



## INSIGHTS INTO THE REPRODUCTIVE BIOLOGY AND AGE AT MATURITY OF CLIMBING PERCH, *Anabas testudineus* (BLOCH, 1792) IN MALAYSIA

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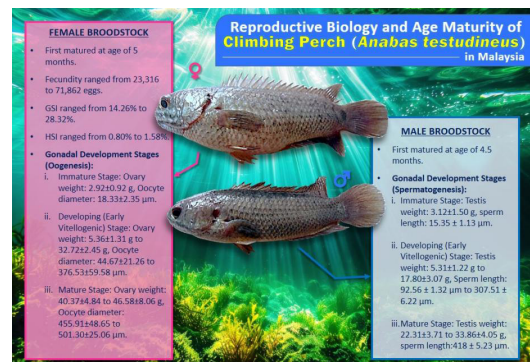
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### HIGHLIGHTS

- Males matured at 4.5 months (12.69 ± 0.75 cm TL), while females reached maturity at five months (12.4 ± 0.96 cm) under controlled conditions.
- Fecundity ranged from 23,316 to 71,862 eggs, with positive linear relationships observed with total length, body weight, and gonad weight.
- Gonadosomatic Index (GSI) values ranged from 14.26% to 28.32% and Hepatosomatic Index (HSI) values ranged from 0.80% to 1.58%.
- Demonstrated that climbing perch can reach sexual maturity and reproduce effectively within five months under captive conditions.

### GRAPHICAL ABSTRACT



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### ABSTRACT

Understanding the reproductive biology and age at maturity of climbing perch (*Anabas testudineus*) is essential for effective aquaculture management and broodstock development. This study examined the species' reproductive traits under laboratory conditions, focusing on fecundity, Gonadosomatic Index (GSI), Hepatosomatic Index (HSI), and age at maturity. Fecundity was assessed in 30 mature females (total length: 11.0 to 15.0 cm; weight: 32.72 to 68.92 g), which exhibited a fecundity range of 23,316 to 71,862 eggs, with a mean of 49,498 eggs. Positive linear relationships were observed between fecundity and parameters such as total length, body weight, and gonad weight. GSI values ranged from 14.26% to 28.32% and HSI ranged from 0.80% to 1.58%. Gonadal development and age at maturity were studied

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in juveniles with histological analysis revealing that males reached maturity at 4.5 months ( $12.66 \pm 1.73$  cm,  $34.54 \pm 10.56$  g,  $n = 30$ ), while females matured at 5 months ( $12.4 \pm 0.96$  cm,  $48.06 \pm 14.59$  g,  $n = 30$ ). The gonads of both sexes progressed through distinct stages of development with maturity being reached during the latter stages. These findings indicate that climbing perch achieve sexual maturity within five months under controlled conditions and these insights provide critical information in optimising broodstock management and seed production strategies. The study offers significant implications for aquaculture practices aimed at enhancing sustainability and productivity.

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## Introduction

The reproductive biology (spawning period, size at maturity) and age at sexual maturity of individual fish species are critical for sustainable fisheries management, conservation strategies, health monitoring, and aquaculture practices (Muchlisin *et al.*, 2010; van Overzee & Rijnsdorp, 2015; Hasan *et al.*, 2022). Among the numerous species that have successfully farmed through aquaculture, the climbing perch stands out as a highly thriving candidate. Climbing perch is a freshwater fish native to Southeast Asia and parts of Africa, and is found in fresh and brackish waters of Nigeria. It is widely traded across several countries in the region, including Malaysia, Thailand, Vietnam, India, Cambodia, the Philippines, and Indonesia (Slamat *et al.*, 2019).

Climbing perch is a widely favoured food and received attention through previous studies exploring its reproduction and spawning behaviour (Zworykin, 2012), captive breeding (Sarkar *et al.*, 2005), breeding and seed production methods (Bhuyan & Hussain, 2018; Kumar & Mohanty, 2023), induced breeding (Zalina *et al.*, 2012), crossbreeding (Ahhammad *et al.*, 2021), nursing technology (Uddin *et al.*, 2021), and alternative feed (Akbar *et al.*,

2016; Vongvichith *et al.*, 2020) as well as its performance in semi-intensive aquaculture systems (Kohinoor *et al.*, 2009; Chakraborty, 2016; Mumu & Hossain, 2021).

In Malaysia, climbing perch farming has gained popularity due to the species' adaptability and economic value. Farmers use various systems, including earthen ponds, concrete tanks, and cages (Choresca *et al.*, 2024), as the species can survive in poor water quality, low oxygen, and high stocking densities, making it suitable for both rural and urban aquaculture (Rahman *et al.*, 2012; Debnath *et al.*, 2022). Breeding involves natural or induced spawning with careful broodstock selection. They are fed commercial pellets or alternative protein sources like insect meal (Vongvichith *et al.*, 2020). Semi-intensive and intensive systems optimise growth and productivity. Valued for its market demand and potential in integrated farming systems, climbing perch contributes to food security and income to Malaysia's aquaculture sector (Sarker, 2015). However, detailed studies on its reproductive biology and age at maturity in Malaysian waters are lacking; with existing research focusing more on general biology or aquaculture performance.

Reproductive biology is essential for understanding fish life history, including growth patterns, reproductive strategies, spawning behaviour, and fecundity (Muchlisin, 2014). Age at maturity is a key parameter, reflecting reproductive investment and its impact on the population (Kuparinen *et al.*, 2011; Asheim *et al.*, 2023). In aquaculture and fisheries management, it is crucial as harvesting immature individuals can reduce population size and recruitment. Analysing traits such as GSI, oocyte development, and spawning periodicity offers insights into species resilience and potential under environmental variability (Stequert *et al.*, 2001). Understanding maturity timing is vital for predicting spawning periods and annual population changes (Froese & Binohlan, 2000; Pius, 2005).

The sizes at mature climbing perch ranges between 8 and 15 cm total length, with fecundity varying from hundreds to tens of thousands of eggs (Amornsakun *et al.*, 2005; Pius, 2005; Marimuthu *et al.*, 2009; Ndobe *et al.*, 2020). Spawning is seasonal with timing and duration varying by location (Behera *et al.*, 2015; Froese & Pauly, 2019; Jalilah, 2021). Pius (2005) observed a short spawning season in India (May-June) with a single group of maturing eggs. This study supports the notion that environmental factors and genetic variation influences the size at maturity, egg size, fertility, and sex. In northern and eastern Peninsular Malaysia, increased ovarian activity in wild females correlates with lower water temperatures and higher rainfall (Jalilah *et al.*, 2021). Most research has focused on wild populations with limited data on captive populations, which may exhibit different reproductive traits.

Previous research by Hafijunnahar (2016) explored the size at first maturity of climbing perch, but the age at maturity has not been studied in detail. While fecundity and spawning behaviour has been examined, the age at sexual maturity remains unclear. This gap is significant

for understanding reproductive strategies, population dynamics, and sustainability, especially in aquaculture and fisheries management (Behera *et al.*, 2015). Determining the age at maturity is crucial for optimising breeding programmes and maintaining healthy populations (Thorpe, 2004). Therefore, this study aims to fill these gaps by investigating the reproductive biology and age at maturity of climbing perch in Malaysia, focusing on fecundity, GSI, HSI, gonadal development, and the age and size at first maturity.

## Materials and Methods

### *Study Site and Broodstock Management*

This research was conducted at the Aquaculture Hatchery, Institute of Tropical Aquaculture and Fisheries (AKUATROP), University Malaysia Terengganu (UMT), Kuala Nerus, Terengganu. One hundred healthy broodstock of climbing perch (50 males and 50 females) were procured from a supplier in Marang, Terengganu, and housed in two circular fiberglass tanks (2-tonnes capacity) filled to three-quarters with freshwater and equipped with adequate aeration. The total length of male *A. testudineus* ranged from 8.0 to 14.0 cm with body weight between 16.81 and 40.11 g, while females ranged from 8.0 to 15.0 cm in total length and were 21.22 to 60.32 g in body weight. Before stocking, the broodstock was sexed based on physical characteristics and kept in separate tanks. Females were identified by their larger size (especially during the spawning condition), swollen genital papilla with a slight pink coloration, bulging and soft abdomen, and the ability to release eggs under slight abdominal pressure. Males were distinguished by their narrow, pointed genital papilla and free-flowing milt upon gentle abdominal pressure.

To prevent escape, the tanks were securely covered. The broodstock was fed commercial pellets at approximately 20% of their body weight daily, distributed in three feedings

(morning, afternoon, and evening). Uneaten feed was removed 30 minutes after feeding to avoid water quality deterioration. Water quality was maintained by replacing 40% of the tank water daily after the morning feeding. Tanks were well-aerated and water parameters were consistently monitored to ensure optimal conditions: Dissolved oxygen above 5 ppm, temperature between 25°C to 29°C, and pH between 6.5 and 7.5. Daily water quality monitoring was conducted using a ProQuatro Multiparameter Meter (Model PRO1030, YSI).

#### **Determination of Fecundity, GSI, and HSI**

A total of 30 females were used to estimate their fecundity, GSI, and HSI. Only mature fish were used for this examination. The fish were opened and their gonads were removed. The ovaries were weighed to calculate the GSI using the formula:  $GSI = (\text{ovary weight} \div \text{body weight}) \times 100$ . The liver was also removed from the fish and weighed to calculate the Hepatosomatic Index (HSI) using the formula:  $HSI = (100 \times \text{liver weight}) \div (\text{body weight} - \text{ovary weight})$ . Afterward, the ovaries were opened, and a subsample of eggs was taken from the ovary and weighed (1 g) using a digital scale. The subsample was placed in a Petri dish to calculate fecundity, using the formula:  $\text{Fecundity} = (\text{number of eggs in subsample} \div \text{gonad weight}) \times \text{weight of the subsample}$ .

#### **Gonad Development and Maturity**

Induced breeding was done by selecting three paired of matured climbing perch broodstock (three females and nine males) and given intramuscular injections of ovaprim hormone (0.5 mL/kg fish) near the caudal peduncle. After that, all the broodstock were released to spawn in three breeding aquaria as breeding

containers which that ratio of female to male was 1:3 until fertilisation occurred. Fertilisation occurred after 7 to 8 hours. After fertilisation, the broodstock was removed from the aquaria while the eggs were left back and incubated. All the aquaria were given continuous aeration, and the temperature was set at 26°C to 29°C during incubation period to aid hatching. After 25 to 27 hours, the eggs hatched.

Five hundred hatched larvae were randomly selected and placed in five aquaria. During the 1<sup>st</sup> to 7<sup>th</sup> day, the larvae were fed freshly hatched *Artemia salina* nauplii, followed by a combination of *Artemia salina* nauplii and powdered dry food from the 8<sup>th</sup> to 30<sup>th</sup> day. Subsequently, juvenile climbing perch were fed commercial pellet food three times a day until they reached adulthood.

Maturity tests were conducted using histological methods. Ten fish were randomly sampled at monthly intervals, starting at 1 month of age and then at 1.5 months, 2 months, 2.5 months, and up to the time full maturity was confirmed. The samples were collected from all aquaria. The histological procedure involved in measuring the length and weight of the sampled fish, cutting them open and removing their gonads. Following the method of Cek and Yilmaz (2007), the gonads were fixed in Bouin's solution, dehydrated, embedded in paraffin, sectioned at 5 µm, and stained with haematoxylin and eosin.

Gonadal maturation stages were determined according to Lubzens (2010) and Schulz (2002), and these stages were summarised in Table 1. Oocyte sizes were measured during each period using an advanced microscope. For each sample, 30 oocytes were measured and the mean oocyte diameter was calculated.

Table 1: Summary of the gonadal maturation stages based on Lubzens (2010) and Schulz (2002)

Gonadal Stages	Oocytes (Lubzens, 2010)	Sperm Cells (Schulz, 2002)
Immature/previtellogenic	Previtellogenic phase (no yolk deposition)	Spermatogonia (undifferentiated cells)
Maturing/vitellogenic	Vitellogenic phase (yolk deposition starts)	Spermatocytes and secondary spermatocytes (undergoing division)
Mature/ready for fertilisation	Maturation phase (final oocyte development)	Spermatids and spermatozoa (fully developed sperm cells)

### *Age and Size at First Maturity*

When the gonad development and maturity examination were done, the age and size (total length and body weight) of juveniles were recorded. Furthermore, observations on their changing body characteristics like bulging of abdomen and genital papilla and colour changes were also recorded. These parameters were used to determine the age and size of maturity for climbing perch. Percentage of oocyte and the cell of testis for each stage in every month was determined using the following formula: Percentage of oocyte for each stage = Number of oocyte in a selected phase ÷ total number of oocyte.

### *Data Analysis*

Data collected from this study included the fecundity and size of the mature females, GSI, HSI, ovary weight, ova diameter, and age. Mean and standard derivations for each parameter for all data collected was calculated using Microsoft Office Excel software. The comparison of mean for fecundity, GSI, and HSI between total length groups was analysed using the Paired Samples T-test through SPSS software version 28. Linear regression between fecundity and total length, body weight, ovary weight, ova diameter, GSI, and HSI was determined, and also the linear regression between age and total length, body weight of male and female fry of climbing perch was performed.

### **Results**

#### *Fecundity, Gonadosomatic Index (GSI), and Hepatosomatic Index (HSI) of Broodstocks*

The mature ovaries of climbing perch were paired, they were yellow in appearance and appeared yellowish-white when ready for spawning. The fecundity, GSI, and HSI of climbing perch was measured across different size categories, and the results are presented in Table 2. From the 30 specimens, with total length ranging between 11.0 and 15.0 cm and weight ranging between 32.72 and 68.92 g, the number of eggs per female ranged between 23,316 and 71,862 eggs with a mean of 49,498 eggs. The compared mean, Paired Samples T-test results showed that the fecundity, GSI, and HSI of climbing perch was significantly different to the total length, with *p*-values of 0.001, 0.003, and 0.001, respectively. Fecundity generally increased with fish size with the smallest fish (11.0 cm) having a fecundity of 33,788.2 eggs, while the largest fish (15.0 cm) exhibited a fecundity of 57,112.3 eggs. This increase in fecundity with an increase in the size of the fish is typical for many fish species, where larger individuals have a higher reproductive capacity.

The GSI values showed variability with the smallest fish (11.0 cm) having a GSI of 18.38% and the largest fish (15.0 cm) showing a lower GSI of 14.26%. The peak GSI value (21.67%) was observed in a fish of 13.08 cm, suggesting the most intense gonadal development occurred in this intermediate-size group. This variation in GSI could reflect different stages of gonadal

maturation, with larger fish potentially investing less in reproductive tissue at the time of sampling.

The HSI, which indicates liver size relative to body weight, also varied across the size groups. The smallest fish (11.0 cm) had the lowest HSI at 0.8304%, indicating lower liver development relative to body weight. The highest HSI (3.44%) was recorded in the 14.18 cm fish, suggesting greater energy storage

in the liver at this size. However, the HSI decreased in the largest fish (15.0 cm) to 0.93%, potentially indicating a shift in energy allocation at larger sizes. These findings demonstrate the physiological differences in reproductive and metabolic indices of climbing perch across various size classes, thus offering important insights into its reproductive biology and energy allocation strategies.

Table 2: The fecundity, GSI, and HSI of the climbing perch, *Anabas testudineus* (n = 30). Data were expressed as mean ± Std. Dev. Different superscript alphabets signify statistically significant difference between groups ( $p \leq 0.05$ )

Sizes (cm)	Fecundity	GSI (%)	HSI (%)
11.00 ± 0.00	33,788.2 ± 12.01 <sup>a</sup>	18.38 ± 0.36 <sup>b</sup>	0.83 ± 0.00 <sup>a</sup>
12.06 ± 0.18	37,939.38 ± 41.50 <sup>b</sup>	20.57 ± 1.50 <sup>c</sup>	1.23 ± 0.35 <sup>c</sup>
13.08 ± 0.18	52,182.01 ± 8,310.97 <sup>c</sup>	21.67 ± 2.39 <sup>c</sup>	1.17 ± 0.23 <sup>c</sup>
14.18 ± 0.27	53,679.02 ± 9,173.06 <sup>cd</sup>	19.52 ± 2.26 <sup>bc</sup>	3.44 ± 6.91 <sup>d</sup>
15.0 ± 0.00	57,112.3 ± 7,919.47 <sup>d</sup>	14.26 ± 3.67 <sup>a</sup>	0.93 ± 0.23 <sup>b</sup>

The relationships between fecundity (F) and total length (TL), body weight (BW), ovary weight (OW), GSI, and HSI, as well as between oocyte diameter (OD) and TL and BW were linear and expressed using the equation  $Y = mx + c$ , with 'r<sup>2</sup>' values indicating correlation strength. These relationships are illustrated in Figure 1.

Fecundity and total length showed a linear relationship with the equation  $F = 7,923 TL - 555,999$  ( $r^2 = 0.503$ ), with fecundity ranging from 23,316 to 71,862 eggs across the length range [Figure 1 (A)]. Similarly, fecundity and body weight were linearly related ( $F = 951.7 BW + 4,085$ ;  $r^2 = 0.513$ ), with fecundity ranging

from 23,316 to 71,862 eggs at corresponding body weights of 32.72 to 68.92 g [Figure 1 (B)].

The relationship between fecundity and ovary weight was also linear ( $F = 64,422 OW - 12,053$ ;  $r^2 = 0.882$ ), with fecundity ranging from 23,316 to 71,862 eggs across ovary weights of 5.8 to 12.18 g [Figure 1 (C)].

Fecundity showed a positive correlation with GSI ( $F = 1,264 GSI + 23,848$ ;  $r^2 = 0.101$ ) and HSI ( $F = 12,619 HSI + 34,934$ ;  $r^2 = 0.064$ ) [Figures 1 (D) and (E)]. Egg diameter ranged from 601.42 to 923.87 μm, with a positive relationship to TL ( $OD = 29.6 TL + 411.2$ ;  $r^2 = 0.155$ ) and BW ( $OD = 2.762 BW + 672.1$ ;  $r^2 = 0.095$ ).

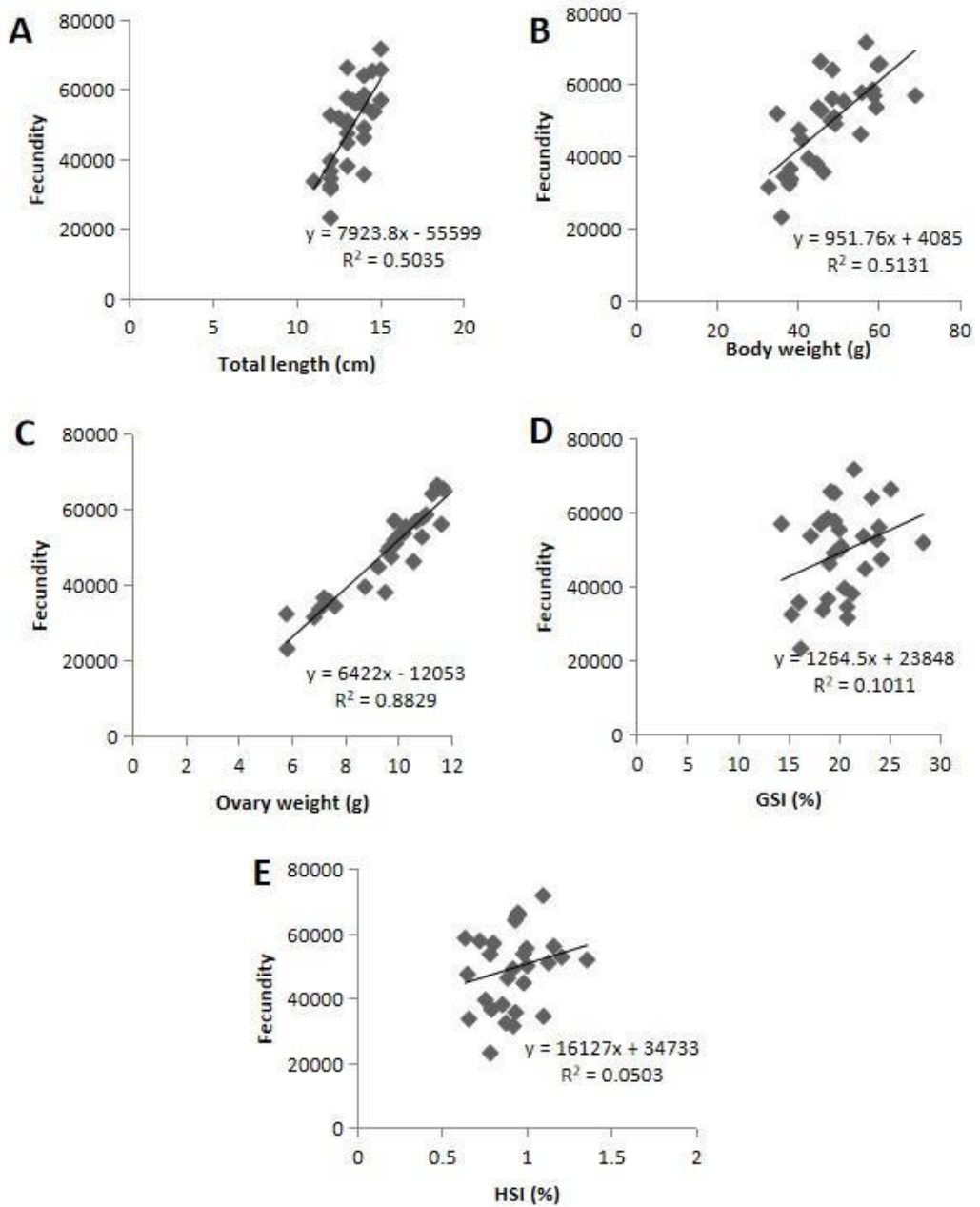


Figure 1: The relationship between fecundity (number of eggs) of climbing perch, *Anabas testudineus* with (A) total length of climbing perch (TL); (B) body weight (BW); (C) ovary weight (OW); (D) Gonadosomatic Index (GSI); and, (E) Hepatosomatic Index (HSI)

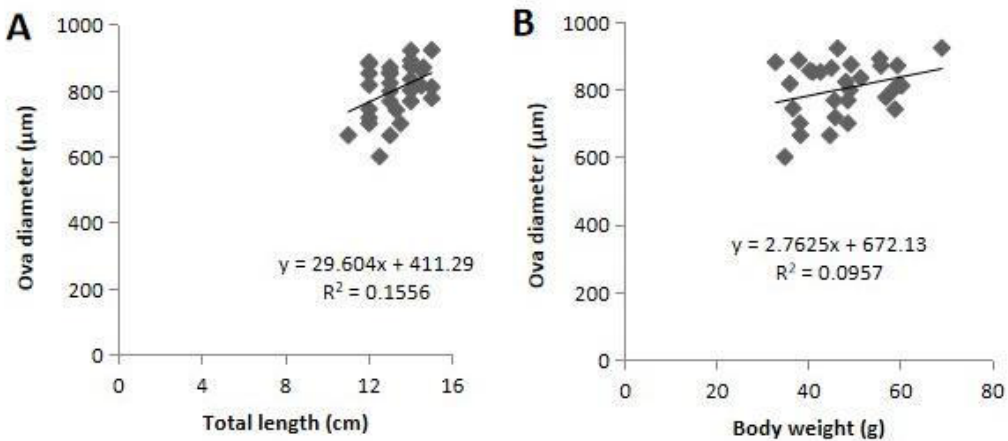


Figure 2: The relationship between ova diameter (µm) of climbing perch, *Anabas testudineus* with (A) total length and (B) body weight

**The Age at First Maturity**

The results from Table 3 shows the growth and reproductive development of male and female climbing perch over time. The male fully matured at the age 4.5 month (135 days after they hatched) with their size is  $12.66 \pm 1.73$  cm in length and  $34.54 \pm 10.56$  g in weight. The female fully matured at the age 5 month (150 days after hatched) with their size is  $12.4 \pm 0.96$  cm in length and  $48.06 \pm 14.59$  g. Maturity was determined based on histological analysis of gonadal development, which showed the presence of fully developed gonads.

For male climbing perch, the total length increased progressively from an average of  $2.4 \pm 0.72$  cm in the first month (Stage 1 maturity) to  $12.69 \pm 0.75$  cm in the fifth month. Males were observed to have five stages of spermatozoa development, with Stage 5 being the final and fully matured stage. By the fifth month, all males had reached Stage 5, indicating full reproductive maturity. The transition from the lower stages (Stages 1 and 2) to higher stages (Stages 4 and 5) occurred steadily as their size increased.

For female climbing perch, total length also showed a steady increase from  $2.4 \pm 0.1$  cm in the first month (Stage 1 maturity) to  $13.2 \pm 4.5$  cm by the fourth month. Females were observed to have six stages of oocyte development, with Stage 6 being the final and fully matured stage. By the fifth month, females reached an average total length of  $12.4 \pm 0.96$  cm and all had progressed to Stage 6. The oocyte diameter increased significantly over time, starting at  $18.33 \pm 2.35$  µm in Stage 1 and reaching  $501.31 \pm 25.06$  µm in Stage 6. Notable increases in oocyte diameter were observed between Stages 4 and 6, reflecting advanced gonadal development.

These findings indicate that male climbing perch achieve reproductive maturity by reaching Stage 5, while females reached full maturity at Stage 6. Males and females showed comparable growth in total length, but their reproductive maturation stages and physiological markers such as oocyte and spermatozoa development, differ significantly. This distinction underscores the sexual dimorphism in reproductive development within climbing perch populations.

Table 3: Monthly change in growth, maturity stages, and oocyte development of male and female of climbing perch, *A. testudineus* based on their stages of maturity. Data are expressed as mean  $\pm$  Std. Dev.

Months	Total Length of Female (cm)	Maturity Stages of Female	Oocyte Diameter ( $\mu$ )	Total Length of Male (Cm)	Maturity Stages of Male
1	2.4 $\pm$ 0.1	Stage 1	18.33014 $\pm$ 2.35	2.4 $\pm$ 0.72	Stage 1
1.5	4.6 $\pm$ 0.78	Stages 1 and 2	44.67443 $\pm$ 21.26	3.1 $\pm$ 1.32	Stages 1 and 2
2	5.57 $\pm$ 1.01	Stages 1, 2, and 3	55.702 $\pm$ 11.95	5.57 $\pm$ 1.01	Stages 1, 2, and 3
2.5	6.07 $\pm$ 0.47	Stages 1, 2, and 3	75.50529 $\pm$ 28.83	8.07 $\pm$ 1.56	Stages 2 and 3
3	9.1 $\pm$ 1.15	Stages 2, 3, and 4	130.2249 $\pm$ 45.12	9.4 $\pm$ 1.54	Stages 3 and 4
3.5	10.07 $\pm$ 1.46	Stages 3, 4, and 5	242.5314 $\pm$ 51.50	11.09 $\pm$ 2.32	Stages 4 and 5
4	12.63 $\pm$ 1.89	Stages 4, 5, and 6	376.5389 $\pm$ 59.58	12.25 $\pm$ 1.99	Stages 4 and 5
4.5	13.20 $\pm$ 4.50	Stages 5 and 6	455.9144 $\pm$ 48.65	12.66 $\pm$ 1.73	Stage 5
5	12.4 $\pm$ 0.96	Stage 6	501.3054 $\pm$ 25.06	12.69 $\pm$ 0.75	Stage 5

### Gonadal Development of Male

The male climbing perch are smaller than female and have an oblong body. The genital papilla of males is rather pointed and narrow with free oozing milt when slight pressure is applied to the abdomen. The monthly changes in gonad development of male climbing perch based on their stages of maturity is shown in Table 4. The males matured fully earlier than female which was at the age of 4.5 months (135 days after hatched), with their size being 12.66  $\pm$  1.73 cm (mean  $\pm$  Std. Dev.) in length and 34.54  $\pm$  10.56 g in weight. At the age of 1 month, the cells of testis in male *A. testudineus* were in Stage 1 and known as spermatogonia. The percentage of Stage 1 cells was 99.98% and the rest 0.02% comprised of Stage 2. About 44.09% cells in testis were at Stage 2 (spermatocytes) at the age of 1.5 months, while 55.91% cells were still at Stage 1. The 2 months old testis consisted of 1.17% of Stage 1, 89.29% of Stage

2, and 9.54% of Stage 3, while at 2.5 months the testis comprised of 19.22% of Stage 2 cell and 80.78% of Stage 3. Stage 3 is also known as secondary spermatocytes.

Stage 4 which is known as spermatid was formed 63.85% when the age of fish was 3 months, and the percentage of Stage 3 reduced to 36.15%. At the age of 3.5 months, the testis comprised of 4.88% of Stage 3, 70.11% of Stage 4, and 25.01 % of Stage 5 (spermatozoa). The percentage of Stages 4 and 5 at 4 months of age was 23.15% and 76.85%, respectively. At the age of 4.5 months, the cells were 97.89% transformed to spermatozoa (Stage 5) and the fish were matured at this time. At the age of 5 months, the cell was 100% spermatozoa.

The histological structure of spermatozoa in climbing perch from 1 month to 4 months and 5-month-old fish can be referred to Figure 3.

Table 4: The stages of gonadal development for male climbing perch, *Anabas testudineus*. Data are expressed as mean ± Std. Dev. (n = 30)

Age (Months)	Total Length (cm)	Weight (g)	Maturity Stages	Sperm Length (µm)	Condition of the Cell in Testis
1	2.4 ± 0.72	3.12 ± 1.50	Stage 1	15.35 ± 1.13	At this time, the cell known as spermatogonia divides mitotically to form spermatocytes
1.5	3.1 ± 1.32	5.31 ± 1.22	Stages 1 and 2	92.56 ± 1.32	At this age, combinations of spermatogonia and spermatocytes appear in testis
2	5.57 ± 1.01	8.921 ± 1.14	Stages 1, 2, and 3	145 ± 1.67	At this age, there are combinations of spermatogonia, spermatocytes, and secondary spermatocytes which appear in testis
2.5	6.07 ± 0.47	13.81 ± 3.71	Stages 2 and 3	284 ± 1.84	At this age, combinations of spermatocytes and secondary spermatocytes appear in testis
3	9.1 ± 1.15	17.80 ± 3.07	Stages 3 and 4	307.51 ± 1.54	At this age, combinations of secondary spermatocytes and spermatid appear in testis
3.5	10.07 ± 1.46	22.31 ± 3.71	Stages 4 and 5	340.82 ± 1.88	At this age, combinations of spermatid and spermatozoa appear in testis
4	12.63 ± 1.89	30.23 ± 2.03	Stages 4 and 5	370.21 ± 1.32	At this age, combinations of spermatid and spermatozoa appear in testis
4.5	12.66 ± 1.73	32.5 ± 3.65	Stage 5	395.11 ± 2.44	At this time, only spermatozoa were found in the testis and the fish were fully mature
5	12.69 ± 0.75	33.86 ± 4.05	Stage 5	418.00 ± 5.23	

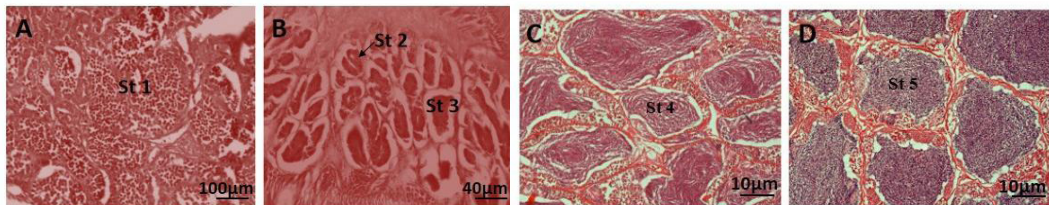


Figure 3: The structure of testis in the male of climbing perch, *Anabas testudineus*; (A) 1 month old fish consist of Stage 1 (spermatogonia cell); St 1 = Stage 1; scale = 100 µm; (B) 2.5 months old fish consist of Stage 2 (spermatocytes) and Stage 3 (secondary spermatocytes); St 2 = Stage 2; St 3 = Stage 3; scale = 40 µm; (C) 3.5 months old fish consist of Stage 4 (spermatid); St 4 = Stage 4; scale 10 µm; (D) 5 months old fish consist of Stage 5 cell (spermatozoa); St 5 = Stage 5; 20 x magnification; scale = 10 µm

**Gonadal Development of Female**

Female climbing perch, based on the stages of maturity are elongated and have a pair of ovaries which is located inside its abdomen and at the posterior half of the body cavity. The monthly changes in gonad development of female climbing perch, based on their stage of maturity is shown in Table 5. The female is fully matured by the age of 5 months (150 days after hatched) with their average size of 12.4 ± 0.96 cm in length and 48.06 ± 14.59 g in weight. At the age of 1 month, the oocyte of climbing perch consists of Stage 1 and Stage 2, with percentage of oocyte for Stage 1 being 80.58%, while Stage 2 is only 19.45%. At the age of 1.5 months, the Stage 2 oocyte were increased to 70.56%, while the Stage 1 oocyte is 29.17%. Stage 3 oocyte also exist in this age but only in small number (0.8%). At the age of 2 months, the number of Stage 1 oocyte was 24.86%, 73.46% for Stage 2, and 1.96% for Stage 3. When the fish is 2.5 months old, the percentage of Stage 1 oocyte

hit 11.05%, and Stage 2 reached 50.86 %, while Stage 3 oocyte was 38.08%.

The ovary of 3 months old fish consists of 2.65% of Stage 2 oocyte, 82.30% of Stage 3, and 14.10% of Stage 4 oocyte. While at 3.5 months ovary, the Stage 2 oocyte was gone and the Stage 3 oocyte is 26.96%, while Stage 4 oocyte is 61.98%. Stage 5 oocyte also appeared at this age 14.06% of the time. At the age of 4 months, the ovary of the fish consisted of 0.62% Stage 3 oocytes, 4.97% Stage 4 oocytes, 85.66 % Stage 5 oocytes, and 8.75% Stage 6 oocytes. When the fish turns 4.5 months, the ovaries are comprising of 66.22% and 33.78% of Stage 5 and Stage 6 oocyte, respectively. At the age of 5 months, the ovary is mature which consists of 99.75% Stage 6 oocytes and only 0.25% of Stage 5 oocytes. Figure 4 illustrates the histological structure of oocytes in climbing perch from 1 to 4.5 months old fish.

Table 5: The stage of gonadal development for females of climbing perch, *Anabas testudineus*. Data are expressed as mean ± Std. Dev. (n = 30)

Age (Month)	Total Length (cm)	Weight (g)	Maturity Stages	Oocyte Diameter (µm)	Condition of the Cell in Ovary
1	2.4 ± 0.1	2.92 ± 0.92	Stage 1	18.33 ± 2.35	At this time, the oocyte is known as a primary oocyte, and it is in previtellogenic phase. Only one nucleolus located in nucleus of oocyte
1.5	4.6 ± 0.78	5.36 ± 1.31	Stages 1 and 2	44.67 ± 21.26	At this age, the ovary still in the previtellogenic phase; however, the oocyte was increased in size and is surrounded by a layer of granulosa cells. The nucleus has several nucleoli at this stage

2	5.57 ± 1.01	9.80 ± 1.96	Stages 1, 2, and 3	55.70 ± 11.95	At this time, some oocyte are still in the previtellogenic phase; however, some of them were entering the vitellogenic phase and the oocyte was continuously increase in size
2.5	8.07 ± 1.56	15.05 ± 2.64	Stages 1, 2, and 3	75.50 ± 28.83	
3	9.4 ± 1.54	19.26 ± 2.47	Stages 2, 3, and 4	130.22 ± 45.12	At this time, the oocyte passes the previtellogenic phase and some of them were enter a new stage and higher cortical alveoli are present in oocyte cytoplasm
3.5	11.09 ± 2.32	23.30 ± 3.08	Stages 3, 4, and 5	242.53 ± 51.50	At this time, the oocyte passes the vitellogenic phase, some of them have cortical alveoli in their cytoplasm and another oocyte enters the exogenous vitellogenesis phase
4	12.25 ± 1.99	32.72 ± 2.45	Stages 4, 5, and 6	376.53 ± 59.58	At this stage, oocyte have cortical alveoli in their cytoplasm, some of them were enter the exogenous vitellogenesis phase and the oocytes were developed in this stage. A few oocytes enter the maturation phase
4.5	13.20 ± 4.50	40.37 ± 4.84	Stages 5 and 6	455.91 ± 48.65	At this time, oocyte passes the exogenous vitellogenesis phase and enter maturation stage. Some of the oocytes were entering the last stage of maturation
5	13.4 ± 0.96	46.58 ± 8.06	Stage 6	501.30 ± 25.06	At this time, the oocyte had reached the final maturation phase, and the ovary is now mature and ready to spawn

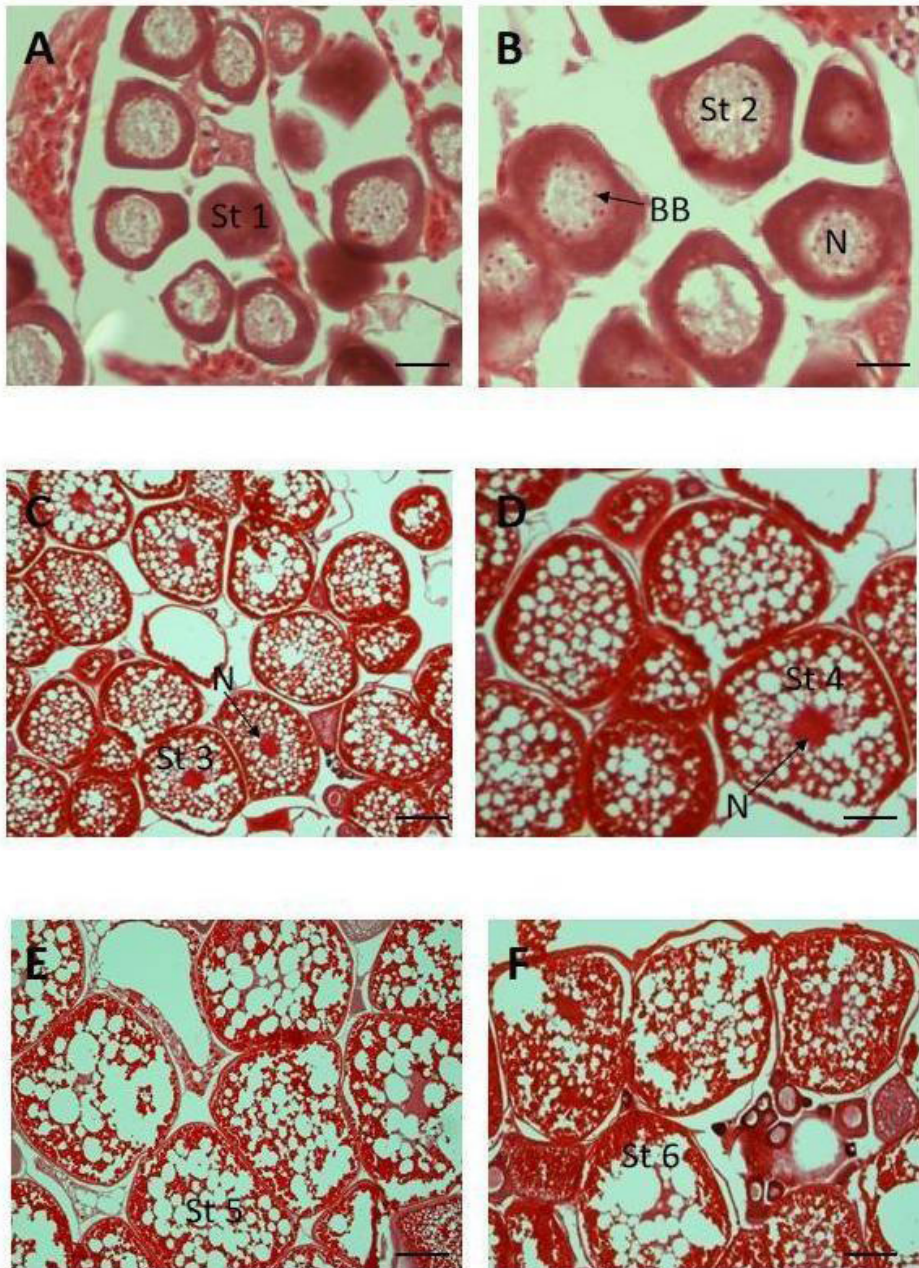


Figure 4: The structure climbing perch, *Anabas testudineus* oocyte; (A) oocyte on 1.5 months old fish (previtellogenic phase); St 1 = Stage 1; C = Connective tissue; (B) oocyte on 2 months old fish (previtellogenic phase); St 2 = Stage 2; (C) oocyte on 3 months old fish (vitellogenic phase); St 3 = Stage 3; N = Nucleus; BB = Balbiani bodies; (D) oocyte on 4.5 months old fish (exogenous vitellogenesis phase); St 4 = Stage 4; and, (E) oocyte on 4.5 months old fish (enter maturation)

## Discussion

Understanding and predicting annual population changes in fish requires a comprehensive understanding of their reproductive biology and breeding patterns, which include key parameters such as fecundity, breeding seasonality, spawning frequency, gonadal maturation, and size at first maturity (Muchlisin, 2014; Asheim *et al.*, 2023). These factors are essential for developing effective management strategies aimed at enhancing fish production and sustainability. While previous studies have examined the reproductive biology of climbing perch in India and Bangladesh (Banu *et al.*, 1985; Pius, 2005), research on this native species in Malaysia remains limited.

### *Size and Weight at Maturity*

In this study, female climbing perch exhibited a larger maximum total length and body weight compared to males, suggesting potential sexual size dimorphism. Comparisons with previous studies reveal differences in size and weight. For example, Pius (2005) reported larger sizes for Indian populations, while Marimuthu *et al.* (2009) observed a range of sizes in Malaysian populations. Such variations likely reflect genetic, environmental, and habitat-related factors, highlighting the influence of local conditions on fish growth and development.

### *Fecundity, GSI, and HSI*

Fecundity defined as the number of eggs produced by a mature female, varied widely in this study, exceeding values reported in other studies. For instance, Marimuthu *et al.* (2009) recorded fecundity ranges in Malaysian populations, while Banu *et al.* (1985) reported fecundity in Bangladeshi populations. Similarly, Pius (2005) found a range in Indian climbing perch, while Amornsakun *et al.* (2005) recorded a mean fecundity in Thai populations. The higher fecundity observed in this study suggests

an adaptive reproductive strategy, potentially influenced by favourable environmental factors or selective breeding practices. Variations in fecundity among individuals of similar size and weight highlight the role of factors such as nutritional status, habitat conditions, and physiological health (Dube, 1993; Muss & Dahlstrom, 1999).

The relationship between fecundity and biological parameters such as total length, body weight, gonad weight, GSI, and HSI was analysed, revealing strong positive correlations. The highest correlation was observed between fecundity and gonad weight ( $r^2 = 0.882$ ), consistent with findings in other fish species such as *Hampala macrolepidota* (Abidin, 1986) and *Mystus bleekeri* (Musa & Abdus, 2007). Variations in correlation strength as observed by Pius (2005) for Indian populations suggest that environmental and genetic factors significantly influence reproductive traits.

Higher GSI values observed in this study were associated with larger fish and more advanced gonadal maturation, aligning with Nichole (2010) who reported that increased GSI signifies the onset of spawning. Similarly, the HSI values were linked to gametogenesis as noted by Cek and Yilmaz (2007). These indices emphasise the physiological readiness of fish for reproduction and highlight variations across sizes and developmental stages.

### *Age and Size at Maturity*

This study provides insight into the age and size at first maturity in male and female climbing perch, reflecting key aspects of their growth and reproductive biology. Male climbing perch reach reproductive maturity earlier than females. These findings highlight sexual dimorphism in growth and reproductive development, with females investing more energy in oogenesis and egg production, which is reflected in their larger

body weight at maturity. This pattern aligns with the reproductive strategies of other freshwater fish species, where females require greater energy investment for gamete production (McBride *et al.*, 2015; Torsabo *et al.*, 2022; Le & Duong, 2023).

This study provides insight into the age and size at first maturity in male and female climbing perch, reflecting key aspects of their growth and reproductive biology. Male climbing perch reach reproductive maturity at 4.5 months (135 days post-hatch), with an average total length of  $12.66 \pm 1.73$  cm and body weight of  $34.54 \pm 10.56$  g. In contrast, females mature at 5 months (150 days post-hatch), with an average total length of  $12.4 \pm 0.96$  cm and body weight of  $48.06 \pm 14.59$  g.

When compared with the reproductive biology of other species such as *Cephalocassis borneensis*, some similarities and differences emerge. Like in *C. borneensis*, male climbing perch mature earlier and at a smaller size than females (Le & Duong, 2023). These findings reinforce the concept that sexual dimorphisms in growth and reproductive strategies is widespread among fish species, shaped by ecological and evolutionary pressures to maximise reproductive success (Mignien & Stoll, 2024).

### ***Gonadal Development and Maturity Stages of Male***

Testicular development in male climbing perch progresses through five stages. At 1 month old, testes were predominantly composed of spermatogonia (Stage 1), transitioning to spermatocytes (Stage 2) by 1.5 months. By 2 months, secondary spermatocytes (Stage 3) begin to appear, dominating by 2.5 months. Spermatids (Stage 4) emerge at 3 months and by 3.5 months, spermatozoa (Stage 5) constitute 25.01%, indicating nearing reproductive maturity. Full maturity is reached at 4.5 months

with 97.89% spermatozoa, and by 5 months, the testes was entirely composed of spermatozoa, marking readiness for spawning.

Spermatozoa length of male climbing perch increases from  $15.35 \pm 1.13$   $\mu$ m at 1 month of age to  $418.00 \pm 5.23$   $\mu$ m at 5 months of age, reflecting advanced testicular development and reproductive readiness. Early male maturity is an adaptive strategy, enhancing reproductive opportunities and success, consistent with findings in other teleost (Tsikliras & Stergiou, 2014; Hasan *et al.*, 2021; Kahar *et al.*, 2024).

### ***Gonadal Development and Maturity Stages of Female***

Female climbing perch progress through three gonadal phases—previtellogenic, vitellogenic, and maturation—spanning six stages. Maturation culminates at 5 months with fully developed oocytes, signalling readiness for spawning. Oocyte development aligns with typical teleost reproductive patterns, as reported in other species (Torsabo *et al.*, 2022; Le & Duong, 2023).

### **Conclusions**

This study provides valuable insights into the reproductive biology and age at maturity of climbing perch in Malaysia. Our findings demonstrate that female climbing perch reach full reproductive maturity at 5 months of age with an average total length of  $13.4 \pm 0.96$  cm and weight of  $46.58 \pm 8.06$  g, while males mature earlier, at 4.5 months, with an average total length of  $12.66 \pm 1.73$  cm and weight of  $32.5 \pm 3.65$  g. The study also highlights the significant size dimorphisms between sexes, with females investing more energy in oogenesis. Gonadal development progresses through distinct stages, with females undergoing six stages of oocyte maturation, while males exhibit five stages of spermatogenesis. Additionally, fecundity was found to be relatively high in the studied

population, suggesting a reproductive strategy adapted to local conditions. These findings not only contribute to a deeper understanding of the reproductive ecology of climbing perch in Malaysia, but also offers essential information for sustainable fisheries and aquaculture management of this species. Further studies are needed to explore environmental influences on reproductive health and to evaluate the impact of different farming practices on the reproductive success of climbing perch.

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### Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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