THE DYNAMIC OF OVARIAN ACTIVITY OF THE HARD-LIPPED BARB (*Osteochilus hasselti* C.V.) UNDER DIFFERENT PHOTOPERIOD REGIMES

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Abstract

Reproductive activity in fish is controlled by several environmental and physiological factors. Many efforts have been directed to understand and use either environmental or physiological factors or a combination of both to increase fish production. One of the major environmental factors involved in cueing reproductive activity is photoperiod. An experimental study has been conducted to evaluate the effect of various photoperiods on ovarian activities in the Hard-lipped Barb (*Osteochilus hasselti* C.V.). Three groups of fish were reared under different photoperiod i.e. 14L:10D, 16L:8D, 18L:6D. The results showed that the gonado-somatic index (GSI) of the 14L:10D.

Key words: Photoperiod, ovarian activity, GSI, 17□-estradiol

Introduction

Reproductive activity in fish is controlled by several environmental and physiological factors (Bromage, 2001). Many efforts have been directed to understand and use either environmental or physiological factors or a combination of both to increase fish production. One of the major environmental factors involved in cueing reproductive activity is photoperiod (El-Naggar 2000). Photoperiod exert it role on reproduction through brain that integrates and conveys input from external and internal cues to the pituitary regulating the synthesis and secretion of gonadotropin (GtHs). The GtHs regulate the two main activities of the gonads i.e. hormone and gamete production (Hansen *et al*., 2001, Rodrigues *et al*., 2004). Ovarian hormone especially estradiol and progesterone play an important role in maintaining and promoting gamete production (Nagahama, 1996; Yaron, 1995).

Photoperiod was recorded as an important cue for the timing of spawning in many fish species, such as sea bream (Devauchelle, 1984), rainbow trout (Duston and Bromage, 1986,1988; Davies *et al*.,1999), sole (Devauchelle *et al*., 1987), turbot (Devauchelle *et al*., 1988), Atlantic salmon (Hansen *et al*., 1992, Endal *et al.*, 2000), striped bass (Blythe *et al*., 1994), Atlantic halibut (Björnsson *et al*., 1994, 1998), sea bass (Devauchelle and Coves, 1988; Carillo *et al*., 1989; Mananos *et al*., 1997; Bayarri *et al*., 2004), thin lipped grey mullet (El-Greisy, 1993), Nile tilapia (Ridha and Cruz, 2000), Arctic char (Frantzena *et al*., 2004) or Atlantic cod (Norberg *et al*., 2004).

The majority of studies were conducted on temperate-zone fishes in which photoperiod strictly differ between seasons. Studies on influence of photoperiod on tropical fishes are still limited. It is interesting to examine whether photoperiod will induce a similar respond in tropical fishes as the case for its temperate-zone counterpart. In this study, Hard-lipped Barb (*Osteochilus hasselti* C.V.) was used as a model since this fish is an indigenous tropical fish and widely cultured in Java (Soeminto, 2004). Therefore studies to improve understanding

of its biological aspect will be benefit not only for research per se but also for the fish farmers.

Hard-lipped Barb is a synchronous batch spawner fish (Wijayanti *et al.*, 2005) capable of spawning several time during the peak of the spawning period. Under a suitable environmental setting, this fish capable to spawn in 60 days after the previous spawning. Hard-lipped Barb has been adapted to a photoperiod of 12L:12D to 14L:10D. The present study examined the effect of different photoperiods on ovarian activity of the Hard-lipped Barb. Special attention was given on, gonado-somatic index (GSI), the proportion of oocytes at various developmental stages and serum concentration of 17β-estradiol.

Material and Method

This study was carried out at the Laboratory of Animal Structure and Development, Faculty of Biology, Jenderal Soedirman University, Purwokerto, Indonesia. Sixty sexually mature female Hard-lipped Barb weighing 100 g in average were purchased from fish farmer in Banyumas Regency. The fish were acclimated at the laboratory for one week then were induced with GnRH analogue (Ovaprim, Syndel Lab., Vancouver, Canada) of 0.5mLxkg^{-1} body weight to spawn. The day of spawning was designated as day 0 post spawned period. The post spawned fish were placed in aquaria of 45 L in volume with density of 3 individuals. Three experimental groups were exposed to photoperiod of 14L:10D, 16L:8D and 18L:6D, as treatments, for 8 weeks. Three aquaria were provided for each experimental group.

Each aquarium was covered by a black light-proof poly bag plastic and lighting was provided by 25 Watt bulb giving a light intensity of approximately 1300 lux at the water surface. Photoperiod was controlled by automatic timer without twilight periods; the light was started at 06.00 am.

The fish were fed commercial dry feed (Sakura) containing 35% protein and 19% lipid, as much as 3% of body weight, twice a day at 08.00 and 17.00. Unconsumed food and waste was siphoned daily between 15.00 and 16.00.

During the experimental period, water dissolve oxygen, CO_2, pH and temperature ranged between 3.5-4.76 ppm, 3.64-4.66 ppm, 6.25-6.75, and 28-28.25 ^oC, respectively.

Data were collected every two weeks starting at week 0 (the starting point of the experiment). On each data collection time, the fish were weighed, bled then were killed by decapitation. Ovaries were dissected out and weighed for GSI calculation (GSI = ovarian weight x total body $weight^{-1}$).

The ovaries were subsequently fixed in neutral buffered formalin for 48 hours and were processed for histological examination using standard paraffin method, cross sectioned at 6 µm and stained with Mayer's haematoxylin and aqueous eosin. The sections were examined under light microscope.

The stages of oocyte development were determined according to (Wijayanti *et al*., 2005). Fifteen sections of each ovary were observed to determine the proportion of oocyte. Proportion of oocyte at a particular stage (O*x*) was calculated by applying the following equation:

$$\text{Proportion of } O_x = \frac{\sum \text{oocytes of x stage}}{\sum \text{observed oocytes}} \times 100\%$$

At the sampling points, 1mL blood was taken from caudal vein of each female. The blood was allowed to clot at room temperature for 15 minutes then was kept overnight at 8°C to optimise clotting. The serum was aspirated by centrifugation at 1,000 g for 10 minutes then was kept at -20°C until further analysis. 200 µL of serum from each sample was used for the assay to determine concentrations of 17β-estradiol using minividas ELISA applying 17β-estradiol Kit (REF 30431, BioMérieux, France) according to Wijayanti and Soeminto (2007). The assay was conducted at Bina Husada Laboratory Purwokerto.

The quantitative data were expressed as mean ± SD. Statistical analysis was performed using two ways ANOVA (p≤0.05) of Minitab version 13 for Windows. Consequently, GSI and the proportion of oocyte were transformed using arcsin transformation. GSI and serum estradiol-17β level and proportion of late vitellogenic oocytes were statistically correlated.

Result and Discusion

The GSI values varied at different photoperiod and time. The GSI values at the starting period were 2.12±0.43% and GSI value at the end of the experiment were ranging from 4.70±0.78% to 7.3±0.85%. The highest GSI value was observed in 14L:10D followed by 18L:6D and 16L:8D (Figure 1), however, their mean GSI values were statistically not significant (p>0.05). The GSI value in this study was lower than previous study (Soeminto *et a*l., 1995) in which GSI value of 40 days post spawned Hard-lipped Barb achieved >16%. This difference could be resulted from a different rearing management. In previous study (?), the fish were kept in a pond while in this study the fish were kept in aquaria. Despite of fulfilment of food requirement and water quality parameters the aquaria might did not provide natural food as that of pond.

Studies in teleost showed that the increase of GSI value during reproductive cycle is mainly resulted from the increase of oocyte diameter due to accumulation of yolk protein during vitellogenesis (Nagahama, 1994; Yaron, 1995). A positive correlation between GSI value and the proportion of vitellogenic oocytes was also identified in this study. Evaluation on ovarian histology showed that the proportion of previtellogenic oocyte slightly decreased throughout the experimental period. The proportion of early vitellogenic oocytes was relatively constant except on 16L:8D group which increased on week 4 then decreased on weeks 8. The proportion of mid and late vitellogenic oocyte of all experimental groups were relatively constant up to week 4 post-spawning but increased on week 8 post-spawning (Figure 3). The increased in proportion of late vitellogenic oocytes was higher in 16L:8D groups compared to 14L:10D and 18L:6D. There was a positive correlation between GSI and the proportion of late vitellogenic oocyte in all treatments (r= 0.6798, r=0.7361 and r=0.9344 for 14L:10D, 16L:8D and 18L:6D, respectively).

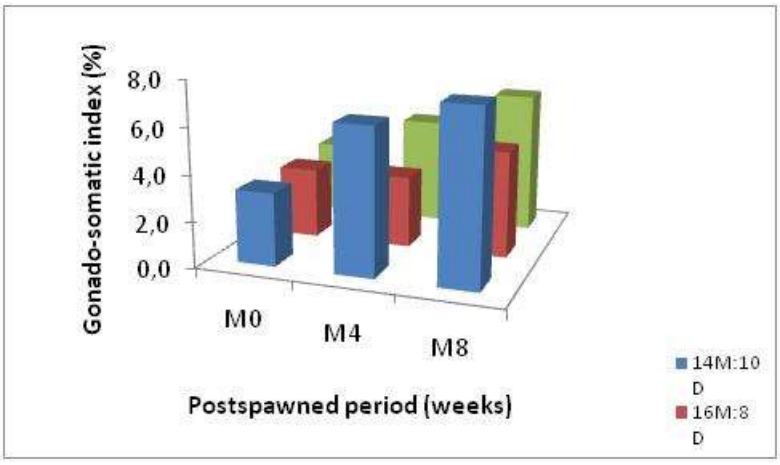

Figure 1. GSI value of female Hard-lipped Barb kept at 14L:10D, 16L:8D and 18L:6D for 8 weeks

Vitellogenesis in the ovary requires vitellogenin, the precursor of yolk protein. This protein is produced in the liver under estradiol stimulation (Arukwe and Goksøyr. 2003; Utoh

at al., 2003). In this study, average serum Estradiol-17β level varied according to time and photoperiod. Serum Estradiol-17β level at the beginning of the experiment was 111.32±25.11 ng.mL^{-1} while at the end of experimental period was 160.89±90.57 ng.mL^{-1} to 301.86±77.42 ng.mL^{-1} (Figure 3). Pattern of fluctuation of Serum Estradiol-17β level in 14L:10D and 16L:8D groups were similar in which the serum estradiol-17β slightly but steadily increased throughout the experimental period; meanwhile serum estradiol-17β level of 18L:6D group increased up to 262.58±83.11 ng.mL^{-1} on weeks 4 post-spawned then decreased to 160.89±90.57 ng.mL^{-1} at the end of the experiment. The variation of serum estradiol-17β levels of 14L:10D and 16L:8D groups were highly correlated to GSI value (r=0.79 and r=0.93 respectively). The correlation was disrupted on 18L:6D group (r=0.083).

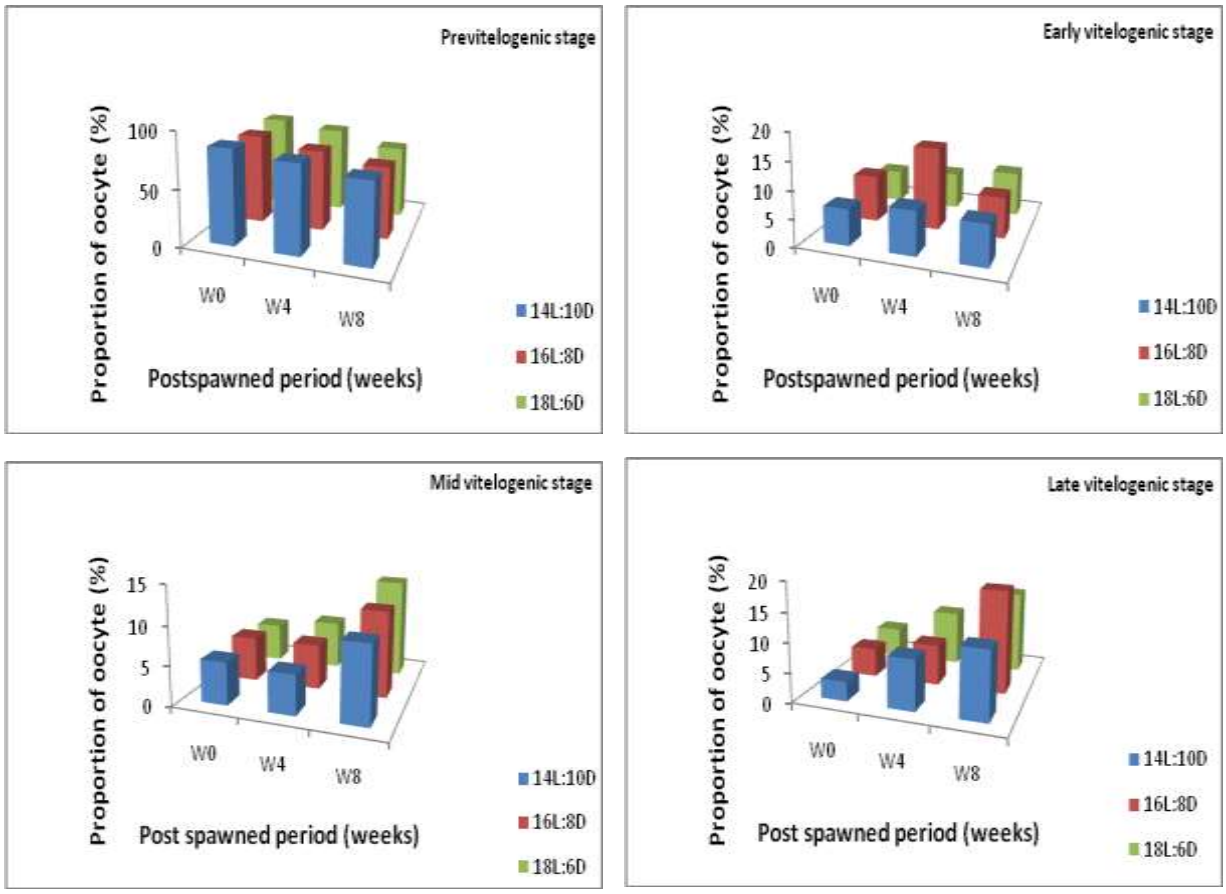

Figure 2 The proportion of oocyte at various developmental stages of Hard-lipped Barb kept at 14L:10D, 16L:8D and 18L:6D for 8 weeks

The effect of photoperiod on ovarian activity of the Hard-lipped Barb as indicated by GSI value, serum estradiol-17β levels and proportion of late vitellogenic oocyte was not significantly seen in this study. Several factors might contribute to this condition, one of them is temperature. A study on *Oreochromis niloticus* showed that photoperiod combined with higher temperature increased GSI, hepatosomatic index, and ova diameter meanwhile the same photoperiod in combination with lower did not produced similar effect (El-Hakim *et al*., 2005).

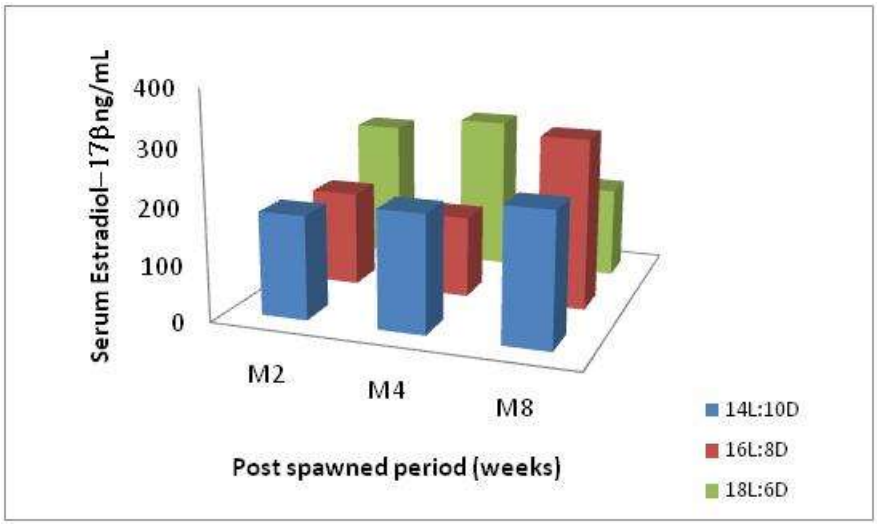

Figure 3. Serum estradiol-17□ of the female Hard-lipped Barb kept at 14L:10D, 16L:8D and 18L:6D for 8 weeks

Other possibility might contributed to the results is time of exposure to extended photoperiod. Exposure to 15L:9D for 90 days to *O. niloticus* produced more significant increased in GSI compared to the same photoperiod for 60 days (El-Hakim *et al*., 2005). Altering photoperiod to *O. niloticus* did not induce any significant effect on reproductive parameter in the first and second reproductive cycles but it did stop reproductive activities after third cycle (Biswas *et al*., 2005). A similar result was also reported in Indian carp, *Catla catla* in which altering photoperiod regime has no effect on reproductive activity at the first cycle (Maitra *et al*., 2005). These results suggested that a certain particular time of exposure is needed for photoperiod to exert it effect on reproductive activities. In this study, the post-spawned Hard-lipped Barb was exposed to longer photoperiod for 8 weeks that equivalent to one reproductive cycle (Djajasewaka, *et al*., 2005, 2007). It would be interesting to evaluate whether extending exposure to long photoperiod could stimulate ovarian activities in the Hard-lipped Barb. Further studies are needed to elucidate the effect of longer photoperiod on reproductive activity in tropical fish and the mechanism involve in the responses.

Conclusion

Based on gametogenetic and steroidogenic activities of the Hard-lipped Barb it was concluded that extension of daily photoperiod up to 18L:6D for 8 weeks was not sufficient to stimulate ovarian activities.

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