# PHOTOPERIOD MANIPULATION IN THE INDUCED BREEDING OF THE RABBIT FISH (*Siganus guttatus*)

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## ABSTRACT

*In teleosts, reproductive development is a continuous process throughout ontogeny and is regulated by pituitary gonadotropins (GtHs). It has been established that fish has two distinct pituitary GtHs that parallel vertebrate follicle stimulating hormone (FSH) and luteinizing hormone (LH) with respective structural similarities. In the present study we investigated the spawning of the Golden rabbit fish Siganus guttatus after exposure to different photoperiod regimes. The role of photoperiod on spawning was studied by exposing female fish to three light regimes: 16h:8h light and dark; 16h:8h dark and light; and 24h continuous light. At every first quarter of the lunar cycle (new moon), the groups exposed to the photoperiod were induced to spawn by the injection of hCG at 2000 IU/kg fish. We observed that only 24 h exposure to continuous light induced successful spawning in female injected with hCG. Fish exposed to the other light: dark regimes could not be induced to spawn. Overall, these findings suggest that continuous light can be a triggering factor for the induction of maturation and spawning of this species under aquaculture conditions.*

*Keywords: Siganus guttatus, photoperiod, induced breeding, spawning.*

## I. Introduction

Reproduction processes are generally regulated by environmental factors that activate internal signals into action leading to the successful production of viable progenies. During seasonal breeding, the release of gametes through spawning are controlled by appropriate environment stimuli or may be induced by appropriate hormones (Zohar et al., 2009). Thus, the internal signals that control breeding are apparently similar in most teleost species. Nevertheless, there are studies showing that the ambient factors that control breeding do significantly fluctuate among fish species (Ingram et al., 2007; Zohar et al., 2009; Melo et al., 2014). Environmental factors that play an integral role in teleost reproductive cycle include water temperature, photoperiod, salinity, water current, diurnal cycles, rainfall and spawning substrate (Weltzien *et al*., 2004). Little attention has been devoted to the role of photoperiodic changes in tropical species.

The reproductive cycle of fishes are closely tied to the environmental changes, particularly seasonal changes in photoperiod and temperature (Nishimura and Tanaka, 2014; Nakane and Yoshimura, 2014). These two environmental variables can directly act together or activate sense organs on the glands that produce hormones, which in turn produce the appropriate physiological or behavioral responses that control breeding (Zohar et al., 2009; Tokarz et al., 2015).The hypothalamus-pituitary-gonadal axis plays an important role in regulating gametogenesis in teleost fishes. In most cases, gonadotropins regulate the biosynthesis of steroid hormones that subsequently control processes of sexual maturation, sexual behavior, fertility, oocyte maturation and ovulation or modifications in other functions that are dependent on the integrity of the reproductive system (Arukwe and Goksoyr, 2003; De Silva et al., 2008; Zohar et al., 2009).

In the Pacific region, the rabbitfish (*Siganus guttatus*) has been considered as a major food fish species in some countries (Lam, 1974), and was farmed using traditional method in the Philippines (Pillai, 1962). However, artificial propagation of this species is yet to be implemented. Previously, an attempt was

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made to reproduce and culture the rabbitfish larvae at the Southeast Asia Fisheries Development Centre (SEAFDEC), Philippine (Juario et al., 1985), but resulted in very low survival rate. Komatsu et al. (2006) studied the maturation process of rabbitfish and showed that gonadotropin-releasing hormone agonist (GnRHa) produced good effects in promoting spermatogenesis in males. Recently in Indonesia, rabbitfish larvae were reared in culture for 35 days, but survival rate of early larval stages was low (<2%) (Rachmansyah et al., 2007). Therefore, for many years, artificial propagation and cultivation of rabbitfish has been a major challenge for marine fish farming. Under natural conditions, the spawning of rabbitfish is synchronously linked to the tide and at specific lunar phases (Duray, 1990). The annual reproductive cycle and the lunar synchronization of ovarian development and spawning in the rabbitfish have been reported (Rahmna et al., 2000a,b), showing that plasma levels of estradiol-17β (E2), 17α,20β-dihydroxy-4-pregnen-3-one (DHP) and 17α,20β,21-trihydroxy-4-pregnen-3-one (20b-S) peaked around the first lunar quarter and decreased rapidly after spawning around the full moon (Rahmna et al., 2000b). Previously, the effects of human chorionic gonadotropin (hCG) and steroid hormones on in vitro induction of GVBD were investigated, showing changes in production of E2 and DHP based on the lunar cycle (Rahman et al., 2002). According to Soletchnik (1984) and Hara et al (1986), *S. guttatus* species in the Philippines can lay eggs all year around (Duray, 1998) and this is a spawning strategy that has not been verified in in natural populations in Vietnam. However, rabbitfish fry from 1.5 - 2.0 cm are often observed in the wild in April - May of the lunar calendar, at least in two regions of Tam Giang - Cau Hai Lagoon (Thua Thien – Hue: 16°19'22"N 107°51'2"E) and Thi Nai Lagoon (Binh Dinh province: 11° 37' 00"N 109° 02' 00"E) (Le and Le, 2006).

Recently, few studies on rabbitfish reproduction have been conducted in order to boost the supply of seed stock through artificial propagation, but reportedly resulted in low quality seed (Le and Le, 2006). One of the reasons for the low quality breeding stock is probably due to the quality of the broodstock or rearing conditions and therapy for stimulating production by hormones. Therefore, the present study was aimed at evaluating the potential of improving maturation, spawning and egg quality of rabbitfish manipulating environmental factors (photoperiod) in conjunction with hormonal therapy.

## II. Materials and methods

### Experimental design

Broodfish of total length and body weight range of 28-30 cm and 450-600 g, respectively were used for the experiments. Fish were cultured in composite tanks at environmental condition that ranged from, water temperature, 27-32 °C, salinity 28-32 ‰, pH 7.8-8.4 and dissolved oxygen (DO) 4.5-6 mg/l. The fish culture density was around 3 kg/m³. Broodfish were fed daily at 3-5 % of their body weight with commercial barramundi pellet with proximate composition of protein (43%), lipid (7%), ash (16%), fiber 5 (3%) and moisture (11%).

***Photoperiod exposures.*** Broodfish were placed in 500 L- composite tanks in 3 treatments. Treatment 1: 16 h: 8h light and dark; Treatment 2: 16 h: 8 h dark and light; and Treatment 3: 24 h continuous light. During the experimental period, every 15th day, fish were checked for maturation status and reproductive parameters using catheter. At every first quarter of the lunar cycle (new moon) fish were induced to spawn by injecting hCG at 2,000 IU per kg of female and 1000 IU per kg of male.

### Sampling reproductive parameters

Prior to sampling, fish were anaesthetized to measure weight and length to the nearest 0.1 g and 0.1 cm, respectively and to check the maturity stages of the eggs using catheter. Every second week fish were checked for maturity. Fish were considered mature using combined criteria that included round and soft abdomen, swollen, protruding and reddish

genital opening and swollen and reddish anus. The diameter of a minimum of 50 spawned eggs and the length of larvae were determined using microscope equipped with a micrometer. Fertilization rate was estimated by examining at least 50 eggs at the 32-cell stage. Eggs were cleared in a solution of glacial acetic acid and saline (1:20 volume/volume), examined under a stereomicroscope and cleaved eggs were classified as fertilized. Eggs were considered normal when cleavage was symmetrical, cells with similar size and cell formation was complete, whereas abnormal eggs were associated with irregular cleavage, poor cell formation with vesicular inclusions, and deformation of blastomeres. The proportion of eggs, that survived to the eyed stage, and until hatch, was assessed relative to the number of fertilized eggs. Duration of embryogenesis (defined as the time between fertilization and hatching) and hatching rate was determined as the number of respective hours or days and frequency from fertilization until 50% of the eggs were hatched. Latent period was estimated as the time to spawning from the time of spawning induction.

Spawning was evaluated in the morning post-spawning induction by checking the number of matured individuals with small and soft bellies, and this was confirmed by gentle stripping without the release of eggs. The maturity rate is the ratio (%) between mature fish and all fish examined in each treatment group while the spawning rate (%) is the ratio between total number of fish spawned and total number of mature fish that were induced. To obtain fecundity, ovaries were removed from the female cavity. Fragments of the ovary from the posterior, middle and anterior parts (0.5–1 g) were counted for all eggs at stage III and IV (yolk formation). Absolute fecundity (AF) is the total egg at stage III and IV in the ovary and relative fecundity (RF) was calculated as $RF=100\times AF\times W-1$ (where AF is absolute fecundity and W is total weight of the female fish).

**Statistical analysis**

The statistical differences in spawning variables including oocyte maturation, egg and larval diameter, and reproductive and spawning parameters were assessed using one-way analysis of variance (ANOVA). Least significant difference and Duncan's multiple range tests at 95 % confident level (Post Hoc Test) was used to compare mean values within individual exposure groups. Linear regression analysis was used to investigate any relationship between oocyte maturation and spawning parameters in individual exposure groups. All computations were performed with the software of Statistical Package for Social Sciences Version 18 (SPSS 18). Values were expressed as mean ± standard error (SE) or standard deviation (SD).

## II. Results

**Biometric data**

The length and weight of broodfish selected for the present study ranged between 28-30 cm and 450-600g, respectively. Males were slightly smaller than females. However. During the experimental period, the broodfish increased in weight and length, and no significant differences (neither in males nor females) in body size were observed after 4 months of rearing under captive condition (Table 1).

**Final oocyte maturation and ovulation (FOMO)**

In order to induce spawn, the broodfish were inspected for final oocyte maturation and ovulation (FOMO) every 15 days. The results indicated that, under captive condition, less than 50% offish reached FOMO and this was not consistent with sampling time. However, percentage of males that achieved maturation status was higher than females (Table 2) and this was consistent at all samplings. The average maturation rate was around 20 and 50% for female and male, respectively, during the main reproductive season from February - May. The highest maturation rate was observed in late April and May for males, while female fish did not show any maturation in February and early March (Table 2).

**Table 1: The changes in body weight and length of the broodfish during the experiment period. Values given as mean ± standard deviation (SD)**

| Sampling date | Female (n=30) | | Male (n=30) | |
|---|---|---|---|---|
| | TL (cm) | BW (g) | TL (cm) | BW (g) |
| 15/02 | 27.6 ± 2.2 | 450 ± 30 | 25.6 ± 2.3 | 350 ± 40 |
| 01/03 | 32.8 ± 1.6 | 430 ± 40 | 26.8 ± 2.8 | 410 ± 48 |
| 15/03 | 28.6 ± 2.5 | 550 ± 35 | 28.3 ± 2.2 | 450 ± 55 |
| 01/04 | 30.7 ± 2.2 | 480 ± 42 | 26. 6 ± 2.8 | 420 ± 62 |
| 15/04 | 32.0 ± 2.6 | 580 ± 66 | 28.1 ± 2.5 | 480 ± 36 |
| 30/04 | 30.0 ± 2.0 | 500 ± 83 | 28.2 ± 2.9 | 460 ± 53 |
| 15/05 | 31.5 ± 2.1 | 620 ± 55 | 27.6 ± 2.4 | 520 ± 52 |
| 30/05 | 30.6 ± 1.9 | 600 ± 68 | 27.2 ± 2.9 | 490 ± 46 |

**Table 2: Maturation status of the broodstock during the experimental period**

| Sampling date | Female (n = 30) | | Male (n=30) | |
|---|---|---|---|---|
| | Number of fish matured | Number of fish immatured | Number of fish matured | Number of fish immatured |
| 15/02 | 0 | 30 | 5 | 25 |
| 01/03 | 0 | 30 | 10 | 20 |
| 15/03 | 6 | 24 | 16 | 14 |
| 01/04 | 10 | 20 | 20 | 10 |
| 15/04 | 8* | 17* | 18 | 12 |
| 30/04 | 12 | 13 | 21* | 5 |
| 15/05 | 10 | 15 | 20 | 6 |
| 30/05 | 11 | 14 | 16 | 10 |

*For female: Immature fish was defined as eggs in stage II or III and not ready to spawn; while mature fish was defined as eggs in stage IV or V and ready to spawn.*
*For male: Mature fish was defined as fish with semen releasing when gently pressing along the abdomen; while immature fish do not.*
**During April fish some broodfish were lost due mortality, reducing overall to 25 and 26 for female and male, respectively.*

**Maturation and spawning performances under photoperiod exposures**

In the present study, reproductive and spawning parameters under different photoperiod regimes were compared and assessed in order to find the better light regime

under captive condition for the Golden rabbit. After 3 months exposure to different light regimes, the average maturation rate was 46 % at the highest (24 h) photoperiod treatment. The other photoperiod treatments produced 20-25 % maturation rates. Fish were sacrificed, ovaries were dissected and eggs counted. Absolute and relative fecundity did not show significant differences between photoperiod treatments ($p>0.05$). The absolute fecundity varied from 560,825 to 820,182 eggs/ female, depending upon fish size. Overall, absolute fecundity increased with increasing fish size. The relative fecundity ranged from 1266 to 1358 eggs/ g

During the study period, at every first quarter of the lunar cycle (new moon), female fish were induced to spawn by hCG injection at 2000 IU/ kg. The results indicated that female fish in the 24 h continuous photoperiod treatment spawned successfully, while fish in the other photoperiod treatments, did not spawn. The corresponding fertilization and hatching rates were 90 and 86 %, respectively. The duration of embryonic development was 18 h after spawning and length of the larvae immediately after hatch was 1.58 mm. The length of larvae at day 2 and 3 were 2.16 and 2.25 mm, respectively (Table 3).

**Table 3: Spawning performance in Golden rabbit fish exposed to different photoperiod regimes and induced to spawn using human choriogonic gonadotropin (hCG). Values given as mean ± standard deviation (SD)**

| Reproductive parameters | Photoperiod regimes | | |
|---|---|---|---|
| | 8 hours light 16 hours dark | 16 hours light 8 hours dark | 24/24 hour light |
| Average matured rate (%) | 20 ± 16 | 25 ± 15 | 46 ± 18 |
| Absolute fecundity (egg/female) | 560,825 ± 46,217 | 820,182 ± 106,200 | 655,4338 ± 88,673 |
| Relative fecundity (egg/ g female) | 1358 ± 185 | 1315 ± 177 | 1266 ± 165 |
| Mean spawning rate (%) | No spawning | No spawning | 50 |
| Latent period (hour) | - | - | 16 ± 2 |
| Ripe egg diameter (μm) | - | - | 475 ± 76 |
| Fertilized egg diameter (μm) | - | - | 530 ± 42 |
| Large oil drop diameter (μm) | - | - | 180 ± 4 |
| Small oil drop diameter (μm) | - | - | 95 ± 6 |
| Fertilization rate (%) | - | - | 90 ± 22 |
| Survival to eyed stage (%) | - | - | 90 ± 25 |
| Hatching rate (%) | - | - | 86 ± 18 |
| Embryogenesis duration (hour) | - | - | 18 ± 2 |
| Length of larvae day 1 (mm) | - | - | 1.58 ± 0.18 |
| Length of yolk-sac day 1 (mm) | - | - | 0.35 ± 0.02 |
| Length of larvae day 2 (mm) | - | - | 2.16 ± 0.16 |
| Length of yolk-sac day 2 (mm) | - | - | 0.26 ± 0.02 |
| Length of larvae day 3 (mm) | - | - | 2.25 ± 0.15 |
| Length of yolk-sac day 3 (mm) | - | - | 0.12 ± 0.04 |

## IV. Discussion

Among the most significant advancements in the field of aquaculture during recent decades is the development of techniques to induce reproduction in fish using hormonal stimulation and environmental factors (Pham et al. 2010, 2013). The interactions between environmental stimuli and gonadotropins activate the secretion of follicle stimulating hormone (FSH) and LH, which regulate hormonal responses that are important for successful reproduction. Thus, environmental variables such as photoperiod play significant roles, because they can act, either directly or indirectly, through sense organs on the glands that produce hormones, which in turn produce the appropriate physiological or behavioral responses that ultimately control the timing of spawning in teleost species. Therefore, an understanding on how these environmental variables may influence reproductive output is important for predictive and reliable estimation of reproductive status and also integral aspects of sustainable fisheries management and aquaculture development. These parameters will also be important for an accurate evaluation of the effects of different treatments on sexual maturation in fish farming. When female fish treated to different light regimes, were induced to spawn with 2000 IU hCG at the beginning of each lunar cycle, 24 h continuous photoperiod increased reproductive performance by accelerating spawning, fertilization and hatching rates, compared with 16h:8h light and dark or 16h:8h dark and light that produced a total spawning inhibition.

In vertebrates, photoperiod has been shown as the main environmental cue that synchronizes daily rhythms and the molecular clock. Alterations in photoperiod have profound physiological effects on reproduction and early development in fish (Mata-Sotres et al., 2015). Under favorable environmental conditions and adequate quality nutrition, Golden rabbitfish has been shown to mature in captivity (Soletchnik, 1984; Juario et al., 1985). For example, Solechnik (1984) reported the first maturation of Golden rabbitfish at 34 cm and 200 g, length and weight, respectively. In nature, the breeding of Golden rabbitfish is closely related with the tide, where they usually lay eggs near the surface of open water at night when tide is low (Lavina and Acala, 1974). According to Soletchnik (1984) and Hara et al. (1986), Golden rabbitfish species in the Philippines lay eggs all year around and this information is yet to be verified for population is Vietnam coastal waters. However, fry of Golden rabbitfish between 1.5 - 2 cm have been observed in least two Vietnam regions of Tam Giang - Cau Hai Lagoon (Thua Thien - Hue) and Thi Nai Lagoon (Binh Dinh) (Le and Le, 2006), usually during April - May of the lunar calendar. In the present study, we observed that Golden rabbitfish treated with hCG spawned successfully under 24 h continuous light condition, while female fish kept under 8 h:1 6h light: dark or 8 h:16 h dark: light photoperiod did not spawn. Overall, these findings suggest that constant light exposure is a determinant spawning factor for the Golden rabbitfish, despite hormonal stimulation.

Our findings are in accordance with previous studies showing that small filefish, *Rudarius ercodesspecies* mature during the spring-summer season that coincide with long photoperiodic phase and increase in water temperature compensation (Asahina and Hanyu. 1983). Other marine fish species such as Olive flounder, *Paralichthys olivaceus* (Kim and Hur, 1991), Rock bream, *Oplegnathus fasciatus* (Kim and Kim, 1990), Red seabream (Jeong et al., 1998) and Rockfish, *Sebastes inermis* (Ko et al., 1998) have successfully spawned when induced by manipulating photoperiod and temperature. Furthermore, male Atlantic salmon reared at 12 h:12 h light and dark cycle under different salinity levels showed that the completion of spermatogenesis was accelerated, irrespective of the salinity levels (Melo et al., 2014). These authors also reported that the accelerated spermatogenesis was associated with higher LH-β (lhb) mRNA and 11-ketotesterone (11-KT) plasma levels, compared to fish reared under 24 h light regime

(Melo et al., 2014), suggesting that salinity and photoperiod modulated different aspects of salmon spermatogenesis. Reproductive success is important for the survival of any organism and most fish species has an optimal breeding season that ensures successful reproductive processes under suitable environmental conditions (Sumpter, 1990). Thus, survival of offspring is secured by using seasonal changes in various cues in the aquatic environments (Dufour et al., 2010). Recently, we reported that in Waigieu seaperch, spawning performances, egg and larval viability were strongly enhanced after exposure to dietary thyroxin (Pham et al., 2010, 2012). In addition, while exogenous gonadotrophs accelerated spawning rate, but resulted in reduced fertilization and hatching rates, forming a strong basis for understanding the reproductive endocrinology of a tropical marine finfish with increasing aquaculture prospects (Pham et al., 2010). The present study showed that photoperiod is a potent and reliable activator of seasonality in reproductive processes of Golden rabbit fish, as has been demonstrated in several other teleost species (Sumpter, 1990).

In conclusion, we have shown that exposure to continuous light stimulation produced successful spawning in the Golden rabbit fish and represents a significant step in achieving optimal seed production for this species. For example, Golden rabbitfish broodstock was cultured for maturation and spawning over six seasons and produced several million larvae with 100% mortality after 4 days (Le and Le, 2006). Although, the authors did not identify the cause of this massive mortality, it was assumed that broodstock nutrition, rearing conditions and reproductive stimulation therapy may have affected the quality of spawns and larvae (Le and Le, 2006). The Golden rabbitfish is a strong candidate for marine aquaculture in Vietnam because of its market high price.

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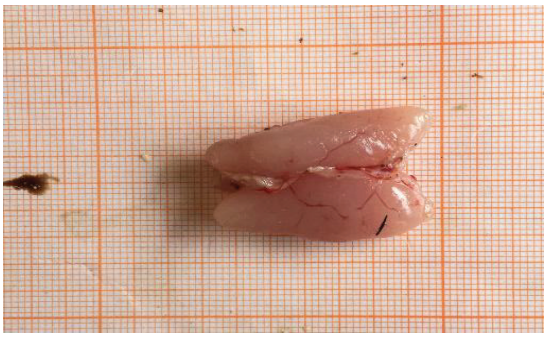
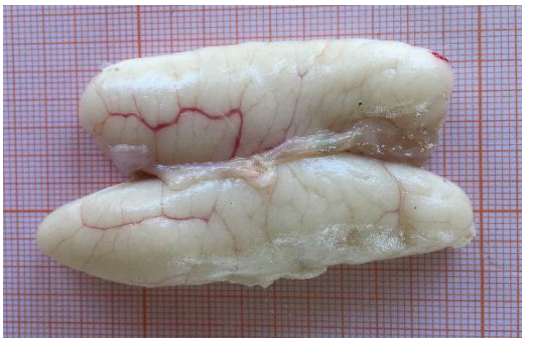
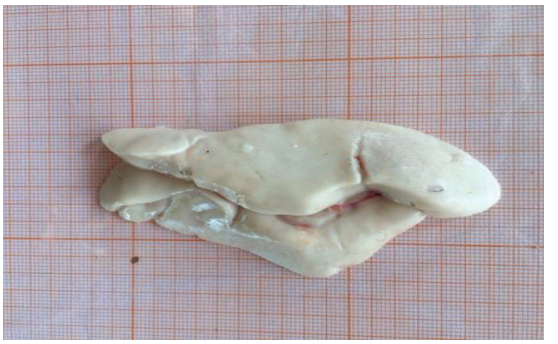


**Immature ovary**



**Mature ovary**

**Rabbitfish *(Siganus guttatus)***



**Mature testis**

**Figure 1: Ovary and testis of Rabbitfish *(Siganus guttatus)***

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