Insect Physiol., *Vol.* 26, *pp.* 315 *to* 320.
Pergamon Press Ltd. 1980. *Printed in Great Britain.*

0022–1910/80/0501–0315 $02.00/0

WATER BALANCE AND OSMOREGULATION IN *ONYMACRIS PLANA*, A TENEBRIONID BEETLE FROM THE NAMIB DESERT

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(*Received* 5 *September* 1979; *revised* 20 *November* 1979)

Abstract—*Onymacris plana*, a tenebrionid beetle from the sand dunes of the Namib desert, lost weight very slowly during 12 days of dehydration at 26°C. Measurement of total lipid showed a gradual decline, the metabolic water produced being sufficient to maintain a constant water content. At the same time the haemolymph volume decreased by 66%. When given water the dehydrated beetles drank rapidly and their weight and haemolymph volume were restored to normal. Haemolymph osmolarity was closely regulated despite the changes in volume. Haemolymph potassium was also well regulated, but sodium was lost from the haemolymph during a cycle of dehydration and rehydration, even though sodium losses in the faeces were small. Water balance in *Onymacris* depends on efficient conservation of water in periods of drought and on water uptake by drinking during the coastal fogs of the Namib.

Key Word Index: Water balance, osmoregulation, *Onymacris*, Tenebrionidae, deserts

INTRODUCTION

THE VEGETATIONLESS sand dunes of the Namib Desert of southwestern Africa support a rich variety of flightless beetles belonging to the family Tenebrionidae (KOCH, 1961; SEELY, 1978). These beetles provide excellent opportunities for research on the adaptations of arthropods to a desert environment. Previous studies of the Namib tenebrionids include those of EDNEY (1971a, b) on water and temperature relations, HOLM and EDNEY (1973) on activity patterns in relation to microclimate, and HAMILTON (1975) on the thermal significance of their black colouration.

Desert arthropods are well known to be very resistant to desiccation (EDNEY, 1974; CLOUDSLEY-THOMPSON, 1975). Their low rates of transpiration have, however, received more attention than their excretory physiology (SHAW and STOBBART, 1972), and there have been few studies of osmoregulation in desert beetles. Riddle *et al.* (1976) compared osmoregulation in three different desert arthropods: a millipede, a scorpion and the tenebrionid beetle *Eleodes hispilabris*. BROZA *et al.* (1976) examined the effects of dehydration and subsequent rehydration on the haemolymph of *Trachyderma*. Most recently, COUTCHIÉ and CROWE (1979b) have studied osmotic and ionic regulation in larvae of the Namib tenebrionid *Onymacris marginipennis*. The present investigation concerns another member of the same genus, *Onymacris plana*, one of the more conspicuous diurnal tenebrionids on the Namib sand dunes.

METHODS

Adult beetles were obtained from the Namib Desert Research Station at Gobabeb. They were kept in glass terraria partly filled with sand at 26±1°C, and all experiments were carried out at this temperature. Beetles were fed fresh lettuce and oatmeal. The weight range of insects used in experiments was 500–1100 mg (mean 864 mg). Although females tend to be larger than males, no difference between the sexes was found for any of the parameters measured. Whenever possible, however, equal numbers of each sex were used in experiments.

Beetles to be dehydrated were weighed and placed in individual glass vials in a desiccator containing silica gel. This provided a relative humidity of 10–15% (measured with a Shaw hygrometer). They were starved for 24 hr before the start of each experiment, in order to empty the gut and minimise water loss via the faeces. After 12 days of dehydration beetles were allowed to drink distilled water to repletion, then maintained at 50–60% r.h. for a further 4 days. All insects not used in the various analyses were apparently healthy at the end of the experimental period.

Insects were weighed every second day to the nearest 0.1 mg. Water content was determined by freeze-drying, the beetles being stored over phosphorus pentoxide before reweighing to obtain dry weights. Lipid content was then estimated by extraction with three changes (24 hr each) of a 2:1 methanol–chloroform mixture (v/v) at room temperature. A final freeze-drying gave fat-free dry weights. This method of lipid analysis was checked by the method of BLIGH and DYER (1959) at the Fishing Industries Research Institute, University of Cape Town.

The haemolymph volume was estimated by a simple gravimetric method first described by RICHARDSON *et al.* (1931). This consists of dissecting the weighed insect and removing the haemolymph with absorbent tissue before reweighing, and has been particularly useful when the haemolymph volume is small or is

reduced by dehydration (NICOLSON, 1976; TUCKER, 1977a; WALL, 1970).

Haemolymph samples for analysis were collected from the coxa in micropipettes (Drummond Scientific Co.). In dehydrated beetles, when very little haemolymph was available, osmolarities were measured by freezing-point depression on a Clifton Nanolitre Osmometer (Clifton Technical Physics). Otherwise osmolarities were determined immediately after haemolymph collection on a Wescor 5100B Vapour Pressure Osmometer. Sodium and potassium concentrations of diluted haemolymph samples were measured on an IL 243 Flame Photometer (Instrumentation Laboratory).

RESULTS

Weight changes during dehydration and rehydration

Dehydration resulted in a very slow weight loss. Figure 1 shows that after 12 days the mean weight of the beetles had decreased by only $11.1 \pm 0.6\%$ (mean $\pm$ S.E.). This is very close to the rate of loss recorded by EDNEY (1971a) for the same species (5.6% in 5 days at 27°C). Approximately 14% of the total weight loss was due to the production of faeces, mostly during the first day of dehydration. This accounts for the initially steeper slope of the curve in Fig. 1. Much of the faecal material consisted of sand grains.

The Namib tenebrionids chosen for EDNEY's (1971a) study showed a negative correlation between original weight and weight loss (as a percentage of original weight). However, no such correlation was apparent in the present experiments on *O. plana*.

When given water on day 12 the dehydrated beetles drank a mean weight of 96 ± 6 mg in only 3–10 min. This quantity was sufficient to restore their weight to its original value (Fig. 1). A rapid gain of weight by drinking after dehydration has also been measured in scorpions (HADLEY, 1971), cockroaches (WALL, 1970; TUCKER, 1977a) and a desert tenebrionid beetle (BROZA *et al.*, 1976).

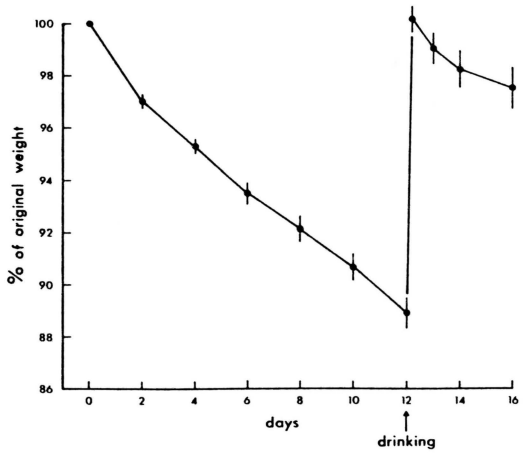

Fig. 1. The effect of dehydration and rehydration on the body weight of *Onymacris*. An arrow marks the time of drinking (after 12 days of dehydration). Vertical lines represent $\pm$ one standard error of the mean. Minimum $N = 10$.

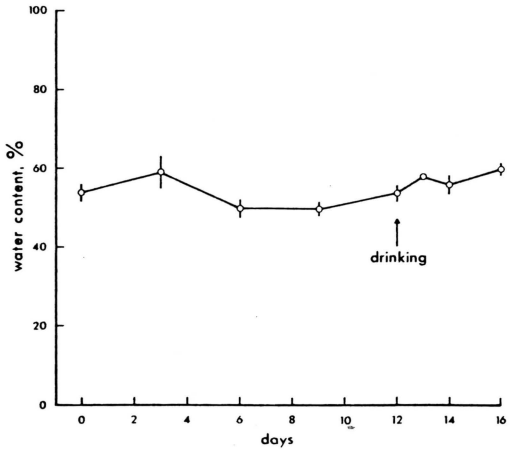

Fig. 2. The water content, expressed as percentage of wet weight, during dehydration and rehydration. Drinking shown by arrow. Vertical lines indicate S.E.'s (except where symbol exceeds size of the S.E.). $N = 6$–8.

Water content

The water content of *O. plana*, measured throughout the cycle of dehydration and rehydration and expressed as a percentage of wet weight, is shown in Fig. 2. The values are low, as expected in an insect possessing a relatively heavy exoskeleton. A one-way analysis of variance showed that there was no significant decrease in water content during dehydration ($P > 0.05$). However, the analysis did show a significant difference between the water content at 6 and 9 days after the start of dehydration and that at 1 and 3 days after drinking.

The constant water content of 53.8% during the period of dehydration means that almost half the weight loss was a loss of dry matter: thus a beetle weighing initially 864 mg lost 52 mg of water and 44 mg of dry matter (of which 13 mg was faecal loss).

Lipid content

The mean weight of lipid extracted from control beetles in methanol–chloroform was $31.5 \pm 2.1\%$ of the dry weight, an average of 126 mg/individual. Analysis of pooled samples of 3 individuals by the method of BLIGH and DYER (1959) gave lower values; 24.0% for males and 23.8% for females. Extraction in methanol–chloroform was, however, considered a satisfactory method for detection of any changes in lipid content during dehydration.

Lipid content, expressed as percentage of dry weight, is plotted against weight loss of individual beetles in Fig. 3. There was a significant negative correlation ($P < 0.01$) between lipid content and weight loss. TUCKER (1977b) also found decreasing lipid reserves in dehydrated cockroaches. The lowest lipid content measured in *O. plana* was 15 mg or 6.9% dry weight, in a beetle which had lost 26% of its original weight during dehydration.

Haemolymph volume

Since the specific gravity of insect haemolymph is only slightly greater than unity (ALTMAN and DITTMER, 1974), the haemolymph weight in mg is approximately equal to its volume in μl. Figure 4

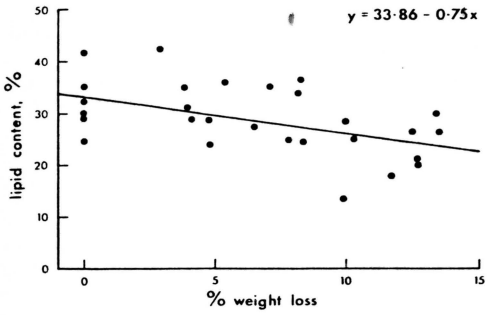

Fig. 3. The relationship between lipid content (as percentage of dry weight) and weight loss during dehydration. The regression line shows a significant negative correlation ($r = -0.52$) between lipid content and percentage weight loss.

shows that the haemolymph volume of the average beetle declined substantially during dehydration, from 98 to 33 μl. However drinking restored the haemolymph volume to normal. Similar changes were found by Broza *et al.* (1976) for haemolymph volume in the tenebrionid *Trachyderma*.

An estimate of the tissue water can be obtained by subtracting the weight of haemolymph from the total body water: this gives values of 367 mg in control beetles and 380 mg in those dehydrated for 12 days. Dehydration affects only the haemolymph water, while the tissue water remains roughly constant.

Haemolymph osmolarity

In spite of the fluctuations in haemolymph volume, the haemolymph osmolarity was regulated within narrow limits during dehydration and rehydration (Fig. 5). After 12 days of dehydration the osmolarity had increased by only 14%, from 435 ± 6 mOsm/l to 498 ± 7 mOsm/l. When measured 24 hr after drinking, the haemolymph osmolarity had returned to its original level (429 ± 12 mOsm/l.)

Sodium and potassium concentrations

Figure 6 gives the results of analyses of sodium and potassium concentrations in the haemolymph. As

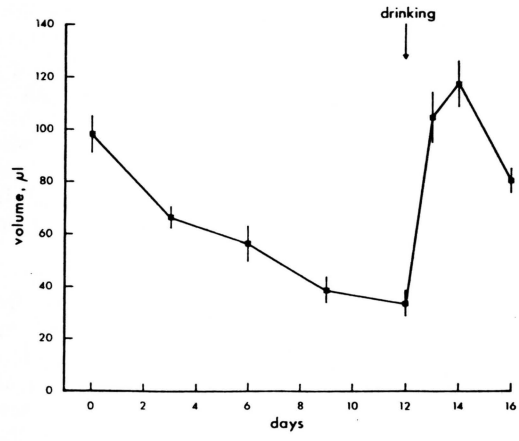

Fig. 4. Changes in the volume of haemolymph (μl) during dehydration and rehydration. The data apply to a standard animal of initial weight 864 mg. Drinking shown by arrow. Vertical lines indicate $\pm$ S.E. $N = 5$–6.

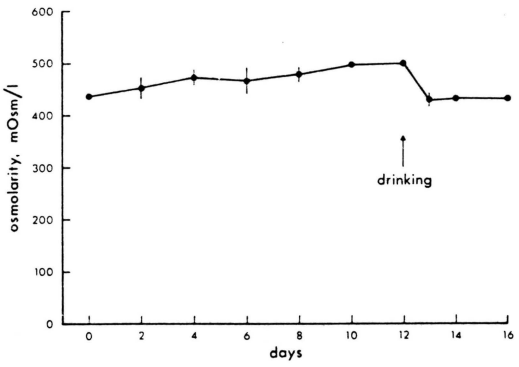

Fig. 5. The effect of dehydration and rehydration on the haemolymph osmolarity in mOsm/l. Drinking shown by arrow. Symbols often exceed the size of the S.E. (vertical lines). $N = 5$–7.

expected from the measurements of osmolarity, these ions were well regulated, particularly potassium. The initial potassium concentration of 19 ± 1 mM rose to 33 ± 3 mM during dehydration, but 24 hr after drinking had returned to 19 ± 2 mM. The sodium concentration, initially 128 ± 2 mM, decreased in the first 2 days of dehydration, then began to rise, the variability between individuals also increasing. After drinking the sodium concentration dropped sharply to 88 ± 3 mM, and did not return to its original level.

Because of this apparent loss of sodium from the haemolymph, a second group of beetles was given food (oatmeal and fresh lettuce) during the rehydration period. Analysis of their haemolymph showed higher sodium concentrations during the 4 days of rehydration (Fig. 6). On day 16 the sodium concentrations of fed and unfed beetles were significantly different ($P < 0.01$). Feeding during rehydration did not affect the haemolymph potassium concentration.

Sodium losses in the faeces were also estimated. A

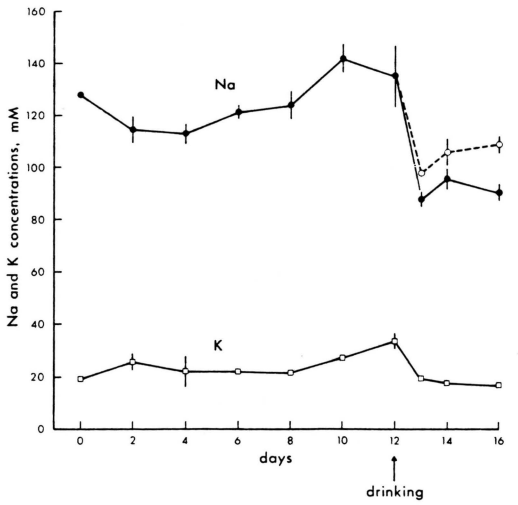

Fig. 6. The effect of dehydration and rehydration on the haemolymph Na (●——●) and K (□——□) concentrations (mM). Na concentrations were higher if beetles were allowed food after drinking on day 12 (○---○). Symbols often exceed the size of the S.E. (vertical lines). Minimum $N = 5$.

Table 1. Sodium and potassium concentrations, expressed as μmole/mg dry weight, of food and faeces of *Onymacris*

	Na	K
Lettuce	0.15	0.82
Oatmeal	0.001	0.02
Faeces	0.08	0.21

sample of faeces collected over the 12 day dehydration period was dried, ashed at 450°C and analysed in the flame photometer. Samples of the beetles' food (oats and lettuce) were treated in the same way. The sodium and potassium concentrations, expressed as μmol/mg dry weight, are given in Table 1.

As expected, sodium gains in the plant food were small. Sodium losses in the faeces were also small, only 0.08 μmol/mg dry weight, which represents a total of 1.04 μmol per individual throughout the period of dehydration. The total quantity of sodium in the haemolymph decreased from 12.53 μmol in control beetles to 9.14 μmol in those rehydrated for 24 hr, so the excreted sodium is less than a third of the sodium lost from the haemolymph.

DISCUSSION

Low rates of evaporative water loss are characteristic of desert arthropods. The present results for *Onymacris plana* are similar to those of EDNEY (1971a), who compared transpiration rates in seven species of Namib tenebrionid, including *O. plana*. For this beetle the weight loss during dehydration appears to be less than for the American desert tenebrionids *Eleodes armata* and *Cryptoglossa verrucosa* (AHEARN and HADLEY, 1969). By separating the total water loss of *E. armata* into different components, AHEARN (1970) found that quinone secretions are a major avenue of water loss in this species, at least under experimental conditions. The fact that the Namib tenebrionids do not produce defensive secretions (SEELY, 1978) must help to minimise their water loss.

In determinations of evaporative water loss it has generally been assumed that weight loss during the experimental period represents a loss of water only (e.g. EDNEY, 1971a). This is not true for *Onymacris*. During 12 days of dehydration, the mean loss of dry weight was 31 mg (excluding faeces). At the same time the total lipid extractable with methanol–chloroform decreased from 34 to 25% dry weight (estimates from regression line, Fig. 3). This represents a decline of 45 mg (from 135 to 90 mg). Even allowing for cuticular lipids, this is more than enough to account for the loss of dry weight. Thus it can tentatively be assumed that 31 mg of lipid is metabolised during dehydration, producing approximately 33 mg of oxidation water, a quantity sufficient to maintain the normal percentage of water (53.8%).

Another insect which draws on reserve lipids during periods of desiccation is the cockroach *Periplaneta*: TUCKER (1977b) measured the lipid content of this insect by extraction in acetone and found a decrease from 24 to 5% dry weight in nymphs dehydrated for 13 days. It is probable that the lipid reserves of *Onymacris* would be adequate for considerably longer periods of desiccation. Under field conditions, however, the lipid stores may be smaller.

In the beetle *Eleodes armata*, tritiated water has been used to measure water exchange in field and laboratory populations (BOHM and HADLEY, 1977). Water loss measured isotopically was found to be greater than that measured by the usual gravimetric methods, the reason being that isotopic estimates include metabolic water production as well as the water lost by evaporation and excretion. A similar discrepancy would be found if both techniques were applied to *Onymacris*, since the estimated 33 mg of metabolic water is substantial when compared to the 52 mg lost by evaporation.

The water lost so slowly during dehydration is very rapidly replenished by drinking. After 12 days' dehydration beetles drank distilled water at an average rate of 18 mg/min, gaining up to 20% of their body weight. Two other desert arthropods are known to drink rapidly to replace the water lost by desiccation, and have also been observed drinking in the field: they are the scorpion *Centruroides sculpturatus* (HADLEY, 1971) and the tenebrionid *Trachyderma* (BROZA *et al.*, 1976; BROZA, 1979). BROZA (1979) has described the widespread use of dew and damp hygroscopic food as water sources for desert arthropods in Israel. In reviewing drinking in land arthropods, EDNEY (1977) suggests that ingestion of moist food in these conditions may be for the sake of the water, rather than the energy contained in the food.

Frequently occurring coastal fog provides water, directly or indirectly, for the animals of the Namib Desert (LOUW, 1972; SEELY, 1978). Larvae of the tenebrionid beetles live beneath the sand, where relative humidities may be high. The larvae of *Onymacris plana* and *O. marginipennis* have recently been shown by COUTCHIÉ and CROWE (1979a) to absorb water vapour from unsaturated air, an ability they share with *Tenebrio* larvae and various other arthropods (EDNEY, 1977). Among beetles only the immature forms possess this ability (EDNEY, 1977): adults of the Namib tenebrionids do not appear able to take up water vapour from unsaturated air (EDNEY, 1971a; LOUW and HAMILTON, 1972). The adults obtain fog moisture in other ways. Three species of *Lepidochora* build tiny trenches along the sand dunes to trap fog water (SEELY and HAMILTON, 1976). On foggy mornings *Onymacris unguicularis* takes up a head-stand posture on the crest of a dune, with the result that fog condenses on its body and trickles down to its mouth (HAMILTON and SEELY, 1976). *O. plana* does not exhibit this fog-collecting behaviour, but instead drinks directly from fog moisture which has condensed on to plants (M. K. SEELY, personal communication).

Drinking restores the haemolymph volume to normal after the 66% decrease resulting from dehydration. Replenishment of the haemolymph volume seems to be under better control than in the beetle *Trachyderma* (BROZA *et al.*, 1976), perhaps because the latter was subjected to more severe desiccation beforehand. In *Onymacris*, as in many other insects, the haemolymph acts as a water reservoir for the tissues. Together with metabolic water production, this ensures that the tissues are protected from the effects of desiccation: in fact, the

absolute weight of tissue water was found to increase slightly during 12 days of dehydration.

Changes in haemolymph osmolarity and cation concentrations during dehydration and rehydration are not proportional to the changes in haemolymph volume. While the average haemolymph volume drops from 98 to 33 μl, the osmolarity increases from 435 to 498 mOsm/l, not to the 1292 mOsm/l to be expected in the absence of osmoregulation. A similar calculation for 24 hr after drinking gives an estimated value of 158 mOsm/l, whereas the measured value is 429 mOsm/l, the same as at the beginning of the experiment. Haemolymph osmolarity of other tenebrionids (both larvae and adults) is also strongly regulated during desiccation and subsequent rehydration (RIDDLE *et al.*, 1976; BROZA *et al.*, 1976; COUTCHIÉ and CROWE, 1979b). Sodium and potassium ions together make up 68% of the cations in *O. plana* haemolymph. Like the total osmolarity, potassium concentrations return to normal within 24 hr of drinking. Sodium, the major cation, is well regulated during dehydration, although the variation between individuals is greater. Rehydration, however, results in a marked drop in haemolymph sodium concentration. COUTCHIÉ and CROWE (1979b) also found haemolymph cations to be more strongly regulated in dehydrated *O. marginipennis* larvae than in hydrated ones.

Regulation of the haemolymph osmolarity while its volume fluctuates indicates that during dehydration osmotically active substances are removed from the haemolymph, to be later returned when rehydration occurs. WALL (1970) suggested that excess sodium ions were sequestered in the tissues of dehydrated *Periplaneta*, to be later mobilised when the insect was able to drink. More recent work on cockroaches supports the hypothesis that the fat body is the major storage site for sodium (TUCKER, 1977b; HYATT and MARSHALL, 1977). An interchange between amino acids and soluble protein might also be involved in the osmoregulation of terrestrial insects (EDNEY, 1977). In the tenebrionid *Trachyderma* amino acids make a major contribution to the total osmolarity and BROZA *et al.* (1976) have provided evidence for such a mechanism.

In the absence of regulation, the sodium concentration of *Onymacris* haemolymph would be expected to fall to 43 mM when the volume increases after drinking. Although the concentration is actually 88 mM on the first day after drinking, this is considerably less than in control insects and does not rise on subsequent days, indicating an apparent loss of sodium from the haemolymph. In cockroaches only a small proportion of the sodium removed from the haemolymph during dehydration is excreted (WALL, 1970; TUCKER, 1977a; HYATT and MARSHALL, 1977). Similarly, analysis of the faeces of *Onymacris* shows that each beetle excretes a total of 1.04 μmol of sodium during dehydration: this is less than one third of the quantity of sodium lost from the haemolymph by the first day of rehydration.

When rehydrating beetles were given food, their haemolymph sodium concentration increased to 109 mM by the fourth day after drinking. Sodium intake in the food did not account for the increase, lettuce and particularly oatmeal having a very low sodium content. It is possible that feeding is necessary for the efficient mobilisation of sodium from storage sites in the tissues. Rehydrating *Periplaneta* also require food if their haemolymph sodium concentration is to return to normal (TUCKER, 1977b).

In terms of its water economy, *Onymacris* is well adapted to the desert environment. Water losses by evaporation and excretion are minimal. A low cuticular permeability is probably combined with spiracular control over respiratory losses, especially since the abdonimal spiracles of desert tenebrionids open into the subelytral cavity rather than directly to the exterior (AHEARN, 1970). The cryptonephric complex of Malpighian tubules and rectum is capable of resorbing most of the water from urine and faeces. During desiccation the haemolymph serves as a water store for the tissues, and the production of metabolic water assumes increased importance.

Water balance is maintained not only by the efficient conservation of water, but also by its uptake when available. All larval instars of *O. plana* except the first can absorb water vapour from relative humidities of 84% or higher (COUTCHIÉ and CROWE, 1979a). In contrast, the adult beetles drink large volumes of water when dehydrated, utilising fog moisture under field conditions. In spite of substantial weight changes in both larvae and adults, the haemolymph osmolarity is closely regulated by the reversible storage of osmotic effectors. Although advantageous for desert life, these aspects of the physiology of *Onymacris* are not unique to desert arthropods.

Acknowledgements—I wish to thank Dr M. K. SEELY, Director of the Namib Desert Research Station, for her advice and assistance. Professor L. C. ISAACSON of the Department of Physiology, University of Cape Town Medical School, kindly allowed me the use of his Clifton Nanolitre Osmometer. I am grateful to Professor G. N. LOUW and Dr. J. P. LOVERIDGE for useful discussions and comments on the manuscript. My thanks are also due to the South African Council for Scientific and Industrial Research for financial assistance, and to the Division of Nature Conservation and Tourism, South West Africa/Namibia, for permission to carry out the research.

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