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

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Running head: Ecophysiology of two Kenyan mangrove crabs

Abstract

The potential long-term effects of altered salinity regimes on the bioenergetics
45 of two ecologically important Kenyan mangrove crabs, Neosarmatium meinerti de
Man, 1887 and N. 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
50 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 four 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
55 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 one month in all
salinity treatments. Nevertheless, their energy budget, was significantly reduced (to
60 below zero) 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 conditions, suggesting that long-term
alteration of mangrove salinity regimes may be detrimental for these ecologically
important mangrove crab populations.

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Keywords

Ecophysiology • energy budget • osmoregulation • salinity tolerance • Sesarmidae

Introduction

70 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
75 (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
80 ecosystem, Smith et al. (1991) considered them keystone species. In Kenya, two burrowing congeners, Neosarmatium meinerti and N. 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;
85 Gillikin, 2000). Therefore, these two species probably play a very significant role in 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
90 and dilute media (concluded from a two 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
95 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, 1997b).
100 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;
105 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
110 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;
115 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; 120 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 125 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).

Neosarmatium meinerti usually inhabits the landward fringing A. marina zone of the mangal, with large fluctuations in salinity and have been found inhabiting 130 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 135 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
140 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.

145 **Materials and methods**

Laboratory methods

Intermolt adult specimens were collected in Gazi Bay, Kenya (S04° 25' E039° 30') (Fig. 1) in September 1999. Pore-water salinity was $\pm 32\text{‰}$ in collection areas. Individuals of both species were captured by hand and were transferred to the field
150 laboratory within three hours, 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 45.59 ± 8.45 g and 35.4 ± 2.1 mm CW (n=63). Crabs were maintained on a 12-hour light-dark cycle in individual chambers (20 cm diameter). Chambers were
155 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 ($\pm 32\text{‰}$) 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.
160 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 four weeks; during the first week, stepwise increments were used to reach the target salinities (e.g. 2‰ to 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 hours, any leaf that showed signs of feeding was considered eaten. Feeding preference studies were carried out for three 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 four weeks of acclimation (three 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 three 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 one hour. Crabs were

allowed to feed for a 24 hour period, after which leaves and leaf fragments were removed, blotted dry, dried at 60°C for 24 hours 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 $DW=0.278*WW+0.0044*Salinity$ ($F=98.05$, $p<0.00001$, $adj R^2=0.92$).

Emmerson and McGwynne (1992) found that all fecal production of N. meinerti ceased after two days without food. Therefore, prior to the feeding experiment, crabs were starved for a minimum of 48 hours 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).

210 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
215 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
220 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 five hours in respirometers with 100
225 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 oxycaloric 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 arthrodial membrane at the base of the 3rd and 4th walking legs with a
230 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 three day stepwise acclimation period. They were held at 65‰ for three days and hemolymph and water samples taken for measurement of the

aforementioned variables. On occasion, obtaining hemolymph samples was difficult,
235 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 Co., Belgium). Using an
ultraviolet endpoint, the fluorescence was read at 340 nm on a Spectra MAX Gemini
240 ultraviolet spectrophotometer (Molecular Devices Corp., 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 Corp., USA).

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Field methods

Fieldwork took place in November 1999 in two Kenyan mangrove areas, Gazi
Bay and Dabaso, Mida Creek (S03° 21' E039° 59') (Fig. 1). Kenya experiences a
monsoonal climate with the majority of precipitation in Mombasa falling between
250 April and June (mean = 192mm 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
255 large difference between pore water (~60‰) and *N. meinerti* burrow water (~20‰)
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
260 from nine bore-holes 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°C to 3°C during field work (for a maximum of three hours), then were treated the same as in the
265 laboratory experiment. Pore water, burrow water and hemolymph were analyzed for urea, ammonia, osmolality and ion concentrations using the methods detailed above.

Statistical analysis

After testing for a normal distribution using the Kolmogorov-Smirnov goodness
270 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, the Student t-test was used. All data analyses were carried out using Statistica 4.3D (Statsoft Inc.). Data are represented as means $\pm$ standard deviations.

275

Results

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
280 treatments, while *N. smithi* had 100% mortality at 65‰ after just five 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.

285 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
290 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-t, $p=0.02$).

At the highest salinity, both species showed significantly lower nitrogen excretion (LSD, $p < 0.05$) (Fig. 3B). Respiration was significantly lower in 16‰
295 compared with 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
300 than N. smithi, over all salinities (Fig. 4A, B). However, N. smithi subjected to 65‰ for three days had lower hemolymph osmolality than N. meinerti that had been in that salinity for one 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 three days, when

305 compared to all other salinities (LSD, $p < 0.05$). The N. smithi in 65‰ for three 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
 310 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 was no overall effect of salinity on ammonia concentrations in N. smithi (ANOVA), however the individuals held in 65‰ for three days maintained lower concentrations (Fig. 5A), which were comparable to N. meinerti
 315 hemolymph (aside from 65‰). Urea concentrations were not significantly different between treatments (ANOVA) and are given in figure 5B.

Field

Table 2 summarizes the results of the variables measured on pore and burrow
 320 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-t, $p < 0.0001$). The highest hemolymph ammonia concentrations in laboratory held N. meinerti (in 65‰) were not significantly different from those at the Dabaso site (student-t).

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Discussion

The results show that N. meinerti is a strong hyper- and hypo-osmoregulator, allowing them to survive in salinities from at least 16‰ to 65‰. N. meinerti appeared

healthy at the end of the experiment as they aggressively ate during the feeding
330 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 one month in hypersaline conditions (Fig. 4), it is not without
335 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 three days
suggest that N. smithi is capable of withstanding hypersaline conditions for a short
340 period (± 1 week). Moreover, they survived well in 16‰ and 48‰ up to three 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
345 (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
350 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
355 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 sesarmids 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
360 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
365 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,
370 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
375 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 would only strengthen our arguments by further
380 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
385 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.

390 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 ± 561 $\mu\text{mol l}^{-1}$ in N. meinerti and from 1137 ± 429 to 1753 ± 54 $\mu\text{mol l}^{-1}$ in N. smithi; the
395 upper values being rare (Calybrook, 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 one 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
400 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 three days maintained a hemolymph osmolality lower than N. meinerti at that salinity, and about the same concentration as N. smithi held in 32‰ for one 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 seven 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
430 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.

435 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
440 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
445 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
450 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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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*.

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 & 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
<i>N. meinerti</i>	Dabaso	PW	491±58	10.5±1.5	546±68	28±64	16±26	1179±37
(n=11)	(37-39)	HL	341±30	11.1±4.8	328±30	807±488	31±42	781±69
<i>N. meinerti</i>	Gazi	BW	637±151	13.3±2.7	714±167	73±120	24±13	nm
(n=9)	(20-65)	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>	Gazi	BW	382±22	8.5±0.6	423±24	19±25	7±4	nm
(n=12)	(23-30)	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>	Dabaso	BW	534±98	11.7±2.2	584±104	71±34	40±31	1122±134
(n=10)	(31-38)	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>	Gazi	BW	423±39	9.2±0.8	472±42	87±61	13±10	1020±105
(n=12)	(27-35)	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.

FIGURE CAPTIONS

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

FIGURE 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‰).

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

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

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