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# Snakehead Fish (*Channa Striata*) : Semi-Induced Breeding and Larval Growth

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## Abstract

**Objectives:**The purposes of the present study are to produce technology of the snakehead fish breeding in Central Kalimantan region of Indonesia and to analyze the growth of larvae produced. **Methods/Statistical Analysis:**The experiments are conducted using twenty four of the broodstock and a pairof the fish isplaced to the hapa.The ovaprim doses injected on dorsal of the fish in this experiment are A: 0 ml/kg (control), B: 0.3 ml/kg, C: 0.5 ml/kg and D: 0.7 ml/kg body weight of fish. The parameters are observed that the natural spawning of the fish and total larval body length of larvae. **Findings:**Spawning is observed 30-37 hours after application of ovaprim with dose 0.5 ml/kg, followed by application of 0.3 ml /kg ovaprim dose with spawning latency time of 4-5 days.During the early stages total length growth is increasedgradually and sharply. It is increased further and after 20 daysfrom hatching until the end of experiment as well as digestive tract development. **Improvement/Applications:** At day 25 after hatching coinciding to presence of pyloric caeca the fish may be introduced artificial feeds.

**Keywords:** Breeding, Larvae, Growth,Snakehead Fish, Semi-Induced

## 1. Introduction

Snakehead fish is a freshwater fish that live in the slow-flowing waters of swamps, rivers, lakes and other small stagnant waters such as irrigation canals, rice fields, and ditches with dense aquatic vegetation. This fish in taxonomy belonging to the family Channidae widely distributed in Central Kalimantan as well as in other Asia region, has a unique ability to live in the peat swamp area with a low pH and low oxygen content because it has additional respiratoryorgan to take oxygen directly from the air[1-4]. This fish can also survive in condition of water with high ammonia content[5-6] and live as carnivorous fish that prey on other types of fish are smaller.

In nature, the snakehead fish spawning occurs once a year during the rainy season.This species is very difficult to spawn naturally in the aquaculture environment. Before conducting spawning, the fish generally prepare their nests on floating plants and grass to place egg producing. Fecundity of the snakehead fish was average 6000 eggs per 100 g of broodstock[7,8] with egg diameter between 1.2 to 1.5 mm[9,5,10,11,12]. Water depth for the snakehead fish spawning is relatively shallow water ranging from 40-80 cm. There are two types of the snakehead fish, namely keeping and without keeping of egg and larvae types during their spawning and early live stages.

Fish spawning is the result of the interactions between environmental signal and central nervous system[13,14,15].

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Firstly, the environmental signals are received by the central nervous system and forwarded to the hypothalamus to release gonadotropin releasing hormone (GnRH), which stimulates the pituitary gland to secrete gonadotropin hormone (GTH) as luteinizing hormone (LH). Furthermore, GTH work on the gonads, ovaries and testes, to release steroid hormones such as progesterone and prostaglandins that stimulate gonadal maturation until the ovulation time since gonadal maturation process reached its peak at the time of ovulation. Meanwhile, the LHRH analogue (LHRHa) is an exogenous hormone to stimulate the pituitary secreting LH which stimulates the final gonadal maturation as well as endogenous hormone acts as a trigger pituitary gland to secrete LH and then stimulates the secretion of steroid hormones to accelerate the spawning time[14,16]. Moreover, the LHRHa has been successfully used to induce final maturation and synchronize ovulation of many commercially cultured fish[13].

In Central Kalimantan, this species is an economically fish along with other species of the genus *Channa* with good taste, market value, and medicinal value. This fish has soft white tasty flesh with a few bones as a favourite food for local community. Moreover, the flesh of the fish has high protein content(> 40%)[17] containing high albumin (>60% of protein) that has an important role in tissue synthesis, wound healing of post-operative patients, and inhibiting free radical production[2,18]. In addition, local government has announced the fish as Central Kalimantan fish icon. However, recently, the snakehead fish production in Central Kalimantan as well as in the world has been highly dependent on catching from the wild, but the attempts to culture the fish are still in level of trials that there is no the fish production from hatchery on a large scale. This occurs due to less understood of breeding and rearing techniques. Meanwhile, the fish habitat has been continuously degradedas result of the waters degradation causing a decrease of snakehead fish production and an increase of difficulty to find the fish in nature. Similarly, the size of the fish found in the natural environment has become smaller.

Based on the advantages of snakehead fish for community of Central Kalimantan, development of the snakehead fish should be conducted through domestication attempts which some stages have been carried out in terms of the snakehead fish nesting and rearing[19,20,21]. The final stage of domestication which is breeding, is less understood. Therefore, the purposes of this study were to investigate utilization of synthetic hormone with OVAPRIM merk to accelerate final gonadal maturation of the snakehead fish and to analyze larval growth from semi artificial breeding in Central Kalimantan environment since in other countries, spawning snakehead fish using LHRH analog can be carried out to accelerate the fish spawning as reported [9,22,23].

## 2. Materials And Methods

### 2.1 Breeding and Larviculture

The experiments were conducted during breeding season of the snakehead fish from July to September 2016 in Fish Breeding Laboratory of Aquaculture Study Program of The University of Palangka Raya. Twenty four the mature male and female fish were collected from brood stock rearing pond (4m X 6m X 1m) and were transferred to the hapa (1m×2m×1m) for the conditioning of the fish. The pond were placed water hyacinths (*Eichhornia crassipes*) to provide shade for the fish. The brood fish were reared in the rearing different pond for female and male fed byartificialfloating feed (floating pellet) at the rate of 5% of their body weight per day. Mature females were selected by their bulging soft abdomen, oval shaped reddish vent slit and smooth pectoral fins and down part of the head while mature males were selected by pale reddish vent slit and rough pectoral fins and down part of the head. The female genital pore was prominent, while the male genital papilla was small and hidden under anal scales. Both sexes of the fish were of about 1 year old and the weights of male and female breeders were 0.352 – 0.405 kg and 0.423-0.516 kg respectively. The length of the male and female breeders varied from 24 to 30 and from 26 to 33 cm respectively.

The experiment of semi-induced spawning was designed with three replicate trials throughout the study period. Before conducting the experiment, female and male of brookstock should be selected carefully in Figure 1. The selected fish of female and male were separately placed.The dose of synthetic hormone, OVAPRIM merk, for female and male similar consisted of 0.0, 0.3, 0.5, and 0.7 ml/kg body weight of the fish. Aquabidest was used to dilute the hormone solution with ratio 1:1. Injections were made intramuscularly at the dorsal regionby means of a hypodermic syringe fitted with needle. Immediately after hormone injection, spawners were returned to the hapa. A pair of male and female wasplaced in the same hapa. After the fish spawned, the breeders were then

transferred into rearing pond and fertilized eggs were maintained an ambient water temperature (27- 29$^0$ C) in the hapa. Moreover, hatched larvae were sampled in days 0, 1, 2, 3, 4, 5, 8, 12, 16, 20, 25, and 30 after hatching for length measurement.

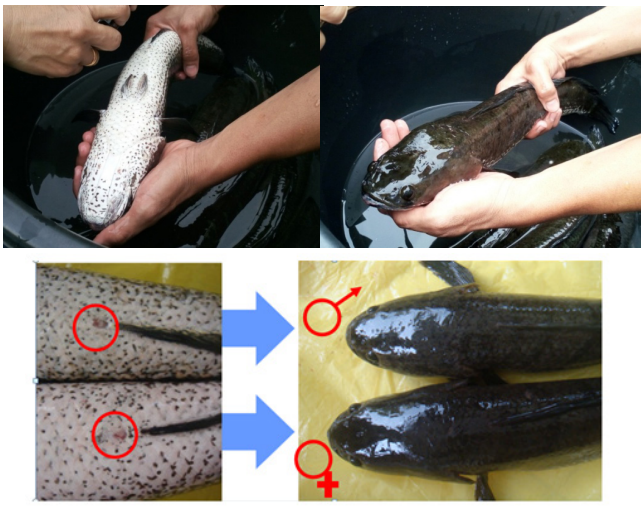

**Figure 1.** a) Selection of Breeders. b) Female Fish with Bulging Soft Abdomen, Oval Reddish Urogenital and Male Fish with Small Genital Papilla and the Round Head Shape.

## 3. Data Analysis

There were two sequent activity measurement conducted. Firstly, parameters of semi-induced breeding were observated such as ovulation time, fertilization rate, incubation time and hatching rate. Moreover, the ovulation time was the time of spawners injected by synthetic hormone to the time to spawning. Fertilization rate was measured after two hours egg released in the spawning. The transparent eggs were considered as fertilized ones whereas the opaque eggs were considered as dead eggs.

The fertilization rate was calculated by employing the following equation:

$$\text{Fertilization rate} = \frac{\text{Number of fertilized eggs}}{\text{Number of the total eggs}} \times 100$$

Incubation of eggs was the time of egg fertilized to the larvae come out from the shell. Meanwhile, the rate of hatching was calculated by employing the following equation:

$$\text{Hatching rate} = \frac{\text{Number of hatching eggs}}{\text{Number of the total fertilized eggs}} \times 100$$

Secondly, there was observation on larval development for 30 days after hatching. The total length of thirty-five larvae after hatching was measured for days 0, 1, 2, 3, 4, and 5, while the rest days the total length of ten larvae was measured.

## 4. Results and Discussion

### 4.1 Semi Induced Breeding

The spawning parameters such as latency time of ovulation, fertilization rate, egg incubation time, and hatching rate were calculated during experiment period. In this experiment, the length of spawning latency time of the snakehead fish was significantly influenced by doses of synthetic hormone application ($p<0.05$). The snakehead fish spawning latency time ranged from 30-37 hours and 4-5 days after the injections were applied in three the fish pairs with the doses 0.5 ml/kg (treatment C) and 0.3 ml/kg (treatment B) in its hapa, respectively. In addition, application of ovaprim dose 0.5 ml/kg to accelerate final gonad maturation of the snakehead fish provided latency time of spawning more than twice that of dose 0.3 ml/kg. The more doses were injected into the spawners, the faster spawning activites were occurred . This seemed that the influence GnRH and anti dopamine caused increasingly gonadotropin hormone (GtH) of pituitary gland to secrete more for maximizing gonadal maturation and accelerating ovulation.Thedoses of ovaprim used to stimulate spawning of the snakehead fish were a bit similar to the dose of ovaprim used to induce spawning of snakehead fish (*Channa* sp.) that was 0.4 ml/kg[24] but spawning latency time of the fish in this study was longer than thatwas reported about 4 – 6 hours after injections[22,25]. It was also longer compared to the ovulation time of different fish species *Anabas testudineus* that varied from 5-6 hours after injection[26]. In addition, in similar fish species of *C. striatus* the ovulation time was about 9-12 hours after of second dose of injection[23] shorter than that of the present study. However, within 5 days the breeders after injection were no spawning activity in treatment D with the highest ovaprim dose of 0.7 ml/kg as well

as in treatment A without any synthetic hormone (0.0 ml/kg)given in Table 1. Injection of ovaprim dose 0.7 ml/kg for breeders may be overdose causing over-ripe eggs and inhibiting ovulation proccess, while no application of GnRH (Gonadotropin Releasing Hormone) analog and anti-dopamine of ovaprim may block ovulation or final gonad maturation so that the spawning was not conducted yet.

**Table 1.** Treatment of semi induced breeding of snakehead fish (*C. Striata*) using different ovaprim dose

| Treatment (dose in ml/kg) | Fish Pair No. | Spawning | Spawning latency time | Egg incubation time |
|---|---|---|---|---|
| A : 0.0 | 1 | No | - | - |
| | 2 | No | - | - |
| | 3 | No | - | - |
| B : 0.3 | 1 | Yes | 4 days | 25-29 hours |
| | 2 | Yes | 5 days | 25-29 hours |
| | 3 | Yes | 5 days | 25-29 hours |
| C : 0.5 | 1 | Yes | 30 hours | 25-29 hours |
| | 2 | Yes | 30 hours | 25-29 hours |
| | 3 | Yes | 37 hours | 25-29 hours |
| D : 0.7 | 1 | No | - | - |
| | 2 | No | - | - |
| | 3 | No | - | - |

The fertilized eggs were swollen, free floating spherical, non- adhesive translucent and bright yellow in colour. The floating eggs due to high lipid content were also reported[27]. After one or two hours, the pale yellow fertilized eggs started to develop, while unfertilized eggs became whittish shown in Figure 2.The embrionic development of the fertilized eggs was showed in Figure 3. During the a few hours preceding hatching, the embrio was mobile and its pigmentation gradually increased. The fertilization rate of the snakehead fish was about 57,4-69,4%. The fertilization rate of the snakehead fish eggs in the present study was similar to that of *Channa striatus* reported about58,83% in average[23], but it was lower than the fertilization rate of *Channa punctatus* ranging 78.3 -97.6%[24].

**Figure 2.** a). The Released Eggs of the Snakehead Fish Natural Spawning after Ovaprim Hormone Injection. b). Unfertilized Whittish Egg Among Fertilized Eggs.

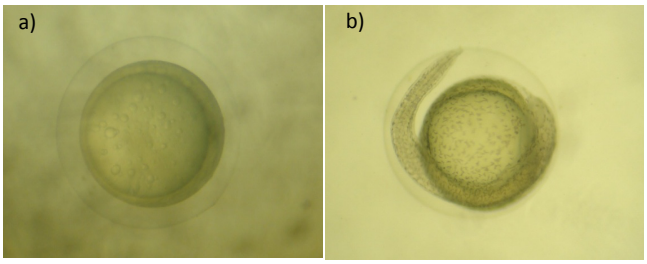


**Figure 3.** a). Six Hours of Fertilized Egg. b). Twenty Hours Fertilized Egg.

After about 25 hours fertilization, the fertilized eggs started to hatch and completed within 4 hours. This egg incubation of the fish was shorter than that of *C. striatus* reported[23]. The fish hatching rate ranged from 23,5-28,2% at temperatures of 27-29°C. However, the hatching rate of the snakehead fish eggs in the present study was lower than that of *C. striatus* and *C. punctatus* reported about 62,33% in average[23] and 90.6% in average[24], respectively. Moreover, both fertilization and hatching rates of the snakehead fish in this present study were low. It might be due to stress in captive condition as reported in Eurasian perch, *Perca fluviatilis* suffered stress during domestication process[28]. The hatching larvae were a bit dark with a distinct round shape of yolk sac, and swimming in schools. The newly hatched larvae were unpigmented eyes and the larvae head was not yet developed as well as its digestive tract, but there was a heart and unpigmented blood circulation inFigure 5a.Besides, the larvae moved passively on the water surface.

## 5. Larval and Juvenile Growth

The growth of the snakehead fish larvae during the study with exponensial curve was described in Figure 4. During the early stages total length development gradually increased and sharp increase in this parameter was observed since

day-20 after hatching until the end of experiment. The total body length ofnewly hatched larvae(day-0 (H0); Figure 5 is ranged from 3.37 to 3.52(mean ± SD: 3,45 ± 0.08) mm (n = 35), reaching 6.06 ± 0.15 mm (n = 20) on day-4 after hatching(H4), 6.43 ±0.16 mm (n = 10) on day-5 after hatching (H5) in Figure5, 11.11 ± 0.37 mm (n = 10) on day-16 after hatching (H16)also in Figure 5,16.11 ± 0.64 mm (n = 10)on day-20 after hatching (H20), 20.77 ± 0.62 mm (n = 10) on day-25after hatching (H25), and 26.32 ± 1.03 mm (n = 10) on day-30 after hatching (H30).

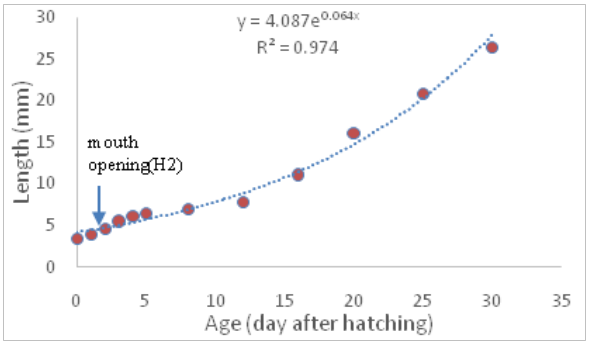


**Figure 4.** Change in Body Length (mm) of Larval and Juvenile the Snakehead Fish from Days 0 to 30 after Hatching. Solid Circle means.

At H0, the digestive tract appeared as a straight tube lying dorsally to the yolk sac, while accessory digestive organs namely liver, pancreas and gall bladder were absent. At H2, (4.63 ± 0.25 mm, n =35) the mouth opened and exogenous feeding began. At day 1-2 after hatching, yolk-sac absorption was very fast and the digestive system of snakehead fish larvae developed and differentiated. It seemed that before mouth and anus opened the larvae highly obtained its nutrition from its yolkas[28] stated that fish larvae acquired endogenous nutrition by endocytosis of yolk sac and oil globule through a syncytium surrounding the yolk sac in early development periods.Moreover, at day 1 after hatching, the eye also started to develop and eye pigmentation was very obvious in Figure 5b and by day-2 after hatching, the eye was fully pigmented suggesting that the larval vision system was functional as full pigmentation of goldlined seabream eye occurred at day 2 after hatching[29]. It was likely that sight was important organ in initial feeding success to support fish larval development and growth.

Mouth opening of the larval snakehead fish was completed at H2 similar to that of the climbing perch[30] and shi drum fish[31] faster than temperate fish species.Presumably, this was due to the higher rearing temperature of the

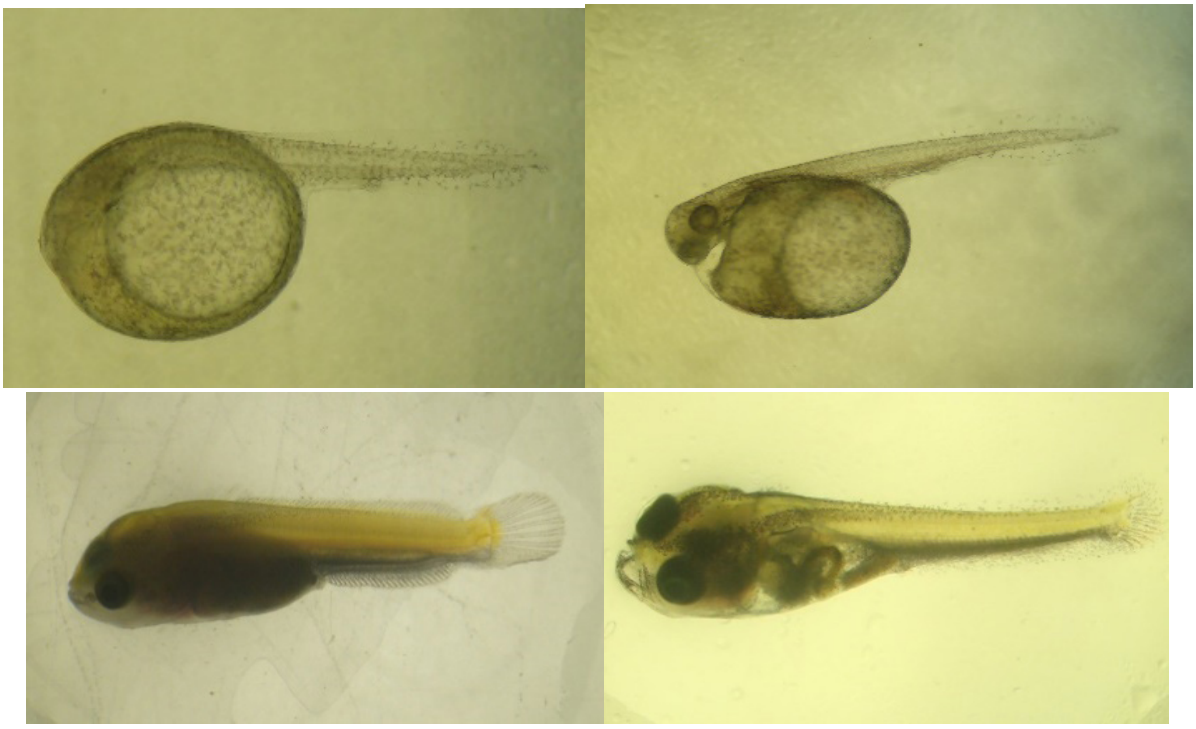

**Figure 5.** Larval Growth of Larval the Snakehead Fish from Days 0 to 16 after Hatching. a) Day-0 after Hatching (H0), b) Day-1 after Hatching (H1), c) Day-5 after Hatching (H5), and d) Day-16 after Hatching (H16).

snakehead(27-29 °C), which is a tropical spawning species. However, in temperate climate with temperature 16-20°C of spawning season, mouth opening of fish gilthead seabream (*Sparus aurata* L.) , common dentex (*Dentex dentex*L.), European seabass (*Dicentrarchus labrax*), and turbot (*Scophthalmus maximus* ), occurred rangingday-4 – day-5 after hatching[32,33,34,35], whereas mouth opening of common pandora (*Pagellus erythrinus*) occurred at day-3 with temperature18.5–20 °C[36].

In addition, based on histology analisis, at H2 there was visible chondrocytes of the rudimentary gill arches under the epithelium of the posterior buccopharynx in Figure 6a. At day 3 after hatching, the buccopharynx channeled with the anterior intestine through a short oesophagus with a rather narrow lumen and prominent liver was observed Figure 6b. However, at H5 the yolk sac was almost completely resorbed in Figure 5c. Changes of the digestive tract of snakehead fish larvae during the first several days development were significantly visible since at hatching, the digestive tract of the larvae was undifferentiated straight tube and rudimentaryclosely the pattern in many small fish larvae such as goldlined seabream[29], sharpsnout sea bream[37,38,39] and common pandora[38].

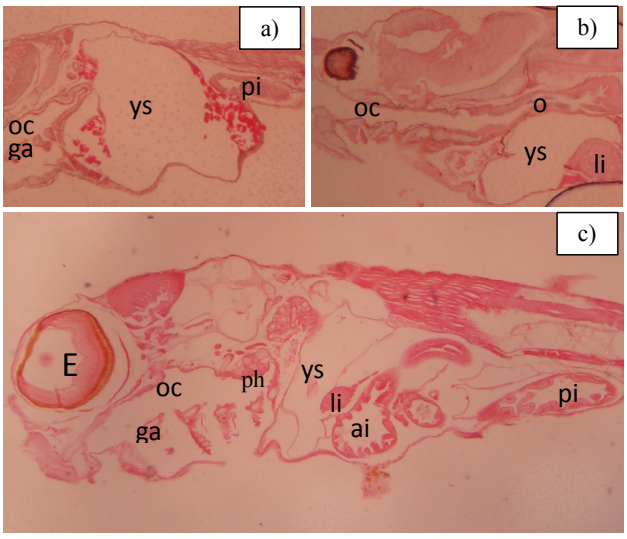


**Figure 6.** Sagittal Sections of the Gastro-Intestinal Tract of Snakehead Fish (*Channa striata*) Larvae : a). At H2 Showing Gill Arches (ga), b). At H3 Showing Oesophagus (o) and Liver (li); c). At H4 Showing Differentiation of Oral Cavity (oc), Pharynx (ph), Anterior Intestine (ai) and Posterior Intestine (pi); 100X; Yolk Sac (ys), Eye (E), Gill Arch (ga).

In addition, with the beginning of exogenous feeding in day 2 and day 3 after hatching at the end of oesophagus there was dilated and small anterior intestine lumen in Figure 6b. In addition, the liver in the snakehead fish differentiated early in development (at day 3 after hatching; Figure 6b. At day 4 after hatching mucosal folding was started with short villi in the anterior and posterior intestine shown in Figure 6c. At day 16 after hatching mucosal folding appeared very well developed in both the anterior and posterior intestine shown in Figure 7a. Similarly, at day 16 after hatching the stomach exhibited a developed shape and it was more developed at day 20 after hatching shown in Figure 7b. This fact was very similar to the development of digestive tract of climbing perch larvae documented[30].

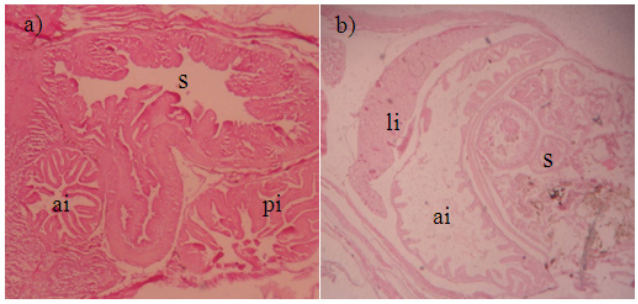


**Figure 7.** Sagittal Sections of Stomach and Intestine of Snakehead Fish (*Channa striata*) Larvae : a). At H16, b). At H20 Showing Well Development; 200X; Stomach (s), Anterior Intestine (ai), Posterior Intestine (pi), Liver (li).

At H16 larvae started to change morphology to be similar to adult type and metamorphosis processesshown in Figure 6d were completed around day 25 after hatching in the most part of larvae. Moreover, two pyloric caeca appeared at day 25 after hatching, indicating the transition from larval to juvenile stage and acquisition of an adult type of digestion so that the weaning phase was started.

In conclusion, the application of ovaprim with doses 0.3 and 0.5 ml/kg of fish in stimulating snakehead fish breeding activity was recommended. Finally, based on the present histological and morphological data, snakehead fish larvae could be weaned to formulated feeds in day 25 after hatching.

## 6. Conclusion

It is concluded from the present study that, semi-artificial breeding of snakehead fish may be stimulated by ovaprim doses of 0.5 and 0.3 ml/kg of fish. According

to larval and juvenile growth of the fish, larvae with age of 25 after hatching could be introduced artificial feeds.

## 7. Acknowledgements

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