

Effect of Light Intensity on Color Performance of False Clownfish, *Amphiprion ocellaris* Cuvier

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Abstract

Color performance of false clownfish, *Amphiprion ocellaris* Cuvier, was examined under three levels of light intensity (20–50, 600–850, and 2700–3500 lx) for 5 wk. The experiment was conducted in nine rectangular glass aquaria (25 × 25 × 20 cm) with three replicates. Each aquarium was stocked with 36 fish, and 3 fish were randomly sampled from each aquarium every other week. Digital images were taken weekly on each individual fish after it was anesthetized in MS-222. The color performance in hue, saturation, and brightness was quantified using image analysis. In addition to the whole-body analysis, each fish image was divided into ventral and dorsal parts to assess the body position-dependent effect. Furthermore, color differences between dorsal fin, anal fin, ventral fin, and caudal fin were also quantified. The whole body was brighter at low light than at medium or at high light intensity. Irrespective of light intensity, the dorsal side was more orange but less bright than the ventral side. Brighter light strengthened overall orange color on fish fins. The dorsal fin and ventral fins appeared more orange than the anal and caudal fins regardless of light intensity and exposure duration. Similar to body color, low light also led to brighter fins, especially for caudal and dorsal fins. Our results indicate that ambient light could regulate fish color performance but could not change the pigment dominance by β -carotene. Light intensity is unlikely to change the contrast between dorsal and ventral sides, but dim light tends to make fish body brighter, and bright light strengthens orange color on fins.

Light intensity is extremely variable and can rapidly change over a wide range (Boeuf and Le Bail 1999). Color 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). Most fishes are visual feeders and need a minimal light intensity for growth and development (Moyle and Cech 2004). As fish often use visual clue to recognize their prey and predators (Bagnara and Hadley 1973), color change has an important implication for many fish species to improve their fitness in nature. Some fish species could camouflage themselves to prevent attack from predators or display conspicuous color to frighten predators.

Color of fish skin is predominantly dependent on the presence of chromatophores containing colored pigments (Fox 1957). The color 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).

Changes in color hue or pattern are crucial for the adaptation of aquatic animals to their environments (Boeuf and Le Bail 1999). A slow color change is usually subject to variation of pigment quantity, while a fast color change is related to hormone regulation (Oshima 2001). A change of light intensity usually provokes a gradual but reversible color change (Oshima et al. 1989). According to Fujii (2000), light intensity is one of the most important factors regulating chromatophore performance through pigment aggregation or dispersion. At present, most research on light and fish interactions has focused on the effect of light on fish growth (Boeuf and Le Bail 1999), vision (Shand and Lythgoe 1987), and behavior (Castro and Caballero 2004;

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Marchesan et al. 2005). Published literature involving light and fish color has been limited to commercial food fish species (Boeuf and Le Bail 1999; Booth et al. 2004; Gines et al. 2004). There is very little research on the impact of light intensity on the color of ornamental fish (Oshima et al. 1989).

False clownfish, *Amphiprion ocellaris*, is an important ornamental fish for the aquarium industry because of its body coloration, swimming behavior, and its symbiotic relationship with anemone (Yasir and Qin 2007). However, the color of farmed clownfish is less attractive than their congener sourced from wild (Allen 1991). Tanaka et al. (1992) reported that the carotenoid composition in the integument has the potential to change the clownfish from yellow orange to orange pinkish when the fish were moved from wild to indoor tanks, but the lighting conditions were not examined.

It has been suggested that, under a given lighting condition, temporary color changes can lead to a long-term color change (Odiorne 1957; Bagnara 1998). Barry and Hawryshyn (1999) found that light intensity could change pigmentation in coral reef fish, but no study has been conducted on clownfish to examine the relationship between color performance and light intensity. The objective of this study was to investigate the effect of light intensity on color performance of the false clownfish through visual analysis and quantification of carotenoid in fish skin. The result could provide insight into the strategies to design appropriate lighting environments for farming ornamental fish.

Materials and Methods

Experimental Fish and Design

Three-month-old juvenile fish with an average weight of 0.21 ± 0.02 g (mean \pm SD) and a standard length of 28.9 ± 3.0 mm (mean \pm SD) were used for this experiment. Fish were hatched and reared under laboratory conditions (Yasir and Qin 2007). During the experiment, all fish were fed twice a day with a gelatin-based diet (sea perch fillet 80%, vitamin C 5%, and gelatin 15%) without any carotenoid addition.

The experiment was conducted in nine rectangular glass aquaria ($25 \times 25 \times 20$ cm) filled with 10 L water. All aquaria were supplied with seawater in a recirculating system treated with a biofilter and a mechanical filter. A screen mesh was used on the outlet pipe in each aquarium to prevent fish escaping. Oxygen level was >6 mg/L with one air stone in each aquarium. Salinity was maintained at 28 ± 2 ‰. Temperature was controlled at 28 ± 1 C, and the photoperiod was 14 h light : 10 h dark.

A total of 332 fish were randomly chosen from the rearing aquaria. Eight fish were collected as the initial sample, while the rest of the fish were randomly distributed into nine aquaria with 36 fish each within 2 h. Three levels of light intensity (20–50, 600–850, and 2700–3500 lx), measured immediately above the water surface of the aquaria, were used with three replicates of each. Light levels were achieved by adjusting the thickness of shading cloth on the top of each aquarium. Light was provided by fluorescent tubes (L18W/840 1350lm; Osram, München, Germany) above the aquaria. Each of the aquaria was wrapped by a black plastic sheet to block the light from the sides. The experiment lasted for 5 wk. Three fish were sampled weekly from each aquarium. Live fish were anesthetized with tricaine methanesulfonate (MS-222) at a concentration of 70 mg/L before visual analysis. The water-soluble MS-222 reduced fish stress but did not change fish color in 30 min. The duration for taking photographs was only 2–5 min. Therefore, neither the esthetic treatment nor the duration for taking images affected fish color measurements. Soon after the fish images were taken, the fish was preserved at -20 C before carotenoid analysis.

Color System and Analyses

Color to human eye is a brain reaction to a specific visual stimulus, and it is extremely subjective because humans describe all colors using three broad bands corresponding to red, green, and blue (RGB). The RGB color model represents colors as they are used in light-emitting objects. In this model, each light beam represents red, green, or blue light. The white color,

for instance, is given by the maximum value of each beam (red = 255, green = 255, and blue = 255), and black is given by the absence of light in each beam (red = 0, green = 0, and blue = 0). The hue–saturation–brightness (HSB) color model is a mathematical representation of color, in a way more similar to human color perception (Georgieva et al. 2005). The HSB model breaks the color into three components: the hue (which would be the “pure” color), the percentage of saturation (“how much” color), and the brightness. The HSB model can be visualized as an upside-down cone (Fig. 1). Hue is the actual color and is measured in angular degrees around the cone starting and ending at red = 0 or 360 (e.g., yellow = 60, green = 120, and blue = 240). Saturation is the purity of the color, measured in percentage from the center of the cone (0) to the surface (100). At 0% saturation, hue is meaningless. Brightness is measured in percentage from black (0) to white (100). At 0% brightness, both hue and saturation are meaningless.

Visual Analysis

Photographs were taken under four natural white color bulbs (NEC, Adelaide, Australia, 18 W with natural white color) mounted on two sides of a table (75 × 75 cm) with a white-mat surface. A digital camera (Nikon Coolpix 4500; Nikon, Tokyo, Japan) was situated on an adjustable arm between the two light sides. A gray card was used to set up the white balance of the camera before any image was taken. The anesthetized fish was then put in a glass container filled to 2-cm depth with MS-222 (70 mg/L). The camera was set up at 25 cm above the fish and could capture the whole fish image along with the two color (yellow and red) reference cards (Kodak, Burbank, CA, USA; CAT 152 7662, Q-14) underneath the container. The HSB values of the reference card were analyzed to examine if between pictures were significantly different. If no significant difference was found, the calibration for the orange–red color of the fish was dismissed.

The image was analyzed with Adobe Photoshop software (version 7.0.1). Four fish fins including caudal fin (CF), dorsal fin (DF), ven-

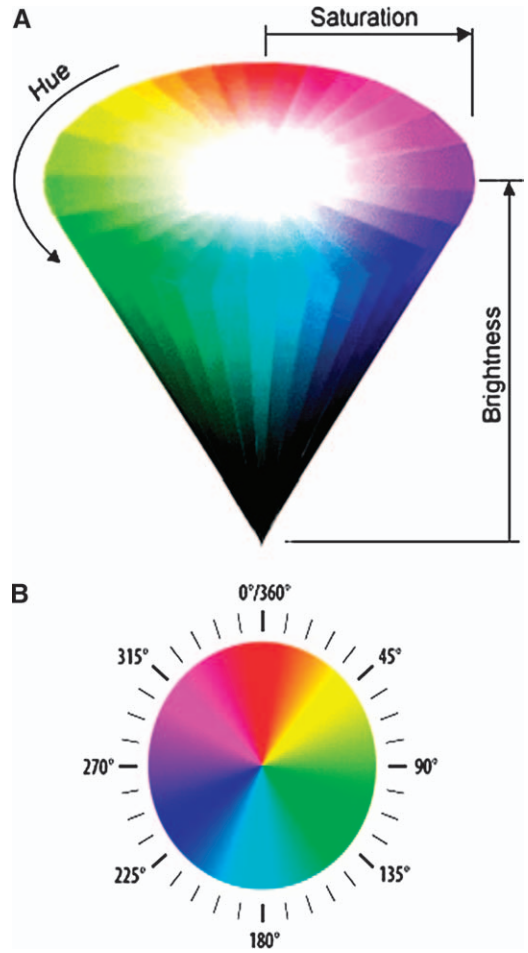


FIGURE 1. The hue–saturation–brightness (HSB) model to illustrate the relationship of hue, saturation, and brightness as a cone with lateral view (A) and top view (B).

tral fin (VF), and anal fin (AF) were separately scanned for color analysis (Fig. 2). In addition, the dorsal body was divided into the middle (MD) and front (FD) parts and the ventral body was divided into the middle ventral (MV) and front ventral (FV) parts. The RGB values were transformed to the HSB value using algorithm transformations (Gardner 2007) before statistical analysis.

Carotenoid Analysis

The frozen fish were thawed at ambient temperature for 5 min before the analysis. The fish skin and all fins were used for

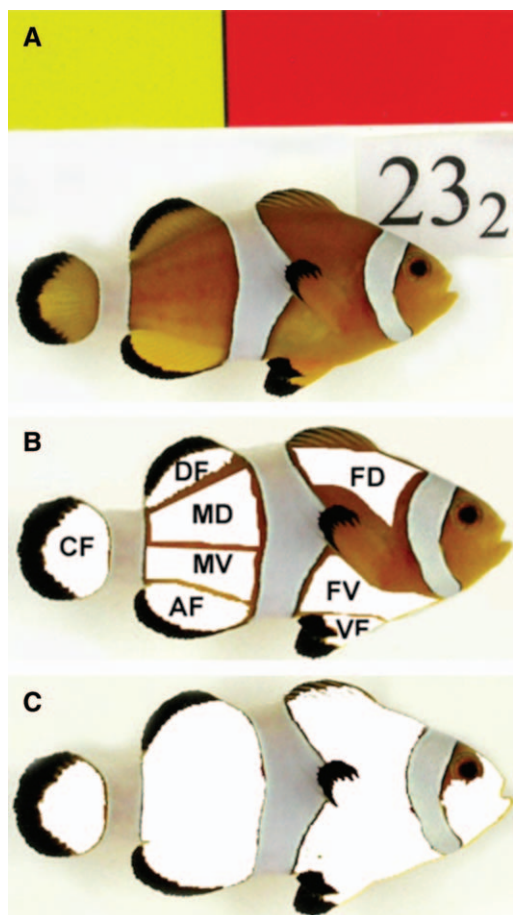


FIGURE 2. Body sections for image analyses. (A) The yellow and red reference card together with fish specimen "232"; (B) a fish image divided into dorsal parts (front dorsal [FD] and middle dorsal [MD]), ventral parts (front ventral [FV] and middle ventral [MV]), and four fins: caudal fin (CF), anal fin (AF), dorsal fin (DF), and ventral fin (VF) fins; (C) parts used for the whole-body analysis.

pigment extraction. The striped fish skin was then transferred to a beaker with 10 mL of solvent acetone : hexane (7 : 3, v/v) and allowed extraction for 10 min. This procedure was repeated three times for pigment extraction. The solvent was then evaporated under vacuum (50 mBar) in a water bath at 50 C. The extracted sample was dissolved in a 10-mL four-solvent mixture of hexane : ethanol : acetone : toluene (10 : 6 : 7 : 7, v/v).

Into the 8-mL extracted solution (after 2 mL used for spectrophotometer), 2 mL of 40% methanolic potassium hydroxide was added. The mixture then was heated at 50 C while being stirred for 10 min. The heated mixture was transferred to a separating funnel by adding 20 mL of the four-solvent mixture. Into it, 30 mL of 3% aqueous sodium sulfate solution was added, and the content was shaken for 30 sec. The top organic layer was separated from the bottom aqueous layer. The aqueous layer was further extracted with another 20 mL of four-solvent mixture. After separation, the organic layer was combined and washed with 40 mL of 3% aqueous sodium chloride solution. The organic layer was then separated and washed with 40 mL distilled water to remove the excess alkali from the organic layer. The organic layer was made up to 40 mL by adding the four-solvent mixture. From here, 2 mL of the mobile phase was added and the solvent was evaporated.

Into the extracted sample, 2 mL of the mobile phase was added and filtered through a 0.25- μ m filter. Normal phase high-performance liquid chromatography (HPLC) was applied using Luna 3 μ -silica (2) 100A (150 \times 4.6 mm) with security guard cartridge silica (4 \times 3.00 mm; Phenomenex, Torrance, CA, USA) and hexane : acetone (81 : 19, v/v). The flow rate was 1.1 mL/min with a 20- μ L injection. The detector was set in a wavelength of 474 nm. Total amount of carotenoids (μ g/g skin) was obtained from the sum of astaxanthin, β -carotene, canthaxanthin, and zeaxanthin. The percentage of each carotenoid type was attained as a relative abundance to the total amount.

Natural sources of canthaxanthin, β -carotene, zeaxanthin, and astaxanthin (Sigma, St. Louis, MO, USA) were used to make the standard solution for the HPLC analysis. The amount of 3.75 mg of each carotenoid was dissolved in a 25-mL volumetric flask with 5 mL of chloroform and sonicated in a water bath to aid solubility. The solution then was made up to 25 mL with acetone as a stock solution. The stock solution was wrapped with aluminum foil and stored in -20 C before use. Standard solution was

TABLE 1. Repeated measures ANOVA results showing procedures of data analysis and the impact of light intensity (LI) on the color responses (hue, saturation, brightness) of the whole body, dorsal-ventral position (D-V), and fins. LI was a between-subject factor, while time, body position, and fins were within-subject factors.¹

Source	Hue			Saturation			Brightness						
	df	MS ²	F	P	df	MS	F	P	df	MS	F	P	
Whole body	Between-subject effects												
	LI	2	8.8213	1.949	0.223	2	11.359	0.369	0.706	2	119.943	10.379	0.011
	Error	6	4.526			6	30.795			6	11.556		
	Within-subject effects												
	Time	2	3.237	0.35	0.712	2	79.154	3.128	0.081	2	1.545	0.159	0.854
	Time × LI	4	4.097	0.443	0.776	4	63.041	2.491	0.099	4	31.771	3.273	0.049
	Error	12	9.25			12	25.303			12	9.707		
	Between-subject effects												
	LI	2	13.18	2.103	0.165	2	5.168	0.090	0.914	2	360.950	15.523	0.0001
	Error	6	6.268			12	57.246			12	23.252		
	Within-subject effects												
	D-V	1	95.642	15.26	0.002	1	0.402	0.007	0.935	1	1974.94	84.935	0.0001
D-V × LI	2	2.592	0.414	0.67	2	96.548	1.687	0.226	2	3.255	0.140	0.871	
Time	2	51.692	5.305	0.012	2	62.107	1.750	0.195	2	41.178	2.777	0.082	
Time × LI	4	7.883	0.809	0.532	4	109.625	3.089	0.035	4	56.466	3.809	0.016	
Time × D-V	2	1.608	0.165	0.849	2	13.211	0.372	0.693	2	2.660	0.179	0.837	
Time × LI × D-V	4	1.244	0.128	0.971	4	11.466	0.323	0.860	4	1.305	0.088	0.985	
Error	12	9.743			24	35.490			24	14.826			
Between-subject effects													
LI	2	104.16	7.696	0.003	2	215.871	4.329	0.025	2	361.229	20.838	0.0001	
Error	24	13.535			24	49.863			24	17.335			
Within-subject effects													
Fins	3	1526.9	112.8	0.0001	3	10058.209	201.716	0.0001	3	3236.475	186.703	0.0001	
Fins × LI	6	20.225	1.494	0.222	6	29.414	0.590	0.735	6	47.659	2.749	0.035	
Time	2	35.137	1.622	0.208	2	150.752	3.513	0.038	2	55.498	4.396	0.018	
Time × LI	4	22.864	1.056	0.389	4	305.411	7.117	0.0001	4	76.801	6.083	0.0001	
Time × fins	6	17.851	0.824	0.557	6	41.979	0.978	0.450	6	7.803	0.618	0.715	
Time × LI × fins	12	27.475	1.269	0.268	12	16.103	0.375	0.966	12	17.285	1.369	0.214	
Error	48	21.659			48	42.911			48	12.625			

¹ Values in bold represent significant differences.

² Mean square.

TABLE 2. One-way repeated measurement ANOVA results for the impact of light intensity (LI) on pigment contents of the fish skin.¹

Source	Astaxanthin			β-carotene			Canthaxanthin			Zeaxanthin		
	df	MS ²	F	P	df	MS	F	P	df	MS	F	P
Between-subject effects												
LI	2	45.166	3.02	0.124	2	37.691	2.567	0.156	2	0.063	0.602	0.578
Error	6	14.956			6	14.683			6	0.105		
Within-subject effects												
Time	2	50.987	5.312	0.022	2	172.761	10.282	0.003	2	7.731	7.282	0.008
Time × LI	4	20.761	2.163	0.135	4	24.603	1.464	0.273	4	1.756	1.654	0.225
Error	12	9.599			12	16.803			12	1.062		

¹ Values in bold represent significant differences.

² Mean square.

made by diluting the stock solution into different concentrations before the HPLC analysis.

Statistical Analysis

The data were analyzed using the SPSS (version 13). Three tests were used to assess how light intensity affected the color expression in different parts of the fish body. The first test was one-way repeated ANOVA to analyze the impact of light intensity on the color expression (HSB) of the whole fish skin and pigment composition (astaxanthin, canthaxanthin, β-carotene, and zeaxanthin) over time (Tables 1 and 2). Secondly, to further explore the effect of light intensity on skin color, the fish body was divided into two parts, that is, middle dorsal (MD) and middle ventral (MV) parts to partition the within-subject variance. In this test, time and body parts were treated as within-subject factors and light intensity was a between-subject factor to examine interactions between time and light intensity and the response of different parts of the body. Last, the impact of light on four fins: caudal fin (CF), dorsal fin (DF), anal fin (AF), and ventral fin (VF) were evaluated using repeated measures ANOVA with time and fins as within-subject factors and light intensity as the between-subject factor (Table 1). If a significant difference between or with subjects was detected, pairwise comparisons with Bonferroni test were used. The significant level of difference was set at $P < 0.05$.

Results

Whole Body

There was no significant interaction between treatment and time on color hue ($P = 0.776$) or saturation ($P = 0.099$) of the whole body. Regardless of time, light intensity had no significant impact on either color hue ($P = 0.223$) or saturation ($P = 0.706$; Table 1). On the other hand, significant interactions between light intensity and time were found on color brightness ($P = 0.049$). In Week 3, fish color at low light intensity was brighter than that at high light intensity ($P = 0.048$; Fig. 3) but was not different from that at medium light ($P = 0.681$). By Week 5, fish were significantly

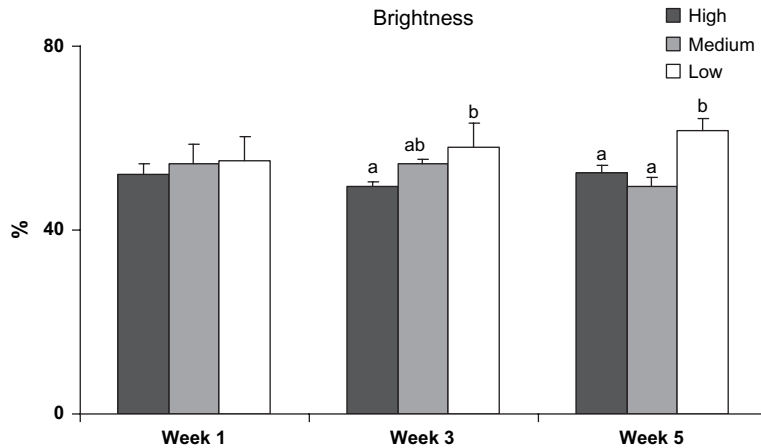


FIGURE 3. Color brightness of the whole fish over time at low, medium, and high light intensities. The values represent the means \pm SEM. Means with different letters denote significant difference ($P < 0.05$).

brighter at low light than those at either high ($P = 0.003$) or medium light ($P = 0.001$).

Body Parts

Because the light came from the top of the aquarium, the amount of illumination received at the dorsal and ventral parts of the fish were different. In the data analysis, after the fish image was divided into dorsal and ventral parts, the treatment effect on color performance of the dorsal and ventral parts was different from the results on the whole body (Table 1; Fig. 4). No significant interaction was found between time and light intensity on color hue of the dorsal and ventral parts ($P = 0.532$). The hue value of the fish dorsal or ventral part increased over time ($P = 0.012$) but was not affected by light intensity ($P = 0.165$). In contrast, color brightness of dorsal and ventral parts was affected by light intensity ($P = 0.0001$) but not by time alone ($P = 0.082$). The effect of light on the brightness of dorsal and ventral parts appeared by Week 3 after the experiment started. Dorsal and ventral parts at low light intensity were significantly brighter than those at high light intensity ($P \leq 0.045$) but did not differ from fish under medium light ($P = 1.000$). After 5 wk, dorsal and ventral parts at low light intensity were significantly brighter than those at medium ($P = 0.001$) or high light intensity ($P = 0.003$).

Dorsal and ventral parts were significantly different in color hue ($P = 0.002$) and brightness ($P = 0.0001$). The dorsal part was more orange (36°) than the ventral side (38°). However, fish color at the ventral side was brighter (58%) than at the dorsal side (46%). Color saturation was not different between dorsal and ventral parts ($P = 0.935$) or between light levels ($P = 0.914$). However, there was an interaction between week and light intensity ($P = 0.035$) on color saturation. At medium light, dorsal and ventral parts were more saturated in Week 1 than in other weeks ($P = 0.045$), but no difference was found between Week 3 and Week 5 ($P > 0.05$).

Fins

Light intensity significantly affected the hue of fish fins ($P = 0.003$; Table 1; Fig. 5). Fins under high light intensity were more orange (low hue value) than those under low light or medium light ($P \leq 0.02$), but the fin hue did not differ between low and medium light ($P \geq 0.118$). Furthermore, the hue was significantly different between fins ($P = 0.0001$). Both dorsal fin and ventral fins were more orange (low hue value) than the anal fin or the caudal fin ($P = 0.0001$), while the anal fin had lower hue value (41°) than the caudal fin (49° ; $P = 0.0001$). No significant difference was found between the dorsal fin and the ventral fin ($P = 0.142$).

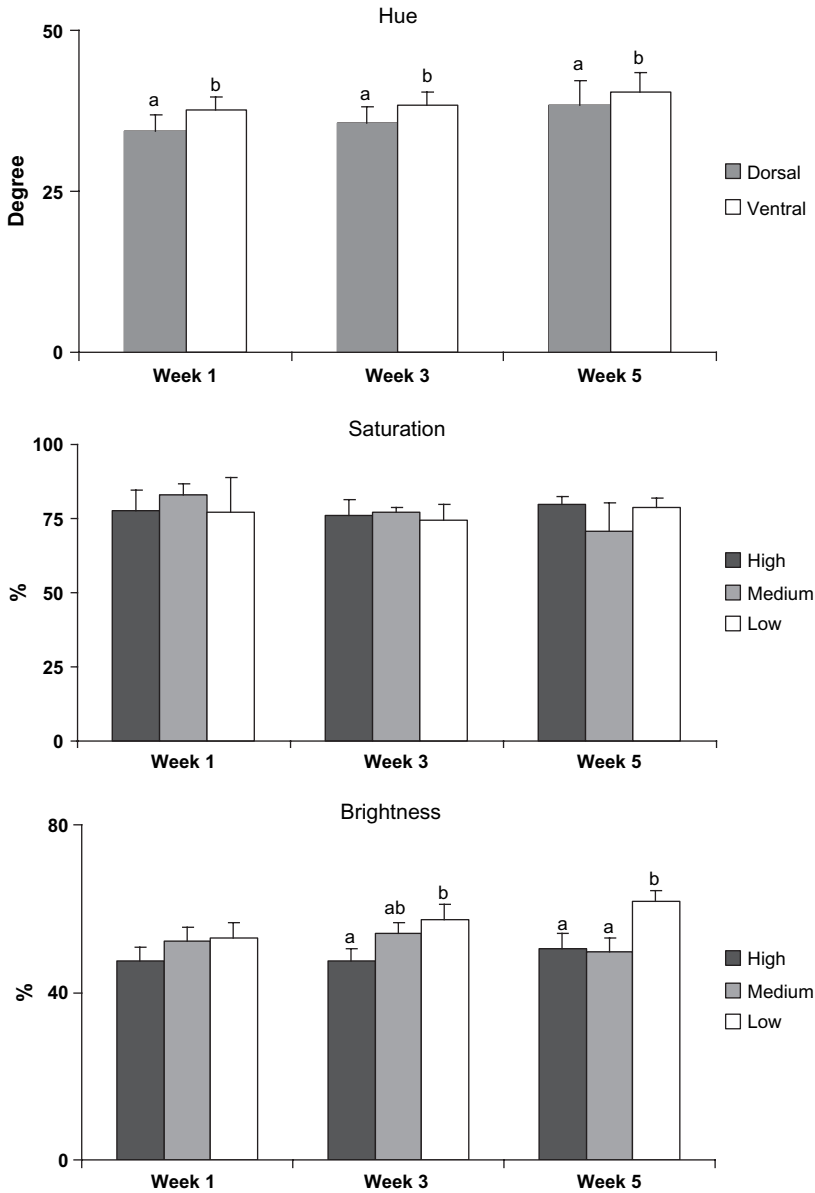


FIGURE 4. Color hue, saturation, and brightness of the ventral and dorsal body parts over time at low, medium, and high light intensities. The values represent the means \pm SEM. Means with different letters denote significant difference ($P < 0.05$).

The impact of light level on fin color saturation was significantly dependent on time ($P = 0.0001$; Table 1; Fig. 6). The fin color became more saturated after 5-wk exposure to high light ($P = 0.030$). In contrast, at medium light, color became less saturated after 5 wk ($P = 0.009$), while under low light, fin color reduced saturation

after 3-wk light exposure ($P = 0.0001$) but slightly gained by Week 5. The highest saturation value occurred on the ventral fin with the descending order of anal fin, caudal fin, and dorsal fin. The fin position significantly affected color saturation ($P = 0.0001$). Regardless of light intensity, colors of the anal fin and ventral fins were more saturated

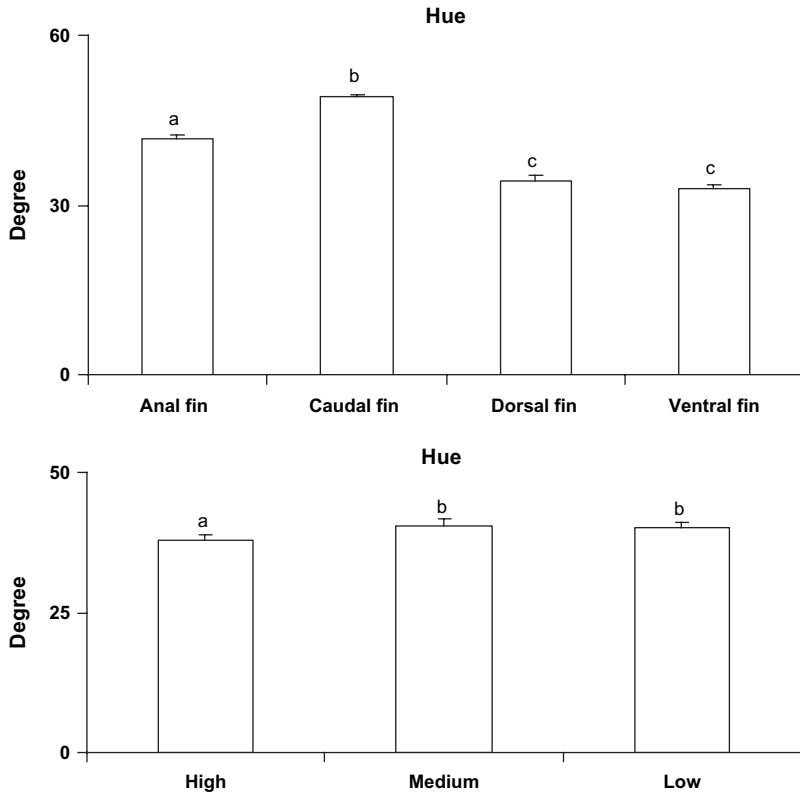


FIGURE 5. Effect of fin position and light intensity on fin color hue. The values represent the means \pm SEM. Means with different letters denote significant difference ($P < 0.05$).

than both caudal fin and dorsal fins ($P < 0.05$), and the caudal fin was more saturated than the dorsal fins ($P < 0.05$). However, color saturation between bottom fins (i.e., anal and ventral fins) was not different ($P > 0.05$).

There was a significant effect of light intensity on fin brightness over time ($P = 0.0001$; Table 1; Fig. 7). Fish under medium or low light for 3 wk had brighter color than fish at high light ($P \leq 0.004$). However, if the same light condition continued until Week 5, fish under low light was significantly brighter than that at medium or high light ($P \leq 0.0001$).

Each of the fins reacted differently when exposed to different light intensities ($P = 0.035$). No effect of light intensity was found on the anal fin or ventral fin ($P \geq 0.092$). In contrary, both caudal and dorsal fins were affected by the light level. Fish under low light had brighter caudal fin than fish under medium

or high light ($P \leq 0.043$), while the dorsal fin of the fish under low or medium light was brighter than the fish under high light ($P \leq 0.010$).

Carotenoid Analysis

The β -carotene content was the principal pigment, accounting for $>80\%$ of the total carotenoids. The zeaxanthin content was ranked the second, and the astaxanthin and canthaxanthin were the two lowest. The impact of light intensity on astaxanthin, β -carotene, and canthaxanthin was not significant ($P > 0.124$; Table 2; Fig. 8) but significant on zeaxanthin ($P = 0.014$). Significant time effects were detected on astaxanthin, β -carotene, and canthaxanthin ($P < 0.05$). However, the pairwise comparison on the percent astaxanthin did not show any changing by time ($P \geq 0.062$).

Canthaxanthin at Week 5 was higher than at Week 3, but β -carotene was lower than at Week

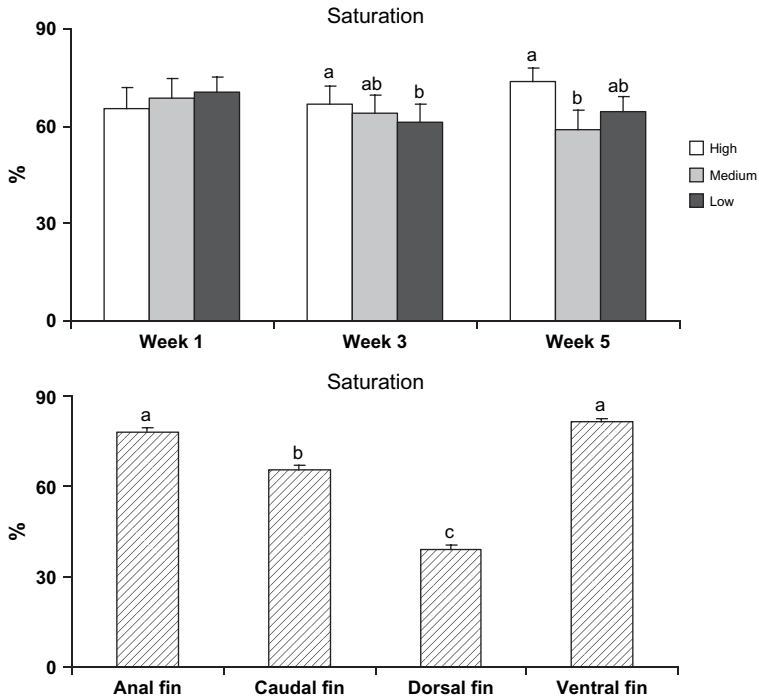


FIGURE 6. Overall fin color saturation over time and specific fin color saturation at each light intensity. The values represent the means \pm SEM. Means with different letters denote significant difference ($P < 0.05$).

3 ($P = 0.018$). Zeaxanthin at low light intensity was higher than at either medium or high light intensity ($P \leq 0.028$), but no difference was found between medium and high light ($P = 1.000$).

Discussion

Color is an important trait in the ornamental fish trade (Hoff 1996). According to Tanaka et al. (1992), false clownfish with reddish-orange color were more preferred by aquarists. The present study showed that ambient light intensity could change color traits not only on the major body but also on fins. In the HSB color system, the red–orange color was between 20 and 36°. In this study, the hue value of the fish skin ranged between 34 and 46°, suggesting that the color of clownfish varies from orange to yellow. The HSB color system offers a quantitative measure for the color expression, which is more objective than the color measurement in other fish color studies based on color charts (Paripatananont et al. 1999).

Light intensity has been considered a limiting factor in aquaculture (Boeuf and Le Bail 1999). For instance, in Chinese longsnout catfish, *Leiocassis longirostris*, growth was reduced when light intensity reached 434 lx (Han et al. 2005). In bright light, fish could increase swimming activity and visual acuity and increase their growth (Puvanendran and Brown 1998). In dim light, growth was compromised but cannibalism was reduced in juvenile barramundi, *Lates calcarifer* (Qin et al. 2004). In this study, light did not change survival in clownfish but significantly affected fish skin brightness. Under low light intensity (20–50 lx), the clownfish displayed brighter color than at higher light intensities (>600 lx). In contrast, the light intensity for Chinese longsnout catfish displaying optimum body color was 434 lx (Han et al. 2005), and the illumination required for sea bream to reach its maximum brightness was 600 lx (Gines et al. 2004). This reversed relationship between light intensity and brightness was also reported

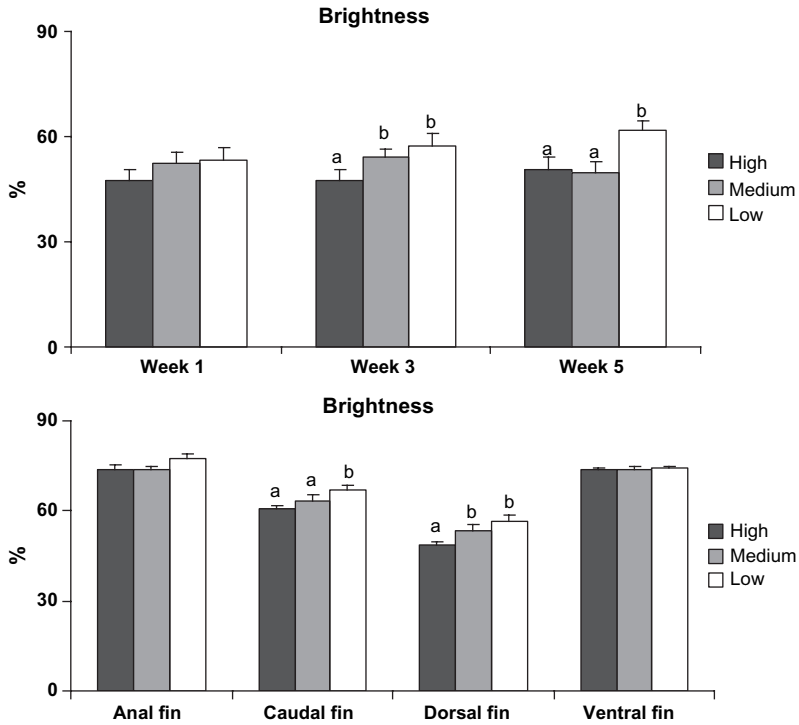


FIGURE 7. Overall fin brightness over time and specific fin brightness at different light intensity. The values represent the means \pm SEM. Means with different letters denote significant difference ($P < 0.05$).

on snapper (Booth et al. 2004). Snapper in cages with 50% light reduction appeared brighter than those in nonshaded cages (Booth et al. 2004). In the present study, when clownfish became brighter under low light level, the content of astaxanthin was not significantly changed. In snapper, however, the reduction of light level significantly reduced the concentration of astaxanthin in the fish skin (Booth et al. 2004).

In this study, neither color hue nor saturation was affected by light-intensity manipulation, which is different from some previous studies. In Chinese longsnout catfish, when light intensity increased from 5 to 434 lx, skin color turned darker (Han et al. 2005). Similarly, in snapper, the addition of shade covers significantly reduced fish skin darkness, but there was no difference between the skin color of fish held under either 50 or 95% shade cover (Booth et al. 2004). Odiorne (1957) suggested that fish skin pigmentation could be changed either by increasing the number of melanophores or by increasing the amount of pigment in the melanophores. The change of

light illumination may alter the morphology of chromatophores (Fujii 2000), but it seems that the response of fish skin color to light intensity depends on species and possibly on age as well. In aquaculture, crowding could elevate concentrations of plasma cortisol and change the color of fish skin because of stress (Rotllant et al. 1997). Van der Salm et al. (2004) reported that a high-density treatment (25 kg/m³) had a darkening effect on the skin color of red porgy, *Pagrus pagrus*, compared with a low-density (10 kg/m³) treatment. In our study, fish density was reduced by 25% at the end because of sampling, which might contribute to the color change over time. The possible impact of stocking density on the color change of clownfish warrants further investigation.

Because of differential exposure to the light source, the dorsal part of the fish skin often appears darker than the ventral side as in red porgy (Chatzifotis et al. 2005). In clownfish, the ventral part of the body was brighter than the dorsal part regardless of light intensity, but the dorsal

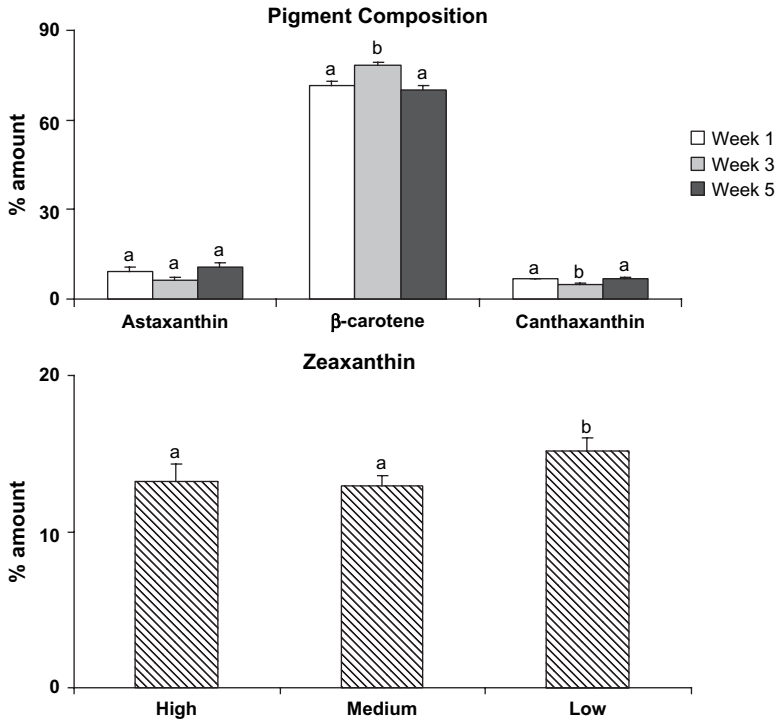


FIGURE 8. Changes of astaxanthin, β -carotene, and canthaxanthin in the fish skin over time and zeaxanthin in the fish skin at different light intensities. Bars represent means \pm SEM. Means with different letters denote significant difference ($P < 0.05$).

part showed more orange than the ventral part. In red porgy, the dark dorsal body was caused by the accumulation of melanophores (Chatzifotis et al. 2005), but the dominant pigment β -carotene in clownfish was not affected by light intensity. Direct illumination on the unpigmented area could trigger the expression of latent melanoblasts and pigments in the light receiving area (Odiome 1957). In this study, the direction of light was not manipulated, but the orange dorsal body seems as a result of more genetic cause than the light direction or intensity. The difference of color hue between dorsal and ventral sides did not change over the experimental period.

In the study of fish taxonomy, fin color can be a useful criterion for identification (Chapman and Fitz-Coy 1997). In the ornamental fish trade, fin color is an important trait for fish hobbyists to choose for breed (Chapman and Fitz-Coy 1997). Previous studies on the impact of light on fish color performance have paid little attention to fish fins. Hatanaka

(1997) reported that the ideal fin color of tiger puffer, *Takifugu rubripus*, was obtained in a black tank with low light condition (1000 lx). Similarly, in this study, low light provoked brighter fins, especially on caudal and dorsal fins. Interestingly, brighter light strengthened orange color on fish fins. In zebrafish, a mutant has been identified controlling the interaction of xanthophores and melanocytes to form the pigment pattern of the adult zebrafish fin (Mellgren and Johnson 2006). In anurans, light-sensitive melanophores were found on the tail fin of *Xenopus* tadpoles (Moriya et al. 1996). Our result suggests that the fin hue showed a more sensitive response to light intensity than the main fish body.

Four types of carotenoid were found on the false clownfish skin in this study, with β -carotene being the most common followed by zeaxanthin. This finding partially agreed with previous observations by Tanaka et al. (1992) for false clownfish reared under the laboratory condition

without addition of carotenoids to the diet. The content of astaxanthin and canthaxanthin accounted for 5–15% and 5–10%, respectively, but was not affected by light intensity. To our knowledge, the content of astaxanthin and canthaxanthin has not been previously reported in clownfish species.

The visual analysis agreed with the result of carotenoid contents on the fish skin. The dominance and paler (high hue value or from red to yellow) of β -carotene on the skin contributed to the yellow component of fish color under the captive rearing condition. Tanaka et al. (1992) reported that pinkish orange false clownfish should contain a high amount of zeaxanthin. Although light manipulation could not alter fish color from yellow orange to red orange by changing the dominant pigment from β -carotene to zeaxanthin, low light did enhance the percentage of zeaxanthin on fish skin in this study.

Fish and other vertebrates cannot synthesize carotenoids, but they have the ability to change color in given circumstances (Matsuno 2001). The false clownfish, like any other clownfish, naturally feeds on plankton (Allen 1991) that supplies all nutrition requirement including carotenoids. Like other animals, fish can metabolize one type of carotenoid and change to another type (Matsuno 2001). Tanaka et al. (1992) reported that clownfish have the ability to convert astaxanthin into canthaxanthin or zeaxanthin. In the present study, the amount of β -carotene, astaxanthin, and canthaxanthin in Week 1 was similar to that in Week 5, suggesting that clownfish could maintain the amount of carotenoids on their skin without carotenoid addition into their feed.

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