ahyaudin (1999) described a poor, disorganized and wavelike GSI value and mature oocyte occurrence compared to the results obtained in captivity. High GSI value as well as the steady ovary growth in captivity is attributed to the good conditions such as good water quality, food availability and palatability. Noticeably, under these conditions, the majority of feed intake and energy is utilized for somatic and gamete growth rather than maintenance and chasing the prey (Mylonas et al. 2010). Furthermore, GSI changes are due to the growth and ripening of the ova while HSI values respond to among others, hepatic protein synthesis such as phosvitin and vitellin which are eventually transferred to the ovary (Tyler et al. 1996).

In the present study, the time taken for snakehead raised in concrete ponds to reach first sexual maturity was at 8 months with a weight of 215.0 g. This is in agreement with previous reports of 9 to 11 months in wild and pond system (Cabrita et al. 2008; Wee 1982; Alikunhi 1953). Maturation together with high GSI values in the current study was probably attributed to food quality and availability. Moreover, in the present study, spawning season was not taken into account and spawning in captivity was induced.

The absolute fecundity reported in previous studies was 5,500 to 11,811 oocytes per wild female (Alikunhi 1953; Parameswaran and Murugesan 1976; Kilambi 1986; Ahyaudin 1999) whereas in the present study it was 33,949 oocyte per captive female. The inconsistency between the findings may be attributed to continuous feeding via high quality feed, energy deposition and captivity condition.

In the present study, the oocyte and egg diameters were found to be 1.21 mm and 1.33 mm, respectively. This result is consistent with the result of previous research on wild population. Parameswaran and Murugesan (1976) reported diameters of 0.97 mm to 1.17 mm to indicate mature oocytes in *C. striatus* whereas Ahyaudin (1999) suggested a diameter range of 1.0mm to 1.5 mm. Alikunhi (1953) revealed that the average diameter of laid *C. striatus* eggs collected from a swamp is 1.53 mm. Cabrita et al. (2008) revealed that the diameter of spawned eggs and mature oocytes are approximately 1.39 mm and 1.0 mm to 1.2 mm respectively. Based on these findings, oocyte diameters of 1.0 mm and above can be used as a maturity criterion. In fish biology, weight-length relationship and its parameters (a and b) are determined to estimate fish weight from fish length or vice versa (Dulčić and Kraljević 1996). According to Sangun et al. (2007), the exponent b usually ranges from 2.5 to 4.0 in many fish species. The calculated value of the exponent b provides information on fish growth; with $b=3$, indicating that the increase in weight is isometric. However, when the value of b is other than 3, weight increase is allometric (positive allometric if $b>3$ and negative allometric if $b<3$). In this study the value of b was approximately 3, indicating that growth of *C. striatus* followed an isometric pattern during the 8 months rearing under captive conditions. The growth pattern in captivity up to first sexual

maturity observed here is similar to that of *C. striatus* in the wild (Ahyaudin 1999). Furthermore, the regression equation with the value of r^2 close to 1 is very useful for culture prediction of this fish. The coefficient of determination of 0.97 suggests that about 97% of the variations in the fish length were explained by the weight of the fish, further justifying the isometric growth pattern prediction of this fish.

Conclusion

The present study, for the first time, documented ovarian development and sexual maturity of *C. striatus* raised in captivity through observation of morphological changes and histological analysis of the ovary. The macroscopic and histological analysis of the ovaries along with examination of oocyte development showed that first sexual maturity could be achieved as early as 8 months, compared to pond-raised and wild fish which took at least 11 months, if sufficient good quality feed is provided to the fish. The observations from this study with regard to ovarian development in *C. striatus* will contribute towards developing strategies for broodstock management in hatcheries.

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