

THE OSMOTIC BEHAVIOUR
OF A NUMBER OF GRAPSOID CRABS WITH
RESPECT TO THEIR DIFFERENTIAL PENETRATION
OF AN ESTUARINE SYSTEM

BY R. S. K. BARNES

Department of Zoology, University of Queensland, Brisbane, Australia

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INTRODUCTION

During the last thirty years there has been a large volume of work published on the osmotic behaviour of invertebrate animals (recently reviewed by Kinne, 1964 and Potts & Parry, 1964), of which much has concerned the Brachyura (see reviews by Robertson, 1960 and Lockwood, 1962). Most of this attention has been directed towards what can be termed 'a purely physiological end-point', comparatively little having been orientated towards ecology, although distributional aspects have been emphasised by Topping & Fuller (1942), Panikkar (1951) and Kinne (1963). The osmotic behaviour of a number of crabs exhibiting differential zonations up a shore, from sublittoral to semi-terrestrial species, has been documented by Pearse (1932). These crabs were not taken from one particular littoral region, however, but were composed of different species from completely separate regions.

Although several workers, e.g. Teal (1958), Snelling (1959) and Ono (1965), have suggested that salinity is a major limiting factor to the distribution of crabs in estuarine or brackish environments, no work has been published on the osmotic behaviour of a group of inter-related crabs showing a differential distribution along a marine-estuarine (-fresh water) series. This lack of data is no doubt to a large degree caused by the absence of such crab series in temperate regions (see Panikkar, 1940).

Teal (1958) stated that salinity was probably one of the two major factors influencing the distribution of *Uca* spp. in a Georgia salt-marsh (the other being habitat preferences). Ono (1965), however, studying the distribution of a number of grapsoid crabs in the Tataru-Umi estuary in Japan, found that the upper limits of the distribution of most species were lower than the upper limits of the distributional range of the respectively suitable substrates. He deduced from field observations and salinity/survival data that chlorinity was the most important factor limiting the upstream penetration of the crabs. Further, Snelling (1959), after a study of the distribution of the intertidal crabs of the Brisbane River, concluded that the limiting factor to their upstream penetration was the salinity, the distributions of the crabs changing in relation to salinity changes in the river caused by the amount of fresh water runoff into the upper reaches.

The following is an investigation, with an ecological bias, of the osmoregulation capabilities of a group of grapsoid crabs, the majority related at the subfamilial level,

that has been shown by Snelling (1959) to exhibit differential penetration of the estuarine system of the Brisbane River and Moreton Bay, Queensland.

MATERIALS AND METHODS

Crabs were collected from various localities in Moreton Bay and the Brisbane River (for location of these sites subsequently mentioned in the text see Fig. 1). The species used were chosen so as to fulfil three requirements as closely as possible:

- (a) they should be taxonomically inter-related, preferably closely;
- (b) they should penetrate the Brisbane River estuary to different levels;
- (c) they should occur in sufficient numbers to provide adequate material for experimental study. (A requirement which unfortunately ruled out a number of otherwise suitable species.)

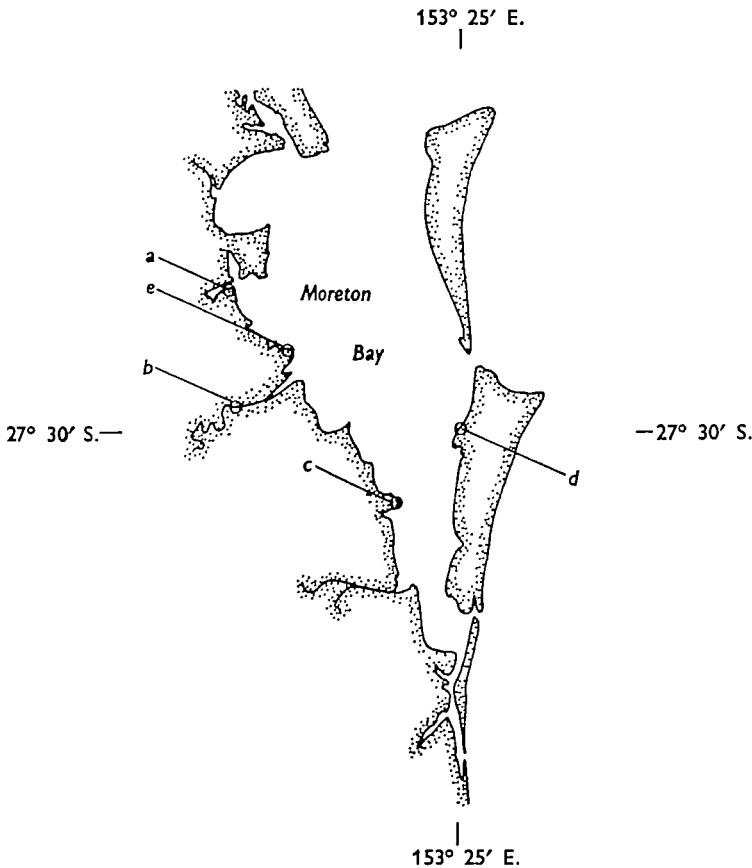


Fig. 1. Sketch-map of Moreton Bay showing the location of collection sites mentioned in the text. *a*, Pine River Estuary; *b*, Bulimba, Brisbane River; *c*, Victoria Point; *d*, North Dunwich, North Stradbroke Island; *e*, Cribb Island.

The specimens of each species collected were also chosen so that:

- (a) Any one species was collected at one time from one locality, and from as small an area as possible from within that locality, in an effort to ensure that the specimens

were all from one population and would all have been subjected to similar, if not identical, environmental conditions prior to collection.

(b) Specimens of any one species were, wherever possible, of the same, or very similar, size; Gilbert (1959) showed that the osmotic pressure of the blood of *Carcinus maenas* varied with size of the individuals, being higher in smaller specimens than in large.

(c) All specimens were in intermoult to eliminate disturbances of the osmotic pressure caused by the moult cycle (Baumberger & Olmsted, 1928; Parry, 1953).

(d) No ovigerous females were collected to avoid any possible changes in the blood osmotic pressure caused by changes in the salinity preferences of the females in response to the presence of the eggs (Kinne, 1964). (It appeared during the course of this study that this precaution may have been unnecessary.)

(e) The sexes were not differentiated, although Gilbert (1959) and Tan & van Engel (1966) have shown a dichotomy in the osmotic pressure of the sexes of *Carcinus maenas* and *Callinectes sapidus* respectively. The marked difference between the osmoregulatory abilities of the two sexes in *Callinectes* is presumably correlated with their differential distribution with respect to the external salinity (Tan & van Engel, 1966). In *Carcinus*, and in the species under study, the sexes occur together throughout the year, and in *Carcinus* the differences between the sexes, under identical conditions, were in the order of a depression of freezing point of 0.05° C. maximum (Gilbert, 1959). It was therefore considered that for the purposes of this study, the sexes, which are ecologically equivalent, need not be separated unless evidence to the contrary appeared during the course of the experiments.

The species used, and their local distributions, are shown below (the information contained therein being derived from Barnes, 1967; Snelling, 1959; and personal observations).

Species	Local distribution
1. <i>Macrophthalmus crassipes</i> M. Edw. (Ocypodidae: Macrophthalminae)	Moreton Bay, but absent from Brisbane River. Occupies burrows in muddy sand, from about mean tide level to low-water neap
2. <i>Mictyris longicarpus</i> Latr. (Mictyridae)	Moreton Bay, and up the Brisbane River as far as Aquarium Passage (2 miles upstream). Occupies burrows in muddy sand, from about mean tide level to high-water neap
3. <i>Macrophthalmus setosus</i> M. Edw. (Ocypodidae: Macrophthalminae)	Moreton Bay, and up the Brisbane River as far as Domain (13.5 miles upstream). Occupies burrows in sandy mud or mud, from low water spring to low water neap (Brisbane River) or from about low-water neap to mean tide level (Moreton Bay)
4. <i>Australoplax tridentata</i> (M. Edw.) (Ocypodidae: Macrophthalminae)	Moreton Bay, and up the Brisbane River as far as St Lucia (18 miles upstream). Occupies burrows in firm mud, from mean tide level to high-water neap (Brisbane River) and from high-water neap to above high-water spring (Moreton Bay)
5. <i>Paracleistostoma mcneilli</i> (Ward) (Ocypodidae: Macrophthalminae)	Apparently absent from Moreton Bay, occurs in the River from Colmslie (5 miles upstream) to St Lucia (18 miles upstream). Occupies burrows in mud, from low-water neap to high-water neap

The distribution of these crabs and the approximate salinity range in Moreton Bay and the Brisbane River is shown in Fig. 2 (salinity data was taken from C.S.I.R.O., 1953; Hodge, 1963; and Bayly, 1965).

Crabs were maintained in the laboratory in sea water of the approximate mean salinity experienced by the respective species in the field, and were not provided with food materials. After capture, crabs were allowed to rest for about 40 hr. in the laboratory to recover from any adverse effects resulting from their mechanical disturbance during capture and the subsequent transportation from the collection site to the laboratory.

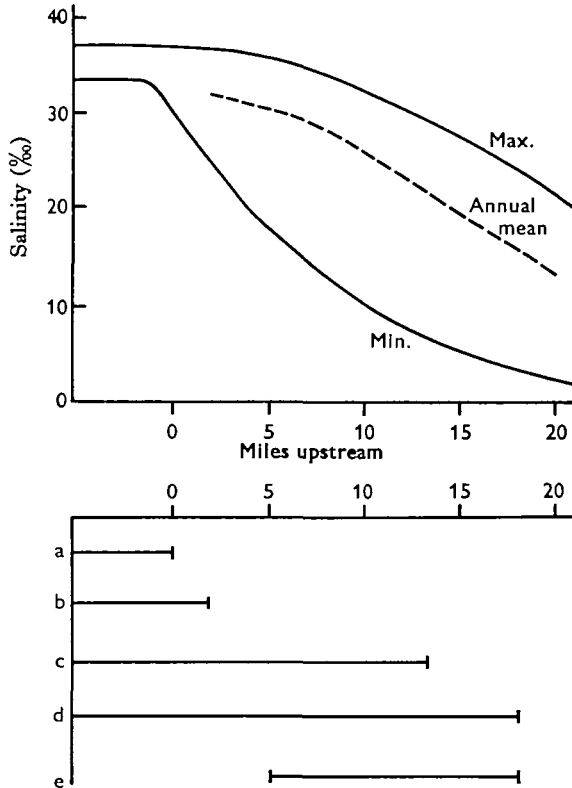


Fig. 2. Graph showing the approximate maximum, mean, and minimum salinity values, and the distribution of the crabs under study, along the Brisbane River Estuary. (Data taken from C.S.I.R.O., 1953; Snelling, 1959; Hodge, 1963; Bayly, 1965.) a, *Macrophthalmus crassipes*; b, *Mictyris longicarpus*; c, *Macrophthalmus setosus*; d, *Australoplax tridentata*; e, *Paracleistostoma mcneilli*.

The largest and smallest specimens of each species (as approximately determined by eye) were blotted dry with absorbent material and then weighed, in order to ascertain the approximate wet-weight range of the experimental animals. Each species was then subjected to three consecutive experimental procedures.

1. Salinity/survival tests

Six specimens of each individual species were placed in each of eight salinities, ranging from about 2 to 70‰ in intervals of approximately 10‰ (= intervals of 28.5‰ sea water approx.), and were kept at a constant temperature of 25° C., to eliminate variations in the osmotic pressure due to temperature (Pannikar, 1940; Dehnel, 1962; Tan & van Engel, 1966). The number of specimens alive in each salinity was recorded every 24 hr. for a total of 8 days.

Experimental salinities were prepared by the dilution of sea water with de-ionized water and by the concentration of sea water by freezing. Approximate salinity determinations were conducted by first recording the specific gravity and then converting to salinity as in Harvey (1945), an approximate method sufficing at this stage.

2. Acclimation time tests

Ten specimens of each species were placed in each of the highest and lowest 'tolerated salinities'—for the purposes of this experiment a 'tolerated salinity' is one in which at least half of the specimens had survived to the end of the 8-day period. Blood was taken through the arthroal membrane at the base of a cheliped from two specimens from both salinities after 24 hr. and its depression of freezing-point was determined. These crabs were discarded and the procedure was repeated with fresh crabs every 24 hr. for a total of 5 days. It was assumed that the times required for acclimation to the intermediate salinities would be less than the longest time found to either of the extremes.

3. Determination of the depression of freezing-point of the blood and media

Four specimens of each species were subjected to each of the 'tolerated salinities' and maintained at 25° C. for a period of time equal to the longer of the two acclimation times previously determined. After acclimation the depressions of freezing-point of the blood (four replicates) and the medium (two replicates) were determined using the method of Jones (1941) with the modifications of Gross (1954). The molarities obtained were converted into equivalent depressions of freezing-point using Parry's (1957) modification of Ramsay's (1949) formula.

The error inherent in the apparatus and method was found to be 2.06% coefficient of variation. This high value, which was probably to a large extent caused by variations in thickness of the glass wall of the melting-point tubes, was, however, considered acceptable in view of the nature of the described investigations.

EXPERIMENTAL RESULTS

The acclimation times required by the five species under study appear to be very similar, in all cases the blood reaching a constant value by 48 hr. Thus this amount of time was allowed in experiments on all the species.

1. *Macrophthalmus crassipes*

Specimens, collected from the margin of a *Zostera* bed at North Dunwich, North Stradbroke Island (see Fig. 1), were taken from a wet-weight range of 2.0–4.8 g.

The range of salinities tolerated by this species in the laboratory (Table 1) is 30–60‰. The range of salinities which this species appears to experience in the field is much narrower, being in the region of from 33.5 to 36.8‰ (C.S.I.R.O., 1953).

As can be seen from Fig. 3 the powers of osmoregulation displayed by *M. crassipes* are very limited. In slightly hyposaline media hyper-osmoregulation is exhibited, but with a maximum maintained difference of only 2.5‰ salinity; whilst in hypersaline media, hypo-osmoregulation is displayed, the difference in concentration between the internal and external media increasing (from 1 to 5‰) with increase in concentration

of the external medium up to about 60‰, after which the salinity is incompatible with further survival. The blood is isosmotic with the external medium at the salinity of normal sea water.

Table 1. *The survival of specimens of Macrophthalmus crassipes in different experimental salinities*

Approx. salinity (‰)	Time period (days)								
	0	1	2	3	4	5	6	7	8
	Nos. alive								
2	6	0	—	—	—	—	—	—	—
10	6	5	4	4	3	3	2	1	1
20	6	6	6	5	5	4	3	2	1
30	6	6	6	6	6	6	5	5	4
40	6	6	6	6	6	6	5	5	5
50	6	6	6	6	6	6	6	6	6
60	6	6	6	6	6	5	5	4	4
70	6	5	5	4	3	2	2	1	0

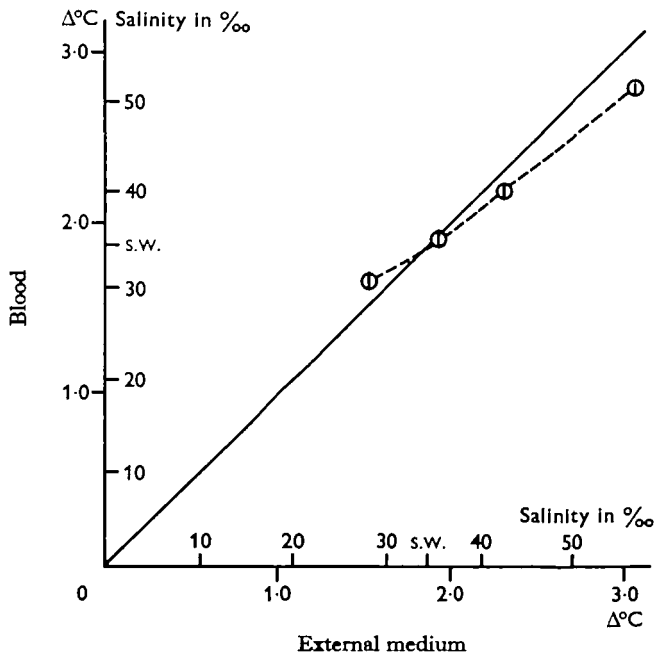


Fig. 3. Graph showing the blood osmo-concentration of *Macrophthalmus crassipes* as a function of salinity. Individual points represent the mean of four replicates, with the range shown as a straight line.

2. *Mictyris longicarpus*

Specimens, collected from the mouth of the Pine River estuary (see Fig. 1), were taken from a wet-weight range of 1.0–3.0 g.

The range of salinities tolerated by *M. longicarpus* without any observable ill effects in the laboratory (Table 2) is 10–50‰. The lowest salinity that could be experienced by *M. longicarpus* in the field, discounting the dilution effects of heavy rainfall, is in

the region of 25‰ (see Fig. 2), and the highest is probably about 37‰, as with *M. crassipes*.

The regulatory powers of this species are quite marked (see Fig. 4). The blood is isosmotic with the external medium at a higher concentration than normal sea water,

Table 2. *The survival of specimens of Mictyris longicarpus in different experimental salinities*

Approx. salinity (‰)	Time period (days)								
	0	1	2	3	4	5	6	7	8
	Nos. alive								
2	6	4	0	—	—	—	—	—	—
10	6	6	6	6	6	5	5	5	5
20	6	6	6	6	6	6	6	6	6
30	6	6	6	6	6	6	6	6	6
40	6	6	6	6	6	6	6	6	6
50	6	6	6	6	6	6	6	6	6
60	6	6	6	5	5	5*	5†	5†	5†
70	6	6	6	6	6	5*	5†	5†	4†

* Animals very sluggish. † Animals extremely lethargic.

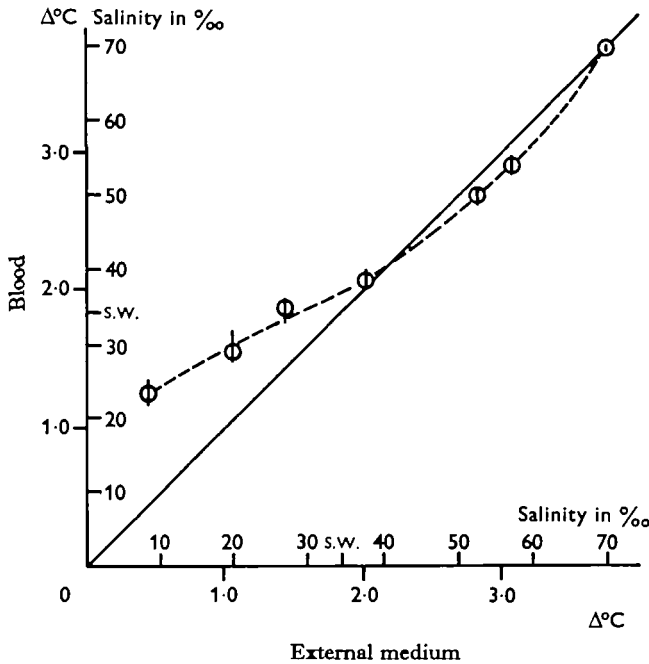


Fig. 4. Graph showing the blood osmo-concentration of *Mictyris longicarpus* as a function of salinity. Individual points represent the mean of four replicates, with the range shown as a straight line.

the isosmotic point being in the region of 40‰. In media hypo-osmotic to normal sea water a considerable degree of hyper-osmoregulation is exhibited; over a range in the external medium of 9–35‰, the blood being maintained from about 25‰ at

the lower value to 38‰ at the higher. *M. longicarpus* is a feeble hypo-osmoregulator, a maximum difference of only 4‰ salinity being maintained, and by twice normal sea water the blood is again isosmotic with the external medium.

3. *Macrophthalmus setosus*

Specimens, collected from the margin of a *Zostera* bed at Victoria Point (see Fig. 1), were taken from a wet-weight range of 5.5–11.8 g.

The range of salinities tolerated by *M. setosus* in the laboratory (Table 3) is 10–60‰. In the field this species does not extend into hypersaline conditions, the maximum

Table 3. *The survival of specimens of Macrophthalmus setosus in different experimental salinities*

Approx. salinity (‰)	Time period (days)								
	0	1	2	3	4	5	6	7	8
2	6	0	—	—	—	—	—	—	—
10	6	4	4	4	3	3	3	3	3
20	6	6	6	6	6	5	5	4	4
30	6	6	6	6	6	6	6	5	5
40	6	6	6	6	6	6	6	6	6
50	6	6	6	6	6	6	6	6	6
60	6	6	6	6	6	5	5	5	5
70	6	6	5	3	3	2	2	1	1

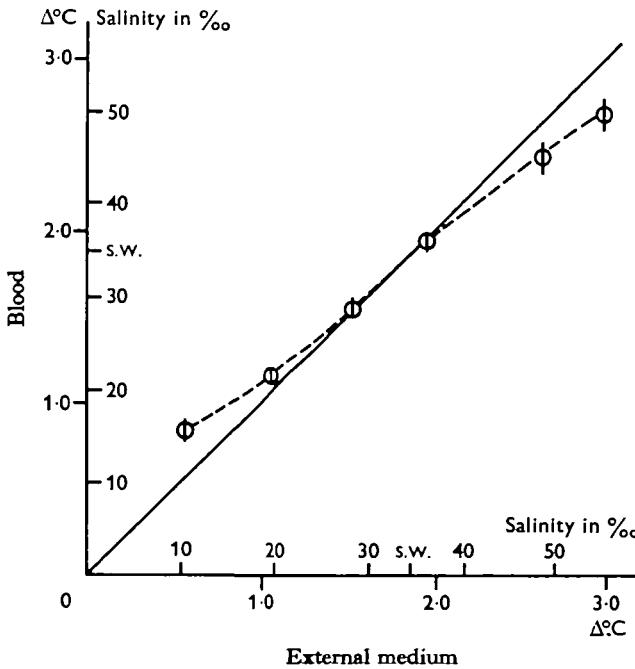


Fig. 5. Graph showing the blood osmo-concentration of *Macrophthalmus setosus* as a function of salinity. Individual points represent the mean of four replicates, with the range shown as a straight line.

experienced salinity probably being in the region of 37‰ (as with the two previous species). In the river *M. setosus* is reduced in numbers in regions experiencing salinities in the order of 15‰ and is absent from regions experiencing salinities of less than 10‰ (data from Snelling, 1959; Hodge, 1963).

M. setosus displays relatively feeble powers of osmoregulation over the relatively wide range of tolerated salinities. The blood is isosmotic with the external medium at the salinity of normal sea water (see Fig. 5), and in media both hypersaline and hyposaline to this isosmotic point regulation is exhibited. The differences maintained between the external salinity and the blood salinity are, however, small, the maximum difference maintained by hyper-osmoregulation being 6‰ and that maintained by hypo-osmoregulation being 5‰.

4. *Australoplax tridentata*

Specimens, collected from under *Avicennia* cover at Cribb Island (see Fig. 1), were taken from a wet-weight range of 0.3–1.1 g.

The range of salinities tolerated by this species in the laboratory (Table 4) is 2–70‰. The two lowest salinities, i.e. 2 and 10‰, and the highest salinity, i.e. 70‰, were only barely tolerated, however, in that 50% mortality occurred; although in the experimental media of approximately 20 and 60‰ salinity no mortality was induced. In the river *A. tridentata* begins to be reduced in numbers in an external salinity of slightly less than 20‰, and then experiences a gradual reduction in numbers down to a salinity of about 3‰ which forms the lower extreme to its distributional salinity range (from data of Snelling, 1959; Hodge, 1963).

Table 4. *The survival of specimens of Australoplax tridentata in different experimental salinities*

Approx. salinity (‰)	Time period (days)								
	0	1	2	3	4	5	6	7	8
	Nos. alive								
2	6	4	4	4	3	3	3	3	3
10	6	6	6	6	6	5	4	4	3
20	6	6	6	6	6	6	6	6	6
30	6	6	6	6	6	6	5	5	5
40	6	6	6	6	6	5	5	5	5
50	6	6	6	6	6	6	6	5	5
60	6	6	6	6	6	6	6	6	6
70	6	4	4	4	3	3	3	3	3

A. tridentata is the only species under study to occur in hypersaline conditions in the field. It has been collected by the author from mangrove-swamp and salt-marsh conditions above mean high-water spring at Noosa (100 miles north of Brisbane), Bribie Island and Cribb Island (Moreton Bay). Badham (1963) records salinities up to 60–70‰ from salt-marsh and mangrove-swamp conditions in Moreton Bay.

This species exhibits the most marked capabilities for osmoregulation of the species under study (see Fig. 6). It shows marked powers of hyper-osmoregulation, although its powers in this direction are less than those of *Paracleistostoma mcneilli*, and considerable powers of hypo-osmoregulation. The blood is isosmotic with the external

medium at a salinity slightly less than that of normal sea water, the isosmotic point being 32.5‰. In media hyposaline to this isosmotic point a quite high degree of homiosmosis is exhibited, *A. tridentata* being comparable in this respect to *Rhithropanopeus harrisii* (Jones, 1941). Over an external salinity range of from 18 to 35‰ the blood is maintained at values from 30 to 32.5‰, while over the external range of 5 to 18‰ the salinity of the blood falls from 30 to 24‰. The value of 18‰ external salinity then marks a change in the abilities of this species to maintain a more or less constant internal osmotic pressure.

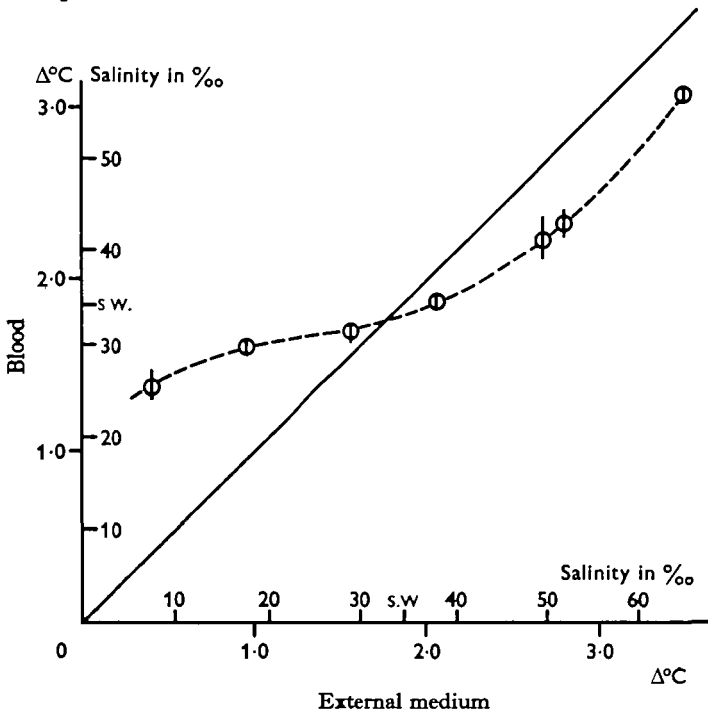


Fig. 6. Graph showing the blood osmo-concentration of *Australoplax tridentata* as a function of salinity. Individual points represent the mean of four replicates, with the range shown as a straight line.

In media hypersaline to the isosmotic point *A. tridentata* displays powers of hypo-osmoregulation almost equivalent to those of *Uca crenulata* and more marked than those of *Pachygrapsus crassipes* (Jones, 1941). Over an external salinity range of from 48 to 65‰ the blood is maintained about 10‰ less than the salinity of the external medium.

5. *Paracleistostoma mcneilli*

Specimens, collected from the Brisbane River at Bulimba (see Fig. 1), were taken from a wet-weight range of 0.3–0.6 g.

The range of salinities tolerated by this species in the laboratory (Table 5) is 2–60‰. The range of salinities which it would experience in the field is in the order of 3–34‰; 3‰ being the approximate lowest salinity recorded by Hodge (1963) from St Lucia (18 miles upstream), whilst 34‰ is the highest salinity occurring in the region of Colmslie (5 miles upstream).

P. mcneilli is a hyper-hypo-osmoregulator, although its powers of hypo-osmoregulation are almost negligible. The blood is isosmotic with the external medium at a salinity of 28‰, a value appreciably less than that of normal sea water. In salinities of less than 28‰ this species displays very marked powers of homoiosmosis, it being comparable

Table 5. *The survival of specimens of Paracleistostoma mcneilli in different experimental salinities*

Approx. salinity (‰)	Time period (days)								
	0	1	2	3	4	5	6	7	8
	Nos. alive								
2	6	6	5	5	5	5	5	5	5
10	6	6	6	6	6	6	6	6	6
20	6	6	6	6	6	6	6	6	6
30	6	6	6	6	6	6	6	6	6
40	6	6	6	6	6	6	6	6	6
50	6	6	6	6	6	6	6	6	6
60	6	6	5	4	4	3	3	3	3
70	6	0	—	—	—	—	—	—	—

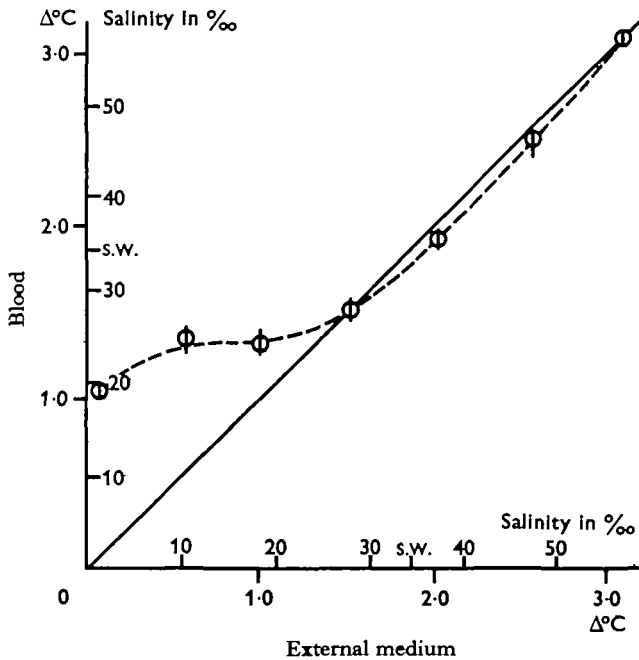


Fig. 7. Graph showing the blood osmo-concentration of *Paracleistostoma mcneilli* as a function of salinity. Individual points represent the mean of four replicates, with the range shown as a straight line.

to species such as *Pachygrapsus crassipes* and *Hemigrapsus oregonensis* (Jones, 1941). Over an external salinity range of from about 6 to 25‰ the blood is maintained at an almost constant value of 25‰ salinity, whilst at an external salinity of 1.5‰ the blood is still markedly hyper-osmotic, having a salinity of about 20‰ (see Fig. 7).

In salinities of 30‰ and higher the blood is slightly hypo-osmotic to the external medium, but the maximum maintained difference is only about 2.5‰. Thus in these salinities *Paracleistostoma mcneilli* probably survives on tolerance alone.

DISCUSSION

The great majority of crabs that have been found to exhibit hyper-hypo-osmoregulation belong to the grapsoid families. Lockwood (1962) predicted that many more grapsoid crabs would be found to exhibit hypo-osmoregulation (and thus also hyper-osmoregulation, since species capable only of hypo-osmoregulation are unknown—Kinne, 1964). The species under study have conformed to Lockwood's prediction in that all are capable of some degree of hypo-osmoregulation, even though, as with *Paracleistostoma mcneilli*, the degree may be slight.

All the species under study are thus osmoregulators, which would seem to be of definite selective advantage to species living in brackish or estuarine environments and thus experiencing fluctuating salinity (Nicol, 1960). However, their range of salinity tolerance and their capabilities for osmoregulation do not always correspond to the environmental conditions in which they are to be found in the field; for example it has been seen that all the species, with the exception of *Australoplax tridentata*, tolerate, and exhibit hypo-osmoregulation in, hypersaline conditions which they are unlikely ever to experience in nature. This may be a result of the organisms having evolved the mechanisms for adjusting themselves to salinity changes in their environments, so that they are to a certain degree independent of these changes, and have tolerance ranges which may exceed the usual range of fluctuations experienced in their immediate environment (Pearse & Gunter, 1957). Or, as Hedgpeth (1957) has suggested, 'Euryhalinity . . . may be a physiological characteristic not of a species or a genus alone, but of a phyletic stock' (p. 696), and species, although thus possessing the capabilities to exist over a wide range of salinities in a euryhaline environment, may become restricted to small portions of their potential range, with respect to salinity, by habitat preferences, etc. Indeed, although Ono (1965) found that habitat preferences did not appear to limit the distribution of grapsoid crabs in the Tataru-Umi Estuary, it will be seen that the probable effect of the substratum upon some of the grapsoid crabs in the Brisbane River Estuary is considerable.

If the species under study are listed in order of decreasing abilities of hyper-osmoregulation, i.e. *Paracleistostoma mcneilli*, *Australoplax tridentata*, *Mictyris longicarpus*, *Macrophthalmus setosus*, and *M. crassipes*, the order so obtained corresponds with that which would be obtained by listing these species in order of decreasing penetration of the Brisbane River, with the exception of the position of *Mictyris longicarpus*.

M. longicarpus has been seen to show comparatively well developed powers of hyper-osmoregulation, and to survive in experimental media of down to 10‰ salinity. Yet it does not penetrate into the Brisbane River beyond Aquarium Passage (2 miles upstream). It would therefore seem unlikely that salinity acts as a limiting factor to the distribution of this species in the area under consideration.

M. longicarpus is found in Moreton Bay in beaches of muddy sand, or in general terms, in substrates in which sand constitutes a large proportion of the total particle

constituents. Most estuaries, including the Brisbane River, are characterised by a general paucity of sandy substrates (Moore, 1958), and where sand does occur it is to be found only near the mouth of the estuary, the upper reaches being characterized by muds of varying texture (Emery & Stevenson, 1957). Snelling (1959) records the presence of this species (and also *Uca marionis*) only in regions near the mouth of the Brisbane River which have a sandy element in the substrate; further upstream, where sand is lacking from the littoral zone, both species are absent.

Thus it would seem that the preference for a sandy substrate, exhibited by this species, limits its distribution in the river to those regions near the mouth where that substrate occurs, although by virtue of its osmotic behaviour it could penetrate further up the river if suitable substrates were there to be found.

Macrophthalmus crassipes occupies very similar substrates to the previous species, although perhaps the mud content of the muddy sand preferred is slightly higher than in *M. longicarpus*. Thus in terms of availability of suitable substrates *M. crassipes* could presumably penetrate up the Brisbane River at least as far as *Mictyris*. This is not found, however, *M. crassipes* being completely absent from the river.

In the laboratory this species survived in experimental salinities of above 30‰ only. It can be seen from Fig. 2 that salinities of below 30‰ occur in the Brisbane River less than half a mile upstream, and thus it seems very probable that the inability of *M. crassipes* to tolerate salinities into the mixopolyhaline range ('Venice System', 1958) would effectively limit its distribution to Moreton Bay and exclude any penetration of the Brisbane River. This species could in fact be considered to inhabit an almost polystenohaline environment, and be regarded as a polystenohaline species which is absent from open oceanic beaches because of the adverse effects of heavy wave action, etc. All polystenohaline decapod crustaceans so far investigated, however, show 'no or negligible osmoregulation' (Kinne, 1963, p. 96) and are thus osmoconformers. Yet although the degree of regulation displayed by this species is limited, it is not negligible. Thus *M. crassipes* can be interpreted as being, to some extent, intermediate between polystenohaline osmoconformers showing negligible regulation and euryhaline osmoregulators with limited tolerated ranges of salinity.

M. setosus occupies a higher zone in the littoral region in Moreton Bay than it does in the Brisbane River, which might indicate that it is a typically marine species invading estuarine habitats (Moore, 1958); and that the limiting factor to its penetration into these conditions will hence probably be salinity. Substrate preference would certainly not seem to be in itself a limiting factor to the distribution of this species, since the muddy substrates preferred extend in distributional range further up the river than the species frequenting them. This substrate, however, may profoundly influence the environmental salinities, and their rate of change, experienced by *M. setosus* (Topping & Fuller, 1942; Smith, 1956).

In the laboratory this species tolerated salinities of down to 10‰ (although barely) and did not survive in media of lower concentration, while in the field *M. setosus* extends into salinities of down to 10‰, albeit in reduced numbers, and is absent from areas experiencing salinities of below that value. From the coincidence of the approximately 10‰ boundary in the experimentally determined range and in the field distribution, it would appear that salinity is indeed probably a limiting factor to the distribution of this species.

The degree of osmoregulation exhibited by this species is relatively feeble, being less than that displayed by any of the other species under study, with the exception of *M. crassipes*. This may indicate that *M. setosus* survives in conditions of reduced salinity primarily by means of tolerance, rather than by effective regulation; or that the osmotic behaviour, as determined by direct transference to the experimental salinities, is not the same as that manifested in the field, as a result of the modifying action of the soft intertidal mud substrate preferred. This substrate may induce hysteresis effects (see Kinne & Rotthauwe, 1952; Anderson & Prosser, 1953) and/or prolonged gradual acclimation effects (see Schlieper, 1929; Beadle, 1943), by virtue of the lowered lability of the interstitial salinity in muddy substrates and the differential retention of high and low salinities by the muds (Topping & Fuller, 1942; Smith, 1956; Moore, 1958).

The distributions of *Australoplax tridentata* and *Paracleistostoma mcneilli* show good correlations with their capabilities for osmoregulation. These species are the most euryhaline of the species under study and show the greatest degrees of regulation; *P. mcneilli* showing the most marked powers of hyper-osmoregulation and *A. tridentata* of hypo-osmoregulation, with hyper-osmoregulatory capabilities second only to *P. mcneilli*.

It can be seen from the data of Snelling (1959) that the Brisbane River estuary can be divided into three zones with respect to the distribution of *A. tridentata*. The first region, in which this species is a dominant organism in its particular intertidal zone, extends from the mouth to about 6 miles upstream and can be characterized by salinities usually in excess of 20‰. The second region in which the numbers of *A. tridentata* are gradually reduced, extends from about 6–18 miles upstream and can be characterized by salinities down to about 3‰ but for most of the year with salinities in excess of 10‰. The third region, from which this species is absent, extends from 18 miles up the river to further upstream and is characterized by lower salinities than those seen in the second region. This tripartite division of the river in terms of the distribution of *A. tridentata* corresponds with the tripartite behaviour of this species with respect to salinity. It has been seen that over an external salinity range of from 18 to 35‰ the blood is maintained almost constant, varying only within 2.5‰ salinity; whilst over the range 18 to 5‰ salinity the blood falls from 30 to 24‰, the homoiosmotic abilities of this species breaking down in these salinities. Thirdly, a salinity of about 2‰ caused 50% mortality after 4 days subjection. Therefore it seems very probable that salinity both limits the penetration upstream of this species and governs its abundance within the tolerated range.

In contrast to the four other species under study *A. tridentata* exhibits marked capabilities for hypo-osmoregulation. Further, it is the only species to occur in hyper-saline conditions above the high-water mark in Moreton Bay. In the laboratory 60‰ was tolerated without mortality, but 50% of the specimens subjected to 70‰ died. It would therefore seem likely that a salinity in the region of 70‰ would act as a limiting factor to the distribution of *A. tridentata*, and salinities of this order do occur in salt-marsh conditions in Moreton Bay (Badham, 1963). However, further studies will be required before the effect of salinity in influencing the distribution of this species in hypersaline conditions can be determined.

Paracleistostoma mcneilli can be described as the most successful of the Macro-phthalminae in the Brisbane River from the point of view of its abundance over com-

paratively large regions of that habitat. It is a dominant organism from about six miles to eighteen miles upstream (cf. *A. tridentata*), and over most of this region *P. mcneilli* is capable of maintaining a constant blood osmotic pressure.

Over an external salinity range of from 6 to 25‰ *P. mcneilli* displays homoiosmosis, the blood having a constant salinity of 25‰. In comparison with *A. tridentata* the homoiosmosis maintained by *P. mcneilli* is more constant and is continued into lower salinities (6‰ instead of 18‰). This ability probably accounts for the continued abundance of *P. mcneilli* in areas experiencing a reduction in the numbers of *A. tridentata*, and for its general abundance in regions of comparatively low salinity.

In the region of 6‰ external salinity a break in the regulatory abilities of this species is seen. Whereas over an external range of 20‰ the blood was maintained constant, over a range of only 4.5‰ below 6‰ the blood falls through 5‰ to 20‰. This break in the regulatory capacity of *P. mcneilli* in very low external salinities can be correlated with its eventual disappearance from the river, which occurs between 18 miles upstream, where it is still fairly common, and 20 miles upstream, where it is absent except during drought conditions (Snelling, 1959).

A further indication of the extent to which *P. mcneilli* has become adapted to estuarine salinities is the low blood/external salinity isosmotic point. This occurs in the region of 28‰, a value 4.5‰ lower than that of *A. tridentata*, and 6.5‰ lower than that seen in any of the other species under study.

P. mcneilli is apparently absent from Moreton Bay and from the Brisbane River from the mouth to Aquarium Passage (2 miles upstream). It has been seen that in salinities of above 30‰, the salinities experienced in these regions, *P. mcneilli* is an osmoconformer—the negligible degree of hypo-osmoregulation being discounted—and thus survives on tolerance alone. Yet it survives on tolerance in the laboratory in salinities of up to almost 60‰. Thus although *P. mcneilli* is fairly obviously primarily an estuarine organism (viz. (a) the low isosmotic point, (b) the high degree of homoiosmosis maintained in hyposaline conditions, (c) the lack of regulation in sea water and hypersaline conditions, etc.), it does not seem plausible to suggest that its absence from Moreton Bay is caused by an inability to osmoregulate, when it survives on tolerance in markedly hypersaline conditions in the laboratory.

It can be observed that the distributions of *P. mcneilli* and *Mictyris longicarpus* in the area under consideration are complementary. In general the Brisbane River from about three miles upstream to the fresh-water regions has a muddy littoral substrate, whilst from 2 miles upstream to the mouth, and in Moreton Bay, the littoral substrate consists of sand with varying degrees of mud intermixed. Mud beaches, equivalent to those from which *P. mcneilli* has been recorded in the river, are scarce in the bay, and are poorly known with respect to their crab fauna. A possibility therefore exists that *P. mcneilli* is confined to its observed region in the Brisbane River by habitat preferences, the preferred mud being absent from the mouth of the river and absent or little known in Moreton Bay. Further research on the mud beach faunas of Moreton Bay will be required before the question of the apparent confinement of *P. mcneilli* to the Brisbane River can be elucidated.

SUMMARY

1. The effect of salinity upon five Australian grapsoid crabs exhibiting differential penetration of an estuarine system has been investigated.
2. The salinity tolerances over a range of 2–70‰ (approx.) salinity, the acclimation times, and the osmoregulatory capabilities of these species have been determined.
3. It is concluded that for four of the five species salinity is probably a limiting factor in hyposaline conditions; whilst for only one species is salinity probably a limiting factor in hypersaline conditions. The relative abundances of two species in the estuary appears to be a function of their capabilities for osmoregulation.
4. Habitat preferences probably limit the upstream penetration of one species, and may limit the downstream penetration of a second.

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