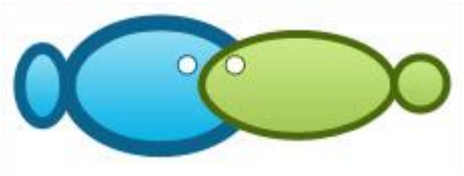

Continuous illumination improves growth and survival in the early stage of snubnose pompano *Trachinotus blochii*

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Abstract. The present study compared the effects of the natural photoperiod (12 hL : 12 hD) with those of continuous illumination (500 Lx) on feeding, growth and survival of the early stage snubnose pompano (*Trachinotus blochii*) larvae from 1 to 10 days post hatch (DPH). Newly hatched larvae were stocked at a density of 15 ind L^{-1} and fed with enriched rotifers (*Brachionus plicatilis*; 50-250 µm) at a density of 30 ind mL^{-1} starting from 2 DPH. The number of rotifers in the digestive organ, feeding incidence and total length of larvae were examined at 3-h interval from 0400H to 2200H at 3 DPH, at 6 h interval from 4 to 5 DPH and once at 8 DPH. Final growth and survival were also determined at the end of the experiment. Results showed that *T. blochii* larvae were visual feeders and exhibited diel rhythm under 24 h light. In contrast, larvae under natural photoperiod (12 hL : 12 hD) normally underwent diurnal rhythms. In conclusion, better larval feeding, growth and survival were obtained under continuous illumination.

Key Words: photoperiod, visual feeder, feeding incidence, diel rhythm, diurnal rhythms.

Introduction. Manipulation of photoperiod can have a major impact on larval growth and survival (Litvak 1999). The relationship between the photoperiod and larval growth is already well known in a number of fish species. In general, an increase in day length improves growth rates in species such as snapper *Pagrus auratus* (Fielder *et al* 2002), putitor mahseer *Tor putitora* (Sawhney & Gandotra 2010) and rabbitfish *Siganus guttatus* (Duray & Kohno 1988). A direct relationship between day length and growth of larvae has been observed in Atlantic cod *Gadus morhua* (Puvanendran & Brown 2002). Cod larvae reared under continuous illumination grew faster than siblings reared under 18 hL : 6 hD photoperiod and both grew faster than those reared under a natural photoperiod (12 hL : 12 hD) during the first 28 days of larval rearing. However, European seabass *Dicentrarchus labrax* larvae exhibited better growth but poorer survival under continuous illumination than under a photoperiod of less than 9 h light period (Ronzani-Cerqueira & Chatain 1991).

Studies have shown that light influences prey detection, feeding ecology, and development of fish larvae (e.g. Cook & Rust 2002). Yoseda et al (2008) have reviewed the influence of continuous light on different kinds of groupers early larval stage. A higher survival of leopard coralgrouper *Plectropomus leopardus* requires optimum photoperiod of 24 hL : 0 hD rather than an ambient condition (13 hL : 11 hD) and 6 hL : 6 hD) repeating 2 cycles of photoperiods a day due to the higher feeding incidence, food intake, growth and survival. In other hatchery-reared groupers (*P. leopardus*, *Epinephelus akaara*, and *Epinephelus malabaricus*), the 3 species possess clear circadian rhythm in spite of the 24 h light conditions. It has been an assumed that 24 h light conditions were efficient from the view of food intake, growth and survival. The ability to catch prey has been shown to be a learned behavior and increasing the photoperiod

provides the larvae an opportunity to develop feeding skills, since longer day lengths allow for longer feeding periods (Fielder et al 2002).

There has been an increasing demand of pompano (*Trachinotus blochii*) both in the Philippine domestic and export market. However, the supply of *T. blochii* fingerlings for aquaculture is problematic due to the low survival in the hatchery phase. There is a need to optimize environmental conditions for a successful larval rearing of this species. One of the first steps towards this is to establish a viable protocol such as possible. The present study aimed to compare the effects of continuous illumination and those of the natural photoperiod on feeding, early survival, and the growth of the early stage *T. blochii* larvae.

Material and Method

Spawning and egg collection. *T. blochii* broodstocks maintained at the SEAFDEC-AQD Igang Mariculture Park, Guimaras Island, Philippines were induced to spawn. Human chorionic gonadotropin (HCG) was administered at 500 IU kg^{-1} fish across 2-day period at 24 h interval. Ovulation took place 36 to 38 h after the first injection and the eggs were released between 00:00H (12 midnight) to 04:00H. Fertilized eggs were collected, transported to the experiment site (SEAFDEC/AQD, Tigbauan, Iloilo), stocked at 250 to 500 eggs L^{-1} in 500 L fiberglass incubation tank. Hatching occurred at around midnight to early morning after approximately 18 to 20 h of incubation.

Experimental set-up. The effects of continuous illumination on feeding, growth and survival of *T. blochii* larvae from 1 to 10 DPH was compared with that of the natural photoperiod (indoor light, 12 hL : 12 hD). Larvae were assigned randomly to 6 tanks (200 L capacity) at three replicates each for the two treatments. Incandescent bulbs (Firefly, frosted standard, 100watts, 230v 60Hz, E27 medium base) were installed 30 cm above the water surface of experimental tanks at light intensity of 500 lx. To remove the effects of any background light on the experiment, each tank was covered completely with black sacks. For the natural photoperiod (control) treatment, the tanks were covered with transparent plastic cellophane and maintained under natural photoperiod of 12 hL: 12 hD (indoor). Surface light intensity was measured using light meter (EXTECT measurements, EA30-EasyView™ Wide Range Light Meter).

Larval culture. The number of newly hatched (day 0) larvae was estimated and determined by aliquot sampling. Two samples (1 L $sample^{-1}$) from different areas of hatching tank were collected, drained through a sieve, transferred onto a Petri dish, the larvae were counted under microscope using Sedgwick-Rafter counting cell. The larvae were stocked randomly at a density of 15 ind L^{-1} in 6 tanks and a single air stone was positioned at the bottom center of each tank. Dissolved oxygen (DO) measured during the study averaged 5 mg L^{-1} while salinity and water temperature ranged between 30 and 35 g L^{-1} and between 28 and 30.5^{o}C, respectively; the latter was maintained using a heater. Other water parameters monitored daily throughout the experimental period.

Following the recommended feeding regime of Riley et al (2009) for *Trachinotus carolinus*, the larvae were fed with enriched rotifers (*Brachionus plicatilis*, 50-250 µm) at an initial density of 30 ind mL^{-1} starting on 2 DPH at 17:00H. Thereafter, the density of rotifer was maintained at 30 ind mL^{-1} and adjusted twice daily at 07:00H and 15:00H until 10 DPH of the experiment. *Nannochloropsis oculata* was added to the experimental rearing tanks at 300,000 cells mL^{-1} twice a day (08:00H and 13:00H).

Seawater used in the experiments was filtered and subjected to ultraviolet radiation. No water change was employed during the first 5 days of the experiment. However, water with the same salinity and temperature was added if necessary to each experimental tank daily to replace the volume of water taken during sampling. After 5 days, water quality was maintained by daily water change of 30 -50% until termination. Fecal waste was siphoned every 2 days starting at 6 DPH. In general, the procedure of Parazo et al (1998) was used for the stocking and feeding of larvae.

Sampling. Samples of 10 larvae were collected from each experimental tank to evaluate food intake (FI) (FI = average number of rotifers in the digestive organ), and total length (TL) of larvae at various sampling period. Larvae were euthanized by brief immersion in cold water (4°C) and preserved in 5% formalin-seawater for gut content analysis (Toledo et al 2002). Samples were photographed and individually dissected under Cole Palmer stereo camera microscope. Digital images were analyzed to obtain standard measurements using Motic Images Plus version 2.0. Percentage of FI and average number of rotifers (ANR) in the digestive organ of the larvae was estimated (Yoseda et al 2008) using the following formula:

$$FI\ (\%) = L/N \times 100$$

Where L represents number of larvae that had eaten one or more rotifer and N is the number of larvae.

$$ANR = \Sigma\ Li/N$$

Where *i* represents number of rotifers in the digestive organ in the larval individual (i = 1,…, *n*), and N is the number of larvae.

On 11 DPH, 20 larvae from each tank were randomly sampled in the early morning for final length and wet weight measurements using Cole Palmer stereo camera microscope and analytical scale, respectively. Total counting was done to determine the remaining larvae and computing the survival rate.

Larval samples for measurements were collected from the water column, from the surface, and from the bottom of the tanks using long polyvinyl chloride pipes (40 mm diameter).

Statistical analysis. Data were subjected to paired Student t-test to determine significant differences on feeding and growth parameters.

Results

Feeding. During the day phase of 3 DPH, both the natural photoperiod (control, 12 hL : 12 hD) and the continuous illumination resulted in no significant differences ($p>0.05$) in the FI and ANR (food intake) of the larvae. During the night phase, however, larvae exposed to continuous illumination exhibited significantly higher FI ($p<0.05$) and higher ANR ($p<0.05$) at both 19:00H and 22:00H (Tables 1, 2). Moreover, larvae under natural photoperiod showed no food intake (i.e. ANR) during the night phase (Tables 2, 3). At 4 and 5 DPH, continuously illuminated larvae exhibited significantly higher FI in the night phase (i.e. 22:00H and 04:00H, respectively) than did those exposed to the natural photoperiod. No significant differences were observed between the two treatments during the day phase at 4 or 5 DPH.

Table 1

Changes in the feeding incidence (%) of early stage *Trachinotus blochii* larvae under natural photoperiod (12 hL : 12 hD) or continuous illumination (24 hL : 0 hD, 500 lx)

Illumination	*3 DPH*						
regime	*04:00H*	*07:00H*	*10:00H*	*13:00H*	*16:00H*	*19:00H*	*22:00H*
Control	0.00±0.00	10.0±5.8	36.7±3.3	66.7±3.3	53.3±3.3	**0.0±0.0**	**0.0±0.0**
Cont. Illum.	13.3±6.7	36.7±6.7	50.0±17.3	60.0±11.5	56.7±8.8	**63.3±8.8**	**70.00±10.0**
Illumination	*4 DPH*				*5 DPH*		
regime	*04:00H*	*10:00H*	*16:00H*	*22:00H*	*04:00H*	*10:00H*	*16:00H*
Control	0.0±0.0	50.0±5.8	50.0±5.8	**0.0±0.0**	**0.0±0.0**	63.3±6.7	80.0±10.0
Cont. Illum.	65.0±5.0	70.0±10.0	75.00±25.0	**45.0±5.0**	**55.0±5.0**	80.0±10.0	90.0±10.0

Bold data's indicates significant differences.

First-feeding larvae (i.e at 3 DPH) were observed in continuous lighting at 04:00H while there was no feeding activity under the natural photoperiod group (Table 1). The continuously illuminated larvae displayed slight increases in the FI from 04:00H on 3 DPH to 16:00H of the following day (i.e. 4 DPH) until it declined to the lowest FI at 22:00H. However, slight changes were apparent on the feeding response of larvae from 3 to 4 DPH. At 5 and 8 DPH of the continuously illuminated treatment, FI and ANR gradually increased during the day phase.

As shown in Tables 2 & 4, the relative feeding responses of continuously illuminated larvae exhibited diurnal variation, which corresponded with that of the daily photoperiod. FI and ANR markedly decreased during the night phase and markedly increased during the day phase despite the 24 h continuous light conditions. In contrast, the normal photoperiod group fed normally during the day phase but no feeding activity was observed during the night phase. FI and ANR gradually increased in the morning and decreased at noontime; highest feeding activity usually occurred between 10:00H and 16:00H. The natural light intensity level under indoor condition possibly affected feeding of larvae since it could change anytime of the day depending on condition. We measured the natural light intensity in the control treatment (indoor) from 1 to 10 DPH and we recorded a range of 20 - 392 lx.

Table 2

Changes in the food intake (ANR) in the early stage of *Trachinotus blochii* larvae under natural photoperiod (12 hL : 12 hD) or continuous illumination (24 hL : 0 hD, 500 lx)

Illumination regime	*3 DPH*						
	04:00H	*07:00H*	*10:00H*	*13:00H*	*16:00H*	*19:00H*	*22:00H*
Control	0.0±0.0	0.1±0.1	0.5±0.0	1.6±0.3	1.3±0.4	**0.0±0.0**	**0.0±0.0**
Cont. illum.	0.1±0.1	0.4±0.1	0.6±0.3	1.2±0.4	1.1±0.2	**1.0±0.2**	**1.3±0.2**
Illumination regime	*4 DPH*				*5 DPH*		
	04:00H	*10:00H*	*16:00H*	*22:00H*	*04:00H*	*10:00H*	*16:00H*
Control	0.0±0.0	0.9±0.0	0.7±0.1	0.0±0.0a	**0.0±0.0**	1.0±0.1	1.9±0.6
Cont. illum.	1.1±0.3	1.4±0.2	1.9±0.9	0.7±0.0b	**0.7±0.1**	2.2±0.6	4.1±1.4

Bold data's indicates significant differences.

Table 3

Comparison of the day phase and night phase food intake (ANR) in the early stage of *Trachinotus blochii* larvae under natural photoperiod (12 hL : 12 hD) or continuous illumination (24 hL : 0 hD, 500 lx)

Illumination regime	*3DPH*		*4 DPH*		*5 DPH*		*8 DPH*
	Day	*Night*	*Day*	*Night*	*Day*	*Night*	*Day*
Control	0.9±0.0	**0.0±0.0**	0.8±0.0	0.0±0.0	**1.5±0.3**	0.0±0.0	2.1*
Cont. illum.	0.8±0.2	**0.8±0.1**	1.6±0.6	0.9±0.2	**3.1±0.5**	0.7±0.1	4.1±0.7

*Control at 8 DAH (n=1); Bold data indicated significant difference.

Table 4

Comparison of the day and the night phase feeding incidence (%) in the early stage of *Trachinotus blochii* larvae under natural photoperiod (12hL : 12hD) or continuous illumination (24 hL : 0 hD, 500 lx)

Illumination regime	*3 DPH*		*4 DPH*		*5 DPH*		*8 DPH*
	Day	*Night*	*Day*	*Night*	*Day*	*Night*	*Day*
Control	41.7±1.7	**0.0±0.0**	50.0±5.0	**0.0±0.0**	72.6±4.4	**0.0±0.0**	70.0±0.0
Cont. illum.	50.8±8.0	**48.9±2.2**	72.5±17.5	**55.0±5.0**	85.0±0.0	**55.0±5.0**	90.0±0.0

Bold data's indicates significant differences.

Growth and survival. The total length of larvae was measured starting at 07:00H of the 3 DPH until 5 DPH (Table 5). The continuous lighting group was considerably longer and exhibited faster length increases over time than did the natural photoperiod group which displayed poorer and slower growth. Larvae exposed to continuous illumination exhibited markedly higher survival (16.22%) than did those exposed to the natural photoperiod group (3.33%).

Table 5

Growth and survival of early stage *Trachinotus blochii* larvae under continuous illumination at different light intensities after 10 DPH

Treatment	*Final length (mm)*	*Final weight (mg)*	*Survival (%)*
Control*	3.79	0.81	3.33
Cont. illum.	4.74±0.06	1.89±0.40	16.22±0.01

*Control (n=1) and continuous illumination (n=3).

Discussion. Most of the fundamental rhythms in nature (diurnal or seasonal) are related to the periodicity of light (Boeuf & Le Bail 1999). In the present study, the presence of light clearly influenced the feeding, growth and survival of early stage *T. blochii* larvae. The experimental fish larvae are particularly receptive to the application of artificial light system and extended day length (photoperiod).

Most fish larvae particularly those in the pelagic habitat use sight as a main sense to locate prey i.e. they are visual feeders (Blaxter 1966) and are highly dependent on light for prey capture and feeding success (Blaxter 1966). Once exogenous feeding begins, a suitable light level is necessary for active feeding. In the present study, larval first-feeding was observed with the presence of continuous illumination on 3 DPH at 04:00H and they exhibited diel rhythm, which enhanced feeding activity and allowed the fish larvae to eat continuously during the night phase. The duration of photoperiod has been shown to affect growth, probably through increasing the period over which the larvae are able to feed (Howell et al 1998). Many animals, including fish exhibit a 24 h cycle of activities (diel rhythm) caused by simple photokinesis (Clarke 1965). In contrast, larvae reared under natural photoperiod (12 hL : 12 hD) normally undergo diurnal rhythms. The fish larvae are active during daylight and less active during darkness. In the natural photoperiod group (12 hL : 12 hD) in the present study, around 10 to 90% of larvae fed during day phase and the highest feeding activity occurred between 10:00H and 16:00H. Despite this, poor growth and very low survival was obtained. This might be due to the ever-changing day light intensity (20-392 lx), which possibly affected the foraging activity. Fish larvae are visual predators with high feeding rate during the day but the rate declines during the night (Heath 1992). First feeding is viewed as a vital period amid which larvae either start feeding or confront starvation. The high mortality rates reported during the first days of larval culture have been related to a non-optimal environmental condition, or to an inadequate development of their visual system at the time of first feeding (Helvik & Karlsen 1996; Planas & Cunha 1999). Survival of larvae is increased when light administrations allow a fast commencement of feeding activity (Gulbrandsen 1991; Huse 1994).

Conclusions. In conclusion, better growth and higher survival of *T. blochii* larvae could be obtained in the hatchery when reared under continuous lighting in a 24 hL : 0 hD photoperiod from 1 to 10 DPH than when reared under natural photoperiod. They exhibited a clear diurnal pattern of feeding or circadian rhythm despite the continuous illumination. The larvae exhibited considerably better FI and ANR during the day phase and significantly higher during night phase than did larvae under the natural photoperiod.

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