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Temperature and salinity tolerance of adult hermit crabs, *Diogenes brevirostris*, Stimpson (Crustacea: Decapoda: Anomura)

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Diogenes brevirostris was found to be tolerant to low salinities $(0,5-4,0^{\circ}/\infty)$ at a wide range of temperatures (12 to 27°C). Tolerance to temperature and salinity were inversely related. At low salinities (0,5 and $1,0^{\circ}/\infty)$ the rate of mortality increased with temperature throughout the range, whereas at higher salinities (1,5 and $4,0^{\circ}/\infty)$ temperature had no effect on mortality. These results are discussed in relation to the distribution of D. brevirostris in estuaries. It appears that temperature and salinity tolerance are not solely responsible for limiting distribution of these crabs to estuary mouths.

Daar is bevind dat *Diogenes brevirostris* lae soutgehalte (0,5 tot 4,0 %) by 'n wye temperatuurbestek (12 tot 27°C) kan verdra. Die verband tussen verdraagsaamheid teenoor temperatuur en soutgehalte is gevind om omgekeerd eweredig te wees. By lae soutgehalte (0,5 en 1,0 %) het die mortaliteitstempo oor die hele bestek toegeneem met stygende temperatuur, maar by hoër soutgehalte (1,5 tot 4,0 %) het temperatuur geen invloed op mortaliteit gehad nie. Die resultate word bespreek ten opsigte van die verspreiding van *D. brevirostris* in getymondings. Dit blyk dat temperatuur- en soutgehalteverdraagsaamheid nie uitsluitlik die verspreiding van hierdie krappe tot getymondings beperk nie.

The South African coastline has a large number of estuaries, each with communities specifically adapted to the variable local environmental conditions. One of the main characteristics of an estuarine population is its ability to tolerate changes in salinity (Day 1981). Estuarine populations are also exposed to wider temperature fluctuations than are marine populations (Hill & Allanson 1971). There has been considerable work on the tolerance of estuarine animals to various temperature and salinity regimes, and speculations regarding the effect these factors have on their distribution (Kinne 1964; Hill 1971; Hill & Allanson 1971; Biggs 1973; Forbes 1974; Aziz & Greenwood 1981; Roddie, Leakey & Berry 1984; Preston 1985). However, no work of this nature has been carried out on Diogenes brevirostris, a common intertidal and estuarine hermit crab found along the southern and eastern Cape coastline of South Africa (Day 1974).

D. brevirostris is very common in the Bushmans River estuary in the Eastern Cape (33°42′S / 26°40′E), where it penetrates upstream to a distance of 3 km from the mouth. In its lower reaches this estuary meanders between intertidal sandbars, the edges of which support fairly extensive stands of Zostera capensis Setchell. These sandbanks extend from the inlet to about 3,5 km upstream and are of marine origin (Baird 1982).

During periods of low rainfall surface salinities of 30 to 32°/_∞ 20 km upstream, and 7°/_∞ 30 km upstream, have been recorded (Robertson 1984). After flooding, however, water at the mouth can be completely fresh (0,35°/_∞) on the outgoing tide, and only slightly saline 20 km upstream (Robertson 1984). As there is only one major impoundment regulating the river, it may be subjected to frequent flooding. Winter water temperatures range from 13,5 to 17°C and summer temperatures from 20 to 24°C, surface and bottom temperatures being almost identical (Robertson 1984).

Despite the extremely low salinities during flooding, D. brevirostris is always common in the lower 2 to 3 km of the Bushmans River estuary. The aim of this investigation was, therefore, to examine the effect of low salinities on the survival of this hermit crab over a wide range of temperatures in an attempt to determine whether this factor limits the distribution of this species to the lower reaches of estuaries.

Materials and Methods

D. brevirostris was collected from the mouth of the Bushmans River estuary on two separate occasions during the first two weeks in April 1982. To ensure that all crabs were from the same population, animals were collected from one sand spit on the east bank. Although larvae and juveniles may be more susceptible to variations in salinity and temperature and thereby set limits on recruitment, they were not included in this study. The average maximum range in weight of whole crabs removed from their 'hermitages' was determined from 10 large and 10 small crabs. Size ranged from 0,126 to 0,558 g. In the laboratory animals were placed in holding tanks with fresh, filtered, aerated dilute sea water (at 24°/_∞) at ambient temperature (17°C). These values were found to be most representative of average conditions experienced in the field. Animals were fed once every three days on fresh mussels (Perna perna (Linn.)). The fixed salinities were obtained by dilution with de-chlorinated water. Chlorinity values were measured with a chloride titrator, and converted to salinity by the method of Harvey (1955).

Pilot experiments using salinities of 4,8 and 10%, at a constant 17°C resulted in negligible mortality, although at salinities below 4%, significant mortalities occurred. As the lowest winter temperature in this estuary is 8°C and the highest summer temperature 30°C, temperatures of 12, 17, 22, and 27°C were used

in this investigation. The effect of these temperatures on survival was investigated at salinities of 0.5; 1.0; 1.5 and $4.0^{\circ}/_{\infty}$, which are known to occur during flooding.

For each of the four temperature treatments, 50 crabs were chosen randomly from the original stock following the method of Allanson & Noble (1964). Each group of 50 animals was transferred to a Gallenkamp water bath in which the water was kept at ambient temperature (17°C) and a salinity of $24^{\circ}/_{\infty}$. Over a period of three days, the temperature in each tank was slowly increased or decreased to the required value, (viz. 12, 17, 22 or 27°C) after which crabs were left for a further two days to acclimatize. On the third day, stock sea water was diluted to the required salinities, and placed in 0.3 1 plastic containers. Two containers, housing five animals each, were used for each of the 16 chosen salinity temperature combinations (4 salinities × 4 temperatures). Thus, in total, 160 hermit crabs were tested during the experiment. A further 10 crabs were maintained at 24% at each of the four experimental temperatures to act as controls. Water in the open containers was 30 mm deep, and both temperature and water level was checked daily, the latter being altered with water of the correct salinity if required. Mortality was monitored daily, and was easily determined as in most cases the crab had left its shell and was found floating on the surface. However, the state of all crabs was checked by prodding one of the chelae and noting any retraction, and observing crabs for 5 to 10 min to see if any movement occurred. Monitoring was terminated after seven days.

The cummulative mortality at day 5 for each salinity and temperature combination was converted to empirical probits by means of a transformation table (Finney 1971). Values at day 5 were used as in most cases no further deaths occurred after this period. These probits of mortality were then plotted against salinity for each temperature by calculating the line of best fit. An estimate of the point at which there was a 50% mortality (LD₅₀) was thus obtained when probit mortality equalled five. To test whether the experimental data were reasonably approximated by the probit lines, a chisquared test was carried out on the values (Finney 1971).

Results

Cumulative percentage mortalities at the four salinities and four temperatures used in the investigation are depicted graphically in Figure 1. It is clear that mortality was inversely related to salinity, with highest cumulative mortalities recorded at $0.5^{\circ}/_{\infty}$, except for the first three days at 17° C where highest mortality was at $1.0^{\circ}/_{\infty}$. The thermal effect was less pronounced, but at $0.5^{\circ}/_{\infty}$ mortality rises with increasing temperature, with 7 day cumulative values of 70, 90, 90 and 100% respectively at 12, 17, 22 and 27°C.

This species of hermit crab appears therefore to be tolerant of salinities of 1,5 and $4,0^{\circ}/_{\infty}$, but intolerant of lower salinities of 1,0 and $0,5^{\circ}/_{\infty}$. To exemplify this trend more clearly, a graph of probit mortality at day five versus salinity is given in Figure 2. Values of

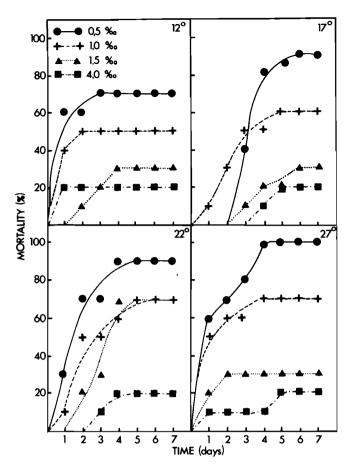


Figure 1 Cumulative percentage mortality at four different temperatures and salinities.

percentage mortality at day five were used because in all cases except at 0.5 and $1.5^{\circ}/_{\infty}$ at 17° C there were no further deaths after the fifth day (see Figure 1). Low salinity tolerance was reduced at higher temperatures, but at higher salinities, temperature had no effect (Figure 2). The slope of the regression equation describing probit mortality as a function of increase in salinity, which rises with temperature (Table 1) reflects this effect. At lower salinities tolerance is inversely related to temperature. At 17 and 27°C this relationship is not significant ($\chi^2 = 4.178$ and 34,3 respectively), whereas at 12 and 22°C it is significant ($\chi^2 = 1.926$; P < 0.05 and 0.745; P < 0.05; see Table 1). Therefore, at higher temperatures, hermit crabs become more stenohaline.

To demonstrate this interaction between temperature and salinity, the relationship between temperature and probit mortality was examined (Figure 3). At salinities of 0.5 and $1.0^{\circ}/_{\infty}$ temperature has a marked influence on mortality, which increases linearly with temperature. At both these salinities, the relationship is significant ($\chi^2 = 2.05$; P < 0.05 and $\chi^2 = 0.11$; P < 0.01 respectively). At a salinity of $1.5^{\circ}/_{\infty}$, temperature appears to have no effect on survival, but values were not significant ($\chi^2 = 6.44$). However, at a salinity of $4^{\circ}/_{\infty}$ temperature had no significant effect on survival ($\chi^2 = 0.00$; P > 0.001).

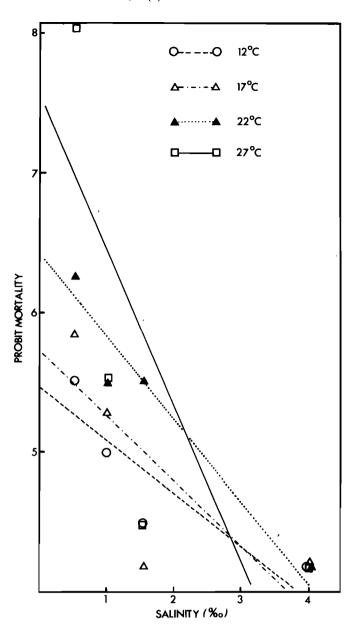


Figure 2 The effect of salinity on probit mortality at Day 5 at four different temperatures.

Discussion

The results of this study show that there is a direct relationship between temperature and salinity on the survival of *Diogenes brevirostris*. Tolerance to low salinity is greater at lower temperatures than at higher temperatures.

It is remarkable that these crabs can tolerate a salinity as low as $0.5^{\circ}/_{\infty}$, with animals still surviving after seven days. A full six days were required for all animals to succumb in fresh water, at a salinity of $0.35^{\circ}/_{\infty}$, and at $4^{\circ}/_{\infty}$ the maximum mortality was only 20%. Wide fluctuations in temperature and salinity occur in estuaries and many euryhaline marine animals are tolerant to low salinity at a range of temperatures (Rothlisberg 1979; Aziz & Greenwood 1981; Robertson 1984).

The tolerance of this hermit crab may be related to the nature of estuaries in the Eastern Cape. Generally

Table 1 Least squares regression equations describing probit cumulative mortality as functions of temperature (upper) and salinity (lower)

Temperature °C	LD ₅₀				
(Figure 2)	Regression	(°/∞)	x ²	p	
12	Y = 5,481 - 0,388X	1,24	1,926	0,05	
17	Y = 5,684 - 0,472X	1,45	4,178	•	
22	Y = 6,401 - 0,596X	2,35	0,745	0,03	
27	Y = 7,513 - 1,110X	2,26	34,300	•	

Salinity °/∞ (Figure 3)		LD ₅₀ (°C)		
0,5	Y = 3,152 + 0,165X	11,2	2,05	0,05
1,0	Y = 4,630 + 0,035X	10,3	0,11	0,01
1,5	Y = 4,660 + 0,000X	_	6,44	•
4,0	Y = 4,160 + 0,000X	_	0,00	0,001

* = Not significant

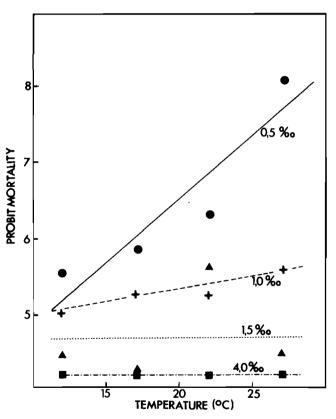


Figure 3 The effect of temperature on probit mortality at Day 5 at four salinities.

salinity gradients along estuaries are a great deal steeper and absolute salinities lower than in the marine environment. However, this variability is usually restricted to defined extremes. In the Eastern Cape 30 to 50% of the annual rainfall may fall over a period of a few days (Macrae 1957) and the resulting floods cause a

sudden and marked decrease in salinity, below the defined extreme values usually experienced. Such an episode was recorded in the Bushmans River in July 1983, when surface salinities close to the mouth on the outgoing tide declined from 34 to $0.35^{\circ}/_{\infty}$ two days after the rains. It took over four weeks before surface salinities in the estuary returned to normal (Robertson 1984). Thus, species inhabiting such estuaries require an osmoregulatory mechanism to ensure survival under these conditions, and this mechanism has been shown to exist in other Crustacea (Hill 1971; Forbes 1974; Roddie et al. 1984) but its existence in D. brevirostris is uncertain.

Robertson (1984) showed that large semidiurnal temperature fluctuations occur in the Bushmans River estuary, owing to its tidal nature and shallowness at low tides. She found that on an outgoing tide in summer, temperatures rise from 17 to 28°C in a few hours, and that these high temperatures may persist for several hours. The incoming tide causes a sudden drop in temperature by as much as 10°C in only 2 to 3 h. In winter water temperatures may rise from 12 to 24°C during one day on an outgoing tide. Hill & Allanson (1971) made similar observations for the Kowie estuary, 29 km east of the Bushmans River. Therefore, a tolerance by estuarine species to high temperatures is advantageous, and has been demonstrated for the estuarine mud prawn, Upogebia africana (Ortmann) (Hill & Allanson 1971).

Thermal influences on feeding, duration of emergence and movement were reported for the crab Scylla serrata (Forskal) by Hill (1980), but these crabs differed in being more sensitive at lower temperatures. Robertson (1984) found a similar trend for the shrimp, Palaemon pacificus (Stimpson), a species which undertakes part of its life cycle in estuaries. She showed that sub-adults tolerated salinities of 1,0 to 79°/ $_{\infty}$ and post-larvae 2 to 60°/ $_{\infty}$ for six days at ambient temperature. Temperatures of 10 to 20°C reduced the low salinity tolerance of post-larvae, but sub-adults were only affected at 30°C.

From the present study, it appears that the effect of extreme levels of salinity and/or temperature in the estuary may not limit the distribution of hermit crabs. Because of their broad tolerance they should be able to survive near the head of the estuary. However, this continuous exposure to low salinity may be intolerable and normal moult limit growth metamorphosis, so although there may not be a catastrophic population crash the population will steadily decline. Other biological or environmental factor/s may also be responsible for limiting their distribution to the lower reaches of the estuary.

Survival on a detritus-based substratum may not be possible as the finer material may clog their filter-feeding mechanism. Nevertheless hermit crabs occur in areas where the substratum has a component of mud, such as the head of the Langebaan Lagoon. Distribution may also be related to reproduction, as larval or juvenile stages may be more sensitive. There is also a possibility that hermit crabs retain water of the original preferred salinity in their body cavities or in their shells. Also,

burrowing into the sand may alter the salinities which they are exposed to during extreme (flood) conditions.

Finally, as these hermit crabs inhabit empty gastropod shells, the availability of shells could also limit their distribution. Emmerson & Alexander (1986) demonstrated that shell availability was important for shell selection by *Diogenes brevirostris* and that most shells were selected from intertidal species. As gastropod zonation and redistribution of empty shells by water also affects shell availability, it is possible that fewer shells are available higher up an estuary.

The results of this study have shown that the estuarine hermit crab, *Diogenes brevirostris*, is extremely tolerant of low salinities, even at high temperatures, over a short period of time. These two environmental factors are probably not solely responsible for limiting their distribution to the lower reaches of the estuary. It is therefore suggested that other factors, as yet not elucidated, may be important in this regard.

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