

## Haemolymph amino acid, sugar and glycerol levels in the Namib Desert tenebrionid *Physadesmia globosa* (Coleoptera: Tenebrionidae) during dehydration and rehydration

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**Abstract.** Haemolymph levels of amino acids, sugars and glycerol were investigated in the tenebrionid *Physadesmia globosa* during dehydration and rehydration. The absolute amount of amino acid decreases during dehydration and increases during rehydration, indicating active regulation of this solute (the osmolal contribution of amino acids is large – approx. 25%). Changes in the amino acid content of the haemolymph during dehydration are not the result of interchange with soluble protein; the possibility exists during rehydration (between 1 h and 48 h). Trehalose and glucose are the only sugars found in appreciable quantity in the haemolymph of this species. Their osmolal contributions (total sugar: 2.6%), and contributions to osmoregulation, are not great. Glycerol is a minor osmolal effector in the haemolymph of *Physadesmia*, and changes in its levels do not contribute importantly to the regulation of haemolymph osmotic pressure.

### INTRODUCTION

Capacities for osmoregulation have been demonstrated in a great variety of mesic and xeric beetles (Machin, 1975; Broza et al., 1976; Riddle et al., 1976; Coutchie & Crowe, 1979; Nicolson, 1980; Cohen et al., 1986; Naidu & Hattingh, 1986, 1988; Riddle, 1986; Naidu, 2001a, b). In most of these studies, the role of major haemolymph cations and anions in osmoregulation has been explored. Despite high concentrations of amino acids and sugars being found in the haemolymph of insects (Mullins, 1985), the literature does not abound with information on the contribution of these organic constituents to haemolymph osmotic pressure (OP) and to osmoregulation. The contribution of amino acids in the regulation of haemolymph OP during dehydration and rehydration has been examined in the Negev desert tenebrionid beetle *Trachyderma philistina* (Broza et al., 1976), in *Onymacris marginipennis* larvae (Coutchie & Crowe, 1979), in the desert meloid beetle *Cysteodemus armatus* (Cohen et al., 1986), and in the Namib desert tenebrionid beetles *Stips stali*, *Onymacris rugatipennis* and *Stenocara gracilipes* (Naidu, 1998, 2001a, b). Sugar contributions as osmolar effectors, and to osmoregulation, have been examined in *O. marginipennis* larvae (Coutchie & Crowe, 1979), and in *S. stali*, *O. rugatipennis* and *S. gracilipes* (Naidu, 1998, 2001a, b). Furthermore, while a number of studies have suggested an osmolar effector role for glycerol (Asahina et al., 1954; Marek, 1979), glycerol levels in the haemolymph have been found to contribute significantly to haemolymph OP and to osmoregulation only in the strictly nocturnal Namib tenebrionid beetle *S. stali* (Naidu, 1998). The present study, therefore, examines haemolymph amino acids, sugars, and glycerol in *Physadesmia globosa* (Haag) during dehydration and rehydration.

### MATERIALS AND METHODS

Adult *Physadesmia globosa* were collected by hand from the dry Kuiseb riverbed, on the edge of the dune field near Gobabeb, Namibia (23°34'S, 15°03'E). They were flown to Johannesburg, where they were kept in glass terraria partly filled with Namib sand, in a controlled laboratory environment (28 ± 2°C, 12 h / 12 h, 35 ± 11% r.h.) for at least 3 weeks prior to investigation. The beetles were fed fresh lettuce and oatmeal.

Both male and female beetles (699.3 ± 28.7 mg, mean ± S.E) were used for study. For dehydration, the beetles were weighed and placed in a desiccator over silica gel (10–15% r.h.), for a period of 10 days at 26°C. After this they were allowed to drink distilled water to repletion, and maintained at 50–60% r.h. for a further 4 days (drinking permitted). Insects not used in analyses by the end of the experimental period (after rehydration), were not all healthy: some were unable to right themselves when turned on their backs (Juliano, 1986).

Beetles were weighed every second day to the nearest 0.1 mg. Haemolymph samples were collected from the coxa or directly from the dorsal vessel (after careful removal of the elytra) into capillary tubes. This was done at approximately the same time every 2 days during the course of dehydration and rehydration. Haemolymph was taken once only from each insect. Haemolymph sugars and glycerol were determined by gas chromatography, as described previously (Naidu, 1998), and total amino acids were determined by a modified ninhydrin assay described earlier (Naidu, 1998).

Results were analysed statistically using a one-way analysis of variance ( $\alpha = 0.05$ ), with pairwise comparison between groups employing Duncan's Multiple Range test.

### RESULTS

#### Amino acid concentration

Changes in haemolymph total amino acid levels in *P. globosa* during dehydration and rehydration are shown in Fig. 1. There was considerable variation in amino acid levels between individuals of a group.

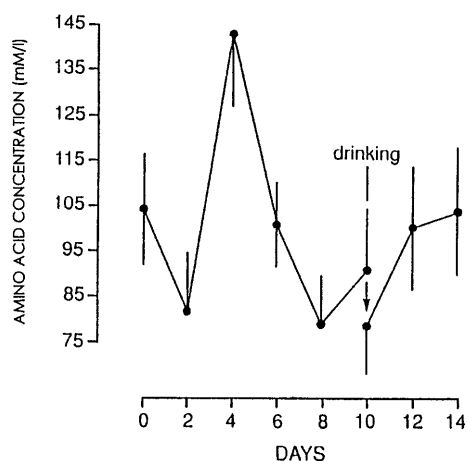


Fig. 1. Effects of dehydration and rehydration on haemolymph free amino acid concentrations (mM/l) in *Physadesmia globosa*. Drinking shown by arrow. Vertical lines indicate  $\pm 1$  S.E.M.  $N = 5-7$ .

Despite the considerable fluctuation in amino acid levels of this species during dehydration, the amino acid concentration on day 10 ( $91.7 \pm 13.5$  mM/l) is not significantly different ( $P > 0.05$ ) from the Control (day 0:  $104.8 \pm 12.5$  mM/l). However, the concentration on day 4 ( $143.1 \pm 16.0$  mM/l) is significantly higher ( $P < 0.05$ ) than concentrations on days 2, 6, 8 and 10. Although a tendency to decrease was apparent after drinking, haemolymph amino acid concentrations during the rehydration period (day 10, 1 h after drinking:  $79.2 \pm 10.8$  mM/l; day 12:  $101.1 \pm 13.7$  mM/l; day 14:  $105.0 \pm 14.1$  mM/l) were not significantly different from either immediate pre-rehydration or Control values ( $P > 0.05$ ).

#### Sugar concentrations

Although traces of other sugars were found in the haemolymph of *P. globosa*, the only carbohydrates present in significant amounts were trehalose and glucose (Fig. 2).

The **trehalose** concentration, initially  $10.4 \pm 1.8$  mM/l, decreased significantly after 2 days of dehydration (day 2:  $5.0 \pm 1.1$  mM/l), after which the concentration increased progressively up to day 8 (day 8:  $8.4 \pm 0.6$  mM/l;  $P > 0.05$ , relative to the Control). However, on day 10 of dehydration, the trehalose concentration in the haemolymph of *P. globosa* was found to be greatly reduced (day 10:  $0.9 \pm 0.3$  mM/l), and significantly lower than all concentrations from day 0 to day 8 ( $P < 0.05$ ). Drinking (1 h) resulted in a significant increase in the haemolymph trehalose concentration (day 10, 1 h after drinking:  $4.0 \pm 0.8$  mM/l;  $P < 0.05$ , relative to immediate pre-rehydration values), and although there was a tendency for further increase on days 12 ( $6.1 \pm 1.1$  mM/l) and 14 ( $7.0 \pm 1.1$  mM/l) of rehydration, the haemolymph trehalose concentration on day 14 was still significantly lower than the Control ( $P < 0.05$ ).

The haemolymph **glucose** level of *P. globosa* is low (day 0:  $0.7 \pm 0.5$  mM/l), and apart from a significantly increased concentration on day 4 ( $1.7 \pm 0.6$  mM/l;  $P <$

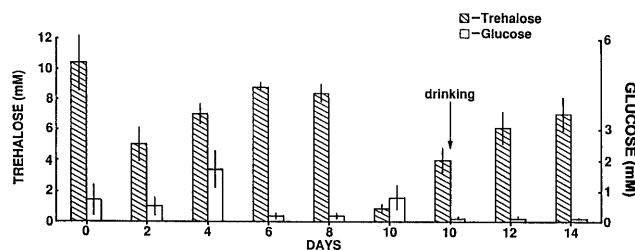


Fig. 2. Haemolymph trehalose and glucose concentrations in *Physadesmia* during dehydration and rehydration (in mM/l). Drinking shown by arrow. Vertical lines represent  $\pm 1$  S.E.M.  $N = 5-7$ .

0.05, relative to the Control), glucose concentrations were found to remain relatively constant during dehydration and rehydration ( $P > 0.05$ , for concentrations on all days except day 4).

#### Glycerol concentration

Glycerol concentrations in the haemolymph of *Physadesmia* during dehydration and rehydration are shown in Fig. 3. The glycerol concentration increases from  $0.5 \pm 0.18$  mM/l on day 0 to a maximum of  $13.7 \pm 1.8$  mM/l on day 6 ( $P < 0.05$ , relative to the Control), after which the concentration decreases to  $0.2 \pm 0.13$  mM/l on day 10, when it is not significantly different from the Control ( $P > 0.05$ ). Although the glycerol concentration appears to increase after drinking (1 h and 48 h), one-way ANOVA shows that concentrations during the rehydration period (day 10, 1 h after drinking:  $2.0 \pm 0.6$  mM/l; day 12:  $3.1 \pm 1.0$  mM/l; day 14:  $0.3 \pm 0.14$  mM/l) are not significantly different from the Control ( $P > 0.05$ ).

#### Regulation of haemolymph amino acids, sugars and glycerol

The observed values for haemolymph amino acids, sugars and glycerol (relative to that expected from simple haemolymph-concentration and -dilution) indicate that all of these substances are removed from the haemolymph of

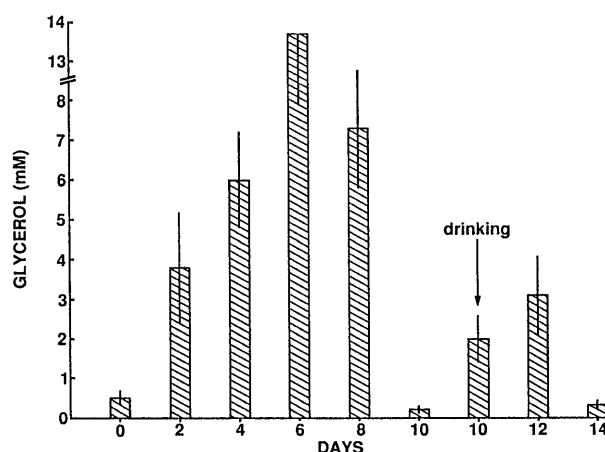


Fig. 3. The effects of dehydration and rehydration on haemolymph glycerol concentrations (mM/l). Drinking shown by arrow. Vertical lines indicate  $\pm 1$  S.E.M.  $N = 5-7$ .

TABLE 1. Regulation of haemolymph amino acids, sugars and glycerol in *Physadesmia globosa* during dehydration and rehydration.

Substance (mM/l)	Haemolymph concentrations (mM/l)				
	Normal Day 0	Dehydration (Day 10)		Rehydration (48 h)	
		Observed	Expected <sup>a</sup>	Observed	Expected <sup>b</sup>
Amino acids	104.8	91.7	267.0	101.1	45.8
Total sugar <sup>c</sup>	11.0	1.7	28.0	6.3	0.8
Glycerol	0.5	0.2	1.3	3.1	0.1

<sup>a</sup> Increase expected from simple haemolymph-concentration; <sup>b</sup> Decrease expected from simple haemolymph-dilution; <sup>c</sup> Total sugar (trehalose + glucose). Haemolymph volumes were obtained from the study of Naidu & Hattingh (1988); the data are for a standard animal of initial weight 699.3 mg.

*P. globosa* during dehydration, and added to it during rehydration (Table 1).

### Osmotic contributions of haemolymph constituents

For assessment of the relative contributions of the haemolymph constituents to osmoregulation during dehydration and rehydration, the measured concentration of each of the constituents was converted to the total millimoles for that constituent (Wall, 1970; Nicolson, 1980; Naidu, 1998):  $\text{mmol/l} \times \mu\text{l haemolymph} = \text{total millimoles}$  (estimates of molal concentrations were projected at 5% above molar values, assuming a haemolymph density of 1.05 g/ml). The osmotic contributions of the measured haemolymph constituents for *Physadesmia globosa* during dehydration and rehydration are shown in Table 2.

After 10 days of dehydration, decreases in the contents of the measured constituents amount to  $25.26 \times 10^{-3}$  mmol. Major contributions to the regulation of osmolality in *P. globosa* are due to decreases in haemolymph free amino acids (40% of the measured total solute decrease), sodium (31%), and chloride (21%). Relatively minor contributions to regulation of haemolymph osmolality are made by the decreases in glucose and trehalose (6%), potassium and glycerol (2.4% and 0.2%, respectively).

After drinking for 1h, the measured total millimoles in the haemolymph of *P. globosa* increases by  $15.57 \times 10^{-3}$  mmol. Major contributors to the solute increase in the haemolymph at this time are sodium (approx. 34%), chloride (approx. 30%) and amino acids (approx. 28%). Relatively slight contributions to regulation of haemolymph osmolality are made by the increases in potassium (4.9%),

trehalose (2.8%) and glycerol (1.5%). There is a slight decrease in the glucose content of the haemolymph after drinking for 1 h ( $0.04 \times 10^{-3}$  mmoles).

### DISCUSSION

Haemolymph amino acids in *P. globosa* are regulated during both dehydration and rehydration, and contribute approximately 40% and 25% to osmoregulation during dehydration and rehydration, respectively. Other beetles which regulate haemolymph amino acids in response to blood volume changes include *T. philistina* (Broza et al., 1976), *Onymacris* larvae (Coutchie & Crowe, 1979), *C. armatus* (Cohen et al., 1986), and the Namib tenebrionids *O. rugatipennis* and *S. gracilipes* (Naidu, 2001a, b). Amino acids were not regulated during rehydration in *Cysteodemus*, however, with approx. 10–40% of the amino acids removed from the haemolymph during dehydration being excreted (Cohen et al., 1986).

The mechanisms proposed to explain haemolymph amino acid regulation during osmoregulation are many and varied (Naidu, 2001b). In *P. globosa*, the decrease in haemolymph amino acid content (absolute amounts) during dehydration appears not to be due to an interchange with haemolymph soluble protein, since the absolute protein content also decreases during dehydration (Naidu & Hattingh, 1988) (Table 3). And, after 1h of drinking, the absolute amount of haemolymph protein increases when haemolymph total amino acids increase. Another beetle which similarly demonstrates no relationship between soluble protein and haemolymph amino acids during osmoregulation is the desert blister beetle *C. armatus* (Cohen et al., 1986). Between 1h and 48 h after drinking, however, there is an inverse soluble protein-amino acid relationship in *P. globosa*, and it is possible that some regulatory mechanism may cleave soluble proteins/peptides to free amino acids (Broza et al., 1976; Collett, 1976a, c; Bosquet, 1977a, b).

The metabolism of amino acids to effect regulation of haemolymph osmotic pressure during dehydration (Coutchie & Crowe, 1979) is worth noting. In *P. globosa*, there is large utilization of lipid reserves during dehydration for 10 days, and the haemolymph total sugar content is virtually depleted at the end of the dehydration period. It is possible, therefore, that amino acid utilization for

TABLE 2. Osmotic contributions of haemolymph constituents in *Physadesmia globosa* during dehydration and rehydration.

Haemolymph Constituents (mmoles $\times 10^{-3}$ )	Normal (day)		Dehydration (day)						Rehydration (hour)		
	0	2	4	6	8	10	1	48	96		
Sodium*	16.98 [122.1]	12.26 [113.2]	10.94 [129.4]	12.10 [132.6]	11.83 [150.6]	9.23 [169.1]	14.45 [125.6]	9.60 [87.8]	9.43 [83.2]		
Potassium*	2.28 [16.4]	2.52 [23.3]	1.94 [23.0]	2.06 [22.6]	2.02 [25.7]	1.68 [30.9]	2.44 [21.2]	1.88 [17.2]	1.83 [16.2]		
Chloride*	14.47 [104.1]	13.51 [124.7]	11.46 [135.5]	12.87 [141.0]	12.66 [161.1]	9.19 [168.3]	13.84 [120.3]	9.55 [87.4]	11.51 [101.4]		
Amino acids	15.30 [110.0]	9.33 [86.2]	12.71 [150.3]	9.70 [106.4]	6.58 [83.7]	5.25 [96.3]	9.57 [83.2]	11.6 [106.2]	12.51 [110.3]		
Glucose	0.10 [0.7]	0.06 [0.5]	0.15 [1.8]	0.02 [0.2]	0.02 [0.2]	0.05 [0.8]	0.01 [0.1]	1 [0.1]	0.01 [0.1]		
Trehalose	1.52 [10.9]	0.57 [5.3]	0.62 [7.4]	0.84 [9.2]	0.69 [8.8]	0.05 [0.9]	0.48 [4.2]	0.01 [6.4]	0.83 [7.4]		
Glycerol	0.07 [0.5]	0.43 [4.0]	0.53 [6.3]	1.31 [14.4]	0.60 [7.7]	0.01 [0.2]	0.24 [2.1]	0.70 [3.3]	0.04 [0.3]		
Unknown	[60.4]	[182.8]	[87.0]	[122.3]	[101.0]	[175.2]	[90.1]	0.36 [59.0]	[53.5]		
Total mmoles ( $\times 10^{-3}$ )	50.72	38.68	38.35	38.90	34.4	25.4	41.03	33.7	36.16		
Osmolality (mOsm/kg)*	[425.1]	[540.0]	[540.7]	[548.7]	[538.8]	6 [641.7]	[446.8]	1 [367.4]	[372.4]		

Molal concentrations in brackets [mmol/kg] – assuming a haemolymph density of 1.05 g/ml. Unknown = Difference between osmolality (mOsm/kg) and summed value of molal concentrations (mmol/kg) of measured constituents. \*Values obtained from the study of Naidu & Hattingh (1988).

TABLE 3. Changes in haemolymph amino acids and proteins in *Physadesmia globosa* during dehydration and rehydration (absolute amounts).

	Dehydration		Rehydration		
	Day 0	Day 10	1 h	48 h	96 h
Amino acids(mmoles $\times 10^{-3}$ )	15.3	5.25	9.59	11.61	12.51
Protein (mg)*	5.23	2.97	4.71	2.31	2.98

\*Protein concentration values from Naidu & Hattingh (1988).

metabolism could have contributed, at least in part, to the observed osmoregulation. The removal of amino acids from the haemolymph during dehydration could also be due, in part, to a generalized tissue uptake for synthesis of cellular protein. Collett (1976b) has shown that amino acid utilization (oxidative metabolism) from the free amino acid pool occurs at a lower rate in *C. erythrocephala* when the fly is maintained on a protein-free diet, and that amino acids appear to be conserved for protein synthesis.

In *Physadesmia*, the total sugar content of the haemolymph at the end of the dehydration period is considerably lower than that expected from simple haemolymph-concentration, indicating removal of sugars from the blood during dehydration. During rehydration, the observed sugar values are higher than expected from simple haemo-dilution, indicating sugar addition to the haemolymph of this species at this time. In both instances, however, the sugar contributions to osmoregulation are not great [being greater during dehydration (approx. 6%) than during rehydration (approx. 2%)]. Coutchie & Crowe (1979) have demonstrated haemolymph sugars (mainly trehalose) to be directly involved in osmoregulation in the tenebrionid *O. marginipennis* larvae. In this species, changes in the absolute amount of trehalose followed changes in the haemolymph volume, indicating its removal during dehydration and subsequent replacement in the haemolymph during rehydration. Active control of the sugar content of the haemolymph was suggested, together with regulation of other solutes, to contribute to the observed regulation of haemolymph OP in *Onymacris* (Coutchie & Crowe, 1979). Further evidence for the regulation of haemolymph sugars in insects, may be found in studies suggesting homeostatic control of blood sugar levels (Hill & Goldsworthy, 1970).

Mechanisms for the removal of sugar from the haemolymph during dehydration and its replacement during rehydration are wide-ranging (Naidu, 2001b). In *P. globosa*, the haemolymph trehalose content increases substantially after drinking for 1h and continues throughout the rehydration period. This could result from a *de novo* synthesis of glucose from amino acids (Naidu, 1998), or from the glycerol released from the catabolism of lipid during rehydration, if significant *glycerol kinase* activity is present (Newsholme & Taylor, 1969). Coutchie & Crowe (1979) have also suggested that if the glyoxalate bypass exists in insects, then sugar synthesis from fatty acid precursors could take place when the insect is rehydrated.

Glycerol contributions to haemolymph osmolality are negligible in *P. globosa*, and while the observed values for haemolymph glycerol (relative to that expected from simple haemolymph-concentration and -dilution) indicate that glycerol is removed from the haemolymph of this species during dehydration, and added to it during rehydration, the contributions to osmoregulation at these times are insignificant. That the glycerol concentration is significantly increased after 6 days of dehydration, however, may confer a stabilizing effect on protein structure. The removal of glycerol from the haemolymph during dehydration may be achieved by its conversion to  $\alpha$ -glycerophosphate (Naidu, 1998), after which it may undergo oxidative degradation (Friedman, 1970), or be converted to glucose and stored as glycogen (Steele, 1981; Candy, 1985; Mullins, 1985). As was suggested for the nocturnal tenebrionid *Stips stali* (Naidu, 1998), glycerol additions to the haemolymph of *P. globosa* during rehydration could result from glucose oxidation (although, like *S. stali*, emphasis appears to be on carbohydrate anabolism after drinking), lipid catabolism [the total lipid content in *P. globosa* decreases significantly during the rehydration period, but not immediately after drinking (1 h) – S. G. Naidu, unpubl.], and also synthesis from glycogen (Steele, 1981; Mullins, 1985).

There is a large increase in the haemolymph content of unknown solute(s) in *P. globosa* between days 0 and 2 of dehydration, which reduces the overall extent of osmoregulation observed in this species at this time. These solutes may represent the greater accumulation of excretory metabolites including allantoin, urea, and ammonia (this does not preclude regulation of some of the unmeasured solute such as  $\text{Ca}^{2+}$  and/or  $\text{Mg}^{2+}$ , however, since an increase in the content of excretory products may have obscured such regulation). Allantoin has been shown to accumulate in the haemolymph of *Dysdercus fasciatus* (Berridge, 1965), as was both urea and ammonia in the haemolymph of *C. armatus* (Cohen et al., 1986), during large blood volume decreases in these species. The drop in the content of these solutes with extended rehydration (between 1 h and 96 h), may be reflected in an increase in the faecal content of these substances over this time.

*Physadesmia globosa* is a strictly diurnal adesmiine tenebrionid, that occurs most commonly in the dry, sandy, vegetated Kuiseb riverbed habitat, but may extend out into the plains via those sandy washes with vegetation or shaded banks (Wharton & Seely, 1982). It avoids extensive activity in the open, i.e. away from the more shaded sectors of its habitat, is psammophilous, and digs beneath the surface to escape the midday heat (activity in this species is greatly decreased during the midday period) or when ambient temperatures are high (Wharton & Seely, 1982). *Physadesmia* maintains lower body temperatures in the field than the *Onymacris* species (29–30°C – Nicolson et al., 1984; Seely et al., 1988), has a metabolic rate approximately one-half that of the dune tenebrionid *Onymacris plana* at rest (at 35°C) and moves at approximately one-quarter the speed of this beetle (Nicolson et al., 1984; Bartholomew et al., 1985). Taxonomically, it is

believed to be intermediate between *Onymacris* and *Physosterna* (*Physosterna cribripes* occurs on the hard gravel plains to the north – one of three major biotopes of the Central Namib) (Penrith, 1979), and like *O. unguicularis* (Louw et al., 1986) it has been shown to respire cyclically (Bartholomew et al., 1985); in both species, the periods of apnoea are considerably increased at rest, and thought to be important to their overall water conservation. *Physadesmia globosa* feeds mainly on fallen *Acacia* flowers (Bartholomew et al., 1985), but they have no noticeable food preferences and are described as opportunistic omnivores. [Wharton & Seely (1982) report movement of this species to *Ficus sycomorus* in large numbers, following fig drop at the end of December.]

Although efficient osmoregulation can afford the insect considerable temporal leverage before the onset of debilitating osmotic stress, *P. globosa* is not a phenomenal osmoregulator at the different levels of haemolymph loss examined. This may be related to a more adroit exploitation of micro-habitat retreats, considerable internal control of water losses, and a possibly higher total water input. Water loss due to faecal production is extremely low in *P. globosa*, and although it has a relatively high surface area-specific water loss rate, its mass-specific loss rate is equable to that of *Stenocara gracilipes* and not greatly different from *Onymacris unguicularis* and *Onymacris rugatipennis* (3 other Namib tenebrionids examined) at similar temperatures (S. G. Naidu, unpublished). That it has a lower body temperature in the field, however, must predispose this species to greater restriction of evaporative water losses, by lowering the effective vapour pressure difference (*P. globosa* also has a lower mass-specific metabolic rate relative to *O. plana* during activity – Bartholomew et al., 1985). And of considerable import to water conservation in the field, and unlike *S. gracilipes*, *P. globosa* spends a substantial proportion of its time submerged beneath the riverbed sand surface (and whenever surface conditions become particularly desiccatory), from whence it may derive considerable hygrothermal relief (Willmer, 1982). On the gain side of the water balance equation for this species, water input in *P. globosa* (between fogs) probably occurs largely in its food (the water content of which may be much higher than that of the dune tenebrionids – the riverbed is thought to represent a mesic corridor through an otherwise harsh environment – Wharton & Seely, 1982). *P. globosa* can reduce its faecal water content to very low values; if the water content of the *Acacia* flowers they feed on is relatively high, then another possibility for increased water input may include increased consumption of food for the water contained therein. *Tenebrio* larvae are thought to consume more food than they need for its water content [Schultz, 1930 (cited in Edney, 1977)], as does the rice weevil *Sitophilus* (Arlian, 1979). In addition, some plant feeders when desiccated, are thought to eat more or unusual food largely for the water contained within (Barton Browne, 1964; Dadd, 1970). Thus, ecologically, *Physadesmia globosa* may be well equipped to survive without the need to osmoregulate perfectly,

because it may rarely experience water stress to any appreciable degree in the field.

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