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Heat production rate of the Baltic amphipod *Gammarus oceanicus* at varying salinities

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Abstract

The heat production rate of *Gammarus oceanicus*, a species of marine origin living in the brackish waters (7 psu (practical salinity units)), was examined by direct calorimetry after acclimation to salinities of 5, 7, 14, 20 and 30 psu (at 10 °C). The mean heat dissipation was greatest at 5 psu with $5.64 \pm 1.60\,J\,h^{-1}\,g^{-1}$ ww (wet weight) and decreased significantly ($P < 0.05$) with increasing salinity down to $2.26 \pm 1.14\,J\,h^{-1}\,g^{-1}$ ww at 30 psu. The high inter-individual variability was of significance for the obtained results. When salinity dropped by only 2 psu from the control (7 psu) the mean heat production rate raised by 18%. A salinity increase from 7 to 30 psu caused a reduction in the average metabolic rate of *G. oceanicus* by 53%. That means that *G. oceanicus* requires more energy for osmotic adjustment at lower salinities.

Keywords: *Gammarus oceanicus*; Metabolism; Direct calorimetry; Salinity

1. Introduction

The Baltic Sea is one of the largest brackish water reservoirs in the world. The low average salinity, which is between 5 and 7 psu (practical salinity units [1]),[1] provides unique conditions for the development of flora and fauna. The fauna, which does occur in the Baltic Sea, is mainly composed of species with a wide range of salinity tolerance and includes freshwater organisms as well as marine species [2]. The amphipod *Gammarus oceanicus* is an example of species of marine origin living in brackish waters of the Baltic Sea. This crustacean accounts for about 20% of the total identified specimens of *Gammarus* Fabricius genus in the Gulf of Gdańsk [3]. *G. oceanicus* exhibited hyperosmotic regulation at low salinities [4]. Thus, this species requires additional energy for osmotic adjustment in a hyposaline environment. According to the literature, it is known that the energy costs of osmoregulation are low and do not exceed 2% of the total animal metabolism [5,6]. However, it is difficult to draw a general conclusion, because not all crustaceans respond in the same way [7] and most studies are based on respirometric measurements [8–10]. Respiration rates measurements do not provide reliable information regarding the energy costs of osmoregulation, because the anaerobic metabolism involved in intracellular osmoregulation is not detected [11,12]. The most appropriate method of metabolic measurements is through the heat produced by an organism [13–15].

Direct calorimetry was used in the present study to determine the metabolic response of the euryhaline amphipod *G. oceanicus* acclimated to various salinities. It allowed to answer the question how big the metabolic costs of osmotic adjustment is for this species in hypo- and hyper-osmotic media.

2. Experimental

Animals were collected in 2002 at a depth of 20 m with the research vessel 'Oceanograf 2' in the Gulf of Gdańst using a bottom dredge. *G. oceanicus* specimens were determined within the Gammaridae group according to characteristics given by Köhn and Gosselck [16]. Only adult males

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[1] The practical salinity scale (psu) connects an obtained salinity with a KCl solution of 32.4356 g in 1 kg solution at 15 °C that has 35 psu.

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of 15–24 mm size were chosen for experiments. They were placed in an aquarium filled with water of the same temperature (10 °C) and salinity (7 psu) as in the place of their collection. Empty shells of the blue mussel *Mytilus trossulus* were additionally placed in the aquarium to render natural conditions. Gammarids were fed twice a week with soft tissue of *M. trossulus*. Specimens were acclimated to each of five experimental salinities: 5, 7, 14, 20 and 30 psu (10 °C, oxygen saturation ca. 100%) in a step-wise way (2 psu per day) up or down from the initial salinity (7 psu). Experimental media were prepared by diluting commercial sea salt (hw-Meersalz, Wiegandt GmbH, Germany) with deionised tap water. Animals were kept at appropriate salinity for 1 week before measurements.

Heat production rates were determined using an isoperibol twin calorimeter (Bioflux, Thermanalyse, France) with vessels of 15 ml. The calibration factor of the calorimeter was 40.7 $\mu V\,m\,W^{-1}$. A single animal was transferred to a vessel containing 12 ml of filtered (0.45 μm pore size) and well oxygenated water and of a proper salinity (at 10 °C). The calorimeter signal was continuously monitored by a recorder (Linseis L6512, Germany) at a full scale sensitivity of 50 mV. Heat production measurements were carried out for 1–2 h starting after an equilibration period. The oxygen content of the medium was determined before and after the experiment with a needle microelectrode (PA2000, Unisense, Denmark). Then the length (millimetre) and wet weight (gram) of each individual were determined. For easier transformation in usual metabolic data in biology, heat production rates were expressed in joules per hour and gram wet weight ($J\,h^{-1}\,g^{-1}$ ww) and not in $mW\,g^{-1}$.

To compare data obtained at different experimental salinities a standardisation to 0.120 g wet weight was done using an equation given by Navarro and Gonzalez [17]:

$$Y_s = \left(\frac{W_s}{W_e}\right)^b Y_e$$

where Y_s is the heat production rate of an animal of standard weight, W_s is standard weight of the animal (0.120 g), W_e is observed weight of an animal, Y_e is uncorrected (measured) heat production rate and b is weight exponent for the heat production rate ($b = 0.68$ for *G. oceanicus*, unpublished data).

Linear ($y = ax + b$) and power regressions ($y = ax^b$), and correlation coefficients (R) were used to describe the relationship between the investigated parameters. The significance of the obtained differences was tested using the Mann–Whitney's U-test at a significance level of 5%.

3. Results

The power–time curves of *G. oceanicus* varied according to the level of activity exhibited by the animals (Fig. 1). Curves of type A, representing the heat production of resting animals, occurred sporadically. In most cases *G. oceanicus* showed locomotor activity during the measurements with peaks of different sharpness. In many cases it was not easy to distinguish between periods of rest and high locomotor activity. Thus, the total metabolic rate measured under the experimental conditions was the sum of both resting and active rates. Animals exhibited high locomotor activity at 7 psu that ranged from 8 to 40% higher than resting metabolism (on average 20 ± 9%) (Fig. 2). When particular activity peaks were analysed, we found that up to 71% of energy were used for locomotor activity.

A high inter-individual variability in heat production rate was observed. The standard metabolic rate of amphipods of the same wet weight differed significantly. Due to the high inter-individual variability as well as to the different levels of activity exhibited a wide range of results were obtained for *G. oceanicus* at a given salinity. The highest values varied from 2.04 to 8.34 $J\,h^{-1}\,g^{-1}$ ww, with an average of 4.77±1.79 $J\,h^{-1}\,g^{-1}$ ww at 7 psu (Fig. 3). On the other hand,

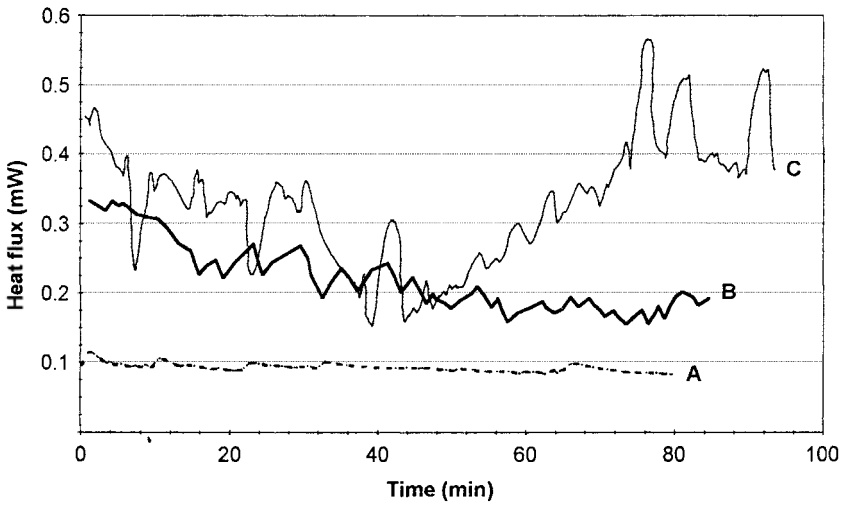

Fig. 1. Power–time curves of *G. oceanicus* exhibiting different levels of activities: (A) resting animal, (B) medium activity and (C) high activity.

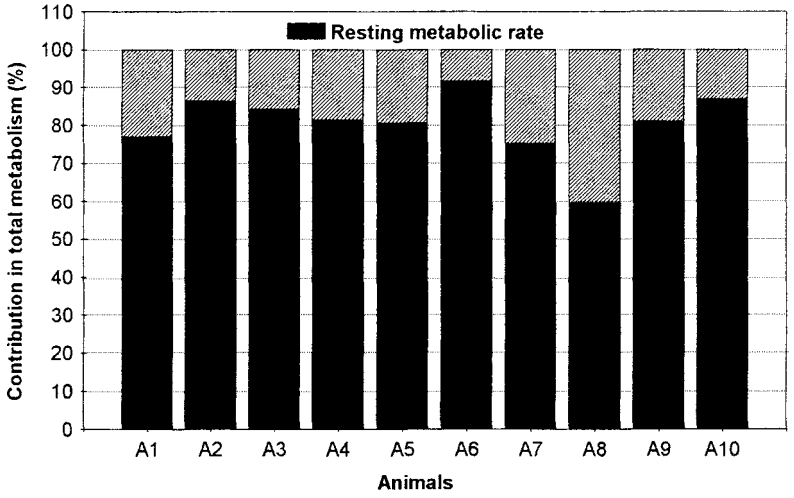

Fig. 2. Contribution of the resting metabolic rate (black bar) to the total metabolism (100%) of 10 animals of *G. oceanicus* at a salinity of 7 psu.

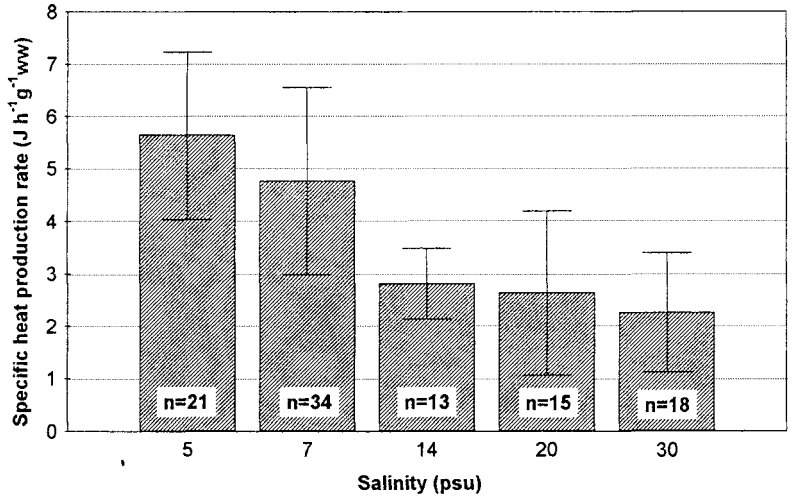

Fig. 3. Mean specific heat production rates (±S.D.) of *G. oceanicus* under different salinities. Numbers inside the bars indicate the number of experiments.

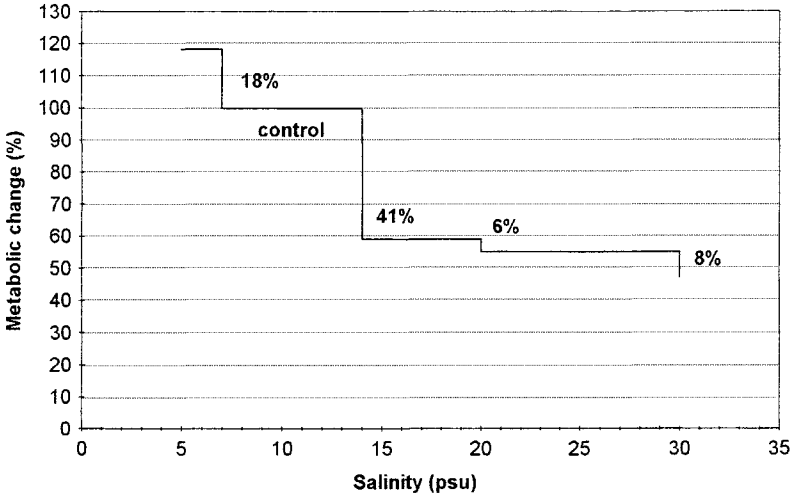

Fig. 4. Percent change of the heat production rate of *G. oceanicus* after exposure to different salinities. An initial 100% value refers to the mean heat production rate at the control (habitat) salinity of 7 psu.

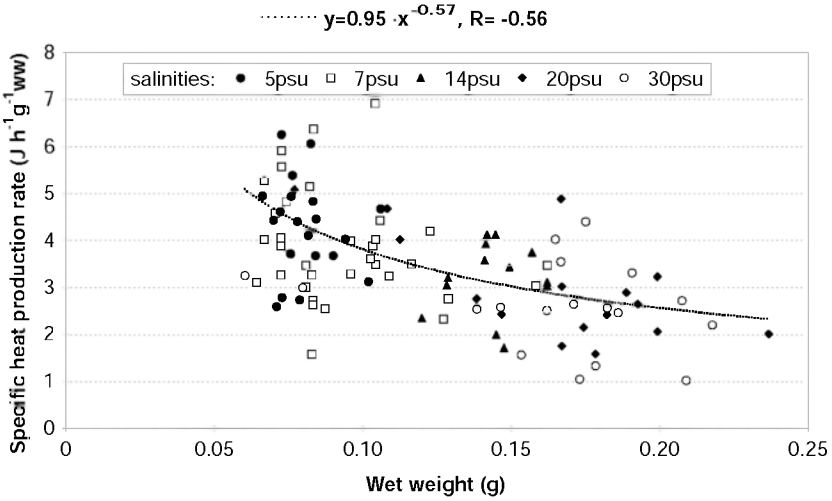

Fig. 5. Relation between the non-standardised mean heat production rate and the wet weight of *G. oceanicus* at different salinities. The solid line represents the mean heat production rate as a function of the wet weight in the range from 0.06 to 0.24 g.

the lowest values spread from 1.48 to 3.67 J h^{-1} g^{-1} ww with an average of 2.81 ± 0.68 J h^{-1} g^{-1} ww at 14 psu. The mean heat production rate (p) was largest at 5 psu (5.64 ± 1.60 J h^{-1} g^{-1} ww) and decreased significantly with increasing salinity (s) following the equation $p = 5.70 - 0.13\,s$ ($R = -0.61$, $P < 0.05$), reaching 2.26 ± 1.14 J h^{-1} g^{1} ww at 30 psu. Statistically significant differences ($P < 0.05$) in the specific heat production rate occurred between the lower (5 and 7 psu) and higher salinities (14, 20 and 30 psu). The mean heat production rate at the highest salinity was about 60% lower than that at 5 psu. When the salinity dropped only by 2 psu from the control value of 7 psu the mean heat production rate raised by already 18% (Fig. 4), while an increase in salinity from 7 to 30 psu caused a reduction of 53%.

Collecting all original, non-standardised values of the mean specific heat production rate as function of animal mass, we obtained a significant ($P < 0.05$) relation between the non-standardised specific heat production rate (p) and the wet weight (ww) of *G. oceanicus* that can be described by the power function $P = 0.95\,\mathrm{ww}^{-0.57}$ within the experimental range from 0.06 to 0.24 g (Fig. 5).

4. Discussion

Salinity is regarded as an important factor modifying the metabolic rate of aquatic invertebrates. At least four different types of metabolic responses are known in marine and brackish-water invertebrates [18]. *G. oceanicus* exhibited a form of response that is typical for a large group of euryhaline invertebrates: its metabolic rate increased in subnormal and decreased in supranormal salinities. This species is a hyper-regulator and maintains its haemolymph osmolality on a level higher than that of the environment. Active transport of Na^+ and Cl^- ions, which takes place against a concentration gradient, requires energy expenditure [19]. Brodie and Halcrow [4] reported that the haemolymph concentration of *G. oceanicus* reaches a new steady state in a relatively short time of about 4 h after the change in salinity. When the salinity decreased from 7 to 5 psu, the difference in osmolality between haemolymph and environment increased from 153 to 239% for *G. oceanicus* from the brackish waters of the Baltic Sea (unpublished data). This may explain the high increase in the metabolic rate between the two salinities. The energetic costs of osmotic adjustment of *G. oceanicus* was highest under these conditions in agreement with our observations of a high mortality of this species at salinities below 5 psu. At 30 psu, the haemolymph osmolality of *G. oceanicus* is almost isoosmotic to that of the environment (unpublished data), so that the amount of energy required for osmoregulation is minimal at these conditions. When juveniles of the lesser crab *Callinectes similis*, a strong regulator, were collected at a salinity of 29 psu and acclimated to 2.5 psu they showed a maximum respiration rate twice as high as under normal conditions [19]. The metabolic rate of *G. oceanicus* was 2.5 times higher at 5 psu than at 30 psu. The semiterrestric fiddler crab *Uca pugilator* is adapted to live in cyclic salinity conditions under tide influence at the Atlantic Coast and experiences increasing salinities due to evaporation and decreasing ones due to dilution by rain. In both directions the heat production rate grows up by more than 100% [20].

The significant reduction in the metabolic rate of *G. oceanicus* at higher salinities results from lower energetic costs for the osmotic adjustment, but it might be intensified by differences in the oxygen content of these waters. As oxygen solubility in water decreases with increasing salinity [21], its concentration at 30 psu is ca. 15% lower

compared to 5 psu. It is known that the oxygen content may considerably modify rates of both metabolism and animal activity [22]. In our studies we observed that the level of activity did not differ significantly in the range of experimental salinities. There was also no relation between the activity level and the duration of the experiment. High inter-individual variability in metabolic rates and in the level of locomotor activity obscured the effects of salinity on heat production rates. Thus, it is difficult to determine the energy metabolism capacity of *G. oceanicus*. Similar conclusions were drawn by Liu et al. [12] when studying the energy metabolism of the oyster drill *Thais haemastoma*.

The weight dependence of the non-standardised specific heat production rate presented in Fig. 5 exhibits an unexpected low exponent. It may result from the different activity states of animals during the calorimetric measurements. It was shown in the literature that higher activity levels tend to depress this exponent [23].

Kinne [18] wrote that many aquatic invertebrates respire at the most economic rate in those salinities to which they are genetically adjusted. Our studies are not in agreement with this statement, because the mean metabolic rate of *G. oceanicus* at the habitat salinity was among the highest rates. But we do not know if there are other physiological mechanisms, which allow to compensate for the high energetic costs required for osmotic regulation under low salinity. The data obtained by Kolding [24] showed that two Baltic populations of *G. oceanicus* living at different salinities are genetically adapted to their local habitats. For example, *G. oceanicus* from the Limfjord (Denmark) does not survive at salinities below 20 psu. Guerin and Stickle [10] found also that tolerance to salinity may differ between geographically separated populations of the same species.

It is difficult to compare the obtained values with data given in the literature because (i) investigated species were of different origin, (ii) exhibited different mechanisms of osmotic regulation and (iii) different methods (mostly respirometric) or (iv) experimental conditions were applied by each researcher. The total metabolic rate of *G. oceanicus* at habitat salinity was similar compared to an other brackish water species *Gammarus tigrinus*, which is characterised by $4.7 \pm 1.2\,J\,h^{-1}\,g^{-1}$ ww [25]. Heat dissipation of *G. tigrinus* increased by 72% after transfer to a K^+ pollution of $10\,mM\,l^{-1}$. Such a response is typical and results from the high energy cost of osmotic adjustment in the initial phase of a salinity stress.

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