

Physiological responses of two ecologically important Kenyan mangrove crabs exposed to altered salinity regimes

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Abstract

The potential long-term effects of altered salinity regimes on the bioenergetics of two ecologically important Kenyan mangrove crabs, *Neosarmatium meinerti* de Man, 1887 and *Neosarmatium smithi* H. Milne Edwards, 1853 were investigated in light of recent findings suggesting that groundwater redirection may alter salinity regimes in Kenyan mangroves. Although changes in groundwater may cause only small increases in salinities, these changes would be chronic and may impact crab populations already living above their optimal salinity. To assess potential impacts, fundamental physiological processes and hemolymph components were measured on animals acclimated to 16‰, 32‰, 48‰ and 65‰ for 4 weeks in a field laboratory. For comparative purposes, crabs were also sampled in the field. *N. smithi* survived poorly in all salinities except the control (32‰). Although high mortality in *N. smithi* did not allow for reliable estimations of an energy budget, mortality and osmoregulatory capacity shows that this species can osmoregulate for a limited time in elevated salinities (± 1 week), but cannot withstand long-term hypersaline conditions. In contrast, *N. meinerti* survived well and was able to osmoregulate for 1 month in all salinity treatments. Nevertheless, their energy budget, was significantly reduced (to below 0) in the 65‰ treatment. Overall, this study shows that these two congeneric species exhibit different long-term responses to variations in salinity. However, they are both negatively effected by hypersaline

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conditions, suggesting that long-term alteration of mangrove salinity regimes may be detrimental for these ecologically important mangrove crab populations.

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1. Introduction

Crabs are the most abundant of the mangrove macro-fauna and are a valuable asset to the mangrove ecosystem. Crabs aerate the sediment by burrowing (Micheli et al., 1991), modify topography and grain size distribution (Warren and Underwood, 1986), reduce pore water salinity by allowing flushing of the sediment via their burrows (Ridd, 1996; Stieglitz et al., 2000), trap energy within the mangrove forest (Robertson, 1986; Robertson and Daniel, 1989; Lee, 1998; Ashton, 2002), create microhabitat for other fauna (Bright and Hogue, 1972; Gillikin et al., 2001), contribute to secondary production (Lee, 1997), and increase the amount of nutrients and decrease the sulfide concentration in the sediment by a plethora of activities (Smith et al., 1991). Due to the critical role burrowing crabs play in the mangrove ecosystem, Smith et al. (1991) considered them keystone species. In Kenya, two burrowing congeners, *Neosarmatium meinerti* and *Neosarmatium smithi*, have opposing distributions across the mangrove forest (with *N. meinerti* inhabiting the high shore and *N. smithi* the mid- and lower shore), relatively large body sizes, deep (~ 1 m) and wide diameter (~ 4 to 5 cm) burrows, and occur in high densities (Micheli et al., 1991; Gillikin, 2000). Therefore, these two species probably play a very significant role in the function and structure of Kenyan mangrove ecosystems.

Despite their importance, data on mangrove grapsoid ecophysiology remains patchy. Gross et al. (1966) showed that the mangrove crabs *N. meinerti* and *Cardisoma carnifex* (Herbst, 1794) are powerful osmoregulators in both concentrated and dilute media (concluded from a 2-day experiment), which would allow them to survive in the landward *Avicennia marina* (Forssk.) Vierh. zone subjected to periodic extreme salinity fluctuations. Although mangrove crabs usually show a distinct zonation, osmoregulatory ability has not been shown to be linked with the observed zonation patterns (Frusher et al., 1994). However, osmoregulatory ability may not be enough to explain actual salinity tolerances. For example, the temperate estuarine crab *Callinectes similis* Williams, 1966, has been shown to be a strong osmoregulator, although scope for growth and long-term actual growth experiments show that the energy remaining for somatic growth is slightly reduced in higher salinities and is reduced by more than half in lower salinities (Guerin and Stickle, 1997a,b). Therefore, the long-term extra energy expenditure due to subtle, sublethal, effects of salinity may dictate long term salinity tolerances, especially for animals already living above their optimal salinity.

High evaporation and natural episodic fluxes of freshwater input into estuaries from meteorological events are common and often result in acute salinity fluctuations; whereas anthropogenically induced changes in freshwater input result in chronic, if not indefinite, changes in estuarine salinity regimes (Christensen et al., 1997). Groundwater has been shown to contribute large amounts of water to estuarine water budgets, generally buffering

salinity (Church, 1996; Moore, 1996). It is especially important in large riverine mangrove forests away from direct river input where water circulation is reduced and evaporation is high. For example, Kitheka (1998) calculated that the backwater residence time was more than 11 days in Mida Creek, Kenya. Anthropogenic changes in groundwater outflow may be leading to changes in mangrove and seagrass distribution, community structure, faunal distribution and species richness (Kitheka, 1998; Kitheka et al., 1999; Tack and Polk, 1999; Kamermans et al., 2002). Furthermore, the degree of community change induced by freshwater flow alterations is difficult to predict quantitatively because of the lack of field-based salinity range data available (Christensen et al., 1997).

Although there have been studies of acute osmotic stress tolerances of mangrove crabs (Gross et al., 1966; Frusher et al., 1994; Schubart and Diesel, 1998; Anger and Charmantier, 2000), few studies have looked at chronic salinity tolerances. Since both growth and osmoregulation are energy requiring processes, the sublethal effects of chronic salinity stress may include changes in the energy budget of the animal. Physiological rates can be measured across a salinity gradient and converted to energetic equivalents to determine what effect salinity has on components of the energy budget. An energy budget can then be estimated from energetic equivalents of fundamental physiological processes such as food uptake, excretion and oxygen consumption (Withers, 1992).

N. meinerti usually inhabits the landward fringing *A. marina* zone of the mangal, with large fluctuations in salinity and have been found inhabiting salinities ranging from 1‰ to 65‰ (Gillikin, 2000). *N. smithi* occupy the lower broad *Rhizophora mucronata* Lam. zone, which is usually inundated daily and thus has a more stable salinity regime, but which may be as low as 21‰ and as high as 53‰ (Gillikin, 2000). Both species are semi-terrestrial and are well suited for aerial respiration. They were chosen for this study due to their abundance, large size, wide geographical distribution (see Davie, 1994) and fossorial mode of life. Much ecological work has been done on *N. meinerti* (see references herein), while little has been done on *N. smithi*. *N. smithi* is widely distributed throughout the Indo-Pacific, but has been wrongly identified in the south-western Pacific (e.g. Giddens et al., 1986; Robertson and Daniel, 1989; Micheli, 1993) where it is replaced by its sister species *N. trispinosum* (Davie, 1994).

The objective of the present study is to give insight into the possible long-term effects of altered salinity regimes on the bioenergetics of two of the potentially most important Kenyan mangrove crab species.

2. Materials and methods

2.1. Laboratory methods

Intermolt adult specimens were collected in Gazi Bay, Kenya (S04°25' E039°30') (Fig. 1) in September 1999. Pore-water salinity was ± 32 ‰ in collection areas. Individuals of both species were captured by hand and were transferred to the field laboratory within 3 h, rinsed with seawater, blotted dry, sexed, weighed (to the nearest 0.01 g) and carapace width (CW) measured. Average weight and size of freshly caught *N. meinerti* was 35.65 ± 11.06 g and 37.6 ± 2.9 mm CW ($n=54$) and for *N. smithi* was

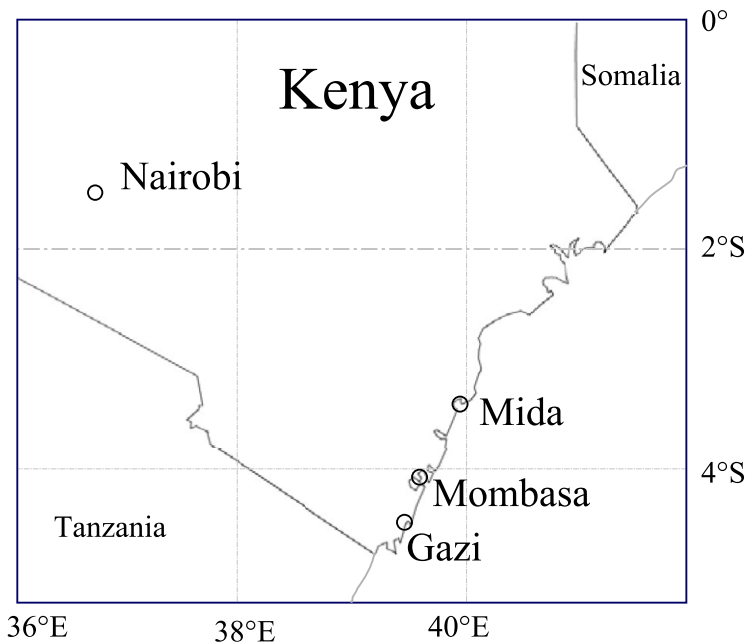


Fig. 1. Map of the Kenyan coast with study areas indicated.

45.59 ± 8.45 g and 35.4 ± 2.1 mm CW ($n=63$). Crabs were maintained on a 12-h light–dark cycle in individual chambers (20 cm diameter). Chambers were filled with approximately one cm of water, which allowed them to replenish their branchial water, but not fully submerge themselves. During the collection period, crabs were maintained in natural seawater (± 32 ‰) and were offered fresh leaves (at least 5 g wet weight (WW)). Water and food was changed daily. Water remained between 24.0 and 26.9 °C with a pH between 7.0 and 8.0 throughout the experiment. In Kenya, all laboratory work was conducted in a basic field station operated by the Kenya Marine and Fisheries Research Institute (KMFRI) located in Gazi Village, Kenya (about 40 km south of Mombasa).

Ten crabs of each species were acclimated to one of four salinities (16‰, 32‰, 48‰ and 65‰). Crabs were selected for the different salinities assuring similar distribution of size and sex throughout the salinity range. Acclimation took place over 4 weeks; during the first week, stepwise increments were used to reach the target salinities (e.g. 2–5‰ per day). Water of different salinity was produced by either diluting filtered natural seawater with local well water or by adding natural sea-salt. Salinity was measured using a WTW multiline P4 conductivity meter.

Food preference studies took place during the first days of the step-wise acclimation. Crabs were offered similar amounts of fresh green *A. marina*, *Cerriops tagal* (Perr.) C.B. Rob. and *R. mucronata* leaves. After 24 h, any leaf that showed signs of feeding was considered eaten. Feeding preference studies were carried out for 3 days. *A. marina* leaves

and *R. mucronata* leaves for *N. meinerti* and *N. smithi*, respectively, were used during the energy consumption experiment.

A simplified energy budget (E) in each salinity was estimated using the following equation:

$$E = FC - (R + U); \quad (1)$$

where FC = energy consumed as food, R = respiration and U = excretion.

After 4 weeks of acclimation (3 weeks at target salinities), energy consumed as food (FC), excretion (U) and oxygen consumption (R) rates were measured on all surviving crabs to allow an estimation of their energy budget.

Energy consumption experiments (FC) were carried out over 3 days, during which the crabs were offered 2–3 g WW of fresh green leaf material of their preferred type. All leaves were collected daily, from the same two trees (from the same areas the crabs were collected) and WW was measured within 1 h. Crabs were allowed to feed for a 24-h period, after which leaves and leaf fragments were removed, blotted dry, dried at 60 °C for 24 h and weighed (leaves needed to be dried due to the water they absorbed during the experiment). To obtain regressions of WW to dry weight (DW), additional leaves were treated in the same manner as above, except were placed in containers without crabs (for all salinities). For each salinity, two controls were used, one with whole leaves and one with shredded leaves to determine the effect of shredding by crabs and salinity on WW/DW conversions. Samples of dried leaves were returned to Belgium for calorimetric analysis (IKA-calorimeter type C400). To determine feeding rates, initial leaf WW was converted into DW and the DW after feeding was subtracted. For *A. marina* leaves, initial WW was converted to DW using $DW = 0.388 * WW$ ($F = 314.9$, $p < 0.00001$, adj $R^2 = 0.93$, $n = 24$). For *R. mucronata* leaves, there was an effect of salinity ($F = 7.4$, $p < 0.01$, $n = 18$), thus $DW = 0.278 * WW + 0.0044 * \text{Salinity}$ ($F = 98.05$, $p < 0.00001$, adj $R^2 = 0.92$).

Emmerson and McGwynne (1992) found that all fecal production of *N. meinerti* ceased after 2 days without food. Therefore, prior to the feeding experiment, crabs were starved for a minimum of 48 h to purge their gut of food. An attempt was made to use the method of Conover (1966) to determine the energy absorbed from food (AE) (i.e., energy in food consumed minus the energy lost as ejecta or feces). This is a simple method, which assumes that inorganic contents of the food are not taken up in the gut and can therefore be used as a tracer; thus, only the ratio of ash-free dry weight to dry weight in both the food and feces is required. However, this method gave very unsatisfactory results (all highly negative absorption efficiencies) and thus AE could not be determined (see Discussion).

During the food consumption experiment, water samples were taken at the end of each day for determination of ammonia and urea excretion rates (U) (a standard method used to determine excretion; e.g., Lee and Chen, 2003). All containers (with crabs and controls) contained 100 ml of water. Ammonia and urea analysis of water samples was performed colorimetrically using the phenol-hypochlorite method of Solorzano (1969) and the diacetyl monoxime method of Rahmatullah and Boyde (1980), respectively. Standards were made up in chemically defined seawater of each salinity (as defined by

Vercauteren and Blust, 1996). Detection limits for ammonium and urea were 6 and 3 $\mu\text{mol l}^{-1}$, respectively. Excretion rates were converted to energy expenditure rates using the factors of 0.0832 cal per μmol of ammonia and 0.1544 cal per μmol of urea, which are based on the standard heat of formation of each compound (Elliot and Davison, 1975).

Oxygen consumption (R) was determined by using the direct aerial method, where CO_2 is absorbed by an alkali (in our case, 20% potassium hydroxide) (Dixon, 1934). Crabs were allowed to respire for at least 5 h in respirometers with 100 ml of water (~ 1 cm deep). Data were collected after the crabs were allowed to settle from handling. Oxygen consumption rates were converted into energy expenditure using the oxy-calorific value of 4.8 cal per ml O_2 consumed (Crisp, 1971).

Immediately after the respiration experiment, 1 ml hemolymph samples were taken from the arthro-dial membrane at the base of the third and fourth walking legs with a sterile 19-gauge hypodermic needle and syringe. Due to high mortality of *N. smithi* in 65‰ (see Results), three additional *N. smithi* were caught fresh from the field and acclimated to 65‰ over a 3-day stepwise acclimation period. They were held at 65‰ for 3 days and hemolymph and water samples taken for measurement of the aforementioned variables. On occasion, obtaining hemolymph samples was difficult, and for a few crabs impossible due to clotting. All hemolymph and water samples were immediately frozen at -20°C until analysis in Belgium. Hemolymph urea was measured using the method above, while ammonia concentrations were determined using the Sigma ammonia test kit 171 (Sigma-Aldrich, Belgium). Using an ultraviolet endpoint, the fluorescence was read at 340 nm on a Spectra MAX Gemini ultraviolet spectrophotometer (Molecular Devices, USA), resulting in a detection limit of 50 $\mu\text{mol NH}_4^+ \text{l}^{-1}$. Osmolality of hemolymph and water samples was measured on a Fiske One-Ten Osmometer (Fiske Associates, USA) on 30 μl of sample. All hemolymph and water samples were analyzed for Na^+ , K^+ and Cl^- on an AVL 9180 Electrolyte Analyzer (AVL Scientific, USA).

2.2. Field methods

Fieldwork took place in November 1999 in two Kenyan mangrove areas, Gazi Bay and Dabaso, Mida Creek ($\text{S}03^\circ21' \text{E}039^\circ59'$) (Fig. 1). Kenya experiences a monsoonal climate with the majority of precipitation in Mombasa falling between April and June (mean = 192 mm month $^{-1}$), with February and January being the driest months (mean = 22 mm month $^{-1}$); November receives 96 mm month $^{-1}$ on average (Lieth et al., 1999). Two sites were chosen in Dabaso, one for each species, whereas three sites were chosen in Gazi. Two of the sites in Gazi were the same as the collection areas of the laboratory experiment. The third site was chosen due to the large difference between pore water ($\sim 60\text{‰}$) and *N. meinerti* burrow water ($\sim 20\text{‰}$) salinity (indicating hypersaline burrow water during the dry season; cf. Stieglitz et al., 2000).

At each field site, at least nine crabs were sampled for hemolymph and three replicate water samples were taken from their burrows (when possible). Pore water from nine boreholes was taken from the area surrounding the sampled crab burrows. Hemolymph was sampled as in the laboratory. Only non-gravid adult intermolt specimens were used (*N. meinerti* = 35.7 ± 3.1 mm CW, $n = 32$; *N. smithi* = 34.6 ± 2.9 mm CW, $n = 22$).

Hemolymph and water samples were stored in a cool box at 2–3 °C during field work (for a maximum of 3 h), then were treated the same as in the laboratory experiment. Pore water, burrow water and hemolymph were analyzed for urea, ammonia, osmolality and ion concentrations using the methods detailed above.

2.3. Statistical analysis

After testing for a normal distribution using the Kolmogorov–Smirnov goodness of fit test and homogeneity of variances using the multivariate Box M test, ANOVA was used to test for significance. The post hoc Least Squares Difference (LSD) test was used to test for differences between individual variables. Where appropriate, Student's *t*-test was used. All

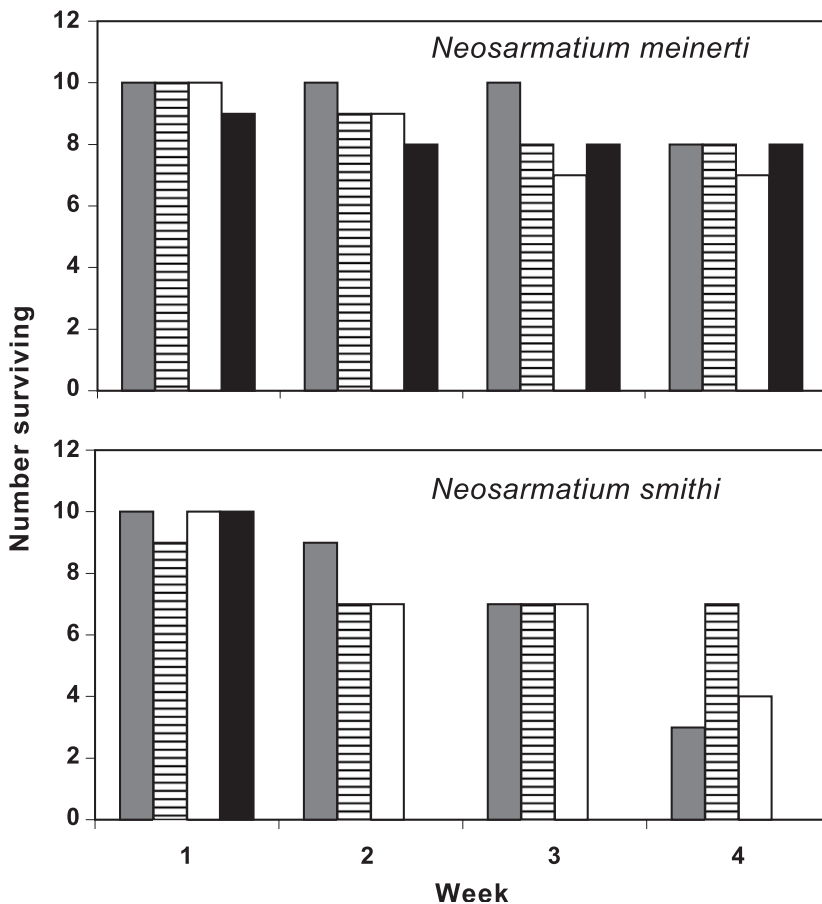


Fig. 2. Number of crabs out of 10 surviving at the end of each week in each salinity during the acclimation period (first week is the step-wise acclimation to target salinity) (gray = 16 ‰, hatched = 32 ‰, white = 48 ‰, black = 65 ‰).

Table 1
Percent of all 'eaten' leaves (see text) measured on consecutive days

Species	Day	Rm, %	Ct, %	Am, %
<i>Neosarmatium smithi</i>	1	73	24	3
	2	88	12	0
	3	50	44	6
<i>Neosarmatium meinerti</i>	1	21	11	68
	2	31	15	54
	3	28	26	46
	4	23	23	54

Rm = *Rhizophora mucronata*, Ct = *Ceriops tagal*, Am = *Avicennia marina*.

data analyses were carried out using Statistica 4.3D (Statsoft). Data are represented as means $\pm$ standard deviations.

3. Results

3.1. Laboratory

A distinct difference was observed in the mortality rates of *N. smithi* and *N. meinerti* during acclimation (Fig. 2). *N. meinerti* survived equally well in all salinity treatments,

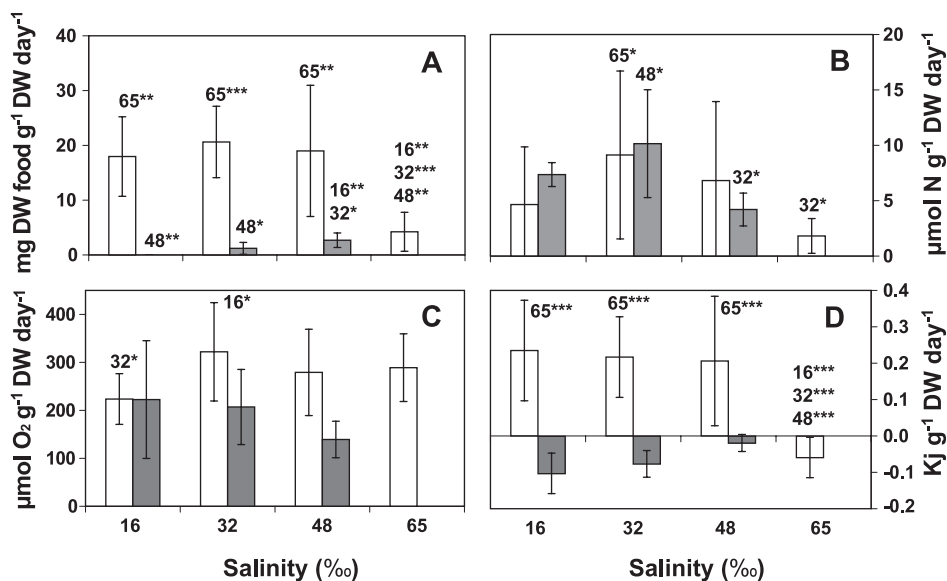


Fig. 3. Means and standard deviations of food consumption (A), nitrogen excretion (B), oxygen consumption (C) and energy budgets (D) in *N. meinerti* (white bars) and *N. smithi* (gray bars) after the 4-week acclimation period in four salinities. Numbers above bars are salinities which were significantly different (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

while *N. smithi* had 100% mortality at 65‰ after just 5 days at the target salinity. Subjecting this species to 16‰ and 48‰ also resulted in high mortality rates, while those in 32‰ had comparable rates with *N. meinerti* in all treatments. There was no significant weight change in any of the animals throughout the experiment, nor did any individual molt.

There was a clear preference in food choice for both species (Table 1). Salinity had a significant effect on food consumption of both species (ANOVA *N. meinerti* $p < 0.001$; *N. smithi* $p < 0.05$) (Fig. 3A). At the commencement of the feeding experiment, *N. meinerti* in all salinities were very active and started to eat within seconds of being offered food, whereas *N. smithi* remained lethargic after being fed and only ate in the 32‰ and 48‰ treatments. Leaves of *A. marina* contained significantly more energy ($17.8 \pm 1.3 \text{ kJ g}^{-1} \text{ DW}$, $n = 31$) than *R. mucronata* leaves ($16.8 \pm 0.4 \text{ kJ g}^{-1} \text{ DW}$, $n = 10$) (Student's *t*-test, $p = 0.02$).

At the highest salinity, both species showed significantly lower nitrogen excretion (LSD, $p < 0.05$) (Fig. 3B). Respiration was significantly lower in 16‰ compared with

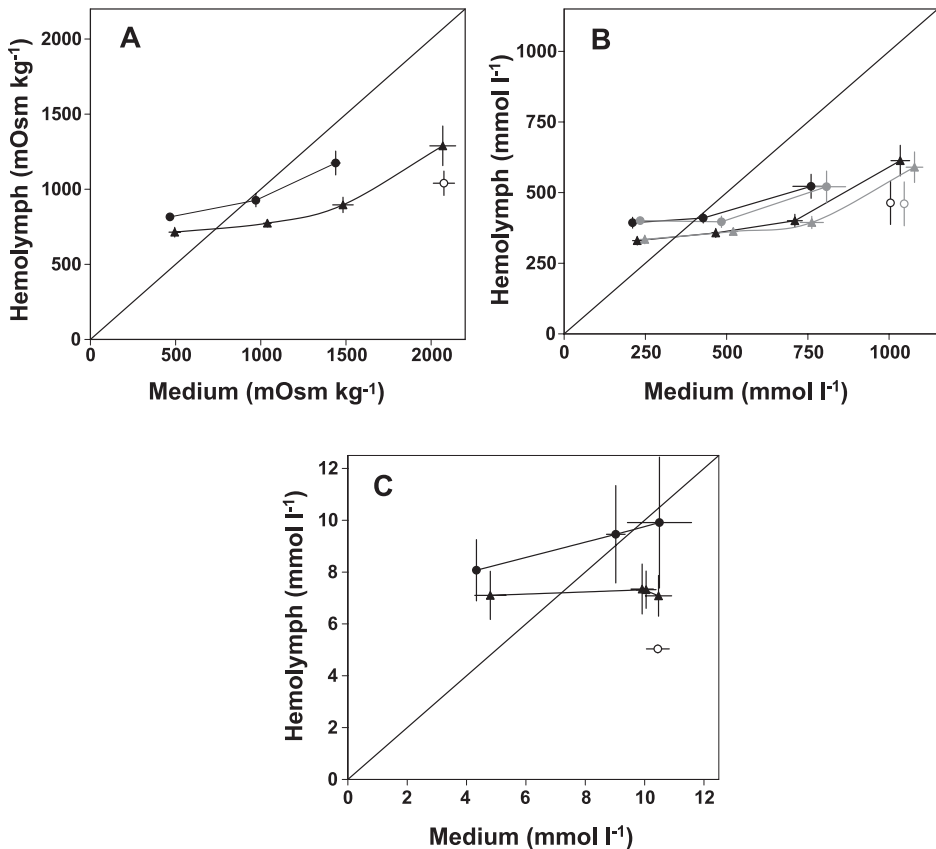


Fig. 4. Means and standard deviations of hemolymph osmotic pressure (A), Na^+ (black) and Cl^- (gray) (B) and K^+ (C) of *N. meinerti* (▲) and *N. smithi* (●) after the 4-week acclimation period in each salinity. Data from *N. smithi* held at 65‰ for 3 days are also given (○). The isosmotic line is shown as diagonal.

32‰ in *N. meinerti* (LSD, $p=0.02$) (Fig. 3C). The energy budget was significantly affected by salinity in *N. meinerti* (ANOVA, $p<0.001$) and was negative in all salinities for *N. smithi* (Fig. 3D).

Both species regulated their internal osmolality at all salinities, except *N. smithi* at 32‰. *N. meinerti* had much lower hemolymph osmolalities and ion concentrations than *N. smithi*, over all salinities (Fig. 4A,B). However, *N. smithi* subjected to 65‰ for 3 days had lower hemolymph osmolality than *N. meinerti* that had been in that salinity for 1 month (ANOVA, $p<0.05$). There was no difference observed in K^+ concentration in either species used in the energy budget experiment, however it was significantly lower in the *N. smithi* subjected to 65‰ for only 3 days, when compared to all other salinities (LSD, $p<0.05$). The *N. smithi* in 65‰ for 3 days had similar Na^+ and Cl^- concentrations compared to individuals in other salinities (Fig. 4A).

Ammonia concentrations in hemolymph were significantly higher in *N. smithi* as compared to *N. meinerti* at all salinities (excluding *N. smithi* at 65‰), ranging from 4.5 to 6.5 times higher (ANOVA, $p<0.0001$) (Fig. 5A). *N. meinerti* had increased levels of ammonia in 65‰ compared to all other salinities (ANOVA, $p<0.001$) (Fig. 5A). There

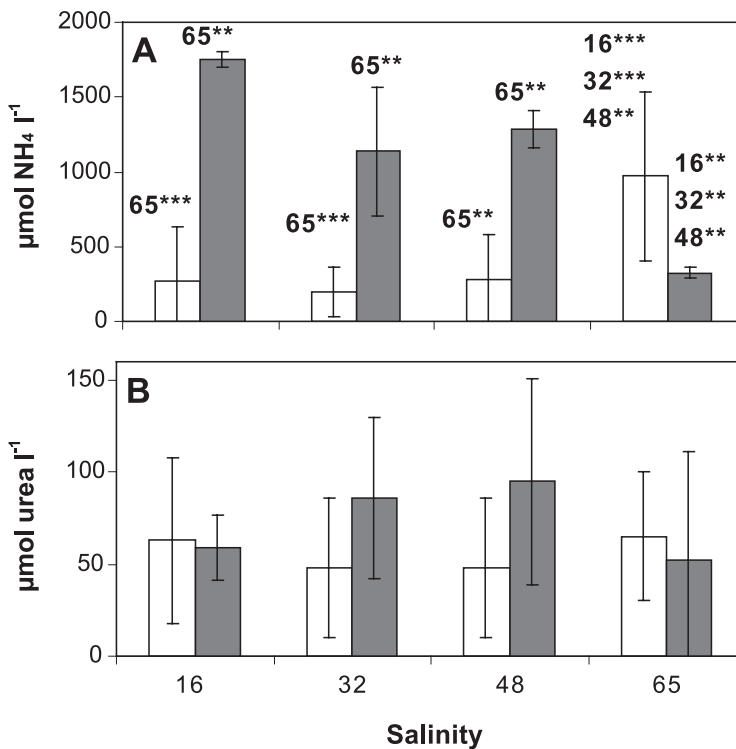


Fig. 5. Means and standard deviations of hemolymph ammonia (A) and urea (B) in *N. meinerti* (white bars) and *N. smithi* (gray bars) after the 4-week acclimation period in each salinity (note that *N. smithi* in 65‰ were held at this salinity for only 3 days). Numbers above bars are salinities which were significantly different (* $p<0.05$, ** $p<0.01$, *** $p<0.001$).

Table 2

Means and standard deviations of ion concentrations, osmotic pressure, ammonia and urea in pore water (PW), burrow water (BW) and hemolymph (HL) measured in the field

Species	Location and salinity	Type	Na ⁺ , mmol l ⁻¹	K ⁺ , mmol l ⁻¹	Cl ⁻ , mmol l ⁻¹	NH ₄ ⁺ , μmol l ⁻¹	Urea, μmol l ⁻¹	Osmolality, mOsm kg ⁻¹
<i>N. meinerti</i> (n = 11)	Dabaso (37–39)	PW	491 ± 58	10.5 ± 1.5	546 ± 68	28 ± 64	16 ± 26	1179 ± 37
		HL	341 ± 30	11.1 ± 4.8	328 ± 30	807 ± 488	31 ± 42	781 ± 69
<i>N. meinerti</i> (n = 9)	Gazi (20–65)	BW	637 ± 151	13.3 ± 2.7	714 ± 167	73 ± 120	24 ± 13	nm
		PW	825 ± 28	16.3 ± 0.7	924 ± 32	2 ± 5	9 ± 6	nm
		HL	340 ± 27	12.0 ± 2.7	331 ± 32	565 ± 313	24 ± 18	752 ± 64
<i>N. meinerti</i> (n = 12)	Gazi (23–30)	BW	382 ± 22	8.5 ± 0.6	423 ± 24	19 ± 25	7 ± 4	nm
		PW	443 ± 48	9.6 ± 1.2	481 ± 43	5 ± 7	10 ± 6	1039 ± 27
		HL	327 ± 10	9.2 ± 1.1	322 ± 12	346 ± 131	14 ± 9	729 ± 40
<i>N. smithi</i> (n = 10)	Dabaso (31–38)	BW	534 ± 98	11.7 ± 2.2	584 ± 104	71 ± 34	40 ± 31	1122 ± 134
		PW	499 ± 38	10.7 ± 0.9	552 ± 41	28 ± 20	9 ± 6	1150 ± 73
		HL	382 ± 11	10.4 ± 1.1	368 ± 17	601 ± 268	52 ± 78	815 ± 61
<i>N. smithi</i> (n = 12)	Gazi (27–35)	BW	423 ± 39	9.2 ± 0.8	472 ± 42	87 ± 61	13 ± 10	1020 ± 105
		PW	428 ± 37	9.2 ± 1.0	477 ± 41	113 ± 59	13 ± 11	896 ± 126
		HL	373 ± 13	10.8 ± 1.9	365 ± 11	499 ± 229	43 ± 47	785 ± 25

n = number of individuals sampled; salinity = salinity range of available water in ‰; nm = not measured.

was no overall effect of salinity on ammonia concentrations in *N. smithi* (ANOVA), however the individuals held in 65‰ for 3 days maintained lower concentrations (Fig. 5A), which were comparable to *N. meinerti* hemolymph (aside from 65‰). Urea concentrations were not significantly different between treatments (ANOVA) and are given in Fig. 5B.

3.2. Field

Table 2 summarizes the results of the variables measured on pore and burrow water and hemolymph. Due to logistical problems, not all water samples could be measured for osmolality. Hemolymph ammonia concentrations of *N. smithi* in the laboratory were significantly higher than those in the field (Student's *t*-test, $p < 0.0001$). The highest hemolymph ammonia concentrations in laboratory held *N. meinerti* (in 65‰) were not significantly different from those at the Dabaso site (Student's *t*-test).

4. Discussion

The results show that *N. meinerti* is a strong hyper- and hypo-osmoregulator, allowing them to survive in salinities from at least 16–65‰. *N. meinerti* appeared healthy at the end of the experiment as they aggressively ate during the feeding experiment and had comparable feeding rates to those in the study of Emmerson and McGwynne (1992), in all salinities but 65‰. Although food consumption (FC) of *N. meinerti* decreased in 65‰, energy expenditure ($R + U$) did not, driving their energy budget below zero. These data

indicate that although *N. meinerti* can iono- and osmoregulate for 1 month in hypersaline conditions (Fig. 4), it is not without significantly increased physiological costs, as indicated by the negative energy budget in 65‰ (Fig. 3D).

Mortality data distinctly show the large difference in salinity tolerance between the two congeners (Fig. 2). Data from the three individuals held at 65‰ for 3 days suggest that *N. smithi* is capable of withstanding hypersaline conditions for a short period (± 1 week). Moreover, they survived well in 16‰ and 48‰ up to 3 weeks in the laboratory, with elevated mortality only during the fourth week. Due to the high mortality rate, a reliable estimation of the energy budget could not be calculated for *N. smithi*. The negative energy budget in the control salinity of 32‰ and the large difference between hemolymph ammonia of *N. smithi* in the laboratory and field (Table 2 and Fig. 5A) indicates another stress factor acted on these animals. Additionally, *N. smithi* had overall much lower consumption rates than its sister species, *N. trispinosum*, fed on a similar diet (Micheli, 1993). In both species studied, Na^+ and Cl^- each contributed about 45% of total osmolality and thus paralleled osmolality (Fig. 4B). K^+ , which is not associated with volume readjustment (Gilles and Pequeux, 1983), remained low and constant across salinities and was within the range reported for other crustaceans (Mantle and Farmer, 1983) (Fig. 4C). Therefore, there does not seem to be a breakdown of ionoregulation in either species. Although our calculations of the energy budget for *N. smithi* do not seem a reliable indication of salt stress alone, mortality and hemolymph osmolality clearly indicate increased salt stress at higher salinities.

As mortality in 16‰ and 48‰ was increased in *N. smithi* only later in the experiment and recent studies suggest that leaves are only a part of the diets of sesamids and that sediment and animal matter contribute significantly to their natural diets (Bouillon et al., 2002; Skov and Hartnoll, 2002), it may be argued that the overall poor condition of these animals was caused by offering an incomplete diet. Furthermore, it has been postulated that mangrove crabs eat decayed litter over senescent leaves due to the increased palatability (lower tannins) and lower C/N ratio (Giddens et al., 1986; Robertson, 1986; Micheli, 1993). However, the diet used in this experiment (green leaves) has a lower C/N ratio than both senescent and decayed leaves because the trees have not yet reabsorbed the nutrients (cf. Robertson, 1988; Rao et al., 1994). Crabs have also been shown to choose green leaves over senescent or decayed leaves (Camilleri, 1989; Greenaway and Raghaven, 1998; Ashton, 2002) and will readily consume green leaves in the field (Ashton, 2002; Gillikin, 2000). Although lack of nutrition may have played a role in the poor condition of *N. smithi*, we argue that it was probably not mostly caused by offering an incomplete diet, but was a result of other stressors reducing food intake.

Mangrove leaves contain high concentrations of inorganic water-soluble salts (Cram et al., 2002) and crabs have been shown to take up salts from leaf diets (Greenaway and Linton, 1995; Greenaway and Raghaven, 1998). Therefore, we believe while attempting to measure AE, the main assumption of Conover (1966), that no inorganic matter is taken up via the gut, was broken. The fact that AE could not be determined challenges the reliability of these results. However, if it is assumed that AE is highest in the crabs' optimal salinity, as was found for other invertebrates (e.g. Moens and Vincx, 2000), AE data would only

strengthen our arguments by further reducing consumed energy of *N. meinerti* in 65‰. Moreover, we calculated that to lose statistical significance of a reduced energy budget in *N. meinerti* in 65‰, as compared to the other treatments, AE would have to be at least 25% higher in the 65‰ treatment, an unlikely scenario. Although AE could not be determined, comparing the energy budget of *N. meinerti* between salinities should still be valid due to the large difference between 65‰ and the lower salinities and the reproducibility within the lower salinities (the energy budget of the 23 crabs in the three lower salinities was statistically indistinguishable; Fig. 3D). However, it is stressed that these results are relative approximations of energy budgets and should not be extrapolated to actual field situations.

Ammonia is a highly toxic substance, partly because it elevates pH, which changes protein structure (Withers, 1992), interferes with amino acid transport and affects many aspects of the nervous system (reviewed in Wright, 1995). In the laboratory, hemolymph ammonia concentrations ranged from 195 ± 164 to $970 \pm 561 \mu\text{mol l}^{-1}$ in *N. meinerti* and from 1137 ± 429 to $1753 \pm 54 \mu\text{mol l}^{-1}$ in *N. smithi*; the upper values being rare (Claybrook, 1983), but not unheard of, especially in more terrestrial crabs (cf. Wood and Boutilier, 1985). The higher hemolymph ammonia concentrations observed in *N. smithi* held in the laboratory for 1 month as compared to those in the field clearly indicate the poor condition of these animals. *N. meinerti* in 65‰ had high hemolymph ammonia concentrations, but still within the range of those at the Dabaso site. The crabs at this site may have been experiencing excretory difficulties as there was no free burrow water present at this site and urine flow is tied to overall water balance (Greenaway, 1991). In high salt concentrations, however, sodium is not reabsorbed from the urine, thus affecting the ion pumps in the gills which could block ammonia excretion (Greenaway, 1991). This suggests *N. meinerti* in 65‰ in the laboratory, with high hemolymph ammonia concentrations and low nitrogen excretion may have been experiencing an impairment of ammonia excretion, thus compounding the effects of salt exposure.

The *N. smithi* held at 65‰ for 3 days maintained a hemolymph osmolality lower than *N. meinerti* at that salinity, and about the same concentration as *N. smithi* held in 32‰ for 1 month. As mortality data show, after about one week at this salinity, they die. This suggests that hemolymph osmolality is maintained at a fairly low concentration for a few days until the animals can no longer osmoregulate, and die. Thus, when discussing long term ecosystem change, and an animal's ability to cope with it, acute studies should only be used in the correct context. Additionally, considering only mortality and osmoregulatory capacity of *N. meinerti* in 65‰, the large energy expenditure needed to survive in this salinity is not evident. This may be a possible reason why Frusher et al. (1994) did not find a connection between osmoregulatory abilities and distribution in the field; they held their animals at different salinities for 7 days and only measured mortality and osmoregulatory capacity.

It is interesting to note that *N. meinerti* in the field had comparable hemolymph osmolality with those in 16‰ and 32‰ in the laboratory. When considering the energy budget, mortality and hemolymph properties, *N. meinerti* seems well adapted to salinities ranging from 16‰ to 48‰. *N. smithi* on the other hand, had highest survival in 32‰ and hemolymph properties comparable to field populations in 16‰ and 32‰ (see Table

2), which suggest an optimal salinity range of 16‰ to 32‰, and not much higher. Although *N. smithi* generally live in areas inundated daily, their burrow water as well as ambient pore water salinity can be as high as 53‰ (Gillikin, 2000). It has been assumed that mangrove crabs commonly have higher salt tolerances than mangrove trees (Jones, 1984), which would make crabs less vulnerable to salinity increases. However, our data suggest the contrary, i.e., that both Kenyan *Neosarmatium* species are more susceptible to increased osmotic stress than are adult mangrove trees in general (see Chen and Twilley, 1998 and references therein for salt tolerances of adult trees), especially when living above their optimal salinity.

Although both *Neosarmatium* species inhabiting Kenyan mangrove forests are closely related congeners (C.D. Schubart pers. com.) and can occur in close proximity to one another (Gillikin, 2000), they differ substantially in their salinity tolerances. *N. meinerti* can withstand high salt concentrations, but it is unlikely that populations could be fully viable in hypersaline areas due to the increased energy expenditure of osmoregulation. Partial groundwater redirection may or may not drastically increase salinities; however, specimens living in their upper salinity tolerance may still be effected by small changes in salinity. Where there is suitable habitat (e.g. shelter and food), crabs will tolerate living above their optimal salinity (cf. McGaw, 2001) making them more susceptible to salinity changes. Both studied species have been recorded in high salinities (*N. meinerti* up to 65‰ and *N. smithi* up to 53‰) in Mida Creek and Gazi Bay mangrove forests (Gillikin, 2000). The increased stress of non-optimal salinities could make them more susceptible to other stressors such as temperature, desiccation, pollution, food shortages or disease, as was probably the case in laboratory held *N. smithi*. Such synergic effects must be taken into account when considering the effects of salinity on field populations.

As previously stated, crabs are important for mangrove ecosystems mostly due to their burrowing activities. Both species studied here are large burrowers, occurring in high densities, therefore probably placing them among the most important faunistic components of Kenyan mangrove ecosystems. If, due to increasing salinity, populations would decrease or disappear, the mangrove forest could be impacted (cf. Smith et al., 1991). Groundwater flow has been shown to affect estuarine salinity regimes (see previous references), if it is altered in East Africa, these crabs may be impacted, in particular, *N. smithi*. Moreover, *N. smithi* inhabit a much larger area in both fringe and riverine forest types (Gillikin, 2000), this and their lower salinity tolerance and adaptational capacity, makes them the more critical species.

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References

- Anger, K., Charmantier, G., 2000. Ontogeny of osmoregulation and salinity tolerance in a mangrove crab *Sesarma curacaoense* (Decapoda: Grapsidae). J. Exp. Mar. Biol. Ecol. 251 (2), 265–274.
- Ashton, E.C., 2002. Mangrove sesarmid crab feeding experiments in Peninsular Malaysia. J. Exp. Mar. Biol. Ecol. 273 (1), 97–119.
- Bouillon, S., Koedam, N., Raman, A.V., Dehairs, F., 2002. Primary producers sustaining macro-invertebrate communities in intertidal mangrove forests. Oecologia 130, 441–448.
- Bright, D.B., Hogue, C.L., 1972. A synopsis of the burrowing land crabs of the world and list of their symbionts and burrow associates. Contr. Sci. Nat. Hist. M220, 1–58.
- Camilleri, J.C., 1989. Leaf choice by crustaceans in a mangrove forest in Queensland. Mar. Biol. 102, 453–459.
- Chen, R., Twilley, R.R., 1998. A gap dynamic model of mangrove forest development along gradients of soil salinity and nutrient resources. J. Ecol. 86, 37–51.
- Christensen, J.D., Monaco, M.E., Lowery, T.A., 1997. An index to assess the sensitivity of Gulf of Mexico species to changes in estuarine salinity regimes. Gulf Res. Rep. 9 (4), 219–229.
- Church, T.M., 1996. An underground route for the water cycle. Nature 380, 579–580.
- Claybrook, D.L., 1983. Nitrogen metabolism. In: Bliss, D.E., Mantel, L.H. (Eds.), The Biology of Crustacea, Internal Anatomy and Physiological Regulation, vol. 5. Academic Press, New York, pp. 163–213.
- Conover, R.J., 1966. Assimilation of organic matter by zooplankton. Limnol. Oceanogr. 11, 338–354.
- Cram, J.W., Torr, P.G., Rose, D.A., 2002. Salt allocation during leaf development and leaf fall in mangroves. Trees-Struct. Funct. 16, 112–119.
- Crisp, D.J., 1971. Energy flow measurement. In: Holme, N.A., McIntyre, A.O. (Eds.), Methods for the Study of Marine Benthos. Blackwell, Oxford, pp. 197–279.
- Davie, P.J.F., 1994. Revision of *Neosarmatium* Serène and Soh (Crustacea: Brachyura: Sesarminae) with descriptions of two new species. Mem. Queensl. Mus. 35, 35–74.
- Dixon, M., 1934. Manometric Methods as Applied to the Measurement of Cell Respiration and other Processes. Cambridge Univ. Press, London.
- Elliot, J.M., Davison, W., 1975. Energy equivalents of oxygen consumption in animal energetics. Oecologia 19, 195–201.
- Emmerson, W.D., McGwynne, L.E., 1992. Feeding and assimilation of mangrove leaves by the crab *Sesarma meinerti* De Man in relation to leaf-litter production in Mgazana, a warm-temperate southern African mangrove swamp. J. Exp. Mar. Biol. Ecol. 157, 41–53.
- Frusher, S.D., Giddins, R.L., Smith, T.J., 1994. Distribution and abundance of Grapsid crabs (Grapsidae) in a mangrove estuary: effects of sediment characteristics, salinity tolerances, and osmoregulatory ability. Estuaries 17 (3), 647–654.
- Giddens, R.L., Lucas, J.S., Neilson, M.J., Richards, G.N., 1986. Feeding ecology of the mangrove crab *Neosarmatium smithi* (Crustacea: Decapoda: Sesarmidae). Mar. Ecol. Prog. Ser. 33, 147–155.
- Gilles, R., Pequeux, A., 1983. Interactions of chemical and osmotic regulation with the environment. In: Bliss, D.E., Vernberg, W.B. (Eds.), The Biology of Crustacea, Environmental Adaptations, vol. 8. Academic Press, New York, pp. 109–177.
- Gillikin, D.P., 2000. Factors controlling the distribution of Kenyan brachyuran mangrove crabs: Salinity tolerance and ecophysiology of two Kenyan *Neosarmatium* species. MSc thesis. Vrije Universiteit Brussel, Belgium.
- Gillikin, D.P., De Grave, S., Tack, J.F., 2001. The occurrence of the semi-terrestrial shrimp *Merguia oligodon* (De Man, 1888) in *Neosarmatium smithi* H. Milne Edwards, 1853 burrows in Kenyan mangroves. Crustaceana 74 (5), 505–508.
- Greenaway, P., 1991. Nitrogenous excretion in aquatic and terrestrial crustaceans. Mem. Queensl. Mus. 31, 215–227.

- Greenaway, P., Linton, S.M., 1995. Dietary assimilation and food retention time in a herbivorous terrestrial crab *Gecarcoidea natalis*. *Physiol. Zool.* 68 (6), 1006–1028.
- Greenaway, P., Raghaven, S., 1998. Digestive strategies in two species of leaf eating land crabs (Brachyura:Gecarcinidae) in a rain forest. *Physiol. Zool.* 71 (1), 36–44.
- Gross, W.J., Lasiewski, R.C., Dennis, M., Rudy, P., 1966. Salt and water balance in selected crabs of Madagascar. *Comp. Biochem. Physiol.* 17, 641–660.
- Guerin, J.L., Stickle, W.B., 1997a. A comparative study of two sympatric species within the genus *Callinectes*: osmoregulation, long-term acclimation to salinity and the effects of salinity on growth and moulting. *J. Exp. Mar. Biol. Ecol.* 218 (2), 165–186.
- Guerin, J.L., Stickle, W.B., 1997b. Effect of salinity on survival and bioenergetics of juvenile lesser blue crabs *Callinectes similis*. *Mar. Biol.* 129 (1), 63–69.
- Jones, D.A., 1984. Crabs of the mangal ecosystem. In: Por, F.D., Dor, I. (Eds.), *Hydrobiology of the Mangal*. Dr. W. Junk Publishers, The Hague, NL, pp. 89–109.
- Kamermans, P., Hemminga, M.A., Tack, J.F., Mateo, M.A., Marbà, N., Mtolera, M., Stapel, J., Verheyden, A., Van Daele, T., 2002. Groundwater effects on diversity and abundance of lagoonal seagrasses in Kenya and on Zanzibar Island (East Africa). *Mar. Ecol. Prog. Ser.* 231, 75–83.
- Kitheka, J.U., 1998. Groundwater outflow and its linkages to coastal circulation in a mangrove-fringed creek in Kenya. *Estuar. Coast. Shelf Sci.* 47 (1), 63–75.
- Kitheka, J.U., Mwashote, B.M., Ohowa, B.O., Kamau, J., 1999. Water circulation, groundwater outflow and nutrient dynamics in Mida creek, Kenya. *Mangroves Salt Marshes* 3, 135–146.
- Lee, S.Y., 1997. Potential trophic importance of the faecal material of the mangrove crab *Sesarma messa*. *Mar. Ecol. Prog. Ser.* 159, 275–284.
- Lee, S.Y., 1998. Ecological role of grapsid crabs in mangrove ecosystems: a review. *Mar. Freshw. Res.* 49, 335–343.
- Lee, W.C., Chen, J.C., 2003. Hemolymph ammonia, urea and uric acid levels and nitrogenous excretion of *Marsupenaeus japonicus* at different salinity levels. *J. Exp. Mar. Biol. Ecol.* 288, 39–49.
- Lieth, H., Berlekamp, J., Fuest, S., Riediger, S., 1999. Climate diagram world atlas. In: Lieth, H. (Ed.), 1st ed. CD-Series: Climate and Biosphere, vol. 7. Backhuys Publishers, Leiden.
- Mantle, L.H., Farmer, L.L., 1983. Osmotic and ionic regulation. In: Bliss, D.E., Mantel, L.H. (Eds.), *The Biology of Crustacea, Internal Anatomy and Physiological Regulation*, vol. 5. Academic Press, New York, pp. 53–161.
- McGaw, I.J., 2001. Impacts of habitat complexity on physiology: purple shore crabs tolerate osmotic stress for shelter. *Estuar. Coast. Shelf Sci.* 52, 865–876.
- Micheli, F., 1993. Feeding ecology of mangrove crabs in North Eastern Australia: mangrove litter consumption by *Sesarma messa* and *Sesarma smithii*. *J. Exp. Mar. Biol. Ecol.* 171, 165–186.
- Micheli, F., Gherardi, F., Vannini, M., 1991. Feeding and burrowing ecology of two East African mangrove crabs. *Mar. Biol.* 111, 247–254.
- Moens, T., Vincx, M., 2000. Temperature, salinity and food thresholds in two brackish-water bacterivorous nematode species: assessing niches from food absorption and respiration experiments. *J. Exp. Mar. Biol. Ecol.* 243 (1), 137–154.
- Moore, W.S., 1996. Large groundwater inputs to coastal waters revealed by ^{226}Ra enrichments. *Nature* 380, 612–614.
- Rahmatullah, M., Boyde, T.R.C., 1980. Improvements in the determination of urea using diacetyl monoxime; methods with and without deproteinisation. *Clin. Chim. Acta* 107 (1–2), 3–9.
- Rao, R., Woitichik, A.F., Goeyens, L., Van Riet, A., Kazungu, J., Dehairs, F., 1994. Carbon, nitrogen contents and stable carbon isotope abundance in mangrove leaves from an East African coastal lagoon (Kenya). *Aquat. Bot.* 47 (2), 175–183.
- Ridd, P.V., 1996. Flow through animal burrows in mangrove creeks. *Estuar. Coast. Shelf Sci.* 43, 617–625.
- Robertson, A.I., 1986. Leaf-burying crabs: their influence on energy flow and export from mixed mangrove forests (*Rhizophora* spp.) in northeastern Australia. *J. Exp. Mar. Biol. Ecol.* 102, 237–248.
- Robertson, A.I., 1988. Decomposition of mangrove leaf litter in tropical Australia. *J. Exp. Mar. Biol. Ecol.* 116, 235–247.
- 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.

- Schubart, C.D., Diesel, R., 1998. Osmoregulatory capacities and penetration into terrestrial habitats: a comparative study of Jamaican crabs of the genus *Armases* Abele, 1992 (Brachyura: Grapsidae: Sesarminae). Mar. Sci., B 62 (3), 743–752.
- 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.
- Smith, T.J., Boto, K.G., Frusher, S.D., Giddens, R.L., 1991. Keystone species and mangrove forest dynamics: the influence of burrowing by crabs on soil nutrient status and forest productivity. Estuar. Coast. Shelf Sci. 33 (5), 419–432.
- Solorzano, L., 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. Limnol. Oceanogr. 14, 799–801.
- Stieglitz, T., Ridd, P., Müller, P., 2000. Passive irrigation and functional morphology of crustacean burrows in a tropical mangrove swamp. Hydrobiologia 421, 69–76.
- Tack, J.F., Polk, P., 1999. The influence of tropical catchments upon the coastal zone: modelling the links between groundwater and mangrove losses in Kenya, India/Bangladesh and Florida. In: Harper, D., Brown, T. (Eds.), The Sustainable Management of Tropical Catchments. Wiley, London, pp. 359–371.
- Vercauteren, K., Blust, R., 1996. Bioavailability of dissolved zinc to the common mussel *Mytilus edulis* in complexing environments. Mar. Ecol. Prog. Ser. 137, 123–132.
- Warren, J.H., Underwood, A.J., 1986. Effects of burrowing crabs on the topography of mangrove swamps in New South Wales. J. Exp. Mar. Biol. Ecol. 102, 223–236.
- Withers, P.C., 1992. Comparative Animal Physiology. Saunders, New York.
- Wood, C.M., Boutilier, R.G., 1985. Osmoregulation, ionic exchange, blood chemistry and nitrogenous waste in the land crab *Cardisoma carnifex*: a field and laboratory study. Biol. Bull. Mar. Biol. Lab., Woods Hole 169, 267–290.
- Wright, P.A., 1995. Nitrogen excretion: three end products, many physiological roles. J. Exp. Biol. 198, 273–281.