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Feeding, selection, digestion and absorption of the organic matter from mussel waste by juveniles of the deposit-feeding sea cucumber, *Australostichopus mollis*

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ABSTRACT

An understanding of the feeding and digestive capabilities of juvenile sea cucumbers is an important step for their aquaculture development. The feeding behaviour, food selectivity, digestion and absorption of total organic matter (TOM) were determined for juveniles (35–40 g wet weight) of the Australasian brown sea cucumber, *Australostichopus mollis*, kept in circular plastic tanks (9 l; 6 replicate tanks per feed treatment). Sea cucumbers were fed with feeds containing different levels of TOM (1, 4, 12 and 20%) prepared from sand mixed with the biodeposits of aquacultured green-lipped mussel, *Perna canaliculus*. No significant diurnal patterns in digestion were detected in all feed treatments. As the TOM level in the feed increased, the selection of organic particles decreased and most importantly overall nutrient absorption increased, which is likely to lead to improved growth rates. However, absorption efficiency declined as organic matter content of the feed increased. This study suggests that palatable-artificial diets rich in organic matter can be used to increase nutrient uptake and potentially facilitate increased stocking densities of sea cucumbers under controlled conditions.

1. Introduction

Despite the commercial importance of sea cucumbers, in both fisheries and aquaculture, information about the feeding behaviour and digestive capabilities of juvenile sea cucumbers is only available for a few species. Most of this information comes from studies on the Japanese sea cucumber, *Apostichopus japonicus* (Liu et al., 2004; Renbo and Yuan, 2004; Xiyin et al., 2004; Huiling et al., 2004; Dong et al., 2008a; Yuan et al., 2006; Dong et al., 2008b; Okorie et al., 2008; Liu et al., 2009) and the sandfish, *Holothuria scabra* (Battaglene et al., 1999; Mercier et al., 1999; Hamel et al., 2001; James, 2004; Giraspy and Ivy, 2008). Very little is known about juveniles of the Australasian brown sea cucumber, *Australostichopus mollis*, which has a great commercial potential. This species is the current focus of commercial aquaculture development such as the improvement of hatchery techniques and the development of co-culture methods with other aquaculture species in New Zealand (Morgan and Archer, 1999; Slater and Carton, 2007; Morgan, 2009a; Morgan, 2009b; Stenton-Dozey and Heath, 2009; Maxwell et al., 2009; Slater and Carton, 2010).

A. mollis is an aspidochirotid sea cucumber that is common in the shallow coastal waters of New Zealand and can be found in many parts of southern Australia in a wide range of habitats from shallow rocky reef to sandy bottoms and mudflats (Pawson, 1970). In New Zealand this species can also be found in large numbers under green-lipped mussel (*Perna canaliculus*) farms (Gribben and Bell, 2000; 2001). The great variety of habitats where this species can be found could be explained by its ability to exploit different food sources. Although juveniles can be found together with the adults in several types of habitats, settlement seems to be restricted to specific areas (Slater and Jeffs, 2010; Slater et al., 2010). As an aspidochirotid, *A. mollis* moves along the seafloor collecting particles (i.e. a mix of minerals, live organisms, decaying material, including faeces of other marine organisms) by extending peltate (shield-shaped) tentacles that surround the mouth. The tentacles trap particles and then take them into the mouth (Roberts et al., 2000). Once inside the mouth the particles are compressed and transported by peristalsis, without further mixing, along a simple tubular digestive system that ends in the anus located in the posterior part of the animal (Feral and Massin, 1982; Penry, 1989). In previous studies, green-lipped mussel biodeposits (faeces and pseudofaeces) have proved to be highly palatable and good for juvenile *A. mollis* growth (Slater and Carton, 2007; Slater et al., 2009). These biodeposits can be used to obtain basic information about the feeding biology of this species which in turn is useful for the development of the aquaculture of sea cucumbers.

Deposit feeding sea cucumbers, such as *A. mollis*, can only digest and utilise the organic component removed from the sediment (Roberts et al., 2000). Typically the naturally occurring organic content in marine sediments, measured as total organic matter (TOM), is very low. Consequently, the foraging behaviour and digestive capabilities of sea cucumbers appears to be modified to improve the intake of nutrients from the organic component in the sediments that are needed for their growth and survival (Roberts et al., 2000). For example, some sea cucumber species search for

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patches of sediment with higher nutritive value on which to feed (Uthicke and Karez, 1999; Mercier et al., 1999; Slater, 2010), or they actively pick out organic rich particles from the sediment (Massin, 1982; Moriarty, 1982; Hammond, 1983; Rainer and Herndl, 1991; Paltzat et al., 2008). They can also alter their ingestion rate to suit their nutrient requirements (Huiling et al., 2004; Yuan et al., 2006; Liu et al., 2009; Slater et al., 2009 and Maxwell et al., 2009), or they can digest and absorb (assimilate) particles with different efficiencies depending on the food that is being consumed (Sibuet et al., 1982; Hammond, 1983; Huiling et al., 2004; Yuan et al., 2006; Liu et al., 2009; Slater et al., 2009; Maxwell et al., 2009; Slater and Jeffs, in press).

Due to the fact that the utilisation of the particles within the sediment by sea cucumbers is a result of the interaction of biotic and abiotic factors, a better understanding of the feeding and digestive capabilities of a deposit feeding sea cucumber species is an important step for their aquaculture development. However, currently this knowledge is lacking for *A. mollis*. From studies in natural habitats it is known that juvenile *A. mollis* can be found on sediments with a TOM content between 5.5 and 7%, but they can also survive on sediments with a TOM content as low as 4% (Slater and Jeffs, 2010). However, this species is also capable of consuming sediments with a higher TOM content, such as biodeposits from green-lipped mussel with 25% TOM (Slater et al., 2009) and uneaten formulated abalone food (*Laminaria japonica* kelp flakes) and abalone faeces with 76% and 55% TOM content respectively (Maxwell et al., 2009).

The aim of the current study is to obtain information useful for understanding the feeding biology of, *A. mollis*, to assist in aquaculture development. The research evaluated how the feeding behaviour, food particle selectivity, digestion, absorption and faecal production, of the sea cucumber, *A. mollis*, changed in response to being fed different levels of organic matter under controlled conditions.

2. Materials and methods

Two experiments were undertaken. The first experiment aimed to determine how different levels of TOM in the feed influenced feeding and digestion in juveniles of *A. mollis*. The second experiment examined the ability of juvenile sea cucumbers to select and absorb organic particles from sediments with different levels of TOM. For both experiments, biodeposits derived from green-lipped mussel aquaculture were mixed with sand in different proportions to produce the experimental feeds. Biodeposits from this mussel contain 19.6% carbohydrates, 5.1% proteins, 1.0% lipids and 72.3% ash (Slater et al., 2009).

2.1. Experimental animals

Juvenile sea cucumbers were collected from the Mahurangi Harbour in northern New Zealand by divers in September 2009 at a depth of 12 m. The sample site is characterised by a substratum dominated by silt/mud with large shell fragments (Slater and Jeffs, 2010). The sea cucumbers were transferred to the nearby Leigh Marine Laboratory and held in tanks with flowing 100 µm filtered seawater at ambient temperature. The sea cucumbers were unfed for 48 h to ensure that the gut contents were fully evacuated. The sea cucumbers were then weighed to the nearest gramme, after the excess water from the respiratory tree was removed by gently squeezing the posterior half of each animal and the external water was blot dried (Sewell, 1990). Only sea cucumbers of similar weight were used in the experiments: 37.56 g ($\pm$2.51 SE) for the feeding and digestion experiment, and 38.24 g ($\pm$2.08 SE) for the food selective ingestion and digestion experiment.

For both experiments the sea cucumbers were maintained in circular plastic tanks (9 l), which had a floor surface area of 0.03 m^2 (water depth of 23 cm) and were supplied with ambient filtered seawater (50 µm) at a rate of 14 l h^{-1}. A natural light cycle was maintained during the experiment, although shaded from direct sunlight (L: 0.2–0.3 µE m^{-2} s^{-1}). For the feeding and digestion experiment a single gut-evacuated juvenile sea cucumber was randomly allocated into each of 24 tanks. For the selective ingestion and digestion experiment, two gut-evacuated juvenile sea cucumbers were randomly allocated to each tank in a second set of 24 tanks. In both experiments six replicate tanks were randomly selected for each of four feed treatments and the sea cucumbers were allowed to acclimate to the respective diet for 6 days. The amount of feed supplied for each treatment was equivalent to 30% of the wet body weight of the sea cucumbers per day (Slater et al., 2009).

2.2. Experimental feed treatments

Four feed treatments were used in both experiments. The feeds were prepared using acid washed sand (Ajax Finechem Pty Ltd), with a grain size between 250 and 500 µm, mixed in different proportions with homogeneous biodeposits, collected from beneath green-lipped mussel (*P. canaliculus*) cultured in tanks on raw ambient seawater. To avoid any size class stratification of grains in the feed treatments, the ingredients were mixed thoroughly to a thick paste then spread to form a thin sheet and rapidly frozen at −80 °C. Each feed treatment contained a different percentage of TOM, nominally 1, 4, 12 and 20% of the dry weight of sediment that was made by mixing the proportions of sand to biodeposits 95.8:4.2, 83.3:16.7, 50:50, and 16.7:83.3 respectively.

Three random samples of each prepared feed treatment were analysed to determine the actual TOM of the four feed treatments by a variation of the combustion method recommended by Byers et al. (1978). The samples were oven dried at 60 °C for 48 h, weighed and then placed in a furnace for 6 h at 500 °C to ensure complete combustion of organic matter, and then re-weighed. The percentage of TOM by dry weight was calculated by sample weight lost after combustion.

2.3. Feeding and digestion experiment

Feed was supplied frozen on Petri dishes (57 cm^2), which were placed in the centre of the circular tank floor. During the experimental acclimation period the feed was changed every 48 h. From a pilot experiment it was determined that the TOM content of all feed treatments did not significantly change in a period of 48 h, either to leaching or bacterial action. After the acclimation period the feed was replaced and the faeces produced by each sea cucumber were carefully collected from the floor of each tank with a plastic pipette every 3 h for a period of 48 h. At each sampling interval the number of sea cucumbers that had produced faeces per treatment was recorded, as well as the number of sea cucumbers located on the floor or on the wall of the tanks. The presence of faeces in a tank was an indirect indicator of food consumption and if a sea cucumber was on the walls of the tank it was an indicator that it was not feeding on the feed provided, which was only available on the floor of the tank. Tanks walls were kept clean and incoming sea water was filtered so it was not possible that the sea cucumbers were feeding on other sources of organic material, such as thick bacterial films.

Faecal samples were stored in small plastic containers and rapidly frozen to −80 °C and then transferred to a −20 °C freezer for storage. The samples were thawed and then filtered on a pre-burnt glass microfiber filter (47 mm diameter). During the filtering procedure the faeces were gently rinsed with deionised water to remove salt residue. Then TOM content was determined as described before, but with a correction for the filter weight, in order to determine if there are changes in the digestion over time expressed as changes in the TOM content of the faeces.

The faeces produced by each sea cucumber in each treatment were pooled together but separated by the faeces produced during day and night in order to estimate the faecal production rate (FPR) and to be able to identify differences in the feeding intensity expressed in