OSMOREGULATION BY AN ESTUARINE AND A MARINE SPECIES OF *UPOGEBIA* (ANOMURA, CRUSTACEA)

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INTRODUCTION

Studies on osmoregulation by decapod Crustacea are largely based upon the Brachyura and Macrura. Little attention has been given to the Anomura, probably due to availability of material. In southern Africa there are two common species of the burrowing anomuran *Upogebia*, namely *U. africana* (Ortmann) and *U. capensis* (Krauss). Barnard (1950) pointed out that *U. africana* might merely be a variety of *U. capensis* although it was possible to distinguish the two on the basis of morphology and distribution. *U. capensis* is exclusively marine whereas *U. africana* is mainly an inhabitant of estuaries.

Along the south-eastern coast rainfall is erratic. There is very little steady runoff and estuarine salinities approximate sea water. Macnae (1957) reported that between one third and one half of the annual rainfall comes over a few days. At such times rivers come down in spate causing flooding of the estuaries with fresh water. The flooding is of short duration and within a few days or weeks at most, the strong tidal flows re-establish the normal salinity regime.

Animals such as *U. africana*, therefore, living in these estuaries must be able to cope with reduced salinities even although they live in a salinity greater than 30‰ for most of the year. In contrast, *U. capensis* lives subtidally and in sheltered areas of the intertidal zone of the sea where they are rarely exposed to low salinities. It appeared that a comparison of the tolerance of these two species to low salinity and a determination of their osmoregulatory ability would be of considerable interest both in extending our knowledge of anomuran osmoregulation as well as clarifying the validity of the taxonomic separation of these two species. In addition experimental studies were undertaken to establish the effect of reduced salinity on moulting in *U. africana*.

MATERIAL AND METHODS

The salinity of water samples was estimated by titration using Harvey's modification of the Mohr method as described by Barnes (1959). Silver nitrate solutions were standardised against sea water from the Hydrographic Laboratory in Copenhagen.

Samples of *U. africana* were collected by digging in the intertidal mudbanks in the Kowie estuary at Port Alfred (33° S, 27° E). Although primarily a west coast species *U. capensis* occurs in the intertidal zone at Port Alfred where it constructs mud lined burrows in shelly grit in rock crevices. Large numbers of *U. africana* were available but only few *U. capensis* and, as

a result, the experiments on moulting and salinity tolerance reported below were conducted using *U. africana*. Measurements of osmotic pressure of blood were performed on both species.

Prawns used in survival experiments were randomised into batches of 10 using the technique described by Allanson and Noble (1964), and kept in covered borosilicate glass dishes containing four litres of aerated water. Experiments were carried out at ambient temperature (18–22° C) and were started within 24 hours of collection of animals. Sea water dilutions were made up from water collected at Port Alfred (salinity 34–35‰), diluted with glass distilled water.

The osmotic pressure was deduced from a measurement of the freezing point depression of blood samples using the method described by Ramsay and Brown (1955). Animals were kept under the same conditions as in survival experiments. Blood samples were collected from prawns by first blotting dry the arthrodial membrane at the base of the fifth periopod and puncturing it with a silica capillary into which the blood was drawn. Samples were stored at −10° C and the freezing point depression determined within 12 hours of sampling.

The effect of low salinity on moulting frequency was determined on a batch of 75 *U. africana* kept in a salinity of 1,7‰ under the same conditions as described for survival experiments except that 25 animals were put into each dish. A control of 37 prawns was kept in sea water. Records were kept of moults and whether the animals died or survived ecdysis. The experiment was terminated after 690 hours when control animals began to die.

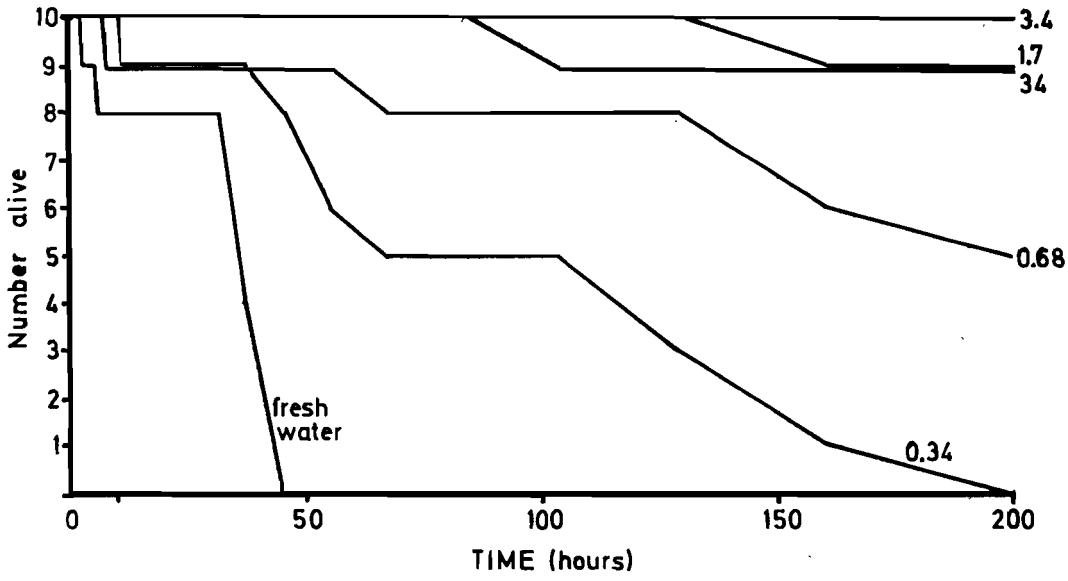

FIGURE 1
Survival of batches of *Upogebia africana* exposed to a series of salinities. The salinity in parts per thousand is shown next to each survival curve.

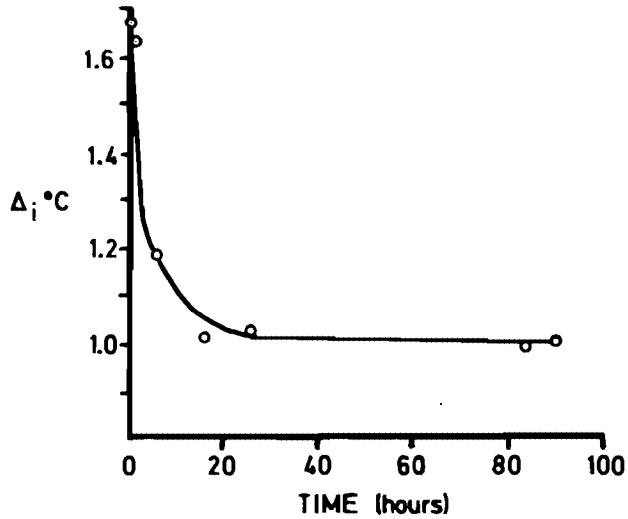

FIGURE 2
Freezing point depression ($\triangle_i$°C) of blood of individual *Upogebia africana* after exposure to a medium of freezing point depression 0,19°C (3,4‰) for various periods of time.

RESULTS

Survival in Low Salinity of U. africana

No difference in survival in a salinity of 1,7‰ was found between males and females, nor was there any size effect. The absence of a size effect could have been due to the lack of small animals used in experiments since prawns between only 10 mm and 20 mm carapace length were used. Survival in salinities ranging from full sea water down to 1,7‰ was similar (Fig. 1), but in salinities of 0,68‰ to fresh water it was reduced. A salinity of 1,7‰ appeared to be a critical value, below it prawns died fairly rapidly, above it at least 80% of the sample survived for 200 hours.

Osmoregulation

The ability of *U. africana* to osmoregulate was deduced from measurement of the freezing point depression of blood samples of animals kept in a salinity of 3,4‰ for varying periods of time. When the animals were put into the dilute medium there was a rapid reduction in osmotic concentration of the blood (Fig. 2). After about 20 hours the osmotic concentration reached a steady state ($\triangle_i$ 1,0° C) which was maintained for at least 90 hours. Since the freezing point depression of the medium ($\triangle_e$) was only 0,19° C, the prawns were maintaining a large osmotic gradient between the blood and medium. *U. africana* clearly regulates hyperosmotically.

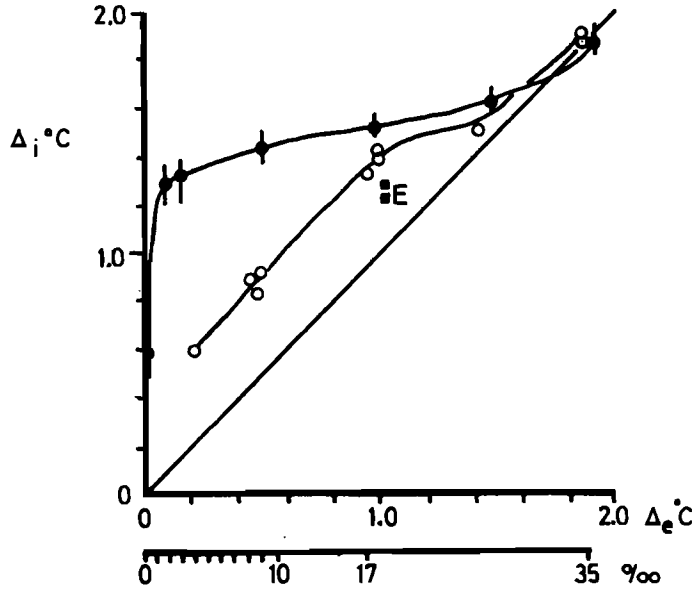

FIGURE 3

Freezing point depression ($\triangle_i$°C) of blood of *Upogebia africana* (● indicates mean of five animals), and individual results of blood of *U. capensis* (O) in a range of salinities ($\triangle_e$°C). Vertical bars indicate range of readings for *U. africana*. Points marked E represent freezing point depression of blood drawn from two specimens of *U. africana* within one hour of ecdysis in a salinity of 19‰.

A comparison of the osmotic concentration of the blood of *U. africana* and *U. capensis* kept in various salinities for at least 24 hours (Fig. 3) shows that in sea water both species were virtually isosmotic but in salinities below 30‰ ($\triangle_e$ 1,5°C) they regulated hyperosmotically. In the case of *U. capensis* a plateau of internal concentration was maintained down to 18‰ ($\triangle_e$ 1,0° C), whereas in *U. africana* this plateau was maintained down to 1,7‰ ($\triangle_e$ 0,1° C). Below these concentrations both species showed a marked fall off in internal osmotic concentration although they were still hyperosmotic to the medium. *U. capensis* survived for a maximum of only 36 hours in salinities below 3,4‰.

A comparison of the $\triangle_i$ of blood of *U. africana* at 3,4‰ in Fig. 2 and Fig. 3 shows a discrepancy. This may be the result of a seasonal effect since the results shown in Fig. 2 were obtained from animals collected in winter (August) whereas those in Fig. 3 were from summer animals (November).

Moulting in Low Salinity

The survival of *U. africana* exposed to 1,7‰ is shown in Fig. 4 in which the percentage survival is plotted on a probit scale. The first moult in this low salinity occurred after 339 hours had elapsed and this individual subsequently died at 355 hours. Thereafter every death in 1,7‰ was associated with moulting, either at ecdysis or within 24 hours of ecdysis. In contrast

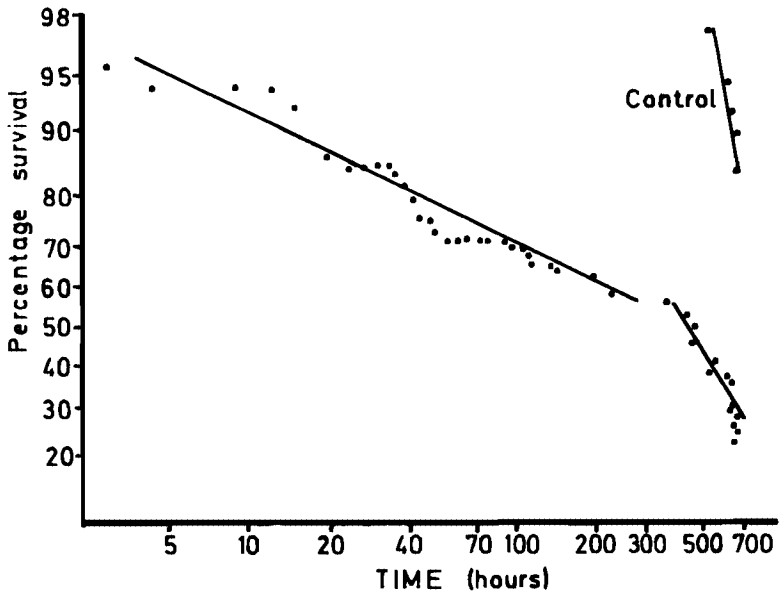

FIGURE 4
Percentage survival (on a probit scale) of *Upogebia africana* in a salinity of 1,7‰. Control kept in sea water.

26% of the control animals in sea water had successfully moulted before 350 hours. No deaths occurred in the control before 540 hours by which time 43% had successfully moulted. The split probit in Fig. 4 suggests two causes of death. Up to 350 hours, deaths were probably due to animals succumbing to the general osmoregulatory stress resulting from the low salinity. Every death in 1,7‰ after 350 hours was associated with ecdysis and this is the point at which the survival curve exhibits a sharp change in slope. Although *U. africana* can live in a dilution of 1,7‰ for a long period (median time of survival 420 hours), it cannot survive a moult in this salinity. In addition it appears that since 26% of the control animals in sea water had successfully moulted before the first moult in 1,7‰, moulting can be delayed in low salinity.

The lowest salinity at which moulting can successfully occur was established by maintaining four batches, each of 18 animals, at different salinities. A record was kept of all moults for a period of 50 days and if the moulted prawn survived, the moult was described as successful. *U. africana* successfully moulted in salinities down to 3,4‰ (Table 1), although the number of prawns which attempted ecdysis was less than in higher salinities. In 3,4‰ only 28% had moulted after 50 days whereas in sea water 72% had moulted after this time. Table 1 supports the suggestion that *U. africana* may delay ecdysis in low salinity since the first moult in 8,5‰ and 3,4‰ only occurred after 17 days in contrast to the two-day delay in full sea water and 17‰.

DISCUSSION

Earlier work on the osmoregulatory capacity of prawns of the genus *Upogebia* has been reviewed by Thompson and Pritchard (1969). These authors showed experimentally that at least two species of *Upogebia*, namely *U. pugettensis* and *U. affinis* were strong hyperosmotic regulators in salinities below 75% sea water, in contrast to earlier suggestions that the genus *Upogebia* was stenohaline (Gross 1957). The results reported in this paper clearly support Thompson and Pritchard's contention that members of the genus *Upogebia* should be considered as euryhaline animals.

A comparison of the four species of *Upogebia* which have been investigated shows that *U. africana* apparently has the most marked osmoregulatory capability. This species is also

TABLE 1

PERCENTAGE FREQUENCY AND SUCCESS OF MOULTING BY *Upogebia africana* IN VARIOUS SALINITIES OVER A PERIOD OF 50 DAYS. EIGHTEEN ANIMALS WERE USED IN EACH SALINITY

Salinity ‰	*First moult* (*days*)	*Percentage of prawns attempting moult*	*Percentage of prawns which successfully moulted*
34	2	72	67
17	2	56	56
8,5	17	44	39
3,4	17	28	17

the most tolerant of lowered salinities surviving a salinity of 1,7‰ for long periods (Median time of survival 420 hours) whereas *U. capensis*, *U. affinis* and *U. pugettensis* all have a lower lethal limit of 3,4‰ or higher. The greater tolerance to low salinity and the better developed osmoregulatory ability of *U. africana* relative to *U. capensis* accords with their distribution, *U. africana* being estuarine and thus periodically exposed to low salinity whereas *U. capensis* is marine.

This of course raises the question, to what extent is a burrowing animal exposed to transient salinity changes in water overlying the burrow. Laboratory observations on *U. africana* indicated that if fresh water is introduced into an aquarium, pumping is only partially interrupted. After a period of about 30 minutes during which pumping is intermittent, the animals pumped fresh water through their burrows. Clearly in a flood, *U. africana* will be exposed to low salinity. Thompson and Pritchard reported that following upon a flood the burrows of *U. pugettensis* contained water of low salinity.

Although *U. africana* can tolerate a salinity of 1,7‰ it can only survive a moult in a salinity of 3,4‰. Similar results have been reported by McLusky (1967) who found that the

amphipod *Corophium volutator* could only successfully moult in a narrower salinity range than that normally tolerated. The cause of death of *Upogebia* at ecdysis in low salinity is probably dilution of the blood. Not only is the new cuticle more permeable but at ecdysis, decapod Crustacea take up water through the gut in order to increase their total volume. Robertson (1937) found that a 50 g *Carcinus* absorbed about 35 g of water at moult and *Cancer* (Drach 1939) showed a ninefold increase in blood volume during ecdysis. If the crustacean is hyperosmotic this uptake of medium would cause a decrease in osmotic pressure of the blood. This decrease is clearly shown by the points marked E in Fig. 3 which represent the freezing point depression of the blood of two specimens of *U. africana* immediately after ecdysis in low salinity. Thus when *U. africana* moults in a low salinity the uptake of medium causes a dilution of the blood. The degree of dilution is obviously related to the osmotic concentration of the surrounding medium and in salinities below 3,4‰ apparently reaches lethal values.

Moulting is delayed in low salinities. The selective advantage of this in view of the sensitivity of the animals to low salinities at ecdysis, is obvious. Passano (1960) stated that moulting is frequently delayed by unfavourable conditions but that salinity does not appear to affect moult. More recent work, however, (McLusky 1967) shows that it does reduce the frequency of moulting in amphipods.

The difference in osmotic properties of the blood indicates that there are physiological differences between *U. africana* and *U. capensis*. This, together with differences in morphology and distribution (Barnard 1950), constitute a strong case for the retention of *U. africana* as a species distinct from *U. capensis*.

Although the lower lethal salinity is different for *U. africana* and *U. capensis*, in both species death occurs at a similar internal osmotic concentration, namely when the Δ_i is about 0,5–0,7° C. Apparently these two species have a common tissue sensitivity to dilution of the blood. In addition, the differences in the curves of osmotic concentration shown in Fig. 2 are quantitative rather than qualitative. Both species are nearly isosmotic in sea water and both are hyperosmotic in a salinity less than 30‰. They both show a "plateau" of fairly constant internal concentration (Δ_i 1,4–1,6° C) in a limited salinity range below 30‰. This condition is different to that seen in *U. affinis* which has no plateau, and *U. pugettensis* where there is only a slight indication of this effect. Only in salinities below 18‰ did the concentration of *U. capensis* blood fall off more rapidly than that of *U. africana*. Despite the differences between them, *U. africana* and *U. capensis* are physiologically and morphologically more similar and thus probably more closely related than either is to *U. affinis* or *U. pugettensis*. It is suggested therefore that *U. africana* probably evolved from that part of the *U. capensis* population which successfully invaded the estuarine habitat and concomitantly became more tolerant of reduced salinity.

SUMMARY

1. *Upogebia africana* (Ortmann), an estuarine species, is more tolerant of low salinities than *U. capensis* (Krauss) which is a marine species. Both species are isosmotic in sea water but regulate hyperosmotically in reduced salinities.

2. *U. africana* can tolerate a salinity of 1,7‰ but can only moult in a salinity of 3,4‰. In addition low salinity causes a delay in the initiation of moulting and a reduction in moulting frequency.
3. Physiological differences between the two species of *Upogebia* investigated support the recognition of *U. africana* as a distinct species. It is suggested that *U. africana* may have evolved from *U. capensis* and that this speciation occurred in association with the invasion of the estuarine habitat.

ACKNOWLEDGMENTS

This work was carried out with financial assistance from the South African C.S.I.R. and Rhodes University. I also wish to thank Professor B. R. Allanson and Dr. R. E. Boltt for their interest.

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