Gonad development and 17β-estradiol profile in the blood serum of female koi (*Cyprinus carpio*) broodstock stimulated with male koi pheromones

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Abstract. This study aimed to evaluate gonadal development and 17β-estradiol profile in the blood serum of female koi broodstock stimulated with male koi pheromones. The research used a completely randomized design, consisting of 4 treatments with 2 replications, namely: treatment A = stimulation through the eyes; B = stimulation through the nose; C = stimulation through the eyes and nose; and D = stimulation through the eyes, nose, and skin. The results showed that the gonad of female koi developed as indicated by an increase in the gonadal maturity index (GMI) value. The 17β-estradiol profile in the blood serum of female koi increased from week 1 to week 4, then decreased until the end of the study at week 7. The results of statistical analysis showed that pheromone stimulation of male koi had a significant effect ($p < 0.05$) on the 17β-estradiol profile and gonadal development of female koi as expressed by increasing the value of the GMI. There is a linear relationship between GMI values and 17β-estradiol levels in the blood serum of female koi broodstock.
Key Words: broodstock, *Cyprinus carpio*, gonad development, GMI, pheromone, 17β-estradiol.

Introduction. The use of synthetic hormones such as salmon gonadotropin-releasing hormone analog (S-GnRHa) in stimulating the processes of gonadal development, ovulation, and spawning of fish is commonly used in fish hatcheries. Its use is quite practical and effective, but less economical because the price is relatively expensive. The quality of the larvae produced also tends to decrease due to excessive use (Ningrum et al 2019). Therefore, an alternative method is required that is more economical, and natural, but still guarantees the quality of the larvae produced.

The process of gonadal development in fish is strongly influenced by internal and external factors. Internal factors are in the form of hormone performance while external factors are environmental conditions including rainfall, light, petrichor, air quality, feed, and the presence of male fish (Kobayashi et al 2002). Internal and external factors jointly affect growth, gonadal development, reproductive performance, quality, and quantity of eggs and larvae produced (Volkoff & London 2018).

The existence of male fish is one of the external factors that influence the process of gonadal development. The pheromones produced by male fish can cause reactions in female fish involving sensory organs such as the eyes, nose, and skin (Kobayashi et al 2002). The problem is, organ sensors can give different effects, depending on the type of stimulant received by the female fish. Photoreceptors in the eyes can receive stimulants in the form of light or color, while chemoreceptors in the nose and skin can detect odors or dissolved compounds in the air (Stacey et al 2003). Stimulants received by organ sensors will be forwarded to the hypothalamus to produce gonadotropin releasing hormone (GnRH), which will then stimulate the pituitary gland to produce gonadotropin hormone (GtH). Through the bloodstream, GtH will go to the gonads and stimulate them to secrete steroid hormones which are direct mediators of spawning (Ahlina et al 2015).

Therefore, the present study was conducted to evaluate gonadal development and 17β-estradiol profile in the blood serum of female koi (*Cyprinus carpio*) broodstock stimulated with male koi pheromones.

Material and Method. The study was conducted from April to July 2020. The study used 8 rectangular wooden tanks covered with dark-colored plastic (length 2.5 m, breadth 1.5 m, and height 0.5 m). The test animals used were male and female koi carp broodstock weighing 1100-1200 g per fish. Each tank was stocked with 8 broodstocks.

Design of the study. The design of the study was a completely randomized design (CRD), consisting of 4 treatments with 2 replications. The four treatments were: treatment A = stimulation through the eye, where the transparent partition is installed between male and female koi broodstocks; treatment B = stimulation through the nose, where a dark partition is placed between the male and female koi broodstock; treatment C = stimulation through the eyes and nose, where a perforated transparent partition is installed between male and female koi broodstocks; treatment D = stimulation through the eyes, nose, and skin, where no partition between male and female koi broodstocks.

Feeding and water exchange. Type of feed used during the research was artificial feed (in the form of pellets). The feed was given 3 times a day (morning, afternoon, and evening) ad libitum. Siphoning was done daily to remove leftover feed and water exchanges were done when deemed necessary.

Observation of estradiol 17–β levels. Observation of 17β-estradiol levels in the blood serum of female koi broodstocks was carried out every week until spawning. Analysis of estradiol hormone concentrationin in hemolymph was performed by enzyme-linked immunosorbent assay (ELISA) method referring to the procedure recommended by Setiadi et al (2014).

Observation of gonad development. Gonad development was observed weekly until spawning. The development of the gonads is expressed as the value of gonadal maturity index (GMI) with the assumption that the weight of the gonads is the difference between the weight of the broodstock at t-time and the weight of the broodstock at the beginning of the study. GMI was calculated based on Effendi's formula (2002):

$$GMI\ (\%) = \frac{GW}{BW - GW} \times 100$$

where: GMI = gonad maturity index (%);
BW = body weight (g);
GW = gonad weight (g).

Water quality observation. The water quality parameters including temperature, pH, dissolved oxygen were measured using a portable DO meter AZ8403, while CO_2 was measured using a portable carbon dioxide CGP-31. Ammonia used an ammonia meter. All water quality measurements were taken daily.

Data analysis. Data were analyzed using analysis of variance (ANOVA) at the 5% level and if the results showed significant differences then it was continued with the Least Significant Difference (LSD) test. The test results are statistically significant if the p value < 0.05 (Steel & Torrie 1989).

Results and Discussion

Profile of 17β-estradiol. The level of 17β-estradiol (pg mL^{-1}) in the blood serum of female koi showed an increase at the beginning of the study (from week 1 to week 4), then decreased until the end of the study at week 7 (Figure 1).

Based on the analysis of variance, pheromone stimulation of male koi had a significant ($p < 0.05$) effect on the 17β-estradiol profile in the blood serum of female koi. The results of the LSD test showed a significant difference between treatments. Treatment A was significantly different from treatments B, C, and D, but there was no significant difference between treatments B, C, and D, indicating, the female koi's sensory organs, nose or eyes, respond differently to pheromone stimuli from male koi given to them. The difference in response to these stimuli affects the 17β-estradiol profile in the blood serum of the female koi broodstock.

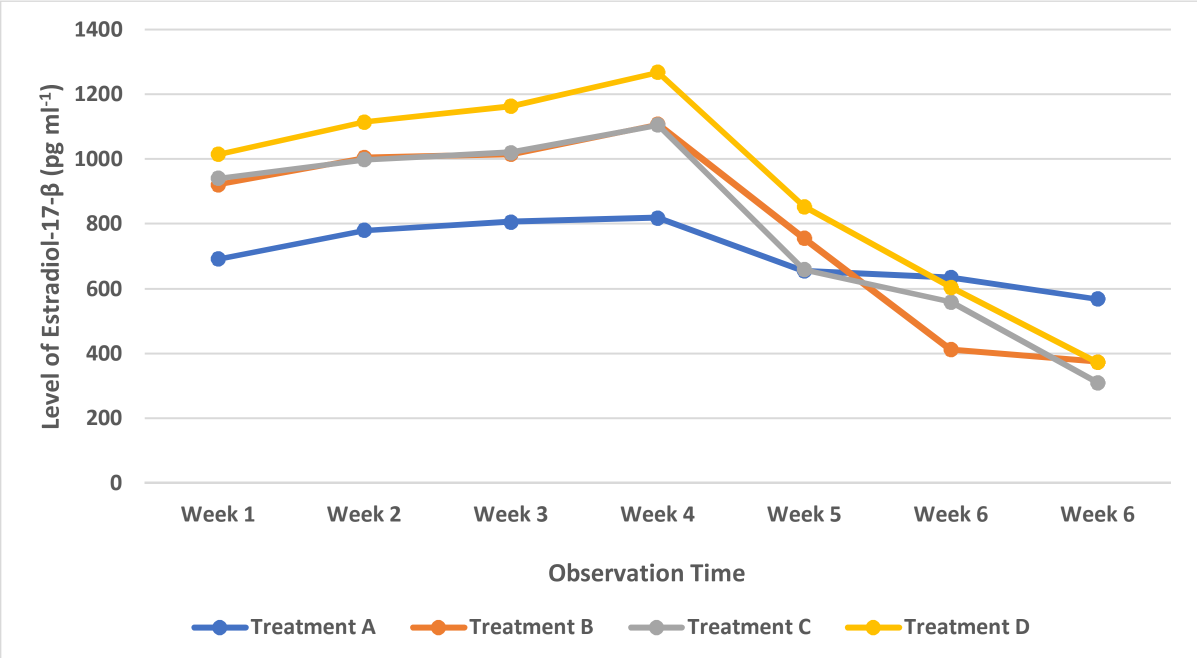

Figure 1. Profile of estradiol-17β in blood serum of female koi broodstock.

The hormone 17β-estradiol plays a role in stimulating the liver to synthesize vitellogenin during the reproductive process. The process of vitellogenin synthesis (vitellogenesis) is a stage of oocyte development before heading to the final maturation stage (Basuki et al 2006). The hormone 17β-estradiol is produced in oocytes in the granulosa layer with the help of the aromatase enzyme (Nagahama et al 1995).

The results showed that from the beginning of the observation (week 1), 17β-estradiol levels increased until week 4 (Figure 1). According to Basuki et al (2006), during oocyte development (vitellogenesis), the hormone 17β-estradiol is produced more by the granulosa layer to stimulate the liver to synthesize vitellogenin. Stimulation of the hormone 17β-estradiol to the liver occurs due to a bond between the hormone 17β-estradiol released from the granulosa layer in the oocyte and the receptors in hepatocyte cells of the liver. Furthermore, hepatocyte cells will synthesize vitellogenin, where this vitellogenin is the precursor of egg yolk. According to Rawung et al (2020), the presence of the hormone 17β-estradiol greatly determines the activity of vitellogenin synthesis by the liver during reproduction. Vitellogenin produced by the liver is then absorbed by the developing oocyte by being transported through blood circulation.

Entering week 5, the production of 17β-estradiol began to decrease until the end of week 7 of observation (Figure 1). The decrease in 17β-estradiol levels is an indicator that the vitellogenesis process has ended, then oocytes enter the final maturation stage (Basuki et al 2006). The same thing was observed in bada fish (*Rasbora argyrotaenia*), where the concentration of 17β-estradiol in blood plasma showed a pattern of fluctuation in all experimental groups. At the beginning of the preparation, 17β-estradiol levels increased to reach a peak during vitellogenesis, then decreased before spawning (Akhdiana et al 2021).

17β-estradiol fluctuations found in broodstock are thought to be an individual response to ongoing reproductive activity (Rawung 2019). At the end of the present study, the levels of 17β-estradiol decreased in all treatments. This is due to the use of 17β-estradiol in the process of forming steroid hormones that play an essential role during reproduction. Meanwhile, according to Wahyuningsih et al (2012), the decrease in cholesterol concentration in the blood is due to the increase in the size of the egg diameter that occurs during oocyte development (vitellogenesis).

Overall, the highest levels of 17β-estradiol were found in treatment D. This shows that as a sensory organ for touch, the skin can receive and transmit stimulation from male koi pheromones, besides that the skin can increase the response of the female koi olfactory sensory organ (nose), as well increase mechanical stimulation, especially in the genital area which encourages spending more male koi urine/milt. Although the results of research by Van Weerd et al (1991) showed that the skin of anosmic female African catfish (*Clarias gariepinus*) showed no response to stimulation from male fish.

The sensory organ of touch (skin) besides playing a role in continuing mechanical stimulation can also produce mucus with a specific smell, which is thought to be able to cause a response to the opposite sex. According to Green & Zielinski (2013), on the skin there is mucus (mucus) with a characteristic odor, which can act as a means of chemical communication between fish species, known as pheromone compounds. The characteristic odor found on the skin of the male koi is thought to contain pheromone compounds. These bioactive compounds can stimulate the release of GtH from the pituitary gland.

It was further stated that pheromones are received by chemoreceptors in the olfactory sensory organ (nose) of female koi, then passed on to the hypothalamus to produce gonadotropin-releasing hormone (GnRH). Furthermore, GnRH stimulates the pituitary gland to produce gonadotropin hormone (GtH), and GtH stimulates follicles in the gonads to produce 17β-estradiol (Green & Zielinski 2013). The more stimulants the female koi broodstock receives, the more it encourages the production of GtH by the pituitary gland through the hypothalamus-pituitary-gonadal axis. GtH stimulates follicles in the gonads to produce 17β-estradiol. 17β-estradiol from the gonads will stimulate hepatocyte cells to synthesize and secrete vitellogenin for the development of oocytes in the gonads themselves. Thus, at that time 17β-estradiol levels in the blood will increase (Figure 2) (Ahlina et al 2015).

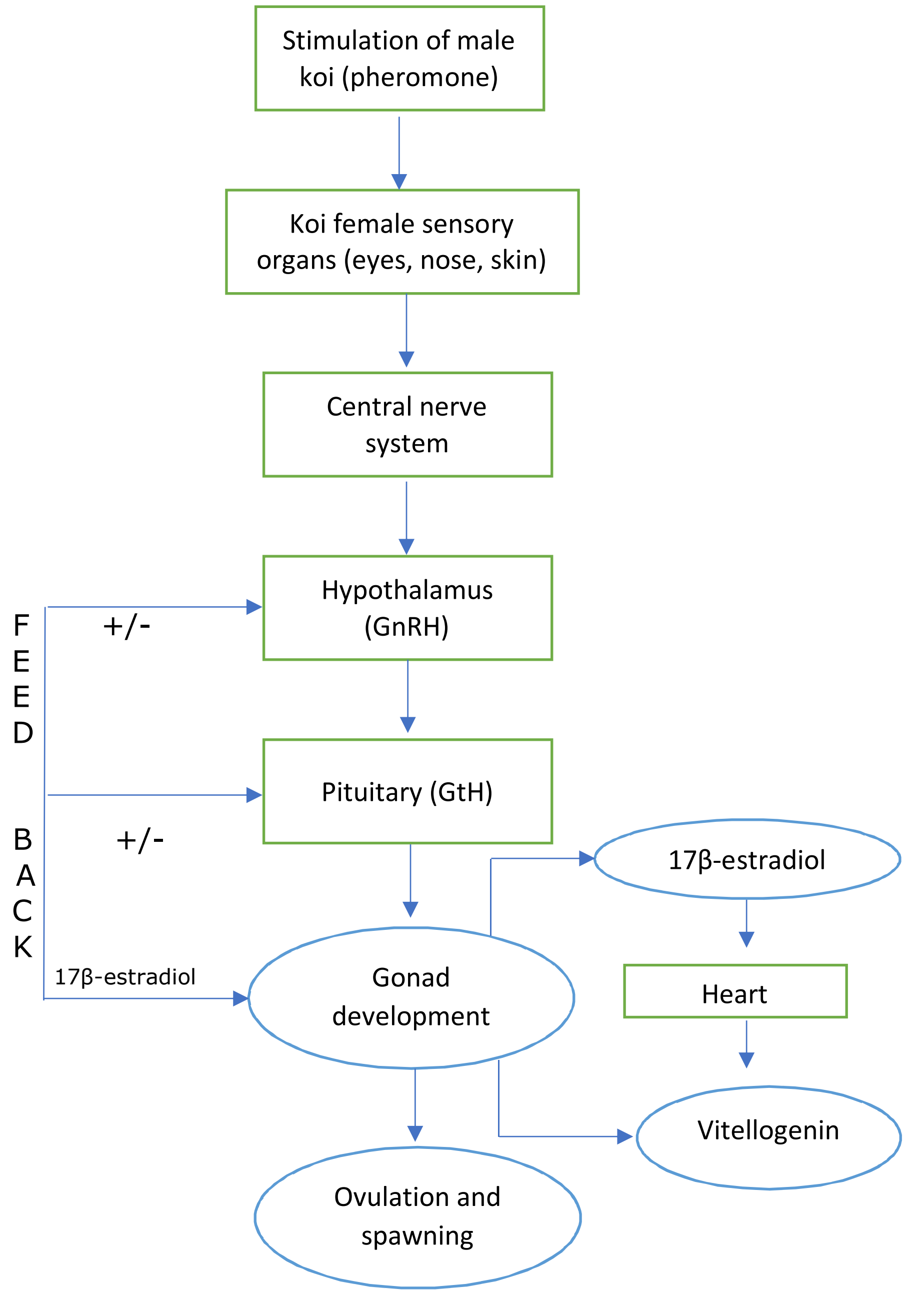

Figure 2. Synthesis of 17β-estradiol by stimulation of male koi via hypothalamic-pituitary-gonadal axis (adapted from Fostier et al 1983).

Gonad maturity index (GMI). Overall, the GMI value of female koi obtained in all treatments increased until the end of the study (Figure 3).

The results of the analysis of variance showed that male koi pheromone stimulation had a significant effect ($p < 0.05$) on GMI values of female koi broodstock. The LSD test results showed a significant difference between the treatments. Treatment A is significantly different from B, C, and D.

The difference in GMI values between treatments is closely related to 17β-estradiol levels. According to Rawung et al (2020), 17β-estradiol is required to induce hepatocyte cells in the synthesis and secretion of vitellogenin. Vitellogenin is a precursor for the synthesis of lipoproteins, which contain about 20% of lipid molecules, mainly phospholipids, triglycerides, and cholesterol. Vitellogenin will then be released into the bloodstream and will be absorbed by oocytes by micropinocytosis. In the oocyte, vitellogenin is converted into egg yolk protein. The oocyte then undergoes a growth period, which includes 2 phases: the first previtellogenesis in which the oocyte enlarges due to increased cytoplasmic volume, and the second phase of vitellogenesis in which the accumulation of egg yolk synthesized by the liver occurs (Jobling et al 1995), and in the end will increase the GMI value so that the GMI values in treatment B, C and D were higher than control (A).

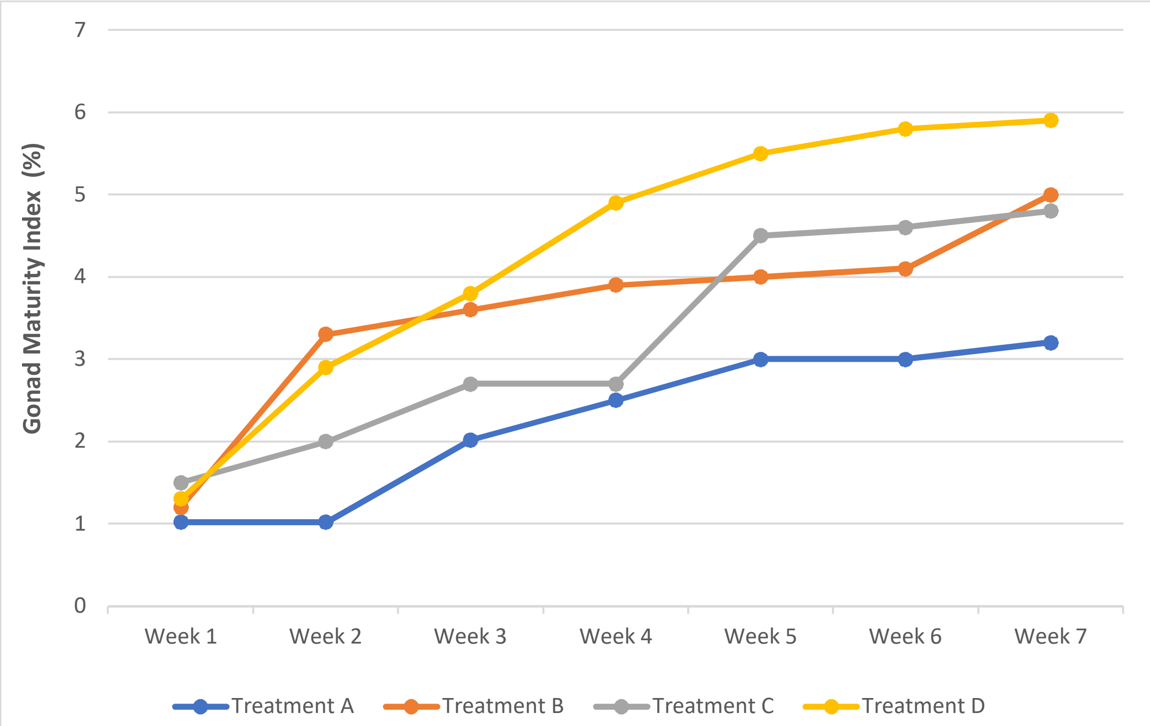

Figure 3. Gonad maturity index (GMI) female koi brodstock stimulated with male koi pheromone.

Overall, the GMI values of all treatments increased during the study. This increase is closely related to 17β-estradiol levels. The findings of Muhazar et al (2019) showed a linear relationship between gonadal development and 17β-estradiol levels in blood plasma. Vitellogenic development is concomitant with increased levels of 17β-estradiol in blood plasma. The results of linear regression analysis using the grafted polynomial model showed a linear relationship between GMI values and 17β-estradiol levels in the blood serum of female koi broodstock, with the regression line equation Y = 833.551 + 9.661x. This means that every 1% increase in the GMI values will respond to an increase in 17β-estradiol levels in the blood by 9.661 pg mL^{-1}.

Observation of the water quality parameters indicated that all water quality parameters measured were within the optimal range and supported the development process of the broodstock gonads. Water temperature ranged from 27 to 31^{o}C, pH ranged from 6.5 to 7.5; O_2 ranged from 7.0 to 7.5 ppm; CO_2 ranged from 3.8 to 4.5 ppm and NH_3 ranged from 0.01 to 0.03 ppm. Inappropriate water temperature has been associated with slow gonadal growth and development (Hermelink et al 2011).

Conclusions. Based on the data obtained, it can be concluded that pheromone stimulation of male koi broodstock has a significant effect on the gonadal development of female koi which is expressed through increasing the value of the gonadal maturation index (GMI), stimulation of male koi. Pheromones have a significant effect on the profile of 17β-estradiol levels in the blood serum of female koi fish. In addition, there is a linear relationship between GMI values and 17β-estradiol levels in the blood serum of female koi broodstock. The results of this study provide a clear direction to overcome the problem of the presence of gonadal mature male fish, especially the pheromones produced by male broodstocks can cause reactions in female broodstocks

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Conflict of interest. The authors declare that there is no conflict of interest.

References

Ahlina H. F., Sudrajat A. O., Budiardi T., Affandi R., 2015 [Hormonally induced gonadal maturation in eels, *Anguilla bicolor bicolor* McClelland 1984 with the use of Pregnant Mare Serum Gonadotropin, anti dopamine, and recombinant Growth Hormone]. Jurnal Iktiologi Indonesia 15(3):209-221. [in Indonesian]

Akhdiana I., Zairin Jr. M., Haryani G. S., Suprayudi A. M., 2021 [Gonad development and blood plasma profile of female bada fish (*Rasbora* sp.) by giving a combination of the hormone estradiol and spirulina in feed. Jurnal Iktiologi Indonesia 21(3):253-265. [in Indonesian]

Basuki F., Zairin Jr. M., Sudradjat A. O., Yusuf T. L., Purwantara B., Toelihere M. R., 2006 [Effect of aromatase inhibitors (IA) on oocyte development in goldfish (*Carassius auratus*)]. Jurnal Ilmu-ilmu Perairan dan Perikanan Indonesia 13(2):171-175. [in Indonesian]

Effendi M. I., 2002 [Fisheries biology methods]. Yayasan Dewi Sri, Bogor. [in Indonesian]

Fostier A., Jalabert B., Billard R., Breton B., Zohar Y., 1983 The gonadal steroids. In: Fish physiology. Volume 9, Part A. Hoar W. S., Randall D. J., Donalson E. M. (eds), Academic Press, New York, pp. 277-372.

Green W. W., Zielinski B. S., 2013 Chemoreception. In: The physiology of fishes. 4th edition. Evans D. H., Claiborne J. B., Currie S. (eds), CRC Press, Boca Raton, pp. 345-373.

Hermelink B., Wuertz S., Trubiroha A., Rennert B., Kloas W., Schulz C., 2011 Influence of temperature on puberty and maturation of pikeperch, *Sander lucioperca*. General and Comparative Endocrinology 172(2):282-292.

Jobling S., Sumpter J. P., Sheahan D., Osborne J. A., Matthiessen P., 1995 Inhibition of testicular growth in rainbow trout (*Oncorhynchus mykiss*) exposed to estrogenic alkylphenolic chemicals. Environmental Toxicology and Chemistry 15(2):194-202.

Kobayashi M., Sorensen P. W., Stacey N. E., 2002 Hormonal and pheromonal control of spawning behavior in the goldfish. Fish Physiology and Biochemistry 26(1):71-84.

Muhazar, Zairin Jr. M., Yulianda F., Suprayudi M. A., Alimuddin, Effendi I., 2019 [Effect of 17β-estradiol hormone administration on the dog conch's *Laevistrombus turturella* gonad development]. Jurnal Ilmu dan Teknologi Kelautan Tropis 11(3):583-593. [in Indonesian]

Nagahama Y., Yoshikuni M., Yamashita M., Tokumoto T., Katsu Y., 1995 Regulation of oocyte growth and maturation in fish. Current Topics in Developmental Biology 30: 103-145.

Ningrum D. R. K., Budi D. S., Sulmartiwi L., 2019 [Spawning induction of silver rasbora (*Rasbora argyrotaenia*) using OvaprimTM with different doses]. Depik: Jurnal Ilmu-Ilmu Perairan, Pesisir dan Perikanan 8(2):117-124. [in Indonesian]

Rawung L. D., 2019 [The effect of the combination of curcumin and thyroxine hormone on the reproductive performance of broodstock and the growth of catfish (*Clarias gariepinus*) larvae]. Dissertation, Institut Pertanian Bogor. [in Indonesian]

Rawung L. D., Ekastuti D. R., Zairin Jr. M., Rahminiwati M., Sunarma A., Manalu W., 2020 Reproductive performances and egg qualities in African catfish (*Clarias gariepinus*) broodstock supplemented with curcumin and thyroxine hormone. Omni Akuatika 16(1):32-47.

Setiadi D. R., Supriatna I., Agil M., 2014 [Validation of commercial Enzyme-Linked Immunosorbent Assay kit for analysis of estradiol and progesterone hormone in blood of kacang goat]. Jurnal Veteriner 15(4):446-453. [in Indonesian]

Stacey N., Chojnacki A., Narayanan A., Cole T., Murphy C., 2003 Hormonally-derived sex pheromones in fish: exogenous cues and signals from gonad to brain. Canadian Journal of Physiology and Pharmacology 81(4):329-341.

Steel R. G. D., Torrie J. H., 1989 Stastistical principles and procedures. PT. Gramedia, 145 pp.

Van Weerd J. H., Eding E. H., Bongers A. B. J., Vermeirsen E. L. M., Richter C. J. J., Lambert J. G. D., Granneman C. J. M., 1991 A possible non-gonadal origin of vitellogenin pheromones in the African catfish *Clarias gariepinus*. In: Proceeding of the Fourth International Symposium on the Reproductive Physiology of Fish. Scott A. P., Sumpter J. P., Kime D. E., Rolfe M.S. (eds), Shelfied, pp. 182-187.
Volkoff H., London S., 2018 Nutrition and reproduction in fish. In: Encyclopedia of reproduction. Skinner M. A. (ed), Elsevier, Amsterdam, pp. 743-748.
Wahyuningsih H., Zairin Jr. M., Sudrajat A. O., Tumbelaka L. I. T. A., Manalu W., 2012 [Changes of blood plasma and gonadal maturity on female Tor soro in pond]. Jurnal Iktiologi Indonesia 12(1):25-34. [in Indonesian]

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