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Adaptation of *Gammarus tigrinus* Sexton, 1939 to new environments—Some metabolic investigations

Monika Normant [a,*], Martin Feike [b], Anna Szaniawska [a], Gerhard Graf [b]

[a] *Department of Experimental Ecology of Marine Organisms, Institute of Oceanography, University of Gdansk, Al. Marszalka J. Pilsudskiego 46, 81-378 Gdynia, Poland*
[b] *Institute for Biosciences, University of Rostock, Albert-Einstein-Straße 3, D-18051 Rostock, Germany*

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Abstract

The heat dissipation rates of the invasive amphipod *Gammarus tigrinus* Sexton, 1939 of the southern Baltic Sea were measured by means of direct calorimetry at the habitat salinity of 7‰ (males and females) and after gradual acclimation (only males) to lower (3‰) and higher (13‰ and 19.5‰) values ($T = 18\,^{\circ}C$). The mean specific metabolic rate at 7‰ amounted to $1.67 \pm 0.86\,mW\,g^{-1}$ ww ($n = 25$, average wet weight 10.9 ± 5.1 mg). Due to the sexual dimorphism and the smaller size of the females their specific metabolic rates were two-fold higher than that of males. Animals exposed to lower than habitat salinity insignificantly increased their specific metabolic rate by 42.5%. A reduction of 37.5% was observed when they were subjected to the highest examined salinity (19.5‰). *G. tigrinus* was thus able to change its metabolic rate by 56% in the studied salinity range from 3‰ to 19.5‰

Keywords: *Gammarus tigrinus*; Metabolism; Salinity; Direct calorimetry; Isothermal calorimetry (ITC); Baltic Sea

1. Introduction

During the last few decades many new species of flora and fauna appeared in the Baltic Sea [1,2]. One of them is the amphipod *Gammarus tigrinus* Sexton, 1939, a species, which originates from the Atlantic coast of North America [3]. It appeared for the first time in Europe around 1918 and since then its area of occurrence extended progressively [4–8]. In 1988, *G. tigrinus* arrived at Poland, where it inhabited mostly fresh- and oligohaline (0.5–5‰) rivers and estuaries [9–11]. Several years later, in 2001, *G. tigrinus* was also found in β-mesohaline (5–10‰) coastal waters of the Gulf of Gdansk, where it is abundant nowadays [12,13].

The high colonization success of *G. tigrinus* observed in different types of habitats results from its wide tolerance of biotic and abiotic factors, e.g. salinity [14,15]. In general, variations in salinity are manifested in changes of different physiological processes of organisms [16]. A good indicator of an animal's adaptation and performance is its total metabolism, which includes all chemical reactions and energy transformations taking place in the cells of living organisms [17]. Among many different methods of metabolic rate determination, direct calorimetry, based on heat dissipation measurements, seems to be the most appropriate one. It gives the net thermal effects of all exothermic and endothermic processes and allows determining of both aerobic and anaerobic metabolism [18,19].

Although the literature concerning *G. tigrinus* deals mainly with its spread to European waters as well as with ecology generally, the data concerning the physiology of the species are considerably less numerous [e.g. 20–28]. Thus, we have performed direct calorimetric studies on the metabolic rate of *G. tigrinus* from the coastal Baltic waters with regard to body size, sex and salinity. The obtained results provide new information on the physiology of the species and, in comparison with the maintenance energy cost of other native or non-native crustaceans, might be used to explain its competitive success in various aquatic biotopes.

2. Experimental

2.1. Animals

Specimens were collected with a hand net in August 2004 in the coastal zone of the Gulf of Gdansk (southern Baltic Sea).

* Corresponding author. Tel.: +48 58 5236868; fax: +48 58 5236678.
E-mail address: ocemn@univ.gda.pl (M. Normant).

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Salinity and temperature in the collection site were 7‰ and 18 °C, respectively. Macroalgae of the genera *Enteromorpha* and *Cladophora*, used as food for the caught amphipods, were sampled at the same time. In the laboratory, the sex of each gammarid was determined according to ref. [29]. Moreover, females with eggs in brood pouches were separated from the non-ovigerous females. Next, the surface water was blotted off the animal with soft tissue paper and the wet weight was determined (±0.01 mg, Sartorius M2P, Germany). Before experiments animals were held in the laboratory for a week at the temperature and salinity of their natural habitat.

To study the effects of size and sex on the metabolic rate 25 specimens of different mass (4.22–22.1 mg) and sex (11 males and 14 females: 8 non-ovigerous and 6 ovigerous) were used (experiment 1), whereas the salinity effect was investigated on 32 adult males (n = 7–10 per salinity) of 9.73–22.1 mg (experiment 2).

2.2. Experimental protocol

Salinity and temperature applied in experiment 1 were 7‰ and 18 °C, respectively. In experiment 2, adult males were acclimated to each of three experimental salinities: 3‰, 13‰ and 19.5‰ (T = 18 °C, full air saturation). Acclimation was conducted in a step-wise manner (2‰ per day) up or down from the habitat salinity of 7‰. Experimental media were prepared by diluting commercial sea salt (hw-Meersalz, Wiegandt GmbH, Germany) with deionised tap water. Before heat dissipation measurements, animals were kept at the appropriate salinity for 5 days to reach their new steady-state. Well-aerated and filtered water (cellulose filter, 0.45 μm) of the proper salinity was used as experimental medium.

2.3. Direct calorimetry

Heat dissipation was measured in a differential LKB 10700-2 batch calorimeter (Bromma, Sweden) described by ref. [30] and modified by refs. [31,32]. The instrument was equipped with two identical vessels (measuring and reference), each of 5 ml volume. Measurements were performed on single animals placed in the vessel with 4 ml of experimental medium. Before and after the measurements, the base line was registered (i.e. the thermal signal of the experimental medium only). The sensitivity of the calorimeter amounted to 69.9 μV mW^{-1}. The calorimetric signal was continuously registered (every 12 s) by a nanovoltmeter (Agilent 34420A, Agilent Technologies, U.S.A.) and stored on a commercial IBM PC computer operated by MS Windows 95. Heat dissipation measurements were conducted over a period of 90 min after an equilibration time of around 60 min. The oxygen tension in the medium was determined before and after each measurement with a needle microelectrode (PA 2000, Unisense, Denmark). The oxygen tension dropped by 10–31% depending on the animal's size and activity level.

The specific metabolic rates (SMR) of rest (level between two peaks) and activity (maximal peak) of a single animal expressed in milliWatt per gram wet weight (mW g^{-1} ww) were calculated in Microsoft Office Excel 2003 following [33].

2.4. Statistics

Linear ($y = ax + b$) and power regressions ($y = ax^b$) were used to describe the relationships between size or salinity (x) and the metabolic rate (y) at a significance level of $P < 0.05$. The significance of the obtained differences was tested with the Mann–Whitney U-test at a level of $P < 0.05$. "Significant" in the text means significant at the 5%-level.

3. Results

The mortality of *G. tigrinus* was very low in all examined salinities. The animals exhibited their usual behaviour in the laboratory consisting of periods of rest or moderate activity randomly interrupted by spontaneous movements. On the basis of the power–time curves' pattern, similar activities were assumed to occur inside the calorimetric vessel. It was easy to distinguish between periods of rest and such of locomotor activity seen as abrupt peaks in most of the power–time curves. Fig. 1 shows that both females were active during the whole experimental period. The larger female (no. 1) moved more frequently inside the calorimetric vessel, but its maximum activity was less pronounced and shorter than that of the smaller female (no. 2). In general, both, larger and smaller studied individuals moved with similar frequency and intensity inside the vessel. The ratios between maximum recorded metabolic rate and the resting level were similar for female nos. 1 and 2 and amounted to 2.0:1 and 1.9:1, respectively. Although, the highest recorded ratio between active and resting metabolic rates for all studied specimens (n = 25) at 7‰ was 2.9:1, the mean value was distinctly lower and amounted to (1.6 ± 0.4):1 only.

The specific metabolic rate (SMR) of *G. tigrinus* at 7‰ varied in a broad range from 0.58 to 3.39 mW g^{-1} ww (mean 1.61 ± 0.86 mW g^{-1} ww) and was significantly related to the specimen's wet weight (M). The relationship can be described within the experimental mass range (4.22–22.1 mg) by the power function $SMR = 0.034M^{-0.81}$ (R^2 = 0.58, n = 25)

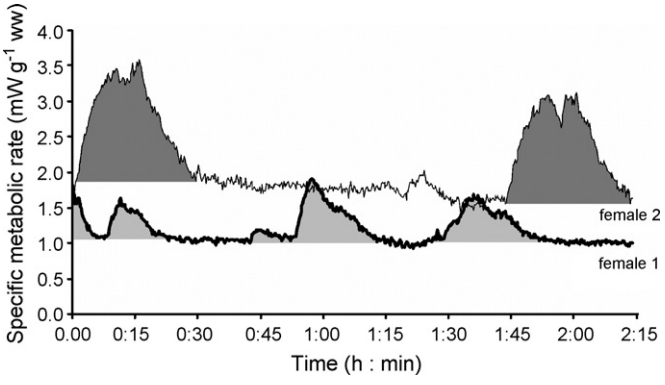

Fig. 1. Typical power–time curves for *G. tigrinus* females at a salinity of 7‰: (1) 10.9 mg and (2) 5.68 mg. Shaded areas represent activity peaks. The curves were taken at different days.

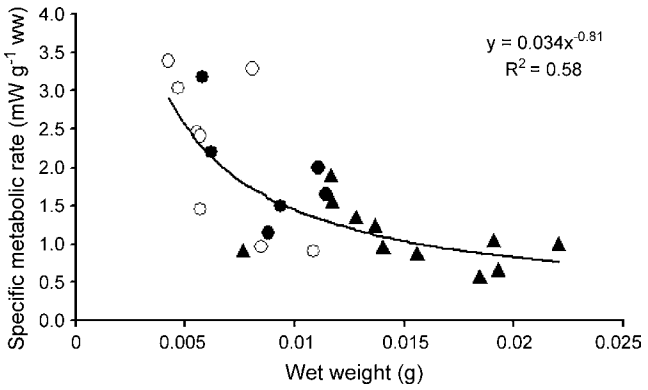

Fig. 2. Relationship between metabolic rate and wet weight of *G. tigrinus* ($n = 25$). Open circles indicate values for non-ovigerous females, black circles for females with eggs in brood pouches and triangles for males.

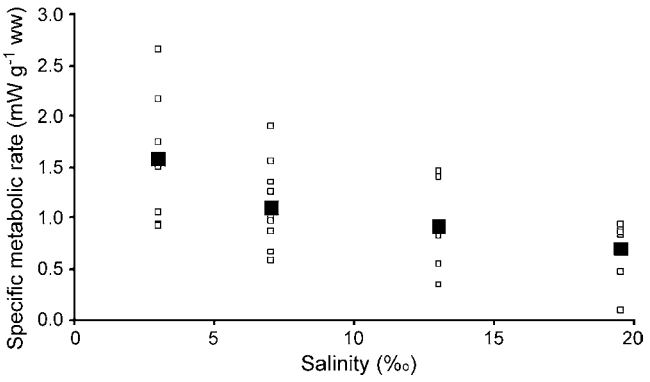

Fig. 4. Metabolic rates (open squares) of *G. tigrinus* males at different salinities. Black squares indicate mean values.

(Fig. 2). The specific metabolic rates of females ($n = 14$) of a mean wet weight of 7.55 ± 2.48 mg were significantly higher than those of males ($n = 11$, mean wet weight 14.5 ± 4.56 mg). The values varied between 0.91 and 3.39 mW g^{-1} ww (mean 2.11 ± 0.88 mW g^{-1} ww) for females and 0.58 and 1.9 mW g^{-1} ww (mean 1.10 ± 0.39 mW g^{-1} ww) for males. This large difference results from both, a larger absolute heat production rate and a distinctly smaller mass. Among the studied females there were also specimens brooding a clutch of eggs ($n = 6$). Although, they had significantly higher weights (5.76–11.4 mg) than non-ovigerous females their metabolic rates were similar reaching means of 2.08 ± 1.00 and 1.94 ± 0.72 mW g^{-1} ww, respectively.

Salinity affected the ratio between the active and resting metabolic rates in *G. tigrinus* significantly. At the lowest salinity of 3‰, the mean value was (1.5 ± 0.2):1, whereas it amounted to (2.4 ± 1.1):1 at the highest salinity (19.5‰; Fig. 3). A salinity decrease from 7‰ to 3‰ caused a non-significant increase of the specific metabolic rate from 1.11 ± 0.42 to 1.58 ± 0.67 mW g^{-1} ww (Fig. 4). On the other hand, a salinity rise up to 19.5‰ was manifested by a significant metabolic rate reduction to 0.69 ± 0.31 mW g^{-1} ww. Thus, *G. tigrinus* was able to reduce its metabolic rate significantly by 56% in the salinity range 3–19.5‰.

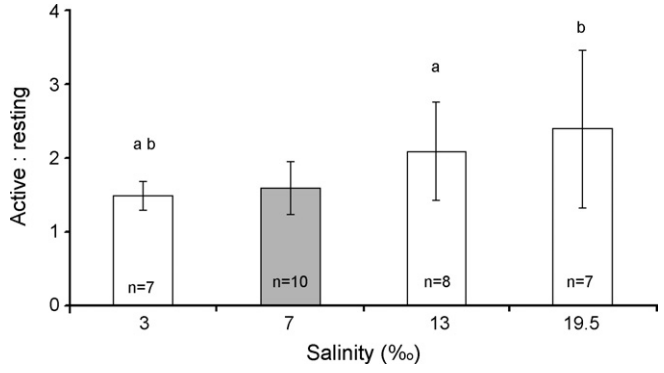

Fig. 3. Ratio between active and resting metabolic rates (mean ± S.D.) in *G. tigrinus* males exposed to different salinities. The grey bar represents the habitat salinity; numbers inside bars indicate the number of specimens and letters above bars statistically significant ($P < 0.05$) differences between salinities.

4. Discussion

The mean specific metabolic rate of *G. tigrinus* at 7‰ was similar to that published for *Gammarus oceanicus* at $T = 10\,^{\circ}C$ (1.57 ± 0.61 mW g^{-1} ww) [34] and lower than that of *Idotea chelipes* at $T = 18\,^{\circ}C$ (2.08 ± 0.94 mW g^{-1} ww) [33]. *G. tigrinus* from the Gulf of Gdansk is characterized by slightly higher metabolic rates than specimens from the German rivers Werra and Weser, where the average value was 1.31 ± 0.33 mW g^{-1} ww ($n = 16$) [24]. The differences might be explained by other experimental conditions (body size, salinity and temperature) applied by the authors as well as by differences (e.g. in morphology, biochemistry or physiology) between the populations [17,35–37].

The specific metabolic rate of *G. tigrinus* varied with body mass. Values were almost six times higher for the smallest specimens than those of the largest individuals. Exponent $b = 0.19$ in the power function is much lower than the value 0.67 observed in many metazoan [38]. Recent findings also show that multiple factors potentially affect metabolic scaling causing that a variety of other scaling relationships have been also observed, although 3/4-power scaling appears to be common [39]. The low exponent values in this study might be explained by high inter-individual variability in heat dissipation rates found in the specimens of lower weights (Fig. 2). This group consisted mostly of females, which can represent different physiological stages with regard to reproduction. In the present studies, only non-ovigerous and ovigerous females were separated. One of the high colonization successes of *G. tigrinus* is the fact that a single female can give several broods in one reproduction season [40]. It means that at the same time when embryos develop in their marsupium (it takes around 1 month till hatching) new eggs are already produced in the ovaries so that a further energetic effort is necessary in these females. Despite no visible "pregnancy" symptoms it might be that some of the studied females were in such a "double" state with an increased metabolic rate. Moreover, it might be important for the total metabolic rate of females, that Gammarid amphipods brood their eggs using lipid stores [41].

The metabolic rates of *G. tigrinus* males and females differed significantly, supposedly caused by differences in wet weight due to a sexual dimorphism, which is well known

among crustaceans [19,34]. *G. tigrinus* males become larger (max 12.5 mm) than females (max 10.0 mm) [4]. But the small numbers of males and females of similar weight did not allow us to draw reliable conclusions from the present experiment.

The maximum energetic cost of locomotor activity of *G. tigrinus* at 7‰ salinity amounted to 66% of the total metabolic rate and was similar to values recorded for *G. oceanicus* [34]. As many other gammarids, *G. tigrinus* lives attached to different substrata. In the laboratory it was sitting and feeding on *Enteromorpha* spp. or *Cladophora* spp. filaments most of the time. Besides spontaneous movements resulting from changing the position, its degree of locomotor activity was rather low. A recently developed calorimeter equipped with an optical system for the observation of animal behaviour showed that the maximal peaks recorded in the power-time curves might be related to different types of activity of *G. tigrinus* inside the measuring vessel. Amphipods are mostly sitting on the bottom and/or contracting their bodies, forming an O-shape. They can also swim, horizontally or vertically, inside the vessel (Normant et al., unpublished data). Nevertheless, the ratio between maximum and resting metabolic rates for *G. tigrinus* are within the range 2–10 given by ref. [17] for invertebrates. Exposure of invertebrates to salinity variations might be manifested by quantitative and qualitative changes in their locomotion and activity [16,42]. In the present study, the movement frequency exhibited by *G. tigrinus* was similar at all examined salinities. Small differences occurred in regard to maximal recorded peaks, which might be explained by various types of performed activities. The significant reduction of the resting metabolic rate in *G. tigrinus* exposed to higher salinity and the almost invariable maximal recorded peaks were the probable reason of the increased ratios between these parameters observed at 13‰ and 19.5‰. It means that gammarids are still able to move with the same power, although the maintenance energy cost at 19.5‰ is half lower on the average than at 3‰.

To become a successful invader, non-native species have to develop a high tolerance to abiotic factors, especially temperature and salinity [25,43]. Metabolic response of *G. tigrinus* as an increase in sub-normal and a decrease in supra-normal salinities is typical for euryhaline osmoregulators [17]. Changes in its metabolic rate after exposure to lower or higher salinities are tightly associated with osmotic regulation. According to Werntz [20], the osmotic concentrations of the haemolymph are correspondingly 5.5- and 2.9-fold higher than that of the environment at low salinities of 3.5‰ and 7‰. The high energetic cost of an ion transport against the salinity gradient increases the metabolic rate. A maximum gill Na^+, K^+-ATPase activity was found in *G. tigrinus* acclimated to freshwater [28]. The osmotic capacity (the difference between the osmotic concentrations of the internal and external medium) decreases along with the salinity reaching isoosmosis around 21‰ [20]. This is manifested by the metabolic rate reduction observed in the present studies. The metabolic response of *G. tigrinus* at higher salinities was opposite to that recorded by Koop et al. for the same species, where animals increased their metabolic rates [24]. These differences might result from the fact that: (i) the above-mentioned authors performed experiments on animals in the initial phase of a salinity stress and that (ii) they exposed animals to a 10 mM l^{-1} KCl pollution, which might disturb the ion proportions.

In the presently studied salinity range, *G. tigrinus* was able to regulate its metabolism with a rate of 3.3% per one salinity unit, whereas according to ref. [20], the osmotic capacity of this species decreased with a higher rate of 5.1% per one salinity unit. The greater change in the osmotic capacity than in the metabolic rate suggests that not only active but also passive processes are involved in osmotic regulation. The reduction in surface permeability to ions and water is an other feature in *G. tigrinus*, which allows decreasing the energy expenditure for the active compensatory transport in dilute environments [21]. The rate of metabolic reduction in *G. tigrinus* was similar to that of *G. oceanicus* [44]. The latter species was able to reduce its metabolic rate with 3.6% per salinity unit in salinities between 5‰ and 20‰, whereas its osmotic capacity decreased with a lower rate of 1.4% per salinity unit [45]. The reduction depends probably on the type of osmoregulation and is supposed to be higher in hyperosmotic than in hyper-hypoosmotic osmoregulators [46].

This study shows that the maintenance energy cost for *G. tigrinus* is highest in freshwater and oligohaline areas. Spreading to the β-mesohaline coastal waters allowed *G. tigrinus* to reduce its metabolic rate, but the observed values are still higher than the metabolic minimum, which would be predicted for a salinity near to the internal isoosmotic concentration. Here, the question arises why *G. tigrinus* does not inhabit α-mesohaline zones (10–18‰) in the Baltic Sea. There are a few possible explanations. First of all its distribution in the native regions of North America is restricted to shallow lagoons, bays and estuaries, although *G. tigrinus* exhibits a wide range of salinity tolerance [8]. It shows the same habitat preferences in the coastal Baltic waters. Another point is that gammarids are exposed to a variety of biotic and abiotic factors in their environment, which act simultaneously. Therefore, single factors like salinity cannot determine an invader's success. Another reason might be the species' minimum (the lowest biodiversity) observed at a salinity of 5–8‰ [47]. These water bodies are more vulnerable to alien species [48]. It would be interesting to perform more detailed morphological, behavioural, biochemical and physiological studies to find out if there are differences between fresh- and brackish water populations of *G. tigrinus* and whether they developed any special mechanism to cope with their environmental salinity.

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