OSMOREGULATORY ABILITY OF *CHIROMANTES ORTMANNI* (CROSNIER, 1965) SUBJECTED TO DILUTE AND HYPERSALINE SEAWATER

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ABSTRACT

The short-term osmoregulatory ability and salinity tolerance of *Chiromantes ortmanni* was studied in the laboratory. *C. ortmanni* is a non-burrowing, high shore mangrove crab often subjected to extreme salinity fluctuations. They were found to be exceptional osmoregulators in salinities ranging from 0‰ to 65‰ after three days. They could also osmoregulate in 80‰ for at least 24 hours, but 96‰ proved to be lethal after just 12 hours. Osmotic capacity suggests that they are best suited for salinities ranging from about 16‰ to 48‰. As they are frequently found in salinities higher than this, it is hypothesized that they tolerate the higher energetic costs of large osmotic gradients as a trade-off for other benefits such as reduced predation and food competition.

RÉSUMÉ

La capacité d'osmorégulation à court terme et la tolérance à la salinité de *Chiromantes ortmanni* ont été étudiées en laboratoire. *C. ortmanni* est un crabe ne creusant pas de terriers, occupant les rives hautes des mangroves souvent soumises à des variations de salinité extrêmes. Ce crabe s'est révélé capable d'une osmorégulation exceptionnelle à des salinités s'étendant de 0‰ à 65‰ après trois jours. Il est également capable d'osmorégulation à une salinité de 80‰ pendant au moins 24 heures, mais une valeur de 96‰ s'est avérée mortelle après 12 heures. Cette capacité osmotique suggère qu'il est le mieux adapté pour des salinités allant environ de 16‰ à 48‰. Comme on le rencontre fréquemment à des salinités supérieures à ces dernières, on émet l'hypothèse qu'il tolère les coûts énergétiques plus élevés dûs aux larges gradients osmotiques en échange d'autres avantages tels qu'une réduction de la prédation et la compétition pour la nourriture.

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INTRODUCTION

Mangrove crabs often live in a harsh environment with drastically varying salinities and water availability. Although the importance of mangrove crabs on mangrove ecosystem structure and function is well known (Jones, 1984; Smith et al., 1991; Lee, 1998), there is still a lack of knowledge regarding mangrove crab physiology.

Six species of semi-terrestrial crabs are associated with the upper shore of Kenyan mangrove swamps, which is inundated only a few times per annum. Due to high amounts of freshwater run-off during the rainy season and high evaporation during the dry season, coupled with infrequent inundation periods, this area can experience large fluctuations in salinity. Two of the crab species present include the small burrowing fiddler crabs *Uca inversa* (Hoffmann, 1874) and *Uca annulipes* (H. Milne Edwards, 1852). The two largest species present are *Cardisoma carnifex* (Herbst, 1794) and *Neosarmatium meinerti* (De Man, 1887), both of which construct burrows down to the water table (Cott, 1929; Hogue & Bright, 1971; Gillikin, 2000). The other two species belong to the genus *Chiromantes*, with *C. eulimene* (De Man, 1895) being much less common than *Chiromantes ortmanni* (Crosnier, 1965) (see Ruwa, 1997; pers. obs.). *C. eulimene* tends to be more associated with fresh water than *C. ortmanni* (see Macnae, 1968). *C. ortmanni* are easily found in large numbers anywhere from the upper terrestrial zone to the lower edge of the mixed *Avicennia marina* (Forsk.) Vierh. / *Ceriops tagal* (Perr.) C. B. Robinson zone. They are often found in the upper shafts of both *N. meinerti* and *C. carnifex* burrows, but do not venture farther down the burrows as both species defend their burrows well (Gillikin, 2000) and *N. meinerti* will readily consume them (pers. obs.). *C. ortmanni* do not seem to construct their own burrows (M. Vannini, pers. comm.; pers. obs.), but have well developed setae between the walking legs, which can probably efficiently extract pore water (Hartnoll, 1988). Therefore, they must rely on surface pools after rains or tidal inundation, water in flooded burrows, or pore water: all with highly varying salinity values. This species has been recorded in areas with access to water of salinities ranging from 0‰ to 70‰ (Gillikin, 2000). To assess the range of salinities that this species can survive and its short-term osmoregulatory abilities, individuals were exposed to salinities ranging from 0‰ to 90‰ after a short acclimation period and mortality recorded and haemolymph sampled.

METHODS

Adult male and adult non-ovigerous female *Chiromantes ortmanni* (17.2 ± 2.7 mm carapace width, n = 61) were collected by hand from the landward edge of the *Avicennia marina* zone close to the village of Gazi, Kenya (04°25′S 039°31′E) in November 1999, during the short rainy season. Salinities in this area ranged from 15‰ (flooded *N. meinerti* burrows) to 70‰ (pore water). In a field station, four crabs were placed into each of 14 bowls (14 cm diameter) with approximately 1 cm of water (salinity of 32‰). As they are mostly herbivores (Dahdouh-Guebas et al., 1999), the crabs were allowed to feed ad libitum on fresh green *A. marina* leaves. Water and food were changed and mortality was recorded every 12 hours. Starting from 32‰, eight crabs (two bowls) were stepwise acclimated over four and a half days to each of the target salinities (0‰, 16‰, 32‰, 48‰, 65‰, 80‰, and 96‰). Just before the target salinities were reached, the salinities were: 7‰, 20‰, 32‰, 44‰, 55‰, 69‰, and 74‰. Natural seawater was diluted with rainwater or natural sea salt was added to reach the target salinities. Salinity was estimated with a hand refractometer. After three days at the target salinity, haemolymph samples (up to 150 μl) were taken from the arthrodial membrane at the base of the 3rd and 4th walking legs with a sterile 19-gauge hypodermic needle and syringe. Water samples were also taken from each bowl (1 ml). Samples were kept below 0°C during transport back to Belgium, where they were centrifuged and analysed for total osmolality using the freezing point depression method (Osmomat 030 cryoscopic osmometer, Gonotec GmbH). Due to 100% mortality in 80‰ and 96‰, nine additional *C. ortmanni* (all male, 20.1 $\pm$ 1.6 mm carapace width) were collected and placed in 80‰ for 24 hours (with no stepwise acclimation) before haemolymph sampling.

RESULTS

Chiromantes ortmanni survived exposures of more than three days to salinities ranging from 0‰ to 65‰. Crabs in 80‰ survived for about 24 hours after the stepwise acclimation and 96‰ was lethal after just 12 hours of exposure (fig. 1). Of the nine crabs in the 80‰ 24-hr treatment (no stepwise acclimation), one died. *C. ortmanni* was shown to be a strong osmotic regulator in hypo- as well as hypersaline external conditions (fig. 2). Leaves in all bowls showed signs of feeding at each water change except for those in the 96‰ treatment. During the first day, a few individuals moulted and were replaced with new crabs. There was no correlation or difference between different sizes or sexes. Two crabs in 80‰ had haemolymph osmolalities about 1.5 times higher than the rest of the 80‰ group and were not included in statistical analyses. There was a significant linear correlation between haemolymph and medium osmotic concentrations ($y = 778.8 + 0.24x$, $R^2 = 0.82$, $p < 0.001$, n = 38; fig. 2). Using this regression equation, the isosmotic point was calculated to be 1025 mOsm kg^{-1} for *C. ortmanni*.

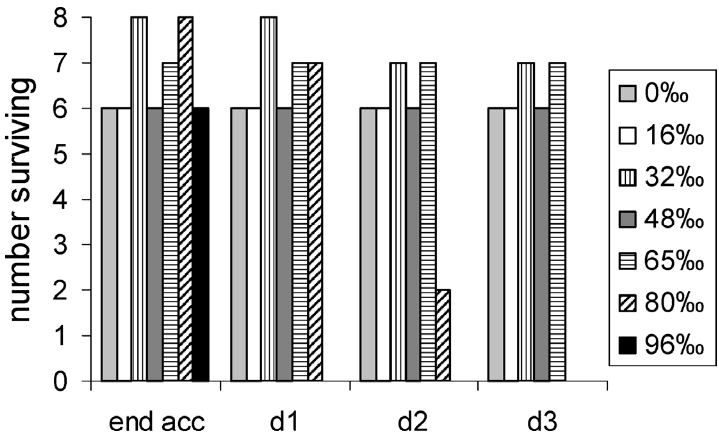

Fig. 1. Number out of eight *Chiromantes ortmanni* (Crosnier, 1965) surviving at the end of the stepwise acclimation period (end acc; see materials and methods for salinities) and at the end of each day in each salinity.

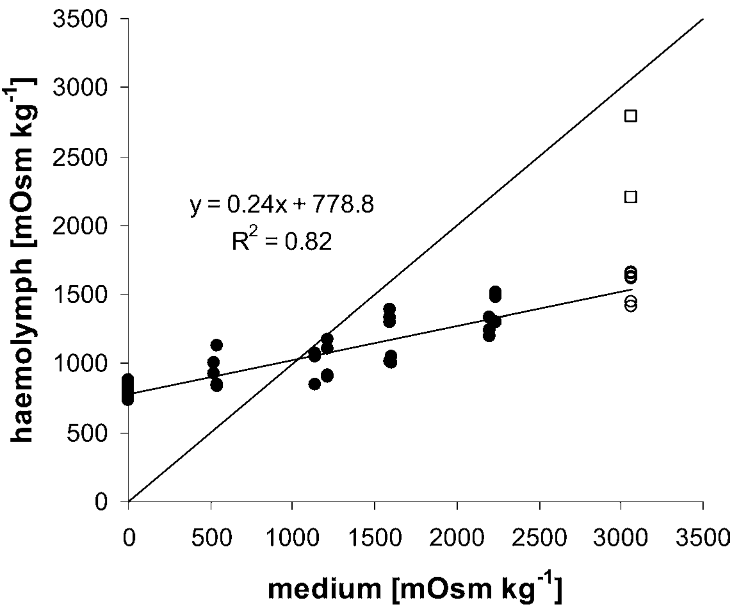

Fig. 2. Individual measurements (n = 40) and linear regression of haemolymph osmotic concentration of *Chiromantes ortmanni* (Crosnier, 1965) after stepwise acclimation to different external salinities. Solid circles represent crabs held at respective salinities for three days; open circles represent crabs held in 80‰ for 24 hours. Open squares are two measurements not included in statistics (see text). The isosmotic line is shown as a diagonal.

DISCUSSION

This study has shown that adult *Chiromantes ortmanni* are euryhaline and strong hyper- hypo-regulators, allowing them to survive in the harsh upper mangal. These crabs can regulate over a range from pure freshwater to 65‰ salinity for at least three days, and for a limited time in 80‰. Strong regulation is often found in crabs from the upper mangal with its widely fluctuating salinities due to evaporation and dilution by freshwater influx (Greenaway, 1988). *Chasmagnathus granulatus* Dana, 1851, another crab inhabiting areas of extreme salinities and considered one of the most efficient osmoregulators among the Grapsoidea, is able to maintain its haemolymph within 306 mOsm kg^{-1} over a range of 1-44‰ (1250 mOsm kg^{-1}) (Charmantier et al., 2002). *C. ortmanni* maintains its haemolymph within 377 mOsm kg^{-1} over a similar range (0-48‰ or 1600 mOsm kg^{-1}) and within 517 mOsm kg^{-1} in waters spanning more than 2000 mOsm kg^{-1}, making it a very efficient regulator.

C. ortmanni can survive short periods (± 1 day) with access to water with a salinity as high as 80‰, which is probably near the maximum salinity this species can survive for more than 12 hours (fig. 1). Due to the high osmotic gradient (-1285 mOsm kg^{-1}), shortly after 24 hours in this salinity, crabs can no longer regulate, and die as indicated by the one dead crab and the two specimens with greatly elevated haemolymph osmolalities. Furthermore, at the time of haemolymph sampling, all crabs in this salinity were rather lethargic. Crabs in 0‰ maintained high internal osmolalities (806 ± 51 mOsm kg^{-1}) and showed low mortality. Similarly, *C. eulimene*, which are more common in lower salinities, have much lower internal osmolalities in freshwater ($\pm$ 730 mOsm kg^{-1}) and a lower isosmotic point ($\pm$ 820 mOsm kg^{-1}) (Boltt & Heeg, 1975). The lowering of the haemolymph osmotic concentration is considered an adaptation to freshwater environments (Schubart & Diesel, 1999). Therefore, the high internal osmolality in freshwater and the high isosmotic point in *C. ortmanni* would indicate that this species is a truly marine species, with the ability to tolerate short periods of strong hypo- and hyper-regulation. Although this would suggest they are poorly suited for fresh water, other factors may play a role. In this study, *C. ortmanni* were held in the target salinities for only three days, which may not have been enough time for the animals to fully adjust; Boltt & Heeg (1975) ran their acclimation for 14 days. Furthermore, Boltt & Heeg (1975) acclimated *C. eulimene* to 34‰ before subjecting them to varying salinities, while in this study *C. ortmanni* were collected from areas where they had access to water sources of different salinities (15‰ to 70‰). Thus, it is possible the different species were acclimated to different salinities before the commencement of the experiments. However, *Neosarmatium meinerti* also has a lower internal osmolality in freshwater (654 ± 78 mOsm kg^{-1})

after only two days with no acclimation period (Gross et al., 1966). A possible reason for the lower osmolality in *N. meinerti* may be that they often have access to lower salinity burrow water, whereas *C. ortmanni* must rely more on higher salinity pore-water. It has been shown that in periods of low rainfall the salinity at the surface increases more than the salinity deeper in the sediment (Drexler & Ewel, 2001).

Although indicative of absolute tolerance, these results say little about long-term tolerances. For example, although *N. meinerti* can osmoregulate in 65‰ for one month, maintaining its haemolymph 780 mOsm kg^{-1} lower than the external medium, the energetic cost of osmoregulation has a severe impact on the overall energy budget of the animal (Gillikin et al., in press). The same study showed that *Neosarmatium smithi* (H. Milne Edwards, 1853), a mid-shore crab, can maintain a difference of more than 1000 mOsm kg^{-1} for one week before experiencing 100% mortality. Considering that *C. ortmanni* in 0‰ and 65‰ were maintaining their haemolymph 800 and 900 mOsm kg^{-1} higher and lower, respectively, than water osmolality, they too were most likely experiencing a negative energy budget in these salinities and may have had high mortality after a few more days. Using osmotic capacity (defined as the difference between haemolymph and medium osmolalities (Charmantier et al., 2002)) as a metric of energy expenditure, *C. ortmanni* seems to be best suited for life in waters ranging from 16‰ to 48‰, where they experience small osmotic gradients (+400 to −400 mOsm kg^{-1}). However, some crabs can maintain large differences between haemolymph and medium, such as the freshwater crab *Armases roberti* (H. Milne Edwards, 1853), which has an osmotic haemolymph concentration of 678 ± 33 mOsm kg^{-1} in fresh water (Schubart & Diesel, 1998). Although it is sure that maintaining increased osmotic gradients requires increased energy expenditure, one must not forget that semi-terrestrial crabs can avoid osmotic challenges behaviourally (Wolcott & Wolcott, 2001). For example, they may select water of a particular salinity when foraging, and burrows and food may be a source of replenishing lost salts in freshwater situations (Greenaway, 1988).

Gillikin (2000) recorded the distribution of crabs along a transect from land to sea in Gazi Bay mangroves and found that *Chiromantes ortmanni* has a wider distribution across the mangal than *Neosarmatium meinerti*, extending into areas of higher salinities, farther from terrestrial run-off. In particular, in two separate years, they were found inhabiting the mixed *Avicennia marina* / *Ceriops tagal* zone that had pore-water salinities ranging from 47 to 59‰. The crabs living in this area are exposed to salinities approaching 60‰ for long periods (within hours of rainfall or tidal immersion, the pore-water salinities are still hypersaline), which must cause additional energy expenditure due to osmoregulation (note that the crabs inhabiting areas of 70‰ in this study were relatively close to less saline areas;

± 10 m). A possible reason they inhabit this particularly harsh environment is that the only other crab species present here is *Uca annulipes*. In other words, there are no predators and the only other competition for food is the mud whelk, *Terebralia palustris* (Linnaeus, 1767) (cf. Fratini et al., 2000). A recent study has shown that crabs will tolerate osmotic stress as a trade-off for other benefits (McGaw, 2001). Another Kenyan species apparently utilizing the strategy of trading off osmotic stress for reduced predation and competition is *Uca inversa*, which inhabits barren areas adjacent to mangroves with pore-water salinities as high as 99.4‰ (unpubl. data). However, populations living above their optimal salinity using this strategy might still be vulnerable to environmental perturbations, e.g., El Niño Southern Oscillation drought, or groundwater redirection (cf. Drexler & Ewel, 2001; Gillikin et al., in press).

In summary, this study has shown that *C. ortmanni* is a powerful osmoregulator, however, extreme salinities probably require extensive energetic output. This species most likely combines its strong osmoregulatory capacity with behavioural strategies to survive in extreme salinities.

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