

THE OSMOREGULATORY ABILITY IN THREE SPECIES OF THE GENUS *IDOTEA* (ISOPODA, CRUSTACEA)

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ABSTRACT

The osmoregulation of *Idotea baltica*, *I. viridis* and *I. granulosa* from Denmark was investigated in an attempt to elucidate their ecological distribution. All were able to keep their haemolymph hyperosmotic relative to the medium in salinities below 30‰. Above this concentration they were isosmotic.

The lower salinity limits tolerated varied, as it was 5, 7 and 9‰ for respectively *I. viridis*, *I. baltica* and *I. granulosa*. *I. viridis* was a significantly better osmoregulator than the two other species.

When transferred from high to low salinity water the haemolymph concentration dropped rapidly in all species within the first three hours. After 24 hours an almost steady-state condition was reached. When exposed to a sudden raise in salinity all regulated to the new steady-state situation within three hours.

The physiological data found in these experiments fit in with the known ecological distribution of the three species.

INTRODUCTION

The ecological distributions of *Idotea baltica* (Pallas), *I. granulosa* Rathke and *I. viridis* (Slabber), have been discussed in several reports.

I. viridis is a brackish water species found in localities where the salinity fluctuates and periodically reaches extremely low values (0‰) (Sywula, 1964b).

Naylor (1955) found *I. viridis* in tidal pools with fluctuating salinities (6.8-38.6‰). Muus (1967) did not find it in salinities less than 4-5‰ in Danish estuaries and lagoons, outside of which it is replaced by *I. baltica*. Naylor (1955) associates the latter species with drifting algae.

Segerstråle (1944) reports that all three species occur far into the Baltic Sea with *I. granulosa* in salinities as low as 6‰, and *I. viridis* and *I. baltica* in 3.5‰. *I. granulosa* has almost the same distribution as *I. baltica*. Naylor (1955), however, found *I. granulosa* among algae in the intertidal zone. The main ecological factors influencing the distribution of these three species are salinity and water movement (Sywula, 1964b, Muus, 1967).

All three species tolerate salinities higher than 35‰. Hence the lower limits are of more interest. Naylor (op. cit.) found that *I. viridis* tolerate 4-6‰ and *I. granulosa* 9‰, which agrees with the findings of Todd (1963). Sywula (1964b) distinguished between minimum absolute salinity which is what a given organism can tolerate periodically, and minimum mean salinity which is what is required to maintain life. *I. baltica* and *I. granulosa* showed the same tolerance, viz. a minimum absolute salinity of 4‰ and the minimum mean salinity should be above 4.25‰, whereas *I. viridis* has a minimum absolute salinity of 0.2‰ and required a minimum mean salinity above 3‰.

According to Muus (1967) *I. viridis* prefers shallow waters with irregular or regular renewal of water. Sywula (1964b) finds *I. viridis* in a few localities with strong water movements, but only near rivers where the salinity is fluctuating. *I. baltica* prefers continuous renewal of water whereas *I. granulosa* prefers strong currents or waves (Muus, 1967).

To further elucidate the ecological distribution of these three species, it was decided to compare their osmoregulatory abilities, under varying salinities. The paper is part of a larger comparative investigation of the ecophysiology of the three species. A comparison of the diel activities of *I. baltica* and *I. granulosa* is published elsewhere in this journal (Hørlyck, 1973).

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MATERIALS AND METHODS

I. baltica and *I. granulosa* were caught north of Helsingør at a depth of 0.5-5 metres. *I. viridis* was caught in Præstø fjord (southern Sjælland) at a depth of 0-1 metre. Both females and males were used. After collection all animals were placed in water of the same salinity as the biotope-water and acclimated to 10°C, at which temperature all experiments were done.

Two types of experiments were performed, steady-state experiments and experiments with sudden changes in the salinity. In the steady-state experiments, the animals were first acclimated as described above for at least 24 hours and then transferred to the experimental salinities. Each animal was placed in a 300 ml glass bowl with a glass lid in 200 ml water and with a piece of algae (*Furcellaria fastigiata*) as food and substrate. After at least 4 days, which was considered long enough for complete acclimation (see Todd, 1963), haemolymph samples were taken.

In experiments where changes in osmotic concentration of the haemolymph were followed, the animals were first acclimated 24 hrs. to 10°C as described above. They were then acclimated for at least one week in water of high/low salinity and then transferred to low/high salinity with one animal in each bowl.

Haemolymph samples were taken just before transfer to the new salinity, then once every hour for three hours, and after that at increasing intervals.

Lower salinity water was made by diluting sea-water with deionized water. Light/dark conditions in the coldroom was the same as the natural light/dark cycle. Only animals in intermoult stages were used.

Before the haemolymph samples were taken, the animals were washed in deionized water and gently dried with a Kleenex Medical Wipe and then placed under liquid paraffin. A haemolymph sample of about $0.03\ \mu\text{l}$ was taken directly from the heart with a capillary glass tube inserted from the dorsal side. The sample was sealed in both ends with liquid paraffin. Each animal was used once only.

The osmotic concentration of the haemolymph was determined by the freezing-point depression method, using a modified Ramsay (1949) apparatus, where the temperature gradient was regulated by a HETO gradient unit 01 PG 623. All haemolymph samples were measured within one hour after they had been taken.

The surface permeability of the animals was studied using the silver-staining technique as described by Croghan (1958). The animals were washed thoroughly in deionized water and then placed for five minutes in a $0.02\ \text{N}\ \text{AgNO}_3$ solution. The animals were then washed again and placed in a photographic developer (PQ universal) to reduce the silverions to metallic silver. To avoid the osmotic stress the process was repeated, but with a $0.02\ \text{N}\ \text{AgNO}_3$ sucrose solution isosmotic with the medium in which the animals were kept. Moreover, staining with KMnO_4 as described by Croghan (1958) was tried. All experiments were performed during the summer 1973.

RESULTS

The blood of *I. baltica* was hyperosmotic to the medium in the salinity range 7-30‰ (Fig. 1). Above 30‰ the blood was isosmotic to the medium. The mean value of the freezing-point depression ($\Delta\ ^\circ\text{C}$) of the haemolymph, corresponding to 7‰ medium, was 0.88, and the difference between the blood osmotic concentration and the medium reaches a maximum at this salinity. It was difficult to keep *I. baltica* in a medium with a lower salinity than 10‰. Still, with gradual changes of the medium over one month, it could be acclimated to 7‰.

When *I. baltica* was transferred from 24.5‰ to 6‰ (Fig. 2) the $\Delta\ ^\circ\text{C}$ of its haemolymph fell from 1.42 to about 1.0 after one hour. It then fell gradually during the next 20 hours to reach a steady-state value (mean $\Delta\ ^\circ\text{C} = 0.8$). After 72 hours all animals were moribund resulting in a drop in the blood concentration. This shows that *I. baltica* is unable to survive a sudden change to 6‰ as the one here performed. When *I. baltica* was transferred from 7 to 25‰ (Fig. 3) the $\Delta\ ^\circ\text{C}$ of its haemolymph rose from 0.71 to about 1.28 within one hour, and during the next 5 hours it reached the steady-state value (mean $\Delta\ ^\circ\text{C} = 1.38$).

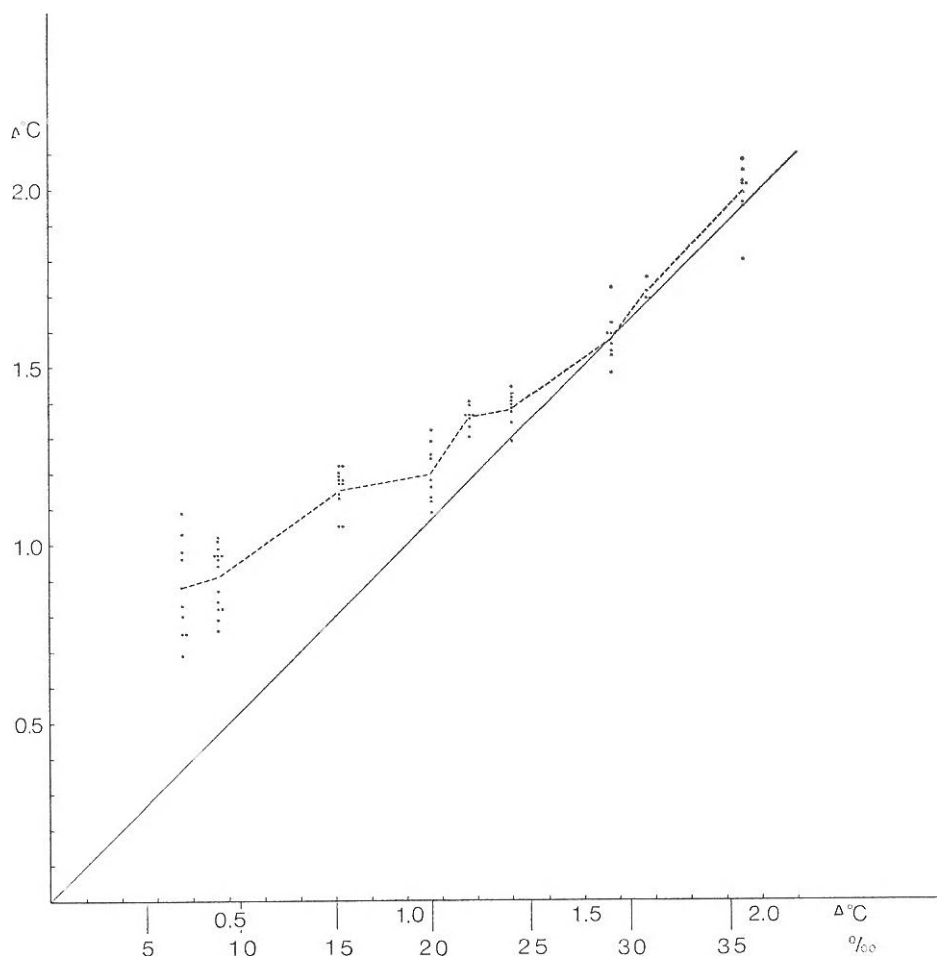


FIG. 1. The haemolymph osmotic concentration in *Idotea baltica* acclimated to different salinities.

The blood of *I. granulosa* was also hyperosmotic to the medium in salinities below 30‰ ($\Delta^{\circ}\text{C}$ at 30‰ = 1.60), and above this value the blood was isosmotic to the medium (Fig. 4). The lowest salinity in which *I. granulosa* could be kept alive under laboratory conditions was 9.3‰ which means that the $\Delta^{\circ}\text{C}$ of the haemolymph was found to be 0.85. The difference between the osmotic concentration of the blood and that of the environment reached a maximum in water of 10‰ ($\Delta^{\circ}\text{C}$ = 1.03).

When *I. granulosa* was transferred from 25 to 7‰ (Fig. 5) the haemolymph concentration dropped rapidly during the first hour (mean $\Delta^{\circ}\text{C}$ = 1.07). During the next 24 hrs it reached a value (mean $\Delta^{\circ}\text{C}$ = 0.7) which was kept until the animals died (LD 50 = 52 hrs.).

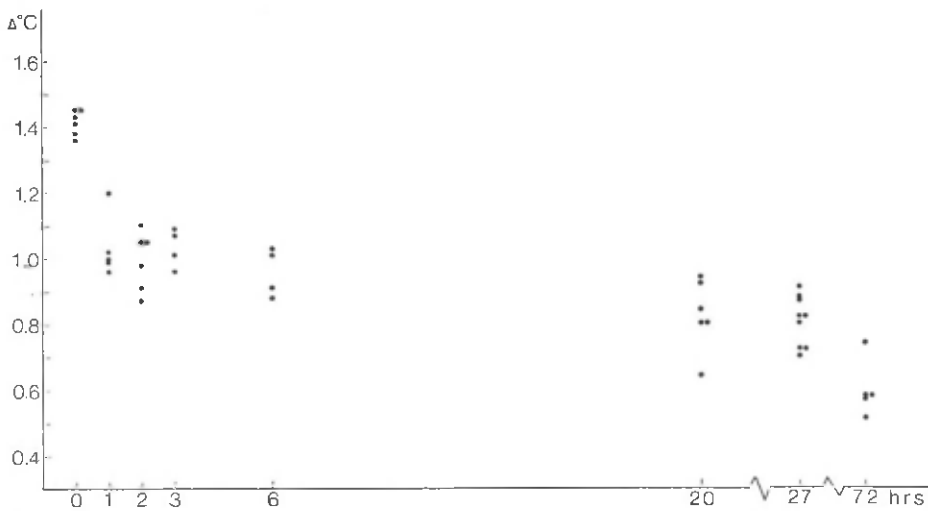


FIG. 2. The changes in haemolymph osmotic concentration of *Idotea baltica* acclimated to 24.5‰ and transferred to 6.0‰ salinity.

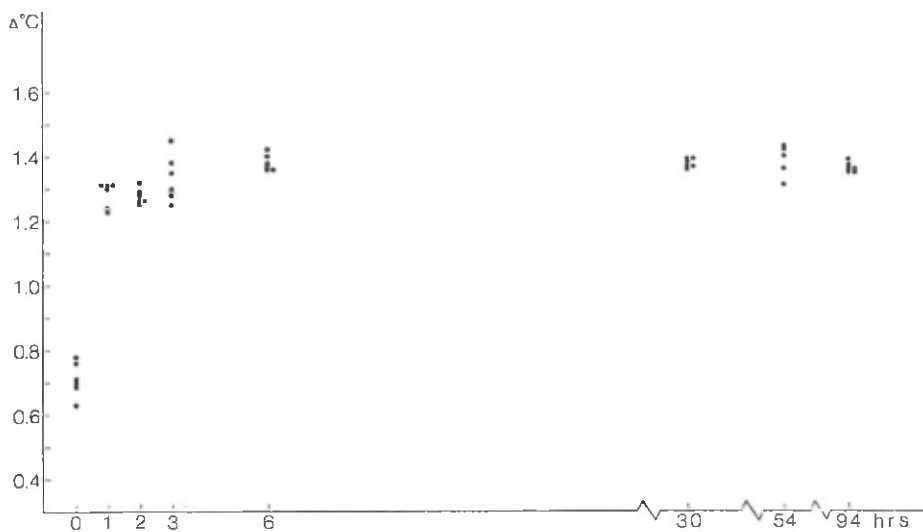


FIG. 3. The changes in haemolymph osmotic concentration of *Idotea baltica* acclimated to 7.0‰ and transferred to 25.0‰ salinity.

In *I. granulosa* acclimated to 9.3‰ and then transferred to 25‰ (Fig. 6) the blood concentration reached a steady-state level (mean $\Delta^\circ\text{C} = 1.38$) after 3 hrs. The animals regulated almost completely after one hour (mean $\Delta^\circ\text{C} = 1.32$).

I. viridis had a hyperosmotic haemolymph when kept in water of a salinity lower than 30‰ (Fig. 7) ($\Delta^\circ\text{C}$ at 30‰ = 1.62). The osmotic concentration of

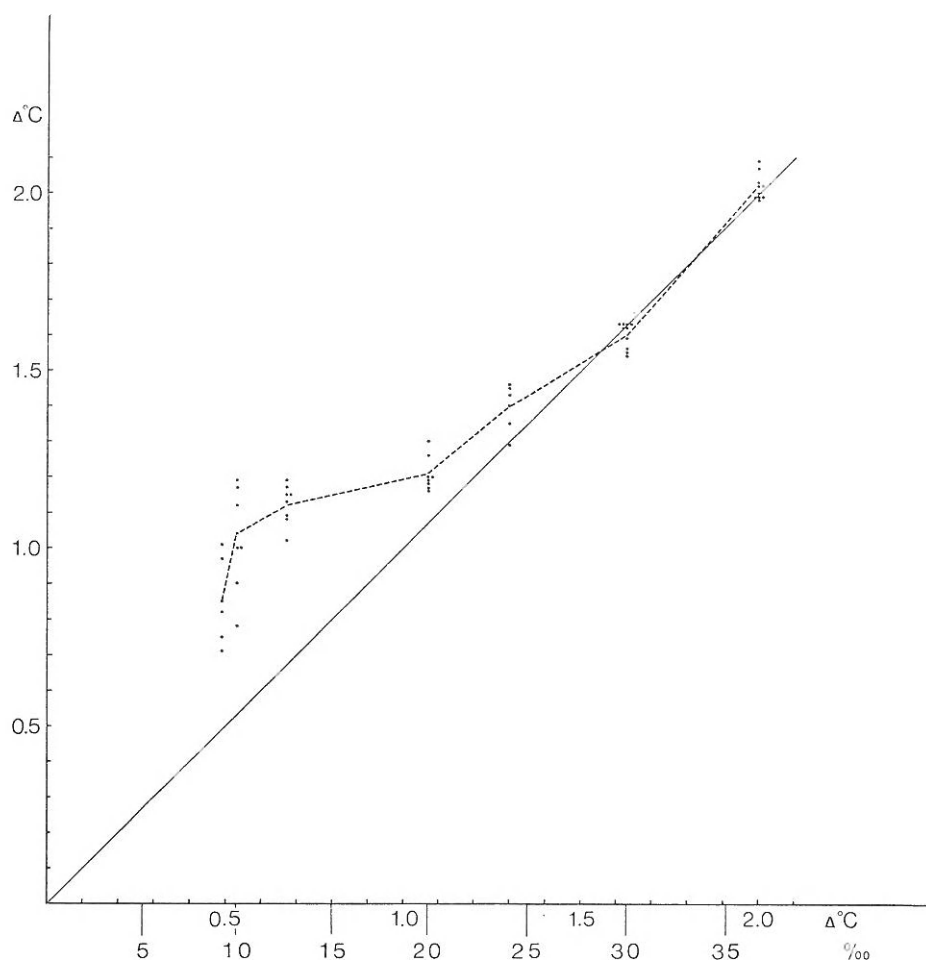


FIG. 4. The haemolymph osmotic concentration in *Idotea granulosa* acclimated to different salinities.

the haemolymph was practically constant in environmental salinities of 9-20‰, i.e. *I. viridis* was a 100% efficient osmotic regulator in this interval. Below 9‰ the blood osmotic concentration dropped, and in water of 5‰ the mean $\Delta^{\circ}\text{C}$ was 0.95. Haemolymph concentration in a medium of higher salinity than 30‰ was not measured, but it is probably isosmotic to the medium.

In the experiment where *I. viridis* was transferred from 25 to 10‰ (Fig. 8) the mean $\Delta^{\circ}\text{C}$ of the blood dropped from 1.5 to 1.35 during the first two hours reaching a steady-state value in 24 hrs (mean $\Delta^{\circ}\text{C} = 1.23$). In this experiment the initial value (time 0) of $\Delta^{\circ}\text{C}$ at 25‰ was slightly higher than the corresponding value found in the steady-state experiment.

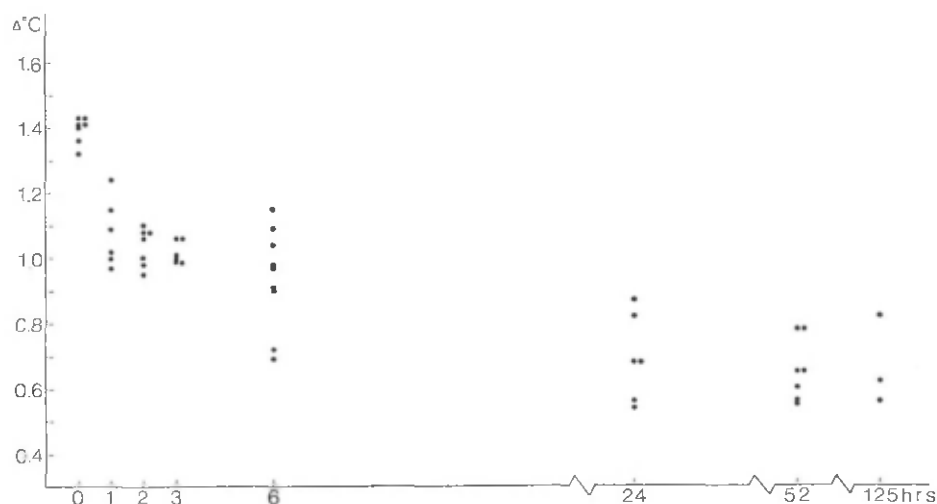


FIG. 5. The changes in haemolymph osmotic concentration of *Idotea granulosa* acclimated to 25.0‰ and transferred to 7.0‰ salinity.

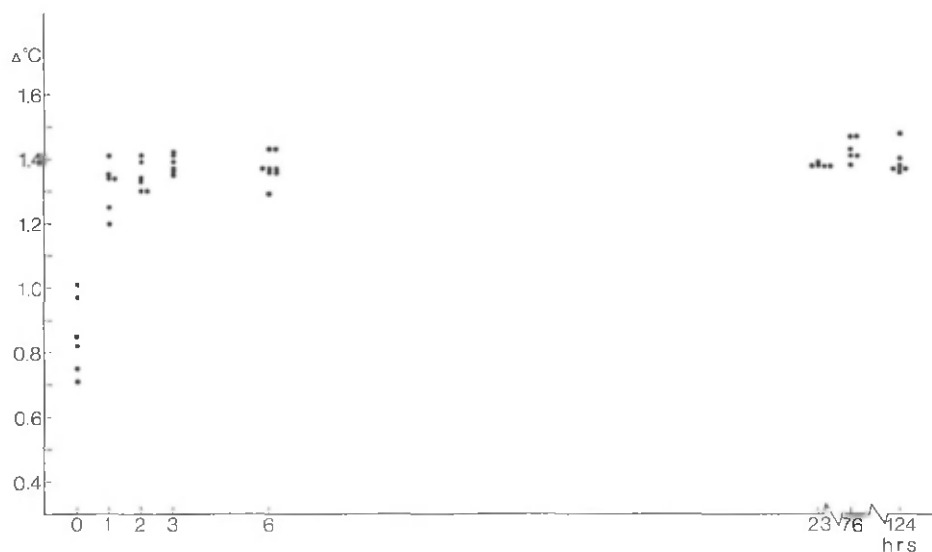


FIG. 6. The changes in haemolymph osmotic concentration of *Idotea granulosa* acclimated to 9.3‰ and transferred to 25.0‰ salinity.

In the opposite experiment where *I. viridis* was transferred from 9.8 to 24.8‰ (Fig. 9), the blood osmotic concentration was almost regulated within one hour from mean $\Delta^\circ\text{C} = 1.15$ to the new steady-state value ($\Delta^\circ\text{C} = 1.37$). During the next 45 hrs the animals hyperregulated slightly, and reached the steady-state value again after 73 hours ($\Delta^\circ\text{C} = 1.43$).

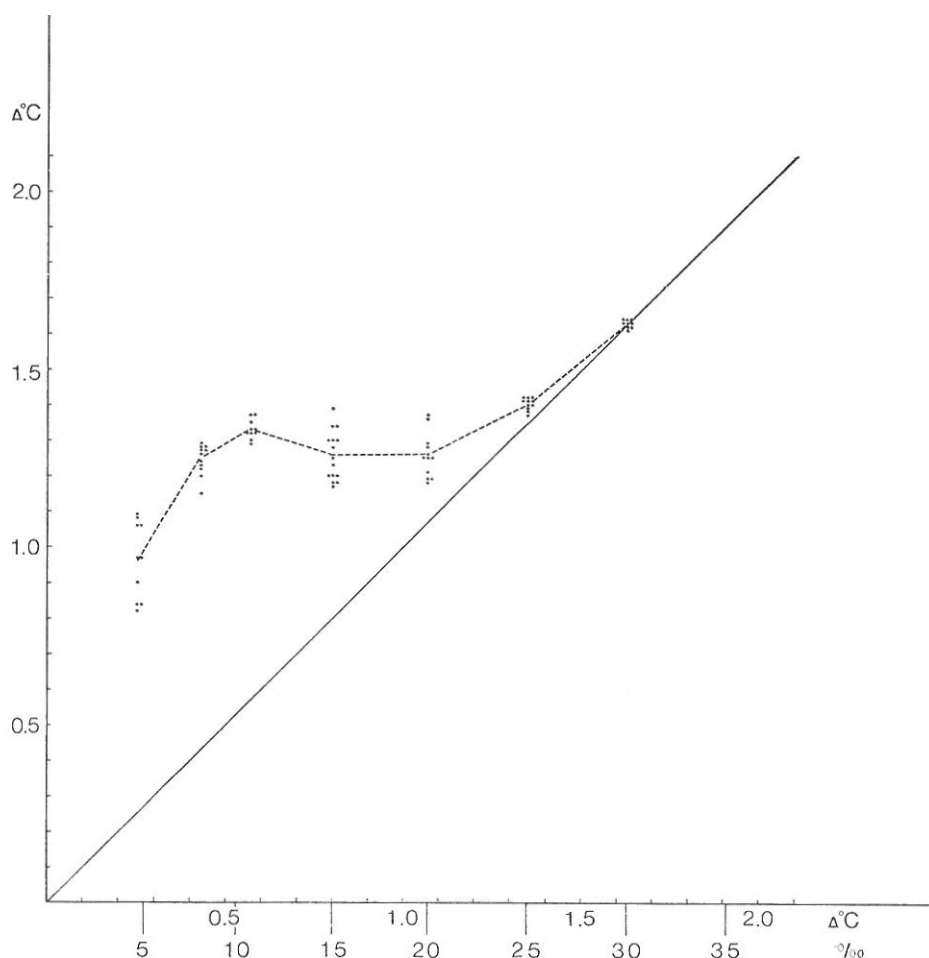


FIG. 7. The haemolymph osmotic concentration in *Idotea viridis* acclimated to different salinities.

Both silver staining experiments gave the same results, and all three species were stained in the same way. Only the endopodites of the three last pleopods and the "upper lip" were coloured. When the animals were treated with KMnO_4 only the endopodites of the last three pleopods were coloured. If the animals were kept for a longer period in AgNO_3 it was possible to stain the exopodites too.

DISCUSSION

All types of osmoregulatory patterns are found among crustaceans living in environments with fluctuating salinities. Animals with hyper-hypo regulation have been investigated by e.g. Wilson (1970), Hagerman (1971) and McLusky &

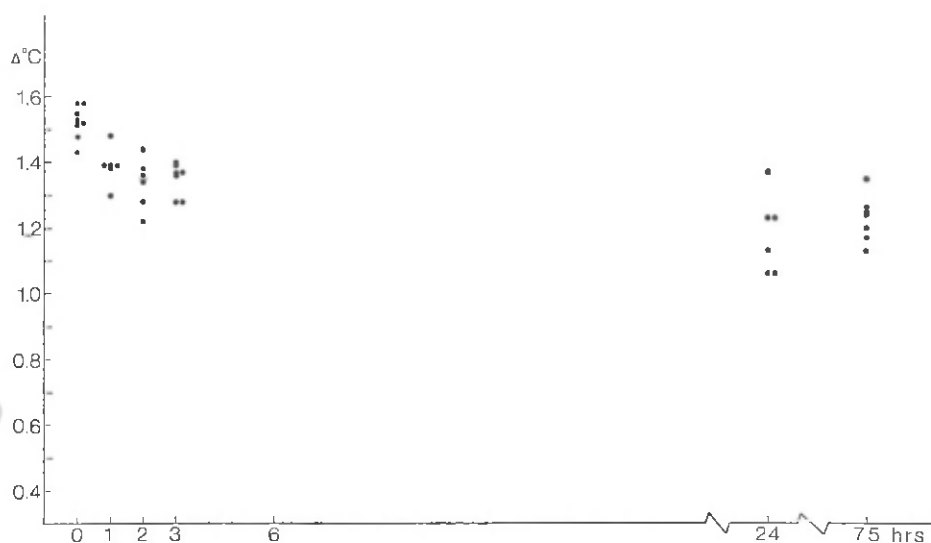


FIG. 8. The changes in haemolymph osmotic concentration of *Idotea viridis* acclimated to 25.0‰ and transferred to 10.0‰ salinity.

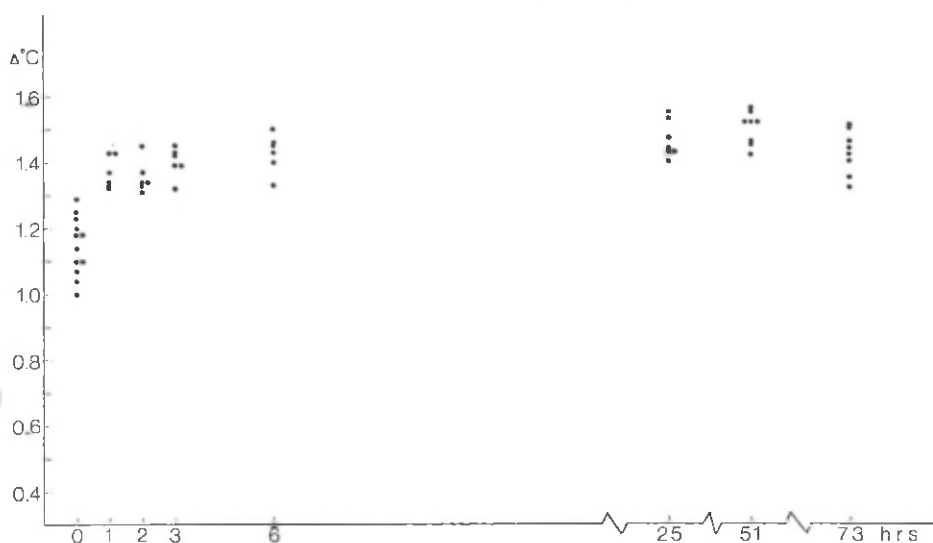


FIG. 9. The changes in haemolymph osmotic concentration of *Idotea viridis* acclimated to 9.8‰ and transferred to 24.8‰ salinity.

Heard (1971). Animals with hyperregulation have been reported on by Todd (1963), and the hyper-isosmotic type has been investigated by e.g. Lockwood (1959), Riegel (1959), Jones (1972), and Harris (1972). It is the investigations dealing with related species which are of special interest in connection with this paper.

Jones (1972) made an investigation on three species of the genus *Jaera*, and found that the osmoregulatory capabilities of these species were well correlated with their known ecological distribution, i.e. *Jaera ischioetosa* and *J. albifrons* are better regulators than *J. prae-hirsuta* and *J. forsmanni* in 5 ‰ sea-water at 17.5°C. This agrees with the fact that the two former species live under less stable conditions of salinity than the two latter. Jones (op.cit.) further found that *J. forsmanni* in 100 ‰ sea-water and at 15°C has a very high haemolymph osmotic concentration. This is a feature which resembles the condition found in *Ligia oceanica* (Todd, 1963). Common to both species is, that this is an adaption to semiterrestrial life. *J. forsmanni* is occasionally exposed to drier environments due to low tides. Wilson (1970) investigated two species of the genus *Ligia* from California, viz. *L. occidentalis* and *L. pallasii*. They were not hypersaline in 100 ‰ sea-water as was found for *L. oceanica* (Todd, 1963), but isosmotic. *L. pallasii* is a better regulator than *L. occidentalis* in water of lower concentration than 100 ‰ sea-water. They are both good hyporegulators in water of higher concentrations than 100 ‰ sea-water. According to Wilson (1970) this corresponds well with their ecology and behavior, i.e. *L. pallasii* is moving slower than *L. occidentalis* and must therefore stay longer under hyposaline conditions, whereas the faster *L. occidentalis* can alternate its activity between periods of foraging in drier terrestrial environments and periods in water of varying salinities to replace water lost by evaporation.

L. occidentalis is found on rocky shores from near the open ocean to almost fresh water, and the temperature varies tremendously where it lives. *L. pallasii* is found 5-20 feet above high tide mark, and the temperature conditions are not extreme. The animals are here often exposed to hyposaline conditions.

The isopods investigated in the present paper are of the hyper-isosmotic type, which seems to be the most common among isopods. All three species are osmoregulators in salinities lower than 30‰. However, there is a marked difference between *I. viridis* and the other two species. *I. viridis* is a significantly better regulator. There is no significant difference in the osmoregulatory capabilities between *I. granulosa* and *I. baltica* in the interval 10-30‰, but for *I. granulosa* there is a drop in blood concentration when the lower threshold of the salinity tolerated by this species is reached. However, contrary to me, Todd (1963) found that *I. granulosa* was hyperosmotic in the range 9-35‰. *I. baltica* continues to increase the difference between the haemolymph osmotic concentration and the concentration of the outer medium until the lower threshold is reached.

The physiological data of *I. baltica*, *I. granulosa* and *I. viridis* correspond well with the known ecological distribution of the three species with respect to salinity (Sywula, 1964b, Muus, 1967) as described in the introduction. The lower survival thresholds with respect to salinity found for these species agree with the theorem made by Muus (1967) which places *I. viridis* as the most tolerant and *I. granulosa* as the least tolerant species. The lower survival limits with respect to the salt

concentration found by me is higher than those found under natural conditions. This may be due to long-time adaptation in the local populations, and/or that the laboratory conditions are not adequate in all respects.

The fact that *I. viridis*, because it is adapted to more dilute water than the others, is a better osmoregulator has parallels in other species as mentioned above. Different populations of the same species may show different osmoregulatory abilities. Thus Riegel (1959) found that *Gnorimosphaeroma oregonensis*, which is widely distributed over the west coast of North America and may be found in estuaries and even in fresh water, was able to hyperregulate in diluted seawater, and that fresh water populations were better regulators than estuarine or bay-forms.

The same phenomenon has been found in two populations of *Mesidotea* (*Saduria*) *entomon*, viz. a fresh-water population from a Swedish lake and a population from the Baltic Sea (Lockwood & Croghan, 1957; Croghan & Lockwood, 1967). Theede (1969) found the same phenomenon for two populations of *Carcinus maenas* when he compared populations from the North Sea and the Baltic Sea.

It would be of interest to compare the osmoregulatory ability of *I. baltica* and *I. granulosa* from the inner Baltic with the Øresund population to see if the phenomenon is found here also.

In the silverstaining experiments only the gills and the "upper lip" were stained. The staining of the lip may be due to some special cuticular structure (porous matter containing water or chemoreceptors and the remaining NaCl will result in a staining). The T-shaped ventral patches found on the sterna of *Corophium volutator* when treated with AgNO_3 (McLusky, 1968) was not seen in any of the treated animals. The stainings indicate that the influx of water and ions mainly takes place through the surface of the endopodites of the last three pleopods, i.e. via the respiratory organs.

The investigations done on the ionic and the osmotic regulation of marine and brackish water isopods show that there is no significant difference between females and males or between animals of different sizes, which indicate that the osmoregulatory ability is fully developed also in the juveniles.

Although the physiological characters of the three *Idotea* species investigated to some extent explain the known ecological distribution of *I. viridis* versus the two other species, the experiments also show that other factors than the salinity must be involved as regards *I. granulosa* and *I. baltica*. Sywula (1964b) and Muus (1967) think there is a connection between water movement and the distribution of these two species. When found on the same locality *I. granulosa* prefers stronger water movements than *I. baltica*.

Where *I. baltica* and *I. granulosa* were collected for these experiments it was seen that they migrate from deeper waters to the coast and back again in an annual cycle, and that the time for this migration is not the same for both species.

Thus, it was noticed that *I. baltica* migrated earlier to the coast in the spring than *I. granulosa*, and that it returned to deeper waters before *I. granulosa*.

A further investigation of this problem is needed to get a better understanding of the ecology of the two species.

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