AN ECOPHYSIOLOGICAL STUDY OF THE EFFECTS OF CHANGES IN SALINITY AND TEMPERATURE ON THE DISTRIBUTION OF *MACROBRACHIUM PETERSI* (HILGENDORF) IN THE KEISKAMMA RIVER AND ESTUARY.

Dissertation

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by

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SUMMARY

The distribution of adult, juvenile, post larval and larval Macrobrachium petersi (Hilgendorf) was studied in relation to temperature and salinity in the Keiskamma river and estuary from May 1979 to May 1981. M. petersi is a subtropical species, which confined activity of all stages in the field to the summer months. Variable freshwater discharge and tidal effects determined the salinity and temperature profiles in the middle and upper reaches of the Keiskamma estuary. Thus, a dry 1979/80 summer and a wet 1980/81 summer markedly influenced the abundance and distribution of adult M. petersi in the river and estuary respectively. Adult M. petersi migrate to the estuary under flooding conditions and upstream in response to elevated salinities.

Although M. petersi has nine larval stages only a preponderance of stage I were caught in the field. These portray a distinct nocturnal and diurnal distribution pattern which is influenced by salinity, especially under stratified conditions. After flooding the larvae show an affinity for salt front regions and reach these by remaining in the water column on the ebbing tide. A substantial drop in abundance downstream from the salt front suggests that the larvae loose their planktonic phase which is an effective retention mechanism, and confines larval development to the middle and upper reaches of the estuary. Post larvae were caught towards the end of the 1981 breeding season which indicates that complete larval development takes place in the estuary. A post larval migration to freshwater, which reaches a peak in February and March, was monitored. Thus both the freshwater and estuarine environments form an inseparable link in the life cycle of M. petersi.

The distribution of stage I larvae in the Keiskamma estuary suggested that salinity played a role in development. The fact that other larval stages were not found emphasised the necessity for a quantitative laboratory investigation to determine the importance of salinity in the developmental history of M. petersi larvae and post larvae. However, the modifying influence of temperature could not be ignored so a multivariable approach was adopted. This, together with a surface response technique, aided the interpretation of the effect of a variety of combinations of salinity and temperature on ecdysis to stage II, larval survival and requirements for metamorphosis to post larvae. It was estimated that the

minimum salinity requirement for complete larval development, within a temperature range from 18 to 30°C was $8^{o}/_{oo}$, although ecdysis to stage II and metamorphosis to post larvae could occur in salinities less than this value. Despite the euryhalinity of the larvae, the behaviour of adult M. petersi to an increase in salinity and the affinity of stage I larvae to salt front regions restricted development to the upper reaches of the estuary. This is discussed as an adaptation which not only ensures retention within the estuary but favours recruitment to the adult population in freshwater.

The osmoregulatory patterns of larval, post larval, juvenile and adult M. petersi correlated with their distribution. These were approximated by a cubic polynomial which enabled the different patterns to be compared. The larval stages investigated (I, II, V & IX) displayed a remarkable capacity to regulate which was strongest in stage I as these could regulate in both freshwater and $35^{o}/_{oo}$. The ability to regulate in freshwater was lost hereafter but regained in the post larvae, which also regulated in $35^{o}/_{oo}$. Juveniles (caught at the ebb and flow) displayed a similar regulatory pattern to the adults and "hyposmoconformed" in salinities beyond the isosmotic point as the need to regulate in $35^{o}/_{oo}$ was no longer necessary. The osmoregulatory capacity of M. petersi larvae in relation to other decapod larvae is discussed.

Marine transport of the euryhaline larval and post larval stages accounts for the distribution of M. petersi along the South African coastline. However, south of 31°S latitude the sea-surface temperature decreases abruptly. This region coincides with the southern limit of the distribution of M. petersi. Although larval M. petersi can tolerate high salinity ($35^{o}/_{oo}$) in combination with low temperature, the post larvae cannot, which is likely to account for their restricted southern distribution.

The genus Macrobrachium are in the process of invading freshwater. The possible course that this might have taken has been discussed in the light of available evidence as well as the findings of this study.

INTRODUCTION

The Republic of South Africa is an arid country. As it is well advanced both agriculturally and economically tremendous demands are made on the meagre water supply. Coupled with this fact, dams have been constructed in many of the major river catchment areas, causing a further reduction in the flow of water to the coastal plain, and consequently to the estuarine environment. It is not unreasonable to assume, due to the escalating rate of development, that in the not too distant future questions regarding the ecological role of freshwater in the estuarine environment will arise.

In South Africa estuarine research has been orientated more towards the mouth and lower regions of the estuary where tidal and marine effects exert a major influence on the extant biota. Very few studies, if any, have centered around the head and upper regions where the tide and freshwater discharge determine changes in composition and abundance of the estuarine fauna. Day (1981a) has mentioned that this region of the estuary is the most variable of all. The variability is to a large extent due to the erratic freshwater discharge which in times of extreme flooding can inundate the entire estuary. A dry and wet summer over the two year study period offered an insight into the hydrological heterogeneity of this region in the Keiskamma river estuary, situated on the south eastern seaboard of South Africa.

The Palaemonidae are a large family with representatives from the marine, estuarine and freshwater environments (Hedgpeth, 1957). Prawns from the genus Macrobrachium have a wide global distribution and species occupy both freshwater and brackish water environments (Johnson, 1967). Holthuis (1950) has recorded over 100 Macrobrachium species from the Indo-Pacific region. Despite the large number and species diversity, relatively little is known about their ecology. It is perhaps due to the commercial emphasis (Hanson & Goodwin, 1977) upon Macrobrachium rosenbergii that relatively few biological or ecological studies have been conducted on other members of the genus in recent years. Important exceptions are the work of Ibrahim (1962), Raman (1967), Rao (1967), Ling (1969), Rajyalakshmi & Ranadhir(1969) and Rasalan et al., (1969) which covered the general biology and fishery of certain Macrobrachium species. Information on detailed integrated field and

laboratory investigations to elucidate some of the interesting biological characteristics of this genus remain, however, few.

There are seven species of Macrobrachium in South Africa (Kensley, 1972) of which Macrobrachium petersi (Hilgendorf) is endemic, and according to Barnard (1950) its distribution is typically subtropical, stretching from Tete on the Zambezi river in the north to the Umtata river in the south. This was the first record of their occurrence in the Keiskamma river and offered a unique opportunity to study the prawn in its natural environment.

Recently, comprehensive laboratory studies have been carried out by Nelson et al., (1977a & b) on the commercially important M. rosenbergii. They investigated the prawns energetic requirements under various temperature and salinity combinations, dietary regimes etc. to determine conditions under which the prawns would respond optimally for purposes of commercial propagation. In addition, the results have also been used to explain the animal's distribution and behaviour in the natural environment, but as Nelson et al., (1977b) have remarked, "detailed studies of the biology and behaviour of the prawn in native habitats are few, and available reports serve to provide an ecological context for interpretations of the metabolic responses to environmental conditions." This state of affairs is not ideal and in this investigation an attempt has been made to integrate field and laboratory studies into an ecophysiological whole so that patterns and causes of distribution of M. petersi, with particular emphasis upon salinity, temperature and their interaction, can be better understood. I have been greatly assisted in this task by the powerful techniques of multivariable analysis and its application to biological studies (Alderdice, 1972). In my view this statistical tool aids immeasurably in the interpretation of complex multivariable responses.

It is hoped that the results of the present study will contribute to a better understanding of the freshwater/salt water interface region and stimulate further research in this much neglected area of South African estuaries.

PART ONE

THE KEISKAMMA RIVER BASIN AND ESTUARY.

1. Physiographic features.

The catchment area of the Keiskamma river, largest river system within the proclaimed boundaries of the Ciskei, covers about 35% of the homeland's surface area. It fans out over an area of 2530 km^2 across the central southern most part of the Ciskei with the narrowest point at the coast. The location of the Ciskei in relation to Southern Africa is shown in Fig. 1 (page 4).

1.1 The Keiskamma River Basin.

Within the boundaries of the basin (Fig. 1) three major zones of relief can be recognised; (a) the Amatola Mountain Range which forms the headwaters of the Keiskamma river and rises to an elevation of 2000 m above sea level, (b) the Central Plateau which ranges in elevation between 200 and 600 m through which the river follows a narrow path and (c) the Coastal Belt which is a relatively narrow strip of land fluctuating in width from 12 to 20 kms. The coastline is reinforced by a ridge of low sand dunes well stabilised by dune vegetation of the Capensis region (Taylor, 1978). Uplift of the coastline during the Tertiary has caused a rejuvenation of the river valley which is deeply incised (Haughton, 1969). The valley is widest at the mouth region where there is a relatively large alluvial flood plain. Most of the Keiskamma River Basin is underlain by sandstones, quartzites and mudstones. Deposits of consolidated and semi-consolidated aeolian sands occur along the coast (Mountain, 1946).

The climate in the Keiskamma River Basin is generally temperate to warm and humid with a definite summer rainy season which reaches a maximum in autumn and a minimum in winter (Schulze, 1965). Fig. 2 (page 5) shows the mean monthly rainfall in the Keiskamma River Basin over a twenty year period. Almost 70% of the annual rainfall occurs in the seven months between October and April, March normally being the wettest month. Mean annual rainfall is highest on the mountain peaks. It ranges between

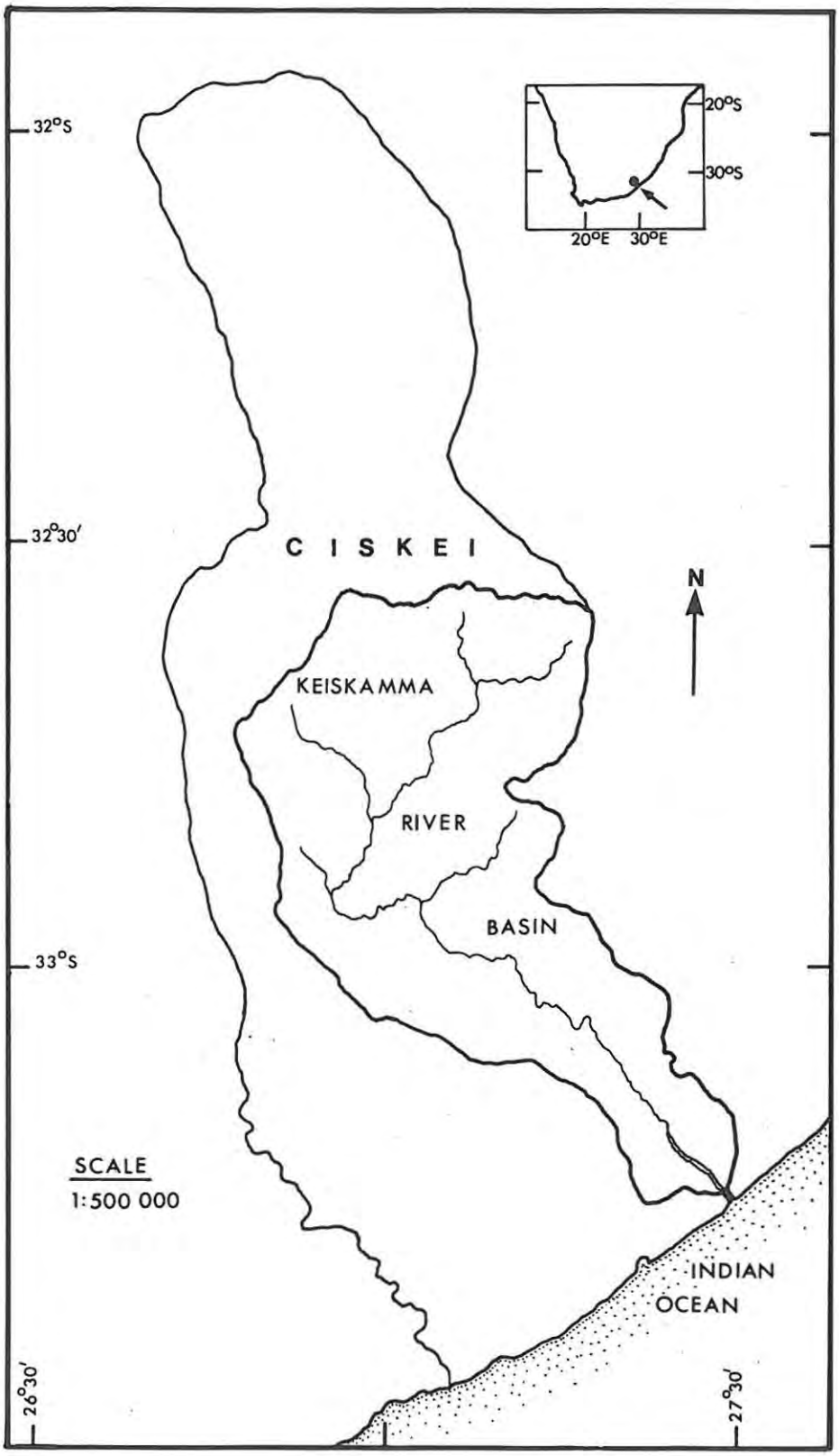


Fig. 1 Keiskamma river basin within the boundaries of the Ciskei. Inset: Southern Africa showing location of the Ciskei.

450 - 650 mm on the Coastal Plateau and 600 - 700 mm along the Coastal Belt. Most of the tributaries draining into the Keiskamma river in the Amatola Mountains have a perennial flow while those that drain the semi-arid Coastal Plateau have a seasonal flow despite relatively large catchments.

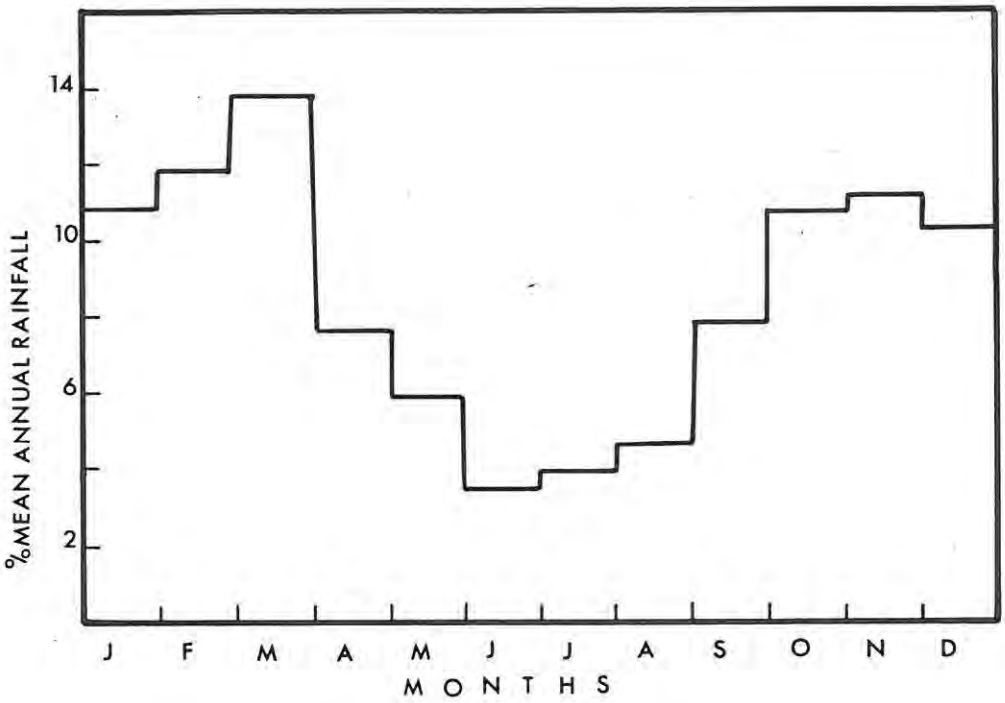


Fig. 2 The mean monthly distribution of rainfall in the Keiskamma River Basin. (Anon, 1977).

1.2 The Keiskamma River at the Ebb and Flow.

The ebb and flow of the Keiskamma river is determined by a gauging weir (Fig. 3) which marks the head of the Keiskamma estuary. Fig. 4 shows spot monthly river temperatures recorded at the weir before 0800 hrs. The minimum temperature recorded was approximately 12.0°C in June 1979 reaching a maximum of 28.0°C in February 1980. No temperatures were taken in June and July 1980; a maximum of 27.8°C was recorded in January 1981. In summer the maximum daily temperature range at the weir between 0800 and 1800 hrs was approximately 1.0°C.

The period of my studies from March 1979 to May 1981 was characterised by erratic rainfall which was somewhat at variance with

Fig. 3 Gauging weir at the ebb and flow.

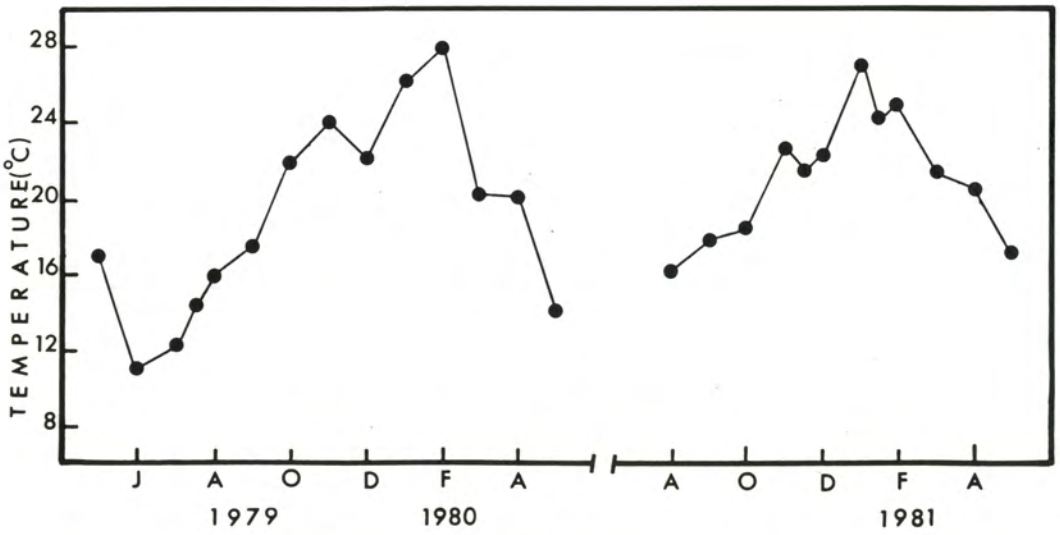


Fig. 4 Spot monthly temperatures taken at the weir before 0800 hrs.

the data reported in Fig. 2. Fig. 5 shows that the summer of 1979/80 was generally dry and that of 1980/81 wet.

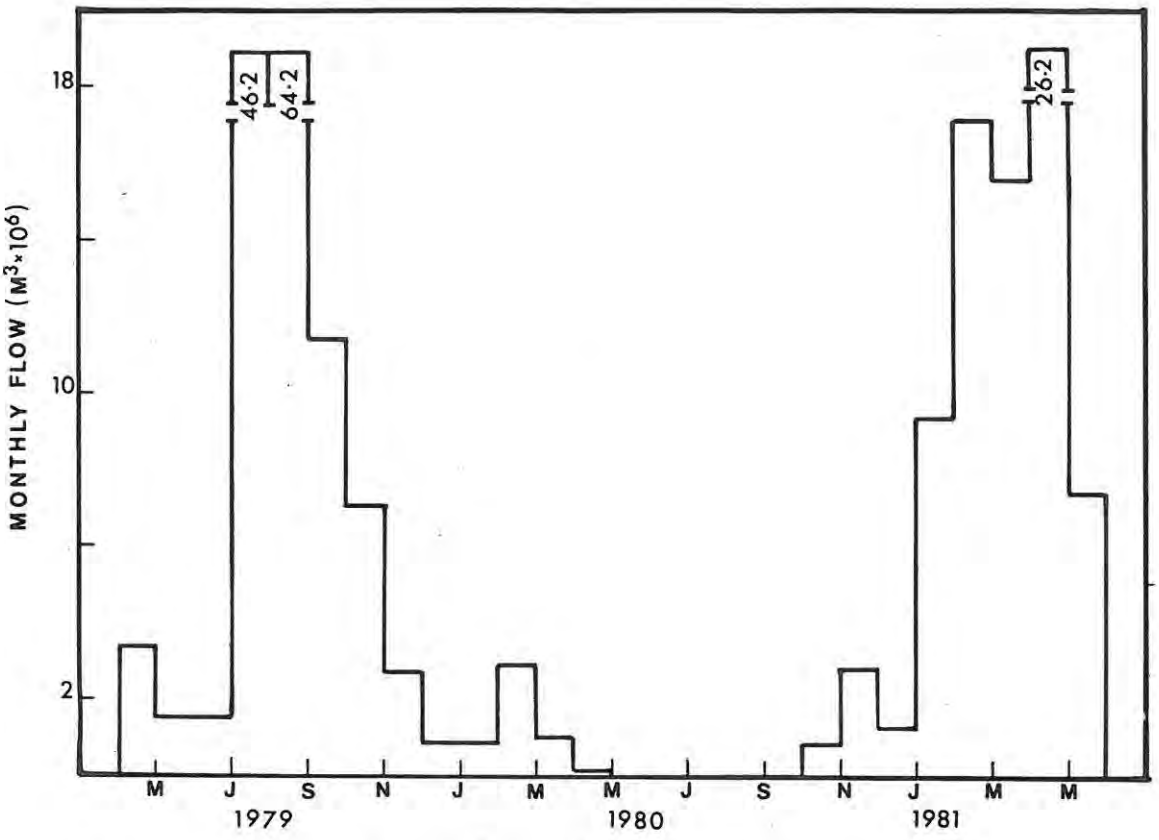


Fig. 5 Monthly flow ($m^3 \times 10^6$) of water into the Keiskamma estuary from February 1979 to May 1981.

During the summer of 1979/80 (November to April) a mean of $1.5 \times 10^6 m^3$ of freshwater discharged into the estuary whereas for the same period in 1980/81, $12.5 \times 10^6 m^3$ was discharged. Fig. 5 indicates that the flow in January, February and March 1981, when four floods occurred, accounted for the increased river discharge. These variable hydraulic conditions made a significant impact on the salinity characteristics of the estuary.

River flow also influenced the total particulate matter (T.P.M.). During non-flooding conditions a mean value of 34.0, SD $\pm$ 18.0 mg ℓ^{-1} (n = 10) was obtained over 10 months. However, during the floods of July and August 1979 (see Fig. 5) the T.P.M. rose to 1847 and 2815 mg ℓ^{-1}

respectively. During July and August a total of 111 x $10^6 m^3$ of water flowed over the gauging weir. At a mean value of 2331 mg ℓ^{-1} T.P.M. the equivalent of 258 000 tons of silt was carried into the estuary. Undoubtedly, most of this was deposited on the flood plain and out at sea but the estuary is likely to have been substantially affected. Possible effects of siltation are referred to later.

Erratic rainfall also affects the concentration of salts, expressed as Cl^-, of the Keiskamma river water. During the dry summer of 1979/80 the mean Cl^- value was 223, SD $\pm$ 96 mg $Cl^-\ell^-$ (n = 13). This rose to a maximum of 576 mg $Cl^-\ell^{-1}$ in October 1980 but with the onset of the first spring rain the concentration dropped to 35.5 mg $Cl^-\ell^{-1}$ and remained in that range for the remainder of the study. Presumably, the elevated Cl^- values were due to the combined effects of low rainfall and evaporation. The pH ranged between 8.1 and 8.4.

1.3 The Keiskamma Estuary.

The mouth of the Keiskamma river (33° 17'S/27° 29'E) opens into the Indian Ocean at Hamburg, 60 kms south west of East London (Fig. 6). The head of the estuary is determined by a gauging weir built in 1969. Prior to this, a rocky outcrop 2 kms upstream formed the natural ebb and flow. The estuary is 18 kms long and navigable to small craft over its entire length. Numerous streams drain into the estuary. At the head and in the upper reaches the river is 15 to 25 m wide and passes through a narrow valley. Thereafter the valley broadens and the river meanders downstream through a 2 to 3 km broad flood plain. The width of the mouth varies but is permanently open between rocks on the northern side, and a sandspit on the southern. The dominant flora in the intertidal region are thickets of Phragmites communis with patches of Scirpus litoralis and Cyperus natalensis (Cowling et al., 1979). The depth of the river varies between 2 and 3.5 m and the water is usually turbid. Siltation is a problem. The faunal biomass and submerged vegetation is impoverished compared with other Eastern Cape estuaries (McLachlan & Grindley, 1974; Branch & Grindley, 1979). Cowling et al.,(1979) and Day (1981b) attribute this to siltation and water turbidity. These phenomena, which affect all warm temperate estuaries of the Eastern Cape to a greater or lesser extent,

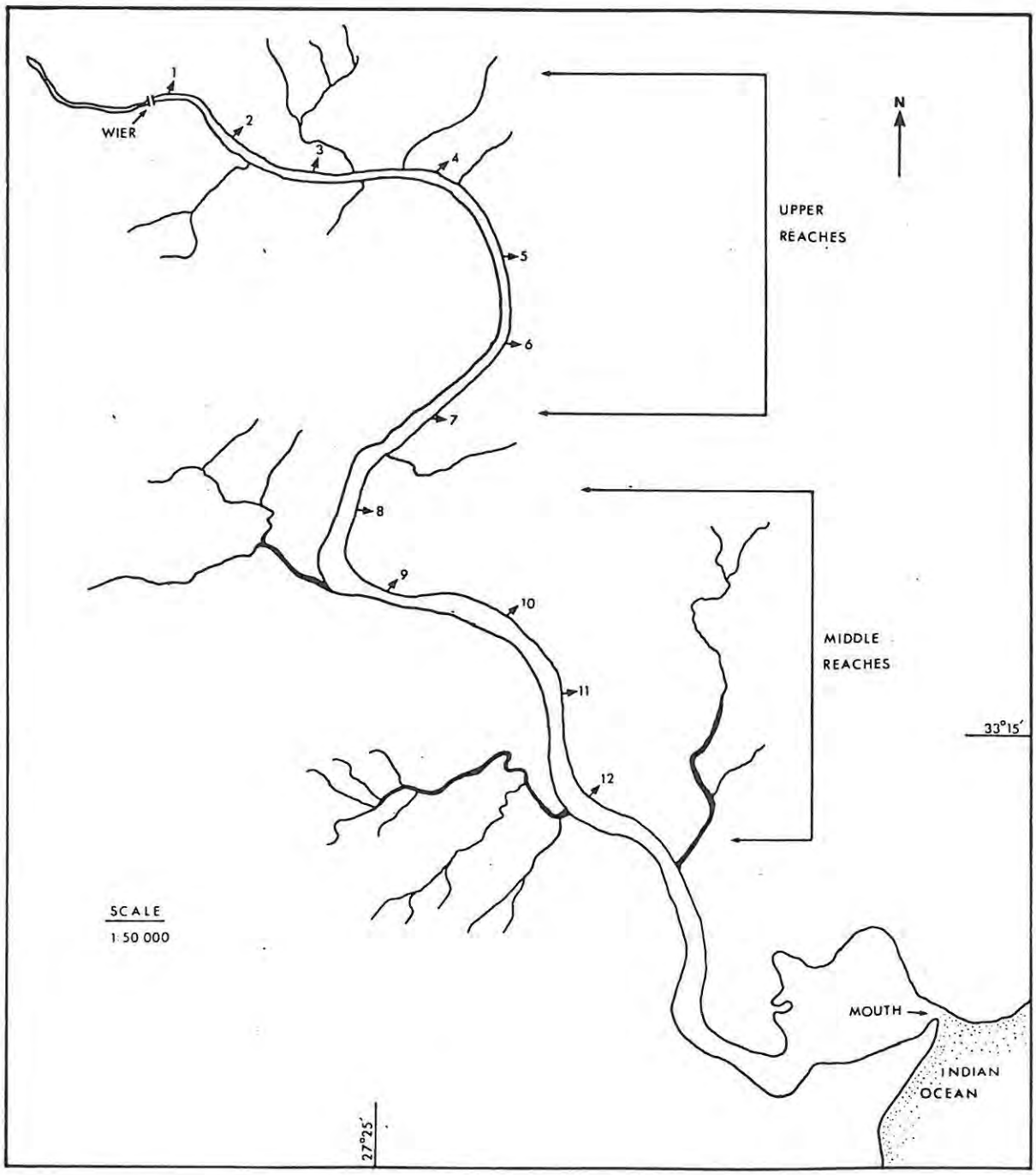


Fig. 6 The Keiskamma estuary and sampling stations.

are undoubtedly due to periodic summer flooding and poor agricultural practices in the catchment area. The problem is chronic in Natal where Begg (1978) has stated that at least 45 out of the 73 estuaries show deterioration through siltation. Begg regards siltation as the principal explanation for the impoverished state of Natal estuaries. Siltation, whether due to urbanisation, agriculture or industry, is ubiquitous but may be exaggerated in the South African context as our estuaries are considerably smaller than those of Europe and North America.

The difference in height above sea level between the mouth and the head is approximately 0.3 m. Therefore, tidal amplitude at the head can be significant and varies between 0.4 and 1.3 m. The duration of the flood tide is less than that of the ebb, a characteristic of South African estuaries (Day, 1981a). The time lag between high and low tide at the mouth and head can vary between 50 and 90 mins.

This brief outline has served to introduce the Keiskamma river and estuary. In the following section attention is focused on two factors, namely freshwater discharge and tidal movement which alter the distribution of salinity and temperature in the upper and middle reaches.

2. The distribution of salinity and temperature in the upper and middle reaches of the Keiskamma estuary.

The estuary has been divided into upper (stations 1 - 6) and middle (stations 7 - 12) reaches (Fig. 6). The stations are approximately 1 km apart and thus the numbers 1 - 12 also indicate distance (kms) downstream from the weir at the head of the estuary. It has not been possible to define the regions more precisely because of the large variation in salinity that each region experiences.

Temperature differences are less pronounced than those of salinity, seasonal variation being greater than that caused by tidal incursion. Although daily changes in these two factors occur, salinity *per se* is the more likely factor to account for variation in faunal distribution patterns. Thus in the following section more emphasis has been placed on salinity than on temperature fluctuation.

2.1 Material and methods.

All data were recorded from a 3 m glass fibre dinghy powered by a 3.5 h.p. Seagull outboard engine. Water samples below the surface were obtained using a Frascati bottle. Salinities $>1.0^{\circ}/_{\circ\circ}$ were read in situ using an optical refractometer. Samples showing $<1.0^{\circ}/_{\circ\circ}$ were stored and salinity determined in the laboratory using a chloride titrator (Radiometer, Copenhagen). The values were read as m-equiv. Cl^{-1} (SD $\pm$ 0.84) and converted to salinity using the expression $S^{\circ}/_{\circ\circ}$ = $0.30 + 1.805\ Cl^{-}{}^{\circ}/_{\circ\circ}$ (Harvey, 1955). Temperature was recorded in °C ($\pm$ 0.1) using a standard mercury thermometer.

2.2 Salinity profiles.

During 1979/80 11, and 1980/81 26 horizontal salinity patterns of varying extent were recorded between stations 1 and 12. More observations were taken in the latter season due to the erratic river flow which regularly altered the prevailing salinity regime.

In terms of the criteria laid down for the classification of estuaries (McLusky, 1981) the Keiskamma estuary can be considered a positive estuary with incoming salt water on the bottom and fresher outgoing water at the surface. However, the pattern of salinity distribution within the estuary varies widely so that at any one time the estuary may be simultaneously highly stratified, partially mixed or homogeneous with respect to salinity. This is due to the nature of Southern African estuaries which differ from their European counterparts in that they are generally shallower, narrower, shorter, not always permanently open and are subject to erratic flooding (Day, 1981c). Thus, although the factors (tidal amplitude, river discharge, width and depth) which affect the current system and the distribution of salinity are essentially the same, the relationships amoung the variables are different. This results in highly variable and dynamic environments. The Keiskamma estuary is no exception.

As previously mentioned, the 1979/80 season was characterised by a greatly reduced freshwater flow. Fig. 7 shows the changes in surface salinity between stations 1 and 10 in November 1979, and January and August 1980. At all stations mixing was complete, so the

salinity readings reflect that of the entire water column. Salinity at station 1 increased from $0.5^{o}/_{oo}$ in November to $5.0^{o}/_{oo}$ in January. By August the salinity had reached $24.0^{o}/_{oo}$ at station 1.

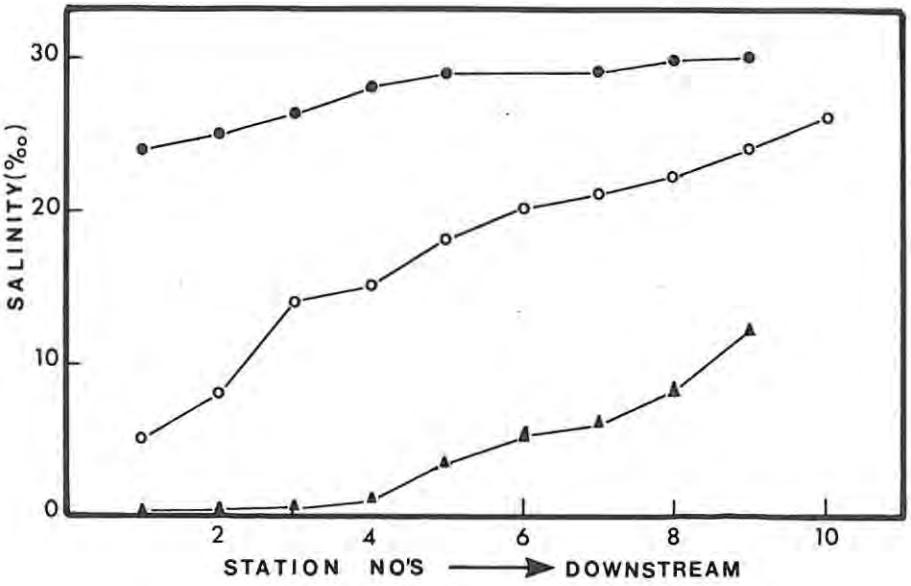


Fig. 7 Surface salinity ($^{o}/_{oo}$) between stations 1 and 10 in November 1979 ▲—▲ ; January 1980 o—o and August 1980 ●—● .

During the 1980/81 season salinity patterns were highly variable due to the erratic freshwater flow. Fig. 8 shows the change in salinity distribution as a result of a flood on 26 March when the river level, measured at the weir, rose 1.35 m. It is appreciated that the differing patterns of salinity stratification shown in Fig. 8 represent a complex set of hydraulic events, in which are included variation in salt wedge distribution during each tidal cycle and the entrainment of dense saline water by the upward movement across the interface, reducing the overall salinity. The sharp salinity profile, (Fig. 8 b & c) which was eventually established, is largely due to the rapid upstream movement of a salt wedge (Table 1) and the maintained freshwater discharge. Fig. 8 d & e shows the mixing effect due to an increased tidal intrusion and reduction in river flow, while Fig. 8 f provides yet another example of the reinstatement of stratification due to an increase in freshwater flow. Thus, within a short period, 38 days,

the estuary between stations 1 and 12 has moved through homogeneous, highly stratified and partially mixed conditions.

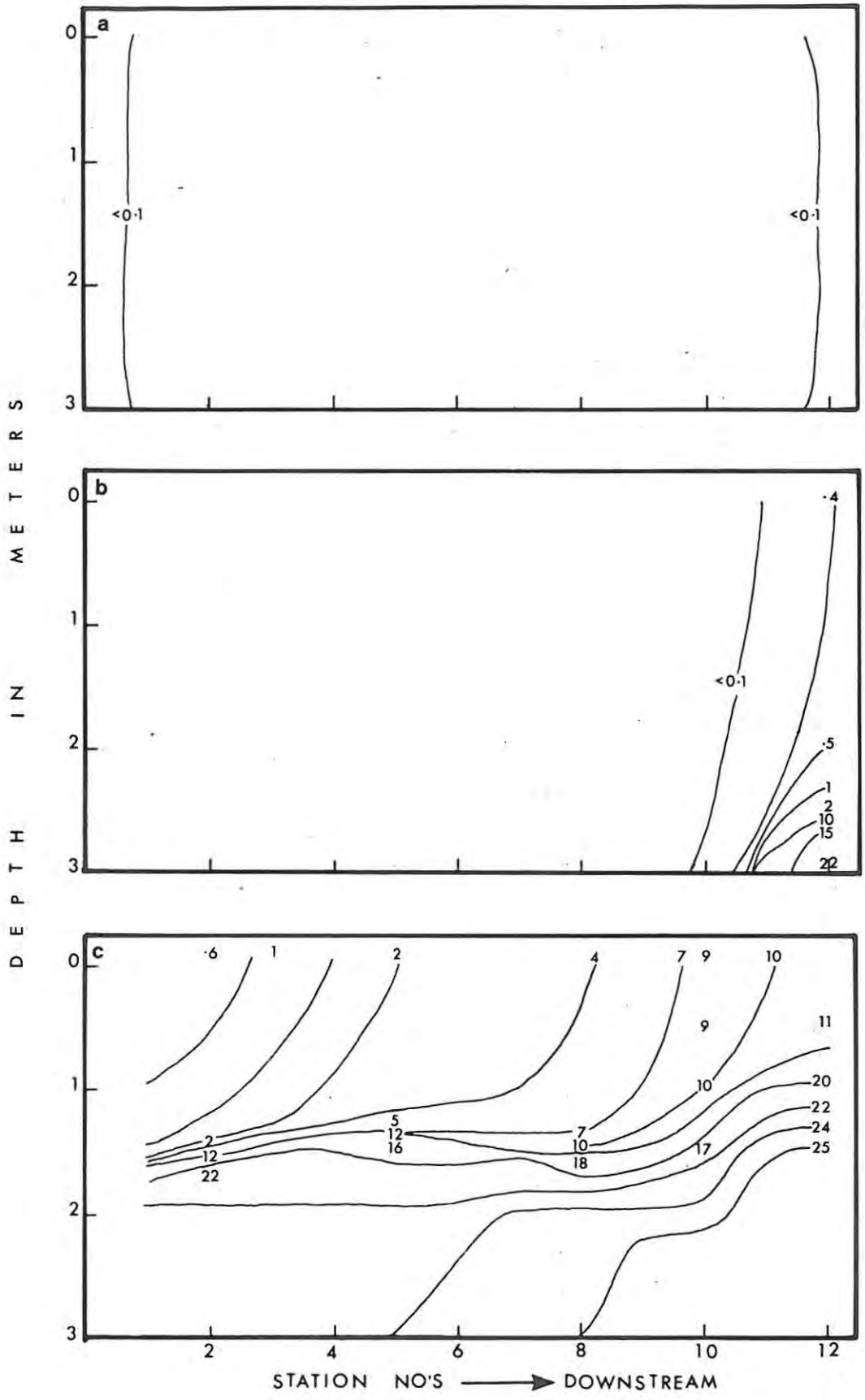


Fig. 8 (pages 13 & 14) Vertical salinity profiles which show the re-establishment of saline water at station 1 after a flood on 26 March 1981.

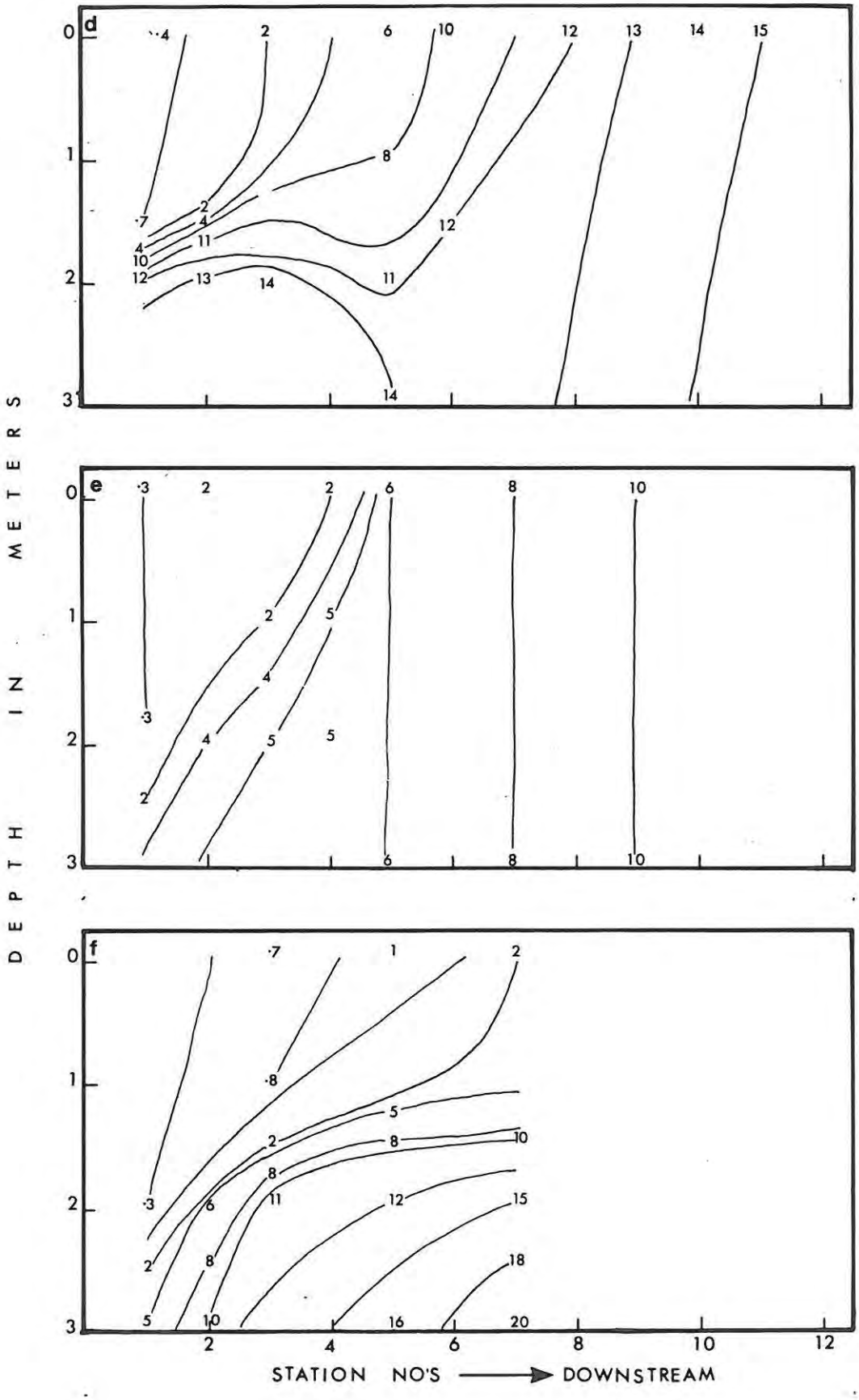

d
e
f
DEPTH IN METERS
STATION NO'S → DOWNSTREAM

Table 1. *In situ* upstream movement of a salt wedge under variable freshwater discharge and tidal regimes. In the first column the two dates indicate the observed positions of the wedge in relation to a particular station from which the interval between observations (column 2) can be determined and the upstream movement (column 3) approximated.

Observed dates of salt wedge	Number of days between observations	Approximate distance moved (kms)
9 - 14 December 1980	5	2
14 - 17 December 1980	3	3
30 January - 5 February 1981	7	6
5 - 12 February 1981	7	8
26 February - 5 March 1981	7	8
5 - 16 March 1981	11	5
8 - 15 April 1981	7	8

Fig. 9 shows four examples of the tidal effect on salinity in homogeneous, partially mixed and stratified conditions. The change can either be quite substantial (Fig. 9 a & b) or less dramatic (Fig. 9 c & d). In the summer of 1979/80 the estuary was homogeneous with respect to salinity and Fig. 10 shows the horizontal change in surface salinity during high and low spring tides in January and April 1980 respectively.

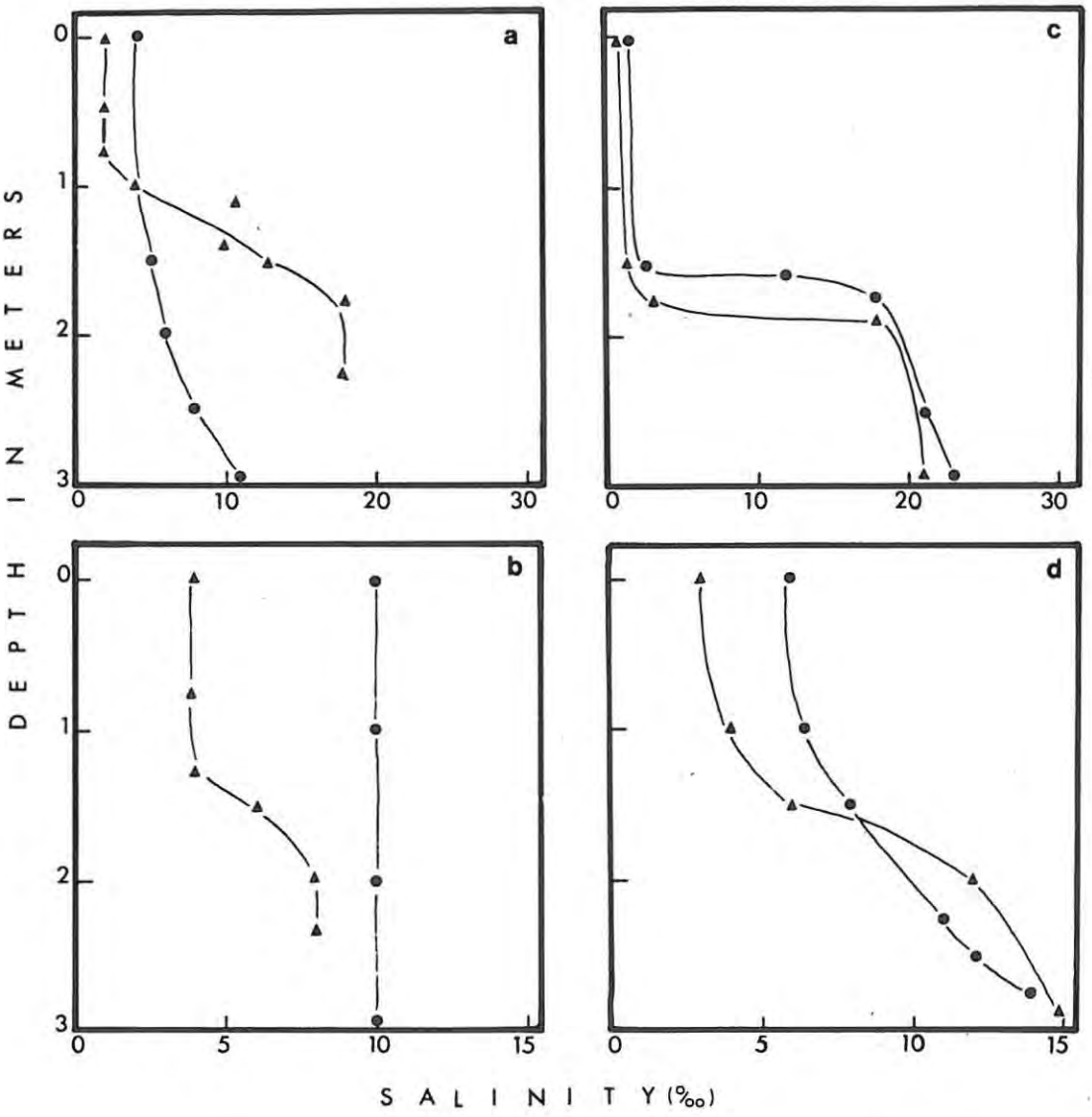


Fig. 9 Vertical salinity profiles at high ●——● and low ▲——▲ tides in partially mixed (a) and (d), homogeneous (b) and stratified (c) conditions.

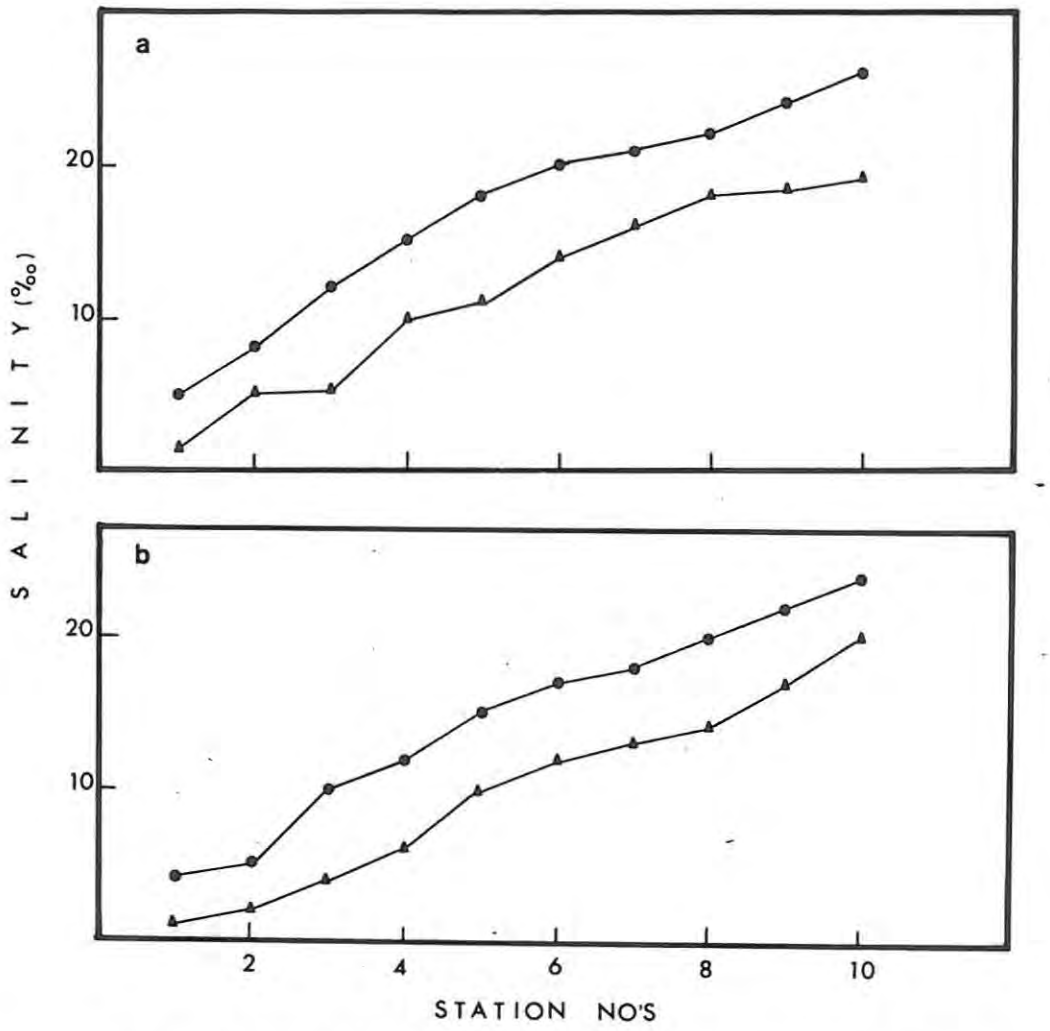


Fig. 10 Horizontal profiles of surface salinity changes during high ●——● and low ▲——▲ spring tides in January (a) and April (b) 1980.

2.3 Temperature profiles.

The patterns of temperature stratification were defined by the relative distribution of seawater and freshwater during the tidal cycle and season of the year. A typical set of these patterns is given in Figs. 11, 12 and 13 and Tables 2 and 3.

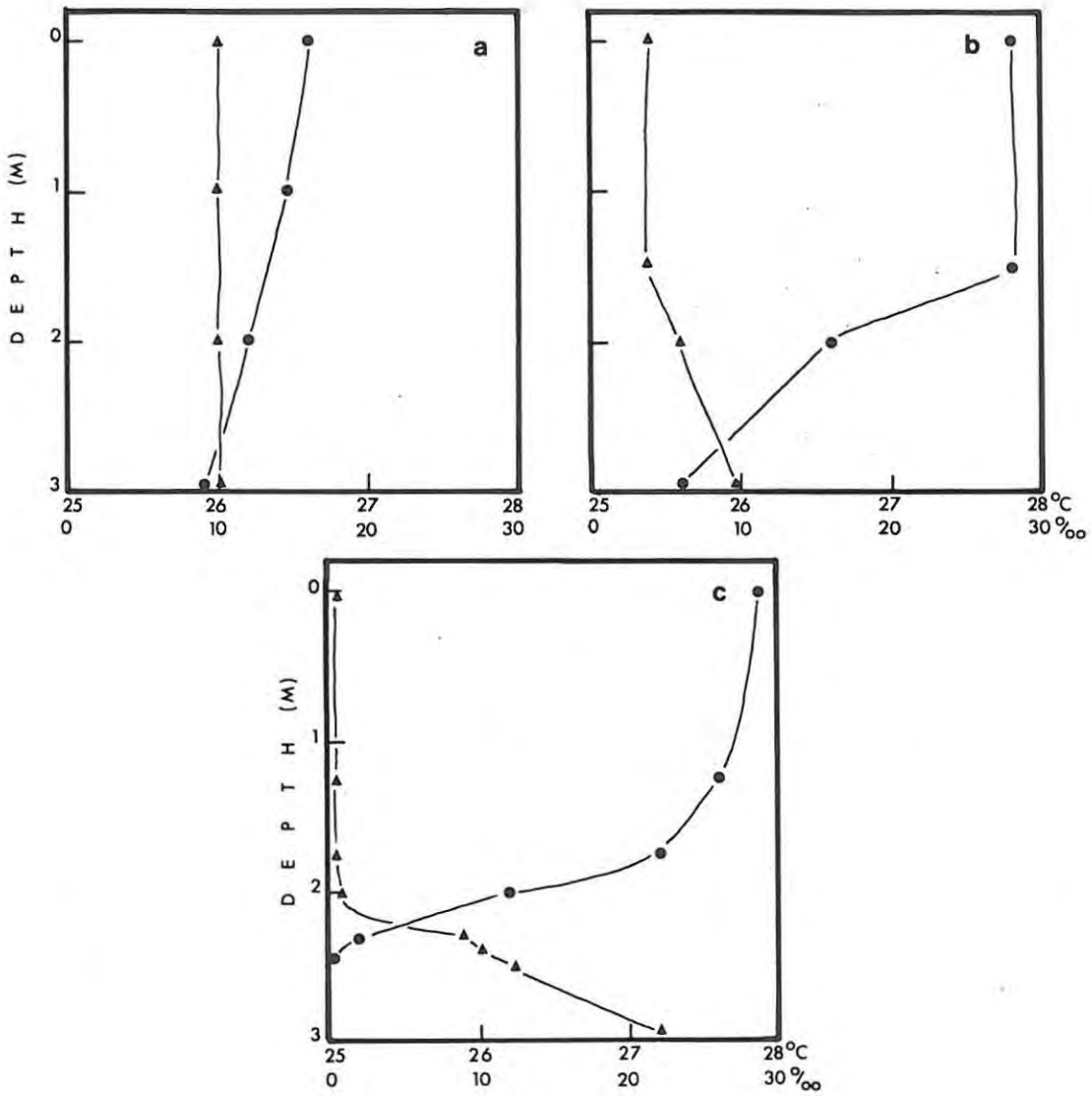


Fig. 11 Vertical temperature (●) and salinity (▲) profiles at stations 5 (a), 3 (b) and 1 (c) on 20 January 1981.

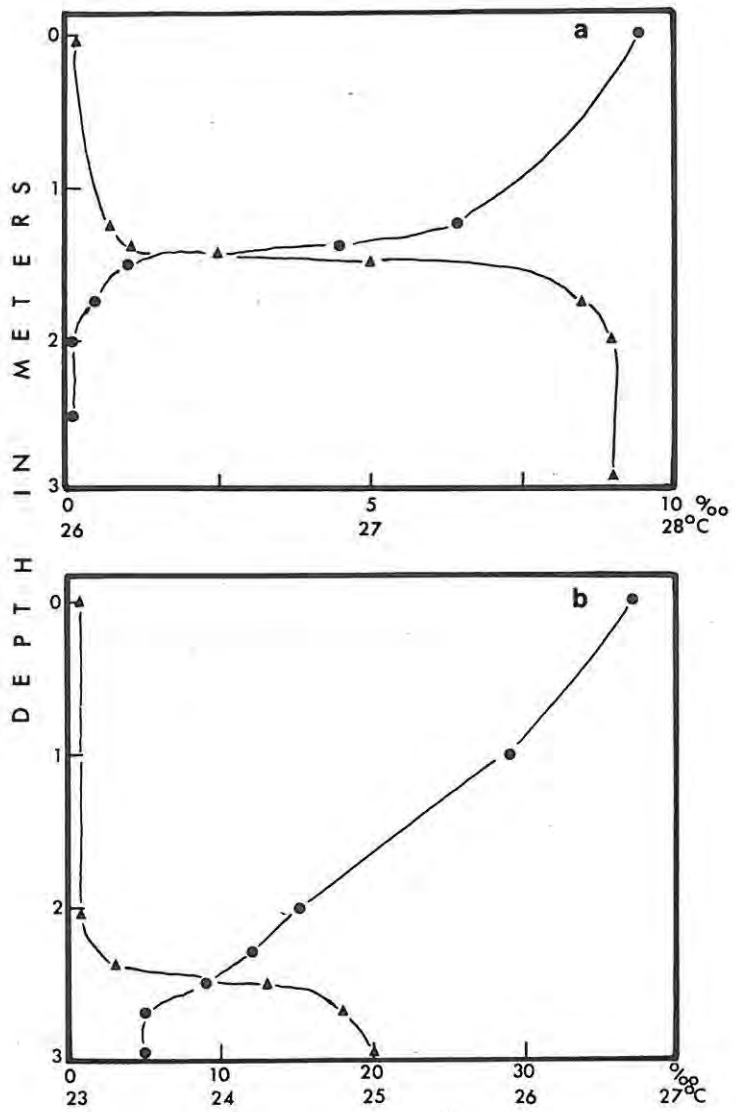


Fig. 12 Vertical salinity (▲) and temperature (●) profiles at station 1 on 13 January (a) and 14 February (b) 1981.

Fig. 11 records the salinity and temperature profiles upstream from station 5 on 20 January 1981. These data demonstrate that temperatures were markedly influenced by salinity discontinuities. In stratified conditions that had been established for a long time (Fig. 12 a), differences in temperature and salinity were not as marked compared with those where the stratification had been recently established (Fig. 12 b). Comparing the vertical temperature profiles

in a stratified region of the estuary in winter, (Fig. 13 a) revealed that the water above the halocline was cooler than that below; in summer (Fig. 13 b) the opposite occurred. Although temperature differences due to tide do occur, these were minimal (<1.0°C) and not likely to be of much consequence ecologically.

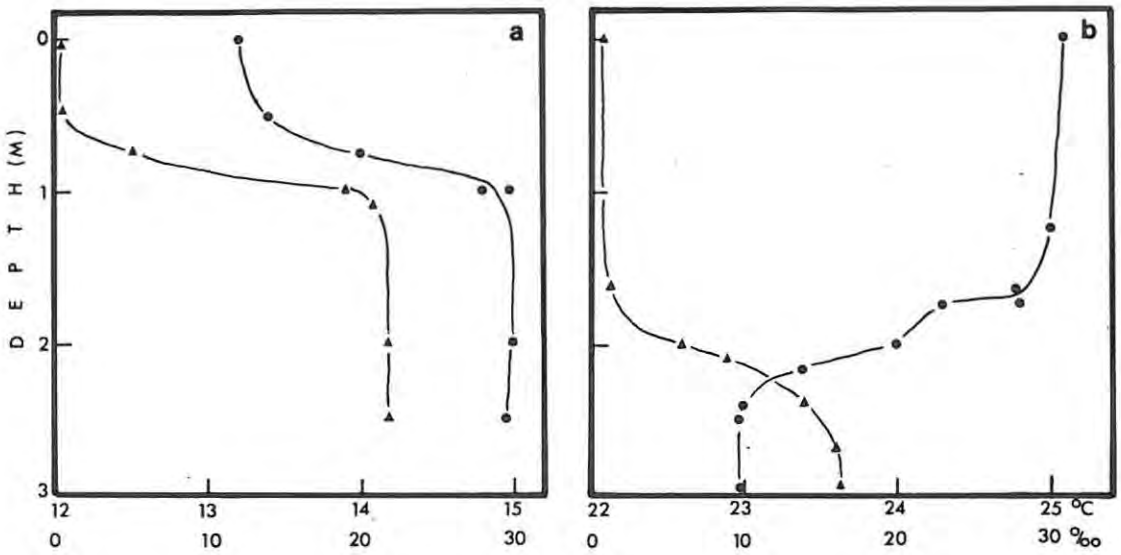


Fig. 13 Vertical salinity ▲——▲ and temperature ●——● profiles on 13 July 1979 (a) and 15 January 1981 (b).

Table 2. Horizontal surface and bottom temperatures (°C) in January, April and August 1980.

Month	Station Number 1	2	3	4	5	6	7	8	9	10
January										
Surface	29.7	29.2	28.3	28.3	28.3	28.3	28.2	28.4	28.3	28.0
Bottom	29.1	28.2	28.0	28.2	28.2	28.3	28.1	28.3	28.4	27.9
April										
Surface	21.8	21.2	21.4	21.2	21.4	21.3	21.2	21.1	21.1	21.1
Bottom	21.4	21.6	21.4	21.4	21.7	21.6	21.7	21.5	21.4	21.4
August										
Surface	16.3	16.5	16.4	16.7	16.6	-	16.6	16.5	16.3	-
Bottom	16.2	16.3	16.5	16.6	16.6	-	16.6	16.4	16.4	-

Table 2 shows the horizontal surface and bottom temperatures during January, April and August 1980 when the estuary was homogeneous with respect to salinity. These temperatures were far more consistent compared with those in February 1981 (Table 3) when the estuary was being alternately influenced by freshwater and seawater respectively.

Table 3. Horizontal surface and bottom temperatures (°C) during February 1981.

	Station number											
	1	2	3	4	5	6	7	8	9	10	11	12
Surface	28.2	28.2	25.0	-	23.0	23.0	22.9	23.5	23.8	23.8	23.7	23.5
Bottom	21.5	21.6	21.6	-	21.8	21.8	21.5	21.8	22.0	22.0	22.1	21.8
Surface	25.2	24.9	-	-	25.3	-	24.5	-	25.0	25.2	-	26.2
Bottom	24.8	24.6	-	-	24.5	-	21.0	-	21.6	21.2	-	20.0
Surface	25.4	-	24.5	-	24.3	-	23.1	23.9	-	23.5	-	23.2
Bottom	25.1	-	24.5	-	24.2	-	22.5	21.9	-	22.5	-	22.5

The data that I have presented on the distribution of salinity and temperature in the middle and upper reaches of the Keiskamma estuary, serve to illustrate what is already well known about estuaries, namely that they are highly variable, dynamic bodies of water. This instability is somewhat different in the Southern African context in that major changes in salinity distribution can take place over a relatively short space of time. This feature was aptly demonstrated by the record of events shown in Fig. 8. Such instability is a characteristic of many South African estuaries.

The low freshwater discharge in the 1979/80 summer has given an indication of the role of freshwater in an estuarine system; it maintains heterogeneity in an environment that would otherwise become homogeneous. The 1979/80 summer salinity profiles attest to this fact. It would appear, then, that freshwater is an essential estuarine component (Schroeder, 1978) necessary to offset the otherwise dominating influence of the sea.

PART TWO

DISTRIBUTION AND ABUNDANCE OF MACROBRACHIUM PETERSI (HILGENDORF) IN THE KEISKAMMA RIVER AND ESTUARY IN RELATION TO CHANGING PATTERNS OF SALINITY AND TEMPERATURE.

Part one has recorded the range of temperature and salinity variation in the head, upper and middle reaches of the Keiskamma river. In part two the ecological significance of this heterogeneous environment is critically examined during the life cycle of M. petersi. Sexually mature adults of certain species from the genus Macrobrachium are known to undergo a spawning migration to estuarine waters at the onset of the breeding season (Gunter, 1937; George,1969; Ling, 1969; Choudhury, 1971b;Monaco, 1975). As the migratory behaviour of M. petersi is as yet unrecorded, I decided to sample the freshwater environment over a two year period both above the weir and in the estuary. It was argued that if both these environments were sampled simultaneously, changes in distribution would be reflected as differences in abundance. The dry and wet summers of 1979/80 and 1980/81 respectively resulted in marked different patterns of distribution both in the freshwater and estuarine environments. These changes are recorded in the first section.

1. Changes in distribution and abundance of adult M. petersi in the Keiskamma river and estuary.

1.1 Capture methods.

Traps used to catch juvenile and adult M. petersi were made of 3 mm thick galvanised diamond mesh (4 mm). A cone with a 1.5 cm hole at the apex was spot welded at each end of a cylindrical trap 30 cm long and 15 cm in diameter, allowing the prawns to enter at either end (Fig. 14). Captured prawns were removed via a 10 x 10 cm aperture, cut into a side of the trap. The aperture was closed by a 12 cm x 12 cm hinged lid of diamond mesh. An airtight plastic ball, which served as a marker, was attached to each trap. Fresh bait was made for each trapping series by adding 1.5 kg of fishmeal to a heated mixture of water (500 ml) and 500 g of maizemeal. Gelatin (50 g), which acted as a binder, was dissolved

in 100 ml of boiling water, thoroughly blended with the fish/maizemeal mix, rolled into approximately 3 cm diameter balls and deep frozen. All traps below the weir between stations 1 and 12 were laid from a boat in the littoral stands of Scirpus and Phragmites approximately 1 m from the bank. Above the weir the traps were laid from the bank. A preliminary exercise showed that the mean number of prawns (2.08, SD $\pm$ 1.4) caught overnight in 12 traps was significantly greater ($t = 4.179$; $P<0.001$) than that caught during the day (0.58, SD $\pm$ 0.66); consequently, all traps were set in the late afternoon and retrieved the following morning.

Fig. 14 Trap used to catch juvenile and adult M. petersi.

1.2 Distribution and abundance in the Keiskamma river, 1979-1981.

In April, May and September 1979, 5, 10 and 12 traps respectively were laid at 'Crossroads', 13 kms upstream from the weir to determine the extent of M. petersi's upstream distribution. An equal number of traps was set above the weir to serve as control. No prawns were caught at 'Crossroads' but the control yielded 4, 8 and 12 prawns in April, May and September respectively. These data suggested that the upstream distribution of M. petersi in the Keiskamma river was restricted to an area in close proximity to the head of the estuary. An aerial view of

the area between the weir and 'Crossroads' revealed six causeways across the river. It was possible that artificial barriers such as these could be responsible for the limited upstream distribution of M. petersi. Sampling in the Keiskamma river therefore was carried out in an area that extended from the weir to 0.75 kms upstream. All traps were set along the western bank only. In 1979/80 the trap number varied from 5 to 13 per month while in the following season, 1980/81, 9 to 25 were used each month. Changes in abundance of M. petersi caught above the weir from May 1979 to May 1980 and from September 1980 to May 1981 were recorded together with mean monthly temperatures (Fig. 15).

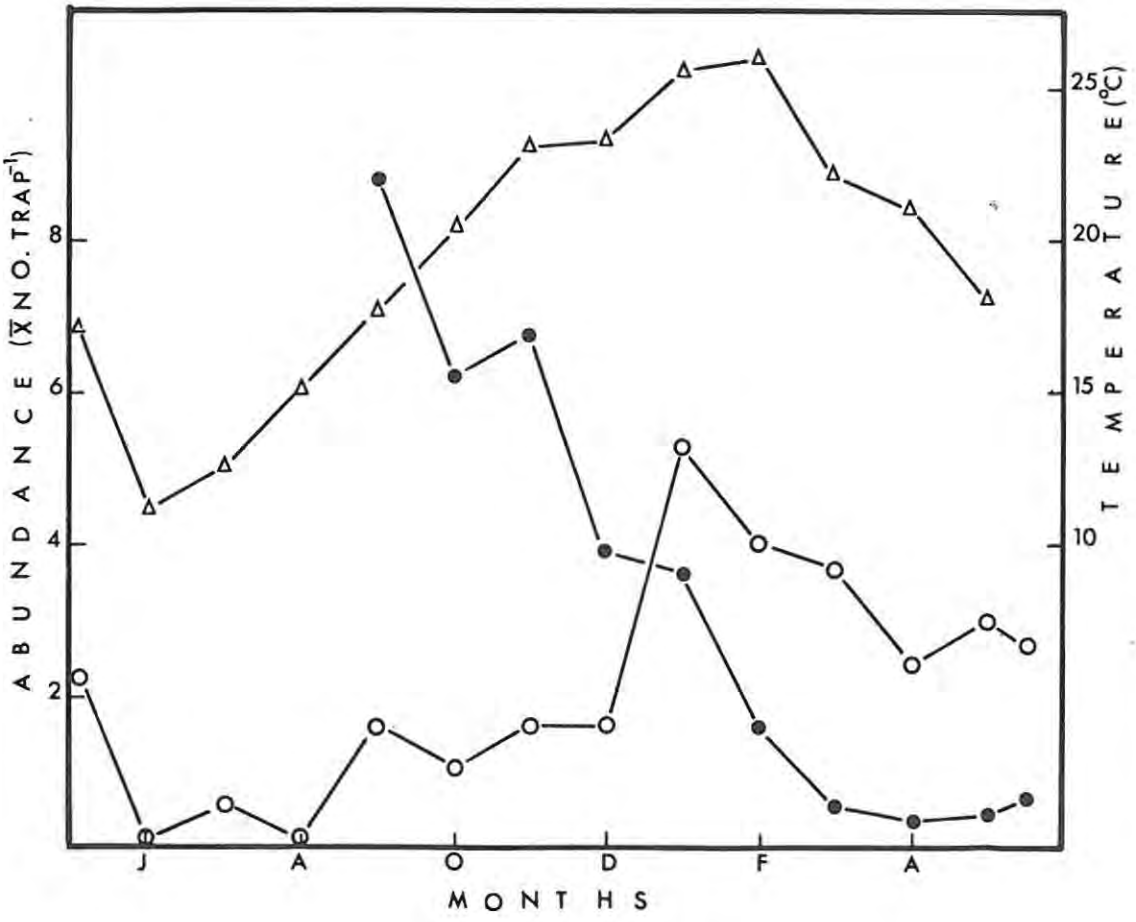


Fig. 15 Mean monthly temperature Δ—Δ and changes in abundance (mean no. trap^{-1}) of M. petersi above the weir in 1979/80 O—O and 1980/81 ●—●

In the 1979/80 season an apparent correlation existed between prawn abundance and temperature which was not evident in the 1980/81

season. This anomaly suggested that the changes in abundance above the weir (Fig. 15) were correlated with the response of the prawns to changes in salinity in the head of the estuary. During the 1980/81 floods, a reduction in salinity below the weir to levels that the adults could tolerate, enabled them to exploit the estuarine environment to maximum advantage; hence the pronounced reduction in abundance from September 1980 to May 1981. Although no adults were caught moving from the freshwater above the weir to the estuary below, I have interpreted this steady reduction in abundance as evidence of a breeding migration in M. petersi. However, in the 1979/80 season, rising salinities at the head of the estuary, in response to a reduced river flow, caused an upstream migration over the weir. This, it was argued, was responsible for the significant increase in abundance from 1.8 in December 1979 to 5.3 in January 1980 ($t = 4.62$; d.f. = 22; $P<0.001$). Utilisation of the favourable estuarine environment during 1981 is shown in Table 4.

Table 4. Analysis of the change in abundance (mean no. $trap^{-1}$) of adult M. petersi above the weir between January and May in 1980 and 1981 respectively.

	MONTHS					
	Jan.	Feb.	Mar.	April	May (early)	May (late)
YEAR	ABUNDANCE (mean no. $trap^{-1}$)					
1980	5.3	4.0	3.9	2.5	3.0	2.7
1981	3.6	1.6	0.58	0.38	0.41	0.66

Statistical analysis

Source of variation	Degrees of freedom	Sum of squares	Mean square	F ratio	Significance
Main effects	5	374.44	74.88	18.37	P<0.001
Year	1	191.72	191.72	47.04	P<0.001
Month	4	209.76	52.44	12.86	P<0.001
Interaction	4	8.12	2.03	0.49	Not significant
Residual	143	582.74	4.07		
Total	152	965.30	6.35		

The mean monthly abundance above the weir from January to May was significantly higher in 1980 compared with 1981. Mean monthly abundance values presented in Table 4 were also significantly different. In 1980 the decrease in abundance from January to May probably reflected emigration as the prawns moved further upstream. In 1981 the reduction in abundance indicated movement to the estuarine environment. It appeared, therefore, that the two patterns of abundance above the weir during 1979/80 could be satisfactorily explained in relation to the variable salinity regimes that characterised the upper reaches of the estuary. This either discouraged (1979/80) or encouraged (1980/81) exploitation of the estuary by adult M. petersi.

In the light of these findings it was not unreasonable to expect that patterns of abundance in the upper estuary were likely to be highly variable and correlated with the changing salinity regime. The estuarine distribution of adult M. petersi is reported in the following section.

1.3 Distribution and abundance in the Keiskamma estuary, 1979/80.

Fig. 16 shows the vertical salinity profiles and estuarine distribution of M. petersi expressed as mean number per trap per month between September 1979 and May 1980 (see pages 27 & 28). There were no data for February. The number of stations sampled varied between months but stations 1, 2 and 3 were sampled every month. The number of traps used for each sampling series varied between 14 and 20. Early in the 1979/80 season the estuary was inundated with freshwater with a resultant high abundance (Fig. 16 a) and downstream distribution (Fig. 16 c). By December 1979 however, a reduced river flow resulted in an increase in salinity at the head of the estuary and as a consequence an upstream migration of M. petersi over the weir. For example,between January (Fig. 16 e) and May (Fig. 16 h) a downstream distribution was no longer apparent and the abundance had dropped to below one prawn per trap. These changes appear to have been brought about by the increase in salinity in this region of the estuary from 6 - 10$^{o}/_{oo}$ in January to 17 - 22$^{o}/_{oo}$ in May.

Changes in abundance which took place at stations 1, 2 and 3 in the upper and head regions of the estuary between September 1979 and May 1980 are shown in Table 5 (page 29). The results were analysed

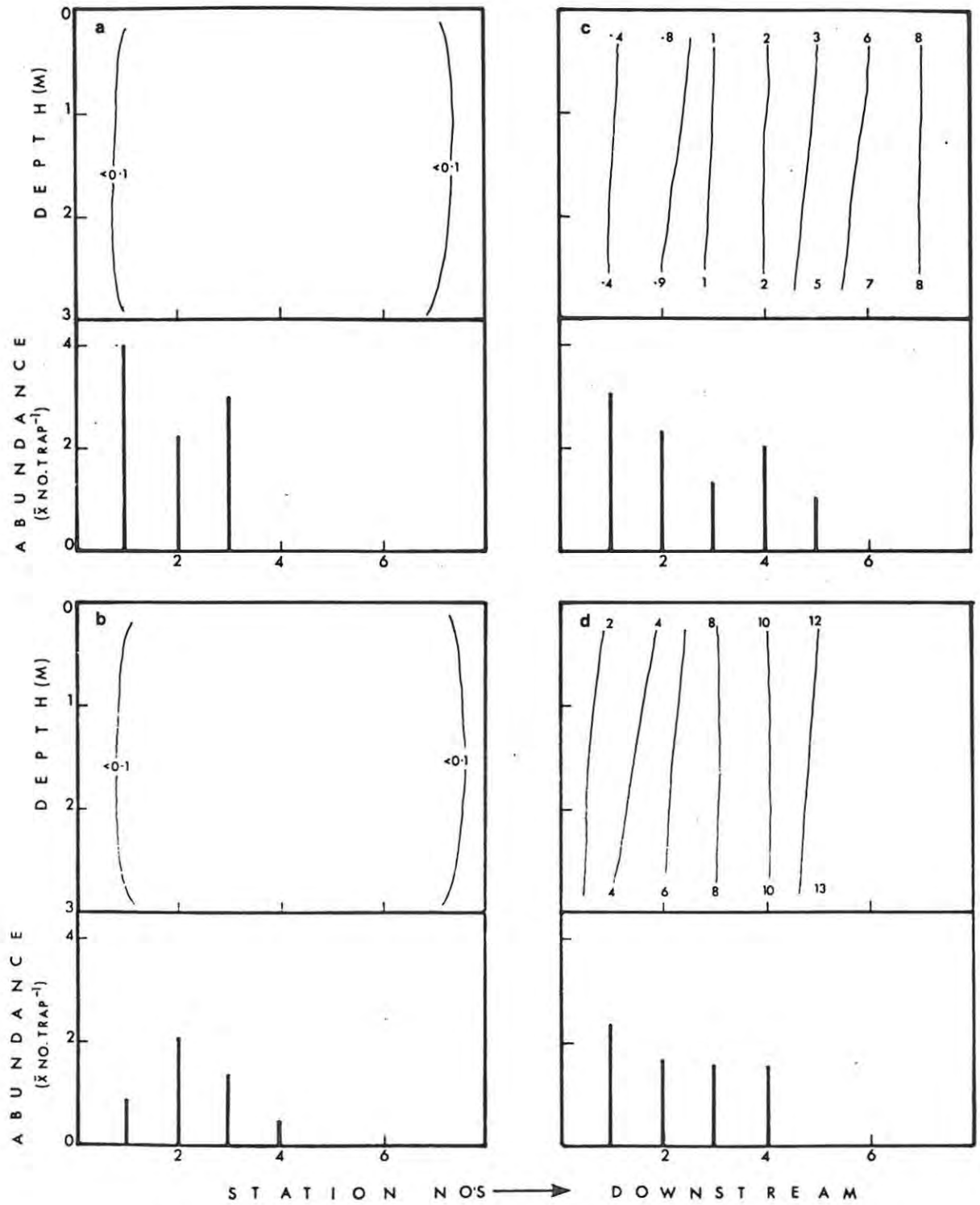


Fig. 16 (pages 27 & 28) Vertical salinity profiles, changes in abundance (mean no. trap^{-1}) and distribution of M. petersi from September 1979 (a) to May 1980 (h) respectively.

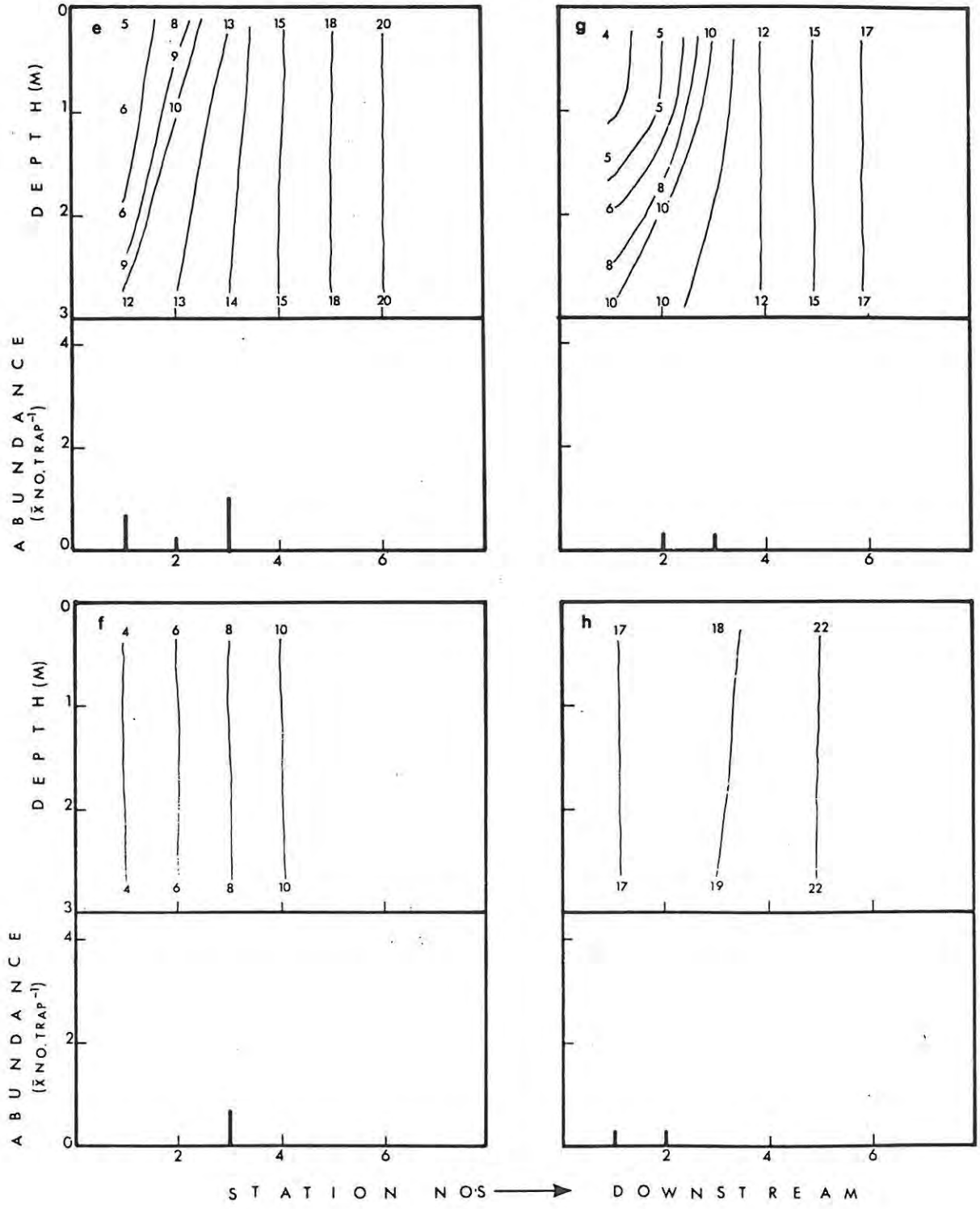

e
g
f
h
DEPTH (M)
ABUNDANCE (x̄ NO. TRAP⁻¹)
STATION NOS → DOWNSTREAM

Table 5. ANOVA of change in abundance (mean no. $trap^{-1}$) of M. petersi at stations 1, 2 and 3 between September 1979 and May 1980. Means underscored by a common line are not significantly different at the 95% confidence level. (L.S.D. $P \leq 0.05$).

MONTHS							
Sept.	Oct.	Nov.	Dec.	Jan.	Mar.	April	May
ABUNDANCE (mean no. $trap^{-1}$)							
3.0	1.81	2.12	1.76	0.66	0.23	0.18	0.25

Statistical analysis

Source of variation	Degrees of freedom	Sum of squares	Mean square	
Between months	7	161.96	23.13	$F_{7,86} = 5.89$
Within months	86	337.50	3.92	$P<0.001$
Total	93	499.46		

using analysis of variance (ANOVA) as four or more traps (replication) were used at each station. As the difference between more than two means was being tested, the least significant difference (L.S.D.) method was used to indicate whether means differed significantly. Results indicate (Table 5) that the data can be treated as two separate groups, one between September and December and the other between January and May. The difference in monthly abundance within each group was not significant but abundance between the two groups was significant ($P<0.001$). These changes in abundance were associated with an increase in salinity at the head of the estuary as a result of the reduced freshwater discharge during the dry 1979/80 summer. The decrease in adult abundance in the estuary matches the marked increase in abundance in January 1980 above the weir. The higher catch in January 1980 has already been discussed in section 1.2.

The reduction in numbers and restriction in distribution suggested that increases in salinity played a major role in regulating the abundance and distribution of adult M. petersi. However, water

temperature had also changed (see Fig. 4 for January and April temperatures) and its possible influence, in combination with salinity, could not be ignored. The interaction between salinity, temperature and abundance of adult M. petersi had been examined by assuming that a second order polynomial would provide an adequate fit to the data (see page 74). The essentials of this analysis are given for each sampling station in Table 6.

Table 6. An analysis of variance of the interaction effect between temperature, salinity and abundance (mean no. trap^{-1}) of adult M. petersi between May 1979 and May 1980.

	Station No. 1			Station No. 2			Station No. 3		
Year	Abundance (mean no. trap^{-1})	Temp. (°C)	Sal. (‰)	Abundance (mean no. trap^{-1})	Temp. (°C)	Sal. (‰)	Abundance (mean no. trap^{-1})	Temp. (°C)	Sal. (‰)
1979	0.5	17.9	12.0	0.0	18.1	15.0	0.0	18.3	15.0
	0.3	15.2	22.5	0.7	12.4	0.4	1.8	15.4	15.0
	0.8	15.3	0.4	0.3	15.4	0.4	5.8	18.3	0.4
	1.7	23.5	0.4	1.8	18.6	0.4	3.3	22.9	0.4
	3.0	26.7	0.4	2.3	23.7	0.4	1.7	25.2	1.7
1980	2.0	26.4	2.7	2.3	25.4	1.3	4.0	26.2	9.3
	0.8	26.6	6.0	2.0	26.4	2.7	1.2	26.6	12.0
	0.0	26.4	6.0	0.3	26.8	10.0	1.6	26.7	12.0
	0.0	21.3	6.0	0.0	26.5	10.0	0.3	20.3	10.0
	0.3	15.6	15.0	0.1	20.9	8.0			

Statistical analysis

Station No.	Coeff. of determination (R^2)	Interaction effect as % of total variation explained	F ratio	Significance
1	89.2	44.2	$F_{1,8} = 32.74$	P<0.001
2	92.6	21.8	$F_{1,8} = 22.79$	P<0.001
3	95.16	53.2	$F_{1,7} = 106.4$	P<0.001

These data, collected over one year, reveal that the interaction between temperature and salinity accounted for a significant proportion of the total variance. Thus, changes in abundance and distribution were due to both temperature and salinity and not salinity per se.

1.4 Distribution and abundance in the Keiskamma estuary, 1981.

Erratic floods characterised the summer of 1981 so that the head and middle reaches were inundated with freshwater for considerable periods of time. The variation in vertical salinity profiles with changes in distribution and abundance of M. petersi between stations 1 and 7 from February to May 1981 is shown in Fig. 17 (pages 32 & 33). It appeared that the freshwater conditions enabled M. petersi to extend its downstream distribution to station 7 compared with station 5 in the previous year. No traps were set beyond station 7 but the high mean abundance recorded (Fig. 17 b & c) suggested station 7 was not the limit of their downstream distribution. As in the previous year unfavourable salinity conditions have influenced the distribution pattern. Fig. 17 c shows that whereas M. petersi were caught at station 7 in April 1981, the distribution had narrowed to between stations 4 and 5 by early May (Fig. 17 d) and at stations 1, 2 and 3 by late May (Fig. 17 e). Associated with this decrease in abundance was an increase in salinity and a drop in water temperature from April to May. Once again the combined effects of increased salinity and reduced temperatures altered distribution. Apparently the prawns responded to the elevated salinities relatively quickly. In April (Fig. 17 c) the total number of prawns caught between stations 4 and 7 was 18. By early May however a salt front (Fig. 17 d) had been established and only one prawn was caught.

Table 7 (page 34) shows the variation in prawn abundance between stations 3 to 7 from February to May. These stations were chosen because they experienced fluctuating salinities and therefore would most likely demonstrate the effect of salinity and temperature on abundance. ANOVA (Table 7) revealed that catches in May were significantly lower than those of February, March or April. Thus adult M. petersi responded to an increase in salinity and decrease in temperature as in the previous year. The high abundance at stations 1 and 2 in late May (Fig. 17 d) suggested an upstream movement away from the salt front.

Confirmation of the response of adult M. petersi to elevated salinity was demonstrated by comparing abundance at stations 1, 2 and 3 during March, April and May in 1980 with that of 1981. A two-way ANOVA (Table 8, page 34) of monthly abundance between 1980 and 1981 showed that

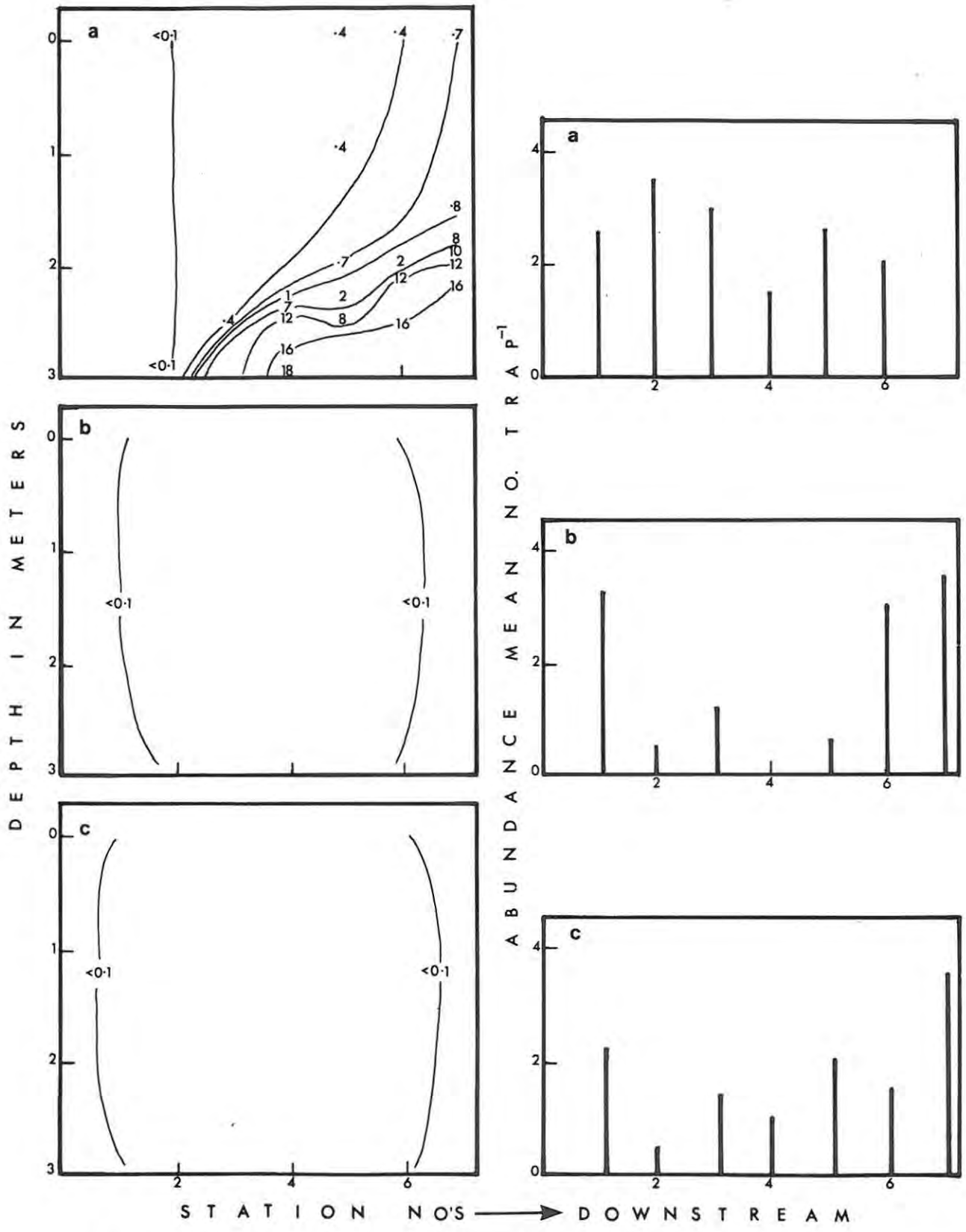
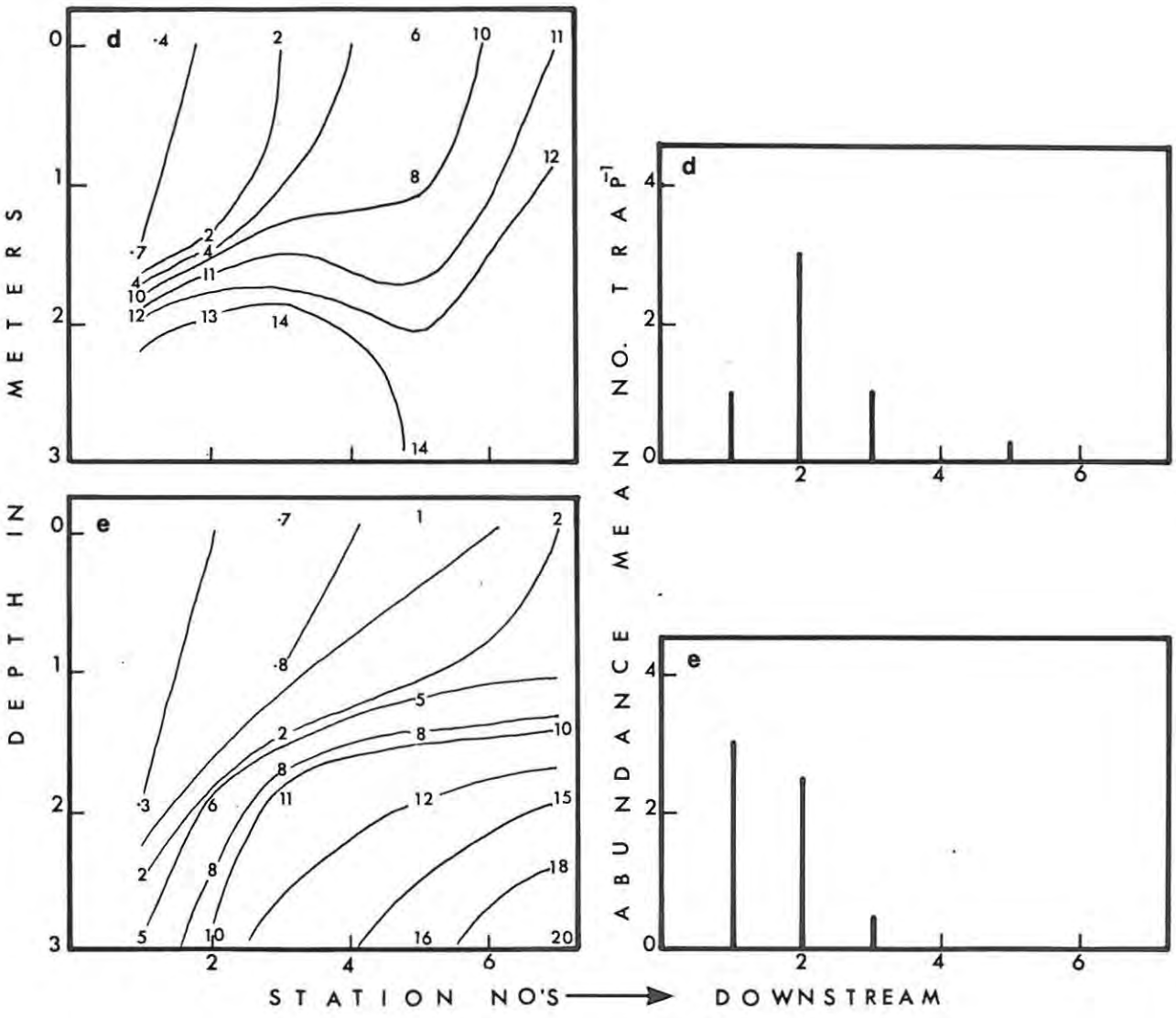


Fig. 17 (pages 32 & 33) Vertical salinity profiles, changes in abundance and distribution of M. petersi during February (a); March (b); April (c); early May (d) and late May (e), 1981. Trap number varied between two and four per station.


d
e
DEPTH IN METERS
ABUNDANCE MEAN NO. TRAP^{-1}
STATION NO'S → DOWNSTREAM

the between month catch was not significantly different in either year but between years the difference was highly significant.

Table 7. ANOVA of change in abundance (mean no. $trap^{-1}$) of M. petersi at stations 3, 4, 5, 6 & 7 from February to May 1981. Means underscored by a common line are not significantly different at the 95% confidence level (L.S.D. $P \leq 0.05$).

February	March	MONTHS April	May (early)	May (late)
		ABUNDANCE (mean no. $trap^{-1}$)		
2.23	1.58	1.92	0.38	0.23

Statistical analysis

Source of variation	Degrees of freedom	Sum of squares	Mean square	
Between months	5	35.05	7.01	$F_{5,59} = 5.59$
Within months	59	94.94	1.60	$0.001<P<0.002$
Total	64	130.00		

Table 8 ANOVA of change in abundance (mean no. $trap^{-1}$) of M. petersi at stations 1, 2 and 3 in March, April and May 1980 and 1981 respectively.

	MONTHS March	April	May
YEAR	ABUNDANCE (mean no. $trap^{-1}$)		
1980	0.23	0.18	0.25
1981	1.77	1.50	1.50

Statistical analysis

Source of variation	Degrees of freedom	Sum of squares	Mean square	F ratio
Main effects	3	30.35	10.11	P<0.001
Year	1	29.94	29.94	P<0.001
Month	2	0.20	0.10	Not significant
Interaction	2	0.34	0.17	Not significant
Residual	61	104.94	1.72	
Total	66	135.64	2.05	

In both years adult M. petersi experienced salinity changes but 1980 was not punctuated by erratic freshwater discharges which allowed the population, in 1981, to utilise the estuarine environment to maximum advantage. As will be demonstrated, M. petersi larvae require saline conditions for survival. For adults to remain in close proximity to the estuarine environment is therefore adaptive.

1.5 Discussion.

The 1979/81 distribution data on the abundance of adult M. petersi in the Keiskamma river and estuary revealed, due to the contrast in 1979/80 and 1980/81 summer conditions, that adult M. petersi migrate to the estuary under flooding conditions and upstream in response to elevated salinities. Of the variety of Macrobrachium species (Holthuis, 1950) a few were known to undergo a spawning migration to estuarine waters at the onset of the breeding season; the most widely documented migration is that of Macrobrachium rosenbergii (Rao, 1967; Raman, 1967; Ling, 1969; George, 1969). Hughes & Richard (1973) demonstrated experimentally that berried M. acanthurus tend to swim with, and unberried females against, the current. This has been interpreted in the light of the "spawning and post-spawning" migration that the prawn undergoes in its natural environment. As the majority of larvae of Macrobrachium species that have been investigated require saline water, the significance of a migration to the estuarine environment is immediately apparent. What is more difficult to explain is the reaction of adult M. petersi to elevated salinities. Direct response to salinity is often mediated through the organisms osmoregulatory capacity and in such species there is usually a parallel between the organisms osmoregulatory ability and distribution. The differential distribution of grapsoid crabs in certain estuaries offer some cogent examples (Snelling, 1959; Barnes, 1967; Boltt & Heeg, 1975). It was impossible to determine the precise salinity to which M. petersi reacted but it appeared that as soon as the salinity increased to between 4 and 10$^{o}/_{oo}$ adults vacated that particular region of the estuary. Osmoregulatory stress was not the cause of this withdrawal since M. petersi hyperosmoregulates strongly at these salinities (see part four). Thus the salinity range that caused withdrawal was well within the animals regulatory capacity.

In order to account for the potamodromous behaviour of M. petersi three hypotheses have been advanced. Two will be discussed in detail here while the third appears in part four.

The effect of low salinity on fecundity, development of eggs and newly hatched larvae in a variety of invertebrates is well known (see Remane & Schlieper, 1971, for response in Baltic Sea invertebrates; also Hynes, 1954; McLusky, 1968; Dybern, 1967; Forbes & Hill, 1969; Hill, 1974 & Forbes, 1978), although Bynum (1978) has recently shown that no difference existed in the reproductive biology of the caprellid amphipod Caprella penantis inhabitating estuarine and marine habitats.

However, information on the reproductive consequences of freshwater invertebrates penetrating brackish water is meagre (Kinne, 1971). The freshwater sponge Ephydatia fluviatilis lost its potential for asexual reproduction in brackish water (Seifert, 1938; op. cit. Remane & Schlieper, 1971) while the freshwater snail Neritina fluviatilis was smaller in brackish than freshwater and produced smaller egg capsules containing fewer eggs (55 - 80) compared with 100 in freshwater (Bondesen, 1938; op. cit. Remane & Schlieper, 1971). I suggest that the avoidance of increasing salinity by adult M. petersi may be due to the effect of salinity on egg development and the hatching of viable larvae. Indeed, a berried female kept in the laboratory at $20^{\circ}/_{oo}$ produced stage I larvae with deformed abdomens, which impaired swimming, and in certain individuals, development of the antennae had not taken place. Equally, comparatively little is known about osmotic and ionic regulation in eggs and embryos of freshwater invertebrates (Greenaway, 1979). Eggs of the freshwater/land crab Sudanonautes absorb ions from water but have a low salt permeability (Lutz, 1969). Water content on the other hand appears to change during development. It was shown (Wear, 1974) that eggs of decapod crustaceans swell significantly during development, and hatching took place due to the bursting of an outer membrane by pressure from within. Furthermore, in the freshwater prawn Potimirim glabra and the brackish water prawn Palaemonetes vulgaris pressure is caused by an osmotic uptake of water across the inner membrane (Davis, 1964 a, b & c). In the stenohaline lobster Homarus americanus however, swelling of the larvae itself bursts the outer membrane. If the osmotic mechanism is used by M. petersi then an increase in salinity would reduce the inflow of water and so decrease swelling which may affect the viability of the hatch.

Kinne (1971) has introduced the term horohalinicum (from the Greek horos: limit, boundary line) to define the ecophysiological boundary line of 5 - 8‰ where the lowest species number occurred compared with that found in freshwater or marine environments. Khlebovich (1968, 1969) demonstrated that changes in ion ratios (Ca: Na & K : Na) and a relative increase in calcium occurred in the salinity range 4 - 7‰. Thus, estuarine water in the critical salinity range could not simply be regarded as dilute sea water as the relative proportion of solutes varies, especially below 5‰ (McLusky, 1981). Since a marked change in abundance of adult M. petersi also occurred within this salinity range, it is suggested that a change in ionic composition of the water rather than the total salinity per se, may be responsible. This may influence ion transport processes either directly through interference with exchange rates or indirectly via action on the permeability to ions and water (Lockwood, 1976).

2. Changes in distribution and abundance of larval M. petersi in the Keiskamma estuary.

Before the distribution of M. petersi larvae in the middle and upper reaches of the Keiskamma estuary could be determined, the reproductive cycle of M. petersi had to be established. This was to ensure that the investigation of larval distribution coincided with the beginning of the breeding cycle. The breeding cycle was monitored over two years but larval distribution for only one season between December 1980 and May 1981. Part one has demonstrated the variable saline conditions that prevailed during this season. Although only one season's data were reported, the larval distribution patterns that emerged were likely to be representative of those occurring in other years.

2.1 Sampling methods.

Since the trawling programme was conducted by myself, methods used had to be tailored to meet this constraint. Larval collection therefore was carried out in the following manner. A zooplankton net (30 cm diameter; 0.475 mm mesh) was attached to the end of a galvanised iron rod 3.5 m in length and graduated at 0.25 m intervals (Fig. 18, page 38). The rod was mounted at the bow of a 3 m glass fibre dinghy powered by a 3.5 h.p. Seagull outboard engine. The rod could be set

Fig. 18 Dinghy showing mounting used to attach zooplankton and D-nets.

at a fixed depth from the surface so that the water column could be sampled at 0.25 m depths. Samples of the water column immediately above the substratum were taken using a D-net (Fig. 19).

Fig. 19 D-net used to trawl immediately above the substratum.

The diameter of the D was 25 cm and the base 45 cm long. This was mounted on a sleigh of two aluminium rods rounded at the anterior ends. The net was attached to the boat using the same mounting as the zooplankton net. The D-net often became ensnared on debris at the bottom and thus its use was restricted to those parts of the estuary which were comparatively free of such obstacles.

2.2 Catch analysis.

It was found that a 5 min trawl was sufficient to catch a meaningful number of larvae. However, expressing larval abundance per 5 min trawl, especially for comparative purposes, was clearly inadequate. A more acceptable method would be number per unit volume of water filtered.

Applying the relationship, $\text{Volume} = \frac{\text{Distance}}{\text{Time}}$ it was possible to determine the volume of water filtered by a net over a fixed period. Since distance = velocity x time, and if the boat velocity and trawling time were known, multiplying these values by the net area would give an estimate of the volume of water filtered. The boat velocity was determined in the following manner: two markers were set up 183 m apart at station 8. With the zooplankton net, set at various depths, the trawling time with and against the current between the two markers was determined over one tidal cycle. Thus the effects of both changing current velocity and direction were taken into consideration. A mark on the throttle of the motor ensured that each trawl was performed at approximately the same speed. A total of 46 trawls (23 with and 23 against the current) was completed with a mean boat velocity of 48.0, SD $\pm$ 14 m min^{-1}. For a trawl of 5 min duration, irrespective of the state of the tide, it was estimated that

$$48.0 \times 5 \times \frac{22}{7} \times (0.15)^2 \quad \text{or} \quad 17.0 \text{ m}^3$$

of water was filtered. Thus for each 5 min trawl the larval number was divided by 17.0 and expressed as abundance per kℓ of water filtered ($k\ell^{-1}$). Despite a 30% error in the estimate, and since relative and not absolute changes in larval abundance were required, it was felt that the method was sufficiently accurate to portray such changes.

2.3 The reproductive cycle of M. petersi.

As stated in section 1, monthly catches of adult animals were recorded above the weir and in the upper reaches of the estuary to monitor changes in distribution. At the same time the reproductive condition of all females was noted. These data were used to determine the breeding cycle of M. petersi.

Before the eggs hatched a number of distinct reproductive stages involving ovaries and eggs could be identified (Table 9), primarily on

Table 9. Stages of ovary and egg development during the reproductive cycle of M. petersi

Ovary	Description
Stage I	Partially distended, yellow to pale green colour.
Stage II	Fully distended into first abdominal segment, dark green colour with discrete eggs visible in the ovary.
Eggs	
Stage I	Brown-green colour.
Stage II	Yellow to grey colour, eye pigment visible.

colour changes during ovary and egg development. The ovary is clearly visible beneath the cephalothorax, and the eggs are attached under the abdomen. Laboratory observations have shown that after the first spawn, when the eggs are deposited on the pleopods, ovary development is re-initiated so that both ovary and egg maturation take place simultaneously. Thus, by the time the eggs hatch the ovary is fully ripe; ecdysis and fertilisation occur and the prawn spawns again. Simultaneous development of ovary and eggs also occurs in M. rosenbergii (Rao, 1967) and M. ohione (Truesdale & Mermilliod, 1979).

Ecdysis occurred during ovary development in lobsters and crayfish (Berry, 1971; Rice & Armitage, 1974; Aiken & Waddy, 1976) but not in penaeid prawns (Emmerson, 1980). This is probably related to the fact that penaeid prawns do not carry berry and can spawn up to three times per moult

cycle whereas in M. petersi and presumably other caridea, only one spawn per moult cycle was possible. Thus the number of spawns during the breeding season is influenced by the frequency of ecdysis.

Fig. 20 shows females in reproductive condition expressed as a percentage of the total number caught in 1979/80 (Fig. 20 a) and 1980/81 (Fig. 20 b) respectively.

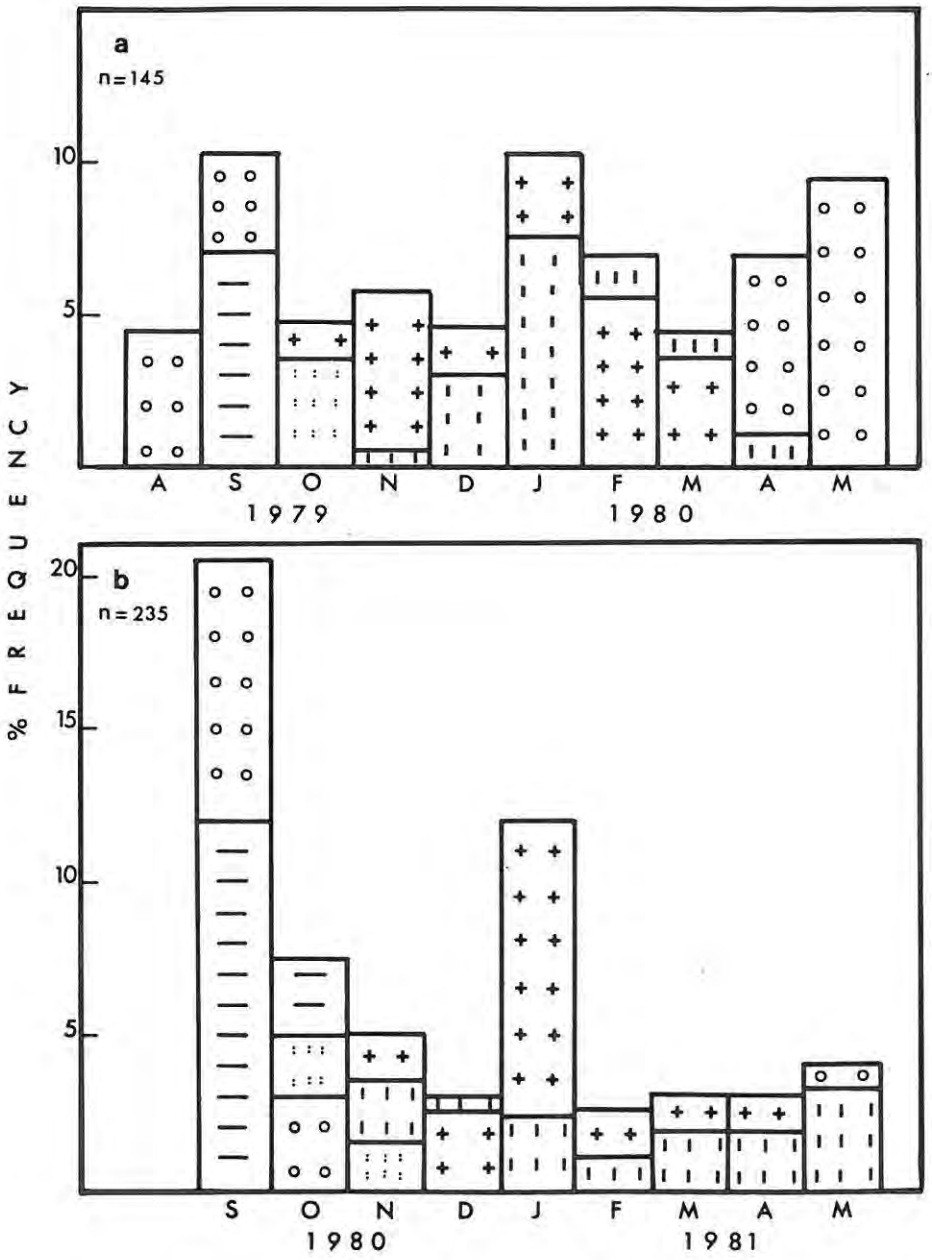


Fig. 20 Percentage females in reproductive condition during (a) 1979/80 and (b) 1980/81; o o no ovaries or berry; — stage I ovary; ::: stage II ovary; + + stage I berry; ||| stage II berry.

M. petersi breeds in summer as both reproductive cycles indicate. In 1979/80 females with stage I ovaries first appeared in September. In October females with stage I berry were present and by November a small percentage (0.6%) had developed stage II berry. Therefore the first hatching could be expected around this time. From December 1979 to March 1980 different proportions of stage I and stage II berry were evident but by April females without ovaries or berry reappeared indicating that the cycle was nearing completion. All reproductive activity had ceased by May. A similar sequence of events occurred in the following year (Fig. 20 b).

The duration of the reproductive cycle in M. petersi varied between eight to nine months, although larvae were only released for six or seven months. In both years the greatest proportion of berried females occurred in January, so it would be expected that larval input into the estuary would reach a maximum during January and February. The breeding period of M. malcolmsonii was similar to that of M. petersi and extends over a period of eight months commencing with the first heavy rains (Ibrahim, 1962). The breeding season of M. rosenbergii appeared to vary but followed the predominant monsoon season of the area. Thus, it breeds from December to July in the Hooghly estuary with peak spawning from March to May (Rajyalakshmi, 1961) and from August to December in the Kerala estuary (Raman, 1967). Lewis et al. (1966) found that the breeding activity in M. carcinus extended over a period of six months during 1962 but this was reduced to four months during 1963. No reason for the difference was given.

The onset of the reproductive cycle in M. petersi coincided with the rise in water temperature and increase in day length. In 1979 the appearance of berried females occurred a month earlier compared with 1980. A linear regression of temperature on time (Fig. 21, page 43) indicates that the slope of the regression line was steeper (b = 1.60) in 1979/80 than in 1980/81 (b = 1.12). The steeper slope implied that the rate of temperature increase was greater which may account for earlier ovary maturation in the 1979/80 population. It was reported (Rao, 1967) from field observations that the onset of reproductive activity in M. rosenbergii was brought about by both an increase in temperature and salinity. With ovigerous M. ohione however, (Truesdale & Mermilliod, 1979) the abundance in the Atchafalaya Basin (Mississippi River) increased

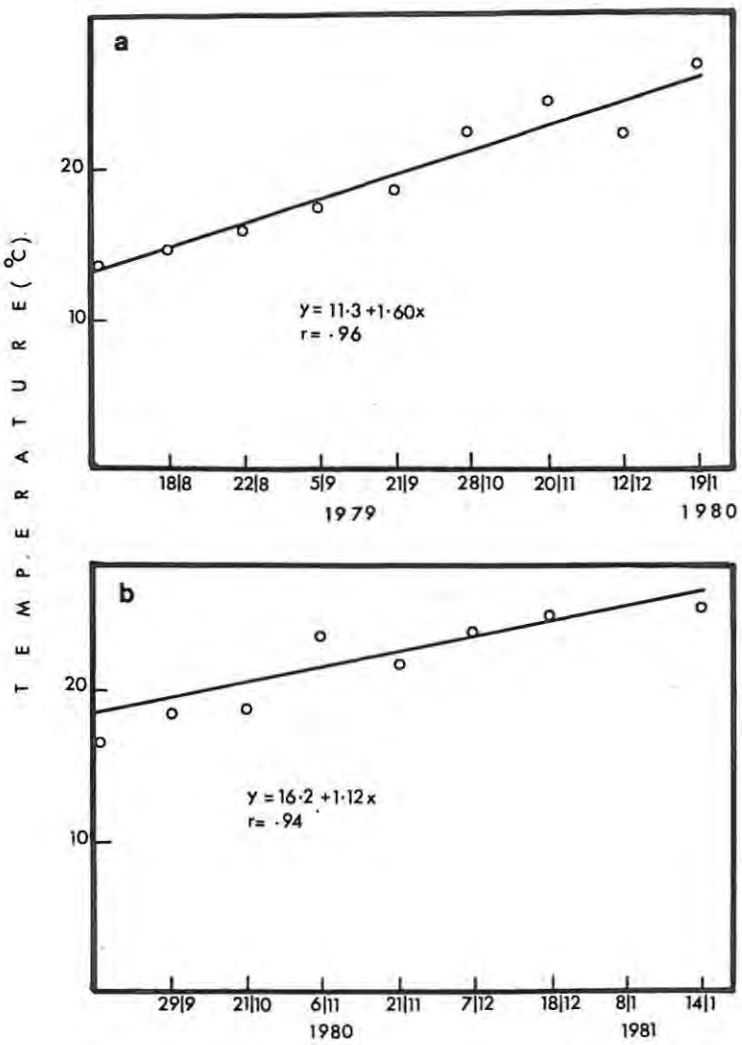


Fig. 21 Spring temperature (°C) increase in the Keiskamma river in (a) 1979/80 and (b) 1980/81.

as temperature rose in spring (February) but greatest numbers were caught before water temperatures had reached a seasonal maximum. By October, when the water temperature had dropped below 20°C no ovigerous females were caught. Little (1968) has shown in the laboratory that although a rise in temperature induced winter breeding in the shrimp Palaemonetes pugio, a temperature-photoperiod regime was more effective than temperature alone. From field data Wood (1966) defined the minimum temperature required to initiate reproduction in P. pugio as 18 - 20°C. He found two spawning peaks, one in early summer and the other in early autumn, which suggests as Little (1968) demonstrated, that photoperiod may be important. In the temperate crayfish Orconectes virilis,

photoperiod and temperature control endocrine function. Changing photoperiod in combination with temperature stimulated ovarian growth while increased water temperature induced egg laying in spring (Aiken, 1969 a, b). It was not possible to determine, (Fig. 20), the effect of temperature on the rate of embryonic development as the time interval between each sampling exercise was too long. A relationship therefore between temperature and the rate of embryonic development in M. petersi eggs was determined in the laboratory using the following experimental procedure. A female that had recently mated was used to supply the eggs. These were carefully stripped from the pleopods and separated individually or into groups of two or three. A batch of 100 eggs was counted and put into containers made from P.V.C. piping (5 cm in diameter and 10 cm in length) with mesh (No. 44, 0.410 mm) at one end to ensure a continuous flow of freshwater over the eggs. These were immersed in six constant temperature baths set at 18.0, 20.0, 22.0, 25.0, 28.0 and 30.0°C respectively. Every day approximately four to six eggs from each temperature were observed under a microscope to determine the time taken to reach a specific stage of embryonic development. The sequence of observed events in M. petersi were similar to those recorded for M. rosenbergii (Ling, 1969), and were as follows: (1) the embryonic cap, a clear area at the pole of the egg, (2) first chromatophores, (3) heart beat, (4) eye pigment, (5) first twitching of the maxillipedes. Since the precise time of spawning was not known, development could only be followed accurately from the time of appearance of the embryonic cap. The time for the freshly laid egg to reach this stage was estimated. The time at which 50% of the eggs had hatched to stage I larvae was taken as the incubation time at that particular temperature.

Table 10 (page 45) records that at 18.0°C embryonic development was so retarded that by day 24 only the chromatophore stage had been reached. By this stage only a few eggs remained, since the others had been removed because of fungal infection. However, at 20.0°C complete development had taken place and 50% of the eggs had hatched after 30.1 days. As temperature increased the number of days before hatching was reduced to 12 at 30.0°C. A similar relationship between developmental time and increasing temperature has been demonstrated for a variety of British decapod crustacea (Wear, 1974).

Table 10. Influence of temperature on incubation period and time (days) taken to reach various stages of embryonic development ($\pm$ 1 standard deviation (SD)).

Temperature (°C)	18.0±0.4	20.0±0.3	22.0±0.2	25.0±0.4	28.0±0.3	30.0±0.4
Stage of embryonic development						
(1) embryonic cap	10.7	6.7	5.9	4.2	3.5	3.2
(2) chromatophores	24.8	12.6	9.0	7.2	5.7	5.3
(3) heart beat	-	14.8	10.5	8.3	6.8	6.0
(4) eye pigment	-	15.8	11.3	8.8	7.2	6.5
(5) twitching	-	19.8	15.0	11.0	9.0	8.7
Incubation period	-	30.1	22.5	15.7	13.2	12.0

The relationship between temperature and development rate expressed as a percentage increase per day is shown in Fig. 22, (page 46). Slight curvature accounts for the quadratic temperature term. By substitution in the equation

$$y = - 16.97 + 1.35x_1 - 0.01x_2$$

where y is the rate of development and x_1 and x_2 the linear and quadratic temperature terms, it is possible to estimate the time (days) taken for development at a particular temperature. At 18.0°C the incubation period was estimated at 52 days. It took approximately one month for ovary development in M. petersi (Fig. 20) so that at 18.0°C it would take 82 days to complete one reproductive cycle. At this temperature the reproductive output of M. petersi would be sufficiently reduced to suggest that the establishment of viable M. petersi populations could only take place in estuaries which have mean summer maxima well in excess of 18.0°C.

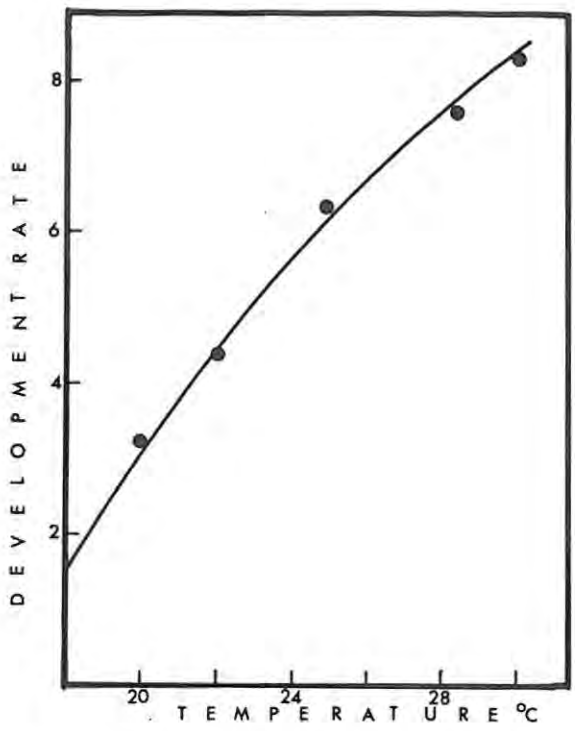


Fig. 22 Relationship between temperature and the rate of development $\left(\frac{100}{\text{incubation period}}\right)$ of M. petersi eggs.

According to Fig. 4 (page 6) 18.0°C is exceeded from September to May which are the months that the breeding cycle begins and ends. Thus 18.0°C is the lower temperature limit at which embryonic development is likely to proceed.

It is difficult to establish whether the population of M. petersi inhabiting the Keiskamma river is large or small. Barnard (1950) regards M. petersi as a subtropical species which suggests that it may breed all year round in that environment. Comparative data on abundance in rivers north of the Keiskamma are not available but it is not unreasonable to assume that the length of the reproductive period would influence the size of the population.

In November 1980, two trapping exercises were carried out on the

5 and 20 November respectively to establish when the first release of larvae into the Keiskamma river occurred. Egg development in females caught on 5 November had not advanced past stage I. However, on the 20 November five females were in stage II berry and one had just released larvae. This female was recognised as the abdominal pleura were abnormally distended and the setae which attach the eggs to the pleopods visible. It was likely therefore, that the first release of larvae into the Keiskamma river took place in late November.

In summary, M. petersi breeds in summer so that a larval study programme was launched in December 1980 to monitor changes in distribution of the larvae in the head and middle reaches of the Keiskamma estuary. The results of this investigation are reported in the next section.

2.4 Diurnal and nocturnal vertical distribution of stage I M. petersi larvae.

Following the terminology of Hart & Allanson (1976) the term diurnal is associated with daytime distribution and nocturnal at night, between sunset and sunrise. Although the larvae of various species of Macrobrachium have been successfully reared in the laboratory (Ling, 1969; Kwon & Uno, 1969; Fielder, 1970; Choudhury, 1971 a, b; Monaco, 1975) data on their distribution and behaviour in the field is limited to Raman (1967) for M. rosenbergii and Ibrahim (1962) for M. malcolmsonii, who both comment on the abundance of stage I larvae in the surface water but the absence of later stages. This led Raman (1967) to state whether their absence "implied that after the first stage the larvae abandon their planktonic habit and sink".

Before changes in the horizontal distribution of M. petersi larvae could be investigated, it was necessary to establish the nocturnal and diurnal vertical distribution patterns of M. petersi larvae. Extremely variable salinity conditions, and the fact that the larvae of Macrobrachium species require saline water for development (Ling, 1969; Kwon & Uno, 1969; Choudhury, 1971 a, b; Monaco, 1975; Greenwood et al., 1976) made it imperative that the larval collection was carried out in conjunction with vertical salinity profiles at the time of sampling.

(i) Larval identification.

To facilitate identification of field samples of M. petersi, larvae were reared in the laboratory and the various stages of development recorded. The details of larval rearing and stage identification are described in part three. Throughout the field study however, only stage I and II larvae were caught. These were readily distinguishable since the eyes of the latter were stalked and the former sessile. On two occasions at the beginning of the survey, over 100 stage II larvae were caught. This catch was never repeated so that the behaviour and distribution patterns refer to stage I larvae only. No other Palaemonidae were breeding at the same time as M. petersi.

(ii) Nocturnal vertical distribution.

Fig. 23 (page 49) shows the migratory response of M. petersi stage I larvae between 1815 hrs on 5 February to 0630 hrs on 6 February 1981. The water column was sampled at four depths, namely 2.5 m, 1.5 m, 0.75 m and just below the surface. Before sunset, although larvae were present in the upper water column, they occurred in greatest abundance at 1.5 and 2.5 m depths. After sunset there was a vertical migration of larvae and by 2030 hrs abundance at 0.75 m and just below the surface had increased substantially. For the remainder of the night abundance fluctuated widely between the four depths and no particular pattern was evident. After sunrise the larvae appeared to undergo a "dawn descent" so that by 0630 hrs the larval concentration at 2.5 m had reached 6.0 larvae $k\ell^{-1}$, compared with 4.5 larvae $k\ell^{-1}$ at 0515 hrs. During the period of the observations the salinity varied between 4.9‰ and freshwater. Due to the shallowness of the estuary and the influence of the tide the migratory patterns were not as distinct as have been obtained for other estuarine taxa, e.g. Pseudodiaptomus hessi (Hart & Allanson, 1976), but notwithstanding, a movement to the surface waters at night was clearly evident and followed that described for other South African estuarine zooplankton (Grindley, 1972; Wooldridge, 1976; Grindley, 1981).

The nocturnal migration was monitored at station 8 over one tidal cycle. It should be possible, therefore, to determine the role, if any, that a change of tide played in the migratory response and whether, as with other zooplankton, (Sandifer, 1975; Wooldridge, 1976; Wooldridge & Erasmus, 1980) utilisation of the tide may aid retention within the estuary. A current meter was not available so current velocity and

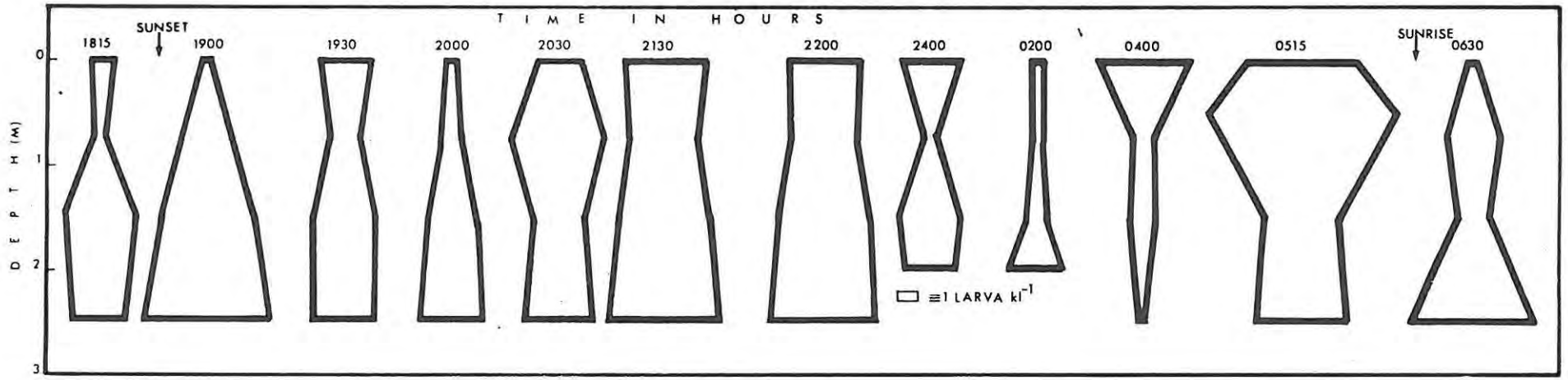


Fig. 23 Nocturnal distribution of stage I larvae at station 8 from 1815 hrs to 0630 hrs.

direction were estimated from the trawling data as the duration of each trawl and the distance travelled were known. Current velocity and direction were estimated by firstly determining the boat velocity with and against the current at each sampling interval. Using these values a velocity relative to the water was calculated and subtracted from the boat velocity. This gave an estimate of the current velocity while a change in sign of this value indicated that the tide had turned.

Plotting current velocity against time, the time at which the tide turned was determined. Fig. 24 shows that at approximately 2220 hrs and 0412 hrs the current direction changed. Thus larvae between 2400 and 0400 hrs were sampled on an incoming tide while all others, on an outgoing tide.

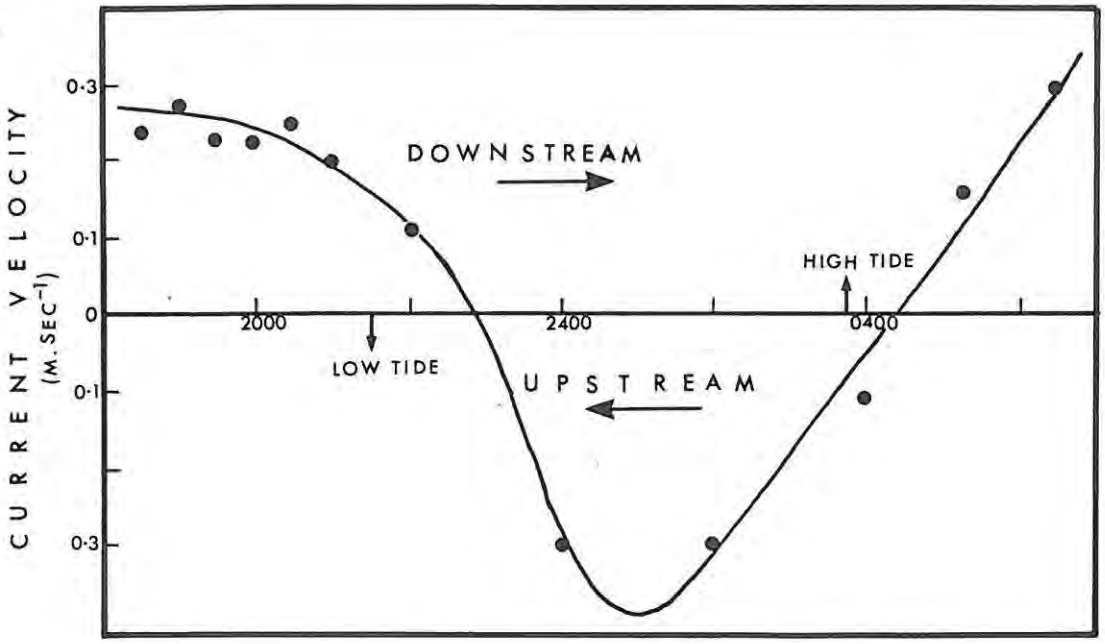


Fig. 24 Changes in current velocity and direction during one tidal cycle at station 8. Line fitted by eye.

Assuming larval abundance to vary with depth and tide direction it was possible to separate these variables into two categories. Depth could be considered either in relation to the surface (0 to 1 m) or the bottom (1.5 to 2.5 m) while tide could be either incoming or outgoing. ANOVA should indicate whether depth or a change in current direction affected larval abundance. In order to avoid the possible effect of light, larval abundance between sunset and sunrise was used in the analysis.

Table 11 shows an analysis of the change in larval abundance in relation to tide and depth. There was no difference between the mean surface or bottom larval abundance during the incoming or outgoing tides. This indicated that the larvae did not respond to an incoming tide by rising to the surface or to an outgoing tide by sinking.

Table 11. Analysis of the relationship between depth, tide and larval abundance (no. $k\ell^{-1}$) at station 8 ($\pm$ 1 SD).

Depth / Tide	Surface			Bottom		
Incoming	2.98	0.75		3.05	2.70	
	0.70	4.82		2.34	1.13	
	0.77	0.91		1.00	0.46	
	$\bar{x} = 1.82 \pm 1.71$			$\bar{x} = 1.78 \pm 1.05$		
Outgoing	2.66	2.04	3.48	3.16	2.40	4.44
	1.48	4.81	3.33	3.14	3.31	5.05
	0.71	4.08	5.15	2.45	4.50	3.71
	1.09	3.30	9.63	3.03	5.31	4.50
	$\bar{x} = 3.48 \pm 2.39$			$\bar{x} = 3.75 \pm 0.98$		

Statistical analysis

Source of variation	Degrees of freedom	Sum of squares	Mean square	F ratio	Significance
Regression	3	26.77	8.92	3.04	$0.01<P<0.05$
Tide	1	26.33	26.33	8.98	$0.001<P<0.01$
Depth	1	0.24	0.24	0.08	Not significant
Interaction	1	0.19	0.19	0.06	Not significant
Residual	32	93.81	2.93		
Total	35	120.59	3.44		

However, a significant tidal difference ($F_{1,32} = 8.98$; $0.001<P<0.01$) was evident since there was a greater abundance of larvae in the water column, independent of depth, on the outgoing than incoming tide.

There were two possibilities which could account for the difference in abundance through tidal change. Firstly, water volume in the estuary increased with the incoming tide so that the reduction in abundance could simply be a dilution effect. It was shown however

(Fig. 23, page 49) that although the tide had turned by 2400 hrs the water at station 8 was at its lowest level as the trawl at 2.5 m had to be elevated to avoid contact with the substratum. This should have the effect of concentrating the larvae. Thus a reduction in abundance due to a dilution effect seems unlikely. Rather, I suggest that by remaining in the water column during an ebbing tide, the larvae were effectively carried to saline water which, as part three will demonstrate, is necessary for development. The significance of this behaviour in post flood conditions, when a large proportion of the estuary is inundated with freshwater, is obvious. It would appear then that the tidal behaviour of stage I larvae was not directly associated with maintenance of station and retention within the estuary. The significance of this behaviour in relation to retention within the estuary will be discussed later.

The effect of salinity discontinuities on the distribution of zooplankton in the field (Hansen, 1951; Grindley, 1964; Wooldridge, 1976) and in the laboratory (Lance, 1962; Harder, 1968; Roberts, 1971) has been well documented. Accordingly the dusk ascent of stage I larvae in homogeneous, partially mixed and stratified conditions was investigated. The results are presented in Figs. 25, 26 and 27 (pages 52, 53 & 54).

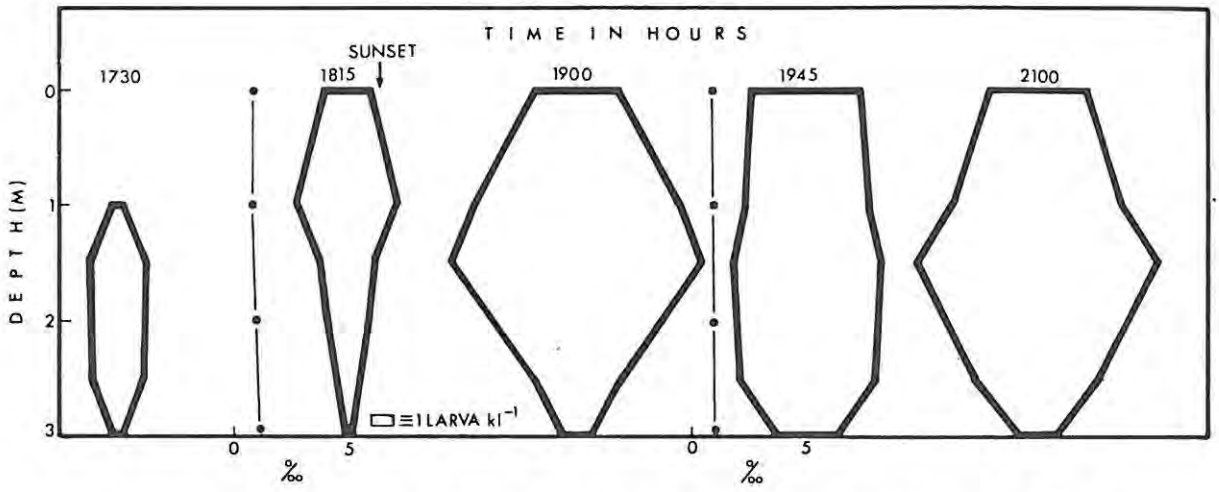


Fig. 25 Dusk ascent of stage I larvae in completely mixed conditions at station 8 on the 20 March 1981. Two vertical salinity profiles were taken at 1815 and 1945 hrs on an ebbing tide.

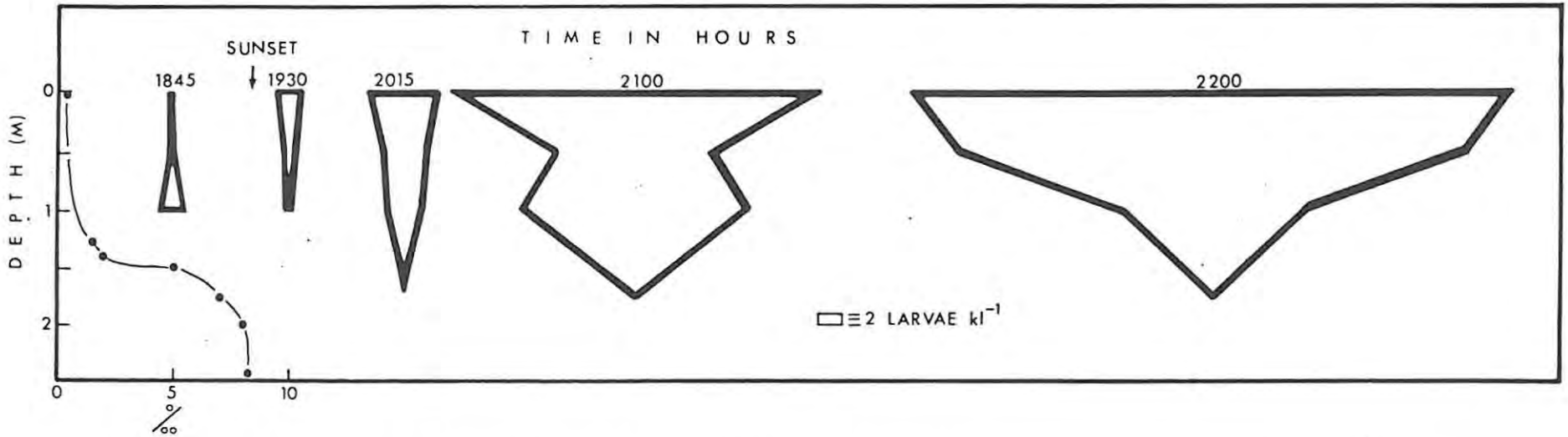


Fig. 26 Dusk ascent of stage I larvae in partially mixed conditions at station 1 on 13 January 1981. The vertical salinity profile was taken at 1845 hrs on an ebbing tide.

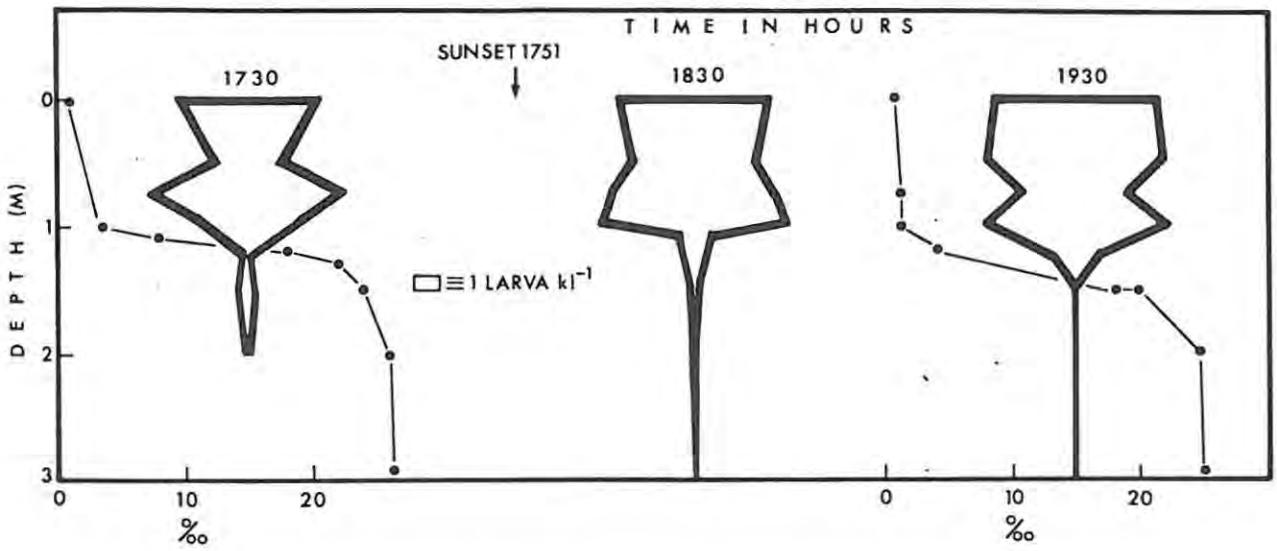


Fig. 27 Dusk ascent of stage I larvae in stratified conditions at station 8 on 12 April 1981. Vertical salinity profiles were taken at 1730 and 1930 hrs on a flowing tide.

In partially and completely mixed conditions the pattern of ascent was essentially the same. Larvae reached the surface just before or soon after sunset (Figs. 25 & 26). However, maximum abundance at the surface was usually only attained in complete darkness, (see Fig. 26 at 2200 hrs) which suggested that the larvae were not responding to a source of low light intensity as do many zooplankton species (see Hutchinson, 1967, for review). Instead, the response could be interpreted either as an endogenous physiological rhythm (Grindley, 1972) or a response to the ebbing tide. Although endogenous activity has been demonstrated in a number of zooplankton species (Harris, 1963; Rudjakov, 1970; Hart & Allanson, 1976) this was more pronounced in adult forms (Hart & Allanson, 1976). Thus a tidal response was the more likely explanation. Stratified conditions (Fig. 27) restricted the utilisable volume of the water column to the low salinity region above the halocline. Since the larvae were concentrated in the top half of the water column no pronounced dusk ascent was evident. By 1930 hrs abundance of larvae between the surface and 0.5 m had increased from 8.5 $k\ell^{-1}$ at 1730 hrs to 13.6 $k\ell^{-1}$. Over the two hour sampling period the level of the halocline dropped from 1.25 m to

1.5 m. Accordingly, larval density at 1.25 m increased from 0.3 $k\ell^{-1}$ to 2.0 $k\ell^{-1}$, but density below the halocline remained unchanged, indicating that the halocline was impenetrable. Both Lance (1962) experimentally, and Grindley (1964) in the field, have shown that the presence of a low salinity layer at the surface inhibited the vertical migration of certain species of estuarine zooplankton. Hansen (1951) noted four different patterns of vertical distribution of zooplankton in a stratified estuary, of which M. petersi stage I larvae fall into the first category, those situated above the discontinuity layer. Even though stage I were able to tolerate the salinity below the halocline (see part three), as do other zooplankton (Lance, 1962), the salinity difference between the two water layers was the inhibitory factor.

(iii) Diurnal vertical distribution.

The daytime distribution patterns of stage I larvae in partially mixed, totally mixed or stratified conditions is shown in Fig. 28 a, b & c (page 56). Generally, the distribution of the larvae tended towards the bottom except in the stratified condition where the existence of a halocline overrided this pattern.

2.5 Horizontal distribution of M. petersi stage I larvae.

Due to the variable diurnal distribution patterns and the difficulty of trawling above the substratum, it was apparent that an investigation of the horizontal distribution of M. petersi larvae should take place at night and that sampling should be restricted to the top 1.5 m of the water column.

(i) Flooding.

As would be expected, the erratic freshwater discharge had a significant effect on the horizontal distribution of stage I M. petersi larvae. Table 12 (page 57) shows the horizontal distribution of stage I larvae at 0.5 m on the 9 and 16 January prior to the first summer flood. The greatest abundance of larvae was found at stations 1 and 2. As the major larval recruitment to the estuary was via the inflowing freshwater, and the distribution of adults in the estuary showed the highest concentration in this region (see Fig. 17), the

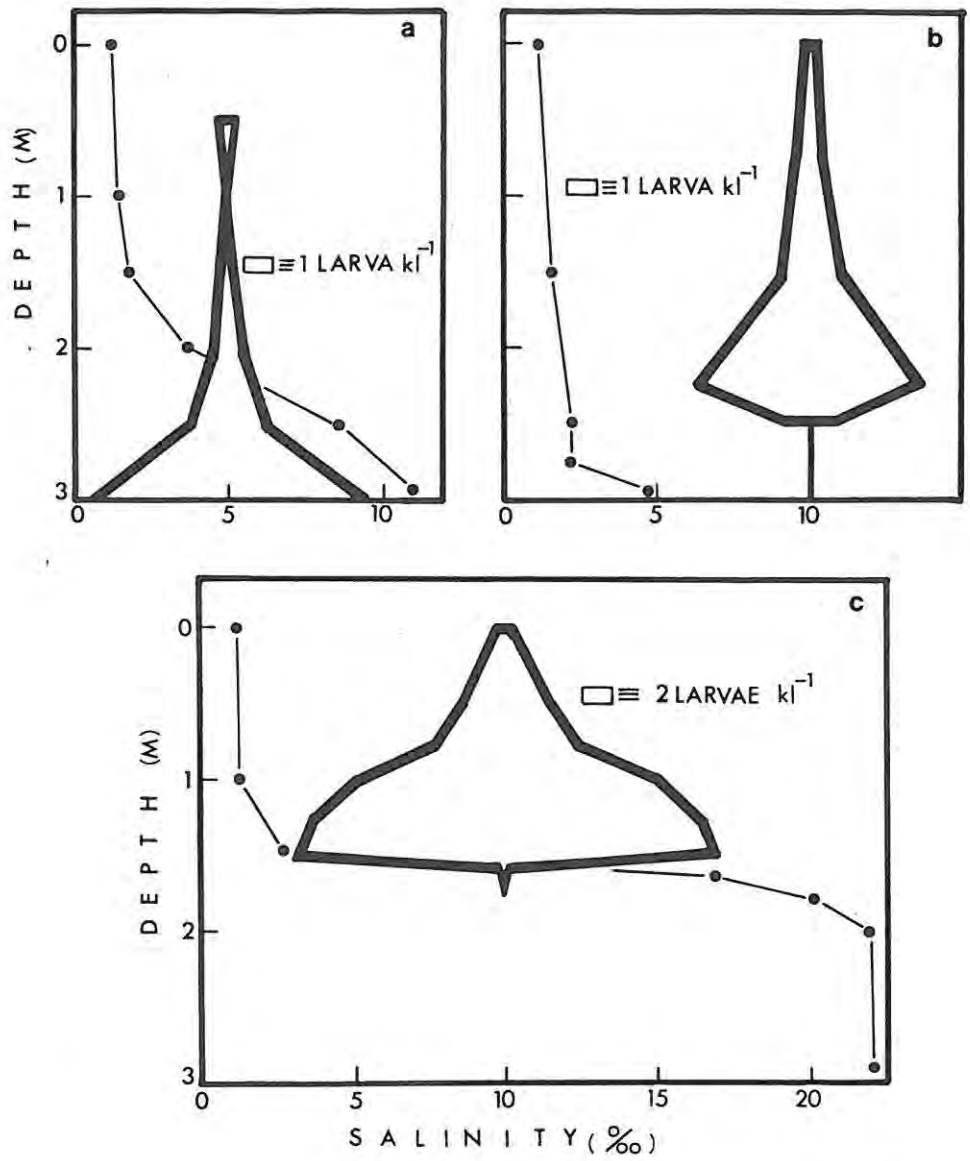


Fig. 28 Daytime distribution of stage I larvae in partially mixed (a), stratified (b) and completely mixed (c) conditions.

results were not surprising. The rapid decrease in abundance downstream from station 2 was the result of favourable salinities which promoted larval development with a resultant drop in stage I abundance.

Table 13 (page 57) shows the distribution and abundance of larvae on the 30 January and 12 February, 2 and 14 days after the first summer flood respectively. As expected, the overall abundance at

all stations on the 30 January was considerably reduced compared with the pre-flood values (Table 12, 16 January). In a relatively short

Table 12. Pre-flood distribution and abundance (larvae $k\ell^{-1}$) of stage I M. petersi larvae at 0.5 m.

9 January 1981		16 January 1981	
Station No.	Larvae $k\ell^{-1}$	Station No.	Larvae $k\ell^{-1}$
1	10.9	1	16.6
2	17.2	2	16.8
3	2.6	3	9.0
4	2.1	4	3.3
5	0.4	5	1.2
6	0.3	6	0.5
		7	1.0
		8	None
		9	None
		10	None

time, however, larval abundance attained pre-flood levels. On the 12 February (Table 13) the mean larval abundance at 0.5 m was 5.2 larvae $k\ell^{-1}$ compared with 4.8 larvae $k\ell^{-1}$ on the 16 January before the flood.

Table 13. Post-flood distribution and abundance (larvae $k\ell^{-1}$) of stage I M. petersi larvae on 30 January and 12 February 1981.

30 January 1981		12 February 1981		
Station No.	Larvae $k\ell^{-1}$	Station No.	Larvae $k\ell^{-1}$	SD
1	-	1	6.7**	±2.4
3	0.5*	2	3.3	±0.5
5	0.6	3	5.2	±2.3
7	0.8	4	13.4	±5.7
9	2.2	5	8.8	±3.8
10	0.5	6	10.6	±3.2
12	0.8	8	1.3	±0.3
		9	2.6	±1.8
		10	3.2	±1.9
		11	1.6	±1.0
		12	1.1	±1.4

* One sample at 0.5 m.

** Mean of three samples at 1.0 m, 0.5m and just below the surface.

Persistent rainfall throughout the month of February prompted an ad hoc investigation into larval distribution beyond station 12. Table 14 shows that larvae were caught at station 16 which was approximately 2 kms from the estuary mouth indicating that during flooding larvae were likely to be lost from the estuary.

Table 14. Distribution and abundance (larvae $k\ell^{-1}$) in the lower reaches of the Keiskamma estuary on 26 February 1981.

Station No.	Larvae $k\ell^{-1}$	SD
10	0.1*	±0.0
11	0.6	±0.3
12	0.2	±0.1
13**	0.3	±0.2
14**	0.0	±0.0
15**	0.5	±0.4
16**	1.3	±1.0

* Mean of three samples at 1.0m, 0.5 m and just below the surface.

** These stations are not shown in Fig. 6 but represent approximately one km distances downstream from station 12.

(ii) Frontal regions.

During the erratic flooding conditions in the 1980/81 summer the formation of salt water frontal areas (salt wedges) were common. Fig. 29 (page 59) shows the abundance of stage I larvae (between the surface and 1.0 m) in relation to the salinity profiles on the 4 February, six days after a flood. There was a noticable increase in abundance in the vicinity of the front (stations 10 & 11) which declined by station 12. ANOVA of the mean larval abundance (Table 15, page 59) between stations 1 and 12 (note that the stations have been ranked in decreasing order of larval abundance) indicated that abundance at station 11 was significantly greater than at all other stations. A similar concentration of stage I larvae was found on the 23 February. Fig. 30 (page 60) shows abundance of stage I larvae between stations 1 and 13.

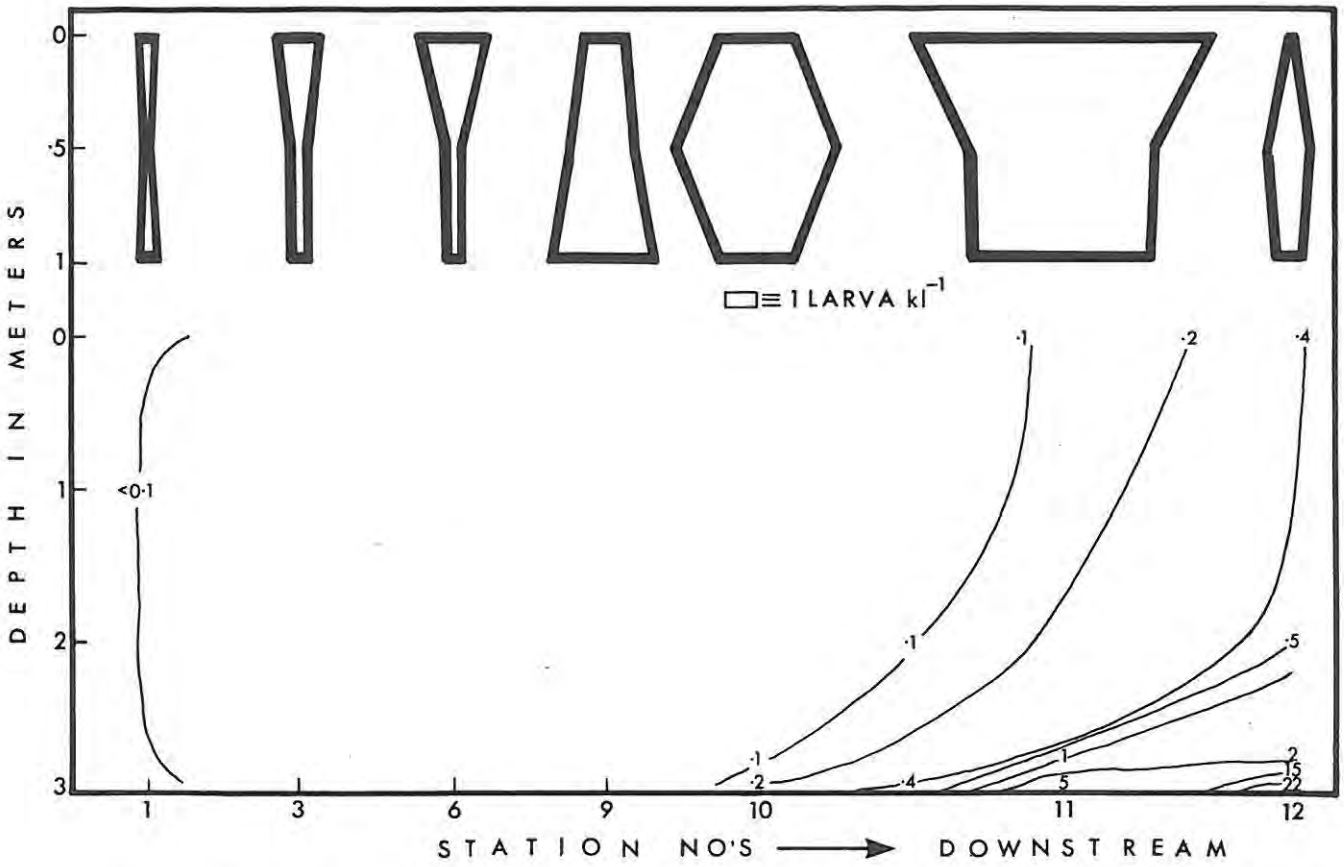


Fig. 29 Changes in the horizontal distribution and abundance (larvae $k\ell^{-1}$) of stage I M. petersi larvae in relation to a salt front on 4 February 1981.

Table 15. Variation in abundance (larvae $k\ell^{-1}$) of stage I M. petersi larvae between stations 1 & 12 on 4 February 1981. Means that are not underscored by a common line are significantly different. (L.S.D. $P \leq 0.05$).

Station No.	1	12	3	6	9	10	11
Mean abundance	0.3±0.2	0.8±0.7	1.0±0.5	1.2±0.9	2.7±0.8	3.5±1.5	7.5±2.2

The greatest concentration of larvae was found in the vicinity of the salt front between stations 7 and 8 and has been statistically verified (Table 16, page 60). The pattern of salinity distribution in the frontal

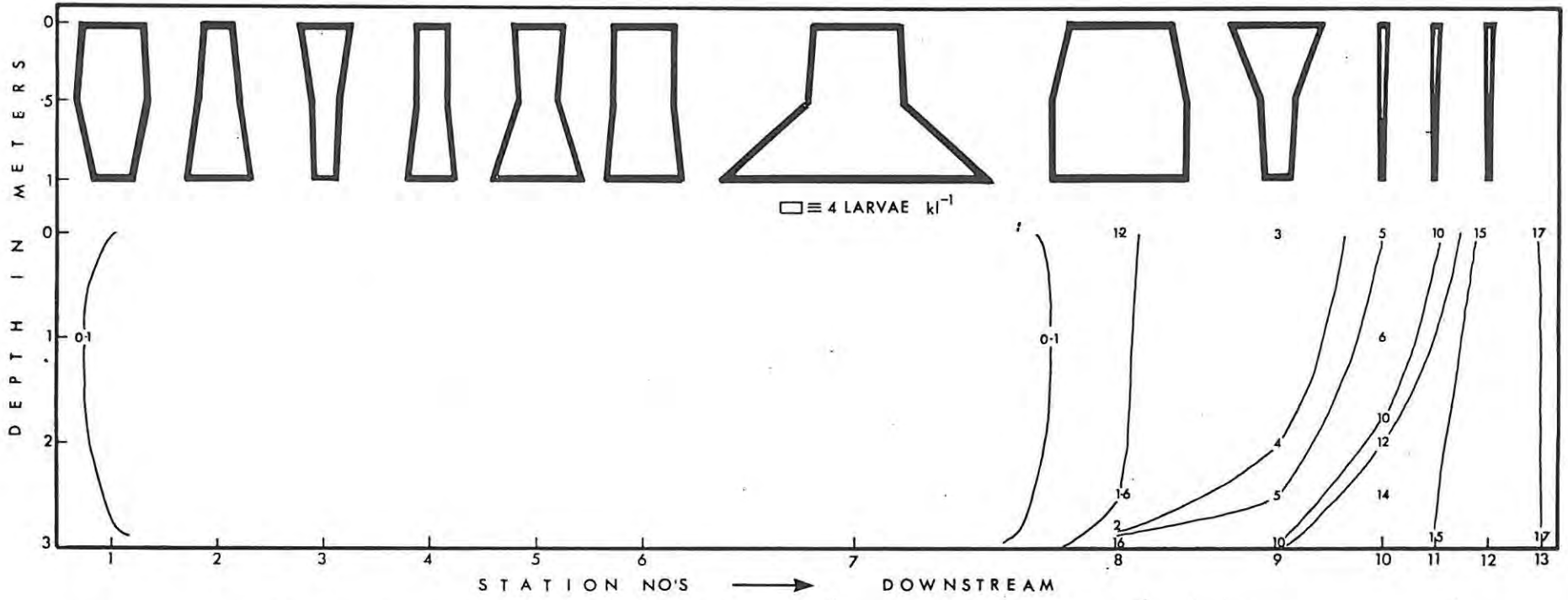


Fig. 30 Changes in horizontal distribution and abundance (larvae $k\ell^{-1}$) of stage I M. petersi larvae in relation to a salt front.

Table 16. Variation in mean abundance (larvae $k\ell^{-1}$) of stage I M. petersi larvae between stations 1 & 13 on 23 February 1981. Means that are not underscored by a common line are significantly different. (L.S.D. P≤0.05).

Station No.	12	13	11	10	3	4	2	9	1	5	6	8	7
Mean abundance	0.1±0.0	0.1±0.0	0.1±0.0	0.5±0.4	3.7±1.9	4.0±0.4	4.2±1.8	5.4±3.5	5.7±1.5	5.9±2.3	6.6±0.8	11.8±1.1	15.2±10.5

region differed from that in Fig. 29 due to a more pronounced frontal gradient and flood waters of lower magnitude. The significance of the reduction in abundance downstream from station 9 will be discussed later.

It could be argued that the correlation between larval abundance and a frontal region reflected a temporal distribution (it took approximately 5 hrs to complete each trawling exercise) which chanced to coincide with a frontal region. Such a coincidence could be avoided if two stations, one in freshwater and the other in a frontal region, were trawled simultaneously. Suitable field conditions for a comparison of this nature occurred on 6 March. A salt front was located at station 8 and station 4 was chosen to represent a freshwater region. Two sets of equidistant markers (183 m apart) were erected at both stations and an identical trawling programme, taking current effects etc. into account, was adopted for each station. All trawling equipment except for boat and engine were identical. Between 2200 hrs and 0515 hrs a total of 20 trawls at four different depths were performed simultaneously at each station. The mean larval abundance at station 8 (2.8, SD $\pm$ 1.8) larvae $k\ell^{-1}$) was significantly different ($t = 3.03$; d.f. 38, $0.001<P<0.01$) from the 1.3, SD $\pm$ 1.1 larvae $k\ell^{-1}$ obtained at station 4. It could not be stated that a correlation between increased larval abundance and a salt front region existed which emphasised a behavioural characteristic of stage I M. petersi larvae, namely an affinity for water of increased salinity.

The affinity for water of an elevated salt content has also been demonstrated during the day using a D-net. Fig. 31 (page 62) shows the change in larval abundance between stations 1 and 6 on the 12 February. Again there was a substantial increase in stage I abundance in the frontal region (station 5) compared with the freshwater region of the estuary. Although no trawls upstream of the frontal region were carried out, Fig. 32(page 62) demonstrates the sharp decrease in numbers downstream from the frontal region. No stage I larvae were caught at station 12. Thus the changes in abundance of stage I larvae above the substratum during the day mimic those at night with respect to salt front regions. While the literature records many examples of the avoidance reaction of crustacea in the laboratory (Lance, 1962; Harder, 1968; Scarratt & Raine, 1967; McLusky, 1970; Roberts, 1971) or in the field (Kinne, 1963, McLusky, 1968; Marsden, 1973; Ruello, 1973; Mills & Fish, 1980) to

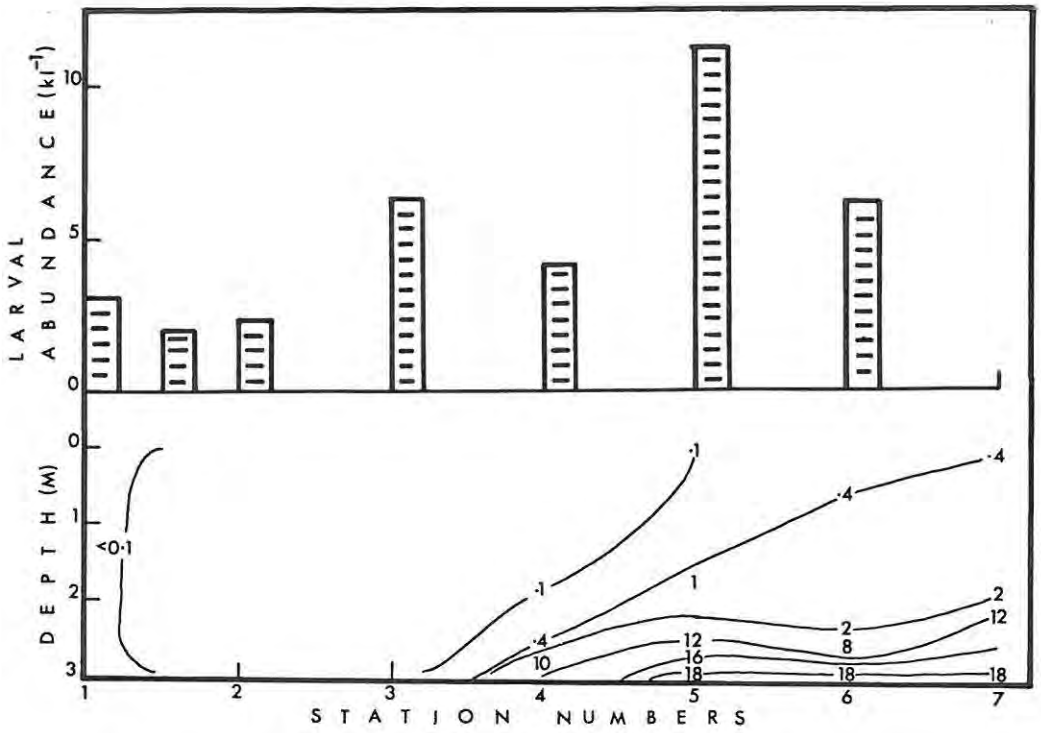


Fig. 31 Daytime distribution and abundance (larvae $k\ell^{-1}$) of stage I M. petersi larvae in relation to a salt front on 12 February 1981.

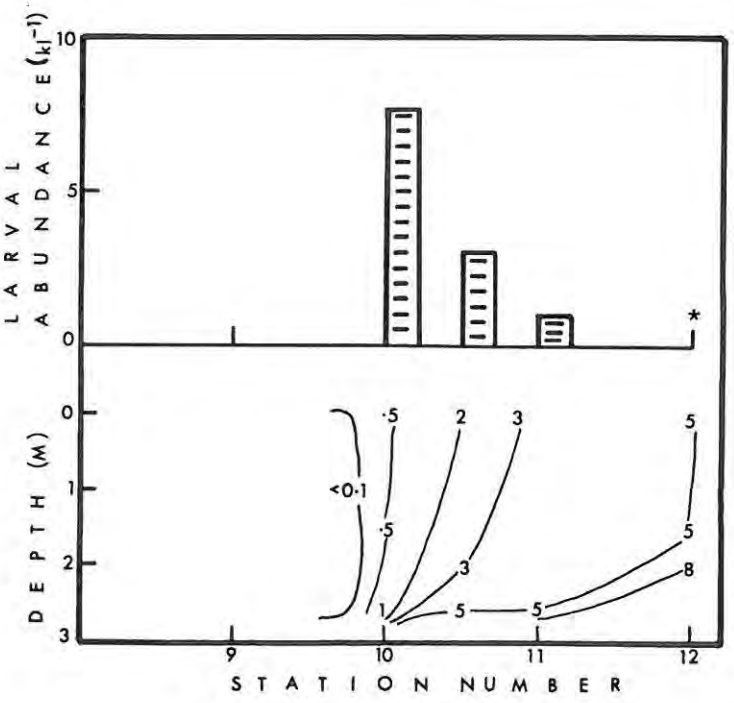


Fig. 32 Daytime distribution and abundance (larvae $k\ell^{-1}$) of stage I M. petersi larvae in relation to a salt front.
* No larvae caught at station 12.

salinity, affinity for a particular salinity regime appears to be less well documented. In penaeid prawns however, during immigration from the sea to the nursery grounds such as estuaries, bays and coastal lagoons, post larvae are drawn to these environments by the reduced salinity gradient (Mair, 1980). Lagerspetz & Mattila (1961) attempted to correlate the capacity of fresh and brackish water isopods and amphipods, to detect changes in salinity which favour survival in the Baltic Sea, in response to low salinity conditions in spring. Although the crustacea reacted to salinity changes, the reaction thresholds were not low enough to allow behavioural selection of salinities in natural conditions.

2.6 Distribution of stage II M. petersi larvae.

Other than on two occasions, only one or two stage II larvae were caught per trawl. This happened irrespective of whether trawling took place in a homogenous, partially mixed or stratified region of the estuary. The D-net used under a variety of conditions yielded similar results. Later in the investigation, a series of 24 hr larval salinity vs. survival experiments revealed that stage II and subsequent stages were intolerant of freshwater. Despite an intensive trawling effort in saline water both in the water column and above the substratum, no other larval stages were located. After a flood on 23 March the only region of the estuary with elevated salinities was that between station 12 and the mouth. The lower estuary was trawled at seven depths both during the ebb and incoming tide. A total of 93 stage I larvae were caught. Using a larger 45 cm diameter zooplankton net to investigate the possibility of net avoidance was unsuccessful. Trawling at various speeds made no difference either. Finally both the standard and 45 cm diameter nets were used to trawl as close to the littoral area as possible. While an abundance of stage I larvae were caught, no other stages were evident. The failure of these efforts was most surprising since laboratory observation of the behaviour of all larval stages gave no indication of any change which could remotely explain their absence in the field.

Only on two occasions, 13 and 17 December 1980, at the beginning of the larval investigation, were stage II larvae caught in substantial

numbers. Table 17 shows the abundance of stage I and stage II larvae sampled at two depths at 1900 and 2100 hrs on the 13 December 1980 at station 5. At all depths the abundance of stage II larvae was greater than that of stage I. A similar result was obtained on 17 December.

Table 17. The abundance (larvae $k\ell^{-1}$) of stage I and II M. petersi larvae at station 5 on 13 December 1980.

	Depth (m)			
Time	0.50		1.75	
(hrs)	I	II	I	II
1900	0.5	0.4	3.0	8.0
2100	13.8	18.0	3.8	8.6

The vertical salinity profiles during this period were not atypical. If stage II had been caught in the process of moulting, in proportion to the vast numbers of stage I that were subsequently caught, this phenomenon should have occurred more frequently. No other palaemonid prawns were reproductively active so the presence of stage II could not be attributed to another species.

Both Raman (1967) and Ibrahim (1962) remarked on the abundance of stage I larvae of M. rosenbergii and M. malcolmsonii in the field respectively as well as the absence of other larval stages. A similar result has been obtained for M. petersi. At one stage I thought that the small population of M. petersi in the Keiskamma river, being the southern most limit of their distribution (see part five), may have been responsible. This does not seem likely since both M. rosenbergii and M. malcolmsonii support a lucrative fishery. The absence of later stages was more perplexing as larval development, for M. rosenbergii, takes between 25 and 45 days to complete (Ling, 1969). For M. petersi the minimum time to post larva was 20 days. Thus, compared with estuarine species, larval life was considerably protracted which should increase the chances of capture. The larvae were obviously in the estuary, so there must be some important facet of their ecology, common to a number

of species, that was being overlooked.

2.7 Distribution of post larval M. petersi.

Post larvae were defined as the stage after larval metamorphosis which occurred in the estuary and underwent a migration to freshwater. Until the latter part of the study no pattern of post larval distribution had emerged. The first post larva was caught at 0.5 m on the 9 January 1981 at station 1 and by the middle of April 11 had been found. The post larvae utilised the water column as well as the substratum since they were caught in both the zooplankton and D-nets.

The post larval distribution and horizontal salinity profiles on the 15 April, when 49 post larvae were caught between stations 1 and 8 in the zooplankton net, are shown in Fig. 33. A narrow salinity

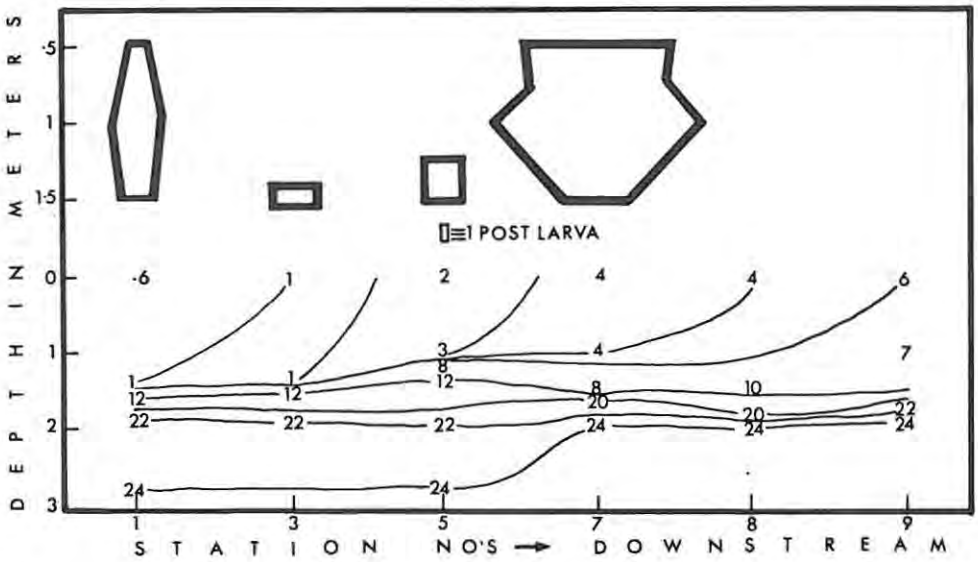


Fig. 33 Horizontal distribution of post larvae and salinity on the 15 April 1981.

stratification was evident at 1.5 m. A D-net trawl at station 3 yielded one post larva, suggesting that post larvae were present on the substratum as well as in the water column.

The vertical distribution of post larvae may be size dependent. The mean carapace length of post larvae caught on the 15 April above the

halocline was 1.6, SD $\pm$ 0.20 mm (n = 49). On 3 May the entire water column at station 1 was sampled at 0.25 m intervals; a halocline occurred at 2.0 m. No post larvae were found in the water column but 95 were caught in a single trawl over the substratum. A further 85 post larvae were caught 2 hrs later. The mean carapace length of these post larvae was 2.3, SD $\pm$ 0.40 mm (n = 30) which was significantly ($t = 8.06$; $P<0.001$) different from the April sample. Thus at a particular size M. petersi post larvae drop out of the water column and utilise the substratum in their upstream migration to freshwater. Contact with the substratum may offer a means of resisting downstream current as well as providing a rheotactic stimulus.

2.8 Migration of M. petersi.

The gauging weir, situated 18 kms from the estuary mouth, was the site chosen to monitor the migration of M. petersi. The weir, built in 1969, was constructed on the upstream side of a concrete causeway which had previously spanned the river (Fig. 34). The causeway was approximately

Fig. 34 Causeway at ebb and flow before weir was built in 1969.

60 m long and 7 m wide with a 15 cm concrete lip on the tidal side. Water flowed over the gauging weir and flooded the causeway before entering the estuary. Animals migrating from the estuary crossed the causeway before entering freshwater. It appeared from the variety of species (pers. obs.) that the causeway and weir did not obstruct such movement.

Fig. 35 shows the design of the trap used to catch migrating M. petersi. It consisted of a 1.5 m x 0.75 m x 0.15 m rectangular metal frame covered on three sides with wire gauze. The entrance consisted of an elevated platform leading to a 0.02 m x 1.5 m aperture. The trap was positioned on the tidal side of the causeway against the concrete lip in such a way that prawns migrating over the lip were caught. Migration took place at night only, so the trap was set after sunset and retrieved after sunrise.

Fig. 35 Trap used to catch migrating M. petersi.

A total of 4022 M. petersi were caught between January and May 1981. Table 18 (page 68) shows that the migration started in

January and reached an abrupt peak in February. The mean number declined in March and dropped sharply in April. By May the migration had effectively ended. No attempt was made to correlate abundance with the tidal cycle or river flow. However, if the trap was set on an incoming tide catches appeared to be greater than when set on an ebbing tide.

Table 18. Changes in mean abundance of migrating M. petersi between January and May 1981.

Month	No. of trappings	Total caught	Mean	Range Min.	Range Max.
January	1	16	16	16	16
February	6	2121	354	46	741
March	5	1487	297	5	1045
April	3	358	119	17	221
May	2	40	20	12	28

The mean carapace length of a random sample (n = 1544) of migrating prawns between January and May was 6.0, SD $\pm$ 0.90 mm which, for the Keiskamma population, characterised the size at which the migrating prawns reached freshwater. A carapace length of 6 mm was used to differentiate post larval from juvenile M. petersi. In the 1979/80 season, the migration trap had not been constructed, but evidence of a migration was nevertheless being sought. The causeway was checked regularly using a dip net. On the 13 April 1980 16 M. petersi (mean carapace length 5.6, SD $\pm$ 0.5 mm) were caught, apparently the first recruits from the 1979/80 breeding season. After this date more M. petersi were caught but compared with the activity in 1981, it could be said that no migration occurred in 1980. The significant difference between the 1979/80 and 1980/81 seasons was the freshwater discharge. It was suggested therefore that a regular discharge which at times reached flooding proportions may be one of the environmental cues which stimulated the migratory behaviour of post larvae M. petersi.

Post larval migratory behaviour has been noted in a few species of Macrobrachium (Raman, 1967; George, 1969), even those thought to be intolerant of saline water (Rajyalakshmi & Ranadhir, 1969) but quantitative data are lacking. The size at which M. rosenbergii started

its upstream migration varied between 9 and 30 mm total length (Rao, 1967). Ibrahim (1962) found the length frequency distribution of migrating M. malcolmsonii to vary between 13 and 60 mm total length with a mode at 30 mm. As with M. petersi migration occurred at night only.

2.9 Discussion.

The Keiskamma river and estuary form an inseparable link in the life cycle of M. petersi as both freshwater and estuarine environments are utilised. Although no larvae more advanced than stage II were caught, all other stages of the life cycle have been identified and their respective positions within these two environments defined.

Although only indirect evidence of an adult migration (a reduction in abundance above the weir in 1980/81) to the estuary was found, it would appear that M. petersi, like other Macrobrachium species, migrates to the estuary during the breeding period which in the case of M. petersi, extended from November to May. Berried females were found in freshwater, indicating that the estuarine environment was not a prerequisite for reproductive development. In favourable estuarine conditions as in the 1980/81 season, adult behaviour was orientated towards the estuary. Similarly, the nocturnal behavioural pattern of the stage I larvae brings them to the surface waters, and, as the nett water displacement was downstream, they eventually reached the estuary. Once in the estuary larvae appeared in the water column in greater abundance on the outgoing than incoming tide, which was interpreted as a further indication of the importance of saline water. This has been confirmed by the horizontal distribution patterns which demonstrated that stage I larvae show an affinity for salt front regions. Such behaviour could not continue indefinitely as the larvae would eventually get washed out of the estuary. Thus, some retention mechanism was likely to come into operation to prevent this.

The drop in abundance of stage I larvae, downstream from the salt front, suggests that such a retention mechanism was operative. It is postulated that the salinities encountered in salt front regions are sufficient to promote ecdysis to stage II. The lack of stage II and subsequent stages in the plankton indicates a loss of the planktonic

phase. I have interpreted the loss of a planktonic existence as the mechanism which retains M. petersi larvae in the Keiskamma river. How the behaviour has changed is unknown but it is initiated at the onset of stage II and must be very effective as no other larval stages were caught.

Loss of a planktonic larval existence is not unique to M. petersi. Forbes (1973) showed that not only had the larvae of the burrowing prawn Callianassa kraussi lost its planktonic existence (development takes place in the parental burrow) but that the number of larval stages had been reduced compared with other Callianassa species (Devine, 1966). Forbes (1973) has attributed the abbreviated larval period (3 - 5 days) and lack of planktonic stages in C. kraussi as adaptive, since Callianassa species that do not live in estuaries do not possess such characteristics. There are a variety of behavioural adaptations that may contribute to the maintenance of larvae or pelagic adults within estuaries. These include (1) a preference for the lower regions of the water column in larvae whose adults are heavily dependent on estuarine habitats (Sandifer, 1975), (2) detection and avoidance of low salinities in highly stratified estuaries (Grindley, 1964), (3) modification of larval behaviour with age (Bousfield, 1955; Sandifer, 1975), (4) vertical migration into the upper layer of a stratified estuary at night and remaining in the deeper inflowing water during the day (Cronin et al., 1962; Wooldridge, 1976), and (5) utilising of lateral currents in the lower regions of the estuary (Wooldridge & Erasmus, 1980).

In view of the behaviour pattern of stage II and subsequent stages, the function of the stage I larvae is distributative and ensures that saline water is reached so that larval development can proceed. Although the intermediate larval stages were not caught, the appearance of post larvae in the plankton confirms that both larval development and metamorphosis is completed in the estuary.

Distribution of post larvae in the estuary did not follow any pattern but the migration to freshwater effectively completed the estuarine life cycle. Migration, whether it involves the movement from one locality to another or simply a change of behaviour in one particular environment, has been documented in the Penaeidea, Reptantia, and Caridea (Allen, 1966 a). Within the Caridea, migration in the family Crangonidae was chiefly concerned with the movement of adults between marine,

estuarine and coastal zone environments in response to changing temperature and salinity conditions, (Broekema, 1942; Haefner, 1969; Heerebout, 1974; Modlin, 1980) although Boddeke (1975) questioned the influence of these variables on migration. Data collected over 14 years indicated that the autumn migration in Crangon crangon was in response to fluctuations in water temperature only. Boddeke (1975) interpreted the autumn migration not as a movement from unfavourable conditions, but rather as an efficient means of re-distributing the population, which leaves sexually unripe animals in the tidal area and richest food supply.

Although representatives from the family Palaemonidae are found in freshwater, estuarine and marine environments, (Hedgpeth, 1957) patterns of migration have mainly been documented in estuarine and marine species (Forster, 1959; Allen, 1963; 1966 b; Antheunisse et al., 1971). Although a breeding and post larval migration back to freshwater has been observed in freshwater species from the genus Macrobrachium, few quantitative migratory studies and factors that may influence it have been carried out. The life cycle of M. rosenbergii however has been extensively researched (Rajyalakshmi, 1961; Raman, 1967; Rao, 1967; George, 1969; Ling, 1969). A post larval migration to freshwater, after the breeding season, has often been observed but the return adult migration, and the factors that affect it, have received scant attention. The unusually dry 1979/80 season has given an insight into the factors that may be responsible for the return migration in adult M. petersi. These prawns reacted to an increase in salinity in the 1979/80 summer which elicited a potamodromous response. The end of the breeding season coincides with a marked reduction in rainfall. In the estuary there is a corresponding increase in salinity in the upper reaches due to the reduced freshwater discharge (see Fig. 17). I am suggesting therefore, that at the end of each breeding season an increase in salinity, as occurred in the dry summer of 1979/80, is responsible for the return breeding migration to freshwater of adult M. petersi. In M. acanthurus current direction appeared to be a factor responsible for the return migration but field evidence is lacking. Hughes & Richard (1973) carried out laboratory studies on the response of gravid and non-gravid M. acanthurus females to currents within a chamber. Gravid females swam primarily in a downstream direction while non-gravid individuals swam almost exclusively in an upstream direction. These workers interpreted this response as evidence of a breeding and return migration.

The results of my field study on the distribution of adult, larval, post larval and juvenile stadia of M. petersi leave no doubt that the estuarine environment is necessary for larval development and metamorphosis to post larva. As only stage I larvae were caught however, I have no idea of the role that salinity plays in development of subsequent larval stages. For instance, are the low salinities encountered in the salt front region sufficient to promote ecdysis to stage II as has been postulated? The following section therefore attempts to determine, in the laboratory, the importance of salinity in the developmental history of M. petersi larvae and post larvae. In such experiments the modifying effect of temperature in combination with salinity cannot be ignored (Kinne, 1964) so that a range of temperatures was included in all experimental designs.

PART THREE

EXPERIMENTAL ANALYSIS OF THE RESPONSE OF M. PETERSI LARVAE AND POST LARVAE TO COMBINATIONS OF SALINITY AND TEMPERATURE.

1. Introduction

It is now accepted that a laboratory analysis of ecological problems can only be confidently evaluated using multivariate techniques. Due to the complexity of the natural environment it is hazardous to infer a response in the field from a simple univariable experimental analysis. If associations in the field are to be tested in the laboratory it is important that the pertinent biological, physical or chemical variables responsible for the association are correctly identified and the right questions asked.

As a result of the field investigation, although only two larval stages were caught, the presence of post larvae indicated that complete larval development must have taken place in the estuarine environment. Equally, the higher concentration of stage I larvae in regions of elevated salinity suggested that saline water was likely to play a role in the larval development of M. petersi. This was not an unreasonable assumption considering the salinity requirements for growth and development shown by other Macrobrachium larvae (Kwon & Uno, 1969; Ling, 1969; Choudhury, 1971 a, b; Monaco, 1975). Since only two larval stages were caught in the field it was difficult to gauge the range of salinity required for complete larval development. It could be argued that larvae originating from a freshwater environment may be more tolerant of low rather than of high salinity, in the same way as low salinities restrict the upstream penetration of the marine component of the estuarine fauna (Day, 1951). As larval development and metamorphosis took place in the estuary, was the whole estuary utilised or did this only occur in a restricted region? The former would imply larval euryhalinity but the latter not. To answer this question, a series of experiments were designed to determine the larval response to a range of salinities and

the extent of larval adaptation to an estuarine existence. Since temperature could modify the effects of salinity and enlarge or reduce the range of salinity tolerance (Kinne, 1964), temperature effects were evaluated in combination with salinity. The analysis also attempted to account for the restricted coastwise distribution of M. petersi.

Alderdice (1972), in an excellent review paper, extended the use of response surface methodology (Box & Wilson, 1951) to enable biological inter-relationships to be quantitatively determined. Alderdice demonstrated that multivariate analysis was not only applicable to combinations of two environmental variables such as salinity and temperature upon growth and survival, but enabled complex interactions between environmental and biological factors such as oxygen concentration, locomotory activity, acclimation, age and sex, to be determined. This method of analysis is now being widely used to aid interpretation of laboratory (Buxton et al., 1981; Tettelbach & Rhodes, 1981) and field (Beukema, 1976) investigations as well as for predictive purposes (Greenwood & Bennett, 1981).

Essentially, standard regression analysis enabled the quantitative relationship between independent variables and a dependent response to be examined simultaneously. Since the true functional relationship between the response y and the independent variables x_1, x_2 ... x_k was unknown, some approximation must be used. Of the available response equations a second order polynomial readily approximates this function. Thus, for two independent variables x_1 and x_2 the response function was approximated by :-

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{12} x_1 x_2$$

where β_0 estimated the response in the absence of x_1 and x_2; β_1 and β_2 estimated the linear effects of x_1 and x_2 respectively; β_{11} and β_{22} estimated the quadratic effects of x_1 and x_2 respectively and β_{12} estimated interaction between x_1 and x_2.

It is necessary to point out, however, that the fitted polynomial should not be over interpreted and that no biological meaning should be attached to the individual terms. For instance, a quadratic temperature effect on survival is biologically meaningless. Rather, the various terms serve to explain complex interactions between factors that occur

within the experimental range defined by the x_1 and x_2 axes. Many variations are possible as the sign of each variable may change or be linear or quadratic (Newell, 1979).

The regression coefficients were calculated by the method of least squares using standard regression analysis. These coefficients which quantify* the underlying biological relationship were used to generate a contour diagram defined by the co-ordinates x_1 and x_2. Although the resultant biological response surface was three dimensional, it was portrayed in the x_1, x_2 plane by contours of equal response. The surface was visualised geometrically and took many forms of which an ellipsoid, a saddle or a rising ridge were the most common. Inspection of the surface yielded sufficient information regarding possible effects of low salinity, high temperature combinations without recourse to more sophisticated techniques which reduce complex polynomial equations to a less complex or canonical form (Box, 1954).

2. Material and methods.

Since the design and execution of many of the experiments was similar, what follows is an overview of experimental method and techniques employed in setting up experiments. Any major alteration of technique will be discussed with the appropriate experiment.

2.1 Maintenance of berried female M. petersi.

Females with developing eggs, caught above the weir in the Keiskamma river, were transported to the laboratory and used to produce larvae for subsequent experiments. They were maintained separately (to prevent larval cannibalism) in eight 20 ℓ circular plastic containers in a closed freshwater (<0.1‰) circulatory system of 300 ℓ capacity in a constant environment room under a 16 hr light (L) and 8 hr dark (D) photoperiod. Water flowed by gravity from a 150 ℓ reservoir through the system and was biologically filtered before being recycled. The temperature was thermostatically controlled at 24.0, range $\pm$ 1.0°C and water circulated through the system at the rate of 1.0 ℓ min^{-1}. Prawns were fed, on alternate days, with a fishmeal/maizemeal mix bound with gelatin

* to determine the quantity of, to measure; Shorter Oxford English Dictionary, 3rd Edition, Oxford 1959.

to prevent food contaminating the system. The eggs usually hatched within one week after introduction of the female into the system.

2.2 Collection and allocation of larvae.

To reduce genetic variation larvae from one female only were used for each experiment. Females with a carapace length in excess of 15.0 mm produced between 1500 and 3000 larvae. Fifty newly hatched stage I larvae were counted into each salinity by the following method. The larvae, being strongly photopositive, were concentrated by light and siphoned from the circular tank into a 5 ℓ glass beaker. A sample of larvae were removed from the 5 ℓ beaker and pipetted into the centre of a 10 cm diameter petri dish containing the test salinity. Thus, salinity change was instantaneous. Larvae that swam to the side of the petri dish were regarded as "healthy" and fifty counted into each test salinity which was randomly assigned to a temperature. Once this was completed the temperature bath was switched on and the starting time taken as the time when the test salinity had attained the experimental temperature. A stocking density of effectively 100 larvae ℓ^{-1} was not considered excessive (Sandifer & Smith, 1979). The outside of each beaker was painted a dark green which aided orientation of larvae towards their food (Caulton, pers. comm.).

2.3 Routine monitoring and feeding.

Temperatures of each test salinity and not the water bath were recorded daily using a standard thermometer graduated in 0.1°C. Salinity was checked every other day, and varied between 0.4 and 1.0$^{o}/_{oo}$. Water baths were covered with glass plates to minimise evaporation. Beakers containing larvae were not aerated but 50% of the water was changed every second day. To prevent loss of larvae during this process contents of the beaker were poured through a 475 micron mesh positioned in a larger bowl containing the test salinity. The mesh was submerged so that larvae were never poured directly onto it. The beaker was rinsed with distilled water, and 250 ml of old water mixed with 250 ml of new water. Larvae were reintroduced into the test salinity by washing the mesh with a jet of water of the same salinity from the wash bottle.

Approximately one gm of Artemia eggs were dispensed into 750 ml of sea water in a conical rain gauge and aerated vigorously for 24 hrs.

After switching off the air the newly hatched nauplii concentrated at the bottom of the gauge which separated them from unhatched eggs. As stage I M. petersi larvae do not feed, in those experiments involving stage I larvae only, no food was given. Otherwise Artemia nauplii were introduced at stage I to ensure that by the time stage II emerged, food was available. Larvae were fed approximately the same number of freshly hatched Artemia nauplii every day by pipetting one ml, from a concentrate of Artemia, into each test salinity.

2.4 Experimental design.

Test conditions were set up according to a factorial design using two variables, salinity and temperature, at three or more levels depending upon the response being investigated. In a factorial design the variables were investigated simultaneously, which improved the overall estimation of the factor effects and permitted interactions between the different variables to be assessed (Finney, 1971).

The experiments were conducted in a constant environment room with a 16 hr L, 8 hr D photoperiod. Each temperature bath was an identical hard plastic 60 ℓ container with a thermostatically controlled heater/stirrer (Haake, Germany). The temperature in the environment room was 2 - 3°C lower than the lowest temperature in the experimental series so that the temperature of each bath was determined by the heater alone. The maximum number of salinities that could be tested in each bath was six. The volume of the salinity experiments was 500 ml.

In all trials the chlorinity of the test water was determined using a chloride titrator (Radiometer, Copenhagen) standardised with a solution of 0.100 M ℓ^{-1} NaCl. The chlorinity was then converted to salinity by :-

$$S\text{‰} = 0.03 + 1.805\ Cl\text{‰} \quad \text{(Harvey, 1955)}$$

In applying this equation it was assumed that the linear relationship between salinity and chlorinity held for the calculation of salinities less than 1‰. All test salinities were prepared from sea water (of known salinity) and diluted with distilled water to the desired salinity.

It is important to mention at this stage that two fundamentally different experimental approaches were adopted. In the first the response

of a particular larval stage to a range of temperatures and salinities was investigated. For example, the effect of temperature and low salinity on the median survival time of stage I larvae was determined. This was possible as the range of salinities employed inhibited ecdysis to stage II. In a further experiment optimal conditions for metamorphosis from stage IX larvae to post larvae were estimated.

The second approach was used in two experiments designed to estimate optimal temperature and salinity conditions for survival of M. petersi larvae. The method I adopted, to answer essentially the same question, differed somewhat from that used by other workers. Costlow et al. (1960, 1962, 1966) examined the effects of salinity and temperature on mortality of individual larval stages of several marine and estuarine decapod crustaceans. Costlow et al. then combined the individual stage responses to give an estimate of the overall larval requirement for successful development. While this approach allows the temperature and salinity requirements to be precisely defined for each larval stage, it omits, probably, the most sensitive event in larval development, ecdysis from one stage to another. Hill (1971) demonstrated that adult Upogebia africana can tolerate a salinity of $1.7^{\circ}/_{\circ\circ}$ but only moult successfully in $3.4^{\circ}/_{\circ\circ}$ or greater. Thus, conditions that favour survival do not necessarily accommodate moulting. I attempted to simulate natural conditions by subjecting stage I larvae to a range of salinities and temperatures and then estimated those which favoured survival after a given period of time. This estimate therefore, was derived from an experimental population that consisted of an array of larval stages, which included temperature and salinity conditions necessary for successful ecdysis as well.

3. Statistical analysis of experimental data.

All data were analysed using a Statistical Package for the Social Sciences (SPSS) computer program run on the Rhodes University I.C.L. 1904 computer. Before analysis was attempted I was advised by the Department of Mathematical Statistics on the correct use and interpretation of the "SPSS" multiple regression program.

This program was used to calculate the regression coefficients of the quadratic polynomial approximation by the method of least squares. The null hypothesis stated that the sample of observations had been

drawn from a population in which the regression coefficients were equal to zero. Rejection of the hypothesis implied that one or more of the population regression coefficients had a value different from zero. Which regression coefficients were non zero was determined by breaking down the regression sum of squares into components attributable to each term in the equation. Summation of the contribution of these terms gave an estimate of total variation explained by the quadratic polynomial. The magnitude of R^2 (square of the multiple coefficient) reflected the goodness of fit and was usually expressed as a percentage.

The regression coefficients were used to draw a response surface by entering these into a Plot Package (Rhodes University Computer Centre) which generated a contour diagram. The individual contours could be drawn at preselected intervals.

3.1 Dose-response experiments.

In these experiments temperature and salinity were regarded as the agents and the different levels, the dosage. Moreover, time was fixed so that the results described the reaction of larvae to a specific range of conditions after a given period of time. Analysis of a typical dose experiment is best illustrated by an example. The example used was a preliminary experiment to gain a broad impression of the range of salinity and temperature tolerated by M. petersi stage I larvae over a given time period. A 48 hr interval was thought sufficient to demonstrate a response which was not complicated by a preponderance of stage II larvae. Furthermore, the lower temperature range used extended beyond that likely to be encountered by stage I larvae but as the experimental period was short, drastic conditions were employed.

The design was a 6 x 6 factorial involving 36 temperature and salinity combinations. The experiment was set up and monitored as described in section 2. Temperatures were checked three times per day, the water was not changed, nor were the larvae fed. Table 19 (page 80) shows the results of the 48 hr survival experiment. A multiple regression analysis of the percentage survival yields the following quadratic polynomial fit :-

$$y = - 52.54 + 6.64x_1 + 8.47x_2 - 0.21x_1^2 - 0.19x_2^2 + 0.07x_1x_2$$

where y is the estimated percentage survival after 48 hrs and x_1 and x_2

the salinity and temperature variables respectively. The null hypothesis was rejected ($F_{5,30} = 8.62$; $0.001<P<0.01$) indicating that one or more of the coefficients has a value different from zero. ANOVA output from the "SPSS" program is given in Table 20. Five terms in the equation

Table 19. Percentage survival of predominantly stage I larvae in 36 salinity-temperature combinations after 48 hrs. Over the 48 hr period the range of any temperature or salinity value did not exceed $\pm$ 0.5°C or $\pm$ 0.4‰ respectively.

Salinity (‰)	Temperature (°C) 11.4	15.0	17.0	21.6	26.0	29.7
2.4	0.0	56.0	36.0	22.0	40.0	52.0
4.6	45.0	100.0	66.0	84.0	98.0	100.0
9.3	92.0	100.0	100.0	100.0	98.0	100.0
18.8	92.0	100.0	100.0	100.0	100.0	100.0
27.0	78.0	100.0	100.0	100.0	100.0	100.0
33.0	0.0	21.0	98.0	100.0	100.0	100.0

Table 20. Statistical analysis (ANOVA) of larval survival in 36 salinity-temperature combinations. S,S^2 & T,T^2 linear and quadratic salinity and temperature terms respectively; S x T, interaction term; *d.f. (N-k-1) where N is the number of observations and k the number of variables in the quadratic polynomial; ** values have been rounded off to the second decimal.

Source of variation	R^2 (%)	R^2 change (%)	Regression coefficients	F ratio (1,34)*	Significance
S^2	19.7	19.7	- 0.21**	13.46	P<0.001
S	39.4	19.7	6.64	16.68	P<0.001
S x T	55.3	15.9	0.07	16.68	P<0.001
T^2	59.1	3.8	- 0.19	3.21	Not significant
T	59.6	0.5	8.47	0.49	Not significant
Constant			-52.54		

account for 59.6% of the total variability. Since the experiment was exploratory the amount of variation explained by the quadratic polynomial was thought adequate. All terms except the linear and quadratic temperature effects contributed significantly to the total variability. Change in R^2 was used to calculate the significance of each regression coefficient. All regression coefficients were retained in the equation irrespective of whether these were statistically significant from zero. The order in which the terms appear was determined by the respective contribution of each to the total R^2. Thus, the linear and quadratic salinity terms explained 39.4% of the total variability and were ranked first and the linear temperature term 0.5%, and was ranked last. The significant contribution of the interaction term underlined the importance of analysing salinity and temperature effects in combination.

Fig. 36 shows the fitted response surface for survival of M. petersi larvae after 48 hrs. The survival contours were drawn at

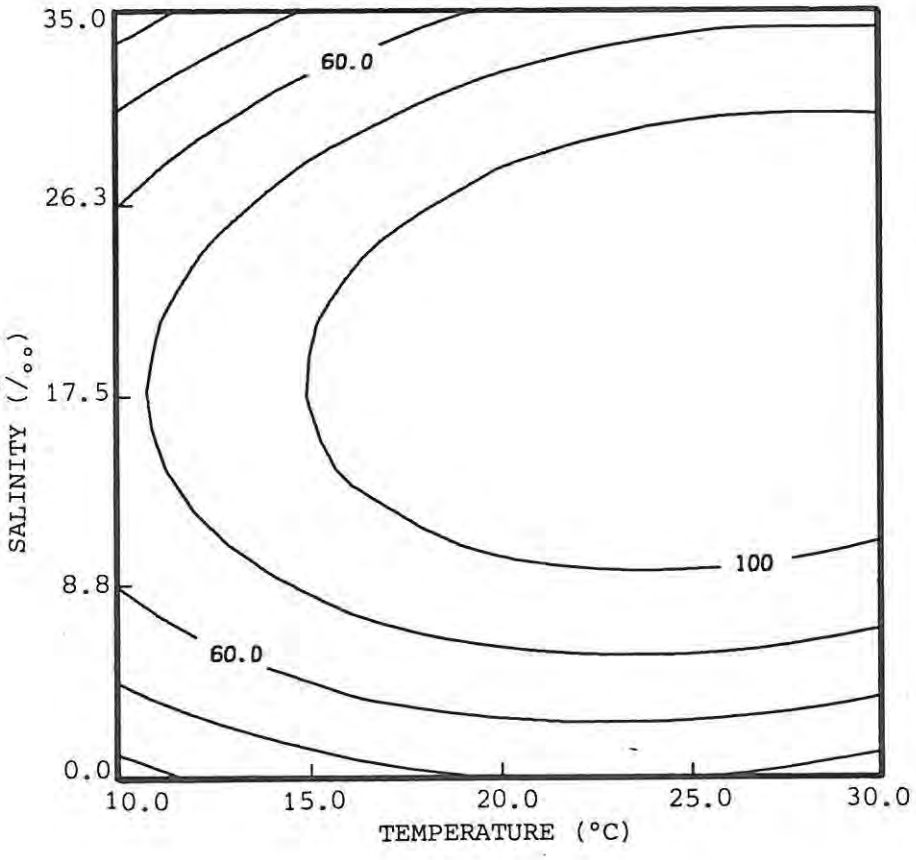


Fig. 36 Surface response estimation of the percentage survival of predominantly stage I M. petersi larvae in 36 salinity-temperature combinations after 48 hrs.

intervals of 20%. The 100% survival contour bounded the 10 to 30$^{o}/_{oo}$ salinity and 16 to 30°C temperature range. Survival fell off rapidly both at high and low salinity in response to a decrease in temperature below 16°C, indicating that temperature below 16°C was too drastic to be of value in long term experiments. However, low temperature (16°C) at higher salinity (30 - 35$^{o}/_{oo}$) appeared to favour survival while high temperature (22 - 28°C) and low salinity (0 - 4$^{o}/_{oo}$) do not. These relationships warrant further investigation with respect to the coastwise distribution of M. petersi and larval distribution in the Keiskamma river per se. Survival at salinities in excess of 10$^{o}/_{oo}$ appear to be independent of temperature in the 20 to 30°C range.

From this experiment it was decided that a meaningful temperature range for future experiments should be between 16 and 30°C. As far as the salinity range was concerned, the larvae appeared particularly sensitive to low salinities (0 - 10$^{o}/_{oo}$) over the whole temperature range whereas at higher salinities (10 - 35$^{o}/_{oo}$) survival was considerably improved. Approximately 10% of the stage I larvae had moulted to stage II after 48 hrs so it was unlikely that a change in the experimental larval population could account for the categorisation of the salinity response into two ranges.

3.2 Time-response experiments.

In these experiments time was not fixed but regarded as the dependent variable. The temperature and salinity range that defined the response of 50% of the larval population was used to determine (1) optimum conditions for larval survival, (2) minimum salinity and temperature levels for ecdysis from stage I to stage II, (3) median survival time in freshwater conditions. Coefficients of determination were then estimated from the median response values for each particular salinity and temperature combination and used to generate a median response contour diagram.

In certain salinity and temperature combinations a proportion of the population may not have responded by the time the experiment was terminated. In such combinations the response was artificially truncated on the assumption that, had the experiment continued long enough, the remaining individuals would have reacted in essentially the same manner

as those that had already responded (Bliss, 1935). Calculation of the time-response equation was more complex in truncated distributions as improved estimates of the mean and standard deviation are obtained by a series of iterations. Those individuals in the truncated part of the distribution were included in the time-response calculation. The standard probit method (Bliss, 1935, 1967) was used to estimate the median response time for both complete and truncated time-response curves. The method of analysis was based on that of accumulative frequencies, which in time-response experiments, were strongly correlated with each other. This could be avoided if the observations were treated not cumulatively, but rather as individual interval frequencies which were not correlated. Thus, in each time-response experiment an observation consisted of the number of responses in consecutive individual time intervals. A programme (see appendix) was developed to compute the median response time using individual frequencies. Failure of the assumptions of the model, namely homogeneity of variance and normality, were checked using a random selection of raw data. In each case the requirements of the model were satisfied. In one experiment error was introduced by aberrant mortality which varied between 2.0 and 6.0% in any test combination. As the time interval in which the mortality occurred was recorded, it was decided to include the mortality with the number that had responded. Clearly, this was an approximation which could be improved by using statistical censoring techniques.

The method of recording and analysing time-response data is best illustrated by an example. The example chosen was an experiment designed to explain the drop in stage I larval abundance downstream from a salt front. For instance, on 4 February 1981 (Table 15, part two), the mean abundance at station 11 decreased from 7.4, SD $\pm$ 2.2 larvae $k\ell^{-1}$ to 0.8, SD $\pm$ 0.70 larvae $k\ell^{-1}$ at station 12. This could be explained if the salinity in the vicinity of the salt front promoted ecdysis to stage II with a concomitant change in behaviour that made them difficult to catch. To verify this hypothesis an experiment was designed to determine the minimum range of salinity and temperature required for 50% of stage I larvae to moult to stage II.

The experimental design was a 3 x 4 factorial with four salinities 0.54, 0.77, 1.05 and 1.56 $^{o}/_{oo}$ and three temperatures 21.8, 25.2 and 28.3°C respectively. The experiment was set up and monitored as

described in section 2. Fifty stage I larvae were allocated to each test salinity and not fed. Time zero was the time when the temperature of each test salinity had attained that of the water bath. Test combinations were checked periodically, stage II larvae removed, counted and the time recorded. The experiment was terminated after 153 hrs. Surviving stage I larvae (the truncated individuals that had not yet responded) were counted and incorporated in the estimation of the median response time. In a duplicate control at 24.1°C and 12.0°/₀₀ all larvae had moulted to stage II within 90 hrs.

Table 21 shows the estimated time taken for 50% of the larval population to change to stage II in 12 salinity and temperature combinations.

Table 21. Median response time (hrs) for ecdysis to stage II at three temperatures and four salinities.
* Mean, ± SD.

Temperature (°C) / Salinity (°/₀₀)	21.8 ± 0.4*	25.2 ± 0.2	28.3 ± 0.3
0.54 ± 0.1*	156.7	92.0	118.2
0.77 ± 0.1	126.7	101.7	76.6
1.05 ± 0.2	106.8	73.4	77.4
1.56 ± 0.2	117.3	71.8	60.3

The median response time decreased with an increase in temperature and salinity which suggested that both salinity and temperature promoted ecdysis to stage II. The response surface is shown in Fig. 37 (page 85). The contours represent 10 hr intervals between 70 and 120 hrs. The curvature of the isopleths suggested that interaction between temperature and salinity was marked. An optimum was reached within the experimental region but the fact that the 70 hr isopleth was not complete suggested that shorter median response times were likely at higher salinities and higher temperatures. The experiment however was designed to yield minimum, not optimum salinity and temperature ranges for ecdysis to stage II. The range of temperature and salinity bounded by the 70 hr isopleth defined the minimum conditions required for 50% of the stage I larvae to change to stage II. The salinity range lay between 1.0 and

$1.75^{\circ}/_{\circ\circ}$, while temperatures greater than 25.0°C were required.

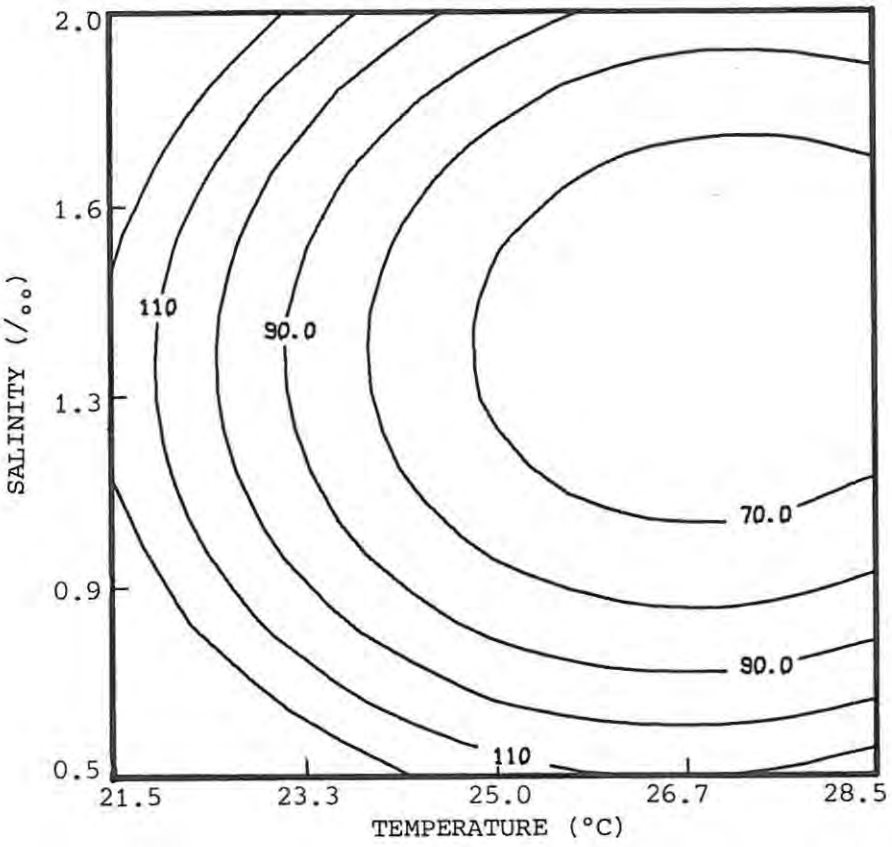


Fig. 37 Response surface estimation of the median response time (hrs) for ecdysis from stage I to stage II larvae as affected by temperature and salinity.

These data demonstrated that ecdysis to stage II can take place at salinities that were likely to be normally encountered at the head of a salt front region. Therefore, the drop in stage I abundance downstream from the salt front was due to ecdysis to stage II which, since they were not caught, appeared to have lost the planktonic mode of existence.

Table 22 (page 86) shows that the fitted quadratic polynomial accounted for 89.9% of the total variability. The null hypothesis was rejected ($F_{5,6} = 10.70$; $0.001<P<0.01$) and of the five terms in the equation, interaction and the quadratic salinity and temperature terms contributed significantly to the overall variability. The effect of

interaction explained 45% of the total variability.

Table 22. Statistical analysis of the change from stage I to stage II larvae at three salinities and four temperatures.

Source of variation	R^2 (%)	R^2 change (%)	Regression coefficients	$F_{(1,10)}$ ratio	Significance
T x S	45.0	45.0	- 1.96	44.64	P<0.001
S^2	75.5	30.5	64.82	30.26	P<0.001
T^2	86.8	11.3	1.82	11.17	0.001<P<0.01
S	89.0	2.2	- 125.29	2.17	Not significant
T	89.9	0.9	- 96.38	0.89	Not significant
Constant			1452.42		

These two examples have been chosen to illustrate the effectiveness of the multiple regression analysis and contour diagram techniques to quantify the observed response and facilitate interpretation of the data. Using these methods it should be possible to obtain a statistically sound assessment of the response of larval M. petersi to salinity and temperature and therefore an insight into whether the larvae are suitably adapted to an estuarine existence.

3.3 Analysis of residual plots.

After completion of each experiment the residuals were checked for failure of two assumptions, namely homogeneity of variance and normality. Residuals are a measure of a deviation of an observed from an estimated y. The assumption of homogeneity of variance was found acceptable if the plot of residual against estimated y resulted in random scatter around the zero line. Fig. 38 (page 87) shows that the assumed homogeneity of variance in this particular experiment was acceptable but this certainly was not so for Figs. 39 (page 87) and 40 (page 88). The scatter in Fig. 39 suggested that the samples were drawn from three separate populations. In this experiment the effect of three temperatures and six salinities on the growth of five day old M. petersi post larvae was determined. Since it was not possible to

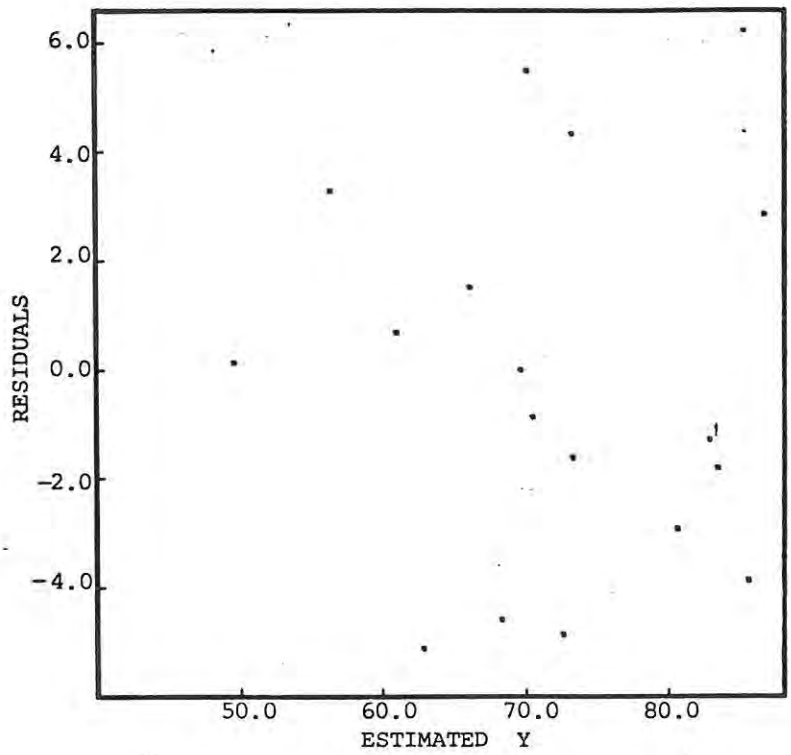


Fig. 38 A residual plot showing homogeneity of variance.

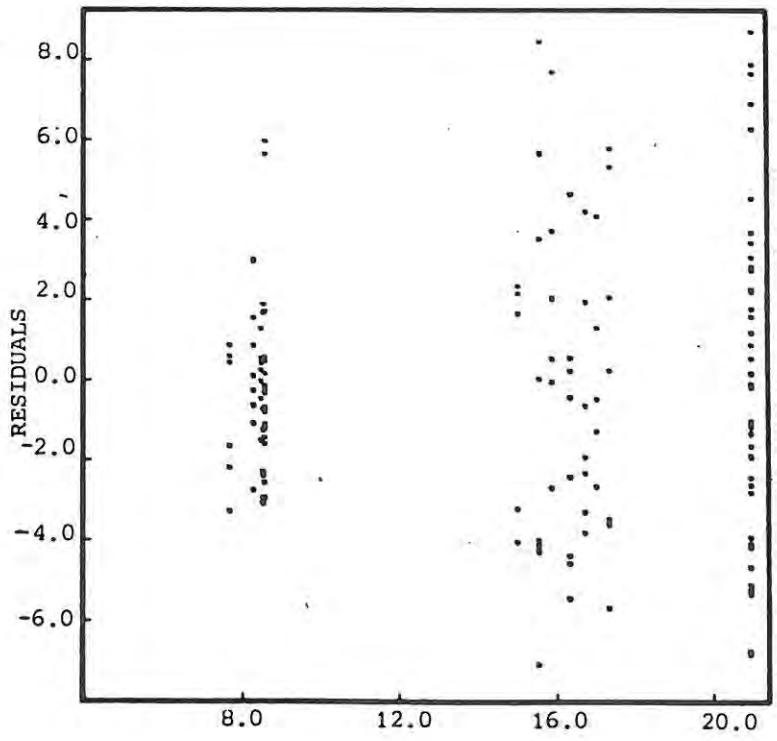


Fig. 39 A residual plot showing non-homogeneity of variance.

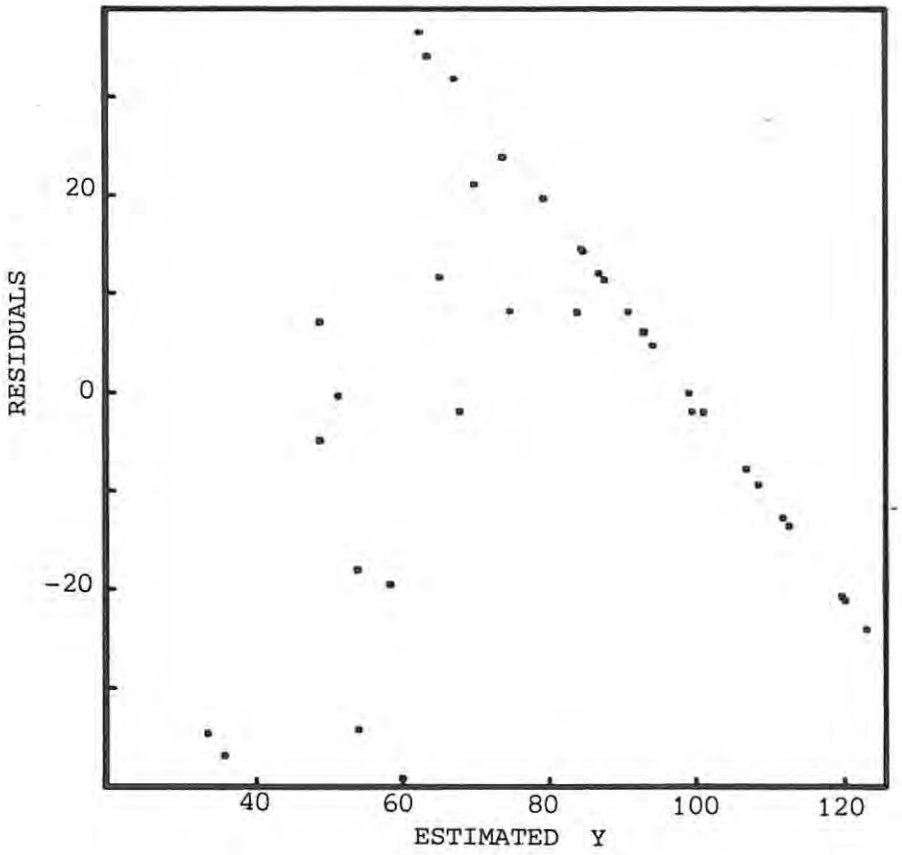


Fig. 40 A residual plot testing for homogeneity of variance in the preliminary 48 hr stage I larval survival experiment.

synchronise production of sufficient five day old post larvae for the entire experiment, post larvae were reared from eggs of a different female for each temperature. Clearly this technique appeared responsible for the resultant non-random scatter and the low (64.1%) coefficient of determination in the experiment. In future growth experiments therefore, this could be avoided by setting up the experiment using post larvae, irrespective of age, from a common pool. In Fig. 40 the residuals were clearly linearly related to the estimated response and not scattered randomly, which probably accounted for the low (58%) R^2 value in this experiment.

The assumption of normality of the error term was found acceptable if the probability plot of the residuals yielded a straight line. Fig. 41 (page 89) shows the residual plot from a median response

time experiment which indicated that distribution of the error term could be regarded as approximately normal. Inspection of the residual plots not only permits a rapid assessment of validity of the assumptions and aptness of the quadratic approximation but also alerts the experimenter to other abnormalities that may have arisen which can be corrected in future designs (Neter & Wasserman, 1974).

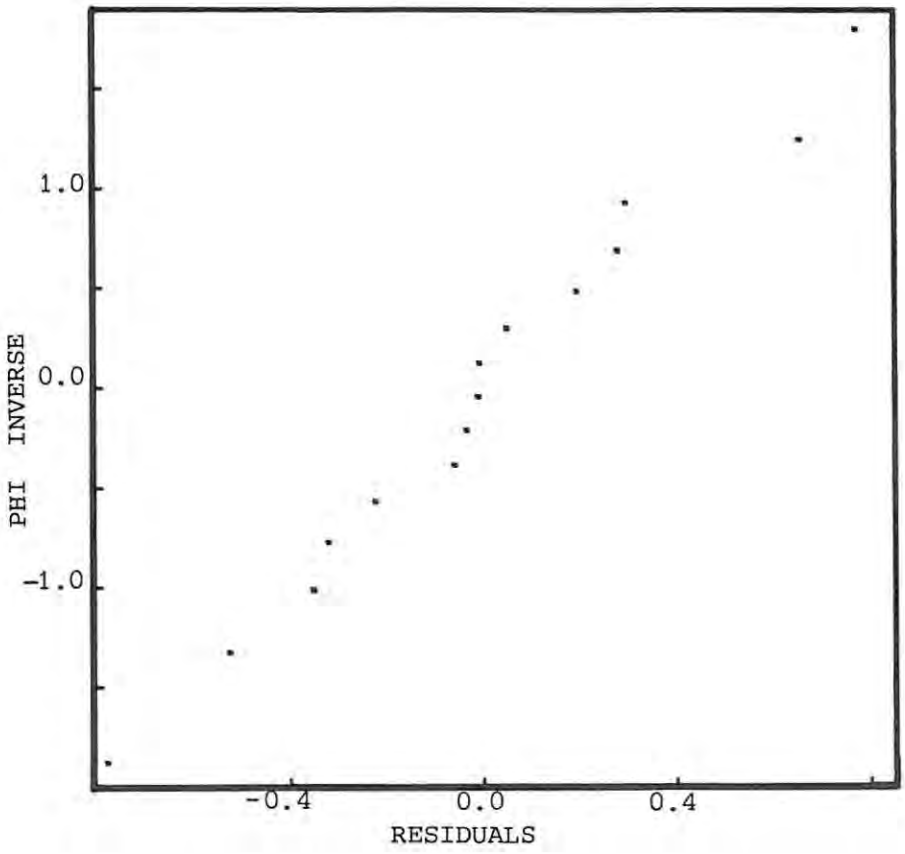


Fig. 41 A probability plot testing for normality of the error term from a median response time experiment.

4. Experimental studies.

Before the laboratory investigation of the response of M. petersi larvae to various combinations of salinity and temperature could proceed, a method of larval rearing had to be developed should the demand for a particular stage arise. Therefore, larval M. petersi were reared en masse

for the following reasons :-

(a) to identify the developmental stages which would aid in the identification of larvae caught in the field;

(b) to supply material for laboratory studies as it was not possible to bring sufficient larvae alive from the field;

(c) to observe larval behaviour which could lead to an alteration of the trawling technique adopted in the field.

4.1 Material and methods.

A closed saline water circulatory system was established under natural photoperiod conditions. Fig. 42 shows the design of the system.

Fig. 42 Design of circulatory system for mass rearing M. petersi larvae.

Saline water gravitated from a 500 ℓ reservoir into six (0.6 x 0.4 x 0.25 m) plastic tanks each with a separate glass tap to regulate water flow; the outlet pipe was covered to prevent loss of larvae. Water flowed from each tank into a biological filter from where it was pumped into the reservoir before being recycled. Flow rates varied between 400 and 600 ml min^{-1}. The water was not aerated. Temperature was controlled

thermostatically at 25.0, range $\pm$ 1.2°C. Sea water was diluted with tap water and a salinity of 14.0, range $\pm$ 2.2‰ maintained. Evaporative losses were compensated for by adding tap water.

Larvae were hatched as outlined in section 2. Stage I larvae were never mixed so that each tank contained larvae from one female only. Tanks were understocked (<30 ℓ^{-1}) which may account for the low mortality experienced. Larvae were fed freshly hatched Artemia nauplii daily.

4.2 Stage identification.

Time taken to metamorphosis of the first post larvae varied from between 20 to 22 days but the process usually carried on for a period of 10 days or more. Post larvae of M. rosenbergii are commonly obtained after day 21 but peaked between 25 and 35 days (Ling, 1969). In M. nipponense the first post larvae were obtained after 20 days (Uno, 1971). Marked variation in the duration of larval development is found in several groups of decapod crustaceans but appears most pronounced in the Palaemonidae (Sandifer & Smith, 1979). Nine larval stages were identified in M. petersi using the criteria of Ling (1969) and Kwon & Uno (1969) for M. rosenbergii and M. nipponense respectively. Table 23 (page 92) shows conspicuous morphological changes used to identify the nine larval stages. Post larvae have not been included as these swim horizontally and are therefore easily recognisable from larvae which swim vertically. Table 23 is not complete but merely meant to serve as a practical guide for rapid identification when live material of a particular stage is required.

4.3 Larval behaviour.

Observation of the laboratory reared larvae during larval development did not reveal any obvious changes in behaviour, swimming ability, position in the water column or extended use of the bottom to account for their absence in the field. All indications from the laboratory behaviour of the larvae pointed to a pelagic and predacious existence and an ability to utilise the water column to maximum advantage. Accordingly, the field results were most surprising as the water column was extensively sampled. Older larvae (>10 days) attached themselves to the side of the tank which might suggest that in the littoral areas of

Table 23. Morphological changes used to identify the nine larval stages of M. petersi.

Larval stage	Changes in morphology of : Telson	Eyes	Carapace	Abdomen
I	Telson broad with spines	Sessile	No setae beneath first rostral tooth	Smooth
II	"	Stalked	"	"
III	Exopods of uropod present, endopods forming	"	"	"
IV	Uropod complete with exo- & endopods; telson much narrower	"	"	"
V	"	"	1 setae beneath first rostral tooth	Rudimentary pleopod buds appear
VI	"	"	2-3 setae beneath first rostral tooth	Pleopods bare and biramous
VII	"	"	2-3 setae beneath first rostral tooth	Pleopods bare, biramous and paddle-like
VIII	"	"	3-4 setae beneath first rostral tooth	4th pleopod with 1-3 setae along margin
IX	"	"	5 or more setae beneath first rostral tooth	Setae on margins of all pleopods

the estuary they attach to stalks of Phragmites and Scirpus. At one stage certain tanks were infested with a small planarian which the larvae devoured which showed that they were not solely reliant on a free swimming food source. From observations of larval behaviour no problems were envisaged catching larvae in the field using conventional zooplankton trawling methods. The fact that this was not so emphasises the danger of predicting patterns of field behaviour from laboratory observations only.

4.4 Effect of temperature and low salinity on median survival time of stage I M. petersi larvae.

Macrobrachium species are known to undergo a downstream breeding migration to the estuarine environment. No direct evidence of such a

migration was obtained for M. petersi, but in the 1979/80 summer movement was in the opposite direction in response to elevated salinities at the head of the estuary. Thus in the 1979/80 breeding season the majority of stage I hatched in freshwater above the weir and relied on downstream flow for transport to the estuary. Current velocity above the weir was not measured but the low discharge would suggest this to be considerably reduced. Under these circumstances a protracted period in freshwater could be expected. An experiment was designed therefore to estimate the median survival time of stage I in freshwater under a range of temperatures.

In the Keiskamma river the salinity content of the freshwater varied between 0.05 and 0.6‰ depending on the extent of freshwater discharge. Three salinity levels, 0.05, 0.10 and 0.40‰ and five temperatures, 18.1, 22.2, 24.1, 28.3 and 29.7°C were therefore selected to represent conditions that existed above the weir. The experimental technique and data analysis were similar to that described in sections 2 and 3 respectively. The stage I larvae were not fed as the salinities were too low to promote ecdysis to stage II, hence all recorded mortalities were of stage I larvae only. The response surface as a function of salinity and temperature is shown in Fig. 43, and the estimated

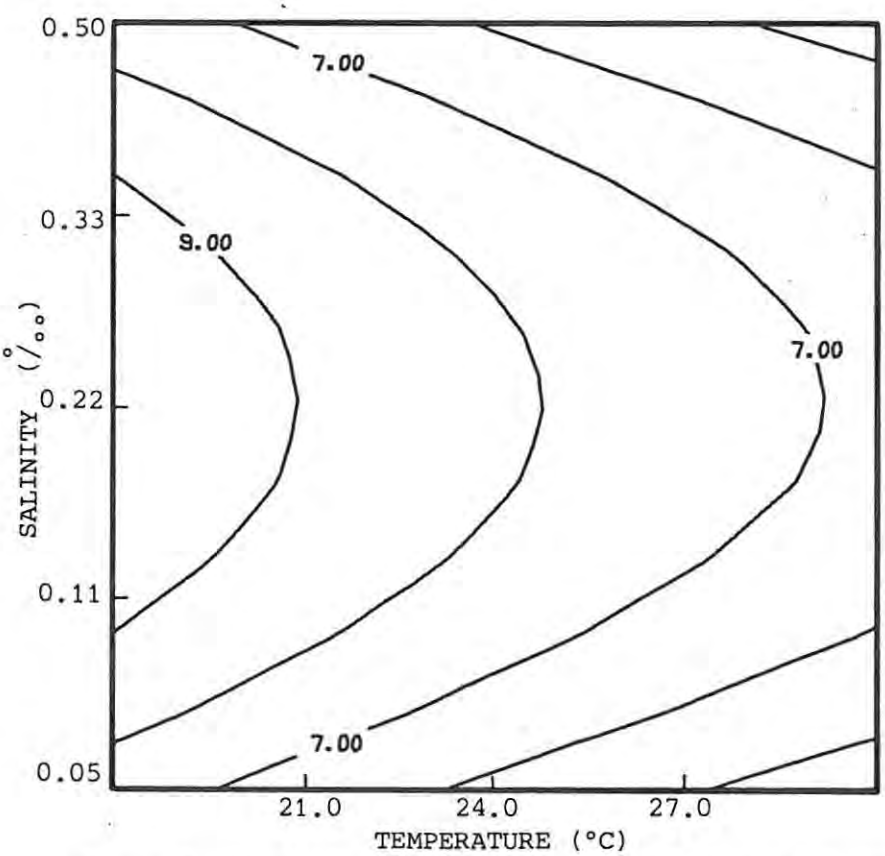


Fig. 43 Response surface calculated for median survival time (days) of stage I M. petersi larvae as a function of temperature & salinity. The contours of equal response were drawn at one day intervals.

median survival time in 15 temperature and salinity combinations shown in Table 24. The pattern of response (Fig. 43) showed a rising ridge of increasing survival time with a decrease in temperature. In relation to

Table 24. Median survival time (days) of stage I M. petersi larvae in three salinities and five temperatures. * Mean ± SD.

Temperature (°C) / Salinity (‰)	18.1±0.1*	22.2±0.3	24.1±0.2	28.3±0.3	29.7±0.2
0.05±0.0*	8.3	5.9	5.4	5.8	3.4
0.12±0.1	7.7	7.2	6.8	6.1	5.6
0.42±0.1	9.1	8.3	7.2	6.5	6.1

an increase in salinity the response was difficult to explain as survival time increased and then decreased. This anomaly may be due to the restricted range of salinity being investigated (we are only looking at a very small region of the total surface) or to the fact that the moult to stage II was inhibited. Furthermore, the spurious conclusions that could be drawn from the shape of this response surface emphasised the importance of subjective judgement when interpreting results of this nature. This is important when polynomial functions are used to describe a biological response as extrapolation may lead to erroneous conclusions.

Table 25 shows that 86.8% of the total variability was explained by the quadratic polynomial. The null hypothesis was rejected

Table 25. ANOVA of median survival time of stage I M. petersi larvae in three salinities and five temperatures.

Source of variation	R^2 (%)	R^2 change (%)	Regression coefficients	$F_{(1,13)}$ ratio	Significance
T	60.6	60.6	- 0.44	60.1	P<0.001
S	81.9	21.3	24.78	21.1	P<0.001
S^2	86.6	4.7	-46.13	4.6	0.01<P<0.05
S x T	86.7	0.1	0.04	0.03	Not significant
T^2	86.8	0.1	0.004	0.01	Not significant
Constant			13.06		

($F_{5,9}$ = 11.93; P<0.001) and of the five terms in the equation the first three contribute significantly to the response of which the linear temperature term accounts for 60.6%. The results indicate that duration of stage I survival in the Keiskamma river between January to March 1980 would have varied between 7 and 8 days unless the stage I larvae were swept over the weir into the estuary. Drought conditions in the catchment area had reduced the flow considerably so that it was not unreasonable to assume that stage I mortality may have been unusually high. It has been suggested (part two, section 2.8) that the absence of a migration during the summer of 1979/80 was due to low freshwater discharge which, under normal conditions, may provide a stimulus for a post larval return migration to freshwater. These findings suggest that an additional factor may have contributed to the absence of a migration, namely an unusually high larval mortality at stage I.

4.5 Effect of low and high salinity in combination with temperature on survival, haemolymph osmotic concentration and development of M. petersi larvae.

The preliminary 48 hr larval survival investigation revealed that the tolerance of predominantly stage I larvae effectively divided response to salinity into two ranges, below and above 10‰. Two experiments were set up to investigate, over a longer time, the relationship between temperature and low and high salinity on survival of M. petersi larvae. These experiments were designed to cover the salinity spectrum that larvae were likely to experience in the Keiskamma estuary and therefore embrace the optimal range for survival and development. From these estimates it should be possible to determine whether the entire estuary or only a particular region was likely to be utilised by M. petersi larvae.

As mentioned in section 2.4, the approach adopted to estimate optimal conditions for survival of M. petersi larvae was different from that of other workers (Costlow et al., 1960, 1962, 1966). An assumption of the method, since requirements from an array of larval stages were being estimated, was that conditions for survival were unlikely to differ markedly between stages. To test the validity of this assumption a series of 24 hr salinity tolerance experiments on different larval stages was carried out and the salinity, at which 50% survived, estimated.

The following procedure was adopted to determine the LD 50 salinity for stages II, III, IV, V, VII and IX. Larvae from a single hatch were reared under conditions described in section 4. Fifty larvae were used in each test salinity, maintained in a constant temperature bath at 25.2, range $\pm$ 0.8°C. Photoperiod, equipment, salinity checks and allocation of larvae were carried out as described in section 2. The larvae were not fed Artemia nauplii. Between five and seven test salinities, within a 1.0 to 4.0°/$_{oo}$ salinity range, were chosen as a preliminary trial revealed that at salinities greater than 4.0°/$_{oo}$, 100% survival was obtained for all stages. Other than stages II and III, it was not possible to select fifty larvae of the same stage. However, in each instance more than 80% of the larvae were representative of the designated stage. Transfer of larvae from rearing (14.0, range $\pm$ 2.0°/$_{oo}$) to the test salinities was instantaneous. After 24 hrs the number of surviving larvae in each salinity was counted and the survival percentage transformed to probits. Regression equations were calculated and the salinity at LD 50 and 95% confidence limit estimated (Finney, 1971).

Table 26 shows that the salinity at LD 50 increased from 2.0°/$_{oo}$ for stage II larvae to 3.0°/$_{oo}$ for stage IV. Hereafter it did not change but decreased again to 2.0°/$_{oo}$ for stage IX. It was not possible to calculate an LD 50 for stage I larvae as mortality was zero after 24 hrs.

Table 26. Probit regression and median lethal salinity of non-acclimated larvae where y is survival in probits corresponding to a given salinity X, between 1.0 & 4.0°/$_{oo}$.

Larval stage	Probit regression	LD 50 (/$_{oo}$)	95% confidence limit
II	y = 5.23 + 2.28 (X - 2.08)	2.0	0.14
III	y = 4.68 + 3.07 (X - 2.63)	2.7	0.20
IV	y = 5.17 + 1.42 (X - 3.21)	3.1	0.25
V	y = 4.84 + 2.47 (X - 3.10)	3.2	0.42
VII	y = 5.06 + 0.84 (X - 3.19)	3.1	0.33
IX	y = 5.28 + 2.25 (X - 2.75)	2.6	0.14

These results demonstrated that the larvae did show a difference in tolerance to low salinity but this was not particularly marked. Thus the

assumption was upheld which enabled the modified experimental approach to be adopted.

(i) Response to low salinity and temperature.

The experiment was designed as a 4 x 5 factorial with four salinities 0.5, 1.4, 2.5 and 5.8‰ and five temperatures, 17.9, 23.4, 24.9, 25.9 and 29.8°C respectively. It was set up and monitored as described in section 2. Fifty stage I larvae were allocated to each test salinity together with Artemia nauplii so when moulting to stage II took place food was immediately available. Survival was determined after four and seven days respectively after which the experiment was terminated and the number of larvae in each development stage noted.

Table 27 shows the percentage survival after four days and Fig. 44 (page 98) the fitted response surface. A combination of high

Table 27. Percentage survival after four days in five temperature and four salinity combinations.

Temperature (°C) / Salinity (‰)	18.0	23.4	24.9	25.9	29.8
0.50	92.0	80.0	70.0	48.0	0.0
1.40	98.0	90.0	92.0	80.0	6.0
2.50	98.0	96.0	94.0	92.0	86.0
5.80	98.0	100.0	96.0	100.0	98.0

temperature and low salinity did not favour survival which decreased markedly with an increase in temperature. An interaction effect was demonstrated by the position of the 100% survival ellipse which was markedly skewed to the right. Thus as 1.5‰ a 100% survival was estimated at 21°C but at 29.5°C this had dropped to 40%. The fitted second degree polynomial accounted for 84.4% of the variability (Table 28, page 98). The null hypothesis was rejected ($F_{5,14} = 15,15$; $P<0.001$) and of the five terms in the equation only the linear effect of salinity was not significant. Although quadratic temperature term had been ranked first, the significant interaction indicates that temperature and salinity

are acting in concert to produce the pattern of response.

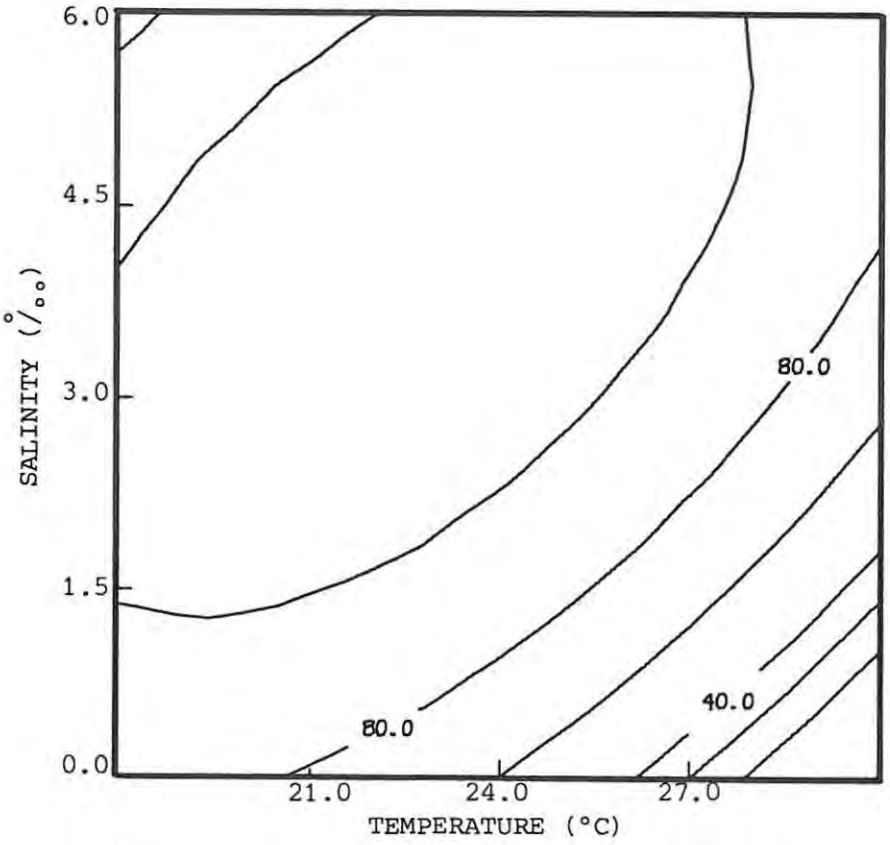


Fig. 44 Response surface of the percentage survival of M. petersi larvae after four days as a function of salinity and temperature. The survival contours were drawn at intervals of 20%.

Table 28. ANOVA of percentage survival of M. petersi larvae after four days.

Source of variation	R^2 (%)	R^2 change (%)	Regression coefficients	$F_{(1,18)}$ ratio	Significance
T^2	30.5	30.5	- 0.54	35.23	P<0.001
T x S	60.8	30.3	1.41	35.04	P<0.001
S^2	78.5	17.7	- 2.73	20.42	P<0.001
T	83.6	5.1	18.01	5.86	0.01<P<0.05
S	84.3	0.7	- 9.50	0.81	Not significant
Constant			-64.68		

Table 29 shows the percentage survival after seven days and Fig. 45 the fitted response surface. A narrower range of temperature and salinity bound the 100% survival contour which indicated that mortality, especially at higher temperature, had taken place. Whereas

Table 29. Percentage survival of M. petersi larvae after seven days in five temperature and four salinity combinations. *Mean ± SD. These values also apply to Table 27.

Temperature (°C) / Salinity (‰)	18.0±0.2*	23.4±0.1	24.9±0.3	25.9±0.2	29.8±0.1
0.50 ± 0.1*	24.0	6.0	8.0	0.0	0.0
1.40 ± 0.10	56.0	50.0	44.0	38.0	0.0
2.50 ± 0.20	74.0	74.0	84.0	72.0	42.0
5.80 ± 0.20	98.0	96.0	86.0	92.0	78.0

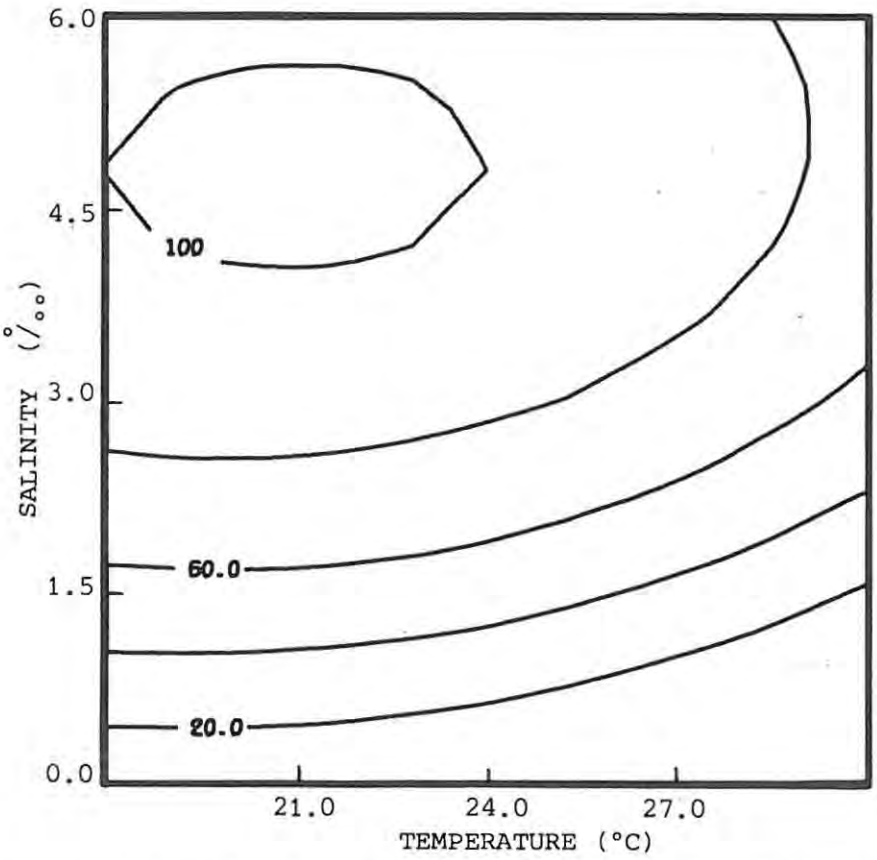


Fig. 45 Response surface of the percentage survival of M. petersi larvae after seven days as a function of temperature and salinity. The survival contours were drawn at 20% intervals.

the temperature range that bounded the 100% survival contour lay between 18 - 27°C after four days, it had now shifted to the left between 18 - 24°C to indicate at low salinity, low temperatures were required for maximum survival. Moreover, the position of the ellipse was parallel to the temperature axis which demonstrated that the interaction effect had been reduced to one of salinity only. This was evident from the number of survival contours crossed in a vertical direction at any temperature. The seven day response was described by fitting a quadratic polynomial which explained 95.2% of the variability (Table 30). The

Table 30. ANOVA of percentage survival of M. petersi larvae after seven days.

Source of variation	R^2 (%)	R^2 change (%)	Regression coefficients	$F_{(1,18)}$ ratio	Significance
S	71.1	71.1	36.66	281.55	P<0.001
S^2	84.2	13.1	- 4.32	51.55	P<0.001
T^2	93.6	9.4	- 0.29	35.69	P<0.001
T	94.9	1.3	10.85	5.15	0.01<P<0.05
T x S	95.2	0.3	0.25	1.34	Not significant
Constant			-95.34		

null hypothesis was rejected ($F_{1,18}$ = 58.8; P<0.001) and only the contribution of the interaction term to the total variability was not significant. Salinity thus played the greatest role and accounted for 71.1% of the total R^2.

These data indicated that not only do temperature and salinity affect larval survival, but age does as well since four, rather than seven day old larvae were more tolerant of both temperature and salinity. Similarly the importance of the terms in the quadratic polynomial changed with time. After four days the interaction and quadratic temperature terms accounted for 61% of the total variability but after seven days salinity per se accounted for 71%. In younger larvae therefore, in low saline conditions, both salinity and temperature were responsible for survival but as the larvae aged the effect of salinity was of overriding importance. Lough & Gonor (1973) reported a similar response in larvae

of the mussel Adula californiensis where 15 day old larvae had a wider salinity but narrower thermal tolerance than three day old larvae.

During the course of this experiment all dead larvae were removed and counted and the time of death recorded. It was argued that if time could be regarded as the dependent variable, then the temperature and salinity conditions bounded by the longest median survival time contour could be regarded as optimal for larval survival. The results were therefore analysed, as a time-response sequence, as described in section 3.2.

Table 31 shows the median survival time of larvae at various salinity and temperature combinations. Generally, median survival time increased with an increase in salinity but decreased with an increase in

Table 31. Median survival time (days) of M. petersi larvae in four salinities and five temperatures. *SD of temperature and salinity same as in Table 29.

Temperature (°C) / Salinity (‰)	18.0*	23.4	24.9	25.9	29.8
0.50*	6.7	6.1	6.1	4.6	2.8
1.40	7.5	8.1	7.0	6.8	4.8
2.50	8.4	10.3	8.6	10.9	7.1
5.80	17.5	16.3	13.9	15.8	13.2

temperature. A fitted response surface (Fig. 46, page 102) illustrated the effect of salinity on median survival time as the contours were almost parallel to the temperature axis. The contribution salinity made to the overall response is listed in Table 32 (page 102). Of the five terms in the quadratic polynomial which accounted for 95.8% of the total variability, salinity per se accounted for 88.3%. The interaction term was not significant which indicated survival could be considered in relation to salinity only. Although the fit was marginally improved by the inclusion of the quadratic temperature term, its contribution could be seen at higher temperatures which tended to increase the curvature of the median survival contours. This suggested that at higher temperatures, the

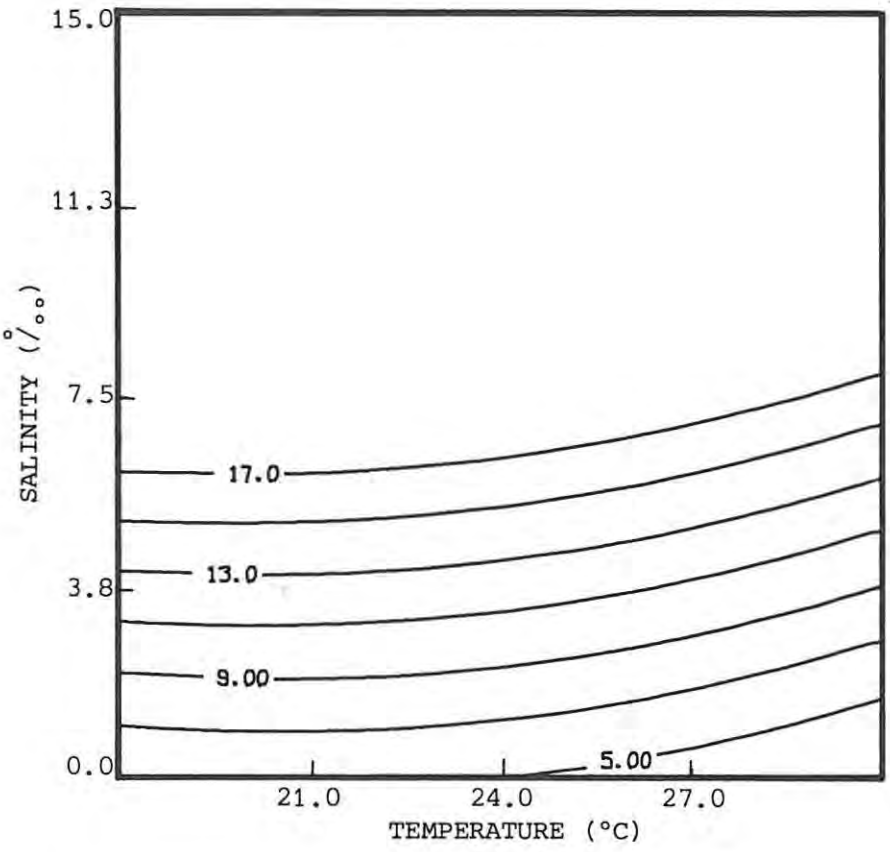


Fig. 46 Response surface estimation of median survival time (days) of M. petersi larvae as a function of temperature and salinity.

Table 32. ANOVA of median survival time of M. petersi larvae.

Source of variation	R^2 (%)	R^2 change (%)	Regression coefficients	$F_{(1,18)}$ ratio	Significance
S	88.3	88.3	2.13	397.60	P<0.001
T^2	94.2	5.9	- 0.03	26.60	P<0.001
T	95.8	1.6	1.55	7.23	0.01<P<0.05
T x S	95.8	0.01	- 0.01	0.31	Not significant
S^2	95.8	0.01	0.01	0.06	Not significant
Constant			-10.80		

larvae required higher salinities to maintain equivalent median survival times. Thus the 17 day median survival time contour cuts the salinity axis at 6.0°/₀₀ at 18°C but at 8.0°/₀₀ at 30°C. Since the time taken to

reach post larvae ranged between 21 and 30 days, it was not unreasonable to assume that the 17 day median survival time contour defined the lower salinity limit within the prescribed temperature range for larval survival. Thus a salinity of $6^{o}/_{oo}$ at a temperature of 18°C and a salinity of $8^{o}/_{oo}$ at 30°C defined the lower limits of the salinity range required for larval survival between these two temperatures.

A small increase in salinity accounted for a dramatic increase in median survival time. At 24°C for every $2.0^{o}/_{oo}$ increment to $6^{o}/_{oo}$ the median survival time increased by approximately five days (Fig. 46). These results emphasised the importance of saline water for larval survival and perhaps explained the increase in concentration of stage I larvae in salt front regions. Indeed, considering the estuary had been subjected to flooding well before the Pleistocene, and the importance of salinity for survival, selection pressure for the development of a mechanism to detect minor increases in salinity was likely to have been great.

The expanded salinity axis of the response surface (Fig. 46) effectively concentrated the median survival time contours in the lower salinity region of the response surface. The fact that the 17 day survival contour had not closed to form an ellipse implied that salinities between 8 and $35^{o}/_{oo}$ also favoured survival. This was in agreement with the guidelines established from the results of the preliminary 48 hr survival requirement.

(ii) Response to high salinity and temperature.

To investigate the survival of M. petersi larvae at high salinities a 5 x 4 factorial design was adopted with five temperatures, 15.6, 19.1, 21.7, 25.1 and 29.3°C and four salinities, 15.5, 21.9, 30.2 and $35.1^{o}/_{oo}$ respectively. The experiment was set up and monitored as described in section 2. Fifty stage I larvae were allotted to each test salinity. A daily diet of Artemia nauplii was introduced into each test salinity as described in section 2.3. The experiment was terminated after ten days and the stage of development and survival recorded.

Table 33 shows the percentage survival after ten days in 20 combinations of salinity and temperature and Fig. 47 the fitted response

surface. Survival contours were almost parallel to the salinity axis which indicated that at higher salinities survival was to a large extent temperature dependent. However, the slightly skewed contours towards high

Table 33. Percentage survival of M. petersi larvae after ten days at five temperature and four salinity combinations. *Mean ±SD.

Temperature (°C) / Salinity (°/₀₀)	15.6±0.1*	19.1±0.1	21.7±0.2	25.1±0.3	29.3±0.1
15.1±0.3*	50.0	94.0	96.0	92.0	94.0
21.9±0.4	35.0	100.0	100.0	88.0	90.0
30.2±0.2	10.0	92.0	92.0	96.0	88.0
35.1±0.1	4.0	98.0	94.0	90.0	90.0

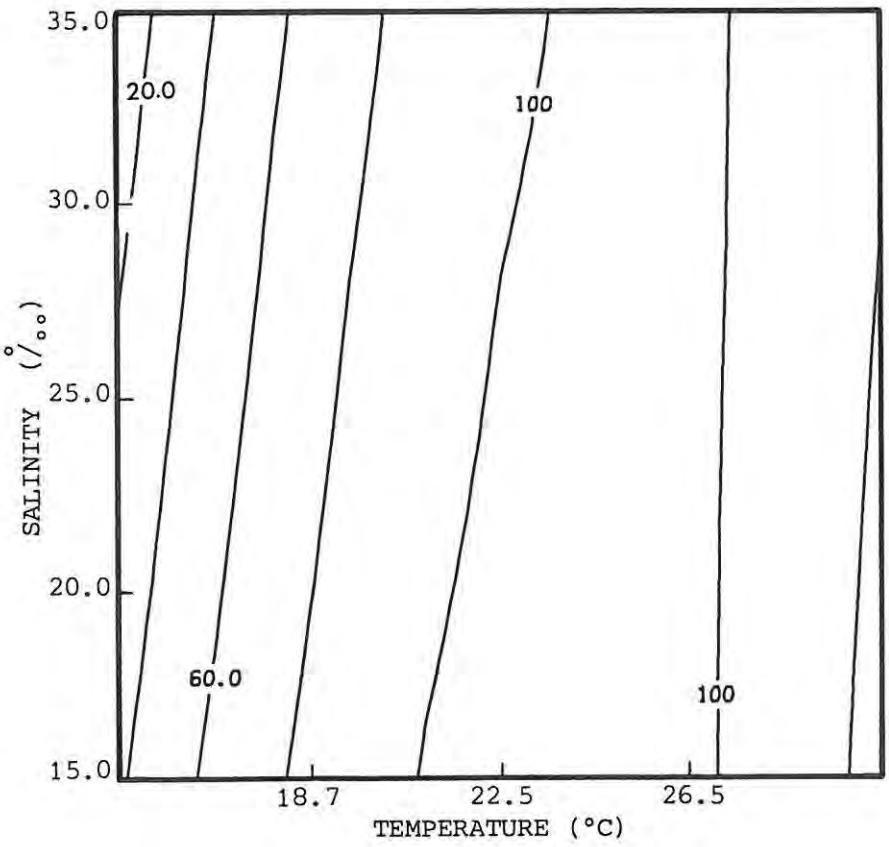


Fig. 47 Surface response estimation of the percentage survival of M. petersi larvae after ten days in relation to temperature and salinity. The survival contours were drawn at 20% intervals.

salinity indicated that mortality, at high salinity, was coupled with low temperature. The position of the 20% survival contour illustrated this point. The 100% survival contour cuts the temperature axis at 20°C and 27°C for salinities between 15 and 35$^{o}/_{oo}$. A shift to a higher temperature range for maximum survival at higher salinities was illustrated by comparison with the previous experiment where a range of 18 to 24°C was optimal for salinities between 0.5 and 6$^{o}/_{oo}$.

Table 34 shows that 81.3% of the variability was explained by fitting a quadratic polynomial to the survival data. The null hypothesis was rejected ($F_{4,15} = 16.34$; $P<0.001$) and the linear and quadratic temperature terms accounted for 76.6% of the total variability. In this

Table 34. ANOVA of survival of M. petersi larvae after ten days.

Source of variation	R^2 (%)	R^2 change (%)	Regression coefficients	$F_{(1,18)}$ ratio	Significance
T	39.8	39.8	41.07	37.69	P<0.001
T^2	76.6	36.8	- 0.90	34.79	P<0.001
T x S	79.2	2.6	0.13	2.02	Not significant
S	81.3	2.1	- 3.41	2.02	Not significant
Constant			-352.59		

example the quadratic salinity regression coefficient did not meet the predetermined statistical constraints ($F<0.10$) and was not included in the quadratic equation. Compared with the results in the low salinity range where salinity determined larval survival, it appeared that at high salinities temperature was of overriding importance. The significance of this finding becomes clear, not in the context of distribution in the Keiskamma estuary, but rather with regard to the geographical distribution of the species along the East African coastline. The relevance of this finding will be discussed in more detail in part five. Furthermore, the prediction of the previous experiment, that a wide range of temperature and salinity favours survival of M. petersi larvae, has been confirmed. Although survival in the 7 to 14$^{o}/_{oo}$ range had not been investigated, since 100% survival was obtained at 6 and 15$^{o}/_{oo}$ respectively, I assumed the larvae to be equally responsive between 7 and 14$^{o}/_{oo}$ within the same

temperature range.

Macrobrachium larvae are generally known to require saline water for survival and development but few studies have determined larval response to temperature and salinity precisely. Guest & Durocher (1979) found that increased salinity to $10^{o}/_{oo}$ reduced the lower thermal tolerance of M. amazonicum larvae. Optimal salinity range for development to post larvae was from 7.5 to $12.5^{o}/_{oo}$ although no response at salinities higher than $15^{o}/_{oo}$ was investigated. However, metamorphosis to post larvae took place at $1.0^{o}/_{oo}$ which indicated that this species was extremely tolerant of low salinities. In M. carcinus (Choudhury, 1971c) and M. holthuisi (Moreira et al., 1979) on the other hand, larvae do not survive in salinities above or below 14 - $17^{o}/_{oo}$ in the temperature range of 20 - 25°C which indicated a stenohaline larval requirement. The optimal salinity range for growth and development of M. rosenbergii larvae was 12 to $14^{o}/_{oo}$ (Ling & Merican, 1961). Despite there being over 100 species of Macrobrachium (Holthuis, 1950) the paucity of clearly defined quantitative laboratory studies was surprising, especially in view of the fact that certain of the species have commercial potential (Hanson & Goodwin, 1977).

In the genus Palaemonetes larval survival and development occurs optimally at high salinities (15 - $30^{o}/_{oo}$) and moderate temperatures (20 - 25°C) but at salinities below $10^{o}/_{oo}$ survival was poor (Broad & Hubschman, 1962; Knowlton, 1965; Sandifer, 1973; Rochanaburanon & Williamson, 1976). These requirements differ from Macrobrachium species whose larvae are more tolerant of lower salinities. Larval tolerance of low salinities probably depends to a large extent on whether adults of the species inhabit freshwater. Of the Palaemonetes species studied all appear to occur in brackish and not freshwater environments.

Certain brachyuran larval responses to a range of salinity and temperature combinations have been quantitatively determined (Costlow et al., 1960, 1962, 1966; Costlow, 1967; Ong & Costlow, 1970). In Sesarma cinereum, Costlow et al., (1960) demonstrated that each larval stage had a particular temperature and salinity preferenda, stage 4 zoea being the least, and megalopa the most tolerant, whilst all larval stages of Rhithropanopeus harrisii (Costlow et al., 1966) were tolerant of a wide salinity and temperature range. In the brachyuran larva, combinations of low temperature and salinity did not favour survival whereas high

temperature in combination with low salinity did. For the larval population of M. petersi in the Keiskamma river the converse was found, namely that low temperature in combination with low salinity favoured survival (see Fig. 45). Considering that M. petersi was a subtropical species from the east coast of Africa (Barnard, 1950) this was surprising. However, viewed in the context of the temperature difference between the warm temperate (Keiskamma river) region and subtropical (Zambezi river) region, tolerance to low temperatures was adaptive for a population living at the most southerly limit of its distribution. The possible mechanism that brings this adaptation about is discussed in the following section.

(iii) Response of the haemolymph osmotic concentration ($\Delta i°C$) of stage VII larvae to a decrease in temperature.

The fact that hatching of M. petersi eggs does take place in low saline water, and larvae survive low temperatures, is adaptive as this effectively broadens the prawns breeding season which ensures a maximum annual recruitment to the adult population. The manner in which temperature effects the relationship between internal and external osmotic concentrations in crustacea, and the range of ecological adaptation, is not yet fully understood (Dorgelo, 1981). Depending on the species, its pattern of osmoregulation and a variety of factors like season, latitude, etc., a decrease in temperature can either raise or lower the haemolymph concentration. In the case of M. petersi larvae, it might be expected, since improved survival occurs in low saline water, that low temperature may depress haemolymph concentration. This would reduce the osmotic gradient and therefore increase the larva's tolerance to low salinity.

I assumed that a similar change in haemolymph osmotic concentration, induced by low temperature, would occur in all larval stages, so stage VII were arbitrarily chosen. As a graded response to an increase in salinity was desired, the difference in concentration between the test salinities was small. Five salinities, 3.0, 4.0, 4.8, 6.0 and 8.1$^{o}/_{oo}$ and three temperatures, 22.1, 24.9 and 28.2°C, which the larvae were likely to experience naturally, were chosen. The larvae were reared as described in section 4 for approximately 15 days by which time the majority had reached stage VII. These were removed from the rearing tank, 20 selected and transferred to each salinity. Salinity change was

therefore instantaneous. The water bath temperature was switched on once all the larvae had been allocated. Larvae were not fed and after 24 hrs the freezing point depression of the blood between four and six stage VII larvae was determined (Ramsay & Brown, 1955). A detailed explanation of the experimental procedure,acclimation period and technique adopted for the determination of haemolymph freezing point depression of M. petersi larvae is given in part four.

The linear relationship between salinity and an increase in haemolymph Δi for stage VII larvae is listed in Table 35. Since the slope of the regression lines at the three temperatures was not significantly different the effect of temperature on Δi can be gauged from the y intercept of each regression equation. $\Delta i°C$ decreased from 0.484 at 28.2°C to 0.371 at 22.1°C, a difference of 23%. Lockwood (1968) has

Table 35. Linear relationship between salinity and $\Delta i°C$ at three temperatures where y is the estimated Δi and x the salinity between 3 and 8‰.

Temperature (°C)	Regression	R^2
28.2	$y = 0.484 + 0.031x$	0.8001
24.9	$y = 0.430 + 0.039x$	0.8817
22.1	$y = 0.371 + 0.047x$	0.9254

pointed out that the change is usually small as the drop in haemolymph concentration activates the sodium uptake mechanism which brings the haemolymph to a new concentration a little below the original level. Thus the reduction in $\Delta i°C$ effectively reduced the concentration gradient between internal and external milieu and so favoured survival in dilute media. Presumably, the increase in larval mortality at high temperature in low salinity was due to the inability of the osmoregulatory mechanism to maintain the required osmotic gradient between haemolymph and medium. The survival pattern obtained in Fig. 45 is readily explained in the light of these findings.

(iv) Larval development in different salinity and temperature combinations.

The results reported in this section were obtained from the

experiments described in (i) and (ii) of section 4.5. They show the composition of larval stages in each temperature and salinity combination after seven (i) and ten (ii) days respectively.

Figs. 48 and 49 (page 110) show the array of larval stages, expressed as a percentage of surviving larvae, in low and high salinity

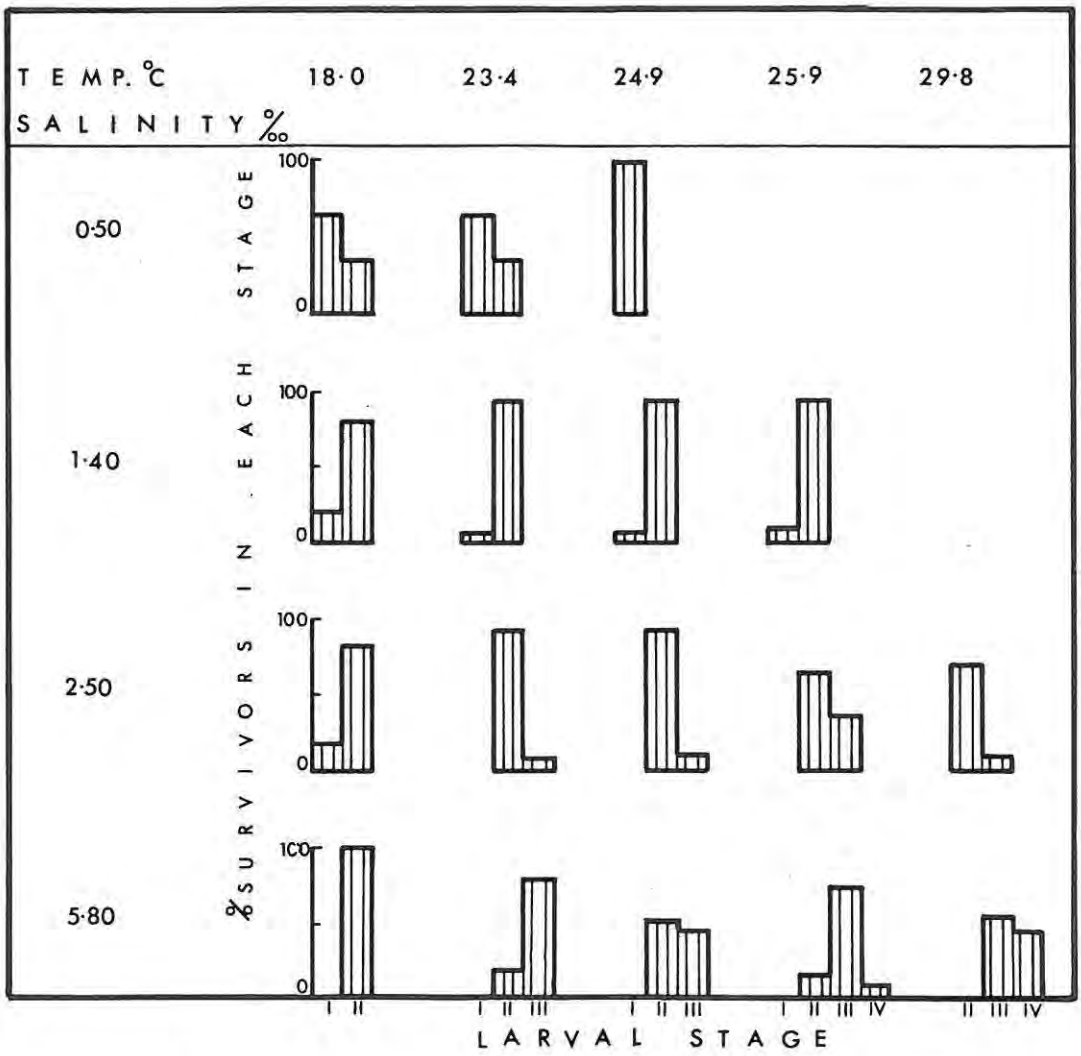


Fig. 48 The effect of low salinity and temperature on larval development after seven days.

and temperature combinations. As the experiments were not of similar duration it was not possible to compare directly the effects of low and high salinity on development. Fig. 48 shows that at the highest salinity ($5.8^{\circ}/_{oo}$) temperature had a direct influence on development; at 18°C all larvae were in stage II but at 25.9°C 15% were in stage II, 78% in stage III and 7% in stage IV. These results were comparable since

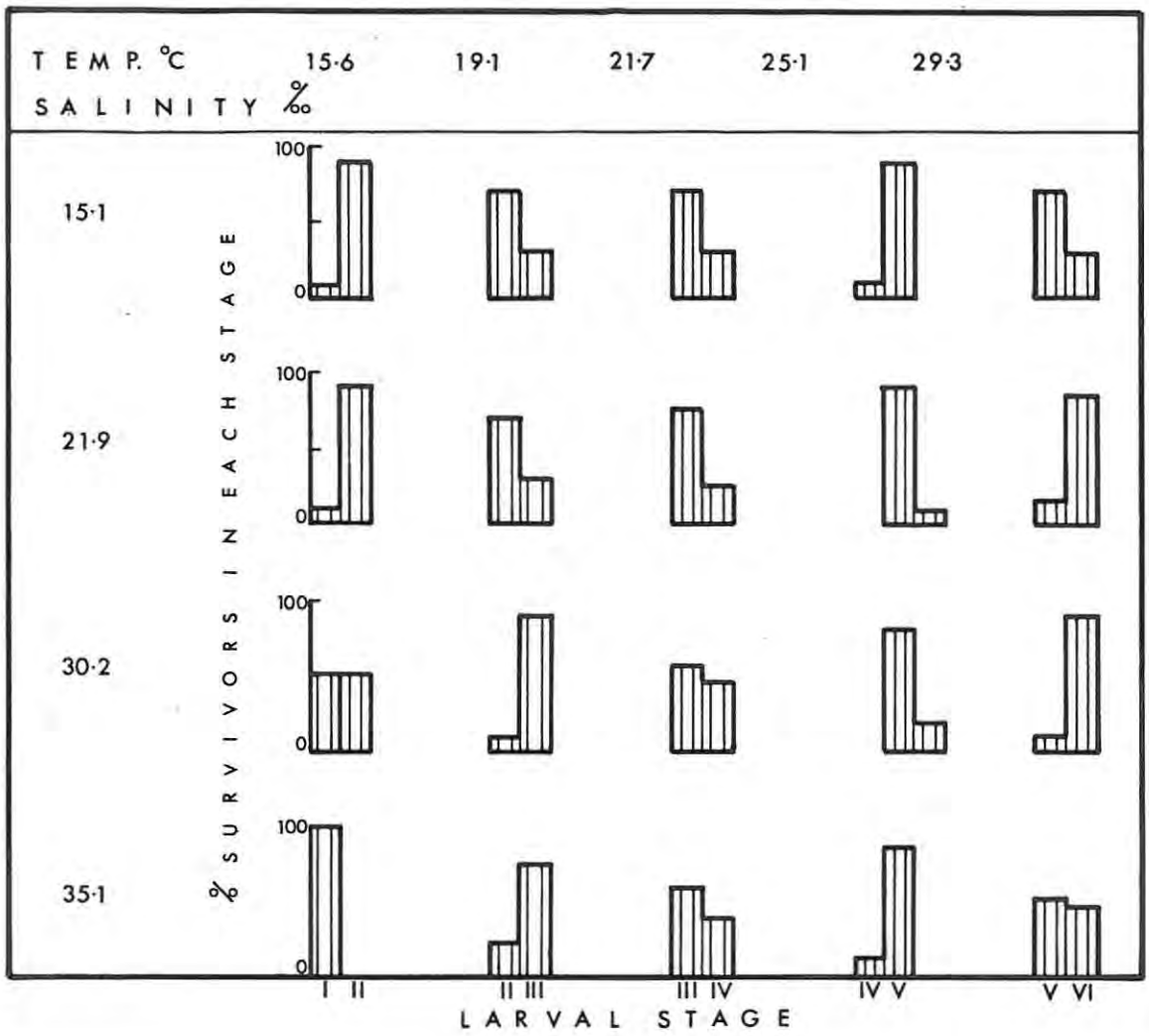


Fig. 49 The effect of high salinity and temperature on larval development after ten days.

survival at 18 and 25.9°C was 98% and 92% respectively. Salinity effects were obscured due to differences in survival and it did not appear to have as marked an effect on development as temperature. For instance, at 18.0°C in 1.4°/₀₀ 20% of the larvae were in stage I and 80% in stage II, but at 5.8°/₀₀, 100% were in stage II. In the lower salinity range therefore temperature, and to a limited extent salinity, affect development.

Fig. 49 shows that temperature was of singular importance for development at high salinities. At 21.7°C in 21.9°/₀₀ 75% of larvae were in stage III and 25% in stage IV; at 29.3°C, 15% were in stage V and 85% in stage VI. Development was independent of salinity since the proportion of stage V larvae in all four salinities at 25.1°C was

approximately the same. A noticable feature was the harmony of change in stage composition that temperature induced. The development pattern contained no anomalies and if it were possible to quantify these data a pattern of response similar to that obtained in Fig. 47 would be likely to emerge. Studies of the effect of temperature and salinity on brachyuran larval development (Costlow et al., 1962, 1966; Ong & Costlow, 1970; Christiansen & Costlow, 1975) and in Palaemonetes vulgaris (Sandifer, 1973) all stressed the importance of temperature, above that of salinity, for development.

It seemed from the development data that the range of salinity in which development took place was as broad as that for survival but the temperature range was considerably narrower. Fig. 49 showed that at all salinities except 35.1‰ the proportion of the most advanced larvae (stage VI) was greater at 29.3°C than at 25.1°C suggesting that this temperature range was optimal for larval development. These series of experiments indicated that conditions required for metamorphosis and growth of post larvae were likely to be equally as broad as those for the larvae. The validity of this assumption is tested in the following section.

4.6 Salinity and temperature requirements for metamorphosis to post larvae.

(i) Minimum requirements.

At times during the 1981 season excessive flooding reduced salinity between stations 1 and 12 to less than 0.1‰ for up to 15 days. Under such conditions excessive larval mortality would be expected. In the light of such events an experiment was designed to determine the minimum salinity and temperature required for 50% of the larval population to metamorphose to post larvae. As it was not possible to obtain sufficient stage IX from one spawn, a total of fifty stage VIII and IX larvae were used in each test combination assuming that the larval proportions, assigned to each test combination, were similar. The larvae were transferred directly from the rearing salinity (15.0, range $\pm$ 1.5‰) to the test salinities. I established that the minimum salinity at which transfer could take place without causing 100% mortality after 24 hrs was 2.9‰. Four salinities, 2.9, 3.5, 4.0 and 5.3‰ and three

temperatures, 22.2, 24.8 and 28.2°C were therefore chosen. Routine monitoring and feeding took place as described in section 2. Each salinity was checked daily for dead larvae and the number of living post larvae removed and counted. Post larvae were considered dead when the scaphognathite did not move. The experiment was terminated after seven days.

Table 36 shows the percentage post larvae obtained in each test combination and Fig. 50 (page 113) the fitted response surface with 10% survival contours. Since the contours were almost parallel to the

Table 36. The percentage post larvae after metamorphosis from stage IX in four salinities and three temperatures. *Mean ±SD.

Temperature (°C) / Salinity ($^{o}/_{oo}$)	22.2±0.3*	24.8±0.2	28.2±0.3
2.9±0.1*	14.0	22.0	12.0
3.5±0.1	24.0	22.0	34.0
4.0±0.2	30.0	34.0	58.0
5.3±0.2	60.0	66.0	60.0

temperature axis salinity exerted the greatest influence on metamorphosis. The 50% survival contour cuts the salinity axis at $4.8^{o}/_{oo}$ at 22.0°C and $4.2^{o}/_{oo}$ at 30°C which indicates, within this salinity range, that metamorphosis to post larvae was independent of temperature. The 50% contour indicated that the minimum salinity for metamorphosis to post larvae lay between 4 and $5^{o}/_{oo}$.

Table 37 (page 113) shows that the fitted quadratic polynomial accounts for 87.9% of the total variability. The null hypothesis was rejected ($F_{3,8} = 19.42$; $P<0.001$) and of the three terms in the equation the linear effect of salinity explained 83.9% of the total variability which confirmed the surface response pattern obtained in Fig. 50 (page 113). The interaction and quadratic temperature terms were excluded since their contribution to the total variability was less than the predetermined statistical requirement for inclusion in the equation. It is noteworthy that the minimum salinity required for metamorphosis was lower than the minimum salinity ($8^{o}/_{oo}$) estimated for completion of larval

development.

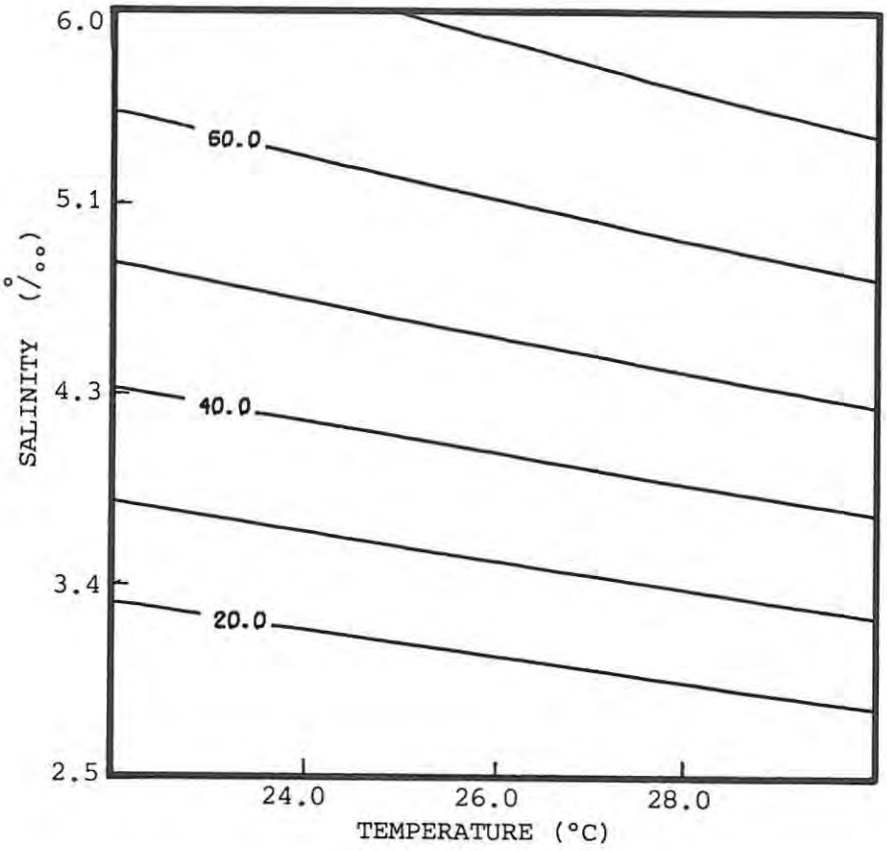


Fig. 50 Response surface estimation of the minimum salinity and temperature requirements for 50% metamorphosis to post larvae in 12 temperature and salinity combinations.

Table 37. ANOVA of the percentage post larvae after metamorphosis from stage IX larvae.

Source of variation	R^2 (%)	R^2 change (%)	Regression coefficients	$F_{(1,10)}$ ratio	Significance
S	83.9	83.9	35.67	69.38	P<0.001
T	87.5	3.6	1.41	3.00	Not significant
S^2	87.9	0.4	- 2.02	0.35	Not significant
Constant			-106.64		

(ii) Optimum requirements.

The low salinity at which metamorphosis to post larvae occurred suggested that metamorphosis could take place over a wide range of salinities. Due to marked variation in the time to metamorphosis shown by M. petersi and other Palaemonidae (Sandifer & Smith, 1979), a time-response method was adopted to define optimal salinity and temperature requirements for metamorphosis to post larvae. The experimental design was a 3 x 6 factorial at three temperatures, 22.2, 24.7 and 28.5°C and six salinities, 8.1, 11.9, 16.0, 24.2, 30.1 and 35.0 °/₀₀. Fifty stage VIII and IX larvae were used as described in section 4.6(i). Routine monitoring and feeding took place as described in section 2. Each test salinity was checked daily and the time of observation and number of post larvae noted. The experiment was terminated after eight days and the number of larvae that had not metamorphosed counted. A median time of metamorphosis to post larvae was estimated for each test combination as described in section 3.2. The temperature and salinity range bounded by the contour of lowest median response time was regarded as optimal for metamorphosis.

Table 38 shows the median time for metamorphosis under three temperature and six salinity combinations and Fig. 51 (page 115) the fitted surface response with contours of equal response time drawn at 20 hr intervals. Although 62.9 hrs was the lowest response time, the 60 hr

Table 38. The median response time for metamorphosis to post larvae in six salinities and three temperatures. *Mean ±SD.

Temperature (°C) / Salinity (°/₀₀)	22.2±1.0*	24.7±0.2	28.5±0.1
8.1±0.3*	90.1	116.8	87.6
11.9±0.1	101.9	105.8	94.6
16.0±0.2	132.4	125.5	62.9
24.2±0.3	139.4	98.0	69.7
30.1±0.4	150.3	121.3	68.5
35.0±0.3	182.3	143.1	91.1

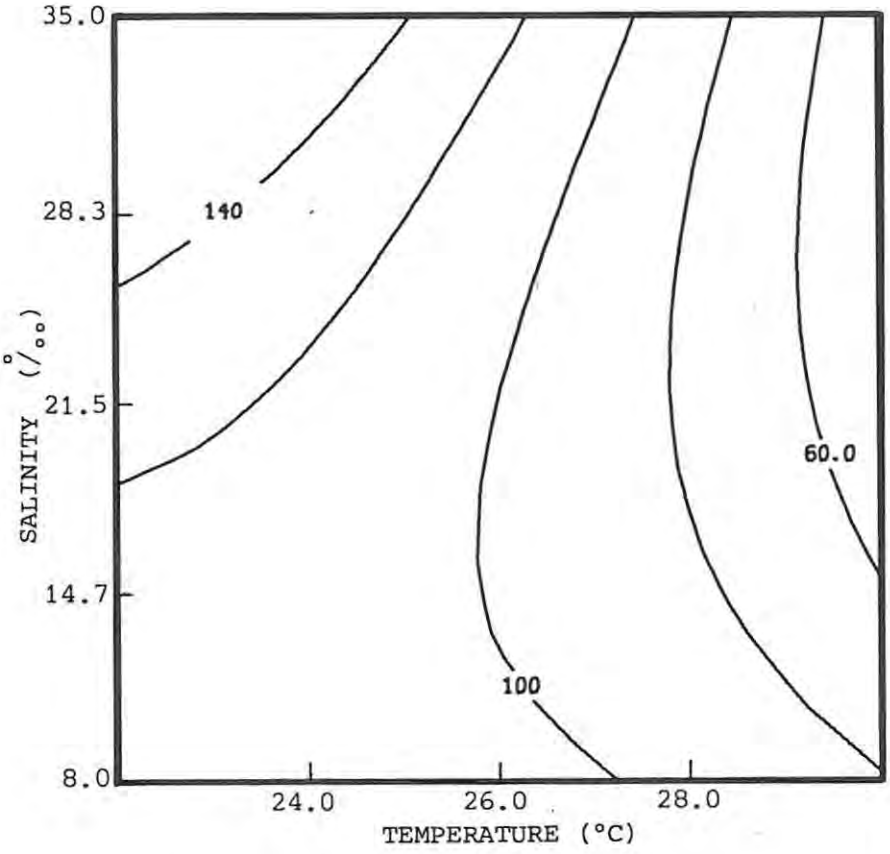


Fig. 51 Response surface estimation of the median response time (hrs) of metamorphosis to post larvae at three temperatures and six salinities.

contour could only be included in the response surface if the temperature axis was expanded to 30°C. Thus, the 100 hr contour which cuts the temperature axis at 27°C was a more realistic temperature from which the requirements for metamorphosis could be estimated. It was not possible to estimate optimal conditions since the contours were not concentric circles. Thus, metamorphosis to post larvae effectively occurred between 8 and 35°/₀₀ at 27°C. Low temperature (22 - 24°C) and high salinity (25 - 35°/₀₀) increased the median response time which indicated an interaction between high salinity and low temperature. The significance of this interaction becomes evident in accounting for the geographical distribution of M. petersi (see part five). Equally, Costlow (1967) found that metamorphosis of megalopa larvae of the blue crab Callinectes sapidus was delayed at low temperature and high salinity. Costlow (1967) suggested that the duration of the megalopa stage in the field was directly associated with the time of hatching and the time taken to reach megalops

in relation to seasonal changes in water temperature and salinity. Table 39 shows that a quadratic polynomial fitted to the data explained 88.1% of the total variability. The null hypothesis was rejected ($F_{5,12}$ = 17.42; P<0.001) and of the five terms in the equation the quadratic temperature term accounted for 53.8% of the variability. The contribution of the interaction term was also significant.

Table 39. ANOVA of the median response time of metamorphosis to post larvae.

Source of variation	R^2 (%)	R^2 change (%)	Regression coefficients	$F_{(1,16)}$ ratio	Significance
T^2	53.8	53.8	- 0.73	71.5	P<0.001
S^2	69.6	15.8	0.08	21.18	P<0.001
T x S	79.7	10.1	- 0.49	13.62	0.001<P<0.01
S	86.9	7.2	10.02	9.57	0.001<P<0.01
T	88.1	1.2	39.06	1.61	Not significant
Constant			-396.36		

From these data and those of the preceeding experiment metamorphosis of M. petersi stage IX larvae took place over a broad salinity and temperature range which confirmed the hypothesis set out at the beginning of this section. Few data, which precisely define the temperature and salinity criteria for metamorphosis, are available for other Macrobrachium species despite the significance of this event in the prawn's life history. Uno (1971) subjected newly hatched M. nipponense larvae to a range of salinities between 3 and 22‰ and temperatures between 20 and 36°C and determined the percentage metamorphosis to post larvae. Low (3‰) and high (22‰) salinities in combination with low temperature did not favour metamorphosis; the optimal salinity and temperature range lay between 8 and 13‰ and 25 to 30°C respectively. Guest & Durocher (1979) reported that the larvae of M. amazonicum all metamorphosed to post larvae between 1 and 15‰ but did not do so in freshwater (presumably <1.0‰). Sandifer et al., (1975) demonstrated that post larvae of M. rosenbergii were unable to withstand direct transfer from 16‰ to 27 or 35‰. Equally, under conditions of a

gradual salinity increase to 35‰ few post larvae were able to tolerate these conditions for longer than 20 days. Although Sandifer et al., (1975) did not investigate the actual event, i.e. metamorphosis to post larvae, it was not unreasonable to assume that the post larval response of M. rosenbergii to salinity would indirectly apply to metamorphosis as well. This indicated that metamorphosis in M. rosenbergii was unlikely to take place over as wide a salinity range compared with M. petersi.

Temperature and salinity conditions for metamorphosis to post larvae in M. petersi appeared broader than those of other Macrobrachium species investigated. M. petersi post larvae undergo a migration to freshwater. The carapace length of newly metamorphosed post larvae was 1.15, SD $\pm$ 0.06 mm. By the time post larvae reached freshwater the carapace length had increased to 6.0, SD $\pm$ 0.94 mm. Presumably growth took place under highly variable saline conditions so that it was likely to be independent of salinity. To test this, an experiment was designed to determine the effect of temperature and a range of salinities on post larval growth.

4.7 Effect of salinity and temperature on growth of M. petersi post larvae.

The experimental design was a 3 x 6 factorial at three temperatures, 21.2, 25.0 and 28.9°C and six salinities, 2.0, 5.1, 10.2, 15.0, 20.1 and 29.9‰. Ten, five day old post larvae, reared in the closed circulatory system described in section 4.1, were randomised into each test salinity. The experiment was staggered due to the difficulty of synchronising the production of sufficient five day old post larvae. Thus the experiment at 21.2°C was set up on 15 February, that at 25.0°C five days later and that at 28.9°C on 26 February 1981. Experimental duration at each temperature was 20 days. The post larvae were not weighed initially as this would be stressful and likely to affect growth performance. Rather, an estimate of the initial mass at each temperature was obtained by weighing a subsample of 15 individuals; the initial mass at 21.2, 25.0 and 28.9°C was 2.4 mg SD $\pm$ 0.4, 2.1 mg SD $\pm$ 0.4 and 2.9 mg SD $\pm$ 0.2 respectively. Routine monitoring and feeding took place as described in section 2. At the completion of each experiment the initial mean mass was subtracted from the final mass of each individual to give an estimate of the mass increase. The post larvae were carefully dried and weighed to the nearest 0.001 mg on a Mettler balance.

Fig. 52 shows the fitted response surface to the growth data. The fact that the growth contours were parallel to the salinity axis indicated that temperature and not salinity affected growth to a greater extent. However, at temperatures greater than 26.0°C a change in the growth pattern, shown by the shape of the 15 mg contour, suggested that at low salinity and high temperature the growth rate decreased.

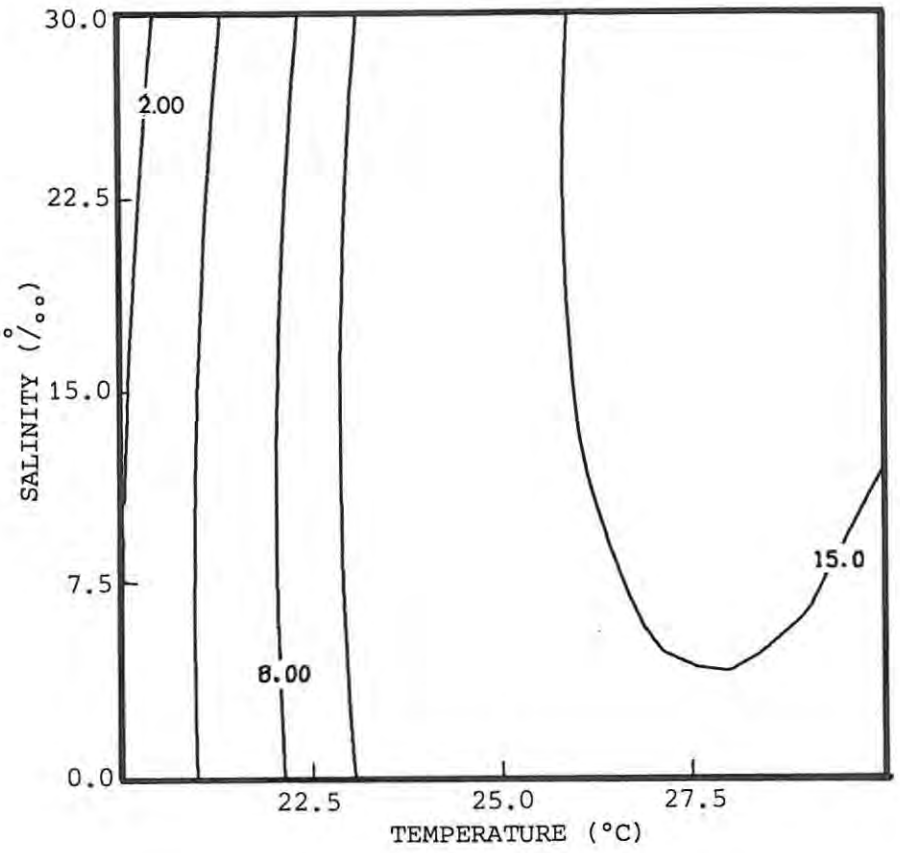


Fig. 52 A surface response estimation of the growth (mg increase) of M. petersi post larvae after 20 days in 18 temperature and salinity combinations.

Table 40 (page 119) shows the statistical analysis of post larval growth. A total of 162 individuals of a possible 180 were used in the analysis; thus the experimental mortality was 9.0%. The null hypothesis was rejected ($F_{5,156} = 49.24$; $P<0.001$) and of the five terms in the equation the linear effect of temperature explained 55.4% of a total of 64.1% of the variability which confirmed the response pattern obtained in Fig. 52. A possible reason for the low R^2 value was discussed

in section 3.3 (page 86).

Table 40. ANOVA of the growth of M. petersi post larvae after 20 days.

Source of variation	R^2 (%)	R^2 change (%)	Regression coefficients	$F_{(1,160)}$ ratio	Significance
T	55.4	55.4	11.96	245.60	P<0.001
T^2	63.3	7.9	- 0.21	35.27	P<0.001
S	63.8	0.5	- 0.29	2.32	Not significant
T x S	64.0	0.2	0.01	1.29	Not significant
S^2	64.1	0.1	- 0.002	0.44	Not significant
Constant			-150.65		

The results of this experiment confirmed that growth of M. petersi post larvae was independent of salinity between 2 and 30‰. Whether growth retardation in low salinity and high temperature was significant, is difficult to assess. Soon after metamorphosis post larvae are likely to be found in a more saline region of the estuary and therefore, at this early stage, it might be expected that growth at low salinity and high temperature is retarded. The cause could simply be osmotic. At higher temperatures and low salinity, energy that would normally be used for growth was now required to compensate for the osmotic gradient between internal and external medium. Perdue & Nakamura (1976) determined the growth rate of 40 day old M. rosenbergii post larvae in four salinities, between freshwater and 15‰. They found no significant difference in growth in freshwater or 2‰ though growth was retarded at 15‰. The difference between these results and those of M. petersi could reflect the age of the post larvae. Zein-Eldin & Aldrich (1965) and Zein-Eldin & Griffith (1969) demonstrated for both post larval Penaeus aztecus and P. setiferus, in a variety of temperature and salinity combinations, that temperature was of overriding importance for post larval growth. At low temperature (<20°C), high salinity retarded growth but at optimal temperatures (25 - 30°C) equivalent growth at salinities between 2 and 40‰ was obtained for P. aztecus. In P. setiferus on the other hand, high salinity at optimal temperatures retarded growth. In

nature the population was not exposed to these conditions as P. setiferus had usually entered estuaries where salinities were considerably lower.

5. Discussion

The precise temperature and salinity values defined for survival, metamorphosis and growth of larval and post larval M. petersi has demonstrated the versatility of the surface response approach in laboratory investigations of this nature. Coupled with residual analyses, a knowledge of the limitations of the technique and the realisation that in certain instances subjective reasoning was warranted, made it possible to interpret the results in an ecologically meaningful way.

On page 73 I asked whether M. petersi larvae could utilise the whole estuary or were likely to be restricted to a specific region. To answer this question a laboratory investigation of salt tolerance during larval development and subsequent metamorphosis to post larvae was required since the majority of larval stages were not found in the field. I can now state that M. petersi larvae are euryhaline, able to tolerate a relatively wide temperature range and therefore well adapted to withstand the rigours of an estuarine environment. In the low salinity range ($0.5 - 6.0^{\circ}/_{\circ\circ}$) salinity per se was of overriding importance for survival whereas in the higher range ($15 - 35^{\circ}/_{\circ\circ}$) temperature played the dominant role. Coupled with the fact that metamorphosis to post larvae took place over a wide range of salinities ($5 - 35^{\circ}/_{\circ\circ}$) indicated that larval development could take place in any region of the estuary. Only during a summer drought, when the river flow was minimal, is larval development confined to a particular region of the estuary. During such periods, as a result of the adult response to an increase in salinity, the majority of stage I larvae hatch in freshwater. In this situation the significance of larval dependence on saline water becomes relevant as many of the larvae may not reach the estuary, which may result in an abnormally high mortality.

Despite the euryhalinity of the larvae, the impression gained from the field investigation was a tendency for larval development to be confined to the middle and upper regions of the estuary. In this respect, the reaction of adult prawns to an increase in salinity deserves comment. The laboratory investigation demonstrated that M. petersi larvae require

saline water in excess of 8$^{o}/_{oo}$ for development which is precisely within the salinity range that induced reduction in adult abundance downstream from the weir. The significance of this behaviour, especially in the light of the larvae's requirement for saline water, is difficult to understand. Not so, however, if the behaviour is regarded as a strategy to confine larval development and subsequent metamorphosis to the upper regions of the estuary. It is noteworthy that the salinity range to which the adults respond also meets the requirements for larval development. Thus, the simultaneous release of larvae from adult females, coupled with the upstream movement in response to elevated salinities effectively confines larval development to the upper reaches of the estuary.

In addition, the increased concentration of stage I larvae in the vicinity of a salt front also confines larval development to lower saline water found in the middle and upper reaches of the estuary. The paucity of stage II larvae downstream from a salt front and the absence of other stages indicates a loss of the planktonic larval phase. This has been interpreted as a strategy for the maintenance of station which not only ensures retention within the estuary, but simultaneously restricts larval development to relatively low saline water found in the middle and upper reaches.

Carriker (1967) stated that larval behaviour patterns which promoted retention within the estuary are probably a step in the evolution of true estuarine species with planktonic larvae. Little is known concerning the mechanisms by which marine decapod crustacea colonise freshwater. Sandifer (1975) speculated that one of the mechanisms may be via recruitment, through retention of larvae within the estuary. I am suggesting, therefore, that the behaviour of adult and stage I M. petersi is an adaptation which favours recruitment through retention of larval development in the middle and upper reaches of the estuary. In a species like M. petersi which inhabits freshwater, selection would favour larval development as close to the adult population as possible. Sandifer (1975) has shown that the larvae of adult species of decapod crustacea that live in the York river estuary, have developed a behaviour pattern which tended to retain them within the estuary. Sandifer (1975) has interpreted this behaviour as favouring recruitment to the adult populations within the estuary.

It would be expected that if the behaviour of both adults and stage I larvae were orientated towards promoting larval development in low saline water, key responses such as ecdysis to stage II and metamorphosis to post larvae could occur in such an environment. Indeed, results of the laboratory investigation have shown that these events do take place in dilute media. For instance, the salinity range for ecdysis to stage II was estimated to be between 1.0 and 1.75$^{o}/_{oo}$. Similarly the minimum salinity required for metamorphosis to post larvae was between 4 and 5$^{o}/_{oo}$. Growth of post larvae was independent of salinity although at lower salinities and high temperatures growth rate decreased to some extent. In the light of this requirement for saline water the upstream intrusion of a salt wedge after a flood must be of considerable ecological significance for the estuarine dependent biota that inhabit the middle and upper reaches. Depending on the magnitude of the freshwater inflow, the middle and upper reaches of the Keiskamma estuary have been known to remain fresh (<0.1$^{o}/_{oo}$) for up to 15 days. Under such conditions the salt wedge is the only means by which fauna in the higher reaches of the estuary can have access to saline water.

In view of the strategy of certain estuarine species to abbreviate larval development (Dobkin, 1969) or reduce the length of time the larvae spend in the plankton (Lockwood, 1976) or a combination of these (Forbes, 1973), the larval developmental period of a minimum of 21 days for M. petersi seems comparatively long for a species living in an estuarine environment where the potential for downward displacement is high. In M. petersi this problem has been largely overcome by confining development to the upper regions of the estuary and the loss of the larval planktonic mode of existence after stage I.

Thorne et al., (1979) carried out an extensive laboratory study on the larval behaviour of Macrobrachium novaehollandiae in response to light, salinity, pressure and temperature. M. novaehollandiae has 12 larval stages but photopositive behaviour was only demonstrated in the first two stages and even then few larvae swam near the surface for long periods of time. The other abiotic factors investigated had no marked influence on orientation of the larvae which led them to conclude that the mechanism that prevented loss from the estuary was the larval behaviour which involved resting on the substratum for long periods of time. Another mechanism to reduce displacement of larvae was the timing of swimming

periods to correspond with tidal currents. Hughes & Richard (1973) found that stage V - VII larvae of M. acanthurus occupied lower levels in the water column when the salinity dropped. This, it was argued, simulated ebb tide and thus prevented larvae from being carried out to sea. Neither of these findings with regard to M. novaehollandiae or M. acanthurus were supported by field evidence. Therefore, in the view of the difference between laboratory larval behaviour of M. petersi compared with that found in the field, an ecological interpretation of the laboratory behaviour patterns should be viewed with caution.

Day (1951) divided estuarine animals into six different components based primarily on their salinity tolerance. It would appear that M. petersi would either fall into the euryhaline freshwater component or the migratory component. Members of the euryhaline freshwater group are "derived from rivers", suggesting that these species are ancient inhabitants of freshwater as the Atyidae for example (Fryer, 1976). Day (1981d) makes no mention of the larval requirements of this group but implies that development occurs in freshwater. The migratory component includes those species that actively migrate out of the estuary during part of their life cycle. In this group larval development takes place in saline water but "the larval stages of many species are less tolerant of low and variable salinities than the adults". I have interpreted this statement to be directed to the stenohaline larvae of those species, like penaeid prawns and portunid crabs (Hill, 1974) which return to the sea to breed. In other words Day (1981d) has not considered species, like M. petersi, which return to the estuary to breed and consequently have euryhaline larvae. In view of the life cycle of M. petersi it would be more suitably placed in the migratory than euryhaline freshwater component of the estuarine fauna but for this to occur Day's definition needs to be modified to include those species with euryhaline larvae.

In summary, not only are M. petersi larvae physiologically adapted to an estuarine existence but behaviourally as well, through maximum recruitment to the adult population. This benefits the species and presumably enables a viable population of M. petersi to be maintained in the Keiskamma river. The method of physiological adaptation to a wide salinity spectrum is discussed in part four.

PART FOUR

INTRASPECIFIC VARIATION IN THE OSMOREGULATORY PATTERN OF *M. PETERSI* : AN ECOLOGICAL INTERPRETATION.

1. Introduction

Caridean prawns inhabit freshwater, estuarine and marine environments and consequently display a variety of osmoregulatory patterns. Marine carideans such as *Pandalus montagui* (Panikkar, 1968) osmoconform while *Crangon septemspinosa* which migrates between the estuarine and offshore environments (Modlin, 1980) is capable of hyposmotic regulation at high salinities and hyperosmotic regulation at low salinities (Haefner, 1969). Those which are restricted to freshwater, for example, *Palaemonetes paludosus* (Dobkin & Manning, 1964) are hyperosmotic at low salinity and either isosmotic or slightly hyperosmotic at higher salinity. Generally, there is a close correlation between osmoregulatory capacity and distribution in relation to salinity in the natural environment.

Holthius (1950) described approximately 100 species in the genus *Macrobrachium* from the Indo-Pacific region of which osmoregulatory patterns in *M. australiense* and *M. equidens* (Denne, 1968), *M. rosenbergii* (Sandifer *et al.*, 1975) and *M. ohione* (Castille & Lawrence, 1981) have been determined. The osmoregulatory pattern of *M. equidens* is strikingly different from the rest in that it is an efficient regulator between 7 and 30$^{o}/_{oo}$ but at salinities <7$^{o}/_{oo}$ the haemolymph osmotic concentration decreases in response to that of the medium. Denne (1968) has suggested that this breakdown of osmoregulatory ability at low salinity accounts for the limited penetration of *M. equidens* into the upper reaches of the estuary compared with *M. australiense* which inhabits freshwater. No osmoregulatory studies have been carried out on the larvae of any of these species although Sandifer *et al.*, (1975) have determined the osmoregulatory pattern in post larval *M. rosenbergii*.

I have shown that both the river and estuary are used during the life cycle of *M. petersi*. Therefore, it is reasonable to assume that the osmoregulatory patterns of stages that inhabit these two environments may differ. *M. rosenbergii* and *M. ohione*, which live in freshwater but undergo a breeding migration to the estuary, are efficient hyperosmotic regulators

(Castille & Lawrence, 1981). Therefore a similar pattern is likely to be evident in juvenile and adult M. petersi. To comment on the osmoregulatory pattern in larval and post larval M. petersi would be speculative but the fact that tolerance to low salinity varies between stages suggests that the osmoregulatory capacity may change as well. Therefore, the wide spectrum of salinity tolerance demonstrated by the different stages in the life cycle of M. petersi is likely to be manifested in equally diverse patterns of osmoregulation. The following section describes quantitatively, the osmoregulatory patterns in larvae, post larvae, juvenile and adult M. petersi.

2. Material and methods

2.1 Freezing point depression determination.

Freezing point depression of the haemolymph in adult, juvenile, post larval and larval M. petersi were determined using the cryoscope described by Ramsay & Brown (1955). The operating procedure was improved by the use of liquid nitrogen instead of dry ice to cool the alcohol-water mixture. Haemolymph was drawn from larvae using the following procedure. After a 24 hr acclimation period (see section 2.2) a larva was removed from the test salinity and placed on filter paper and carefully dried. If, at this stage, the larva's heart was not beating, the larva was discarded. A fine soda glass capillary, drawn from a 10 lambda disposable micro-pipette, was used to extract the haemolymph. The abdomen of the larva was bent and the capillary inserted through the membrane between the carapace and first abdominal segment. Haemolymph immediately flowed into the capillary by capillary action. It was not possible to bracket the haemolymph sample with mineral oil (Foskett, 1977) as the capillary action was lost so an alternative procedure was adopted. The capillary containing the haemolymph was submerged under mineral oil, mounted in a larger glass tube and sealed at both ends. The sample was frozen in liquid nitrogen and rapidly inserted into the fluid bath in the cryoscope. Although Ramsay & Brown (1955) recommended the use of silica capillary tubing to avoid contamination of the haemolymph, I found it impossible to draw a sufficiently fine capillary using this material. Since no change in the freezing point of distilled water, using a soda glass capillary, was observed within one hour, all determinations were carried out within this time.

The same procedure, used to extract haemolymph from larvae, was used for the post larvae and juvenile M. petersi as well. Haemolymph was drawn from adults by inserting a capillary into the base of the fifth walking leg. Only adult males were used and haemolymph was never taken more than once from any animal. Four to six replicates of the freezing point depression ($\Delta i°C$) of the haemolymph were taken at each test salinity. The freezing point depression of the test salinity ($\Delta e°C$) was determined during the course of the experiment.

2.2 Acclimation trials.

In measuring osmotic concentration of the haemolymph it was necessary to determine the time required for acclimation to a particular salinity. Therefore, rates of salinity acclimation for larvae, post larvae and adult M. petersi were determined. Fig. 53 a, (page 127) shows that the acclimation time required for the haemolymph of stage V larvae to reach osmotic equilibrium was one hr. Haemolymph however, was only removed after 24 hrs. The equilibrium time for other larval stages was not determined but presumed to fall within the 24 hr period. Fig. 53 b, (page 127) shows the time required (4 - 6 hrs) for the haemolymph of post larval M. petersi to equilibrate after being transferred from 16 to $35/_{oo}^{o}$. Therefore, as with the larvae, a period of 24 hrs was allowed for post larval acclimation to the test salinities. Haemolymph of juvenile M. petersi was also sampled after 24 hrs.

Time required for the haemolymph of adult M. petersi to reach osmoregulatory equilibrium after being kept at $20/_{oo}^{o}$ for four days and then transferred directly into freshwater was 24 hrs (Fig. 54, page 128). I measured the downward change and presumed acclimation time for the reverse to be the same. Foskett (1977) measured the change in haemolymph concentration after transferring adult Sesarma reticulatum from $25/_{oo}^{o}$ to 5 and $50/_{oo}^{o}$ respectively and found that the acclimation time of the former (downward) was considerably shorter than the latter. Although adjustment occurred within 24 hrs adults were acclimated for 48 hrs prior to measurement. All the acclimation time tests were carried out at 25.0, range $\pm$ 0.7°C.

2.3 Influence of the moult cycle.

The effect of the moult cycle on haemolymph osmotic concentration

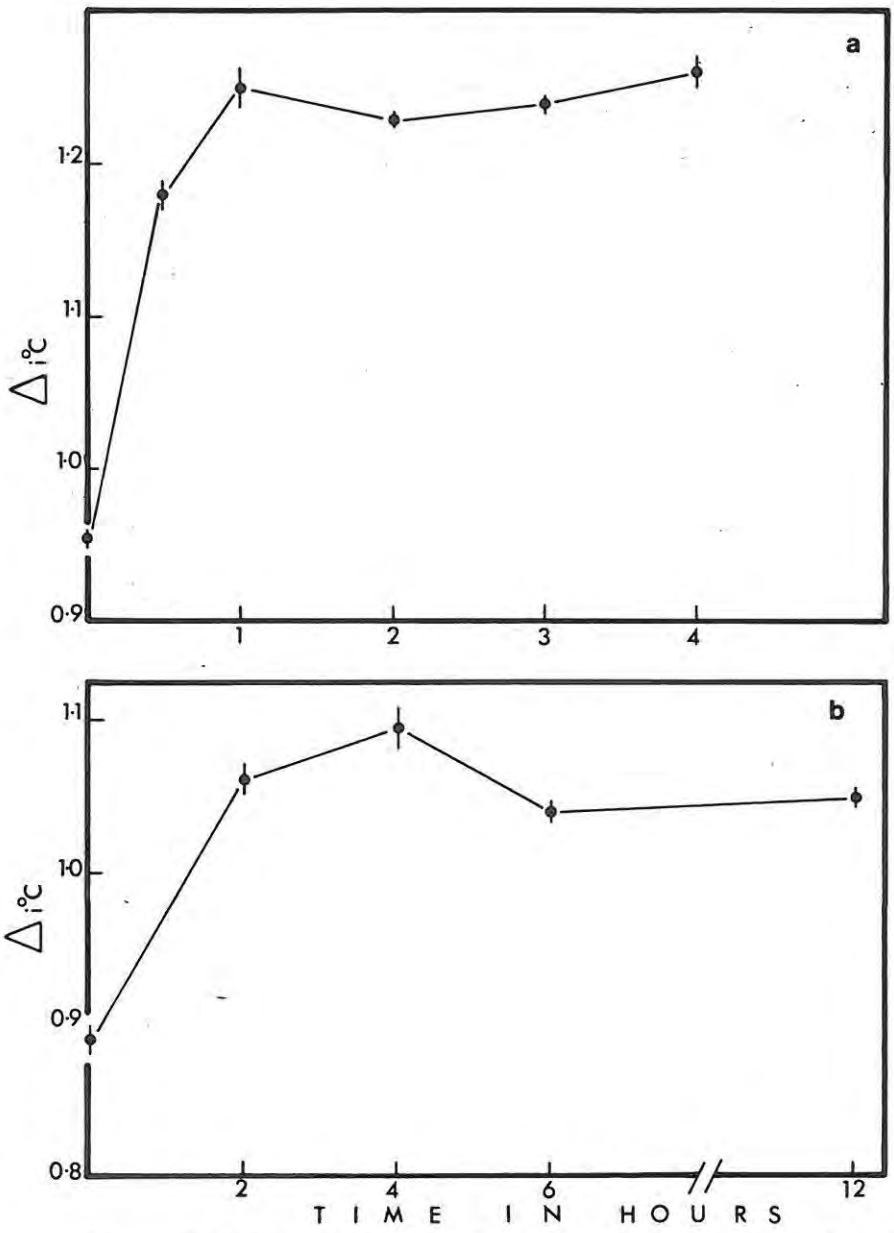


Fig. 53 Time course acclimation for the haemolymph osmoconcentration (Δ i°C) of stage V larvae (a) and post larvae (b) following transfer from 16.0°/₀₀ to 35°/₀₀. Each dot represents the mean of four individuals. Vertical lines represent 1 SD.

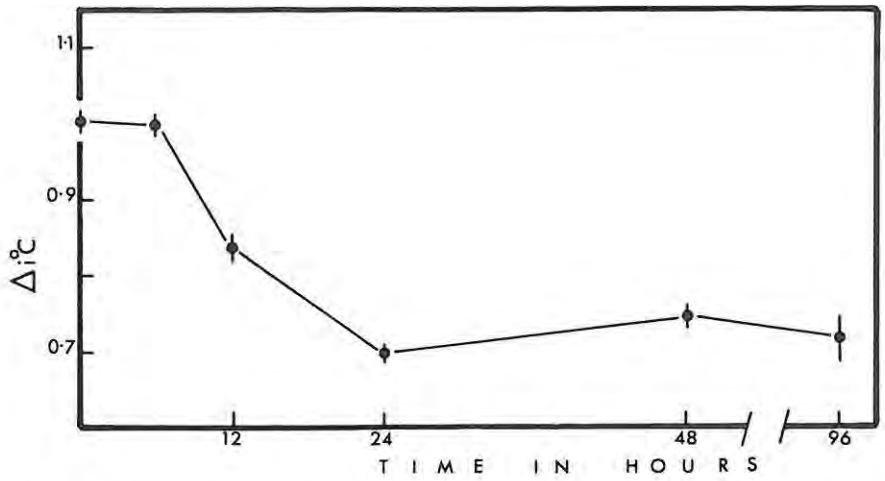


Fig. 54 Time course acclimation for the haemolymph osmoconcentration (Δi°C) of adult M. petersi following direct transfer from 20.0 to 0.5‰. Each dot represents the mean of three individuals. Vertical lines represent 1 SD.

in larval crustacea is not precisely clear. Kalber & Costlow (1966, 1968) and Kalber (1970) have reported an increase in haemolymph osmotic concentration preceding each larval moult in Rhithropanopeus harrisii and Cardisoma guanhumi. The significance of their work was difficult to assess as graphs of the freezing point depression of body fluids were presented but values of means and variation were not. In an earlier paper (Kalber & Costlow, 1966), although changes in osmotic concentration during metecdysis, diecdysis and proecdysis were tabulated, and curves, fitted by the method of least squares, no statistical analysis was supplied to support their claims. Foskett (1977), in an elegant analysis, demonstrated that the moult cycle, in the larvae of Sesarma reticulatum, did not affect haemolymph osmotic concentration. In my study, possible changes that could be attributed to larval ecdysis were avoided by using larvae approximately mid-way through the different stages (Young, 1979). For juveniles and adults only hard (intermoult) prawns were used.

2.4 Experimental design.

Four larval stages, I, II, V and IX, post larvae, juveniles and adults were used in the experiments. Larvae were obtained from ovigerous

females collected from the Keiskamma river. The larvae were hatched in freshwater and reared to the required stage as described in part three. Once the desired larval stage had been reached, between 10 and 15 larvae were transferred from the rearing salinity (14.0, range $\pm$ 2.2$^{o}/_{oo}$) to the test salinities at the same temperature. The test salinities numbered between eight and ten and ranged between freshwater (<0.1$^{o}/_{oo}$) and 35.0$^{o}/_{oo}$. Juveniles (carapace length 6.0, SD $\pm$ 0.9 mm) were caught from the population migrating over the weir into freshwater. Adults (carapace length 20.2, SD $\pm$ 1.2 mm) recently caught in the Keiskamma river, were kept together with the juveniles in freshwater before being transferred directly to the test salinities.

2.5 Data analysis.

A wide variety of osmoregulatory curves describe the relationship between the internal (Δi°C) and external (Δe°C) osmotic concentration of aquatic animals. These vary in complexity depending on the type of regulation (Lockwood, 1962). Spaargaren (1976) expressed these different osmoregulatory patterns using a cubic polynomial function of the form :

$$y = \beta_0 + \beta_1 X + \beta_2 X^2 + \beta_3 X^3$$

where y is the estimated osmotic concentration of the haemolymph (Δi°C) at a particular (X) medium concentration (Δe°C). The regression coefficients β_0, β_1, β_2 and β_3 define a particular regulation pattern within a given range of X. The advantage of polynomial functions as models of regulation patterns is their elementary nature: the least square method produces estimates of the parameters without complex calculations. Furthermore, the mathematical properties of the curves can be used to describe physiological responses within the experimental region defined by the particular function (Studier et al., 1975).

Following Spaargaren (1976), certain properties of the regulation curve could be calculated for each stage; (1) the Δe°C at the isosmotic point where the concentration of Δi°C and Δe°C were the same, (2) the "regulation extent" at the isosmotic point. If the slope of the tangent to the regulation curve was 45° ($\tan\alpha = 1$) then the animal was conforming at that point while an angle of 0° meant perfect regulation ($\tan\alpha = 0$). Spaargaren defined "regulation extent" as 1 - $\tan\alpha$ which ranged between 0 (complete conformity) and 1 (complete regulation), (3) the lower and

upper regulation limits within which regulation occurs. These limits were bounded by zero "regulation extent" beyond which conformity set in. Since stage I larvae, post larvae, juvenile and adult M. petersi regulate in freshwater, the zero "regulation extent" value ($\Delta e°C$) could not be calculated. In these four stages the lower regulation limit was obviously freshwater ($\Delta e°C = 0.02$).

After characterising the regulatory curve of Crangon crangon in this way, Spaargaren (1976) used the cubic polynomial to describe data from 34 crustacea and two teleost species. Due to the linear correlations obtained between β_1 and the remaining three coefficients, he concluded that β_1 sufficed to describe any one curve. He then proceeded to interpret, biologically, the meaning of different values of β_1 and suggested that these demonstrated an evolutionary trend in the development of the regulation pattern. Burton (1980) pointed out that the correlations between the coefficients were spurious and had no physiological significance, and furthermore, that Spaargaren had over-interpreted their meaning. Burton (1980) proposed a different set of equations, the individual coefficients of which reflected obvious features of the curves. I interpreted Burton's criticisms, not as being directed against the use of the cubic polynomial function, but rather against the emphasis that Spaargaren placed on interpreting the meaning of the individual coefficients used to describe the function.

I have consequently used the cubic polynomial to describe my data as it provided a satisfactory fit and allowed certain biological properties, which Spaargaren (1976) outlined, to be determined. No significance has been placed on the value of β_1 other than its contribution to the cubic polynomial per se. The regression coefficients and associated statistics of each regulatory curve were computed from the "SPSS" package on an ICL 1921 computer. Separate programs were used to plot the regulation curves and calculate the properties of the polynomial function. Since the number of observations at each test salinity were replicated it was possible to determine pure and lack of fit error.

3. Hyposmoconform : a comment.

Lockwood (1962) divided the change in haemolymph concentration in relation to the medium in crustacea into three well known categories of regulation; (1) osmoconformers; the haemolymph was almost isosmotic with

the medium over the salinity range tolerated by the animal, (2) hyper-osmotic regulators; the haemolymph was hyperosmotic to the medium in low salinities and isosmotic at high salinities, (3) hyper-hyposmotic regulators; the haemolymph was hyperosmotic in low salinity and hyperosmotic in high salinity. Lockwood (1977) added a further category, solely hyperosmotic regulation, to describe the osmoregulatory pattern shown by the praniza larva of the isopod Paragnathia formica. Haemolymph of the larva remained markedly hyperosmotic in both dilute and concentrated media. This pattern of regulation also characterised certain decapod larvae (Kalber, 1970; Foskett, 1977; Young, 1979). Young (1979) used the term "hyperosmoconform" to describe this regulation pattern. Two distinguishing features of this type of regulation were (a) an almost constant gradient between haemolymph and medium over a wide salinity range and (b) a simultaneous rise in haemolymph concentration as that of the medium increased.

Lockwood (1962, 1977) included the regulation patterns of crabs such as Uca crenulata and Pachygrapsus crassipes in the hyper-hyposmotic regulation category. Clearly, these crustacea are powerful hyperosmotic regulators as the concentration of the haemolymph is stable despite the increase in salinity up to the isosmotic point. Beyond this point however, although the crabs were hyposmoregulating, the concentration of the haemolymph increased with that of the medium to give a regulation pattern parallel, but below, the isosmotic line. Following Young (1979) I propose the term "hyposmoconform" to describe this type of regulation beyond the isosmotic point. Thus, an animal is said to hyposmoconform when the haemolymph beyond the isosmotic point is hyposmotic to the medium and rises, with an increase in medium concentration, parallel, but below, the isosmotic line. I have introduced this term to avoid ambiguity in the definition of hyposmoregulation. Crustacea that hyposmoregulate or hyposmoconform both regulate haemolymph at a level less concentrated than the medium. However, the regulating mechanism is more powerful in hyposmoregulators as the haemolymph remains within a narrow concentration range despite an increase in medium concentration. In hyposmoconformers on the other hand, the capacity to regulate is reduced as the haemolymph concentration increases with that of the medium. The applicability of "regulation extent" (Spaargaren, 1976) to differentiate between hyposmoconformers and hyposmoregulators is immediately apparent. Since

the regulation extent at any point along the regulation curve can be calculated, osmoregulatory curves, at precisely the same external salinities can be compared. Thus, in salinities where crustacea hyposmoconform, regulation extent values close to zero would be expected while in areas of hyposmoregulation, values close to one were likely to be obtained.

4. Results.

The polynomial functions used to approximate the relationship between the osmotic concentration of the haemolymph ($\Delta i°C$) and the osmotic concentration ($\Delta e°C$) of the medium for larval, post larval, juvenile and adult M. petersi are given in Table 41 (page 133). Generally the R^2 was high (except for stage I and post larvae) which confirmed the aptness of the cubic approximation. In no instance was the fit rejected ($P<0.001$) which indicated that variation due to lack of fit remained within acceptable limits. Closer inspection of the pure error mean square (variation within replicates) for stage I and post larvae revealed this to be large which probably accounted for the low R^2 values.

Fig. 55 (pages 134 & 135) shows the osmotic concentration of the haemolymph of larval, post larval, juvenile and adult M. petersi as a function of the osmotic concentration of the medium. The marked differences in regulation patterns between stages have been quantified (Table 42, page 136). The isosmotic value (1.046) was highest in stage I which decreased to 0.871 in stage II and then increased again (1.014) in stage V, only to decrease after metamorphosis when it remained below 0.958. The regulation extent at the isosmotic point showed that both stage I and II larvae regulate maximally (Fig. 55 a & b). The regulation extent was weakest (0.8) in stage V larvae which increased to 1.0 in post larva. Juveniles and adults showed the weakest regulation at the isosmotic point. This was reflected in Fig. 55 f & g as juveniles and adults hyposmoconformed beyond this point. The lower regulation limit for stage I, post larva, juvenile and adult M. petersi was freshwater (0.020) as these stages were powerful hyperosmoregulators (Fig. 55 a, e, f & g). However, this ability was lost in larval stages II, V and IX and the lower regulation limits of 0.119, 0.298 and 0.278 revealed that the ability to hyperosmoregulate in low salinity decreased

Table 41. Cubic polynomial regression equations describing the relationship between Δi°C of the haemolymph and Δe°C of the medium for various stages of the life cycle of M. petersi y and X are the freezing point depression values of the haemolymph and external medium respectively.

* Neter & Wasserman, (1974) p. 119.

Stage of life cycle	Polynomial equation	R^2 (%)	$F = \frac{\text{Regression mean square}}{\text{Residual mean square}}$	Significance	$F = \frac{\text{*Mean square lack of fit}}{\text{Mean square pure error}}$	Significance
Larva I	$y = 0.78 + 0.84X - 0.84X^2 + 0.26X^3$	60.0	$F_{3,36} = 18.42$	P<0.001	$F_{3,33} = 1.98$	P>0.05
Larva II	$y = 0.46 + 1.33X - 1.34X^2 + 0.44X^3$	85.2	$F_{3,34} = 65.56$	P<0.001	$F_{5,29} = 2.74$	0.01<P<0.05
Larva V	$y = 0.25 + 1.59X - 1.13X^2 + 0.29X^3$	90.0	$F_{3,30} = 90.68$	P<0.001	$F_{3,27} = 4.6$	P=0.01
Larva IX	$y = 0.30 + 1.59X - 1.19X^2 + 0.29X^3$	83.0	$F_{3,52} = 84.26$	P<0.001	$F_{7,45} = 3.5$	0.001<P<0.01
Post larva	$y = 0.81 + 0.54X - 0.61X^2 + 0.22X^3$	67.6	$F_{3,40} = 27.48$	P<0.001	$F_{4,36} = 1.56$	P>0.05
Juvenile	$y = 0.82 - 0.04X + 0.10X^2 + 0.07X^3$	93.8	$F_{3,35} = 178.2$	P<0.001	$F_{4,31} = 4.51$	0.001<P<0.01
Adult	$y = 0.90 - 0.27X + 0.28X^2 + 0.02X^3$	94.0	$F_{3,31} = 163.2$	P<0.001	$F_{2,29} = 6.71$	0.001<P<0.01

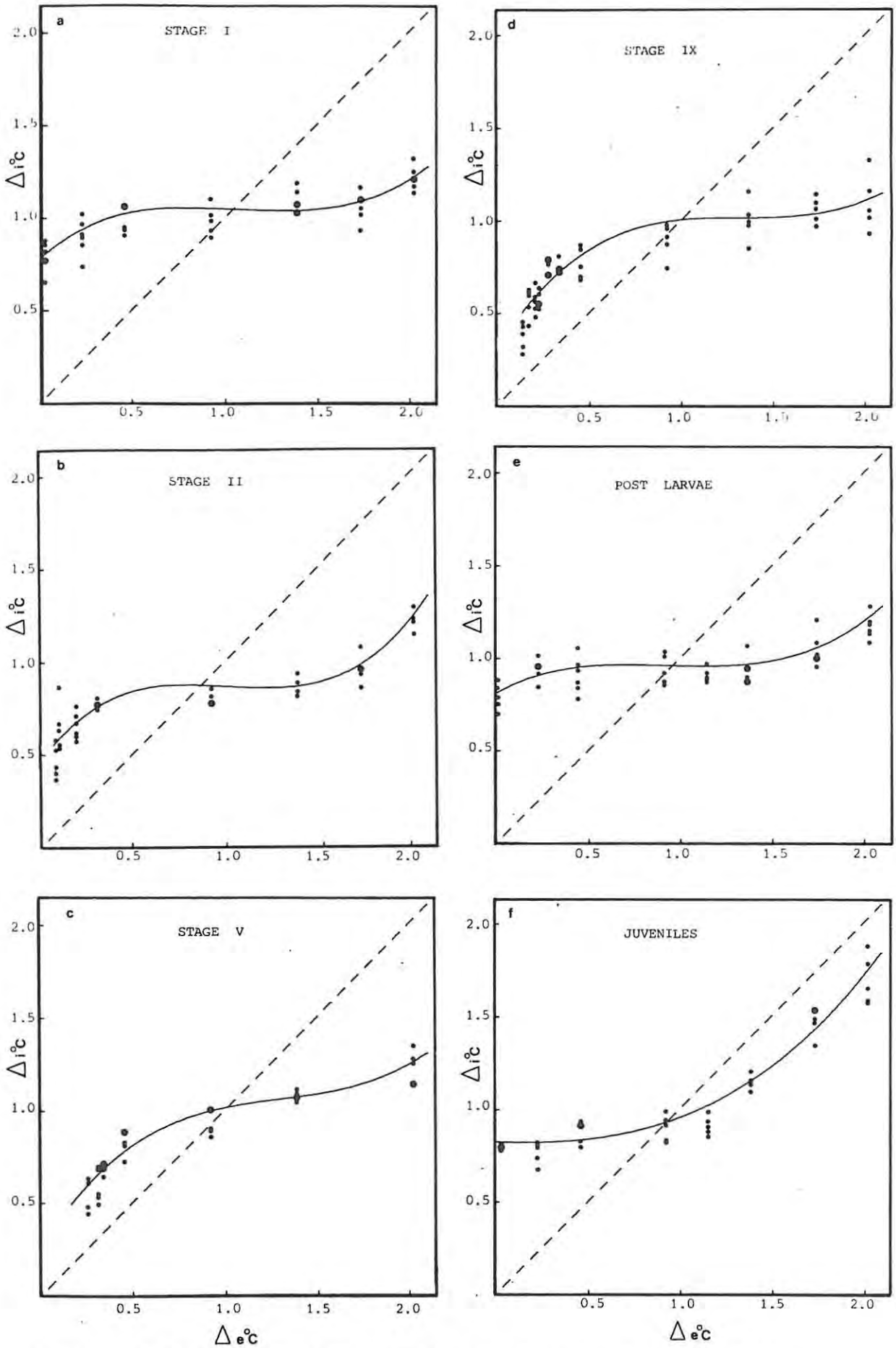
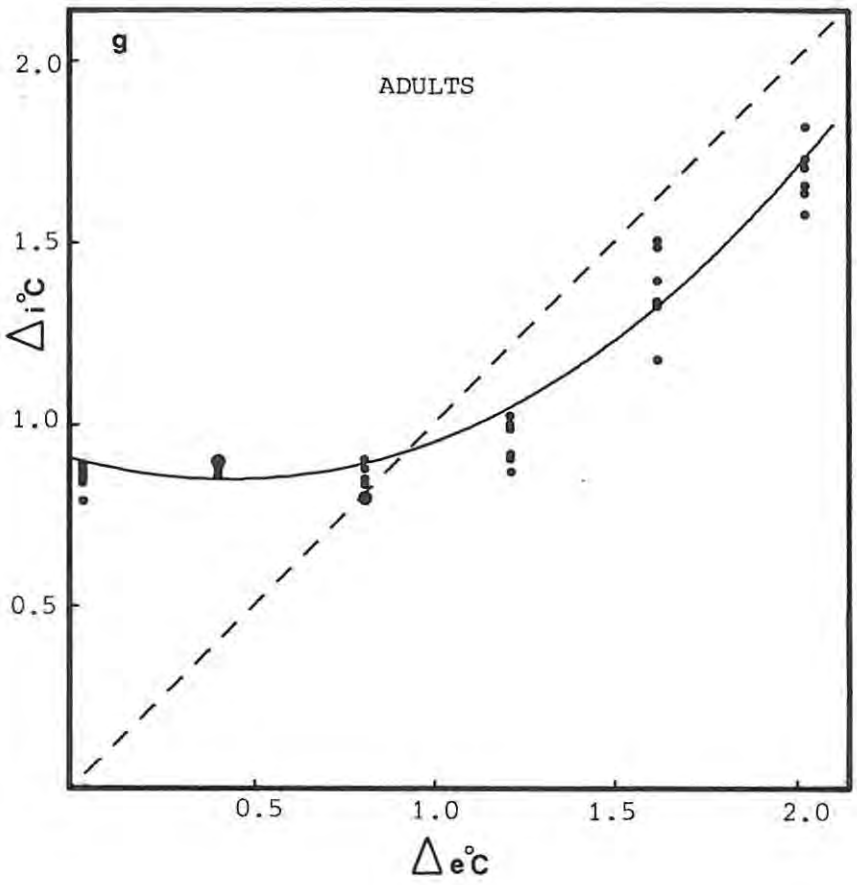


Fig. 55 Osmotic concentration of the haemolymph (Δi°C) in larval, post larval, juvenile & adult M. petersi as a function of the osmotic concentration of the medium (Δe°C). The isosmotic line has been included for comparison. Larger dots represent two identical (Δi°C) values.



from stage II to V but then increased again at stage IX. Fig. 55 b, c & d shows that the osmoregulatory patterns of stages II to IX resulted in an overall displacement of the curve to the right. In salinities below the lower regulation limits in larval stages II to IX the haemolymph concentration parallelled, but remained consistently above, that of the medium. The upper regulation limits of larval and post larval stages were greater than those of juveniles and adults. This was expected as larval and post larval stages showed a marked capacity to hyposmoregulate beyond the isosmotic point. Equally, the regulation range of juveniles and adults was considerably narrower compared with the other stages. Stage I larvae had the widest range (2.210) as, even at $35/_{oo}^{o}$, hyposmoregulation was very pronounced (Fig. 55 a).

5. Discussion.

Compared with the osmoregulatory pattern in other decapod larvae

Table 42. Characteristics of the osmoregulatory curves for the different life cycle stages of M. petersi.

Stage of life cycle	Isosmotic point (Δe°C)	Regulation extent at isosmotic point	Lower and upper regulation limits (Δe°C)	Regulation range (Δe°C)
Larva I	1.046	1.0	0.020-2.230	2.210
Larva II	0.871	1.0	0.119-1.916	1.797
Larva V	1.014	0.80	0.298-2.300	2.003
Larva IX	1.005	0.90	0.278-2.395	2.117
Post larva	0.958	1.0	0.020-2.170	2.153
Juvenile	0.927	0.67	0.020-1.770	1.750
Adult	0.921	0.68	0.020-1.810	1.790

(Kalber & Costlow, 1966, 1968; Kalber, 1970; Foskett, 1977; Young, 1979), those of M. petersi exhibited marked regulatory capabilities. Generally, the osmotic response varied little throughout development in the larvae of Rhithropanopeus harrisii, Callinectes sapidus, Libinia emarginata, Hepatus epheliticus, Sesarma reticulatum and Clibanarius vittatus. All these larvae hyperosmoconformed between 10 and 35‰ (the response to salinities <10.0‰ was not investigated) except the early larval stages of L. emarginata and the seventh zoeal stage of C. sapidus which osmoconformed. In the larvae of Cardisoma guanhumi however, a degree regulation was evident (Kalber & Costlow, 1968). Although stage I and II larvae hyperosmoconformed later stage larvae hyposmoconformed in salinity as low as 20‰. It was perhaps significant that C. guanhumi was a semi-terrestrial crab whereas all the other species were from estuarine or marine environments. This suggested that the habitat frequented by adults may influence the osmoregulatory capability of the larvae. Thus, the difference in osmoregulatory pattern in the larvae of M. petersi compared with the other species, may be due to the fact that the larvae often hatch in freshwater. A powerful form of regulation is required for larvae to survive in such environments.

Kalber & Costlow (1966, 1968) and Kalber (1970) attempted to trace the ontogeny of osmoregulation during larval development in species where

the pattern of adult osmoregulation differed from that of the larvae. As Foskett (1977) pointed out, evidence for this was not convincing and has shown, for example, that in Sesarma reticulatum, even 18 day old megalopa larvae hyperosmoconformed in the same way as stage I zoea. Young (1979) obtained a similar osmoregulatory pattern in the larvae and megalopa of C. vittatus and concurred with Foskett (1977) that the pattern of osmoregulation "was apparently established before hatching and varied little throughout larval development". This statement, however, did not apply to the larval and post larval stages of M. petersi which differed markedly. Furthermore an apparent correlation between osmoregulatory capacity and distribution was evident. For instance, the osmoregulatory capacity of stage I was quite extraordinary and explained their distribution in both fresh and saline water. With such extensive osmoregulatory powers the larvae were able to cope with any salinity change and therefore, suitably adapted for the role as the major dispersal phase within the estuarine environment. The nocturnal behaviour of the stage I larvae also emphasised this role. Stage II larvae however have lost the ability to live in freshwater. The osmoregulatory breakdown in low salinity did not alter during subsequent larval development which effectively confined these stages to the estuarine environment. Although osmoregulatory patterns in four of the nine larval stages were determined, the larvae were nevertheless physiologically well equipped to cope with the salinity variation likely to be experienced during the 20 to 30 day developmental period. The larval osmoregulatory response satisfactorily explained the pattern of survival in low and high salinities obtained in part three.

After metamorphosis post larvae regained the ability to regulate in freshwater (Fig. 55 e). This ability is never lost and placed the post larvae in that small group of crustacea that regulate in both fresh and sea water. Thus, a physiological change took place, preparatory to the freshwater migration, as during this stage of the life cycle a migration occurs. As Fig. 55 f shows, once the post larvae reach freshwater and are regarded as juveniles, the ability to regulate in sea water is lost, and a typical adult osmoregulatory pattern adopted. At low salinities a significant degree of homeosmosis is exhibited, as in the adult pattern, which is obviously linked to a protracted existence in freshwater. Natural selection would have favoured such a change as juveniles have no need to retain the ability to regulate in sea water. Castille & Lawrence

(1981) commented on the difference in osmoregulatory response to high salinity between juvenile M. rosenbergii in their study and post larval M. rosenbergii (Sandifer et al., 1975). Post larval M. rosenbergii maintained haemolymph hyposmotic to the medium, while in juvenile M. rosenbergii the haemolymph was slightly hyperosmotic. Castille & Lawrence (1981) attributed this difference to the fact that post larval M. rosenbergii were found in brackish water whereas juveniles had left this environment and migrated to freshwater where little selective advantage existed for the maintenance of a highly developed hyporegulatory capability. The results that I obtained with post larval and juvenile M. petersi with regard to field behaviour, and change in osmoregulatory pattern, support Castille & Lawrence's argument.

There is no doubt that the pattern of osmoregulation changed throughout development and these changes could, to a large extent, be correlated with the prawns' distribution in the Keiskamma river and estuary. It is noteworthy that the change in adult distribution in response to water of increasing salinity (demonstrated in part two) was not due to osmoregulatory stress as the adults regulate strongly between 4 and 12‰.

The osmoregulatory pattern of adult M. petersi was intermediate between M. equidens on the one hand (Denne, 1968) and M. rosenbergii, M. ohione and M. australiense (Denne, 1968; Sandifer et al., 1975; Castille & Lawrence, 1981) on the other. Denne (1968), and this study, demonstrated that osmoregulatory ability was correlated with habitat. M. equidens was typically found in brackish water whereas the other species, including M. petersi, were primarily freshwater. Within this latter group it would appear that the ability of M. petersi to hyposmoconform may favour survival in higher salinity compared with the other species. To date there is no information on the mechanism of osmoregulation in larval decapod crustacea. The size of the larvae however, may impose certain restrictions on the available mechanisms. Lockwood (1962) argued that as the surface to volume ratio became greater with a decrease in size for species of similar surface permeability, the smaller the size the greater the turnover of ions and water. Therefore, osmotic work per unit mass of body tissue will be greater in small than in large crustacea. This could be reduced if the permeability of the surface or the osmotic gradient between haemolymph and medium was

altered. Lockwood (1962) demonstrated that small freshwater crustacea generally maintained a reduced haemolymph concentration than those of larger body size. This has been interpreted as a means of reducing the osmotic load due to the unfavourable surface to volume ratio. Apparently this does not apply in the case of stage I M. petersi larvae as these have a higher haemolymph osmotic concentration at the isosmotic point than adults (see Table 42). Although this argument is strictly valid for stage I larvae, as only these tolerate freshwater, even stages II and IX that inhabit the estuary do not show a reduced haemolymph concentration compared with adult M. petersi. The lack of correlation between size and reduction in haemolymph osmotic concentration does not apply only to M. petersi larvae. I compared the concentration of haemolymph in stage I zoea and adult Sesarma reticulatum (Foskett, 1977) at the same medium concentration ($\Delta e°C = 1.0$) and estimated this to be 1.37 and 1.41 respectively. Although the values were considerably higher than those of M. petersi, which is to be expected since S. reticulatum is a true estuarine species, there is still no reduction in haemolymph osmotic concentration with size. It is possible, for larvae that require the estuarine environment for survival, that the salt content of the water is sufficiently high to offset the osmotic cost of osmoregulation despite the unfavourable surface to volume ratio. This, however, does not explain the ability of stage I M. petersi larvae to regulate in freshwater without lowering the haemolymph osmotic concentration. It is unlikely that the conservation of osmotic energy, if indeed this is a problem, is not as important in stage I larvae as in small freshwater crustacea. A reduction in surface permeability seemed an unlikely route as the stage I larvae had no gills so conditions which promoted maximum oxygen diffusion were likely to take priority. Whatever the osmoregulatory mechanism, it appears to differ from that found in small freshwater crustacea.

Foskett (1977) suggested that the general decapod larval trend of hyperosmosity may be a mechanism to increase the density of the larvae which enables them to remain close to the substratum and so ensure retention within the estuary. Sandifer (1975) has shown that larvae of a variety of estuarine decapods occurred above the substratum. However, this was based on daytime sampling only. Sandifer (1973) established a 24 hr station to monitor changes in abundance of decapod larvae in

relation to the ebb and flow tide. Unfortunately, the surface sample at 0400 hrs was lost but changes in abundance suggested an increase in surface activity at night despite larval hyperosmosity. Lance (1963) demonstrated that the copepod Acartia tonsa hyperosmoconformed over a wide salinity range yet these were known to actively migrate in the water column (Lance, 1962). Therefore, although hyperosmosity may confine larvae above the substratum, it is unlikely to have developed specifically for this purpose.

In hyposmoconforming larvae the concentration of the haemolymph dropped in proportion to the degree of dilution of the medium, which suggested that the larvae had limited control of water entering the body. Despite this, they tolerated a wide range of salinity. Lockwood (1962) emphasised that species which tolerated large changes in haemolymph concentration, as hyposmoconformers do, must be able to regulate cellular osmotic and ionic levels. It is reasonable to assume, since the cellular sodium pump has survived millions of years of evolution (Rankin & Davenport, 1981), that these larvae may be relying on the efficiency of the sodium pump, adopted by animal cells at a very early stage in evolution, to meet the challenge of living in a variable saline environment. The maintenance of a constant osmotic gradient, over a wide salinity range, ensured that larvae had the problem of coping with cellular swelling only. Furthermore, the rate of influx and efflux of water and ions remained constant as the concentration gradient between haemolymph and medium did not change appreciably. Therefore, the development of a capacity to cope with any variation in the internal and external concentration gradient was not necessary. I suggest that the capacity for cellular osmoregulation is highly developed in hyposmoconformers. In the larvae of M. petersi however adjustment of intracellular concentration was not necessary as the haemolymph remained stable over a relatively wide medium concentration. Although both these methods of osmoregulation differed considerably there was one factor in common, namely, that irrespective of which method was adopted, the larvae tolerated a wide range of salinity. However, I suggest that the mechanism of osmoregulation is more advanced in the larvae of M. petersi compared with the hyperosmoconformers. For instance, M. petersi larvae were obviously able to regulate the osmotic inflow of water in diluted media extremely efficiently. In fact, the ability to hyposmoconform may be seen as an

intermediate step in the evolutionary development of larval osmoregulation in those decapod crustacea in the process of migrating to freshwater. The fact that M. petersi larvae could regulate haemolymph concentration within a narrow range indicated that they were better adapted to tolerate freshwater where haemolymph regulation was an essential prerequisite. Possible evolutionary steps during migration to freshwater in the Palaemonidae are discussed in part six.

In summary, it is not surprising that, with the development of such an elaborate osmoregulatory capability in both larval and post larval M. petersi, factors favouring redistribution and recolonisation in other estuaries are favourable. Thus, the following section describes the geographical distribution of M. petersi along the Southern African coastline and discusses its causes.

PART FIVE

INFLUENCE OF HIGH SALINITY AND LOW TEMPERATURE ON THE GEOGRAPHICAL DISTRIBUTION OF M. PETERSI.

1. Introduction

M. petersi is a subtropical, endemic species, ranging from Tete on the Zambezi river in the north to the Umtata river in the south (Barnard, 1950). Fig. 56 (page 143) shows their distribution south of 30°S latitude along the south eastern coast of Southern Africa. Low numbers (1 per 10 traps) of M. petersi were caught at the ebb and flow in the Great Fish and Kowie rivers, south of the Keiskamma river. The greater abundance in the Keiskamma river suggests that this is probably the last viable population at the southern limit of its distribution. This is the first documented record of M. petersi in these three rivers.

2. Physical features of the coastal waters south of latitude 26°S.

Abiotic conditions that occur along the East African coast are conducive to the southward distribution of tropical planktonic organisms and pelagic invertebrate larvae of marine or estuarine origin. The east coast is bathed by tropical waters from the Mocambique and Agulhas currents. Although the water cools with increase in latitude, the core remains at a sufficiently high temperature (25°C) to effectively disperse tropical pelagic organisms (Brown & Jarman, 1978). Eckman (1967) supports the view that temperature is an important factor in understanding the zoogeography of marine fauna. Along the South African coastline temperature is of primary importance in controlling the distribution of marine plants and animals (Stephenson, 1947; Day, 1974).

Despite the importance of temperature in limiting the distribution of marine biota along the South African coastline, only recently has a detailed seasonal sea temperature pattern of this region been compiled. Christensen (1980), using 10 day sea-surface temperature charts over a 10 year period (1968 - 1978), drew up 12 monthly mean sea temperature data for the region south of latitude 26°S. The data show that sea temperatures decline slowly from the 26°S latitude southwards, but in the area between Port St. Johns (31°S) and East London (33°S) (the Keiskamma river is 60 kms south west of East London) there is a rapid decline in temperature. Christensen (1980) identified three areas along the

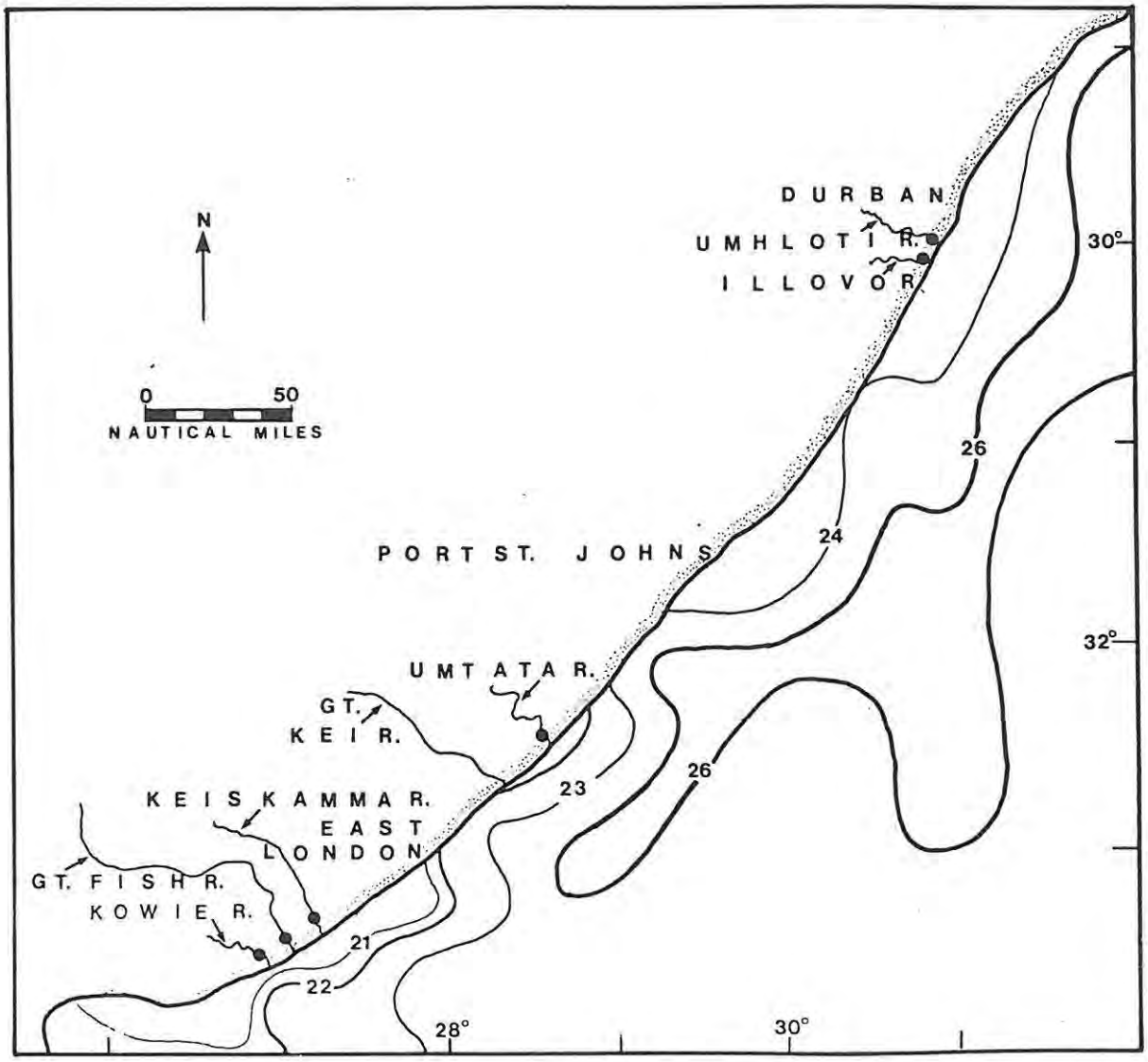


Fig. 56 Southern limit of the geographical distribution of M. petersi (redrawn and updated from Barnard, 1950) and typical coastwise mean (January 1981) sea-surface temperature (°C) patterns. Temperatures for February, March and April 1981 have been omitted. Temperatures from the Maritime Weather Office, Cape Town.

South African coastline where marked changes in temperature occur and in each case these areas were identified by a decline in species richness. In the Port St. Johns - East London area Stephenson (1947) noted a decrease in warm water species but did not have the temperature data to support it; Christensen's data offer an explanation for the reduction in species in this region.

Table 43 (page 144) shows the monthly change in sea temperature

between 31°S and 33°S latitude from January to April 1981. Temperature changes during these months were of interest as it is during this time that larvae and post larvae are abundant in the estuary in greatest numbers.

Table 43. Changes in monthly coastwise sea temperature from 31°S latitude to 33°S latitude.

Latitude	January	February	March	April
31°	24	22	23	23
31° 30'	23	21	21	23
32°	22	20	20	22
32° 30'	21	19	19	20
33°	21	18	18	19
Change (°C)	3	4	5	4

These data were compiled from the same temperature charts that Christensen used and clearly reflect the rapid temperature change that Christensen (1980) identified. In Fig. 56 the mean monthly January 1981 temperature pattern has been drawn. Only the temperature means during one month are shown for reasons of clarity. In addition to the rapid temperature changes in summer, the region is subjected to strong south easterly winds which result in upwelling, so that the sea temperature may drop as low as 10°C (Zoutendyk, 1973; Heydorn & Tinley, 1980). I have stressed the upwelling aspect, as Day (1974) suggested that upwelling along the Atlantic coast of South Africa accounts for the significant reduction in estuarine species diversity compared with estuarine diversity along the Southern Cape coast. At the mouths of Atlantic coast estuaries temperatures are as high as 19.5°C but when the upwelled water flows in at flood tide the temperature may drop as low as 10.5°C. Day remarks on the significance of summer upwelling (as in the East London, Port St. Johns region), as this is the season when larvae of invertebrates and juvenile fishes are normally recruited from the sea.

3. Testing the hypothesis

Generally, the coastwise distribution of a particular estuarine or freshwater species is to a large extent determined by its degree of salt

tolerance. In this respect, not only should the salt tolerance of adults of the species be considered but other stages of the life cycle as well. This is especially relevant in genera like Macrobrachium, as species often have numerous developmental stages. Thus, M. sintangense, M. pilimanus and M. trompii are all freshwater species whose entire life cycle takes place in freshwater. Their distribution is restricted to the Malay Peninsula and Sumatra, Borneo and Java which constituted the Malay subregion during the Pleistocene (Darlington, 1957). Their distribution therefore, has not changed since this time. On the other hand those species like M. rosenbergii, M. equidens, M. idae, M. mirabile and M. scabriculum which, either as adults or as larvae, demonstrate a measurable degree of salt tolerance, range from East Africa to India and the Malay Archipelago (Holthuis, 1950).

Adult M. petersi cannot survive $35/_{oo}^{o}$ for longer than 60 hrs at room temperature so it is unlikely that they play a role in the southerly distribution of the species. I have not determined the salinity tolerance of juvenile M. petersi to $35/_{oo}^{o}$ but a similarity in osmoregulatory pattern suggests they are equally as intolerant as adults. Attention therefore centres on the larvae or post larvae as potential agents of dispersal.

I have demonstrated that larval M. petersi can survive and develop at $35/_{oo}^{o}$ in combination with a range of temperature between 20 and 27°C and furthermore, that metamorphosis to post larvae can also take place under these conditions. The tolerance of larval and post larval M. petersi to $35/_{oo}^{o}$, in conjunction with the abiotic conditions that occur off the Southern African coast, leave no doubt that the southerly distribution has come about by marine transport. Frequent flooding, which is a characteristic of estuaries along the East coast of Southern Africa, increases the chance of larval and post larval M. petersi being washed out of the estuary. What then is the reason for the restricted coastwise distribution of M. petersi? I suggest that a cooler sea temperature due to the sharp decline between 31°S and 33°S latitude, in combination with high salinity ($35/_{oo}^{o}$), restricts the distribution of this species. Larval survival over 10 days (page 103) in salinities between 15 and $35/_{oo}^{o}$ and a temperature range between 16 and 29°C was described by the following relationship :

$$y = - 352.6 - 3.4x_1 + 41.1x_2 - 0.9x_2^2 + 0.13x_1x_2$$

where y = percentage survival after 10 days at x_1 salinity and x_2

temperature. By substituting 19.0°C (mean temperature over four months at 33°S latitude) and 35‰ in the above equation, survival of larvae after 10 days was estimated to be 70%. Assuming metamorphosis to post larvae could take place at this temperature, the 70% survival value casts some doubt on the proposed high salinity, low temperature effect on larval survival. At lower temperatures survival declines rapidly but it is unlikely, due to the time of the year, that the temperature will fall much below this value. Indeed, at 20°C predicted survival increased to 80%, which indicated that an investigation of the effect of high salinity in combination with low temperature on post larval survival was warranted.

The experimental design was a 4 x 6 factorial at four temperatures, 11.0, 12.0, 14.9 and 20.7°C and six salinities, 10.0, 14.9, 20.0, 25.2, 30.0 and 35.1‰ respectively. Post larvae were reared from larvae as described in part three and after metamorphosis maintained at 15.0‰ in the closed circulatory system and fed Artemia nauplii every day. Temperature in the system was gradually decreased (approximately 1.0°C every two days) over a 20 day period to 15.0°C. The post larvae were acclimated for a further five days. The experiment was set up under a 16 hr D and 8 hr L photoperiod. Ten post larvae were randomised into the test salinities by drawing numbers and allocating each post larva to a numbered salinity. Salinity change was instantaneous while temperature change was gradual as the water baths were only switched on after all post larvae had been allocated. Routine monitoring and feeding was carried out as described (see part three). Dead post larvae (no scaphognathite activity) were counted and removed and the experiment terminated after seven days.

4. Results and discussion.

Table 44 (page 147) shows the percentage survival of post larvae after seven days in 24 combinations of salinity and temperature and Fig. 57 (page 147) the fitted response surface. The distorted ellipse suggested an interaction between high salinity and low temperature. This was most noticable at salinities >20.0‰. As temperature decreased and salinity increased survival declined rapidly. Thus, at 21°C, 80% survival was obtained at approximately 28‰ but at 13.5°C at the same salinity survival had dropped to approximately 55%. The position of the

100% survival contour indicated that at salinities <20.0‰, post larval survival was independent of temperature over the seven day period.

Table 44. Percentage survival of M. petersi post larvae after seven days in four temperature and six salinity combinations. *Mean ± SD.

Temperature Salinity	11.0±0.2*	12.9±0.1	14.9±0.2	20.7±0.3
10.0±0.4*	90.0	100.0	90.0	80.0
14.9±0.3	80.0	90.0	100.0	90.0
20.0±0.5	90.0	100.0	100.0	90.0
25.2±0.4	50.0	60.0	100.0	80.0
30.0±0.2	0.0	0.0	80.0	80.0
35.1±0.5	0.0	0.0	0.0	50.0

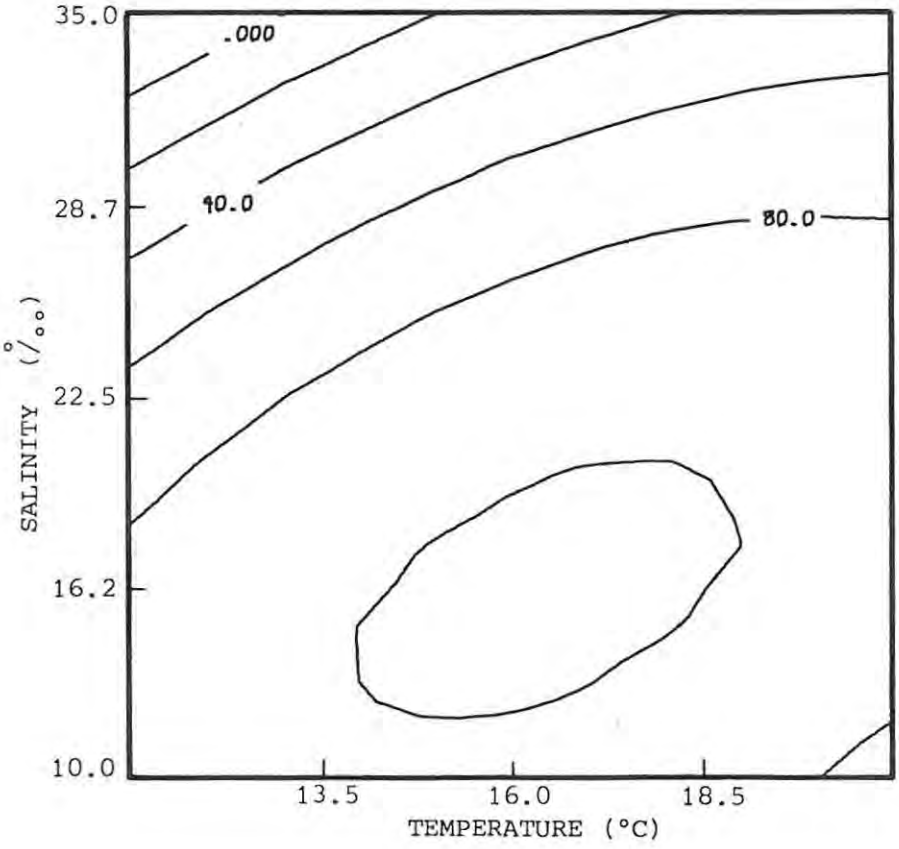


Fig. 57 The estimated response surface of the % survival of post larval M. petersi after seven days in 24 combinations of temperature and salinity. Contours of equal survival are drawn at intervals of 20%.

In an analysis of variance of post larval survival (Table 45) the null hypothesis was rejected ($F_{5,18} = 27.24$ $P<0.001$) and of the five terms in the equation, the quadratic salinity term accounted for 61.8% of the total variability. The interaction term made a significant contribution which confirmed the shape of the survival response surface.

Table 45. ANOVA of the percentage survival of M. petersi post larvae after seven days.

Source of variation	R^2 (%)	R^2 change (%)	Regression coefficients	$F_{1,22}$ ratio	Significance
S^2	62.1	62.1	- 0.21	96.03	P<0.001
T x S	77.9	15.8	0.35	23.69	P<0.001
T^2	84.5	6.6	- 0.59	10.22	0.001<P<0.01
T	85.6	1.1	13.89	1.76	Not significant
S	85.7	0.1	0.71	0.07	Not significant
Constant			-17.06		

By substituting 19.0 or 20.0°C with $35/_{oo}^{o}$ in the quadratic polynomial, (regression coefficients in Table 45) predicted survival values of 38 and 41% respectively were obtained. Thus, at similar temperatures the estimated survival of post larvae was considerably lower than that predicted for the larvae, suggesting that low temperatures reduce the tolerance of the post larvae to high salinity. It would appear then that the post larval phase is the more vulnerable and therefore likely to be responsible for the restricted southern distribution of M. petersi along the South African coastline.

While it is known (Day,1981d) that tropical Indo-Pacific species decrease as they spread southwards down the coast of South Africa and endemic species increase, few studies have been carried out on zoogeographic distribution in estuaries. Day (1981d) analysed the affinities of the fauna in the Morrumbene estuary 24°S, the Mlalazi estuary 29°S, the Mngazana estuary 31°S and the Knysna estuary 34°S. His analysis confirmed that already known about the shore fauna, namely that the percentage of tropical species decreased in more southern estuaries while the percentage of endemic species increased. It was

noteworthy that the decline in tropical species was very marked between the Mngazana (44%) and Knysna (19%) estuaries. This is significant, as the region of rapid temperature change occurs between these two estuaries. The distribution of M. petersi therefore contributes to the change from a preponderance of subtropical to warm temperate fauna that occurs in this region.

Similar changes in faunal assemblages occur offshore of Cape Hatteras, North Carolina, U.S.A. (Vernberg & Vernberg, 1970). In this region the warm northerly flowing waters of the Gulf Stream meet the colder, southerly flowing water of the Virginia coastal current. Fauna of this region have predominantly cold water or warm water affinities. Vernberg & Vernberg (1970) demonstrated that temperature was the primary limiting factor accounting for the zoogeographical distributions between the northern affinity species which do not survive high temperature and the southern affinity species which do. They emphasised the fact that larvae of certain tropical decapod species were more resistent to cold temperature than adults which may account for the establishment of adult populations in areas beyond which the laboratory determined lethal temperature would indicate. Clearly, in the case of M. petersi, larval and post larval tolerance of salinity in excess of that of adults, made a significant contribution to the distribution of M. petersi along the east and southern coast of Southern Africa.

As the geographical distribution of M. petersi can be accounted for in terms of larval salt tolerance, there is no doubt that the ubiquitous global distribution of the genus Macrobrachium can also be attributed to marine transport. They are therefore a reasonably successful diverse genus which occupy both fresh and brackish water environments. Possible factors that may have contributed to this success, in an evolutionary context, are discussed in part six.

PART SIX

PHYSIOLOGICAL AND BEHAVIOURAL ADAPTATIONS IN THE COLONISATION OF FRESHWATER BY THE GENUS MACROBRACHIUM.

The Atyidae are an ancient freshwater group with no extant marine representatives (Fryer, 1976), but related Palaemonidae are found in freshwater, brackish water and marine habitats (Hedgpeth, 1957). As long ago as 1902 Ortmann pointed out that Macrobrachium was a relatively recent genus apparently in the process of emigrating to freshwater. Ortmann(1902) based his conclusion on the fact that members of the genus were found in a variety of habitats ranging from fresh to brackish water. Thus, M. quelchi was found in the mountainous region of British Guiana at an altitude of 2500 m, M. pilimanus in fast torrential stream regions on hilly countryside in Malaya and M. trompii lower down in forest streams in and about leafbeds and peaty detritus in water of pH<4.5 (Johnson, 1967). M. petersi was found in freshwater in the upper reaches of estuaries while M. equidens may be regarded as a truly estuarine species (Denne, 1968). Whereas crabs from the genus Potamon are extremely abundant and well established in a variety of freshwater habitats, there are no estuarine or marine representatives. Therefore, there is little evidence to suggest that, in contrast to the genus Macrobrachium, they are in the process of emerging from the sea (Shaw, 1959b).

Table 46 (page 151) summarises the proportionate distribution in freshwater (FW), brackish water (BW) or sea water (littoral, LITT) or different combinations thereof, of species from 16 palaemonid genera in the Indo-Pacific region. Table 46 has been prepared from Holthuis (1950) who gave a systematic account of the Palaemonidae collected by the Siboga and Snellius expeditions. A total of 161 species constitutes Table 46, of which 55.2% are freshwater representatives from the genus Macrobrachium while the remainder of the genus, 5.6%, inhabits both fresh and brackish water environments. M. petersi falls into this latter category. The genus Palaemonetes which is closely related to Macrobrachium (Hedgpeth, 1949) has the next largest proportion (6.8%) of freshwater representatives while the genus Palaemon has members from fresh and brackish water environments as well as the largest littoral component (6.2%). Thus, of

the genera represented in Table 46, Macrobrachium have colonised freshwater to the greatest extent. How has this taken place? To answer this question I propose to use examples from the Palaemonidae to outline a probable course of both behavioural and physiological adaptation that Macrobrachium may have adopted to successfully invade freshwater. It is necessary to emphasise that the discussion is based on very few species and even on these, physiological and behavioural studies are far from complete. The genus Macrobrachium is however ideally suited for a study of this nature as they are adapting to life in freshwater. Therefore, osmoregulation and the mechanisms of salt and water balance can be considered in the process of evolving and not, as is the case with the genus Potamon, as a final solution to the problem of life in freshwater.

Table 46. Percentage of species from 16 genera inhabiting different environments in the Indo-Pacific region (adapted from Holthuis, 1950).

Genus	FW	FW + BW	FW + BW + LITT	LITT	LITT + BW	BW
Desmocaris	0.6	-	-	-	-	-
Creaseria	0.6	-	-	-	-	-
Leander	-	-	-	1.8	-	-
Leandrites	-	-	-	1.8	-	-
Palaemon	1.2	1.8	1.8	6.2	-	-
Palaeander	-	-	-	1.8	0.6	-
Nematopalaemon	-	-	0.6	0.6	-	0.6
Exopalaemon	1.2	-	0.6	-	-	-
Palaemonetes	6.8	0.6	-	0.6	-	2.5
Troglocubanus	2.5	-	-	-	-	-
Leptocarpus	-	0.6	0.6	-	-	-
Cryphiops	0.6	-	-	-	-	-
Pseudopalaemon	0.6	-	-	-	-	-
Brachycarpus	-	-	-	0.6	-	-
Macrobrachium	55.2	5.6	-	-	-	-

Ortmann (1902) refers to Macrobrachium as a recent genus and if this is true the large proportion of freshwater species (Table 46), compared with the other genera, would not be expected. To explain this it is not unreasonable to suggest that euryhalinity may have been a physiological

characteristic of the ancestral stock(s), a trait not easily come by, and which once attained is very conservative (Hedgpeth, 1957). The adaptive value of this physiological trait early on in the evolution of the genus might have presented ecological advantages which favoured natural selection and therefore a high evolutionary rate. A consequence of populations restricted to estuaries and brackish water environments in the drive to fresh water is genetic isolation (Sandifer, 1975). Carriker (1967) has suggested that natural selection for behavioural patterns which promote retention within this environment is likely to be great in those species colonising the estuary. This would reinforce the physiographic isolation already in existence. A powerful isolating effect would thus operate which would promote speciation resulting in a radiation from the euryhaline ancestor.

In the genus Macrobrachium invasion of freshwater via estuaries probably involved numerous adaptations of which, (a) reproductive behavioural adaptations, (b) osmoregulatory adaptation of adults and larvae and (c) adaptations for egg development in variable saline and freshwater environments, are likely to be the most important. It is unlikely that these processes occurred simultaneously but it is possible to envisage a stepwise invasion of freshwater where physiological adaptations allow behavioural changes which slowly bring different stages of the life cycle closer to the freshwater environment.

On entering the estuarine environment an animal is immediately faced with a variable saline regime and therefore the problem of internal regulation. I suggest that the pattern of osmoregulation of M. equidens (Denne, 1968) indicates that this species is a recent invader of the estuarine environment. M. equidens is capable of both hyper and hyposmoregulation but has not yet developed the capacity to regulate in water of low salinity ($<5.0\,^{o}/_{oo}$). Palaemonetes intermedius (Dobkin & Manning, 1964) shows a similar regulation pattern as well as P. varians, Palaemon serratus and P. elegans (Panikkar, 1941). All of these species are euryhaline, and adults are found in estuarine and littoral environments. Due to the fluctuating salinity it is unlikely that egg and larval development could also occur successfully in this environment. It is conceivable that this problem may have been avoided by the development of a reproductive behavioural pattern where adults continued to reproduce in the sea. Demonstrated examples of this, in another family, are Crago franciscorum and C. nigricauda which move out of the estuaries off the

Californian coast as the spawning season approaches and the larvae hatch in water of high salinity (Israel, 1936). The more distantly related Penaeidae have evolved a similar reproductive behavioural pattern where adults leave the estuary and return to the sea to spawn. After larval development a post larval migration from the sea, back to the estuary, takes place (Allen, 1966a; Champion, 1970). The post larvae have very well developed powers of osmoregulation (Dall, 1981) and are therefore suitably adapted for an estuarine existence. This reproductive strategy offers a temporary solution to the problem of the inability of eggs and larvae to tolerate estuarine conditions. The situation was partially satisfactory, but as recruitment to the adult population in the estuary was probably hazardous, selection for a life cycle that could be completed within the estuary, adjacent to the adult population, was likely to be in progress. The development of an egg membrane to isolate the developing embryo from the variable saline environment, and the ability of larvae to hyperosmoconform (Foskett, 1977; Young, 1979) would ensure that this could take place. As mentioned in part four (page 140) hyperosmoconforming is probably a primitive form of regulation, but is nevertheless very effective. In this regard the osmoregulatory pattern of the larvae of M. equidens would most likely give a more realistic indication of the pattern that the early invaders of the estuarine environment may have adopted. Palaemonetes pugio is an example of a palaemonid that completes the entire life cycle in an estuarine environment (Wood, 1966).

Assuming that the stage has been reached where the species is truly estuarine in every respect, the next step is the emigration from brackish to freshwater. The principle osmotic problems are the continuous inflow of water that has to be expelled and the paucity of ions which have to be actively absorbed or derived from the food. Beadle & Cragg (1940) and Beadle (1943) have suggested that invasion of freshwater proceeded in two stages, the first characterised by the development of a mechanism for the maintenance of a high, but less than normal, haemolymph concentration as represented by Eriocheir (Krogh, 1939) and the second, characterised by a reduction in haemolymph concentration and the production of hyposmotic urine as represented by Astacus (Shaw, 1959a, 1960). However, Shaw (1959b & 1961) argued that the development of a low haemolymph concentration and production of a hyposmotic urine would only have selective value in those crustacea that were essentially semi-permeable,

i.e. permeable to water but not to salts. Thus the acquisition of a low haemolymph concentration and the production of hyposmotic urine were not obligatory requirements for entering freshwater. The mechanism adopted by Potamon niloticus, a successful freshwater crab, illustrates this point. In P. niloticus, a reduction in surface permeability to both salts and water and the development of an ion uptake mechanism which operated at an extremely low external salt concentration, enabled it to retain a relatively high haemolymph concentration and produce a limited volume of isosmotic urine (Shaw, 1959b). Thus, other mechanisms of freshwater adaptation, which differed from Astacus, could also be considered as a solution to the problem of life in freshwater. Unfortunately no information is available on permeability changes or the development of salt uptake mechanisms in Macrobrachium species. Only haemolymph, and in certain cases urine concentrations, are known. Therefore, I have used these as a guide to indicate the possible mechanisms of adaptation to freshwater that Macrobrachium species may have undergone.

The available data suggests that different species of Macrobrachium may have independently adopted either the semi-permeable (like Astacus) or the all round reduction in permeability approach, like Potamon, to colonise freshwater. Evidence for the former mechanism is found in M. australiense which has a haemolymph concentration of 0.96 in freshwater, compared with 0.81 for Astacus, and produces copious quantities of hyposmotic urine (Denne, 1968). M. australiense is a true freshwater species, since the larvae are not dependent on saline water for survival. It produces relatively few eggs (0.8 to 1.1 mm in diameter) and has reduced larval development to three stages lasting five days. The larvae do not feed (Fielder, 1970). It is not unreasonable to assume, from its osmoregulatory and urinary pattern, that M. australiense is semi-permeable and has therefore adapted to freshwater along similar lines to that of Astacus (Shaw, 1959a, 1960). The energy saving implications of this method of osmoregulation have been outlined (Potts, 1954). However, Shaw (1961) has suggested that the condition of semi-permeability may prove the exception rather than the rule. Until more detailed information is gained on the mechanism of osmoregulation in M. australiense Shaw's comment cannot be verified.

Denne (1968) has shown that M. equidens, in low saline water, produces a hyposmotic urine but not as dilute as that of M. australiense

Although M. equidens cannot regulate in freshwater, the production of hyposmotic urine may be the first of several stages required to achieve this ability. It is noteworthy that the haemolymph concentration of M. equidens (1.03) is considerably higher than all other Macrobrachium species studied. Castille & Lawrence (1981) have recorded values of 0.86 and 0.84 for M. ohione and M. rosenbergii juveniles respectively. A value of 0.88 was obtained for M. rosenbergii juveniles by Sandifer et al., (1975) while the haemolymph concentration of M. petersi adults in freshwater at 25°C was 0.904, SD $\pm$ 0.04 (n = 5). The high haemolymph concentration of M. equidens suggests that it may be a recent invader of the estuarine environment. It is possible therefore, that the mechanism of osmoregulation in M. equidens could be the forerunner of that operating in M. australiense

The osmoregulatory patterns of M. rosenbergii and M. ohione (Castille & Lawrence, 1981) are very similar to that of Potamon niloticus (Shaw, 1959b). All three species are strong hyperosmotic regulators in freshwater but as the external concentration approaches the original haemolymph concentration, the haemolymph rises hyperosmotically with the increase in external concentration of the medium. However, the osmoregulatory pattern of M. petersi differs from the above in that the haemolymph crosses the isosmotic line and furthermore, rises hyposmotically with an increase in the medium concentration. This may indicate a tolerance of higher salinities compared with M. rosenbergii and M. ohione and if so, suggests that the latter are the older species. Indeed, the widespread distribution of M. rosenbergii in the Indo-Pacific region (Holthuis, 1950) and M. ohione in North America (Hedgpeth, 1949) compared with the restricted distribution of M. petersi along the east coast of Southern Africa, supports this view. The haemolymph concentration of M. petersi in freshwater is 0.904 and furthermore the urine is isosmotic (0.895, SD $\pm$ 0.04, n = 5). There is no data on the urine concentration or volume produced in either M. rosenbergii or M. ohione but I suggest that an all round reduction in permeability to both salt and water and a high ion affinity similar to that found in P. niloticus may be the means by which M. rosenbergii and M. ohione have invaded freshwater and furthermore, the mechanisms operational in M. petersi may represent the intermediate step. If this is so, although adult M. australiense, M. rosenbergii, M. ohione and M. petersi all live in freshwater, different mechanisms are

employed to achieve this, which suggests that the problem of entering freshwater in the genus Macrobrachium is being solved independently in different species. Fig. 58 shows a diagramatic representation of the possible changes in the haemolymph and urinary patterns that may have taken place during the colonisation of freshwater in the genus Macrobrachium.

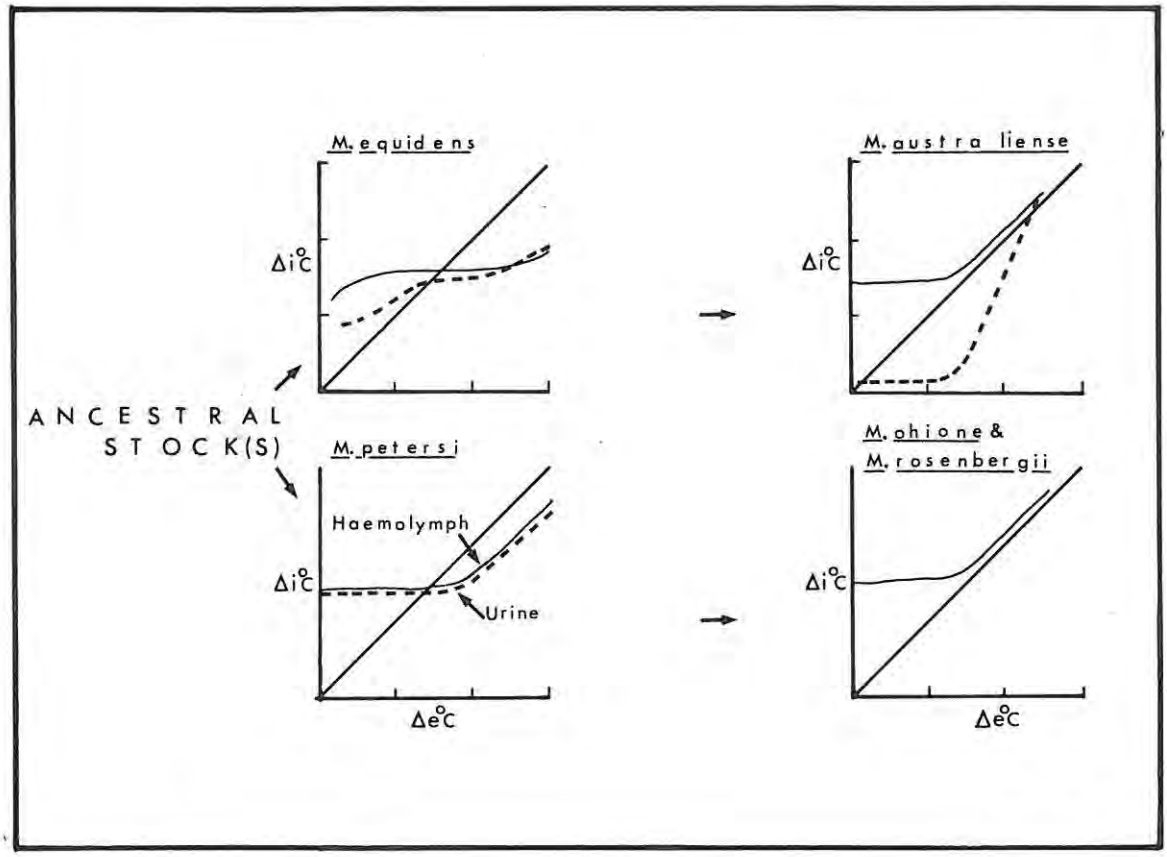


Fig. 58 Changes in the haemolymph and urinary patterns during the colonisation of freshwater. The arrows indicate the possible directions the colonisation might have taken.

The final stage, assuming that the adults have adapted to freshwater, is that egg and larval development take place in this medium. The breeding migration to the estuary of M. petersi and other Macrobrachium species suggests that egg and larval adaptations for development in freshwater do not occur simultaneously. Pearse & Gunter (1957) point out that for adults that have recently adapted to freshwater, the accumulation of sufficient food and salts in the egg, to render development independent of the medium, is the next critical step. Presumably, membrane permeability around the egg mass was further altered to accommodate the

much reduced ionic environment. However, that egg development could take place in freshwater did not imply that larval development must occur in this medium as well. This alleviated, to some extent, the physiological demands of such a transition. The fact that egg development occurs successfully in freshwater in M. petersi, M. rosenbergii (Ling, 1969), M. acanthurus (Choudhury, 1971b), M. amazonicum (Guest & Durocher, 1979) and M. nipponense (Uno, 1971) but the larvae require saline water, attests to this fact. In other words, the estuarine environment is being used as a substitute, to overcome deficiencies which prevent complete larval development within the egg in freshwater. Thus, development of behaviour patterns in the estuary to retain the larvae and adult breeding population close to freshwater would have a pronounced selective value. In M. petersi, the larvae have lost their planktonic existence which minimises downward displacement. Larval development therefore, takes place in close proximity to the adult population which favours recruitment. In addition, the upstream movement of adults, in response to an increase in salinity, ensures that reproduction and larval development occur in the middle and upper reaches of the estuary. Sandifer (1975) has suggested that retention of larvae within an estuary may be one of the steps involved in the gradual colonisation of freshwater. The response of adult M. petersi to an increase in salinity indicates that an adult behavioural pattern may also be needed to effect this process. In M. petersi, a post larval migration to freshwater is an additional mechanism which ensures recruitment to the adult population and therefore a further step towards the successful colonisation of freshwater.

Larval development is not totally confined to the estuary as stage I survive successfully in freshwater, and do not feed. These two characteristics indicate preliminary adaptations to complete larval development in freshwater and abbreviate the number of larval stages (as these can now take place in the egg), leading to a reduction in developmental time, strategies which distinguish many freshwater invertebrates. For obvious reasons larvae that hyperosmoconform would be unable to survive in freshwater therefore some form of regulation is required. Although stage I larvae are strong hyperosmoregulators, this capacity is lost in the subsequent stages. However, it may be significant that the haemolymph concentration of stage II larvae, at the isosmotic point, is lower compared with all other stages investigated (see Table 42, page 136). This is adaptive as it enables the second larval stage to withstand lower salinity

and therefore may well herald another step in the evolutionary adaptation of the larval life cycle to freshwater. As the post larvae have the ability to regulate in freshwater all that is required for M. petersi to loose contact with brackish water is a mechanism enabling larval stages II to IX to regulate in freshwater.

The entire life cycle of M. australiense takes place in freshwater. Eggs are reduced in number but have a larger diameter (0.8 to 1.1 mm) compared with (0.54 to 0.67 mm) in M. petersi and larval development is abbreviated to three non-feeding stages. These metamorphose into post larvae within five to seven days of hatching (Fielder, 1970) compared with M. petersi that requires from 20 to 30 days. Sandifer & Smith (1979) speculated that natural selection, acting on genetic traits that favoured a reduced planktonic period and fewer larval stages, would eventually give rise to true freshwater species. Differential changes along these lines can be traced in extant Macrobrachium populations. Although there is no direct evidence that complete larval development occurs in the egg and young hatch as minature adults in true freshwater Macrobrachium species, indirectly, the size of the egg may give an indication of the degree of development that takes place within it. Holthuis (1952) noted egg size in certain freshwater Macrobrachium from South America. Many were considerably larger than that of M. australiense - which, to date, is the only recorded species with such advanced freshwater larval adaptations. For instance, M. jelskii produced a few large eggs between 1.3 and 2.3 mm in diameter while M. quelchi, that inhabited mountainous regions in British Guiana, had eggs of 2.5 mm in diameter. These eggs are considerably larger than those of M. petersi and twice as large as the eggs of M. australiense It is not unreasonable to assume that in eggs of this diameter complete larval development has been achieved.

As I mentioned at the outset, these ideas are largely speculative but could be tested by locating a river and estuary where the distribution of a number of Macrobrachium species could be correlated with that of salinity. This would effectively simulate an evolutionary progression into freshwater. A detailed field investigation correlating changes in adult abundance with that of salinity, and laboratory analysis of changes in haemolymph and urinary concentration, as well as the mechanisms by which this was achieved, would show possible

physiological and behavioural mechanisms that the genus might have adopted in the colonisation of freshwater. As Shaw (1961) has stated, important physiological aspects that require investigation are changes in the permeability of the body surface to salt and water and the categorisation of the salt uptake mechanism in relation to an affinity for sodium.

Bickerton (pers. comm.), in a preliminary survey of the Siyaya estuary in Zululand (120 kms north of Durban, see Fig. 56, page 143), noted an overlap in distribution between M. equidens and M. petersi in the middle and upper reaches of the estuary, and M. lepidactylus and M. lar at the head and in freshwater regions of the estuary. This situation could be used to advantage to verify the hypothesis. In addition, the role of the larvae and the adaptations that these have undergone both physiologically and behaviourally in the colonisation process, provides a formidable challenge for the estuarine ecophysiologist.

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APPENDIX

Let p(t) be the proportion in the population that responded in the time interval (0,t). Assume that p(t) varies with log t like a normal distribution function i.e.

$$p(t) = N(a + b \log t)$$

for some a and b. If

$$p_i = p(t_i) - p(t_{i-1})$$

is the proportion change in the interval (t_{i-1}, t_i), then

$$p_i = N(a + b \log t_i) - N(a + b \log t_{i-1})$$

(take $\log 0 = -\infty$, $\log \infty = \infty$).

Let n_i be the observed number responding in (t_{i-1}, t_i). Then the log likelihood of the data is

$$\log L = \text{constant} + \sum_{i=1}^{k} n_i \log (p_i)$$

(take $t_0 = 0$, $t_k = \infty$).

To get the maximum likelihood estimators for a and b we take the derivatives of log L with respect to a and b, put these equal to zero and solve the resulting two equations for a and b. This was done iteratively using a Newton-Raphson procedure.