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PROOF RESEARCH PAPAER

Effect of Light Levels on Growth, Survival and Skin Colour Enhancement of Marine Angelfish, *Apolemichthys xanthurus* (Bennett, 1833)

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Abstract

In the present study, effect of various levels light on growth performances and skin colour enhancement of marine smoke angelfish *Apolemichthys xanthurus* was investigated. The fishes were reared in tanks with three different levels (250 - 500, 750 - 1000, and 1500 - 2000 lux) and control (without additional light) for 120 days and the growth, survival and carotenoid content were investigated. The results of the growth performances studies suggested that the fishes reared under low light level (250-500 lux) exhibited higher weight gain (73.90±0.06), specific growth rate (0.616±0.01), and survival rate (90%) and feed conversion ratio (1.00±0.01). The colour enhancement studies suggested that the carotenoid content of the fishes reared under low, medium, high and control was about 6.84±0.03, 6.19±0.04, 5.48±0.06 and 3.57±0.04 mg g^{-1} respectively. Thus, the result obtained from the present study indicates that the low light level (250-500 lux) was more suitable for better growth and skin colour enhancement of *Apolemichthys xanthurus* which could be recommended for the successive production of this high priced species.

Keywords: Smoke Angelfish, *Apolemichthys xanthurus*, light intensity, growth, skin colour, carotenoids.

Introduction

Environmental factors play an important role in regulating reproduction of different animals including fish (Maitra *et al*., 2006). As light is an important environmental factor for animals living in water, many studies have been undertaken on its effect and there are significant differences in behaviour, food intake and growth of aquatic animals appeared under different light conditions (Blaxter 1968; Gehrke 1994; Giri *et al*., 2002). Light is one of the most important culture management factors in that it synchronizes from embryo development to sexual maturation of fish (Guo *et al*., 2012; Villamizar *et al*., 2011). Studies have shown that most marine fish are visual feeders and need a minimal threshold light intensity to be able to develop and grow normally (Blaxter 1986; Hunter 1981; Gehrke 1994). Several investigations have been focused on the combined influence of light quality (meaning the different wavelengths which are absorbed by water to various extents), light quantity (different light intensities) and light periodicity (different photoperiod) (Boeuf and Le Bail 1999). Recently, there are many studies have reported on the effect of light on larval growth performance and physiology (Yasir and Qin 2009; Villamizar *et al*., 2011). Gardner and Maguire (1998) used only two light intensity treatments and concluded that further research was required to clarify the effect of light intensity on survival and growth, especially with higher intensities. Goa *et al*. (2016) reported that both light quality and intensity had significant influences on the embryo development of larva of *Haliotis discus hannai* Ino and the growth of juveniles.

Colour changes in fish are often related to environmental stress, and illumination could be a primary factor regulating pigment distribution through hormone regulation (Van der Salm, *et al*., 2004). Colour of fish skin is predominantly dependent on the presence of chromatophores containing coloured pigments (Fox 1957). The colour of fish skin is generated by the absorption, reflection, and scattering of light by the pigments and microstructures within the fish integument (Fujii 2000). Six types of chromatophores have been reported and each chromatophore contains specific pigments (Fox 1957), but the most dominant pigments in fish are carotenoids, melanin, and purines (Moyle and Cech 2004). Carotenoids are naturally occurring pigments that range in hues from yellow to red (Hill 2002) which are lipid soluble pigments, are responsible for skin colour of ornamental fish, and can determine their

commercial value (Paripatananont *et al*., 1999). The light intensity is one of the most important factors regulating chromatophore performance through pigment aggregation or dispersion (Fujii 2000).

As coloration of ornamental species is considered as an important factor for marketing of the product (Gouveia and Empis 2003; Gouveia and Rema 2005), most research on focused on the effect of light on fish growth (Boeuf and Le Bail 1999), vision (Shand and Lythgoe 1987) and behaviour (Marchesan *et al*., 2005). However, no specific studies have been made on the high priced marine angelfish, *Apolemichthys xanthurus* (Bennett 1833). Keeping this mind, the present study was carried out to understand the effect of various light levels on growth, survival, and skin colour enhancement of marine angelfish *Apolemichthys xanthurus*.

Materials and Methods

Experimental Design

The experiment was carried out at the Aquaculture breeding centre, Centre of Advanced Study in Marine Biology, Annamalai University, India. Juveniles of marine angelfish *Apolemichthys xanthurus* were obtained from Sun shine Aquarium (Chennai, India) and acclimatized for 4 weeks before the trial. The experiment was conducted in (20 liters) rectangular fibreglass tanks connected with a biofilter and a mechanical filter with temperature between 26 and 30^{o} C, salinity 28-30 PSU, pH 7.4 -7.8, dissolved oxygen(DO) 4.2 - 5.6 mg L^{-1} and the photoperiod was set at 12 h light/ 12 h dark cycle to mimic the natural light cycle. A total of 30 fish seeds (average weight 15.64 ± 0.31g) were maintained in each experimental tank throughout study and each experiment was conducted in triplicate. The light was provided by fluorescent tubes (Philips, Kolkata, India) and intensities of light were achieved by adjusting the distance of lamps and water surface. The experimental (250 - 500, 750 - 1000, and 1500 - 2000 lux) and control (without additional light) tanks were covered by black nylon screens, and the energy-saving lamps (Philips, Kolkata, India) were placed 15 cm above the tanks with digital timer controls. Light intensities were tested at the water surface (including the water centre and edge).

The fishes in experiments and control were fed with pellet feed formulated according to the method described by Sun *et al*. (2010) with the feeding rate of 3% of biomass per day provided in equal rations at 8.00 AM, 1.00 PM, 6.00 PM for 120 days and the excess diet was collected and dried at 60ºC, put in room temperature for 3 days to restore the natural moisture and then weighed. Daily feed was adjusted every 30 days by batch weighing of fish in each tank after a 24 h period of starvation. Experimental tanks were cleaned and water exchange was done once a week.

Growth Studies

The growth parameters of the experimental and control fishes such as weight gain, specific growth rate (SGR), survival rate and feed conversion ratio (FCR) were assessed at 30, 60, 90 and 120 days. The weight gain (WG), specific growth rate (SGR) and feed conversion ratio (FCR) was evaluated based on standard formula as follows.

$$\text{Weight gain (g)} = (\text{Final body weight (g)} - \text{Initial body weight (g)})$$

$$\text{Specific growth rate, SGR (\% d}^{-1}) = 100 \times (\text{In (final body weight (g)} - \text{In (initial body weight (g))} \times \text{rearing time}^{-1} \text{ (days)}$$

$$\text{Feed conversion ratio, FCR} = \text{weight of feed consumed (g)} \times (\text{final stock biomass (g)} - \text{initial stock biomass (g)})^{-1}$$

Colour Enhancement and Carotenoid Content Estimation

The color enhancement was monitored by visual examination and estimation of carotenoid content in the skin of experimental fishes. The carotenoid content of the experimental fish skin was extracted according to the method of Torrissen and Naevdal (1984). Three fishes from each experiment were randomly sampled, anesthetised using clove oil (dissolved in 95% ethanol at 1:10) and used for carotenoid content analysis in triplicate. Briefly, 2 mg of skin were collected from both sides between the abdominal and dorsal regions of the fish and then transferred to 10 mL of pre - weighed glass tubes after the fat layer had been removed from the skin and ground well with acetone containing anhydrous sodium sulphate and made up to 10 mL with acetone. The samples were stored for 3 days at 4ºC in a refrigerator, and then extracted three times till no further colour could be obtained and centrifuged at 5000 x *g* for 5 min. The total carotenoid content of the samples was determined using a spectrophotometer (Shimadzu, UV mini 1240) using extinction coefficients (E1%, 1 cm) of 2000 for astaxanthin (Hata and Hata 1971) at 475 nm, and 2500 for carotenoids from alfalfa at 450 nm (Schiedt and Jensen 1995).

Data Analysis

In the present study, all the data were analyzed by statistical methods. The one way analysis of variance (ANOVA) was performed using SPSS (Statistic Package for social science) version 11.5 software to determine the significant differences among means. For all tests, a criterion of $P<0.01$ was used to determine statistical significance.

Results

Growth Analysis

The effect of various light levels on growth of marine smoke angelfish *Apolemichthys xanthurus* was investigated and the results were presented in Table.1. The results revealed that the weight of fishes increased with the increase in days of culture in all experimental groups. At the end of the experiment, it was found that there was significant weight gain in experiments and control where fishes were reared in tanks provided with different light levels (250 - 500, 750 – 1000 and 1500 – 2000 lux). The weight gain was comparatively less in the control group (without light). The weight gain was the highest in the fishes reared at 250 – 500 lux (73.90 ± 0.06g) followed by 750 – 1000 lux (53.22 ± 0.12g), 1500 – 2000 lux (44.60 ± 1.10g) and control groups (30.88 ± 1.10g). The specific growth rate (SGR) was significantly ($P<0.01$) higher in 250-500 lux group (0.616 ± 0.01) compared with fishes in the group of 750 - 1000 lux (0.444 ± 0.01), 1500 – 2000 lux (0.372 ± 0.02) and control (0.257 ± 0.02). Furthermore, the most advantageous value for feed conversion ratio (FCR) classified as follows: 250 - 500 > 750 - 1000 > 1500 – 2000 > control group (Table 1). The survival rate of the fishes was 80 - 90% (in control and 250 – 500 lux, respectively) ($P<0.01$).

Carotenoid Content Analysis

The results of the carotenoid analysis revealed that the initial carotenoid content of the fish skin varied between 1.30 and 1.32 mg g^{-1} in all experimental groups and control and increased gradually with increasing days of culture. At the end of the experimental period, carotenoid content was varied between 3.57 - 6.84 mg g^{-1} (in control and 250 - 500 lux group) (Table 1) ($P<0.01$). Thus, these results clearly indicates that the skin colour as well as carotenoid content of fish group reared at 250-500 lux light intensity for 12 h photoperiod was the highest when compared to fishes reared under medium and high light intensity levels as well as absence of light.

Discussion

Light intensity can be a limiting factor in aquaculture depending on turbidity and depth, and different responses are exhibited in different species and different developmental stages (Boeuf and Le Bail 1999). Recently, several studies have been undertaken to evaluate the effect of light intensity on

Table 1. Growth, survival and carotenoid content of smoke angel fish *Apolemichthys xanthurus* reared under various light levels (250-500, 750-1000 and 1500-2000 lux) and control (without light). Values are presented in mean ± SD, n = 3, FCR - feed conversion ratio; SGR - specific growth rate (% d^{-1})

Days of culture	Growth Parameters	Light level (lux)			
		Control	250-500	750-1000	1500-2000
0 - 30	Initial weight (g)	15.13 ± 0.31	15.53 ± 1.13	15.60 ± 1.10	15.81 ± 0.21
	Final weight(g)	23.92 ± 1.10	29.98 ± 0.30	26.96 ± 1.30	25.18 ± 1.36
	Weight gain (g)	8.79 ± 0.21	14.45 ± 0.28	11.36 ± 0.11	9.37 ± 1.20
	SGR (% d^{-1})	0.293 ± 0.04	0.482 ± 0.03	0.379 ± 0.03	0.312 ± 0.02
	FCR	2.10 ± 0.02	1.28 ± 0.03	1.62 ± 0.03	1.97 ± 0.01
	Survival rate (%)	80	90	90	90
	Carotenoid content (mg g^{-1})	1.32 ± 0.05	1.31 ± 0.03	1.32 ± 0.02	1.3 ± 0.03
0 - 60	Initial weight (g)	15.13 ± 0.31	15.53 ± 1.13	15.60 ± 1.10	15.81 ± 0.21
	Final weight(g)	31.19 ± 0.09	49.57 ± 1.20	37.36 ± 0.23	35.23 ± 1.10
	Weight gain (g)	16.06 ± 0.13	34.04 ± 1.40	21.76 ± 0.10	19.42 ± 1.03
	SGR (% d^{-1})	0.268 ± 0.02	0.567 ± 0.01	0.363 ± 0.03	0.324 ± 0.01
	FCR	2.30 ± 0.02	1.08 ± 0.02	1.70 ± 0.04	1.90 ± 0.01
	Survival rate (%)	80	90	90	90
	Carotenoid content (mg g^{-1})	2.11 ± 0.06	3.58 ± 0.04	3.54 ± 0.03	3.57 ± 0.04
0 - 90	Initial weight (g)	15.13 ± 0.31	15.53 ± 1.13	15.60 ± 1.10	15.81 ± 0.21
	Final weight(g)	38.57 ± 0.12	68.13 ± 1.30	49.16 ± 1.24	44.39 ± 0.11
	Weight gain (g)	23.44 ± 1.10	52.60 ± 0.19	33.56 ± 0.08	28.58 ± 1.13
	SGR (% d^{-1})	0.260 ± 0.01	0.584 ± 0.03	0.373 ± 0.03	0.318 ± 0.02
	FCR	2.36 ± 0.01	1.05 ± 0.01	1.62 ± 0.02	1.94 ± 0.03
	Survival rate (%)	80	90	90	90
	Carotenoid content (mg g^{-1})	2.89 ± 0.04	5.94 ± 0.05	4.89 ± 0.06	4.48 ± 0.05
0 - 120	Initial weight (g)	15.13 ± 0.31	15.53 ± 1.13	15.60 ± 1.10	15.81 ± 0.21
	Final weight(g)	46.01 ± 0.03	89.43 ± 0.08	68.82 ± 0.06	60.41 ± 0.12
	Weight gain (g)	30.88 ± 1.10	73.90 ± 0.06	53.22 ± 0.12	44.60 ± 1.10
	SGR (% d^{-1})	0.257 ± 0.02	0.616 ± 0.01	0.444 ± 0.01	0.372 ± 0.02
	FCR	2.39 ± 0.01	1.00 ± 0.01	1.39 ± 0.03	1.65 ± 0.03
	Survival rate (%)	80	90	90	90
	Carotenoid content (mg g^{-1})	3.57 ± 0.04	6.84 ± 0.03	6.19 ± 0.04	5.48 ± 0.06

survival, growth, swimming activity and cannibalism of larvae or juveniles in various fish species under culture conditions (Petrell and Ang 2001; Downing and Kitvak 2002; Kestemont *et al*., 2003; Han *et al*., 2005). The growth of Chinese longsnout catfish was affected significantly by light intensity and the best growth was obtained with medium light intensity (74–312 lux) (Han *et al*., 2005). Contrarily, some species were reported to show improved growth at very intense light levels such as sea bass larvae at 1400–3500 lux (Barahona–Fernandes 1979), Atlantic cod larvae at 2400 lux (Puvanendran and Brown 2002), and black porgy juvenile at 3000 lux (Kiyono and Hirano 1981). Thus, the results of the earlier studies evidenced that the fishes need precise light levels rather than high or low light intensity for their growth and survival and also that they are species-specific (Puvanendran and Brown 2002).

The present study was carried out to find out the optimum level of light required for the better growth and colour of commercially important marine angelfish *Apolemichthys xanthurus*. The results of the present study suggested that the weight of fishes reared under low light level (250-500 lux) was significantly higher than the fishes reared under medium (750-1000 lux), high (1500-2000 lux) light levels and control. Similarly, weight gain, SGR, FCR and survival rate were also significantly higher in fish group reared under low light (250-500 lux) than other light levels and control. However, no significant variation was in survival rate between various light levels suggested that the light does not cause any legal effect. Han *et al.* (2005) reported that the SGR of juveniles of Chinese longsnout catfish was significantly higher when reared with 74 -312 lux light level. Boeuf and Le Bail (1999) reported that the cause for faster growth in medium light was improved feed conversion efficiency not feed intake. As a crepuscular feeding fish, Chinese longsnout catfish had less activity at suitable light intensity and more food energy could be used for growth (Trippel and Neil 2003).

Colour is an important trait in the ornamental fish trade (Hoff 1996). Fish and other vertebrates cannot synthesize carotenoids, but they have the ability to change colour under given circumstances (Matsuno 2001). The results of the colour enhancement study suggested that the fishes reared under low level were brighter in colour than other light levels and control. It is also observed that the ventral side of the fishes in all groups were brighter than the dorsal side. Han *et al*. (2005) reported that the chinese longsnout catfish also displayed optimum body colour at 434 lux of light intensity and Chatzifotis *et al*. (2005) suggested that the dark dorsal body of red porgy was caused by the accumulation of melanophores.

The carotenoid content analysis suggested that the fishes reared under low light level (250-500 lux) contain significantly higher carotenoid content than other medium and high light intensities. It is clearly evidenced that the fish skin and carotenoid content exhibited sensitive response to light intensity. Likewise, Yasir and Qin (2009) reported that the colour of the clownfish reared under the laboratory condition was significantly affected by light intensity.

The results confirmed that the fishes reared at low light (250-500 lx) exhibited improved growth performance, survival and skin colour. It is clearly evidenced that the growth and skin colour was significantly affected by the light levels and it is also suggested that relatively low light environment (250-500 lux) is most suitable for successful production of the high priced marine angelfish, *Apolemichthys xanthurus*. However, the mode and mechanism of actions about carotenoid content increase in skin by light intensity is in progress.

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