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Patterns of Hemolymph Osmoregulation in Three Desert Arthropods

W.A. Riddle, C.S. Crawford, and A.M. Zeitone

Department of Biology, University of New Mexico, Albuquerque, New Mexico 87131, USA

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Summary. Hemolymph osmoregulation was examined in three species of desert arthropods: a tenebrionid beetle, *Eleodes hispilabris*, a vejovid scorpion, *Paruroctonus aquilonalis*, and a spirostreptid millipede, *Orthoporus ornatus*. During desiccation, beetles regulated hemolymph osmolality and scorpions tolerated increasing osmolality. Millipedes displayed both osmotic regulation and tolerance patterns depending on sex and on duration of desiccation. Rehydration after desiccation depressed blood osmolality of male scorpions and beetles below levels found for freshly collected specimens. This was not the case in millipedes. Seasonal osmolality changes were studied and recorded among field-collected scorpions and beetles. Patterns of regulation and tolerance of hemolymph osmolality appear to vary among different kinds of desert arthropods.

Introduction

Desert insects, arachnids, and myriapods have evolved a variety of physiological means of dealing with severe temperature and desiccation stresses to which they are potentially exposed (Cloudsley-Thompson, 1975). Osmotic stress, produced as a result of desiccation should also be considered as a potentially important problem in survival among desert arthropods, and one which might be dealt with physiologically in a number of ways. Although potentially important, osmoregulation of these groups of desert arthropods has only been minimally studied.

Among insects, the desert cockroach, *Arenivaga*, regulates hemolymph osmolality strongly following both desiccation and rehydration (Edney, 1966, 1968). Evidence for comparable regulation in the domestic cockroach *Periplaneta americana* (Edney, 1968; Wall, 1970) and in locusts (Djajakusumah and Miles, 1966; Shaw and Stobbart, 1972) supports the generalization that insects as a group may be strong hemolymph osmoregulators.

Osmoregulation has not been studied in desert myriapods. Significantly increased hemolymph osmolality during midwinter was demonstrated but not

accounted for in the large centipede *Scolopendra polymorpha* by Crawford et al. (1975). Woodring (1974) showed that the woodland millipede *Pachydesmus crassicutis* regulates hemolymph osmolality poorly following rapid dehydration, although regulation improves after slower dehydration. Regulation seems to be less efficient than in insects studied.

Among scorpions, a relationship of blood osmolality to body size was reported for the tropical *Heterometrus fulvipes* by Padmanabhanaidu (1966). Largely unexplained seasonal changes in hemolymph osmolality of the semimontane scorpion *Diplocentrus spitzeri* were reported by Crawford and Riddle (1975). Hadley (1974) suggested that the desert scorpion *Hadrurus arizonensis* simply tolerates increased hemolymph osmolality during desiccation.

In the present investigation we examined the hypothesis that arid-land arthropods can utilize various physiological means in solving problems of osmotic stress. In doing this we studied hemolymph osmoregulation in three common, sympatric species of desert-grassland arthropods during their active seasons. The species included a tenebrionid beetle, *Eleodes hispilabris* Say.; a vejovid scorpion, *Paruroctonus aquilonalis* (Stahnke); and a spirostreptid millipede, *Orthoporus ornatus* (Girard). Adults of all 3 species were studied; immature stages were only examined in scorpions.

Materials and Methods

Determination of Hemolymph Osmolality

All specimens were collected near Albuquerque, New Mexico. Hemolymph samples either were taken soon after collection or as indicated below. Removal of hemolymph was achieved using 1- or 5-µl micropipettes from a shallow puncture in the pronotum (beetles), from beneath the membrane along the posterior carapace margin (scorpions); and from a dorsal, intersegmental puncture (millipedes). Micropipettes were sealed with vaseline and frozen at −10 °C until melting points were determined using an apparatus similar to that of Gross (1954), equipped with a thermometer graduated to 0.1 °C.

Osmolality of samples was estimated from melting points by using a linear regression equation. Melting points of 30 NaCl solutions of known osmotic concentration were determined and an equation $Y=32.552–521.443\,X$ ($n=30$, $r=-0.99$) was calculated in which Y represented osmolality in milliosmoles (mOsm) and X the sample melting point in degrees Celsius. This equation gave only slightly higher osmolality estimates than a regression equation calculated from published tables relating freezing-point depression to osmolality.

Seasonal Estimates of Hemolymph Osmolality

Possible influences of season on hemolymph osmolality were examined in beetles and scorpions, which were collected periodically. Beetles were taken in the morning, weighed, and sampled for hemolymph within 4 h of collection. Scorpions were collected at night and kept in plastic bags at 17 °C until the next day when hemolymph was removed. Millipedes were collected on 12 July, 1975, within a week of their annual emergence from subterranean hibernacula.

Desiccation Experiments

In order to uniformly desiccate animals, a water-loss device used in previous experiments (Riddle, 1975) was employed. Individual beetles and scorpions were kept in small glass containers covered

with screening. Millipedes were kept in small screen-top petri dishes. All feces produced were weighed before hemolymph sampling and considered in water-loss estimates.

Freshly collected scorpions varied widely in water content; they failed to take up water from moist soil, sponges or filter paper. We could not standardize the hydration state of scorpions, and for consistency did not pre-hydrate any animals before experiments.

Water loss in scorpions and beetles was expressed as per cent loss of original water present before desiccation. This was determined by measuring non-fecal weight loss, and then dry weight after specimens were killed by freezing and dried at 60 °C (hemolymph was sampled after weight-loss measurements). An estimate of original water content was made by subtracting final dry weight from pre-desiccation live weight. Non fecal weight loss was then divided by the estimated original water content and expressed as a percentage. This method was superior to weight loss expressed as a percentage of original live weight because it better represented the severity of desiccation among animals displaying similar weight loss but having different original water contents. Water loss in millipedes was most conveniently expressed as percentage of original weight lost.

Hydration state of scorpions was expressed in mg of H_2O present per mg tissue dry weight rather than as percentage water content (this approach was suggested by Coutchié and Crowe, pers. comm.). It was not used in beetles and millipedes because of the highly variable contribution of gut and fecal dry matter to total live weight.

For desiccation experiments most scorpions were collected in June; mature males were collected in late August and early September. Beetles were collected in early May. Desiccation for about 13 and 20 days was fatal to beetles and scorpions, respectively.

In experiments involving millipedes, hemolymph from 10 males and 10 females was sampled following collection. Eighty other specimens, divided equally as to sex, were then desiccated, with 40 being removed after 20 days and 40 more after 40 days. From each group we took hemolymph samples of 10 males and 10 females, leaving the balance for rehydration experiments. Weight loss due to metabolic use of food reserves was not estimated for any animals. As a result, water-loss values overestimated weight loss due to water loss alone.

Rehydration and Feeding Experiments

Effects of feeding and variable hydration on hemolymph osmolality of beetles were tested using freshly collected specimens. The first experiment involved 2 groups of 40 beetles (20 of each sex) collected 3 May. One group was kept in an insectary (26 °C, 85% r.h., and natural photoperiod) for 3 days on moist soil without food. The other group was treated similarly but also given lettuce and laboratory rat food ad lib. The second experiment utilized another 2 groups of 40 beetles collected 3 July and kept in shaded, outdoor containers for 3 days. Container soil was kept moist, as before, but only one group was fed.

The effect of rehydration following desiccation was tested for beetles collected 5–7 April 1976. Following collection, 3 groups (20 males, and 20 females each) were established: one group was sampled immediately, while the others were desiccated for 4 days. After desiccation, blood samples for one group were taken, and the remaining beetles tested after rehydration for 24 h on wet soil.

Effects of rehydration on hemolymph osmolality in scorpions were tested following water uptake from moist soil. Severely desiccated adult specimens (9 females, 14 males, mean live weight loss, 27.8%) were placed on wet soil and weighed 12, 24, 48, and 96 h later. After the final weighing they were placed in clean containers at 24 °C and 100% r.h. overnight prior to hemolymph sampling the next day.

Possible rehydration of scorpions in near-saturated air was tested using another group of severely desiccated adults (8 females, 9 males, mean live weight loss, 18.6%). High humidity (98% r.h. at 24 °C) was established in the water-loss apparatus using a saturated solution of aqueous potassium dichromate ($K_2Cr_2O_7$) (Winston and Bates, 1960). After 60 h of exposure resulting in no weight gain due to water vapor uptake specimens were put on lightly moistened soil presumably simulating field conditions, and water uptake was measured after 36, 72, and 100 h.

Effects of rehydration on millipede hemolymph osmolality were tested following rehydration of previously desiccated groups for 3 days on wet gravel in the insectary. The influence of starvation

on osmolality was separately examined using 14 females and 18 males kept outdoors for 20 days in a shaded container with moist soil and no food.

Comparisons of mean levels of osmolality and hydration state were made using Student's *t*-tests. In correlation analyses the significance of the correlation coefficient (r) was tested.

Results

Beetles

The effect of season on hemolymph osmolality of beetles is illustrated in Figure 1. Osmolality levels for females remained statistically similar during the year. Males collected 17 June displayed significantly higher ($P<0.01$) levels than females. Male osmolality was significantly higher on 17 June than 3 July or 17 July ($P<0.001$ and $P<0.01$, respectively), and also higher on 3 May than 3 July ($P<0.01$).

The effect of continuing desiccation on hemolymph osmolality (Fig. 2) shows considerable osmoregulation which is similar in both sexes.

Results of laboratory and outdoor feeding and hydration experiments (Table 1) indicate a strong elevation in blood osmolality with feeding in both cases. In the laboratory group and outdoor group (beetles collected 3 May and 3 July respectively) watering was not associated with any significant change in osmolality from levels shown for field animals collected on those dates (Fig. 1).

The influence of rehydration following desiccation in beetles is summarized in Table 2. For beetles sampled immediately following desiccation, percentage water loss of original water present was similar for males and females (30.9% and 29.4%, respectively). For beetles to be rehydrated, water loss among male beetles (36.7%) was greater than that of females (30.3%). Rehydration was effective in depressing osmolality significantly below field levels for males but not for females.

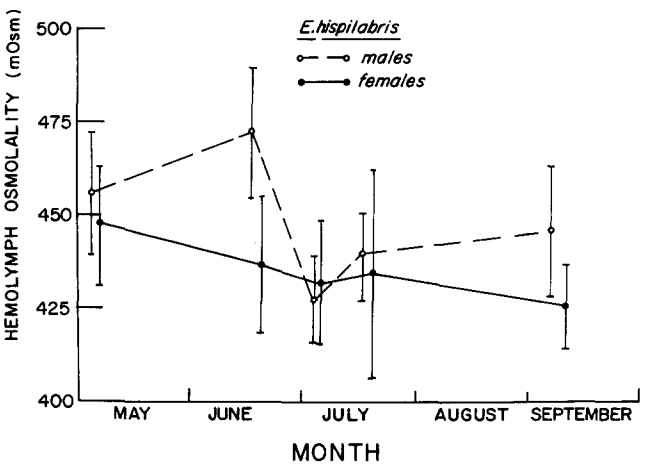

Fig. 1. Seasonal variations in hemolymph osmolality for field-collected *Eleodes hispilabris* beetles. Vertical limits are 95% confidence intervals. $N=15$–22

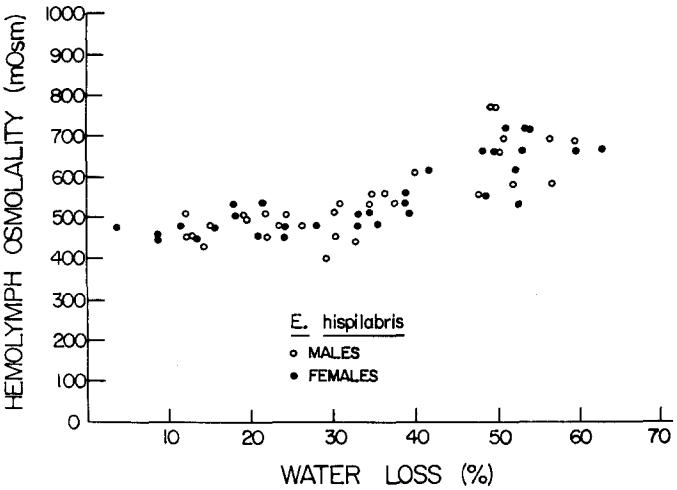

Fig. 2. Effect of prolonged water loss on the hemolymph osmolality of *Eleodes hispilabris* beetles. Water loss is expressed as a percentage lost of original water present

Table 1. Effect of laboratory and outdoor feeding and hydration exposure on hemolymph osmolality of the beetle *Eleodes hispilabris*

Treatment group	Hemolymph osmolality mOsm (N) ±S.E.			
	males[a]	*P*	females[a]	*P*
Laboratory				
watered	455.4±21.6 (19)	<0.001	440.7±12.5 (20)	<0.001
fed and watered	544.1±16.3 (13)		537.4±10.7 (20)	
Outdoor				
watered	422.3± 5.6 (16)	<0.001	416.2± 5.9 (17)	<0.001
fed and watered	527.0± 7.4 (19)		540.1±11.9 (15)	

[a] No significant differences were found between males and females following similar exposure

Table 2. Effect of dehydration and rehydration exposure on hemolymph osmolality of *Eleodes hispilabris* beetles collected April, 1976

Treatment group	Hemolymph osmolality mOsm (N) ±S.E.			
	males[a]	*P*	females[a]	*P*
Field collected[b]	461.7±5.7 (20)	<0.001	461.7±3.9 (20)	<0.001
Dehydrated	554.2±9.1 (20)	<0.001	542.5±8.5 (20)	<0.001
Rehydrated[b]	432.6±7.9 (18)		450.0±4.9 (20)	

[a] No significant differences were found between males and females following similar exposure
[b] Osmolality of field-collected and rehydrated beetles differed ($P<0.01$) only for males

Table 3. Effect of season on hemolymph osmolality and hydration state in the scorpion *Paruroctonus aquilonalis*

Collection date and state of maturity	Hemolymph osmolality mOsm (N) ±S.E.		Hydration state mg H_2O/mg dry wt (N) ±S.E.	
	males[a]	females	males[a]	females
31 March (mature)	–	566.2±16.5 (14)	–	2.105±0.082 (14)
25 April (mature)	–	532.2±12.3 (20)	–	–
7 June (immature)	502.1±7.0 (24)[b]	492.3± 8.2 (16)[b]	2.079±0.076 (24)[c]	1.984±0.110 (16)[c]
27 July (immature)	573.4±8.6 (11)	559.7± 8.1 (15)	1.765±0.056 (11)	1.650±0.53 (15)
1 September (mature)	570.7±7.3 (30)	–	2.205±0.051 (30)	–

[a] No significant differences were found in hemolymph osmolality or hydration state between males and females; means of both parameters between mature and immature specimens were not computed
[b] Significantly different from mean value immediately below at $P<0.001$
[c] Significantly different from mean value immediately below at $P<0.05$

Scorpions

The influence of season on osmolality of scorpions is summarized in Table 3. A significantly higher ($P<0.001$) blood osmolality was found among immatures of both sexes taken 27 July than among those collected 7 June. A significant ($P<0.05$) decrease in hydration state was also noted during this period among immatures.

For immature scorpions (16 females and 24 males) collected 7 June the following correlations were found to be significant ($P<0.05$): (i) hydration state and osmolality males: $r=-0.45$, females: $r=-0.60$), (ii) hydration state and dry weight (males: $r=-0.48$, females: $r=-0.58$), and (iii) osmolality and dry weight (females only: $r=+0.60$). Combining data from males and females ($n=40$) increased significance levels of r to $P<0.01$ for these correlations. Neither hydration state nor osmolality were correlated with live weight.

The effect of continuing desiccation on scorpion hemolymph osmolality (Fig. 3) illustrates a relatively poor ability to osmoregulate in comparison with the tenebrionid beetles.

Rehydration rates of adult scorpions (females collected 7 June, males collected late August) on saturated or lightly moistened soil are presented in Figure 4. Prior to rehydration males had lost an average of 31.7% and females 21.8% of initial body weights. After 100 h exposure to saturated soil, hemolymph osmolalities (±SE) were 522.0±15.4 mOsm (males) and 540.0±15.7 mOsm (females). Corresponding hydration states (±SE) were 2.706±0.063 mg H_2O/mg dry wt (males) and 2.555±0.115 mg H_2O/mg dry wt (females).

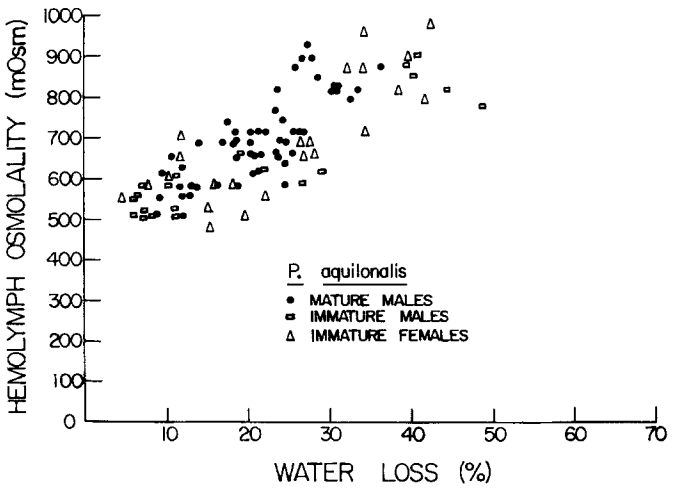

Fig. 3. Effect of prolonged water loss on the hemolymph osmolality of *Paruroctonus aquilonalis* scorpions. Water loss is expressed as a percentage lost of original water present

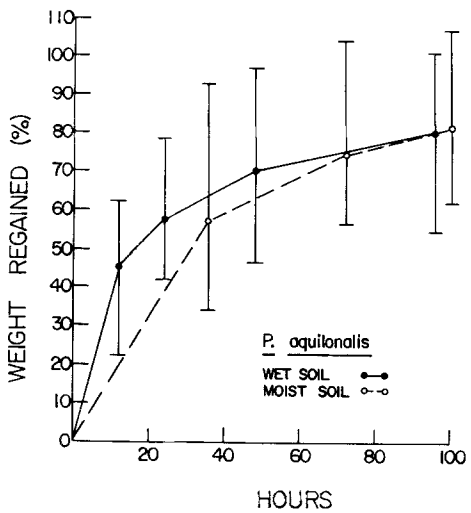

Fig. 4. Effect of exposure to moist or wet soil on water uptake of severely desiccated *Paruroctonus aquilonalis* scorpions. Ordinate represents percentage of pre-desiccation weight regained. Vertical limits are ranges

The rehydrated adult males described above were compared with adult males collected 1 September (Table 3), and were found to have significantly lower mean hemolymph osmolality ($P<0.01$) and greater hydration states ($P<0.001$). Rehydrated adult males displayed significant correlations between osmolality and hydration state and between osmolality and dry weight ($r=-0.71$, $P<0.01$ and $r=+0.63$, $P<0.05$, respectively), while such correlations were not found among adult males collected on 1 September.

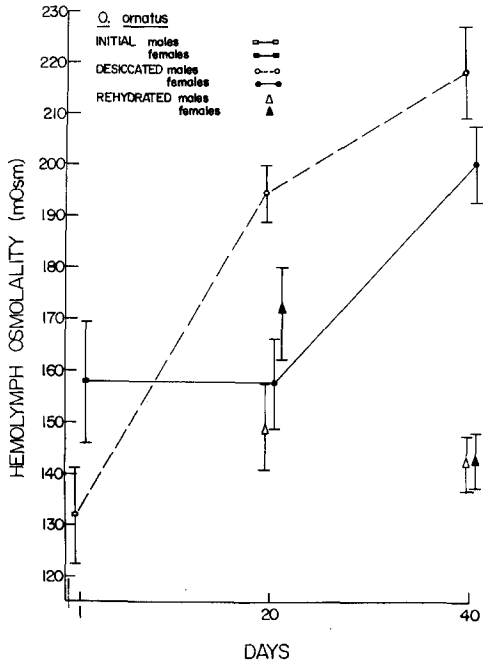

Fig. 5. Effect of dehydration and rehydration on hemolymph osmolality of *Orthoporus ornatus* millipedes. Duration of desiccation exposure is given in days. Vertical limits are standard errors. $N = 9$–10

Millipedes

Results of desiccation and rehydration experiments appear in Figure 5. Initial values of hemolymph osmolality for freshly collected specimens of each sex were not significantly different; however, after 20 days of desiccation, values from males were significantly greater ($P < 0.01$) than those from females, although mean weight losses were about the same (males: 18.2%, females: 15.2%). Subsequent rehydration depressed male osmolality significantly below desiccation levels ($P < 0.001$); osmolality in rehydrated females did not change significantly.

After 40 days of desiccation male osmolality did not increase significantly over that of the 20-day group; however, female osmolality rose to a point where it became statistically similar to that of males. Again, mean weight losses by both sexes were similar (males: 28.5%, females 25.8%). Subsequent rehydration now depressed hemolymph osmolalities significantly in both sexes ($P < 0.001$) to a point where both means were statistically similar to those from freshly collected millipedes. In females, rehydration osmolalities were significantly greater after 20 days than after 40 days ($P < 0.01$). Osmolality levels measured after twenty days of starvation on moist soil were not significantly different from those of freshly collected specimens in either sex (Fig. 5), final values ($\bar{X} \pm SE$) being 145.4 ± 6.3 mOsm for males and 144.3 ± 5.1 mOsm for females.

Discussion

Patterns of osmotic regulation and osmotic tolerance are evident in the arthropods we studied. Regulation appears typical of the tenebrionid beetle (*Eleodes hispilabris*), while tolerance is found in the vejovid scorpion *Paruroctonus aquilonalis*. The spirostreptid millipede *Orthoporus ornatus* appears somewhat intermediate in its capacity to regulate or tolerate, regulation being more evident in adult females than in males.

Sexual differences in osmolality also occurred in June-collected *E. hispilabris*, with osmolality of males rising during the warm, very dry weather. Onset of summer rains in early July corresponded well with the sharp depression of male osmolality shown in Figure 1. In spite of the loss of one-third of total body water (Fig. 2), osmolality levels remained stable; increasing only from about 450 to about 500 mOsm. These results are similar to those reported for fed and hydrated *Arenivaga*, which only increased its hemolymph osmolality from 433 to 452 mOsm after a similar desiccation (Edney, 1966). Slightly poorer regulation during dehydration is apparent among beetles tested April, 1976 (Table 2) than during the previous year. After losing about 30% of original water, osmolality increased from about 460 mOsm to about 550 mOsm. This yearly variation in regulation ability may have been due to differences between years in the extent of natural dehydration prior to laboratory desiccation.

Rehydration results for beetles (Table 2) indicated that even after severe desiccation and short-term rehydration, blood osmolality was regulated at nearly field levels, especially among females. Blood osmolality during desiccation was possibly regulated at least in part by removal of excess blood solutes by excretion, as suggested by continuing feces production during desiccation. More severe dehydration among males (36.7%) than for females (30.3%) prior to rehydration may have been associated with greater solute loss. Higher solute loss for males would have contributed to the dilution evident in their lower post-hydration osmolality (Table 2).

Among arthropods, scorpions as a group are comparatively resistant to water loss (Hadley, 1974), although overall resistance can be a function of size and habitat (Crawford and Wooten, 1973). In *P. aquilonalis*, water loss occurring during desiccation results in a steady elevation in hemolymph osmolality. Without osmoregulation, adult males beginning desiccation exposure at about 570 mOsm (Table 3) and losing one-third of their total body water should achieve an osmolality of 950 mOsm. This estimate is close to the level of about 850 mOsm that a fitted curve of the results in Figure 3 would predict. Such a pattern of osmotic tolerance as similar to that described for terrestrial isopods undergoing desiccation (Berridge, 1970; Lindqvist and Fitzgerald, 1976).

Water uptake by severely desiccated adult *P. aquilonalis* on moist soil contrasts with our unsuccessful attempts to hydrate freshly collected scorpions. Water uptake from moist soil has been demonstrated for desiccated immature *Diplocentrus spitzeri* scorpions, but not for adults (Crawford and Wooten, 1973). Water uptake from moist sponges in desiccated *Hadrurus arizonensis* scorpions was very limited (Hadley, 1970).

Positive correlations between dry weight and osmolality among freshly

collected immature and recently hydrated adult male *P. aquilonalis* are consistent with the size-osmolality relationship in male *Heterometrus fulvipes* reported by Padmanabhanaidu (1966). However, any adaptive significance associated with such a correlation remains unclear. In the isopod *Porcellio scaber* an inverse relationship was found, while among other arthropods a variety of size-osmolality patterns have been noted (see discussion by Lindqvist, 1970).

The desert millipede *Orthoporus ornatus* seems to have evolved a different set of emphases with regard to hemolymph osmoregulation. Because they are large (2–4 g) as adults in the population studied, as well as unusually resistant to desiccation (Crawford, 1972), it is understandable that only about one-fourth of the water in body and gut contents in both sexes was lost after 40 days of desiccation. During this time hemolymph osmolality also rose by about one-fourth, although females exhibited much greater regulation than males for at least the first 20 days. Therefore, both sexes are tolerant of an osmolality increase, as in the case of scorpions, but females are also capable of considerable regulation, which is true in both sexes of tenebrionids.

Conceivably, *O. ornatus* uses water and solutes present in its large and fairly turgid gut to regulate hemolymph osmolality. This is suggested by an unchanging osmolality during 20 days of access to water but not to food, during which time anal drinking (Crawford, 1972) was often observed. Virtual lack of defecation during 40 days of dehydration, and strong post-hydration regulation of osmolality, also point to possible gut involvement. Shaw and Stobbart (1972) speculate that regulation of solute uptake by the insect midgut might increase the flexibility of the excretory system during dehydration.

Our results indicate that there are different physiological responses to desiccation and rehydration in different kinds of arid-land arthropods. The extent of tolerance or regulation of hemolymph osmolality may be a consequence of phylogenetic status and largely unrelated to desiccation resistance, which is generally well developed in such animals.

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