The Dynamic Or Testicular Activity Of The Hard-Lipped Barb (*Osteochilus Hasselti* C.V.) Under Extended Photoperiod

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Abstract

Hormonal manipulation has been widely used in aquaculture to promote fish reproduction. However some concern has been raised in accordance to it effect of human health as well as environmental issues. Therefore alternative approaches in promoting fish reproduction need to be continuously studied. Photoperiod manipulation offers a favorable alternative since it is one of the most crucial environmental cues necessary for fish reproduction. An experimental study has been conducted to evaluate the effect of various photoperiods on testicular activities in the hard-lipped barb (*Osteochilus hasselti* C.V.). Four groups of fish were reared under different photoperiod i.e. 14L:10D, 16L:8D, 18L:6D and the control group were reared under natural photoperiod for eight weeks period. Forth nightly three fish of each group were stripped to aspirate milt for measurement of milt volume and sperm concentration, they were decapitated for gonado-somatic index calculation and spermatogenic activities. The results showed that the gonado somatic index (GSI) of the control group, 14L:10D and 18L:6D increased throughout the experimental period, meanwhile GSI of the fish reared under 16L:8D increased up to 4th week then decreased until the end of the study. The fluctuation of the GSI values was followed by the fluctuation of milt volumes and sperm concentration. This can be concluded that rising daily photoperiod up to 16 hours could improve reproduction in male hard-lipped barb.

Key words: photoperiod, spermatogenesis, *Osteochilus hasselti*

Introduction

Hormonal manipulation has been widely used in aquaculture to promote fish reproduction. The most frequently hormones used are GnRH or LHRH analog, gonadotropin analog and pituitary extract (Nagahama 1994). The hormones had been used to induce spawning when environmental cues were insufficient to stimulate spontaneous spawning. Such hormones had also been used to shorten the reproductive cycle (Soeminto, 2004). However, some concern has been raised in accordance to their effect of human health as well as environmental issues. Therefore alternative approaches in promoting fish reproduction need to be continuously studied. Photoperiod manipulation offers a favorable alternative since it is one of the most crucial environmental cues necessary for fish reproduction (Norberg *et al.*, 2004).

Many temperate-zone fishes such as Atlantic cod (*Gadus morhua*) spawn at a specific period of the year (Brander, 1994). This event is mainly controlled by photoperiod resulting in synchronous spawning within each population at approximately the same time evry year (Norberg *et al.*, 2004). The mechanism underlying this process is that brain 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 gamet production. In the male teleost production of testosterone and 11-ketotestosterone (11-KT) are elevated during spermatogenesis (Nagahama 1994; Norberg *et al.*, 2004).

The majority of studies were conducted on temperate-zone fishes in which photoperid is strictly differ from season to season. Studies on influence of photoperiod on tropical fishes is 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, the hard-lipped barb (*Osteochilus hasselti* C.V.) was used as a model since this

fish is an indogenous tropical fish and widely cultured in Jawa (Soeminto, 2004). Therefore studies to get a better understanding of its biological aspect will be benefit not only for reasearch perse but also for the fish farmers.

The hard-lipped barb is a synchronous batch spawner fish (Wijayanti *et al*., 2005) capable of spawning several times during the peak spawning period. Under a suitable environmental setting, this fish capable to pawned in 60 days after the previous spawning. Based on fluctation of milt production and sperm concentration, male hard-lipped barb could spawn more frequently then the female as optimal milt volume and sperm concentration can be obtained by stripping every 16 days (Wijayanti and Sugiharto, 2006). As a tropical fish, the hard-lipped barb, has been adapted to a photoperiod of 12L:12D to 14L:10D. The present study examined the effect of various photoperiod up to 18L:6D on testicular activity of the hard-lipped barb. Special attention was given to milt volume, sperm concentration and gonado-somatic index (GSI). Histological evaluation of the testis was performed to examine the dynamic of spermatogenic activities.

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 male hard-lipped barb weighted of 100g in average were purchased from local fish farmer. Upon one week aclimatisation, they were induced to spawned using 0.5ml.kg^{-1} BW GnRH analog (Ovaprim, Syndel Laboratory, Vancouver, Canada). The day of spawning was considered as day 0 of post-spawned period.The post spawned fish were placed in aquaria with density of 3 fish/45L. Three aquaria were provided for each experimental group.

Each aquarium was shield by a black light-proof poly bag plastic and lighting was provided by 25Watt bulb giving a light intensity of approximately 1300 lux at the water surface. Photoperiod was controlled by automatic timer without twilight period, the light was started at 06.00. Three experimental groups were exposed to photoperiod of 14L:10D, 16L:8D and 18L:6D for 8 weeks, while the control group was exposed to natural photoperiod.

The fish were fed commercial dry feed with nutritional value of 35% protein and 19% lipid. Food as much as 3% of fish body weight was offered at 08.00 and 17.00. Unconsumed food and waste was siphoned daily between 5.00 and 16.00. Water quality was monitored by measuring dissolve oxygen, CO_2, pH and temperature.

Data was collected every two weeks starting at week 0 (the starting point of the experiment). At each data collection time, the fish were weight, stripped to collect the milt then were killed by decapitation. Milt volume was measured using a graded spuit and sperm concentration was counted using haemocytometer. Testes were dissected out and weight for GSI measurement (GSI = testes weight x total body weight^{-1}). The testes were subsequently fixed in neutral buffered formalin for 48 hours and processed for histological examination using standard paraffin method, cross sectioned at 6μm then were stained with haematoxylin and eosin. The sections were examined under light microscope with 100x objective and spermatogenic stages were determined according to Hibiya and Takashima (1985). Proportion of each spermatogenic stage (Sp_x) was calculated by applying the following equation:

$$\text{Proportion of } Sp_x = \frac{\sum \text{spermatogenic of x stage}}{\sum \text{observed spermatogenic cells}} \times 100\%$$

GSI, sperm volume, sperm concentration and proportion of lobules containing particular spermatogenic stage were analyzed using two-ways ANOVA.

Result and Discussion

The GSI of the control and the treated groups were fluctuated throughout the experimental period. The highest GSI value was found in the 8th week even though statistically not significant (p=0.05) (Table 1.). The range of GSI value was considered normal. Previous finding showed that under laboratory condition GSI value of male hard-lipped bard were 2,45±0,15% on day 0 post-spawning then increased to 6,63±0,85% on day 16 and 6,54±1,01% on day 32 post-spawning and subsequently decrease up to 0,08±0,13 on day 64 post-spawning (Wijayanti and Sugiharto, 2006). Under natural condition, GSI value increase as spermatogenesis proceed and reach the highest value prior to spawn (Guraya 1994, Nagahama, 1994).

This study suggested that exposure to photoperiod up to 18L:6D for 8 weeks has no significant effect on GSI of hard-lipped barb. This result was in the contrary to that in Indian carp (*Catla catla*) in which exposure to photoperiod of 16L:8D stimulate gonadal maturation (Bhattacharryya *et al*., 2005). It is possible that 8 weeks exposure to extended photoperiod insufficient to promote testicular development. Experiment of Biswas *et al.* (2005) showed that exposure of 6L:6D, 12L:12D and 14L:10D photoperiod to *Oreochromis niloticus* induced significant effect after three spawning cycles. It would be interesting to evaluate the effect of extended photoperiod for a longer time.

Table 1. SGI of post-pawned hard-lipped barb exposed to extended photoperiod for 8 weeks

Photoperiod	Experimental period (week)				
	0	2	4	6	8
Control	4.15±2.86	6.02±3.43	3.80±2.23	2.76±0.89	4.29±2.44
14L:10D	3.21±0.69	3.44±0.93	3.27±1.59	3.26±1.39	4.89±0.62
16L:8D	3.57±0.90	6.60±4.64	7.19±2.50	5.78±2.13	4.28±2.64
18L:6D	6.02±3.43	5.61±2.84	6.87±3.74	4.56±0.73	8.32±1.56

Even though the GSI value was not affected by tested photoperiod, milt volume and sperm concentration suggested that photoperiod of 16L:8D stimulate spermiation in the hard-lipped barb. This is supported by histological features showing large proportion of testicular lobules containing spermatozoa. Milt volume at the starting point of the experiment was ranging from 0.083±0.029 to 0.567±0.703mL (Figure 1), no significant different was observed between experimental groups. At the second week, fish exposed to 16L:8D produce the highest milt volume.

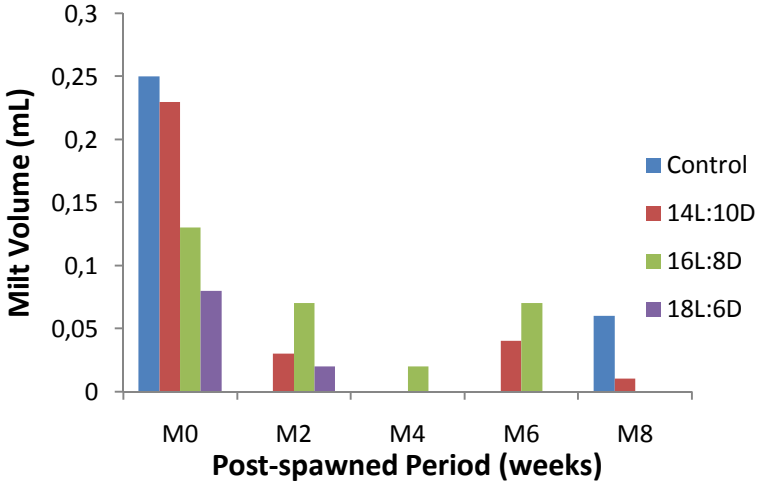

Figure 1. Milt volume of post-spawned hard-lipped barb exposed to extended photoperiod for 8 weeks

At the forth week milt was obtained only from fish exposed to 16L:8D. At the sixth week a similar pattern to that of the second week was observed. At the eighth week, milt was obtained only from control group and those exposed to 14L:10D.

Sperm concentration at the initial stage of the experiment was not significantly different amongst the experimental groups (p<0.05). From the second week to the sixth week, the highest sperm concentration was obtained from fish exposed to 14L:10D and 16L:8D while at the eighth week the highest sperm concentration was obtained from fish exposed to 14L:10D (Figure 2).

Histological features showed that the testes contained lobules with all spermatogenic stages. Lobules containing spermatogonia were characterised by narrow cyste enclosing a number of large cells with lighter stain compare to other spermatogenic cells.

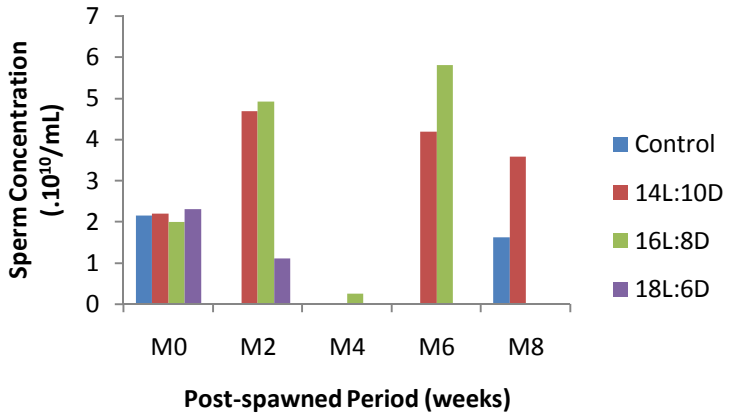

Figure 2. Sperm concentration of post-spawned hard-lipped barb exposed to extended photoperiod for 8 weeks

Table 2. Proportion of testicular lobules containing spermatogonia, spermatocyes, spermatids and spermatozoa of male nilem exposed to various photoperiod

Spermatogenic stages	Photo-period	Experimental period (week)				
		0	2	4	6	8
Spermatogonia (%)	Control	0.98±1.26	0.91±1.41	0.78±0.53	0.71±0.61	3.20±1.58
	14L:10D	0.64±0.71	0.24±0.31	0.24±0.17	0.50±0.31	0.32±0.18
	16L:8D	1.96±1.47	0.72±1.07	0.71±0.11	1.02±0.49	0.17±0.07
	18L:6D	0.58±0.76	0.27±0.23	0.64±0.39	0.68±0.52	0.12±0.08
Spermatocytes (%)	Control	11.81±12.52	2.68±1.59	0.95±0.89	4.09±6.58	12.72±14.69
	14L:10D	7.47±5.31	0.63±0.59	1.05±1.25	6.23±0.62	11.01±6.89
	16L:8D	14.21±12.18	1.83±1.78	10.17±3.25	12.46±3.81	1.47±2.05
	18L:6D	13.99±16.92	2.93±1.81	2.97±1.46	0.97±0.61	3.75±1.22
Spermatids (%)	Control	18.86±25.11	1.17±0.38	1.79±1.72	2.78±3.76	5.50±5.63
	14L:10D	24.67±23.84	0.87±0.23	1.26±0.94	4.38±0.99	9.82±3.43
	16L:8D	11.96±13.21	1.79±2.45	2.92±2.33	9.96±6.64	0.94±1.31
	18L:6D	5.24±0.19	1.94±0.76	1.87±1.36	2.57±1.91	1.64±0.45
Spermatozoa (%)	Control	68.71±27.72	96.18±2.67	98.93±0.91	92.93±10.27	79.40±22.77
	14L:10D	67.97±29.02	98.50±0.01	97.37±2.51	88.90±1.67	81.37±12.42
	16L:8D	72.67±20.32	96.18±4.46	86.31±4.24	76.58±4.16*	97.59±3.43
	18L:6D	78.56±20.79	95.00±2.97	95.41±1.23	97.27±0.32	95.22±1.49

The proportion of testicular lobules containing spermatogonia was not significantly different amongst groups throughout the experimental period (p>0.05). Lobules containing

spermatocytes were characterised by cyste containing larger cell compared to spermatogonia with bigger nucleus. The proportion of testicular lobules containing spermatocytes was not significantly different amongst experimental groups up to second week of exposure (p>0.05). On the forth and sixth week, the proportion of lobules contaioning spermatocytes of the 16L:8D group was significantly higher to other experimental groups and control (p<0.05), while on the eighth week it slightly lower than other groups (p>0.05). The proportion of lubules containing spermatids were not significantly different amongst all groups throughout the experimental period (p<0.05). The lubules containing spermatozoa were characterised by large dictinctive irregular lubules containing homogenous tipe of cell. The proportion of lubules containing spermatozoa of all experimental groups increased on the second week but were not significantly different amongst them. In the forth and sixth week, however, the proportion of lubules containing spermatozoa was significantly lower in 16L:8D group compared to other groups (p<0.05). In the eighth week, the proportion of lobules containing spermatozoa in 16L:8D and 18L:6D were higher than control and 14L:10D groups (p<0.05).

The high proportion of lobules containing spermatozoa indicating that the testes of all groups were actively underwent spermatogenesis. The dynamic of spermatogenesis was in line to the gonadotrophin secretion from the pituitary (Wijayanti and Soeminto, 2007). Under natural photoperiod, 14L:10D and 16L:8D, serum gonadotropin of male hard-lipped barb steadily increased up to eight weeks post-spawning; while under 18L:6D serum gonadotrophin sharply increased on the second week post-spawning then slowly increased up to eight weeks post-spawning (Wijayanti and Soeminto, 2007). The acceleration of gonadotrophin increased in 18L:6D groups could be resulted from the reduction of melatonin secretion. Studies in some teleost species showed that long photoperiod inhibit the secretion of melatonin which allows increase in gonadotrophin production (Amano *et al*., 2001; Bayarri *et al*., 2004; Migaud *et al*., 2006). It was likely that the increased of gonadotropin did not sufficient to induce hydration in the testis which is necessary for milt production (Peter dan Yu, 1997) even though the spermatozoa production was increased. Another possibility is that extended photoperiod in some degree induced stress which resulted in an increased of cortisol secretion. Studies in male brown trout, *Salmo trutta* L., and tilapia, *Oreochromis mossambicus,* showed that increase of cortisol suppressed plasma level of testosterone in mature male (Pickering *et al*., 1987; Foo and Lam, 1993). It would be interesting to study the effect of extended photoperiod on cortisol level in hard-lipped barb.

Conclusion

In conclusion, the exposure of male hard-lipped barb to photoperiod of 16L:8D for 8 weeks stimulate spermiation but has less prominent effect on GSI and milt production.

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