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Research Article

Carotenoid Accumulation in Common and Orange-Muscle Mutant of Abalone, *Haliotis gigantea*, Fed with Different Macroalgae

Xiaohui Wei,[1,2] **Bingye Yang**,[3] **Wencui Zeng**,[1,2] **Bin Tang**,[1,2] **Miaoqin Huang**,[1,2] **Xuan Luo**,[1] **Weiwei You**,[1,2] **and Caihuan Ke**[1,2]

[1]*State Key Laboratory of Marine Environmental Science, College of Ocean and Earth Sciences, Xiamen University, Xiamen 361102, China*
[2]*Fujian Collaborative Innovation Center for Exploitation and Utilization of Marine Biological Resources, Xiamen 361002, China*
[3]*Xiamen Key Laboratory Of Marine Medicinal Natural Products Resources, Ximen Medical College, Xiamen 361023, China*

Correspondence should be addressed to Weiwei You; wwyou@xmu.edu.cn and Caihuan Ke; chke@xmu.edu.cn

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Aquatic animals cannot synthesize carotenoids, thus they must come from diet or from symbionts. Previous studies have found that orange-muscle abalones are rich in carotenoids, but the effects of different diets on the accumulation of carotenoids are unknown. In this study, the effects of macroalgae (*Gracilaria lemaneiformis*), fresh and dried kelp (*Laminaria japonica*) on the contents of the predominant carotenoids, including zeaxanthin, β-carotene, and fucoxanthin, between the common and orange-muscle mutant of *Haliotis gigantea* were compared through a one-year culture experiment. Our study confirmed that carotenoids in abalones mainly come from diets, and the carotenoid types in the muscles were similar to their diets. We also found that feeding on *G.lemaneiformis* played an important role in maintaining a stable carotenoid content over time, especially zeaxanthin in *H.gigantea*. Our data also provided that abalones had a good growth performance under the feeding conditions of *G.lemaneiformis*. Finally, compared with common abalones, orange-muscle abalones had a notably enhanced ability to accumulate carotenoids through their diet, especially zeaxanthin ($P < 0.01$). However, the growth performance of orange-muscle abalones was lower than those of common abalones fed the same diets. These results inferred that the content of carotenoid in abalone may be controlled by genetic factors, and diet had a significant influence on the accumulation of carotenoid in abalone to some extent.

1. Introduction

Carotenoids are the predominant pigments in animals, bringing beautiful color to organisms and enhancing their economic and nutritional value. Although animals need carotenoids as precursors of retinoid metabolites (including vitamin A, bioactive molecules such as retinoic acid and visual pigments) and for coloring, they cannot synthesize carotenoids de novo. Generally, animals accumulate them in specific tissues directly or through partial modification after obtaining carotenoids from food or symbionts. For example, salmon cannot synthesize astaxanthin from other carotenoids, so the astaxanthin in their bodies comes from crustaceans in their food [1–3].

Color plays an important role in the overall acceptability of food. The color of seafood determines the final choice of consumers to some extent [4]. Carotenoids are the main pigments of many aquatic animals. The unique colors of edible fish (salmon and trout), crustaceans (shrimp and lobster), and mollusks (mussels, clams, and marketable urchins) are also caused by carotenoids [5–7]. The muscles and skin of fish,

exoskeletons and gonads of crustaceans, and other tissues such as the foot and adductor muscles of mollusks are rich in carotenoids [8, 9]. The grading pricing of shrimp, salmon, grouper, and snapper is directly related to the intensity of the red hue [10]. Ostrander found that consumers think color is the most important factor in distinguishing salmon from trout [11]. A variety of carotenoids have been isolated and identified in bivalves such as scallops and oysters [12, 13]. Both *Patinopecten yessoensis* and *Chlamys nobilis* have orange adductors due to carotenoids [14, 15]. As an important food source for marine animals and human beings, shellfish play an important role in transferring nutrients, including carotenoids, through the food web to higher vertebrates.

Many crustaceans can convert β-carotene in algae into astaxanthin through the processes of echinenone, 3-hydroxyechinenone, cantharidin, and marigold [1, 2]. The carotenoid species in bivalves include fucoxanthin, diatomaceous xanthene, phytochrome, and isoflavones, all of which are derived from microalgae. Gastropods such as sea slugs, sea rabbits, and snails feed on brown and red algae. A series of metabolites of β-carotene, lutein, and zeaxanthin have been identified in their bodies [16].

Abalone, a marine herbivorous shellfish, feeds mainly on macroalgae. The carotenoids identified in vivo include β-carotene, α-carotene, zeaxanthin, lutein, and fucoxanthin [17]. The macroalgae ingested include the brown algae *Laminaria japonica* and *Undaria pinnatifida*, as well as the red algae *Gracilaria lemaneiformis*. Different seaweeds contain different carotenoids. Xishi abalone (*Haliotis gigantea*) is distributed throughout the coast of Japan and Korea. These individuals are large with strong disease resistance and high breeding potential [18]. The muscles of *H. gigantea* are usually brownish-yellow, but a small proportion of mutant individuals were found in the same breeding group with bright orange muscles. Previous studies have demonstrated that abalones with orange muscles are not only bright in color but also rich in zeaxanthin and β -carotene. The amount of zeaxanthin and β-carotene in orange-muscle abalones (OA) was about 16.5 and 3.1 times than those of common abalones (CA) [19].

Previous studies identified that the main carotenoids in abalones were zeaxanthin and β-carotene. Some studies have shown that β-carotene and zeaxanthin were also the predominant carotenoids in *G. lemaneiformis*. Zeaxanthin was the most important carotenoid in *G. lemaneiformis*, accounting for 59.9–78.6% of the total carotenoids [20]. It is unknown whether carotenoids in *H. gigantea* come directly from its diet, whether there is a transformation process among different carotenoids, and whether different diets will affect the type and amount of carotenoid in *H. gigantea*. Answering these questions may be important for finding suitable diets to optimize pigment accumulation in cultured abalone. It also will provide the basis for breeding abalones with high carotenoid content benefit for human health in the future.

In this study, we compared the effects of different diets on carotenoid accumulation in *H. gigantea*. We identified the species and quantity of different carotenoids in *H. gigantea* cultured with different diets, aiming at clarifying the sources of different carotenoids in *H. gigantea* and the process of metabolic transformation. This study provided the theoretical information to understand the physiological mechanisms of carotenoid accumulation in *H. gigantea*, leading to different muscle pigmentation.

2. Materials and Methods

2.1. Animal Treatment. The abalones used in this study were cultured in Fuda Abalone Factory, Fujian Province, and fed mainly *G.lemaneiformis*. All individuals were 2 years old (the shell lengths of CA and OA were 42.46 ± 3.44; 43.16 ± 3.95), which they were raised in a culture pond under the same feeding conditions. During the temporary rearing period, the seawater was completely changed every day, and the feed was fed every two days. The normal light was maintained day and night, the salinity was stable at 33, and the pH was stable at 7.8. The dead abalones and the extra baits were removed in time. The temporary rearing time was 12 months, and the water temperature varied with the season. There were six treatment groups in the experiment, with three replicates in each treatment and 60 abalones in each replicate. After fasting for one week, the abalones were randomly divided into groups and fed with different diets. The total number of abalones used in the experiment was 1080 (Figure 1). Before the experiment, 50 OA and 50 CA were randomly selected. The shell length and total weight of the abalones were measured, and the lightness value (L^*), red-green value (a^*), and yellow-blue value (b^*) of the foot were detected using a colorimeter NR110 (3nh Shenzhen Sanenshi Technology Co., Ltd.). Fifteen OA and CA were randomly selected for dissection. The amount of zeaxanthin, β-carotene, and fucoxanthin in the muscles was measured using HPLC. The a^* value is usually used as an index to evaluate muscle color [21]. The average red-green values (a^*) of OA and CA were 15.17 ± 2.04 and 6.04 ± 1.21, respectively. The facilities used in each replicate were family barrels with identical specifications. Each barrel had a volume of 600 L ($100\,\text{cm} \times 100\,\text{cm} \times 60\,\text{cm}$). The experimental water was flowing seawater. During the experiment, the abalones were fed at 17 : 00 p.m. every day over a period of 12 months.

2.2. Sample Collection and Analyses. At the end of the experiment, all abalones were starved for three days to empty the intestinal contents. Samples were taken from all the treatment groups at 0, 2, 4, 6, and 12 months after the beginning of the experiment. At the each step of the experiment during 12 months, fifty abalones were taken from each treatment at the each step; the shell length and total weight of each abalone were measured. The lightness value (L^*), red-green value (a^*), and yellow-blue value (b^*) of the foot were detected using a colorimeter NR110. Ten abalones were taken from each treatment at each sampling point, which all abalones were starved for three days to empty the intestinal contents, and the amounts of zeaxanthin, β-carotene, and fucoxanthin were measured using HPLC.

2.3. Determination of the Predominant Carotenoid Contents. The carotenoid content of abalones was determined by normal phase high performance liquid chromatography with a silica gel column (YMC-Pack SIL column) ($250 \times 4.6\,\text{mm}$,

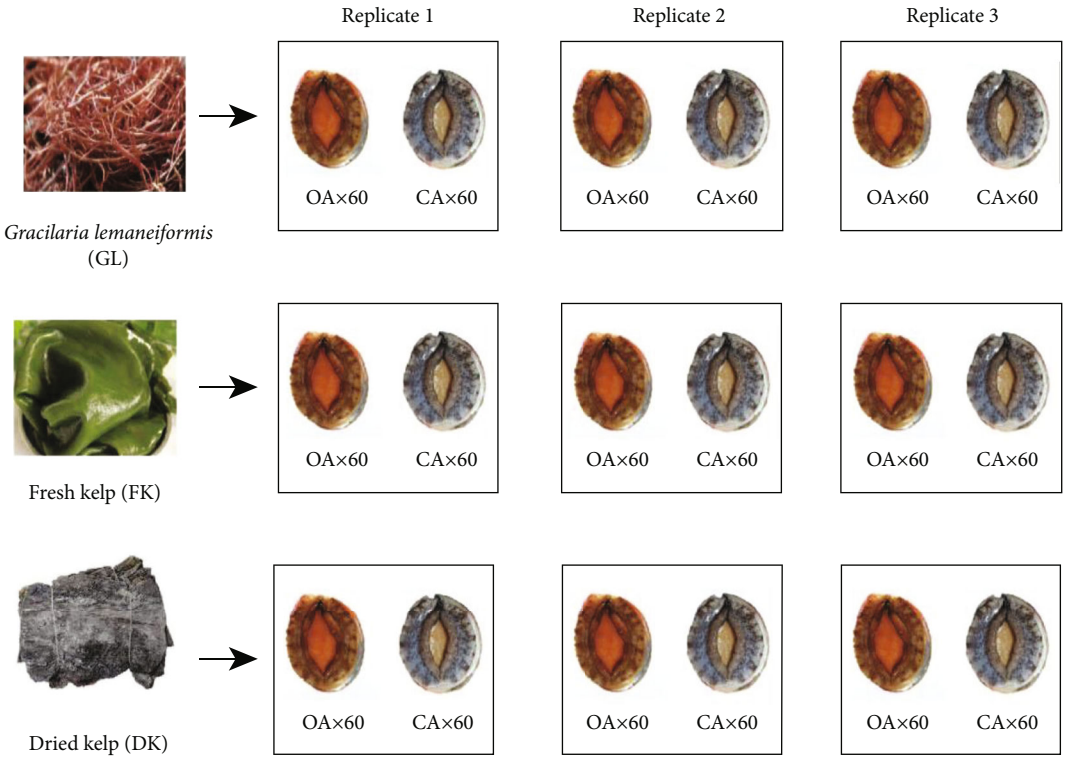

Figure 1: Experimental design.

5 μm, 12 nm, YMC CO., Ltd., Kyoto, Japan). The mobile phase consisted of n-hexane (a) and ethyl acetate (b), which were eluted by linear gradient step by step: (1) 40% A and 60% B were eluted for 10 min, (2) 100% A was eluted for 5 min, and then (3) 40% A and 60% B were balanced for 5 min. The total elution time was 20 min. The injection volume was 20 μL, the flow rate was 1.5 mL/min, the detection wavelength was 446 nm, and the column temperature was 25°C.

The 1.0 mg analytical standard was dissolved in 5.0 mL ethyl acetate to prepare 200 μg/mL zeaxanthin stock solutions. The stock standard solution was diluted with ethyl acetate to prepare a standard curve working solution. The concentrations of the working standard diluents were 1.25, 2.5, 5, 10, 25, 50, 100, and 200 μg/mL. A 1.0 mg analytical standard was dissolved in 20 mL ethyl acetate to prepare 50 μg/mL β-carotene stock solutions. The concentrations of the working standard diluent were 0.25, 1.25, 2.5, 5, 10, 15, 25, and 50 μg/mL. A 1.0 mg analytical standard was dissolved in 20 mL ethyl acetate to prepare 50 μg/mL fucoxanthin stock solutions. The concentrations of the working standard diluents were 0.05, 0.25, 1.25, 2.5, and 5 μg/mL. The concentrations of zeaxanthin, β-carotene and fucoxanthin were determined using a standard curve established by normal phase high performance liquid chromatography.

2.4. Increase Rates. After one year of the experiment, the abalones were sampled and measured. The shell length and total weight of 50 OA and CA were measured. The main carotenoids (zeaxanthin, β -carotene, and fucoxanthin) in the muscles of 10 abalones were determined using HPLC. The increase rates of the shell length, total weight, zeaxanthin, β-carotene, and fucoxanthin amounts in abalone muscles at each sampling point were calculated using the following formula:

$$Y_n(\%) = \frac{X_n - X_{n-1}}{X_{n-1}} \times 100. \quad (1)$$

in which Yn is the increase rate of each index in N sampling period, Xn is the average value of each index at N sampling points, and $Xn - 1$ is the average value of each index at the previous sampling point N.

2.5. Data Analysis. The data were preprocessed in Excel. GraphPad Prism 6.0 was used for drawing figures, and SPSS 20.0 was used to analyze the experimental results statistically. The results were expressed as mean ± standard deviation. The analysis methods of one-way ANOVA, independent t -tests and nonparametric tests were used to compare differences among the experimental groups. The level of significance was set at $P < 0.05$ (marked by * in the figures) and the level of extreme significance was set at $P < 0.01$ (marked by ** in the figures). At $P > 0.05$, there was no significant difference among the experimental groups (marked by ns in the figures).

3. Results

3.1. Effects of Various Diets on the Muscle Pigmentation of H. gigantea. At the end of the experiment, the chromatism indices of the foot muscle of OA were significantly higher than

Table 1: Effects of diet on the muscle chromatic value in *H. gigantea* for 12months culture (Mean ± SD).

	L^*		a^*		b^*	
	OA	CA	OA	CA	OA	CA
GL	-50.67 ± 5.98^a	-41.37 ± 6.83^a	27.66 ± 6.56^a	8.80 ± 3.64^a	38.98 ± 5.13^a	22.36 ± 4.44^a
FK	-43.98 ± 6.30^b	-40.41 ± 5.09^a	11.19 ± 3.08^b	6.73 ± 2.52^a	28.15 ± 3.70^b	19.93 ± 3.82^a
DK	-43.98 ± 3.80^b	-38.28 ± 3.87^a	9.94 ± 2.71^b	5.83 ± 1.79^b	25.73 ± 3.35^b	18.59 ± 2.64^b

Note: Different letters in the same column show significant difference ($P < 0.01$).

those of CA ($P < 0.01$). The red-green value (a^*) was the main index differentiating OA and CA. The red-green value (a^*) of OA fed with *G. lemaneiformis* was significantly higher than abalones fed with fresh kelp and dry kelp ($P < 0.01$). There was no significant difference between OA fed with fresh kelp and those fed with dry kelp ($P > 0.05$), as shown in Table 1. The red-green value (a^*) of CA fed *G. lemaneiformis* was not significantly different from abalone fed with fresh kelp ($P > 0.05$), but it was significantly higher than that of abalones fed with dry kelp ($P < 0.01$).

The change trend in the yellow-blue value (b^*) was the same as that for the red-green value (a^*). The yellow-blue value (b^*) of orange-muscle abalones in the *G. lemaneiformis* group was significantly higher than it was in the fresh kelp and dried kelp groups ($P < 0.01$). The b^* value of common abalone in *G. lemaneiformis* group was not significantly different from that of fresh kelp ($P > 0.05$), but it was significantly higher than that of dry kelp ($P < 0.01$). The lightness value (L^*) of orange-muscle abalones in *G. lemaneiformis* group was significantly higher than that of fresh kelp group and dry kelp group ($P < 0.01$), while the lightness value (L^*) of common abalones was higher than other groups, but the difference was not significant ($P > 0.05$).

3.2. Effects of Various Diets on Carotenoid Contents of H. gigantea with Different Color Muscles. The quantities of three predominant carotenoids (β-carotene, zeaxanthin, and fucoxanthin) in the muscles of *H. gigantea* fed three different diets (*G. lemaneiformis*, fresh, and dry kelp) were compared at 12month after the beginning of the experiment, as shown in Table S1. At 12 month, the quantities of the three main carotenoids in the muscles of orange-muscle abalones were significantly higher than in common abalones ($P < 0.01$). The amounts of zeaxanthin, β-carotene, and fucoxanthin were significantly higher in the muscles of orange-muscle abalones fed *G. lemaneiformis* than in those fed with fresh kelp and dry kelp ($P < 0.05$). There was no significant difference in the quantity of carotenoids between the fresh kelp group and the dry kelp group ($P > 0.05$) (Figure 2). For common abalones, *G. lemaneiformis*, fresh kelp, or dried kelp groups had no significant difference in the amount of zeaxanthin and fucoxanthin in their muscles ($P > 0.05$). The amounts of zeaxanthin in the muscles of common abalones followed the order of *G. lemaneiformis* > fresh kelp > dried kelp. The amount of β-carotene in the muscles of common abalones fed *G. lemaneiformis* was significantly higher than in common abalones fed dried kelp ($P < 0.05$) (Figure 2).

3.3. Effects of Various Diets on the Carotenoid Increase Rates of H. gigantea with Different Color Muscles. The effects after 12 months of various diets on the increase of carotenoid in abalones with different colors are shown in Figure S1. The increase rates of β-carotene content in common and orange-muscle abalones in the *G. lemaneiformis* group, fresh kelp group, and dry kelp group were all negative. With respect to zeaxanthin, the only group that showed an increase was the orange-muscle abalones fed *G. lemaneiformis*. The other groups of abalones showed a negative trend in zeaxanthin. With respect to fucoxanthin, common abalones fed with *G. lemaneiformis* and fresh kelp had a positive trend but the other groups of abalones all showed a negative trend.

3.4. Effects of Various Diets on Increase Rates of Growth Traits of H. gigantea with Different Color Muscles. The effects after 12 months of various diets on the growth traits of abalones are shown in Figure S2. The increase rates of each growth index (shell length and total weight) for common abalones fed *G. lemaneiformis*, fresh kelp, and dry kelp was higher than those for orange-muscle abalones. As shown in Figure S2, the increase rates of each growth index for orange-muscle and common abalones fed *G. lemaneiformis* for 12 months was higher than those associated with feeding with fresh and dry kelp. However, there was no obvious difference between fresh and dry kelp groups.

3.5. Composition and Contents of Carotenoids in Various Diets. The composition and quantities of various carotenoids in *G. lemaneiformis,* fresh kelp, and dried kelp are shown in Table 2. The main carotenoids in *G. lemaneiformis* were β-carotene and zeaxanthin, with a small amount of fucoxanthin. The major carotenoids in fresh kelp and dried kelp were β-carotene and fucoxanthin, with a small amount of zeaxanthin. The β-carotene content was significantly higher in *G. lemaneiformis* than in fresh kelp and dry kelp ($P < 0.01$). The β-carotene content was higher in fresh kelp than in dry kelp, but the difference was not significant ($P > 0.05$). The amount of zeaxanthin was significantly higher in *G. lemaneiformis* than in fresh kelp and dry kelp ($P < 0.05$). The amount of β-carotene and zeaxanthin in the three diets was as follows: *G. lemaneiformis* > fresh kelp > dried kelp. The amount of fucoxanthin was higher in fresh kelp than in dried kelp or *G. lemaneiformis* ($P < 0.01$). The fucoxanthin content was significantly higher in dried kelp than in *G. lemaneiformis* ($P < 0.01$). The order of fucoxanthin in the three diets was fresh kelp > dried kelp > *G. lemaneiformis*.

3.6. Effects of Various Diets on Changes in Carotenoid Content of H. gigantea with Different Color Muscles. As shown in Figure 3, after one year of feeding with *G. lemaneiformis,* the amount of β-carotene in abalone muscles decreased. After

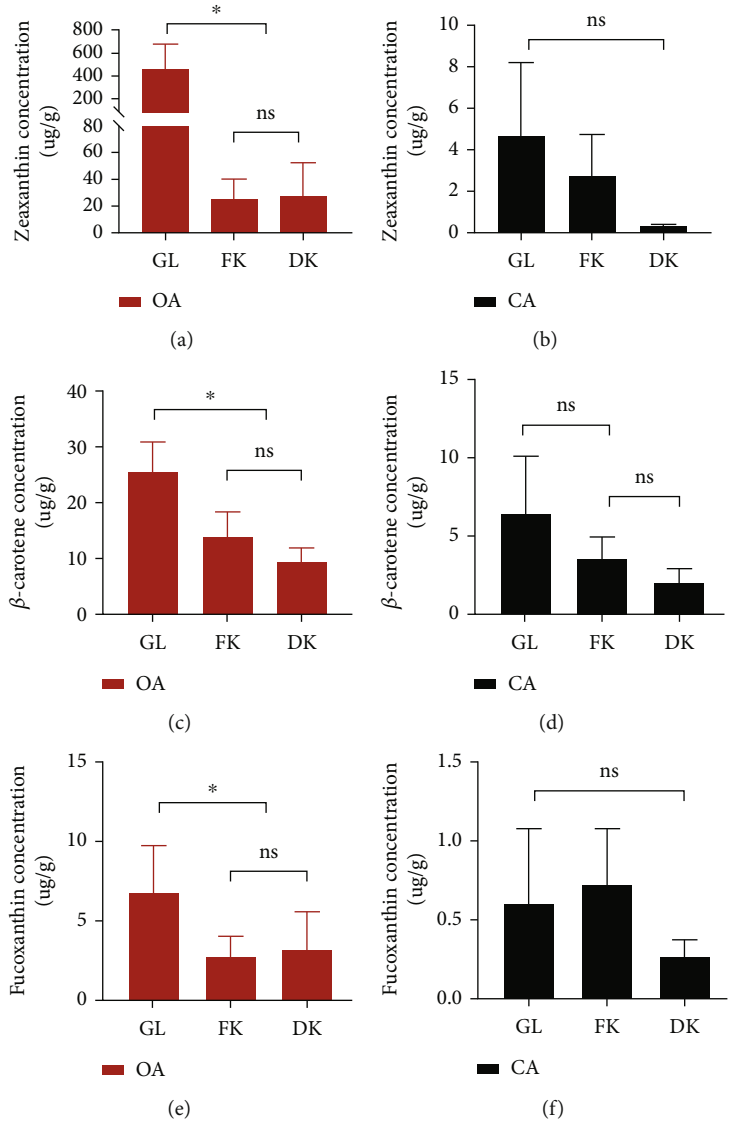

Figure 2: Quantities of three carotenoids in *H. gigantea* muscles with different colors under various diets after 12 months.

six months of feeding with fresh or dry kelp, the amount of β-carotene in abalone muscles (both orange-muscle abalones and common abalones) tended to decrease in general. The changes leveled off after 6 to 12 months. After 12 months, the amount of β-carotene in abalone muscles was significantly lower than the initial experimental values ($P < 0.01$). The order of β-carotene content in abalone muscles was *G. lemaneiformis* group > fresh kelp group > dry kelp group. The β-carotene content was significantly higher in the muscle of orange-muscle abalones than in common abalones ($P < 0.01$) (Figure 3(a)). The amount of zeaxanthin in the muscle of orange-muscle abalones fed with *G. lemaneiformis* increased significantly after one year ($P < 0.05$), while the amount of zeaxanthin in the muscle of common abalones showed no significant change ($P > 0.05$). The amount of zeaxanthin in the muscles of orange-muscle abalones and common abalones fed with fresh kelp and dried kelp decreased during the first six months and then leveled off between six to 12 months. After 12 months, the amounts of zeaxanthin were significantly lower than the initial values ($P < 0.01$). In all of the feeding groups, the amount of zeaxanthin was significantly higher in the muscle of orange-muscle abalones than in common abalones after one year ($P < 0.01$) (Figure 3(b)). After feeding *G. lemaneiformis* to orange-muscle abalones and common abalones for 12 months, there was no significant difference between the initial and final values of fucoxanthin in the muscles ($P > 0.05$). However, in the fresh kelp and dry kelp groups, the fucoxanthin content in the muscles of orange-muscle abalones decreased during the first six months and leveled off between six to 12 months. After culturing for 12 months they were significantly lower than the initial values ($P < 0.01$). After one year of culture, the amount of fucoxanthin was significantly higher in the muscles of orange-muscle abalones than in common abalones ($P < 0.01$) (Figure 3(c)).

3.7. Effects of Various Diets on Changes in the Growth Traits of H. Gigantea with Different Color Muscles. Changes in the growth traits (including shell length and total weight) of *H.*

Table 2: The quantity of carotenoids in different diets (Mean ± SD).

Diets	β-Carotene (μg/g)	Zeaxanthin (μg/g)	Fucoxanthin (μg/g)
GL	$\mathbf{46.77 \pm 20.46^a}$	$\mathbf{7.33 \pm 3.06^a}$	$\mathbf{0.54 \pm 0.30^a}$
FK	$\mathbf{21.16 \pm 8.56^b}$	$\mathbf{3.63 \pm 3.62^b}$	$\mathbf{90.55 \pm 66.32^b}$
DK	$\mathbf{15.38 \pm 10.33^b}$	$\mathbf{0.232 \pm 0.16^c}$	$\mathbf{2.08 \pm 1.29^c}$

Note: Different letters in the same column show significant difference ($P < 0.05$).

gigantea with different color muscles in various feeding groups after culturing for 0, 2, 4, 6, and 12 months are shown in Figure 4. Each growth index showed a continuous positive trend. After culture for 12 months, the values of each growth index for common abalones with three feedings were higher than for orange-muscle abalones.

4. Discussion

Our study confirms that the carotenoids in abalones come directly from their diets, and the carotenoid types in the muscles are the same as in their diets. The types and quantities of carotenoids in different diets directly or indirectly affect the types and quantities of carotenoids in animals. In this study, the effects of different diets on the composition and amount of carotenoid in *H. gigantea* with different color muscles were compared and analyzed.

After one year of culture, the amount of zeaxanthin and β-carotene were significantly higher in the muscles of orange-muscle abalones fed with *G. lemaneiformis* than in those fed with fresh kelp and dried kelp ($P < 0.01$). The orange-muscle abalones fed with *G. lemaneiformis* had the largest positive growth rate of zeaxanthin, whereas the other groups of abalones showed a negative trend (Figure S1). This trend seems partly due to the fact that the amount of β-carotene and zeaxanthin is higher in *G. lemaneiformis* than in fresh kelp and dry kelp. The amount of zeaxanthin in *G. lemaneiformis* was the highest among the three kinds of food. The results verified that the type of diets affected the ability of accumulating carotenoids in orange-muscle abalones to a certain extent. Except for environmental factors such as diets, the change of biological phenotype may also be caused by the difference of gene expression caused by environmental factors. The same genotype has different phenotypes in different environments, which may be due to a change in gene expression. For example, changes in the color of snowshoe hares, whose fur was brown in summer and white in winter, were caused by differences in gene expression [22]. The quantities of carotenoids were higher in the muscles of orange-muscle abalones fed with *G. lemaneiformis* than in those fed other diets. Except for the different contents of carotenoids in the diets, this may be caused by the different expression of related genes in orange-muscle caused by different diets. Abalones with the same genotype showed phenotypic differences under various diets, but the specific situation needs further exploration.

There was no significant difference in zeaxanthin content in common abalones cultured for 12 months with three kinds of diets ($P > 0.05$). The β-carotene content was significantly higher in the muscle of common abalones fed with *G. lemaneiformis* than in abalones fed dry kelp, but there was no significant difference compared to abalones in fresh kelp group. Especially, although the amount of fucoxanthin was significantly lower in *G. lemaneiformis* than in fresh kelp and dry kelp, the fucoxanthin content was significantly higher in orange-muscle abalones fed with *G. lemaneiformis* than in abalones fed with fresh kelp or dry kelp ($P < 0.01$). And there was no significant difference in the fucoxanthin content in common abalones with three kinds of diets after culturing for 12 months ($P > 0.05$). These results may indicate there was a significant difference between orange-muscle and common abalones in terms of their ability to accumulate carotenoids. In addition, the one-year culture experiment confirmed that the three predominant carotenoid types were significantly higher in orange-muscle abalones than in common abalones ($P < 0.01$). The differences between orange-muscle abalones and common abalones did not change with various diets. In summary, the orange-muscle abalones exhibit an enhanced ability for accumulating carotenoids, especially zeaxanthin. The body color of the animal is influenced not only by diet but also by genetic factors, and the genetic factors can be inherited by offspring. Genetic factors such as gene mutation and gene recombination are generally considered to be the main mechanisms for maintaining the genetic diversity that is needed to adapt to a changing environment. For example, there was a MuPKS gene encoding synthetic yellow pigment in budgerigar, and a single base mutation on MuPKS caused the color change [23]. At the same time, the variation of noncoding sequences will also affect the phenotype. Toomey et al. found that the SCARB1 gene could encode a membrane receptor of high-density lipoprotein to mediate the absorption of carotenoids, thus making canary feathers yellow. When there was a single regulatory mutation at a splice donor site of the SCARB1 gene, it could lead to abnormal cutting of this gene. If this happens, the gene lost its original function and the canary changed from yellow to white [24]. With respect to marine shellfish, there was a large accumulation of carotenoids in the adductor muscle of cultured scallop. The genes responsible for this trait could be stably inherited. This provided a theoretical basis for obtaining new shellfish varieties with high carotenoid content by means of genetic breeding. Related studies have confirmed that the carotenoid-enriched trait of scallop *Patinopecten yessoensis* was a stable heritable quality trait [14]. In our study, the difference in carotenoids between orange-muscle abalones and common abalones did not change with various diets. The quantities of three predominant carotenoids were significantly higher in orange-muscle abalones than in common abalones ($P < 0.01$) regardless of diet. Compared with common abalones, orange-muscle abalones exhibited an enhanced ability for accumulating carotenoids, especially zeaxanthin. It could be inferred that the carotenoid contents in abalone's muscle may be mainly controlled by genetic factors, although the type of diets affected its change to some extent. However, the absorption, transformation and metabolism of natural pigments in animals are the rather complicated process. It

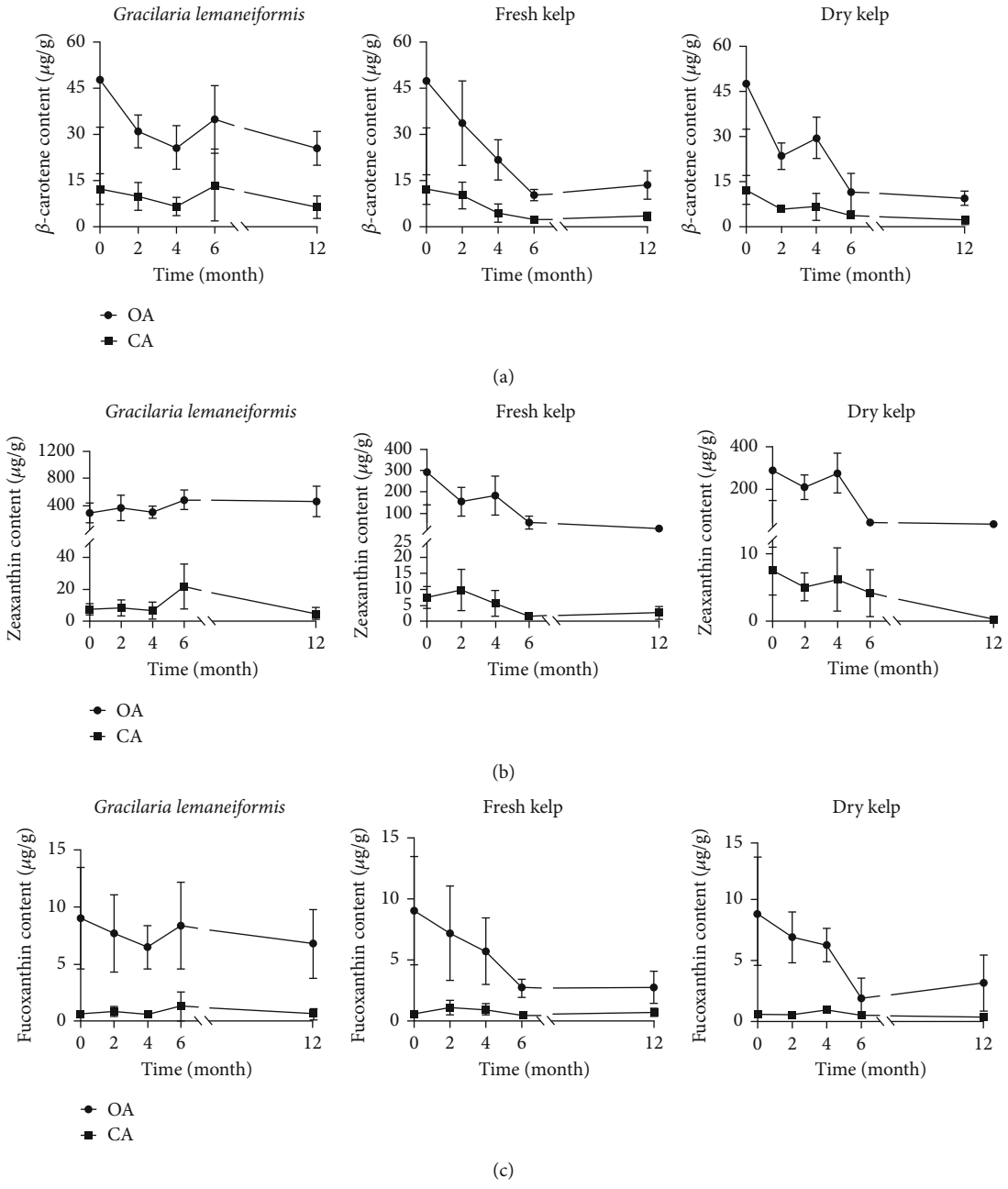

Figure 3: Variations in carotenoid content in *H. gigantea* muscles with different colors under various diets for 12 months.

is not clear what genetic factors are responsible for the different colors in abalones. We will explore this topic further in the following research. The results of this study provide strong support for exploring genetic mechanisms in the future.

After one year of culture, the amount of β-carotene in orange-muscle abalones and common abalones fed *G. lemaneiformis* decreased significantly and remained higher than the amount of β-carotene in abalones fed fresh kelp or dried kelp, but the amounts of zeaxanthin and fucoxanthin increased or did not change significantly. However, in fresh kelp and dry kelp groups, the contents of three kinds of carotenoids in abalones all decreased significantly, except that the content of fucoxanthin in common abalones fed with fresh kelp changed little. It could be inferred that after being fed fresh kelp and dried kelp for one year, the carotenoid content in abalones showed an obvious downward trend whereas the carotenoid content in abalones fed with *G. lemaneiformis* remained steady. Feeding with *G. lemaneiformis* played an important role in maintaining carotenoid content, especially zeaxanthin, in orange-muscle abalones or common abalones. This may be related to the fact that the abalones in the experimental groups were fed *G. lemaneiformis* before the experiment. Once the abalone's diet was changed, its accumulation ability changed and the carotenoid contents in abalones would decrease for a period of time, and it would tend to be flat after reaching certain content. However, the absorption and accumulation of carotenoids in animals are affected by many

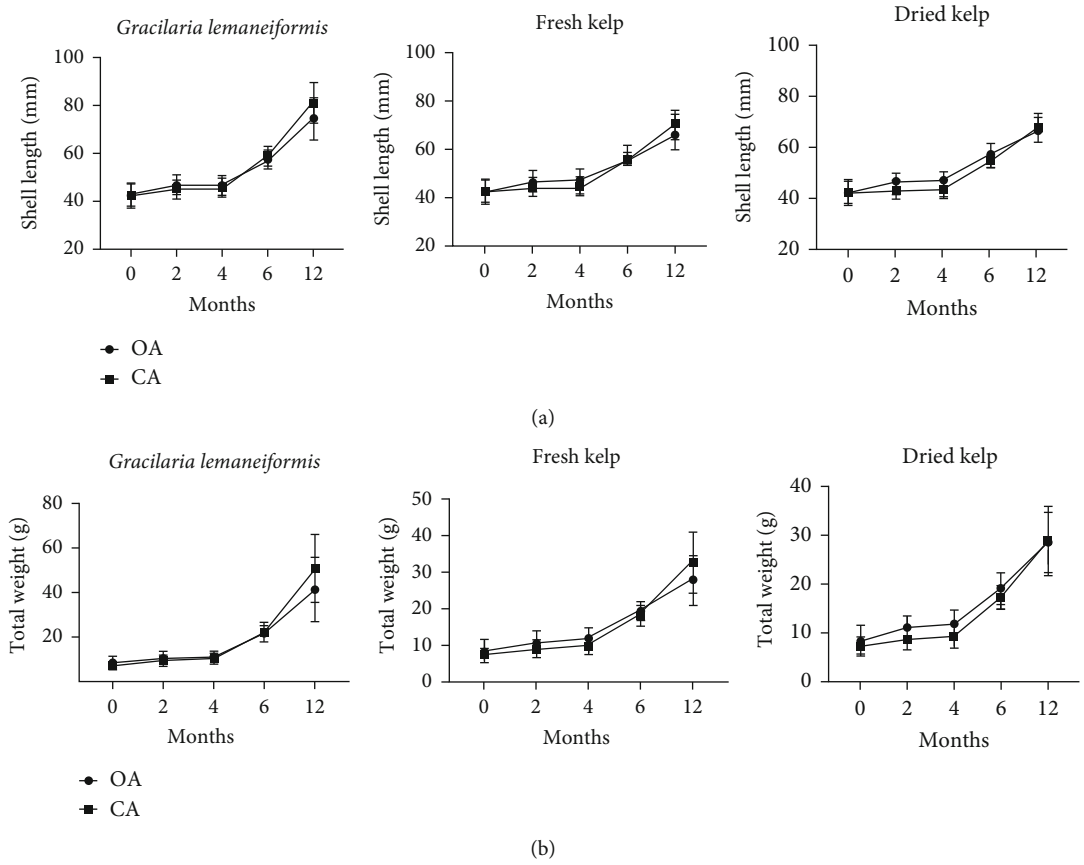

Figure 4: Changes in the growth traits of *H. gigantea* with different colors under various diets for 12 months.

factors, including food matrix, food composition (lipid, fiber), and carotenoid species. Since carotenoids are fat-soluble, dietary fat content directly affects the bioavailability of carotenoids. In humans, dietary fat can stimulate the secretion of bile to promote the absorption of carotenoids and increase the lumen concentration of bile salts, which play the role of surfactant in mixed micelles [25]. Adding avocado or avocado oil to a low-fat diet based on fresh vegetables can significantly improve the absorption of carotenoids (β-carotene, lutein, and lycopene) [26]. Different seaweeds contain different kinds and quantities of nutrients (seaweed polysaccharide, fatty acid, polypeptide, and alginate) and secondary metabolites (diterpenes, seaweed polyphenols, and sulfated polysaccharides), which determine their bioavailability to abalones. Related studies have shown that the fat content of brown algae is generally lower than that of red algae [27]. The fresh kelp and dried kelp used in this experiment are brown algae, while *G. lemaneiformis* is red algae. We inferred that the higher fat content of *G. lemaneiformis* compared with brown algae kelp increased the bioavailability of carotenoids and their accumulation. However, the absorption, transformation, and metabolism of natural pigments in abalones may be the rather complicated process. It is unclear what causes the accumulation of zeaxanthin in *G. lemaneiformis* by abalones, and the related mechanism is still unclear. How the diets affect the composition and content of carotenoids in abalones need further exploration.

By comparing and analyzing differences in the growth traits of *H. gigantea* from different groups after being cultured for 12 months with different diets, we found that the changes in shell length and total weight of abalones with various diets showed a continuous upward trend (Figure 4). The shell length and total weight of abalones fed with *G. lemaneiformis* were higher than those of abalones fed with fresh kelp or dry kelp (Figure 4). The selection and utilization effects of abalones on different seaweeds are different. For example, it has been reported that feeding *Undaria pinnatifida* to *Haliotis discus hannai* resulted in better growth than with other diets [28]. Mai et al. [29] reported that abalones (*H. tuberculata*) fed red algae (*Palmaria palmata*) had better growth than those that were not [29]. This study was similar to Mai et al. [29], which proved that abalones grow well when fed the red algae *G. lemaneiformis*. Related studies showed that cellulase activity in the digestive gland of *Haliotis discus hannai* would increase when fed with *G. lemaneiformis* [30]. The protein content of red algae is higher than that of brown algae, which could affect the growth of abalones. The nitrogen content of *G. lemaneiformis* is significantly higher than that of kelp [31], which may be the reason for the higher growth of abalones fed with *G. lemaneiformis* as compared to those fed kelp. After culture for 12 m, the growth rates of shell length and total weight of common abalones in *G. lemaneiformis* group, fresh kelp group, and dried kelp group were higher than that of orange-muscle abalones, as shown in

Figure S2. Although the carotenoid contents of orange-muscle abalones were significantly higher than that of common abalones, the increased rates of growth traits of orange-muscle abalones were lower than that of common abalones after feeding the same diets. Some studies have reported that carotenoids could promote the growth of marine organisms. However, this experiment found that increases in carotenoid contents did not improve the growth ability of orange-muscle abalones. The relationship between carotenoid accumulation and growth traits, the causes of this phenomenon, and whether it is related to energy distribution in abalones is still unclear and needs further exploration.

5. Conclusion

In summary, our study confirmed that carotenoids in abalones come from their diets directly, and the carotenoid types in the muscles were basically the same as in their diets. The one-year culture experiment showed that the amounts of zeaxanthin, β-carotene, and fucoxanthin were significantly higher in the muscles of orange-muscle abalones fed with *G. lemaneiformis* than in those fed with fresh kelp or dried kelp ($P < 0.01$), and the amounts of zeaxanthin were the biggest difference. In addition, the quantities of the three main carotenoids were significantly higher in orange-muscle abalones than in common abalones ($P < 0.01$). Orange-muscle abalones exhibited an enhanced ability for accumulating carotenoids, especially zeaxanthin. Our results verified that the carotenoid contents in abalone's muscle may be mainly controlled by genetic factors, although the type of diets affected its change to some extent. After being fed fresh kelp or dried kelp for one year, the amount of carotenoid in abalones showed an obvious downward trend. The amount of carotenoid in abalones fed with *G. lemaneiformis* was stable. Consuming *G. lemaneiformis* played an important role in maintaining carotenoid contents, especially zeaxanthin contents in *H. gigantea*. Our study also proved that abalones had a good growth performance under the feeding conditions of *G. lemaneiformis*. Although the carotenoid contents of orange-muscle abalones were significantly higher than that of common abalones, the increased rates of growth traits of orange-muscle abalones were lower than that of common abalones after feeding the same diets. The specific cause of this phenomenon is still unclear, which needs further exploration.

Data Availability

The authors declare that the data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors have no conflicts of interest to declare. All coauthors have seen and agree with the contents of the manuscript, and there is no financial interest to report.

Authors' Contributions

Xiaohui Wei and Bingye Yang contributed equally to this work.

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Supplementary Materials

Supporting Information associated with this article listed below. Supplementary Tables and Figures. *(Supplementary Materials)*

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