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RESEARCH PAPER

Vitellogenesis of Giant Gourami (*Osphronemus goramy*) Examined by the Measurement of Estradiol-17β, Vitellogenin Concentration and the Size of Ovary

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Abstract

This study aims at observing the rate of estradiol-17β synthesis on vitellogenin and the ovary size of the Giant gourami broodstock candidates and female broodstock. The female broodstock candidates were reared in a pond, while the female broodstock were reared in several ponds with the male broodstock (2:1). The blood sample taking was done every week during the three-week observation. The purpose is to measure the concentration of estradiol-17β and vitellogenin. The observation on the broodstock candidates and the broodstock's ovary size was done in the last week of the observation using veterinary ultrasound. Most of the estradiol-17β synthesis on the broodstock candidates is for vitellogenesis on the initial cycle of reproduction to reach maturation. In contrary, most of estradiol-17β synthesis on the female broodstock is not for vitellogenesis in the next spawning cycle. The observation shows that the size of the broodstock candidates and broodstock's ovary is small.

Introduction

Giant gourami or *Osphronemus goramy* is one of Indonesia's fishery commodities having rivers and swamps as its natural habitat. The high interest of the Giant gourami consumption makes it a superior aquaculture commodity in Indonesia. Thus, fish farmers are required to be able to maximize the production of Giant gourami to meet the demand on the market. It is therefore necessary for them to understand the reproduction of Giant gourami.

The slow rate of Giant gourami's growth affects its genital maturation. The range of female Giant gourami's genital maturation is approximately 1 year, while gonad maturation and spawning are around 2-3 years. Giant gourami with 300 grams approximate weight starts to show several sexual behaviors and changes on shape and size which can be analyzed through morphometric study Sularto *et al*. (2016), Giant gourami has asynchronous type of oocyte growth. There are various stages of oocyte growth in the ovary without the domination of an oocyte growth population (Lubzens *et al*., 2010). The uneven type of oocyte growth influences the frequency of Giant gourami spawning that can last throughout the year, even though spawning happens unpredictably sometimes.

The main factor of obtaining gonad maturation and spawning throughout the year is to fulfill the availability of vitellogenic oocyte. This is explained by Patino and Sullivan (2002), who found that fish oocyte can reach its maturation and be ovulated once it is fully filled with yolk or reaches the last stage of post vitellogenesis. Vitellogenesis is known as a process of yolk precursor synthesis or vitellogenin triggered by estradiol-17β inserted to oocyte through bloodstream. Vitellogenin or phospholipoglycoprotein has 300-640 kDa molecular

weight and consists of lipovitellin (Lv), phosvitin (Pv), β' component (β'-c) and C-terminal peptide (Garnayak *et al*., 2013). Vitellogenin has a great contribution to the growth of oocyte. It is as much as 80-90% of the density of yolk ovulated on some fish (Reading and Sullivan, 2011).

The previous studies on Giant gourami only discussed the profile of reproductive hormones after spawning, such as testosterone, estradiol-17β, and progesterone on female broodstock. However, studies on the main hormonal mechanism that plays a key role in the stages of oocyte growth or vitellogenesis on Giant gourami broodstock candidates and female broodstock have never been undertaken to date. Besides, previous analysis on the profile of Giant gourami's hormones generally involved observation on the morphology of ovary either macroscopically or microscopically which had to sacrifice the fish. On the contrary, in this study, the observation on the morphology of ovary was done non-invasively to Giant gourami broodstock candidates and and female broodstock using ultrasound. The use of ultrasound on fish becomes the newest breakthrough that can be utilized to expand knowledge.

Based on the above explanation, the major purpose of this study is to find out the hormonal mechanism of vitellogenesis through analysis on the correlation between estradiol-17β and vitellogenin concentration in obtaining the stage of oocyte main growth, and to find out the morphology of ovary through veterinary ultrasound observation which included the measurement of Giant gourami (*Osphronemus goramy*) broodstock candidates and female broodstock's ovary.

Additionally, the analysis of estradiol-17β and vitellogenin correlation in this study was carried out by measuring the concentration of estradiol-17β and vitellogenin in the blood serum of the broodstock candidates and female broodstock. The utilization of blood serum to measure the concentration of hormones is considered to be more effective compared to the use of bood plasma since blood serum has more antigens compared to blood plasma. The method used in measuring estradiol-17β and vitellogenin is sandwich ELISA. Meanwhile, ultrasound was employed as a method of observing the morphology and the size of ovary. It refers to grayscale image on ultrasound screen displaying echogenicity or characteristics of a tissue.

Materials and Method

Selecting Giant Gourami Broodstock Candidates and Female Broodstock

Giant gourami broodstock candidates and female broodstock were obtained from and reared in Freshwater Fish Germination and Cultivation Center or *Balai Perbenihan dan Budidaya Ikan Air Tawar* (BPBIAT) Muntilan, Central Java. The selection for broodstock candidates and broodstock took place in September. In each observation group, both broodstock candidates and broodstock, there was repetition for 9 individuals. The criteria for selecting female broodstock candidates are having 500-600 grams weight, not having any physical disabilities and healthy. In addition, the criteria for selecting female broodstock are having 2-3 kilograms weight, not having any physical disabilities and healthy. The female broodstock candidates group was reared in one rearing pond, while the female broodstock group was paired with male broodstock (2:1) and reared in several rearing ponds. The broodstock candidates group was fed with *Sente* or Taro leaves and other additional fodder twice a day, in the morning and in the evening. Additionally, the broodstock group was only given *Sente* or Taro leaves twice a day, in the morning and in the evening. The experience of the local Giant gourami fish farmers demonstrated that fish feeding using Taro leaves affects the eggs laid. The eggs will not contain excessive oil and the fertilization success rate is higher compared to using high animal protein fodder.

Collecting the Blood Sample of the Selected Broodstock Candidates and Broodstock

The initial step in blood sample taking on Giant gourami broodstock candidates and broodstock was to anesthetize each individual from the broodstock candidates group and the broodstock group. The material used for anesthesia was 20 ppm clove oil for 15 liter water. Rahim (2017) stated that the active substance in clove oil, namely eugenol, is proven to weaken the nerve and disturb the nervous system. The work mechanism starts when absorption in the blood throughout the body parts takes place, then the inhibition of acetylcholinesterase formation that can reduce the performance of chemical mediator happens. After each individual from broodstock candidates and broodstock group was half conscious, they were taken out of the anesthesia container. After that, blood sample taking or phlebotomy was done. The blood sample taking on each individual of the observation group was 2 ml using non-heparine syringe.

Furthermore, the location of blood sample taking or phlebotomy on Giant gourami broodstock candidates and broodstock is on caudal vein. It is located near to the lateral side or the ventral midline (Dyer and Cervasio, 2008). The insertion point of syringe is below the caudal and all the way to the linear lateralis with 30^0 tilt. After syringe gets closer to linear lateralis, blood sample taking can be initiated. The blood sample obtained was moved into 3 ml plain vacuum tube afterwards. Next, blood sample centrifugation was undertaken using 5000 g speed for 15 minutes to get the blood serum. Then, it was moved into 1.5 ml micro tube, and saved in -20^0C until it was about to be used for estradiol-17β and vitellogenin concentration testing using sandwich ELISA

method based on the manufacturer instruction (Bioassay Technology Laboratory).

Testing Estradiol-17β and Vitellogenin Concentration Using Sandwich ELISA Method

The procedure of estradiol-17β concentration testing in the blood serum of Giant gourami broodstock candidates and broodstock was performed within room temperature. All of the reagent, standard solution and testing sample were prepared first. Then, the total of testing strips used was determined. After that, strips were put into frames. Meanwhile, the unused strips were stored in 2-8^{0}C temperature. The following step was pouring 50 µl standard solution into standard well, and putting 40 µl sample and 10 µl anti-E antibody into it for estradiol-17β concentration testing purpose, and 10 µl anti-VTG antibody into sample wells for vitellogenin concentration testing. Next, 50 µl streptavidin-HRP was added to sample wells and standard wells (except blank control well). Then, plate was shut with sealer and solution was mixed using fortex mixer. After that, solution was incubated for 60 minutes in 37^{0}C temperature.

After incubation, plate wrapping was opened. Plate or every well in the plate was washed 5 times using wash buffer. At least 0.35 ml wash buffer was required to rinse the wells for 30 seconds up to 1 minute for each washing process. Automatic washing machine was employed in the well washing in this study. Besides, paper towel and other absorbent material were used to dry the plate. Next, 50 µl substrate solution A and B were added to every well. Then, plate was shut and incubated for 10 minutes in a dark room with 37^{0}C temperature. 50 µl stop solution was added to every well to change the blue color of the solution into yellow. Last, optical density (OD) value of every well was determined using microplate reader with 450 nm wavelength within 30 minutes after stop solution was added.

Analyzing the Ovary Morphology and Size of the Broodstock Candidates and Broodstock Using Veterinary Ultrasound

The observation on Giant gourami's ovary done non-invasively using veterinary ultrasound requires good understanding on the location of ovary and the use of veterinary ultrasound. Giant gourami's ovary is located in the anterior part of the body inside the visceral cavity. After perceiving the location of the ovary, ultrasound probe can be directed to the predicted position. The suggested distance between probe and the object that will be observed is approximately 3 cm to obtain image optimization. The ultrasound frequency used in this study was 7.5 MHz. The higher the frequency wave of the ultrasound, the higher the reflectivity will be. Therefore, weighing Giant gourami with thick scale needs higher ultrasound frequency. After having good interpretation about the location of the ovary and the use of ultrasound, the observation on Giant gourami's ovary morphology and size can be initiated by directing ultrasound probe on the anterior part of the body close to the pectoral fin. Then, the image of the ovary obtained can be saved, so that the length and width of the object on the ultrasound can be calculated.

Measuring the Water Quality of the Broodstock Candidates and Broodstock's Rearing Pond

The measurement of the water quality in the broodstock candidates and the broodstock's rearing pond was done once a week during 3 week observation. The parameters of the water quality were temperature, pH, and DO. These three parameters have important contribution in the success of fish growth and reproduction. In this study, no special treatment was given to control the quality of water. The quality of the rearing pond water had been adjusted with the quality of water in the natural habitat of Giant gourami.

Data Analysis

Shapiro-Wilk test was used as a normality test of the overall data in this study. Meanwhile, the data analysis employed to find out the comparison between estradiol-17β and vitellogenin concentration in every week of the observation was One Way Anova and Least Significant Different (LSD) test. Also, the analysis on the correlation between estradiol-17β and vitellogenin concentration test result utilized Pearson test.

Results

Estradiol-17β and Vitellogenin Concentration of the Female Broodstock Candidates

Based on the overall result of the measurement of estradiol-17β and vitellogenin concentration of every 9 individuals of Giant gourami female broodstock candidates within 3 week observation period, it is shown that on average, broodstock candidates individual experienced fluctuation of estradiol-17β and vitellogenin concentration every week of the observation (Figure 1). The result of the measurement on estradiol-17β and vitellogenin concentration of each individual of Giant gourami female broodstock candidates group is shown on Table 1. The average of estradiol-17β and vitellogenin concentration of the broodstock candidates on the first week is 8.936±2.579^a ng/ml and 64.984±27.973^a µg/L. Based on the average of estradiol-17β and vitellogenin concentration on the second week, no significant difference is found (P≥0.05). Nevertheless, a significant difference is seen (P≤0.05) with the average of estradiol-17β and vitellogenin concentration on the third week. The average of

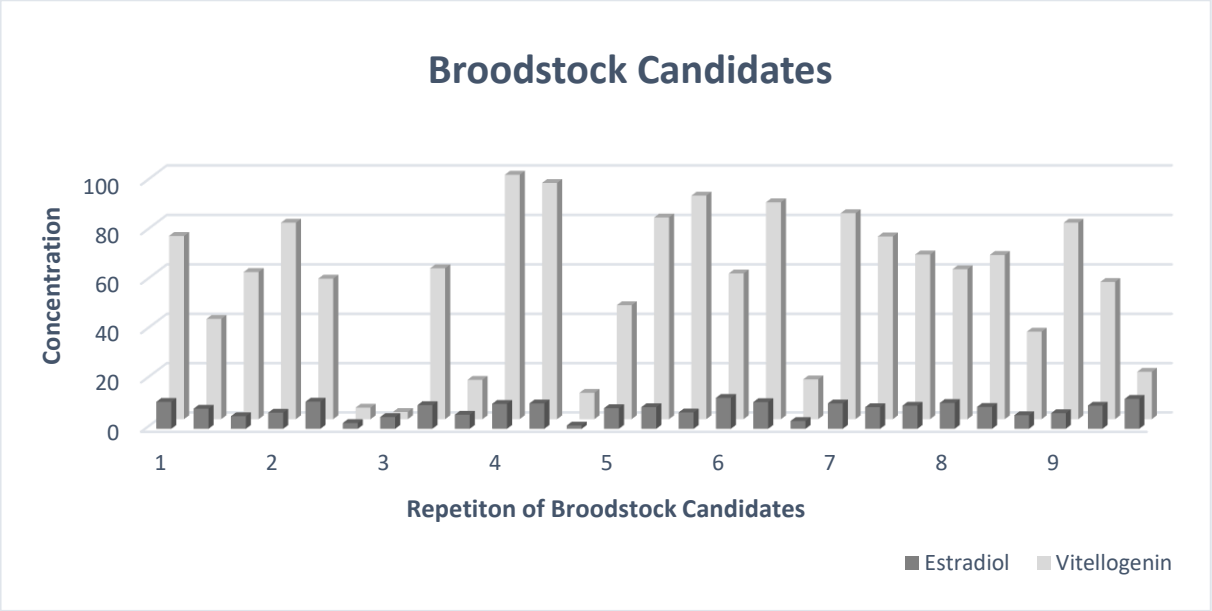


Figure 1. Fluctuation of estradiol-17β and vitellogenin concentration of Giant gourami female broodstock candidates

Table 1. The measurement on estradiol-17β and vitellogenin concentration of Giant gourami female broodstock candidates group

Sample ID	Conc. Estradiol-17β	Conc. Vitellogenin
C1.1	10.92	74.225
C2.1	6.532	79.591
C3.1	4.797	2.933
C4.1	10.104	98.957
C5.1	8.369	46.244
C6.1	12.553	59.085
C7.1	10.308	83.424
C8.1	10.512	60.809
C9.1	6.328	79.591
C1.2	8.165	40.687
C2.2	11.022	56.977
C3.2	9.593	61.193
C4.2	10.308	95.65
C5.2	8.777	81.699
C6.2	10.818	87.831
C7.2	8.777	74.033
C8.2	8.879	66.559
C9.2	9.389	55.635
C1.3	5.103	59.66
C2.3	2.347	4.657
C3.3	5.715	15.964
C4.3	1.327	10.686
C5.3	6.634	90.515
C6.3	3.164	16.156
C7.3	9.389	66.75
C8.3	5.511	35.512
C9.3	12.145	19.222

estradiol-17β and vitellogenin concentration of the broodstock candidates on the second week is 9.525±0.996[a] ng/ml and 68.918 17.475[a] µg/L, no significant difference is shown (P≥0.05) with the average of estradiol-17β and vitellogenin concentration on the first week. However, a significant difference is found (P≤0.05) with the average of estradiol-17β and vitellogenin concentration on the third week. The average of estradiol-17β and vitellogenin concentration of the broodstock candidates on the third week is 5.714±3.413[b] and 35.458±29.947[b] µg/L, showing a significant difference (P≤0.05) to the average of estradiol-17β and vitellogenin concentration on the first and second week (Table 2). Besides, the result of the data analysis using Pearson Correlation test (Table 3) shows that estradiol-17β concentration has correlation with vitellogenin concentration (P≤0.05) on Giant gourami broodstock candidates.

The Concentration of Estradiol-17β and Vitellogenin on Female Broodstock

Based on the overall result of the measurement on estradiol-17β and vitellogenin concentration of every 9 individuals of Giant gourami female broodstock within 3 week observation period, it is shown that on average, broodstock individual experienced an increase on estradiol-17β and vitellogenin concentration every week of the observation (Figure 2). The measurement result of estradiol-17β and vitellogenin concentration of each individual from Giant gourami female broodstock group is shown on Table 4. The average of estradiol-17β

Table 2. The significant difference and average of estradiol-17β and vitellogenin concentration of the broodstock candidates

(I)Test of Estradiol-17β Broodstock Candidates	(J) Test of Estradiol-17β Broodstock Candidates	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Inverval Lower Bound	95% Confidence Inverval Upper Bound
1st week	2nd week	-.61211	1.19778	.614	-3.0842	1.8600
	3rd week	3.20933*	1.19778	.013	.7372	5.6814
2nd week	1st week	.61211	1.19778	.614	-1.8600	3.0842
	3rd week	3.82144*	1.19778	.004	1.3494	6.2935
3rd week	1st week	-3.20933*	1.19778	.013	-5.6814	-.7372
	2nd week	-3.82144*	1.19778	.004	-6.2935	-1.3494

(I)Test of Vitellogenin Broodstock Candidates	(J) Test of Vitellogenin Broodstock Candidates	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Inverval Lower Bound	95% Confidence Inverval Upper Bound
1st week	2nd week	-3.933889	12.125076	.748	-28.95882	21.09104
	3rd week	29.526333*	12.125076	.023	4.50141	54.55126
2nd week	1st week	3.933889	12.125076	.748	-21.09104	28.95882
	3rd week	33.460222*	12.125076	.011	8.43529	58.48515
3rd week	1st week	-29.526333*	12.125076	.023	-54.55126	-4.50141
	2nd week	-33.460222*	12.125076	.011	-58.48515	-8.43529

* a significant difference

Repetition	Estradiol-17β (ng/L)	Vitellogenin (µg/ml)
1st week	8.946±2.579[a]	64.984±27.973[a]
2nd week	9.525±0.996[a]	68.918±17.475[a]
3rd week	5.714±3.413[b]	35.458±29.947[b]

Table 3. The result of the data analysis of estradiol-17β and vitellogenin using Pearson Correlation test

		Estrogen BroodstockCandidates	Vitellogenin BroodstockCandidates
EstrogenBCs	Pearson Correlation	1	.572**
	Sig. (2-tailed)		.002
	N	27	27
VitellogeninBCs	Pearson Correlation	.572**	1
	Sig. (2-tailed)	.002	
	N	27	27

** Correlation is significant at the 0.01 level (2-tailed).

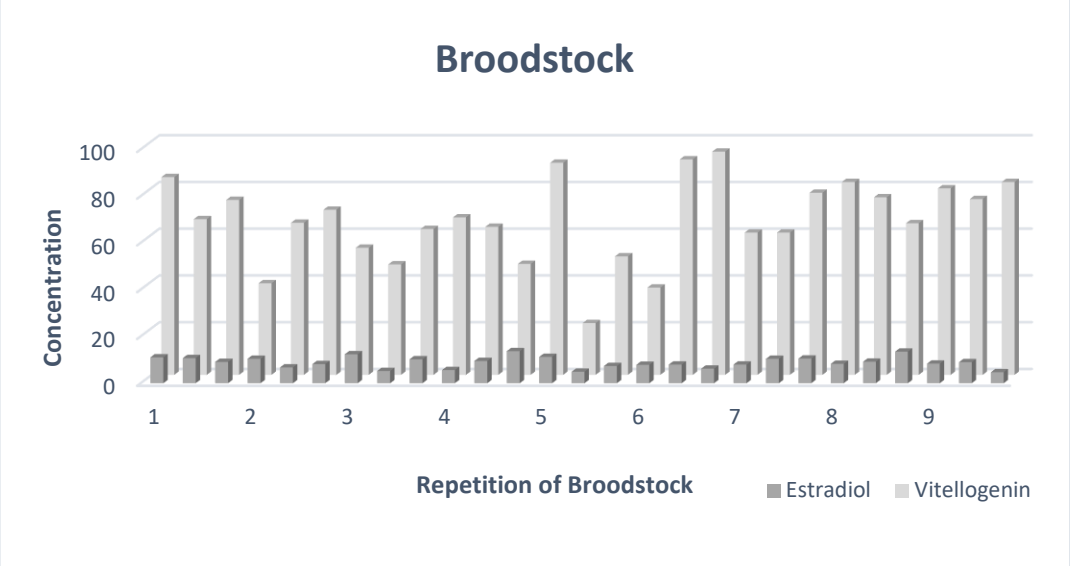


Figure 2. Fluctuation of estradiol-17β and vitellogenin concentration of Giant gourami female broodstock

Table 4. The measurement on estradiol-17β and vitellogenin concentration of Giant gourami female broodstock group

Sample ID	Conc. Estradiol-17β	Conc. Vitellogenin
I1.1	11.022	84.574
I2.1	10.410	39.154
I3.1	12.349	54.294
I4.1	5.613	67.343
I5.1	11.226	90.706
I6.1	7.858	37.272
I7.1	7.961	60.809
I8.1	8.267	82.465
I9.1	8.369	79.782
I1.2	10.716	66.559
I2.2	6.736	65.026
I3.2	5.205	47.203
I4.2	9.491	63.301
I5.2	4.899	22.097
I6.2	7.961	92.18
I7.2	10.41	60.809
I8.2	9.185	75.949
I9.2	8.981	75.183
I1.3	9.083	74.8
I2.3	8.165	70.583
I3.3	10.206	62.343
I4.3	13.676	47.394
I5.3	7.348	50.652
I6.3	6.226	95.482
I7.3	10.512	77.866
I8.3	13.472	64.834
I9.3	4.695	82.47

and vitellogenin concentration of the broodstock on the first to the third week is not significantly different (P≥0.05) (Table 5). Also, the result of the data analysis using Pearson Correlation test shows that estradiol-17β concentration has no correlation to vitellogenin concentration (P≥0.05) (Table 6).

The Morphology and Size of Giant Gourami Broodstock Candidates and Broodstock's Ovary

Based on the result of the observation done non invasively, the morphology of the ovary was seen as grey image on the ultrasound screen (Figure 3). Gonzalez-Bulnes *et al*. (2010), discovered that a tissue like ovary or uterine stroma reflects intermediate ultrasound wave called echoic or echogenic which displays grey color on the screen. The location of Giant gourami's ovary is in the visceral cavity, specifically on the anterior part of the body or close to the pectoral fin (Figure 4). The average of the ovary length and width of Giant gourami female broodstock candidates with an average ±940 gram body weight, ±36 and 13 cm length and weight is around ±18 mm length and ±19 mm width. On the one hand, the average of the ovary length and width of Giant gourami female broodstock with an average ±2.1 kg body weight, ±50 cm length and 18 cm width is approximately ±34 mm and ±24 mm. The overall observation result of Giant gourami female broodstock candidates and broodstock's ovary can be found on Table 7.

Table 5. The significant difference and average of estradiol-17β and vitellogenin concentration of the broodstock

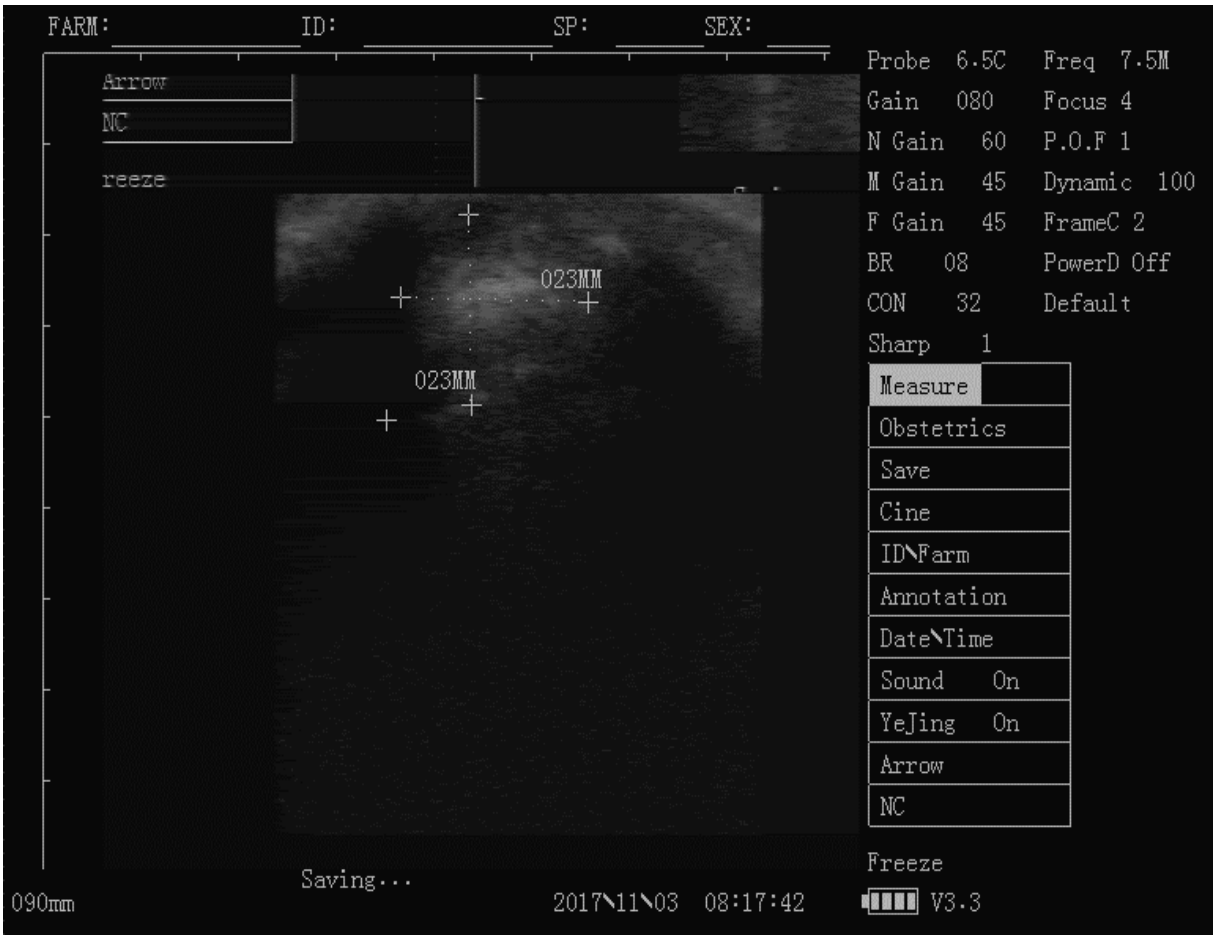
(I)Test of Estradiol-17β Broodstocks	(J) Test of Estradiol-17β Broodstocks	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Inverval Lower Bound	95% Confidence Inverval Upper Bound
1st week	2nd week	1.05456	1.16951	.376	-1.3592	3.4683
	3rd week	-.03422	1.16951	.977	-2.4480	2.3795
2nd week	1st week	-1.05456	1.16951	.376	-3.4683	1.3592
	3rd week	-1.08878	1.16951	.361	-3.5025	1.3250
3rd week	1st week	.03422	1.16951	.977	-2.3795	2.4480
	2nd week	1.08878	1.16951	.361	-1.3250	3.5025

(I)Test of Vitellogenin Broodstock	(J) Test of Vitellogenin Broodstock	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Inverval Lower Bound	95% Confidence Inverval Upper Bound
1st week	2nd week	3.121333	8.659492	.722	-14.75098	20.99365
	3rd week	-3.336111	8.659492	.703	-21.20842	14.53620
2nd week	1st week	-3.121333	8.659492	.722	-20.99365	14.75098
	3rd week	-6.457444	8.659492	.463	-24.32976	11.41487
3rd week	1st week	3.336111	8.659492	.703	-14.53620	21.20842
	2nd week	6.457444	8.659492	.463	-11.41487	24.32976

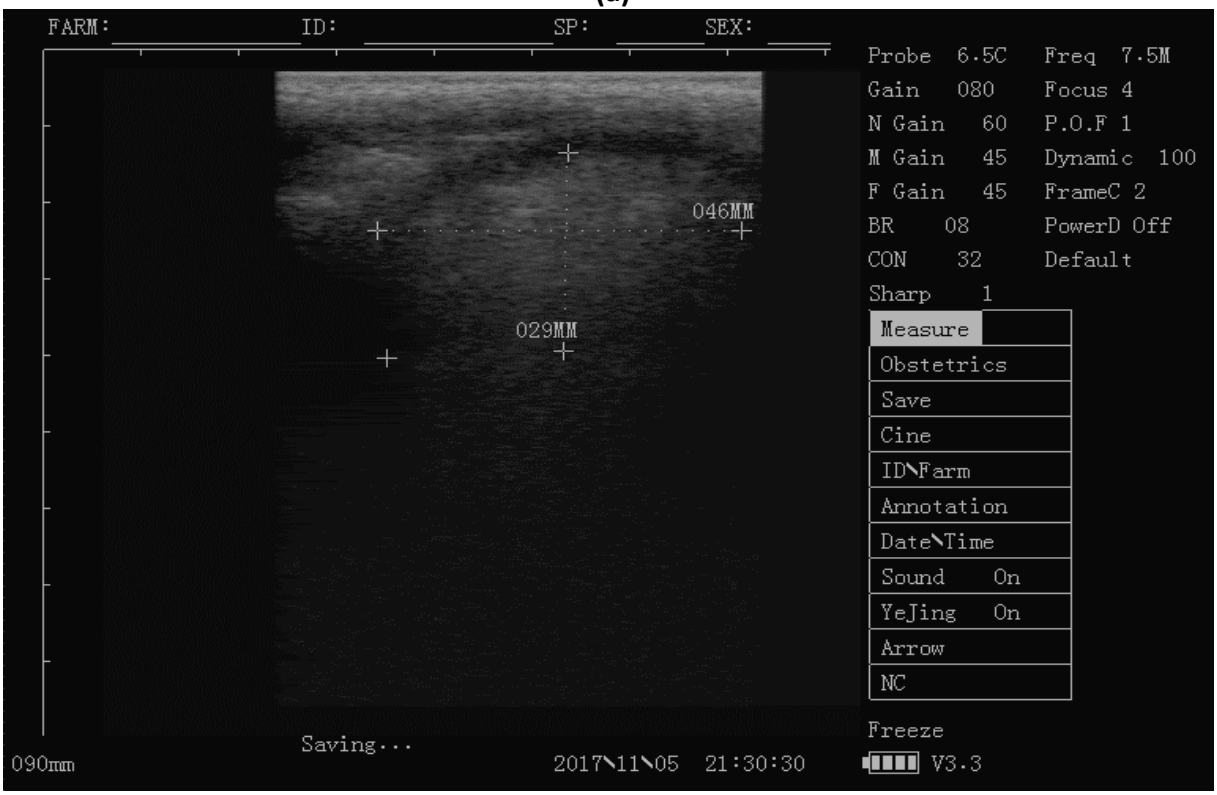
Repetition	Estradiol-17β (ng/L)	Vitellogenin (µg/ml)
1st week	9.116[a]±2.225	66.267[a]±19.772
2nd week	8.187[a]±2.142	63.145[a]±19.722
3rd week	10.285[a]±2.865	69.603[a]±15.245

Table 6. The result of the data analysis of estradiol-17β and vitellogenin using Pearson Correlation test

		Estrogen Broodstock	Vitellogenin Broodstock
EstrogenB	Pearson Correlation	1	.036
	Sig. (2-tailed)		.859
	N	27	27
VitellogeninB	Pearson Correlation	.036	1
	Sig. (2-tailed)	.859	
	N	27	27



(a)



(b)

Figure 3. Grey image of Gourami broodstock candidates and broodstock's ovary on the ultrasound screen, respectively.

Table 7. The size of Giant gourami female broodstock candidates and broodstock's ovary

	Female broodstock candidate (mm)		Female broodstock (mm)	
	Ovary Length	Ovary Width	Ovary Length	Ovary Width
1	20	20	28	24
2	23	23	30	25
3	23	19	33	31
4	15	22	39	18
5	19	22	47	25
6	12	17	27	14
7	17	14	27	19
8	16	18	45	27
9	18	18	46	29
	$\bar{x}_l$=18	$\bar{x}_w$=19	$\bar{x}_l$=36	$\bar{x}_w$=24

Figure 4. The location of Giant gourami's ovary.

The Measurement of the Rearing Pond Water Quality of Giant Gourami Female Broodstock Candidates and Broodstock

The measurement results of the rearing pond water quality covering temperature, pH, and DO are 27-29^{0}C, 8-9, and 2-3, subsequently. The overall results can be found on Table 8.

Discussion

The Concentration of Estradiol-17β and Vitellogenin of Female Broodstock Candidates

The measurement on estradiol-17 and vitellogenin concentration of Giant gourami (*Osphronemus goramy*) female broodstock candidates and broodstock was done during the highest peak of the spawning season in September-October. Wijayanti (2009) believes that the frequency of Giant gourami spawning hits its peak in January-April, and August-February. As a result, the measurement on estradiol-β and vitellogenin concentration in the blood done during this period is hoped to generate maximum value of the two variables.

Based on the result of the correlation analysis, it is found that estradiol-17β and vitellogenin concentration on Giant gourami female broodstock candidates show a positive correlation. This explains that estradiol-17β and vitellogenin synthesis rate have reciprocal relationship. On the initial reproduction cycle of the broodstock candidates, most of estradiol-17β synthesis was directed to the liver to trigger the synthesis process of vitellogenin which was then transferred to the ovary through the bloodstream to support the development of oocyte. Meanwhile, the reduction of estradiol-17β and vitellogenin concentration on the initial reproduction cycle of the broodstock shows that oocyte has reached the last stage of vitellogenesis and post vitellogenesis oocyte growth will stop for several months. The growth will continue after the oocyte receives influences from

Table 8. The measurement results of the rearing pond water quality

Water quality	Temperature			Average	pH			Average	DO			Average
	1st week	2nd week	3rd week		1st week	2nd week	3rd week		1st week	2nd week	3rd week	
Broodstock candidate's pond	29	26	27	27	8.74	9.37	10.7	9.6	2	4.4	4	3.5
Broodstock pond 1	29	28	28	28	8.79	4.17	10.2	7.72	2	3.4	2.8	2.7
Broodstock pond 2	29	29	29	29	8.8	4.6	9.99	7.8	2	3	2.6	2.5
Broodstock pond 3	29	28	29	29	8.8	10.1	10	9.63	3	4.4	2.6	3.3
Broodstock pond 4	29	28	31	29	8.75	10.2	10	9.65	2.6	3.2	3.3	3
Broodstock pond 5	29	28	31	29	8.77	10.22	10	9.7	2.6	3	2.6	2.7

the environment to start the final maturation process, namely meiosis division resumption.

As seen on Table 1, it is explained that estradiol-17β and vitellogenin concentration of the broodstock candidates on the first and the second week increased. It is contradictory to what happened on the third week where estradiol-17β and vitellogenin concentration dropped. The significant decrease on estradiol-17β and vitellogenin concentration was assumed to be the result of the ovary completing the last stage of vitellogenesis. This can be predicted considering that October-September becomes the highest peak of the spawning season period for Giant gourami. At the peak of the spawning season, the oocytes at their final vitellogenesis would be recruited to continue the oocyte maturation stage. The final stage of vitellogenesis in Giant gourami in this research took place at one year or 365 days after hatching. It is almost similar to the puberty period of mackerel chub, which starts at 7 months, or 213 dph (day post hatching) characterized by the presence of cotical alveolus until at the final stage of the vitellogenesis of the oocyte (Nyuji *et al., 2014*). In the species of *Rhamdia quelen*, the finishing stage of the vitellogenesis takes place for three months, from August to September, and it is characterized by the increase of estradiol-17 and the presence of oocytes at their final stage of vitellogenesis or oocytes at their maturation stage (Barcellos *et al., 2014*).

Similar to asynchronous fish, *Metynnis maculatus* during its highest spawning season showed a positive correlation between estradiol-17β and density of oocytes at the final stage of vitellogenesis. Estradiol-17β concentrations were seen to be lower in June during the highest spawning period than in August, the highsest vitellogenin period was the highest. The period of highest vitellogenin, in August, shows a slight increase in the oocytes population at the final stage of vitellogenesis, which are stable during the highest spawning period (Pereira *et al.*, 2013).

The Concentration of Estradiol-17β and Vitellogenin in Female Broodstock

The synthesis rate of the estradiol-17β and vitellogenin in female Giant gourami broodstock is different from the prospective broodstock, in which both synthesis rates showed an increase from the beginning to the end of the observation (Figure 2). However, based on the results of Pearson correlation test, no positive correlation between estradiol-17β concentration and vitellogenin concentration was shown. This explains that there are only a few possible reciprocal relationships between the two synthesis rates. It is said that there are a few possible reciprocal relationships that exist because the synthesis rate of vitellogenin is still ongoing, but it is most likely that the majority of the estradiol-17β synthesis rate is not directed towards the liver in order to secrete vitellogenin. In addition to playing an important role in the vitellogenesis process, estradiol-17β plays other roles, including supporting the proliferation of oogonia and acting as negative feedback signals to the hypothalamus to establish oocyte maturation. According to Korta *et al.* (2010), it is stated that the total number of oocyte contributions to support fecundity is ensured by the surge in oogonia proliferation and the recruitment of oocytes to the maturation stage.

The Giant gourami broodstock in this study had previously been spawned. Therefore, it was suspected that there might be a recovery in the number of oocytes in the ovary. This is similar to the content of ovaries in zebra fish, which become asynchronous after the spawning. According to Connolly *et al.* (2014), the asynchronous ovary content in ovaries (*Danio rerio*), after 1-32 days of spawning, consists of stage 1 or primary oocyte growth as much as 70.8%, stage 2 or formation of cortical alveolus (previtellogenesis) as much as 15.8%, stage 3 or vitellogenesis 2.7%, and stage 4 or post vitellogenesis as much as 10.7%.

Another possibility that causes the absence of reciprocal relationship between the rate of estradiol-17β synthesis and vitellogenin is the fact that the Giant gourami broodstock shows an indication of skipping its spawning time. According to Rideout and Tomkiewicz (2011), most of the asynchronous fish have the potential to pass spawning by showing a small amount of secondary oocytes or vitellogenesis.

According to Rideout and Tomkiewicz (2011), most of the asynchronous fish have the potential to skip their spawning period by showing secondary oocytes or small amount of vitellogenesis. In addition, if there is atresia from either the primary or the secondary oocytes, there will be no potential for additional oocyte recruitment in order to perform spawning. Groups of fish that have the potential in skipping the spawning period are characterized by the amount of time in beginning the redevelopment and absorption of egg yolks. It is also characterized by the longer time to start spawning compared to the period of the fish spawning season.

There are both internal and external factors might influence the development of the fish oocyte. They include hormonal and genetical factors and stress condition towards their environment, which can influence the success of gametogenesis process.

Gonadotrophin-inhibitory hormone (GnIH) in fish has two effects on the synthesis and the release of gonadotrophin. It acts as a stimulator and an inhibitor. In addition, several studies revealed that GnIH is also involved in the regulation of the release of growth hormone (GH) and mRNA *gh*. Based on the results of research by Di Yorio *et al.* (2016), which was conducted in vitro, it is shown that the cd-LPQRFa-1 or GnIH in *cichlid fish* could inhibit the release of LH and FSH, but was able to stimulate GH release in pituitary tissue culture.

In addition to the GnIH influence, other influences such as Neuropeptide Y (NPY), which is one of the metabolic signals, has a role as in determining of the relationship of energy balance between somatic cell growth and fish reproduction. NPY can directly affect somatotrophs and can indirectly affect gonadotrophs. In other words, the effect of NPY on GH is greater than that of gonadotropins. NPY meets the proximal pars distalis or it is near *somatotropic cells*. This shows that there is a close relationship between NPY and GH. On the contrary, there is only a little closeness between the NPY and FSH, and there is no closeness at all to LH. However, in vitro observations showed that there is an increase in GH and β-FSH release in culture media even though it did not show changes in GH and β-FSH expression. Conversely, the presence of NYP is able to increase the release of β-LH and β-LH mRNA expression (Di Yorio *et al.*, 2015). Further research on NPY needs to be carried to find out the specific effects that are generated, especially in Giant gourami.

In addition to GnIH and NPY, Cortisol is classified as the substance that influences fish reproduction. The role of cortisol in the development and maturation of fish oocytes is still mazy. The increase of maternal plasma cortisol is thought to reflect an increase in cortisol content in oocytes. Research by Mao Li *et al.* (2012) was able to prove that glucocorticoid and cortisol metabolism can be formed into less potent glucocorticoid and cortisone (cortisol catabolism products) in all oocytes and YF (yolk free) follicles, naturally ovulated ovum, and embryonic tissue indicates the presence of 11β-HSD activity (cortisol regulator) and glucocorticoid sulphotransferase (GTS). In vitro observation of a number of oocytes and follicular YF (yolk free) shows little sulfate activity or cortisol biotransformation when compared to ovulated ovum. This explains that oocytes and YF follicle, compared to ovulated ovum, are more susceptible to increased levels of cortisol. However, a further research needs to be carried to find out the specific effects in the oocytes and ovum of certain fish, especially in Giant gourami.

The Morphology and Ovarium Size of the Broodstock Candidate and Broodstock Using Ultrasound

Various types of ultrasound have been widely used for gender identification and sex maturation. However, there are advantages and disadvantages of using ultrasound in detecting gender and maturation of the fish. According to Kohn (2013), the advantages of using ultrasound include the ease and speed of sampling, the non-invasive approach, which can minimize stress on fish, and immediately obtained results. Ultrasound also allows identification of females that have not sexually developed. Meanwhile, the shortcomings derived from the use of ultrasound include requiring repeated scanning, needing expertise, inconclusive images, and operation-dependant.

The ultrasonography scan results appear on a screen that displays a *grey-scale* or black-and-white image. Differences in *echotexture* from various observed tissues have special designations depending on tissue characteristics (*echogenicity*). Substances such as urine, follicular fluid, or other fluids by sound waves that do not produce *echo* or sound are called *anechoic* or *anechogenic*. Whereas tissues such as the bone or uterine cervix that strongly reflect ultrasound waves resulting in a strong *echo* are called *hyperechoic* or *hyperechogenic*, which is portrayed by the white color on the screen. In addition, tissues such as ovaries or *uterine stroma* that reflect intermediate ultrasound waves are called *echoic* or *echogenic*, which is portrayed by the gray color on the screen (Gonzalez-Bulnes *et al.*, 2010).

Ultrasonography image in this study can be used as a reference for the length and width of the ovary of the prospective broodstock and sexually mature Giant gourami broodstock. Based on the results of measurements, the length and width of the ovary of the prospective broodstock and female Giant gourami broodstock are considered small. According to Wijayanti (2009), the average diameter of pre-vitellogenesis oocyte should be about 39-90 µm, average diameter of mid-vitellogenesis oocytes should be about 222-558 µm, and average diameter oocyte at the late - vitellogenesis should be about 874-1709 µm. Gonadal maturation index of the female Giant gourami broodstcok is considered small, which is only 4.5%.

The Water Quality in the Rearing Pond of the Prospective Broodstock and Matured Broodstock

The water quality in the rearing pond of the prospective broodsctock and female Giant gourami broodstock, including the temperature, pH, and *dissolve oxygen* (DO) has an important role in supporting reproductive productivity during the spawning season. Based on the results of the quality review of the water in the rearing pond conducted every week during the observation, it is shown that the water quality of the rearing pond has not reached the optimal range needed to support growth and reproduction. According to Nirmala and Rasmawan (2010), the optimal temperature able to support the growth of Giant gourami is around 24.9-28°C. The higher is the temperature; the lower is the value of dissolve oxygen (DO). The optimal range of dissolved oxygen to support Giant gourami growth is 4-6 mg/l. Moreover, the pH range with less than 6.5 or more than 9.5 will affect the growth and reproduction of fish in the long run.

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