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Metabolic Rate Characteristics and Sediment Cleaning Potential of the Tropical Sea Cucumber *Holothuria scabra*

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Abstract

The oxygen consumption rate (OCR) and ammonium excretion rate (AER) of a tropical sea cucumber, *Holothuria scabra*, were determined in laboratory experiments. OCR and AER exhibited a significant negative correlation to body weight (BW), expressed as a power function of BW: $OCR = 0.09 \times BW^{-0.58}$ (mgO_2/g/h, r^2=0.89, n=15) and $AER = 0.38 \times BW^{-0.19}$ (μmolN/g/h, r^2=0.54, n=15). These values were comparable to those in previous studies on other sea cucumber species. The OCR of shrimp tank sediment was reduced to less than half (4.5 ± 0.3 to 1.0 ± 0.1 mgO_2/gdry/h) by the ingestion and excretion process of *H. scabra*. Acid volatile sulfide (AVS-S) concentration was also decreased to less than half (0.67 to 0.31 mgS/mgdry); despite the low reduction rates of organic carbon and nitrogen contents (0.19 to 0.14 mgC/mgdry and 0.022 to 0.019 mgN/mgdry, respectively). These results suggest that components in the sediment with high oxygen consumption potential were removed by *H. scabra*. These findings also provide fundamental information with which to evaluate the quantitative role of *H. scabra* in polyculture with shrimp.

Discipline: Aquaculture

Additional key words: ammonium excretion, feces, oxygen consumption, sediment, shrimp aquaculture

Introduction

Intensive aquaculture has been widely practiced in many Southeast Asian countries, including Thailand, Indonesia and the Philippines. Black tiger shrimp, *Penaeus monodon*, is one of the most important aquaculture species in this region (Briggs et al. 2004). However, since shrimp production has soared, the shrimp industry has faced many problems, particularly frequent viral disease outbreaks such as yellowhead disease, infectious hypodermal and hematopoietic necrosis and white spot disease, resulting in mass mortalities (Bondad-Reantaso et al. 2001). Although several measures have been taken to cope, such as using antibiotics and switching to relatively disease-resistant species (e.g. *Litopenaeus vannamei*, Briggs et al. 2004), no fundamental solution has been reached. In many cases, disease outbreaks are triggered by deterioration of the shrimp pond environment due to excessive accumulation of organic matter on the bottom sediment derived from shrimp feces and leftover feed (Avnimelech & Ritvo 2003).

In this regard, the development of polyculture methods has been attempted to mitigate nutrient and carbon load and remediate waste materials in the pond, and the use of deposit feeding sea cucumbers, which are of high commercial value, has been drawing much attention (Purcell et al. 2012, Watanabe et al. 2012). *Holothuria scabra* is the most valuable and commonly cultured tropical sea cucumber species (Hamel et al. 2001, Purcell et al. 2012). Several experimental studies on the polyculture of *H. scabra* with shrimps in a shrimp pond recommended various rearing conditions and practical culture methods (Pitt et al. 2004, Bell et al. 2007,

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Purcell et al. 2012).

Mathematical models incorporating dynamic energy budgets for cultured species have been introduced as a quantitative tool to determine the environmental effects and carrying capacity of aquaculture systems (Ren et al. 2012). However, quantitative studies about the impact and role of *H. scabra* in material cycling, such as oxygen, carbon and nitrogen, in the shrimp pond system remain limited due to the lack of physiological information.

Oxygen consumption and nitrogen excretion rates are important aspects of bioenergetics and fundamental parameters; required not only to understand the physiological features of animals but also to evaluate their energetic role, as well as material cycling in the related ecosystem. These parameters have been studied for several species of sea cucumber, including *Stichopus japonicus* (Yang et al. 2006), *Holothuria leucospilota* (Yu et al. 2012), *Holothuria atra* (Mukai et al. 1989), *Australstichopus mollis* (Zamora & Jeffs 2012) and *Scotoplanes globsa* (Smith 1983). Although there has also been a study on *H. scabra* (Mukai et al. 1989), it only involved a small range of body weights, and information is lacking on the effect of body size on these physiological parameters.

In this study, oxygen consumption and ammonium excretion rates of *H. scabra* were investigated in relation to body size under captivity. The effects of *H. scabra* ingestion on the oxygen consumption rate and sulfide level of black tiger shrimp tank sediment were also examined to ascertain the bioremediation capability of *H. scabra*.

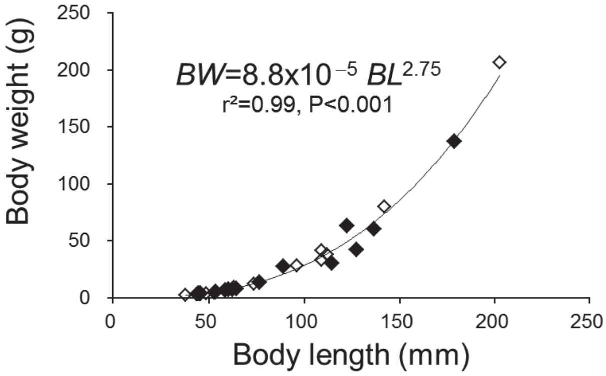

Fig. 1. Relationship between body length and body weight of *H. scabra* used in OCR (◆) and AER (◇) experiments

Materials and Methods

1. Experimental animals

Small *H. scabra* ranging from 37.8 to 76.7 mm in body length (11.6 to 22.9 g in body weight) were obtained from the Leganes hatchery laboratory of the University of the Philippines Visayas, and larger individuals ranging from 89.2 to 202 mm (27.2 to 206 g) were collected from the wild in Suclaran, Guimaras, the Philippines. The relationship between body length and body weight of *H. scabra* used in the experiment is shown in Fig. 1.

2. Oxygen consumption and ammonium excretion measurements

H. scabra smaller than 60 g were placed individually in a 300 mL Erlenmeyer flask filled with filtered (1 µm) and oxygen-saturated seawater pumped off the coast of the Aquaculture Department of the Southeast Asian Fisheries Development Center (SEAFDEC/AQD). Flasks were sealed with a rubber stopper to avoid gas exchange, placed in an incubator set at a constant temperature of 27°C and incubated under dark conditions. *H. scabra* exceeding 60 g were placed individually in a sealed 1 L plastic container and incubated under conditions equivalent to those of the flasks. The salinity of the rearing seawater was 32 ppt, and the initial concentrations of dissolved oxygen (DO) and ammonium were 6.5 mgO_2/L and 2.9 µmolN/L, respectively. DO concentrations in the flasks were measured every hour using a calibrated portable dissolved oxygen meter (DO-55G, DKK-TOA Corporation, Japan). To measure excretion, a 5 mL water sample was collected with a small syringe every 3 hours and ammonium concentrations were analyzed using an Autoanalyzer TrAAcs 800 (Bran + Luebbe, Germany).

The individual rates of DO consumption (OCR_i, mgO_2/h) and ammonium excretion (AER_i, µmolN/h) of the sea cucumber were calculated as follows:

$$OCR_i,\ AER_i = (C_0 - C_t)\ V/\ t$$

where C_o is the initial DO concentration (mgO_2/L) or ammonium concentration (µmolN/L) in the flasks and containers corrected by subtracting the control value from the measurement, C_t is the DO concentration or ammonium concentration after incubation in the container, V is the volume of seawater in the container (L), and t is the time duration (h). These values were converted to body weight specific rates by dividing by the animal's body weight: *OCR* ($mgO_2/g/h$) and *AER* (µmolN/g/h).

The atomic ratio of OCR to AER (O/N ratio), an index of the catabolic balance of protein substrates against carbohydrate and lipid substrates, was calculated from the overall mean of OCR and AER.

3. Oxygen consumption rate of sediment and feces

To determine the ability of *H. scabra* to clean the sediment in shrimp aquaculture ponds, the organic carbon and nitrogen contents were compared between sediment from shrimp culture tanks at SEAFDEC/AQD and the feces of *H. scabra*, which were fed with the shrimp tank sediment. The DO consumption rates of the shrimp tank sediment and *H.*

scabra feces were also compared.

Prior to collecting feces, *H. scabra* were starved and allowed to defecate for 24 hours. Shrimp tank sediment was fed to *H. scabra* in a 50-L fiber glass tank with a bare bottom, and feces were collected immediately after defecation. The samples were kept in the dark at 4°C until analysis. About 3 g of shrimp tank sediment and feces were incubated under the same conditions described for OCR and AER experiments. DO consumption rates were also calculated via the same procedure.

The shrimp tank sediment and fecal samples were oven-dried at 50°C, soaked in 1N HCl overnight for decarbonation, rinsed with distilled water and oven-dried again. Finally, dried samples were ground into a fine powder and organic carbon and nitrogen contents were measured by an elemental analyzer FLASH EA1112 (Thermo Finnigan, USA).

Acid volatile sulfides (AVS-S) concentration of fresh shrimp tank sediment and *H. scabra* feces were measured with an H_2S absorbent column (GASTEC, Kanagawa prefecture, Japan).

4. Statistical analysis

All relationships between body weight (BW) and metabolic rates (OCR_i, AER_i, OCR and AER) of *H. scabra* obtained in this study were fitted to a power function expressed as $R = aBW^b$, where R is the metabolic rate, and a and b are constants obtained by the least squares method and a t-test was used to evaluate the relationships obtained. Differences among the values obtained for oxygen consumption rates of shrimp tank sediment and feces were tested using a two tailed t-test. The relationships and differences were considered statistically significant if P values <0.05 for all tests.

Results

1. Oxygen consumption and ammonium excretion rates of *H. scabra*

DO consumption and ammonium excretion rates of individual *H. scabra* ranged from 0.08 to 0.74 mgO_2/h and 0.55 to 25.7 µmolN/h, respectively, and significant positive correlations were observed between body weight (BW) and the following parameters (Fig. 2):

OCR_i (mgO_2/h) $= 0.09 \times BW^{0.42}$,
(r^2=0.81, n=15, P<0.001)

AER_i (µmolN/h) $= 0.38 \times BW^{0.81}$,
(r^2=0.96, n=15, P<0.001).

In contrast with individual rates, body weight specific OCR and AER significantly decreased with increasing *H. scabra* body weight (Fig. 3):

OCR (mgO_2/g/h) $= 0.09 \times BW^{-0.58}$,
(r^2=0.89, n=15, P<0.001)

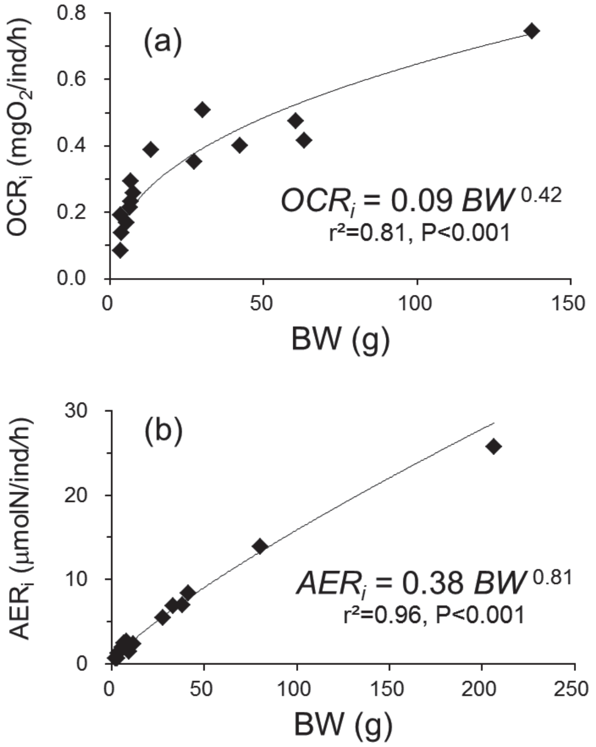

Fig. 2. Relationships between body weight (BW) and (a) oxygen consumption rate (OCR_i) and (b) ammonium excretion rate (AER_i) of *H. scabra*

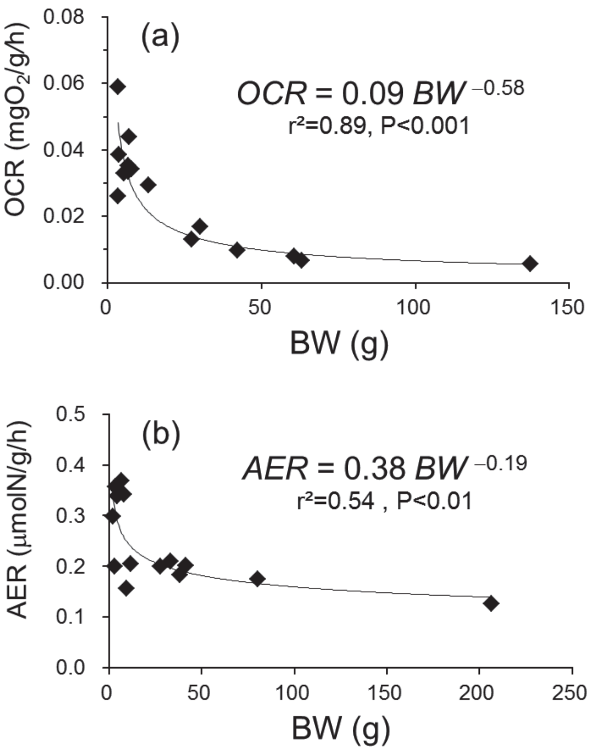

Fig. 3. Relationships between body weight (BW) and weight-specific oxygen consumption rate (a) OCR and ammonium excretion rate (b) AER of *H. scabra*

Table 1. Water content, organic carbon and nitrogen content, and acid volatile sulfides (AVS-S) of shrimp tank sediment and feces of *H. scabra* fed the sediment

	Water content (%)	Carbon (mgC/mgdry)	Nitrogen (mgN/mgdry)	AVS-S (mgS/mgdry)
Sediment	85	0.19	0.022	0.672
Feces	83	0.14	0.019	0.308

AER (µmolN/g/h) = $0.38 \times BW^{-0.19}$,

(r^2=0.54, n=15, P<0.01).

The atomic ratio of OCR to AER (O/N ratio) of *H. scabra* was 27.4.

2. Oxygen consumption and sulfide content of sediment and feces

DO consumption rate, organic carbon and nitrogen content and AVS-S concentrations of shrimp tank sediment and fresh *H. scabra* feces are summarized in Table 1. The DO consumption rate of the feces (1.0 ± 0.1 mgO_2/gdry/h, n=3) was significantly reduced (P<0.001) to less than 25% of that of the sediment (4.5 ± 0.3 mgO_2/gdry/h, n=3) (Fig. 4).

AVS-S concentration in the feces (0.31 mgS/mgdry) was also reduced to less than half that of the sediment (0.67 mgS/mgdry). Reduction rates of organic carbon (from 0.19 to 0.14 mgC/mgdry in the sediment and feces, respectively) and nitrogen content (from 0.022 to 0.019 mgN/mgdy) were relatively smaller than those for the DO consumption rate and AVS-S. No statistical tests were performed due to the small number of samples (n=2).

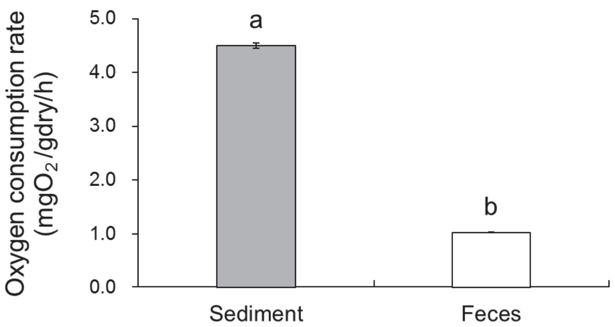

Fig. 4. Oxygen consumption rate of shrimp tank sediment (n=3) and feces of *H. scabra* fed the sediment (n=3). Error bars indicate standard deviation. Different superscripts indicate significant differences between the mean values (P<0.001)

Discussion

The relation between body size and metabolic rate is a fundamental piece of physiological information necessary to assess organisms' impact on the environment. Specifically, respiration and excretion rates are often used as important metabolic indices for bioenergetic measurements (Yang et al. 2006). OCR can be also used to indicate energy production in echinoderms (Talbot & Lawrence 2002, Yuan et al. 2010). It is well known that respiration and excretion rates of marine animals, including echinoderms, tend to increase with body weight but decrease on a per-unit-weight basis (Cai & Summerfelt 1992, Mukai & Koike 1984, Dy et al. 2002). This is consistent with the decrease in the surface area to volume ratio of the animals (Conover 1978). Our results with *H. scabra* conformed to this pattern.

Although it is difficult to directly compare these metabolic indicators among different species since there are various factors modifying metabolic rates, such as the temperature, salinity and nutritional condition of the animals (Yang et al. 2006, Yu et al. 2012, Zamora & Jeffs 2012), the OCR and AER of *H. scabra* obtained in this study were comparable to those in previous studies for sea cucumbers, such as *Stichopus japonicus* (Yang et al. 2006), *Holothuria leucospilota* (Yu et al. 2012), *Holothuria atra* (Mukai et al. 1989), *Australstichopus mollis* (Zamora & Jeffs 2012), *Scotoplanes globsa* (Smith 1983), as well as *H. scabra* (Mukai et al. 1989). The bodyweight specific oxygen consumption and ammonium excretion rates of other animals, including shrimps and fish, generally slightly exceed those of sea cucumbers (Beamish 1964, Bray et al. 1988, Cai & Summerfelt 1992, Carvalho & Pham 1997, Mukai et al. 1989, Walker et al. 2009, Weymouth et al. 1944) perhaps due to the sedentary and inactive nature of sea cucumbers. In polyculture of shrimp and sea cucumber where shrimp is the main commodity with much higher stocking biomass, the impact of sea cucumbers on oxygen consumption and ammonium excretion to the system would be minor compared to that of shrimp.

The O/N ratio value, atomic ratio of the OCR to AER, obtained in this study was 27.4, which was comparable to the previously reported value of 32.0 for *H. scabra* (Mukai et al. 1989). It is well documented that a low O/N ratio indicates proteinaceous materials being primarily catabolized, while one that is high indicates lipid catabolism dominating in an animal's metabolism (Ikeda 1974). The result in the present study suggested the importance of lipid catabolism for *H. scabra* as reported by Mukai et al. (1989).

This is the first study to report OCR and AER of *H.*

scabra as a function of body size. Mukai et al. (1989) reported these values for a limited size range of *H. scabra*. The regression formulae obtained in this study can be used to evaluate the energetic role of *H. scabra* polycultured with shrimp, as well as their potential in terms of oxygen consumption and nitrogen regeneration.

The DO consumption rate and AVS-S of the shrimp tank sediment were reduced to less than half after excretion by *H. scabra* as feces, which suggests that components in the shrimp tank sediment with high oxygen consumption potential, including easily degradable organic matter and sulfides, are removed by the ingestion and excretion processes of *H. scabra*. The DO consumption rates and AVS-S levels of the sediment are important indicators to evaluate the deterioration of aquaculture farm sediments (Yokoyama 2003). Accordingly, although *H. scabra* consume oxygen and release ammonium, it is considered to contribute in terms of remediating sediment quality.

A reduction in organic carbon and nitrogen concentrations was also observed in the feces, but at rates relatively lower than those of DO and AVS-S, indicating that nitrogen and carbon may not be selectively assimilated against other atoms by *H. scabra*. A feeding trial using black tiger shrimp tank sediment, shrimp feces and *Navicula ramossisima* as food sources showed that *H. scabra* grew fastest with the feces (Watanabe et al. 2012). The digestibility of animal based organic matter can be as high as 89% in *H. scabra* (Orozco et al. 2012). Therefore, *H. scabra* is supposed to be efficient in removing leftover shrimp feed high in animal protein content and shrimp feces from the shrimp pond sediment. It can be said that *H. scabra* has the ability to improve the sediment quality of shrimp ponds. However, there is a need to further calculate all processes and budgets of oxygen, carbon and nitrogen to evaluate the potential role of *H. scabra* within the whole shrimp pond system.

Practically, OCR and AER comprise important parameters in ecosystem models, and the present study provides regression formulae for OCR and AER as a function of body size, which are useful for the time series ecosystem model analysis of *H. scabra*. However, it is also well known that OCR and AER of sea cucumbers are strongly influenced by various factors, such as temperature, salinity and nutritional conditions (Yang et al. 2006, Yu et al. 2012, Zamora & Jeffs 2012). Measurements of metabolic rates under various temperature and salinity conditions should be further conducted to develop highly reproducible ecosystem models and design an effective polyculture method for shrimp and *H. scabra.*

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