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WATER VAPOUR ABSORPTION IN TERRESTRIAL ISOPODS

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Summary

Continuous and intermittent gravimetric measurements have identified active water vapour absorption (WVA) in three species of terrestrial Isopoda. Water activity thresholds for uptake lie in the range 0.92–0.95. Above the threshold, WVA shows non-saturated kinetics; the rectum apparently serves as a supplementary avenue for fluid resorption during rapid uptake. Standardized uptake fluxes, corrected for vapour pressure deficit, can be varied, allowing animals to balance water losses accurately over long periods.

Blocking experiments have localised the ventral pleon as the uptake site. The pleopods display ventilatory cycling during WVA. Cycle frequency increases with humidity, compensating for changes in activity deficit between uptake fluid and air, and allowing uptake rate to be maximised. Freezing-point depression studies reveal hyperosmotic fluid in the ventral pleon. Osmolalities are compatible with prior uptake rates of the specimens studied.

WVA would allow terrestrial isopods to regulate their water balance in external activities below the haemolymph activity (approximately 0.99) and above the uptake threshold. Liquid water – an alternative source for hydration – is rapidly absorbed across the hydrophilic cuticle, posing severe danger of drowning.

Introduction

Active water vapour absorption or WVA (Mellanby, 1932; O'Donnell and Machin, 1988) is a widespread phenomenon in terrestrial insects and acarines but remains to be demonstrated in the other invertebrate taxa showing major terrestrial radiations: remaining arachnid orders, Myriapoda, Onychophora and Gastropoda. In Crustacea, it has only been suggested for the desert isopod *Hemilepistus afghanicus* (Coenen-Stass, 1981). WVA provides an important adaptive means of colonizing habitats of irregular or unpredictable water supply and/or the absence of liquid water. It also provides an important supplementary water source for species feeding on materials of low water content: dermophagous ectoparasites, and feeders on wood, grain and stored products. Familiar examples are Psocoptera and related Mallophaga (Rudolph, 1982*a*,*b*, 1983), dust mites (Arlian and Wharton, 1974), ticks (Rudolph and Knulle, 1974; Needham and Teel, 1986), tenebrionid larvae (Mellanby, 1932; Ramsay, 1964; Machin, 1976), lepisma-

Key words: water vapour absorption, Isopoda, Oniscidea, water activity, pleopods.

tid Thysanura (Noble-Nesbitt, 1970, 1975) and Blattodea (O'Donnell, 1977, 1982*a*,*b*).

Four major mechanisms of WVA have been postulated. All require that the water activity (a_w) of the air exceeds that of the absorbing fluid, permitting water to enter the animal along an osmotic gradient. Haemolymph activities for terrestrial animals lie in the range 0.985–0.995. For lower external activities, WVA requires the maintenance of a fluid compartment of correspondingly reduced activity, isolated from the haemolymph. Proposed mechanisms are: (i) to elevate the vapour pressure of air to the dew point, using compression within a sealed chamber, and thereby condense water onto a surface from which it can be absorbed or transported (Corbet, 1988); (ii) to alter the radius of curvature of a fluid surface by surface tension and hence depress its activity below that of the haemolymph, as proposed by Beament (1964, 1965); (iii) to effect changes in the water affinity of proteins by moving them away from the iso-electric point *via* alterations in ionic strength or voltage [strong supportive evidence for such a mechanism has been demonstrated for the desert cockroach *Arenivaga* (O'Donnell, 1982*a*,*b*)]; (iv) to utilise an uptake fluid of elevated osmolality and correspondingly reduced activity.

The first mechanism was originally advanced by Maddrell (1971) to explain the uptake mechanism in *Thermobia* but lacks supporting experimental evidence. It has since been proposed as a widespread means of active vapour exchange in the insect tracheal system and in other terrestrial organisms (Corbet, 1988). Production of a hyperosmotic uptake fluid has been demonstrated in several species and appears to be the most frequently employed mechanism. The activity of the absorbing fluid determines the minimum humidity at which vapour uptake is possible and this constitutes the uptake threshold (Machin, 1979). Since absorption rate is proportional to the activity gradient between air and absorbing fluid, uptake rates will increase with humidity above the threshold. To achieve net uptake, absorption rate must at least balance water losses incurred from transpiration and active processes (respiration ventilation, excretion, salivation); this is satisfied at the critical equilibrium humidity or CEH (Knulle and Wharton, 1964), alternatively expressed as critical equilibrium activity or CEA (Wharton and Devine, 1968), which typically will be just above the threshold activity of the absorption mechanism.

Use of hyperosmotic uptake fluids poses the problems of generating elevated osmotic pressures, transporting fluids to the absorbing surface without dilution, and fluid resorption following vapour uptake. Limited physiological tolerance of elevated solute concentrations restricts the choice of osmolites to K^+, Na^+, Cl^-, some amino acids, and possibly other organic molecules. Saturation concentrations of NaCl and KCl set lower limits to the activities of uptake fluids based on these solutes.

Terrestrial Isopoda and WVA

The terrestrial isopods (Crustacea, Isopoda, Oniscidea) are the most successful

terrestrial crustaceans, whether judged by species numbers, numerical abundance or ecophysiological adaptation and ecological diversity. Much of their success must be attributed to osmoregulatory and water balance control. The extensive studies of Edney (1951, 1954, 1964*a*,*b*, 1968) concentrate on these aspects. Although acknowledging physiological adaptation, Edney and subsequent authors have come to stress the great significance of behavioural adaptation in the radiation of the Oniscidea. The cutaneous permeability barriers of isopods are considered inferior to the epicuticular waxes of insects and arachnids and desiccation is exacerbated by the need to maintain moist respiratory surfaces (Edney and Spencer, 1955; Lindqvist, 1971, 1972; Hoese, 1981) and retention of ammoniotely as the major means of nitrogenous excretion (Dresel and Moyle, 1950; Hartenstein, 1968; Weiser *et al.* 1969; Weiser and Schweitzer, 1970). Although improved experimental techniques have shown cutaneous transpiration in oniscideans to be an order of magnitude lower than previously thought (J. C. Wright and J. Machin, unpublished data) and respiratory and excretory water losses to be surprisingly low (Hadley and Quinlan, 1984; J. C. Wright, personal observations), overall losses are still higher than for typical insects or arachnids and Edney's major thesis is of undoubted significance.

It was probably the relatively high water losses sustained by terrestrial oniscideans that led Edney (1954) and Spencer and Edney (1954) to investigate the possibility of vapour uptake. Using aqueous salt solutions to control humidity at predetermined values, they concluded, from intermittent weighing experiments, that isopods continue to lose water in humidities below 98 %. They discounted the existence of active vapour uptake. The subject was resurrected by Coenen-Stass (1981), who showed that the desert species *Hemilepistus afghanicus* attained equilibrium mass in 93 % relative humidity (RH) and gained mass in 98 %. WVA would appear to be involved here, although discussion is restricted to mechanisms of vapour condensation in saturated air. Subsequently, the same author has shown that three xeric isopod species attain equilibrium between vapour absorption and net transpiration in 97 % RH (Coenen-Stass, 1989). The author proposes the pleon as a possible uptake site but does not distinguish between active and passive mechanisms. Discontinuous uptake rates in near-saturated humidities are attributed to hydration-dependent changes in cuticle permeability rather than to the intervention of an active uptake process. Clearly, such changes could not account for net uptake when external water activity falls below that of the haemolymph.

Improved techniques for continuous monitoring of transpiration in living animals, in precisely controlled temperature and humidity regimes, led the present authors to re-investigate these conflicting conclusions. The validity of results drawn from intermittent weighing experiments may sometimes be questionable since the long equilibration times of aqueous vapour–liquid systems can result in substantial errors if a humidity chamber is regularly perturbed for specimen weighing. Even if inter-weighing intervals greatly exceed equilibration times for a chamber, WVA may not be detectable if uptake rates are insufficient to balance losses associated with weighing and re-equilibration.

Materials and methods

Isopods were collected locally and maintained in culture with deciduous litter and rotting wood. Since humidity in the litter fluctuated around 99%, it was assumed that experimental animals removed from this environment were well hydrated. Three common oniscideans were studied: *Armadillidium vulgare* Latreille (xeric–mesic), *Porcellio scaber* Latreille (mesic) and *Oniscus asellus* L. (mesic–hygric). Studies fall into four categories.

Continuous gravimetric monitoring

Individual animals were allowed to crawl freely in cylindrical, pre-weighed cages (25 mm × 10 mm) constructed from 2 mm mesh aluminium gauze. These were weighed in moving air (800 $cm^3 min^{-1}$) on a Sartorius 4410 digital microbalance (Sartorius GMBH, Gottingen) sensitive to 10 μg. Temperature and humidity in the balance chamber were accurately controlled with a Hewlett Packard 71B computer and 3421A data acquisition/control unit; details are given in Machin (1976). All experiments were conducted at 20.0°C. Following various periods of dehydration, isopods were exposed to a range of high humidities to study the relationship between transpiration and vapour pressure deficit, and the possible intervention of WVA. Mass losses were corrected for vapour pressure deficit by dividing by the sum of the vapour pressure of aqueous air at 20.0°C (2.334 kPa) and the activity deficit between the air and haemolymph. Haemolymph activity was calculated using the osmolality measurements of R. T. Barrett (unpublished report, Department of Zoology, University of Bristol) for *P. scaber* (701 mosmol kg^{-1}); this value was assumed for all three species.

Intermittent weighing in sealed relative humidity chambers

The use of saturated salt solutions in humidity control is discussed in detail by Winston and Bates (1960). Three humidities were used here: 98.0% (K_2SO_4), 93.5% (KNO_3) and 88.0% (K_2CrO_4). Laboratory temperature varied between 21.0 and 22.5°C during the course of experiments. Saturated aqueous solutions (0.5 l) were contained in 2.0 l Plexiglas chambers with lids which could be slid off horizontally to minimise simultaneous pressure changes. Above the level of the regulating solution, an inner shelf supported a 4 mm mesh steel grid. Single isopods were contained in 20 ml glass bottles with gauze lids to allow free gaseous exchange. These were stood, inverted, on the steel grid, bringing the animal to within 1.0 cm of the vapour–liquid interface. Faecal pellets fell through the gauze lids and thus contributed to mass changes, but gravimetric studies show that such losses would be small (<10%) compared to transpiratory losses incurred over a 24 h period. Control bottles, with or without lids, were included to quantify adsorption errors. The chambers were given 24 h of equilibration and bottles were weighed, without lids, every 24 h on a Mettler analytical balance; use of a mechanical balance precludes errors from the long-term drift suffered by

electromagnetic models. These experiments enable the role of WVA in long-term mass regulation in sub-saturated humidities to be assessed.

Location of the uptake site: blocking experiments

Identification of the vapour uptake site was studied by blocking likely surfaces/apertures with 2:1 beeswax:colophony following Krogh and Weis-Fogh (1951). Animals were anaesthetised with CO_2, and molten wax was applied to the areas with a fine soldering iron at the minimum temperature for effective melting (approx. 41°C). Controls were allowed to recover from anaesthesia without blocking. Three sites were investigated: mouth, rectum and the total pleopodal surface. Water loss/uptake in blocked animals was monitored using both intermittent and continuous weighing. Possible movements associated with uptake were studied with a ×20 binocular microscope during continuous monitoring.

Identification of uptake fluid: freezing-point depression studies

Controlled warming of frozen sections permits melting points of various body fluids to be differentiated; freezing-point depression (dt) can then be converted to osmolality using the standard formula $dt = 1.86\,°C \times$ osmolality (osmol kg^{-1}).

Moderately dehydrated (approx. 10 % mass loss) isopods were attached dorsally to a wire loop, using beeswax:colophony as before, and mass changes were monitored continuously. Following a sustained period of WVA, they were fast-frozen in liquid hexane at approx. −90°C, transferred to a cryostat at −20°C, and embedded in Tissue-Tek (Miles Inc., Indiana). Sections of 3–5 μm were cut and mounted in kerosene on pre-cooled glass slides. They were then warmed by increments from −12°C in a Mettler FP2 cooled stage during high-power (×160) observation.

Results

Continuous gravimetric monitoring

Hydrated isopods weighed during desiccation in high RH (>90 %) show normal patterns of water loss; when corrected for vapour pressure and surface area to give standardized flux (permeability) estimates, these are comparable to values calculated from low RH transpiration. Details of cutaneous transpiration and additional components of water loss have been discussed elsewhere (Coenen-Stass, 1981, 1989; Hadley and Quinlan, 1984).

Animals dehydrated to about 10 % mass loss modify this situation, regulating their water content (as estimated from the total mass) to near constancy over long periods when exposed to humidities above a certain level (CEA). A typical plot of net flux with time for *Armadillidium vulgare* is shown in Fig. 1, illustrating uptake and loss components in a range of external humidities. More severely dehydrated animals (>15 % mass loss) show a gain in mass above the CEA, the rate of vapour uptake increasing at higher activities. Below the CEA, but above the uptake threshold, animals often continue to employ WVA to reduce net losses. Occasion-

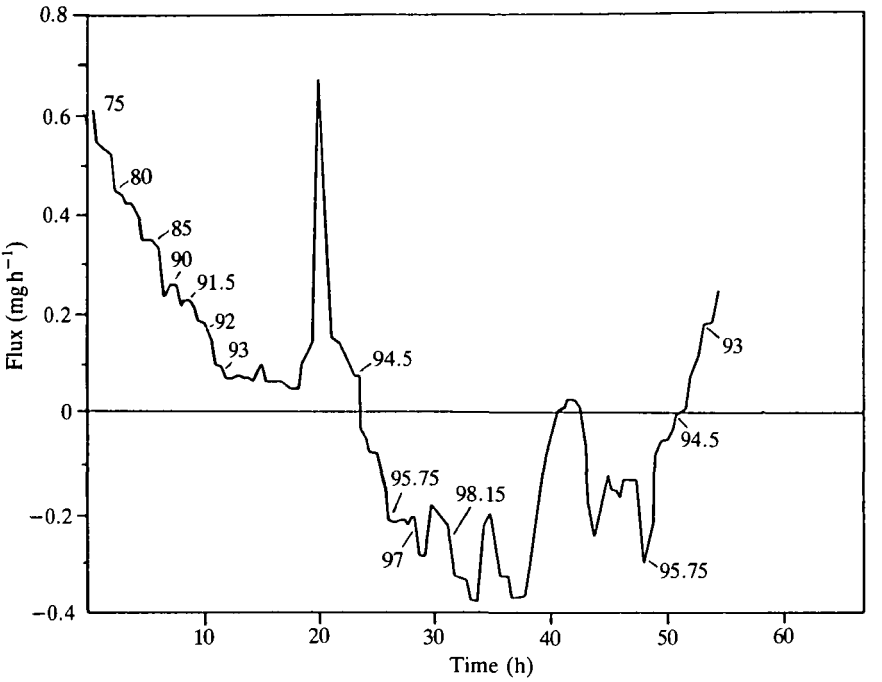

Fig. 1. Example of changes in net flux ($mg\,h^{-1}$) with time for a specimen of *Armadillidium vulgare* exposed to successive changes in relative humidity (RH). Brief periods of augmented flux are attributable to locomotion and/or excretion of maxillary fluid into the ventral capillary system. Negative fluxes (water vapour absorption: WVA) are observed in humidities exceeding 93 %. Changes in RH (%) are marked on the trace.

ally, animals dehydrated to more than 20 % mass loss failed to demonstrate WVA, even above 98 % RH, and became lethally desiccated. This may reflect an inability to synthesise an uptake fluid of sufficiently high osmolality under physiologically stressful conditions.

The relationship between WVA and external humidity (a_w) was studied for the three species by comparing uptake rates in a sequence of different humidities. Sequences were chosen randomly to avoid possible biases from the effects of rehydration on ensuing WVA. Mean mass gains ($mg\,h^{-1}$) were calculated for 15 min or 30 min intervals. A representative plot for an individual of *A. vulgare* is shown in Fig. 2. Above an external a_w of about 0.92, passive losses are countered by WVA. Although maximum uptake rates apparently increase linearly with a_w, these are only occasionally employed; rates of WVA can clearly be regulated to compensate for simultaneous losses and net dehydration of the animal.

By selecting measurements corresponding to the most rapid WVA at a given a_w, regression analysis gives the mean maximum uptake flux and the intercept with the regression line describing passive losses gives the threshold for WVA. The CEA is

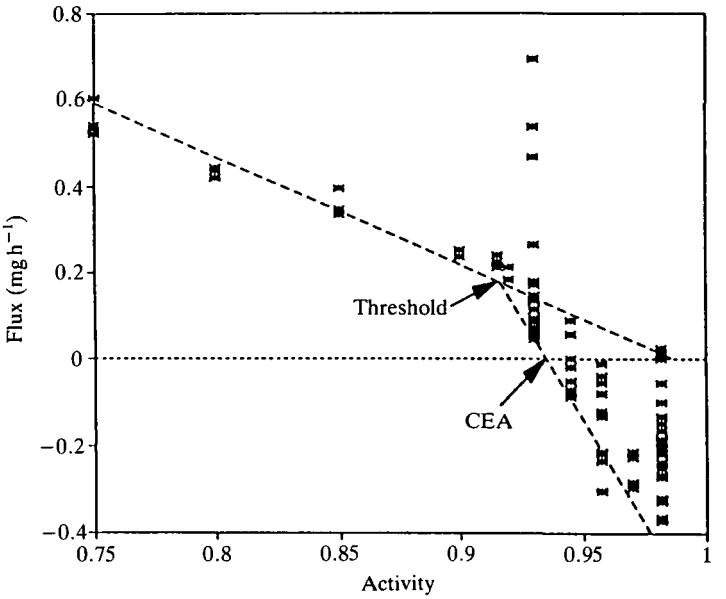

Fig. 2. Example plot of variation in net flux with external water activity for *Armadillidium vulgare*. Positive fluxes (passive losses) vary as a direct function of external activity, indicating near-stable, gradient-independent permeability. Haemolymph and external vapour are in dynamic equilibrium (stable mass) at an activity of about 0.99. Below the regression line for passive losses, flux may be reduced (above the threshold) or reversed (above the CEA) by WVA. Uptake rates are variable in a given external activity, but maximum rates suggest a linear increase in WVA with relative humidity; the uptake mechanism thus shows non-saturated kinetics.

Table 1. *Collective water vapour absorption data from continuous gravimetric monitoring*

Species	*N*	Maximum uptake flux ($mg\,h^{-1}$)	CEA	Threshold activity
Armadillidium vulgare	5	0.514±0.0350	$0.933\pm2.028\times10^{-3}$	$0.913\pm2.433\times10^{-3}$
Porcellio scaber	4	0.488±0.0162	$0.942\pm1.293\times10^{-3}$	$0.921\pm2.867\times10^{-3}$
Oniscus asellus	3	0.609±0.0784	$0.962\pm1.886\times10^{-3}$	$0.935\pm2.867\times10^{-3}$

Values are mean±S.E.
CEA, critical equilibrium activity.
Threshold is the minimum activity at which uptake can occur.

calculated as the intercept between the regression line for maximum uptake rates and the ordinate value where net flux is zero. These values were determined for replicate animals for each species and are listed in Table 1.

An alternative way of expressing these data is to describe uptake in terms of the pump threshold. For a threshold of a_t, and external activity of a_x: standardized

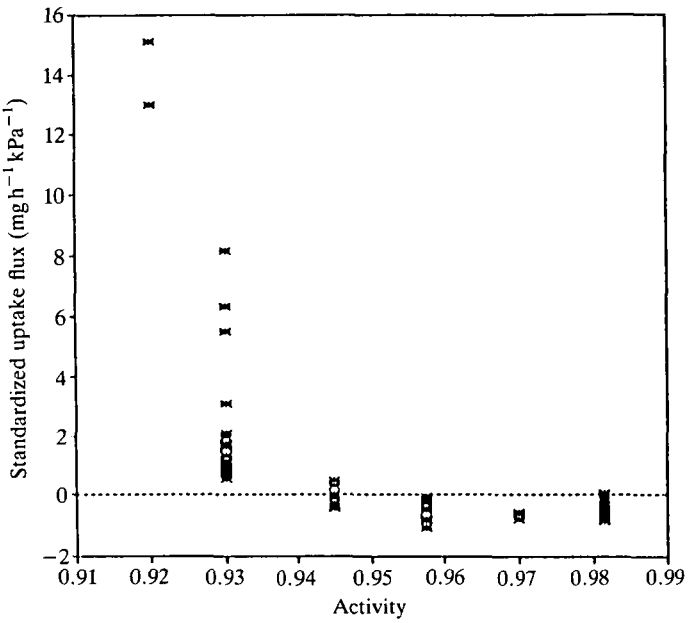

Fig. 3. The relationship between standardized uptake flux ($mg\,h^{-1}\,kPa^{-1}$) and external activity for the data set in Fig. 2. Uptake fluxes ($mg\,h^{-1}$) have been corrected for passive losses and divided by the vapour pressure deficit between external air and uptake fluid, assuming a fluid activity of 0.918. This activity determines the uptake threshold. Standardized uptake fluxes above this activity show near-constant maxima (dotted line), indicating a non-saturating uptake mechanism.

uptake flux ($mg\,h^{-1}\,kPa^{-1}$)=uptake flux ($mg\,h^{-1}$)/$[(a_x-a_t)VP_0]$, where VP_0 is the saturated vapour pressure at the experimental temperature. Uptake flux is here calculated as the measured uptake flux minus the mean cuticle flux (losses). The relationship between standardized uptake flux and a_w is illustrated in Fig. 3, assuming a threshold activity of 0.918. This represents the uptake capacity of the pump (O'Donnell and Machin, 1988), corrected for vapour pressure difference. Maximum values, corresponding to the maximum uptake fluxes plotted in Figs 1 and 2, approximate to constancy above the threshold (a_t). Thus, the uptake mechanism does not saturate at high humidities – for example, because of increasing dilution of uptake fluid prior to resorption – and the pump can exploit very high humidities for rapid WVA.

Intermittent weighing

Mass changes of isopods incubated in controlled humidities for several days are illustrated in Fig. 4A,B; control bottles (without lids) showed no long-term mass changes during the course of experiments. All three species survived prolonged exposure to 98 % RH and utilised WVA (liquid water was not available) to balance passive losses closely. *Armadillidium vulgare* was also capable of WVA in 93.5 %

RH, maintaining near-constant mass, although the other species died in this humidity (Fig. 4B). No species was capable of WVA in 88% RH. Results for replicates are summarized in Table 2.

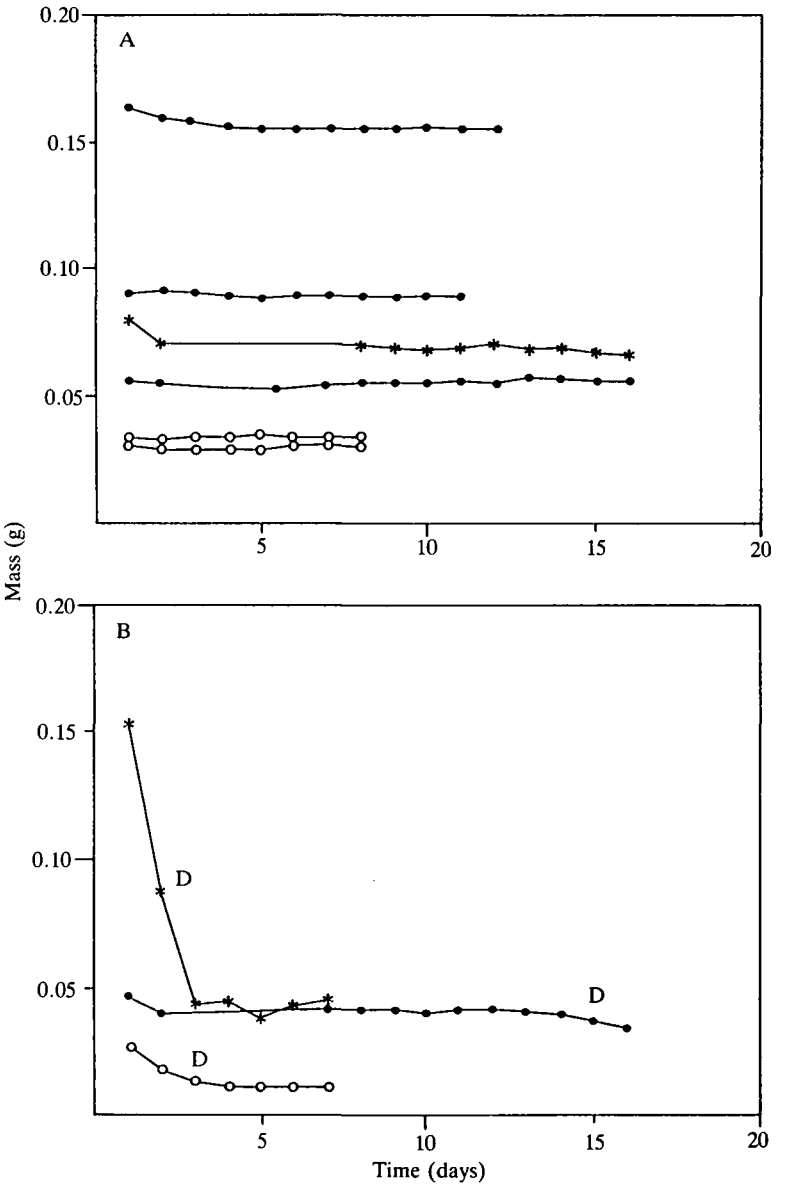

Fig. 4. Mass changes of individual animals of the three study species in controlled humidities as revealed by intermittent (daily) weighing. (A) In 98% RH, each species regulates its mass closely using WVA. (B) In 93.5% RH, only *Armadillidium vulgare* (●) demonstrates a capacity for prolonged WVA, the remaining species dehydrating lethally (D) after 1–2 days. ○ *Porcellio scaber;* * *Oniscus asellus.*

Table 2. *Survival of isopods in controlled humidities (intermittent weighing)*

Species	Humidity (%)	*N*	% surviving more than 2 days	% surviving more than 6 days
Armadillidium vulgare	98.0	5	80.0	60.0
	93.5	6	66.7	33.3
Porcellio scaber	98.0	4	50.0	50.0
	93.5	3	0	0
Oniscus asellus	98.0	4	75.0	50.0
	93.5	3	0	0

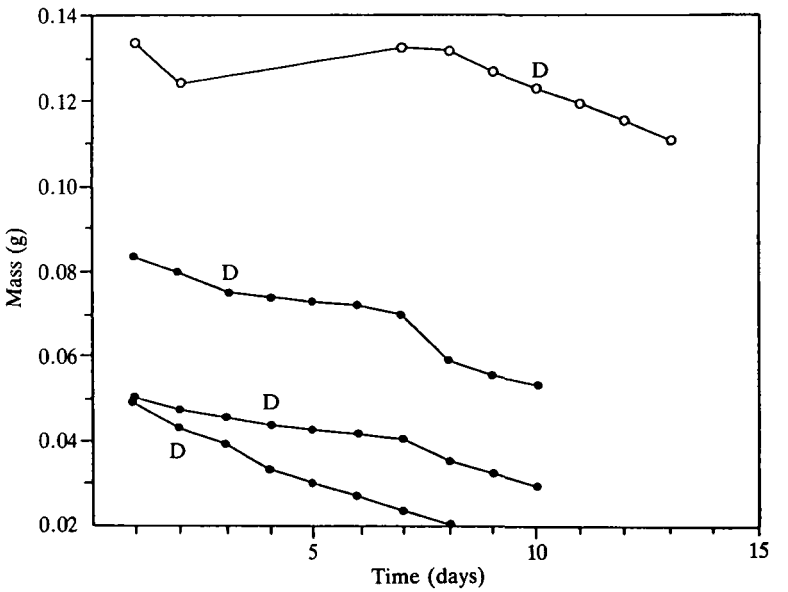

Fig. 5. Mass changes of *Armadillidium vulgare* in 98% RH following blocking of pleopods (closed symbols) and anus (open symbols) with beeswax:colophony. Mass regulation by WVA is only inhibited by complete blocking of the pleopodal surface, resulting in lethal dehydration (D) after 1–3 days.

Blocking experiments

Wax-blocking of the oral and rectal apertures had no distinguishable effect on WVA; dehydrated animals utilised vapour uptake to maintain water balance in the usual way. Blocking the pleopodal surface, however, prevented uptake. Example plots of mass change during intermittent weighing experiments are shown in Fig. 5 and results are summarized in Table 3. These results were amply confirmed by continuous gravimetric monitoring. Partial blocking of the pleopodal surface (anterior or posterior halves) did not inhibit uptake and had no determinable effect on uptake rates with continuous monitoring. Inhibition of WVA by

Table 3. *Survival of isopods in 98.0% relative humidity following blocking treatments*

Species	Blocked site	*N*	% surviving more than 2 days	% surviving more than 6 days
Armadillidium vulgare	Mouth	2	100.0	100.0
	Rectum	4	75.0	25.0
	Pleopods (total)	4	0	0
	Pleopods (anterior)	1	100.0	100.0
	Pleopods (posterior)	1	100.0	100.0
Porcellio scaber	Mouth	1	100.0	100.0
	Rectum	2	50.0	50.0
	Pleopods	2	0	0
Oniscus asellus	Rectum	2	50.0	50.0
	Pleopods	6	0	0

complete blocking may thus act by halting gaseous or liquid transfer between the pleon (endopods?) and the exterior. Control animals with blocked pleopods survived for several weeks in culture.

Activities associated with water vapour absorption

Low-power observation of isopods utilising WVA during continuous monitoring reveals a clear association between uptake and pleon ventilation. 'Pumping' of the pleopodal exopods was always visible during uptake and only occasionally observed at other times, when it probably serves to remove excess liquid from the water transport system (Wasserleitungssystem) during maxillary excretion (J. C. Wright, unpublished observations). Each pump cycle comprises a shallow posterior (retrograde) wave of pleopodal depression, lasting 1–3 s, an intermediate period of about 1 s, and an anterior (direct) wave of elevation of similar duration to the depression wave. Consecutive cycles are separated by varying intervals. Unrestrained animals remain motionless during WVA and adopt a hunched posture with the mid-ventral surface lifted from the substratum. This probably facilitates gaseous exchange in the pleon.

During a prolonged bout of uptake, the ventilation rhythm is regular and cycle frequencies were counted for *P. scaber* and *A. vulgare* to study the relationship between ventilation, humidity and absorption rate. Results are summarized in Table 4. Animals show faster and more conspicuous cycling as humidity is increased, with concurrent increases in uptake flux. Two of the animals interrupted WVA for a significant period during observations, and the relationship between ventilation, uptake flux and humidity was subsequently altered. However, in the remaining animals (*P. scaber* 1 and 3), the increase in ventilation rate with humidity is very clear.

These results also confirm the observations from continuous monitoring that animals vary uptake fluxes widely above the threshold activity. This may be

Table 4. *Pleopodal ventilation frequency and uptake flux of isopods in different humidities*

Animal	RH (%)	Mean ventilation frequency (min^{-1})	S.E.	Mean uptake flux ($mg\,h^{-1}$)	Standardized uptake flux ($mg\,h^{-1}\,kPa^{-1}$)
Porcellio scaber 1	96.3	8.00	0.143	−0.050	−0.93
	97.5	8.05	0.116	0.015	0.18
	98.2	8.80	0.050	0.055	0.54
P. scaber 2	97.0	13.38	0.145	0.020	0.29
	98.0	14.70	0.259	0.110	1.18
	97.5	16.58	0.084	0	0
P. scaber 3	98.5	9.70	0.126	0.150	1.43
	95.0	8.57	0.046	−0.240	−10.29
	97.0	9.00	0.128	0.050	0.71
Armadillidium vulgare 1	96.9	6.88	0.265	−0.115	−1.01
	97.5	6.80	0.098	−0.120	−0.93
	98.2	16.73	0.375	−0.120	−0.83

achieved by changes in ventilation, whilst always employing uptake fluid of fixed (threshold) activity, or by secreting an uptake fluid of activity appropriate to local needs and adjusting pleopodal ventilation to maximize uptake in differing humidities. Employment of the latter mechanism would be energetically efficient, utilising uptake fluid of minimum osmolality with maximum efficiency, rather than utilising fluid of maximum (invariable) osmolality with variable efficiency. Support for such a mechanism is provided by the approximately linear relationship between uptake flux and external humidity during an uninterrupted bout of WVA (Fig. 6). Maximum uptake flux can be attained by exploiting a large activity gradient between air and uptake fluid (high RH) for brief periods and ventilating the system frequently, rather than absorbing the maximum quantity of water available per ventilation by allowing air and uptake fluid to reach equilibrium. This can explain the positive relationship between ventilation rate and external humidity. However, the observation that ventilation frequencies can change between isolated bouts of WVA, other factors remaining unchanged, suggests that uptake fluid activity is also variable.

Pressure changes in the pleon during cycling cause 'shunting' of fluid in the water transport system, and fluid is often visible bulging from the pleon during pleopodal depression. Immediately prior to this, the apposed uropods may be briefly separated, exposing the anal plates, and small movements of these suggest rectal resorption of fluid. Such movements were only associated with rapid vapour uptake, implying that the rectum provides a supplementary avenue for resorption.

Freezing-point depression studies

Transverse sections of the pleon in vapour-absorbing *P. scaber* and *A. vulgare* were observed and photographed sequentially during warming. Melting points of

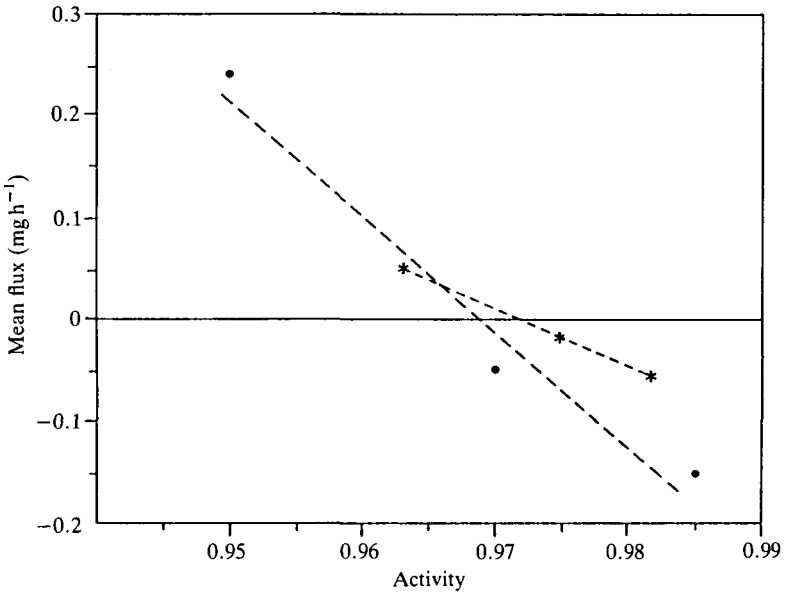

Fig. 6. Plots of uptake flux ($mg\,h^{-1}$) against external water activity for two specimens of *Porcellio scaber* employing sustained WVA during continuous gravimetric monitoring. Increased uptake fluxes were accompanied by concurrent increases in pleopodal ventilation frequency (see Table 4). The observed linear relationships suggest uptake fluid of constant osmolality, but differing in each case. The CEA estimates (at zero uptake flux) show that neither animal is employing WVA at the maximum rate. Lines are fitted by eye.

fluids (temperatures at which complete dissolution of crystals occurs) were estimated and are listed in Table 5. The calcified isopod cuticle and fragility of tissues in non-impregnated specimens prevented fine structural preservation. However, a reliable distinction between dorsal trunk and pleopodal cavity could be made.

Calculated osmolalities of fluids fall into two classes. Haemolymph and cytoplasm have osmolalities of 0.38–0.65 osmol kg^{-1}, showing reasonable agreement with the measurements of R. T. Barrett (unpublished report, Department of Zoology, University of Bristol). Other fluids, confined to the pleopodal region, show more pronounced freezing point depression, corresponding to osmolalities of 1.72–2.15 osmol kg^{-1}. Following O'Donnell and Machin (1988), the relationship between osmolality and a_w is:

$$a_w = [55.5\,\text{mol}\,kg^{-1}]/[55.5 + \text{osmolality (osmol}\,kg^{-1})].$$

From this, the calculated activities of the pleopodal fluids lie in the range 0.963–0.970. Assuming that these represent uptake fluids, the activities represent the pump thresholds. These differ significantly from the thresholds calculated from continuous gravimetric monitoring (Table 1). Both animals studied were frozen

Table 5. *Freezing-point depressions of body fluids in animals utilising water vapour absorption*

Fluid sample	*N*	Freezing point depression (°C)	Osmolality (osmol kg^{-1})	Activity
Porcellio scaber				
Uptake fluid	1	3.2–4.0	1.72–2.15	0.970–0.963
Haemolymph	2	0.7–1.2	0.38–0.65	0.993–0.988
Armadillidium vulgare				
Uptake fluid	6	3.60 (S.E. 0.190)	1.94 (S.E. 0.102)	0.966 (S.E. 1.72×10^{-3})
Haemolymph	7	1.04 (S.E. 0.057)	0.56 (S.E. 0.031)	0.990 (S.E. 5.47×10^{-4})

whilst maintaining approximately constant mass in 98.2 % RH. Since uptake and losses are balanced in this instance, the pump thresholds will be a little below 0.98. The calculated fluid osmolalities indicate that variability of pump threshold might be achieved by varying the concentration of uptake fluid. Minimum pump thresholds, as estimated from continuous gravimetric monitoring (Table 1), would be employed for WVA in lower humidities or in response to more severe dehydration.

Discussion

Complementary techniques provide clear evidence for the presence of WVA in the terrestrial isopods studied. Identification of the ventral pleon as the region of uptake confirms the suggestion of Coenen-Stass (1989) and constitutes a new site for WVA, uptake structures in other Arthropoda being confined to the oral and rectal cavities. Like these, the pleopodal cavity of isopods provides a ventilated chamber containing moistened cuticle surfaces.

Although the origin of the uptake fluid has not been elucidated, the transporting epithelia of the pleopodal endopods (Kummel, 1981) seem likely to be involved in the secretion and/or resorption processes. The proposal of Kummel (1984) that the endopods are organs of active ion exchange would pre-adapt them for WVA. This is currently under investigation. At least two processes influence the uptake flux generated at a given a_w. The ventilation rate of the pleopodal cavity determines the duration of exposure of uptake fluid to air, allowing animals to exploit large activity gradients for short periods, or lower gradients for longer periods, and hence maximize uptake flux for uptake fluid of a given osmolality. Specific activity gradients are, in turn, affected by the osmolality of uptake fluid. The low variability of osmolalities measured by freezing-point depression supports the assumption that these values represent freshly secreted uptake fluid rather than fluid undergoing progressive dilution during vapour uptake. Continuous

gravimetric monitoring reveals that animals can regulate uptake fluxes to balance losses in activities exceeding the CEA. This is most easily explained by the production of variably hyperosmotic uptake fluid. Ventilation rate may then provide a short-term means of maximising uptake in fluctuating humidities. The observation that ventilation rate is not constant for given vapour-pressure deficits and uptake rates supports this idea.

The significance of uropodal movements during rapid WVA suggests rectal resorption of fluids. This is probably not the usual avenue, since corresponding movements were only rarely associated with lower uptake fluxes and anal blocking did not inhibit uptake. The rectum could therefore provide a supplementary resorption pathway. The uptake mechanism shows non-saturated kinetics which may depend on the intervention of rectal resorption during rapid uptake.

The use of WVA in water balance physiology requires compensatory osmoregulation and ion regulation and/or wide tolerance of osmotic and ionic variation. Horowitz (1970) showed that *P. scaber* regulates haemolymph osmolality to near constancy during substantial dehydration, and Price and Holdich (1980) obtained similar results for *A. vulgare*, though no regulation was evident in *O. asellus*. Studying *P. scaber*, Lindqvist and Fitzgerald (1976) produced evidence to suggest that ions are actively absorbed into the gut during dehydration. The reverse process could control osmolality during water uptake by imbibation or WVA. However, although *P. scaber* shows a rapid increase in haemolymph osmolality when dehydrated below 90 % hydrated mass, animals continue to survive a 120 % increase in osmolality during further drying (Horowitz, 1970). Tolerance of hypo-osmosis awaits investigation.

The calculated minimum uptake thresholds are high by the standards of other vapour absorbers (see O'Donnell and Machin, 1988), only permitting WVA in humidities above about 91.3 % for *Armadillidium vulgare* and above about 92.5 % for the more hygric *Porcellio scaber* and *Oniscus asellus*. In addition, the higher integumental permeabilities of the latter species mean that WVA can only balance losses (the CEH) in considerably higher humidities (94–96 %). These uptake capacities would be of only modest adaptive benefit in the colonization of thermic and xeric habitats. The study species may also seem unlikely candidates for WVA since they do not, at first sight, suffer from irregular or unpredictable supplies of liquid water, being characteristic of temperate deciduous litter, and are popularly associated with 'damp' habitats. However, unlike many other macro-invertebrates of woodland litter, oniscideans regularly forage in exposed habitats, venturing over the litter surface or climbing trees to graze on algae and lichens (Paris, 1963, 1965; Den Boer, 1961; Warburg, 1968). Nocturnal habits of temperate species help confine activity to periods of high humidity (Paris, 1963), but ambient humidities will often fall well below saturation (Cloudsley-Thompson, 1956; Edney, 1968, 1977). Given the limited abilities of these isopods to restrict water losses, such foraging patterns may result in substantial dehydration. Diurnal WVA would then provide a means of replenishing water supplies whilst resting in high-humidity litter.

Den Boer (1961), studying *P. scaber*, showed that vertical activity is negatively correlated, and horizontal activity positively correlated, with vapour pressure deficit. Having assumed that vertical migration is not governed by dietary requirements, Den Boer suggested that it serves for the removal of excess water accumulated in cryptozoic habitats during the day. His ideas have not received further investigation. Daily accumulation of a water burden seems unlikely given that *P. scaber* survives indefinitely in saturated air (J. C. Wright, personal observation) yet, like the other species studied, is seriously threatened by standing water. The water-transport system and respiratory surfaces both necessitate hydrophilic cuticle and thus preclude efficient waterproofing; these species die within a few minutes when immersed. Behavioural adaptations will be critical in avoiding free water, either by vertical migration (following rainstorms) or by selection of absorbent and well-drained substrata during the day. These aspects require further investigation, but would be additional selective factors in the evolution of vapour absorption.

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