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Research Note

Oxygen Consumption and Ammonia Excretion Rates of the Sea Cucumber *Holothuria spinifera,* (Theel, 1886) in Relation to Reproductive Stages

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The knowledge on oxygen consumption and ammonium excretion rates of aquaculture species are important aspects useful in their captive management. It will help in understanding their energetic role and material recycling in an ecosystem. A study was conducted to assess the rates of oxygen consumption (OCR) and ammonia excretion (AER) in *Holothuria spinifera* with respect to reproductive stages following standard techniques. The OCR was comparatively higher for the indeterminate animals, and the mean OCR was maximum of 0.025 ± 0.021 ml O_2 $hr^{-1}g^{-1}$dry wt in the indeterminate stage-two. Whereas the AER was comparatively higher in the spent stages of both the sexes, and the mean value was highest of 20.03 ± 0.15 μg atom N hr^{-1} g^{-1} dry wt for the spent male. In this study the smallest individual exhibited the highest rate of oxygen consumption and ammonia excretion. The body size and metabolic rate are important in assessing the organism impact on the environment.

Keywords: Physiology, indeterminate animals, spent male, *Holothuria spinifera*, environmental impact

Introduction

The quantitative information on oxygen consumption and ammonia excretion is of primary importance in assessing the energy and material flow through food web. Interactions of both exogenous factors (temperature, salinity, humidity and dissolved oxygen) and endogenous factors (body size, activity, gametogenic stage and sex) and other dependent variables such as season affect metabolic energy expenditure (Newell & Bayne, 1983). Energy losses can be calculated from the oxygen consumed per unit time and the carbon dioxide released or the liberation of heat.

The utilization of oxygen is a direct measure of the degree of activity and heat production in animals. Excretion rates have been known to be a function of body size (Emerson, 1969), temperature (Bayne & Scullard, 1977) and season (Widdows & Shik, 1985). Previously, the relationships between ammonia excretion and the body size of organisms have been studied (Mace & Ansell, 1982; Stickle & Bayne, 1982).

Sea cucumbers are commercial echinoderms and were once an important trade item in India, owing to their high commercial value and export demand. They constituted a significant source of livelihood for the dependent fishermen until the ban on their fishery was enforced by the Ministry of Environment and Forest, Government of India, since 2001. *H. spinifera* was a medium valued sea cucumber species in the commercial market with restricted distribution in Arabian Gulf, India, Sri Lanka, Philippines, Western Australia, and Southern China (Purcell et al., 2012b; Ding et al., 2020). *H. spinifera* was one of the main constituents of the beche-de-mer industry that once existed along the Gulf of Mannar and Palk Bay of the south east coast of India. Apart from the commercial value, this species is also reported to contain the metabolites of anti-fouling and anti-cancerous properties (Abdelhameed et al., 2020). The international Union for Conservation of Nature (IUCN) has classified *H. spinifera* as data deficient (www.iucnredlist.org). Owing to its com-

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mercial and conservation importance in Indian waters, attempts were being made to standardize the hatchery technology of this species (Asha & Muthiah, 2002; 2007).

Many studies were conducted on the fluctuations in oxygen consumption and excretion rates of the sea cucumbers like *Stichopus japonicus* (Yuan et al., 2010; Yuan et al., 2006), *Holothuria leucospilota* (Yu et al., 2012), *H. atra* (Mukai et al., 1989), *Australstiatoplus mollis* (Zmora & Jeffs, 2012) and *Scotoplane globsa* (Smith, 1993), *Eupentacta quinquesemita* (Sabourin & Stickle, 1981) and *Holothuria scabra* (Mercier et al., 1999). Results of these studies indicated that physiological factors are associated well with the health conditions of sea cucumbers. The use of commercially valued deposit-feeding sea cucumbers in polyculture systems to mitigate the nutrient, carbon-load and to remediate waste materials has been sought (Purcell, 2012a; Watanabe et al., 2014). The oxygen consumption and ammonium excretion rates are important aspects to evaluate energetic role and material recycling of the ecosystem. The present study focuses on the rates of oxygen consumption and ammonia excretion in *H. spinifera* with respect to reproductive stages. The study results will provide valuable knowledge for improving the aquaculture management of this species in tropical and subtropical coastal areas.

H. spinifera specimens of length 89-304 mm collected from the skin diver's collections of Kalavasal, Tuticorin coast were used for the experiments. After collections the live specimens were transported to the wet laboratory and were acclimatized in tanks with sand-filtered natural seawater of temperature 29–31.8 °C and salinity of 35 ppt.

The animals were starved for 48 h to void their gut contents before the experiment. The animals of various reproductive stages were randomly selected for the study and the experiment was conducted by keeping single specimens in 3000 ml PVC jars filled with sand-filtered seawater. The jars were closed air tight with plastic tops carefully preventing the trapping of air bubbles. Before the introduction of each animal, seawater was siphoned into a 300 ml BOD bottle to estimate the initial oxygen content. The jars with sea cucumber were kept for three hours in seawater at room temperature of 29 °C. After incubation, samples of seawater were withdrawn every hour and the oxygen content was estimated by Winkler's method (Strickland & Parson, 1972). The difference in the dissolved oxygen content between the two samples was taken as the quantity of oxygen (ml O_2 hr^{-1} g^{-1} dry wt) utilized for respiration by the sea cucumber for an hour. A correction was made for the reduction of seawater volume in the jar, caused by the displacement of the experimental animal and control without the animal too was maintained.

Ammonium excretion rate of the samples were also estimated. Ammonia content was estimated from the samples before and after incubation by following Phenol hypochlorite method (Solarzano, 1969). The increase in ammonia content was taken as the quantity of ammonia excreted (μg atom N hr^{-1} g^{-1} $dry.wt^{-1}$) by the animal.

After the experiment, the relaxed body length and dry weight of the animals were recorded, and the maturity conditions were noted. The dry weight was calculated after drying the animal without gut at 80 °C for 48 h for expressing the rates of oxygen consumption and ammonia excretion in terms of the gram dry meat weight of the animal.

A total of 36 animals (length 89-304 mm) of various reproductive stages of both sexes were used for the experiment. The mean difference in the values of oxygen consumption and ammonia excretion concerning the reproductive stages was used to interpret the results.

The Oxygen Consumption Rate (OCR) was higher for the individual indeterminate animals than both males and females and varied between 0.0043 and 0.095 ml O_2 hr^{-1} g^{-1} dry.wt for the indeterminate stages. Similarly, females reported higher OCR than males and ranged from 0.0029 and 0.015 ml O_2 $hr^{-1}g^{-1}$dry.wt in females and 0.00065 and 0.012 ml O_2 $hr^{-1}g^{-1}$dry.wt in males. In both sexes, spent animals recorded the lowest OCR than the maturing and mature stages. The mean OCR was minimum of 0.004 ± 0.002 ml O_2 $hr^{-1}g^{-1}$dry.wt in the spent stage of male and maximum of 0.025 ± 0.021 ml O_2 $hr^{-1}g^{-1}$dry.wt in the indeterminate stage-two (Table 1). The oxygen consumption rates of *H. spinifera*, with a length of 89, 195, 245 and 304 mm were 0.095, 0.005, 0.0082, 0.0079 ml O_2 hr^{-1} g^{-1} dry.wt and the average consumption with a dry weight of 11.6, 32.0, 46.2 and 65.5 g were 0.015, 0.012 and 0.008 and 0.003 ml O_2 hr^{-1} g^{-1} dry. wt respectively.

The Ammonium Excretion Rate (AER) was higher in the spent stages of both sexes. The value ranged from

Table 1. Mean (± S.E) values of oxygen consumption and ammonia excretion rates of *Holothuria spinifera* with respect to reproductive stages.

Sex	Stages	Nos. of animals tested	OCR (ml O_2h^{-1} g^{-1} dry.wt)	AER (μg atom N h^{-1} g^{-1} dry.wt)	Length Range (mm)
Indeterminate	I	3	0.01 ± 0.004	14.9 ± 2.1	142-190
	II	6	0.025 ± 0.021	9.3 ± 6.1	89-304
Male	Maturing	4	0.009 ± 0.004	11.1 ± 3.1	165-255
	Mature	3	0.008 ± 0.0009	10.3 ± 4.2	195-282
	Spent	5	0.004 ± 0.002	20.03 ± 0.15	232-274
Female	Maturing	8	0.010 ± 0.003	13.05 ± 8.2	185-296
	Mature	3	0.008 ± 0.002	6.2 ± 0.7	165-190
	Spent	4	0.005 ± 0.0006	21.0 ± 3.6	175-215

2.90 to 27.12 μg atom N $hr^{-1}g^{-1}$dry.wt among the individuals of the indeterminate stages; from 2.44 to 20.2 μg atom N $hr^{-1}g^{-1}$ dry.wt among males and 1.41 to 24.02 μg atom N hr^{-1} g^{-1} dry.wt among females. The mean value was highest of 20.03 ± 0.15 μg atom N hr^{-1} g^{-1} dry.wt for the spent male and the lowest of 6.2 ± 0.7 μg atom N hr^{-1} g^{-1} dry. wt for the mature females (Table 1).

The rates of ammonia excretion of *H. spinifera*, with a length of 89,195, 245 and 304 mm were 19.81, 13.04, 10.62, 4.07 μg atom N hr^{-1} dry.wt^{-1} and for the dry weight of 11.6, 32, 46.2 and 65.5 g were 11.5, 6.4, 4.1 and 2.1 μg atom N hr^{-1} g^{-1} dry.wt respectively.

The mean values of oxygen consumption and ammonia excretion in *H. spinifera* were 0.012 ml O_2 hr^{-1} g^{-1} dry.wt and 12.45 μg atom N hr^{-1} g^{-1} dry.wt respectively, irrespective of maturity conditions. The oxygen consumption rate was slightly lower than that of 0.1 ml O_2 hr^{-1} g^{-1} ash free dry.wt of *H. scabra* and 0.331 ml O_2 $hr^{-1}g^{-1}$ ash free dry.wt of *H. atra* (Mukai et al., 1989). Thus, comparatively the low level of oxygen consumption in *H. spinifera* may be due to the heavy dependence on anaerobic respiration, as indicated for *Leptosynapta inharens,* since both are highly burrowing species (Webster, 1975). The low values of oxygen consumption and ammonium excretion in holothurian, when compared to other invertebrates may be because of the inefficient oxygen transport to the internal organs, and the larger volume of perivisceral fluid and the low metabolic activity of the animal in general (Webster, 1975).

The seasonal variations in oxygen consumption and ammonium excretion are linked with the gametogenic cycle in invertebrates (Newell & Bayne, 1973). Ru et al. (2018) also reported that *Apostichopus japonicus* change the energy budget patterns to meet the energy demand of gonad growth, limiting energy investment in somatic growth and self-maintenance functions. Similarly Ru et al. (2018) also observed the reduction in metabolism (i.e., lower oxygen consumption and ammonium excretion) in *H. leucospilota* under stressed conditions and opined that this may be because of a possible mechanism of energy conservation by the species.

It was observed that in *H. spinifera* that the rate of respiration and ammonia excretion decreased with an increase in body weight and the smallest individual exhibited the highest rate of oxygen consumption and ammonia excretion. This observation is in conformity with similar observations in bivalves like *Martesia striata* (Nagabhushanam, 1966) and *Placopecten magellanicus* (Mac Donald & Thompson, 1986); sea urchins like *Stronglocentrotus purpuratus*, (Webster & Giese, 1974), *Allocentrotus fragilis* (Webster, 1975); sea cucumbers like *H. scabra* and in *H. atra* (Mukai et al., 1989). The relationship between body size and metabolic rate is of greater importance in assessing the organism impact on the environment. Dy et al. (2002) & Kodama et al. (2015) also noticed a decrease in oxygen consumption rate and ammonium excretion rate per unit weight basis in sea urchin and sea cucumber respectively. The study recommends the development of models based on the metabolic rates under different environmental and nutritional conditions of sea cucumber species for its effective utilization in polyculture systems.

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