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Impact of Background on Color Performance of False Clownfish, *Amphiprion ocellaris*, Cuvier

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Abstract

Color performance of false clownfish, *Amphiprion ocellaris*, Cuvier was first examined at four color backgrounds (blue, green red, and white) for 4 wk, then all fish were transferred to a white background for another 4 wk to test whether the impact of background colors on fish skin could have a lasting effect when the environment colors are changed. The experiment was conducted in 10-L rectangular plastic buckets with three replicates. Thirty fish were stocked in each bucket and three fish were randomly sampled from each tank in Weeks 1, 4, and 8. The color hue, saturation, and brightness were quantified using image analysis. In addition to the whole body analysis, each fish image was divided into ventral and dorsal parts to examine the body position-dependent response. Furthermore, color differences among the dorsal fin, anal fin, ventral fin, and caudal fin were also quantified. Blue or green background enhanced red orange color on fish skin, whereas white background made fish color brighter. Irrespective of background color, the dorsal side of fish exhibited more red orange, but the color was less bright and less saturated than that of ventral side. Upper fins (dorsal and caudal fins) were more red orange in a blue background than in a white background. Transferring fish from colored backgrounds to a white background made the fish skin and fins brighter, the color of ventral body and ventral fins less saturated, and the bottom fins more yellow orange. The results indicate that blue or green background could strengthen the orange color, whereas white background made fish color less saturated but brighter. The impact of background on the performance of fish color is temporary and likely to disappear when environmental color changes.

Fish color can be altered by environmental factors through the rearrangement of chromatophores in the skin (Bagnara and Hadley 1973; Sugimoto 1993). Changes in color hue and pattern are considered as a result of physiological adaptation through chromatophore reallocation or a result of morphological adaptation by varying the amount of pigmentary materials under skin. According to Bagnara (1998), the change of background color could provoke physiological color adaptation, commonly known as camouflage, to lessen predator–prey interactions. Cryptic coloration enables animals to change their color property and patterns to blend into the background (Moyle and Cech 2004). Most color changes in fish result from the migration of melanophores, dark brown pigment cells, xanthophores, and iridophores (Oshima et al. 1989).

Tank color is known to influence survival and growth of fish larvae (Duray et al. 1996; Oshima et al. 1989; Tamazouzt et al. 2000), probably because a contrasting background enables better prey visualization and capture efficiency (Martin-Robichaud and Peterson 1998). Previous studies have mainly focused on the comparison of tank colors as a contrasting background, or between black and white tanks (Oshima et al. 1989; Duray et al. 1996; Downing and Litvak 2000), but have not been conducted across a broad color background. Background color could not only impact the feeding success but also affect the fish color performance. During the culture of aquatic

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animals, changes in pigmentation pattern and intensity are induced by changing the tank color (Fujimoto et al. 1991; Baker et al. 2002). The color of the aquaculture environment has reportedly affected the color of red porgy, *Pagrus pagrus* (Van der Salm et al. 2005), Atlantic cod, *Gadus morhua* (Bransden et al. 2005), sole, *Solea solea* (Ellis et al. 1997), and medaka, *Oryzias latipes* (Sugimoto 1993).

The response of fish skin color to background color varied from fish species and age. Red porgy, *P. pagrus*, kept in a white background had lighter color than when they were kept in a red background (Van der Salm et al. 2005). Similarly, sole, *S. solea,* could match their skin color "tone" to the surrounding background under captivity (Ellis et al. 1997). However, the larvae of Atlantic cod, *G. morhua*, kept in red and gray backgrounds showed no significant difference on their skin color (Bransden et al. 2005). Fox (1957) reported that no variation of xanthophyll was observed in juvenile trout, *Fundulus parvipinnis*, when fish were transferred from dark to light containers or even to total darkness for 6 wk. Inconsistent results were found with immature opaleye, *Girella nigricans,* where the rate of color loss was more rapid when the fish were kept in a white background than in a yellow or red background (Sumner and Fox 1935). Despite the discrepancy among fish species, these reports support the idea that fish kept in a particular background color for a certain period of time could not only change their skin color but also alter the responsiveness of melanophores to neural and hormonal factors that control the chromatophores motility (Oshima et al. 1989).

False clownfish, *Amphiprion ocellaris*, are one of the most popular ornamental fishes because of their color pattern, behavior, and ease of handling in aquaria (Yasir and Qin 2007). According to Wabnitz et al. (2003), during 1997–2002, false clownfish was the most common marine aquarium species covering 15.6% of the total number of fish exported worldwide and over 25% into European countries. Thus, clownfish have been considered the "goldfish" of marine aquaria and its value is judged by skin color (Hoff 1996). Yasir and Qin (2009) reported that low light intensity could brighten the skin color of clownfish, and bright orange color could add to the commercial value in an aquarium trade. Based on the color composition of clownfish, we further provided four background colors, blue, green, red, and white, to the fish for 4 wk, and then separately moved all fish to a white background. With this design, we aimed to investigate the impact of background colors and their lasting effect on color performance of the clownfish. In this study, we not only analyzed the color response of the whole fish, but also quantified the color of the dorsal body, ventral body, anal fin, caudal fin, dorsal fin, and ventral fin. The result could provide insight into the strategies to design appropriate background environments for farming and keeping ornamental fish.

Materials and Methods

Experimental Design and Procedure

Three month-old, *A. ocellaris* (29.02 ± 3.36 mm standard length), were used in the experiment. All fish were hatched and reared in brown tanks under laboratory conditions at 26–27 C. The experimental fish were acclimatized by feeding on a gelatine-based diet without carotenoid addition. The experiment was conducted in twelve 10-L plastic buckets with four background colors (red, green, blue, and white) with white as control. Three replicates were used in treatment and each bucket was stocked with 30 fish. After the fish were allocated to each bucket, three fish were randomly collected from each bucket as the initial sample in Week 1. Three fish were then subsequently sampled each time at the end of Weeks 4 and 8 using the same sampling protocol as in Week 1.

The buckets were randomly suspended inside two 600-L tanks that were supplied with seawater in a recirculation system treated with a bio-filter and a mechanical filter. An airstone was placed into each tank for water circulation and aeration. The bottom of each bucket was cut open and replaced with a white plastic screen (1 mm mesh) allowing water exchange. In

addition, another airstone was placed inside the bucket to facilitate water exchange. Each bucket was capped with a plastic mesh to prevent fish escaping. Light intensity was 200–250 lx, provided by four bulbs (36 W, Osram, Germany) located 2 m above the tanks. Oxygen level was 6 mg/L, salinity was 27–28%, and temperature was 26–27 C. The light period was controlled at 14 h light: 10 h dark.

During the 8-wk experiment, the fish were fed twice a day with a gelatine-based diet containing sea perch, *Lutjanus malabaricus*, flesh (80%), vitamin C (5%), and gelatin (15%) by weight. In Week 4, all fish in the red, green, blue, and white buckets were separately transferred into new white buckets to test whether fish color gained in the colored backgrounds could be retained.

Prior to visual analysis, live fish were anesthetized with tricaine methanesulfonate (MS-222) at a concentration of 70 mg/L. MS-222 is a water soluble compound which could reduce fish stress but did not produce any visually detectable color change. To further assess the impact of MS-222 on fish color during anesthesia, fish images were taken in a 5-min interval for 30 min, and no significant change was detected over time through image analysis. The duration of taking photographs for each experimental fish was only 2–5 min; therefore, neither the anesthetic treatment nor the duration for taking images affected fish color measurements. Soon after the fish images were taken, each fish was placed in a sealed plastic bag covered with aluminum foil. The specimen was frozen at −20 C until carotenoid analysis. The same protocol was used throughout the experimental sampling.

Procedures for image and carotenoid analyses were described in detail in Yasir and Qin (2008). In brief, photographs were taken under four natural white color bulbs. A digital camera was situated on an adjustable arm between the two light sides. The camera was set up 25 cm above the fish and could capture the whole fish image along with yellow and red reference cards (Kodak, CAT 152 7662, Q-14) underneath the container. The image was analyzed with Adobe Photoshop software (version 7.0.1). In addition to the analysis for the whole body skin, the fish body was further divided into the dorsal part and the ventral part for color analysis. Furthermore, four fish fins–caudal fin, dorsal fin, ventral fin, and anal fin–were separately scanned for color analysis to test the fin position-dependent effect.

The hue–saturation–brightness (HSB) color model was used to quantify the color property (Georgieva et al. 2005). The HSB model breaks the color into three components: the hue (i.e., how "pure" the color is), the percentage of saturation (i.e., how "much" the color is), and the brightness. Hue is the actual color and is measured in angular degrees around the cone starting and ending at red, which is equal to 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.

Carotenoids were extracted from fish skin and all fins with solvent acetone: hexane (7:3 v/v) for 10 min (Yasir and Qin 2009). Normal phase high performance liquid chromatography (HPLC) was applied using Luna 3 μ silica (2) 100 Å(150 × 4.6 mm) with security guard cartridge silica (4 × 3.00 mm; Phenomenex) and hexane : acetone (81:19 v/v). The flow rate was 1.1 mL/min with a 20-μL injection. The detector was set in a wavelength of 474 nm. Total amount of carotenoids (μg/g skin) was obtained from the sum of astaxanthin, β-carotene, canthaxanthin, and zeaxanthin. Natural sources of canthaxanthin, β-carotene, zeaxanthin, and astaxanthin (Sigma) were used to make the standard solution for the HPLC analysis. Standard solution was made by diluting the stock solution into different concentrations prior to the HPLC analysis.

Statistical Analysis

The data were statistically analyzed using SPSS (version 13) with three protocols. The first test was a one-way repeated ANOVA to analyze the impact of background color on the

color property (hue, saturation, and brightness) of the whole fish skin and the pigment composition (astaxanthin, canthaxanthin, β-carotene, and zeaxanthin) over time. To further explore the effect of background on skin color, the fish body was divided into dorsal and ventral parts. In this test, time and body parts were treated as within-subject factors and background was a between-subject factor to examine interactions among time, background color, and the response of different body parts. Finally, the impact of background on four fins–caudal fin, dorsal fin, anal fin, and ventral fin–was evaluated using a repeated measure ANOVA with time and fins as within-subject factors and the initial background color as the between-subject factor (Table 2). If a significant difference between or within subjects was detected, pairwise comparisons with Bonferroni test were used. The significant level of difference was set at $P < 0.05$.

Results

Whole Body

Repeated measure ANOVA showed that the background color significantly affected the color hue of whole fish skin ($P = 0.004$; Table 1; Fig. 1). Fish color was more red orange (low hue value) in a blue or green background than in a white background ($P < 0.05$, Bonferroni test), whereas no color difference between red and white backgrounds or between blue and green backgrounds was detected ($P > 0.05$). For color saturation, there was an interaction between background color and time ($P = 0.038$; Table 1). After 4-wk exposure to colored backgrounds, fish did not alter color saturation ($P > 0.05$), but when these fish were separately moved from the color background to a white background for 4 wk, fish showed higher color saturation in the white background than those previously exposed to colored backgrounds ($P < 0.05$; Bonferroni test; Fig. 1).

TABLE 1. *Repeated measure ANOVA table showing procedures of data analysis and the impact of background color on the color response (hue, saturation, and brightness) of the whole body and dorsal–ventral body parts. Background color was the between-subject factor, while time and body position were within-subject factors. The values with bold numbers represent significant differences.*

		Hue				Saturation				Brightness			
	Source	df	MS	*F*	*P*	df	MS	*F*	*P*	df	MS	*F*	*P*
Whole body	Between-subjects effects												
	BC	3	90.907	10.198	**0.004**	3	38.801	3.044	0.092	3	38.71	3.492	0.07
	Error	8	8.914	—	—	8	12.745	—	—	8	11.086	—	—
	Within-subjects effects												
	Week	2	26.87	1.862	0.187	2	8.375	0.682	0.52	2	260.137	15.719	**0.0001**
	Week × BC	6	22.682	1.572	0.219	6	36.477	2.972	**0.038**	6	31.06	1.877	0.147
	Error (week)	16	14.429	—	—	16	12.275	—	—	16	16.549	—	—
Body parts (dorsal-ventral)	Between-subjects effects												
	BC	3	173.474	11.2713	**0.003**	3	118.924	9.456	**0.005**	3	72.258	3.439	0.072
	Error	8	15.391	—	—	8	12.577	—	—	8	21.01	—	—
	Within-subjects effects												
	Body part	1	301.952	203.398	**0.0001**	1	548.681	450.761	**0.0001**	1	2385.283	3388.954	**0.0001**
	Body part × BC	3	3.287	2.214	0.164	3	2.746	2.256	0.159	3	1.366	1.941	0.202
	Error (body part)	8	1.485	—	—	8	1.217	—	—	8	0.704	—	—
	Week	2	32.544	1.103	0.356	2	129.008	9.128	**0.002**	2	433.119	29.657	**0.0001**
	Week × BC	6	29.533	1.001	0.458	6	50.184	3.551	**0.02**	6	44.787	3.067	**0.034**
	Error (week)	16	29.506	—	—	16	14.133	—	—	16	14.604	—	—
	Body part × week	2	1.973	1.272	0.307	2	17.23	7.502	0.105	2	6.563	3.419	0.058
	Body part × week × BC	6	1.087	0.7	0.653	6	2.286	0.995	0.461	6	1.25	0.651	0.689
	Error (body part × week)	16	1.552	—	—	16	2.297	—	—	16	1.92	—	—

BC = background color.

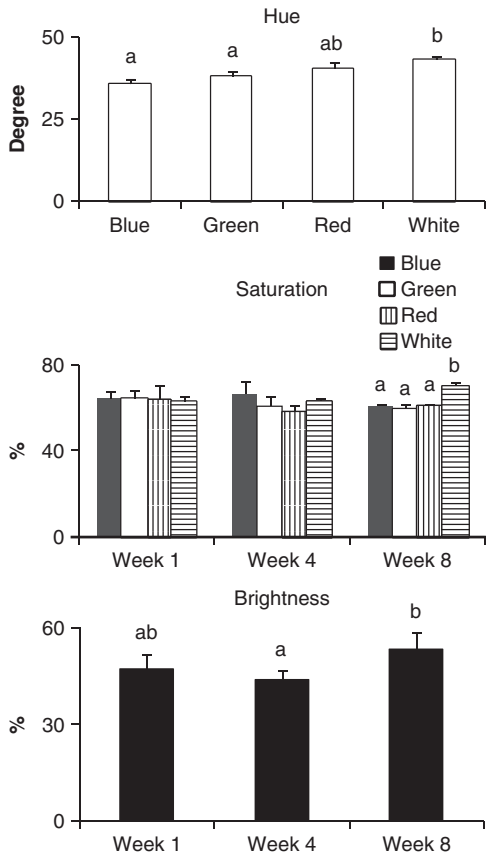

FIGURE 1. *Responses of whole fish skin color hue, saturation, and brightness to background color (blue, green, red, and white) over time. All fish were separately transferred from color background to white on Week 4. The values represent the means ± SE. Means with different letters denote significant differences (*$P > 0.05$*).*

Fish brightness was not changed by the background color ($P = 0.07$; Table 1), but was significantly influenced by time ($P = 0.0001$). After fish were exposed to color background for 4 wk, fish brightness was slightly reduced, but when fish were moved from color backgrounds to a white background for 4 wk, fish brightness was significantly increased ($P < 0.05$; Bonferroni test) regardless of previous exposure to colored backgrounds.

Dorsal and Ventral Body

Regardless of background color, the dorsal part showed more red orange (i.e., low hue value) than the ventral part ($P = 0.0001$; Table 1; Fig. 2). Considering both dorsal and ventral parts, the background color significantly affected fish color hue ($P = 0.003$; Table 1; Fig. 2). The dorsal or ventral parts were more red orange in the blue, green, or red background than in the white background ($P < 0.05$), whereas no color difference between blue and green, or between blue and red backgrounds was detected ($P > 0.05$; Bonferroni test; Fig. 2).

The ventral color was more saturated than the dorsal color throughout the experimental period ($P < 0.0001$; Table 1; Fig. 2). However, the impact of background on the color saturation of both dorsal and ventral parts depended on time ($P = 0.02$; Table 1; Fig. 2). After fish were exposed to different color backgrounds for 4 wk, no significant difference in fish color saturation was observed between fish in various backgrounds ($P \geq 0.11$; Bonferroni test; Fig. 2). After these fish were separately transferred to a white background for 4 wk, the fish having been continuously kept in a white background exhibited more saturated color than fish previously exposed to a blue, green or red background ($P \leq 0.006$). In other words, more saturated fish color was only observed in fish that had been in white background for 8 wk. Fish color saturation did not differ among blue, green, and red backgrounds ($P \geq 0.64$).

Regardless of background color, the ventral body was brighter than the dorsal part and this status remained unchanged throughout the experimental period ($P = 0.0001$; Table 1; Fig. 2). However, the impact of background on the color brightness of both dorsal and ventral parts depended on time ($P = 0.034$; Table 1; Fig. 2). One week after the experiment started, fish in the white background was significantly brighter than fish kept under a green background ($P = 0.031$; Bonferroni test), but did not differ from fish kept under a blue or green environment ($P \geq 0.165$). In comparison, after fish were exposed to different background colors for 4 wk, no difference in fish color brightness was detected among white, blue, red, and green backgrounds ($P > 0.05$) and this status did not change after these fish were separately

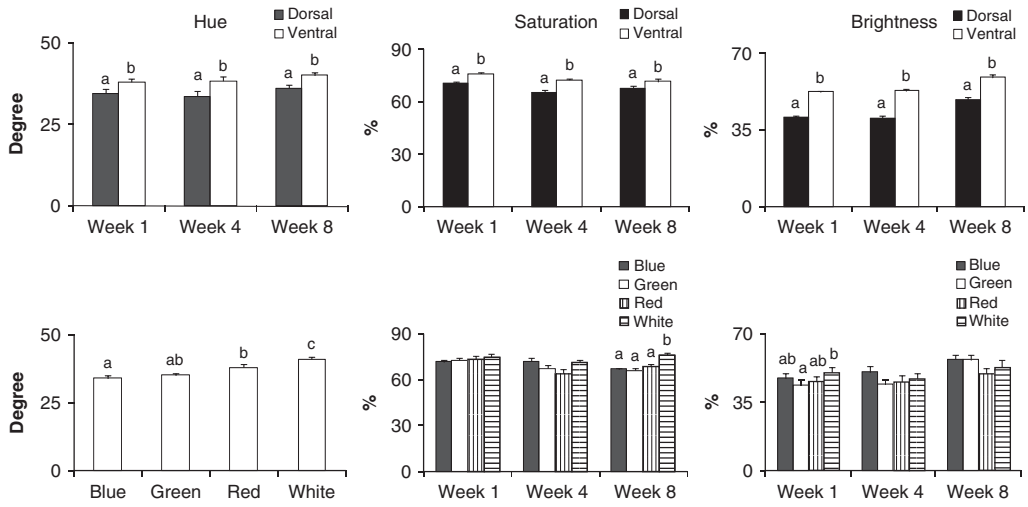

FIGURE 2. *Impact of background colors on fish color hue, saturation, and brightness between ventral and dorsal body over time. The values represent the means ± SE. Means with different letters denote significant differences* ($P < 0.05$).

transferred to a white background ($P > 0.05$; Fig. 2).

Fins

The hue value of fish fins ranged from 34° (red orange) to 52° (yellow orange) and were significantly affected by the interaction of fin position and background ($P = 0.03$; Table 2; Fig. 3). The dorsal fin of fish in a blue background was significantly more red orange (low hue) than that in a white or red background ($P \leq 0.017$; Bonferroni test; Fig. 3), whereas no hue difference of the dorsal fin was detected among the white, green, and red backgrounds ($P > 0.05$). Similarly, the caudal fin of the fish in a blue background was more red orange than in a white background ($P \leq 0.02$; Fig. 3), but no hue difference of the caudal fin was detected among the white, green, and red backgrounds ($P > 0.05$). Background color did not affect the hue of either anal fin or ventral fin ($P \geq 0.19$).

The color hue of fish fins was also significantly affected by the interaction of fin position and time ($P = 0.0001$; Table 2; Fig. 3). In contrast to the background effect, the hue of bottom fins (anal and ventral fins), rather than upper fins (dorsal and caudal fins), was significantly affected by the time of exposure to color backgrounds. Both anal and ventral fins became yellowish (i.e., high hue value) after fish were kept in a color background for 4 wk. Interestingly, the yellow orange on these fins continued increasing and became significant after these fish were separately transferred to a white background for four more weeks ($P \leq 0.005$).

The change of overall fin color saturation depended on time ($P = 0.001$; Table 2). The fin saturation did not change in the first 4 wk ($P > 0.05$; Bonferroni test; Fig. 3), but decreased after these fish were transferred to a white background for 4 wk ($P < 0.05$). Regardless of background color, the anal fin, caudal fin, and dorsal fin were slightly less saturated by the end of the Week 4, but became significantly less saturated by Week 8 when these fish were transferred from colored backgrounds to a white background for 4 wk ($P < 0.09$; Fig. 2).

After being kept in color backgrounds for 4 wk, the overall fin brightness increased after fish were transferred to a white background for 4 wk ($P < 0.05$; Fig. 3). Background color did not significantly affect the brightness of fins ($P = 0.327$; Table 2), but it seemed that bottom fins were brighter than upper fins with anal fin being the brightest (73%) followed by ventral fin (69%), caudal fin (60%), and dorsal fin (49%). The variation of fin brightness depended

TABLE 2. *Repeated measure ANOVA table showing procedures of data analysis and the impact of background color on the color response (hue, saturation, and brightness) of the whole body, dorsal–ventral body parts (D–V), and fins. Background color was the between-subject factor, while time, body position and fins were within-subject factors. The values with bold numbers represent significant differences.*

		Hue				Saturation				Brightness			
	Source	df	MS	*F*	*P*	df	MS	*F*	*P*	df	MS	*F*	*P*
	Between-subject effects												
	BC	3	154.641	5.063	**0.03**	3	124.083	5.325	**0.026**	3	26.336	1.343	0.327
	Error	8	30.541	—	—	8	23.3	—	—	8	19.604	—	
	Within-subjects effects												
	Fins	3	607.479	200.633	**0.0001**	3	5608.119	707.873	**0.0001**	3	4099.311	797.44	**0.0001**
	Fins × BC	9	7.885	2.604	**0.03**	9	11.784	1.487	0.209	9	8.417	1.637	0.161
Fins	Error (fins)	24	3.028	—	—	24	7.922	—	—	24	5.141	—	—
	Week	2	42.161	1.308	0.298	2	668.702	10.462	**0.001**	2	267.925	6.679	**0.008**
	Week × BC	6	41.859	1.299	0.313	6	132.454	2.072	0.114	6	98.154	2.447	0.072
	Error (week)	16	32.222	—	—	16	63.916	—	—	16	40.116	—	—
	Fins × week	6	51.207	19.779	**0.0001**	6	29.589	2.595	**0.029**	6	31.568	4.068	**0.002**
	Fins × week × BC	18	4.91	1.896	**0.04**	18	9.475	0.831	0.657	18	8.311	1.071	0.407
	Error (fins × week)	48	2.589	—	—	48	11.401	—	—	48	7.76	—	—

BC = background color.

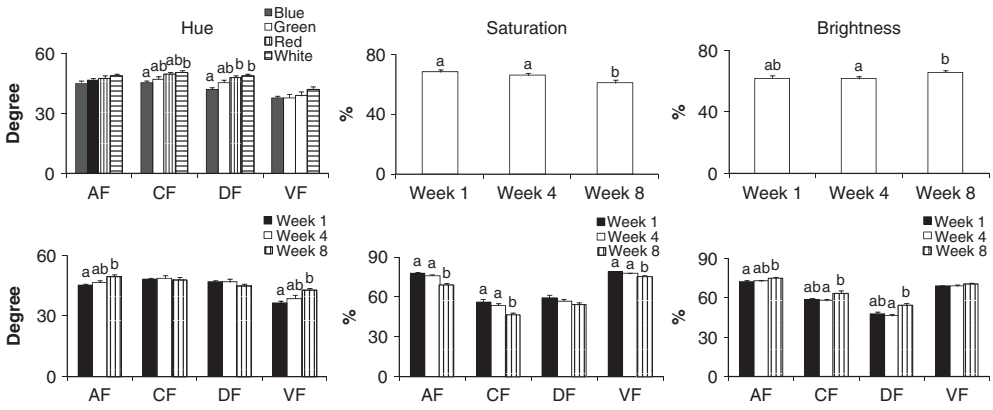

FIGURE 3. *Impact of background on the color hue, saturation, and brightness of anal fin, caudal fin, dorsal fin, and ventral fin. The values represent the means ± SE. Means with different letters denote significant differences (*$P < 0.05$*).*

on the fin position and time of exposure to the background color ($P = 0.002$; Table 2). The brightness of ventral fin did not vary over time ($P > 0.05$), but the anal fin, caudal fin, and dorsal fin became brighter after fish were transferred from color backgrounds to a white background for 4 wk ($P < 0.05$).

Carotenoid Analysis

Carotenoids composition analysis showed that β-carotene was the dominant pigment and accounted for >85% of the carotenoids during the experiment (Fig. 4). No pigments were affected by the background color ($P \geq 0.449$; Table 3), but all pigments except β-carotene were significantly affected by time ($P \leq 0.007$; Table 3). The amount of astaxanthin in fish skin significantly increased after 4 wk in the color backgrounds, but significantly reduced when fish were moved from colored backgrounds to a white background for 4 wk ($P < 0.05$). The amount of canthaxanthin was significantly

TABLE 3. *One-way repeated measurement ANOVA results for the impact of background color on pigment contents of the fish skin. The values with bold numbers represent significant differences.*

	Astaxanthin				β-carotene				Canthaxanthin				Zeaxanthin			
Source	df	MS	F	*P*	df	MS	*F*	*P*	df	MS	*F*	*P*	df	MS	*F*	*P*
Between-subjects effects																
BC	3	0.591	0.98	0.449	3	4.252	0.485	0.702	3	0.813	0.05	0.984	3	0.388	0.096	0.960
Error	8	0.603	—	—	8	8.765	—	—	8	16.312	—	—	8	4.044	—	—
Within-subjects effects																
Week	2	15.21	16.222	**0.0001**	2	43.683	6.806	**0.007**	2	174.079	11.841	**0.001**	2	47.975	16.069	**0.0001**
Week × BC	6	0.595	0.635	0.701	6	1.874	0.292	0.932	6	0.453	0.031	0.990	6	0.113	0.038	0.990
Error (week)	16	0.938	—	—	16	6.419	—	—	16	14.701	—	—	16	2.986	—	—

BC = background color.

reduced by 4-wk exposure to the colored backgrounds ($P < 0.05$; Fig. 3), and the canthaxanthin reduction was not recovered after fish were moved from the colored backgrounds to the white background for 4 wk. In contrast, the amount of zeaxanthin was slightly increased by the 4-wk exposure to the colored backgrounds, but its amount continued increasing despite the change from colored backgrounds to a white background for 4 wk ($P < 0.05$).

Discussion

The present study demonstrated that background color could affect the color expression of clownfish after a short term exposure. The reduction of hue value in a blue or green background enhanced red orange color of the whole fish skin, while fish kept in a white background appeared yellowish. The reaction of clownfish to the background color is similar to Australian snapper (Doolan et al. 2007). The skin color was significantly lighter in snapper held in white cages compared with snapper held in black cages. The monochromatic color of the experimental cages used by Doolan et al. (2007) proved to be the overwhelming factor governing the skin lightness of snapper. In another study, Van der Salm et al. (2004) reported that red porgy, *P. pagrus*, kept on a white background were significantly lighter than those kept in a red background. However, in our study, the red background did not seem to differ from the white background in regulating fish hue and brightness. Doolan et al. (2007) hypothesized that the cage color appears to have directly influenced the neuroendocrine system of snapper, which has in turn influenced the physiological state of the melanophores contained in their skin. Although the mechanisms involved for color adaptation to the background remain to be resolved, Rotllant et al. (2003) reported that red porgy, *P. pagrus*, were much less stressed in a white background than a gray or black background as shown by the level of stress hormones. The choice of tank color for the aquaculture use should consider the growth and stress responses of fish to the color background. The white tank seems more suitable for growing snapper because of low stress to fish and the light fish color it produces (Rotllant et al. 2003), whereas blue or green tanks may be more suitable to produce red orange clownfish, the favorite color for most aquarium hobbyists.

One of the motivations of this study was to test whether the gained color traits in fish from colored backgrounds would last if the clownfish were moved to a neutral color environment (i.e., white). In snapper, Doolan et al. (2007) reported that fish held in white cages became lighter in 2 d, but we found that the clownfish took much longer to significantly fade their color hue after removal to a white background. The transfer of fish from blue buckets to white buckets increased the hue value, and fish became paler (i.e., changing from reddish to yellowish), which means a reduction of color quality in clownfish. Van der Salm et al. (2004) found that a fast paling response

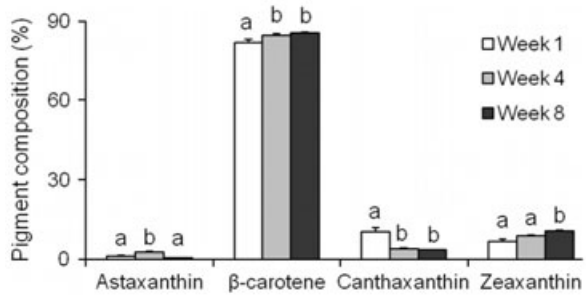

FIGURE 4. *The change of percent contents of astaxanthin, β-carotene, canthaxanthin, and zeaxanthin over time. The values represent means ± SE.*

to a white background is a form of neural regulation of the melanophores. Rodrigues and Sumpter (1984) recognized that pigmentation is controlled both hormonally and neuronally. In fish, fast color changes usually result from neuronal control and longer-term morphological color changes from hormonal control (Fujii 2000). Generally, fast color changes involve a quick reallocation (dispersion or aggregation) of pigment granules (melanosomes) within the dermal melanophores, while the morphological color change involves proliferation or apoptosis of melanophores, which may be combined with increased or decreased sensitivity of the melanophores to regulatory signals (Sugimoto et al. 1997). A 4-wk process of paling in clownfish may be more related to hormone control but further study is needed to confirm this presumption.

One of the most successful chromatic adaptations in fish is the dorsal–ventral pigment pattern in which the dorsal skin is darkly colored, whereas the ventrum is light. In this study, the dorsal body of clownfish had a lower hue value (more reddish orange) than the ventral body, but the ventral body color was brighter and more saturated. This status did not change over time and the response of both sides to background color was similar. In a recent study, Yasir and Qin (2009) reported that the ventral part of clownfish was brighter than the dorsal part regardless of light intensity, but the dorsal part was always more orange than the ventral part. Similarly, the color difference between dorsal part and ventral body in red porgy was not affected by the light level manipulation (Chatzifotis et al. 2005). Cerda-Reverter et al. (2005) suggested that the development of the dorsal–ventral pigment pattern in fish is achieved by a melanization inhibition factor which inhibits melanoblast differentiation and supports iridophore proliferation in the ventrum. However, in clownfish, neither light level manipulation nor color background seems able to change the dorsal–ventral color traits.

Fin color is an important trait in ornamental culture and brood selection (Chapman and Fitz-Coy 1997). However, most attention to fin color has been paid in taxonomy studies. For instance, the color pattern of the caudal fin is a useful criterion for identification of two species of tilapia and their hybrids (Nobah et al. 2006). In aquaculture practice, Hatanaka (1997) identified that the ideal fin color of tiger puffer, *Takifugu rubripus*, was obtained in a black tank. Yasir and Qin (2009) noted that low light provoked brighter fins especially on caudal and dorsal fins and brighter light strengthened orange color on fish fins. In this study, blue background enhanced red orange on both dorsal fin and caudal fin compared with white background. Interestingly, in the first 4 wk, the anal fin and ventral fin showed more red orange, but their color became yellow orange after being kept to a white background for 4 wk. The transfer from colored backgrounds to a white background also reduced color saturation on the anal fin, caudal fin, and ventral fin, but increased color brightness on the anal fin, caudal fin, and dorsal fin. Our result suggests that responses of

fish body skin and fins to color background are similar, although the bottom fins (anal and ventral) are relatively less sensitive to the environmental manipulation. The white screen (1 mm mesh) on the bottom of each bucket might be a potential factor influencing the response of the bottom fins to the color background. Nevertheless, despite the quick response of fish skin and fins to the color background manipulation, the color change derived from the colored background seems to be temporal rather than a permanent alternation.

β-carotene was the most dominant pigments in clownfish, followed by zeaxanthin, canthaxanthin, and astaxathin, but none of these pigments was significantly affected by background manipulation. Tanaka et al. (1992) suggested that pinkish orange of clownfish should contain a high amount of zeaxanthin. Through manipulating light intensity, Yasir and Qin (2009) found that zeaxanthin was enhanced in clownfish by low light illumination, but there was no evidence that zeaxanthin was affected by the color background change in this study. Fish and other vertebrates cannot synthesize carotenoids (Matsuno 2001) and have to rely on feed that contains carotenoids (Allen 1991). Although clownfish could temporarily change the color expression through changing light intensity (Yasir and Qin 2009) or background color (this study), it is unlikely to change the pigment composition through environmental manipulation. It is, therefore, necessary to further test the response of color expression of clownfish to diet manipulation.

In aquaculture, a high fish density could alter the color expression of the skin of red porgy, *P. pagrus*, as a result of stress (Rotllant et al. 2003). Van der Salm et al. (2004) further reported that a high density (25 kg/m^3) could darken the skin color of red porgy, compared to the low density (10 kg/m^3) treatment. In our study, the fish density was reduced by 20% as a result of sampling before the end of the experiment, which might contribute to the color change over time. The possible impact of stocking density on the color change of clownfish needs further investigation.

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