Effect of different light regimes on the maturational progress of the whitespotted rabbitfish (*Siganus sutor*)

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Received:December 2012 Accepted:July2013

Abstract

In this study, the effects of different light regimes on the reproductive activity of a typical Indo-Pacific coral reef rabbitfish, *Siganus sutor*, were evaluated. Forty- five adult fish were exposed to nine different photoperiod (8L: 16D, 12L: 12D, 16L: 8D) and light intensity (1000, 2000, 3000 lux) combinations with three replicates and five other fishes reared under indoor light condition (Control). Gonadosomatic Index (GSI) and Hepatosomatic Index (HSI) were calculated after 60 days and compared among different experimental regimes in males and females. In the control group, GSI and HSI mean values were 4.67 and 3.24%, respectively, for females and 10.05 and 2.10%, respectively, for males, and these fish showed differences in comparison with the exposed fish. Females kept under 1000 and 2000 lux light intensities had a higher GSI mean value (9.26 and 10.39%, respectively) and also lower average HSI (2.10 and 2.31%, respectively) in 16L: 8D treatment. A similar result was also obtained for males, whereas the 3000 lux light intensity, 8L: 16D day length combination led to more gonadal development (GSIs of 16.41% in females and 12.03% in males). A comparison of results among different photoperiods also confirmed that maturation was induced better in fish maintained under 16L: 8D in both sexes. This investigation revealed the visible role of both photoperiod and light intensity on inducing maturity in the whitespotted rabbitfish, *S. sutor*. Thus, rearing of adults exposed to an artificial light regime, including 16L: 8D and 2000 lux light intensity, promotes more gonadal development than that occurring in the wild.

Keywords: Photoperiod, Light intensity, GSI, HSI, rabbitfish, *Siganus sutor*, Persian Gulf

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[DOR: 20.1001.1.15622916.2013.12.4.16.5]

Introduction

The environment plays an important role in the regulation of reproduction in different animals including fish (Maitra et al., 2006). A number of environmental factors such as temperature, salinity, water current, rainfall, spawning substrate, food supply, and lunar cycle have been implicated as possible proximate cues for the reproductive activity of different species. It is the diurnal and seasonally changing pattern of light, including day length (photoperiod) and light intensity, which is probably responsible for the cueing and timing of reproduction in the majority of fishes (Bromage et al., 2001; Miranda et al., 2009; Pham et al., 2010). It is envisaged that each fish species would respond in a different manner to photoperiodic treatment. Thus, allowing gonadal development and spawning to occur at time of the year that is characteristic for that species (Bromage et al., 2001). The photoperiod specification is also an important driver in warm temperate and tropical systems (Pankhurst and Porter, 2003). Therefore, the manipulation of the light parameters is currently being used in aquaculture to induce maturation, control spawning, and stimulate growth in many species from a wide range of families that inhabit both temperate and tropical latitudes such as the Atlantic salmon, *Salmo salar* (Taranger, 1993; Porter et al., 1999); European seabass, *Dicentrarchus labrax* (Rodriguez et al., 2001); gilthead bream, *Sparus aurata* (Kissil et al., 2001); Atlantic cod, *Gadus morhua* (Davie et al., 2007); Atlantic halibut, *Hippoglossus hippoglossus* (Norberg et al., 2001); Nile tilapia, *Oreochromis niloticus* (Campos-Mendoza et al., 2004; Rad et al., 2006); grey mullet, *Mugil cephalus* (Kelly et al., 1991); Indian carp, *Catla catla* (Dey et al., 2005; Maitra and Chattoraj, 2007); and red sea bream, *Pagrus major* (Biswas et al., 2010).

Rabbitfishes (Siganidae) are typical Indo-Pacific coral reef fishes, which occupy all types of coastal habitats, from estuaries and mangroves to the reef front, the reef flat, and seaweed mats in the lagoons (Borsa et al., 2007). Siganids are an important human food source for the tropical Indo- Pacific region and the Mediterranean (Saoud et al., 2008; Kamukuru, 2009). The fast growth rate, diurnal schooling behavior, and shallow browsing habits of siganids make them ideal for aquaculture, as evidenced by numerous studies on their growth and reproduction. Colorful species are also popular in the aquarium trade (Kamukuru, 2009). Although, it is reported that rabbitfish species are definite lunar-synchronized spawners (Rahman et al., 2001; Rahman et al., 2003; Park et al., 2006; Kamukuru, 2009), it has been confirmed that the fish of tropical origin can respond flexibly to regional changes in various environmental cues to extend their breeding period and increase their chances of reproductive success (Bapary and Takemura, 2010). In this regard, it has been reported that the little spinefoot *Siganus spinus* inhabiting the tropical regions has two reproductive seasons, whereas the same species inhabiting the subtropical regions actively reproduces only once a year when the regions

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experience increases in photoperiod and temperature (Bapary and Takemura, 2010). Thus, one of the most important factors that has yet to be determined is how light can alter the timing of puberty and breeding activity of siganids. For this reason, in this study, brooders of the whitespotted rabbitfish, *S. sutor* were exposed to different combinations of day length and light intensity, and the effects on gonadal and hepatic indices were evaluated.

Material and methods

A random sample of 100 individual *S. sutor* (total length: 306.4 ± 2.12 mm; body weight 401.5 ± 8.45 g) were collected using gillnet from the northern reefs of Lavan Island, Iran, during autumn, 2011. The fishes were subsequently transported to the Aquaculture department, Persian Gulf and Oman Sea Ecological Research Institute, where they were equally divided into two round fiberglass stock tanks (5000-l capacity) with gentle aeration and 80% daily sea water exchange to acclimate. They were hand- fed two times a day to become saturated with a commercial shrimp pellet (EX SF; Beyza Feed mill).

One day before the initiation of the experiment, fish were randomly distributed into ten 300-l tanks (n = 5). The fish in the control tank were exposed to indoor light condition and the fish in each of the other tanks received combinations of photoperiod and light intensity (Table 1). The experiment started on January 2012, and lasted 60 days. Along this period, water temperature, pH, dissolved oxygen, and light intensity (control tank) were measured.

Table 1: Light conditions of different tanks

Tank code	Control	T1	T2	T3	T4	T5	T6	T7	T8	T9
Photoperiod (Light: Darkness)	Indoor condition	16:8	16:8	16:8	12:12	12:12	12:12	8:16	8:16	8:16
Light intensity (Lux)	Indoor condition	3000	2000	1000	3000	2000	1000	3000	2000	1000

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At the end of the experiment, the animals were anesthetized with the clove powder, weighed [total body weight (TW), to the nearest 0.1 g], and measured [total length (TL), to the nearest 0.1 cm]. Then paired gonads and liver were excised and weighed to the nearest 0.01 g (GW and LW, respectively). To determine the effects of light exposure on reproductive features, the gonadosomatic index (GSI= GW/ TW× 100) and hepatosomatic index (HSI= LW/ TW× 100) were estimated for females and males.

Data are presented as the mean ±SD. The statistical significance of the differences between groups were analyzed using one-way analysis of variance (ANOVA) followed by the Duncan test. Differences were considered statistically significant at $P< 0.05$.

Results

The effect of various photoperiod and light intensity regimes on gonadal and hepatic indices of whitespotted rabbitfish were examined at the end of the experiment. The GSI and HSI mean values of females and males are summarized in Table 2.As shown in Table 2, it is obvious that the GSI mean values varied at different combinations of photoperiod and light intensity compared with the control group in females and males.

In females, the lowest value of GSI was recorded at a short photoperiod and median intensity (i.e., 8L, 2000 lux) that only had significant difference with females in tanks 2 and 7 ($P < 0.05$). However, in that day length, when the light intensity increased to 3000 lux (T7), the mean GSI was raised to its highest value, 16.41%. In comparison with females in tank 7, the differences were significant with females in tanks 1, 4, 5, 6, 8, and control ($P<0.05$). Figure 1 show that fishes exposed to different photoperiods combined with 1000 lux light intensity did not have significant differences on GSI mean values ($P>0.05$). In this instance, 2000 and 3000 lux treatments showed significant differences between fishes kept under 8 h and 16 h day length ($P<0.05$). Furthermore, a comparison among various light intensities arranged on the basis of three types of photoperiods (Fig.1, Right) indicated that the differences were only significant between fishes reared under 3000 and 2000 lux light intensities combined with 8 h day length (tanks 7 and 8, respectively).

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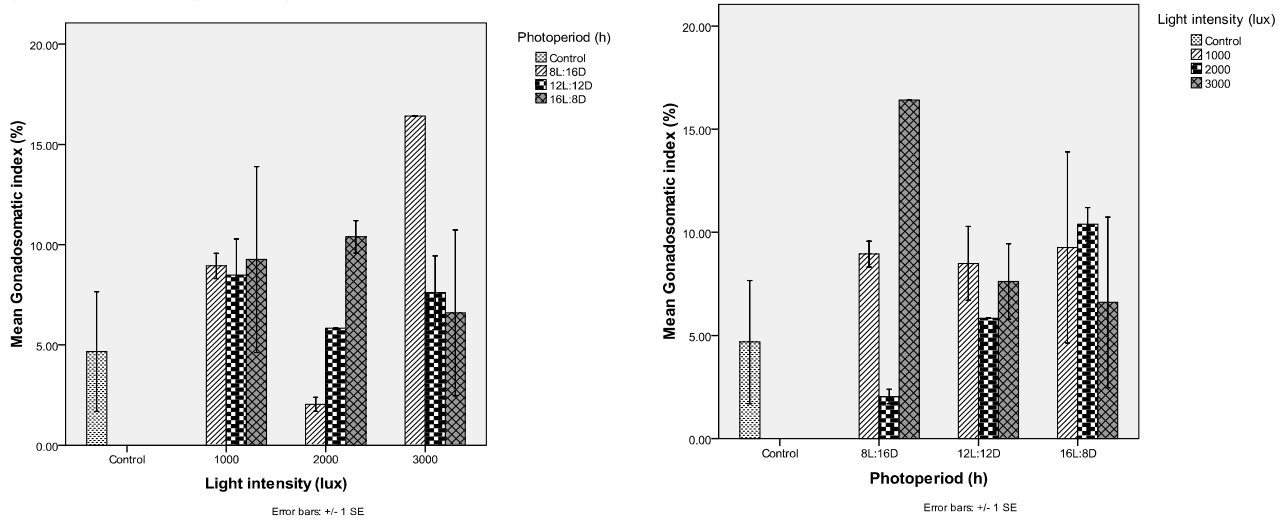
Table 2: The GSI and HSI mean values of *S. sutor* exposed to different light conditions

Tank code		Control	T1	T2	T3	T4	T5	T6	T7	T8	T9
GSI (%)	Female	4.67±2.98	6.60±4.13	10.39±0.80	9.26±4.63	7.61±1.83	5.83±0	8.49±1.79	16.41±0	2.03±0.34	8.94±0.62
	Male	10.05±4.12	5.32±1.95	14.56±0.41	13.44±1.26	11.16±0	13.91±1.52	13.07±1.89	12.03±1.35	8.15±0.43	13.46±1.21
HSI (%)	Female	3.24 ± 0.10	2.81 ± 0.18	2.31 ± 0.22	2.10 ± 0.58	2.32 ± 0.16	2.49 ±0	2.66 ±0.03	2.54 ± 0	2.87±0.12	2.45±0.03
	Male	2.10 ± 0.27	2.28 ± 0.40	1.59 ± 0.21	1.49±0.17	1.4±0	1.63±0.11	1.91±0.19	2.02±0.36	1.99±0.06	1.75±0.24

Figure 1: Changes of gonadosomatic index (GSI) in females of *S. sutor* subjected to different combinations of day length and light intensity. Left: among three types of light intensities; Right: among three types of photoperiods. GSI values are given as mean ±SE .



In males, the highest and lowest values of GSI were taken in 16L: 8D treatments where fishes in tanks 2 and 1 were reared under 2000 and 3000 lux, respectively. Although, differences among fishes in the control group and other tanks were not significant, the light regime including 16L:8D and 2000 lux (tank 2) was significantly different compared with the regimes used for fishes in tanks 1 and 8 (P<0.05) (Table 2). The arrangement of treatments based on constant light intensity (Fig. 2, Left) showed the highest males that had been kept under 1000 lux light intensity.

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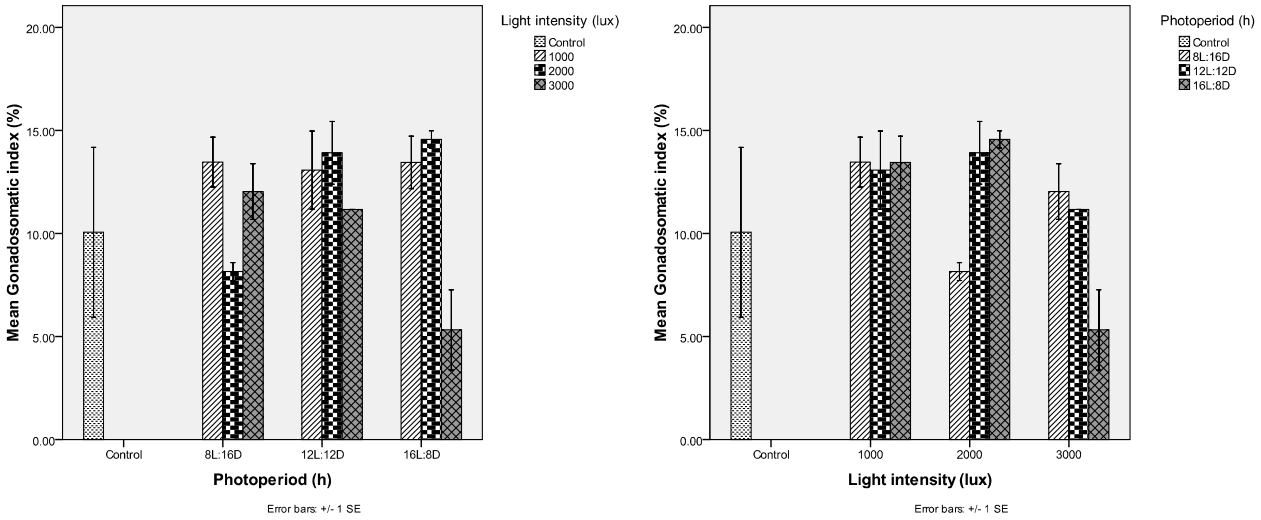

Figure 2: Changes of gonadosomatic index (GSI) in males of *S. sutor* subjected to different combinations of day length and light intensity. Left:Among three types of light intensities; Right: Among three types of photoperiods. GSI values are given as mean ±SE.

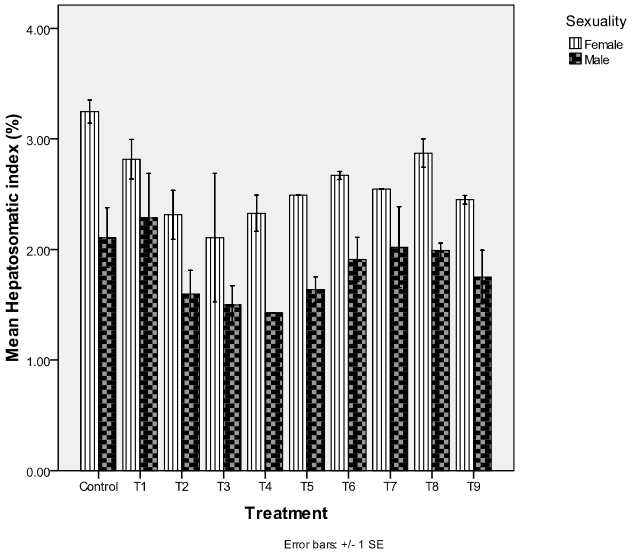

Figure 3: Changes of hepatosomatic index (HSI) in whitespotted rabbitfish (*S. sutor*) subjected to different combinations of day length and light intensity. HSI values are given as mean ±SE.

At the end of the experiment, females of *S. sutor* had larger liver than males, so the HSI mean values of females were all higher (Table 2). Figure 3 shows that females kept under various light conditions had lower HSI values versus the control group (3.24%). These differences were

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significant with most of the tanks except T1, T6, and T8. Although, changes among males subjected to different conditions were not significant ($P > 0.05$), fishes belonging to tanks 1 and also the control had higher HSI values than others (2.28% and 2.10%, respectively). According to Figure 3, fluctuations of HSI mean values were the same among different light regimes in both sexes, particularly when treatments were arranged based on photoperiod alternations in each light intensity.

Discussion

It is well known that environmental factors such as light cues can have profound effects on the timing of gametogenesis, vitellogenesis, and maturation in fish. Although, the precise mechanisms involved have not been yet fully elucidated, the neuroendocrine system is clearly involved (Miranda et al., 2009). It is believed that melatonin is the key hormone involved in regulation of endogenous rhythms by photoperiod cues. Melatonin action is linked to other reproductive endocrinology and its synthesis by photoreceptors of the pineal gland is increased during the hours of darkness (Ridha and Cruz, 2000). An alternative explanation is that the annual cycle of reproduction is controlled by an endogenous rhythm or clock whose periodicity is circannual. Under ambient conditions, the periodicity of this clock is closely entrained by the seasonally changing pattern of day length (Bromage et al., 2001). This advantage led us to apply different light regimes to modify the timing and quality of reproduction process of fish species inhabiting different latitudes. In this study, we thus manipulated day length and light intensity to examine the effect of these factors on the brain- pituitary- gonadal axis of whitespotted rabbitfish (*S. sutor*) by comparison of GSI and HSI among fishes illuminated with different light regimes.

GSI is one of the most functional indicators to investigate reproductive biology that demonstrates gonadal development and increased sharply during spawning season. Therefore, for many studies on rabbitfishes in tropical and the subtropical waters, the initiation and termination of the reproductive season have been determined by following changes in GSI values (Ntiba and Jaccarini, 1990; Al-Ghais, 1993; Yeldan and Avsar, 2000; Bariche et al., 2003; Park, 2004; Kamukuru, 2006).

The results of GSI values and their comparison among fishes that had been kept under various treatments has shown that the fish reared under indoor conditions, 12D: 12L photoperiod (approximately) and 0- 200 lux light intensity, had lower values than fish exposed to altered light regimes, expect for males in tank 1 (16L, 3000 lux) and fishes in tank 8 (8L, 2000 lux). These results clearly demonstrated that fishes of both sexes had shifted the timing of their maturation cycle in response to the altered light regimes in most of tanks. This acceleration role of light on gonadal development was similar to the results obtained on species such as pejerrey, *Odontesthes bonariensis* (Miranda et al., 2009); damselfish, *Chrysiptera cyanea* (Bapary and Takemura, 2010); goldfish, *Carassius auratus* (Sarkar and Upadhyay, 2011); Atlantic salmon,

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Salmo salar (Taranger et al., 2003); and Atlantic halibut, *Hippoglossus hippoglossus* (Norberg et al., 2001). It is also noted that seasonal changes in the gonads of *S. canaliculatus* is positively correlated with photoperiod rather than rainfall or prevailing wind (Duray, 1998).

As it was mentioned above, day length in the control group was moderately equal with the photoperiod used in tanks 4, 5, and 6 (i.e., 12L: 12D). Thus, increased values of GSI may have been maintained due to constant high light intensities (1000- 3000 lux). This observation is in agreement with the report of Ridha and Cruz (2000) for the Nile tilapia, *Oreochromis niloticus*. In that species, 2500 lux light intensity led to improved reproductive performance while lower one (500 lux) did not do so even when photoperiod was similar for both treatments. The mechanism by which light intensity acts was described by Sumpter (1990), who indicated that low light intensity stimulates the secretion of the antigonadotropin melatonin by the pineal gland. In contrast, we found that testes and ovaries in *S. sutor* were better developed at 1000 and 2000 lux light intensities rather than 3000 lux. The effects of increased light intensity were also revealed for fishes in tanks 7 and 9, where superior gonadal development was observed compared with the control, albeit in a photoperiod that had been decreased to 8 h. However, for fishes kept in tanks 1, 2, and 3, both parameters of light had been increased. In the case of photoperiod, it is reported that 18 h of light and 6 h of darkness retarded the gonadal maturation in the whitespotted spinefoot (*S. canaliculatus*) in Singapore (Duray, 1998). Similar results were obtained in 3000 lux treatments where maturation decreased along with the extension of light duration, whereas there was positive correlation between mean GSI values and photoperiods in fish illuminated with 2000 lux light intensity. These observations suggest that the physiological responses to light depend on the interaction between photoperiod and light intensity.

The liver size and consequently HSI values were higher in females than in males at the end of lightening. It is not a permanent pattern and depends on the puberty stage. Kamukuru (2009) reported that in *S. Sutor* high HSI value in males takes place along with GSI at the 5^{th} developmental stage (ripe),whereas in females the highest values of HSI and GSI occur during fourth (late developing) and fifth developmental phases, respectively. Albeit in mature males, metabolic requirements other than gamete formation during the reproductive season (such as migration to mating grounds and resistance to starvation) was believed to be related to high HSI values (Henningsen, 1999), the liver of mature females produces vitellogenin, the precursor of exogenous yolk, during oogenesis that leads to liver weight gain. In addition, some liver lipids are used in yolk anabolism (El-Gamal and El-Greisy, 2005; Chen and Liu, 2006). Then vitellogenin, which is synthesized by hepatocytes, released into the circulation of the blood stream and is sequestered by the oocytes (El-Gamal and El-Greisy, 2005). Thus, gonad weight and consequently GSI are gradually increased and reached the highest value after HSI. These

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findings may help to explain the occurrence of higher HSIs in mature females compared with mature males and also with immature females. On the basis of these results, a high GSI value along with reduced HSI is the sign of promoted maturity, particularly for females. In this study, therefore, fishes that were influenced by artificial light regimes had been more mature, because they had higher and lower values of GSI and HSI, respectively, than individuals reared under indoor condition.

It has been confirmed that gonadal development and spawning in siganids are synchronized by lunar periodicity; although, the combined results of these experiments indicate that both photoperiod and light intensity are also two important environmental determinants of reproduction in whitespotted rabbitfish. Al-Ghais (1993) reported that the major reproductive season of this species lasts from March to May in the Persian Gulf. Thus, on the basis of this study if mature fish were reared under a light regime including 16L: 8D photoperiod and constant 2000 lux light intensity throughout winter, it is possible that gonadal development would be induced earlier than in the wild.

Acknowledgments

The authors wish to express their deepest gratitude to Dr. Mortazavi (Head of Persian Gulf and Oman Sea Ecological Research Institute) for his useful advice and technical assistance and to Mr. Daghooghi (Manager of Marine Biology Department of Persian Gulf and Oman Sea Ecological Research Institute) for his abundant help with the experiment and data proposition. The authors also sincerely thank H. Rameshi, A. Esmaeilzadeh, and H. Sareban (Persian Gulf Molluscan Research Station) for help during fish sampling.

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[DOR: 20.1001.1.15622916.2013.12.4.16.5]