Physiological responses to salinity changes of the isopod *Idotea chelipes* from the Baltic brackish waters

Tomasz Łapucki, Monika Normant *

*Department of Experimental Ecology of Marine Organisms, Institute of Oceanography, The University of Gdańsk,
Al. Marszałka J. Piłsudskiego 46, 81-378 Gdynia, Poland*

Abstract

Survival, haemolymph osmolality and the specific metabolic rate of the isopod *Idotea chelipes* from the brackish waters of the southern Baltic Sea (salinity 7 psu) were investigated after acclimation to different salinities in the range of 3–30 psu keeping other parameters constant ($T=15$ °C, full air saturation). Haemolymph osmolality of *I. chelipes* increased from 379 ± 33 mmol kg^{-1} at 3 psu to 935 ± 12 mmol kg^{-1} at 30 psu. In the salinity range of 3–11 psu *I. chelipes* exhibited a hyperosmotic pattern of haemolymph regulation, whereas from 14 to 25 it behaved like an osmoconformer. Isoosmosis occurred at 28.5 psu (920 mmol kg^{-1}). The specific metabolic rate of *I. chelipes* was significantly ($P<0.05$) related to salinity, with maximum (2.4 ± 0.8 J g^{-1} wm h^{-1}) and minimum values (0.6 ± 0.3 J g^{-1} wm h^{-1}) at 7 and 20 psu, respectively. The changes in the specific metabolic rate and in the difference between the osmolality of haemolymph and external medium were significantly correlated for *I. chelipes* ($P<0.05$). Rates of change per salinity unit were, however, unequal varying according to salinity transition. These differences indicate that osmotic adjustment may be a more or less costly in terms of energy according to salinity.
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1. Introduction

The semi-enclosed, non-tidal Baltic Sea is one of the few water basins of the world with a salinity gradient over relatively large distances (Leppäkoski and Bonsdorff, 1989; Zettler et al., 2007). In the southern parts of this basin the average salinity amounts to of 5–7 psu – the so called 'horohalinicum' or critical salinity zone – which forms a barrier for many aquatic organisms (Khlebovich, 1990). Changes in physico-chemical water properties observed at this range are the reason of the significant reduction in species diversity, especially pronounced when moving from the western to the north-eastern parts of the Baltic Sea (Remane, 1940; Bonsdorff, 2006). The aquatic Baltic fauna mainly consists of salinity tolerant species of different ecological (fresh and brackish water or marine) and geographical origin (e.g. Pono-Caspian, North America, Asia) (Bonsdorff, 2006).

It is essential, that the restricted daily and seasonal salinity fluctuations conspicuously differentiate the Baltic Sea from most other brackish areas, especially estuaries on open oceanic coasts (Segerstråle, 1971). To cope with low, but rather stable and predictable salinity, many euryhaline Baltic organisms developed either behavioral, structural or physiological mechanisms that enable them to regulate cell and body volume in the most efficient way (Kinne, 1971; Remane and Schlieper, 1971). The most common osmoregulatory strategies observed in brackish fauna include: (i) decrease membrane permeability to water, (ii) lowering the flux rate by the decreasing the gill epithelia surface, (iii) increase in activity of Na$^+$/K$^+$-ATPase or carbonic anhydrase, (iv) liberation of osmotic effectors (amino acids and peptides) to the haemolymph and (v) production of ammonia or hypoosmotic urine (Mantel and Farmer, 1983; Rosas et al., 1999; Henry, 2001). The diversity of the developed mechanisms is reflected by the variety of osmoregulation patterns exhibited by brackish water invertebrates, which even may differ within the same species in regard to life cycle, physiological state and environmental conditions (e.g. hydrochemical

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* Corresponding author. Tel.: +48 58 523 68 68; fax: +48 58 523 66 78.
E-mail address: ocemn@univ.gda.pl (M. Normant).

properties of the surrounding water, temperature, nutrition) (Kinne, 1971).

However, little is known about salinity tolerance as well as about the capacity for osmoregulation of the Baltic crustaceans the hitherto existing data showed that they may differ compared to populations inhabiting marine waters (e.g. of the adjoining North Sea) (Todd, 1963; Theede, 1969; Bulnheim, 1985; Postel et al., 2000). One of the probable explanations seems to be the long-term geographic isolation of the Baltic forms. Moreover, differences in salinity tolerance between subgroups of the same population were even observed within the Baltic Sea what is assumed to be the result of local acclimatization or the limited gene flow (Kolding, 1985).

One of the boreal species, widely distributed in different types of aquatic biotopes ranging from the estuarine to marine waters is the isopod *Idotea chelipes* Pallas, 1766 (Naylor, 1955; Sywula, 1964; Kamermans et al., 2002; Bamber, 2004; Polte et al., 2005). In the Baltic Sea its distribution is limited to the southern and central parts of the basin, where, unlike to other idoteids, it was sometimes found at localities of very low salinity (Sywula, 1964; Korheina, 1981). Studies performed by Hørlyck (1973) on *I. chelipes* from the Danish coast (Præstø Fjord, Arkona Basin) showed efficient osmotic regulation at salinities of 9–20 psu what is expected to be manifested in the change of the total metabolism as result of expenditures for 'osmotic work'. Although many papers have demonstrated the effect of salinity on metabolic changes in aquatic invertebrates, only few concern the osmotic work, which is estimated to make a rather low contribution in the total energy expenditures (e.g. Potts, 1954; Styczyńska-Jurewicz, 1970; Siebers et al., 1972; Sutcliffe, 1975; Goolish and Burton, 1989; Aarset and Aunas, 1990). The majority of these studies are based on respiration measurements which include only the aerobic part of metabolism. Since some aquatic invertebrates under stress by environmental factors (temperature, salinity, oxygen depletion, pollutions, e.g.) are able to use anaerobic pathways as energy source (Pamatmat, 1978; Conte et al., 1980; Oeschger, 1990). Therefore, respiration measurements might not always provide reliable information regarding the energy metabolism (Potts and Parry, 1964; Liu et al., 1990; Hardewig et al., 1991; Normant et al., 1998). All chemical reactions and the energy turnover (aerobic and anaerobic) taking place in cells of living organisms are accompanied by heat transformations, so that heat dissipation measurement seems to be the most appropriate method of metabolic rate determination (Pamatmat, 1980; Lamprecht, 1998).

In this paper we studied tolerance and the osmotic response to salinity changes of *I. chelipes* from the southern Baltic Sea in order to find out its osmoregulatory abilities. The second objective of this work was to determine the effect of salinity on the total metabolism of this species based on heat dissipation measurements. Simultaneous studies of osmoregulation and total metabolic rate were performed in order to assess the relationship between both processes.

Due to the fact that there is already information in literature concerning osmoregulation in *I. chelipes* from Danish (Præstø Fjord, Arkona Basin) and Dutch waters (Lake Veere) it was possible to compare the obtained data and find out if intraspecific

differences exist (Hørlyck, 1973; Vlasblom et al., 1977). As only aerobic respiration rate studies of *I. chelipes* have been performed previously on specimens inhabiting British and Dutch waters (Jones, 1974; Vlasblom et al., 1977), there is no information on the effect of salinity on the total energy metabolism of this species.

2. Materials and methods

2.1. Animal collection

Adult males 11–15 mm in length (wet mass 0.0121–0.0429 g) were collected in June 2005 from the phytal zone (depth of 0.5–1 m) in the Gulf of Gdańsk (southern Baltic Sea, $S = 7$ psu, $T = 15$ °C). Experimental animals were kept for 1 week in the laboratory ($S = 7$ psu, $T = 15$ °C, full air saturation). They were fed with macroalgae of the genera *Enteromorpha* and *Cladophora*.

2.2. Experimental protocol

Metabolic rate and haemolymph osmotic concentration were measured at the following salinities: 3, 7, 14, 20 and 30 psu with other parameters kept constant ($T = 15$ °C, full air saturation). To obtain better osmoregulation pattern the haemolymph osmotic concentrations were measured also at three additional salinities of 11, 17 and 25 psu. The experimental media were prepared by dissolving commercial sea salt (hw-Meersalz, Wiegandt GmbH, Germany) in tap water. From 15 to 26 males were acclimated gradually (2 psu per day) to each experimental salinity. After attaining the appropriate salinity, the individuals were kept in an aquarium for 5 days to reach a new steady state. During the acclimation time as well as the 5 days of exposure to new conditions mortality was recorded.

2.3. Metabolic rate

The total metabolic rate at a temperature of 15 °C was determined by heat dissipation measurements conducted in an isothermal calorimeter of the Calvet type described by Normant et al. (2007a). A single animal was placed in the measuring vessel (diameter 28 mm, height 50 mm) filled with 15 mL of filtered (cellulose filter, 0.45 µm) and fully aerated water of the appropriate salinity. Additionally, a piece of artificial sea grass was placed inside the vessel to reduce the stress and activity of the animal during the measurements. At each salinity 7–9 specimens were studied. Before and after the measurements, the calorimetric signal of a vessel only filled with water was determined (base line). Heat dissipation measurements were conducted during 120 min after an equilibration time of about 60 min. The oxygen tension of the medium was monitored with a needle microelectrode (PA 2000, Unisense, Denmark) before and after the measurement. Application of an optical system for long-term video registration incorporated into the calorimeter (Normant et al., 2007a) allowed for monitoring the animal behavior during the measurements, essential at analyses of the obtained calorimetric power time curves. After the measurement surface water of specimens was blotted by means of soft
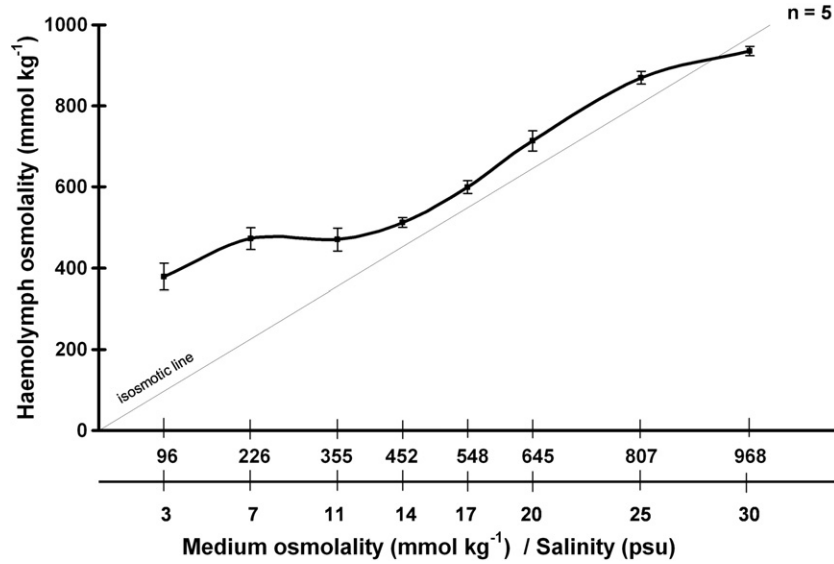
Fig. 1. Mean (±SD) haemolymph osmolality of *I. chelipes* in relation to the medium osmolality (the psu equivalent of salinity is given in the bottom panel).

paper tissue and the wet mass determined to the nearest 0.1 mg. The specific metabolic rate (SMR) of a single resting animal was calculated according to an equation given by Lapucki et al. (2005) and expressed in Joules per gram wet mass and hour (J g^{-1} wm h^{-1}). As the here used metabolic unit J g^{-1} wm h^{-1} for the metabolic rate transforms to 0.278 mW g^{-1}, the given values can be also read as mW g^{-1}.

2.4. Haemolymph osmolality

A haemolymph sample (five per salinity) of about 0.10 µL was taken from the animal with a capillary glass tube inserted directly into the heart of the idoteid at the dorsal side. The sample was sealed at both ends with liquid paraffin. At the same time a sample of the experimental medium was collected. The osmotic pressure of the haemolymph and the external medium were determined by the freezing-point depression method as

described by Ramsay (1949) and modified by Dobrzycka and Szaniawska (1995). The haemolymph osmolality was expressed in millimoles per kilogram (mmol kg^{-1}). The symbol ΔOSM used in the text refers to the difference between osmolality of the haemolymph and medium.

2.5. Statistical analyses

Values were expressed as the mean together with the standard deviation (mean±SD) for a specified number (*n*) of repetitions. The normality of the data distribution was verified with the Shapiro–Wilk test at significance level of 5%. The Kruskal–Wallis analysis of variance ANOVA was applied to test the effect of salinity on haemolymph osmolality and specific metabolic rates, whereas the non-parametric Mann–Whitney *U* test was used to compare the specific metabolic rates between experimental salinities. "Significant" in the text
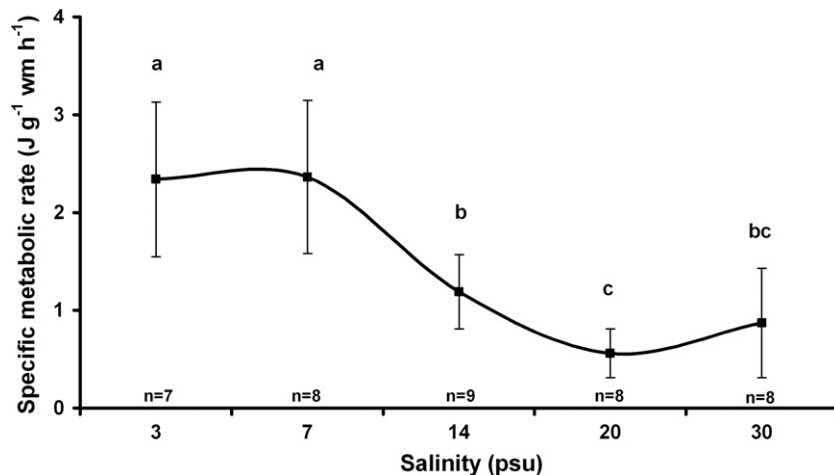

Fig. 2. Mean (±SD) specific metabolic rate of *I. chelipes* exposed to different salinities (*n* indicates the number of specimens). Means with different superscripts are significantly different (*P* < 0.05).
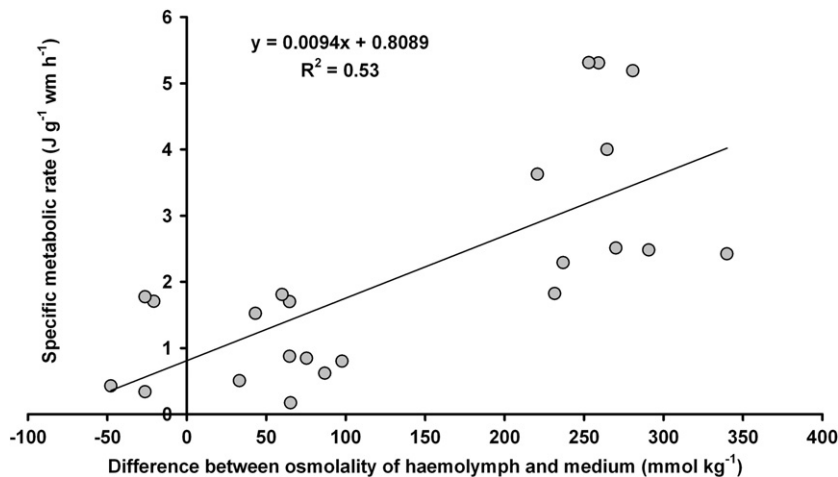
Fig. 3. Relationship between the specific metabolic rate and the difference between osmolality of haemolymph and medium for *I. chelipes*.

means significant at the 5%-level. The analyses were performed with the STATISTICA 7 PL program (StatSoft, Poland).

3. Results

Recording of survival of *I. chelipes* was terminated after 16 days showing influence of both, the period of acclimation and the salinity. The survival rate decreased significantly with increasing salinity ($P < 0.05$, $R^2 = 0.70$, $n = 75$) attaining 71% after 11 days of acclimation to 30 psu. On the other hand only 78% of the animals survived after 3 days of acclimation to a salinity of 3 psu. During 5 days of subjection to each of the final experimental salinities the survival rate additionally decreased by 6% in habitat (control) salinity and by 13–14% at 3, 20 and 30 psu.

Salinity had significant effect (ANOVA, $P < 0.05$) on the haemolymph osmolality of *I. chelipes*, which increased from 379 ± 33 mmol kg^{-1} at 3 psu to 935 ± 12 mmol kg^{-1} at 30 psu (Fig. 1). Hyperosmotic regulation took place in the salinity range 7–11 psu, whereas *I. chelipes* behaved like osmoconformer between 14 and 25 psu. Isoosmosis occurred at 28.5 psu (920 mmol kg^{-1}).

The insignificant differences ($P > 0.05$) of the body masses of *I. chelipes* males acclimated to different salinities allow for comparison of their metabolic rates. The Kruskal–Wallis analysis of variance (ANOVA) showed that the effect of salinity on the specific metabolic rate of *I. chelipes* was significant ($P < 0.05$). The mean values were greatest at 3 and 7 psu and decreased significantly ($P < 0.05$) by 75% with increasing salinity reaching a minimum of 0.6 ± 0.3 J g^{-1} wm h^{-1} ($n = 8$) at 20 psu (Fig. 2). At a salinity of 30 psu (above the isoosmotic point) the mean metabolic rate of *I. chelipes* increased by 55% ($P > 0.05$), attaining a value similar to that observed at 14 psu.

The results presented in Fig. 1 for the haemolymph as function of the medium osmolality and in Fig. 2 for the specific metabolic rate as function of salinity can be combined in a graph showing the metabolic rate in dependence ($P < 0.05$, $R^2 = 0.53$, $n = 23$) of the difference between osmolality of the haemolymph and medium (Fig. 3). The latter one is represented by 3 clusters in this

graph: (i) high values for salinity 3 and 7 psu at the right side, connected with the maximum metabolic level (left side in Fig. 2); (ii) small values for the transition salinities 14 and 20 psu near the origin of the axes; and (iii) a few negative points for 30 psu, connected with the low metabolic level.

4. Discussion

Despite many ecological changes in the Gulf of Gdańsk observed over the last few decades (Wiktor and Pliński, 1992; Szaniawska et al., 1999), *I. chelipes* is still the most abundant component of the phytal zone, while many other species have disappeared from this area (Jażdżewski et al., 2005; Łapucki et al., 2007). Any changes of biotic and abiotic factors might be critical for organisms which live under permanent hypoosmotic stress as far as they affect all physiological processes necessary for a normal functioning. Among them, osmoregulation and metabolic rate might play an essential role as indicators of animal adaptation and performance during exposure to different factors (Lignot et al., 2000).

Inhabiting diverse ecological and geographical regions, *I. chelipes* exhibits high tolerance towards a wide range of salinity, but there might be a shift in lower and upper salinity tolerance limits between populations. In the Baltic specimens the highest survival rate was observed at 7 psu, whereas Jones (1974) and Vlasblom et al. (1977) showed that *I. chelipes* adapted to salinity of about 20 psu was characterized by the highest survival rate at conditions above 16 psu. Such an intra-specific difference may result from acclimatization or long-term adaptation to habitat salinity. Studies on subpopulations of *I. chelipes* from the southern English coast showed significant levels of genetic differentiation despite small spatial scale; this was explained by the physical barrier created by salinity (Jolly et al., 2003). In the populations from western Mediterranean and Atlantic the genetic differences were also found (Charfi-Cheikhrouha et al., 1998). One of the good indicators of the long-term adaptation to low salinity in crustaceans resulting from the selective gene expression might be the activity

of carbonic anhydrase (Henry, 2001). Genetic speciation caused by some ecological (including salinity) or geographical barriers, manifested in different behavior, morphology, physiology or biochemistry, was observed in many other crustaceans also (Koehn et al., 1980; Bulnheim, 1985; Kolding, 1985; Harris and Thuet, 1987; Laughlin and French, 1989).

Intraspecific differences are not only evident in the survival rates, but also in the case of osmoregulation. The haemolymph osmolality of *I. chelipes* from the Gulf of Gdańsk is 15–30% lower within the salinity range of 3–17 psu and higher by 6–14% higher at salinities of 25–30 psu than that observed for *I. chelipes* from more saline (~10 psu, $T = 10 \,°C$) Danish waters (Hørlyck, 1973). Similar conclusions might be drawn for *I. chelipes* from the Gulf of Gdańsk and from Dutch waters (20 psu, $T = 5 \,°C$ and 15 °C): at lower salinities haemolymph osmolalities are lower, whereas at more saline waters they are higher (Vlasblom et al., 1977). Discrepancies might be due not only to the lower experimental temperatures applied by these authors, but also to differences in membrane permeability, which decreases in low salinities and increases in marine waters (Lockwood, 1962; Lockwood et al., 1976). Analogous observations were made for brackish and marine forms of *Saduria* (*Mesidotea*) *entomon*, *Corophium volutator* and *Gammarus oceanicus* (McLusky, 1967; Percy, 1985; Dobrzycka and Szaniawska 1995; Normant et al., 2005). Moreover, the osmoregulation patterns in *I. chelipes* from less and more saline waters look different. Specimens from the Gulf of Gdańsk are less effective regulators compared to those inhabiting Danish waters, which maintain haemolymph osmotic pressure at the similar level at a wider salinity range of 9–20 psu (Hørlyck, 1973). While there are differences in both, osmotic pressures and osmoregulation patterns, isoosmosis takes place at almost similar salinities in specimens of Gulf of Gdańsk (28.5 psu) and Danish waters (30 psu).

The high metabolic rate observed in *I. chelipes* at habitat salinity is in agreement with a statement of Glazier and Sparks (1997) that the evolution of resistance to hypoosmotic stress has involved an increase, not a decrease, in the metabolic rate. On the other hand reduction of the values following exposure to higher salinities is the typical response for many brackish invertebrates resulting from the reduced cost of osmotic adjustment (Kinne, 1971). The exposure from 7 to 20 psu induced decrease to one fourth in specific metabolic rate of *I. chelipes*. The rate of metabolic reduction was, however, different at particular salinity transitions indicating changes in the contribution of some adaptive mechanisms developed by *I. chelipes*. One of them which allows reducing the metabolic cost of osmoregulation seems to be the lower membrane permeability (Potts, 1954; Smith, 1967). A hard and well mineralized exoskeleton protects *I. chelipes* against the environment, but its physical properties may vary according to the moulting stage. Differences in the membrane permeability as resulted from moulting are supposed to be the reason of the highest inter-individual variability in the specific metabolic rates observed at lowest salinities of 3 and 7 psu. Presumably this process was overlooked in the laboratory due to the fact that freshly moulted specimens often consume their own old exoskeletons (Sywula, 1964). This hypothesis is strengthened by a higher variability in the haemolymph osmolality recorded among

I. chelipes at the lowest studied salinities. The permeability of the membrane may be also modified by a diet rich in highly unsaturated fatty acids (HUFA) – it decreases as the fatty acid length increases (Turner et al., 2003; Gong et al., 2004; Palacios et al., 2004; Hurtado et al., 2007; Maazouzi et al., 2007). It is assumed that the diet of *I. chelipes* which consists mainly of macrophytes in the Baltic Sea (e.g. *Enteromorpha*, *Cladophora*, *Pilayella*) and of detritus might be a good source of HUFA (Sywula, 1964; Jansson and Mathiesen, 1971). Moreover, the tissues of macroalgae of the genus *Enteromorpha* contain $20.6 \pm 4.6\%$ of mineral compounds in the dry weight (Haroon and Szaniawska, 1995). Besides quality, also the quantity of the consumed food can play an essential role in the osmoregulation by providing necessary ions (Kinne, 1971). Animals can consume more food at lower than at higher salinities as it was observed in *Gammarus oceanicus* (Normant and Lamprecht, 2006).

On the other hand the enlargement of metabolic cost in *I. chelipes* exposed to dilute media may be caused by the increase in Na^+/K^+-ATPase activity at pleopod endopodites as it was recorded for *Idotea baltica* and *Idotea wosnesenskii* (Holliday, 1988; Postel et al., 2000). Another reason might be an active ammonia excretion resulting from a greater catabolism of amino acids involved in osmotic regulation (Regnault, 1987). Moreover, it was found that the NH_4^+ played a role as a counter ion in Na^+ transport through membranes (Gonçalves et al., 2006).

The minimum thermodynamic cost of osmotic regulation in brackish water animals has been estimated theoretically from 1 to 5% of the total metabolic energy, however it is expected to be greater as far as the process of ion transporting itself is less than 100% efficient (Potts, 1954). As it was mentioned before osmoregulation is not only an ion and water exchange, but also a complex process involving many different mechanisms to maintain the ion homeostasis requiring energy in many cases. For example the theoretical cost of amino acid production following a hypoosmotic stress of the copepod *Tigriopus californicus* was estimated to be 11.6% of the daily energy use (Goolish and Burton, 1989). Metabolic cost of osmoregulation is supposed to vary in a wide range according to species-specific features as well as to combinations of biotic and abiotic factors (Potts, 1954; Davenport, 1985; Pequeux, 1994). Practical calculations of the energy expenditures resulting from 'osmotic work' following salinity changes might be performed precisely based on heat dissipation measurements, but only if all factors affecting energy turnover, other than salinity, are taken into account (e.g. sex, size, nutrition, locomotor activity, temperature, oxygen tension). Our studies show that changes in the specific metabolic rate and in the difference between the osmotic pressure of haemolymph and external medium are closely related in *I. chelipes*. Within a salinity range of 3–20 psu *I. chelipes* reduced both, the difference in osmolality between haemolymph and medium (ΔOSM) and metabolism with a rate of 4.5% per 1 psu. However the energy spent for maintaining the ion homeostasis can be different at the particular salinity transitions. It seems that osmoregulation is more efficient in *I. chelipes* at lower salinities as far as the rate of changes in metabolism is lower compared to that in ΔOSM. For example, they amounted to 7.1 and 13.3% per 1 psu between 7 and 11 psu, respectively, whereas there was no change in

metabolic rate at salinity transition from 7 to 3 psu; the rate of change in ΔOSM was 3.6% per 1 psu. *I. chelipes* seems to be a less effective osmoregulator compared to the co-existing amphipod *Gammarus tigrinus* – a recent colonizer of the Gulf of Gdańsk – which reduces metabolism and ΔOSM with rates of 3.3 and 5.1% per 1 psu in the salinity range 3–20 psu, respectively (Normant et al., 2007b). On the other hand, it copes better with low salinity than another amphipod *G. oceanicus* – a species of marine origin living in the Gulf of Gdańsk – which reduces its total metabolism and ΔOSM with a rate of 3.6 and 1.4 % per 1 psu in the range of 5–20 psu (Normant et al., 2004; Normant et al., 2005).

Although the comparative studies on physiological responses as well as on mechanisms evolved by euryhaline organisms in the adaptation to a hypoosmotic stress become more complete continuously there are still many doubts in regard to intraspecific differences. Therefore further investigations on *I. chelipes* as well as on other Baltic crustaceans are on the way.

Acknowledgements

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