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Direct calorimetric studies on the metabolic rate of *Gammarus oceanicus* from the brackish waters of the Baltic Sea[☆]

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Abstract

Heat production rates of the benthic amphipod *Gammarus oceanicus* from the brackish waters of the Gulf of Gdansk were measured by means of direct calorimetry at their usual ambient (“habitat”) salinity (7‰) and 10 °C. Animals exhibited locomotor activity during the measurements, so that the total metabolic rate was the sum of both resting and active metabolism. The mean specific metabolic rate amounted to 1.57 ± 0.61 mW g^{-1} wet weight (ww) (n = 73, average wet weight 67.2 ± 34.0 mg). Smallest animals with a mean wet weight of 24.5 ± 3.4 mg exhibited the highest specific metabolic rates with 2.24 ± 0.57 mW g^{-1} ww (n = 10), whereas the largest ones with wet weights of 117.7 ± 19.4 mg showed the lowest values with 1.10 ± 0.32 mW g^{-1} ww (n = 15). There was a significant negative correlation ($R^2 = 0.49$, $P < 0.05$) between the specific metabolic rate of *G. oceanicus* and its wet weight. The metabolic rate of females was higher by 29% ($P < 0.05$) than that of males of the same length due to the differences in wet weight.
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Keywords: *Gammarus oceanicus*; Metabolism; Direct calorimetry; Baltic Sea; Locomotor activities

1. Introduction

Physiological ecologists regard the metabolic rate as a significant element in the studies of energy flow in marine organisms and populations [1]. The total energy metabolised by an animal per unit time is also immensely useful in comparative studies of animal adaptation and performance [2]. Among many different methods of metabolic rate determination, direct calorimetry, based on heat production measurements, seems to be the most appropriate one [3]. It gives the net thermal effects of all exothermic and endothermic processes taking place in an organism [4,5] and allows for a determination of both aerobic and anaerobic metabolism [6–9]. Moreover, it shows the instantaneous heat flow in contrast – e.g. to manometry, that renders integral values about gas consumption or production in the past experimental period. Many benthic organisms exhibit great metabolic fluctuations, which might be caused for example by different physical activities. Strong locomotor activity may even force them to additionally apply anaerobic metabolism [10].

The metabolic rate of an organism depends on both intrinsic (size, sex, physiological stage) as well as on environmental factors (temperature, and – e.g. on salinity or dissolved gasses in aquatic environment). But the relationship between metabolic rate and body mass remains one of the most important and controversially discussed issues in comparative animal physiology [2,11].

The amphipod *Gammarus oceanicus*, a species that belongs to the *Gammarus* Fabricius genus, is wide-spread in all types of aquatic biotopes, ranging from the open sea to brackish waters of estuaries [12]. This benthic crustacean is abundant in the Polish coastal waters (Gulf of Gdansk), where

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it most frequently occurs in the presence of the blue mussel *Mytilus trossulus* [13]. *G. oceanicus* is omnivorous and itself is a food item for several fish species.

There are a number of studies on physiology of this species inhabiting different regions of the Baltic Sea [14,15]. However, only few information on the total metabolism of *G. oceanicus* and on the effects of body size and sex on it are found in the literature. But such quantities might provide more knowledge on physiological adjustments of this species to the brackish waters of the Baltic Sea. Therefore, our interest was to determine calorimetrically the different levels of activity, the mean heat production rates of this species in total and of female and male individuals separately together with their metabolic scope under the experimental conditions.

2. Experimental

G. oceanicus was collected in November 2002 in the Gulf of Gdansk (southern Baltic Sea, Poland) at a depth of 20 m using a bottom dredge. The wet weight and the body length of the animals varied between 14.2 and 160.2 mg and 6.5 and 20.0 mm, respectively. After the catch the specimens were determined within the Gammaridae group according to characteristics given in [16]. The amphipods were maintained in the laboratory for 1 week at their usual (“habitat”) environmental temperature ($T = 10\,^{\circ}\mathrm{C}$) and salinity ($S = 7‰$). They were fed with fresh soft tissue of the blue mussel *M. trossulus*.

Calorimetric studies were performed by means of two isoperibol twin calorimeters (Bioflux, Thermanalyse, France) with perpex and stainless steel vessels of 12 and 15 ml, respectively. The sensitivities amounted to 40.65 and 44.73 $\mu\mathrm{V\,mW^{-1}}$. Single animals were transferred to a vessel containing 10 or 12 ml of filtered (cellulose filter, 0.45 μm) water ($T = 10\,^{\circ}\mathrm{C}$, $S = 7‰$) well oxygenated by bubbling air for at least 30 min. Heat production measurements were carried out during 120 min after an equilibration time of about 90 min. Mean heat production rates were determined by electronic integration (DIGIKON, Kontron, Munich/Germany) of the area under the obtained power-time curve and division by the time of the experimental period (120 min). The resting metabolic rate (level with no locomotor activity) was defined as the mean value calculated for the areas under the smooth lines between activity peaks. The oxygen tension of the medium was monitored in the vessel directly before and after the experiment by a needle microelectrode (PA2000, Unisense, Denmark). In this way, it can be assumed that the obtained tension values correspond to those experienced by the animals during investigation. The oxygen tension dropped by about 25% during the experimental time. Sex and length of the specimens (from the rostrum to the end of the telson) were determined at the end of the experiment. Surface water was taken away from the animals by means of soft tissue papers and the sample wet weight was evaluated with a precision balance (SAUTER 414/13, Germany) to the nearest 0.1 mg. The specimens were dried at 55 °C for 48 h and weighed again. Specific metabolic rates were expressed in milliwatt per gram wet ($\mathrm{mW\,g^{-1}}$ ww) or dry weight ($\mathrm{mW\,g^{-1}}$ dw).

The significance of differences was tested using the Mann–Whitney*U*-test at a probability level of 5%.

3. Results

The metabolic rates of the animals were affected by levels of rest and activity. In most cases *G. oceanicus* exhibited strong locomotor activity seen as fluctuations in the calorimetric signal (Fig. 1). Therefore, the results presented for the metabolic rate of animals include both resting and active behaviour. The maximal energetic cost of locomotor activity of *G. oceanicus* amounted to around 60% of the total metabolic rate.

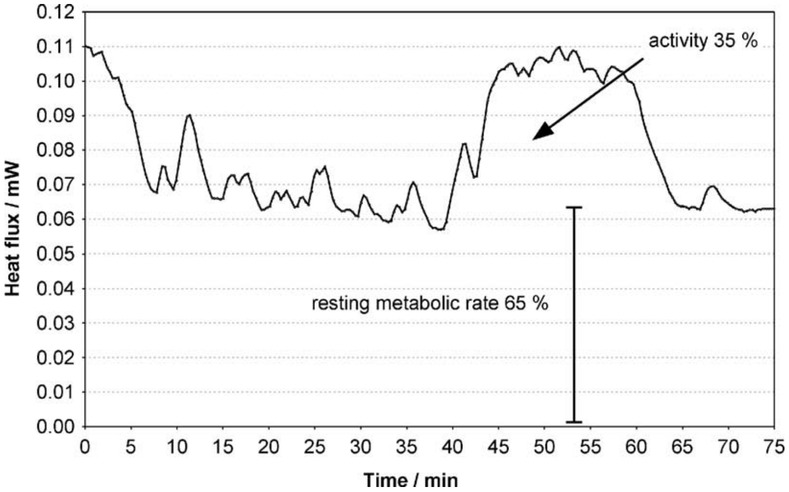

Fig. 1. Power-time curve of a *G. oceanicus* female of 49.2 mg wet weight at 10 °C and without substratum showing different levels of locomotor activity. The registration started after a thermal equilibration time of about 90 min. The indicated percentages give the partition of energy between resting metabolism and activity during the registration period.

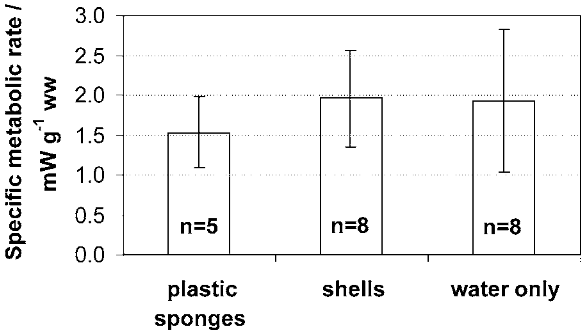

Fig. 2. Mean specific metabolic rate (± S.D.) of *G. oceanicus* specimens kept in vessels with plastic sponges, blue-mussel shells or in vessels with water only (n = number of repetitions).

Bearing in mind that this species lives among blue-mussels, often even attached to their shell surface in the natural environment, we decided to perform additional experiments to reduce animal activity. In order to attain our aim, we placed pieces of a blue mussel shell or a woven plastic kitchen sponge into the measuring vessel to allow animals to attach to them. The lowest heat production rate was observed among specimens kept in vessels with a plastic sponge: 1.53 ± 0.45 mW g^{-1} ww (n = 5), the highest one in vessels with a blue mussel shell: 1.96 ± 0.61 mW g^{-1} ww (n = 8) (Fig. 2). The largest range occurred in vessels filled with water only: 1.93 ± 0.89 mW g^{-1} ww (n = 8). Since there were no statistically significant differences ($P > 0.05$) between these alternative designs further experiments were performed in vessels without substratum.

The mean specific metabolic rate of *G. oceanicus* amounted to 1.57 ± 0.61 mW g^{-1} ww (n = 73, average wet weight 67.2 ± 34.0 mg). The specific metabolic rate of females (n = 11) with an average length of 14.45 ± 0.82 mm (average wet weight 47.2 ± 13.2 mg) varied between 1.29 and 2.29 mW g^{-1} ww (average 1.72 ± 0.34 mW g^{-1} ww), whereas that of males (n = 11) with an average length of 14.73 ± 0.65 mm (average wet weight 65.3 ± 9.7 mg) changed from 1.06 to 1.94 mW g^{-1} ww (average 1.34 ± 0.26 mW g^{-1} ww). The differences were statistically significant ($P < 0.05$) (Fig. 3). Length and mass of *G. oceanicus* significantly affect heat production. Mass specific metabolic rates were higher in smaller than in larger individuals (Fig. 4). This relationship between the specific metabolic rate (SMR) and the wet weight (WW) may be described within the experimental mass range from 0.0142 to 0.1602 g by a power function SMR = a ww^{b-1} as introduced by Kleiber long time ago [11]. The slope a represents a constant with the dimension mW g^{-1} and b a dimensionless broken exponent between 0 and 1. In the present case, the power function reads SMR = 0.40 $ww^{-0.45}$ ($R^2 = 0.49$, $P < 0.05$) in agreement with Kleiber's prediction.

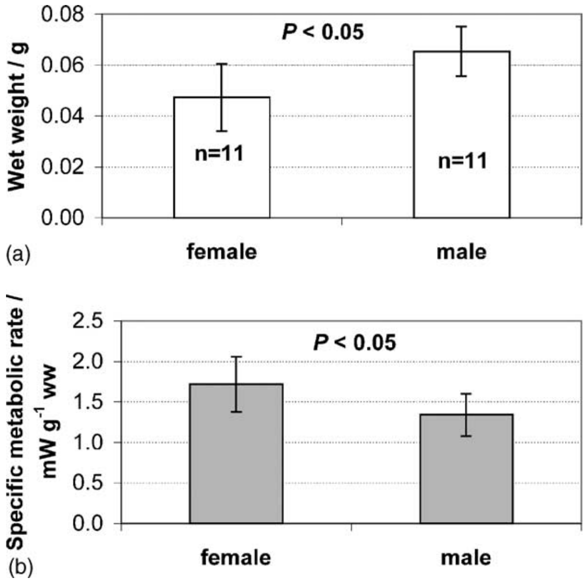

Fig. 3. Comparison of the wet weights (a) and the specific metabolic rates (b) for females and males of *G. oceanicus* at 10 °C (mean ± S.D., n = number of repetitions).

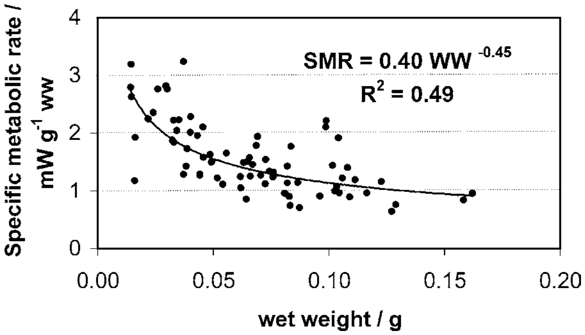

Fig. 4. Power function for the relationship between the specific metabolic rate (SMR) and the wet weight (ww) of *G. oceanicus* for the points given in the graph (n = 73).

4. Discussion

The majority of studies on metabolic rate of aquatic invertebrates, determined both by oxygen consumption and by heat production measurements, has been carried out in the absence of a living substratum like blue mussels [10]. This was done as a precaution due to the fact that other respiring organisms (e.g. microbes attached to the substratum) may create large errors. On the other hand, the lack of a substratum provides an additional stress to the studied animals, that may significantly affect the obtained results. For example, the burrowing polychaete *Neanthes virens* showed large random, and apparently abnormal, fluctuations of activity when kept only in water, in contrast to a slightly rhythmic steady average activity in the presence of sediments [10]. Similar findings were made for the benthic isopod *Saduria entomon* [8].

Our observations on *G. oceanicus* under laboratory conditions showed that this species exhibits a rather low degree of locomotor activity although it belongs to the mobile fauna.

G. oceanicus is used to living attached to natural (shells of *M. trossulus*) or artificial (woven plastic kitchen sponges) substrata. Both types of substratum were placed in the aquaria, where animals were kept before the heat production measurements. The idea to apply the woven plastic kitchen sponges, used by the gammarids for shelter was taken from [17]. On the basis of obtained power time curves we can conclude that the animals were in a stressful state during the measurements. *G. oceanicus* exhibited moderate to high degrees of locomotor activity during nearly the whole measuring period. The time spent actively was much longer than that without movement. Even placing both natural or artificial substrata inside the calorimetric vessel did not reduce this state. This increase in locomotor activity of *G. oceanicus* was probably an attempt to escape, a first reaction to unfavourable conditions observed in many invertebrates and vertebrates [18,19]. It was possible to differentiate between active and resting metabolic rates in some cases and to evaluate the ratio between them. The highest value was 2.4:1, the lowest one 1.5:1. They are in agreement with data given by Opalinski [20] for another amphipod, *Paramoera walkeri*, where swimming and resting specimens respired 3.02 and 1.88 $mm^3\ O_2\ h^{-1}$ per animal (1.6:1), respectively. According to [2,21] the ratio between maximum and basal metabolic rate in invertebrates varies in a range between 2 and 10.

The duration of measurements may also play an important role for the obtained results. The small volume of the measuring vessel as well as the decreasing oxygen tension inside make long-term measurements impossible. It is known from literature that the respiration values of *G. oceanicus* obtained from short-term measurements are higher by 22% than those obtained during long-term ones [22].

A power-function dependence of metabolic rates on body size has been reported by many researches for various crustaceans [23–26]. It mostly concerned resting or immobile animals. The low value of the exponent in the power function ($b = 0.55$ or $b - 1 = -0.45$ for the relation between the metabolic rate or weight specific metabolic rate and the weight itself) obtained in the present studies may result from the different activity states of animals during the calorimetric measurements. It was shown that higher activity levels tend to depress the exponent b in the case of two decapods, *Panulirus interruptus* and *Hommarus gammarus*, where the values of the allometric exponent b were 0.49 and 0.48, respectively. In another Baltic crustacean, *Idotea balthica*, the value $b = 0.68$ was closer to the expected one ($0.67 < b < 1$) when the activity effects due to locomotion were excluded by the small size of the respiration chamber and the presence of a substratum for the animals to attach [27]. An especially low value ($b = 0.3$) was observed by [28] for the bivalve *Pisidium amnicum* suggesting a strong reduced heat output in larger animals. However, that situation was explained by more frequent parasitic infections of larger animals. Previous studies showed that the great variety of coefficient b occurred in many groups of animals, including Crustacea [29].

It was suggested [30] that the power-function dependence of metabolic rates on body size may be different in juvenile and adult specimens. In small animals of low mass the allometric exponent approaches $b = 1$, whereas body size has minimal effects on metabolic rates in the largest individuals in the post-reproductional stage, and b equals 0.4 or even less. In the weight range between 0.0142 and 0.0492 g the relationship between the specific metabolic rate (SMR) and the wet weight (ww) for *G. oceanicus* is described by the power function $\mathrm{SMR} = 0.70\,\mathrm{ww}^{-0.30}$ in agreement with the above hypothesis. The ocurence of very low metabolic rates of the largest specimens of *G. oceanicus* might reflect considerable changes, which do not completely depend on the mass, but rather are due to the age of the organism [29]. Physiological degradation of body tissues as well as of particular organs of post-reproductive adults were observed in some invertebrates [31]. An another explanation and point in this direction might be that old individuals of *G. oceanicus*, a species of a short life span of about 1 year and only one breeding period, may grow less intensively than younger ones. Low metabolic rates of biggest animals might also support the hypotesis that adult specimens are more resistant to unfavourable environmental conditions than are younger ones [19,32,33].

On the basis of the allometric relation for several Baltic crustaceans we can conclude that *G. oceanicus* has a low specific metabolic rate [29]. Compared to other gammarids from the Baltic Sea this species occurs at the greatest depths [34], where the temperature and salinity fluctuations are not as high as in the shallow waters. *G. oceanicus* is also resistant to oxygen deficiency and hydrogen sulphide [35] as well as to diesel oli and heavy metals [12]. The low energy demand of this species is probably one of the features which may help *G. oceanicus* to withstand unfavourable conditions.

The statistically significant differences in the specific metabolic rates between males and females of *G. oceanicus* of the same lengths are probably caused by differences in the wet weights. Females of the same length as males are characterised by a significantly lower mass probably due to the discrepancy in the water content. Females contain 73% of water and males 76%. The higher metabolic rate would probably occur in females during the reproduction period, when energy reserves have to be accumulated in form of egg yolk for the next generation. Since we had only females in a non-reproductive stage it was impossible to draw any certain conclusions in this direction. The isopod *I. balthica* as well as the Antarctic krill *Euphasia superba* show no differences in metabolic rates between sexes [27,36].

In Table 1 we compare mean specific rates of aerobic metabolism in different invertebrates measured under habitat conditions. Tedengren et al. [37] found that the respiration rates of *G. oceanicus* were the lowest at habitat salinities. Most of these data were achieved using indirect calorimetric methods (respirometry). Using conversion factors given by Gnaiger and Forstner [38], the values were recalculated to energy units, to facilitate the comparison. But it has to be kept in mind that it is difficult to compare these data, mostly

Table 1
Comparison of specific metabolic rates (mean ± S.D.) of different invertebrates determined on the basis of the heat production (hp) or oxygen uptake (r) and recalculated using conversion factors of 20.08 J cm^{-3} O_2 and 450 kJ mol^{-1} O_2 given by Gnaiger and Forstner [38]

Species	Specific metabolic rate		Reference
	mW g^{-1} ww	mW g^{-1} dw	
Bivalves			
Astarte borealis	–	5.86 ± 0.31 (hp)	[41]
Arctica islandica	–	4.97 ± 1.69 (hp)	[41]
Worms			
Nephtys hombergii	–	1.84 (r)	[1]
Halicryptus spinulosus	–	0.48 (r)	[42]
Crustaceans			
G. oceanicus			
(6.5‰) Baltic Sea	–	5.74 ± 0.27 (r)	[37]
(7‰) Baltic Sea	1.57 ± 0.61 (hp)	6.17 ± 2.73 (hp) 3.81 (r)	Present study
(15‰) Baltic Sea	–	6.17 ± 0.08 (r)	[22]
(24‰) North Sea	–	2.37 (r)	[37]
(32‰) North Sea	–	5.74 ± 0.27 (r)	[15]
G. oceanicus (6.5‰) Baltic Sea	–	6.17 ± 2.73 (hp) 3.81 (r)	[37]
Gammarus tigrinus	1.31 ± 0.33 (hp)	–	[39]
Corophium volutator	–	5.19–13.84 (r)	[43]
Idotea chelipes	–	3.34–4.41 (r)	[43]
Saduria entomon	1.39–2.79 (r)	–	[44]

because of methodological differences between the various approaches. Mean specific metabolic rates of *G. oceanicus* calculated per unit of wet weight did not differ significantly from those of another brackish water amphipod, *Gammarus tigrinus*, and of the isopod *S. entomon* [8,39]. The mean specific metabolic rate calculated per unit of dry weight in our study for *G. oceanicus* at 7‰ salinity is slightly higher than those of the same species inhabiting different salinities. These differences might be related to the fact that our data concern the total, that means resting plus active metabolism. It is also known from previous studies that an increase of environmental salinity depresses the metabolic rate of *G. oceanicus* [40]. This may help to explain the lower values obtained by [15] and [22] for populations living at salinities of 15 and 32‰, respectively. As *G. oceanicus* belongs to the fauna of marine origin and inhabits brackish water of the Baltic Sea it lives under a permanent osmotic stress. Thus, it has to spend additional energy for active hyper-regulation of body fluids (maintaining the haemolymph osmotic concentration on a higher level than that of the external environment). It was reported, that different populations of *G. oceanicus* occurring in the Baltic Sea show habitat specialisation, which is believed to be the result of local adaptation to salinity [15]. Therefore, differences in metabolic rates between various populations of *G. oceanicus* may occur eventually.

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