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Profile of 17ß-estradiol, vitellogenin, and egg diameter during gonad maturation process of striped catfish *Pangasianodon hypophthalmus*

Profil 17ß-estradiol, vitelogenin dan diameter telur pada proses pematangan gonad ikan patin siam *Pangasianodon hypophthalmus*

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ABSTRACT

This study was conducted to evaluate the profile of 17ß-estradiol (E2) and vitellogenin (Vtg) in plasma and egg diameter during gonad maturity process of striped catfish *Pangasianodon hypophthalmus*. Blood samples were collected from immature striped catfish, male and female with different stage of gonad maturity (stage I, II, III, and IV) to measure the concentrations of E2 and Vtg. Gonad maturity development of striped catfish was observed based on egg diameter. Result showed that E2 concentrations were the highest (843.65 pg/mL) on female with maturity stage III, the lowest on the male (26.34 pg/mL), and immature female fish (29.37 pg/mL). The protein band of Vtg was obtained on the plasma of the mature female (stage I, II, III, and IV) with a molecular weight (MW) between 140−180 kDa, but it was not obtained on immature female dan male striped catfish. The highest concentration of Vtg was found in the plasma of the female fish with maturity stage III (87.34 mg/mL), then on the stage II (74.83 mg/mL), I (68.58 mg/mL), and IV (33.45 mg/mL). It showed that egg yolk formation occurred in the female mature. The average egg diameter was 0.107 ± 0.052 mm, 0.318 ± 0.086 mm, 0.864 ± 0.099 mm, and 1.041 ± 0.058 mm on the maturity stage I, II, III, and IV respectively. The increase of egg diameter along with development of gonad maturity stage indicated that egg development occurred due to the process of vitellogenesis and the addition of egg yolk on oocyte.

Keywords : egg diameter, gonad maturity, striped catfish, 17ß–estradiol, vitellogenin

ABSTRAK

Penelitian dilakukan untuk mengevaluasi profil 17ß–estradiol (E2), vitelogenin (Vtg) dalam plasma dan diameter telur pada proses pematangan gonad ikan patin siam *Pangasianodon hypophthalmus*. Sampel darah untuk pengukuran konsentrasi E2 dan Vtg plasma diperoleh dari ikan patin siam betina yang belum matang gonad, ikan jantan, ikan betina dengan tahap kematangan gonad yang berbeda (tahap I, II, III, dan IV). Perkembangan kematangan gonad ikan patin siam diamati berdasarkan diameter telur. Hasil penelitian menunjukkan bahwa konsentrasi E2 tertinggi (843,65 pg/mL) pada ikan betina dengan kematangan tahap III, terendah pada ikan jantan (26,34 pg/mL), dan ikan betina tidak matang gonad (29,37 pg/mL). Pita protein Vtg pada sampel plasma diperoleh dari betina matang gonad (tahap I, II, III, dan IV) dengan berat molekul antara 140−180 kDa, tetapi tidak diperoleh pada ikan patin siam betina yang belum dewasa dan jantan. Nilai konsentrasi tertinggi Vtg ditemukan dalam plasma darah ikan betina dengan tingkat kematangan III (87,34 mg/mL) kemudian pada tahap II (74,83 mg/mL), I (68,58 mg/mL) dan IV (33,45 mg/mL). Hal ini menunjukkan bahwa pada ikan betina dewasa terjadi proses pembentukan kuning telur (vitelogenesis). Rata-rata diameter telur adalah 0,107 ± 0,052 mm, 0,318 ± 0,086 mm, 0,864 ± 0,099 mm dan 1,041 ± 0,058 mm pada tingkat kematangan I, II, III, dan IV secara berurutan. Peningkatan nilai diameter telur seiring dengan perkembangan tahap kematangan gonad menunjukkan bahwa perkembangan telur terjadi karena proses vitelogenesis dan penambahan bahan kuning telur pada oosit.

Kata kunci : diameter telur, 17ß–estradiol, kematangan gonad, patin siam, vitelogenin

INTRODUCTION

The striped catfish *Pangasianodon hypophthalmus* is a superior freshwater species in South East Asia, especially in Vietnam (Phan *et al*., 2009; Bui *et al.,* 2010), Malaysia (Asdari *et al.,* 2010), India (Singh & Lakra, 2012; Kumar *et al.,* 2013), and Bangladesh (Ahmed *et al*., 2013). This species is also recognized as a superior aquaculture species in tropical regions, as well as a major aquaculture product on world markets (McGee, 2014). Striped catfish *Pangasianodon hypophthalmus*, which is a high economic value commodity, has been widely produced in Indonesia for many years. According to the Ministry of Fisheries and Marine Affairs Republic of Indonesia (2018), the market price ranges between Rp 20,000 and Rp 27,000 per kg for consumption size. The striped catfish fillet industry continues to grow as the demand increases on both local and global markets. However, striped catfish culture faces several crucial problems, such as the continuity of seed availability, also the quality and quantity of mature broodstock resulted in unsustainable seed production. It potentially happens because naturally, striped catfish spawns in a certain seasons. The main spawning season of catfish commonly occurs in the rainy season, whereas in the dry season it is demanding to find the mature female (Moses *et al*., 2016). Scientific information about the reproductive physiology is limited. Scientific research about striped catfish reproduction is required to improve production. In the recent years, the reproductive biology of many fish species have been studied by analyzing the vitellogenin (Vtg) and 17ß-estradiol (E2) levels in the blood plasma (Muhammad *et al.,* 2011; Tirado *et al.,* 2017; Luck *et al*., 2019). Variations in steroids have been shown to be correlated with the reproductive cycle (Liu *et al.,* 2008; Yan *et al*., 2011; Ni *et al.*, 2013).

The vitellogenin, as an egg yolk protein precursor, is generated in the liver under the control of estrogen hormone and transported to ovary through the bloodstream (Reading *et al.*, 2017). Furthermore, it is absorbed by vitellogenin receptors (VtgRs) on the surface of growing oocytes (Dominguez *et al*., 2012). In several species, Vtg has a molecular weight (MW) between 140–200 kDa (Komatsu & Hayashi, 1997). Ding (2005) reported that Vtg has a MW between 200–700 kDa, similar to killifish, *Fundulus heteroclitus* (200 kDa). In mature female of teleost oviparous species, the Vtg is an essential nutrition source for oocyte and embryo development through vitellogenesis process (Lubzens *et al.,* 2010; Ding, 2005). In the natural condition, the level of Vtg indicates the stage of gonad maturation in female species (Hara *et al.,* 2016). The Vtg was devoid in male as well as in immature females, but exist in mature vitellogenic females (Fenske *et al.,* 2001). However, these organisms will generate the Vtg if they are carried out with synthetic estrogens, mainly 17ß-estradiol (Leonardi *et al.,* 2010; Boucard *et al.,* 2008). Basically, the Vtg is able to be found in matured females and is identified in a least amount which can be acknowledged insignificant in males and immature females (Muhammad *et al.,* 2011).

Previous studies reported that 17ß-estradiol successfully induced the synthesis of vtg in many fish species (Mendoza *et al.,* 2011). The 17ß-estradiol stimulates the liver to synthesize and secrete Vtg which is concentrated in the oocyte. The correlation between changes in gonadal steroid plasma levels and the development of oocytes has been noticed in a number of freshwater species including catfish *Hemibagrus nemurus* (Adebiyi *et al.,* 2013), kutum *Rutilus frisii* (Sabet *et al.,* 2009), mahseer *Tor tambroides* (Ismail *et al.,* 2011), Indian shad *Tenualosa ilisha* (Pramanick *et al.,* 2013), marine species including gilthead seabream *Sparus aurata* (Pozo *et al*., 2008), and pejerrey *Odontesthes bonariensis* (Elisio *et al.,* 2014). Scientific information about reproductive physiology including vitellogenesis is strictly valuable in fish broodstock management.

Study on vitellogenin and 17β-estradiol level in striped catfish and scientific information about its reproduction and vitellogenesis are definitely required. In farm management, an advance understanding of vitellogenesis is very crucial to determine the maturity status of the species. Thus, this study purposed to evaluate the profile of Vtg, 17ß-estradiol levels in plasma and egg diameter on gonad maturation process of striped catfish *(Pangasianodon hypophthalmus).*

MATERIALS AND METHODS

Experimental fish

This study was done at the Research Institute for Fish Breeding, Ministry of Marine Affairs and Fisheries, West Java, Indonesia. The striped catfish (*Pangasianodon hypophthalmus)* were obtained from a broodstock population at the Institute. Males, mature, and immature females (N= 30; mature female 3302.55 ± 276.82 g, 53.875 ± 1.07 cm; male 3099.6 ± 981.04, 56.08

± 5.68 cm; immature female 104.8 ± 2.28 g, 17.9 ± 0.22 cm) were used as the experimental fish. The mature female (gonad maturity stage I, II, III, and IV), male and immature female were measured 17ß-estradiol and Vtg concentration in blood plasma. The male and immature female were used as negative control of this parameters. Egg diameter was measured on each of the gonad maturity stage to observed gonadal development of mature females.

Plasma 17β-estradiol and vitellogenin concentrations

Blood samples were collected for measurements of plasma 17ß-estradiol and Vtg concentrations. Three millilitres of blood was collected from each tested fish using heparinized syringe containing phenylmethylsolfonyl fluoride, PMSF (Roche, Germany) (100 µL, 1 mM). The blood was stored on ice and centrifuged at 3000 rpm for 15 min at 4°C. The supernatant (plasma) was stored at -20°C prior to analyze by ELISA (EIA1561 DRG International Inc., Marburg, Germany) to measure 17ß-estradiol level and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) method to measure Vtg.

Detection of the Vtg from the plasma of tested catfish was conducted using SDS polyacrylamide gel electrophoresis (SDS-PAGE) (Bio-Rad, Hercules, California, USA) (Walker, 2002). The quantification of the detected Vtg was measured using the Bradford method (Kruger, 2002).

Egg diameter

Egg diameter was measured using an binokuler microscope Olympus BX 21. For each fish, the diameter of thirty eggs were measured and the average egg diameter was calculated. The egg development was characterized based on the diameter size of the eggs on a particular stage. Photomicrographs of various stages of egg development were taken using a Olympus BX 21 microscope. For the histological observation, eggs were fixed in Bouin's solution for 24 hours and then embedded in paraffin. The paraffin embedded specimens were sectioned in 5 to 6 µm thick sections. The sections were stained with Mayer's hematoxylin-eosin, and observed under a light microscope (Olympus BX 21).

Data analysis

The data of the 17ß-estradiol and Vtg level were presented descriptively to describe the changes of 17ß-estradiol and Vtg level development. Data of egg diameter was statistically analyzed using Microsoft Excell 2016 and SPSS version 25.

RESULTS AND DISCUSSIONS

Results

17ß-estradiol concentration

The concentrations of 17ß-estradiol hormone on several maturity stage of female, male and immature female are presented in Figure 1. The highest concentration value of 17ß-estradiol was shown in the blood plasma of the female fish with maturity stage III (843.65 pg/mL) and the lowest concentration at the male fish (26.34 pg/mL). The 17ß-estradiol concentration value in blood plasma increased from stage I (102.50 pg/mL) to stage II (326.45 pg/mL) and III (843.65 pg/mL) then decreased at stage to IV (80.45 mg/mL) of ovarian development. Male and immature female fish have low concentration value of 17ß-estradiol (26.35 pg/mL and 29.37 pg/mL).

Vitellogenin concentration

Result of electrophoresis showed that protein band of Vtg was obtained on the plasma of the mature female (stage I, II, III, and IV) with a molecular weight between 140–180 kDa, but it was not obtained on immature female and male striped catfish (Figure 2). Estimation of molecular weight of Vtg protein band was calculated by comparing with molecular weight of marker.

The highest concentration value of Vtg was found in the blood plasma of the female fish with maturity stage III and the lowest concentration at stage IV (Figure 3). Vtg content in blood plasma increased gradually from stage I (68.58 mg/mL) to stage II (74.83 mg/mL) and III (87.34 mg/mL) then decreased at stage IV (33.45 mg/mL) of ovarian development. Concentration value of vitellogenin in blood plasma were not found in male and immature female.

Egg diameter

The egg development process was proved by the size of egg diameter. Egg diameter for each maturation stage was 0.107±0.052 mm (stage I), 0.318 ± 0.086 mm (stage II), 0.864 ± 0.099 mm (stage III), and 1.041 ± 0.058 mm (stage IV). A steady increase was observed in the egg diameter throughout the maturation stages. Statistical analysis showed significant differences between maturation stage ($P<0.05$). Egg diameter (1.041 ± 0.058 mm) was found to be greater at stage IV of ovarian development. There was a gradual increase in egg diameter from stage I to IV which reflects egg growth leading to vitellogenesis in the final stage of ovarian development IV (Figure 4 and 5).

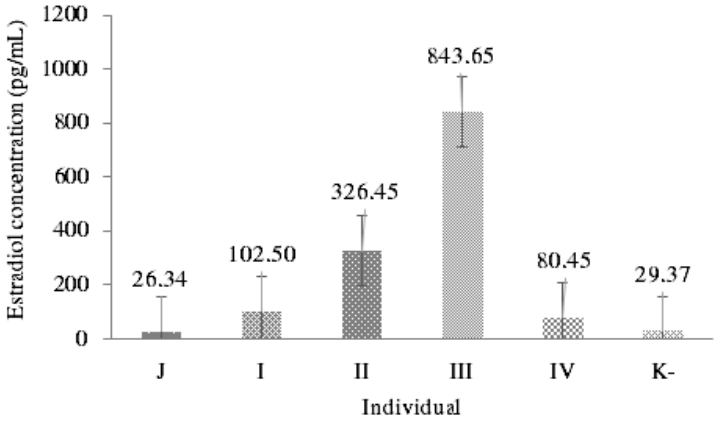


Figure 1. Estradiol concentration (pg/mL) of J (male), IV (female on maturity stage IV), III (female on maturity stage III), II (female on maturity stage II), I (female on maturity stage I), and K- (immature female as a negative control).

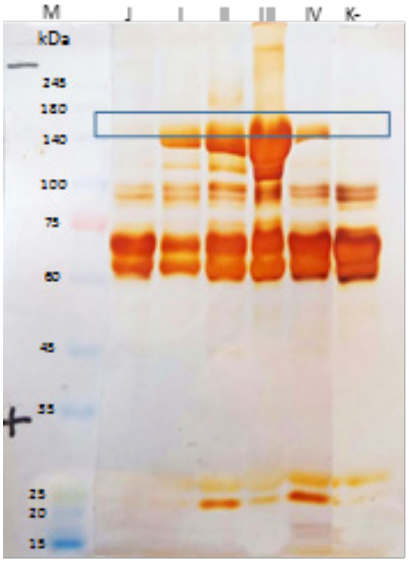


Figure 2. The profile of protein band of Vtg plasma of the female and male of various maturity stages on SDS-PAGE (7.5% SDS) stained with silver staining. Lane M : molecular weight markers (Fermentas, USA), Lane J : Male as a negative control, Lane I : maturity stage I, Lane II : Maturity stage II, Lane III : maturity stage III, Lane IV : vitellogenic female on matur stage IV, Lane K- : Immature female as a negative control.

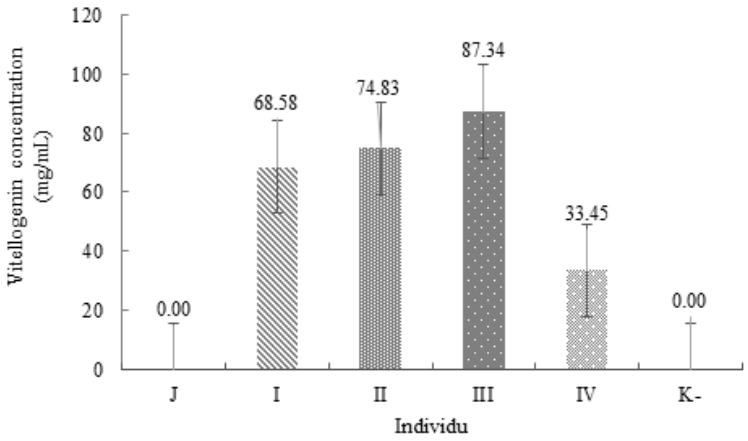


Figure 3. Vitellogenin concentration (mg/mL) of J (male), IV (female on maturity stage IV), III (female on maturity stage III), II (female on maturity stage II), I (female on maturity stage I), and K- (immature female as a negative control)

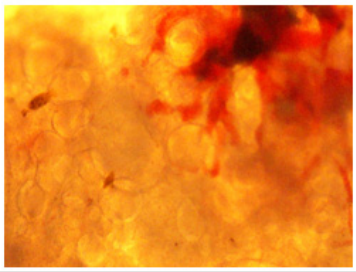
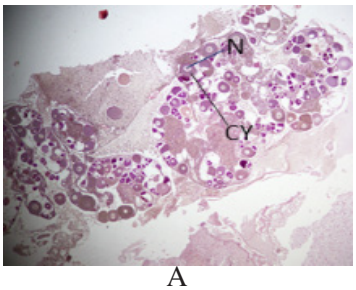
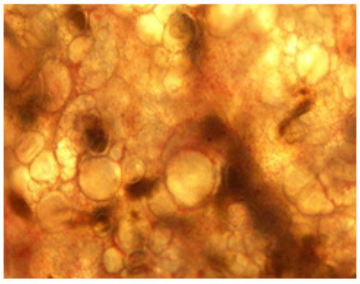
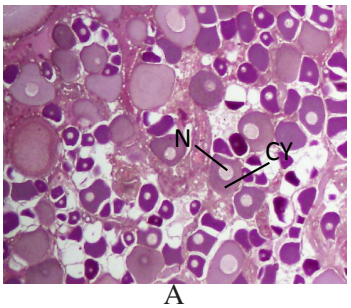
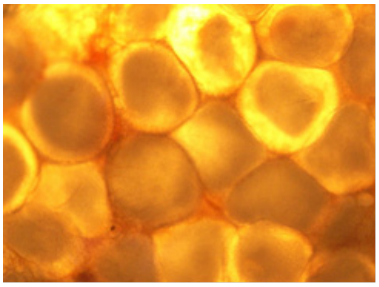
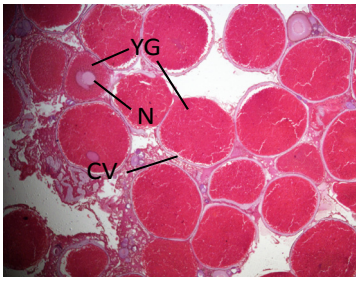
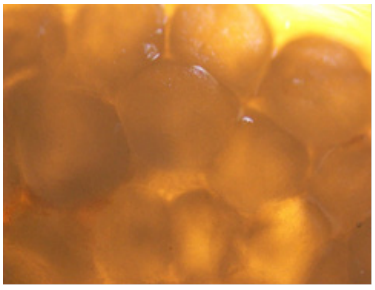
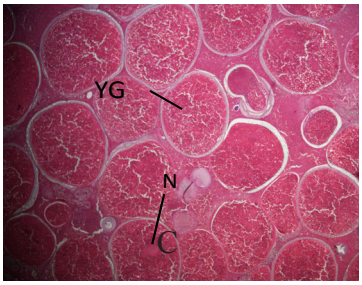
Maturity stage	Whole eggs	Histological slide
Stage I		 A
Stage II		 A
Stage III		 A
Stage IV		 B

Figure 4. Egg development on four maturity stages of striped catfish *Pangasianodon hypophthalmus* (n = nucleus, cy = cytoplasma, cv = corticol vesicle, yg = yolk globule, A = previtellogenesis, B = vitellogenesis, C= mature).

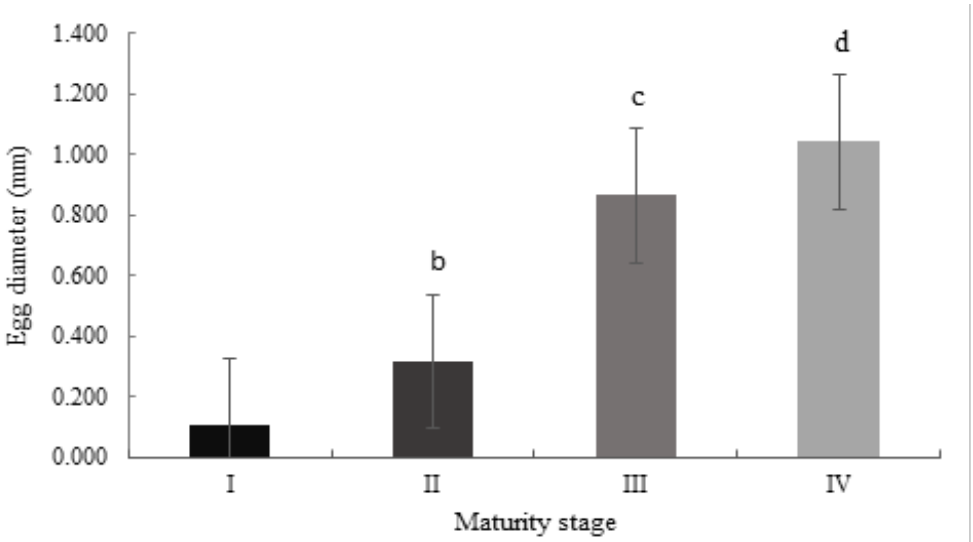


Figure 5. Egg diameters on four maturity stages of striped catfish *Pangasianodon hypophthalmus*

Discussion

The primary aim of the present study was to evaluate the profile of 17ß-estradiol, Vtg levels in plasma, and egg diameters on gonad maturation process of striped catfish *Pangasianodon hypophthalmus*. In this study, we evaluated the transformation in plasma levels of 17ß-estradiol and Vtg during gonad maturation process in striped catfish. Gonad maturity development of striped catfish was observed based on egg diameter. The 17ß-estradiol and Vtg levels in the blood plasma were closely related to the stage of ovary maturity (Lubzens *et al*., 2010; Baumann *et al*., 2013; Chatakondi and Kelly, 2013). The 17ß-estradiol is the primary steroid in vitellogenesis and play a role to induce hepatic vitellogenin which synthesizes vitellogenin (Vtg), an essential precursor for the oocytes formation, ovarian growth, and steroidogenesis (Amaral *et al*., 2019, Samaee *et al*., 2009; Shappell *et al*., 2010; Tortolero *et al*., 2010; Dammann *et al*., 2011). In addition, the increase of steroid hormone at the maturation stage is followed by high GSI value at the mature stage of female. Ghosh *et al*. (2016) reported that the gradual increase of 17ß-estradiol in plasma during vitellogenesis, increased Vtg level and ovarian weight consistently, reaching the peak in the pre-spawning. The 17ß-estradiol is particularly detected in mature females and not normally detected in juveniles and males, whereas injection of exogenous estrogen can induce the Vtg expression.

Result of this study showed that Vtg band was detected in blood plasma of mature female (stage I, II, III, and IV), but not detected on male and immature female of striped catfish. Currylow *et al.* (2013) reported that Vtg band was not detected among any male samples of Eastern box turtle *Terrapene carolina* as negative sample and found Vtg band in female blood plasma as positive sample. Muntaziana *et al.* (2011) revealed that it is difficult to measure the level of Vtg in male and immature fish, but the administration of 17ß-estradiol to male and juvenile teleost evidently induced accumulation of Vtg in the blood. The Vtg protein band of mature female was observed between 140–180 kDa of MW. In some fish species, the molecular weight of Vtg is between 140–200 kDa (Komatsu & Hayashi, 1997). Ding (2005) reported that the molecular weight of Vtg ranged between 200–700 kDa, for example killifish, *Fundulus heteroclitus,* which has monomeric Vtg about 200 kDa.

Gonad maturation processes correlated to Vtg and 17ß-estradiol levels. Plasma level of gonadal steroids can be used as gonadal activity indicator during the reproductive cycle (Nagahama & Yamashita, 2008). Based on Figure 2 and 3, it showed increasing level of Vtg and 17ß-estradiol in blood plasma of mature female. It was predicted that the peak of vitellogenesis process occurred on maturation stage III, thus, Vtg has been absorbed by oocytes on maturation stage IV resulted in decreased concentration of 17ß-estradiol. The 17ß-estradiol concentration in fish naturally decreased after gonad maturation. The completion of vitellogenesis is followed by a sharp decline in 17ß-estradiol levels and usually occurs with the onset of full oocyte maturation (Zupa *et al.,* 2017). High concentrations of Vtg and 17ß-estradiol hormone were associated with gonad maturity stages. Reading *et al.* (2017)

reported that 17ß-estradiol stimulates the hepatic synthesis and secretion of vitellogenin, which is assimilated into developing oocytes. Quinitio *et al*. (1994) reported that differences in serum Vtg levels during the stage of gonadal maturity correlated with fluctuations in 17ß-estradiol and progesterone levels in the hemolymes of *Penaeus monodon*. The ovary produces 17ß-estradiol and releases it to the blood plasma and reaches the hepatopancreas to stimulate Vtg synthesis. Revathi *et al*., (2012) revealed that the variations in the vitellogenin content in hepatopancreas and hemolymph differed significantly during different ovarian stages of freshwater female prawn *Macrobrachium rosenbergii* (De Man).

The absorption of Vtg by oocytes affects the increase of egg diameter. Egg diameter increases when the ovarian development stage progressed, resulting in an increase in ovarian volume (Figures 4 and 5). The highest average of egg diameter was at maturity stage IV. This is in accordance with Tsukimura (2001) which state that the deposition of egg yolk in the oocytes when vitellogenesis occured, resulted in an accelerated increase in oocyte diameter. The oocytes and ovaries increase in size and volume along with ovary differentiation stages (Islam *et al*., 2010). Therefore, it can be stated that the determination of oocyte diameter provides important information about the classification of ovary differentiation stages (Peixoto *et al*., 2005).

This study demonstrated that the classification of ovary differentiation stages can be based on the size of the eggs and the levels of 17ß-estradiol and Vtg during gonad maturation process. On the other hand, it evidenced that biochemical constituents are also closely associated with the ovarian development. Vitellogenesis, which is a biomarker of female reproductive activity, shows increased vitelin accumulation in oocytes and fluctuated in Vtg content in blood plasma during the fish ovary development stages.

CONCLUSION

The highest concentration value of 17ß-estradiol and vitellogenin was shown in the blood plasma of the female fish with maturity stage III. Vtg band was detected in blood plasma of mature female (stage I, II, III and IV) but was not detected on male and immature female of striped catfish. The changes of 17ß-estradiol and vitellogenin level were followed by a gradual increase in the egg diameter throughout the maturity stages.

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