



Energetic metabolic differences between tropical (*Lytechinus variegatus*) and polar (*Sterechinus neumayeri*) echinoderms

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ABSTRACT

The objective of the present study is to evaluate and compare the oxygen consumption of individuals of two species of sea urchins, one tropical (*Lytechinus variegatus*) and the other polar (*Sterechinus neumayeri*). Measurements were held in respirometric sealed chambers. It was observed that individuals of *L. variegatus*, in spite of being larger and heavier, have higher oxygen consumption rates (per unit of body weight) than *S. neumayeri*. This is probably an adaptation to polar conditions as a result of a long evolutionary history of polar invertebrates in an environment with low temperatures and high seasonality of food production. The O:N ratio of *S. neumayeri* was the same as that of *L. variegatus*, which indicates that both species were using a similar substratum for metabolic demands.

Key words: oxygen consumption, metabolism comparisons, *Sterechinus neumayeri*, *Lytechinus variegatus*.

INTRODUCTION

Many authors consider temperature a crucial factor for the selection of adaptive strategies of organisms in the Antarctic environment. Scholander et al. (1953) proposed a theory known as “Metabolic Cold Adaptation”, later endorsed by Wohlschalg’s studies (1960, 1964). According to this theory, polar marine ectothermic animals would have metabolic rates relatively more elevated than the expected for the low temperatures, when the rates of temperate species were compared with the polar ones. Nowadays such theory is under discussion in different studies. According to Clarke (1991), for instance,

the difficulty in transferring data obtained in laboratories for natural environments and the evolutionary history of the biota are a barrier to this kind of comparisons.

Despite of the large number of studies held on laboratories in order to evaluate the effect of temperature on vital functions of aquatic ectothermic animals, the short period during which they were conducted are certainly very different from those of evolutionary adaptations (Clarke, 1983). Moreover, studies that led to the development of “the metabolic cold adaptation” hypothesis were conducted, in vast majority, in eurythermic animals, more specifically with fishes not very well acclimated to laboratory conditions. The author discus-

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sed that, in many experiments, acclimation to temperature and experimental care to avoid stress were not considered.

During the acclimation in short scale (3 to 4 weeks), a fish can develop physiological responses to increase the synthesis of enzymes that work more efficiently in specific conditions.

Besides that, structural alterations can be present after longer periods, such as the size and quantities of mitochondria, quantity of plasmatic reticulum and the distance of diffusion. Individuals of some species also accumulate lipids (triacylglycerol) which can be used as a source of energy. In fact, the most conspicuous adaptation of fish to temperature at Earth's Polar Regions is the presence of some glycoproteins which are used as antifreeze solution for body fluids. Clarke (1983, 1987) and Hazel (1989) made comprehensive reviews on this subject. Thermal variation associated with large spatial scales is thought to significantly affect the physiology of organisms in marine systems and consequently limit their distribution. To examine this phenomenon, Osovitz and Hofmann (2005) compared the heat shock protein (*hsp70*) mRNA expression of purple sea urchins (*Strongylocentrotus purpuratus*) collected from different geographic locations and those acclimated to different temperatures. The suggestion was that the thermal variation across *S. purpuratus* range of occurrence is enough to affect the regulation of thermally sensitive genes, such as *hsp70*. Thus it is expected that the changes of cellular metabolism can be demonstrated by comparisons between the physiologies of similar phylogenetic species in different environments.

Brockington and Clarke (2001) and Brockington and Peck (2001) discuss the influence of seasonality on the metabolism of Antarctic sea urchins *Sterechinus neumayeri*, demonstrating that these animals present alterations in the metabolic rates in different periods of the year, associated not only to the temperature variation but mainly to the food availability. The authors verified that gonads decreased in size after long periods of starvation. Sim-

ilar phenomenon was also observed in *Lytechinus variegatus* (Brockington and Clarke 2001).

Bioenergetics studies have been widely used to understand the ecological role of organisms, the adaptations to the specific environmental conditions where they live and the transformations of energy related to their functional processes (Brett and Groves 1979).

A consensus among physiologists exists showing that routine metabolism corresponds to the necessary cost for an organism to stay alive, maintaining the spontaneous activity that does not demand efforts (Clarke 1990). Once it is almost impossible to perform precise measurements of basal requirements, the routine metabolism is a good approximation to the basic energy demand of an organism. Oxygen consumption is one of the easiest and most efficient methods to assess metabolic expenditure (Cech Jr 1990).

Previous studies dealing with phagocytes indexes of Antarctic sea urchin *S. neumayeri* showed that these indexes are lower than those found in individuals of the tropical species *L. variegatus* (Borges et al. 2002). These results indicate that physiological and immunological differences are present in these animals due to their different evolutionary history. To tackle this issue, the routine metabolism of individuals belonging to the two species of sea urchin *L. variegatus* (tropical) and *S. neumayeri* (Antarctic) was evaluated to study the physiological differences between them. For that, oxygen consumption and ammonia excretion were measured in animals previously acclimated to the experimental conditions.

MATERIAL AND METHODS

ANIMAIS BREEDING

Lytechinus variegatus

In the winter of 2002 (July) sea urchins ($n = 35$) of the specie *L. variegatus*, 162.46 ± 37.32 g and volume of 139.43 ± 34.40 ml were collected at 3 to 7 meters depth in the rocky coast around the Tiasussê island ($23^{\circ}49.530'$ South and $0.45^{\circ}26.394'$

West), Baraqueçaba beach, São Sebastião, north coast of São Paulo state. The animals were transported to the station of Oceanographic Institute – University of São Paulo and kept in tanks of 500 l with seawater constantly renewed, salinity 35, temperature $20 \pm 1^\circ\text{C}$, artificial aeration and feed *ad libitum* with algae collected in the region.

Sterechinus neumayeri

During the Brazilian Antarctic Expedition, in the summer of 2002 (January), sea urchins ($n = 35$) *S. neumayeri*, 68.48 ± 22.82 g and volume of 64.14 ± 20.95 mL were collected at a depth of 5 to 10 m with small drag net. The sampling site was at the surroundings of Antarctic Brazilian Station Comandante Ferraz, Admiralty Bay, King George Island ($62^\circ 10', 168^\circ \text{S}$; $058^\circ 26', 959^\circ \text{W}$), Antarctica. The animals were kept in laboratories under controlled temperature ($0 \pm 1^\circ\text{C}$) in 500 l tanks, artificial aeration, and salinity 35 s. They were fed *ad libitum* with algae collected in the region and the water in the tanks was partially renewed once a day.

RESPIROMETRY

Routine metabolism variations and ammonia excretion measurements were performed in a sealed chamber for respirometry in the temperatures of $0 \pm 1.0^\circ\text{C}$ for *S. neumayeri* and $20.0 \pm 1.0^\circ\text{C}$ for *L. variegatus*. For both species the starving period was 48 hours.

Ten respirometric chambers of inert plastic of 1010 and 1030 mL were used. Before the chambers were closed the animals were kept inside, with continuous circulation of water for 12 hours, to minimize the stress caused by the manipulation. Afterwards, the water supply was suspended and chambers were closed in a way that animals could consume the oxygen of the water in the chamber with a known volume. Ali seawater was previously filtered in a CUNO filter with $1\mu\text{m}$. Preliminary tests showed that alterations caused by small organism were not significant.

Samples of the water inside the respirometer

were collected at the beginning and at the end of the experimental period, and dissolved oxygen was determined by the Winkler's method (Grasshoff 1983). The experimental periods were 12 hours for Antarctic sea urchin *S. neumayeri* and 4 hours for the tropical sea urchin *L. variegatus*. At the end of all experiments the oxygen concentration was always above 70% of the initial saturated water, to avoid the effect of the gas depletion on the metabolic rates. Samples of 10 mL of water were also collected at the beginning and at the end of the experiments for ammonia determination by the Koroleff method (1970).

Oxygen consumption by individual animals ($\mu\text{lO}_2/\text{h}$); the weight specific oxygen consumption ($\mu\text{lO}_2/\text{g/h}$); the ammonia excretion by individual animals (lIatgN/h) and the weight specific ammonia excretion (lIatgN/g/h) were calculated and analyzed in relation to temperature and body mass of the animals. The O:N (oxygen/nitrogen) ratio was calculated from the data of oxygen consumption and ammonia excretion transformed in atomic equivalents, to estimate the substratum used to provide energy to the organisms (Maysaud and Conover 1988).

Data were compared between species but to make a better comparison it is necessary to correct the effects of body weight on the metabolism. There is a general biological rule that animals of lower body weight have higher metabolic rates per unit of body weight. This variation is indicated by the inclination (b) of the regression lines of metabolism as function of the variation of body weight of the population. To make this adjustment a medium oxygen specific consumption was calculated for each species as if all the individuals had 100 g of total body weight, as described by Steffensen et al. (1994), using the equation (1). Original data of each individual employed was then corrected and a hypothetical average was calculated

$$VO_{2(wh)} = VO_{2(1)} * (Wt/Wh)^{(1-b)} \quad (1)$$

where, $VO_{2(wh)}$ is the oxygen specific consumption as if the individual had 100 g (W) calculated from the consumption really measured $VO_{2(1)}$, during an experiment with an individual of total body weight

Wt ; the letter b represents the exponent of regression between body mass and the range of weight of the population, assumed as 0.8.

For statistical analysis T tests were used ($\alpha = 0.01$) and data were compared under constant temperatures. Test of multiple comparisons of Newman-Keuls (Zar 1996) were also performed, after ANOVA, to search significant difference between averages.

RESULTS

OXYGEN CONSUMPTION

Significant differences between individual oxygen consumption and also between weight specific oxygen consumption were observed when the average of each parameter, for the different sea urchin species, was compared. Consumption of O_2 was higher for tropical sea urchin *L. variegatus*.

AMMONIA EXCRETION

Significant differences between individual ammonia excretion and also between weight specific ammonia excretions were observed when the average of each parameter, for the different sea urchin species, was compared. Excretion was higher for tropical sea urchin *L. variegatus*.

O:N RATIO

There were no significant differences in the O/N ratio between both species.

DISCUSSION

Oxygen consumption is one of the best ways to evaluate the aerobic metabolism of animals (Becker et al. 1992). In sedentary bottom species, as sea urchins, the routine metabolism covers the majority of the daily metabolic demand. Even when considering more active species, as fishes, the routine metabolism can represent more than 40% of the daily oxygen consumption (DuPreez et al. 1990).

Many systems can be employed to measure the metabolism of aquatic animals. The systems using sealed respirometric chambers are still in use due

to its operative facilities, with good results, comparable to the continuous flux system. The main disadvantage of the method of sealed respirometry is the depletion of dissolved oxygen and the waste accumulation during the experiments. The hypoxia and the waste metabolites can interfere on the results, inducing to wrong interpretations. For that reason the confining period in the sealed chamber must be adjusted so as to maintain the final concentration of dissolved oxygen above 70% of the initial concentration (Di Pardi 2000).

The concept of “metabolic cold adaptation” has been investigated and is still controversial among many authors. This concept is based mainly on Wohlschlag (1960) studies on routine metabolism of Antarctic ectotherms. The author stated that the metabolic demands of resting polar fish were similar to those of fish of temperate regions near the minimum limit of the temperature variation. Nevertheless, some authors suggest that the measurements of oxygen consumption of polar organisms were probably overestimated caused by manipulation stress, which was not always considered in previous studies. Wells (1986 *apud* Forster et al. 1987) observed a great interspecific variability of data in accordance with the habitat. The resting metabolism of the Antarctic fish *Pagothenia borchgrevinki* obtained by Forster et al. (1987) was always under the values reported by Wohlschlag (1964). On the other hand, metabolism of the more active mesopelagic fish *Electrona antarctica* was about 80% over the metabolism estimated for *P. borchgrevinki* (Torres et al. 1985).

Torres and Somero (1988) observed interspecific variations on the O_2 consumption among species living at small depth differences, probably reflecting the different strategies for feeding and for locomotion (Sullivan and Somero, *op. cit.*; Siebenaller et al., *op. cit.*). Interspecific variations of the O_2 consumption on the muscle enzymatic activity can also be influenced by the nutritional state and by the size (Sullivan and Somero 1983; Lowery et al. 1987).

Our results agree with Jovito (1998) and Di Pardi (2000) data about physiological adaptations

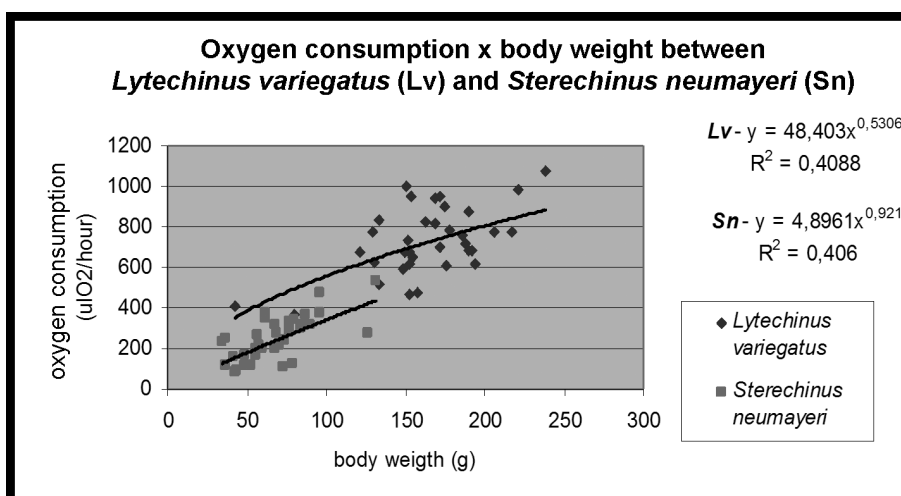


Fig. 1 – Regression analysis between oxygen consumption and body weight of *Lytechinus variegatus* and *Sterechninus neumayeri*.

of Antarctic crustaceans at Admiralty bay. It was also observed a lower oxygen consumption of the Antarctic invertebrate when compared to tropical ones. Results indicate that *S. neumayeri* metabolism is significantly lower than *L. variegatus*. This result is still more evident if the average were corrected for body weight differences (Table I). This kind of procedure is currently used in the literature to allow better comparisons between different species or populations (Steffesen et al. 1994; Glazier 2005).

Low metabolic levels in Antarctic invertebrates were also observed by other authors (Clarke 1991; Chapele and Peck 1995; Gomes et al. 1995).

Studies with sea urchins showed that different factors can change their metabolism, such as the size of the animals (McPherson 1968), nutritional state (Farmanfarmaian 1966), reproductive cycle state (Giese et al. 1966), oxygen tension (Johansen and Vadas 1967), salinity (Giese and Farmanfarmaian 1963) and habitat (Lewis 1968), but there is little information available on the effects of temperature. Farmanfarmaian and Giese (1963) studied the relation between metabolic rates and the temperature in *Strongylocentrotus purpuratus* according to the acclimation temperature. They observed that animals acclimated at lower temperatures had higher metabolic rates. For the sea urchin *Eucidaris tribuloides*, McPherson (1968) had the higher metabolic rates at higher temperatures during winter than the

correspondent temperatures in summer. Brockington and Peck (2001) measured the metabolism of *S. neumayeri* juveniles along the year and verified that the metabolism variation is directly related to temperature of acclimation and the food availability. The O:N ratio increased in summer, probably as a result of diet changes. Brockington and Clarke (2001) verified that food availability is more important to metabolism variations than water temperature. The authors suggested that when food resources are abundant, more elevated is the metabolism. This is probably an adaptation to Antarctic marine conditions where variations of temperature along the year are much less pronounced than variations of food availability. Marine primary production in the water column is low or absent during winter when light is weak and the sea surface is covered with ice. During relatively long periods benthic animals may depend on resources that were accumulated during periods of higher production. Food availability would also explain the decay of metabolic rates of several species which were measured in winter. Animals with low energetic demands and a seasonal variation of metabolic demands established during evolution would have advantages over those with higher energetic needs as, in fact, the water temperature is stable and variations throughout the year are very small to control these processes. Brockington and Clarke (2001)

TABLE I
Means of the data obtained for individuals of Tropical (*Lytechinus variegatus*, n = 35)
and Antarctic (*Sterechinus neumayeri*, n = 35) species of sea urchins.

Metabolic differences between Tropical and Antarctic sea urchins		
Parameters	<i>L. variegatus</i>	<i>L. neumayeri</i>
Animal volume (ml)	139.43 ± 34.4*	64.14 ± 20.95
Wet weight (g)	162.46 ± 37.32*	68.48 ± 22.82
O ₂ consumption/hour (μlO ₂ /h)	728.23 ± 170.58*	253.17 ± 117.03
Specific consumption of O ₂ (μlO ₂ /g/h)	4.65 ± 1.26*	3.75 ± 1.53
Steffensen factor (O ₂ consumption – 100 g wet weight)	5.12 ± 1.03*	3.43 ± 0.97
Ammonia excreted/hour (μatgN/g/h)	0.09 ± 0.02*	0.14 ± 0.093
Specific ammonia excreted/hour (μatgN/g/h)	0.0006 ± 0.0002*	0.0021 ± 0.001
Oxygen/Nitrogen relation	9.02 ± 2.03	10.34 ± 5.25

*p<0.01.

discussed that even the 010 values calculated for *S. neumayeri* until today need to be interpreted under the light of food availability, since only 15-20% of metabolic variations are due to temperature.

Smith et al. (1998) also verified that temperature and food availability are the two main factors that influence metabolism and larval development of echinoderms. The larval development of *Sterechinus neumayeri* occurs during summer (water temperatures over +0.2°C), whereas embryos of *Odontaster meridionalis* and *O. validus* are released during winter when the temperature is more constant and below -1.6°C. Temperature did not influence the development of embryos of *O. validus*, but embryos of *O. meridionalis* and *S. neumayeri* suffered a reduction of the development with temperature decrease. The geographic distribution of these species can be related to this fact: *O. meridionalis* has a limited distribution at the south of Georgia islands (54°8, 36'W) and Kerguelen (49°8, 63'W) in depth between 15 and 590 m, whereas *O. validus* occurs in depth between 0 and 914 m being found in Bhang Rocks (53°8, 42'W) and Bouvetoya (54°8, 03'E).

Ulbricht and Pritchard (1972), studying the relations between metabolic rates and temperature in different species of sea urchins of different habitats, suggested that organisms that occurs in more exposed habitats have oxygen consumption relatively independent of temperature, possibly as an adaptive strategy that allows these animals to keep

their metabolic rates constant under the considerable environmental temperature variation. The authors measured the metabolic rates of the purple sea urchin (*Strongylocentrotus purpuratus*) at the intertidal zone, and concluded that rates were independent of the temperature on a wide range (12 to 21°C). On the other hand, the red sea urchin (*S. franciscanus*) that occurs at the subtidal zone, presented a high 010 value (8.26) at the range of temperature studied. In a more wide vision, it is evident that the plasticity of sea urchins in relation to temperature depends on the ecological habitat.

The sea urchins *S. purpuratus* did not showed any correlation between the reproductive process and oxygen consumption (Giese et al. 1966). Oxygen consumption variations were detected neither during the gonadal development nor during the development of specific female glands in the fertile period. However, authors verified a direct correlation between oxygen consumption rate and temperature, but these results were considered as a consequence of the stress provoked by acute temperature changes, without previous acclimation of the organisms.

The results obtained indicate that the metabolism of *S. neumayeri* and *L. variegatus* presented significant differences, considering that the specific consumption of the last one is 1.24 larger than that of the Antarctic species, even when difference in size is not considered. It is widely known as a bio-

logical rule that smaller individuals have higher metabolic rates when compared to bigger ones (Steyermark 2002). Thus, considering the metabolic rates of both species, relative to the respective temperatures of their natural environments, a “metabolic cold adaptation” was not observed for *S. neumayeri*.

The present results indicate that the excretion of *S. neumayeri* is about 3 times larger than that of *L. variegatus*. Ammonia excretion rates are much more variable, once it is a very complex process which is exposed to the influence of variables of difficult control, such as the nutritional state or the kind of substratum that has been used for the oxidative processes (Echevarría et al. 1993; Gomes et al. 1995). Nevertheless, the O:N ratio can be used to estimate the substratum that is being used to supply energy demands (Maysaud and Conover 1988). Rates between 3 and 16 are indicative of protein utilization; rates until 60 of protein plus lipid utilization and rates above 60 of lipid utilization only. The O:N rates are similar for both species, indicating that a similar substratum is normally used to provide energy to *S. neumayeri* or to *L. variegatus*. The discussion about metabolic adaptation in relation to temperature must consider the acclimation and starvation period of the animals, stress, the methodology used, among other factors. For that reason, it is difficult to compare our results with data available in the literature, because many times the methodology is not completely described.

For the first time the metabolism of the Antarctic sea urchin *S. neumayeri* that was studied comparing with that of the tropical sea urchin *L. variegatus*, using exactly the same methodology. The O:N rates are not easy to explain once they demonstrate the use of similar energetic substratum but not the same quality of the substratum.

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RESUMO

O presente trabalho descreve diferenças metabólicas entre equinodermos tropicais e polares. O objetivo deste estudo foi avaliar o consumo de oxigênio dos ouriços-do-mar tropicais *Lytechinus variegatus* e o antártico *Sterechinus neumayeri*, por meio do protocolo experimental utilizando-se câmaras respirométricas seladas. Observou-se que as espécies tropicais, com tamanhos e pesos maiores, apresentaram maiores taxas quanto ao consumo de oxigênio e menores quanto à excreção de elementos nitrogenados em relação aos antárticos. Essas diferenças podem demonstrar que as variáveis encontradas são adaptações resultantes de uma longa história evolutiva de invertebrados polares em um ambiente com baixas temperaturas e com alta sazonalidade na produção de recursos alimentares. A razão oxigênio-nitrogênio (O:N) entre ambas as espécies foi a mesma, indicando que utilizam semelhantes substratos energéticos para suprir suas demandas metabólicas.

Palavras-chave: consumo de oxigênio, diferenças metabólicas, *Sterechinus neumayeri*, *Lytechinus variegatus*.

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