

Salinity tolerance and osmoregulation in several subtropical decapods

By

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Declaration

I hereby declare that this study contains original work and has not been submitted for any degree at another university. The work of others used in this study has been acknowledged in the text.

SN Khanyile

2012

Dedicated to

My family:

In loving memory of my parents (Nomvula MaMgaga Khanyile and Lokothwayo Khanyile). I thank the Mgaga family as the whole, my sisters Shongishilo, Nokuthula and my brother Mbekezeli Sfiso Khanyile. Special thanks to my cousin Soka Caiphas Mgaga and his family, I would not be here if it was not for you. I would like to thank my husband (Vusi) and my daughter (Wenziwe) for their support. Lastly to my grandmother MaShoba Ntombi kaMpikela thank you.

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Abstract

This study investigated salinity tolerances and osmoregulatory strategies of several subtropical brachyuran mangrove crabs and an anomuran prawn, with particular reference to *Uca vocans*, *Uca urvillei*, *Uca chlorophthalmus*, *Uca annulipes*, *Dotilla fenestrata*, *Macrophthalmus depressus*, *Macrophthalmus grandidieri*, *Metopograpsus thukuhar*, *Chiromantes eulimene* and *Callianassa kraussi*. All species investigated were either directly exposed or acclimated to salinities between 0-75 and their tolerance to these salinities and osmoregulatory strategies monitored over a 4 day period. Other experiments conducted included an investigation of the time dependant responses of species following direct transfer to various salinities, and for one species also the influence of temperature on salinity tolerance and osmoregulatory strategy. All the species were shown to be euryhaline, as would be expected for species inhabiting an estuarine environment. However, the degree of euryhalinity varied between species. The general salinity range they could tolerate was between 0-55, but species like *U. annulipes*, *D. fenestrata*, *C. eulimene* and *C. kraussi* tolerated salinity as high as 65. Direct exposure was shown to be more stressful than acclimation, especially in low and high salinities. Out of seven species that were directly exposed and acclimated, *C. eulimene* was the only species able to tolerate freshwater (salinity 0) following direct exposure. All crab species followed an osmoregulation strategy by hyper-regulating at low salinities and hypo-regulating at higher salinities. The hyper-regulatory ability of most species was stronger than the hypo-regulatory ability, as this was shown by the hemolymph osmolality line which was much closer to the isosmotic line at salinities above the isosmotic point and also by the lower osmotic capacity (OC) at comparable salinity differences below and above the isosmotic point. *Callianassa kraussi* osmoregulated at salinities lower than 25 and osmoconformed at salinities above 25.

All *Uca* species investigated were able to tolerate direct transfer to freshwater for up to eight hours without experiencing any mortality. All specimens of *U. vocans*, which occurs lowest in the intertidal zone, died within 24 hours of exposure. All *U. urvillei* died within two days of exposure. *Uca annulipes*, which lives in the highest region of the intertidal zone, was the most tolerant to rapid freshwater exposure, with 70% of crabs surviving up to 72 hours. *Uca annulipes* regulated its hemolymph osmolality more efficiently than *U. urvillei* and *U. vocans*, which live on the lower level of the intertidal zone. The ability of *Uca* crabs to survive as well as regulate their hemolymph osmolality when directly transferred to freshwater was closely linked to the level they occupy in the intertidal zone.

The third part of this study looked at the influence of temperature on the salinity tolerance and osmoregulation of *C. eulimene*. Temperatures between 14-22°C had no effect on salinity tolerance or osmoregulatory capability of *C. eulimene* at salinities between 0-45. Exposure of *C. eulimene* to lowered temperatures had no effect on the salinity tolerance and osmoregulation capacity of this species. Lower temperatures do not inhibit the distribution of this species from South and West Coast of Africa.

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Chapter 1

General Introduction

1.1. General Introduction

Estuaries usually have a gradient of salinity along their length, ranging from seawater at the mouth to near freshwater at the head. Salinity is one of the primary factors affecting the distribution of organisms in estuaries (Kennish 1986), and the distribution of many animals in estuaries can be correlated with salinity gradients (Branch & Branch 1981; Day 1981). At any specific location within an estuary there are short-term changes in salinity associated with tidal action, and longer-term changes associated with seasonal changes in rainfall. These changes are in turn superimposed on changes brought about by episodic events, such as floods and droughts. Inhabitants of the intertidal are also exposed to other changes, since high salinities can develop in mangrove sediments through evaporation during low tide and may be followed by sudden dilution to near freshwater levels following heavy rainfall. Organisms that live permanently in estuaries must be able to tolerate and cope with these changes in salinity. They do this through a combination of physiological and behavioural means.

Osmoregulation is the process by which organisms regulate their osmotic balance, maintaining an equal proportion of solutes (dissolved substances) both inside and outside of the cells (in interstitial fluid) in order to function properly. All organisms must carefully regulate the volume and chemical composition of fluid in their internal environment (Branch & Branch 1981; Mantel & Farmer 1983; Thurman 2003). Cells cannot afford to either lose water, or gain excess water. Another major requirement for cell survival is the presence, in appropriate concentrations, of various solutes (e.g. salts) in the extracellular and intracellular compartments. A number of mechanisms are used by organisms to handle osmotic (water and solute) problems and to regulate the differences between intracellular and extracellular compartments and the external environment. These are collectively referred to as osmoregulatory strategies.

Invertebrates living in the open sea are seldom exposed to osmotic fluctuations because the ocean is a highly stable environment (Warner 1977). Thus, the amount of salt in the oceans waters is always constant, at about 35 grams per litre (the amount of salt in water is referred to as the salinity - the salinity of seawater is 35). Because of this stable environment, oceanic invertebrates have very limited abilities to withstand osmotic changes. If they are exposed to diluted seawater, most die quickly because their cells cannot tolerate dilution and cannot prevent it. These animals are thus restricted to living in a narrow salinity range and are said to be stenohaline. Most marine invertebrates are in isosmotic equilibrium with their seawater environment, that is, their body fluids have a similar solute concentration to that of the surrounding seawater (Robertson 1960). Because of this similarity in concentration, water neither flows into or out of their bodies by osmosis. These animals have body surfaces that are permeable to salts and to water, and their body fluid concentration rises or falls in conformity with changes in the concentration of the surrounding seawater (Lockwood 1962).

Because such animals are not able to regulate their body fluid osmotic pressure as the osmotic pressure of the surrounding water changes, they are referred to as osmotic conformers (osmoconformers).

Invertebrates living in freshwater environments are generally also stenohaline, and most are unable to tolerate even a small increase in the solute concentration of the surrounding medium. Freshwater invertebrates however maintain their body fluid concentrations above that of the surrounding medium. To do so they must actively expel excess water entering through osmosis into their bodies, by excreting large quantities of dilute urine. Even though this urine is dilute, the urine is isosmotic to the body fluids and large amounts of important ions are lost. Ions must be replaced by active uptake of salts from the surrounding medium by special cells, the ionocytes, usually situated in the gill epithelia but often also in the branchial chamber epithelium. Because these salts are being moved against a concentration gradient, these animals expend a considerable amount of energy on maintaining body fluid homeostasis (Péqueux 1995).

Conditions in estuaries and intertidal environments are very different compared with those in freshwater and marine environments. Here, as previously stated, animals must be able to withstand the large and often abrupt changes in salinity that is characteristic of the estuarine environment. Not surprisingly, most permanent estuarine residents are able to withstand wide ranges of salinity, that is, they are euryhaline. Most are also osmoregulators, usually maintaining their body fluid concentrations very different to those of the external medium.

Estuarine intertidal crabs are faced with two major problems, fluctuating temperatures and salinities (Bliss 1968, Day 1981). *Ocypodid* and *Sesarmid* crabs that are found high up the intertidal zone tend to be more physiologically adapted to cope with stress posed by combinations of increase or decrease in temperature and salinity (Macintosh 1988). Sasekumar (1974) recorded salinity extremes of 3.5 to 47.6 for sediment water, a minimum salinity being measured after a rainy day and a maximum salinity measured after several days of low tide without rainfall.

1.2. Objectives of this research

The overall objective of this study was to evaluate the salinity tolerances and osmoregulatory strategies of several subtropical brachyuran crabs and an anomuran prawn, in order to understand how they are physiologically adapted to the estuarine and mangrove environments in which they live.

1.3. Relevance of this study

Although the salinity tolerances and osmoregulatory strategies of several brachyuran crabs and anomuran prawns from temperate South African waters have been investigated, there is little information in this regard on subtropical crabs (findings of these studies are discussed in chapter 2). In South African estuarine species a salinity tolerance range of 5 – 55 is considered non-lethal (de Villiers & Hodgson 1999). The large

swimming crab *Scylla serrata* has a salinity tolerance range from 2 - 89 (Hill 1979). Similar results on *Scylla serrata* has been published from Australia (Heasman & Fielder 1983, Hyland *et al* 1984, Heasman *et al* 1985). *Upogebia africana*, which is found in the intertidal mud banks of estuaries, can penetrate upper reaches of an estuary where salinities are as low as 1.7 and it could survive moulting in salinities down to 3.4 (Hill 1971, 1981). *Upogebia africana* has greater osmoregulatory ability and is more tolerant of low salinity than *U. capensis*, a marine species (Hill 1971). The sand prawn *Callinassa kraussi* is very tolerant of low salinity, having being found in salinities down to 1 (Forbes 1974). Bolt & Heeg (1975) found that *Cyclograpsus punctatus* was less tolerant to lower salinities, tolerating salinities from 20 and above. *Parasesarma catenata* and *Chiromantes eulimime* were more tolerant to low salinities, occurring in salinities of 7 and 2.7, respectively (Bolt & Heeg 1975). The osmoregulatory abilities of these three grapsoid crabs correspond to their salinity tolerance, with *C. eulimene* regulating strongly and *C. punctatu* regulating less than the other two crabs. South African studies on salinity tolerance to date only focused on the ability of these species to tolerate salinities less than 35. This lack of information forms an important motivation for this study. This study provides information on the salinity tolerance ranges of the various crabs and prawns that are investigated, as well as the underlying osmoregulatory mechanisms that these crustaceans follow.

An understanding of the salinity tolerances and preferences of estuarine organisms is important from a South African perspective, since many estuaries along the South African coastline close off periodically from the sea. This will disturb the distribution of organisms within an estuary, because each species can tolerate a particular range of salinity. In response to these estuaries that periodically close off from the sea, the Department of Water Affairs and Forestry has implemented Estuarine Flow Requirement studies to determine how much freshwater must flow into an estuary in order to preserve the fauna and flora in that estuary, based partly on the physiological tolerances of the fauna and flora. Very little information is however available on the salinity tolerances and preferences of most estuarine organisms in South Africa. This study addresses this lack of information, and provides data on which estuarine management decisions can be based.

1.4. General ecology and biology of species investigated in this study

Crabs are the most abundant of the mangrove macro-fauna and are a valuable asset to the mangrove ecosystem, forming an important link between primary producers and predators. The removal and processing of mangrove leaves by crabs helps to trap energy stored in the leaves within the mangal before the tide can carry them away (Gillikin 2004). They aerate the sediment by burrowing (Micheli *et al.* 1991; Ridd 1996), modify topography, and distribute the sediment according to grain size (Warren & Underwood 1986). They create microhabitats for other fauna (Gillikin *et al.* 2001), contribute to secondary production (Lee 1997), and increase the amount of nutrients and decrease the sulphide and ammonium concentrations in the sediment (Smith *et al.* 1991). Crab larvae are the major source of food for juvenile fish inhabiting estuarine and adjacent nearshore waters. Crabs are also a food source for many bird species and other animals inhabiting mangroves (Seys *et al.* 1995; Zimmerman *et al.* 1996).

Nine brachyuran crabs and one anomuran prawn were investigated in this study (Table 1.).

Table 1. Taxonomy, habitat preference and collection sites (CS) of crab species and one anomuran prawn that were investigated in this study (see) Figure 2.1 for location of sites and Plate 1 for photos of different species used in this study).

Family	Species	Habitat	Collection site
Ocypodidae	<i>Uca vocans</i>	Estuarine / intertidal	CS 7
Ocypodidae	<i>Uca urvillei</i>	Estuarine / intertidal	CS 9
Ocypodidae	<i>Uca chlorophthalmus</i>	Estuarine / intertidal	CS 8
Ocypodidae	<i>Uca annulipes</i>	Estuarine / intertidal	CS 3
Ocypodidae	<i>Dotilla fenestrata</i>	Estuarine / intertidal	CS 5
Ocypodidae	<i>Macrophthalmus depressus</i>	Estuarine / intertidal	CS 4
Ocypodidae	<i>Macrophthalmus grandidieri</i>	Estuarine / intertidal	CS 4
Grapsidae	<i>Metopograpsus thukuhar</i>	Estuarine / intertidal	CS 2
Grapsidae	<i>Chiromantes eulimene</i>	Estuarine / intertidal	CS 1
Thalassidea	<i>Callianassa kraussi</i>	Estuarine / intertidal and subtidal	CS 6

Brachyuran crabs associated with mangroves are highly diverse with an estimated 275 species from six families worldwide (Warren 1977; Lee 1998; Gillikin 2000). Most mangrove crabs are fiddler crabs (Family: Ocypodidae, genus *Uca*) or sesarimid crabs (Family: Grapsidae, subfamily Sesarminae) (Hartnoll *et al.* 2002). Fiddler crabs are widely distributed throughout the tropics and subtropics. Currently, there are 97 recognized species or subspecies of fiddler crabs worldwide (Rosenberg 2001). The west coast of tropical America (eastern Pacific) is by far the richest area in number of species. The eastern coast of Africa is poor in number of species compared with tropical America, having only 6 species (*Uca annulipes* (H. Milne Edwards), *Uca chlorophthalmus* (H. Milne Edwards), *Uca inversa* (Hoffman), *Uca vocans* (Linnaeus), *Uca tetragonon* (Herbst) and *Uca urvillei* (H. Milne Edwards) (Hartnoll 1975; Skov & Hartnoll 2002).

Almost all fiddler crabs inhabit different zones with different sediment types in the intertidal of sheltered bays and estuaries, creating burrows up to 60 cm deep in the muddy substrate to which they retreat during high tides (Macnae 1968; Icely & Jones 1978; Gillikin 2000). Macnae (1968) noted that a change in sediment often shows a change in fauna. Gillikin (2000) found that percentage organic matter was positively correlated with species richness, but species like *U. annulipes* and *U. inversa* preferred areas of low organic content (higher level of the intertidal zone), this may be reason to escape competition for food space or even predation. All *Uca* species are surface deposit feeders; they rely for food on organic matter, either fine or coarse, associated with sediment surface (Ólafsson & Ndaro 1997; Skov & Hartnoll 2001; Skov *et al.* 2002). Therefore, the sediment type can be expected to be an important factor governing their abundance and distribution and mouthparts of *Uca* species have been shown to be closely linked to the habitats in which these crabs occur (Miller 1961; Icely & Jones 1978). Fiddler crabs feed by scooping small portions of sediment with the minor cheliped, which is then transferred to the mouth where it is sifted for algae, microbes, fungus, or other decaying detritus. The sediment is then deposited as a small ball. The action of feeding fiddler crabs is thought to play a vital role in the preservation of wetland environments (Smith *et al.* 1991); by sifting through the sands, they aerate the substrate and prevent anaerobic conditions.

There are 166 species of Grapsidae that are associated with mangrove systems worldwide (Warren 1977; Lee 1998). Grapsid crabs, especially the family Sesarmidae, are reported as being species rich (Warren 1977; Lee 1998). They are frequent inhabitants of semi-terrestrial habitats between the marine intertidal and adjacent freshwater and terrestrial zones. Brachyuran crabs are one of the most important taxa with regard to number of species, density and biomass, in mangrove forests (Lee 1998; Hartnoll *et al.* 2002). Grapsid crabs are widely distributed in African estuaries and mangroves. East Africa has a limited number of brachyuran crabs associated with mangroves. Hartnoll (1975) listed 35 species compared with South East Asia with Malaysia alone having more than 100 species (Tan & Ng 1994). The primary food source for grapsid is believed to be leaf litter (*e.g.* Robertson & Daniel 1989; Robertson 1991), supplemented with algae and animal matter (Leh & Sasekumar, 1985; Cannicci *et al.* 1999; Dahdouh-Guebas *et al.* 1999).

Uca vocans (Linnaeus) (Plate 1.) is usually found in exposed habitats with little mangrove cover (Icely & Jones 1978). It has a preference for the lower region on the intertidal zone, extending up only as far as the mean low water neap level (MLWN), where it overlaps with *U. annulipes*. *Uca vocans* is found in muddy sediment with high organic content. The geographic distribution of *U. vocans* extends from the Red Sea to Tanzania, Zanzibar, Madagascar, Indonesia and the central Pacific (Vannini & Valmori 1981).

Uca urvillei (H. Milne Edwards) (Plate 2.) inhabits a slightly higher region of the intertidal zone which often overlaps with *U. vocans*, but may also extend quite far up the intertidal zone along the banks of creeks draining mangroves, provided that the sediment is of a suitable nature and retains a high moisture and organic content (Macnae 1963; Hartnoll 1975 Vannini & Valmori 1981; Ruwa 1997; Gillikin 2000). The geographic distribution of *U. urvillei* extends from KwaZulu-Natal to Somalia, Madagascar, the coast of Pakistan and western India (Vannini & Valmori 1981).

Uca chlorophthalmus (H. Milne Edwards) (Plate 3.) inhabits the middle region of the intertidal zone and is restricted to regions where mangroves dominate and which are regularly covered with water at high tide. It is said to be the least heat tolerant, therefore inhabits shaded areas. *Uca chlorophthalmus* is found usually in mud or muddy and with a high organic content (Macnae 1963; Icely & Jones 1978; Gillikin 2000). The geographic distribution of *U. chlorophthalmus* is from Port Elizabeth to Somalia; Mauritius and Madagascar (Vannini & Valmori 1981).

Uca annulipes (H. Milne Edwards) (Plate 4.) is the most common of all *Uca* species, forming dense aggregations on open sand flats in estuaries and lagoons. This species occupies the highest region on the intertidal zone, reaching high densities in the drier landward part (Hartnoll 1975). It also extends into areas with strong freshwater influence (Hartnoll 1975; Icely & Jones 1978). It inhabits sandy areas with a larger particle size than is found in areas occupied by other species and can survive in sediment with low organic content (Gillikin 2000). The geographic distribution of *U. annulipes* is from South Africa to Madagascar, Thailand, Indonesia, Malaysia, Philippines (Vannini & Valmori 1981).

Dotilla fenestrata (Hilgendorf) (Plate 5.) (commonly known as the soldier crab) is the only east African representative of the Scopimerinae (Brachyura: Ocypodidae). It occurs mainly between MLWN and mean high water neap (MHWN), where the sediment surface is well drained (Hartnoll 1975), but can occasionally also be found among the lowermost zone mangrove swamps (Macnae 1963). It lives in dense aggregations on sheltered sandbanks, burrowing shallowly and emerging in countless numbers to feed during low tide, sucking organic material from the sediment and depositing tiny pellets of processed sand. It is also a detritus feeder feeding on microalgae on sand flats adjacent to mangroves (Hartnoll 1975). The geographic distribution of *D. fenestrata* is from South Africa to Somalia, Madagascar and the Comore Islands (Vannini & Valmori 1981).

Macrophthalmus depressus (Rüppell) (Plate 6.) and *M. grandidieri* (Milne Edwards) inhabit muddy sand and mud from about mean low tide (MLT) down to MLWN, where the surface does not drain completely. These species inhabit the mid tide level (Gove & Paula 2000) and prefer sediment with high organic matter. It is distributed throughout the West Indian Ocean (Richmond 1997).

Metopograpsus thukuhar (Owen) (Plate 7.) is very common in Indo-Pacific mangroves (Macnae 1968; Gillikin 2000) but in South Africa also inhabits rocky areas in estuaries. It does not burrow, but may exploit burrows of other species as temporary refuge (Vannini *et al.* 1997; Fratini *et al.* 2000). *Metopograpsus thukuhar* occurs on the lower level of the intertidal zone (Icely & Jones 1978; Ruwa 1997). The diet of this species is less dependent on leaves. It mainly feeds on macro-algae (Dahdouh-Guebas *et al.* 1999). Its geographic range extends from Japan, Tahiti, Hawaii, the Red Sea to South Africa (Vannini & Valmori 1981).

Chiromantes eulimene (De Man) (Plate 8.) (previously known as *Sesarma eulimene*) is an intertidal crab found in estuaries and mangroves along the east coast of Africa from Kenya to South Africa. *Chiromantes eulimene* is found up to the highest level of the intertidal zone, and also extends into areas where there is a strong freshwater influence. It does not burrow but uses burrows of other crab species (Macnae 1963; Hartnoll 1975; Gillikin 2004). It prefers muddy areas of saltmarshes and mangroves. This species is omnivorous, feeding on both leaf litter and animal matter (Gillikin 2000). Its geographic distribution is from South Africa to Somalia, Tanzania and Madagascar (Vannini & Valmori 1981).

Callianassa kraussi (Stebbing) (Plate 9.) (commonly known as the sand prawn) occurs around the southern African coast from Lamberts Bay on the west coast Day (1959) to San Martinho in Mozambique on the east coast (Forbes 1973). It is able to burrow in a variety of substrates including mud, muddy sand, coarse gravel, and even clay, but has a strong preference for sandy substrates (Wooldridge 1968). *Callianassa kraussi* is typically found on sandflats and sandbanks of lagoons, sheltered bays and estuaries that may be open or temporarily closed from the sea (McLachlan & Grindley 1974, Day 1981). It is generally found to be absent from areas with strong water currents and high sediment transport (Forbes 1973). *Callianassa kraussi* is a

low shore species, which dominates the bottom and most of the subtidal areas of the lower reaches of the estuarine intertidal. It builds deep burrows and sifts the sediment for food, removing particles from the burrow entrance to create miniature ‘volcanoes’, oxygenating and turning over large volumes of sediment (Branch & Branch 1981). It profoundly affects other organisms, promoting bacteria but burying diatoms and reducing the meiofauna. It is a subsurface feeder, feeding on small organic particles that it sifts from the sands (Branch & Branch 1981).

This dissertation consists of five chapters, each of which has been written in the form of scientific papers, as a result some issues have been repeated. Chapter 1 provides the general introduction to this research as well as the description of the general ecology of the decapods that were investigated. Chapter 2 investigated salinity tolerances and osmoregulatory strategies of several brachyuran crabs and an anomuran prawn, in order to understand how they are adapted physiologically to the estuarine environment. Chapter 3 investigates salinity tolerance and hemolymph osmotic responses in several crabs following osmotic shock. Chapter 4 investigates the influence of low temperature on salinity tolerance and osmoregulation of the crab *Chiromantes eulimene*. Chapter 5 summarises the findings of all chapters, and gives directions for further research.

Plate 1. Photos showing different species of crabs and prawns used in this study





Plate 1. *Uca vocans*

Plate 2. *Uca urvillei*

Plate 3. *Uca chlorophthalmus*

Plate 4. *Uca annulipes*

Plate 5. *Dotilla fenestrata*

Plate 6. *Macrophthalmus depressus*

Plate 7. *Metopograpsus thukuhar*

Plate 8. *Chiromantes eulimene*

Plate 9. *Callinassa kraussi*

(Photos from 'A Field guide to Kenyan mangroves', Chris Lukhaup and Stefano Cannicci).

1.5. References

- BLISS, D.E. 1968. Transition from water to landing decapod crustaceans. *Americana Zoologica* **8**: 355 – 392.
- BRANCH, G. & BRANCH M. 1981. *The living shore's of Southern Africa*. Struik Publishers, Cape Town.
- CANNICCI, S., FRATINI, S. & VANNINI, M. 1999. Use of time, space and food resources in the mangrove climbing crab *Selatium elongatum* (Grapsidae: Sesarminae). *Marine Biology* **135**: 335-339.
- CRANE, J. 1975. *Fiddler crabs of the world: Ocypodidae: Genus: Uca*. Princeton University Press, New Jersey.
- DAHDOUH-GUEBAS, F., GIUGGIOLI, M., OLUOCH, A., VANNINI, M. & CANNICCI, S. 1999. Feeding habits of non-ocypodid crabs from two mangrove forests in Kenya. *Bulletin of Marine Science* **64**: 191-297.
- DAY, J.H. 1981. The estuarine fauna. In: *Estuarine Ecology with particular reference to southern Africa*, (ed) J.H. Day, Ch 9. A A Balkema, Cape Town.
- DE VILLIERS, C.J. & HODGSON, A. 1999. Studies on estuarine macroinvertebrates - The macrobenthos. In: *Estuaries of South Africa* (eds) B.R. Allanson & A.D. Baird, Ch. 8. Cambridge University Press, UK.
- FORBES, A.T. 1973. An unusual abbreviated larval life in the estuarine burrowing prawn *Callinassa kraussi* (Crustacea: Decapoda; Thalassinidea). *Marine Biology* **22**:361-365.
- FRATINI, S., CANNICCI, S. & VANNINI, M. 2000. Competition and interaction between *Neosarmatium smithi* (Crustacea: Grapsidae) and *Terebralia palustris* (Mollusca: Gastropoda) in a Kenyan mangrove. *Marine Biology*. **137**: 309-316.
- GOVE, D. & PAULA, J. 2000. Rhythmicity of larval release in three species of intertidal brachyuran crabs (Crustacea: Brachyura) from Inhaca Island (Mozambique). *Marine Biology* **136**: 685 – 691.
- GILLIKIN, D.P. 2000. Factors controlling the distribution of Kenyan brachyuran mangrove crabs: salinity tolerance and ecophysiology of two Kenyan *Neosarmatium* specie. M.Sc. thesis, Vrije Universiteit Brussel, Brussels.
- GILLIKIN, D.P., DE GRAVE, S. & TACK, J.F. 2001. The occurrence of the semiterrestrial shrimp *Merguia oligodon* (De Man, 1888) in *Neosarmatium smithi* (H. Milne Edwards, 1853) burrows in Kenyan mangrove. *Crustaceana* **74**: 505-508.
- GILLIKIN, D.P. 2004. Osmoregulatory ability of *Chiromantes ortmanni* (Crosnier, 1965) subjected to dilute and hypersaline seawater. *Crustaceana* **77**:67-74.
- HARTNOLL, R.G. 1975. The grapsidae and ocypodidae (Decapoda: Brachyura) of Tanzania. *Journal Zoology of London* **177**: 305-328.
- HARTNOLL, R.G., CANNICCI, S., EMMERSON, W.D., FRATINI, S., MACIA, A., MGAYA, Y., PORRI, F., RUWA, R.K., SHUNILA, J.P., SKOV, M.W. & VANNINI, M. 2002. Graphic trends in mangrove crab abundance in East Africa. *Wetlands Ecology and Management* **10**: 203-213.
- * HEASMAN, M.P. & FIELDER, D.R. 1983. Laboratory spawning and mass rearing of the mangrove crab, *Scylla serrata* (Forsk.) from first zoea to first crab stage. *Aquaculture* **34**: 303 – 316.
-

-
- * HEASMAN, M.P., FIELDER, D.R. & SHEPHERD, R.K. 1985. Mating and spawning in the mud crab, *Scylla serrata* (Forsk.) (Decapoda: Portunidae) in Morton Bay, Queensland. *Australian Journal of Marine and Freshwater Research* **36**:773 – 783.
- HILL, B.J. 1971. Osmoregulation by an estuarine and marine species of *Upogebia* (Anomura, Crustacea). *Zoologica Africana* **6**: 229 – 236.
- HILL, B.J. 1979. Biology of the crab *Scylla serrata* (Forsk.) in the St Lucia system. *Transitional Royal Society of South Africa* **44**: 55 – 62.
- HILL, B.J. 1981. Adaptation to temperature and salinity stress in southern African Estuaries. In: *Estuarine Ecology with particular reference to Southern Africa*, (ed) J.H. Day, Ch 11. A A Balkema, Cape Town.
- * HYLAND, S.J, HILL, B.J. & LEE, C.P. 1984. Movement within and between different habitats by the portunid crab *Scylla serrata*. *Journal of Marine Biology*, Berlin 80: 57 – 61.
- ICELY, J.D. & JONES, D.A. 1978. Factors affecting the distribution of the genus *Uca* (Crustacea: Ocypodidae) on an East African shore. *Estuarine and Coastal Marine Science* **6**: 315-325.
- KENNISH, M. J. 1986. *Ecology of estuaries. (Physical and Chemical Aspects)*. CRC Press. Boca Rator, Florida.
- LEE, S.Y. 1997. Potential trophic importance of the faecal material on the mangrove crab *Sesarma messa*. *Marine Ecology Progress Series* **159**: 275.284.
- LEE, S.Y. 1998. Ecological role of grapsid crabs in mangrove ecosystems: a review. *Marine and Freshwater Research* **49**: 335-343.
- LEH, C.M.U. & SASEKUMAR, A. 1985. The food of Sesarmid crabs in Malaysian mangrove forests. *Malayan Nature Journal* **39**: 135-145.
- LOCKWOOD, A.P.M. 1962. The osmoregulation of crustacea. *Biological Review* **37**: 257-305.
- MACINTOSH, D.J. 1984. Ecology and productivity of Malaysian mangrove crab populations (Decapoda: Brachyura). In Proceedings of the Asian symposium on mangrove environment, research and management: 354 – 377. (Eds Soepadmo, E., Rao, A.N. & M
- MACINTOSH, D.J. 1988. The ecology and physiology of decapods of mangrove swamps. *Symposia. Zoologica Society of London* **59**: 315 – 341.
- MACLACHLAN, A. & GRINDLEY, J.R. 1974. Distribution of soft substrata in Swartkops estuary with observations on the effect of floods. *Zoological Africana* **9**: 211-233.
- MACNAE, W. 1963. Mangrove swamps in South Africa. *Journal of Ecology* **51**: 1-25.
- MACNAE, W. 1968. A general account of the fauna and flora of mangrove swamps and forests in the Indo-West-Pacific Region. *Advanced Marine Biology* **6**: 73-270.
- MANTEL, L.H. & FARMER, L.L. 1983. Osmotic and ionic regulation. In: *The biology of crustacea, Internal Anatomy and Physiological Regulation*, (eds) D.E. Bliss & L.H. Mantel. Ch. 2. Academic Press, New York .
- MICHELI, F., GHERARDI, F. & VANNINI, M. 1991. Feeding and burrowing ecology of two Eastern African crabs. *Marine Biology* **111**: 247-254.
-

- MILLER, D.C. 1961. The feeding mechanism of fiddler crabs, with ecological consideration of feeding adaptations. *Zoologica* **46**: 89-101.
- ÓLAFSSON, E. & NDARO, S.G.M. 1997. Impact of mangrove crabs *Uca annulipes* and *Dotilla fenestrata* on meiobenthos. *Marine Ecology Progress Series* **158**: 225-231.
- PÉQUEUX, A. 1995. Osmotic regulation in Crustaceans. *Journal of Crustacean Biology* **15**: 1-60.
- RICHMOND, M.D. 1997. A guide to the seashores of Eastern Africa and the Western Indian Ocean Islands. Sida / Department for Research Cooperation, SAREC. Zanzibar, Tanzania. 448.
- RIDD, P.V. 1996. Flow through animal burrows in mangrove creeks. *Estuarine, Coastal and Shelf Science* **43**: 617-625.
- ROBERTSON, J.D. 1960. Ionic regulation in the crab *Carcinus maenas* (L.) in relation to the moulting cycle. *Comparative Biochemistry and Physiology*. **1**: 183-212.
- *ROBERTSON, A.I. & DANIEL, P.A. 1989. The influence of crabs on litter processing in high intertidal mangrove forests in tropical Australia. *Oecologia* **78**: 191-198.
- ROBERTSON, A.I., 1991. Plant-animal interactions and the structure and function of mangrove forest ecosystems. *Australian Journal of Ecology* **16**: 433-443.
- ROSENBERG, M.S. 2001. The systematics and taxonomy of fiddler crabs: A phylogeny of the genus *Uca*. *Journal of Crustacean Biology* **21**: 839-869.
- RUWA, R.K. 1997. Zonation of burrowing crabs in the mangroves of the East Coast of Kenya. In: *Mangrove ecosystem studies in Latin America and Africa*, (eds) B. Kjerfve., L.D. de Lacerda. & E.H.S. Diop. UNESCO Technical Papers in Marine Science, Paris, France.
- SASEKUMAR, A.1974. Distribution of macrofauna on Malayan mangrove shore. *Journal of Animal Ecology* **43**: 51 – 69.
- *SEYS, J.G., BOERA, M.P. & NGOA, M. 1995. Distribution and abundance of birds in tidal creeks and estuaries of Kenyan Coast between the Sabaki River and Gazi Bay. *Scopus* **19**: 47-60.
- SKOV, M.W. & HARTNOLL, R.G. 2001. Comparative suitability of binocular observation, burrow counting and excavation for the quantification of the mangrove fiddler crab *Uca annulipes* (H. Milne Edwards). *Hydrobiologia* **449**: 201-212.
- SKOV, M.W. & HARTNOLL, R.G. 2002. Paradoxical selective feeding on a low nutrient diet: why do mangrove crabs eat leaves? *Oecologia* **131**: 1-7.
- SKOV, M.W., VANNINI, M., SHUNULA, J.P., HARTNOLL, R.G. & CANNICCI, S. 2002. Quantifying the density of mangrove crabs: Ocypodidae and Grapsidae. *Marine Biology* **141**: 725-732.
- SMITH, T.J., BOTO, K.G., FRUSHER, S.D. & GIDDENS, R.L. 1991. Keystone species and mangrove forests dynamics: the influence of burrowing by crabs on soil nutrient status and forest productivity. *Estuarine, Coastal and Shelf Science* **33**: 419-432.
- TAN, C.G.S. & NG, P.K.L. 1994. An annotated checklist of mangrove brachyuran crabs from Malaysia and Singapore. *Hydrobiologia* **285**: 75-84.
- THURMAN, C. 2003. Osmoregulation by six species of fiddler crabs (*Uca*) from the Mississippi delta area in the northern Gulf of Mexico. *Journal of Experimental Marine Biology and Ecology* **291**: 233-253.

- *VANNINI, M. & VALMORI, P. 1981. Researchers on the coast of Somalia. The shore and the dune of Sar Uanle. 31. *Ocypodidae* and *Gecarcinidae* (Crustacea Brachyura). *Monitore Zoologico Italiano* **6**: 57-101.
- *VANNINI, M. & VALMORI, P. 1981. Researchers on the coast of Somalia. The shore and the dune of Sar Uanle. 30. Grapsidae (Decapoda Brachyura). *Monitore Zoologico Italiano* **14**: 199-226.
- VANNINI, M., OLUOCH, A. & RUWA, R.K. 1997. Tree-climbing decapods of Kenyan mangroves. In: *Mangrove Ecosystem studies in Latin American and Africa*, (eds) B. Kjerfve., L.D. de Lacerda. & E.H.S. Diop. UNESCO, Paris, France.
- WARNER, G.F. 1977. *The biology of crabs*. Elek Science Publishers, London.
- WARREN, J.H. & UNDERWOOD, A.J. 1986. Effects of burrowing crabs on the topography of mangrove swamps in South Wales. *Journal of Experimental Marine Biology and Ecology* **102**: 223-236.
- WOOLDRIDGE, T.H. 1968. The study of the distribution of *Upogebia africana* (Ortmann) and *Callianassa kraussi* (Stebbing) in estuaries on the basis of substrate. Honours, Rhodes University, South Africa.
- ZIMMERMAN, D.A., TURNER, D.A. & PEARSON, D.J. 1996. *Birds of Kenya and northern Tanzania*. Princeton University Press, Princeton, NJ. USA.
- * Original not seen

Chapter 2

Salinity tolerance and osmoregulatory strategies of several subtropical brachyuran crabs and an anomuran prawn

2.1. Introduction

Day (1980) gave the definition of an estuary as “a partially enclosed coastal body of water which is either permanently or periodically open to the sea and within which there is a measurable variation of salinity due to the mixture of sea water with freshwater derived from the land drainage”. The mixing of riverine and marine water leads to a gradient of salinity along the length of an estuary. At any particular location within a permanently open estuary there are short-term changes in salinity associated with tidal action, and longer-term changes associated with seasonal changes in rainfall. Inhabitants of the intertidal are exposed to other salinity variations, because high salinities can develop in intertidal sediments and in intertidal pools through evaporation during low tide and may be followed by sudden dilution to near freshwater levels following heavy rainfall. Sasekumar (1974), for example, reported a sediment pore water salinity of 3.5 in the intertidal zone of a Malaysian mangrove during a neap tide following heavy rainfall, while Gillikin (2000) reported sediment pore water salinity as high as 90 in a Kenyan mangrove. Organisms that live permanently in estuaries must be able to tolerate and cope with these changes in salinity. They do this through a combination of physiological and behavioural adaptations.

Aquatic organisms must regulate the volume and/or chemical composition of their body fluid (Branch & Branch 1981, Mantel & Farmer 1983, Thurman 2003). Cells cannot afford to either lose too much water, or gain excess water, but must maintain an equal proportion of solutes both inside and outside of the cells, a condition called osmotic balance (Warren *et al.* 1966). The process by which organisms regulate their osmotic balance is termed osmoregulation.

According to their salinity tolerance, aquatic organisms can be divided into two categories. Stenohaline organisms are able to tolerate only a narrow range of external salinity (e.g. organisms inhabiting marine and freshwater environments), while euryhaline organisms can withstand a wide range of external salinity (e.g. organisms inhabiting estuaries). When there is a change in the osmotic concentration of the surrounding medium an organism may respond in one of two ways. One is to change the osmotic concentration of its body fluids to conform to that of the surrounding medium, thereby remaining isosmotic with the medium. Such organisms are referred to as osmoconformers. The second way is for an organism to maintain or regulate the osmotic concentration of its body fluids in spite of changes in the osmotic concentration of surrounding medium. Such organisms are referred to as osmoregulators. Most marine crustaceans are stenohaline osmoconformers (Péqueux 1995). Euryhaline osmoregulators have representatives over the complete range of salinities from seawater to brackish water to freshwater. They are divided into two broad categories, one being “weak osmoregulators” that exhibit limited capabilities of regulation when the salinity

of the surrounding medium changes and the other being “strong osmoregulators” that regulate their hemolymph osmolality very different to that of the surrounding medium (Péqueux 1995).

Although southern African estuarine invertebrates are generally considered to be able to tolerate salinities between 5-55 (e.g. Hill 1981, de Villiers & Hodgson 1999), this conclusion is based on laboratory-derived data for relatively few species such as the ocypodid crab *Uca annulipes* (Edney 1961), the hymenosomatid crab *Hymenosoma orbiculare* (Millard & Broekhuysen 1970), the portunid crab *Scylla serrata* (Hill 1979), and the sesarmid crabs *Parasesarma catenata* and *Chiromantes eulimene* (Alexander & Ewer 1969; Boltt & Heeg 1975). The actual salinity tolerance ranges and osmoregulatory strategies are in fact not known for most species. *Paratyloidiplax blephariskios* (Owen & Forbes 2002), a South African ocypodid crab can tolerate salinities as high as 65. Previous salinity tolerance and osmoregulation studies also usually only examined salinities less than 35. As a result, the response of species to higher salinities is not known. Exposure of experimental animals to salinities higher than seawater provides important information. Firstly, as previously stated intertidal organisms may be exposed to salinities in excess of seawater on a regular basis, and this provides an indication of how they tolerate these high salinities. Secondly, important differences in salinity tolerance and osmoregulatory capability usually only become apparent near an animal's physiological limit of tolerance (Thurman 2003), and these provide important information on differences in physiology between organisms inhabiting similar environments. Temperature has been shown to influence tolerance to salinity of decapod crustacea, and occasionally also osmoregulatory capability (e.g. Dehnel 1962, Dorgelo 1981, Kirkpatrick & Jones 1985, Lemaire *et al.* 2002).

The present study investigates the salinity tolerances and osmoregulatory strategies of several sub-tropical brachyuran crabs and an anomuran prawn, in order to understand how they are adapted physiologically to life in the estuarine intertidal environment.

2.2. Materials and methods

2.2.1. Study area

The Mhlathuze estuary (Figure 2.1) is located at 28° 47' S, 32° 05' E and covers an area of approximately 11.5 km² of the 30 km² of the original estuary (Begg 1978). It is regarded as permanently open estuarine bay based on the classification by Whitfield (1992). All crabs and the prawn species were collected in Richards Bay Harbour and the Mhlathuze River estuary on the east coast of South Africa.

2.2.2. Collection and handling of crabs and prawns

Crabs were collected from the intertidal zone at various sites in Richards Bay and the Mhlathuze River estuary by hand or with the aid of small hand-held nets. Prawns were collected from Richards Bay using a hand-operated suction pump. Crabs and prawns were rinsed in the field to remove sand and mud before they were brought to the laboratory within a few hours of collection, in plastic containers filled with water from Richards Bay (full strength seawater).

In the laboratory (environmental controlled rooms) the crabs and prawns were placed in tanks filled with about 18 litres of aerated and filtered (0.45 µm) seawater of a salinity of 35 and left for a period of between 12-16 hours to acclimate. Between 30-40 individuals were placed into each tank. There was little mortality that was noticed in tanks, and dead animals were removed to prevent water fouling. Mortality was not recorded in the holding tanks because crabs were not yet exposed to experimental salinities, but they were left for a day before the actual experiments. An exception to this was for fiddler crabs, which were immediately isolated from one another on return to the laboratory since aggressive interactions between males in containers often resulted in death. The tanks were covered to limit evaporation of water. Crabs and prawns were fed fish flakes, but during the experiments they were not fed. The tanks were held in an environmentally controlled chamber at a constant temperature of 22°C (±1°C) and a photoperiod of 12 hours light: 12 hours dark was maintained in all experiments. Salinity, temperature and mortality were checked in the tanks daily. For all experiments, seawater was diluted with filtered (0.45 µm) dechlorinated tap water to obtain salinities lower than 35, and by adding Instant Ocean Synthetic Sea Salts (Aquarium Systems, Inc.) artificial sea salt to get salinities higher than 35. Salinities were checked using an Atago refractometer.

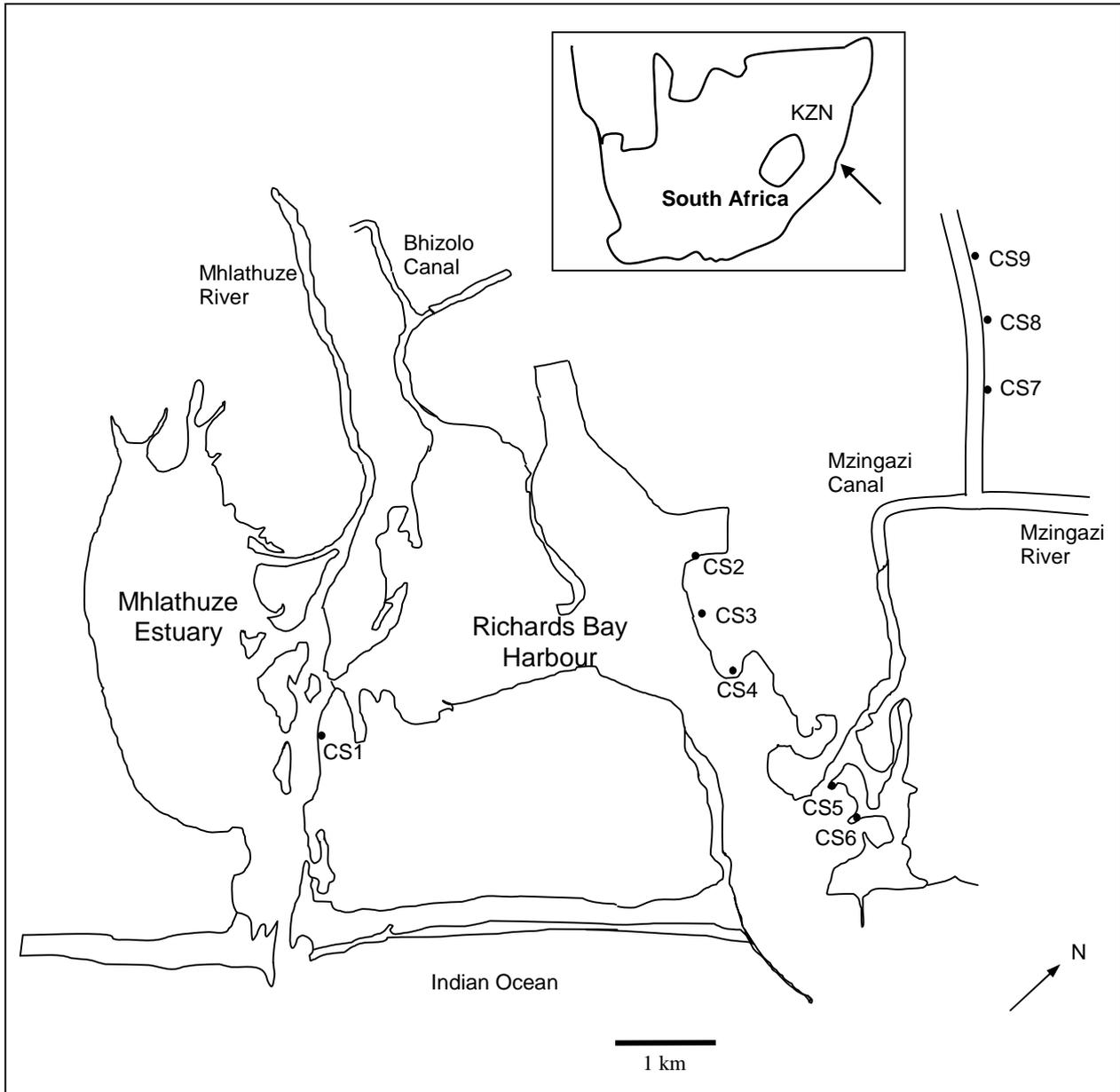


Figure 2.1. The Mhlathuze Estuary and Richards Bay Harbour showing nine sampling sites.

2.2.3. Salinity Tolerance Experimental Procedure

The influence of ten different salinities was examined, namely 0, 1, 3, 9, 18, 26, 35, 45, 55, 65 and 75. Organisms inhabiting the intertidal zone are exposed to wide variations in environmental factors, including temperature and salinity in a short period of time. Lower salinities were used because during a rainy day or the rainy season salinity in intertidal pools can be as low as 0. Forbes (1974) showed that *Callinassa kraussi* can tolerate salinities as low as 1, therefore freshwater was used to determine whether these decapods can tolerate such low salinities. During evaporation rock pools or sediment water can become hypersaline, often to salinities far exceeding that of seawater, therefore higher salinities were employed to determine the highest salinity these decapods can tolerate.

Experiments were conducted in an environmentally controlled chamber at 22°C ($\pm 1^\circ\text{C}$). These different salinities were employed as they are likely to be encountered by crabs in South African estuaries. Crabs inhabiting the intertidal are more likely to be exposed to low or high salinities caused by heavy rainfall and evaporation of water from the pools. The temperature of 22°C was used because it is the average water temperature on east Coast of South Africa. Usually between 7-10 crabs were exposed to each salinity, but there were cases where fewer crabs were exposed due to the low number of crabs available in the field. Crabs and prawns were held individually in containers filled with between 200 ml and 400 ml of exposure water depending on the animal's size. The containers were loosely covered to limit evaporation and to prevent crabs escaping, because of this the experimental containers were not aerated.

There were two types of experiments, namely direct exposure and acclimation experiments. In the direct exposure experiments, crabs and prawns were directly transferred from the holding salinity of 35 to experimental salinities. There was little mortality that was observed during acclimation experiments as shown from (Figure 2.2 – 2.11) except in lower salinity of 0 and higher salinity of 75. When comparing direct and acclimation experiments, there was a significant difference in salinity tolerance of decapods, with acclimation showing the highest survival. In the acclimation experiments, crabs were exposed to experimentally increased salinities at a rate of between 2-10 per day. For those species where low numbers in the field prevented the collection of sufficient animals for both experiments, only acclimation experiments were conducted (i.e. *Uca chlorophthalmus*, *Macrophthalmus depressus* and *Macrophthalmus grandidieri*).

The crabs were monitored at 24 hour intervals for a period of 96 hours, this monitoring period in the acclimation experiments only starting after all animals had reached the target salinities. Dead animals were identified by the lack of movement of appendages from prodding for less than 3 minutes. Salinity and temperature of water in the containers was checked daily using a hand-held refractometer and a mercury thermometer respectively. Water in the containers was changed at every two days, but daily for *Metopograpsus thukuhar* since water quality decreased rapidly for this crab.

2.2.4. Osmoregulatory Capacity Experimental Procedure

Hemolymph osmolality in all crabs and prawns that survived the salinity tolerance experiments was measured, with the exception of *Dotilla fenestrata* and *Uca chlorophthalmus*, which were not measured because their small body size did not provide sufficient hemolymph for measurement. In crabs, hemolymph was extracted from the arthrodistal membrane at the base of a pereopod (usually fifth) using a 19 gauge sterile 1.0-ml tuberculin syringe. Hemolymph for *Callinassa kraussi* was extracted by inserting the syringe in the membrane of the linea thalassinica. The hemolymph was placed onto a piece of parafilm and taken up by a 20 μl sampler and the osmolality immediately measured using a freezing point depression osmometer (Advanced Model 3300 Micro-Osmometer). The osmolality of experimental water (surrounding medium) was measured following a similar procedure.

2.2.5. Data analysis

Survival between individuals exposed to the same salinity was compared by means of a z-test for pairwise comparisons and a Tukey-type multiple comparison of proportions test for multiple comparisons. For osmoregulation, data are expressed either as hemolymph osmolality (in mOsm.kg⁻¹) or as osmoregulatory capacity (OC) (OC, also in mOsm.kg⁻¹), the latter defined as the difference between the osmotic pressure of the hemolymph and of the surrounding medium at a given salinity (Charmantier *et al.* 1989). Data distributions of hemolymph osmolality and OC often deviated from normality (Kolmogorov Smirnov test) and/or had unequal variance (Bartlett's test). Data transformation (log and ln) often failed to approximate either data normality or variance homogeneity, and thus despite these violations of parametric test assumptions untransformed hemolymph osmolality and OC data were analysed using more robust parametric statistical procedures; Students t-tests for pairwise comparisons and one-way ANOVA followed where appropriate by a Tukey multiple comparison test for multiple comparisons. Different letters on graphs indicate a statistically significant difference between mean values.

Salinity tolerance is represented by three graphs (Figure 2.2 – 2.11). The first two graphs represent direct and acclimation experiments of all the decapods that were investigated in this study. Different letters represent significant difference between salinities in one exposure (direct or acclimation). The third graph represents the comparison between two experiments and different letters indicate significant difference in salinity tolerance between two exposures.

2.3. Results

2.3.1. Salinity Tolerance

Uca vocans: The range of salinity tolerated by *U. vocans* in the direct exposure experiment was 3-45 (Figure 2.2). There was no statistical difference in survival over this salinity range. In the acclimation experiment *U. vocans* tolerated a wider range of salinity, between 0-55 (Figure 2.2). Survival at a salinity of 55 was significantly lower than that at salinities of 0, 18, 26, 35 and 45, but not between salinities of 3 and 9. There was no statistical difference in survival between the direct exposure and acclimation experiments at salinities between 3-45, but survival in the direct exposure experiment at salinities of 0 and 55 was significantly lower than that in the acclimation experiment (Figure 2.2).

Uca urvillei: The range of salinity tolerated by *U. urvillei* in the direct exposure experiment was 3-45 (Figure 2.3). Survival at a salinity of 45 was significantly lower than that at 9, 26 and 35, but not at 3 and 18. In the acclimation experiment *U. urvillei* tolerated a wider range of salinity, between 0-55 (Figure 2.3). Survival at a salinity of 55 was significantly lower than that at 3, 9, 26, 35 and 45, but not at 0 and 18. There was no statistical difference in survival between the direct exposure and acclimation experiments at salinities between 3-45, but survival in the direct exposure experiment at 0 and 55 was significantly lower than that in the acclimation experiment (Figure 2.3).

Uca annulipes: The range of salinity tolerated by *U. annulipes* in the direct exposure experiment was 3-65 (Figure 2.4). Survival at a salinity of 65 was significantly lower than that at 18, 26, 35 and 45, but not at 3, 9 and 55. The salinity tolerance range in the acclimation experiment was slightly wider, between 0-65 (Figure 2.4). Survival in freshwater was significantly lower than that at all other salinities, while survival at a salinity 45 was significantly lower than that at 9, 18, 26, and 35, but not at 55 and 65. There was no statistical difference in survival between the direct exposure and acclimation experiments at salinities between 0-65 (Figure 2.4).

Uca chlorophthalmus: *Uca chlorophthalmus* tolerated salinities from 0-55 in acclimation experiment (Figure 2.5). Survival in freshwater was significantly lower than all other salinities.

Macrophthalmus depressus: The salinity tolerance range of *M. depressus* was 3-65. Survival at salinities of 3, 9 and 65 was significantly different to the survival in salinities from 18-55. (Figure 2.6). *Macrophthalmus grandidieri*: The salinity tolerance range for *M. grandidieri* was from 9-35 (only salinities from 0-35 were investigated). There was no statistically significant difference in survival at the salinities examined (Figure 2.7).

Dotilla fenestrata: *Dotilla fenestrata* tolerated salinities from 3-65 in direct exposure experiments (Figure 2.8). There was no statistical difference in survival over the salinity range tolerated. In the acclimation experiment the salinity tolerance range was 0-65 with no statistical difference in survival over this salinity range. There was no statistical difference in survival between the direct exposure and acclimation experiments at salinities between 3-65 (Figure 2.8).

Metopograpsus thukuhar: The salinity tolerance range of *M. thukuhar* was 18-55 in the direct exposure experiment (Figure 2.9). Survival at salinities of 18 and 35 was significantly lower than at 26, but not at 45, whilst survival at 55 was significantly lower than the survival in all other salinities (Figure 2.9). The salinity tolerance range in the acclimation experiment was 3-55. Survival at salinities of 3, 35 and 55 was significantly lower than that at 18 and 26, but not at 9 and 45. There was no statistical difference in survival between the direct and acclimation experiments at salinities between 18-55 (Figure 2.9).

Chiromantes eulimene: The salinity tolerance range of *C. eulimene* in the direct exposure experiment was 0-65 (Figure 2.10). The salinity tolerance range in the acclimation experiment was 0-65 (Figure 2.10). Survival at a salinity of 65 was significantly lower than survival in other salinities, and survival at 55 was lower than that at 3, 9 and 35, but not at 0, 18, and 26. There was no statistical difference in survival between the direct and acclimation experiments at salinities between 0-65 (Figure 2.10).

Callinassa kraussi: The salinity tolerance range for *C. kraussi* in the direct exposure experiment was 1-55 (Figure 2.11). There was no statistical difference in survival over the salinity range tolerated. In the

acclimation experiment the salinity tolerance range was 3-65 (Figure 2.11). Survival at a salinity of 65 was significantly lower than survival salinities between 3-55. Survival at a salinity of 5 was significantly lower than that at 3-45, and survival at salinities of 9 and 35 was significantly lower than that at 3, 18 and 45, but not at 26. There was no statistical difference in survival between the direct exposure and acclimation experiments at salinities between 3-55 (Figure 2.11).

2.3.2. Osmoregulation

Uca vocans: There was no significant difference in hemolymph osmolality between crabs directly exposed or acclimated to experimental media (t-tests, $p > 0.05$). Only the acclimation experiment data are therefore discussed further. *Uca vocans* is a strong hyper-hypo-osmoregulator (Figure 2.12). The change from hyper to hypo-regulation, that is the isosmotic point, was reached at an external medium salinity of about 27 (about 783 mOsm.kg⁻¹). Hemolymph osmolality increased significantly from a salinity of 0 to 3, and was then maintained within a range between salinities of 3-26. There was no significant difference in hemolymph osmolality over this salinity range. Hemolymph osmolality increased significantly with each further increase in the salinity of the external medium. The hyper-regulatory ability of *U. vocans* is stronger than the hypo-ability. This can be seen by the fact that the hemolymph osmolality line is much closer to the isosmotic line at salinities above the isosmotic point in (Figure 2.12) and also by the lower Osmotic Capacity (OC) at comparable salinity differences below and above the isosmotic point.

Uca urvillei: There was a significant difference in hemolymph osmolality between crabs directly exposed or acclimated to a salinity of 45 (t-tests, $p > 0.05$), but no significant difference at salinities of 3, 9, 18, 26 and 35. Only the acclimation experiment data are therefore discussed further. *Uca urvillei* is a strong hyper-hypo-regulator (Figure 2.13). The change from hyper to hypo-regulation, that is the isosmotic point, is reached at a medium salinity of about 27 (about 783 mOsm.kg⁻¹). Hemolymph osmolality increased significantly from a salinity of 0 to 3, and was then maintained within a very narrow range between salinities of 3-9, hemolymph osmolality increased significantly from a salinity of 9 to 18 and was maintained within a narrow range between salinities of 18-26. Hemolymph osmolality increased significantly with each further increase in the salinity of the external medium. The hyper-regulatory ability of *U. urvillei* is stronger than the hypo-regulatory ability. This can be seen by the fact that the hemolymph osmolality line is much closer to the isosmotic line at salinities above the isosmotic point in Figure 2.13 and also by the lower OC at comparable salinity differences below and above the isosmotic point (Figure 2.13).

Uca annulipes: There was a significant difference in hemolymph osmolality between crabs directly exposed or acclimated to a salinity of 55 (t-tests, $p > 0.05$), but no significant difference at salinities of 9, 18, 26, 35 and 65. Only the acclimation experiment data are therefore discussed further. *Uca annulipes* is a strong hyper- hypo-regulator (Figure 2.14). The change from hyper to hypo-regulation, that is the isosmotic point, is reached at a medium salinity of about 30 (about 870 mOsm.kg⁻¹). Hemolymph osmolality increased significantly from a salinity of 9 to 18, and was then maintained within a narrow range between salinities of

18-26. Hemolymph osmolality increased significantly again from a salinity of 26 to 35 and was then maintained within a narrow range between salinities of 35-45. Hemolymph osmolality increased significantly with each further increase in the salinity of the external medium. The hyper-regulatory ability of *U. annulipes* is stronger than the hypo-regulatory ability. This can be seen by the fact that the hemolymph osmolality line is much closer to the isosmotic line at salinities above the isosmotic point in Figure 2.14 and also by the lower OC at comparable salinity differences below and above the isosmotic point (Figure 2.14).

Macrophthalmus depressus: *Macrophthalmus depressus* is a weak hyper-hypo-osmoregulator, with an isosmotic point reached at a surrounding medium salinity of about 33 (about 957 mOsm.kg⁻¹) (Figure 2.15). Hemolymph osmolality increased significantly with each increase in the salinity of the surrounding medium.

Macrophthalmus grandidieri: *Macrophthalmus grandidieri* is a weak hyper-regulator in salinities equal or greater than 35. Hemolymph osmolality increased significantly with each increase in the salinity of the surrounding medium, and was isosmotic at a surrounding medium salinity of 35 (Figure 2.16).

Metopograpsus thukuhar: There was no significant difference in hemolymph osmolality between crabs directly exposed or acclimated to experimental media (t-tests, $p > 0.05$). Only the acclimation experiment data are therefore discussed further. *Metopograpsus thukuhar* is a strong hyper-hypo-osmoregulator (Figure 2.17). The change from hyper to hypo-regulation, that is, the isosmotic point, was reached at a surrounding medium salinity of about 26 (about 750 mOsm.kg⁻¹). Hemolymph osmolality increased but not significantly from a salinity of 3-18, followed by a significant increase of hemolymph osmolality from a salinity of 18 to 26 and maintained for a narrow range between salinities of 26-35 and further significant increase of hemolymph osmolality from a salinity of 35 to 45. The hyper-regulatory ability of *M. thukuhar* is stronger than its hypo-regulatory ability. This can be seen by the fact that the hemolymph osmolality line is much closer to the isosmotic line at salinities above the isosmotic point in Figure 2.17 and also by the lower OC at comparable salinity differences below and above the isosmotic point (Figure 2.17).

Chiromantes eulimene: *Chiromantes eulimene* is a strong hyper-hypo-osmoregulator (Figure 2.18). The change from hyper to hypo-regulation, that is, the isosmotic point, was reached at a surrounding medium salinity of about 26 (about 780 mOsm.kg⁻¹). There was a significant difference in hemolymph osmolality between crabs directly exposed or acclimated to 0, 3, 45 and 55 salinity (t-tests, $p > 0.05$), but no significant difference in hemolymph osmolality between crabs directly exposed or acclimated to salinities of 9, 18, 26 and 35. In direct experiments hemolymph osmolality increased but not significantly between salinities of 0-18. Hemolymph osmolality at a salinity of 26 was significantly similar to the osmolality at salinities of 3, 9, 18 and 35, but not at a salinity of 0 (Figure 2.18). Above a salinity of 35 hemolymph osmolality increased significantly up to a salinity of 55 and decreased at a salinity of 65, but not significantly, to hemolymph osmolality at a salinity of 55. In acclimation experiments hemolymph osmolality increased significantly from a salinity of 0-3 and was maintained for a narrow range from a salinity of 3-26, there was no significant

difference in hemolymph osmolality over this salinity range (Figure 2.18). The hyper-regulatory ability of *C. eulimene* is stronger than the hypo-regulatory ability. This can be seen by the fact that the hemolymph osmolality line is much closer to the isosmotic line at salinities above the isosmotic point in Figure 2.18 and also by the lower OC at comparable salinity differences below and above the isosmotic point (Figure 2.18).

Callinassa kraussi: There was a significant difference in hemolymph osmolality between prawns directly exposed or acclimated to salinities of 9, 35 and 45 (t-tests, $p > 0.05$), but no significant difference in hemolymph osmolality between prawns directly exposed or acclimated to salinities of 3, 18, 26 and 55. *Callinassa kraussi* is a hyper-osmoregulator in salinities below a salinity of 26 and osmoconforms with increasing salinity of the surrounding medium (Figure 2.19). Hemolymph osmolality increased but not significantly from a salinity of 1-3 and 3-9 in direct and acclimation experiments respectively, and then hemolymph osmolality increased significantly with each further increase in the salinity of the external medium in both experiments up to a salinity of 25, and was then maintained isosmotic to the surrounding medium in higher salinities. This species is a strong osmotic regulation in dilute media as compared to hypersaline media shown by the high Osmotic Capacity in dilute media and lost its ability to regulate its body fluid in salinities above 25 where the Osmotic Capacity is also low.

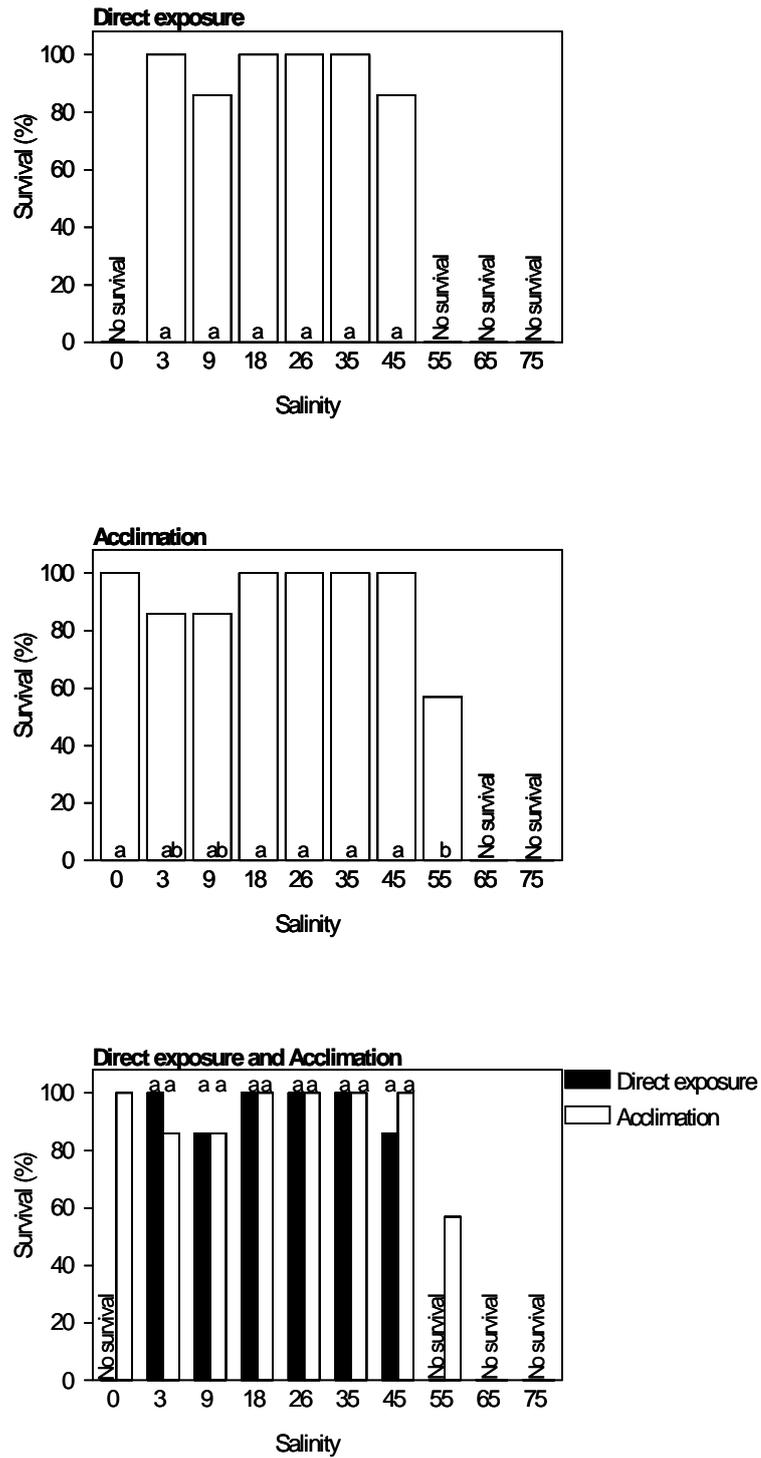


Figure 2.2. Survival of *Uca vocans* after 96 hrs following direct exposure or acclimation to various salinities. Different letters indicate a statistically significant difference in survival (based on multiple comparison of proportions tests or z-tests, $p < 0.05$).

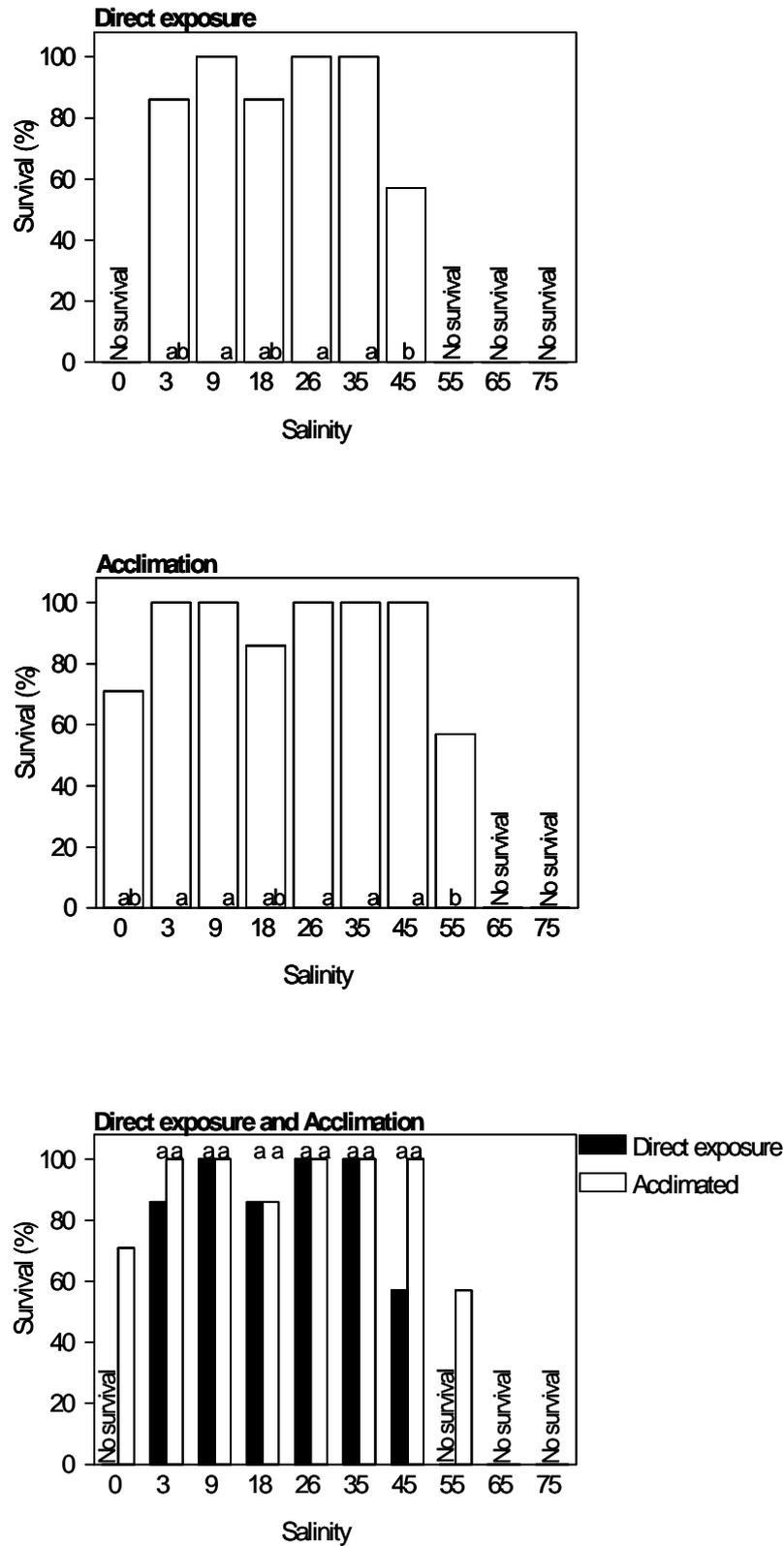


Figure 2.3. Survival of *Uca urvillei* after 96 hrs following direct exposure or acclimation to various salinities. Different letters indicate a statistically significant difference in survival (based on multiple comparison of proportions tests or z-tests, $p < 0.05$).

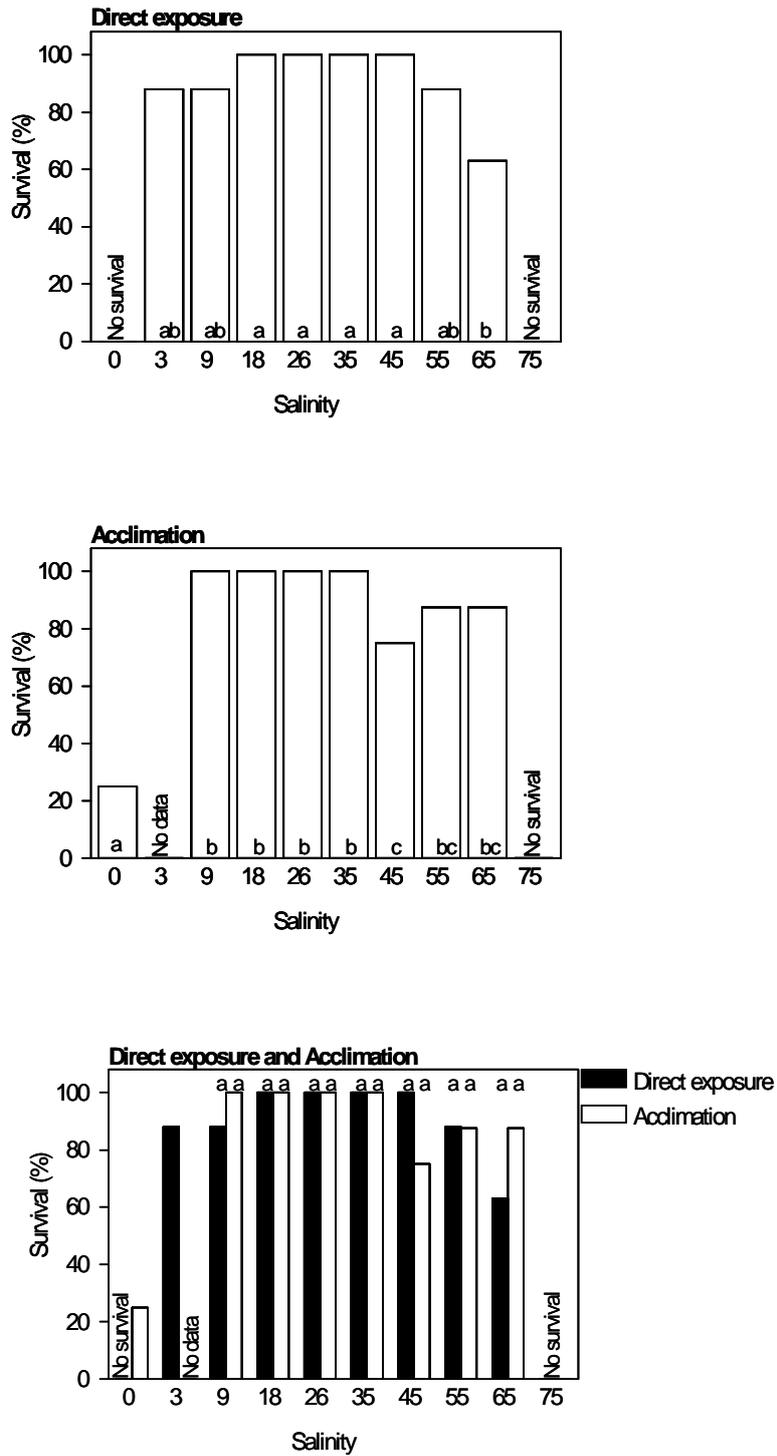


Figure 2.4. Survival of *Uca annulipes* after 96 hrs following direct exposure or acclimation to various salinities. Different letters indicate a statistically significant difference in survival (based on multiple comparison of proportions tests or z-tests, $p < 0.05$).

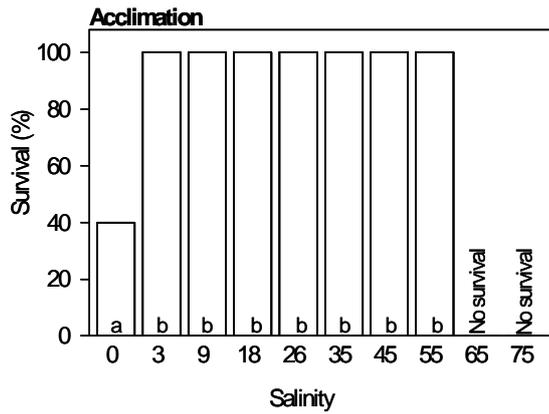


Figure 2.5. Survival of *Uca chlorophthalmus* after 96 hrs following acclimation to various salinities. Different letters indicate a statistically significant difference in survival (based on multiple comparison of proportions test, $p < 0.05$).

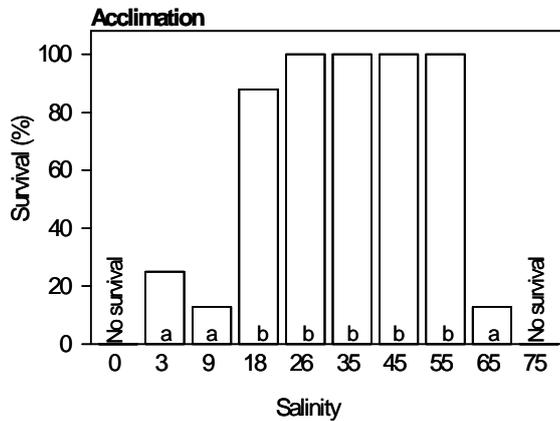


Figure 2.6. Survival of *Macrophthalmus depressus* after 96 hrs following acclimation to various salinities. Different letters indicate a statistically significant difference in survival (based on multiple comparison of proportions test, $p < 0.05$).

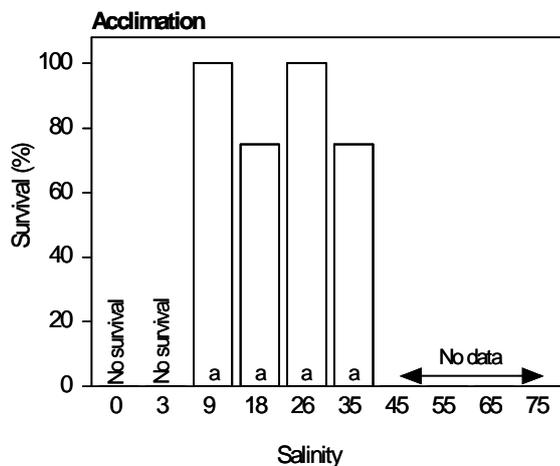


Figure 2.7. Survival of *Macrophthalmus grandideri* after 96 hrs following acclimation to various salinities. Different letters indicate a statistically significant difference in survival (based on multiple comparison of proportions test, $p < 0.05$).

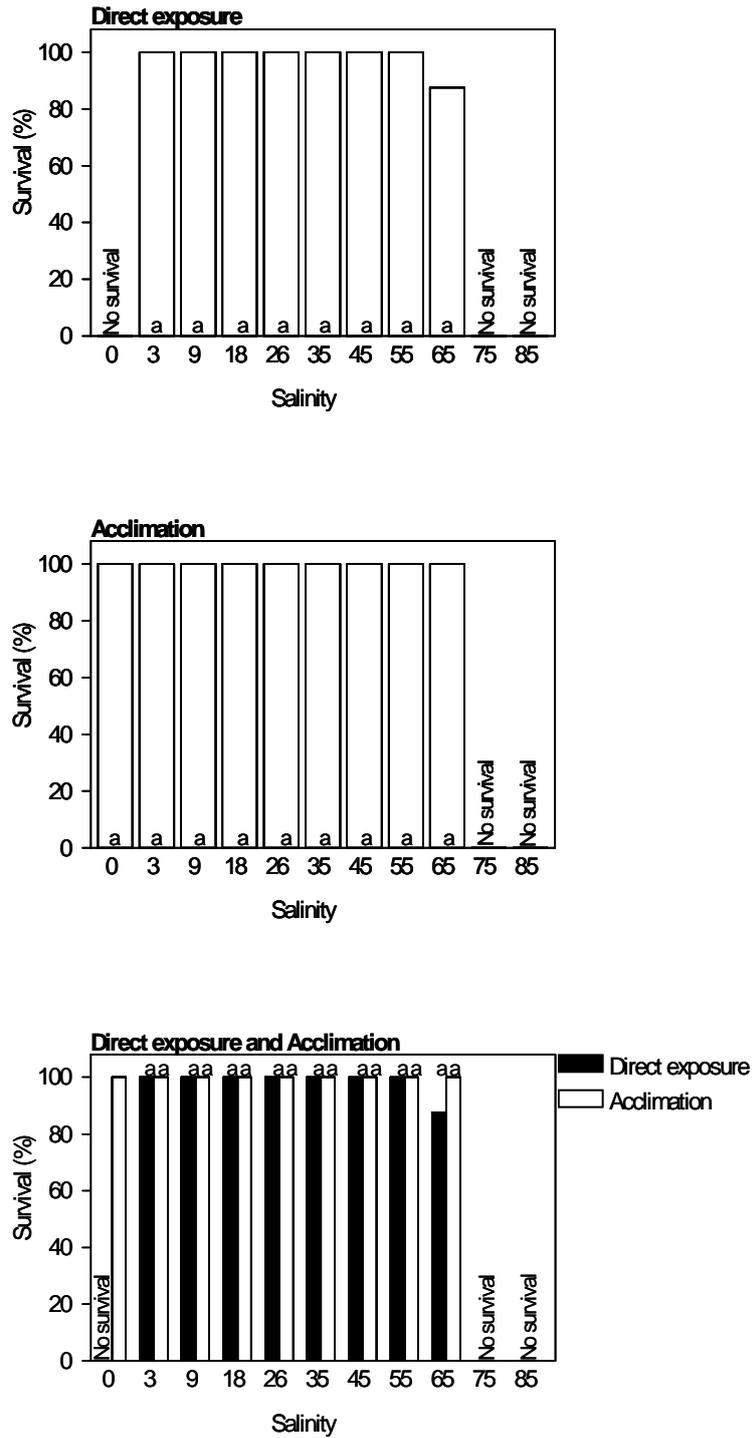


Figure 2.8. Survival of *Dotilla fenestrata* after 96 hrs following direct exposure or acclimation to various salinities. Different letters indicate a statistically significant difference in survival (based on multiple comparison of proportions tests or z-tests, $p < 0.05$).

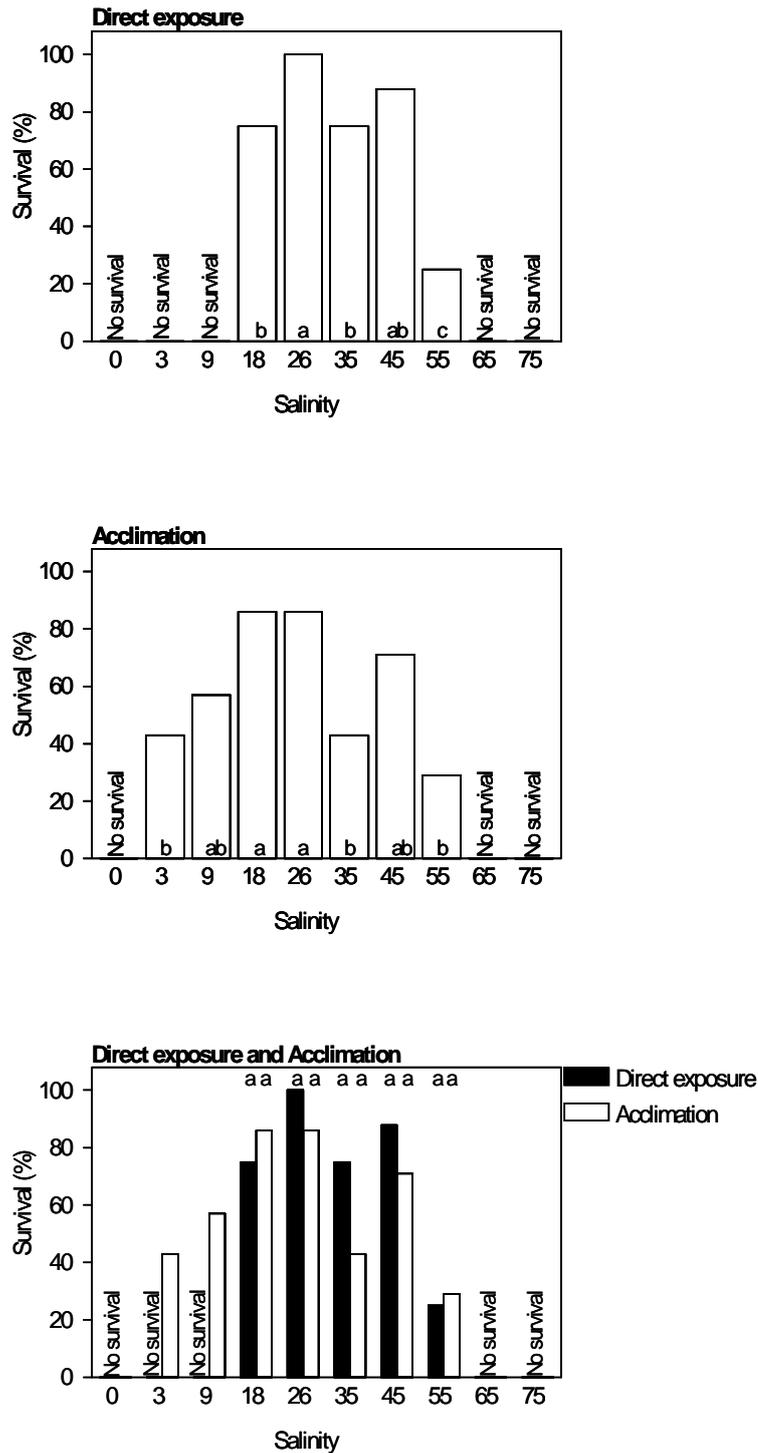


Figure 2.9. Survival of *Metopograpsus thukuhar* after 96 hrs following direct exposure or acclimation to various salinities. Different letters indicate a statistically significant difference in survival (based on multiple comparison of proportions tests or z-tests, $p < 0.05$).

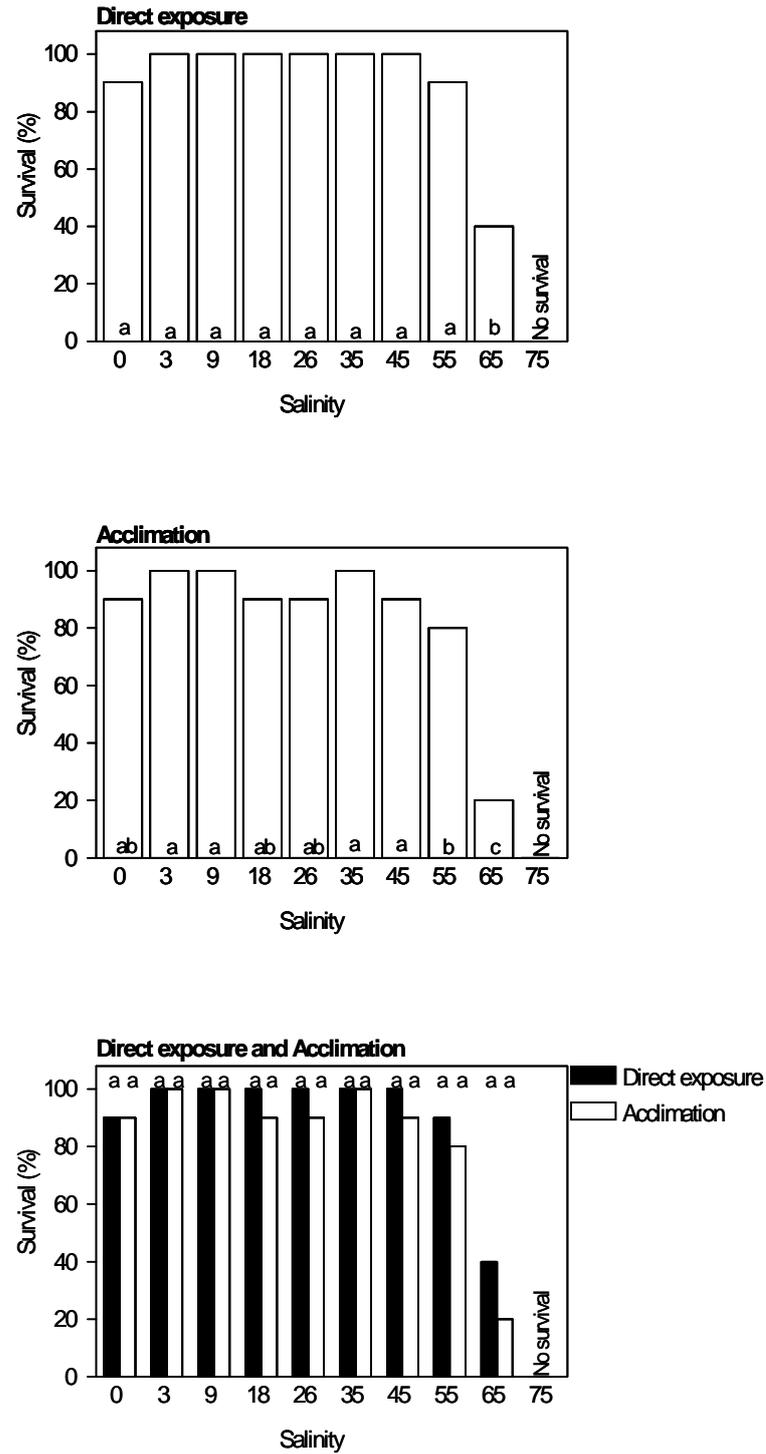


Figure 2.10. Survival of *Chiromantes eulimene* after 96 hrs following direct exposure or acclimation to various salinities. Different letters indicate a statistically significant difference in survival (based on multiple comparison of proportions tests or z-tests, $p < 0.05$).

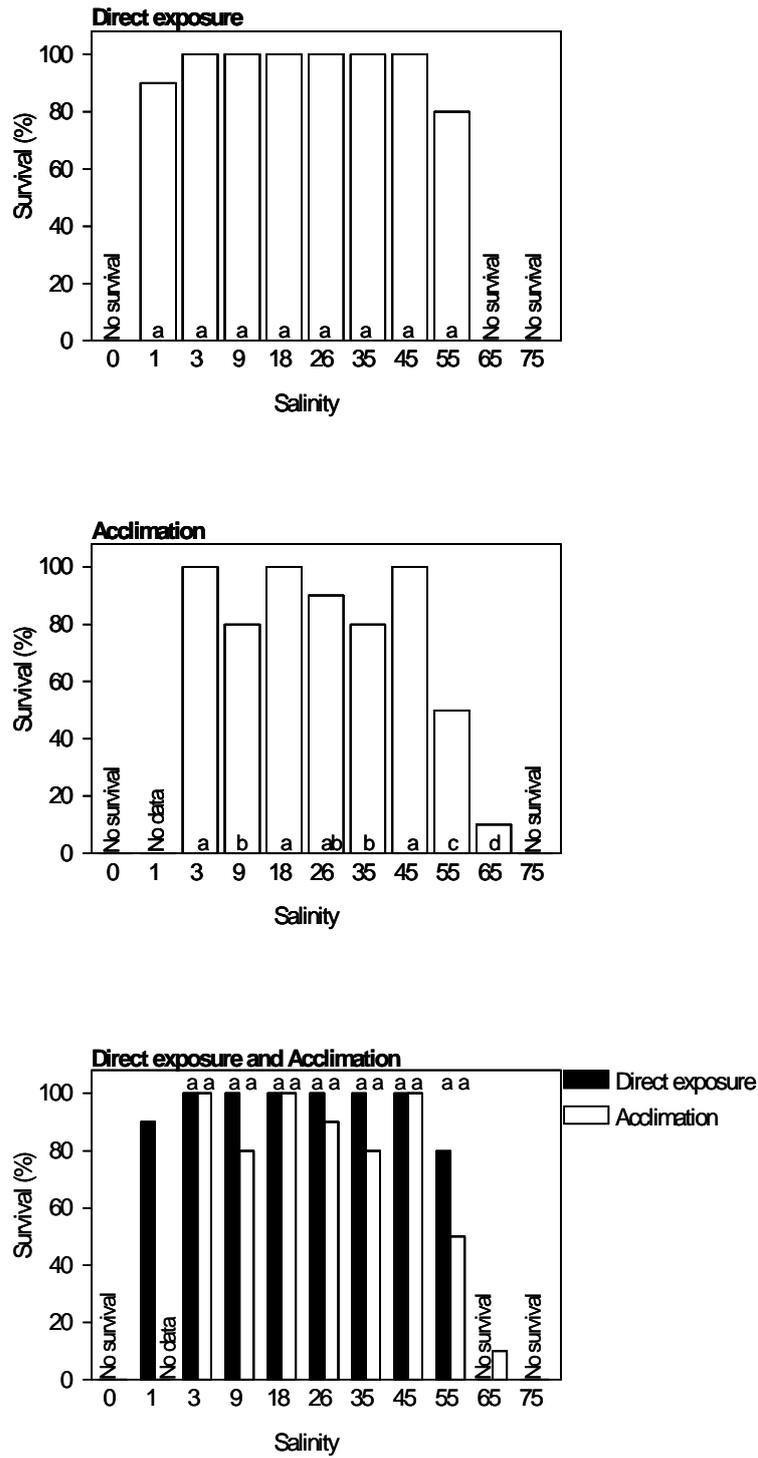


Figure 2.11. Survival of *Callianassa kraussi* after 96 hrs following direct exposure or acclimation to various salinities. Different letters indicate a statistically significant difference in survival (based on multiple comparison of proportions tests or z-tests, $p < 0.05$).

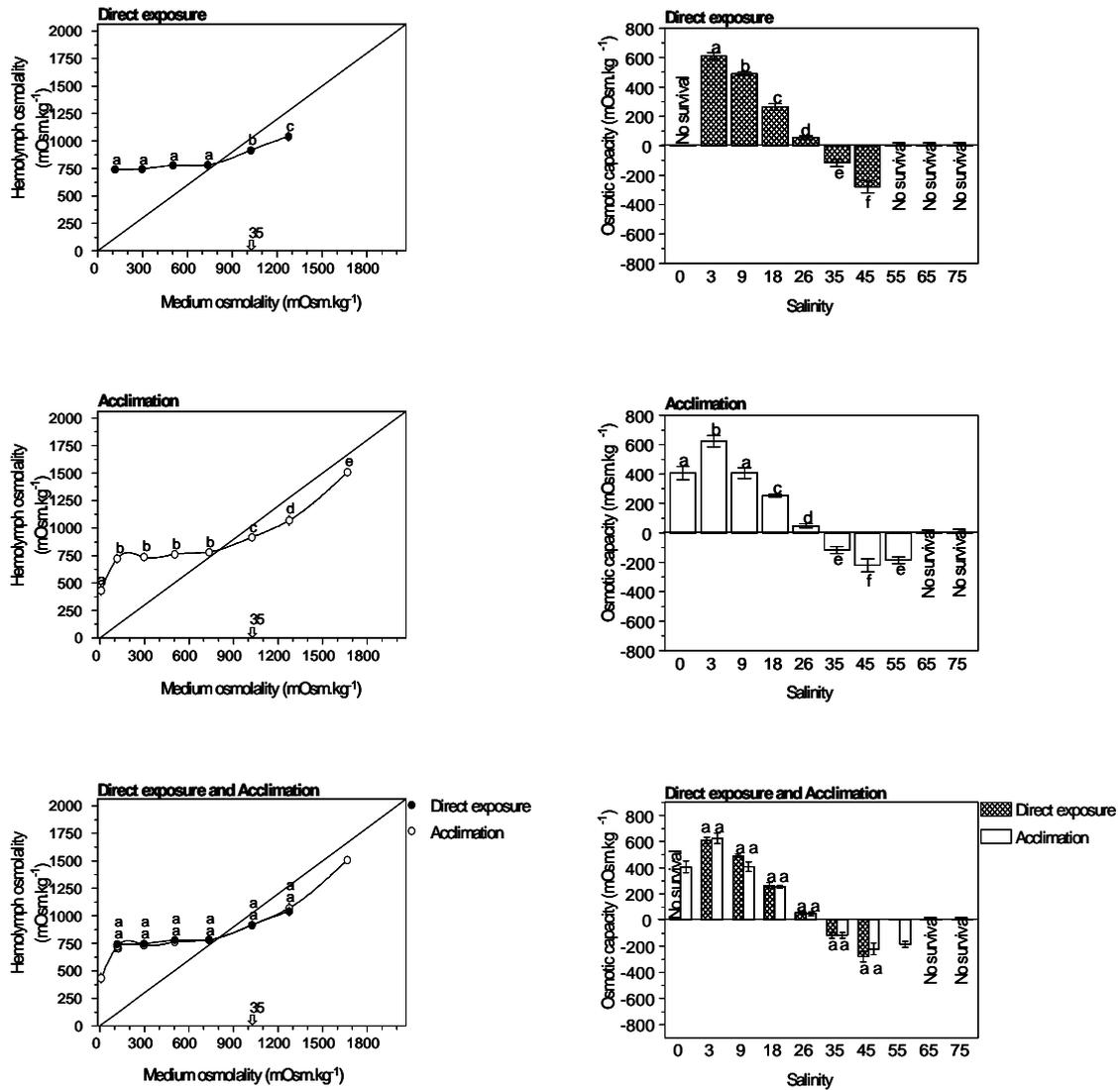


Figure 2.12. *Uca vocans*. (left) Relationship between hemolymph osmolality (mOsm.kg⁻¹ ± 1 SD) and osmolality of the surrounding medium after 96 hrs following direct exposure or acclimation to various salinities. The diagonal line is the isosmotic line. The arrow pointing downward represents a salinity of 35 (seawater). Different letters indicate significant differences between mean values (either one-way ANOVA or t-tests, p < 0.05). (right) Osmoregulatory capacity (mOsm.kg⁻¹ ± 1 SD) after 96 hrs following direct exposure or acclimation to various salinities. Different letters indicate significant differences between mean values (either one-way ANOVA or t-tests, p < 0.05).

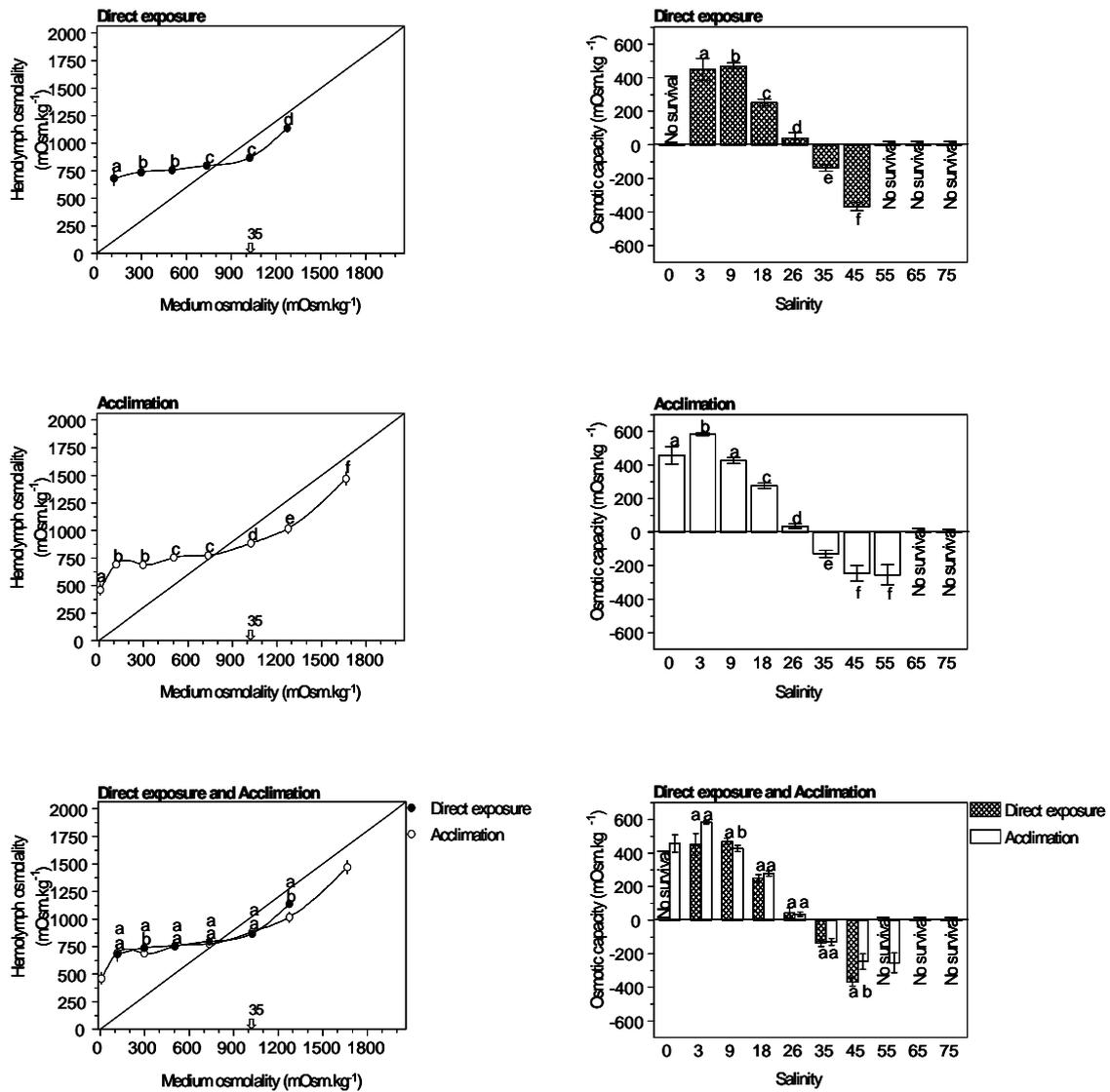


Figure 2.13. *Uca urvillei*. (left) Relationship between hemolymph osmolality ($\text{mOsm.kg}^{-1} \pm 1 \text{ SD}$) and osmolality of the surrounding medium after 96 hrs following direct exposure or acclimation to various salinities. The diagonal line is the isosmotic line. The arrow pointing downward represents a salinity of 35 (seawater). Different letters indicate significant differences between mean values (either one-way ANOVA or t-tests, $p < 0.05$). (right) Osmoregulatory capacity ($\text{mOsm.kg}^{-1} \pm 1 \text{ SD}$) after 96 hrs following direct exposure or acclimation to various salinities. Different letters indicate significant differences between mean values (either one-way ANOVA or t-tests, $p < 0.05$).

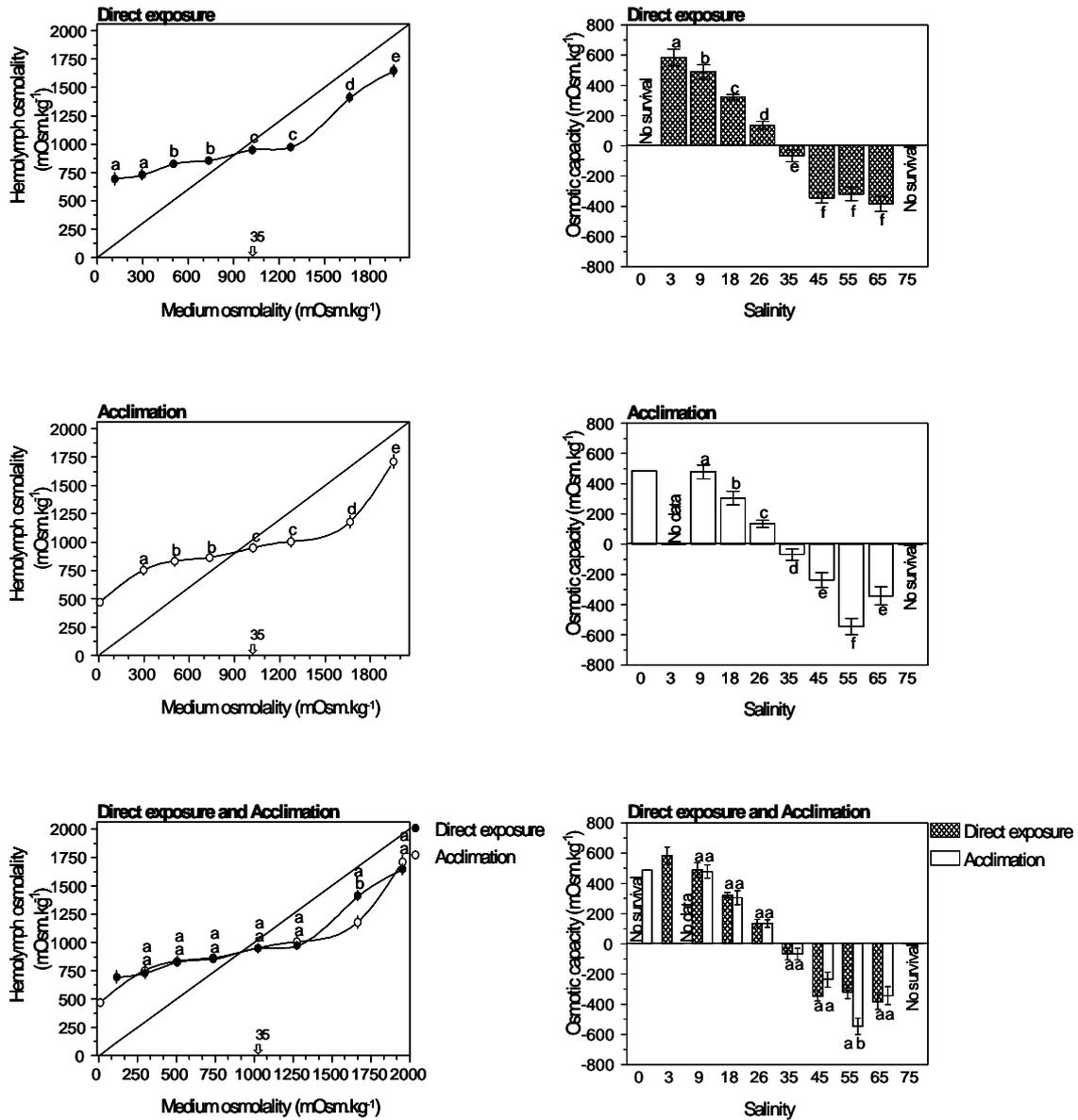


Figure 2.14. *Uca annulipes*. (left) Relationship between hemolymph osmolality (mOsm.kg⁻¹ ± 1 SD) and osmolality of the surrounding medium after 96 hrs following direct exposure or acclimation to various salinities. The diagonal line is the isosmotic line. The arrow pointing downward represents a salinity of 35 (seawater). Different letters indicate significant differences between mean values (either one-way ANOVA or t-tests, p < 0.05). (right) Osmoregulatory capacity (mOsm.kg⁻¹ ± SD) after 96 hrs following direct exposure or acclimation to various salinities. Letters show significance of data at the same medium salinity. Different letters indicate significant differences between mean values (either one-way ANOVA or t-tests, p < 0.05).

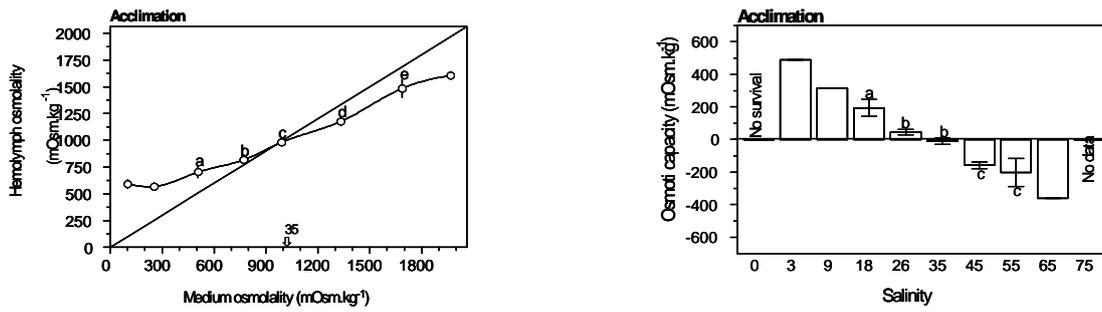


Figure 2.15. *Macrophthalmus depressus*. (left) Relationship between hemolymph osmolality ($\text{mOsm.kg}^{-1} \pm 1$ SD) and osmolality of the surrounding medium after 96 hrs following acclimation exposure to various salinities. The diagonal line is the isosmotic line. The arrow pointing downward represents a salinity of 35 (seawater). Different letters indicate significant differences between mean values (either one-way ANOVA or t-tests, $p < 0.05$). (right) Osmoregulatory capacity ($\text{mOsm.kg}^{-1} \pm$ SD) after 96 hrs following acclimation exposure to various salinities. Letters show significance of data at the same medium salinity. Different letters indicate significant differences between mean values (either one-way ANOVA or t-tests, $p < 0.05$).

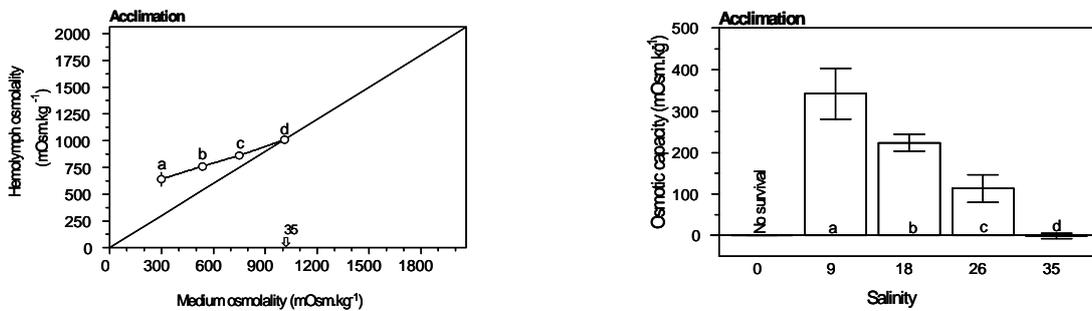


Figure 2.16. *Macrophthalmus grandidieri*. (left) Relationship between hemolymph osmolality ($\text{mOsm.kg}^{-1} \pm 1$ SD) and osmolality of the surrounding medium after 96 hrs following acclimation exposure to various salinities. The diagonal line is the isosmotic line. The arrow pointing downward represents a salinity of 35 (seawater). Different letters indicate significant differences between mean values (either one-way ANOVA or t-tests, $p < 0.05$). (right) Osmoregulatory capacity ($\text{mOsm.kg}^{-1} \pm$ SD) after 96 hrs following acclimation exposure to various salinities. Letters show significance of data at the same medium salinity. Different letters indicate significant differences between mean values (either one-way ANOVA or t-tests, $p < 0.05$).

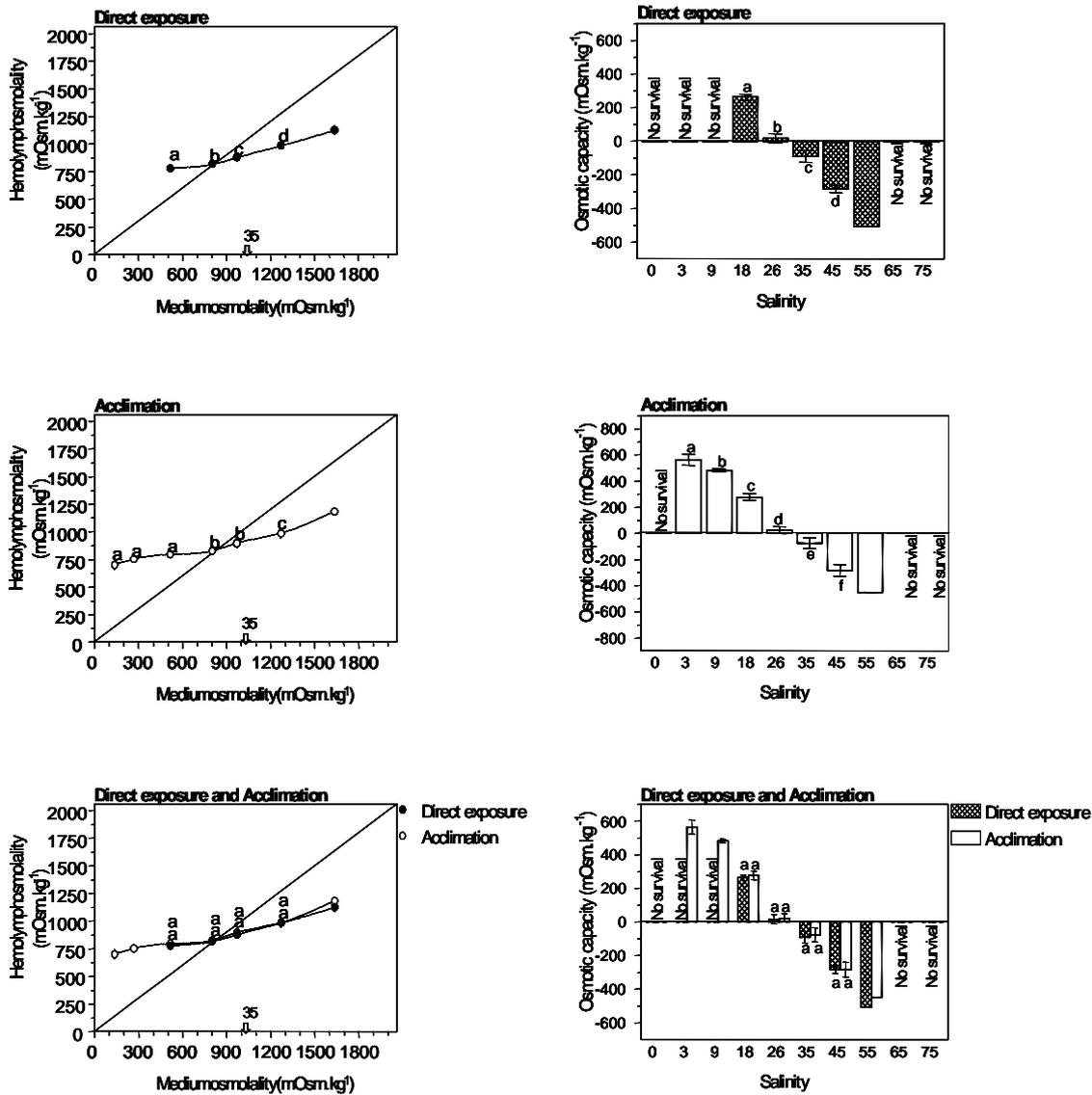


Figure 2.17. *Metopograpsus thukuhar*. (left) Relationship between hemolymph osmolality ($\text{mOsm.kg}^{-1} \pm 1$ SD) and osmolality of the surrounding medium after 96 hrs following direct exposure or acclimation to various salinities. The diagonal line is the isosmotic line. The arrow pointing downward represents a salinity of 35 (seawater). Different letters indicate significant differences between mean values (either one-way ANOVA or t-tests, $p < 0.05$). (right) Osmoregulatory capacity ($\text{mOsm.kg}^{-1} \pm \text{SD}$) after 96 hrs following direct exposure or acclimation to various salinities. Letters show significance of data at the same medium salinity. Different letters indicate significant differences between mean values (either one-way ANOVA or t-tests, $p < 0.05$).

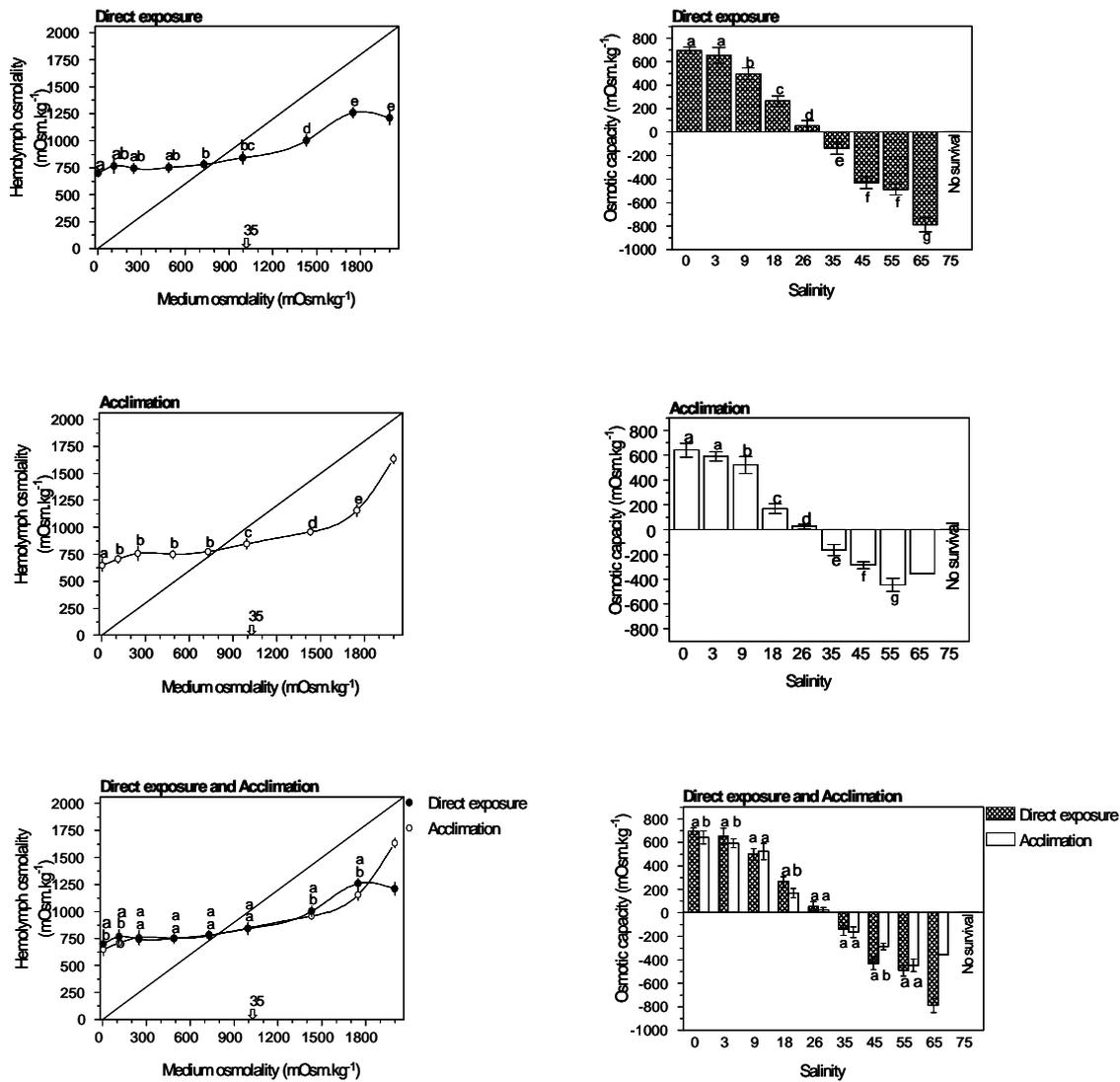


Figure 2.18. *Chiromantes eulimene*. (left) Relationship between hemolymph osmolality (mOsm.kg⁻¹ ± 1 SD) and osmolality of the surrounding medium after 96 hrs following direct exposure or acclimation to various salinities. The diagonal line is the isosmotic line. The arrow pointing downward represents a salinity of 35 (seawater). Different letters indicate significant differences between mean values (either one-way ANOVA or t-tests, $p < 0.05$). (right) Osmoregulatory capacity (mOsm.kg⁻¹ ± SD) after 96 hrs following direct exposure or acclimation to various salinities. Letters show significance of data at the same medium salinity. Different letters indicate significant differences between mean values (either one-way ANOVA or t-tests, $p < 0.05$).

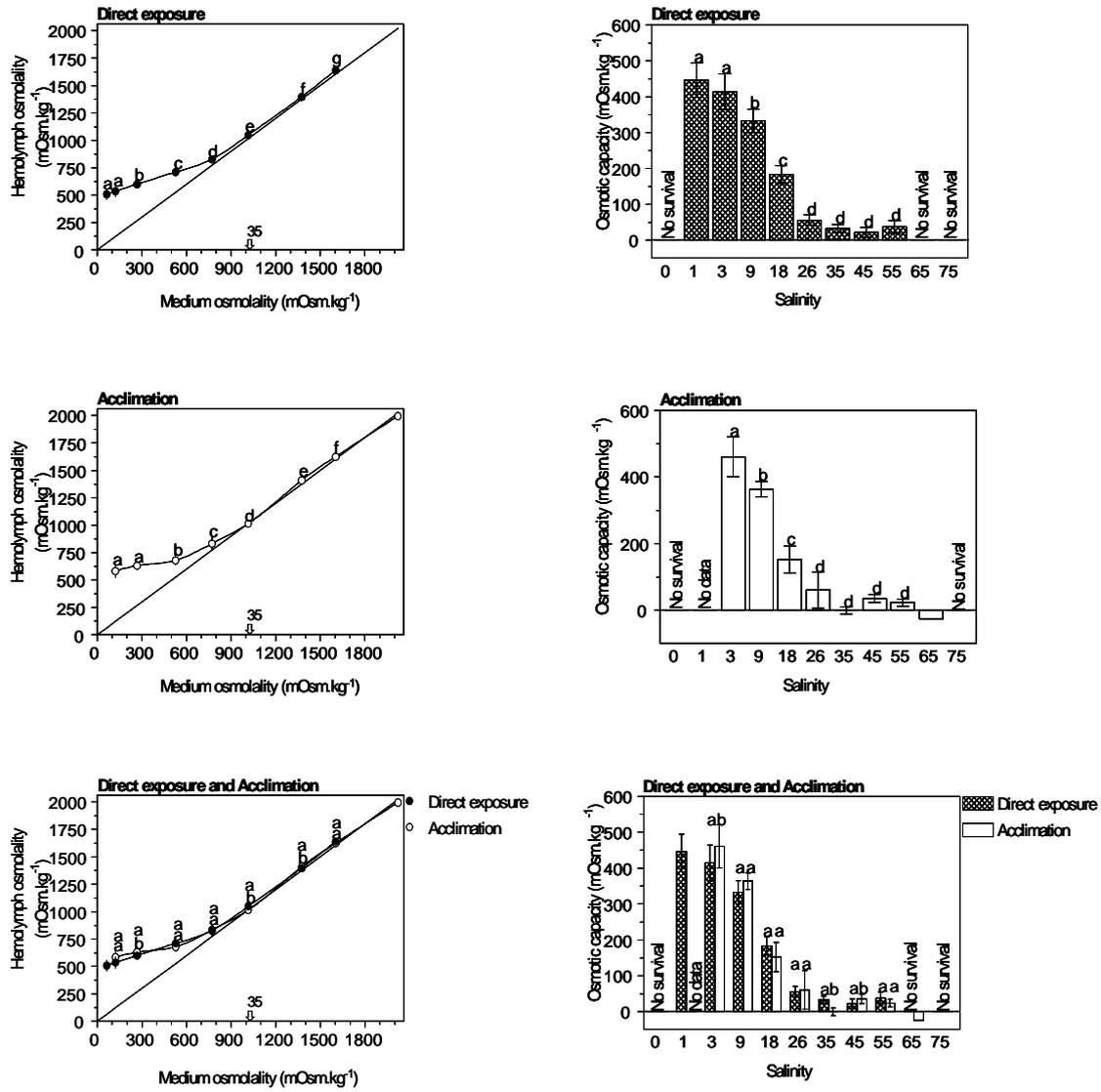


Figure 2.19. *Callinassa kraussi*. (left) Relationship between hemolymph osmolality (mOsm.kg⁻¹ ± 1 SD) and osmolality of the surrounding medium after 96 hrs following direct exposure or acclimation to various salinities. The diagonal line is the isosmotic line. The arrow pointing downward represents a salinity of 35 (seawater). Different letters indicate significant differences between mean values (either one-way ANOVA or t-tests, p < 0.05). (right) Osmoregulatory capacity (mOsm.kg⁻¹ ± 1 SD) after 96 hrs following direct exposure or acclimation to various salinities. Different letters indicate significant differences between mean values (either one-way ANOVA or t-tests, p < 0.05).

2.4. Discussion

Each of the species investigated in the present study have two common physiological adaptations. Firstly, they are all euryhaline since they are able to tolerate wide ranges of salinity, and secondly they are all osmoregulators. Euryhalinity is considered to be an important adaptation for organisms inhabiting estuaries, since they must be able to withstand the highly variable salinities that characterise this environment. The degree of euryhalinity varied between the different species investigated, and this is discussed further below. Osmoregulation is also an important adaptation for organisms inhabiting estuaries (Vernberg & Vernberg 1962, Mantel & Farmer 1983; Péqueux 1995). The ability to osmoregulate also varied between the different species investigated, and is also discussed further below.

Chiromantes eulimene is a euryhaline, very strong hyper-hypo-osmoregulator. In the direct exposure and acclimation experiments *C. eulimene* was able to tolerate salinities between 0-65 (Figure 2.10). Bolt & Heeg (1975) evaluated salinity tolerance and osmoregulatory capability of a population of *C. eulimene* from the Umgeni River estuary, which is about 180 km south of Richards Bay. These crabs tolerated salinities between 0.7 - 34 for up to 14 days in the laboratory. Bolt & Heeg (1975) only investigated the latter salinity range. The only mortalities recorded in the crabs from the Umgeni River estuary was at salinities between 0.7 - 2, where between 60 - 80% of survived. Survival after 96 hours in the study by Bolt & Heeg (1975) was very similar to that found in the present study at similar salinities. The present study shows that *C. eulimene* is also able to tolerate a salinity of 0 for at least 96 hours, and also that the upper limit of its salinity tolerance is somewhere between 65-75 (Figure 2.10). Bolt & Heeg (1975) found an identical isosmotic point for *C. eulimene* to that found in the present study (about a salinity of 26), and the trend in hemolymph osmolality after 96 hrs exposure was almost identical at similar salinities.

Chiromantes eulimene can be found in the upper regions of estuaries, where the salinity is low (e.g. Macnae 1968, Alexander & Ewer 1969, Day 1974, Gillikin 2000) and in areas of mangroves where there is a strong freshwater influence (Gillikin 2000). Although *C. eulimene* is able to tolerate freshwater for 96 hrs in the laboratory, it is not known to live permanently in freshwater. The high tolerance of low salinities permits it to live in the upper regions of estuaries, where the salinity is low. *Chiromantes eulimene* inhabits the intertidal zone, and can be found in areas that are only covered by tides for a few days at spring high tide. This crab does not burrow, and it must therefore depend on water that remains on the surface in small pools after rainfall or after the tide has receded, and also on water in other crab burrows. *Chiromantes eulimene* may possibly also use sediment pore water, since it has very fine setae at the base of the legs. Other crabs use these setae to take sediment pore water up (Branch & Branch 1981; Gillikin 2000). The salinity of these different water sources in the intertidal zone can vary widely. When it rains, the salinity can decrease rapidly, and when it is hot evaporation can lead to high salinities. Gillikin (2000) measured sediment pore water salinities between 1-90 in a Kenyan mangrove. In the direct exposure experiment, *C. eulimene* was able to survive direct exposure to a salinity of 0, from a salinity of 35 at which the crabs were held in the laboratory

before the experiment began. This crab is therefore able to tolerate a wide, fast decrease in salinity, such as that which might occur during heavy rainfall.

Chiromantes eulimene is a very strong osmoregulator. The ability to osmoregulate is much better at salinities below the isosmotic point compared to salinities above the isosmotic point. Hemolymph osmolality was very well regulated at salinities between 3-26 (Figure 2.18). This salinity range is equivalent to a range in osmolality of about 770 mOsm.kg⁻¹, but over this range the mean hemolymph osmolality of *C. eulimene* differed by only about 70 mOsm.kg⁻¹. Strong regulation of hemolymph is often found in crabs from the upper mangal with its widely fluctuating salinities (Greenway 1988). The benefit of this efficient regulation of the hemolymph osmolality is that metabolic reactions that depend on a stable hemolymph environment will not be adversely affected during variations in the salinity of the surroundings.

The sesarmid crab *Parasesarma catenata* occurs in the same estuaries as *C. eulimene* along the east Coast of South Africa. *Chiromantes eulimene* penetrates further into the low salinity upper reaches of estuaries compared with *S. catenata* (Bolt & Heeg 1975). Bolt & Heeg (1975) reported that *P. catenata* tolerates salinities from 0.7- 34. Affat (2000) reported that *P. catenata* tolerated salinities between 0-49 (the range investigated) at a temperature of 20°C, and as high as salinity of 70 at 28°C (latter salinity only investigated at latter temperature). Mortality only occurring at a salinity of 0 only when the temperature was 12°C, thus combination of low salinity and low temperature does not favour the survival of *P. catenata*. Bolt & Heeg (1975) reported that the capability to regulate body fluid is more efficient in lower salinities for *C. eulimene* than *P. catenata*, and they suggested that this explains why *C. eulimene* penetrates further into estuaries compared to *P. catenata*. Species common in freshwater have lower internal osmolalities in freshwater and lower isosmotic points (Gillikin 2004), this conforms to the isosmotic point of *C. eulimene* at a salinity of 26 and *P. catenata* at a salinity of 28. The lowering of the hemolymph is considered as an adaptation to freshwater environments (Schubart & Diesel 1998).

Gillikin *et al.* (2004) compared tolerance with salinity and osmoregulation in two sesarmid crabs in Kenya, namely *Neosarmatium meinerti* inhabiting high shore in the intertidal and *Neosarmatium smithi*, by exposing them to salinities between 16-65. *Neosarmatium meinerti* tolerated the whole range of salinity for four weeks. These results correspond to a previous study by Gross *et al.* (1966), who found the salinity tolerance of *N. meinerti* to be from a salinity of 0-65. *Neosarmatium smithi* survived a salinity of 65 for one week, and salinities from 16-48 for three weeks with elevated mortality in the fourth week. Both species were also shown to be strong hyper-hypo-osmoregulators.

Gillikin (2004) found *Chiromantes ortmanni*, a closely related species to *C. eulimene*, to be extremely euryhaline, tolerating salinities from 0- 65 and for limited time of 24 hours also a salinity of 80. *Chiromantes eulimene* tends to be associated with freshwater compared with *C. ortmanni* (Macnae 1968). In freshwater its body fluid was at 806 ± 51 mOsm.kg⁻¹ and isosmotic at a salinity of 33 whereas *C. eulimene* had hemolymph osmolality ± 730 mOsm.kg⁻¹. *Chiromantes ortmanni* is a strong hyper-hypo-regulator allowing them to

survive in a harsh environment of rapid fluctuation in terms of salinity and maintains its body fluid and isosmotic point higher than *C. eulimene* which is common in lower salinities.

Sesarmid crabs inhabiting east African estuaries and mangroves have common physiological adaptations. These species are all “semiterrestrial” and are exposed to similar environmental conditions. They are all euryhaline which is an important adaptation to intertidal and estuarine environment with fluctuating salinity. They also follow hyper-hypo-osmoregulation strategy. Belonging to the same family and coming from the same ancestor may be the reason why they show these similar adaptations.

Metopograpsus thukuhar tolerated salinities from 3-55 in the acclimation experiments (Figure 2.9). In the direct exposure experiments however this crab was only able to tolerate salinities between 18-55 (Figure 2.9). Spaargaren (1977) also investigated salinity tolerance in *M. thukuhar* from the Red Sea, and found that crabs tolerated salinities between 10-50. Spaargaren (1977) did not provide survival data however, so it is not possible to compare the actual levels of survival at different salinities. The slight difference in the salinity tolerance range found in the present study and the study by Spaargaren (1977) may be due to the fact that different populations were used for experiments. Stanton & Felder (1992) and Thurman (2003) found differences in the salinity tolerance ranges of spatially separated populations of the same crab species. Owen & Forbes (2002) studied *Paratyloidiplax blephariskios*, a crab endemic to southern Africa, from two different estuaries, St. Lucia and Mhlathuze. They found that the salinity tolerance ranges of this crab from the two estuaries differed by a salinity of 5. The Mhlathuze population had an upper tolerance limit of 60 compared with 55 in the St. Lucia population. This difference was attributed to the fact that the Mhlathuze population is exposed to a wider range of salinity fluctuations arising from greater tidal exchange volumes and freshwater input compared with St. Lucia, which is deprived of its most essential freshwater source due to being artificially separated from Mfolozi River mouth (Whitfield & Taylor 2009).

According to its salinity tolerance range in the acclimation experiments, *M. thukuhar* cannot inhabit the upper reaches of the estuary where the salinity is permanently below 3. *Metopograpsus thukuhar* was unable to survive at salinities below 18 for 96 hr in the direct exposure experiments (Figure 2.9). This does not mean that it cannot tolerate low salinities in the intertidal zone that might be caused by rainfall, as survival in freshwater was still quite high after 24 hrs. This crab lives in areas of the intertidal zone that are covered by tides each day, and it will not need to tolerate freshwater for fairly long periods.

Metopograpsus thukuhar is a strong hyper-hypo-osmoregulator (Figure 2.17). Spaargaren (1977) reported the hemolymph osmolality of *M. thukuhar* at medium osmolalities of 290, 870, 1160 and 1450 mOsm.kg⁻¹ to be 810, 860, 940, 1100 mOsm.kg⁻¹ respectively. This was very similar to the present study, where hemolymph osmolality was 750, 820, 938 and 1070 mOsm.kg⁻¹ when the surrounding medium was 290, 870, 1160 and 1450 mOsm.kg⁻¹ respectively (Figure 2.17). Results for *M. thukuhar* can also be compared to the related species *Metopograpsus messor* that was investigated by Kamemoto & Kato (1969), who found that this crab tolerated salinities between 9-34. *Metopograpsus messor* is a stronger regulator than *M. thukuhar* as

it kept its hemolymph osmolality constant at 965 mOsm.kg^{-1} in all tolerated salinities. Hemolymph osmolality of *M. messor* was also therefore kept higher than that of *M. thukuhar* at comparable salinities.

Macrophthalmus depressus can be classified as euryhaline since it tolerated a wide range of salinity, from a salinity of 3-65 (Figure 2.6). There was a preference for salinities between 18-55, survival in these salinities >90%. *Macrophthalmus depressus* is a weak osmoregulator, exhibiting hyper-regulation at low salinities and hypo-regulation at higher salinities, with low Osmotic Capacity values (Figure 2.15). *Macrophthalmus grandidieri* is difficult to classify as only salinities between 0-35 were investigated (Figure 2.7). *Macrophthalmus grandidieri* was unable to tolerate salinities below 9, and is also a weak hyper-osmoregulator at the salinities investigated (Figure 2.16). The findings for these species can be compared to other species of the genus *Macrophthalmus* for which information is available. The range of salinities tolerated by *Macrophthalmus setosus* is 10-60, and the range tolerated by *Macrophthalmus crassipes* is at a salinity of 30-60 (Barnes 1967). *Macrophthalmus setosus* and *M. crassipes* are also weak osmoregulators, and are isosmotic to their surrounding environment in seawater of a salinity of 35, as is *Macrophthalmus depressus*. *Macrophthalmus grandidieri* and *M. depressus* live in the lower region of the intertidal and will thus be exposed for shorter periods to rainfall-induced low salinities during low tide.

Callinassa kraussi is euryhaline, tolerating salinities between 1-65 (Figure 2.11). Previous studies have also identified this species as euryhaline (Day 1951; Allanson *et al.* 1974; Forbes 1974; Day 1981; Hill 1981). Day (1951) provided a salinity tolerance range of 1.25-59.5. Forbes (1978) reported a population of *C. kraussi* living in upper reaches of the Keurbooms River estuary at a salinity of 1. *Callinassa kraussi* is a strong hyper-regulator at salinities between 1-25, and isosmotic at salinities above a salinity of 25 (Figure 2.19). Forbes (1974) found that *C. kraussi* is a hyper-regulator at salinities between 3.5-21 and is isosmotic at higher salinities. This wide tolerance of salinity agrees with the wide distribution of adult *C. kraussi* in estuaries, from the lowest reaches and also in the extreme upper reaches (e.g. Forbes 1974). Thompson & Pritchard (1969) found *C. californiensis* to osmoconform at salinities between 10-34 and Felder (1978) found *C. major* and *C. islagrande* to osmoconform at salinities of about 10-35, and *C. jamaicaense* to hyperregulate at salinities between 3.5-20 and osmoconform from there until a salinity of 35, which is similar to this study for *C. kraussi*.

Previous work by Newman & Khanyile (unpublished data) on *C. kraussi* larvae and the decapodid (first juvenile stage) allows for the comparison of salinity tolerance between the early life history stages and the adults. *Callinassa kraussi* has two non-planktonic larval stages, which remain in parent burrows. Changing water conditions in an estuary may expose larvae to variations in salinity and this could influence their survival. These variations in environmental conditions may be due to tidal rhythms, rainfall or unseasonal weather patterns may apply stress that may affect feeding, development or survival ability (Johns 1981).

Many investigations involving decapod crustaceans have focused on demonstrating that early life history stages tend to be less tolerant of environmental variables than the adults (e.g. Kinne 1964, Robert 1971;

Anger & Charmantier 2000; Lemaire *et al.* 2002; Cieluch *et al.* 2004). Larvae and immature prawns are more sensitive to low salinities than the adult stages (Forbes 1978). Salinity tolerance is often different at different life cycle stages (Kinne 1964). All of these findings correspond with the results presented in Figure 2.20 for the two zoeal stages and the decapodid of *C. kraussi*. The zoeae 1 tolerated salinities between 17-52. There was an increase in salinity tolerance in the zoeae 2 and decapodid to salinities of 9-52 (only a single individual of the zoeae 2 survived a salinity of 61). This wide tolerance of the larval stages is important, since the larval stages complete development in the parents burrow, and have no way of avoiding changes in the salinity of the water column. This is different from the larvae of most estuarine crustaceans, which are unable to tolerate low salinities and therefore leave the estuary and complete development in the sea, and then return to estuaries immediately before metamorphosis to the first crab stage. Vorsatz (2000) also found that zoeae 1 and zoeae 2 of *C. kraussi* could not tolerate salinities below 8 across all temperature ranges from 16-28°C and survival of less than 30% for both stages at higher temperatures. No salinities higher than 35 were investigated by Vorsatz (2000).

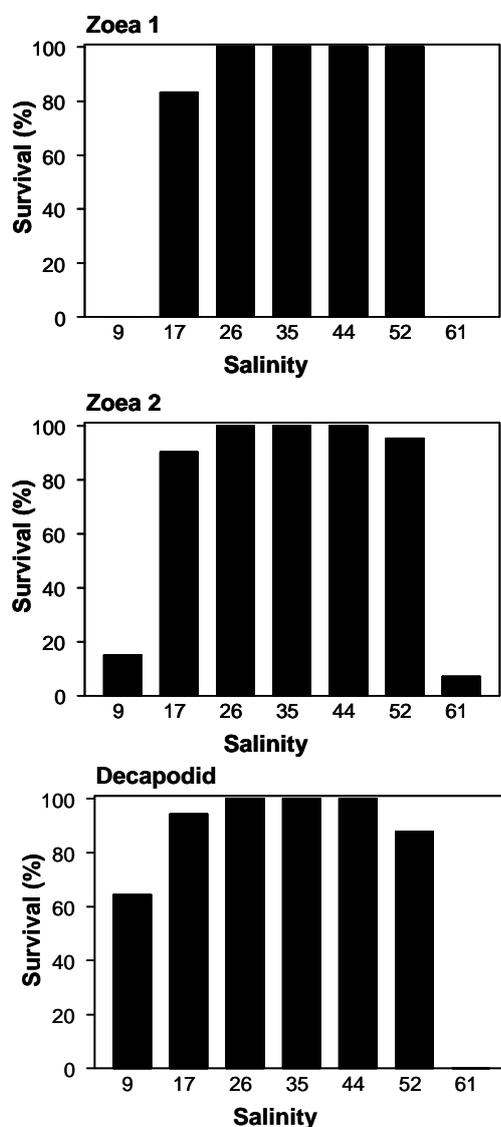


Figure 2.20. *Callianassa kraussi*. Ontogenetic changes in the salinity tolerance of successive larval stages and the decapodid (Newman & Khanyile unpublished data)

No previous work has reported on the salinity tolerance or osmoregulation of *Dotilla fenestrata*, but Day (1981) described this species as euryhaline. Data from the present study agree with this, since this crab was able to tolerate salinities between 0-65 in the acclimation experiments (Figure 2.8). Salinity tolerance of *D. fenestrata* can be compared with that of *D. myctiroides* which was exposed to salinities between 7-35 by Matsumasa *et al.* (2001). *Dotilla fenestrata* was more tolerant to lower salinities than *D. myctiroides* with the lowest tolerance at a salinity of 10.5 and higher salinities can not be compared as they were not investigated in this study. *Dotilla myctiroides* is a hyper-osmoregulator at salinities below a salinity of 30 and an osmoconformer at and above the latter salinity.

Four species of fiddler crabs were investigated in the present study, and their salinity tolerances and osmoregulatory strategies can be compared directly. As was discussed in Chapter 1, these crabs inhabit different areas of the intertidal zone. It is important to re-examine these differences again because abiotic conditions differ at different levels of the intertidal zone, and these differences may help explain differences in salinity tolerance and osmoregulation in the fiddler crabs. *Uca vocans* is generally restricted to the lowest regions of the intertidal zone that are exposed for only a short period at low tide. Although *Uca urvillei* usually inhabits a slightly higher region of the intertidal zone it often overlaps with *U. vocans*, but may also extend quite far up the intertidal zone along the banks of creeks draining mangroves provided that the sediment is of a suitable nature and retains high moisture content. *Uca chlorophthalmus* inhabits the middle region of the intertidal zone, which is regularly covered with water at high tide. *Uca annulipes* is the most 'terrestrial' of the species, extending from about the mid-tide level through to the upper regions of the intertidal zone, often reaching high densities on unvegetated sand flats landward of mangroves that may be covered by tides only a few times in the year (Hartnoll, 1975). These fiddler crabs are exposed to large changes in osmotic stress in the intertidal zone. Gillikin (2000) measured sediment pore water salinities and crab community composition along a transect through the intertidal zone of a Kenyan mangrove and found *U. annulipes* at maximum pore water salinity of 60.5 (mean \pm SD; $44.7 \pm$ a salinity of 8.0), *U. chlorophthalmus* at a salinity of 54.5 ($40.5 \pm$ a salinity of 6.1), and *U. urvillei* and *U. vocans* at a salinity of 35.4 ($34.3 \pm$ a salinity of 1.4) and a salinity of 40.0 ($35.1 \pm$ a salinity 3.1) respectively.

All of the fiddler crabs investigated in the present study are euryhaline. In the acclimation experiments *U. vocans*, *U. urvillei* and *U. chlorophthalmus* tolerated salinities between 0-55, while *U. annulipes* tolerated salinities between 0-65 (Figure 2.2 – 2.5). The ability of *U. annulipes* to tolerate a salinity of 65 while the other species cannot agrees with the higher position that this crab occupies in the intertidal zone. In this higher area, which is drier, receives full sun and is subject to high evaporation rates, it may be exposed to sediment pore water salinities as high as 60.5 for long periods (Gillikin 2000).

Crabs that live in the upper region of the intertidal zone are more likely to be exposed to low salinity and wide salinity variation due to rainfall. It may therefore be expected that crabs from this region of the intertidal zone are better able to tolerate exposure to freshwater. Macintosh (1984) found a positive

correlation between the ability to tolerate exposure to freshwater and the region of intertidal zone occupied by several Malaysian fiddler crabs. The opposite was found in the acclimation experiments in the present study, where the species from the lower region of the intertidal zone (*U. vocans* and *U. urvillei*) showed a better ability to tolerate a salinity of 0 compared to the species from the higher region of the intertidal zone (*U. chlorophthalmus* and *U. annulipes*) (Figure 2.2 – 2.5). In the direct exposure experiments however all *U. vocans*, *U. urvillei* and *U. annulipes* died within 48 hrs of exposure, while 40% of *U. chlorophthalmus* were alive after 96 hrs. The trend in mortality for *U. vocans*, *U. urvillei* and *U. annulipes* agrees with the respective area they inhabit in the intertidal zone, with 0, 14 and 63% of crabs alive after 24 hrs respectively. This aspect is investigated further in chapter three of this study. All species had a similar tolerance (>85% survival) of a salinity of 3 following both direct exposure and acclimation from a salinity of 35. All of the fiddler crabs investigated are therefore able to tolerate a wide, fast reduction in salinity in the intertidal zone, as might occur during heavy rainfall.

Crab species of the genus *Uca* have well-developed powers of osmotic and ionic regulation (Holliday 1985, Zanders & Rojas 1996, Thurman 2003). All of the *Uca* crabs investigated in the present study adopt a hyper-hypo-osmoregulatory strategy, hyper-regulating at salinities below the isosmotic point and hypo-regulating at salinities above the isosmotic point. There was almost no difference in the hemolymph osmolality of *U. vocans* and *U. urvillei* at different external salinities. *Uca annulipes* however maintained its hemolymph osmolality slightly higher at salinities below 35, and lower at higher salinities (Figure 2.4). Thurman (2003) has reported habitat having a greater impact on osmoregulation among *Uca* species than taxonomic relationship. Salinity tolerance and osmoregulation of these species conform to the position they occupy in the intertidal zone. *Uca annulipes* has a wide salinity tolerance and is the strong osmoregulator - it lives high up in the intertidal zone where it has to withstand fluctuating salinities due to rainfall during low tide. *Uca vocans* and *U. urvillei* tolerate a narrow range of salinity and are weaker regulators than *U. annulipes*, they inhabit the lower and middle levels in the intertidal area.

An interesting comparison can be made between two unrelated species that live in the extreme upper reaches of the intertidal, namely *U. annulipes* and *C. eulimene*. Since they live in this same area, both of these crabs are exposed to similar environmental conditions. It would be expected therefore that they would show similar adaptations. Both species tolerate salinities from 0-65 and are strong hyper-hypo-regulators (Figure 2.4 and 2.10). *Uca annulipes* is however less tolerant to reduced salinities compared to the *C. eulimene* species, whereas *C. eulimene* is less tolerant to elevated salinities compared to *U. annulipes* (Figure 2.14 and 2.18). The isosmotic point of *U. annulipes* is at a salinity of 30 whereas for *C. eulimene* it is at a salinity of 26. Previously it was mentioned that species that can tolerate low salinities best have a lower hemolymph osmolality and lower isosmotic point (Gillikin 2004). Hemolymph osmolality of *U. annulipes* was kept a little higher and isosmotic point was at higher salinities than that of *C. eulimene*, suggesting that *U. annulipes* is not well adapted to withstand low salinities for long periods. The limited ability of *U. annulipes* to tolerate freshwater exposure is due to the fact that it is unlikely that it will experience complete freshwater

and rainfall does not decrease pore water salinity to a salinity of 0, and in any case it is able to withstand a salinity of 0 salinity for longer than a single tidal period. An important difference between these crabs is that *U. annulipes* is a burrower while *C. eulimene* does not burrow. In the upper reaches of the intertidal zone, when conditions become unfavourable *C. eulimene* can move and look for a more suitable environment while *U. annulipes* will have to tolerate those conditions as it seeks shelter from predators in its burrow. This may explain why *U. annulipes* is able to better tolerate very high salinities of 65 compared to *C. eulimene*.

Salinity and temperature are important environmental variables in the natural environment, and changes in both variables often occur together. Combinations of salinity and temperature frequently interact in nature affecting organisms differently than when acting separately. For animals to survive these changes they must accommodate, tolerate or avoid fluctuations. The estuarine environment is a highly variable environment with daily fluctuations in both salinity and temperature (Day 1981). The fluctuating physicochemical conditions of estuaries limit the diversity of organisms, with few organisms able to adapt to life in estuaries. Although salinity is the most significant variable factor, its effect is frequently modified by temperature (Kinne 1964).

2.5. Conclusions

The principal characteristic of an estuarine organism is its ability to accommodate changes in salinity. All species investigated in this study have this adaptation, that is, they are euryhaline. The species followed different osmoregulatory strategies however. Although most were hyper-hypo-osmoregulators, *C. kraussi* is a hyper-regulator at low salinities and an osmoconformer at high salinities. The ability to hyper and hypo-regulate differed widely among the different crab species tested. Therefore, euryhalinity is not necessarily associated with a strong hyper-hypo-osmoregulatory strategy.

The determination of an animal's estuarine distribution based on its salinity tolerance range in the laboratory is often criticised, as it does not always correspond to the actual distribution range of the animal in the natural environment (Dorgelo 1976). According to the results obtained in this study, most species investigated (except *M. grandidieri*) can theoretically inhabit almost the entire length of the estuary. Previous studies (e.g. Mclachlan & Grindley 1974; Hill 1981; Teske & Wooldridge 2003) have however shown that salinity is not the only factor that governs the distribution of organisms along the estuary, with other factors such as substrate, food availability and competition also being involved.

The information obtained in the present study does however tend to rule out the influence of salinity for most species, and any differences in their penetration of the estuarine environment must be due to factors other than salinity alone.

2.6. References

- AFFAT, S.V. 2000. Salinity tolerance and osmoregulation as a function of ambient temperature in the marsh crab, *Sesarma catenata*. Honours thesis, University of Port Elizabeth, South Africa.
- ALEXANDER, S.J. & EWER, D.W. 1969. A comparative study of some aspects of the biology and ecology of *Sesarma catenata* Ort and *Cyclogras punctatus* M. Edw. with additional observations on *Sesarma meinerti* De Man. *Zoologica Africana* **4**: 1-35.
- ALLANSON, B.R., BRENNON, M.N. & HART, R.C. 1974. The plants and animals of Lake Sibaya, KwaZulu, South Africa: A checklist. *Review of Zoologica Africana*. **88**: 507-532.
- ANGER, K. & CHARMANTIER, G. 2000. Ontogeny of osmoregulation and salinity tolerance in a mangrove crab, *Sesarma curacaoense* (Decapoda: Grapsidae). *Journal of Experimental Marine Biology and Ecology* **251**: 265-274.
- BARNES, R.S.K. 1967. The osmotic behaviour of a number of grapsoid crabs with respect to their differential penetration of an estuarine system. *Journal of Experimental Biology* **47**: 535-551.
- BEGG, G. (1978). The estuaries of Natal. Natal Town and Regional Planning Commission Report, Pitermaritzburg, 41.
- BOLTT, G. & HEEG, J. 1975. The osmoregulatory ability of three grapsoid crab species in relation to their penetration of an estuarine system. *Zoologica Africana* **10**: 167-182.
- BRANCH, G. & BRANCH M. 1981. *The living shore's of Southern Africa*. Struik Publishers, Cape Town.
- CIELUCH, U., ANGER, K., AUJOLAT, F., BUCHHOLZ, F., CHARMANTIER-DAURES, M & CHARMANTIER, G. 2004. Ontogeny of osmoregulation structures and functions in the green crab *Carcinus maenas* (Crustacea: Decapoda). *The Journal of Experimental Biology* **207**: 325-336.
- *DAY, J.H. 1951. The ecology of South African estuaries. Part 1. A review of estuarine conditions in general. *Transactions of the Royal Society of South Africa* **23**: 53-91.
- DAY, J. H. 1974. The ecology of Morrumbene estuary, Mozambique. *Transactions of the Royal Society of South Africa* **41**: 43-97.
- DAY, J. H. 1980. What is an estuary? *South African Journal of Science* **76**: 198.
- DAY, J.H, MILLARD, N.A.H. & HARRISON, A.D. 1952. The ecology of South African estuaries. Part III. Knysna: A clear, open estuary. *Transactions of the Royal Society of Africa* **33**: 367-413.
- DAY, J.H. 1981. The estuarine fauna. In: *Estuarine Ecology with particular reference to southern Africa*, (ed) J.H. Day, Ch 9. A A Balkema, Cape Town.
- DE VILLIERS, C.J. & HODGSON, A. 1999. Studies on estuarine macroinvertebrates - The macrobenthos. In: *Estuaries of South Africa* (eds) B.R. Allanson & A.D. Baird, Ch. 8. Cambridge University Press, UK.
- DORGELO, J. 1976. Salt tolerance in crustacea and the influence of temperature upon it. *Biological Review* **51**: 255-290.
- EDNEY, E.B. 1961. The water and heat relationship of fiddler crabs (*Uca* spp.). *Transaction of Royal Society of South Africa* **36**: 71-91.

- FELDER, D.L. 1978. Osmotic and ionic regulation in several western Atlantic *Callinassidae* (Crustacea, Decapoda, Thalassinidea). *Biological Bulletin* **54**: 409-429.
- FORBES, A.T. 1974. Osmotic and ionic regulation in *Callinassa kraussi* Stebbing (Crustacea: Decapoda: Thalassinidea). *Marine Biology* **22**: 361-365.
- GILLIKIN, D.P. 2000. Factors controlling the distribution of Kenyan brachyuran mangrove crabs: salinity tolerance and ecophysiology of two Kenyan *Neosarmatium* specie. M.Sc. thesis, Vrije Universiteit Brussel, Brussels.
- GILLIKIN, D.P. 2004. Osmoregulatory ability of *Chiromantes ortmanni* (Crosnier, 1965) subjected to dilute and hypersaline seawater. *Crustaceana* **77**: 67-74.
- GILLIKIN, D.P., DE WACHTER, B. & TACK, J.F. 2004. Physiological responses of two ecological important Kenyan mangrove crabs exposed to altered salinity regimes. *Journal of Experimental Biology and Ecology* **301**: 93-109.
- GREENWAY, P. 1988. Ion and water balance. In: *Biology of land crabs*, (eds) W.W. Burggren & B.R. McMahon, Cambridge University Press, Cambridge, UK.
- GROSS, W.J., LASIEWSKI, R.C., DENNIS, M. & RUDY Jr, P. 1966. Salt and water balance in selected crabs of Madagascar. *Comparative Biochemistry and Physiology* **17**: 641-660.
- HARTNOLL, R.G. 1975. The grapsidae and ocypodidae (Decapoda: Brachyura) of Tanzania. *Journal Zoology of London* **177**: 305-328.
- HILL, B.J. 1979. Biology of the crab *Scylla serrata* (Forsk.) in the St. Lucia system. *Transaction of Royal Society of South Africa* **44**: 55-62.
- HILL, B.J. 1981. Adaptation to temperature and salinity stress in southern African Estuaries. In: *Estuarine Ecology with particular reference to Southern Africa*, (ed) J.H. Day, Ch 11. A A Balkema, Cape Town.
- HOLLIDAY, C. W. 1985. Salinity-induced changes in Na, K-ATPase activity in the mud fiddler, *Uca pugnax*. *Journal of Experimental Zoology* **223**: 199-208.
- ICELY, J.D. & JONES, D.A. 1978. Factors affecting the distribution of the genus *Uca* (Crustacea: Ocypodidae) on an East African shore. *Estuarine and Coastal Marine Science* **6**: 315-325.
- JOHNS, D.M. 1981. Physiological studies on *Cancer irroratus* larvae. I. Effects of temperature and salinity on survival development rate and size. *Marine Ecological Progress Series* **233**: 575-583.
- KINNE, O. 1964. The Effect of Temperature and Salinity on Marine and Brackish Water Animals. *Oceanography and Marine Biology Review* **2**: 281-339.
- *KAMEMOTO, F.I & KATO, K.N. 1969. The osmotic and chloride regulative capabilities of five Hawaiian decapod crustaceans. *Pacific Science*. **23**: 232-237.
- LEMAIRE, P., BERNARD, E., MARTINEZ-PAZ, J.A. & CHIM, L. 2002. Combined effect of temperature and salinity on osmoregulation of juvenile and subadult *Penaeus stylirostris*. *Aquaculture* **209**: 307-317.
-

- MACINTOSH, D.J. 1984. Ecology and productivity of Malaysian mangrove crab populations (Decapoda: Brachyura). In: *Proceedings of the Asian symposium on mangrove management*, (eds) E. Soepadmo, A.N. and D.J. Macintosh, University of Malaysia and UNESCO, Malaysia.
- MACINTOSH, D.J. 1988. The ecology and physiology of decapoda of mangrove swamp. *Symposia. Zoological Society of London* **59**: 315-341.
- MACLACHLAN, A. & GRINDLEY, J.R. 1974. Distribution of soft substrata in Swartkops estuary with observations on the effect of floods. *Zoologica Africana* **9**: 211-233.
- MACNAE, W. 1968. A general account of the fauna and flora of mangrove swamps and forest in the Indo-West-Pacific region. *Advances in Marine Biology* **6**: 73-270.
- MANTEL, L.H. & FARMER, L.L. 1983. Osmotic and ionic regulation. In: *The biology of crustacea, Internal Anatomy and Physiological Regulation*, (eds) D.E. Bliss & L.H. Mantel. Ch. 2. Academic Press, New York.
- MATSUMASA, M., KIKUCHI, S., TAKEDA, S., POOVACHIRANON, S., YONG, H. & MURAI, M. 2001. Blood osmoregulation and ultra structure of the gas windows ('tympana') of intertidal Ocypodid crabs: *Dotilla* vs. *Scopimera*. *Benthos Research* **56**: 47-55.
- MILLARD, N.A.H. & BROEKHUYSEN, G.J. 1970. The ecology of South African estuaries. Part X. St. Lucia: A second report. *Zoologica Africana* **5**: 277-307.
- OWEN, R.K. & FORBES, A.T. 2002. Salinity tolerance of the burrowing ocypodid crab, *Paratyloidiplax blephariskios*, in the St. Lucia and Mhlathuze estuaries, KwaZulu-Natal, South Africa. *African Journal of Aquatic Science* **27**: 21-29.
- PÉQUEUX, A. 1995. Osmotic regulation in Crustaceans. *Journal of Crustacean Biology* **15**: 1-60.
- ROBERT, M. H. 1971. Larval development of *Pagurus longicarpus* Say reared in the laboratory. II. Effects of reduced salinity on larval development. *Biological Bulletin* **140**: 104-116.
- SASEKUMAR, A. 1974. Distribution of macrofauna on a Malaysian mangrove shore. *Journal of Animal Ecology*. **43**: 51-69.
- SCHUBART, C.D. & DIESEL, R. 1998. Osmoregulation capacities and penetration into terrestrial habitats: a comparative study of Jamaican crabs of the genus *Armases* Abele, 1992 (Brachyura: Grapsidae: Sesarminae). *Bulletin of Marine Science* **60**: 743-752.
- SPAARGAREN, D. H. 1977. On the water and salt economy of some decapod crustaceans from the Gulf of Aqaba (Red Sea). *Netherlands Journal of Sea Research* **11**: 99-106.
- STANTON, J.L. & FELDER, D.L. 1992. Osmoregulatory capacities in disjunct western Atlantic populations of the *Sesarma reticulatum* complex (Decapoda: Grapsidae). *Journal of Crustacean Biology* **12**: 335-341.
- TESKE, P.R. & WOOLDRIDGE, T. 2003. What limits the distribution of subtidal macrobenthos on permanently open and temporarily open/closed South African estuaries? Salinity vs. sediment particle size. *Estuarine, Coastal and Shelf Science* **57**: 225-238.
- THOMPSON, L.C. & PRITCHARD, A.W. 1969. Osmoregulation capacities of *Callinassa* and *Upogebia* (Custacea: Thallassinidea). *Biological Bulletin. (Woods Hole, Mass)* **136**: 114-129.
-

- THURMAN, C. 2003. Osmoregulation by six species of fiddler crabs (*Uca*) from the Mississippi delta area in the northern Gulf of Mexico. *Journal of Experimental Marine Biology and Ecology* **291**: 233-253.
- VERNBERG, W.B & VERNBERG, J.V. 1972. *Environmental physiology of marine animals*. Springer-Verlag. New York.
- VORSATZ, J. 2000. Life history strategies in the estuarine sandprawn *Callinassa kraussi*. Masters thesis, University of Port Elizabeth, South Africa.
- WHITFIELD, A.K. 1992. A characterization of southern African estuarine system. *Southern African Journal of Aquatic Sciences*. **18**: 89-103.
- ZANDERS, I.P. & ROJAS, W.E. 1996. Osmotic and ionic regulation in the fiddler crab *Uca rapax* acclimated to dilute and hypersaline seawater. *Marine Biology* **125**: 315-320.

* Original not seen

Chapter 3

Salinity tolerance and hemolymph osmotic responses in several crabs following osmotic shock

3.1. Introduction

The intertidal zone is a narrow fringe of land between ocean and land, which is alternately exposed to air and covered by water (Vernberg & Vernberg 1972). Organisms inhabiting the intertidal zone are exposed to wide variations in environmental factors, including temperature, salinity, and oxygen concentration (Vernberg & Vernberg 1972; Lohrer *et al.* 2000). Temperature has been shown to influence tolerance to salinity of decapod crustacea, and occasionally also osmoregulatory capability (e.g. Dehnel 1962, Dorgelo 1981, Kirkpatrick & Jones 1985, Lemaire *et al.* 2002). Because of these fluctuating environmental factors, it is not surprising to find that intertidal organisms can usually tolerate more extreme conditions than sub-tidal organisms. Crabs inhabiting the upper region of the intertidal zone are more likely, and for a longer period, to be exposed to low or high salinities and also wide salinity variations caused by heavy rainfall compared to crabs from the lower intertidal zone. It might therefore be expected that crabs from the upper intertidal zone should be able to tolerate low salinities better than crabs from the low intertidal zone. Indeed, Macintosh (1984) found a correlation between the ability to tolerate exposure to freshwater and the region of the intertidal zone occupied by several Malaysian fiddler crabs.

Euryhalinity and a strong hemolymph regulatory capability are considered advantageous in environments where there is a wide variation in salinity. One reason is that metabolic reactions that are dependant on a stable hemolymph environment will be only minimally influenced during such variation. The ability to maintain a slow change to the new hemolymph osmotic state following osmotic shock is probably also advantageous, for the same reason. In weakly hyper-regulating decapod crustaceans direct exposure to salinities far below that at which individuals were acclimated often results in a rapid decrease in hemolymph osmolality, and is often accompanied by an ‘undershoot’ before hemolymph osmolality stabilises (e.g. *Callinassa kraussi*, Forbes 1974). Metabolic processes dependent on a stable hemolymph environment may be adversely affected by such variation in hemolymph osmolality.

This study was conducted based on salinity tolerance results of *Uca vocans*, *Uca urvillei* and *Uca annulipes* following direct exposure to a salinity of 0 (see Figure 2.2 – 2.4). These three *Uca* crab species did not survive in direct exposure to freshwater. The present study investigates survival and changes in hemolymph osmolality in *U. vocans*, *U. urvillei* and *U. annulipes* following direct exposure from a salinity of 35 to freshwater, to determine whether their responses can be related to the position of the intertidal zone occupied. Data used in this chapter for *Uca* species is the same data from chapter two.

A similar study is done for *Chiromantes eulimene*, but here crabs were transferred from a salinity of 35 to salinities of 3 and 17. These salinities were employed because 90% of *C. eulimene* survived in freshwater

following direct exposure (see Figure 2.10) and also to compare whether hemolymph osmolality will change between these two salinities. Bolt and Heeg (1975) directly transferred *Parasesarma catenata*, which is closely related to *C. eulimene*, from a salinity of 34 to a salinity of 3.4. Results for *C. eulimene* can then be compared to those for *P. catenata*.

3.2. Materials and methods

3.2.1. Collection and handling of crabs

Crabs were collected from the intertidal at various sites in Richards Bay and the Mhlatuze River estuary (Figure 2.1) with the aid of small hand-held nets. They were rinsed in the field to remove sand and mud before they were brought back to the laboratory within a few hours of collection, in plastic containers filled with water from Richards Bay.

3.2.2. Experiments with fiddler crabs

In the laboratory crabs were held individually in 400 ml of water of 35 salinity for 24 hours before experiments started. Crabs were then directly exposed to freshwater. The containers were loosely covered to limit evaporation and to prevent crabs escaping, because of this the experimental containers were not aerated. Survival and hemolymph osmolality measurements were taken after 0, 1, 2, 4, 6, 8, 12, 24, 48 and 72 hours in batches of between 8 - 10 individuals. Hemolymph extraction and measurement followed the same procedure as that in Chapter 2 (see Section 2.3).

3.2.3. Experiments with *Chiromantes eulimene*

In the laboratory the crabs were held in five tanks filled with 10 litres of seawater 35 salinity for 2 days. Between 35-40 crabs were placed into each tank. They were not fed during this period and the water was aerated. After two days, crabs were directly exposed to salinities of 3 and 17. Survival and hemolymph osmolality was measured in batches of crabs (8 - 10 individuals) after 0, 2, 4, 6, 12, 48 and 72 hours. Hemolymph extraction and measurement followed the same procedure as that in Chapter 2 (see Section 2.3).

3.2.4. Data analysis

Survival was compared by means of a z-test for pairwise comparisons and multiple comparison of proportions test for multiple comparisons. For osmoregulation One-way ANOVA (Tukey test) and t-tests were performed using SigmaStat, 2.03 version. $P < 0.05$ was taken as the level of significance. Data are shown as mean \pm SD. For osmotic capacity each crab species and anomuran prawn was calculated by subtracting the osmotic concentration of the external medium from the osmotic concentration of the hemolymph. Figure 3.1 represents time course change in survival of each *Uca* species i.e. *Uca vocans*, *Uca urvillei* and *Uca annulipes* after direct transfer from 35 to 0 salinity for 72 hours. Different letters represent statistical significant difference in survival between hours for each *Uca* species. Figure 3.2 represents the comparison between three *Uca* species i.e. *Uca vocans*, *Uca urvillei* and *Uca annulipes* after direct transfer

from 35 to 0 salinity for 72 hours. Different letters represent statistical significant difference between three *Uca* species for each time course.

3.3. Results

3.3.1. Survival

***Uca vocans*:** *Uca vocans* survived for less than 48 hours in freshwater. Survival after 12 and 24 hours was significantly similar whereas it is significantly different with the survival from 0-8 hours (Figure 3.1).

***Uca urvillei*:** *Uca urvillei* could tolerate direct exposure to freshwater for less than 72 hours. After 12 hours survival decreased to 80% and after 48 hours to 60%. Survival after 12, 24 and 48 hours was significantly similar whereas it is significantly different with the survival from 0-8 hours (Figure 3.1).

***Uca annulipes*:** *Uca annulipes* tolerated direct exposure to freshwater for more than 72 hours. After 8 hours survival decreased from 100 to 90%, at the end of 24 hours survival decreased again to 80% and the lowest survival was 70% after 48 and 72 hours. Survival after 12 hours is significantly similar to survival after 24, 48, 72 and survival from 0-8 hours, whereas survival after 24, 48 and 72 is significantly different with the survival from 0-8 hours (Figure 3.1).

***Chiromantes eulimene*:** There was 100% survival of *C. eulimene* over the entire exposure period at both salinities of 3 and 17.

3.3.2 Osmoregulation

***Uca vocans*:** Hemolymph osmolality decreased significantly from 0-1 hour and stabilized for an hour (Figure 3.3). From 2 hours it decreased significantly again to 4 hours and stabilized for two hours up to 6 hours. After 8 hours it decreased significantly until 24 hours, after which all crabs were dead. Hemolymph osmolality before exposure to freshwater (0 hour) was 930 mOsm.kg⁻¹, but after 24 hours it had decreased to 480 mOsm.kg⁻¹ (Figure 3.3).

***Uca urvillei*:** Hemolymph osmolality decreased from 0-2 hours but not significantly from 2 hours to 4 hours hemolymph osmolality decreased significantly and stabilized up to 6 hours (Figure 3.3). From 8-24 hours hemolymph osmolality decreased but not statistically significantly. After 48 hours hemolymph osmolality decreased significantly (Figure 3.3).

***Uca annulipes*:** Hemolymph osmolality decreased from 0-1 hour but not significantly and stabilized for an hour, from 2 hours it decreased but not significantly to 4 hours and stabilised for 4 hours (Figure 3.3). From 8-12 hours it decreased but not significantly. From 12-24 hours hemolymph osmolality decreased significantly, from 24-48 it decreased but not significantly and from 24-72 hemolymph osmolality decreased significantly (Figure 3.3).

Comparison among *Uca* species

Hemolymph osmolality at the start of the experiments was statistically similar between the three *Uca* species. *Uca annulipes* maintained a higher hemolymph osmolality over the entire exposure period followed by *U. urvillei* and *U. vocans*. From 1-6 hours hemolymph osmolality of *U. urvillei* and *U. annulipes* was not statistically different. After 8 hours hemolymph osmolality of *U. urvillei* dropped to be slightly higher than that of *U. vocans*, but not statistically different. After 12 hours all of the species had different hemolymph osmolality. After 24 hours hemolymph osmolality of *U. vocans* was significantly different to *U. urvillei* and *U. annulipes*, whereas after 48 hours hemolymph osmolality of *U. urvillei* and *U. annulipes* was significantly similar (Figure 3.4).

***Chiromantes eulimene*:** At a salinity of 3, hemolymph osmolality decreased significantly from 840 to 710 mOsm.kg⁻¹ after two hours exposure. Between 2-72 hours hemolymph osmolality stabilized around 710 mOsm.kg⁻¹ (Figure 3.5). Hemolymph osmolality at salinity of 17 followed the same trend in a salinity of 3, hemolymph osmolality decreasing significantly after 2 two hours from 840 to 740 mOsm.kg⁻¹, and was maintained at this level for the remainder of the experiment (Figure 3.5). When comparing hemolymph osmolality at salinities of 3 and 17, hemolymph osmolality was only significantly different at 2 hours with crabs kept at a salinity of 17 maintaining a higher concentration of hemolymph osmolality than those kept at 3 salinity (Figure 3.6).

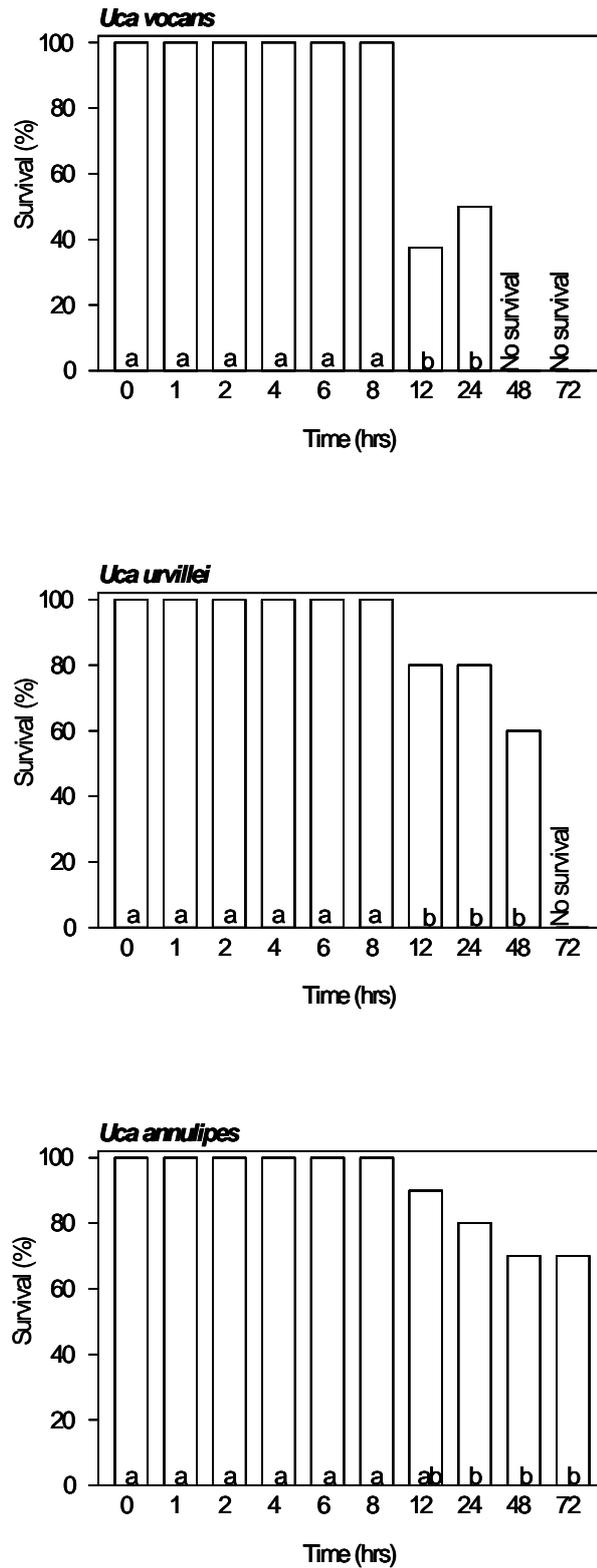


Figure 3.1. Time course of change in survival of *Uca vocans*, *Uca urvillei* and *Uca annulipes* after direct transfer from a salinity of 35 to a salinity of 0. Different letters indicate a statistically significant difference in survival (based on multiple comparison of proportions tests, $p < 0.05$).

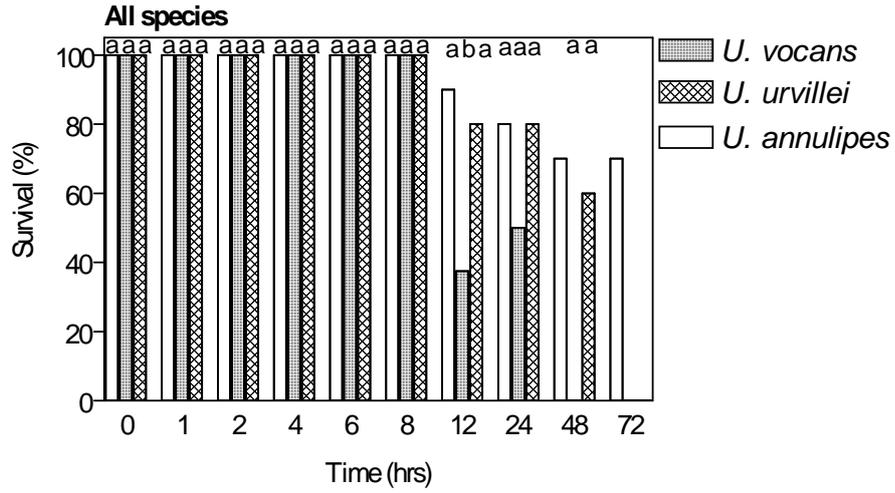


Figure 3.2. Time course of changes in survival among three *Uca* species i.e. *Uca vocans*, *Uca urvillei* and *Uca annulipes* after direct transfer from a salinity of 35 to a salinity of 0. Different letters indicate a statistically significant difference in survival between species at each salinity (based on multiple comparison of proportions tests, $p < 0.05$).

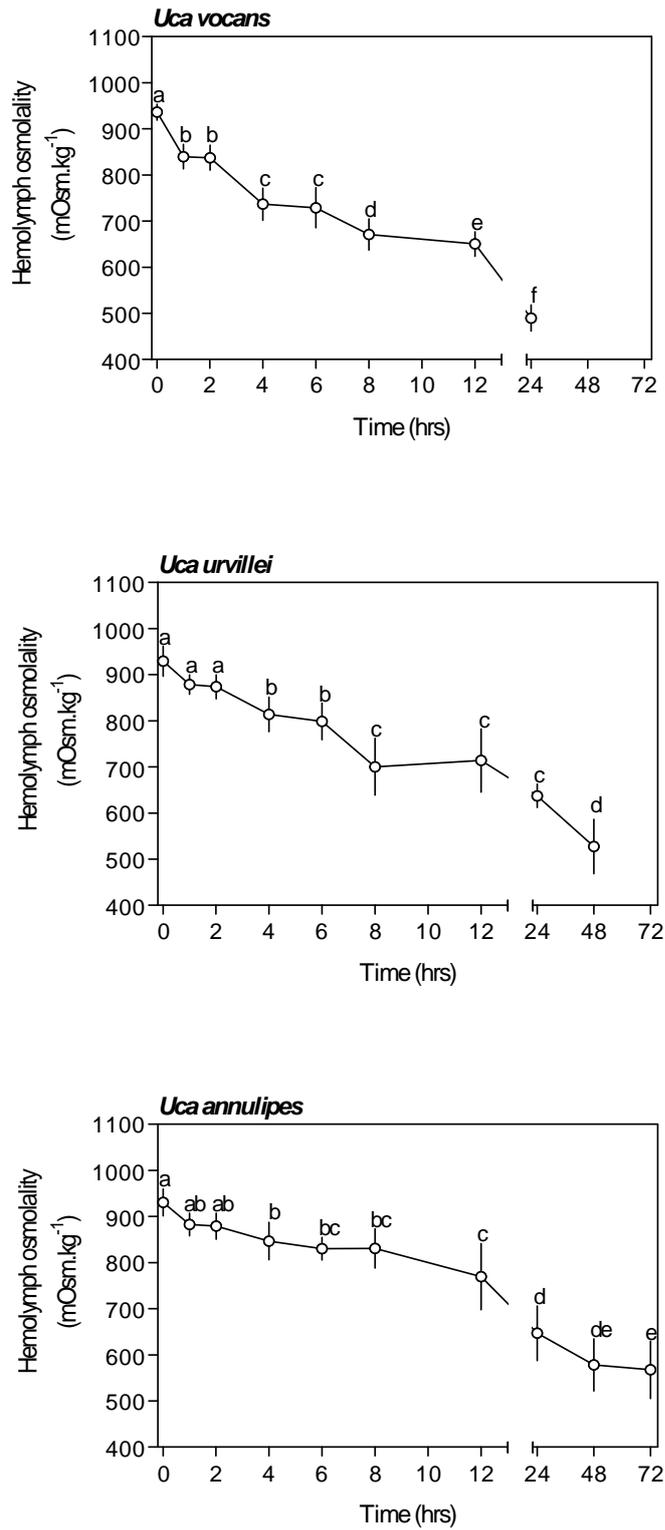


Figure 3.3. Time course of changes in hemolymph osmolality (mOsm.kg⁻¹, mean \pm 1 SD) in *U. vocans*, *U. urvillei* and *U. annulipes* after direct transfer from a salinity of 35 to a salinity of 0. Different letters indicate a statistically significant difference between mean values of hemolymph osmolality (one-way ANOVA, $p < 0.05$).

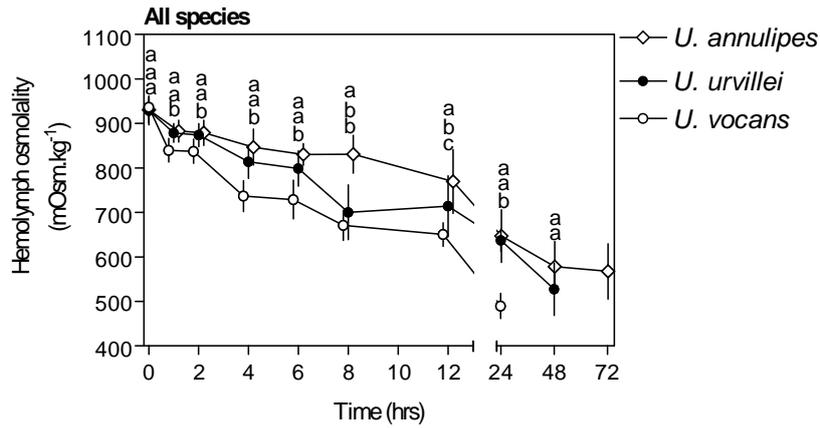


Figure 3.4. Time course of changes in hemolymph osmolality (mOsm.kg⁻¹, mean \pm 1 SD) among three *Uca* species *U. vocans*, *U. urvillei* and *U. annulipes* after direct transfer from a salinity of 35 to a salinity of 0. Different letters indicate a statistically significant difference between mean values of hemolymph osmolality (one-way ANOVA, $p < 0.05$).

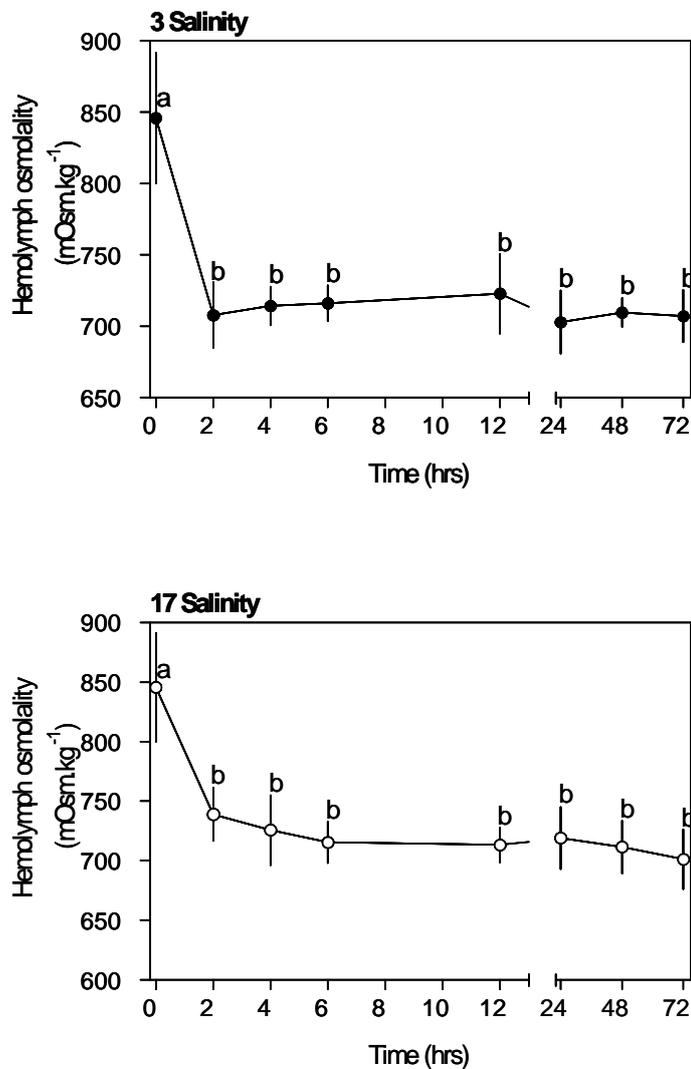


Figure 3.5. Time course of changes in hemolymph osmolality (mOsm.kg⁻¹, mean \pm 1 SD) in *Chiromantes eulimene* after transfer from a salinity of 35 to salinities of 3 and 17. Different letters indicate a statistically significant difference between mean values of hemolymph osmolality (one-way ANOVA, $p < 0.05$).

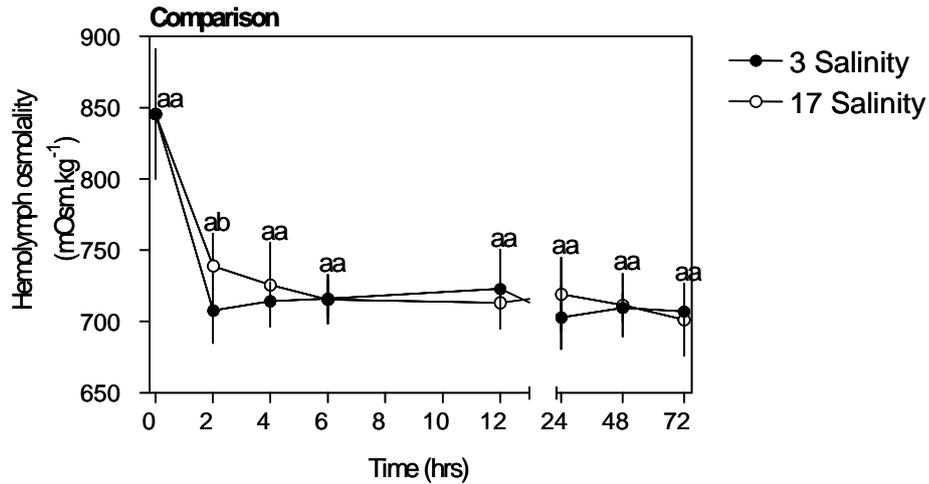


Figure 3.6. Comparing time course of changes in hemolymph osmolality (mOsm.kg⁻¹, mean \pm 1 SD) in *Chiromantes eulimene* between two salinities after transfer from a salinity of 35 to salinities of 3 and 17 salinity. Different letters indicate a statistically significant difference between mean values of hemolymph osmolality (t-tests, $p < 0.05$).

3.4. Discussion

Crabs inhabiting the intertidal area are faced with an environment that poses several physiological challenges. One important challenge is that the salinity of the surrounding environment can vary widely. During low tide the only water that is available to crabs in the intertidal is that which remains in small pools after the tide has receded, that in their burrows, and sediment pore water. The salinity of these different sources of water can vary widely. When it is hot, high rates of evaporation can result in high salinities. For example, Gillikin (2000) recorded sediment pore water salinity as high as 90 in a Kenyan mangrove. Evaporation is a slow process, and crabs have time to acclimatise to changes in salinity due to evaporation. During heavy rainfall at low tide in contrast, the salinity may decrease extremely rapidly, and animals have little time to acclimatise to these changes. Sasekumar (1974), for example, reported a sediment pore water salinity change of greater than 20 in the intertidal of a Malaysian mangrove during a neap tide following heavy rainfall.

Crabs occupying the highest level of the intertidal zone face more physiological problems compared with crabs in the lower intertidal zone, since they are exposed for longer periods. In the present study, the ability of the *Uca* species to tolerate a sudden decrease in salinity from 35 to freshwater was investigated (Figure 3.1 and 3.2). This was done to simulate a sudden decrease in salinity as could occur at low tide when it rains heavily.

All *Uca* species investigated were able to tolerate direct transfer to freshwater for up to 8 hours without experiencing any mortality (Figure 3.2). In other words, each of these species is physiologically well adapted to tolerate wide, rapid decreases in salinity as may occur during low tide when it rains. Survival for periods longer than eight hours showed a relationship to the region of the intertidal occupied (Figure 3.2). All *U. vocans*, which occurs lowest in the intertidal zone, died within 24 hours of exposure (Figure 3.2). All *U. urvillei* died within two days of exposure (Figure 3.1). *Uca annulipes*, which lives in the highest region of the intertidal zone, was the most tolerant to rapid freshwater exposure, with 70% of crabs surviving up to 72 hours (Figure 3.1). Although *U. chlorophthalmus*, which lives in a region of the intertidal zone between *U. urvillei* and *U. annulipes*, was not examined in the present study, as already stated in Chapter 2 when this crab was directly exposed to freshwater 40% of crabs were alive after 72 hrs, which is intermediate between *U. urvillei* and *U. annulipes* (Figure 2.5). Therefore, the long-term ability to tolerate exposure to freshwater appears to be related to the region of the intertidal occupied by these fiddler crabs.

A strong hemolymph regulatory capability is advantageous in habitats where there are wide variations in salinity, since metabolic reactions dependant on a stable hemolymph environment will be only minimally affected during such variation (Greenway, 1988). The ability to maintain a slow change to the new hemolymph osmotic state following osmotic shock is probably also advantageous, for the same reason. As was already shown, each of the fiddler crab species investigated are strong hyper-osmoregulators at salinities below the isosmotic point (Figure 3.4). When the salinity of the surrounding medium decreases from a salinity of 3 to freshwater however their hemolymph osmolality decreases significantly. This suggests that hemolymph regulatory breaks down in freshwater. When *U. vocans*, *U. urvillei* and *U. annulipes* were transferred from a salinity of 35 to freshwater, their hemolymph osmolality decreased sharply (Figure 3.4). Hemolymph osmolality continued to decrease with time and did not stabilise within the experimental period, and probably will continue to decrease until animals died. However, the rate at which hemolymph osmolality decreased showed a relationship to the region of the intertidal occupied. The rate was fastest in *U. vocans*, which lives in the low intertidal zone, and slowest in *U. annulipes*, which lives in the upper intertidal zone, and intermediate in *U. urvillei*, which lives in the region of the intertidal between these species.

The responses of the *Uca* species investigated following direct transfer from a salinity of 35 salinity to freshwater suggests that the ability of these fiddler crabs to inhabit different regions of the intertidal is in part a function of their ability to tolerate exposure to very low salinities. Crabs living in the highest region of the intertidal zone tolerated low salinities much better and were able to slow the rate of hemolymph dilution much better compared to crabs living in the lowest level of the intertidal zone. This agrees with the probability that crabs from the upper region of the intertidal will more likely be exposed to low salinities, and for longer periods, compared with crabs from the low intertidal zone.

Direct transfer of *C. eulimene* from a salinity of 35 to salinities of 3 and 17 had no effect on its survival. *Chiromanthes eulimene* also did not suffer much mortality when directly transferred to a salinity of 0 (see

Chapter 2) with 90% survival. This provides a clear indication that this crab is extremely well adapted to tolerate wide, rapid decreases in salinity in the intertidal during low tide. Hemolymph osmolality of *C. eulimene* decreased sharply within the first 2 hours of transfer from a salinity of 35 to salinities of 3 and 17, and had stabilised within 6 hours already at both of the latter salinities. This rapid stabilisation of hemolymph osmolality means that metabolic reactions dependent on a stable hemolymph environment will be only minimally affected during such variation. Bolt & Heeg (1975) acclimated *Parasesarma catenata* to a salinity of 34 before directly transferring them to a salinity of 3.4 salinity, a very similar situation to that examined in the present study which permits comparison between these two closely related crabs. Bolt & Heeg (1975) did not provide any data on survival of crabs in their experiments. Hemolymph osmolality in *P. catenata* decreased sharply following exposure, and it took about 16-24 hours before hemolymph osmolality stabilised. This is obviously much longer than the 6 hour period it took for hemolymph osmolality to stabilise in *C. eulimene*. This difference may explain the fact that *C. eulimene* is able to penetrate further into the low salinity upper reaches of estuaries compared to *P. catenata*.

Temperature has been shown to influence tolerance to salinity of decapod crustacea, and occasionally also osmoregulatory capability (e.g. Dehnel 1962, Dorgelo 1981, Kirkpatrick & Jones 1985, Lemaire *et al.* 2002). Combinations of salinity and temperature frequently interact in nature, affecting organisms differently than when acting separately (McKenney 1996). Temperature can increase, decrease, or even shift, the salinity tolerance of an aquatic organism (Bookhout 1964, Dorgelo 1976; de Villiers *et al.* 1999).

3.5. Conclusion

This study has shown that the tolerances of *U. vocans*, *U. urvillei* and *U. annulipes* to low salinities and their ability to regulate hemolymph osmolality following osmotic shock is closely related to the region of the intertidal that they occupy. The sesarmid crab *C. eulimene* is able to rapidly stabilise its hemolymph osmolality following osmotic shock, permitting metabolic reactions that rely on a stable hemolymph environment to proceed with little interference.

3.6. References

- BOLTT, G. & HEEG, J. 1975. The osmoregulatory ability of three grapsoid crab species in relation to their penetration of an estuarine system. *Zoologica Africana* **10**: 167-182.
- *BOOKHOUT, C. G. 1964. Salinity effects on larval development of *Pagurus bernhardus* (L) reared in the laboratory. *Ophelia* **1**: 275-294.
- DE VILLIERS, C.J., HODGSON, A.N. & FORBES, A.T. 1999. Studies on estuarine macrobenthos. In: *Estuaries of South Africa* (eds) Allanson, B.R. & Baird, A.D., Ch. 8. Cambridge University Press.
- DEHNEL, P.A. 1962. Aspects of osmoregulation in crab *Hemigrapsus nudus*. *Canadian Journal of Zoology* **52**: 923-937.
- DORGELO, J. 1976. Salt tolerance in crustacea and the influence of temperature upon it. *Biological Review* **51**: 255-290.
- FORBES, A.T. 1974. Osmotic and ionic regulation in *Callinassa kraussi* Stebbing (Crustacea: Decapoda: Thallassinidea). *Journal of Experimental and Marine Biology* **16**: 301-311.
- GILLIKIN, D.P. 2000. Factors controlling the distribution of Kenyan brachyuran mangrove crabs: salinity tolerance and ecophysiology of two Kenyan *Neosarmatium* specie. M.Sc. thesis, Vrije Universiteit Brussel, Brussels.
- GREENWAY, P. 1988. Ion and water balance. In: *Biology of land crabs*, (eds) W.W. Burggren & B.R. McMahon, Cambridge University Press, Cambridge, UK.
- ICELY, J.D. & JONES, D.A. 1978. Factors affecting the distribution of the genus *Uca* (Crustacea: Ocypodidae) on an East African shore. *Estuarine, Coastal and Shelf Science* **6**: 315-325.
- KIRKPATRIC, K. & JONES, M.B. 1985. Salinity tolerance and osmoregulation of a prawn, *Palaemon affinis* (Milne Edwards) (Caridea: Palaemonidae). *Journal of Experimental Marine Biology and Ecology* **93**: 61-70.
- LOHRER, A.M., FUKUI, Y., WADA, K. & WHITLATCH, R.B. 2000. Structural complexity and vertical zonation of intertidal crabs, with focus on habitat waquirements of the invasive Asian shore crab, *Hemigras sanguineus* (de Haan). *Journal of Experimental Marine and Ecology* **244**: 203-217.
- MACINTOSH, D.J. 1984. Ecology and productivity of Malaysian mangrove crab populations (Decapoda: Brachyura). In: *Proceedings of the Asian symposium on mangrove management*, (eds) E. Soepadmo, A.N & D.J. Macintosh, University of Malaysia and UNESCO, Malaysia.
- *MCKENNEY JR, C.L., 1996. The combined effects of salinity and temperature on various aspects of the reproductive biology of the estuarine mysid, *Mysidopsis bahia*. *Invertebrate Reproduction and Development* **29**: 9-18.
- RABALAIS, N.N. & CAMERON, J.N. 1985. Physiological and morphological adaptations of adult *Uca subcylindrica* to semi-arid environments. *Biological Bulletin* **168**: 135-146.
- SASEKUMAR, A. 1974. Distribution of macrofauna on a Malaysian mangrove shore. *Journal of Animal Ecology* **43**: 51-69.

VERNBERG, W.B. & VERNBERG, J.V. 1972. *Environmental physiology of marine animals*. Springer-Verlag, New York.

WHITFIELD, A.K. & TAYLOR, R.H. 2009. A review of the importance of freshwater inflow to the future conservation of Lake St Lucia. *Aquatic conservation: Marine and freshwater Ecosystems* **19**: 838 – 848.

* Original not seen

Chapter 4

Influence of low temperature on salinity tolerance and osmoregulation in *Chiromantes eulimene*

4.1. Introduction

Salinity and temperature are important environmental variables in the natural environment, and changes in both variables occur together (de Villiers *et al.* 1999). In an estuary, salinity and temperature vary over the short term with each tidal cycle, but drastic changes may occur between seasons, when rainfall patterns and temperature change (de Villiers *et al.* 1999). Combinations of salinity and temperature frequently interact in nature, affecting organisms differently than when acting separately (McKenney 1996). Estuarine water temperatures vary seasonally, but many estuaries experience long or short term fluctuations in temperature (Day 1981, Taylor 1992). The south Western Cape is a winter rainfall area, and estuarine organisms inhabiting these cool temperate estuaries encounter simultaneous low salinity and low temperature every year (de Villiers *et al.* 1999). Organisms in subtropical estuaries along the east coast of South Africa are rarely exposed to low salinities and temperature due to most rainfall falling in the warmer summer months (de Villiers *et al.* 1999). Temperature can increase, decrease, or even shift, the salinity tolerance of an aquatic organism (Bookhout 1964). Experiments testing an organism's response to the combined effects of temperature and salinity are important because they more accurately reflect natural conditions than do single-factor experiments.

Although *Chiromante eulimene* experiences tropical or subtropical temperature conditions over most of its geographical range, the southern portion of its range in South Africa extends into warm temperate waters, where the temperature of estuarine water falls as low as 12-14°C during winter (e.g. Robertson 1984). Temperature has been shown to influence tolerance to salinity of decapod crustacea, and occasionally also osmoregulatory capability (e.g. Dehnel 1962, Dorgelo 1981, Kirkpatrick & Jones 1985, Lemaire *et al.* 2002). The reason for the southern limit of the geographical range of *C. eulimene* is not known. Since temperature is such an important factor affecting estuarine animals and because low temperature has been shown to influence the salinity tolerances of many estuarine organisms, the present study investigates the influence of low temperature on tolerance to salinity and osmoregulatory capability in *C. eulimene* to determine whether low temperature explains the southern limit of this crabs geographical range.

4.2. Materials and Methods

4.2.1. Collection and handling of crabs

Crabs were collected from the intertidal at various sites in Richards Bay and the Mhlatuze River estuary (Figure 2.1) by hand or with the aid of small hand-held nets. They were rinsed in the field to remove sand and mud before they were brought back to the laboratory. Crabs were transferred to the laboratory within a few hours of collection, in plastic containers filled with seawater. In the laboratory the crabs were placed in

tanks filled with about 18 liters of aerated and filtered seawater, and left for a period of between 12-16 hrs. Between 60-70 individuals were placed into a tank.

The tanks were covered with aluminium foil to prevent crabs escaping and to limit evaporation of water. Crabs were fed fish flakes in the holding tanks, but during the experiments they were not fed. The holding tanks were held in an environmentally controlled chamber at a constant temperature of 22°C and a photoperiod of 12 hours light: 12 hours dark for all the experiments. Salinity, temperature and mortality were checked in the tanks daily. There was little mortality that was noticed in holding tanks whenever there was dead animal it was removed from the holding tank to reduce water quality fouling too quickly. To obtain salinities lower than that of seawater a salinity of 35, the seawater was diluted with (0.45µm) filtered dechlorinated freshwater, and salinities higher than that of seawater was obtained by adding Instant Ocean Synthetic Sea Salts (Aquarium Systems, Inc.) artificial sea salt to seawater. The salinities for accuracy were measured using a refractometer.

To evaluate the effect of temperature on hemolymph osmotic response at different salinities in *C. eulimene* crabs were exposed to different salinities at temperatures of 14, 18, and 22°C. For each temperature treatment nine salinities (0, 3, 9, 18, 26, 35, 45, 55, and 65 salinity) were examined. The crabs were firstly acclimated to test temperature at a rate of 4°C per day, after that they were than acclimated to nine salinities at a rate of between 6-10 per day in their test temperatures. During the acclimation process crabs were kept in 35 salinity seawater.

Ten different crabs were each placed into a jar filled with 500ml of water of a known salinity. The crabs were monitored at 24 hour intervals for a period of 96 hours. The osmolality of the crabs and the water which they were in were measured using an osmometer. The osmolalities of the crabs were obtained by withdrawing the hemolymph of the crab from the arthroal membrane at the base of fourth and fifth walking legs using a sterile 1.0ml tuberculin syringe and after that the hemolymph was measured using the osmometer to obtain the crab's osmolality. The data was recorded and the osmoregulatory strategy of the crabs was determined by plotting and comparing hemolymph osmolality to the osmolality of the surrounding water.

4.2.2. Data analysis

Survival between individuals exposed in the same salinity was compared by means of a z-test for pairwise comparisons and multiple comparison of proportions test for multiple comparisons. For osmoregulation, data are expressed either as hemolymph osmolality (in mOsm.kg⁻¹) or as osmoregulatory capacity (OC) (OC, also in mOsm.kg⁻¹), the latter defined as the difference between the osmotic pressure of the hemolymph and of the surrounding medium at a given salinity (Charmantier *et al.* 1989). The Student t-test was used for pairwise comparisons and one-way ANOVA followed where appropriate by a Tukey multiple comparison

test for multiple comparisons. Different letters on graphs indicate a statistically significant difference or similarities between mean values.

4.3. Results

4.3.1. Survival in different temperatures

14°C: The salinity tolerance range in this temperature was between salinities of 0-55, with a salinity of 55 having the lowest survival. Survival other than at a salinity of 55 was greater than 80% (Figure 4.1). There was no survival at salinities of 65 and 75. Survival in salinities from 3-45 was similar, whilst the survival in freshwater was similar with that at a salinity of 3 and 45 (Figure 4.1).

18°C: *Chiromantes eulimene* survived exposures of more than four days to salinities ranging from 0 to 65 under temperature of 18°C, with no survival in salinity of 75. No survival rate was less than 80% at salinities from 0-65. Survival at salinities of 45 and 65 was similar to each other with no statistically significant difference with the survival in 18 salinity and statistically different to the rest of the salinities (Figure 4.1).

22°C: *Chiromantes eulimene* survived 96 hour exposure to salinities ranging from a salinity of 0 to 65 under temperatures of 22°C. The lowest survival in this temperature was observed at a salinity of 65 and no survival was less than 80% from a salinity of 0-55 (Figure 4.1).

4.3.2. Osmoregulation

Chiromantes eulimene was a strong hyper – hypo-regulator. It followed the same strategy in all three temperatures tested (14-22°C). It was hyper-regulating in salinities below isosmotic point and above isosmotic point it was hypo-regulating. In all temperatures there was an initial increase of hemolymph osmolality from salinity of 0-3 and after that maintaining its hemolymph osmolality over a wide range of salinity from a salinity of 3-35 at 14°C, a salinity of 3-45 at 18°C and a salinity of 3-26 at 22°C. After that there was an increase in hemolymph osmolality with increasing salinity of the medium. The isosmotic point of these crabs was at a salinity of 27 at all temperatures (Figure 4.2).

When comparing the hemolymph of different temperatures at the same salinity, at salinities between 0 and 55 hemolymph at 18°C was significant different to the hemolymph osmolality in other temperatures. At a salinity of 3 hemolymph at 14°C was significantly different to the hemolymph osmolality at other temperature. At salinities of 9 and 35 it was significantly similar in all temperatures. At salinities of 18, 26 and 45 hemolymph osmolality at 22°C was significantly different to the hemolymph osmolality in other temperatures (Figure 4.2).

4.3.3. Osmotic Capacity

At 14°C Osmotic Capacity (OC) ranged between 100 and 650 mOsm.kg⁻¹ during hyper-regulation and during hypo-regulation it ranged between 180 and 500 mOsm.kg⁻¹(Figure 4.3). At 18°C OC was between 10-700

mOsm.kg⁻¹ during hyper-regulation 200 and 650 mOsm.kg⁻¹ during hypo-regulation. Lastly at 22°C Osmotic Capacity ranged from 10 and 650 mOsm.kg⁻¹ during hyper-regulation and during hypo-regulation it was between 100 and 480 mOsm.kg⁻¹. Osmotic Capacity followed the same strategy in all temperatures (Figure 4.3). When *C. eulimene* was hyper-regulating Osmotic Capacity was maintained at the first two salinities and then decreased with salinity until the 26. When it was hypo-regulating Osmotic Capacity increased with salinity.

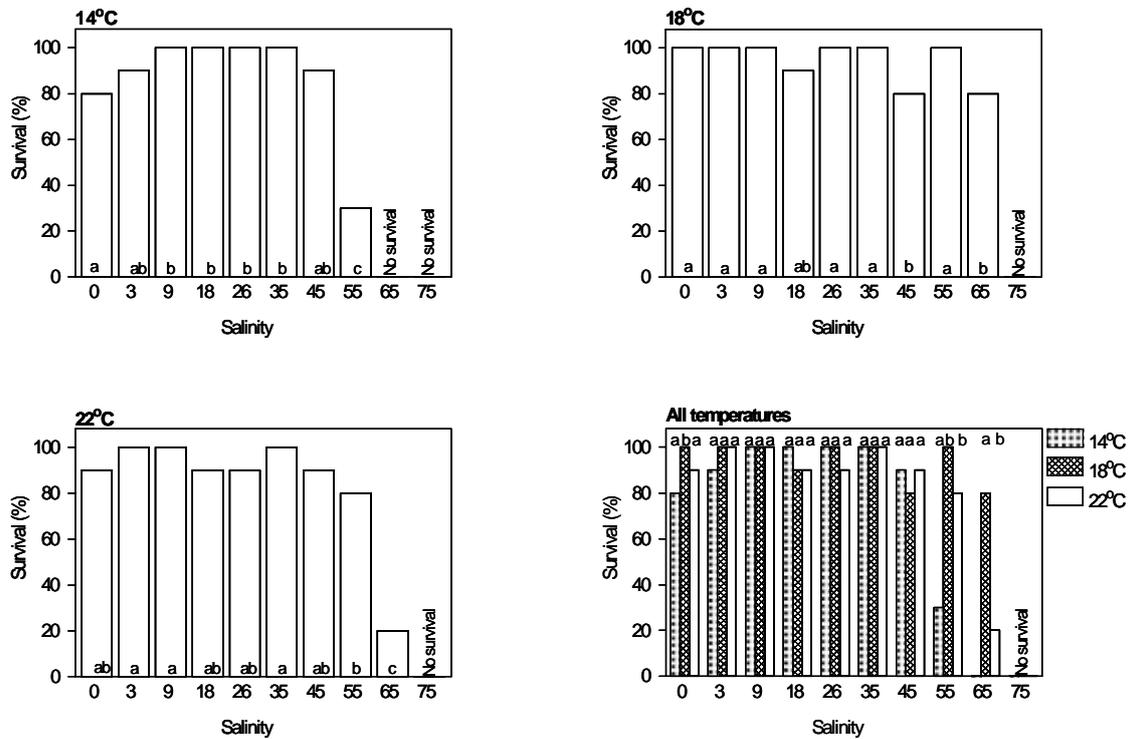


Figure 4.1. Survival of *Chiromantes eulimene* after 96 hrs in various salinities at 14, 18 and 22°C. In graphs of individual temperatures, different letters indicate a statistically significant difference in survival between salinities (based on multiple comparison of proportions tests, $p < 0.05$). In graph of all temperatures combined, different letters indicate a statistically significant difference in survival between temperatures (based on multiple comparison of proportions tests or z-tests, $p < 0.05$).

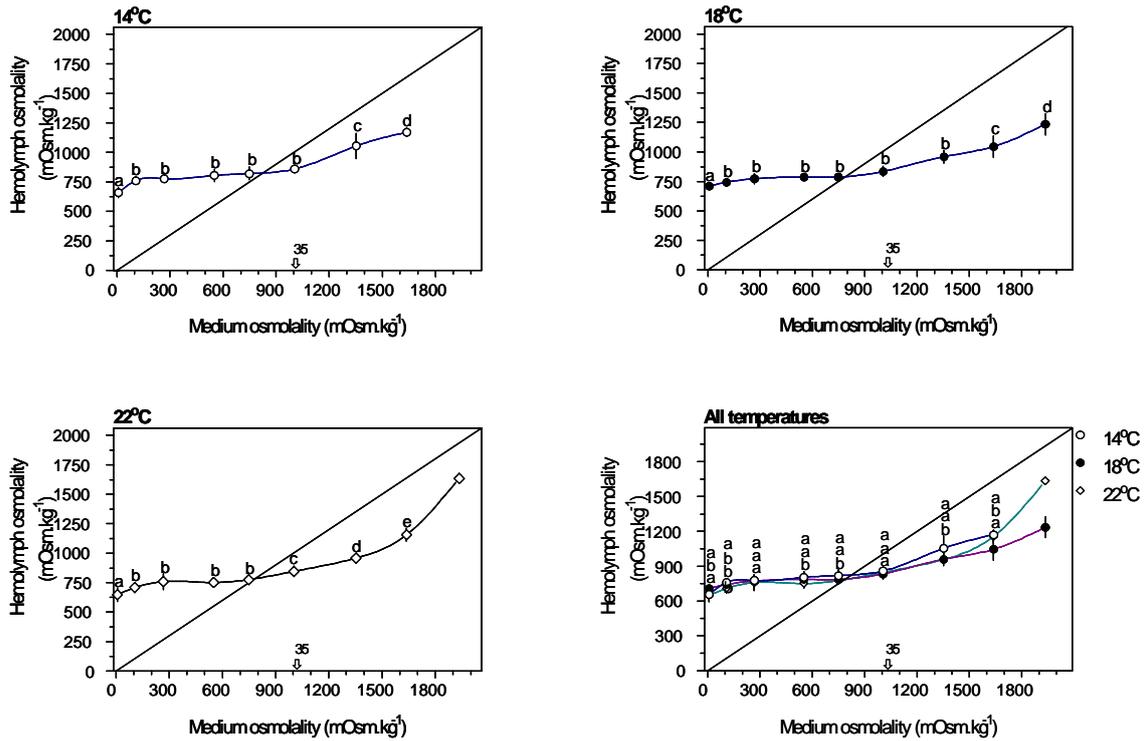


Figure 4.2. Relationship between hemolymph osmolality (mOsm.kg⁻¹; mean ± 1 SD) and osmolality of the surrounding medium measured in *Chiromantes eulimene* after 96 hrs exposure to various combinations of salinity and temperature at 14°C, 18°C and 22°C. The diagonal straight line is the isosmotic line. Different letters indicate significant differences between mean values (either one-way ANOVA or t-tests, p < 0.05). In graph of all temperatures combined, different letters indicate a statistically significant difference in hemolymph osmolality between temperatures (based on one-way ANOVA, p < 0.05).

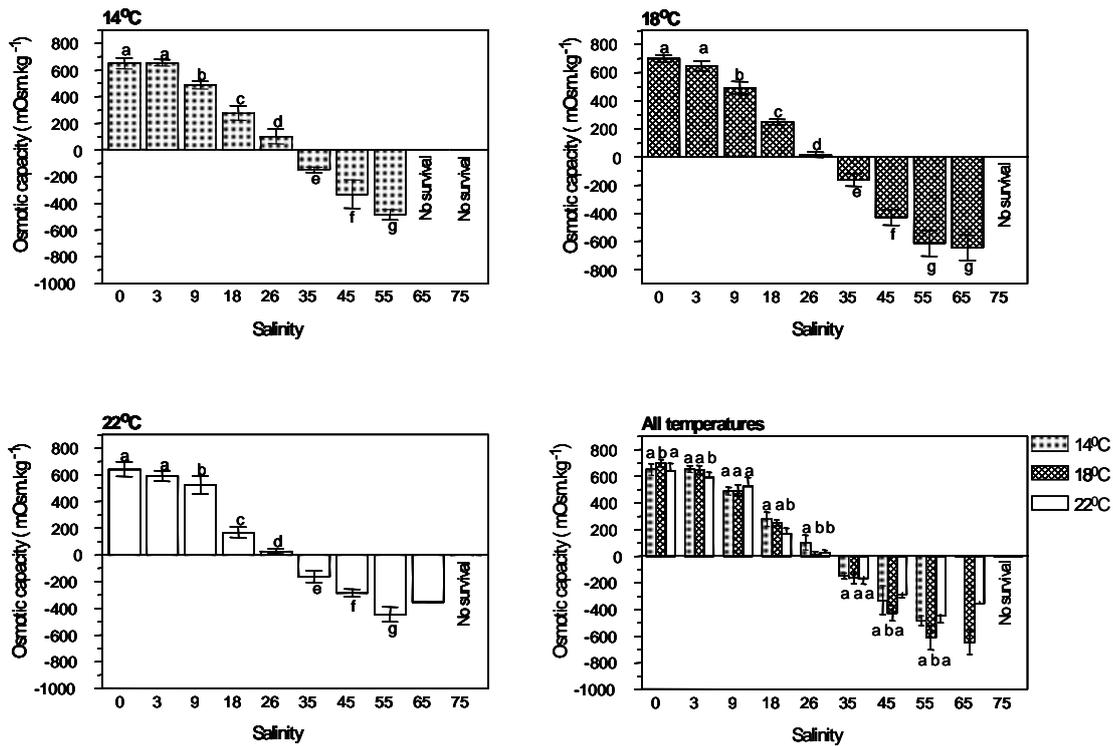


Figure 4.3. Osmotic capacity (OC); in mOsm.kg⁻¹; mean \pm 1 SD of *Chiromantes eulimene* after 96 hours exposure to different salinities and temperatures. Different letters indicate a statistically significant difference between mean values (one-way ANOVA, $P < 0.05$). In graph of all temperatures combined, different letters indicate a statistically significant difference in osmoregulatory capacity between temperatures (based on one-way ANOVA, $p < 0.05$).

4.4. Discussion

Temperature is one of the most important environmental factors limiting the distribution of aquatic organisms and most animals have several limiting temperatures (Kinne 1971). Temperature may affect the animal's growth, feeding, reproduction and other physiological functions individually or in combination with salinity (de Villiers *et al.* 1999). Temperature modifies the range of salinity tolerated while salinity has a strong influence on survival (Dorgelo 1976; de Villiers *et al.* 1999). Since an estuary is an area of mixing between river and sea water, estuarine temperatures are determined by the amount of tidal inflow and river discharge to an estuary (Hill 1981).

At the southern portion of the geographical range of *C. eulimene* the lowest temperatures experienced by this crab are about 12-14°C. Indeed Day (1981b) gives minimum temperatures of 12-14°C for warm-temperate estuaries. Temperatures between 14-22°C had no effect on the tolerance to salinity or osmoregulatory capability of *C. eulimene* at salinities between 0-45 (Figure 4.1). Considering that this range of salinity is that most likely to be experienced by estuarine crabs, temperature does not appear to be an important factor determining the southern limit of the geographical range of *C. eulimene*. The absence of *C. eulimene* from the south and west coasts of South Africa might be as a result of the inability of the larval stages or juvenile

crabs to tolerate low temperatures. High salinity and low temperature in contrast interacted strongly, reducing the upper limit of tolerance to 55 at 14°C compared to 65 at 18°C and 22°C (Figure 4.1). There was however no associated effect on osmoregulatory capability. Survival at a salinity of 65 was significantly higher at 18°C compared to 22°C, a finding that was unexpected considering that *C. eulimene* is generally regarded as a subtropical to tropical crab. The reason for this difference is uncertain.

Combination of low temperature and low salinities did not affect the survival of *C. eulimene*. This correspond to previous studies done by Avis (1988) who found that the hermit crab *Diogene brevisrostris* tolerated low salinities at low temperatures and also Hill (1974) who found that the zoeae of *Scylla serrata* increased its survival from 50 to 90% if temperatures were decreased from 22.5-25°C to 7-15°C. Affat (2000) investigated the influence of temperature (12-28°C) on the salinity tolerance (salinities of 0-49) of the sesarimid crab *Parasesarma catenata*. Affat (2000) found that no mortality occurred at any temperature when the salinity was above a salinity of 7. There was a strong interaction between temperature and exposure to freshwater however, all crabs dying in freshwater at 12 °C and 16°C, survival increasing as the temperature increased above this. The ability of *C. eulimene* to tolerate combination of lower temperatures and lower salinities may be the reason why it occurs further up into estuaries than *P. catenata*. Under conditions of exposure to decreased salinity and temperature both factors have a cumulative acute effect, dictating the magnitude and time taken for the animal to re-acclimate (e.g. de Villiers *et al.* 1989b, de Villiers 1990).

In contrast to the present study, other studies on crustaceans have shown that the combination of low salinities and low temperatures result in high mortality rates. A study done by Rome *et al.* (2005) showed that the blue crab *Callinectes sapidus* is less tolerant to cold temperatures at low salinities. The lowest temperature it survived was 1°C and the lowest salinity being a salinity of 8. Another study done by Lemaire *et al.* (2002) shows that a combination of temperature and salinity does have an effect on osmoregulatory capacity of the juveniles and subadults of *Penaeus stylirostris*. The drop in temperature had a greater effect on hemolymph osmolality especially at higher salinities (salinities above 43) and lower salinities (salinities below 10). This explained why these species died during the cold season.

Chiromantes eulimene is an intertidal crab so its is unlikely that it will be exposed to a single temperature and single salinity, but will respond to short term fluctuations within the tidal period by moving away from unfavorable conditions to a suitable area. So it is important not to consider survival only as both factors may fluctuate within the animal's tolerance range, as the previous chapters have shown that it has a wide salinity range and can tolerate low temperatures. The composition, distribution and abundance of estuarine macrofauna is a result of the combined effects of numerous factors e.g. estuarine sediment, salinity fluctuations, temperature, river flow etc. All these factors occur together thus affecting the animal differently than when they occur individually and an animal's success depends upon its ability to accommodate, tolerate or avoid unfavorable conditions. These fluctuations may influence the animal's performance at a sub-lethal level, thus excluding it in areas where unfavorable conditions occur simultaneously (de Villiers *et al.* 1999).

Temperature determines the geographical distribution of organisms along the coast and salinity determines which region in the intertidal zone they will occupy. However, temperature and salinity are not the only environmental factors that play a role in the distribution of brachyuran crabs. Different species have different environmental requirements and behaviour patterns (Elliot 1977). Owen (2003) studied the distribution of the burrowing ocypodid crab *Paratyloidiplax blephariskios* looking at the factors affecting its distribution further south of Mngazana estuary in the Eastern Cape, where species like *P. edwardsii* and *P. algoensis* occur as far down as the Eerste estuary and Langebaan Lagoon respectively. *Paratyloidiplax blephariskios* in the laboratory survived temperatures between 12.5°C – 35°C and should be able to survive at least as far as the Swartkops (Owen 2003). Insufficient areas of suitable muddy habitat have prevented *P. blephariskios* from establishing viable populations in estuaries south of the Mngazana. Sediment texture and shore level play a major role in distribution of brachyuran crabs (Macintosh 1988). Previous studies (e.g. Mclachlan & Grindley 1974; Hill 1981; Teske & Wooldridge 2003) have also shown that salinity is not the only factor that governs the distribution of organisms along the estuary, with other factors such as substrate, food availability and competition also affecting their distribution.

4.5. Conclusion

This study has shown that exposure of *Chiromantes eulimene* to lowered temperatures has no effect on its salinity tolerance and osmoregulation capacity. Lower temperatures do not appear to be an important factor eliminating this species on the South and West Coasts of South Africa. Reviews on the effect of temperature on the distribution of macroinvertebrates also point out that sub-lethal effects such as slow growth and reduced reproductive output, rather than temperature alone influences establishment and long-term success of populations (Hill 1981; de Villiers & Hodgson 1999).

4.6. References

- AFFAT, S.V. 2000. Salinity tolerance and osmoregulation as a function of ambient temperature in the marsh crab, *Sesarma catenata*. Honours. thesis, University of Port Elizabeth, South Africa.
- AVIS, A.M. 1988. Temperature and salinity tolerance of adult hermit crab *Diogenes brevisrostris* (Stimpson) (Crustacea: Decapoda: Anomuran). *South African Journal of Zoology* **23**: 351-355.
- *BOOKHOUT, C. G. 1964. Salinity effects on larval development of *Pagurus bernhardus* (L) reared in the laboratory. *Ophelia* **1**: 275-294.
- BRANCH, G. & BRANCH, M. 1981. *The living shore's of Southern Africa*. Struik Publishers, Cape Town.
- DAY, J.H. 1981. The estuarine fauna. In: *Estuarine Ecology with particular reference to southern Africa*, (ed) J.H. Day, Ch 9. A A Balkema, Cape Town.
- DAY, J.H. 1981 b. Summaries of current knowledge of 43 estuaries in southern Africa. In: *Estuarine Ecology: With particular reference to Southern Africa*, (ed) JH Day, AA Balkema Cape Town.
- DEHNEL, P.A. 1962. Aspects of osmoregulation in crab *Hemigrapsus nudus*. *Canadian Journal of Zoology* **52**: 923-937.
- DE VILLIERS, C.J., HODGSON, A.N. & ALLANSON, B.R. 1989. Effect of salinity and temperature on the filtration rate and distribution of *Solen cylindraceus* (Hanley). In: *Reproduction, genetics and distribution of marine organisms*, (eds) J.S. Ryland & P.A. Tyler, Olsen and Olsen, Denmark.
- DE VILLIERS, C.J. 1990. Aspect of the biology of the infaunal bivalve mollusc *Solen cylindraceus* (Hanley) in the Kariega estuary. PhD. thesis, Rhodes University, Grahamstown.
- DE VILLIERS, C.J. & HODGSON, A.N. 1999. Studies on estuarine macrobenthos: The macrobenthos: In: *Estuaries of South Africa* (eds) B.R. Allanson & D. Baird, Ch. 8. Cambridge University Press, Cambridge.
- DE VILLIERS, C.J., HODGSON, A.N. & FORBES, A.T. 1999. Studies on estuarine macrobenthos. In: *Estuaries of South Africa* (eds) Allanson, B.R. & Baird, A.D., Ch. 8. Cambridge University Press.
- DORGELO, J. 1976. Salt tolerance in crustacea and the influence of temperature upon it. *Biological Review* **51**: 255-290.
- * ELLIOT, J.M. 1977. Some methods for the statistical analysis of samples of benthic invertebrates. Second edition *Freshwater Biology Association of Scientific Publication*.
- HILL, B.J. 1974. Salinity and temperature tolerance of zoeae of the portunid crab *Scylla serrata*. *Marine Biology* **25**: 21-4.
- HILL, B.J. 1981. Adaptation to temperature and salinity stress in southern African Estuaries. In: *Estuarine Ecology with particular reference to Southern Africa*, (ed) J.H. Day, Ch 11. A A Balkema, Cape Town.
- KINNE, O. 1964. The effect of temperature and salinity on marine and brackish water animals. *Oceanography and Marine Biology Review* **2**: 281-339.
- KIRKPATRIC, K. & JONES, M.B. 1985. Salinity tolerance and osmoregulation of a prawn, *Palaemon affinis* (Milne Edwards) (Caridea: Palaemonidae). *Journal of Experimental Marine Biology and Ecology* **93**: 61-70.
-

- LEMAIRE, P., BERNARD, E., MARTINEZ-PAZ, J.A. & CHIM, L. 2002. Combined effect of temperature and salinity on osmoregulation of juvenile and subadult *Penaeus stylirostris*. *Aquaculture* **209**: 307-317.
- MACINTOSH, D.J. 1988. The ecology and physiology of decapods of mangrove swamps. *Symposia. Zoologica Society of London* **59**: 315 – 341.
- *MCKENNEY JR, C.L., 1996. The combined effects of salinity and temperature on various aspects of the reproductive biology of the estuarine mysid, *Mysidopsis bahia*. *Invertebrate Reproduction and Development* **29**: 9–18.
- MACLACHLAN, A. & GRINDLEY, J.R. 1974. Distribution of soft substrata in Swartkops estuary with observations on the effect of floods. *Zoologica Africana* **9**: 211-233.
- OWEN, R.K. 2003. A short note on factors affecting the geographic distribution of the burrowing ocypodid crab, *Paratyloplitax blephariskios*: temperature, habitat and competition. *African Journal of Aquatic Science* **28(1)**: 57 – 60.
- ROBERTSON, W.D. 1984. Aspects of ecology of the shrimp *Palaemon pacificus* (Stimpson) (Decapoda: Palaemonidae) in the Bushmen River Estuary. M. Sc. thesis, Rhodes, Grahamstown.
- ROME, M.S., YOUNG-WILLIAMS., A.C., DAVIS, G.R. & HINES, A.H. 2005. Linking temperature and salinity tolerance to winter mortality of Chesapeake Bay blue crabs (*Callinectes sapidus*). *Journal of Experimental Marine Biology and Ecology* **319**:129-145.
- TAYLOR, D.I. 1992. The influence of upwelling and short term changes in concentrations of nutrients in the water column on fluxes across the surface of a saltmarsh. *Estuaries* **15**: 68-74.
- TESKE, P.R. & WOOLDRIDGE, T. 2003. What limits the distribution of subtidal macrobenthos on permanently open and temporarily open/closed South African estuaries? Salinity vs. sediment particle size. *Estuarine, Coastal and Shelf Science* **57**: 225-238.

* Original not seen

Chapter 5

General conclusion and opportunities for future research

5.1. General conclusion

This study examined the tolerances to salinity and osmoregulatory strategies of several brachyuran crabs and an anomuran prawn, in order to understand how they are adapted physiologically to the estuarine environment. All of the species investigated are euryhaline. This is an important adaptation within the estuarine environment, where the salinity fluctuates frequently and widely. All of the species achieve this wide salinity tolerance by means osmoregulation. All of the crabs examined were hyper-hypo-osmoregulators, while *C. kraussi* was a regulator only at low salinities, becoming isosmotic to the surrounding medium at high salinities.

The salinity tolerances of the most of the species investigated appear to correspond to the position that they occupy in the intertidal zone. Those species that occur in the lower region of the intertidal zone have a narrow tolerance range compared with those species that occupy the higher region of the intertidal zone. This may be due to the fact that species in the lower region of the intertidal zone are exposed to extreme salinities for shorter periods compared to species in the higher region of the intertidal zone. A similar study done in Australia by (Dunbar & Coates 2004) showed that differences in tolerance to dilute seawater may influence the habitat preferences of these species within the same geographical area. Indeed, in this study this was shown by two unrelated species that occupy the same intertidal zone where *Uca annulipes* was less tolerant to dilute seawater than *Chiromantes eulimene*. Gillikin *et al* (2004) on Kenyan mangrove crabs has also shown two related species found in same geographic area when exposed to higher salinities of 65 *Neosarmatium smithi* could osmoregulate for a limited time than *Neosarmatium meinerti*. In the present study *U. annulipes* tolerated higher salinity of 65, but could not tolerate freshwater, whereas *C. eulimene* is less tolerant to higher salinities of 65 and more tolerant to freshwater. This is attributed to the fact that *U. annulipes* is a burrower and *C. eulimene* is not, thus it can escape unfavourable conditions.

Uca species that inhabit estuarine environment have been shown to be euryhaline and strong osmoregulators (Thurman 2003). *Uca* species from the Northern Gulf of Mexico are excellent osmoregulators between salinities of 3 and 65 (Thurman 2003). Mantel & Farmer (1983) presented osmoregulation and salinity tolerance data from 15 ocypodid crabs worldwide, including the genera *Macrophthalmus*, *Ocypodid*, *Paracleistoma* and *Uca*, which showed that ocypodids are generally able to tolerate a wide range of salinities ranging from a minimum salinity of 1.5 in *Paracleistoma mcheilli* to a maximum salinity of 70 in *Uca mordex*.

A study done in Asia observed that *U. annulipes* was significantly more abundant on the upper shore on sandy substrate, while *U. vocans* was significantly more abundant on the normally muddier lower shore

levels (Lim *et al* 2005). *Uca vocans* moved to the upper shore levels only where the substrate had higher mud content. Crane (1975) observed that the distribution and abundance of *Uca* species is also controlled by the ability of crabs to make and maintain their burrows. Fiddler crabs (genus *Uca*) are highly dependent on their burrows, they use them to hide during high tide, escape from predators, buffer from temperature and water physiology (Crane 1975, Lim *et al* 2005). Distribution of these two Asian *Uca* species along the shore agrees with the salinity tolerance range in this study, which indicates that *U. vocans* can inhabit the lower intertidal zone, whereas *U. annulipes* can inhabit the higher intertidal zone. Low shore crab *Metaplex elegans* could not tolerate salinities below 10, whereas *Chiromantes onychophorum* and *Uca* species inhabiting the middle and upper shore zones, could survive at a salinity of 5 (Macintosh 1984).

General salinity tolerance range of estuarine invertebrates found internationally agrees with the salinity tolerance range of South African invertebrates, with each species exhibiting different degrees of euryhalinity as mentioned before. Euryhalinity is enabled by abilities of hyper and hypo-regulate in both high and low salinities respectively. Very few species show tolerance to low salinities especially freshwater only *C. eulimene* could tolerate freshwater exposure in both direct and acclimation experiments.

The influence of low temperatures on salinity tolerance and osmoregulation of adult *C. eulimene* appear to be limited and suggests that low temperature is not the reason why this species does not occur in estuaries on the South and West Coasts of South Africa.

The findings on salinity tolerance are critical from a South African perspective. South Africa is facing a freshwater crisis, and the Department of Water Affairs and Forestry (DWAF) has thus implemented plans for the construction of several new dams and inter-catchment transfer schemes. These water storage reservoirs in catchments will alter natural patterns of river flow, thus disturbing natural patterns in the downstream riverine and estuarine ecosystems (Reddering & Rust 1990). Due to dams and other impoundments the scouring action of floods has been decreased and many estuaries are now closing more frequently and for longer periods (Wooldridge 1999), leading to salinity alterations in a number of estuaries. These hydrodynamic impacts have the capacity to negatively affect estuarine populations that have a marine phase of development. In response, DWAF has implemented Estuarine Flow Requirements studies to quantify the amount of water that has to flow into an estuary in order to preserve the fauna and flora. The amount of water that is required to be released into estuaries will in part be based on the physiology of estuarine organisms. The present study provides some information that will be useful in this regard.

5.2. Opportunities for further research

Chapter 2 provided data regarding the salinity tolerances and osmoregulatory strategies of the species investigated. These experiments were however conducted at only a single temperature. Water temperature in an estuary, and especially geographically, does not remain constant, at least not for long periods, and future

studies will need to investigate whether or not the crabs and prawns alter their salinity tolerance and osmoregulatory strategy under different temperature conditions.

This study only looked at crabs and prawns collected from a single population. Some workers have shown that salinity tolerance and osmoregulatory strategy can differ between separated populations. Future studies will need to determine whether different populations of the crabs and prawn collected from different regions of their geographical ranges show the same salinity tolerances and osmoregulatory strategies.

Osmoregulation is an energy demanding process, and to fully understand how the crabs and prawn investigated are adapted to their environment, it will be necessary to determine the metabolic costs of osmoregulation by examining oxygen consumption at different salinities and temperatures.

Adult crabs of *C. eulimene* exposed to 14°C were less active than those at higher temperatures, so temperatures lower than 14°C need to be investigated. It is possible that crabs at these temperatures are more vulnerable to predators thus excluding these species from the South and West Coasts of South Africa. This issue needs to be investigated. The temperature tolerances of larval and juvenile *C. eulimene* also needs to be investigated to determine whether these stages can explain the reason why this crab does not extend into the South and West Coasts of South Africa, since temperature is not the limiting factor for the adults of this species.

5.3. References

- CRANE, J. 1975. *Fiddler crabs of the world: Ocypodidae: Genus: Uca*. Princeton University Press, New Jersey.
- DUNBAR, S.G & COATES, M. 2004. Differential tolerance of body fluid dilution in two species of tropical hermit crabs: not due to osmotic / ionic regulation. . *Journal of Experimental Biology and Ecology* **137**: 321-337.
- GILLIKIN, D.P., DE WACHTER, B. & TACK, J.F. 2004. Physiological responses of two ecological important Kenyan mangrove crabs exposed to altered salinity regimes. *Journal of Experimental Biology and Ecology* **301**: 93-109.
- LIM, S.S.L., LEE, P.S. & DIONG, C.H. 2005. Influence of biotope characteristics on the distribution of *Uca annulipes* (H Milner Edwards, 1837) and *Uca vocans* (Linneus, 1758) Crustacea: Brachyura: Ocypodidae. *The Raffles Bulletin of Zoology* **53(1)**: 111 – 114.
- MANTEL, L.H. & FARMER, L.L. 1983. Osmotic and ionic regulation. In: *The biology of crustacea, Internal Anatomy and Physiological Regulation*, (eds) D.E. Bliss & L.H. Mantel. Ch. 2. Academic Press, New York .
- REDDERING, J.S.V. & RUST, I.C. 1990. Historical changes and sedimentary characteristics of southern African estuaries. *South African Journal of Science* **86**: 425-428.
- THURMAN, C. 2003. Osmoregulation by six species of fiddler crabs (*Uca*) from the Mississippi delta area in the northern Gulf of Mexico. *Journal of Experimental Marine Biology and Ecology* **291**: 233-253.
- WOOLDRIDGE, T.H. 1999. Estuarine zooplankton community structure and dynamics. In: *Estuaries of South Africa* (eds). B.R. Allanson & D. Baird, Ch. 7. Cambridge University Press, Cambridge.