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The Effect of Day Length and Lunar Phases on the Spawning Activity in the Nile Tilapia *Oreochromis niloticus*

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ABSTRACT : Nile tilapia (*Oreochromis niloticus*) are mouth-brooders so that the females holding eggs in their mouth sacrifice their somatic growth for reproduction. For this reason, artificial control of reproduction for the culture of this species has been of interest. Manipulation of photoperiod is an emerging technique for such purpose, but little information is available to establish appropriate photoperiod regime. To obtain necessary basic information, sexually mature females were individually accommodated to glass aquarium, and the spawning activity of these females were monitored for two years under natural photoperiod regime. Female tilapia spawned most frequently on March, April and May when the day length gradually increased from 11 hours to 14 hours and least frequently on September, October, November and December when the day length gradually decreased from 13 hours to less than 10 hours in the first year. The decrease of spawning frequency as day length decreased was also observed in the second year, although the increase of spawning frequency as day length increased was less clear. Spawning of female tilapia was less active when the night was dark due to the disappearance of moonlight (Dark Phase), compared to the Phase of Getting Lighter, Light Phase and Phase of Getting Darker. Results from this study suggest that long day length, particularly increasing phase, is favoured for active spawning of Nile tilapia, and that this species, as a tropical fish species, may utilize changing lunar phases as a secondary environmental cue for reproduction.

Key words : Photoperiod, Moonlight, Lunar phase, Spawning, Reproduction, Tilapia, *Oreochromis niloticus*.

INTRODUCTION

Animals persue to deliver their genetic information to the next generation through successful reproduction. In some cases, however, such reproductive successes are not welcomed. In the field of fish culture, precocious sexual maturation and reproduction cause many problems, including growth retardation, deterioration of flesh quality and changes in body color (McAndrew, 1993; Bromage et al., 2001).

Nile tilapia (*Oreochromis niloticus*) are mouth-brooders so that the females holding eggs in their mouth could not take food properly. They just sacrifice their somatic growth for reproduction. Various different techniques have been employed to overcome this biological constraint in tilapia farming including hormonal sex reversal (Kwon et al., 2000), temperature-induced sex reversal (Kwon et al., 2002), hybridization, high stocking density, sterilization, selection and use of YY male broodstock (Longalong et al., 1999). Manipulation of photoperiod have been found to be influential on many physiological functions in fish species such as growth, reproduction and gonadal maturation, and are now widely used in aquaculture to alter spawning season, manipulate maturation and stimulate growth (Boeuf & Le Bail, 1999; Bromage et al., 2001; Randall et al., 2001; Rodriguez et al., 2001; Biswas et al., 2005). Long photoperiod regimes have been used in Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), Atlantic halibut (*Hippoglossus hippoglossus*), sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) to enhance growth and/or to delay sexual maturity (Boeuf & Le Bail, 1999; Oppedal et al., 1999; Simensen et al., 2000; Randall et al., 2001; Rodriguez et al., 2001; Gines et al., 2004).

Nile tilapia has been originated from Egypt which belongs to tropic to sub-tropical area. Bromage et al. (2001) suggested that reproduction of many sub-tropical and tropical species

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could also be cued by photoperiod. Studies carried out by Ridha and Cruz (2000) and Campos-Mendoza et al. (2004) demonstrate that reproductive performance and seed production in tilapia species (*O. niloticus* and *O. spilurus*) are influenced by photoperiod manipulation. Thus, photoperiod manipulation could be developed into a useful tool for the control of reproduction in tilapia. However, a lot more information is required to increase our understanding on the reproductive process of this species with regards to photic conditions since the results from different researchers are inconsistent. Rad et al. (2006) suggested, based on their results, that long day or continuous artificial lighting could be influential on enhancing somatic growth and delaying gonadal development in Nile tilapia during fingerling stage. Contrary to this, Campos-Mendoza et al. (2004) found long day length could improve some important reproductive traits in the same species. Biswas et al. (2005) suggested that reproductive activities in this species can be paused by photoperiod manipulation (by reducing the length of both day and night). The data from all of these studies were produced for less than 6 month period.

To identify the cause of such discrepancies and find effective photic conditions for the control of reproduction, spawning activity of this species is required to be monitored for longer period. In this study, we have monitored the spawning activity for two years and studied the effect of day length and lunar phases on the spawning frequency in female Nile tilapia *O. niloticus*.

MATERIALS AND METHODS

Fish (Nile tilapia, *O. niloticus*) were obtained from a local fish farm (Chungju, Chung Buk in Korea) and acclimatized in fish rearing facilities in Sunmoon University (Asan, Chung Nam in Korea) for a month before the initiation of monitoring fish spawn. To monitor the spawning of fish, sexually mature females (400-800 g in body weight per fish) were individually accommodated to two recirculating fish culture systems that were made of partitioned glass

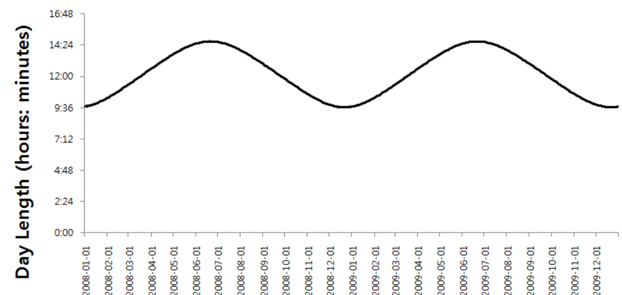

Fig. 1. Changes of regional day length during the monitoring of spawning activity in Chung Nam, Korea. Day length were calculated by utilizing the time of sunrise and set in the region.

aquaria, filtering units (biofilters and sedimentation chambers) and reservoirs. The number of female fish monitored was 8 in 2008 and 19 in 2009. The monitoring of spawning activity began from January, 2008 and continued for two years until the end of December, 2009. Female fish were individually checked everyday for the presence of eggs in their mouths, and the eggs were removed when found. The date of spawning and the identification number of the fish were recorded and kept until analysis. For the two years of monitoring, fish were fed daily with commercial freshwater fish pellets. Water temperature was kept constant at 27±1℃ throughout the whole monitoring period with heaters that were equipped with sensors and automatic controllers.

Natural photoperiod regime was subjected to the fish under the monitoring for the two years (Fig. 1). Changes of regional day length were calculated as follows by utilizing the open data from Korea Astronomy and Space Science Institute (KASI, http://astro.kasi.re.kr).

Regional Day Length =
The time of regional sunset − The time of regional sunrise

The effect of moonlight on spawning activity was estimated by investigating the frequency of spawning at different lunar phases. For this, the date of spawning based on solar calendar was converted into the date of lunar calendar (Day 1 - Day 30). Lunar phase was categorized into four groups as Dark phase (Day 1, 2, 3 and Day 27, 28, 29, 30), the Phase of Getting Lighter (Day 4, 5, 6, 7, 8, 9, 10, 11), Light phase (Day 12, 13, 14, 15, 16, 17, 18) and the Phase of Getting Darker (Day 19, 20, 21, 22, 23, 24, 25, 26). The spawning frequencies at each lunar date within each lunar phase were put together and compared with the frequencies of other lunar phases. At this, to equalize the number of date in the four lunar phases (to make them 7 days each), the frequency data of Day 4 and 11 were put together and then divided by 2 before applying to the Phase of Getting Lighter, and the data of Day 19 and 26 were also processed in the same way for the Phase of Getting Darker.

RESULTS

1. Effect of Day Length Changes on the Spawning Activity of Tilapia

The annual cycle of day length changes in the region where the monitoring had been taken place for the two years (2008 and 2009) was shown in Fig. 1. In this region, the longest day length was 14 hours 42 minutes (June 20, 2008) and 14 hours 41 minutes (June 17-24, 2009), respectively, and the shortest day length was 9 hours and 38 minutes (December 22, 2008 & 2009). Female tilapia spawned most frequently on March, April and May when the day length gradually increased from 11 hours to 14 hours and least frequently on September, October, November and December when the day length gradually decreased from 13 hours to less than 10 hours in 2008 (Fig. 2). The decrease of spawning frequency as day length decreased was also observed in 2009, although the increase of spawning as day length increased was less clear (Fig. 2).

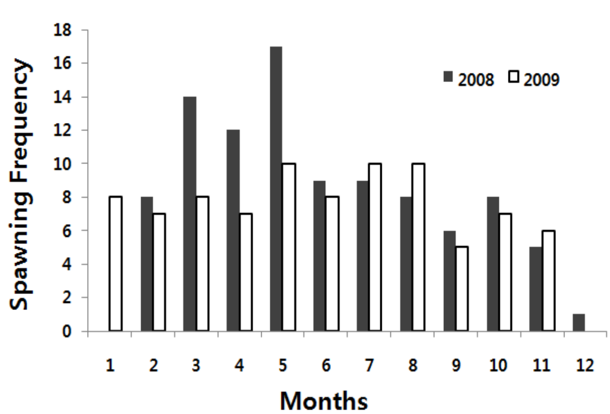

Fig. 2. Monthly changes of spawning frequency in Nile tilapia for two years. Number of mature female fish monitored was 8 in 2008 and 19 in 2009.

2. Effect of Lunar Phases on the Spawning Activity of Tilapia

Spawning of female tilapia was less active when the night was dark due to the disappearance of moonlight (Fig. 3). The spawning took place 25.5 times at the Phase of Getting Lighter, 24 times at Light Phase, 22.5 times at the Phase of Getting Darker and 19 times at Dark Phase in 2008. The difference became more dramatic in 2009 when the females spawned 20.5 times at the Phase of Getting Lighter, 24 times at Light Phase, 21 times at the Phase of Getting Darker and only 11 times at Dark Phase. Putting the data from two years still shows clear difference between the spawning frequency at Dark Phase and those of other lunar phases (Fig. 3).

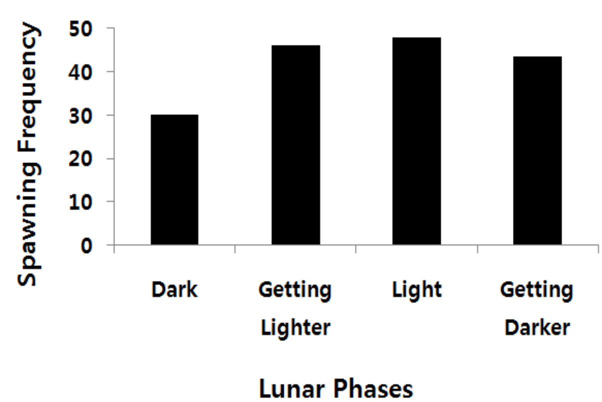

Fig. 3. Spawning frequency of Nile tilapia at different lunar phases. Days in lunar calendar were divided into 4 phases (Dark, Getting Lighter, Light, Getting Darker) depending on the status of moon. Spawning frequencies at each lunar phase were put together and then compared to each other. See Materials and Methods for details.

DISCUSSION

The spawning activity of Nile tilapia was affected by both changing day length and lunar phases in this study. In a number of fish species, gonadal development and final maturation are known to be associated with photic information (Boeuf & Le Bail, 1999; Oppedal et al., 1999; Simensen et al., 2000; Randall et al., 2001; Rodriguez et al., 2001; Gines et al., 2004). The accumulated data from previous studies suggest that fish reproduction is largely dependent on the annual changes of photoperiod (Bromage et al., 2001). However, fish with different ecological niche may require different and more complicated photic information for the reproductive process.

Long day length appears to enhance some important reproductive traits (eg., total fecundity, spawning periodicity and spawning synchrony) in Nile tilapia (Ridha and Cruz, 2000; Campos-Mendoza et al., 2004). Ridha et al. (1998) also found that tilapia *O. spilurus* produced significantly more seeds under 14 hour-day length than did under 13 hour-day length. Considering the fact that Ezypt, where Nile tilapia was originated, has distinct 4 seasons, it is logical to postulate that physiological functions including reproductive process in this species has been adapted to the annual cycle of day length change. Results from our study here also support such postulation and the findings of Ridha et al. (1998), Ridha and Cruz (2000) Campos-Mendoza et al. (2004). It seems quite obvious that photoperiod is one of the most strong environmental regulator of reproduction in tilapia, and that the spawning frequency is in favour of long day length.

However, other previous works introduce some complication in understanding the photic response of tilapia reproduction. Biswas et al. (2005) could not find any significant differences in reproductive performance between 14 hour-day length and 12 hour-day length, although they found short day (6 hour-day length) suppressed reproductive activities in Nile tilapia. Rad et al. (2006) came up with even more confusing results. They found that long day or continuous artificial lighting could delay gonadal development in Nile tilapia during fingerling stage. Authors of these works tried to explain the cause of the discrepancies on the base of the differences in species, life stages and light intensities that were employed to the respective previous studies.

Most tilapia species were originally distributed to tropic and sub-tropic areas where the change of day length was mild or insignificant. Fish that lives in such areas might have different environmental cues for the control of their reproduction. Golden rabbitfish *Siganus guttatus*, a well known tropical fish, spawns at a specific lunar phase (Takemura et al., 2004). According to Thresher (1984), many other tropical reef fishes such as Acanthuridae, Apogonidae, Balistidae, Blenniidae, Carangidae, Chaetodontidae, Epinephelinae, Labridae, Lutjanidae, Mugilidae, Mullidae, Opistognathidae, Pomacentridae, Pseudochromoids, Pteroidae, Scaridae, Siganidae, Sphyraenidae and Sparidae exhibit moon-related reproductive patterns. In the present study, we also found that the spawning of female Nile tilapia were less active at Dark Phase, indicating a possible association of reproduction with moonlight change.

In support of this, Martinez-Chavez and Migaud (2009) reported that eyes (for retinal perception of light) were necessary to maintain night time melatonin production in Nile tilapia. When both eyes were completely removed by surgery, the pineal glands did not produce any melatonin. In this respect, tilapia is unique and differs from those fish that live temperate zone.

In conclusion, findings in this study suggest that long day length, particularly increasing phase, is favoured for active spawning of Nile tilapia, and that this species, as a tropical fish species, may utilize changing lunar phases as a secondary environmental cue for reproduction. In addition, our results also suggest that one should consider not only the day length, but also the change of lunar phase when develops new techniques to control the reproduction of this species.

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