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The Effect of Different Photoperiods on The Oogenesis of Fighting Fish (*Betta Splendens*)

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Abstract

The demand for fighting fish Betta splendens continues to increase every year, this encourages farmers to develop technology in the production process. Accelerating reproduction is one solution to support production demand. One technology prior to this is photoperiod. The aim of this research was to evaluate different photoperiod treatments on the stages of betta fish ovarian development. The method used is completely randomized design (CRD) to test 3 different treatments in quintuplicates. Treatment P1 (10-h Light L :14-h Dark D), P2 (14-h L :10-h Dark D), and P3 (Control, ambient lights). Observation of the oogenesis stages of betta fish was carried out through histological preparations classic and calculating the proportions of oogenesis stages. Data were then analyzed statistically (ANOVA) with 0.05 level. The research results showed that treatment P2 was significantly different from P1 and P3, due to domination of previtellogenic oocytes (22.05%), oocyte exogenous vitellogenin (22.89%), and hydrated vitellogenin oocytes (25.24%). Then followed by treatment P1 (10L; 14D) presented previtellogenic oocytes (20.86%), exogenous vitellogenin oocyte (20.17%), and hydrated vitellogenin oocytes (21.82%). In Control, ovaria contained previtellogenic oocytes (19.43%), exogenous vitellogenin oocytes (18.79%), and hydrated vitellogenin oocytes (19.21%). This indicated that under such photoperiod treatments fish kept on adept to develop their ovaria. Moreover, fish matured their ovaria and were apt to spawn.

Keywords: *Betta fish, Betta splendens, Photoperiod, Oogenesis*

1. Introduction

Betta fish is one of the popular commodities in Indonesia. The demand for Betta fish continues to increase every year (Pattiasina et al., 2021; Syaifudin et al., 2016; Ayuningthias et al., 2021). This encourages farmers to increase production of betta fish. Hatchery is one of the processes in increasing betta fish production (Saputra et al., 2020). Where in this seeding the seeds produced must be of high quality. The quality of the seeds produced comes from quality parents too. One of the characteristics of quality brood stock is looking at the level of gonad maturity or readiness for the spawning process (Hartami et al., 2022; Patmadev et al., 2022). In general, many efforts have been made to mature the gonads of Betta fish, both from the nutritional aspect and hormone injections. (Nurhayati et al., 2018). However, this is still not enough to support the maturation of betta fish gonads, technology is needed that can be done at any time. The application of environmental manipulation technology deals a solution to this.

Photoperiod is an environmental manipulation technology that applies lighting periods when rearing brood betta fish (Reni & Handayani, 2023). The length of time the photoperiod is applied in the cultivation process can influence the growth and reproduction levels of fish (Veras et al., 2013). Photoperiod signals stimulation of the endocrine system, which lengthens the spawning time process, thereby increasing the number of eggs produced (Choi et al., 2023; Lee et al., 2017). Almeida et al., (2009), stated that photoperiod has a major influence on the brain and pituitary gland to control gonad maturity and activity in vertebrates. In female teleost fish, testosterone and 17β-estradiol are the dominant steroids in the oogenesis process (El-Gamal & El-Greisy, 2005). Besides, in African catfish, Solomon & Okomoda (2012) demonstrated that photoperiod might not affect the condition factor, while

higher shoot composition was considered one of the causes of increased mortality and could be reduced to a large extent when light phase was reduced, thus improving survival rate.

The oogenesis process in several teleost fish species has been proven to be related to photoperiod (Sari et al., 2017). In gonad maturation, environmental cues in the form of photoperiod are considered the most important in influencing endogenous rhythms, thereby triggering spawning in a population at the same time each year. (Elisio et al., 2015). In several studies, photoperiod manipulation was carried out to accelerate the development of fish gonads. On research of Lee et al., (2017), the use of photoperiod treatment with longer light periods can accelerate the oogenesis stage in damselfish. Shahjahan et al., (2020) also studied that photoperiod treatment with a longer dark period was able to increase the maturation of *Labeo Rohita* ovaria. Previously, in Eurasian perch *Perca fluviatilis*, Migaud et al (2004) concluded that seasonal variation in day length appeared to be related to the onset of gametogenesis in Eurasian perch, while the continuous photoperiod inhibited gonadal development. Photoperiodic variations might play an important role in the initiation of spawning in perch. The curruent research, therefor, different photoperiod treatments on the stages of betta fish ovarian development were evaluated.

2. Materials And Methods

Experimental design and sample preparation

Female halfmoon fighting fish (6-7 mos. old) were collected from fish farmers in Beji village, Banyumas Regency, Indonesia. Betta fish were kept for 40-d with lighting period treatment (photoperiod). A completely randomized design (CRD) was applied to examine 3 different treatments in quintuplicates each. The Treatments were P1 (10-h Light L : 14-h Dark D), P2 (14-h L : 10-h D), and P3 (Control, ambient lights).

Preparing histological classic

Sampling Ovaria

Ovaria samples, obtained at final experiment from each treatment, were subjected for histological classic preparations. The samples, after weighing, were then fixed in Bouin's Holland for further process and analysis. After fixation, the samples were subsequently run to histological classic process, i.e. fixation, dehydration, clarification, embedding in paraffin, cutting in 6 µm of thickness, mounting in object glass, clearing from paraffin, staining with H&E, and balm attachment for covering. (Sulistyo, 2008).

Preparation Analysis and Calculation of Oogenesis Stages

Ovarian preparations were observed and counted using a light microscope and documented. Observation of proportion of oocyte development stages were based on Rinchard and Kestemont (1996), and Sulistyo (1998). Each ovarian preparation was observed in quintuplicates field of view, and 20 oocytes were noted from each field of view. Proportion of each stage development oocytes was calculated using the formula according to Santo et al., (2014) as follows:

$$\text{Proportion of stages (\%)} = 100 \times \frac{\sum development stage\ x}{\sum total\ oocytes}$$

Water Quality Monitoring

Water quality monitoring involved temperature, pH and dissolved oxygen. Temperature and pH were daily controlled, while dissolved oxygen measurements were taken at the beginning and the end of experiments.

Data analysis

Data on the proportion of oogenesis stages was presented in the form of pictures and graphs then analyzed descriptively and compared with the literature. To compare the treatments data were previously subjected to ANOVA at 0.05 levels, however they were transformed first in arc-sin**.**

3. Results and Discussion

Proportions of Oogenesis Stages

The research results obtained showed that proportion of oogenesis stages was dominated by 3 stages of oogenesis, namely Oocyte previtellogenin OP, Exogenous vitellogenin oocyte ExO, and fully developing vitellogenin FDV. Where the highest value was obtained in treatment 2 (14L; 10D), since OP was 22.05%, ExO was 22.89%, and FDV was 25.24%. From treatment P1 (10L; 14D) and control,

OP were 20.86% and 19.43%, respectively, ExO were 20.17% and 18.79%, respectively, and FDV were 21.82% and 19.21%, respectively. Proportion of oogenesis stages was presented in Figure 1.

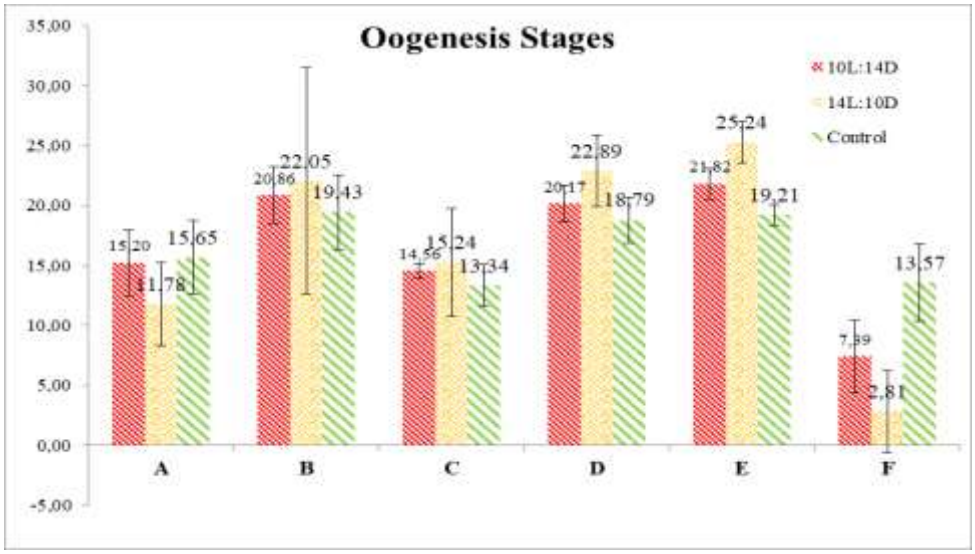

Figure 1. Proportion of oogenesis stages. A (Oogonia); B (Oocyte Previtellogenin); C (Endogenous Vitellogenin Oocyte); D (Exogenous vitellogenin oocyte); E (fully developing vitellogenin oocyte); F (Atretic Oocyte).

Water Quality

Water quality data were presented in Table 1. Temperature varied 27.69 - 27.84°C, pH was 7, and dissolved oxygen was 7.2 mg/L.

Table 1. Water quality during the maintenance process

Parameter	Measurement results	Quality Standards*	Tolerance Range**
Temperature (°C)	27.73±0.35 (P1) 27.84±0.17 (P2) 27.69±0.25 (control)	Deviation 3	24 - 28
pH	7	6 – 9	5.5 - 7.0
Dissolved oxygen (mg/L)	7.2	≥ 6	≥ 5

In fish, histology of gonads was studied to determine the peak fish spawning period, as well as to understand effective methods used to increase brood stock efficiency, and could be adopted to increase fish production (Milton et al., 2018; Insivitawati et al., 2015; Hayati et al., 2023). In our study, ovarian histology of fighting fish was observed to detect oogenesis stages under different photoperiods. In gonadal maturation, photoperiods played an important role (Shahjahan et al., 2020). Photoperiod regulated endogenous rhythms, as well as the synthesis and secretion of sex hormones (Elisio et al., 2015). In fish, light stimulated central nervous system to increase pituitary secretion, which subsequently affected gonadal and oocyte developments (Qiang et al., 2021). Furthermore, ample lighting might boost immune system function, growth, gonad development, food absorption, enzyme activity, and the build-up of lipids and crude protein, all of which served as stressors. (Choi et al., 2023).

The average oogenesis stage in the current study had entered the stage vitellogenin, indicating the impact of photoperiod manipulation on gonad maturation. The oocytes grew larger, spherical cortical vesicles started to form at the cytoplasm's edge, and the quantity and size of yolk vesicles increased during the vitellogenin stage. When an oocyte reached the vitellogenin stage, its density expanded toward the center of the alveolar cortex area, the development of the vitelline membrane took place, and the granular structure that formed in the alveolar cortical phase was larger and the nucleus was asymmetrical in shape. (Koç et al., 2008). The quantity of oocytes reaching the vitellogenin stage signified that the oocytes progressed to the stage preceding ovulation (Nabila et al., 2022). The oocytes passed on the ovary or abdominal cavity through the lumen after ovulation, suggesting that the fish prepared to spawn (Mahdaliana et al., 2015; Carman et al., 2023).). Oocytes developed from oogonia to atresia oocytes during the oogenesis. The process of creating these phases took times. Therefore, the

purpose of applying this photoperiod was to accelerate oogenesis. Ovarian endocrine variables, such as progestins and estrogens, as well as pituitary gonadotropins (GTH) had an impact on oogenesis (Miura et al., 2007; Agustin et al., 2020).

The growth of the ovarian in fighting fish was influenced by the duration of the dark and light lighting phases. In this investigation, treatment 2 proved to be the most effective photoperiod (14L:10D). Three phases — OP, ExO, and FDV — dominated the development of oogenesis in treatment 2. The results in treatment 2 showed that the length of the light period influenced the oogenesis of fighting fish. As demonstrated by results of Lee et al., (2017), that the duration of photoperiod (14L:10D) influenced the damselfish gonad maturation, with the ovaries reached the vitellogenin stage. According to (Hansen et al., 2001; Zhu et al., 2014), lengthy durations of darkness had the effect of delaying ovarian maturation, while lengthy durations of light brought the opposite effect. But according to a number of studies, fish gonad maturation could also be impacted by the duration of the dark period. Further, research of Sulistyo et al (1998), and Shahjahan et al., (2020) explained that as darkness increases, the ovaries of fish such as *Labeo rohita*, *Perca fluviatilis*, *Oreochromis niloticus*, and *Cheirodon interruptus* would mature more. This indicates that fluctuations in the effects of photoperiods were also impacted by the fish's physiological state and the environment in which they resided. The length of the lighting period applied could increase fecundity (El-Sayed & Kawanna, 2004) and stimulated the maturation of fish ovaries (Zhu et al., 2014).

Water quality during the current experiment was also one of the environmental factors influencing the oogenesis. According to Reading et al., (2018), numerous variables, including the environment (photoperiod, temperature, pH, and dissolved oxygen) and endogenous factors (species and hormones), had an impact on oogenesis. Aside from that, other significant factors affecting the stages of oogenesis were fish diet and living environment. Apart from the photoperiod treatments, temperature, pH, and dissolved oxygen presented significant water qualities that might promote the development of the oogenesis phases in this study, and however they were within normal limits to support the life and growth of fighting fish.

4. Conclusion

The stages of oogenesis development were influenced by photoperiod treatments. Treatment 2 exhibits the best results, with a lighting period of 14L and 10D. The stages of oogenesis development were dominated by previtellogenic oocytes, exogenous vitellogenin oocytes, and fully development oocytes. When the ovary of fighting fish reached this value, it indicated that they were mature and prepared for spawning.

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Conflict of interest:

The authors declare that there are no conflicts of interest

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