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Experimental studies on physiological and behavioural response mechanisms of *Nitocra spinipes* (Crustacea: Harpacticoidea) from brackish-water rockpools

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Abstract

Volume regulation and salinity-preference tests have been made on *Nitocra spinipes* Boeck (Crustacea, Harpacticoidea). This species is often dominant in Baltic brackish-water rockpools. The investigation attempts to evaluate the relative importance of some of the different response mechanisms of this species to salinity changes in relation to the unstable environmental conditions of the rockpools. Volume-regulation experiments have shown that *N. spinipes* is capable of hypoosmotic and probably hyperosmotic regulation in the tested salinity range of 1 to 20 ‰ S. In laboratory cultures, reproduction, hatching and moulting occurred in salinities ranging from 0.5 to 30 ‰ S. Preference experiments showed that *N. spinipes* has a very weak behavioural response even to very large variations in salinity concentrations between the alternatives; a significant choice could only be found under conditions which never occur in the natural biotope. It is therefore concluded that, in a biotope such as the rockpool, where salinity changes will affect the whole biotope rather than produce microclimatic variation, regulation and adaptation must have a higher ecological importance than escape responses.

Introduction

In the vicinity of the Askö Laboratory in the northern Baltic Sea proper, *Nitocra spinipes* Boeck (Crustacea, Harpacticoidea) is very common in several types of brackish-water rockpools, especially those with a luxurious growth of algae and with the bottom covered with a detrital layer. These pools have been thoroughly studied by Ganning (1971).

In brackish and estuarine habitats, salinity has often been regarded as the main factor which controls the distribution of the animals. Dussard (1967) considered *Nitocra spinipes* to be euryhaline, with a salinity optimum below that of marine water; this view corresponds with the opinion of Noodt (1956) and others. However, their conclusions are drawn from field distributions alone, and controlled laboratory experiments must be performed in order to distinguish between behavioural and physiological responses of the species to a single environmental factor such as salinity.

In a survey by Parry (1964), 5 different response mechanisms to osmotic changes in the environment are distinguished: (1) animals which survive only in an isotonic and isoosmotic medium, cannot tolerate any changes, and have no capacity to compensate for such changes; (2) animals which have some tolerance to slight changes but cannot control them; (3) animals which have a great tolerance to changes and which function as osmometers; (4) impermeable animals, with tissues which are not affected by external changes; (5) animals with an osmoregulatory capacity which protects the tissues from changes. This physiological approach is complemented by the more general ecological view of Kinne (1964) stating (1) escape, (2) reduction of contact, (3) regulation, (4) adaption, as the four different possibilities for animals to compensate for environmental changes.

This investigation attempts to evaluate the relative importance of the different response mechanisms of *Nitocra spinipes* in relation to the salinity conditions of the rock-pool biotope; it forms part of a research project concerning the ecology of brackish-water rockpools carried out at the Askö Laboratory.

Materials and methods

Most of the present information on osmotic regulation of crustaceans relates to the larger species, especially decapods. This is partly due to the difficulties in developing adequate techniques for studying the small, and especially the microscopic, forms. Of basic interest is information on the osmotic pressure of the body fluids in relation to the external medium. Although kryoscopic methods for direct measurement of the osmotic pressure of very small quantities of fluid (10^{-6} ml) have been described (Potts, 1952), indirect methods involving measurements of changes of body volume or weight are more convenient and give adequate results, at least for ecological purposes. The latter measurements can also be made on living animals under continuous observation. The volume-regulation techniques have been widely used and throughly discussed (e.g. Lange and Moostad, 1967; Lockwood, 1970). In the case of *Nitocra spinipes*, even very small changes of body volume can be detected due to the specific external morphology of the harpacticoid body. The body can be described as an elongated cylinder of segments of non-elastic cuticulae, connected with elastic membranes. If the volume is changed, the distances between the segments vary but not their diameters. Thus, the total length-variation is a very suitable and easily measurable parameter, which reflects even very small changes in body volume.

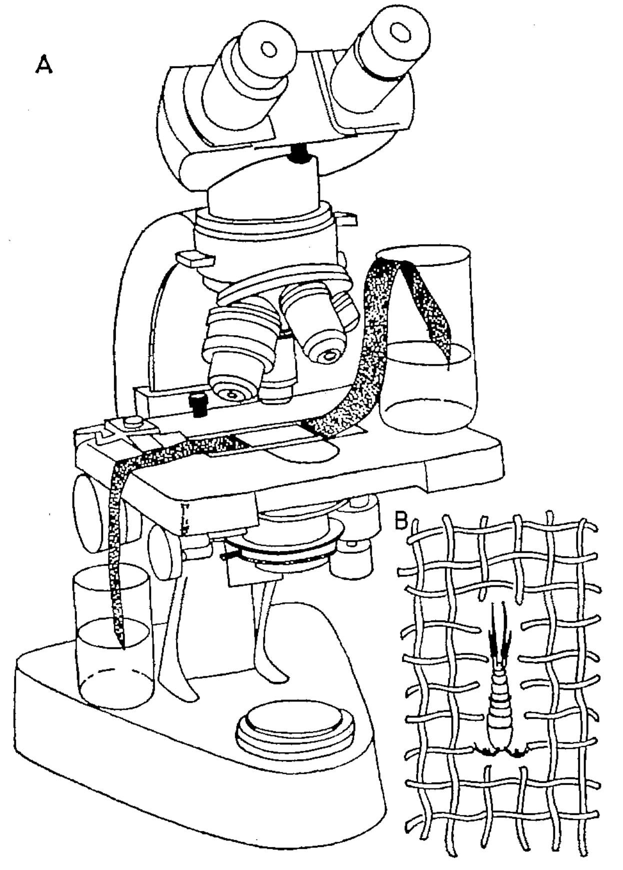

Fig. 1. (A) Experimental set-up of flow-through microaquarium on microscope table; (B) *Nitocra spinipes* placed in opening cut out of plankton gauze

The length variations of the crustaceans were measured through a microscope equipped with an eyepiece micrometer at 60X. In order to ensure a continuous supply of water at the desired salinities and to avoid lack of oxygen and accumulation of waste products, a special "microaquarium" was used, similar to that described by GUSTAFSON and KINNANDER (1956) (Fig. 1A). The device consists of a piece of plankton gauze, mesh size 150 μ, placed between a glass slide and a coverglass on the microscope table. Single parallel threads are cut out of the gauze, until a rectangular opening is formed. When the coverglass is placed in position, a microaquaria is formed with roughly the same space as the maximum volume of the animal to be tested and preventing it from bending or turning around during observation. Water is gently passed through the plankton gauze and the microaquaria through thick strips of filter paper connected to opposite ends of the gauze. If the salinity of the water is to be changed, both the filter-paper strips and the supply container are replaced with a new set; this makes it possible to obtain the new desired salinity concentration inside the microaquaria within 15 sec.

The salinity preference of *Nitocra spinipes* was tested in a chamber especially constructed for use with small, motile, aquatic animals. The apparatus and working procedures has been described by GANNING and WULFF (1966).

Five separate chambers were used simultaneously with 10 individuals each. The animals were originally taken from a brackish-water rockpool on the island of Vrångskär, in the vicinity of the laboratory. Laboratory cultures were set up containing animals, algae and detritus from the biotope and with varying salinities obtained by diluting sea water (35‰ S) from the Swedish West Coast with distilled water. All individuals used in the experiments were taken from these laboratory cultures which were maintained at constant salinities for 1 week prior to each experiment. The animals were held at room temperature (20 °C) both in the cultures and during the experiments. The salinity was measured with the aid of a wheatstone bridge connected with an immersion cell, as described by JANSSON (1962).

Results and discussion

Salinity tolerance

In the laboratory cultures, reproduction, hatching and moulting occurred at all the salinity concentrations used, e.g. between 0.5 and 30.0‰ S. CLARK (1968) investigated the upper tolerance limit for *Nitocra spinipes* and found it to lie between 40 and 50‰ S at 10° to 15 °C. The survival time was only slightly shorter in 0.5‰ than in 30‰ S. GANNING (1971) has studied the environmental conditions in a number of brackish-water rockpools in the northern Baltic Sea. In pools where *N. spinipes* could be found, salinity normally varied between 3 and 25‰ S; he recorded a maximum alteration of 5‰ S. day^{-1} in the smallest pools.

Volume regulation

Fig. 2 shows a typical result from one of numerous volume-length regulation experiments with *Nitocra spinipes*. The length of the crustacean is expressed as scale units of the eyepiece micrometer (ordinate) but in the further discussion, the length variations are referred to as volume variations. When the animal, adapted to 10.6‰ S, is transferred to 5.6‰ S, it rapidly swells due to osmotic water-inflow; it reaches a maximum volume and then begins to regain its original body volume. This may be accomplished by excreting hypotonic urine together with an active uptake of ions from the external medium. On the other hand, the return to the original body size can be accomplished solely by passive processes: by outward diffusion under pressure through the membranes; this will stop at approximately the original body size, when the hydrostatic pressure of the internal medium is at the same level as that of the external medium. Fig. 2 also shows that the animal immediately shrinks when finally exposed to its original medium. This indicates that the animal has a lower internal osmotic pressure than at the beginning of the experiment. Apparently, the membranes must be permeable both to water and, to a lesser extent, to ions. Passive diffusion under pressure is possible, and *N. spinipes* is, therefore, not a perfect osmometer. According to POTTS and PARRY (1964), this is the normal condition of most invertebrate animals.

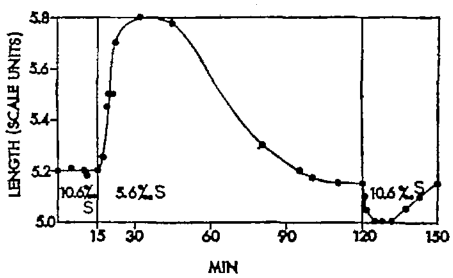

Fig. 2. *Nitocra spinipes.* Volume alterations of individual adapted to 10.6 ‰ S, exposed to 5.6 ‰ S, and finally to 10.6 ‰ S

These volume regulation experiments could only be conducted for a few hours because of the possible damage to animals after longer exposure. Reactions to sudden exposure to various salinities do not explain if crustaceans are able to regulate the osmotic pressure of their body fluids. Long-term responses to salinity alterations relevant to natural environmental conditions must be studied. However, by using animals well adapted to different salinities and by observing only their initial change in body volume when exposed to other salinity concentrations, it is possible to determine the relative osmotic pressure of the body fluids in relation to that of the external medium.

Results from such experiments are shown in Fig. 3. Individuals from 7 laboratory cultures with salinities between 0.7 and 19.4‰ S were used. The salinity-concentration interval within which the crustaceans neither swelled nor shrunk was determined. The extent of this interval is quite large, partly because of the difficulties in observing the smallest changes in body volume which actually occur, and partly because of the animals possible capacity of immediate adjustment of the volume at minor changes of the salinity. Despite this, the results clearly show that *Nitocra spinipes* have a lower internal osmotic pressure than that of the culture water to which they were adapted in the salinity range of 8‰ S to at least 20‰ S. Individuals adapted to salinities less than 6‰ S showed no change in volume when exposed to test media with salinities inside the range of 6.2 and 0.4‰ S. However, this must be attributed to a highly effective and immediate osmoregulatory capacity rather than to isotonicity, as it is not likely than the body fluids are isotonic at such low salinities; even fresh-water

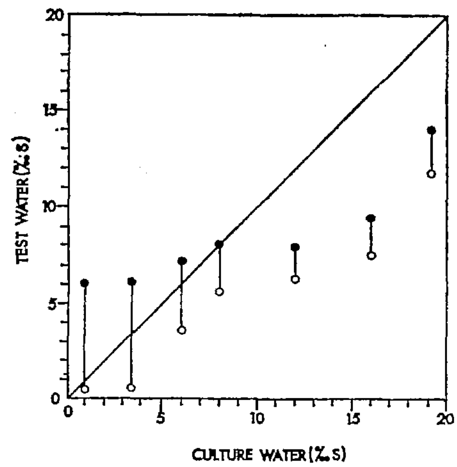

Fig. 3. *Nitocra spinipes.* Salinity intervals within which no swelling (open circles) or shrinking (filled circles) was found upon exposure to varying test salinities (ordinate) and adaptation to different salinites (abscissa)

crustaceans have an internal osmotic pressure corresponding to salinities higher than 7‰ S (POTTS and PARRY, 1964). It is, therefore, likely than *N. spinipes* is capable of hyperosmotic regulation as well as of hypoosmotic regulation.

Salinity preference

Numerous experiments were run to discover the preferred salinity range of *Nitocra spinipes*. Tests were performed with individuals adapted to different salinities in order to see whether salinity preference changed with biotope salinity. However, no clear picture could be obtained due to the weak behavioural response of this species to salinity gradients. To illustrate this, some of the results are presented in Fig. 4A, B, C, D. One of the alternatives had the same salinity as the biotope in all 4 experiments. The graphs show the number of animals in the correspond-

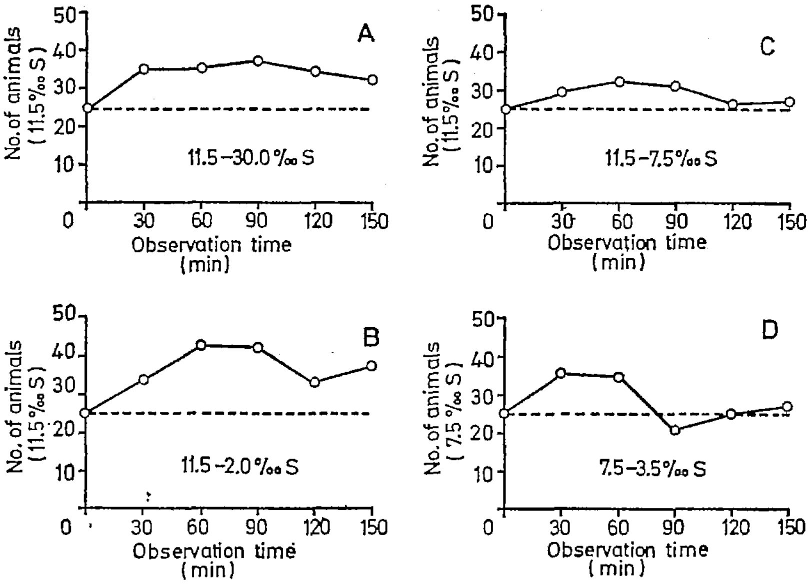

Fig. 4. *Nitocra spinipes*. Salinity-preference tests. Five chambers with 10 individuals each were used simultaneously. For explanations see text

ing half of the alternative chamber (ordinate) plotted against time of observation (abscissa).

One of the basic concepts concerning preference tests is to allow the animals to choose between adequate alternative concentrations. The animals must be able to move around freely within the whole chamber and neither of the alternatives must cause inactivity. With *Nitocra spinipes*, a difference in salinity of 18.5‰ S could be used without any harm to the animals, as shown in Fig. 4A. If a greater difference between the alternatives was used, some of the animals became inactive when approaching the salinity gradient. However, in this experiment, all the animals moved freely between the alternatives, and a clear preference ($\chi^2 > 3.9$, $p < 0.05$ at all observations) for the 11.5‰ S alternative was shown. In Fig. 4B, a rather clear choice of 11.5‰ S ($\chi^2 > 2.3$, $p < 0.20$ at all observations) was shown by the crustaceans when offered an alternative of 2.0‰ S: a salinity difference of 9.5‰ S. However, if the difference in salinity between the alternatives is further reduced, as shown in Fig. 4C with the alternatives of 11.5 and 7.5‰ S, an initial preference for the biotope salinity ($\chi^2 > 3.9$, $p < 0.05$ after 60 min) is progressively diminished. The same tendency is illustrated in Fig. 4D, where the animals are adapted to 7.5‰ S, and are offered the alternative of 3.5‰ S with an initial, but later vanishing preference for the biotope salinity ($\chi^2 > 8.0$, $p < 0.01$ at 60 min). Apparently, a difference of 4‰ S is well within the crustaceans osmoregulatory capacity, even upon sudden exposure (as shown by the volume-regulation experiments), and an escape reaction is not necessary.

These experiments show that, in order to get a clear, lasting behavioural response of this species to salinity gradients, very large differences in salinity between the alternatives must be used. However, from an ecological point of view, such experiments are quite useless, since the animals will never meet with such conditions in the field.

Studies on the environmental conditions in various rockpools in the Askö area by Ganning (1971) showed that salinity stratification seldom occurs. The vertical salinity gradients which are sometimes found in the pools during calm weather, after heavy rain, are very unstable, and the difference in salinity between surface and bottom water never exceeds a few permille. The salinity-preference tests presented here can be compared with those made by Jansson (1968) on harpacticoids from a sandy beach at Askö. These interstitial species had a much more characteristic behavioural response towards salinity than *Nitocra spinipes*, and they distinguished between much smaller differences in salinity concentrations in an alternative chamber. *Scizopera baltica* Lang, for instance, showed a significant choice between 2.5 and 1.2‰ S, and *Nitocra fallaciosa* Klie significantly chose between various combinations of salinities with a difference of 5‰. In contrast to the rock-pool biotope, a sandy beach has not only a highly unstable microenvironment, but is also characterized by strong gradients within short distances (Jansson, 1966, 1967). In such a biotope it must be advantageous for animals to respond to changes in the salinity gradient by migration. In the rockpools, such escape responses are of no use, since salinity changes will affect the whole biotope in the same magnitude. The salinity preference tests also show that *N. spinipes* lacks this ability to respond to different salinity concentrations, at least within the ecologically important range. On the other hand, rearing in laboratory cultures, salinity resistance and volume-regulation experiments show this species' ability to survive and even breed in a salinity range of 0.5‰ S to at least 30‰ S, due to its great adaptational and osmoregulatory ability.

Summary

1. In brackish-water rockpools in the northern Baltic Sea, *Nitocra spinipes* BOECK is often the dominating species in pools of a salinity varying between 3 and 35‰ S.

2. This species was shown to survive and reproduce in laboratory cultures with salinity concentrations well beyond the range found in the natural biotope.

3. Volume-regulation experiments revealed a strong osmoregulatory capacity in *N. spinipes*, but in salinity-preference experiments, behavioural responses occurred only after salinity variations so great that they never occur in the rockpools studied.

4. The results of these laboratory experiments indicate that, in a biotope such as a rockpool, where salinity alterations affect the whole biotope rather than produce microclimatic variation, regulation and adaptation are more important than escape responses.

Literature cited

CLARK, M. E.: The ecology of supra-littoral rockpools with special reference to the copepod fauna, Ph. D. thesis. 108 pp. University of Aberdeen, Scotland 1968.

DUSSART, B.: Les copepodes des eaux continentales, pp 212—214. Paris: N. Boubée & Cie 1967.

GANNING, B.: Studies on chemical, physical and biological conditions in Swedish rockpool ecosystems. Ophelia **9**, 51—105 (1971).

— and F. WULFF: A chamber for offering alternative conditions to small motile aquatic animals. Ophelia **3**, 151—160 (1966).

GUSTAFSON, T. and H. KINNANDER: Microaquaria for time lapse cinematographic studies of morphogenesis in swimming larvae and observations on sea urchin gastrulation. Expl. Cell Res. **11**, 36—51 (1956).

JANSSON, B.-O.: Salinity resistance and salinity preference of two oligochaetes *Aktedrilus monospermatecus* KNÖLLNER and *Marionina preclitellochaeta* n.sp. from the interstitial fauna of marine sandy beaches. Oikos **73**, 292—305 (1962).

— Microdistribution of factors and fauna in marine sandy beaches. Veröff. Inst. Meeresforsch. Bremerh. (Sdbd) **2**, 77—86 (1966).

— Diurnal and annual variations of temperature and salinity of interstitial water in sandy beaches. Ophelia **4**, 173—201 (1967).

— Quantitative and experimental studies of the interstitial fauna in four Swedish sandy beaches. Ophelia **5**, 1—71 (1968).

KINNE, O.: Physiologische und ökologische Aspekte des Lebens in Ästuarien. Helgoländer wiss. Meeresunters. **11**, 131—156 (1964).

LANGE, R. and A. MOOSTAD: Cell volume regulation in osmotically adjusting marine animals. J. exp. mar. Biol. Ecol. **7**, 209—219 (1967).

LOCKWOOD, A. P. M.: The involvement of sodium transport in the volume regulation of the amphipod crustacean, *Gammarus duebeni*. J. exp. Biol. **53**, 737—751 (1970).

NOODT, W.: Verzeichnis der im Eulitoral der schleswigholsteinischen Küsten angetroffenen Copepoda Harpacticoidea. Schr. naturw. Ver. Schlesw.-Holst. **28** (1), 42—64 (1956).

PARRY, G.: Organ systems in adaption: the osmotic regulating system. *In:* Adaption to the environment, pp 245—257. Baltimore: Williams & Wilkins Co. 1964.

POTTS, W. T. W.: Measurement of osmotic pressure in single cells. Nature, Lond. **169**, p. 834 (1952).

— and G. PARRY: Osmotic and ionic regulation in animals, 402 pp. London: Pergamon Press 1964.

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