# OSMOREGULATION IN THE PRAWN *PALAEMON LONGIROSTRIS* (CARIDEA, PALAEMONIDAE)

-by-

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This work has not been accepted and is not concurrently being submitted for any other degree, and is a record of work carried out by the candidate himself.

Signed P.A.S. Campbell

Candidate

Date 28/4/88

This is to certify that the work submitted here was carried out by the candidate himself. Due acknowledgement has been given to any assistance received.

Signed P.A.S. Campbell

Candidate

Date 28/4/88

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Supervisor of Studies

Date 28/4/88.

# Osmoregulation in the prawn *Palaemon longirostris* (Caridea, Palaemonidae)

Peter J Campbell

## ABSTRACT

Salinity tolerance, and several aspects of osmoregulation, ionic regulation and permeability were measured for *Palaemon longirostris* at three temperatures (4, 12 & 20°C). Influence of ontogenetic stage on salinity tolerance and osmoregulation was investigated by testing separately individuals sorted, using carapace length, into 'small' (10-18mm), 'medium' (18-24mm), 'large' (>24mm) and 'ovigerous' (>24mm) size groupings. Effect of seasonal acclimatization on salinity tolerance and osmoregulation was taken into account by comparing responses of summer- with winter-collected prawns.

Irrespective of temperature and size, summer *Palaemon longirostris* were extremely euryhaline and had >90% survival in various salinities from 0.5-34‰ . For summer prawns, survival in 43‰ was reduced, particularly at 4°C. Salinity tolerance of winter prawns was generally less than that of summer individuals, this difference being marked at salinity extremes in combination with low temperature.

Over the salinity range 0.5-34‰ , prawns were very efficient hyper-hypo-osmoregulators at each temperature. At 43‰, blood osmolalities tended towards the isosmotic, indicating that osmoregulation was breaking down. There was no clear effect of prawn size or season on osmoregulation, however, low temperature appeared to be disruptive. Transfer of prawns from 14‰ to either 5‰ or 34‰, and from 1‰ to 34‰, resulted in a new steady blood osmolality within 6-12h. Transfer from 34‰ to 1‰, caused blood osmolality to drop significantly within 12h, and a new equilibrium was not reached until 72h. The inorganic ions sodium, chloride, potassium, calcium and magnesium accounted for >94% of total blood osmolality over the salinity range 0.5-34‰ . There was no consistent effect of temperature on the regulation of these ions.

Prawns showed reduced permeability (to water) at low salinity and low temperature. Transfer of prawns from 34‰ to 1‰ had no immediate effect on permeability, however, transfer of prawns from 1‰ to 34‰ caused an immediate increase in permeability. The permeability of *Palaemon longirostris*, acclimated to a range of salinities between 0.5-34‰, was lower than that of *Palaemonetes varians*, *Crangon crangon* and *Palaemon elegans*

These results are discussed in terms of their adaptive significance and in relation to the evolution of the family Palaemonidae. It is suggested that *Palaemon longirostris* represents a evolutionary link between brackish-water and freshwater palaemonids.

TABLE OF CONTENTS

# CHAPTER 1

# GENERAL INTRODUCTION

# 1. GENERAL INTRODUCTION

Species within the family Palaemonidae occur throughout the world in a wide variety of aquatic habitats. The majority of palaemonids inhabit fresh water, whereas relatively few are marine and only a minority are brackish-water species (Kirkpatrick & Jones, 1985).

Surprisingly, freshwater palaemonids can tolerate a relatively wide range of salinities, even though their habitats generally vary little with regard to salinity (Sandifer et al., 1975; Guest & Durocher, 1979; Moreira et al., 1986). The osmoregulatory characteristics of freshwater palaemonids are typical of those reported for freshwater crustaceans in general. Thus, all freshwater palaemonids studied to date have isosmotic points well below the osmotic concentration of sea water, and are hyper-osmoregulators (Parry, 1957, 1961; Dobkin & Manning, 1964; Denne, 1968; Castille & Lawrence, 1981c; Moreira et al., 1983).

Marine and brackish-water palaemonids have similar osmoregulatory characteristics irrespective of whether they occur in stable saline habitats or in environments where salinity fluctuations are pronounced. All are euryhaline, have isosmotic points below that of sea water, and have the ability to hyper-hypo-osmoregulate (Panikkar, 1941; Parry, 1954, 1957; Dobkin & Manning, 1964; Potts & Parry, 1964a; Born, 1968; Denne, 1968; Spaargaren, 1972; Hagerman & Uglow,

1983; Knowlton & Kirby, 1984; Ramirez de Isla Hernandez & Taylor, 1985; Kirkpatrick & Jones, 1985). Hyper-hypo-osmoregulation is thought to be the most sophisticated genetic adaptation to salinity change associated characteristically with species living under extensive salinity fluctuations (Kinne, 1963a,b; Hagerman, 1971). Unfortunately, relatively few species from such environments have been studied in any detail, and therefore the general applicability of this correlation remains to be confirmed.

*Palaemon longirostris* (H. Milne-Edwards, 1837) has a geographical distribution ranging from the Mediterranean and Black Seas northwards to the coasts of Britain and Germany (Smaldon, 1979). In Britain, this species is generally restricted to localized occurrences around the southern coasts of England, and is relatively common in the South West where it occurs predominantly in the upper reaches of large river estuaries. *P. longirostris* is regarded as an estuarine prawn (Smaldon, 1979), however, there are reports of ovigerous females migrating downstream to the sea at times of egg hatching (Gurney, 1923). Such migration was thought necessary to release the larvae into a more suitable saline environment for their development. Recent evidence tends to support this theory, as larval *P. longirostris* require high rather than low salinities for optimal development (Antonopoulou, 1987). Although, high salinities provide the best conditions for larval development in *P. longirostris*, migration of ovigerous prawns does not

appear to be the universal method of ensuring that the larvae develop in high salinity. Some populations, occupying almost fresh water, have ovigerous females present throughout the breeding season (Fincham & Furlong, 1984; Antonopoulou, 1987). An alternative method for the dispersal of *P. longirostris* larvae into saline waters has thus been proposed which helps to explain this apparent contradiction. The second method involves nocturnal swimming at high tide by ovigerous females and the release of larvae into the water column; the larvae being subsequently carried out to sea on the ebb tide (Fincham & Furlong, 1984). Thus, the mechanism by which larvae of *P. longirostris* are dispersed downstream appears to vary, and may well be related to differences in the strengths of water currents. For instance, under conditions of low water movement ovigerous females act as biological transporters, whereas in habitats with strong currents moving seawards, water velocity would carry the larvae to the sea.

Apart from these studies on larval tolerances and swimming rhythms of ovigerous females, little is known of the ecology of *P. longirostris*. Similarly, apart from some preliminary measurements of blood and urine osmolality in prawns acclimated to one salinity (Parry, 1957), no aspect of the eco-physiology of this species has been studied previously. Therefore, the present work set out to investigate the osmoregulatory adaptations of *P. longirostris*, concentrating on post-larval stages. Particular attention was placed on the influence of prawn

size, temperature and season, as the influence of intrinsic and enviromental factors on osmotic response has been relatively little studied in crustaceans.

Estuarine crustaceans are typically euryhaline (Lockwood, 1976), therefore Chapter 2 of this thesis reports on the degree of salinity tolerance shown by *Palaemon longirostris*. The criterion of tolerance used was to record survival in a wide range of external salinities over a seven day period.

Due to the continuous osmotic flow of water and the diffusional flow of salts that occur between an aquatic animal and its surrounding medium, species inhabiting fluctuating salinity conditions are faced with problems of water and salt balance. To overcome these osmotic problems, euryhaline organisms have adopted one of two separate physiological strategies. Euryhaline animals either conform osmotically (osmoconformers) to their surrounding medium or maintain an internal osmotic concentration different from that of the external medium (osmoregulators). In order to determine which of these strategies is employed by *Palaemon longirostris*, the osmotic concentrations of individual prawns acclimated to a wide range of salinities were analysed and the results are reported in Chapter 3. Since salinity fluctuations within an estuary can occur quite rapidly (Lockwood, 1976), Chapter 3 reports also on the time-based osmotic response of the haemolymph of individual *P. longirostris* exposed to sudden, acute salinity changes.

It is well established that inorganic ions are the most important effectors of total blood osmolality in crustaceans, however, a discrepancy between the total ionic concentration and the total osmotic concentration has been recorded for some species at specific salinities (Hagerman, 1973). These discrepancies are thought to be due to the involvement of organic osmotic effectors such as proteins in the regulatory process. Consequently, Chapter 4 investigates the degree of importance of ionic regulation to osmoregulation in *Palaemon longirostris*.

The crustacean cuticle acts as an interface between the animal and its surrounding external medium. Therefore, any movement of water and salts that occurs between an aquatic crustacean and its surrounding medium must take place across the cuticle. Cuticular permeability to water is thus a very important aspect of the osmoregulation of euryhaline crustaceans. For this reason, the effect of salinity on apparent permeability to water was measured for *Palaemon longirostris* and compared with values obtained for three other caridean prawns which show characteristic horizontal distributions within the same estuary as *P. longirostris*. The results are presented in Chapter 5.

Finally, the general findings of this study are discussed in Chapter 6, and are compared with the literature values reported for other marine, brackish-water and freshwater palaemonids in order to determine to which grouping *Palaemon longirostris* belongs. The data are used also to comment on the phylogeny of this very interesting family of prawns.

# CHAPTER 2

## SALINITY TOLERANCE

This chapter was presented as a paper at the 22nd European Marine Biology Symposium (Barcelona, 1987).

## 2.1. INTRODUCTION

Estuarine organisms have evolved several adaptations to the abrupt and pronounced fluctuations of salinity and temperature which are dominant features of their demanding environment (for reviews see, Vernberg & Vernberg, 1975; Lockwood, 1976). In general, estuarine crustaceans are euryhaline (Dorgelo, 1976), but their salinity tolerance limits may be modified by both intrinsic and environmental factors. For example, body size is known to influence the salinity tolerance of some crustaceans (Kinne, 1971), however, salinity tolerance may either increase (Marsden, 1973; Kirkpatrick & Jones,1985) or decrease (Gable & Croker, 1978; Jones, 1981) with increasing animal size. Temperature may also influence the salinity tolerance of crustaceans (Dorgelo, 1976; Kinne, 1970), and some species show improved salinity tolerance at low temperatures (McLusky, 1979), whereas others have improved salinity tolerance at higher temperatures (Hagerman & Uglow, 1983). Season has also been shown to affect the salinity tolerance of some crustacean species (Lance, 1963; Hicks, 1973; McLusky, 1979; Marsden, 1980; Jones,1981), however, the effect of season on salinity tolerance is inconsistent between species. Information as to the direction and adaptive significance of the effects of these variables on salinity tolerance is rather limited and somewhat contradictory. The influence of each of these

factors on the salinity tolerance of *Palaemon longirostris* (Milne Edwards 1837) (Caridea, Palaemonidae) is reported here.

*Palaemon longirostris* is a shallow-water species which occupies the upper reaches of large river estuaries (Smaldon, 1979). As all post-larval stages of *P. longirostris* occur throughout the year in the dilute saline regions of estuaries (per. obs.), this species is of particular interest with regards to the adaptive significance of any intra-specific variability in salinity tolerance. Any intra-specific variation in the salinity tolerance of this species cannot be related to major differences in adult distribution. In addition, as *P. longirostris* has a geographical range centred around the Mediterranean and Black Seas and reaches its northern limit in southern England (Smaldon, 1979), the effect of low temperature on the salinity tolerance of an English population of *P. longirostris* may explain its absence from estuaries at higher latitudes.

## 2.2. MATERIALS AND METHODS

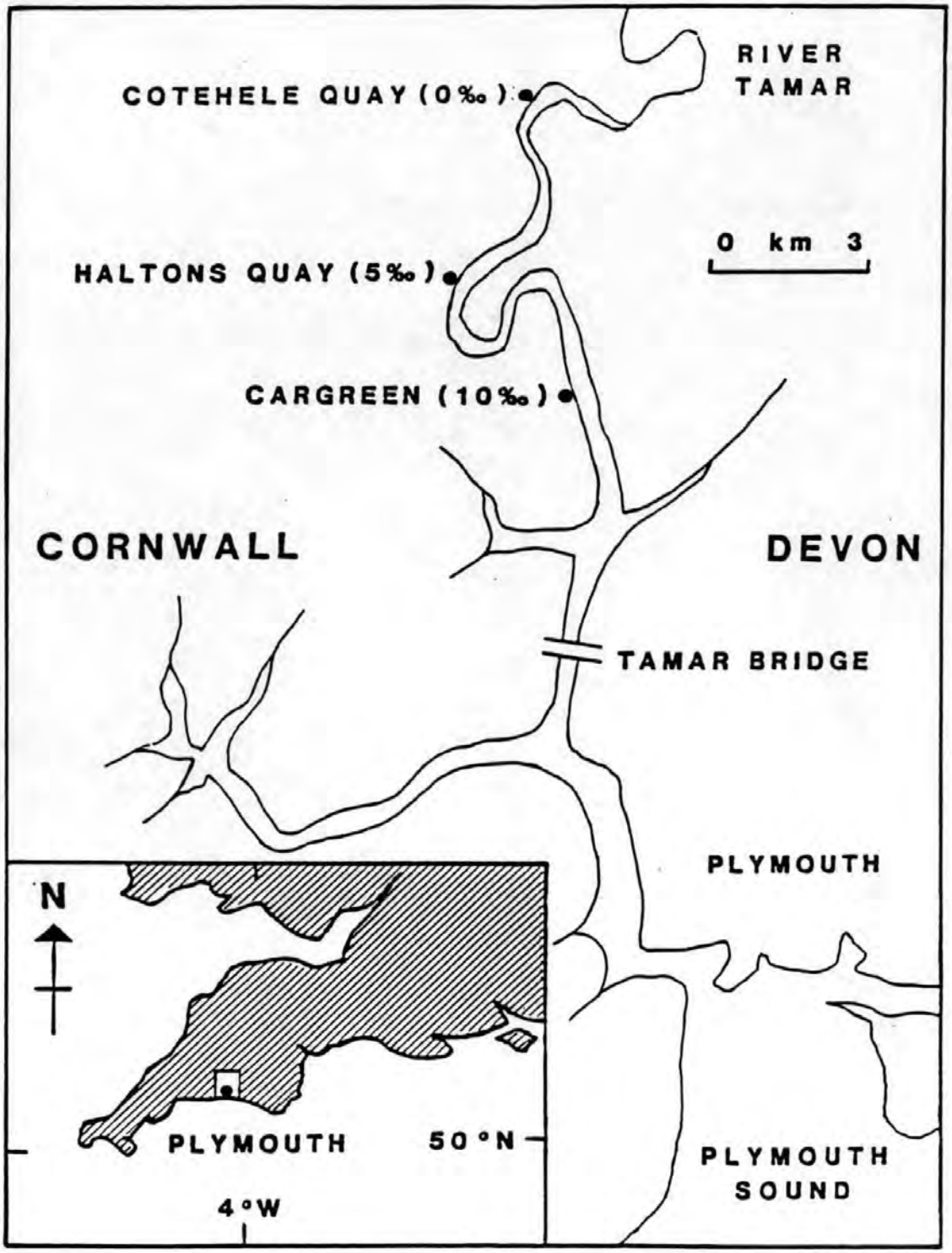
### 2.2.1. Collection of Animals

*Palaemon longirostris* were collected from the upper reaches of the River Tamar Estuary, Plymouth between June and September ('summer acclimatized' prawns) at Cotehele Quay, and November to March ('winter acclimatized') between Haltons Quay and Cargreen (Fig. 1). Individuals were hand-netted at low tide and transported back to the laboratory in habitat water.

### 2.2.2. Animal Husbandry

Prawns were maintained in plastic aquaria supplied with continuously flowing sea water (S, 34‰ ; T, 12 °C) and fed once a week on mussel (*Mytilus edulis L.*) or fish (*Gadus morhua L.*). Seven days prior to the tolerance experiments, prawns were transferred to dilute saline aquaria (S, 14‰ ; T, 12 °C), and held under a 12h light : 12h dark cycle. They were not fed from three days before being tested, nor during the tolerance experiments.

Figure 1. Map of the study area showing the position and representative low tide salinities of prawn collection sites in the River Tamar.


RIVER
TAMAR
COTEHELE QUAY (0‰)
0 km 3
HALTONS QUAY (5‰)
CARGREEN (10‰)
CORNWALL
DEVON
TAMAR BRIDGE
PLYMOUTH
N
PLYMOUTH
50°N
4°W
PLYMOUTH
SOUND

### 2.2.3. Experimental Protocol

Prawns were sorted, based on carapace length (cl) (measured from rostral tip to posterior carapace edge) and presence of eggs, into 'small' (cl 10-18 mm), 'medium' (cl 18-24 mm), 'large' (cl >24 mm) and ovigerous (cl >24 mm) groupings, and each was studied separately. The sex ratio in the small class was about equal, but the medium class was dominated by males and the large class by females. A similar biased sex distribution with prawn size was reported for the New Zealand estuarine prawn *Palaemon affinis* (Kirkpatrick & Jones, 1985). Only intermoult prawns were used and two replicates, each of six prawns, were set up for each class of summer acclimatized individuals at every combination of salinity and temperature. Due to fewer prawns being available during winter, one replicate of six prawns was set up, and small and ovigerous groupings were not included for winter acclimatization experiments.

Prawns were held in salinities of 0.5, 3.5, 7, 15, 22, 34 and 43‰ at 4, 12 and 20 °C (±1 °C) in aerated, plastic containers (approx. 2.8l) in constant temperature water baths. Each container was divided into six compartments by clear perspex partitions, one prawn was placed into each compartment and the containers were covered. All prawns were held at the appropriate experimental temperature for 24h in 14‰ before the start of the experiment. Salinities were prepared by adding distilled water to filtered aquarium

water (S, 34‰) and checked using a hand-held refractometer. Solutions were changed after 72h or immediately after a prawn moulted or died.

### 2.2.4. Salinity Tolerance Criterion

Survival (based on scaphognathite beat viewed through the transparent carapace) was checked hourly for the first 6h, at 12h and, thereafter, at 24-h intervals over 7 days. Tolerance is presented as 'survival days' and is the area below the cumulative survival curve at each salinity over 7 days (Jones, 1972). This single value, expressed as a percentage of the maximum survival possible ('percentage survival days'), allows statistical comparison of the different prawn classes in each salinity at different temperatures and seasons. All statistical analyses were performed using two-way analysis of variance (ANOVA) (programme held on Plymouth Polytechnic Prime 'A' system).

## 2.3. RESULTS

### SUMMER ACCLIMATIZED PRAWNS

All prawn groupings showed high tolerance to salinities between 0.5 and 34‰ at each temperature (Table 1, Figs 2-5). Medium, large and ovigerous prawns also showed high survival when exposed to 43‰ at 12 and 20°C (Figs 3, 4 & 5). Tolerance to this salinty, however, was reduced for small prawns at all three temperatures (Fig. 2), and for medium and ovigerous prawns at 4°C (Figs 3 & 5). Large prawns were the only size grouping which showed high tolerance to 43‰ at all three temperatures (Fig. 4). Prawn size, however, had no significant effect on survival (Table 2 , ANOVA, $P>0.05$). Therefore, the data were combined and the different prawn classes were used as extra replicates to study the influence of temperature on salinity tolerance. Two-way analysis of variance with replication revealed that temperature had no significant effect on salinity tolerance (Table 3, $P>0.05$). Salinity did have a significant effect on survival (Table 3, $P<0.001$) and examination of the data in Table 1 indicates that tolerance to 43‰ was significantly reduced. These results show that *Palaemon longirostris* is very euryhaline over a relatively wide range of temperatures but has reduced tolerance to hypersaline conditions .

Table 1. Variation in salinity tolerance (based on percentage survival days) of *Palaemon longirostris* of different ontogenetic stages under different environmental conditions.

| Prawn category | Temperature (°C) | Salinity (‰) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 0.5 | 3.5 | 7 | 15 | 22 | 34 | 43 |
| 'SUMMER' PRAWNS (12 individuals per combination) | | | | | | | | |
| Small | 20 | 93.2 | 90.6 | 95.2 | 98.9 | 100 | 96.4 | 58.1 |
| | 12 | 100 | 98.4 | 100 | 100 | 100 | 100 | 46.4 |
| | 4 | 98.8 | 100 | 100 | 100 | 100 | 98.8 | 30.9 |
| Medium | 20 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | 12 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | 4 | 93.3 | 92.9 | 98.9 | 100 | 98.9 | 100 | 70 |
| Large | 20 | 100 | 93.6 | 97.6 | 98.9 | 100 | 100 | 100 |
| | 12 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | 4 | 100 | 100 | 100 | 100 | 100 | 100 | 97.6 |
| Ovigerous | 20 | 100 | 100 | 97.6 | 100 | 100 | 100 | 100 |
| | 12 | 100 | 100 | 100 | 100 | 100 | 100 | 82 |
| | 4 | 100 | 100 | 100 | 100 | 100 | 100 | 60.6 |
| 'WINTER' PRAWNS (6 individuals per combination) | | | | | | | | |
| Medium | 20 | 72.7 | 80.7 | 80.2 | 72.8 | 96.6 | 100 | 76.1 |
| | 12 | 100 | 86.4 | 86.4 | 97.6 | 100 | 100 | 89.8 |
| | 4 | 54.7 | 100 | 100 | 100 | 100 | 100 | 16.9 |
| Large | 20 | 80.7 | 89.8 | 93.2 | 100 | 83 | 100 | 100 |
| | 12 | 100 | 100 | 100 | 93.2 | 100 | 100 | 100 |
| | 4 | 97.6 | 100 | 96.6 | 93.2 | 100 | 100 | 85.6 |

Figure 2. Survival patterns for summer acclimatized small *Palaemon longirostris* at 4, 12 and 20°C.

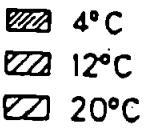
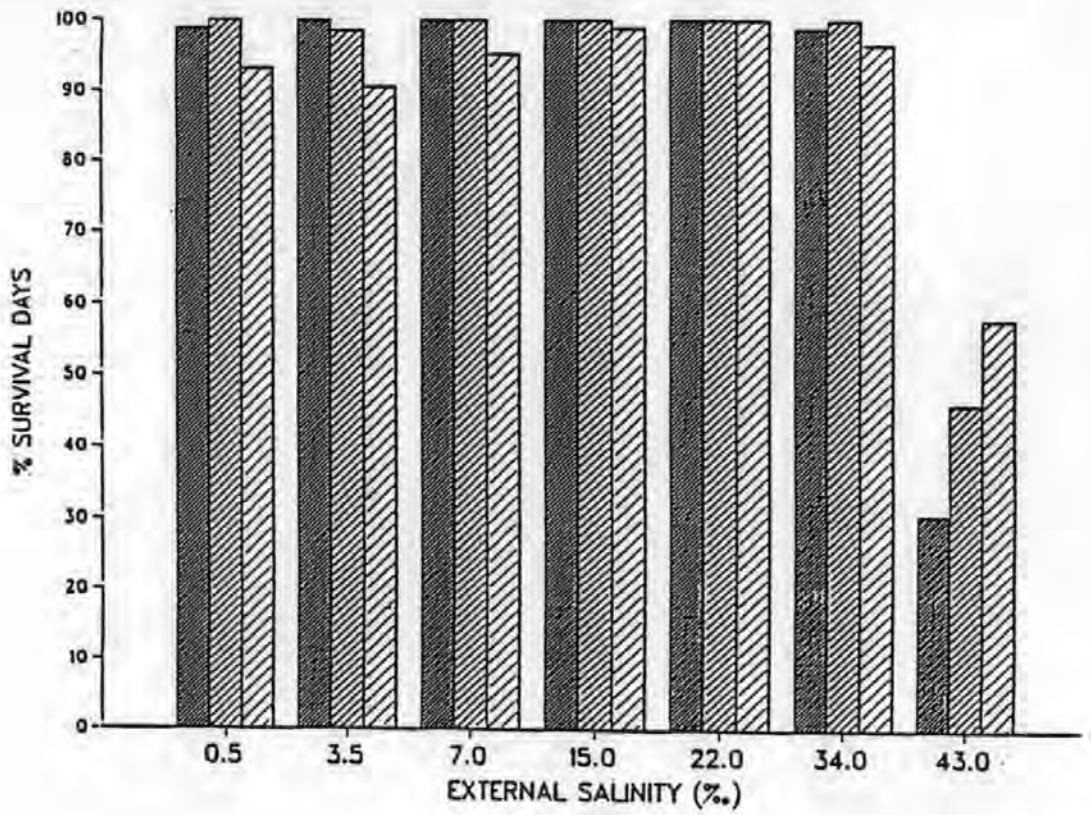



Figure 3. Survival patterns for summer acclimatized medium *Palaemon longirostris* at 4, 12 and 20°C.


% SURVIVAL DAYS
100
90
80
70
60
50
40
30
20
10
0
0.5
3.5
7.0
15.0
22.0
34.0
43.0
EXTERNAL SALINITY (‰)

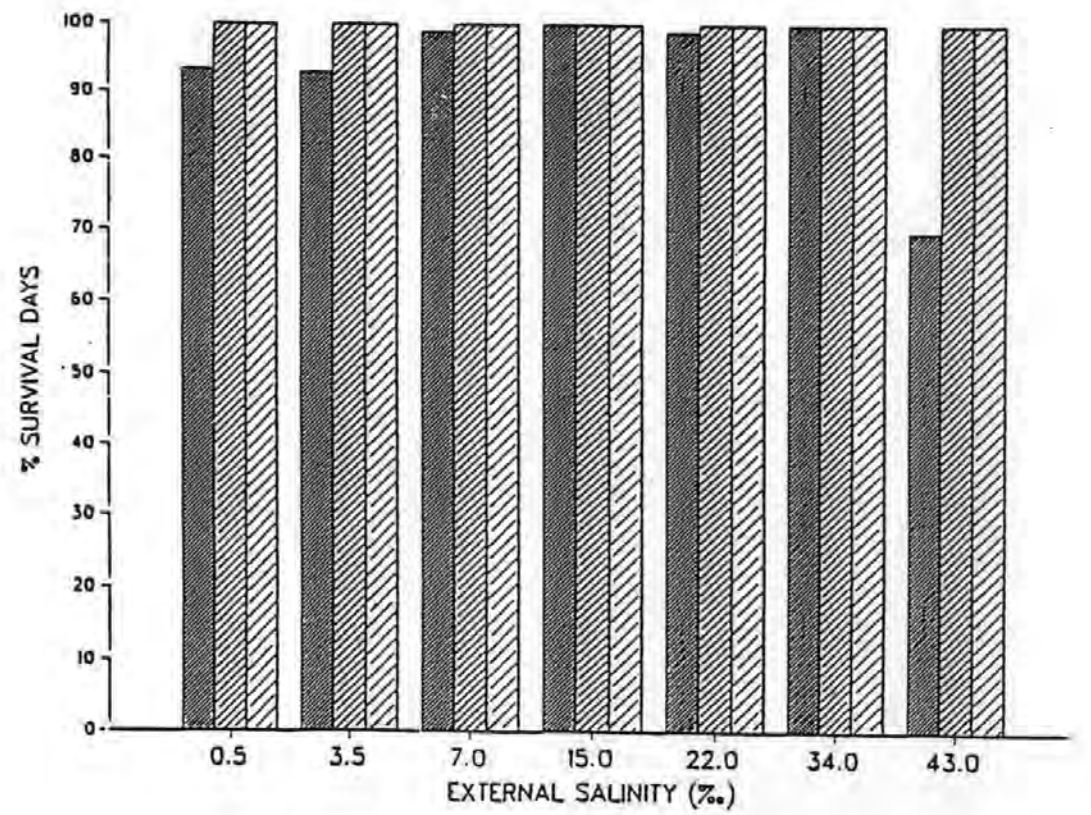

% SURVIVAL DAYS
100
90
80
70
60
50
40
30
20
10
0
0.5
3.5
7.0
15.0
22.0
34.0
43.0
EXTERNAL SALINITY (‰)

Figure 4. Survival patterns for summer acclimatized large *Palaemon longirostris* at 4, 12 and 20°C.

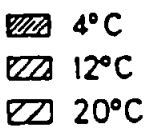
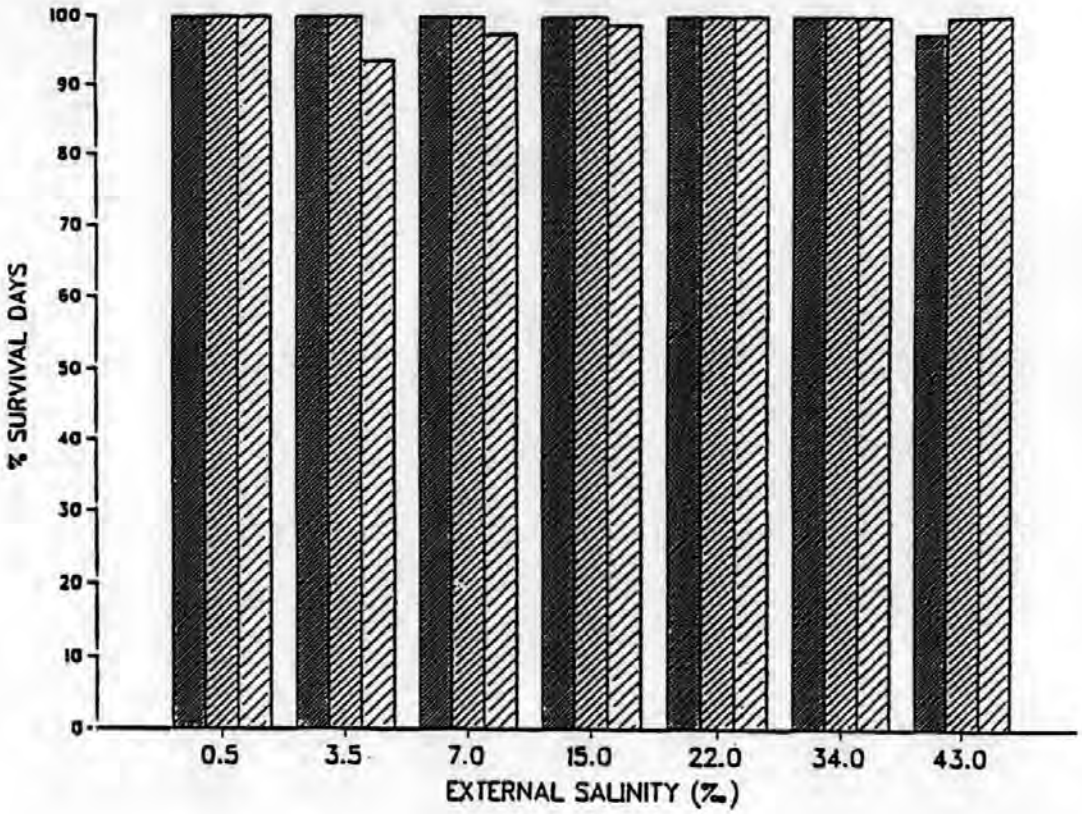



Figure 5. Survival patterns for summer acclimatized ovigerous *Palaemon longirostris* at 4, 12 and 20°C.


% SURVIVAL DAYS
100
90
80
70
60
50
40
30
20
10
0
0.5
3.5
7.0
15.0
22.0
34.0
43.0
EXTERNAL SALINITY (‰)

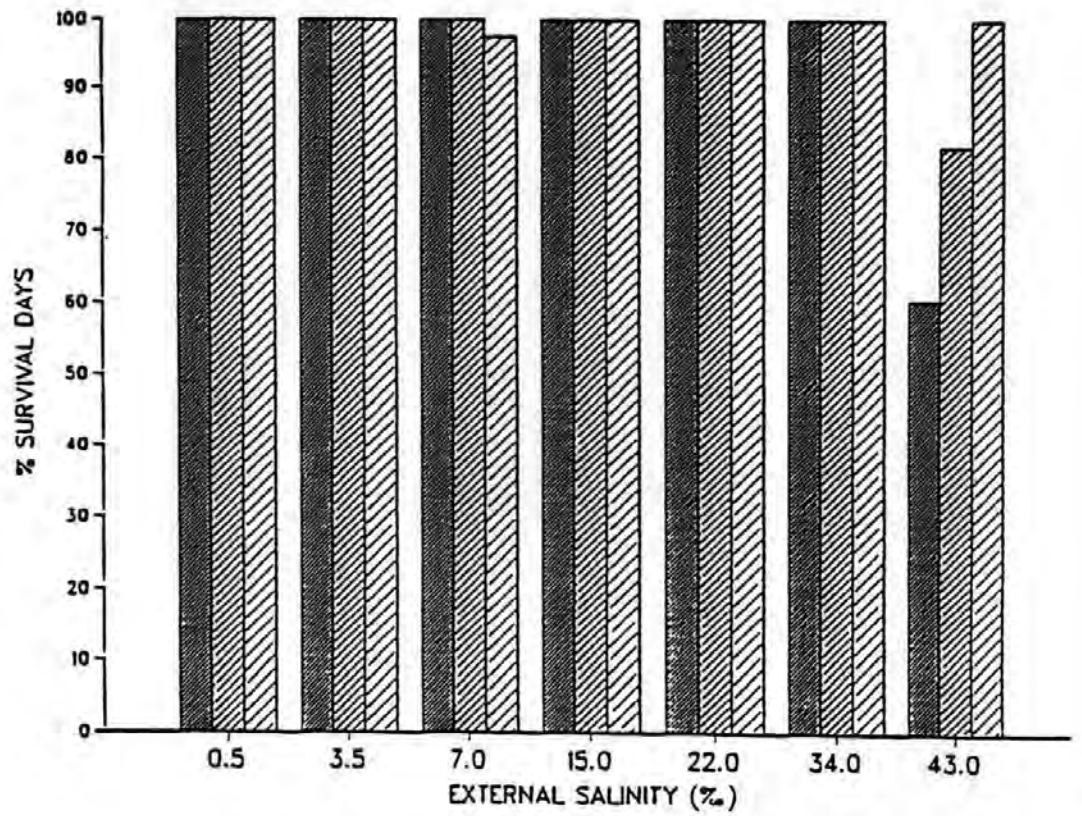

% SURVIVAL DAYS
100
90
80
70
60
50
40
30
20
10
0
0.5
3.5
7.0
15.0
22.0
34.0
43.0
EXTERNAL SALINITY (‰)

Table 2. Two-way analysis of variance of the effect of prawn size on survival (as % survival day) of Palaemon longirostris (d.f., degrees of freedom; S.L., significance level; n.s., not significant).

| Source of variation | d.f. | F-ratio | S.L. |
|---|---|---|---|
| 'SUMMER' PRAWNS | | | |
| 4°C | 3,18 | 1.05 | n.s. |
| 12°C | 3,18 | 1.07 | n.s. |
| 20°C | 3,18 | 2.63 | n.s. |
| 'WINTER' PRAWNS | | | |
| 4°C | 1,6 | 1.71 | n.s. |
| 12°C | 1,6 | 2.77 | n.s. |
| 20°C | 1,6 | 2.77 | n.s. |

Table 3. Two-way analysis of variance with replication (using the different size groupings as replicates) of the effect of salinity and temperature on survival (as % survival days) of *Palaemon longirostris* (d.f., degrees of freedom; S.L., significance level; n.s., not significant)

| Source of variation | d.f | F-ratio | S.L. |
|---|---|---|---|
| 'SUMMER' PRAWNS | | | |
| Salinity | 6,63 | 7.94 | 0.001 |
| Temperature | 2,63 | 0.90 | n.s. |
| S x T | 12,63 | 1.09 | n.s. |
| 'WINTER' PRAWNS | | | |
| Salinity | 6,21 | 1.57 | n.s. |
| Temperature | 2,21 | 1.59 | n.s. |
| S x T | 12,21 | 1.14 | n.s. |

## WINTER ACCLIMATIZED PRAWNS

Fewer data are available for winter prawns (Table 1), although the general patterns of survival are similar to those described for summer individuals. Winter prawns were also euryhaline (Table 1, Figs 6 & 7), and salinity tolerance was not influenced significantly by either size or temperature (Tables 2 & 3 , ANOVA, $\underline{P}>0.05$). Interestingly, survival in 43‰ was not significantly different from that in the other salinities used (Tables 2 & 3 , ANOVA, $\underline{P}>0.05$). In general, salinity tolerance was reduced in winter compared with summer (Fig. 8), although the difference between seasons was significant only at 20 °C (Table 4 , ANOVA, $\underline{P}<0.001$).

Figure 6. Survival patterns for winter acclimatized medium *Palaemon longirostris* at 4, 12 and 20°C.

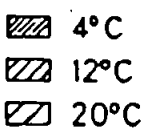
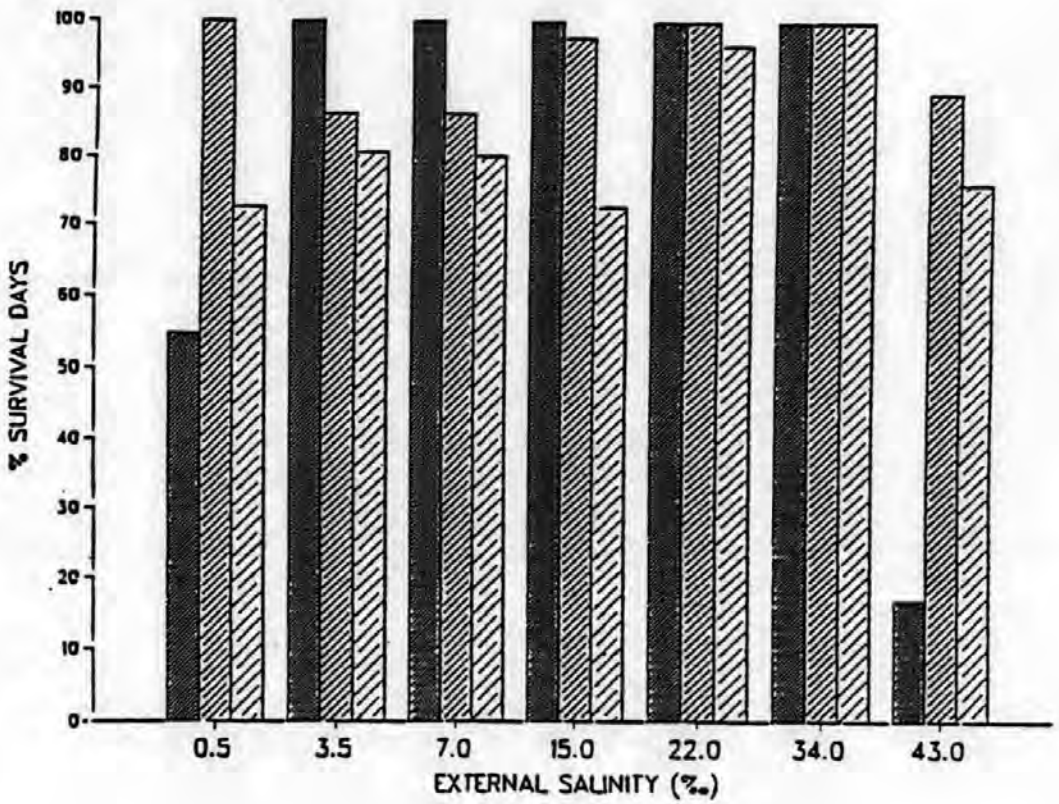



Figure 7. Survival patterns for winter acclimatized large *Palaemon longirostris* at 4, 12 and 20°C.


% SURVIVAL DAYS
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80
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3.5
7.0
15.0
22.0
34.0
43.0
EXTERNAL SALINITY (‰)

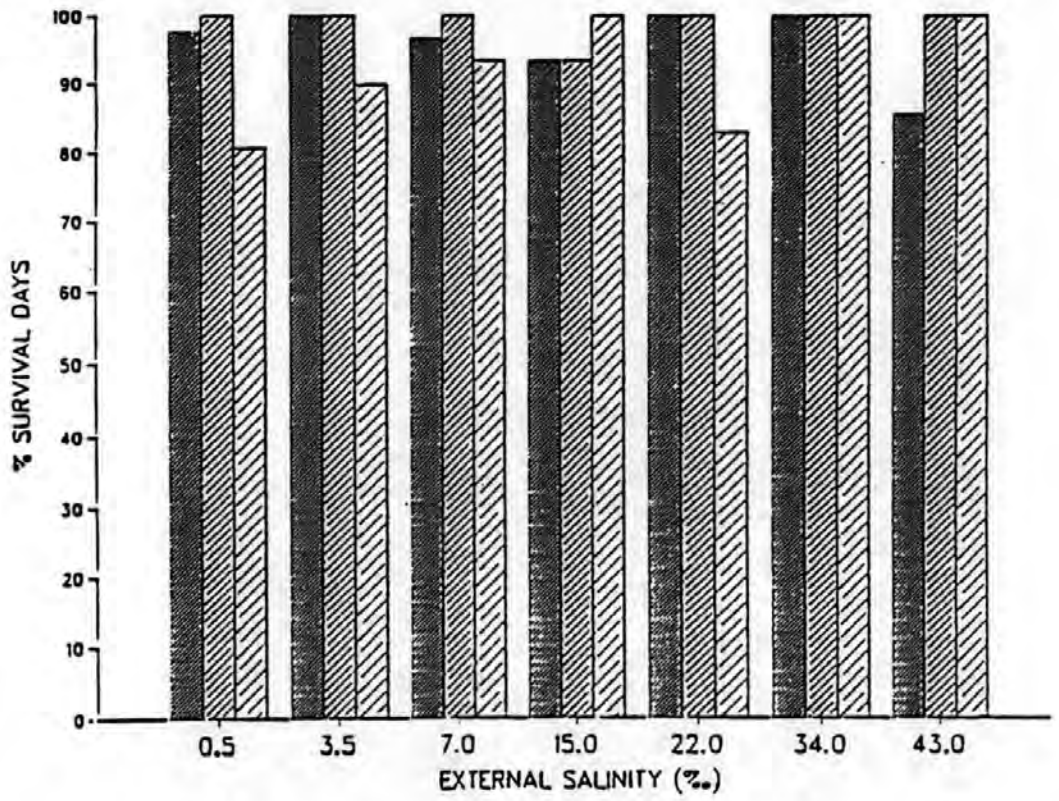

% SURVIVAL DAYS
100
90
80
70
60
50
40
30
20
10
0
0.5
3.5
7.0
15.0
22.0
34.0
43.0
EXTERNAL SALINITY (‰)

Figure 8. Survival of *Palaemon longirostris* at different combinations of salinity and temperature for summer (joined heavy lines) and winter prawns (stipled area).

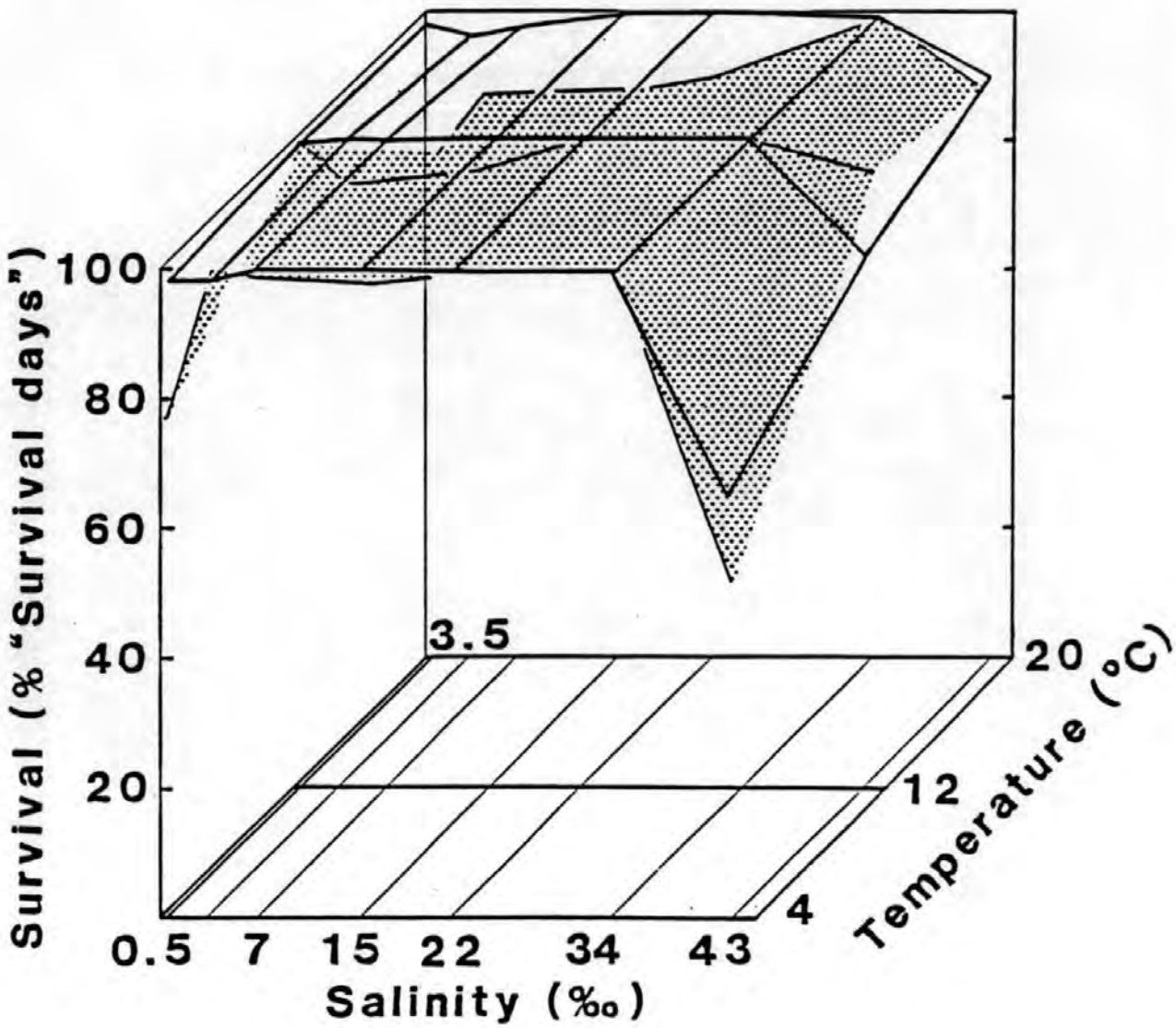

Survival (% "Survival days")
100
80
60
40
20
0.5
7
15
22
34
43
Salinity (‰)
3.5
4
12
20
Temperature (°C)

Table 4. Two-way analysis of variance with replication (using 'medium' and 'large' size groupings as replicates) of the effect of season and salinity on survival (as % survival days) of *Palaemon longirostris* (d.f., degrees of freedom, S.L., significance level; n.s., not significant).

| Source of variation | | d.f. | F-ratio | S.L. |
|---|---|---|---|---|
| 4°C | | | | |
| | Salinity | 6,14 | 2.19 | n.s. |
| | Season | 1,14 | 1.53 | n.s. |
| | S x S | 6,14 | 0.69 | n.s. |
| 12°C | | | | |
| | Salinity | 6,14 | 0.59 | n.s. |
| | Season | 1,14 | 4.41 | n.s. |
| | S x S | 6,14 | 0.59 | n.s. |
| 20°C | | | | |
| | Salinity | 6,14 | 0.79 | n.s. |
| | Season | 1,14 | 14.58 | 0.01 |
| | S x S | 6,14 | 0.70 | n.s. |

## 2.4. DISCUSSION

Irrespective of body size, reproductive condition, temperature and season, *Palaemon longirostris* is very euryhaline and has high tolerance to a range of salinities between 0.5-43‰. These findings support an earlier comment on *P. longirostris* that its "indifference to salinity can be equalled by very few animals and surpassed by none" (Gurney, 1923). Thus, *P. longirostris* is another of the relatively few prawn species that can tolerate equally well the entire salinity range from marine to limnetic conditions (Dorgelo,1976); others include *Palaemonetes varians* (Hagerman & Uglow, 1983) and *Palaemonetes pugio* (Knowlton & Kirby, 1984).

Wide salinity tolerance is an obvious adaptation to the estuarine environment and is a feature of many estuarine crustaceans (Lance, 1963; McLusky, 1967, 1979; Preece, 1970; McLusky & Heard, 1971; Jones, 1972, 1981; Marsden, 1973, 1980, Hagerman & Uglow, 1983; Knowlton & Kirby, 1984; Kirkpatrick & Jones, 1985; De Lisle & Roberts, 1986). Not surprisingly, salinity tolerance ranges often correlate with the ecological distributions of crustaceans, particularly at the upper end of the range (Dorgelo, 1976). For example, *Gammarus palustris* has a salinity tolerance range between 7 and 50‰ and occurs in hyper-saline environments such as salt marshes (Gable & Croker, 1978). Similarly,

*Palaemon serratus* has a salinity tolerance range between 16 and 39‰ and is essentially a marine species (Spaargaren, 1972). *Crangon crangon*, on the other hand, has a salinity tolerance range between 5 and 39‰ and is a marine species which penetrates into the lower reaches of river estuaries (Spaargaren, 1971). field observations for *P. longirostris* ,however, suggest that this species has a more restricted distribution than its salinity tolerance range would allow. For, although having high tolerance to salinities between 0.5-43‰ , it occurs predominantly in the upper regions of estuaries (Smaldon, 1979). Different salt tolerances exhibited by closely related species could play a very important role in partitioning resources and avoiding inter-specific competition. It may be that *P. longirostris* only occupies the lower part of its wide salinity tolerance range because it comes into contact with other prawns and shrimps such as *Crangon crangon* and *Palaemon serratus* in higher saline conditions. These species may be better competitors and would therefore exclude *P. longirostris* from such environments. For example, it has been suggested that competitive exclusion is also responsible for the separation of *Palaemonetes varians* and *Palaemon adspersus* within a brackish-water Danish fjord (Hagerman & Uglow, 1983).

Over the salinity range 0.5 to 43‰ there was no significant effect of size on salinity tolerance of *Palaemon longirostris*. Body size is known to influence the salinity tolerance of some crustaceans, but the direction of the effect is not predictable, and salinity tolerance may

either increase or decrease with increasing animal size. Previous studies on this topic for euryhaline crustaceans are limited. Panikaar (1941) observed that juvenile *Palaemon serratus* had better salinity tolerance than adults and that ovigerous females were particularly intolerant to dilute sea water. Similar findings that juvenile stages were more euryhaline than adults were reported for estuarine crabs (Jones, 1981) and saltmarsh amphipods (Gable & Croker, 1978). On the other hand, other animals from the same habitats show opposite trends with regard to size and salinity tolerance. Thus, Marsden (1973) showed that adults of a saltmarsh isopod were more euryhaline than juveniles, and Kirkpatrick & Jones (1985) reported that the salinity tolerance of an estuarine prawn (*Palaemon affinis*) increased directly with body size, with ovigerous females having the widest tolerances. The present results for *P. longirostris*, showing no effect of prawn size on salinity tolerance, are in agreement with the findings of Dobkin & Manning (1964) for another estuarine prawn, *Palaemon intermedius*. At present, it is difficult to identify consistent patterns for the size effect on salinity tolerance even for different species of the same genus occupying similar habitats. For members of the genus *Palaemon*, the effect of size appears to be complicated further by differences in the sex ratio associated with prawn size (Kirkpatrick & Jones, 1985). Unfortunately, no general conclusion on the adaptive significance of intra-specific variation in salt tolerance of estuarine crustaceans can be made. It is clearly

adaptive, however, for a species like *P. longirostris*, which is a permanent resident of the upper reaches of estuaries, for all post-larval life-history stages to be very euryhaline.

Short-term temperature change had no significant effect on the salinity tolerance of summer and winter acclimatized *Palaemon longirostris*. Lack of sensitivity to temperature has been reported previously amongst temperate euryhaline species living in dilute saline habitats (Dorgelo, 1976). There is, however, some variation amongst euryhaline invertebrates as to the effect of temperature on salinity tolerance (for reviews see, Kinne, 1970, 1971; Dorgelo,1976). High latitude species generally have their salinity tolerance enhanced at low rather than high temperatures (Marsden, 1973, 1980; Gable & Croker, 1978; McLusky, 1979; Hicks, 1980; Ramirez de Isla Hernandez & Taylor, 1985), although there are exceptions (eg, Hagerman & Uglow,1983). Other species show no consistent temperature effect on salinity tolerance even between different stages of the life cycle of the same species (Jones, 1981). Until more emphasis is given to the geographical position of the study population relative to the entire latitudinal range of the species, the adaptive nature of the effect of temperature on salinity tolerance will remain unresolved. Absence of a temperature effect on the salinity tolerance of post-larval *P. longirostris* is, however, rather surprising. This species is at the northern limit of its distribution in South West England, and species living at their geographical

limits are generally very sensitive to temperature (Vernberg & Vernberg, 1964). Temperature, however, does influence the salinity tolerance of larval *P. longirostris*. Reduced larval salinity tolerance coupled with impaired larval development were recorded for this species at low water temperatures (Antonopoulou, 1987). This reduction in salinity tolerance of larval *P. longirostris* at low temperatures, may be one factor which limits the northern distribution of this species.

Although all post-larval stages of *Palaemon longirostris* are eurythermal, there were interesting differences in salinity tolerances between summer and winter individuals which can be correlated with differences in estuarine distribution between seasons. Winter prawns were less euryhaline than summer prawns and had reduced survival at very low salinity. During winter, *P. longirostris* are found further downstream in relatively more saline regions of the River Tamar Estuary such as at Cargreen, whereas in summer, prawns occur in essentially fresh water at Cotehele Quay (Fig. 1) (pers. obs.). These findings suggest that prawns migrate into more saline waters in winter to avoid the detrimental effect of combination of low temperature and low salinity on survival. Other authors have reported similar findings for estuarine crustaceans. For example, Haefner (1969a) observed the migration of the prawn *Crangon septemspinosa* towards more saline regions of estuaries in autumn when temperatures were decreasing. Similarly, Hicks (1973) stated that higher salinities

provided more suitable media to survive the lower winter temperatures for estuarine crabs. An alternative strategy to life in estuaries is not to migrate but to show seasonal acclimatization. Several temperate estuarine crustaceans have better ability to survive dilute salinity in winter compared with summer (Lance, 1963; Hicks, 1973; McLusky, 1979; Marsden, 1980; Jones, 1981). This seasonal shift in salinity tolerance is clearly adaptive for species which live in estuaries where temperatures and dilution of sea water by freshwater drainage show clear seasonal patterns. *P. longirostris* does not possess this adaptation and therefore has to migrate during winter from the upper reaches of the River Tamar Estuary to more saline regions. This seasonal difference in ability to tolerate very low salinities at low temperatures may help explain the absence of *P. longirostris* from estuaries at higher latitudes. In more northern areas, low temperatures occur over a longer time scale and would mean that *P. longirostris* spends more time in lower estuarine regions. These areas typically would be occupied by presumed better competitors and thus competitive exclusion may be a further explanation for the lack of colonization of estuaries at higher latitude by *P. longirostris*. It seems likely that a combination of inter-specific competition associated with adult response to low temperature, together with reduced larval recruitment at low temperature, contribute to setting the limits for the northern distribution of *P. longirostris*.

# CHAPTER 3

## OSMOREGULATION

This chapter was presented as a poster at a meeting of the Society for Experimental Biology (York, Spring 1987).

## 3.1. INTRODUCTION

Estuarine and brackish-water crustaceans have evolved different physiological responses to the osmotic demands placed upon them by their demanding environment. The majority are euryhaline species (Chapter 2), however, the osmotic concentration of their haemolymph can respond in one of two different ways to any change in the osmotic concentration of the surrounding medium. Some species have no control over their body fluid osmolality. Their haemolymph remains isosmotic to the external medium in all salinities down to the lethal limits. Such species are termed "osmoconformers" and the porcelain crab *Porcellana platycheles* is an example (Davenport, 1972, 1985). Many crustaceans which have successfully invaded dilute saline environments, however, have evolved the ability to regulate their haemolymph osmolality. These animals are known as "osmoregulators" (Gilles, 1975). The most common type of osmoregulation shown by crustaceans is that of hyper-osmoregulation. Crustaceans which exhibit this form of osmoregulation maintain their haemolymph isosmotic to the external medium at high salinities and hyper-osmotic at low salinities. The majority of euryhaline decapod and peracarid crustaceans which have moved away from the sea into littoral, estuarine and brackish-water environments are hyper-osmoregulators. Examples are legion, but in Britain

include the common shore crab *Carcinus maenas* (Shaw, 1961) and the amphipod *Corophium volutator*(McLusky, 1968).

A small number of crustaceans have evolved a different type of osmoregulation. In these forms, not only are body fluids maintained hyper-osmotic to the environment when in low salinities, but are also kept hypo-osmotic in high salinities. Such species are called hyper-hypo-osmoregulators (Kinne, 1971). Hyper-hypo-osmoregulation is the most advanced form of genetic adaptation to osmotic change possessed by invertebrates (Kinne, 1963a,b), and is thought to be typical of species which live in environments characterized by extreme fluctuations in salinity (Hagerman, 1971). Examples include the estuarine mud crab *Uca crenulatus* (Jones, 1941) and the brine shrimp *Artemia salina* (Croghan, 1958).

*Palaemon longirostris* is an extremely euryhaline species (Chapter 2) which occupies the upper reaches of large river estuaries. In order to determine which type of osmoregulatory response is employed by this species, the present work set out to describe the osmoregulatory curve of *P. longirostris* over a wide range of salinities. Since salinity fluctuations within an estuary may be both frequent and rapid, the magnitude and speed of the blood osmotic responses to sudden salinity change are important physiological features of animals which successfully colonise such areas (Haefner, 1969b; Hagerman & Uglow, 1983; Kirkpatrick & Jones, 1985). Thus, the rate of osmotic response to sudden salinity change by *P. longirostris* is

reported here also.

As all post-larval life-history stages of *Palaemon longirostris* occur within the confinements of the estuary, any effect of body size on osmoregulation would clearly have ecological significance to the species. Body size has been shown to influence the osmoregulatory response of some estuarine crustaceans, however, osmoregulation may improve with either increasing (Kelley & Burbanck, 1972) or decreasing body size (Haefner, 1969b; Kirkpatrick & Jones, 1985). Information as to the direction of size effects on osmoregulation is thus not predictable. The effect of body size on osmoregulation in *P. longirostris* is reported here.

Other features typical of estuaries include short-term tidal fluctuations and long-term seasonal fluctuations in temperature. Since temperature influences most biological processes, it is not surprising that osmoregulation responds to this ecological master factor (Vernberg & Silverthorn, 1979). Studies on the influence of temperature on osmoregulation of estuarine crustaceans, however, have revealed that effect of temperature is not consistent between species (Dorgelo, 1981). Some species show improved regulation at low temperatures (Weber & Spaargaren, 1970; Kirkpatrick & Jones, 1985), while others show improved osmoregulation at high temperatures (Castille & Lawrence, 1981a; Hagerman & Uglow, 1983). In this study, *P. longirostris* is living at the northernmost limit of its latitudinal distribution. As animals living at their geographical limits are generally sensitive to temperature

(Vernberg & Vernberg, 1964), the effects of temperature and season on osmoregulaton, and the effect of temperature on rate of osmotic response in *P. longirostris*, are reported here.

## 3.2. MATERIALS AND METHODS

Collection of animals and holding procedures used for *Palaemon longirostris* are described in detail in the previous Chapter (Sections 2.2.1. and 2.2.2.).

### 3.2.1. OSMOREGULATION

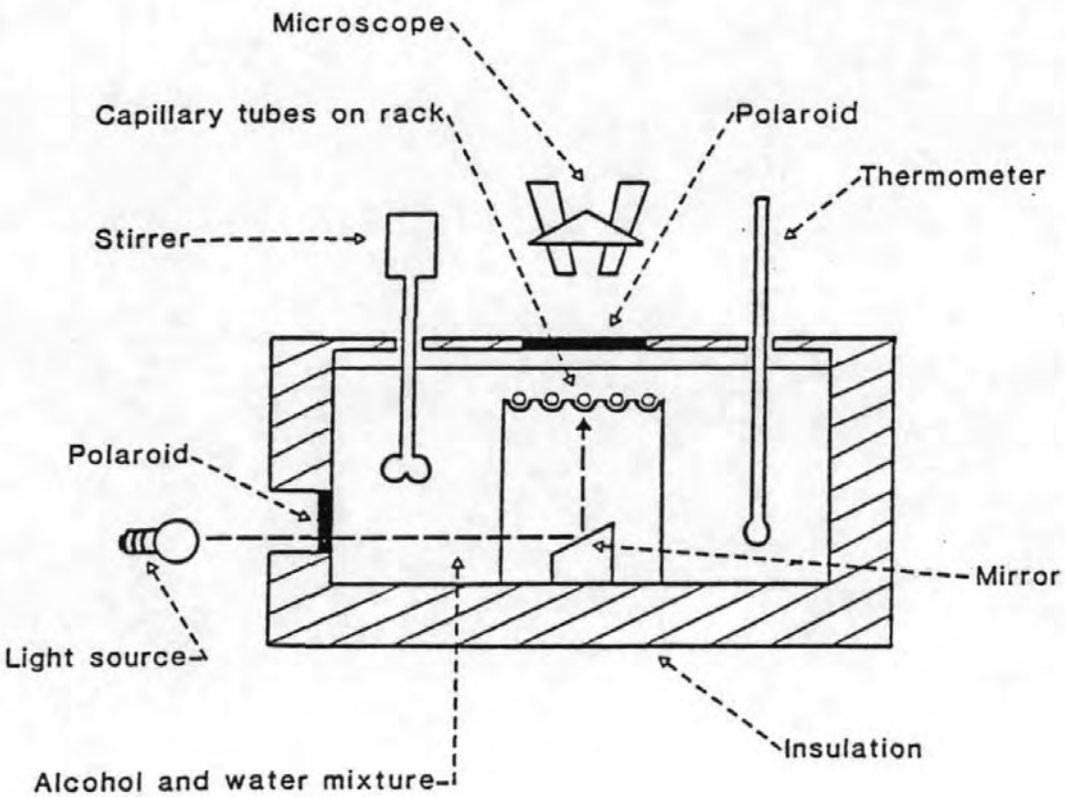
Prawns were held in salinitis of 0.5, 3.5, 7.0, 15.0, 22.0, 34.0 and 43‰ at 4, 12 and 20°C. Twelve prawns (two replicates of six) were held at each salinity/temperature combination for summer acclimatized experiments, and six prawns for winter acclimatized experiments. The experimental protocol is described in detail in the previous Chapter (2.2.3.).

After 7 days, haemolymph was sampled from the pericardial cavity (between the thorax and the first abdominal segment) of individual prawns using a drawn out Pasteur pipette with an attached suction tube. The point of insertion and the general cuticle was dried carefully with absorbent paper before the sample was taken. Haemolymph osmolality (mOsmol/kg [water]) of an 8µl sample from each prawn was measured immediately after collection using a Wescor Model 5100C Vapour Pressure Osmometer.

It was not possible to obtain an 8µl haemolymph sample

from individual small prawns, therefore a freezing-point depression technique modified from that described by Ramsay & Brown (1955) was used. The method of obtaining haemolymph from small prawns was the same as that described for larger prawns. Once collected, however, the haemolymph was expelled under liquid paraffin, and approximately 0.5µl was drawn into a glass Einmal-Mikropipetten capillary tube and encapsulated at both ends with liquid paraffin. Each end of the capillary tube was sealed with Cristaseal putty and the sample was stored in a deep freeze for subsequent analysis. The freezing-point depression of the blood contained in each capillary tube was determined using an apparatus based on that described by Ramirez de Isla Hernandez (1984) (Fig. 9). The frozen blood samples were immersed in a solution of 70% alcohol held within an insulated perspex tank (Fig. 9). The temperature of this solution was lowered by adding solid carbon dioxide and raised by using a small immersion heater. The blood samples were frozen to -30°C in a solution of acetone cooled with solid carbon dioxide and then transferred to the perspex tank which was also cooled using solid carbon dioxide to -15°C. The crystals in the frozen blood sample were viewed through a microscope with the aid of two polarising filters. The surrounding alcohol mixture was gently heated and the temperature at which the last crystal in the sample melted was read to the nearest 0.01°C using a Hortvet-2to+1C nitrogen filled thermometer. To allow direct comparison of the data for small prawns with the other size groupings, the freezing-point depression (°C)

Figure 9. Schematic diagram of the apparatus used for the measurement of the freezing-point depression of the blood of *Palaemon longirostris* (taken from Ramirez de Isla Hernandez, 1984).


Microscope
Capillary tubes on rack
Polaroid
Thermometer
Stirrer
Polaroid
Mirror
Light source
Insulation
Alcohol and water mixture

values were converted to mOsmol/kg using the relationship that a 1000 mOsmol/kg solution freezes at -1.86°C. The osmolalities of the experimental media were also measured at the end of each experiment for all prawn size groupings.

Statistical analyses of the results were performed using two-way analysis of variance with replication (ANOVA) (package held on Plymouth Polytechnic Prime 'A' mainframe computor) and the Student t-test (BBC micro-computor statistical package).

### 3.2.2. RATE OF OSMOTIC RESPONSE TO SUDDEN SALINITY CHANGE

Generally, experiments were carried out at 4, 12 and 20°C using summer acclimatized medium and large prawns. At each temperature, prawns were maintained at a salinity of 14‰ for 7 days before being transferred directly to salinities of either 5‰ or 34‰. The experimental protocol used for these experiments was identical to that descibed in detail elsewhere (Chapter 2.2.3.). For each of these direct salinity transfer experiments, the haemolymph of six prawns was sampled after 0,1,2,3,4,5,6,12,24,48 and 72h of immersion in each new salinity. Methods of haemolymph sampling and haemolymph osmotic analysis were identical to those described in detail for medium and large prawns in Section 3.2.1.

To further examine the ability of *Palaemon longirostris* to respond to extensive osmotic change, another set of

transfer experiments were carried out at 12°C, using only summer acclimatized medium prawns. In these experiments, prawns were maintained at salinities of 1‰ and 34‰ for a period of 7 days before being transferred directly to salinities of 34‰ and 1‰ respectively. Experimental protocol, methods of haemolymph sampling and haemolymph osmotic analysis were identical to those outlined in the previous section. Statistical analysis of the data was performed using an analysis of variance (ANOVA) package held on the Plymouth Polytechnic Prime 'A' mainframe computor.

## 3.3. RESULTS

### 3.3.1. OSMOREGULATION

#### 3.3.1.1. SUMMER ACCLIMATIZED PRAWNS

Osmoregulation curves for all prawn size groupings at each temperature were similar in general form and show *Palaemon longirostris* to be an extremely efficient hyper-hypo-osmoregulator (Figs 10-13). Although ANOVA showed that salinity had a significant effect on blood osmoregulation (Table 5, $P<0.001$), osmoregulation was very efficient over the salinity range 0.5 to 34‰ at all three temperatures. Thus, over an external osmotic range of 1000 mOsmol/kg, blood osmolality varied by less than 100 mOsmol/kg for prawns tested at 12 and 20°C, and by less than 180 mOsmol/kg for prawns tested at 4°C (Table 6). In 43‰ , prawns showed a rise in blood osmolality and the haemolymph osmotic concentration approached the isosmotic line suggesting a breakdown in blood osmoregulation at this hyper-saline salinity (Figs 10-13).

Prawn size had a significant effect on osmoregulation (Table 7, ANOVA, $P<0.001$). In general, small prawns had lower blood osmolalities than the other size groupings (Figs

Figure 10. Osmotic concentrations of the haemolymph of summer acclimatized small *Palaemon longirostris* in relation to salinity at 4 (triangles and irregular dashed line), 12 (circles and solid line) and 20°C (squares and regular dashed line) (vertical lines represent ±1 Standard Deviation).

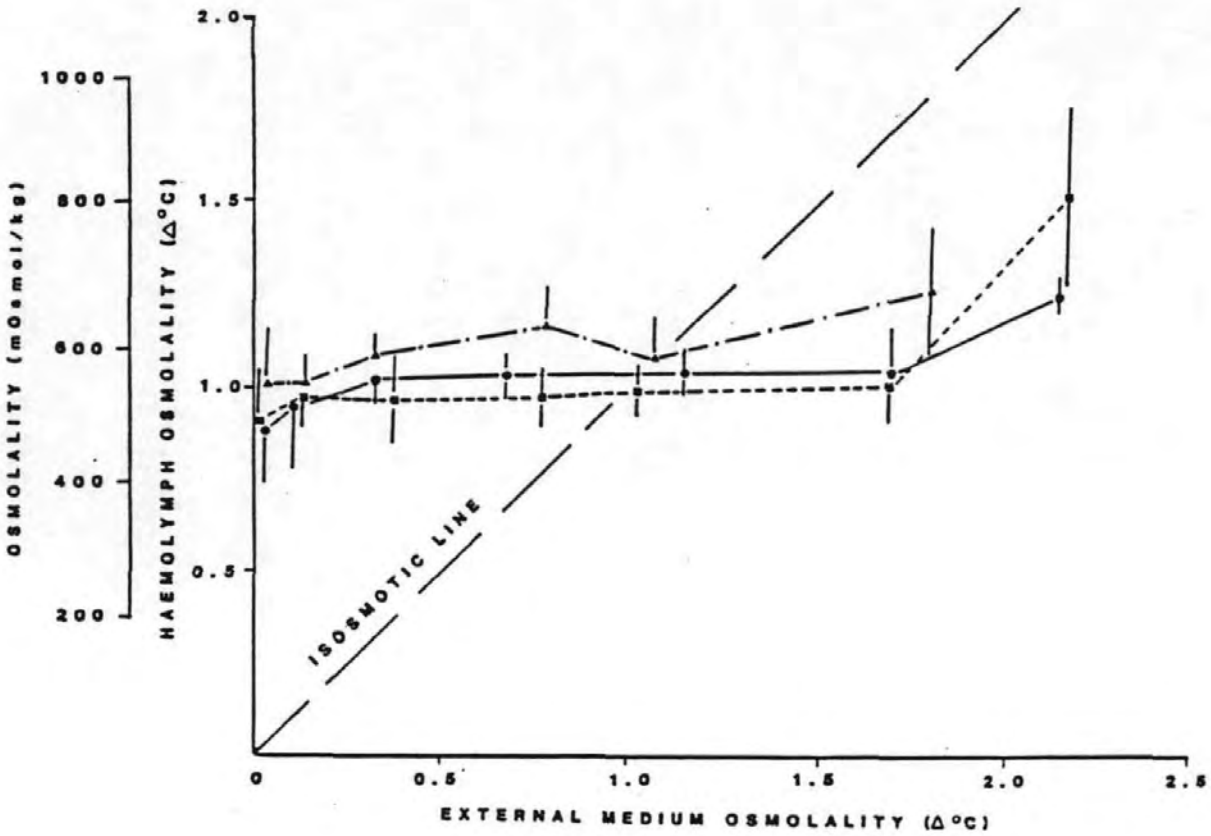

OSMOLALITY (mOsmol/kg)
1000
800
600
400
200
HAEMOLYMPH OSMOLALITY (Δ°C)
2.0
1.5
1.0
0.5
ISOSMOTIC LINE
0
0.5
1.0
1.5
2.0
2.5
EXTERNAL MEDIUM OSMOLALITY (Δ °C)

Figure 11. Osmotic concentrations of the haemolymph of summer acclimatized medium *Palaemon longirostris* in relation to salinity at 4 (triangles and irregular dashed line), 12 (circles and solid line) and 20°C (squares and regular dashed line) (vertical lines represent ±1 Standard Deviation).

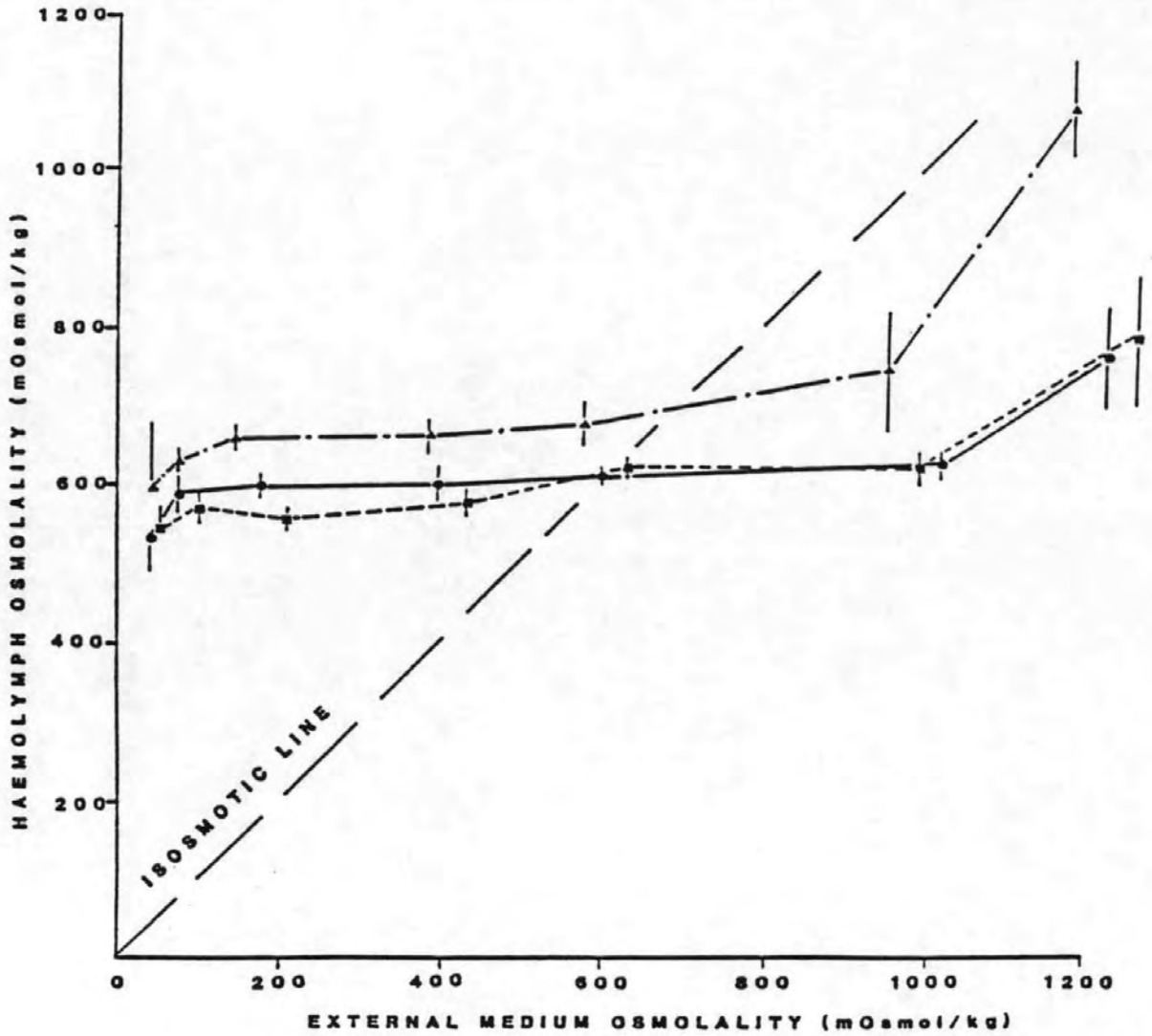

1200
1000
800
600
400
200
HAEMOLYMPH OSMOLALITY (mOsmol/kg)
ISOSMOTIC LINE
0
200
400
600
800
1000
1200
EXTERNAL MEDIUM OSMOLALITY (mOsmol/kg)

Figure 12. Osmotic concentrations of the haemolymph of summer acclimatized large *Palaemon longirostris* in relation to salinity at 4 (triangles and irregular dashed line), 12 (circles and solid line) and 20°C (squares and regular dashed line) (vertical lines represent ±1 Standard Deviation).

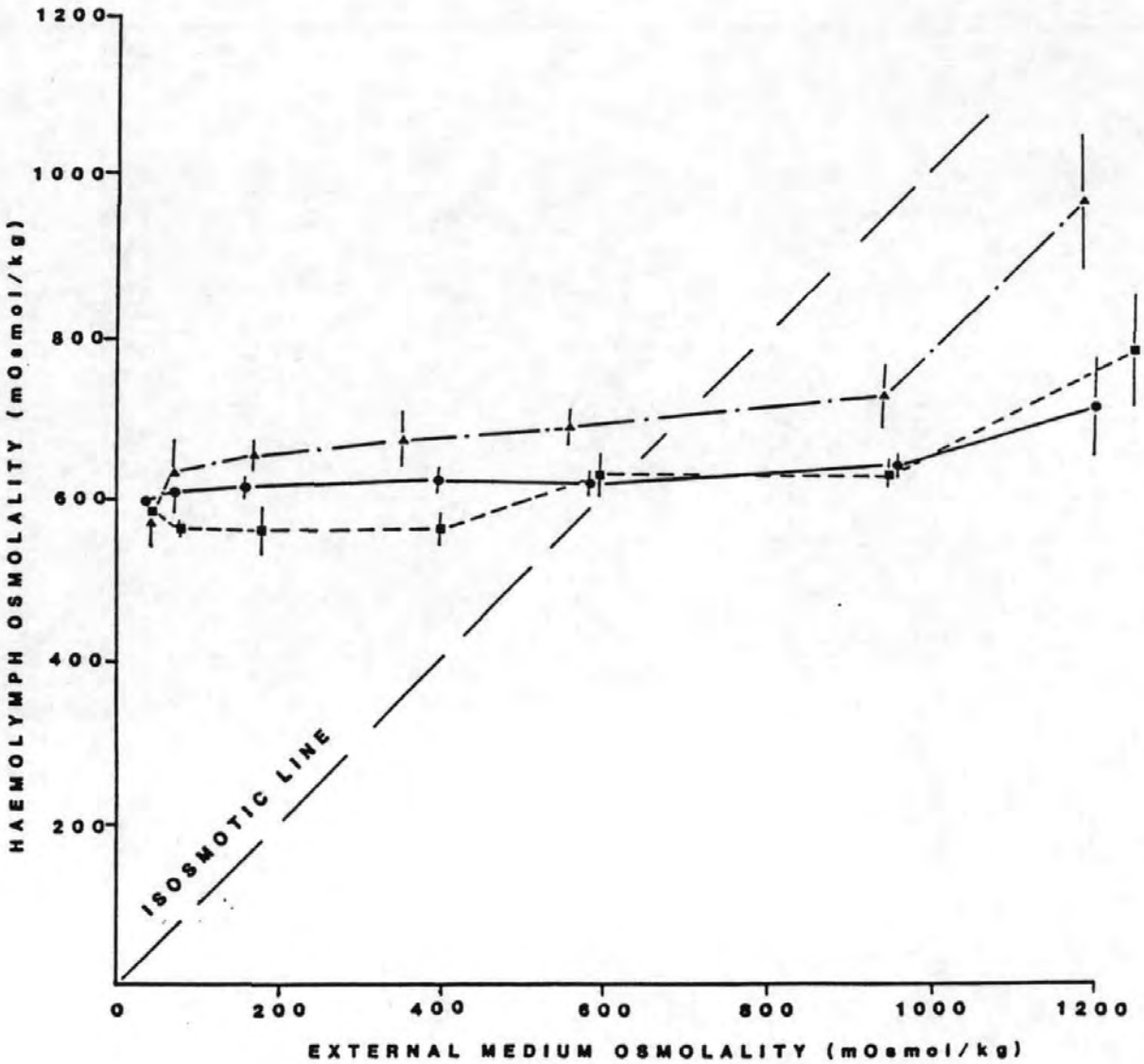

1200
1000
800
600
400
200
HAEMOLYMPH OSMOLALITY (mOsmol/kg)
ISOSMOTIC LINE
0
200
400
600
800
1000
1200
EXTERNAL MEDIUM OSMOLALITY (mOsmol/kg)

Figure 13. Osmotic concentrations of the haemolymph of summer acclimatized ovigerous *Palaemon longirostris* in relation to salinity at 4 (triangles and irregular dashed line), 12 (circles and solid line) and 20°C (squares and regular dashed line) (vertical lines represent ±1 Standard Deviation).

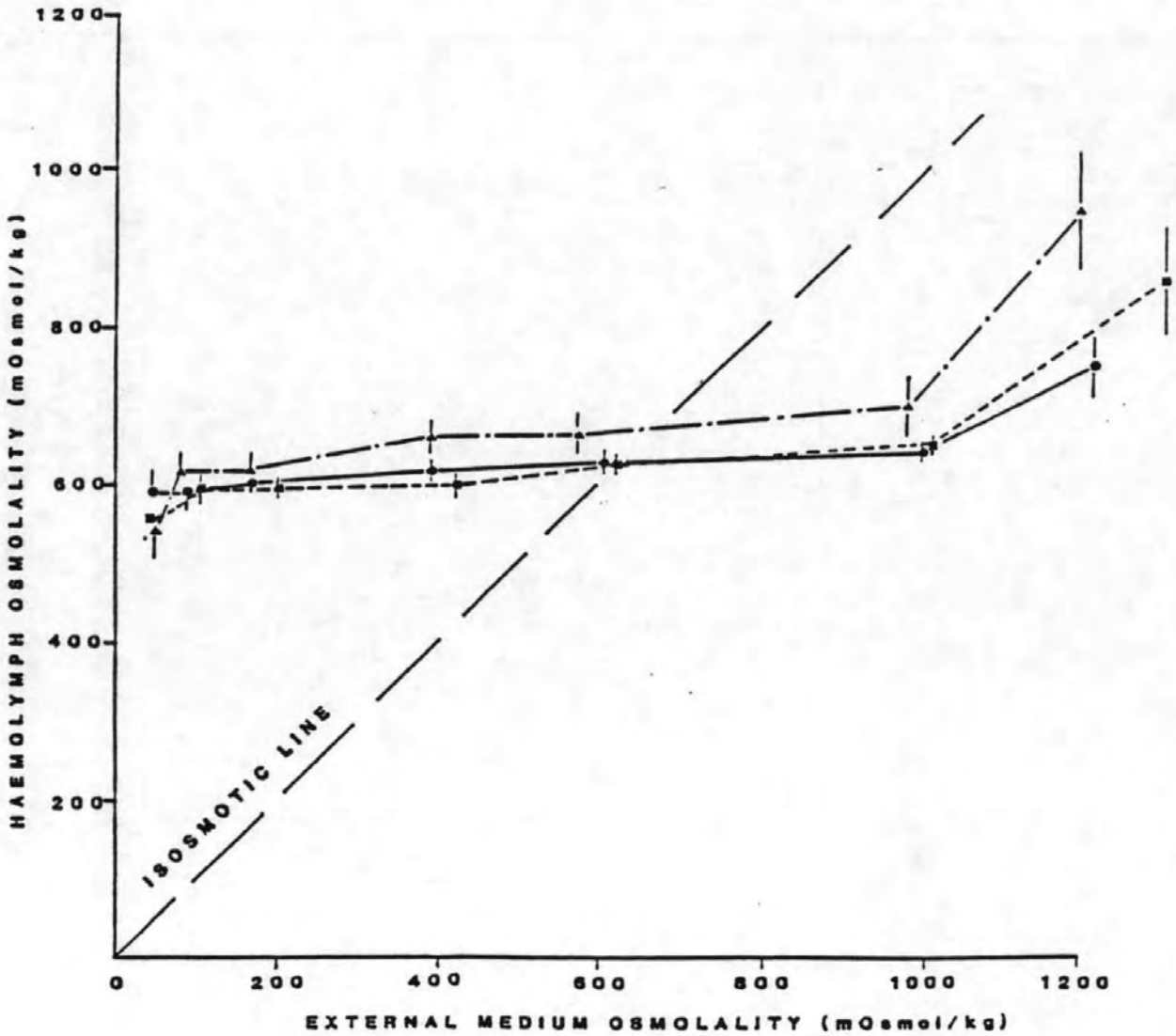

1200
1000
800
600
400
200
HAEMOLYMPH OSMOLALITY (mOsmol/kg)
ISOSMOTIC LINE
0
200
400
600
800
1000
1200
EXTERNAL MEDIUM OSMOLALITY (mOsmol/kg)

Table 5. Two-way analysis of variance with replication (using 8 individual prawns as replicate values) of the effect of salinity and temperature on the blood osmotic concentration of summer acclimatized Palaemon longirostris exposed to a range of salinities between 0.5 and 34‰. ( d.f., degrees of freedom; S.L., significance level; n.s., not significant).

| Source of variation | | d.f. | F-ratio | S.L. |
|---|---|---|---|---|
| Small | | | | |
| | Salinity | 5,126 | 7.18 | 0.001 |
| | Temperature | 2,126 | 19.94 | 0.001 |
| | S x T | 10,126 | 1.09 | n.s. |
| Medium | | | | |
| | Salinity | 5,126 | 32.67 | 0.001 |
| | Temperature | 2,126 | 88.28 | 0.001 |
| | S x T | 10,126 | 2.57 | 0.01 |
| Large | | | | |
| | Salinity | 5,126 | 43.25 | 0.001 |
| | Temperature | 2,126 | 118.90 | 0.001 |
| | S x T | 10,126 | 12.73 | 0.001 |
| Ovigerous | | | | |
| | Salinity | 5,126 | 53.94 | 0.001 |
| | Temperature | 2,126 | 26.41 | 0.001 |
| | S x T | 10,126 | 5.78 | 0.001 |

Table 6. Difference in mean blood osmolalities (mOsmol/kg) of Palaemon longirostris in 0.5‰ and 34‰ S media.

| Season | Prawn size | Temperature (°C) | | |
|---|---|---|---|---|
| | | 4 | 12 | 20 |
| Summer | Small | 137 | 83 | 55 |
| | Medium | 138 | 91 | 83 |
| | Large | 180 | 40 | 50 |
| | Ovigerous | 146 | 57 | 88 |
| Winter | Medium | 200* | 106 | 198 |
| | Large | 180 | 125 | 279 |

* Due to high prawn mortality in 0.5‰ at 4°C, difference in mean blood osmolality was calculated for prawns in 3.5 and 34‰.

Table 7. Two-way analysis of variance with replication (using 8 individual prawns as replicate values) of the effect of salinity and prawn size on the blood osmotic concentration of summer acclimatized Palaemon longirostris exposed to a range of salinities between 0.5 and 34‰ (d.f., degrees of freedom; S.L., significance level; n.s., not significant).

| Source of variation | d.f. | F-ratio | S.L. |
|---|---|---|---|
| 4°C | | | |
| Salinity | 5,168 | 42.40 | 0.001 |
| Size | 3,168 | 10.76 | 0.001 |
| S x S | 15,168 | 1.06 | n.s. |
| 12°C | | | |
| Salinity | 5,168 | 16.10 | 0.001 |
| Size | 3,168 | 29.47 | 0.001 |
| S x S | 15,168 | 1.12 | n.s. |
| 20°C | | | |
| Salinity | 5,168 | 19.71 | 0.001 |
| Size | 3,168 | 28.37 | 0.001 |
| S x S | 15,168 | 1.83 | n.s. |

10-13), and, at all three temperatures, the blood isosmotic points of small prawns were substantially lower than those for the other size classes (Table 8). Osmoregulation in 43‰ was also directly related to prawn size. Thus the osmotic gradient between the blood and the external medium in 43‰ was smallest for medium prawns (Fig. 11), and largest for large and ovigerous prawns (Figs 12 & 13). These observations indicate that osmoregulation in 43‰ improved directly with increasing prawn size. Furthermore, all small prawns died within 7 days in 43‰ (Fig. 10, Chapter 2) and it is assumed that this mortality was due to a complete breakdown in the osmoregulatory mechanism at this hyper-saline salinity.

Temperature had a significant effect on the blood osmolality of all prawn size groupings (Table 5, ANOVA, $P<0.001$). In general for each size category, the osmoregulatory gradient between haemolymph and external medium was less at 20°C than at 12°C. A reduction in temperature to 4°C resulted in an elevation of blood osmolality over the entire salinity range (Figs 10-13). For each size group, the blood isosmotic point was higher at 4°C than at 12 or 20°C (Table 8). This temperature effect enhanced the observed breakdown of blood regulation in 43‰ S media (Figs 10-13).

Table 8. Variability of the blood isosmotic point in *Palaemon longirostris*.

| Prawn size | Temperature (°C) | Isosmotic Points (mOsmol/kg) | |
|---|---|---|---|
| | | Summer | Winter |
| Small | 4 | 591 | — |
| | 12 | 559 | — |
| | 20 | 532 | — |
| Medium | 4 | 695 | 720 |
| | 12 | 610 | 620 |
| | 20 | 615 | 565 |
| Large | 4 | 700 | 680 |
| | 12 | 620 | 630 |
| | 20 | 625 | 595 |
| Ovigerous | 4 | 665 | — |
| | 12 | 625 | — |
| | 20 | 625 | — |

### 3.3.1.2. WINTER ACCLIMATIZED PRAWNS

Osmoregulatory curves for medium and large winter prawns at 4, 12 and 20°C were similar in general form to those of summer prawns (Figs 14 & 15). Generally, winter prawns also showed efficient hyper-hypo-osmoregulation and over an external range of 1000 mOsmol/kg (0.5-34‰), blood osmolality varied by less than 280 mOsmol/kg (Table 6). Salinity had a significant effect on blood osmolality and this effect was most pronounced at the upper and lower salinity limits. Thus at 43‰, blood osmolality rose towards isosmotic concentrations and, at 0.5‰, blood osmolality declined towards isosmotic concentrations (Figs 14 & 15). Although prawn size had no significant effect on blood osmolality (Table 10, ANOVA, $\underline{P}>0.05$), only large prawns were able to survive and osmoregulate in 0.5 and 43‰ at 4°C (Figs 14 & 15). At these salinity/temperature combinations, all medium prawns died within 7 days (Chapter 2; Fig. 6).

Temperature also had a significant effect on osmoregulation of winter prawns (Table 9, ANOVA, $\underline{P}<0.001$). Generally, low temperature (4°C) resulted in elevated blood osmolalities compared with higher temperatures (Figs 14 & 15). This temperature effect enhanced a breakdown in osmoregulation at 43‰ , such that the blood osmolality of large prawns increased sharply towards isosmotic concentrations and medium prawns all died within 7 days (Figs 14 & 15; see also Chapter 2). All medium prawns held

Figure 14. Osmotic concentrations of the haemolymph of winter acclimatized medium *Palaemon longirostris* in relation to salinity at 4 (triangles and irregular dashed line), 12 (circles and solid line) and 20°C (squares and regular dashed line) (vertical lines represent ±1 Standard Deviation).

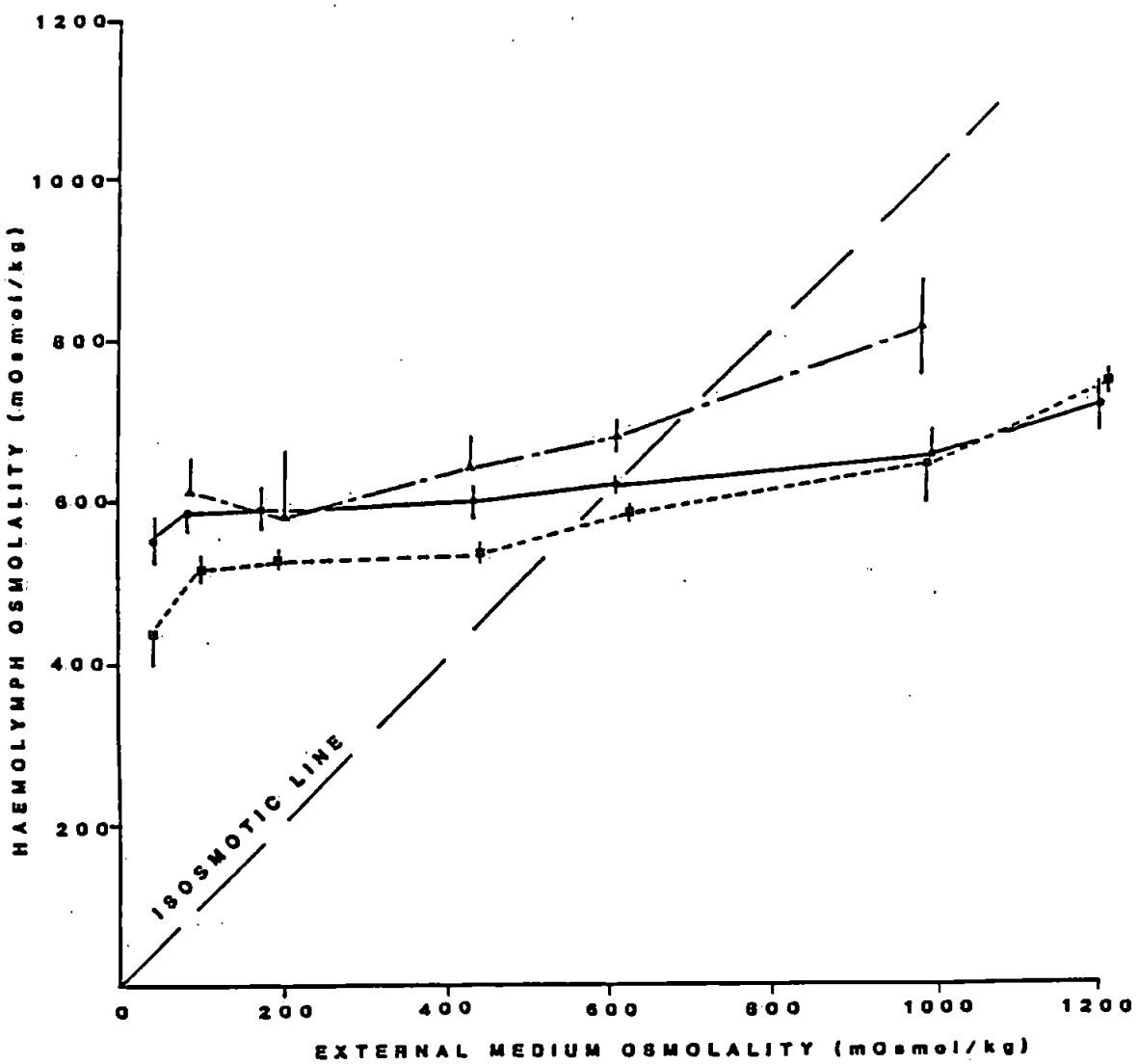

1200
1000
800
600
400
200
HAEMOLYMPH OSMOLALITY (mOsmol/kg)
ISOSMOTIC LINE
0
200
400
600
800
1000
1200
EXTERNAL MEDIUM OSMOLALITY (mOsmol/kg)

Figure 15. Osmotic concentrations of the haemolymph of winter acclimatized large *Palaemon longirostris* in relation to salinity at 4 (triangles and irregular dashed line), 12 (circles and solid line) and 20°C (squares and regular dashed line) (vertical lines represent ±1 Standard Deviation).

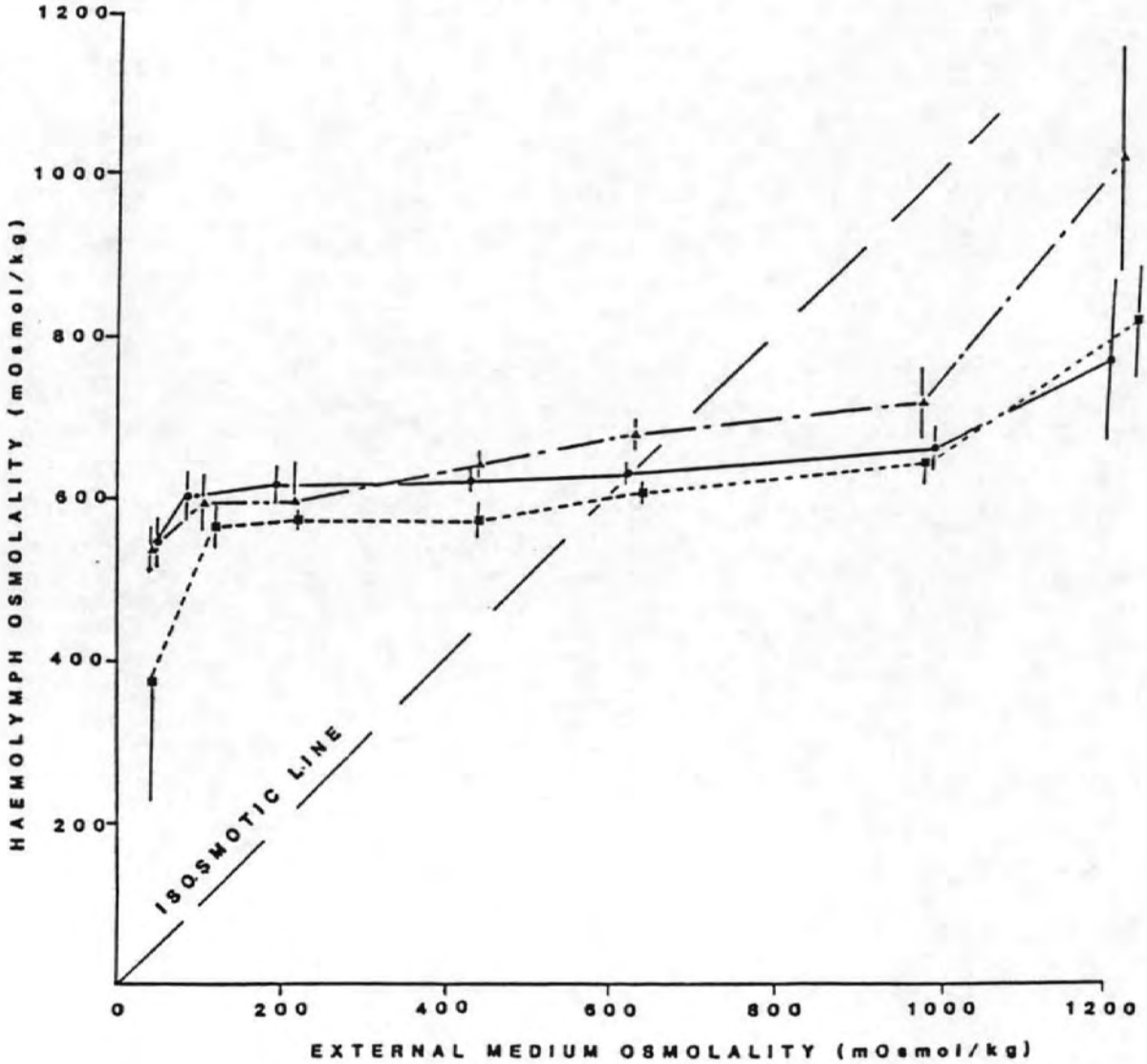

1200
1000
800
600
400
200
HAEMOLYMPH OSMOLALITY (mOsmol/kg)
ISOSMOTIC LINE
0
200
400
600
800
1000
1200
EXTERNAL MEDIUM OSMOLALITY (mOsmol/kg)

Table 9. Two-way analysis of variance with replication (using 4 individual prawns as replicate values) of the effect of salinity and temperature on the blood osmotic concentration of winter acclimatized Palaemon longirostris exposed to a range of salinities between 0.5 and 34‰ (d.f., degrees of freedom; S.L., significance level; n.s., not significant).

| Source of variation | | d.f. | F-value | S.L. |
|---|---|---|---|---|
| Medium | | | | |
| | Salinity | 4,45 | 33.60 | 0.001 |
| | Temperature | 2,45 | 49.79 | 0.001 |
| | S x T | 8,45 | 3.41 | 0.01 |
| Large | | | | |
| | Salinity | 5,54 | 24.59 | 0.001 |
| | Temperature | 2,54 | 19.82 | 0.001 |
| | S x T | 10,54 | 2.49 | 0.05 |

Table 10. Two-way analyses of variance with replication (using 4 individual prawns as replicate values) of the effect of salinity and prawn size on the blood osmotic concentration of winter acclimatized Palaemon longirostris exposed to a range of salinities between 0.5 and 34‰ (d.f., degrees of freedom; S.L., significance level; n.s., not significant).

| Source of variation | d.f. | F-ratio | S.L. |
|---|---|---|---|
| 4°C* | | | |
| Salinity | 4,30 | 19.31 | 0.001 |
| Size | 1,30 | 2.37 | n.s. |
| S x S | 4,30 | 2.57 | n.s. |
| 12°C | | | |
| Salinity | 5,36 | 22.32 | 0.001 |
| Size | 1,36 | 4.04 | n.s. |
| S x S | 5,36 | 0.43 | n.s. |
| 20°C | | | |
| Salinity | 5,36 | 21.02 | 0.001 |
| Size | 1,36 | 0.15 | n.s. |
| S x S | 5,36 | 1.84 | n.s. |

* Due to high prawn mortality in 0.5‰ at 4°C, ANOVA was calculated on prawns exposed to a range of salinities between 3.5 and 34‰.

in 0.5 ‰ at 4°C also died within 7 days (see Chapter 2). At 20°C, all prawn size groupings showed a drop in blood osmolality compared with the other temperatures studied (Figs 14 & 15). This effect of temperature was most pronounced at 0.5‰, where both medium and large prawns showed a large drop in blood osmotic concentration (Figs 14 & 15). The blood isosmotic point of both medium and large prawns was also inversely related to temperature. The highest isosmotic point was recorded at 4°C and the lowest isosmotic point was recorded at 20°C (Table 8).

### 3.3.1.3. SEASONAL DIFFERENCES IN OSMOREGULATION

Summer prawns acclimated to a range of salinities between 0.5 and 34‰ demonstrated lower mean blood osmolality ranges than winter prawns (Table 6). These data suggest that summmer prawns are more efficient hyper-hypo-osmoregulators than winter prawns. There were also some interesting seasonal differences in blood osmoregulation for medium prawns at 4°C. For example, the rise in blood osmolality observed for medium prawns in 34‰ at 4°C was significantly higher in winter than in summer (Table 11, *t*-test, $P<0.01$, Figs 11 & 14). Medium prawns also showed a seasonal difference in the ability to osmoregulate in 0.5 and 43‰ at 4°C (Figs 11 & 14). In addition, medium winter prawns all died within 7 days in these salinity/temperature conditions (Fig. 14), whereas medium

Table 11. Statistical comparison of the blood osmolalities of medium *Palaemon longirostris* between seasons (*d.f.*, degrees of freedom; *S.L.*, significance level; *n.s.*, not significant ).

| Temperature (°C) | Salinity (‰) | *t*-value | *d.f.* | *S.L.* |
|---|---|---|---|---|
| 4 | 0.5 | – | – | * |
| | 22.0 | 0.18 | 15 | *n.s.* |
| | 34.0 | 3.37 | 16 | 0.01 |
| 12 | 0.5 | 0.85 | 16 | *n.s.* |
| | 22.0 | 2.23 | 15 | 0.05 |
| | 34.0 | 2.09 | 16 | 0.05 |
| 20 | 0.5 | 3.11 | 14 | 0.01 |
| | 22.0 | 3.74 | 15 | 0.001 |
| | 34.0 | 1.81 | 16 | 0.05 |

* 100% prawn mortality after 7 days.

summer prawns were able to regulate blood osmolality and thus survive under the very same salinity/temperature conditions (Fig. 11).

The osmoregulation of winter prawns was more severely affected by high temperature (20°C) than that of summer prawns. The decline in blood osmolality observed at 20°C was significantly lower in winter than in summer for medium prawns at 0.5, 22 and 34‰ (Table 11, t-test, $P<0.05$), and for large prawns at 0.5‰ (Table 12, t-test, $P<0.001$). Although there were other significant differences in the blood osmotic concentration between seasons for both medium and large prawns at 12°C (ANOVA, $P<0.05$, Tables 11 & 12), it is not possible to identify or explain these differences at this time.

### 3.3.2. RATE OF OSMOTIC RESPONSE TO SUDDEN SALINITY CHANGE

The time-based osmoregulation curves obtained for prawns transferred directly from 14 to 34‰ at 4, 12 and 20°C are shown in Figures 16 & 17. Time of immersion in the new salinity had a significant effect on blood osmolality (Table 13 & 14, ANOVA, $P<0.05$). In general at each temperature, there was an initial increase in blood osmolality within the first 4 to 5 h, followed by a decrease to a new steady state. This new steady state of blood osmolality was normally reached within 6h. For most individuals, there was no significant change in blood

Table 12. Statisitical comparison of the blood osmolalities of large Palaemon longirostris between seasons (d.f., degrees of freedom; S.L., significance level; n.s., not significant).

| Temperature (°C) | Salinity (‰) | t-value | d.f. | S.L. |
|---|---|---|---|---|
| 4 | 0.5 | 0.43 | 14 | n.s. |
| | 22.0 | 1.59 | 16 | n.s. |
| | 34.0 | 0.71 | 16 | n.s. |
| 12 | 0.5 | 6.34 | 16 | 0.001 |
| | 22.0 | 2.43 | 16 | 0.05 |
| | 34.0 | 3.86 | 16 | 0.01 |
| 20 | 0.5 | 5.41 | 14 | 0.001 |
| | 22.0 | 1.34 | 15 | n.s. |
| | 34.0 | 0.68 | 16 | n.s. |

Figure 16. Osmotic concentration of the haemolymph of summer acclimatized medium *Palaemon longirostris* transferred directly from 14‰ to 34‰ at 4 (triangles and irregular dashed line), 12 (circles and solid line) and 20°C (squares and regular dashed line). Vertical lines represent ±1 Standard Deviation; over first 6h period standard deviations were less than 40 mOsmol/kg and have been omitted for clarity.

Figure 17. Osmotic concentration of the haemolymph of summer acclimatized large *Palaemon longirostris* transferred directly from 14‰ to 34‰ at 4, 12 and 20°C. Symbols as for Fig. 16.

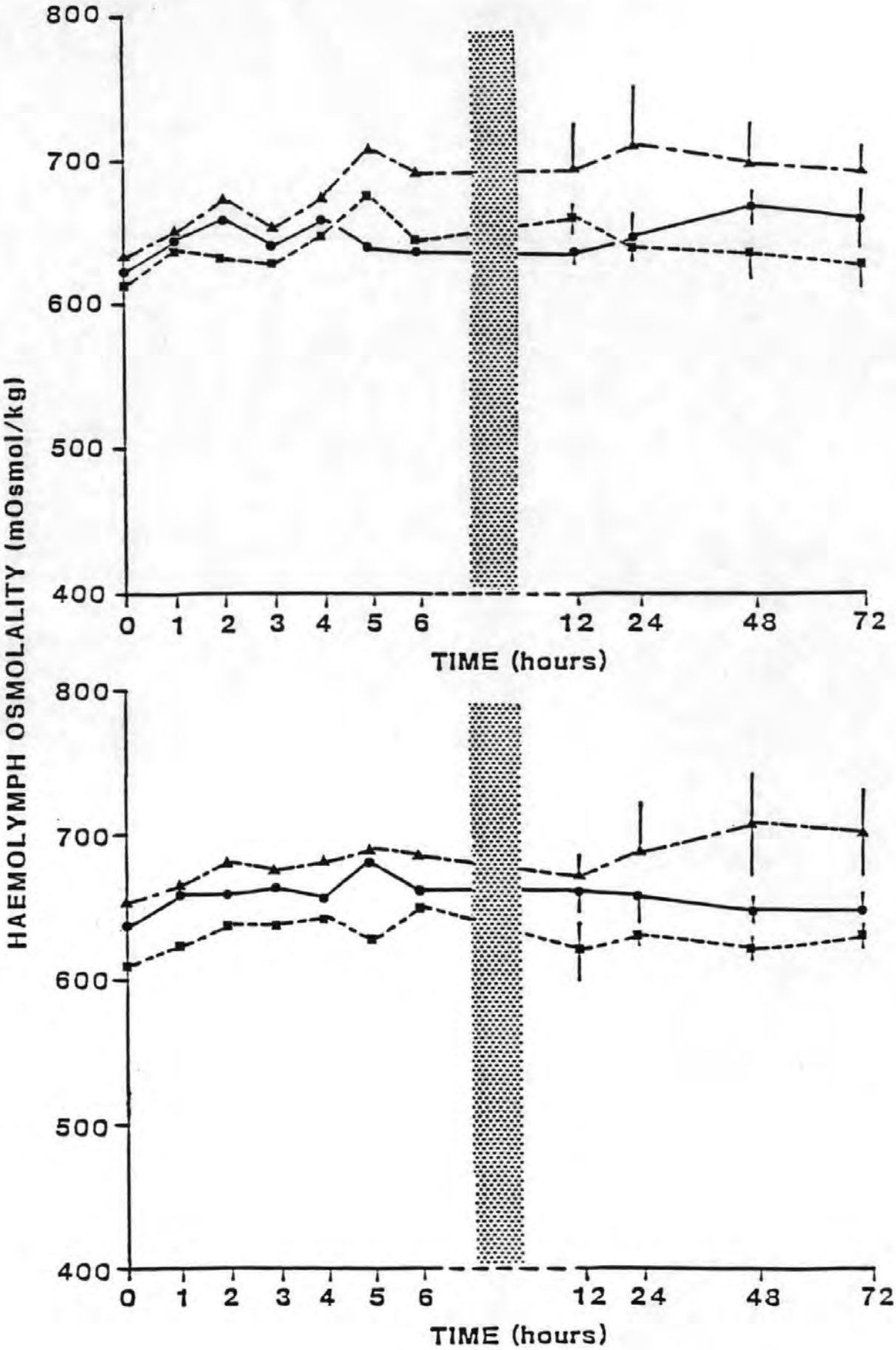

800
700
600
500
400
HAEMOLYMPH OSMOLALITY (mOsmol/kg)
0 1 2 3 4 5 6 12 24 48 72
TIME (hours)
800
700
600
500
400
0 1 2 3 4 5 6 12 24 48 72
TIME (hours)

Table 13. Two-way analysis of variance with replication (using 6 individual prawns as replicate values) of the effect of time and temperature on the blood osmotic concentration of summer acclimatized Palaemon longirostris transferred directly from 14‰ to 34‰ (d.f., degrees of freedom; S.L., significance level; n.s., not significant).

| Source of variation | d.f. | F-ratio | S.L. |
|---|---|---|---|
| Large | | | |
| Time | 10,165 | 5.02 | 0.001 |
| Temperature | 2,165 | 129.20 | 0.001 |
| T x T | 20,165 | 2.39 | 0.001 |
| Medium | | | |
| Time | 10,165 | 9.62 | 0.001 |
| Temperature | 2,165 | 75.60 | 0.001 |
| T x T | 20,165 | 3.77 | 0.001 |

Table 14. Two-way analysis of variance with replication (using 6 individual prawns as replicate values) of the effect of time and prawn size on the blood osmotic concentration of summer acclimatized Palaemon longirostris transferred directly from 14‰ to 34‰ (d.f., degrees of freedom; S.L., significance level; n.s., not significant).

| Source of variation | d.f. | F-ratio | S.L. |
|---|---|---|---|
| 4°C | | | |
| Time | 10,131 | 7.63 | 0.001 |
| Size | 1,131 | 0.05 | n.s. |
| T x S | 10,131 | 1.66 | n.s. |
| 12°C | | | |
| Time | 10,131 | 6.55 | 0.001 |
| Size | 1,131 | 16.01 | 0.001 |
| T x S | 10,131 | 3.26 | 0.001 |
| 20°C | | | |
| Time | 10,131 | 6.11 | 0.001 |
| Size | 1,131 | 24.85 | 0.001 |
| T x S | 10,131 | 1.93 | 0.05 |

osmolality from 6-72 h (Table 15, ANOVA, $P<0.05$). There was no clear effect of temperature on rate of osmotic response, however, blood osmolalities of both medium and large prawns were significantly higher at 4°C than at 12 or 20°C (Table 13, ANOVA, $P<0.001$, Figs 16 & 17). Prawn size also did not have a significant effect on rate of osmotic response. Although for some time intervals, medium prawns showed significantly different blood osmolalities to those of large prawns (ANOVA, $P<0.05$, Table 14), it was not possible to identify or explain this effect.

The time-based osmoregulation curves for prawns transferred directly from 14 to 5‰ at 4, 12 and 20°C are shown in Figures 18 & 19. Exposure time had a significant effect on blood osmolality (Tables 16 & 17, ANOVA, $P<0.05$). For some groups there was a gradual decrease in blood osmolality with time, whereas for others there was an initial drop in blood osmolality, usually within the first 6 to 12 h. This latter decline was follwed by a rise and a second, more gradual, decline which continued to the end of the experiment. The second pattern was more obvious at 20°C than at 4 or 12°C. Unlike prawns transferred from low to high salinity, the new blood osmolality steady state for prawns transferred from 14 to 5‰ was not normally reached within the first 6h. From 6 hours after transfer onwards, time still had a significant effect on blood osmolality (Table 18, ANOVA, $P<0.05$). Thus, prawns transferred from 14 to 34‰ reached a new steady state more quickly than prawns transferred from 14 to 5‰ (Table 15 & 18). Temperature had

Table 15. One-way analysis of variance of the effect of time (from 6h onwards) on the blood osmotic concentration of summer acclimatized Palaemon longirostris transferred directly from 14‰ to 34‰ (d.f., degrees of freedom; S.L., significance level; n.s., not significant).

| Source of variation | d.f. | F-ratio | S.L. |
|---|---|---|---|
| Medium | | | |
| 4°C | 4,25 | 0.43 | n.s. |
| 12°C | 4,25 | 4.15 | 0.05 |
| 20°C | 4,25 | 1.27 | n.s. |
| Large | | | |
| 4°C | 4,25 | 1.57 | n.s. |
| 12°C | 4,25 | 2.05 | n.s. |
| 20°C | 4,25 | 3.60 | 0.05* |

* not significant after 12 hours.

Figure 18. Osmotic concentration of the haemolymph of summer acclimatized medium *Palaemon longirostris* transferred directly from 14‰ to 5‰ at 4 (triangles and irregular dashed line), 12 (circles and solid line) and 20°C (squares and regular dashed line). Vertical lines represent ±1 Standard Deviation; over first 6h period standard deviations were less than 28 mOsmol/kg and have been omitted for clarity.

Figure 19. Osmotic concentration of the haemolymph of summer acclimatized large *Palaemon longirostris* transferred directly from 14‰ to 5‰ at 4, 12 and 20°C. Symbols as for Fig. 18.

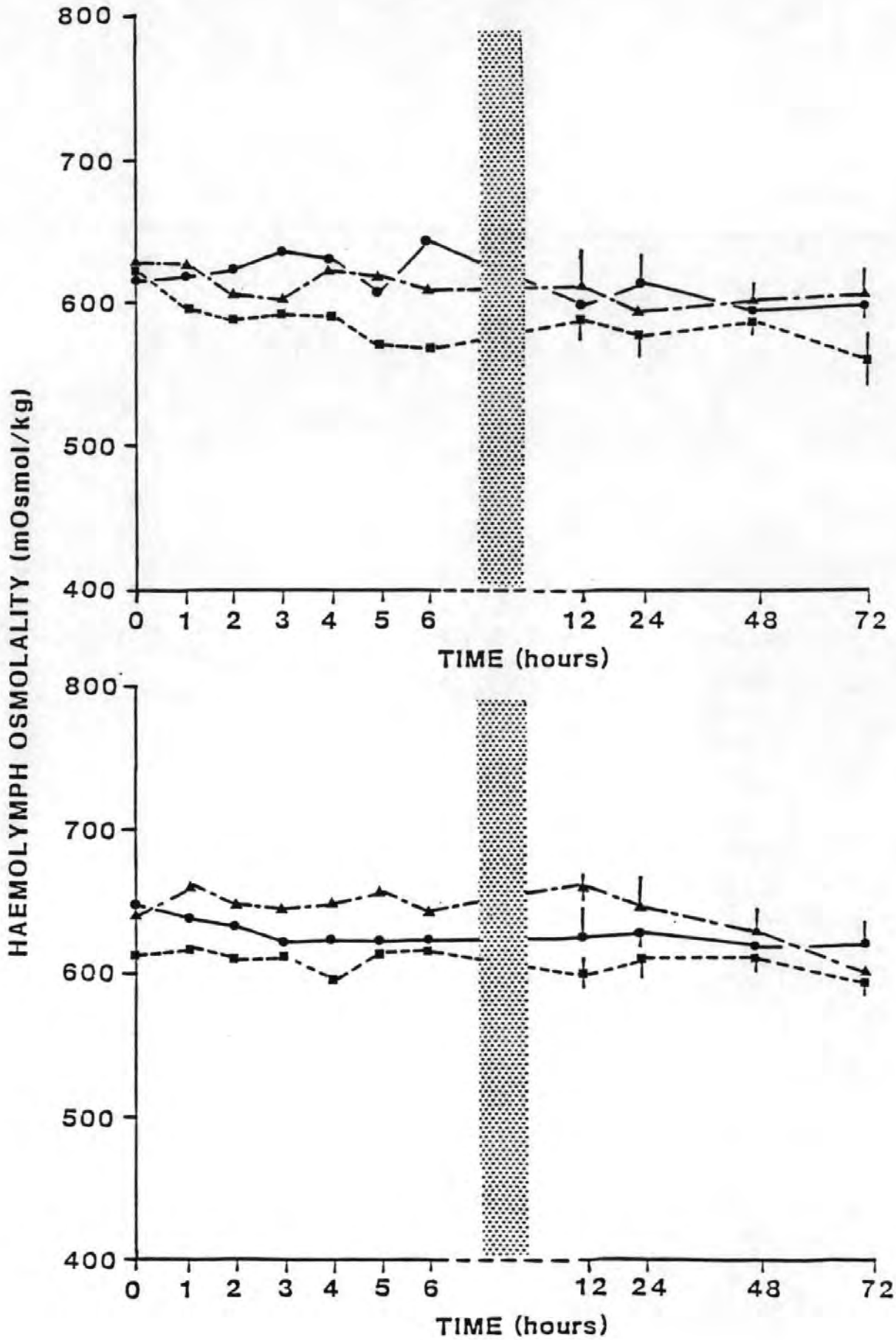

800
700
600
500
400
HAEMOLYMPH OSMOLALITY (mOsmol/kg)
0 1 2 3 4 5 6 12 24 48 72
TIME (hours)
800
700
600
500
400
0 1 2 3 4 5 6 12 24 48 72
TIME (hours)

Table 16. Two-way analysis of variance with replication (using 6 individual prawns as replicate values) of the effect of time and temperature on the blood osmotic concentration of summer acclimatized Palaemon longirostris transferred directly from 14‰ to 5‰ ( d.f., degrees of freedom; S.L., significance level; n.s., not significant).

| Source of variation | d.f. | F-ratio | S.L. |
|---|---|---|---|
| Large | | | |
| Time | 10,165 | 7.05 | 0.001 |
| Temperature | 2,165 | 80.10 | 0.001 |
| T x T | 20,165 | 2.64 | 0.001 |
| Medium | | | |
| Time | 10,165 | 4.48 | 0.001 |
| Temperature | 2,165 | 39.80 | 0.001 |
| T x T | 20,165 | 2.67 | 0.001 |

Table 17. Two-way analysis of variance with replication (using 6 individual prawns as replicate values) of the effect of time and prawn size on the blood osmotic concentration of summer acclimatized Palaemon longirostris transferred directly from 14‰ to 5‰ (d.f., degrees of freedom; S.L., significance level; n.s., not significant).

| Source of variation | d.f. | F-ratio | S.L. |
|---|---|---|---|
| 4°C | | | |
| Time | 10,131 | 3.32 | 0.001 |
| Size | 1,131 | 65.80 | 0.001 |
| T x S | 10,131 | 1.57 | n.s. |
| 12°C | | | |
| Time | 10,131 | 4.63 | 0.001 |
| Size | 1,131 | 13.47 | 0.001 |
| T x S | 10,131 | 3.16 | 0.001 |
| 20°C | | | |
| Time | 10,131 | 6.87 | 0.001 |
| Size | 1,131 | 76.74 | 0.001 |
| T x S | 10,131 | 5.23 | 0.001 |

Table 18. One-way analysis of variance of the effect of time (from 6h onwards) on the blood osmotic concentration of summer acclimatized Palaemon longirostris transferred directly from 14‰ to 5‰. (d.f., degrees of freedom; S.L., significance level; n.s., not significant).

| Source of variation | d.f. | F-ratio | S.L. |
|---|---|---|---|
| Medium | | | |
| 4°C | 4,25 | 0.76 | n.s. |
| 12°C | 4,25 | 13.56 | 0.001* |
| 20°C | 4,25 | 4.56 | 0.01 |
| Large | | | |
| 4°C | 4,25 | 5.42 | 0.01* |
| 12°C | 4,25 | 0.77 | n.s. |
| 20°C | 4,25 | 6.27 | 0.01 |

* not significant after 12 hours.

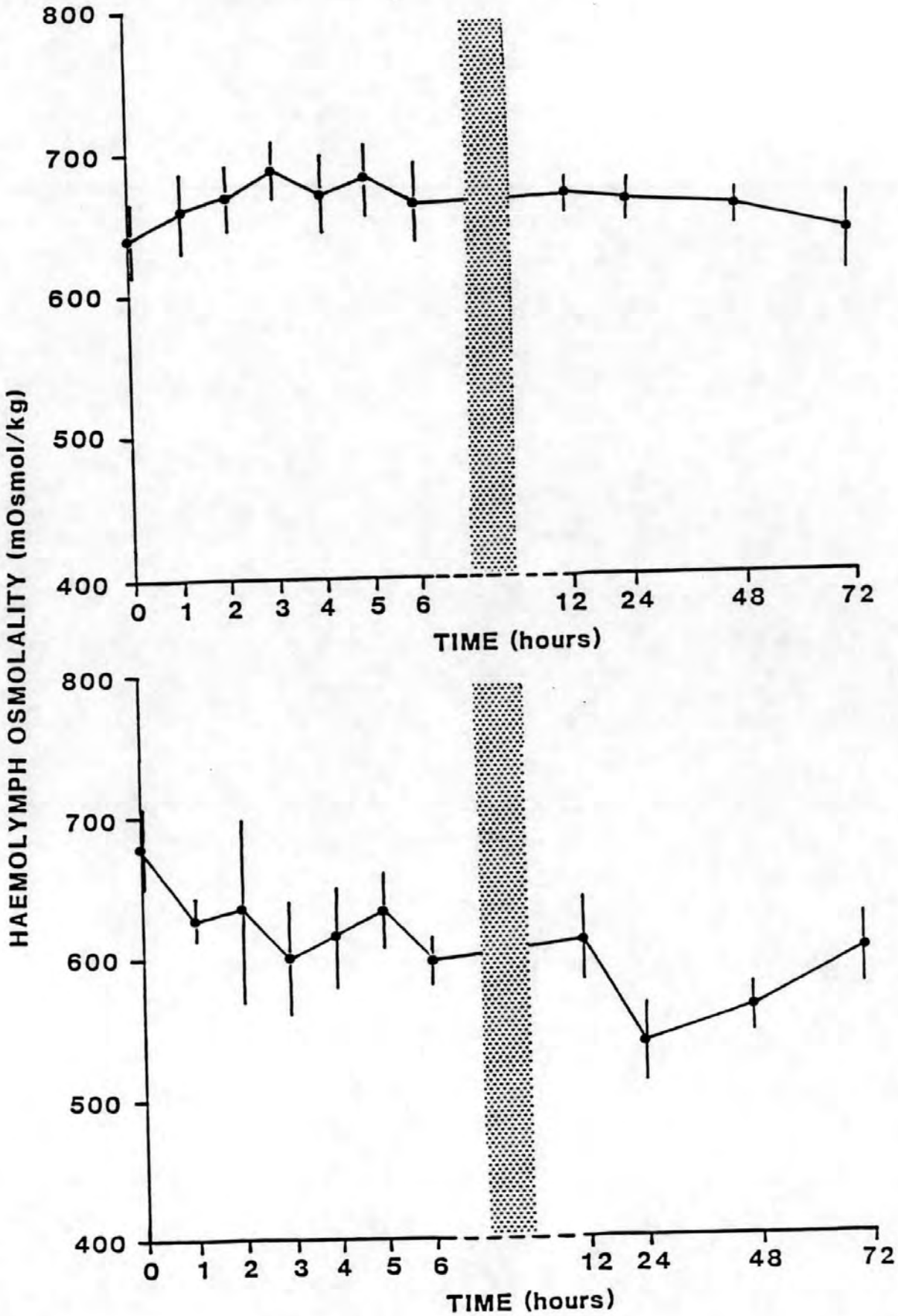
no clear effect on rate of osmotic response, however, blood osmolalities for both medium and large prawns were significantly higher at 4°C than at 12 or 20°C (Table 16, ANOVA, $P<0.001$, Figs 18 & 19). There was also no obvious effect of prawn size on speed of osmotic response, although medium prawns transferred from 14 to 5‰ had significantly lower blood osmolalities than large prawns at each temperature (Table 17, ANOVA, $P<0.001$, Figs 18 & 19).

Since *Palaemon longirostris* appeared to have such an efficient osmoregulatory mechanism with which to respond to relatively extensive salinity change, medium prawns were subjected further to even more acute salinity changes to test the response limits. Figures 20 & 21 show the time-based osmoregulation curves for medium prawns transferred from 1 to 34‰ , and from 34 to 1‰ at 12°C respectively. Prawns transferred from 1 to 34‰ showed an initial rise in blood osmolality within 6 h, followed by a gradual decline to a new steady state (Fig. 20). The magnitude of this response was no larger than the response shown for prawns exposed to a much smaller salinity change of 14 to 34‰ (Figs 16 & 17).

Prawns transferred from 34 to 1‰ showed a large drop in blood osmolality which reached its lowest point 24h after transfer. This drop was followed by a gradual rise to a new steady state (Fig. 21). The magnitude of this response was much larger than that shown for prawns transferred from 14 to 5‰ (Figs 18 & 19).

Figure 20. Osmotic concentration of the haemolymph of summer acclimatized medium *Palaemon longirostris* transferred directly from 1‰ to 34‰ at 12°C. Vertical lines represent ±1 Standard Deviation; over first 6h period standard deviations were less than 40 mOsmol/kg.

Figure 21. Osmotic concentration of the haemolymph of summer acclimatized medium *Palaemon longirostris* transferred directly from 34‰ to 1‰ at 12°C. Symbols as for Fig. 20.


HAEMOLYMPH OSMOLALITY (mOsmol/kg)
800
700
600
500
400
0 1 2 3 4 5 6 12 24 48 72
TIME (hours)
800
700
600
500
400
0 1 2 3 4 5 6 12 24 48 72
TIME (hours)

## 3.4. DISCUSION

*Palaemon longirostris* has the capacity to hyper-osmoregulate in low salinities and hypo-osmoregulate in high salinities. Hyper-hypo-osmoregulation is a typical feature of many euryhaline palaemonid species (Panikkar, 1941; Dobkin & Manning, 1964; Born, 1968; Denne, 1968; Spaargaren, 1972; Hagerman & Uglow, 1983; Kirkpatrick & Jones, 1985; Ramirez de Isla Hernandez & Taylor, 1985) but is not restricted to this group alone. Other euryhaline prawns such as the penaeids (Williams, 1960; Bursey & Lane, 1971; Castille & Lawrence, 1981a,b,e; Ferraris et al., 1987) and the crangonids (Haefner, 1969b; Weber & Spaargaren, 1970; Hagerman, 1971; Spaargaren, 1971; McLusky et al., 1982) also show patterns of hyper-hypo-osmoregulation of body fluids. Although hyper-hypo-osmoregulation is characteristically associated with euryhaline prawns, this type of osmoregulation has been reported also for other euryhaline crustaceans such as amphipods (Moore & Francis, 1985), isopods (Kelley & Burbanck, 1972), mysids (McLusky, 1979), and grapsid crabs (Dehnel, 1962).

The salinity range over which *Palaemon longirostris* regulates its haemolymph osmolality (0.5-43‰) is very wide and compares favourably with that of other euryhaline crustaceans. Only one other prawn regulates its blood osmolality over a wider salinity range (1-60‰) and that is

the closely related *Palaemon macrodactylus* (Born, 1968). Although some hyper-hypo-osmoregulating prawns can regulate their haemolymph osmolality over a similar range of salinities to *P. longirostris*, few species can maintain comparable stable blood osmolalities over their entire salinity tolerance range as *P. longirostris*. The small variation in blood osmolality demonstrated for *P. longirostris* over a relatively wide range of salinities, is one indication of the extremely efficient mechanism of osmoregulation possessed by this species.

Another major difference in the osmoregulation of *Palaemon longirostris* compared with other hyper-hypo-osmoregulating prawns is its ability to maintain a large osmotic gradient between the haemolymph and the external medium at very dilute salinities. Only one other group of prawns can osmoregulate in almost fresh water and that is the various species of *Macrobrachium* (Castille & Lawrence, 1981c; Moreira et al., 1983). *Macrobrachium* species are also palaemonids and are essentially a freshwater group of prawns. *Macrobrachium* species, however, show reduced hypo-osmoregulatory capacity in high salinities compared with hyper-hypo-osmoregulating species such as *P. longirostris*.

*Palaemon longirostris* also shows extremely strong hypo-osmoregulation when exposed to high salinities. Indeed, the degree of hypo-osmoregulation illustrated by this species is equivalent to that reported for marine palaemonids such as *Palaemon serratus* (Spaargaren, 1972) and

*Palaemon elegans* (Ramirez de Isla Hernandez & Taylor, 1985). These marine palaemonids, however, show reduced hyper-osmoregulatory ability in low salinties compared with *P. longirostris*.

The level of hyper-hypo-osmoregulation measured for *Palaemon longirostris* is thus extremely high and is virtually unparalleled amongst other decapod crustaceans. Efficient hyper-hypo-osmoregulation has obvious physiological advantages for *P. longirostris*, as it produces relatively stable conditions within the animal and thus minimises cellular damage (Verwey, 1957).

The salinity at which haemolymph osmolality becomes isosmotic with the external medium differs between prawn species, depending on whether they occupy marine, estuarine or freshwater habitats. Thus, marine species such as *Crangon allmanni* (Spaargaren, 1971) and *Lymsata seticaudata* (Spaargaren, 1972) are isosmotic at 35‰; estuarine species such as *Crangon vulgaris* (Hagerman, 1971) and *Palaemonetes varians* (Hagerman & Uglow, 1983) are isosmotic in salinities between 21-27‰ , and freshwater species such as *Macrobrachium australiense* (Denne, 1968) and *Macrobrachium potiuna* (Moreira et al., 1983) are isosmotic at salinities of 15-18‰. The isosmotic point for *Palaemon longirostris*, although varying between 18-23‰ depending on prawn size and temperature, falls between the estuarine and freshwater ranges. Reduction in the blood isosmotic point is thought to be an essential adaptation to life away from the sea, because lowering the isosmotic point effectively lowers the

osmotic gradient between the blood and the external medium at dilute salinities. Consequently, lowering the isosmotic value reduces the osmotic work load on the animal which is clearly adaptive (Dorgelo, 1981). The data presented here for *P. longirostris*, however, show that the isosmotic point of a crustacean is not necesarily fixed but can vary depending on environment and intrinsic factors such as water temperature and body size. For example, the isosmotic point for *P. longirostris*, was elevated by a reduction in the water temperature, and was depressed by a reduction in the body size. Unfortunately, in order to study inter-specific variation of isosmotic points between crustacean species, the isomotic point data are generally presented as a mean value, thus masking the intra-specific variation. Until more examples of variation of isosmotic points are available, the adaptive nature of the variation remains unknown.

Intrinsic factors, such as body size, may influence the osmoregulatory ability of crustaceans (Haefner, 1969b). Improved osmoregulation by juveniles rather than adults has been reported for some species such as *Crangon septemspinosa* (Haefner, 1969b) and *Palaemon affinis* (Kirkpatrick & Jones, 1985). Similarly, juvenile stages of migrating prawns such as *Penaeus setiferus* and *Penaeus stylirostris* have a more enhanced osmoregulatory ability than adults (Castille & Lawrence, 1981b). For migrating penaeids, improved osmoregulation in juveniles is essential, as it is this stage of the life cycle which generally matures within the more dilute regions of estuaries where salinity fluctuations

are pronounced. Adult penaeids, migrate to sea as they mature and thus do not need strong osmoregulatory powers to survive in this relatively stable saline environment. Alternatively, improved osmoregulation by adults rather than juveniles has been reported for the isopod *Cyathura polita* (Kelley & Burbanck, 1972). This is somewhat surprising as this species usually spends its entire life cycle within an area of a few metres and both juveniles and adults are exposed to the same environmental conditions (Burbanck et al., 1964).

The results for *Palaemon longirostris* show yet another type of size effect on osmoregulation. Although all prawn sizes showed strong hyper-hypo-osmoregulation and ANOVA indicated that size had no significant effect on blood osmolality ranges, at each temperature, small prawns had significantly lower blood osmolalities than larger prawns over the entire salinity range between 0.5 and 34‰. All post-larval life-history stages of *P. longirostris* are normally found in the upper reaches of large river estuaries where salinities are generally very low (pers. obs.). Small *P. longirostris*, due to their larger surface area to volume ratio, are exposed to more extensive water and salt balance problems than are adults. Depressed blood osmolalites, as reported here for small prawns, would result in a smaller osmotic gradient between the haemolymph and the external medium. This reduction in the osmotic gradient presumably compensates for any extra osmotic work imposed on small prawns by their large surface area to volume ratio.

Therefore, small individuals use less metabolic energy for osmoregulation than larger prawns, and have more metabolic energy for growth. As small prawns generally grow faster than adults, this size response shown by *P. longirostris* is interpreted as an adaptation to life in the dilute saline environment.

Temperature is known to affect the osmoregulation of crustaceans (see reviews of Kinne, 1971; Vernberg & Silverthorn, 1979; Dorgelo, 1981; Mantel & Farmer, 1983). Due to extensive inter-specific and intra-specific variation, however, generalizations and adaptive interpretations of the nature of these temperature effects on osmoregulation are difficult to isolate.

For *Palaemon longirostris*, reduction in temperature from 12 or 20°C to 4°C resulted in elevations of blood osmolality and of the blood isosmotic points. These results support the widely held view that low temperatures are associated with high blood osmolalities (Kinne, 1971) but the adaptive significance of this temperature effect on osmoregulation remains unresolved. Elevated blood osmolalities at low temperatures are not universal amongst crustaceans. Some estuarine species, including the prawns *Palaemonetes varians* (Hagerman & Uglow, 1983) and *Crangon septemspinosa* (Haefner, 1969b), some gammarids (Dorgelo,1977) and some mysids (McLusky, 1979) show no effect of temperature on osmoregulation. It is argued that lack of sensitivity to temperature is adaptive to these species, as they occupy environments characterised by large

ranges in water temperature (McLusky, 1979). Another temperature effect on osmoregulation revealed by this study of *Palaemon longirostris* was a reduction of the osmotic gradient between the blood and the external medium at high temperature (20°C). This type of temperature effect on osmoregulation has been described previously for temperate crustaceans (Dorgelo, 1981), and occurs in the prawns *Crangon crangon* (Weber & Spaargaren, 1970), *Palaemon adspersus* (Larsen, cited by Hagerman & Uglow, 1983), *Palaemon elegans* (Ramirez de Isla Hernandez & Taylor, 1985) and *Palaemon affinis* (Kirkpatrick & Jones, 1985), and the isopods *Ligia occidentalis*, *Ligia pallasii* (Wilson, 1970) and *Sphaeroma serratum* (Charmantier, cited by Dorgelo, 1981). Since these species are either coastal or estuarine, any temperature effect which depresses their blood concentrations in dilute salinities, is often interpreted as favouring life in dilute saline environments (Dorgelo, 1981).

Not surprisingly, tropical crustaceans show the opposite temperature effect on osmoregulation, to that shown by temperate crustaceans (Dorgelo, 1981). Thus, the penaeid prawns *Penaeus aztecus* and *Peneaus duorarum* (Williams, 1960; Castille & Lawrence, 1981a) showed improved osmoregulation at high rather than low temperatures. The effect of temperature on osmoregulation is complicated further by the fact that geographically separated populations of the same species can show different responses. Panikkar (1941) reported improved regulation at lower temperatures for

*Palaemonetes varians* but a later study on the same species from a different geographical location reported improved regulation at higher temperatures (Hagerman & Uglow, 1983). Temperature effects on osmoregulation in crustaceans are thus complex and can even differ between two populations of the same species. Such inter-specific and intra-specific variation in the temperature response of crustaceans is propably due to phenotypic acclimation to different environmental regimes (Weber & Spaargaren, 1970; McLusky et al., 1982). The present results shown here for *P. longirostris* are yet another example of improved regulation at lower compared with high temperatures, for a population living at relatively high latitudes (Kirkpatrick & Jones, 1985).

Season has also been shown to affect the osmoregulatory ability of crustaceans (Dorgelo, 1981). Species such as *Palaemon serratus* (Panikkar, 1941) and *Paranephrops zealandacus* (Wong & Freeman, 1976) have higher blood osmolalities in winter than in summer. This seasonal difference is consistent with the effect of temperature on the osmoregulation of these species, ie, high blood osmolalities at low temperatures. Although, *Palaemon longirostris* did not show any elevation or reduction in blood osmolality between seasons, there were some interesting seasonal differences in osmoregulation at specific salinity/temperature combinations. Generally, winter prawns had larger blood osmolality ranges and were more sensitive to changes in temperature than summer prawns.

Thus, at high temperatures, haemolymph regulation by winter prawns appeared to breakdown in low salinities. This effect was probably due to the extreme difference between the experimental temperature (20°C) and the environmental temperature (3°C) at the time of prawn collection. A combination of very low temperature and very low salinity, enhanced osmoregulatory breakdown for winter prawns and resulted in high prawn mortality (Chapter 3.). Since *P. longirostris* used in the present study is at the northernmost part of its latitudinal range, it is perhaps not surprising that the cold winter temperatures reduce osmoregulatory efficiency in this warmer water species.

The pattern of haemolymph osmotic response to sudden salinity change shown by *Palaemon longirostris* included an initial erratic overshoot, followed by a gradual attainment of a new steady state. This type of response pattern is typical of all osmoregulating Crustacea (Panikkar,1941; Kinne, 1964) and reflects the time lag involved in both the switching on and switching off of the osmoregulatory process. The time scale and magnitude of the resulting blood osmotic changes, however, differs between species. Most penaeid prawns (Bursey & Lane, 1971; Castille & Lawrence, 1981a), *Palaemon affinis* (Kirkpatrick & Jones, 1985) and *Crangon septemspinosa* (Haefner, 1969b), when exposed to a sudden change in salinity, attain a new steady state within 2-3 days. As penaeid prawns occur in inshore coastal waters which are relatively stable with regards to salinity, the time taken to reach a new steady state is consistent with

their environment. The caridean prawn species cited, however, occupy either estuarine or inter-tidal habitats which are characterised by both rapid and large fluctuations in salinity. Therefore the speed of osmotic response to sudden salinity change exhibited by these species (2-3 days) is not well adapted to cope with the salinity fluctuations that can occur within their environments. It is suggested that perhaps behavioural rather than physiological adaptations are involved in avoiding acute osmotic shock in these species.

*Palaemon longirostris* attained a new blood osmolality steady state within 6-12h after exposure to a sudden salinity change. Some palaemonids achieve a new steady state following transfer within an even shorter time period. For example, *Palaemonetes varians* (Hagerman & Ugow, 1983) and *Palaemon serratus* (Spaargaren, 1972) reach a new steady state within 2 and 4 h respectively. As *P. longirostris*, *P. varians* and *P. serratus* occur in habitats characterized by short term salinity change, a quick osmotic response to sudden salinity change is clearly adaptive. When *P. longirostris* was transferred from nearly fresh water (1‰) to full strength sea water and <u>vice versa</u>, new blood osmolality steady states were reached within 6 and 72 h respectively. Such strong and quick osmotic responses to extreme, acute salinity transfer appears to be unique amongst decapod crustaceans. Interestingly, the rate of osmotic response in *P. longirostris* was faster in prawns transferred to a more concentrated salinity than in prawns

transferred to a more dilute salinity. This effect has not been reported for any other hyper-hypo-osmoregulating crustacean, but may give some indication of the evolutionary origins of the family Palaemonidae (see Chapter 6). Not only did *P. longirostris* show a more rapid osmotic response than most other estuarine shrimps and prawns, but also showed considerably smaller changes in blood osmolality. Such strong blood regulation could be attributed to low cuticle permeabilities, and the involvement of permeability changes in the osmoregulation of *P. longirostris* is reported in Chapter 5.

Temperature can also affect the rate at which crustaceans respond to sudden salinity change (Kinne, 1964; Dorgelo, 1981). When the coastal prawns *Crangon crangon* (Weber & Spaargaren, 1970), *Palaemon serratus*, *Lymsata seticaudata* (Spaargaren, 1972) and *Palaemon elegans* (Ramirez de Isla Hernandez & Taylor, 1983) were transferred to more concentrated media, temperature had no effect on the rate of osmotic response. However, when these prawns were transferred to more dilute salinity media, a new steady state of blood osmolality was reached faster at higher rather than lower temperatures. The adaptive significance of this temperature response in these crustaceans remains unclear. The present results for *Palaemon longirostris*, show no effect of temperature on rate of osmotic response and are in aggreement with the findings of Kirkpatrick & Jones (1985) for another estuarine prawn (*Palaemon affinis*). As the estuarine environment is characterized by both

fluctuations in temperature and salinity, it is clearly adaptive for a species which occupies such an environment not to have the speed of its osmotic response adversely affected by temperature change.

# CHAPTER 4

# IONIC REGULATION

## 4.1. INTRODUCTION

It is now well established that inorganic ions are the main effectors of the total osmolality of the body fluids of aquatic crustaceans (see reviews by Potts & Parry, 1964b; Gilles, 1975; Gilles & Pequeux, 1983; Mantel & Farmer, 1983). Sodium and chloride account for about 95% of the total osmotic concentration of sea water and of the haemolymph of crustaceans, and regulation of these ions is an extremely important aspect of the efficient osmoregulation of crustaceans. Compared with the contribution of sodium and chloride, other ions such as potassium, calcium and magnesium account for a small part of the extra-cellular fluid osmotic concentration of crustaceans. Regulation of these relatively minor haemolymph ions is, however, also essential to crustaceans for several reasons. Potassium is important in the control of neuromuscular efficiency in active species (Robertson, 1960), calcium is the basic material used in crustacean exoskeletons and also plays an essential role in cell membrane function (Robertson, 1960), and magnesium inhibits neuromuscular transmission (Robertson, 1953).

Even marine crustaceans, which are generally isosmotic with their environment, regulate the ionic concentrations of their haemolymph. For example, the spiny lobster *Panulirus longipes* and the rock shrimps *Sicyonia brevirostris* and

*Sicyonia dorsalis* maintain blood sodium concentrations higher, and blood chloride concentrations lower, than the external medium (Dall, 1974; Castille & Lawrence, 1981d). These species maintain a small but consistent sodium and chloride concentration gradient between the blood and the external medium over their entire salinity tolerance range. In addition, *P. longipes* shows strong hyper-regulation of blood calcium, strong hypo-regulation of blood magnesium and weak hyper-hypo-regulation of blood potassium (Dall, 1974). For these osmoconformers, the pattern and strength of ionic regulation varies with the specific ion concerned and is not consistent between species. For example, the spider crab *Maia squinado* maintains a haemolymph magnesium concentration four times higher than that of the Dublin Bay prawn *Nephrops norvegicus* (Potts & Parry, 1964b), even though both decapods are marine stenohaline species.

Crustaceans from environments characterized by fluctuating salinities have drawn considerable attention due to the variety of methods used by such species to maintain ionic stability (Dehnel & Carefoot, 1965). Osmoregulating species, in particular, have well developed ionic regulatory mechanisms. Not surprisingly, the regulation of sodium and chloride generally follows the same pattern as the osmoregulatory response curve for the species. The majority of osmoregulating crustaceans which have penetrated into the littoral, estuarine and brackish-water environments are hyper-osmoregulators (Kirschner, 1979), and thus show hyper-regulation of haemolymph sodium and chloride. Examples

include the crabs *Carcinus maenas* (Zanders, 1980) and *Helice crassa* (Bedford, 1972), the isopod *Sphaeroma serratum* (Harris, 1972) and the amphipod *Corophium volutator* (McLusky, 1968). There is, however, considerable variation amongst crustaceans from these habitats in the strength and pattern of regulation of other haemolymph ions such as potassium, calcium and magnesium. Over a wide range of salinities, each of these haemolymph ions may be variously hypo-regulated, hyper-regulated or hyper-hypo-regulated by different crustacean species (Mantel & Farmer, 1983).

A small number of crustaceans from fluctuating salinity type environments, however, have evolved the ability to hyper-hypo-regulate haemolymph osmolality (Kirschner, 1979). The previous chapter reported on the osmoregulation of *Palaemon longirostris* and showed that it was an extremely strong hyper-hypo-osmoregulator. Nothing is known, however, regarding the role of ions in the osmoregulatory process of this species. The present work thus set out to describe the regulation of sodium, chloride, potassium, calcium and magnesium ions in the haemolymph of *P. longirostris* acclimated to a wide range of salinities. Inter-specific variation in haemolymph concentrations of specific ions may not be entirely salinity-related. Temperature, in particular, can affect osmoregulation of crustaceans (Dorgelo. 1981; Mantel & Farmer, 1983; Chapter 3) and, not surprisingly, ionic regulation is also susceptible to thermal effects (Vernberg & Silverthorn, 1979). Unfortunately, the effect of this factor on ionic regulation

has been little studied in crustaceans, and the restricted information available indicates that ionic regulation may improve with elevated (Hagerman & Uglow, 1983) or lowered temperature (Dehnel & Carefoot, 1965). To increase understanding of the thermal effects on ionic regulation in crustaceans, the influence of temperature on the regulation of sodium, chloride, potassium, calcium and magnesium ions has been studied for *P. longirostris*, and is reported here.

## 4.2. MATERIALS AND METHODS

The methods of collection and the holding procedures used were identical to those described elsewhere for *Palaemon longirostris* (Chapter 2; Sections 2.1 & 2.2). Only summer acclimatized medium prawns were used for all the experiments reported in this chapter.

Four replicates, each of six prawns, were held in salinities of 0.5, 3.5, 7.0, 15, 22, 34 and 43‰ at 4, 12 and 20°C. The salinity and temperature acclimation protocol was the same as that described for the salinity tolerance experiments (Chapter 2; Section 2.3.). After a seven-day acclimation period at each salinity/temperature combination, a blood sample was obtained from individual prawns using the sampling technique described in Chapter 3 (Section 2.1.). Unfortunately, the volume of blood obtained from a single prawn was often not large enough for complete ionic analyses. To overcome this problem of insufficient volume, the blood obtained from individual prawns within each replicate was pooled until a volume greater than 0.1 ml was obtained. Generally, between four and six prawns were needed to provide sufficient blood for ionic analyses.

The pooled blood was transferred into a plastic Eppendorf tube (0.5ml volume) and centrifuged in a MSE Microcentaur centrifuge for 5 minutes at 1000g to remove blood cells and any coagulated protiens. The ionic analyses

were carried out on the haemolymph supernatant (cell-free fluid). 0.1ml of supernatant was pipetted into a plastic Eppendorf tube (1.5ml volume) and diluted with 0.9ml of distilled water. The haemolymph and distilled water were mixed thoroughly within the plastic tube using a Fisons Whirlmixer and then covered with a thin layer of liquid paraffin to prevent evaporation. The plastic tubes containing the diluted blood samples were then capped and stored overnight in a refrigerator at 3°C. At the end of the salinity acclimation period, a sample (1.5ml) of the external medium, at each salinity/temperature combination, was pipetted into a plastic eppendorf tube and held under identical storage conditions to the diluted blood samples.

The day following collection, the concentrations of sodium, potassium, calcium and magnesium ions in the haemolymph samples, and in the external media, were measured on a Varian-975 Atomic Absorption Spectrophotometer; the results were recorded on a Epson RX 8 F/T series printer. The haemolymph samples were variously diluted depending on which ion was being measured (for sodium, X 10000; for potassium, X 200; for calcium, X 200; for magnesium, X 600). Since some of these ions occurred in markedly different concentrations in the external medium compared with the haemolymph, different dilutions were used for the ionic determinations of the external medium (for sodium, X 10000; for potassium, X 100; for calcium, X 100; for magnesium, X 1000-5000). The concentrations of chloride ions in the blood and the external media were determined using a Corning 920

series Chloride Titrator. Chloride determinations were carried out on undiluted external medium samples and on diluted haemolymph samples (X 10).

Statistical analysis of the data was carried out using a two-way analysis of variance with replication package (ANOVA) held on the mainframe computor, Plymouth Polytechnic. The ionic concentrations recorded from the four pooled blood samples were used as replicates.

## 4.3. RESULTS

### 4.3.1. CHLORIDE REGULATION

The pattern of chloride regulation in the haemolymph of *Palaemon longirostris* is shown in Figure 22. In salinities lower than 22‰, haemolymph chloride was hyper-ionic to the external medium, while at higher salinities chloride was hypo-ionic to the external medium. Regulation of chloride ions was extremely efficient. For example at 12°C, blood chloride concentrations varied by less than 38mEq/l over an external chloride concentration range of 536mEq/l (0.5-34‰) (Table 19). At 43‰, however, regulation appeared to breakdown and haemolymph chloride concentrations tended towards isoionic values (Fig. 22).

Temperature and salinity had significant independent and interactive effects on blood chloride (Table 20, ANOVA, $P<0.001$). In general, blood chloride concentrations were higher at 4°C than at 12 and 20°C, and the blood isoionic point was raised from 18.5 to 22‰ (Fig. 22). The optimum temperature for chloride regulation appeared to be 12°C, since at this temperature the concentration difference was smaller than at 20 or 4°C (Table 19), indicating little variation of chloride ions between 0.5 and 34‰.

Figure 22. The concentration of chloride ions in the haemolymph of *Palaemon longirostris* exposed to a range of external salinities at 4 (triangles and irregular dashed line), 12 (circles and solid line) and 20°C (squares and regular dashed line) (vertical lines represent ±1 Standard Deviation).

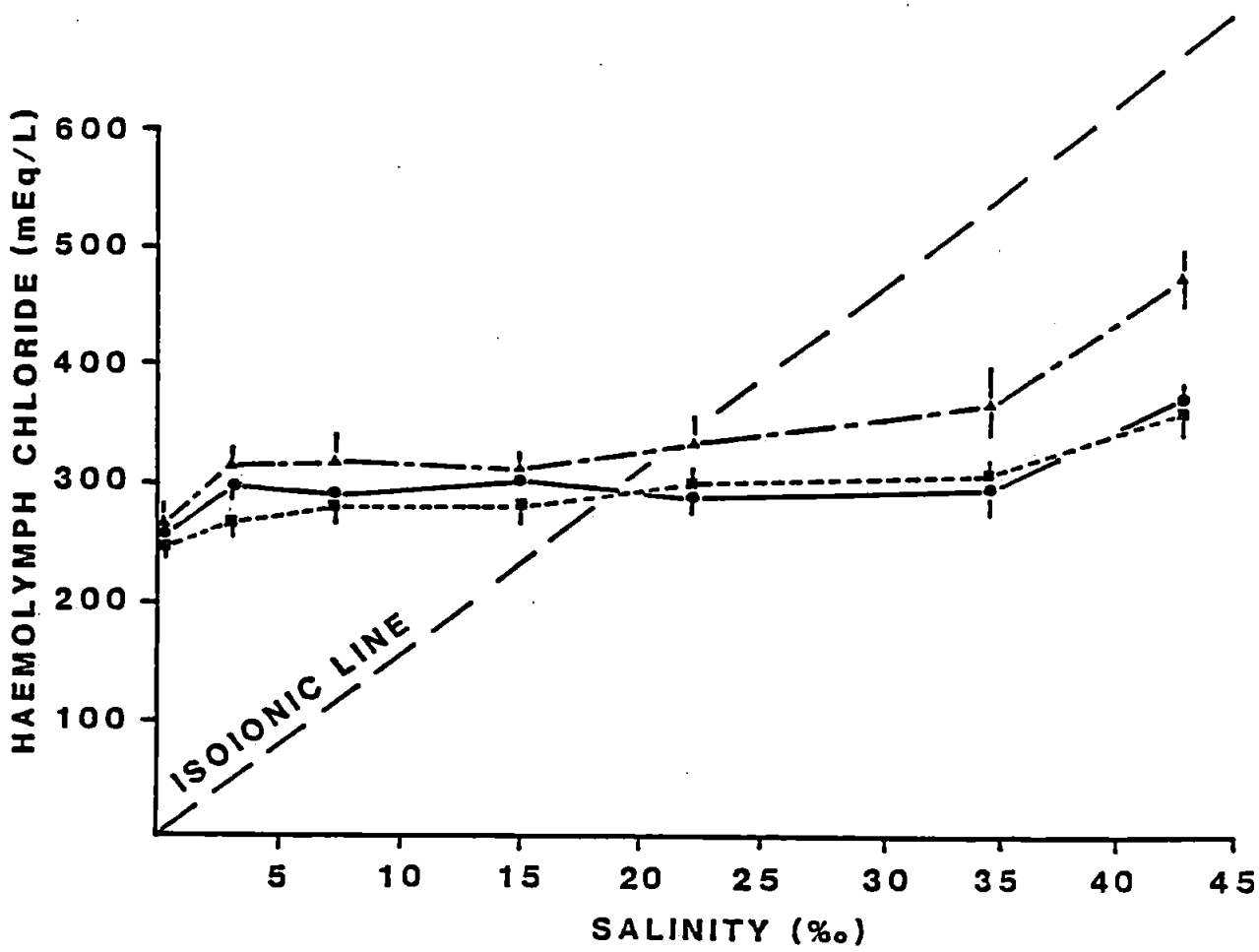

HAEMOLYMPH CHLORIDE (mEq/L)
600
500
400
300
200
100
ISOIONIC LINE
5
10
15
20
25
30
35
40
45
SALINITY (‰)

Table 19. Differences in mean blood ionic concentrations (mEq/L) of Palaemon longirostris in 0.5‰ and 34‰. Figures in parenthesis represent the haemolymph ionic difference of prawns in 0.5‰ and 34‰ expressed as a % of the external ionic range.

| Ion | External Medium | Temperature °C | | |
|---|---|---|---|---|
| | | 4 | 12 | 20 |
| Chloride | 536 | 108 (20) | 38 (7.1) | 53 (9.9) |
| Sodium | 445 | 45 (10) | 9.0 (2.0) | 34 (7.6) |
| Potassium | 11 | 0.9 (7.7) | 0.9 (8.0) | 1.9 (17) |
| Calcium | 36 | 5.2 (14) | 7.3 (20) | 12 (35) |
| Magnesium | 117 | 25 (21) | 17 (9.4) | 4.1 (3.5) |

Table 20. Two-way analysis of variance with replication (using 4 pooled blood samples as replicates) of the effect of salinity and temperature on the plasma ionic concentrations of Palaemon longirostris exposed to a range of salinities between 0.5‰ and 43‰ (d.f., degrees of freedom; S.L., significance level; n.s., not significant).

| Source of Variation | | d.f. | F-ratio | S.L. |
|---|---|---|---|---|
| Sodium | Salinity | 6,63 | 5.61 | 0.001 |
| | Temperature | 2,63 | 2.94 | n.s. |
| | S x T | 12,63 | 1.01 | n.s. |
| Chloride | Salinity | 6,63 | 57.50 | 0.001 |
| | Temperature | 2,63 | 45.30 | 0.001 |
| | S x T | 12,63 | 5.26 | 0.001 |
| Potassium | Salinity | 6,63 | 6.08 | 0.001 |
| | Temperature | 2,63 | 1.96 | n.s. |
| | S x T | 12,63 | 1.11 | n.s. |
| Magnesium | Salinity | 6,63 | 26.10 | 0.001 |
| | Temperature | 2,63 | 16.10 | 0.001 |
| | S x T | 12,63 | 9.10 | 0.001 |
| Calcium | Salinity | 6,63 | 7.52 | 0.001 |
| | Temperature | 2,63 | 7.78 | 0.001 |
| | S x T | 12,63 | 0.41 | n.s. |

### 4.3.2. SODIUM REGULATION

Not surprisingly, the general pattern of sodium regulation was very similar to that shown for blood chloride regulation. In salinities below 22‰, sodium was regulated hyper-ionically and in higher salinities it was regulated hypo-ionically (Fig. 23). Hyper-hypo-regulation of blood sodium was extremely efficient. For example at 12°C, blood sodium concentrations varied by only 9mEq/l over an external sodium concentration range of 445mEq/l (0.5-34‰ ) (Table 19). However, at 43‰ , sodium regulation appeared to breakdown, and blood sodium concentrations were significantly higher at this salinity compared with other salinities (Table 20, ANOVA, $P<0.001$).

The optimum temperature for sodium regulation appeared to be 12°C. At this temperature, the smallest sodium concentration difference was measured (Table 19); however, temperature did not have a statistically significant effect on sodium regulation (Table 20, ANOVA, $P>0.05$). In addition, the isoionic point for blood sodium regulation was unaffected by temperature (Fig. 23).

### 4.3.3. POTASSIUM REGULATION

Blood potassium was also regulated hyper-hypo-ionically (Fig. 24). In salinities below 22‰, the concentration of haemolymph potassium was hyper-ionic to the external medium

Figure 23. The concentration of sodium ions in the haemolymph of *Palaemon longirostris* exposed to a range of external salinities at 4 (triangles and irregular dashed line), 12 (circles and solid line) and 20°C (squares and regular dashed line) (vertical lines represent ±1 Standard Deviation).

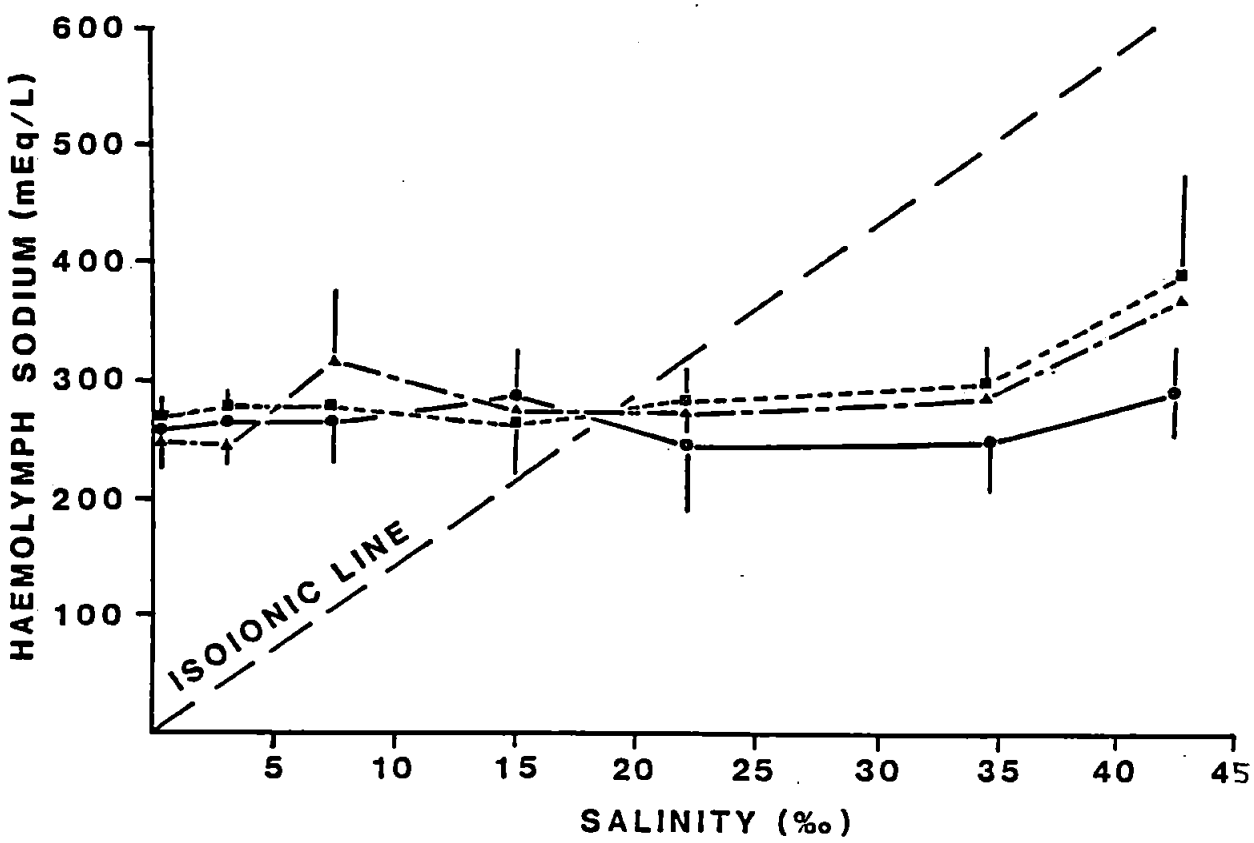
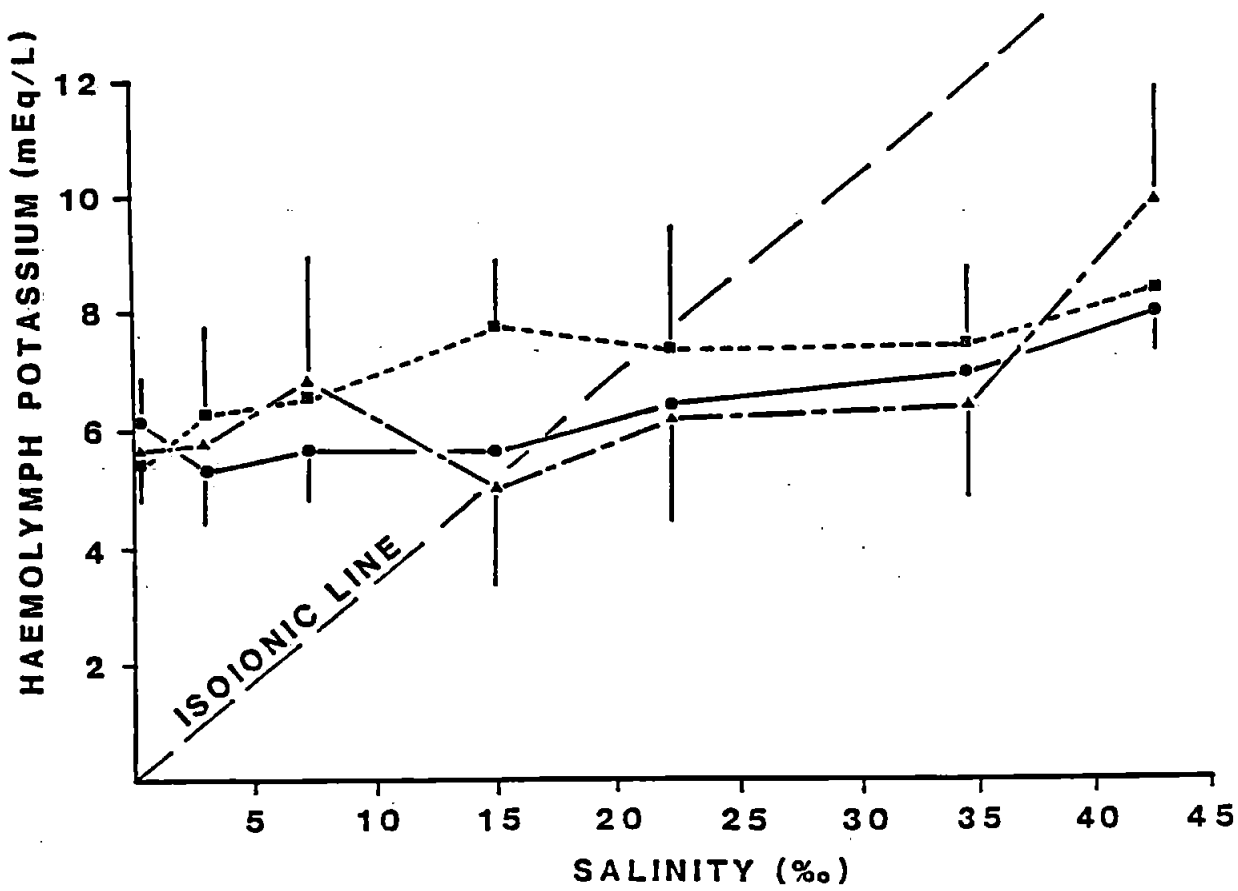

HAEMOLYMPH SODIUM (mEq/L)
600
500
400
300
200
100
ISOIONIC LINE
5
10
15
20
25
30
35
40
45
SALINITY (‰)

Figure 24. The concentration of potassium ions in the haemolymph of *Palaemon longirostris* exposed to a range of external salinities at 4 (triangles and irregular dashed line), 12 (circles and solid line) and 20°C (squares and regular dashed line) (vertical lines represent ±1 Standard Deviation).


HAEMOLYMPH POTASSIUM (mEq/L)
12
10
8
6
4
2
ISOIONIC LINE
5
10
15
20
25
30
35
40
45
SALINITY (‰)

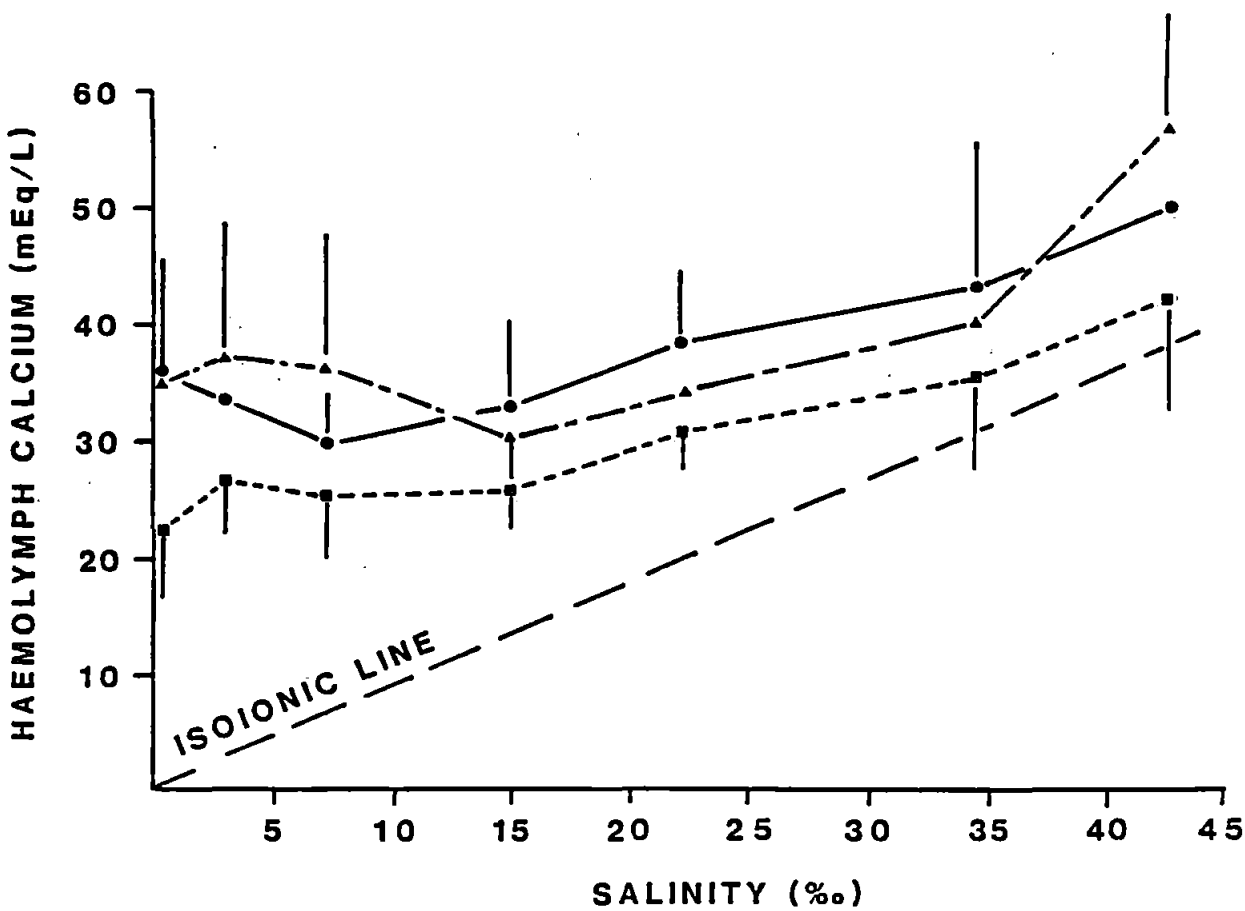
and in higher salinities potassium was hypo-ionic to the external medium. At each salinity, however, there were large standard deviations, indicating that there was considerable individual variation in the level of potassium ions in the blood of prawns. In general, potassium was efficiently regulated, and at 12°C, blood potassium varied by only 0.9mEq/l over an external potassium concentration range of 11mEq/l (0.5-34‰) (Table 19). At 43‰, blood potassium concentrations rose towards isoionic values, and this effect probably accounts for the significant influence of salinity on potassium regulation identified by ANOVA (Table 20).

Blood potassium was generally better regulated at 4 and 12°C than at 20°C, as indicated by the difference in ion concentrations between prawns held in 0.5 and 34‰ (Table 19). Generally, prawns acclimated to 20°C showed elevated blood potassium concentrations, and the isoionic point was raised from 15 to 22‰ at this temperature (Fig. 24). Statistical analysis, however, showed that these temperature effects were not significant (Table 20, ANOVA, $P>0.05$).

### 4.3.4. CALCIUM REGULATION

The pattern of haemolymph calcium regulation for *Palaemon longirostris* is shown in Figure 25. Calcium was consistently hyper-regulated over the entire salinity range tested. At 4 and 12°C, blood calcium concentrations varied by less than 8mEq/l over an external calcium concentration

Figure 25. The concentration of calcium ions in the haemolymph of *Palaemon longirostris* exposed to a range of external salinities at 4 (triangles and irregular dashed line), 12 (circles and solid line) and 20°C (squares and regular dashed line) (vertical lines represent ±1 Standard Deviation).


HAEMOLYMPH CALCIUM (mEq/L)
60
50
40
30
20
10
ISOIONIC LINE
5
10
15
20
25
30
35
40
45
SALINITY (‰)

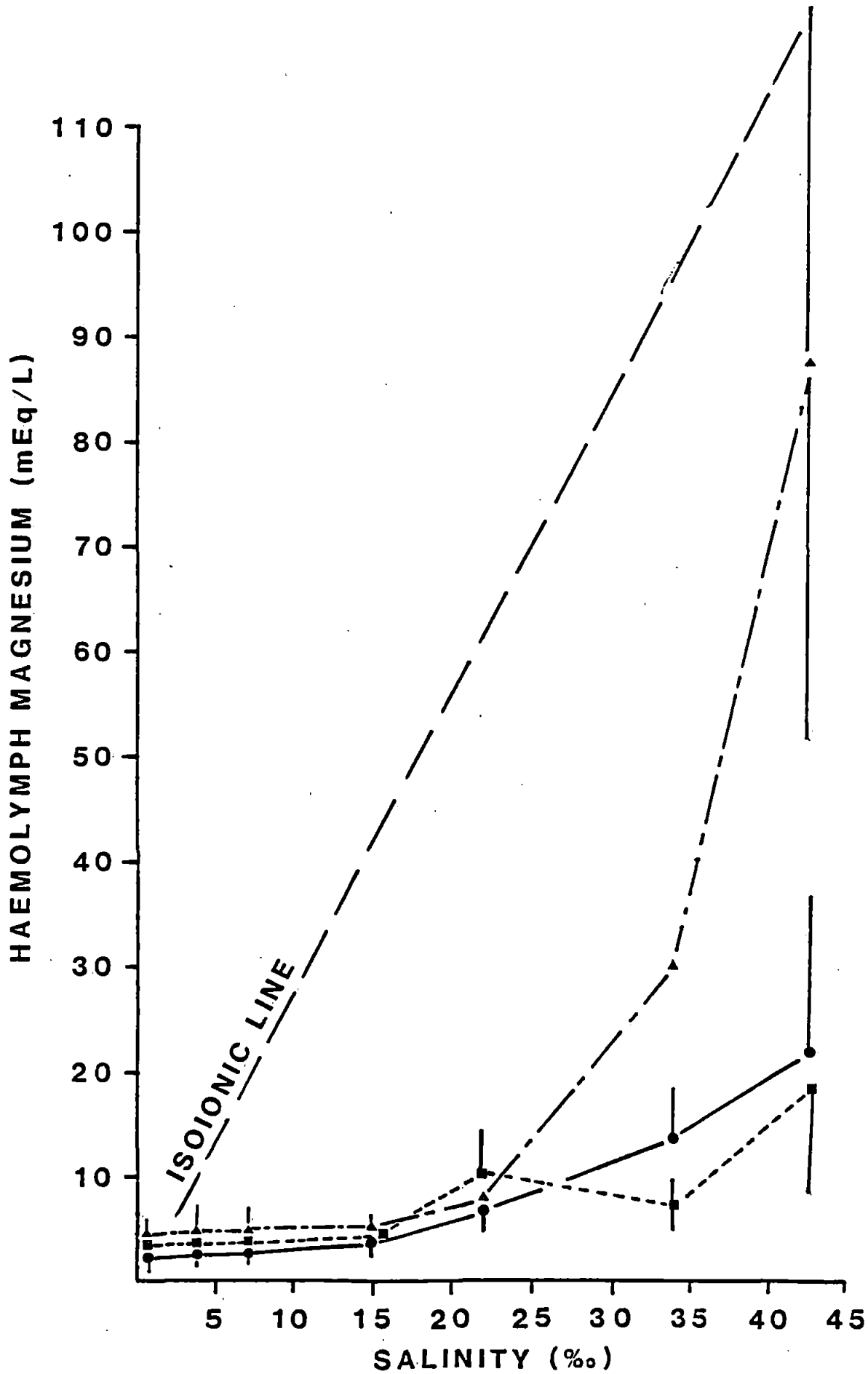
range of 36mEq/l (0.5-34‰) (Table 19). At low salinities (0.5-7.0‰), the calcium concentration gradient between the blood and the external medium was highest, indicating very efficient regulation (Fig. 25). Salinity had a significant effect on calcium regulation (Table 20, ANOVA, $P<0.001$) and, in general, as the external salinity increased there was an increase in the blood calcium concentration.

Temperature had a significant effect on calcium concentrations (Table 20, ANOVA, $P<0.001$). Calcium was more strongly hyper-regulated at 4 and 12°C than at 20°C over the entire salinity range (Fig. 25). This depression of blood calcium concentrations at 20°C was most obvious at both low and high salinities, where the calcium concentration gradient between the blood and the external medium was greatly reduced (Fig. 25).

### 4.3.5. MAGNESIUM REGULATION

The blood magnesium regulatory curves for *Palaemon longirostris* show that this ion was strongly hypo-regulated over the external salinity range 3.5-43‰ (Fig. 26). In salinities below 22‰, magnesium was regulated at a concentration which was generally lower than 10mEq/l. Prawns acclimated to salinities greater than 22‰ (and particularly 43‰), however, showed an apparent breakdown in blood magnesium regulation, as indicated by movement of the regulatory curve towards the isoionic line (Fig. 26).

Figure 26. The concentration of magnesium ions in the haemolymph of *Palaemon longirostris* exposed to a range of external salinities at 4 (triangles and irregular dashed line), 12 (circles and solid line) and 20°C (squares and regular dashed line) (vertical lines represent ±1 Standard Deviation).


HAEMOLYMPH MAGNESIUM (mEq/L)
110
100
90
80
70
60
50
40
30
20
10
ISOIONIC LINE
5
10
15
20
25
30
35
40
45
SALINITY (‰)

Temperature interacted significantly with salinity to influence magnesium regulation (Table 20, ANOVA, $P<0.001$). There was no obvious effect of temperature on blood magnesium regulation in prawns acclimated to salinities below 22‰. However, prawns acclimated to higher salinities showed more efficient hypo-regulation of blood magnesium concentrations at 12 and 20°C than at 4°C (Fig. 26). The data suggest that low temperature (4°C) greatly enhances the breakdown of blood magnesium regulation at 43‰.

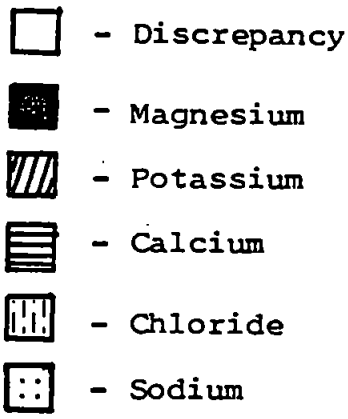
### 4.3.6. DISCREPANCIES BETWEEN TOTAL IONIC AND TOTAL OSMOTIC CONCENTRATIONS

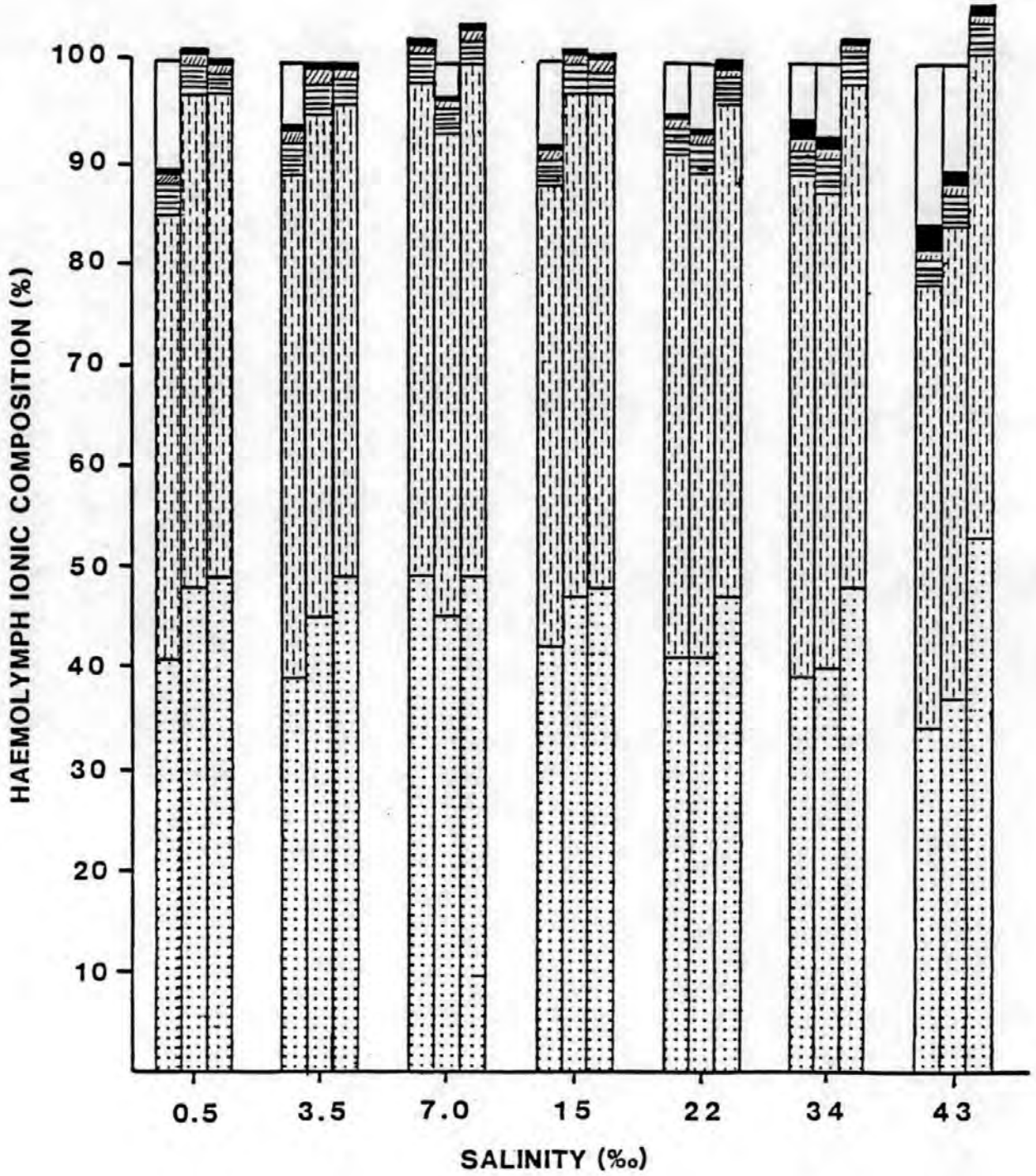
The haemolymph concentrations of sodium, chloride, potassium, calcium and magnesium ions have been expressed in terms of their proportional contribution to total blood osmolality in *Palaemon longirostris* (Table 21, Fig. 27). In prawns acclimated to salinities between 0.5 and 34‰, at each experimental temperature, inorganic ions generally accounted for greater than 90% of total blood osmolality (Table 21, Fig. 27). The discrepancy between total blood ionic concentration and total blood osmotic concentration at each salinity/temperature combination was generally less than 7% for prawns at 12 and 20°C, and less than 10% for prawns at 4°C. At 43‰, however, the largest discrepancies occurred, with a maximum difference of 16% being measured at 4°C (Fig. 27). At each salinity, discrepancies were also

Table 21. The concentration of sodium, chloride, potassium, calcium and magnesium ions in the haemolymph and their contribution to total haemolymph osmolality in Palaemon longirostris exposed to a range of salinities between 0.5‰ and 43‰ at 4, 12 and 20°C.

| Salinity of External Medium (‰) | Temp (°C) | Haemolymph Osmolality (mOsmol/kg) | Haemolymph Ionic Concentration (mMol/L) | | | | | Total Ions | Discrepancy | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Na | CL | K | Ca | Mg | mMol/L | mMol/L | % |
| 0.5 | 4 | 595 | 244 | 261 | 5.6 | 17.4 | 2.4 | 530 | 65 | 10.9 |
| | 12 | 539 | 261 | 264 | 6.2 | 18.0 | 1.4 | 551 | 12 | 2.0 |
| | 20 | 535 | 264 | 256 | 5.5 | 11.4 | 1.7 | 539 | 4 | 0.0 |
| 3.5 | 4 | 628 | 247 | 314 | 5.8 | 18.5 | 2.1 | 587 | 41 | 6.5 |
| | 12 | 593 | 267 | 297 | 5.3 | 16.7 | 2.5 | 588 | 5 | 0.7 |
| | 20 | 568 | 279 | 271 | 6.3 | 13.4 | 2.1 | 572 | 4 | 0.0 |
| 7.0 | 4 | 645 | 317 | 370 | 6.8 | 18.1 | 2.1 | 664 | 19 | 3.0 |
| | 12 | 598 | 265 | 289 | 5.7 | 14.8 | 1.8 | 579 | 19 | 3.3 |
| | 20 | 564 | 278 | 291 | 6.6 | 12.7 | 1.8 | 590 | 26 | 4.0 |
| 15 | 4 | 665 | 278 | 309 | 5.0 | 15.0 | 2.5 | 609 | 56 | 8.4 |
| | 12 | 600 | 282 | 304 | 5.7 | 16.5 | 2.4 | 611 | 11 | 3.0 |
| | 20 | 575 | 276 | 286 | 7.8 | 13.0 | 1.8 | 585 | 10 | 2.0 |
| 22 | 4 | 670 | 278 | 336 | 6.2 | 17.0 | 3.7 | 641 | 29 | 4.3 |
| | 12 | 610 | 252 | 297 | 6.4 | 19.3 | 3.4 | 578 | 32 | 5.2 |
| | 20 | 611 | 285 | 300 | 7.4 | 15.4 | 5.2 | 613 | 2 | 0.0 |
| 34 | 4 | 733 | 288 | 369 | 6.4 | 20.0 | 15.2 | 699 | 34 | 4.7 |
| | 12 | 630 | 252 | 301 | 7.0 | 21.6 | 6.9 | 589 | 41 | 6.6 |
| | 20 | 618 | 298 | 309 | 7.5 | 17.7 | 3.7 | 636 | 18 | 3.0 |
| 43 | 4 | 1075 | 367 | 473 | 9.9 | 28.4 | 43.6 | 922 | 153 | 14.0 |
| | 12 | 784 | 293 | 370 | 8.0 | 25.0 | 10.8 | 707 | 77 | 9.8 |
| | 20 | 746 | 392 | 363 | 8.4 | 21.0 | 9.3 | 794 | 48 | 6.0 |

Figure 27. The percentage contribution of haemolymph ions to total haemolymph osmolality in *Palaemon longirostris* exposed to a range of external salinities at 4 (left-hand column), 12 (middle column) and 20°C (right-hand column) (Osmotic values were taken from Chapter 3).




HAEMOLYMPH IONIC COMPOSITION (%)
100
90
80
70
60
50
40
30
20
10
0.5
3.5
7.0
15
22
34
43
SALINITY (‰)

generally larger for prawns at 4°C than at 12 or 20°C (Fig. 27).

Ionic and osmotic measurements at each salinity/temperature combination were not determined from the same prawn, thus, in some cases the ionic contribuion to total blood osmolality exceeded 100% (Fig. 27).

## 4.4. DISCUSSION

The present results for *Palaemon longirostris* show that this prawn has a very strong capacity for hyper-hypo-regulation of blood sodium and chloride concentrations over a wide range of external salinities. Indeed, the levels of sodium and chloride in the blood of *P. longirostris* were almost constant over an impressive salinity range (0.5-34‰). Hyper-hypo-regulation of blood sodium and chloride is not unusual and has been reported for other hyper-hypo-osmoregulating prawns; examples include *Crangon crangon* (Hagerman, 1971, 1973), *Penaeus duorarum* (Bursey & Lane, 1971), *Penaeus monodon* (for chloride) (Ferraris et al.,1987), *Penaeus merguiensis* and *Metapenaeus bennettae* (Dall & Smith, 1981). What sets *P. longirostris* apart from the other species cited, is that only *P. longirostris* maintains stable blood sodium and chloride concentrations over its entire salinity tolerance range. The only other prawns which hyper-hypo-regulate blood sodium and chloride as efficiently as *P. longirostris* and over comparable salinity ranges, are other species of estuarine and brackish-water palaemonids. For example, *Palaemon macrodactylus* (Born, 1968) has strong hyper-hypo-regulation of blood chloride over the salinity range 1-61.2‰, *Palaemonetes pugio* (Knowlton & Kirby, 1984) over the salinity range 1-40‰ , and *Macrobrachium equidens*

(Denne, 1968) and *Palaemonetes varians* (Potts & Parry, 1964a; Hagerman & Uglow, 1983) over salinity ranges of 5-35‰ and 0.5-35‰ respectively.

Hyper-hypo-regulation of blood sodium and chloride is considered to be an adaptation to environments characterized by fluctuating salinity conditions, since this type of regulation produces relatively stable ionic conditions within the animal, regardless of the external salinity (Potts & Parry, 1964b). It is very surprising, therefore, that this pattern of sodium and chloride regulation occurs in marine palaemonids, which occupy relatively stable salinity environments. Examples include *Palaemon serratus* (Parry, 1954) and *Palaemon elegans* (Ramirez de Isla Hernandez & Taylor, 1985). That these marine prawns actively maintain lower sodium and chloride concentrations in their haemolymph compared with sea water is particularly interesting and unusual. This apparent anomaly may hold evolutionary significance for the family Palaemonidae and is discussed in detail in Chapter 6.

In almost fresh water, *Palaemon longirostris* maintains large sodium and chloride concentration gradients between the haemoplymph and the external medium. The only other crustaceans that can maintain similar gradients for these ions in comparable salinities are species of freshwater prawns and the freshwater crayfish *Paranephrops zealandicus* (Wong & Freeman, 1975); prawns include *Syncaris pacifica* (Born, 1968), *Macrobrachium australiense* (Denne, 1968), and *Macrobrachium rosenbergii* and *Macrobrachium ohione* (Castille

& Lawrence, 1981c). In dilute saline environments, it is essential to strongly hyper-regulate these two major haemolymph ions. Such strong hyper-hypo-regulation of blood sodium and chloride accounts for the ability of *P. longirostris* to maintain its blood osmolality within narrow limits (100mOsmol/kg range) over a wide salinity range (1000mOsmol/kg osmotic range) (Chapter 3). Thus, *P. longirostris* is one of the relatively few euryhaline crustaceans which possesses a similar sodium and chloride regulatory ability to typical freshwater species. This feature means that *P. longirostris* possesses ionic regulatory adaptations which are well suited to life in the most dilute regions of estuaries.

Compared with the contribution of sodium and chloride to total blood osmolality, the concentration of potassium in the extra-cellular fluid of crustaceans is very small. This ion, however, is strongly regulated by most euryhaline crustaceans. The majority of crustaceans show strong hyper-regulation of blood potassium in salinities lower than 35‰ . Examples include the semi-terrestrial beach-flea *Orchestia gammarelus* (Spicer & Taylor, 1987), the inter-tidal crab *Carcinus maenas* (Zanders, 1980), the brackish-water amphipod *Corophium volutator* (McLusky, 1968), and the estuarine mysid *Praunus flexuosus* (McLusky, 1979). In hyper-saline salinities, most euryhaline crustaceans show weak hypo-regulation of blood potassium. Example include the inter-tidal crabs *Hemigrapsus nudus* and *Hemigrapsus oregonensis* (Dehnel & Carefoot, 1965), the estuarine crab

*Helice crassa* (Bedford, 1972) and the estuarine prawns *Crangon crangon* (Hagerman, 1973), *Penaeus plebejus* and *Penaeus merguiensis* (Dall & Smith, 1981). *Palaemon longirostris*, however, shows strong hypo-regulation of blood potassium in salinities above 22‰ . This regulation is better than previously reported for other hyper-hypo-regulating prawns such as the euryhaline penaeids *Penaeus duorarum* (Bursey & Lane, 1971), *Penaeus esculentus* and *Metapenaeus bennettae* (Dall & Smith, 1981). In these penaeids, the isoionic points for potassium were also higher (30-33‰ ) than that of *P. longirostris* (15-22‰). Apart from *P. longirostris*, only the marine prawns *Palaemon serratus* (Parry, 1954) and *Palaemon elegans* (Ramirez de Isla Hernandez & Taylor, 1985) strongly hypo-regulate blood potassium in salinities approaching full strength sea water. Thus, strong hypo-regulation of blood potassium in high salinities seems to be a unique trait of the palaemonids; however, the adaptive significance of this feature remains unresolved.

Generally, crustaceans accumulate calcium to offset the large proportion of body calcium lost during ecdysis. Aquatic crustaceans make up this loss by active reabsorption from the surrounding water (Robertson, 1960). Even poikilosmotic species such as *Panulirus longipes* accumulate calcium by this process (Dall, 1974). Although all aquatic crustaceans hyper-regulate blood calcium, the strength of regulation varies between species. The inter-tidal crab *Carcinus maenas* (Zanders, 1980) and the estuarine amphipod

*Corophium volutator* (McLusky, 1968) show weak hyper-regulation of blood calcium. More efficient hyper-regulation of blood calcium, however, is shown by other inter-tidal crabs such as *Hemigrapsus nudus* and *H. oregonensis* (Dehnel & Carefoot, 1965), and the estuarine prawn *Crangon crangon* (Hagerman, 1973). These latter species maintain larger calcium concentration gradients between their blood and the external medium than the previously mentioned group of crustaceans. At salinities greater than 35% ,however, the concentration of calcium in the blood of all crustaceans tends towards isoionic concentrations.

*Palaemon longirostris* showed extremely strong hyper-regulation of blood calcium, maintaining a very high, and almost constant calcium concentration, over its entire salinity tolerance range (0.5-43‰). In particular, a large calcium concentration gradient was maintained between the blood and the external medium at very low salinities (0.5‰ ). Similar patterns of blood calcium regulation were reported for other palaemonid prawns (*Palaemon serratus* [Parry, 1954] and *Palaemon elegans* [Ramirez de Isla Hernandez & Taylor, 1985]). The only other species which maintains such a constant level of calcium in its blood, over an equivalent salinity range, however, is *Metapenaeus bennettae* (Dall & Smith, 1981). Other penaeid prawns, such as *Penaeus esculentus*, *P. merguiensis* and *P. plebejus* (Dall & Smith, 1981), show only weak hyper-regulation of blood calcium over an equivalent salinity range.

As calcium affects cell membrane function (Robertson,

1960), which in turn may affect cell permeability and thus indirectly influences the osmoregulatory abilty of a species, it is not surprising that crustaceans with efficient osmoregulation show strong hyper-regulation of blood calcium. The strength of blood calcium regulation shown by *Palaemon longirostris*, especially in dilute salinity, is matched by few species and surpassed by none. Such high blood calcium concentrations, and such strong hyper-regulation of blood calcium may be one of the factors responsible for the efficient osmoregulation of *P. longirostris* , particularly in very dilute saline environments.

In crustaceans, magnesium inhibits neuromuscular transmission and, in excess, induces a state of narcosis (Boardman & Collier, 1946). The heart rate and general activity of crustaceans are inversely related to blood magnesium concentrations (Robertson, 1953; Walters & Uglow, 1981). Thus, species with high levels of blood magnesium (40-50mEq/l) such as *Maia squinado* and *Hyas* sp. are generally sluggish and may live in a semi-narcotised state (Robertson, 1953). Other species, which maintain low levels of blood magnesium such as the rock lobster *Panulirus longipes* (Dall, 1974) and the inter-tidal crabs *Hemigrapsus nudus* and *H. oregonensis* (Dehnel & Carefoot, 1965), are more active.

*Palaemon longirostris* maintained blood magnesium levels well below those of the external medium in a wide salinity range (1-34‰). Maintainance of such low magnesium levels is

not surprising, since *P. longirostris* is capable of sudden bursts of intense activity. Similar low blood magnesium levels over a wide range of salinities have been reported for other active prawns such as *Penaeus esculentus*, *P. plebejus*, *P. merguiensis*, *Metapenaeus bennattae* (Dall & Smith, 1981), *Crangon crangon* (Hagerman, 1973), *Palaemon serratus* (Parry, 1954) and *Palaemon elegans* (Ramirez de Isla Hernandez & Taylor, 1985). At 43‰ , blood magnesium regulation by *P. longirostris* appeared to breakdown; levels rose by over 400% and prawns became very sluggish. Such failure of magnesium regulation, rather than osmotic effects, appeared to be the cause of death at this high salinity. Breakdown of blood magnesium regulation at high salinity has been reported for *Panulirus longipes* (Dall, 1974), *Palaemon elegans* (Ramirez de Isla Hernandez & Taylor, 1985) and some *penaeid* species (Dall & Smith, 1981).

Interestingly, mud-dwelling or semi-terrestrial crustaceans such as *Orchestia gammarellus* (Spicer & Taylor, 1987), *Helice crassa* (Bedford, 1972) and *Corophium volutator* (McLusky, 1968) have much higher blood magnesium concentrations than estuarine or brackish-water species, especially in sea water. Recent studies have shown that magnesium affects functional properties of the respiratory pigment haemocyanin (Mangum, 1983). Thus, a high blood magnesium concentration would increase the affinity of haemocyanin for oxygen and could be advantageous for burrowing and mud-dwelling animals (Felder, 1978).

There is little information on the effect of

temperature on the ionic regulation of prawns, although some data are available for other crustaceans (see review by Mantel & Farmer, 1983). In the majority of crustaceans studied, there is an inverse relationship between temperature and blood ion concentration (Vernberg & Silverthorn, 1979), although the specific effect of temperature on decapod ionic regulation varies between specific ions and between different species. In *Palaemon longirostris*, blood sodium regulation was unaffected by temperature. Lack of sensitivity to temperature for the regulation of this ion has been reported previously for *Hemigrapsus nudus* (Dehnel & Carefoot, 1965) and *Palaemon elegans* (Ramirez de Isle Hernandez & Taylor, 1985). Such temperature independence, however, is not universal amongst crustaceans, and improved sodium regulation at low temperatures was reported for the crabs *Hemigrapsus oregonensis* (Dehnel & Carefoot, 1965) and *Callinectes sapidus* (Engel et al., 1974). It appears that the effect of temperature on sodium regulation in euryhaline crustaceans is varied and unpredictable. For *P. longirostris*, however, lack of a temperature effect on blood sodium regulation is rather surprising, as temperature affects the general osmoregulatory response of this species (Chapter 3). On the other hand, blood chloride levels were elevated at 4°C compared with 12 and 20°C over the salinity range 0.5-34‰ . These increased chloride levels appear to account for most of the rise in total blood osmolality observed for *P. longirostris* at low temperatures (Chapter 3). Elevated

blood chloride concentrations at low temperatures appear to be typical of euryhaline estuarine crustaceans, and examples are *Callinectes sapidus* (Engel et al., 1974) and *Palaemonetes varians* (Hagerman & Uglow, 1983). Inter-tidal or marine crustaceans, however, show different temperature effects on blood chloride regulation from that described for estuarine species. Thus, *Palaemon elegans* has elevated blood chloride concentrations at high temperatures (Ramirez de Isla Hernandez & Taylor, 1985), whereas *Palaemon serratus* and *Lymsata seticaudata* have no effect of temperature on blood chloride regulation (Spaargaren, 1972).

*Palaemon longirostris* has efficient regulation of haemolymph potassium at all three study temperatures, however, at 20°C potassium concentrations were increased compared with 4 and 12°C. Elevated potassium levels at high temperature have been reported for *Hemigrapsus nudus* and *Hemigrapsus oregonensis* (Dehnel & Carefoot, 1965). Some crustaceans, for example *Palaemon elegans* show elevated blood potassium levels at low temperatures (Ramirez de Isla Hernandez & Taylor, 1985). Others, such as the blue crab *Callinectes sapidus* show no effect of temperature on blood potassium regulation (Engel et al., 1974) . Based on the limited information available the effect of temperature on the regulation of blood potassium in euryhaline crustaceans is also variable and unpredictable.

Increase in temperature resulted in an impairment of blood calcium hyper-regulation for *P. longirostris*. At 20°C, blood calcium concentrations were depressed and this

response seems to be universal amongst crustaceans, having been reported in *Hemigrapsus nudus* and *H. oregonensis* (Dehnel & Carefoot, 1965), and *Palaemon elegans* (Ramirez de Isla Hernandez & Taylor, 1985). Low blood calcium levels at high temperatures correlate with increased moulting frequencies which occur at high temperatures.

Blood magnesium regulation in *Palaemon longirostris* was unaffected by temperature over the salinity range 0.5-22‰, however, at higher salinities low temperature caused an elevation of magnesium concentrations. This rise was most prominent at 43‰ and probably accounted for the prawn mortality measured at this salinity (Chapter 2). An identical temperature effect on magnesium regulation was reported for *Palaemon elgans* (Ramirez de Isla Hernandez & Taylor, 1985). Increase in blood magnesium concentrations at low temperature and high salinity (35‰) was reported also for *Callinectes sapidus* (Engel et al., 1974); additionally, a rise in blood magnesium concentration at very high temperatures (28°C) was also recorded for this species. Temperature had no effect on blood magnesium regulation of the crabs *Hemigrapsus nudus* and *H. oregonensis* (Dehnel & Carefoot, 1965).

The effects of temperature on the ionic regulation of decapod crustaceans are thus complex and subject to both inter-specific and intra-specific variation. Presently, there is no universal explanation for mechanisms that account for the effect of temperature on ionic regulation. For *Palaemon longirostris*, however, the optimum temperature

for ionic regulation was generally 12°C, and this was the experimental acclimation temperature used. Thus acclimation temperature probably influences the direction of temperature effects on ionic regulation in crustaceans.

Although inorganic ions account for the majority of osmotic activity in crustaceans, some species show small discrepancies between the total blood ionic concentration and the total blood osmotic concentration at certain salinities. For example, discrepancies were found at all salinities for the euryhaline prawn *Crangon crangon* (Hagerman, 1973). These discrepancies are thought to be due to the involvement of non-electrolytes, such as ninhydrin positive substances (NPS) and free amino acids (FAA), in osmoregulation (Weber & Van Marrewijk, 1972). Discrepancies were also observed for the euryhaline crab *Hemigrapsus nudus* (Dehnel, 1966) and the euryhaline amphipod *Corophium volutator* (McLusky, 1968). In these latter species, the size of the discrepancy increased with reduction of the salinity of the external medium. Sutcliffe (1961) suggested that at low salinities, the ionic concentrations in the haemolymph drop, and osmotically active substances such as FAA and NPS are liberated into the haemolymph from a protein resevoir. These movements compensate for the loss of ions and maintain the haemolymph osmolality. This type of intracellular regulation is of particular importance to osmoconforming and weakly osmoregulating species which cannot efficiently regulate haemolymph ionic concentrations at low salinities. Not surprisingly, the discrepancy between total osmotic and

total ionic concentrations for a strong osmotic and ionic regulator such as *Palaemon longirostris* was generally less than 10% at all salinities. Thus, although organic osmotic effectors such as FAA and NPS are present in the blood of *P. longirostris*, they do not appear to be important in the osmoregulation of this species.

# CHAPTER 5

# WATER PERMEABILITY

## 5.1. INTRODUCTION

Irrespective of habitat, aquatic crustaceans constantly exchange body inorganic ions and water with their environment, and maintain an ionic gradient between their body fluids and the external medium (Robertson, 1949; Gilles, 1975; Kirschner, 1979; Mantel & Farmer, 1983). The extent of the ionic (and resulting osmotic) gradient is, however, correlated with habitat and increases as less saline environments are occupied. Thus, mechanisms exist in non-marine crustaceans for the maintainance of hyper-osmotic body fluids, as all estuarine, brackish-water and, particularly, freshwater crustaceans maintain hyper-osmotic body fluids in dilute media (Greenaway, 1979; Spaargaren, 1979).

Due to passive water and ion fluxes which occur between the animal and its surounding medium when in an hypo-osmotic environment, there is a loss of salts which must be compensated for by active uptake, and a gain of water which must be expelled. One obvious adaptation to reduce the rate of water gain and salt loss is to lower integumental permeability (Lockwood, 1976). Clearly, it is impractical for aquatic crustaceans to maintain a completely impermeable body surface, since some permeable surfaces (eg, gills) are needed to exchange gases for respiration; a surface which is permeable to gases is generally permeable to water as well

(Lockwood et al., 1982). Any reduction in body surface permeability to water, without too great a reduction in respiratory efficiency, however, would confer a strong physiological advantage to species living in dilute saline habitats. Consequently, it is not surprising to find that the integumental water permeability of some estuarine, brackish-water and freshwater crustaceans is less than that of marine species, and reduction of integumental permeability is considered a major adaptation to life in dilute saline water (Potts & Parry, 1964b; Rudy, 1967; Bolt, 1983; Bolt, 1986).

Individual animals, living under conditions of fluctuating salinity, would also benefit from an ability to reduce integumental water permeability, particularly at times of large osmotic gradients between the body fluids and the external medium. It has been suggested that such flexibility of integumental permeability would require a mechanism sensitive to the concentration gradient between the blood and the external medium (Bolt, 1983). There is evidence to support this hypothesis as some euryhaline crustaceans alter their water permeability when the concentration gradient of the external medium changes. Examples include the crabs *Carcinus maenas* (Smith, 1970), *Rhithropanopeus harrisi* (Smith, 1967) and *Hemigrapsus nudus* (Smith & Rudy, 1972), and the amphipods *Gammarus duebeni* (Lockwood & Inman, 1973; Bolt et al., 1980; Bolt, 1983) and *Corophium volutator* (Taylor, 1985).

*Palaemon longirostris* is an extremely euryhaline

estuarine prawn (Chapter 2) which maintains an almost constant haemolymph osmotic and ionic concentration over a wide range of external salinities (Chapters 3 & 4). The present study investigates the water permeability of *P. longirostris* using tritiated water (THO), and compares the half time of exchange of body water with the environment ($T^1/_2$) for prawns acclimated to different salinities and prawns exposed to sudden salinity change. Since temperature affects the osmotic and ionic regulation of *P. longirostris* (Chapters 3 & 4), the influence of temperature on the water permeability of *P. longirostris* is reported also. In addition, to investigate any adaptive relationship which may exist between habitat and water permeability, the permeabilities of three other prawn species [*Palaemonetes varians* (Leach, 1814), *Crangon crangon* (Linnaeus, 1758) and *Palaemon elegans* (Rathke, 1837)] were studied and compared with *P. longirostris*. All four species ocur in the River Tamar Estuary, but have different horizontal distributions and different salinity tolerance ranges (Broekema, 1941; Hagerman & Uglow, 1983; Ramirez de Isla Hernandez & Taylor, 1985).

### 5.1.1. CRITICISMS OF TRITIATED WATER TECHINIQUE

In the permeability experiments reported here, tritiated water (THO) was used as a marker to measure water fluxes. However, do measured differences in water flux

represent real differences in hydraulic permeability ? The following are three possible interpretations of any observed changes in water fluxes when THO is used as a marker:

1) the differences measured could be due to artefacts of the experimental technique and do not represent real changes in cuticular permeability;

2) water flux variations could be caused by variations in the surface area over which water movement occurs due to circulatory variations (ie. changes in heart rate); and

3) variations in hydraulic permeability are real and are reflected in the $T^1/_2$ for THO exchange.

If the first possibility is the explanation for any permeability differences, the results can have no biological significance. If either the second or the third interpretation is the explanation for any permeability differences, then the results do have biological significance. Each of these possibilities is discussed in detail below.

(1) Experimental artefacts

Dainty & House (1966) showed that changes in permeability to water could be caused by variations in the thickness of unstirred layers at either side of a membrane, and that if passage of water occurred through narrow pores in a membrane then distortions in the theoretical diffusional exchange rates at either side of a membrane may occur. Thus, the ratio of osmotic permeability (*Pos*, determined by net water transfer) to diffusional

permeability (*Pdiff*, determined by THO fluxes) would differ depending on the efficiency of irrigation. For example, Pos/Pdiff ratios for poorly irrigated tissue (frog skin) are higher [27.2 for *Bufo regularis* and 9.3 for *Xenopus laevis* (Maetz, 1968)] than those measured for well irrigated tissues such as crustacean gills [1.0 for *Libinia emarginata* and 2.5 for *Carcinus maenas* (Cornell, 1979b)]. The lower Pos/Pdiff ratios, as illustrated by crustacean gills, can be accounted for by the adequate irrigation of the gills externally by respiratory currents and internally by blood circulation, thus diminishing unstirred layers. Thus the first possible explanation of observed changes in water permeability can be ignored.

(2) Circulatory variations

The water permeabilitiy changes observed for some species acclimated to different salinities may be attributed to a change in heart rate. Cornell (1979a) reported a drop in heart rate on transfer from 100% to 80% sea water for the crab *Libinia*; this heart rate reduction was correlated with a corresponding decrease in water permeability. However, in the more euryhaline amphipod *Gammarus duebeni*, changes in heart rate due to salinity were minimal and often showed an inverse relationship with apparent water permeability (ie, higher heart rate with lower apparent water permeability) (Bolt et al., 1980). Since heart rate can therefore affect the water permeability of a species, the effect of salinity and temperature on heart rate of *Palaemon longirostris* was

monitored in the present study.

(3) Hydraulic permeability changes

In order to confirm the validity of using the THO method to determine water permeability, Bolt (1985) compared THO flux measurements with calculated fluxes obtained from urine flow rates measured using radio-active labelled Chromium $^{51}$ Ethylenediaminetetra-acetic acid (Cr E.D.T.A.) as a marker for the euryhaline amphipod *Gammarus duebeni*. Measurements could only be taken when the animal was urinating (ie, when the animal was hyper-osmotic to the external medium). Bolt (op. cit.) assumed that the urine flow was equal to the net flow of water into the animal; thus the theoretical $T^1/_2$ could be calculated. The results showed that the measurement of $T^1/_2$ using THO was comparable to the calculated $T^1/_2$ using urine measurements.

In summary, it would appear from published work that changes in water permeability, using THO as a marker, do reflect real changes in the water permeability of an animal. However, when referring to water permeability measurements calculated from THO fluxes, the term "apparent water permeability" will be used following Smith (1967), as the method is an indirect measure of real permeability changes.

## 5.2. MATERIALS AND METHODS

*Palaemon longirostris* were collected and maintained as described in detail in Chapter 2 (Sections 2.1. & 2.2.). *Crangon crangon* and *Palaemonetes varians* were hand-netted from Cargreen and St Johns lake, River Tamar Estuary, Plymouth. *Palaemon elegans* was hand netted at times of low tide from inter-tidal rockpools at Wembury, near Plymouth.

### 5.2.1. SALINITY AND TEMPERATURE ACCLIMATION

For each prawn species, ten individuals (carapace length, 18-24mm = medium size grouping of *Palaemon longirostris*) were acclimated to a range of experimental salinities for 7 days at 12°C $\pm$1°C (temperature was controled with the use of constant temperature water baths). During this period, prawns were held in plastic aquaria (50l) supplied with continuously aerated, recycled sea water at the appropriate salinity. They were subjected to a 12h light : 12h dark cycle, and were not fed three days prior to or during the experiments. Only intermoult individuals were used, and these were acclimated to the appropriate experimental temperature (4, 12 & 20°C) for 24h prior to experimentation.

## 5.2.2. WATER PERMEABILITY IN *PALAEMON LONGIROSTRIS*

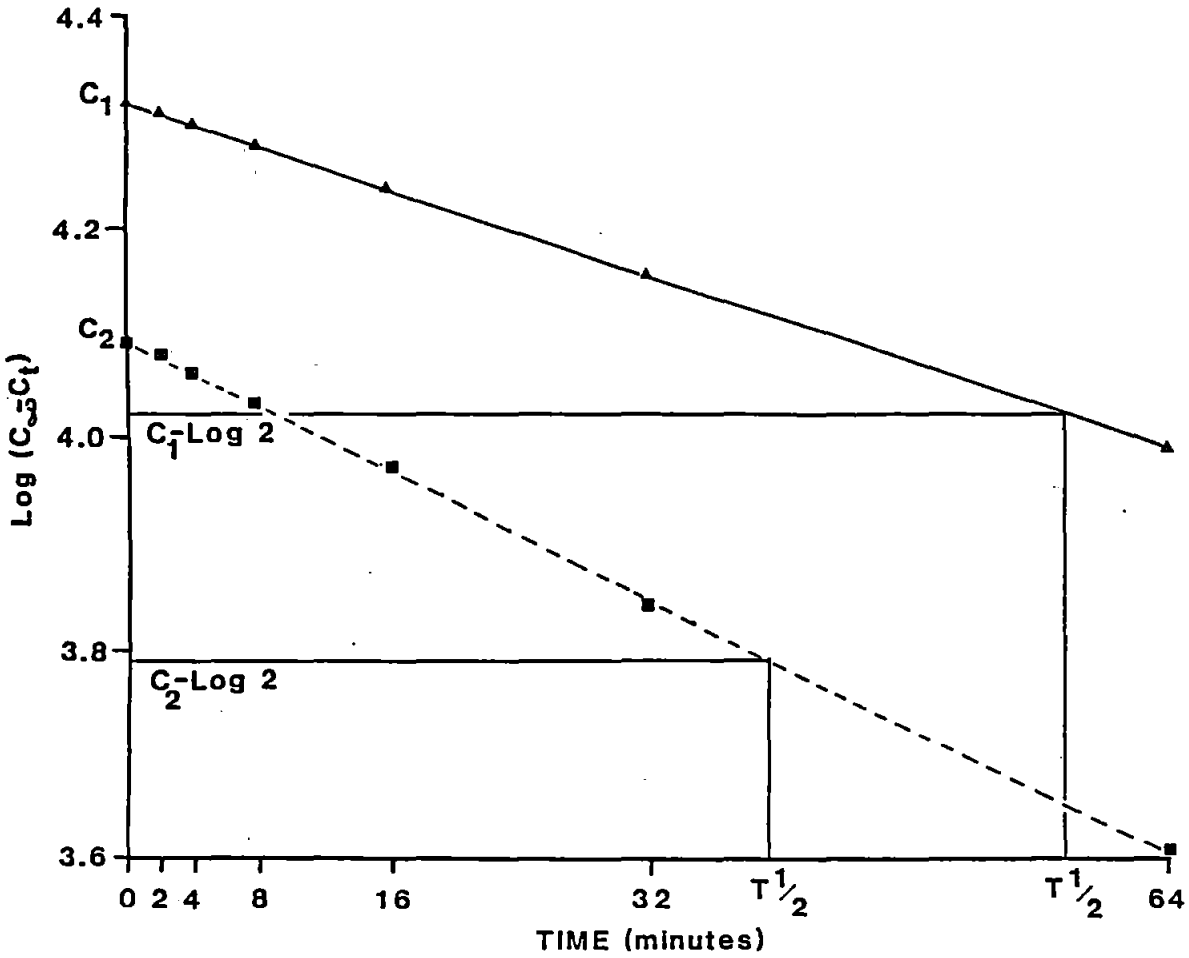
### 5.2.2.1. STEADY STATE SALINITY EXPERIMENTS

Water permeability was measured at 0.5, 7.0, 22.0, 34.0 and 43.0‰ at 12°C, and at 0.5 and 34‰ at 4 and 20°C. Permeability was determined by measuring the time taken for half the body water to exchange with the external medium ($T^1/_2$), using tritiated water (THO) as a tracer. The method used was a modification of the outflux technique described originally by Lockwood et al. (1973).

Prawns were transferred from the acclimation aquaria into glass screw top jars containing 250ml of a tritiated water (THO) solution (50μCi/ml) at the same salinity and temperature as the acclimation medium, and left to load for a minimum period of 5h. They were then removed and thoroughly rinsed with 600ml of water of the same salinity and temperature as the loading medium. This was done to remove any superficial tracer. Prawns were subsequently blotted dry with absorbent tissue and transferred to a blank unloading medium containing 250ml of water at the same salinity and temperature as the loading medium. This unloading medium was contained within screw top jars to prevent any exchange of THO with water vapour in the air. The time of immersion in the unloading medium was noted and 100μl aliquots of this medium were taken at 2, 4, 8, 16, 32

and 64 minutes after immersion (*Ct*). The aliquots were placed into 4ml of a liquid scintilation cocktail (Cocktail 'Ex' Scintran, BDH) and counted using a liquid scintilation counter (Philips, model PW 4700). Tests by Lockwood et al. (1973) showed that quenching of the counts due to differences in the salinity of the external medium were negligable. The last aliquot was taken when the THO in the animal was in equilibrium with the external medium (>10 $T^1/_2$). This last reading was taken as being the $C_\infty$ reading. The Log($C_\infty$ -Ct) was plotted against time and, if the permeability was consistent throughout the experiment, the result was a straight line. A regression line ($y=mx+C$, where $x$ and $y$ are the axis, $m$ is the gradient, and $C$ is the $y$ intercept) was fitted to the data points. Using this equation, the time taken for half the body water to be exchanged with the external medium ($T^1/_2$) was calculated by taking the $x$ value of the $y$ point corresponding to $C$-Log.2. A complete determination of $T^1/_2$ using this technique is shown in Figure 28. It was found that the correlation coefficient of the previously described straight line was generally greater than 0.98. This correlation was sufficiently good, that only 2 *Ct* values needed to be taken at a time close to that of the $T^1/_2$, to fit a regression line to the data. However, this short cut could only be taken when the approximate $T^1/_2$ was known (eg. replication for prawns at same salinity/temperature combination). When this method was used the $T^1/_2$ could be calculated as follows:

Figure 28. A complete determination of $T^1/_2$ by outflux of tritiated water for individual *Palaemon longirostris* acclimated to 0.5‰ (triangles and solid line) and 34‰ (squares and dashed line) at 12°C.


4.4
C1
4.2
C2
4.0
C1-Log 2
3.8
C2-Log 2
3.6
Log (C∞-Ct)
0 2 4 8 16 32 T1/2 T1/2 64
TIME (minutes)

$$K = 1/t \times \ln[C_{\infty} / (C_{\infty} - Ct)]$$

and

$$T^{1}/_{2} = \ln 2/K$$

Where $K$ is the rate constant (% body water/hr), $Ct$ is the count at time $t$ and $C_{\infty}$ is the count at equilibrium.

### 5.2.2.2. SUDDEN SALINITY CHANGE EXPERIMENTS

Changes in permeability to water for 10 prawns acclimated to 34‰ and 0.5‰ for seven days and transferred directly to 0.5‰ and 34‰ respectively were measured at 12°C. Water permeability was determined by measuring the $T^{1}/_{2}$ for THO outflux using the same method as described in the previous section, however, prawns that were loaded in 34‰ were unloaded in 0.5‰ and vice-versa.

### 5.2.3. WATER PERMEABILITY IN SELECTED CARIDEAN PRAWNS

Water permeability was measured for the prawns *Palaemonetes varians* at 0.5 and 34‰, *Crangon crangon* at 7, 22 and 34‰ and *Palaemon elegans* at 22 and 34‰. Experiments were conducted at 12°C using 10 prawns of a similar size to

*Palaemon longirostris* (cararpace length, 18-24mm). Water permeability for these prawns was determined by calculating the $T^1/_2$ for THO outflux as described earlier.

### 5.2.4. HEART RATE IN *PALAEMON LONGIROSTRIS*

The heart rates of 5 medium prawns were measured in salinities of 0.5 and 34‰ at 4, 12 and 20°C. Salinity and temperature acclimation procedures were identical to those already described in this chapter (Section 2.1.).

#### 5.2.4.1. IMPEDANCE TECHNIQUE

Heart rate was recorded using the impedance technique described by Johnson (1985). Two "Classic H7 Impedance Pneumograph" transducers (Scientific Instruments Centre) were coupled to a 2 channel George Washington Ltd Oscillograph. A diagrammatical representation of the experimental arrangement is shown in Figure 29. Heart rate was measured by implanting a small pin (recording) electrode close to the pericardium. One large common earth electrode was placed in the same tank as the prawns, and both electrodes were connected to a single transducer (Fig. 29). The pneumograph produced a small oscillating current between the two electrodes, and any movements occurring close to the pin electrode (eg. heart beat) were detected, filtered and

Figure 29. Diagram of the system for monitoring the activity of the heart in individual *Palaemon longirosris*. The pin electrode (P.E.) is attached near the heart. The large metal electrode (E) is used as a common earth electrode for the impedance pneumograph (I.P.), the output from which is passed through an amplifier (A) before being recorded. Detail shows the site of electrode insertion

P.- Pericardium,

A.M.- Arthrodial membrane,

1.A.- First abdominal segment,

C.W.- Coated wire,

B.W.- Bared wire,

W.G.- Wax and glue mixture.

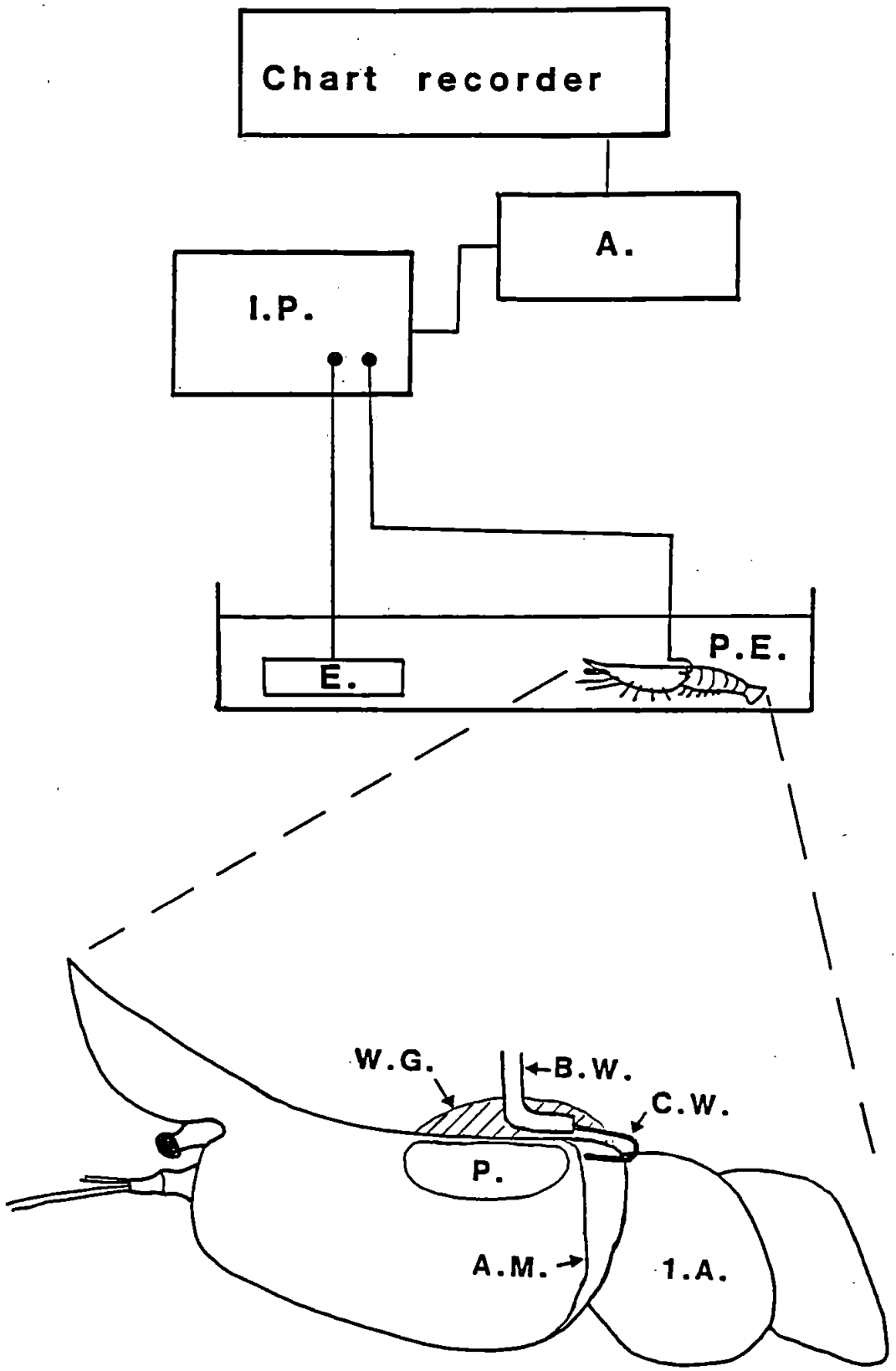


Chart recorder
A.
I.P.
E.
P.E.
W.G.
B.W.
C.W.
P.
A.M.
1.A.

amplified. The output of each transducer was recorded on a chart recorder (George Washington Ltd), so that a quantitative analysis of the heart rate could be carried out.

5.2.4.2. ELECTRODE ATTACHMENT

Each animal was removed from the water and the carapace dried thoroughly with absorbant tissue. The recording electrode (made from 0.25mm plastic coated miniature solid wire, which had the last 4mm bared and bent through 180°) was inserted, without puncturing the arthrodial membrane, between the carapace and the 1st abdominal segment, and placed close to the pericardium (Fig. 29). The wire was then bent back and anchored on to the carapace using a combination of super glue and a low melting point wax. Animals were returned to the aquaria as soon as the wax had dried. This techinique is a modification of the techinique originally described by Dyer & Uglow (1977).

5.2.4.3. HEART RATE RECORDING

Five prawns with electrodes attached were held at the appropriate salinity in covered plastic, aerated aquaria (volume = 2.5l). Prawns were separated by plastic dividers. Aquaria were covered to prevent any shadow stimilus on heart rate. After electrode attachment, the prawns were left to

recover for 24h before readings were taken, thereby enabling the heart rate to return to a non stressed level (Cumbridge & Uglow, 1977). A recording of heart rate from each prawn at each salinity/temperture combination was taken for approximately 2 minutes.

### 5.2.5. STATISTICAL ANALYSIS

Data were analysed using either one-way analysis of variance (statistical package held on the Plymouth Polytechnic 'Prime A' mainframe computor) or a Student's *t*-test held (BBC microcomputor software package).

## 5.3. RESULTS

### 5.3.1. APPARENT WATER PERMEABILITY IN *PALAEMON LONGIROSTRIS*

#### 5.3.1.1. STEADY STATE SALINITY

The apparent water permeabilities (AWP) of *Palaemon longirostris* acclimated to a range of salinities between 0.5 and 43‰ at 12°C are shown in Figure 30. Apparent water permeability varied from a $T^1/_2$ of exchange of 58.7 min for prawns at 0.5‰ to a $T^1/_2$ of exchange of 37.7 min for prawns at 43‰. Analyses of these data showed that the APW of *P. longirostris* was significantly reduced as the external salinity was lowered (Table 22, ANOVA, $\underline{P}<$ 0.001).

#### 5.3.1.2. SUDDEN SALINITY CHANGE

The APWs of *Palaemon longirostris* before and immediately after sudden salinity change at 12°C are shown in Figure 31. Prawns acclimated for seven days to 0.5‰ and transferred to 34‰ showed a rise in APW, as indicated by a reduction in the $T^1/_2$ of exchange from 58.7 min to 47.0 min after salinity transfer. This difference in $T^1/_2$ times was

Figure 30. Apparent permeability to water ($T^1/_2$) in *Palaemon longirostris* exposed to a range of salinities at 12°C (vertical line represent ±1 Standard Deviation).

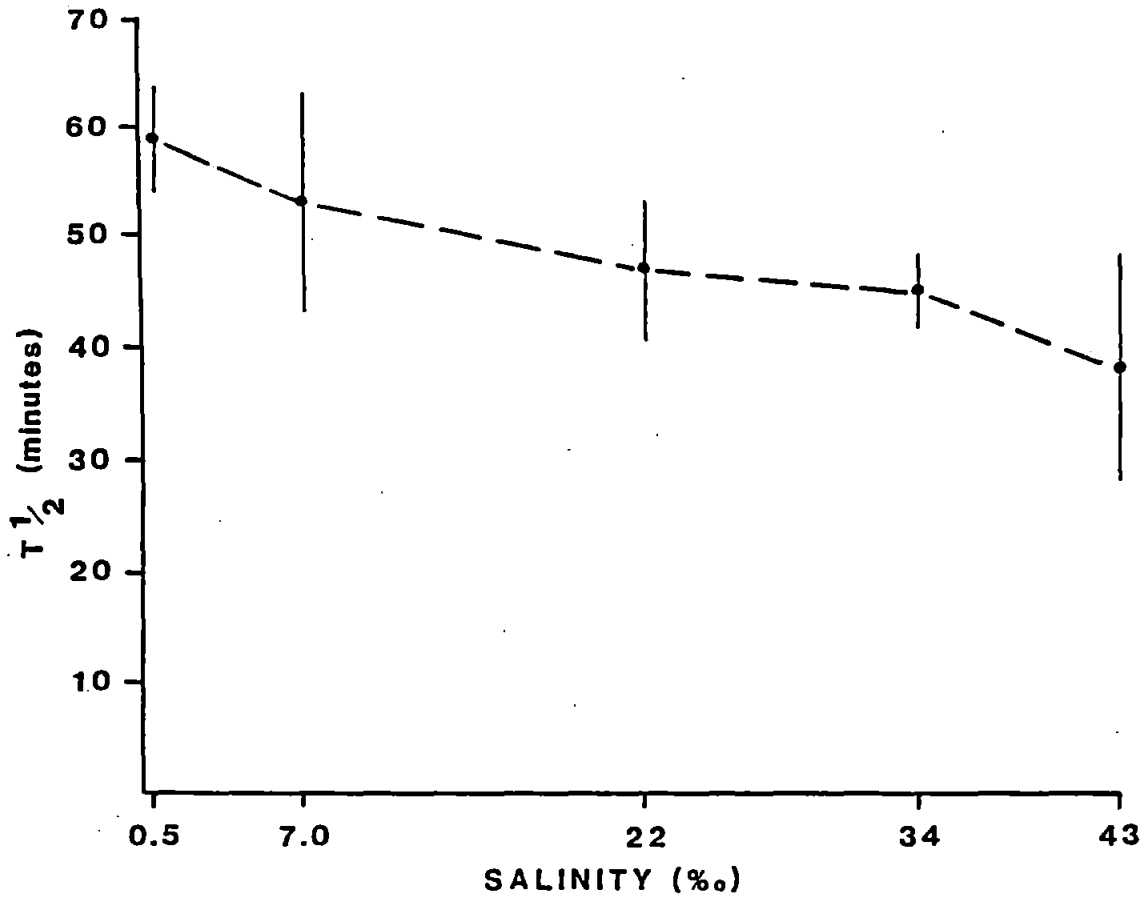
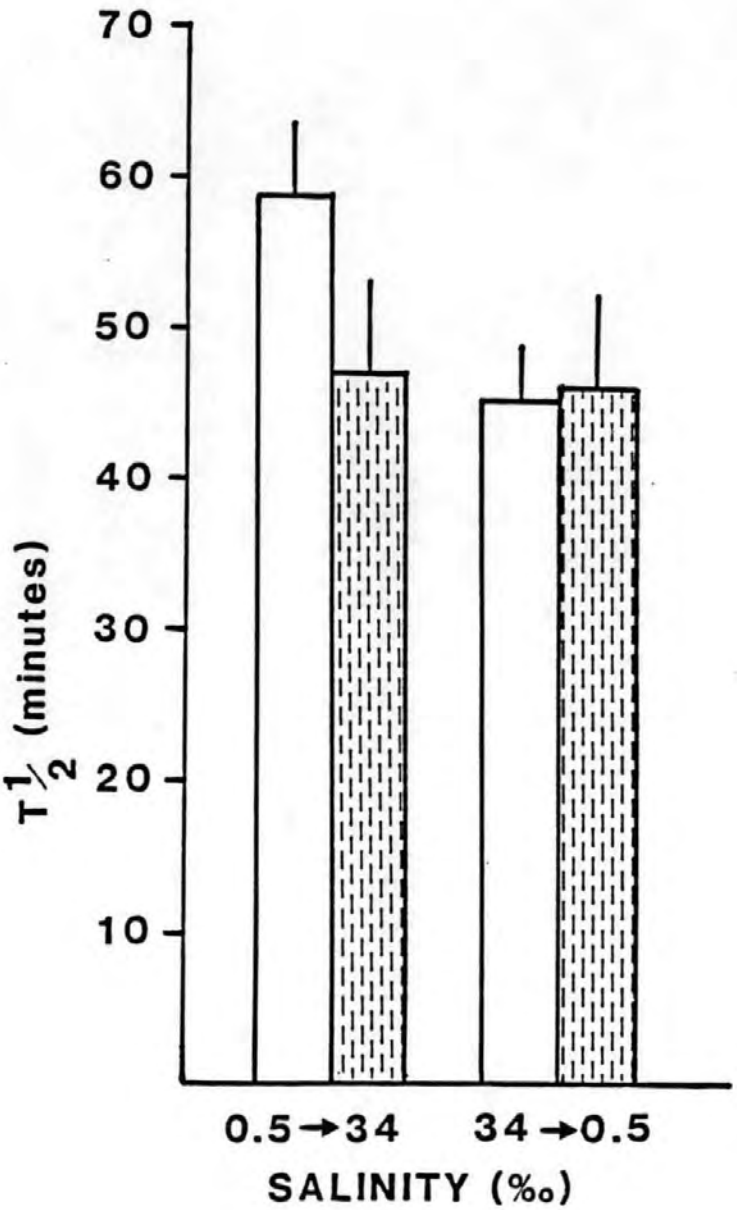

70
60
50
40
30
20
10
T 1/2 (minutes)
0.5
7.0
22
34
43
SALINITY (‰)

Table 22. One-way analysis of variance on the effect of salinity on the apparent water permeability ($T^1/_2$) of Palaemon longirostris exposed to a range of salinities between 0.5 and 43‰ at 12°C, and the effect of temperature on apparent water permeability ($T^1/_2$) of Palaemon longirostris exposed to 0.5 and 34‰ at 4, 12 and 20°C (d.f., degrees of freedom; S.L., significance level; n.s., not significant).

| Source of variation | Temperature (°C) | Salinity (‰) | d.f. | F-ratio | S.L. |
|---|---|---|---|---|---|
| Salinity | 12 | – | 4,45 | 13.5 | 0.001 |
| Temperature | – | 0.5 | 2,27 | 49.5 | 0.001 |
| Temperature | – | 34 | 2,27 | 51.7 | 0.001 |

Figure 31. Apparent permeability to water ($T^1/_2$) in *Palaemon longirostris* before and immediately after salinity change from 0.5 to 34‰, and from 34 to 0.5‰ at 12°C. Shaded area represents prawns tested immediately after salinity change and white area represents prawns tested after seven days salinity acclimation (vertical lines represent ±1 Standard Deviation).


70
60
50
40
30
20
10
$T^{1}/_{2}$ (minutes)
0.5→34
34→0.5
SALINITY (‰)

significantly different (t= 4.86, d.f. 18, P< 0.001). The APW remained unchanged for prawns acclimated to 34‰ and transferred to 0.5‰ (Fig. 31), and $T^1/_2$ times were not significantly different (t= 0.28, d.f. 18, P> 0.05). The $T^1/_2$ of exchange for prawns transferred directly to 0.5‰ from 34‰ was also significantly lower than the $T^1/_2$ of exchange for prawns acclimated for seven days to 0.5‰ (t= 5.10, d.f. 18, P> 0.001). From these data, it would appear that acclimation plays an important role in the reduction of apparent permeability to water of *P. longirostris* at low salinities. On the other hand, the $T^1/_2$ of exchange for prawns transferred directly to 34‰ from 0.5‰ , was not significantly different from the $T^1/_2$ of exchange for prawns acclimated for seven days at 34‰ (t= 0.81, d.f. 18, P> 0.05). Thus acclimation appears not to affect the APW in *P. longirostris* at high salinities.

### 5.3.1.3. TEMPERATURE EFFECTS ON APPARENT PERMEABILITY TO WATER

APWs for *Palaemon longirostris* acclimated to 0.5‰ and 34‰ at 4,12 and 20°C are shown in Figure 32. At each temperature the APW was significantly lower for prawns acclimated to 0.5‰ than for prawns acclimated to 34‰ (Table 23, student t-test, P<0.01). In addition, at each salinity, APWs were lower at 4°C compared with 20°C. Hence, the $T^1/_2$ of exchange rose from 41.8 min at 20°C to 82.8 min

Figure 32. The effect of temperature on the apparent permeability to water ($T^1/_2$) of *Palaemon longirostris* acclimated to 0.5‰ (triangles and solid lines) and 34‰ (squares and dashed line) (vertical lines represent ±1 Standard Deviation).

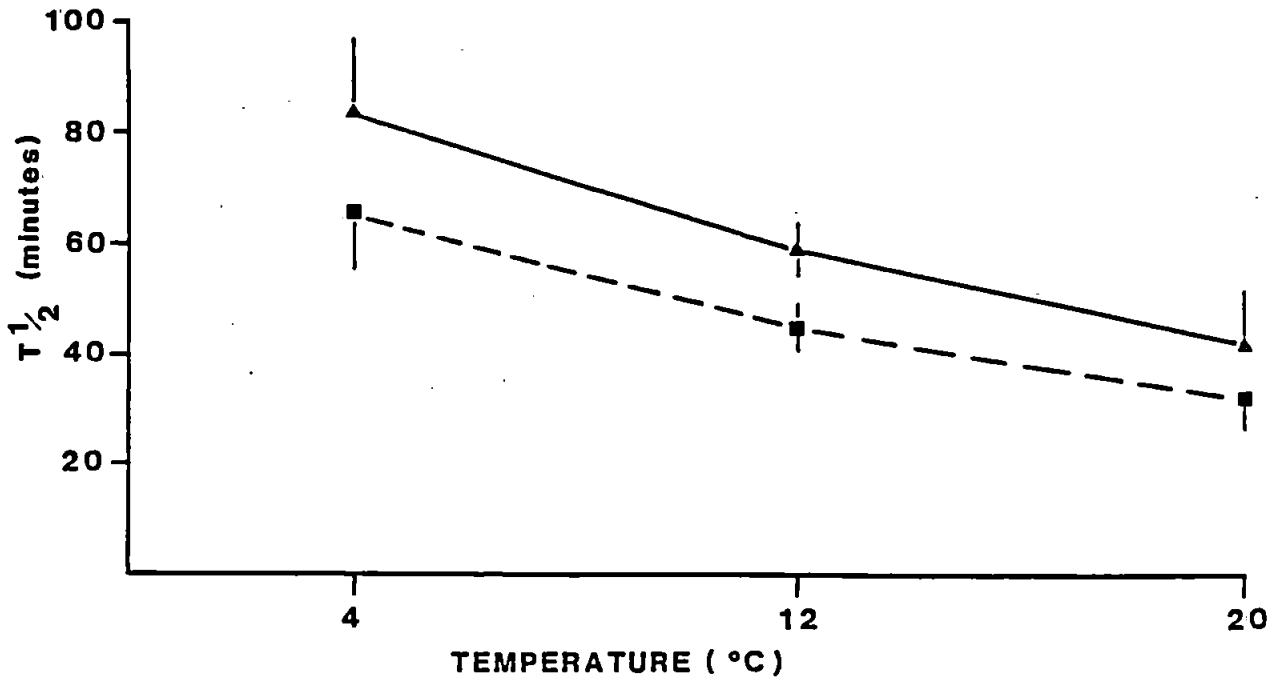

100
80
60
40
20
$T^{1}/_{2}$ (minutes)
4
12
20
TEMPERATURE ( °C )

Table 23. Differences in the apparent water permeabilities ($T_{\frac{1}{2}}$) of *Palaemon longirostris* acclimated to 0.5‰ and 34‰ at 4, 12 and 20°C (*d.f.*, degrees of freedom; *S.L.*, significance level; *n.s.*, not significant).

| Source of variation | Temperature (°C) | *t*-value | *d.f.* | *S.L.* |
|---|---|---|---|---|
| Salinity (0.5 and 34‰) | 4 | 3.30 | 18 | 0.01 |
| " | 12 | 7.14 | 18 | 0.001 |
| " | 20 | 3.17 | 18 | 0.01 |

at 4°C for prawns acclimated to 0.5‰, and from 32.1 min at 20°C to 64.6 min at 4°C for prawns acclimated to 34‰ (Fig. 32). Thus a decrease in temperature resulted in a significant drop in APW of *P. longirostris* acclimated to both 0.5‰ and 34‰ (Table 22, ANOVA, $\underline{P}< 0.001$). The relative magnitude of the temperature related changes in APW could be demonstrated by calculating the $Q_{10}$ values (magnitude of decrease in the $T^1/_2$ of exchange for a 10°C rise in temperature) at each salinity. The $Q_{10}$ values were almost identical at 0.5‰ (1.54) and 34‰ (1.56) and showed a rise in the $T^1/_2$ of exchange in, the order of 1.5 for a drop in temperature from 20 to 10°C (Fig. 32).

### 5.3.2. NET OSMOTIC FLOW IN WHOLE ORGANISMS

Knowing the $T^1/_2$ of exchange, the osmotic concentration of the haemolymph and the osmotic concentration of the external medium, the net osmotic fluxes in an animal can be calculated. Net water movements into or out of prawns (expressed as percentage of body water exchanged per minute) were calculated from the following equations:

$$F = 100 \times (ln2/T^1/_2)$$

Where $F$ is the water flux, and $ln$ is the natural logarithm. Net osmotic water flow ($Os$) was determined from the water flux data and was based on the difference in the mole

fraction of water in the medium and haemolymph of the animal:

$$Os = (Mm-Ma/Mm)xF$$

Where *Mm* is mole fraction of water in the external medium, and *Ma* is the mole fraction of water in the haemolymph. Mole fraction of the blood and the external medium = 55.56/(55.56+*L*), where *L* is the osmolal concentration of the medium or haemolymph between 0-1.

The calculated net fluxes for *Palaemon longirostris* over the salinity range 0.5-43‰ at 12°C are shown in Table 24. When the prawns were hyper-osmotic to the external medium (0.5 & 7‰), the net flux was into the animal, and when the animal was hypo-osmotic to the external medium (34 & 43‰) the net flux was out of the animal. In salinities of 0.5, 7 and 34‰, the osmotic gradient between the haemolymph of *P. longirostris* and the external medium varied from 492 mOsmol/kg at 0.5‰ to 395 mOsmol/kg at 34‰. The net water fluxes at these three salinities were, however, relatively constant at around 15% body weight/day (Table 24). At 22‰, *P. longirostris* was isosmotic with the external medium, thus the net water flux was almost zero (Table 24). At 43‰, although the osmotic gradient between the blood and the external medium was no greater than at 0.5‰, the net water flux rose to 21.7% body water/day (Table 24). It was assumed that net fluxes into the animal would be matched by urine flow out of the animal if the volume of the animal was to

Table 24. Half times of exchange ($T_{\frac{1}{2}}$), derived permeability constants (K), osmotic gradients and net water fluxes in Palaemon longirostris exposed to a wide range of external salinities at 12°C.

| | Salinity (‰) | | | | |
|---|---|---|---|---|---|
| | 0.5 | 7.0 | 22.0 | 34.0 | 43.0 |
| $T_{\frac{1}{2}}$ (mins) | 58.7 | 53.3 | 46.7 | 45.3 | 37.7 |
| K (hourly water exchange fraction) | 0.71 | 0.78 | 0.89 | 0.92 | 1.10 |
| Osmotic Gradient (mOsmol/kg) | 492 | 423 | 13 | 395 | 451 |
| Net Water Flux (% body weight/day ) | 15.3 | 15.0 | 0.4 | 15.7 | 21.7 |

remain constant. Similarly, it would be expected that net fluxes out of the animal would be matched by drinking of water. Thus, these net water fluxes can be used as indirect estimates of the rate of urine production and water drinking.

Another expression of the APW of an animal is the *K* constant. The *K* constant is the hourly water exchange fraction and is dimmensionless. The *K* constant can be calculated from the $T^1/_2$ of exchange using the equation:

$$K = ln2/T^1/_2$$

The rate constants (*K*) were calculated for *Palaemon longirostris* acclimated to 0.5, 7.0, 22.0, 34.0, and 43‰ at 12°C and are shown in Table 24. The values for *K* increased from 0.71 for prawns acclimated to 0.5‰ to 1.10 for prawns acclimated to 43‰ (Table 24). Thus the *K* constants reflected the same trend of reduced APW at low salinities for *P. longirostris* , as shown by the $T^1/_2$ of exchange for this species (Table 24).

### 5.3.3. HEART RATE IN *PALAEMON LONGIROSTRIS*

#### 5.3.3.1. EFFECT OF SALINITY

In order to investigate whether the changes in APW of

*Palaemon longirostris* at low salinities were real or were due to circulatory changes, the heart rate was measured in prawns acclimated to 0.5‰ and 34‰ at 4, 12 and 20°C (Fig. 33). At each temperature, there was no significant change in heart rate between prawns in 0.5‰ and 34‰ (Table 25, student t-test, $P > 0.05$). Thus it can be concluded that a change in heart rate is not responsible for the observed reduction of APW in *P. longirostris* at low salinities.

5.3.3.2. EFFECT OF TEMPERATURE

Figure 33 also shows the effect of temperature (4, 12 & 20°C) on the heart rates of *Palaemon longirostris* acclimated to salinities of 0.5‰ and 34‰. Heart rate increased from 45.6 bts/min at 4°C to 139.6 bts/min at 20°C for prawns acclimated to 0.5‰, and from 48.2 bts/min at 4°C to 143.6 bts/min at 20°C for prawns acclimated to 34‰ (Fig. 33). Statistical analyses revealed that these temperature-related differences in heart rate were significant (Table 26, ANOVA, $P < 0.001$), and suggest that the observed temperature-related changes in APW of *Palaemon longirostris* are probably due to changes in heart rate. At lower temperatures, heart rate slows and causes a reduction in the blood flow through the gills. Such a reduction in gill blood flow effectively reduces the surface area to volume ratio available for water exchange across the gills and results in a lower APW.

Figure 33. The effect of salinity and temperature on the heart rate of *Palaemon longirostris*. Shaded area represents prawns tested at 0.5‰ and white area represents prawns tested at 34‰ (vertical lines represent ±1 Standard Deviation).

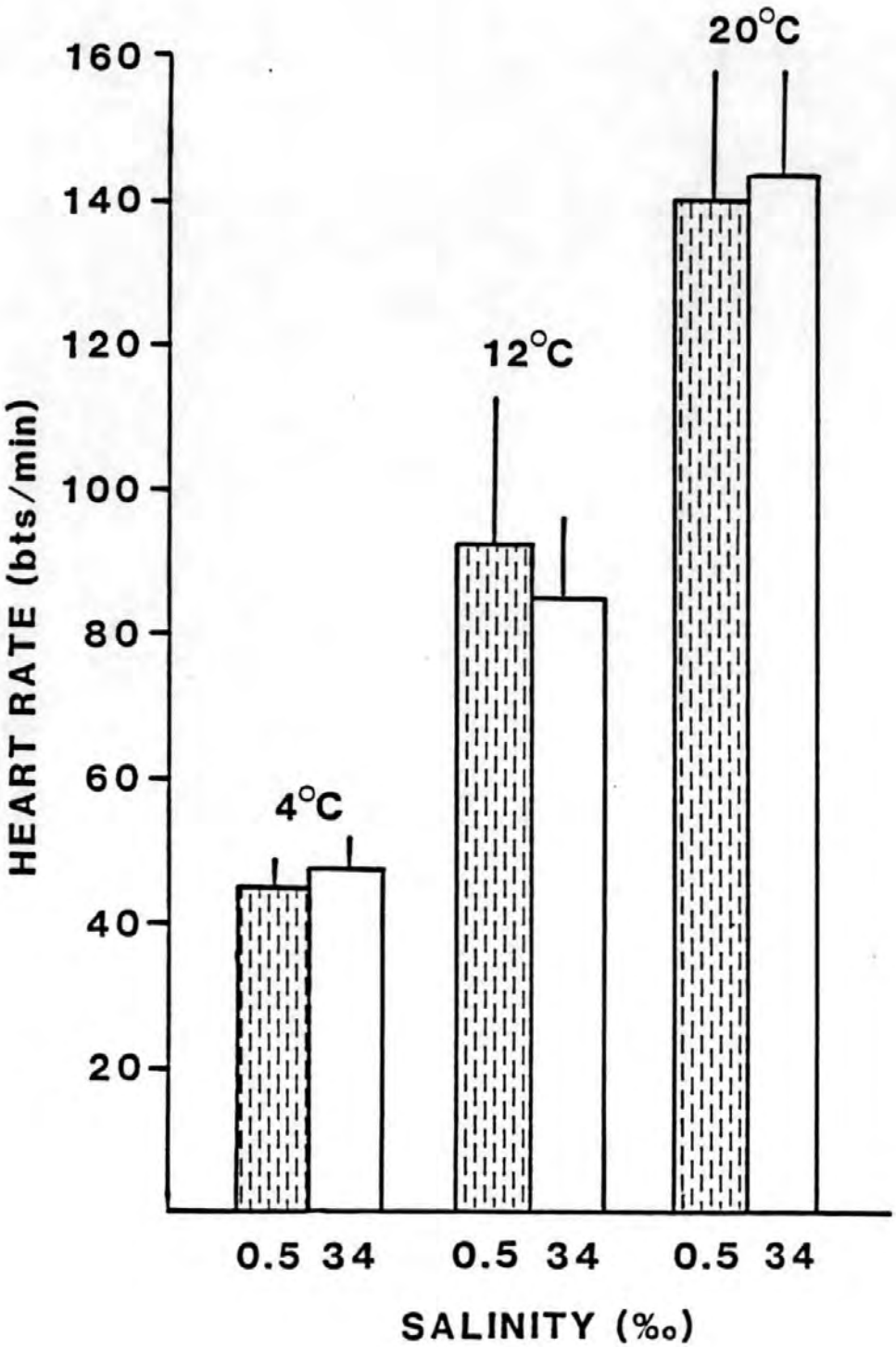

20°C
160
140
120
12°C
100
80
60
4°C
40
20
HEART RATE (bts/min)
0.5 34
0.5 34
0.5 34
SALINITY (‰)

Table 25. Differences in the heart rate of Palaemon longirostris acclimated to 0.5‰ and 34‰ at 4, 12 and 20°C (d.f., degrees of freedom; S.L., significance level; n.s., not significant).

| Source of variation | Temperature (°C) | t-value | d.f. | S.L. |
|---|---|---|---|---|
| Salinity | 4 | 0.99 | 8 | n.s. |
| Salinity | 12 | 0.69 | 8 | n.s. |
| Salinity | 20 | 0.02 | 8 | n.s. |

Table 26. One-way analysis of variance as to the effect of temperature (4, 12 and 20°C) on heart rate (beats/minute) of Palaemon longirostris at 0.5 and 34‰. (d.f., degrees of freedom; S.L., significance level; n.s., not significant).

| Source of variation | Salinity (‰) | d.f. | F-ratio | S.L. |
|---|---|---|---|---|
| Temperature | 0.5 | 2,12 | 39.3 | 0.001 |
| Temperature | 34 | 2,12 | 51.1 | 0.001 |

### 5.3.4. APPARENT WATER PERMEABILITY IN SELECTED CARIDEAN PRAWNS

The APW of *Palaemonetes varians* acclimated to 0.5‰ and 34‰ , *Crangon crangon* acclimated to 7, 22 and 34‰ and *Palaemon elegans* acclimated to 22‰ and 34‰ at 12°C are shown in Figure 34.

*Palaemonetes varians* showed a reduced apparent permeability to water at 0.5‰ compared with 34‰ (Fig. 34). For example, $T^1/_2$ of exchange was significantly higher at 0.5‰ (47.4 min) than at 34‰ (41.0 min) (Table 27, student t-test, $P < 0.05$).

For *Crangon crangon*, the APW varied from a $T^1/_2$ of exchange of 34.4 min at 34‰ to a $T^1/_2$ of exchange of 43.7 min at 7‰ (Fig. 34). These data show that this species can significantly reduce its apparent permeability to water at low salinities (Table 27, ANOVA, $P < 0.01$).

*Palaemon elegans* showed a slight increase in $T^1/_2$ of exchange at 34‰ (28.9 min) compared with 22‰ (32.5 min)(Fig. 34); however, this reduction of APW was not statistically significant (Table 27, student t-test, $P > 0.05$).

Figure 34. Apparent permeability to water ($T^1/_2$) in *Palaemon longirostris*, *Palaemonetes varians*, *Crangon crangon* and *Palaemon elegans* exposed to a range of salinities at 12°C (vertical lines represent $\pm 1$ Standard Deviation).

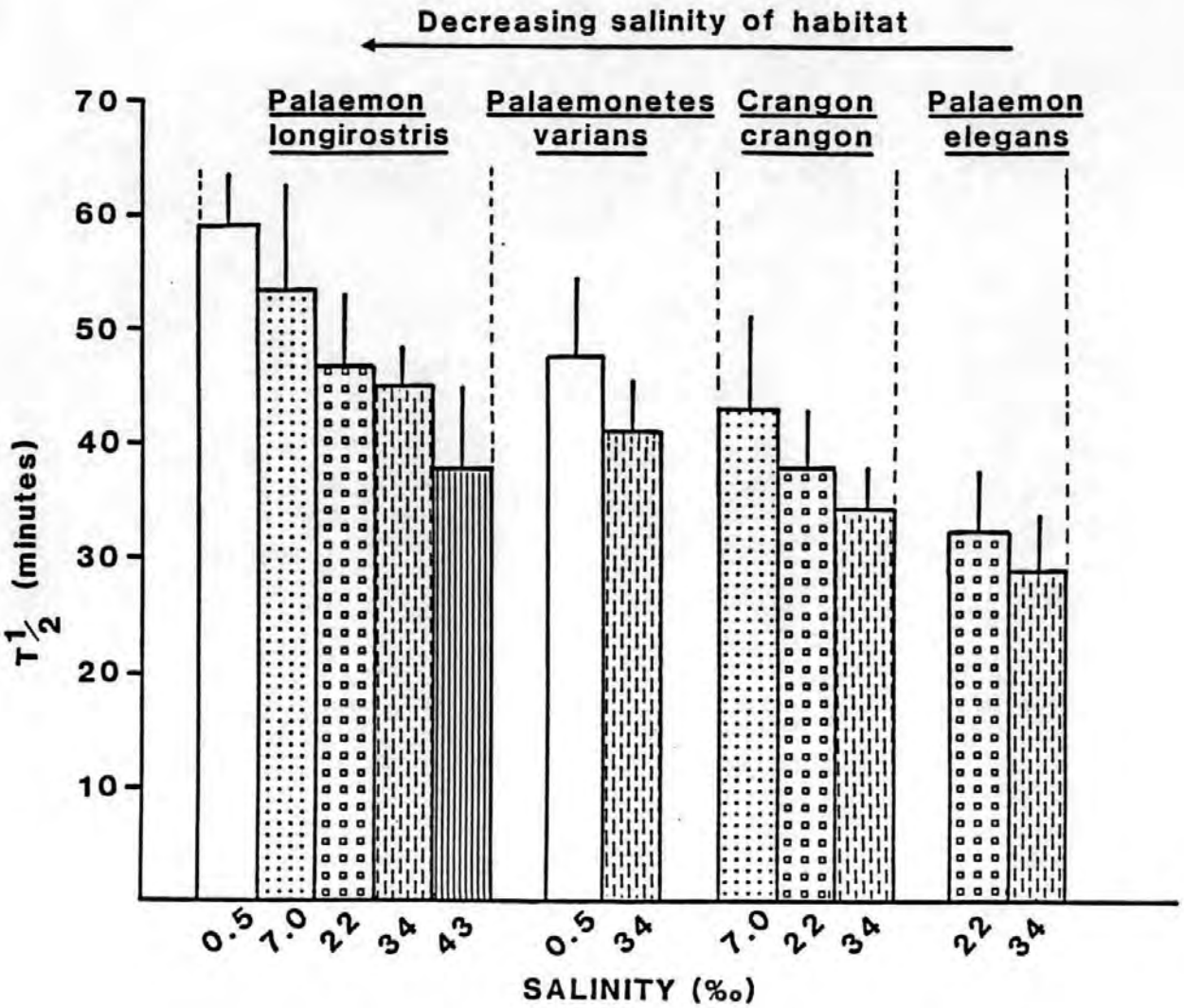

Decreasing salinity of habitat
Palaemon longirostris
Palaemonetes varians
Crangon crangon
Palaemon elegans
70
60
50
40
30
20
10
T 1/2 (minutes)
0.5
1.0
2.2
3.4
4.3
0.5
3.4
1.0
2.2
3.4
2.2
3.4
SALINITY (‰)

Table 27. Differences in apparent water permeabilities ($T_{\frac{1}{2}}$) of *Palaemonetes varians* at 0.5‰ and 34‰, *Palaemon elegans* at 22‰ and 34‰, and *Crangon crangon* at 7, 22 and 34‰. All experiments at 12°C (*d.f.*, degrees of freedom; *S.L.*, significance level; *n.s.*, not significant).

| Species | Source of variation | *t*-value | *F*-ratio | *d.f.* | *S.L.* |
|---|---|---|---|---|---|
| *P. varians* | Salinity | 2.42 | – | 18 | 0.05 |
| *P. elegans* | Salinity | 1.65 | – | 18 | *n.s.* |
| *C. crangon* | Salinity | – | 6.00 | 2,27 | 0.01 |

### 5.3.5. COMPARISON OF APPARENT PERMEABILITY TO WATER BETWEEN SPECIES

The APWs of *Palaemon longirostris*, *Palaemonetes varians*, *Crangon crangon* and *Palaemon elegans* over a range of salinities within their optimal salinity tolerance ranges are shown in Figure 34. At 0.5‰, the $T^1/_2$ of exchange for *P. longirostris* (58.7 min) was significantly longer than that for *P. varians* (47.4 min) (Fig. 34) (Table 28, student t-test, $P < 0.001$). Similarly, at 7‰, the $T^1/_2$ of exchange for *P. longirostris* (53.3 min) was significantly longer than that for *C. crangon* (43.2 min) (Fig. 34) (Table 28, student t-test, $P < 0.05$). Comparison of the $T^1/_2$ of exchange for *P. longirostris* - *C. crangon* - *P. elegans* acclimated to 22‰ revealed a reduction in $T^1/_2$ in the order *P. longirostris* (46.7 min), *C. crangon* (38.0 min) and *P. elegans* (32.5 min) (Fig. 34). Analyses showed that these differences in the APW between species at 22‰ were also statistically significant (Table 29, ANOVA, $P < 0.001$). At 34‰, the $T^1/_2$ of exchange for all four prawn species showed a general reduction between species in the order *P. longirostris* (45.3 min) - *P. varians* (41.0 min) - *C. crangon* (34.3 min) - *P. elegans* (28.9 min) (Fig. 34). ANOVA again showed that these differences were significant (Table 29, $P < 0.001$).

These results show that at each salinity *Palaemon longirostris* had the lowest APW, *Palaemonetes varians* had a lower APW than *Crangon crangon*, and *Palaemon elegans* had the highest APW.

Table 28. Differences in apparent water permeability ($T_{\frac{1}{2}}$) between *Palaemon longirostris* and *Palaemonetes varians* at 0.5‰ and between *Palaemon longirostris* and *Crangon crangon* at 7‰. All experiment at 12°C (d.f., degrees of freedom; S.L., significance level; n.s., not significant).

| Source of variation | Salinity (‰) | t-value | d.f. | S.L. |
|---|---|---|---|---|
| *Palaemonetes varians* | 0.5 | 4.18 | 18 | 0.001 |
| *Crangon crangon* | 7 | 2.46 | 18 | 0.05 |

Table 29. One-way analysis of variance on the effect of different species on the apparent water permeabilities ($T_{\frac{1}{2}}$) of prawns acclimated to 22‰ (*Palaemon longirostris*, *Crangon crangon*, *Palaemon elegans*) and 34‰ (*Palaemon longirostris*, *Palaemonetes varians*, *Crangon crangon*, *Palaemon elegans*) at 12°C. (d.f., degrees of freedom; S.L., significance level; n.s., not significant).

| Source of variation | Salinity (‰) | d.f. | f-ratio | S.L. |
|---|---|---|---|---|
| Species | 34 | 3,36 | 29.8 | 0.001 |
| Species | 22 | 2,27 | 17.7 | 0.001 |

## 5.4. DISCUSSION

Present results for the apparent permeability to water (APW) of four euryhaline prawns with different ecological distributions show that only the inter-tidal prawn *Palaemon elegans* did not have a significant change in the APW as the external salinity was varied. The estuarine prawns *Palaemon longirostris* and *Crangon crangon*, together with the brackish-water prawn *Palaemonetes varians*, however, demonstrated ability to reduce APW when acclimated to dilute sea water. Shaner et al. (1985) have reported a similar reduction in APW at low salinities (5‰) for the estuarine prawn *Crangon fransiscorum*. The findings for *P. varians* contradict earlier work as both Parry (1955) and Rudy (1967) reported *P. varians* did not alter its APW as the concentration of the external medium was changed. Such intra-specific variation in the APW response to salinity, as shown for *P. varians*, is probably due to acclimatization of geographically separated populations to their specific habitat salinity conditions (Shaner et al., 1985). The results of this study, and those of Shaner et al. (1985), suggest strongly that a reduction of APW at low salinities is a response which may be typical of estuarine prawns.

Investigations into the APW of estuarine crabs show a similar trend to that reported here for estuarine prawns. Thus, Smith (1967) demonstrated that *Rhithropanopeus harrisi*

lowered its APW on acclimation to lower salinities. Similarly, *Carcinus maenas* (Smith, 1970) and *Hemigrapsus nudus* (Smith & Rudy, 1972) showed a reduction in APW in salinities below 50% and 60% sea water respectively. Reduced APW has been reported also for isolated gill and gut tissues from the estuarine crabs *Callinectes sapidus* and *Cancer irroratus* acclimated to low salinities (Cantelmo, 1977). In contrast to the general trend shown for estuarine crabs, salinity had no effect on the APW of the semi-terrestrial crabs *Uca pugilator*, *Uca minax* and *Uca rapax* (Hannan & Evans, 1973). This is perhaps not surprising, since semi-terrestrial crabs maintain a permanent very low integumental water permeability in order to reduce water loss by evaporation, (Herried, 1969).

Another crustacean group which has been studied with respect to salinity induced changes in APW is the euryhaline amphipods. Taylor (1985) reported that *Corophium volutator* lowered its APW on acclimation to 15% sea water. A similar response was shown by the brackish-water and salt-marsh amphipod *Gammarus duebeni*, which reduced its APW on acclimation to 2% sea water (Lockwood et al., 1973; Bolt et al., 1980). Alternatively, salinity was found to have no effect on the APW of the inter-tidal amphipods *Chaetogammarus marinus* (Bolt et al., 1980; Bolt, 1983) and *Gammarus locusta* (Bolt, 1983). Thus as was concluded for prawns and crabs, only amphipods which inhabit extremely fluctuating salinity type environments, such as estuaries,

brackish-water and salt-marsh pools have the ability to reduce APW on acclimation to low salinities. This is not surprising, as inter-tidal crustaceans are rarely exposed to prolonged periods of very low salinity conditions, whereas estuarine, brackish-water and salt-marsh crustaceans often experience considerable periods in dilute saline waters.

Cornel (1973) suggested that a drop in heart rate at low salinities may account for the associated drop in APW of a species. The present results for *Palaemon longirostris*, however, showed that salinity had no significant effect on heart rate. Similarly, salinity was found to have no effect on the heart rate of the freshwater prawn *Palaemon adspersus* (Hagerman & Uglow, 1979), the shore crab *Carcinus maenas* (Hume & Berlind, 1976) and the brackish-water and salt-marsh amphipod *Gammarus duebeni* (Bolt et al., 1980). Thus, the salinity induced changes in APW of *P. longirostris* and other estuarine crustaceans, do appear to reflect real changes in integumental permeability.

Animals living under dilute saline conditions gain water primarily by osmotic uptake. Expulsion of this water takes place mainly in the form of urine production, which also involves ion loss to the environment. Reduction of the APW decreases osmotic entry of water, which in turn reduces urine production and ion loss to the environment (Kirschner, 1979). Reduction of integumental APW is regarded as a significant physiological adaptation to life in low salinity environments, as it effectively reduces the energy required to maintain stable blood ionic concentrations by active ion

uptake. Estuarine crustaceans such as *Palaemon longirostris*, which live permanently under very dilute saline conditions, thus benefit energetically from the ability to reduce their APW. It has also been postulated that maintainance of a comparatively high APW at high salinities is also adaptive for such estuarine crustaceans, since maximization of water gain across the integument ensures sufficient urine for excretory purposes (Lockwood & Inman, 1973).

*Palaemon longirostris* maintained a net water flux of approximately 15% body weight/day when acclimated to salinities of 0.5, 7 and 34‰. When prawns were isosmotic with the external medium (22‰ ), net water fluxes were reduced to almost zero. The net water fluxes calculated for *P. longirostris* are comparable with those for the other euryhaline prawns *Palaemonetes varians*, *Crangon crangon* and *Palaemon elegans* (Table 30). Net water fluxes of similar magnitude (20.5% body weight/day) have been reported also for another estuarine prawn, *Crangon fransiscorum* (Shaner et al., 1985). Thus one very important adaptation possessed by these estuarine prawns is an ability to maintain a relatively low net water flux even when experiencing a large osmotic gradient between the blood and the external medium. The net water flux data summarized in Table 30 show how well *P. longirostris* is adapted to life in dilute salinities compared with the other euryhaline prawns. For example, when *P. longirostris* and *P. varians* were both acclimated to 0.5‰ , *P. longirostris* was able to maintain a net water flux almost identical to that of *P. varians*, even though the

Table 30. Comparison of the osmotic gradients, net water fluxes and permeability constants (K) for four euryhaline prawns species in different salinities. Mole fraction data used to calculate net water fluxes for prawns other than Palaemon longirostris was obtained from the literature cited. Figures in parenthesis represent urinary flow rates (at 18°C) measured by direct cannulation of the excretory pore by Parry (1955).

| | Palaemon longirostris | | | | | Palaemonètes varians | | Crangon crangon | | | Palaemon èlegans | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Salinity (‰) | 0.5 | 0.7 | 22.0 | 34.0 | 43.0 | 0.5 | 34.0 | 7.0 | 22.0 | 34.0 | 22.0 | 34.0 |
| Osmotic gradient (mOsmol/kg) | 492 | 493 | 13 | 395 | 451 | 378 | 304 | 201 | 16 | 81 | 75 | 220 |
| Net water flux (% body weight/day) | 15.3 (43.7) | 15.0 | 0.4 | 15.7 | 21.7 | 14.8 (18.0) | 12.4 (12.1) | 13.9 | 4.3 | 2.95 | 1.8 (2.4) | 14.1 |
| K (hourly water exchange fraction) | 0.71 | 0.78 | 0.89 | 0.92 | 1.10 | 0.87 | 1.01 | 0.96 | 1.09 | 1.21 | 1.28 | 1.44 |
| Mole fraction data source | Chapters 3 and 5 | | | | | Hagerman and Uglow, 1983. | | Spaargaren, 1971. | | | Ramirez de Isla Hernandez and Taylor, 1985. | |
| Temperature (°C) | 12 | | | | | 12 | | 12 and 15 | | | 10 and 12 | |

osmotic gradient between its blood and the external medium was 100mOsmol/kg larger than that for *P. varians* (Table 30). A similar adaptation to dilute salinities was reported for the extremely euryhaline amphipod *Gammarus duebeni* which maintained much lower net water fluxes than the inter-tidal amphipods *Chaetogammarus marinus* and *Gammarus locusta* over identical salinity ranges (Bolt, 1983).

When prawns are hyper-osmotic to the external medium, the calculated net water fluxes have been taken to represent a minimum estimate of urinary flow rates. Estimates of urine production in *Palaemonetes varians* and *Palaemon elegans*, using the THO outflux techinique, were comparable with those obtained for the same species by Parry (1955) using direct cannulation of the excretory pore (Table 30). However, the urine flow rate obtained by Parry (1955) for *Palaemon longirostris* acclimated to 7% sea water was three times larger than the urine flow rate predicted in this study (Table 30) and appears artificially high.

Although the majority of estuarine crustaceans studied to date have the ability to reduce their APW on acclimation to low salinities, only one species has been found which shows a rapid reduction in its APW on exposure to a sudden salinity change. Thus, Lockwood et al. (1973) and Bolt (1983) reported an almost instantaneous drop in APW in *Gammarus duebeni* transferred from 2 to 100% sea water, and from 100 to 50% sea water. In contrast, an increase in APW was reported for *Palaemon longirostris* following transfer from 0.5 to 34‰ . Interestingly, the blood osmotic

concentration of *P. longirostris*, following such a transfer, was almost unchanged (Chapter 3). These results for *P. longirostris* suggest that strong ionic regulation (Chapter 4) may be more important in maintaining constant blood osmolalities at high salinities than permeability to water.

*Palaemon longirostris* acclimated to 34‰ and transferred to 0.5‰ showed no immediate change in APW. After seven days acclimation to 0.5‰ , however, the APW was lowered. Similarly, results have been reported for the estuarine amphipod *Corophium volutator* transferred from 85% sea water to 15% sea water (Taylor, 1985), and the estuarine prawn *Crangon fransiscorum* transferred from 25‰ to 5‰ (Shaner et al., 1985). However, *Corophium volutator* showed a reduction in APW after seven days acclimation (Taylor, 1985) and *C. fransiscorum* after 5-6 weeks acclimation to these lower salinities (Shaner et al., 1985). Interestingly, the unchanged APW reported for *P. longirostris* after transfer from 34‰ to 0.5‰ was also accompanied by an initial large drop in blood osmolality (Chapter 3). The corresponding reduction in APW that occurred during the next seven days was also accompanied by a gradual rise in blood osmolality to a new steady state (Chapter 3). Hence, ionic regulation alone is not responsible for the maintainance of a large osmotic gradient between the blood and the external medium in *P. longirostris* acclimated to very low salinities. A reduction in APW thus seems to play an important role in the osmoregulation of *P. longirostris* at low salinities.

Although these APW changes occurred too slowly to be effective within a tidal cycle, they would be of considerable advantage to estuarine species such as *Palaemon longirostris* which live predominantly in dilute saline regions of estuaries. Estuarine prawns are continuously subjected to low salinity conditions and thus can acclimate to them in the form of reducing their APW. Surprisingly, not all euryhaline crustaceans show this ability to reduce APW after exposure to a salinity change. For example, sudden salinity transfer had no long-term or short-term effect on the APW of the inter-tidal amphipods *Chaetogammarus marinus* or *Gammarus locusta* (Bolt, 1983); these species, however, rarely encounter very low salinity conditions in their natural habitat (Bolt, 1983).

Salinity induced changes in the APW of animals acclimated to specific salinities do not always reflect the APW changes that occur in animals exposed to a cycling salinity regime. For instance, *Gammarus duebeni* acclimated to a selection of salinities showed a reduction in APW at lower salinities (Lockwood et al., 1973; Bolt, 1983). On the other hand, *G. duebeni* exposed to a cyclic salinity regime displayed a high APW at isosmotic salinities, an intermediate value at very low salinities and a low APW at high salinities (Bolt, et al., 1980; Bolt, 1983). Thus *G. duebeni* exhibits two quite different integumental water permeabilty responses to salinity change; a salinity acclimation response and a continuous salinity cycle response. This salinity cycle-related APW response aids the

maintainance of a stable blood osmolality in *G. duebeni* exposed to continuously fluctuating salinity conditions, and is a significant adaptation to life in the fluctuating salinity type environments normally occupied by this species. Not surprisingly, the inter-tidal amphipods *Chaetogammarus marinus* and *Gammarus locusta* showed no significant change in APW when exposed to the same salinity cycle as *G. duebeni*. Thus, the ability to alter APW as the salinity of the external medium is varied appears to be a physiological adaptation restricted to species which occupy widely fluctuating salinity habitats, such as estuaries, brackish-water pools and salt marshes.

The effect of temperature on APW has been little studied in crustaceans, and the limited data available suggest that APW is lowered by a reduction in temperature. $Q_{10}$ values (magnitude of the increase in APW caused by a 10°C rise in temperature), however, have been calculated for a small number of euryhaline crustaceans. Smith and Rudy (1972) reported $Q_{10}$ values (between 10-20°C) of 1.51 and 1.63 for the estuarine crab *Hemigrapsus nudus* acclimated to 95% and 60% sea water respectively. The results of this study reported a mean $Q_{10}$ value (over any 10°C temperature range between 4 and 20°C) of 1.55 in *Palaemon longirostris* acclimated to 100% sea water and 1.53 in prawns acclimated to 5% sea water. Similarly, Tun (1975) reported $Q_{10}$ values (between 4-14°C) of 1.57 for *Palaemonetes varians* acclimated to 100% sea water and 1.60 for *P. varians* acclimated to 2% sea water. However, $Q_{10}$ values for *P. varians* over a higher

temperature range (14-24°C) were elevated to 2.11 in prawns acclimated to 100% sea water and to 2.04 in prawns acclimated to 2% sea water (Tun, 1975). Similarly, over the same temperature range (between 14-24°C), $Q_{10}$ values for the semi-terrestrial crab *Uca pugilator* were 2.10 for crabs acclimated to 100% sea water and 1.73 for crabs acclimated to 3% sea water (Hannan & Evans, 1973). Therefore, higher temperatures appears to have a more pronounced effect on APW than lower temperatures.

Since so few crustaceans have been studied with respect to temperature induced changes in APW, the adaptive significance of these changes remains unresolved. However, temperature induced changes in APW, as shown for *Palaemon longirostris*, are probably responsible for the observed rise in blood osmolality at low temperatures and the observed drop in blood osmolality at high temperatures for prawns acclimated to low salinities. In other words, at low temperature APW drops and water flow into the animal is restricted at low salinities causing an increase in blood osmolality; the opposite occurs for prawns at high temperature/low salinity combinations.

The temperature-induced changes in APW reported here for *Palaemon longirostris* were also correlated with temperature-induced changes in heart rate. At low temperature, a drop in APW was associated with a drop in heart rate, whereas at higher temperatures the reverse occurred. Therefore, such changes in heart rate probably account for the associated changes in APW. Although these

temperature induced changes in APW do not represent real hydraulic changes in cuticular permeability, the resulting effect is a drop in the permeability to water of the animal, which still has biological significance to the energetics of the animal (Lockwood et al., 1982). At low temperatures, heart rate drops and blood circulation through the gills is reduced, which in turn effectively reduces the surface area for water and ion transport across the gills.

Integumental APW has also been found to vary between different aquatic groups of crustaceans. Rudy (1967) showed that the marine swimming crab *Macropipus depurator* (acclimated to 100% sea water) was three times more permeable to water than the inter-tidal shore crab *Carcinus maenas*, and that *C. maenas* (when acclimated to 40% sea water) was three times more permeable to water than the freshwater crayfish *Astacus fluviatilis* (acclimated to fresh water). Reduction of the APW of animals inhabiting less saline areas is a useful physiological adaptation, as it effectively reduces the osmotic work load on such animals.

Although estuarine and brackish-water crustaceans have been studied with regards to salinity induced changes in APW, the relationship between the salinity of the natural habitat and the relative APW for such crustaceans has been neglected. The results of this study show a clear relationship between habitat salinity and the relative APW in the prawns *Palaemon longirostris*, *Palaemonetes varians*, *Crangon crangon* and *Palaemon elegans*. The APW of each species was directly related to the natural salinity range

of its ecological niche. Hence, *Palaemon longirostris* which was found living in the most dilute regions (almost fresh water), showed the lowest APW at all salinities. *Palaemonetes varians* which was found inhabiting brackish-water pools indirectly connected to the sea showed a higher APW than *P. longirostris* but a lower APW than the lower estuarine prawn *C. crangon*. The most marine species, *Palaemon elegans*, which occurs in the inter-tidal zone, showed the highest APW at all salinities (for summary see permeability constants K, Table 30). Thus, these prawns show a general trend of a reduction in APW for species which occupy less saline areas. Similarly, Bolt (1983) showed that the extremely euryhaline amphipod *Gammarus duebeni*, which often occupied very dilute saline habitats, possesed a lower APW than the inter-tidal amphipods *Chaetogammarus marinus* and *Gammarus locusta*.

These observations suggest that permeability to water may be the determining factor in separating species which possess overlapping salinity tolerance ranges. The data also support the theory that one of the major physiological adaptations to life in very dilute saline environments is reduction of integumental water permeability (Lockwood, 1976; Greenaway, 1979; Vernberg & Vernberg, 1983).

# CHAPTER 6

# GENERAL DISCUSSION

# 6. GENERAL DISCUSSION

Any attribute of an animal which enhances its probability of survival in a specific environment is called an adaptation (Prosser, 1975). Consequently, the ability of an animal to survive in any environment is related to its degree of adaptation to that special environment. The present study has investigated various aspects of the osmoregulation of *Palaemon longirostris* and the adaptive significance of the results are discussed in this final chapter.

All post-larval life-history stages of *Palaemon longirostris*, including juveniles and ovigerous females, are extremely euryhaline, with a general salinity tolerance range of between 0.5 and 43‰ being recorded. Based on these results, *P. longirostris* is well adapted to survive in the range of salinities which are characteristic of its natural environment. The mechanism underlying this high degree of euryhalinity in *P. longirostris* is an ability to control haemolymph osmotic concentration. *P. longirostris* is a very strong hyper-hypo-osmoregulator and, over an external osmotic range of 1000mOsmol/kg, its haemolymph osmotic concentration generally varies by less than 100mOsmol/kg. Indeed, the strength of osmoregulation shown by *P. longirostris* is such that prawns transferred directly from 14‰ to either 5‰ or 34‰, show only minimal changes in

blood osmotic concentrations, and reach a new steady state level within 6-12h. Hyper-hypo-osmoregulation is thought to represent one of the most advanced forms of genetic adaptation to osmotic change possessed by invertebrates (Hagerman, 1971). Present work has also shown that extremely strong hyper-hypo-regulation of sodium and chloride, the major haemolymph inorganic ions, together with the maintainance of a relatively low apparent permeability to water are the mechanisms responsible for hyper-hypo-osmoregulation in *P. longirostris*. In addition, efficient hyper-osmoregulation in this species was facilitated by its ability to reduce apparent permeability to water on acclimation to low salinities. The maintainance of low cuticular permeability in *P. longirostris* may well be related to the fact that blood calcium ions are strongly hyper-regulated; calcium affects cell membrane function (Robertson, 1960), which in turn may indirectly affect cell permeability. Strong hyper-regulation of blood calcium thus ensures high concentrations of this ion in the blood, regardless of external salinity. In general, inorganic ions contributed more than 94% of the total haemolymph osmolality in *P. longirostris* at each salinity, suggesting that organic osmotic effectors, such as free amino acids, probably play a minor (if any) role in the osmoregulation of this species.

Although most of the descriptions of osmoregulatory mechanisms employed by *Palaemon longirostris* were based on results from static salinity conditions, examination of blood osmolality and apparent water permeability changes in

prawns exposed to acute salinity change have been reported also. Prawns transferred directly from 34‰ to 1‰ show an initial drop in blood osmolality within the first 12h, followed by a gradual rise in blood osmolality to a new steady state over a period of 3 days. The apparent water permeability for prawns exposed to the same salinity transfer does not change immediately, however, declines by 20% after 7 days. This change in apparent permeability to water reduces the osmotic gain of water across the cuticle and thus decreases the osmotic workload of the animal. Thus, it appears that reduction in apparent permeability to water plays an important part in osmotic adjustment to low salinity in *P. longirostris*. When *P. longirostris* was transferred directly from 1‰ to 34‰ , blood osmolality remained relatively unchanged, while the apparent permeability to water rose by 20% immediately after transfer. These results suggest that permeability to water does not play a role in the osmoregulation of *P. longirostris* at high salinities. It appears, therefore, that *P. longirostris* operates a dual mechanism of osmoregulation. Low permeability to water plays a dominant part in hyper-osmoregulation, whereas strong regulation of sodium and chloride plays a dominant role in hypo-osmoregulation.

It is well established that estuaries are characterised by tidal and seasonal fluctuations in water temperature (Lockwood, 1976; Vernberg & Silverthorn, 1979), and that temperature and season may influence the osmotic response of

euryhaline crustaceans (Dorgelo, 1976, 1981, Mantel & Farmer, 1983). Over the salinity range 0.5 to 34‰ , short-term temperature change (between 4 and 20°C) had no measurable effect on the salinity tolerance nor on the osmoregulatory efficiency of *Palaemon longirostris*. Reduction in temperature to 4°C, however, caused increased mortality for prawns acclimated to 43‰. This mortality was correlated with a breakdown in osmoregulation associated mainly with a large increase in blood magnesium. Although the osmoregulatory ability of *P. longirostris* was unaffected by short-term temperature change, low temperatures generally resulted in higher blood osmotic concentrations. At high salinities, these osmotic increases could be accounted for entirely by increases in blood chloride, calcium and magnesium concentrations. At low salinities, however, high blood osmolality could not be totally accounted for by increased ionic concentrations. Elevated blood osmolalities at low temperature /low salinity combinations appear to result from changes in water permeability aswell as small increases in ionic concentrations. At 4°C, apparent permeability to water was reduced at both 0.5 and 34‰ compared with 12 and 20°C. Such changes would effectively reduce the osmotic flow of water between the animal and its surrounding medium. Due to the larger osmotic gradient present between the blood and the external medium at very low salinities (eg, 0.5‰), this reduction of osmotic flow of water would be most pronounced in prawns acclimated to lower salinities. Hence, reduction of apparent water

permeability at low salinities, in combination with low temperature, results in reduced osmotic flow of water into the animal and probably accounts for the rise in blood osmolality recorded. These observations provide further evidence for the dual role of low water permeability and strong ionic regulation in the osmoregulation of *P. longirostris* at low and high salinities respectively.

Winter acclimatized *Palaemon longirostris* were less euryhaline and had reduced survival in dilute salinities compared with summer prawns. Mortality of winter prawns at low salinities could be explained by a breakdown in osmoregulation. Presumably, to overcome the osmotic problems posed by low salinity/low temperature conditions, prawns migrate downstream to more saline regions of estuaries in winter (Fincham & Furlong, 1984; pers.obs.). In this study, *P. longirostris* occurs at the northern limit of its distribution and therefore it is not surprising that individuals are particularly sensitive to cold water. Seasonal differences in the salinity tolerance of *P. longirostris* may explain the absence of this species from river estuaries at higher latitudes. At more northern locations, low water temperatures occur over a longer time scale and would cause *P. longirostris* to spend extended periods in more saline estuarine regions, which will be occupied by presummed better competitors. For example in the River Tamar, during the summer *P. longirostris* is found upstream in dilute regions unoccupied by any other prawn species, whereas in winter, *P. longirostris* occurs

downstream in more saline regions occupied by the prawns *Crangon crangon* and *Palaemon serratus* (pers. obs.).

The foregoing summary of the osmoregulatory characteristics of *Palaemon longirostris* has shown this species to be well adapted to the salinity and temperature conditions found in the upper regions of estuaries. Some of these osmotic features, however, can be interpreted as favouring life in a fluctuating salinity environment, and others as favouring life under very low salinity or freshwater conditions. Indeed, the very nature of the upper estuarine habitat normally occupied by *P. longirostris*, stimulates the question as to whether this prawn is essentially a brackish- or freshwater species. The same question has been addressed to the ancestry of the family Palaemonidae, and has caused considerable debate and some controversy. For example, Panikkar (1941) suggested that because marine palaemonids exhibited a strong pattern of hyper-hypo-osmoregulation, they must have acquired this mechanism in fresh water at some time in their evolutionary past. Consequently, Panikkar (1941) suggested that the family Palaemonidae has already evolved from the sea into fresh water and is secondarily invading the marine environment. Several other authors (Ortmann, 1902; Hedgpeth, 1949, 1957; Born, 1968; Moreira et al., 1983), however, have put forward an alternative interpretation. They believe that because palaemonids occur in a wide variety of aquatic habitats, with the majority inhabiting fresh water, the family is a very recent one, is probably in a transitionary

status, and is in the process of invading fresh water. Although it is impossible to resolve the problem of the ancestry of palaemonids based solely on physiological adaptations, some comment on the phylogeny of the family Palaemonidae can be made using the present data for *P. longirostris*, a species whose distribution and physiological adaptations place it somewhere between the brackish- and freshwater groupings.

In general, all palaemonid prawns are very euryhaline although salinity tolerance limits are correlated with habitat (Reynolds. 1975; Sandifer et al., 1975; Thorp & Hoss, 1975; Guest & Durocher, 1979; Hagerman & Uglow, 1983; Knowlton & Kirby, 1984; Moreira et al., 1986; Kirkpatrick & Jones, 1985). Thus, marine palaemonids show better tolerance to high rather than low salinities, freshwater palaemonids have improved tolerance to low rather than high salinities, and brackish-water palaemonids have good tolerance to salinities ranging between almost fresh water and sea water (Table 31). The salinity tolerance range reported here for *Palaemon longirostris* compares well with those reported for brackish-water palaemonids, however, the lower lethal salinity limit demonstrated for *P. longirostris* is more typical of freshwater than brackish-water palaemonid species (Table 31).

Two osmoregulatory patterns have been reported for palaemonid prawns. Marine and brackish-water species are hyper-hypo-osmoregulators (Panikkar, 1941; Dobkin & Manning, 1964; Born, 1968; Denne, 1968; Spaargaren, 1972; Hagerman &

Table 31. A comparison of the salinity tolerance and osmoregulatory characteristics of marine, brackish-water and freshwater groups of palaemonids with *Palaemon longirostris*. Data on other palaemonids were extracted from references cited in the text and are quoted as means (between species) or as examples from species. Figures in parenthesis represent ± 1 Standard Deviation.

| Habitat | Salinity tolerance range (‰) | Mean lower lethal salinity limits (‰) | Osmoregulation type | Mean blood osmolality differences* (mOsmol/kg) | Blood isosmotic point ranges (‰) | Mean blood isosmotic points (‰) |
|---|---|---|---|---|---|---|
| Marine | 10-47.5 | 14.0 (3.7) | Hyper-hypo-osmoregulation | 134 (52) | 25-27.5 | 25.6 (1.3) |
| Brackish water | 1-61.2 | 2.5 (1.9) | Hyper-hypo-osmoregulation | 119 (82) | 18-25 | 22.7 (2.1) |
| Fresh water | 0-21 | 0 | Hyper-iso-osmoregulation | 15 (14) | 15-19 | 16.3 (1.7) |
| *Palaemon longirostris* (upper estuarine) | 0.5-43 | 0.5 | Hyper-hypo-osmoregulation | 39 (25) | 18-23 | 21.0 (1.3) |

* Between prawns in isosmotic salinities and lower lethal salinities.

Uglow, 1983; Kirkpatrick & Jones, 1985; Ramirez de Isla Hernandez & Taylor, 1985; Table 31), whereas freshwater palaemonids are hyper-osmoregulators (Parry, 1957, 1961; Dobkin & Manning, 1964; Denne, 1968; Sandifer et al., 1975; Castille & Lawrence, 1981c; Moreira et al., 1983; Table 31). The osmoregulatory response shown by *Palaemon longirostris* is typical for marine and brackish-water palaemonids (Table 31). A further difference in the osmoregulatory features of marine, brackish- and freshwater palaemonids is shown by the salinity at which the blood becomes isosmotic with the external medium. The data, summarized in Table 31, show a trend of reduction in the isosmotic point for species penetrating into very dilute salinities, as proposed for crustaceans in general (Verwey, 1957). Although the isosmotic point for *P. longirostris* is within the range reported for brackish-water palaemonids, the mean value for this species is lower than the mean isosmotic point shown by brackish-water palaemonids (Table 31). The degree of hyper-osmoregulation also differs between palaemonid species. One method of quantifying this difference is to compare blood osmolality values for prawns acclimated to their isosmotic salinities with those for individuals acclimated to their lower lethal salinity limits. The data show a clear trend of a reduction in blood osmolality differences for palaemonids as they occupy less saline regions (Table 31). The blood osmolality difference for *Palaemon longirostris* is more typical of freshwater than of marine and brackish-water palaemonids (Table 31).

Patterns of blood ionic regulation, particularly of sodium and chloride, show similar trends between palaemonids occupying different environments to those described for osmoregulation. Thus, marine and brackish-water species are hyper-hypo-regulators of sodium and chloride (Parry, 1954; Potts & Parry, 1964a; Born, 1968; Dene, 1968; Spaargaren, 1972; Hagerman & Uglow, 1983; Knowlton & Kirby, 1984; Ramirez de Isla Hernandez & Taylor, 1985; Table 32). Freshwater palaemonids are hyper-iso-regulators of sodium, and may both be hyper-hypo-regulators or hyper-iso-regulators of chloride (Denne, 1968; Castile & Lawrence, 1981c; Table 32). The pattern of regulation of blood sodium and chloride shown for *Palaemon longirostris* is therefore typical of marine and brackish-water palaemonids. The salinities at which blood sodium and chloride concentrations became isoionic with the external medium also show a general trend of reduction for prawns as they colonise less saline waters (Table 32). The isoionic point for blood chloride for *P. longirostris* is typical of brackish-water palaemonids (Table 32). On the other hand, the isoionic point for blood sodium is more typical of freshwater than marine and brackish-water palaemonids (Table 32). The degree of hyper-ionic regulation of blood sodium and chloride, however, does vary between palaemonid species and can be quantified in the same way as that described for osmoregulation. There is a general reduction in the blood sodium and chloride concentration differences as species leave the sea and enter more dilute saline habitats (Table

Table 32. Comparison of the sodium and chloride regulatory characteristics of marine, brackish-water and freshwater palaemonids with those of *Palaemon longirostris*.

| Habitat | Species | Ionic regulation type | Isoionic point (‰) | | Lower lethal salinity limit (‰) | Blood ionic concentration differences * (mEq/L) | | Source |
|---|---|---|---|---|---|---|---|---|
| | | | Na | Cl | | Na | Cl | |
| Marine | *Palaemon elegans* | Hyper-hypo (Na & Cl) | 23 | 20 | 5 | 140 | 150 | Ramirez de Isla Hernandez & Taylor, 1985. |
| Brackish water | *Palaemonetes varians* | Hyper-hypo (Na & Cl) | 22 | 19 | 1 | 104 | 95 | Potts & Parry, 1964a; Hagerman & Uglow, 1983. |
| | *Palaemonetes pugio* | " | 21 | – | 1 | 90 | – | Knowlton & Kirby, 1984. |
| | *Palaemonetes macrodactylus* | " | – | 22 | 1 | _ | 120 | Born, 1968. |
| Fresh water | *Macrobrachium australiense* | Hyper-iso (Na & Cl) | 20 | 12 | 0 | 50 | 20 | Denne, 1968. |
| | *Macrobrachium rosenbergii* | Hyper-iso (Na) Hyper-hypo(Cl) | 15 | 13 | 0 | 20 | 25 | Castille & Lawrence, 1981c. |
| | *Macrobrachium ohione* | Hyper-iso (Na) Hyper-hypo(Cl) | 15 | 14 | 0 | 25 | 60 | Castille & Lawrence, 1981c. |
| Upper estuarine | *Palaemon longirostris* | Hyper-hypo (Na & Cl) | 17.5 | 19 | 0.5 | 25 | 35 | Present study. |

* Between prawns in isomotic salinities and lower lethal salinities.

32). The blood sodium and chloride concentration differences shown for *P. longirostris* are similar to those of freshwater palaemonid species (Table 32).

The degree of adaptation of crustaceans to fresh water is reflected in their permeability to water (Rudy, 1967). A comparative study, reported here, of the apparent water permeability changes in the euryhaline prawns *Palaemon longirostris*, *Palaemonetes varians*, *Palaemon elegans* and *Crangon crangon*, revealed that only the estuarine and brackish-water prawns (*P. longirostris*, *P. varians* and *C. crangon*) could reduce their apparent permeability to water in low salinities; the inter-tidal prawn, *P. elegans*, could not alter its apparent permeability to water when exposed to dilute salinities. Reduced permeability to water effectively decreases trans-integumentary flow of water, and is clearly adaptive for both fresh- and brackish-water crustaceans which are exposed to low salinities in their natural environment (Greenaway, 1979; Spaargaren, 1979). Although both *P. longirostris* and *P. varians* were able to reduce apparent permeability to water on acclimation to low salinities, the resulting reduction was 25% greater for *P. longirostris* than the brackish-water species. Furthermore, comparison of the absolute apparent water permeabilities of *P. longirostris*, *P. varians*, *C. crangon* and *P. elegans* , acclimated to a wide range of salinities, revealed that *P. longirostris* exhibited the lowest apparent permeability to water at all salinities. Based on these values, *P. longirostris* possesses a high degree of

adaptation to fresh water. Interestingly, the apparent permeability to water of each species was related directly to the salinity range normally encountered in nature in the following order of decreasing apparent permeability to water: *P. elegans* (inter-tidal), *C. crangon* (lower estuarine), *P. varians* (brackish-water), and *P. longirostris* (upper estuarine/fresh water). Permeability to water may therefore play an important role in determining the ecological distribution of crustacean species along a naturally occurring salinity gradient such as in a river estuary.

These comparisons of osmotic characteristics of palaemonids, place *Palaemon longirostris*, with wide salinity tolerance, hyper-hypo-osmotic/ionic-regulation and reduced apparent permeability to water, within the brackish-water palaemonid grouping. However, some of the more refined osmotic characteristics reported here for *P. longirostris*, such as reduced lower lethal salinity limit, reduced isosmotic point, extremely efficient hyper-osmotic/ionic-regulation and lowered apparent permeability to water, place the species closer to the freshwater rather than brackish-water palaemonids. *P. longirostris*, however, does not produce hypo-tonic urine (Parry, 1957) nor is it able to complete its larval development in fresh water (Antonopoulou, 1987), and thus appears to be incomplete in its adaptation to the freshwater environment (Greenaway, 1979; Vernberg & Vernberg, 1983). Consequently, it appears that *P. longirostris* may represent

a link between brackish- and freshwater palaemonid species. *P. longirostris* also has high tolerance to hyper-saline salinities, a feature which could have evolved only in the marine environment. Since freshwater palaemonids do not show high tolerance to hyper-saline salinities (Sandifer et al., 1975; Guest & Durocher, 1979; Moreira et al., 1984), this is further evidence that *P. longirostris* is in evolutionary terms a "young species" which has recently evolved from the sea. Since the majority of brackish-water palaemonids studied to date also show high tolerance to hyper-saline salinities (Panikkar, 1941; Dobkin & Manning, 1964; Born, 1968; Denne, 1968; Knowlton & Kirby, 1984; Kirkpatrick & Jones, 1985), this suggests that the family Palaemonidae is a "recent evolutionary group" which is still in the processs of invading fresh water. This suggestion is supported by the fact that *P. longirostris*, and many freshwater palaemonid species, require brackish-water for successful larval development. Further evidence for a recent evolutionary migration from the sea for *P. longirostris* is found in the type of temperature effect on osmoregulation recorded for this species. Prawns acclimated to high temperatures generally showed a reduction in blood osmolality. Interestingly, this species was observed to migrate upstream to more freshwater regions during such high water temperatures. As a reduction in blood osmolality is thought to be one of the most important adaptations to life in the freshwater environment (Verwey, 1957), reduction in blood osmolality at high temperatures, would enhance penetration

of fresh water by animals living at relatively low latitudes (Dorgelo, 1981). This effect being particularly significant for *P. longirostris*, as it is a relatively low latitude species. A study of the effect of temperature on the osmoregulation of low latitude freshwater palaemonid species, may thus help resolve the ancestry of the family Palaemonidae.

The results of this thesis have shown that *Palaemon longirostris* is extremely well adapted to life under very dilute and/or fluctuating salinity conditions. The physiological adaptations to osmotic change demonstrated for *P. longirostris*, further suggest that this species represents an evolutionary link between brackish- and freshwater palaemonids. It is the conclusion of this author that the evidence reported in this thesis supports the hypothesis that the family Palaemonidae is in a transitionary status and is still in the process of invading freshwater (Ortmann, 1902; Hedgpeth, 1949, 1957; Born, 1968; and Moreira et al., 1983) rather than is secondarily invading the marine environment (Panikkar, 1941).

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