Reproductive Biology of *Anabas testudineus* From The Bencah Kelubi Village, Kampar Regency, Riau

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ABSTRACT

Anabas testudineus is a type fish that commonly inhabit blackwater area such as swamp, stream and canals in Riau. Eventhough this fish can be found easily, information on its biological aspect is rare. To understand the reproductive biology of this fish, a study has been conducted. The *A. testudineus* was sampled in irrigation canals in the palm tree plantation area in the Bencah Kelubi Village, Kampar Regency, Riau, from January to March 2013. There were 153 fishes (68 males and 85 females) captured. The size of fish is various, female ranged from 61 – 145 mm TL and 11 – 126 gr BW , and that of the male was 49 – 131 mm TL and 4 -79 gr BW. In January, almost all of fish were in the 1st to 2nd maturity stages and there was no mature fish. In February and March, however, there were mature males and females captured. It is predicted that mature male released pheromone that attract females and causing sex ratio between male and female was around 1 : 2.4 in the last 2 months. The male can be distinguished by longer dorsal fin, slimmer and darker body color. Study on histological structure of gonads shown that the ovary of mature female is dominated by mature eggs, indicated that this fish is a total spawner. In the testes tissue, surprisingly, there is trace of egg remains, indicated in special condition this fish might be able to reverse their sex .

***Keywords**: Reproductive biology, Anabas testudineus, Kampar Regency, Riau*

INTRODUCTION

Climbing perch *(Anabas testudineus)* or "*betok fish*" is freshwater fish that commonly inhabit stream, swamp and canals in the plantation areas in Riau. This fish also known as *blackwater fish,* as it has ability to inhabit blackwater and able to live in the area that has relatively low water quality such as low O_2, low pH and it is survive in the water with relatively high salinity (up to 20 ppm).

In Riau, the climbing perch has relatively high economical values. This fish commonly sold as dried-salted fish (Rp 60.000- Rp 80.000/ kg) or fresh (Rp 20.000 – Rp 40.000/ kg). Many traditional Riau culinaries are made from this type of fish.

Anabas testudineus is belonged to Anabantidae family. It is characterized by dark greenish or brownish in the dorsal area, yellowish in the lateral area and there are irregular vertical black stripes along the body of the fish. A black spot present behind the operculum and there are small spines along the rear edge of the operculum.

Even though *A. testudineus* is a common protein source in Riau, and demand of this fish is relatively high, culture of this fish is never been done. This fish is captured from natural waters throughout the year. As this fish can be found easily and the population of this fish in the nature is relatively high, there is no regulation in capturing the fish. There is no minimum legal size and no time limitation and no area boundary in exploiting this fish. This uncontrolled exploitation of this fish may lead to decrement fish population in the future. To prevent the occurence the future problem in *A. testudineus* population, several efforts, especially fish culture is needed. However, basic biological information needed for fish culture, such as nformation on reproductive biology of this fish is almost none. To provide the information needed, a study titled " Reproductive biology of *Anabas testudineus* from the Bencah Kelubi Village, Kampar Regency, Riau" is conducted.

METHODS

In this study, fish samples were captured from irrigation canals located in tha palm trees plantation area, in the Bencah Kelubi Village, Kampar Regency. Samplings were conducted once/ 2 weeks for a 3 months period. The fish was captured using bamboo traps, nets and line fishing. Macroscopic and microscopic studies on the reproductive biology of the fish were then conducted. The macroscopic characters studied are as follows:

a. Morphological characteristics of male and female fish (body size, body form, colour and specific sex-related characters)
b. Maturity level of the fish
c. Gonado Somatic Index (GSI, proportion of gonad weight toward body weight)
d. Fish fecundity (using a gravimetric method)

To study the microscopic characters of the reproductive biology of the fish, the fish gonads were processed for histological study. The gonad of 3 males and 3 females from fish in each maturity level were processed for histological study. The tissue were alcohol series processed, paraffin embedded, 6 µ sectioned and Haematoxylin – Eosin stained (Darjono *et al.*, 2001). The tissues were then studied using a binocular microscope (Olympus CX 21). The characters of gonad tissue in each maturity stage were then identified.

RESULTS AND DISCUSSION

In this study, total number of fish capured was 153 (68 males and 85 females). *A. testudineus* is heterosexual fish. The size of fish captured was various and in general female was bigger than the male. The size of female ranged from 61 – 145 mm TL and 11 – 126 gr BW , while that of the male was 49 – 131 mm TL and 4 -79 gr BW. Anonimous (2014) stated that the female Anabas is slightly bigger than the male.

General morphological characteristic of male and female fish is similar, but male has darker color, slimmer and longer body than that of the female, while female body tend to be rounded. Male has relatively longer anal fin. Axelrod *in*

Haloho (2008) stated that commonly male has darker body color than that of the female.

a) Male b) Female

Figure 1. Morphological characteristics *A.testudineus*

Gonad of *A. testudineus* is located in the abdominal cavity. The gonad consist of a pair of gonad lobes and each lobe is located in the outer side of the swim bladder. Putra *et al*. (2011) stated in the fish gonad located in the left and right side of the swim bladder.

Morphological characteristic of male and female gonads are different. During the early development period (1st maturity stage), male and female gonads are small, transparent, thread-like form and it is very difficult to be distinguished between ovary and testes. As the gonad developed, testes and ovary has its own characteristics and they can be distinguished easily. The ovary of female is bigger than that of males in the same maturity stage and egg granules can be identified easily. The ovary was grey whitish and become orange as the eggs mature. The testes, however, having smooth surface and whitish color. Its color is almost constant as the gonad developed.

During the sampling period, the number of male and female captured was fluctuated. In January, the number of males is higher than that of the females. In February and March, however, the number of male decreased and more female captured (Table 1).

Table 1. Sex ratio of *A. testudineus*

No	Month	Sex ratio of *A. testudineus*				Sex ratio (M: F)
		Male		Female		
1	January	43	63%	25	37%	1 : 0.58
2	February	16	29%	39	71%	1 : 2.4

3	March	9	30%	21	70%	1 : 2.3
	Average	68	41%	85	59%	1 : 1.25

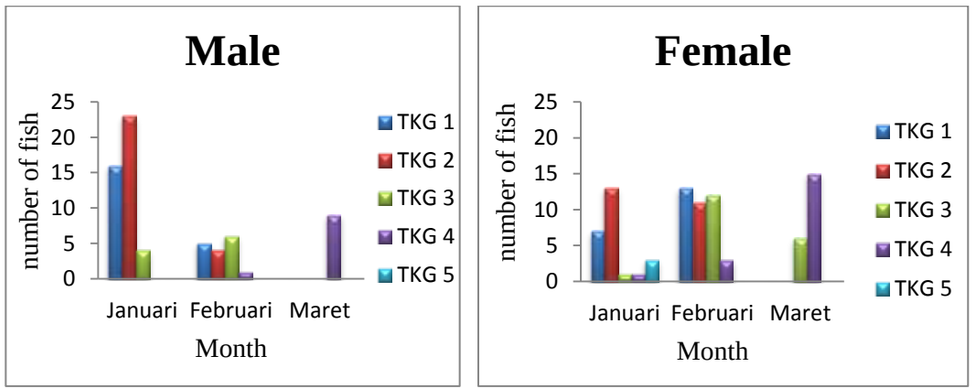


Figure 2. The number of fish captured, based on sex and gonad maturity levels

The Gonad Maturity Level (GML) of the fish captured during sampling period was vary. The males were in the 1st - 4th maturity level, while that of the females were the 1st to 5th. In January, most fish captured are immature (1st-3rd), while in February and March mature males and females are present. The differences of fish sex ratio during the sampling may related to the maturity level of the fish and pheromone produced by mature fish. In January, the number of male was higher than that of females, while in the next following 2 months, the number of females was greater than that of the males. As there was no mature fish in January, there was no pheromone released by the fish and as a consequence there was no fish come to the study area for conducting reproductive related activities.The fish present in the study area may conduct other activity such as feeding. Matty (1985) stated that the pheromone released with the urine of male will trigger the ovary of the female to develop. The pheromone also increase the unity of sexual activity in the fish population. In this recent study, high number of female present in the study area increase the possibility of that fish to be captured.

In February and March, in contrast, there was a number of mature males. It is predicted that the mature males released pheromone and attract mature females to come. As a result, the number of female increased and become more than the number male. Data on the sex ratio of *A. testudineus* also indicate that this fish may a polygamous fish, a single male may be able to fertilize several females.

In *A. testudineus*, the weight of gonad is increasing as the gonad maturity improve. Gonads become larger, heavier and thus the GSI increase as the gonad developed. In males, the GSI was 0.07% of BW in the 1st maturity level, and reach 0.63% of BW in the 4th maturity level. In female, however, the GSI increase drastically and peaked in the 4th (3.58% of BW). As the eggs are ovulated in this

stage, the ovary size reduced and the GSI drop into 0.12% of BW in the 5t^{h} maturity stage (Table 2 and Figure 3). Tang and Affandi (2001) stated that male body weight may increase by 10-15% as the gonad mature, while that of the female increase by 15-25%.

Table 2. Gonado Somatic Index (GSI) of *A. testudineus*

Maturity level	Male				Female			
	Number of fish	GSI (%)			Number of fish	GSI (%)		
		Min	Max	Average		Min	Max	Average
I	21	0.01	0.24	0.07	20	0.01	0.24	0.10
II	27	0.01	0.29	0.10	24	0.03	0.59	0.20
III	10	0.06	0.36	0.16	19	0.13	1.80	0.74
IV	10	0.43	0.88	0.63	19	1.37	5.86	3.58
V	0	-	-	-	3	0.10	0.15	0.12

In this present study, the GSI of male in general is lower than that of the female. The female gonad is relatively big and heavy as there is yolk accumulation in the mature eggs. Pellokila (2009) stated that in *Anabas* sp, the GSI value of the male is lower than that of the female.

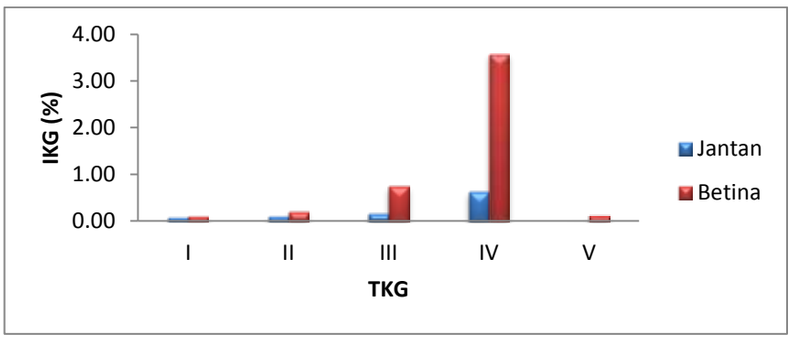


Figure 3. Gonado Somatic Index values of *Anabas testudineus*

The fecundity of *A. testudineus* is relatively high. In female with 74-145 mm SL and 16-111 gram BW, the fecundity ranged from 4,116-13,998 eggs. This result is lower that the fecundity of *A. testudineus* studied by Pellokila (2009), which is 964 – 30,208 eggs in female with 91 - 183 mm SL and 13 - 81 gram BW. Differences in fish size might causing the low fecundity of fish in this recent study. Effendie (2002) stated that fecundity is strongly related to body weight, while Ali (2005) stated that body size, age, environmental condition and egg size also affect the fecundity. In this study, body weight strongly affect the fecundity of *A. testudineus* (Figure 4).

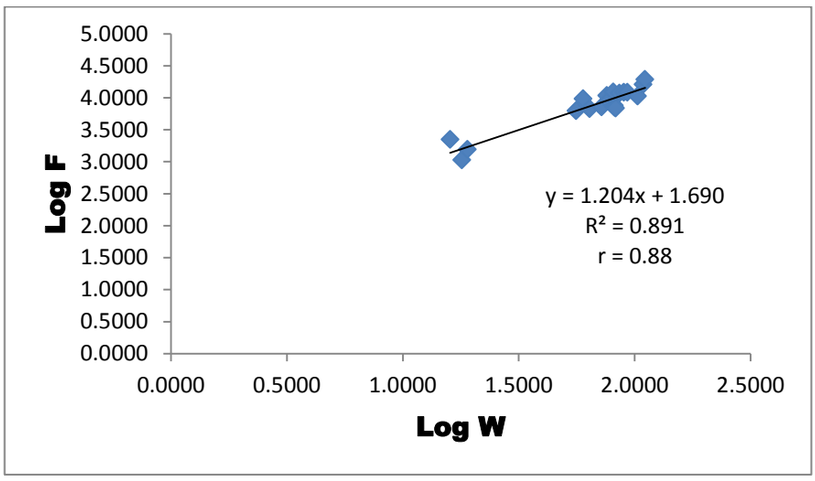


Figure 4. Relationship between body weight and fecundity of *A. testudineus*

Histological study of the ovary of female *A. testudineus* (Figure 5 and 6) showing that there is significant changing in the ovarian structure as the gonad developed. The ovary of fish in the 1st maturity stage filled with immature non-vittelogenic eggs and few eggs that are in early vittelogenic stage. The egg diameter range from 0.04-0.09 mm (non vittelogenic) and 0.05-0.10 mm (early vittelogenic stage). In the 2nd maturity stage, the early vittelogenic egg diameter increase into 0.08-0.12 mm. In the 3rd maturity stage, there are non vittelogenic, early vittelogenic and vittelogenic eggs (0.19-0.63 mm in diameter) present in the ovary. As the fish mature (4th maturity stage), the ovary is dominated by vittelogenic eggs that reach its maximum size, 0.45-0.69 mm in diameter. In the 5th maturity level, the mature eggs have been ovulated, the ovary become empty, the un-ovulated eggs become deteriorated (atretic eggs) and the non vittelogenic eggs present. In general, developmental stage of eggs in each maturity level is almost homogenous, indicated that *A. testudineus* is a total spawner.

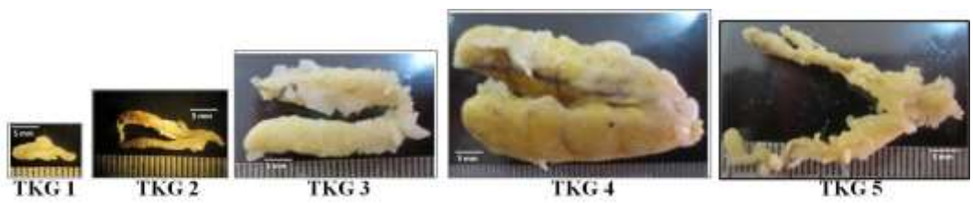
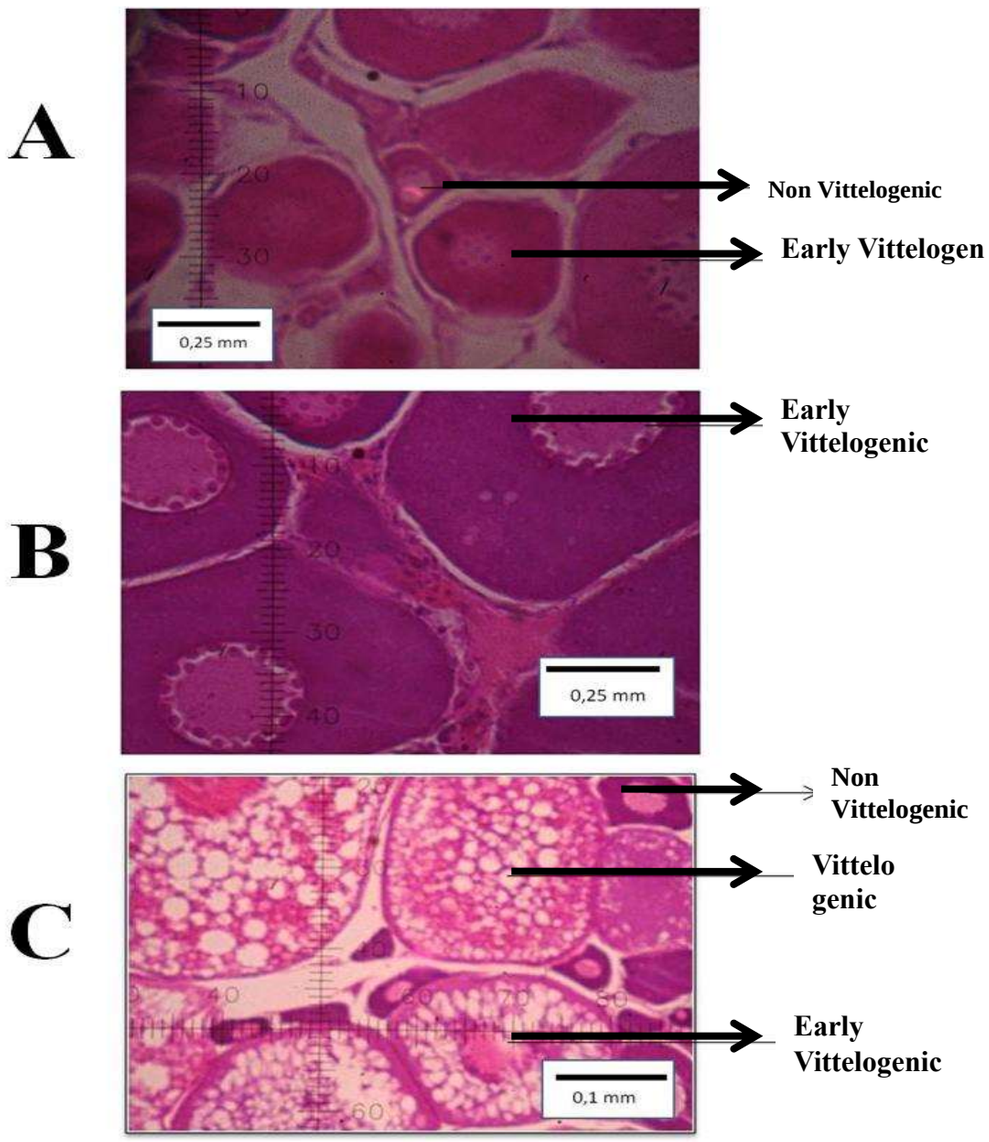


Figure 5. The morphology of *A. testudineus* ovary, in the 1st, 2nd, 3rd,4th and 5th maturity stages


A
Non Vittelogenic
Early Vittelogen
0,25 mm
B
Early
Vittelogenic
0,25 mm
C
Non
Vittelogenic
Vittelo
genic
Early
Vittelogenic
0,1 mm

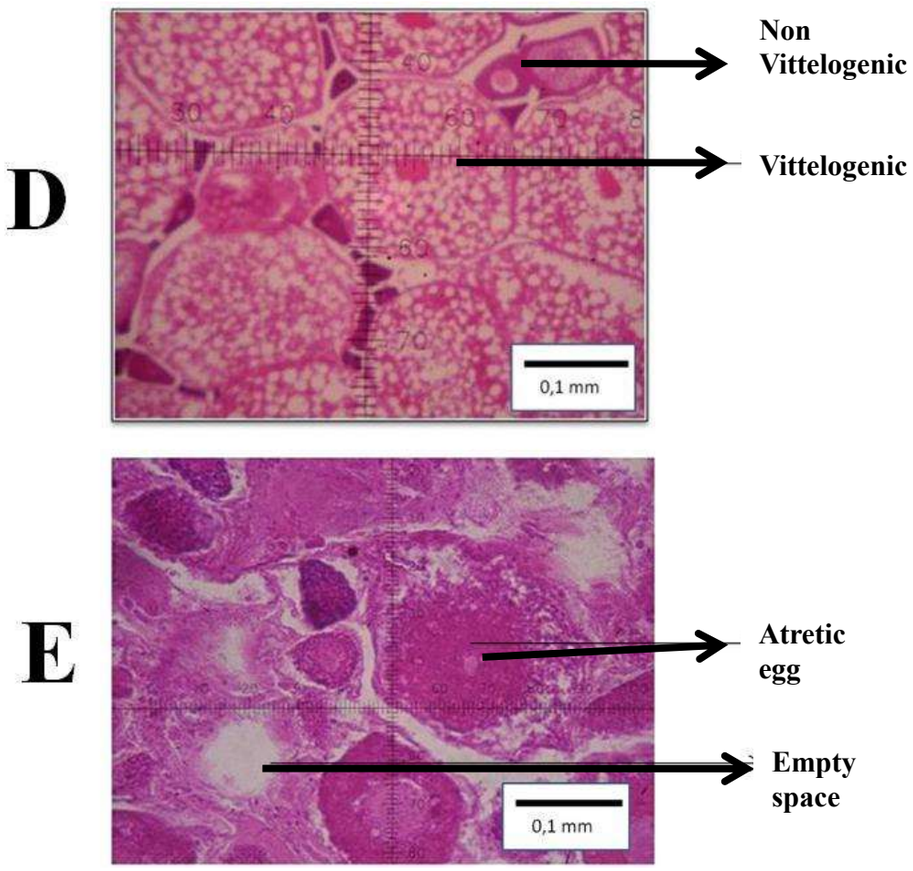


Figure 6. Ovarian structure of *A. testudineus* ovary, in the 1st, 2nd, 3rd,4th and 5th maturity stages

In male *A. testudineus*, testes structure also changing as the maturity of gonad improve (Figure 7 and 8). In the 1st maturity stage testes is small and transparent. As the testes developed, its size increase and the color become milky whitish. Putra *et al*. (2011) stated that the color of testes become milky whitish as the gamet developed.

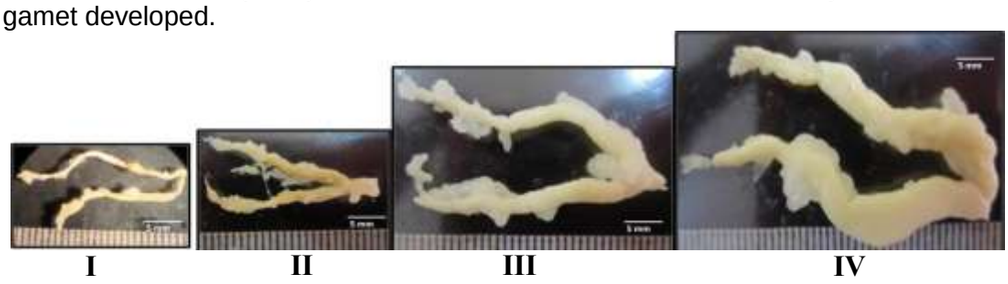


Figure 7. The morphology of *A. testudineus* testes, in the 1st, 2nd, 3rd and 4th maturity stages

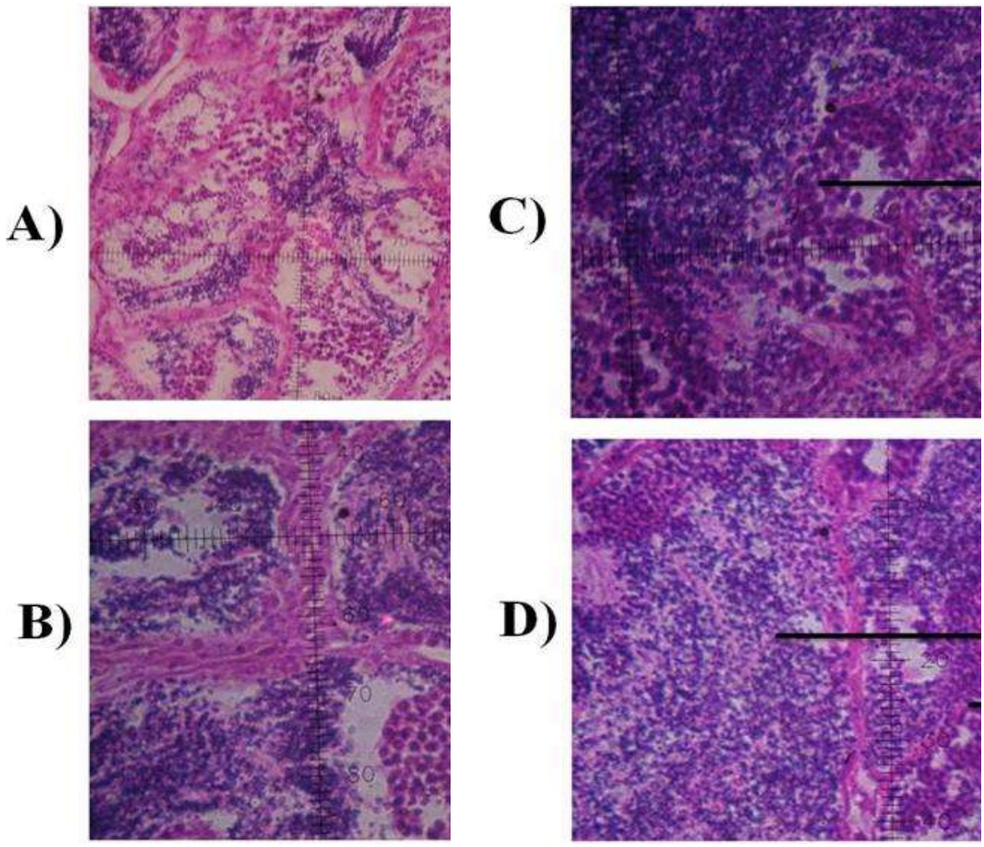


Figure 8. Testes tissue structure of *A. testudineus*, in the 1st, 2nd, 3rd and 4th maturity stages.

In the 1st maturity stage, the lobula of testes filled early developed sperm, the spermatogonia and spermatocyte. As the testes developed (2nd and 3rd maturity stages), the developed sperm (spermatids and mature sperms) occur. In the 4th maaturity stage, testes filled with mature sperm that are ready to be spawned.

Among the males sampled, surprisingly, there are remains of ovarian structure present in testes tissue of 2 fish (Figure 9). There are several undeveloped eggs (non vittelogenic eggs) present. This fact indicate that *A. testudineus* might be able to undergo sex reversal. Guraya (2000) stated that spontaneus sex reversal might be occur in several fish species and non-mammalian vertebrate. Warner *et al*. (1975) and Oldfield (2005) stated that the ability of fish in changing their sex might be trigger by various condition such as lack of alpha male and hormonal imbalance or genetic factor. Sex reversal in non hermaphrodite fish might be occur in fish with *sexual lability*. This fish may reverse their sex before or after reach the maturity stage (Oldfield, 2005).

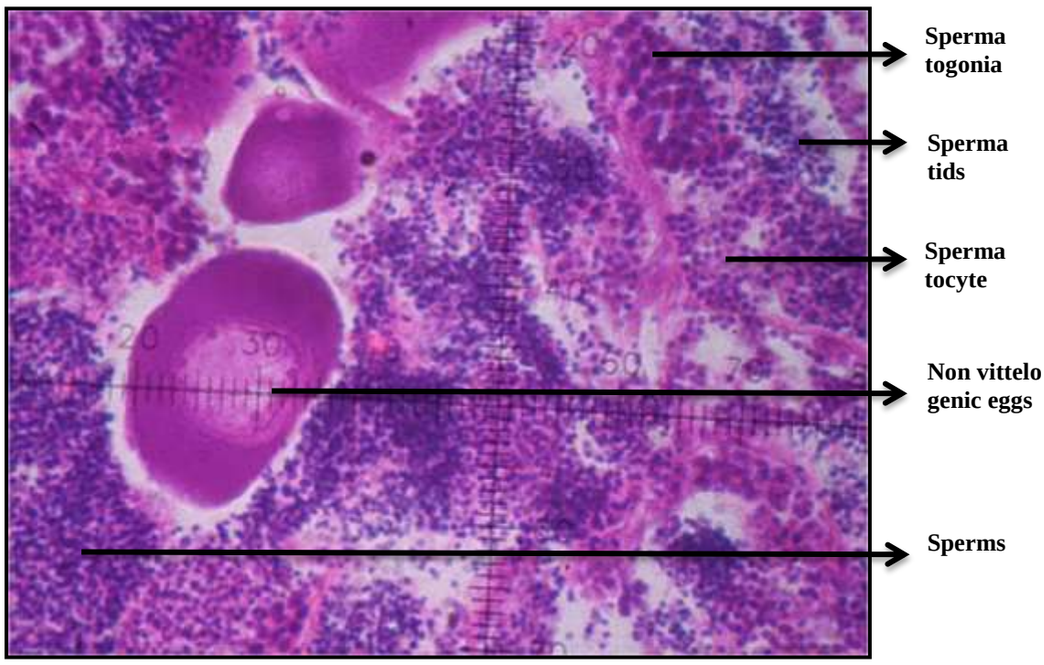


Figure 9. Testes structure with egg remains among the sperms.

CONCLUSION

A. testudineus start to spawn in February and March. This fish is a polygamous fish with sex ratio between male and female is 1: 2.4. The GSI of male is lower than that of the female (0.0088% - 0.4288% in male and 0.0140% - 5.8567% in female). Fecundity of this fish ranged from 4,116-13,998 eggs, with egg diameter ranged from 0.62-0.69 mm. The maturity level of egg in the ovary is almost homogenous, indicated that this fish is a total spawner. The fecundity is related to Standard Length (r = 0.84) and body weight (r = 0.88). There is ovarian remains in the male testes, indicates that *A. testudineus* might be able to reverse their sex.

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