THE OSMOREGULATORY ABILITY OF THREE GRAPSOID CRAB SPECIES IN RELATION TO THEIR PENETRATION OF AN ESTUARINE SYSTEM

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ABSTRACT

The osmoregulatory abilities of the grapsoid crabs *Cyclograpsus punctatus*, *Sesarma catenata* and *Sesarma eulimene* were studied comparatively in an attempt to explain, at least in part, their distribution in estuaries. Both survival and haemolymph osmotic pressure were used as indices of hyperosmotic regulatory ability.

All three species were shown to be capable of regulating their haemolymph osmotic pressure against lowered external salinities. *S. eulimene* was found to be better able to control its haemolymph concentration in very low salinities than *S. catenata*, which would account for the former's occurrence in the upper reaches of estuaries. *C. punctatus* was shown to be capable of maintaining its haemolymph osmotic pressure above that of the environmental medium in lowered salinities, but without the degree of constancy characteristic of the two *Sesarma* species. It does, however, show a marked ability to tolerate lowered internal osmotic pressure, an ability which appears to develop in response to partial dilution of the haemolymph. These adaptations, together with its semi-terrestrial habit as a shore crab, suffice to enable *C. punctatus* to penetrate an estuary to almost the same extent as *S. catenata*.

INTRODUCTION

Sesarma catenata Ortmann and *Sesarma eulimene* de Man both occur in many estuaries along the eastern seaboard of South Africa, the latter species extending further upstream into fresh water than the former (Alexander & Ewer 1969; Macnae 1968). Neither species is known to occur outside the estuarine habitat. *Cyclograpsus punctatus* Milne-Edwards is typically a shore crab, occurring in the upper intertidal zones on rocky shores, and probably having a circum-polar distribution (Barnard 1950). It has, however, also been recorded from a number of South African estuaries (Alexander & Ewer 1969; Day, Millard & Harrison 1952; Macnae 1957, 1963; Millard & Scott 1955; Scott, Harrison & Macnae 1952).

The ranges of *C. punctatus* and *S. catenata* overlap to a significant extent in the estuary of the Kowie River at Port Alfred (Alexander & Ewer 1969). *S. catenata* is absent from the area inhabited by *C. punctatus* near the mouth of the estuary, but where the estuarine mud-flats commence these two species co-exist, to the extent of sharing the same burrows, over a distance of 10 km. The range of *S. catenata* extends only 2 km further upstream, where it overlaps with *S. eulimene* before giving over entirely to the latter species.

Penetration of an estuary must, at least in part, be governed by the crabs' abilities to with-

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stand osmotic stress. Preliminary studies on *C. punctatus* have shown this species to be capable of both hypo- and hyperosmotic regulation (Heeg & Cannone 1966). The present investigation compares the osmoregulatory ability of *C. punctatus*, primarily a marine shore crab, with that of the two truly estuarine *Sesarma* species in an attempt to evaluate the role of osmoregulatory ability in governing the distribution of these species within an estuary.

MATERIAL AND METHODS

Specimens of *C. punctatus* and *S. catenata* used in the investigation were collected from the West Lagoon of the Kowie Estuary at Port Alfred. The *S. eulimene* came from the mangroves at the mouth of the Umgeni River. While it would have been preferable to have used *S. eulimene* from the Kowie River, its habitat in the upper reaches of the estuary was not readily accessible without a boat. Macnae (1968) throws some doubt on the identity of the crabs collected as *S. eulimene*, since the habitat from which they were obtained corresponds more closely with that described for the very similar *Sesarma ortmanni*. However, the crabs used do not agree with Crosnier's (1965) description of *S. ortmanni* but do fit Barnard's (1950) description of *S. eulimene*. In the absence of a reference specimen of *S. ortmanni*, these crabs have been accepted as *S. eulimene*.

All experimental animals were kept in the laboratory in batches of 15. Different species were kept separately, and each batch comprised crabs of similar size, though no attempt was made to separate the sexes. Each batch was housed in a plastic bath measuring 50 × 32 × 15 cm containing 500 ml of sea water.

The baths were tilted by placing a block of wood, 2 cm high, under one end; this allowed the crabs to leave the water at will. The baths were covered with plastic sheeting to curb evaporation and were housed in an air-conditioned room at a near constant temperature of 20 °C. Fresh fish was fed to the crabs every three to four days and the water in the baths was changed on the day after feeding. The crabs moulted successfully under the above conditions and females came into berry from time to time, suggesting that the artificial habitat provided was at least adequate for the adults.

The crabs used in an experiment were not fed during the 24-hour period preceding its commencement, nor during its duration. Individual crabs were placed singly in plastic containers measuring 20 × 10 × 7 cm, each containing 500 ml of an experimental medium and fitted with a lid to curb evaporation. The animals were immersed in the medium and could not leave it, although they had free access to the surface. Aeration was found to be unnecessary even for prolonged survival under these conditions.

The experimental media used were sea-water of salinity 34‰, volumetrically diluted with deionized water to give salinities of 27,0; 20,0; 14,0; 7,0; 3,4; 2,0; 1,4 and 0,7‰. Normal sea-water (S 34,0‰) was used for control experiments. The medium in the experimental vessels was changed every three days; no changes in salinity could be detected over this period.

The crabs were kept in the experimental media over a period of 14 days. Mortality was recorded daily, and samples of haemolymph were drawn from the arthrodial membrane at the base of the first pereiopod of each crab after two, four, six and fourteen days. The haemolymph samples, of the order of 10 μl, were collected between liquid paraffin into fine silica capillaries.

Each filled capillary was sealed into a liquid paraffin-filled pyrex capillary of larger bore by means of sealing wax. The freezing points of the haemolymph samples were determined using the method and apparatus described by Ramsay & Brown (1955).

The freezing point of the haemolymph collected from the arthrodial membrane at the base of the first pereiopod of any particular crab did not differ from that collected from its heart or from sites on other parts of the body.

Sealed capillaries could be stored in a deep freeze at —10 °C for prolonged periods without any measurable change in the freezing point of the contained haemolymph. Gilbert (1959) has shown that size and sex significantly affect the conductivity and freezing point depression of the haemolymph of *Carcinus maenas* in the range of 3–110 g. No such effect was apparent in the present investigation, where the size range of the animals used was small (3–15 g live weight) and only very few females were used. Female crabs in berry were not used at all in the investigation.

RESULTS

From the mortalities recorded in the various media, shown in Table 1, the two *Sesarma* species appear to be more tolerant of lowered salinities than *C. punctatus*. The latter species shows a marked increase in mortality at salinities below 10‰, and experiments were not carried out on it at salinities below 3,4‰ because of low survival over even very short periods. *S. eulimene*, on the available evidence, seems slightly better able to withstand lowered salinities than *S. catenata*, but the evidence is far from conclusive.

Changes in the osmotic pressure of the haemolymph of the crabs, when exposed to media of lowered salinity, confirmed the above findings. Figure 1 shows the relationship between the freezing point depression of the haemolymph of *C. punctatus* and that of the medium (Δ_i/Δ_e curve) after 2, 4, 6 and 14 days immersion in various lowered salinities. While this species is able to osmoregulate over the whole range of salinities tested, there is a tendency for the Δ_i/Δ_e curve to come closer to the isosmotic line with prolonged exposure. This suggests that regulation of the intercellular compartment is not sustained and gives way to at least partial conformation in time. Figures 2 and 3 are comparable Δ_i/Δ_e curves for *S. catenata* and *S. eulimene* respectively. There is clear evidence for both hyposmotic and hyperosmotic regulation in both species, confirming the trends indicated by the mortality records in Table 1. Regulation of the intercellular compartment is sustained at a constant level over at least 14 days, since there is no tendency for the Δ_i/Δ_e curve to approach the isosmotic line, or to change in any way.

A comparison between Figures 2 and 3 also supports the suggestion that *S. eulimene* is better able to withstand very low salinities than *S. catenata*, since the Δ_i/Δ_e curve for the former shows a remarkable degree of constancy even at the lowest salinities tested.

In view of the comparatively poor osmoregulatory ability shown by *C. punctatus*, and since Alexander & Ewer (1969) had shown that specimens of this species collected from the limit of its range in the Kowie Estuary were more tolerant of lowered salinities than those from the West Lagoon, it was necessary to determine whether *C. punctatus* could be acclimated to better survival at such lowered salinities. Attempts at acclimating the crabs by complete submergence

TABLE 1

Mortalities of *C. punctatus*, *S. catenata* and *S. eulimene* during 14 days exposure to various lowered salinities.

	Cyclograpsus punctatus			*Sesarma catenata*			*Sesarma eulimene*		
Salinity (‰)	*No. of animals used*	*Time to first mortality (days)*	*No. of survivors after 14 days*	*No. of animals used*	*Time to first mortality (days)*	*No. of survivors after 14 days*	*No. of animals used*	*Time to first mortality (days)*	*No. of survivors after 14 days*
34,0	5	14+	5	13	14+	13	5	14+	5
27,0	5	14+	5	5	14+	5	5	14+	5
20,0	5	14+	5	5	7	4	5	14+	5
14,0	5	6	3	5	14+	5	5	14+	5
10,0	5	5	3	—	—	—	—	—	—
7,0	5	5	1	5	14+	5	5	14+	5
3,4	5	2	2	15	1	12	5	14+	5
2,7	—	—	—	15	1	13	5	14+	5
2,0	—	—	—	15	1	9	5	1*	3
1,4	—	—	—	15	1	8	5	10	3
0,7	—	—	—	15	1	10	5	1†	4

* Two *S. eulimene* died on Day 1 as a result of unsuccessful moult, not necessarily connected with lowered salinity.
† One *S. eulimene* died on Day 1 as a result of unsuccessful moult.

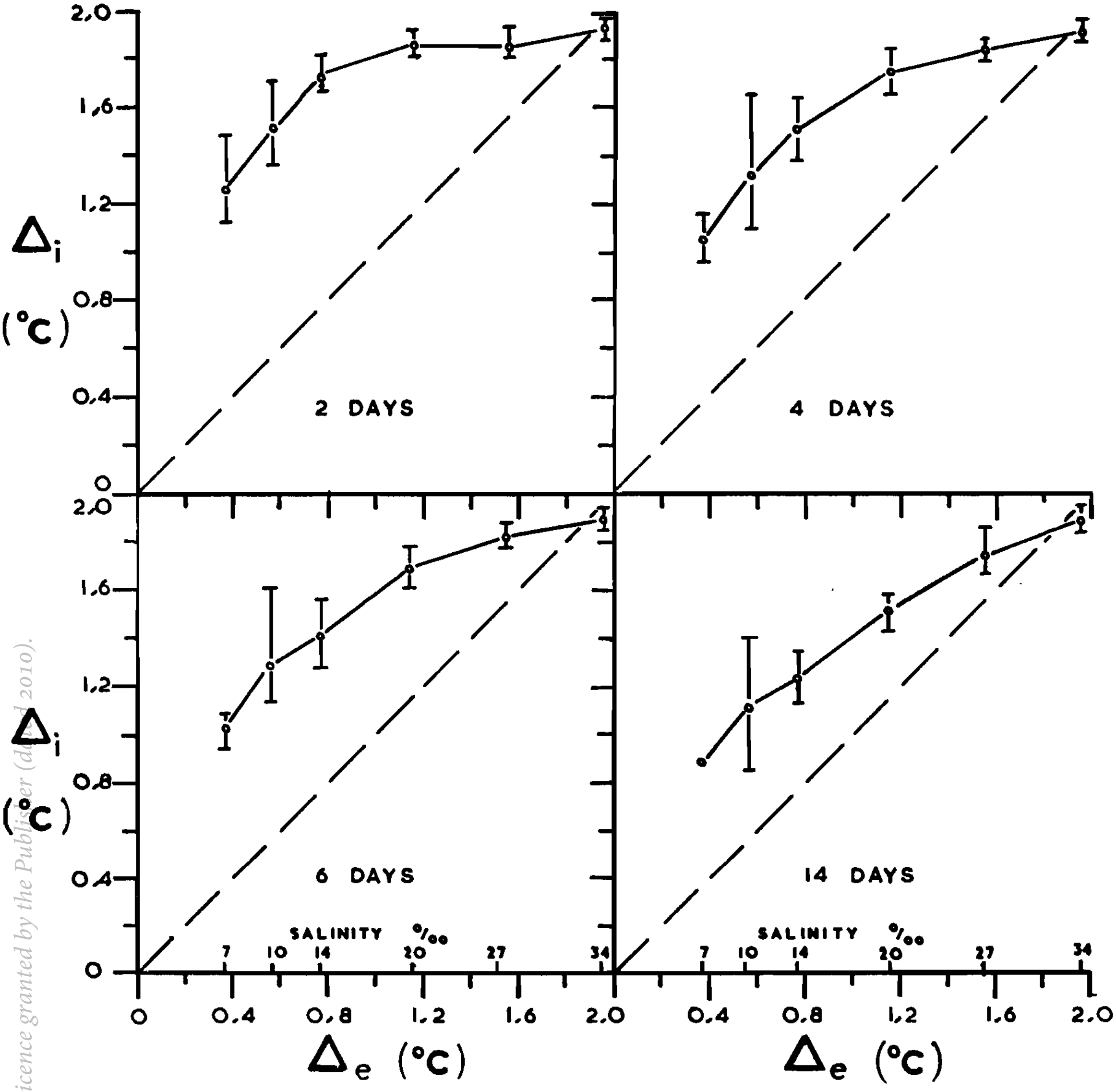

FIGURE 1
Relationship between the freezing point depression of the haemolymph of *C. punctatus* (Δ_i) and that of the experimental medium (Δ_e) after 2, 4, 6 and 14 days total immersion. Co-ordinates denote mean values, offsets denote ranges.

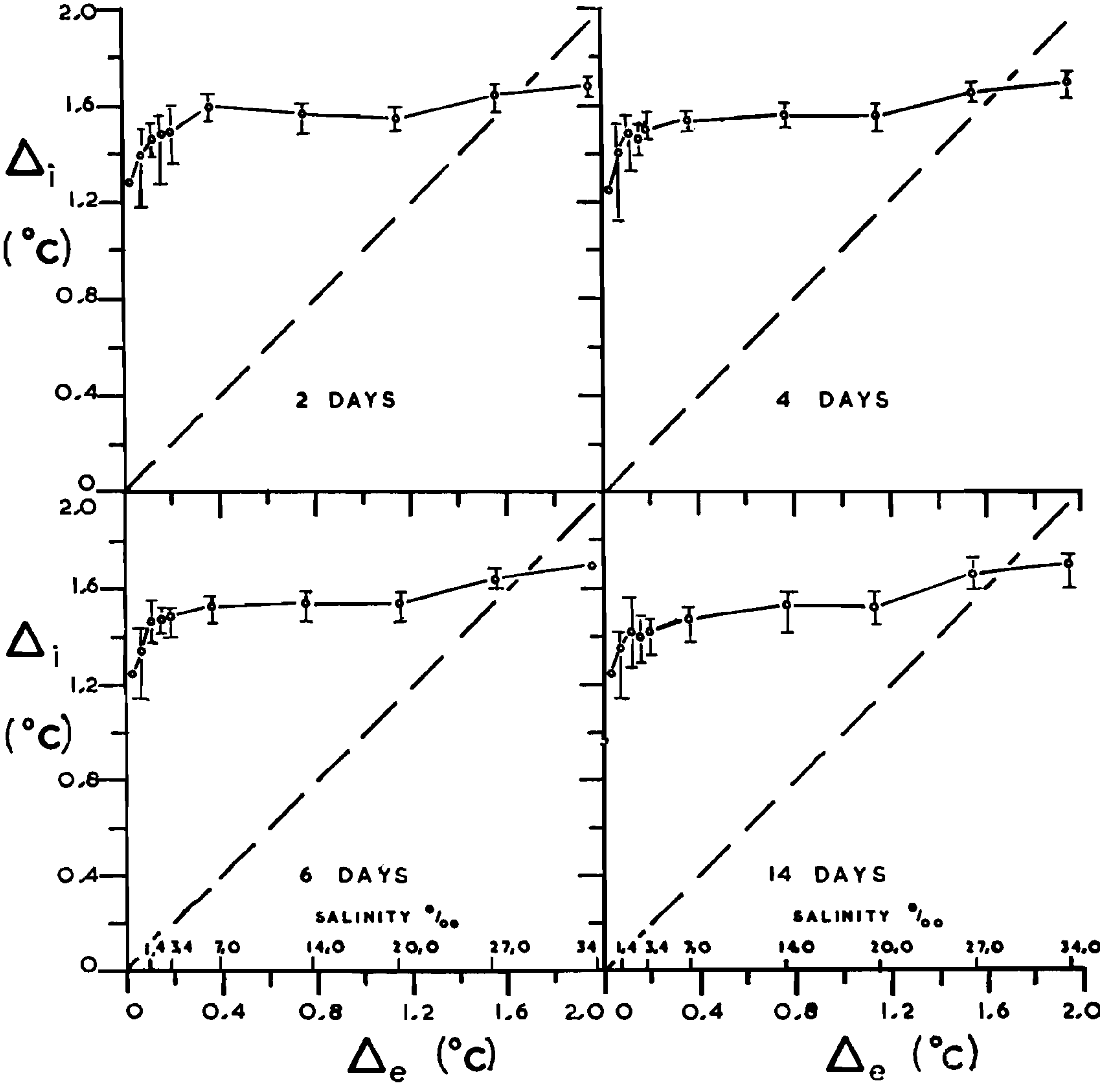

FIGURE 2
Relationship between the freezing point depression of the haemolymph of *S. catenata* and that of the experimental medium after 2, 4, 6 and 14 days total immersion. Details as for Fig. 1.

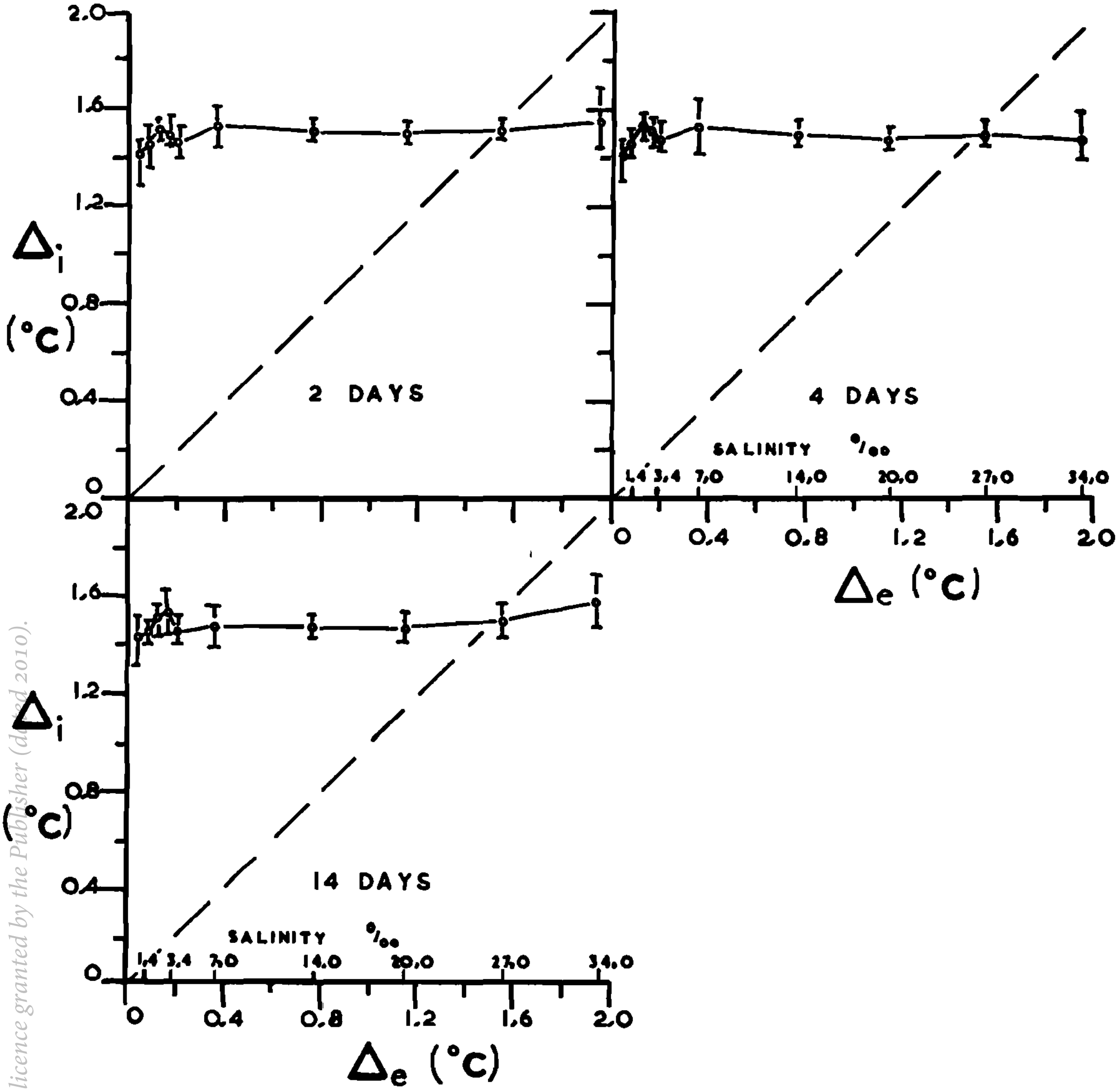

FIGURE 3
Relationship between the freezing point depression of the haemolymph of *S. eulimene* and that of the experimental medium after 2 4 and 14 days total immersion. Details as for Fig. 1.

in water of salinity 17‰ for a protracted period proved unsuccessful; the carapaces of the animals became mottled, as if suffering progressive decalcification, and mortality was high. A further attempt at acclimation, which more closely corresponded to the field conditions to which the crabs would normally be subjected, was to replace the sea-water in normal culture baths with acclimation media of 14‰ and 7‰ salinity. The crabs could now enter and leave the acclimation medium at will, and survived under these acclimation conditions for 44 days, after which they were subjected to 14 days submergence in the various experimental media, with haemolymph samples being taken for freezing point determination as described earlier. The survival pattern of these acclimated crabs was similar to that of crabs kept with access to normal sea-water (Table 2). However, acclimation did result in a change in the $\triangle_i/\triangle_e$ curves, as shown in Figures 4 and 5. Prior exposure to lowered salinities resulted in curves closer to the isosmotic line after two days total immersion in the experimental media, the difference being most marked in salinities higher than the acclimation medium. This difference between the curves for acclimated and non-acclimated crabs becomes progressively less after four and six days, to be all but non-existent after 14 days. While the use of only five crabs in each salinity did not allow for statistical analysis, the fact that both acclimation media elicited the same result suggests that the difference between acclimated and non-acclimated crabs is real, a suggestion which is further supported by the ranges of the internal osmotic pressures not overlapping between 14 and 28‰. Such a difference implies a reduction in osmoregulatory activity by the acclimated crabs.

The experiments described here, while demonstrating regulation of haemolymph concentration, do not distinguish between active regulation involving ion transport and/or water excretion, and passive control of the movement of water and salts through structural impermeability.

Heeg & Cannone (1966) have provided evidence for active, extra-renal water excretion by way of the posterior diverticulum of the gut in *C. punctatus*, but nothing is known about regulation in the *Sesarma* species. Active regulation can be simply detected by comparing the rate of change in haemolymph concentration in crabs transferred from a high to a low salinity with that of crabs transferred from a low to a high salinity. An active regulatory mechanism, whether water excretion or ion uptake, will show itself in a more rapid concentration of the haemolymph following the upward transfer, when compared with the rate of dilution following the downward transfer. *C. punctatus* kept in sea-water (S 34‰) for 21 days were transferred to a salinity of 7‰, and specimens kept totally immersed in 17‰ for 21 days were transferred to 34‰. For *S. catenata* both upward and downward transfers were between 3,4‰ and 34‰. A lower salinity of 17‰ was used for the upward transfer of *C. punctatus* since this species could not survive total immersion in 7‰ for 21 days. This discrepancy would only affect interpretation in the absence of a regulatory mechanism, when fluxes would be entirely due to the concentration gradients. Since lowering of the haemolymph osmotic pressure in media of lowered salinity had been shown not to be a linear function of time, the highest rate of change occurring during the first two days, more frequent sampling was required during the early stages of this experiment. Seven sampling times were therefore set to fall within the first 24 hours after transfer, with further sampling times at 36, 48 and 72 hours. Each crab had its haemolymph sampled for freezing point determination immediately before transfer and again at two of the set sampling times allocated by

TABLE 2

Survival, during 14 days total immersion in media of lowered salinities, of *C. punctatus* acclimated to lowered salinities through having been kept with access to water of salinity 14‰ and 7‰ over a period of 44 days. The data for non-acclimated animals presented in Table 1 are included for comparison.

	Control: Access to 34‰			*Acclimated: Access to 14‰*			*Acclimated: Access to 7‰*		
Salinity (‰)	*No. of animals used*	*Time to first mortality (days)*	*No. of survivors after 14 days*	*No. of animals used*	*Time to first mortality (days)*	*No. of survivors after 14 days*	*No. of animals used*	*Time to first mortality (days)*	*No. of survivors after 14 days*
34,0	5	14+	5	5	14+	5	5	14+	5
27,0	5	14+	5	5	14+	5	5	14+	5
20,0	5	14+	5	5	14+	5	5	14+	5
14,0	5	6	3	5	9	4	5	14+	5
10,0	5	5	3	5	10	2	5	10	3
7,0	5	5	1	5	4	0	5	5	1
3,4	5	2	2	5	2	0	5	2	0

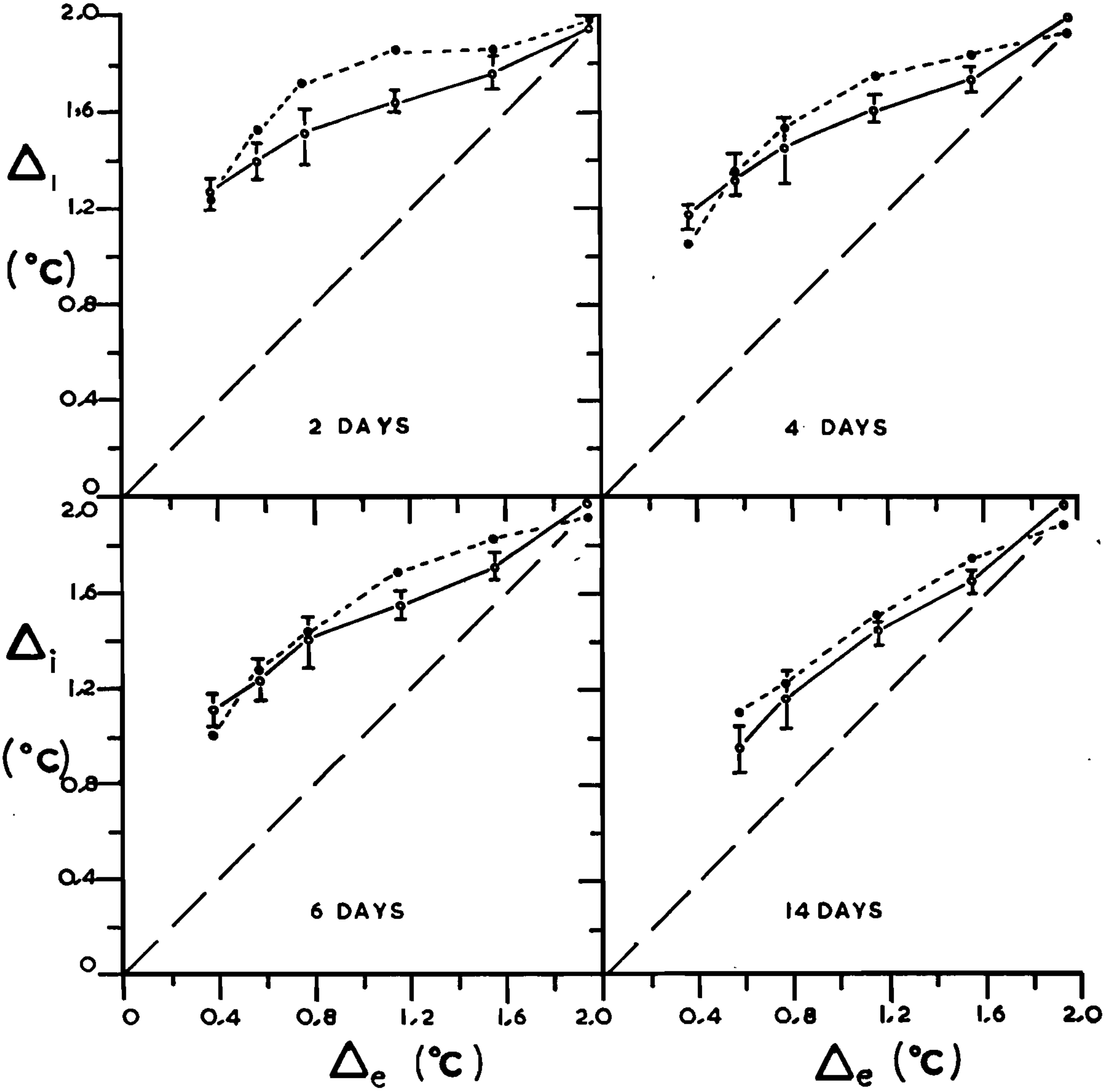

FIGURE 4

Δ_i/Δ_e curves for *C. punctatus* acclimated to lowered salinity through having been kept with access to water of salinity 14°/oo over a period of 44 days. Co-ordinates denote mean values, offsets denote ranges. Comparable curves from Fig. 1 (broken line) are included for comparison.

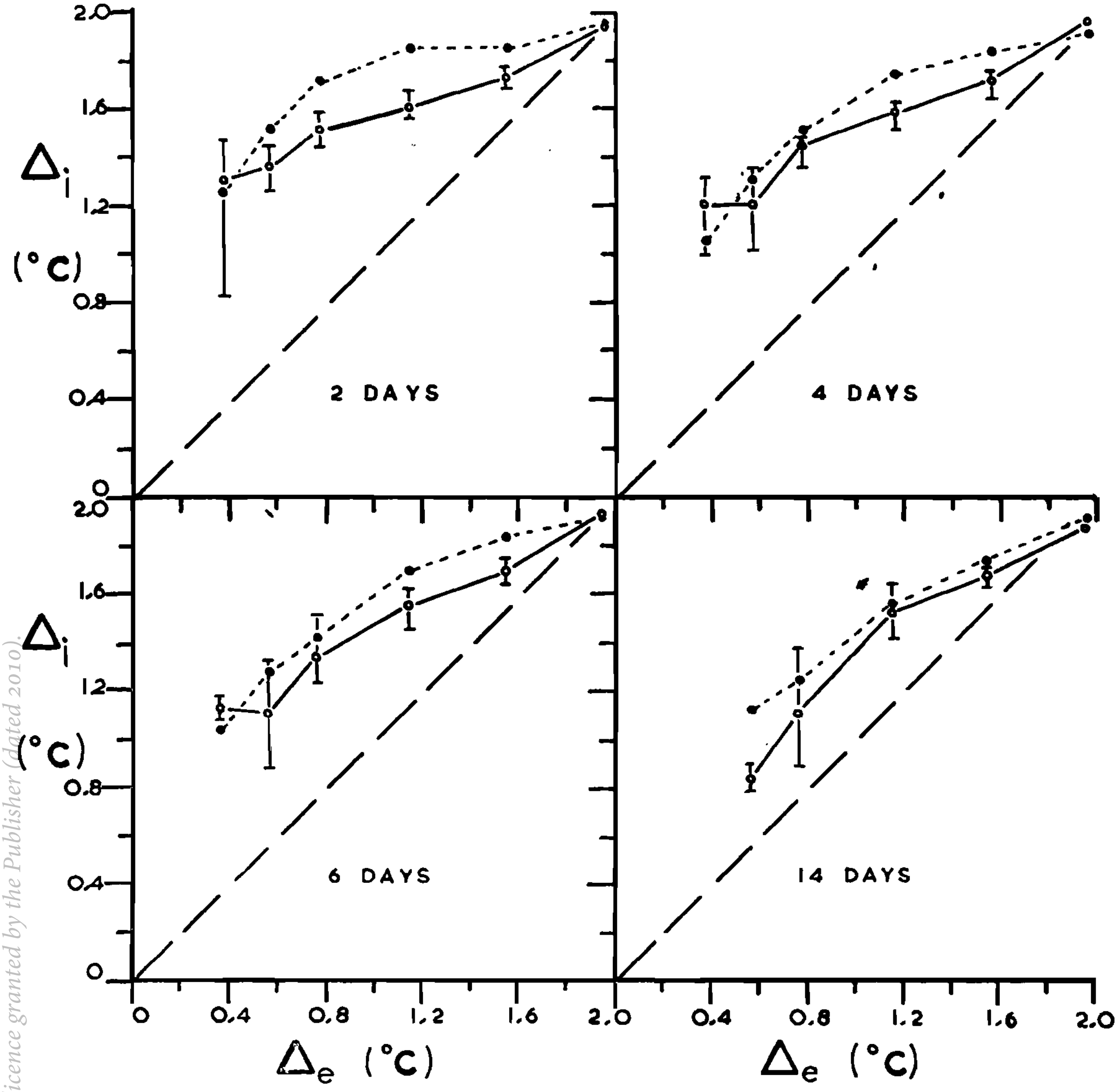

FIGURE 5

Δ_i/Δ_e curves for *C. punctatus* acclimated to lowered salinities through having been kept with access to water of salinity 7°/₀₀ over a period of 44 days. Details as in Fig. 4.

means of a table of random numbers.

The results of these experiments are shown in Figures 6 and 7. In both species the increase in haemolymph concentration on transfer from a medium of low salinity to normal sea-water is much more rapid than the decrease on transfer to a lowered salinity. This cannot be explained in terms of the respective magnitudes of the osmotic gradients between media and haemolymph; in the case of the upward transfer the gradient is less than in the downward transfer and one would thus expect slower equilibration. An active osmoregulatory mechanism is, therefore, operative in both species, and survival in lowered salinities is not simply a result of impermeability, but nothing further can be said about the nature of the mechanism on the basis of these results. The relatively smaller difference between dilution and recovery rates in *S. catenata*, when compared with *C. punctatus*, may be indicative of a greater degree of impermeability to salts and water in the former.

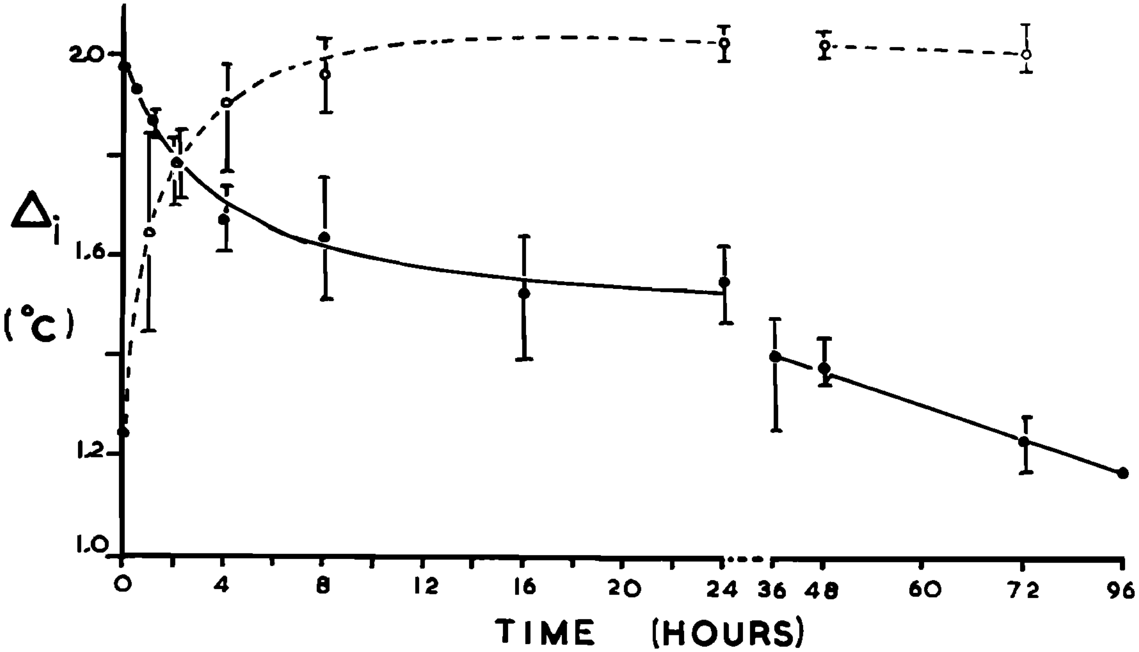

FIGURE 6

Changes with time in the freezing point depression of the haemolymph of *C. punctatus* (Δ_i) on transfer from 34°/oo to 7°/oo (closed circles) and from 17°/oo to 34°/oo (open circles). Co-ordinates denote mean values, offsets denote ranges.

DISCUSSION

Estuaries, by virtue of their characteristically fluctuating physico-chemical conditions, constitute a stress habitat requiring considerable plasticity in the adaptations of the organisms which inhabit them. Kinne (1967) has pointed out that, physiologically, few organisms find optimal conditions in estuaries, and that the availability of mechanisms for protection and increase of tolerance, as well as regulation and acclimation, are therefore necessary prerequisites for successful establishment in estuarine waters. The main environmental variable in an estuary must certainly be salinity. While most estuaries can be expected to have salinity gradients which persist for most of the time, seasonal and less predictable short-term variations must also occur. Salinity most certainly does govern the distribution of organisms within an estuarine system to a large extent. Barnes (1967) has compared the osmotic adaptation of five grapsoid species in the Brisbane River and has found a clear correlation between osmoregulatory ability of four of these species and the extent of their penetration of the estuary; the fifth had its range within the limits of its osmoregulatory ability narrowed by substrate preference.

Similar correlations between osmoregulatory ability and penetration of estuaries have been shown by Teal (1958) for three species of *Uca* and by Ono (1965) for several ocypodid species.

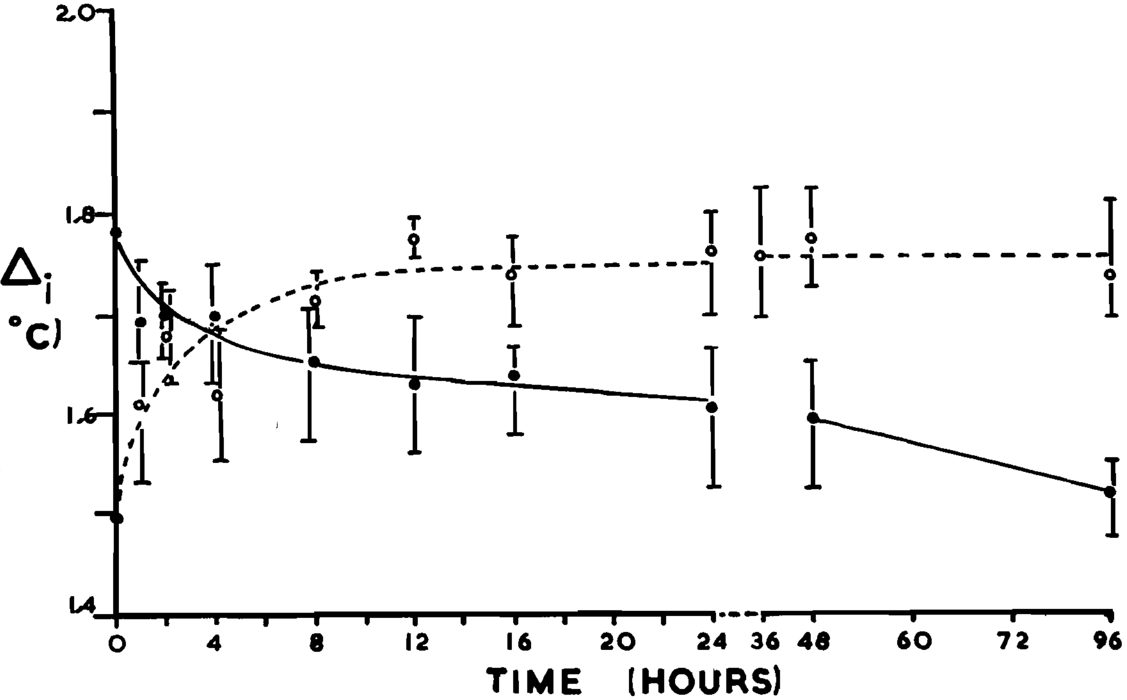

FIGURE 7

Changes with time in the freezing point depression of the haemolymph of *S. catenata* on transfer from 34°/oo to 3,4°/oo (closed circles) and from 3,4°/oo to 34°/oo (open circles). Details as in Fig. 6.

Alexander & Ewer (1969) have shown that, although their ranges overlap over a short distance, *S. eulimene* extends further up the Kowie Estuary than does *S. catenata*. This agrees well with the findings in the present investigation, where *S. eulimene* has been shown to be able to regulate its haemolymph osmotic pressure more effectively than *S. catenata* in very low salinities. Both these *Sesarma* species live within the limits of their osmoregulatory ability. The presence of *S. catenata* between *S. eulimene* and the mouth of the estuary may well limit the latter's seaward distribution, while lack of a suitable substrate is very likely to limit that of *S. catenata* to seaward. Day *et al* (1952) report that the abundance of *S. catenata* in the Knysna Estuary increases as the proportion of mud in the sand increases, thus the deep water and boulder-littered sides of the artificially deepened channel leading into the Kowie Estuary are likely to present a barrier to this species, while affording *C. punctatus* a habitat similar to that which it inhabits on the seashore.

The penetration of *C. punctatus* into the Kowie Estuary, however, is such that the crab finds itself in salinities where its osmoregulatory ability can no longer maintain a constant haemolymph osmotic pressure. It does not compete with *S. catenata* for food (Alexander & Ewer 1969), and this must, at least in part, permit the overlap in ranges, but, in spite of being a much poorer osmoregulator than *S. catenata*, it penetrates the estuary almost as far. Day *et al* (1952) have shown that there is a correlation between the levels which animals inhabit on the shore and their penetration into estuaries, those animals which inhabit the upper levels of the intertidal zone occurring further into the estuarine environment. This can be explained by those animals which normally only experience brief submergence on the sea-shore, and which select similar levels in an estuary, being exposed to lowered salinities only for brief periods at high tide when dilution is minimal. In the laboratory, where the animals could leave the water in their culture baths at will, all three species spent a considerable part of the time out of water, and in the estuary they do not follow the outgoing tide. This ensures minimal contact with low salinity water.

Adaptations evolved in the intertidal zone, where inundation by fresh water following rain would subject the animals to osmotic stress, would enable animals to survive brief periods of exposure to lowered salinities and the degree of osmoregulatory ability shown by *C. punctatus* would constitute an effective resistance adaptation in the intertidal zone of both the sea-shore and an estuary.

However, the results obtained by Alexander & Ewer (1969) are indicative of a further, and more long-term, adaptation, in that *C. punctatus* from the limits of its range were better able to survive lowered salinities than specimens from the West Lagoon. The latter is fully tidal and therefore deviates little from sea-water in its salinity. While survival experiments in this investigation did not confirm the implied acclimatory ability, possibly due to the small number of crabs subjected to each salinity, acclimation does affect the regulatory process. Crabs acclimated to reduced salinities responded by allowing the internal osmotic pressure to fall much more rapidly than did non-acclimated crabs. This could explain why the mortalities of the two categories were so similar; the non-acclimated crabs, in all probability, acclimated to the same extent as the acclimated crabs during the 14 days duration of the experiment.

The tolerance of a lowered haemolymph osmotic pressure, as shown by *C. punctatus*, implies a shift of regulation from between external medium and haemolymph to between haemolymph and the cytoplasm of the body cells. Osmoregulation at the cellular level is not uncommon among euryhaline animals, and some Brachyura, among them *Carcinus maenas* (Shaw 1958) and *Eriocheir sinensis* (Duchateau-Bosson & Florkin 1962) have been shown to achieve this by regulating the amount of free amino-acids present in the cells in order to maintain the water content of the cytoplasm. While such biochemical regulation is effective, it is not instantaneous, and is therefore subject to an acclimatory period for its manifestation. However, once established, it allows for a reduction in active transport and hence constitutes a saving in metabolic activity.

Selection by the estuarine environment can, therefore, be for osmotic regulation at either the organismic or the cellular level, or both, since all will enhance survival. Both *S. catenata* and *S. eulimene* have a near perfect mechanism for maintaining a constant haemolymph concentration which is hyposmotic to sea-water, suggesting a permanent adjustment at the cellular level earlier in their evolution.

Such a reduced haemolymph concentration must perforce reduce the gradient over which the crabs need to regulate in the estuarine environment, and the greater impermeability, inferred from the results obtained in this investigation, would further reduce the demand on the osmoregulatory mechanism. These adaptations suggest that, compared with *C. punctatus*, the two *Sesarma* species have a long history of estuarine existence, a suggestion which finds support in their being exclusive to estuaries. *C. punctatus*, by contrast, is a successful opportunist in the estuarine environment which has capitalized on its modest osmoregulatory ability, its capacity for adjusting to lowered haemolymph concentrations and its behaviour as a shore crab to invade the habitat of the more fully adapted *Sesarma* species.

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