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Feeding rates and absorption efficiencies of four species of sea urchins (genus *Echinometra*) fed a prepared diet

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Abstract

Four closely related species of sea urchins belonging to the genus *Echinometra*, *Echinometra* sp. A (Ea), *E. mathaei* (Em), *E.* sp. C (Ec), and *E. oblonga* (Eo), occur sympatrically but in different microhabitats on Okinawan coral reefs. Feeding rates and absorption efficiencies of the four species were investigated in the laboratory by feeding sea urchins *ad libitum* a diet prepared from turf algae and agar over a 7-day period. Feeding rates differed significantly among the four species of *Echinometra* (Ea > Em ≈ Ec > Eo). Absorption efficiencies of protein and lipid did not differ significantly among the four species. Carbohydrate, a major nutrient component in the diet, was absorbed by the four species at significantly different efficiencies (Eo > Ec ≈ Em > Ea), which resulted in similar interspecific differences in absorption efficiencies of dry matter, total organic matter, and energy. The amount of nutrients absorbed from the diet was directly related to the feeding rate, indicating that the increase in absorption efficiency was not sufficient to completely compensate for low feeding rate. The interspecific difference in physiological performance in relation to feeding and absorption is consistent with taxonomic differentiation among the four species.

Keywords: Absorption; Artificial diet; *Echinometra*; Feeding; Nutrition; Okinawa; Sea urchins

1. Introduction

Sea urchins of the genus *Echinometra* are commonly found in tropical and subtropical shallow waters. Among them, *Echinometra mathaei* had been recognized as the only species of the genus *Echinometra* in the Indo–Pacific (Mortensen, 1943). However, recent studies on morphology, gamete incompatibility, and mitochondrial DNA of *E. mathaei* on Okinawa have revealed that Okinawan *E. mathaei* can be classified into four different species: *Echinometra* sp. A, *E. mathaei*, *Echinometra* sp. C, and *E. oblonga* (Uehara and Shingaki, 1985; Uehara, 1990; Matsuoka and Hatanaka, 1991; Palumbi and Metz, 1991; Metz and Palumbi, 1996).

Ecological investigations have shown that these four species occur sympatrically but in different microhabitats on Okinawan coral reefs (Nishihira et al., 1991; Aslan, 2000; Suzuki and Kan, 2004). In general, *Echinometra* sp. A is common in submerged areas, such as tide pools, moats, and reef slopes, whereas *E. mathaei* occurs in lower intertidal areas near shore and behind reef margins. The other two species, *Echinometra* sp. C and *E. oblonga*, are abundant in the upper intertidal areas near shore and on reef margins. Because of this microhabitat segregation, the four species are subjected to different physical and biological environments, and therefore can be expected to have different morphological, physiological, and life-history traits. This expectation is supported by Arakaki and Uehara (1991) who showed that tolerance to extreme conditions such as high and low temperatures, and salinity changes differ significantly among the four species, and that these interspecific differences in physiological tolerance reflect their microhabitat differences.

In this study, feeding rates and absorption efficiencies of the four species fed on an artificially prepared diet containing turf algae and agar were measured to evaluate the physiological performance of these closely related species.

2. Materials and methods

2.1. Collection of sea urchins

Similar-sized adult individuals of *Echinometra* (30–35 mm test length; $n = 10$ per species) were collected from a fringing

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reef at Onna located in the west coast of the central part of Okinawa Island (26°30′N; 127°51′E) in January 12, 2005. The sea surface temperature at the collection site was 21.6 °C. *Echinometra* sp. A (Ea) were collected from tide pools and moats. *E. mathaei* (Em) were collected from shallow burrows in the lower intertidal area near shore, while *Echinometra* sp. C (Ec) and *E. oblonga* (Eo) were collected from deep burrows in the upper intertidal areas near shore and on reef margins. Care was taken not to damage the tube feet, spines, and epidermis of the sea urchins. The collected sea urchins were immediately transported to the laboratory. Each species was separately maintained at a seawater temperature of 25 °C in a recirculating seawater aquarium without food for 5 days prior to the experiment to promote uniform nutritional state.

2.2. Diet

An artificial diet containing turf algae and agar was used in the feeding experiments. Turf algae are a multi-specific assemblage of diminutive and filamentous algae that overgrow hard substrate on the reef. Ecological investigations have shown that the gut content of *Echinometra* collected from Onna is generally occupied by turf algae (37.5–44.9%) and sediment (28.0–33.0%) regardless of species and season (Hiratsuka and Uehara, unpublished data). Turf algae were collected from the same area as in the case of sea urchins. The turf algae collected were composed mainly of species in the genus *Ectocarpus*, *Centroceras*, *Hydrolithon*, *Jania*, *Polysiphonia*, *Sphacelaria*, *Enteromorpha*, *Lobophora*, *Laurencia*, *Cladophora*, *Ceramium*, and *Leveillea*. They were cut into pieces, and washed carefully in seawater to remove $CaCO_3$ fragments found with the turf algae. The cleaned turf algae were dried for 24 h at 50 °C, and ground to fine powder in a blender. The algal powder was mixed well with heated agar solution (5 g algal powder: 3 g of agar in 100 mL seawater). The mixture was subsequently poured into small Petri dishes to produce disks (30 mm in diameter × 10 mm in height) with a wet mass of approximately 4.0–4.1 g.

2.3. Biochemical analysis of diet

To ascertain the nutritional composition of the artificial diet, 10 samples of the diet were analyzed using the following techniques. Each sample was freeze-dried and ground to fine powder using a mortar and pestle. Ash content was determined by placing 20 mg samples into a muffle furnace for 4 h at 500 °C (Paine, 1971). Total sodium hydroxide-soluble protein was determined on 10 mg samples after extraction in 1 N NaOH by the method of Lowry et al. (1951) using BSA as the standard. Total trichloroacetic acid (TCA)-soluble carbohydrate was determined on 10 mg samples after extraction in hot 5% TCA by the method of Dubois et al. (1956) using glucose as the standard. Total lipid was determined on samples of 50–100 mg after extraction in chloroform–methanol solution using the modified method of Bligh and Dyer (1959) described in Meziane and Tsuchiya (2000). Insoluble carbohydrate was determined by subtraction. Conversion into energy units was made using the following equivalences: 1 mg protein = 23.65 J, 1 mg carbohydrate = 17.16 J, and 1 mg lipid = 39.55 J (Crisp, 1984).

2.4. Experimental design

Following the starvation period of 5 days, each of the 40 individuals ($n = 10$ per species) was placed in a mesh cage (90 mm in diameter × 100 mm in height) suspended in a 1.5-L plastic container. Each container was filled with filtered seawater at salinity of 35‰ and supplied with moderate aeration. All individuals were exposed to a photoperiod of 12 h: 12 h light–dark cycle and a seawater temperature of 25 °C. All containers and mesh cages were cleaned and refilled with fresh filtered seawater daily to prevent bacterial growth.

2.5. Feeding rates

Feeding rates for sea urchins offered the prepared diet were recorded every 24 h over a 7-day period. A known amount of food (approximately 4.0–4.1 g wet mass) was placed in each mesh cage daily. Food left over from the previous day was retrieved, blotted with paper towels, and weighed. The wet mass of food ingested over 24 h was calculated by subtraction, and converted to dry mass using the following equation: Dry mass of food (mg) = Wet mass of food (mg) × [1 − Water content of the diet (i.e., 89.6%)/100]. The feeding rate of each individual (mg dry mass) was expressed as the average of the amount of food ingested daily over a 7-day period. Ten control containers that contained food but no sea urchin were set up to examine whether autogenic weight change of the food occurred over a 24-h period.

2.6. Absorption efficiencies

Absorption efficiency is defined as the percentage of ingested material moving across the intestinal wall (Lawrence, 1975). Absorption efficiencies of the four species of *Echinometra* for the prepared diets were measured using the direct method expressed as the following equation: Absorption efficiency (% dry weight) = [(Ingested material − Egested material)/Ingested material] × 100. It should be noted that this direct method has the advantage of rapid and easy estimation of absorption efficiency, but requires precise qualitative recovery of uneaten food and feces (Lares, 1999). Absorption efficiencies were expressed in terms of dry matter, total organic matter, protein, soluble and insoluble carbohydrates, total carbohydrate, lipid, and energy. The amount of nutrients ingested was estimated by multiplying fractions of nutrients in the diet by the amount of food consumed. The amount of nutrients egested was estimated by quantifying the nutrients in representative samples of feces using the techniques described above (see Biochemical analysis of diet). Feces were collected daily until they were no longer produced. Feces accumulating at the bottom of the container were siphoned into a large Petri dish, and uneaten food fragments, tube feet, spines, and pedicellariae were carefully removed. Uneaten food fragments collected were added to the uneaten food recovered on that day. Feces were then collected on a filter paper to remove excess seawater, freeze-dried, weighed, and kept in a freezer until analyses.