HAEMOLYMPH CONCENTRATION AND APPARENT PERMEABILITY IN VARYING SALINITY CONDITIONS OF *GAMMARUS DUEBENI*, *CHAETOGAMMARUS MARINUS* AND *GAMMARUS LOCUSTA*

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SUMMARY

Haemolymph and external medium sodium concentrations have been investigated in three euryhaline amphipods, *Gammarus duebeni* (Lilljeborg), *Chaetogammarus marinus* (Leach) and *Gammarus locusta* (Linnaeus). These were subjected to various salinity regimes, including sudden and cycling salinity changes. The apparent permeability to water of these three species was investigated in various salinity regimes using THO as a marker. Of the three species, only *G. duebeni* demonstrated large and significant changes in apparent permeability to water. The bulk flow into and out of the animals was calculated showing a variation of responses in the three species, *G. duebeni* exhibiting the smallest bulk flow and *G. locusta* the largest.

These results were related to the ecology of the three species.

INTRODUCTION

Fully marine invertebrate organisms inhabit a stable environment where the external medium is comparable with the internal osmotic concentration of their body fluids, causing few osmotic problems for the cells. Organisms tolerating a variable salinity environment (euryhaline) face the problem of having to vary their regulatory responses as the external medium changes. This problem is heightened in small animals with a relatively large surface area to volume ratio, where small changes in volume can cause large changes in body fluid concentrations. The development of a highly impermeable body surface is impractical for an aquatic animal which respires through part or all of its body surface. However, it would be advantageous for fresh and brackish water animals to reduce their permeability to water and hence limit ion and water fluxes, if this could be done without too great a reduction in respiratory efficiency. Animals experiencing salinity changes would benefit from the ability to restrict the passage of water and ions when large osmotic gradients are present between the body fluids and the external medium. This would necessitate a mechanism

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controlling the permeability of the body surface in relation to the concentration gradient between the haemolymph and external medium.

Several species of euryhaline Crustacea have been demonstrated to change their apparent permeability to water when the external medium concentration is altered. The euryhaline crabs *Rhithropanopeus harrisi* (Smith, 1967), *Carcinus maenas* (Smith, 1970), the arctic amphipod *Gammarus setosus* (Bolt, 1982) and the euryhaline amphipod *Gammarus duebeni* (Lockwood & Inman, 1973) all display varying degrees of apparent permeability when acclimated to different salinities.

More recent work on *G. duebeni* (Bolt, Dawson, Inman & Lockwood, 1980) has shown that this species exhibits large, rapid changes in apparent permeability when exposed to sudden or cycling salinity changes, often associated with periods of salinity when the haemolymph is approaching isosmocity with the external medium. A particular feature is that the half-time of exchange of labelled water decreases dramatically in conditions around isosmocity. The purpose of the present paper is to compare these results with the corresponding changes in apparent permeability in less euryhaline species.

G. duebeni is found in a wide range of salinities from fresh water streams (Hynes, 1950) to 60‰–70‰ salt water rock pools in Norway (Davenport, 1979). The present work was carried out on populations from Totton Marsh, Southampton, where the animals are found in small pools in a salt marsh. In this region *G. duebeni* are found in three localities: (i) in drainage creeks, subject to cyclical salinity changes from 1–22‰ (Lockwood & Inman, 1973); (ii) in low salinity water in fresh water drainage channels where they only encounter saline conditions at high water springs; (iii) in small pools at the extreme high water mark where animals are covered at extreme high water springs and are subjected to extremes of salinity due to evaporation and precipitation. During high water springs the animals from these populations intermix, ensuring that there are no ecologically distinct races on the marsh. In contrast, *C. marinus* and *G. locusta*, although occurring well into estuaries (Spooner, 1947), are not generally subjected to long periods of low salinity. The *G. locusta* and *C. marinus* used in this paper are found co-existing under *Fucus* sp. in the middle region of a muddy shoreline on Hayling Island, near Portsmouth. These animals were selected as being less euryhaline than *G. duebeni*, though still being able to tolerate relatively short periods of reduced salinity.

MATERIALS AND METHODS
Salinity cycle generation

A microprocessor-controlled cycling salinity system was used for the regulation of external medium salinity (Lockwood *et al.* 1982). The standard cycle used in the present experimental series was 12 h 25 min, varying from 1–31‰ salinity. The temperature was maintained at 15 °C.

Water permeability

Tritiated water was used to determine water fluxes of the amphipods. Measurements of flux in both directions were made on animals in a steady state. However, only

influx can be used in experiments in non-steady state systems. These methods are modified from Lockwood, Inman & Courtenay (1973). Permeability to water of the amphipods was expressed as a half-time of exchange of tritiated water into or out of the animal. Tritiated water samples were counted in a Liquid Scintillation cocktail in a Beckman Series 3000.

Influx

The half-time of exchange of influx is calculated by comparing the tritiated water uptake by an animal in a 5-min loading period with the amount taken up when the animal is fully loaded.

Outflux

Outflux was measured by loading the animals with tritiated water, transferring them to unloading medium and monitoring the loss of tritiated water to the external medium.

These techniques (influx and outflux) compare counts at time *t* with counts at equilibrium. This enables counts per minute (c.p.m.) to be used directly without the necessity of finding the efficiency of the counter using quench curves.

Determination of sodium concentrations

Haemolymph was collected using a drawn out Pasteur pipette. It was possible routinely to remove 1–5 µl of haemolymph from a 100 mg animal. Aliquots of haemolymph (usually 1 µl) were added to 5–10 ml deionized water and analysed using an emission flame spectrophotometer (Pye Unicam SP900 or SP90). Sodium concentrations were determined from a standard curve.

Net fluxes

The net fluxes are calculated by the method of Lockwood *et al.* (1973). All experiments were carried out at 15 °C.

Animals

All three species were kept at 15 °C prior to and during the experiments. *G. duebeni* and *G. locusta* varied from 50–100 mg, and *C. marinus* varied from 50–150 mg.

All the animals were fed on Bemax prior to experiments and starved throughout any period of acclimatization and experimentation.

RESULTS

Sodium concentration

The haemolymph sodium concentration of *G. duebeni*, *G. locusta* and *C. marinus*, after acclimation to different salinities are compared in Fig. 1 using data from Lockwood (1964) and Haywood (1970). Comparison of haemolymph of the three species exposed to at least eight cycles in the salinity system is given in Fig. 2.

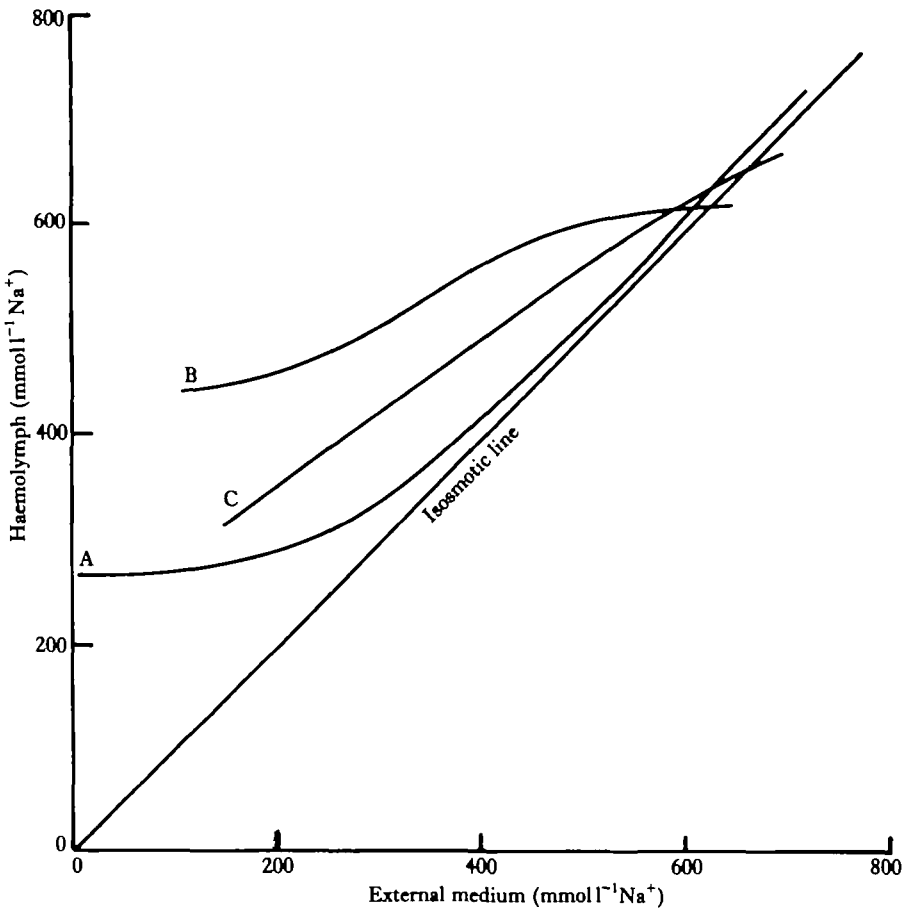These three species demonstrate a variation of responses in animals exposed to both

Fig. 1. Haemolymph Na$^+$ concentrations in *Gammarus duebeni*, *Chaetogammarus marinus* and *Gammarus locusta* acclimated to a range of salinities. A, *G. duebeni*; B, *C. marinus* and C, *G. locusta* (after Lockwood, 1964; Haywood, 1970).

steady-state and cycling conditions. In steady state conditions, *G. duebeni* maintains its haemolymph concentration strongly hyperionic at low salinities, while *C. marinus* and *G. locusta* are less hyperionic (Fig. 1). This trend is echoed when the animals are exposed to the cycling salinity conditions, where again *G. duebeni* maintains its haemolymph relatively constant (295 ± 15 mM-Na$^+$), *C. marinus* fluctuates from 350 mM-Na$^+$ to 450 mM-Na$^+$ and *G. locusta* varies from 200 mM-Na$^+$ to 450 mM-Na$^+$. *G. locusta* and *C. marinus* remain hyperionic or isionic throughout the cycle, while *G. duebeni* is hypoionic for a period of approximately 4 h during maximum salinity in the cycle.

These three species therefore exhibit a gradation of responses which correspond to their range of habitats and relative mortality in the experimental regime. *G. duebeni* do not appear to be adversely affected by the cycling salinity, even when maintained for periods extending up to 2 months. *C. marinus* are less able to maintain a constant haemolymph concentration, which is reflected by an initial mortality of up to 15 % when exposed to the salinity cycle. *G. locusta* is the least able to cope with cycling
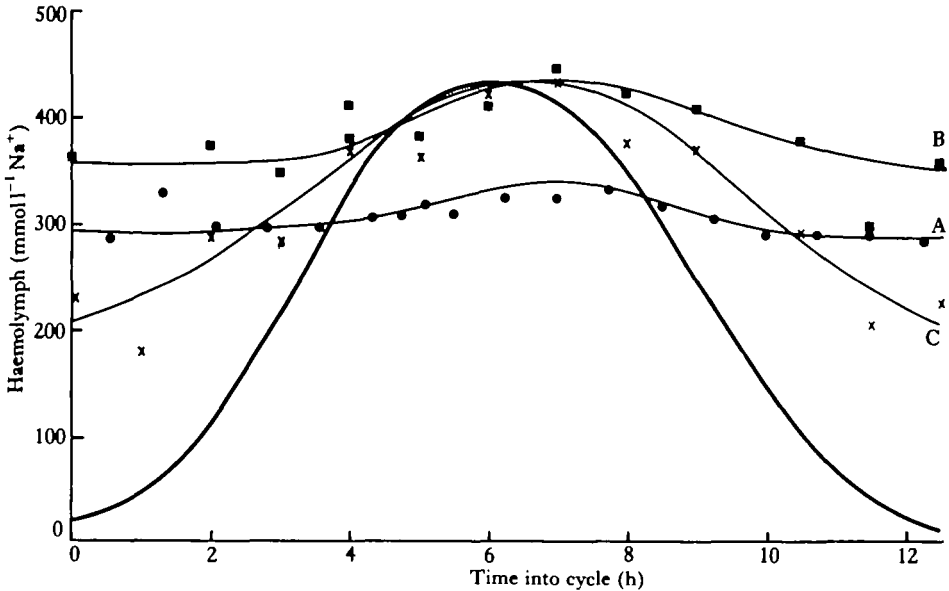
Fig. 2. Haemolymph Na$^+$ concentration in *Gammarus duebeni*, *Chaetogammarus marinus* and *Gammarus locusta* exposed to the salinity cycle. A, *G. duebeni* (solid circles); B, *C. marinus* (solid squares) and C, *G. locusta* (crosses). Lines are fitted by eye.

salinity conditions of this degree of severity and specimens died throughout the experiment. Nevertheless, *C. marinus* and *G. locusta* appeared to show a considerable range of individual tolerance, some animals of both species surviving for several weeks in the cycling regime.

Apparent permeability to water

Cycling salinity

G. duebeni exposed to the cycling salinity system exhibit distinct increases in apparent permeability which coincide with the two periods of isoionicity at 4 and 8 h into the cycle (Figs 2, 3). The apparent permeability increases to a $t_{1/2}$ of exchange of 10 min and 5 min at the isionic points, while the lowest apparent permeability ($t_{1/2}$ of 24 min) occurs when *G. duebeni* is hypotonic to the medium (Bolt *et al.* 1980).

C. marinus and *G. locusta* do not significantly vary their apparent permeability to water during the salinity cycle. *C. marinus* maintains a $t_{1/2}$ of 9 ± 2 min throughout the cycle and *G. locusta* maintains a $t_{1/2}$ of 4 ± 1 min.

Rapid changes in salinity

Apparent permeability and haemolymph sodium concentrations were measured in the three species before and after sudden changes in salinity of the external medium. Animals transferred from 2 % sea water to 100 % sea water are initially forced hypotonic to the external medium. In *G. duebeni* the haemolymph concentration then slowly increases over a period of approximately 16 h until the animal becomes isotonic and finally slightly hypertonic to the external medium (Fig. 4). During this period the
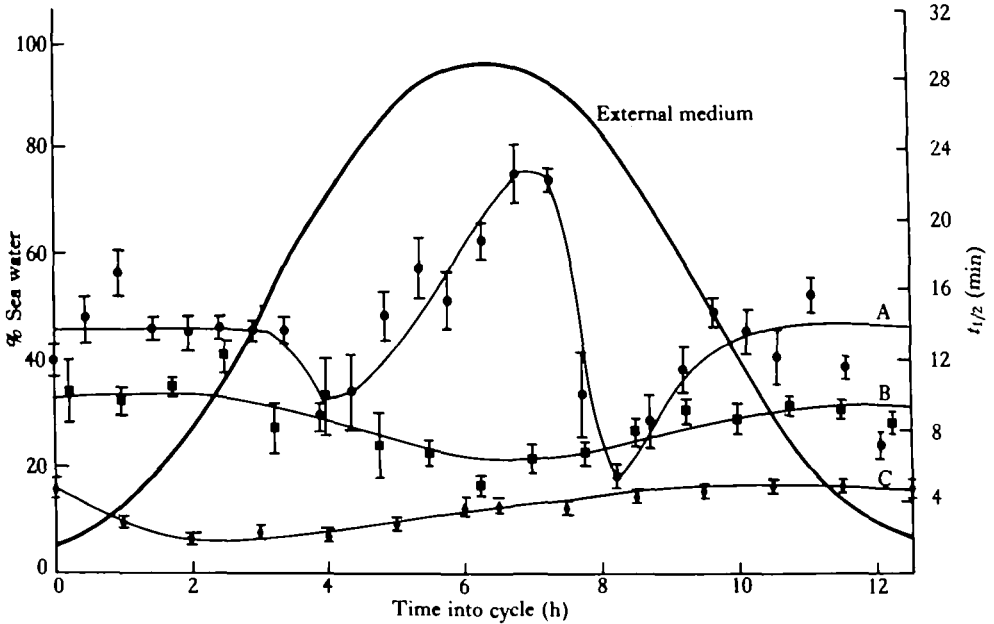
Fig. 3. Apparent permeability to water in *Gammarus duebeni*, *Chaetogammarus marinus* and *Gammarus locusta* exposed to cycling salinity conditions. A, *G. duebeni* (solid circles); B, *C. marinus* (solid squares) and C, *G. locusta* (solid diamonds). Vertical bars = 1 × s.e.m.

apparent permeability decreases slightly (increase in $t_{1/2}$ of exchange) after the initial transference to 100 % sea water. The change is from approximately 18 min to 21 min and the latter value is then monitored up to the isoionic point, where there is a sudden increase in apparent permeability ($t_{1/2}$ drops to 5 min). Thus *G. duebeni* appears to be least permeable during periods of hypotonicity when the osmotic bulk flow is out of the animal.

In contrast, *C. marinus* and *G. locusta* acclimated to 10 % sea water and transferred

Table 1. *Apparent permeability to water of* Chaetogammarus marinus *and* Gammarus locusta *exposed to steady state salinity and a sudden change from 10 % to 100 % sea water*

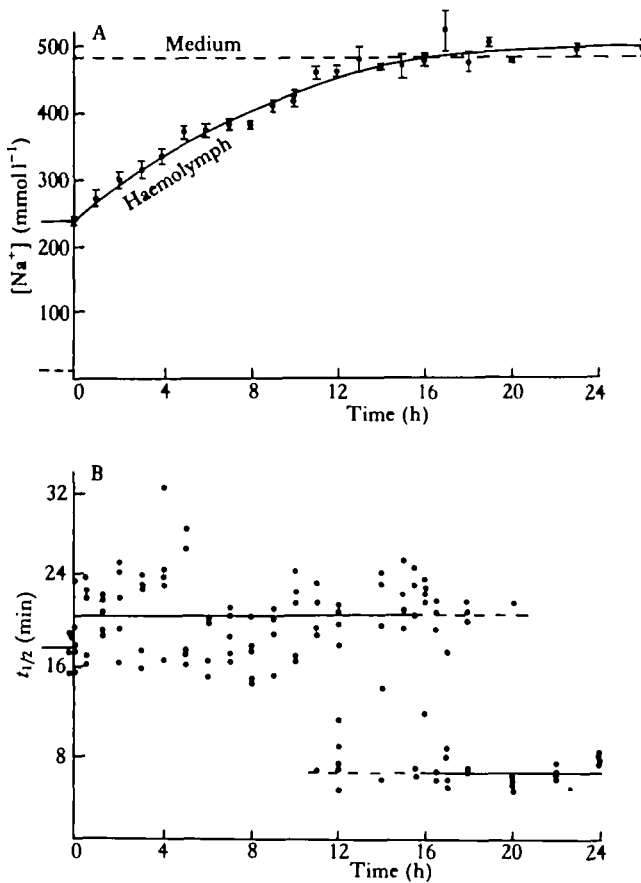|	Sea water concentration (%)	$t_{1/2}$ (min)	s.e.m.	Blood/med gradient (mM grad)	N
		Chaetogammarus marinus			
Steady state salinity	10	9·0	1·6	+320 (Hypertonic)	5
Sudden change in salinity	10–100	6·1	1·0	−160 (Hypotonic)	6
		Gammarus locusta			
Steady state salinity	10	4·21	0·24	+176 (Hypertonic)	5
Sudden change in salinity	10–100	3·2	0·7	−305 (Hypotonic)	6

Fig. 4. (A) Haemolymph and medium Na$^+$ concentrations in *Gammarus duebeni* exposed to a sudden change in salinity of the medium. Vertical bars = 1×s.e.m. Lines fitted by eye. (B) Half-time of exchange (THO) in *G. duebeni* exposed to a sudden change in salinity of the medium. Lines fitted by eye.

to 100 % sea water do not exhibit significant changes in apparent permeability associated with hypotonicity. The $t_{1/2}$ of exchange in *C. marinus* changes from 9·0 to 6·1 min, and the $t_{1/2}$ of *G. locusta* shows a small decrease from 4·21 to 3·2 min (Table 1). Thus *C. marinus* and *G. locusta* do not exhibit large changes in apparent permeability even when they are forced hypotonic to the external medium.

Net flow calculations

If the half-time of exchange of water, the osmotic concentration of the haemolymph and the osmotic concentration of the medium are all known, it is possible to calculate the net fluxes in the animal. When the haemolymph is hypertonic to the external medium, the bulk flow of water is into the animal and if the haemolymph is hypotonic, then the flow is outwards.

The net flow is calculated using the following formulae (Lockwood & Inman, 1973).

$$\frac{100 \ln 2}{t_{1/2}} = F \tag{1}$$

$$\frac{\text{Mm} - \text{Ma } F}{\text{Mm}} = Os, \tag{2}$$

where Mm is the mole fraction of medium, Ma is the mole fraction of haemolymph, F is the water flux and Os is the net water flow. The mole fractions of the blood and medium are calculated as:

$$M = \frac{55 \cdot 56}{55 \cdot 56 + x},$$

where x is the osmolal concentration between 0 and 1. Calculating the net flow of water throughout the cycling salinity system in the three amphipods, G. duebeni, G. locusta and C. marinus (Fig. 5) demonstrates the different degree of adaptation to salinity change in these three species. G. duebeni never exceeds a flux of 100 % body water per day, C. marinus reaches the rate of 120 % body water per day, while G. locusta peaks at a rate of nearly 300 % body water per day.

The difference between the three species is further emphasized by calculations of the percentage water uptake or loss of body water over the period of one cycle (= 12 h). This is achieved by integrating the area under the curve in Fig. 5 for each animal. G. duebeni has a % body water uptake of +15 % during the cycle, C. marinus +31·9 % and G. locusta +60·6 % body water per cycle. It is assumed that these osmotic fluxes into the animal are matched by urine flow out of the animal if the volume of the animal is to remain constant.
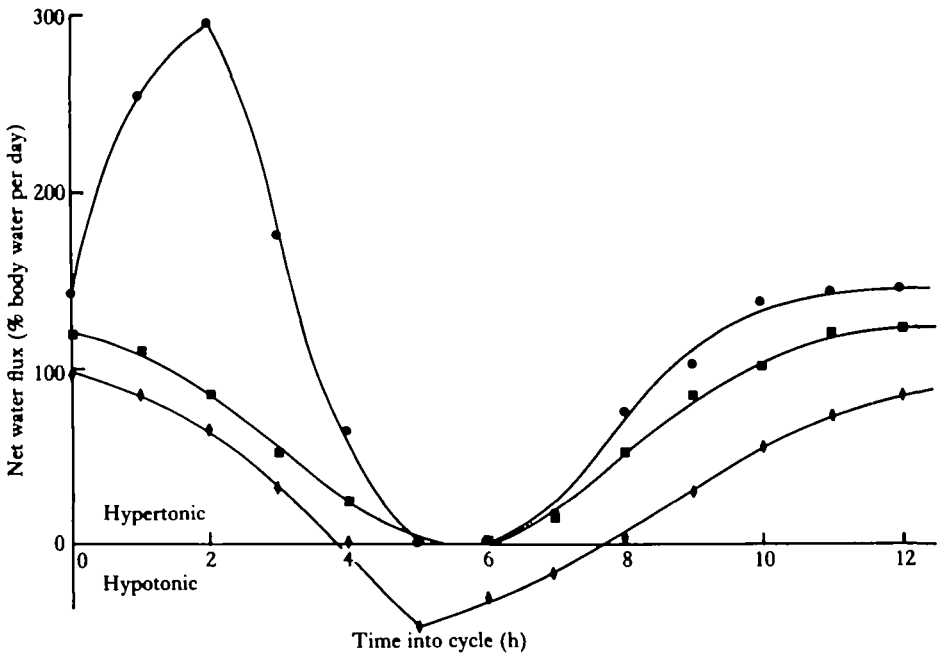

Fig. 5. Net water flux in *Gammarus duebeni* (diamonds), *Chaetogammarus marinus* (squares) and *Gammarus locusta* (circles) exposed to the cycling salinity regime.
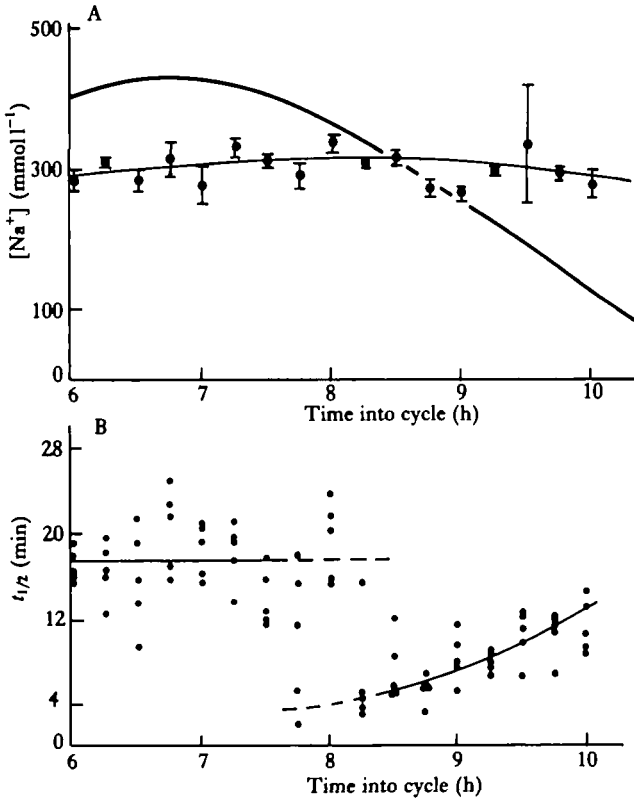
Fig. 6. (A) Haemolymph and medium Na$^+$ concentrations in *Gammarus duebeni* exposed to the cycling salinity regime. (B) Half-time of exchange (THO) in *G. duebeni* exposed to the cycling salinity regime. Lines fitted by eye.
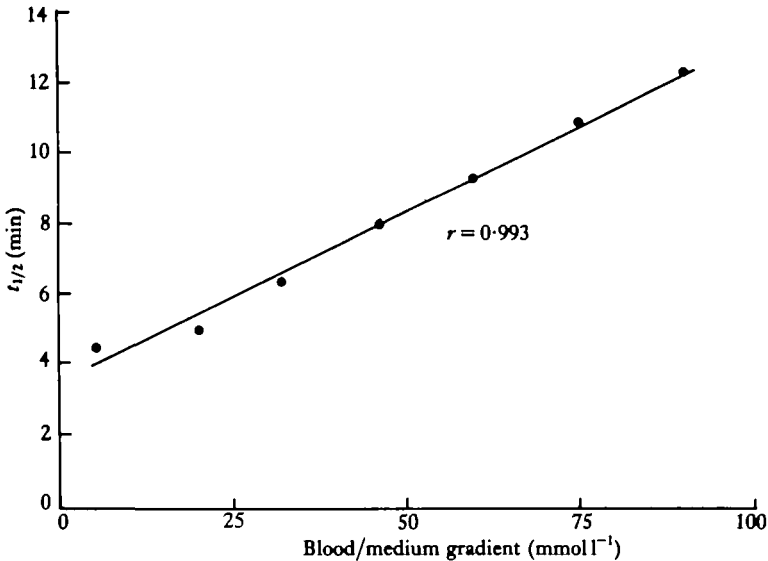

Fig. 7. Half-time of exchange (THO) against haemolymph/medium concentration gradient in *Gammarus duebeni* exposed to cycling salinity regime. Animals always hypertonic to the medium. Line fitted by linear regression.

A more detailed study of the apparent permeability of *G. duebeni* during a period of large permeability change in the cycling salinity system demonstrates that the change in $t_{1/2}$ is extremely rapid (Fig. 6). Apparent permeability and blood sodium were measured over the 6- to 10-h period of the cycle. During this period, the animals were changing from being hypotonic to hypertonic to the external medium, and accompanying this transition there was a rapid change in permeability. This is manifested by a rapid transition from high $t_{1/2}$ (low apparent permeability) to a low $t_{1/2}$ as the animals gradually approach isionicity from hypoionicity and a gradual return to a high $t_{1/2}$ (low apparent permeability) as the degree of hypertonicity increases. The $t_{1/2}$ drops rapidly from 16 min to 4 min, subsequently returning gradually to 13 min.

The $t_{1/2}$ of exchange appears to be proportional to the gradient between the haemolymph and external medium, when the animals are hypertonic to the medium (Fig. 7) (linear regression gives a correlation coefficient of 0·993). During hypotonicity the $t_{1/2}$ is not proportional to the gradient.

DISCUSSION

Examination of the water fluxes in *Gammarus duebeni*, *Chaetogammarus marinus* and *Gammarus locusta* has shown that, of these three species, only *G. duebeni* demonstrates a major change in apparent permeability as the salinity of the medium is varied. Detailed study of these changes shows that in all cases investigated, the change in apparent permeability is extremely rapid when the animal is in transition from being hypotonic to isotonic. Conversely if the animal is in transition from isotonicity to hypertonicity, the $t_{1/2}$ of exchange appears to be correlated directly to the gradient between haemolymph and external medium. These observations appear to hold in cycling salinity conditions (Fig. 6), steady state condition (Fig. 1) and in non steady state experiments (Fig. 4). In animals transferred from 2 % sea water to 100 % sea water (Fig. 4) the change of apparent permeability from a $t_{1/2}$ of 21 min to a $t_{1/2}$ of 6 min appears extremely rapid, even though the change in the gradient from haemolymph to external medium is relatively slow. Again, this rapid change in apparent permeability occurs as the animal is approaching isosmocity with the external medium. Unfortunately, experimental techniques preclude the measurement of apparent permeability and blood sodium simultaneously and it is thus impossible to know the ionic concentration and $t_{1/2}$ in an individual animal.

These results were obtained using THO as a marker to measure water exchange rates. Until such changes in water permeability have been demonstrated by a different approach, the possibility that the permeability change is an artefact of the experimental method must linger. Smith (1967) accepted this limitation and noted the necessity of using the term 'apparent' when discussing permeability changes. However, studies using ^{51}Cr EDTA to monitor urine flow rates during apparent permeability changes have largely removed this doubt (S. R. L. Bolt, in preparation; Bolt, 1982).

It is thus proposed that the ability of *G. duebeni* to become less permeable in conditions of high water fluxes guards the animal against physiologically embarrassing conditions. This is envisaged as being especially important when the net flux is out of the animal during periods when the body fluids are hypotonic to the medium. This

corresponds with the rapid changes in $t_{1/2}$ as the animal goes from being isotonic to ▌ypotonic to the medium and *vice versa*.

The above conclusions are not applicable in *C. marinus* and *G. locusta*, neither of which inhabit such euryhaline conditions as those occupied by *G. duebeni*. It would be interesting to know whether other highly euryhaline forms adopt similar physiological responses as *G. duebeni*. The isopod *Sphaeroma rugicauda* co-exists with *Gammarus duebeni* in the salt marsh pools in Totton, Southampton (Harris, 1967). These animals are of similar size and experience identical osmotic conditions as *G. duebeni*. Under experimental conditions, *S. rugicauda* shows an extremely large individual variation of water permeability due to the animals' behavioural response of rolling up into a tight ball when disturbed (S. R. L. Bolt, unpublished observations). Further work is needed to overcome this problem and to determine if a crustacean of the Order Isopoda living in the same conditions as *G. duebeni* has evolved a similar response to osmotic stress as *G. duebeni*.

The three species, *G. duebeni*, *C. marinus* and *G. locusta* clearly show a variation in response to a varying external salinity which can be correlated to their ecological tolerance to osmotically stressful conditions.

G. duebeni inhabits shallow salt marsh pools where salinity change can be unpredictable and extreme. Furthermore, the salinity in the pools can be extremely low (2   ) for protracted periods of up to 14 days. These osmotically demanding conditions necessitate an effective osmoregulatory mechanism. In contrast *C. marinus* and *G. locusta* only experience reduced salinity for relatively short periods due to fresh water run off and precipitation at low tides and there is correspondingly less selection pressure for the presence of a mechanism to cope with protracted low salinities. It is thus proposed that the ability to vary apparent permeability is an important facet of osmoregulation, which is advantageous to a species which successfully survives in osmotically stressful conditions.

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