The effect of salinity on osmoregulation in *Corophium volutator* (Pallas) and *Saduria entomon* (Linnaeus) from the Gulf of Gdańsk*

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Osmoregulation
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Gulf of Gdańsk

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Abstract

Material for the study was collected in the summer of 1994 in the Gulf of Gdańsk where specimens of *Corophium volutator* and *Saduria entomon* – organisms living in a zone of critical salinity (5–8 psu) – commonly occur.

The high osmolarity of their body fluids is indicative of their adaptation effort to the salinity in their habitat. A species of marine origin, *Corophium volutator* maintains its osmotic concentration of haemolymph at a high level, as other species in brackish waters do; however, this is not the case with *Corophium volutator* specimens living in saline seas. *Saduria entomon* – a relict of glacial origin, originally from the Arctic Sea – also maintains a high osmotic concentration of haemolymph in comparison with specimens of this species living in the Beaufort Sea.

1. Introduction

The 5–8 psu salinity zone is the boundary separating the marine world from the freshwater world, a fact stressed by many authors, *e.g.* Remane (1934), Khlebovich (1989, 1990a,b) or Styczyńska-Jurewicz (1972, 1974). The critical salinity is defined by Khlebovich (1990a,b) as a narrow zone where massive mortality of fresh- and salt-water forms occurs, in both estuaries and laboratories. It limits the life activity of isolated cells and tissues,

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and of forms without osmoregulatory capabilities. The critical nature of the 5–8 psu salinity is reflected in the reduced number of marine, fresh- and brackish-water taxa in waters of this salinity (Khlebovich, 1989).

Corophium volutator is a typically euryhaline marine organism (Jażdżewski, 1970, 1971), occurring in waters with a salinity of 2–50 psu (McLusky, 1967), the most favourable being 10–30 psu, and 20 psu in particular (McLusky, 1970b). This species is geographically widespread: its range of occurrence includes the Atlantic coast of North America between the 40th and 50th parallels, and the coasts of Europe from the Mediterranean, the Black Sea, the Sea of Azov and the Baltic, to the coasts of Norway, Great Britain and Ireland (Schellenberg, 1942; Guryanova, 1951). However, it is most probable that the *orientalis* form of *Corophium volutator* is also present off the Mediterranean and Black Sea coasts, even to the exclusion of other forms (Schellenberg, 1942). In the Baltic this species is found down to a depth of 30 m, more or less to the 2–3 psu isohaline in the Gulf of Bothnia and Gulf of Finland (Schellenberg, 1942). It generally inhabits the near-shore zones of seas, gulfs, estuaries and river mouths, preferring a loose muddy or sandy-muddy bottom.

Saduria entomon, also an euryhaline species, is equally widespread, inhabiting the Arctic and temperate zones of the northern hemisphere along the Arctic coasts of Siberia, the White Sea, Beaufort Sea and Bering Strait. Regarded as a glacial relict, it first appeared at the end of the last glaciation in the Baltic Sea and then in many Swedish lakes and Lake Ladoga (Ekman, 1919; Segerstråle, 1957; Percy, 1985). Generally, two forms of this species are distinguished in our zone:

- the fresh-water form, found in lakes (*e.g* Vättern, Mälaren, Ornö, Stora Färgen, Sävelången, Mjörn, Vänern, Anten in Sweden, and Ladoga in Russia),
- the Baltic-brackish form (Haahtela, 1975).

Its occurrence in lakes is limited principally to waters below the thermocline; the higher salinity in the western Baltic is responsible for the smaller numbers of this species there.

The aim of this paper was to study the method by which the osmotic pressure of the body fluids of these two species have adapted to the waters of the Gulf of Gdańsk, a zone of critical salinity, where the salinity and inorganic ion concentration are both low. It would thus be interesting to learn about the strategy of adaptation to salinity in the euryhaline species inhabiting the brackish waters of the Gulf of Gdańsk. It should be emphasized that no studies of this kind have been conducted before on organisms from the southern Baltic, not to mention the Gulf of Gdańsk, despite the great interest in osmoregulation problems among foreign authors, *e.g.* Virtanen (1987), Zanders and Rodriquez (1992), and many others.

2. Material and methods

- *Corophium volutator* was collected at station S1, located at a distance of 0.5 km in the direction of Puck from the outfall of the waste-water-treatment plant at Swarzewo. The material was obtained from the near-shore zone (about 20 m from the shore) using an Ekman grab.
- *Saduria entomon* was sampled from a depth of 60 m by means of a bottom drag at a station located near the village of Świbno from on board the r/v 'Oceanograf'.

After moving the specimens to a laboratory, they were acclimatized by keeping them for seven days in thermostatted tanks of aerated water at a salinity and temperature (10°C), similar to that of their habitat. During this period, the specimens of *Corophium volutator* were fed with mud containing organic matter, and *Saduria entomon* with frozen fish. Following acclimatization, when the animals' metabolic processes had become stabilized, they were moved in batches to waters of various salinities (Tab. 1).

Table 1. The experimental salinity (S = psu) at which animals were acclimatized

Corophium volutator		*Saduria entomon*	
salinity	number of individuals	salinity	number of individuals
0	12	0	6
7	12	4.5	6
7.5	12	5	6
8.5	12	8	10
18	20	10	6
39	20	32	6
50	20	45	6
total	108		46

The animals were kept in aerated water of a given salinity for ten days. They were not fed, although in the case of *Corophium volutator* organic matter was present in the substrate, which is not only a trophic factor, but also makes acclimation to salinity easier (McLusky, 1967, 1970a). This 10-day period allowed the animals to become completely acclimated to the given salinities, as *Corophium volutator* attains steady osmolarity of body fluids after two days (McLusky, 1967), and *Saduria entomon* after three – five days (Percy, 1985).

The mortality rate of *Corophium volutator* and *Saduria entomon* specimens was estimated. Before the osmotic concentration of haemolymph was determined, the animals were delicately dried on blotting paper. Adult specimens of known sex were used in the experiments.

In the case of *Corophium volutator*, the specimens were cut laterally and following the insertion of a glass capillary tube into the haemocoel, about 1 μl of haemolymph was taken from each specimen. After having been immersed in liquid paraffin, a capillary tube was filled with this fluid, then in the haemocoel (so that it would fill with haemolymph), and then again in paraffin, as in McLusky (1967). In addition to the haemolymph sample, a sample of distilled water was placed in each capillary tube as an indicator.

Specimens of *Saduria entomon* were placed on their ventral side and with a syringe about 20 μl of haemolymph were usually removed from the heart of each specimen, an amount sufficient for several to a dozen or so subsamples. Further procedures were as for *Corophium volutator*.

The samples were kept in a refrigerator until the osmotic concentrations were measured.

The osmotic concentration of haemolymph was determined on the basis of its coagulability – by measuring the melting temperature of haemolymph samples in spirit, as Ramsay (1949) and Klekowski (1963) did. A thermometer with a PT 100 platinum resistance sensor and an ELT 101 electronic display, with a measurement range of –50÷100°C and a resolution of 0.01°C, were used for this purpose. The thermometer had a HOLD function, allowing the measurement to be memorized.

KALTE 75 KONTAKT-CHEMIE fluid was used for freezing the samples to –42°C before the experiment. The melting samples were viewed under a stereoscopic microscope (magnification: 1.6 lens × 6.3 ocular = 10) fitted with a polarization device. The instant of melting was regarded as the moment the last crystal melted. The melting temperatures of two samples were read at a time, and the osmotic pressure of the haemolymph was calculated on the basis of the relationship –0.6°C = 10 psu.

3. Results

3.1. Mortality

The mortality of specimens in different salinity variants was determined:

- *Corophium volutator* (Tab. 2),
- *Saduria entomon.*

Table 2. Mortality of *Corophium volutator* (% of individuals) in water of different salinity

Salinity [psu] days	0	7	7.5	8.5	18	39	50
1	0	0	0	0	0	0	0
2	25	0	0	0	0	15	15
3	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0
5	0	0	0	8.3	15	35	50
6	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0
9	25	16.7	16.7	16.7	35	10	0
total after 10 days of experiment	50	16.7	16.7	25	50	60	65

In the case of *Saduria entomon* no dying specimens were observed; cannibalism was noted only at salinities of 5, 32 and 45 psu (one animal at each of these salinities).

3.2. Osmoregulation

The results of the experiments with *Corophium volutator* are presented in Figs. 1 and 2. Acclimatized to different salinities (0–50 psu) at a temperature of 10°C, the animals exhibited a hyperosmotic level of body fluids in relation to the environment (Fig. 1). Only in the ambient salinity of 50 psu was the haemolymph of the specimens isoosmotic in relation to the water outside the organisms. At lower salinities, the animals exhibited a higher hyperosmotic haemolymph level in relation to their environment. Both males and females exhibited similar osmotic reactions to salinity. No significant effect of sex on the osmolarity of body fluids was observed.

The results of acclimatization to different salinities (0–45 psu) in *Saduria entomon* are presented in Figs. 3 and 4. The animals exhibited a hyperosmotic body fluid level; only at a salinity of 32 psu did they tend towards a hypoosmotic haemolymph level with their environment. Higher mean osmotic concentrations of haemolymph were observed in females at all salinities (Fig. 4).

4. Discussion

Corophium volutator is a well-known species from the point of view of osmoregulation and ionic regulation (McLusky, 1967, 1968, 1970a,b;

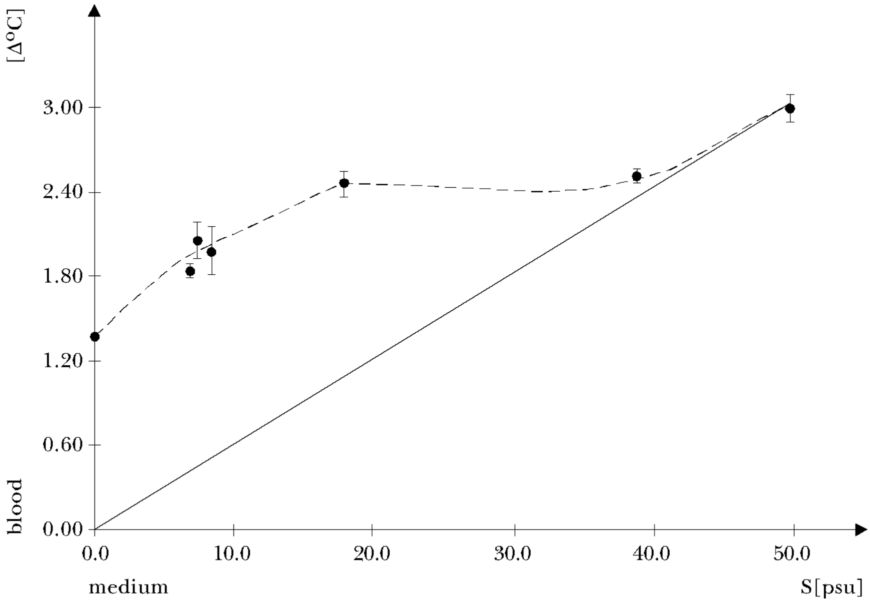

Fig. 1. The effect of salinity on the osmotic concentration of blood in *Corophium volutator*

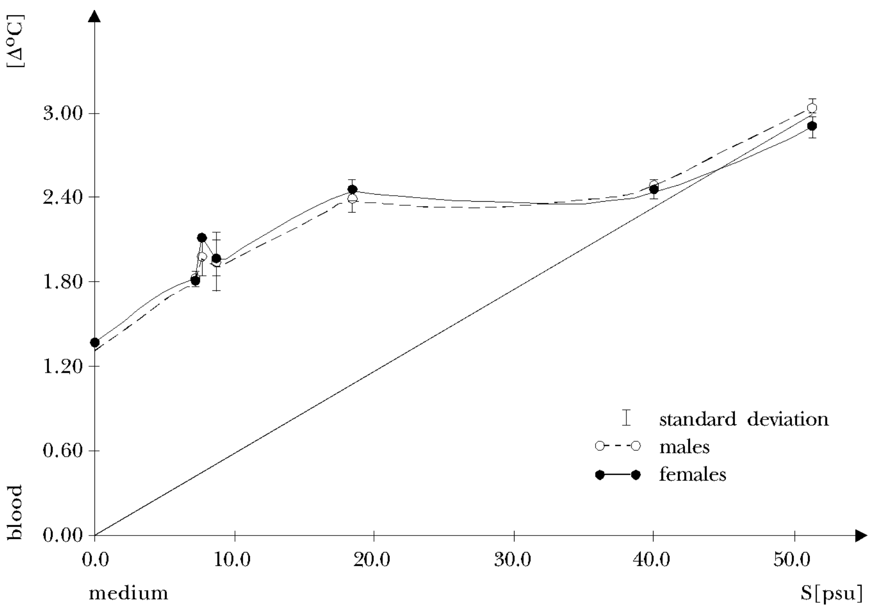

Fig. 2. The effect of salinity on the osmotic concentration of blood in males and females of *Corophium volutator*

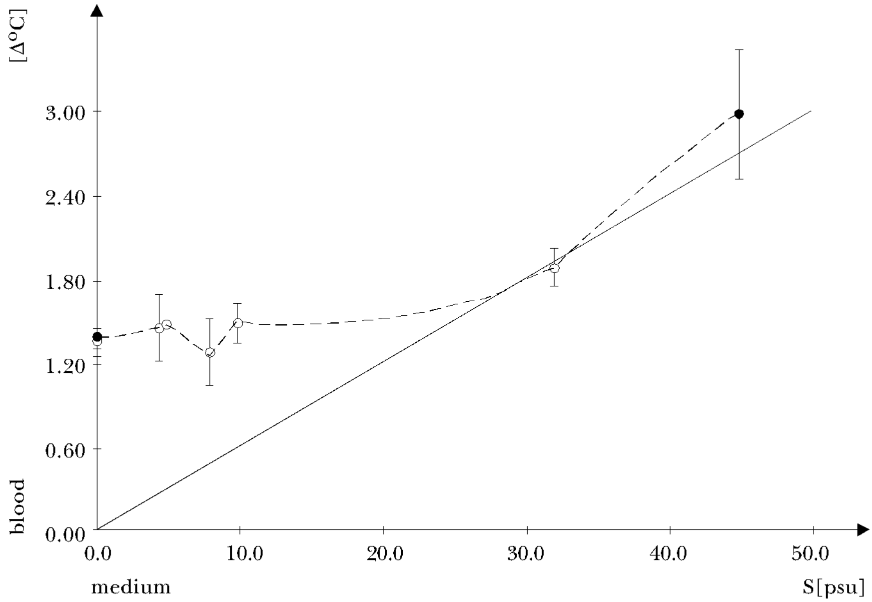

Fig. 3. The effect of salinity on the osmotic concentration of blood in *Saduria entomon*

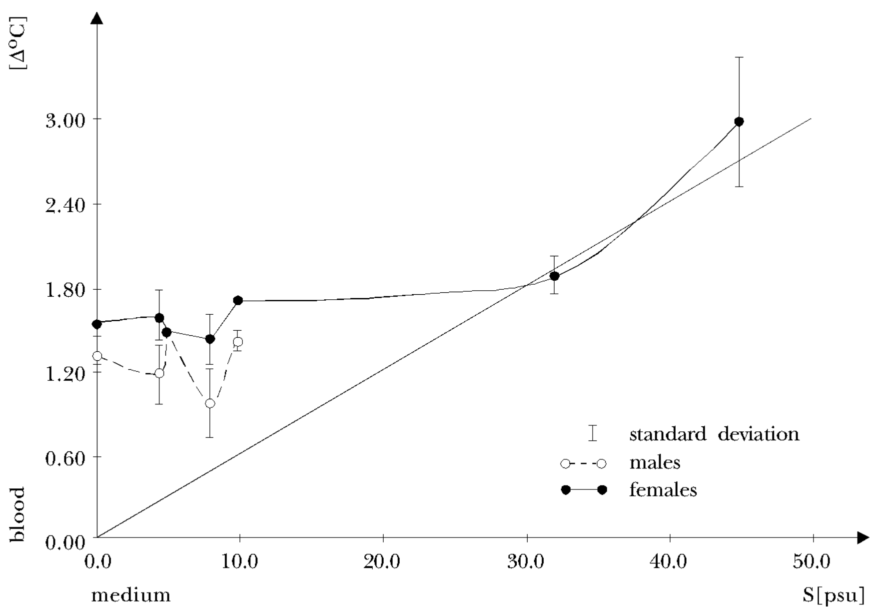

Fig. 4. The effect of salinity on the osmotic concentration in males and females of *Saduria entomon*

McLusky *et al.*, 1982). Even so, no studies have been conducted before on the osmotic and ionic relations between this organism and the Gulf of Gdańsk environment.

The pattern of osmotic regulation in *Corophium volutator* specimens, determined on the basis of the melting temperature of haemolymph samples, reflects the adaptation strategy of these animals to different water salinities. *Corophium volutator* is a hyperregulator over the entire range of prepared water salinities, although at a salinity of 50 psu it becomes isoosmotic in relation to its environment. However, it may be seen that the hyperosmotic haemolymph level is greater at lower than at higher salinities (Fig. 1).

In comparison with the osmotic regulation pattern of *Corophium volutator* from the estuary of the river Ythan, Aberdeenshire, Scotland (8°C) (McLusky, 1967), where the water salinity ranges from 5 to 35 psu in its central zone, the pattern for *Corophium volutator* from the Gulf of Gdańsk is slightly different. Despite the fact that specimens from various populations of the same species exhibit a hyperosmotic haemolymph level in relation to the environment, this level in *Corophium volutator* from the Gulf of Gdańsk is greater.

The following factors influence the specific nature of the adaptation strategy of *Corophium volutator* from the Gulf of Gdańsk:

- the more stable and relatively low salinity in the Inner Bay of Puck,
- the proximity of the biological sewage treatment plant at Swarzewo, which, by discharging large quantities of nutrients (nitrates, phosphates) and other pollutants from partially-treated waters, leads to considerable eutrophication.

Trophic conditions are of great importance in the osmotic adaptation of *Corophium volutator* to the environment (McLusky, 1970a), so the habitat favours the maintenance of a high hyperosmotic level of body fluids by its specimens.

The adaptation strategy of maintaining a higher osmotic concentration by animals adapted to lower salinities in comparison with animals inhabiting more saline waters is well-known, *e.g.* in *Carcinus maenas* (Theede, 1984), or *Crangon crangon* (McLusky *et al.*, 1982). No significant differences between the osmolarity of males and females in *Corophium volutator* were observed (Fig. 2).

The osmotic regulation pattern in *Saduria entomon* is also indicative of the hyperosmotic adaptation mechanism of this species. At lower salinities, these animals likewise maintain haemolymph concentration at a higher level, while at higher salinities, the difference in concentrations between the water in the environment and body fluids is smaller (Fig. 3). Despite this, *Saduria* *entomon* generally maintains its haemolymph concentration at a lower level than *Corophium volutator*.

The Baltic form of *Saduria entomon* is characterized by hyperosmotic regulation at a salinity below 20 psu and is isoosmotic or slightly hypoosmotic at higher salinities (Bobowicz 1968, 1970; Bogucki, 1932; Lockwood and Croghan, 1957; Croghan and Lockwood, 1968; Lockwood *et al.*, 1976; Hagerman and Szaniawska, 1992). Such a regulation pattern is almost identical with that observed during the present study.

Saduria entomon from the Gulf of Gdańsk is a brackish-water form of this species. The adaptation mechanism of these organisms resembles that in marine *Saduria entomon* from the Beaufort Sea, where the salinity is about 35 psu. It may be observed that *Saduria entomon* specimens from the Gulf of Gdańsk behave like brackish-water species of marine origin in comparison with their counterparts from more saline waters. This points to the marine origin of *Saduria entomon*.

Percy (1985) studied osmotic regulation in specimens of the *Saduria (Mesidotea)* complex from the Beaufort Sea (temperature 5°C), revealing differences between the freshwater form and the typically marine form. He recorded a distinctly higher osmotic concentration of haemolymph in the freshwater form over the entire salinity range tested, in comparison with the marine form, despite the hyperosmotic haemolymph level in both forms. He explained this by differences in their physiological adaptation. Croghan and Lockwood (1968) also agree that many freshwater forms have a lower blood concentration than the corresponding forms inhabiting brackish waters. These authors point out two features of typical brackish-water animals: the high osmotic concentration of blood, and the ability to tolerate a saline environment (even the critical salinity of sea water). There is no doubt that these features characterize *Saduria entomon* from the Gulf of Gdańsk. It is interesting to note the evolution of the freshwater form of *Saduria entomon* from the brackish-water form. The freshwater form may, however, adapt to brackish waters and is capable of surviving in fully saline sea water. The maintenance of the haemolymph concentration at a lower level with respect to the brackish-water form indicates a more efficient osmoregulatory mechanism in this form (Lockwood and Croghan, 1957).

Differences in body fluid osmolarity between males and females in specimens of this species were observed (Fig. 4): females exhibited a higher osmotic level of the blood, which may signify the lower efficiency of the osmoregulatory organs in males.

5. Conclusions

- Osmo-regulation patterns in *Corophium volutator* and *Saduria entomon* from the Gulf of Gdańsk, determined on the basis of measurements of melting temperature of haemolymph samples, exhibit a hyperosmotic character over the entire range of experimental salinities.
- Generally, osmotic haemolymph concentrations are maintained at a relatively high level, which is due to the adaptation of specimens of both species to life in the critical salinity of the Gulf of Gdańsk.
- Specimens of *Corophium volutator* from the Gulf of Gdańsk maintain their osmotic concentration of haemolymph at a higher level than *Corophium volutator* specimens from the estuary of the river Ythan, Aberdeenshire, Scotland (an estuary with a generally higher salinity than the Inner Bay of Puck).
- Specimens of *Saduria entomon* maintain haemolymph osmotic pressure at a higher level than do specimens of this species from the Beaufort Sea.

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