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Feeding preferences of the sea cucumber *Apostichopus japonicus* (Selenka) on various seaweed diets

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ABSTRACT

Food choice experiments were conducted to study the preferred seaweed diet of the sea cucumber *Apostichopus japonicus* (Selenka). Six diets containing *Sargassum thunbergii*, *Sargassum polycystum*, *Zostera marina*, *Ulva lactuca*, fresh *Laminaria japonica* and boiled *L. japonica* were offered separately in a mixture with sea mud and rare earth oxides (as inert markers). A feeding preference model was constructed to calculate the feeding preference index of sea cucumbers on the seaweed diets above. Sea cucumbers showed clear preferences for the *L. japonica* diets over the other diets. Fresh and boiled *L. japonica* diets made up about 54.73% of the entire intake. The sea cucumber preference indices changed with time. The preference for *S. thunbergii* rose significantly, changing from being rejection to somewhat preferred over a 30 day period. Our study suggests that a diet containing about 50% *L. japonica* of the total algal is very suitable for sea cucumber culture.

1. Introduction

All organisms must obtain food for survival, growth and reproduction. Most animals have evolved senses that enable them to discriminate foodstuffs, resulting in dietary preferences. Feeding-preference experiments are a useful tool for assessing important ecological processes (Roa, 1992), such as estimating complexity of food webs in nature (Ho and Pennings, 2008). Furthermore, knowledge of feeding preference enables enhancement of artificial diets used in aquaculture to stimulate feeding (Dworjanyn et al., 2007).

Feeding preferences are influenced by a complex of species-specific factors including food itself, the animal's physiological requirements, the environment and prey profitable. Food influence of feeding preference may be a function of nutritional characteristics (Goff and Klee, 2006; Watson and Norton, 1985), its morphology (Jensen, 1983), and availability (Arrontes, 1990). The animal's physiological requirements may affect feeding preference depending on ontogeny (Lyons and Scheibling, 2007), the nutritional requirements, during periods such as reproduction, or according to digestive capabilities and mode of feeding (Lowe and Lawrence, 1976). Environmental conditions may also affect feeding preference according to, for example, seasonality or different habitat types providing a range of different prey capture efficiencies (Vilas et al., 2008). A fourth factor influencing feeding preference is prey profitable which means that a predator consumes prey of higher profitability (ratio of energy gain per handling time) (Figueiredo et al., 2008; Wong and Barbeau, 2005). Studies have been reported on the impacts of the factors above on marine animals feeding on algae, including the sea urchin *Strongylocentrotus droebachiensis* (Lyons and Scheibling, 2007; Simoncini and Miller, 2007), the saltmarsh crab *Armases cinereum* (Pennings et al., 1998), and the blackfoot abalone *Haliotis iris* (Allen et al., 2006). Other feeding preference studies have been conducted for marine organisms, including mollusks (Doropoulos et al., 2009), amphipods (Duffy, 1991), mysids (Vilas et al., 2008), fish (Russo et al., 2009). Feeding studies on food preferences of holothurians, however, are comparatively rare.

Holothurians are active sediment "reworkers" that alter the stability of the sea floor by reducing volatile sulfide concentrations and increasing oxidation–reduction potentials (Dar and Ahmad, 2006). Furthermore, sea cucumbers form the basis of important, long established fisheries in Russia, China, Japan, and Korea. Of the commercially exploited species, *Apostichopus japonicus* (Selenka) is considered to be the most valuable (Okorie et al., 2008). Depletion of natural sea cucumber stocks has led to stock enhancement and aquaculture programs for many holothurians, especially *A. japonicus* (Yuan et al., 2006).

Gut contents analysis and observations on live holothurians show that their diet comprises of mainly inorganic compounds, organic detritus of macroalgae, decaying animals, and microorganisms (Dar and Ahmad, 2006). In littoral benthic ecosystems, most holothurians select for sediments containing the highest amounts of macroalgae

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detritus (Uthicke and Karez, 1999). Powdered macroalgae and sea mud have been used as the main components of formulated feeds in semi-intensive sea cucumber culture systems (Liu et al., 2010). *S. thunbergii*, *S. polycystum*, *Z. marina*, *U. lactuca* and *L. japonica*, are the most common seaweeds in the north of China, and all have been used in feed for cultured sea cucumbers (Liu et al., 2010; Seo et al., 2011; Yuan et al., 2006). To potentially improve productivity of sea cucumber culture, identifying dietary preference for common north China seaweed is an important step.

Food items may be individually presented to an organism or in combination with other such items. Experiments using individual food items do not address the issue of feeding preference because no food choice is provided (Peterson and Renaud, 1989) and a less preferred food type may be consumed at higher rates to compensate for its low nutritional value (Roa, 1992). Therefore, true feeding preference experiments can only be performed when two or more food types are offered simultaneously (Roa, 1992). Peterson and Renaud (1989) simultaneously offered one experimental and one control food items to a consumer to analyze food preference in multiple-choice experiments. They considered that finding a proper procedure for such experiments presented "one of the biggest current challenges in ecological methodology". In our study rare earth oxides were used to label the different food types, which were placed into containers in a specific order, and offered to the experimental sea cucumbers. A feeding preference model was then constructed based on the excretion of these rare earth oxides to determine feeding preference indices. The aim of the present study was to determine food preference for sea cucumbers offered various seaweed diets.

2. Materials and methods

2.1. Experimental diets

Six experimental diets were prepared from powdered mixtures of sea mud, a single seaweed species, and one kind of rare earth oxide (as an inert marker). The diets and markers are described in Table 1: ST) *S. thunbergii*, La_2O_3; SP) *S. polycystum*, Dy_2O_3; ZM) *Z. marina*, Eu_2O_3; UL) *U. lactuca*, Tb_2O_3; LJf) fresh *L. japonica*, Sm_2O_3; and LJb) boiled *L. japonica*, Y_2O_3. The five species of fresh seaweed (ST, SP, ZM, UL, and LJf), and sea mud, were collected from the Yellow Sea near Rongcheng City, Shandong Province, China in May 2010, sun dried, and then pulverized into ultra-fine powder (<75 μm). The boiled *L. japonica* (LJb) used in this study was discarded from a kelp food factory, and consisted mainly of the edges of *L. japonica* fronds and some salt. Kelp process involved gathering fresh *L. japonica* and then boiling it in salted water. Samples of this product were taken from the factory, stored in a refrigerator, sun dried and ground into superfine powder (<75 μm) for use in our experiments. Each experimental diet type contained 30% powered seaweed because this proportion has been shown to produce the best growth performance in sea cucumbers (Yuan et al., 2006). In this experiment, each seaweed diet was mixed with an equal amount of water to which 6 g of seaweed mixture was added and made into a 4 cm diameter patch. Three patches for each of the six seaweed diets under test were placed into white plastic aquaria (Fig. 1), so that each diet patch was surrounded by patches with different diets. Analyses of feed samples were made following the Association of Official Analytical Chemists (AOAC, 1990).

Table 1
Ingredients and chemical composition of six algal diets.

Ingredient (100 g)	Algal diets					
	ST	SP	ZM	UL	LJf	LJb
Seaweed powder	30	30	30	30	30	30
Sea mud powder	69.99	69.99	69.99	69.99	69.99	69.99
Rare earth indicator	0.01	0.01	0.01	0.01	0.01	0.01
Diet composition						
Dry matter (%)	96.86	96.25	96.48	97.05	96.98	97.82
Protein (%)	4.65	5.97	5.34	3.42	3.50	3.23
Lipid (%)	0.77	0.85	0.80	0.50	0.65	0.47
Ash (%)	74.93	73.37	74.01	76.30	77.11	80.86

ST) *S. thunbergii*, SP) *S. polycystum*, ZM) *Z. marina*, UL) *U. lactuca*, LJf) fresh *L. japonica*, LJb) boiled *L. japonica*.

2.2. Experimental procedures

Sea cucumbers were obtained from the semi-intensive culture system of Shandong Oriental Ocean Sci-Tech Co. Ltd., Yantai City, Shandong Province, China. They were acclimated to laboratory conditions after placement in square concrete tanks (4×4×1.5 m). After 24 h starvation, 48 sea cucumbers with an average body weight of 9.4 g were randomly selected from the acclimated animals and eight were placed into each of six white, round plastic aquaria. The aquaria contained 20 L of aerated water with a salinity of 30±2 PSU, and a dissolved oxygen level >5 mg L^{-1}. The tanks were maintained at 15±2 °C and the water changed at a rate of 0.45 L/min. Food was replenished every 2 days. Fecal matter from each tank was collected every 10 days and rinsed in distilled water to remove the salt. Fecal matter was stored at −20 °C for analysis. At the end of 30 days, all of the sea cucumbers in each tank were weighed.

Trace element analysis of fecal matter was conducted by drying samples at 65 °C until constant weight, after which 40 mg of powdered sample was dissolved with 2 ml HF, 0.6 ml HNO_3 and 0.25 ml $HClO_4$ in Teflon beakers at a temperature of 150 °C for about 72 h. The residues were then dissolved in 1 mL HNO_3 and 1 mL pure water. The solutions were cooled and diluted with 1000 times their volume of pure water, and analyzed for trace elements using an Inductively Coupled Plasma Mass Spectrometer (ICP-MS) (ELAN DRC II, American PE Company) at the Institute of Oceanology Chinese Academy of Sciences (IOCAS), Qingdao. A series of deep-sea sediment standard GBW07315, GBW07316, GBW07101, GBW07102, DTS-2b, BCR-2 and BHVO-2 were used as reference materials for the analyses. The detection limits of the ICP-MS for rare earth elements (REE) and other trace elements are 10^{-8} g/g, and the analytical precisions (RSD, n=3) for REE and other trace elements are <5%. Analyzed uncertainties (2σ) of ICP-MS data at the ppm level are better than ±10% for trace elements and REE. The analyses of the standards were in agreement with the recommended values.

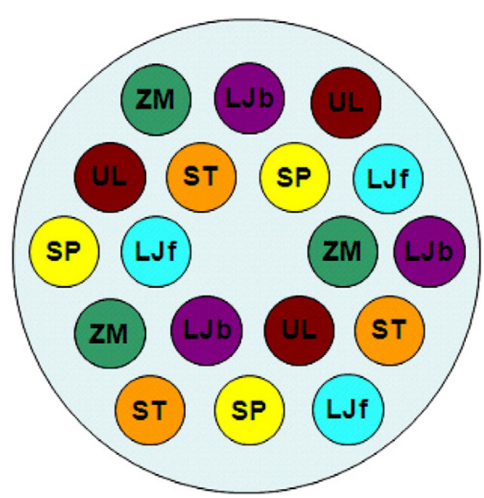

Fig. 1. Spatial distribution of eighteen food patches in the test aquaria.