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Changes of body colour and tissue pigments in greenlip abalone (*Haliotis laevigata* Donovan) fed macroalgal diets at different temperatures

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Abstract

In Australia, commercially farmed greenlip abalone (*Haliotis laevigata* Donovan), fed formulated diets display a faint lip and dark foot colour in comparison with wild abalone. This study evaluates the impact of macroalgal diets and water temperatures on the colour and tissue pigment deposition in farmed greenlip abalone. Three diets are (a) fresh *Ulva* sp., (b) a commercial diet and (c) a formulated diet of 30% of dried *Ulva* sp. meal supplement were used to feed 3-year-old greenlip abalone at either 22 or 26°C for 38 days. Abalone fed 30% dried *Ulva* sp. meal supplement had significantly higher the shell colour saturation than those fed fresh *Ulva* sp. The lip colour was deeper in abalone fed fresh *Ulva* sp. and was significantly more pronounced than those fed the diet containing 30% *Ulva* sp. meal. Abalone at 22°C had a significantly higher lip colour saturation value than those at 26°C. The foot of abalone fed fresh *Ulva* sp. was light gold, whereas abalone fed the 30% *Ulva* sp. meal or the commercial diet had a dark brown foot at 22°C. The foot colour was paler in abalone at 26°C than at 22°C. The content of β-carotene was significantly higher in abalone fed fresh *Ulva* sp. than those fed the other diets. Abalone contained a higher amount of β-carotene at 22°C than at 26°C. This study indicates that high water temperatures make foot colour paler and the fresh *Ulva* sp. diet can make the foot colour lighter at 22°C.

KEYWORDS

abalone, algae, colour, pigments, temperature, *Ulva* sp.

1 | INTRODUCTION

Greenlip abalone (*Haliotis laevigata* Donovan) is one of the main species for commercial aquaculture in Australia (Stone et al., 2013). Previous studies have shown that greenlip abalone lose their natural colour under artificial culture conditions (Hoang et al., 2016, 2017). Wild greenlip abalone that consume macroalgae typically have a green lip, yellow foot and a variety of shell colours, whereas cultured abalone fed the commercial formulated diets exhibit a milky lip, brown foot and light green shell (Hoang et al., 2016, 2017). Farmed abalone fed formulated diets have a higher growth rate, but may have less colour on their body than in wild abalone (Bautista-Teruel & Millamena, 1999; Ju et al., 2015). The altered colour may influence market acceptability as greenlip abalone with a milky lip and darker foot fetch a lower price (Freeman, 2001; Hoang et al., 2017). Feeding certain fresh macroalgae, such as *Gracilariopsis bailinae* and *Gracilaria cliftonii* or a supplementation of enriched and dried macroalgae as *Gracilaria cliftonii*, *Ulva* sp. in formulated diets is a way to provide both nutrients and pigments in order to obtain natural colour and grow healthy abalone (Bansemer et al., 2016; Bautista-Teruel &

Millamena, 1999; Hoang et al., 2016, 2017; Ju et al., 2015; Lim & Lee, 2003; Qi et al., 2010). For example, the ass's-ear juvenile abalone (*Haliotis asinine*) fed formulated diets produced light bluish-green shells while those fed seaweed showed original brown colour (Bautista-Teruel & Millamena, 1999). Bansemer et al. (2016) recommended a dietary addition of 10% *Gracilaria* sp. meal or 5% *Ulva* sp. meal to improve the growth of greenlip abalone compared with those fed a formulated diet. Hoang et al. (2017) reported that the addition of 20% *Gracilaria cliftonii* in the formulated diet improved green colour of the lip and produced a brown shell in greenlip abalone. In land-based culture systems, *H. laevigata* fed formulated diets normally need up to 3 years to reach market size, and it is essential to maintain the natural colour, or a desirable colour to meet consumers' requirements before harvesting.

Green macroalgae such as *Ulva* sp., a secondary dietary preference of wild greenlip abalone, contain a variety of pigments including chlorophyll, β-carotene, lutein, violaxanthin, neoxanthin and zeaxanthin that may change the colour of abalone tissue (Chandini, Ganesan, Suresh, & Bhaskar, 2008; El-Baky, El-Baz, & El-Baroty, 2008). Recently, Hoang et al. (2016) found that 1-year-old greenlip abalone fed fresh *Ulva* sp. produced a light-yellow foot colour, similar to wild abalone. However, an addition of 20% enriched and dried *Ulva* sp. meal in the formulated diet did not affect the colour of 1-year-old greenlip abalone (Hoang et al., 2017). It is possible that a feed consisting of 20% dried *Ulva* sp. may be too low to induce a colour change.

Water temperature is an important environmental factor, not only governing physiological activities but also affecting the colour of animals (Gines, Valdimarsdottir, Sveinsdottir, & Thorarensen, 2004; Harris, Burke, Edwards, & Johns, 2005; Morash & Alter, 2015; Olsen & Mortensen, 1997; Stone et al., 2013). For example, the flesh of Arctic charr (*Salvelinus alpinus*) fed a commercial diet at 10°C had a more intense orange colour than those fed at 15°C (Gines et al., 2004). Arctic charr fed diets ranging from 0 to 192 mg/kg astaxanthin at 8°C, had significantly more pigmentation than those at 12°C (Olsen & Mortensen, 1997). According to Storebakken, Foss, Huse, Wandvik, and Lea (1986), the extent of pigmentation varied according to geographical location in yearlings of Atlantic salmon (*Salmo salar*), rainbow trout (*Salmo gairdneri*) and sea trout (*Salmo trutta*) due to temperature differences between regions. Lin, Lin, and Huang (2009) also reported that skin colour change in 10-day-old juvenile seahorses (*Hippocampu erectus*) differed significantly among three different temperatures, 23, 26 and 29°C. In abalone, the influence of water temperature on growth, survival, food intake, metabolism and reproduction are well documented (Bansemer et al., 2015; Grubert & Ritar, 2004; Harris et al., 2005; Lange, Currie, Howarth, & Stone, 2014; Moss, 1998; Stone et al., 2013, 2014), but there are no published reports on the effect of temperature on the colour of abalone. We, thus, hypothesised that temperature can affect abalone colour as it governs dietary pigment intake, absorption and digestion.

Most studies on abalone colour have focused on feeding abalone with various pigment sources such as fresh macroalgae, synthetic pigments or algae pigments in formulated diets (Canales-Gómez, Correa, & Viana, 2010; Hoang et al., 2016, 2017; Horiguchi, Kimura, & Ueno, 1987; Ju et al., 2015; Lim & Lee, 2003; Liu, Wu, Zhao, Zhang, & Guo, 2009; Olsen, 1968; Qi et al., 2010). However, the impact of water temperature on body colour and tissue pigment deposition in abalone is little known. Australian greenlip abalone are cultured predominantly in land-based systems, where they experience large seasonal water temperature fluctuations and suffer high mortality during summer months (Stone et al., 2013). Stone et al. (2014) reported that a fresh *Ulva* sp. diet improved the survival of *H. laevigata* at 26°C. We further hypothesise that feeding abalone fresh *Ulva* sp. or including 30% dried *Ulva* sp. in the abalone diet can improve survival at high temperatures and also help maintain their natural colour. Therefore, the aim of this study was to evaluate the effects of diet and water temperature on the colour change of abalone lip, foot and shell and pigment deposition in whole abalone tissue at 22°C (a preferable growth temperature) compare against 26°C (a suboptimal water temperature).

2 | MATERIALS AND METHODS

2.1 | Experimental animals and system

The experimental animals were obtained from a local farm and held at the South Australian Research and Development Institute (SARDI), Aquatic Sciences Centre in round tanks connected to a flow-through, UV-treated and seawater system. The commercial abalone feed (Eyre Peninsula Aquafeed Pty Ltd (EPA)) was used to feed abalone ad libitum daily prior to the experiment.

The trial was conducted in the SARDI Nutrition Laboratory and the experimental system has been previously described in Stone et al. (2013). Briefly, the system comprised a series of blue plastic culture tanks and a thermal controlling component. Water level was set at 5 cm deep using a standpipe providing a water volume of 5.4 L in each tank. The water flow was kept at 300 ml/min, and flow-through UV-treated water was supplied to tanks from a saltwater system. Photoperiod was controlled at 12 hr low intensity and following by 12 hr dark. Air temperature was maintained at 20 ± 1°C while water temperature was controlled at 22 ± 1.5°C or 26 ± 1.5°C. Abalone were fed with respective diets as described below.

This research was based on the results of a summer mortality model of Stone et al. (2014). At 26°C, mortality occurred in 3-year-old abalone fed the formulated diet which is similar to the mortality pattern on South Australian abalone farms. The experiment lasted 38 days as the mortality was above 50% in the abalone fed a formulated diet at 26°C.

2.2 | Experimental diets

Three experimental diets including (a) a control diet (the commercial diet—EPA Abgrow premium diet), (b) fresh *Ulva* sp. and (c)

a formulated diet with 30% dried *Ulva* sp. meal were used to fed abalone (26.8 ± 14.7 g, 57.9 ± 11.6 mm shell length) at a water temperature of 22 or 26°C. The fresh *Ulva* sp. was collected from Gulf St., and outdoor cultured in a 4,000-L tank, enriched with 8 L of modified F2 nutrient media in seawater every 15 days. The details of dietary preparation and experimental design were fully described in Lange et al. (2014).

Freshly enriched *Ulva* sp. was sun-dried for 8 hr and then oven-dried at 60°C for 48 hr. Dried *Ulva* sp. was homogenized into a fine powder using a blender and stored at −20°C until the abalone meals were prepared.

To produce the diet of 30% *Ulva* sp. meal addition, 30% of the EPA diet mash from the commercial diet was replaced by a similar amount of *Ulva* sp. meal. The supplemental level was calculated to meet the same amount of fresh *Ulva* sp. that would be consumed daily by 3-year-old abalone (Stone et al., 2014). All ingredients were weighed out and mixed in a Hobart mixer for 5 min. Water (30% of the total ingredient weight) was added to the diet mash and mixed. The 5-mm flat sinking pellet meal was made using a TR110 pasta machine. The pellets were then oven-dried at 45°C for 48 hr and frozen at −20°C until use. The proximate and pigment compositions of experimental diets were analysed and presented in Table 1.

TABLE 1 Ingredients and biochemical composition of experimental diets fed to greenlip abalone (*Haliotis laevigata*)

Diets	Commercial diet	Enriched fresh *Ulva* sp.	30% enriched dried *Ulva* sp. meal
Ingredients[a] (g/kg diet as fed)			
Commercial diet mash	983.0	na	683.0
Enriched dried *Ulva* sp.	0.0	na	300.0
Vitamin-mineral premix	2.0	na	2.0
Salmon oil (ml/kg)	15.0	na	15.0
Fresh *Ulva* sp.	0.0	1,000.0	0.0
Sum	1,000.0	1,000.0	1,000.0
Proximate composition[a] (g/kg diet as fed)			
Moisture	100.0	780.0	100.0
Crude protein	306.0	59.8	295.7
Lipid	45.0	9.3	44.1
Ash	62.0	36.7	93.4
Carbohydrate	518.7	42.9	421.7
NFE	587.0	114.3	566.7
Gross energy (MJ/kg)	17.9	3.7	16.0
Diet pigments (μg/g)			
Astaxanthin	-	5.50	1.50
β-carotene	0.13	3.50	1.35
Chlorophyll *a*	-	4.26	2.17
β-cryptoxanthin	-	1.63	-
Zeaxanthin	0.05	1.07	0.51

Note: Denotes 'na' as not analysed and '-" as not detectable.
[a]Reported by Lange et al. (2014).

2.3 | Stocking and feeding

Greenlip abalone (26.8 ± 14.7 g; shell length 57.9 ± 11.6 mm) were taken from a large population and randomly stocked at 12 abalone per tank with three replicates in each treatment. The water temperature was slowly adjusted from ambient (23°C) to the required treatment water temperatures of 22 and 26°C over a period of 7 days. Tank water temperature was then maintained within ± 1.5°C of the treatment temperatures until the end of the experiment.

The feeding rate was based on the report by Stone et al. (2014) on the 3-year-old greenlip abalone fed fresh *Ulva* sp. or a formulated diet. Rations were based on stocking biomass and adjusted by weight loss due to mortality and were supplied in excess of the animal's daily intake. The feeding rate was 1.0%–1.2% body weight per day for the formulated diets. In the fresh *Ulva* sp. treatment, the abalone were fed at a rate of 1.5% body weight per day for the first week and then increased to 2.5% body weight per day for the rest of the experiment due to the significant increase in feed intake of abalone fed *Ulva* sp. at 22°C from the second week. Abalone were fed daily at 16:00 hr after the day of stocking throughout the trial while cleaning and collecting uneaten feed were done at 08:30 daily by sieving the entire tank contents with a fine mesh collector.

2.4 | Sample preparation and analyses

Twenty abalone were collected for photograph prior to the trial while three animals from each tank were photographed for image analysis at the end of experiment. All samples were then kept at −80°C prior to pigment analysis.

2.4.1 | Colour analysis

The method of image study was based on the colour research protocol of Hoang et al. (2016). In brief, a light table equipped with a white mat surface and two white colour bulbs was used for illumination. A digital camera (Canon IXUS 230HS, 12.1 Megapixel Sensor Resolution) held at 25 cm above the specimen on the adjustable arms between two lights above the table. The reference colour card was used during this period to ensure the colour and exposure was correctly set. All images were analysed with Gimp2 software. The hue, saturation and brightness (HSB) values were obtained via the conversion of mean values of red, green and blue (RGB), respectively through an online software programme (www.colorizer.org at http://colorizer.org/).

2.4.2 | Pigment analysis

Pigment extraction was performed according to the method described in Hoang et al. (2016). All experimental diets and abalone tissues (excluding gut) were defrosted at room temperature before freeze-dried for 48 hr. The tissues were then separately ground into fine powder. Subsequently, 0.35 g of abalone tissue powder was used to extract with 10 ml ethanol-hexane (1:1, v/v) three times. The liquid extraction was centrifuged for 5 min at 16,000 *g* then transferred to the 2-ml HPLC vials. The solution was dried completely under a stream of pure nitrogen gas. The dried extractions were then dissolved in 200 μl heptane and acetone (1:1, v/v) and vortexed for 20 s. The pigments were identified by the HPLC and quantified by the Shimadzu software. Solvent A consisted of 80% acetonitrile and 20% water and solvent B was acetone. The flow rate of 1 ml/min was used, and the injection volume was 5 μl. The detection wavelength was set at 450 nm for astaxanthin, zeaxanthin, β-carotene and β-cryptoxanthin and was set at 630 nm for chlorophyll *a*. The standard concentrations of zeaxanthin (Fluka, 14681), astaxanthin (Sigma, SML0982), β-carotene (Sigma, C4582), β-cryptoxanthin (Sigma, C6368) and chlorophyll *a* (Sigma, C6144) were used for developing the calibration curves. The minimum level of detection for HPLC was 0.3 μg/ml. The chromatographic peak identification or retention time was 4.7 min for astaxanthin, 6.1 min for zeaxanthin, 9.9 min for β-cryptoxanthin, 10.2 min for chlorophyll *a* and 12.1 min for β-carotene.

2.4.3 | Ethics statement

The use of experimental animals in this study was complied with the protocol approved by the Animal Ethics Committee at Flinders University, Australia.

2.5 | Statistical analysis

The data were analysed using SPSS (version 22) with the significance level set at $p < .05$. Two-way ANOVA was used to determine the interactive effects between dietary type and water temperature on colour and pigment contents of abalone. When no significant interaction between dietary type and water temperature was observed, Tukey's HSD post hoc test was used to detect significant differences between treatment means. However, when a significant interaction between those two factors was observed, Tukey's HSD test was used to detect significant differences between diets at 22 and 26°C.

3 | RESULTS

3.1 | Shell colour properties

Dietary type (two-factor ANOVA; $p = .625$; Table 2; Figure 1), water temperature ($p = .612$) and the interaction between these two factors ($p = .451$) did not significantly affect shell hue. Shell hue values ranged from 54.84 ± 2.04 to 60.35 ± 3.03 degrees.

Shell saturation was only significantly influenced by dietary type (two-factor ANOVA; $p = .020$; Table 2; Figure 1). Abalone fed the commercial diet had a significantly higher shell saturation than those fed fresh *Ulva* sp. ($p = .011$), but not from those fed 30% *Ulva* sp. meal ($p = .243$).

Shell brightness was only significantly influenced by water temperature (two-factor ANOVA; $p = .007$; Table 2; Figure 1). Shell brightness was significantly lower at 26°C than at 22°C ($p = .007$).

3.2 | Lip colour properties

Among lip colour components, only lip colour saturation was significantly affected by dietary type (two-factor ANOVA; $p = .045$; Table 2; Figure 1) and water temperature ($p = .001$). Abalone fed fresh *Ulva* sp. had a significantly higher lip colour saturation than those fed 30% of *Ulva* sp. meal ($p = .035$), but not from those fed the commercial diet ($p = .738$). Saturation was similar between abalone fed the commercial diet and the 30% *Ulva* sp. diet ($p = .218$). Abalone had significantly lower lip colour saturation at 26°C compared with those at 22°C ($p = .001$).

3.3 | Foot colour properties

Foot hue was significantly influenced by dietary type ($p < .001$; two-factor ANOVA; Table 2; Figure 1) and water temperature ($p < .001$), but not by their interaction ($p = .513$). Abalone fed fresh *Ulva* sp. had a light gold foot, while the foot was brown in abalone fed the commercial diet or the 30% *Ulva* sp. diet. The foot hue was a darker shade of brown at 22°C than at 26°C.

There was significant effect from water temperature on foot colour saturation ($p = .02$; two-factor ANOVA; Table 2). However, dietary type ($p = .246$) and the interaction between these two factors ($p = .051$) had no significant effect on foot saturation. Abalone showed a significantly reduced foot colour saturation at 26°C compared with those at 22°C ($p = .002$).

Foot colour brightness was only significantly impacted by dietary type ($p = .001$; two-factor ANOVA; Table 2; Figure 1). Abalone fed the 30% enriched and dried *Ulva* sp. had significantly lower foot brightness than those fed the commercial diet ($p = .012$) and fresh *Ulva* sp. ($p < .001$). Foot brightness value was similar between abalone fed the commercial diet and fresh *Ulva* sp. ($p = .318$).

3.4 | Pigment contents in diets and abalone tissue

The pigment contents of abalone tissue are presented in Table 1.

All five pigments as zeaxanthin, astaxanthin, β-carotene, β-cryptoxanthin and chlorophyll *a* were detected in enriched fresh *Ulva* sp. Similar pigments, except β-cryptoxanthin, were also found in the 30% enriched and dried *Ulva* sp. meal diet. The commercial diet contained a small amount of β-carotene and zeaxanthin.

TABLE 2 Pigment contents and colour components of greenlip abalone, *Haliotis laevigata* fed graded levels of macroalgae inclusion[a]

	22°C			26°C			ANOVA (*p* value)		
Temperatures Diets	**Comm. (A)**	**Fresh *Ulva* sp. (B)**	**30% dried *Ulva* sp. (C)**	**Comm[b] (A)**	**Fresh *Ulva* sp. (B)**	**30% dried *Ulva* sp. (C)**	**Diet A B C**	**Temp. (°C)**	**Interaction**
Whole abalone body pigments									
β-carotene (μg/g)	1.43 ± 0.09	6.37 ± 0.24	3.71 ± 0.06	0.27 ± 0.05	2.63 ± 0.12	1.22 ± 0.06	X Z Y***	22°C > 26°C***	NS
Colour components of shell									
Hue (degree)	59.96 ± 3.59	58.63 ± 2.40	57.15 ± 2.65	54.84 ± 2.04	60.35 ± 3.03	57.16 ± 1.26	0.625	0.612	NS
Saturation (%)	55.23 ± 1.91	47.30 ± 2.31	53.19 ± 2.03	53.25 ± 2.72	45.51 ± 2.92	46.98 ± 3.49	Y X XY*	0.131	NS
Brightness (%)	48.62 ± 2.62	50.03 ± 1.01	49.43 ± 1.52	49.45 ± 1.22	43.95 ± 2.49	39.16 ± 2.86	0.122	22°C > 26°C*	NS
Colour components of foot									
Hue (degree)	23.19 ± 1.21	30.95 ± 1.23	20.64 ± 1.45	30.41 ± 2.23	36.77 ± 0.79	29.80 ± 2.00	X Y X***	22°C < 26°C***	NS
Saturation (%)	66.79 ± 1.62	66.79 ± 0.94	68.98 ± 1.00	66.99 ± 1.47	60.72 ± 2.39	59.06 ± 3.04	0.246	22°C > 26°C**	NS
Brightness (%)	52.92 ± 2.55	60.71 ± 1.54	48.23 ± 2.01	58.93 ± 1.27	56.86 ± 3.98	49.00 ± 3.62	Y Y X***	0.668	NS
Colour components of lip									
Hue (degree)	43.86 ± 0.94	46.37 ± 0.67	44.99 ± 1.21	45.75 ± 0.45	45.43 ± 0.47	45.87 ± 0.81	0.449	0.382	NS
Saturation (%)	53.20 ± 1.91	54.06 ± 2.05	51.08 ± 1.78	45.70 ± 2.61	49.93 ± 2.66	41.18 ± 2.79	XY Y X*	22°C > 26°C***	NS
Brightness (%)	56.40 ± 1.50	59.93 ± 0.87	54.68 ± 1.70	58.30 ± 2.10	54.86 ± 3.10	51.99 ± 2.76	0.103	0.279	NS

Note: A significance level of $p < .05$ was used for all statistical tests. Post hoc tests were used to determine differences between means (two-factor ANOVA; Tukey's HSD test; $p < .05$). X, Y, Z: for variables with a significant effect of diet (X indicates the lowest value; $p < .05$).

[a]Data are presented as mean ± SE, $n = 3$.

[b]Indicated $n = 2$.

*Significant at $p < .05$;

**Significant at $p < .005$;

***Significant at $p < .001$. NS, no significant interaction ($p > .05$).

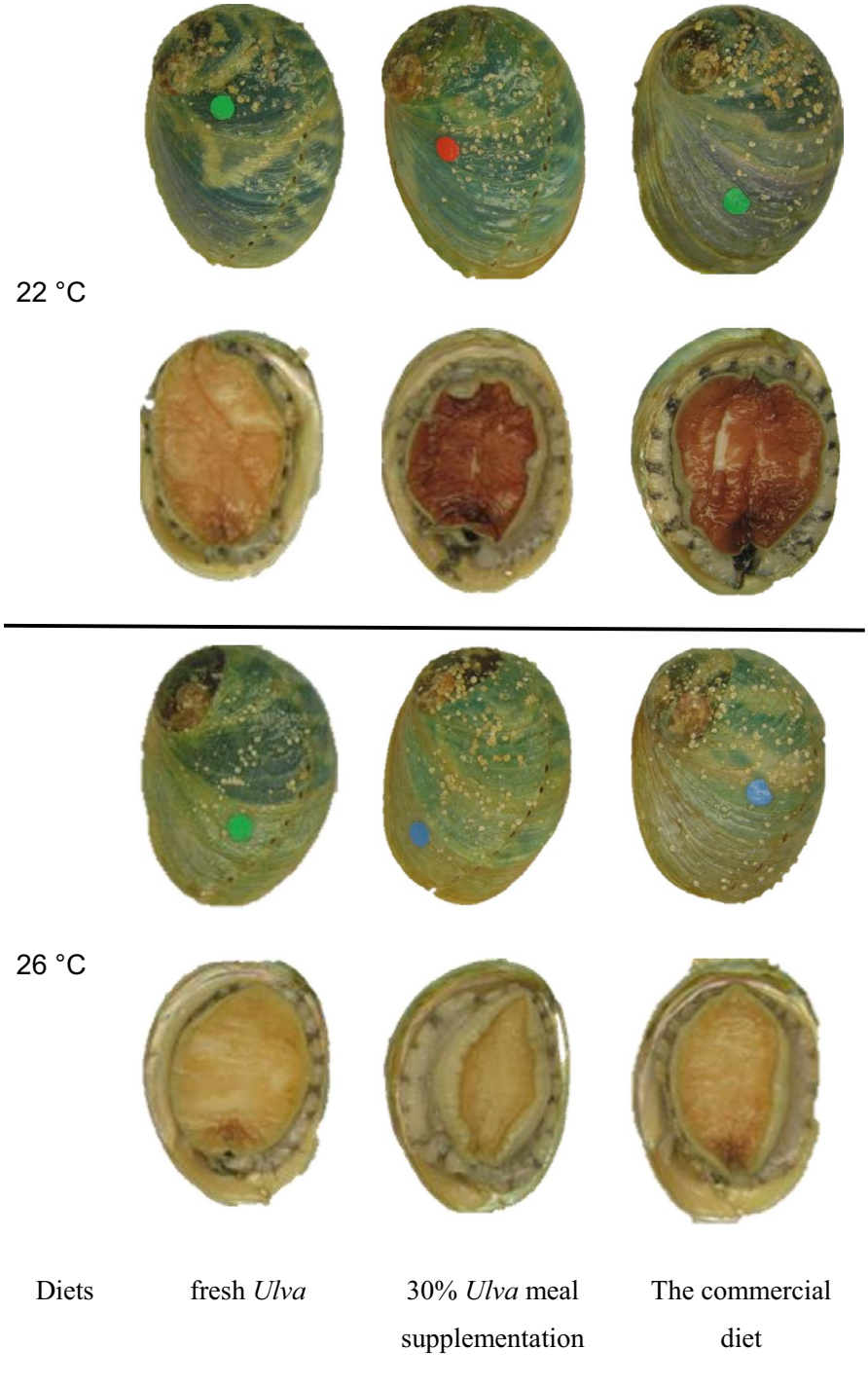

FIGURE 1 Shell, foot and lip colour of greenlip abalone (*Haliotis laevigata*) fed different diets at 22 or 26°C water temperature

Among of four pigments, only β-carotene was detected in the whole tissue of abalone and it was significantly affected by both dietary type ($p < .001$) and water temperature ($p < .01$). Abalone fed fresh *Ulva* sp. had a significantly higher amount of β-carotene than those fed the commercial diet ($p < .001$) and 30% *Ulva* sp. ($p < .001$).

4 | DISCUSSION

The aim of current study was to evaluate the colour change and pigment deposition of abalone during thermal stress (26°C) compared with the optimal water temperature at 22°C. According to Lange et al. (2014) who used the same system, but studied the survival and growth performance, there was no mortality across treatments at 22°C and the growth rate of abalone fed 30% *Ulva* sp. inclusion was higher than those fed fresh *Ulva* sp. At 26°C, the survival was improved in abalone fed fresh *Ulva* sp. or 30% *Ulva* sp. inclusion compared with those fed the commercial formulated diets. Daily growth rate of abalone fed 30% *Ulva* sp. inclusion was highest among all diets.

This study demonstrates that the foot hue of 3-year-old greenlip abalone was affected by both diet and water temperature. Abalone

fed fresh *Ulva* sp. had a light gold foot, while the foot was dark brown in abalone fed the commercial diet or the diet containing 30% enriched *Ulva* sp. meal at 22°C. The foot of abalone was paler at 26°C than at 22°C. Foot colour of some abalone species is one of the most important characteristics in determining abalone quality, price and attractiveness at market. Abalone with a light colour foot generally fetch a better price, while more pre-market treatment is required to sell abalone with a darker foot (Allen, Marsden, Ragg, & Gieseg, 2006; Brown, Sikes, Elliott, & Tume, 2008; Freeman, 2001; Oakes & Ponte, 1996). For example, Allen et al. (2006) suggested to exclude algal stimulants from the diet of blackfoot abalone (*Haliotis iris*) for a period prior to sale due to producing darker foot.

In the present study, foot colour was dependent on diet type. The 3-year-old abalone fed fresh *Ulva* sp. had a light gold foot at 22°C, which was slightly different from the light-yellow foot of 1-year-old abalone fed the same diet in our previous study (Hoang et al., 2016). The slight difference in foot colour between studies may have been due to abalone size and age because the same enriched *Ulva* sp. were used to feed greenlip abalone at the same temperature (22°C) and under the same environmental conditions (Hoang et al., 2016).

The 3-year-old greenlip abalone exhibited a brown foot when fed the diet containing 30% enriched and dried *Ulva* sp. meal or the commercial diet at 22°C, whereas the foot was light gold in abalone fed only enriched fresh *Ulva* sp. in the current study. Pigment analysis showed that the contents of β-carotene, astaxanthin, β-cryptoxanthin, zeaxanthin and chlorophyll *a* in the enriched fresh *Ulva* sp. diet was higher than in the diet containing 30% enriched and dried *Ulva* sp. It is likely that the pigments in the 30% enriched and dried *Ulva* sp. diet were lost due to processing and therefore resulted in weak influence on foot colour. Similarly, Choubert and Heinrich (1993) reported a degradation of green alga *Haematococcus pluvialis* carotenoids during the pelleting process (~3.3%) even at a low temperature (42°C), as well as during the 15-day storage at 7°C ambient temperature (~5.2%).

Water temperature significantly affected the abalone foot hue. At 22°C, abalone had lower foot hue than those at 26°C in all treatments. The foot colour of greenlip abalone at a high water temperature was paler. Generally, within the optimal temperature range, feed intake of greenlip abalone increases with an increase in water temperature. Nevertheless, it declines when water temperature is beyond this range as abalone may be under thermal stress (Bansemer et al., 2015; Lange et al., 2014; Stone et al., 2013, 2014). According to Stone et al. (2014), the feed intake of abalone at 22°C significantly increases, whereas it is suppressed at 26°C. Similarly, the low feed acceptance under thermal stress condition (26°C) resulted in the reduction of pigment intake through food, which may be a cause of the paler foot in current study.

The green pigment on the lip is more relevant to a market trait than the colour of other body parts in greenlip abalone (Bansemer, Qin, Harris, Howarth, & Stone, 2014; Hoang et al., 2017). In this study, although diet and water temperature had some effects on foot colour, neither had any impact on the green pigmentation of the lip. The 3-year-old greenlip abalone exhibited a milky lip colour across all treatments, which is similar to the results of Hoang et al. (2017), where the lip colour of 1-year-old greenlip abalone fed enriched and dried *Ulva* sp. meal or the same formulated diet was also milky. The previous study shows that abalone can develop a green lip when fed ≥10% of red macroalgae *Glacilaria cliftonii* meal, which is similar to the colour of wild abalone (Hoang et al., 2017). The green pigment of the lip is presumably associated with the red macroalgae pigment (e.g. carotenoids) or the melanin pigment, which are bio-accumulated from a dietary source or endogenously biosynthesised by abalone from algae precursors (Hoang et al., 2017). Since the green pigmentation of the lip is an important factor to assess product quality in greenlip abalone, further research should investigate the role of red macroalgae pigments on lip colour of 3-year-old greenlip abalone.

High water temperature beyond the optimal range can also decrease digestion in aquatic animals, related to a reduction in residence time of food in the gut (Ytrestøyl, Struksnæs, Koppe, & Bjerkeng, 2005). In greenlip abalone, gastrointestinal evacuation time has been shown to decrease with increasing water temperatures from 14 to 26°C in 2-year-old (6.7 g) and 3-year-old (25.7 g) animals (Currie, Lange, Herbert, Harris, & Stone, 2015). The reduction of food residence time leaves less time for absorption, whereas a longer residence time may increase nutrient digestibility and absorption (Bansemer et al., 2016; Ytrestøyl et al., 2005). In addition, most carotenoids are lipophilic molecules and their absorption from the gut is a relatively slow process compared with that of other essential nutrients (Ytrestøyl et al., 2005). In the present study, the content of β-carotene in abalone tissues was significantly lower at 26°C than at 22°C. Therefore, it is possible that high water temperature had a negative effect not only on pigment intake, but also on digestibility and absorption of carotenoids. However, the metabolism of carotenoids and other pigments at different water temperature needs further investigation to understand the pigment pathways from the diet to animal tissues.

Changes in body colour or pattern are crucial adaptations of aquatic animals to their environments (Yasir & Qin, 2009). According to Miura, Nishi, and Chiba (2007), temperature is likely to be the most significant factor causing shell colour change in the intertidal gastropod *Batillaria* sp. The dark shell colour was an adaptation to cold while light shell adapted to hot temperature. However, shell colour of greenlip abalone was not influenced by water temperature. It is possible that no difference in shell colour of abalone cultured at 22 and 26°C due to short experimental period.

Colour change is subject to not only variation of pigment quantity, but also due to hormone regulation relevant to environmental stress (Van der Salm, Martinez, Flik, & Bonga, 2004). In the present study, greenlip abalone were chronically exposed to an elevated water temperature (26°C) for 38 days during the experimental period. Therefore, it is possible that the difference in foot colour of greenlip abalone between 22 and 26°C is a stress response to high water temperature.

In conclusion, water temperature and diets had a tangible impact on foot colour and the amount of β-carotene in the whole tissue

of 3-year-old greenlip abalone, but had little influence on shell and lip colour. Foot colour was paler and the β-carotene content in the whole tissue was lower at the higher water temperature. Three-year-old greenlip abalone fed a diet of 30% *Ulva* sp. or a commercial diet exhibited dark brown foot coloration which may potentially affect product quality and consumer choice, whereas abalone fed fresh *Ulva* sp. had a light gold foot colour, similar to wild greenlip abalone.

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CONFLICT OF INTEREST

The authors declare that this greenlip abalone colour research was conducted and submitted to the publication without any potential conflict of interest.

AUTHOR CONTRIBUTIONS

Thanh H. Hoang conducted the experiment, performed the analysis and wrote the manuscript. David Stone and Jian G. Qin contributed animal material, laboratory facilities, experimental design and the manuscript preparation while Duong N. Duong and James O. Harris contributed in data analysis and provided comments on the draft of the manuscript.

DATA AVAILABILITY STATEMENT

The authors declare that this is the original research, the colour and pigment data sharing not applicable and no colour and pigment datasets were used during the current study.

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