Vol. 9: 281–291, 2017
https://doi.org/10.3354/aei00228

AQUACULTURE ENVIRONMENT INTERACTIONS
Aquacult Environ Interact

Published August 9

Optimal mud to sand ratios in sediment for sea cucumber aquaculture as revealed by carbon stable isotopes

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ABSTRACT: Sediment, which is central as a sink for organic material and nutrient cycling, provides habitat for feeding, resting, and nesting of sea cucumbers in aquaculture systems. We conducted a 2 mo experiment to investigate the optimal sea mud to sand ratios in sediment for sea cucumber *Apostichopus japonicus* (Selenka, 1867) aquaculture. Sea mud (<0.08 mm) and sand (0.08–0.5 mm) were used to prepare 5 different sediment formulations with different sea mud:sand ratios: 3:1, 1:1, 1:3, 1:0, and 0:1. Sea cucumbers were cultured in 20 fiberglass tanks (50 l) with 5 cm thick sediment spread on the bottom. Results showed that the specific growth rate of sea cucumbers and the contribution of the seaweed *Sargassum muticum* to food uptake by sea cucumber in the 3:1 ratio group were significantly higher than in the 1:0, 0:1, and 1:3 ratio groups, while no significant differences were observed between the 3:1 and 1:1 ratio groups. Furthermore, the total nitrogen, total organic carbon, and ammonium contents of the 1:1 and 3:1 ratio groups significantly decreased with time. These findings suggest that sea mud:sand ratios of 1:1 and 3:1 are optimal in sediment for *A. japonicus* aquaculture and effectively promote growth and bioremediation.

KEY WORDS: *Apostichopus japonicus* · Sediment · Sea cucumber aquaculture · Carbon stable isotope

INTRODUCTION

The sea cucumber *Apostichopus japonicus* (Selenka, 1867) (Liao 1980), which belongs to the Echinodermata, Holothuroidea, is an epibenthic and temperate species inhabiting coastal areas of Asia (Yang et al. 2005). Among the 1100 known sea cucumber species in the world, *A. japonicus* is one of the most commercially valuable aquaculture species (Okorie et al. 2008) and has long been exploited as an important fishery resource in Russia, China, Japan, and Korea (Sloan 1984). Aquaculture of *A. japonicus* has rapidly expanded in China over the past 20 yr (Chen 2004, Xia et al. 2012). The total production of *A. japonicus* exceeded 201 000 t in 2014, representing a 3.8% increase relative to 2013 (MOAC 2015).

Sea cucumbers, being deposit feeders, mainly ingest microorganisms, organic detritus, and animal feces including their own feces (Yingst 1976, Moriarty 1982, Uthicke & Klumpp 1998). *A. japonicus* plays an important role in the removal, recycling, and repackaging of nutrients, especially of organic

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matter (Jumars & Self 1986). Their trophic position and ability to process sediment enriched and affected by aquaculture activities has led to strong interest in their use in integrated multitrophic aquaculture systems (IMTAs) worldwide (Zamora et al. 2016). In IMTAs, *A. japonicus*, being an important production unit, can remove pollutants from the sediment and promote the recycling of aquaculture waste (Yuan 2005). Kang et al. (2003) observed that in co-culture with the abalone *Haliotis discus hannai* in a static culture system, *A. japonicus* could effectively reduce the food residue and feces in sediment and improve water quality. In a suspension aquaculture system with filter-feeding bivalves, co-cultured *A. japonicus* could effectively utilize particulate waste and act as a scavenger with great potential for bioremediation (Yuan et al. 2012). This sea cucumber is therefore not only an important economic aquaculture species, but also an important potential species for bioremediation in aquaculture systems (Yuan 2005).

Sediment and the overlying water column constitute habitat for sea cucumbers in aquaculture ponds. Sediment, which is central as a sink for organic material and nutrient cycling, provides habitat for feeding, resting, and nesting of sea cucumbers. The residual materials, mainly uneaten food, feces, and organic detritus, accumulate at the sediment–water interface by settlement, complexation, and adsorption. A number of studies on holothurians have demonstrated selectiveness for particle size and habitat (Rhoads & Young 1971, Hauksson 1979, Roberts 1979, Sloan & von Bodungen 1980, Wiedemeyer 1994, Dar & Ahmad 2006). Zhou et al. (2008) determined that approximately 60% of the intestinal contents of wild *A. japonicus* consisted of sand particles ranging in size from 0.0625 to 0.5 mm. However, Zhao & Yang (2010) reported from a field investigation that 90% of the intestinal contents of the *A. japonicus* gut were fine particles (1–80 µm).

Stable isotope analysis had been widely used as an effective tool to trace food sources (Fry 2006, Michener & Lajtha 2008, Sun et al. 2013) and the fate of fish-farming waste (Sarà et al. 2004, Yokoyama 2013). In the present study, we used stable isotope analysis to reveal the utilization and assimilation of the seaweed *Sargassum muticum.* We also used sea mud (<0.08 mm) and sand (0.08–0.5 mm) to produce different sediment formulations with different sea mud:sand ratios. The interactions between sea cucumbers and each specific sediment were investigated to find an optimized sediment formulation for *A. japonicus* aquaculture.

MATERIALS AND METHODS

Sediment preparation

The compositions of experimental sediments with 5 different ratios of sea mud and sand are presented in Table 1. The experimental sea mud and sand were obtained from the coastal zone of Qingdao, China. After having dried in the sun, the sea mud was ground and sieved through a 0.08 mm mesh. Sand was ground and sieved using 0.08 and 0.5 mm meshes. After being ground and sieved, the sea mud (<0.08 mm) and sand (0.08–0.5 mm) were used to prepare 5 different sediment formulations with different sea mud:sand ratios, i.e. 1:0, 3:1, 1:1, 1:3, and 0:1 (represented as M, MMMS, MS, MSSS, and S, respectively).

Culturing experiment

The experiment was conducted at the laboratory of the Qingdao National Ocean Scientific Research Center, Ocean University of China. The experimental sea cucumber (*Apostichopus japonicus*) specimens were collected from a local commercial farm. The experimental diets were composed of the seaweed *Sargassum muticum*, which was dried and ground to pass through a 0.063 mm mesh. Prior to the beginning of the experiment, *A. japonicus* were cultured in 50 l fiberglass tanks for 7 d to acclimatize them to the laboratory conditions. After acclimating, sea cucumbers with initial weights of 7.49 ± 0.23 g (mean ± SD) were cultured in glass aquarium tanks (30 cm × 40 cm × 50 cm) with 5 sediment formulations in 4 replicates for each formulation. The bottoms of all 20 experimental aquarium tanks were covered with 5 cm sediment, and the tanks were filled with seawater. Sea cucumbers were fed every 2 d at 16:00 h with experimental diets of 5% of the body weight. The diets were well mixed with water and evenly poured into every tank. The water tempera-

Table 1. Ingredients of the experimental sediments

Sediment Treatments	Ingredient (%)	
	Sea mud	Sand
M	100	0
MMMS	75	25
MS	50	50
MSSS	25	75
S	0	100

ture was maintained at 16.5 ± 0.5°C, and salinities ranged from 29 to 31‰. Two-thirds of the volume of the water in each tank was replaced with filtered seawater every 2 d. The experiment was conducted for 2 mo.

Sample collection

Before the experiment, 10 sea cucumbers were dissected as initial samples to collect body wall materials for the analysis of carbon stable isotope ratios ($\delta^{13}C$). At the end of the experiment, *A. japonicus* individuals were starved for 48 h to empty the gut and then were weighed. After weighing, all *A. japonicus* individuals in each aquarium were dissected on ice to separate the body walls. All separated body walls were placed in plastic bags and kept in a freezer at –80°C. The same was done with sea mud, sand, and the seaweed *S. muticum* samples, before the analysis of carbon stable isotope ratios ($\delta^{13}C$).

During the 2 mo experiment, 3 sampling points were randomly selected in each of the 20 tanks, and 0–1 cm surface sediment samples were collected with a plastic pipe measuring 2 cm in diameter before the experiment began, and then again during Weeks 2, 4, and 8. All sediment samples from the same tank were well mixed, dried in a lyophilizer (CHRIST LYO Alpha 1-4 LD plus), ground to powder in a mortar to pass through a 100 µm mesh sieve, and kept in a freezer at –80°C before further analyses of the total nitrogen (TN), total carbon (TC), total organic carbon (TOC), exchangeable NH_4^+, and NO_3^- concentrations.

Laboratory analyses

The growth performance of *A. japonicus* in terms of specific growth rate (SGR) was calculated as:

$$\text{SGR}\ (\%\ d^{-1}) = (\ln W_f - \ln W_i)/t \times 100 \quad (1)$$

where W_f and W_i are the final and initial weights of *A. japonicus* individuals in each aquarium, respectively, and t is the duration of the experiment in days.

Inorganic carbon was removed as CO_2 from samples of sea mud (<0.08 mm) and sand (0.08–0.5 mm) by adding 1:1 HCl. Then, together with samples of body tissues and the seaweed *S. muticum*, the sand and sea mud were dried at 60°C for 48 h to a constant weight for carbon stable isotope measurements. $\delta^{13}C$ and carbon content of sea mud, sand, *S. muticum*, and body tissues of sea cucumbers were determined using an elemental analyzer coupled with an isotope ratio mass spectrometer (EAIRMS, ThermoFinnigan MAT Delta-plus). Results of the stable isotope ratios were expressed in standard δ-unit notation, which is defined as follows:

$$\delta^{13}C = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000 \quad (2)$$

where R is the ^{13}C:^{12}C ratio. The values were reported relative to the Vienna Pee Dee Belemnite standard. Glycine was employed as the laboratory working standard and was run every 10 samples. Analytical precision was ± 0.1‰.

TN and TC concentrations of sediment samples were determined using a FlashEA 1112 Series NC Analyzer. TOC concentrations were also determined using the element analyzer after inorganic C as CO_2 was removed by adding 1:1 HCl and oven-dried to a constant weight.

The sediment samples were extracted with KCl solution (2M) to extract exchangeable NH_4^+ and NO_3^- (Bremner 1965). Sediment samples of 0.2 g were placed into 50 ml centrifuge tubes and treated with 20 ml KCl solution (2M), then equilibrated at 25 ± 2°C in an end-over-end shaker for 2 h before being centrifuged at 5000 × *g* (10 min). The supernatant was then filtered through a 0.45 µm glass fiber filter (Whatman GF/C) and determined for NH_4^+ and NO_3^- contents.

Stable isotope mixing model and statistical analysis

For sediment groups M and S, the 2-source concentration-weighed isotope mixing model was used to evaluate the respective contributions of the sediment ingredient and *S. muticum* to the food uptake of *A. japonicus* (Phillips 2001, Phillips & Koch 2002):

$$(\delta^{13}C'_X - \delta^{13}C_M)[C]_X f_{X,B} + (\delta^{13}C'_Y - \delta^{13}C_M)[C]_Y f_{Y,B} = 0;\ f_{X,B} + f_{Y,B=1} \quad (3)$$

where $f_{X,B}$ and $f_{Y,B}$ represent the fractions of assimilated biomass (B) of sources X and Y, respectively, in the mixture M. $[C]_X$ and $[C]_Y$ represent the carbon concentrations in food sources X and Y. Isotope signatures for the sources were corrected for trophic fractionations as designated by the prime (') symbol.

For sediment groups MS, MSSS, and MMMS, the stable isotope mixing model Isosource procedure was conducted to calculate the contributions of sea mud, sand, and *S. muticum* to the food uptake of *A. japonicus* (Phillips & Gregg 2003, Wen et al. 2016). An average fractionation effect of 1‰ for carbon isotopes was used to correct stable isotope shifts for

Table 2. Growth performance of *Apostichopus japonicus* in different sediment groups. Data are presented as mean ± SD (n = 3). Different letters in the same row mean significant differences (ANOVA with Duncan's test for multiple comparisons, p < 0.05). IBW (FBW): initial (final) body weight, SGR: specific growth rate. We used 5 different sediment formulations with different sea mud:sand ratios, i.e. 1:0, 3:1, 1:1, 1:3, and 0:1 (represented as M, MMMS, MS, MSSS, and S, respectively)

	M	MMMS	MS	MSSS	S
IBW (g)	7.49 ± 0.14	7.56 ± 0.24	7.61 ± 0.14	7.47 ± 0.30	7.46 ± 0.22
FBW (g)	11.49 ± 0.73^{b}	13.40 ± 0.51^{c}	13.61 ± 1.09^{c}	11.25 ± 1.27^{b}	9.52 ± 0.20^{a}
SGR (% d−1)	0.76 ± 0.15^{bc}	1.02 ± 0.12^{c}	1.04 ± 0.16^{c}	0.72 ± 0.20^{b}	0.44 ± 0.04^{a}

each trophic level (Peterson & Fry 1987, Gao et al. 2006, Sarà 2007, Sun et al. 2013).

All data were subjected to 1-way analysis of variance (ANOVA) followed by Duncan's test for multiple comparisons to determine the differences in each parameter between different months for each specific group at a significance level of 0.05 ($p < 0.05$). Prior to statistical analysis, raw data were assessed for normality of distribution and homogeneity of variance using Kolmogorov-Smirnov and Levene's tests, respectively (Zar 1999). Data were presented as mean ± SD (n = 3). All statistical analyses were performed using the statistical software SPSS for Windows (Release 22.0).

RESULTS

Growth performance

Growth performance of the sea cucumber *Apostichopus japonicus* on different sediments is shown in Table 2. At the beginning of the experiment, we observed no significant difference in the initial body weights (IBW) of *A. japonicus* between 5 different sediment groups (1-way ANOVA, $F_{4,10} = 0.247$, $p > 0.05$). After the 70 d feeding trial, significant differences in final body weight (FBW) ($F_{4,10} = 11.816$, $p < 0.05$) and SGR ($F_{4,10} = 8.643$, $p < 0.05$) were observed between different sediment groups. The FBW of *A. japonicus* in sediment groups MS and MMMS were significantly higher than in the sediment groups M, S, and MSSS ($F_{4,10} = 11.816$, $p < 0.05$). The SGR (% d^{-1}) of *A. japonicus* in sediment groups MS and MMMS were significantly higher than in the sediment groups S and MSSS ($F_{4,10} = 8.643$, $p < 0.05$). However, no significant differences in the FBW ($F_{4,10} = 11.816$, $p > 0.05$) and SGR ($F_{4,10} = 8.643$, $p > 0.05$) between groups MS and MMMS were observed. The FBW ($F_{4,10} = 11.816$, $p < 0.05$) and SGR ($F_{4,10} = 8.643$, $p < 0.05$) of *A. japonicus* in sediment group S were significantly lower than in any other sediment group. There were no significant differences in FBW ($F_{4,10} = 11.816$, $p > 0.05$) and SGR ($F_{4,10} = 8.643$, $p > 0.05$) values between sediment groups M and MSSS.

Carbon stable isotope ratios and nutritional contributions

Carbon stable isotope ratios ($\delta^{13}C$: ‰) and carbon content (%) of seaweed *Sargassum muticum* and potential food sources, including sea mud and sand, are listed in Table 3. Isotopic signatures of the seaweed *S. muticum*, sea mud, and sand were significantly different (ANOVA, $F_{2,6} = 104.628$, $p < 0.05$).

The $\delta^{13}C$ values of *A. japonicus* at the end of the experiment were remarkably different from the initial values (Table 3; $F_{5,12} = 23.565$, $p < 0.05$). The mean $\delta^{13}C$ values of *A. japonicus* in the 5 different sediment groups were all located within those of the corresponding 2 (for sediment groups M and S) or 3 (for sediment groups MS, MMMS, and MSSS) food ingredients after correction for isotope fractionation, indicating the simultaneous absorption of 2 or 3 food sources by *A. japonicus.*

Table 3. Carbon stable isotope ratios ($\delta^{13}C$) and carbon content of *Sargassum muticum*, sedimentary ingredients, and sea cucumbers *Apostichopus japonicus*. Data are presented as mean ± SD (n = 3); other details as in Table 2

Sample	$\delta^{13}C$ (‰)	C content (%)
Food source		
S. muticum	−16.06 ± 0.21^{c}	26.53 ± 1.03
Sea mud	−18.57 ± 0.44^{b}	0.26 ± 0.03
Sand	−21.29 ± 0.60^{a}	0.04 ± 0.01
Sea cucumber		
Initial	−17.56 ± 0.09^{a}	26.45 ± 2.56
M	−16.56 ± 0.28^{c}	22.65 ± 3.92
MMMS	−16.23 ± 0.21^{d}	24.15 ± 2.53
MS	−16.40 ± 0.11^{cd}	25.47 ± 1.98
MSSS	−16.64 ± 0.21^{c}	25.94 ± 1.77
S	−17.04 ± 0.07^{b}	27.45 ± 3.11

Table 4. Nutritional contributions (%) of *Sargassum muticum* and sedimentary ingredients to the food uptake of sea cucumber *Apostichopus japonicus*. Data are presented as mean ± SD (n = 3); other details as in Table 2

	M	MMMS	MS	MSSS	S
S. muticum	86.25 ± 1.75[ab]	94.27 ± 5.80[c]	90.75 ± 2.25[bc]	83.00 ± 6.06[a]	80.23 ± 1.41[a]
Sea mud	13.75 ± 1.75	1.73 ± 1.80	1.50 ± 0.50	5.33 ± 2.02	
Sand		4.00 ± 4.00	6.75 ± 1.75	11.67 ± 4.04	19.77 ± 1.41

Analyses were conducted using the 2-source concentration-weighted isotope mixing model, and the stable isotope mixing model Isosource procedure revealed the relative contribution of *S. muticum* and potential food sources, including sea mud and sand, to the food uptake of *A. japonicus* in the 5 different sediment groups (Table 4). In each of the 5 different sediment groups, the sea mud and sand contributed a mere fraction to the food uptake of sea cucumber relative to *S. muticum*. The proportional contributions of *S. muticum* to the food uptake of *A. japonicus* in sediment groups MS and MMMS were significantly higher than in groups S and MSSS ($F_{4,10}$ = 6.015, p < 0.05), while no significant differences in the proportional contributions of *S. muticum* between sediment groups MS and MMMS were observed ($F_{4,10}$ = 6.015, p > 0.05). The seaweed *S. muticum* contributed more in sediment group MMMS than in group M ($F_{4,10}$ = 6.015, p < 0.05). However, the proportional contributions of *S. muticum* in sediment groups MS and M showed similar values ($F_{4,10}$ = 6.015, p > 0.05).

Sedimentary TN, TC, and TOC content

Significant temporal decreases in TN content of sediment groups M ($F_{3,8}$ = 18.935, p < 0.05), MS ($F_{3,8}$ = 3.290, p < 0.05), MSSS ($F_{3,8}$ = 6.415, p < 0.05), and MMMS ($F_{3,8}$ = 10.115, p < 0.05) were observed over the 8 wk experimental period (Fig. 1). In Week 8, TN content of sediment groups M ($F_{3,8}$ = 18.935, p < 0.05), MS ($F_{3,8}$ = 3.290, p < 0.05), MSSS ($F_{3,8}$ = 6.415, p < 0.05), and MMMS ($F_{3,8}$ = 10.115, p < 0.05) was significantly lower than the corresponding initial content in Week 0. In contrast, the sedimentary TN content of sediment group S did not show significant temporal changes throughout the experimental period ($F_{3,8}$ = 0.841, p > 0.05).

Over the 8 wk experimental period, we observed significant increases in the TC content in sediment groups M ($F_{3,8}$ = 7.071, p < 0.05), S ($F_{3,8}$ = 10.934, p < 0.05), and MSSS ($F_{3,8}$ = 31.320, p < 0.05; Fig. 2). A significant declining trend in TC content was found in sediment group MS ($F_{3,8}$ = 3.976, p < 0.05), while no significant temporal changes occurred in TC content of sediment group MMMS throughout the experimental period ($F_{3,8}$ = 1.415, p > 0.05).

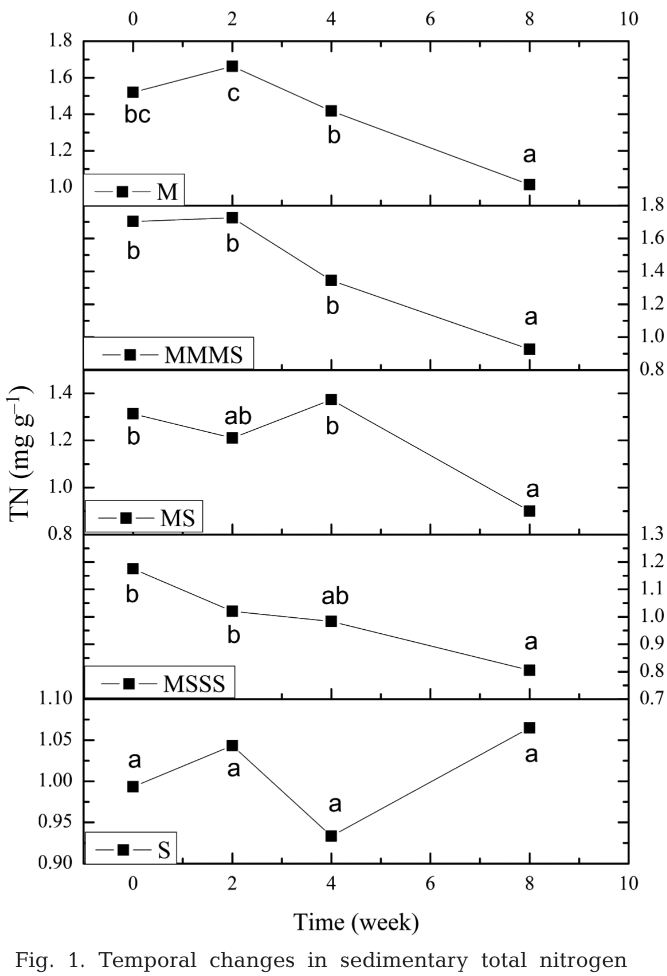

Fig. 1. Temporal changes in sedimentary total nitrogen (TN) content of different sediment treatments during the sea cucumber *Apostichopus japonicus* culturing experiment. Different letters in the same block mean significant differences between different sampling time points for each specific group (ANOVA with Duncan's test for multiple comparisons, p < 0.05). We used 5 different sediment formulations with different sea mud:sand ratios, i.e. 1:0, 3:1, 1:1, 1:3, and 0:1 (represented as M, MMMS, MS, MSSS, and S, respectively). Note different scales on the *y*-axes

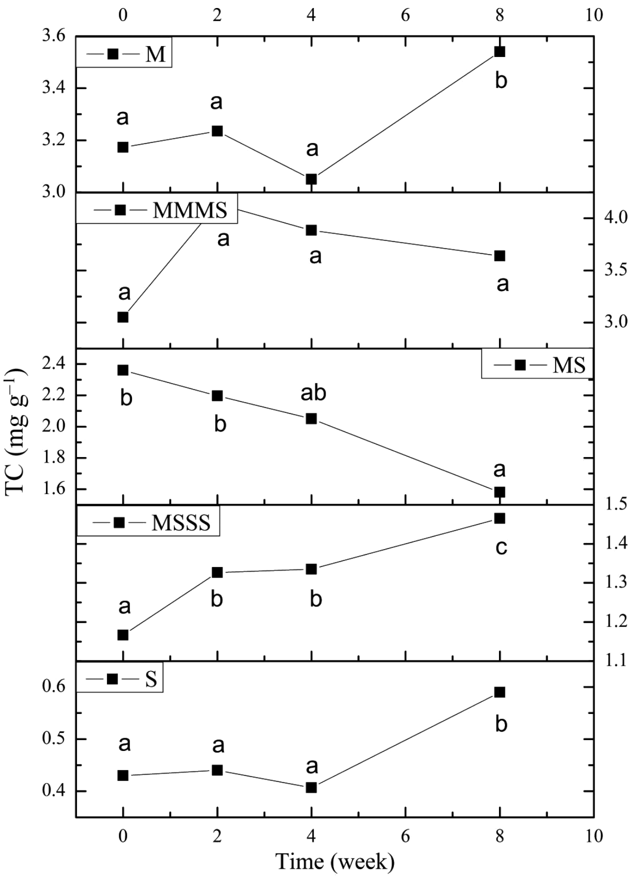

Fig. 2. Temporal changes in total carbon (TC). Details as in Fig. 1

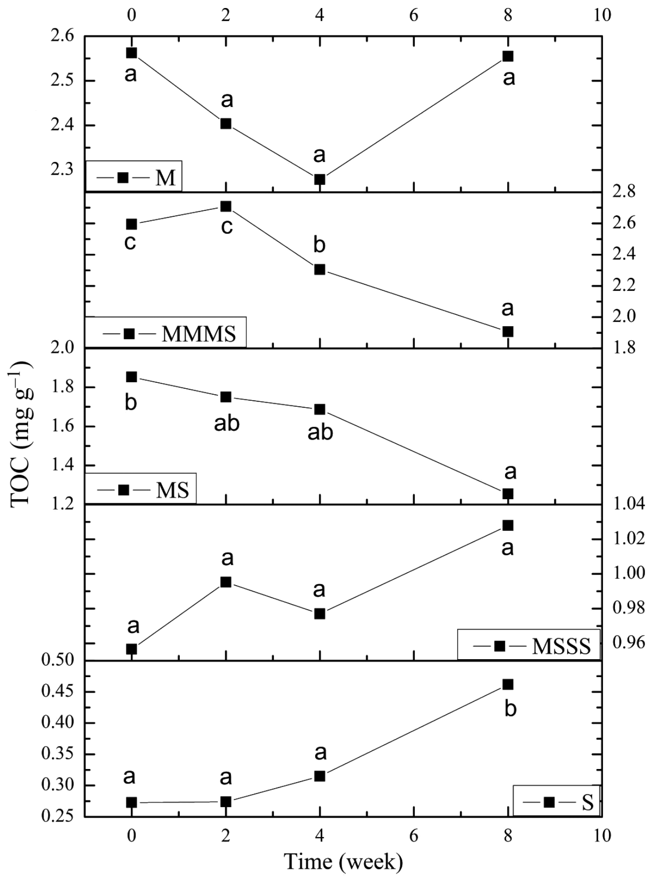

Fig. 3. Temporal changes in total organic carbon (TOC). Details as in Fig. 1

The TOC content of sediment groups MS ($F_{3,8}$ = 2.474, $p < 0.05$) and MMMS ($F_{3,8}$ = 34.726, $p < 0.05$) significantly decreased over the 8 wk experimental period (Fig. 3). The TOC content of sediment group S increased significantly with time ($F_{3,8}$ = 12.838, $p <$ 0.05); in contrast, no significant temporal changes occurred in the TOC content of sediment groups M ($F_{3,8}$ = 0.367, $p > 0.05$) and MSSS ($F_{3,8}$ = 0.047, $p >$ 0.05).

NH_4^+ and NO_3^- content in sediment

Significant temporal decreases in NH_4^+ content of sediment groups MS ($F_{3,8}$ = 3.128, $p < 0.05$) and MMMS ($F_{3,8}$ = 5.150, $p < 0.05$) were observed (Fig. 4). In Week 8, the sedimentary NH_4^+ content of sediment groups MS ($F_{3,8}$ = 3.128, $p < 0.05$) and MMMS ($F_{3,8}$ = 5.150, $p < 0.05$) was significantly lower than the corresponding initial values in Week 0. However, no significant temporal changes were found in the NH_4^+ content of sediment groups M ($F_{3,8}$ = 0.366, $p > 0.05$) and MSSS ($F_{3,8}$ = 0.579, $p > 0.05$) throughout the experimental period ($p > 0.05$). The NH_4^+ content of sediment group S in Weeks 4 and 8 was significantly higher than in Week 0 ($F_{3,8}$ = 58.754, $p < 0.05$). Although no significant difference was observed between the NH_4^+ content of sediment group S in Weeks 4 and 8 ($F_{3,8}$ = 58.754, $p > 0.05$), the NH_4^+ content of sediment group S in Weeks 4 and 8 was significantly higher than in Week 0 ($F_{3,8}$ = 58.754, $p < 0.05$). The temporal changes implied a significant increasing trend with time ($F_{3,8}$ = 58.754, $p < 0.05$).

We observed significant temporal increases in NO_3^- content of sediment groups M ($F_{3,8}$ = 3.959, $p <$ 0.05), S ($F_{3,8}$ = 13.747, $p < 0.05$), and MMMS ($F_{3,8}$ = 13.957, $p < 0.05$; Fig. 5). The NO_3^- content of sediment groups MS ($F_{3,8}$ = 14.474, $p < 0.05$) and MSSS ($F_{3,8}$ = 11.332, $p < 0.05$) initially increased over time to a maximum value in Week 4, then decreased significantly ($F_{3,8}$ = 14.474, $p < 0.05$; $F_{3,8}$ = 11.332, $p < 0.05$). The NO_3^- content of sediment groups MS ($F_{3,8}$ = 14.474, $p < 0.05$) and MSSS ($F_{3,8}$ = 11.332, $p < 0.05$) in Week 8 was significantly lower than those in Week 4.

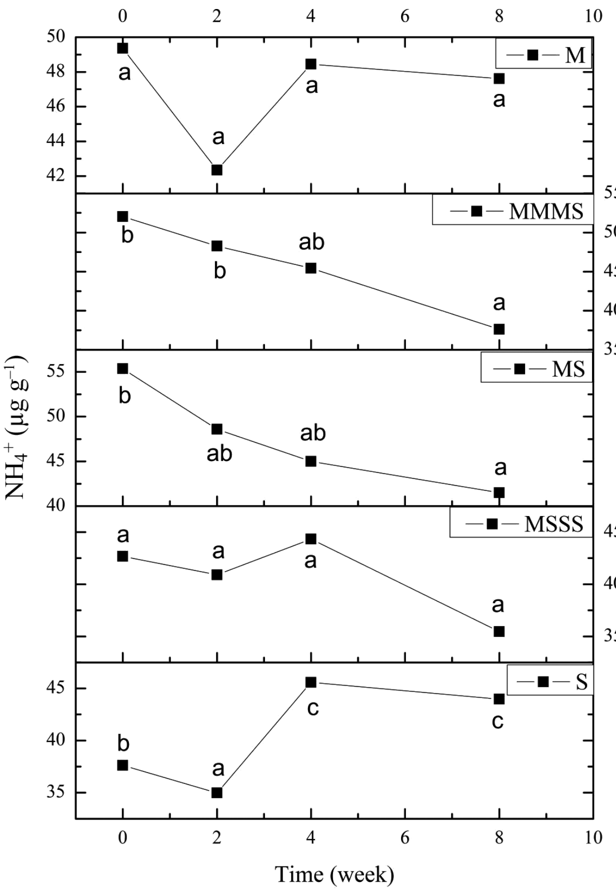

Fig. 4. Temporal changes in NH_4^+. Details as in Fig. 1

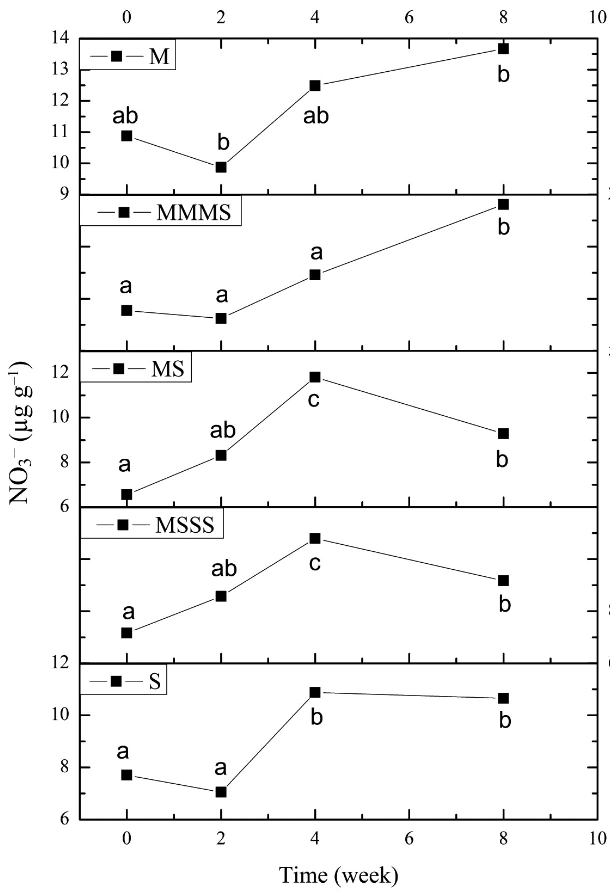

Fig. 5. Temporal changes in NO_3^-. Details as in Fig. 1

DISCUSSION

Growth performance and nutritional contribution

Previous studies have demonstrated that the half-life of carbon isotopic turnover in the body tissue of the sea cucumber *Apostichopus japonicus* (weighing 5.14 ± 0.17 g and 15.30 ± 0.43 g) was 21.39 and 54.15 d, respectively (Sun et al. 2013). Hence, in the present experiment, *A. japonicus* (7.49 ± 0.23 g) completed its carbon isotopic turnover after a feeding trial of 70 d, and the shifts in $\delta^{13}C$ values of *A. japonicus* toward those of the corresponding diets after isotopic fractionation correction were pronounced. The significant temporal changes indicated the incorporation of potential food items and subsequent isotopic effects on *A. japonicus*. Results from mixing model analyses revealed the relative contributions of the seaweed *Sargassum muticum*, sea mud, and sand to the food uptake of *A. japonicus* in 5 different sediment groups.

Significant differences in proportional contributions of *S. muticum* and growth performance of sea cucumber between sediment groups MMMS, MS, and M might illustrate that the coarse sediment in groups MMMS and MS could effectively improve the feeding and absorption of *S. muticum* by sea cucumber and subsequently enhance the growth and production of sea cucumber to a greater extent than the pure muddy sediment in group M. In each sediment group, the sea mud and sand contributed a mere fraction to the food uptake of sea cucumber relative to *S. muticum*, which was consistent with the proportional contributions of sea mud and yellow mud (terrestrial soil obtained from the freshwater pond) to the food uptake of sea cucumber determined by Jin et al. (2013). The nutritional value of sediment was low, as only a small proportion was readily assimilable, and a large fraction was refractory and not available to most consumers (Heip et al. 1995, MacIntyre et al. 1996, Venturini et al. 2012). Therefore, sediment might help in some other vital functions, not just as a food source (Dar & Ahmad 2006, Zhao & Yang 2010).

Previous studies have indicated that *A. japonicus* preferentially selects habitats with a high proportion of coarse particles (Zhao 2010). A similar preference has been reported in other holothurian species. Dar (2004) observed a strong selective behavior in the feeding habits of some holothurians in the Red Sea: *Holothuria atra*, *Bohadschia marmorata*, and *H. leucospilota* scavenge through coarse sediments much more than through medium or fine grained sediment. Unstable muddy areas like the shipping channels in Hamilton or St. George's Harbours or the deep areas of Harrington Sound in Bermuda had no holothurians (Sloan & von Bodungen 1980). During the contraction/motion process, which regulates the dynamics of sea cucumbers, the tube feet require stable points on the surface to enable suction. Fine particles are probably unsuitable to provide enough surface area for effective suction. Sea cucumbers therefore might find locomotion more difficult on soft muddy sediments than on coarse sediment (Sloan & von Bodungen 1980, Dar & Ahmad 2006, Zhao & Yang 2010). Coarse sediment might also help in the digestion process (Dar & Ahmad 2006).

Although *A. japonicus* preferentially selected coarse sediment, likely owing to important functions in motion and digestion processes, the proportional contributions of *S. muticum* and the growth performance of sea cucumber in sediment groups MSSS and S was much lower than in sediment groups MS and MMMS. An excessively high proportion of sand might have negative effects on the feeding and assimilation of *S. muticum*, and could retard the growth and production of sea cucumber.

As noted by Mezali & Soualili (2013), *H.* (*Panningothuria*) *forskali* and *H.* (*Platyperona*) *sanctori* show a preference for fine sediments. *A. japonicus* also generally selected fine particles (<0.08 mm) rather than coarse particles (Zhao & Yang 2010). Furthermore, several studies focused on particle selection by other deposit feeders, including polychaetes (Whitlatch 1974, 1976, Hylleberg 1975, Cadee 1976), amphipods (Fenchel et al. 1975), sipunculids (Hansen 1978), gastropods (Fenchel et al. 1975), bivalves (Reid & Reid 1969, Hylleberg & Gallucci 1975), and freshwater oligochaetes (Davis 1974, Meadows & Bird 1974, Kikuchi & Kurihara 1977, Taghon et al. 1980). In most studies, small grains were preferentially ingested. This is understandable given the higher surface area to volume ratios for the smaller particles. As the surface area to volume ratios increase with small particles, the surface area to which organic matter can adsorb increases as well (Belbachir et al. 2014). However, since bacterial quantity per unit surface area of sedimentary particles seems surprisingly constant (Dale 1974, Hargrave 1972), the total amount of bacteria, being directly related to the total surface area of the sedimentary particles, increases with smaller particle size (Newell 1965, Odum & de la Cruz 1967, Fenchel 1970, Hargrave 1972, Dale 1974). Hence, the smaller particles represented more food per unit volume and were selectively ingested by sea cucumbers. Another possible reason for the selective ingestion of fine particles rather than coarse (sand) particles was the smooth surface of sand, which was difficult for organic matter to adhere to.

Accordingly, in our experiment, sea cucumbers tended to select the smaller particles (sea mud) with more available food in order to gain a maximum amount of energy with a minimum loss of time and minimum loss of energy, which is consistent with optimal foraging theory (Taghon et al. 1978, Taghon 1982). Coarse sand particles might help in the digestion process and motion process, which shortens foraging time and reduces energy consumption during motion more effectively than pure, muddy sediment. The sediments in groups MMMS and MS could provide enough small sea mud particles for selective ingestion by sea cucumbers as well as coarse sand particles for mechanically assisting locomotion.

Temporal changes in organic and nutrient loads

Sea cucumbers are considered the most important macrofaunal consumers and processors of surface sediments in a wide variety of sub-tidal marine systems. Previous studies have shown that sea cucumbers can reduce nutrient and organic content in sediment through consumption, digestion, physical displacement, and bioturbation (Moriarty et al. 1985, Levin 1999, Uthicke 1999, 2001, Roberts et al. 2000, Michio et al. 2003). Given the environmental advantages of sea cucumbers for sediment, *A. japonicus* can act as an important scavenger with great potential for bioremediation which could effectively remove the aquaculture waste and inhibit the accumulation of sedimentary nutrients and organic matter in aquaculture systems (Kang et al. 2003, Işgören-Emiroğlu & Günay 2007, Ren et al. 2010, Yuan et al. 2012).

Temporal changes in TN, TC, TOC, and nutrient content of different sediment groups indicated that the sea cucumbers in sediment groups MS and MMMS could effectively stop the accumulation of organic matter and ammonium by means of ingestion, digestion, and bioturbation. However, organic matter accumulated remarkably in sediment groups S, MSSS, and M, especially in sediment group S, which showed high ammonium accumulation. *A. japonicus* cultured in groups MSSS, S, and M could not effectively remove the aquaculture waste and inhibit the accumulation of sedimentary nutrients and organic matter in the aquaculture system. This was probably due to the negative effects on sea cucumber derived from unsuitable sediments in these groups. The sediments in groups S and MSSS were unable to supply enough small sea mud particles, so the sea cucumbers may have spent more energy and time on foraging, ingestion, and digestion, which probably ultimately would have resulted in starvation. This in turn might reduce overall activity and might represent an adaptation of sea cucumbers to minimize the use of metabolic reserves (Newell 1973, Féral 1985). During starvation, the bioturbation derived from sea cucumbers in sediment groups S and MSSS was weaker than in the sediment groups MMMS and MS. Although the sediment in group M could provide enough small sea mud particles, the absence of coarse sand particles made it difficult for sea cucumbers to move normally (Sloan & von Bodungen 1980, Dar & Ahmad 2006, Zhao & Yang 2010), which may subsequently have interfered with foraging, ingestion, and bioturbation. Hence, the sea mud:sand ratios in sediments of groups S, MSSS, and M were unsuitable for sea cucumber aquaculture in terms of ecological benefits. The unsuitable sediment formulations significantly interfered with the bioremediation of *A. japonicus* and damaged the environmental sustainability in the aquaculture system.

In conclusion, sediments with suitable sea mud: sand ratios ranging from 1:1 to 3:1 could provide enough small sea mud particles (<0.08 mm) that were preferred by *A. japonicus* because of their organic matter and abundant bacterial biomass. These sediment formulations would also include enough sand particles to help during locomotion; thus, sea mud:sand ratios ranging from 1:1 to 3:1 would provide a sediment range that should lead to the best growth performance. Furthermore, sea cucumbers cultured on these sediments could effectively prevent organic matter and nutrient accumulation. Hence, this sediment composition might be the best choice for sea cucumber culture in aquaculture ponds.

Acknowledgements. This study was funded by the National Natural Science Foundation of China (grant nos. 31672657 and 31372549).

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Editorial responsibility: Gianluca Sará, Palermo, Italy

Submitted: November 28, 2016; Accepted: May 21, 2017
Proofs received from author(s): July 27, 2017