STUDIES ON THYSANURA. I. THE WATER ECONOMY OF *MACHILOIDES DELANYI* WYGODZINSKY AND *CTENOLEPISMA LONGICAUDATA* ESCHERICH

J. HEEG

*Zoology Department, Rhodes University**

INTRODUCTION

The water economy of the Thysanura has been little studied. Sweetman (1938) has considered the survival of *Thermobia domestica* (Packard) when under water stress, and Beament *et al* (1964) have investigated the cuticular permeability of this species. Lindsay (1940) has studied the general biology of *Ctenolepisma longicaudata* Escherich and gives some information on survival times at different humidities, water content and water uptake. These investigations are all concerned with the Lepismatida. Other than the observations of Willem (1924) the water relations of the Machilida have not been investigated at all.

The primitively apterous Thysanura represent the closest living relatives of the Pterygota. Of the two orders† comprising the subclass, the Lepismatida show the greater affinity to the Pterygota (Snodgrass 1938, Barnhart 1961), the Machilida having a number of characters in common with the Symphyla. Even though the protosymphylan ancestry of the Hexapoda is now doubted (Manton 1964), it may nevertheless be inferred that the Machilida are less removed from the ancestral hexapod than are the Lepismatida.

Although much of the success of the Pterygota is generally, and quite justifiably, attributed to their having evolved the ability to fly, physiological adaptations, particularly those concerned with water economy, must have been of considerable consequence in the evolution of flight. The importance of the humus/litter complex of forest floors in the evolution of the terrestrial arthropod fauna has been stressed by Lawrence (1953, 1954). It is the only habitat in which the initially ill-adapted ancestors of most land Arthropoda could have survived, but this sheltered environment does not meet the requirements for the evolution of flight (Wigglesworth *et al* 1963). Physiological adaptations which enabled the ancestors of the Pterygota to leave the shelter of the forest floor are, therefore, a necessary prerequisite in the evolution of flight, and should be manifested to some degree in the living Thysanura which have emancipated themselves from the ancestral habitat (Heeg in preparation), but have stopped short of the final step in hexapod evolution.

The present investigation constitutes a comparative study of the water economy of a lepismatid, *Ctenolepisma longicaudata* Escherich with that of a machilid, *Machiloides delanyi* Wygodzinsky. The behavioural adaptations and ecology of these two species, together with zoogeographical considerations are described elsewhere (Heeg 1967 and in preparation).

*Present address: Zoology Dept., University of Natal, Pietermaritzburg.

† Although most authorities now agree that the two thysanuran sub-groups should be given ordinal status, no satisfactory nomenclature is, as yet, agreed upon. The terms Thysanura (=machilids) and Zygentoma (=lepismatids) imply that the latter now becomes a distinct sub-class, which overemphasises the differences between the two groups. Since this is not a systematic paper, and since the former family designations Machilidae and Lepismatidae are familiar through usage, I have provisionally coined the terms Machilida and Lepismatida.

MATERIAL

Machiloides delanyi is the commonest machilid species occurring in the Grahamstown area. Specimens were collected from under stones in natural forest, plantations and even in open country in the proximity of permanent water. These animals can successfully be kept in the laboratory if fed on strips of bark supporting a good thallophyte microflora. Water was given daily, but with care, since high humidities were found to be detrimental in that they fostered fungal attack.

Ctenolepisma longicaudata is a widespread household pest. It was collected from various undisturbed niches in the laboratory, where the animals subsist on book bindings, paper and miscellaneous organic debris. In laboratory culture they will thrive and even reproduce on a diet of dry rolled oats and dried yeast. Water was given occasionally, but this was not usually necessary.

WATER LOSS AND ITS CONTROL

THE RATES OF WATER LOSS

A comparative study of the rates of water loss was carried out under conditions of extreme desiccation. Preliminary investigations showed that *M. delanyi* was well able to withstand, for moderate periods of time, relative humidities of 0% at room temperature, and that such desiccation gives rise to easily measurable water losses.

The experimental animals were subjected to a standard pretreatment before the commencement of an experiment. They were given free access to abundant food and water over a period of at least 48 hours, after which they were starved, but given water for a further 48 hours. This ensured that the animals were in good condition, being well fed, and also that the alimentary canal was empty, thus precluding the possibility of the animals defaecating during the course of the experiment and thus upsetting the results. A further 24 hours in a dry container ensured that most of the moisture adsorbed onto the animals had evaporated. The treatment outlined here was followed in all experiments concerned with water loss.

After pretreatment the animals were placed in clean, disiccator dry specimen tubes which had been previously weighed. In the case of *M. delanyi* the tube mouth was closed by means of a wide mesh nylon net held in place by an elastic band. *C. longicaudata*, however, was able to bite through the net, and plastic window gauze had to be used in experiments with this species. With normal care the handling of the tubes during closing had no measurable effect on the weight, and the gauze, net and elastic bands were found to be non-hygroscopic.

The tubes plus contained animals were weighed prior to desiccation. Desiccation was effected over anhydrous fused granular calcium chloride in a standard desiccator in a constant temperature room at 20C. At intervals of eight and sixteen hours (at 9 a.m. and 5 p.m. daily) the tubes were weighed and the loss in weight expressed as a percentage of the initial body weight of the animal concerned.

The use of still air desiccators for experiments of this nature has been subject to criticism. It has been pointed out that the rate of water loss from an animal in still air is both membrane and vapour limited since an humidity gradient is set up between the surface of the animal and the desiccant. The rate of water loss from the animal will therefore reflect not only the degree of permeability of the cuticle (the membrane limited system) but also the resistance offered to the water molecules by the region of high humidity surrounding the animal due to evaporation (the vapour limited system). Ramsay (1935) has, however, pointed out that whilst this criticism is most certainly valid in the case of a large animal which loses water rapidly,

the vapour limited system may be negligible in the case where the evaporating surface is small relative to the volume of the desiccator and where water is lost very slowly to the surroundings by virtue of cuticle impermeability. In the present investigation from four to six animals, each with a surface area of approximately 150 square millimetres, were housed in a desiccator with a capacity of 1·5 l, and the animals were found to lose weight very slowly. These considerations, together with the fact that the main aim of the experiments was to obtain comparative rather than absolute values, minimised the importance of any errors due to the use of still air desiccators. A more serious criticism which may be levelled against the use of desiccators as described above is the disturbance of the humidity within when the desiccator is opened to remove tubes for weighing. It was found that the dry atmosphere is only re-established after some 45 minutes, depending, of course, on the ambient relative humidity. For this reason the animals were only weighed twice daily, thus keeping the number of times that the desiccator had to be opened to a minimum.

Desiccation of *M. delanyi* was continued until a constant weight had been attained, thus giving a measure of the rate of water loss before and after death of each animal. *C. longicaudata*,

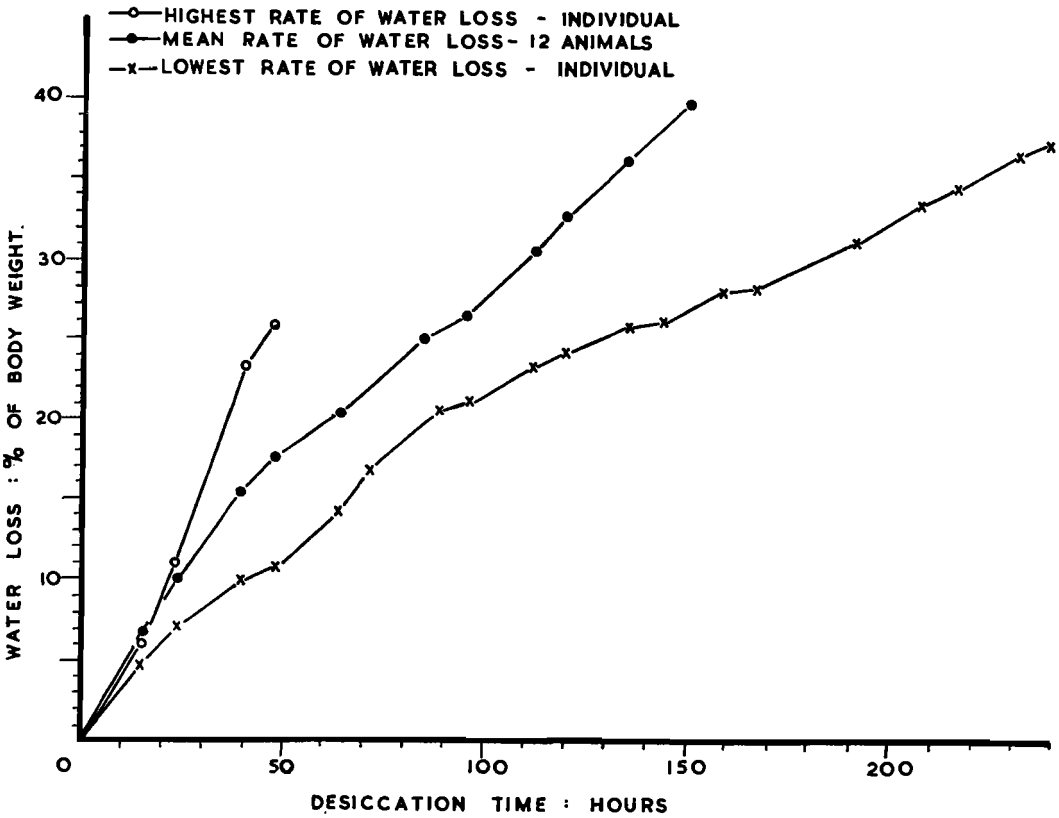

Figure 1. Rate of water loss from a random sample of *M. delanyi* desiccated over anhydrous calcium chloride at 20°C.

however, proved much more resistant to desiccation and would die of excessive water loss only after prolonged exposure to a dry atmosphere. In order to obtain a comparison between the rates of water loss from living and dead specimens it was therefore necessary to kill twelve specimens by means of coal gas and then to desiccate these to constant weight.

Figure 1 shows the mean rate of water loss for a random sample of twelve specimens of *M. delanyi* compared with the individual rates for the longest and shortest lived specimens included in the sample. This species showed a high degree of variability in this respect, some specimens losing water rapidly and succumbing after a comparatively short period of desiccation, whilst others displayed a remarkable resistance to drying. The rate of water loss from a random sample of *C. longicaudata*, shown in Figure 2, is both lower and less variable than that of *M. delanyi*.

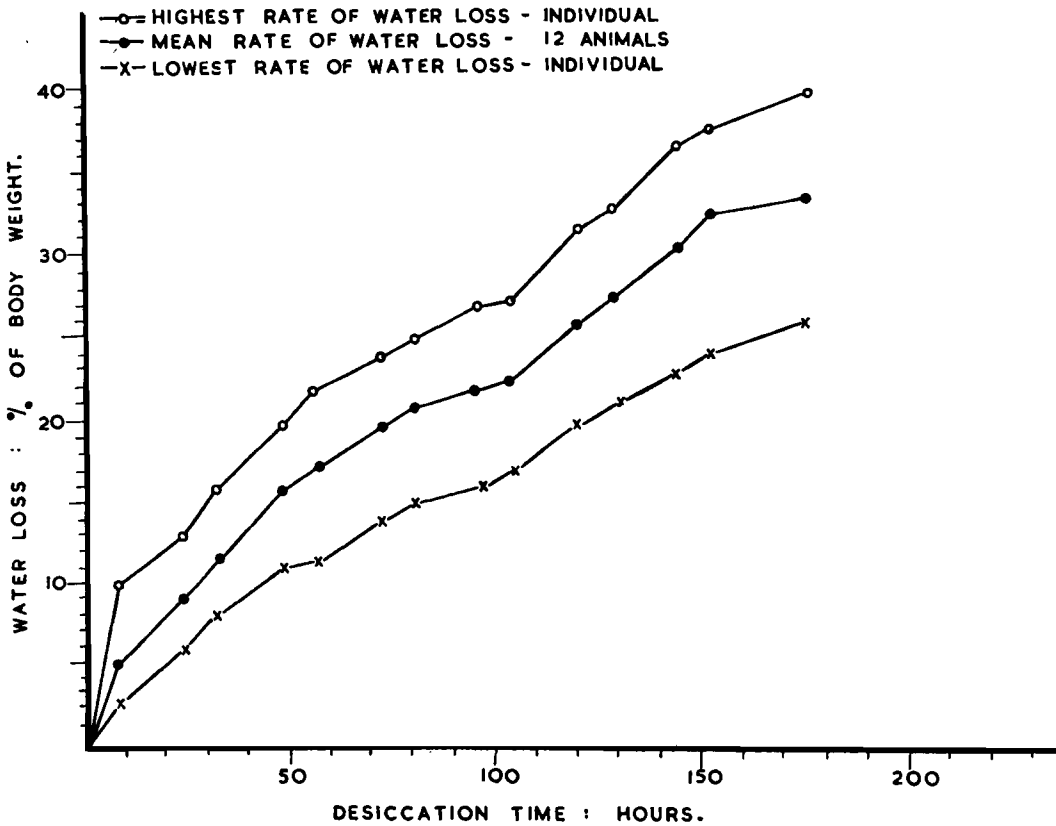

Figure 2. Rate of water loss from a random sample of living *C. longicaudata* when desiccated over anhydrous calcium chloride at 20°C.

Both species showed definite evidence for an active control over the rate of water loss in that the rate increased sharply after death. Figure 3 shows the individual rates of water loss from four specimens of *M. delanyi* desiccated to constant weight. Whilst the rate of water

loss from the live animals showed considerable variability, there was, in each case, an increase to a higher rate after death, and this increased rate was approximatley the same for all animals tested. Figure 4 shows the rate of water loss from twelve dead specimens of *C. longicaudata* compared with twelve live specimens; the highest individual rate for a live specimen is also shown. The dead animals again lost weight more rapidly than did the living specimens. This is contrary to the findings of Lindsay (1940) who claims that no such difference exists in this species.

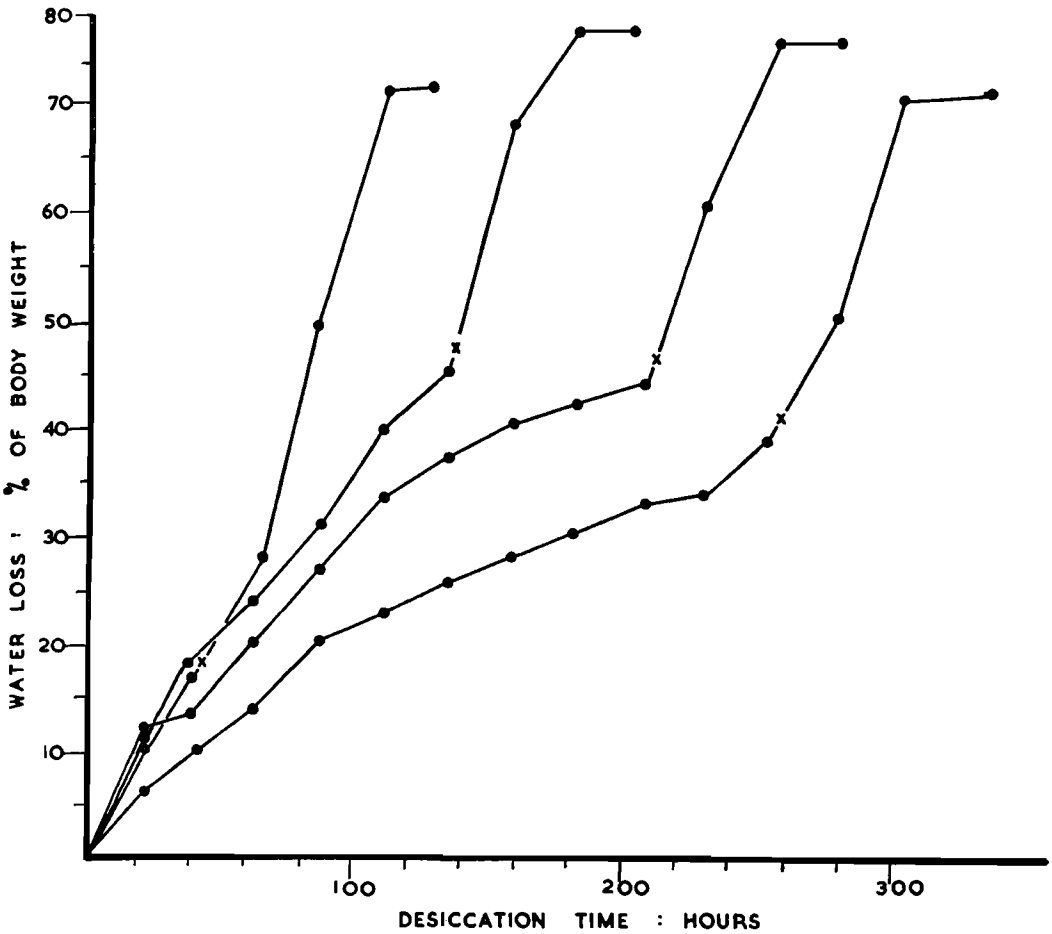

Figure 3. Rate of water loss from four specimens of *M. delanyi* desiccated to constant weight over calcium chloride at 20°C, showing the change in rate after death. "X" indicates the point where the animal died.

The falling off of the rate of water loss as desiccation progressed was a consistent feature of all live animals tested. This suggests that in both species the permeability decreases during desiccation. Since this phenomenon was not apparent in dead specimens, it suggests a pro-

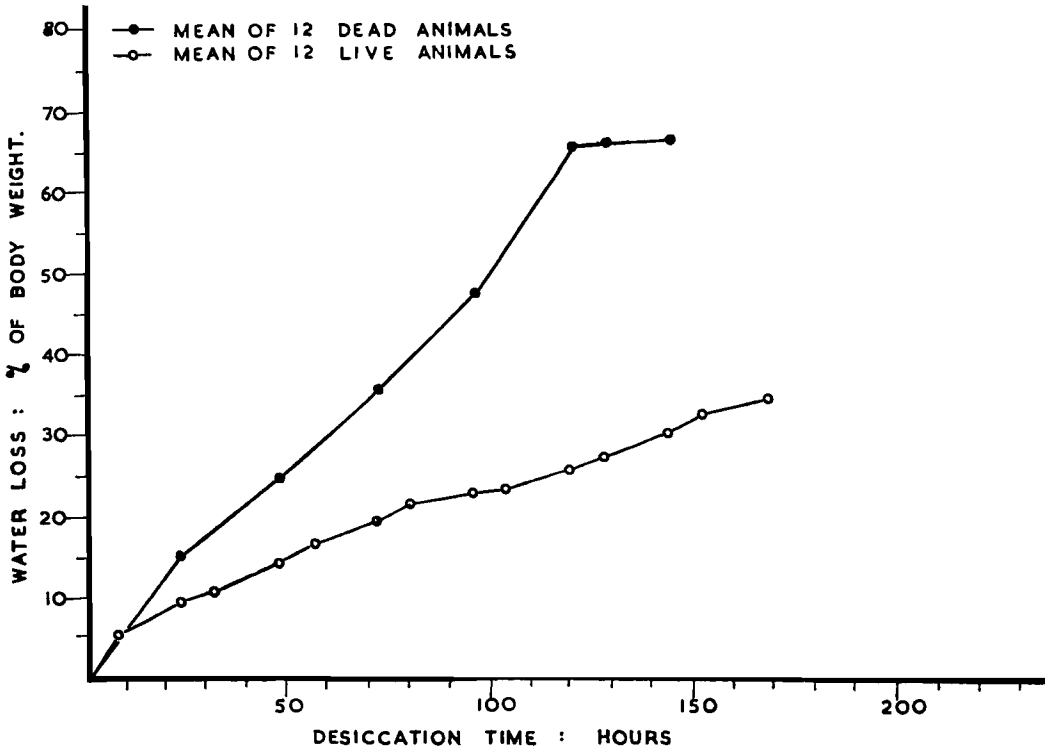

Figure 4. Comparison between the rates of water loss from living and dead specimens of *C. longicaudata* desiccated over calcium chloride at 20°C. (Co-ordinates for regressions B and C as in Fig. 2).

gressive increase in active water retention, possibly governed by the internal water content of the animals. A preliminary investigation of the effect of desiccation on the rate of oxygen uptake in *C. longicaudata* showed a possible correlation between water content and oxygen consumption. This would, in part, support the above suggestion, but to date insufficient results have been obtained to determine whether the correlation is significant. Figure 5 summarises all the data relating to water loss rates, but omits the initial high rate of water loss from live specimens in order to facilitate comparisons.

Size and stage in instar were two possible sources of the variability in the rate of water loss observed above. In order to investigate this, experimental animals were desiccated at three and ten days after moulting, desiccation having been preceded by the standard pretreatment previously outlined. The results, which are shown in Table 1, were analysed by means of the product-moment method for a possible correlation between size and rate of water loss.

The results show a significant difference in both rate of water loss and survival time between specimens of *M. delanyi* desiccated three days and ten days after moulting. Although the difference between three and ten day *C. longicaudata* is statistically not significant, animals were only used in subsequent experiments when ten days had elapsed after moulting. It seems likely that the variability in the rate of water loss from *M. delanyi* noted in the previous experi-

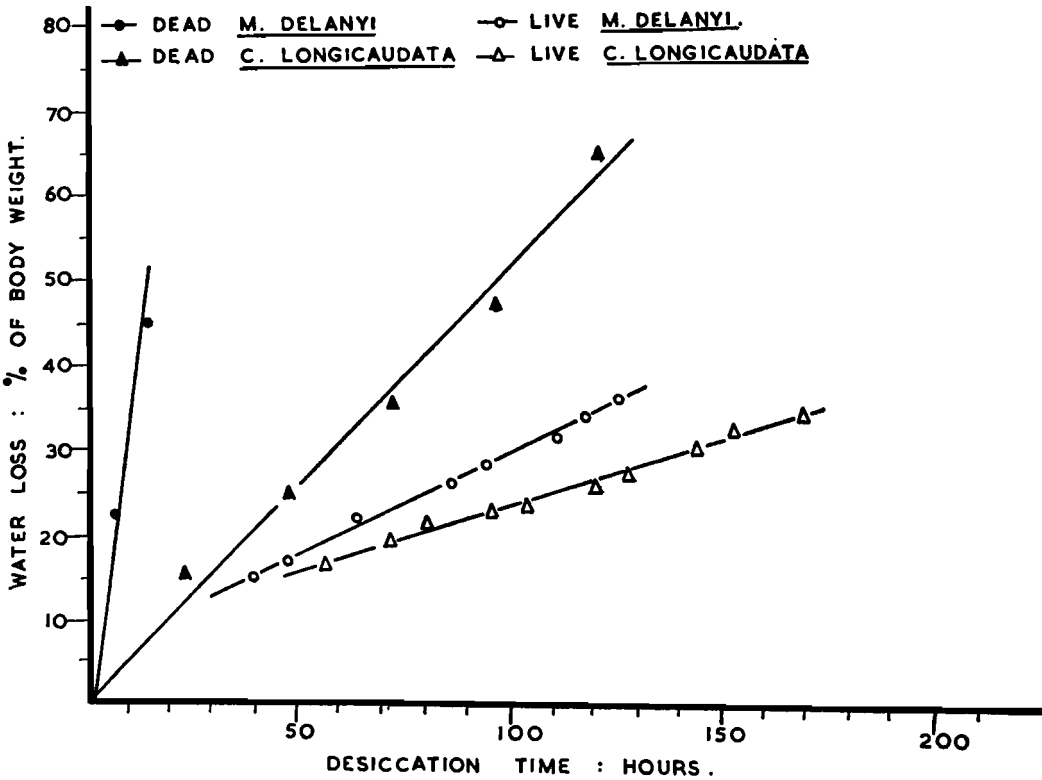

Figure 5. Summary of the data relating to water loss rates from *M. delanyi* and *C. longicaudata*. Only the linear parts of the regressions are shown in respect of the live animals.

TABLE 1

MEAN WATER LOSS (% OF BODY WEIGHT/24 HR.) FROM *M. delanyi* AT 28C AND *C. longicaudata* AT 20C WHEN DESICCATED OVER ANHYDROUS CALCIUM CHLORIDE AT THREE AND TEN DAYS AFTER MOULTING. (P=PROBABILITY OF THE DIFFERENCE BEING DUE TO CHANCE.)

Species	*No. of animals per category*	*Mean daily water loss*		*P*	*Mean survival time*		*P*
		3 *days*	10 *days*		3 *days*	10 *days*	
M. delanyi	15	17·4±1·0	11·7±0·95	0·001	47 hr.	98 hr.	0·001
C. longicaudata	15	5·4±0·24	4·6±0·26	0·1	Whole duration of experiment		

ment can be in part attributed to the animals being at different stages within an instar. However, as the standard errors reflect, there is still a tremendous amount of variability within each category in the experiment here under discussion, so there must be other factors involved as well.

In neither species was a correlation apparent between rate of water loss and size, but such a correlation might well be masked by the considerable variability within the material. The calculated difference between the largest and smallest specimens in the ten day sample of *M. delanyi*, based on surface to volume ratio, was only 1·9% of the mean for the sample, and so small a difference would be impossible to detect. In the case of *C. longicaudata* the difference would be smaller still.

CONTROL OF WATER LOSS

The ability to retain water is dependent upon two components. One of these is the mechanical barrier presented by the cuticle, but as the present investigations has shown, since the rate of water loss of dead specimens is higher than that of living ones, some second and active process is also involved.

Cuticular barriers to water loss must be of importance. Beament *et al* (1964) have shown that the scales of *Thermobia domestica* play a minor role in reducing water loss through the cuticle. These experiments were undertaken on dead animals. The effects of scale removal on the water loss of living animals has been studied in the present investigation.

Scale removal was effected by cooling the animals to a state of coma and then applying a strip of adhesive tape to the body. The scales adhere to the tape and may thus be removed from sections of the animal's surface without risk of abrading the underlying cuticular layers. Scales were removed from the tergites only, since the presence of the abdominal styles does not allow the clearing of the ventral surface of *M. delanyi* by this method. After scale removal the animals were subjected to the normal pretreatment prior to desiccation. A batch of control animals which had been chilled but did not have their scales removed was similarly treated. The results, set out in Table 2, show that the scales play a minor role, if indeed they play any, in the conservation of water.

TABLE 2

MEAN RATE OF WATER LOSS (% OF BODY WEIGHT/24 HR) FOR NORMAL AND DESCALED SPECIMENS OF *M. delanyi* AND *C. longicaudata* DESICCATED OVER ANHYDROUS CALCIUM CHLORIDE AT 20C.

Species	*No. of animals used*	*Rate of water loss*	
		Normal	*Descaled*
M. delanyi..	8	8·2 ± 0·51	9·3 ± 0·63
C. longicaudata	10	4·7 ± 0·26	4·7 ± 0·16

Lower (1958) has described the presence of a very resistant sudanophil layer as forming the outermost part of the epicuticle of *C. longicaudata*. This layer is Millon negative and insoluble in boiling wax solvents. It could clearly act as a barrier to the outward passage of water. To study the action of this layer, animals were descaled as before and their individual rates of water loss observed over a period of 51 hr. The animals were then chilled and their dorsal surfaces abraded either with fine aluminium dust or very fine sandpaper, after which the rates of loss were again determined. A control batch, which was not abraded, showed that the second chilling did not itself affect the rate of water loss.

The results of these experiments are shown in Table 3. It is clear that the rate of water loss is unaffected by abrasion. Such a result is open to several possible interpretations. It would appear to imply that if there is any waxy or greasy layer upon the outer surface of these animals, such a layer does not make a significant contribution to lowering the rate of water loss through the cuticle. It might imply that the sudanophil layer of Lower is also an unimportant element in the cuticle from the view point of water loss, but it has not been demonstrated histologically that this layer was removed by abrasion and it may have resisted the experimental treatment.

TABLE 3

MEAN RATES OF WATER LOSS (% OF BODY WEIGHT/24 HR.) FROM *M. delanyi* and *C. longicaudata*, DESICCATED OVER ANHYDROUS CALCIUM CHLORIDE AT 20C, BEFORE AND AFTER ABRASION OF THE CUTICLE.

Species	*No. of animals used*	*Abrasive*	*Rate of water loss*	
			Before abrasion	*After abrasion*
M. delanyi	7	Aluminium dust	8·2 ± 0·71	7·8 ± 0·82
M. delanyi	5	Sandpaper	7·9 ± 0·97	8·4 ± 0·67
C. longicaudata	8	Aluminium dust	4·4 ± 0·11	4·6 ± 0·17
C. longicaudata	6	Sandpaper	4·5 ± 0·20	4·1 ± 0·24

In pterygotes it has been found that resistance to the passage of water through the cuticle is in fact often dependent upon an outer cuticular layer of wax or grease (Wigglesworth 1945) and further, that in many cases the rate of water loss rises abruptly at a "critical temperature" which probably corresponds with a change of state in this outermost layer of the epicuticle (Beament 1959). The rate of water loss at different temperatures and at a constant saturation deficit was therefore examined over the temperature range of 7C to 33C. The results are shown in Fig. 6 where it can be seen that there is not a very clear discontinuity in the rate of water loss in living specimens of either *M. delanyi* or *C. longicaudata* with increasing temperature. Such a result could again be interpreted as being due to the absence of any waxy or greasy epicuticular layer which acted as a water barrier.

It has been stressed that there is a second and active process concerned with the control of water loss. The only vital processes aiding water retention which suggest themselves are spiracular occlusion and an active retention or resorption of water by the cells of the hypodermis.

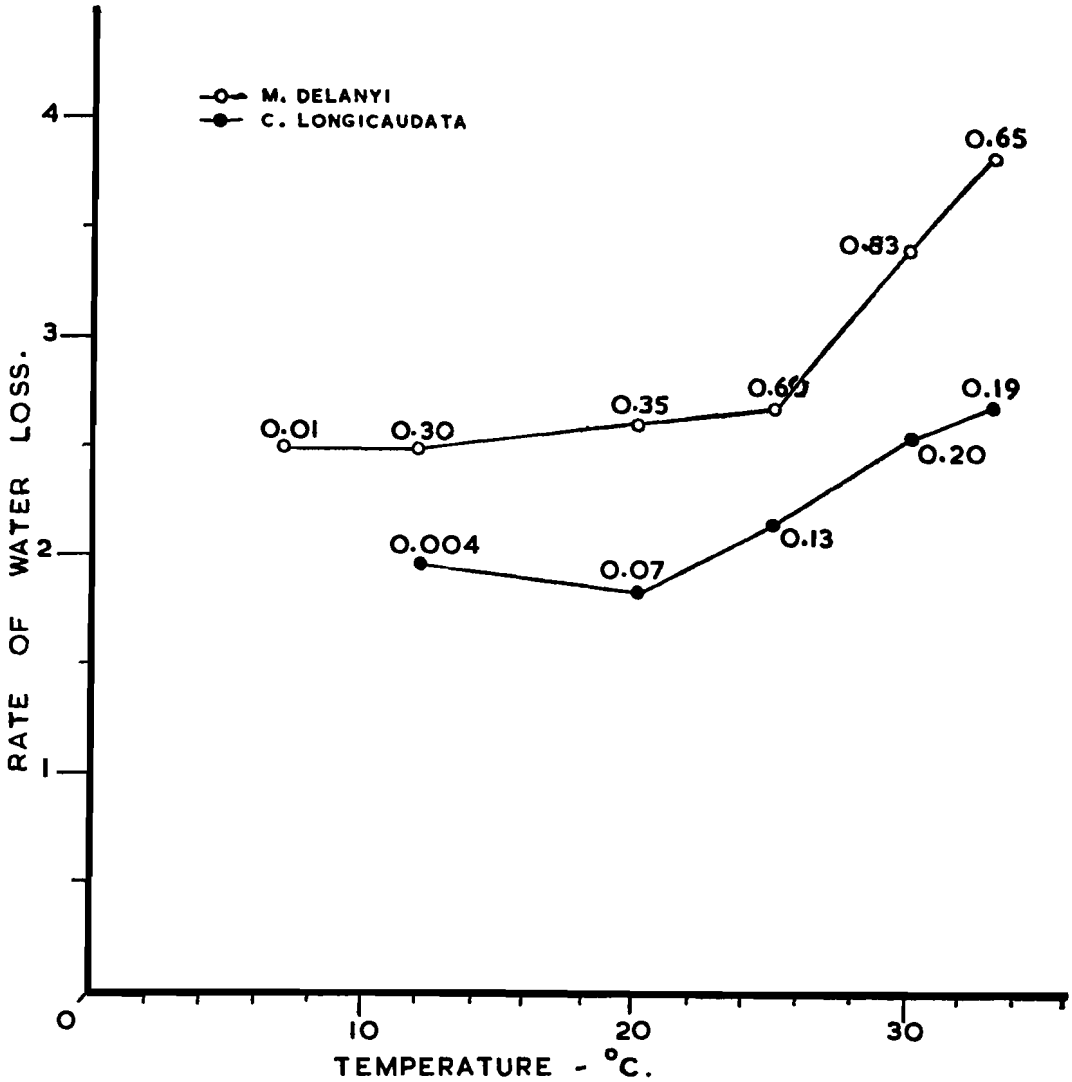

Figure 6. The effect of temperature on the rate of water loss (% of body weight/24 hr.) at a near constant saturation deficit. Saturation deficit = 7.16 — 7.96 mm Hg for *M. delanyi* and 10.52 — 10.33 mm Hg for *C. longicaudata*. Standard errors are shown for each of the co-ordinates.

Although no spiracular closing mechanism has been found in the Thysanura (Oudemans 1888, Snodgrass 1927, 1931), the possibility of an indirect method of closure cannot be overlooked. Since carbon dioxide in concentrations as low as 2% and 5% will cause permanent opening of the spiracles of *Xenopsylla* (Wigglesworth 1935) and *Rhodnius* (Wigglesworth and Gillett 1936) respectively, the effect of this gas upon the rate of water loss was investigated. In this experiment the animals were desiccated in an air flow desiccator, the rate of water loss

being determined before and after the addition of 10% CO_2 to the dry air stream. The apparatus was housed in a constant temperature room at 20C and the air stream passed through a heat exchanger to bring it to room temperature before being led into the apparatus. The rate of air flow was kept constant throughout the experiment.

The results, which are set out in Table 4 show that CO_2 has no effect upon the rate of water loss. These results cannot be regarded as fully critical evidence that no spiracular closing mechanism exists, since such a mechanism might not be CO_2 sensitive; but taken together with the anatomical evidence, they make such a mechanism seem unlikely. This would suggest that water retention is effected, at least in part, by some active integumental process rather than spiracular occlusion.

TABLE 4

MEAN RATES OF WATER LOSS (% OF BODY WEIGHT/24 HR.) FROM *M. delanyi* AND *C. longicaudata* IN MOVING DRY AIR, BEFORE AND AFTER THE ADMIXTURE OF 10% CARBON DIOXIDE.

Species	*No. of animals used*	*Rate of water loss in dry air stream*	
		without CO_2	*With CO_2 added*
M. delanyi	8	8·56 ± 0·93	8·33 ± 0·67
C. longicaudata	8	4·30 ± 0·27	4·27 ± 0·30

Carbon *mon*oxide anaesthesia causes an increase in the rate of water loss comparable with that brought about by death of the animals. This effect may persist some days after the animals have otherwise recovered from the effect of the anaesthetic. This is shown for *C. longicaudata* in Table 5. *M. delanyi* succumbs very rapidly to CO poisoning, therefore the rate of only two specimens under CO anaesthesia was measured. Of these one showed a fourfold increase in the rate of water loss over a period of 12 hours after apparent recovery. The second showed a sevenfold increase, which is greater than the increase brought about by death of the animal and must therefore be regarded as incorrect. Both these animals died within 12 hours of anaesthesia. These results lead to the tentative suggestion that the cytochrome system of the hypodermis is affected by the CO and that, as a result, an energy-dependent water retaining or -resorbing mechanism in the hypodermis is inactivated. In this connection it is interesting to note that Browning (1954) found that very high concentrations of CO_2, in the range of 30–45%, caused a sharp increase in the rate of water loss from living unfed nymphs of the tick *Ornithodorus moubata*. This was interpreted by Browning as due to a direct action of CO_2 on an active water retaining mechanism in the hypodermal cells. It may be regarded as a parallel case to the effect of CO upon *M. delanyi* and *C. longicaudata*, although the effect of CO, unlike that of CO_2, is persistent.

The present results conflict in several particulars with the observations made by Beament *et al* (1964) upon transpiration through the cuticle of *Thermobia domestica*. In considering these differences, it is important to realise that while the present experiments were undertaken

TABLE 5

EFFECT OF CO ANAESTHESIA ON THE RATE OF WATER LOSS (% OF BODY WEIGHT/24 HR.) FROM LIVING *C. longicaudata* COMPARED WITH THAT FROM DEAD AND FROM LIVING UNANAESTHETISED SPECIMENS.

Rate of water loss from CO anaesthetised specimens			*Rate of water loss from dead specimens*	*Rate of water loss from living specimens*
Period after apparent recovery				
0–24 hr	24–48 hr.	48–72 hr.		
$10{\cdot}7 \pm 0{\cdot}36$	$8{\cdot}3 \pm 0{\cdot}27$	$7{\cdot}0 \pm 0{\cdot}28$	$12 \pm 0{\cdot}09$	$5 \pm 0{\cdot}24$

with living animals, the specimens of *Thermobia* studied by Beament *et al* (1964) had been killed by exposure to H_2S before their rates of water loss were determined.

The first difference lies in the effect of temperature upon the rate of water loss. In *Thermobia* a sharp transition temperature at about 28C is found, the rate of water loss increasing about fivefold between 28C and 31C. In the present experiments, the results shown in Fig. 5 would suggest the possible presence of a transition point at about 20C for *C. longicaudata* and at 26C for *M. delanyi*. But the effect is very slight and it was earlier suggested that the lack of any striking change in the rate of water loss with temperature spoke against the presence of any epicuticular layer of fat or grease. If, however, there is an active process of water retention arising from the activity of the hypodermal cells, it is to be expected that the efficiency of any "water pumps" will also increase with temperature and as a result the presence of a transition point will be partly or wholely masked in a living animal.

The second difference lies in the results obtained with abrasives. While Beament *et al* (1964) found that abrasion caused an immediate increase in cuticular permeability, no comparable increase in the rate of water loss has been obtained in the present experiments. Beament *et al* (1964) too, were unable to demonstrate an increase in water loss from living *Thermobia* of which the mesonotum had been abraded, but this involved only a small fraction of the animal's surface. The present result led to the view tentatively expressed above that there is no outer layer of wax or grease. It seems possible that the difference in the experimental findings may reflect differences in technique of abrasion. If this is correct, then they are not in conflict, for Beament *et al* (1964) concluded, upon the basis of extraction experiments and studies on the wetting properties of the cuticle that the epicuticle of *Thermobia* is built up of a stiff, epicuticular grease overlain by a resistant and imperforate lamella. It is possible that such a cement layer is common to at least the Lepismatida among the Thysanura. Lawrence (1959) reports that a lepismatid species in the Namib Desert of South West Africa" . . . wriggles or almost swims with fish-like movements through the sand." and *Hyperlepisma australis* Wygodzinsky (possibly the same species as that referred to by Lawrence) is described by its collector as "*fouisseur dans dune*" (Wygodzinsky 1959). These records of lepismatids which burrow in loose desert sand suggest a lipid epicuticle which, if present, is not readily damaged by abrasion. It is therefore suggested that, in the absence of adequate histological control, the abrasion

methods used in the present experiments may not have disrupted this outer cement layer.

The third difference lies in the findings concerning the relative rates of water loss at different stages within an instar. Beament *et al* (1964) find that the newly moulted firebrat has a lower rate of water loss than have specimens older within an instar. This result is to be expected, since it seems probable that the newly formed and perfect epicuticular layer will offer a better barrier to water loss than an older and slightly damaged epicuticle. In the present experiments, however, the opposite effect has been observed. The rate of water loss was found to be higher from specimens at the third day of an instar than those from the tenth day. The explanation of this difference is not apparent. It may be tentatively suggested that the postulated hypodermal pumps do not become fully efficient until some time after a moult; in other words, that the process of secretion of a new cuticle by the hypodermal cells interferes with their active water-retaining role, but it is clear that this point requires further and more detailed examination before it can be satisfactorily resolved.

WATER UPTAKE

However well developed the water conserving mechanisms of an animal may be, transpiration still takes place and to survive it is therefore necessary, from time to time, to replenish water lost in this way. Water taken in with food can adequately supply the needs of many arthropods, but those feeding on comparatively dry foods must obtain additional water to replenish their internal water store. *C. longicaudata* certainly falls into this category, and, whilst *M. delanyi* may gain some moisture from the plant material on which it feeds, it seems unlikely that this would be adequate for its needs.

Water, other than that taken in with food, can be acquired in a number of ways. Oral drinking commonly occurs among arthropods, but this requires the presence of free water. A surface film of water on a stone, or capillary water in the soil cannot normally be taken in without special structural modifications, but such uptake has been shown to occur. The spiders *Tarantula barbipes* and *Lycosa radiata* are able to suck up capillary water from a bed of fine graphite particles (Parry 1953) and various terrestrial Isopoda take in capillary water by way of both mouth and anus (Spencer & Edney 1954). Absorption of water through the cuticle is also of common occurrence among terrestrial arthropods; here eversible vesicles, located on the abdomen or on the coxae of abdominal appendages, are commonly important sites of water uptake. Among the Hexapoda, the "collophore" or ventral tube of the Collembola has been shown to be the main water absorbing organ (Nutman 1941, Noble-Nesbitt 1963) and the eversible vesicles of *Campodea* (Diplura) serve a similar function (Drummond 1956). Eversible vesicles are also known to be concerned with water uptake in other arthropods (Tiegs 1947, Alexander & Ewer 1955, Manton 1958) and have been shown to be capable of extracting capillary water (Tiegs 1947, Alexander & Ewer 1955). These organs would also be capable of absorbing water from a surface film. Water vapour provides another source for replenishment (Mellanby 1932, Lees 1946, Edney 1947, Browning 1954).

In order to investigate which means of gaining water are available to the two species of Thysanura under consideration, and under what conditions they are used, the experimental animals were first desiccated over calcium chloride in the normal manner. *M. delanyi* was subjected to between 24 and 48 hours desiccation, while *C. longicaudata* was desiccated for

as long as 120 hours to give an approximately comparable water loss. The desiccated animals were divided into three batches, each of which was given water in one of the following ways:

(i) Free water; water droplets scattered over stones in a plastic dish.

(ii) Water film; stones which had been wetted by immersion in water and then shaken to remove all but a thin film of water covering each stone.

(iii) Capillary water; an unglazed china plate which had been soaked in water.

After 12 hours access to these respective water sources, the animals were subjected to a further three hours desiccation over calcium chloride to remove the adsorbed moisture and then weighed again.

Both species gained weight under all experimental conditions as shown in Table 6.

TABLE 6

MEAN WATER GAIN FROM THREE DIFFERENT WATER SOURCES BY *M. delanyi* AND *C. longicaudata*. WATER LOST DURING DESICCATION AND GAINED FROM WATER SOURCE ARE EXPRESSED AS A PERCENTAGE OF INITIAL BODY WEIGHT.

Species and water source	*No. of animals used*	*Mean water loss*	*Mean water gain*	*Percentage of water lost recovered*
M. delanyi				
Free water	6	9·5	14·8	156%
Water film	7	20·1	15·9	79%
Capillary water	7	13·5	10·9	81%
C. longicaudata				
Free water	6	18·1	18·6	103%
Water film	5	17·6	11·0	63%
Capillary water	6	19·4	16·2	84%

The experiments were repeated using a weak aqueous solution of Light Green instead of water. After each experiment had been completed, the animals were killed by means of ethyl acetate vapour and dissected in order to ascertain the distribution of the dye within each animal. The results are set out in Table 7 and show the modes of water uptake to be different in the two species. Whilst *M. delanyi* uses its vesicles to obtain water not readily available for drinking, *C. longicaudata*, which has no such structures, showed no uptake of the dye at all. The weight gain in the experiments was comparable with those shown in Table 5.

Both species are, therefore, able to drink water, and do so readily when water is available in drinkable form. *M. delanyi*, however, uses its vesicles for the uptake of water from sources where drinking is not possible. *C. longicaudata*, under these conditions, shows no visible evidence of water uptake through any part of its cuticle, although it must gain water in some manner. The only way in which water could enter the animals is in the form of water vapour.

Lindsay (1940) claims that *C. longicaudata* is able to absorb water from an atmosphere at 99% relative humidity, but experiments carried out so near saturation are open to the objection that slight temperature changes can bring about condensation and thus make water available

TABLE 7

DISTRIBUTION OF DYE (LIGHT GREEN) IN *M. delanyi* AND *C. longicaudata* AFTER WATER UPTAKE FROM THREE DIFFERENT WATER SOURCES.

Water Source	*M. delanyi*			*C. longicaudata*	
	No. of animals used	*No. with dye*		*No. of animals used*	*No. with dye in gut*
		in gut	*in vesicles*		
Free water	8	8	5	7	7
Water film	6	1	6	6	0
Capillary water	8	0	8	7	0

for oral drinking. This question was reinvestigated. The possibility of *M. delanyi* sharing this ability could not be rejected merely on the grounds of the presence of an alternative water absorbing mechanism, so this species was also studied.

The experimental animals were desiccated as previously described and then exposed to various relative humidities, at a constant temperature of 20C, for periods of 24 hours. They were then desiccated again for a period of three hours before weighing. The animals were subsequently exposed to the experimental humidity for a further 24 hours to ascertain whether any further gain in weight would occur.

TABLE 8

WATER GAIN FROM SATURATED AND SUBSATURATED ATMOSPHERES BY *M. delanyi* AND *C. longicaudata*. WATER LOSS AND WATER GAIN ARE EXPRESSED AS A PERCENTAGE OF THE ORIGINAL BODY WEIGHT.

Species and relative humidity	*No. of animals used*	*Mean water loss*	*Mean water gain after*		*Percentage of water lost recovered*
			24 hr.	48 hr.	
M. delanyi					
100%	7	26·4	−0·6	—	0
98%	8	27·2	−1·3	—	0
C. longicaudata					
100%	6	34	+25	+25	75%
98%	8	27·3	+20	+21	78%
90%	7	26·0	+18	+21	78%
75%	6	17·8	+16	+17	93%
60%	7	23·1	+ 6	+ 6	27%
60%	3*	21·3	+15	+17	80%

*Includes only those animals from the preceding batch which were able to absorb water at 60% relative humidity.

While *M. delanyi* proved incapable of absorbing water from even a saturated atmosphere, *C. longicaudata* was found to be able to replenish its water store in this way from a relative humidity as low as 60%. The results are shown in Table 8. It is not clear why Lindsay (1940) claims the limit of this ability to be at 99% relative humidity, since nowhere in the text of her paper does she mention having conducted experiments at any lower humidity.

That the ability to take up water from the atmosphere gives *C. longicaudata* a greater freedom of movement in the terrestrial environment can be demonstrated in a choice chamber, using fused granular anhydrous calcium chloride and distilled water as humidity controlling agents. Normal and desiccated specimens of both species were introduced into choice chambers and left undisturbed over a long period; their distribution was noted at regular intervals. Figures 7 and 8 show the reaction intensities towards the dry side of the chamber for *M. delanyi* and *C. longicaudata* respectively. Undesiccated specimens of *M. delanyi* initially showed a high reaction intensity towards the dry side of the chamber, which is their normal response under these conditions (Heeg 1967). However, as the animals lost water, this reaction was reversed, and the reaction intensity towards the dry side became negative. Normal

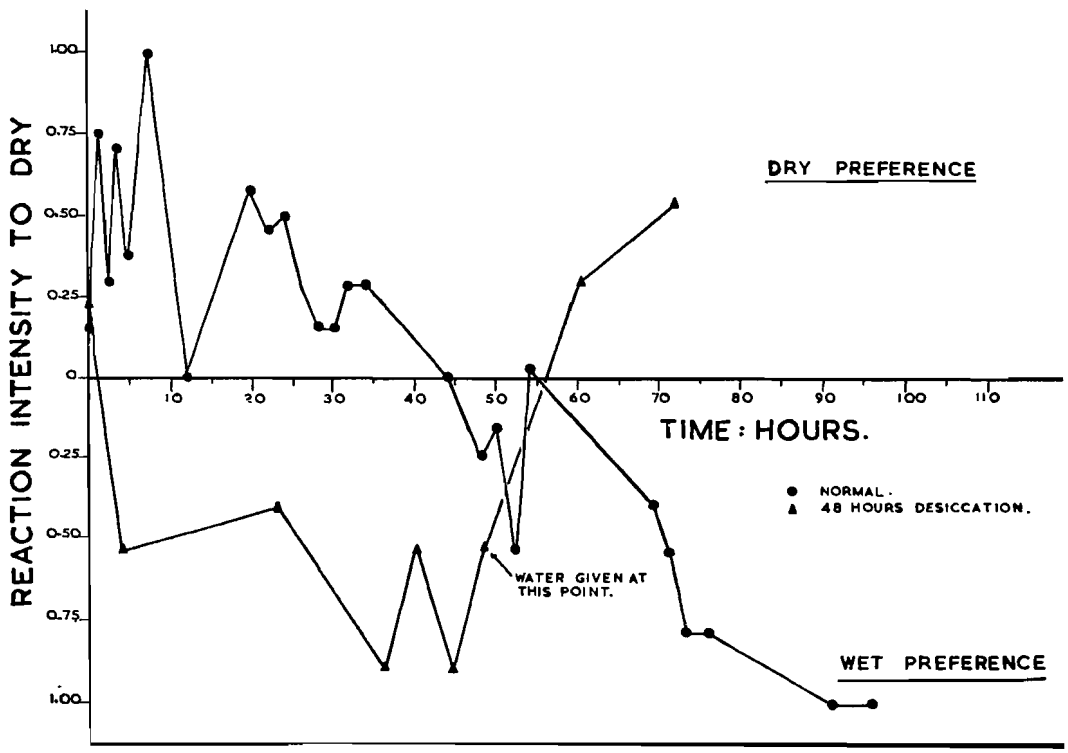

Figure 7. Reaction intensity of normal and desiccated *M. delanyi* to the dry side, as it varied with time, during a prolonged stay in a wet/dry alternative choice chamber.

$$\text{Reaction Intensity} = \frac{\text{No. of animals in dry} - \text{No. in wet}}{\text{Total no. of animals used.}}$$

C. longicaudata, on the other hand, showed a random distribution throughout, since the presence of water vapour in the wet side ensured that the internal water content never falls to a level where a reversal is elicited. Desiccation of the experimental animals prior to their introduction into the choice chamber confirms these findings. Both species showed an initial wet response, which is normal for animals with a depleted internal water store (Heeg 1967) but whilst the distribution of *C. longicaudata* soon became random, *M. delanyi* remained in the wet side of the choice chamber until such time as liquid water was made available, after which its normal dry response was re-established.

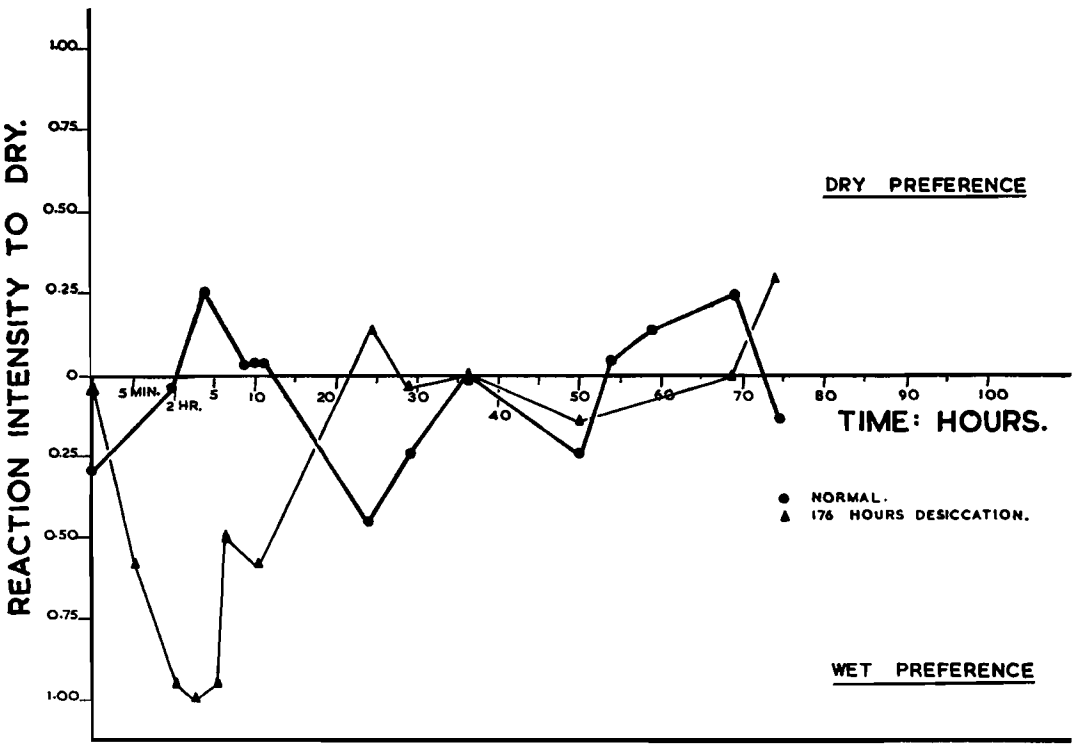

Figure 8. Reaction intensity of normal and desiccated *C. longicaudata* to the dry side, as it varied with time, during a prolonged stay in a wet/dry alternative choice chamber.

DISCUSSION

M. delanyi typically inhabits a microhabitat where conditions of low saturation deficit prevail (Heeg in preparation). The present investigation shows that whilst *M. delanyi* loses water at a very variable rate, it is able to withstand, for comparatively long periods of time, considerably more severe conditions of desiccation than those encountered in its typical microhabitat. This unexpected resistance to desiccation has not been reported for any other machilid species, although Kühnelt (1961) describes all machilids as " . . . extraordinarily resistant to drying." Preliminary experiments conducted on other species of *Machiloides* showed these to differ but little, if at all, from *M. delanyi*. This resistance to desiccation enables *M. delanyi*, and other

machilid species, to survive under humidity conditions far harsher than those prevailing in the humus/litter complex of forest floors. The amphipod *Talitrioides eastwoodae*, a typical inhabitant of this horizon in South African indigenous forests, survives desiccation over calcium chloride for a maximum of 110 minutes. *Orthomorpha gracillis*, an introduced diplopod also commonly found in this habitat in the Grahamstown area, is somewhat more resistant, surviving up to 12 hours (Perttunen 1953, Brunhuber 1964). Brunhuber (1964) has also shown that water retention in this species is not an active process, and that the rate of water loss is approximately the same as that for dead specimens of *M. delanyi*. The active water retaining mechanism of *M. delanyi*, and, presumably, other Machilida, may therefore well be the main factor which allows them to survive outside the shelter of the forest floor.

C. longicaudata is much better able to withstand extreme conditions of desiccation, and this can be attributed to further improvement in the waterproofing mechanism. Physical barriers to evaporation in the cuticle seem to contribute largely to this decreased permeability, since the mean rate of water loss from dead individuals of this species is 15% of body weight per 24 hr, compared with 50% from dead *M. delanyi*. However, it is not water retention alone that is responsible for the success of *C. longicaudata* as a cosmopolitan household pest; the ability to replenish a depleted internal water store from a subsaturated atmosphere is of considerably greater importance, since it enables the species to survive in habitats where water in its liquid state is seldom, if ever, encountered.

The mechanism by means of which active uptake of water vapour is effected has been suggested by Lees (1947, 1948) to be a metabolically driven pump in the hypodermis, which effects, in some way, an inward secretion of water molecules against a concentration gradient. Active water retention against a concentration gradient would require a similar pump and it seems not improbable that the mechanism in both cases is the same. The difference between the performances of the pumps of *M. delanyi* and *C. longicaudata* would then be attributable to either actual differences in their respective efficiencies or to differences in cuticular properties. In the present investigation, differences in cuticular properties have been demonstrated in terms of general permeability, but in the absence of information regarding possible asymmetrical permeabilities and hygroscopic properties no definite conclusions can be drawn.

Whatever its mechanism, active water retention is expensive in terms of energy, and a reduction in energy expenditure can be obtained by improving physical barriers to evaporation. Sufficiently high structural impermeability, together with spiracular occlusion and a locomotory efficiency which permits easier access to water allow for the dispensing with the pump altogether. These latter requirements are all met by the Pterygota, but even here the phenomenon of active uptake of water vapour is still encountered in some instances (Lees 1947, Edney 1947, Beament 1954).

SUMMARY

1. *Machiloides delanyi* shows a considerable advance over typical members of the forest cryptofauna in its ability to retain water. This is largely due to an active water retaining mechanism.

2. *Ctenolepisma longicaudata* is more resistant to desiccation than *M. delanyi.* Although it, too, depends on an active water retaining mechanism, its advance over *M. delanyi* seems to be largely due to a less permeable cuticle.
3. *M. delanyi* is able to replenish a depleted internal water store from water sources which cannot be drunk, such as thin water films on stones or soil capillary water. It absorbs this water by way of eversible vesicles situated on the abdominal coxosternites. *C. longicaudata* lacks these structures, but is able to actively take up water from a sub-saturated atmosphere, an ability not shared by *M. delanyi.* Independence from water in its liquid state gives *C. longicaudata* greater freedom of movement, and consequently a wider range than that of *M. delanyi.*

ACKNOWLEDGEMENTS

This work was initiated while I was in receipt of a Research Studentship awarded to me by Messrs. African Explosives and Chemical Industries. Prof. D. W. Ewer supervised the project and has also criticised the manuscript; his interest and enthusiasm have been a constant stimulus for which I am extremely grateful.

REFERENCES

ALEXANDER, A. J. and EWER, D. W. 1955. A note on the function of the eversible sacs of the Onychophoran *Opisthopatus cinctipes* Purcell. *Ann. Natal Mus.* 13: 217–222.

BARNHART, C. S. 1961. The internal anatomy of the Silverfish *Ctenolepisma campbelli* and *Lepisma saccharinum* (Thysanura, Lepismatidae). *Ann. ent. Soc. Amer.* 54: 177–196.

BEAMENT, J. W. L. 1954. Water transport in insects. *Symp. Soc. exp. Biol.* 8: 94–117.

BEAMENT, J. W. L. 1959. The waterproofing mechanism of Arthropods. I. The effect of temperature on cuticle permeability in terrestrial insects and ticks. *J. exp. Biol.* 36: 391–422.

BEAMENT, J. W. L., NOBLE-NESBITT, J. and WATSON, J. A. L. 1964. The waterproofing mechanism of arthropods. III. Cuticular permeability in the firebrat *Thermobia domestica* (Packard). *J. exp. Biol.* 41: 323–330.

BROWNING, T. O. 1954. Water balance in the tick *Ornithodorus moubata* Murray, with particular reference to the influence of carbon dioxide on the uptake and loss of water. *J. exp. Biol.* 31: 331–340.

BRUNHUBER, B. S. 1964. Unpublished observations.

DRUMMOND, F. H. 1956. The eversible vesicles of *Campodea. Proc. R. ent. Soc. Lond.* (A) 28: 145–148.

EDNEY, E. B. 1947. Laboratory studies on the bionomics of the rat fleas *Xenopsylla brasiliensis* Baker and *X. cheopis* Roths. II. Water regulation during the cocoon period. *Bull. ent. Res.* 38: 263–280.

HEEG, J. 1967. Studies on Thysanura II. Orientation reactions of *Machiloides delanyi* Wygodzinsky and *Ctenolepisma longicaudata* Escherich to temperature, light and atmospheric humidity. *Zool. afr.* 3: 43–57.

HEEG, J. (in preparation). Studies on Thysanura, III. Factors governing the distribution of South African Thysanura.

KÜHNELT, W. 1961. *Soil Biology.* Faber, London.

LAWRENCE, R. F. 1953. *The Biology of the Cryptic Fauna of Forests*. Balkema, Cape Town.

LAWRENCE, R. F. 1954. The role of forests in the evolution of terrestrial life. *J. ent. Soc. S. Afr.* 17: 3–8.

LAWRENCE, R. F. 1959. The sand-dune fauna of the Namib Desert. *S. Afr. J. Sci.* 55: 233–239.

LEES, A. D. 1946. The water balance in *Ixodes ricinus* L. and certain other species of ticks. *Parasitology* 37: 1–20.

LEES, A. D. 1947. Transpiration and the structure of the epicuticle in ticks. *J. exp. Biol.* 23: 379–410.

LINDSAY, E. 1940. The biology of the Silverfish, *Ctenolepisma longicaudata*, with particular reference to its feeding habits. *Proc. roy. Soc. Victoria* 52: 35–83.

LOWER, H. F. 1958. The integument of two Apterygota—*Ctenolepisma longicaudata* Esch. (Thysanura, Lepismatidae) and *Smynthurus viridis* Lubbock (Collembola, Smynthuridae). *J. Morph.* 103: 331–352.

MANTON, S. M. 1958. The evolution of arthropodan locomotory patterns. Part 6—Habits and evolution of the Lysiopetaloidea (Diplopoda), some principles of leg design in the Diplopoda and Chilopoda, and limb structure of the Diplopoda. *J. Linn. Soc. (Zool.)* 43:487–556.

MANTON, S. M. 1964. Mandibular mechanisms and the evolution of Arthropods. *Philos. Trans.* 247: 1–183.

MELLANBY, K. 1932. The effects of atmospheric humidity on metabolism of the fasting mealworm (*Tenebrio molitor* L. Coleoptera). *Proc. roy. Soc.* (B) 111: 376–390.

NOBLE-NESBITT, J. 1963. A site of water and ionic exchange with the medium in *Podura aquatica* L. (Collembola, Isotomidae). *J. exp. Biol.* 40: 701–712.

NUTMAN, S. R. 1941. The function of the ventral tube in *Onychiurus armata* (Collembola). *Nature, Lond.* 148: 168–169.

OUDEMANS, J. T. 1888. Beitrage zur Kenntnis der Thysanura und Collembola. *Bijdr. Dierk.* 1888: 147–226.

PARRY, D. A. 1953. On the drinking of soil capillary water by spiders. *J. exp. Biol.* 31: 218–227.

PERTTUNEN, V. 1953. The reactions of diplopods to the relative humidity of the air. Investigations on *Orthomorpha gracillis, Iulus terrestris* and *Schizophyllum sabulosum*. *Ann. Zool. Soc. "Vanamo"* 16: 1–69.

RAMSAY, J. A. 1935. Methods of measuring the evaporation of water from animals. *J. exp. Biol.* 12: 355–372.

SNODGRASS, R. E. 1927. Morphology and mechanism of the insect thorax. *Smithson. misc. Coll.* 80 (1): 1–108.

SNODGRASS, R. E. 1931. Morphology of the insect abdomen. Part 1. General structure of the abdomen and its appendages. *Smithson. misc. Coll.* 85 (6): 1–128.

SNODGRASS, R. E. 1938. The evolution of the Annelida, Onychophora and Arthropoda. *Smithson. misc. Coll.* 97 (6): 1–159.

SPENCER, J. O. and EDNEY, E. B. 1954. The absorption of water by woodlice. *J. exp. Biol.* 31: 491–496.

SWEETMAN, H. L. 1938. Physical ecology of the firebrat *Thermobia domestica* (Packard). *Ecol. Monogr.* 8: 285–311.

TIEGS, O. W. 1947. The development and affinities of the Pauropoda based on a study of *Pauropus silvaticus*. *Quart. J. micr. Sci.* 88: 275–336.

WIGGLESWORTH, V. B. 1935. Regulation of respiration in the flea *Xenopsylla cheopis* Roths. *Proc. roy. Soc.* (B) 118: 397–419.

WIGGLESWORTH, V. B. 1945. Transpiration through the cuticle of insects. *J. exp. Biol.* 21: 97–114.

WIGGLESWORTH, V. B. and GILLETT, J. D. 1936. The loss of water during ecdysis in *Rhodnius prolixus* Stal. *Proc. R. ent. Soc. Lond.* (A) 11: 104–107.

WIGGLESWORTH, V. B., HINTON, H. E., JOHNSON, C. G. and LESTON, D. 1963. The origin of flight in insects. *Proc. R. ent. Soc. Lond.* (C) 28: 23–32.

WILLEM, V. 1924. Observations sur *Machilis maritima*. I° note. *Bull. biol. France Belg.* 58: 306–320.

WYGODZINSKY, P. 1959. Contribution to the knowledge of the "Thysanura" and "Machilida" (Insecta). *Rev. Brasil. Biol.* 19: 441–457.