OXYGEN CONSUMPTION AND AMMONIA EXCRETION OF THE ANTARCTIC AMPHIPOD *Bovallia gigantea* PFEFFER, 1888, AT DIFFERENT TEMPERATURES AND SALINITIES

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**A B S T R A C T**

The energy budget of Antarctic stenothermic and/or stenohaline ectotherms is modulated by variations of temperature and salinity. The joint effects of these latter on polar organisms have been but little studied. Data on this subject are of great importance for an understanding of the energy demand of Antarctic animals such as amphipods, especially when considering their ecological importance and the possible impacts of global changes. Experiments were carried out at the Brazilian Antarctic Station "Comandante Ferraz" under controlled conditions. Specimens of *Bovallia gigantea* were collected in Admiralty Bay and acclimated to temperatures of 0°C; 2.5°C and 5°C and to salinities of 35, 30 and 25. Thirty measurements were taken for each of the nine possible combinations of the three temperatures and the three salinities. Metabolism rates were assessed based on oxygen consumption and total ammonia nitrogenous excretion in sealed respirometers. At 0°C and 2.5°C, the metabolic rates of the animals that were acclimated to salinities of 30 or 35 were similar, indicating a possible mechanism of metabolic independence of temperature. However, the metabolic rates were always higher at 5.0°C. The effects of temperature on oxygen consumption and on ammonia excretion rates were intensified by lower salinities. Individuals of *B. gigantea* have a temperature-independent metabolic rate within a narrow temperature window that can be modified in accordance with salinity.

**R E S U M O**

O orçamento energético de animais ectotérmicos antárticos estenotérmicos e/ou estenohalinos é fortemente modulado pelas variações da temperatura e da salinidade. O efeito conjunto desses fatores em animais polares é pouco estudado. Dados sobre esse assunto são necessários para avaliar a demanda energética de organismos antárticos, como os anfípodes, principalmente devido a sua importância ecológica e aos possíveis impactos ocasionados por mudanças globais. Os experimentos foram realizados na Estação Antártica Brasileira "Comandante Ferraz", em condições controladas. Indivíduos de *Bovallia gigantea* coletados na Baía do Almirante foram aclimatados às temperaturas de 0°C, 2,5°C e 5°C e às salinidades de 35, 30 e 25. Foram realizadas 30 medições para cada uma das nove combinações possíveis entre os fatores. Foram mensurados o consumo de oxigênio e a excreção de produtos nitrogenados, em câmaras respirométricas seladas. A 0°C e 2,5°C, as taxas metabólicas de indivíduos aclimatados às salinidades 30 ou 35 foram muito semelhantes, indicando um possível mecanismo de independência metabólica à temperatura. A 5,0°C, as taxas metabólicas foram sempre mais elevadas. Os efeitos da temperatura sobre as taxas de consumo de oxigênio e de excreção de produtos nitrogenados foram intensificados pela diminuição da salinidade. Indivíduos de *B. gigantea* apresentam independência térmica das taxas metabólicas em uma pequena janela de variação, que pode ainda ser modificada pela salinidade.

Descriptors: Antarctica, Amphipods, Metabolic adaptations, Temperature, Salinity, *Bovallia gigantea*.

INTRODUCTION

The marine Antarctic ecosystem is characterized by low temperatures, seasonal light cycles, seasonal phytoplankton productivity and the presence of sea ice. Water temperature is low but stable all year long and roughly varies between -2°C and +3°C. Salinity is also stable, generally between 34 and 35. In Admiralty Bay, where the present study was undertaken, summer temperatures range from -1.6°C to 5.3°C; salinity ranges from 16 to 34 for the first 35 meters of depth due to constant solar heating and ice melting (PRUSZAK, 1980; SZAFRANSKI; LIPSKI, 1982; JAZDZEWSKI et al., 1986). The summer pycnocline is mainly determined by salinity but can also be determined by both temperature and salinity in the inner parts of inlets (WEBER; MONTONE, 2006).

Marine polar organisms are adapted to an environment that is always cold, with a narrow range of temperature fluctuation. The stability of the sea water temperature is one of the main reasons for the large number of stenothermic species (CLARKE, 1991). Their influence on the physiology of Antarctic marine ectotherms has far-reaching applications for the understanding of their resistance to changes determined whether by natural causes or anthropic interference, which can have a profound impact on the ecosystem (FLORES et al., 2012). The determination of oxygen consumption and nitrogen waste as a function of environmental factors is extensively employed for this kind of study as these factors are directly related to the general metabolism of these organisms and, consequently, to their energy expenditure.

Amphipods are abundant in marine Antarctic waters. They occupy habitats of different characteristics and are essential components of coastal and open-sea food webs (NYSSSEN et al., 2005). Amphipods are the main components of the coastal fauna of Admiralty Bay on the Antarctic King George Island, where the Brazilian Station “Comandante Ferraz” is located (JAZDZEWSKI et al., 1991; SICINSKI et al., 2011). The amphipod Boavilia gigantea Pfeffer, 1888, is a circumpolar species that is very common in Admiralty Bay. They are considered giant amphipods as they can reach 54mm (DE BROYER; JAZDZEWSKI, 1993). They occur in shallow waters, from the sub-tidal zone to 40-90 meters depth, where they live close to algal beds or among pebbles that give them shelter and nourishment. In summer, their diet is composed of copepods, small amphipods and other crustaceans (70%), as well as diatoms and macroalgae (30%) (BONE, 1972). Their importance to coastal food webs is recognized as they are the main diet components for a large number of fish species that live in shallow waters (RICHARDSON, 1975; LINKOWSKI et al., 1983). They are suitable for experimental studies for the following reasons: they are easy to collect; they are resistant to captivity, transportation and handling; they are representative of amphipods living in well illuminated shallow water areas; they are exposed to greater environmental variability than deeper water species; data on the physiology, ecology and general biology of the species, including those obtained by our team, are already available in the literature.

The aim of the present study was to contribute to the understanding of the energy demand and adaptive ecophysiological mechanisms of B. gigantea in relation to temperature and salinity, abiotic factors of great importance in Antarctic marine environments.

MATERIAL AND METHODS

The amphipods were collected from Martel Inlet, Admiralty Bay, King George Island, Antarctica (62°5’S, 58°22’W) during the XXIII Brazilian expedition (2005). They were collected at approximately 50cm depth at low tide using hand nets. Small amphipods of other species and macroalgae were also collected to be offered as nourishment. Individuals that were moving spontaneously, with an intact body and appendages, were selected and maintained in 500 liter tanks for 10 days at ambient temperature, ranging from 1.0°C to 2.5°C. The water was artificially aerated and partially changed every day. The animals remained active, moving, capturing and eating prey during the entire maintenance period. No mortality was registered either during this period or during the experiments. After resting, each group of 30 amphipods was placed in three aquaria containing 30 liters of sea water (10 individuals per aquarium) and acclimated for 5 days to each combination of the experimental temperatures and salinities in rooms with controlled temperatures. The water temperature was adjusted over 24 hours using thermostat baths. The salinity was reduced from 35 by adding fresh water over 24 hours to reach a salinity of 30, and over 48 hours to reach a salinity of 25. Sea water was passed through a 1 µm pore filter. The acclimation and experimental temperatures used were 0°C, 2.5°C and 5.0°C and the salinities used 35, 30 and 25. The temperatures and salinities were selected taking into consideration the possible variations that could occur, including their limits. Adjustments were made to allow all the individuals to remain at the experimental temperature for 5 days and at the experimental salinity for 24 hours. Feeding was interrupted 48 hours before measurements. The combinations of temperature and salinities studied were as follows: 0°C–25; 0°C–30; 0°C–35; 2.5°C–25; 2.5°C–30; 2.5°C–35; 5°C–25; 5°C–30 and 5°C–35. A different group of thirty individuals was submitted to each one of those
established combinations in order to obtain 10 measurements at a time, with 3 replicates, resulting in a total of 30 sets of data for each situation and 270 overall. The mean weight of the individuals was 0.64mg (± 0.20mg).

Depending on the size of the animal, sealed respirometric chambers of 150 to 200ml were used for the measurements, as described by GOMES et al. (2013). In summary, each acclimated amphipod was placed inside one chamber for 3 hours with running water to reduce the effects of handling. Circulating water was then halted and the chambers were sealed for previously estimated periods, ranging from 3 to 6 hours. A particular time was established for each experimental condition according to the size of the animal in relation to the chamber volumes in such a way as to prevent dissolved oxygen dropping below 70% of saturation. The dissolved oxygen concentrations were determined by Winkler’s method, modified by FOX and WINGFIELD (1938) for small samples of water. The dissolved nitrogen concentrations were determined by the method developed by KOROLEFF (1970). Samples were taken immediately before sealing and immediately after opening the chambers. Differences between the samples multiplied by the chamber water volume, corrected for an hour, corresponded to consumption and excretion during this period. No animal was measured twice. At the end of the experiment, the animals were sacrificed and their body mass was measured with a precision of 0.001g.

Data were checked for normal distribution using the Shapiro-Wilk test and for variance homogeneity using Levene’s test. Two-way ANOVA, followed by Tukey’s test for multiple comparisons, were used (p˂0.05) to assess the differences between means.

The ratio between the oxygen consumed and nitrogen excreted (O: N) was calculated for each group of the 9 combinations of temperature and salinity, using the adjusted data (MAYZAUD; CONOVER, 1988). The O: N ratio gives an indication of the kind of substrate that was being used for catabolism under a given condition.

The Van’t-Hoff temperature coefficient (Q_{10}) was calculated as described by MUSATOV (1994), using the oxygen consumption data obtained for each level of salinity for the temperature intervals 0.0°C – 2.5°C and 2.5°C – 5.0°C. This coefficient was used to determine the amplitude of the oxygen consumption variations between different temperatures, at certain levels of salinity.

RESULTS

The mean values of oxygen consumption as a function of temperature, at different salinities, indicated that there were no significant differences between consumption at any salinity at 0.0°C and 2.5°C and that both of these were significantly lower when compared to consumption at 5.0°C (Fig. 1). Upon analysis of the mean values of oxygen consumption as a function of salinity at each temperature, it was noticeable that they were similar at 0.0°C and 2.5°C, although consumption at 5.0°C was significantly lower at salinity 35 than at 30 and 25 (Fig. 2).

Fig. 1. Mean (±SEM) oxygen consumption rate (µLO₂ g⁻¹ h⁻¹) of Bovallia gigantea as a function of temperature at different salinities. Different letters denote significant differences (p<0.05) at different temperatures.

Fig. 2. Mean (±SEM) oxygen consumption rate (µLO₂ g⁻¹ h⁻¹) of Bovallia gigantea as a function of salinity at different temperatures. Different letters denote significant differences (p<0.05) at different salinities.

At salinity 25, there were no significant differences between mean ammonia excretion at 0.0°C and 2.5°C, although both of the values were significantly lower than that for excretion at 5.0°C. At salinity 35, excretion was significantly different as a function of temperature: lower at 2.5°C; intermediate at 0°C and higher at 5°C (Fig. 3). The mean values of
ammonia excretion as a function of salinity were significantly different at every temperature studied (Fig. 4). At 0.0°C, excretion was lower at salinity 35, intermediate at salinity 25 and higher at salinity 30. At both the temperatures 2.5°C and 5.0°C, excretion decreased from the lower to the higher salinity.

![Fig. 3](image1.png)

Fig. 3. Mean (±SEM) ammonia excretion rate (µM-N g⁻¹ h⁻¹) of Bovallia gigantea as a function of temperature at different salinities. Different letters denote significant differences (p<0.05) at different temperatures.

![Fig. 4](image2.png)

Fig. 4. Mean (±SEM) ammonia excretion rate (µM-N g⁻¹ h⁻¹) of Bovallia gigantea as a function of salinity at different temperatures. Different letters denote significant differences (p<0.05) at different salinities.

O: N rates ranged from 10.52 to 32.21, which indicates a metabolism mainly supported by proteins for a mixture of protein and lipids (Table 1). The values were variable but there was a trend towards an increasing ratio with increasing salinity and higher values at 2.5°C.

The temperature coefficients Q₁₀ were lower for the temperature interval of 0.0°C – 2.5°C, ranging from 0.98 to 1.34, and higher for the interval of 2.5°C – 5.0°C, ranging from 3.04 to 10.66. Lower values always occurred at salinity 35 (Table 2).

Table 1. O:N rates obtained from the means of oxygen consumption and ammonia excretion of Bovallia gigantea, acclimated at 0.0°C, 2.5°C and 5.0°C and salinities of 25, 30 and 35.

<table>
<thead>
<tr>
<th>Salinity</th>
<th>Temperature °C</th>
<th>O:N ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>0.0</td>
<td>14.61</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>15.80</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>13.46</td>
</tr>
<tr>
<td>30</td>
<td>2.5</td>
<td>10.52</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>18.27</td>
</tr>
<tr>
<td>35</td>
<td>0.0</td>
<td>19.97</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>32.21</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>20.13</td>
</tr>
</tbody>
</table>

Table 2. Q₁₀ values for different intervals of temperature obtained from means of oxygen consumptions of Bovallia gigantea acclimated at 0.0°C, 2.5°C e 5.0°C and salinities of 25, 30 and 35.

<table>
<thead>
<tr>
<th>Salinity</th>
<th>Temperature range (°C)</th>
<th>Q₁₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>0.0 – 2.5</td>
<td>1.25</td>
</tr>
<tr>
<td></td>
<td>2.5 – 5.0</td>
<td>0.67</td>
</tr>
<tr>
<td>30</td>
<td>0.0 – 2.5</td>
<td>1.34</td>
</tr>
<tr>
<td></td>
<td>2.5 – 5.0</td>
<td>10.66</td>
</tr>
<tr>
<td>35</td>
<td>0.0 – 2.5</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>2.5 – 5.0</td>
<td>3.04</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The oxygen consumption and, consequently, the energy demand of B. gigantea, were always higher at 5°C than at other temperatures for all of the acclimation salinities. This result was already expected as temperature plays a key role in the regulation of the metabolic rate of ectotherms. Nevertheless, it is noticeable that the metabolism was always very similar at 0°C and 2.5°C, for all of the acclimation salinities, as is easily verifiable in Figure 1 and by examining the Q₁₀ values given in Table 2. The two lower temperatures studied are the most frequent in summer in the shallow coastal areas where B. gigantea lives. Temperatures higher than these are rare in the stable situation of Antarctic sea water. In an energy saving environment, it is highly probable that temperature-independent metabolic rates are very well adjusted to the common variation expected, regulating metabolism in such a way that energy demands remain stable and continuous, even within a narrow temperature window. From a physiological perspective, this seems to be a very comfortable trait of Antarctic marine ectotherms which may be due to the physical characteristics of the ecosystem.
On the other hand, a temperature increase of a similar range, from 2.5°C to 5°C, exerted an extra energy demand that was significant at all the salinities studied. The metabolic increase was much more evident at lower salinities (25 and 30) than at 35, as reflected in the Q10 values (Table 2). Animals from temperate and subtropical waters are frequently less sensitive to this range of temperature variation, since a broad range of temperature-independent metabolic rates usually adjusts the organisms to the temperature window of their habitats (STICKLE; BAYNE, 1987; CHEUNG; LAM, 1995; PÖRTNER, 2001; EZGETA-BALI et al., 2011). Although B. gigantea can cope with a temperature increase from 0°C to 2.5°C with no change in energy requirement, the effects of an extra increase to 5°C always result in a higher energy expenditure, as is clearly represented in Figure 1. In Figure 2, it is also noticeable that there is a synergistic effect of temperature and salinity in the fine adjustment of the metabolic rates of B. gigantea. At lower temperatures, salinity variations have less effect on metabolism. At 5°C, these effects are pronounced. It is clear that mechanisms of thermal regulation are highly effective between 0°C and 2.5°C, especially at salinity 35. Relatively small variations in temperature and salinity above the usual, even those that currently exist in Antarctic waters, as is the case of the maximum value tested herein (5°C), have a significant effect on physiological systems. Referring to fish, MITENBECK et al. (2012) called attention to the fact that as a consequence of the generally narrow thermal tolerance windows, sensitivity to combined stressors is enhanced among Antarctic ectothermic species.

Nitrogenous excretion is usually more variable and more difficult to interpret than oxygen consumption as it depends on a great many elements of the individual’s physiological condition. However, the data indicated that there is a trend to enhanced excretion at 5°C, more evident at salinity 25, as can be confirmed in Figure 3. In general, excretion decreases with increasing salinity, with the exception of 0°C, which exhibited exceptional data at salinity 30, due to the higher values recorded for certain individuals. These data were not discarded since there was no conceivable explanation for them. Ammonia excretion depends on the metabolic substrate that is being used according to energy demand and is also related to certain processes such as osmoregulation. At higher salinities, free amino acids are retained in tissues to maintain osmotic balance, reducing excretion as a consequence (FRICK; WRIGHT, 2002; ROCHA et al., 2005). At higher temperatures, an accelerated metabolization may result in a higher excretion rate even if the balance detected by the O:N ratio indicates variable amounts of protein and lipids being used as substrate. At higher salinities, the metabolism may be mainly dependent on proteins, thus increasing the O:N ratio. At lower salinities, the higher energy demand may cause a partial shift on the part of the metabolic substrate from almost pure protein to a mixture of proteins and lipids, reducing nitrogen excretion.

Similar features were also found for individuals of the Antarctic amphipods Gondogeneia antarctica (GOMES et al., 2013). They were even more sensitive to salinity variations, probably by virtue of their reduced body mass. Nevertheless, the highly stable temperature conditions of Antarctic waters and habitat similarities directed their metabolic adaptations to the same range of resistance. The metabolic rates of B. gigantea and G. antarctica (GOMES et al., 2013), both of which live in well illuminated shallow waters, are relatively higher than the same rates for other amphipods such as Waldeckia obesa, a scavenger of dark, deep waters (CHAPELLE; PECK, 1995; GOMES et al., 1995). At salinity 35 and 0°C, correcting for the effects of body mass using the method described by STEFFENSEN et al. (1994), the mean oxygen consumption rate of B. gigantea was 56 µlO₂/g/h, whereas the mean oxygen consumption rate of G. antarctica is 65 µlO₂/g/h and that of W. obesa is only 16 µlO₂/g/h. These results are highly congruent since B. gigantea are sit-and-wait large predators, whereas G. antarctica are much smaller and more active and W. obesa have thick exoskeletons and are usually not so active. In the past, it was considered that the metabolic rates of Antarctic ectotherms were relatively higher than expected when compared to species of lower latitudes using the Q10 index and other methods (SCHOLANDER et al., 1953). In spite of their importance as pioneers, a number of authors considered that methodological restrictions were responsible for these overstated results (CLARKE, 1991). Nowadays, however, new data indicate that certain variations may occur and are dependent on the life cycle strategies of the species rather than on low temperatures (PÖRTNER et al., 2006). Following this reasoning, metabolic rates may differ between species and be dependent, among other reasons, on the activity of the organisms. The magnitude of the regulatory mechanisms and the resistance to environmental changes can be similar across many species, leading one to suppose that they were established during the evolutionary process to fit the corresponding narrow ranges of sea water temperature variations, with very slight temperature-compensation ability (STROBEL et al., 2013). Nevertheless, significant differences are to be expected between them, at the genetic level too (MITENBECK et al., 2012; BAIRD; STARK, 2013).

Preliminary experiments made by our team indicate that B. gigantea can survive for a short period in temperatures as high as 10°C and salinity as low as 14. Long term resistance, however, is not known. In spite of these relatively broad ranges, after analysing a
series of available data, a number of authors are in agreement that mortality after longer exposures may not be caused directly by the kinetic effects of temperature on metabolic reactions. The same authors believe that the main causes of this mortality are due to the fact that the requirements of an increased metabolism are greater than the organism’s capacity to capture and distribute enough oxygen to produce the energy, since their systems are adapted to lower environmental demands (PÖRTNER et al., 2006).

The data obtained thus far are useful for the understanding of the adaptive, ecological and physiological aspects of B. gigantea and the implications of expected environmental changes, whether of natural or anthropological origins. Further studies are needed to complete the data available.

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