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Effects of the sizes of mud or sand particles in feed on growth and energy budgets of young sea cucumber (*Apostichopus japonicus*)

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ABSTRACT

The feed effects of sea mud (<0.008 mm in particle size), fine sand (0.074–0.25 mm) and medium sand (0.25–0.5 mm) as ingredients in fresh diatom *Cylindrotheca fusiformis* or macroalgae *Sargassum polycystum* on growth and energy budgets of the sea cucumber *Apostichopus japonicus* were studied. The results showed that algae species (*C. fusiformis* and *S. polycystum*), sizes of mud or sand particles and their interactions significantly modulated the specific growth rates (SGR) and ingestion rates (IR) of the sea cucumbers ($P < 0.001$). Larger mud or sand particles in diatom feed led to significant lower SGR, IR and energy consumption ($P < 0.05$). Nevertheless, the energy lost in feces elevated with the increasing particle sizes in all treatments. The defecation rates of the sea cucumbers also increased with the increasing particle sizes significantly in the first 3 h after ingestion ($P < 0.05$). These results provide evidences that i) the sea mud and sands, as feed ingredients, might regulate the residence time of feed in the digestive tract of the sea cucumber; and ii) the sea mud and sands dilute nutrients in feeds, leading to better growth performance. Sea mud smaller than 0.008 mm has better growth effects on sea cucumber *A. japonicus*. Different mud or soil proportions should be applied for different food ingredients in diets of the sea cucumber *A. japonicus*.

1. Introduction

The sea cucumber *Apostichopus japonicus* is an important mariculture species in China (Chen, 2004). Macroalgae and microalgae which contain high ratio of organic matter are the main nutrient sources of cultured *A. japonicus* (Jin et al., 2013), however, the sea cucumber *A. japonicus* fed solely on macroalgae or microalgae showed poor growth performances (Liu et al., 2009; Shi et al., 2013a; Yuan et al., 2006). At present, the mixture of macroalgae *Sargassum* spp. powder and sea mud is often provided as the main component of formulated feeds for *A. japonicus*. The rapid development of sea cucumber farming industry has led to the massive macroalgal powder input, causing potential environmental risk. It has been reported that the use of sea mud may affect the benthic communities in or on the sediment (Liu et al., 2009). Therefore, it is critical to find substitutes for the macroalgae and sea mud in order to maintain the sustainable development of sea cucumber farming. Previous studies suggested that diatom *Cylindrotheca fusiformis* could be used as a substitute for macroalgae *Sargassum thunbergii* (Shi et al., 2013a,b), while yellow soil which has similar nutrient content, could be used as a substitute for sea mud (Liu et al., 2009).

Though it is already a consensus among aquaculturists to add a certain proportion of sea mud to feed of *A. japonicus*, the role of such ingredients is still uncertain. The main function of sea mud has been inferred as providing microbes (Fenchel and Blackburn, 1979; Gao et al., 2011; Moore et al., 1995; Moriarty, 1978; Yingst, 1976), mineral components (Xu et al., 1999), certain nutrients (Gong et al., 2012) or digestion regulators (Yuan et al., 2006). To ascertain the main function of sea mud in feed of *A. japonicus* is essential in developing its substitute.

The yellow soil (96.21% ash, 0.25% lipid, 0.41% protein) from farm land and the sea mud (95.43% ash, 0.39% lipid, 0.60% protein) from offshore have similar nutritional profile (Liu et al., 2009), but they may carry distinct microbiota respectively. It has been reported that sea mud provided microbes that were vital to the digestion of sea cucumber (Fenchel and Blackburn, 1979; Gao et al., 2011; Moore et al., 1995). However, Liu et al. (2009) and Shi et al. (2013b) found that there was no significant difference in growth and energy allocation of the sea cucumbers fed diets containing the same proportion of sea mud and yellow soil. Such studies indicate that different microbiota from sea mud and yellow soil may not be the main contributor in modulating the digestion of the sea cucumber.

Other than the microbial perspective, it was also suggested that the sea mud provided the organic matter for the sea cucumbers directly (Gong et al., 2012). Some researchers argued that the microbes in sea mud itself were important food sources for the sea cucumbers (Moriarty, 1978; Yingst, 1976). However, Rublee (1982) and Cammem

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(1982) revealed that the microbes accounted only for 2% total organic carbon in sediment, which was inadequate for the nutrient requirement of deposited-feeders. The organic contents of yellow soil and sea mud were both around 4% (Liu et al., 2009), and their direct contributions to the growth of the sea cucumbers were less than 10% (Jin et al., 2013). Therefore, sea mud may have other functions rather than providing nutrients. The particle size of sediments in natural habitat is different from the sea mud used in sea cucumber farming industry (Choe, 1963; Zhang et al., 1995; Zhou et al., 2008). Some sea cucumbers prefer to feed certain sizes of particles (Dar and Ahmad, 2006; Zhao and Yang, 2010; Zhou et al., 2008). Dar and Ahmad (2006) found that larger sea cucumbers *Holothuria atra*, *Holothuria hawaiiensis* and *Bohadschia vitiensis* preferred to feed on smaller particles in reproduction period, and the authors presumed that smaller particles were more nutrient. Nevertheless, other studies showed that the nutrient contents and particle sizes of the marine sediments were not significantly relevant (Cammem, 1982). Zhou et al. (2008) found that approximate 60% sand particles in the digestive tract of wild sea cucumber *A. japonicus* were 0.0625–0.5 mm. The same study suggested that *A. japonicus* preferred particles of 0.125–0.25 mm. Therefore, it is interesting to hypothesize that sea mud contributes to the digestion of sea cucumbers by regulating the speed of food passing through the digestive tract. To test the hypothesis, the growth and energy budget of the sea cucumber *A. japonicus* fed with sea mud (<0.008 mm), fine sand (0.074–0.25 mm) and medium sand (0.25–0.5 mm) as feed fillers of fresh diatom *C. fusiformis* or *S. polycystum* were measured.

2. Materials and methods

2.1. Diet preparation

The diatom (*C. fusiformis*) used in this experiment was cultured at Aoshanwei Experimental Station of Ocean University of China. The diatom solution was centrifuged to a density of 5.0×10^7 cell mL^{-1}, about 25 g L^{-1}. Sea mud (94.33 ± 0.02% ash, 0.53 ± 0.02% lipid, 0.45 ± 0.01% protein) and sand were collected from an intertidal zone at Aoshan Bay, Qingdao, dried at 65 °C to constant weight. Sea mud was ground and sieved by a 0.008 mm mesh. Sands were sieved by 0.074, 0.25 and 0.5 mm mesh respectively to collect fine sand (0.074–0.25 mm) and medium sand (0.25–0.5 mm).

Concentrated diatom fluid and sea mud or sand were mixed thoroughly in certain proportions. The mixtures were then put in ice cube trays and frozen at −20 °C before use.

The formulated diets used in this study were consisted of *S. polycystum* powder and sea mud or sand. The *S. polycystum* were dried at 65 °C for 48 h, ground and sieved by a 0.15 mm mesh, then well mixed with sea mud or sands. A small amount of water was added to the mixture, the slurry was slightly stirred, then made into a cylindrical form, dried at 65 °C for 36 h and stored at 4 °C until use.

2.2. Experimental design

2.2.1. Growth experiment

The experiment consisted of ten treatments, with four replicates in each treatments: CS, *C. fusiformis* filled with sea mud; CSF, *C. fusiformis* filled with sea mud and fine sand; CSM, *C. fusiformis* filled with sea mud and medium sand; CF, *C. fusiformis* filled with fine sand; CM, *C. fusiformis* filled with medium sand; SS, *S. polycystum* filled with sea mud; SSF, *S. polycystum* filled with sea mud and fine sand; SSM, *S. polycystum* filled with sea mud and medium sand; SF, *S. polycystum* filled with fine sand; and SM, *S. polycystum* filled with medium sand. The ingredients and nutrient contents of the experimental diets were given in Tables 1 and 2.

When offering the feeds, the frozen feeds sank immediately and softened in 1–2 min. Formulated diets also sank immediately.

Table 1
Ingredients of the experimental diets (mean).

Diet codes	Ingredient (%)				
	C. fusiformis	*S. polycystum*	Sea mud < 0.008 mm	Fine sand 0.074–0.25 mm	Medium sand 0.25–0.5 mm
CS	14	–	86	–	–
CSF	14	–	43	43	–
CSM	14	–	43	–	43
CF	14	–	–	86	–
CM	14	–	–	–	86
SS	–	80	20	–	–
SSF	–	80	10	10	–
SSM	–	80	10	–	10
SF	–	80	–	20	–
SM	–	80	–	–	20

Data were calculated on a dry matter basis. CS, *C. fusiformis* filled with sea mud; CSF, *C. fusiformis* filled with sea mud and fine sand; CSM, *C. fusiformis* filled with sea mud and medium sand; CF, *C. fusiformis* filled with fine sand; CM, *C. fusiformis* filled with medium sand; SS, *S. polycystum* filled with sea mud; SSF, *S. polycystum* filled with sea mud and fine sand; SSM, *S. polycystum* filled with sea mud and medium sand; SF, *S. polycystum* filled with fine sand; and SM, *S. polycystum* filled with medium sand.

The experiment was carried out from May 25th to July 24th, 2013. The sea cucumbers used in the present study were collected from a sea cucumber farm in Qingdao. Prior to the experiment, the animals were fed with macroalgal powder (*Laminaria japonica*) and acclimated at 16.5 °C for two weeks. After 24 h of starvation, the initial body weights of the sea cucumbers were measured individually as described in Dong et al. (2006). The sea cucumbers were allocated into 40 groups randomly, cultured in a glass aquarium (50 cm × 30 cm × 40 cm, water volume of 45 L). Each group contained six sea cucumbers with the initial wet body weight of about 5 g. During the experiment, sea cucumbers were fed 5% wet body weight once a day at 16:00. The feces and uneaten feed were collected by siphon after 22 h.

2.2.2. Defection rate experiment

The sea cucumbers used in this experiment were the same batch of those in growth experiment and reared under the same circumstances. The sea cucumbers were fed 10 kinds of feed as described above. Each treatment had three replicates with four sea cucumbers (mean body weight 5 ± 0.2 g). Before the experiment, the sea cucumbers were starved for 48 h to ensure empty stomach. During the experiment, the sea cucumbers were fed 10% of body weight at 16:00. The residual feeds were removed once feces appeared in each tank. Feces were collected by siphon every hour during the next 12 h, desalted, dried and weighted. The experiment last 12 h. During the experiment the light was on from 8:00 to 22:00, with the intensity of 300 lx on the surface of water.

2.3. Rearing condition

During the experiment, aeration was provided continuously. 2/3 volume of the water in each aquarium was changed every day. Seawater used in the experiment was filtered by composite sand filter. Seawater temperature was controlled at 16.5 ± 0.5 °C. Dissolved oxygen was maintained above 5.0 mg L^{-1}. The levels of ammonia in the water of aquaria were less than 0.25 mg L^{-1}, salinity ranged from 28 to 30 g L^{-1}, pH ranged from 7.8 to 8.2. A photoperiod of 14:10 h (light/dark) was used.

2.4. Sample collection and data calculation

Twenty sea cucumbers were collected as an initial sample before the experiment. Feces and residual feed collected during the experiment were desalted with fresh water and then dried at 65 °C to constant weight. The sea cucumbers were starved for 48 h at the end of experiment (Xia et al., 2012), and then weighed, dried at 65 °C until constant