A comparative study on growth, protein turnover and energy budget of green and white color morphs of sea cucumber *Apostichopus japonicus* (Selenka)

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ABSTRACT: A 98 d experiment was conducted to compare the growth performance, protein turnover and energy budget between green and white color morphs of the sea cucumber *Apostichopus japonicus*. During the experiment, sea cucumbers were fed a single natural diet of *Sargassum thunbergii* and a premixed artificial diet. Results showed that there was no significant interaction of diet, species or experimental time on growth performance and feed utilization of *A. japonicus*. Specific growth rate (SGR) of green sea cucumber was significantly higher than that of white sea cucumber. Green sea cucumber fed *S. thunbergii* exhibited much higher food conversion efficiency (FCE) and protein efficiency ratio (PER) compared to those on the artificial diet, indicating preferential absorption of *S. thunbergii*. There were also significant discrepancies in body wall production rate (BWPR), crude protein and ash content between green and white sea cucumber. The observed $\delta^{15}N$ values gradually increased owing to the assimilation of the diets containing higher nitrogen stable isotope ratios. Green sea cucumber reflected and approached $\delta^{15}N$ values of the experimental diets much more quickly and with shorter half-lives than white sea cucumber. The relatively constant metabolic contributions in intestine (71 to 78%) and body wall (46 to 52%) of sea cucumber indicated a stable bioenergetic distribution strategy of tissue. According to the time-based turnover model, the estimated fractionation ($\Delta^{15}N$) ranged from 2.37 to 3.30‰. The average formulas of energy allocation for green and white sea cucumber were: $100C = 7.8G + 53.1F + 6.2U + 32.8R$, $100C = 6.3G + 57.9F + 8.9U + 27.0R$, respectively. Taken together, our results suggest that white sea cucumber may have higher dietary quality requirements for growth and metabolism than green sea cucumber.

KEY WORDS: *Apostichopus japonicus* · Growth · Nitrogen stable isotope · Protein turnover · Energy allocation

INTRODUCTION

The sea cucumber *Apostichopus japonicus* (Selenka) (Echinodermata: Holothuroidea) traditionally has been used as a remedy for wound healing due to its curative and aphrodisiac effects (Sloan 1984, Xia et al. 2013a). With increasing market demand and decreasing natural resources available to *A. japonicus*, the farming scale has been expanded rapidly in the last few decades, especially in Shandong, Liaoning and Fujian provinces in China (Sun et al. 2012). Total production of this species reached 206 000 t in 2015, a 101% increase compared to that in 2009 (MOAC 2016). However, long-term inbreeding and unsustainable brood-stock culture has resulted in serious problems, including breed deterioration and disease (Gao et al. 2011, Zheng et al. 2012). Therefore, selecting fine breeding lines has become the top

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priority for the sustainable culture of sea cucumber (Bai et al. 2015).

Color variation is an important and particular characteristic for sea cucumber. Previous studies have shown that sea cucumber can be divided into green, red and black morphs (Kanno et al. 2006, Kang et al. 2011). White sea cucumber, a genetic albino variant, is rare in nature (Ma et al. 2014a) and extensively believed to be rich in the element selenium, and consequently has a high market value. Therefore, it would be fascinating to foster this commercial species in aquaculture practice. With successful hatchery and semi-intensive culture techniques that are currently used, the production of white sea cucumber is increasing year by year in China. Previous studies have mainly focused on physiological performance (Pei et al. 2012, Lin et al. 2013) and genetics (Ma et al. 2014a,b); however, there is little information on nutritional characteristic and energy allocation of white sea cucumber.

As a typical deposit-feeding species, sea cucumber mainly take up detritus of sea grass and macroalgae, benthic microalgae, protozoa and bacteria as food sources in the sediment of natural seawater (Xia et al. 2015a). In aquaculture practice, macroalgae powder consisting of *Sargassum thunbergii*, *Gracilaria lemaneiformis* or *Laminaria japonica* is generally provided as a supplement to sea cucumber (Gao et al. 2011). Recently, a range of physicochemical and biological tests were conducted to assess the suitability of terrestrial plant protein sources, e.g. soybean meal (Seo & Lee 2011, Seo et al. 2011) and corn leaf (Wu et al. 2015), as a replacement for seaweeds. Sea cucumber generally requires a relatively low energy supply for optimum growth (Sun et al. 2004, Zhu et al. 2005). Stable isotope analysis can effectively yield valuable information on the incorporation of micro- and macronutrients (Le Vay & Gamboa-Delgado 2011, Martin-Perez et al. 2013, Xia et al. 2015b). In the present study, we compared the discrepancies of growth, protein turnover and energy budget of green and white sea cucumber under different dietary regimes, to provide scientific basis for further study on the physiological ecology and nutriology of sea cucumber.

MATERIALS AND METHODS

Experimental design

Juvenile green and white sea cucumbers of initial dry weight 0.44 ± 0.01 g were collected from a commercial farm in Yantai City. To ensure complete turnover of nitrogen stable isotope and subsequent homogeneous isotopic composition at the beginning of the experiment, the sea cucumbers were fed the same diet for 6 mo (Xia et al. 2015a). The sea cucumbers were then immediately transported to the laboratory and acclimated to the experimental conditions for 3 wk at salinity 30 to 32, dissolved oxygen 6.5 ml l^{-1}, temperature 20 ± 0.5°C and a photoperiod of 14 h light:10 h dark (Sun et al. 2012, Xia et al. 2017). After acclimation, the green and white sea cucumbers were equally divided into 2 groups, respectively. Each group contained 120 sea cucumbers that were randomly allocated into 3 glass aquaria (50 × 60 × 60 cm^3, 180 l), i.e. 40 ind. aquarium^{-1}.

Sea cucumbers in each group were fed a single natural diet (80% *Sargassum thunbergii* and 20% sea mud) and premixed artificial diet, respectively. The artificial diet contained fish meal, soybean meal and kelp powder as protein sources (Table 1). To comply with the feeding habits of sea cucumber, sea mud was collected from an intertidal zone, dried and ground to fine powder. Then it was combusted at 550°C for 6 h to remove organic matter. All ingredi-

Table 1. Formulation and nutritional composition of the experimental diets (dry matter, %)

Ingredient	Single natural diet	Premixed artificial diet
Sargassum thunbergii	80	/
Fish meal	/	6.0
Soybean meal	/	22.0
Gelatinized cornstarch	/	20.0
Kelp powder	/	8.0
Wheat starch	/	6.0
Squid liver oil	/	0.5
Lecithin	/	0.5
Mineral premix[a]	/	1.0
Vitamin premix[b]	/	1.0
Sea mud	20	35.0
Proximate analysis		
Crude protein	15.71	16.03
Energy (kJ g^{-1})	11.58	11.76
$\delta^{15}N$ (‰)	9.78	4.12

[a]Mineral premix (g kg^{-1}): $MgSO_4 \cdot 7H_2O$, 90.0; ferric citrate, 18.0; $ZnSO_4 \cdot 7H_2O$, 3.0; $MnSO_4 \cdot H_2O$, 2.5; $Ca(H_2PO_4)_2$, 160.0; Ca-lactate, 25.8; $CuCl_2$, 0.8; $AlCl_3 \cdot 6H_2O$, 0.18; NaCl, 2.0; KIO_3, 0.04; $CoCl_2 \cdot 6H_2O$, 0.07
[b]Vitamin premix (g kg^{-1}): L-ascorbic acid, 150.0; DL-α-tocopherol acetate, 10.0; thiamin hydrochloride, 6.0; riboflavin, 8.0; pyridoxine hydrochloride, 5.0; niacin, 40.0; myo-inositol, 100.0; D-biotin, 0.3; folic acid, 1.5; *p*-amino benzoic acid, 10.0; menadione, 4.0; retinyl acetate, 1.5; cholecalciferol, 0.005; cyanocobalamin, 0.005

ents were blended thoroughly with 30% distilled water. Pellets were made with a moist pelletizer, dried for 24 h in an oven at 60°C, ground into desirable sizes (180 meshes), and stored at −20°C for use.

The experiment lasted for 98 d. During the experiment, the sea cucumbers were fed up to 5% of their total biomass once per day at 17:00 h. This ration size ensured satiation and minimized feed waste (Liu et al. 2009, Xia et al. 2015a). Uneaten feed and feces were collected separately by siphoning 22 h after feeding, and dried at 60°C to a constant weight for further analysis. The weight of uneaten feed was adjusted by the leaching ratio of the diet in water (Shi et al. 2015).

Sample collection and determination

On Day 0, 7, 14, 28, 42, 70 and 98, 3 sea cucumbers from each aquarium were randomly sampled. After being weighed, all individuals were dissected on ice, and the intestine and body wall were separated immediately. All samples were dried, ground and stored at −80°C for further analysis.

Nitrogen stable isotope values were measured by an EA-IRMS (ThermoFinnigan MAT Delta-plus). Results are expressed in standard δ-unit notation, defined as follows:

$$\delta^{15}N = [(R_{sample} / R_{standard}) - 1] \times 1000‰ \qquad (1)$$

where R is $^{15}N{:}^{14}N$. The ratios were reported compared to air N_2. Analytical precision was ±0.1‰.

At the end of the experiment, the sea cucumbers were fasted for 24 h, and collectively weighed and counted for calculations of survival, growth performance and feed utilization. Proximate composition was determined by the standard methods of AOAC (1995). The Kjeldahl method after acid digestion was used to analyze crude protein (N × 6.25); crude lipid was measured by the ether-extraction method. The energy and nitrogen contents of all samples were determined by a calorimeter (PARR Instrument) and a CHNS/O Analyzer (PE2400 Series II; PerkinElmer), respectively.

Time-based turnover model

Temporal changes in nitrogen stable isotope ratios of sea cucumber were modeled as an exponential function (Sun et al. 2012), represented as follows:

$$\delta_t = \delta_f + (\delta_i - \delta_f) \, e^{-(k + m)t} \qquad (2)$$

where δ_t is the measured isotopic signature at experimental time t, δ_f is the expected isotopic signature in equilibrium with the diet, δ_i is the initial isotopic signature, and m is the metabolic turnover constant. The growth rate constant (k) was calculated using the following equation (Xia et al. 2013b):

$$k = \ln(W_f / W_i) / t \qquad (3)$$

where W_f is the final dry weight of sea cucumber at sampling time t and W_i is the initial dry weight at $t = 0$. Expected isotopic change due to growth alone was determined by Eq. (2) where m was set to 0, while isotopic turnover due to metabolism was derived by comparing expected isotopic turnover owing to growth with the observed turnover during the experiment. Any isotopic turnover in excess of what was contributable to growth was caused by metabolic tissue replacement (m), estimated using non-linear regression.

The relative contributions of growth (P_g) and metabolism (P_m) to nitrogen isotopic turnover were expressed as the following equation (Buchheister & Latour 2010):

$$P_g \text{ (or } P_m) = k \text{ (or } m) / (k + m) \qquad (4)$$

Nitrogen isotopic fractionation was calculated as follows (Gamboa-Delgado et al. 2013):

$$\Delta = \delta_f - \delta_d \qquad (5)$$

where δ_d is the $\delta^{15}N$ value of the diet. The period needed to achieve an α percent isotopic turnover (Hesslein et al. 1993) was calculated as follows:

$$T_{\alpha / 100} = -\ln(1 - \alpha / 100) / (k + m) \qquad (6)$$

To determine half-life ($T_{0.5}$), the equation was solved for α = 50.

Energy budget

Energy allocation was constructed by the following equation (Liu et al. 2009):

$$C = G + F + U + R \qquad (7)$$

where C represents energy consumed, G is energy for growth, F is energy of feces produced, U stands for energy loss as ammonia excretion, R is energy loss as respiration (kJ g^{-1} d^{-1}). U was estimated by the nitrogen budget equation (Shi et al. 2013):

$$U = (C_N - G_N - F_N) \times 24830 \qquad (8)$$

where C_N is nitrogen consumed from diet, G_N is nitrogen deposited in body, F_N is nitrogen lost in feces, and 24830 is the energy content in excreted ammonia (J g^{-1}). The value of R was calculated by $R = C - G - F - U$.

Statistical analysis

A 3-way analysis of variance (ANOVA) was used to examine the interaction of diet, color morph and experimental time on growth, survival, feed utilization and proximate composition of *Apostichopus japonicus*, while the parameters based on the turnover model of $\delta^{15}N$ (e.g. m, P_g, $T_{0.5}$, etc.) and energy parameters of sea cucumber were analyzed by 2-way ANOVA. The discrepancies in parameters between the diet treatments and between the color morphs were compared using paired Student's *t*-tests. A probability level of 0.05 was used for rejection of the null hypothesis. Prior to analysis, raw data were examined for normality of distribution and homogeneity of variance with Kolmogorov-Smirnov and Levene's tests, respectively (Zar 1999). All statistical analyses were performed with software SPSS (2008). Data are mean ± SD.

RESULTS

Growth performance and feed utilization

Three-way ANOVA showed that there was no significant interaction of diet, species and experimental time on growth performance and feed utilization of *Apostichopus japonicus* (Table 2). However, significant interactions of species and experimental time on W_f, weight gain and protein efficiency ratio (PER) of sea cucumber were found during the 98 d experiment (p < 0.05). Individuals increased in average dry weight by 61.09 to 66.92% and 45.46 to 47.91% for green and white sea cucumber, respectively. Green sea cucumber had higher specific growth rates (SGRs) compared to white sea cucumber, and the SGRs of green sea cucumber fed *Sargassum thunbergii* were significantly higher than those fed the artificial diet (p < 0.05). Food conversion efficiency (FCE), feed intake (FI) and PER all showed significant discrepancies between green and white sea cucumber (p < 0.05). However, no significant difference between diet treatments was observed for white sea cucumber (p < 0.05). Three-way ANOVA also showed no significant interaction of the factors on proximate composition of sea cucumber (p > 0.05), while crude protein was significantly affected by the interaction of species and experimental time (p < 0.05) (Table 3). The body wall production rate (BWPR) and crude protein content of white sea cucumber were significantly higher than those of green sea cucumber (p < 0.05). There was no significant difference in moisture or crude lipid content between green and white sea cucumber or between the diet treatments (p < 0.05).

Nitrogen isotopic turnover

Temporal changes in the observed $\delta^{15}N$ values of green and white sea cucumber fed the single natural diet of *S. thunbergii* and premixed artificial diet are shown in Figs. 1 & 2, respectively. All the observed $\delta^{15}N$ values in sea cucumber tissues gradually increased owing to assimilation of the experimental

Table 2. Growth performance and feed utilization of green and white sea cucumber fed the experimental diets. W_f: final dry weight; weight gain = $(W_f - W_i) / W_f \times 100$; specific growth rate, SGR = ln $(W_f / W_i) / t \times 100$; food conversion efficiency, FCE = $(W_f - W_i) / $ dry diet consumed $\times 100$; protein efficiency ratio, PER = $(W_f - W_i) / $ protein intake; feed intake, FI = dry diet consumed / $([W_f + W_i] / 2 \times t)$. Different superscript capital letters within the same column indicate significant differences between the diets for the same color morph (p < 0.05); different lowercase letters indicate significant differences between the color morphs fed the same diet (p < 0.05). *p < 0.05; **p < 0.01; ***p < 0.001; ns: non-significant

	Species	Survival (%)	W_f (g)	Weight gain (%)	SGR (% d^{-1})	FCE (%)	PER	FI (g g^{-1} d^{-1})
Sargassum thunbergii	Green	90.76 ± 4.18	1.36 ± 0.08[Aa]	66.92 ± 1.94[Aa]	1.13 ± 0.06[Aa]	5.99 ± 0.12[Aa]	0.29 ± 0.01[Aa]	0.21 ± 0.02[a]
diet	White	91.02 ± 5.50	0.86 ± 0.07[b]	47.91 ± 3.15[b]	0.67 ± 0.06[b]	4.37 ± 0.31[b]	0.21 ± 0.02[b]	0.15 ± 0.01[b]
Artificial diet	Green	92.24 ± 6.69	1.17 ± 0.05[Ba]	61.09 ± 0.95[Ba]	0.98 ± 0.03[Ba]	5.29 ± 0.20[Ba]	0.25 ± 0.01[Ba]	0.19 ± 0.02[a]
	White	90.33 ± 3.57	0.83 ± 0.06[b]	45.46 ± 3.53[b]	0.62 ± 0.07[b]	4.02 ± 0.33[b]	0.19 ± 0.03[b]	0.14 ± 0.02[b]
Three-way ANOVA								
Diet		ns	*	*	ns	*	ns	ns
Species		ns	***	**	*	**	*	*
Time		ns	*	**	ns	ns	ns	ns
Diet × Species		ns	ns	ns	ns	ns	ns	ns
Diet × Time		ns	ns	ns	ns	ns	ns	ns
Species × Time		ns	*	*	ns	ns	*	ns
Diet × Species × Time		ns	ns	ns	ns	ns	ns	ns

Table 3. Proximate composition of green and white sea cucumber (wet weight,%) fed the experimental diets. Body wall production rate, BWPR = (body wall weight / total weight) × 100. Different lowercase letters indicate significant differences between the color morphs fed the same diet (p < 0.05). *p < 0.05; **p < 0.01; ns: non-significant

	Species	BWPR (%)	Moisture	Crude protein	Crude lipid	Ash
Sargassum thunbergii diet	Green	61.08 ± 1.35^a	90.23 ± 1.08	4.21 ± 0.12^a	0.26 ± 0.06	2.98 ± 0.11^a
	White	66.64 ± 2.07^b	91.18 ± 1.45	4.76 ± 0.07^b	0.22 ± 0.08	2.54 ± 0.10^b
Artificial diet	Green	60.12 ± 3.12^a	90.37 ± 1.34	4.17 ± 0.09^a	0.27 ± 0.03	2.89 ± 0.08^a
	White	66.31 ± 1.97^b	92.04 ± 2.19	4.65 ± 0.13^b	0.23 ± 0.02	2.61 ± 0.06^b
Three-way ANOVA						
Diet		ns	ns	ns	ns	ns
Species		*	ns	**	ns	*
Time		*	ns	*	ns	ns
Diet × Species		ns	ns	ns	ns	ns
Diet × Time		ns	ns	ns	ns	ns
Species × Time		ns	ns	*	ns	ns
Diet × Species × Time		ns	ns	ns	ns	ns

diets with higher nitrogen stable isotope ratios. There were significant shifts between the sampling time points (p < 0.05). Significant differentiations in the nitrogen stable isotope signatures between green and white sea cucumber also commenced from the first week. Green sea cucumber had shorter half-lives (for intestine, $T_{0.5}$ = 14.98 to 15.23 d; for body wall, $T_{0.5}$ = 29.66 to 33.68 d) compared to white sea cucumber (for intestine, $T_{0.5}$ = 25.77 to 34.25 d; for body wall, $T_{0.5}$ = 51.73 to 60.80 d) according to the calculations of the time-based turnover model (Table 4). Sea cucumbers fed *S. thunbergii* showed shorter half-lives than those fed the artificial diet, except for the intestine of green sea cucumber, and the half-lives were significantly affected by the interaction of diet and species for intestine and body wall

(p < 0.05). The turnover rates in the intestine of sea cucumber fed *S. thunbergii* and artificial diet ($T_{0.5}$ = 14.98 to 34.25 d) were faster than that of the body wall ($T_{0.5}$ = 29.66 to 60.80 d).

In Figs. 3 & 4, the turnover rates with the contribution of growth and metabolism (solid lines) were significantly faster relative to those owing to growth alone (dotted lines) (p < 0.05). Metabolic contributions to the nitrogen isotopic turnover reached approximately 71 to 78% and 46 to 52% for intestine and body wall of sea cucumber, respectively. Non-significant differences in the P_m values between the diets and between the color morphs were found (p > 0.05). The final asymptotic nitrogen isotopic values (δ_f) of the sea cucumbers fed *S. thunbergii* were significantly higher than those fed the artificial diet (p <

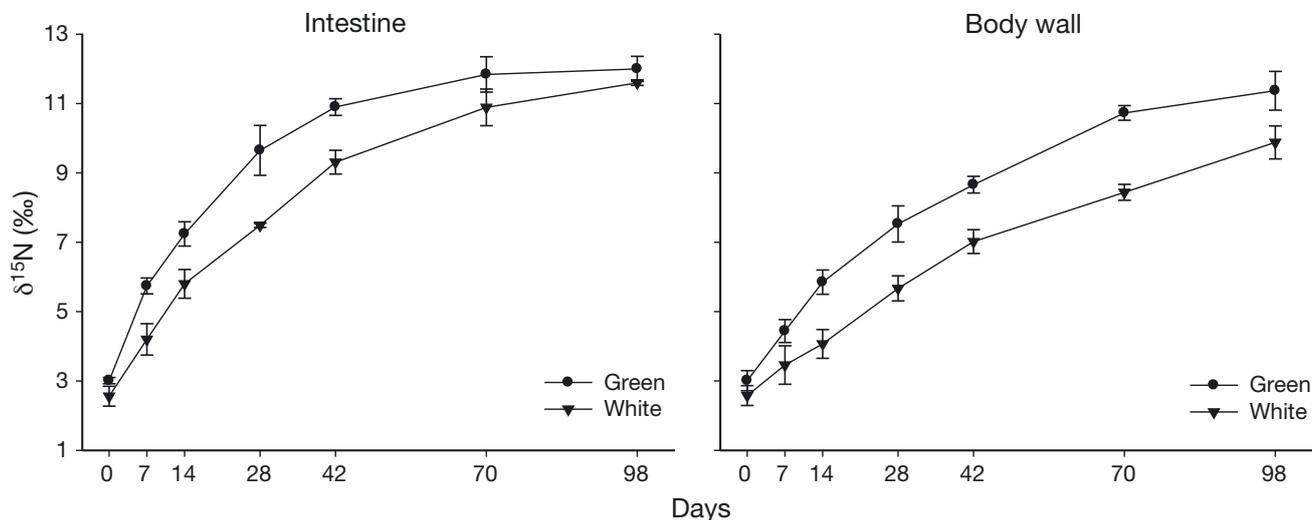Fig. 1. Temporal changes in the observed $\delta^{15}N$ values for intestine and body wall of green and white sea cucumber fed single natural diet of *Sargassum thunbergii*
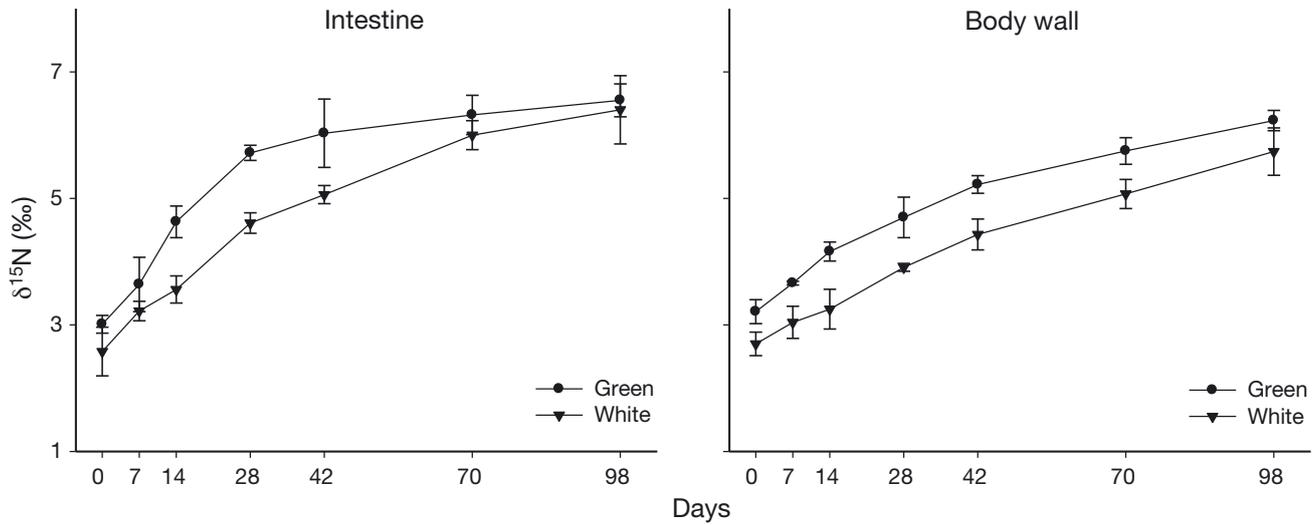
Fig. 2. Temporal changes in the observed $\delta^{15}N$ values for intestine and body wall of green and white sea cucumber fed a premixed artificial diet

0.05). According to the time-based turnover model, the estimated fractionation ($\Delta^{15}N$) ranged from 2.37 to 3.30‰ in the present study (Table 4).

Energy budget

Energy parameters and budget of green and white sea cucumber fed *S. thunbergii* and artificial diet are shown in Table 5. During the experiment, the consumed energy of green sea cucumber was significantly higher than that of white sea cucumber (p < 0.05). Two-way ANOVA indicated significant interaction between diet and species in energy for growth (p < 0.05). Green sea cucumber fed *S. thunbergii* had higher energy for growth than those fed the artificial diet (p < 0.05). The energy of feces produced and lost

through ammonia excretion of green sea cucumber was significantly lower than those of white sea cucumber, which had lower energy deposited for respiration (p < 0.05). The average formulas for energy allocation in green and white sea cucumber were: $100C = 7.8G + 53.1F + 6.2U + 32.8R$, and $100C = 6.3G + 57.9F + 8.9U + 27.0R$, respectively.

DISCUSSION

In the present study, green and white sea cucumber both exhibited obvious growth and nutritional suitability of various protein sources. The relatively lower growth performance and feed utilization of white sea cucumber was likely due to its lower digestive and immune enzyme activities (Xia 2012). Our

Table 4. Parameter estimate and calculation from the time-based model of $\delta^{15}N$ turnover for green and white sea cucumber fed the experimental diets. m: metabolic turnover constant; k: growth constant; P_m: proportion of metabolic contribution to nitrogen turnover; $T_{0.5}$: time-based half-life; $T_{0.95}$: time needed to reach 95% nitrogen turnover; δ_f: final asymptotic nitrogen isotopic value; Δ: fractionation between diet and tissue. Different superscript capital letters within the same row indicate significant differences between diets for the same color morph (p < 0.05); different lowercase letters indicate significant differences between color morphs fed the same diet (p < 0.05)

| Tissue/Diet	Species	m (d^{-1})	$k + m$ (d^{-1})	P_m	$T_{0.5}$ (d)	$T_{0.95}$ (d)	δ_f (‰)	Δ (‰)
Intestine								
Sargassum thunbergii	Green	0.0351 ± 0.0022[a]	0.0463 ± 0.0013[a]	0.75 ± 0.03	14.98 ± 0.61[a]	64.71 ± 3.20[a]	12.14 ± 0.26[A]	2.37
	White	0.0201 ± 0.0015[Ab]	0.0269 ± 0.0018[Ab]	0.75 ± 0.02	25.77 ± 0.52[Ab]	111.37 ± 7.34[Ab]	12.34 ± 1.60[A]	2.56
Artificial diet	Green	0.0357 ± 0.0009[a]	0.0455 ± 0.0024[a]	0.78 ± 0.03	15.23 ± 0.97[a]	65.83 ± 4.37[a]	6.57 ± 0.19[B]	2.45
	White	0.0141 ± 0.0012[Bb]	0.0203 ± 0.0008[Bb]	0.71 ± 0.07	34.25 ± 0.61[Bb]	148.33 ± 7.67[Bb]	7.04 ± 0.23[B]	2.93
Body wall								
S. thunbergii	Green	0.0121 ± 0.0035[a]	0.0234 ± 0.0014[a]	0.51 ± 0.06	29.66 ± 0.39[Aa]	127.39 ± 5.91[Aa]	12.29 ± 0.71[A]	2.59
	White	0.0067 ± 0.0020[b]	0.0134 ± 0.0021[b]	0.50 ± 0.04	51.73 ± 1.28[Ab]	223.56 ± 6.60[Ab]	12.51 ± 0.28[A]	2.77
Artificial diet	Green	0.0108 ± 0.0018[a]	0.0206 ± 0.0022[a]	0.52 ± 0.09	33.68 ± 1.47[Ba]	145.57 ± 8.93[Ba]	6.64 ± 0.51[B]	2.54
	White	0.0047 ± 0.0005[b]	0.0104 ± 0.0008[b]	0.46 ± 0.10	60.80 ± 2.01[Bb]	271.49 ± 11.48[Bb]	7.42 ± 0.53[B]	3.30
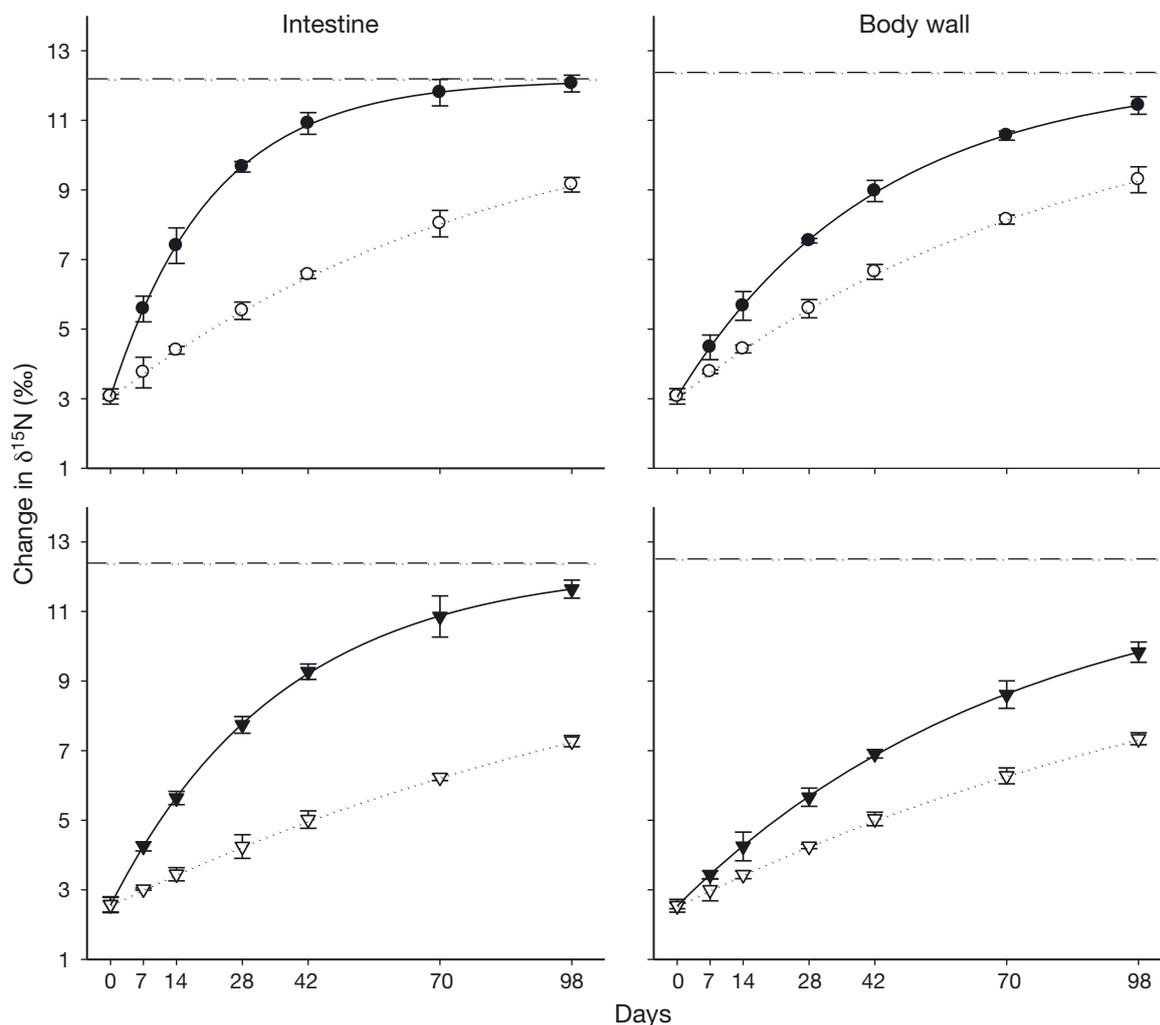
Fig. 3. Changes in the $\delta^{15}N$ values estimated with time-based turnover model for intestine (left column) and body wall (right column) of green (circles) and white sea cucumber (triangles) fed a single natural diet of *Sargassum thunbergii*. Solid lines/filled symbols: $\delta^{15}N$ values with the contribution of growth and metabolism; dotted lines/open symbols: $\delta^{15}N$ values with the contribution of growth alone (metabolic turnover constant, $m = 0$). Horizontal dash-dotted line: expected $\delta^{15}N$ values when tissues are completely equilibrated to *S. thunbergii*

results found that feed intake and energy deposited for growth in white sea cucumber were significantly lower than those of green sea cucumber. The higher SGR, FCE and PER of green sea cucumber demonstrated preferential absorption of *Sargassum thunbergii*, while white sea cucumber, which was also fed the 2 experimental diets, showed no preference. The significant interaction of species and experimental time revealed that weight gain and PER of sea cucumber increased as its body weight increased. Experimental time also had significant effect on BWPR and crude protein content, as reported by Song et al. (2009).

Stable isotope analysis can be used for studying the protein catabolism and anabolism of aquatic organisms through straightforward procedures with rapid sample analysis and high accuracy (Abimorad et al.

2014). Generally, the turnover rate of stable isotopes in tissue reflects the rate at which the isotopic signature of a consumer changes with diet (Guelinckx et al. 2007, Xia et al. 2015a). In the present study, green sea cucumber approached the $\delta^{15}N$ values of the experimental diets much more quickly, hence had shorter half-lives compared to white sea cucumber. We speculate that protein turnover might be responsible for the discrepancies in crude protein between green and white sea cucumber, which should be further discussed in a future study. Due to variation in biochemical components and enzyme systems, the turnover rate of nitrogen stable isotope in the intestine of sea cucumber was faster than that of the body wall. Previous studies have found much faster isotopic turnover rates and shorter half-lives of stable
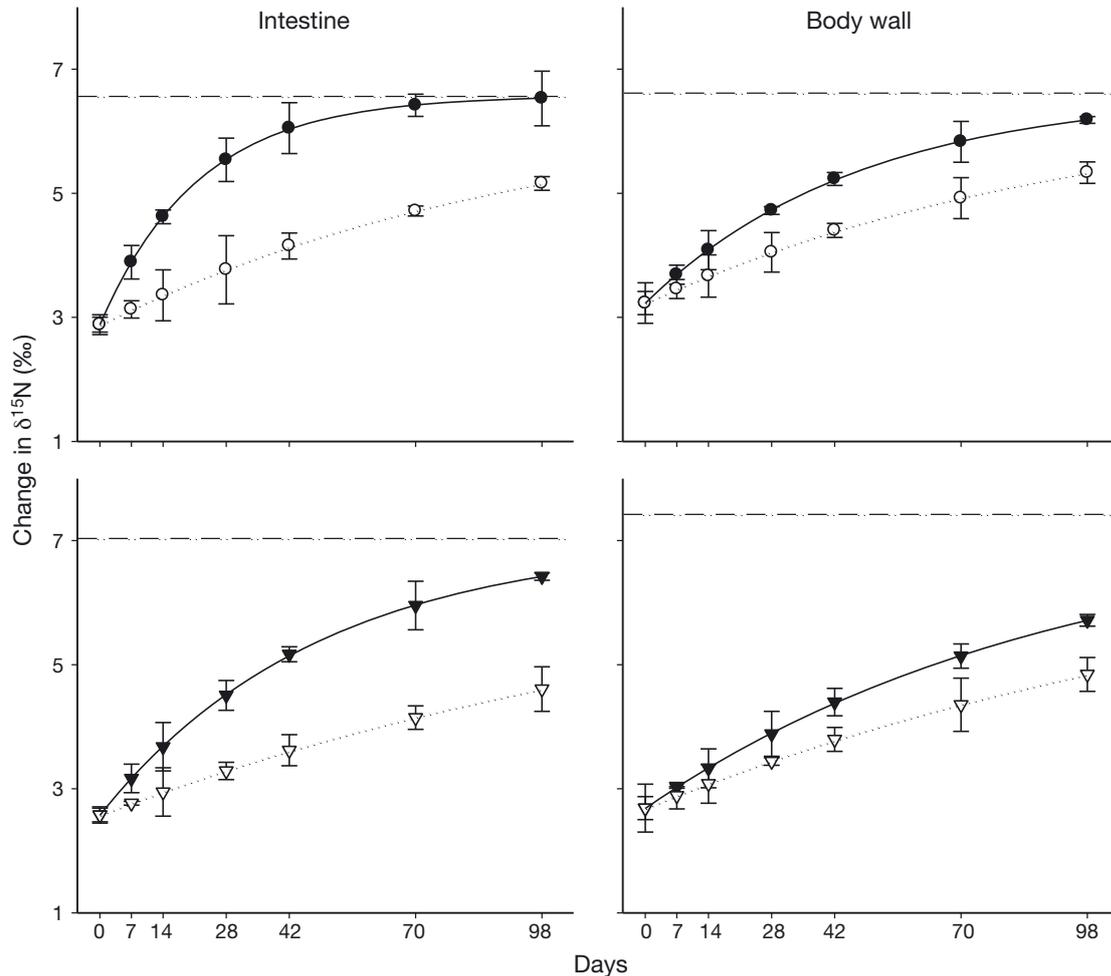
Fig. 4. Changes in the δ^{15}N values estimated with time-based turnover model for intestine (left column) and body wall (right column) of green (circles) and white sea cucumber (triangles) fed a premixed artificial diet. Solid lines/filled symbols: δ^{15}N values with the contribution of growth and metabolism; dotted lines/open symbols: δ^{15}N values with the contribution of growth alone (metabolic turnover constant, $m = 0$). Horizontal dash-dotted line: expected δ^{15}N values when tissues are completely equilibrated to artificial diet

isotopes in vigorous metabolic tissues (Tarboush et al. 2006, Sun et al. 2012). Our results also proved that variable growth and metabolic contributions to the isotopic turnover process resulted in the differentiations in turnover rate of nitrogen stable isotope (Arneson & MacAvoy 2005).

Tissue turnover is mainly driven by catabolic breakdown and anabolic replacement processes, which depend on growth (synthesis in excess of breakdown) and metabolism (a balanced rate of breakdown and resynthesis) (Hesslein et al. 1993). Previous studies have demonstrated that the relative importance of growth versus metabolism in isotopic turn-

Table 5. Energy allocation of green and white sea cucumber fed the experimental diet. C: energy consumed; G: energy for growth; F: energy of feces produced; U: energy lost through ammonia excretion; R: energy lost through respiration. Different superscript capital letters within the same row indicate significant differences between diets for the same color morph ($p < 0.05$); different lowercase letters mean significant differences between color morphs fed the same diet ($p < 0.05$)

Parameter	*Sargassum thunbergii*		Artificial diet	
	Green	White	Green	White
C (kJ g^{-1} d^{-1})	2.01 ± 0.11[a]	1.50 ± 0.12[b]	2.11 ± 0.08[a]	1.66 ± 0.06[b]
G (% C^{-1})	8.24 ± 0.35[Aa]	6.36 ± 0.27[b]	7.39 ± 0.11[Ba]	6.23 ± 0.12[b]
G (kJ g^{-1} d^{-1})	0.17 ± 0.01[a]	0.10 ± 0.02[b]	0.15 ± 0.01[a]	0.10 ± 0.02[b]
F (% C^{-1})	52.43 ± 2.07[a]	57.55 ± 2.21[b]	53.84 ± 1.54[a]	58.19 ± 1.80[b]
F (kJ g^{-1} d^{-1})	1.05 ± 0.12	0.86 ± 0.07	1.14 ± 0.10	0.97 ± 0.08
U (% C^{-1})	5.99 ± 0.31[a]	8.89 ± 0.16[b]	6.39 ± 0.22[a]	8.87 ± 0.18[b]
U (kJ g^{-1} d^{-1})	0.12 ± 0.02	0.13 ± 0.02	0.14 ± 0.03	0.15 ± 0.01
R (% C^{-1})	33.33 ± 1.42[a]	27.20 ± 1.85[b]	32.35 ± 2.27[a]	26.72 ± 3.04[b]
R (kJ g^{-1} d^{-1})	0.67 ± 0.15[a]	0.41 ± 0.04[b]	0.68 ± 0.11	0.44 ± 0.07

over is related to the bioenergetic allocations of organisms (e.g. Xia et al. 2015a,b). In the present study, non-significant differences in the P_m were found between green and white sea cucumber and between the diet treatments, suggesting that sea cucumber maintained a relatively stable bioenergetic distribution strategy in their tissues. According to Beltrán et al. (2009) and Le Vay & Gamboa-Delgado (2011), greater fractionation, low growth rate and high protein mobilization can occur in fish and crustaceans under conditions of low dietary protein quality. Therefore, the high $\Delta^{15}N$ values in the tissues of the white sea cucumber might imply high dietary quality requirement.

The energy budget provides a framework for the evaluation of various ways in which nutrients are utilized (Lawrence & Lane 1982). The energy deposited for growth accounted for 7.39 to 8.24% of energy intake for green sea cucumber and 6.23 to 6.36% for white sea cucumber. Energy losses as feces and respiration were the main proportion of energy intake. White sea cucumber spent more energy in excretion and feces compared to green sea cucumber, whereas it deposited less energy in growth performance with lower metabolic activities. Previous studies found that higher percentage of energy loss resulted in a lower percentage of energy deposition in growth of Holothurians (Yuan et al. 2006, Zhang et al. 2012, Xia et al. 2013a). The higher energy deposited for growth and relatively lower energy loss of feces and excretion for green sea cucumber fed *S. thunbergii* also demonstrated the high dietary quality of *S. thunbergii*.

CONCLUSIONS

The single natural diet of *Sargassum thunbergii* and premixed artificial diet supplied sufficient energy for sea cucumber growth and metabolism. Both green and white sea cucumber fed the experimental diets maintained a relatively stable bioenergetic distribution strategy in their tissues. Our results also revealed that white sea cucumber might have higher dietary quality requirements for growth and metabolism compared to green sea cucumber, which implies increased nutrient pollution in waste discharge. To reverse this trend, more effective water treatment measures and more attention to detail in culture management are warranted. The present study yielded valuable information that should be of great interest to the culture of green and white sea cucumber.

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